ICOHAR International Conference on One Health Antimicrobial Resistance
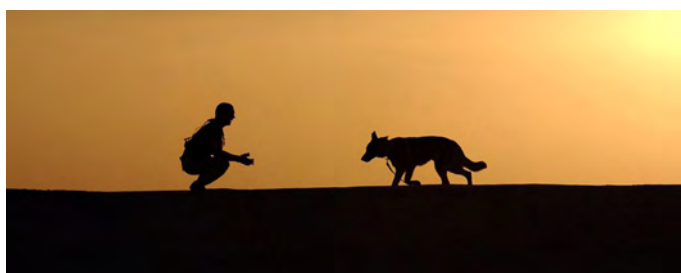

ICOHAR 2019
16-18 April 2019, Utrecht, Netherlands

FINAL PROGRAM

PROGRAM

Day 1 - Tuesday, 16 April 2019
Venue: Utrecht University Hall, Academic Building, Dom Square, Utrecht

15.00-19.00	**Registration**
18.30-18.40	**Welcome speech** by Luca Guardabassi (DK/UK)
18.40-19.05	**Opening keynote lecture** by **Marc Sprenger (World Health Organization):** One Health and AMR: taking stock 4 years after approval WHO Global Action Plan in AMR
19.05-19.30	**Jorge Pinto Ferreira (F): The OIE strategy on antimicrobial resistance**
19.30-20.30	**Welcome reception**

Day 2 - Wednesday, 17 April 2019
Venue: Jaarbeurs MeetUp, Jaarbeursplein, Utrecht

8.30-9.00	**Keynote lecture** by **Jaap Wagenaar (NL):** One Health in antimicrobial resistance: who should care?

	Room: Johan Friso Foyer	**Room 117**
9.00-9.45	**One health integrated surveillance (organized by ESGARS)** *Chair: Arjana Tambic (CR)* **Luis Martinez- Martinez (ES):** Supranational Networks for surveillance of human multiresistant pathogens **Frank Møller Aarestrup (DK):** The relative contribution by livestock to resistance problems in human medicine	**New approaches for precision antimicrobial therapy (organized by EPASG).** *Chair: P.L. Toutain (F)* **Leonardo Pagani (I):** PK/PD-driven antimicrobial therapy in ICU **Alain Bousquet-Mélou (F):** New approaches for precision antimicrobial therapy
9.45-10.30	**MDR in Mycobacteria and Mycoplasma of human and animal origin (organized by ESGMYC and ESGMI).** *Chair: Nir-Oaz Ran (IL)* **Miguel Viveiros (P):** AMR in Tuberculosis and Non-Tuberculosis Mycobacteria: a slow selective process in progress in One-Health, One-World **Sabine Pereyre (F):** Resistance and multi-drug resistance in mycoplasmas of human and animal origin	**The use of animal models for assessing antimicrobial impact on the gut microbiome (organized by ESGVM and ESGHAMI).** *Chair Ed J. Kuijper (NL)* **Hauke Smidt (NL):** In vitro and animal models of the gut microbiome **Luis Pedro Coelho (ROC):** The gut microbiome of dogs and cats shares genes and species with the human gut microbiome.
10.30-10.45	**Coffee break**	
11.00-11.30	**Invited lecture** by **Lloyd Reeve -Johnson (AUS):** One Health and AMR: taking stock 4 years after approval WHO Global Action Plan in AMR	
11.30-12.15	**AMR in Clostridium difficile lineages shared by humans and animals (organized by ESGCD).** *Chair: John Coia (DK)* **Maja Rupnik (SL):** AMR and C. difficile from different reservoirs **Kate Dingle (UK):** A role for tetracycline selection in recent evolution of the agriculture-associated C. difficile PCR-ribotype 078	**Education on responsible antibiotic use in European medical and veterinary universities (organized by ESGAP).** *Chair: Jeroen Schouten (NL)* **Carmen Espinosa Gongora (DK):** Training the next generation of veterinary prescribers **Oliver Dyar (S):** Are we preparing medical students to prescribe antibiotics responsibly?
12.15-13.00	**Zoonotic transfer of AMR: the case of E. coli (organized by ESGMD).** *Chair: John W.A. Rossen (NL)* **John WA Rossen (NL):** Next generation sequencing: first diagnostic one-stop shop in one-health microbiology **Adrian Tett (IT):** Cultivation-free detection and characterisation of pathogens by metagenomics	**The impact of vaccination programmes on reduction of antimicrobial use (organized by EVASG).** *Chair: Johanna Fink-Gremmels (NL)* **Susanna Esposito (I):** Impact of influenza and pneumococcal vaccines on antimicrobial resistance **Anders Fabio Antenucci (DK):** How vaccination prevented an emerging E. coli epidemic and antimicrobial overuse in the Scandinavian broiler production
13.00-14.00	**Lunch break & Poster viewing**	
14.00-14.30	**Invited lecture** by **Willem van Schaik (UK):** The complex dynamics of antimicrobial resistance and microbiomes	

	Room: Johan Friso Foyer	**Room 117**
14.30-15.15	**The role of the environment in the spread of AMR (organized by EFWISG).** *Chair: Teresa Coque (SP)* **Luísa Vieira Peixe (P):** Key players driving environmental emergence and spread of AMR **Johan Bengtsson-Palme (S):** The environment and the antibiotic resistance development – now and in the future	**Nosocomial infections: a One Health perspective (Organized by ESGNI).** *Chair: Margreet C. Vos (NL)* **Laurent Poirel (CH):** Emergence of acquired polymyxin resistance in Gram negatives; perfect example of a One-Health issue **Dorina Timofte (UK):** Infection control in veterinary hospitals: why it matters
15.15-16.00	**One Health issues related to AMR in staphylococci (organized by ESGS).** *Chair: Jodi Lindsay (UK)* **Francois Vandenesch (F):** Antibiotic pollution as potential driving force of CA-MRSA expansion **Anette Loeffler (UK):** MRSP carriage in dogs: A risk to people?	**Management of critically ill patients (organized by ESGCIP).** *Chair: David Dockrell (UK)* **Jeroen Schouten (NL):** The role of Antimicrobial stewardship programs in intensive care units **Joris Robben (NL):** The challenge of infection control in a small animal intensive care unit
16.00-16.30	**Coffee break**	
16.45-17.15	**Roundtable discussion on the lessons learned from emerging resistances in the past (e.g. VRE, ESBL, MRSA, carbapenem and colistin resistance).** *Chair: Patrick Butaye (B/SKN)* Participants: Lina Cavaco (DK), Laurent Poirel (F), Willem van Schaik (UK), Patricia Poeta (PT) and Youjun Feng (CN)	
17.15-18.00	**Antimicrobial use guidelines for urinary and respiratory tract infections in human and veterinary medicine: a One Health perspective** **Invited lecture** by **Joseph Blondeau (CA)** and **Michael Lappin (USA)**	
18.00-18.30	**Keynote lecture** by **Paul Flowers (UK):** Antimicrobial resistance: a biopsychosocial problem requiring innovative interdisciplinary and imaginative interventions	
18.30-20.00	**Programme break**	
20.00-22.15	**Social Dinner** Stadskasteel Oudaen, Oudegracht 99, Utrecht	

PROGRAM (continued)

Day 3 - Thursday, 18 April 2019
Venue: Jaarbeurs MeetUp, Jaarbeursplein, Utrecht

8.30-9.00	**Keynote lecture: Sensitive pathogen detection and rapid AST in the One Health future** by Alex Van Belkum (F)
9.00-9.30	**Invited lecture: Antifungal use in veterinary practice and emergence of resistance.** by Amir Seyedmousavi (International Society for Human and Animal Mycology)
9.30-10.00	**Invited lecture: CBPs, ECOFFs, Intermediate, and all that jazz** by John Turnidge (European Committee on Antimicrobial SusceptibilityTesting)
10.00-10.30	**Roundtable discussion on the future of antimicrobial susceptibility testing** *Chair: Luca Guardabassi (DK/UK);* participants: **John Turnidge** (EUCAST secretary), **Peter Damborg** (VetCAST chair), **Hilde Moyaert** (Animal Health Europe/Zoetis), **Jordi Torren** (European Medicines Agency) and **Sakurako Marchand** (bioMerieux)
	Coffee break
11.00-11.30	**Invited lecture: What can we learn from One Health evaluation of interdisciplinary AMR research projects?** by Liza Rosenbaum Nielsen (DK)
11.30-12.15	**Presentations from selected abstracts**
12.15-12.45	**Closing keynote lecture: Alternatives to conventional antimicrobial agents: present and future** by David Gally (UK)
12.45-13.00	**Closing remarks** by Johanna Fink-Gremmels (NL) and Ed J. Kuijper (NL)
13.00-13.30	**Lunch package & refreshments**

The ICOHAR Organizing Committee:

Prof. Luca GUARDABASSI (ESGVM)
University of Copenhagen (DK) and Royal Veterinary College (UK)

Prof. Patrick BUTAYE (ESGVM)
Ross University School of Veterinary Medicine (SKN) & Ghent University (B)

Prof. Johanna FINK-GREMMELS (NCOH and EAVPT)
Utrecht University (NL)

Prof. Ed J. KUIJPER (ESGHAMI and NCOH)
Leiden University Medical Center (NL)

Important Information

Opening Hours Registration:
Tuesday 16 April: 15.00 – 19.00 in the Utrecht University Hall, Academic Building, Dom Square
Wednesday, 17 April: 8.00 – 12.00 in the Jaarbeurs MeetUp, Jaarbeursplein

> ### It will be possible to register onsite: €250 for one day and €500 for the entire conference

Currency
All official prices are indicated in Euro (€). The official currency in The Netherlands is EURO (€). All major credit cards are accepted in most hotels, restaurants and shops.

Insurance
The congress organisers cannot accept liability for personal injuries sustained, or for loss or damage of property belonging to conference participants, either during, or as a result of the meeting. Please check the validity of your own insurance.

Language
The congress language is English. Simultaneous translation will not be provided.

Sponsors

ESCMID (supporting organization)

bioMérieux (platinum sponsor)

Zoetis (supporter)

Organization:

ICOHAR 2019 is supported by the European Society for Clinical Microbiology and Infectious diseases (ESCMID) and organized by the ESCMID Study Group for Veterinary Microbiology (ESGVM). As part of its mission, ESGVM promotes One Health by facilitating joint research and training collaborations between medical and veterinary microbiologists within areas of common interests, namely zoonoses and antimicrobial resistance. The event is organized in collaboration with other organizations:

The central theme of ESCMID Study Group for Host and Microbiota Interactions (ESGHAMI) focuses on clinical significance of and therapeutic interventions on host-microbiota associated diseases and health in both humans and animals.

AMR and One Health are priority topics for research and education at the Department of Veterinary and Animal Sciences of the University of Copenhagen. Among the various One Health activities, the Department coordinates a large multidisciplinary research center on control of AMR in people and animals (www.uc-care.ku.dk) and is involved in the organization of MSc course on AMR shared by veterinary and medical students.

The European Association of Veterinary Pharmacology and Toxicology (EAVPT) is the leading organisation of veterinarians active in the field of pharmacology and pharmacotherapuetics in Europe. The Association is strongly committed to the development of safe and effective therapeutics in the context of One health initiatives cognisant of the impact on animal, human and environmental health.

Edinburgh Infectious Diseases (EID) is the network of infectious disease researchers and clinicians in Edinburgh. AMR is a priority research topic for this network, which fosters infectious disease teaching and training at all levels within the University, including the development of a new MSc in One Health.

Netherlands Center for One Health aims for an integrated One Health approach to tackle the global risk of infectious diseases, and commits to create durable solutions for this major challenge by bundling world-leading academic top research in the Netherlands in the area of One Health.

The World Small Animal Veterinary Association (WSAVA)

Conference Venue

The Welcome reception on April 16th will be held at the **Utrecht University Hall, Dom Square,** in the historic city centre of Utrecht. The Academic Building is located directly next to the main Cathedral (The Dom).

The rest of the conference will be held at **Jaarbeurs MeetUp, Jaarbeursplein, Utrecht** (see map below)

Navigation address: Graadt van Roggenweg 400 | 3531 AH Utrecht
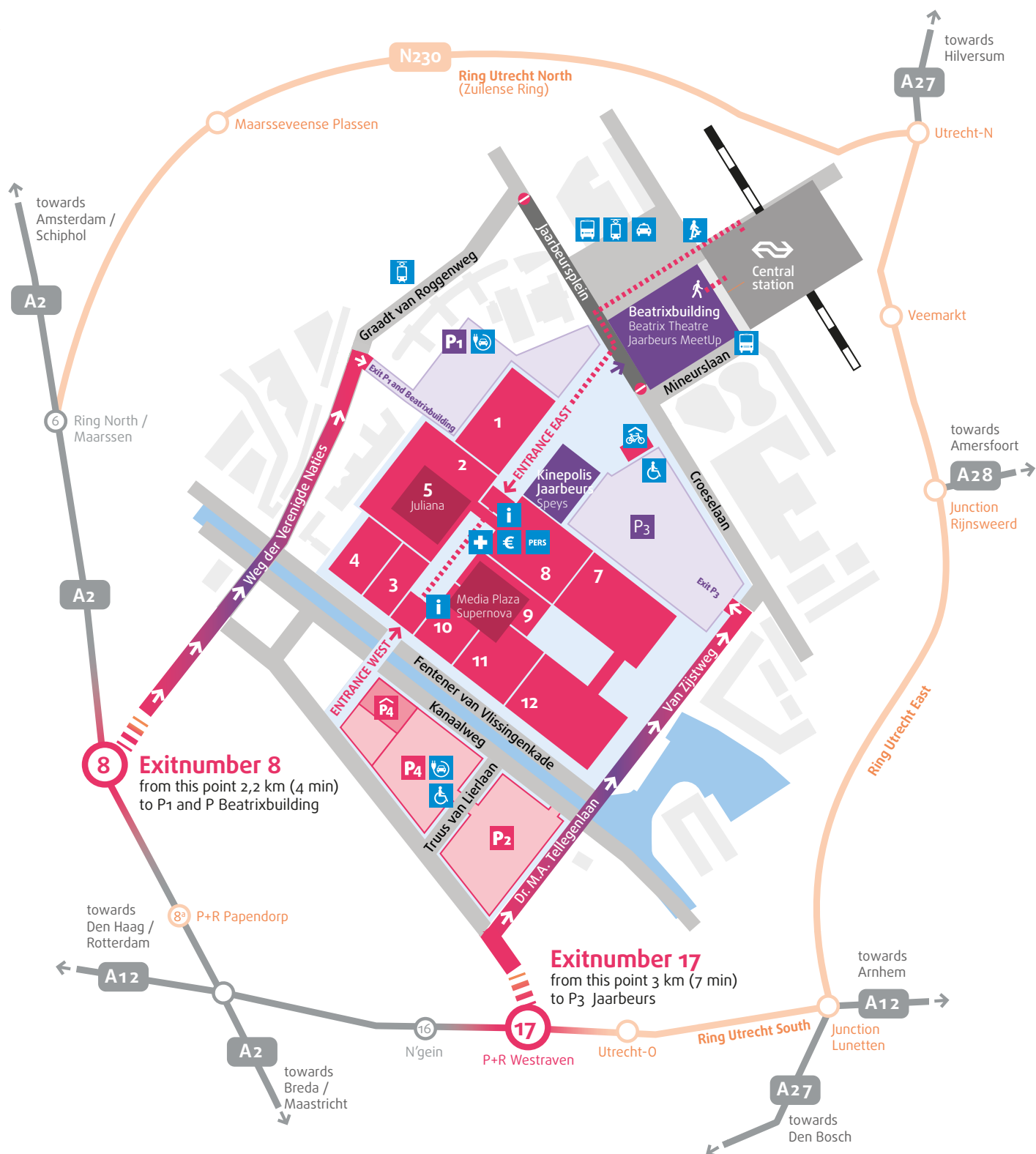
Dear conference participant, dear reader,

It is generally recognized that control and prevention of antimicrobial resistance (AMR) requires a holistic One Health approach involving specialists from different sectors. In line with this concept, the 2ⁿᵈ International Conference on One Health Antimicrobial Resistane (ICOHAR) aims at bringing together representatives from all relevant sectors (e.g. public health, human and veterinary medicine, livestock production, food safety and environmental sciences) to share research and education strategies for understanding and reducing the risks of AMR at the interphase between humans, animals and the environment. The programme does not only focus on zoonotic transfer of AMR but also on the numerous AMR-related challenges shared by clinicians, clinical microbiologists, infectious disease specialists and researchers working in these sectors.

In this conference, the second "International Conference on One Health and Antimicrobial resistance" ICOHAR, we have included as much as possible diverse ecosystems, and updated the audience on contemporary perspectives. It was also an ideal forum to present new data, foster new collaborations and challenge existing.

This online abstract book is a compilation of most of the talks and all poster abstracts presented at the conference. We thank all presenters who have duly contributed their presentations and helped to shape a better collective understanding on the One Health implications of antimicrobial resistance.

We are also very thankful to our sponsors, ESCMID, Biomerieux and Zoetis. This conference would not have been possible without their contributions.

We hope to see you all at the next ICOHAR! Information will follow in due time.

Best regards,

The organizing and scientific committee.
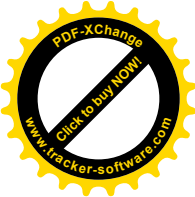
ICOHAR
One Health and AMR: Taking stock 4 years after approval of WHO Global Action Plan on AMR

Marc Sprenger, MD PhD , Director AMR
16 April 2019

World Health Organization

AMR is the Greatest Threat to Modern Medicine

Profound health consequences
- Individuals, health systems, food systems, and practice of medicine

Economic and other intersectoral implications
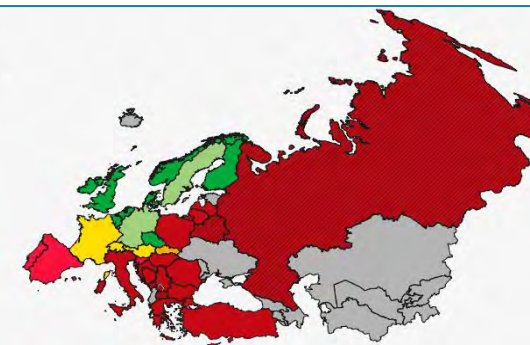- Development, agriculture, food, business, etc.

Long-term threat with no end in sight unless fundamental changes are made

World Health Organization

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Multidrug-resistant *Acinetobacter spp.*
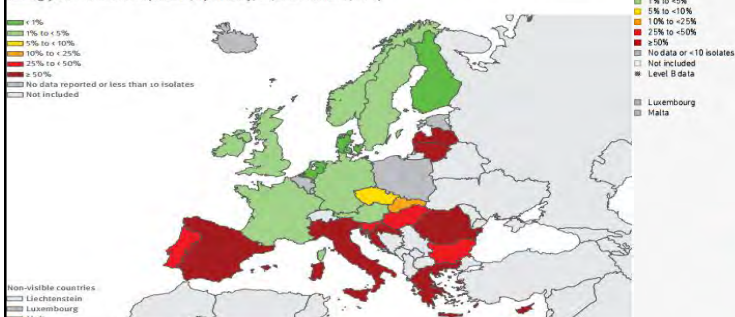
2014 EARS-net

2016 CAESAR

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3 |

Magnitude of Resistance

OECD: 40%-60% of infections in Indonesia, Brazil, Russian Federation resistant
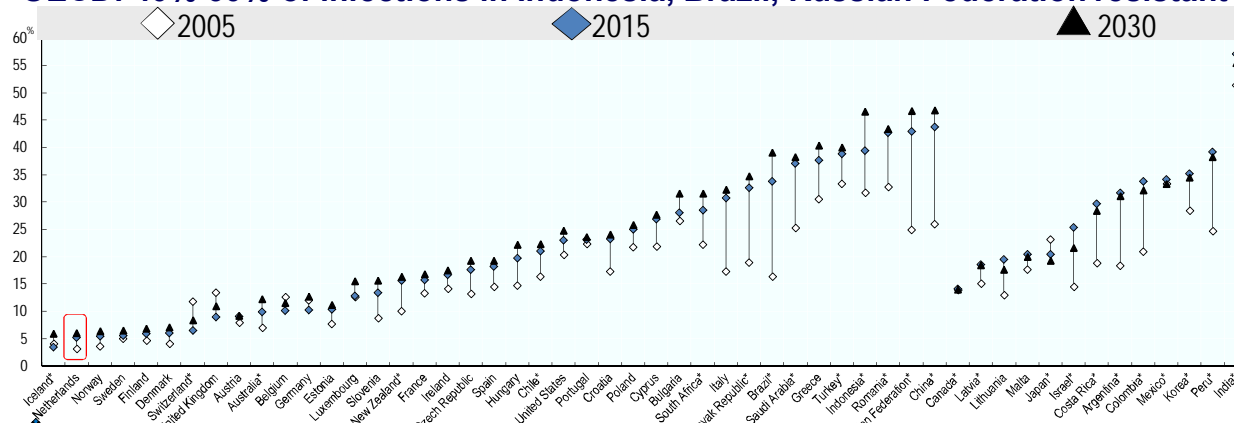
Source: OECD. Stemming the Superbug Tide: Just a Few Dollars More (p. 92). (2018). Available at: https://www.oecd-ilibrary.org/docserver/9789264307599-en.pdf?expires=1546597697&id=id&accname=ocid195767&checksum=3DDA307F378357E85A08C17542ED65F2 (p. 92)

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OECD Analysis: Key Findings

- Between 2015-2050, **2.4 million people could die** in Europe, North America and Australia due to superbug infections

  - **75% of these deaths can be averted** by spending **USD 2** per person/year on selected health policies

- A package of policies to promote hospital hygiene and policies to reduce over-prescription of antibiotics could **save up to 1.6 million lives by 2050** in countries included in the OECD analysis

  - Investment in these policies would pay for themselves within one year's time!

Source: OECD. (2018). Stemming the Superbug Tide: Just a Few Dollars More. *OECD Publishing, Paris.*

World Health Organization

OECD Modelling: Cost Effectiveness of Antimicrobial Resistance Control Policies

Healthcare-based interventions		
Improved hand hygiene	Stewardship programmes	Environmental hygiene
Reduction in mortality due to AMR		
58%	54%	53%

Community-based interventions		
Mass media	Delayed prescribing	RDTS
Reduction in mortality due to AMR		
9%	16%	25%

- A policy package including all 3 hospital-based policies would save on average **USD PPP 1.2 million per 100,000 persons per year**

- A community-based policy package would result in in average reductions in health care expenditure of approximately **USD PPP 275,000**

- Hand hygiene policy has an implementation cost that is on average **10 times lower** than enhanced environmental hygiene policy, and generates savings that are, on **average 15 times higher** than the implementation cost! *(implementation cost of USD PPP 8,500 per 100,000 persons for a net return of around USD PPP 140,000)*

Source: OECD. (2018). Stemming the Superbug Tide: Just a Few Dollars More. *OECD Publishing, Paris.*

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Modelling Analysis: Attributable Deaths and DALYs by Antibiotic-Resistant Bacteria in EU and EEA in 2015

- **671 689** infections with antibiotic-resistant bacteria
  - 33 110 attributable deaths
  - 874 541 DALYs

- **An estimated 63.5%** of antibiotic-resistant infection cases are associated with health care, leading to
  - **72.4%** attributable deaths
  - **74.9%** of DALYs per 100,000 population

- **Infection prevention and control in health care facilities is KEY!**

Source: Cassini, A., Hogberg, L. D., Plachouras, D., Quattrocchi, A., Hoxha, A., Simonsen, G. S.,...the Burden of AMR Collaborative Group. (2019). Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: A population-level modelling analysis. *The Lancet Infectious Diseases*, *19*(1), p. 56–66.

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A Complex and Multisectoral Problem

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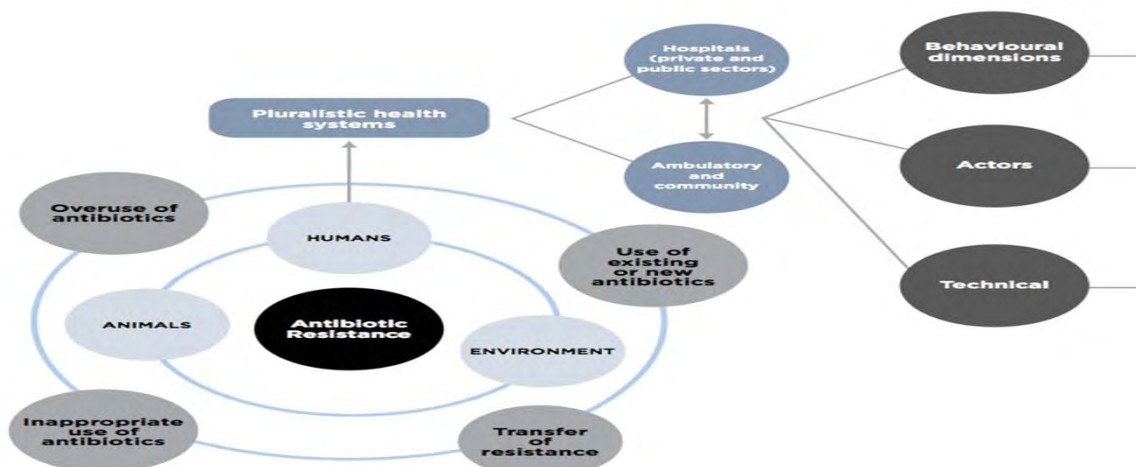
Magnitude Problem: Communicable Disease Burden

Figure 21: Burden of disease by broad cause group and region, 2004

World Health Organization
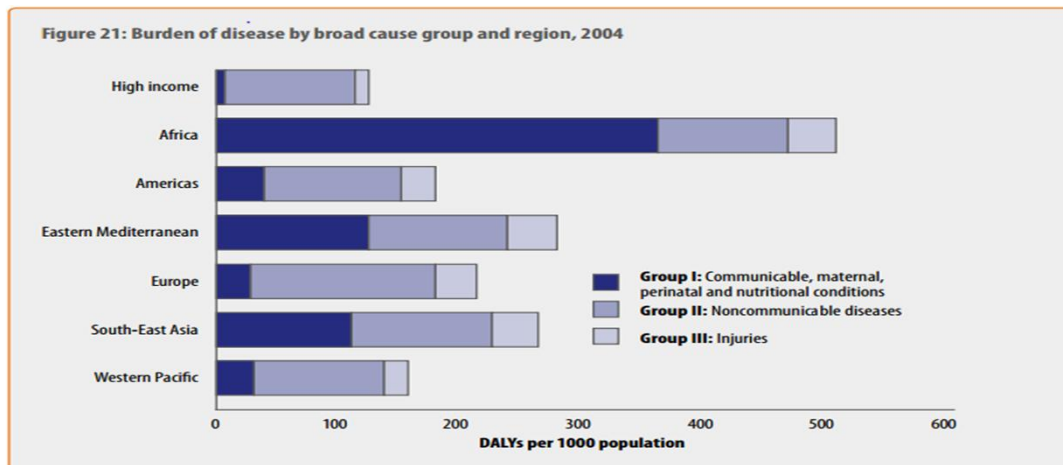
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Factors Contributing to AMR

Holmes at al., 2016

World Health Organization
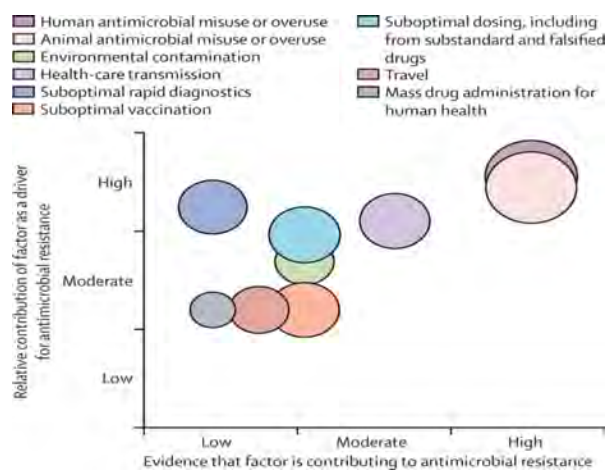
10 |

Use of Antibiotics Is On The Rise

Total global antibiotics consumption increased 30%
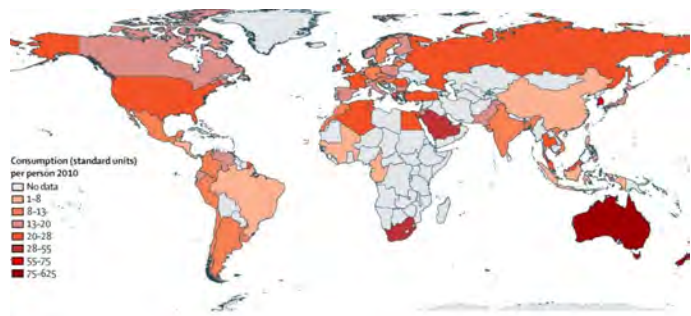
Van Boeckel et al. The Lancet Infectious Diseases 2014 14, 742-750DOI: (10.1016/S1473-3099(14)70780-7)

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Antibiotics Consumption

In food prod animals & humans mg/kg biomass
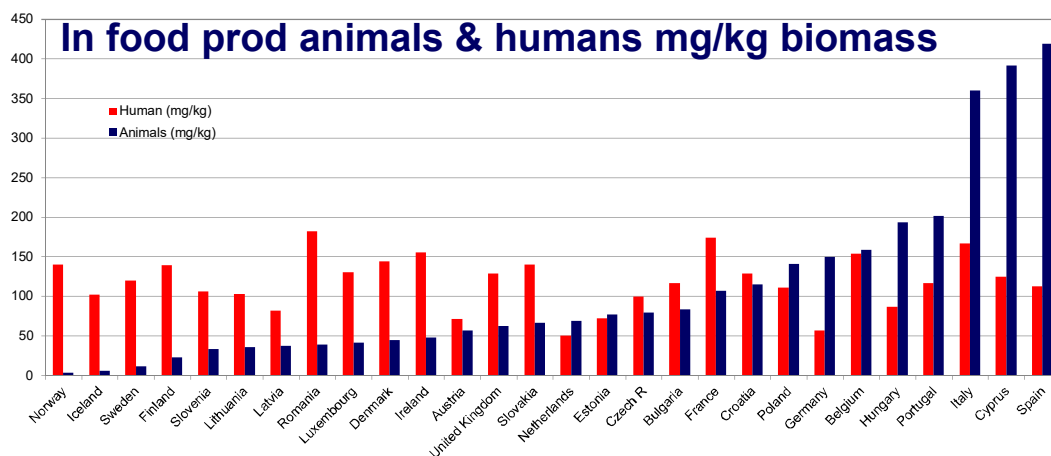
(JIACRA ECDC/EFSA/EMA, 2017)

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Global, projected consumption of protein from animal-source foods in 2030: (a) per square kilometre

Consumption (kg/sqkm)

0	100 - 500
0 - 10	500 - 5 000
10 - 50	> 5 000
50 - 100	No Data

No individual country data available for Europe

http://www.fao.org/d 2425e00.htm

Antibiotic Excretion by Farm Animals (kg/10km2)
(modelled)

Log10 [(mg/pixel)+1]

0 - 1	
0 - 4	
4 - 5	
5 - 6	
6 - 7	
7 - 8	
8 - 9	
9 - 10	
10 - 11	

Source: Adapted from Van Boeckel et al., 2015;
Assumption: 50% of consumption excreted

Use of antimicrobials in plant production

Food and Agriculture Organization of the United Nations

T.Smith

Erwinia amylovora, bacterium causing fire blight,
A disease of apples and pears in many parts of the world
Pulverization with streptomycin

Injections with tetracycline
in tree stems (how common?)

AMR and the SDGs

1 NO POVERTY **AMR hardest on the poor**	**6 CLEAN WATER AND SANITATION** **Antibiotic residues (hosp, pharma & agri contaminate water**
2 ZERO HUNGER **Untreatable infections in animals threaten food prod**	**8 DECENT WORK AND ECONOMIC GROWTH** ***Cumulative costs AMR $120 trillion by 2050**
3 GOOD HEALTH AND WELL-BEING **AM core components health systems**	**12 RESPONSIBLE CONSUMPTION AND PRODUCTION** **Balance access, innovation and conservation of AM**
17 PARTNERSHIPS FOR THE GOALS **Require multi-stakeholder partnerships**	

*World Bank Group Report on Drug-Resistant Infections (March 2017)

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Global Action Plan Antimicrobial Resistance

- Adopted by World Health Assembly in 2015

- Recognized & supported by FAO (Resolution 4/2015) and OIE (Resolution 26) governing bodies in 2015

- AMR Resolution in January 2019

- AMR Resolution to be presented at World Health Assembly in May 2019

GLOBAL ACTION PLAN
ON ANTIMICROBIAL
RESISTANCE

World Health Organization

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Global Action Plan's 5 Strategic Objectives

1. Improve awareness and understanding
2. Strengthen knowledge through surveillance & research
3. Reduce the incidence of infection
4. Optimize the use of antimicrobial medicines
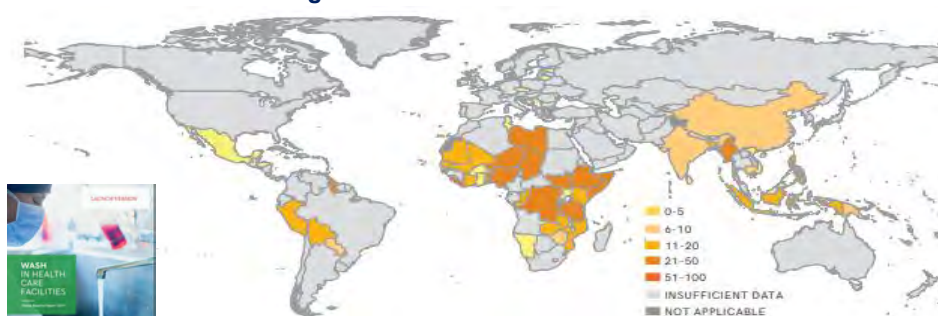5. Ensure sustainable investment

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RESISTANCE

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Develop National Action Plans

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Country Progress on Development of NAP

Source: TrACSS

World Health Organization
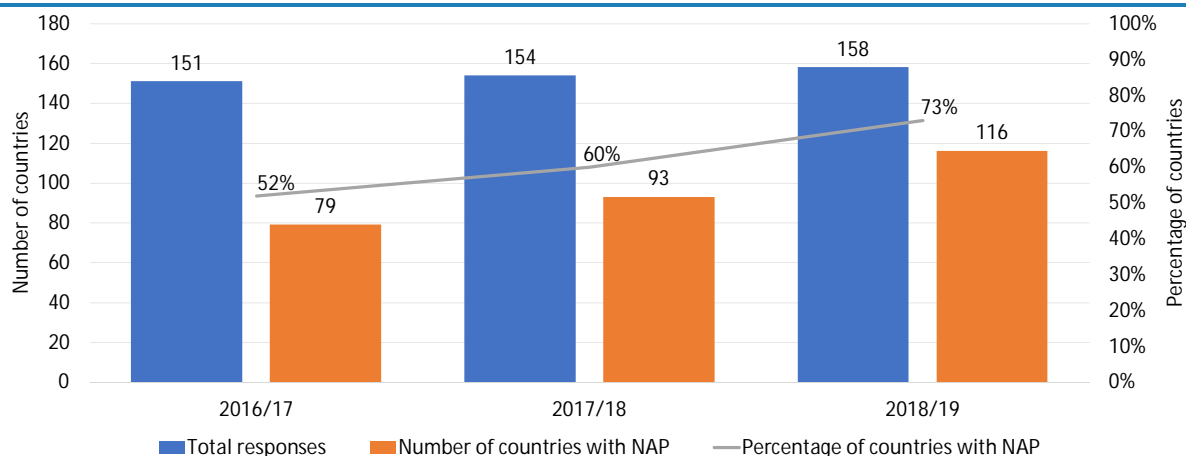
Proportion of Low-Income Countries with NAPs

Source: TrACSS

World Health Organization
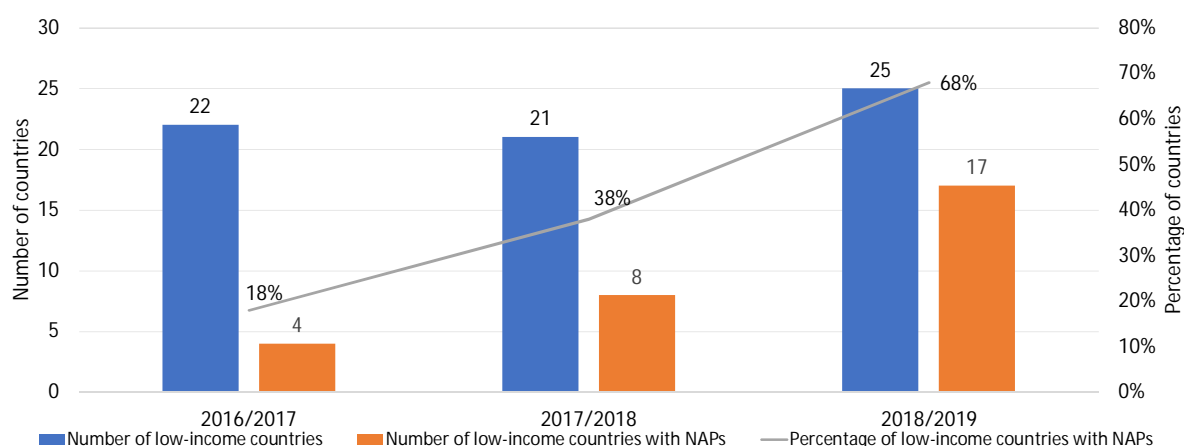
Country Progress on Multisectoral Working Group on AMR
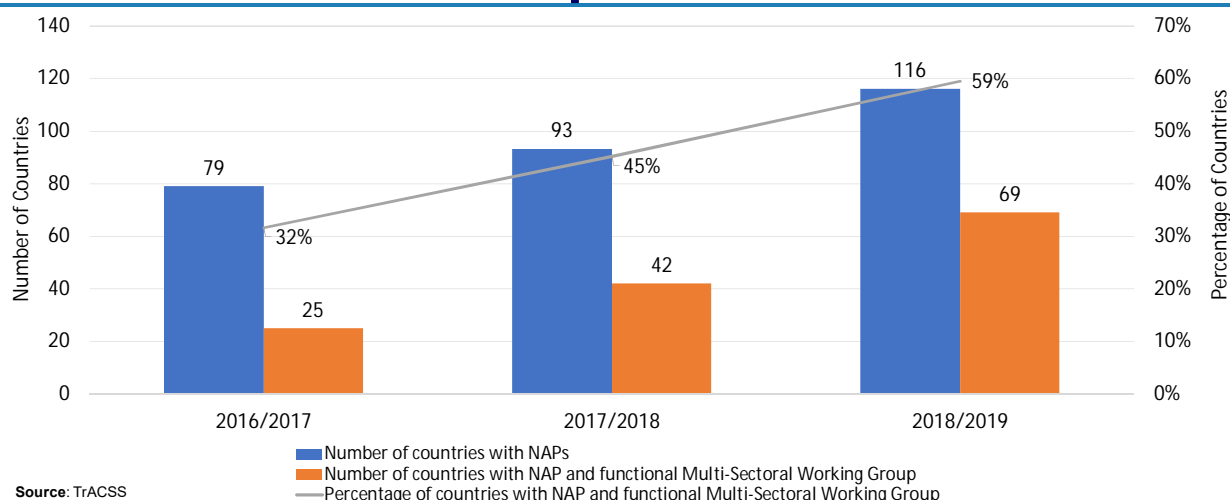
Source: TrACSS

Legend:
- Number of countries with NAPs
- Number of countries with NAP and functional Multi-Sectoral Working Group
- Percentage of countries with NAP and functional Multi-Sectoral Working Group

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# Global Action Plan's 5 Strategic Objectives

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GLOBAL ACTION PLAN
ON ANTIMICROBIAL
RESISTANCE

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Awareness & Behavior Change

World Antibiotic Awareness Week

HANDLE ANTIBIOTICS WITH CARE

Competency Framework

WHO COMPETENCY FRAMEWORK FOR HEALTH WORKERS' EDUCATION AND TRAINING ON ANTIMICROBIAL RESISTANCE

Participation from 130 countries in 2018, and nearly 500 events reported globally
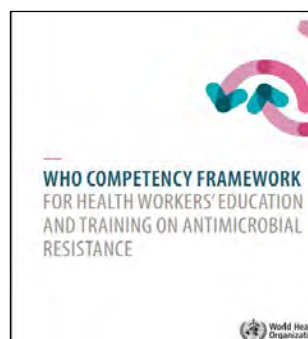
Includes training module on infection prevention and control for AMR

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Global Action Plan's 5 Strategic Objectives

1. Improve awareness and understanding
2. **Strengthen knowledge through surveillance & research**
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GLOBAL ACTION PLAN ON ANTIMICROBIAL RESISTANCE

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Information for Action: Global Antimicrobial Resistance Surveillance System

77 countries ENROLLED in GLASS

64% increase in country enrolment compared to previous year!

More than double the number countries are SUBMITTING DATA through GLASS compared to 2016-2017 Report   (**48 vs. 22**)

Enrolment completed (n=71)
Enrolment in progress (n=6)
* Call for country enrolment issued on 21 March 2016
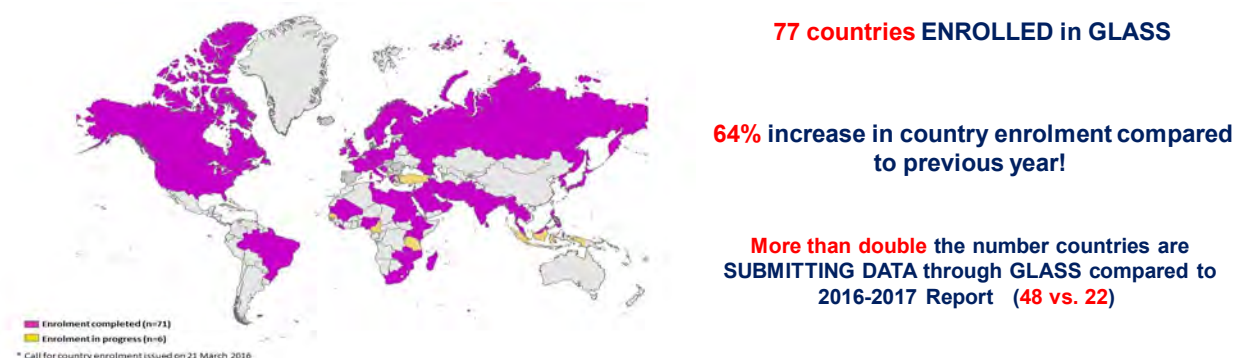
Ongoing data call for countries to provide AMR data files according to the GLASS format and upload them in the GLASS IT platform→ next report is scheduled for January/February 2020

Source: World Health Organization. (2019). Global Antimicrobial Resistance Surveillance System (GLASS) Report: Early implementation 2017-2018. Available at:
https://www.who.int/glass/resources/publications/early-implementation-report-2017-2018/en/:

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Information for Action: Bacteria & Antibiotics

Regional Reporting of Surveillance Data through GLASS* vs. Reporting of Consumption Data**
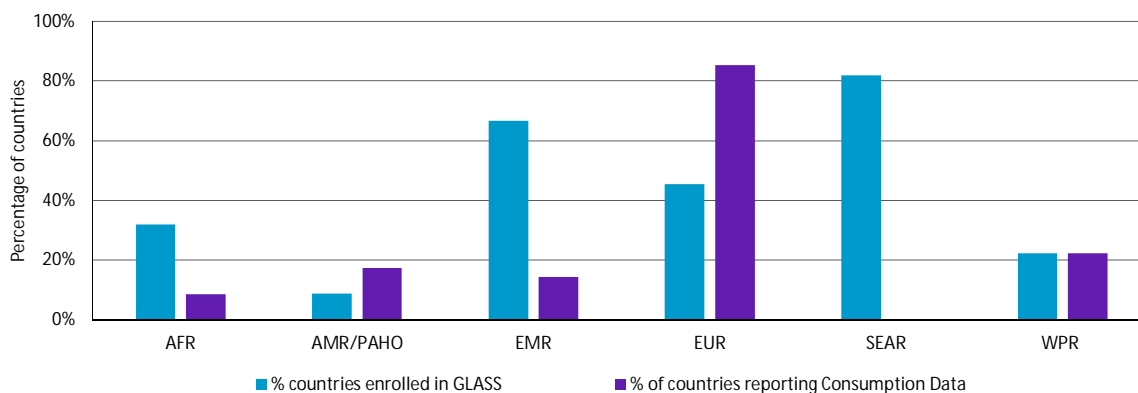
■ % countries enrolled in GLASS   ■ % of countries reporting Consumption Data

*Source: World Health Organization. (2019). Global Antimicrobial Resistance Surveillance System (GLASS) Report: Early implementation 2017-2018
**Source: World Health Organization. (2018). WHO Report on Surveillance of Antibiotic Consumption

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# Global Action Plan's 5 Strategic Objectives

1. Improve awareness and understanding
2. Strengthen knowledge through surveillance & research
3. Reduce the incidence of infection
4. Optimize the use of antimicrobial medicines
5. Ensure sustainable investment

GLOBAL ACTION PLAN
ON ANTIMICROBIAL
RESISTANCE

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Key: Reduce Spread of Infections and AMR

Water, Sanitation and Hygiene (WASH) is KEY for Infection Prevention and Control!

Percentage of health facilities without access to water

WHO WATER, SANITATION AND HYGIENE STRATEGY 2018-2025

WASH IN HEALTH CARE FACILITIES

- 0-5
- 6-10
- 11-20
- 21-50
- 51-100
- INSUFFICIENT DATA
- NOT APPLICABLE

In Least Developed Countries, only 55% of health care facilities had basic water service
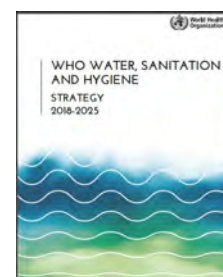
Source: WHO & UNICEF. (2019). WASH in health care facilities: Global baseline report 2019. Retrieved from https://www.who.int/water_sanitation_health/publications/wash-in-health-care-facilities-global-report/en/

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Infection Prevention and Control Programmes in Human Healthcare and Animal Production
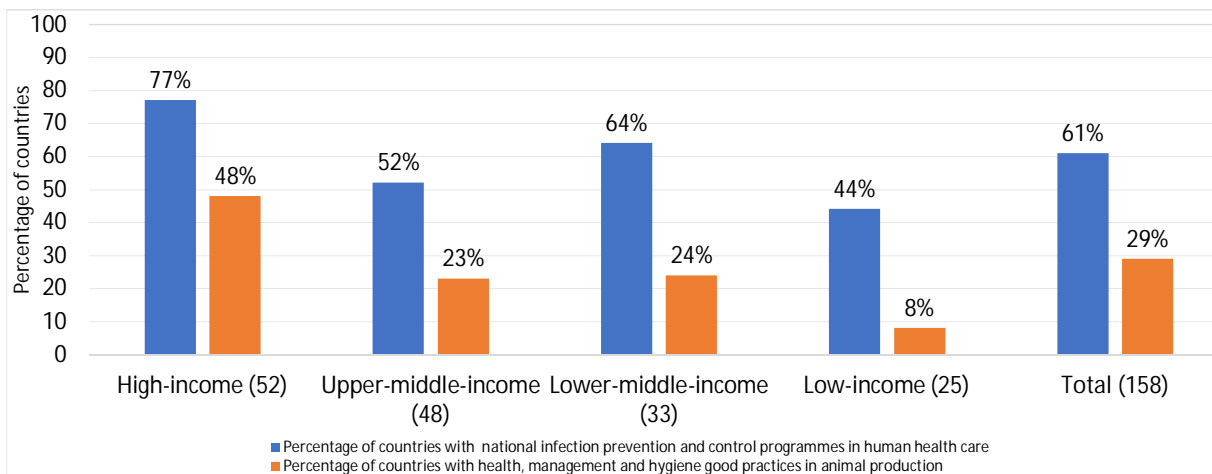
Source: Preliminary TrACSS 2018/2019 data

World Health Organization

Global Action Plan's 5 Strategic Objectives

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2. Strengthen knowledge through surveillance & research
3. Reduce the incidence of infection
4. Optimize the use of antimicrobial medicines
5. Ensure sustainable investment

GLOBAL ACTION PLAN
ON ANTIMICROBIAL
RESISTANCE

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Policies and Regulations on Antimicrobial Use
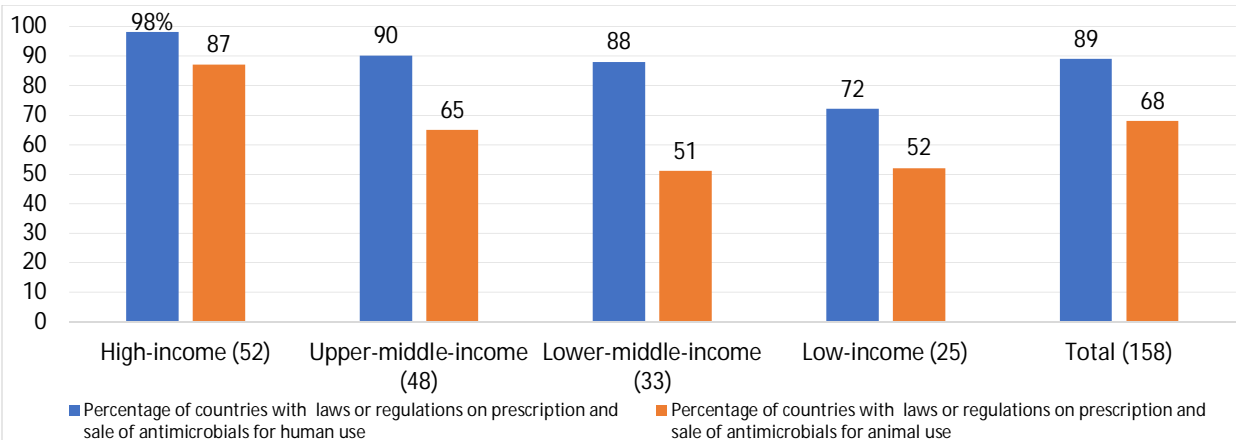
Source: Preliminary TrACSS 2018/2019 data

World Health Organization

Optimize the Use of Antimicrobial Medicines

AWaRe Categorization of Antibiotics
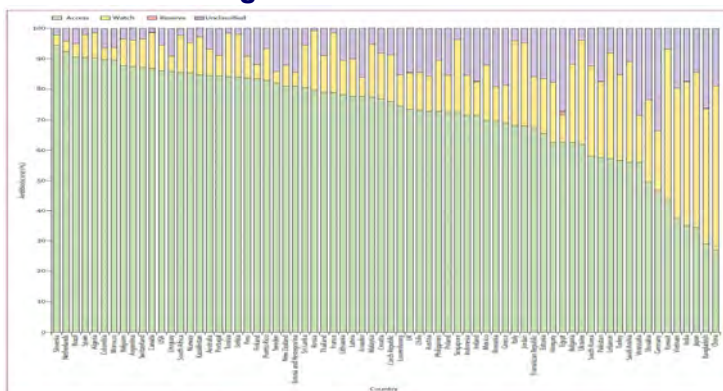
ACCESS Antibiotics that should be available at all times

WATCH Antibiotics recommended as first- or second-choice treatments for a small number of infections

RESERVE Antibiotics that are last-resort options

UNCLASSIFIED

Source: Hsia, Y., Sharland, M., Jackson, C., Wong, I., Magrini, N., Bielicki, J. (2018). Consumption of oral antibiotic formulations for young children according to the WHO Access, Watch, Reserve (AWaRe) antibiotic groups: an analysis of sales data from 70 middle-income and high-income countries. *The Lancet, 19(1)*, p. 67-75.

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Netherlands: results so far
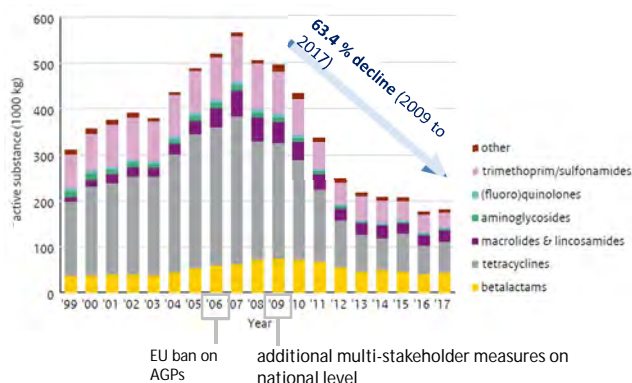
63.4 % decline (2009 to 2017)

EU ban on AGPs

additional multi-stakeholder measures on national level

- 67.9% reduction (2007-2017)
- 63.4% reduction (2017 to reference year 2009)
- Fluoroquinolones and 3rd/4th-gen cefalosporines usage strongly reduced
- 80.7% reduction in use of colistin (2011-2017)

Source: Maran, 2018

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Global Action Plan's 5 Strategic Objectives

1. Improve awareness and understanding
2. Strengthen knowledge through surveillance & research
3. Reduce the incidence of infection
4. Optimize the use of antimicrobial medicines
5. **Ensure sustainable investment**

GLOBAL ACTION PLAN ON ANTIMICROBIAL RESISTANCE

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Identification of Priority Pathogens for R&D

Critical needs:

- Drug-resistant TB

- Gram-negative bacteria:

    - Carbapenem-resistant *A. baumannii*

    - Carbapenem-resistant *P. aeruginosa*

    - Carbapenem-resistant and 3rd generation cephelosporin resistant *Enterobacteriaceae*
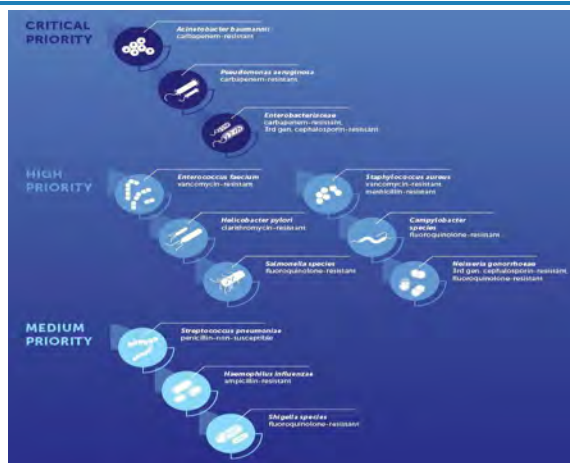
Source: WHO. (2017). Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis. Available at: http://www.who.int/entity/medicines/areas/rational_use/PPLreport_2017_09_19.pdf?ua=1

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Way Forward

Tripartite Monitoring Framework

World Health Organization
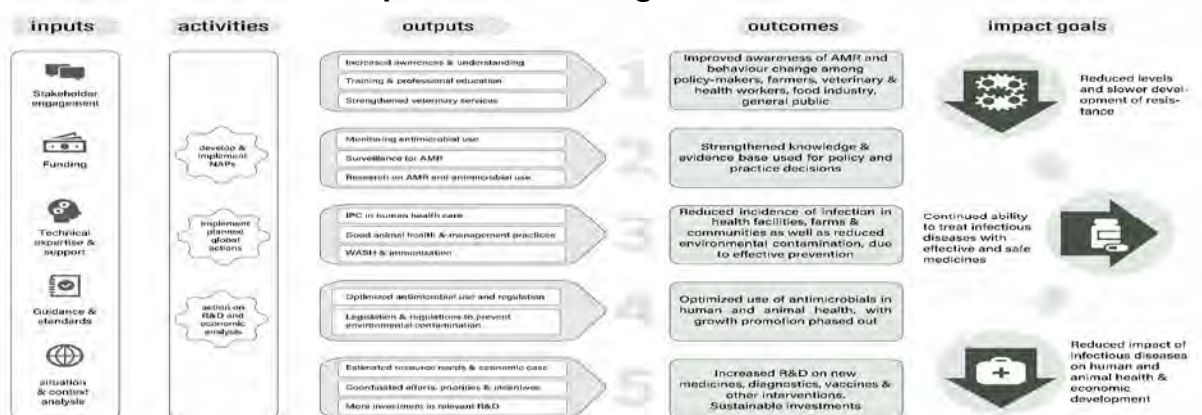
Can you make a difference?...Yes!

1. Political commitment essential!

2. Support multi-sectoral AMR coordination team

3. Support AMR as part of the Universal Health Coverage agenda & Health Security Agenda & Good animal husbandry

4. Reach out to Civil society and non-state-actors

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Way Forward

Five critical areas of focus:

- **Urgency: local political commitment**

- **One Health Approach (more agri/animal)**

- **Stakeholder Engagement**

- **Implementation of National Action Plans**

- **Reduction use of AB in Human/Agriculture/Animal**

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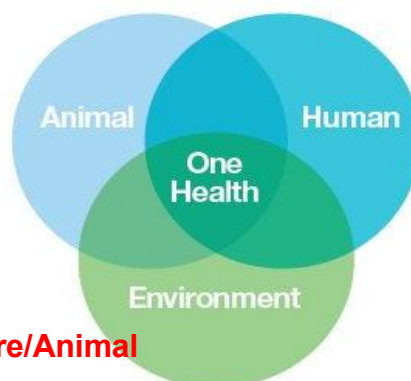
Antimicrobial resistance and One Health: who cares?

Bridging the gap between policy and practice

Prof. Jaap Wagenaar DVM, PhD
David Speksnijder DVM, PhD

Department of Infectious Diseases and Immunology,
Faculty of Veterinary Medicine, Utrecht University, Utrecht - NL

Wageningen Bioveterinary Research, Lelystad - NL
j.wagenaar@uu.nl

Outline

- One Health Action Plans

- Attribution: transmission of resistance between animals and humans

- Geographical differences

- Challenges to bridge the gap between policy and practice

- Take home messages

AMR: antimicrobial resistance
AMU: antimicrobial use

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One Health collaboration / coordination

Global (n=154)

F 1%
E 17%
A 15%
D 4%
C 13%
B 50%

Europe (n=50)

E 24%
A 12%
D 12%
C 14%
B 38%

EU/EEA (n=29)

A 10%
E 42%
B 24%
C 14%
D 10%
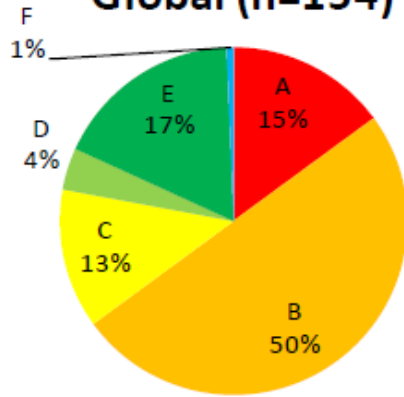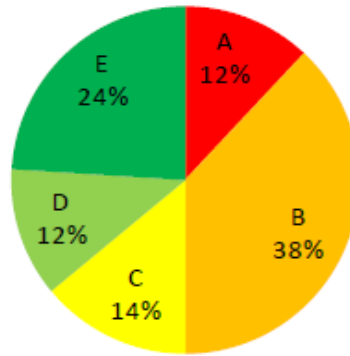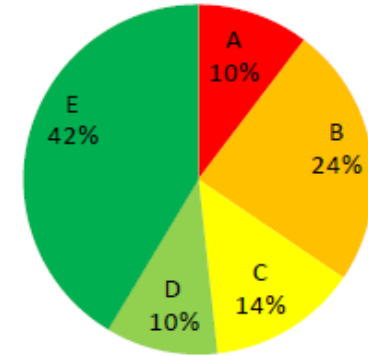
A - No formal multi-sectoral governance or coordination mechanism exists.
B - Multi-sectoral working group(s) or coordination committee on AMR established with Government leadership.
C - Multi-sectoral working group(s) is (are) functional, with clear terms of reference; regular meetings, and funding for working group(s). Activities and reporting/accountability
D - Joint working on issues including agreement on common objectives, including restriction of use of critically important antimicrobials.
E - Integrated approaches used to implement the national AMR action plan.

From the 2nd global Tripartite self-assessment
Respons: 154/194 (79%)

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Quantification of AMR-attribution in humans from animals

Human illness source attribution methods

Methodologies for attribution of human illness to specific sources

Approaches	Methods
Microbiological approaches	Microbial subtyping
	Comparative exposure assessment
Epidemiological approaches	Analysis of sporadic cases
	Analysis of data from outbreak investigations
Intervention studies	
Expert elicitation	

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Challenges for AMR attribution

- <u>Epidemiological</u> approach: exposure does not lead to immediate respons/symptoms

- Effect in humans of AMU <u>intervention</u> in animals under-explored and difficult because of parallel interventions

- <u>Microbiological approach</u>: typing is complex

Extended Spectrum Beta-Lactamase (ESBL)

1. ESBL gene

2. Mobile elements
   - Plasmid
   - Insertion sequence
   - Transposons

3. *E. coli* carrier/host

Gene(s)

Plasmid(s)

Single strain
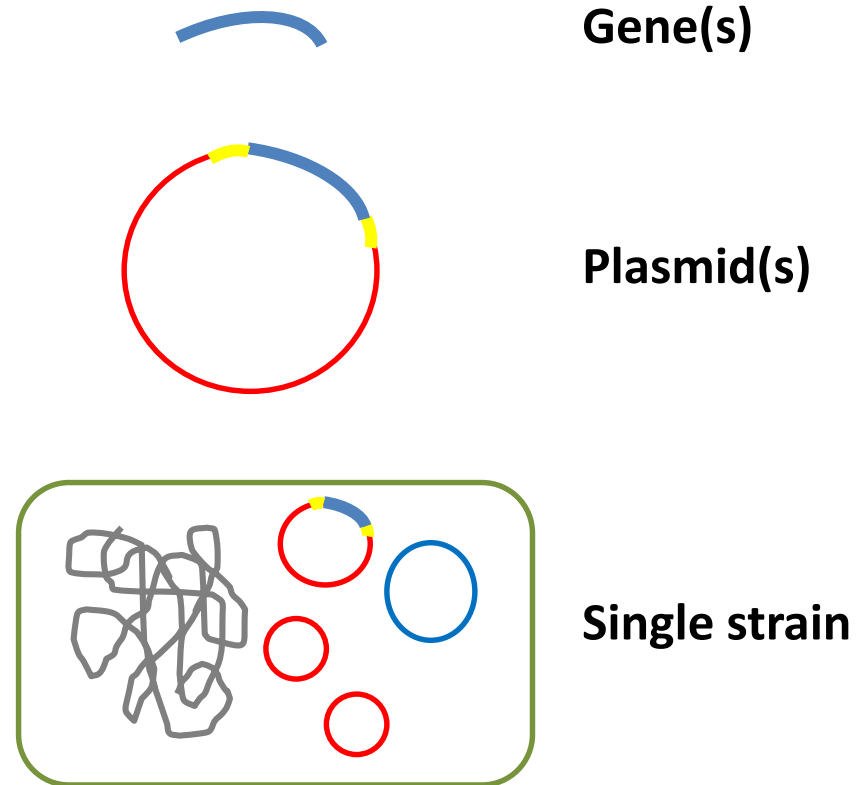
Molecular relatedness of ESBL/AmpC-producing *Escherichia coli* from humans, animals, food and the environment: a pooled analysis

Alejandro Dorado-García[1,2]*†, Joost H. Smid[1]†, Wilfrid van Pelt[3], Marc J. M. Bonten[3,4], Ad C. Fluit[4], Gerrita van den Bunt[3,5], Jaap A. Wagenaar[2], Joost Hordijk[2], Cindy M. Dierikx[3], Kees T. Veldman[6], Aline de Koeijer[3,6], Wietske Dohmen[1], Heike Schmitt[1], Apostolos Liakopoulos[6], Ewa Pacholewicz[1], Theo J. G. M. Lam[7], Annet G. Velthuis[6], Annet Heuvelink[7], Maaike A. Gonggrijp[7], Engeline van Duijkeren[3], Angela H. A. M. van Hoek[3], Ana Maria de Roda Husman[1,3], Hetty Blaak[3], Arie H. Havelaar[1,8], Dik J. Mevius[2,6] and Dick J. J. Heederik[1]

[1]Institute for Risk Assessment Sciences (IRAS), Utrecht University, PO Box 80175, 3508 TD Utrecht, The Netherlands; [2]Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, PO Box 80165, 3508 TD Utrecht, The Netherlands; [3]Centre for Infectious Disease Control, National Institute for Public Health and the Environment, PO Box 1, 3720 BA Bilthoven, The Netherlands; [4]Department of Medical Microbiology, University Medical Centre Utrecht, PO Box 85500, 3508 GA Utrecht, The Netherlands; [5]Julius Centre for Health Sciences and Primary Care, University Medical Centre Utrecht, PO Box 85500, 3508 GA Utrecht, The Netherlands; [6]Wageningen Bioveterinary Research, PO Box 65, 8200 AB Lelystad, The Netherlands; [7]GD Animal Health, PO Box 9, 7400 AA Deventer, The Netherlands; [8]Institute for Sustainable Food Systems, Emerging Pathogens Institute and Animal Sciences Department, University of Florida, PO Box 100009, Gainesville, FL 32610, USA

*Corresponding author. Tel: +31-30-2539499; E-mail: A.DoradoGarcia@uu.nl
†Both authors have contributed equally to this work.

Background: In recent years, ESBL/AmpC-producing *Escherichia coli* (ESBL/AmpC-EC) have been isolated with increasing frequency from animals, food, environmental sources and humans. With incomplete and scattered evidence, the contribution to the human carriage burden from these reservoirs remains unclear.

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Colour legend for PCA, reservoir numbers in panels, type of reservoir (human [H], environmental [E], food [F] and animal [A])

- 1 H-general population
- 2 H-clinical UTIs
- 3 H-clinical blood
- 4 H-clinical faecal
- 5 H-clinical respiratory, wounds, other
- 6 H-broiler farming community
- 7 H-pig farming community
- 8 E-wastewater
- 9 E-surface water non-recreational
- 10 E-surface water recreational
- 11 F-chicken meat at retail
- 12 F-chicken meat at slaughterhouse
- 13 F-beef at retail
- 14 F-veal calf meat at slaughterhouse
- 15 F-turkey meat at retail
- 16 A-broilers
- 17 A-laying hens
- 18 A-dairy cattle
- 19 A-veal calves
- 20 A-pigs
- 21 A-wild birds
- 22 A-dogs

PCA on ESBL/AmpC gene frequencies
PCs, principal components (% variance explained)

Taken from: Dorado-Garcia et al, JAC, 2017

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PCA on ESBL/AmpC gene frequencies
PCs, principal components (% variance explained)

Colour legend for PCA panels, type of reservoir environmental [E], food

- 1 H-general population
- 2 H-clinical UTIs
- 3 H-clinical blood
- 4 H-clinical faecal
- 5 H-clinical respiratory
- 6 H-broiler farming
- 7 H-pig farming community
- 8 E-wastewater
- 9 E-surface water
- 10 E-surface water
- 11 F-chicken meat
- 12 F-chicken meat
- 13 F-beef at retail
- 14 F-veal calf meat
- 15 F-turkey meat at
- 16 A-broilers
- 17 A-laying hens
- 18 A-dairy cattle
- 19 A-veal calves
- 20 A-pigs
- 21 A-wild birds
- 22 A-dogs

- Limited similarity between farm animals and humans in clinical settings or general population

- Farmers/family members very similar to animal reservoirs (occupational ESBL transmission)

- Human to human attribution overall highly relevant

- Animal to human attribution in the 1-10% range for some specific livestock associated genes and animal species

Taken from: Dorado-Garcia et al, JAC, 2017

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PCA on ESBL/AmpC gene frequencies
PCs, principal components (% variance explained)

−0.15   −0.10   −0.05   0.00   0.05   0.10   0.15   0.20

Colour legend for PCA panels, type of reservoir, environmental [E], food

- 1 H-general populat
- 2 H-clinical UTIs
- 3 H-clinical blood
- 4 H-clinical faecal
- 5 H-clinical respirato
- 6 H-broiler farming c
- 7 H-pig farming com
- 8 E-wastewater
- 9 E-surface water no
- 10 E-surface water
- 11 F-chicken meat a
- 12 F-chicken meat a
- 13 F-beef at retail
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- Limited similarity between farm animals and humans in clinical settings or general population

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- For ESBL-producing *E. coli* in the Netherlands

Taken from: Dorado-Garcia et al, JAC, 2017

−2   −1   0   1   2   3
PC1 (49%)

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Effect in humans of reduced AMU in animals

Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis

Karen L Tang, Niamh P Caffrey, Diego B Nóbrega, Susan C Cork, Paul E Ronksley, Herman W Barkema, Alicia J Polachek, Heather Ganshorn, Nishan Sharma, James D Kellner, William A Ghali

Summary

Background Antibiotic use in human medicine, veterinary medicine, and agriculture has been linked to the rise of antibiotic resistance globally. We did a systematic review and meta-analysis to summarise the effect that interventions to reduce antibiotic use in food-producing animals have on the presence of antibiotic-resistant bacteria in animals and in humans.

Methods On July 14, 2016, we searched electronic databases (Agricola, AGRIS, BIOSIS Previews, CAB Abstracts, MEDLINE, Embase, Global Index Medicus, ProQuest Dissertations, Science Citation Index) and the grey literature. The search was updated on Jan 27, 2017. Inclusion criteria were original studies that reported on interventions to reduce antibiotic use in food-producing animals and compared presence of antibiotic-resistant bacteria between intervention and comparator groups in animals or in human beings. We extracted data from included studies and did meta-analyses using random effects models. The main outcome assessed was the risk difference in the proportion of antibiotic-resistant bacteria.

Findings A total of 181 studies met inclusion criteria. Of these, 179 (99%) described antibiotic resistance outcomes in animals, and 81 (45%) of these studies were included in the meta-analysis. 21 studies described antibiotic resistance outcomes in humans, and 13 (62%) of these studies were included in the meta-analysis. The pooled absolute risk reduction of the prevalence of antibiotic resistance in animals with interventions that restricted antibiotic use commonly ranged between 10 and 15% (total range 0–39), depending on the antibiotic class, sample type, and bacteria

Interpretation Interventions that restrict antibiotic use in food-producing animals are associated with a reduction in the presence of antibiotic-resistant bacteria in these animals. A smaller body of evidence suggests a similar association in the studied human populations, particularly those with direct exposure to food-producing animals. The implications for the general human population are less clear, given the low number of studies.

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Triggers for reduction policy

Humans

Animals
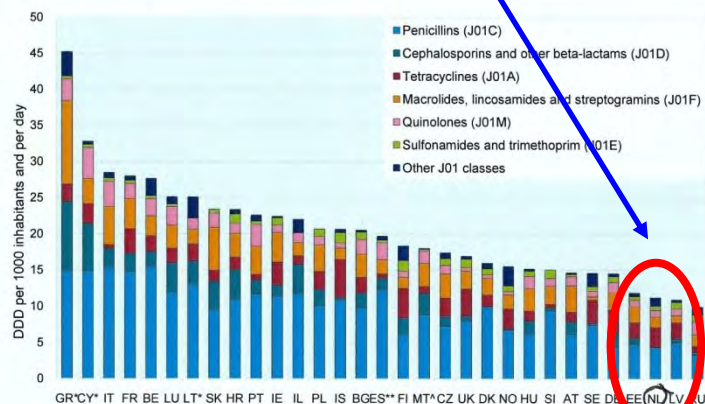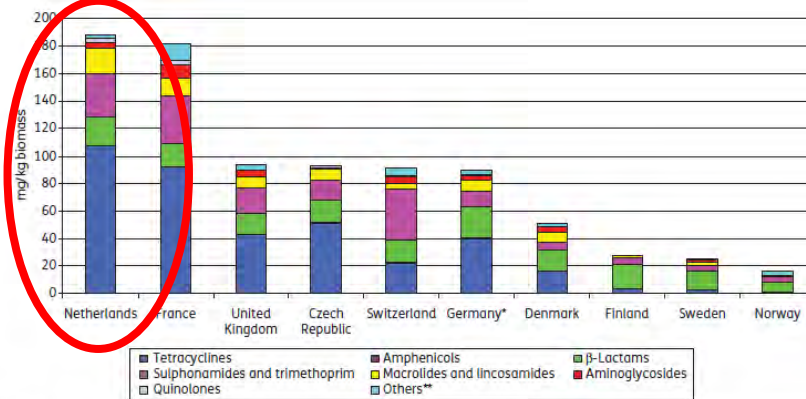
Figure 1. Amounts, in mg, of veterinary antibacterial agents sold in 2007 per kg biomass of pig meat, poultry meat and cattle meat produced plus estimated live weight of dairy cattle. *2005 data. **The substances included vary from country to country.

The Netherlands
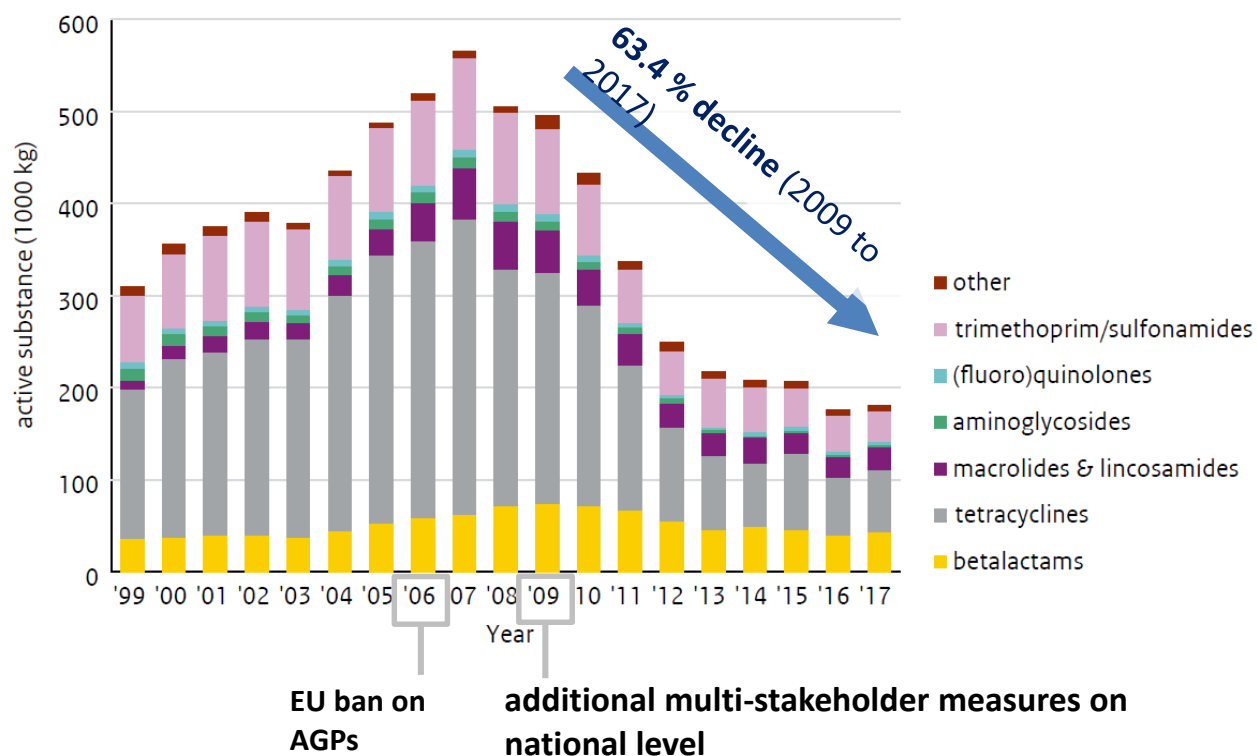
- 67.9% reduction (2007-2017)

- 63.4% reduction (2017 to reference year 2009)

- Fluoroquinolones and 3rd/4th-gen cefalosporines usage strongly reduced

- 80.7% reduction in use of colistin (2011-2017)

Source: Maran, 2018
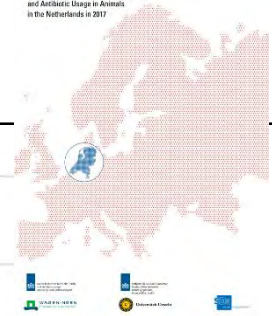
Effect on antimicrobial resistance
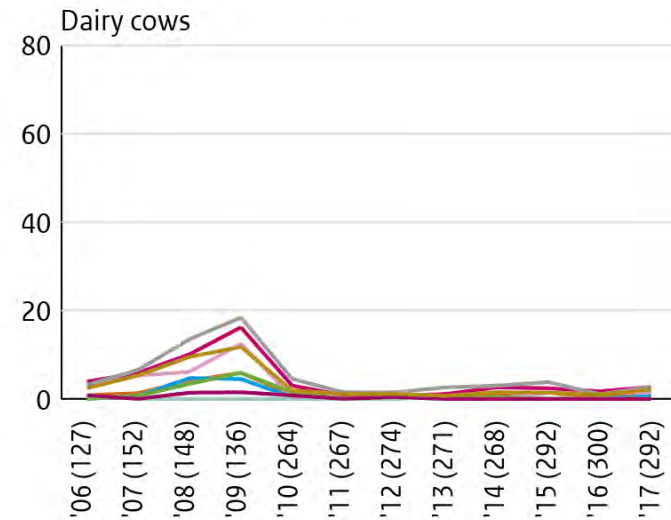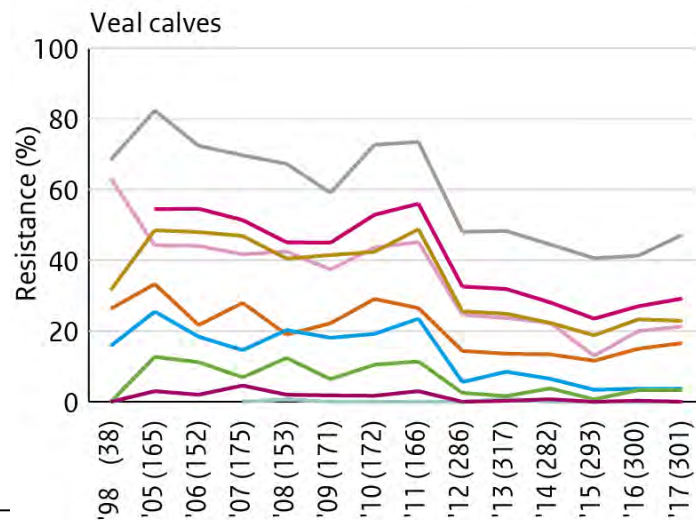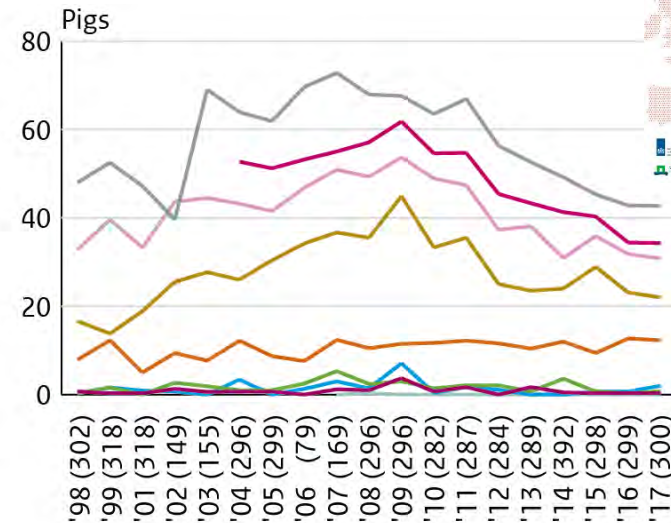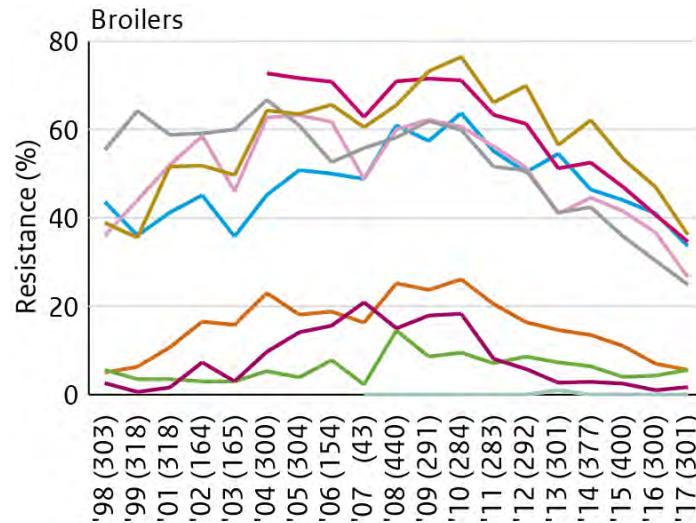
Figure 5.14: *Escherichia coli*: proportion of third-generation cephalosporin resistance in 2009

- █ < 1%
- █ 1% to < 5%
- █ 5% to < 10%
- █ 10% to < 25%
- █ 25% to < 50%
- █ ≥ 50%
- ▨ No data reported or less than 10 isolates
- ☐ Not included

Non-visible countries
- █ Luxembourg
- █ Malta
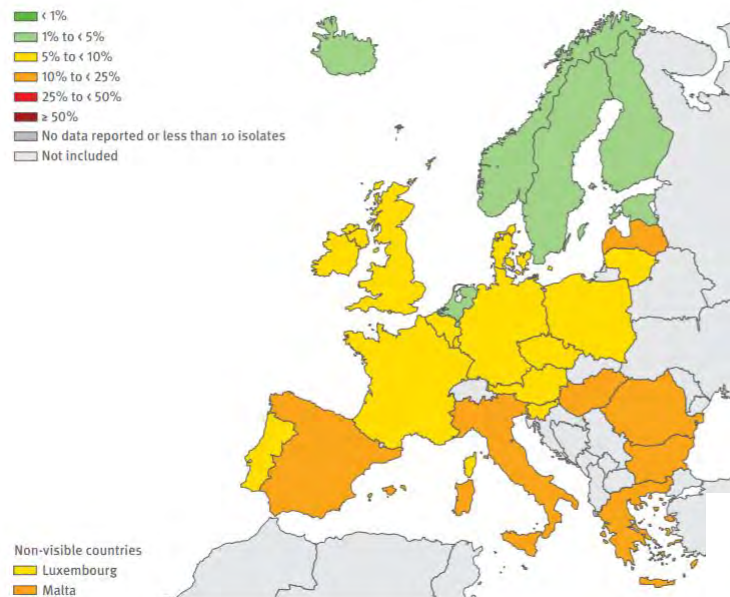
Figure 3.3. *Escherichia coli*. Percentage (%) of invasive isolates with resistance to third-ger country, EU/EEA countries, 2017

- █ < 1%
- █ 1% to < 5%
- █ 5% to < 10%
- █ 10% to < 25%
- █ 25% to < 50%
- █ ≥ 50%
- ▨ No data reported or fewer than 10 isolates
- ☐ Not included

Non-visible countries
- ☐ Liechtenstein
- █ Luxembourg
- █ Malta

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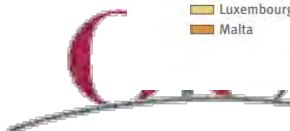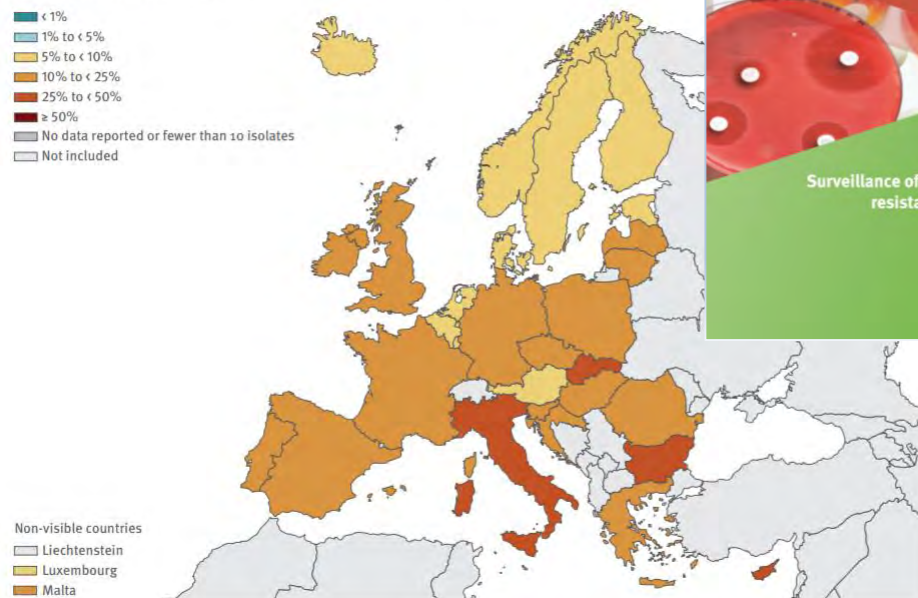
Nethmap 2018 (human surveillance)

Figure 4.2.1 Trends in antibiotic resistance (from left to right 2013 to 2017) among diagnostic urinary isolates of E. coli, K. pneumoniae, P. mirabilis, and P. aeruginosa from selected general practitioners' patients in ISIS-AR, by age category.
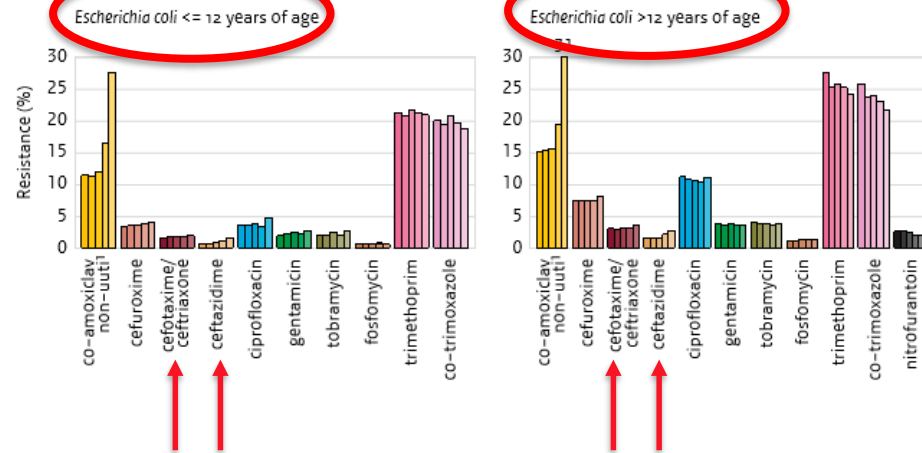
Figure 4.3.1.1 Trends in antibiotic resistance (from left to right 2013 to 2017) among diagnostic isolates of E. coli, K. pneumoniae, P. mirabilis, and P. aeruginosa from patients attending outpatient departments in ISIS-AR
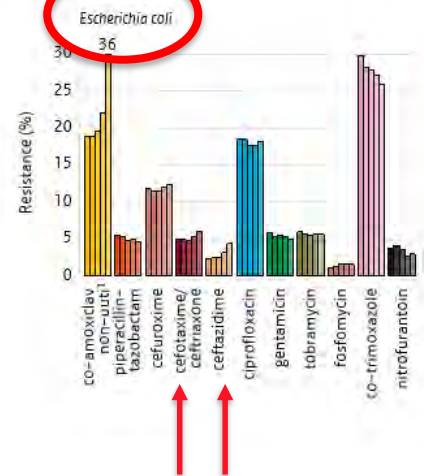
Trends in prevalence of ESBL-producing bacteria as a cause of bloodstream infections in ISIS-AR
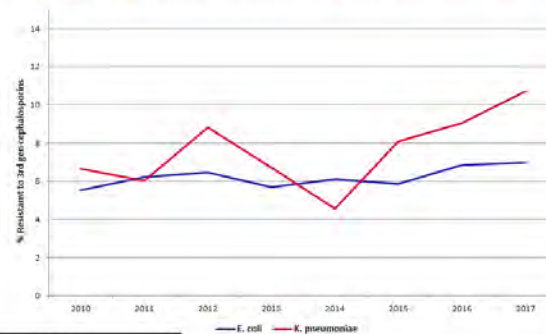
Data from 21 labs
E. coli: 3,257-4,035/yr
K. pneumoniae: 507-719/yr

Prof. Marc Bonten, ESBLAT-symposium, 2018

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Pillars of containment of resistance

- Prevent selection => reduce use

- Prevent spread => infection control

Pillars of containment of resistance

- Prevent selection => reduce use
  - Reduce use in humans and animals

- Prevent spread => infection control
  - humans: quarantine in hospitals, hand washing, disinfection etc
  - Animals: biosecurity (on-farm, within production chain); companion animal clinics (management of multiresistant micro-organisms)

Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage

Rene S. Hendriksen[1], Patrick Munk[1], Patrick Njage[1], Bram van Bunnik[2], Luke McNally[3],
Oksana Lukjancenko[1], Timo Röder[1], David Nieuwenhuijse[4], Susanne Karlsmose Pedersen[1], Jette Kjeldgaard[1],
Rolf S. Kaas[1], Philip Thomas Lanken Conradsen Clausen[1], Josef Korbinian Vogt[1], Pimlapas Leekitcharoenphon[1],
Milou G.M. van de Schans[5], Tina Zuidema[5], Ana Maria de Roda Husman[6], Simon Rasmussen[7],
Bent Petersen[7], The Global Sewage Surveillance project consortium[#], Clara Amid[8], Guy Cochrane[8],
Thomas Sicheritz-Ponten[9], Heike Schmitt[6], Jorge Raul Matheu Alvarez[10], Awa Aidara-Kane[10], Sünje J. Pamp[1],
Ole Lund[7], Tine Hald[1], Mark Woolhouse[2], Marion P. Koopmans[4], Håkan Vigre[1], Thomas Nordahl Petersen[1] &
Frank M. Aarestrup[1]

Antimicrobial resistance (AMR) is a serious threat to global public health, but obtaining representative data on AMR for healthy human populations is difficult. Here, we use metagenomic analysis of untreated sewage to characterize the bacterial resistome from 79 sites in 60 countries. We find systematic differences in abundance and diversity of AMR genes between Europe/North-America/Oceania and Africa/Asia/South-America. Antimicrobial use data and bacterial taxonomy only explains a minor part of the AMR variation that we observe. We find no evidence for cross-selection between antimicrobial classes, or for effect of air travel between sites. However, AMR gene abundance strongly correlates with socio-economic, health and environmental factors, which we use to predict AMR gene abundances in all countries in the world. Our findings suggest that global AMR gene diversity and abundance vary by region, and that improving sanitation and health could potentially limit the global burden of AMR. We propose metagenomic analysis of sewage as an ethically acceptable and economically feasible approach for continuous global surveillance and prediction of AMR.

We what we should do….

main aim: reduction of antimicrobial use

with special attention for Highest Prioritized Critically Important Antimicrobials for human medicine (CIA-list)

3rd/4th gen cephalosporines – fluoroquinolones – colistin - glycopeptides - macrolides
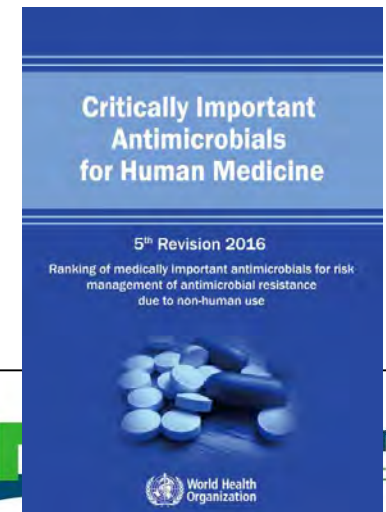
The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017

European Food Safety Authority and
European Centre for Disease Prevention and Control

Abstract

The data on antimicrobial resistance in zoonotic and indicator bacteria in 2017, submitted by 28 EU Member States (MSs), were jointly analysed by EFSA and ECDC. Resistance in zoonotic *Salmonella* and *Campylobacter* from humans, animals and food, and resistance in indicator *Escherichia coli* as well as meticillin-resistant *Staphylococcus aureus* in animals and food were addressed, and temporal trends assessed. 'Microbiological' resistance was assessed using epidemiological cut-off (ECOFF) values; for some countries, qualitative data on human isolates were interpreted in a way which corresponds closely to the ECOFF-defined 'microbiological' resistance. In *Salmonella* from humans, as well as in *Salmonella* and *E. coli* isolates from fattening pigs and calves of less than 1 year of age, high proportions of isolates were resistant to ampicillin, sulfonamides and tetracyclines, whereas resistance to third-generation cephalosporins was uncommon. Varying occurrence/prevalence rates of presumptive extended-spectrum beta-lactamase (ESBL)/AmpC producers in *Salmonella* and *E. coli* monitored in meat (pork and beef), fattening pigs and calves, and *Salmonella* monitored in humans, were observed between countries. Carbapenemase-producing *E. coli* were detected in one single sample from fattening pigs in one MS. Resistance to colistin was observed at low levels in *Salmonella* and *E. coli* from fattening pigs and calves and meat thereof and in *Salmonella* from humans. In *Campylobacter* from humans, high to extremely high proportions of isolates were resistant to ciprofloxacin and tetracyclines, particularly in *Campylobacter coli*. In five countries, high to very high proportions of *C. coli* from humans were resistant also to erythromycin, leaving few options for treatment of severe *Campylobacter* infections. High resistance to ciprofloxacin and tetracyclines was observed in *C. coli* isolates from fattening pigs, whereas much lower levels were recorded for erythromycin. Combined resistance to critically important antimicrobials in both human and animal isolates was generally uncommon but very high to extremely high multidrug resistance levels were observed in *S.* Typhimurium and its monophasic variant in both humans and animals. *S.* Kentucky from humans exhibited high-level resistance to ciprofloxacin, in addition to a high prevalence of ESBL.

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

SEARCH

CDC A-Z INDEX

National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS)

NARMS

About NARMS

CDC's Role in NARMS

Partners in Antibiotic Resistance

Antibiotics Tested by NARMS

Antibiotic Resistance

NARMS in Action

Reports and Interactive Data

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24/7 Outbreak Notices

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About NARMS

Tracking Trends in Resistance

The National Antimicrobial Resistance Monitoring System - Enteric Bacteria (NARMS) is a US public health surveillance system that tracks antimicrobial resistance in foodborne and other enteric bacteria.

NARMS is an interagency partnership among the US Centers for Disease Control and Prevention (CDC), the US Food and Drug Administration (FDA), the US Department of Agriculture (USDA), and state and local health departments. Human surveillance began in fourteen sites in 1996 and became nationwide in 2003.

NARMS monitors antimicrobial resistance among enteric bacteria from three sources:

- humans (CDC)
- retail meats (FDA)
- food animals (USDA)

Learn more about the roles of federal and state agencies who track antibacterial resistance in support of food safety.

Download NARMS 20th Anniversary Timeline [PDF – 1 page]

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OIE

World Health Organization

WAGENINGEN UNIVERSITY & RESEARCH

LMIC

ANTIBIOTICS USE ON SMALL AND MEDIUM SCALE BROILER FARMS IN WEST JAVA, EAST JAVA AND SOUTH SULAWESI PROVINCES, INDONESIA

Food and Agriculture Organization of the United Nations

USAID FROM THE AMERICAN PEOPLE

Directorate General of Livestock and Animal Health Services
Ministry of Agriculture
Republic of Indonesia

N.M.R. Isriyanthi[1], E. Setyawan[2], D.M. Pangaribuan[1], R. Telussa[2], E.R. Fitriastuti[1], G.B. Utomo[2], A. Kompudu[2], A. Harja[2], I. N. Agustina[1], J. Wagenaar[3, 4], D.C. Speksnijder[3], L. Schoonman[2], J. McGrane[2].

Top Ten Antibiotics (and combinations) used on 360 Surveyed Farms

Universiteit Utrecht

Oie

World Health Organization
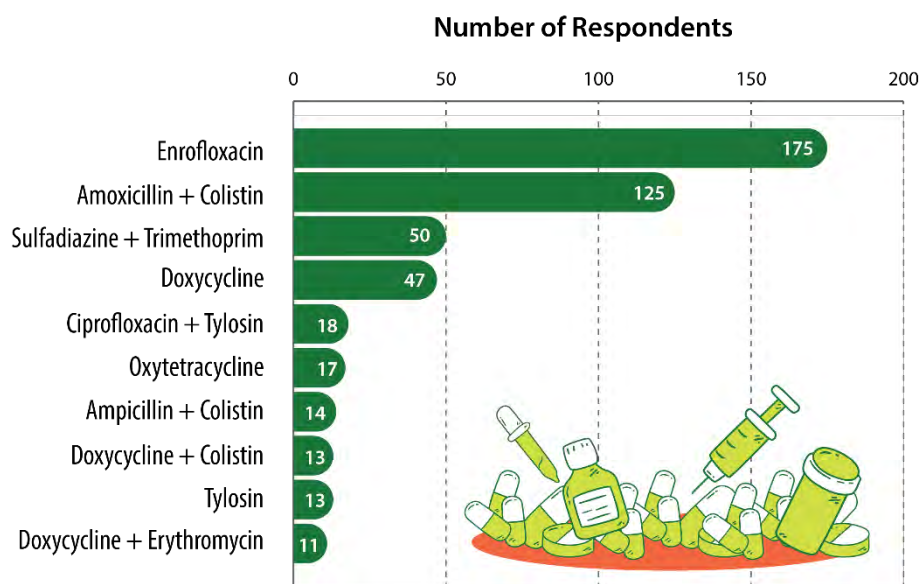
WAGENINGEN UNIVERSITY & RESEARCH

Purpose of Antibiotic Usage on Surveyed Farms
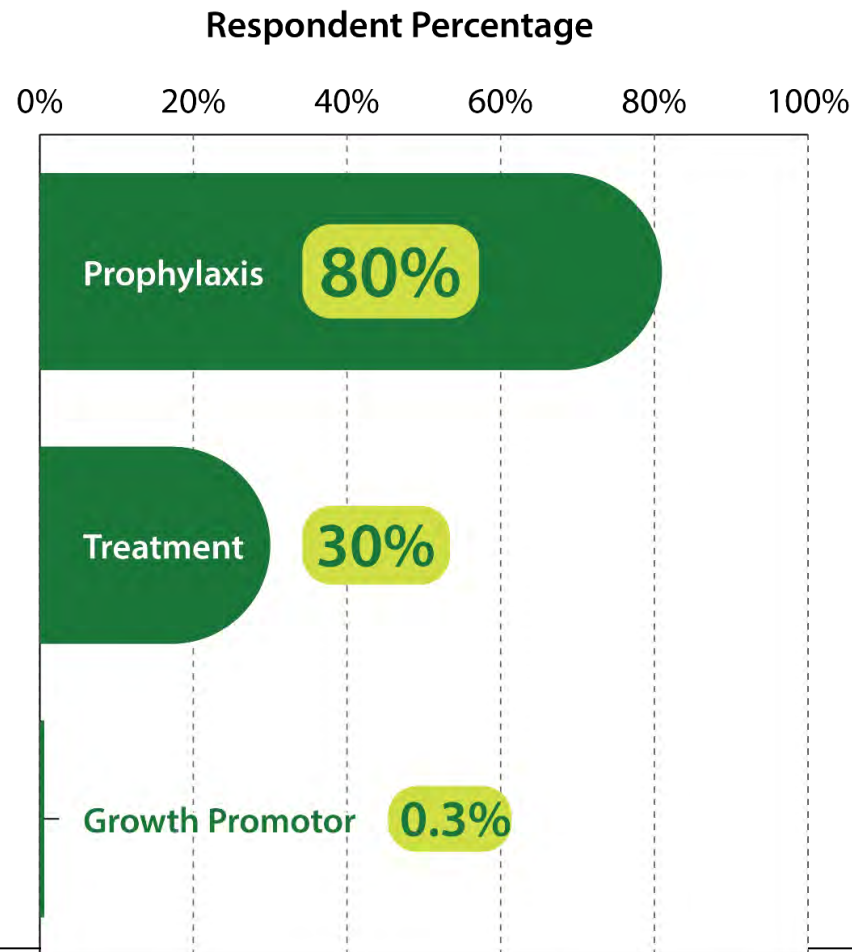
Why are antimicrobials used on poultry farms?

How much can we reduce and what is the effect of reduced use?

Poultry

- *Mycoplasmata*

- *E. coli*

- *Salmonella*

- *Avibacterium paragallinarum* (Coryza)

- *Clostridium*

- Vaccination

- Biosecurity

- Primarily: improvement of animal health

- Secundary: reduction of AMU

Challenges in LMIC

- Knowledge and awareness
- Surveillance of AMR, AMU and residues in animals
- Over the counter availability
- Systematic use of antimicrobials
- Biosecurity, housing conditions, feed quality
- Enforcement of the regulations?
- Good governance
- The need for veterinarians as professional advisors
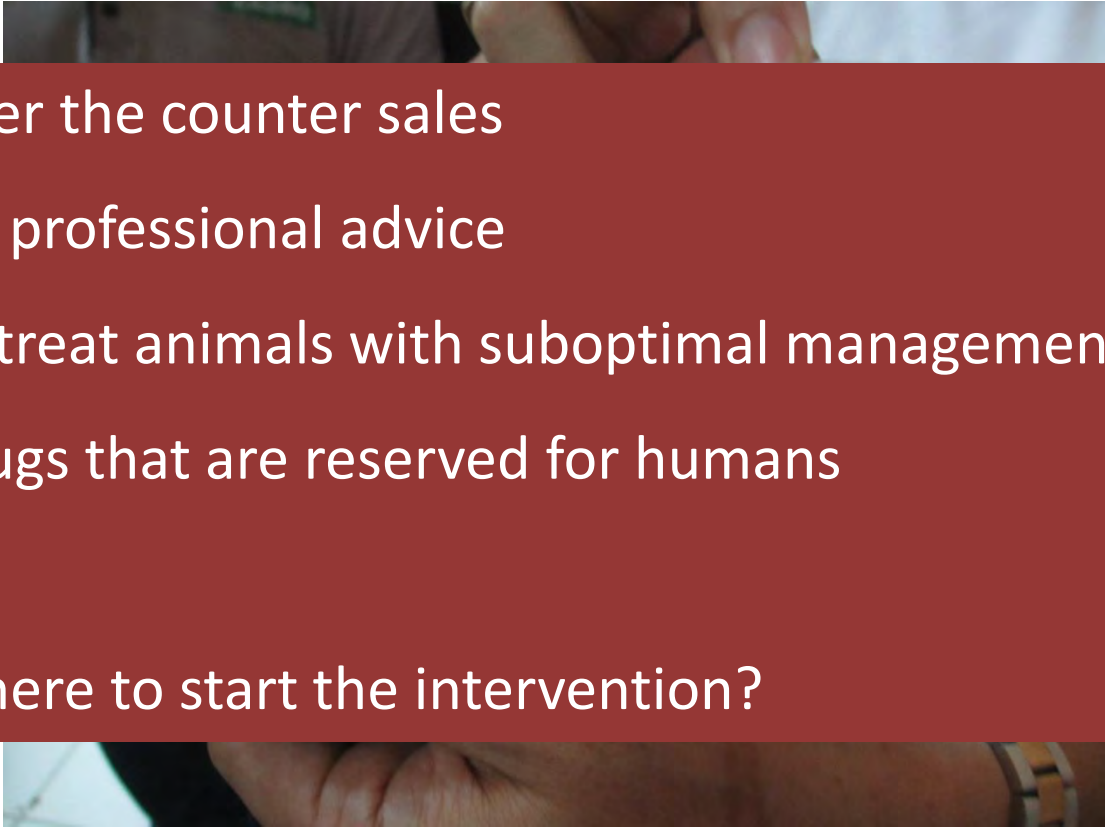
- Over the counter sales

- No professional advice

- To treat animals with suboptimal management

- Drugs that are reserved for humans

- Where to start the intervention?

What can motivate a farmer to change?

What's in for me?

Antimicrobials….

One of the few drugs that have a positive effect for you (or your animals) and a negative effect for the society

Decision to use should not be at the individual level but by prescription only and according to guidelines developed by professionals

What's in for me?

- No reduction of costs expected

- Are there alternatives?

- Meeting requirements set by law?

- Corporate responsibility (e.g. McDonalds banned specific antimicrobials from production)

- Branding (consumer requests)

- Export/trade: levels of resistance, levels of residues

- Reduction of resistance in poultry pathogens

How can we come to a reduction in AMU in the field?

''20 years ago we did not reach the political level''

Marc Sprenger, 16 April 2019

Take home messages

- Quantification of AMR transfer from animals to humans remains weak.

- Any attribution should be addressed! (food borne pathogens, LA-MRSA)

- A clear and honest story is needed towards farmers

- Implementation of the NAPs: fiction and reality

- How can we implement policy into practice: the steps to achieve reduction are urgently needed in particular in LMIC

- Investment in animal health is required to pave the road for AMU reduction

Acknowledgements
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Panos Mallioris
Imron Suandy
Pim Sanders

ICOHAR International Conference on One Health Antimicrobial Resistance

One health integrated surveillance

Supranational Networks for surveillance of human multiresistant pathogens

Luis Martínez Martínez
Dept. Microbiology, University of Cordoba
Unit of Microbiology, Univ. Hosp. Reina Sofía
IMIBC
Cordoba, Spain

Utrecht, April 17 2018

ESGARS
ESCMID STUDY GROUP FOR ANTIMICROBIAL RESISTANCE SURVEILLANCE
European Society of Clinical Microbiology and Infectious Diseases

ESCMID
EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

SURVEILLANCE (…of Resistance)

Ongoing generation, capture, assembly, analysis and interpretation of all information on the evolving nature, spread, and distribution of [infecting microbes and their] resistance to antimicrobial agents, the results of which are disseminated for public-health actions and to assess the effects of any intervention program

NEEDS FOR SURVEILLANCE AMR

- **Identifying, understanding and predicting trends in and spread of resistant microorganisms (impact in guidelines)**

- **Detecting new resistance mechanisms**

- **Identifying the need for new diagnostic tests**

- **Identifying outbreaks of resistant organisms and infection control**

- **Identifying the need for new antibiotics**

- **Monitoring the impact of new empirical antibiotic prescribing**

- **Monitoring the impact of interventions to improve antimicrobial use and control the spread of infectious agents**

- **Identifying needs for sentinel laboratories in low-resources areas**

- **Public health and clinical guidelines**

- **Educating health care providers, patients and the general public**

AMR SURVEILLANCE LEVELS

- **Local**

- **Regional**

- **National**

- **SUPRANATIONAL**

ONGOING AMR SURVEILLANCE NETWORKS
Public funding

NETWORK	PATHOGENS or AGENT	FUNDING	NUMBER COUNTRIES	STARTING YEAR
ANSORP	Bacteria	Academic T/F	14	1996
BIRDY	Bacteria	Academic T/F	3	2012
CAESAR	Bacteria	WHO/ESCMID/Gov.	20	2013
CARPHA	Bacteria	WHO/Gov.	25	2013
CDDEP	Bacteria	Academic T/F	N.A.	1999
INICC	Bacteria	Academic T/F	43	2002
ERAS-Net	Bacteria	WHO/ESCMID/Gov	29	1999
FWD-Net	Bacteria	WHO/Gov.	29	2007
GASP	*N. gonorrhoeae*	WHO/Gov.	70	1992
SIREVA (+ SIREVA II)	Bacteria (Pneumonia-Meningitis	WHO/Gov	19	1993
[Global-PSP]	Bacteria	Academic T/F	63	2015
ReLAVRA	Bacteria	WHO/Gov.	19	1996

Supranational AMR surveillance networks involving LMICs (2000-2017)

Ashley EA et al. JAC 2018:1737
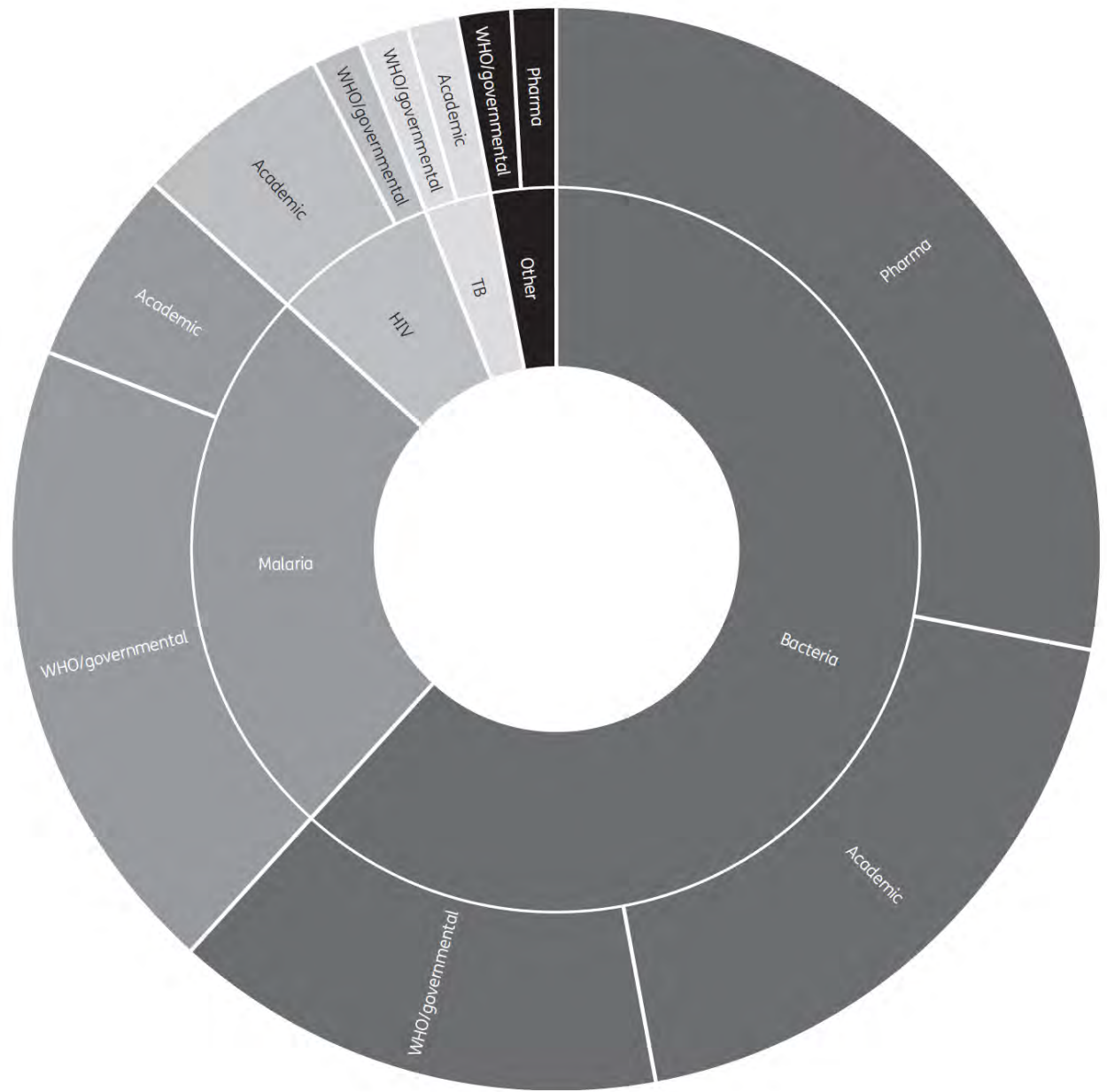
ONGOING AMR SURVEILLANCE NETWORKS
Pharma Programs

NETWORK	PATHOGENS or AGENT	FUNDING	NUMBER COUNTRIES	STARTING YEAR
AWARE	Ceftaroline	Pharma/CRO	7	2012
Int. Daptomycin S.P.	Daptomycin	Pharma/CRO	33	2011
PACTS	Ceftolozane-tazobactam	Pharma/CRO	16	2012
SARISA	*S. aureus*	Pharma	18	1996
SENTRY	Bacteria-fungi	Pharma/CRO	40	1997
SOAR	Bacteria	Pharma	48	2002
Int. Solythro-mycin Prog.	Solythromycin	Pharma	27	2011
TARGETed	Bacteria	Pharma/CRO	7	2003
TEST	Tygecycline	Pharma/CRO	65	2004
ZAAPS	Zyvos®	Pharma/CRO	42	2004

European Food- and Waterborne Diseases and Zoonoses Network (FWD-Net)

In 2007, the EU-funded dedicated surveillance network for enteric pathogens – *Salmonella, E. coli* and *Campylobacter* (Enter-net) was transferred to ECDC from the Health Protection Agency in the United Kingdom. Subsequently, the scope of the disease network was broadened to cover 21 food- and waterborne diseases and zoonoses, and nomination of disease experts followed the ECDC policy on Coordinating Competent Body (CCB).

FWD-Net is coordinated by ECDC with the support of a coordination committee (CC) consisting of representatives from the EU Member States. The committee advises ECDC on ways to strengthen and improve FWD surveillance and prevention in Europe and reviews technical documents relevant to the network.

FWD-Net also collaborates with partners, such as European Food Safety Authority (EFSA), World Health Organisation, relevant European Union Reference Laboratories and public health authorities of non-EU countries, e.g. US CDC. Furthermore, ECDC is actively collaborating with PulseNet International, the global network of public health laboratory networks, to ensure comparability of data and linkage to the global public health community.

The ECDC network co-ordinators can be contacted at fwd@ecdc.europa.eu.

Campylobacteriosis

Annual Epidemiological Report for 2017

SCIENTIFIC REPORT

ECDC/EFSA/EMA second joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals

Joint Interagency Antimicrobial Consumption and Resistance Analysis (JIACRA) Report

European Centre for Disease Prevention and Control (ECDC),
European Food Safety Authority (EFSA) and
European Medicines Agency (EMA)
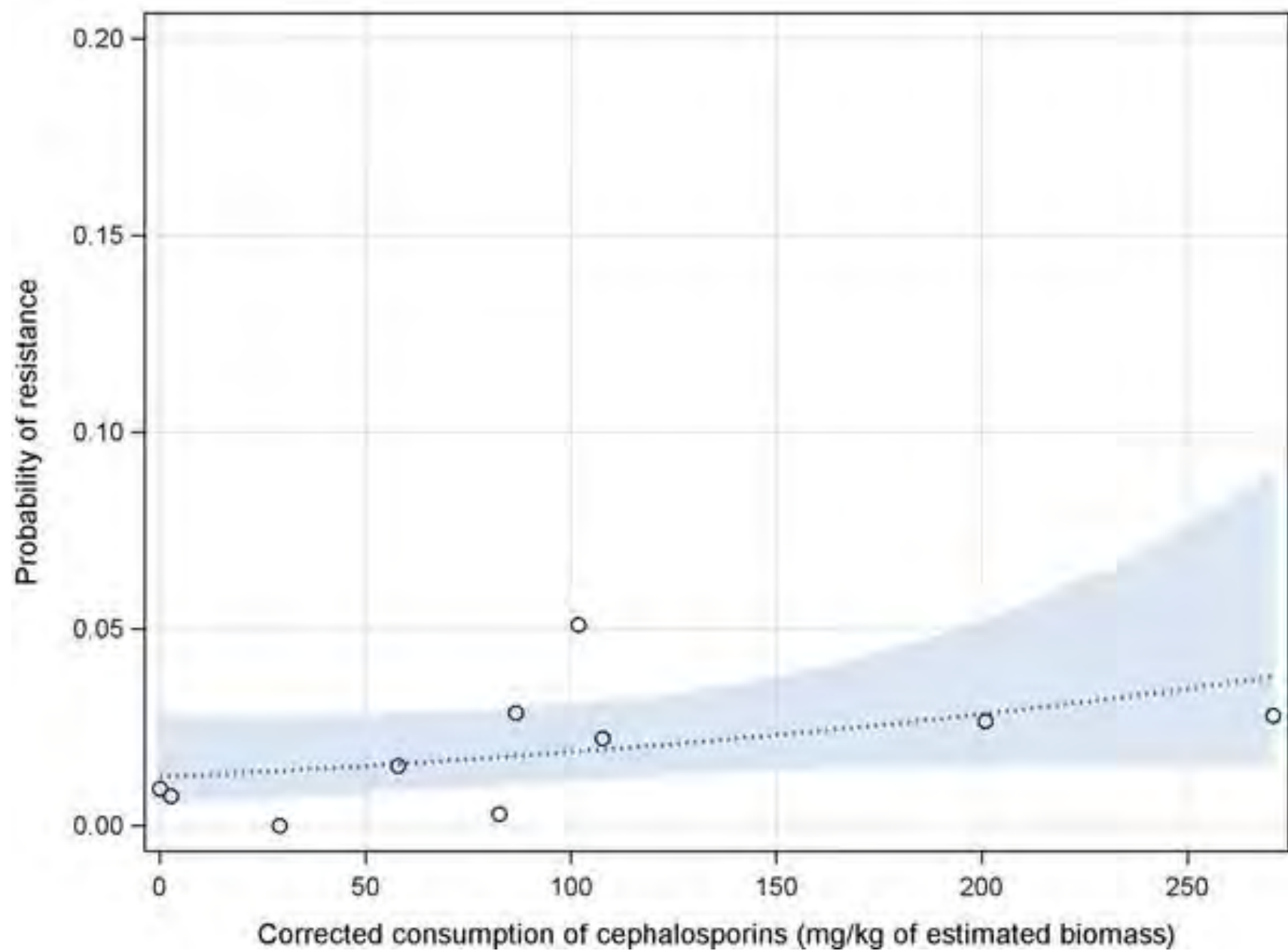
EARS-Net

European Antimicrobial Resistance Surveillance Network (2010…)
It continues the European Antimicrobial Resistance Surveillance System (EARSS) establsihed in 1998.

30 countries (most in EU)
900 microbiological laboratories, 1500 hospitals

Managed and coordinated by the European Centre for Disease Prevention and Control (ECDC)

Objectives :
- Collect comparable, representative and accurate AMR data
- Analyse temporal and spatial trends of AMR in Europe
- Provide timely AMR data for policy decisions
- Encourage the implementation, maintenance and improvement of national AMR surveillance programs
- Support national systems in their efforts to improve diagnostic accuracy by offering annual external quality assessments (EQA)

EARS-Net

Surveillance of indicator pathogens:
Escherichia coli, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium*

Bloodstream infections and meningitis

Variations in AMR over time and place

Web-accessible database (maps, graphs,…)
https://atlas.ecdc.europa.eu/public/index.aspx

Last report available in 2018 for data referring to 2017
https://ecdc.europa.eu/sites/portal/files/documents/EARS-Net-report-2017-update-jan-2019.pdf
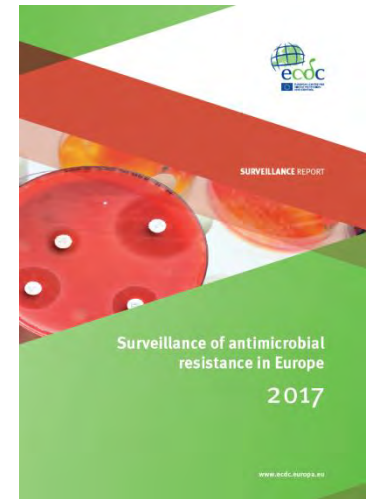
Netherlands

Coverage and representativeness of population, hospitals and isolates included in EARS-Net, Netherlands, 2014–2017

	2014	2015	2016	2017
Estimated national population coverage (%)	65	65	65	65
Population sample representativeness	High	High	High	High
Hospital sample representativeness	High	High	High	High
Blood culture sets/1000 patient-days	Unknown	Unknown	Unknown	Unknown
Isolate sample representativeness	High	High	High	High

Laboratories contributing data to EARS-Net: participation in EARS-Net EQA and use of clinical guidelines, Netherlands, 2014–2017

	2014	2015	2016	2017
Percentage laboratories participating in EARS-Net EQA	82	73	85	85
Percentage laboratories using EUCAST or EUCAST harmonised guidelines	96	93	100	100

Annual number of reporting laboratories*, number of reported isolates and proportion of isolates reported from patients in intensive care units (ICU), Netherlands 2014–2017

Pathogen	2014			2015			2016			2017		
	Laboratories (N)	Isolates (N)	Isolates from ICUs (%)	Laboratories (N)	Isolates (N)	Isolates from ICUs (%)	Laboratories (N)	Isolates (N)	Isolates from ICUs (%)	Laboratories (N)	Isolates (N)	Isolates from ICUs (%)
E. coli	35	6514	10	27	5380	10	32	6398	9	33	6687	7
K. pneumoniae	35	926	14	27	908	13	32	1135	11	33	1190	11
P. aeruginosa	35	555	24	27	502	22	31	543	14	33	657	17
Acinetobacter spp.	26	75	22	21	74	19	31	108	13	30	122	21
S. pneumoniae	35	1406	16	27	1301	15	32	1517	12	33	1511	10
S. aureus	35	2580	15	27	2107	15	32	2702	11	33	2695	11
E. faecalis	35	721	23	27	648	22	32	783	21	33	895	19
E. faecium	34	535	50	27	572	53	32	686	50	33	808	47

* Number of laboratories reporting at least one isolate during the specific year. Total number of laboratories participating in EARS-Net might be higher.

EARS-NET MRSA
1998-2017
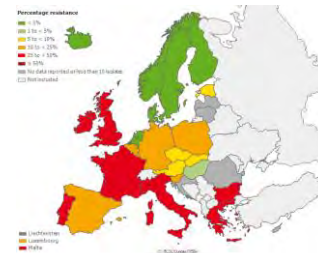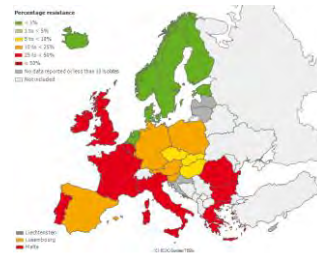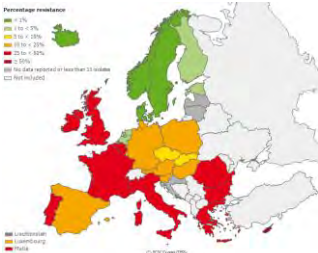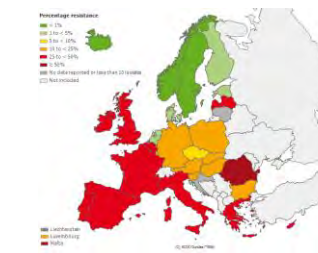
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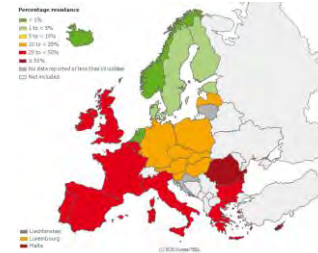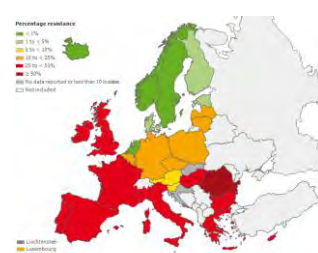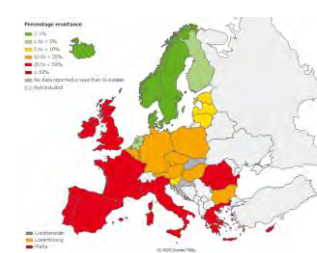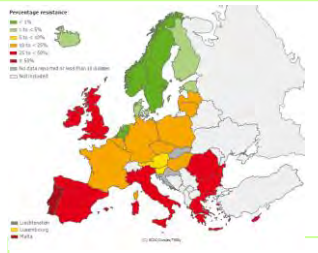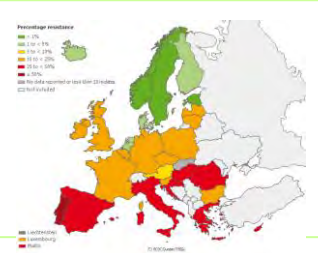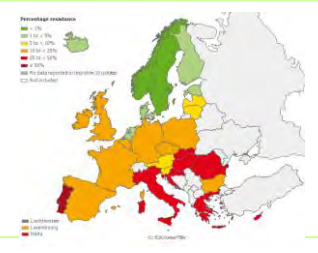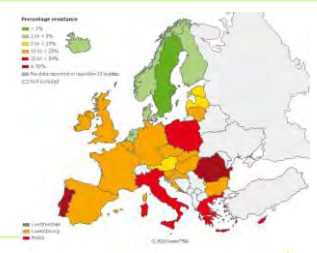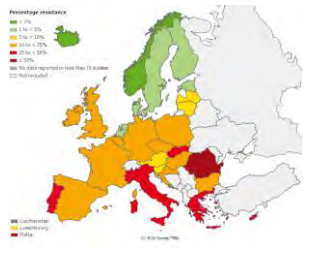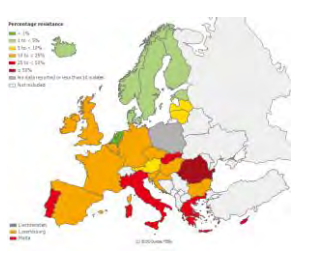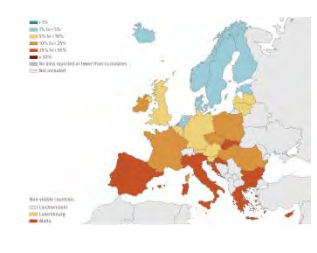
Surveillance Atlas of Infectious Diseases

Antimicrobial resistance ▼   Klebsiella pneumoniae ▼   Carbapenems ▼   Resistant (R) isolates proportion ▼   ▶ ◀◀ 2017 ▼ ▶▶   ⋮

Region	Resistant (R) isolates proportion (%)
Austria	1.0
Belgium	1.1
Bulgaria	12.4
Croatia	0.0
Cyprus	15.5
Czech Republic	0.4
Denmark	0.3
Estonia	0.0
Finland	0.3
France	0.7
Germany	0.5

⤢ ⊖ ⊕   Clear ✕   Filter ✕

Resistant (R) isolates proportion (%)

- 🟩 <1%
- 🟨 1-<5%
- 🟧 5-<10%
- 🟧 10-<25%
- 🟥 25-<50%
- 🟥 50-<75%
- 🟥 >=75%
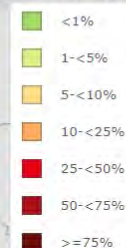
Resistant (R) isolates proportion, by age ▼   Bar ▼
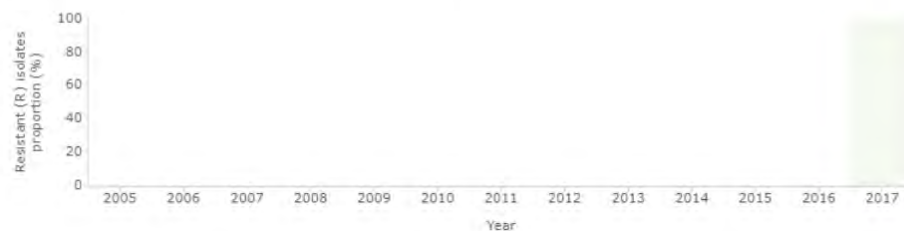
CAESAR

Central Asian and Eastern European Surveillance of Antimicrobial Resistance

Network of national surveillance systems for AMR

Joint initiative:

>**WHO**
>**European Society of Clinical Microbiology and Infectious Diseases (ESCMID-ESGARS)**
>**Dutch National Institute for Public Health and the Environment (RIVM).**

Countries of the region not integrated into EARS-Net

EARS-Net methodology

Central Asian and Eastern European Surveillance of Antimicrobial Resistance

Annual report 2018

ReLAVRA

Latin American Antimicrobial Resistance Surveillance Network

Created in 1996

Led by
    WHO Regional Office for the Americas/
    Pan American Health Organization (AMRO/PAHO)

    1996-2000: Community-acquired pathogens
    2000-…: Both community and nosocomial pathogens

Aggregated data provided by national reference laboratories (NRLs)

NRLs from 25 countries in Latin America plus Canada and the USA
720 Sentinel laboratories (2015)
Quality external control: Administración Nacional de Laboratorios e
Institutos de Salud (ANLIS), "Dr. C. G. Malbrán", Buenos Aires, Argentina

2000: 72 000 isolates
2010: >150 000 isolates

Last available Report on AMR: 2014

ReLAVRA. Considered Microorganisms

Community Pathogens

Noscomial Pathogens

- *Enterococcus* spp.
- *Klebsiella pneumoniae*
- *Acinetobacter* spp.
- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*
- *Escherichia coli*
- *Enterobacter* spp.

- *Salmonella* spp.
- *Shigella* spp.
- *Vibrio cholerae*
- *Escherichia coli*
- *Neisseria meningitidis*
- *Neisseria gonorrhoeae*
- *Streptococcus pneumoniae*
- *H. influenzae*
- Campylobacter
- *S. β hemolítico*
- *S. aureus*

SUPRANATIONAL INITIATIVES RELATED TO AMR SURVEILLANCE IN ASIA

1980s. WHO-Western Pacific Region.
Agreement of 14 Member States to share AMR data
20 pathogens (hospital --- community)
Annual reports for network participants
Interrupted in the early 2000s due to other emergencies)

2011, Jaipur Declaration on Antimicrobial resistance
Commitment to combat AMR (health ministers, 11 Member States)
6 Member States already have national surveillance systems
Regional database and consultative process
http://www.searo.who.int/entity/antimicrobial_resistance/sea_cd_273.pdf

Asian Network for Surveillance of Resistant Pathogens (ANSORP)
Independent, non-profit nongovernmental international collaborative
research group on AMR and infectious diseases in the Asian- Pacific
region. Based in the Republic of Korea, which is a member of the Asia
Pacific Foundation for Infectious Diseases (APFID)
Collaborators from 123 hospitals in 14 countries, territories and areas

ANSORP

Korea
19 centers
9 cities

Japan
3 centers
3 cities

China
36 centers
13 cities

Taiwan
8 centers
7 cities

Hong Kong
1 center

India
2 centers
2 cities

Vietnam
8 centers
3 cities

Philippines
6 centers
5 cities

Saudi Arabia
1 center
1 city

Thailand
5 centers
4 cities

Malaysia
12 centers
10 cities

Sri Lanka
6 centers
4 cities

Singapore
4 centers

Indonesia
2 centers
2 cities

Song J-H. AMR CONTROL 2015

RESISTANCE TO ANTIMICROBIAL AGENTS (ANSORP)
Highest reported prevalence of resistance

Pathogen	Disease	Antibiotic	Resistance[1]%	Focus area
Community				
Streptococcus pneumoniae	CAP[2]	Macrolide	73%	Asia
Escherichia coli	UTI[3]	3rd cephalosporins	95%	Asia
Salmonella Typhi	Enteric infection	Ciprofloxacin	84%	Asia
Hospital				
Staphylococcus aureus	HAP[4], bacteremia	Methicillin	82%	Asia
E. coli	HAP, bacteremia	Ciprofloxacin	96%	Asia
Klebsiella pneumoniae	HAP, bacteremia	3rd cephalosporins	81%	Asia
Pseudomonas aeruginosa	HAP	Carbapenem	30%	Asia
Acinetobacter baumanii	HAP	Carbapenem	68%	Asia

INITIATIVES RELATED TO AMR SURVEILLANCE AFRICA

Limited Information

Surveillance only in a few countries.

No formal framework for collaboration among surveillance programs

No common strategy for tracking and containing the emergence of resistant organisms, and to systematically evaluate trends and resistance-containment activities

[WHO guide to facilitate the establishment of laboratory-based surveillance for priority bacterial diseases in the region]

Journal of Antimicrobial Chemotherapy

Antimicrobial drug resistance among clinically relevant bacterial isolates in sub-Saharan Africa: a systematic review

Stije J. Leopold[1], Frank van Leth[1], Hayalnesh Tarekegn[1] and Constance Schultsz[1,2]*

Perspectives
Antimicrobial resistance in the WHO African region: current status and roadmap for action

S.Y. Essack[1], A.T. Desta[2], R.E. Abotsi[1], E.E. Agoba[1]

BMC Infectious Diseases

RESEARCH ARTICLE   **Open Access**

CrossMark

Antimicrobial resistance in Africa: a systematic review

Birkneh Tilahun Tadesse[1,2,3]*, Elizabeth A. Ashley[4], Stefano Ongarello[1], Joshua Havumaki[1], Miranga Wijegoonewardena[1], Iveth J. González[1] and Sabine Dittrich[1]

Page 1 of 7   ■   Lessons from the Field

Stepwise approach for implementation of antimicrobial resistance surveillance in Africa

Olga Perovic[1]
Constance Schultsz[2]

Number of studies in African countries on AMR [Surveillance]

GLOBAL ANTIMICROBIAL RESISTANCE SURVEILLANCE SYSTEM (GLASS)

World Health Organization
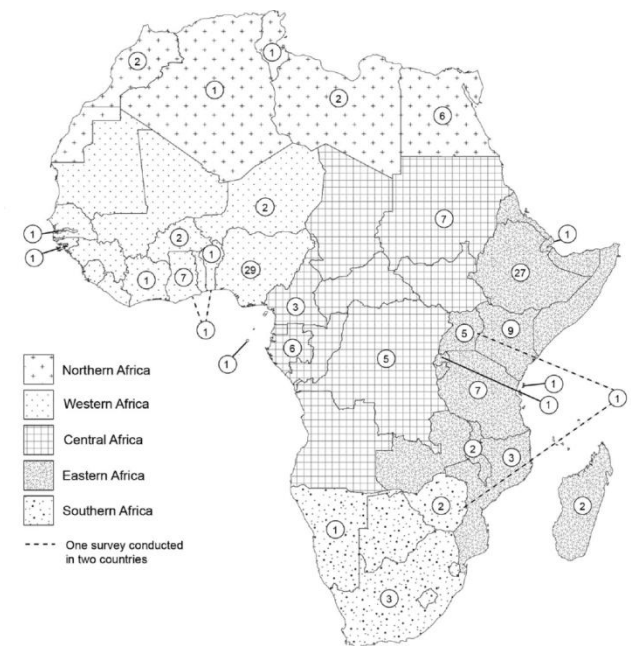
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Global Antimicrobial Resistance Surveillance System (GLASS)

GLASS

Country participation

IT Platform

Data collection

Data visualization

GLASS reports

Resource centre

GLASS-EAR

Laboratory

WHO Collaborating Centres Network

Partnerships

Events

Antimicrobial resistance

Global Antimicrobial Resistance Surveillance System (GLASS)

Road map | Scope | Collaboration | Call for country participation

Launched in October 2015, the Global Antimicrobial Resistance Surveillance System (GLASS) is being developed to support the global action plan on antimicrobial resistance. The aim is to **support global surveillance and research in order to strengthen the evidence base on antimicrobial resistance (AMR)** and help informing decision-making and drive national, regional, and global actions.

– Global action plan on antimicrobial resistance

GLASS promotes and supports a **standardized approach to the collection, analysis and sharing of AMR data at a global level** by encouraging and facilitating the establishment of national AMR surveillance systems that are capable of monitoring AMR trends and producing reliable and comparable data.

GLASS objectives

- Foster national surveillance systems and harmonized global standards;
- estimate the extent and burden of AMR globally by selected indicators;
- analyse and report global data on AMR on a regular basis;
- detect emerging resistance and its international spread;
- inform implementation of targeted prevention and control programmes; and
- assess the impact of interventions.

Road map

GLASS
Global AMR
Surveillance System

Key publications

Available now
Global antimicrobial resistance surveillance system (GLASS) report Early implementation 2017-2018

Global AMR Surveillance System (GLASS): Manual for early implementation 2015

GLASS OBJECTIVES

- **Foster national surveillance systems and harmonized global standards**

- **Estimate the extent and burden of AMR globally by selected indicators**

- **Analyse and report global data on AMR on a regular basis, and detect emerging resistance and its international spread**

- **Inform implementation of targeted prevention and control programmes; and**

- **Assess the impact of interventions**

Global Antimicrobial Resistance Surveillance System

Manual for Early Implementation

World Health Organization

Global Antimicrobial Resistance Surveillance System (GLASS) Report

Early implementation

2017-2018

World Health Organization

Netherlands

Population 17.04 million

The country is enrolled in GLASS since 2017.

Current status of the national AMR surveillance system

42 participating laboratories*

42 laboratories performing AST
No EQA provided

Surveillance Sites   Surveillance Sites   Surveillance Sites

NRL
selected
AST standard
EUCAST
EQA
not provided

National Reference
Laboratory

National
Coordinating
Centre

NCC
established
National AMR surveillance plan
in place (with budget)
National Focal Point
appointed

GLASS

in 2018 data call
Participating Laboratories providing data to GLASS
(42 laboratories)

* The identification of the total number of surveillance sites submitting specimens to participating laboratories was not possible due to the set up of the national surveillance system

Data submission

Specimen type	Data on number of tested patient	Pathogen	AST results	Age	Gender	Infection origin
Blood	●	Acinetobacter spp.	●	●	●	●
		E. coli	●	●	●	●
		K. pneumoniae	●	●	●	●
		Salmonella spp.	●	●	●	●
		S. aureus	●	●	●	●
		S. pneumoniae	●	●	●	●
Urine	●	E. coli	●	●	●	●
		K. pneumoniae	●	●	●	●
Stool	●	Salmonella spp.	●	●	●	●
		Shigella spp.	●	●	●	●
Genital	●	N. gonorrhoeae	●	●	●	●

● 100% data collected   ● 99-70% data collected   ● <70% data collected

---

Klebsiella pneumoniae

■ ≤ 30% unknown AST results   □ > 30% unknown AST results

Blood

Antibiotic:
- 3 gen cephalosporins
- Fluoroquinolones
- Carbapenems

0%   25%   50 %   75%   100%

Proportion of non–susceptible isolates

Staphylococcus aureus

■ ≤ 30% unknown AST results   □ > 30% unknown AST results

Blood

Antibiotic:
- PSB-lactams

0%   25%   50 %   75%   100%

Proportion of non–susceptible isolates

Streptococcus pneumoniae

■ ≤ 30% unknown AST results   □ > 30% unknown AST results

Blood

Antibiotic:
- Penicillins

0%   25%   50 %   75%   100%

Proportion of non–susceptible isolates

Gambia

Population 2.1 million

The country is enrolled in GLASS since 2018.

Current status of the national AMR surveillance system
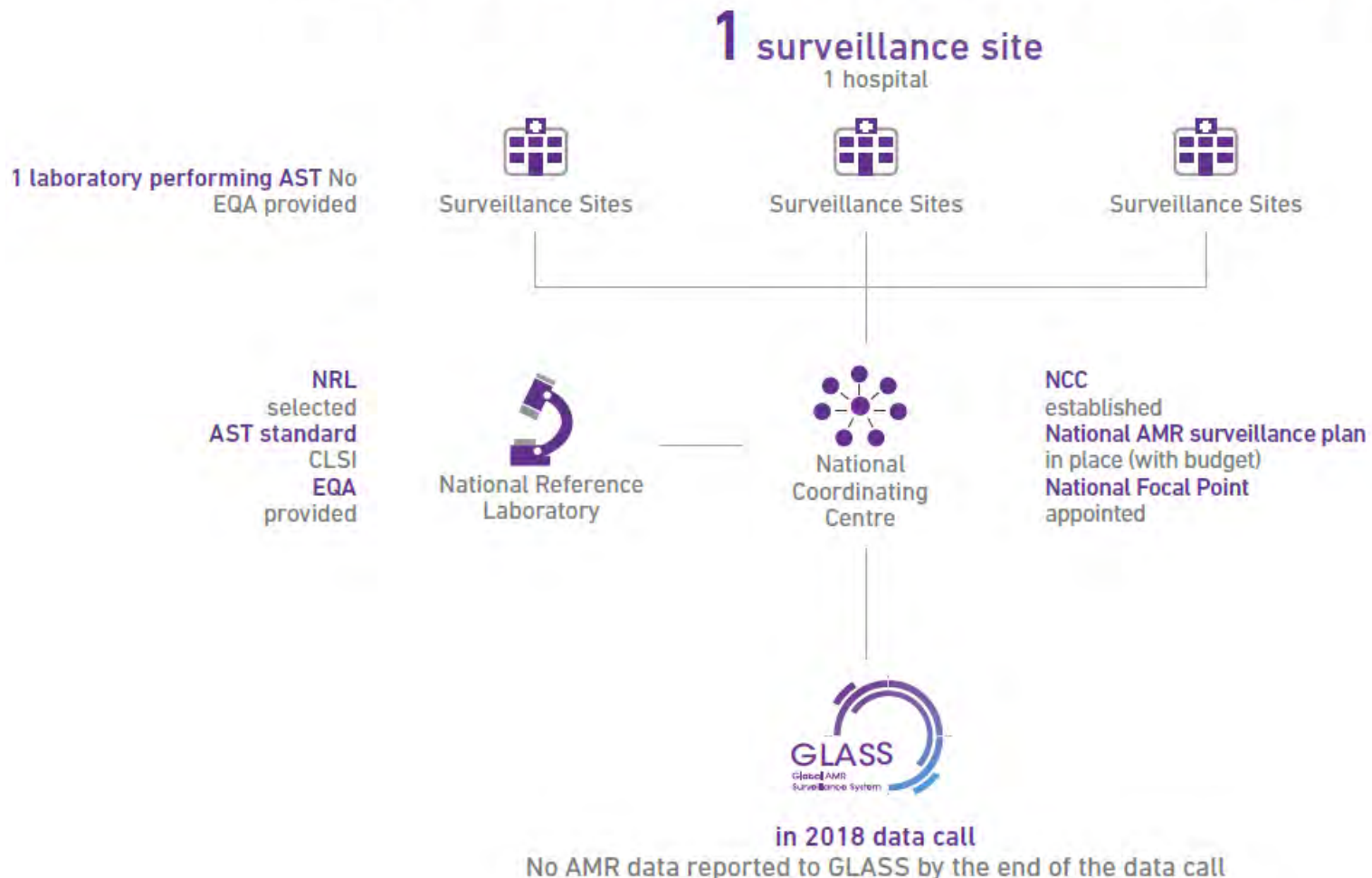
1 surveillance site
1 hospital

1 laboratory performing AST No EQA provided

Surveillance Sites

Surveillance Sites

Surveillance Sites

NRL
selected
AST standard
CLSI
EQA
provided

National Reference Laboratory

National Coordinating Centre

NCC
established
National AMR surveillance plan
in place (with budget)
National Focal Point
appointed

GLASS
Global AMR
Surveillance System

in 2018 data call
No AMR data reported to GLASS by the end of the data call

CENTER FOR DISEASE DYNAMICS, ECONOMICS & POLICY

The State of the World's Antibiotics in 2018

Ella Pringle Lecture
Royal College of Physicians of Edinburgh

Ramanan Laxminarayan
Twitter @CDDEP

CDDEP THE CENTER FOR
Disease Dynamics,
Economics & Policy
WASHINGTON DC · NEW DELHI

ResistanceMap

ResistanceMap is an interactive collection of charts and maps that summarize national and subnational data on antimicrobial use and resistance worldwide.

Column charts compare rates of antibiotic use and resistance between countries
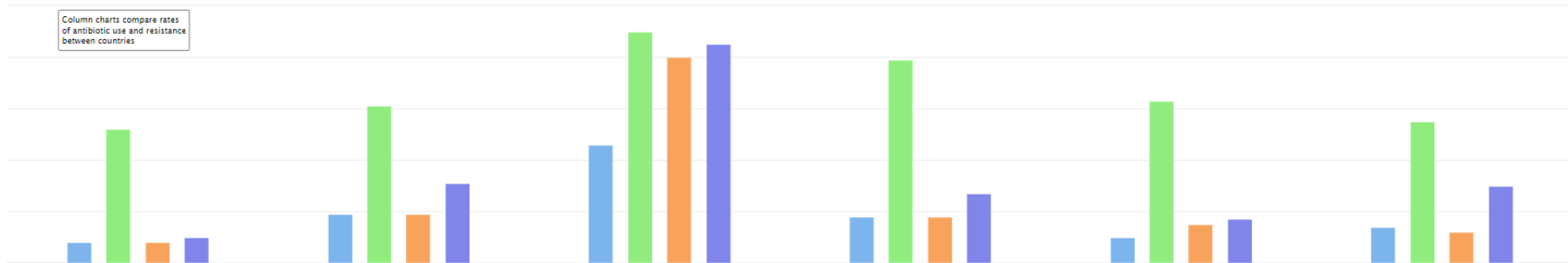
Start exploring the data by selecting a category below. ⬇

Antibiotic Resistance

Choose a pathogen and compare resistance to different antibiotics across countries. World map, in-country trends over time, and charts to compare between countries.

Antibiotic Use

Compare use rates between countries and over time. World map, charts, and breakdowns by antibiotic class.

Explore by Country

Focus on a single country and explore maps and charts on either antibiotic use or antibiotic resistance. Sub-national data is available for the United States.

About Resmap

ResistanceMap is a collection of tools summarizing national and subnational data on antimicrobial use and resistance around the world. Since its launch in 2010, ResistanceMap has helped inform researchers, policy makers and the public of important trends in drug resistance and antibiotic use. In 2015, ResistanceMap re-launched with a new design interface, expanded tools and the addition of antibiotic use and resistance data from several low- and middle-income countries in Africa, Asia and South America. Learn more here.

About CDDEP

The Center for Disease Dynamics, Economics & Policy (CDDEP) produces independent, multidisciplinary research to advance the health and wellbeing of human populations in the United States and around the world. For more information, visit CDDEP's main website.

'ONE HEALTH' SURVEILLANCE

- **Antibiotic usage/consumption PLUS Antibiotic Resistance data**

- **Humans, animals and the environment**

- **International collaboration and capacity**

Quennan K et al IJAA 2016, 422
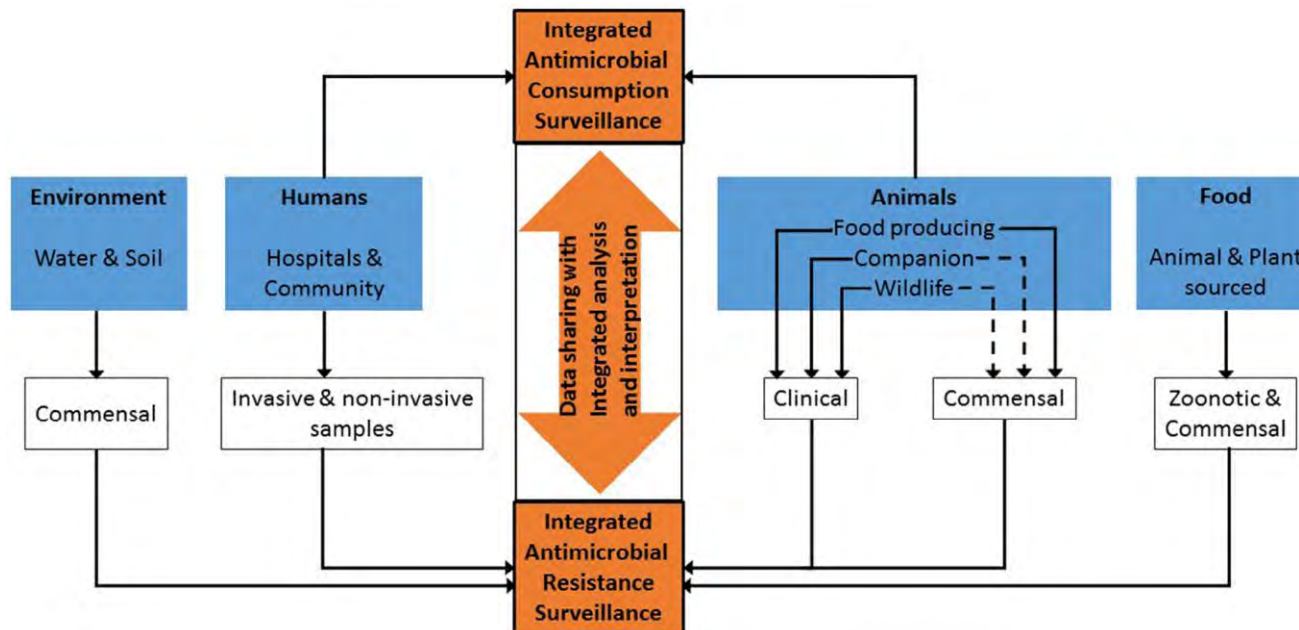
GLOBAL SHARING AND COMPARISON OF DATA

GENOMETRAKR

    2012. US Food and DrugAdministration PLUS NCBI

    www.fda.gov/Food/FoodScienceResearch/WholeGenomeSequencingProgramWGS

    Freely available. >61,000 isolates sequenced, >100 genomes closed

    Data can be uploaded into NCBI

    A phylogenetic tree can be generated by NCBI with all uploaded data

    Additional analysis can be performed locally

COMPARE

    2014. European Commission-funded

    www.compare-europe.eu

    "Integrate state-of-the-art strategies, tools, technologies and methods for collecting, processing and analysing sequence-based pathogen data in combination with associated (clinical, epidemiological and other) data"

    Bacteria, parasites, and viruses, single genomes and metagenomes

    Support release into the public domain (temporary 'quarantine' allowed)

CONCLUSIONS:
FUTURE NEEDS FOR AMR SURVEILLANCE

- To organize an integrated surveillance of AMR system considering humans, [food-producing] animals and the food chain

- To ensure collaboration between existing surveillance networks and surveillance centers towards coordinated regional and global surveillance

- To elaborate strategies for population-based surveillance of AMR

- To report data on resistance together with data on antimicrobial use in humans and animals

- To develop tools and standards for harmonized global surveillance of AMR

- To periodically evaluate both methods used and data collected to ensure their usefulness for public health purposes

- To increase the timeliness of data collection and reporting

- To collect and report subtyping data (e.g. genomic sequence) for important resistant pathogens

- To provide more extensive information on emerging and ongoing public health issues related to resistant pathogens and their health and economic impact

- To improve and continue economical-political-social support to AMR surveillance

THANK YOU!!

PK/PD-driven antimicrobial therapy in ICU

Leonardo Pagani, MD

Antimicrobial Stewardship Program

Infectious Diseases Unit

Bolzano Central Hospital, Bolzano (Italy)

WHO Expert Advisor on AMR and AS programs

Utrecht, 17.04.2019

Effective antibacterial dosing

- Early and effective appropriate antibacterial therapy is a significant determinant of clinical outcome

- Once correct antibacterial has been selected, dose selection occurs

- The aims of antibiotic dosing are to:
  - Maximise rate and extent of bacterial kill
  - Minimise the development of antibacterial resistance
  - Minimise possibility of drug toxicity

$\rightarrow$ Enhances likelihood of positive clinical outcomes

"One size fits all" policy is inappropriate
for patients in ICU

How to maximise positive outcomes?

Dosing complexities

- High level of sickness severity increases importance of achieving optimal therapy and exposure BUT also decreases the likelihood

- The pathophysiological status in ICU affecting antimicrobial disposition

Healthy volunteer

Patient in ICU

ESBL vs. AmpC β-lactamases vs. MβL

	AmpC	ESBL	MBL
Penicillins	Resistant	Resistant	Resistant
Cephalosporins	Resistant (except cefepime)	Resistant	Resistant
Aztreonam	Resistant	Resistant	Sensitive
β-lactamase inhibitors (tazobactam, clavulanic, etc)	Resistant	Sensitive	Resistant

Pharmacokinetic parameters

- Absorption and Bioavailability: how much drug will be available (when given orally)

- Volume of distribution (Vd): where the drug distributes

- Protein binding: the unavailable fraction of a drug

- Half-life ($t_{1/2}$): how long the drug circulates

- Clearance: how the body clears the drug

Not so easy to get there....
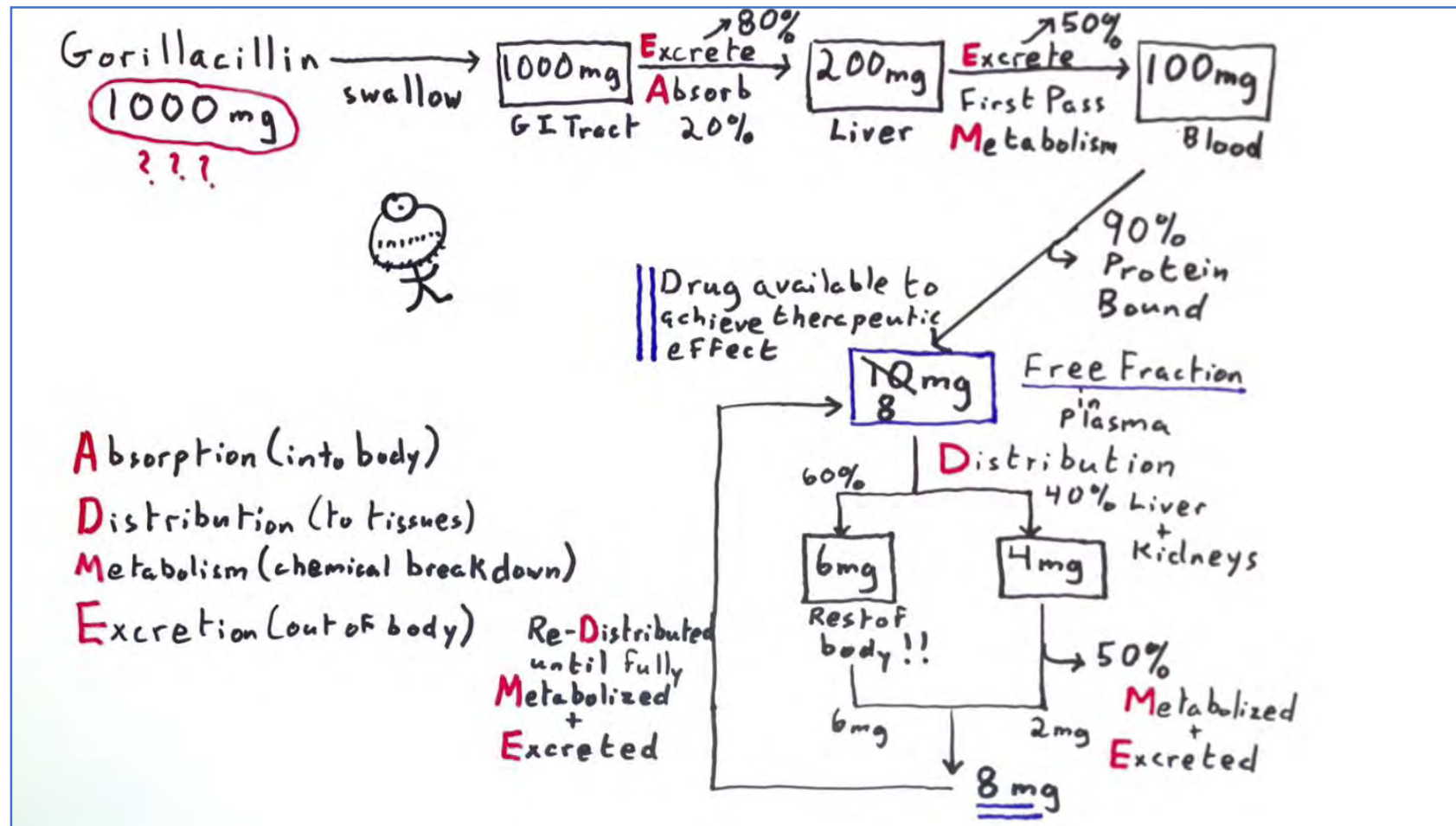
Pharmacokinetics of antimicrobials

- HYDROPHILIC

- β-lactams

- Glycopeptides

- Carbapenems

- Aminoglycosides

- Unable to cross cell membranes

- Limited Vd

- Inactive against intracellular pathogens

- Usually renal clearance

- LIPOPHILIC

- Oxazolidinones

- Rifampin

- Quinolones

- Azalides & Tetracyclins

- Free diffusion across cell membranes

- Wide Vd

- Active against intracellular pathogens

- Usually liver metabolism

The relevant role of apparent V_d in drug disposition

Drug	Vd (L/kg)	Vd for 70 kgs	Vd for 70 kgs + 5 l	% of loss or dilution
Flucloxacillin	0.1	7	12	> 70
Gentamicin	0.25	17.5	22.5	~ 30
Ciprofloxacin	1.8	126	131	< 1
Azithromycin	32	2240	2245	< 0.001
Linezolid	30-50	2100-3500	NR	NR

Main factors potentially responsible for alterations in drug-albumin binding
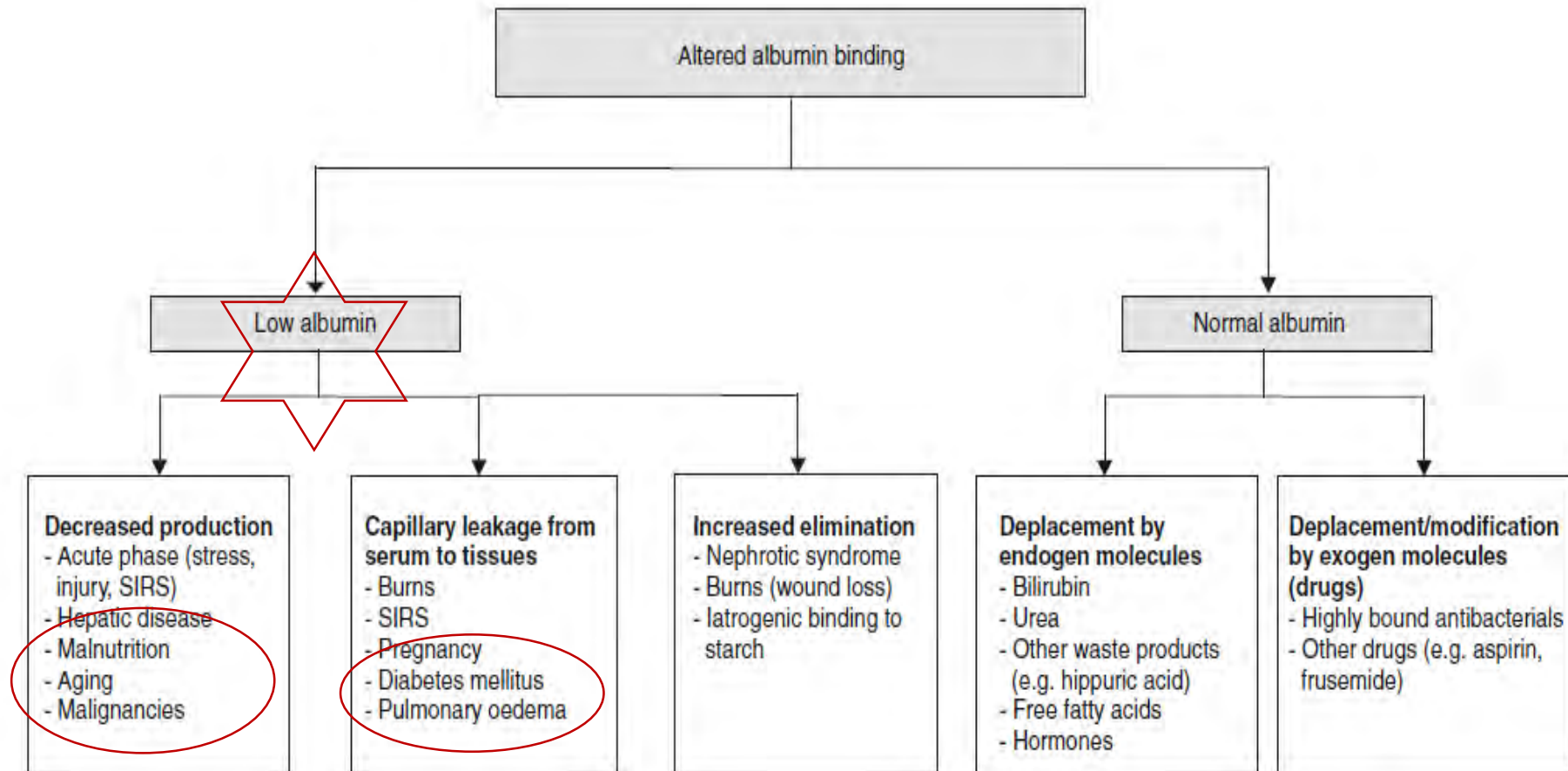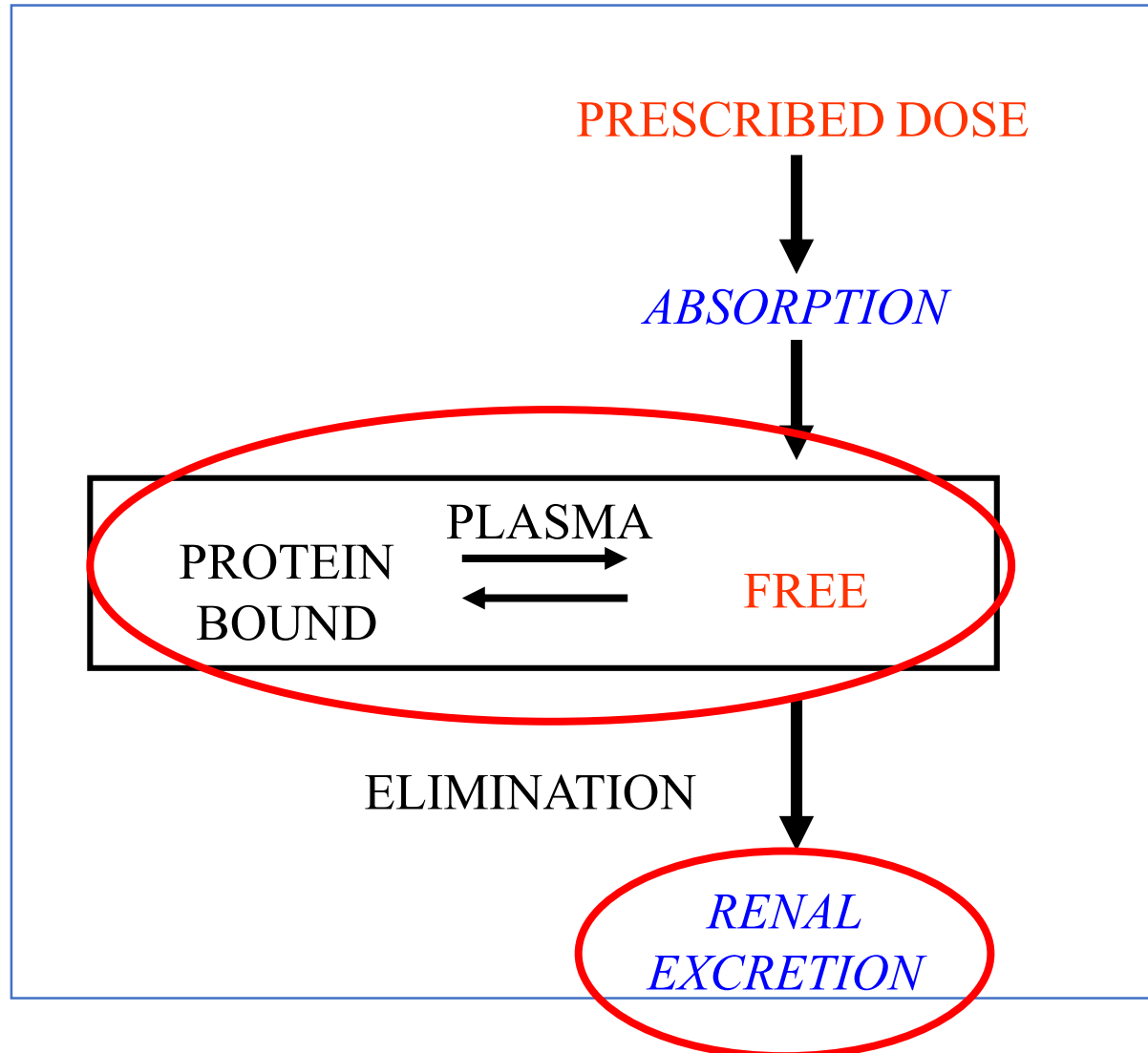
Roberts JA et al. *Clin Pharmacokinet* 2013; 52: 1-8

Sources of PK variability

CRITICAL ILLNESS				
Hyperdynamic ↑ Cardiac output	**Altered fluid balance** Third spacing &/or altered protein binding	**No organ dysfunction**	**Renal &/or hepatic dysfunction**	**Organ support** RRT &/or ECMO
↑ CL	↑ Vd	Unchanged Vd and CL	↑ Vd & ↓ CL	↑ Vd and ?CL
↓ Plasma concentrations	↓ Plasma concentrations	'Normal' plasma concentrations	↑ Plasma concentrations	↓ Or ↑ Plasma concentrations
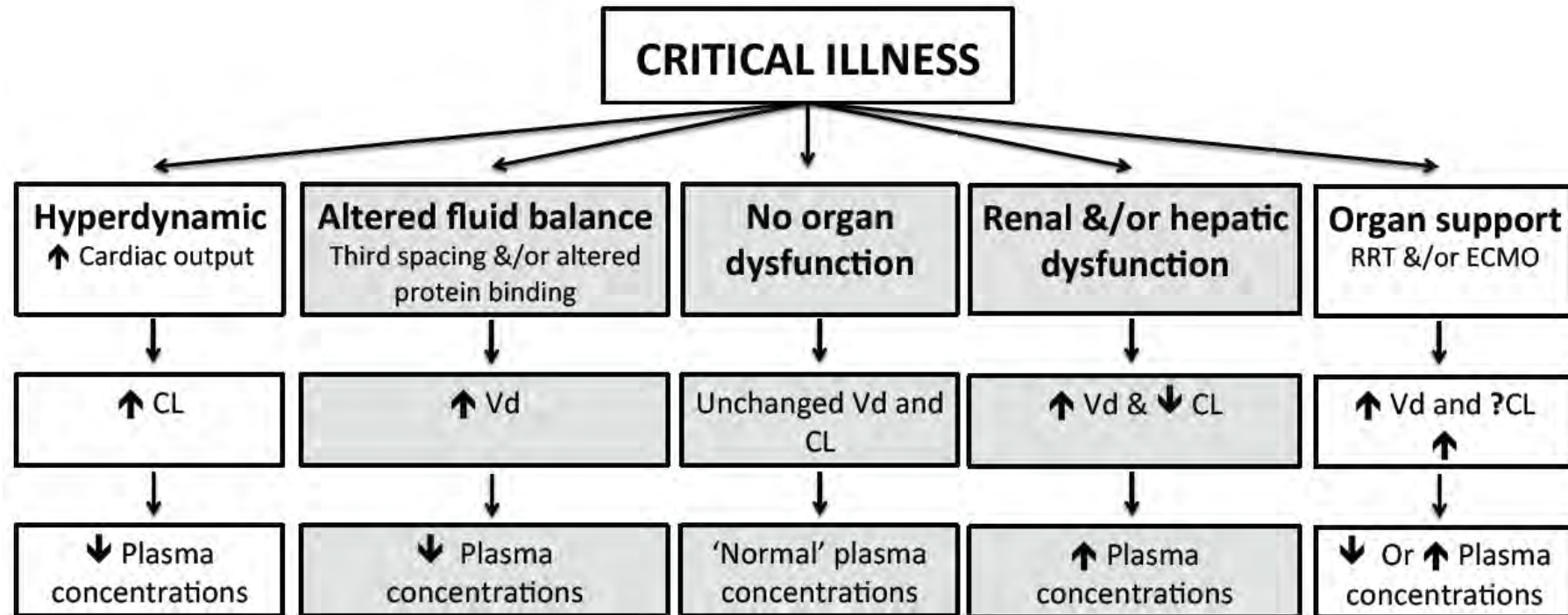
If dosing does not account for these changes – sub-optimal therapy! → Sub-optimal patient outcomes

RESISTANCE !!!

Augmented renal clearance, low β-lactam concentrations and clinical outcomes in the critically ill: An observational prospective cohort study

Angela Huttner [a,*], Elodie Von Dach [a], Adriana Renzoni [a], Benedikt D. Huttner [a], Mathieu Affaticati [b], Leonardo Pagani [a], Yousef Daali [c], Jerôme Pugin [d], Abderrahim Karmime [e], Marc Fathi [e], Daniel Lew [f], Stephan Harbarth [a]

[a] Infection Control Programme, Geneva University Hospitals and Faculty of Medicine, Rue Gabrielle Perret-Gentil 4, 1211 Geneva 4, Switzerland
[b] University Hospitals and Faculty of Medicine, Rue Gabrielle Perret-Gentil 4, 1211 Geneva 4, Switzerland
[c] Division of Pharmacology, Geneva University Hospitals and Faculty of Medicine, Rue Gabrielle Perret-Gentil 4, 1211 Geneva 4, Switzerland
[d] Division of Critical Care Medicine, Geneva University Hospitals and Faculty of Medicine, Rue Gabrielle Perret-Gentil 4, 1211 Geneva 4, Switzerland
[e] Department of Laboratory Medicine, Geneva University Hospitals and Faculty of Medicine, Rue Gabrielle Perret-Gentil 4, 1211 Geneva 4, Switzerland
[f] Division of Infectious Diseases, Geneva University Hospitals and Medical School, Rue Gabrielle Perret-Gentil 4, 1211 Geneva 4, Switzerland

ARC is common in the critically ill and strongly predicts diminished β-lactam plasma concentrations.

Beta-lactam PK/PD variability in ICU
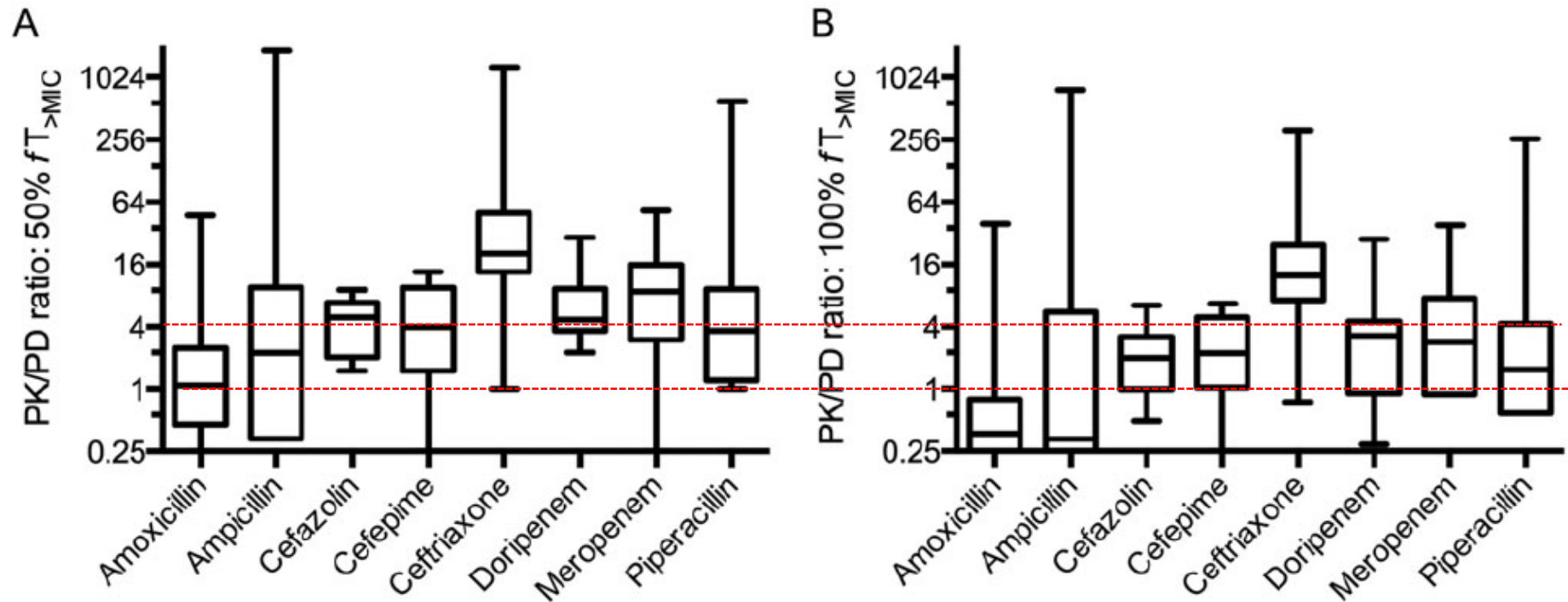
DALI: Defining Antibiotic Levels in Intensive Care Unit Patients: Are Current β-Lactam Antibiotic Doses Sufficient for Critically Ill Patients?

Thomas Jefferson
drafting
an audit on
ceftriaxone use

Fluoroquinolones

Resistance, dosing and target attainment

Labreche MJ, Frei CH.
Am J Health-Syst Pharm 2012;
69:1863-1870

Figure 1. Resistance of selected gram-negative organisms to ciprofloxacin and levofloxacin, 1999–2008, based on surveillance data from Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) program.[9]

Agent (Targeted Organism)
- Ciprofloxacin (*Escherichia coli*)
- Ciprofloxacin (*Enterobacteriaceae*)
- Ciprofloxacin (*Klebsiella pneumoniae*)
- Levofloxacin (*E. coli*)
- Levofloxacin (*Enterobacteriaceae*)
- Levofloxacin (*K. pneumoniae*)

Table 1.
Cumulative Fraction of Response (%) and Minimum Inhibitory Concentration (MIC) for Standard Dosing Regimens Against Three Gram-Negative Bacteria[19,a]

Antimicrobial, Dosing Regimen, and MIC	Escherichia coli 2002	2004	2006	Klebsiella species 2002	2004	2006	Pseudomonas aeruginosa 2002	2004	2006
Ciprofloxacin									
400 mg i.v. every 12 hr	91.6	78.3	71.3	93.6	91.3	79.8	62.1	61.1	63.5
400 mg i.v. every 8 hr	92.1	78.6	71.8	95.6	92.8	80.9	65.6	65.5	67.0
MIC_{90}(µg/mL)	0.125	4	4	0.125	0.125	4	4	4	4
Levofloxacin									
750 mg i.v. every 24 hr	...[b]	78.6	72.1	...	91.8	80.4	...	52.9	55.8
MIC_{90}(µg/mL)	...	16	8	...	0.5	0.25	...	16	8

[a]MIC_{90} = minimum inhibitory concentration required to inhibit the growth of 90% of organisms.
[b]Not reported.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The efficacy target of AUC/MIC>125 is based on the study of Forrest in 1993.
- Recent studies have shown that in ICU patients the ciprofloxacin efficacy target of AUC/MIC>125 is often not reached.

WHAT THIS STUDY ADDS

- The efficacy targets of ciprofloxacin in patients in general wards are often not reached. Most patients have low AUC with current iv dosing regimens. We suggest increasing the standard dose of ciprofloxacin to 1200 mg intravenously per 24 hours.
- Patients in general wards have high interindividual variability of pharmacokinetic parameters and therapeutic drug monitoring could be useful to support dosing.

The Ciprofloxacin Target AUC/MIC Ratio Is Not Reached in Hospitalized Patients with the Recommended Dosing Regimens

Haeseker M et al. *Br J Clin Pharmacol* 2013; 75: 180-185
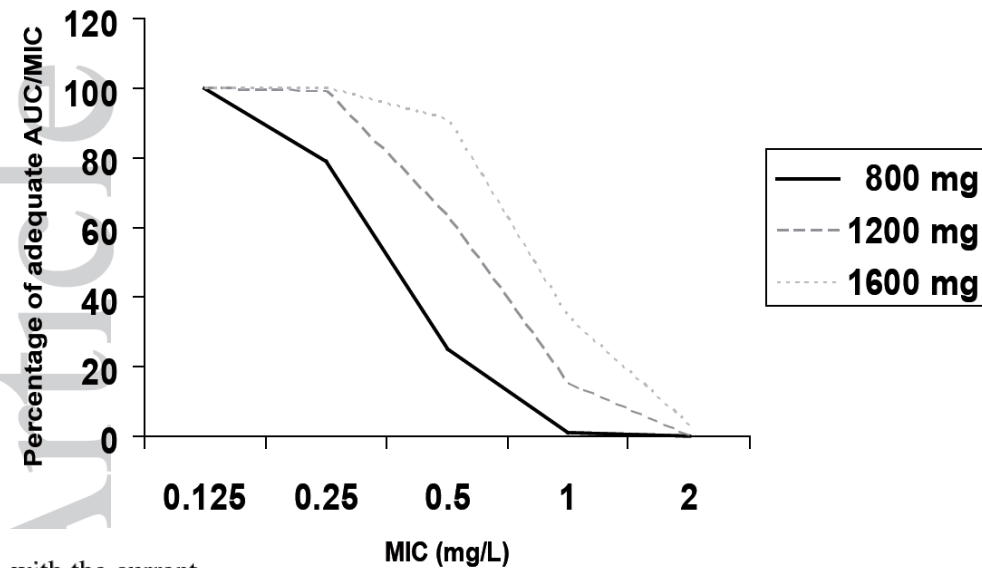
The majority of hospitalized patients did not reach the target AUC/MIC ratio with the current iv doses. Taken into account the increasing resistance for ciprofloxacin worldwide, TDM and subsequent dose adjustment may decrease development of ciprofloxacin resistance. A large randomized clinical trial of ciprofloxacin treatment is needed to confirm the AUC/MIC>125 or higher AUC/MIC ratios (>250) are needed for good clinical and microbiological outcome.

Low antibiotic exposures can lead to emergence of resistance

MIC...?
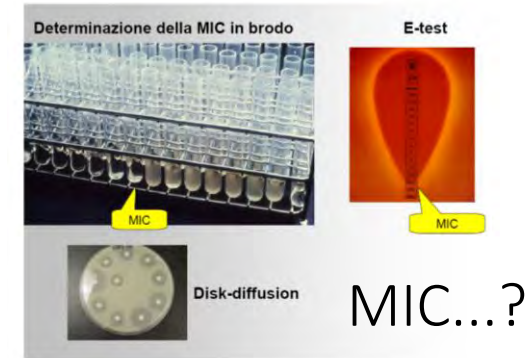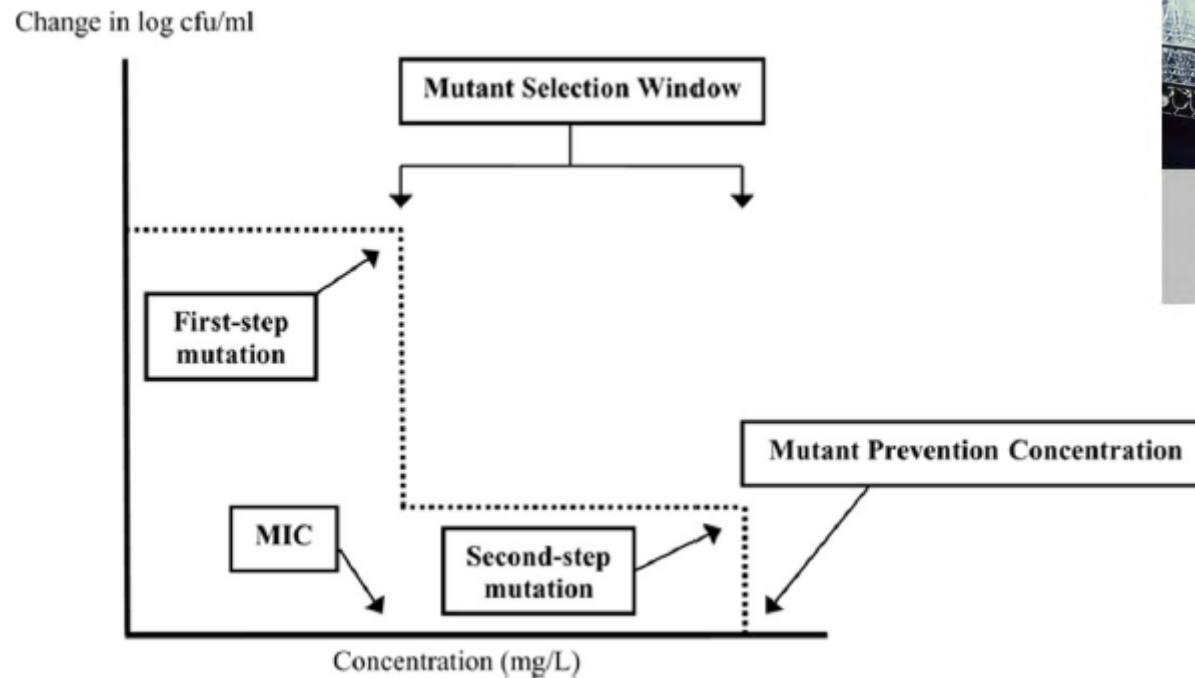
Minimal

Interest

for the **C**linicians..!!

Review Article

Antibiotic resistance—What's dosing got to do with it?

Jason A. Roberts, B Pharm (Hons); Peter Kruger, MBBS, FJFICM; David L. Paterson, MBBS, FRACP, PhD; Jeffrey Lipman, MBBCh, FJFICM, MD

(Crit Care Med 2008; 36:2433–2440)

Shape does matter: short high-concentration exposure minimizes resistance emergence for fluoroquinolones in *P. aeruginosa*

- High CIP concentrations over 1–10 h yielded more rapid and extensive initial killing compared with 16 and 24 h exposures at the same fAUC/MIC. No resistance emerged for 1–10 h exposures, although regrowth of susceptible bacteria was extensive.

- CIP exposure over 24 h yielded less regrowth, but CIP-resistant bacteria at 5× MIC amplified by over 5 $\log_{10}$ and almost completely replaced the susceptible bacteria by 24 h

- Pre-existing resistant subpopulations amplified extensively with 24 and 16 h exposures, but not with shorter durations.

- The shape of the CIP concentration profile was critical to minimize resistance emergence.

Rees VE et al, J Antimicrob Chemother 2015; 70: 816-826

Antimicrobial pharmacodynamics:
Interaction between antibiotics and their effects on pathogens

- The study of antimicrobial pharmacodynamics has proved useful for

  - establishing newer optimal dosing regimens for currently available drugs

  - developing new antimicrobials and new formulations

  - establishing susceptibility breakpoints

  - formulating guidelines for empirical therapy of infections

2016-03-30

Submission of comments on Guideline on the use of pharmacokinetics and pharmacodynamics in the development of antibacterial medicinal products (EMA/CHMP/594085/2015)

Comments from:

Name of organisation or individual
EPASG - ESCMID PK/PD of Anti-Infectives Study Group

Please note that these comments and the identity of the sender will be published unless a specific justified objection is received.

Approaching the end...

- Clear concentration-effect relationships exist for antibiotics

- We are commonly underdosing our patients because we don't understand the PK in individual patients

- Underdosing leads to resistance…and we are here just for that..

- One solution may be TDM

- Clinical utility of antibacterial TDM still being quantified

And if you don't have TDM?

Do your best! Better outcome, less resistance…

- TZP 12-16 g/24h
- Ciprofloxacin 750 mg/d PO; 2 x 600 mg/d IV
- Gentamicin 5-7 mg/kg OD
- Amikacin 20-25 mg/kg OD
- Meropenem 3-4 g/24h
- Vancomycin 30 mg/kg/24h
- …and so on..
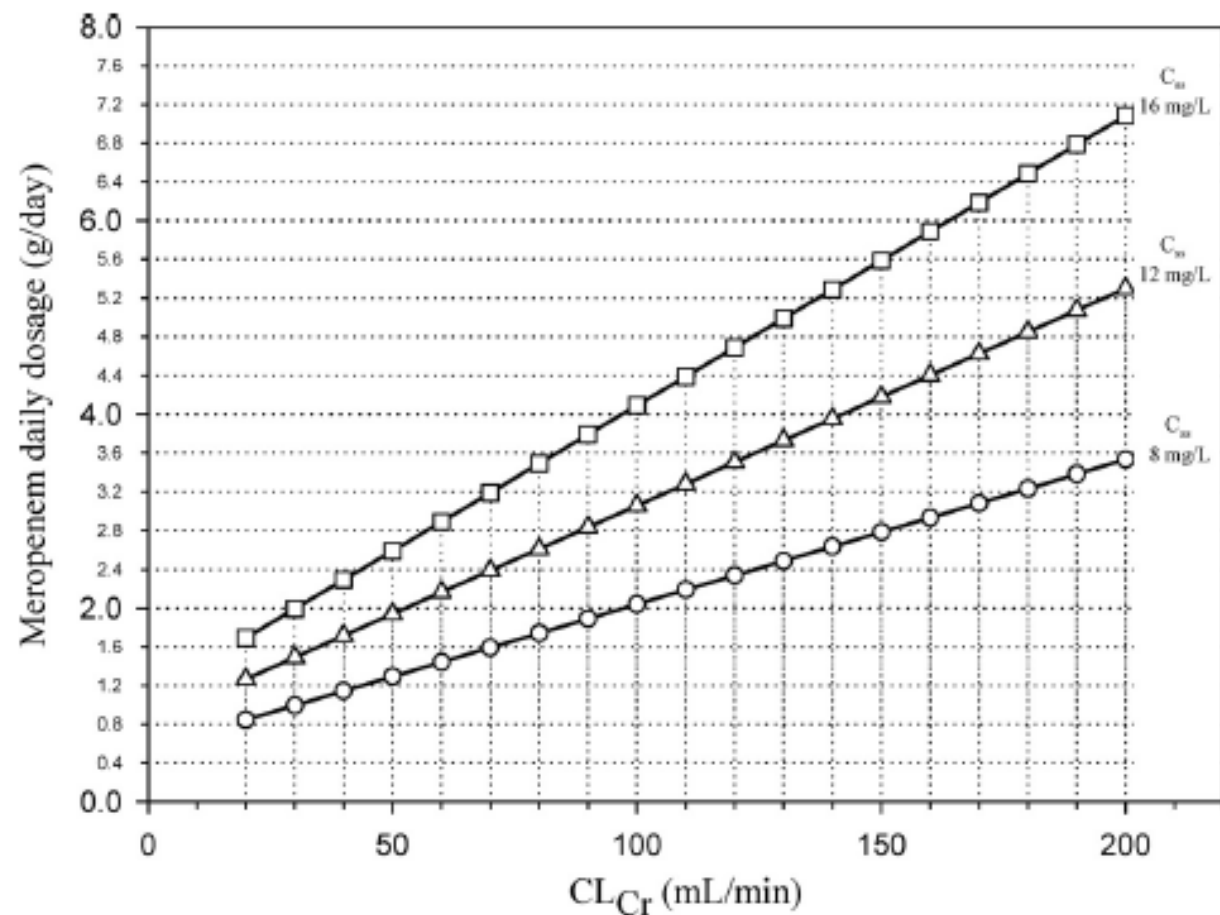
Dosing Nomograms for Attaining Optimum Concentrations of Meropenem by Continuous Infusion in Critically Ill Patients with Severe Gram-Negative Infections: a Pharmacokinetics/Pharmacodynamics-Based Approach

Federico Pea, Pierluigi Viale, Piergiorgio Cojutti and Mario Furlanut

A mind is like a parachute:

It does not work if it is not open

(*Franck Zappa*)

thanks for your attention

Precision Antimicrobial Therapy in food-producing animal

Alain Bousquet-Mélou

UMR 1436 INTHERES

Innovations Thérapeutiques et Résistances

One World, one Health : bacterial ecosystems
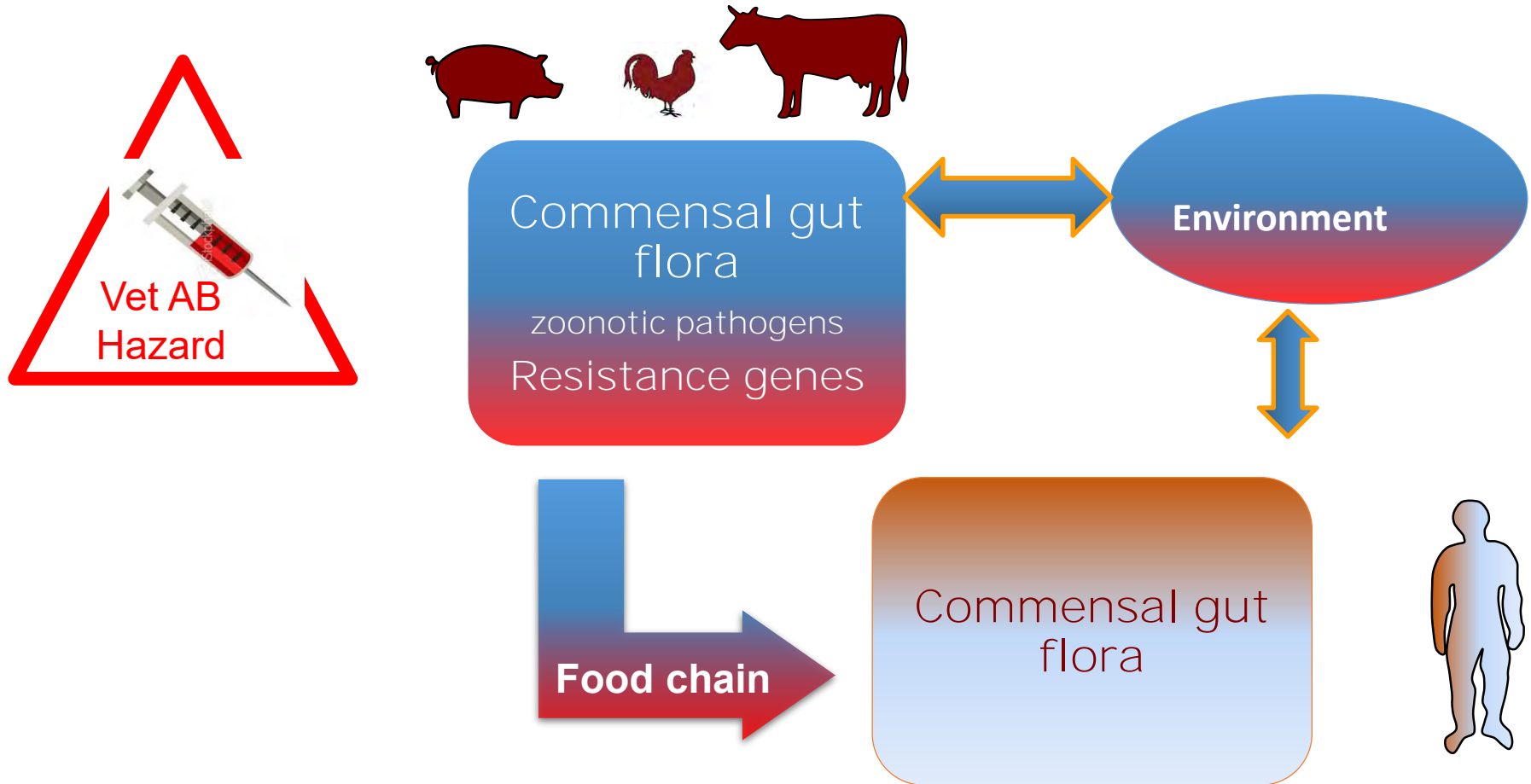
Objective : To reduce AB selective pressure on intestinal microbiota

Antibiotic Oral

Digestive Tract

Resistant zoonotic Pathogens
(*Salmonella, Campylobacter* …)
Non-pathogenic flora (resistance genes)

Antibiotic Parenteral

Blood

Food chain

Environment

Contact

Pathogen for the animal

ANIMAL HEALTH

Genes Flux

HUMAN

HUMAN HEALTH

Objective : To reduce AB selective pressure on intestinal microbiota

Antibiotic Oral

Digestive Tract

Resistant zoonotic Pathogens
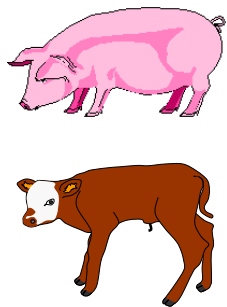(*Salmonella, Campylobacter* …)
Non-pathogenic flora (resistance genes)
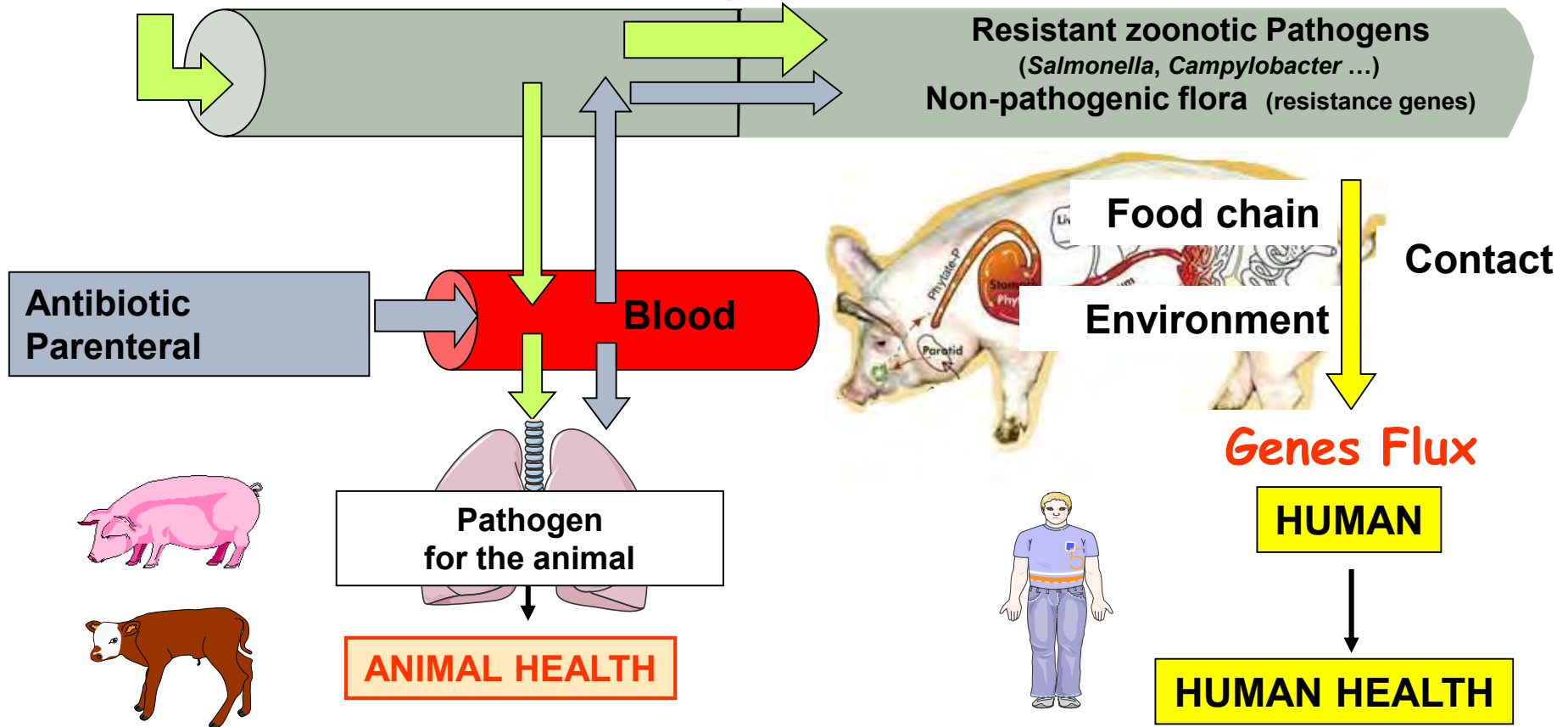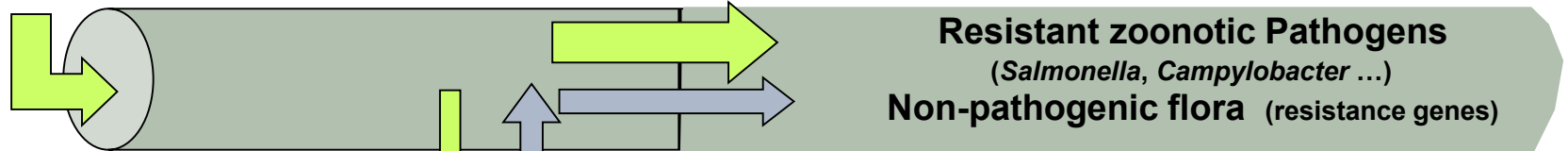
Antibiotic Parenteral

Blood
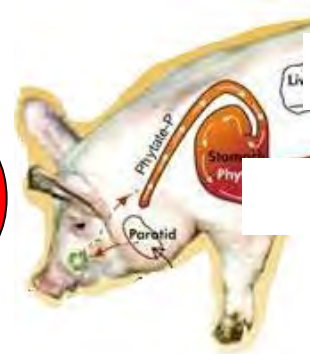
Pathogen for the animal

ANIMAL HEALTH

HUMAN HEALTH
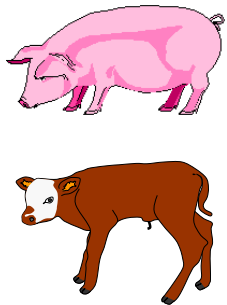
X

?

Objective : To reduce AB selective pressure on intestinal microbiota

Antibiotic Oral

Digestive Tract

Resistant zoonotic Pathogens
(*Salmonella, Campylobacter* …)
Non-pathogenic flora (resistance genes)

Antibiotic Parenteral

Blood
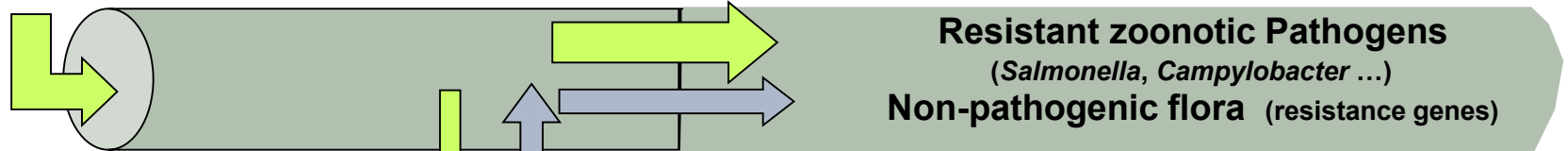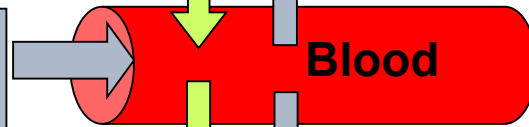
- **Optimization of existing antibiotics**

Pathogen for the animal
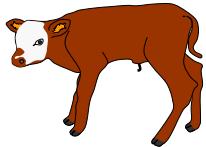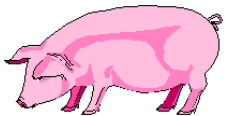
- **New "Green" or "Eco-friendly" antibiotics**

ANIMAL HEALTH

- **Alternatives to antibiotics**

Objective : To reduce AB selective pressure on intestinal microbiota

Antibiotic Oral

Digestive Tract

Resistant zoonotic Pathogens
(*Salmonella, Campylobacter* …)
Non-pathogenic flora (resistance genes)

Antibiotic Parenteral

Blood

- **New "Green" or "Eco-friendly" antibiotics**

To minimize environmental pollutions by
- **Resistance genes**
- **Active antibiotics**

Pathogen

Figure 3: Waste-water treatment facilities can be hotspots for horizontal transfer of resistance

Objective : To reduce AB selective pressure on intestinal microbiota

Antibiotic Oral

Digestive Tract

Resistant zoonotic Pathogens
(*Salmonella, Campylobacter …*)
Non-pathogenic flora (resistance genes)

Antibiotic Parenteral

Blood

Blood
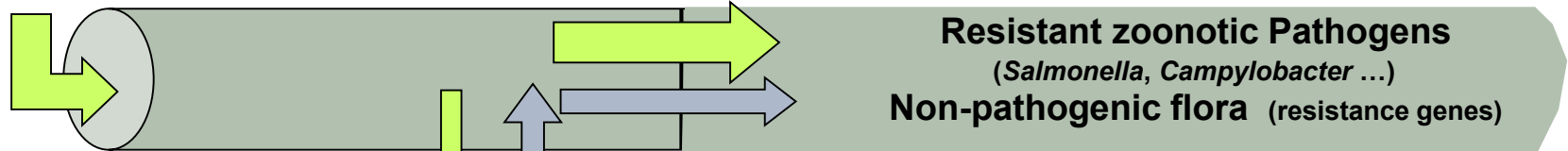
Pathogen for the animal
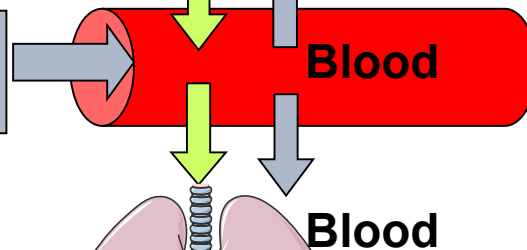
ANIMAL HEALTH

- **New "Green" or "Eco-friendly" antibiotics**

To minimize environmental pollutions by
- **Resistance genes**
- **Active (stable) antibiotics**
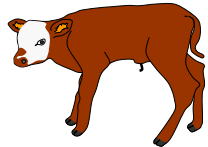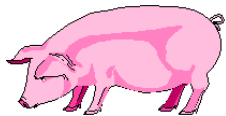
Veterinary Medicine Needs New Green Antimicrobial Drugs

Pierre-Louis Toutain[1]*, Aude A. Ferran[1], Alain Bousquet-Melou[1], Ludovic Pelligand[2] and Peter Lees[2]

[1] Ecole Nationale Vétérinaire de Toulouse, Institut National de la Recherche Agronomique, TOXALIM, Université de Toulouse, Toulouse, France, [2] Comparative Biomedical Sciences, The Royal Veterinary College, Hatfield, UK

Objective : To reduce AB selective pressure on intestinal microbiota

Antibiotic Oral

Digestive Tract

Resistant zoonotic Pathogens
(*Salmonella, Campylobacter* …)
Non-pathogenic flora (resistance genes)

- **Optimization of existing antibiotics**

Antibiotic Parenteral

Blood

Pathogen for the animal

ANIMAL HEALTH

Microbiology Spectrum
American Society for Microbiology Press

Optimization of Antimicrobial Treatment to Minimize Resistance Selection

LUCA GUARDABASSI,[1] MIKE APLEY,[2] JOHN ELMERDAHL OLSEN,[1] PIERRE-LOUIS TOUTAIN,[3] and SCOTT WEESE[4]

[1]Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, 1870 Frederiksberg C, Denmark; [2]Kansas State University College of Veterinary Medicine, Manhattan, Kansas, 66506; [3]INTHERES, Université de Toulouse, INRA, ENVT, Toulouse, France; [4]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Canada

PRECISION LIVESTOCK FARMING

Precision Livestock Farming (PLF)

Monitoring and Regulation of breeding systems /productivity
- Climate, housing conditions
- Feeding
- Animal behaviour : **welfare** and **production process**

Real-time monitoring of physiological, behavioural, environmental data

Actimetry data
Drinking, feeding behaviors
Acoustic / video signals

Swine and Poultry productions
Bovine production /Dairy cattle
Aquaculture

Precision Livestock Farming (PLF)

Actimetry data, drinking /feeding behaviors

HOW LIVESTOCK RFID WORKS
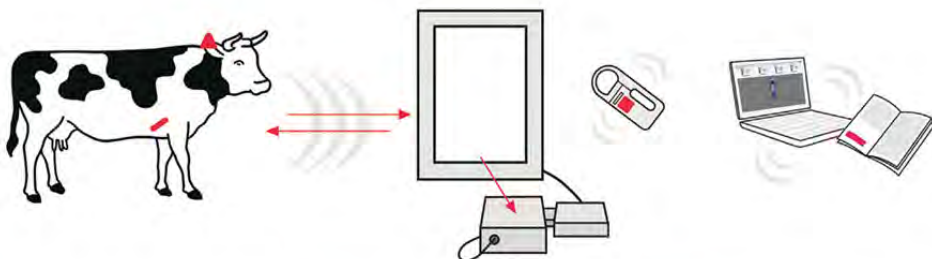
1. Apply ear tags to, and/or implant boluses in your animals

2. Read RFID identification codes to track individual animals using Datamars portable readers or raceway antennas

3. Use the included software to integrate data seamlessly to your livestock management system

Process

1. The Reader transfers energy to the antenna which in turn emits electromagnetic waves through the air to the ear tag.

2. The RFID ear tag uses the energy (electromagnetic waves) to return a signal in form of the information saved to the transponder chip to the antenna.

3. The antenna/reader, which received the ear tag transponder response, processes the information accordingly with its IT system (back end).

Precision Livestock Farming (PLF)

Acoustic / video signals

K.U.Leuven
University of Milan

SoundTalks
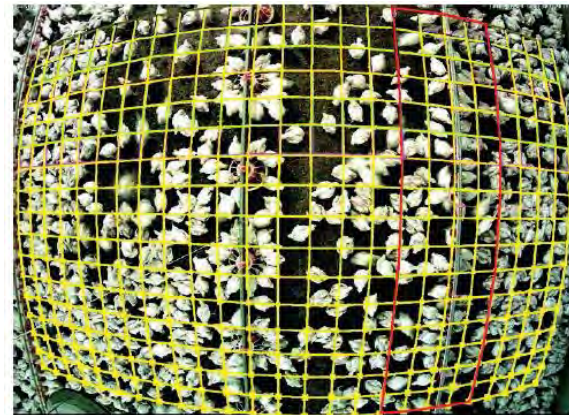
Fancom®
forward thinking

Analysis of poultry eating and drinking behavior by software eYeNamic

A. De Montis,[1] A. Pinna,[1] M. Barra,[1] E. Vranken[2,3]

[1]University of Sassari, Dipartimento di Agraria, Sassari, Italy; [2]Fancom B.V., Panningen, The Netherlands; [3]KULeuven, Division M3-BIORES, Heverlee, Belgium

PRECISION LIVESTOCK FARMING

⬇

EARLY DETECTION OF DISEASE

⬇

PRECISION ANTIMICROBIAL THERAPY

Metaphylaxis

A strategy of antimicrobial therapy tailored to food-producing animals ?

REGULATION (EU) 2019/6 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 11 December 2018

on veterinary medicinal products and repealing Directive 2001/82/EC

(Text with EEA relevance)

Metaphylaxis

- means the administration of a medicinal product to a **group of animals** after a diagnosis of clinical disease in part of the group has been established, with the aim of treating the clinically sick animals and controlling the spread of the disease to animals in close contact and at risk and which may already be subclinically infected.

Prophylaxis

- means the administration of a medicinal product to an animal or group of animals before clinical signs of a disease, in order to prevent the occurrence of disease or infection;

Metaphylaxis in the time course of a disease

- ***Phylaxis* = PROTECTION**

  - From *phylax* = guard, watchman
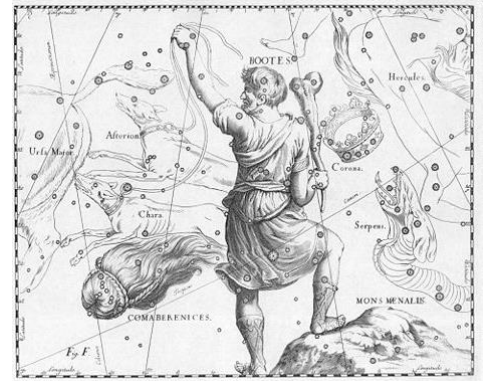
Arctophylaxis (Boote)
the « bear-driver »
(Ursus Minor, Ursus Major)

- **In relation with the start of the aggression**
  - *Pro* - : before the aggression begins
  - ***Meta* - : after the aggression begins**

The time course of the bacterial disease

Health

Start
of the
infectious disease

Disease

No "visible" symptoms   "Visible" symptoms
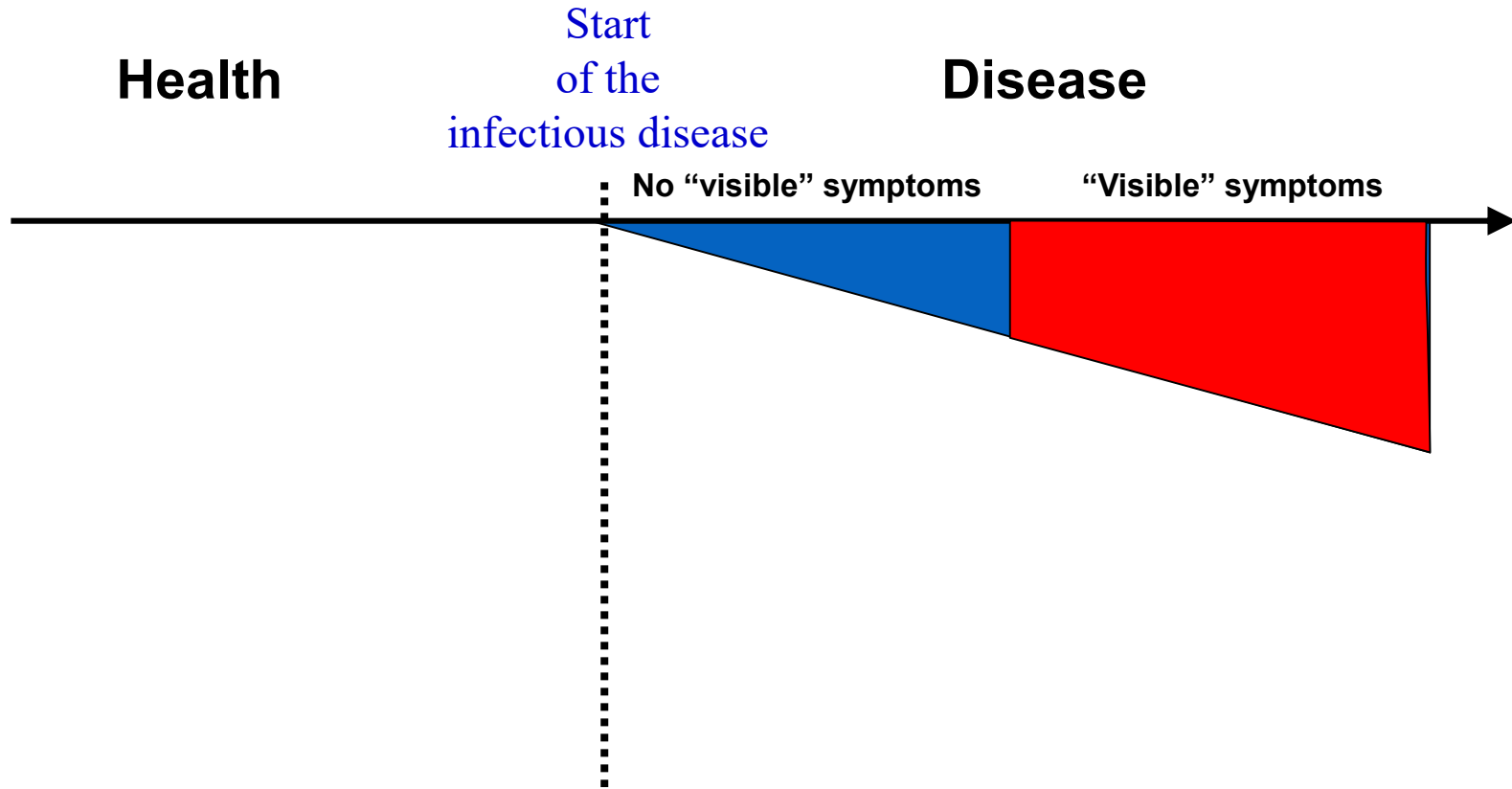
Bacterial contamination / Host defenses
Growth of the initial inoculum

The time course of the bacterial disease

Health

Start
of the
infectious disease

Disease

No "visible" symptoms   "Visible" symptoms

?

METAPHYLAXIE

The time course of the bacterial disease

Health

Start of the infectious disease

Disease

No "visible" symptoms   "Visible" symptoms

?

METAPHYLAXIS

EARLY

(few)
conventional

(few ?)
PREVENTIVE

CURATIVE TREATMENT
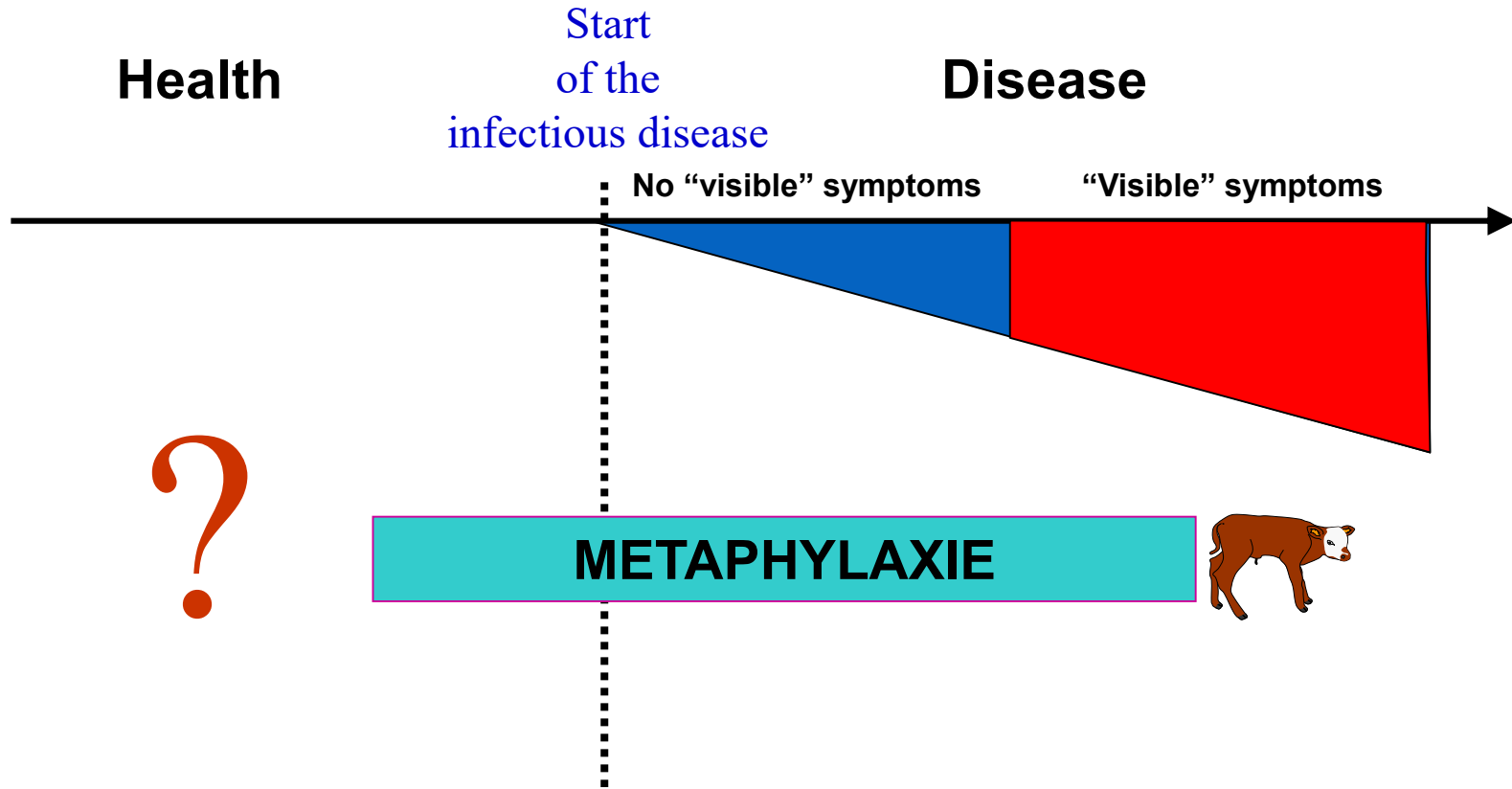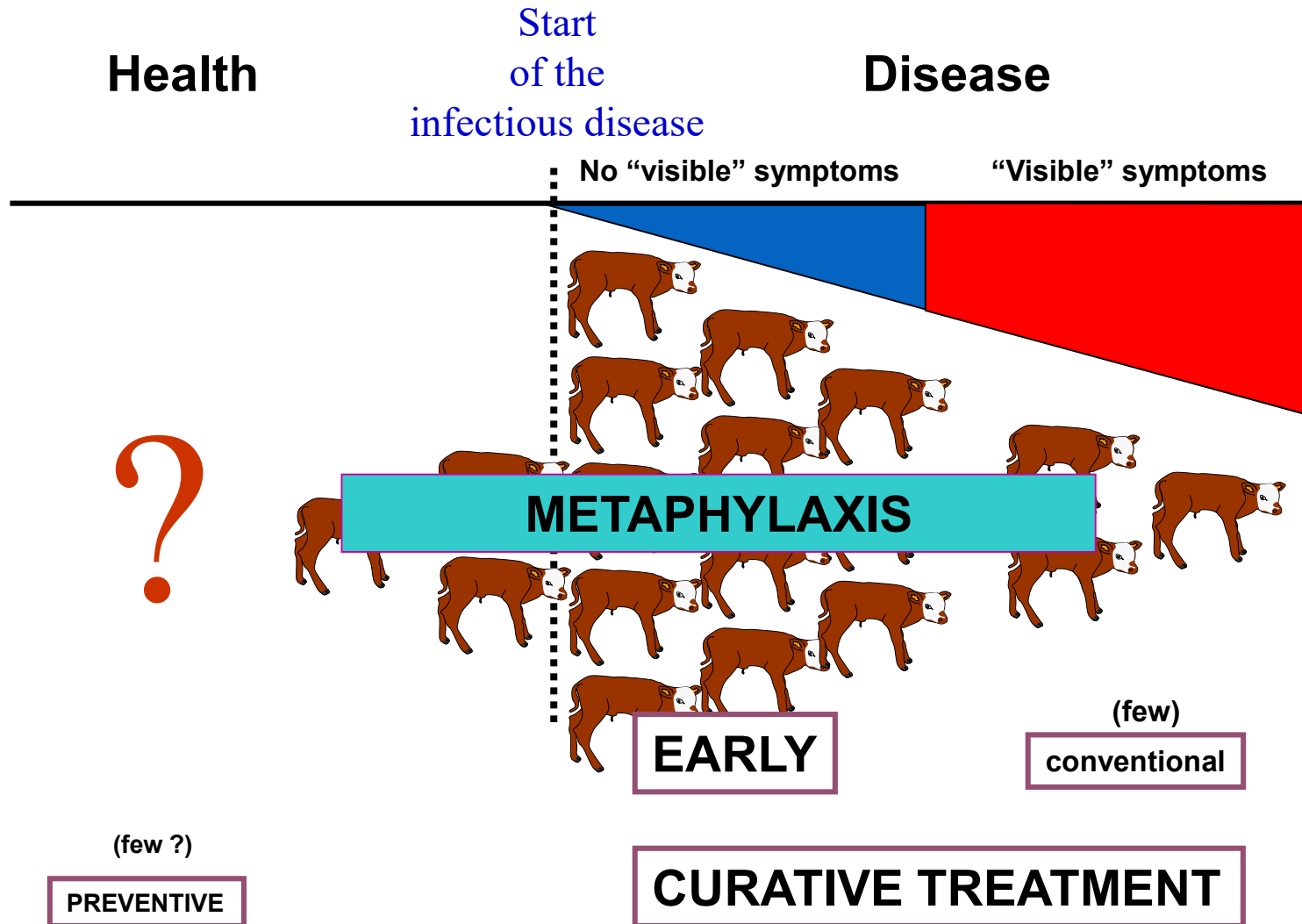
Antibiotics are more active against lower bacterial load

The time course of the bacterial disease
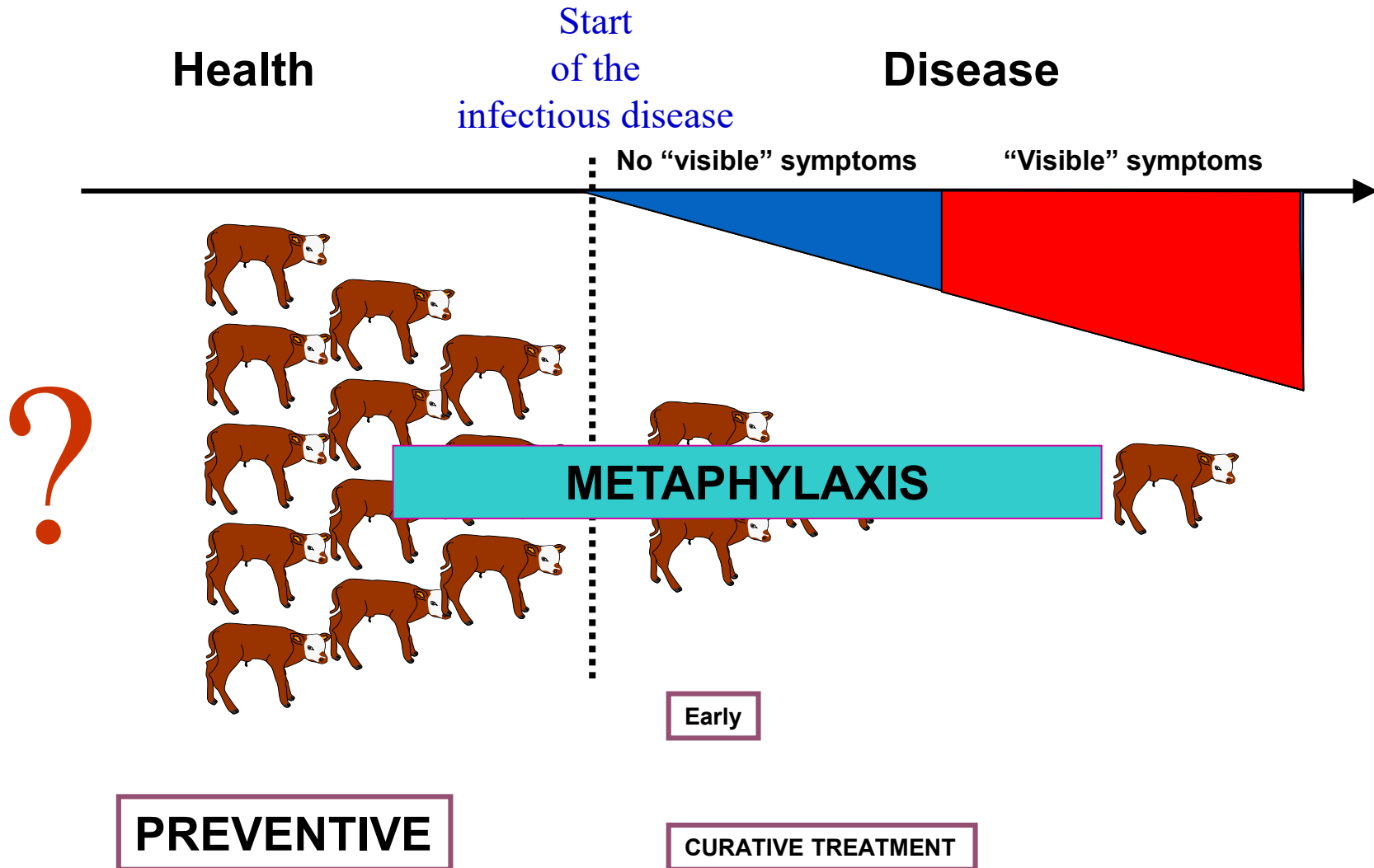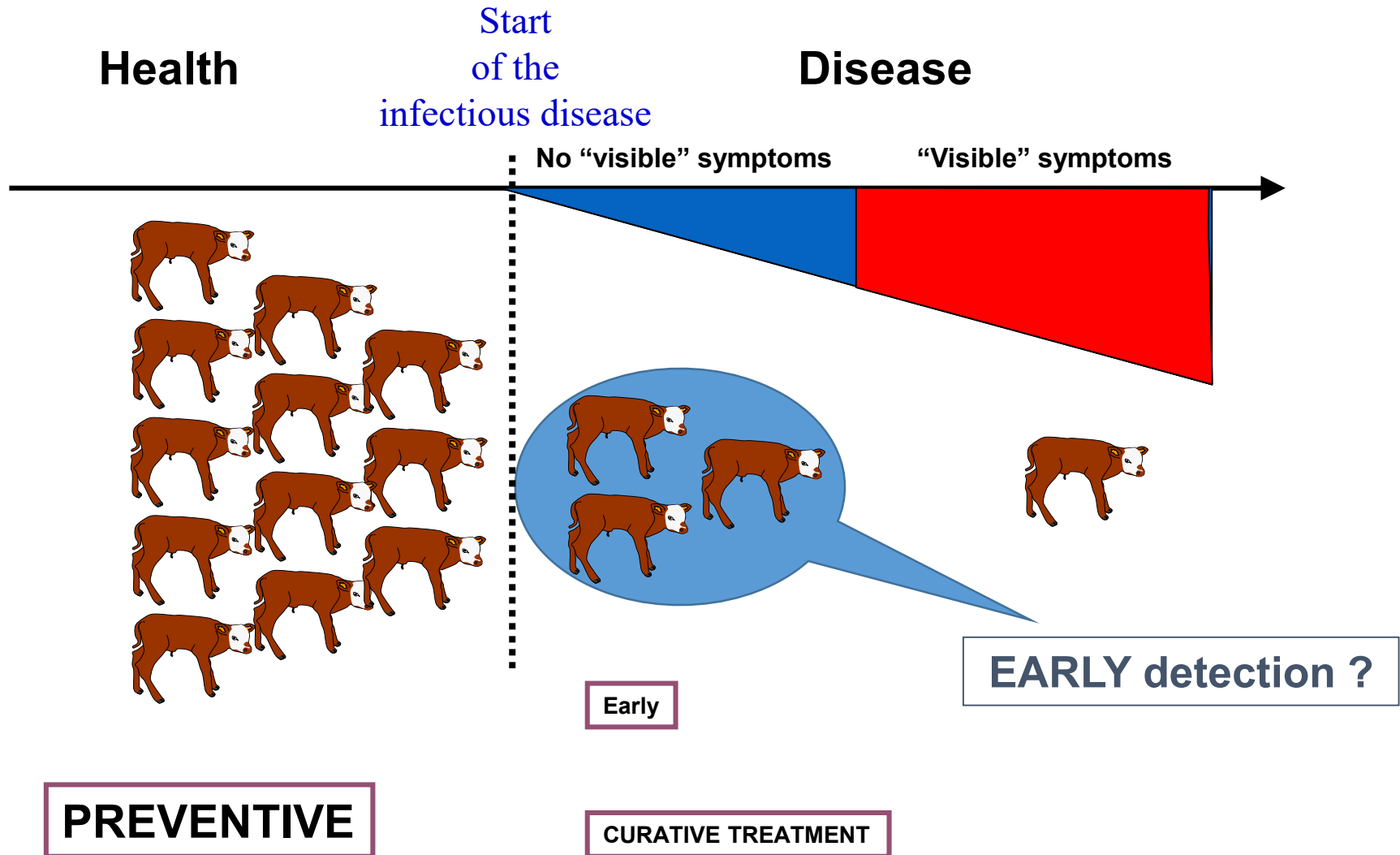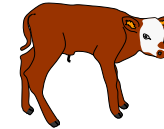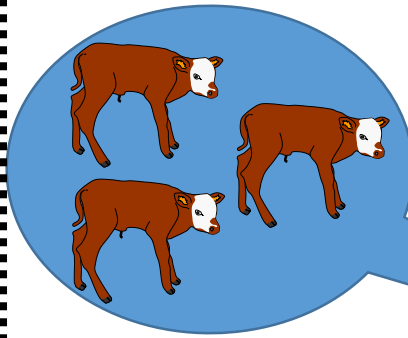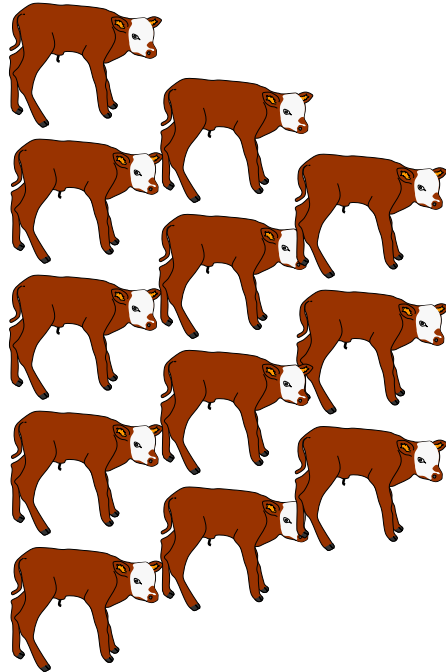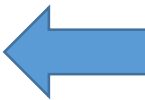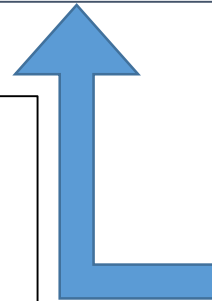
PLF Tools
Early / Individual Detection ➡️ Precision Medication

PigletDetect

Food and water real-time monitoring of pigs to perform early disease detection

HealthyLivestock

Tackling Antimicrobial Resistance through improved livestock Health and Welfare
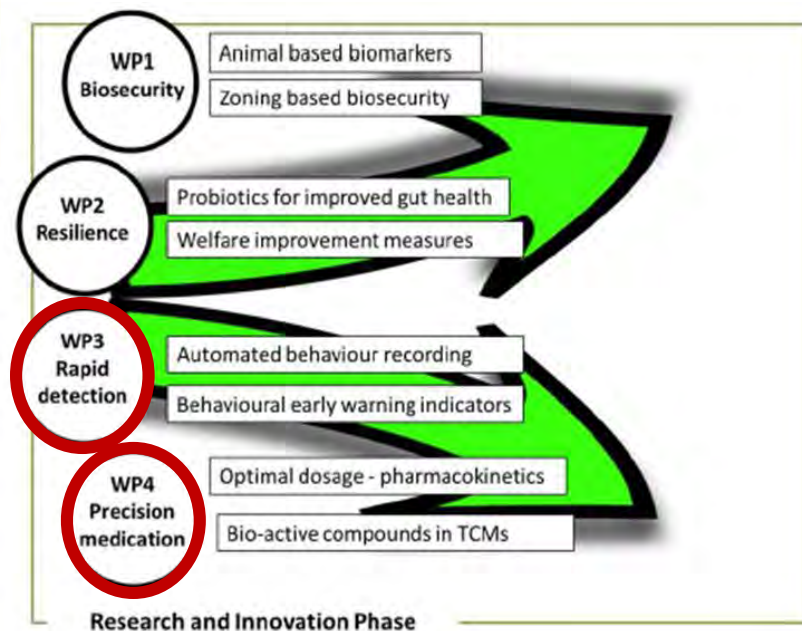
PLF Tools
Early / Individual Detection ➡ Precision Medication

1. Early detection (ED)

1. PLF use continuous recording of biological data
2. Mathematical modelling of healthy and diseased situations
3. Algorithm for **automatic and early** detection of diseased status

Individual water consumption monitoring
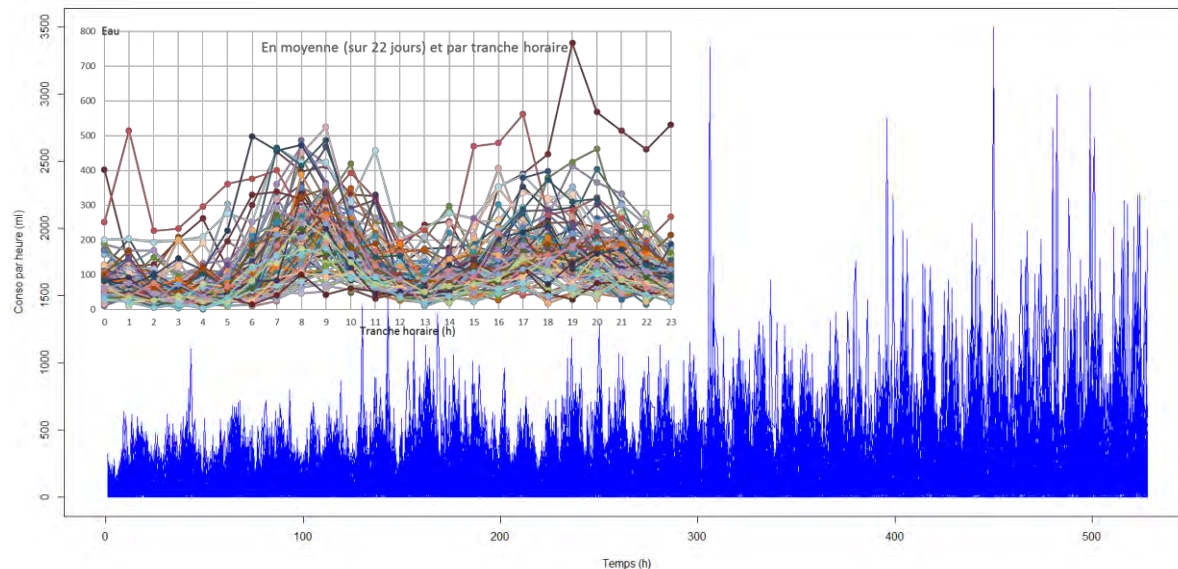
PLF Tools
Early / Individual Detection ➡ Precision Medication

1. ## Early detection (ED)
   1. PLF use continuous recording of biological data
   2. Mathematical modelling of healthy and diseased situations
   3. Algorithm for **automatic and early** detection of diseased status

Machine learning
Supervised learning

4. Increased ED performances when combining several variables :
individual drinking behaviour / *feeding behaviour / actimetry …*

ROC Curve

Sensitivity

1 - Specificity
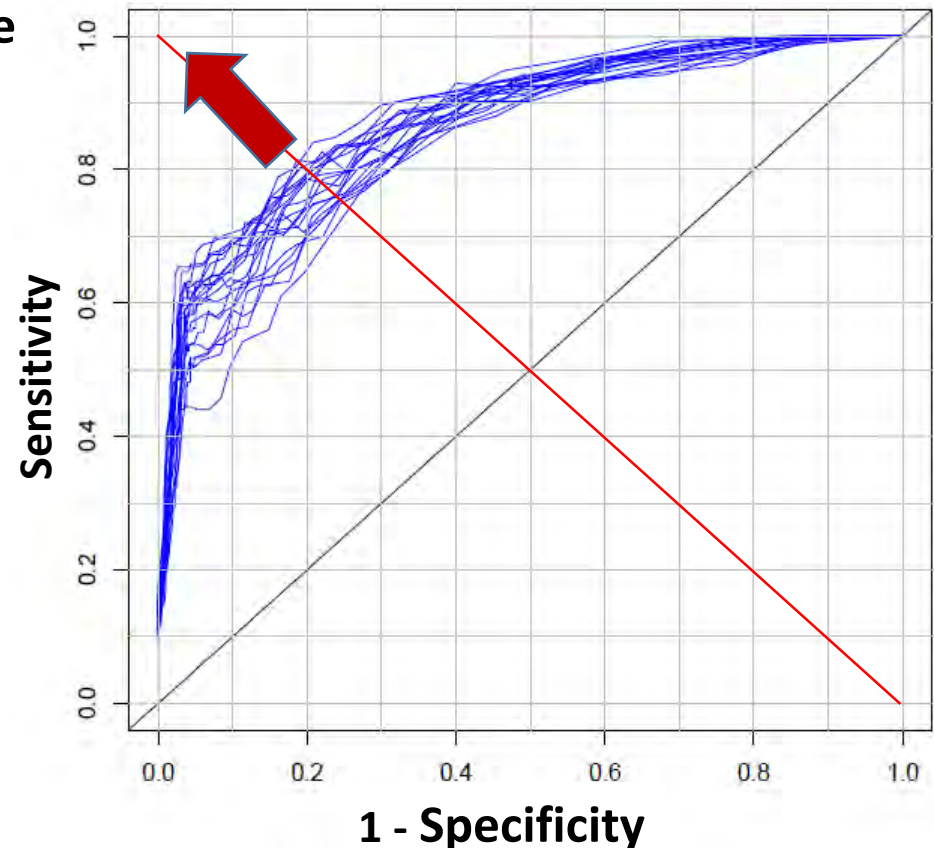
PLF Tools
Early / Individual Detection ➡ Precision Medication

1. Early detection (ED)

Machine learning
Supervised learning

PLF Tools
Early / Individual Detection ➡ Precision Medication

1. Early detection (ED)
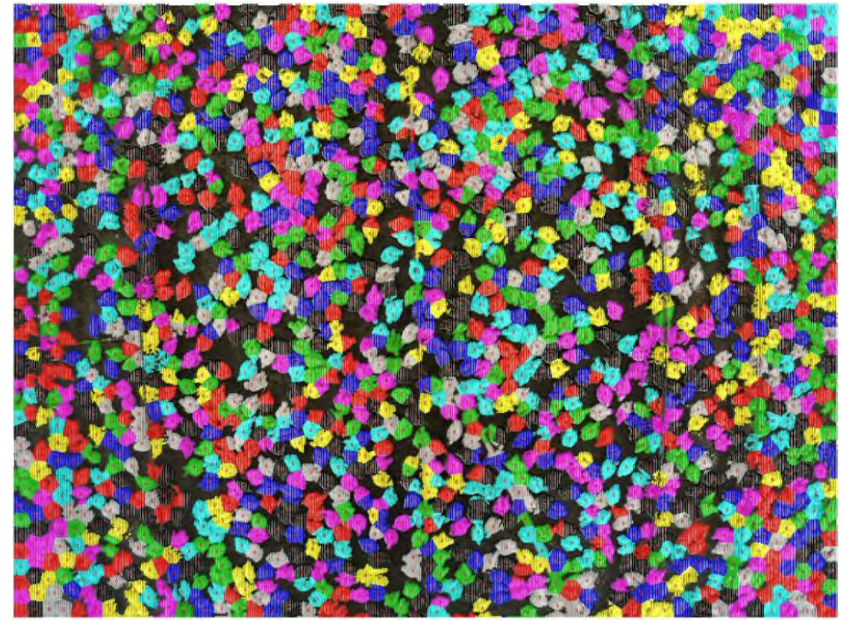
Algorithm that computes activity/movement (distance, velocity) at the individual level

Early / Individual Detection ➡ Precision Medication

1. Early detection (ED)

Detection of a problem is not Diagnostic of a specific disease
- **Absence of pathology** : Breeding system dysfunction / Welfare indicator
- **Presence of pathology** : **EARLY detection allows saving time for TARGETED intervention : Diagnostic / Treatment**

PLF Tools
Early / Individual Detection ➡ Precision Medication

1. Early detection (ED)

2. Precision medication
    1. Selecting animals to treat : moving from "mass medication" to ***"pen medication"*** or "individual medication" (when possible)
    2. Treating smaller groups (pen) permitted by **drugs delivery in water** using dosing pumps
    3. Optimizing therapy in the case of collective distribution of drugs in water : by taking into account ***individual drinking behaviour***

Doxycycline in medicated food
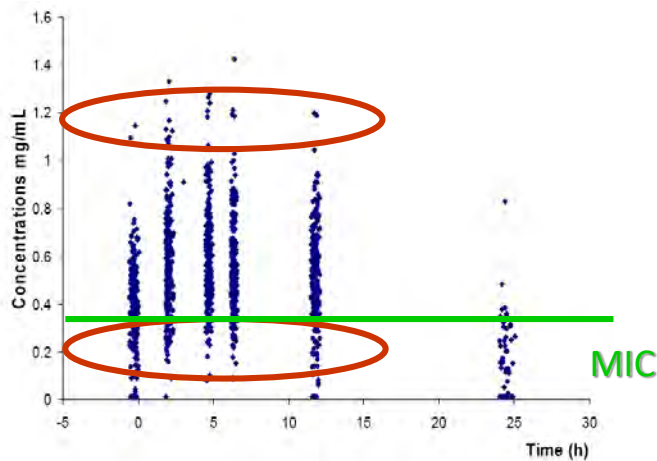
Exposure variability of fosfomycin administered to pigs in food or water: Impact of social rank

Alejandro L. Soraci[a,*], Fabián Amanto[b], María O. Tapia[a], Eulalia de la Torre[a], Pierre-Louis Toutain[c]

n = 215

**AB in medicated food**

F

MIC

AB in drinking water

MIC

MIC
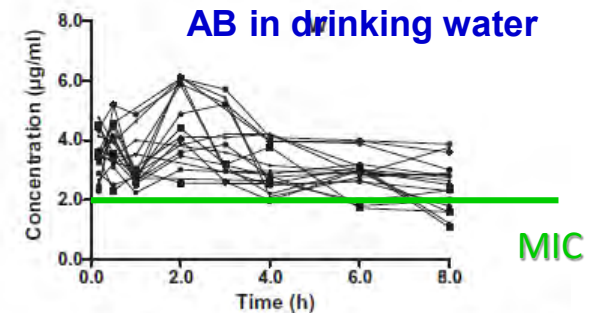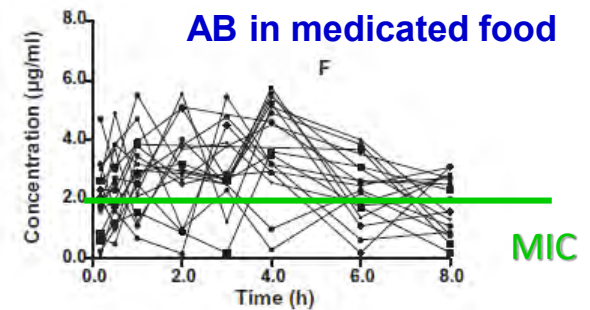
Fig. 4. Plasma concentrations of fosfomycin obtained after fosfomycin administration at a dose of 20 mg/kg in the food (F) or water (W) (groups F & W) for 36 pigs under farm conditions (n = 18 per group).

Modelling for prediction of individual concentration profiles after collective delivery in water
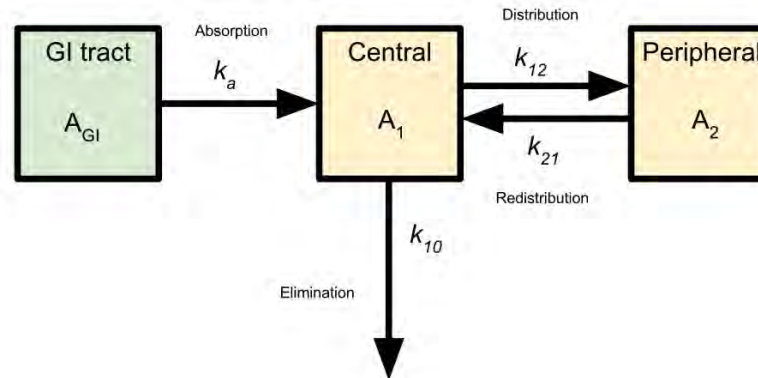
Individual water consumption
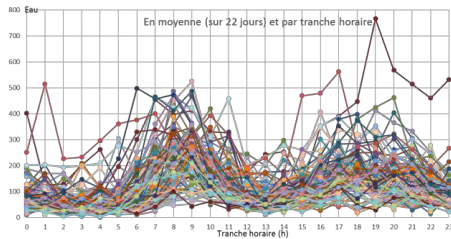
EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

19 July 2018
EMA/CVMP/849775/2017
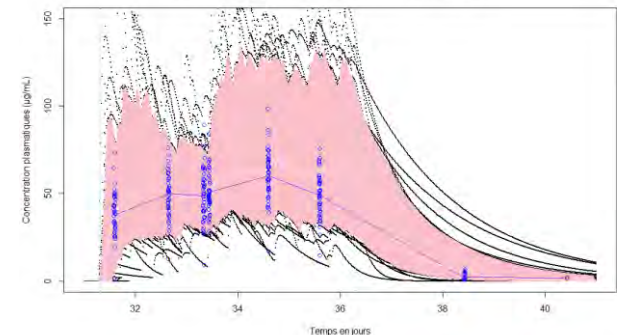Committee for Medicinal Products for Veterinary Use (CVMP)

Reflection paper on dose optimisation of established veterinary antibiotics in the context of SPC harmonisation

Pharmacokinetic (population) model

PREDICTION
Individual antibiotic plasma concentrations

Prédiction des concentrations en sulfadimethoxine en IPRED

Conclusion

- Precision antimicrobial therapy in food-producing animals should take advantage of using tools of PLF and Smart Farming

- Moving Metaphylaxis to "As early / More targeted" treatments
  - Should benefit from development of rapid and in-field diagnostic

Thank you for your attention

Global progress in TB care and control 2000–2012

2000	Year	2012
148	Incidence (per 100 000 population per year)	122
263	Prevalence (per 100 000 population)	169
22	Mortality (per 100 000 population per year)	13
42	Case detection rate (%)	66
69	Treatment success rate (%)	87

Global Tuberculosis Report 2013

GLOBAL TUBERCULOSIS REPORT 2015 — 20th edition

GLOBAL TUBERCULOSIS REPORT 2018

Stop TB Partnership 2011-2015

THE END TB STRATEGY 2015-2030

FIG. 3.4 Estimated TB incidence rates, 2017

Fact: The more we detect – the more we treat – the more we cure ! - but we also select DR-TB !

Incidence per 100 000 population per year
0–24
25–99
100–199
200–299
≥300
No data
Not applicable

Source: WHO Global TB Program – 2013 and 2015, http://www.who.int/tb/publications/global_report/en/

Global progress in TB care and control 2000–2012

2000	Year	2012
148	Incidence (per 100 000 population per year)	122
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GLOBAL TUBERCULOSIS REPORT 2015

Global Tuberculosis Report 2013

20th edition

GLOBAL TUBERCULOSIS REPORT 2018
World Health Organization

Stop TB Partnership
2011-2015

THE END TB STRATEGY
2015-2030

FIG. 3.20
Percentage of new TB cases with MDR/RR-TB[a]

Percentage of cases
- 0–2.9
- 3–5.9
- 6–11
- 12–17
- ≥18
- No data
- Not applicable
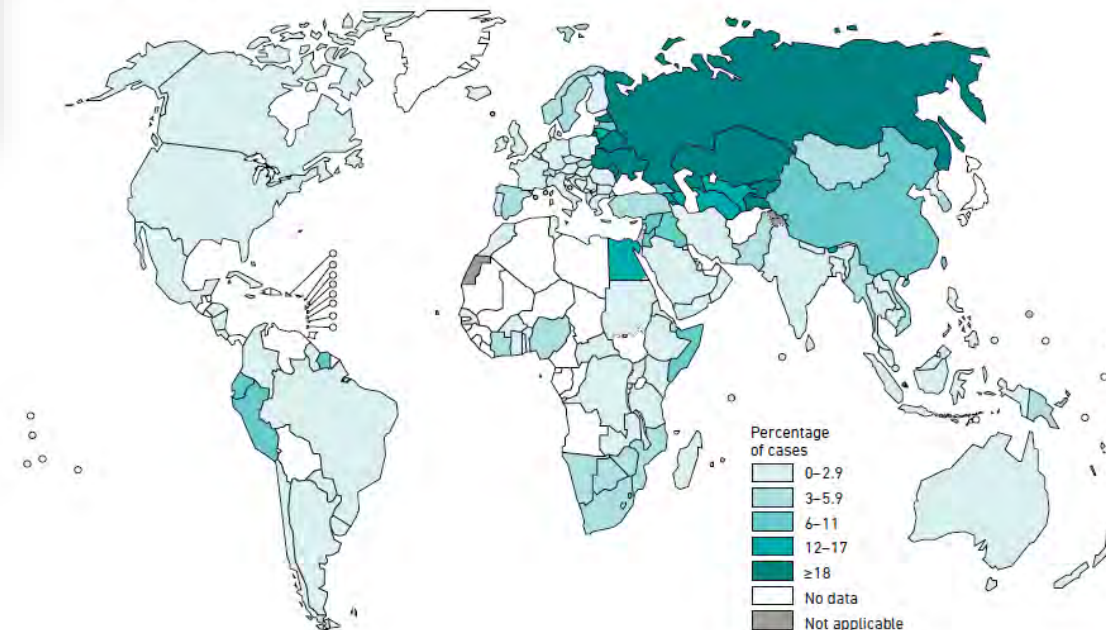
[a] Figures are based on the most recent year for which data have been reported, which varies among countries. Data cover the period 2002–2018.

Fact: *The more we detect – the more we treat – the more we cure ! - but we also select* **DR-TB** *!*

Source: WHO Global TB Program – 2013 and 2015, http://www.who.int/tb/publications/global_report/en/

Global progress in TB care and control 2000–2012

2000	Year	2012
148	Incidence (per 100 000 population per year)	122
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GLOBAL TUBERCULOSIS REPORT 2015
Global Tuberculosis Report 2013
GLOBAL TUBERCULOSIS REPORT 2018

Stop TB Partnership 2011-2015

THE END TB STRATEGY 2015-2030

FIG. 2.5
Countries in the three high-burden country lists for TB, TB/HIV and MDR-TB being used by WHO during the period 2016–2020, and their areas of overlap

Fact: The more we detect – the more we treat – the more we cure ! - but we also select DR-TB !

TB
Cambodia*
Sierra Leone*

Bangladesh
DPR Korea
Pakistan
Philippines
Russian Federation
Viet Nam

Brazil
Central African Republic*
Congo*
Lesotho*
Liberia*
Namibia*
UR Tanzania
Zambia*

Azerbaijan
Belarus
Kazakhstan
Kyrgyzstan
Peru
Republic of Moldova
Somalia
Tajikistan
Ukraine
Uzbekistan

Angola
China
DR Congo
Ethiopia
India
Indonesia
Kenya
Mozambique
Myanmar
Nigeria
Papua New Guinea*
South Africa
Thailand
Zimbabwe*

Botswana
Cameroon
Chad
Eswatini
Ghana
Guinea-Bissau
Malawi
Uganda

MDR-TB

TB/HIV

* Indicates countries that are included in the list of 30 high TB burden countries on the basis of the severity of their TB burden (i.e. TB incident cases per 100 000 population per year), as opposed to the top 20, which are included on the basis of their absolute number of incident cases per year. Also see Table 2.4.

Source: WHO Global TB Program – 2013, 2015 and 2018, http://www.who.int/tb/publications/global_report/en/

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UNIVERSIDADE NOVA DE LISBOA

ICOHAR International Conference on One Health Antimicrobial Resistance

Distribution of NTM

Legend:
- MAC
- *M. kansasii*
- *M. xenopi*
- *M. malmoense*
- RGM
- *M. gordonae*
- Other SGM
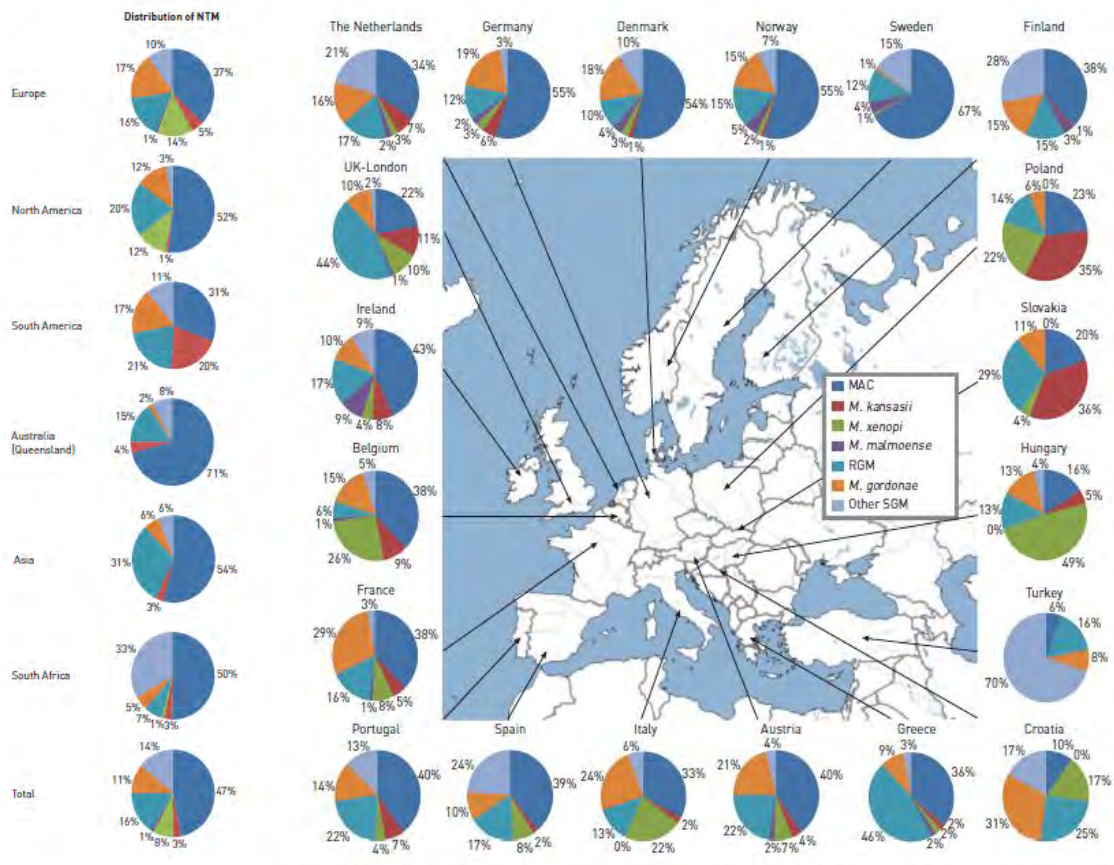
FIGURE 3 Distribution of different nontuberculous mycobacteria from pulmonary samples in 2008 in Europe. MAC: *Mycobacterium avium* complex; RGM: rapid-growing mycobacteria; SGM: slow-growing mycobacteria.

ORIGINAL ARTICLE
RESPIRATORY INFECTIONS

The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples

An NTM-NET collaborative study

Wouter Hoefsloot¹, Jakko van Ingen¹, Claire Andrejak, Kristian Ängeby,

Eur Respir J 2013; 42: 1604–1613 | DOI: 10.1183/09031936.00149212

Pulmonary
MAC
- *M. avium*
- *M. intracellulare*
- *M. chimaera*

- *M. kansasii*
- *M. abscessus*
- *M. abscessus* sensu stricto
- *M. massiliense*
- *M. boletii.*
- *M. simiae*
- *M. xenopi*
- *M. malmoense*

Cervical Lymphadenitis
- *M. scrofulaceum*
- *M. avium*
- *M. malmoense*
- *M. lentiflavum*
- *M. bohemicum*

Bone and Joints
MAC
- *M. haemophilum*
- *M. kansasii*
- *M. xenopi*
- *M. goodii*
- *M. terrae complex*

Disseminated Infections
- *M. avium*
- *M. intracellulare*
- *M. haemophilum*
- *M. genavense*

Skin & Soft Tissue
- *M. ulcerans* (Buruli ulcer)
- *M. fortuitum*
- *M. marinum*
- *M. haemophilum*
- *M. abscessus*
- *M. chelonae*

NTMs

PLOS Neglected Tropical Diseases | https://doi.org/10.1371/journal.pntd.0007083   February 14, 2019

REVIEW

https://doi.org/10.4046/trd.2018.0026
ISSN: 1738-3536(Print)/2005-6184(Online) · Tuberc Respir Dis, Respir Dis 2019;82:94-101

trd

Infection Source and Epidemiology of Nontuberculous Mycobacterial Lung Disease

Doosoo Jeon, M.D.
Department of Internal Medicine, Pusan National University Yangsän Hospital, Pusan National University School of Medicine, Yangsan, Korea

Legend:
- TB prevalence (notification rate)
- TB incidence (new case notification rate)
- NTM prevalence
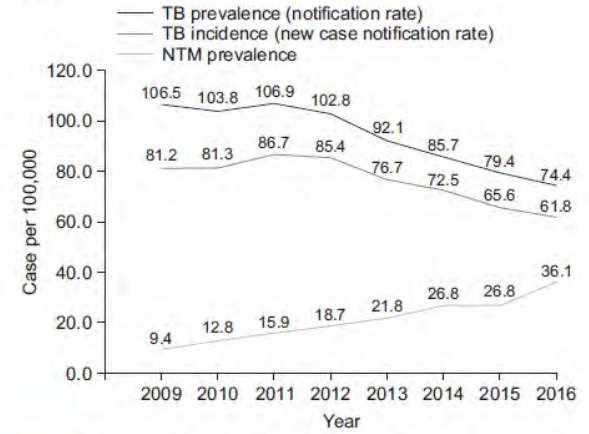
Figure 1. Trend in the prevalence of tuberculosis (TB) and nontuberculous disease from 2009 to 2016 in South Korea. NTM: nontuberculous mycobacteria. Adopted from Yoon et al. BMC infect Dis 2017;17:432, according to Creative Commons license[60].

Figure. Prevalence of positive test results for NTM and MTB in respiratory specimens from patients in US-affiliated Pacific Island jurisdictions, 2007–2011. *Data for 2007 were extrapolated from data for August–December 2007. MTB, *Mycobacterium tuberculosis*; NTM, nontuberculous mycobacteria.

Increasing Prevalence of Nontuberculous Mycobacteria in Respiratory Specimens from US-Affiliated Pacific Island Jurisdictions¹

Chunrong Lin,¹ Chad Russell, Bruce Soll, Dominic Chow, Sapna Bamrah, Richard Brostrom, Wesley Kim, Jerry Scott, Matthew J. Bankowski²

*Fact: The more we detect – the more we treat – the more we cure ! - but we also select **DR-NTM** !*

488   Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 24, No. 3, March 2018
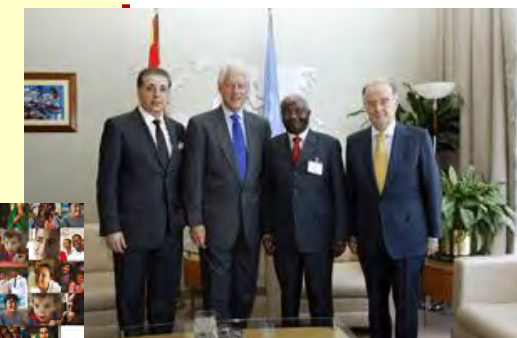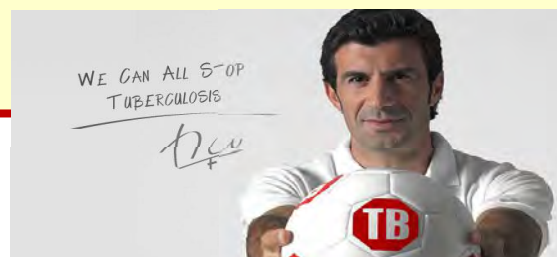
HOW M/X/TDR-TB and DR-NTMs IS GENERATED – THE CURRENT DOGMA ?

Spontaneous mutation
Mycobacterium tuberculosis

MUTATION
⇓

Man-made Selection
- Wrong prescription (Doctor)
- Non-adherance (Patient)
- Patient pharmacokinetics and pharmacogenetic characteristics

AMPLIFICATION
⇓

Man-made transmission
- Delay in diagnosis (Health system)
- Deficient control of transmision and infection (Health system)

TRANSMISSION
⇓

Wild-type Mycobacterium tuberculosis / NTM

Resistant strain of M. tuberculosis / NTM

DR

MDR

AQUIRED RESISTANCE (Relapse or retreatment)

TDR

XDR

PRIMARY RESISTANCE (New case)

EXTENSIVELY DRUG-RESISTANT TUBERCULOSIS

Acquired DR during treatment – Similar to TB and more often in NTMs

Source:
• The WHO/IUTALD Global Proj. on Anti-TB Drug Resistance Surveillance 1994-2000 , 1999-2002 e 2002-2007
• WHO (2005) Anti-tuberculosis drug resistance in the world. Report nº 3.
• Raviglione M. XDR-TB: entering the post-antibiotic era? Int J Tuberc Lung Dis. 2006 Nov;10(11):1185-7

With molecular epidemiology it became possible to study the phylogenetic relationships of the Mycobacterium tuberculosis complex species and how they evolved and adapted to humans and animals !!

In contrast to other bacterial pathogens, where genetic diversity arises as a result of recombination, duplication, insertion and exclusion events, *M. tuberculosis* complex presents a **clonal evolution from its common ancestor losing ancestral genomic regions without any genetic exchange with other species.**
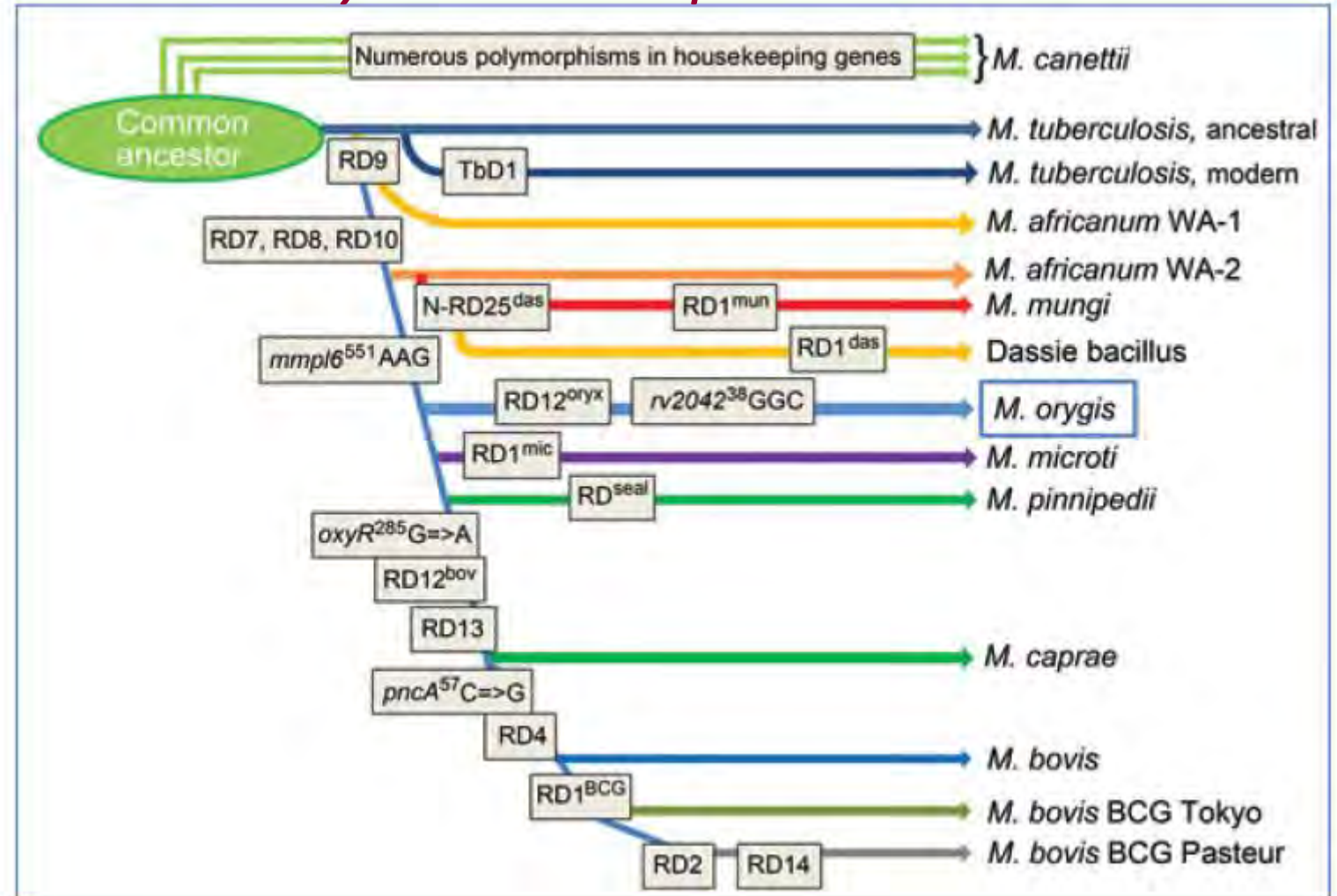
Schematic illustration of phylogenetic relationships between members of the M. tuberculosis complex according to van Ingen et al. (2012), based on the initial study by Brosh et al. (2002).

van Ingen J, Rahim Z, Mulder A, Boeree MJ, Simeone R, Brosch R, van Soolingen D. Characterization of M. orygis as M. tuberculosis complex subspecies. Emerg Infect Dis. 2012 Apr;18(4):653-5.
Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, Eiglmeier K, et al. A new evolutionary scenario for the Mycobacterium tuberculosis complex. Proc Natl Acad Sci U S A. 2002;99:3684–9.

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ICOHAR International Conference
on One Health Antimicrobial Resistance

The mycobacterial phylogeny and the lineages of *M. tuberculosis*

Microbiology Spectrum
The Evolutionary History, Demography, and Spread of the Mycobacterium tuberculosis Complex
MAXIME BARBIER and THIERRY WIRTH
Laboratoire Biologie Intégrative des Populations, Evolution Moléculaire, Institut de Systématique, Evolution, Biodiversité, UMR-CNRS 7205, Muséum National d'Histoire Naturelle, Univ. Pierre et Marie Curie, EPHE, Sorbonne Universités, 75231 Paris cedex 05, France

nature REVIEWS

Nature Reviews Genetics | AOP, published online 25 March 2014; doi:10.1038/nrg3664

DISEASE MECHANISMS
Genomic insights into tuberculosis
James E. Galagan
Abstract | Prevalent since pre-history, human tuberculosis — caused by the pathogen Mycobacterium tuberculosis — remains a major source of death worldwide. Moreover, increasing drug resistance poses the threat of disease resurgence. However, the expanding application of genomic techniques is providing new avenues for combating this old foe. Whole-genome sequencing, comparative genomics and systems biology are generating new insights into the origins and ongoing evolution of M.tuberculosis, as well as the molecular basis for its pathogenicity. These have important implications for our perspective of the disease, development of new drugs and vaccines, and treatment of patients using existing therapeutics.

Microbiology Spectrum
Paleomicrobiology of Human Tuberculosis
HELEN D. DONOGHUE
Centre for Clinical Microbiology, Division of Infection and Immunity, University College London, United Kingdom

FIGURE 3 Whole-genome phylogeny of 261 strains belonging to the MTBC. Animal and *M. africanum* specific deletions are indicated, as well as mutations affecting the PhoPR virulence regulator. Adapted from Bos et al. (55) and Gonzalo-Asensio et al. (34).

Large sequence polymorphisms (LSPs) led to the identification of seven distinct strains of *Mycobacterium tuberculosis*, which were categorized as **"old"** or **"modern"** according to the presence or absence of the **TbD1** region. **"Old"** lines 1, 5, 6 and 7 are geographically restricted, while **"Modern"** Lineages 2, 3 and 4 **form a monophyletic group, with lineages 2, 3 and 4 being frequently isolated from patients with widespread global spread and an enormous capacity to cause cavitary disease and acquire resistance** - After all *Mycobacterium tuberculosis* are not all the same - some strains are worse **[L2, L3 & L4]** than others!

FIGURE 2 Evolutionary relationship between selected mycobacteria and members of the *Mycobacterium tuberculosis* complex (MTBC). The MTBC was thought to arise as a clonal expansion from a smooth tubercle bacillus (STB) progenitor population. The animal-

Gagneux S, DeRiemer K, Van T, Kato-Maeda M, de Jong BC, Narayanan S, Nicol M, Niemann S, Kremer K, Gutierrez MC, Hilty M, Hopewell PC, Small PM. 2006. Variable host-pathogen compatibility in Mycobacterium tuberculosis. Proc Natl Acad Sci USA. 103(8): 2869-73.

Galagan JE. 2014. Genomic insights into tuberculosis. Nat Rev Genet 15:307–320.
Donoghue HD. 2016. Paleomicrobiology of Human Tuberculosis. Microbiol Spectr. 4(4). doi: 10.1128/microbiolspec.PoH-0003-2014

Barbier M, Wirth T..2016. The Evolutionary History, Demography, and Spread of the Mycobacterium tuberculosis Complex. Microbiol Spectr. 2016 Aug:4(4).

We know how *Mycobacterium tuberculosis* traveled on the globe and in modern history !!
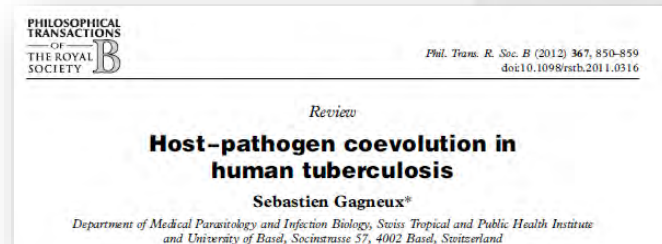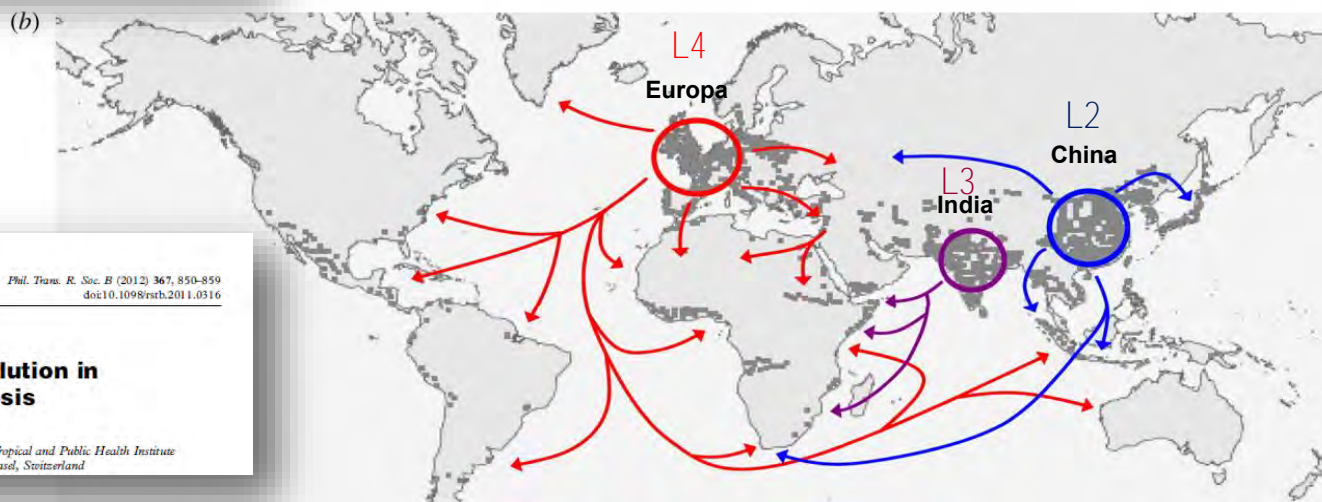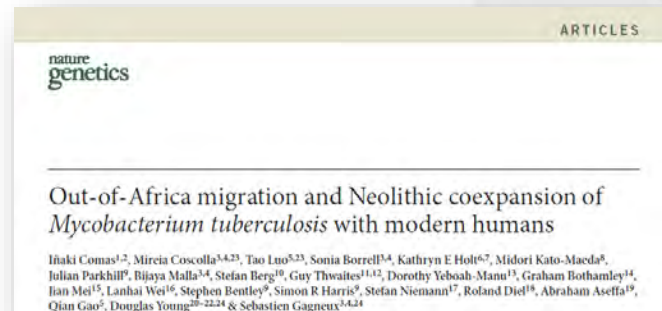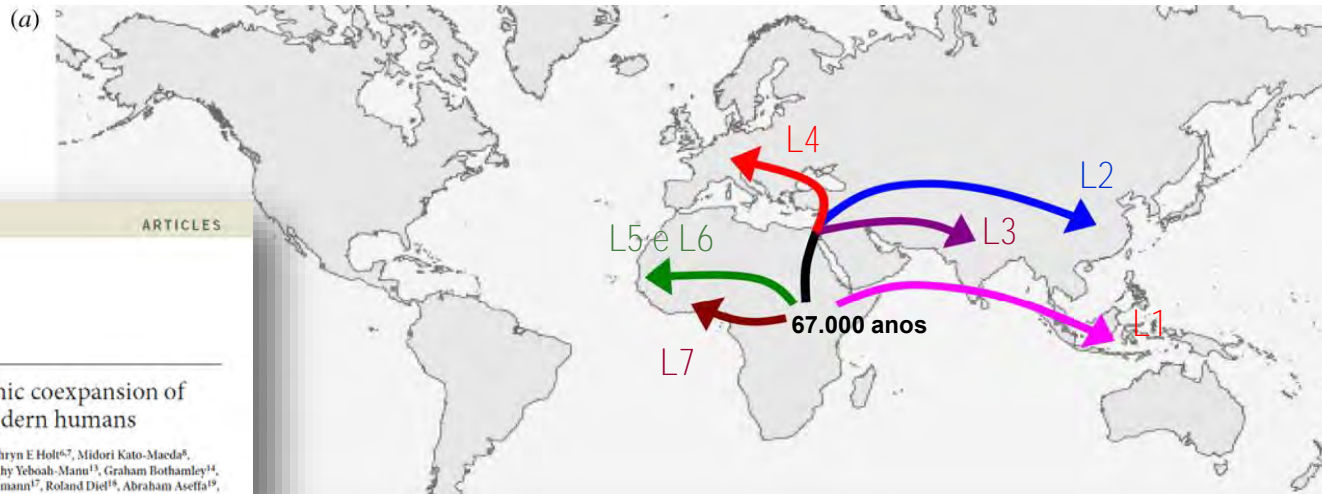
Hershberg's famous **out-of-Africa - back to Africa** theory supported by recent molecular epidemiology studies.

The three modern lineages travel and "re" colonize Africa in the "expansions" **Indian (Sec. I-X), Chinese (Sung Sec.X-XII Dynasty) and European (Sec. XV-XVI)**

Comas I, *et al.* (2013) Out-of-Africa migration and Neolithic coexpansion of *Mycobacterium tuberculosis* with modern humans. *Nat Genet.* 2013 Oct;45(10):1176-82.

Gagneux S. (2012) Host-pathogen coevolution in human tuberculosis. *Philos Trans R Soc Lond B Biol Sci.* 2012 Mar 19;367(1590):850-9.

The more we detect ! – the more we treat! – the more we cure ! – but we also select DR-TB !

We have seen this in Portugal !

Tuberculose: casos por 100 mil habitantes
Taxa - Por cem mil

1960 - **200**/100,000inh

1990 - **63**/100,000inh

2018
15,4/100,000inh

Casos de tuberculose por 100 mil hab.

Fontes/Entidades: INE, INE | DGS/MS, PORDATA

Journal of Antimicrobial Chemotherapy

J Antimicrob Chemother 2013; **68**: 27–33
doi:10.1093/jac/dks371 Advance Access publication 10 October 2012

From multidrug-resistant to extensively drug-resistant tuberculosis in Lisbon, Portugal: the stepwise mode of resistance acquisition

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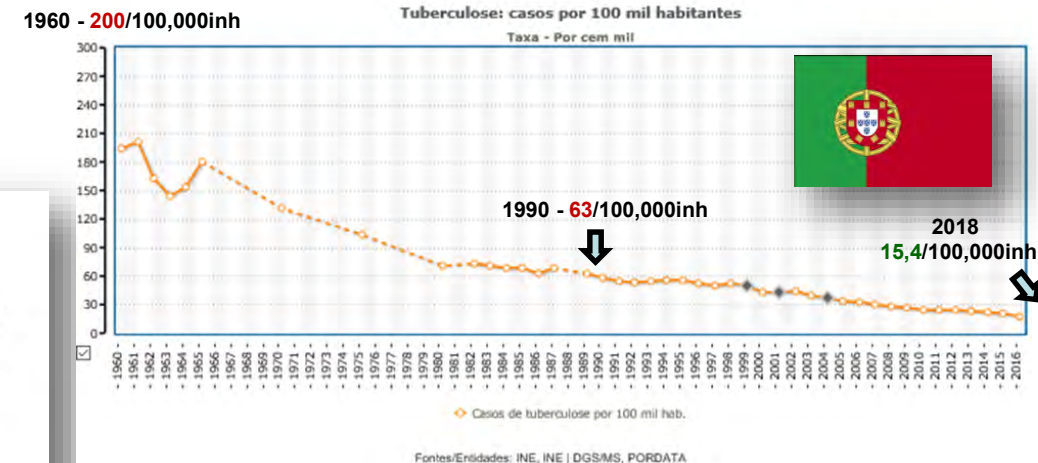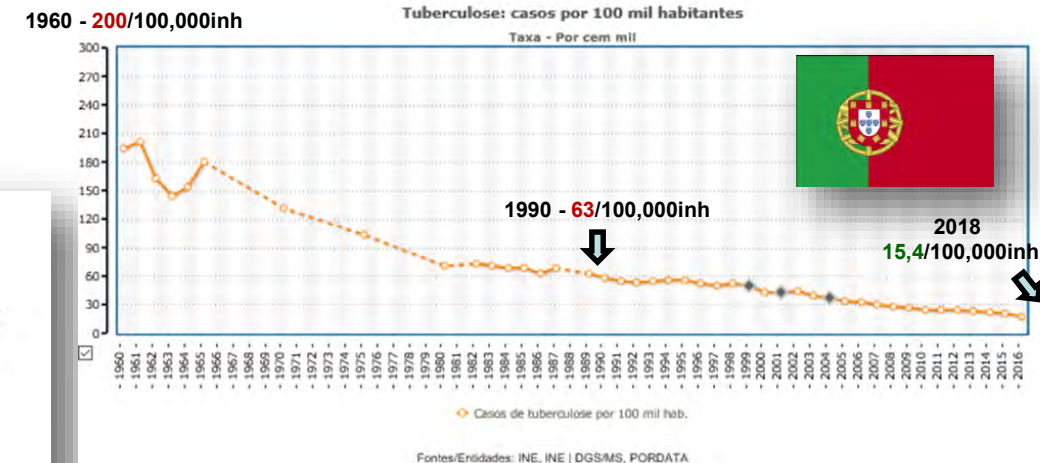
Almost all M/XDR [2000-2015] were from one clade –The Lisboa strain - with two main 2 clusters Lisboa 3 and Q1 – All Lisboa strains are from Lineage L4

Global Tuberculosis Report 2013
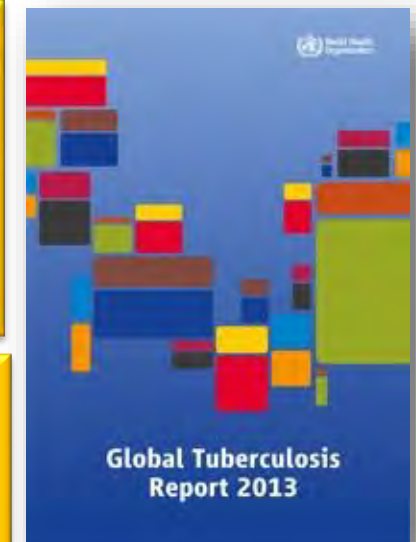
L4 – Lisboa strains are more virulent, more cavitary, reaching high bacterial loads with increased mutational frequency, more prone to acquire resistance and transmit into the community – Evolutively adapted to the "Portuguese and Lusophone Human Environment"

Source: Direcção Geral de Saúde. 2013 and 2019. Programa Nacional de Luta Contra a Tuberculose (PNT). Ponto da situação epidemiológica e de desempenho ano 2011/2012 and 2017/2018 , p. 1-33. DGS, Lisboa, Portugal ; Perdigão *et al J Antimicrob Chemother*. 2013 Jan;68(1):27-33..

M/XDR-TB !!!
The example of Portugal

EXPERT REVIEWS

Molecular tools for rapid identification and novel effective therapy against MDRTB/XDRTB infections
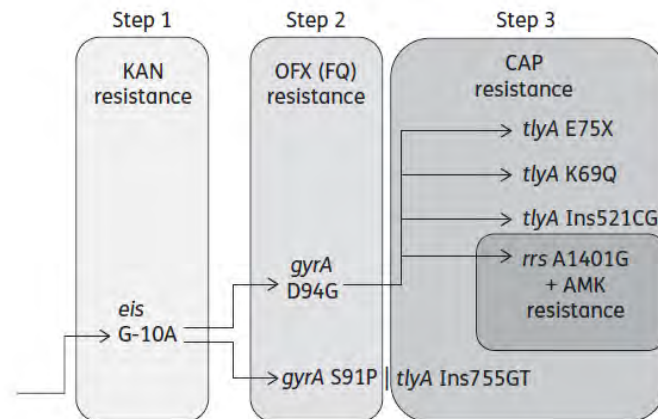
Expert Rev. Anti Infect. Ther. 8(4), 465–480 (2010)

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Marta Martins,
Isabel Couto,
Liliana Rodrigues,
Diana Machado,
Isabel Portugal and
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Tuberculosis (TB) is mainly an intracellular infection of the lung alveolar macrophages, and any anti-TB agent must therefore be active at the macrophage. Among the available therapies, isoniazid and rifampicin are the most effective drugs against susceptible Mycobacterium tuberculosis, but they are ineffective against multidrug-resistant TB (MDRTB) strains. Rates of MDRTB in Portugal are the highest in Western Europe, demanding effective measures for their control. Our application of molecular techniques for the early identification of MDRTB assisted in the reduction of these rates. Further examination revealed that a large number of MDRTB cases were extensively-drug resistant (XDRTB), providing evidence for the urgent need of new and effective anti-MDRTB/XDRTB therapeutic strategies. This review describes in detail: the characteristics of the main M. tuberculosis strains circulating in Portugal; the creation of a Task Force for TB control, based on molecular tools that allow 1-day identification of an MDRTB patient; the usefulness of evaluating the ex vivo activity of anti-tubercular agents against the M. tuberculosis isolated from the patient's sputum; and the mode of action by which phenothiazines have been shown to promote the killing of intracellular MDRTB/XDRTB by nonkilling macrophages.

KEYWORDS: macrophages • MDRTB • multidrug resistance • phenothiazines • tuberculosis • XDRTB

From multidrug-resistant to extensively drug-resistant tuberculosis in Lisbon, Portugal: the stepwise mode of resistance acquisition

João Perdigão[1], Rita Macedo[1,2], Carla Silva[1], Diana Machado[3], Isabel Couto[3,4], Miguel Viveiros[3], Luisa Jordao[5] and Isabel Portugal[1]*

Figure 1. Multistep process of resistance acquisition dynamics in the Lisboa3 cluster. The scheme represents the process through which XDR has most likely been acquired multiple independent times in a maximum of three steps. Step 1 consists of the acquisition of low-level...
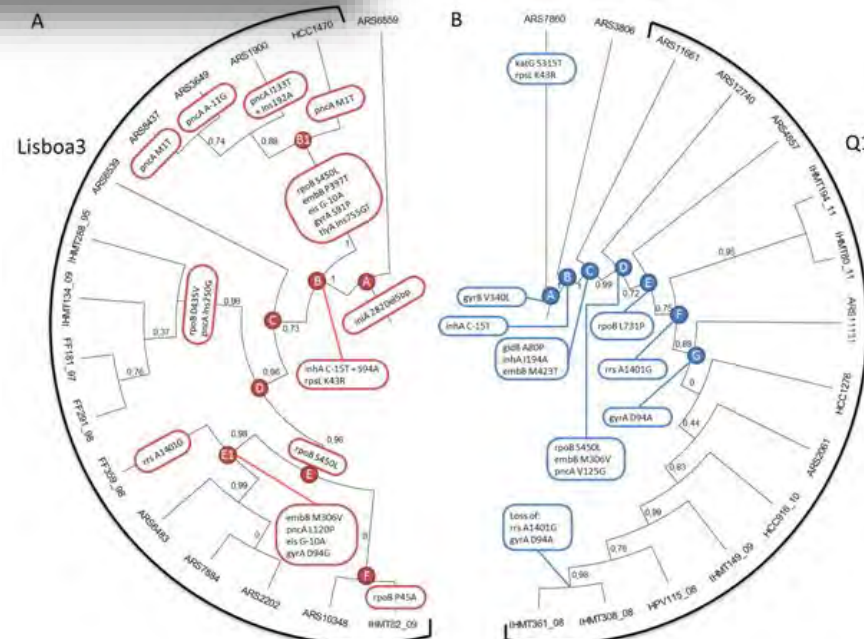
Empirical, non-effective 2nd line anti-TB treatment led to stepwise selection of mutations for Resistance + transmission of M/XDRTB Lisboa Strains
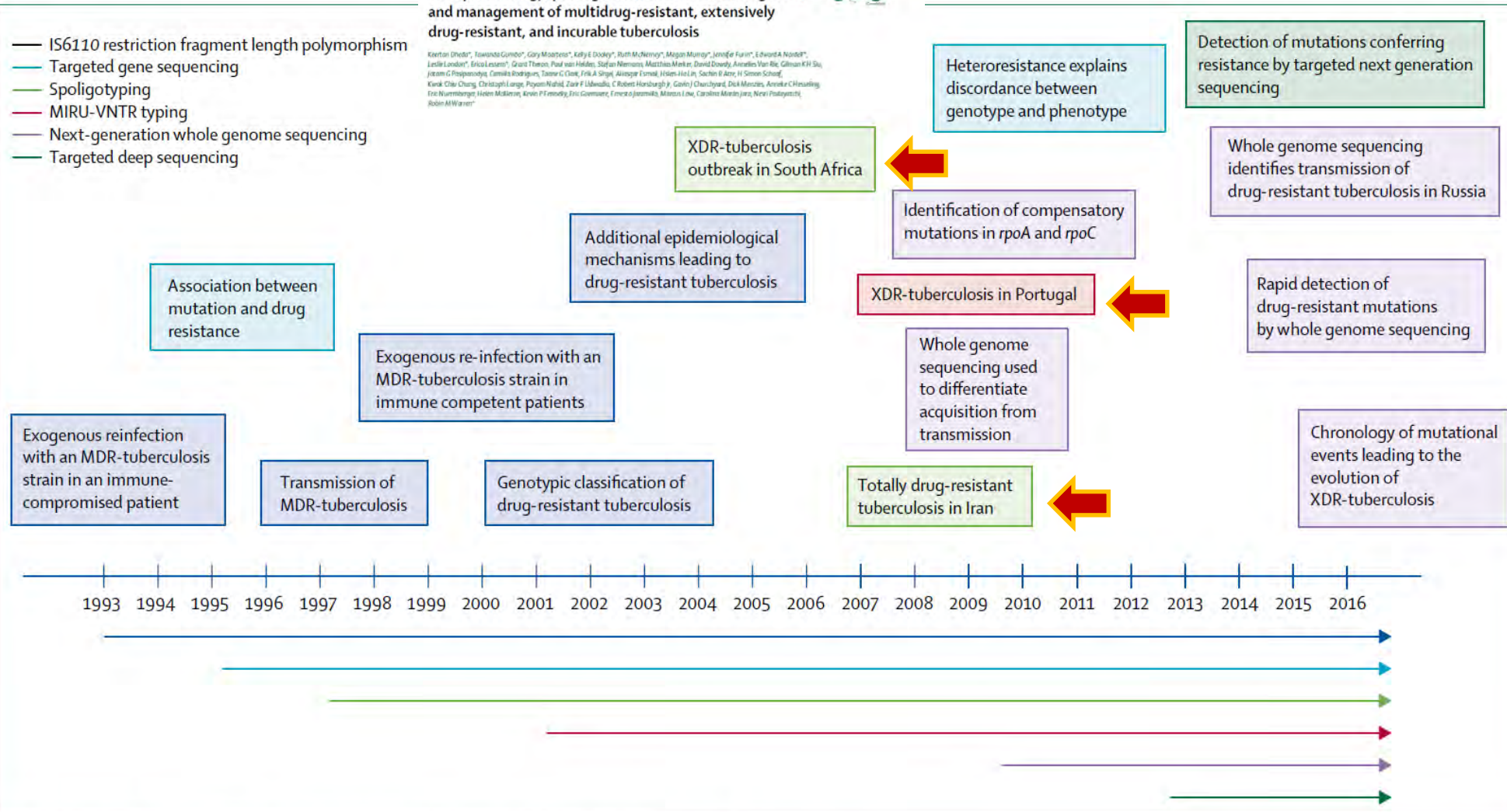
Figure 2: Timeline of key molecular epidemiological findings using different genotyping tools

Genotyping tools used for each finding are indicated by different colours. MDR=multidrug resistant. MIRU–VNTR=mycobacterial interspersed repetitive units–variable numbers of tandem repeat. XDR=extensively drug-resistant.

Lisboa strain is now part of the history and story of the M/XDRTB evolution along side with the KZN XDRTB and the iranian TDR strains !!

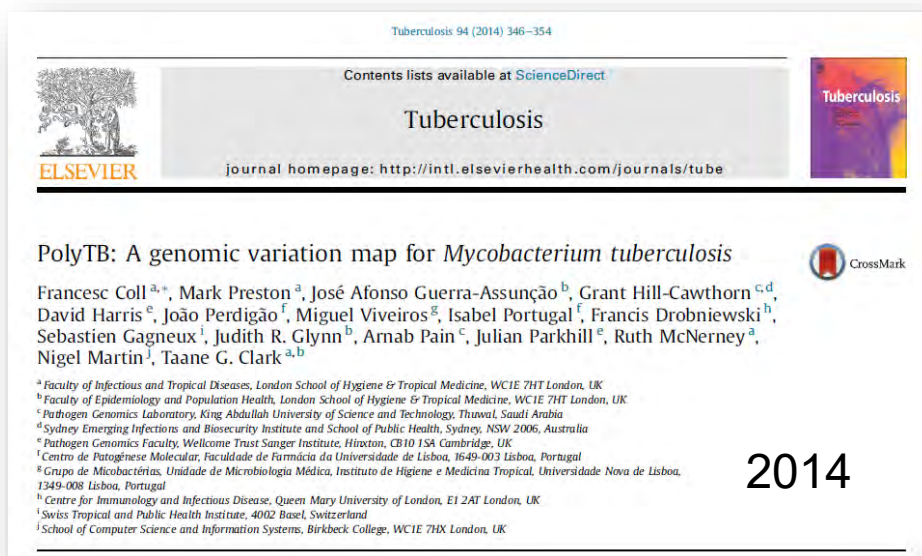Dheda K, et al.. The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. Lancet Respir Med. 2017 Mar 15. pii: S2213-2600(17)30079-6.
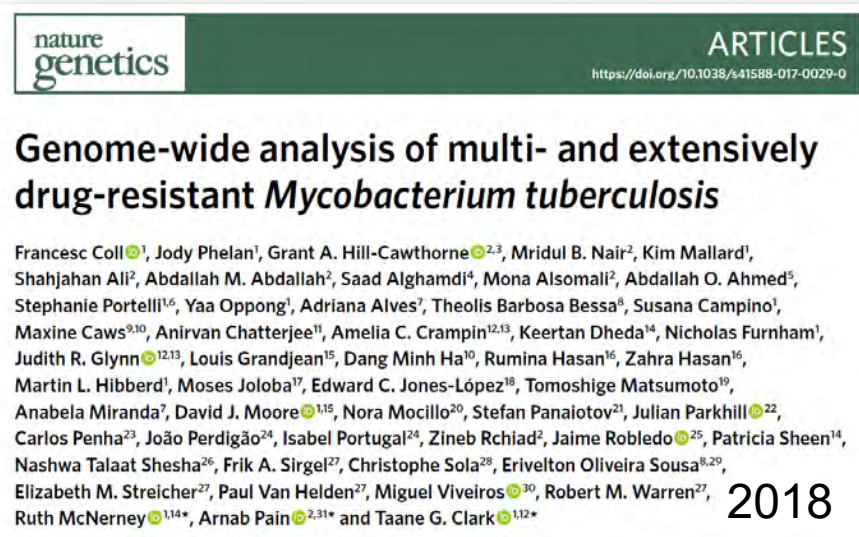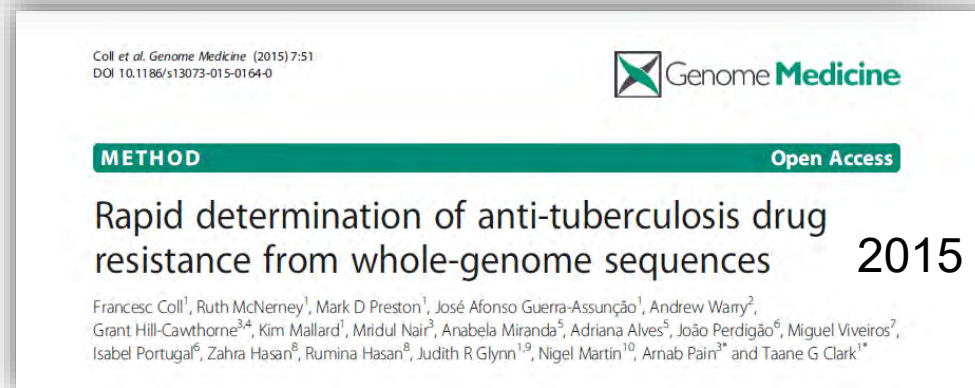
WHOLE GENOME SEQUENCING - COMPLETE GENOME IN 24 HOURS! – BUT!!!
MultiCenter Studies and Global Genome-Wide Association Studies revealed many lineage specific associations with DR and many genes correlated with the phenotypic DR levels of *M. tuberculosis* – *other mechanisms of resistance than simply mutations*.

Source: Viveiros e Atouguia, 2008; Da Silva et al, 2011; Wilson, 2011; O'Grady et al, 2011

Anti-TB drugs	Gene	Protein	Frequency of clinically resistant strains (%)		
Rifampicin (RIF)	rpoB	RNA polimerase B- subunit	96-98%	?	
Isoniazid (INH)	KatG ahpC inhA ndh	Catalase Peroxidase Alquil hidroxireductase Enoil ACP reductase NADH desidrogenase	42-68% 21-34%	?	1st line
Ethambutol (EMB)	embCAB	arabinosil transferase	47-65%	?	
Pyrazinamid (PZA)	pncA	amidase	72-97%		
Streptomycin (SM)	rpsL rrs	Ribossomal protein S12 16S rRNA	52-59%	?	
Fluoroquinolones	gyrA	DNA gyrase	50-70%		
Aminoglicosídes	rrs	16S rRNA	90-95%	?	2nd line
Ethionamid	inhA ethA ethR	Enoil ACP redutase Flavinamonooxigenase Transcripcional repressor	50-60%	?	
D-cicloserin	Alr ddl	D-alanin racenase D-alanin ligase	30-50%	?	
Viomicin	rrs	16S rRNA	nd		

Mutational targets for anti-TB Drugs do not explain all DR-TB NOR THE LEVEL OF RESISTANCE!

A possible and plausible explanation ?

Targeting efflux pumps of MDR

Mycobacterium tuberculosis.

Aínsa *et al*. (1998) *J Bacteriol*., 180 : 5836-43.

Viveiros *et al*. (2002), *Antimicrob Agents Chemother*, 46 : 2804-10.

De Rossi E *et al*. (2002) *Mol Med*., 8 : 714-24.

Viveiros *et al*. (2003), *International J*. of *Antimicrob Agents*, 22 : 274-8.;

Pasca *et al*. (2005), *Antimicrob Agents Chemother*. 49 : 4775-7;

Colangeli *et al*. (2005), *Mol Microbiol*. 55 : 1829-40.;

De Rossi E, *et al*. (2006). *FEMS Microbiol Rev* 30: 36–52;

Amaral L*, et al* (2007) *J Antimicrob Chemother*. 59:1237-46.

Danilchanka O, *et al.* (2008). *Antimicrob Agents Chemother*. 52:2503-11;

Louw GE, *et al*. (2009). *Antimicrob Agents Chemother*. 53(8):3181-9.;

Ramón-García S, *et al*. (2009). *Antimicrob Agents Chemother*. 53(9):3675-82.

Gupta AK,.*et al* (2010) *Microb. Drug Resist*. 16(1):21-8.

Pasipanodya JG & Gumbo T. (2011) *Curr Opin Pharmacol*. 11(5):457-63.

da Silva PE *et al*. (2011) *FEMS Immunol Med Microbiol*. 63(1):1-9.

Viveiros *et al*. (2012) *Expert Rev Anti Infect Ther*. 10 :983-98.

Black *et al*. (2014) *Antimicrob Agents Chemother*. 2014 Mar 10.

(a) Small hydrophilites (nutrients) — Porins — Trehalose dimycolate — Arabinogalactan mycolate — Peptidoglycan — Plasma membrane (5 nm)

Barrier of hydrophilites (10 nm)

Barrier of lipophilites

Nguyen, L. & C. J. Thompson. 2006. TRENDS in Microbiology.14: 304-312

Since 1998 several efflux-pumps and export systems have been characterized and many were shown to be correlated with increased antibiotic resistance in *M. tuberculosis* !!

M. tuberculosis genome

The first evidences of the role of efflux-pumps on INH resistance!

We were able to reverse **induced resistance to INH by overexpression of efflux-pumps** in clinical and laboratory TB and MDR-TB strains by the use of known inhibitors of efflux pumps – Verapamil, Chlorpromazine, Thioridazine and Reserpine ⇒ **A reversible resistance to INH !!!** ⇒

Non mutational resistance !!!

Isoniazid-Induced Transient High-Level Resistance in *Mycobacterium tuberculosis*

Miguel Viveiros,[1] Isabel Portugal,[2] Rosário Bettencourt,[1] Thomas C. Victor,[3] Annemarie M. Jordaan,[3] Clara Leandro,[1] Diane Ordway,[1] and Leonard Amaral[1]*

Unit of Mycobacteriology, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, P-1349-008 Lisbon,[1] and Department of Microbiology, Faculdade de Farmácia da Universidade de Lisboa, P-1649-019 Lisbon,[2] Portugal, and MRC Centre for Molecular and Cellular Biology, Department of Medical Biochemistry, University of Stellenbosch, Stellenbosch, South Africa[3]

Viveiros *et al.* (2002) Antimicrob. Agents. Chemother. 46 : 2804-2810
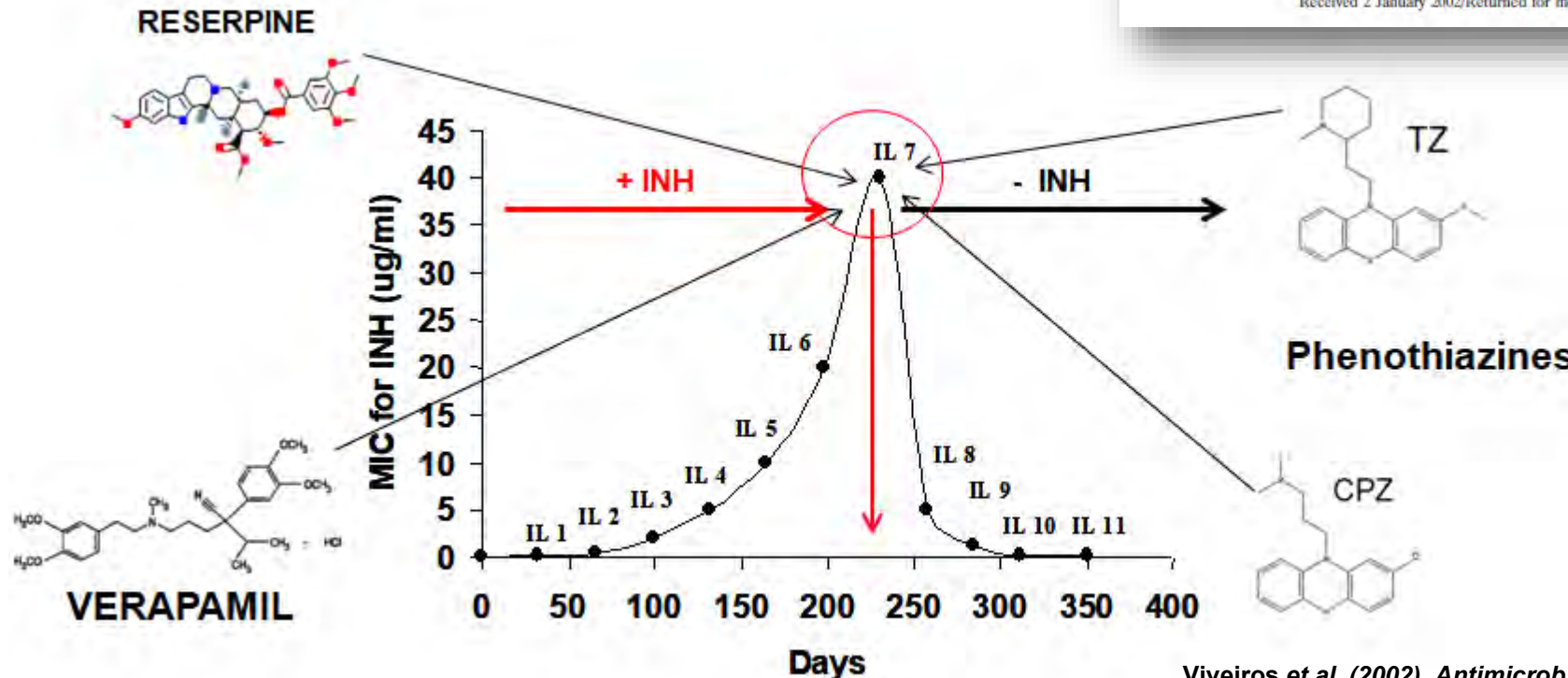
Real-time EP activity measurements and its correlation with antibiotic exposure!

Result 1) Demonstration of efflux pumps involved in INH increased resistance in *M. bovis* BCG, *M. tuberculosis*, *M. avium*, *M. abcessus* etc..
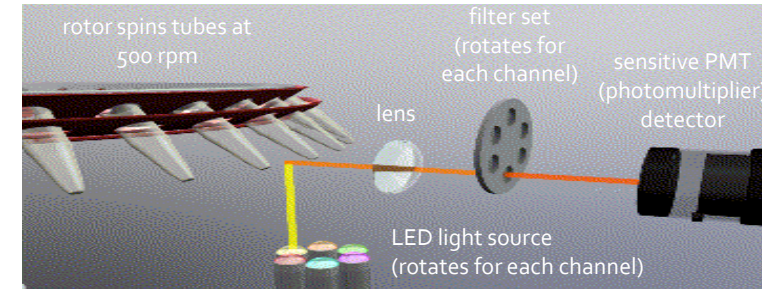
M. bovis BCG

M. bovis BCG$_{INH}$

RealTime visualization of EtBr efflux and inhibition in *Mycobacteria*! Correlation with INH resistance and with many other antibiotics!
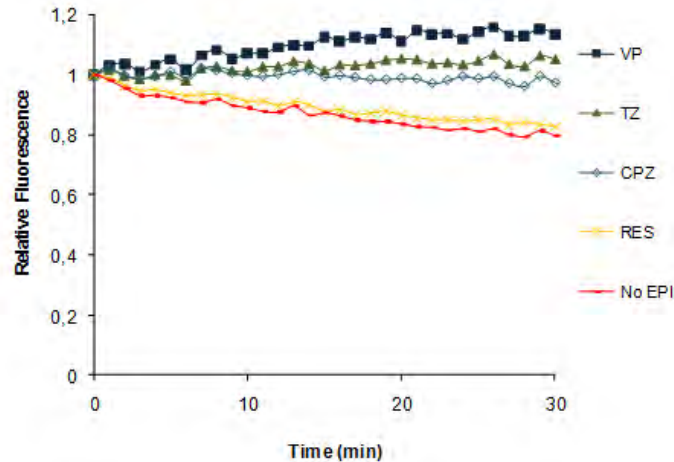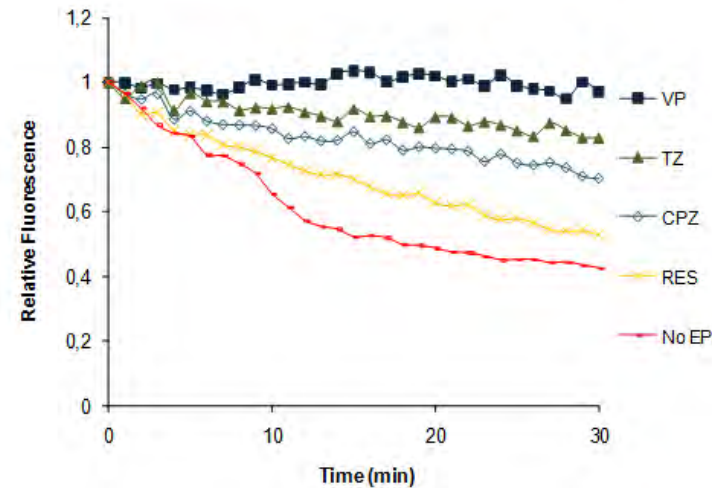
M. bovis BCG$_{INH}$ presents increased efflux of EtBr

Efflux of EtBr is inhibited by EPIs – Verapamil has the strongest inhibitory effect

Viveiros M*, et al.* (2010) *Methods Mol Biol*. 642:159-72. ;   Rodrigues L, *et al.* (2011). *BMC Microbiol. Feb 18;11:35*.   Rodrigues L, *et al.* 2012. *Infect Genet Evol. 2Jun;12(4):695-700.*

Result 3) INH exposure=> ↓ EtBr accumulation => ↑Efflux

The *in vitro* induction of an isoniazid resistant phenotype by prolonged serial exposure of *M. tuberculosis* strains to the **critical concentration of isoniazid** lead to an **enhanced real-time efflux of ethidium bromide**.

A clear relation between **overexpression of the EP genes and increased efflux pump function** was found.

Further exposure to isoniazid resulted in the **selection and stabilization of spontaneous mutations and deletions in the *katG* gene along with sustained increased efflux activity**.

Month #1   Month #1-3   Month #6
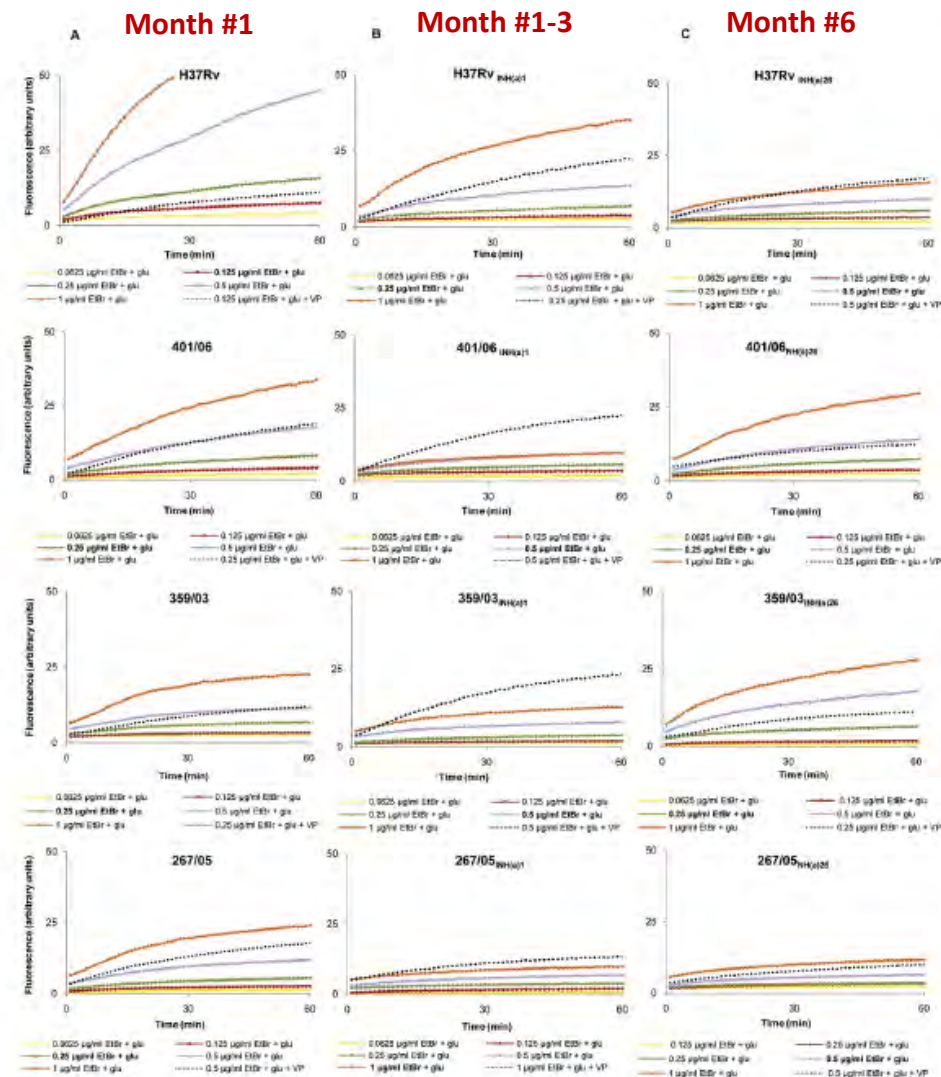
Figure 3. Accumulation of EtBr by the *M. tuberculosis* strains tested. The figure shows the accumulation of EtBr by the strains from adaptation process A as an example. The values at bold type correspond to the higher concentration of EtBr that cells can handle without detectable accumulation. The dotted line corresponds to the assay run using the EtBr concentrations for which influx-efflux are at equilibrium, in the presence of the EI verapamil, at sub-inhibitory concentrations. Panel (A): Parental strains (passage #0); Panel (B) strains after first passage with INH and Panel (C): strains after 26 passages with INH. INH: isoniazid.
doi:10.1371/journal.pone.0034538.g003

Machado D. *et al.* 2012. *PLoS One.* 2012;7(4):e34538. .
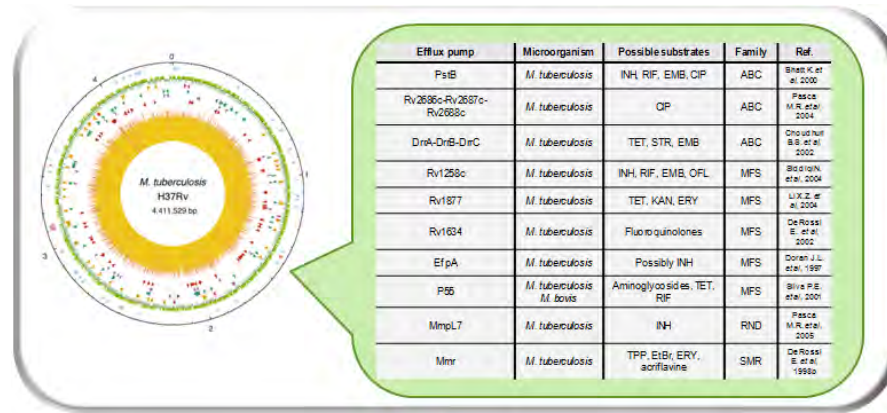
Selection of efflux pumps associated with INH efflux

MmpL7, EfpA, Tap, P55, Mmr

qRT-PCR

Result 5) Overexpression of Efflux-pumps

Relative expression = 1: expression level identical to the INH non-induced strain

- mmpL7 (Rv2942)
- tap (Rv1258c)
- P55 (Rv1410c)
- efpA (Rv2846c)
- mmr (Rv3065)
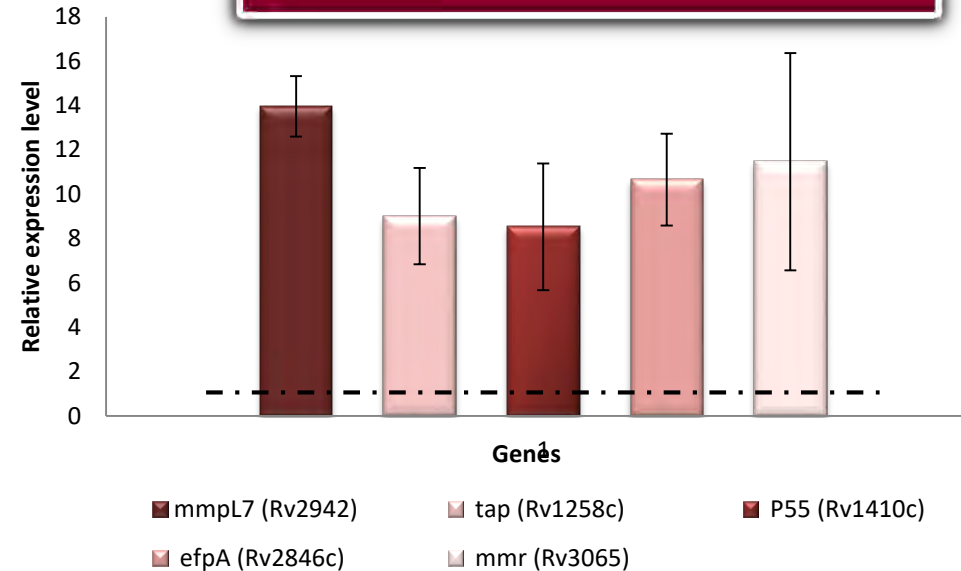
Overexpression of all the efflux pumps previously associated with INH efflux – none was specifically overexpressed ! After a few months of exposure the INH mutants emerge with **natural spontaneous and stable mutations in KatG**.

Conclusion : Enhancement of a "**multipump response**" by INH exposure at critical concentration – **a pheno-genotypic stress/survival response** !!!

Machado D. et al. 2012. PLoS One. 2012;7(4):e34538.

The clinical model !:

The results so far support the hypothesis that activity of efflux pumps allows the maintenance of anti-TB drugs resistant/tolerant populations in a sub-optimally treated patient from which genetically resistant mutants emerge**. Therefore, the use of inhibitors of efflux should be considered in the development of new therapeutic strategies for preventing the emergence of M/X/TDR-TB during treatment.**

Non-compliance
Immunosupression
Errors in prescription or drug quality
Patient personal pharmacodinamics

Inspired by Gillespie SH, *et. al.* (2002). *Microb Drug Resist.* 8:273-9.

Conclusions so far !!!

….on drug resistance…

antibiotics MDPI

Article

**Efflux Activity Differentially Modulates the Levels of
Isoniazid and Rifampicin Resistance among
Multidrug Resistant and Monoresistant
Mycobacterium tuberculosis Strains**

Diana Machado [1], João Perdigão [2], Isabel Portugal [2], Marco Pieroni [3,4], Pedro A. Silva [5],
Isabel Couto [1] and Miguel Viveiros [1,*]

1. Inhibition of efflux pumps lead to isoniazid and rifampicin intracellular accumulation and increased susceptibility to this drug despite the presence of a mutation leading to resistance

2. Isoniazid and rifampicin act like an inducer that stimulates a general stress response via overexpression of efflux pumps

3. Different levels of resistance to isoniazid and rifampicin are a balance between the induction of several efflux pumps that regulate the intracellular level of isoniazid and the mutation
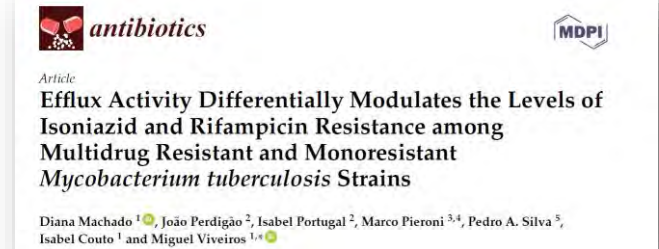
…on the therapeutic usefulness of efflux inhibitors…

Future **MICROBIOLOGY**

Research Article

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**Adjuvant therapies against tuberculosis:
discovery of a 2-aminothiazole targeting
Mycobacterium tuberculosis energetics**

Diana Machado[1], Elisa Azzali[2,3], Isabel Couto[1], Gabriele Costantino[2], Marco Pieroni[2] &
Miguel Viveiros[*,1]

4. In vitro therapeutic value for compounds that have the capacity to inhibit mycobacterial efflux pumps via the retention of co-administered antibiotics that are subject to efflux

5. Neuroleptics, antipsychotics and anti-hypertension drugs (ion-channel blockers!!) demonstrate synergistic effect when combined with the anti-tuberculosis drugs such as isoniazid (and rifampicin!).

6. However, they are noxious at these concentrations *in vivo*, yet, they can be used at tolerable concentrations as efflux inhibitors.

Viveiros et al. 2002 Antimicrob. Agents. Chemother. 46 : 2804-2810 ; Machado D. *et al.* 2012. *PLoS One 2012;7(4):e34538; Antibiotics (Basel) 2018 Mar 3;7(1); Front Microbiol. 2017 Apr 27;8:711; Future Microbiol. 2018 Sep;13:1383-1402.*
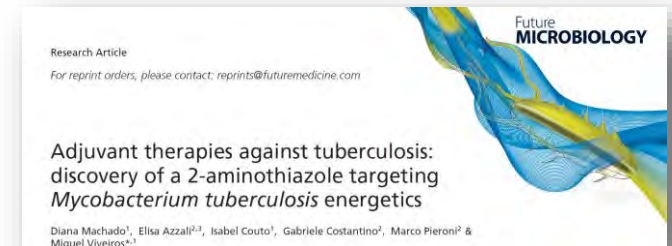
Future perspectives and ongoing competition!

M. avium

M. tuberculosis

"We propose that induction of several efflux pumps is the first step in a general pathway to drug resistance that eventually leads to high-level chromosomal-mutation-related resistance in mycobacteria as ordered events in an "antibiotic resistance arrow of time.""

M. avium

M. tuberculosis

• **Mycobacteria lineages are not all the same – Some are more virulent and prone to be resistant.. The ones that evolved via adaptation, active disease and global dissemination *versus* those that are ancient, latent and locally restricted;**

• **Immediately after antibiotic exposure there is a stress response via activity and over-expression of efflux pumps that allows the maintenance of anti-TB drugs [resistant/tolerant] populations in a sub-optimally treated patient from which genetically resistant mutants emerge;**

•**Efflux-pump inhibitors enhance the activity of *effluxable* antibiotics against mycobacteria**

• **Anti-tubercular therapy employing efflux-pump inhibitors (e. g. thioridazine, verapamil) in combination with conventional anti-tubercular drugs will certainly provide advantages over conventional therapy and will contribute to prevent the emergence of *Multi - Extensively - Totally - Drug Resistant Tuberculosis* . – A clear case of the "*Red Queen hypothesis*"!! - *Bacteria constantly adapt, evolve, and proliferate not merely to gain reproductive advantage, but also simply to survive against ever-evolving opposing organisms in an ever-changing environment.***
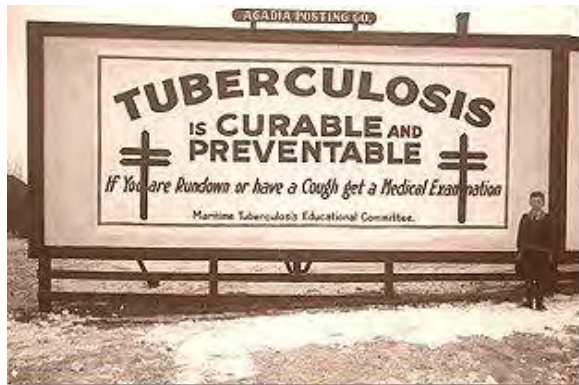
Anti-Microbial Resistance in Tuberculosis and Non-Tuberculosis Mycobacteria is a slow selective and adaptative process in progress in One-Health, One-World

"The Red Queen has to run faster and faster in order to keep still where she is. That is exactly what you all are doing!"

Leigh Van Valen. (1973). "A new evolutionary law". Evolutionary Theory 1: 1-30. ;Lewis Carroll. 1960 (reprinted). The Annotated Alice: Alice's Adventures in Wonderland and Through the Looking-Glass,

University Paris Diderot,
APHP, Saint Louis-Lariboisière Hospital,
NRC mycobacteria, Paris, France

Thank you for your attention !

Multidrug resistance in human and animal mycoplasmas

Sabine Pereyre

[1]**USC EA 3671 Mycoplasmal and chlamydial infections in humans**
INRA - University of Bordeaux - Bordeaux University Hospital
French National Reference Center for bacterial STIs

Mycoplasmas

- **Minimal bacteria with high host specificity**

  - **No zoonotic transmission**
  - **116 *Mycoplasma* species and 7 *Ureaplasma* species** (Bergey's 2014)
  - **Shared tissue tropism: respiratory tract, urogenital tract and joint**

- **Human pathogenic species**

  - ***M. pneumoniae*** → respiratory tract infections

  - ***M. hominis***
  - ***Ureaplasma* spp.**
  - ***M. genitalium***

    Urogenital tract infections
    Sexually transmitted infections (Mg)
    + extra-genital infections (arthritis) :
    immunocompromised patients +++

2

- **Many important animal pathogenic species**
  - Internationally regulated diseases (listed by the world organization for animal health OIE) + diseases associated with significant economic losses
  - **Cattle**
    - ✓ *M. bovis*
    - ✓ *M. mycoides* subsp. *mycoides* (Contagious bovine pleuropneumonia CBPP)
  - **Small ruminants**
    - ✓ *M. capricolum* subsp. *capripneumoniae* (Contagious caprine pleuropneumonia CCPP)
    - ✓ *M. agalactiae, M. mycoides* subsp. *capri, M. capricolum* subsp. *capricolum, M. putrefaciens* (Contagious agalactia)
  - **Swine** : *M. hyopneumoniae* (enzootic pneumonia)
  - **Poultry**
    - ✓ *M. gallisepticum* (chronic respiratory infection)
    - ✓ *M. synoviae* (joint, bone and respiratory infection)

3

Intrinsic resistance

- **Several families of antibiotics !**

- **Resistance related to the *Mollicutes* class**
  - β-lactam antibiotics, glycopeptides, fosfomycin : no cell wall
  - Rifampicin, polymixin, sulfonamides

- **Intrinsic resistance to macrolides in some species**
  - 14 and 15-membered macrolides (e.g. *M. hominis*)

4

"Assumed" active antibiotics

- **Used in human _and_ animal infections**
  - Macrolides and related antibiotics (MLSK)
  - Tetracyclines (TC)
  - Fluoroquinolones (FQ) ⚠ restricted use in animals ⚠

- **Used specifically in animal mycoplasmal infections**
  - Pleuromutilins (tiamulin, valnemulin)
  - Phenicols (florfenicol)
  - Aminoglycosides (spectinomycin)

5

Acquired resistance in mycoplasma

☞ **Same mechanisms of resistance in human and animal mycoplasmas**

One noteworthy exception = Tet(M)

- **Biochemical mechanisms of resistance**
  - ✓ Target modification (MLSK, FQ, pleuromutilins) or target protection (TC)
  - ✓ Efflux (few _in vitro_ evidence only)
  - ✓ No enzymatic inactivation

6

Acquired resistance (1)

- **Genetic support**
  1. **Chromosomal MUTATIONS +++**
     - ✓ Lack of some DNA repair systems : high mutation rates
     - ✓ Mutation sites
       - 23S rRNA (MLSK, pleuromutilins, phenicols)
       - Gyrase, topoisomerase IV (fluoroquinolones)
       - 16S rRNA (tetracyclines)

  2. **Acquisition of mobile genetic elements +/-**

7

Mutations associated with resistance to MLSK pleuromutilins and phenicols

Mutations in the antibiotic target : 23S rRNA

Human MLSK
- Erythromycin (14)
- Clarithromycin (14)
- Roxythromycin (14)
- Azithromycin (15)
- Spiramycin (16)
- Josamycin (16)
- Clindamycin
- Pristinamycin
- Quinupristin-Dalfopristin
- Telithromycin

Animal MLS
- Erythromycin (14)
- Azithromycin (15)
- Gamithromycin (15)
- Tulathromycin (13/15)
- Tylosin (16)
- Tilmicosin (16)
- Spiramycin (16)
- Lincomycin
- Clindamycin

Animal pleuromutilins
- Tiamulin
- Valnemulin

Animal phenicols
- Florfenicol
- Thiamphenicol
- Chloramphenicol
  (dogs, cats and horses)

Ban et al, Science, 2000
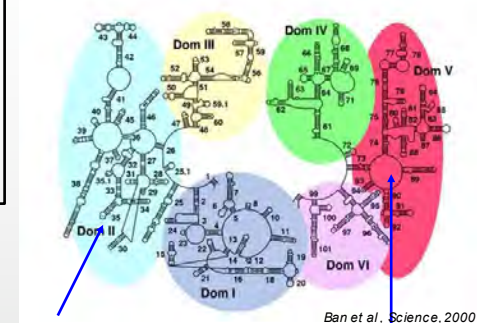
Hairpin 35 of domain II

Peptidyl transferase loop of domain V

☞ **Cross resistance to several antibiotic categories**
☞ **L4 and L22 ribosomal protein mutations : slight MIC increase**

4

Fluoroquinolone resistance by mutations of the target

Human fluoroquinolones
- Ofloxacin
- Ciprofloxacin
- Levofloxacin
- Moxifloxacin

Animal fluoroquinolones
- Enrofloxacin
- Danofloxacin
- Marbofloxacin

Topoisomerase II

DNA Gyrase (ATP-dependent negative supercoiling of DNA)

Topoisomerase IV (chromosomal segregation)

Subunit A *gyrA*

Subunit B *gyrB*

Subunit C *parC*

Subunit D *parE*
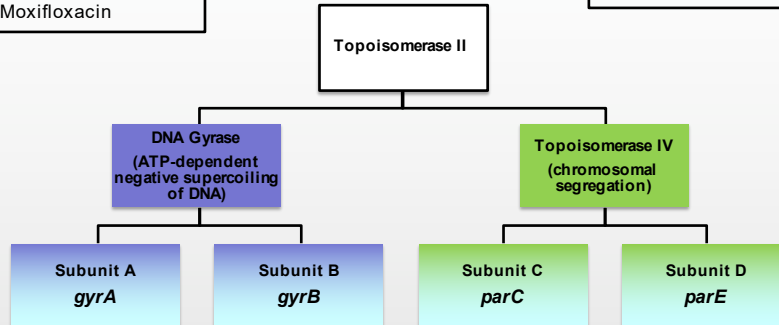
Mutations in QRDR (Quinolone-Resistance Determining Region) in *gyrA*, *gyrB*, *parC* and/or *parE*

9

Tetracycline resistance by mutation in the 16S rRNA target

Human tetracyclines
- Tetracycline
- Doxycycline
- Minocycline

Animal tetracyclines
- Tetracycline
- Oxytetracycline
- Doxycycline
- Chlortetracycline

Tétracycline

Brodersen *et al.*, Cell, 2000.

Human mycoplasmas (in vitro)
Animal mycoplasmas (clinical isolates)
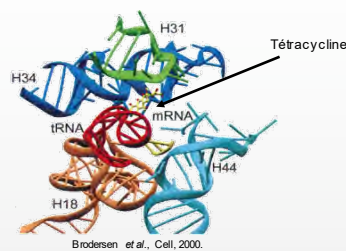
Helix 34

Helix 31

- Mutations in helix 31 and 34
- Humans *in vitro* only
  - Reduced susceptibility : no resistant mutants
- Animals : clinical isolates
  - High MIC isolates in *M. bovis*
  - Some high MIC isolates in *M. agalactiae*

10
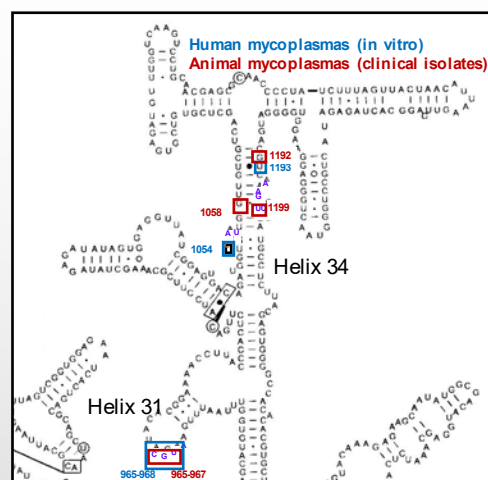
Acquired resistance (2)

- **Genetic support**

    1. **Chromosomal MUTATIONS +++**

    2. **Mobile genetic elements +/-**
        - ✓ Few plasmids (limited to the *M. mycoides* cluster)

        - ✓ Mycoplasma Integrative Conjugative Elements

        Not involved in antibiotic resistance to date

        - ✓ **Transposons : *Tn916 - tet*(M)**

11

Acquisition of the *Tn916 - tet*(M) transposon

- **High-level resistance to all tetracyclines**

- ***Tet*(M) : Target protection**

- **In some human mycoplasmas only**
    - **Not reported in animal species**
    - **Never reported in *M. pneumoniae* nor *M. genitalium***
    - **Frequent in *Ureaplasma* spp. and *M. hominis***

12

Multidrug, eXtensively, pan-drug resistant bacteria

MDR

Not MDR

XDR **PDR**

MDR = multi drug resistant
Non-susceptibility to at least one agent in ≥3 antimicrobial categories (excluding intrinsic R)

XDR = eXtensively resistant
Non-susceptibility to at least one agent in all but 2 or fewer antimicrobial categories
i.e. bacterial isolates remain susceptible to only 1 or 2 categories

PDR = pan-drug resistant
Non-susceptibility to at all agents in all antimicrobial categories

Magiorakos *et al.* Clin Microbiol Infect. 2012, 18, 268-281   13

Example of MDR in animal mycoplasma
M. bovis

14

Mycoplasma bovis

- **Emerging pathogen in cattle in many industrialized countries**
  - **Distributed worldwide**

- **Calves**   **Adult cattle**
  - **Pneumonia**   - **Mastitis**
  - **Arthritis**   - **Pneumonia**
  - **Otitis + neurological disorders**   - **Arthritis, keratoconjuntivitis**

© MA Arcangioli

© Guda Van der Burgt 2008

- **First line treatment :**
  - **Oxytetracycline, tilmicosin or spectinomycin**
  - **More recently long lasting macrolides (tulathromycin)**

15

Resistance in *M. bovis* around the world

No clinical breakpoints for animal mycoplasmas ➔ no S, I, R prevalence

MIC$_{50}$ µg/mL (MIC that inhibited 50% of the tested strains)

	Macrolides (tilmicosin)	Tetracyclines (oxytetracycline)	Phenicols (florfenicol)	Fluoroquinolones (enrofloxacin)	Reference
Pasteurella susceptibility (CLSI)	≤ 8	≤ 2	≤ 2	≤ 0.25	
France	>128	>32	8	0.5	Gautier-Bouchardon et al 2014
UK	>32	8	8	0.5	Ayling et al 2014
NL	512	4	nd	0.25	Heuvelink etal 2016
Israël	128	4	nd	0.125	Gerchman et al 2009
Japan	>128	32	nd	0.5	Kawai et al 2013
China	8	0.5 (doxy)	2	0.125	Kong et al 2016
USA	64	2	1	0.25	Rosenbusch etal 2005

☞ **High MICs of most molecules around the world, except MICs of fluoroquinolones**
☞ **decreased susceptibility to at least one agent in ≥3 antimicrobial categories = MDR**

8

Evolution of susceptibility over time

- **A shift towards higher MICs happened around year 2000**
  - **Especially for macrolides and spectinomycin**
    - ✓ Low MICs in the 80'
    - ✓ High MICs in 2010-2012…
  - **Moderate MIC increase for fluoroquinolones**

- **Associated with modification of molecular subtype (MLST)**
  - **ST1 disappeared**
  - **ST2 emerged, poorly variable strains**

  ➜ **High prevalence of MDR strains due to the spread of one multiresistant clone**
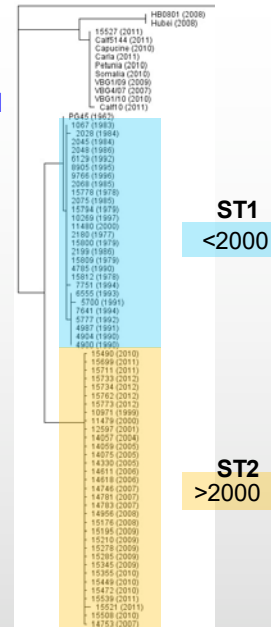
Gautier-Bourchardon, PloS One, 2014; Khalil, MDR, 2017, Becker, Infect Genet Evol, 2016

ST1
<2000

ST2
>2000

17

Evolution of susceptibility over time

- **In 2011, detection of a new ST in France, ST3**

- **ST3 is more likely to gain high level FQ resistance[1]**
  - **Rapid accumulation of mutations in QRDR under selective pressure *in vitro***

- **In 2013, the first ST3 clinical isolate with increased MIC to fluoroquinolones in France**
  - **Enrofloxacin MICs =16 µg/mL and marbofloxacin MIC= 64 µg/mL**
  - **Mutations in *par*C, *gyr*B and *gyr*A**

  M. bovis, from MDR ➜ XDR?

[1]*Khalil et al. AEM, 2016*

Example of MDR human mycoplasma
M. genitalium

Tully, Int J Syst Bacteriol 1983

19

Mycoplasma genitalium

- **STI agent**
  - 1- 3% in general population
  - 4-38% in high sexual-risk population

- ♂   ♀
  - Uretritis   - Cervicitis, PID
                              - Preterm birth, spontaneous abortion

- **First line treatment : Azithromycin**

d1   d2   d3   d4   d5

20

**2016 European guideline
on *Mycoplasma genitalium* infections**

Jørgen Skov Jensen*[1], Marco Cusini[2], Mikhail Gomberg[3]

[1]Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark.

[2]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

[3]Chief Researcher, Moscow Scientific and Practical Centre of Dermatovenereology and Cosmetology.

JEADV 2016 DOI 10.1111/jdv.13849

Avoid azithromycin 1 gram

Uncomplicated infection
First line. No macrolide resistance : Azithromycin 500 mg (day 1) then 250 mg (4 days)
Complicated infections (PID, epididymitis)

21

Prevalence of macrolide resistance in *M. genitalium*

100%(26/26)

56-61%

18%

58%

41%

57%

53%

4.6-6,7%

48%

35%

42%

No data
<10%
10%-20%
20%-40%
40-60%
>60%

9,8%

83% in MSM

43-63%

72-77%

Anagrius, PloS one 2013 ; Tagg, J. Clin. Microbiol. 2013 ; Pond, Clin. Inf. Dis. 2014 ; Salado-Rasmussen, Clin. Inf. Dis, 2014; Kikuchi, J. Antimicrob. Chemother. 2014; Hay, Sex. Transm. Dis. 2015; Gushin, BMC Infect. Dis. 2015; Nijhuis, J. Antimicrob. Chemother. 2015; Gesink, Can. Fam. Physician, 2016; Getman, J. Clin. Microbiol. 2016; Gossé, J. Clin. Microbiol. 2016; Shipitsina, Plos One, 2017 ; Basu, J. Clin. Microbiol. 2017; Tabrizi, J. Clin. Microbiol. 2017; Barbera, Sex. Transm. Dis. 2017; Dumke, Diagn Microbiol infect Dis , 2016; Coorevits, J. Glob . Antimicrob. Resist. 2017; Anderson, J. Clin. Microbiol. 2017; Unemo, Clin. Microbiol. Infect 2017

**2016 European guideline
on *Mycoplasma genitalium* infections**

Jørgen Skov Jensen*[1], Marco Cusini[2], Mikhail Gomberg[3]

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[2]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[3]Chief Researcher, Moscow Scientific and Practical Centre of Dermatovenereology and Cosmetology.

IUSTI
INTERNATIONAL UNION AGAINST SEXUALLY TRANSMITTED INFECTIONS

Avoid azithromycin 1 gram

Uncomplicated infection

First line. No macrolide resistance :
Azithromycin 500 mg (day 1) then 250 mg (4 days)

Second line OR macrolide resistance :
Moxifloxacin 400 mg/d for 7-10 days

Complicated infections (PID, epididymitis)
Moxifloxacin 400 mg/d for 14 days

23

Prevalence of fluoroquinolone resistance-associated mutations

20%
11.1%
0.2%
4.1%
4.5%
6%
8%
6.2%
31- 53%
12-19%
23%

- No data
- <10%
- 10%-20%
- 20%-40%
- 40-60%
- >60%
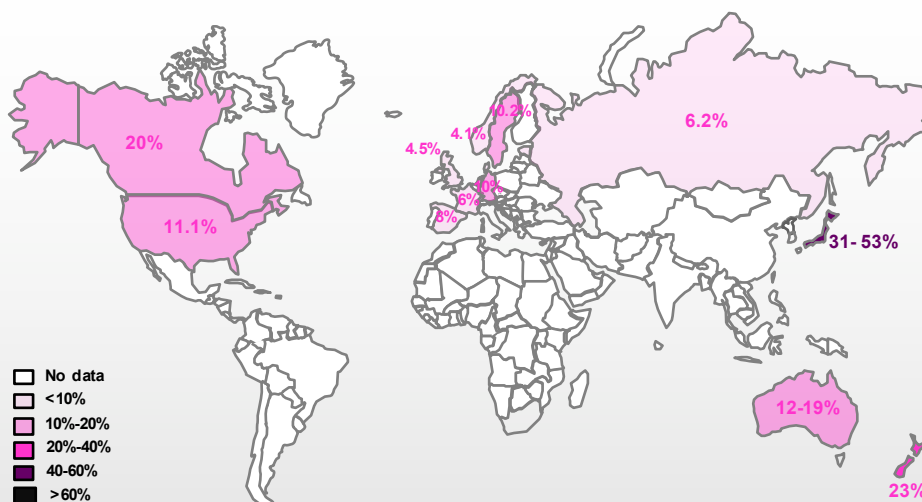
Bissessor Clin Infect Dis 2015; Deguchi, Clin Infect Dis 2016; Dumke, DMID 2016; Kikuchi J Antimicrob Chemother 2014; Le Roy Emerg Infect Dis 2016; Pond Clin Infect Dis 2014; Shipitsina PLoS one 2017; Couldwell Int J STD and AIDS 2013; Gesink Can family Physian 2016; Tagg J Clin Microbiol 2013; Murray Emerg Infec Dis 2017; Barbera Sex Transm infect 2017; Anderson, J Clin Microbiol 2017, Unemo, Clin Microbiol Infect 2017.

Dual class resistance
Macrolides AND fluoroquinolones

- **Prevalence of dual resistance**
  - **France : 1.2%**
  - **Australia : 8.6%**
  - **Japan : 17-30%**

- **Mainly due to successive treatment failures of macrolides then fluoroquinolones**

- **Tremendous clinical implications**

Murray Emerg. Infect. Dis. 2017, Kikuchi J Antimicrobiol Chemother 2014; Le Roy Emerg. Infect. Dis. 2016; Degushi Clin Infect Dis 2016; Kikuchi J Antimicrobiol Chemother 2014

25

2016 European guideline
on *Mycoplasma genitalium* infections

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[1]Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark.
[1]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[2]Chief Researcher, Moscow Scientific and Practical Centre of Dermatovenereology and Cosmetology.

JEADV 2016 DOI 10.1111/jdv.13849

IUSTI
INTERNATIONAL UNION AGAINST
SEXUALLY TRANSMITTED INFECTIONS

Uncomplicated infection

First line. No macrolide resistance :
Azithromycin 500 mg (day 1) then 250 mg (4 days)

Second line OR macrolide resistance :
Moxifloxacin 400 mg/d for 7-10 days

Third-line
- Doxycycline 100 mg X2 daily for 14 days ← Low potency. 70% treatment failure
- Pristinamycin 1g X4 daily for 10 days ← Not available in many countries

Complicated infections (PID, epididymitis)
Moxifloxacin 400 mg/d for 14 days

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MDR or XDR *M. genitalium* ?

Antimicrobial category	Antimicrobial agent	Resistance in *M. genitalium*
Macrolides	Azithromycin	X
Fluoroquinolones	Moxifloxacin	X
Tetracyclines	Doxycycline	2/3 treatment failure
Streptogramins	Pristinamycin	

X: non susceptible

Non-susceptibility to ≥ 3 antimicrobial categories = MDR	Susceptibility to only one or two antimicrobial categories = XDR

27

BBC, July 2018

28

Conclusions

- **Few antibiotic families are active on animal and human mycoplasmas**
- **Same mechanisms of resistance to antibiotics**
  - **Mutations +++**
  - **Exception : *tet*(M) gene : human urogenital mycoplasmas**
- **MDR-XDR in some human and animal species**
  - ***M. bovis***
  - ***M. genitalium***

➢ **Need for new antimicrobial compounds and trials**
➢ **Need for interpretive criteria in veterinary medicine**

29

Acknowledgments

Florence Tardy

University of Lyon, Anses, VetAgro Sup, JRU
Mycoplasmoses in ruminants

30
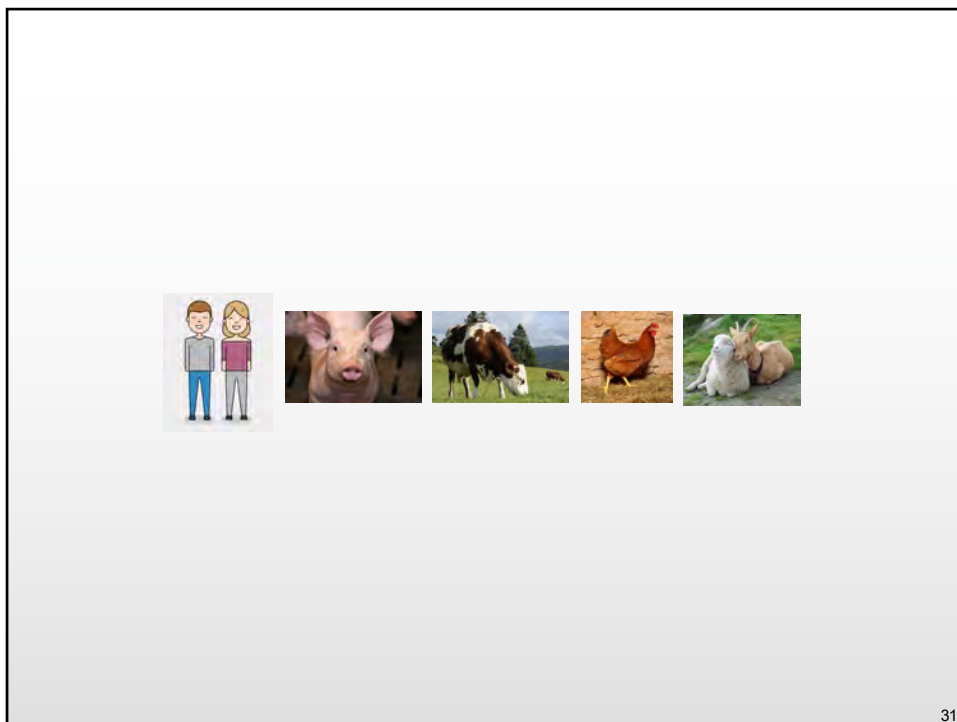
THE GUT MICROBIOME OF DOGS AND CATS SHARES GENES AND SPECIES WITH THE HUMAN GUT MICROBIOME

LUIS PEDRO COELHO

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🐦 @luispedrocoelho

ISTBI 复旦大学类脑智能科学与技术研究院
Institute of Science and Technology for Brain-Inspired Intelligence

FUDAN UNIVERSITY 1905

Pets matter

- People value their pets emotionally and this translates into economic expenditures.
- In US, ca. 30% of households own pets, spending an average of 183 USD per month on their health (Einav et al., Am Econ Review, 2017).

Pets' problems mirror human problems

- More than half of dogs in Western world above their ideal weight (Courcier et al., J. Small An Prac., 2010; McGreevy et al., Vet Rec, 2005; Sandøe et al., Vet Rec, 2014).
- The obesity of a pet and its owner have a positive correlation (at least for dogs, maybe not for cats, see Kienzle et al., J Nutrition, 1998; Nijland et al., Pub Health Nut, 2010).
- Pets consume antibiotics.

(Waiting for the vet, Normal Rockwell)

2017-18: THE SIMILARITY BETWEEN THE DOG GUT MICROBIOME AND THE HUMAN GUT MICROBIOME

- Gene catalog of the dog gut microbiome with 1.24 million genes.
- Compared to publicly-available catalogs for human, mouse, and pig gut microbiomes.
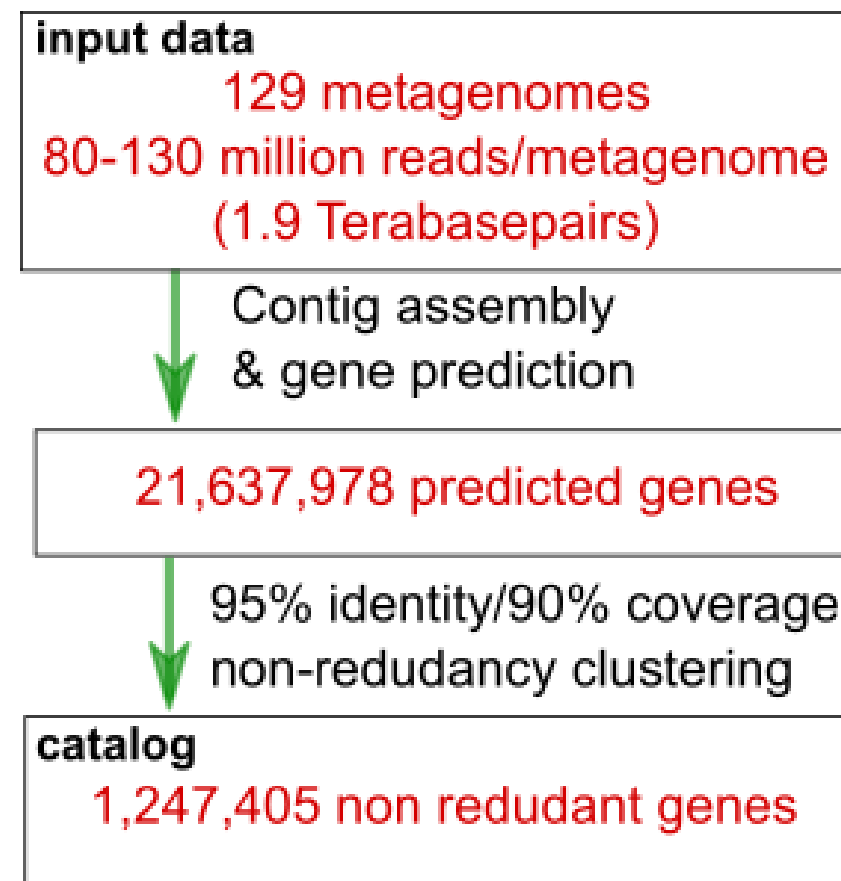
This is published work as (Coelho et al., Microbiome, 2018)

WE OBSERVE GENUS/SPECIES SHARING, BUT STRAIN SPECIFICITY

Fraction of genera

Fraction of genes

SNP-based strain clustering
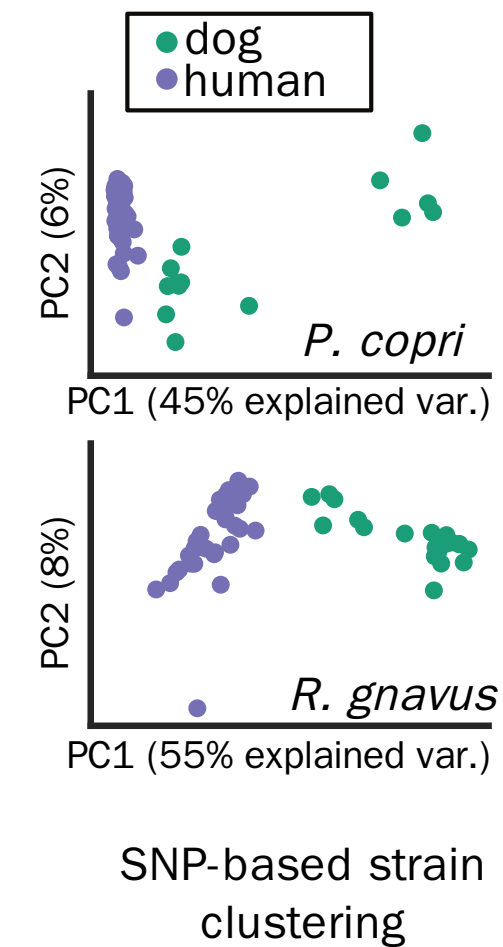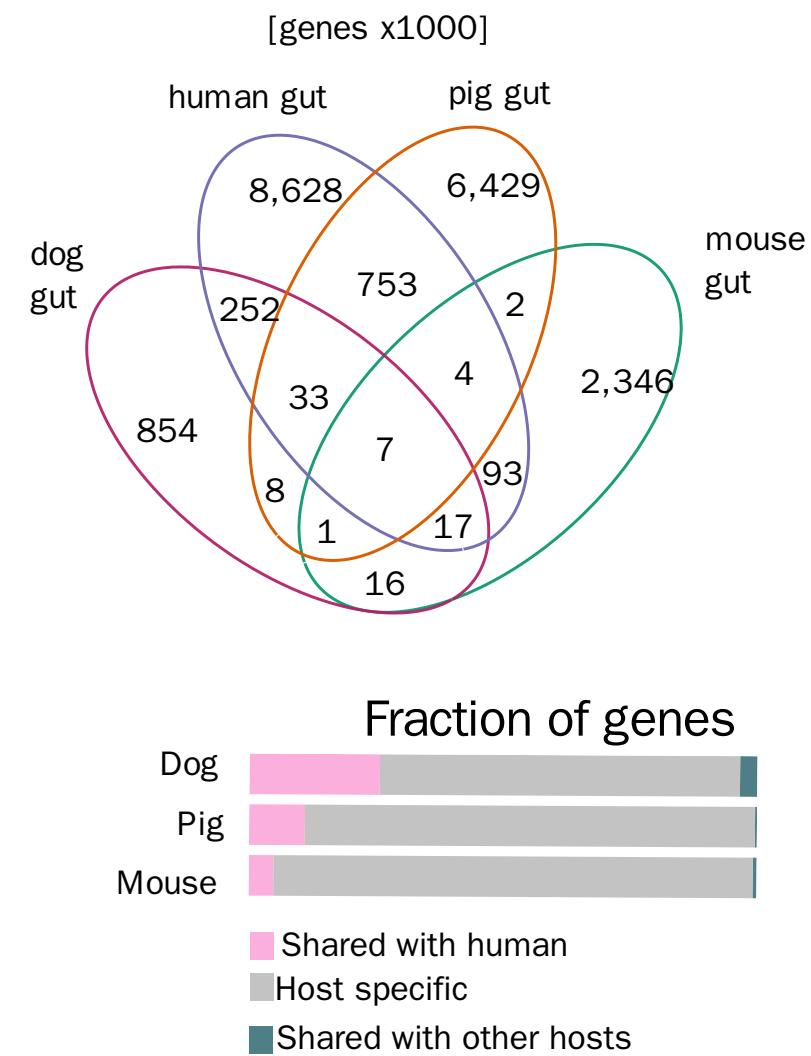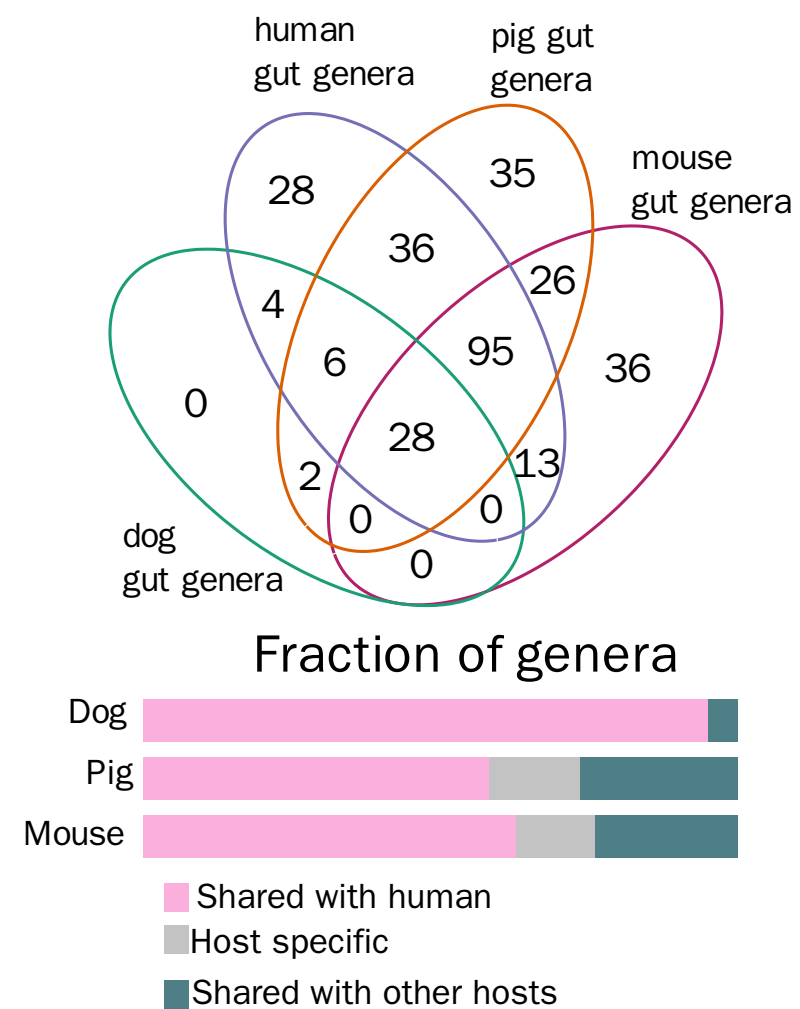
Profiled with metaSNV
(Costea*, Munch*, et al., Plos One, 2017)

- Genera (and even species) are widely shared
- Strains are host specific

THE DOGS WERE SUBJECT TO A RANDOMIZED DIETARY INTERVENTION

- There is an overall community shift; larger for the HPLC diet
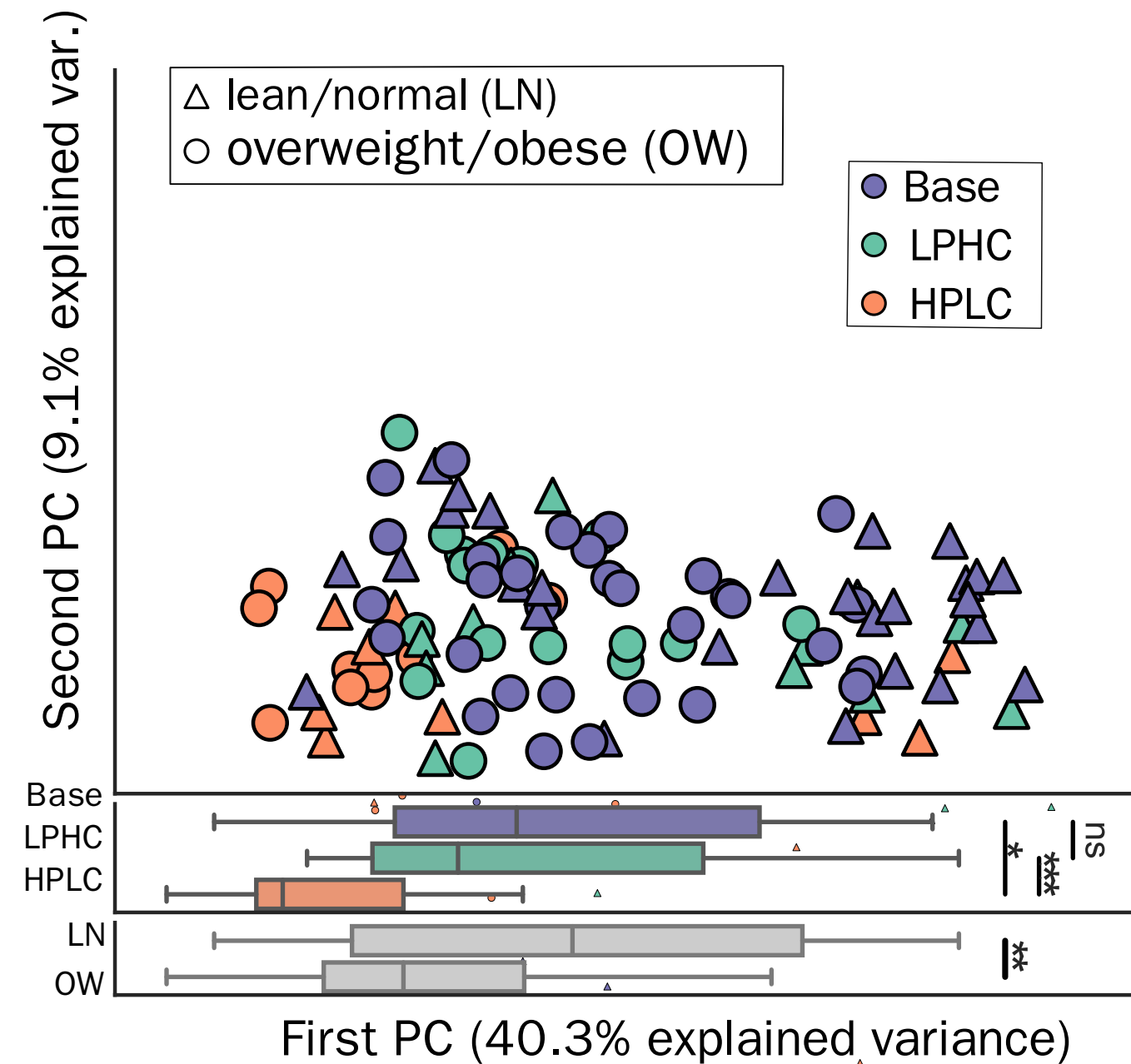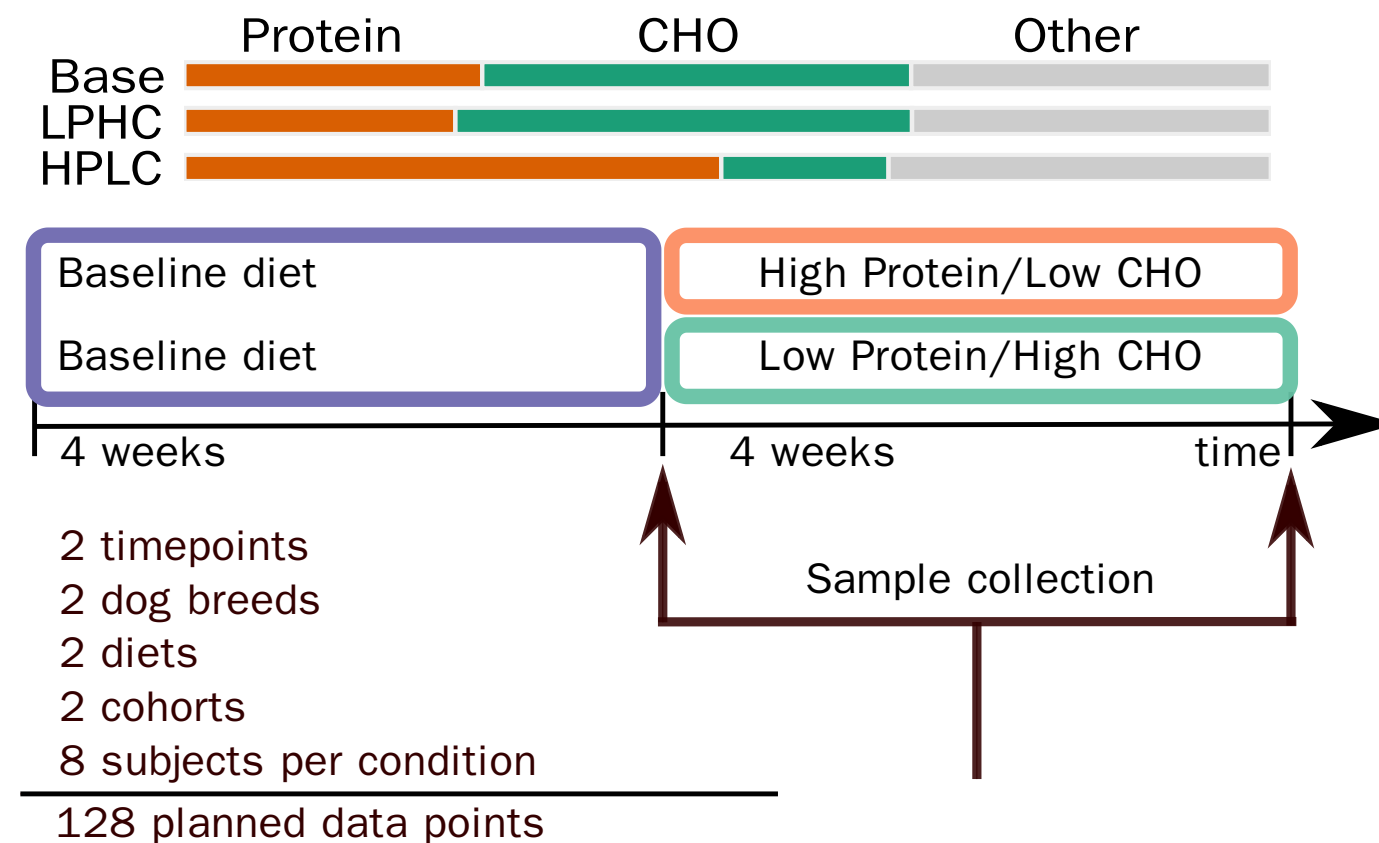
THE MICROBIOME OF OBESE DOGS **SHIFTS MORE**

Because we had two samples (pre- and post-intervention), we *compared the two samples from the same dog.*
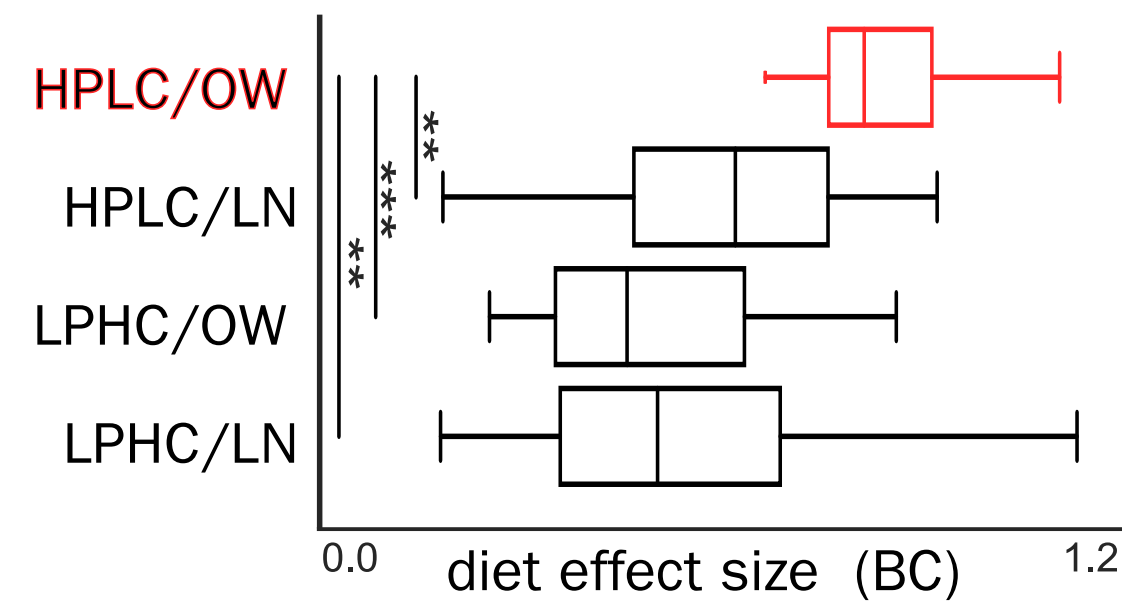
LACTOBACILLUS RUMINIS DISAPPEARS IN THE HLPC DIET

Taxa showing statistically significant prevalence changes

Legend: HPLC, LPHC
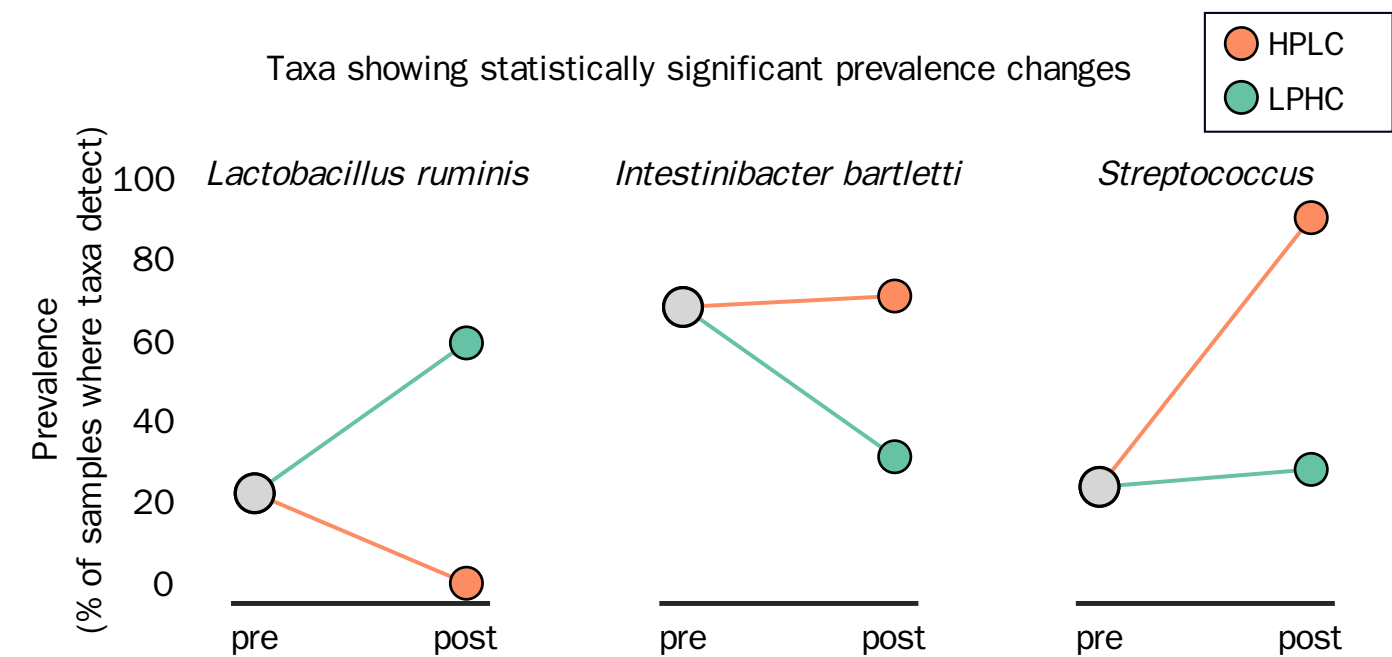
- In the HPLC diet, *L. ruminis* cannot be detected.
- *Streptoccocus* species are almost universally prevalent in the HPLC diet, but only detected in <⅓ of LPHC-fed dogs.
- This happened in **both cohorts**, so it is not a effect of direct transmission.
- Demonstrates the possibilities of *prebiotics* in a controlled study.

Ongoing work (since 2017): more habitats, more data

- Human gut (>7,000 samples, many projects).
- Mouse gut (230 samples, Xiao et al., Nat Biotech, 2015).
- Pig gut (195 Xiao et al., Nat Micro, 2016).
- Dog gut (129 samples, Coelho et al., Microbiome, 2018).
- Kittens gut (124 samples, Deusch et al., PlOS One, 2014; Deusch et al., PlOS One, 2015).

Reworked data from scratch *using a consistent methodology*.

THE CAT MICROBIOME SHARES A LOT OF SPECIES WITH HUMANS
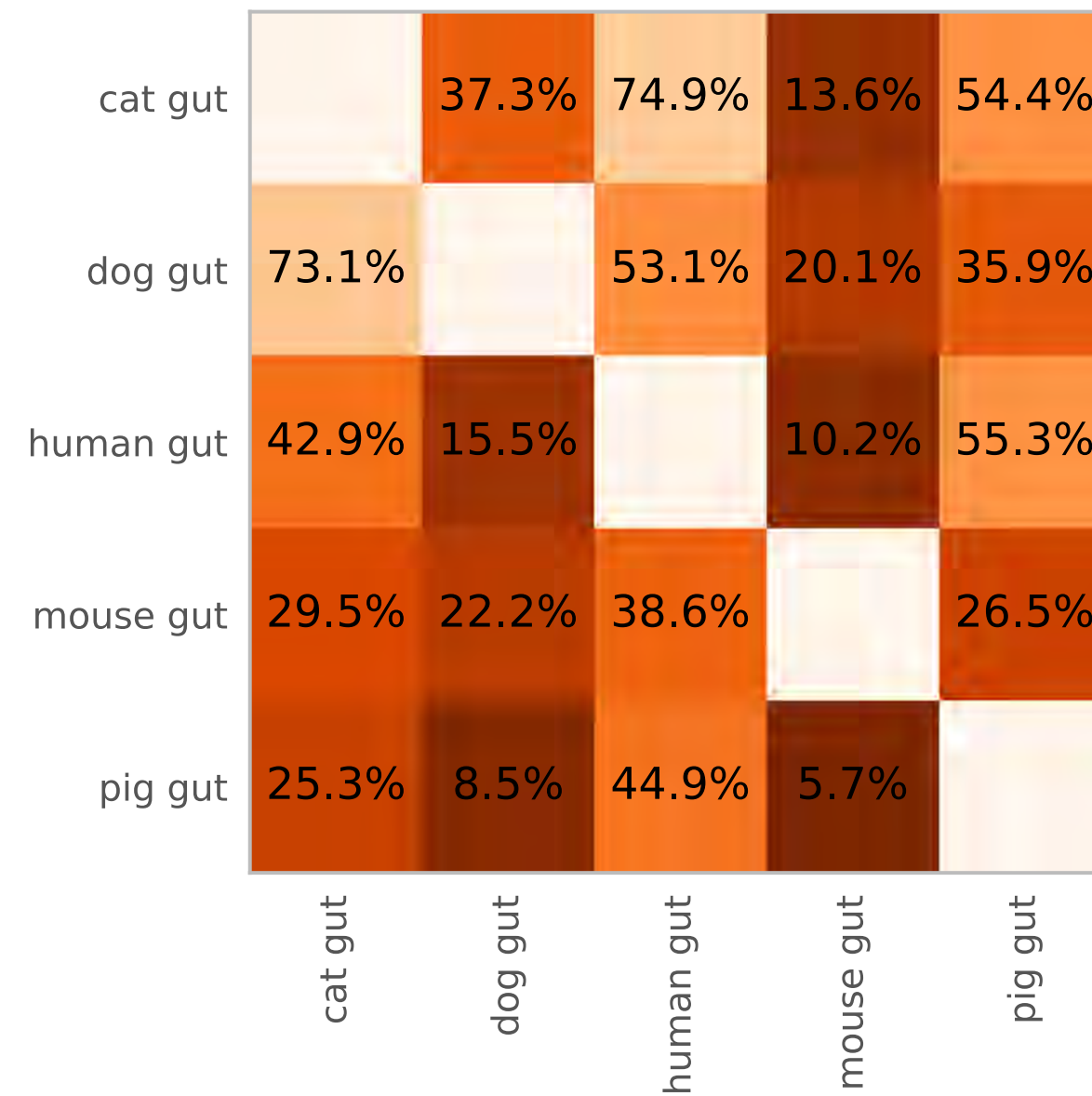
Fraction of species shared between gut microbiomes (using a single-copy marker genes as proxies for species).

Both the dog and the cat microbiome contain human-related genes
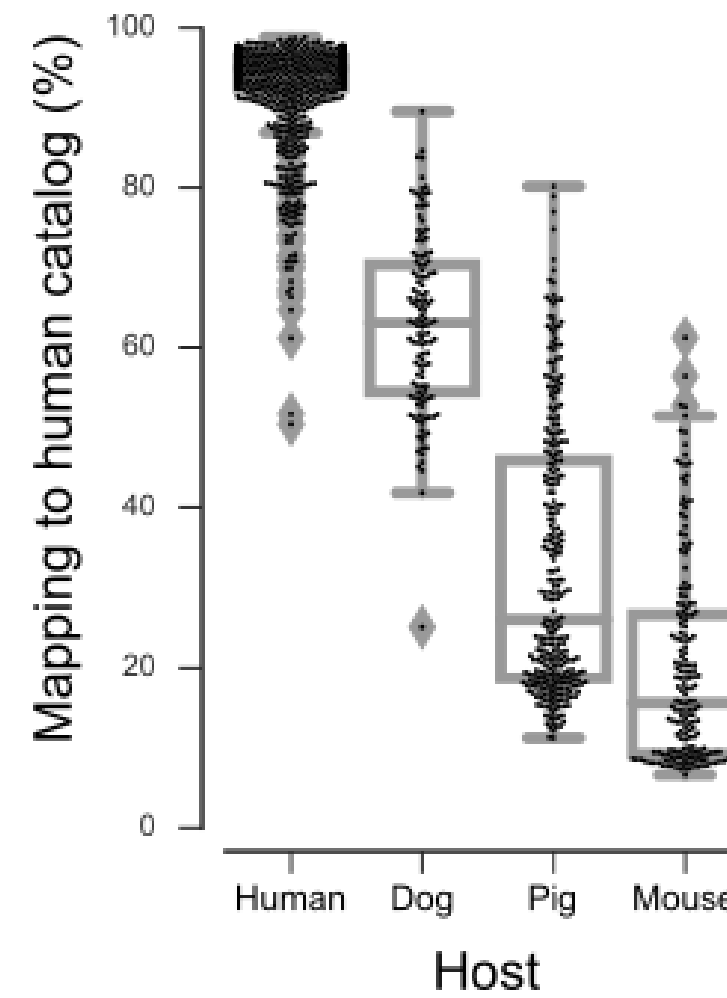
(This is the published figure in 2018)

BOTH THE DOG AND THE CAT MICROBIOME CONTAIN HUMAN-RELATED GENES
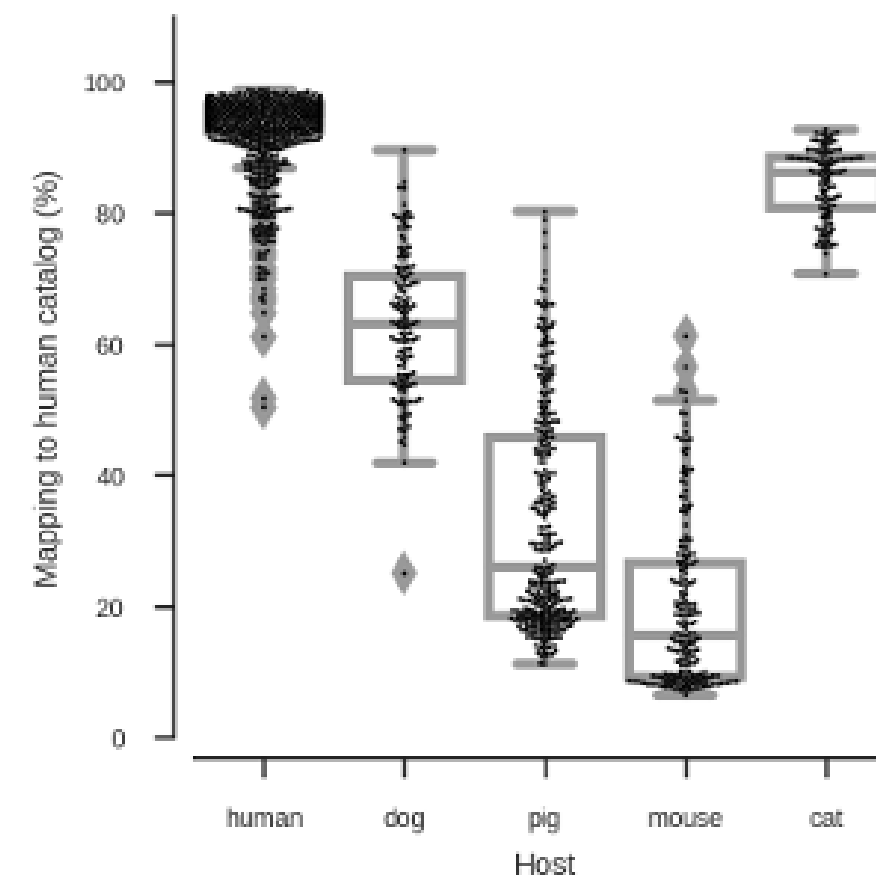
(This now includes the cat data)

Genes are shared between environments (traveling genes)
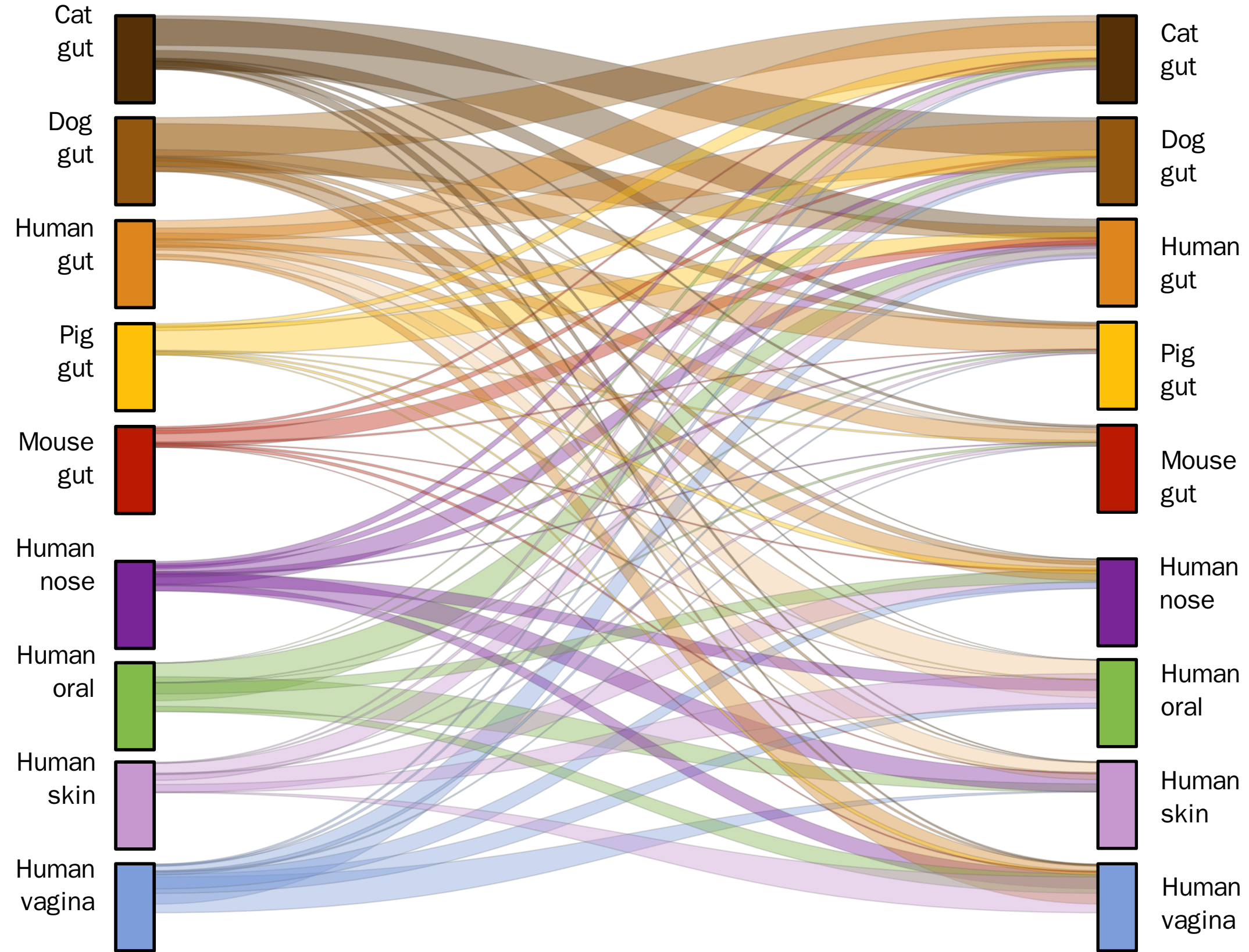
AMR GENES ARE MORE LIKELY TO TRAVEL
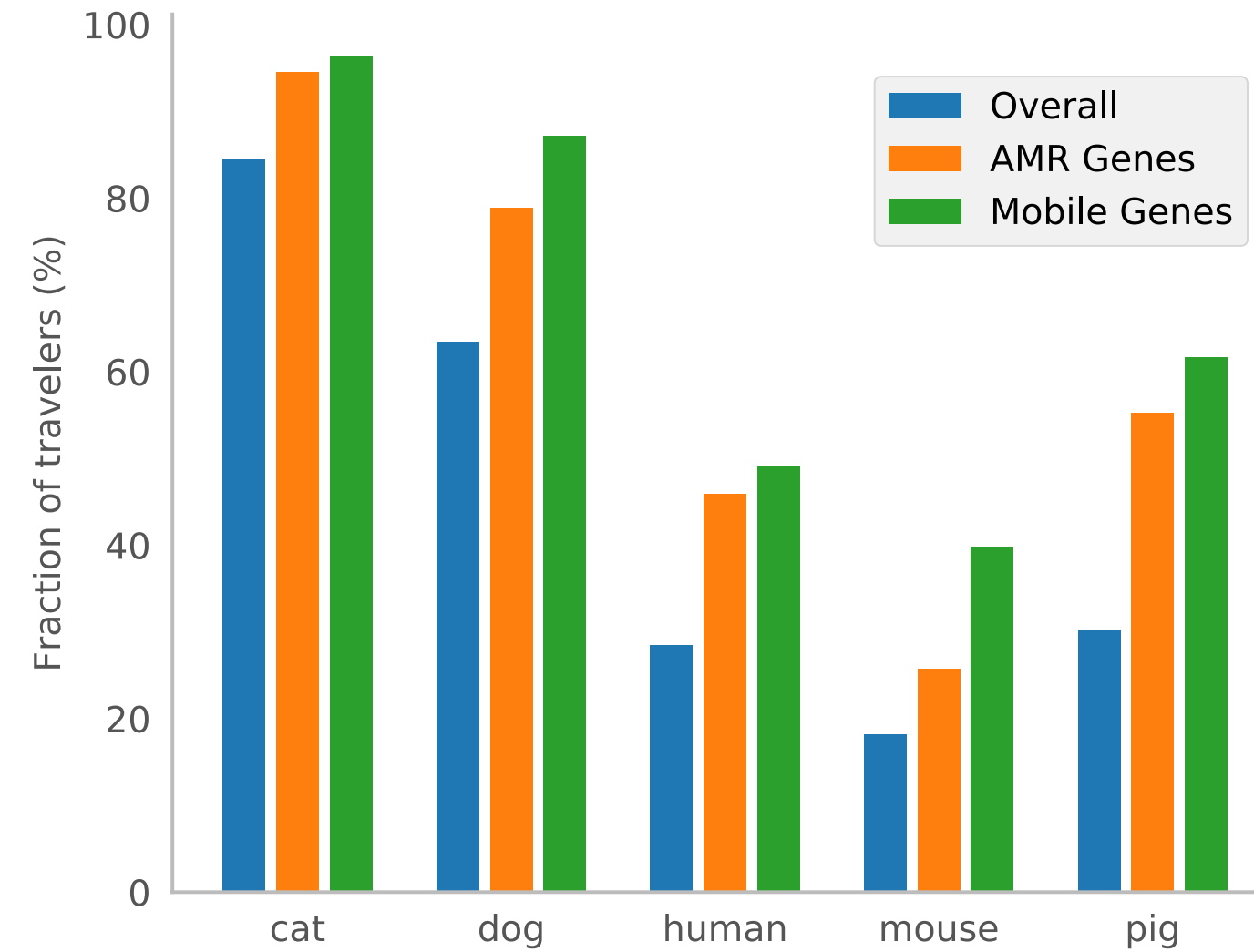
There are millions of genes represented in each bar, all comparisons are highly significant.

SUMMARY

- The dog and cat microbiomes are *similar to that of humans* (although a gap remains).
- Thus, we can use pet studies to formulate hypothesis on how the microbiome may work in humans.
- Pet studies provide a triple-benefit:
  1. good for pets (which is very valuable in itself),
  2. possibly translatable to humans,
  3. how we treat animals may rebound to human health (One Health) as **genes travel and AMR genes travel the most**.
- Our animal data is still very limited (one or two populations).

Acknowledgements

Thank You

JOIN US IN SHANGHAI!

Several postdoc positions open: http://big-data-biology.org/positions/
Talk to meet during the break
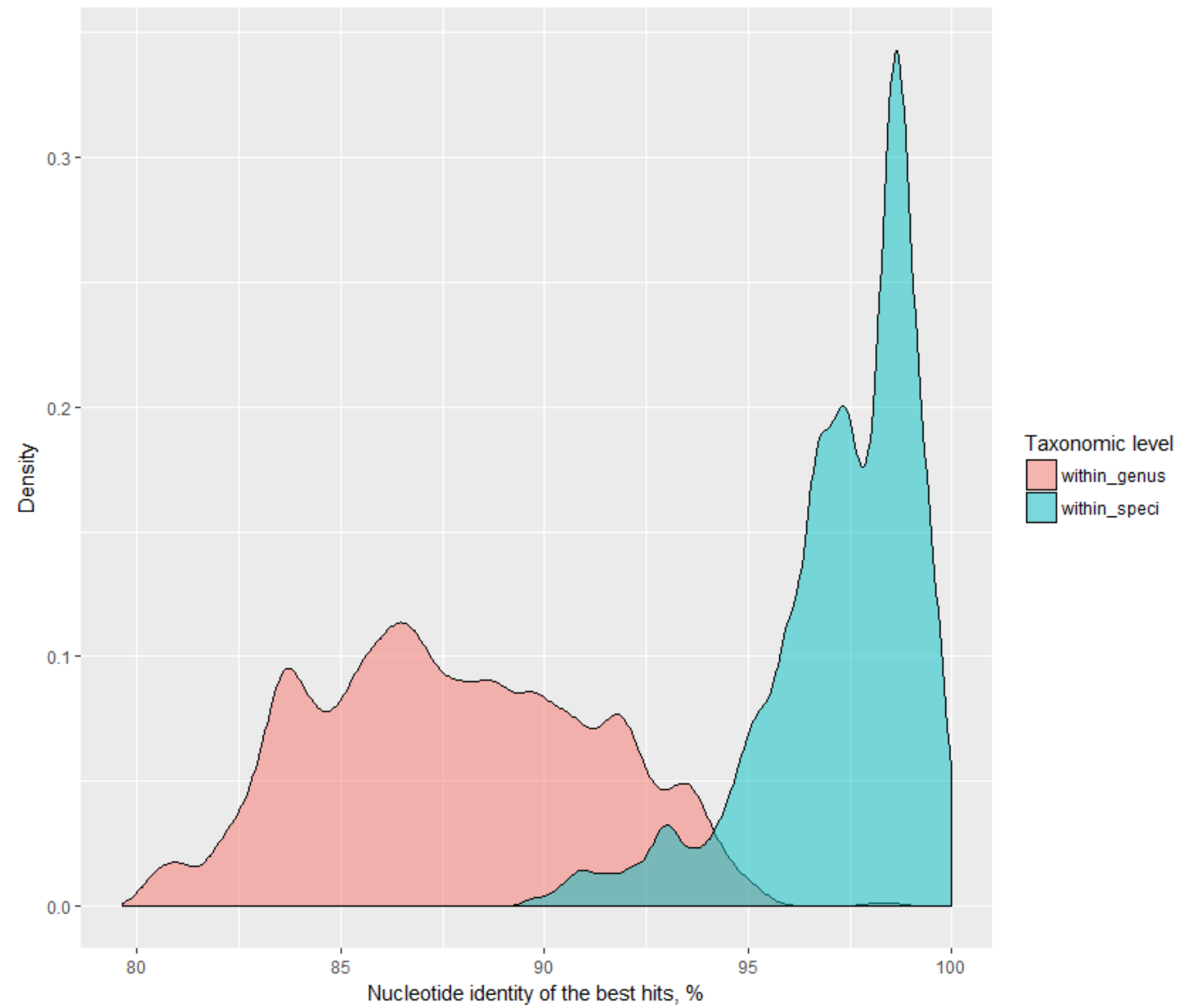
Prevalence changes are seen in **BOTH EXPERIMENTAL COHORTS**
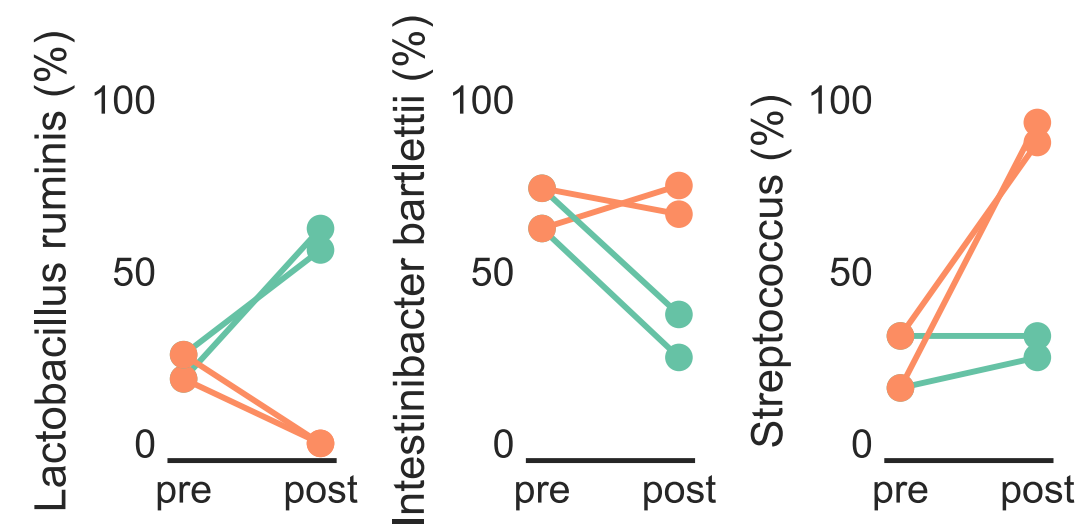
Gene flow (abundance weighted) between mammalian guts

	cat gut	dog gut	human gut	mouse gut	pig gut
cat gut	92.6	45.5	22.6	3.04	4.67
dog gut	34.5	94.0	14.5	6.90	4.80
human gut	58.0	55.1	99.8	20.0	34.7
mouse gut	1.70	2.55	8.97	97.6	0.90
pig gut	20.3	19.3	16.5	2.24	99.0

TRAVELING GENES ARE MORE LIKELY TO BE AMR (MOBILE) GENES
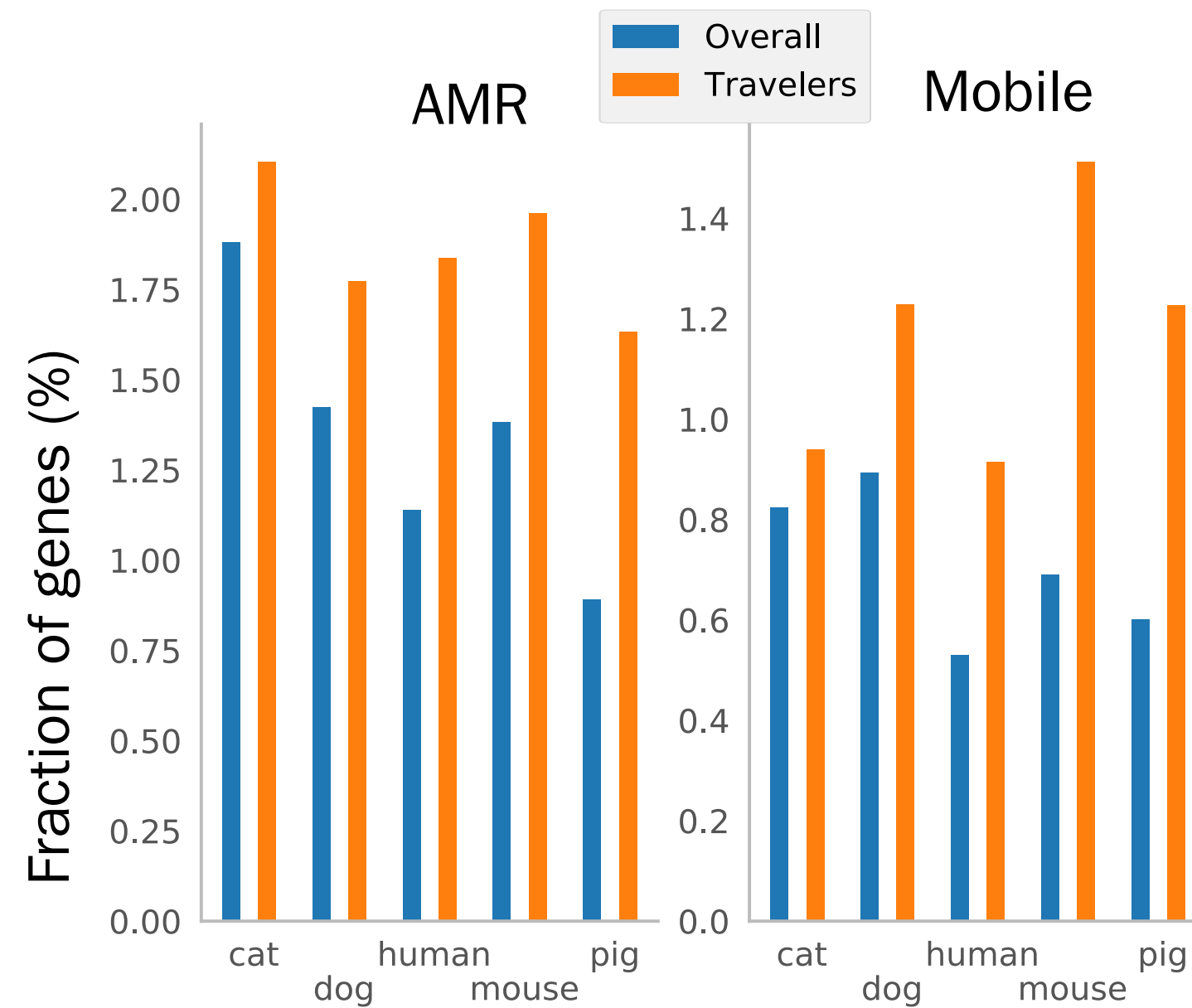
There are millions of genes represented in each bar, all comparisons are highly significant.

The economic drivers for One Health approaches and predictive modelling approaches to altering the usage patterns of antimicrobials in clinical practice

Lloyd Reeve-Johnson

Professor of One Health, Faculty of Science, Health, Education and Engineering, University of Sunshine Coast, Queensland, Australia

The 'One Health' approach implies multiple stakeholders with different perceptions of utility interacting daily in the highly complex system of healthcare delivery. As we adopt increasingly holistic views to major health care challenges, "complexity economics" principles include integrating change in the interaction between disease reservoirs, antimicrobial resistance patterns in multiple species , environmental change, political and economic challenges to sustainability of health services and media generated perc eptions to name a few. One Health approaches with successive iterations continue to evolve to ever greater complexity of interaction between human health, veterinary health, plant and environmental factors which in turn are continually impacted by the wider issues of politico-economic stability and consumer perception. The interplay between social, psychological, environmental and physical determinants of health mean that for health literacy to improve, it has to be targeted to cater to messages that are easily communicated, disease signs that are easily recognisable and messages that are contiguous with belief systems and perceptions that may not relate directly to the current healthcare issue.

A decision model is illustrated showing typical clinical decisions made in delivery of a prescription antibiotic agent. A reductionist approach is also discussed identifying the key stakeholders in healthcare delivery and general perspectives that each may have when interacting or making healthcare-related decisions. Probability interactions and game theory approaches are increasingly used to predict demand and to influence outcomes and cost with real-time dynamic predictive models.

AMR and Clostridioides (Clostridium) difficile from different reservoirs

Prof. dr. Maja Rupnik

National laboratory for health, environment and food, NLZOH, Maribor, Slovenia
and
University of Maribor, Faculty of Medicine, Maribor, Slovenia

maja.rupnik@nlzoh.si

Clostridium difficile and CDI (*C. difficile* infection)
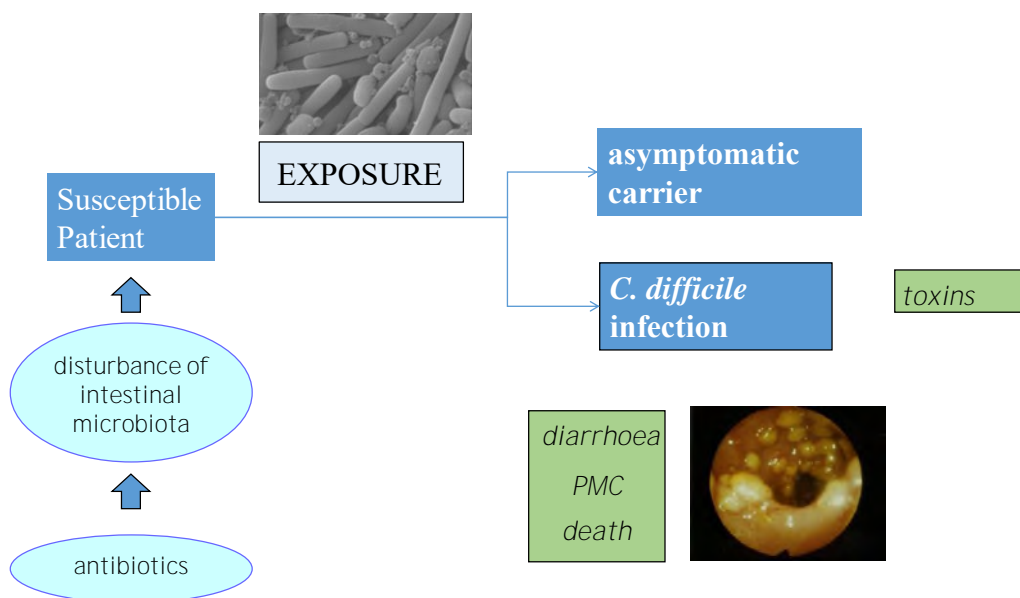
C. difficile – transmissions between reservoirs and typing

- UBIQUITOUS
- natural reservoir - gut of young individuals
- HOSPITALS

RIBOTYING – ribotypes 001, 002…

WGS - MLST – ST (1, 2….)
SNV

Clostridium difficile in food and environment

K. Warriner et al.

Warriner et al., J Appl Micro, 2016

Slide 1

Advances in Experimental Medicine and Biology 1050
Advances in Microbiology, Infectious Diseases and Public Health

Paola Mastrantonio
Maja Rupnik *Editors*

Updates on Clostridium difficile in Europe

Advances in Microbiology, Infectious Diseases and Public Health Volume 8

Springer

Non-human *C. difficile* Reservoirs and Sources: Animals, Food, Environment

Cristina Rodriguez Diaz, Christian Seyboldt, and Maja Rupnik

Slide 2

C. difficile and animals

- any animal species can be colonized
- young animals
- different sensitivity for infection
- microbiological diagnostic rarely done

- variability at farm
  - single type (pigs, dairy farms)
  - multiple types (poultry, veal production)

- importance (for human CDI)
  - farm animals
  - pets

Warriner et al., J Appl Microbiol. 2017
Rodriguez et al., Adv Exp Med Biol, 2016
Bloomfield and Riley, Infect Dis Ther, 2016
Bauer and Kuijper, Infect Dis Clin North Am., 2015
Rodriguez-Palacious et al. Anim Health Res Rev, 2013
Weese, CMI, 2010
Hansgens et al., CMI 2012

Humans
N isolates: 1310
N ribotypes: 96

44

13   5

34

40   4   8

Environment
N isolates: 753
N ribotypes: 91

Animals
N isolates: 522
N ribotypes: 51

Clostridium difficile **Infection in Humans and Piglets: A 'One Health' Opportunity**

Michele M. Squire and Thomas V. Riley

Antibiotic use in animal production
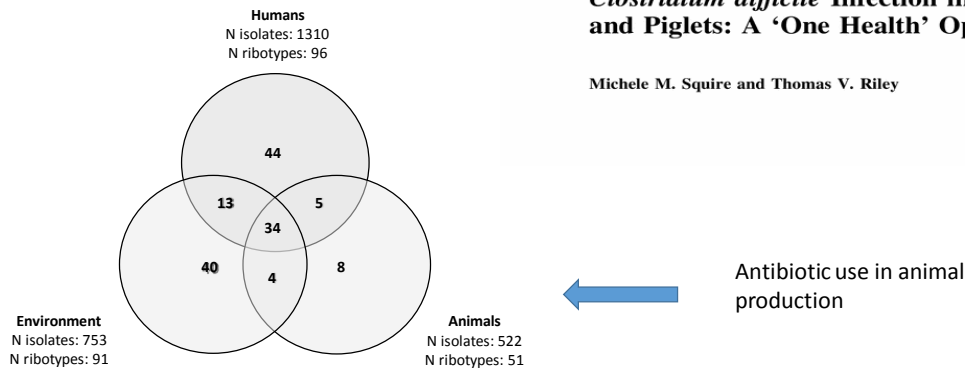
Substantial overlap between PCR ribotypes – humans, animals, food - Europe

Overall ribotype prevalence

- 027 (19%)
- 001/072 (11%)
- 014/020 (10%)
- 140 (4%)
- 002 (4%)
- 010 (3%)
- 078 (3%)
- 018 (3%)
- 015 (2%)
- 176 (2%)
- Other (n = 484)
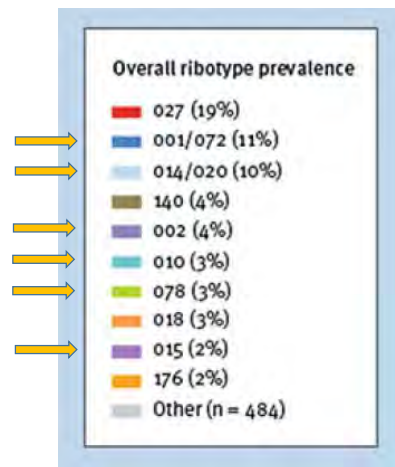
MOST PREVALENT PCR RIBOTYPES
Davies et al., Eurosurveill, 2016; Rodriguez Diaz, AIMI, 2018
Rodriguez-Palacios et al. Aim Health Res Rev, 2013

C. difficile and antibiotic resistance

AB for treating CDI	AB associated with CDI risk
• metronidazol • vankomycin • fidaxomicin	• different groups
• no or very low resistance	• high resistance • MDR • Acessory genome (transposons, prophages)

C. difficile and antibiotic resistance – selective advantage
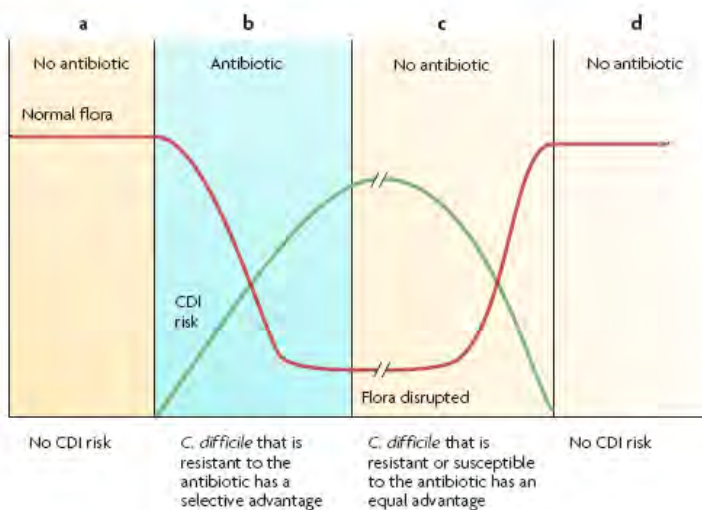
MAIN OUTBREAK TYPES

Before 2003

Clindamycin resistance

After 2003

Fluoroquinolone resistance

Table 1 Antibiotic susceptibility of *C. difficile* clinical isolates as reported in 46 papers published between 2012 and 2017

Antibiotic[a]	Number of strains analyzed		Number of resistant strains	% of resistance
CFs				
	CTT	212	24	11.2
	FOX	423	404	95.5
	CRO	1252	393	31.4
	CTX	95	95	100
	CAZ	86	65	76.0
MLS_B				
	ERY	2316	1138	49.1
	CLI	5839	2982	51.1
FQs				
	CIP	1326	1312	99.0
	MXF	6053	2161	35.7
	GAT	199	136	68.3
MTZ		6724	114	1.7
VAN		5760	134	2.3
RIF		3450	525	15.2

[a]*CFs* cephalosporins, *CTT* cefotetan, *FOX* cefoxitin, *CRO* ceftriaxone, *CTX* cefotaxime, *CAZ* ceftazidime, *MLS_B* macrolide-lincosamide-streptogramin B, *ERY* erythromycin, *CLI* clindamycin, *FQs* fluoroquinolones, *CIP* ciprofloxacin, *MXF* moxifloxacin, *GAT* gatifloxacin, *MTZ* metronidazole, *VAN* vancomycin, *RIF* rifampin

Spigaglia et al., 2018, AIMI

Table 3 Antibiotic susceptibility patterns most frequently observed in MDR *C. difficile* clinical isolates as reported in 19 papers published between 2012 and 2017

Year of publication	Number of strains analyzed	% of MDR strains	Main antibiotic susceptibility patterns (n. of strains)[a]										PCR-ribotype	References
2015	525	66	ERY	CLI	MXF	GAT						(85)	018, 369	Lachowicz et al. (2015), Senoh et al. (2015), Kuwata et al. (2015), Spigaglia et al. (2015), Krutova et al. (2015) and Shayganmehr et al. (2015)
			CLI	CIP	CRO							(51)	DTM	
			ERY	CLI	MXF	RIF						(48)	018, 027, 356/607	
			ERY	MXF	CIP	IMP						(34)	027	
			CIP	CAZ	IMP	AMK						(25)	nd	
			ERY	CLI	MXF	CIP	IMP					(20)	176	
			ERY	MXF	RIF							(15)	027	
			CIP	CAZ	IMP							(14)	nd	
			ERY	MXF	CIP	RIF						(13)	176	
			ERY	CLI	GAT							(11)	018, 369	
			ERY	CLI	MXF							(11)	046, 078, 126	
			CIP	CAZ	AMK							(10)	nd	
2017	276	62	ERY	MXF	RIF							(81)	017	Alvarez-Perez et al. (2017), Kullin et al. (2017) and Ramírez-Vargas et al. (2017)
			CLI	MXF	CIP	LVX	RIF	TET	CHL	TGC	LZD	(12)	012	
			CLI	MXF	CIP	LVX	RIF					(12)	012	
			ERY	MXF	LVX	TET						(5)	078, 126	
			ERY	LVX	TET							(5)	078, 126	
			CLI	MXF	CIP	LVX	TET	CHL	LZD			(4)	012	
			MXF	LVX	TET							(4)	078, 126	
			TET	LVX	ETP							(4)	126	

Spigaglia et al., 2018, AIMI

Original article

The ClosER study: results from a three-year pan-European longitudinal surveillance of antibiotic resistance among prevalent Clostridium difficile ribotypes, 2011–2014

J. Freeman [1,2,*], J. Vernon [2], S. Pilling [2], K. Morris [1], S. Nicholson [2], S. Shearman [2], C. Longshaw [3], M.H. Wilcox [1,2], the Pan-European Longitudinal Surveillance of Antibiotic Resistance among Prevalent Clostridium difficile Ribotypes Study Group

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The ClosER study: results from a three-year pan-European longitudinal surveillance of antibiotic resistance among prevalent Clostridium difficile ribotypes, 2011–2014

Table 4
Proportions of sensitive, intermediately sensitive and resistant Clostridium difficile isolates in the 3 years of the ClosER study (July 2011 to July 2014)

	Years	M	V	FDX	RIF	MXF	CLINDA	IMI	CHLOR	TIG
Sensitive (%)	Y1	97.9	96.7	100.0	80.5	58.7	37.6	62.7	92.9	100.0
	Y2	98.1	98.8	100.0	82.1	64.5	29.1	77.3	93.1	100.0
	Y3	96.9	99.8	100.0	86.8	66.0	18.3	78.1	91.5	100.0
	All years	97.9	98.6	100.0	83.2	63.1	29.0	72.6	92.8	100.0
Intermediately sensitive (%)	Y1	2.0	2.4		6.0	1.8	12.4	30.1	3.5	
	Y2	1.3	0.6		3.7	1.0	13.7	19.7	2.6	
	Y3	2.6	0.1		1.5	0.5	17.4	19.7	5.1	
	All years	1.9	1.1		3.9	1.1	14.4	23.3	3.7	
Resistant (%)	Y1	0.1	0.9		13.5	39.5	49.8	7.2	3.6	
	Y2	0.1			13.7	34.1	56.7	2.3	3.7	
	Y3	0.5	0.1		11.6	33.5	64.3	2.2	3.4	
	All years	0.2	0.1		13.0	35.8	56.6	4.0	3.6	

Abbreviations: CHLOR, chloramphenicol; CLINDA, clindamycin; FDX, fidaxomicin; IMI, imipenem; M, metronidazole; MXF, moxifloxacin; RIF, rifampicin; TIG, tigecycline; V, vancomycin.

Pirs et al.,   Krutova et al.,   Spigaglia et al., 2018, AIMI

C. difficile – reservoir overlap

2008-2010

Humans
N isolates: 601
N ribotypes: 77

42

16   8

11

9   0   4

Environment
N isolates: 81
N ribotypes: 36

Animals
N isolates: 104
N ribotypes:23

2008-2014

Humans
N isolates: 1310
N ribotypes: 96

44

13   5

34

40   4   8

Environment
N isolates: 753
N ribotypes: 91

Animals
N isolates: 522
N ribotypes: 51
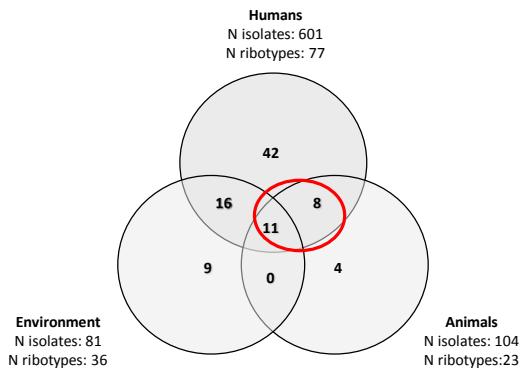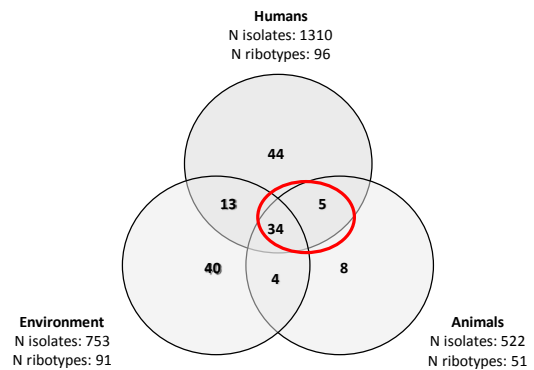
Janezic et al., BMC Microbiol, 2012

Janezic and Rupnik, unpublished

HUMAN STRAINS

ANIMAL STRAINS

Ribotypes
078
126
237
281
288
033

(a) MXF   (b) MXF

MXF

TET   TET

TET

CLI   CLI

CLI

ERY   ERY

ERY
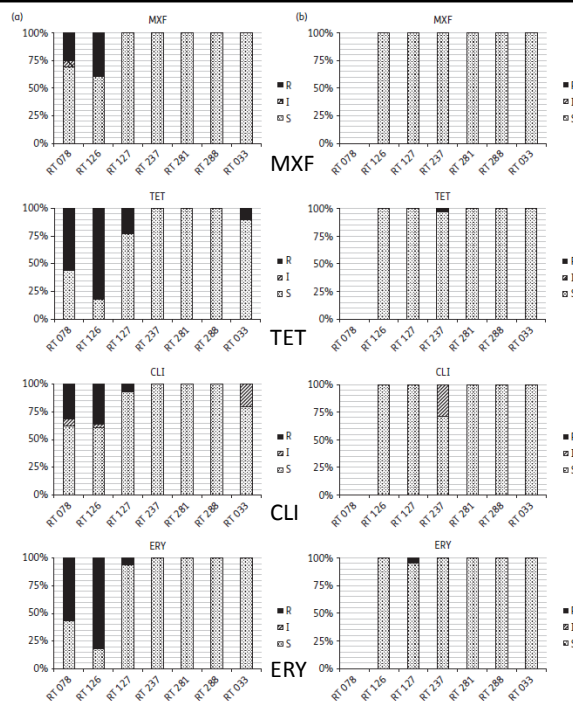
Susceptible phenotype more likely to be shared than resistant phenotype

Knight & Riley, JAC, 2016
Pirs et al., JMM, 2013
Krutova et al., IJMM, 2018

WGS – clonal relationship of animal and human strains

PCR ribotype 014
Australia
PORCINE AND HUMAN STRAINS

Knight et al., Front Microbiol, 2017

- No geographical or temporal clustering
- Persistent community reservoir with long-range dissemination
  piggery effluent recycled in agriculture
  compost used in community settings

Δ SNVs
0-2
3-4
5-19
20-49
≥50
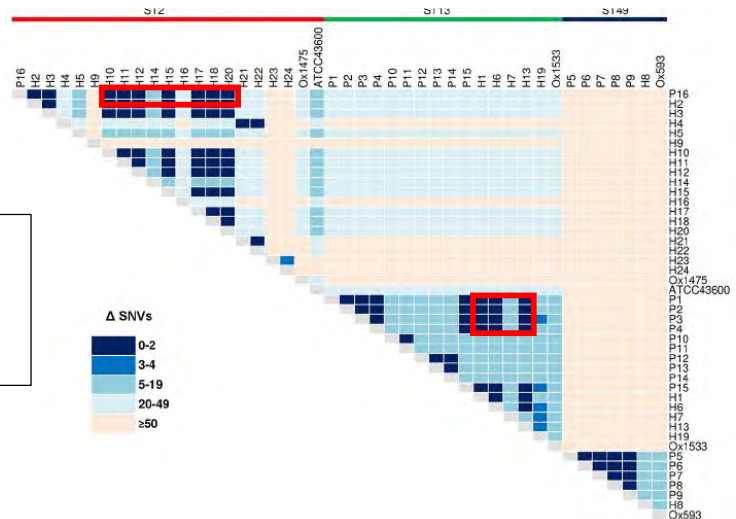
0-2 SNP recent clonal transmission
>10 SNPs are defined for genetically distant strains
(Eyre *et al*. N Engl J Med 2013;369:1195-205)

Agent	Human RT014 (n = 24)[†]		Porcine RT014 (n = 16)		P-value[‡]
	%S	%NS	%S	%NS	
VAN[a]	100	0	100	0	p > 0.05
MTZ[b]	100	0	100	0	p > 0.05
FDX[c]	100	0	100	0	p > 0.05
RFX[d]	100	0	100	0	p > 0.05
AMC[b]	100	0	100	0	p > 0.05
CLI[b]	88	12	31	69	**p < 0.05**
ERY[b]	96	4	31	69	**p < 0.05**
CRO[b]	79	21	81	19	p > 0.05
MEM[b]	100	0	100	0	**p < 0.05**
MXF[b]	100	0	100	0	**p < 0.05**
TET[b]	100	0	31	69	**p < 0.0001**
TZP[b]	100	0	100	0	p > 0.05
TMP	NR	NR	NR	NR	p > 0.05
GEN	NR	NR	NR	NR	p > 0.05
TOB	NR	NR	NR	NR	p > 0.05
SPC	NR	NR	NR	NR	p > 0.05

VAN, vancomycin; MTZ, metronidazole; FDX, fidaxomicin; RFX, rifaximin; AMC, amoxicillin-clavulanate; CLI, clindamycin; ERY, erythromycin; CRO, ceftriaxone; MEM, meropenem; MXF, moxifloxacin; TET, tetracycline; TZP, piperacillin-tazobactam; TMP, trimethoprim, GEN, gentamicin; TOB, tobramycin; SPC, spectinomycin; S, susceptible; NS non-susceptible (intermediate and resistant breakpoints).

WGS – global epidemiology of 078 strains
Repeated international transmissions
Bidirectional spread between humans and animals

PCR ribotype 078
22 countries
 (N America, Europe, Australia)

Knetsch et al., J Clin Micro, 2018

Legend

Host
- Human
- Animal
- Food
- Environment

Continent
- Europe
- North America
- Asia
- Australia

Tree scale: 0.001
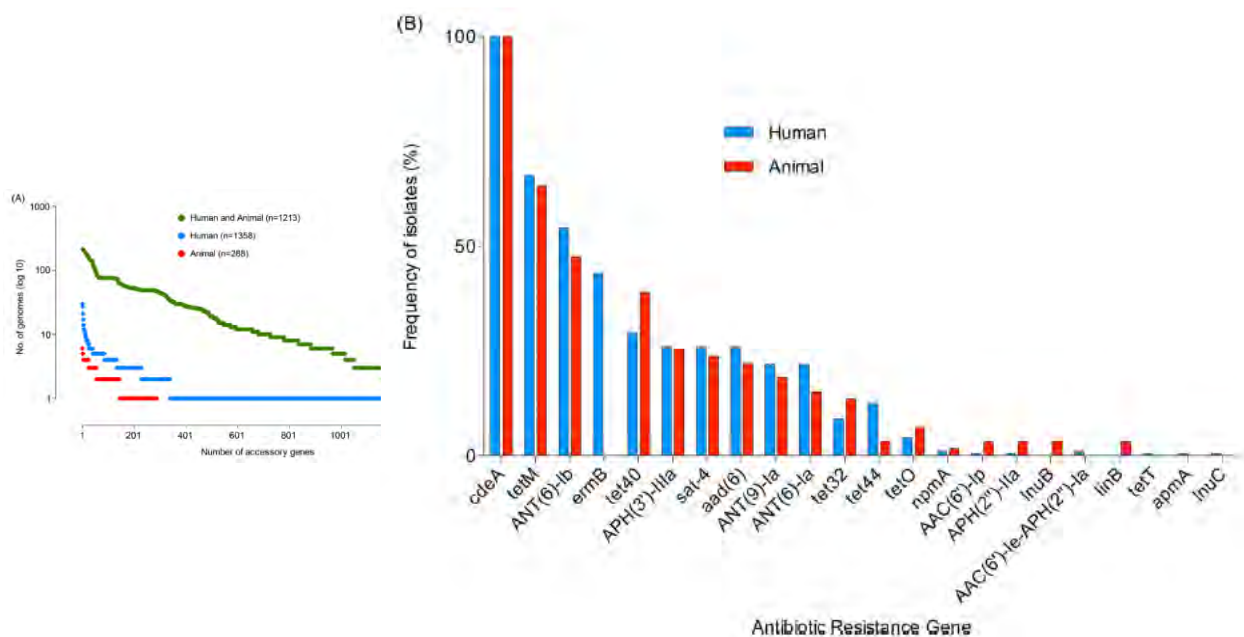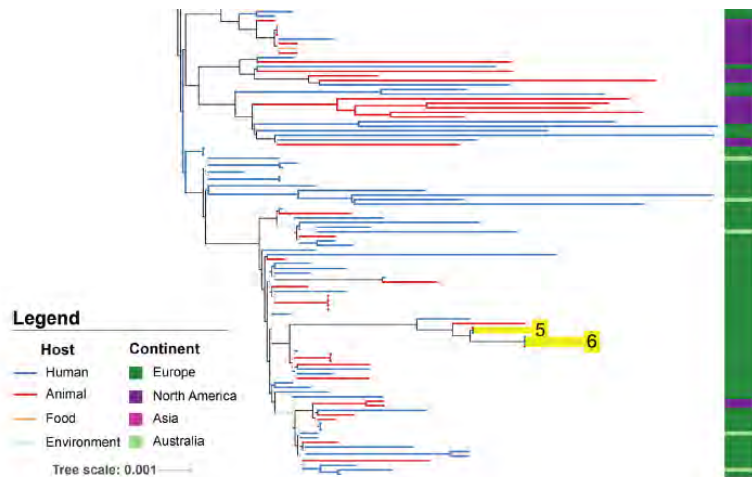
TABLE 2 Distribution of isolated *C. difficile* strains in positive animals at different sampling points[a]

Description of isolate(s) at sampling point (calf age) [strain-toxinotype/PCR ribotype/PFGE type/type of *tet*(M) determinant-antibiotic resistance]

Calf	1 (14 days)	2 (18 days)	3 (25 days)	4 (32 days)	5 (46 days)
A			S21-V/126/C4a/*tet*(M)12-Erm[r], S22-V/126/C4a/*tet*(M)12-Tet[r] Erm[r]		
B		S36-tox−/010/C12-Erm[r], S5-tox−/010/nt-Erm[r]			
C		S14-V/126/C4a/*tet*(M)12-Tet[r] Erm[r], S15-V/126/C4a/*tet*(M)11-Tet[r] Erm[r]			
D	S34-XI/033/C3/*tet*(M)9-Tet[r], S1-V/045/C8, S35-V/045/C8	S6-V/045/C8			
E	S12-V/078/C5	S7-V/078/C5, S16-V/078/C5			
F	S2-XI/033/C2/*tet*(M)10-Tet[r]				
G	S3-XI/033/C1/*tet*(M)11				
H			S13-0/012/C7/*tet*(M)8-Tet[r] Erm[r] Dc[r]	S9-0/012/C7/*tet*(M)8-Tet[r] Erm[r] Dc[r], S38-0/012/C7/*tet*(M)8-Tet[r] Erm[r] Dc[r], S10-V/126/C10/*tet*(M)7-Tet[r] Erm[r]	
I			S23-V/078/C4b/*tet*(M)12-Tet[r] Erm[r], S24-V/078/C4b/*tet*(M)11-Tet[r] Erm[r]		
J		S37-V/045/C8			
K		S17-V/078/C4b/*tet*(M)12-Tet[r] Erm[r]			
L		S18-V/126/C10/*tet*(M)7-Tet[r] Erm[r], S19-V/126/C10/*tet*(M)7-Erm[r]			
M			S25-V/126/C6/*tet*(M)7-Tet[r] Erm[r]		S11-V/126/C6/*tet*(M)7-Tet[r] Erm[r], S31-V/126/C6/*tet*(M)7-Tet[r] Erm[r], S32-V/126/C6/*tet*(M)7-Tet[r] Erm[r], S33-V/126/C6/*tet*(M)7-Tet[r] Erm[r]
N			S26-V/126/C6/*tet*(M)7-Tet[r] Erm[r], S29-V/126/C11/*tet*(M)7-Tet[r] Erm[r], S27-V/078/C9/*tet*(M)12-Tet[r] Erm[r], S28-V/078/C9/*tet*(M)12-Tet[r] Erm[r]		
O	S4-V/078/C4b/*tet*(M)12-Tet[r] Erm[r]	S20-V/078/C4b/*tet*(M)12-Erm[r]	S8-V/078/C4b/*tet*(M)12-Tet[r] Erm[r], S30-V/078/C4b/*tet*(M)12-Tet[r] Erm[r]		

[a] The following antibiotic treatments were given: at 19 days of age, enrofloxacin; at 21 to 31 days of age, colistin plus amoxicillin; at 27 to 32 days of age, doxycycline; and at 27 to 34 days of age, tylosin. If the animal had diarrhea at the time of sampling, the cell within a table is shadowed. nt, this strain was not typeable by PFGE.

Zidaric et al., AEM, 2012

C. difficile – environmental strains

2008-2010

Humans
N isolates: 601
N ribotypes: 77
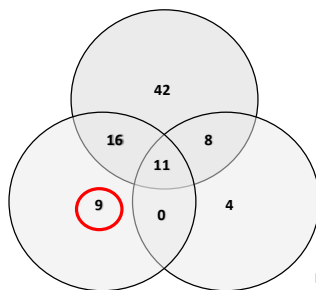
42

16   8

11

9   0   4

Environment
N isolates: 81
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Animals
N isolates: 104
N ribotypes:23

2008-2014

Humans
N isolates: 1310
N ribotypes: 96

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13   5

34

40   4   8

Environment
N isolates: 753
N ribotypes: 91

Animals
N isolates: 522
N ribotypes: 51

Janezic et al., BMC Microbiol, 2012

Janezic and Rupnik, unpublished

11

C. difficile environmental strains can be unusual

- human isolates
- animal isolates
- environmental isolates

- distinct and highly divergent clades
- atypical insertions replacing the PaLoc
- atypical PaLoc only *tcdA* or only *tcdB* at atypical location
- divergent sequences of *tcd*A, *tcd*B, *cdt*
- phage encoded binary toxin genes

- environmental isolates
- few human isolates (CDI patients)

- environmental isolates (soil, rural areas)

Reduced susceptibility:
imipenem, erythromycin, tetracycline

clade 1
clade 2
clade 3
clade 4
clade 5
clade C-I
clade C-II

Elliott *et al.*, 2009; Dingle *et al.*, 2014; Monot *et al.*, 2015, Janezic *et al.*, 2016; Riedel et al., 2017
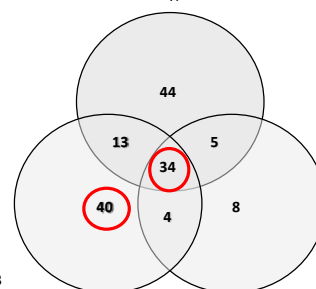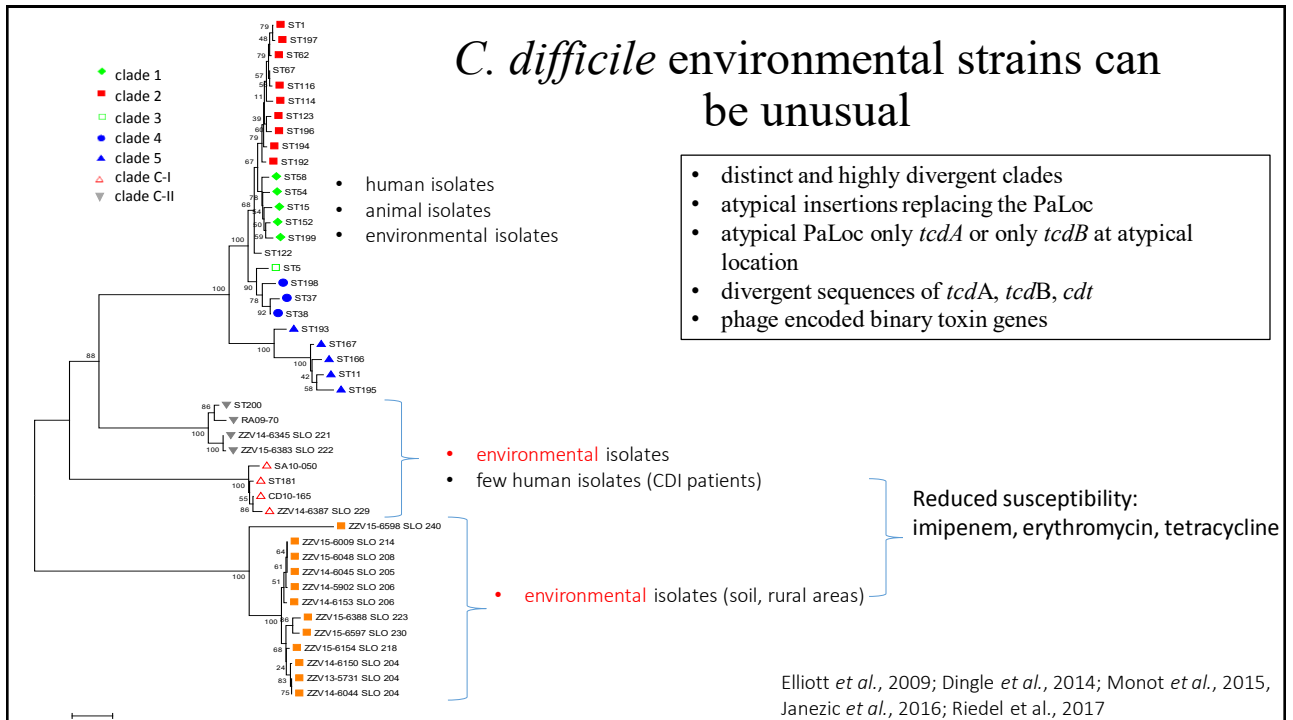
Summary

- One Health approach is important in understanding and controlling CDI

- *C. difficile* has also zoonotic potential (diverse transmission routes - food)

- *C. difficile* is a MDR pathogen (but specific!)

- Similarities in resistance profiles between reservoirs differ among ribotypes and studies

Tetracycline mediated emergence of *Clostridioides (Clostridium) difficile* PCR-ribotype 078

Kate Dingle

University of Oxford

ICOHAR 2019

Session: AMR in *Clostridium difficile* lineages shared by humans and animals

Insights that whole genome sequencing offers One Health

- Antimicrobials used to treat animals frequently overlap those used for humans, often being either identical or related.

- Strategies are required to understand the evolution and spread of antibiotic-resistance bacteria in humans, animals or the environment spread irrespective of species or geography.

- Bacterial whole genome sequencing (WGS) is an ideal frontline tool.

Bacterial Whole Genome Sequencing Insights

- **Understanding the genetic basis of resistance** to key antimicrobials; **predicting resistance phenotype** from genotype.

- Genotyping isolates at the highest possible resolution to improve **understanding of transmission**.

- Combining these strands of evidence informs our understanding of the **evolution and spread of resistance (including MDR lineages)** within bacterial populations and the human and animal species they colonise.

Introduction to *C. difficile* PCR-ribotype 078

- *C. difficile* ribotype 078 first reported as a clinical problem in The Netherlands, rising from 3% to 13% of CDI cases during 2005-2008.

  Goorhuis A, Bakker D, Corver J, Debast SB, Harmanus C, Notermans DW, Bergwerff AA, Dekker FW, Kuijper EJ. 2008. Emergence of *Clostridium difficile* infection due to a new hypervirulent strain, polymerase chain reaction ribotype 078. Clin Infect Dis 47:1162-1170.

- Similarly, a ten fold increase was noted in North America, and increases and occasional outbreaks were reported elsewhere in Europe.

- Three distinctive features of 078-associated CDI have heightened concern:
  - Severity of symptoms.
  - High genotype-specific mortality rate.
  - Higher proportion of community and younger age group infections compared to other genotypes.

Variety of Natural 078 Reservoirs

- **Agricultural settings**; sick and healthy animals (frequently pigs), bird droppings, vermin and the farm environment.

- **Variety of retail meat products** including pork, beef, poultry.

- Can be carried asymptomatically by human infants and adults.

- **What caused 078 to emerge as a clinical problem in humans?**

Phylogenetic Approach using WGS

- WGS used to construct phylogenies.

- Independently from the phylogenetic analysis, resistance genotype for each genome determined.

- Resistance genotype data used to annotate the phylogeny.

- Enables visualisation of *potential* evolutionary impact of antimicrobial selection.

- Time-scaling the phylogeny allows 'look back in time' to examine evolutionary events affecting lineage before, during and after emergence.

Phylogenetic Approach using WGS

Fluoroquinolone resistant lineage

Geographic structure and recent clonal expansions consistent with rapid, localised nosocomial transmission.
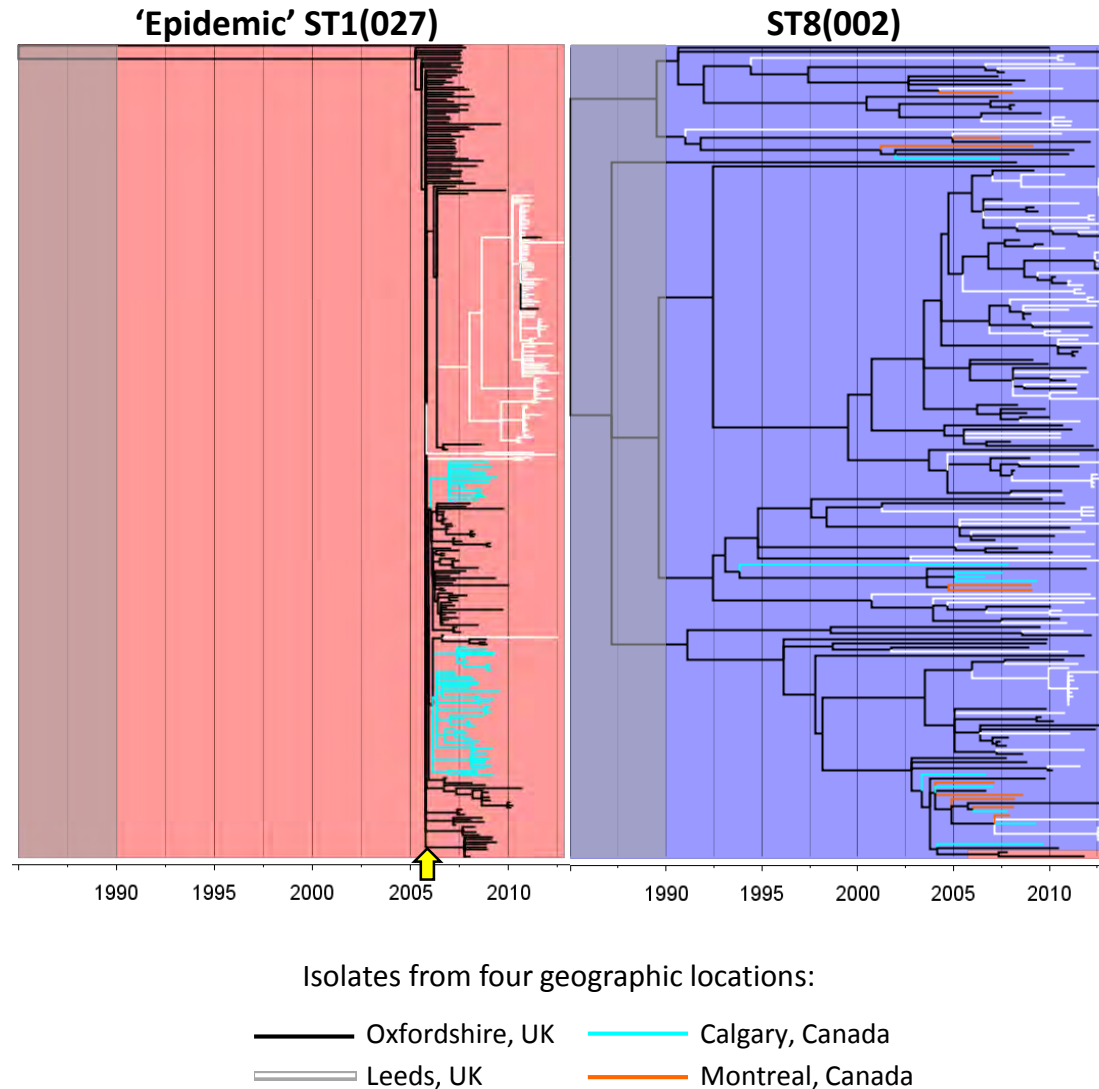
Fluoroquinolone susceptible lineage

Absence of geographic structure or recent clonal expansions consistent with frequent independent introductions to the clinical environment and absence of large scale nosocomial transmission.

'Epidemic' ST1(027)   ST8(002)

Isolates from four geographic locations:

— Oxfordshire, UK   — Calgary, Canada
— Leeds, UK   — Montreal, Canada

See Lancet Infect Dis. 2017 Apr;17(4):411-421.

WGS-approach applied to *C. difficile* 078

Resistance-associated genes and point mutations searched for included:

Tetracycline resistance: *tetM*, *tetO*, *tetW*, *tetO/32/O*, *tetB(P)* (Ribosomal protection proteins)
Tetracycline resistance: *tet40*, *tetA(P)*, *tet*L (efflux)

Aminoglycoside resistance: *aphA1, AAC(6')-APH(2')*
Macrolide-lincosamide-streptogramin B (MLSB) antibiotics, including clindamycin: *ermB*

Fluoroquinolone resistance: *gyrA T[82]I* DNA gyrase subunit A
Fluoroquinolone resistance: *gyrB D[426]N* DNA gyrase subunit
Rifampicin resistance: *rpoB R[505]K* Beta subunit RNA polymerase.

tetM RPP gene by far the most frequently identified in 078.

Prevalence of tetracycline resistance RPP *tetM* in RT078 and clinically relevant *C. difficile* genotypes
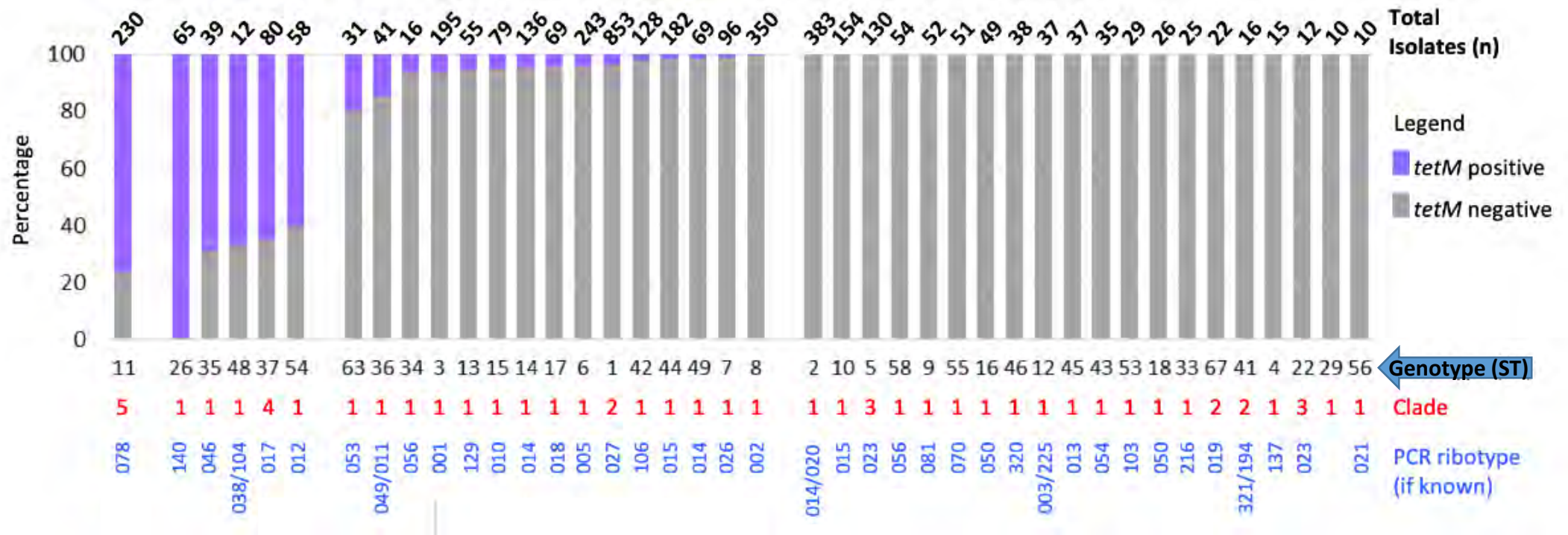
Proportion (%) of each genotype positive RPP encoding gene *tetM*

- Isolate collections: Oxfordshire (EIA positives, negatives, infant and farm), Leeds, North American and European clinical isolates.
- Total number of isolates of each genotype is shown above the bar.
- Data for genotypes having 10 genomes or more.

078 WGS phylogenies were annotated for presence of *tetM* to highlight any association between resistance acquisition and recent clonal expansion.

078 WGS Phylogeny for the UK

- Lack of geographic structure, illustrated by branch colours.

- Strong structuring of *tetM* variants, different variants indicated by coloured bars.

- Recent clonal expansions post *tetM* acquisition, independent of geographic location.

- Clonal expansions followed multiple, independent *tetM* acquisition events.

Geographic areas from which the RT078 *C. difficile* genomes were obtained.

Oxfordshire n=78
Leeds n=104
Scotland n=110

Key to branch colours
Locations within UK

■ Oxfordshire
■ Leeds
■ Scotland

Scotland

Leeds

Oxfordshire

~175 miles
~360 miles

Each branch represents one genome
Branch colours indicates location

RPP allele
tet40
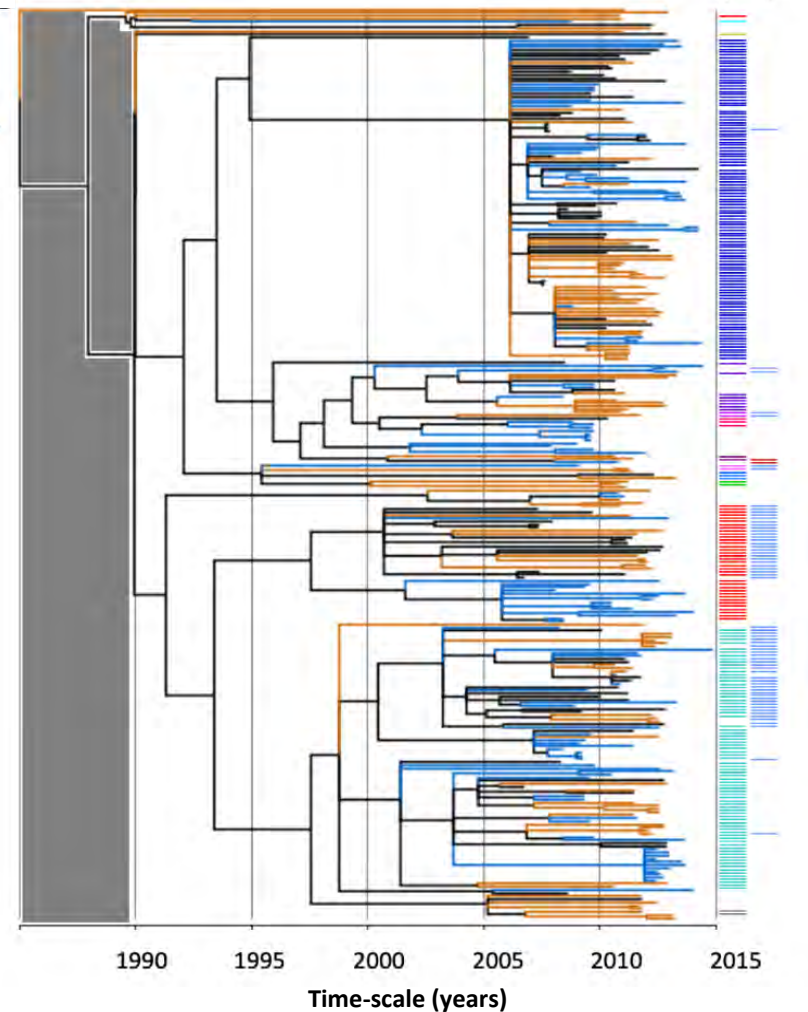
tetM and *tet40* variants indicated by coloured bars.

Time-scale (years)

1990   1995   2000   2005   2010   2015
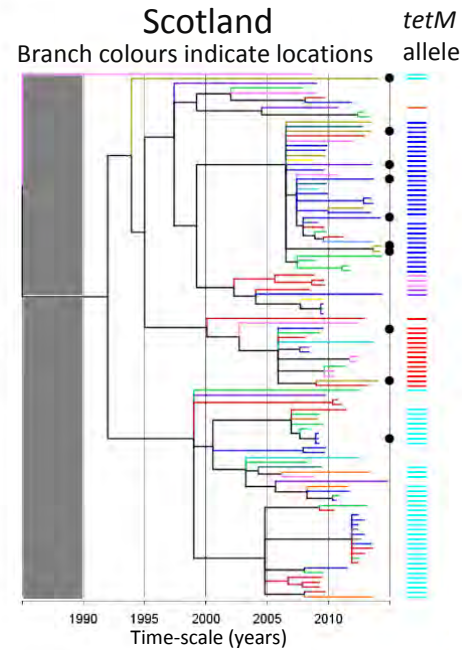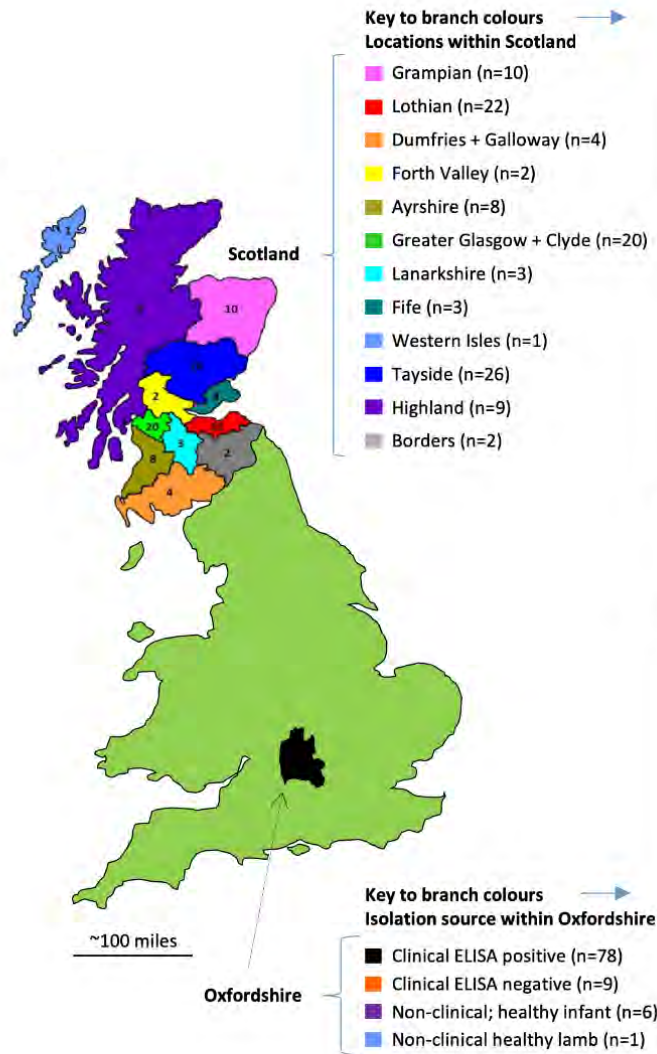
078 WGS Phylogeny for the UK Regions

- Lack of geographic structure, illustrated by branch colours.

- Strong structuring of *tetM* variants, different variants indicated by coloured bars.

- Recent geographically unstructured clonal expansions, post *tetM* acquisition.

078 WGS Phylogeny Representing UK, Europe and N. America
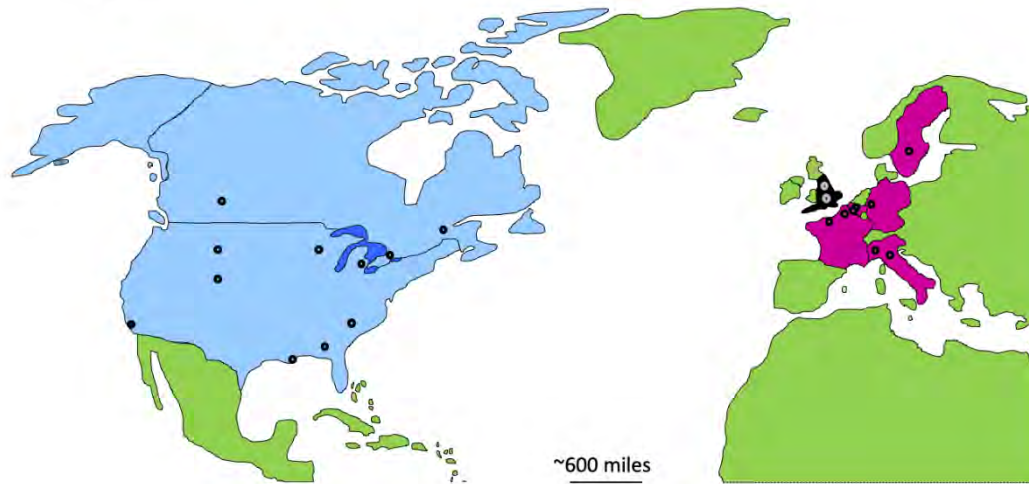
Key to branch colours

■ England:
Oxfordshire EIA positives (n=78)
Leeds (n=104)

□ North America:
USA (n=15)
Canada (n=4)

■ Europe (n=13)

•°° Dots represent location of cities.

~600 miles

RPP   *tetM* allele

Time-scale (years)

- Lack of geographic structure, illustrated by branch colours: N. American and European genomes mixed with English WGS.

- Strong structuring of *tetM* variants, different variants indicated by coloured bars.

Evidence of Multiple, independent *tetM* acquisitions

tetM alleles 10, 16 and 19 carried on closely related Tn916-like conjugative transposons.

Conjugation   Resistance   Regulation   Recombination
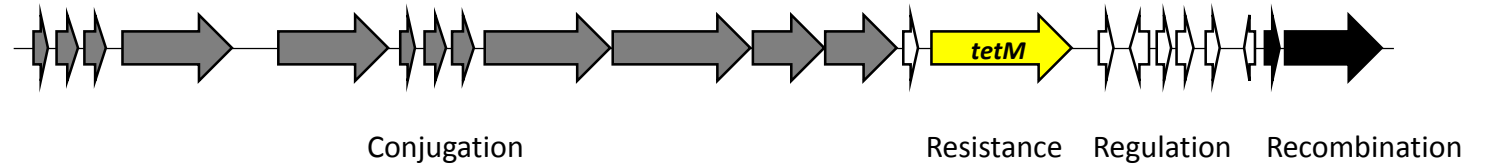
Chromosomal insertion sites for *tetM* alleles 10, 16, 19

C. difficile M120 chromosome 4,047,729 bp (He at al., 2010)

10 →
19
16
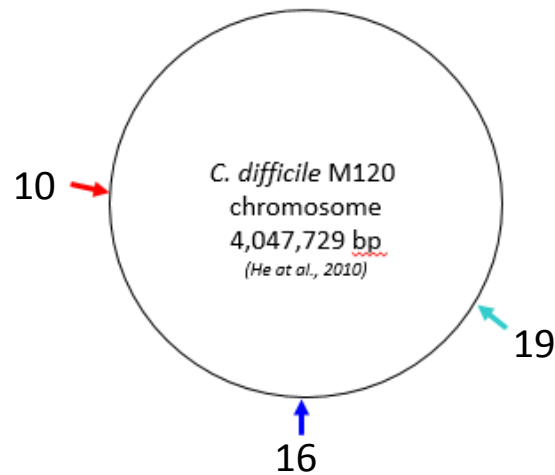
Three most prevalent RPP alleles

— *tetM* 10
— *tetM* 16
— *tetM* 19

ACT comparison of Tn916 like elements carrying tetM allele 10 and allele 16, and their chromosomal ionsertion sites.

Tn916 *tetM* 10

Chromosomal insertion site   100%   97%   87%   95%   99%   Chromosomal insertion site   100%   91%

Tn916 *tetM* 16
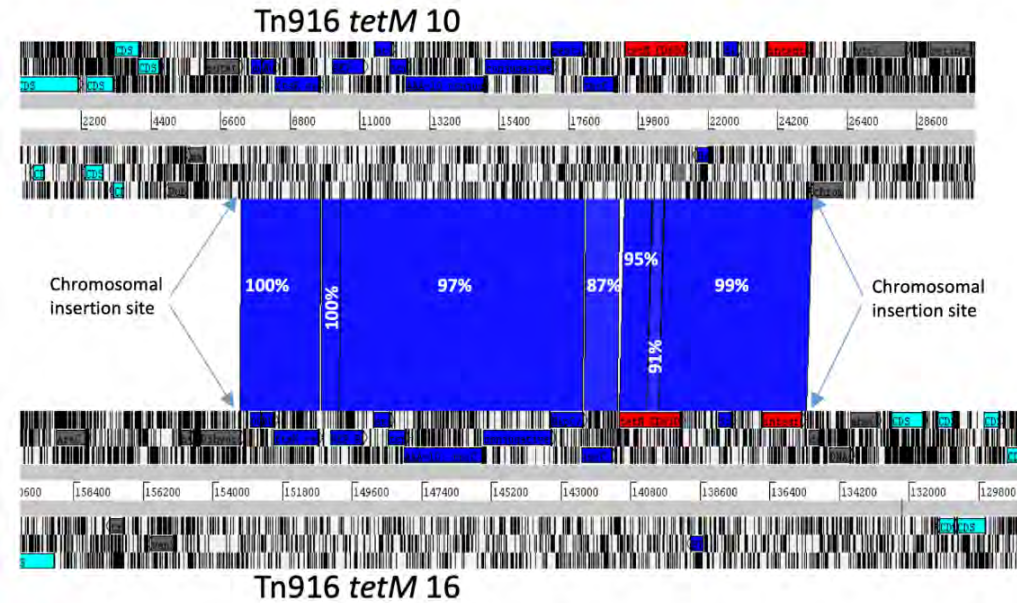
The absence of identity outside the (blue) sequence of the mobile elements highlights their insertion into completely unrelated regions of the chromosome, ie. independent acquisition events.

Non-tetracycline antimicrobial resistance determinants in 078 (UK phylogeny)

Aminoglycoside resistance; *aphA1* aminoglycoside 3'-phosphotransferase

Fluoroquinolone resistance conferred by non-synonymous *gyrA* or *gyrB* substitutions

Clindamycin resistance; *ermB*

aphA1 detected ——
aphA1 not detected ——

Fluoroquinolone resistant (*gyrA*) ——
Fluoroquinolone resistant (*gyrB*) ——
Fluoroquinolone susceptible ——

ermB detected ——
ermB not detected ——
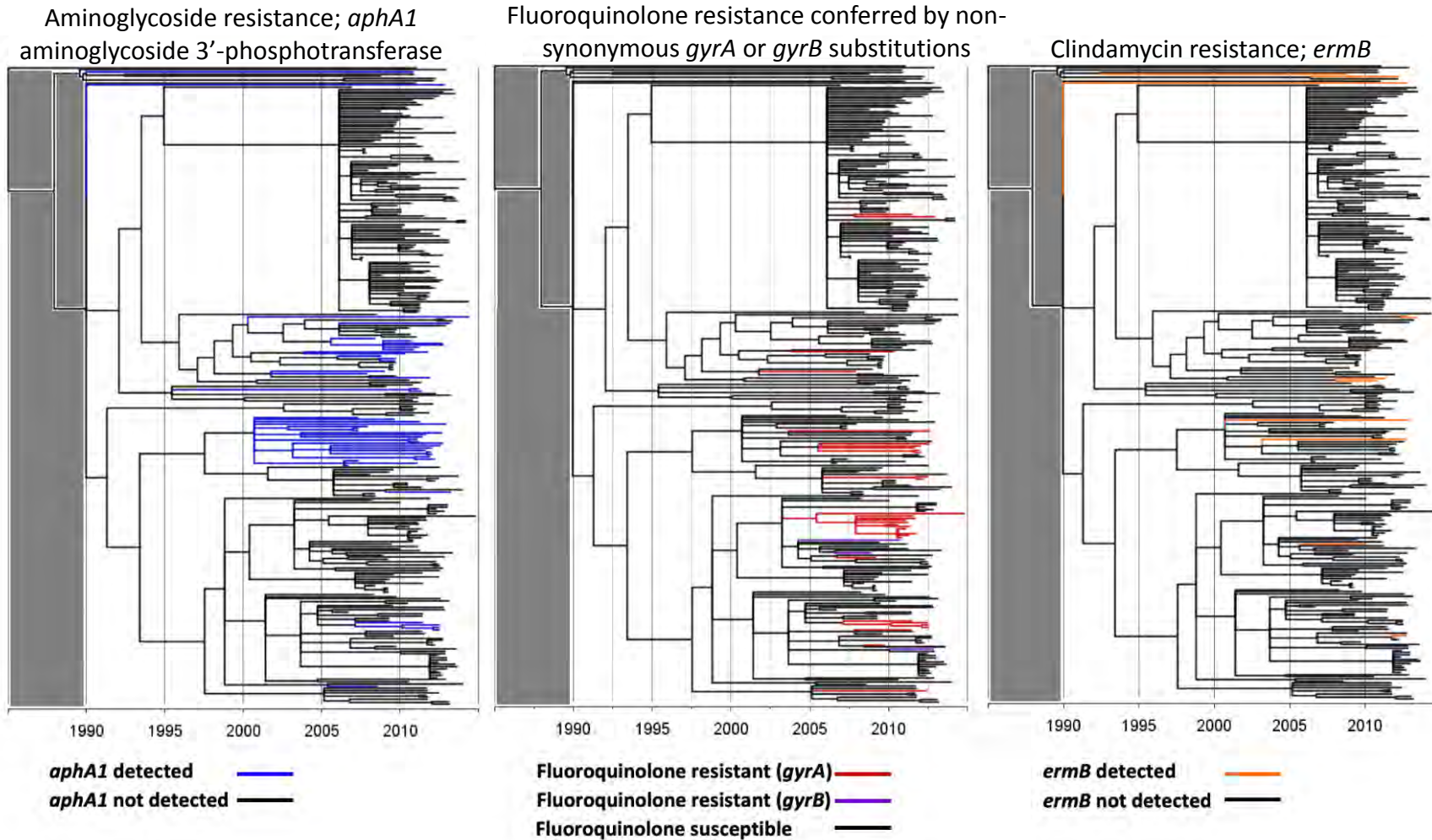
How much tetracycline is used in agricultural settings?

Veterinary use of Tetracyclines: U.S.

2017 Summary Report on Antimicrobials Sold or Distributed for Use in Food-Producing Animals (December 2018)

Tetracyclines, which represent the largest volume of domestic sales (3,535,701 kg in 2017), decreased by 40% from 2016 through 2017.

Antimicrobial drugs approved for use in food-producing animals[1]
Actively marketed in 2017
Domestic sales and distribution data
Reported by medical importance and drug class

	Drug Class	Annual Totals (kg)[2]	% Subtotal	% Grand Total
Medically Important[3]	Aminoglycosides	259,184	5%	2%
	Amphenicols	49,321	1%	<1%
	Cephalosporins[1]	29,369	<1%	<1%
	Fluoroquinolones	22,904	<1%	<1%
	Lincosamides[1]	152,497	3%	1%
	Macrolides	468,794	8%	4%
	Penicillins[1]	690,889	12%	6%
	Sulfas	274,112	5%	3%
	Tetracyclines[1]	3,535,701	64%	32%
	NIR[1,4]	76,440	1%	1%
	Subtotal	**5,559,212**	**100%**	**51%**
Not Medically Important[5]	Ionophores	4,394,850	82%	40%
	NIR[6]	979,306	18%	9%
	Subtotal	**5,374,156**	**100%**	**49%**
	Grand Total	**10,933,367**		**100%**

Ingredient Class	Species	Estimated Annual Totals (kg)[3]	% Subtotal
Tetracyclines[2]	Cattle	1,560,542	44%
	Swine	1,579,145	45%
	Chicken	153,621	4%
	Turkey	192,976	5%
	Other[4]	49,416	1%
	Subtotal	3,535,701	100%

Veterinary use of Tetracyclines: Europe

Sales of veterinary antimicrobial agents in 30 European countries in 2016

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

2.4.2.1. Tetracyclines

Figure 11. Spatial distribution of sales of tetracyclines for food-producing animals, in mg/PCU, by country, for 2016

Figure 4. Sales of antimicrobial agents by antimicrobial class as percentage of the total sales for food-producing species, in mg/PCU, aggregated by 30 European countries, for 2016

Pie chart legend:
- Tetracyclines — 32%
- Penicillins — 26%
- Sulfonamides — 12%
- Trimethoprim — 2%
- Macrolides — 7%
- Lincosamides — 3%
- Polymyxins — 5%
- Aminoglycosides — 5%
- Pleuromutilins — 3%
- Fluoroquinolones — 2%
- Others* — 3%
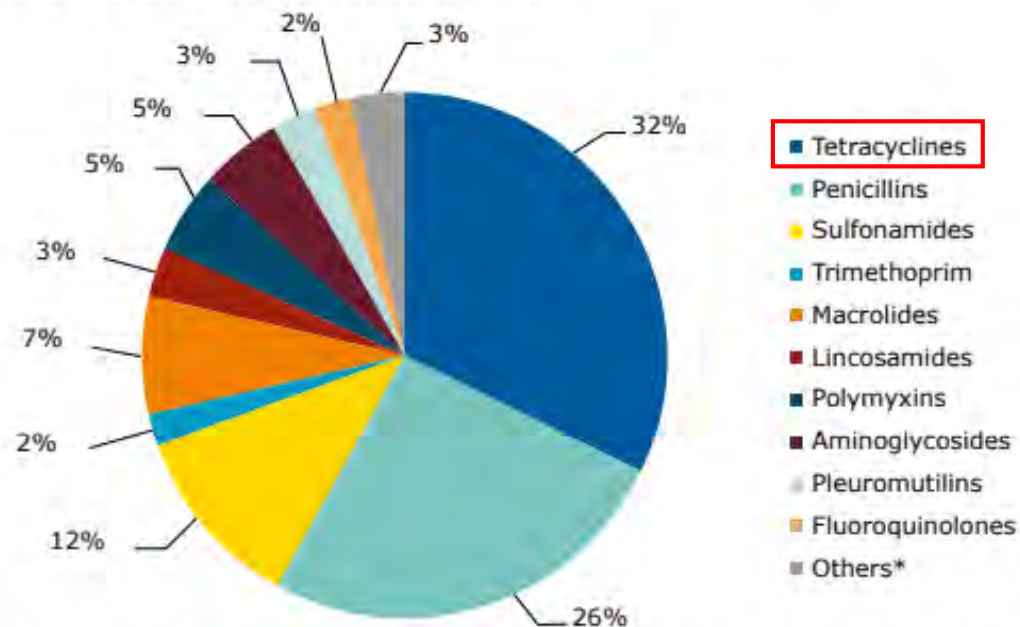
mg/PCU - unit of measurement developed by the European Medicines Agency to monitor antibiotic use and sales across Europe.
PCU refers to the '**Population Correction Unit**' and takes into account the animal population as well as the estimated weight of each particular animal at the time of treatment with antibiotics.

Veterinary use of Tetracyclines: UK
Data for Recent Tetracycline Sales (2013-2017)

UK Veterinary Antibiotic Resistance and Sales Surveillance Report
UK-VARSS 2017
Published October 2018

Figure 1.3: Active ingredient (% weight) of antibiotics sold for all animal species by antibiotic class, 2017
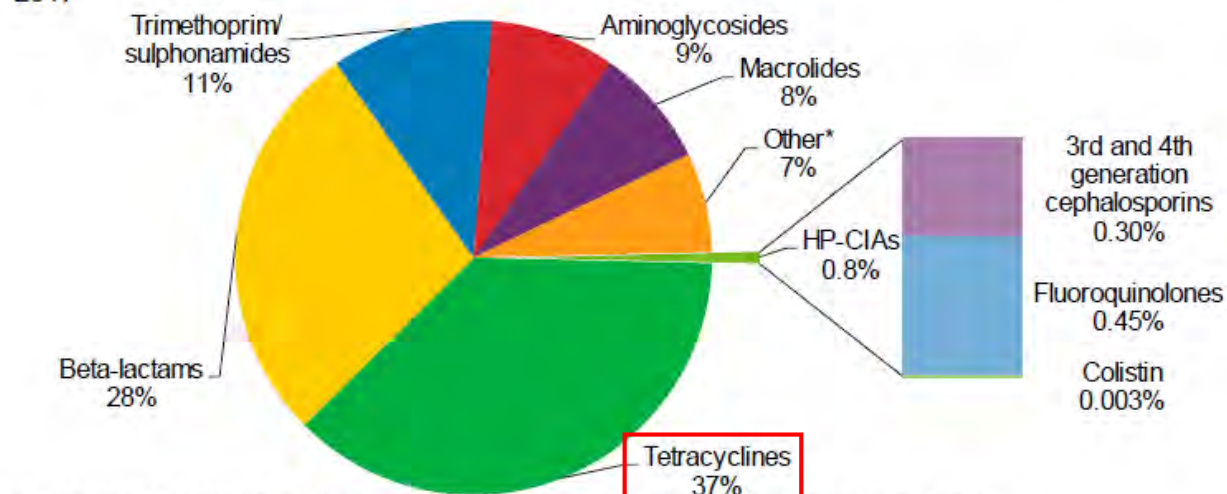
* Amphenicols, lincomycin, pleuromutilins, polymyxins (excluding colistin) and steroidal antibiotics.

Figure 1.4: Active ingredient (mg) of antibiotic sold per kg of food-producing animal species by antibiotic class, 2013–2017
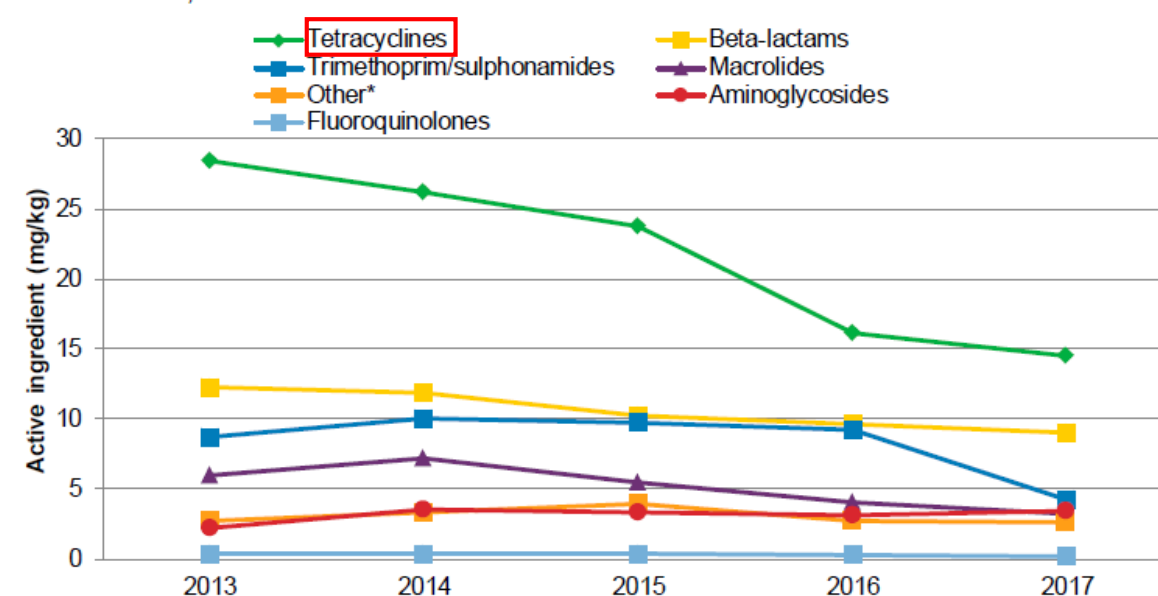
* Amphenicols, lincomycins, pleuromutilins, polymyxins (including colistin) and steroidal antibiotics.

"Tetracyclines remain the most sold antibiotic class (representing 37% of total sales) despite these sales falling by 89 tonnes (46%) since 2013. Beta-lactams are the second most sold class (representing 28% of total sales) and their sales have fallen at a slower rate, by 16 tonnes (17%) since 2013".

Summary: *C. difficile* 078

- Numerous lines of evidence indicate an important role for selection pressure *via* agricultural tetracycline use in the recent evolution of tetracycline resistant *C. difficile* 078.

    Dingle et al., 2019 A Role for Tetracycline Selection in Recent Evolution of Agriculture-Associated *Clostridium difficile* PCR Ribotype 078. MBio. 2019 Mar 12;10(2). pii: e02790-18.

- Studies using whole genome sequencing of Dutch and international RT078 isolates provide further data consistent with the rapid spread of RT078 both internationally, and between animals and humans.

    Knetsch CW, et al., 2014. Whole genome sequencing reveals potential spread of *Clostridium difficile* between humans and farm animals in the Netherlands, 2002 to 2011. Euro Surveill 19:20954.
    Knetsch CW, et al., 2018. Zoonotic Transfer of *Clostridium difficile* Harboring Antimicrobial Resistance between Farm Animals and Humans. J Clin Microbiol 56(3). pii: e01384-17

- These studies support the hypothesis first proposed in 2012, that humans become colonised by RT078 via the food chain and/or the environment.

    Hensgens MP, et al. 2012. *Clostridium difficile* infection in the community: a zoonotic disease?
    Clin Microbiol Infect 18:635-645.

Summary: One Health

- WGS-based approaches can inform the One Health Approach.

- As costs have fallen, WGS could realistically be more widely implemented for local, national and international surveillance.

- Key requirements - capacity to store, exchange and interrogate large volumes of genomic data and bioinformatic tools to manage and interpret the data at the routine level.

Acknowledgements

Training the next generation of veterinary prescribers

Carmen Espinosa-Gongora
DVM PhD
Assistant professor at
Department of Veterinary and Animal Sciences
University of Copenhagen

UNIVERSITY OF COPENHAGEN

Veterinarians in the battlefield

Competencies

World Organisation for Animal Health
Protecting animals, preserving our future

September 2013

≫PVS Pathway

OIE recommendations on the Competencies of graduating veterinarians ('Day 1 graduates') to assure National Veterinary Services of quality
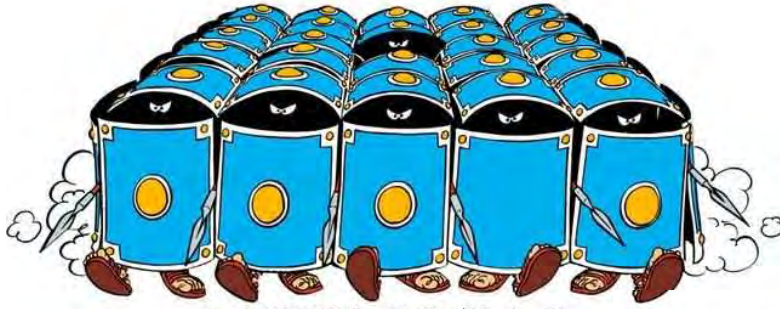
Veterinary Education
Core Curriculum
OIE Guidelines
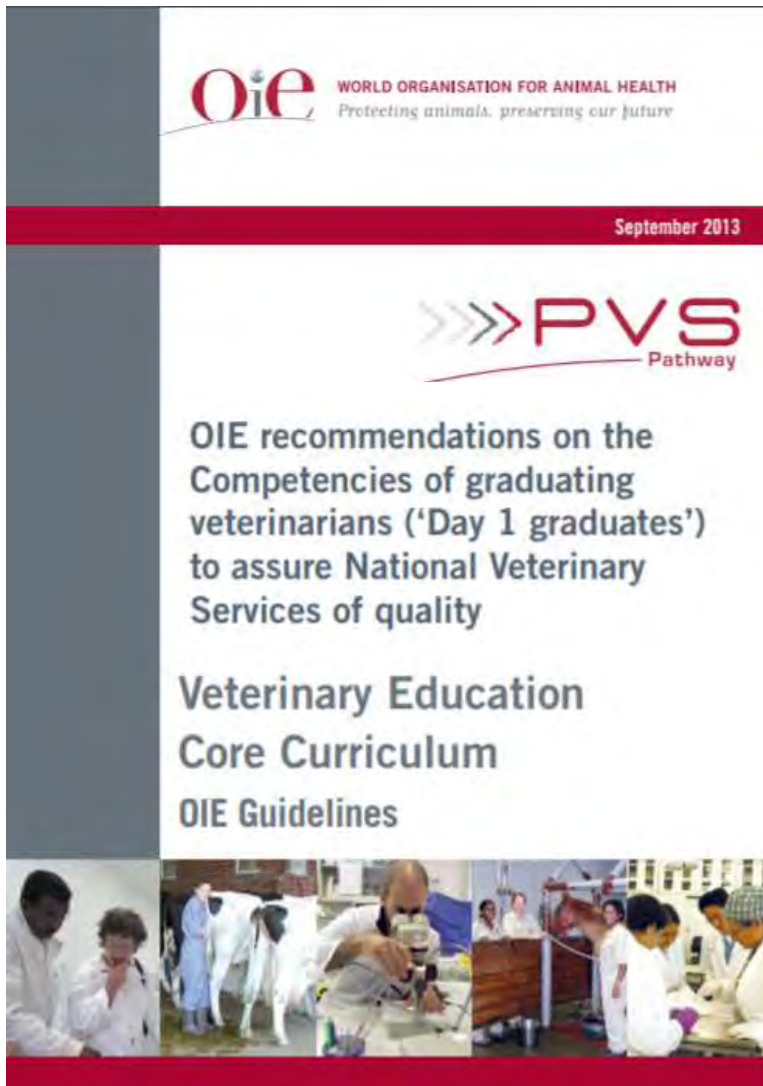
- Article 38 of EU Directive 2013/55/EU

- List of subjects and **Day One Competences** (as approved by the ECCVT on 26 March 2015 and proposed to the EU DG Grow as Annex 5.4.1 of the EU Directive 2013/55/EU)

PREPARE-VET

ESGVM — ESCMID STUDY GROUP FOR VETERINARY MICROBIOLOGY
European Society of Clinical Microbiology and Infectious Diseases

ESGAP — ESCMID STUDY GROUP FOR ANTIBIOTIC POLICIES
European Society of Clinical Microbiology and Infectious Diseases

Aim - To evaluate the need of further **education of European veterinary students** in the field of **antimicrobial stewardship** and to provide guidance to those responsible for educational programs and course development across Europe.

SURVEY

PREPARE-VET

✓ 12 languages

✓ 28 questions

❖ Students (clinical rotations, performance, target professional field)

❖ Perception of preparedness

1. Pharmacology
2. Clinical use of antimicrobials
3. Antimicrobial resistance

❖ "Test" questions

"What have you been taught to…"
Use of guidelines

❖ Opinion: *What is the contribution of veterinary use of AM in AMR in humans?*

❖ Teaching methods

❖ Again, overall perception of preparedness

Enrolment

- 30 Countries
- 89 Vet schools
- 3422 Complete answers

category
- Enrolled
- No vet school
- No coordinator found
- Withdrew
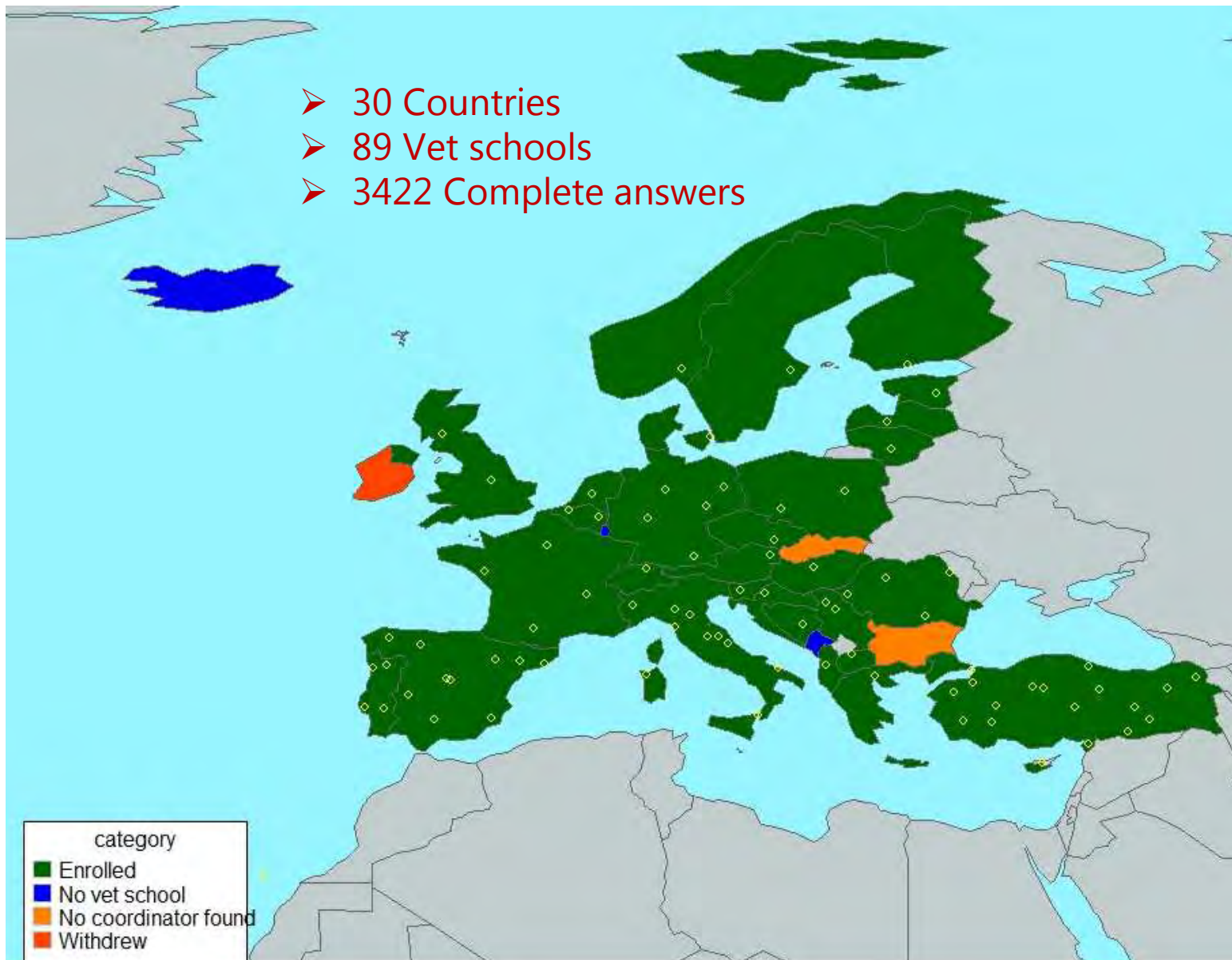
PERCEPTION OF PREPAREDNESS
How prepared do you feel in the following topics ... ?

1. Pharmacology of antibiotics

2. Clinical use of antibiotics

3. Antimicrobial resistance (AMR)

Answers

4 — Well prepared

3 — Sufficiently prepared

2 — Poorly prepared

1 — Not at all prepared

0 — I haven't received any teaching/training

I don't know

I don't understand the question
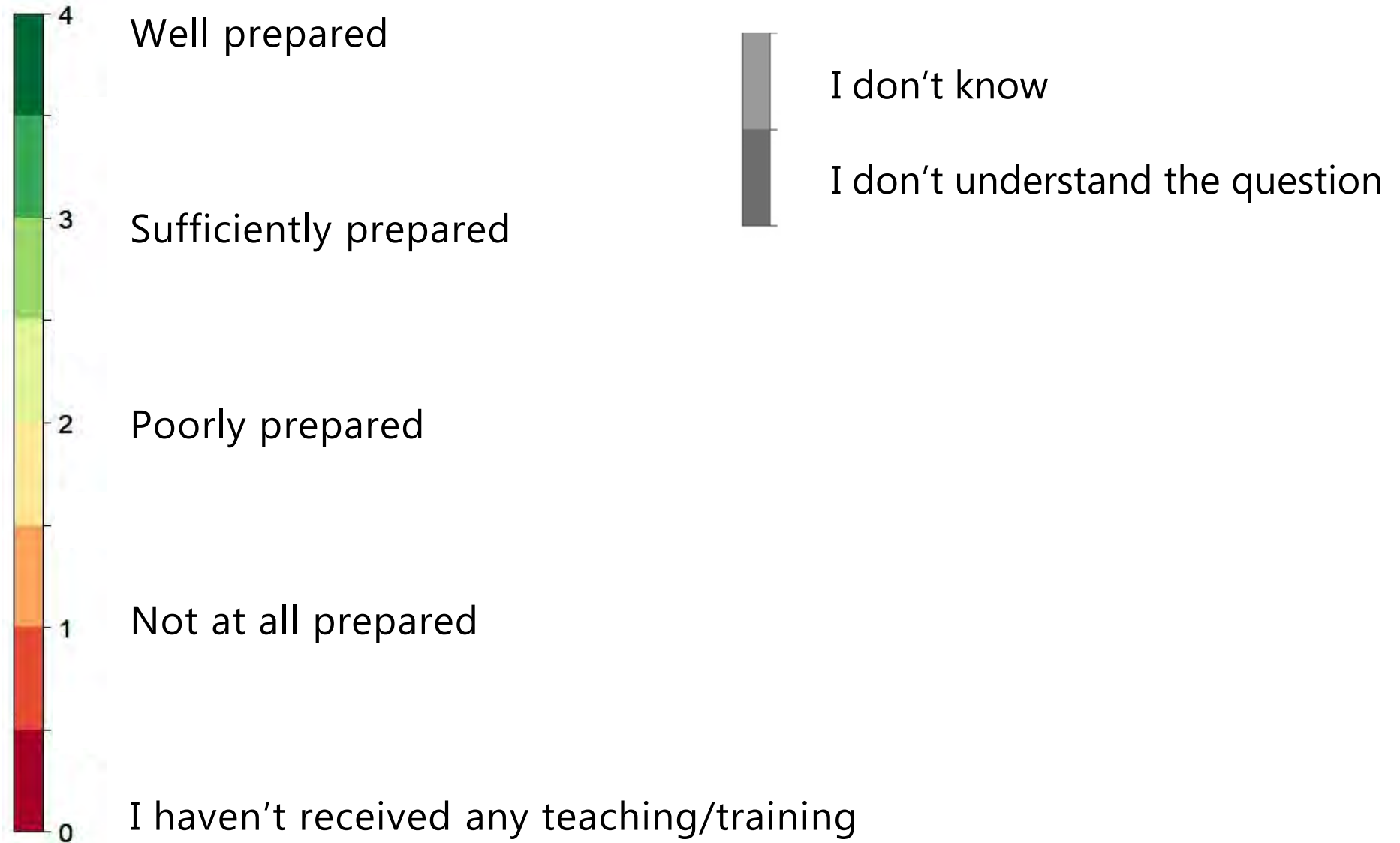
PERCEPTION OF PREPAREDNESS BY COUNTRY

How prepared do you feel in the following topics … ?

Pharmacology   Clinical use   AMR

1   1.5   2   2.5   3   3.5   4
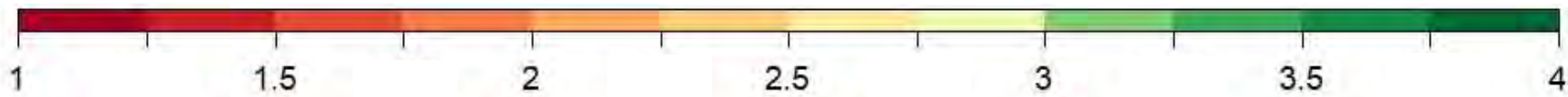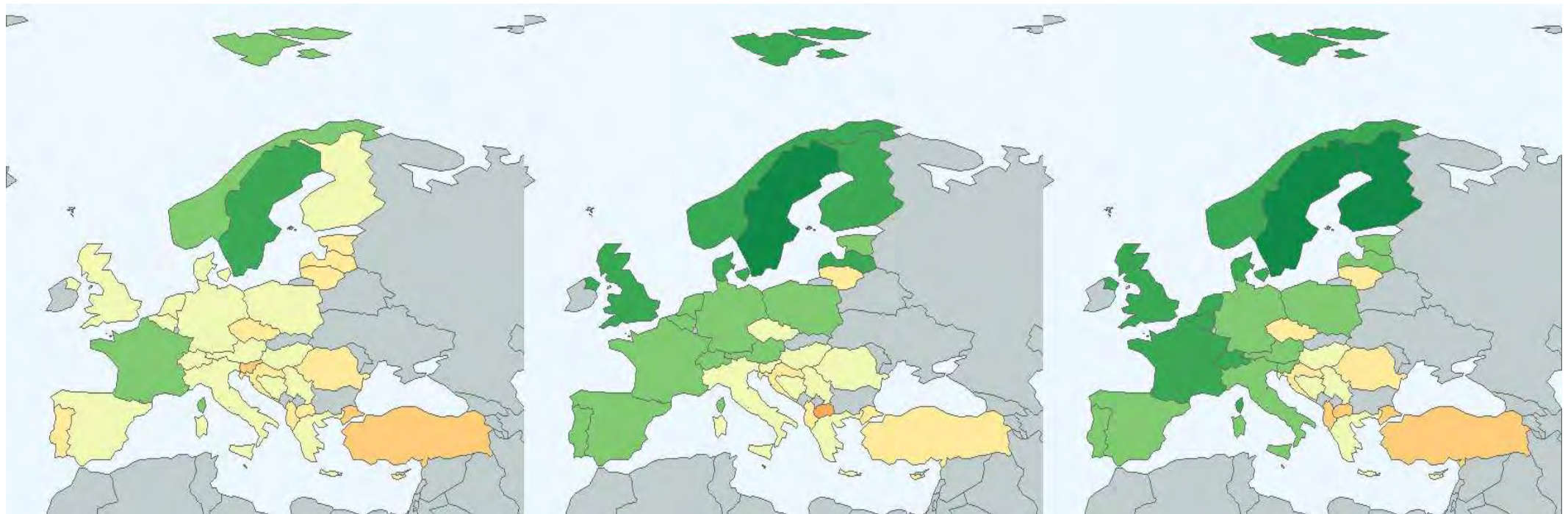
Not at all   Poorly   Sufficiently   Well

=Hardefeldt et al. 2018
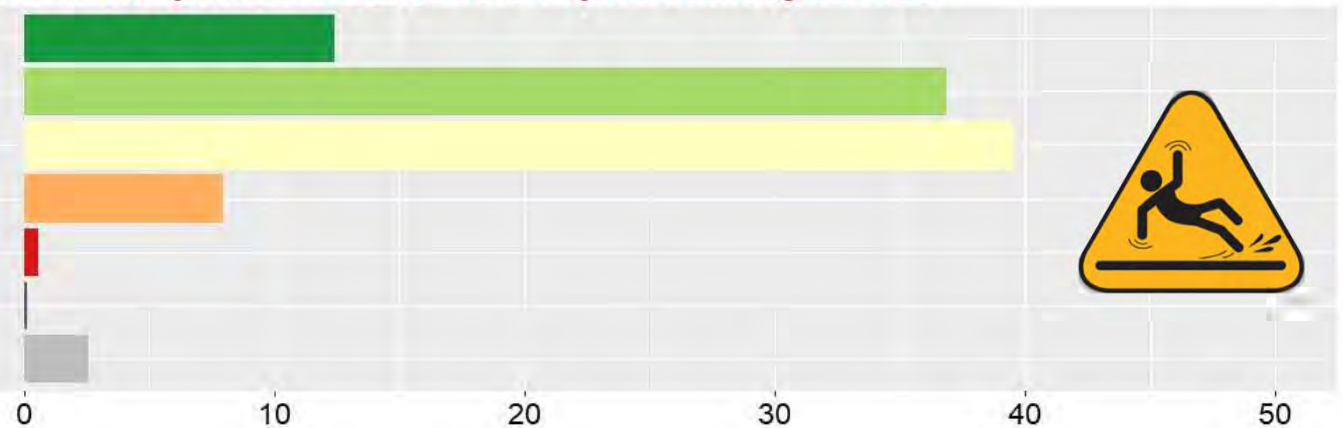
PERCEPTION OF PREPAREDNESS BY COUNTRY

How prepared do you feel in the following topics … ?

Clinical use

...Deciding which bacterial infections need or do not need systemic antibiotic therapy
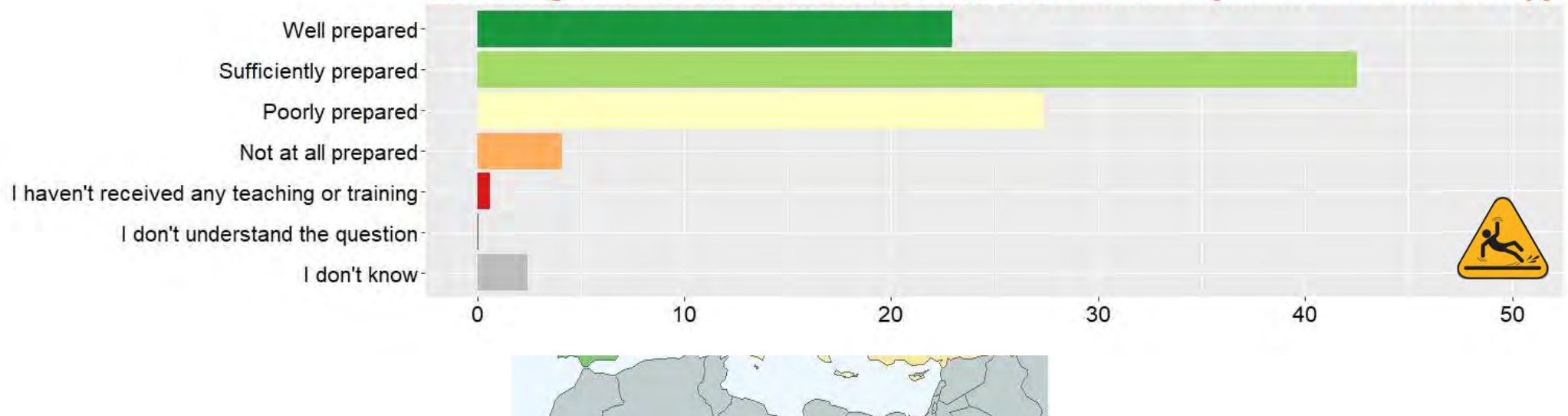
PERCEPTION OF PREPAREDNESS BY COUNTRY

How prepared do you feel in the following topics ... ?
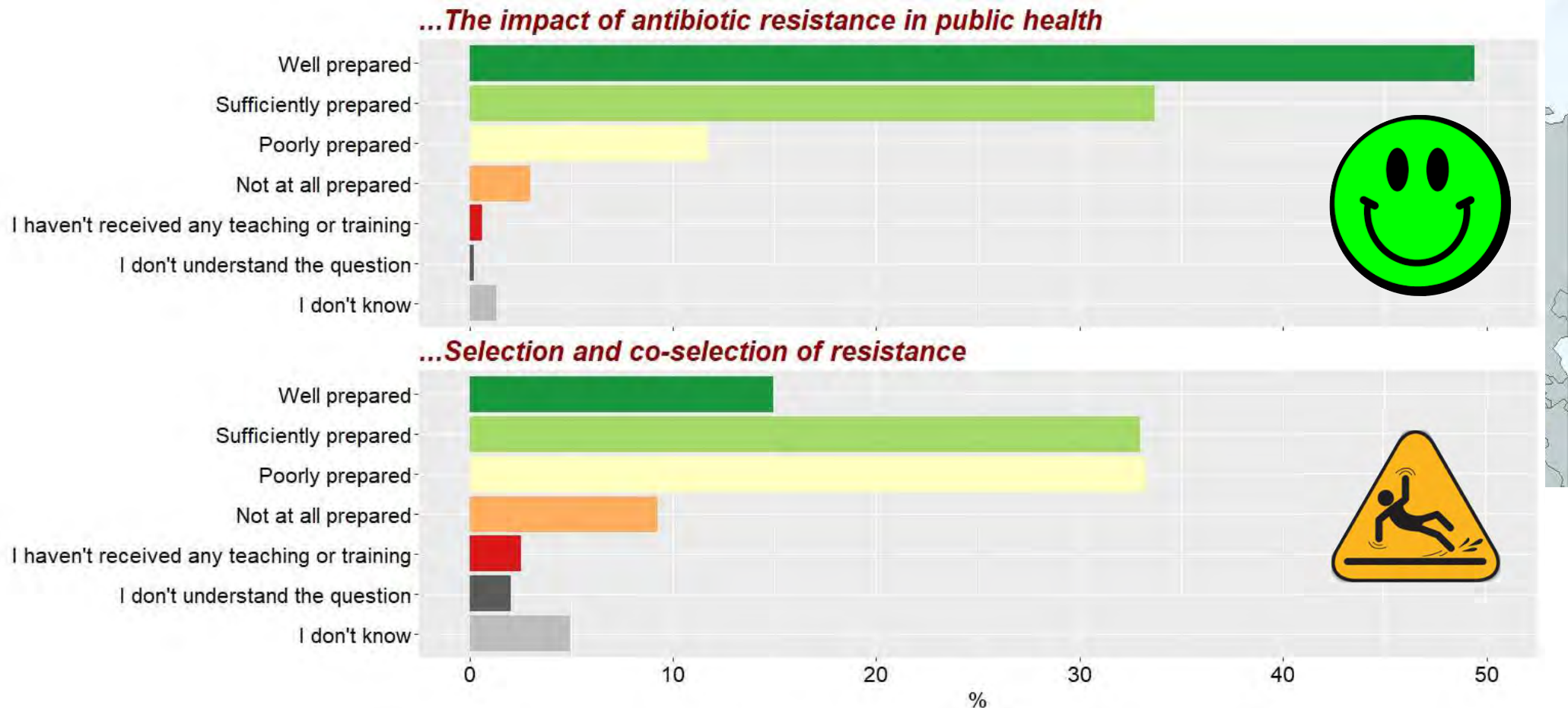
Does **perception of preparedness** correlate with **teaching methods**?

1. Clinical rotations

2. Other teaching methods

27. How often the following methods have been used to teach you on antibiotics, antibiotic resistance and antibiotic use?

	Very often	Sometimes	Rarely	Never	I don't know
Lectures	○	○	○	○	○
Small group teaching	○	○	○	○	○
Discussions of clinical cases	○	○	○	○	○
Active learning assignment (article review, oral presentation)	○	○	○	○	○
E-learning	○	○	○	○	○

Do **clinical rotations** provide students a better **perception of preparedness**?
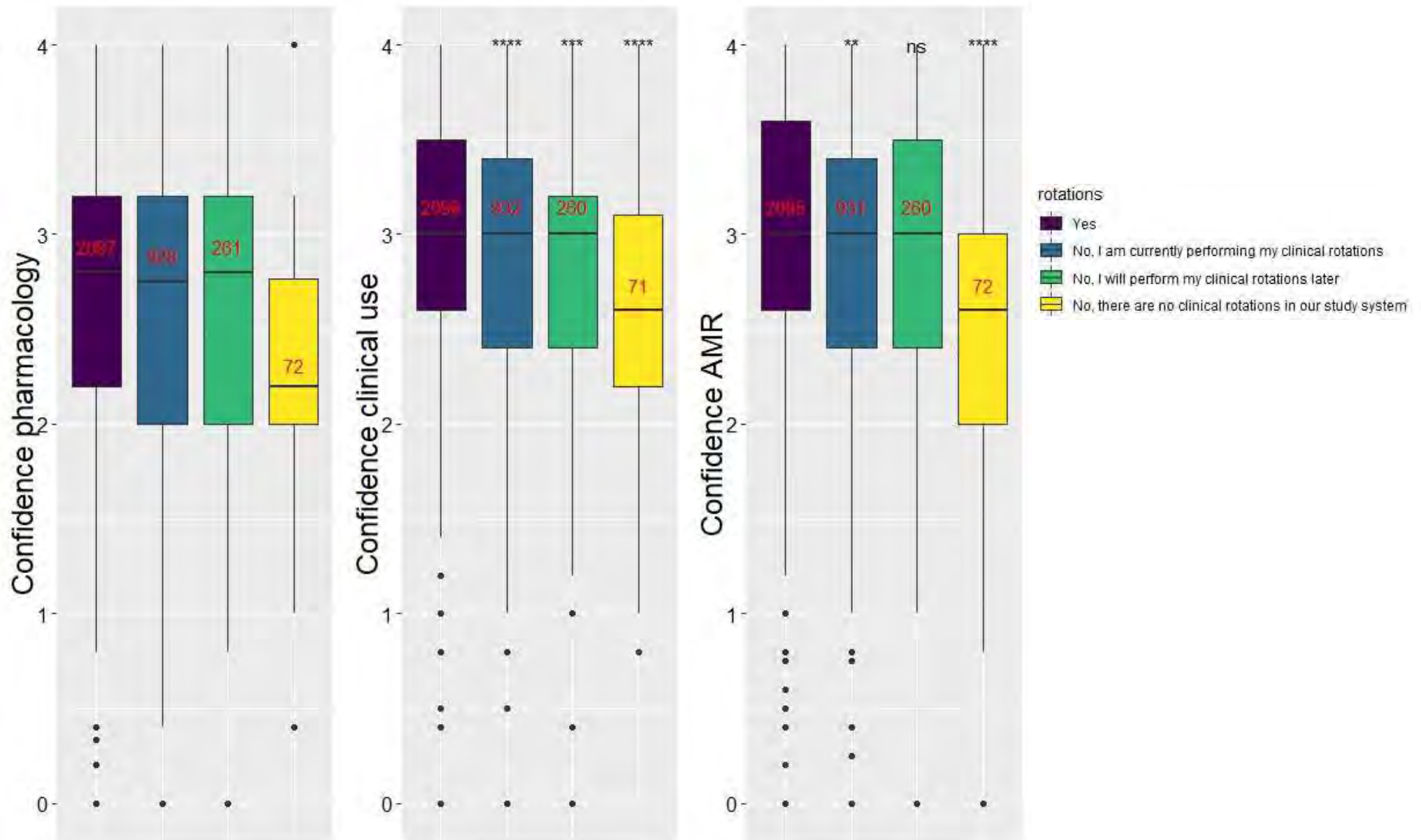
Overall do you think you receive **adequate teaching to face** antibiotic and resistance issues in clinical practice**?**
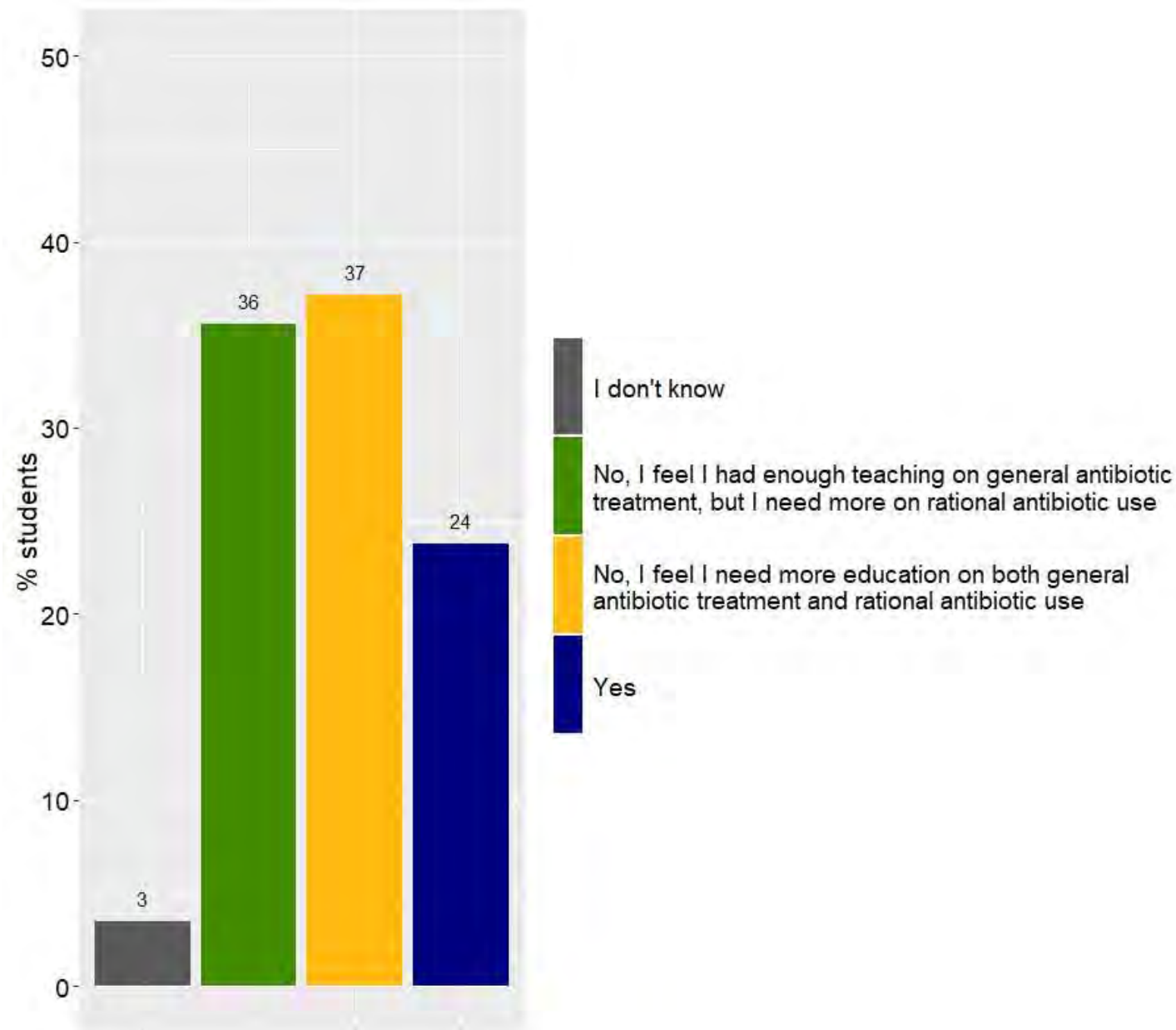
In your opinion what is the relative contribution of **veterinary use of antibiotics** to the clinical problems of **resistant bacteria in humans?**

- ❑ Very low (<0.1%)
- ❑ Low (<5%)
- ❑ Medium (10-20%)
- ❑ High (>50%)
- ❑ Uncertain
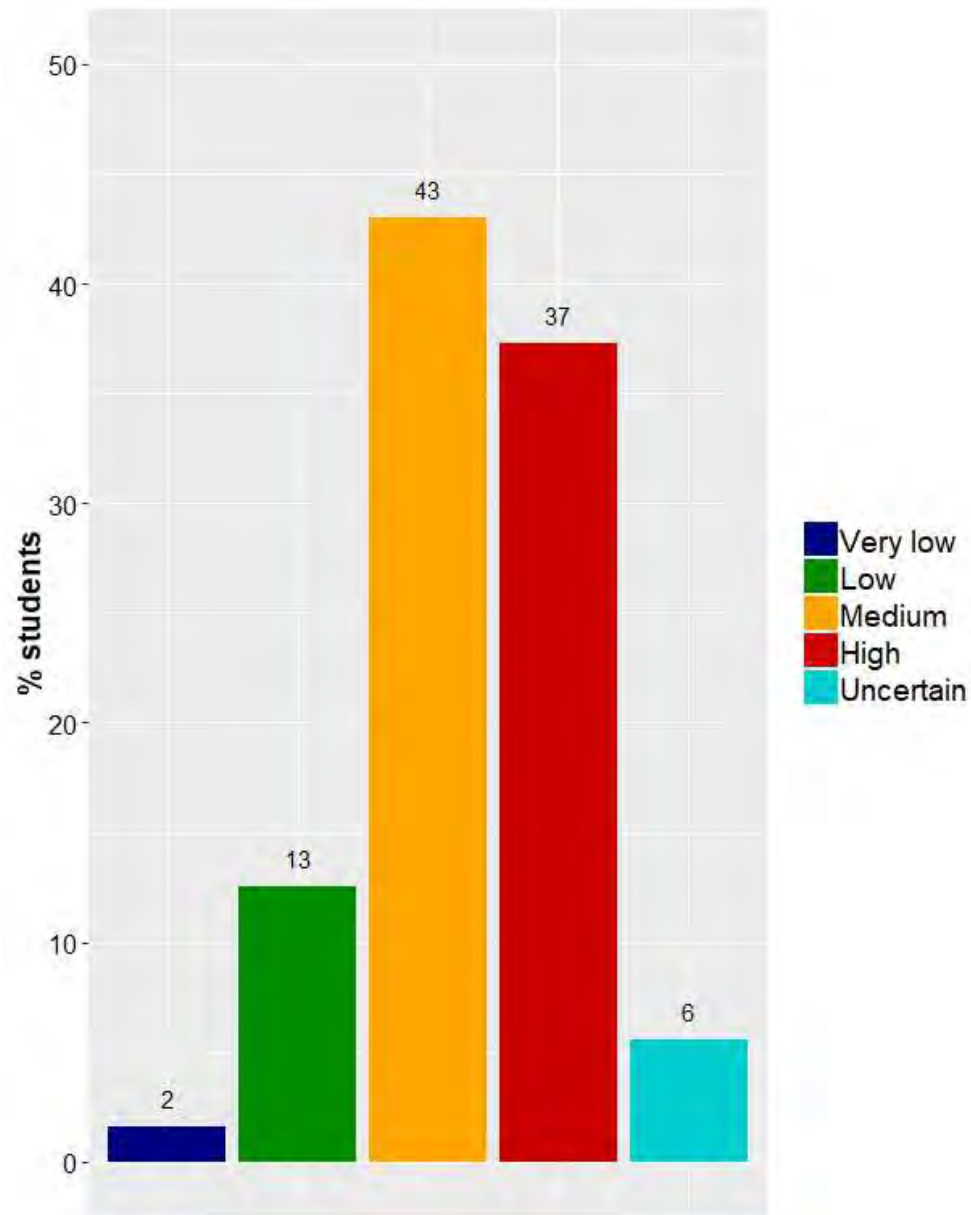
In your opinion what is the relative contribution of **veterinary use of antibiotics** to the clinical problems of **resistant bacteria in humans?**

Very low <- 0
Low <- 1
Medium <- 2
High <- 3
Uncertain <- NA

Proportion

0   0.5   1   1.5   2   2.5   3
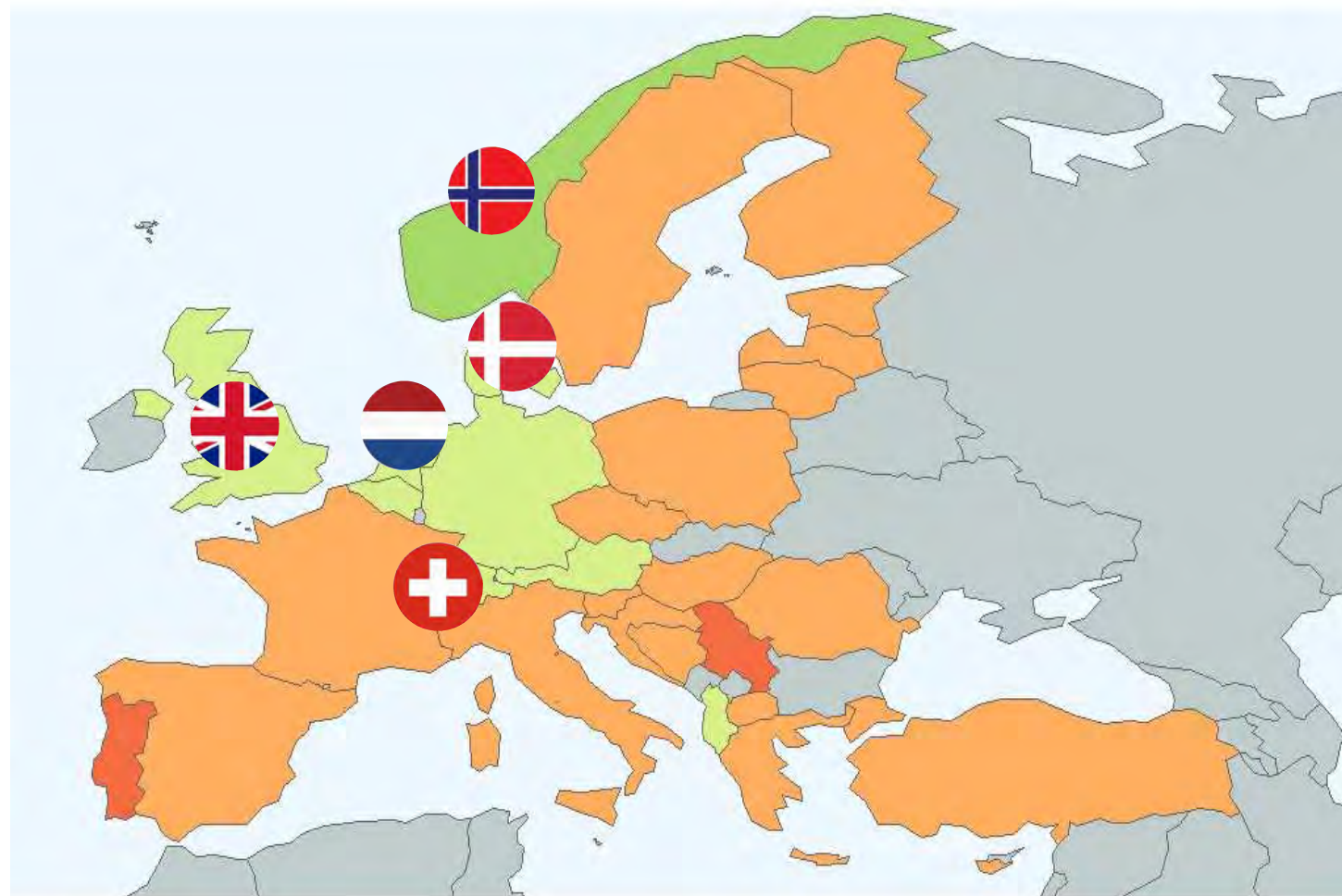
Sales of antibiotics...?

Figure 2. Sales for food-producing species, in mg/PCU, of the various veterinary antimicrobial classes, for 30 European countries, in 2016[1]
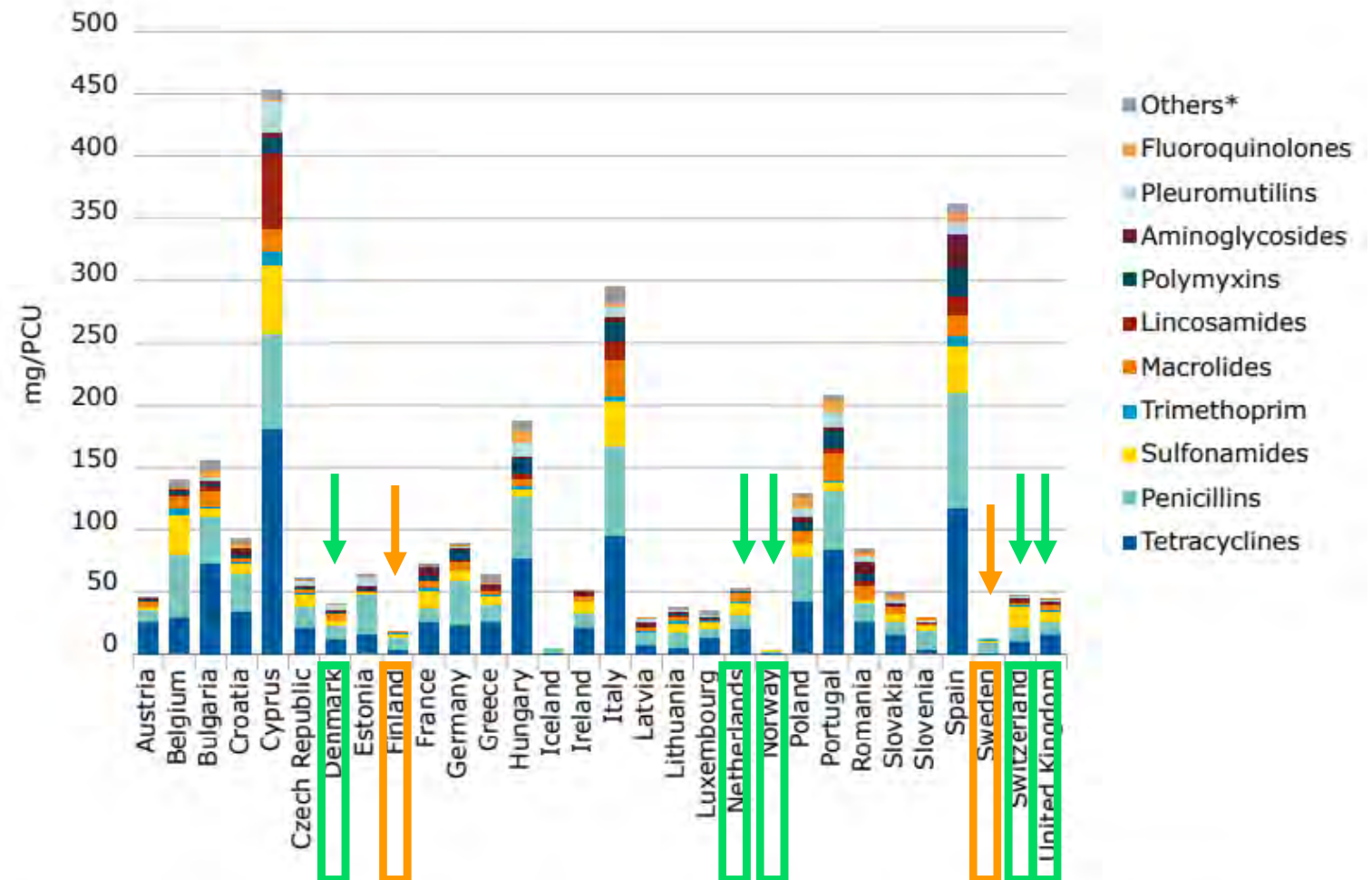
*Amphenicols, cephalosporins, other quinolones and other antibacterials (classified as such in the ATCvet system).
[1] Differences between countries can be partly explained by differences in animal demographics, in the selection of antimicrobia in dosage regimes, in type of data sources, and veterinarians' prescribing habits.

Prevalence of MRSA...?
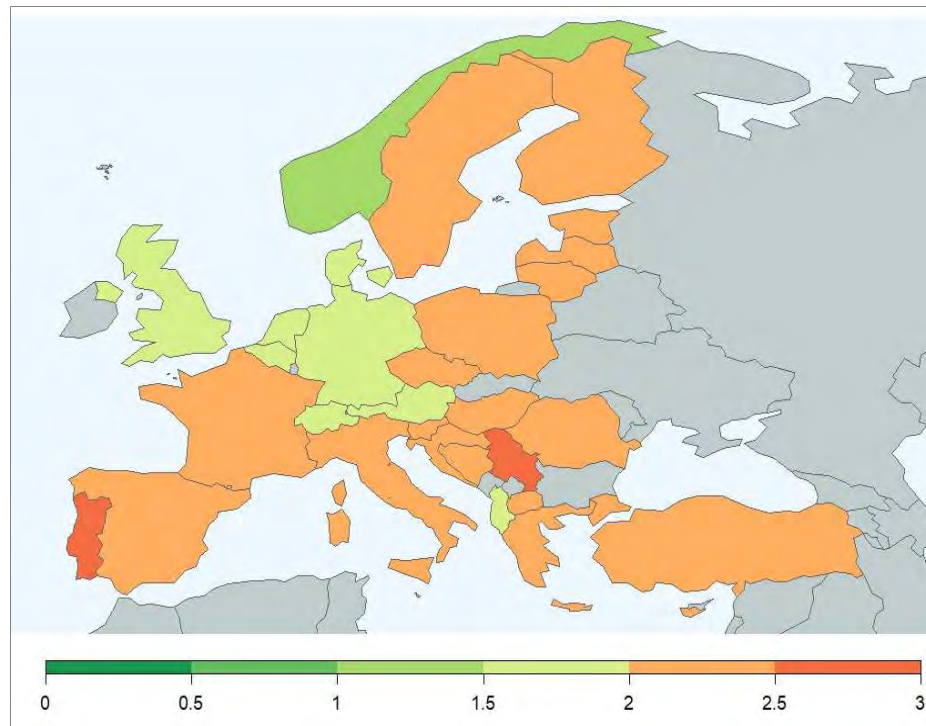
Surveillance of antimicrobial resistance in Europe 2017
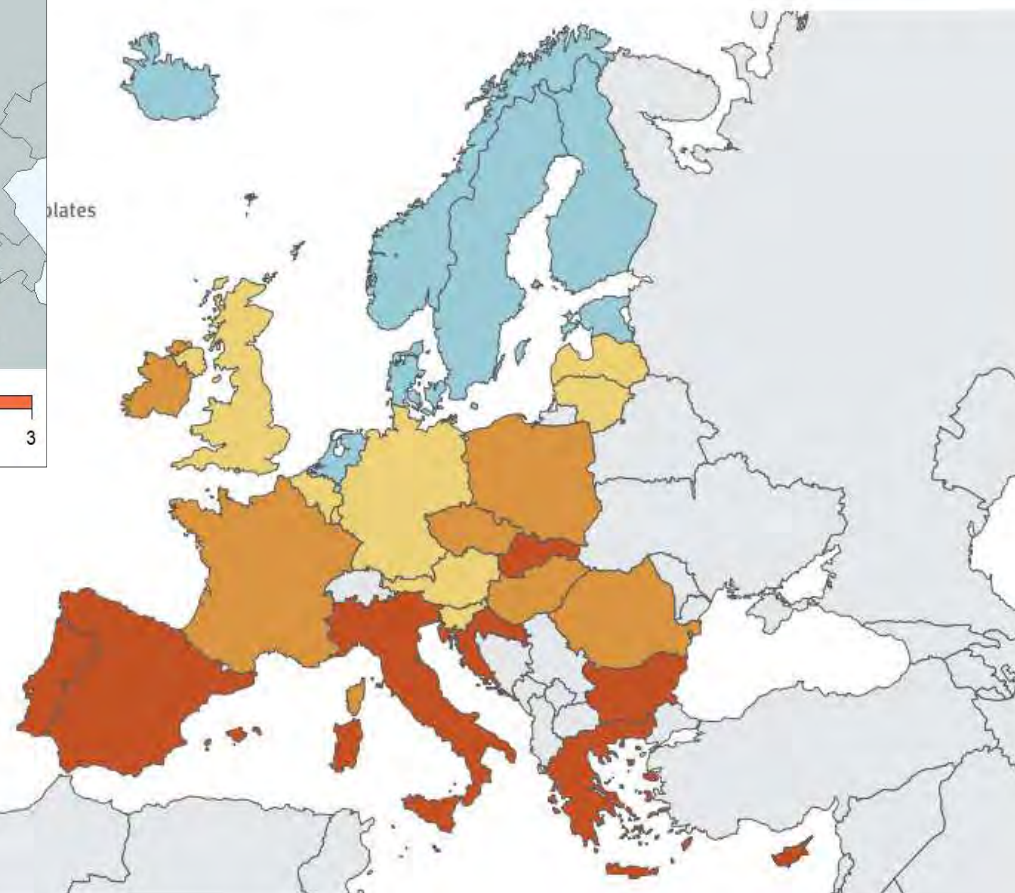
Conclusions

- Veterinary students demand an improvement (75%)
- Harmonisation of curricula in AMS
- More interesting questions to analyse
  - "Test" questions
  - Teaching methods
- Fact-based communication of the veterinary role in human AMR

Thank you

ESGVM ESCMID STUDY GROUP FOR VETERINARY MICROBIOLOGY
European Society of Clinical Microbiology and Infectious Diseases

Luca Guardabassi; Lisbeth Rem Jessen

ESGAP ESCMID STUDY GROUP FOR ANTIBIOTIC POLICIES
European Society of Clinical Microbiology and Infectious Diseases

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- Modestas Ružauskas

- Plamen Trojacanec
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- Dorota Chrobac
- Costanca Pomba
- Dorina Timofte
- Dušan Mišić
- Irena Zdovc; Silvestra Kobal
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- Susanna Stenbjerg Lewerin
- Hanspeter Naegeli; Cedric Muentener
- Naim Deniz Ayaz; Muammer Goncuoglu
- Dorina Timofte

AND ALL VET SCHOOL COORDINATORS

Why should students be prepared?

Obstetrics

Haematology

Urology

Paediatrics

Colorectal surgery

Geriatrics

Orthopedic surgery

Internship
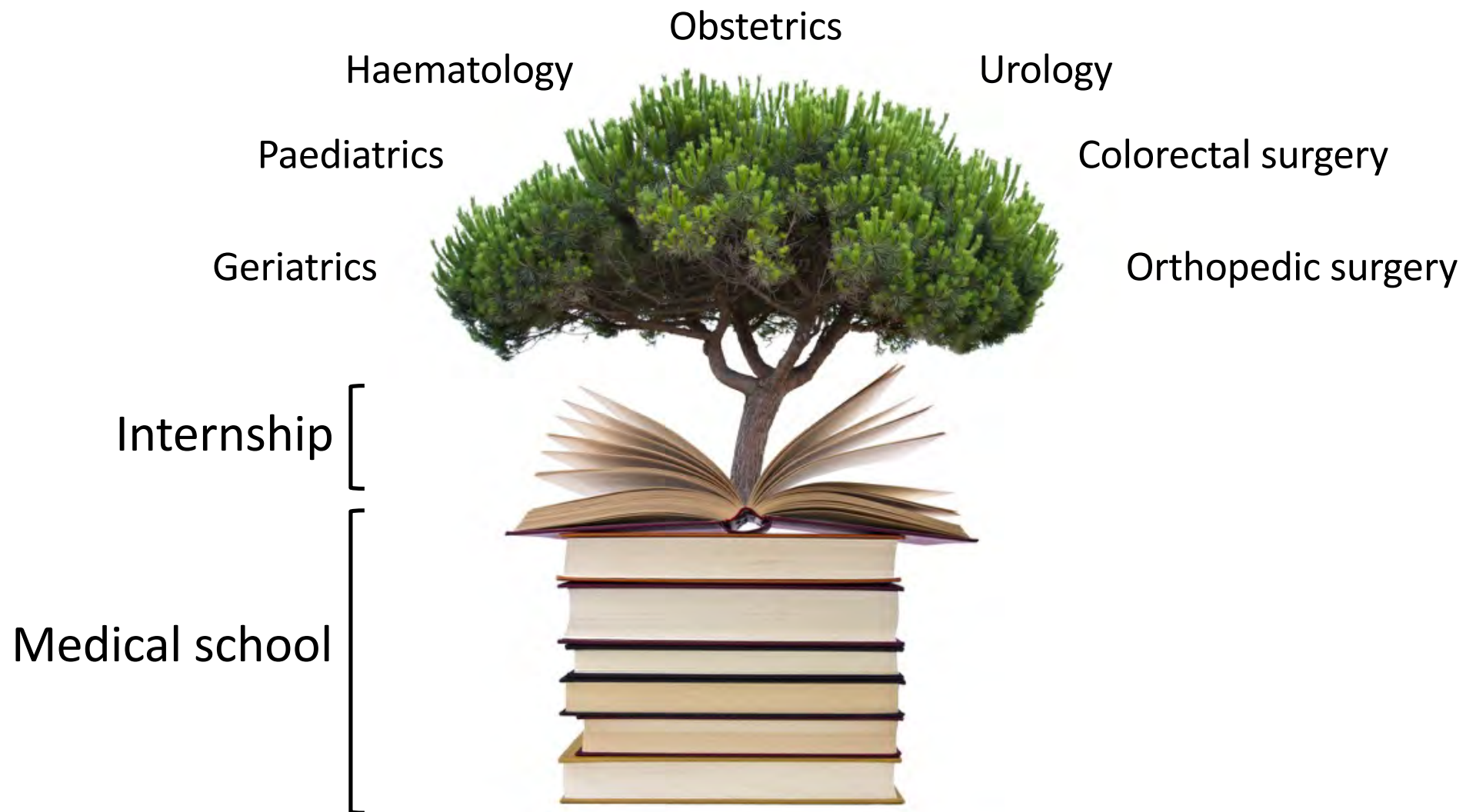
Medical school

Are we preparing students?

"Students graduate from medical school using antimicrobial agents as a substitute for diagnostic acumen"

Harold C. Neu, 1978

Crossing the gap

Skills that may be lacking:

Prescribing

Decision making, treatment planning

Taking responsibility for own learning

Managing stress in the workplace

Teamworking

Interpersonal skills

Competence in carrying out clinical procedures

Alexander C et al Clin Teach 2014; 11:188-192

DOES

SHOWS HOW

KNOWS HOW

KNOWS
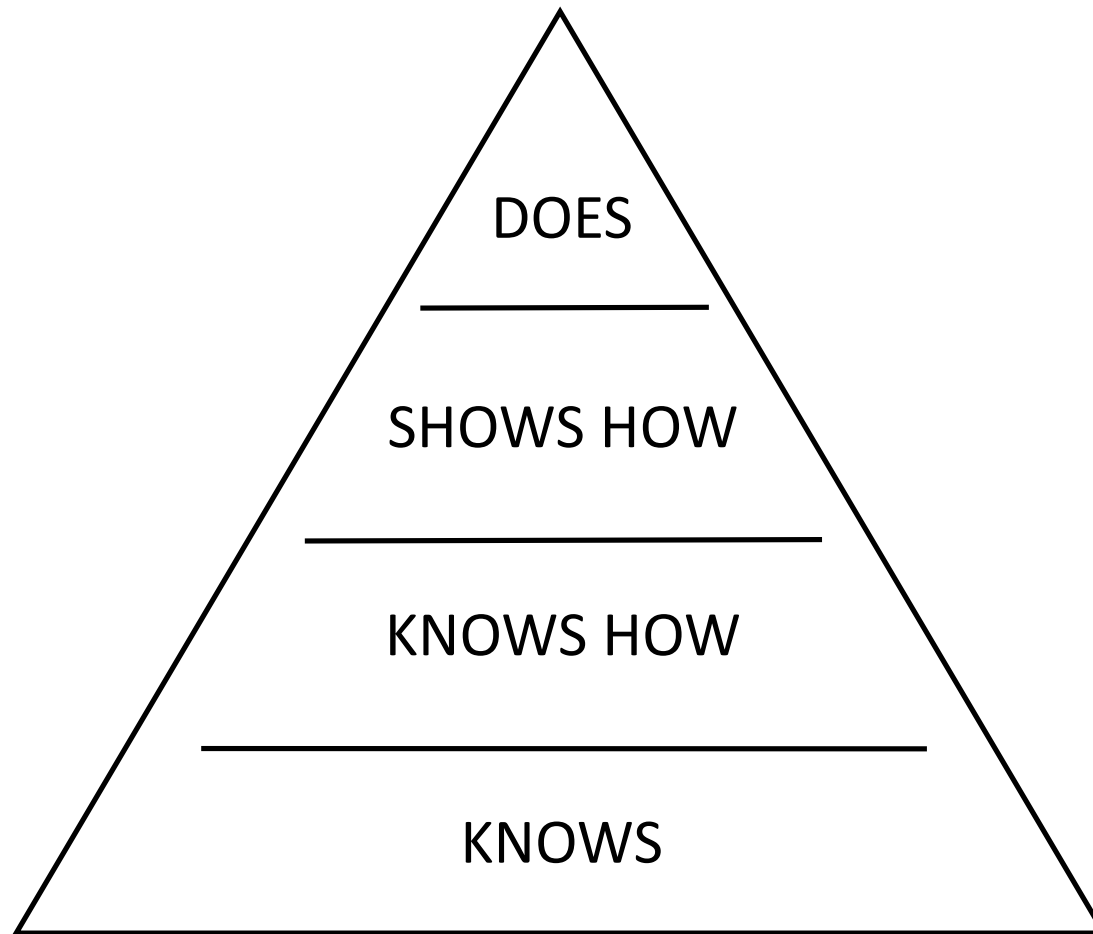
Miller G. Acad Med 1990;65:S63-S67

Do junior doctors feel confident?

Pulcini C et al. CMI 2011;17:80-87
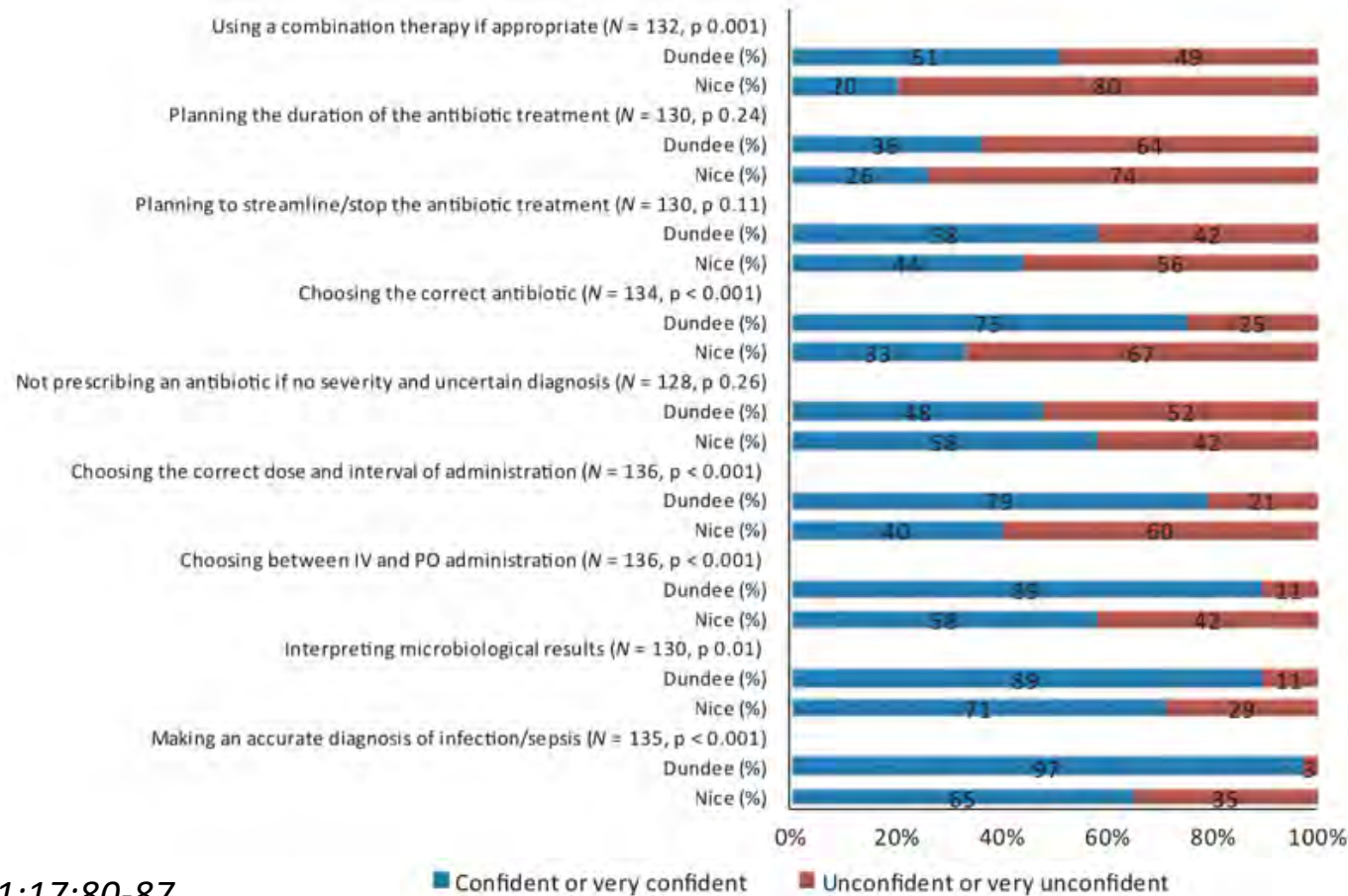
Curriculum study (2013)

Wide variations in content and structure

Some important principles poorly covered

National framework in only 4 countries

Pulcini C et al. CMI 2015 21(4):354-61

Do students feel prepared?

Study	Region	Percentage who would like or feel they need more education
Minen M et al 2010	USA	78%
Abbo L et al 2013	USA	90%
Huang Y et al 2013	China	89%
Dyar OJ et al 2014	Europe	74%
Haque M et al 2016	Malaysia	88%
Wasserman S et al 2017	South Africa	95%
Dyar OJ et al 2018	Europe	64%

Student-PREPARE (2015)

Cross-sectional questionnaire

All final year medical students eligible

Aim: identify specific unmet needs

More education on responsible antibiotic use — 37%

No need for more education — 31%

Unsure — 5%

More education on general antibiotics and responsible antibiotic use — 27%
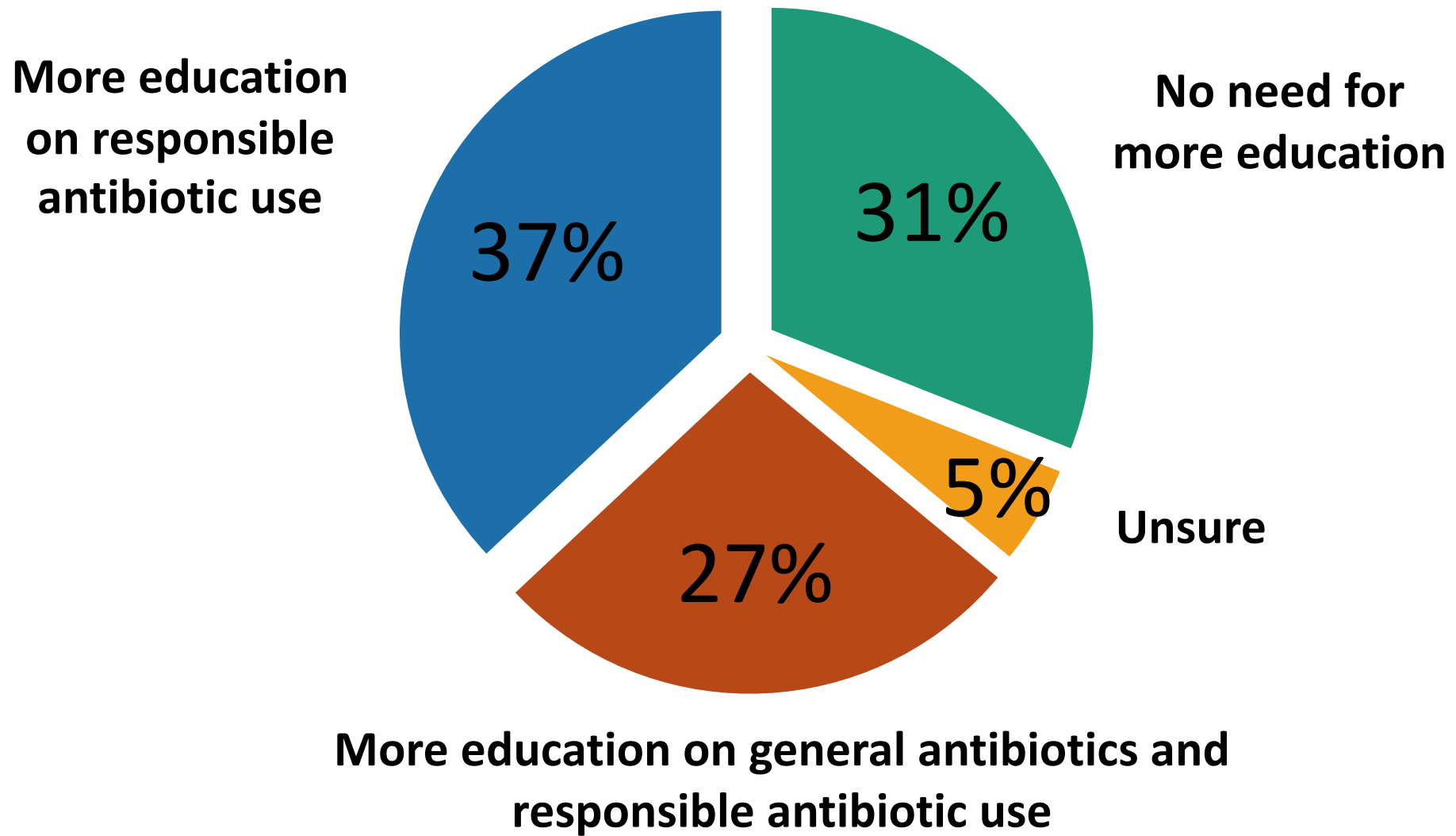
Students who feel they need more education on antibiotics

25-35%

36-50%

51-60%

61-70%

71-80%

81-90%
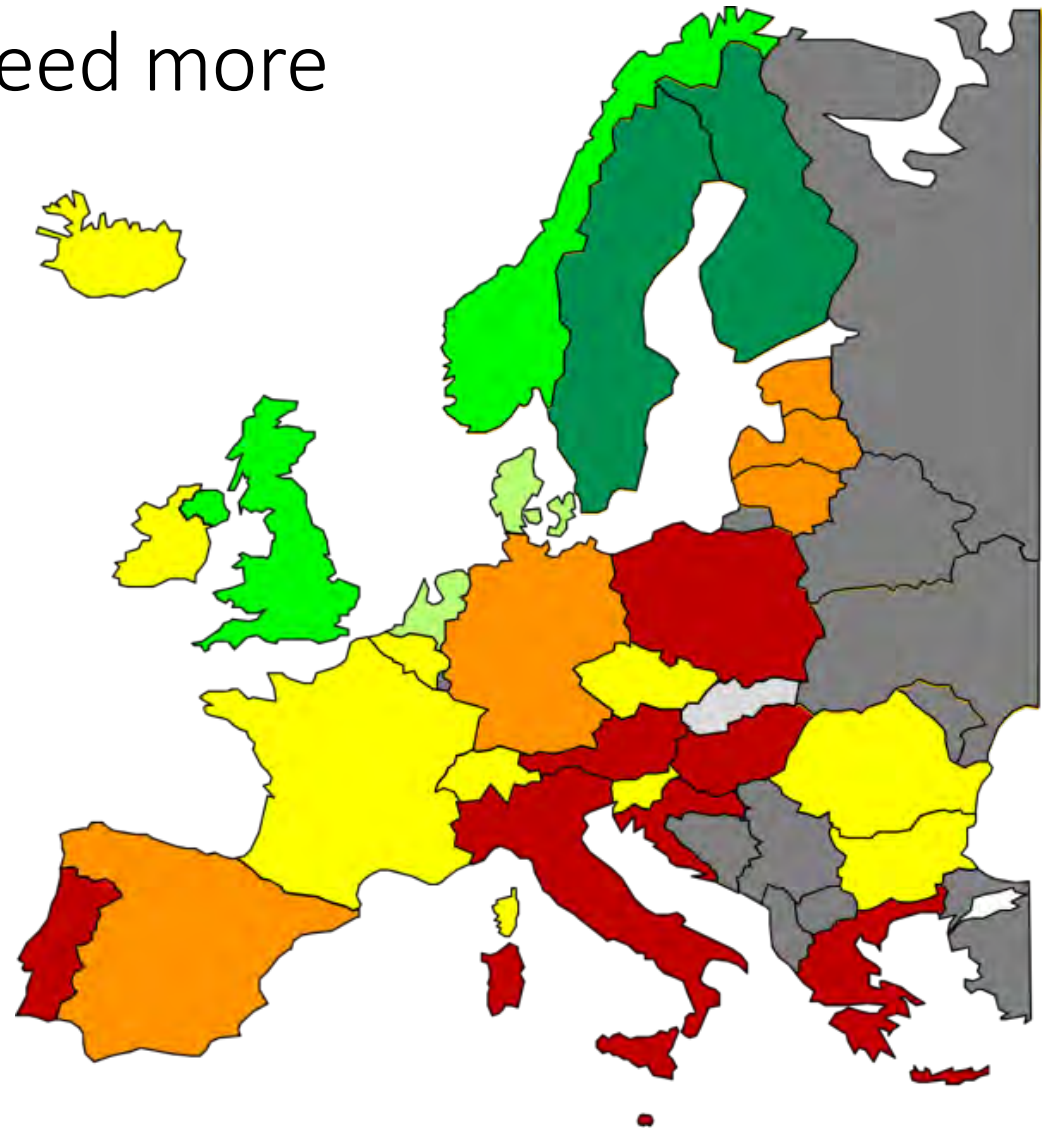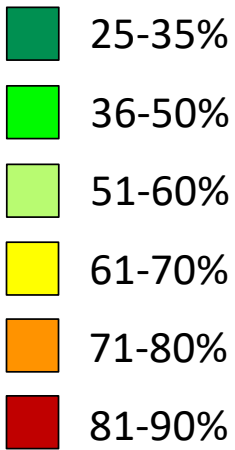
Country grouping by susceptibility score

- **High** (green)
- **Medium** (yellow)
- **Low** (red)
- **Data unavailable** (grey)
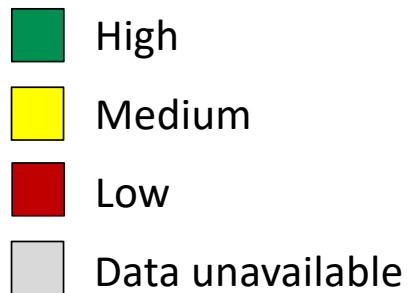
Score using 2014 EARS-NET data:

Methicillin – *S. aureus*

3rd generation cephalosporins – *E. coli*

Fluoroquinolones – *E. coli*

Macrolides – *S. pneumoniae*
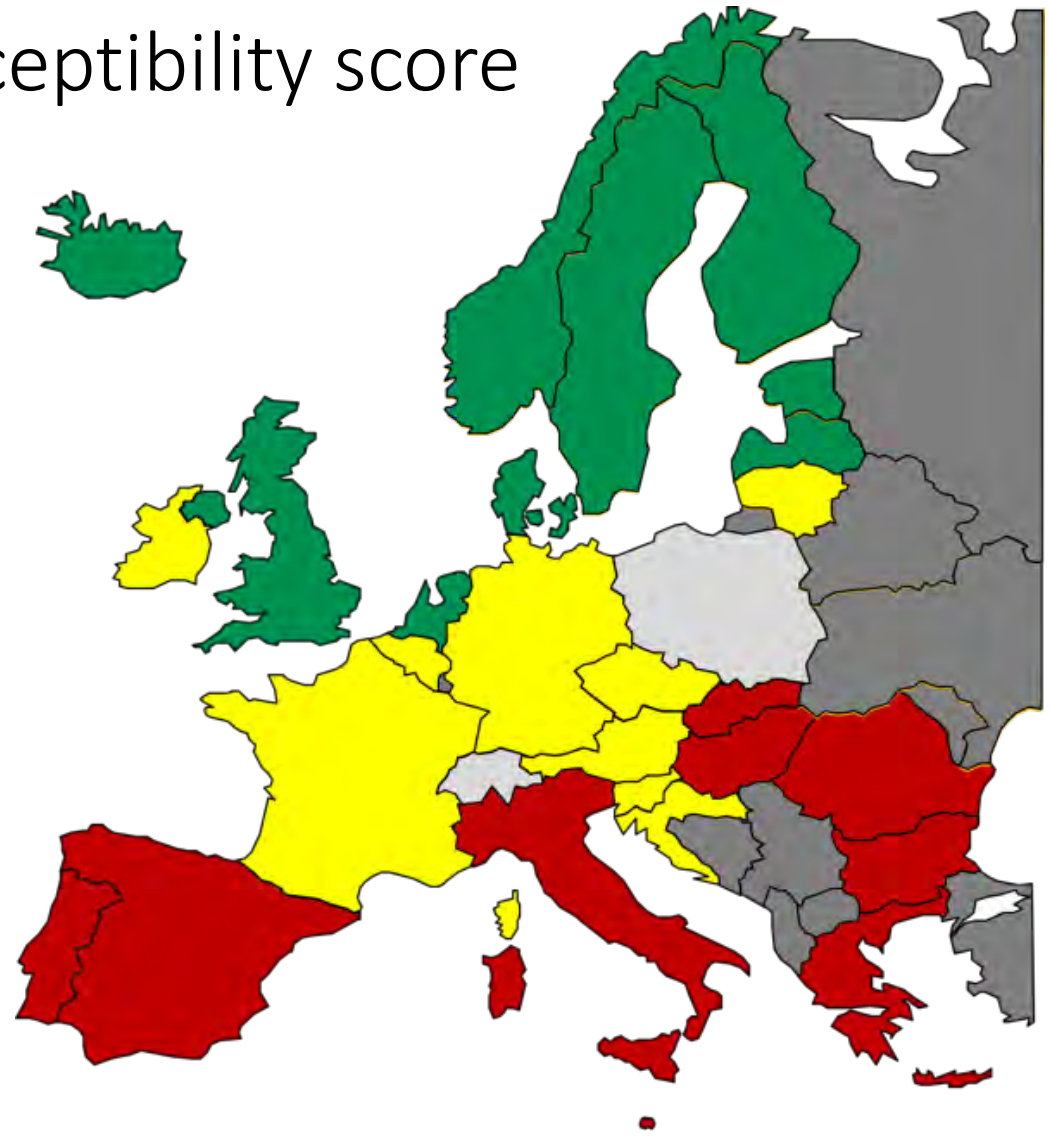
Susceptibility score, education needs and preparedness
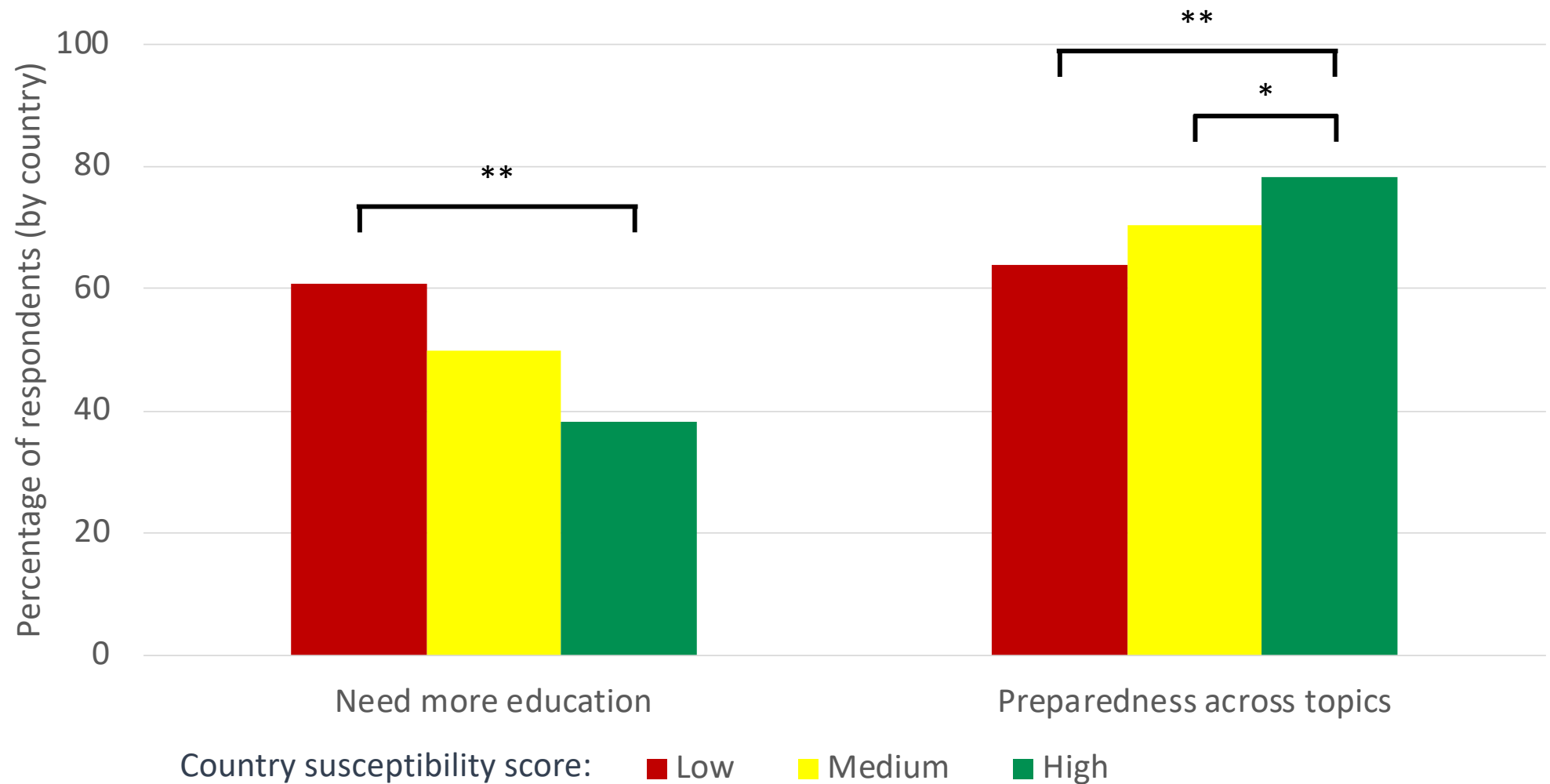
Students feel well prepared to

Recognise clinical signs of infection

Assess the severity of an infection

Differentiate between bacterial and viral upper respiratory tract infections

■ Not sufficiently prepared   ■ Unsure   ■ Sufficiently prepared
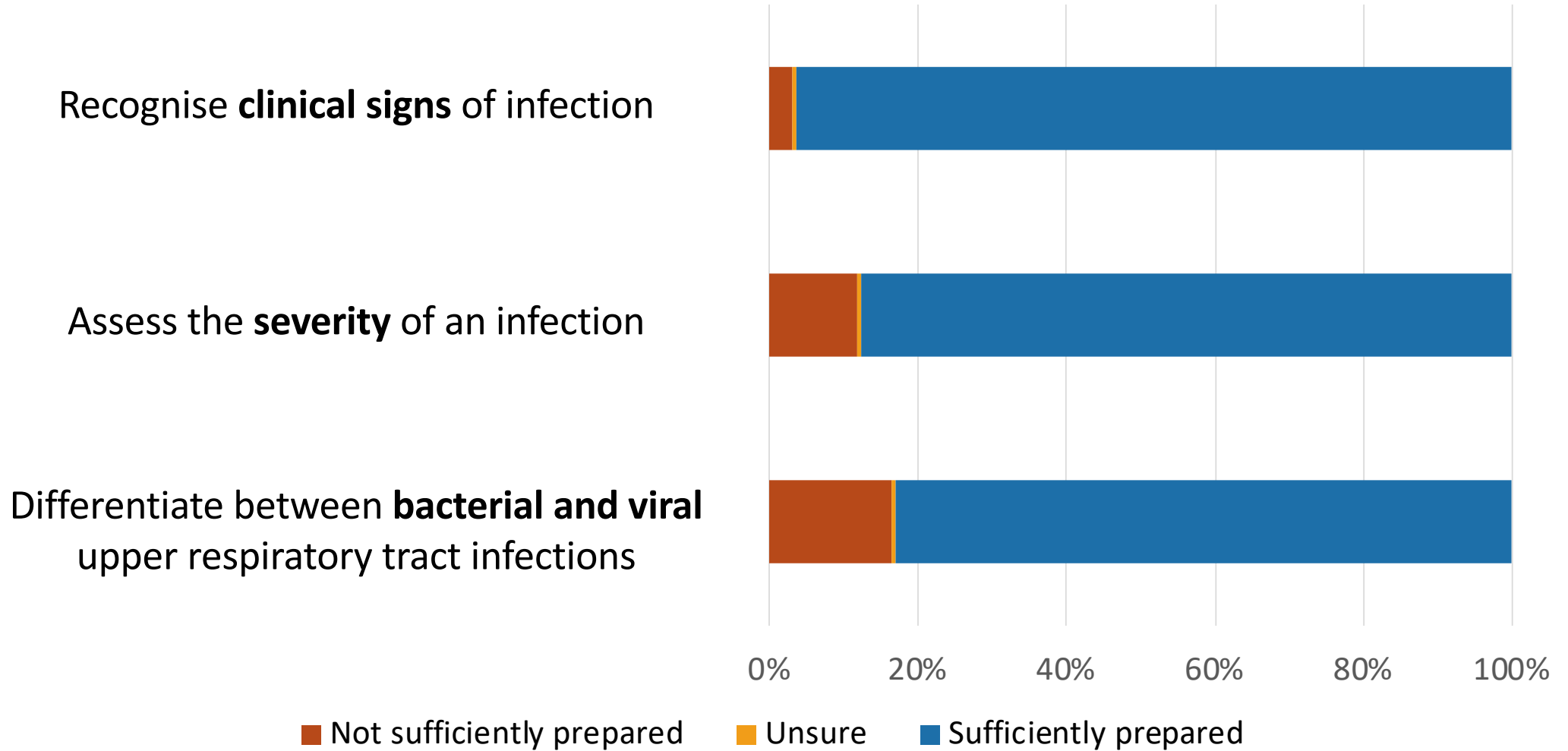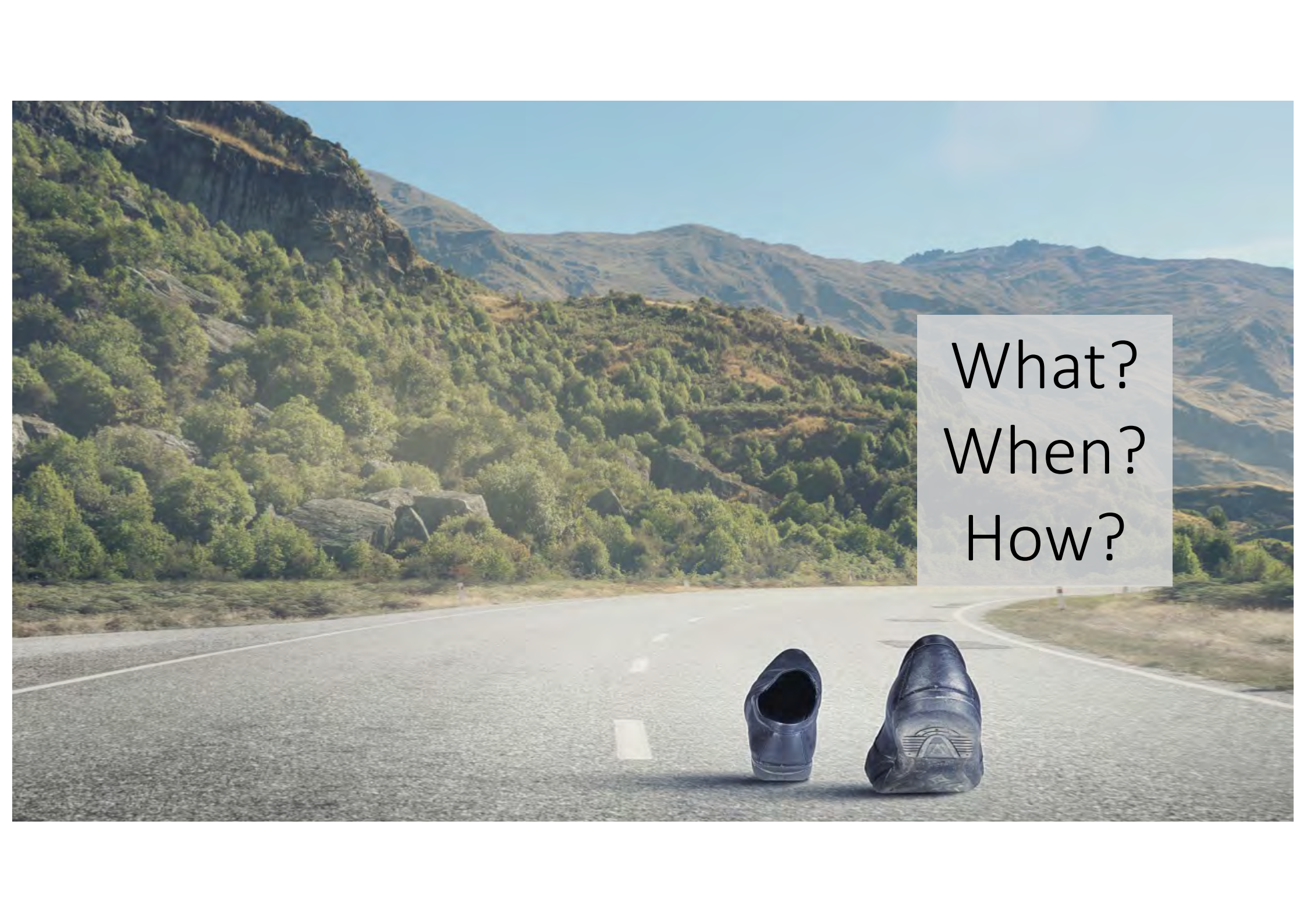
Students feel poorly prepared to

Decide the shortest adequate treatment duration for an infection

Use **surgical antibiotic prophylaxis** principles

Measure antibiotic use and **interpret** the results of such studies

0%   20%   40%   60%   80%   100%

■ Not sufficiently prepared   ■ Unsure   ■ Sufficiently prepared
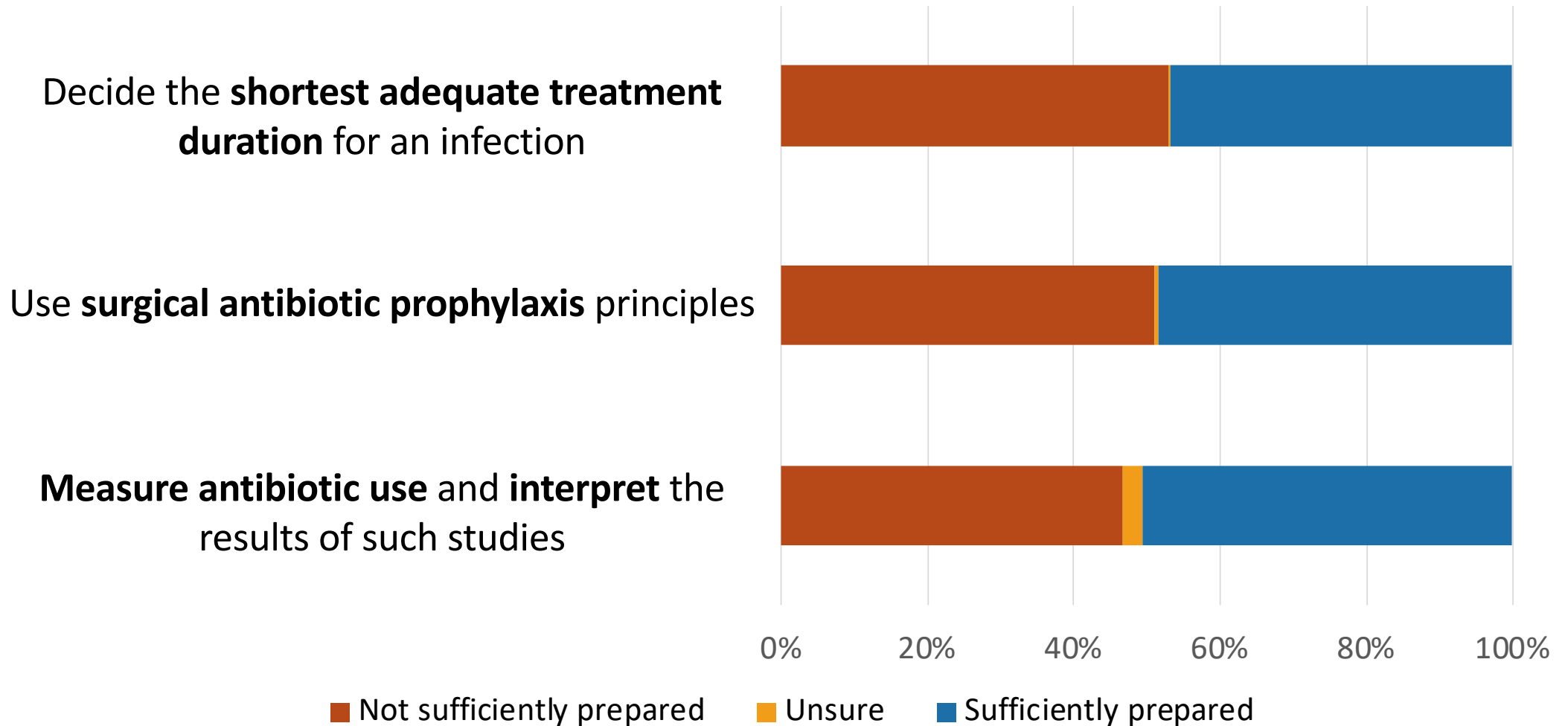
Communicating about antibiotics
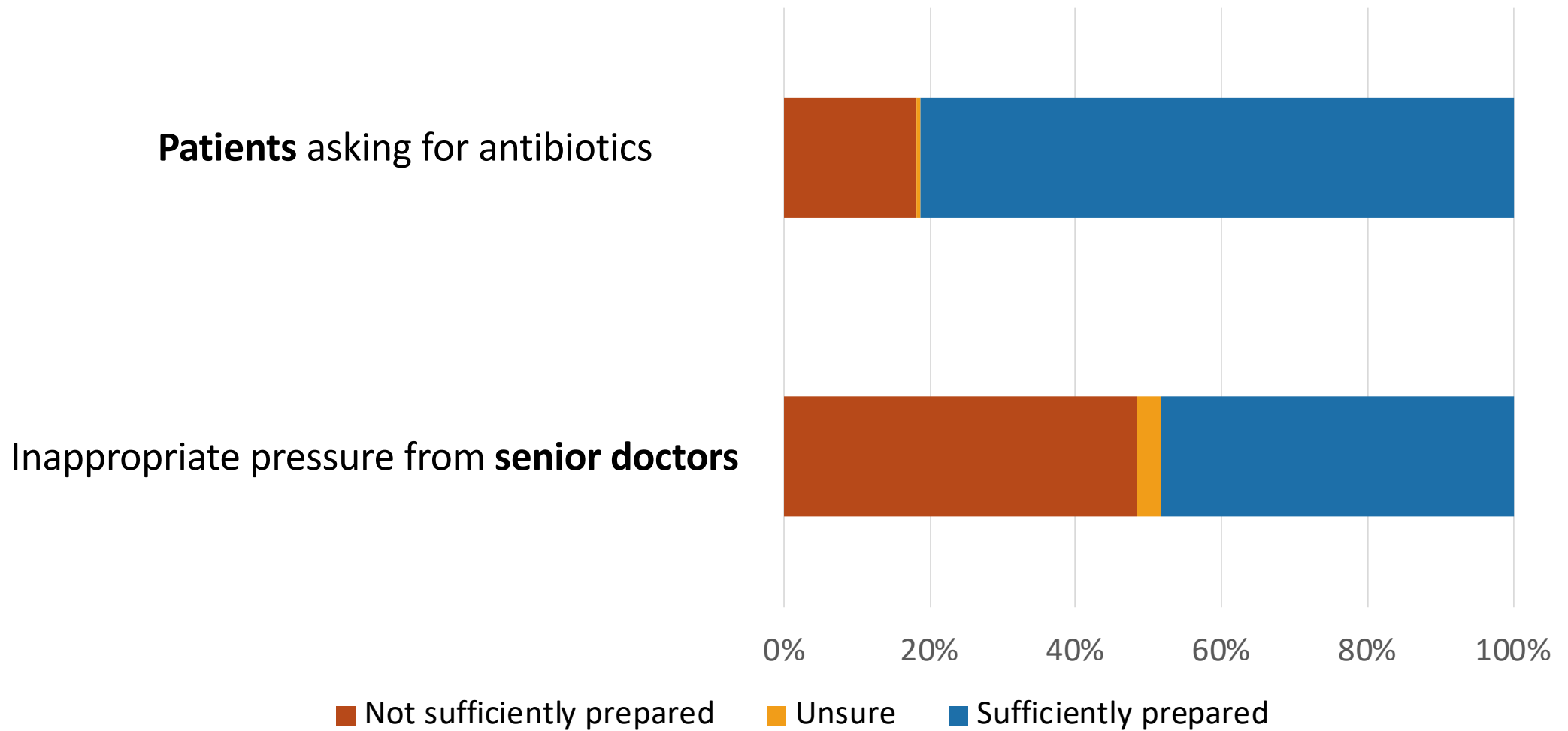
Patients asking for antibiotics

Inappropriate pressure from **senior doctors**

0%   20%   40%   60%   80%   100%

■ Not sufficiently prepared   ■ Unsure   ■ Sufficiently prepared

What?
When?
How?

WHO COMPETENCY FRAMEWORK
FOR HEALTH WORKERS' EDUCATION
AND TRAINING ON ANTIMICROBIAL
RESISTANCE

World Health Organization

Table 1. AMR competency framework

Antimicrobial resistance domains	Category 1: All health workers	Category 2: Prescribers	Category 3: Non-prescribers			Category 4: Public health officers/ health service managers
			Nurses	Pharmacists	Laboratory scientists/ technicians	
Foundations that build awareness of antimicrobial resistance **Competency statement:** Health worker demonstrates that they have the knowledge and awareness of effective approaches to control AMR, and has the skills/attitudes to implement change according to role and level of training.	Relevance: High Knowledge: 1. Understand the development and main causes of AMR. 2. Understand the basic principles of infection prevention and control, i.e. hand hygiene to prevent transmission of infections. 3. Understand the impact of resistance on choice of antimicrobial therapy for treating infections. 4. Understand the morbidity, mortality and economic threat of AMR to human health. 5. Know the importance of optimizing use of antimicrobials in the human and animal sector to prevent development of resistance. Skills: 1. Ability to interpret, communicate the appropriate policies on AMR. Attitudes: 1. Promote awareness of appropriate antimicrobial use amongst all health patient communities, general public. 2. Act to protect the use of antimicrobials as imperative and a	Relevance: High Knowledge: 1. Understand the importance of antimicrobial choice, dosage, interval, duration, preparation and administration of antimicrobials. 2. Know the principles of microbiology in identifying pathogens from clinical samples. 3. Know the basic diagnostic role of the microbiology laboratory. 4. Understand local AMR epidemiology, resistance and susceptibility patterns and use of guidelines. 5. Patient counselling etiquettes, discussion techniques and psychology for patient communication.	Relevance: High Knowledge: 1. Understand the role of bedside nursing in antimicrobial stewardship programmes. Skills: 1. Assess the source of infection and identify appropriate measures. 2. Obtain allergy history, perform medication reconciliation, and record this in the medical record. Attitudes: 1. Contribute to a patient-centred focus in the clinical	Relevance: High Knowledge: 1. Understand the significance of antimicrobial choice, dosage, duration and preparation in the treatment of infections. Skills: 1. Advise patients and prescribers on the appropriate use of antimicrobials. 2. Practise safe disposal of unused antimicrobial medicines. Attitudes: 1. Advocate for patient safety and contribute to the	Relevance: High Knowledge: 1. Understand the diagnostic role of the microbiology laboratory in detecting infections, resistance patterns, guiding patient management and informing AMR control strategies. Skills: 1. Collect and report data on antimicrobial product quality and sensitivity to national drug registration bodies. 2. Advise prescribers on correct microbiological testing procedures. 3. Ability to carry out bacterial	Relevance: High Knowledge: 1. Understand the use of quality improvement frameworks to address gaps in AMR education. 2. Understand the potential for cost savings and health gains associated with effective infection control and appropriate antimicrobial use. 3. Understand the roles and responsibilities of different stakeholders in antimicrobial stewardship teams. Members of the team could include, but are not limited to, the roles of physicians, pharmacists, infection prevention, microbiologists, nurses and health epidemiologists.
Foundations that build awareness of antimicrobial resistance		2. According to settings and where appropriate, encourage adherence to antimicrobial formulary/ protocol restrictions. 3. Understand basic principles of behaviour change in the context of prescribing antimicrobials and model good prescribing behaviour to colleagues.				2. Establish and enforce compliance with antimicrobial formulary/ protocol restrictions at local and national levels according to country policies. 3. Together with civil society, advocate for the responsible development of new antimicrobials and ensure the correct promotion of existing ones.

¹ This framework assumes that knowledge contents are similar for pre-service education and in-service training though emphasis shifts to improving skills and attitudes for in-service training.
² Denotes the basic AMR competencies that all health care workers should have.
³ Includes medical doctors and dentists. Note that pharmacists, nurses and midwives and other health care workers are also included in this category in settings where they are allowed to prescribe antimicrobials by regulation. The extent to which the prescribing competencies are relevant to the different cadres may vary according to scopes of practice and local regulation.
⁴ Non-prescribers include health workers that are not allowed by regulation to prescribe antimicrobials. (Note that in some settings, pharmacists, nurses and midwives are allowed by regulation to prescribe antimicrobials.)
⁵ This category may include personnel from the prescribing and non-prescribing occupational groups who have a leadership role or authority in managing AMR control.

Generic competencies in antimicrobial prescribing and stewardship (2018)

66 expert panel members
24 countries

High acceptance
98% of expert panel

ESCMID — EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES
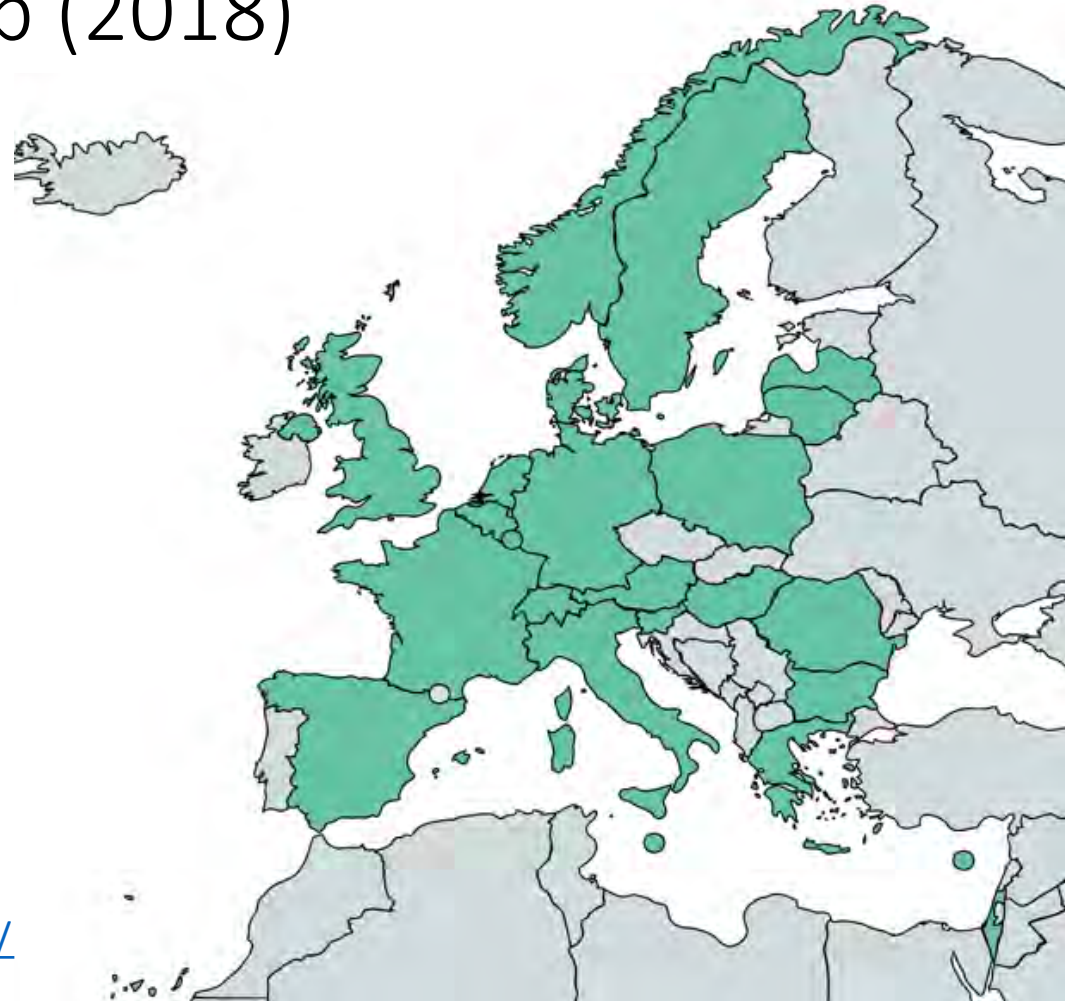
Dyar OJ et al. CMI 2019 25(1):13-19
https://www.escmid.org/escmid_publications/white_papers/
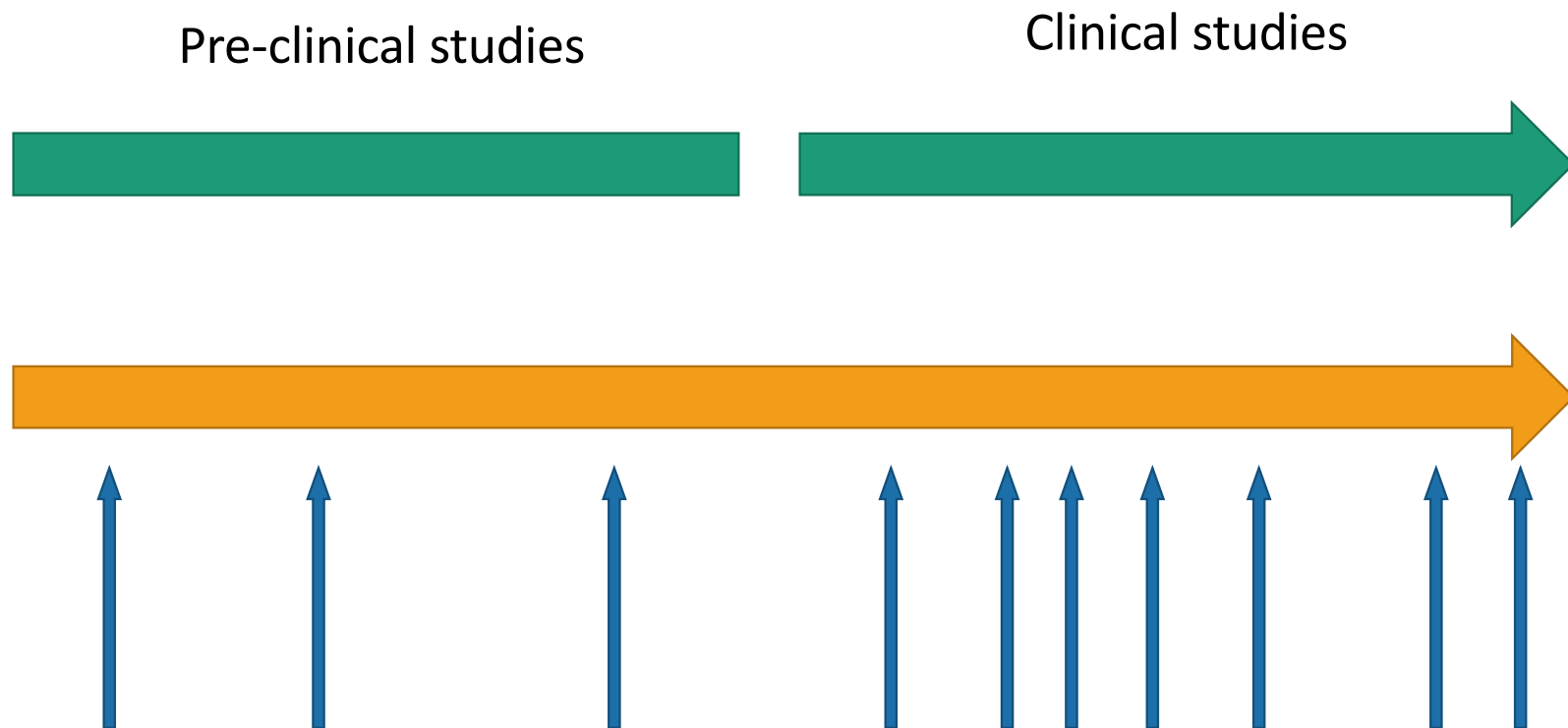
How much time?

Castro-Sanchez E et al. PLoS One 2016 11(2):e0150056

Portfolios

Outcome		E-learning	Control	Δ	95% CI	P	n
Short-term							
- knowledge		4.97	4.47	0.50	(0.04–0.95)	0.032	124
Long-term							
- overall		7.07	6.76	0.31	(0.08–0.53)	0.007	356
- knowledge		6.87	6.55	0.32	(0.03–0.62)	0.031	356
- drug choice		7.04	6.54	0.51	(0.04–0.97)	0.034	356

Difference between groups
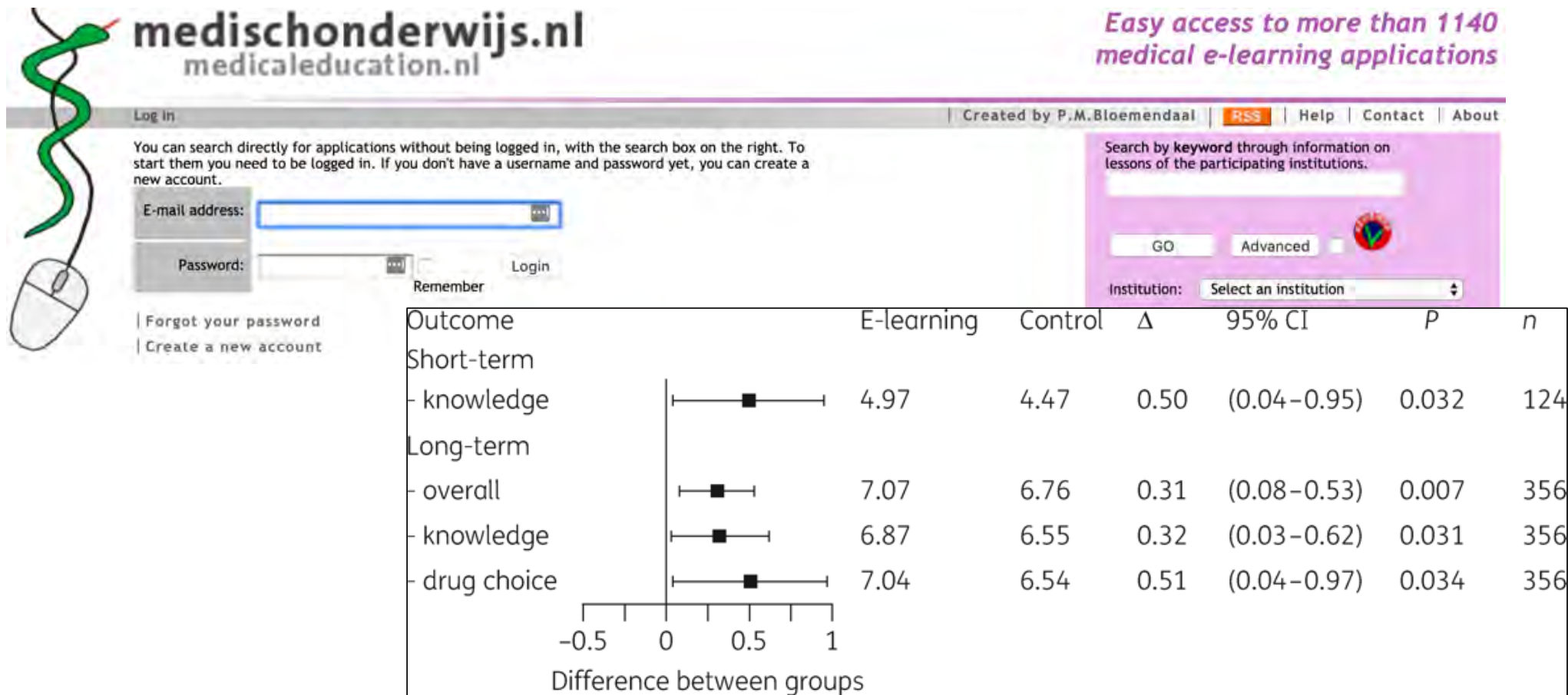
Sikkens J et al. JAC 2018 73(8):2243-2246

Karbownik MS et al. FEMS Microbiol Lett 2016 363(7): pii: fnw045

Miller G. Acad Med 1990;65:S63-S67

Tomorrow's prescribers (and patients) need us to:

- Improve undergraduate education on responsible antibiotic use

- Reach a greater consensus on the what, when and how

- Develop, evaluate and share educational resources

Next generation sequencing: first diagnostic one-stop shop in one health microbiology

John WA Rossen

Personalised Microbiology

Department of Medical Microbiology and Infection Prevention, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

Human (AZU)

Animal (VET)

John's PhD space (ENV)

ESGMD ESCMID STUDY GROUP FOR GENOMIC AND MOLECULAR DIAGNOSTICS

European Society of Clinical Microbiology and Infectious Diseases

Rossenlab Personalised Microbiology

Solidness Surveillance Of mobiLome meDiated aNtibiotic rEsiStance Spread

@SolidnessJPIAMR

Solidness.eu

info@solidness.eu

@rossenlab

WGS as 'One-Stop Shop'

Traditional microlab

Prep
- Sample processing
- Culture or direct detection

ID
- Determine genus/species
- Phenotype (e.g. serogroup)

AST
- Measure MIC or surrogate
- PCR for specific genes

Vir/Tox
- Phenotypic assays / animal
- PCR for specific genes

Typing
- DNA fingerprinting
- Sanger seq – MLST

WGS era

Prep
- Sample processing, culture
- Extraction, lib prep, seq

ID
- Genomospecies calling
- Further taxonomy

AST
- Map resistome, infer
- Detect new variants

Vir/Tox
- Map virulome, infer
- Gene expression?

Typing
- Extract current schemes
- Variant calling / extended MLST

By courtesy of Prof. dr. Jacob Moran-Gilad

WGS to map Outbreaks

- outbreak STEC young infants at a nursery
- repeated animal contact (animal farming and petting)
- Whole genome sequencing (WGS) → real-time investigation and revealed a unique strain of STEC O26:H11 carrying stx2a and intimin
- first STEC outbreak reported from Israel

Moran-Gilad et al., Epidemiol. Infect. (2017), 145, 2998–3006.

Outbreaks and MGE

VRE

ST80

ST262
CT60

CT104

CT16

CT106

Same ward

Same patient

ST117

CT 103

CT 24

CT 105

Long read sequencing
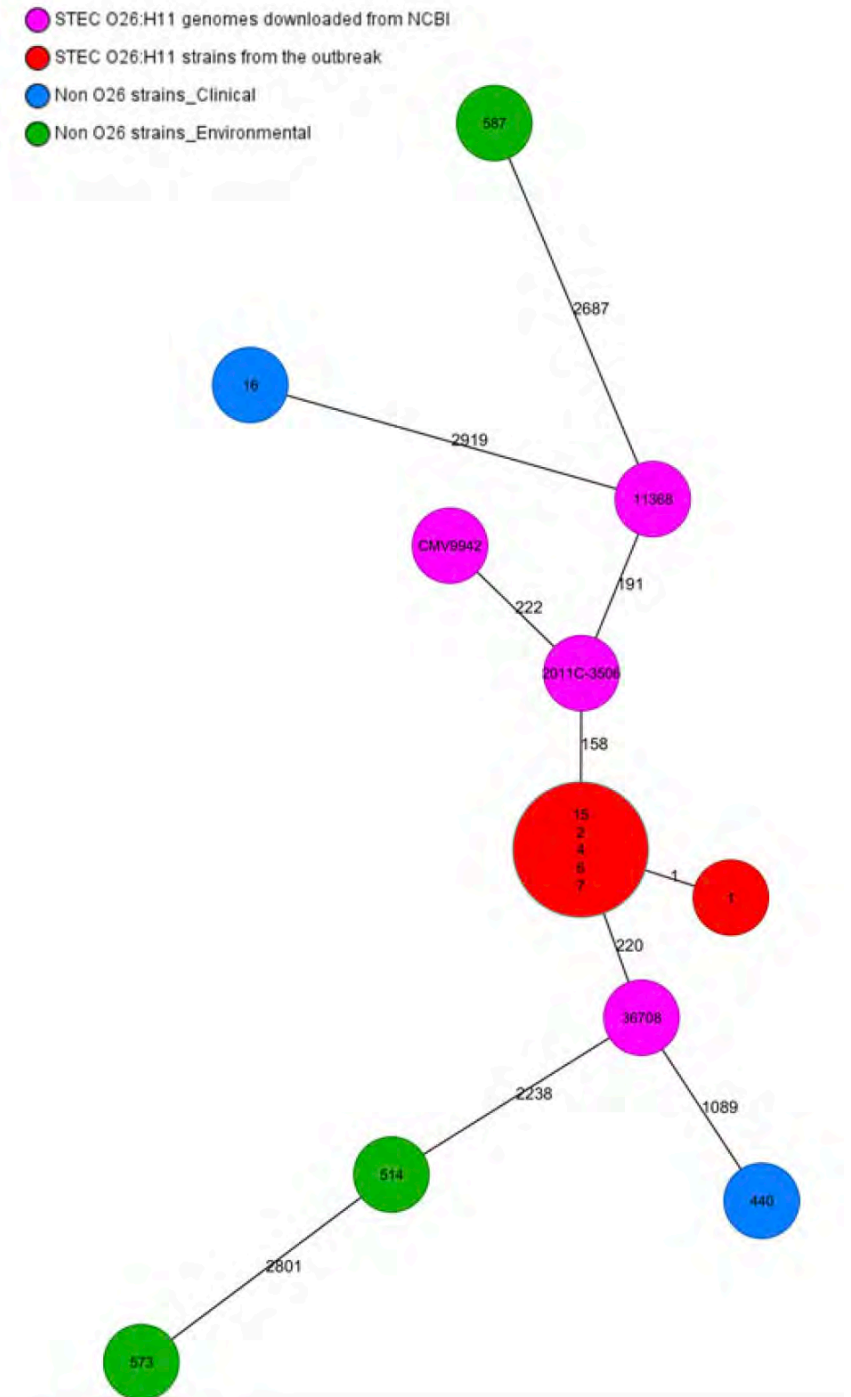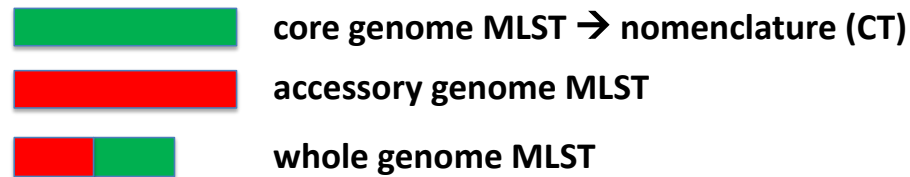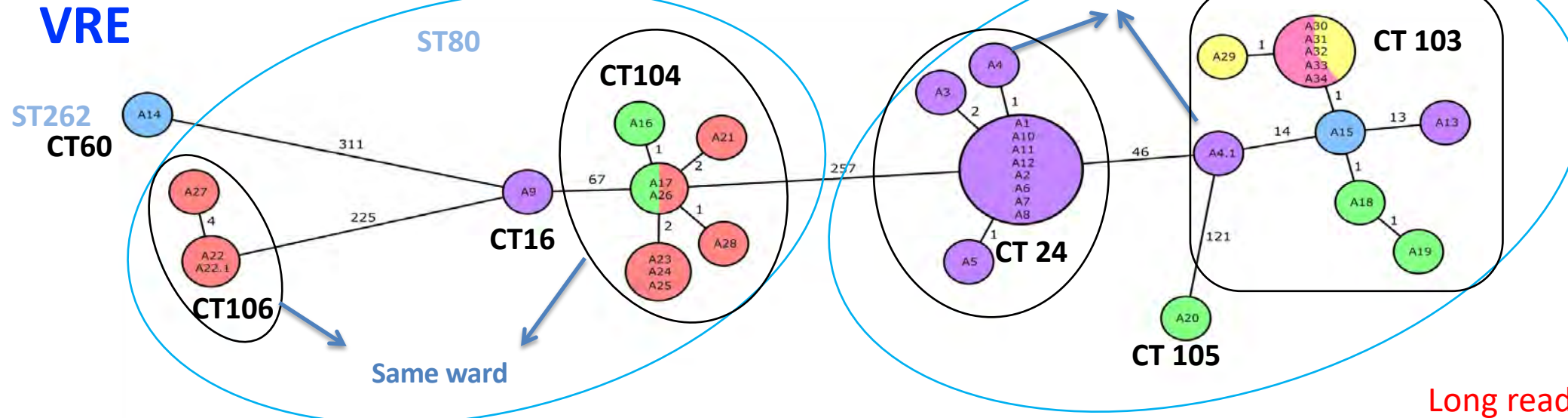
- Outbreak apr 2014 ward 1
- Outbreak jul 2014 ward 1
- Outbreak jul 2014 ward 5, 6, 7
- Outbreak nov 2014 ward 2
- Outbreak nov 2014 ward 8
- Outbreak dec 2014 ward 4
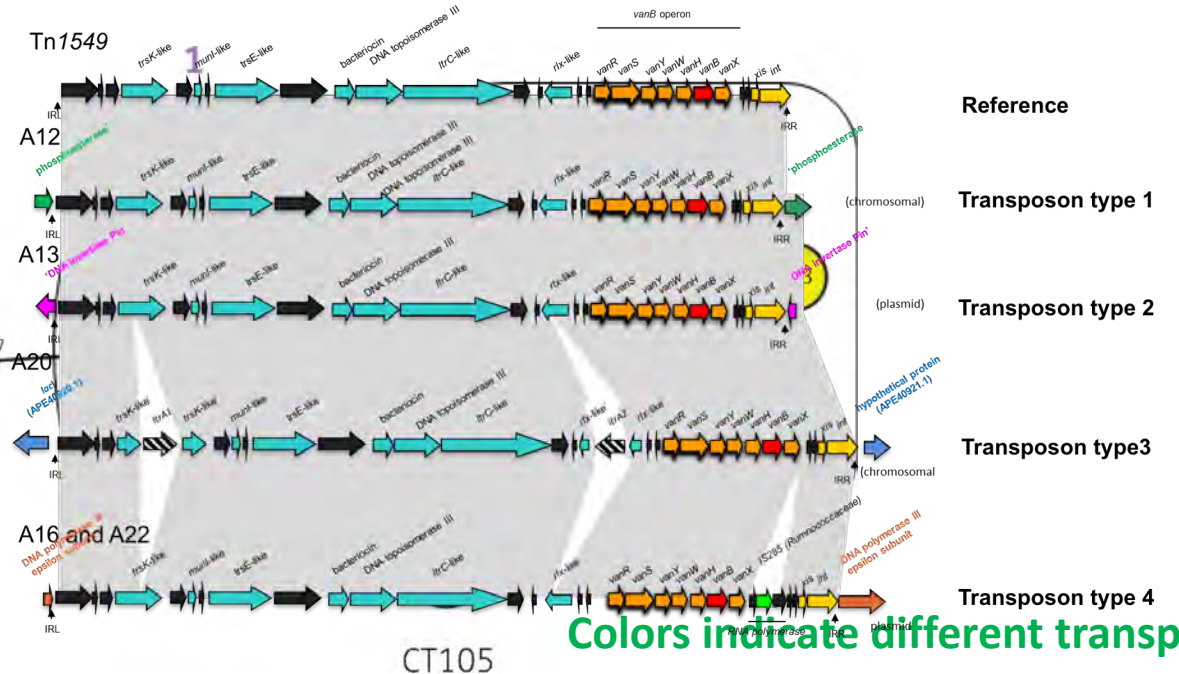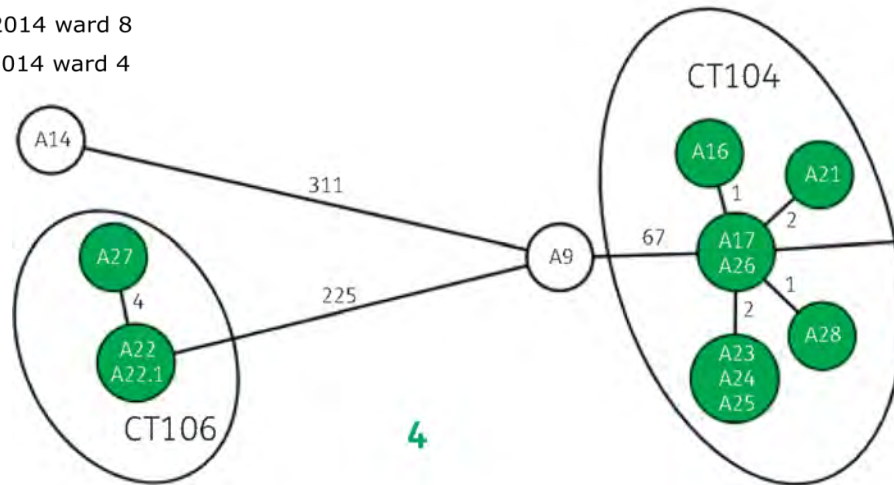
Zhou X et al. J Antimicrob Chemother. 2018 Sep 14. doi: 10.1093/jac/dky349

Colors indicate different transposons

Plasmid-mediated colistin resistance

Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study

Yi-Yun Liu*, Yang Wang*, Timothy R Walsh, Ling-Xian Yi, Rong Zhang, James Spencer, Yohei Doi, Guobao Tian, Baolei Dong, Xianhui Huang, Lin-Feng Yu, Danxia Gu, Hongwei Ren, Xiaojie Chen, Luchao Lv, Dandan He, Hongwei Zhou, Zisen Liang, Jian-Hua Liu, Jianzhong Shen

Presence of *mcr-1*-positive Enterobacteriaceae in retail chicken meat but not in humans in the Netherlands since 2009

Marjolein F.Q. Kluytmans - van den Bergh[1,2], Pepijn Huizinga[3], Marc J.M. Bonten[1,4], Martine Bos[5], Katrien De Bruyne[6], Alexander W. Friedrich[7], John W.A. Rossen[7], Paul H.M.Savelkoul[8,9], Jan A.J.W. Kluytmans[1,3]
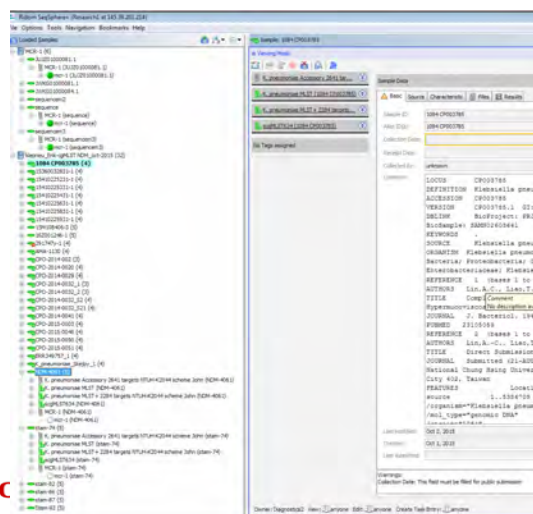
In silico resistance screening

SOLIDNESS - Surveillance Of mobiLome meDiated aNtibiotic rEsiStance Spread

The main goal of SOLIDNESS is to establish a network of excellence for surveillance of MGE- mediated antibiotic resistance and virulence spread

**Natacha Couto,
scientific coordinator**

solidness.eu

JPI-AMR 7th call project – supported by ZonMw

SOLIDNESS - Surveillance Of mobiLome meDiated aNtibiotic rEsiStance Spread

Sequencing harmonization

Team leader: Stefan Schwarz
Objective 1: to establish a protocol for sequencing of MGEs

Sample

DNA extraction

Library preparation

Cluster generation and sequencing

Bioinformatics workflow

Team leader: João A. Carriço
Objective 2: to define a bioinformatics pipeline for MGEs sequence analysis

Sequence-based typing

Team leader: Henrik Westh
Objective 3: to define new sequence-based typing methods, with improvement of nomenclature, for plasmids

"Can we control the dissemination of antibiotic-resistant/virulent bacteria by optimizing the surveillance of mobile genetic elements?"

John Rossen, University of Groningen Netherlands **– Lead partner**
Stefan Schwarz Freie Universität Berlin Germany
Silke Peter University of Tübingen Germany
Alban Ramette University of Bern Switzerland
Adam Roberts Liverpool School of Tropical Medicine
Spyros Pournaras University of Athens Greece
Martin Sundqvist Örebro University Hospital Sweden
Paulo Damasco Federal University of Rio de Janeiro Brazil
João A. Carriço Universidade de Lisboa Portugal
Annamari Heikinheimo University of Helsinki Finland
Stefano Morabito Istituto Superiore di Sanità Italy
Edward Feil University of Bath UK
Jacob Moran-Gilad Ben-Gurion University of the Negev Israel
Henrik Westh Hvidovre Hospital Denmark
Hajo Grundmann University of Freiburg Germany
Teresa Coque Ramón y Cajal University Hospital Spain
Tjaša Cerar Kišek University of Ljubljana Slovenia
Engeline van Duijkeren, RIVM, The Netherlands
Adam P. Roberts, Liverpool School of Tropical Medicine, United Kingdom
Holger Rhode, Universitätsklinikum Hamburg-Eppendorf, Germany
Pieter-Jan Ceyssens, Sciensano, Belgium
Joana Azeredo, University of Minho, Portugal

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Solidness
Surveillance Of mobiLome meDiated aNtibiotic rEsiStance Spread

ZonMw   jpiamr   umcg

WGS for discovering new resistance genes/mechanisms

- Three metronidazole resistant *P. bivia* strains

  - UMCG-3721 gluteal infiltrate of a 75-year-old patient resistant to amoxicillin, clindamycin and metronidazole, susceptible for amoxicillin-clavulanic acid and meropenem

  - UMCG-93105 abdominal infection of a 68-year-old patient resistant for amoxicillin, clindamycin and metronidazole, susceptible for amoxicillin-clavulanic acid, piperacillin-tazobactam and meropenem

  - UMCG-8631 from a previously healthy 27-year-old patient treated with cefotaxime, metronidazole, and teicoplanin (later vancomycin) → finally antibiotic treatment was switched to piperacillin/tazobactam and vancomycin

Three metronidazole resistant *P. bivia* strains

Fig 1. An alignment of the amino acids of the new NimK and other Nim proteins.
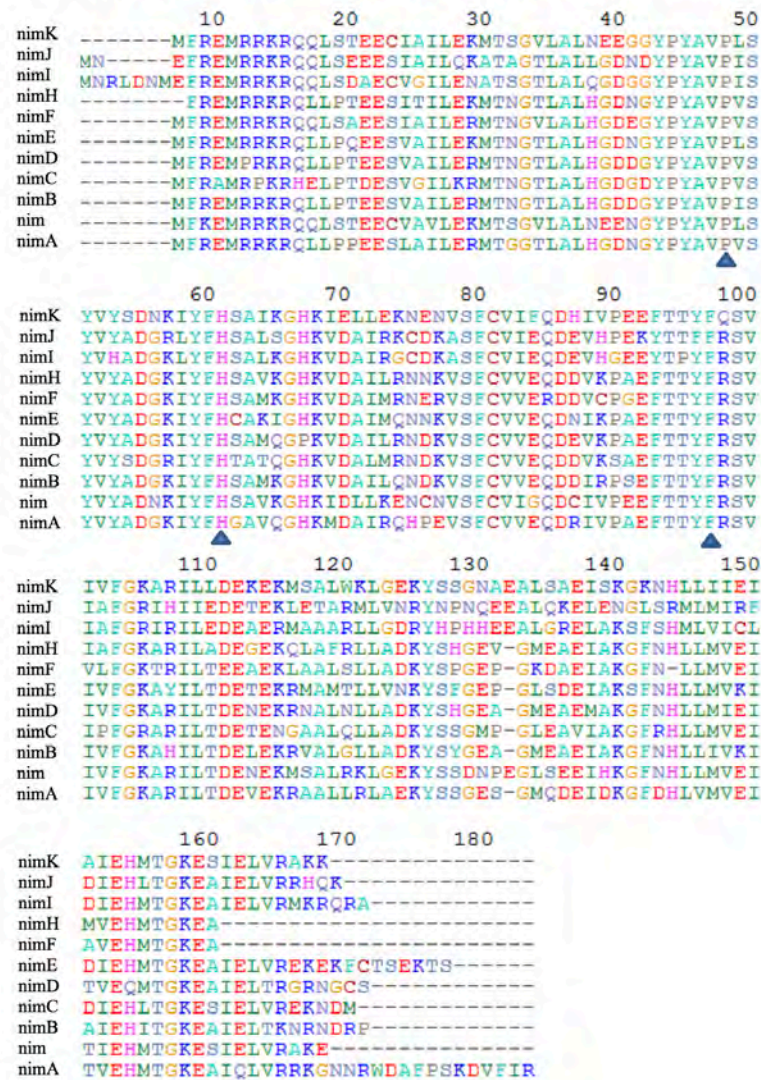
First description of a novel *nim* gene in metronidazole resistant *P. bivia* clinical isolates
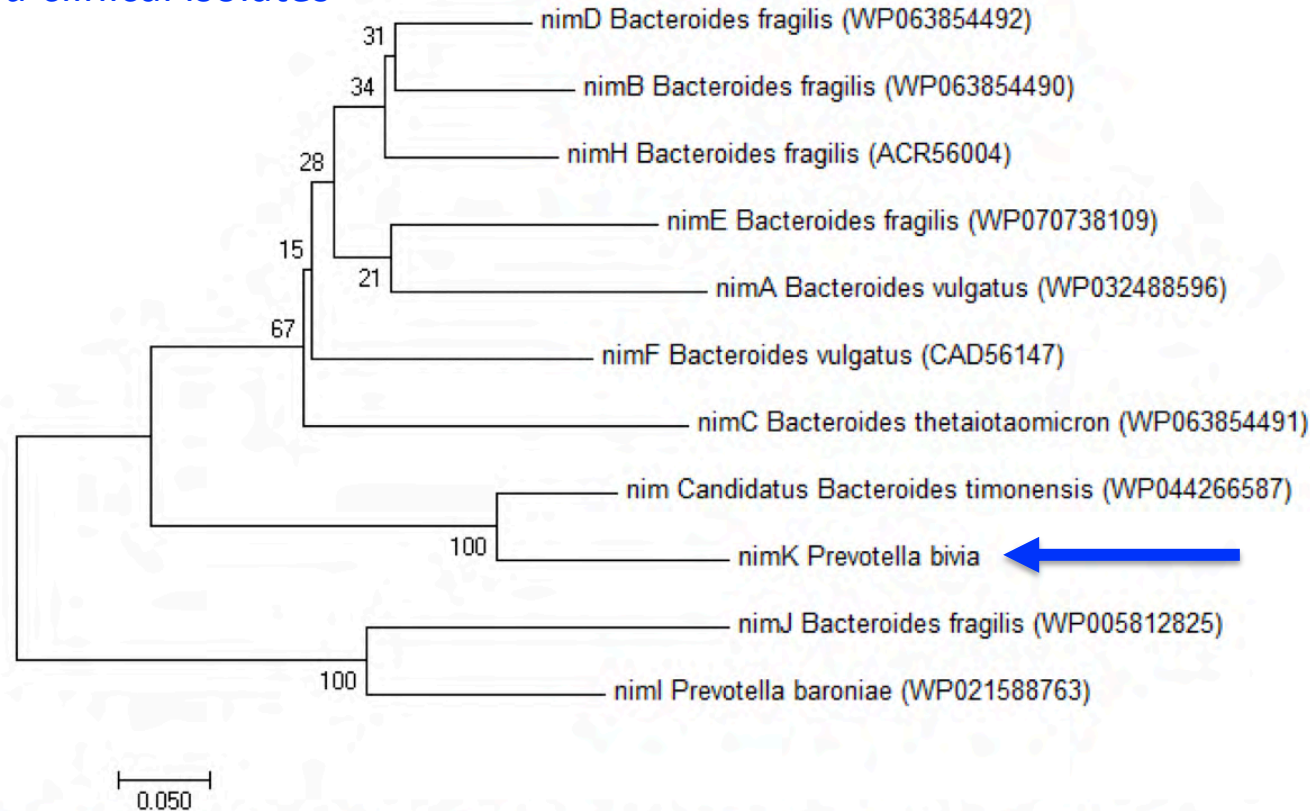
Fig 2. A phylogenetic analysis of the *nimK* gene was performed using the maximum likelihood method. Amino acid sequences were aligned using the MUSCLE method in MEGA7. A consensus tree was calculated from 500 bootstraps. The final dataset consisted out of 151 positions.
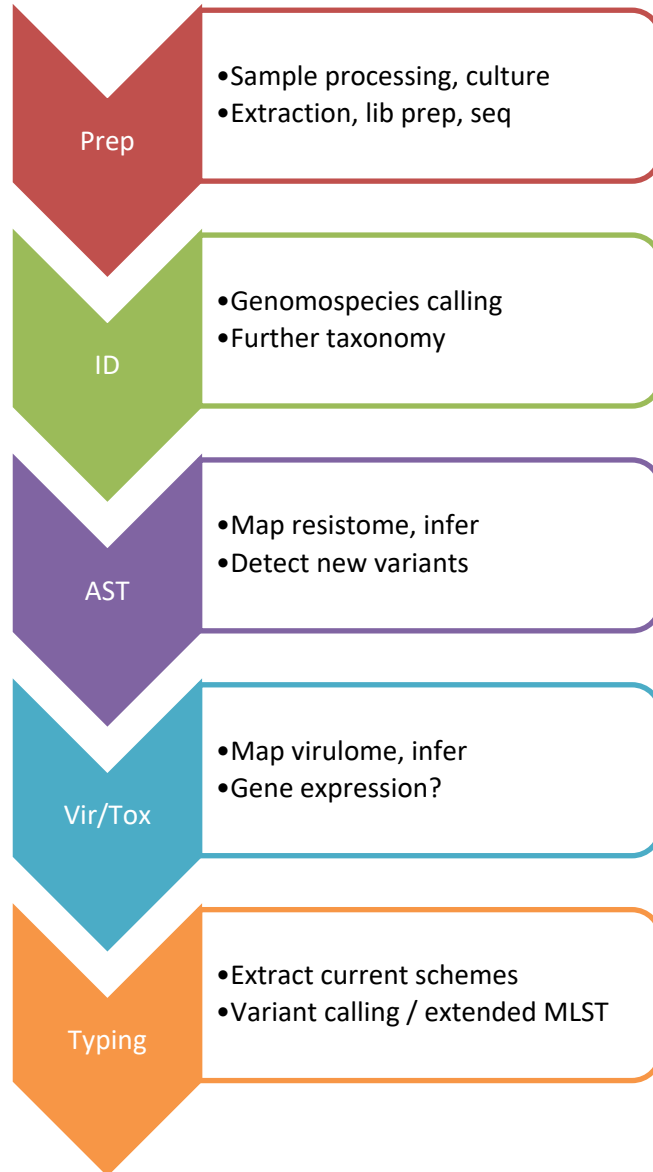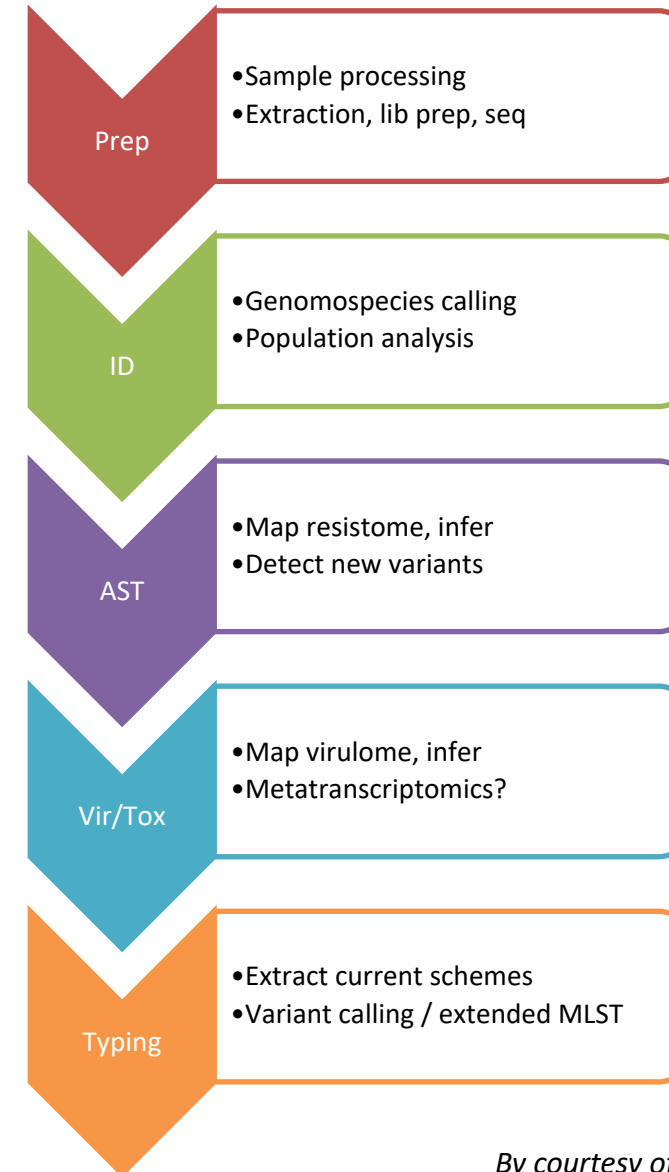
Metagenomics

Targeted-amplicon sequencing

- Taxonomical assignment

- Relative quantification

- Change over time

No Human DNA
More sensitive
Less data per sample
$

Shotgun metagenomics

- Taxonomical assignment

- Relative quantification

- Change over time

- Genomes

- Functions &
pathways
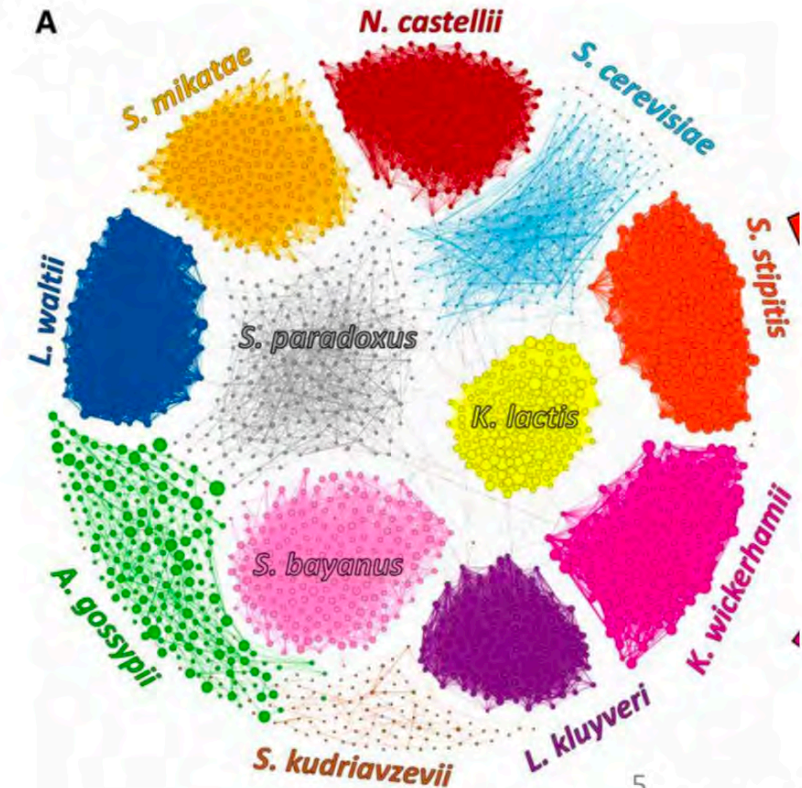
Human DNA
Less sensitive
More data
$$$$$

By courtesy of Claire Bertelli

Burton 2014 G3

5

Targeted metagenomics using 16S-23S NGS

- 16S sanger sequencing: not suitable for ID of multiple pathogens in one sample
- 16 S NGS: not always ID to the species level
- 16-23S: higher discriminatory power?

Dr. Mirjam Kooistra-Smid, Certe

Sabat et al, Sci Rep. *2017* Jun 13;7(1):3434. doi: 10.1038/s41598 017-03458-6.

Challenges

Opportunities

16S-23S rDNA NGS

interpretation within a clinical context

validation time-to-result ISO15189

overcomes limitations current tests

data storage data analysis database

increase diagnostic yield of bacteria

contaminating background reads

Dengue virus detection and typing from blood samples

17 DENV positive patients

Workflow
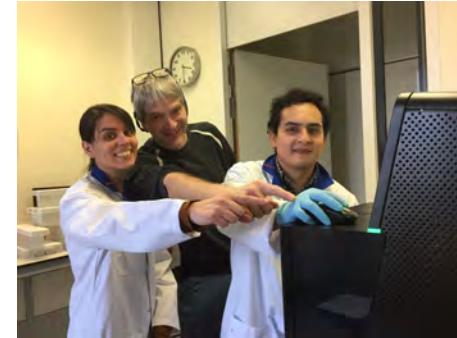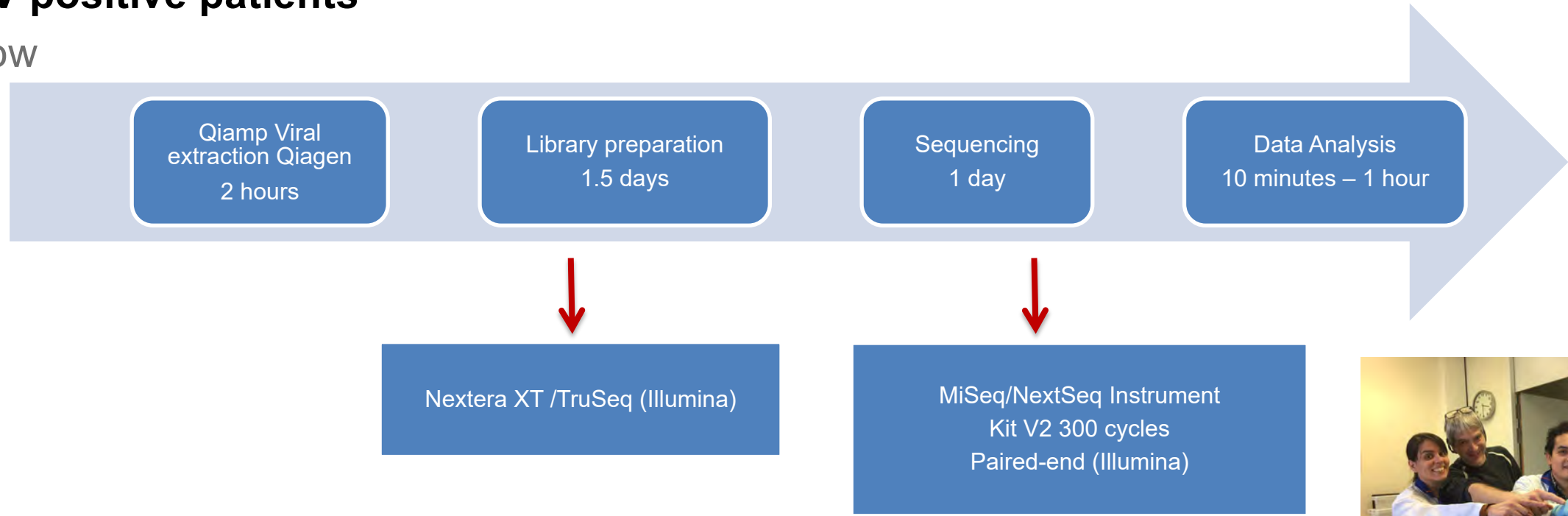
Classic approach: Sanger sequencing

Bioinformatics analysis

CLC Genomics Workbench v10.1.1

QA/QC and Host-reads removal — Viral genome recovery

Raw reads → QA/QC & Trimming → Map against hg18 → Unmapped reads

De novo assembly / Map against DENV reference genome

DENV identification

Blast

MEGA v7.0

Alignment (MUSCLE)
Maximum Likelihood Tree
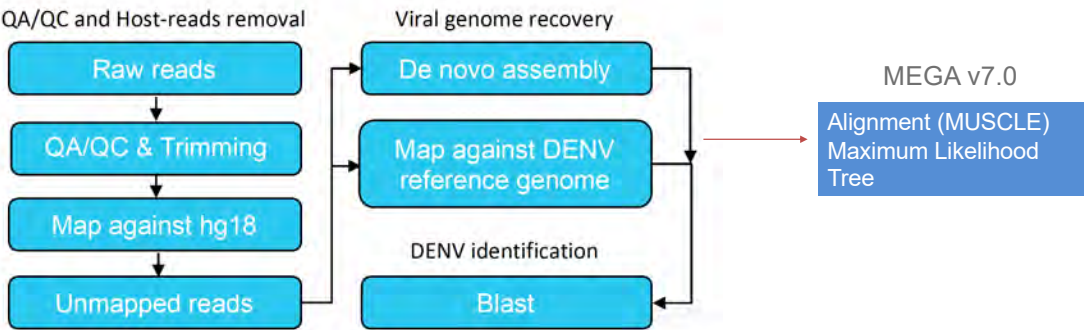
Sample	Total number of reads	Mapped reads against hg18	Unmapped reads against hg 18	Dengue-2 mapped reads	Average coverage
91-0109a	1,243,122	834,830 (67.2%)	408,292 (32.8%)	238,112 (19.2%)	3,301.7
91-0105b	1,427,648	1,215,507 (85.1%)	212,141 (14.9%)	51,914 (4.0%)	668.4
91-0121	2,802,530	2,220,772 (79.2%)	581,758 (20.8%)	288,694 (10.3%)	3,747.2
91-0131a	9,743,154	8,313,085 (85.3%)	1,430,069 (14.7%)	721,536 (7,4%)	10,053.8
91-0135b	562,914	302,336 (53.7%)	260,578 (46.3%)	87,679 (16.0%)	1,114.3
92-1095	3,640,058	3,038,591 (83.5%)	601,467 (16.5%)	165,422 (4.5%)	2,146.9
92-1096	3,918,662	3,671,259 (93.7%)	247,403 (6.3%)	110,770 (2.8%)	1,480.6
92-1099	2,810,772	2,251,377 (80.1%)	559,395 (19.9%)	53,517 (1.9%)	704.6
cc0007	2,654,296	2,144,891 (80.8%)	509,405 (19.2%)	60,185 (2.3%)	604.3

hg18: human genome

DENV-1   DENV-2
DENV-3   DENV-4
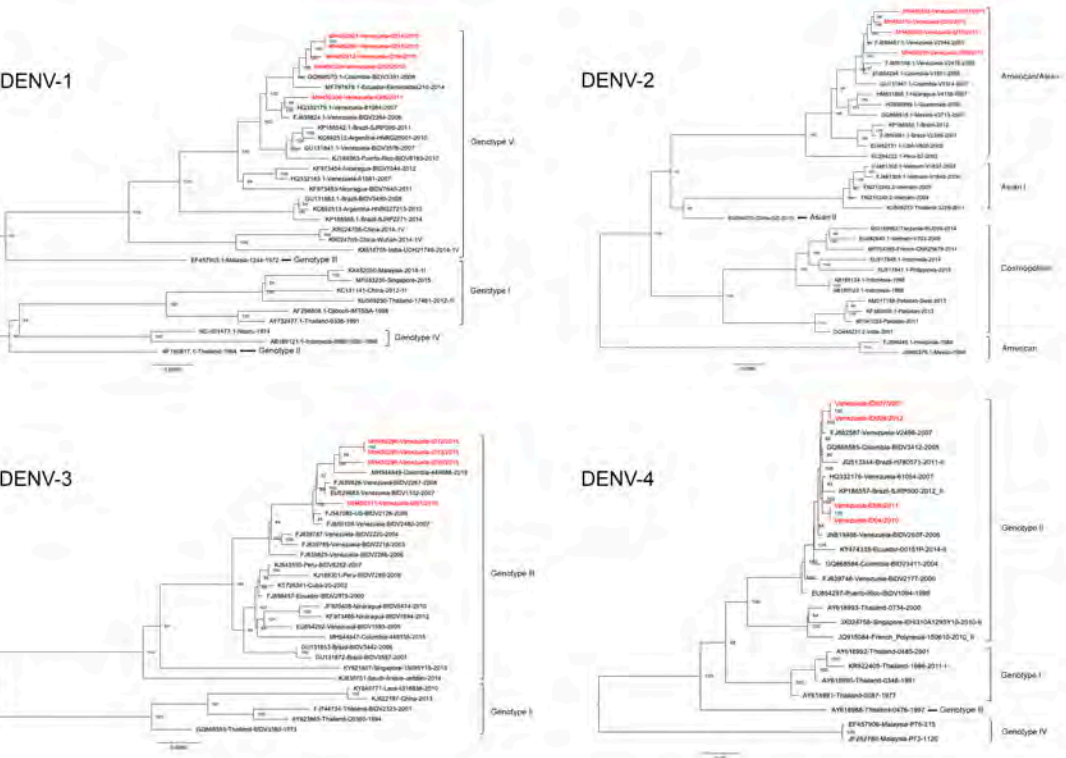
Detection and visualization Taxonomer IDbyDNA)

Dengue 3
Dengue 2
Dengue 1

Spiked sample
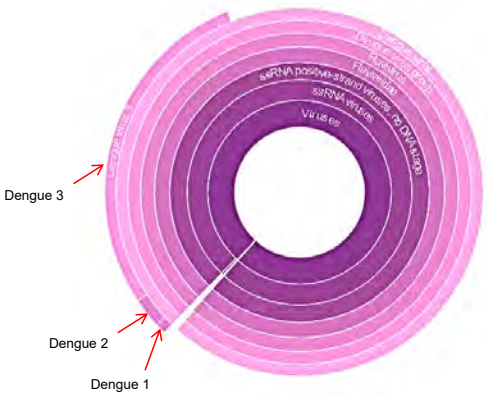
CLC Genomics Workbench v10.1.1

Virus	Mapped reads	Coverage	Consensus (bp)	De Novo Assembly
DENV1	1180	15.45	10614	2796
DENV2	2514	32.4	10675	6736
DENV3	55952	733.66	10675	10555

Manuscript in revision, Erley Lizarazo, Natacha Couto, UMCG

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Pathogen enrichment

ID	Viral reads SISPA	Viral reads SISPA+ViroCap
1	104,363	7,391,047
2	139,453	22,851,254
3	22,093	650,270
4	1,090,222	89,334,846
5	32,472	3,032,847
6	68,895	8,274,203
7	57,723	8,812,685
8	26,751	756,992
9	191,573	37,481,234
10	168,037	12,564,721
11	116,148	20,913,469
12	42,257	1,999,588

Sequence-Independent Single-Primer Amplification (SISPA); ViroCap: enrich nucleic acid from DNA and RNA viruses from 34 families that infect vertebrate hosts

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AMR detection in the WGS era a no go?

- published evidence for using WGS as a tool to infer antimicrobial susceptibility accurately --> poor or non-existent
- for most bacterial species major limitations are
  - current high-cost
  - limited speed
  - dependency on previous culture
- for most bacterial species there is currently insufficient evidence to support the use of WGS-inferred AST to guide clinical decision making

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Antimicrobial resistance

Sample number	Conventional identification (MALDI-TOF)	Conventional susceptibility testing (VITEK 2)[b]	WGS CLC Genomics Workbench	Shotgun metagenomics	
				ReMatCh (Unix)	CLC Genomics Workbench[a]
1	E. faecium S. haemolyticus	LEV, ERY, CLI OXA, GEN, CIP, FOS, ERY, CLI	erm(B), msr(C), ant(6')-Ia, aph(3')-III, dfrG blaZ, mecA, ant(6')-Ia, aph(3')-III, aac(6')-aph(2"), erm(C), mph(C), msr(A)	erm(B), msr(C), ant(6')-Ia, aph(3')-III, aac(6')-aph(2"), blaZ, mecA, erm(C), mph(C), msr(A), dfrG	erm(B), msr(C), ant(6')-Ia, aph(3')-III, aac(6')-aph(2"), blaZ, mecA, erm(C), mph(C), msr(A), dfrG
2	E. avium E. coli Anaerobes	DOX, CLI susceptible —	—[#] —[#] —[#]	Not detected Not detected catS, lnu(D), lsa(C), cepA-44, tet(Q)	Not detected Not detected catS, lnu(D), lsa(C), cepA-44, tet(Q), fusA
3	S. epidermidis	OXA, GEN, TEC, FUS, CIP, ERY, CLI	—[#]	Not detected	Not detected
4	S. aureus	PEN, ERY	blaZ, spc, erm(A)	Not detected	Not detected
5	E. coli K. oxytoca S. anginosus E. faecalis Anaerobes	susceptible AMX susceptible DOX, CLI —	—[#] blaOXY-1–3 —[#] tet(M), lsa(A) —[#]	— Not detected — tet(M) cfxA4, tet(Q)	— Not detected — tet(O) cfxA4, tet(Q)
6	E. faecium	PEN, AMX, CFX, IMP, GENhl, STRhl, LEV, ERY, CLI, AMP/SUL	erm(B), msr(C), ant(6')-Ia, aph(3')-III, aac(6')-aph(2"), dfrG	Not detected	Not detected
7	S. aureus	PEN	blaZ	blaZ, norA	blaZ
8	O. intermedium	AMX, PIP/TAZ, CFX, CFT, CTZ, IMP, FOX, TOB, FOS, NIT, TMP	blaOCH-2	blaOCH-5	blaOCH-2
9	S. aureus	PEN	—[#]	blaZ	blaZ
10	S. marcescens	AMX, AMC, CFX, FOX, NIT, POL	—[#]	blaSST-1, tet(41), oqxB, aac(6')-Ic	tet(41), oqxB, aac(6')-Ic

- 1, 7 and 9 genotypes and phenotypes correlated well
- Other samples not all AMR genes explaining phenotypic resistance identified
- 1, 5, 7 and 10 different results ReMatCh vs CLC Genomics workbench

Couto N et al., Sci Rep. 2018 Sep 13;8(1):13767. doi: 10.1038/s41598-018-31873-w

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There is hope…

- WGS-based MIC prediction allows reliable MIC prediction for five gonorrhoea antimicrobials *Eyre et al. J Antimicrob Chemother 2017; 72: 1937–1947*

- WGS can aid in the timely diagnosis of *Mycobacterium tuberculosis* drug resistance and guide clinical decision-making *Ruesen et al., scientific reports| (2018) 8:9676 | DOI:10.1038/s41598-018-27962-5*

- Whole-genome sequencing effective tool for predicting antibiotic resistance in nontyphoidal *Salmonella*, although the use of more appropriate surveillance breakpoints and increased knowledge of new resistance alleles will further improve correlations *McDermott et al. Antimicrob Agents Chemother 60:5515–5520. doi:10.1128/AAC.01030-16.*

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Metagenomics and the microbiome

- changes in the intestinal microbiota in samples from patients with Shiga Toxin-producing E. coli (STEC) infection compared to healthy and healed controls
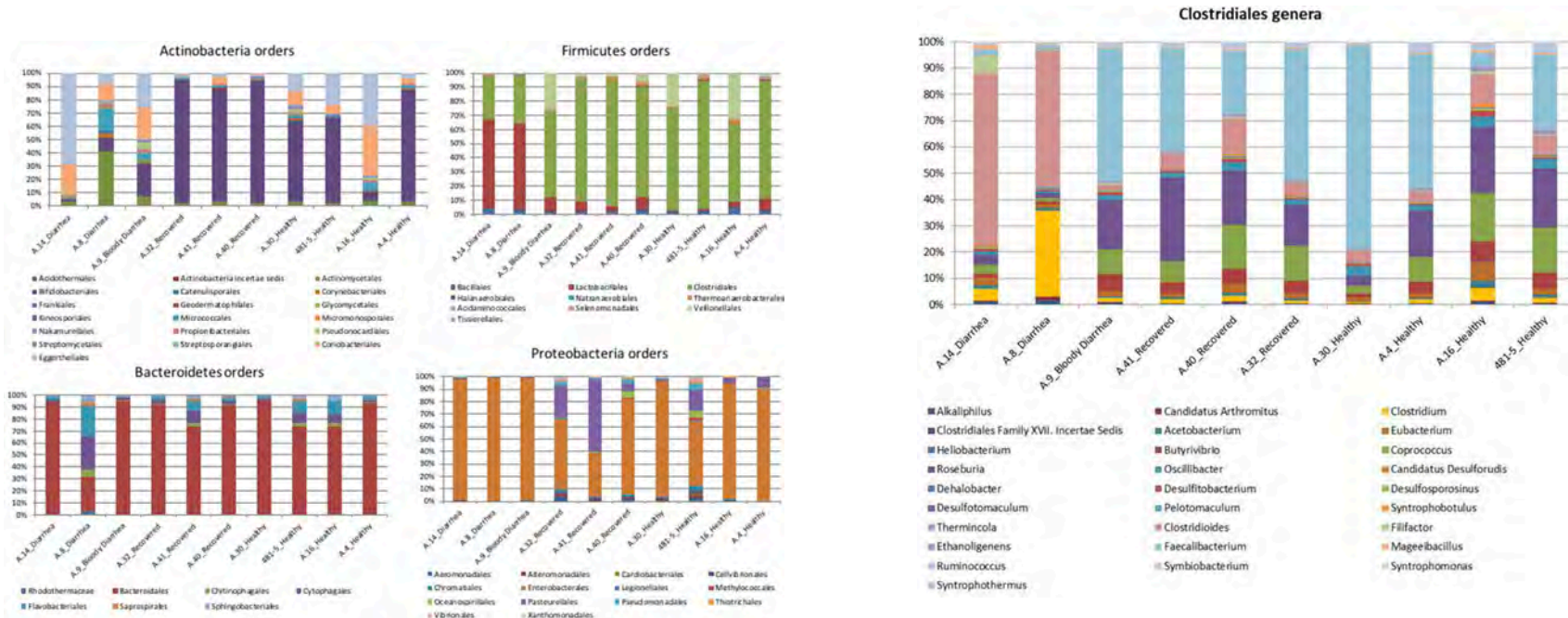
- Inducing immune respons
- Protecting against GI pathogens colonization (competition of binding to EC)
- *in vitro* protection of host epithelial cells from the effect of Shiga toxin

Higher abundance of Bifidobacteriales and
Clostridiales in STEC negative persons (healthy, healed)

Challenges

- Clinical sensitivity and specificity → depletion human DNA /enrichment microbial DNA/RNA

- Genotype not always phenotype (AMR) → RNAseq?

- Which genes belong to which pathogen ? → Single cell sequencing

- Presence of contaminant DNA → reagents, sample taking

- Persistence of DNA from dead microbes → RNAseq

- Presence of microbial DNA in healthy individuals → host response?

- Colonization versus infection → host response, RNAseq?

Collaborations and acknowledgements

UMCG – Guide

Natacha Couto
Kai Zhou
Silvia Garcia-Cobos
Monika Chlebowicz
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Linda Veloo
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Ana Carolina da Cruz Campos
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Mithila Ferdous
Erley Lizarazo Forero
Kathleen Boiten
Sigrid Rosema
Brigitte Dijkhuizen
Erwin Raangs
Fenna Bosma
Yvette Bisselink
Ruud Deurenberg

UMCG – Guide cont.

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Ana Claudia Rosa

Metanet (not yet listed)

Henrik Torkil Westh
Alexander Melmann
Dag Harmsen
Robert Schlaberg

I apopologize in advance if I forget to mention people – please contact me afterwards if you think your name should be on this slide

Rossenlab
Personalised Microbiology

Toward strain-level comparative genomics from metagenomics
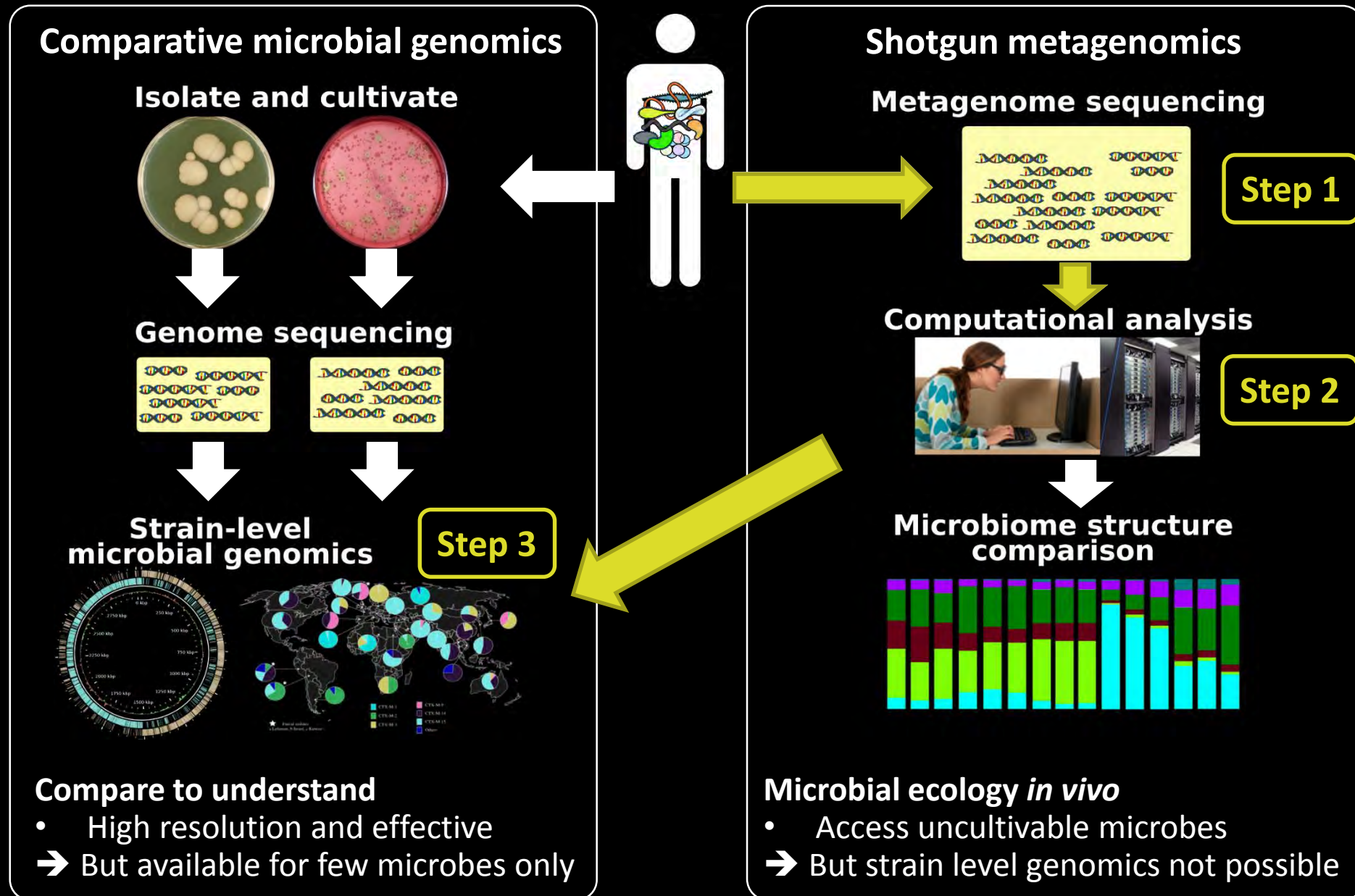
Comparative microbial genomics

Isolate and cultivate

Genome sequencing

Strain-level microbial genomics

Step 3

Compare to understand
- High resolution and effective
- ➔ But available for few microbes only

Shotgun metagenomics

Metagenome sequencing

Step 1

Computational analysis

Step 2

Microbiome structure comparison

Microbial ecology *in vivo*
- Access uncultivable microbes
- ➔ But strain level genomics not possible

The non-culturable revolution

rRNA gene Profiling
Targeted amplification of universal marker gene (commonly 16S)

- Not genome-wide
- **Limited taxonomic resolution**
- **No functional insights**
- Cannot catch viruses and eukaryotes
- **Cost-effective**
- **Avoids host DNA contamination**

- Several (usually underestimated) biases
- Almost impossible for cross-study comparisons
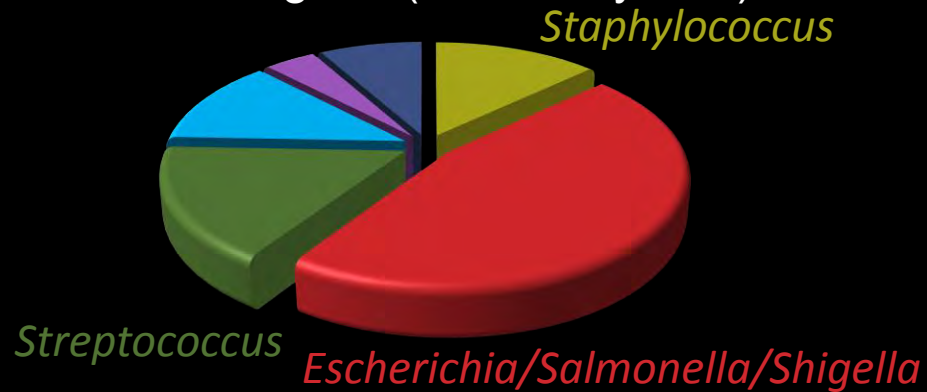
Shotgun metagenomics
Sequencing of the total community DNA
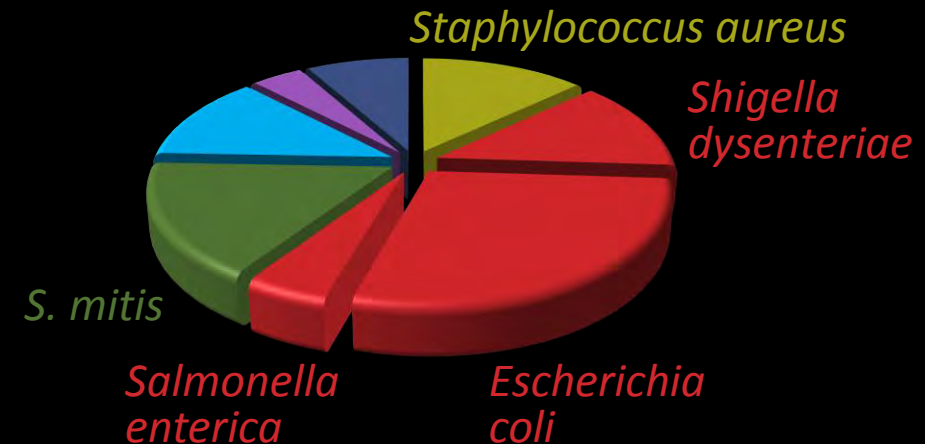
- Genome-wide
- **High taxonomic resolution**
- **Functional profiling**
- Can survey all domains of life simultaneously
- **More expensive**
- **Host DNA contamination**
- **Computational challenges**

Moving toward strain-level metagenomics

ESCHERICHIA COLI OUTBREAK
Hemolytic-uremic syndrome (HUS) is a serious complication of a type of E. coli known as Shiga toxin-producing E. coli (STEC)
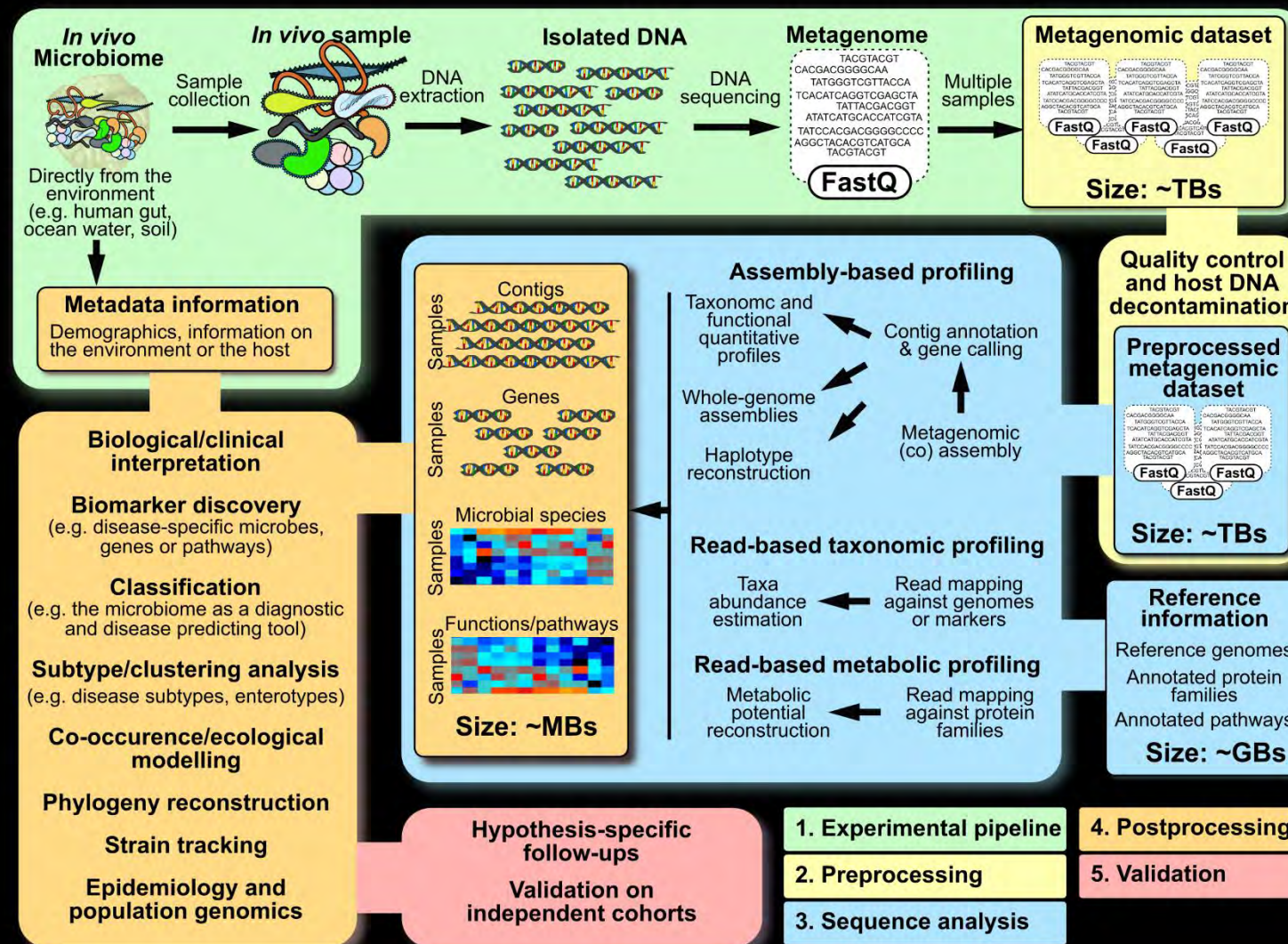
Over 3000 cases
And over 50 deaths
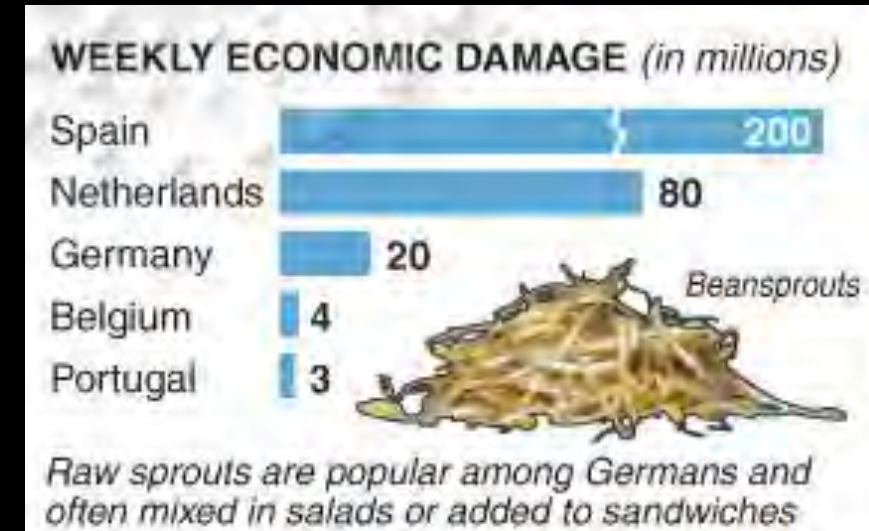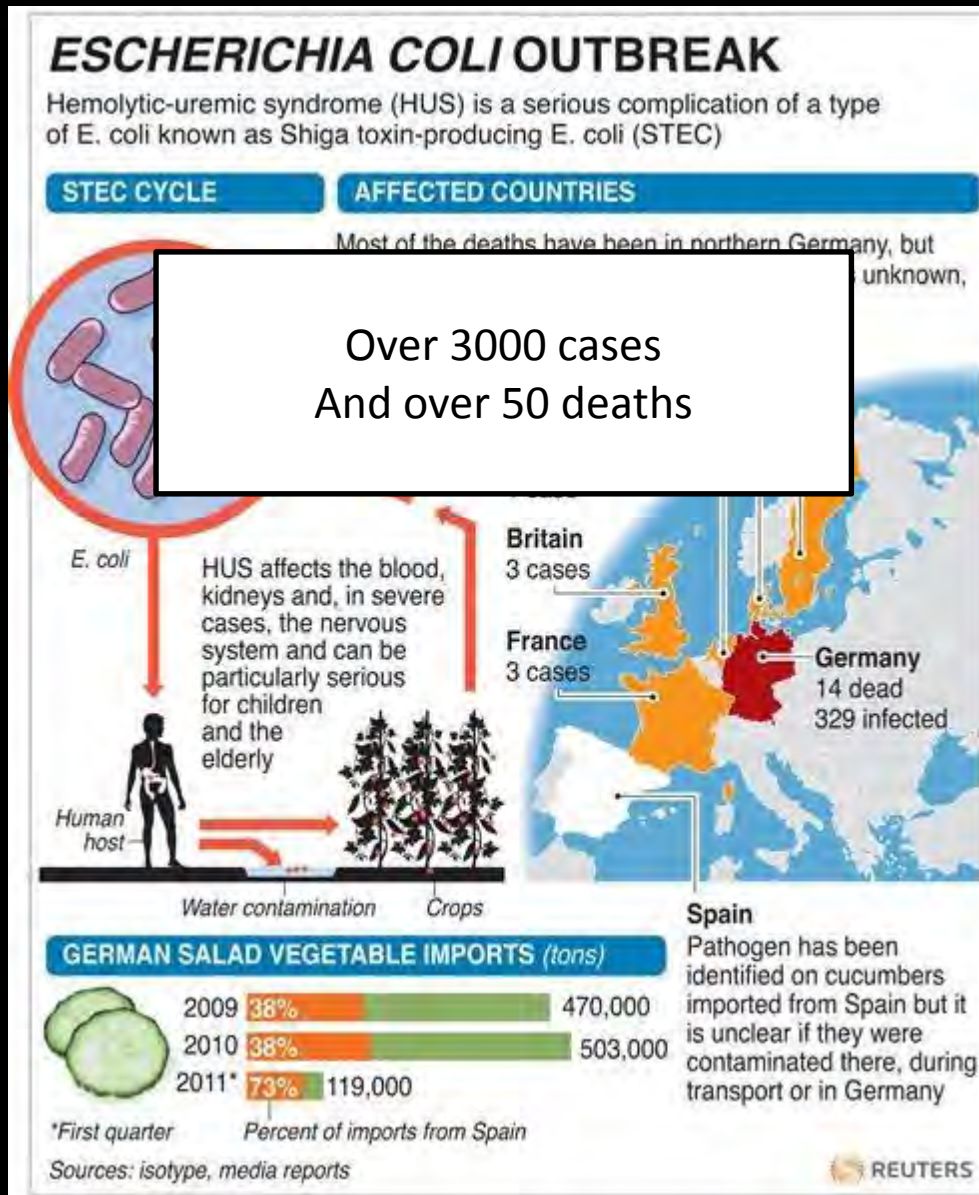
The outbreak strain is a (re)combination of:
- an enteroaggregative (EAEC) strain: pAA plasmid
- an enterohemorrhagic (EHEC) factors: Shiga-toxin gene
- an antibiotic resistence factor: beta-lactamase

Metagenomes from [Loman et al., 2013]

Recent Metagenomic tools

MetaMLST

Nucleic Acids Research

MetaMLST: multi-locus strain-level bacterial typing from metagenomic samples

Moreno Zolfo[1], Adrian Tett[1], Olivier Jousson[1], Claudio Donati[2] and Nicola Segata[1,*]

Assumption: Each strain has a unique combination of......

GENOME RESEARCH

Method

Microbial strain-level population structure and genetic diversity from metagenomes

Duy Tin Truong,[1] Adrian Tett,[1] Edoardo Pasolli,[1] Curtis Huttenhower,[2,3] and Nicola Segata[1]

.....SNPs in the core genome, StrainPhlAn
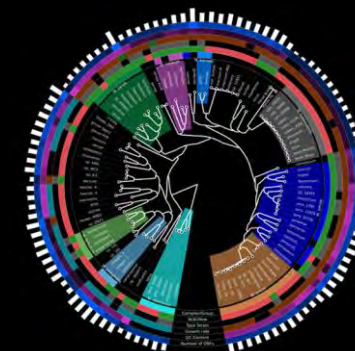
.....genes from the overall species pangenome, PanPhlAn
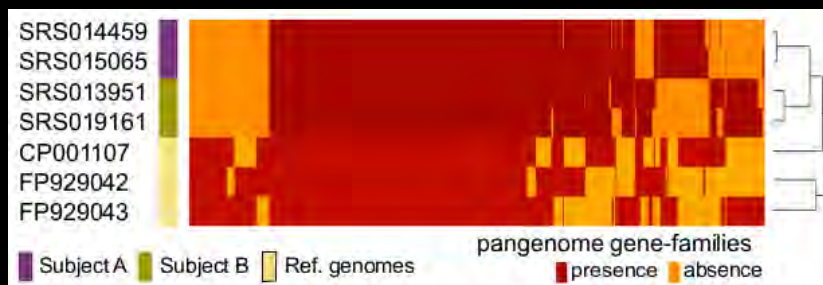
NATURE METHODS

Strain-level microbial epidemiology and population genomics from shotgun metagenomics

Matthias Scholz[1,4], Doyle V Ward[2,4], Edoardo Pasolli[1,4], Thomas Tolio[1], Moreno Zolfo[1], Francesco Asnicar[1], Duy Tin Truong[1], Adrian Tett[1], Ardythe L Morrow[3] & Nicola Segata[1]

http://segatalab.cibio.unitn.it/

MetaMLST tracks *E. coli* in gut microbiome

Zolfo et al., 2017

MetaMLST tracks *E. coli* in gut microbiome

Escherichia coli | **101 Subjects** | **88 STs**

Detection of pathogenic O104:H4 *E. coli*

ST 678 confirmed to be associated to *E. coli* Germany outbreak in 2011.

German *E. coli* outbreak
Chinese Diabetes
HMP

Hub Sequence Type
Sequence Type ID
Clonal Complex 10

Number of samples in the ST
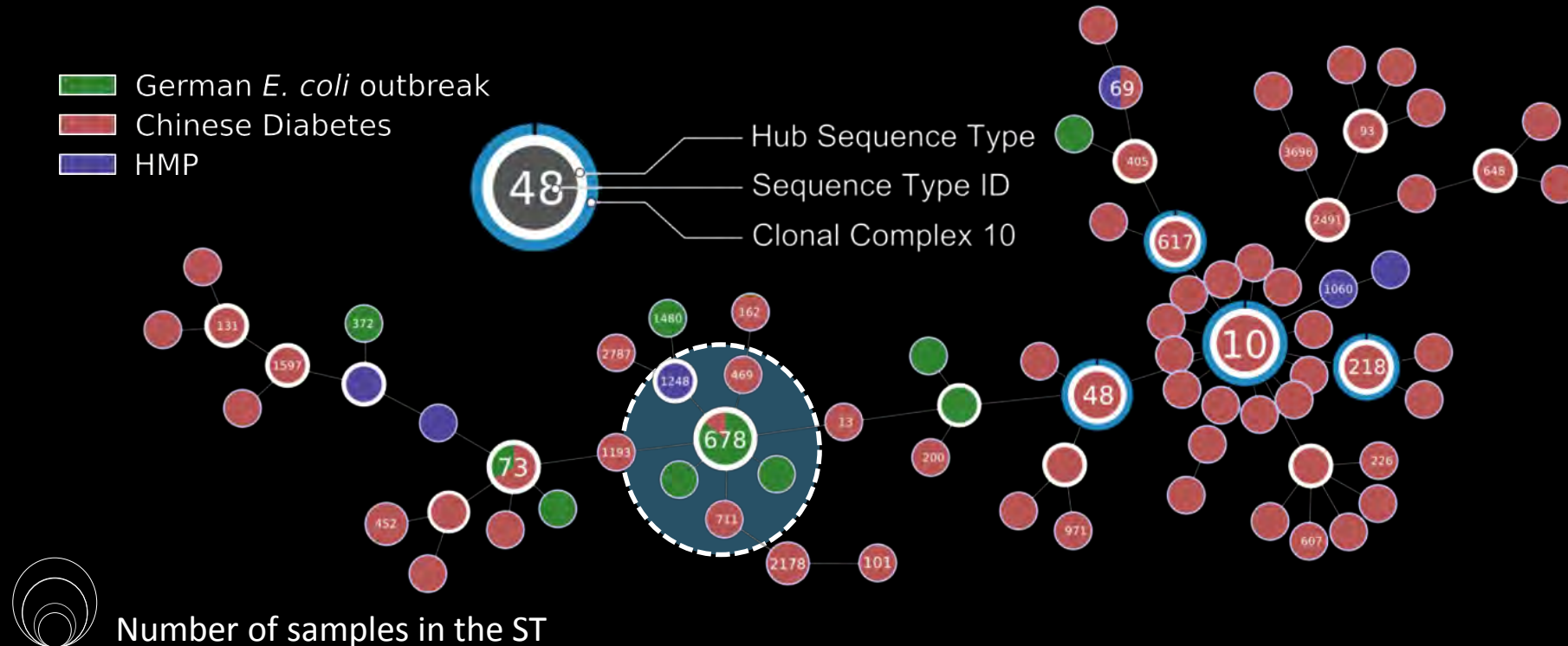
Strain level approaches

PanPhlAn for "meta-epidemiology"
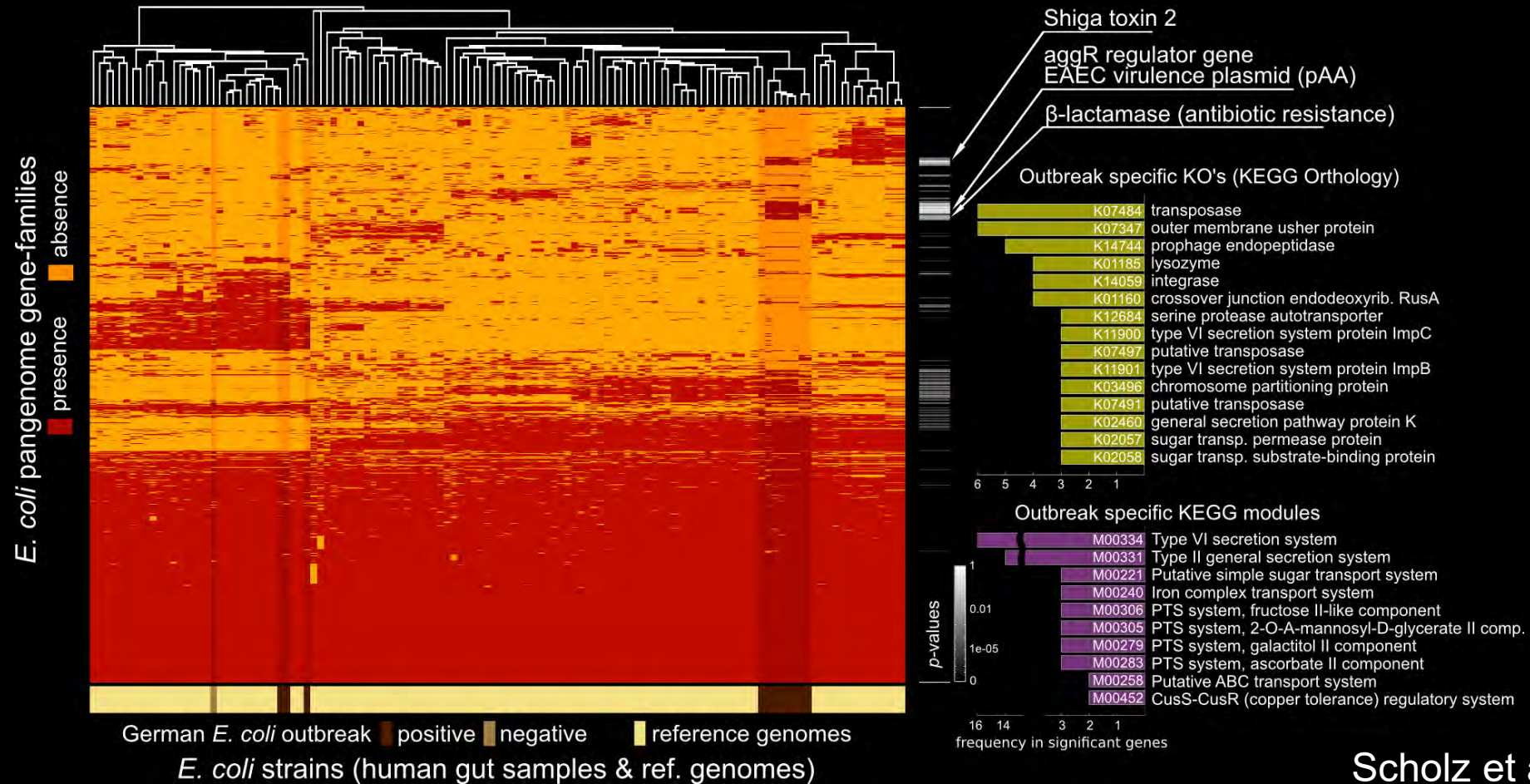
Scholz et al., 2016

Metagenomes from [Loman et al., 2013]

E. coli population genomics with PanPhlAn

E. coli profiling from 1478 shotgun metagenomes

E. coli pangenome gene-families

presence ■ absence

Chinese Diabetes (Qin et al, Nat. 2012)   Women T2D (Karlsson et al., Nat, 2013)   reference genomes
German *E. coli* (Loman et al., JAMA 2013)   HMP (HMP Consortium, Nat, 2012)
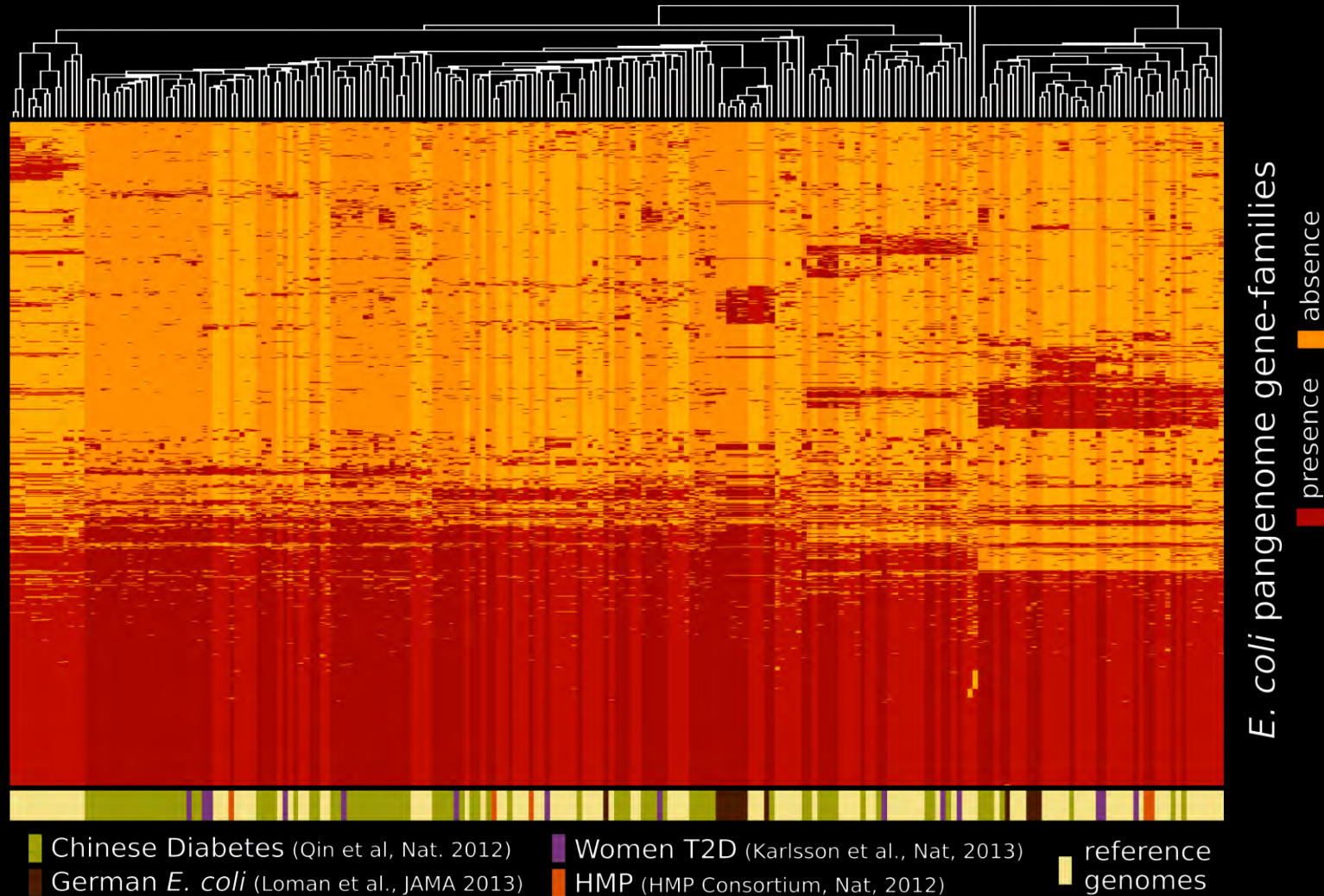
E. coli strains (human gut samples & ref. genomes)

E. coli population genomics with PanPhlAn

- 99.8% genes in common with the outbreak strain
- But missing the Shiga toxin gene
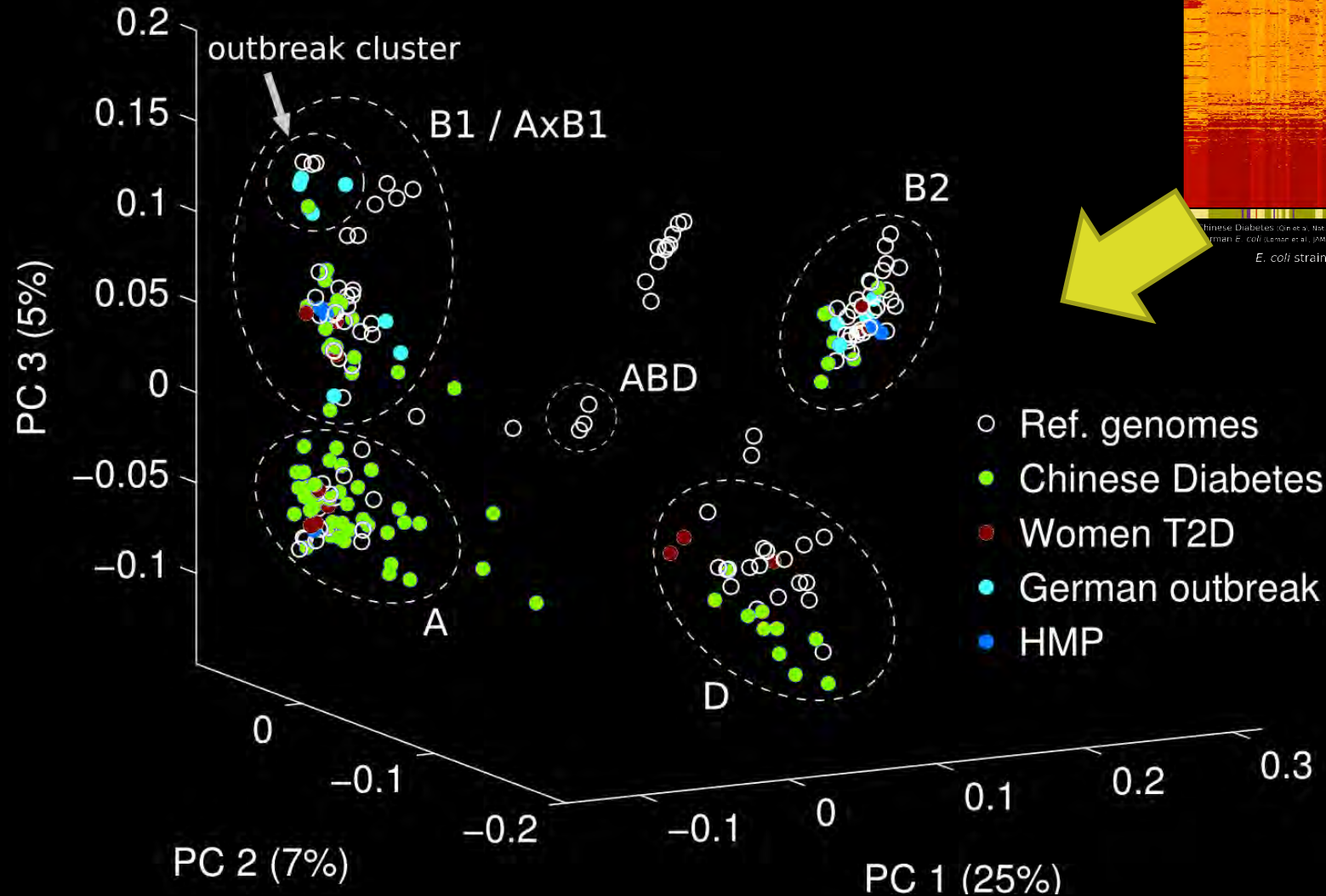
E. coli profiling from 1478 shotgun metagenomes
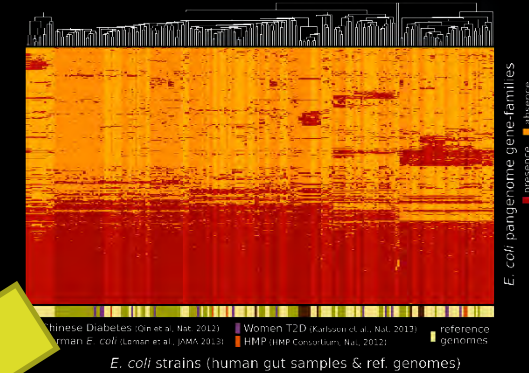
Cystic Fibrosis microbiome – with StrainPhlAn

Clinical colleagues have collected sputum and saliva samples for many CF patients in Northern Italy

Cultivation

Isolation

- ✓ Pathogen specific
- ✗ Non-cultivable microbes
- ✗ Co-infection
- ✗ Unknowns
- ✗ Virus

Sequence metagenome

Computational analysis

- ✓ Pathogen specific
- ✓ Non-cultivable microbes
- ✓ Co-infection
- ✓ Unknowns
- ✓ Virus
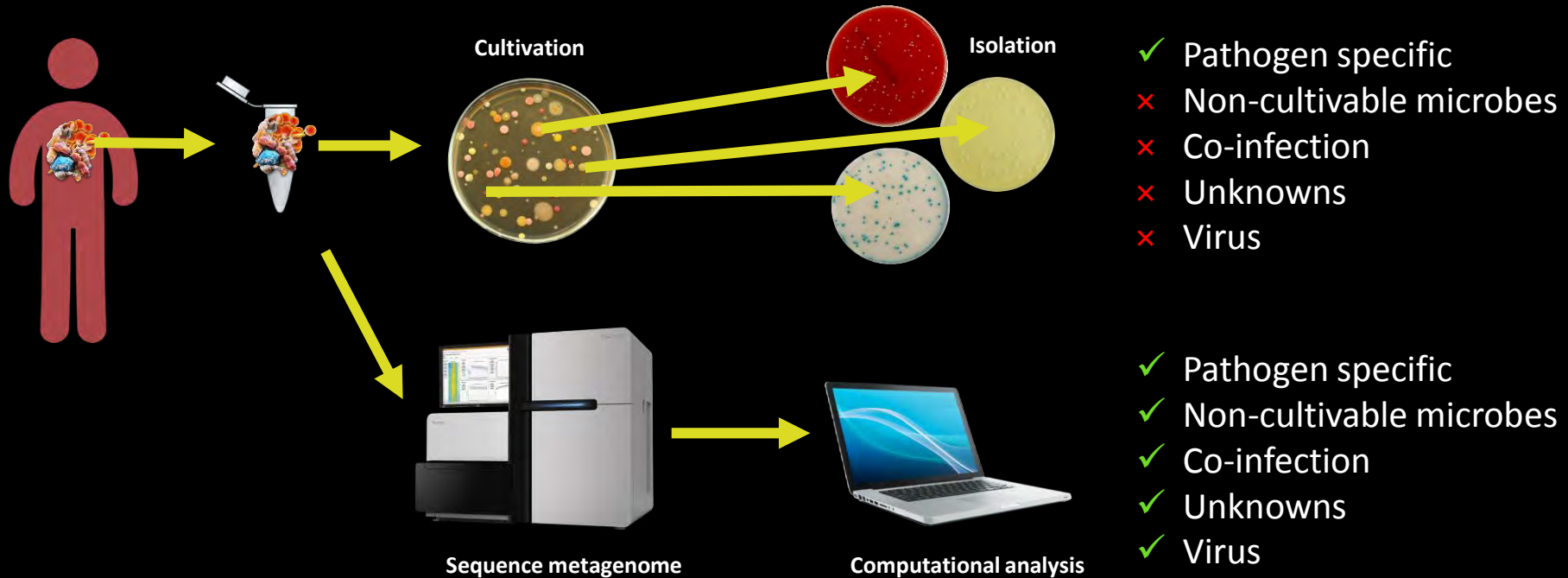
We have currently sequenced metagenomes for 25 patients (4-9 timepoints)

Microbiome-pathogen interaction in CF: hypothesis testing for antibiotic resistance

Is the pathogen acquiring resistance horizontally from commensal microbes?
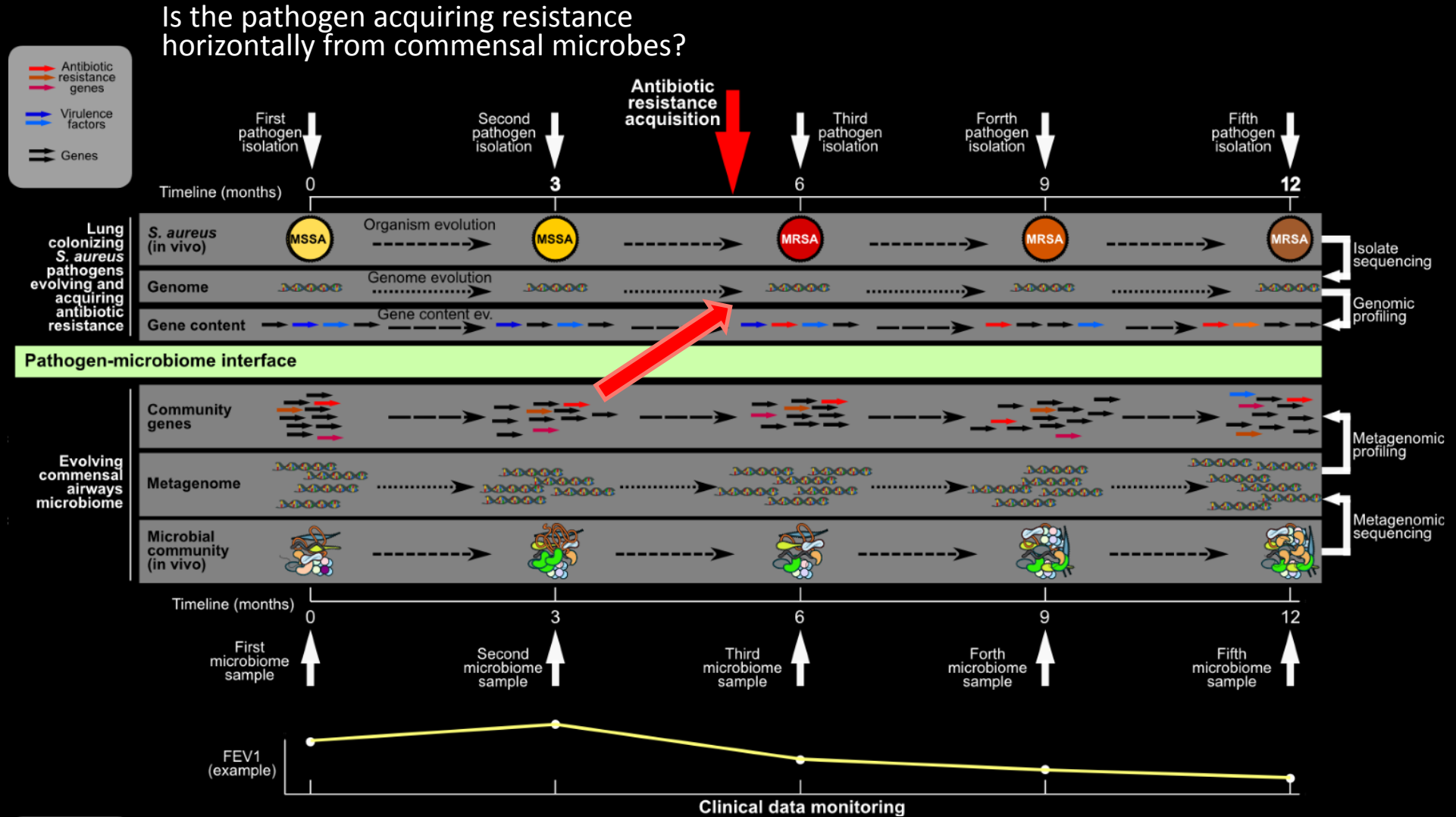
CF Strain stability? *S. aureus*

Eubacterium infirmum
Prevotella oris
Haemophilus parahaemolyticus
Prevotella oulorum
Actinomyces viscosus
Scardovia wiggsiae
Campylobacter gracilis
Atopobium rimae
Prevotella maculosa
Selenomonas flueggei
Dialister micraerophilus
Peptostreptococcus stomatis
Dialister invisus
Mitsuokella sp
Escherichia sp
Rhodopseudomonas palustris
Atopobium vaginae
Haemophilus paraphrohaemolyticus
Lachnoanaerobaculum saburreum
Capnocytophaga ochracea
Selenomonas noxia
Actinobacillus sp
Gardnerella vaginalis
Neisseria meningitidis
Actinomyces naeslundii
Granulicatella elegans
Prevotella denticola
Kingella oralis
Moraxella catarrhalis
Staphylococcus aureus

Timepoint 1
Timepoint 2
Timepoint 3
Timepoint 4

P41 T3
P41 T1
P41 T1Q
P41 T2
P8 T2
P8 T3
P38 T2
P38 T3
P1 T2
P1 T1
P1 T3
P17 T2
P17 T1
P40 T3
P38 T1
P48 T1
P23 T1
P23 T2
P7 T2
P7 T1
P7 T4
P13 T2
P39 T2
P48 T3
P5 T1
P39 T1
P5 T3
P65 T2
P6 T1
P5 T2
P5 T4
P13 T1

Staphylococcus aureus

CF Strain stability? *P. aeruginosa*
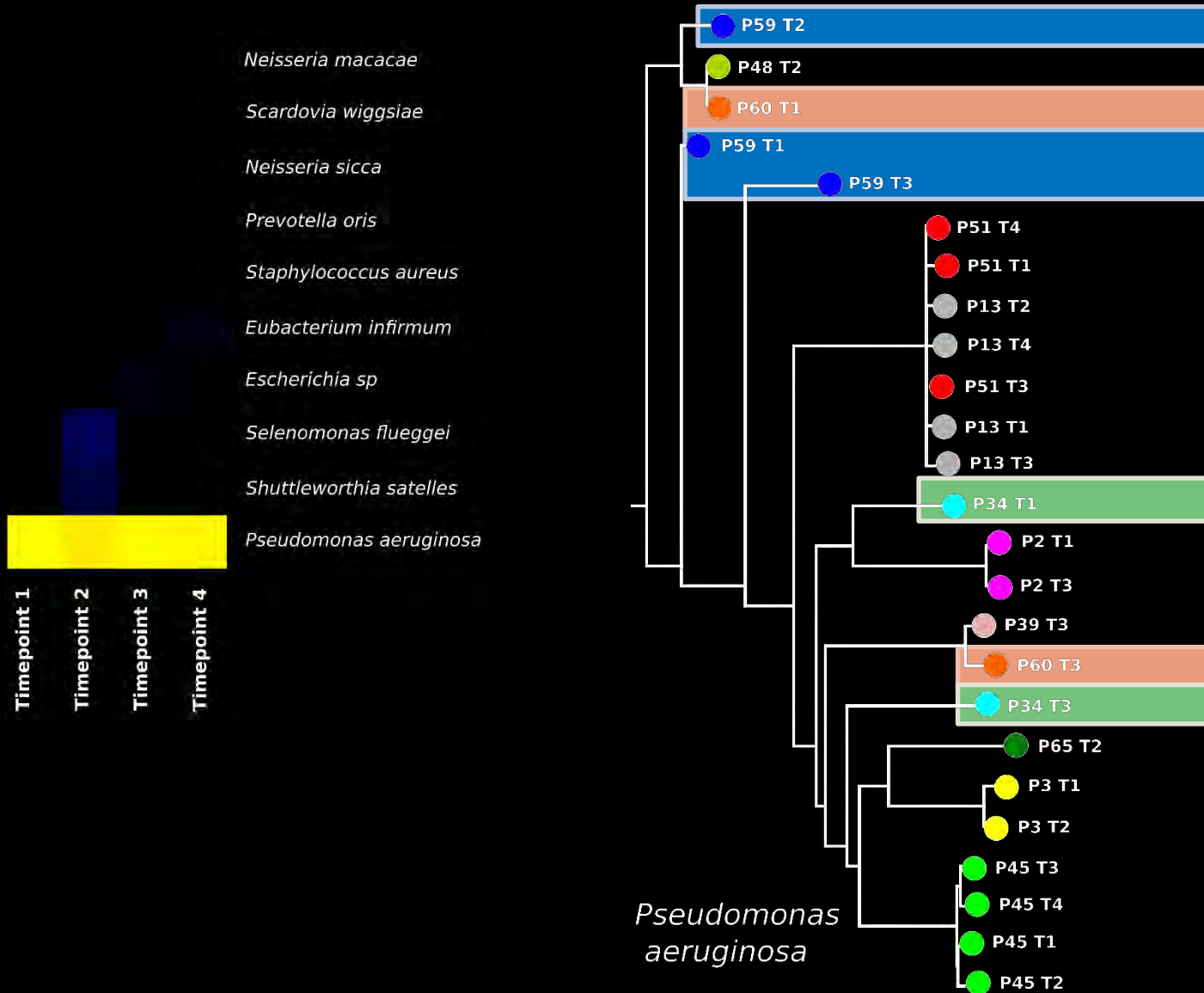
Phages in cystic fibrosis

The skin Microbiome
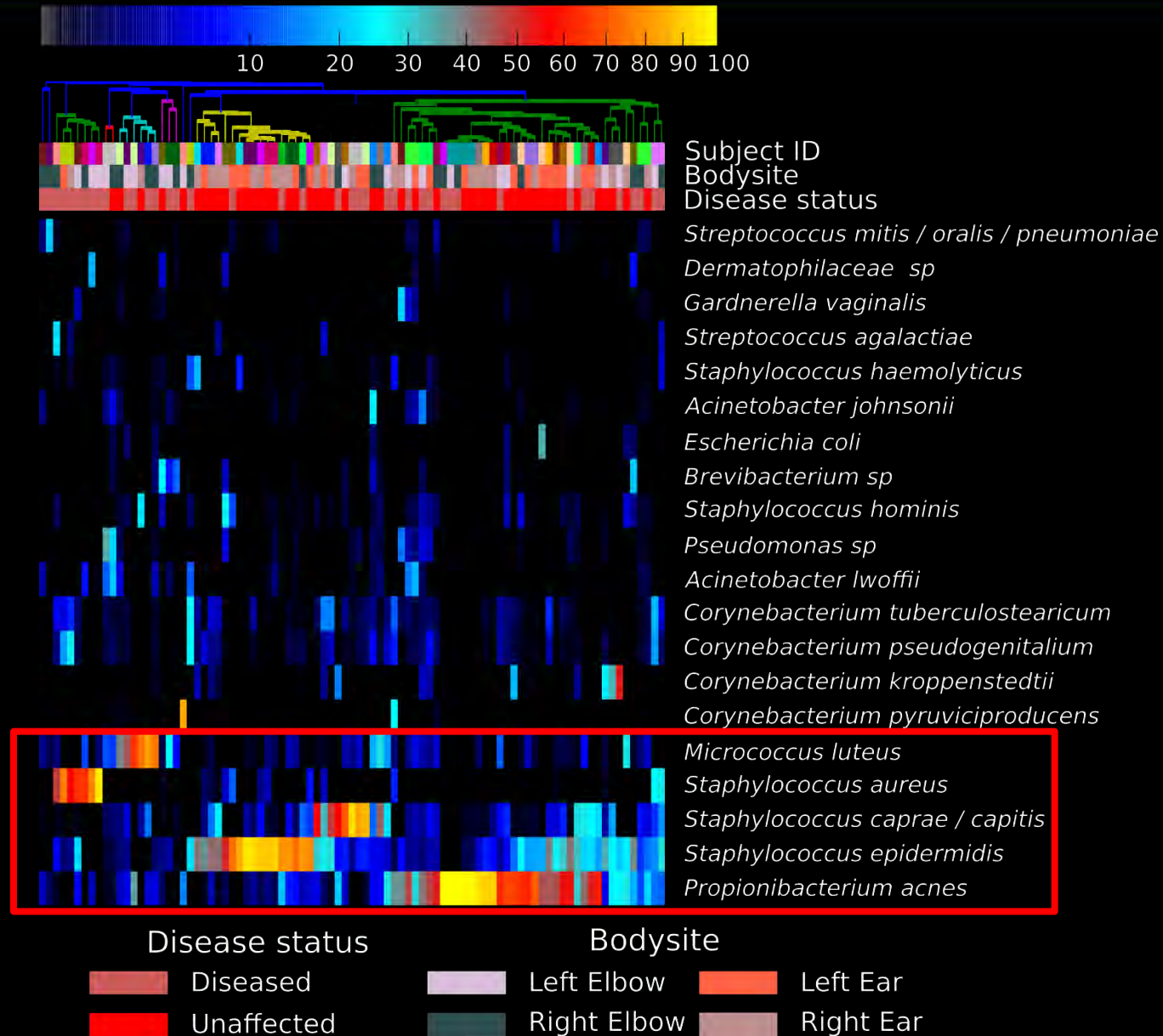
Tett et al., 2017

Strain level differences

Staphylococcus epidermidis   *Propionibacterium acnes*

S. epidermidis   *P. acnes*
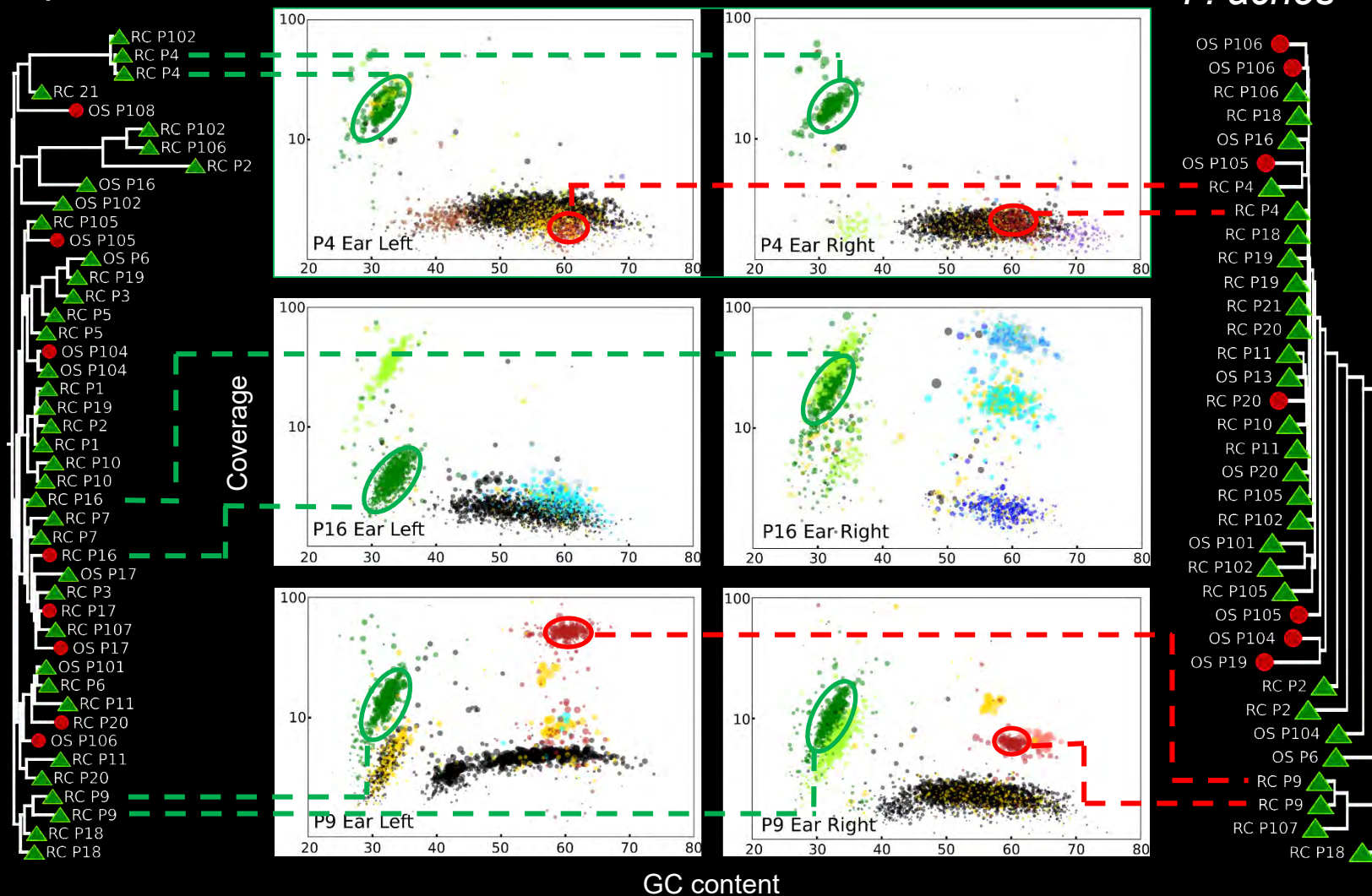
Analysing the strain specific functional repertoire
S. epidemidis
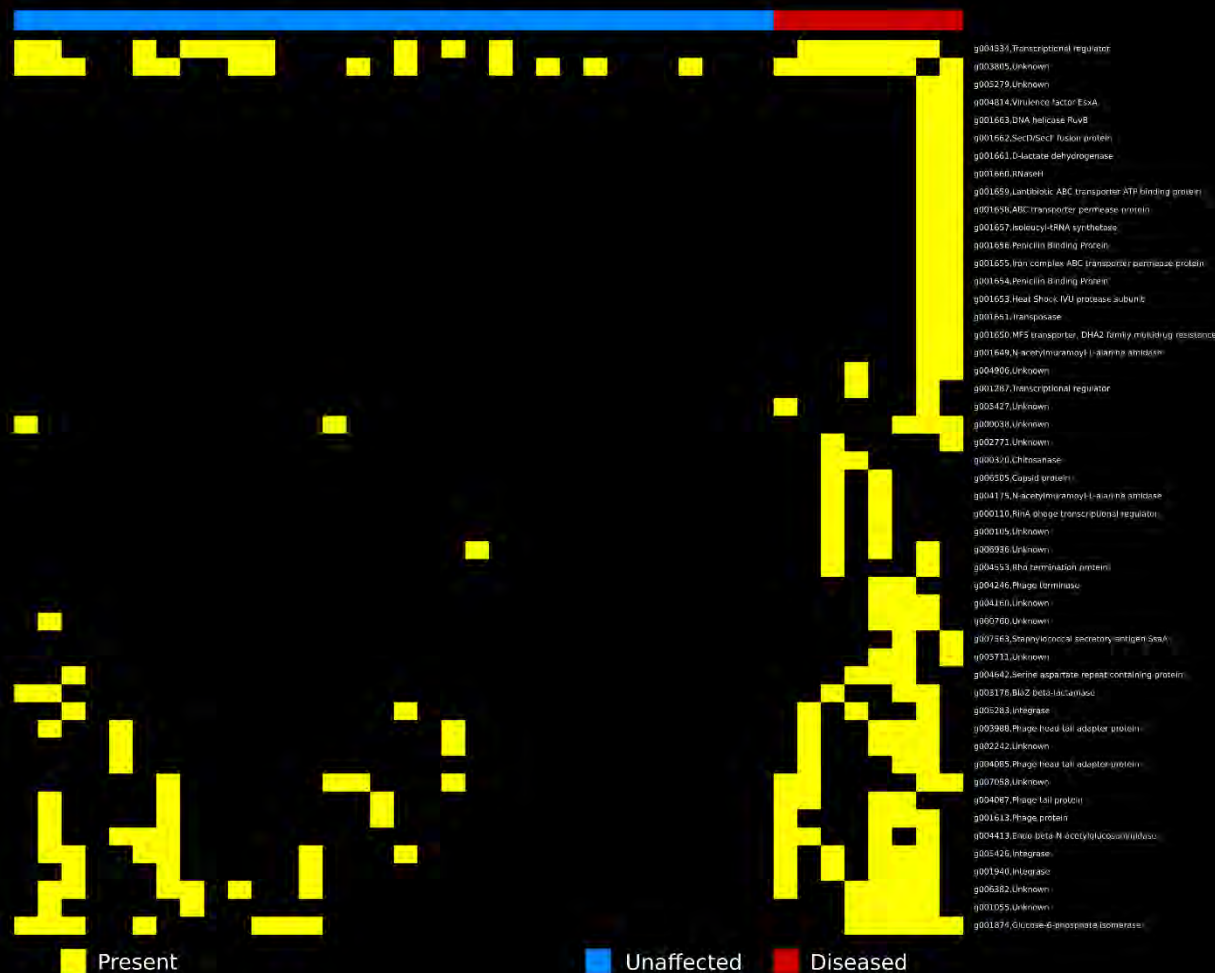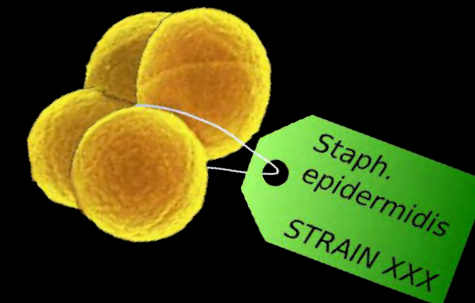
In total 50 genes were significanly more prevalent in strains occupying Psoriatic skin

These include known pathogenic determinants (e.g. SsaA, EsxA)

Markers of HGT events, integrases and transposases

Function commonly associated with mobile genetic elements

Multidrug resistance transporters, penicillin binding proteins

Many gene of unknown function that could be environmentally relevant

Unknowns, the dark matter!

Unknown clusters-the skin's hidden diversity
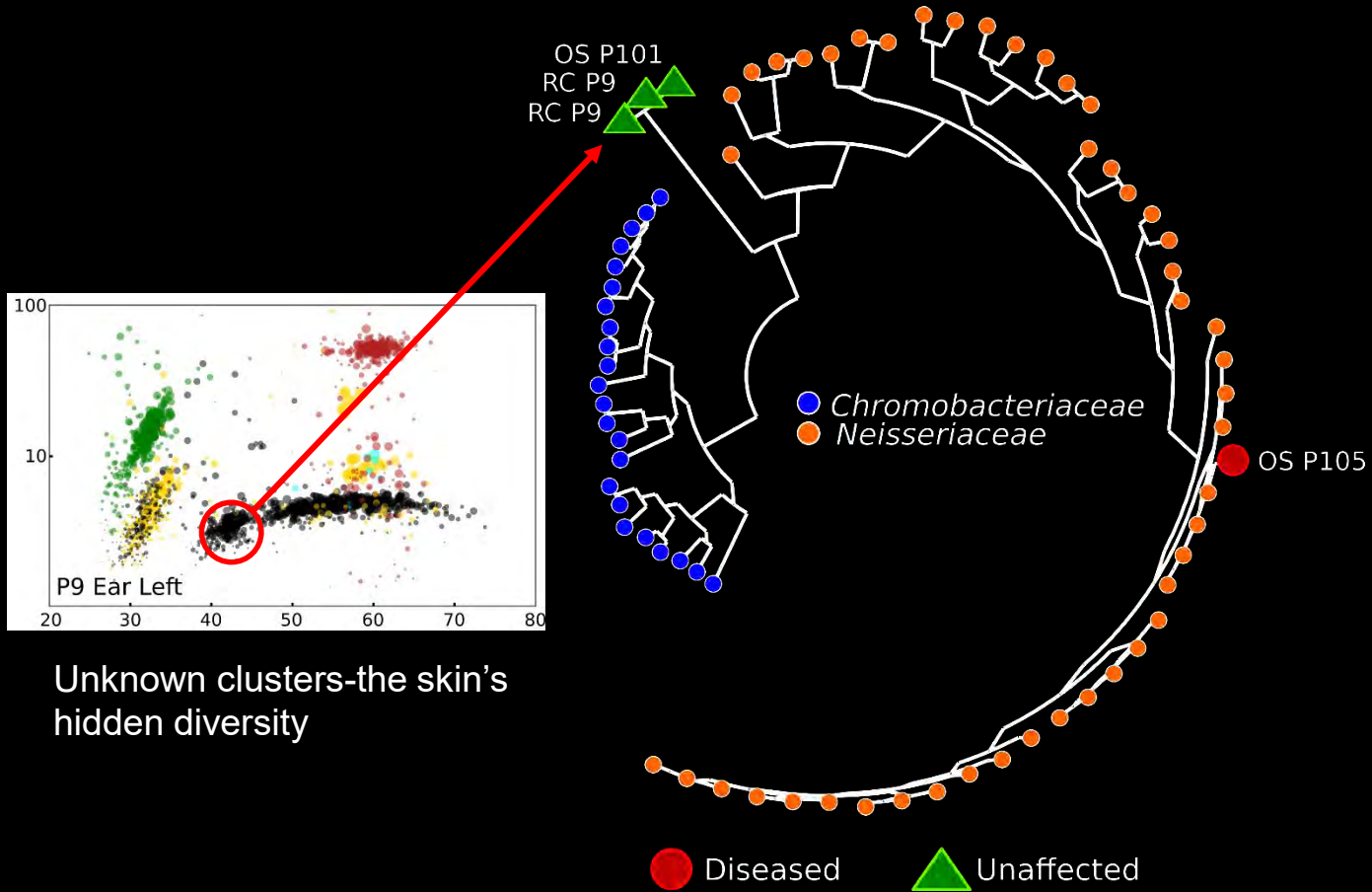
Unknowns, the dark matter!
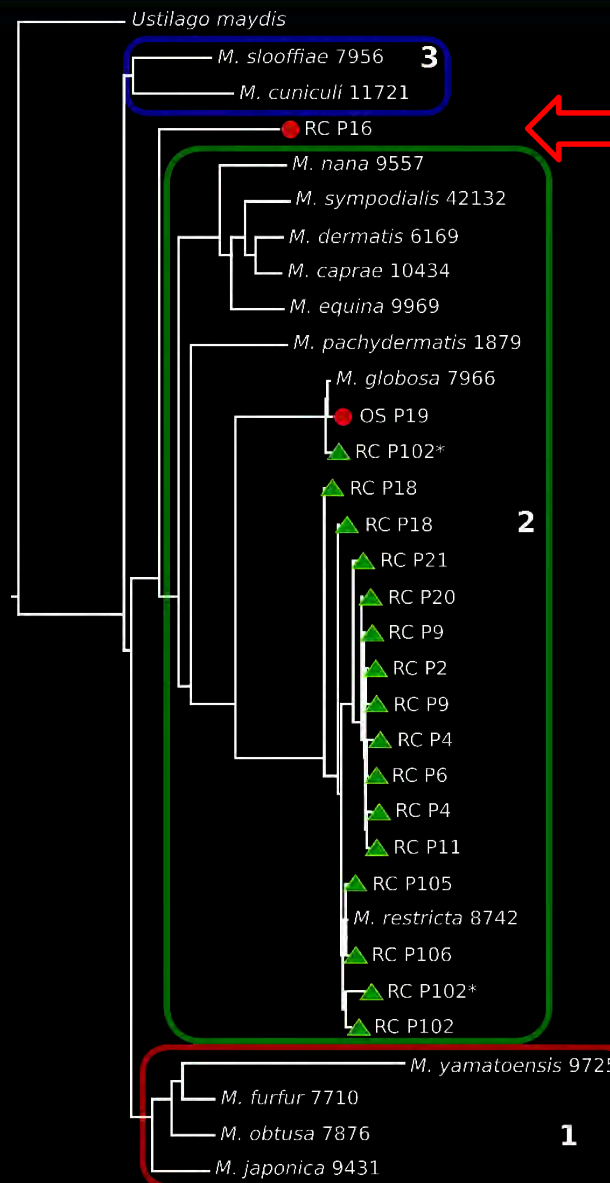
Unknown Fungi!

14 accepted *Malassezia* species

Cluster 2 the most common found on human skin

ANI score suggests potentially new species in Cluster 2

Perhaps a potentially more divergent *Malassezia* species

Perhaps the dark matters!!

Metagenomic assembly at a large scale
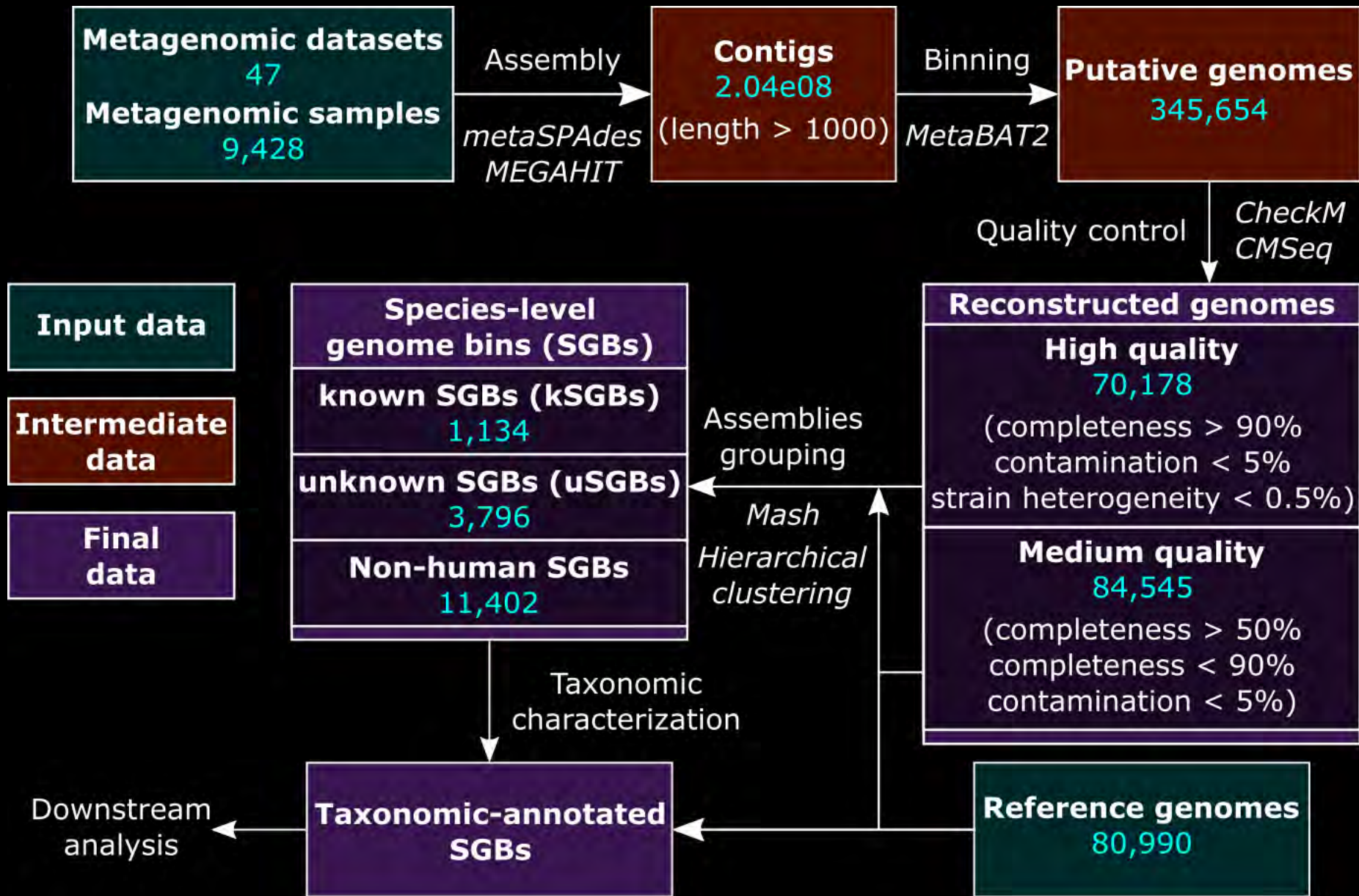
Reconstructing and cataloguing >150,000 human microbiome genomes

A very large assembly effort:
- \> 9,500 metagenomes
- \>154,000 reconstructed genomes
- \> 5,000 species-level bins

➔ Expanding the context for metagenomic studies!
➔ 77% of the SGBs are unknown!!
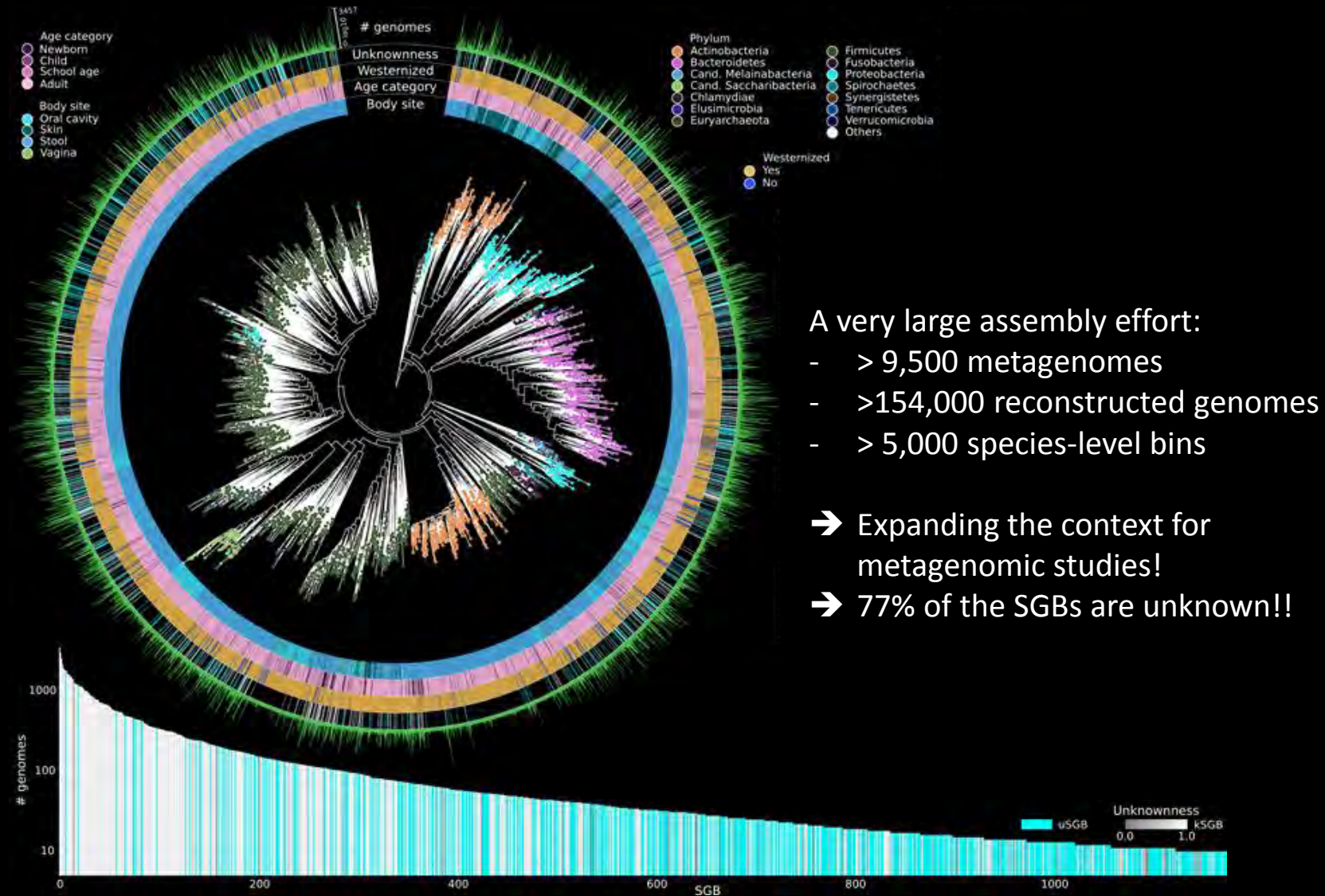
Toward fully mapping the human microbiome
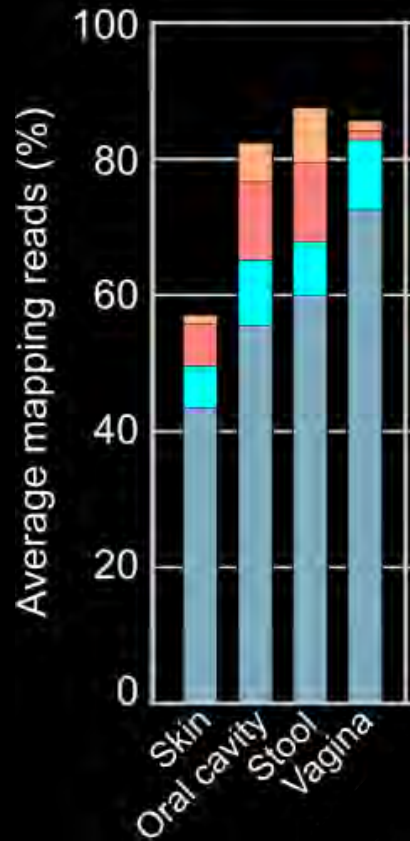
Increased mappability of the gut microbiome
Avg 67.76% to 87.51%

Increased mappability of under-sampled categories and populations

Mappability increase due to new strains of known species and to novel species

Thanks!

The Laboratory of Computational Metagenomics
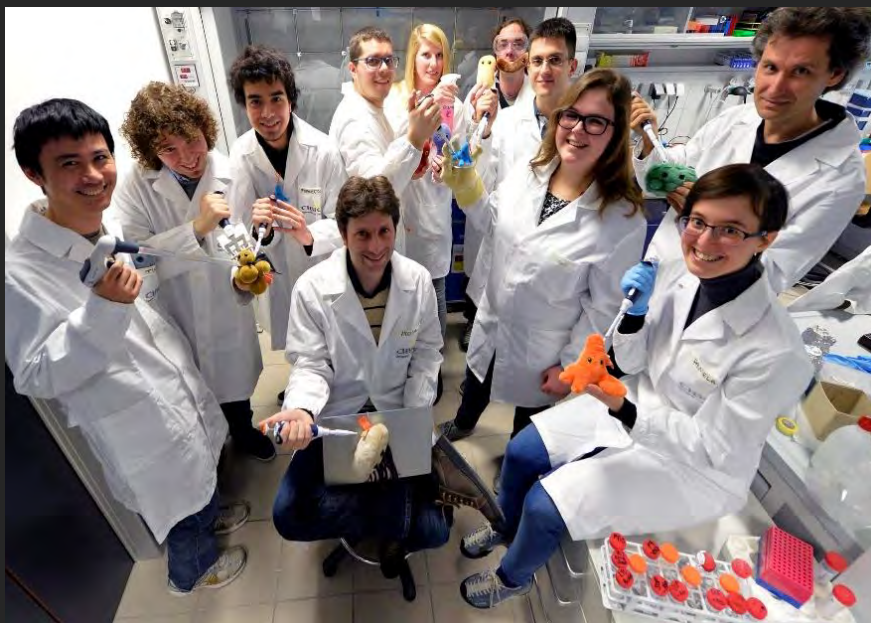
Adrian Tett
Tin Truong
Edoardo Pasolli
Federica Pinto
Federica Armanini
Francesco Asnicar
Serena Manara
Paolo Ghensi
Moreno Zolfo
Francesco Beghini
Kun Huang
Nicolai Karcher
Paolo Manghi
Andrew Thomas

http://segatalab.cibio.unitn.it - nicola.segata@unitn.it

@nsegata   @cibiocm

The complex dynamics of antimicrobial resistance and microbiomes
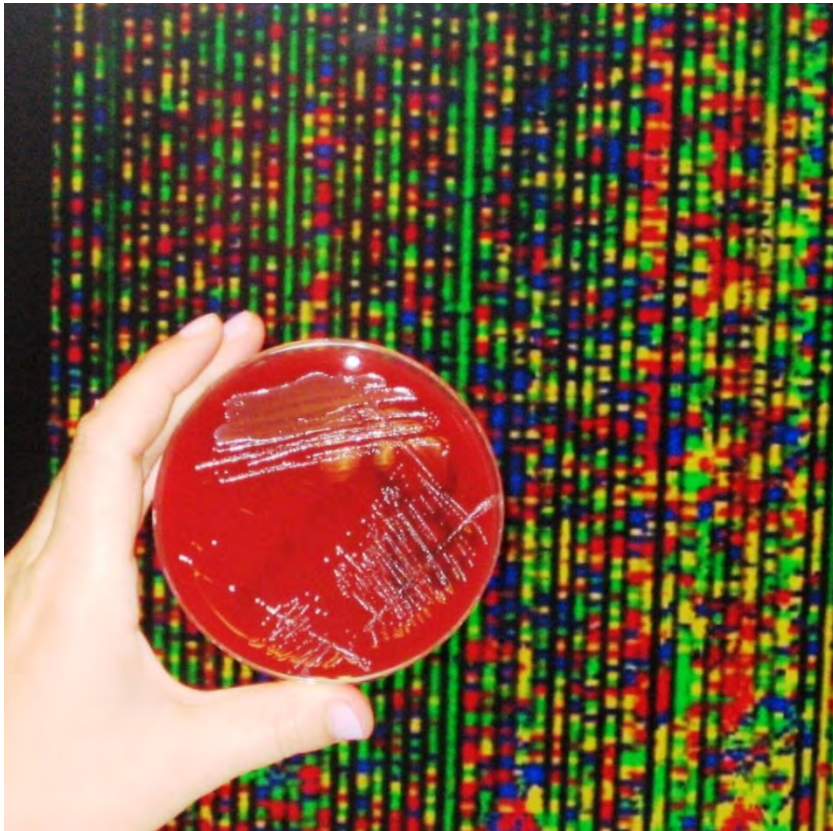
Willem van Schaik

Institute of Microbiology and Infection
University of Birmingham, United Kingdom

w.vanschaik@bham.ac.uk
Twitter: @WvSchaik
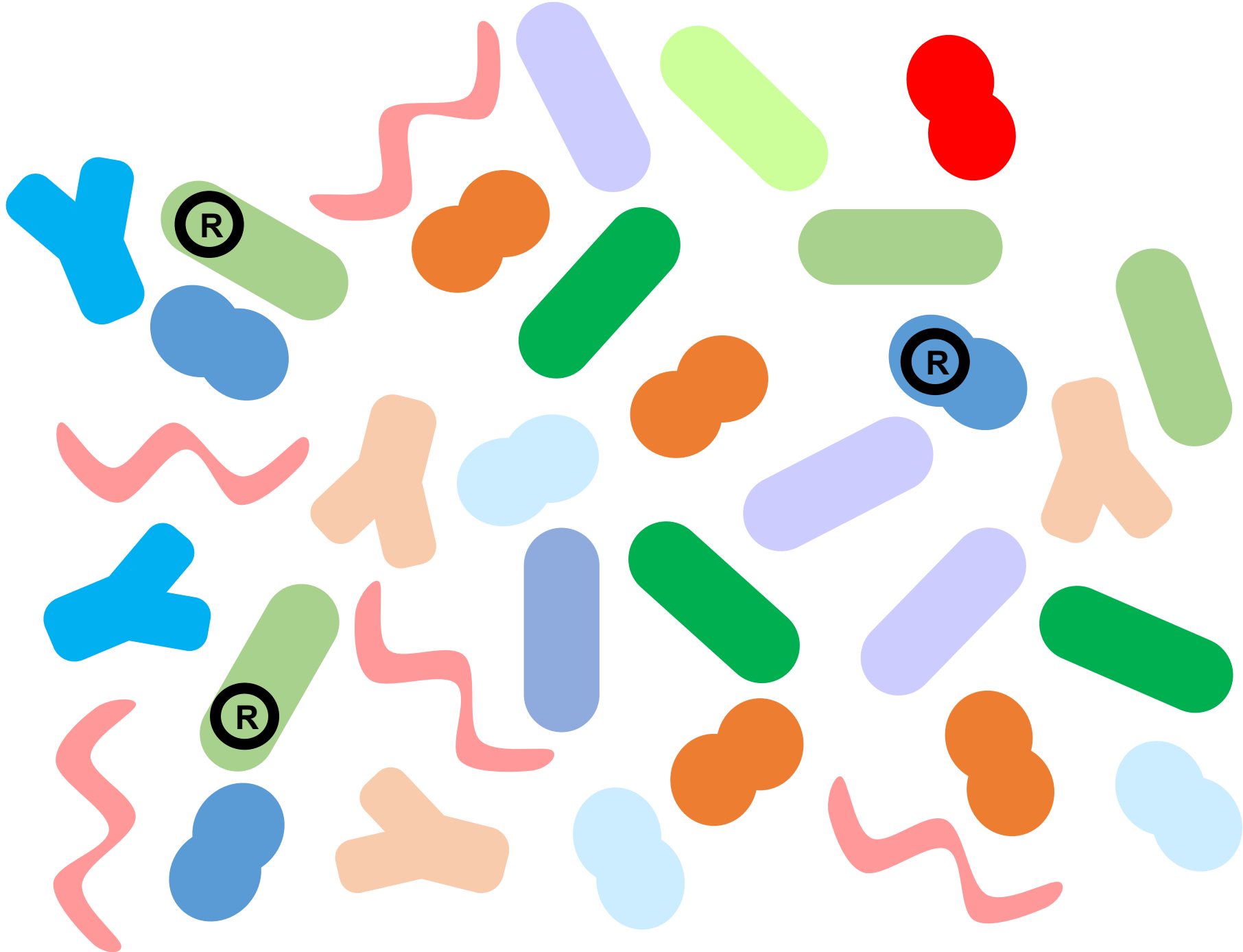
Antibiotics and microbiomes

Intensive antibiotic therapy and the human gut microbiome

Sewage and antibiotic resistance

Antibiotic resistance in rural and urban environments

Dynamics of the microbiome and resistome of patients in Intensive Care Units (ICUs)

Selective digestive tract decontamination

Decontamination of oropharynx and intestinal tract

Mix of two antibiotics (colistin, tobramycin) and an antifungal (amphotericin) as a paste/suspension in throat and intestinal tract + intravenous cefotaxime for first 4 days at ICU

Widely used in ICUs

Lowers patient morbidity, mortality

Minimal effect on resistance, based on diagnostic cultures
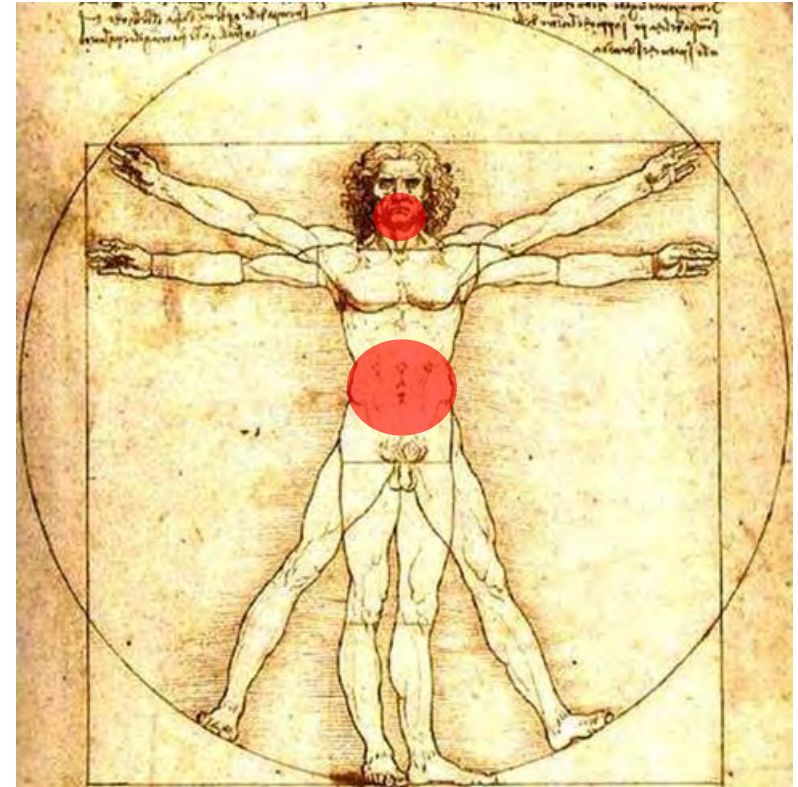
van der Waaij *et al.*, 1972. J Hyg (Lond) 70:605
De Smet *et al.*, 2009. N Engl J Med 360:20
Daneman *et al.*, 2013. Lancet Infect. Dis. 13:328
Oostdijk *et al.*, 2014. JAMA 312:1429

10 ICU-patients, acutely admitted
no history of hospitalisation or antibiotic use
stay ≥10 days, all treated with SDD
sampled at different time points

10 healthy volunteers, two samples, 1 year apart
no antibiotic therapy

Gut microbiome of healthy controls

PC2: 15.5%

PC1: 39%

Buelow, Bello Gonzalez *et al.*, 2017. Microbiome 5:88

Gut microbiome of ICU patients (≤ 5 days)
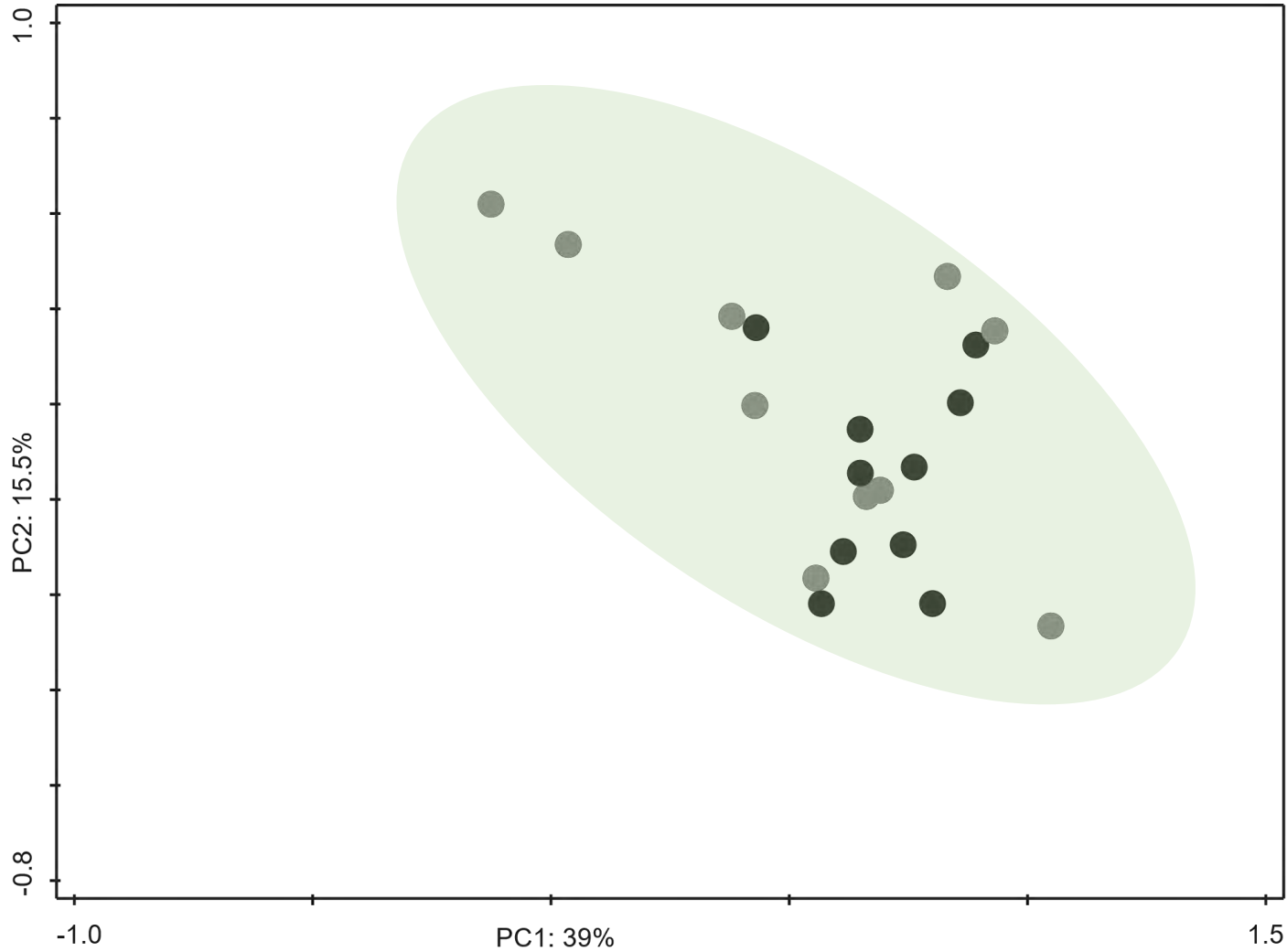
Buelow, Bello Gonzalez *et al.*, 2017. Microbiome 5:88
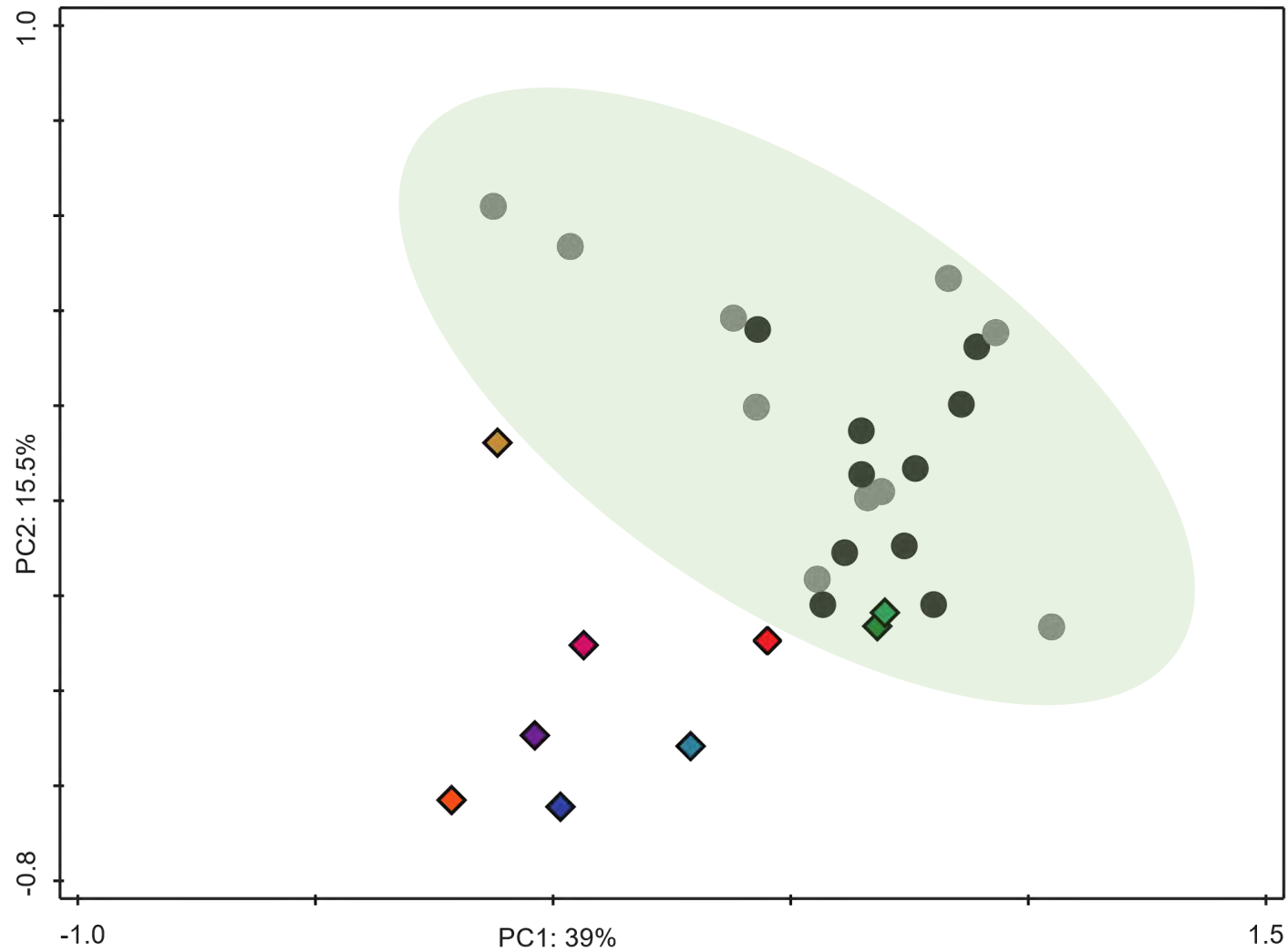
Gut microbiome
of ICU patients

Lower diversity

Overgrowth by
Enterococcus

Buelow, Bello Gonzalez *et al*.,
2017. Microbiome 5:88
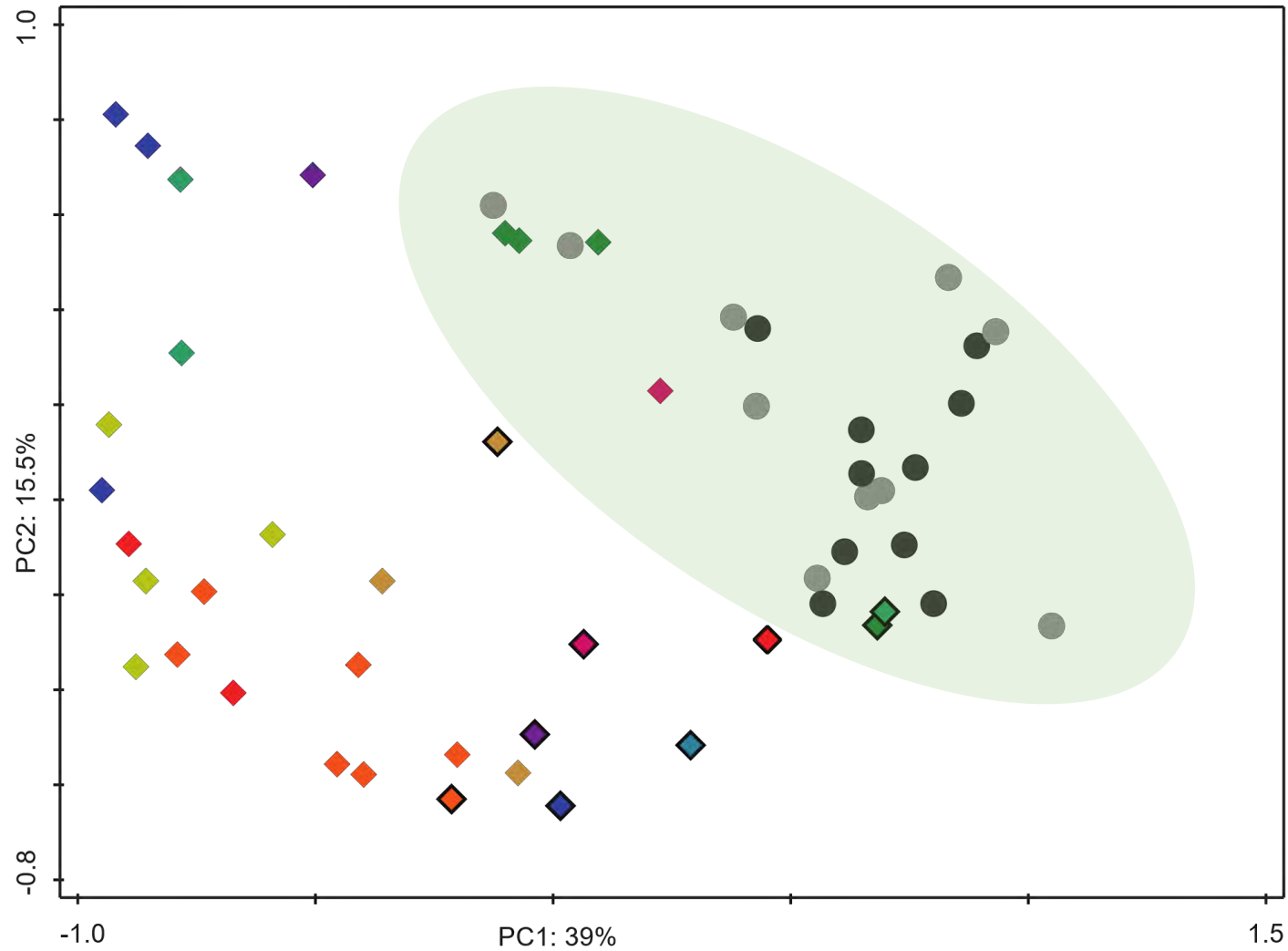
The gut resistome

nanolitre-scale qPCRs (Fluidigm Biomark)
88 samples x 96 targets
higher dynamic range vs shotgun sequencing
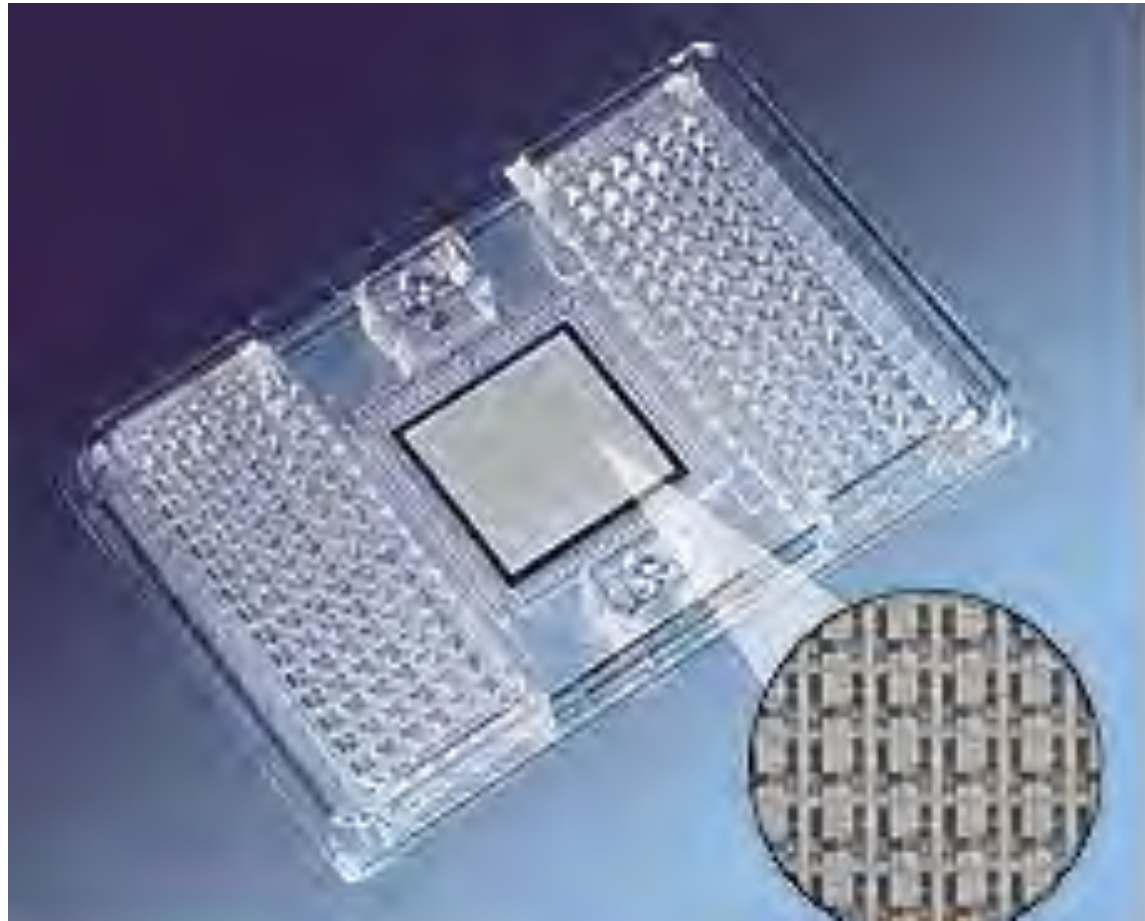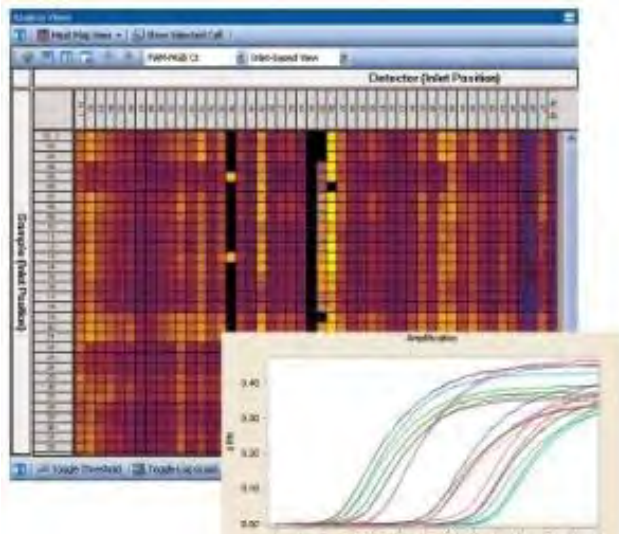
The gut resistome

16S rRNA for relative quantification of abundance

Primers for 81 resistance genes

Most common resistance genes in gut microbiota
Forslund *et al.*, 2013. Genome Res. 23:1163; Hu *et al.*, 2014. Nat. Commun 4:2151

Clinically relevant resistance genes
ESBLs, carbapenemases, *mecA*, vancomycin resistance genes

Buelow, Bello Gonzalez *et al.*, 2017. Microbiome 5:88
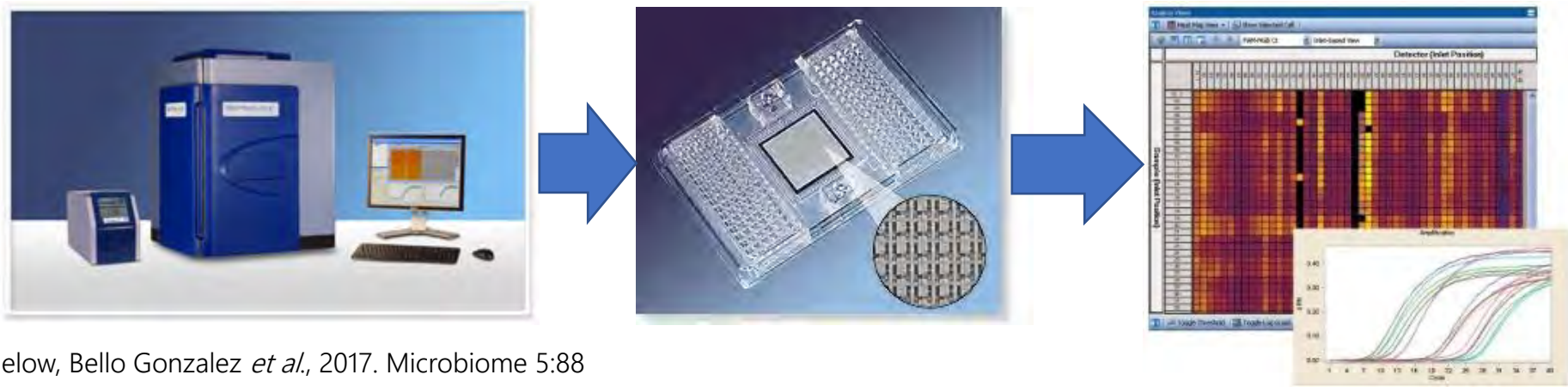
The gut resistome

Large diversity of antibiotic resistance genes

normalized abundance

-10.6 11.8

Buelow, Bello Gonzalez *et al.*, 2017. Microbiome 5:88
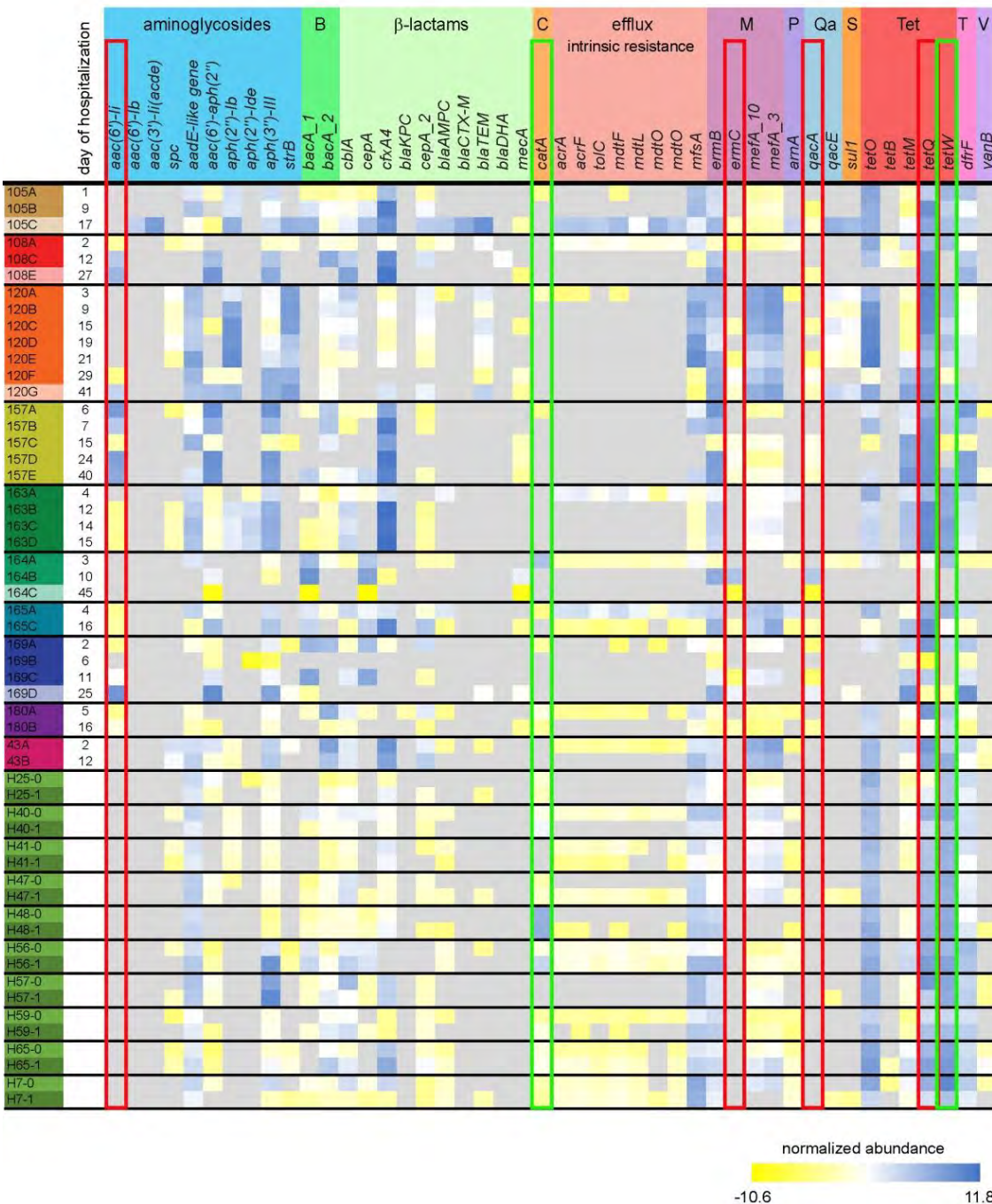
The gut resistome
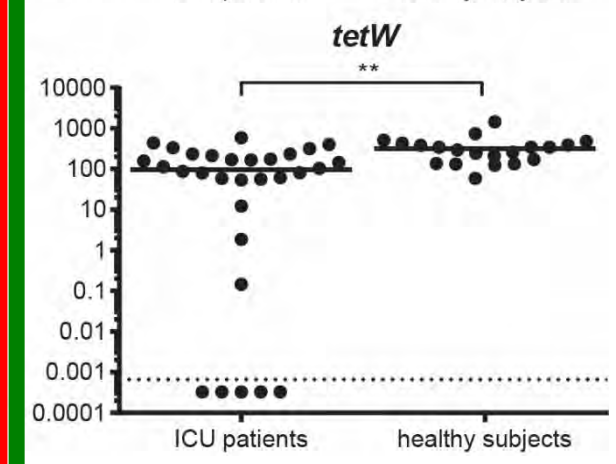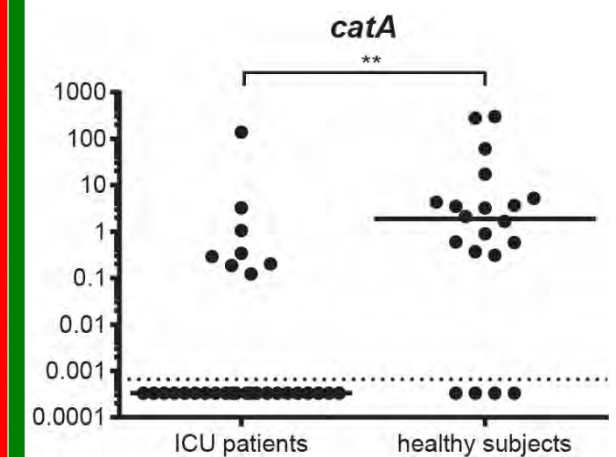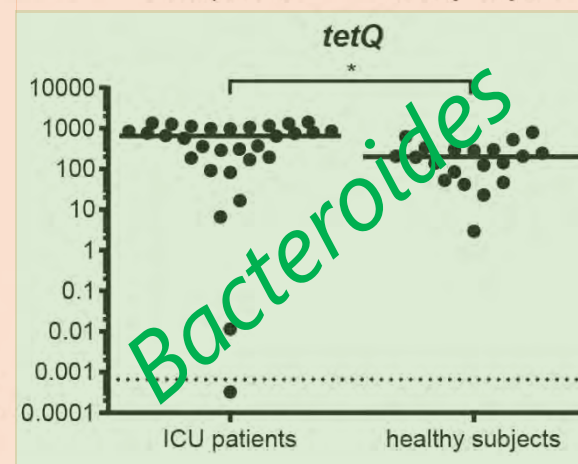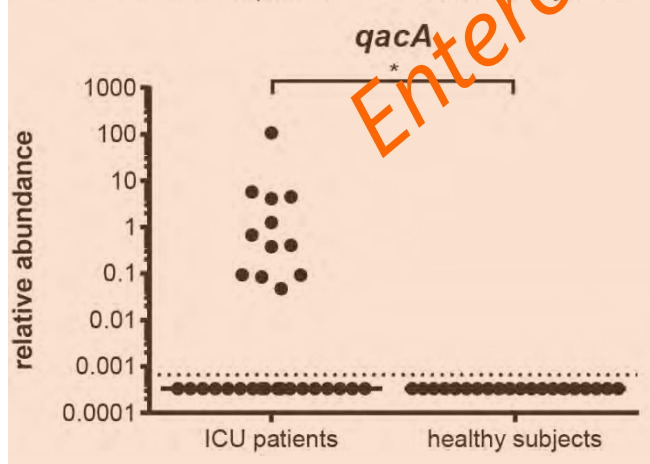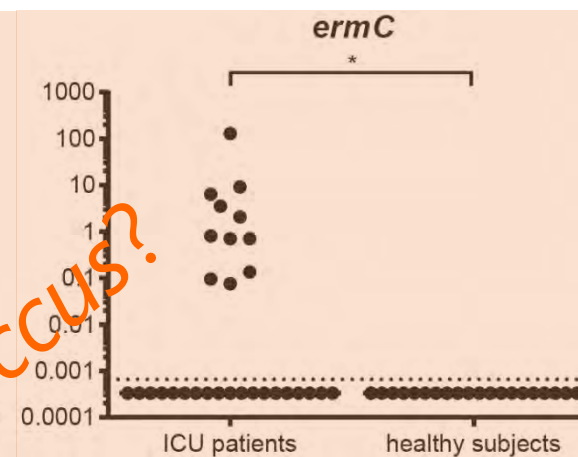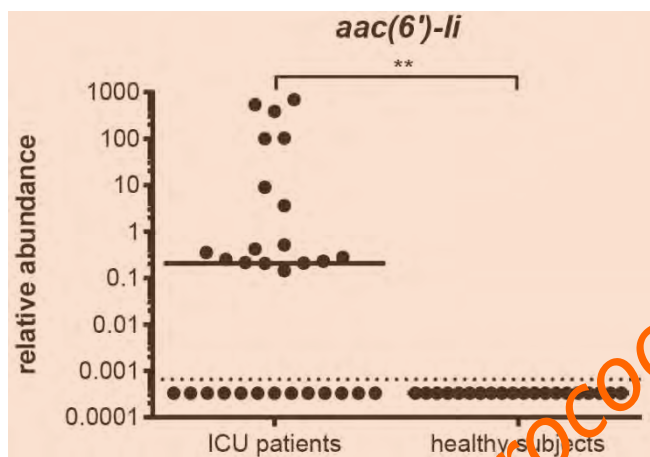
No selection for antibiotic resistance genes conferring resistance to antibiotics used in SDD; selection for resistance genes in bacteria that are (intrinsically) resistant

Sewage microbiome and resistome

Samples collected in March – April 2014, 4 samples/site, 1 wk apart
16S rRNA gene sequencing on Illumina MiSeq; Resistome analysis by qPCR

Buelow *et al.,* FEMS Microbiol Ecol 2018 94:fiy087

Sewage microbiome

prior to treatment

after treatment
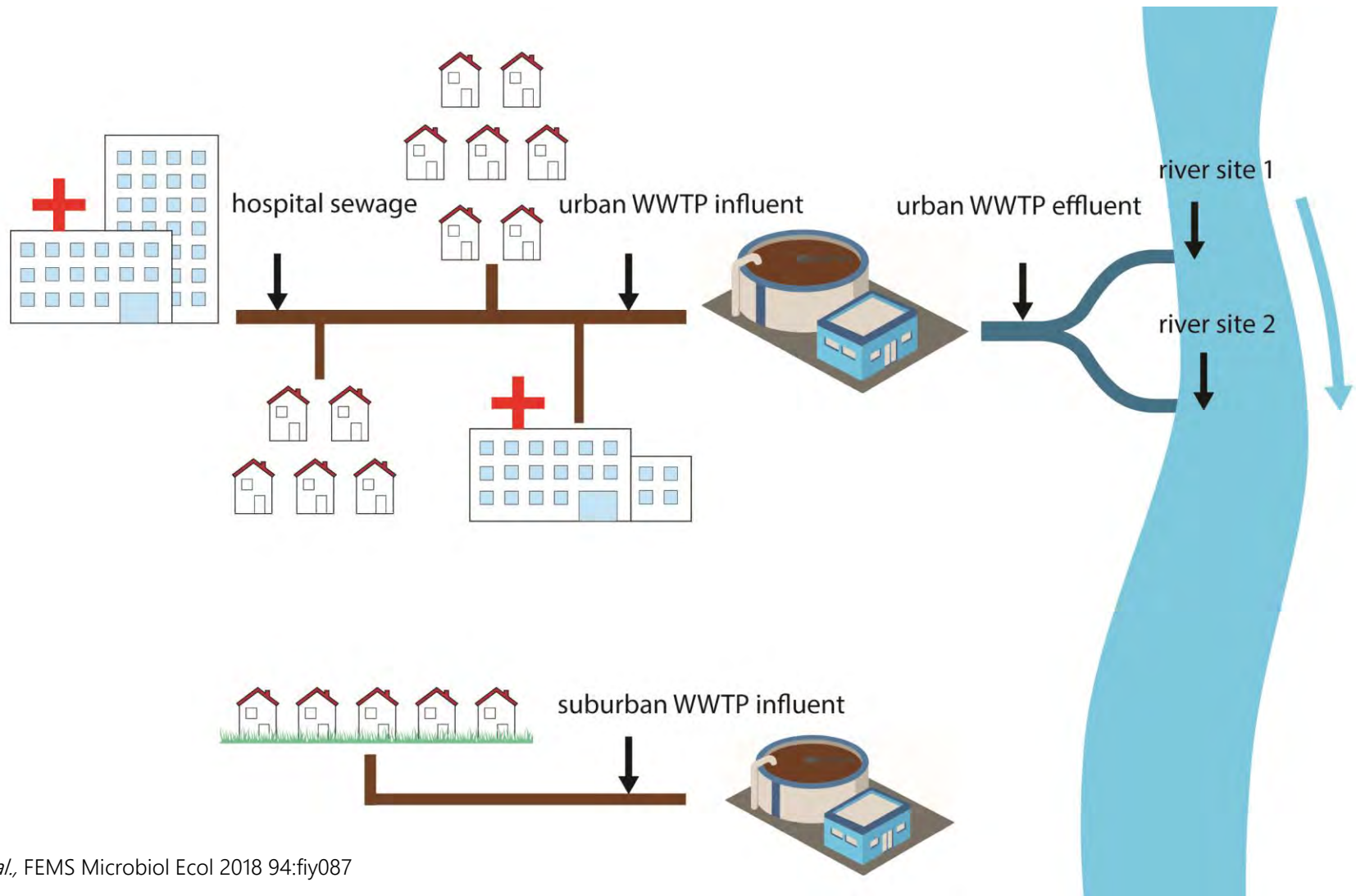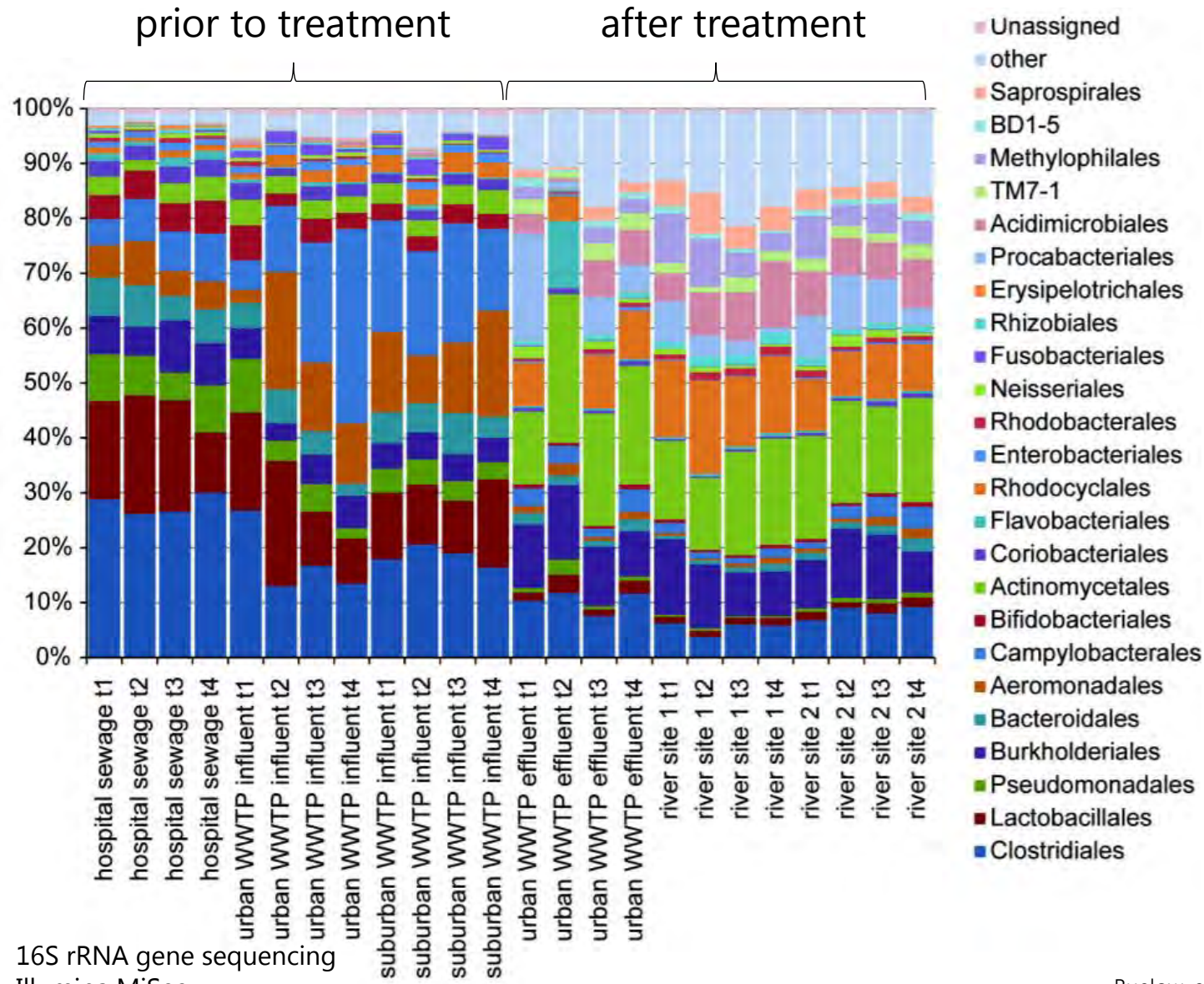
Extremely complex ecosystem

Human gut bacteria replaced by environmental taxa

Major impact of sewage treatment

Legend:
- Unassigned
- other
- Saprospirales
- BD1-5
- Methylophilales
- TM7-1
- Acidimicrobiales
- Procabacteriales
- Erysipelotrichales
- Rhizobiales
- Fusobacteriales
- Neisseriales
- Rhodobacterales
- Enterobacteriales
- Rhodocyclales
- Flavobacteriales
- Coriobacteriales
- Actinomycetales
- Bifidobacteriales
- Campylobacterales
- Aeromonadales
- Bacteroidales
- Burkholderiales
- Pseudomonadales
- Lactobacillales
- Clostridiales

X-axis labels: hospital sewage t1, hospital sewage t2, hospital sewage t3, hospital sewage t4, urban WWTP influent t1, urban WWTP influent t2, urban WWTP influent t3, urban WWTP influent t4, suburban WWTP influent t1, suburban WWTP influent t2, suburban WWTP influent t3, suburban WWTP influent t4, urban WWTP effluent t1, urban WWTP effluent t2, urban WWTP effluent t3, urban WWTP effluent t4, river site 1 t1, river site 1 t2, river site 1 t3, river site 1 t4, river site 2 t1, river site 2 t2, river site 2 t3, river site 2 t4

16S rRNA gene sequencing
Illumina MiSeq

Buelow *et al.,* FEMS Microbiol Ecol 2018 94:fiy087

Sewage resistome

High levels of antibiotic resistance genes in hospital sewage
Decrease upon passage through sewerage system and treatment

Buelow *et al.,* FEMS Microbiol Ecol. 2018 94:fiy087

Sewage resistome
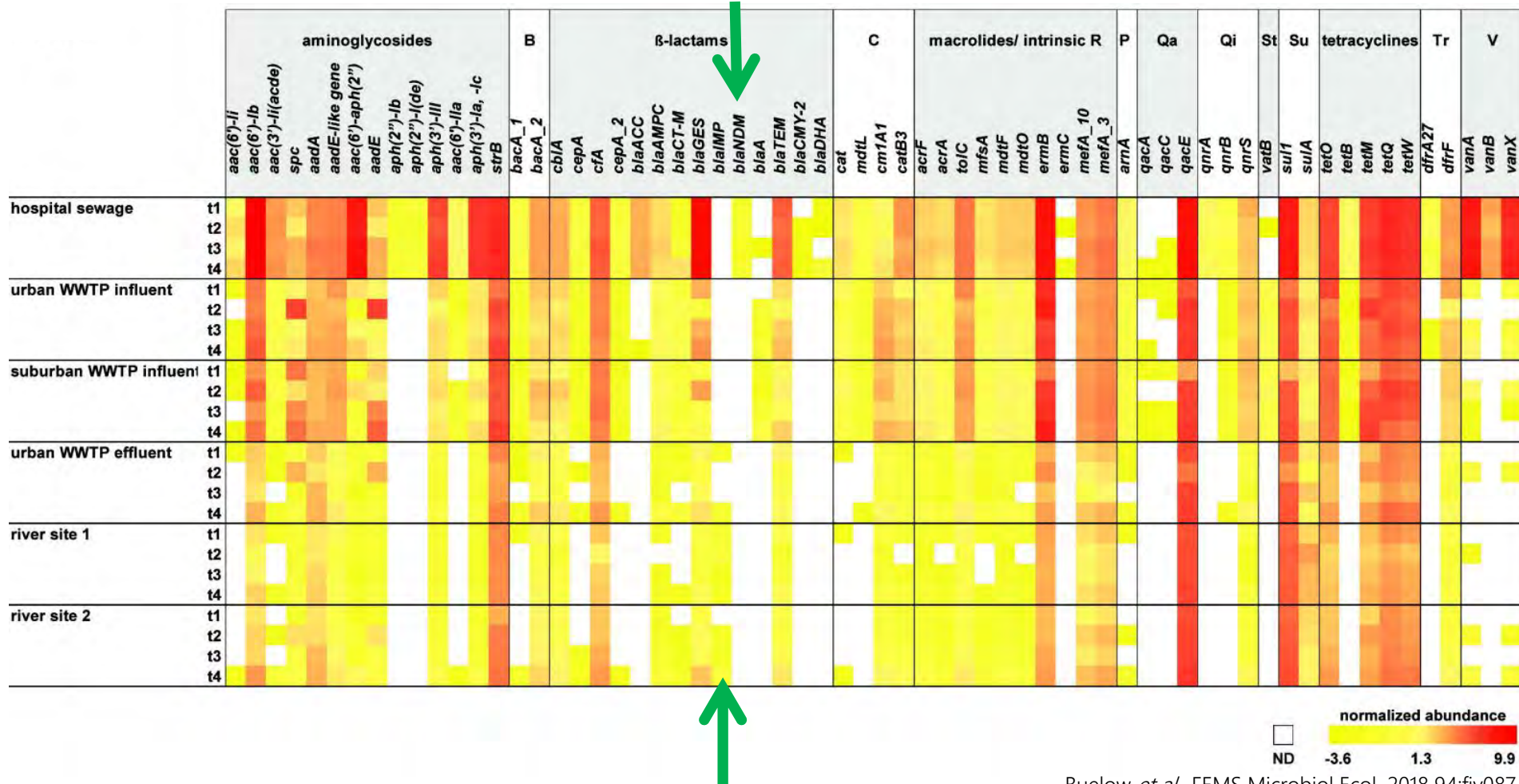
Buelow *et al.,* FEMS Microbiol Ecol. 2018 94:fiy087

Carbapenemases
*bla*NDM in hospital sewage
*bla*IMP in effluent and river water, but not in sewage

Microbiomes as reservoirs for antibiotic resistance genes

The human gut microbiome is a reservoir of antibiotic resistance genes

Selection for resistance is complex, particularly upon exposure to multiple antibiotics

Human-associated microbiomes provide opportunities for horizontal gene transfer of resistance determinants

Acknowledgements

Collaborators

KEY PLAYERS DRIVING ENVIRONMENTAL EMERGENCE AND SPREAD OF AMR

LUÍSA VIEIRA PEIXE

UNIVERSITY OF PORTO. PORTUGAL

Bacteria in the earth - appeared $3,5 \times 10^9$ years ago

One gram of soil: up to 1010 bacterial cells

& Species diversity of 4×10^3 to 5×10^4 species

Antibiotics production: tens (daptomycin, vancomycin) to hundreds (erythromycin, streptomycin) of millions of years ago

Raynaud & Nunan, Pone. 2014

2

Extensive Natural Collection of AMR Genes

Soil, fresh and marine water phyla contain
a huge diversity of ARG genes.

>> More diverse than the clinical ARG pool

Antibiotic resistance is ancient:
- *TetM* and *VanA* in DNA 30,000-year-old;
- Metallo-β-lactamases emerged one billion years ago.

Psychrobacter psychrophilus
MR29-12

Permafrost Siberian
15 000-35 000 anos
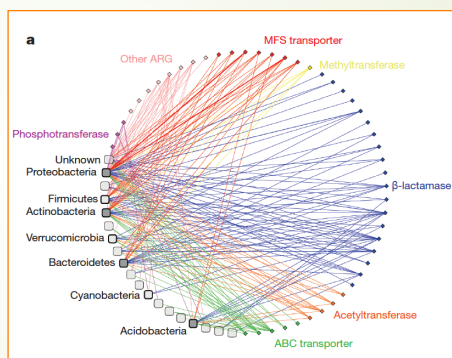
New MBL in soil

Network of predicted bacterial phyla for each AMR used in cross-soil comparisons (n=880) (Forsberg et al., Nature. 2014)
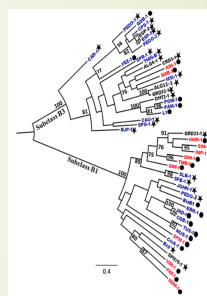
Without human interference, selection for resistance already occurs naturally in microbial populations in soil, water and other habitats

3

Gudeta et al., Frontiers Microb. 2016; D'Costa et al, Nature. 2011; Forsberg et al, Nature.2014; Martinez J.L. Science.2010; FEMS Microbiol Lett 296.2009; Riesenfeld et al, Envir. Microbiol. 2004

What are AMR genes doing in these Bacteria?

Protection against antibiotics

ABR
genes
silencing

Physiological functions
E.g. detoxification; virulence, signal trafficking, intra-domain communication. 2' N-acetyltransferase of *Providencia stuartii* - acetylation of peptidoglycan and gentamycin

Antibiotic-producing microorganisms
...Streptomyces...synthesize over half of all known antibiotics...
... Resistance elements clustered in antibiotic biosynthetic operons
Screening of 480 soil organisms:

Co-habitants of
AB producers

catA
tet
tetM
cml
vanA
sul1
rph
vanA
strA
bla$_{IMI}$
vanA
strA
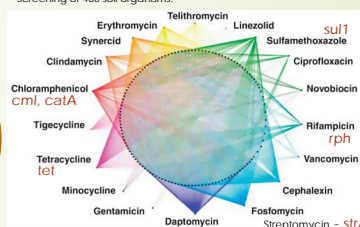
4

Surette and Wright. Annu. Rev. Microbiol. 2017; D'Costa et al, Curr. Opinion Microbiol. 2007; D'Costa et al, Nature, 2011; Fdantas et al, Science. 2008; EMS Microbiol Lett 296. 2009

Is Horizontal Gene Transfer (HGT) shaping natural Resistome ?

HGT of R genes is a key driver for acquisition of antimicrobial resistance in the clinical setting

Study of soils bacteria by metagenomic
Forsberg et al., Science.2014.

Mobility Elements are Rare in Bacteria from Natural Environments

HGT not relevant

Phylogeny, Key role in soil resistome content

Antibiotics have been shaping the bacterial kingdom for millions of years and proviking the evolution of counter-strategies

Fondi et al, Genome Biol Evol. 2016

5
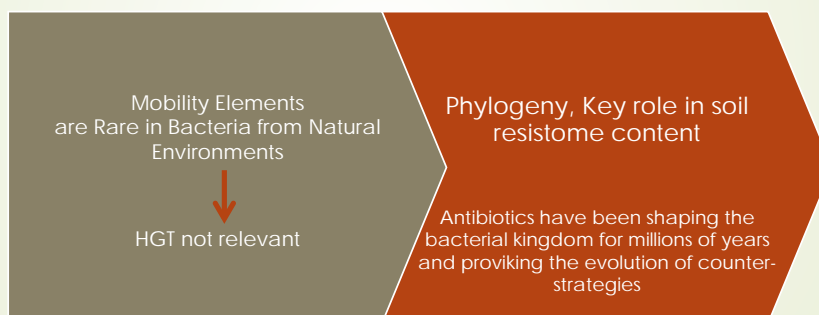
AMR Genes Mobilization and Transfer from Environmental microorganisms to humans/animals bacteria

HGT events mainly involve molecules of the same kind (i.e. either plasmids or chromosomes).

ICE/cTn

Environmental microorganism

IS/Tn

R Plasmid
(co & cross R)

HGT

Pathogenic

Commensal

HGT

Selective Pressure

Frequent Rare?
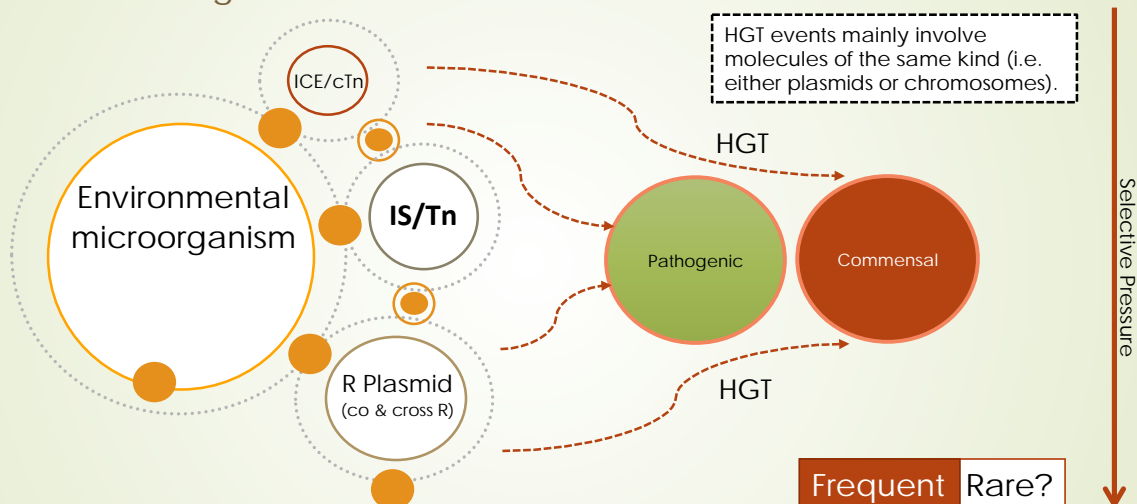
IS - insertion sequence; ICE - integrative conjugative element; Tn - conjugative transposon

Fondi et al, Genome Biol Evol. 2016

6

3

Who Are the R Genes Donors?

Bacteria Subsisting on Antibiotics

Dantas et al., Science 2008

Different antibiotics added to soil:

- Few soil bacterial groups able to survive:
 Mostly Proteobacteria closely related to human pathogens
(e.g. *Enterobacteriaceae, Pseudomonas, Burkholderia,
Vibrio*), but also Actinobacteria, Bacteroidetes and
Firmicutes.

Bacterial Host

Certain environmental bacterial groups more related
with opportunistic & human pathogens more prone to
act as R gene donors to those bacteria

Vaz-Moreira, I. et al. (2014); FEMS Microbiol. Review.

7

Origin of relevant horizontally transferred R genes

Bacterial Host

Gene	Antibiotic resistance	Bacterial species	MGEs*
bla_{CTX-M}	3rd generation Cephalosporins	*Kluyvera* spp.	IS*Ecp1*, ISCR1
qnrA	↓ susceptibility to Fluorquinolones	*Shewanella algae*	ISCR1, IS26
qnrS	↓ susceptibility to Fluorquinolones	*Vibrio splendidus*	IS2, IS26, IS*EcI2*, Tn3-like, mic
fosA3	Fosfomycin	*Kluyvera georgiana* (CTX-M-8 origin)	IS26
vanA	Glycopeptide	*Paenibacillus thiaminolyticus*	Tn1546
bla_{SBM-1}**	Carbapenems	Gene from soil metagenome	ISCR1
strA, strB	Streptomycin	*Streptomyces* spp.	Tn5393

* MGE = Mobile genetic elements; ** just one report

Guardabassi L et al. AAC 2004; Wachino J et al. AAC 2011; Ohnuki T et al. J Bac 1985; Cantón R et al. Front Micr 2012; Hooper DC. Ann N Y Acad Sci 2015; Ito R et al. JAC 2018

8

Origin of relevant horizontally transferred R genes

Dispersed host & MGE + Antibiotics ? = R mobilization likely to occur in different locations

Gene	Antibiotic resistance	Bacterial species	MGEs
cfr	Oxazolidinones, Phenicols, Lincosamides, Pleuromutilins, Streptogramin A	Bacillus spp.?/ Staphylococcus spp.	IS21–558, IS26, ISEnfa5, Tn558
optrA	Oxazolidinones, Phenicols	Staphylococcus sciuri	IS1216, Tn6261
oqxAB	↓ susceptibility to Fluorquinolones	Klebsiella pneumoniae	Tn3, IS26
mcr-1	Colistin	Moraxella novel species	ISApl1, Tn6330
qnrB	Fluorquinolones	spp.	ISCR1, IS26, ISEcp1, ISEcp1C, IS3000, IS6100
bla$_{CMY-2-like}$	3rd generation Cephalosporins	Citrobacter spp.	ISEcp1, IS26, IS5, ISkpn26

Setting where mobilization occurred is unknown

Dai L et al. AAC 2010; Zhang WJ et al, AAC 2015; Wang Y et al. JAC 2015; Sun C et al, JAC 2018; Fan R et al., Vet Microbiol 2017; Rodriguez-Martinez JM et al., Drug Resist Updat 2016; Snesrud E et al., MBio, 2018; Ribeiro TG et al., AAC 2015

9

Conditions influencing environmental emergence and spread of resistance

- Human and animal use
- Antibiotic production

Antimicrobial load

Amount/ Diversity/ Persistence

- Sublethal concentrations
- FQ & tetracyclines: months to years; Mobility
- Most antibiotics are excreted unmetabolized

* Persistence of monophasic variant of S. Typhimurium copper tolerant (sil+pco clusters + ABR genes) in pigs & pig environment (Mourão et al., IJAA 2015).

International Journal of Antimicrobial Agents 45 (2015) 610–616

Metal tolerance in emerging clinically relevant multidrug-resistant Salmonella enterica serotype 4,[5],12:i:– clones circulating in Europe

Joana Mourão

10

Mourão et al, IJAA. 45(6):610-6. 2015; Grave et al, JAC 2014

Conditions influencing environmental emergence and spread of resistance

Slide 11:
- Human and animal use
- Antibiotic production

Antimicrobial load → Amount/ Diversity/ Persistence
- Sublethal concentrations
- FQ & tetracyclines: months to years; Mobility
- Co-selection by disinfectants & metals*

Microbes & Mobilome composition & density → Diversity/spectra R/ amount
- Survival to environmental stressors
- Contact between H, A and E diverse bacteria
- Large & diverse pool of MGE
- Ability to persist in food chain/colonize/infection

Integrative conjugative elements (ICE)

Carbapenemases on the move: it's good to be on ICEs
Botelho et al. Mobile DNA (2018) 9:37
https://doi.org/10.1186/s13100-018-0141-4

Mourão et al, IJAA. 45(6):610-6. 2015: Grave et al, JAC 2014

11

Conditions influencing environmental emergence and spread of resistance

Slide 12:
- Human and animal use
- Antibiotic production

Antimicrobial load → Amount/ Diversity/ Persistence
- Sublethal concentrations
- FQ & tetracyclines: months to years; Mobility
- Co-selection by disinfectants & metals*

Microbes & Mobilome composition & density → Diversity/spectra R/ amount
- Survival to environmental stressors
- Contact between H, A and E diverse bacteria
- MGE: Host range & maintenance-Fitness cost
- Ability to persist in food chain/colonize/infection

Other factors → Socio-economic
- Lack of hygiene & sanitation
- Agricultural intensification & land-use changes

Others
- Education
- Cultural (food habits & open defecation)

Extension of R genes environmental contamination

Emergence of new R genes

Mourão et al, IJAA. 45(6):610-6. 2015: Grave et al, JAC 2014

12

Where is favoured the emergence and spread of resistance?
Hot spots of AMR environmental contamination

Hospital wastewater

High level of different
antibiotics
Accumulation of R pathogens
& commensals

General sewage waste

High Income countries:
30% of wastewater goes untreated
Urban runoff to beaches

Low Income countries:
Few % sewerage
Open defecation
Illegal dumping in landfills, sea

Animal waste

High level of different antibiotics
Accumulation of R pathogens
& commensals

Low efficiency of composting
to ↓ concentrations of
antibiotics and R bacteria in
manure.

Antimicrobial Production Waste

High levels of Ab,and R genes
downstream discharge site-drugs
WWTP. India. Patancheru, Hyderabad
(Kristiansson et al. 2011)

"No company publishes its
environmental audit results,
or its discharge levels."
https://amrbenchmark.org

13

Le Devendec et al, Vet. Microbiol. 2015; Kristiansson et al, Plos One. 2011; Schluter et al, 2007; Zhang et al, 2011

Where is favoured the emergence and spread of resistance?

The Farms That Make Pharma Happy
Largest antimicrobial consumers in 2013 and 2030

Russia 17.1%
Germany 5.2%
U.S. 21.9%
Spain 6.2%
China 59.1%
Iran 29.5%
India 82.1%
Mexico 64.7%
Brazil 41.1%
Argentina 20%

120K tons
10K
2K

Source: Van Boeckel et al, "Reducing antimicrobial use in food animals," *Science*, Sept. 29, 2017

Bloomberg

-50 – -30 -29 – -20 -19 – -10 -9 – 0 1 – 10 11 – 20 21 – 40 41 – 80 >80 No data

Percentage change in antibiotic consumption per capita 2000-2010,
by country

BRICS- Brasil, Russia, India, China, South Africa

65% increase in 2000-2015
Double the consumption in Low to middle income countries: BRICS -largest contributors

14

https://www.cddep.org/wp-content/uploads/2017/06/swa_edits_9.16.pdf

7

Where is favoured the emergence and spread of resistance?

Previous data: highest rates of Ab resistance in Asia, South America, Rep. of South Africa

Global monitoring of antimicrobial resistance based
on metagenomics analyses of urban sewage. Hendriksen R.S et al., *Nature Communication*. 2019

Domestic sewage was collected from 79 sample locations, covering 7 geographical regions from 74 cities in 60 countries
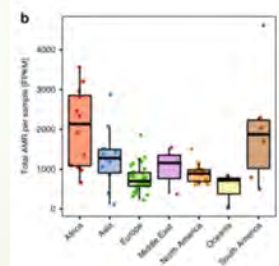
Boxplots of the total AMR fragments per kilo base per million fragments per sample, stratified by region.

How to explain recent data from Hendriksen R.S et al., *Nature Communication*. 2019**? Study design/ Methods flaws?**

15

https://www.cddep.org/wp-content/uploads/2017/06/swa_edits_9.16.pdf

Possible routes of transmission of Antibiotic-resistant bacteria
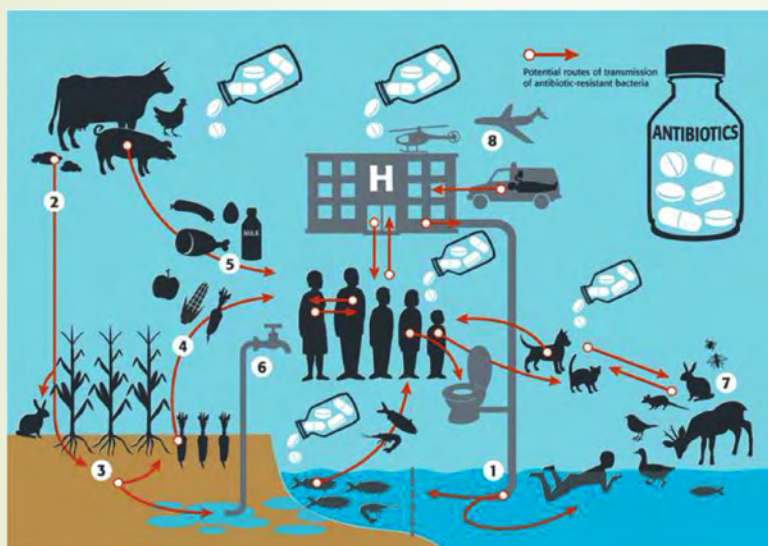
Human exposition to environmental R bacteria:
-Directly: animals, water consumption, air, or recreative activities;
-Indirectly: food-chain

Data that quantitatively link R genes uptake and human health effects is lacking

16

Stephan Harbarth et al, Antimicrobial Resistance and Infection Control. 2015

8

Origins, reservoirs and routes of transmission of MCR-like determinants

Transmission routes of ESBL Producers – Portugal (2001-2018)
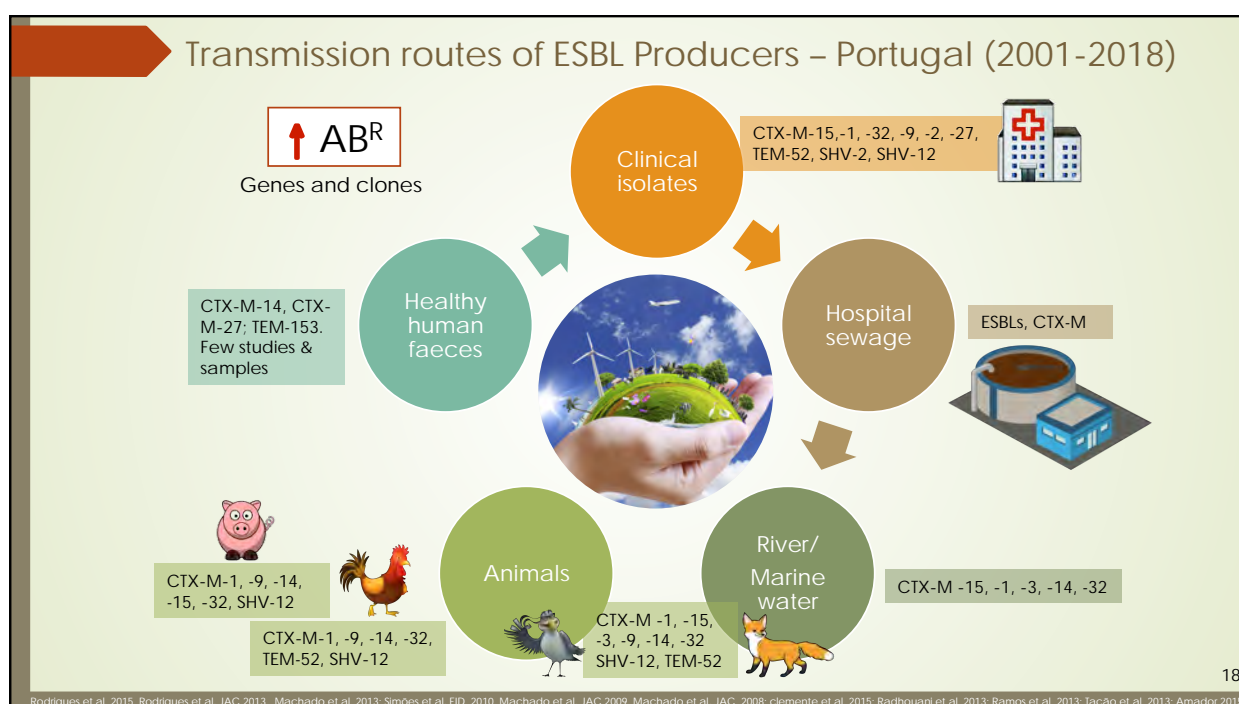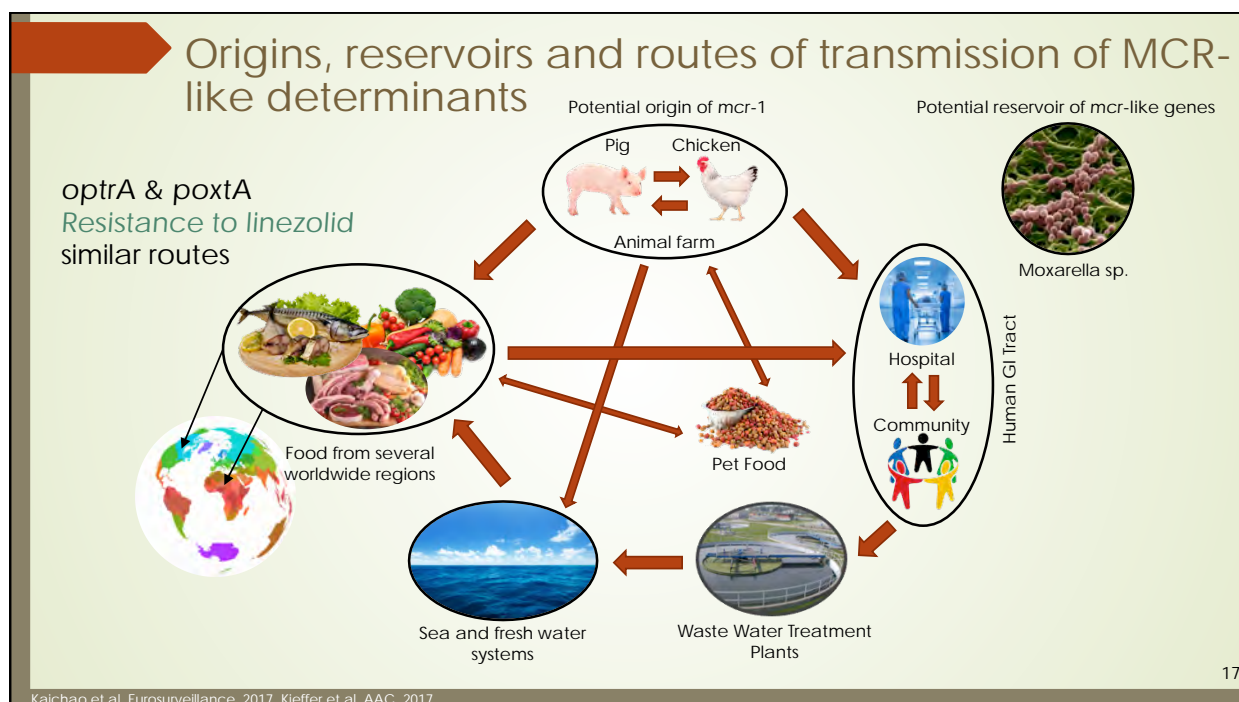
Challenges and Future Perspectives

To avoid emergence and spread of Antibiotic Resistance is critical for human health.
Environment plays an important role on Antibiotic R burden

How?

⇧ Predicted increase

⇧ Treatment of residual wastes

Alternatives to contain infectious diseases

New Ab families needed

Access to Vaccines

Antibiotic Consumption Reduction & Stewardship

Cultural changes

Barriers to change
Food habits, religion

Political & Society Commitment

Research & Innovation

Engagement of governments & society

Prioritization of topics & funds

19

Political commitment

GEOPOLITICS IN HEALTH

CONFRONTING OBESITY, AIDS, AND TUBERCULOSIS IN THE EMERGING BRICS ECONOMIES

EDUARDO J. GÓMEZ

"leaders **aspiring to build their reputations** among **elite** nations demonstrate a **quick** and **effective** public health responses; those who **scorn** the international community tend to **react slowly** and **ineffectively** to the same type of crises"

20

Civil Society Engagement

An experimental educational project for improving antibiotic resistance awareness, and for the discovering of new antibiotic producers in soil

Hunting for Microbes

Informing about Antibiotic resistance & bacterial diversity
Inspiring Students

Discovering Antibiotics

21

Thank you

Luísa Peixe
lpeixe@ff.up.pt

Enjoy
ICOHAR
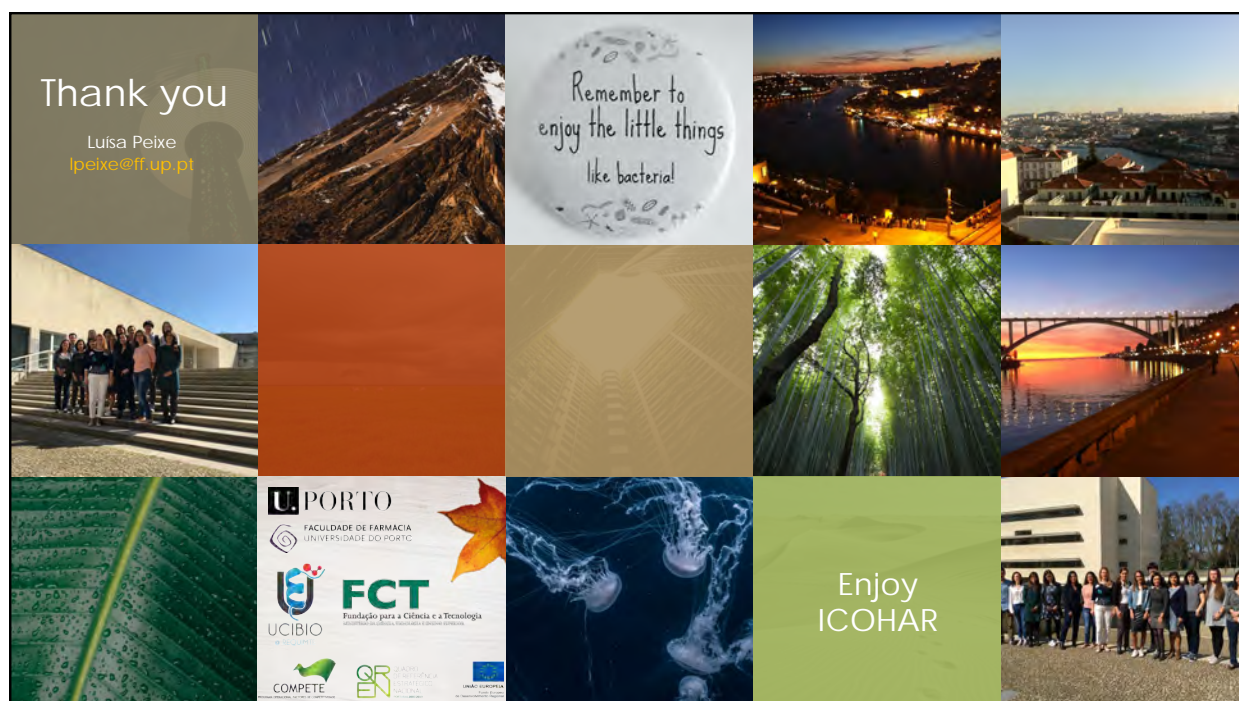
The environment and antibiotic resistance development – now and in the future

Johan Bengtsson-Palme

The roles of the environment in antibiotic resistance development

Human population

Sewage

Dispersal route for resistant pathogens

Industrial pollution

Maintenance of resistant pathogens

Source of opportunistic pathogens

Evolution reducing fitness costs of resistance genes

Recruitment of novel (and known) resistance genes

Animal agriculture

Aquaculture

(1) Sources of antibiotics and resistant bacteria

(2) Evolutionary processes in the environment

(3) Exposure routes to humans

(4) Possible intervention barriers

Bengtsson-Palme et al. 2018
FEMS Microbiol Reviews
Larsson et al. 2018
Environ Int

CARe

University of Gothenburg

There are lots of potential sources

~ 1 000 000 000 000 000 000 000 000 000 000 000 000

Johan Bengtsson-Palme | www.microbiology.se | johan.bengtsson-palme@microbiology.se

From the environment to pathogens
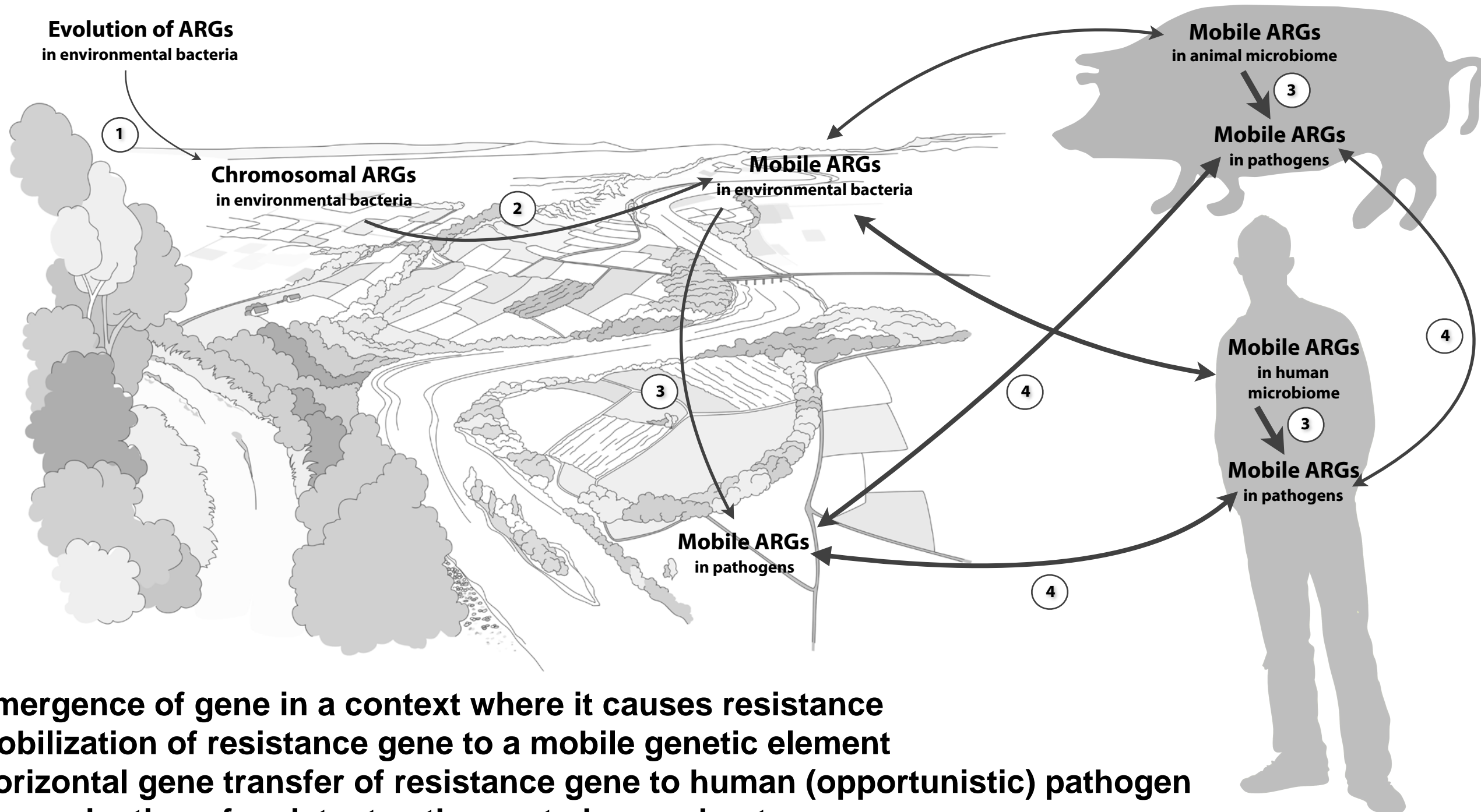
1. **Emergence of gene in a context where it causes resistance**
2. **Mobilization of resistance gene to a mobile genetic element**
3. **Horizontal gene transfer of resistance gene to human (opportunistic) pathogen**
4. **Dissemination of resistant pathogen to human host**

Johan Bengtsson-Palme | www.microbiology.se | johan.bengtsson-palme@microbiology.se

Minimal Selective Concentrations

Gullberg et al. 2011
PLoS Pathogens

Johan Bengtsson-Palme | www.microbiology.se | johan.bengtsson-palme@microbiology.se

Minimal Selective Concentrations Tetracycline

Lundström et al. 2016
Sci Tot Env

Johan Bengtsson-Palme | www.microbiology.se | johan.bengtsson-palme@microbiology.se
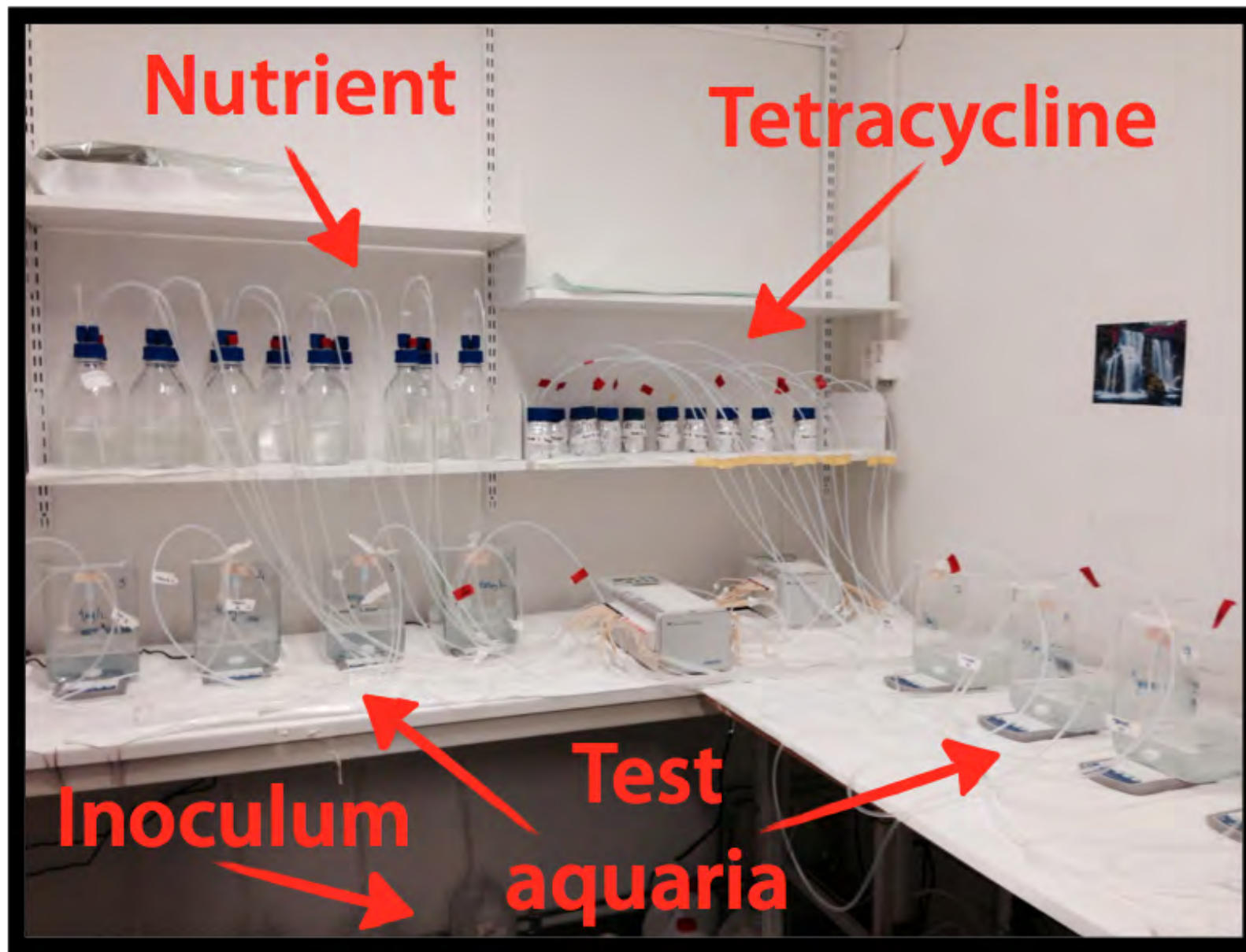
Minimal Selective Concentrations
Tetracycline
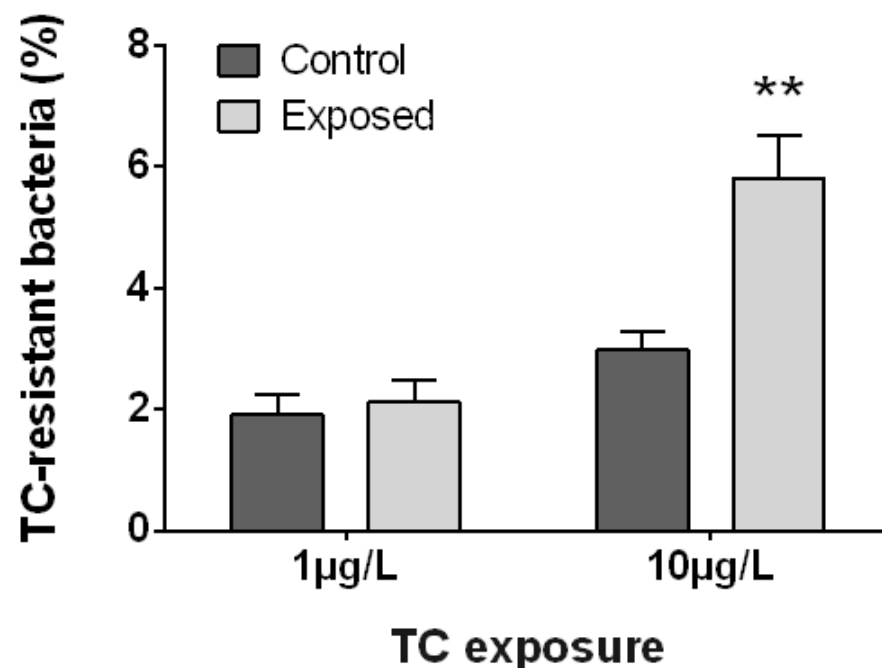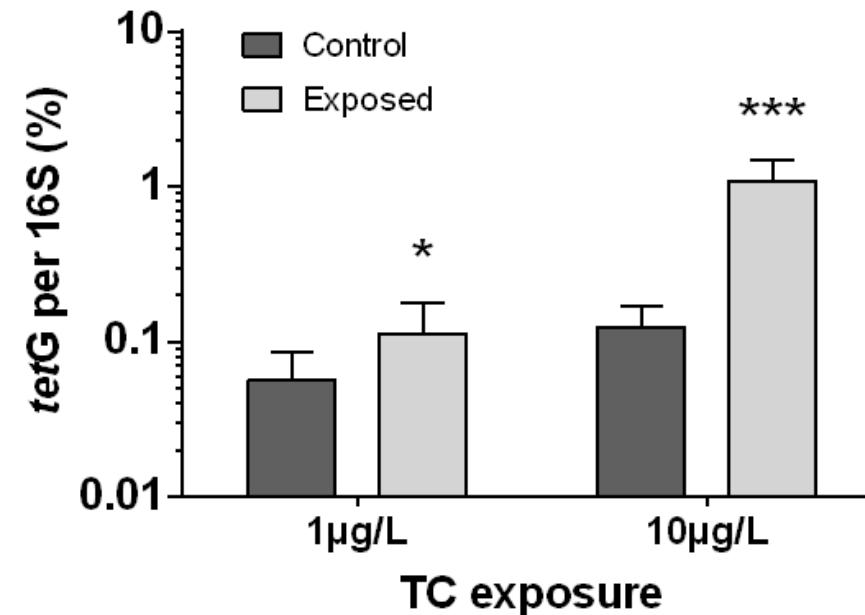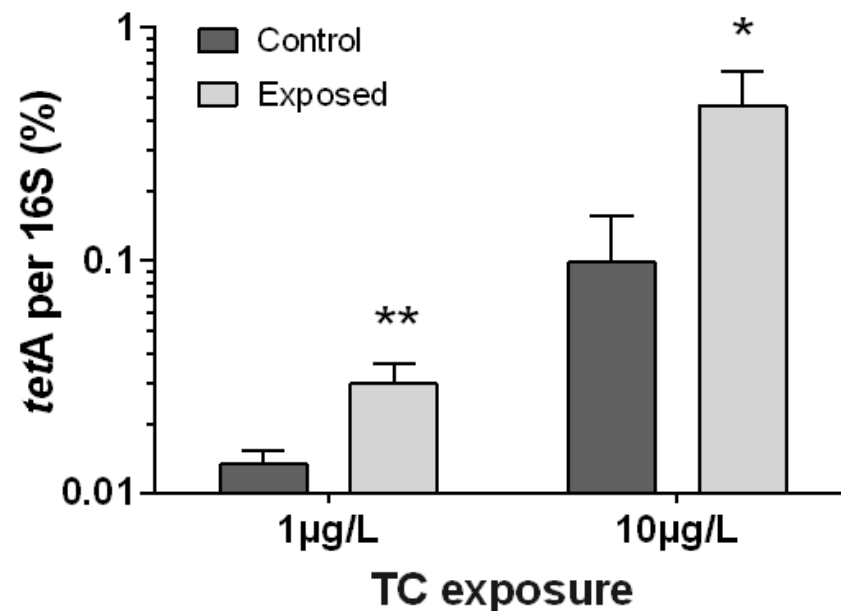
MSC for resistance gene enrichment:
1 µg/L

MSC for phenotypic resistance:
10 µg/L

Lowest observed MIC:
16 µg/L

Lundström et al. 2016
Sci Tot Env

Johan Bengtsson-Palme | www.microbiology.se | johan.bengtsson-palme@microbiology.se

Minimal Selective Concentrations Ciprofloxacin
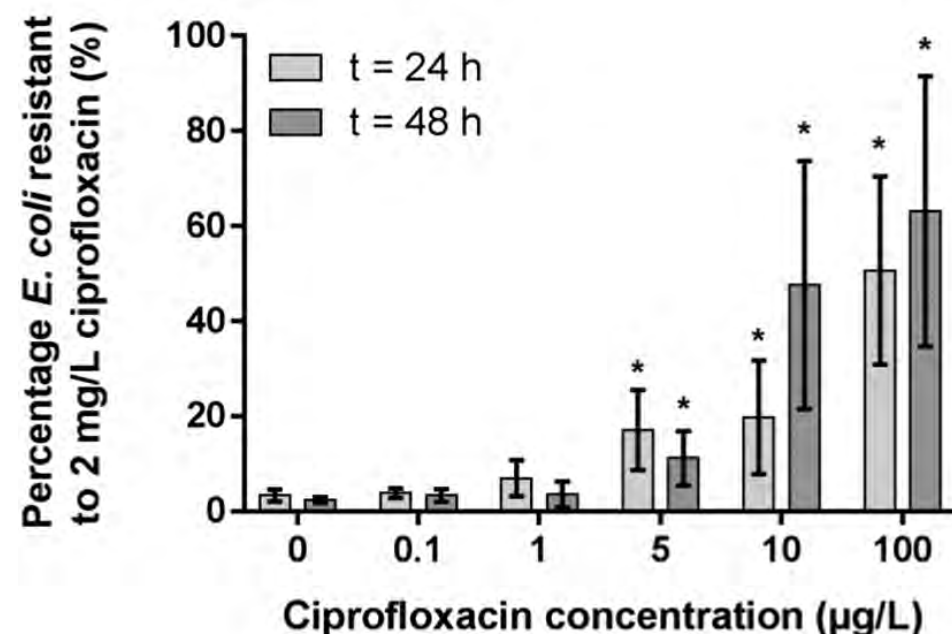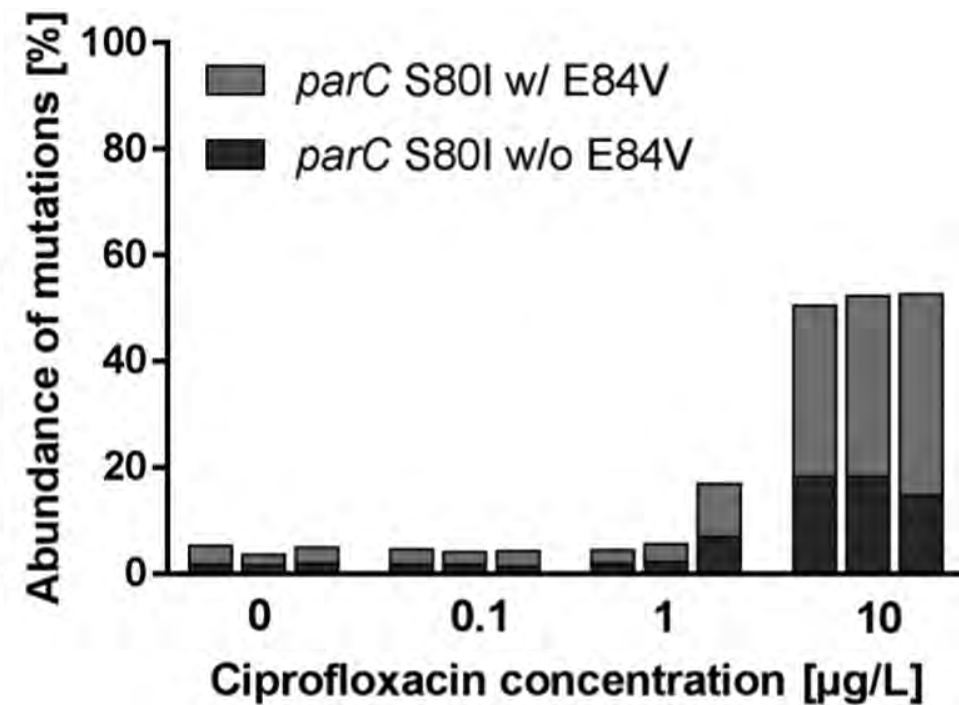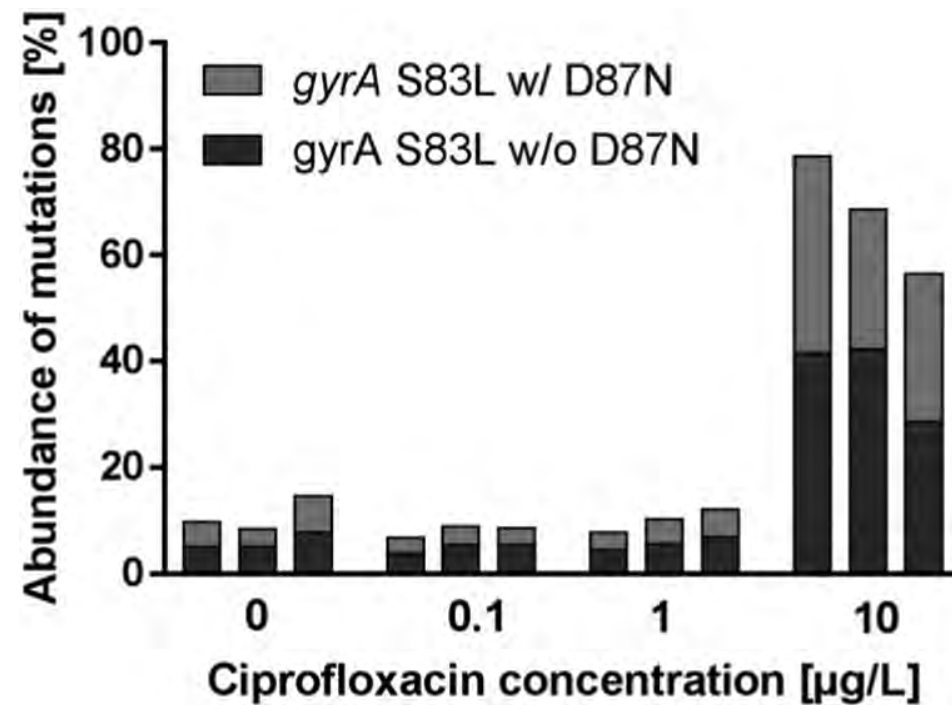
MSC for resistance mutations:
10 µg/L

MSC for phenotypic resistance:
5 µg/L

Lowest observed MIC:
2 µg/L

Kraupner et al. 2018
Env Int

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Minimal Selective Concentrations

Estimated minimal selection concentration boundaries and predicted no-effect concentrations for 26 (of 111) commonly used antibiotics

Antibiotic	Antibiotic class	N[1]	Covered genera (families)	Observed lowest MIC (µg/L)	Size-adjusted predicted lowest MIC (µg/L)[2]	PNEC (incl. assessment factor) (µg/L)	STP Effluent conc. (µg/L)[3]
Gentamicin	Aminoglycosides	68	27 (14)	16	16	1	1.3
Tobramycin	Aminoglycosides	31	15 (8)	16	8	1	
Co-trimoxazole	Antifolate combinations	36	22 (13)	8	4	0.5	
Ertapenem	Carbapenems	36	20 (12)	2	1	0.125	
Cefalexin	Cephalosporins (1st gen.)	10	7 (5)	250	32	4	1.8
Cefaclor	Cephalosporins (2nd gen.)	11	7 (6)	32	8	0.5	1.8
Cefdinir	Cephalosporins (3rd gen.)	5	4 (4)	32	2	0.25	
Benzylpenicillin (G)	Narrow-spectrum penicillins	47	12 (11)	4	4	0.25	
Phenoxymethylpenicillin (V)	Narrow-spectrum penicillins	8	5 (5)	4	0.5	0.064	2
Amoxicillin	Extended spectrum penicillins	29	19 (12)	4	2	0.25	0.05
Ampicillin	Extended spectrum penicillins	64	25 (15)	4	4	0.25	0.126
Vancomycin	Glycopeptides	42	10 (9)	125	125	8	0.04
Daptomycin	Lipopeptide	16	6 (6)	32	8	1	
Azithromycin	Macrolides	12	6 (6)	16	4	0.25	0.38
Clarithromycin	Macrolides	15	10 (10)	8	2	0.25	0.61
Erythromycin	Macrolides	39	14 (13)	16	8	1	0.62
Linezolid	Oxazolidinones	29	9 (9)	125	64	8	
Chloramphenicol	Amphenicols	29	18 (11)	125	64	8	
Colistin	Polypeptides	16	10 (4)	64	16	2	
Ciprofloxacin	Fluoroquinolones (2nd gen.)	70	29 (18)	2	1	0.064	0.742
Levofloxacin	Fluoroquinolones (3rd gen.)	43	24 (16)	4	4	0.25	
Moxifloxacin	Fluoroquinolones (4th gen.)	53	21 (14)	2	2	0.125	0.017
Rifampicin	Rifamycins	19	12 (12)	2	0.5	0.064	
Tigecycline	Glycylcyclines	54	26 (16)	16	16	1	
Doxycycline	Tetracyclines	29	20 (11)	32	16	2	0.915
Tetracycline	Tetracyclines	66	30 (18)	16	16	1	0.62

Notes: [1] These numbers correspond to the number of different species present in EUCAST that could be matched to a valid species name in SILVA. [2] The size-adjusted predicted lowest MIC correspond to the estimated upper boundary for the minimal selective concentrations. [3] The highest concentration observed in effluents from conventional STPs

Bengtsson-Palme & Larsson 2016
Env Int

Johan Bengtsson-Palme | www.microbiology.se | johan.bengtsson-palme@microbiology.se

Risk environments are predictable

Figure S4.
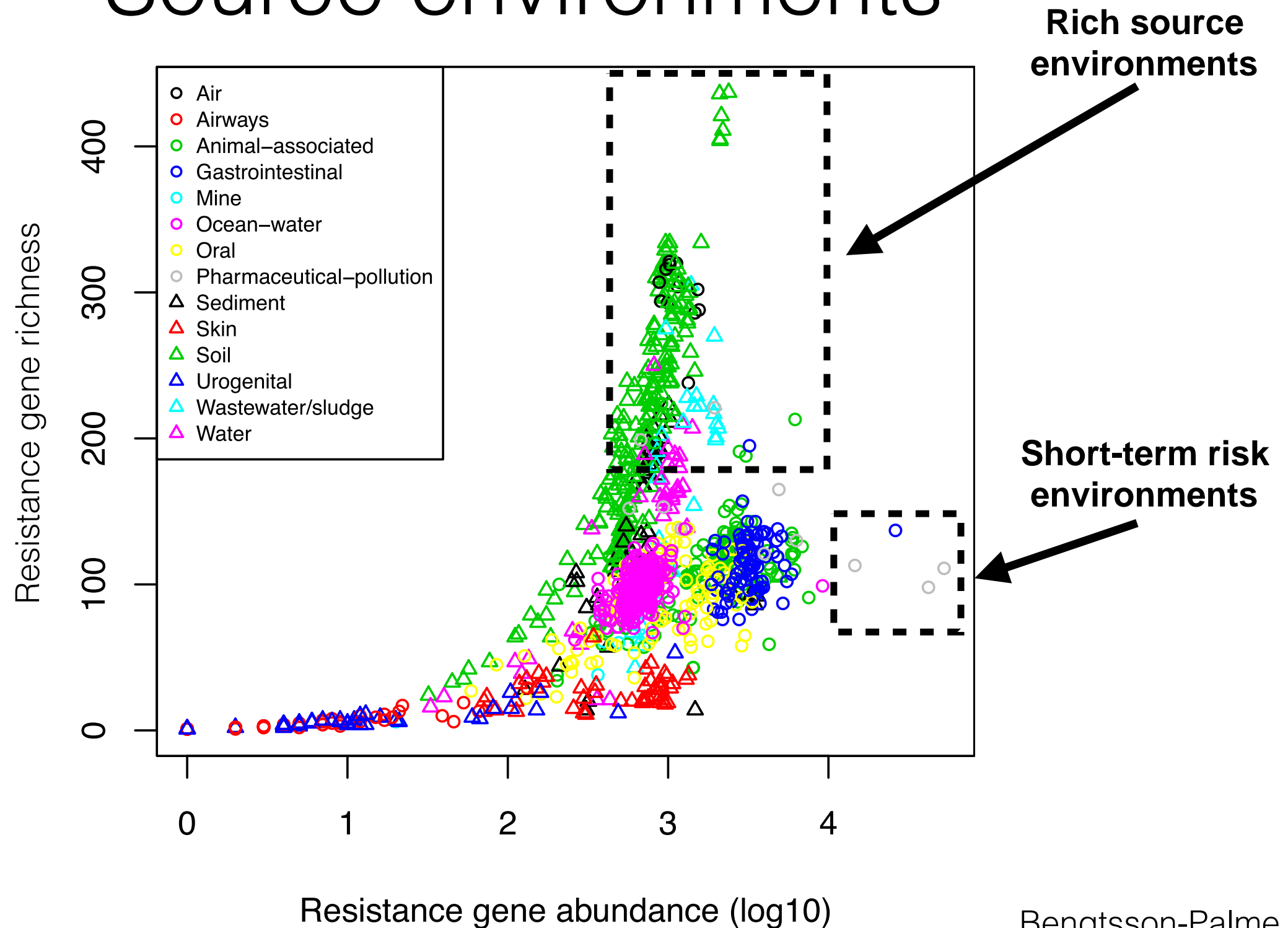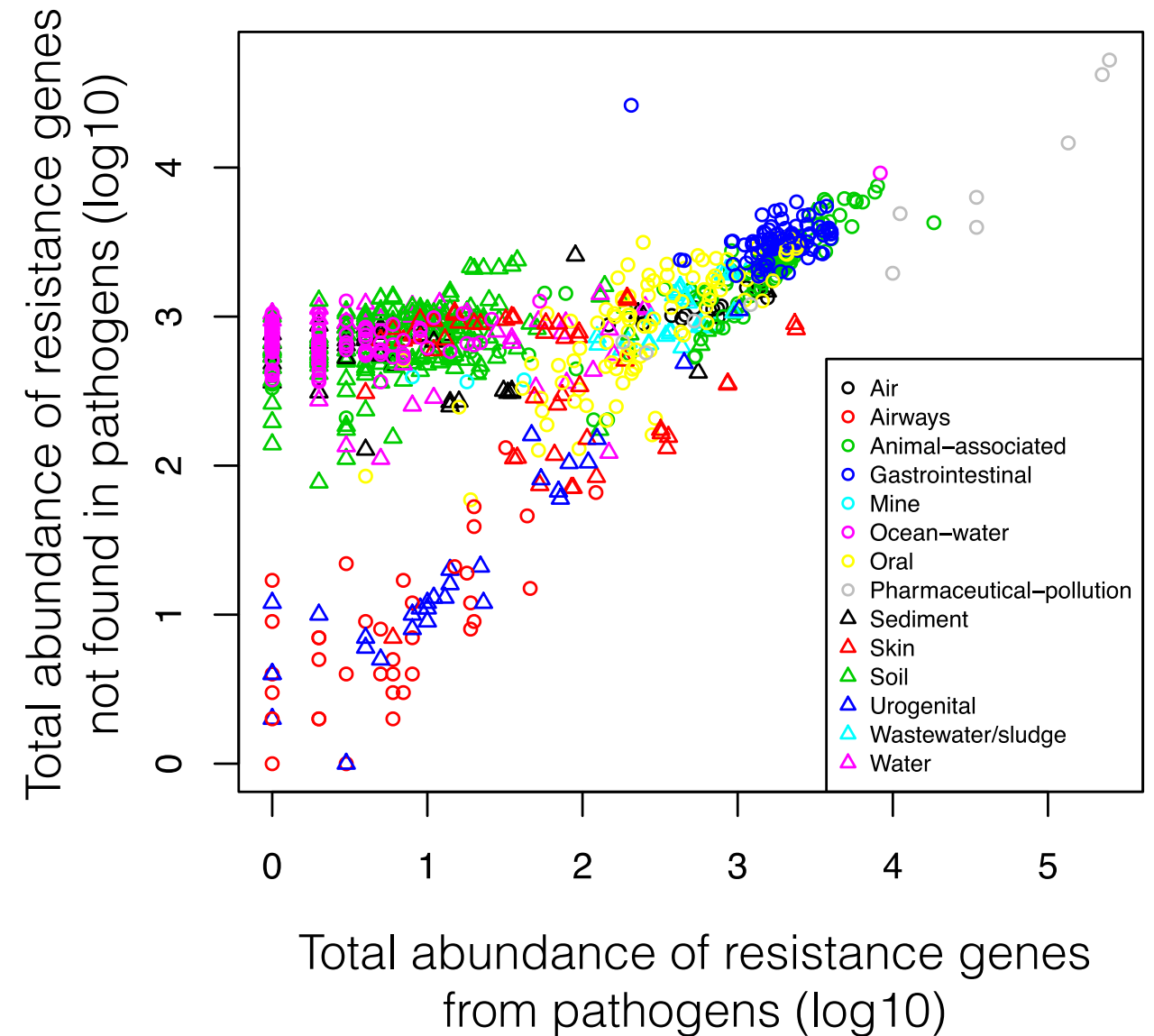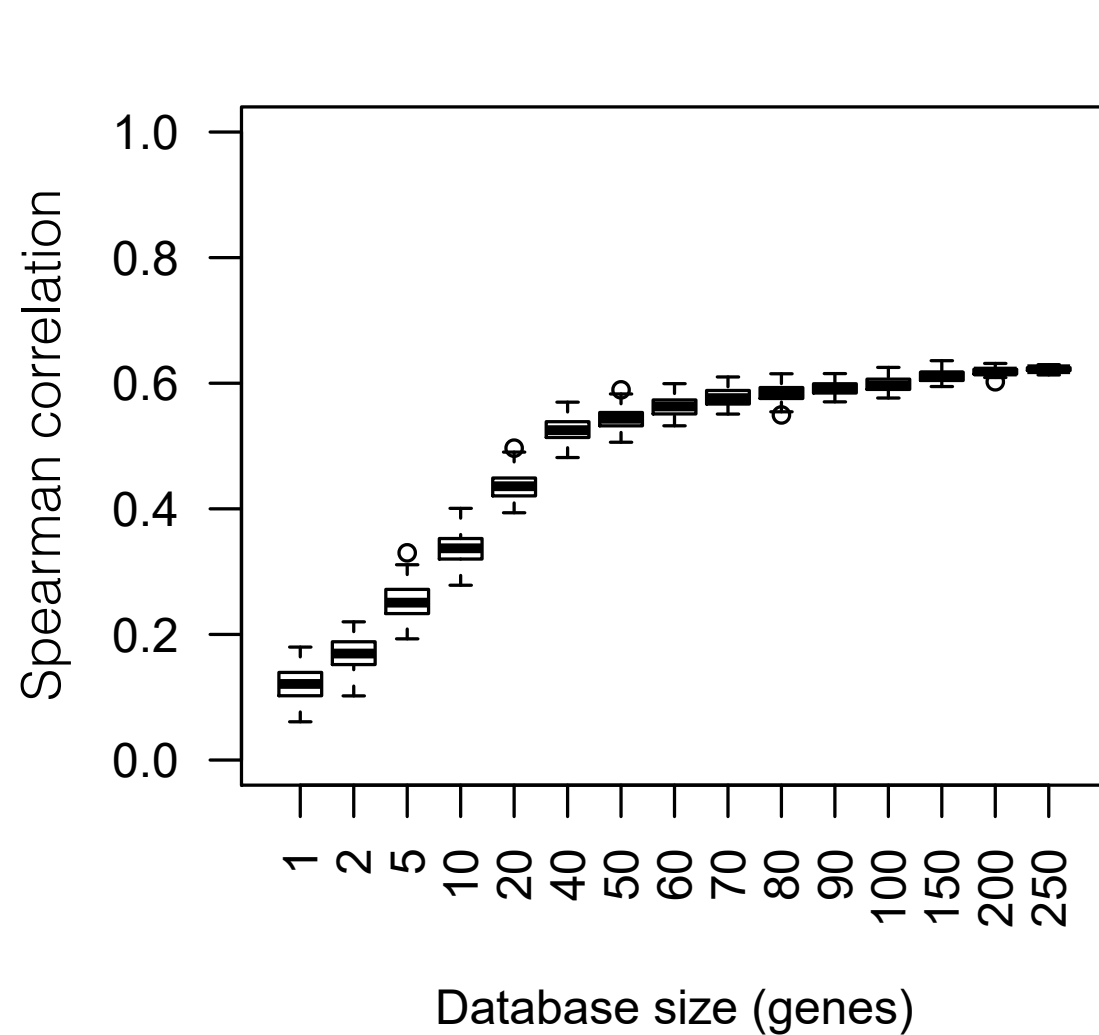
Bengtsson-Palme 2018
Microbiome

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...especially with the right targets

**The ten most predictive genes
in the full database**

Johan Bengtsson-Palme | www.microbiology.se | johan.bengtsson-palme@microbiology.se

The Future

"The ongoing release of selective agents into the biosphere is **likely to affect bacterial evolvability** on a global scale, and include environmental, commensal and pathogenic species"

– Gillings & Stokes Trends Ecol Evol 2012

Antibiotics select for mobile genes

Indian lake subjected to pharmaceutical production waste dumping
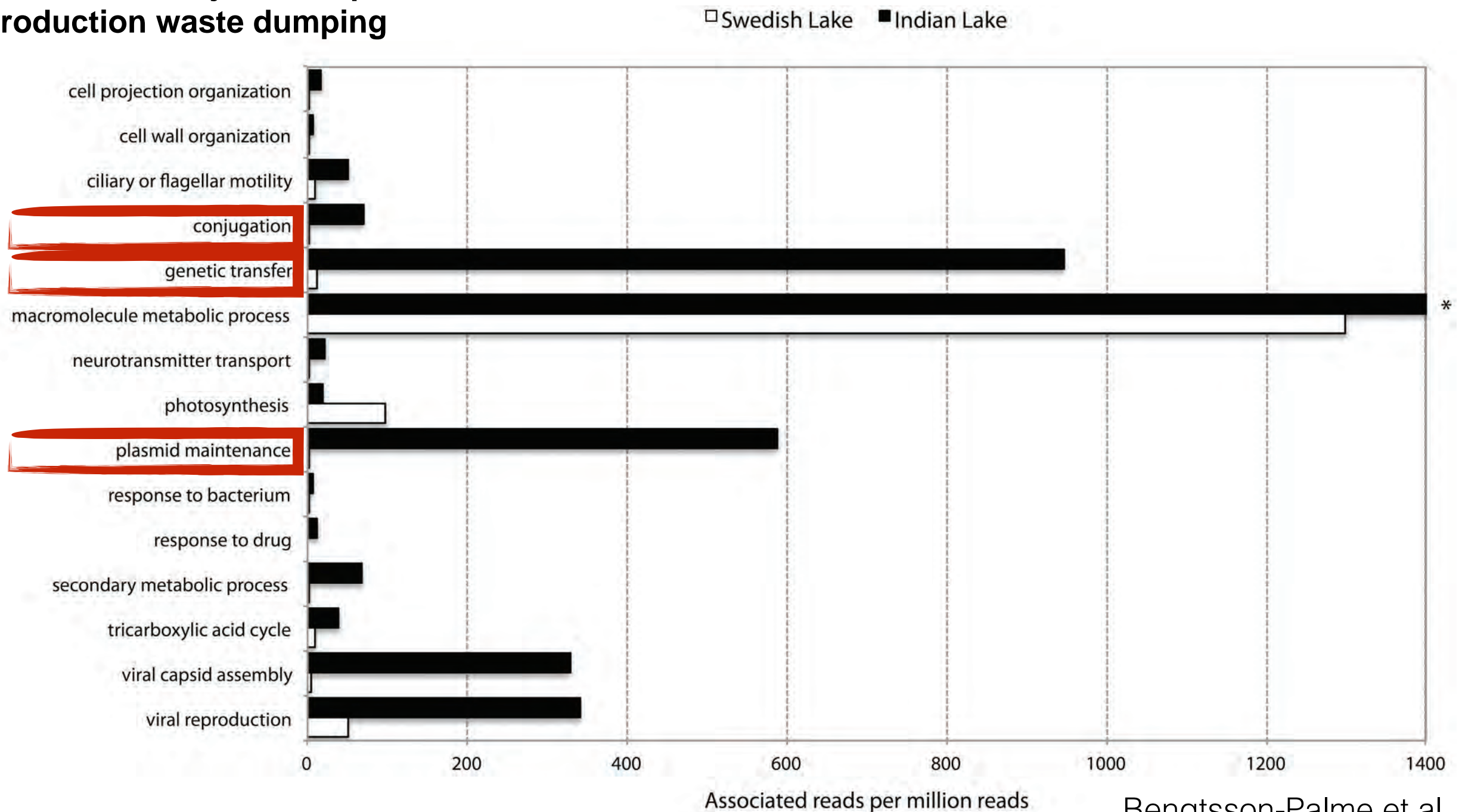
Bengtsson-Palme et al. 2014
Front Microbiol

Secondary effects of antibiotics

- What type of genes?

- At what concentrations?

- What are the timeframes?

Selective advantage of other genes

- Genes that provide a fitness advantage in changing environments

  - Detoxification

  - Utilize alternative carbon sources

  - Competition

  - Surface adhesion

  - Spore formation

  - Colonization and invasion

  - Virulence, transmission and pathogenicity

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Antibiotic exposure is ancient

$r = 0.475$
$P = 5 \times 10^{12}$
$n = 189$

ARG abundance

2×10^4

1×10^4

0.0016   0.0034   0.0057

Ascomycota abundance

Bahram et al. 2018
Nature
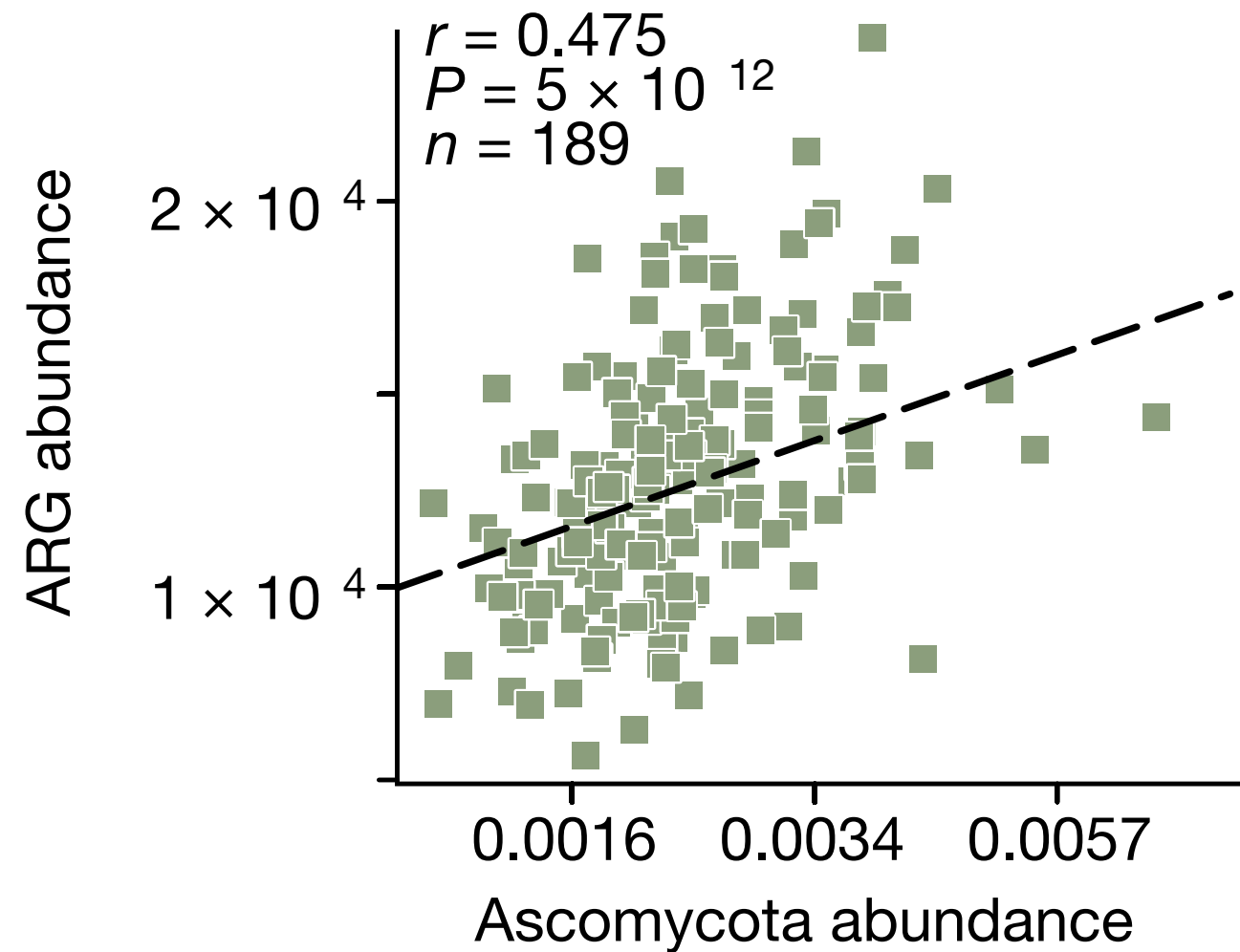
Johan Bengtsson-Palme | www.microbiology.se | johan.bengtsson-palme@microbiology.se

Colonization and Invasion

The THOR model community

Peptidoglycan

Bacillus cereus

?

Plant-like alkaloid

Flavobacterium johnsoniae

Pseudomonas koreensis

Pseudomonas aeruginosa
Transposon mutants

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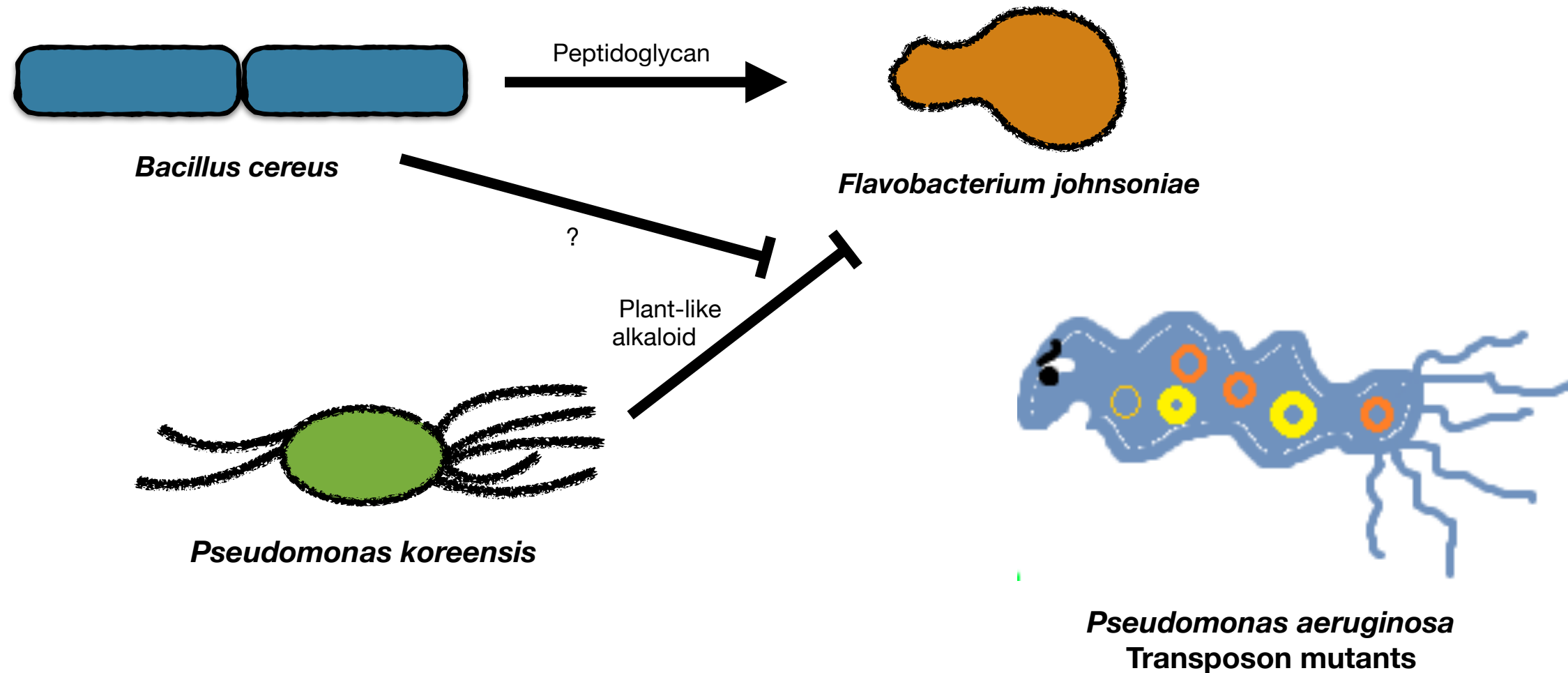
Summary

- Anthropogenic antibiotic exposure selects for resistance in the environment

- Antibiotics increases mobilization, but also genes involved in pathogenesis and invasion

- Are we breeding multiresistant, hypervirulent superbugs?

Acknowledgements

Joakim Larsson Lab
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Jo Handelsman Lab
Jo Handelsman
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Manuel Garavito
Amanda Hurley
J Rajendhran
Jennifer Heinritz

Nikolina Udikovic-Kolic
Mohammad Bahram

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Emergence of acquired polymyxin resistance in Gram negatives; perfect example of a One-Health issue

Laurent Poirel

Emerging Antibiotic Resistance Unit, Medical and Molecular Microbiology, Department of Medicine, University of Fribourg, Fribourg, Switzerland

French INSERM European Unit, University of Fribourg (LEA-IAME), Switzerland

National Reference Center for Emerging Antibiotic Resistance (Switzerland)
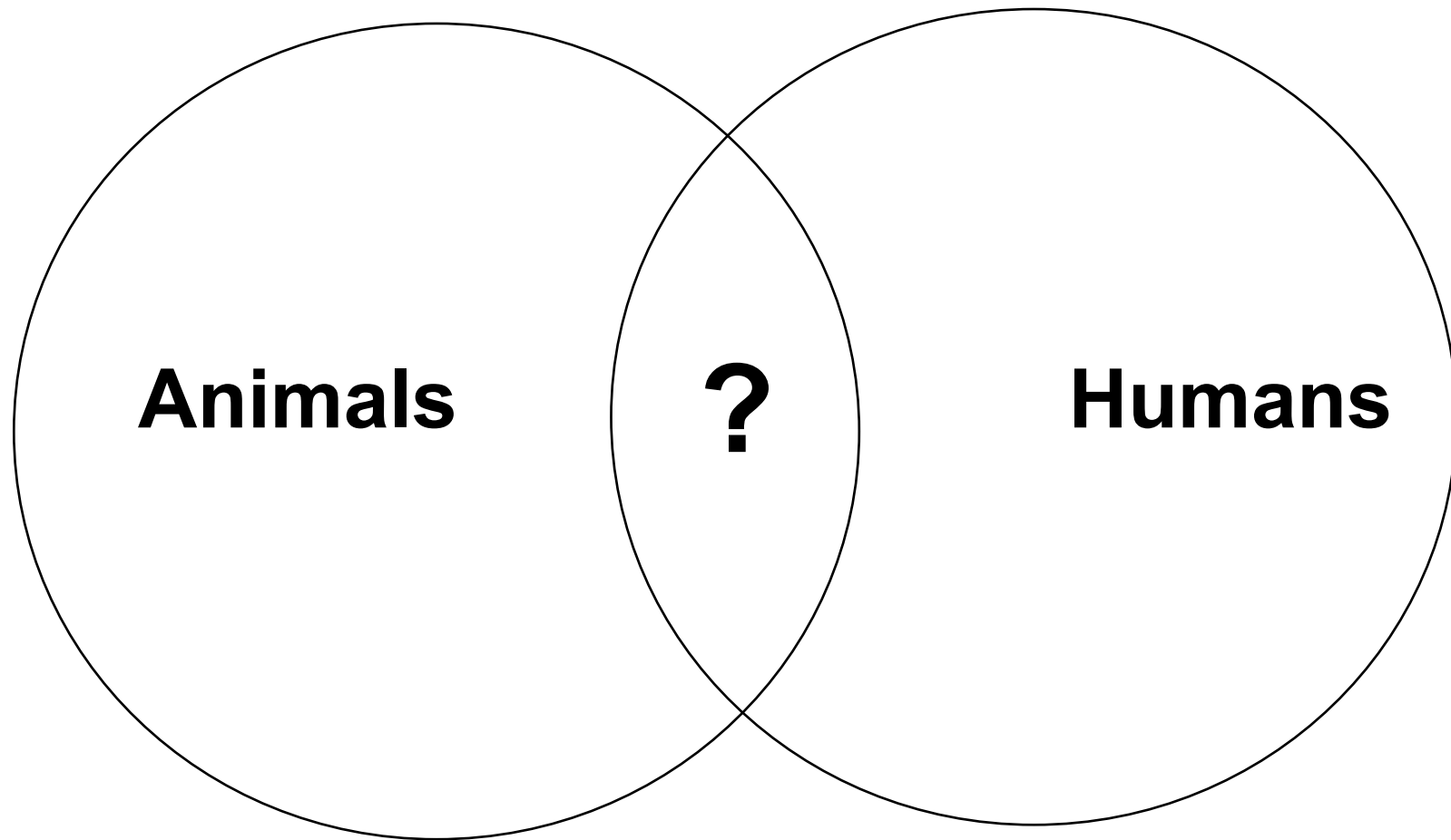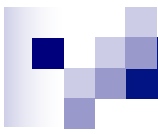
The carbapenemase threat in the animal world: the wrong culprit

Laurent Poirel[1]*, Roger Stephan[2], Vincent Perreten[3]
and Patrice Nordmann[1]

Human medicine is guilty

Emerging plasmid-encoded colistin resistance: the animal world as the culprit?

Laurent Poirel[1]* and Patrice Nordmann[1,2]

Veterinary medicine is (partially) guilty

Colistin (Polymyxin E)
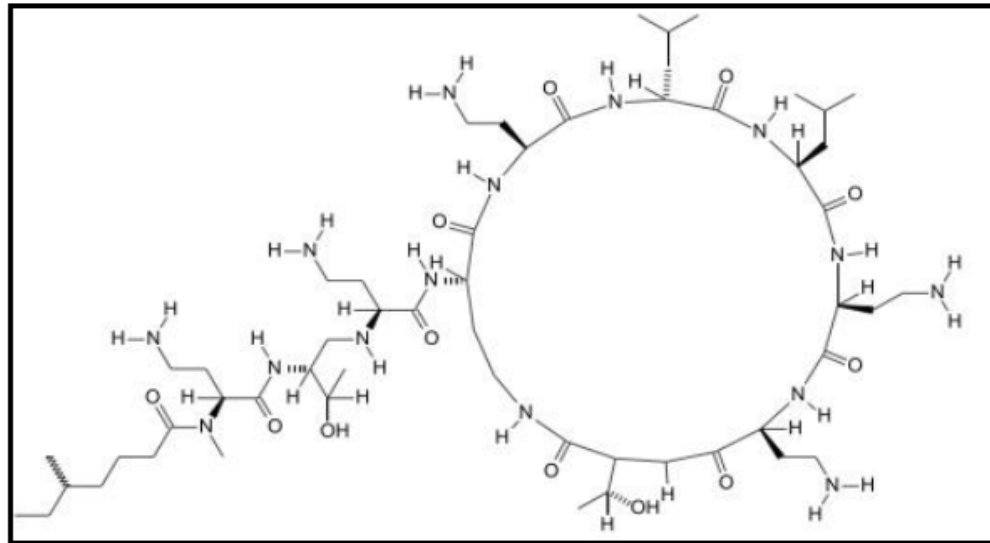
- Synthesis by *Bacillus polymyxa* spp colistinus
- Discovered in the 1940's
- High rates of toxicity (mainly nephrotoxicity) : replacement by newer antibiotics in 1980s
- Widely used in veterinary medicine for decades
- Renewed interest in human medicine in mid-1990s to treat MDR Gram-negative bacteria

Mechanism of action

Colistin
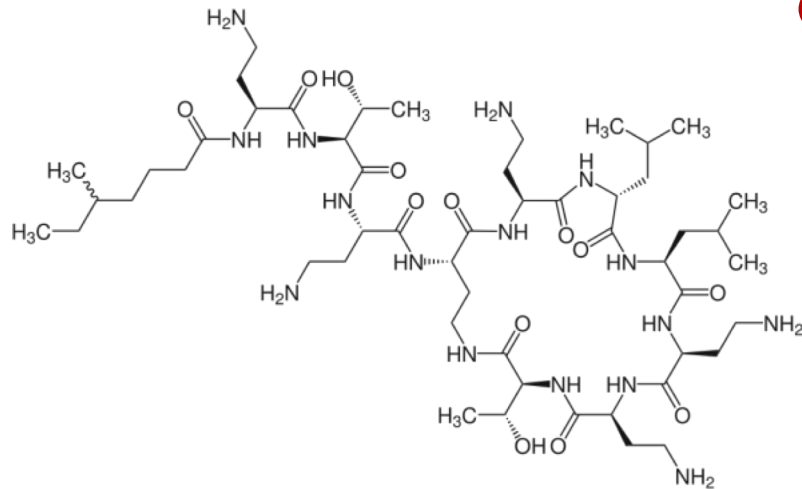
Lipid A
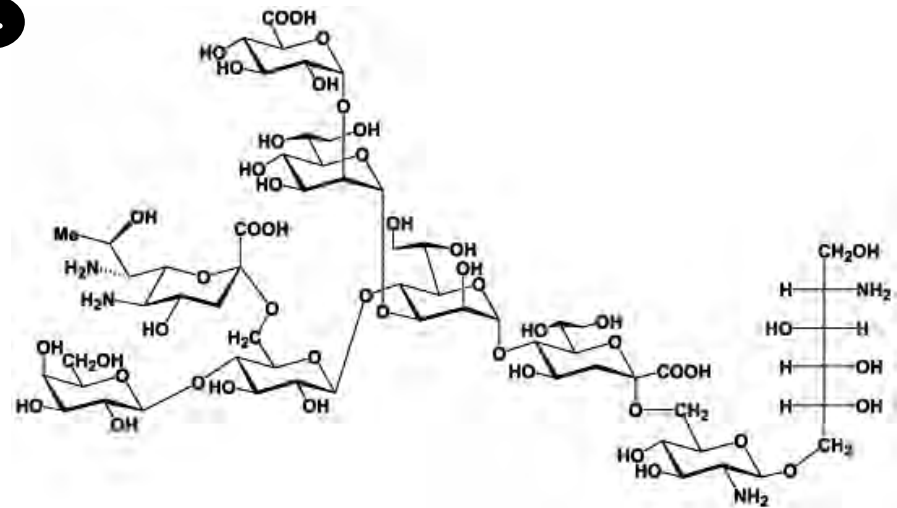
Colistin is a cationic antibiotic that is composed of a cyclic heptapeptide covalently attached to a fatty acyl chain

Lipopolysacharide (LPS) of Gram-negative bacteria is composed by :
- Lipid A
- Core
- Oligosaccharide O

Mechanism of action (2)
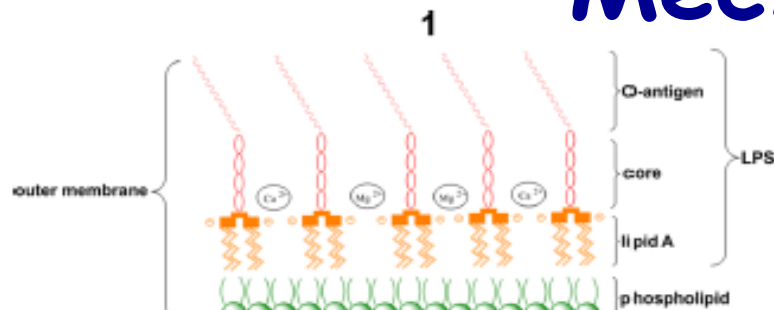
1. Fixation
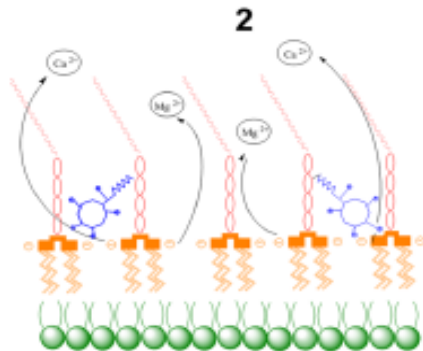
2. Displacement of divalent cation (Ca^{2+} et Mg^{2+})
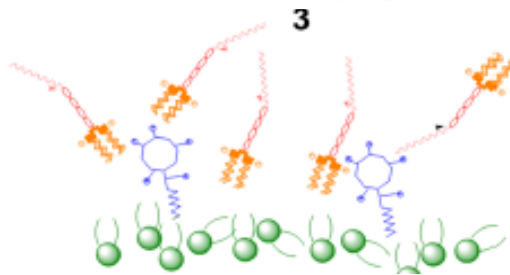
3. Destabilisation of the outer membrane of Gram negatives
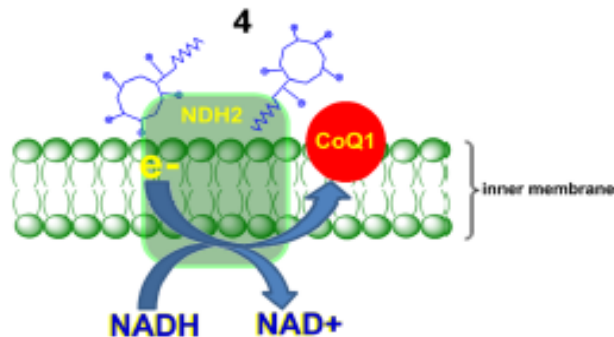
4. Penetration throughout the inner membrane and inhibition of the respiratory enzymes NDH2

Falagas et al, Clin Infect Dis. 2005
Deris et al, J Antibiot. 2013

Spectrum of activity

Susceptible bacteria :

▸ *Pseudomonas aeruginosa, A. baumannii, E. coli, Klebsiella* spp., *Enterobacter* spp.

▸ *H. influenza, Bordetella pertussis*
▸ *Salmonella* spp., *Shigella* spp.
▸ *Legionella, Stenotrophomonas maltophilia*
▸ Some *Mycobacterium* species, and in particular *M. tuberculosis*

Non-susceptible bacteria :

▸ All gram positives

▸ Gram neg cocci: *N. gonorrhoeae, N. meningitidis*
▸ *Proteus* group, *Serratia* spp., *Burkholderia* spp., *Brucella* spp.
▸ Anaerobes

Role of LPS in polymyxin resistance

❖ **LPS modifications : the main mechanism of resistance to colistin :**

Addition of 4-amino-4-deoxy-L-arabinose (LAra4N) and / or phosphoethanolamine (pEtN) to lipid A ➜ Increase of positive charges ➜ decreased affinity for LPS

Synthesis of L-Ara4N and pEtN mediated by PmrA / PmrB, PhoP / PhoQ, and *mgrB* gene

Interplay of resistance mechanisms in *Klebsiella pneumoniae*
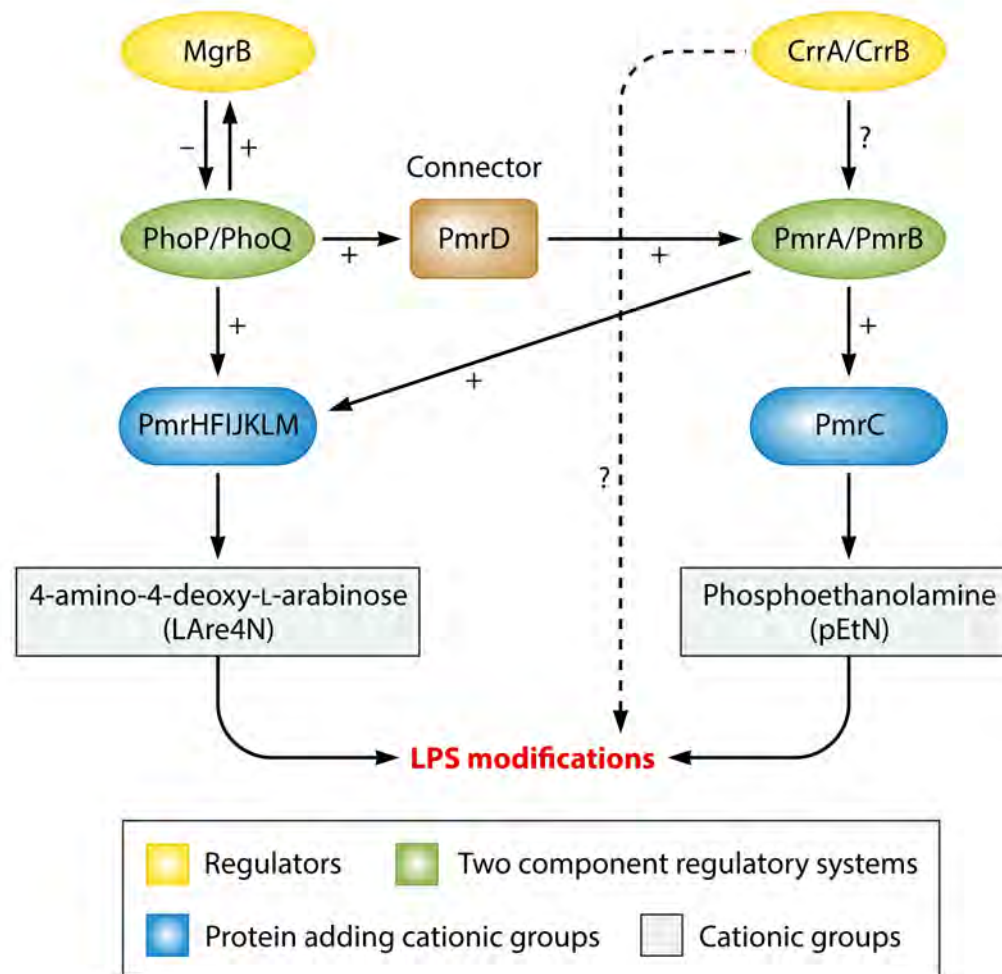
Plasmid-mediated resistance

Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study

Yi-Yun Liu*, Yang Wang*, Timothy R Walsh, Ling-Xian Yi, Rong Zhang, James Spencer, Yohei Doi, Guobao Tian, Baolei Dong, Xianhui Huang, Lin-Feng Yu, Danxia Gu, Hongwei Ren, Xiaojie Chen, Luchao Lv, Dandan He, Hongwei Zhou, Zisen Liang, Jian-Hua Liu, Jianzhong Shen
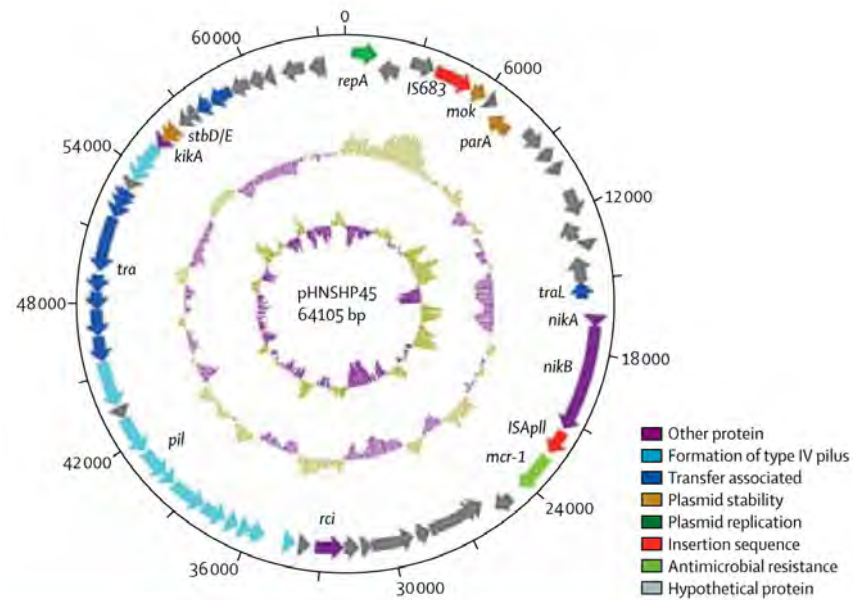
	Year	Positive isolates (%)/number of isolates
Escherichia coli		
Pigs at slaughter	All	166 (20·6%)/804
Pigs at slaughter	2012	31 (14·4%)/216
Pigs at slaughter	2013	68 (25·4%)/268
Pigs at slaughter	2014	67 (20·9%)/320
Retail meat	All	78 (14·9%)/523
Chicken	2011	10 (4·9%)/206
Pork	2011	3 (6·3%)/48
Chicken	2013	4 (25·0%)/16
Pork	2013	11 (22·9%)/48
Chicken	2014	21 (28·0%)/75
Pork	2014	29 (22·3%)/130
Inpatient	2014	13 (1·4%)/902
Klebsiella pneumoniae		
Inpatient	2014	3 (0·7%)/420

Table 2: Prevalence of colistin resistance gene mcr-1 by origin

MCR : Mobilizable Colistin Resistance
→ Phosphoethanolamine transferase (permanent modification of the lipid A)

Countries where the *mcr-1* gene has been detected from Enterobacteriaceae in pigs and other farm animals

Country	Year of report	Animal production	Bacterial species
Laos	2015	Pigs	*Escherichia coli*
Denmark	2015	Chicken	*E. coli*
China	2016	Pigs, chicken	*E. coli*
Algeria	2016	Chicken	*E. coli*
Vietnam	2016	Pigs	*E. coli*
France	2016	Veal calves	*E. coli*
Germany	2016	Pigs	*E. coli*
Malaysia	2016	Pigs	*E. coli*
Japan	2016	Cattle, pigs	*E. coli, Salmonella*
UK	2016	Pigs	*E. coli*
Belgium	2016	Pigs, calves	*E. coli*

Rhouma M et al. *Int J Antimicrob Agents* 2016;48:119-26

The MCR-1 protein; a phosphoethanolamine transferase
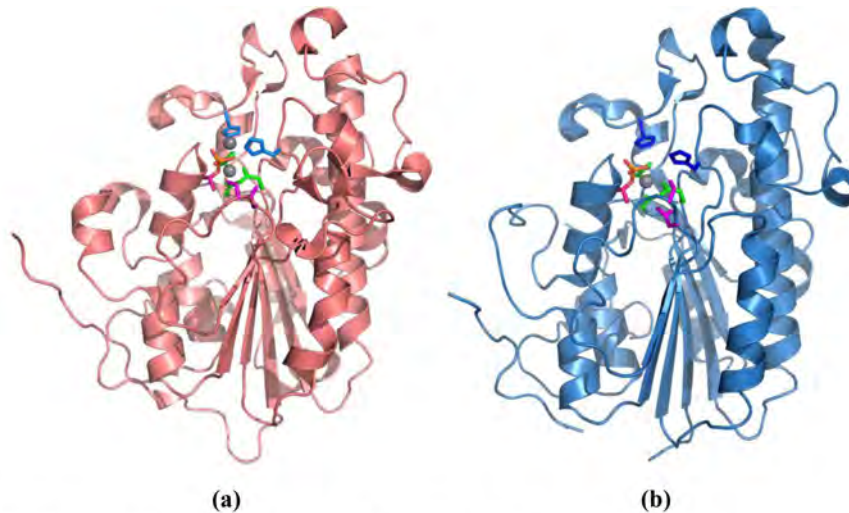
Figure S5. The MCR-1 sequence resembles those of two phosphoethanolamine transferases, **a)** LptA from *Neisseria meningitidis* (pdb ids 4KAY) and **b)** EptC from *Campylobacter jejuni* (pdb ids 4KAY and 4TNO).

- A 16-fold increase in MIC of polymyxins (colistin and polymyxin B)

- From 0.5 μg/ml (recipient *E. coli*) to 8 μg/ml (transconjugant)

Contribution of MCR-1 in resistance to colistin

Wild-type *K. pneumoniae*

+ plasmid encoding MCR-1

MIC of colistin   0.25 µg/ml   4 µg/ml

K. pneumoniae with a PmrB mutation (Leu110-stop)

+ plasmid encoding MCR-1

MIC of colistin   2 µg/ml   8 µg/ml

K. pneumoniae with a PmrB mutation (Trp114Pro)

+ plasmid encoding MCR-1

MIC of colistin   8 µg/ml   32 µg/ml

Jayol, Nordmann, André, Dubois & Poirel, Int J Antimicrob Agents 2017
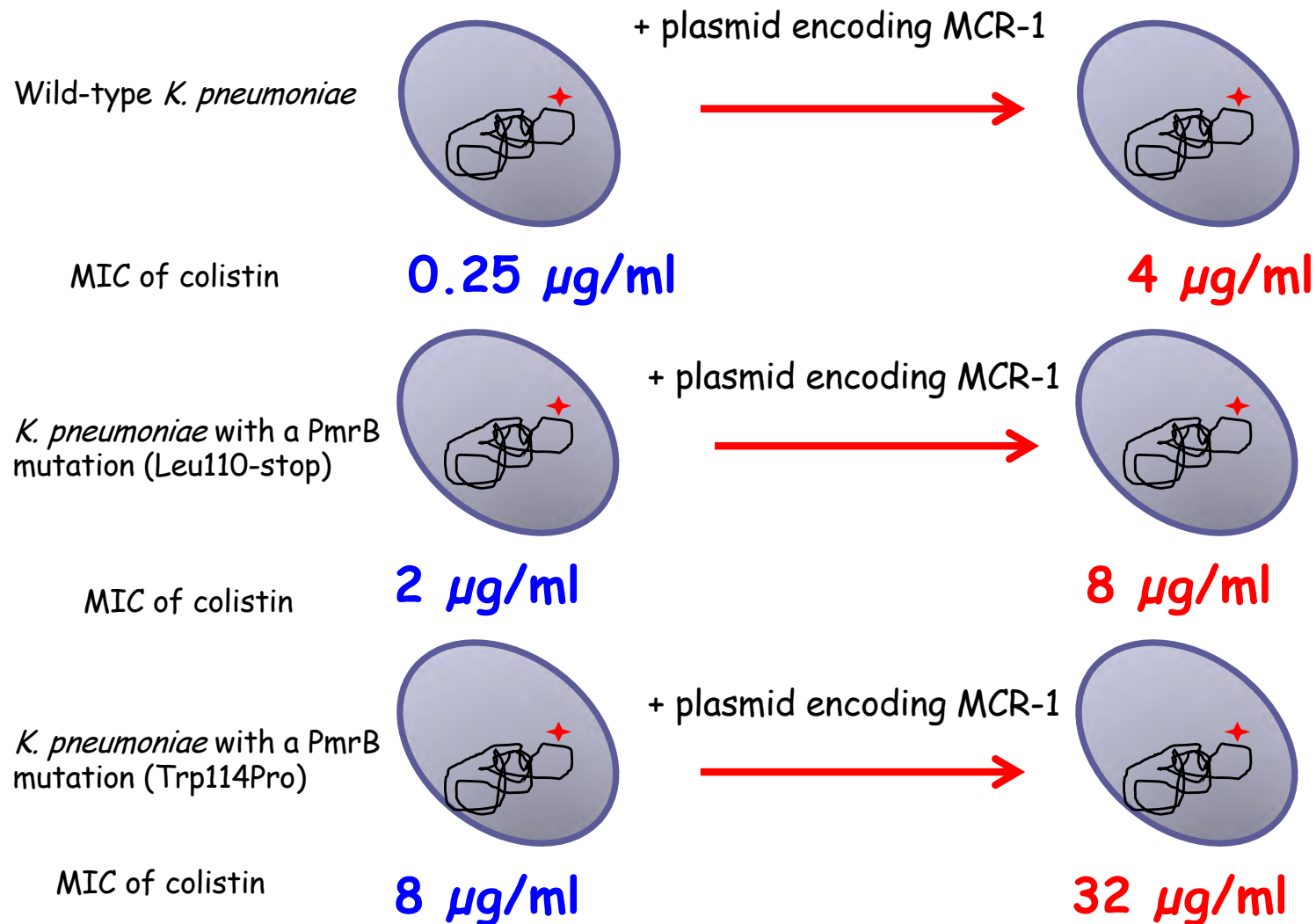
Where do MCR enzymes come from ?

Moraxella spp. common inhabitant of pigs

Selection pressure with colistin

IS*Apl1* originates from *Actinobacillus pleuropneumoniae* reponsible for porcine pleuropneumonia

E. coli also a common inhabitant of pigs

Moraxella species as sources of MCR-like determinants

Kieffer, Nordmann and Poirel, Antimicrob Agents Chemother; 2017
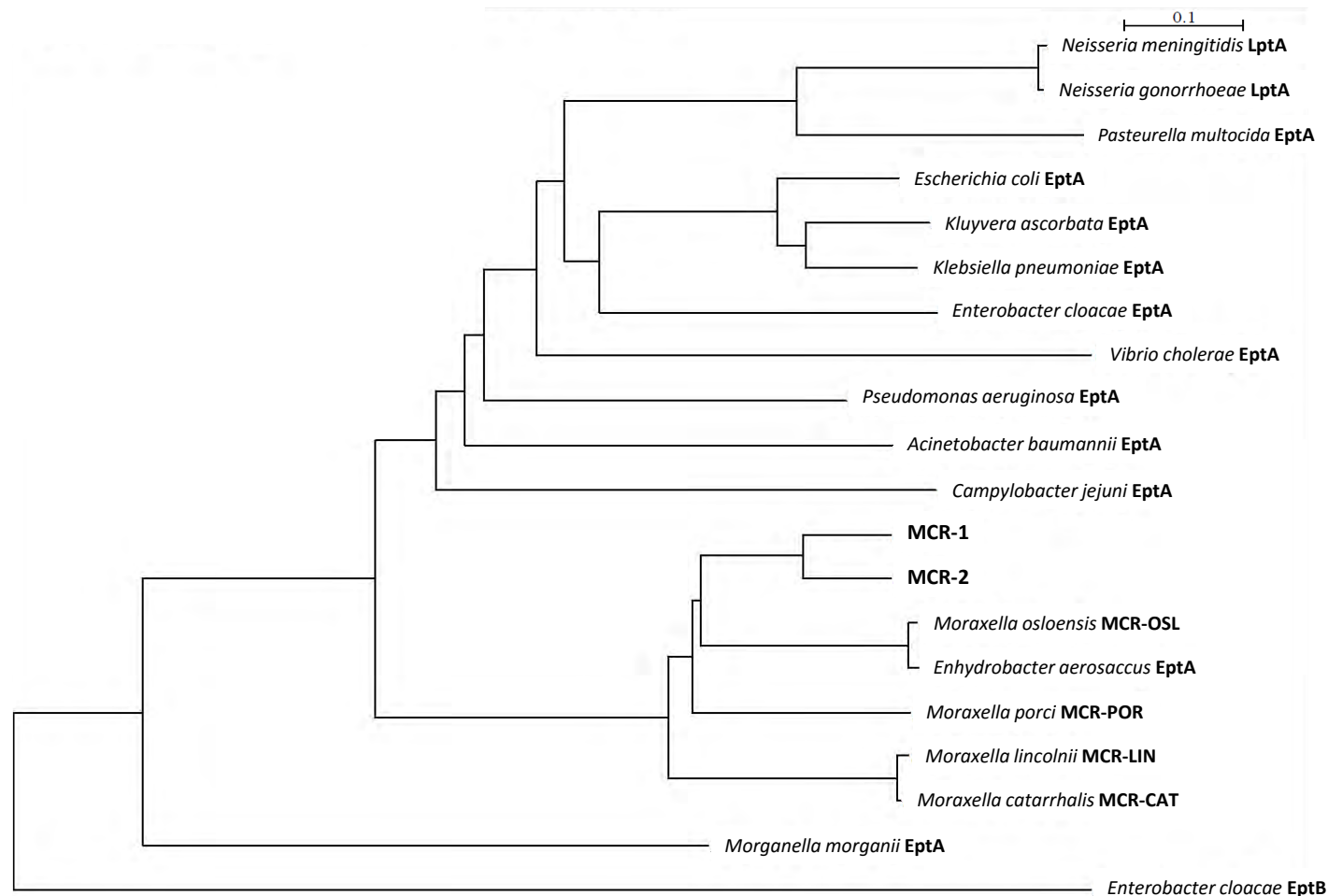
Moraxella pluranimalium is the progenitor of MCR-2

→ *M. pluranimalium* is an aerobic, catalase- and oxydase-positive Gram-negative cocci

→ It harbors an intrinsic gene encoding an MCR-2-like enzyme (99% amino acid identity), with only 8 amino acids difference out of the 538 constituting the MCR-2 enzyme, and 82% amino acid identity with MCR-1

→ Strains belonging to that species have been recovered from pigs being either healthy or suffering from pleuritis and polyserositis (nose, pleura, and peritoneal cavity fluids), and from the brain of a sheep presenting with meningitis

Poirel et al. 2017. J Antimicrob Chemother.

Epidemiology of MCR-1 producers
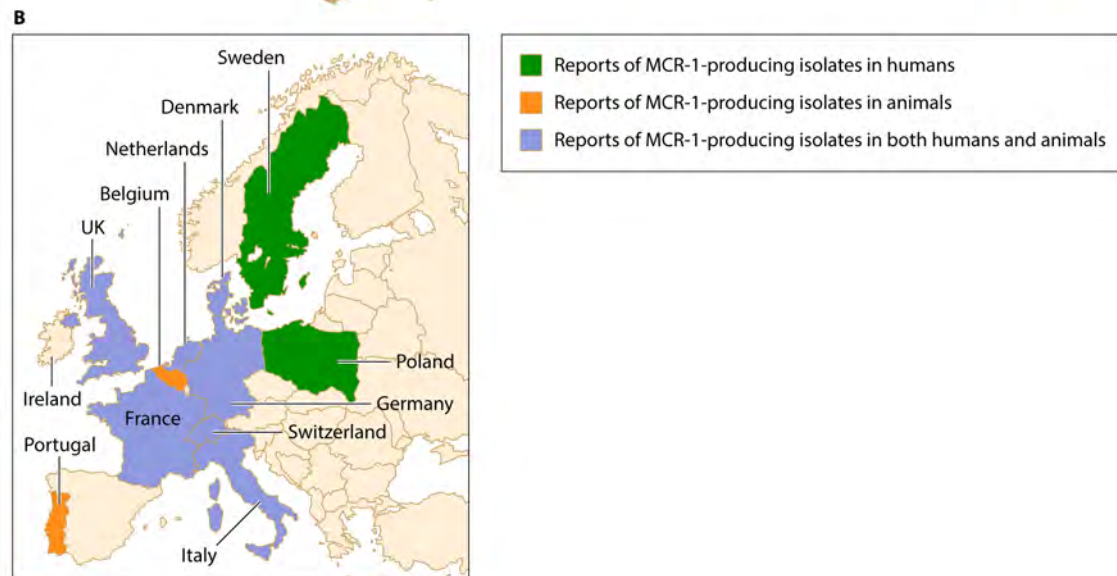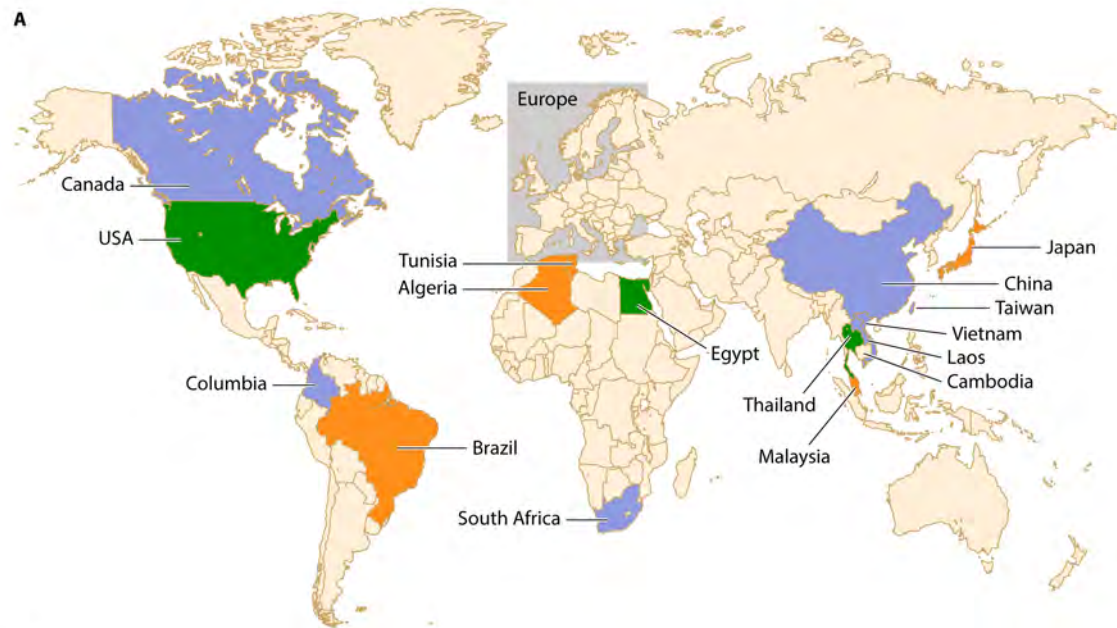
Global Distribution of plasmid-mediated *mcr-1* colistin-resistant strains from Environments, Foods, Animals and Humans (20 countries) (November 2015 to April 2016)- 1,010 Isolates

■ Food   *n* = 174

■ Animal   *n* = 577

■ Human   *n* = 184

■ Environment   *n* = 75

Total no. of bacteria bearing *mcr-1*, *n* = 1010

Courtesy Po-Ren Hsueh

Baron S et al. *Int J Antimicrob Agents* 2016;48:583-591
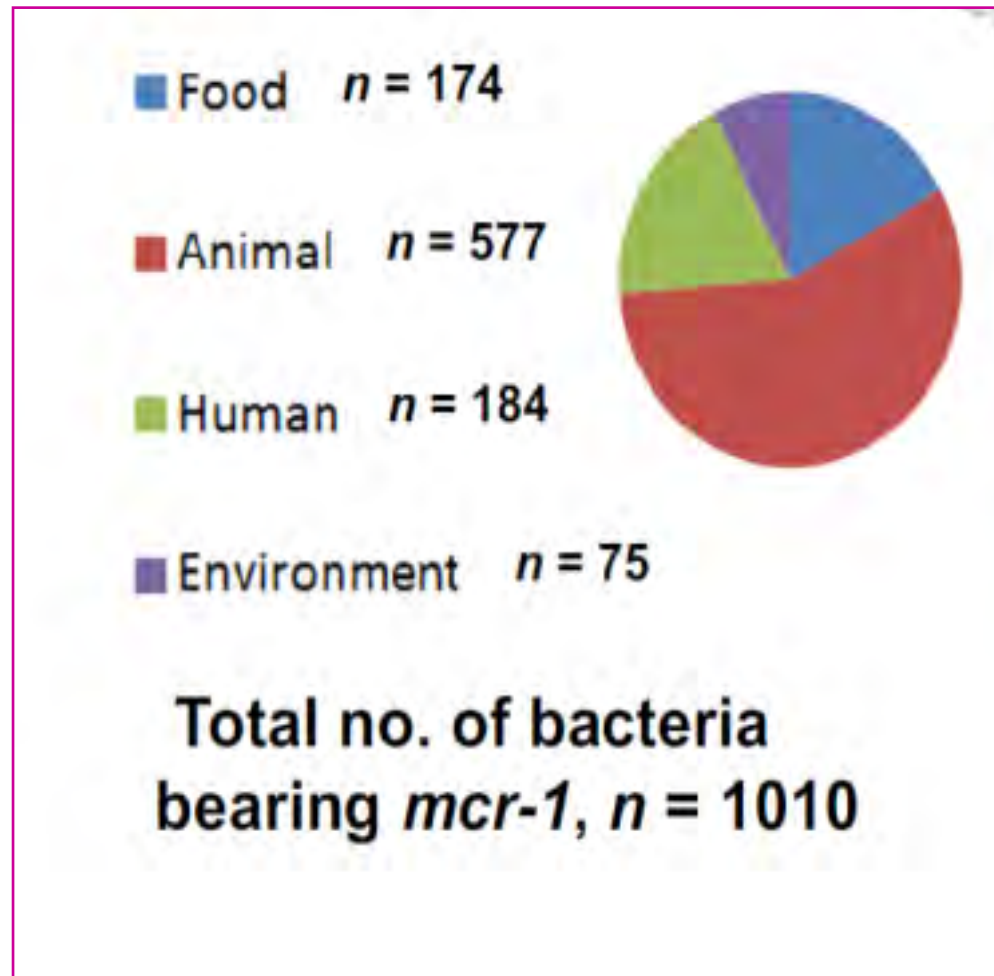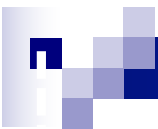
Need for a selective medium allowing selection of bacteria being resistant to polymyxins

Composition of the SuperPolymyxin medium

- ❑ EMB medium; 3.75%

- ❑ Colistin sulfate; 3.5 μg/ml

- ❑ Daptomycin; 10 μg/ml (cannot be substituted by vancomycin)

- ❑ Amphotericin B; 5 μg/ml

☐ SuperPolymyxin medium = screening medium aimed to detect any polymyxin-resistant Gram negative bacteria regardless of its resistance mechanism and of its level.

☐ May be used in :

- human medicine for detecting carriers (stools, rectal swabs)

- in veterinary medicine for epidemiological surveys

- May be used for isolated bacteria, but also clinical samples including tools

☐ Is now commercialized (ELITech company, France)

☐ May help to contain outbreaks due to polymyxin-resistant isolates and thus at least in part preserve the efficacy of polymyxins as last resort antibiotics.

Nordmann P, Jayol, Poirel L. A universal culture medium for screening polymyxin-resistant gram negatives. J Clin Microbiol. 2016
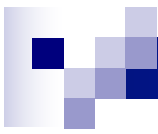
Some applications

Prospective survey in pig farms in Portugal - design

- Two farms in Portugal; 50 pigs sampled in each farm; total 100 pigs

- Rectal swabs collected in 2016

- Pigs receiving colistin in feeding regimen

- Screening on SuperPolymyxin selective plates

- Isolates were confirmed to be resistant to colistin using the Rapid Polymyxin NP test

- Characterization of the resistance mechanisms

Prospective survey in pig farms in Portugal - results

- 108 colistin-resistant isolates recovered

- 98 *mcr-1*-positive enterobacterial isolates !! (94 *E. coli*, 4 *K. pneumoniae*)

- 92 different *E. coli* clones !

- Different plasmid types (IncP, IncX4, IncHI2,

- Different genetic contexts (heterogeneity of the associated transposon)

Extremely high rate of MCR-1-producing and clonally-unrelated *E. coli*

Prevalence of colistin resistance in *Enterobacteriaceae*

- Prevalence of colistin resistance among
  - *MDR Enterobacteriaceae / non Enterobacteriaceae*
  - *Different collections of Enterobacteriaceae /*
  non Enterobacteriaceae from different origins
    - ✓ *Clinical / carriage strains*
    - ✓ *Patients /Animals*

  Always biased

- But what is the prevalence of colistin resistant *Enterobacteriaceae* **in the gut of asymptomatic carriers**?

Aim of the study

- **Question**: what is the prevalence of colistin resistant *Enterobacteriaceae* (and in particular *Escherichia coli*) in the gut of asymptomatic carriers?

- **Answer:**
  - a multicenter prospective study among hospitals of the Assistance Publique - Hôpitaux de Paris - bacteriology labs belonging to the IAME Resistance study group
  - The use of a (pre)-marketed screening culture media
  - The use of a marketed confirmation test

The COLI-RED study: population study

- 6 hospitals in the Paris area
- 3-month period (2016-2017)
- all patients screened systematically upon admission
  - to an intensive care unit
  - anywhere in the hospital if the patient showed risk for carriage of emerging extensively drug-resistant bacteria such as carbapenemase-producing *Enterobacteriaceae* or vancomycin-resistant enterococci (French regulalory action)

  Rectal swab (Eswab®)

- Direct inoculation of one drop of transport medium on **Superpolymyxin®** plate
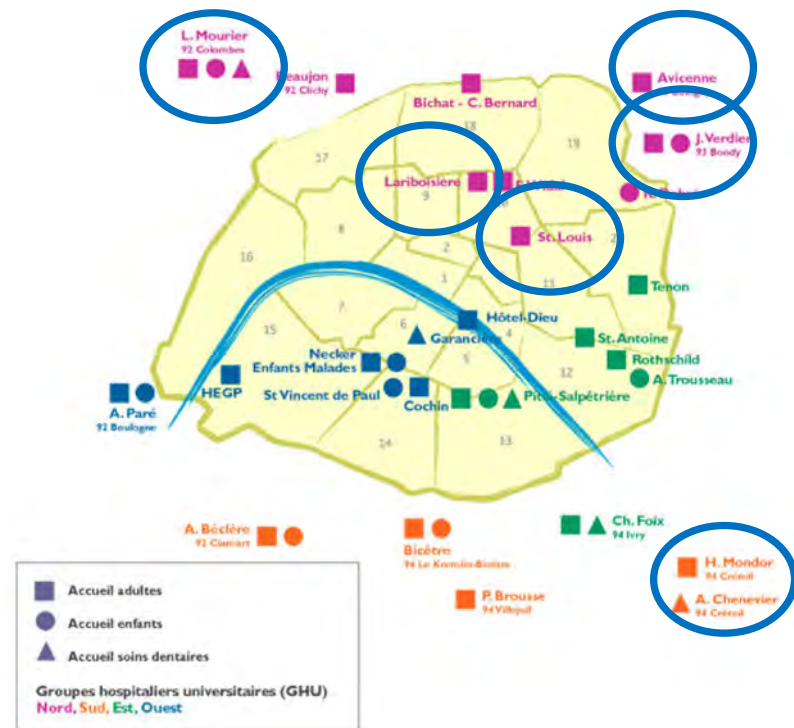
Results (1)

- **1,217** rectal swabs originating from **a relevant snapshot of the colistin resistance prevalence mostly from the community setting**

- 168 colistin-resistant *E. coli* isolates recovered

  ✓ 7 *mcr*-1 positive

  ✓ No other *mcr* gene detected

  ✓ 161 mcr-negative and colistin-resistant strains

Results (2): Analysis of genotypes

- 7 *mcr*-1 positive *E. coli* isolates identified, and submitted to whole genome sequencing

- The strain backgrounds corresponded to commensal phylogroups (A, B1, E, and Clade I)

- The ST types were all different and all but one corresponded to *E. coli* backgrounds always identified from animal sources

- The plasmid scaffolds bearing the *mcr-1* gene were diverse, corresponding to the formerly identified *mcr-1*-positive plasmids (IncHI2, IncX3, IncP)

Results (3): Analysis of genotypes

- Almost all colistin-resistant and non-MCR producing *E. coli*

possess a background corresponding to human commensal strains

- Most of those isolates possess mutations in chromosomal genes

involved in LPS modification

Origin of this high rate of colistin resistant *E. coli?*

☐ Antimicrobial selective pressure? Unlikely owing to:

- The community origin for a large part of the patients (>80%)

- The low consumption of polymyxin in/out hospital setting

☐ Co-selection of colistin resistance through another way /mechanism beyond the use of colistin?

- **Clinical consequence**?

  - So far limited owing to the low probability of colistin therapy

General conclusion

- Plasmid-mediated resistance to polymyxins seems to represent a minor threat for humans, but is very widespread in food-producing animals

- Monitoring the resistance rates to colistin is crucial

- This must be done for human but also animal isolates

- Selective pressures leading to co-selection of resistance to colistin must be identified

Infection Control in Veterinary Hospitals

Why it matters....lessons from Liverpool

Dr Dorina Timofte

DVM PhD MRCVS DiplECVM

Sen Lecturer in Clinical Veterinary Microbiology

Human/Veterinary Hospital environment

Different hospitals - same nosocomial agents

- **Methicillin-Resistant *Staphylcoccus aureus* (MRSA)**

    - **Dog and cats** colonisation/infection: **MRSA E15**

    - **Equine and farm animals**: infections are associated with MRSA isolates *uncommon in humans*

- **Methicillin Resistant *Staphylococcus pseudintermedius* (MRSP)**

    - *S. pseudintermedius* (dog-adapted)

    - Thought to be involved in 95% of all canine pyoderma cases

ESBL-producing *E. coli:*
Hospital dissemination
in Small Animals (dogs)

Clinical isolates

Environmental
door handle and fridge handle, the ward computer keyboard

Veterinary Hospital Dissemination of CTX-M-15
Extended-Spectrum Beta-Lactamase–Producing
Escherichia coli ST410 in the United Kingdom

Dorina Timofte,[1–3] Iuliana Elena Maciuca,[1] Nicola J. Williams,[4] Andrew Wattret,[1] and Vanessa Schmidt[1,2]

Acinetobacter baumannii

in companion animals

Birgit Walther[1,2*], Katja-Sophia Klein[3], Ann-Kristin Barton[3], Torsten Semmler[4], Charlotte Huber[2], Silver Anthony Wolf[4], Karsten Tedin[1], Roswitha Merle[5], Franziska Mitrach[6], Sebastian Guenther[7,8], Antina Lübke-Becker[1], Heidrun Gehlen[3]

- Opportunistic pathogen associated with HAIs

- Little data available on *A. baumannii* as a nosocomial pathogen in veterinary hospitals

MDR *Acinetobacter* spp isolate study:

- *Clinical:* small animals, equine and exotic species (n=98)

- *Veterinary hospital environments* (Env; n=51)
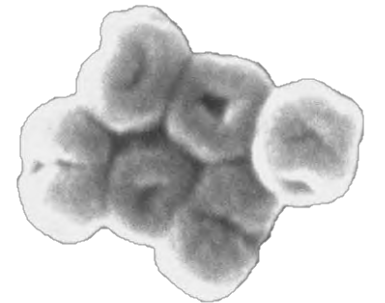
A. baumannii: molecular typing

PCR-based group typing and cgMLST

- 44% of *A. baumannii* isolates were typed to **IC-I (ST1)**

- *Few (6%) of A. baumannii* isolates typed to **IC-II** (ST2, ST427, ST739)

All **IC-I** isolates were Equine (Clinical or Env)

Minimum Spanning Network of animal and human *A. baumannii* SNP profiles

IC-I

IC-II

Equine
Canine
Bovine
Feline
Unknown Animal

Human (from SNPT*Ab* DB)
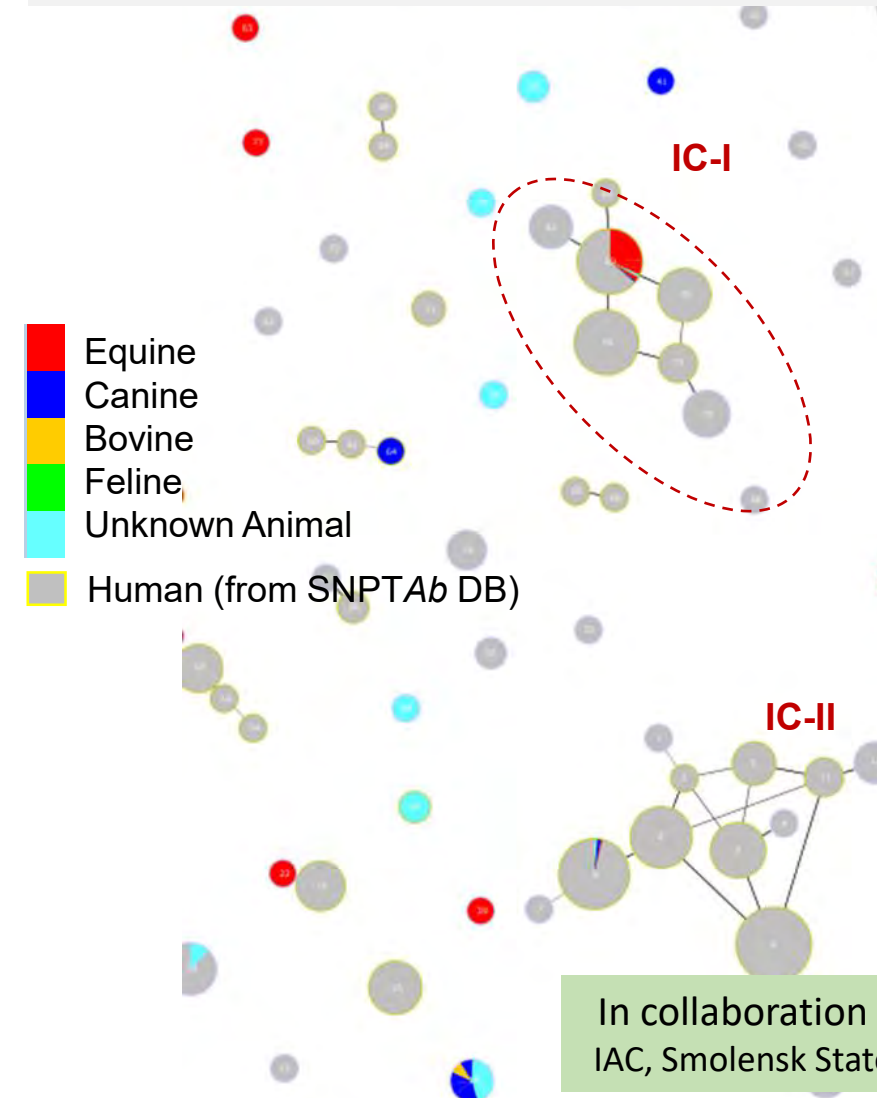
In collaboration with M. Edelstein
IAC, Smolensk State Medical University

Infection Control at UoL - What we do

Active surveillance

Screening patient
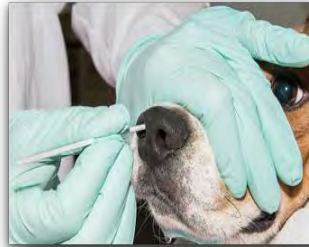
Environmental surveillance

Hand-plates

- **Monitor microbiology diagnostic results**

- *Infection control working group involving clinicians, nurses and microbiologists*

Active surveillance:

Hand-plate sampling

- Provides an effective visual way of disseminating results
- Results fed back to staff as CFU/plate and images
  - Weekly emails
  - Departmental seminars

Active surveillance:

Swab (gauze swabs)

Buffered peptone water (24hr 37°C)

MRSA/MRSP

MDR/HLGR Enterococcus spp

ESBLs

MDR Gram-negatives

Salmonella spp
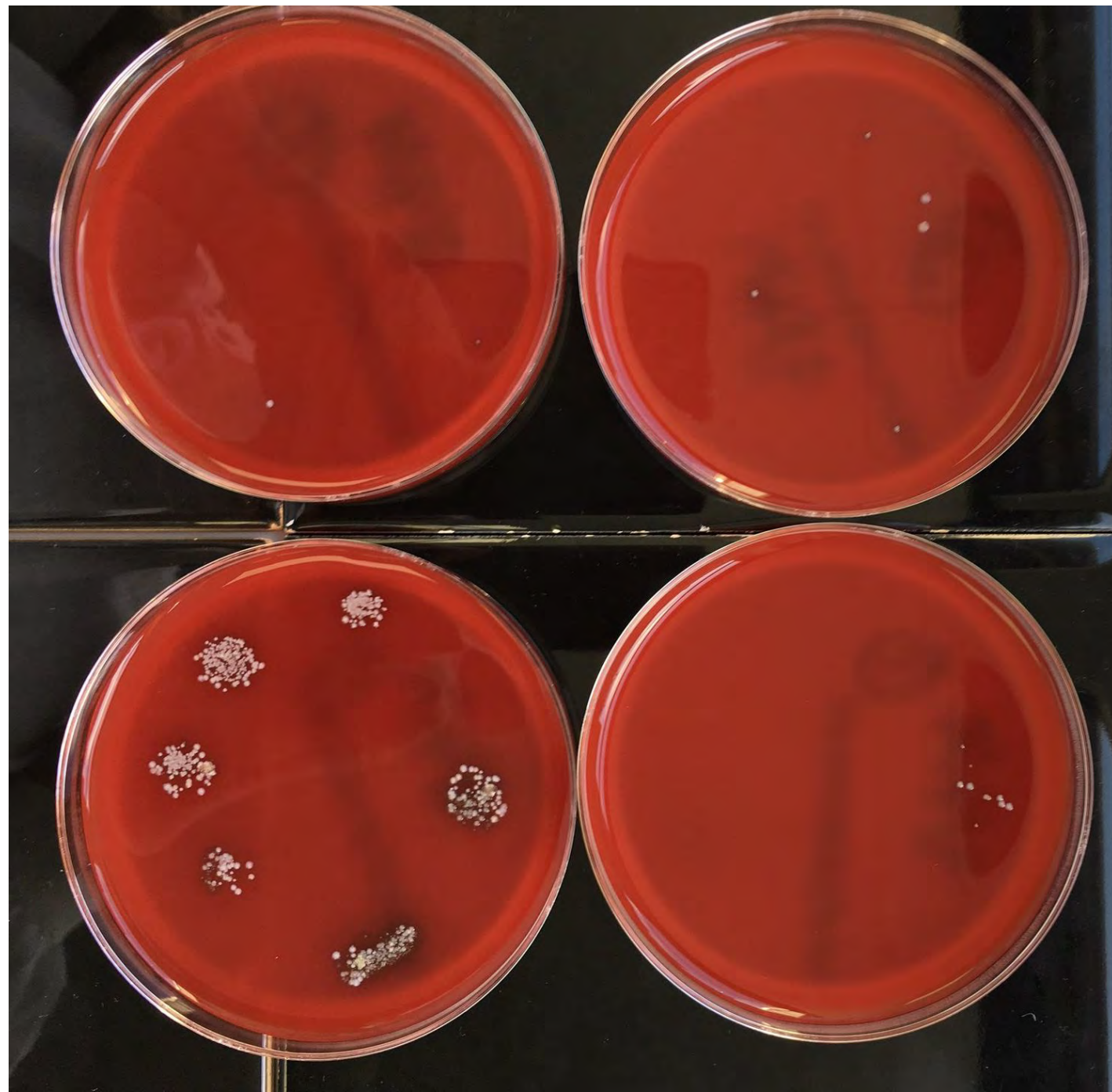
MDR Pseudomonas spp

- **Environmental surveillance**
  - **Screening** MRSA/MRSP, MDR Gram-negative bacteria
    (*E. coli, Acinetobacter* spp, *Pseudomonas* spp)

  - **Action when found:** *cleaning-disinfection-*
    re-swabbing
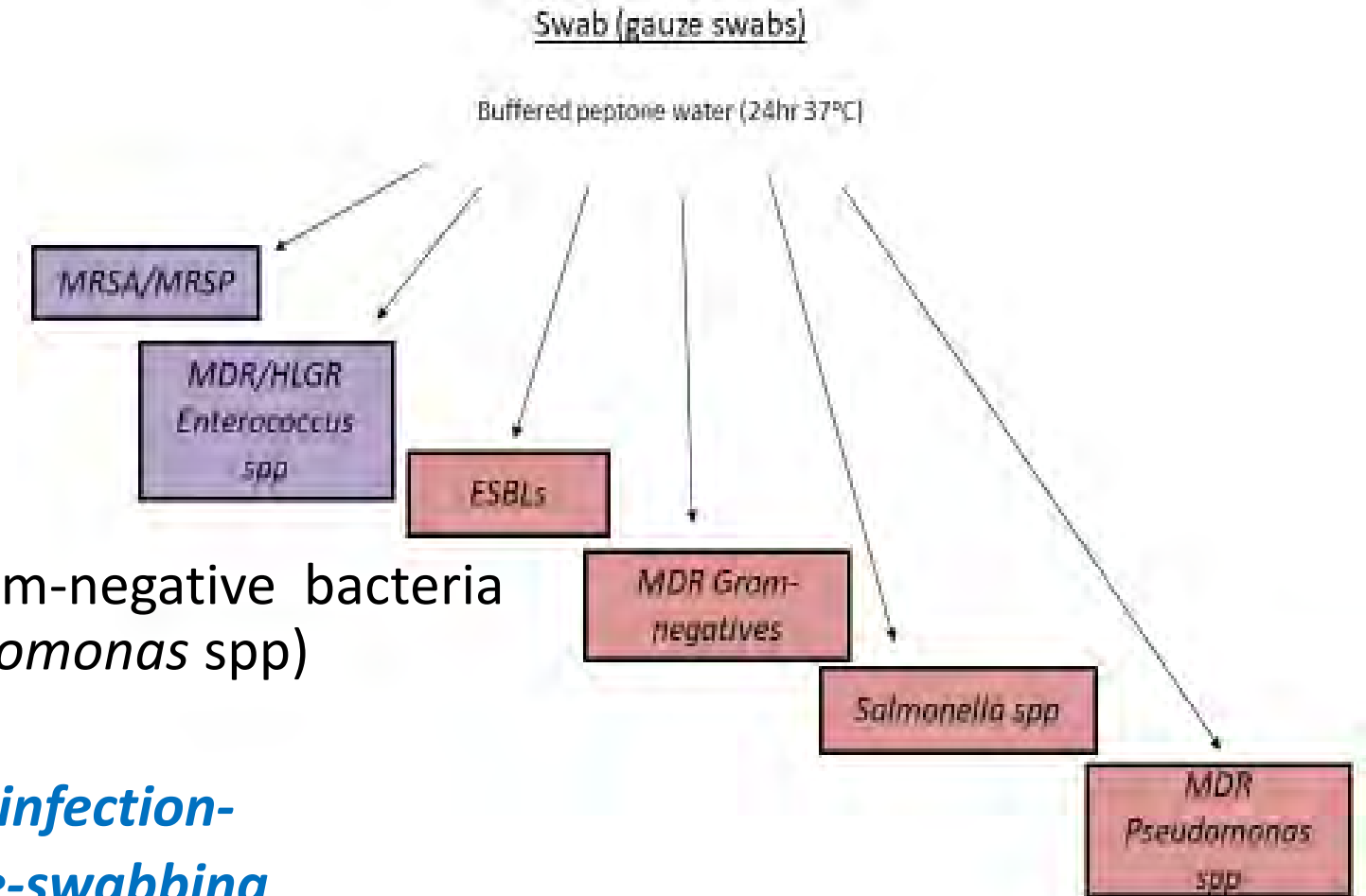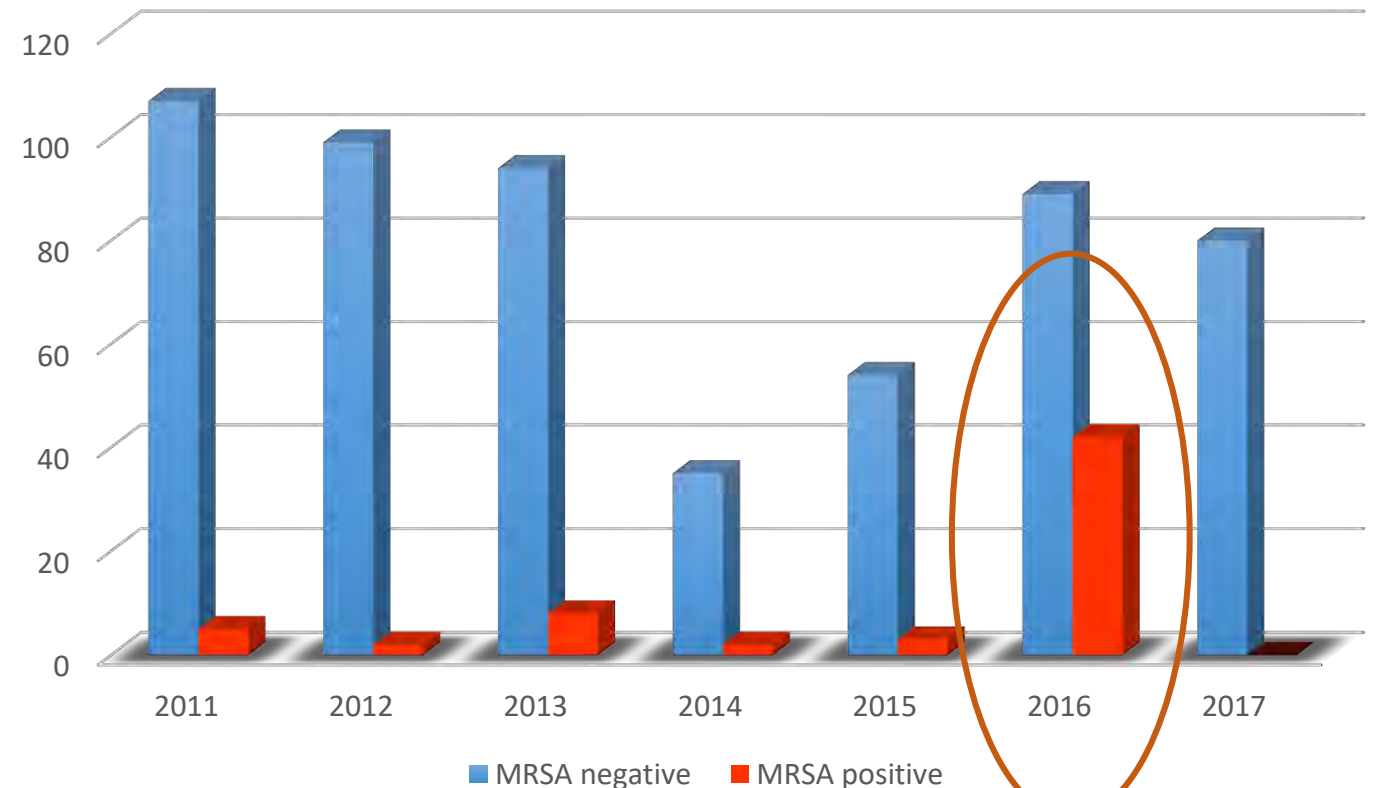
Equine Hospital MRSA outbreak: 2016

- Previous history of intermittent cases
- Feb - April 2016 – 6 individual MRSA SSI (surgical site infection) in ICU cases

Timeline of MRSA isolation from PLEH environment and clinical isolates (Feb-April 2016)

Arrows indicate point of isolation

MRSA outbreak:
Molecular characterisation

Identified:
Livestock-associated MRSA (LA-MRSA) CC398

80% MRSA isolates obtained in the past 5 years belonged to CC398

SCIENTIFIC REPORTS

OPEN Environmental surveillance identifies multiple introductions of MRSA CC398 in an Equine Veterinary Hospital in the UK, 2011–2016

Strain	Year	Site (Location)	CC398	SCCmec type	spa-type	spa CC	Resistance phenotype*
M 1	2011	SSI	+	IVa	t011	spa CC011/3423	Gen, Tet
M 2	2011	ENV-(Stable floor)	+	IVa	t011	spa CC011/3423	Gen, Tet
M 3	2011	ENV- (Stable floor)	+	IVa	t011	spa CC011/3423	Gen, Tet
M 4	2013	ENV- (Stable floor)	+	IVa	t073	Singleton	Enr, Tet
M 5	2013	ENV (Stable floor)	+				
M 6	2013	ENV- (Stable floor)	+				
M 7	2014	SSI	+				
M 8	2014	ENV- (Staff Keyboard)	+				
M 9	2015	ENV (Y-piece no 1)	+				
M 10	2015	ENV (Stable floor)	+				
M 11	2015	SSI	+				
M 12	2015	SSI	+				Tet
M 13	2015	SSI	+	IVa	t011	spa CC011/3423	Gen, Enr, Tet, Neo
M 14	2016	SSI	+	IVa	t588	spa CC011/3423	Gen, Enr, Tet
M 15	2016	SSI	+	IVa	t3423	spa CC011/3423	Gen, Tet
M 16	2016	SSI	+	IVa	t011	spa CC011/3423	Gen, Tet
M 17	2016	SSI	+	IVa	t011	spa CC011/3423	Gen, Tet
M 18	2016	SSI	+	IVa	t011	spa CC011/3423	Gen, Tet
M 19	2016	SSI	+	IVa	t011	spa CC011/3423	Gen, Tet
M 20	2016	ENV (Stable floor)	+	IVa	t588	spa CC011/3423	Gen, Ery, Tet
M 21	2016	ENV (Stable floor)	+	IVa	t011	spa CC011/3423	Gen, Tet
M 22	2016	ENV (Stable floor)	+	IVa	t011	spa CC011/3423	Gen, Tet
M 23	2016	ENV (Stable floor)	+	IVa	t3423	spa CC011/3423	Gen, Tet
M 24	2016	ENV (Stable floor)	+	IVa	t588	spa CC011/3423	Gen, Enr, Tet
M 25	2016	ENV (Stable drain)	+	IVa	t011	spa CC011/3423	Gen, Tet
M 26	2016	ENV (Stable floor)	+	IVa	t011	spa CC011/3423	Gen, Tet
M 27	2016	ENV (Stable wall)	+	IVa	t011	spa CC011/3423	Gen, Tet
M 28	2016	ENV (Stable brush)	+	IVa	t011	spa CC011/3423	Gen, Tet
M 29	2016	ENV (Stable floor)	+	IVa	t011	spa CC011/3423	Gen, Tet
M 30	2016	ENV- (Stable floor)	+	IVa	t011	spa CC011/3423	Gen, Tet
M 31	2016	ENV (ICU Keyboard)	+	IVa	t011	spa CC011/3423	Gen, Tet
M 32	2016	ENV (Y-piece)	+	IVa	t011	spa CC011/3423	Gen, Tet
M 33	2016	ENV (Recep keyboard)	+	IVa	t1985	spa CC011/3423	Gen, Tet
M 34	2016	ENV (Student keyboard)	+	UT	t011	spa CC011/3423	Gen, Tet
M 35	2016	ENV (Hand plate)	+	IVa	t1985	spa CC011/3423	Gen, Tet
M 36	2016	ENV (Hand plate)	+	IVa	t011	spa CC011/3423	Gen, Tet
M 37	2016	ENV (Hand plate)	+	IVa	t011	spa CC011/3423	Gen, Tet
M 38	2016	ENV (Hand plate)	+	IVa	t011	spa CC011/3423	Gen, Tet

Alessio Bortolami[1], Nicola J. Williams[2], Catherine M. McGowan[1,3], Padraig G. Kelly[1], Debra C. Archer[1,2], Michela Corrò[4], Gina Pinchbeck[2], Christine J. Saunders[1] & Dorina Timofte[1,2]

MRSA persistence within the hospital stables

- Routine cleaning and disinfection protocols were not effective

- MRSA was identified in dust samples from higher levels

Lessons learnt

Study highlighted weaknesses

- Cross contamination between patients
  - Poor hand hygiene
  - Poor glove use

- Training and re-training, especially new people

Woa... gloves are single-use
Change 'em

Infection Control Research:

Trialing new MDR typing technologies for Infection control

- Current surveillance program: not providing immediate molecular typing information…….

- New MDR typing technologies are available for strain identification

Molecular typing tools - 1:

Acuitas Resistome (OpGen, US) for typing MDR-GN isolates

- Combines detection of AMR genes/ID/susceptibility to give a unique isolate profile in real-time
- Results provided 48 hours from isolate submission

ESBL
13 Genes
557 Subtypes

Carbapenemases
25 Genes
208 Subtypes

AmpC
11 Genes
147 Subtypes

Organism ID Code :

Organism's genus species abbreviation

Top 3 Resistome Test Gene Codes :

Most severe Resistome Results

AST Result Profile Code:

Unique numerical code for AST results and how often observed

K1:CRE:KPC_NDM1_VIM5:[R70]52-8_[A17]90-1

Phenotype Code :

Classification of the observed Phenotypes

Resistome Result Profile Code:

Unique numerical code for Resistome results and how often observed

Sample

MDR-GN
Selective Culture

24 Hours

Bacterial Isolate

Organism ID &
Antimicrobial
Susceptibility
Testing

Acuitas®
Resistome Test

48 Hours

Results

72 Hours

Results

Molecular typing tools - 2:

IR Biotyper (Bruker) for Microbial Strain Typing (GN)

- Bench-top system based on **Fourier transform infrared (FT-IR) spectroscopy** technology

- Provides unique protein fingerprint

- High discriminatory power to recognize the clonal relationship of isolates

- Fast turnaround times and low costs per sample
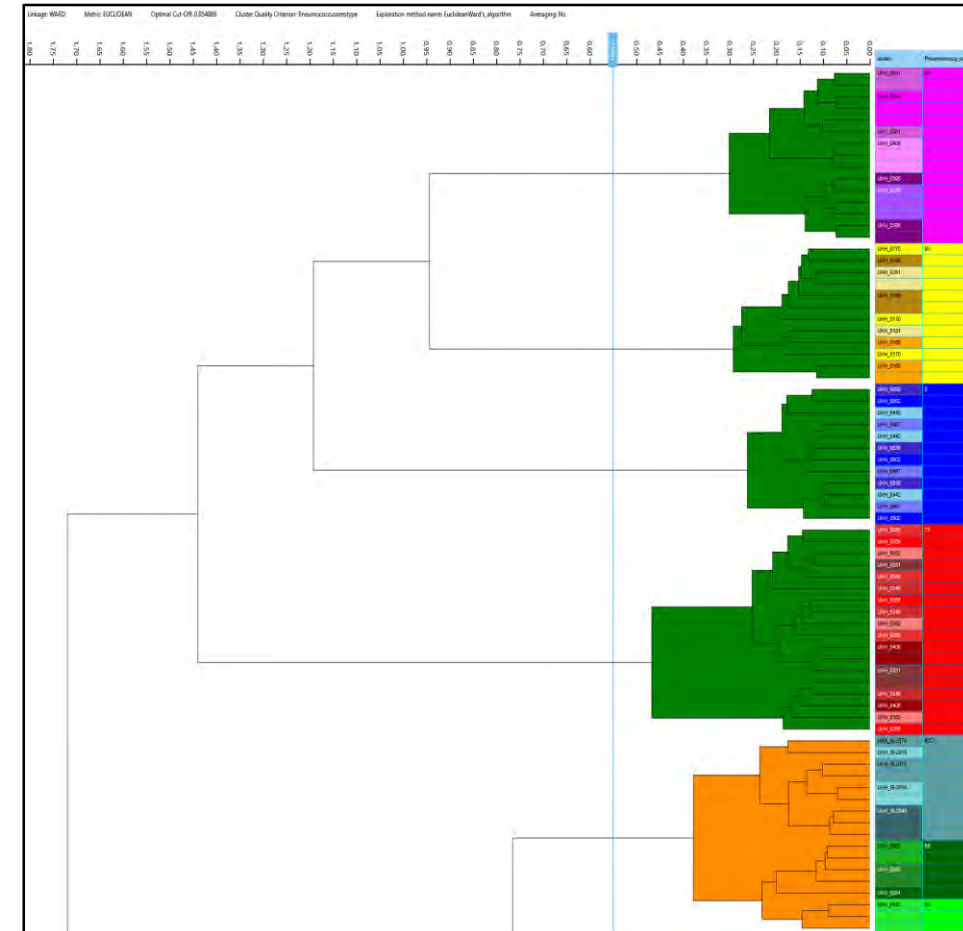
MDR Screening in Veterinary Hospital ICUs: Equine and Small Animal

ESGVM — ESCMID STUDY GROUP FOR VETERINARY MICROBIOLOGY — European Society of Clinical Microbiology and Infectious Diseases

Environmental samples

Faecal samples

T_1 → Admission – 48h

T_2 48 hrs →

All samples – screened for MDR Gram-negative bacteria and isolates posted to OpGen (US) for Resistome Testing

Major Acuitas Resistome profiles identified

Pattern types	Lighthouse Profile Organism Gene-pattern	Equine Hospital				Small Animal Hospital			
		F1	Env1	F2	Env2	F1	Env1	F2	Env2
I	*A. baumannii* A4:CephR-GNB:OXA51:[R70]136_[A17]221			▮	▮		▮		▮
II	*E. cloacae* E10:S-GNB:TEM7_TEM3_TEM1:[R70]286_[A17]457		▮	▮	▮				
III	*E. coli* E1:CephR-GNB:CTXM1:[R70]56_[A17]135	▮		▮	▮				
IV	*K. pneumoniae* K1:CephR-GNB:SHV1_SHV5_DHA1:[R70]147_[A17]180					▮	▮	▮	▮
V	*P. aeruginosa* P1:CephR-GNB:OXA50:[R70]96_[A17]181	▮	▮	▮	▮		▮		

E. coli isolates analysed by Bruker IR Biotyper

34 *E. coli* isolates typed to investigate strain relatedness
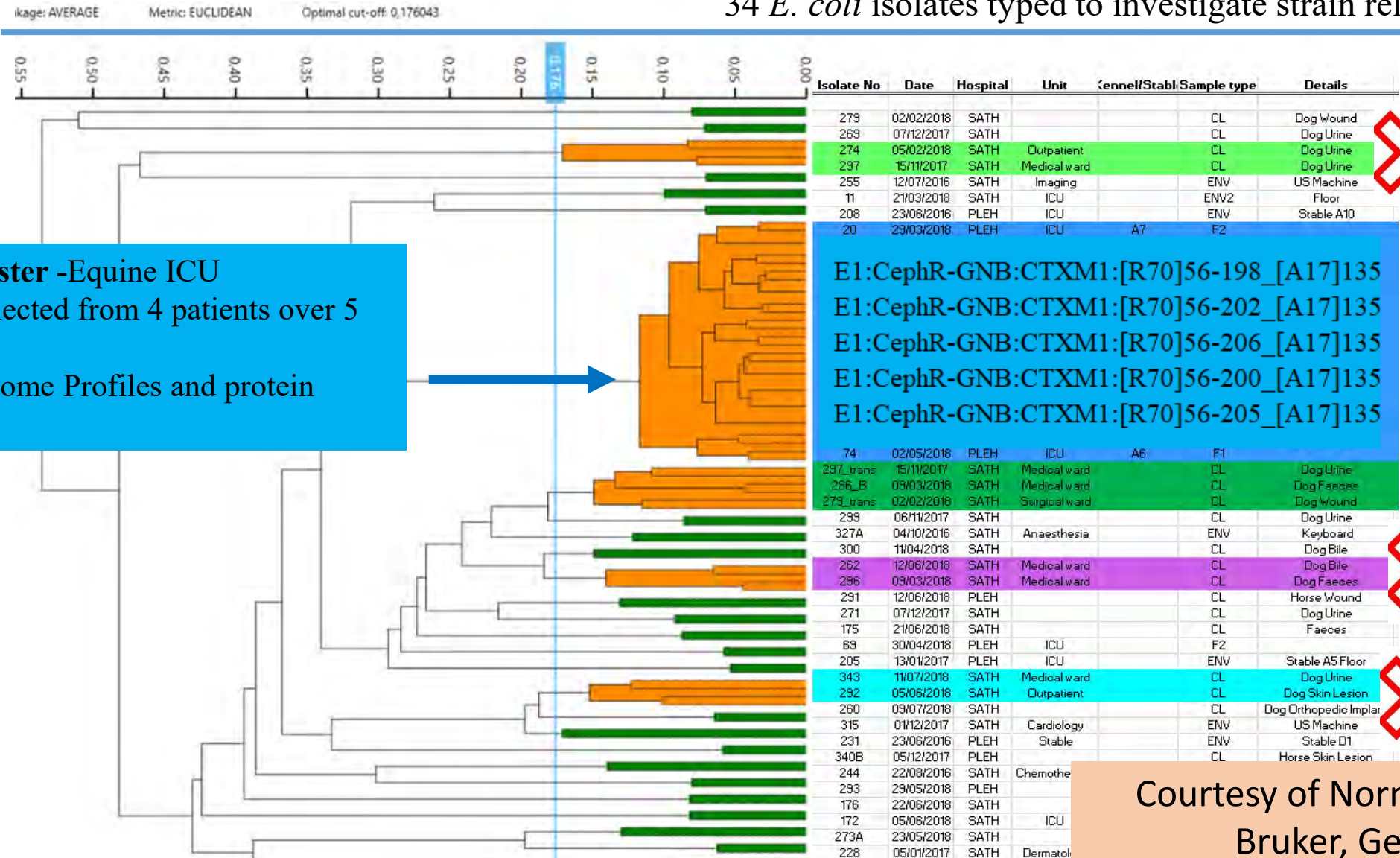
One large cluster -Equine ICU
- Isolates collected from 4 patients over 5 weeks
- same Resistome Profiles and protein fingerprints

E1:CephR-GNB:CTXM1:[R70]56-198_[A17]135
E1:CephR-GNB:CTXM1:[R70]56-202_[A17]135
E1:CephR-GNB:CTXM1:[R70]56-206_[A17]135
E1:CephR-GNB:CTXM1:[R70]56-200_[A17]135
E1:CephR-GNB:CTXM1:[R70]56-205_[A17]135

Courtesy of Norman Mauder, Bruker, Germany

Infection control in Veterinary Hospitals
Conclusions

- **Improves biosecurity** by minimizing the risk of infectious disease transmission within the veterinary facilities

- Reduces potential **financial loss**

- **Targeted environmental monitoring** can be a useful tool for detecting reservoirs and enabling early interventions

- **Important to feedback and inform staff**

- **Hand plates** are cost effective tool for monitoring and reinforcing hand hygiene and are simple to use

- IC programmes are **critical to protect** **patients, veterinary staff, students and animal owners**

Demographic fluctuation of community-acquired antibiotic-resistant Staphylococcus aureus lineages: potential role of a low level antibiotic and heavy metal exposures

F. Vandenesch, MD, PhD
INSERM U1111, University of Lyon, National Reference Center for Staphylococci

MRSA = MSSA + SCC*mec* element

SCC*mec* classification

(old fashioned)

Hiramatsu, Infect & Chemother. 2013
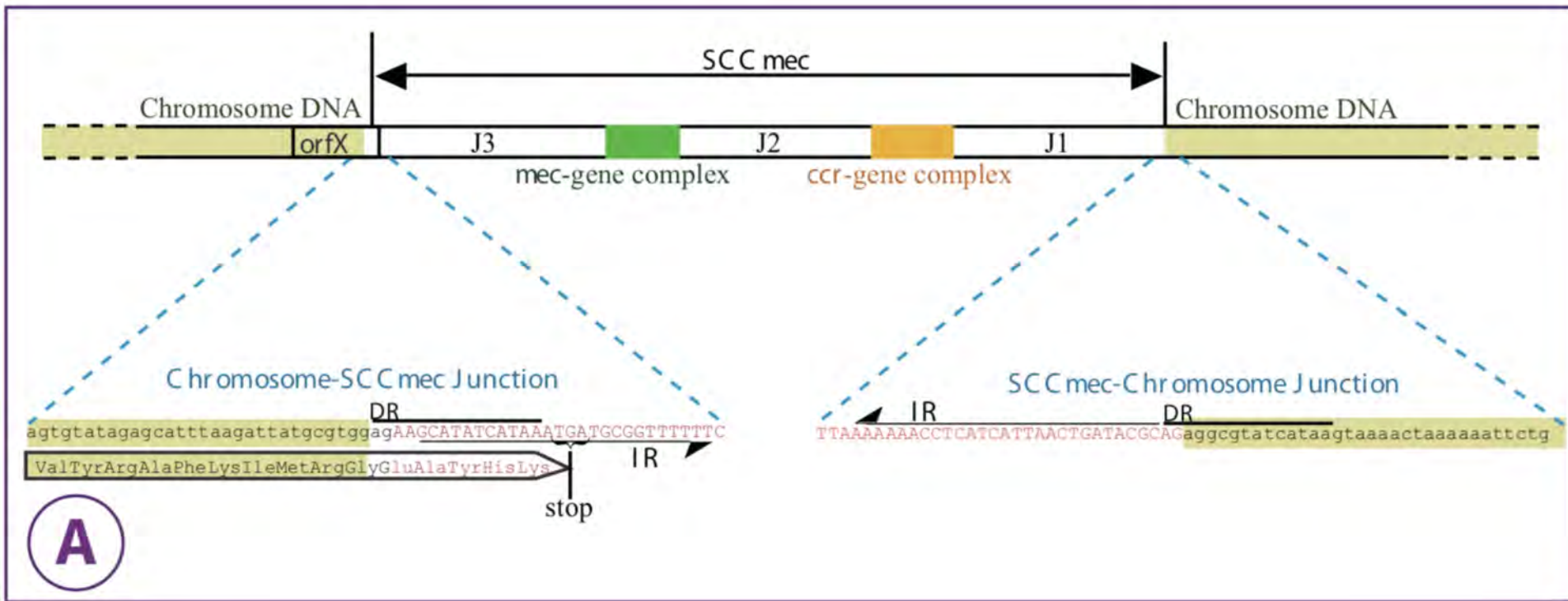
SCC*mec:* a convenient vehicle for virulence genes
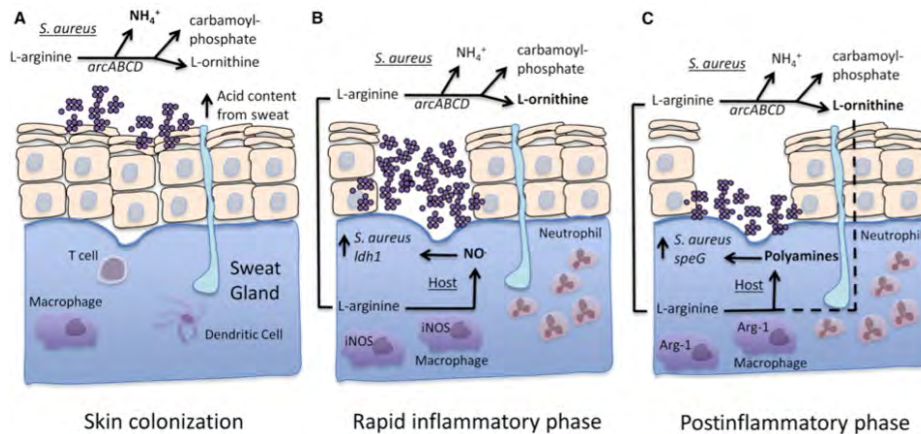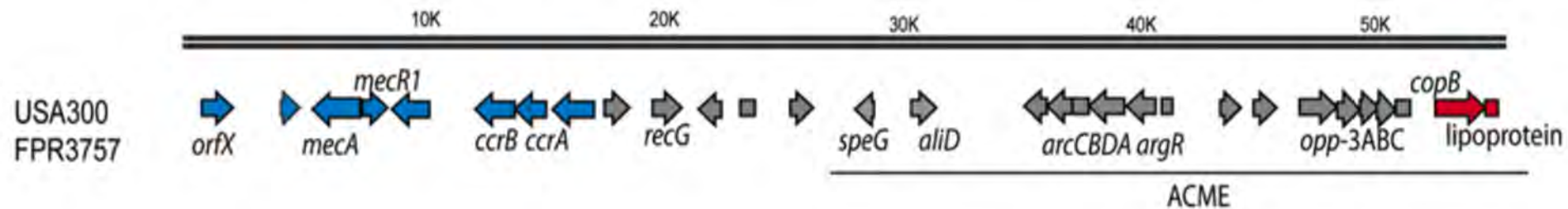
ACME contributes to *S.aureus* survival on and within human skin

Planet et al, JID 2015
Thurlow et al. Cell Host & Microbe 2013
Alonzo et al, Cell Host & Microbe 2013

SCC*mec:* a convenient vehicle for other resistance genes
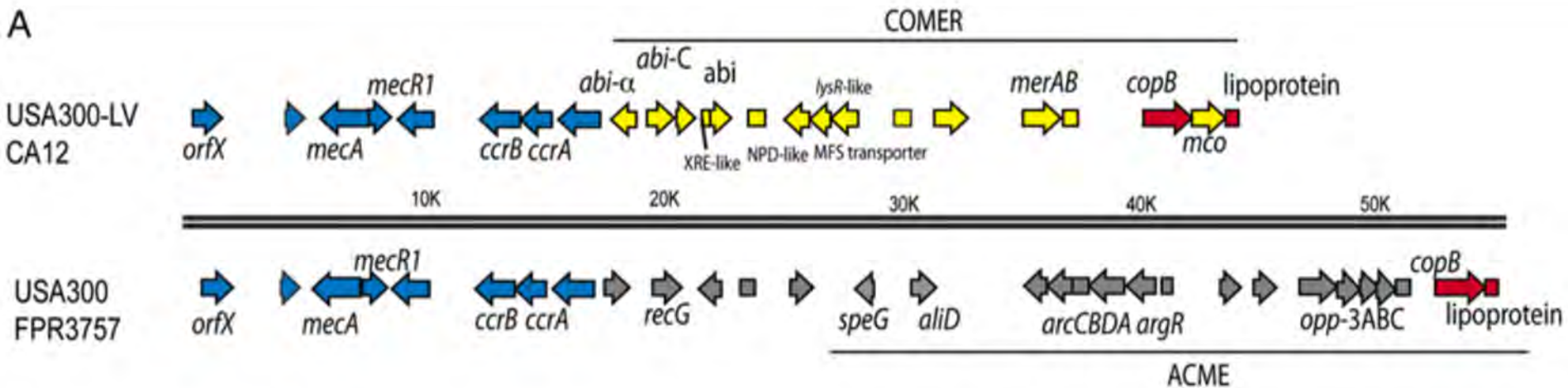
USA300 NA and LV SCCmec share an hyperesistance locus to copper

Planet et al, JID (2015)
Purves J, Environ Microbiol (2018)

SCC*mec:* a convenient vehicle for other resistance genes
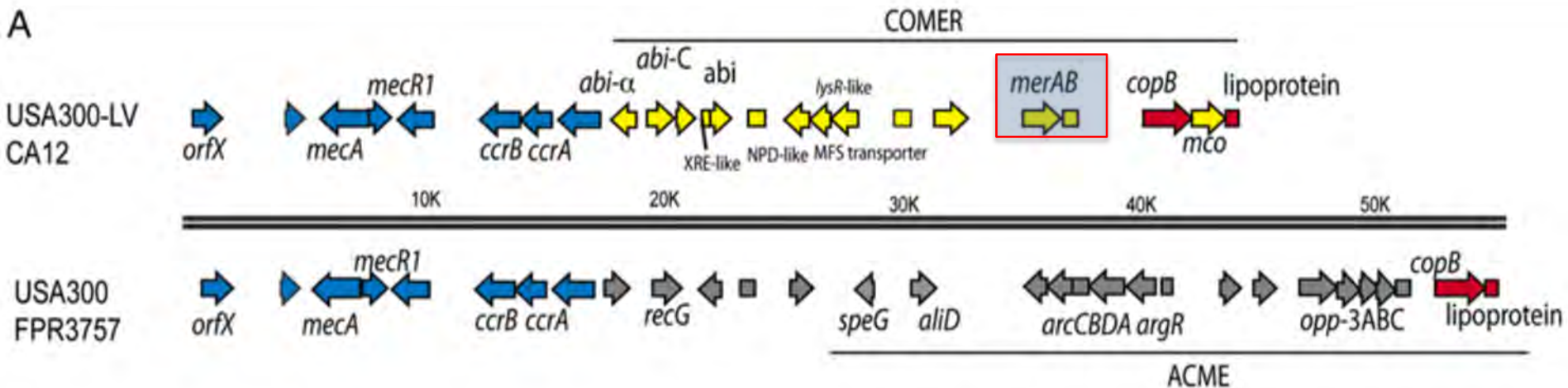
USA300 NA and LV SCCmec share an hyperesistance locus to copper
USA300 LV SCCmec encodes a mercury resistance gene
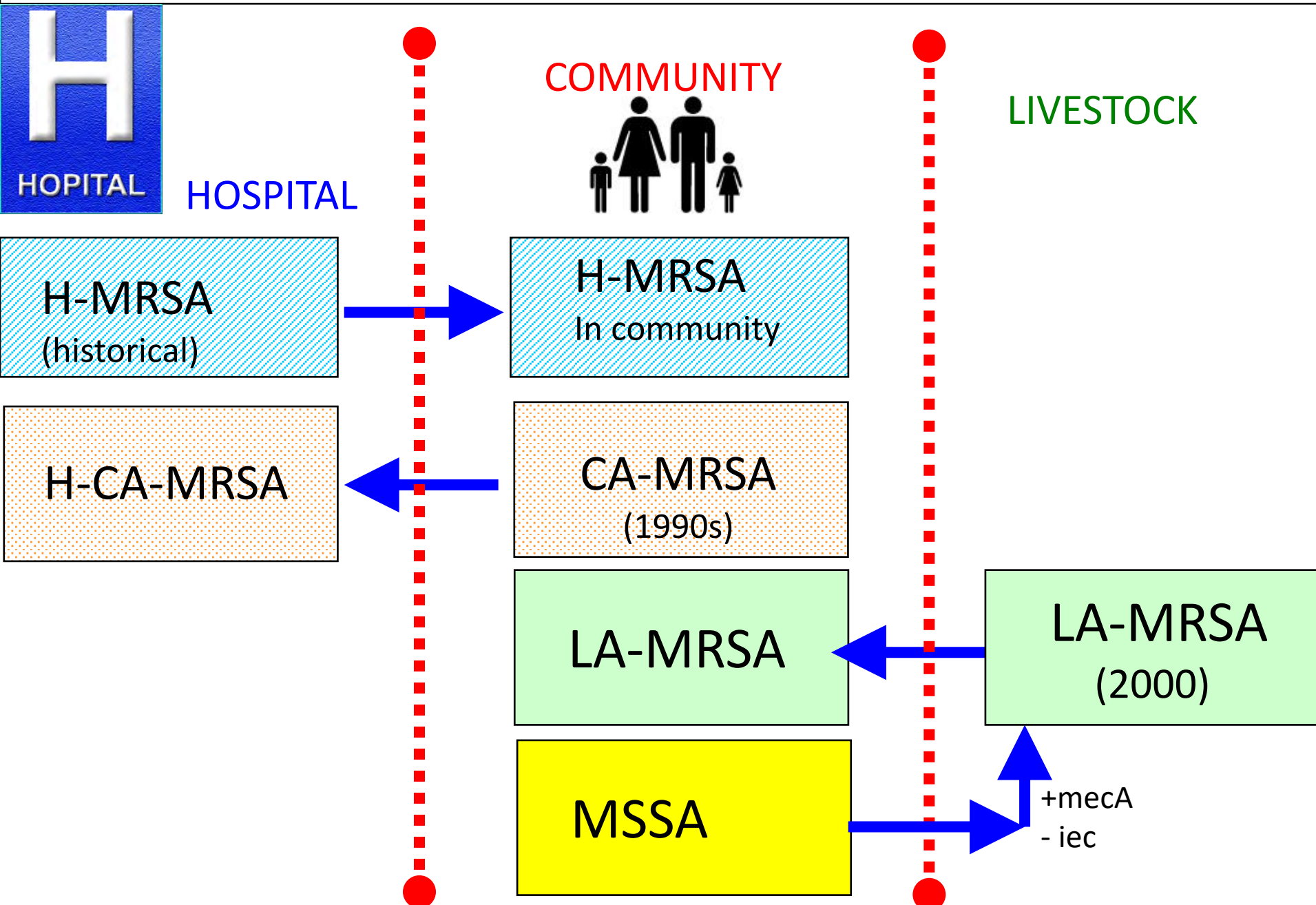
Planet et al, JID (2015)
Purves J, Environ Microbiol (2018)

The categories of MRSA

(historical) CA MRSA build up at genomic level
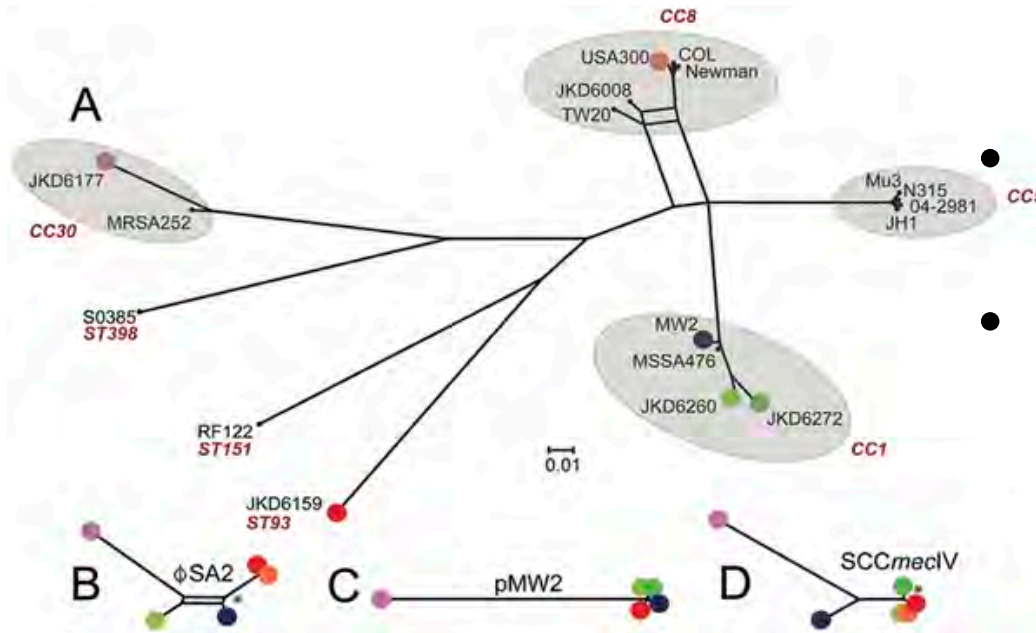
- Genetically distinct lineages
- conserved repertoire of accessory elements
  - PVL harbouring phage
  - SCC*mec* type IV or V
  - pMW2

Vandenesch et al. Emerging Infect Dis (2003)
Li et al. J Infect Dis (2010)
Chua KYL et al. PLoS ONE (2011)
Carpaij et al. PLOS One (2011)

(historical) CA MRSA build up at genomic level
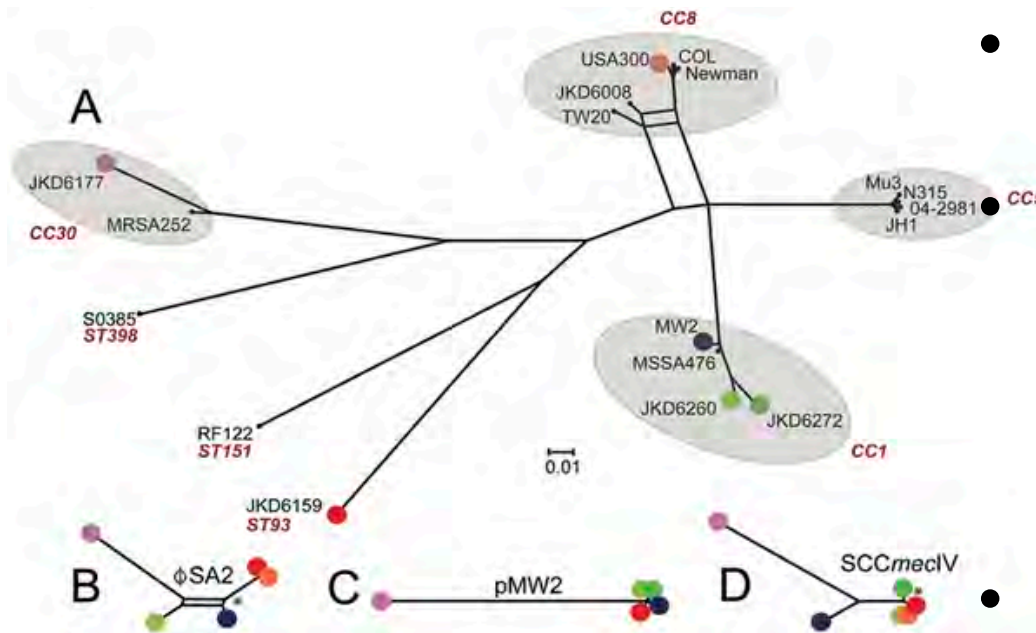
- Genetically distinct lineages
- conserved repertoire of accessory elements
  – PVL harbouring phage
  – SCC*mec* type IV or V
  – pMW2
- Increased expression of core-genome-encoded virulence factors

Li et al. J Infect Dis (2010)
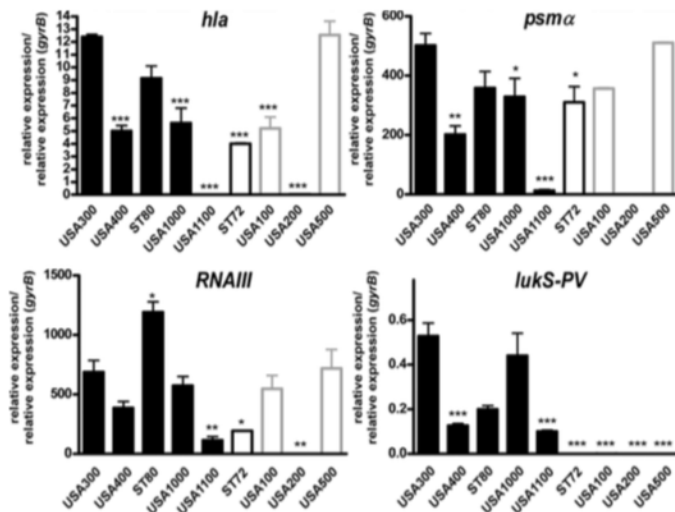
Community-acquired MRSA: independent emergence

Deleo et al. Lancet (2010)
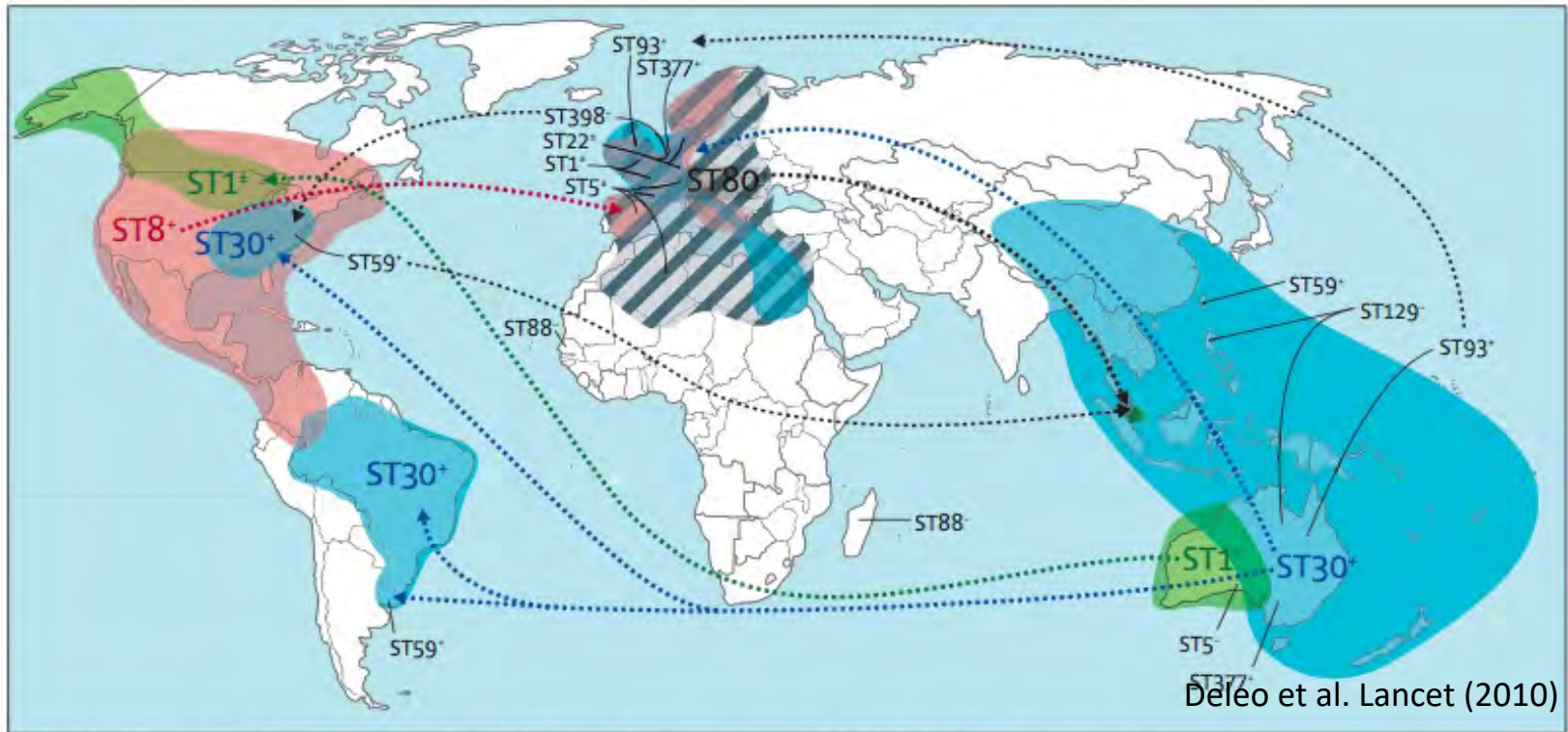
- Lessons from phylogeographic studies ?

ST 80 lineage

97 genomes, 27 countries,
Bayesian coalescent methods -
> Time-trees orientated
phylogenies

– Ancestral strains (TMRCA
1982) = sub-Saharan

– Derived (TMRCA 1985)
(SCCmecIV, AgrcL184I) =
North Africa, Europe and
middle Est

M.Stegger et al. MBIO 2014

Lineage through time (LTT) and skyline

(a) Tree A   Tree B

(b) Number of lineages vs Time (Past ← → Present)

(c) M_i vs Time (Past ← → Present)
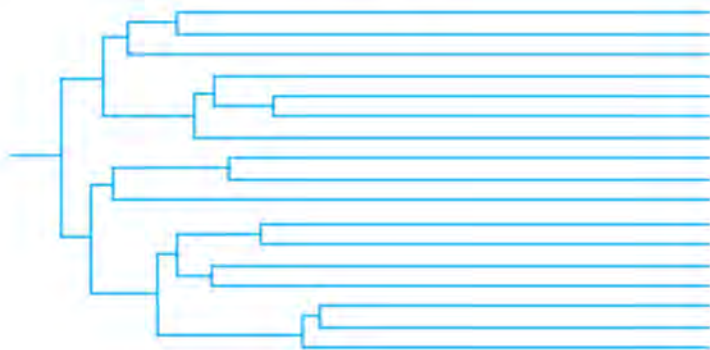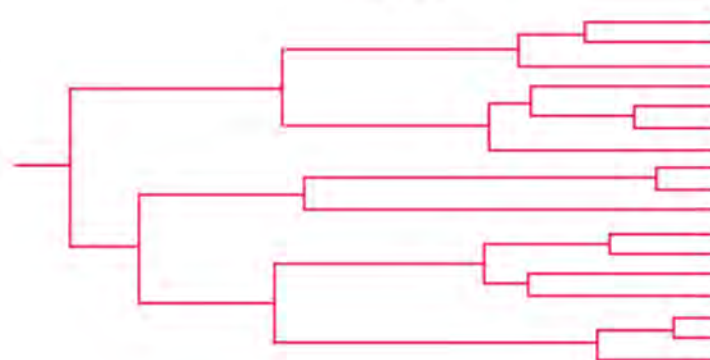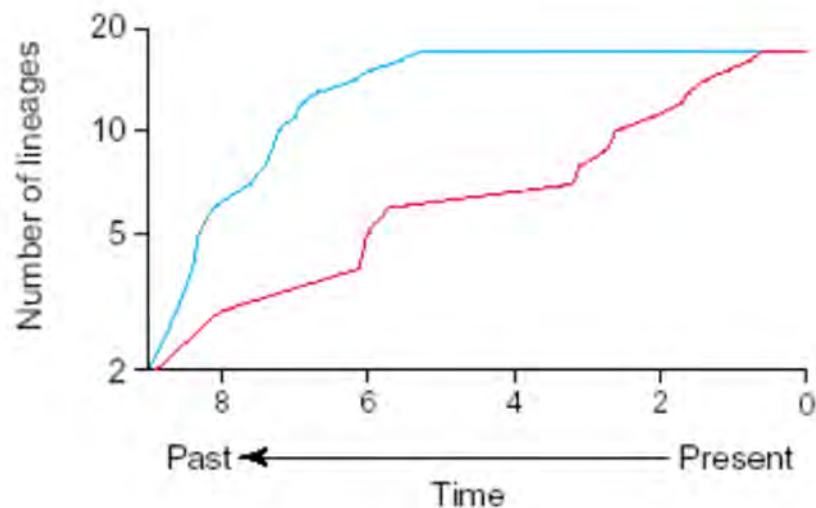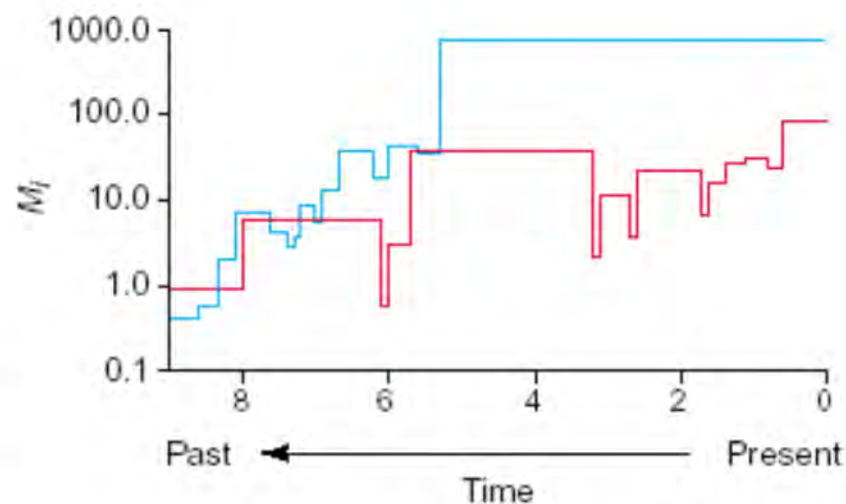
Bayesian skyline plot: effective population size
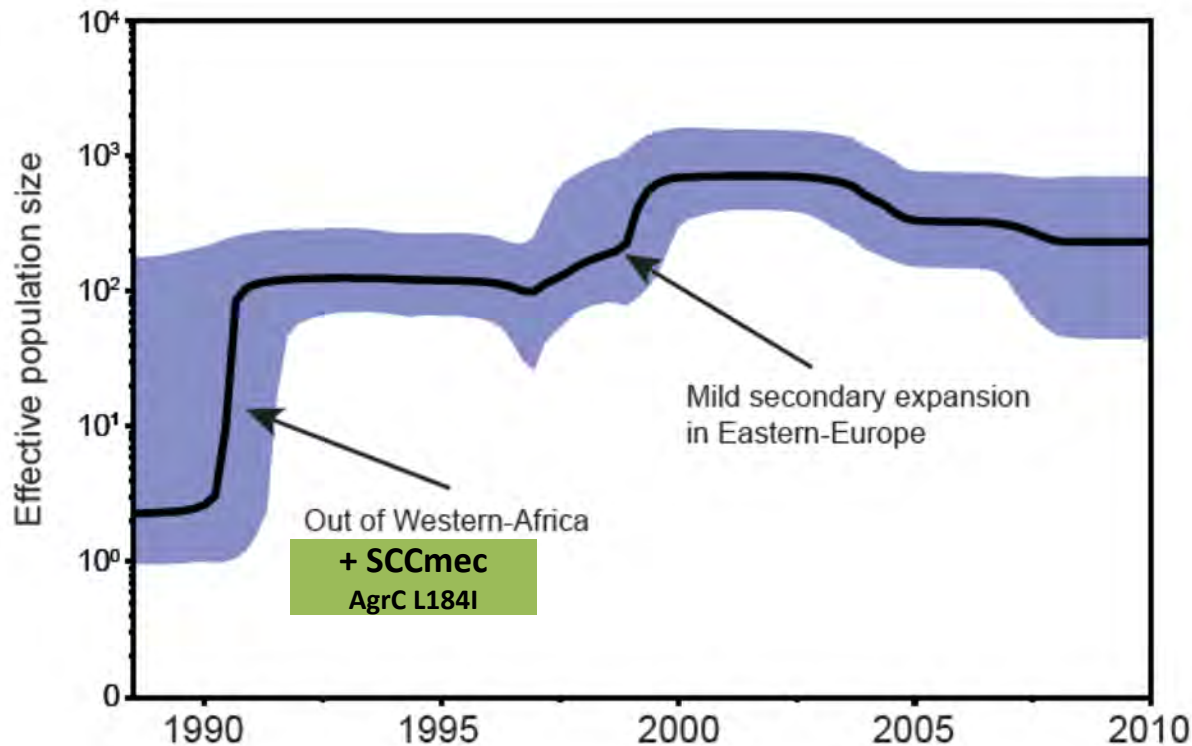
- sharp increase in the early 90's
- secondary mild expansion (in 2000)
- recent stepwise slow decrease

→ in agreement with the first reports of CC80 Isolates around Europe and the later observed increase and spread

ST80 lineage

Deleo et al., Lancet, 2010, Stegger et al. M bio 2014
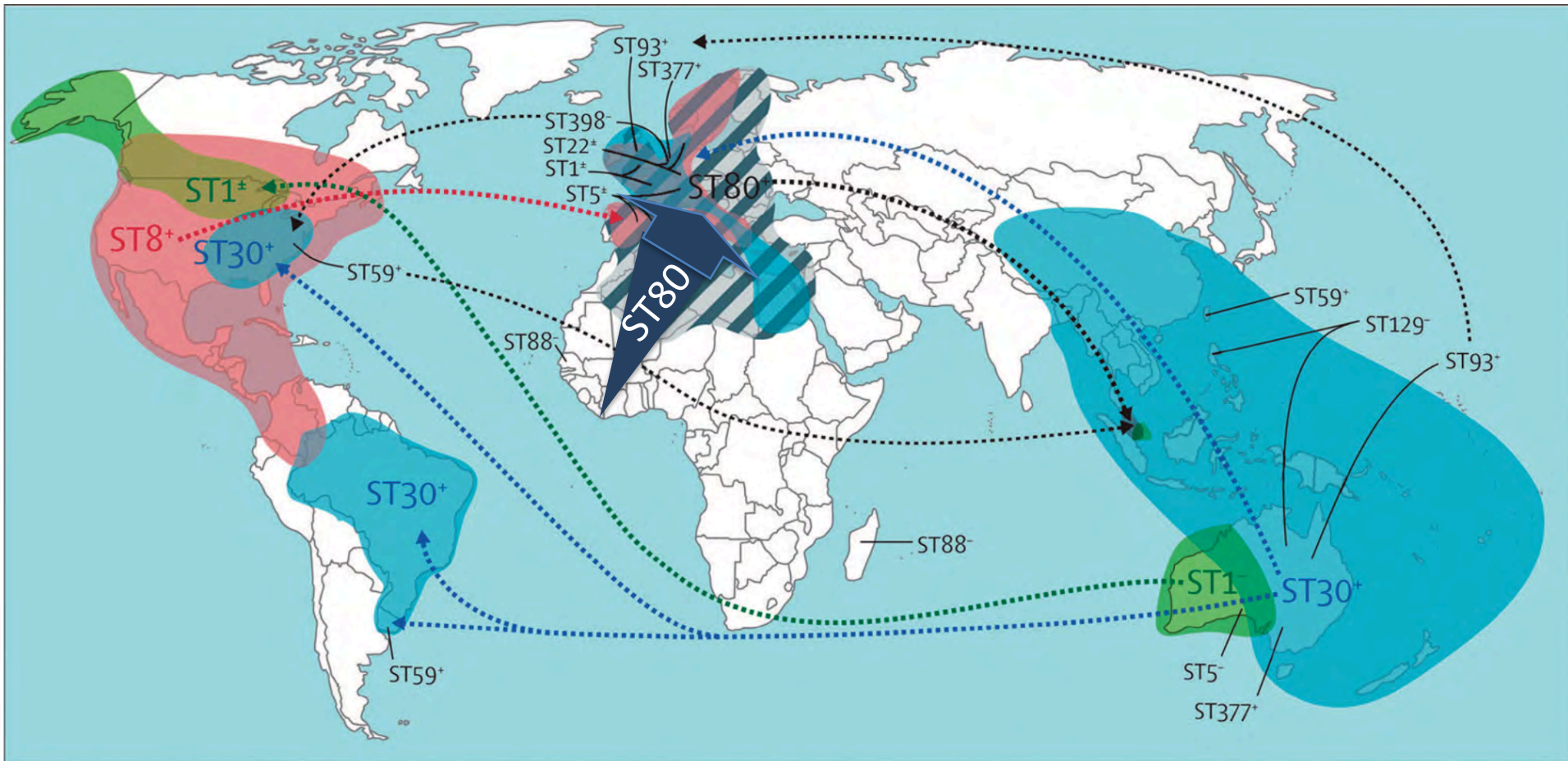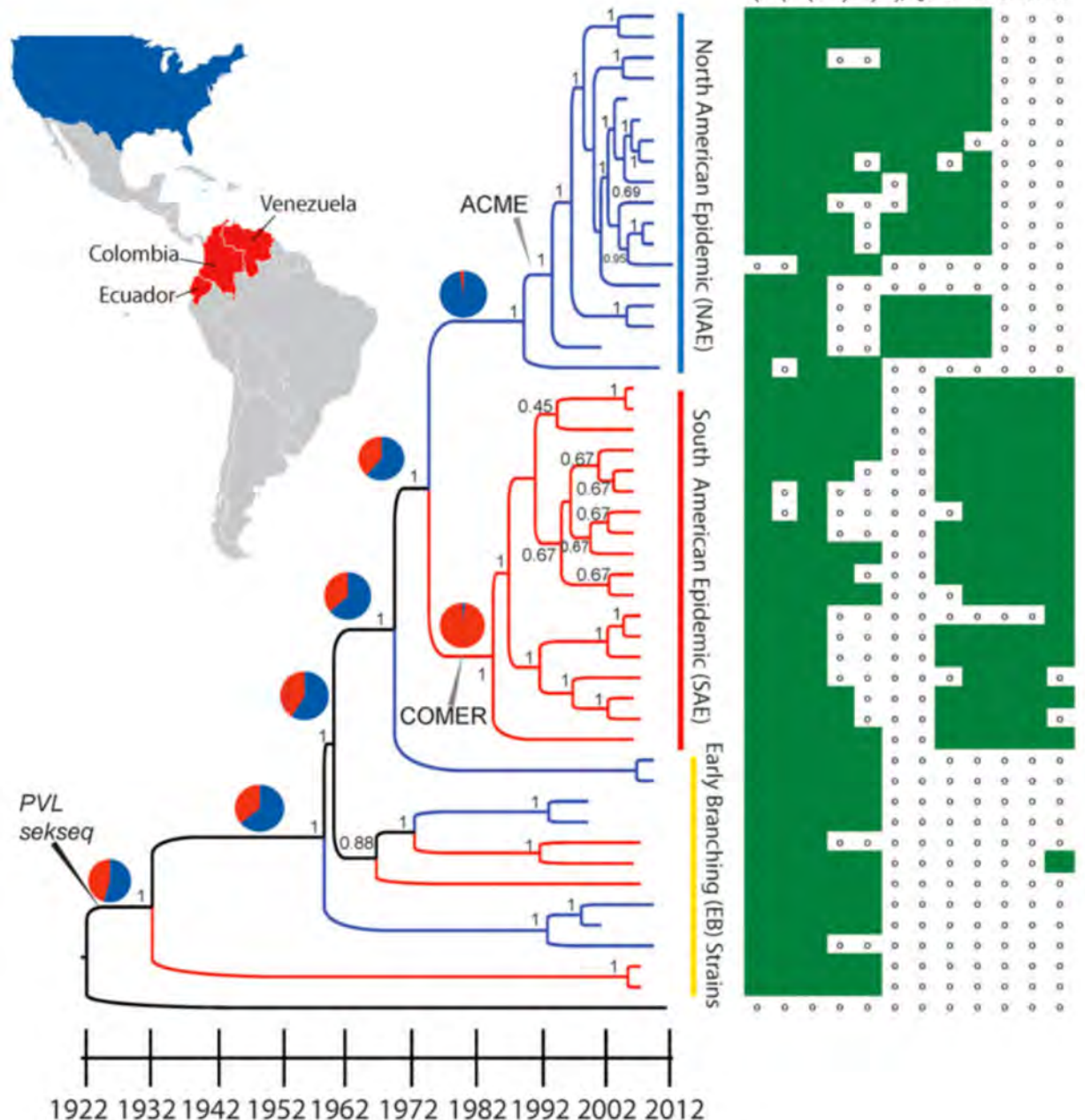
USA300

2 distinct clades

North America

and South America

that segregate by geographical region

ACME in NA clade

COMER in SA clade

Planet et al. J Infect Dis 2015
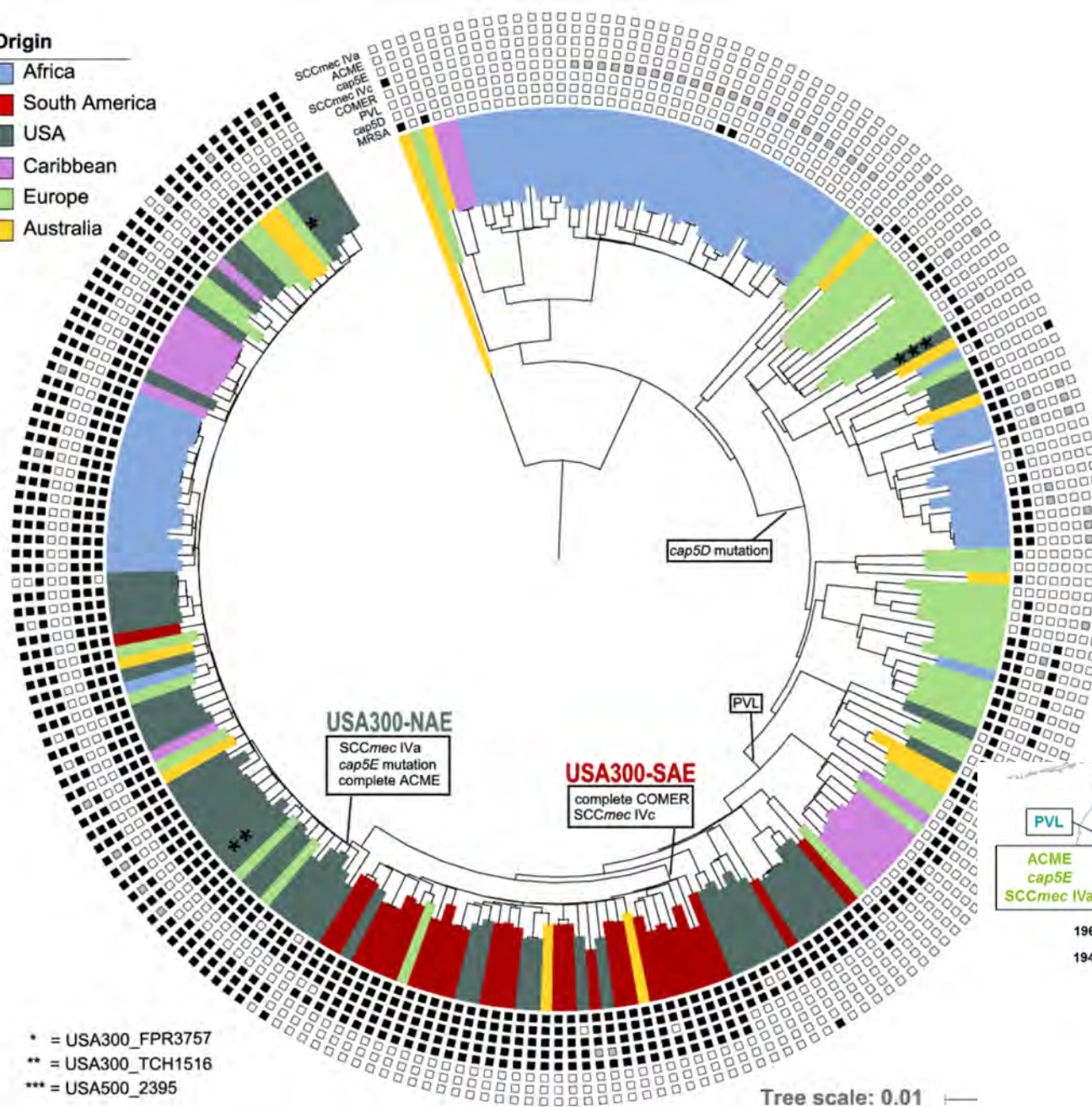
- 224 genomes
- Ancestor of all ST8 in central Europe (mid 19th century)
- Export to America
- Acquisition of PVL
- Acquisition of ACME or COMER
- Worldwide dissemination
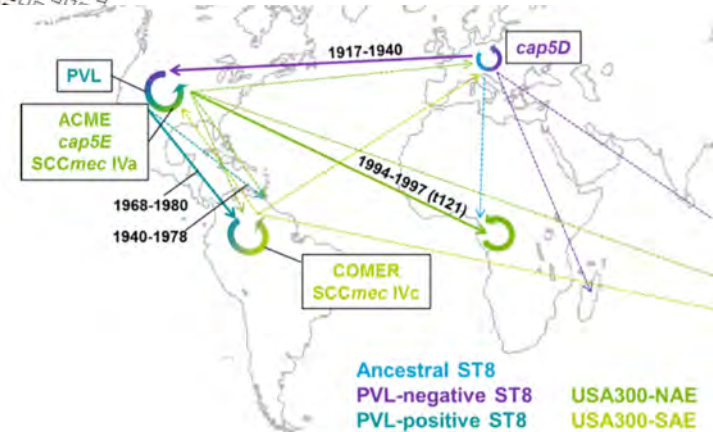
Fig. 1. Maximum-likelihood phylogeny of 224 ST8 *S. aureus* isolates based on 12,403 core genome single nucleotide polymorphisms. The colour indicates the region of origin of each sample. Phylogenetic positions of the included NCBI RefSeq genomes (two USA300 and one USA500) are highlighted with asterisks. Information about the complete presence (black squares), partial presence (grey squares), or absence (white squares) of USA300-specific genetic features is given for each sample. Major genetic introduction events are indicated on the respective phylogenetic branches. Scale bar indicates substitution per site.

Strauß L et al PNAS 2017

Demography ?

World: Total Population

Source: United Nations, Department of Economic and Social Affairs, Population Division (2017).
World Population Prospects: The 2017 Revision. http://esa.un.org/unpd/wpp/
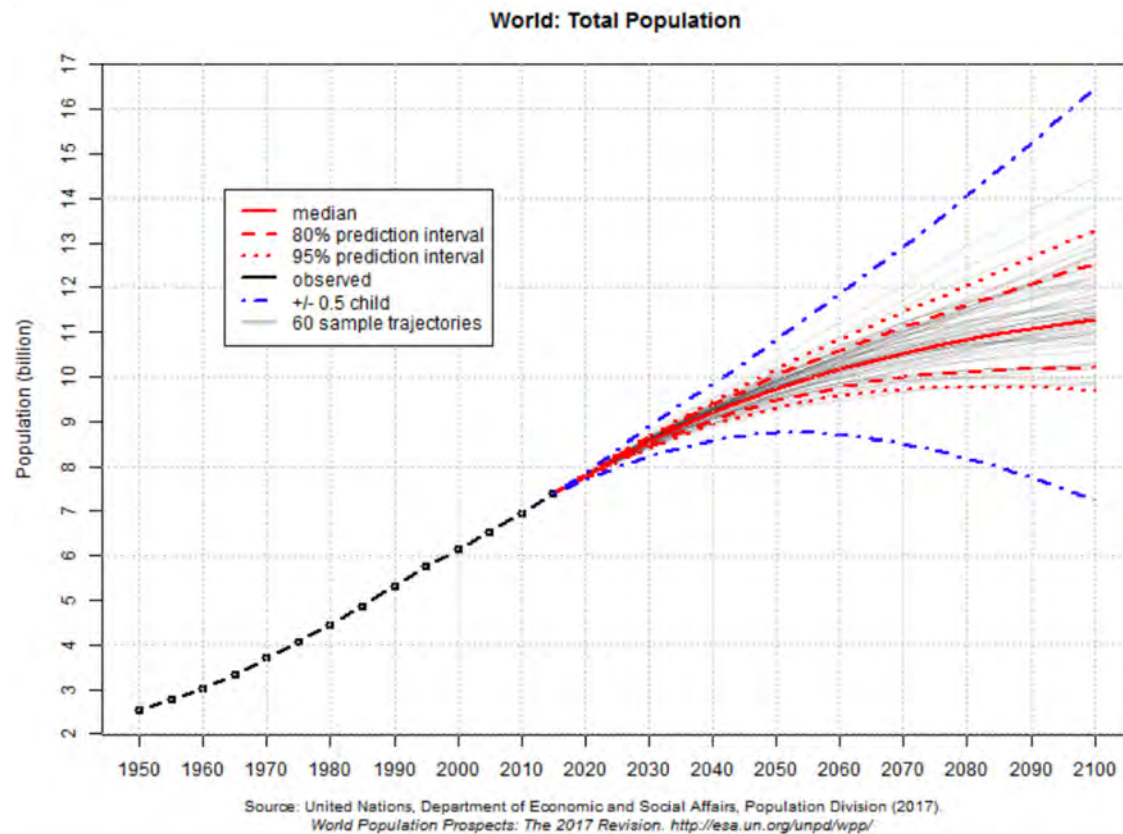
USA300 demography

- 497 genomes, Mostly NA variant from USA and Europe
- Sharp increase upon acquisition of SCCmec & ACME
- Second step of expansion upon FQ resistance
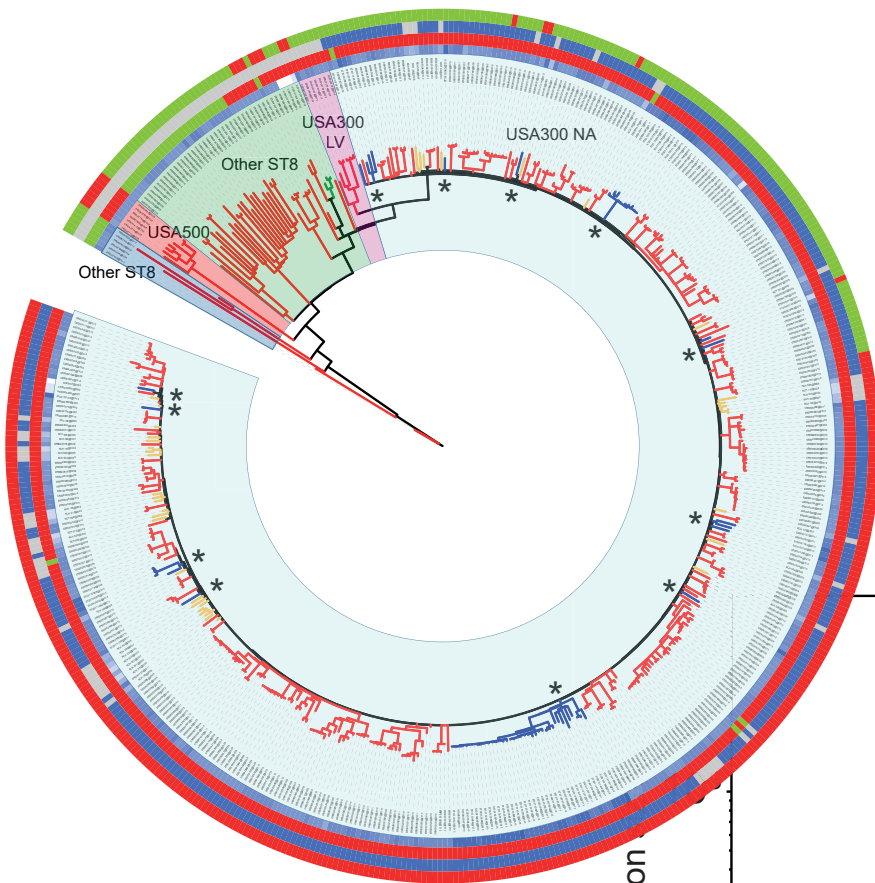
From outer to inner circle
First circle : Fq resistance mutation

- 🟩 Susceptible
- 🟥 Resistant

Second circle : ACME

- ⬜ Absence
- 🟦 Presence

Third circle : Methicillin resistance

- 🟩 Susceptible
- 🟥 Resistant

Fourth circle : Year of isolation in blue gradients
Darkest blue (2014) Lightest blue (2000)

∗ Putative intercontinental transmission event
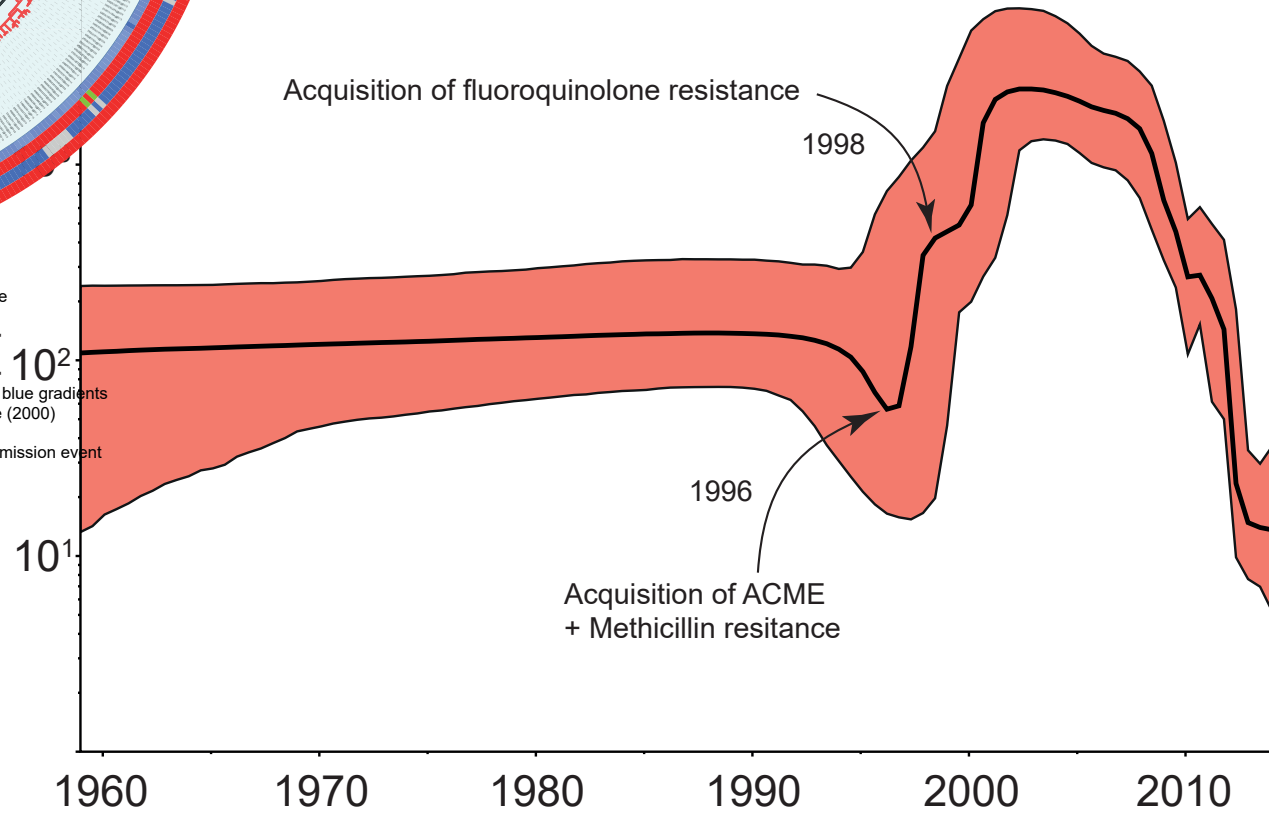
Ph Glaser et al, Mbio 2016

Population dynamic ?

- Which factors govern the expansion of ST80 and USA300?

- What is the link between the identified genetic events and population variation?
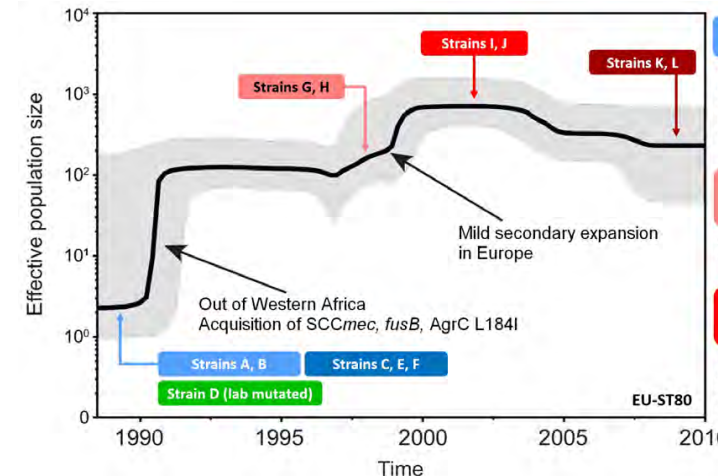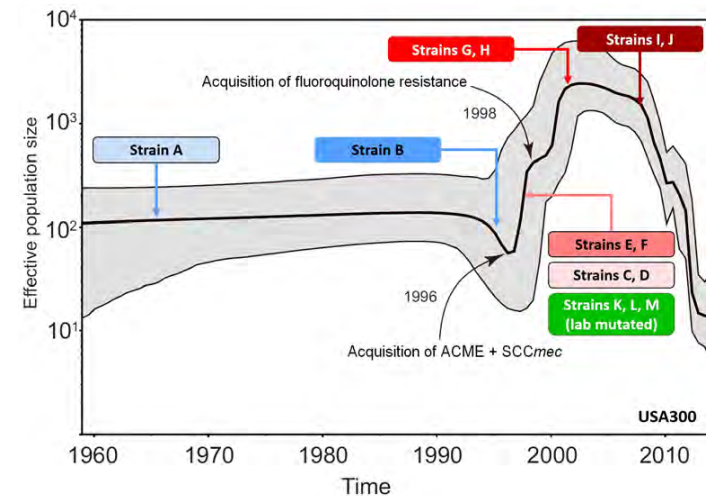
Population dynamic ?

- Which factors govern the expansion of ST80 and USA300?

- What is the link between the identified genetic events and population variation?

Claude-Alexandre Gustave
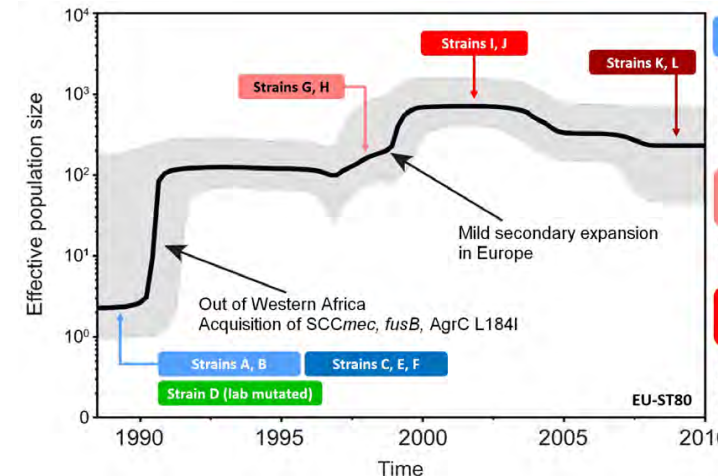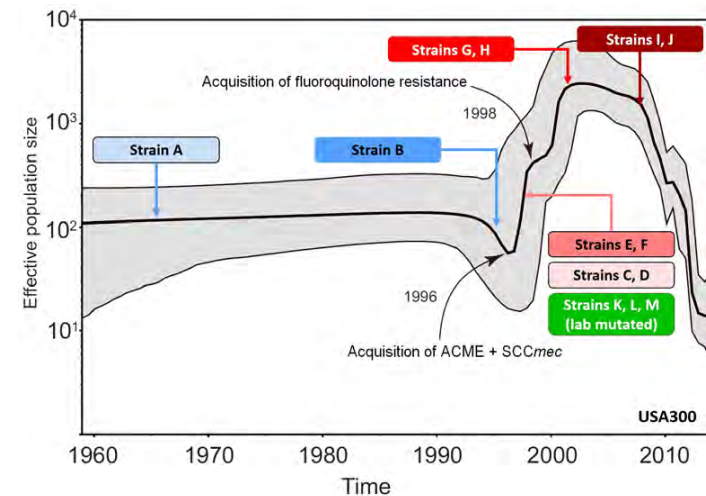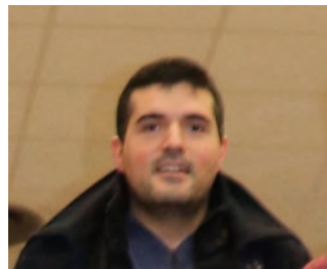
Expansion and virulence

- Do the expanding phases correlate with increased expression of virulence factors?

- -> assessment
  - By qRT-PCR
  - Hla, RNAIII, PSM alpha…

Li et al. J INFECT DIS 2010
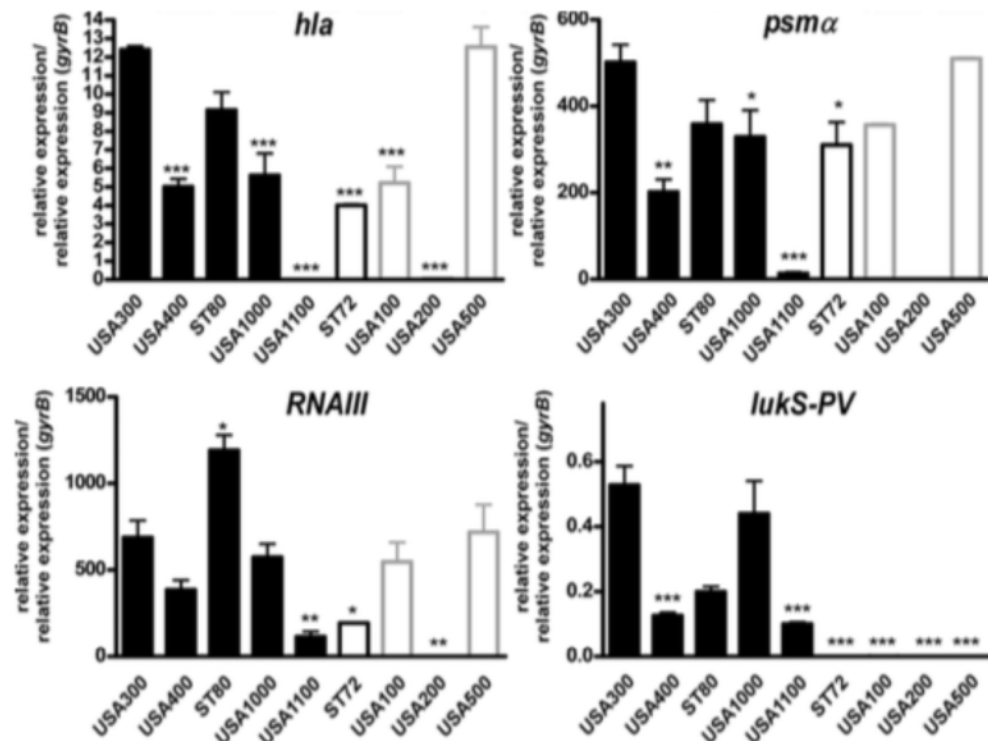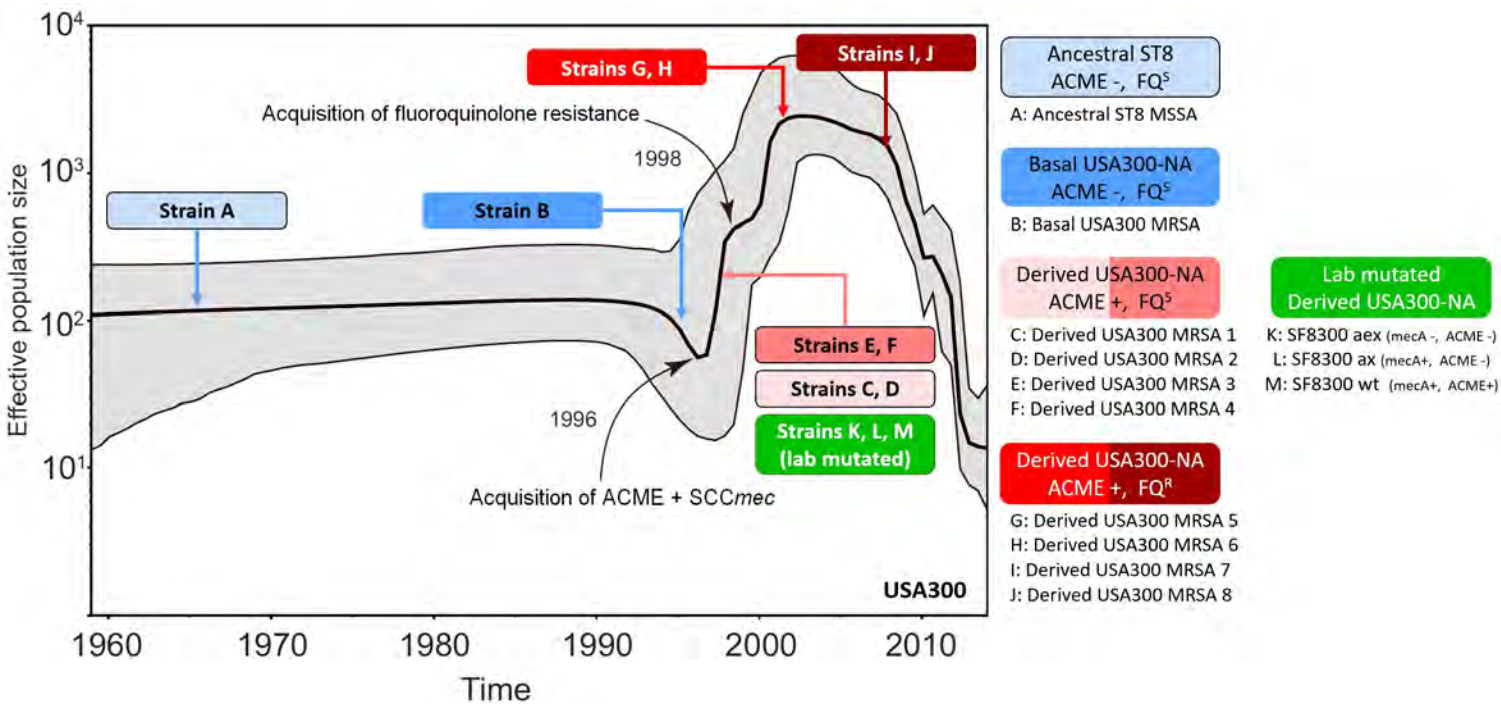
Strain selection

USA300-Virulence factor expression
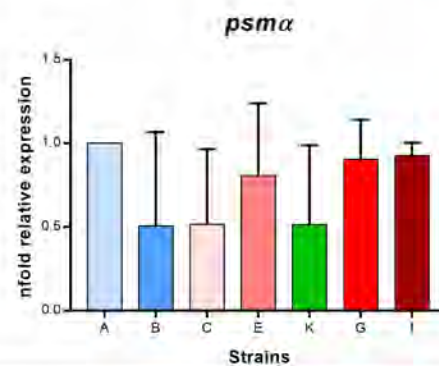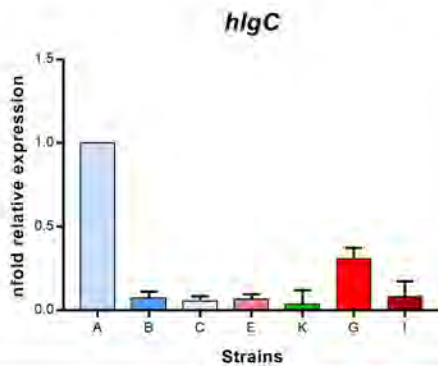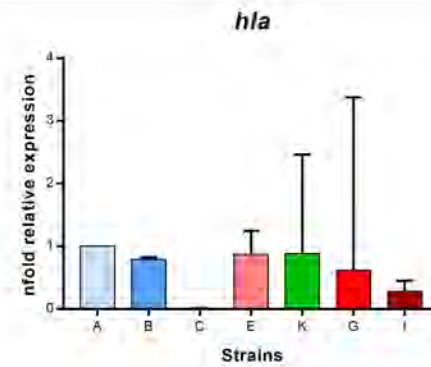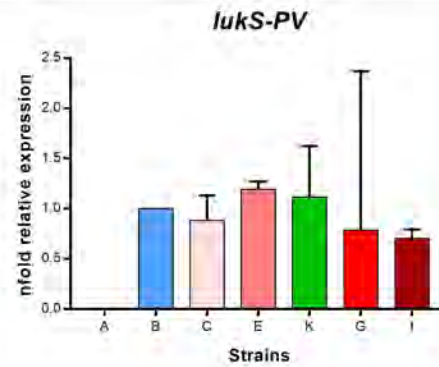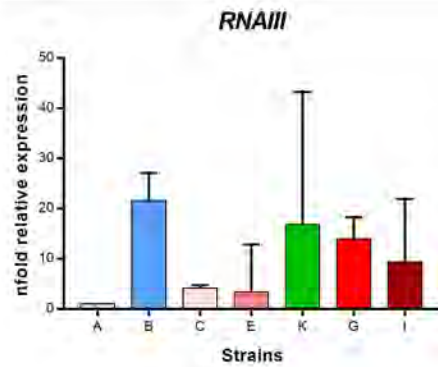
- One outlier with no Hla
- All variations < 2-fold level
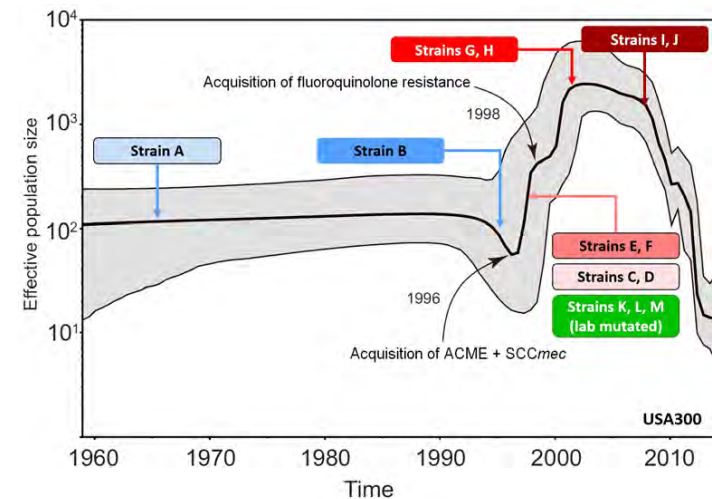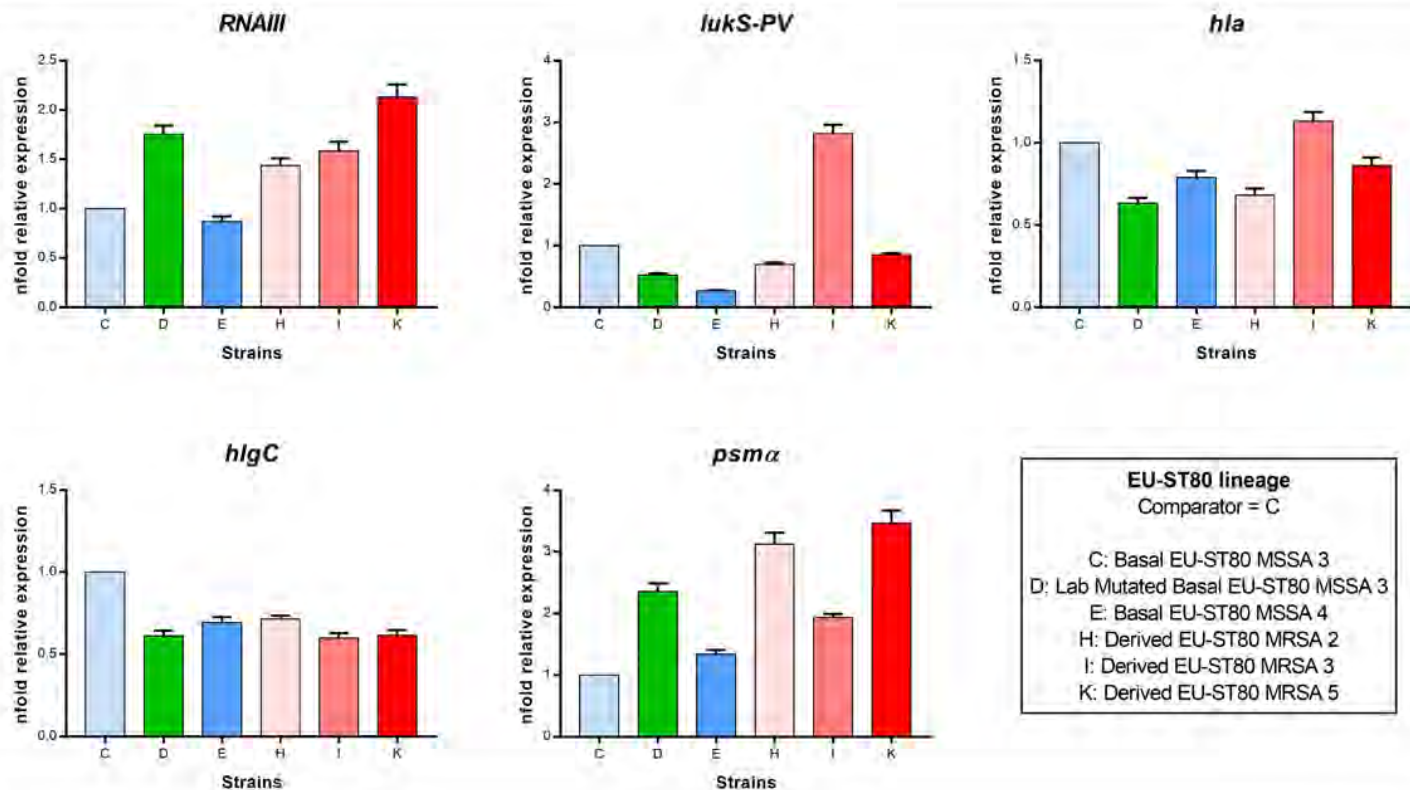
EU-ST80-Virulence factor expression

- RNAIII, reaching a 2.1-fold increase for one strain
- *luk*SF-PV increasing by a factor of 2.8-fold in one strain
- *psmα* increasing by 3 - 3.5-fold in 2 strains
- AgrC mutation had only a 2.2-fold increase in *psmα* expression
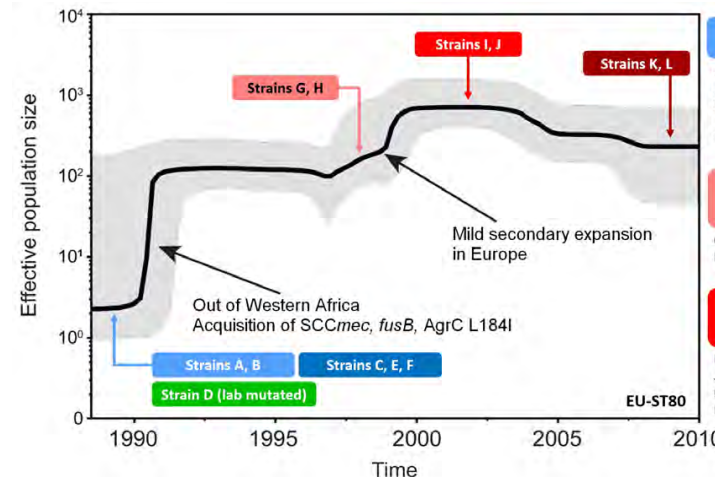
Expansion and virulence

- only slight variation of expression along time
- -> at the population level may have enhanced the success of the lineages by increasing cutaneous infection rate and thus human-to-human transmission by skin contact
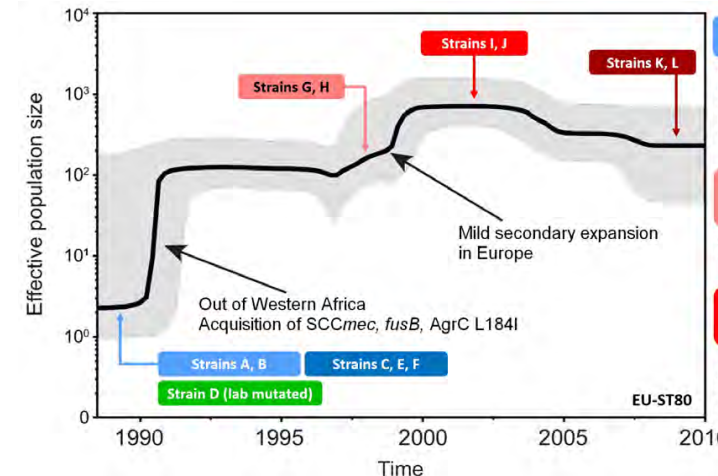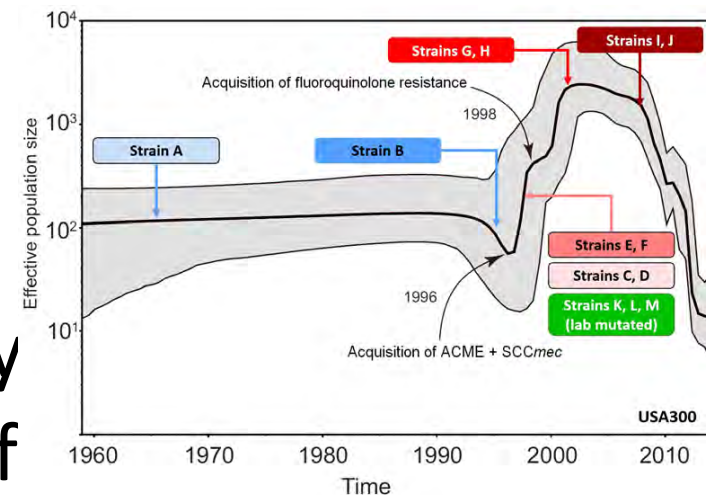
Expansion and fitness

- Do the expanding phases correlate with increased fitness ?

- -> assessment of
  - doubling time
  - competitive fitness in vitro

Doubling time

ST8/USA300 lineage

Oxacilline R

Strain ID	SCCmec	ACME	Antibiotic resistance	Clade
A	Neg	Neg	P, F	Ancestral ST8
B	POS	Neg	P, Oxa	Basal USA300
C	POS	POS	P, Oxa	Derived USA300
D	POS	POS	P, Oxa	Derived USA300
E	POS	POS	P, Oxa, K, E	Derived USA300
F	POS	POS	P, Oxa, K, E	Derived USA300
G	POS	POS	P, Oxa, K, E, O	Derived USA300
H	POS	POS	P, Oxa, K, E, O	Derived USA300
I	POS	POS	P, Oxa, K, E, O, T	Derived USA300
J	POS	POS	P, Oxa, K, E, O, T	Derived USA300
K	Neg	Neg	P, E, C, T, Cip, Mup	Ref. strain mutant
L	POS	Neg	P, Oxa, E, C, T, Cip, Mup	Ref. strain mutant
M	POS	POS	P, Oxa, E, C, T, Cip, Mup	USA300 ref. strain

-> ATB resistance Oxacilline increased doubling time

Doubling time

ST8/USA300 lineage

Strain ID	SCCmec	ACME	Antibiotic resistance	Clade
A	Neg	Neg	P, F	Ancestral ST8
B	POS	Neg	P, Oxa	Basal USA300
C	POS	POS	P, Oxa	Derived USA300
D	POS	POS	P, Oxa	Derived USA300
E	POS	POS	P, Oxa, K, E	Derived USA300
F	POS	POS	P, Oxa, K, E	Derived USA300
G	POS	POS	P, Oxa, K, E, O	Derived USA300
H	POS	POS	P, Oxa, K, E, O	Derived USA300
I	POS	POS	P, Oxa, K, E, O, T	Derived USA300
J	POS	POS	P, Oxa, K, E, O, T	Derived USA300
K	Neg	Neg	P, E, C, T, Cip, Mup	Ref. strain mutant
L	POS	Neg	P, Oxa, E, C, T, Cip , Mup	Ref. strain mutant
M	POS	POS	P, Oxa, E, C, T, Cip, Mup	USA300 ref. strain

-> ATB resistance Oxacilline increased doubling time

-> ACME is associated with reduced doubling time

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Doubling time

ST8/USA300 lineage

Kana, Ery R

Strain ID	SCCmec	ACME	Antibiotic resistance	Clade
A	Neg	Neg	P, F	Ancestral ST8
B	POS	Neg	P, Oxa	Basal USA300
C	POS	POS	P, Oxa	Derived USA300
D	POS	POS	P, Oxa	Derived USA300
E	POS	POS	P, Oxa, K, E	Derived USA300
F	POS	POS	P, Oxa, K, E	Derived USA300
G	POS	POS	P, Oxa, K, E, O	Derived USA300
H	POS	POS	P, Oxa, K, E, O	Derived USA300
I	POS	POS	P, Oxa, K, E, O, T	Derived USA300
J	POS	POS	P, Oxa, K, E, O, T	Derived USA300
K	Neg	Neg	P, E, C, T, Cip, Mup	Ref. strain mutant
L	POS	Neg	P, Oxa, E, C, T, Cip, Mup	Ref. strain mutant
M	POS	POS	P, Oxa, E, C, T, Cip, Mup	USA300 ref. strain

-> ATB resistances (Oxacilline, aminoglycosides, macrolides, fluroquinolones, tetraccylcine) increased doubling time

-> ACME is associated with reduced doubling time

Doubling time

ST8/USA300 lineage

Cipro R

Strain ID	SCCmec	ACME	Antibiotic resistance	Clade
A	Neg	Neg	P, F	Ancestral ST8
B	POS	Neg	P, Oxa	Basal USA300
C	POS	POS	P, Oxa	Derived USA300
D	POS	POS	P, Oxa	Derived USA300
E	POS	POS	P, Oxa, K, E	Derived USA300
F	POS	POS	P, Oxa, K, E	Derived USA300
G	POS	POS	P, Oxa, K, E, O	Derived USA300
H	POS	POS	P, Oxa, K, E, O	Derived USA300
I	POS	POS	P, Oxa, K, E, O, T	Derived USA300
J	POS	POS	P, Oxa, K, E, O, T	Derived USA300
K	Neg	Neg	P, E, C, T, Cip, Mup	Ref. strain mutant
L	POS	Neg	P, Oxa, E, C, T, Cip , Mup	Ref. strain mutant
M	POS	POS	P, Oxa, E, C, T, Cip, Mup	USA300 ref. strain

-> ATB resistances (Oxacilline, aminoglycosides, macrolides, fluroquinolones, tetraccylcine) increased doubling time

-> ACME is associated with reduced doubling time

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Doubling time

ST8/USA3

SCCmec - ACME-

SCCmec + ACME-

SCCmec + ACME +

Strain ID	SCCmec	ACME	Antibiotic resistance	Clade
A	Neg	Neg	P, F	Ancestral ST8
	POS	Neg	P, Oxa	Basal USA300
	POS	POS	P, Oxa	Derived USA300
	POS	POS	P, Oxa	Derived USA300
	POS	POS	P, Oxa, K, E	Derived USA300
F	POS	POS	P, Oxa, K, E	Derived USA300
G	POS	POS	P, Oxa, K, E, O	Derived USA300
H	POS	POS	P, Oxa, K, E, O	Derived USA300
I	POS	POS	P, Oxa, K, E, O, T	Derived USA300
J	POS	POS	P, Oxa, K, E, O, T	Derived USA300
K	Neg	Neg	P, E, C, T, Cip, Mup	Ref. strain mutant
L	POS	Neg	P, Oxa, E, C, T, Cip , Mup	Ref. strain mutant
M	POS	POS	P, Oxa, E, C, T, Cip, Mup	USA300 ref. strain

-> Isogenic strains confirm the opposite effect of ACME and SCC*mec* on doubling time

C.A. Gustave et al, ISME J 2018

Impact of ACME and SCCmec in competitive fitness

Basal USA300 MRSA (SCC*mec* + ACME -)
Ancestral ST8 MSSA (SCC*mec* - ACME -)

no ATB
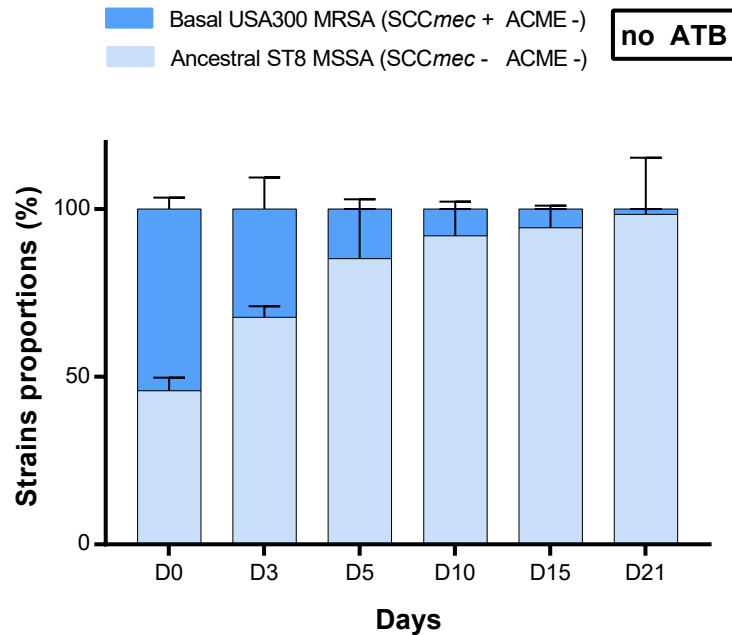
-> the basal SCCmec+ is outcompeted by the ancestral SCCmec-

C.A. Gustave et al, ISME J 2018
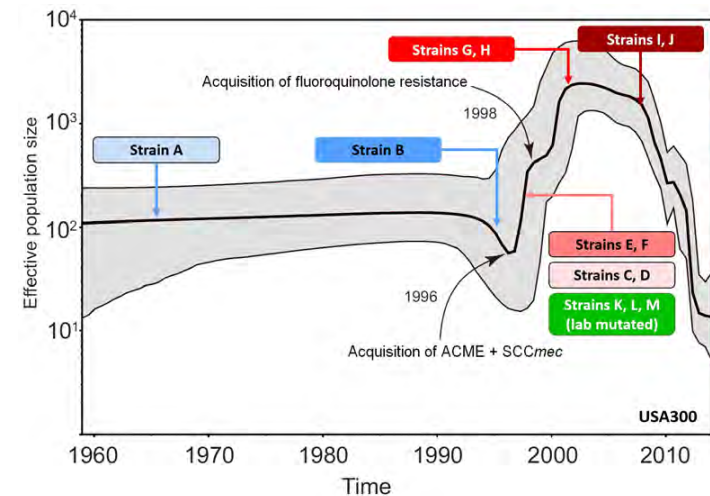
Impact of ACME and SCCmec in competitive fitness
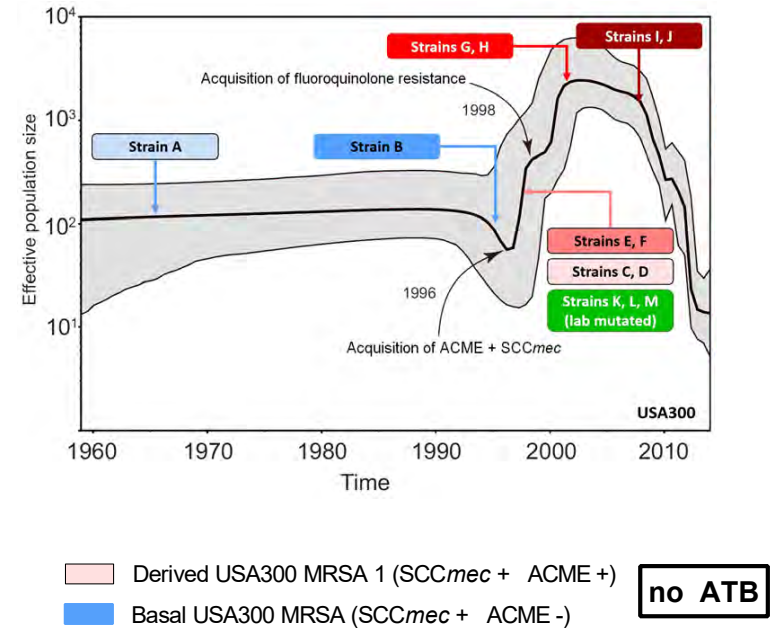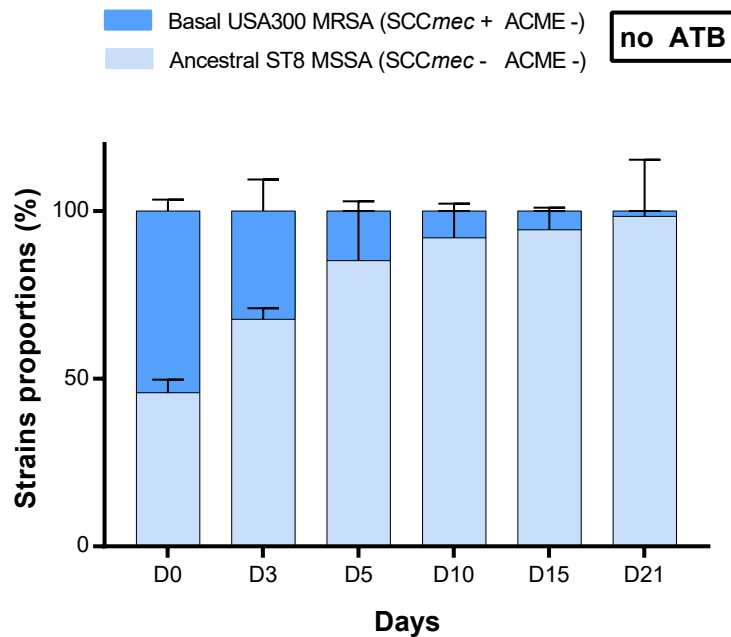
-> the derived ACME+ outcompeted the basal ACME-

C.A. Gustave et al, ISME J 2018

Impact of ACME and SCCmec in competitive fitness

Basal USA300 MRSA (SCC*mec* + ACME -)
Ancestral ST8 MSSA (SCC*mec* - ACME -)   no ATB
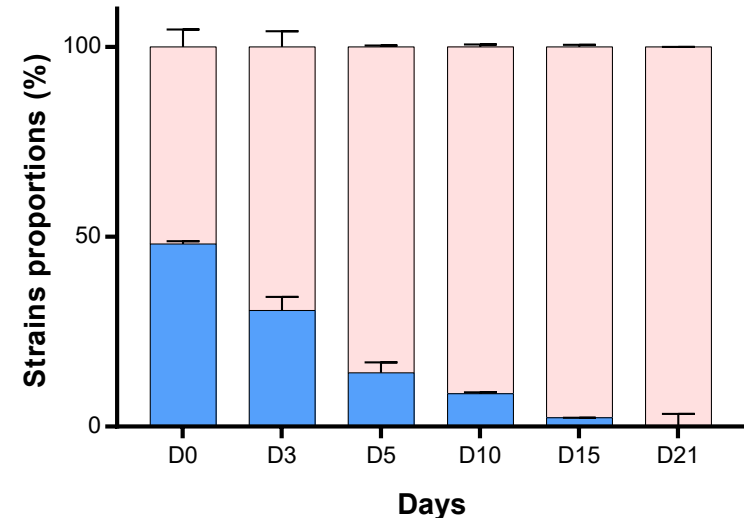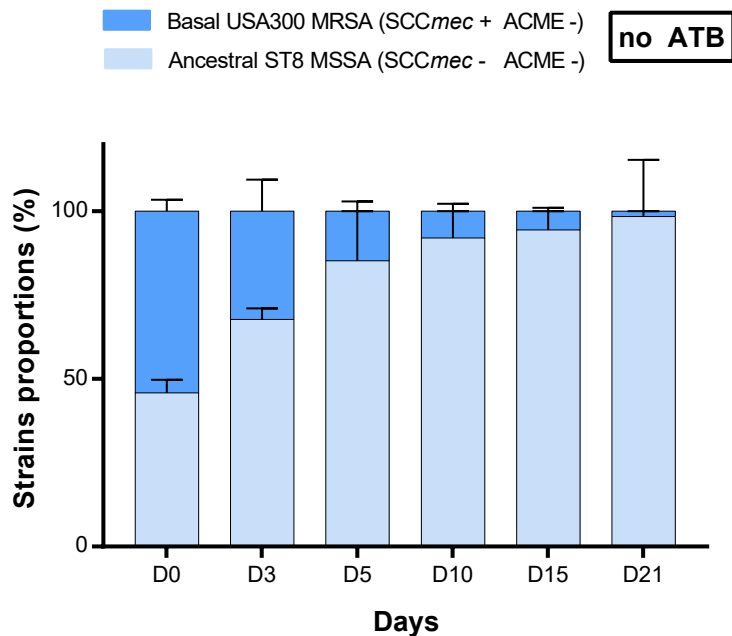
-> ACME is not sufficient to restore the competitive fitness of the ancestral ST8 MSSA

-> ACME + SCCmec do not explain the expansion

a   Derived USA300 MRSA 1 (SCC*mec* + ACME +)   no ATB
Ancestral ST8 MSSA (SCC*mec* - ACME -)

C.A. Gustave et al, ISME J 2018
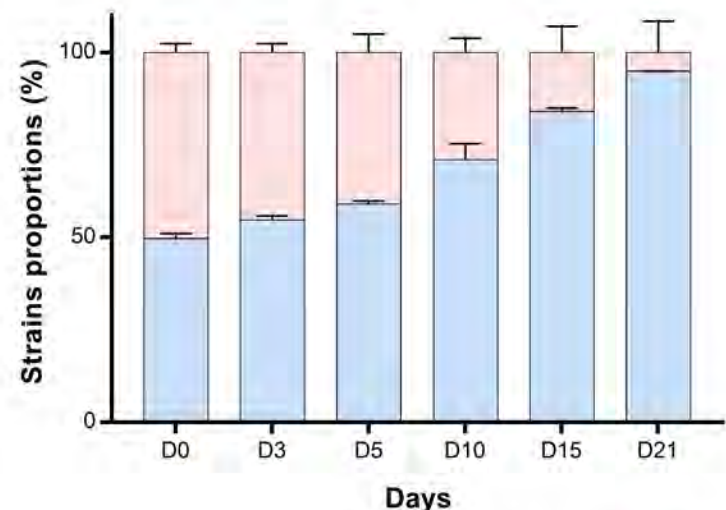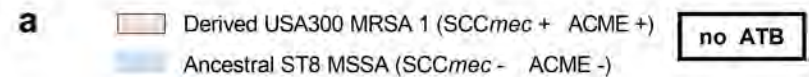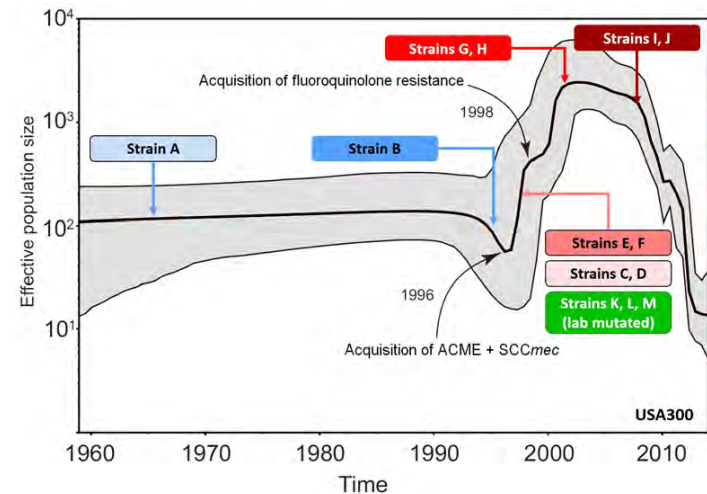
Effect of beta-lactams in competitive fitness
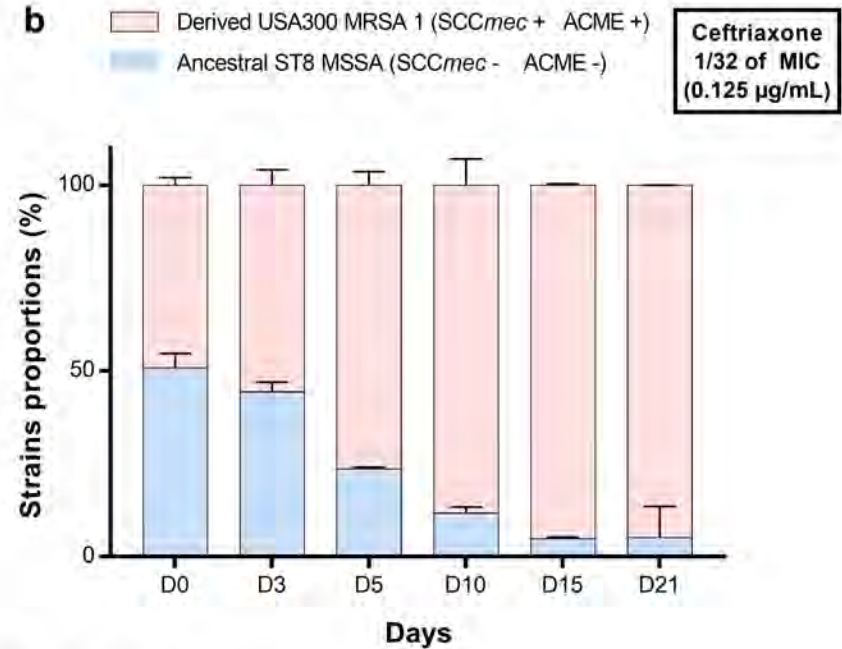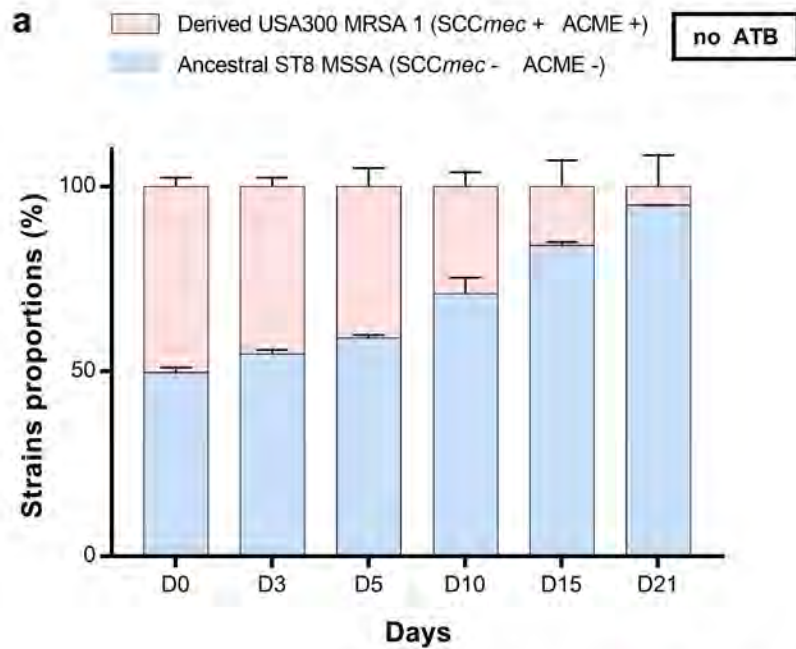
Effect of beta-lactams in competitive fitness
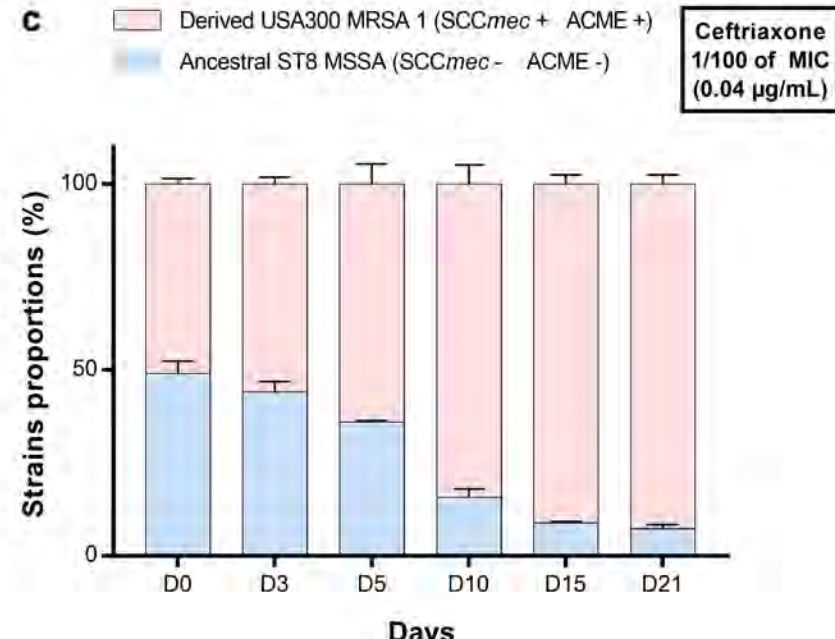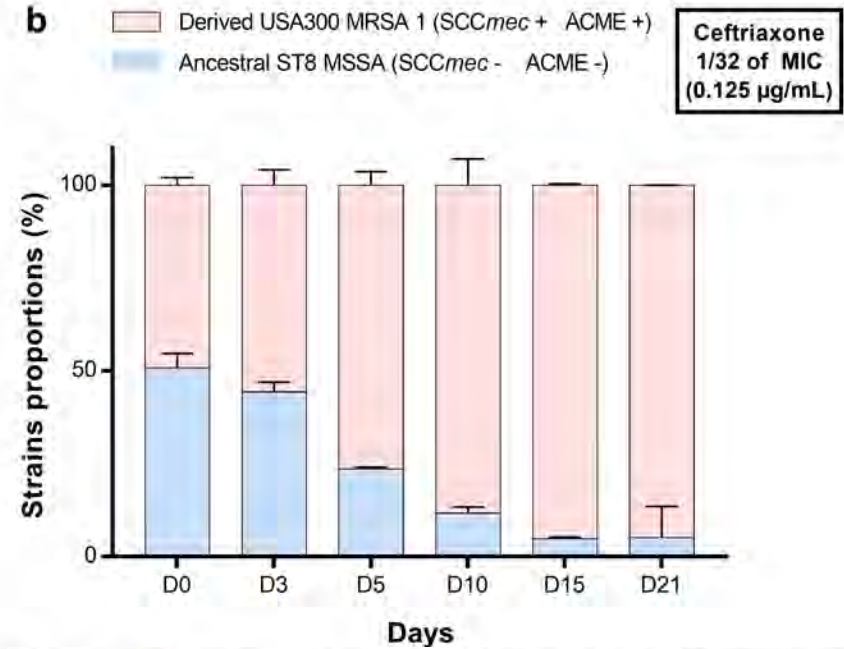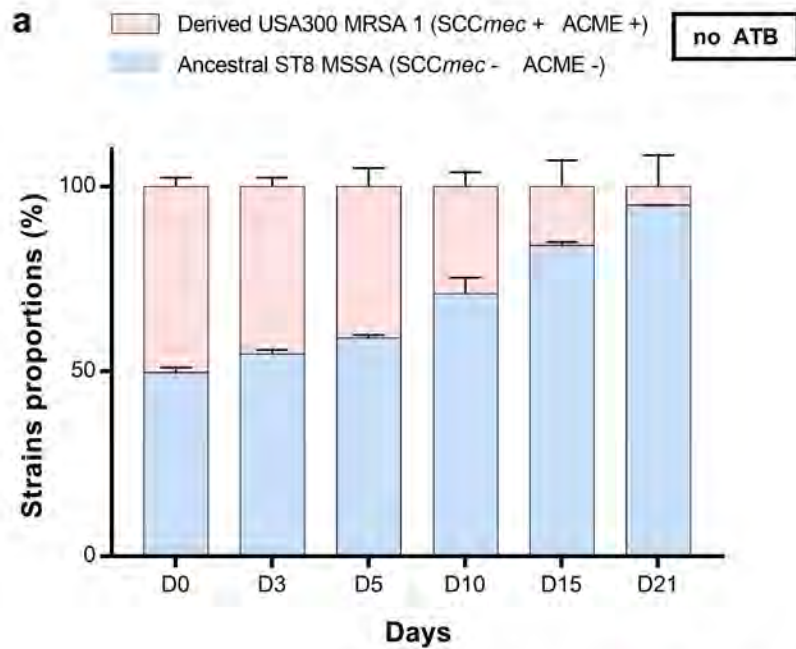
-> AB exposure reverse the fitness cost

C.A. Gustave et al, ISME J 2018

Effect of fluroquinolones in competitive fitness
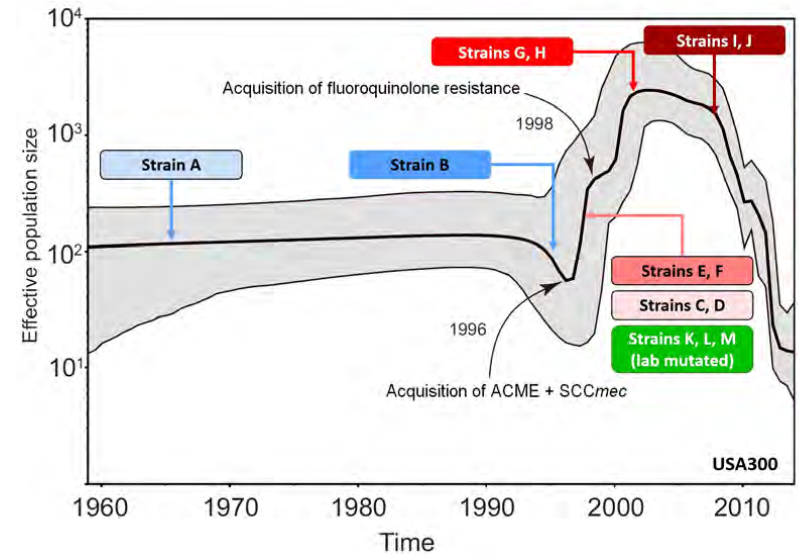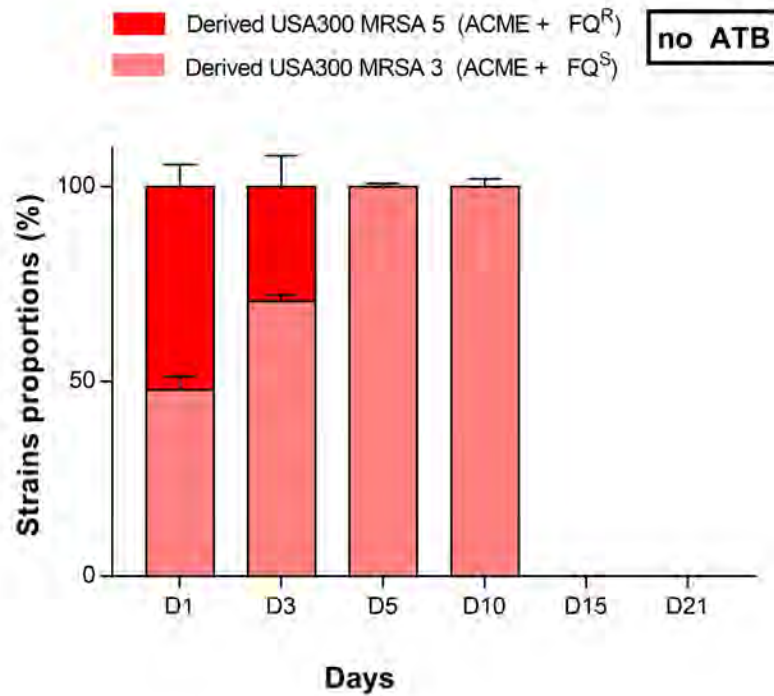
-> FQ resistance impairs the fitness cost

C.A. Gustave et al, bioRxiv 2017

Effect of fluroquinolones in competitive fitness
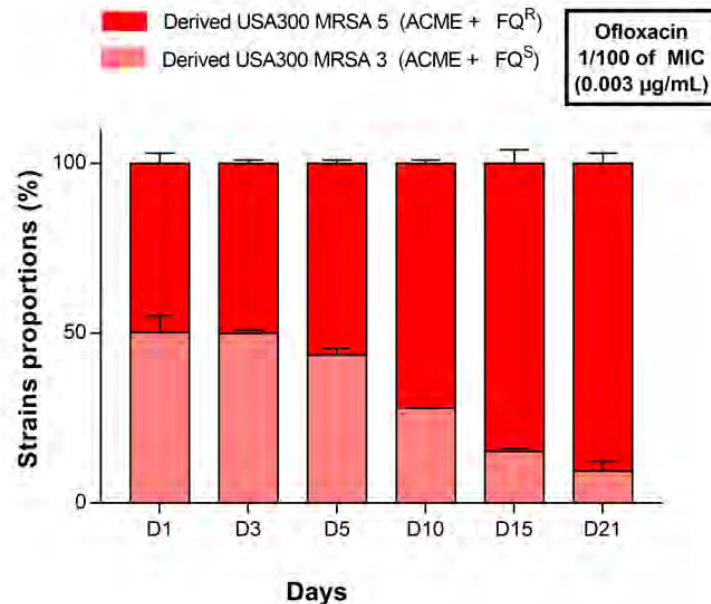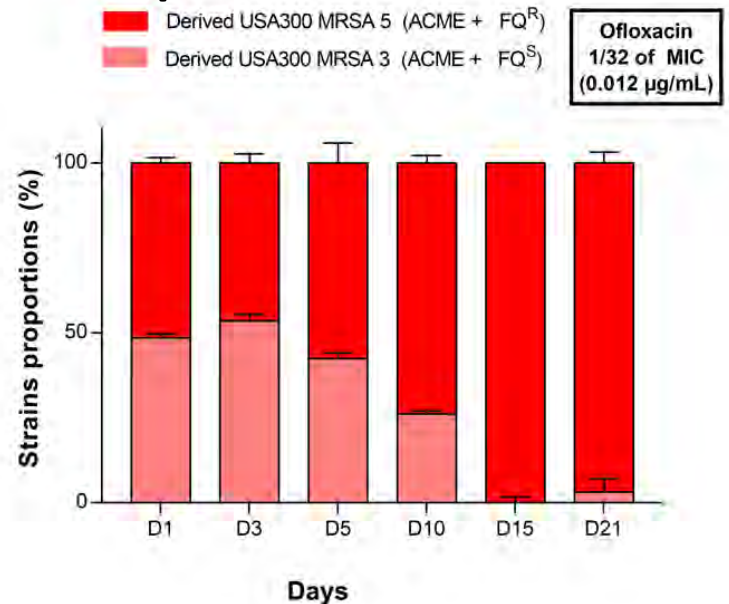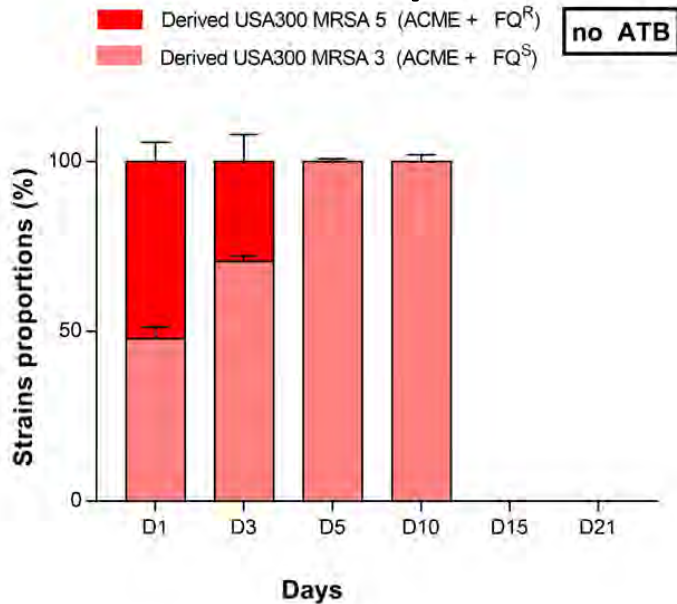
-> FQ exposure reverse the fitness cost

ST80
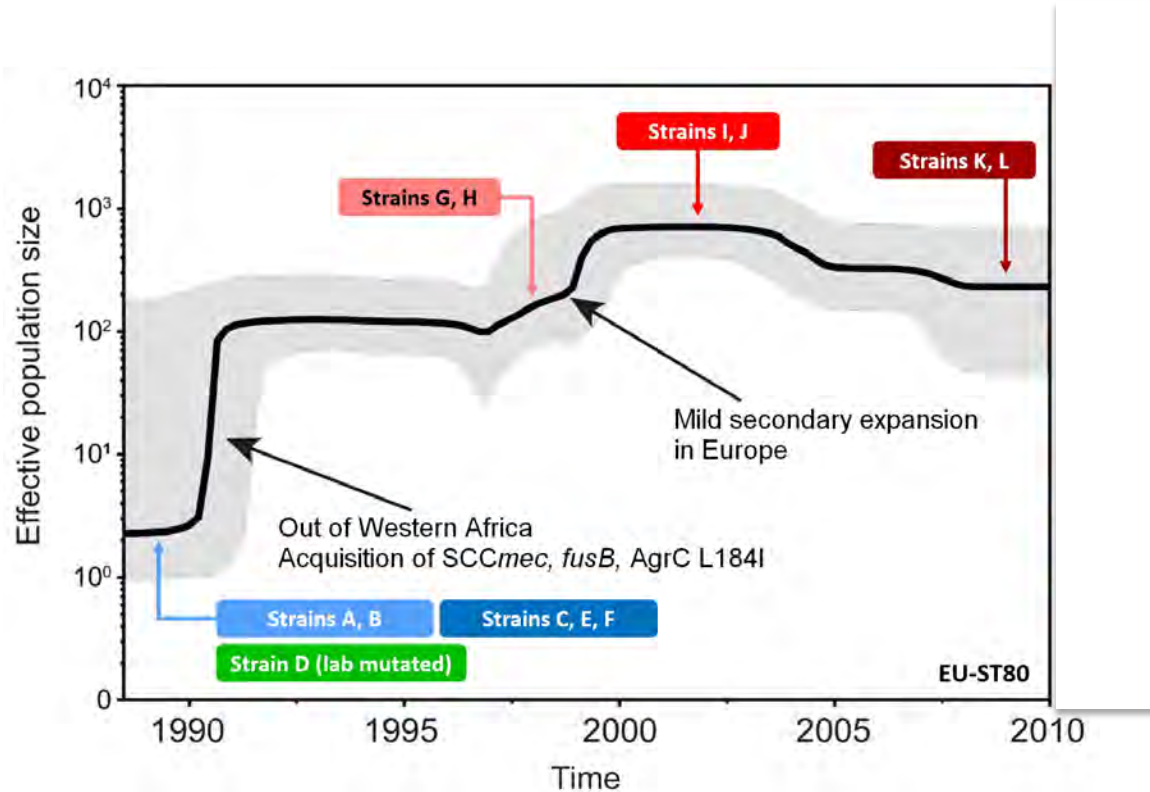
Competitive fitness of ST80 strains

- Impact of SCCmec, fusB and AgrC L184I
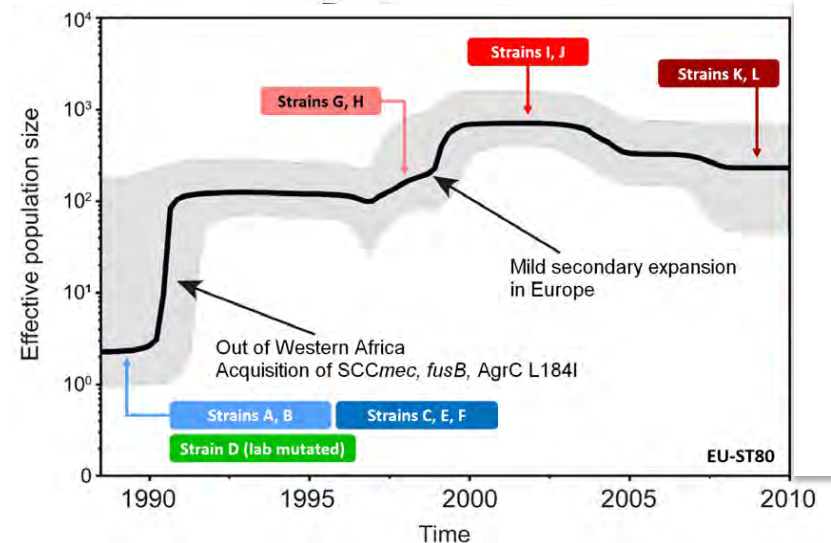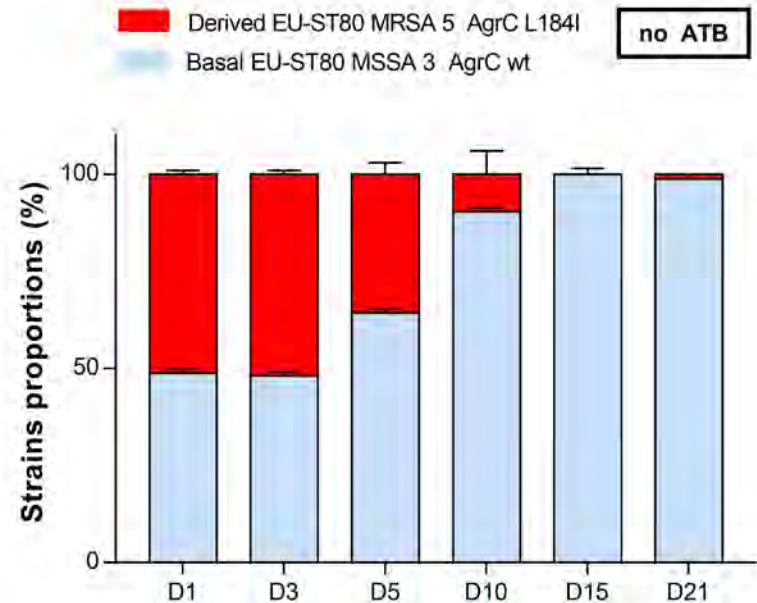
Competitive fitness of ST80 strains

- sub-MIC beta-lactams or fusidic acid reverse the fitness cost

Legend: Derived EU-ST80 MRSA 5 AgrC L184I (red); Basal EU-ST80 MSSA 3 AgrC wt (light blue). no ATB. Strains proportions (%) vs Days (D1, D3, D5, D10, D15, D21).

Legend: Derived EU-ST80 MRSA 5 AgrC L184I (red); Basal EU-ST80 MSSA 3 AgrC wt (light blue). Ceftriaxone 1/100 of MIC 0,03 µg/mL. Strains proportions (%) vs Days.

Legend: Derived EU-ST80 MRSA 5 AgrC L184I (red); Basal EU-ST80 MSSA 3 AgrC wt (light blue). Fusidic acid 1/100 of MIC 0,0009 µg/mL. Strains proportions (%) vs Days.

Link with emergence of CA-MRSA outside hospital setting ?

Review

Fluoroquinolone antibiotics: An emerging class of environmental micropollutants

Xander Van Doorslaer, Jo Dewulf, Herman Van Langenhove, Kristof Demeestere *

Research Group EnVOC, Department of Sustainable Organic Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

CrossMark

CLEAN
Soil Air Water

479

Ritu Gothwal
Thhatikkonda Shashidhar

Department of Civil Engineering,
Indian Institute of Technology
Hyderabad, Ordnance Factory Estate,
Yeddumailaram, Andhra Pradesh,
India

Review

Antibiotic Pollution in the Environment: A Review

Antibiotics have been extensively and effectively used in human and veterinary medicines. Their benefits have been recognized in agriculture, aquaculture, bee-keeping, and livestock as growth promoters. This paper collects information from

PROCEEDINGS
—OF—
THE ROYAL
SOCIETY

Review

The role of natural environments in the evolution of resistance traits in pathogenic bacteria

Jose L. Martinez*

Departamento de Biotecnología Microbiana, Centro Nacional de Biotecnología,
Consejo Superior de Investigaciones Científicas, Darwin 3, Cantoblanco, 28049 Madrid, Spain
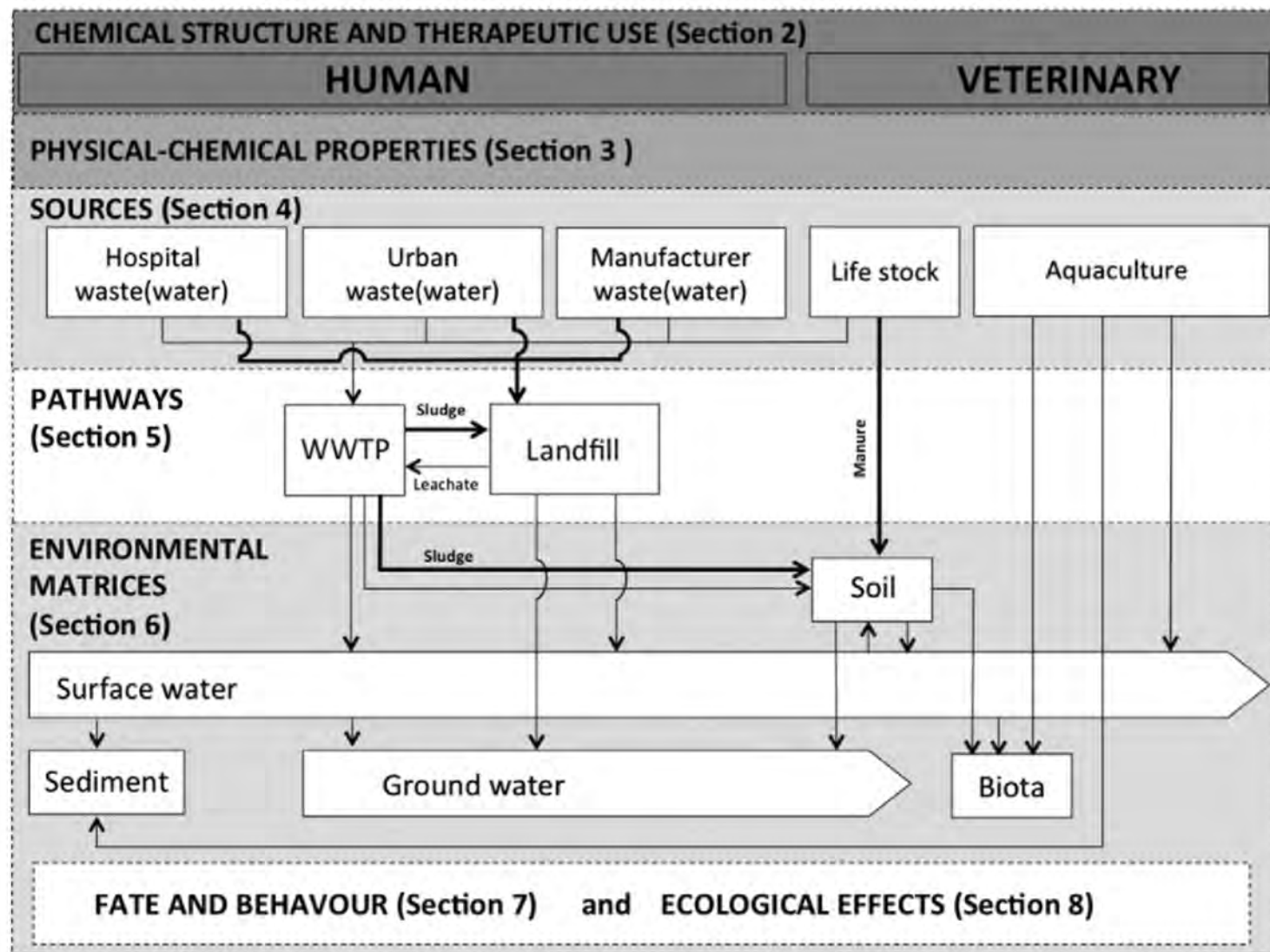
-> e.g. FQ concentration can be as high as 240 ug/L in HWW and 5.7 ug/L in surface water
-> Favour AB entry AND accumulation into biota: vegetables, crops, aquatic plants, and animals

Gothwal et al. CSAWAC 43 (2015)
Van Doorslaer et al., Sci Total Environ 2014

A trivial scenario

- ACME acquisition (USA300) and slight increased expression of virulence factors may have contributed to the success via enhanced inter-human transmission
- The most striking event is the acquisition of resistance genes
  - \> biological cost of antibiotic resistance genes is totally reversed in the presence of trace amount of antibiotics
  - \> Inappropriate antibiotic use / antibiotic in the environment may have driven the expansion
- A novel link between effective population size and a selective advantage conferred by antibiotic resistance

B
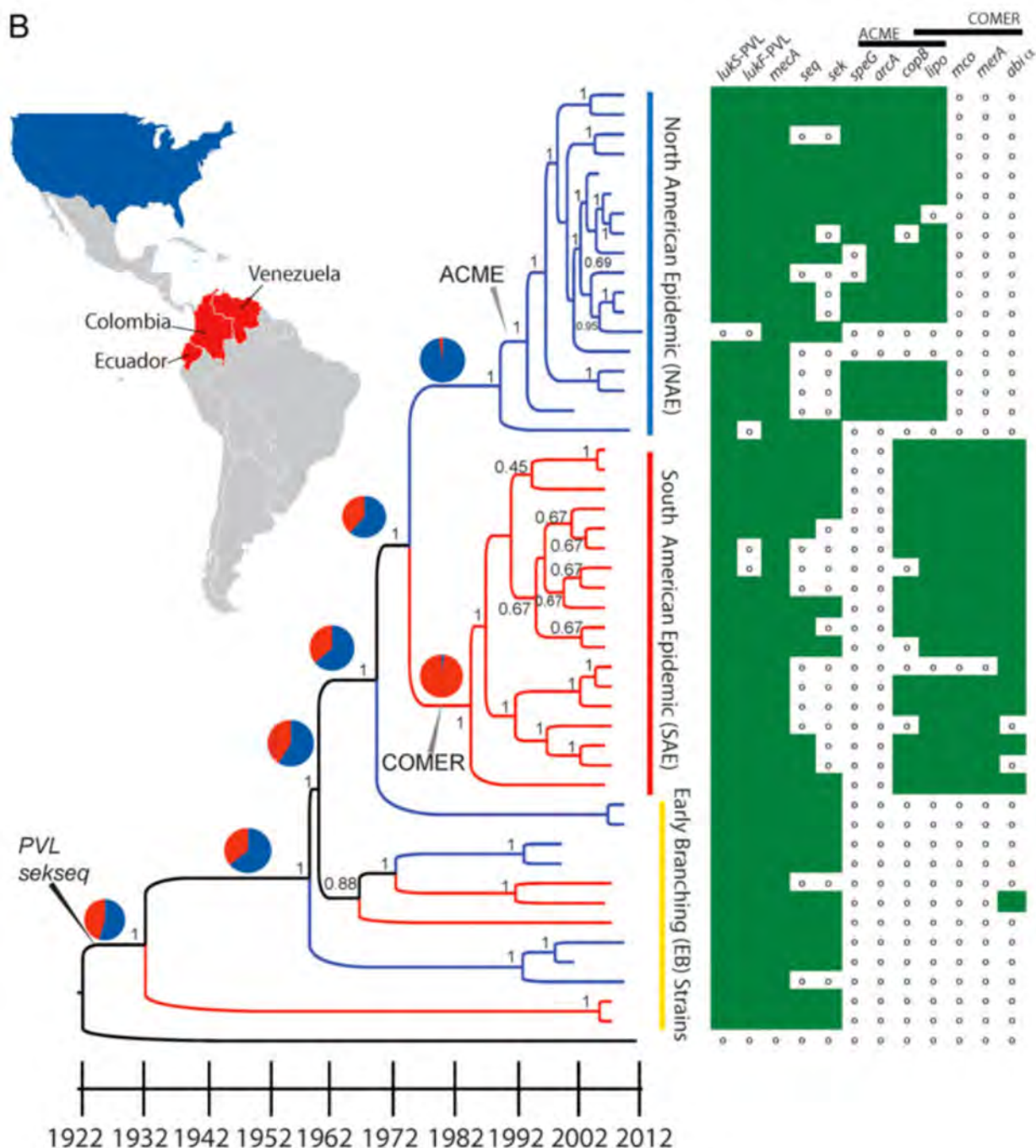
2 distinct clades (South America and North America) that segregate by geographical region

ACME in NA clade
COMER in SA (or LV) clade

SA and NA clades diverged before the emergence of the USA300 epidemic in NA

Planet et al. J Infect Dis 2015

USA300 LV in Latin America

- USA300-LV is the dominant CA-MRSA clone in Colombia, Ecuador, Peru, Trinitad and Venezuela

USA300-0114
USA300-P
USA300-LV

Reyes, Clin Infect Dis 2009
Akvarez, Am J Infect Control 2010
Garcia, J Infect 2011
Monecke, Eur J Clin Microbiol Infect Dis 2011
Sola, Plos One 2012
Nimmo, Clin Microbiol Infect 2012
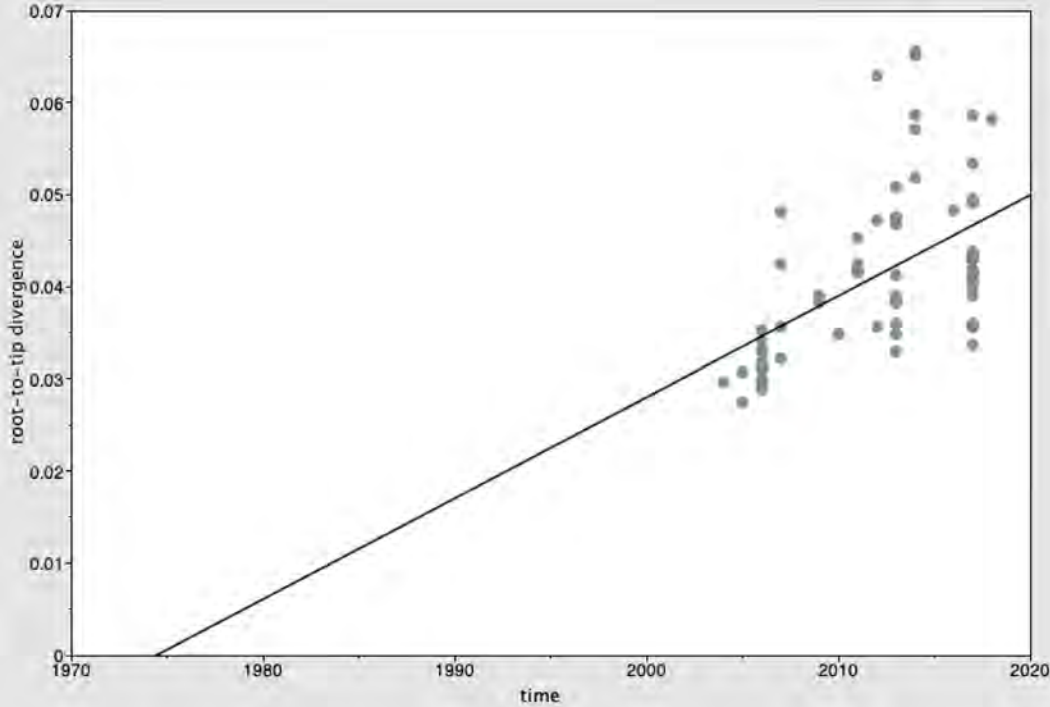
Plotting genetic distance against sampling time
-> Measurably Evolving Population
-> TRMCA 1978
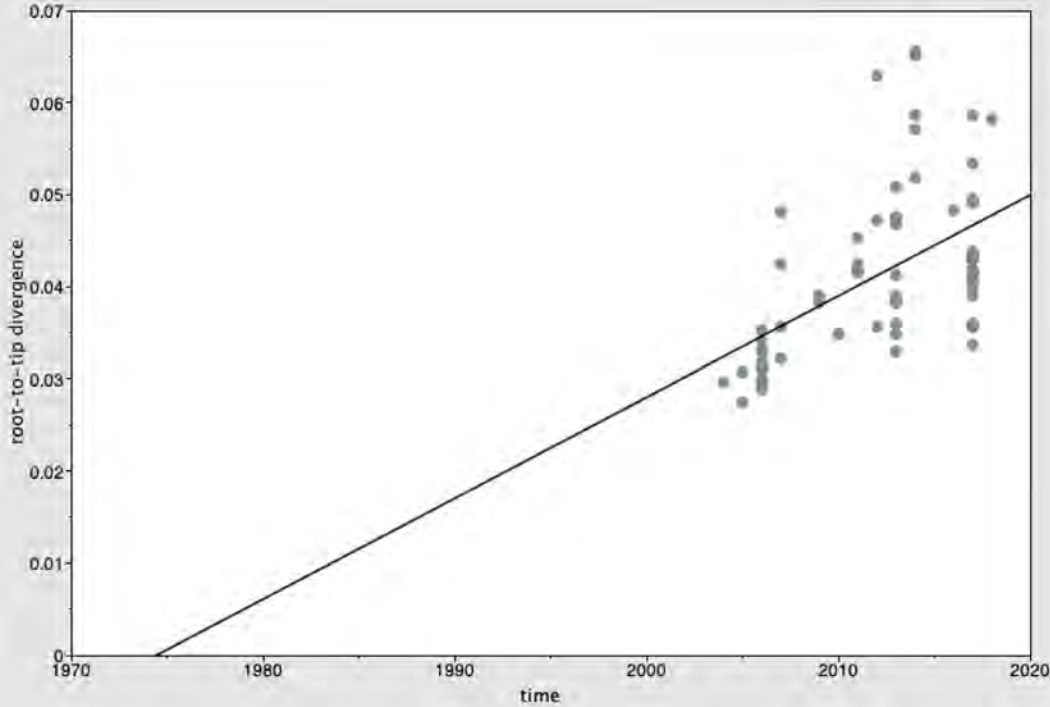
Plotting genetic distance against sampling time
-> Mesurably Evolving Population
-> TRMCA 1978
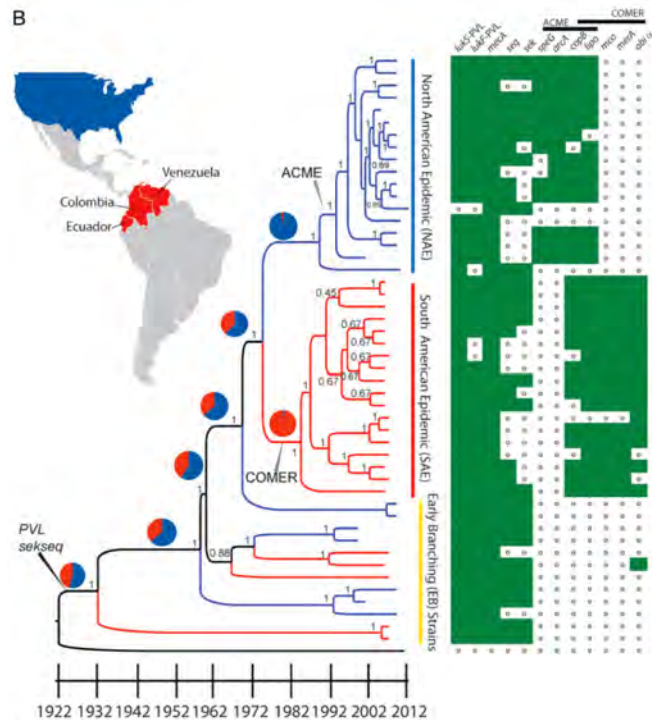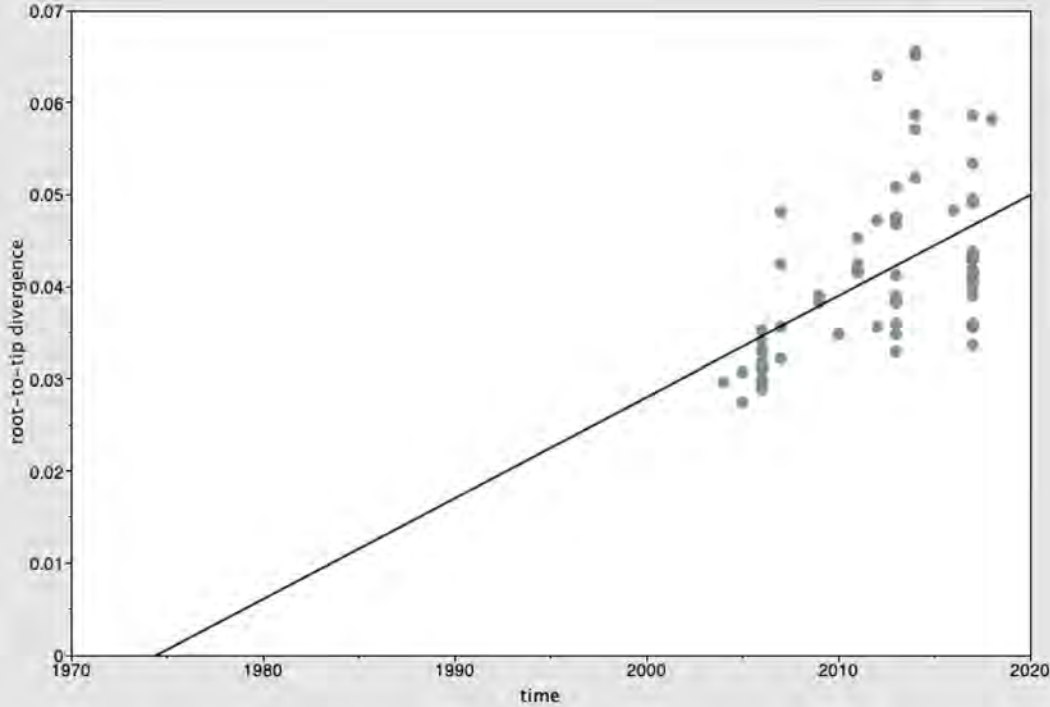
USA300-LV bayesian demography reveals limited expansion

TRMCA 1978
Mesurable Evolving Population
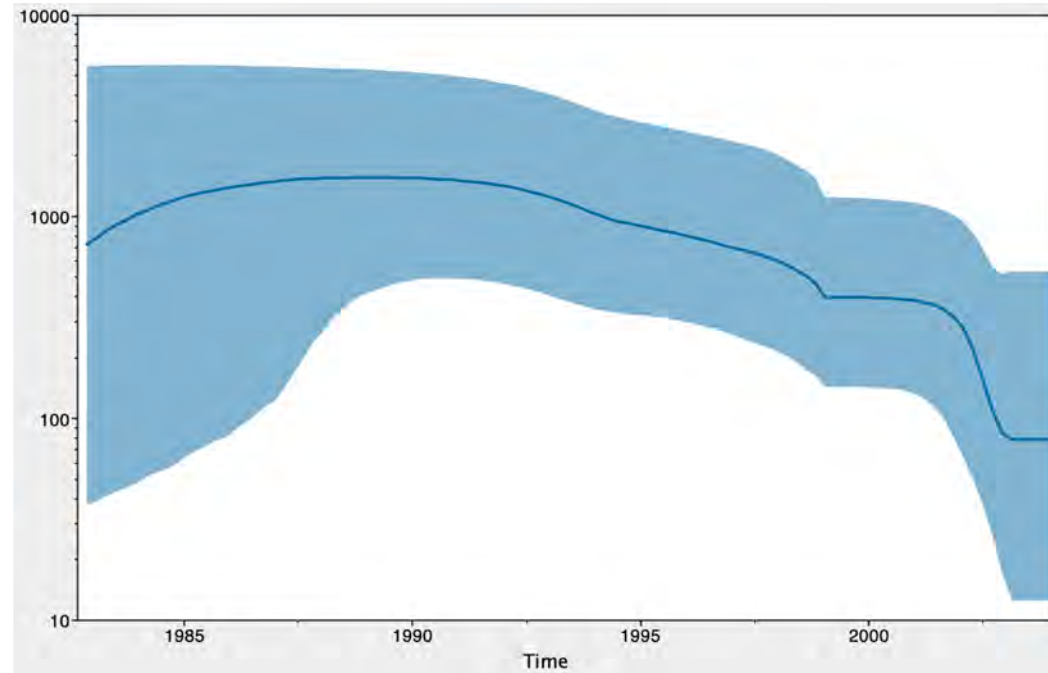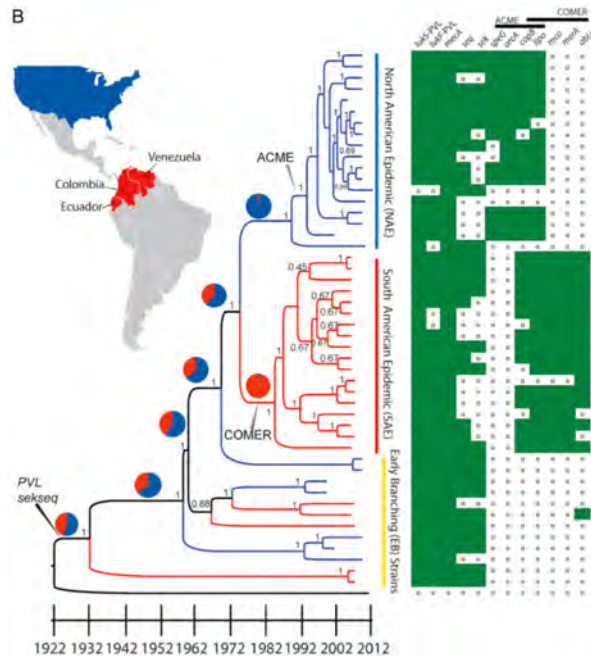
USA300 LV in Latin America

- USA300-LV is the dominant CA-MRSA clone in Colombia, Ecuador, Peru, Trinitad and Venezuela

- -> Why such success in these countries ?

Reyes, Clin Infect Dis 2009
Akvarez, Am J Infect Control 2010
Garcia, J Infect 2011
Monecke, Eur J Clin Microbiol Infect Dis 2011
Sola, Plos One 2012
Nimmo, Clin Microbiol Infect 2012

USA300-0114
USA300-P
USA300-LV

USA 300 SA possesses a unique mercury resistance locus

Purves J, Environ Microbiol (2018)
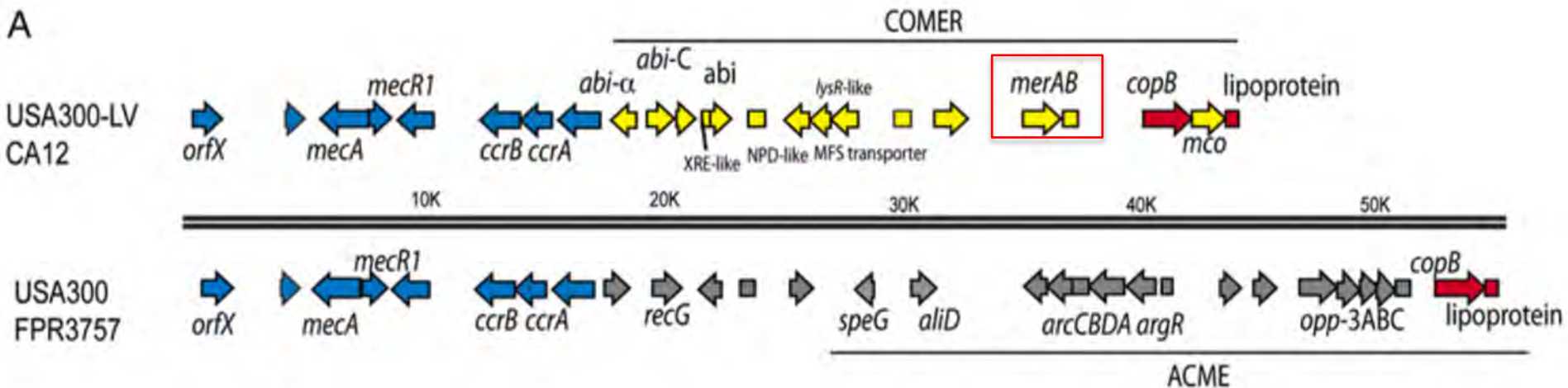
MIC

Strain designation	SCCmec content	MIC HgCl2
Ancestral ST8**ST20172183**	*mec*A(-) ACME(-) COMER(-)	0,57 mg/L
Basal USA300 **ST20172178**	*mec*A(+) ACME(-) COMER(-)	0,57 mg/L
USA300 NA **ST20111414**	*mec*A(+) ACME(+) COMER(-)	0,57 mg/L
USA300 NA **ST20170558**	*mec*A(+) ACME(+) COMER(-)	0,57 mg/L
USA300 SA **HT20030343**	*mec*A(+) ACME(-) COMER (+)	1,70 mg/L
USA300 SA **ST20172176**	*mec*A(+) ACME(-) COMER(+)	1,70 mg/L
USA300 SA **ST20172184**	*mec*A(+) ACME(-) COMER(+)	1,70 mg/L

-> Comer confers a moderate (3 fold increase) resistance toward mercury

Mercury resistance & fitness cost
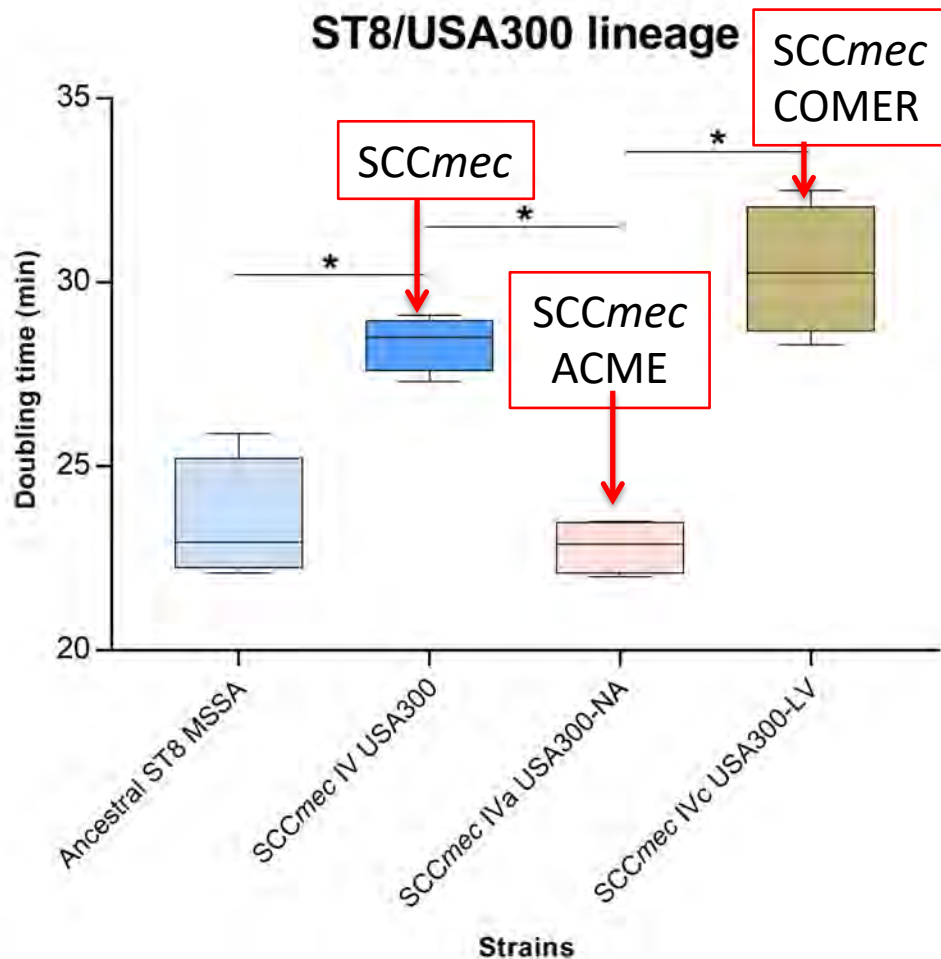
- Acme compensates the fitness cost of SCCmec
- COMER increases the fitness cost
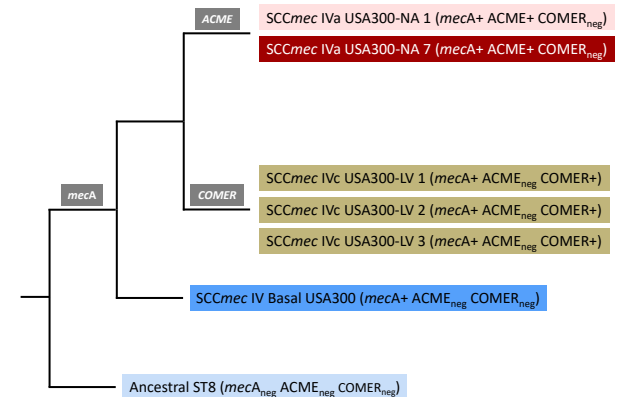
Mercury resistance & fitness cost
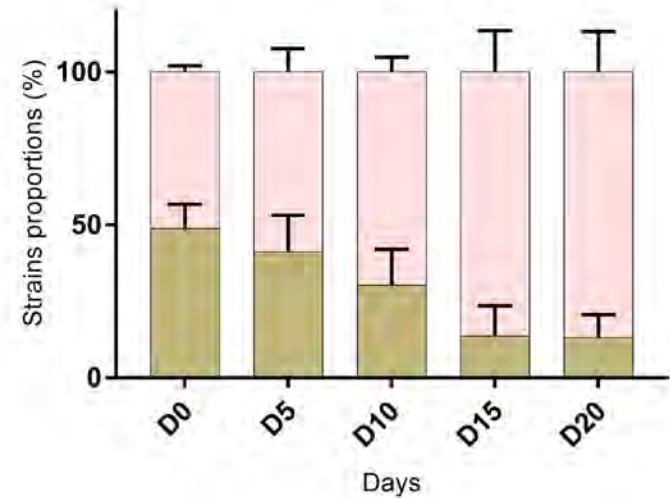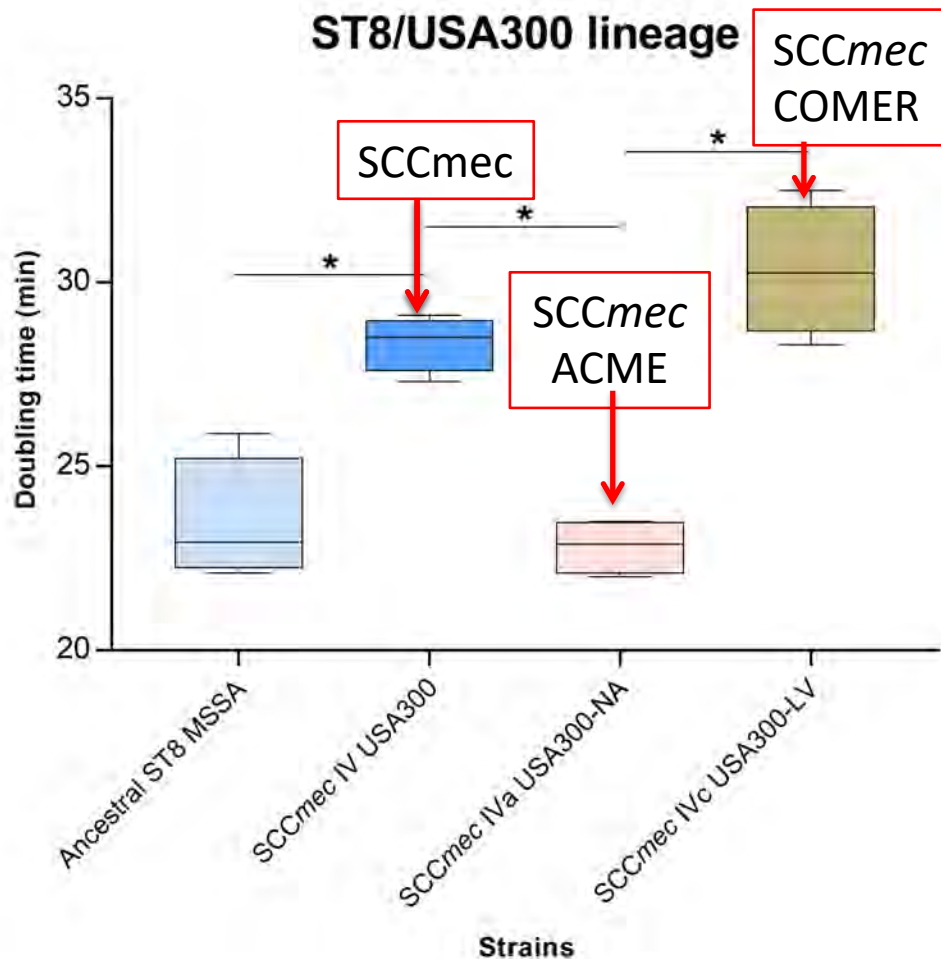
The NA variant outcompete the LV

NA versus LV: Mercury exposure
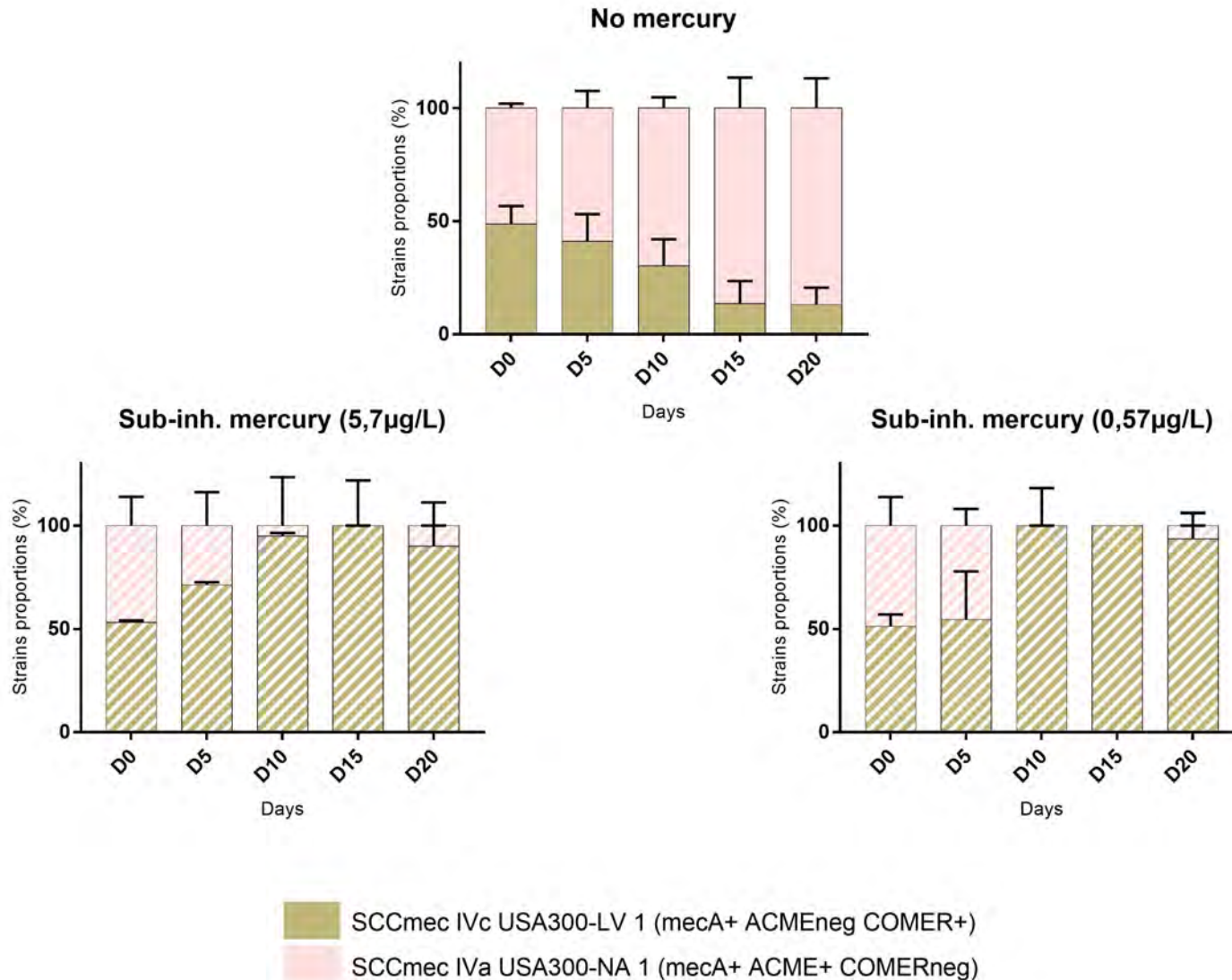
-> In the presence of mercury, USA300-LV outcompete USA300-NA
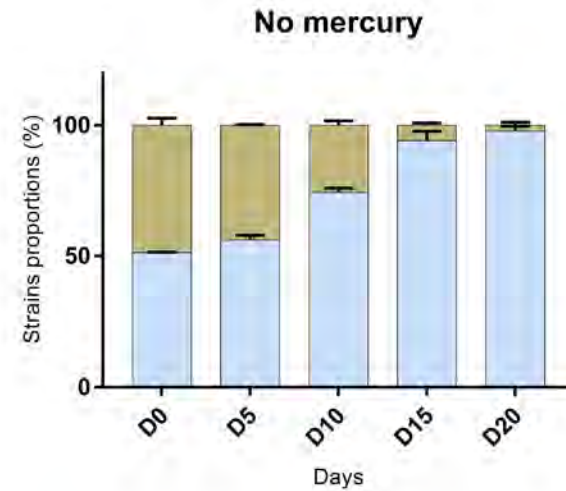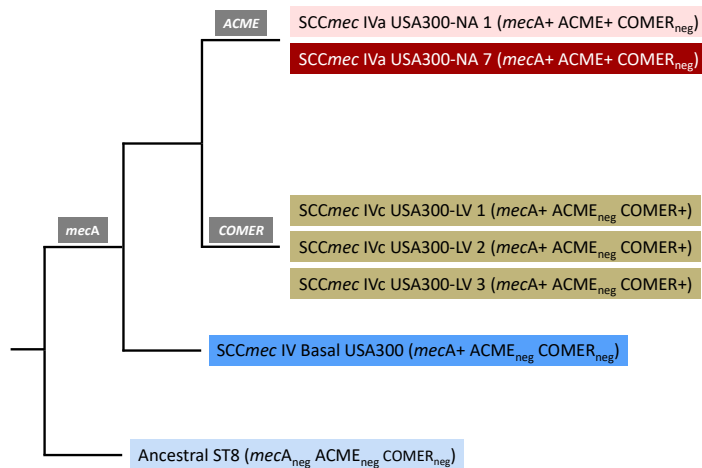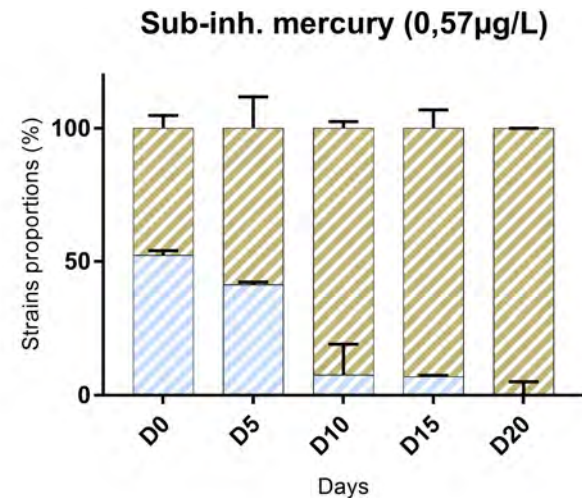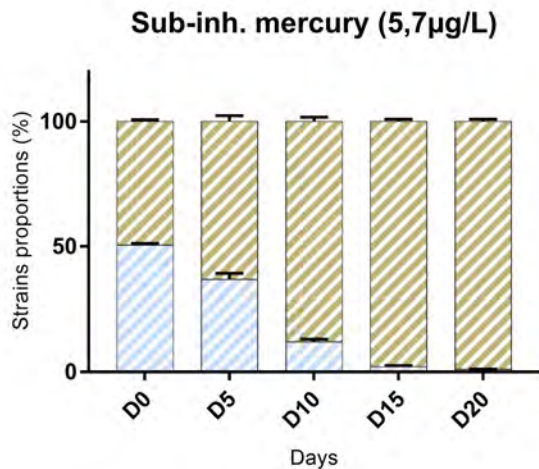
-> In the presence of mercury, USA300-LV outcompete the ancestral multi-susceptible strain

What could be the link between mercury and USA300-LV ?

Golden mines !

Mercury Pollution from Mining and Ore Processing

Population at Risk

- · 100 - 6000
- ● 6001 - 14000
- ● 14001 - 33500
- ◯ 33501 - 70000
- ◯ 70001 - 150000

Illegal gold mining in Peru dumps approximately 30 tons of mercury into the ecosystem every year.

https://www.worstpolluted.org/projects_reports/display/87

Mercury Pollution from Mining and Ore Processing

https://www.worstpolluted.org/projects_reports/display/87
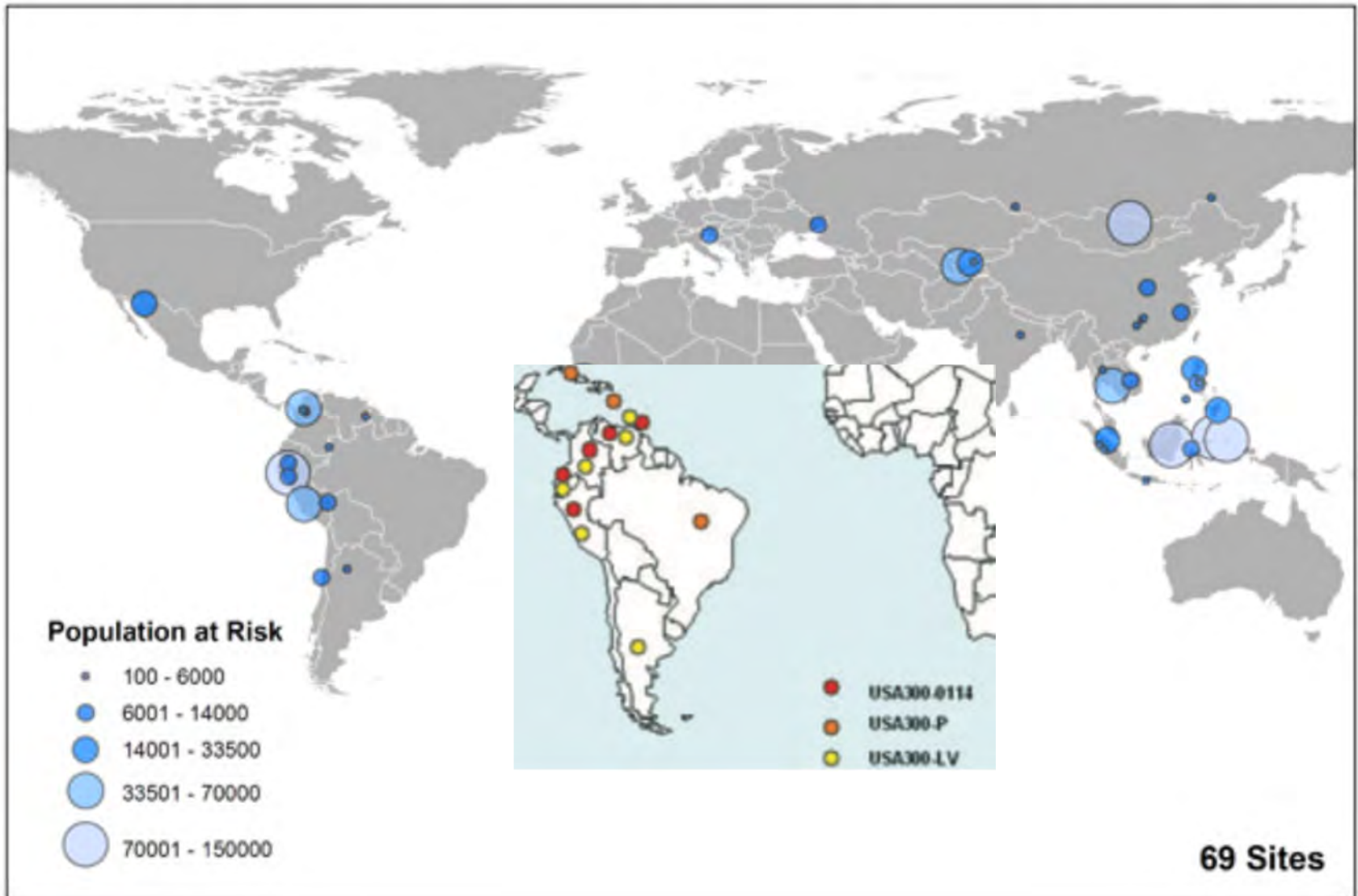
Another trivial scenario

- USA300 LV contains a mercury resistance element
- USA300 LV is prevalent in countries were mercury pollution from mining and ore processing is important
- The biological cost of mercury resistance genes is totally reversed in the presence of trace amount of mercury
  - > Anthropogenic activities leading to environmental pollution may have driven the expansion of USA300-LV
  - > environmental pollutants as a driving force of pathogen success

Pollution & Pathogen emergence: another Darwin's nightmare

Acknowledgements

STAPHYLOCOQUES
Centre National de Référence

CIri
Centre International de Recherche en Infectiologie

Instituts thématiques **Inserm**
Institut national de la santé et de la recherche médicale

CNrs

UCB Lyon 1

InVS
INSTITUT DE VEILLE SANITAIRE

ENS
ENS DE LYON

Hôpitaux de Lyon

MRSP carriage in dogs: A risk to people?

Anette Loeffler

Reader in Veterinary Dermatology

Email: aloeffler@rvc.ac.uk

"The scene" : Small animal veterinary practice

- Every patient comes with an owner
- Owners pay (or private insurance)
- Emotional bond
- Individual animal medicine (not health or flock prescribing)
- Competition amongst practices

Close contact and…

…same antimicrobial classes in people & pets

Sales figures for antimicrobials do not reflect true use in small animals

BMC Veterinary Research

RESEARCH ARTICLE   **Open Access**

Prescribing practices of primary-care veterinary practitioners in dogs diagnosed with bacterial pyoderma

Jennifer F Summers[1*], Anke Hendricks[2] and David C Brodbelt[1]

- 54,600 dogs in the UK (2010)-electronic records
- 683 (1.3%) dogs with **pyoderma**

- **97% received antimicrobials, 92% systemic therapy**
- **Co-amoxiclav, cefalexin, clindamycin, cefovecin**

RVC

The impact of MRSA emergence in pets

Experts warn of epidemic as actress tells how MRSA killed her beloved dog

Now the hospital superbug spreads to pets

DEVOTED: Jill Moss and Bella

By **Matt Nixson**

THE deadly hospital superbug MRSA has spread to pets, experts warned last night.

Now scientists believe that the bug, which kills 5,000 NHS patients every year, could become just as widespread in veterinary clinics.

An expert at the Royal Veterinary College called for urgent action to alert vets and pet owners to the danger.

Professor David Lloyd said: 'Vets may not be looking out for MRSA, and more and more infected animals are being referred to us.

'If we're not careful, veterinary hospitals will become as badly affected as NHS hospitals. There must be more research.'

The risk of infection between animals and humans is slim, but a sick pet is more likely to contract MRSA from a human than vice versa.

Scores of pets have been hit by MRSA but only one, a ten-year-old pedigree Samoyed dog, is known to have died. The animal, called Bella, suffered blood poisoning, pneumonia and organ failure caused by MRSA after an operation on a hind leg.

Bella's owner, actress Jill Moss, 34, said last night: 'It has been a terrible experience. Bella was my companion for more than eight years. She was a real personality and my best friend. I lost my partner in a plane crash four years ago and Bella and I were inseparable.

'In July she ruptured a cruciate ligament while chasing a squirrel. Fixing the problem is a routine operation – a lot of footballers have it – but the wound became infected.

'I kept getting conflicting opinions about what was wrong with her and she was given various drugs. By the time they identified MRSA, it was too late.

'Having her put to sleep was the hardest decision of my life, but she was in agony. By that stage, veterinary nurses didn't want to treat her because they were scared of becoming infected.'

Bella died in August and Miss Moss, who has appeared in TV shows including The Bill, Birds Of A Feather and EastEnders, has created a website warning other pet owners to be on their guard. She added: 'Vets aren't waking up fast enough to the possibility of animal infections like this. I can't bring back Bella but I can warn other owners.'

Sixty years ago, most post-operative infections in humans could be controlled by antibiotics, but bugs have evolved to become resistant to methicillin, the synthetic form of penicillin.

The staphylococcus aureus bacteria that causes MRSA is harmless to healthy people – it is carried by many in the nose and armpits – but it can prove fatal for those with a weakened immune system, such as the sick and the elderly.

Dogs and cats do not commonly carry the bacteria, making it harder for them to pick up MRSA.

Dr Alistair Gibson, of the British Small Animal Veterinary Association, said last night: 'There is little risk of MRSA spreading from pets to humans if owners take common-sense hygiene precautions.'

● Jill Moss's website is www.pets-mrsa.com

MRSA: How big a burden in pets?

- Prevalence amongst pets generally low
- No epidemic spread over past 20 years
- Pet isolates ➜ human hospital-associated lineages (HA-MRSA)
- Considered **"spill-over" from human hospitals**

MRSA isolated from:
9% of referred dogs (n=45)
No cats (n=12)
18% of veterinary staff (n=78)
10% of environmental sites (n=30)

RVC

Methicillin-**r**esistant *S*taphylococcus *p*seudintermedius

- Veterinary nosocomial pathogen (Risk factors & dog adapted)
- In US since 1999 (Gortel et al.), Europe & Japan since 2007
- Skin, ear, wound infections, urinary tract, chest infections etc.
- *mecA* but SCCs different to MRSA
- Multidrug-resistant & zoonotic

RVC

MRSP typical resistance pattern

MRSP Strains	1	2	3	4	5	6	7	8	9	10	11	12
Penicillin	R	R	R	R	R	R	R	R	R	R	R	R
Ampicillin	R	R	R	R	R	R	R	R	R	R	R	R
Amoxicillin-clavulanic acid		R	R	R	R	R	R	R		R	R	R
Oxacillin*	R	R	R									R
Cefalexin*	R	R	R	R								R
Cephalothin	R	R	R	R								R
Enrofloxacin	R	R	R	R	R							R
Clindamycin	R	R	R	R	R							R
Erythromycin	R	R	R	R	R	R					R	R
Gentamycin	S	S	S	S	S	S	S	S	S	S	S	S
Rifampicin	S	R	S	S	S	S	S	S	S	S	R	S
Tetracycline	R	R	R	S	R	R	R	S	R	R	R	R
Trimethoprim-sulfamethoxazole	R	R	R	R	R	R	R	R	R	R	R	R
Fusidic acid	S	S	S	S	I	S	S	S	R	S	S	S

Pet MRSA typically susceptible to tetracyclines & TMPS (and often clindamycin)

RVC

Rapid evolution of multidrug-resistance in *S. pseudintermedius*

Journal of Antimicrobial Chemotherapy

Genomic insights into the rapid emergence and evolution of MDR in *Staphylococcus pseudintermedius*

Alex J. McCarthy[1]†, Ewan M. Harrison[2], Kinga Stanczak-Mrozek[1], Bernadette Leggett[3], Andrew Waller[4], Mark A. Holmes[2], David H. Lloyd[5], Jodi A. Lindsay[1] and Anette Loeffler[5]*

[1]Institute of Infection and Immunity, St George's, University of London, London, UK; [2]Department of Veterinary Medicine, University of Cambridge, Cambridge, UK; [3]Pathobiology Unit, University Veterinary Hospital, School of Veterinary Medicine, University College Dublin, Dublin, Ireland; [4]Centre of Preventive Medicine, Animal Health Trust, Newmarket, UK; [5]Veterinary Clinical Sciences and Services, Royal Veterinary College, Hawkshead Campus, University of London, London, UK

From THEN-*S. pseudintermedius* to NOW-MRSP:

Only 3 genetic events (*mec*A, transposon acquisition, point mutations)

- Selection pressure! Need for antimicrobial stewardship
- Epidemiology similar to MRSA: successful lineages spreading

RVC

How big a problem?

North American university/derm:

17% 2003-2004 (Morris et al. 2006)

40.5% 2012 (Beck et al. 2012)

43.1% 2012 (Bryan et al. 2012)

Europe:

27% 2005-2006 Derm (Loeffler et al. 2007)

21% 2008 Italy vet lab (De Lucia et al. 2010)

2.6% 2007-2012 **UK** RVC (Beever et al. 2015)

5% UK vet diagnostic laboratory (Maluping et al. 2015)

Asia: Up to **70%** from Japan (Kasai et al. 2016)

Australia: **12-13%** in 2013/2014 (Saputra et al. 2017)

RVC

Treatment of canine MRSP infections

- Surface & superficial
- Topical therapy (chlorhexidine, fusidic acid, bleach, multipharma ear drops)
- Compliance (!)

- Deep infections
- Occasionally tetracyclines or fluoroquinolones (authorized for use in dogs)
- Off license: rifampicin, amikacin

RVC

Zoonotic potential of MRSP ?

- **Infections** reported in people but rare
- Mostly associated with dog contact (bites?)

- MORE DETAIL

- **Carriage** associated with infected dogs
- In-contact people & vet staff (Morris et al. 2010)
- Dog owners: identical strains recovered from dogs (MSSP), more frequently if pyoderma (Guardabassi et al. 2004)

RVC

S. pseudintermedius & *S. aureus*:
Similar, but different but different host-preferences

- *S. pseudintermedius* carried by 46-94% of healthy dogs (reviewed by Bannoehr & Guardabassi 2012)

- *S. aureus* found in >50% people at least temporarily

- *S. pseudintermedius* carriage in <10% vet staff, vet students, dog owners (Mahoudeau et al. 1997, Talan et al. 1989, Harvey et al. 1994)

- *S. aureus* <10% canine pyoderma

Veterinary Dermatology 2005, 16, 156–161

Species specificity in the adherence of staphylococci to canine and human corneocytes: a preliminary study

CHRISI SIMOU*‡, PETER B. HILL*, PETER J. FORSYTHE† and KEITH L. THODAY*

Gene transfer *S. aureus* – *S. pseudintermedius*?
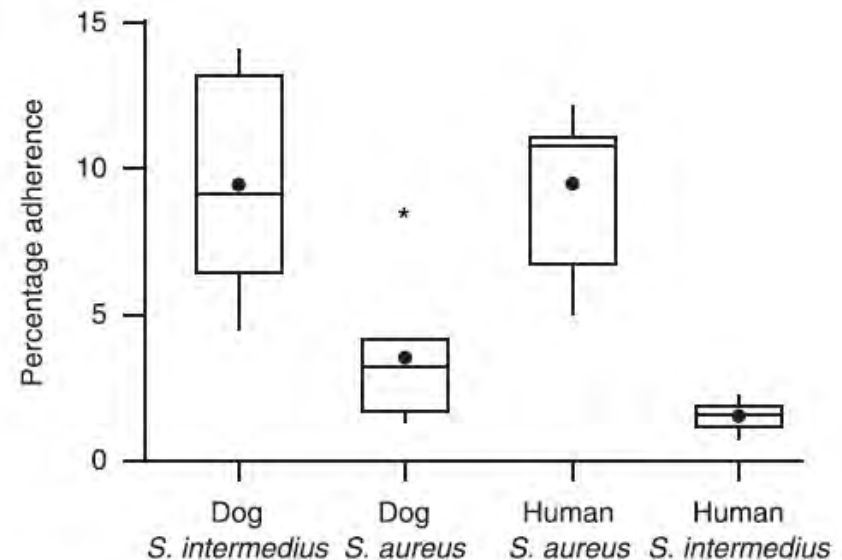
Zoonotic risk & epidemiology varies

- **MRSA** well adapted to humans
- If diagnosed in a pet, owners to inform their medical practitioner

- **MRSP** = veterinary nosocomial pathogen
- Owner advice on zoonotic potential
- Upgrade vet practice & personal hygiene

Mistaken identities?

Clinical Isolates of *Staphylococcus intermedius* Masquerading as Methicillin-Resistant *Staphylococcus aureus*

Sudha Pottumarthy,[1] Jeffrey M. Schapiro,[1] Jennifer L. Prentice,[1] Yolanda B. Houze,[1] Susan R. Swanzy,[1] Ferric C. Fang,[1,2] and Brad T. Cookson[1,2*]

ARTICLE

Staphylococcus pseudintermedius can be misdiagnosed as *Staphylococcus aureus* in humans with dog bite wounds

S. Börjesson · E. Gómez-Sanz · K. Ekström · C. Torres · U. Grönlund

- 13/101 *S. aureus* isolates from human dog bite wounds re-identified as *S. pseudintermedius*

RVC

MRSP carriage

80% of *S. pseudintermedius* isolates from lesional skin were identical to those carried orally by the dog (Pinchbeck et al. 2006)

MRSP carriage & contamination in dogs

RESEARCH ARTICLE **Open Access**

Carriage of methicillin-resistant *Staphylococcus pseudintermedius* in dogs–a longitudinal study

Ulrika Windahl[1*], Elin Reimegård[2†], Bodil Ström Holst[1,3†], Agneta Egenvall[3†], Liselotte Fernström[4†],
Mona Fredriksson[4†], Gunilla Trowald-Wigh[4†] and Ulrika Grönlund Andersson[1]

Current RVC study (ongoing)

- Clinical signs of infection resolved
- 6 carriage sites sampled
- Contact plates for house

- 31 dogs previously diagnosed with MRSP infection

- Sampled at 5 mucosal sites over time

- **Median length of MRSP carriage = 11 months**

- 3/5 dogs treated with an antimicrobial to which their MRSP-isolates were susceptible (tetracycline) were still MRSP-positive at the end of treatment

- **18 / 27 (67%) dogs MRSP +ve**
- 2-14 months after resolution
- MRSP +ve index dog household:
  - 69% environmental sites +ve
  - 90% in-contact dogs +ve

- MRSP –ve index dog household:
  - 14% environmental sites +ve
  - 20% in-contact dogs +ve

"Decolonisation"

- Naturally occurring

- Resistance = cost to fitness in staphylococci (Berger-Bächi 2004)

- Clean environment

- Prevent recurrent opportunistic infections

- For how long?

- Using antimicrobials

- No vet studies, only case reports

- Ethical considerations (healthy animals)

- No products licensed for decolonization of pets

- Poor long-term effect in human medicine for MRSA (Cochrane) mainly done pre-operatively

RVC

Ben

MRSP transmission/acquisition

- Dog-to-dog likely ✔

- Environment-to-dog (+/- humans) via surfaces possible (human carriage during vet hospital outbreaks (e.g van Duijkeren et al. 2011)

- Not easily transmitted between humans & transient: not recovered after two months (Frank et al. 2009)

- In dogs: no dog-to-dog transmission of MRSA:
- 11 dogs sharing a kennel with MRSA carrier dogs
- Daily environmental cleaning
- All MRSA-carriage negative at repeat sampling 2 weeks later

RVC

Is MRSP carriage a problem?

- Low risk to healthy people
- Problem for canine patients & vets
- Case by case discussion/decision

The Human–Companion Animal Bond: How Humans Benefit

Erika Friedmann, PhD*, Heesook Son, MPH, RN

Vet Clin North Am Small Anim Pract. 2009;39:293-326.

KEYWORDS
- Animal assisted therapy • Pet therapy
- Animal-assisted activities • Stress reduction • Pets
- Assistance animals • Assistance dogs • Companion animals

- Opportunity
- Improve awareness for AMS
- Improve hygiene
- Adjust regulations

Sales of antimicrobials for oral use in dogs
(Swedish Veterinary Antimicrobial Resistance Monitoring 2011)

Dog population has not changed

Legend:
- Aminopenicillins
- Macrolides & lincosamides
- Aminopenicillins +inhibitor
- Fluoroquinolones
- Cefalosporins 1st gen

Aminopens + inhib – 44%

Cefalosporins – 62%

Fluoroquinolones – 44%
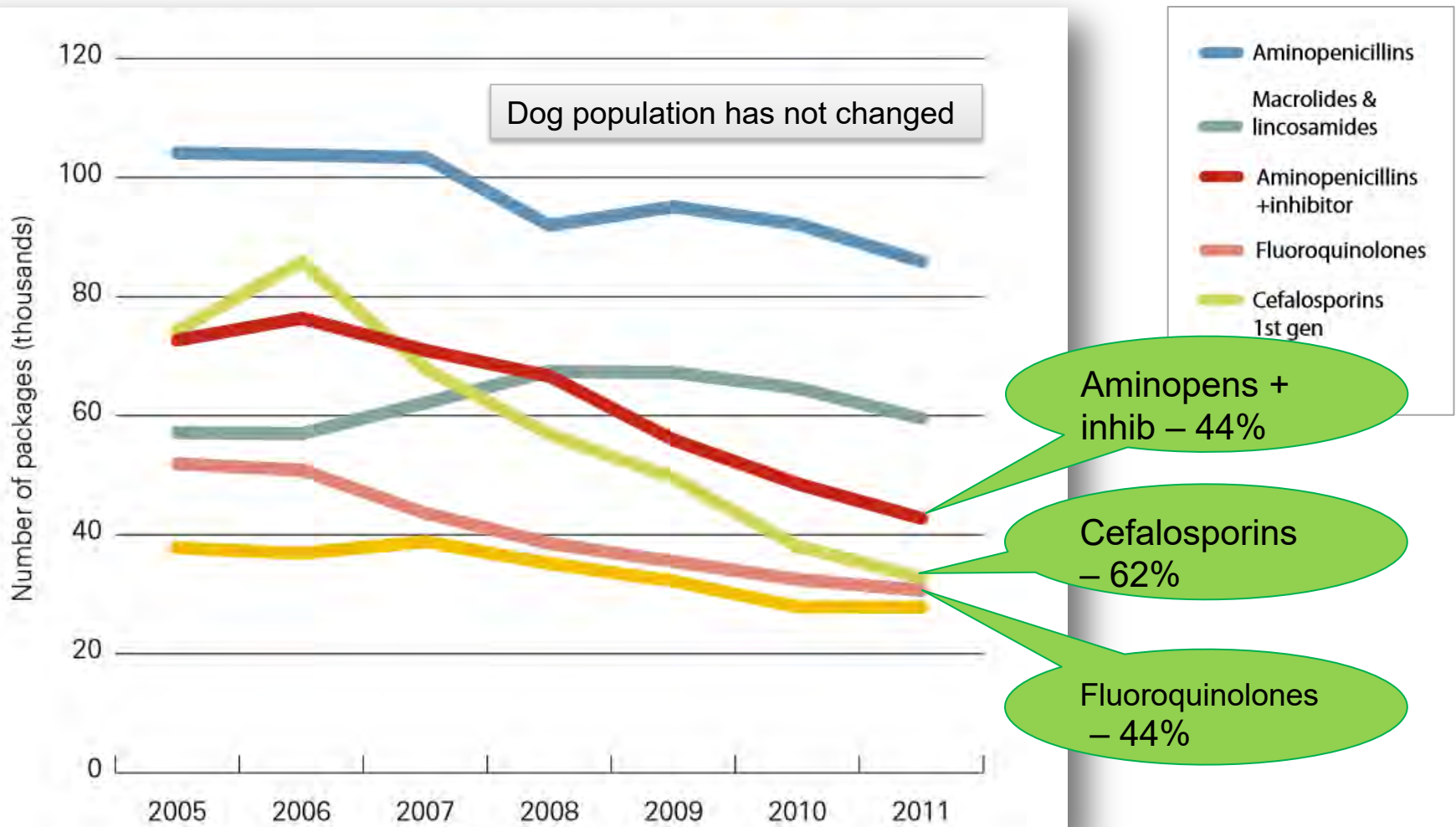
Downward trends associated with appearance of MRSA & MRSP

The bigger picture

'ESKAPE' : Clinically relevant multidrug-resistant pathogens (human med.)

- ***E**nterococcus faecium*
- ***S**taphylcooccus aureus (S. pseudintermedius)*
- ***K**lebsiella pneumonia*
- ***A**cinetobacter baumannii (& other spp.)*
- ***P**seudomonas aeruginosa*
- ***E**nterobacter* species

Boucher et al. Clin Infect Dis 2009; 48: 1-12.
Rice LB.. J Infect Dis 2008; 197: 1079-81

RVC

Footpad of a Cocker spaniel
with MDR-Klebsiella and MRSP

MDR pathogens in pets

OPEN ACCESS Freely available online

PLOS | ONE

Extended-Spectrum-Beta-Lactamases, AmpC Beta-Lactamases and Plasmid Mediated Quinolone Resistance in *Klebsiella* spp. from Companion Animals in Italy

Valentina Donati[1], Fabiola Feltrin[1], Rene S. Hendriksen[2], Christina Aaby Svendsen[2], Gessica Cordaro[1], Aurora García-Fernández[3], Serena Lorenzetti[1], Raniero Lorenzetti[1], Antonio Battisti[1]*, Alessia Franco[1]

1 Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Rome, Italy, 2 Technical University of Denmark, National Food Institute (DTU-Food), Kongens Lyngby, Denmark, 3 Istituto Superiore di Sanità, Department of Infectious, Parasitic and Immune-Mediated Diseases, Rome, Italy

OPEN ACCESS Freely available online

PLoS one

Dogs Leaving the ICU Carry a Very Large Multi-Drug Resistant Enterococcal Population with Capacity for Biofilm Formation and Horizontal Gene Transfer

2011

Anuradha Ghosh[1], Scot E. Dowd[2], Ludek Zurek[1,3]*

Multidrug-Resistant *Acinetobacter baumannii* in Veterinary Clinics, Germany

Sabrina Zordan, Ellen Prenger-Berninghoff, Reinhard Weiss, Tanny van der Reijden, Peterhans van den Broek, Georg Baljer, and Lenie Dijkshoorn

frontiers in MICROBIOLOGY

Antimicrobials, Resistance and Chemotherapy

< Archive

This article is part of the Research
Emerging antimicrobial resistance

ORIGINAL RESEARCH ARTICLE

2013

High prevalence of fecal carriage of extended-spectrum β-lactamase/AmpC-producing *Enterobacteriaceae* in cats and dogs

Joost Hordijk[1]*, Anky Schoormans[1], Mandy Kwakernaak[1], Birgitta Duim[1], Els Broens[1], Cindy Dierikx[2], Dik Mevius[1,2] and Jaap A. Wagenaar[1,2]

1 Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands
2 Central Veterinary Institute of Wageningen University and Research Center, Lelystad, Netherlands

RVC

Acknowledgements & Affiliations

Thanks to:

- RVC Dermatology Group
- David Lloyd (RVC)
- Jodi Lindsay, St Georges
- Dirk Pfeiffer (RVC)
- David Grant (RSPCA)

Figure 5:15. A. Breeches for giant breeds affected by elbow callus pyoderma. They are made of tough stretchable jersey material with foam rubber pad inserts in the region of the elbow. A strap and buckle (suspenders) attach over the back to hold them up. They are comfortable and worn constantly until the lesions heal, and then "nighttime only" usually suffices. These breeches are also useful in hygromas. B. Irish wolfhound with his breeches in place. C. Side view shows strap over the shoulders. (Fig. B and C, E. M. Farber's Irish wolfhound "Finnegan.")

Breeches instead of antibiotics for skin infections?

RVC

Veterinary Dermatology text book from 1983 (Muller, Kirk & Scott)

Role of Antimicrobial Stewardship in the intensive care unit

Jeroen A. Schouten, MD
Intensive Care
IQ Healthcare

Center for Infectious Diseases
Radboudumc

here a problem?

- *Increasing antimicrobial resistance worldwide*
- *Increased morbidity and mortality attributed to untreatable infections*
- *No new drugs in the pipeline, especially not for gram negatives*
- *Need for responsible use of existing antibiotics, but poor adherence*

WHO, AMR global report on surveillance 201

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Why is the problem in ICU?

- *38-50% of ICU patients have an infection*
- *60-70% of ICU patients on antimicrobial therapy*
- *transmission of resistant micro organisms more likely*
- *loss of physiological barriers*
- *poor host response*

AMR not necessarily develops but emerges in ICU

in the largest point prevalance study on infections in ICU (EPIC II) data from thousands of patients
70% on AB,the highest in any healthcare setting- increasing the total

Vincent 2009, EPIC II data
Vincent 2006, SOAP data

Why are we having this conference?

"2nd ICOHAR aims at bringing together representatives from all relevant sectors (e.g. public health, human and veterinary medicine, livestock production, food safety and environmental sciences) to share research and education strategies for understanding and reducing the risks of AMR at the interphase between humans, animals and the environment. The programme does not only focus on zoonotic transfer of AMR but also on the numerous AMR-related challenges shared by clinicians, clinical microbiologists, infectious disease specialists and researchers working in these sectors"

Resistance of *Aspergillus fumigatus* in the Netherlands: One – Health issue in the ICU

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Intensive Care
IQ Healthcare

Center for Infectious Diseases
Radboud umc

"Home sweet home"

Routes of resistance selection

Verweij et al. Lancet Infect Dis 2009;9:789-95

Routes of resistance selection

cavity
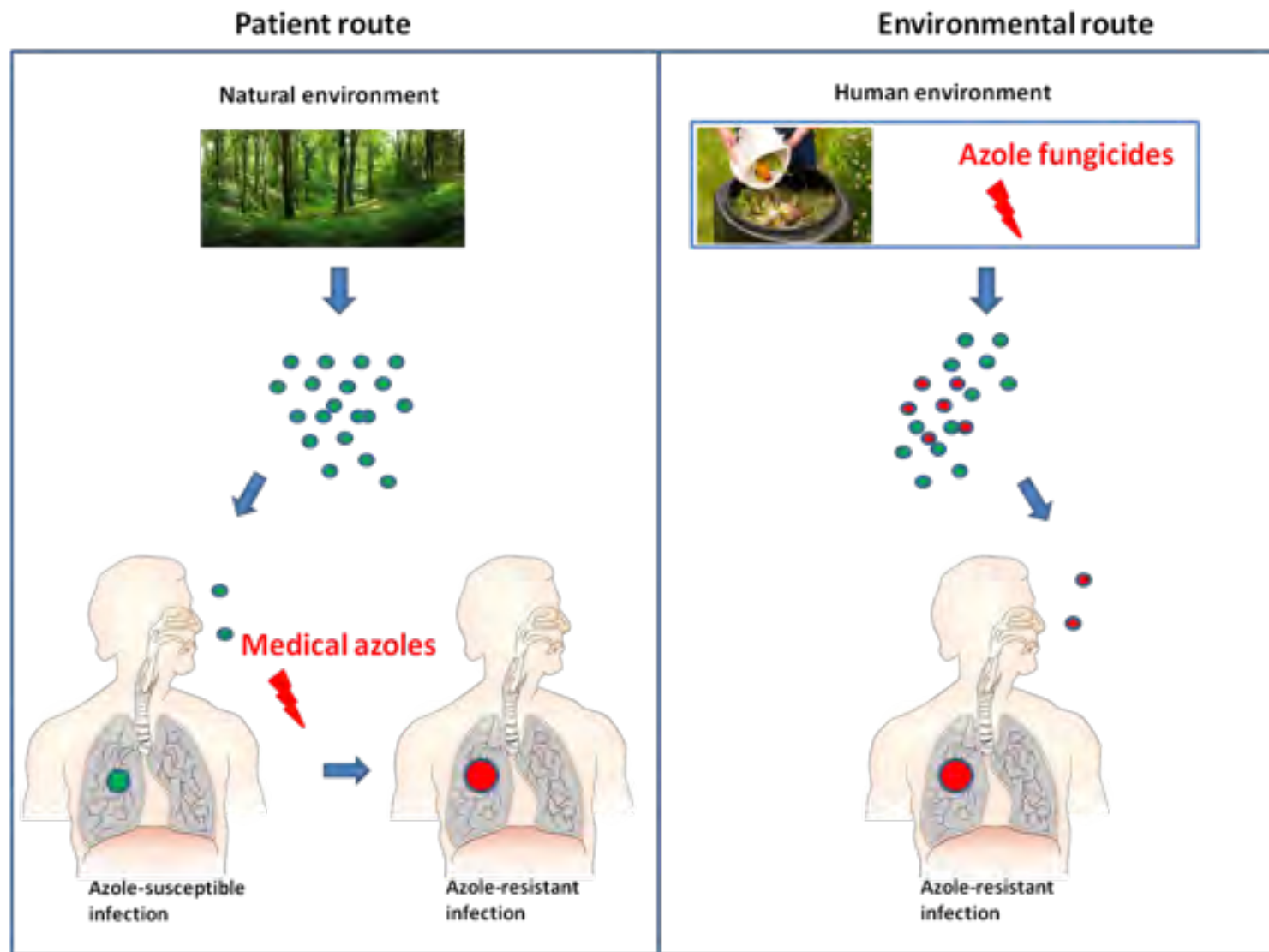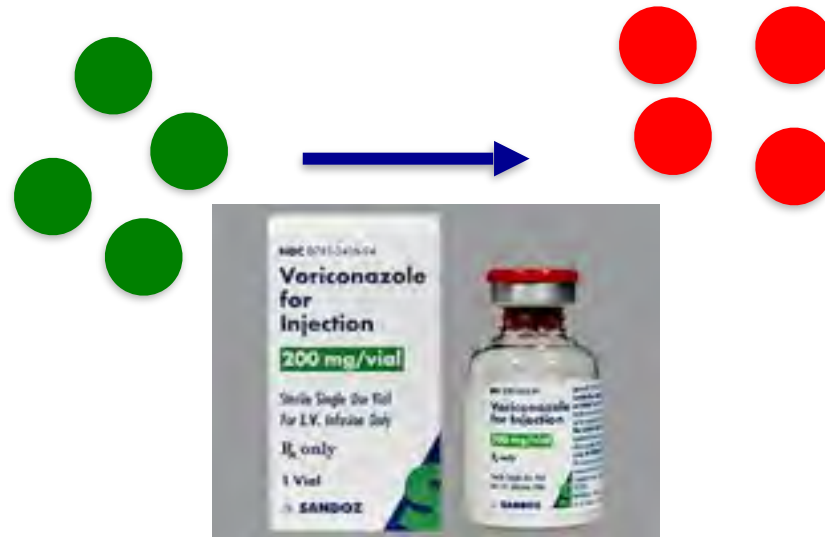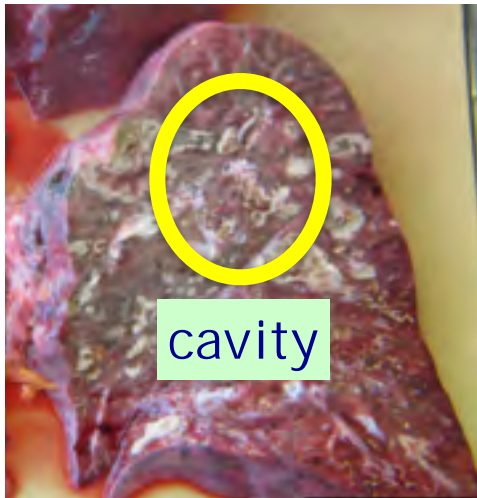
G54
M220
G138
P216
etc.

TR_{34}/L98H
TR_{53}
TR_{46}/Y121F/T289/

How? where?
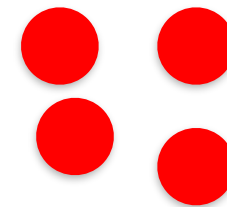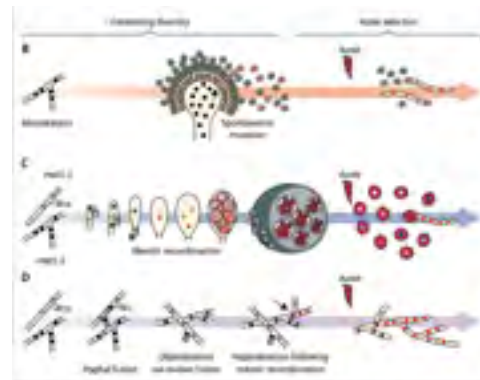
Environmental resistance – one health problem

Medical triazoles

Propiconazole; tebuconazole; epoxiconazole; difenoconazole; bromuconazole
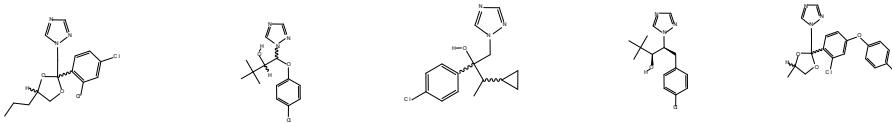
$TR_{34}/L98H$

TR_{53}

$TR_{46}/Y121F/T289A$

Verweij et al. Lancet Infect Dis 2009;9:789-95
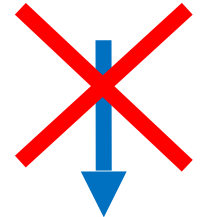
Azole resistance in *A. fumigatus*: Under the radar ...
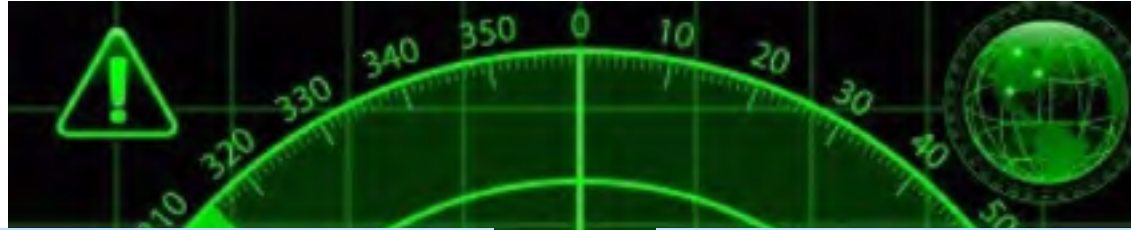
Medical
Not a public health threat....

Agricultural
Not a plant pathogen....

Lack of awareness

Aspergillus disease and development of resistance

Disease	Route of resistance	Characteristics
Cystic Fibrosis	E > P	
ABPA	?	
Aspergilloma	P >> E	cavity, multiple R-mutations, fitness cost
CPA	P >> E	cavity, multiple R-mutations, fitness cost
IA - pulmonary	E	TR_{34} > TR_{46}, mixed infections S/R – R/R
Influenza Associated Aspergillosis	E	TR_{34} > TR_{46}, mixed infections, tracheo bronchitis
CNS-IA	E	TR_{34} & TR_{46}, sanctuary site

P = patient route   E = environmental route

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Azole resistance in *A. fumigatus* associated with increased mortality?

Table 4. Characteristics of patients with azole-resistant invasive aspergillosis, the Netherlands, 2007–2009*

Patient age, y/ sex	Underlying disease	Disease	No. positive cultures†	Resistance mechanism	VCZ MIC, mg/L	Prior azole treatment (duration)‡	Treatment§	Outcome at 12 wk
66/M	Lung carcinoma	Proven pulmonary aspergillosis	1	TR/L98H	4	None	VCZ	Died
59/M	Hematologic malignancy, allo-SCT, GvHD	Proven pulmonary aspergillosis	4	TR/L98H	8	VCZ (>1 mo)	VCZ	Died
54/M	Acute myeloid leukemia, relapse, allo-HSCT	Proven pulmonary aspergillosis	1	TR/L98H	8	ITZ (2–4 wk)	VCZ	Died
50/M	Non-Hodgkin lymphoma, allo-SCT, GvHD, lung cavities	Probable pulmonary aspergillosis	2	TR/L98H	16	VCZ (>1 mo)	VCZ	Died
36/F	Breast carcinoma with metastasis	Probable pulmonary aspergillosis	1	TR/L98H	1	None	VCZ	Died
13/F	Non-Hodgkin lymphoma	Proven pulmonary and CNS aspergillosis	1	TR/L98H	16	None	VCZ, CAS, AMB	Died
58/M	Liver transplantation for hepatic failure after methotrexate treatment for arteritis	Proven pulmonary and CNS aspergillosis	5	TR/L98H	2	None	AMB, VCZ	Died
60/M	Acute myeloid leukemia, allo-SCT, GvHD	Proven pulmonary and CNS aspergillosis	3	TR/L98H	4	FCZ (1–2 wk)	VCZ, CAS, AMB, POS	Survived

Azole R IA in the ICU
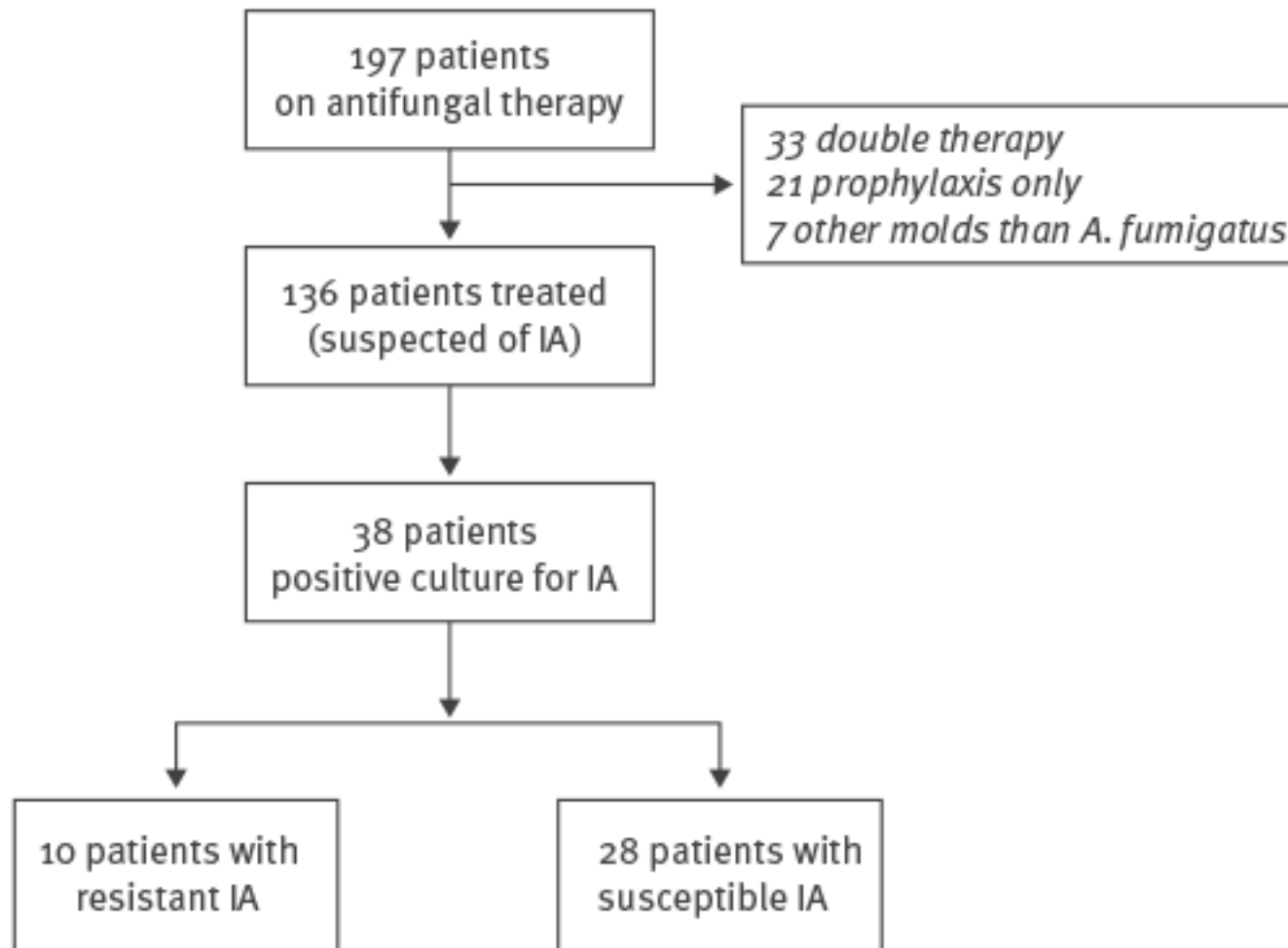
Influenza-associated aspergillosis – azole resistance

Patient ID/age	Underlying disease	Phenotype first culture (specimen)	Azole resistant isolate	MIC (mg/l)(interpretation)ᵀ						Resistance mutation	Initial antifungal therapy*	Subsequent treatment regimens*	Outcome*
				AmB	ITZ	VCZ	POS	ISA	AFG				
2-1 / 34	None	Azole-resistant (BAL)	First culture	0.5 (S)	>8 (R)	2 (I)	0.5 (R)	>8 (R)	0.016	TR₃₄/L98H	Voriconazole (+7)	Li-AmB (+22)	Died (+27)
4-2 /52	None	Mixed (sputum)	First culture	0.25 (S)	2 (I)	>8 (R)	0.5 (R)	>8 (R)	0.031	TR₄₆/Y121F/ T289A	Voriconazole (0)	L-AmB (+4)	Died (+13)
5-5 /38	None	Wild type (sputum)	At autopsy	0.5 (S)	8 (R)	2 (I)	0.5 (R)	8 (R)	0.031	TR₃₄/L98H	Voriconazole (-5)	VCZ+AFG (0); VCZ+Li-AmB (+5)	Died (+16)
2-3 /44	Asthma, sinusitis	Mixed (sputum)	First culture	1 (S)	>8 (R)	4 (R)	0.5 (R)	>8 (R)	0.016	TR₃₄/L98H	Voriconazole (+11)	Li-AmB (+16); CAS (+26); Li-AmB (+30)	Survived
5-1 / 71	Lung cancer, COPD	Azole-resistant (sputum)	First culture	0.5 (S)	>8 (R)	4 (R)	0.5 (R)	>8 (R)	0.016	TR₃₄/L98H	Voriconazole (+5)	VCZ+AFG (+9); Li-AmB (+11); VCZ+AFG (+14)	Survived

3 mixed Azole-S and Azole-R infection

environmental mutations

VCZ

3 died 2 survived

Van de Veerdonk F, et al. Am J Resp Crit Care Med 2017;196:524-7

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PCR diagnosis of resistance-associated mutations (RAMs)

Total patients
n = 78

65 with WT A. fumigatus

13 with A. fumigatus with RAMs
8 TR34/L98H
3 TR34/L98 H combined with WT
2 TR46/T289A/Y121F

20 excluded
3 mixed infections
2 had <5 days azole therapy
3 non-azole combined with azole therapy
7 non-azole therapy from start
3 antifungal therapy or duration unknown
2 untreated

5 excluded
3 TR34/L98H combined with WT
2 had <5 days azole therapy

45 with WT A. fumigatus

8 with A. fumigatus with RAMs
6 TR34/L98H
2 TR46/T289A/Y121F

Six week mortality 2.6 times higher in patients with detected RAM (17.8% without versus 50.0%; P = 0.07).

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Characteristics of azole R aspergillosis

Environmental route

Patient route

Any aspergillus disease

64% no previous azole exposure

High mortality

Specific mutations

Azole S and azole R co-infection

Selection of azole R during VCZ monotherapy

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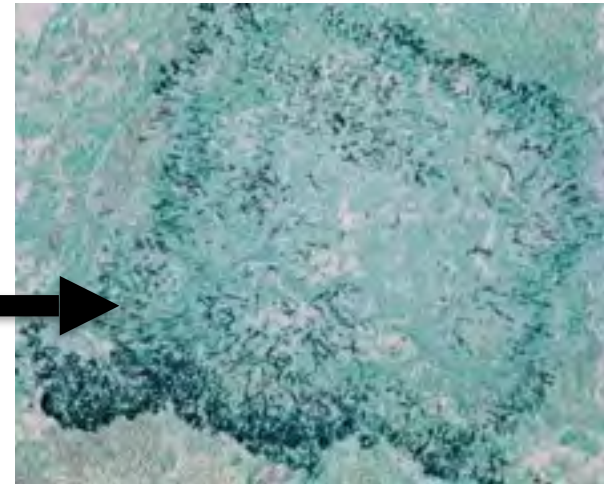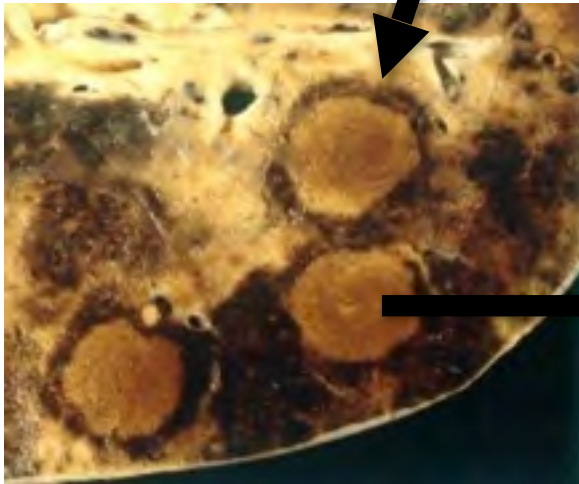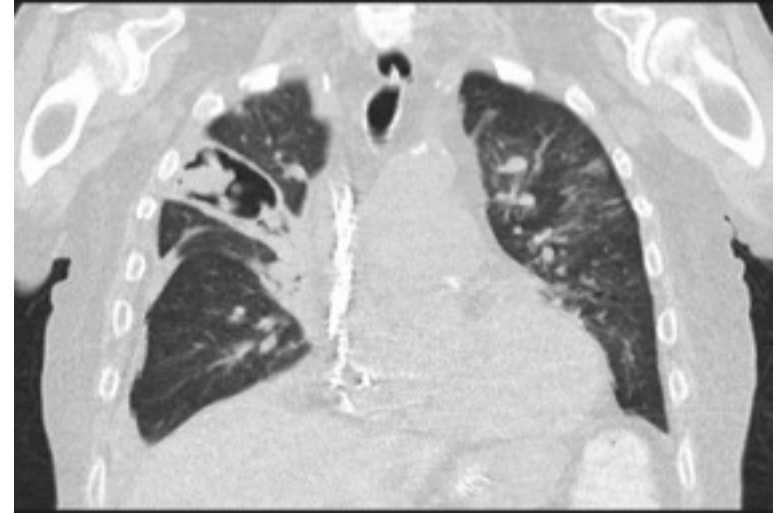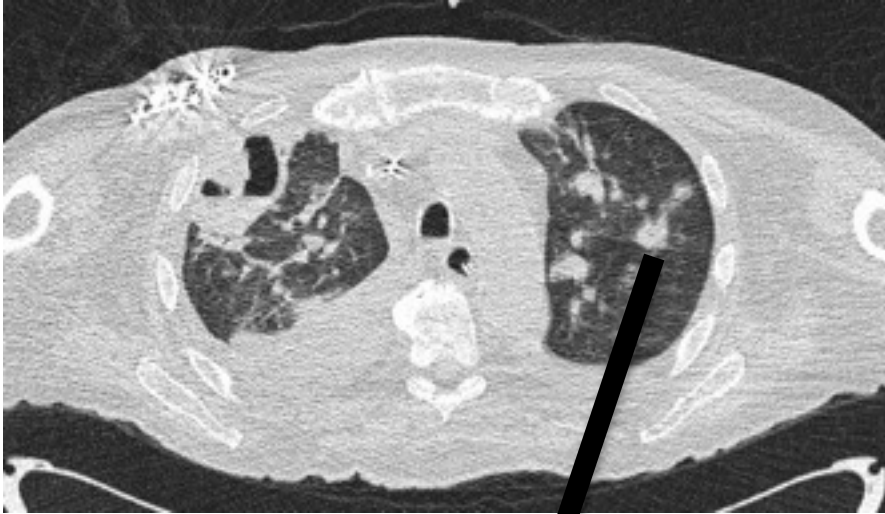
Pathogenesis of mixed infection
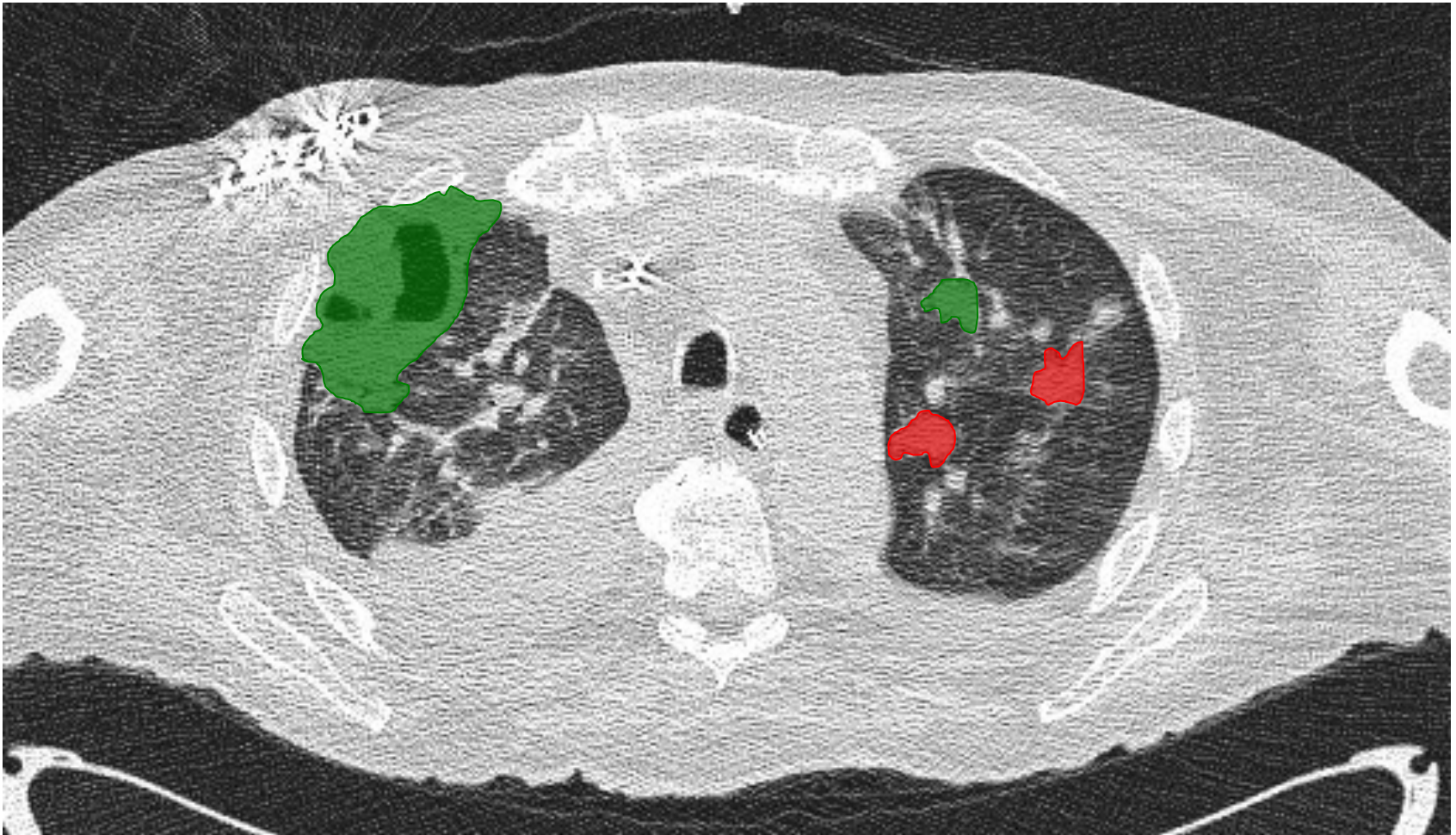
Azole susceptible   Azole resistant

Clinical implications of resistance
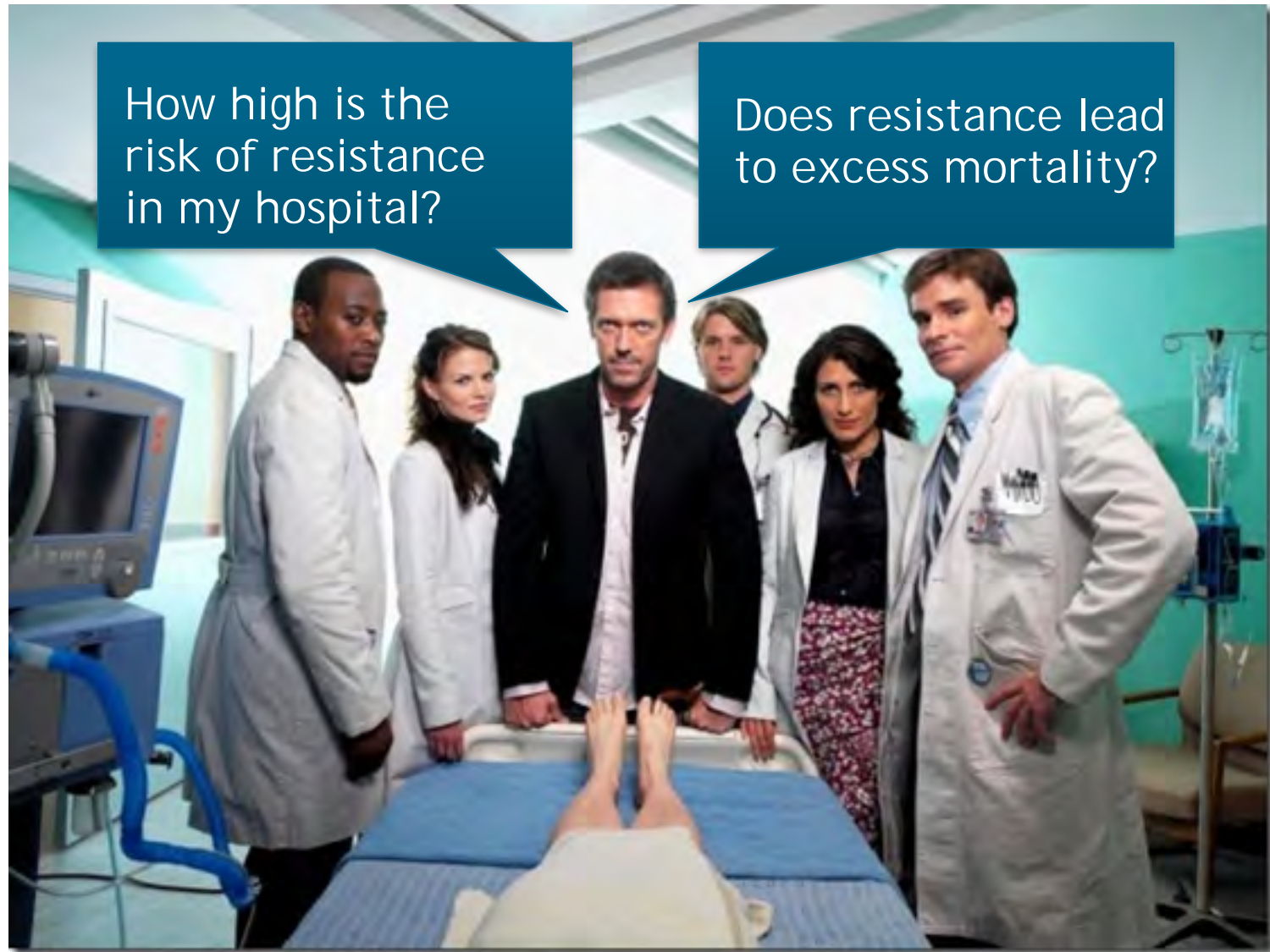
Geographic spread: clinical and environmental

Resistance %
- >10
- Unknown/not yet detected
- Environmental only
- 1–5
- 5–10
- 0–1

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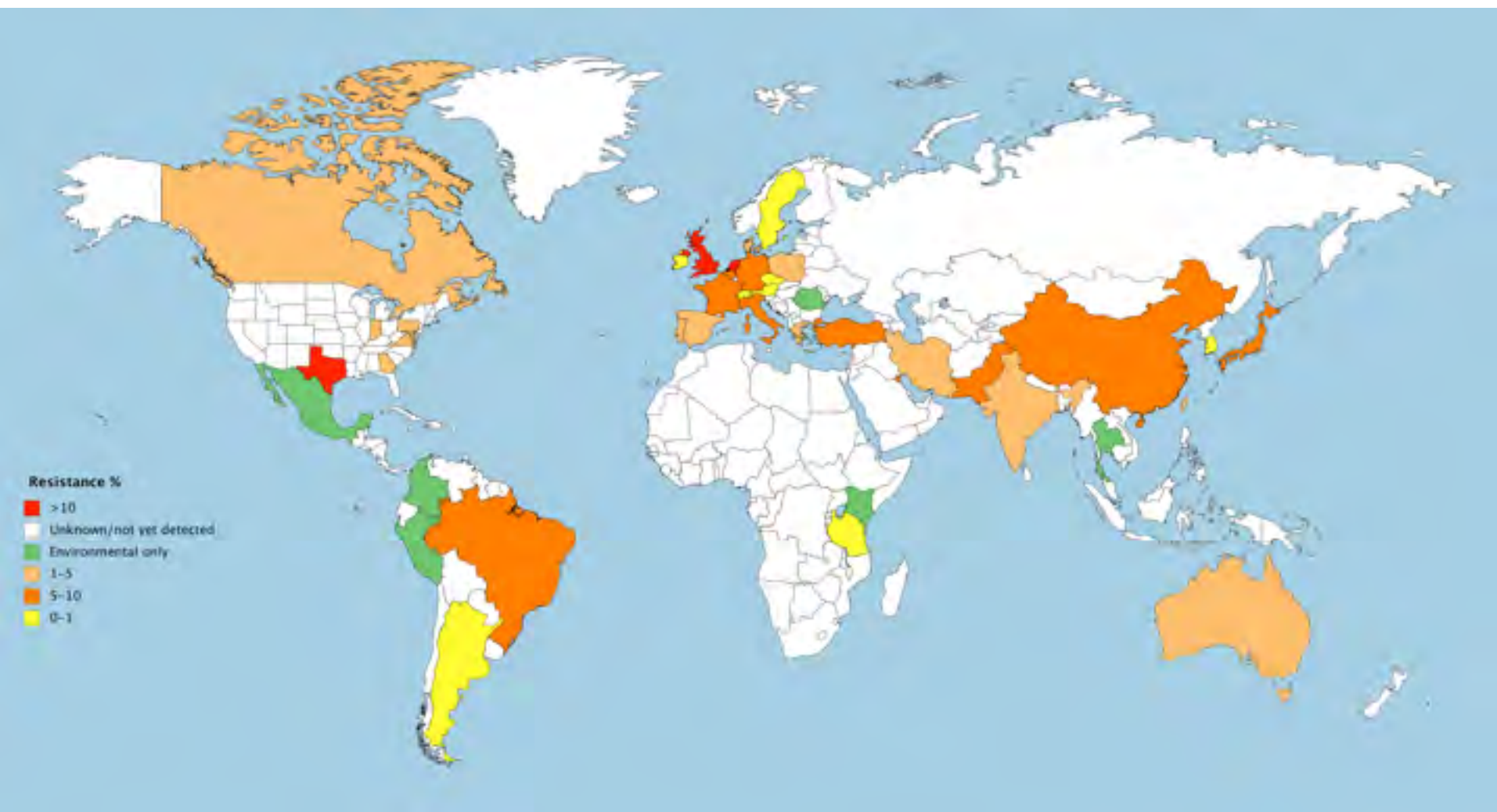
Acquired resistance frequency *A. fumigatus* 2013 - 17

5 university medical centers

Screening for resistance of unselected clinical isolates using VIPcheck™

Includes clinically not-relevant isolates

Number of patients screened 600 to 814 per annum

$$\text{Resistance frequency} = \frac{\text{number of patients with Azole-R isolate}}{\text{number of screened patients}}$$

Netmap 2017:128-31

Acquired resistance frequency *A. fumigatus* 2013 -17
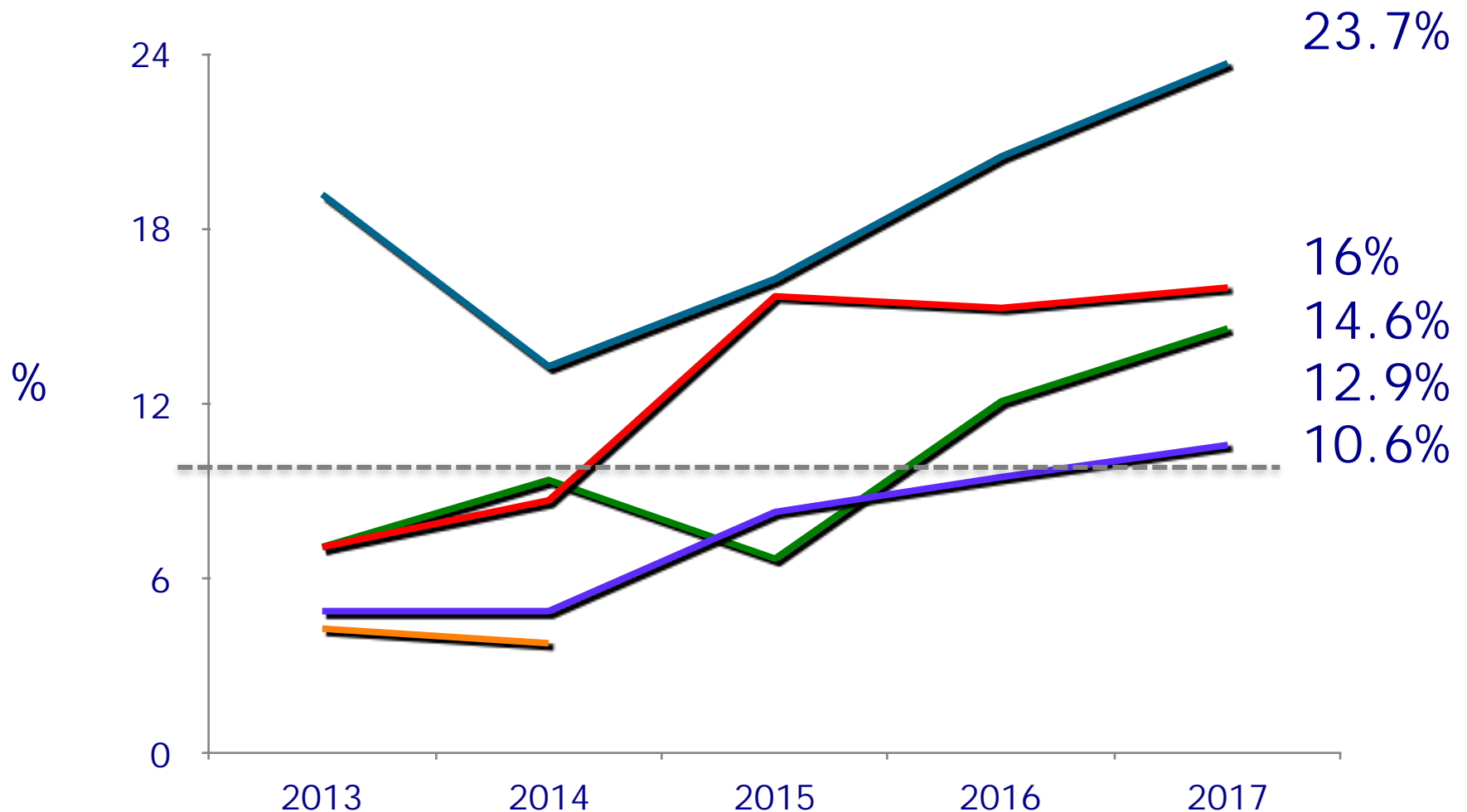
Does resistance lead to excess mortality?

Mortality of voriconazole R IA > voriconazole S IA?

Radboudumc – LUMC – ErasmusMC

2011 - 2015

All patients with *A. fumigatus* in culture

All isolates screened with VIPcheck™

Compare mortality in R versus S

2,266 patients with positive *Aspergillus fumigatus* culture

Study group

2,070

196

● No IA ● IA

EORTC/MSG
Blot et al

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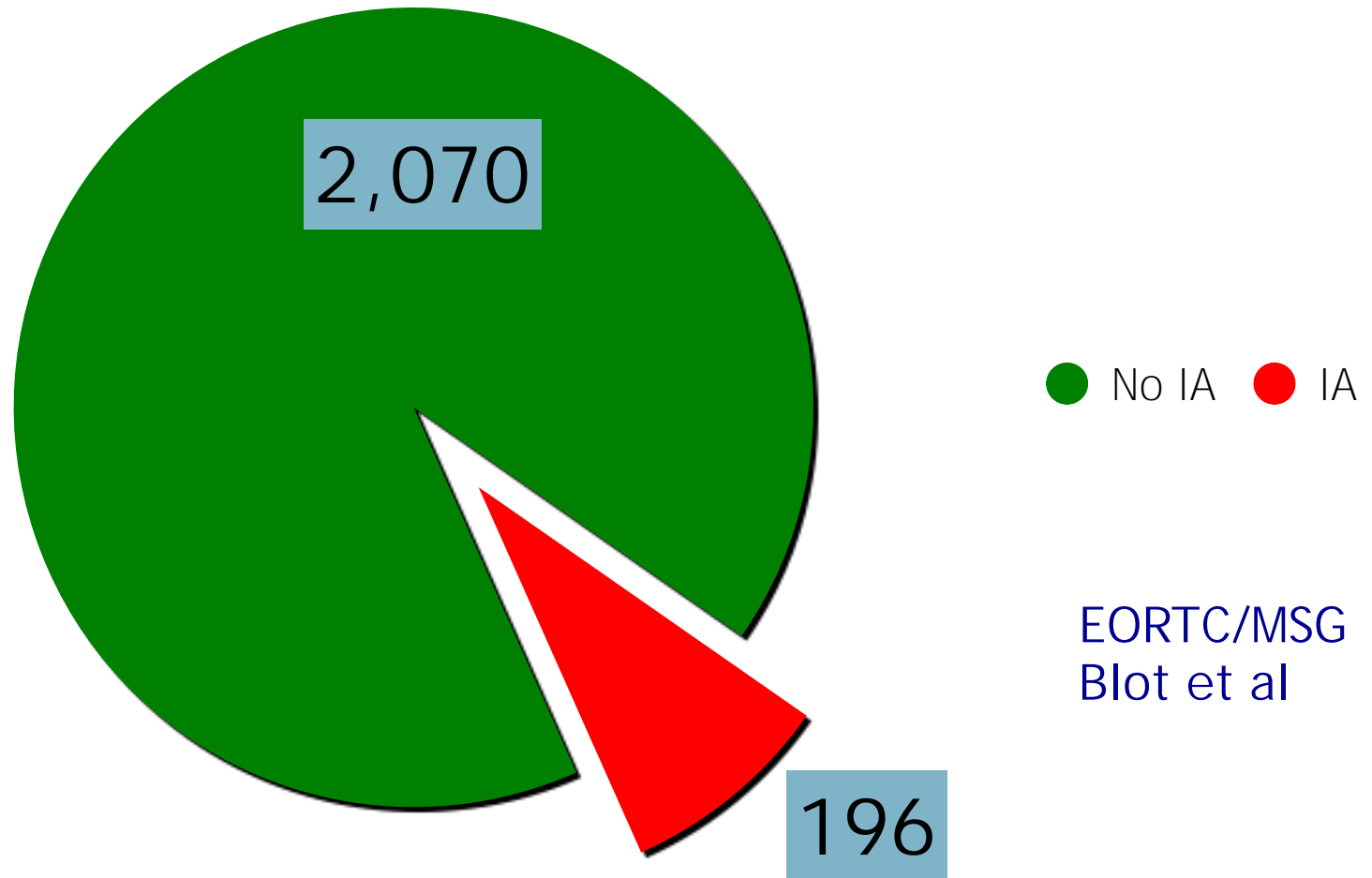
196 patients with invasive aspergillosis

Resistance phenotype

159

37
19%

VCZ-S
VCZ-R
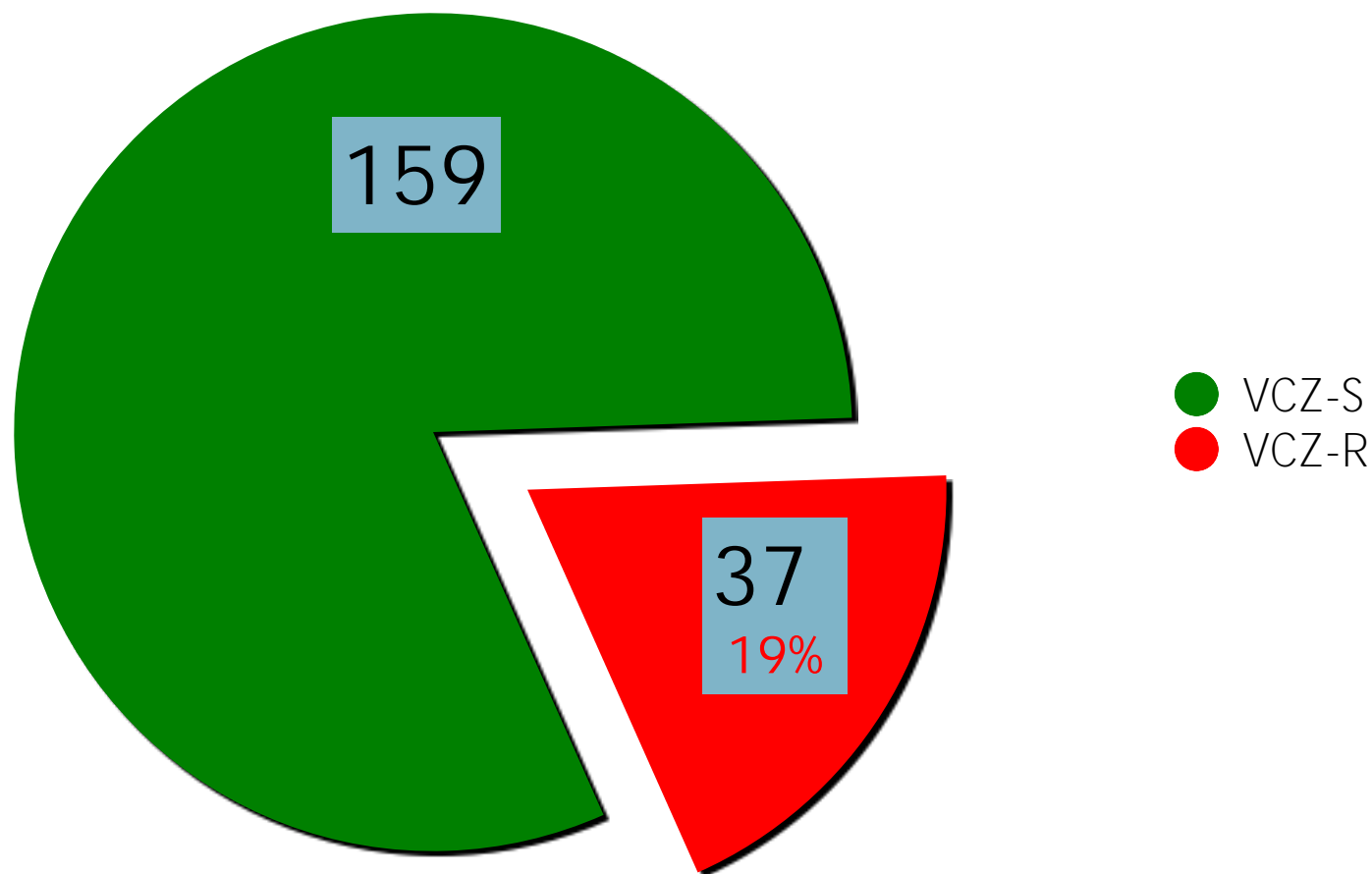
37 patients with VCZ-R invasive aspergillosis

Cyp51A resistance mutations
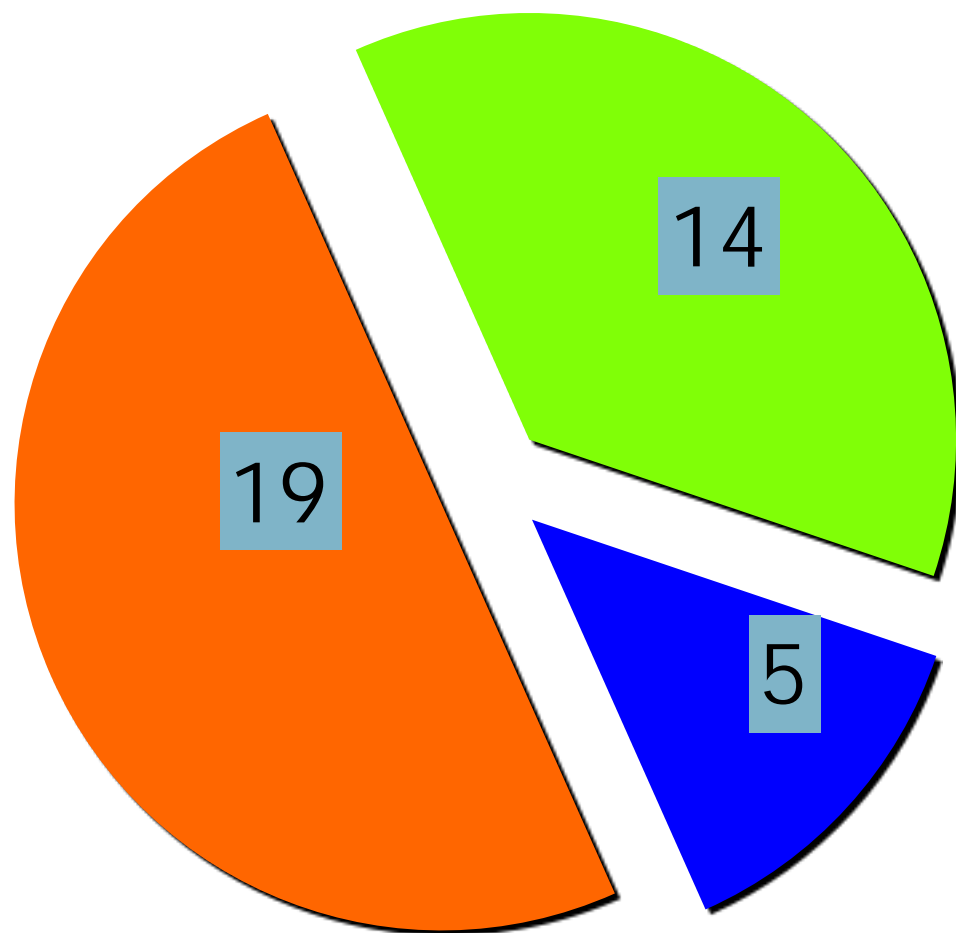
7 pts (19%) with mixed infection:

6 x S/R
1 x R/R

- 🟠 TR34
- 🟢 TR46
- 🔵 WT

19

14

5

87% environmental

A. fumigatus resistance phenotypes

VCZ (>2 mg/l)
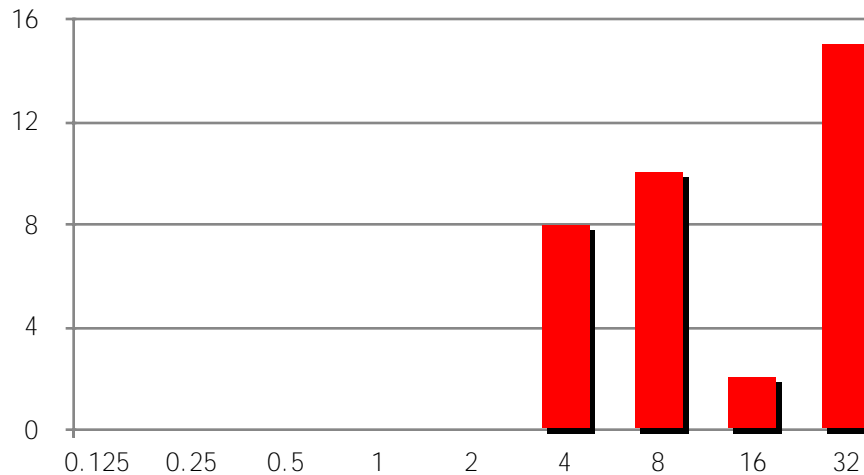
100% cross-resistance with isavuconazole
All susceptible to AmB

ITZ (> 2 mg/l)
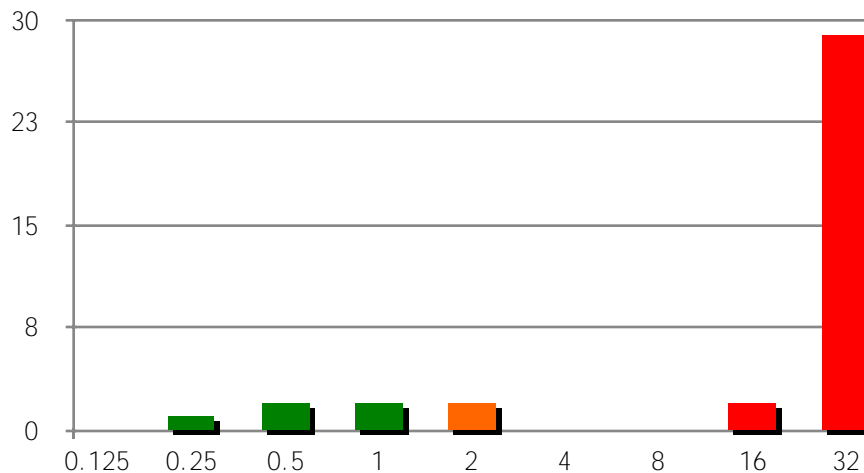
POS (>0.25 mg/l)
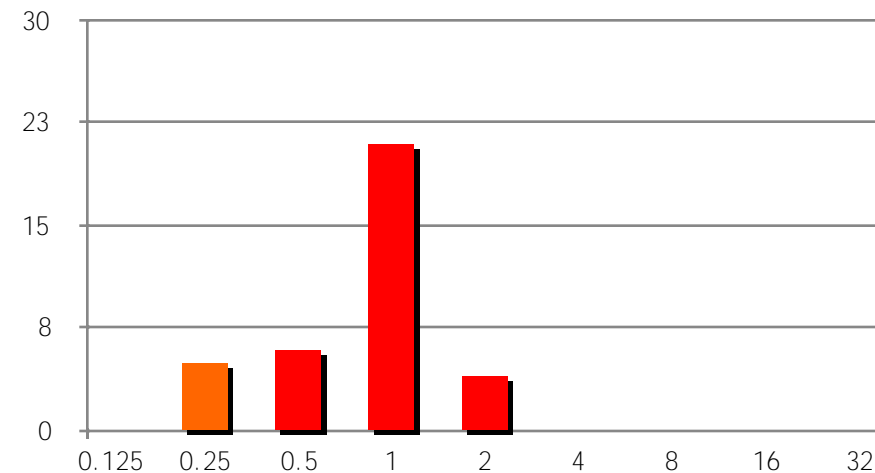
196 patients with invasive aspergillosis: underlying disease

Legend:
- Hematology
- SOT
- Autoimmune
- Lung disease
- Cancer
- Congenital ID
- Other
- None

103 53%
24
24
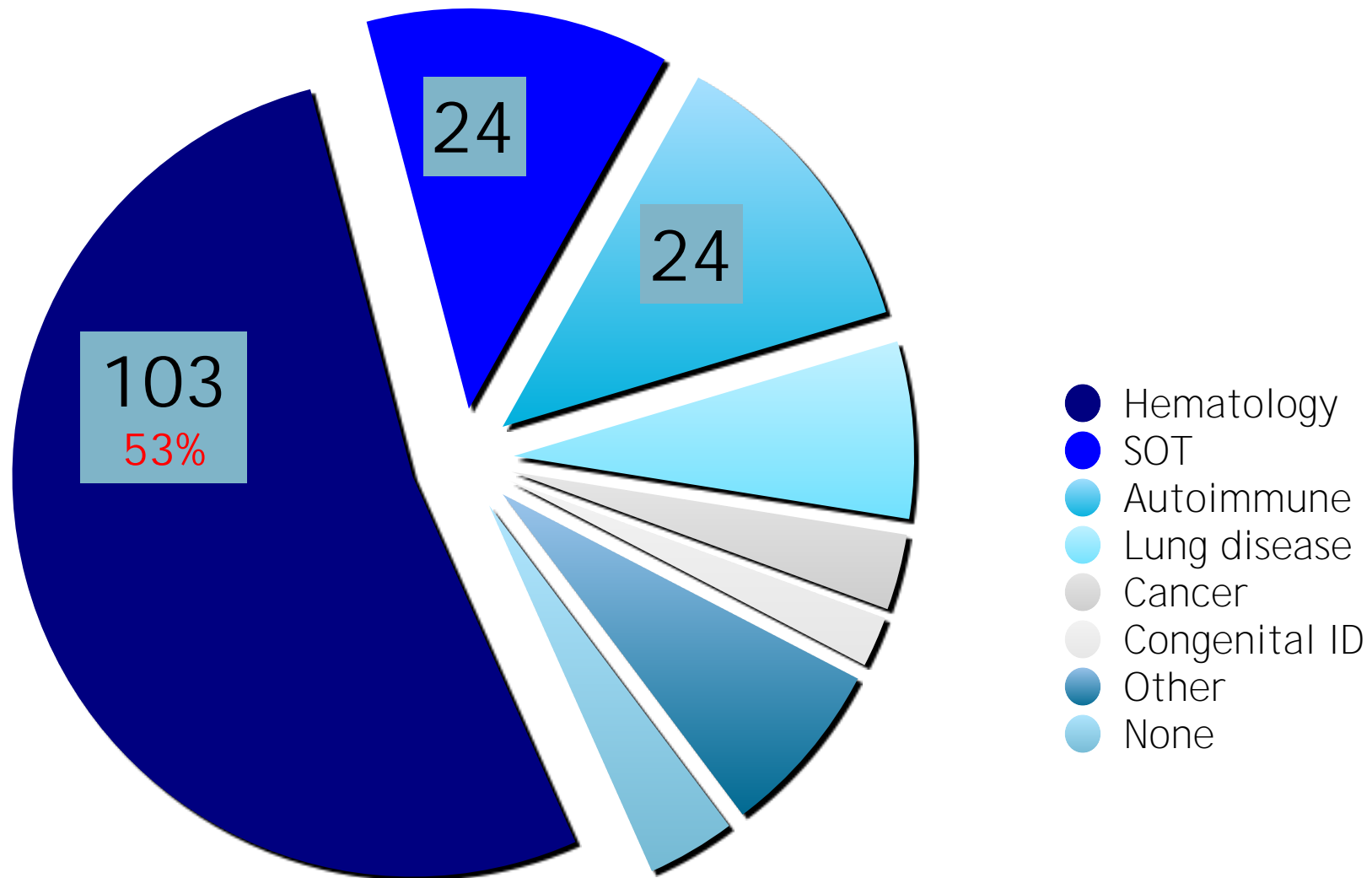
196 patients with invasive aspergillosis

ICU admission

non-ICU
ICU

NB AF therapy was started on the ICU in 59 pts
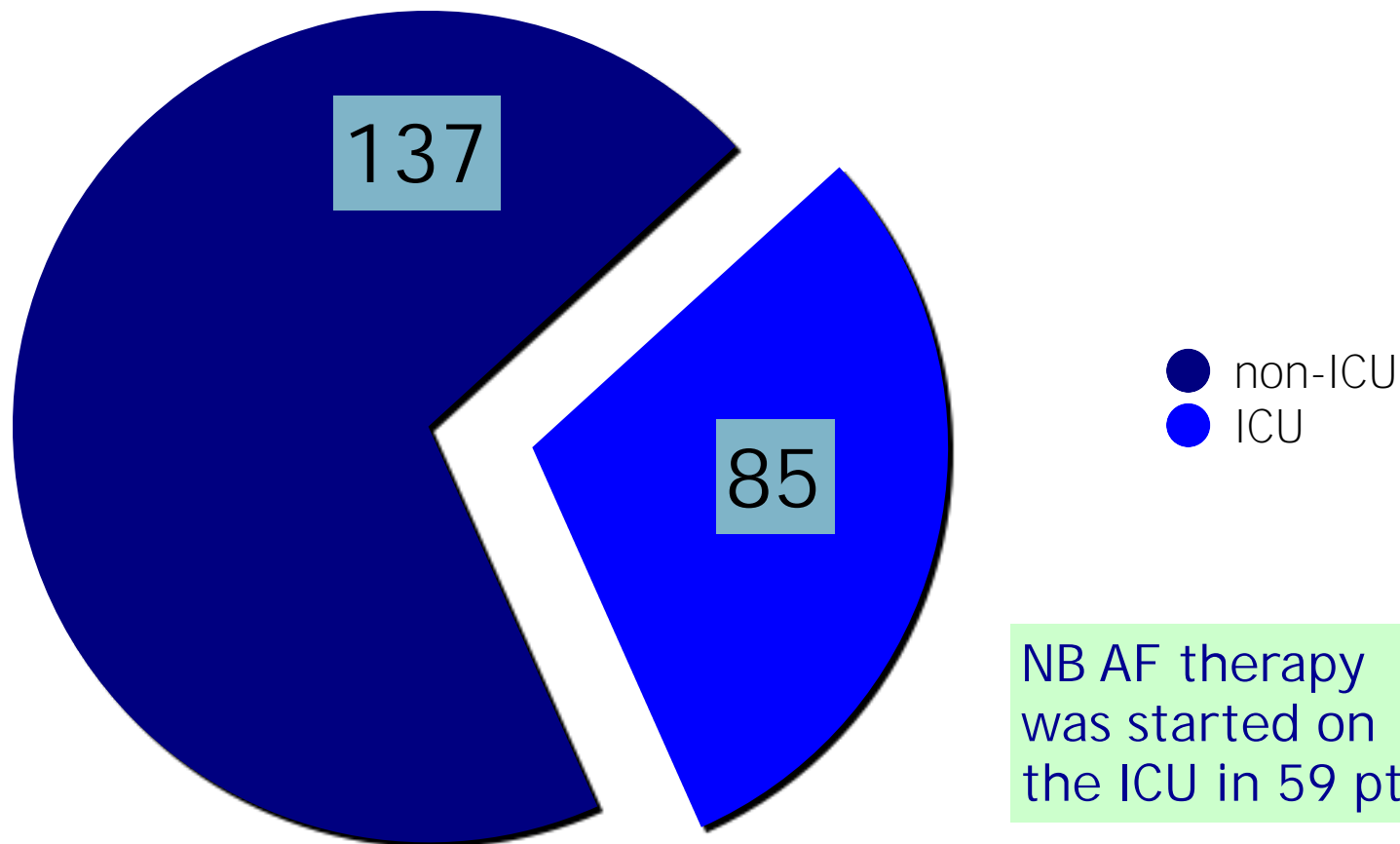
196 patients with invasive aspergillosis

Initial AF therapy

154
79%

27

- VCZ
- L-AmB
- VCZ+Ecand
- VCZ+L-AmB
- POS
- Other

P. Lestrade et al. Clin Infect Dis 2018; Epub Oct 11th
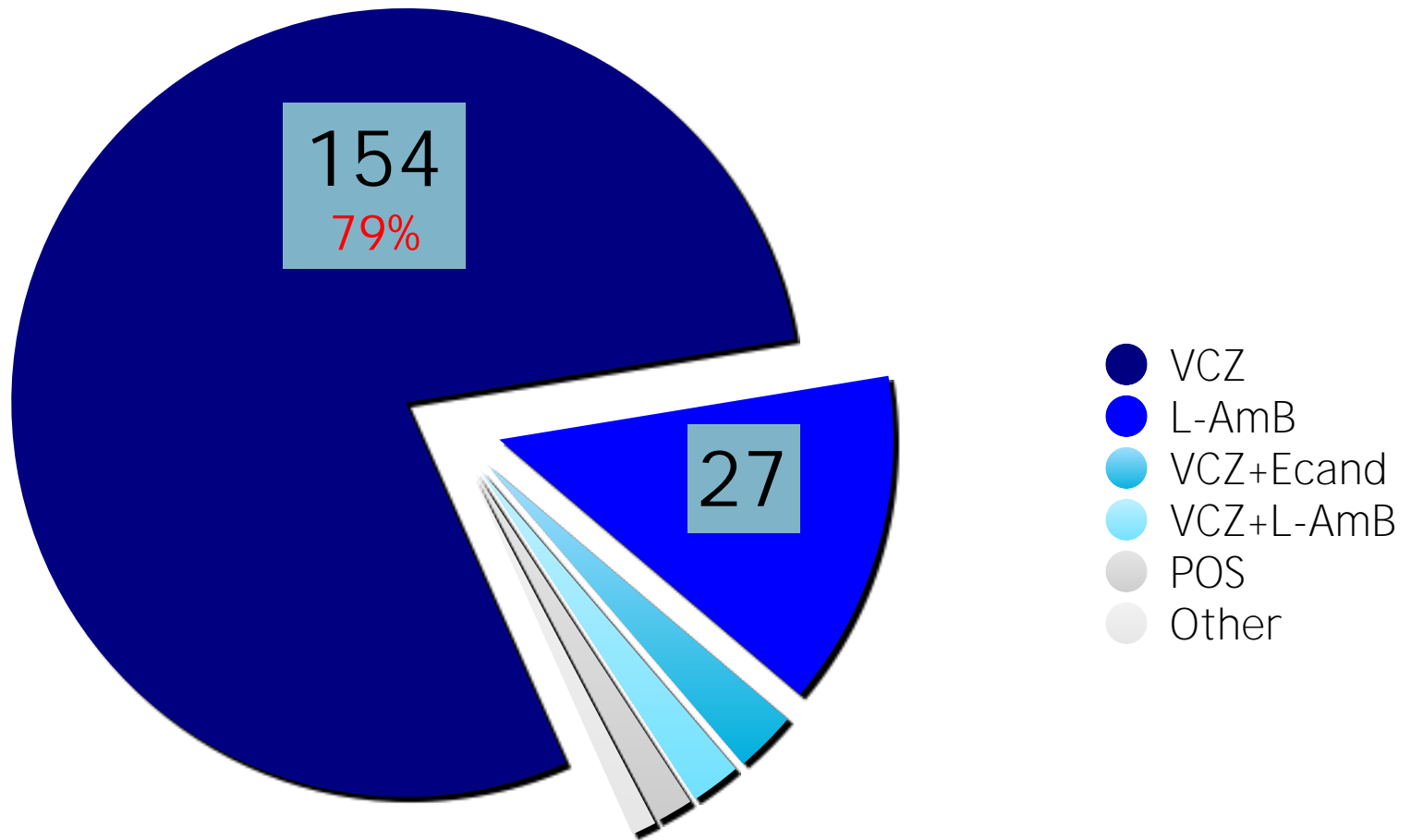
Overall mortality in vori R versus vori S
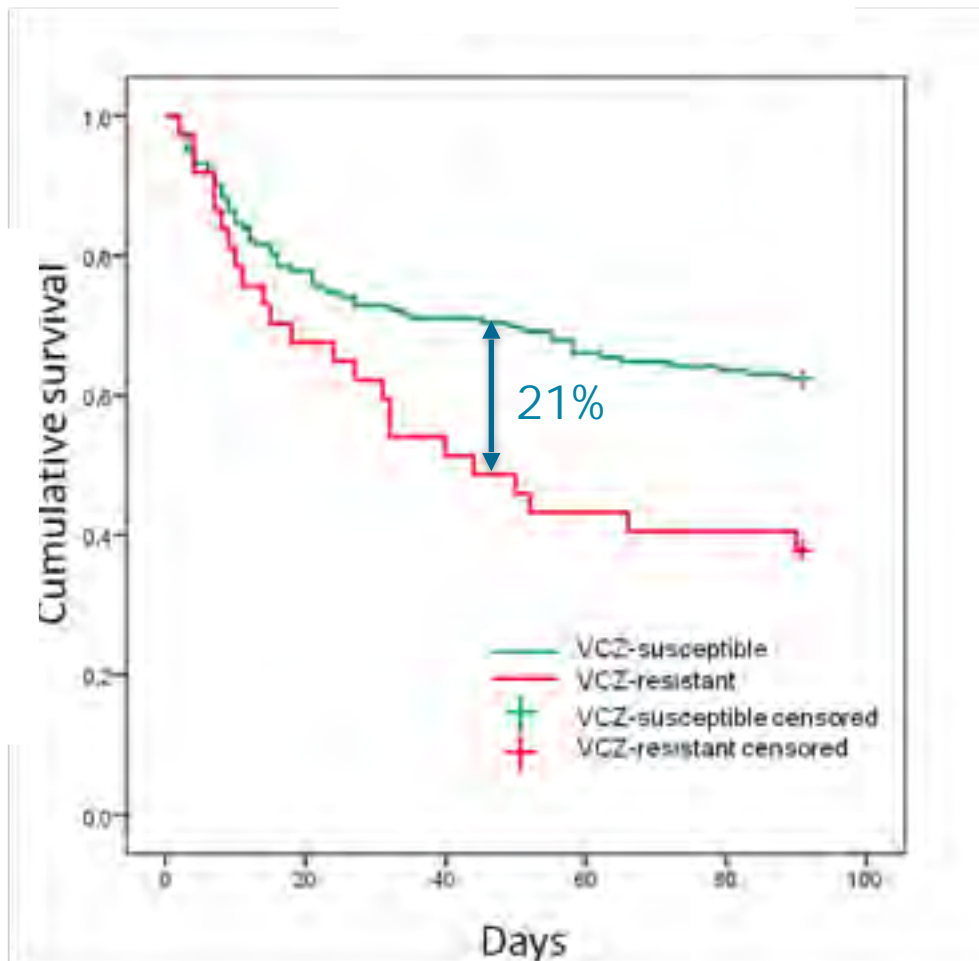
Mortality

Day 42

VCZ-S   28%
VCZ-R   49%
p=0.017

Day 90

VCZ-S   37%
VCZ-R   62%
p=0.0038

P. Lestrade et al. Clin Infect Dis 2018; Epub Oct 11th

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Overall mortality in vori R versus vori S in 59 ICU-patients
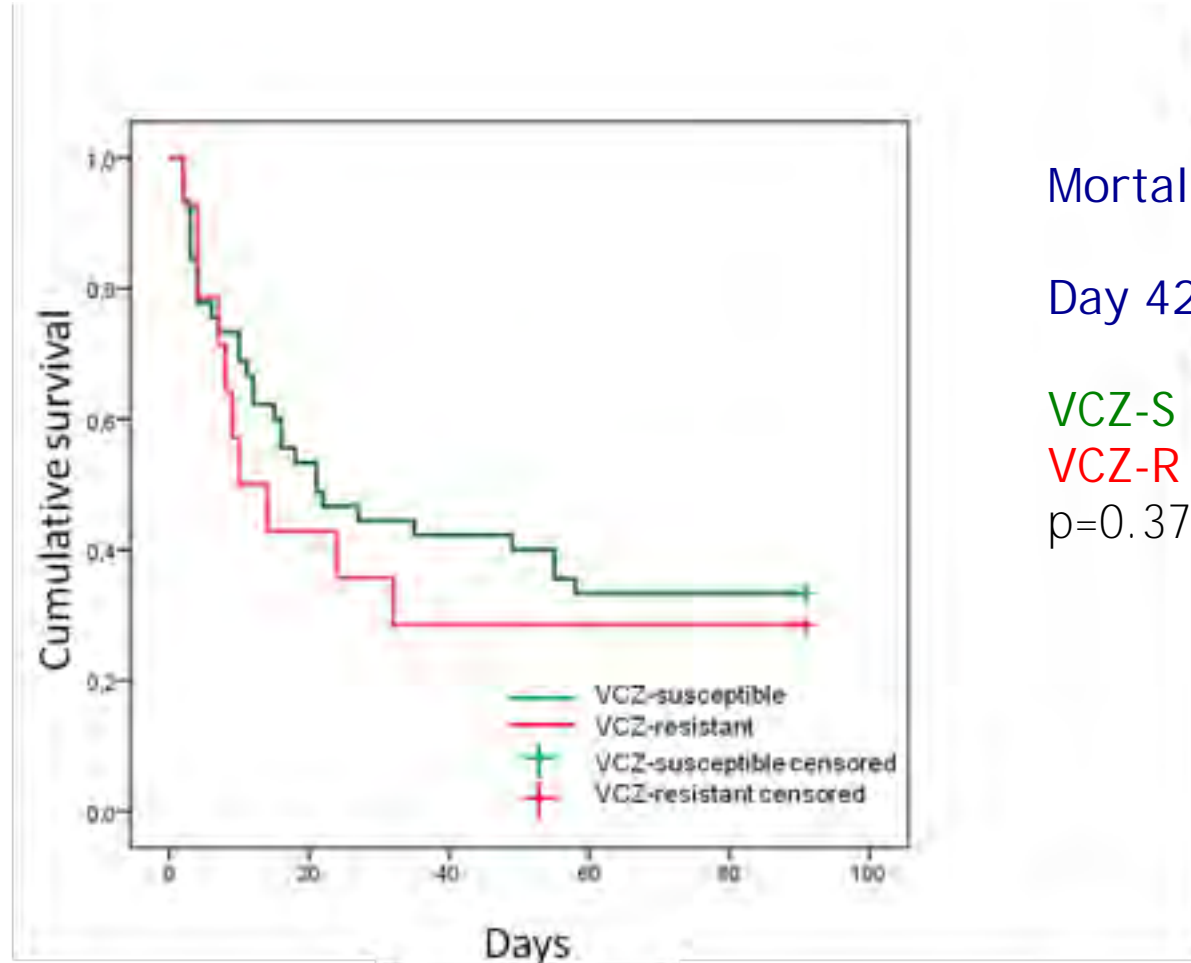
Mortality

Day 42

VCZ-S   65%
VCZ-R   71%
p=0.37

P. Lestrade et al. Clin Infect Dis 2018; Epub Oct 11th

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Overall mortality in vori R versus vori S in non-ICU patients
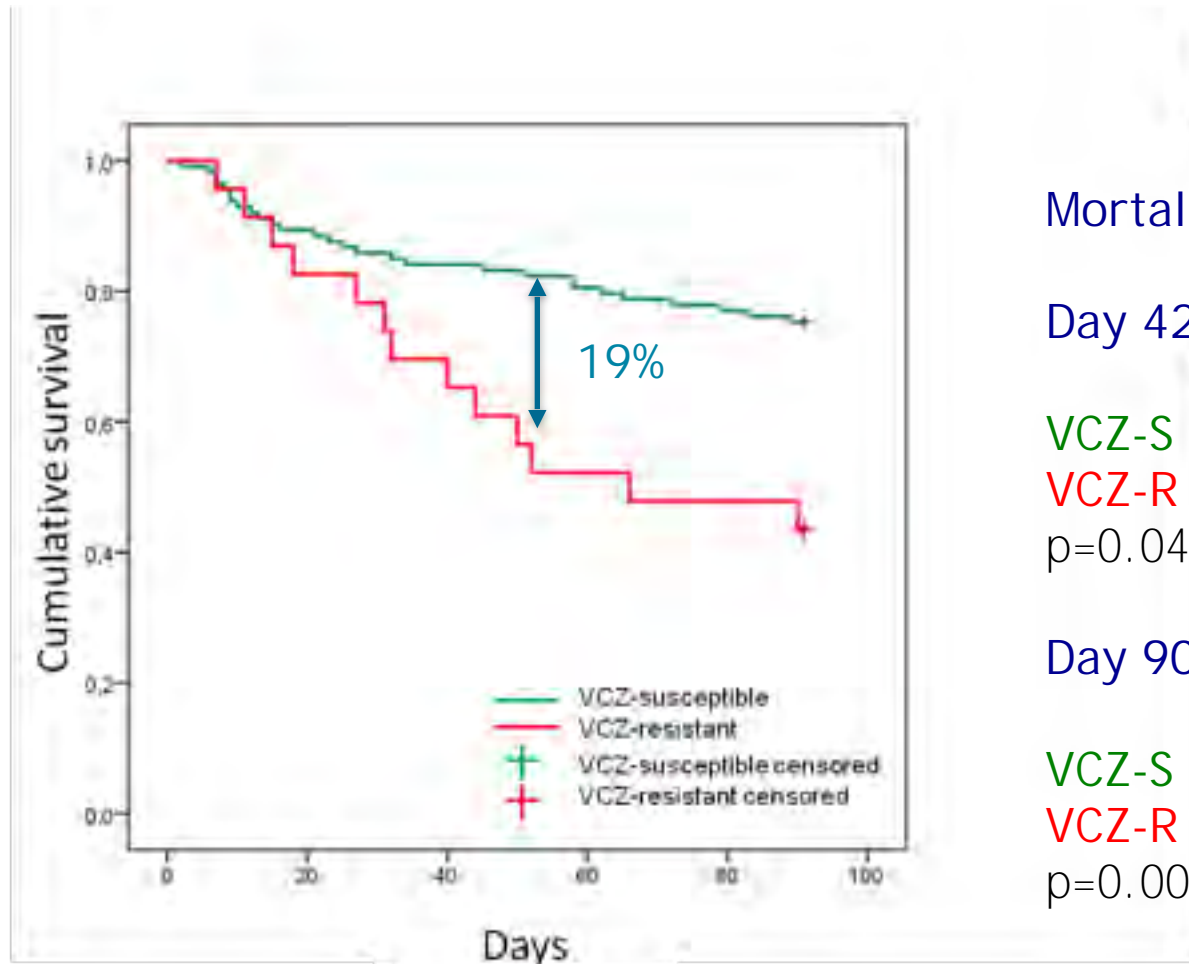
Mortality

Day 42

VCZ-S   16%
VCZ-R   35%
p=0.045

Day 90

VCZ-S   25%
VCZ-R   47%
p=0.002

P. Lestrade et al. Clin Infect Dis 2018; Epub Oct 11th

Azole-resistant associated 12-week mortality of IA in perspective

cumulative survival

voriconazole

70.8%   70.2%   70.7%   69.2%   72.3%   68%   60.6%   S

57.9%   54.9%   43%   R

back to pre-azole era

OLAT VCZ OLAT VCZ VCZ VCZ+AFG VCZ ISA VCZ-S VCZ-R

VCZ vs OLAT day 84
NEJM 2002

VCZ vs OLAT day 84
CID 2015

VCZ vs VCZ+AFG day 84
AIM 2015

VCZvs ISA day 84
Lancet 2016

day 84
CID 2018

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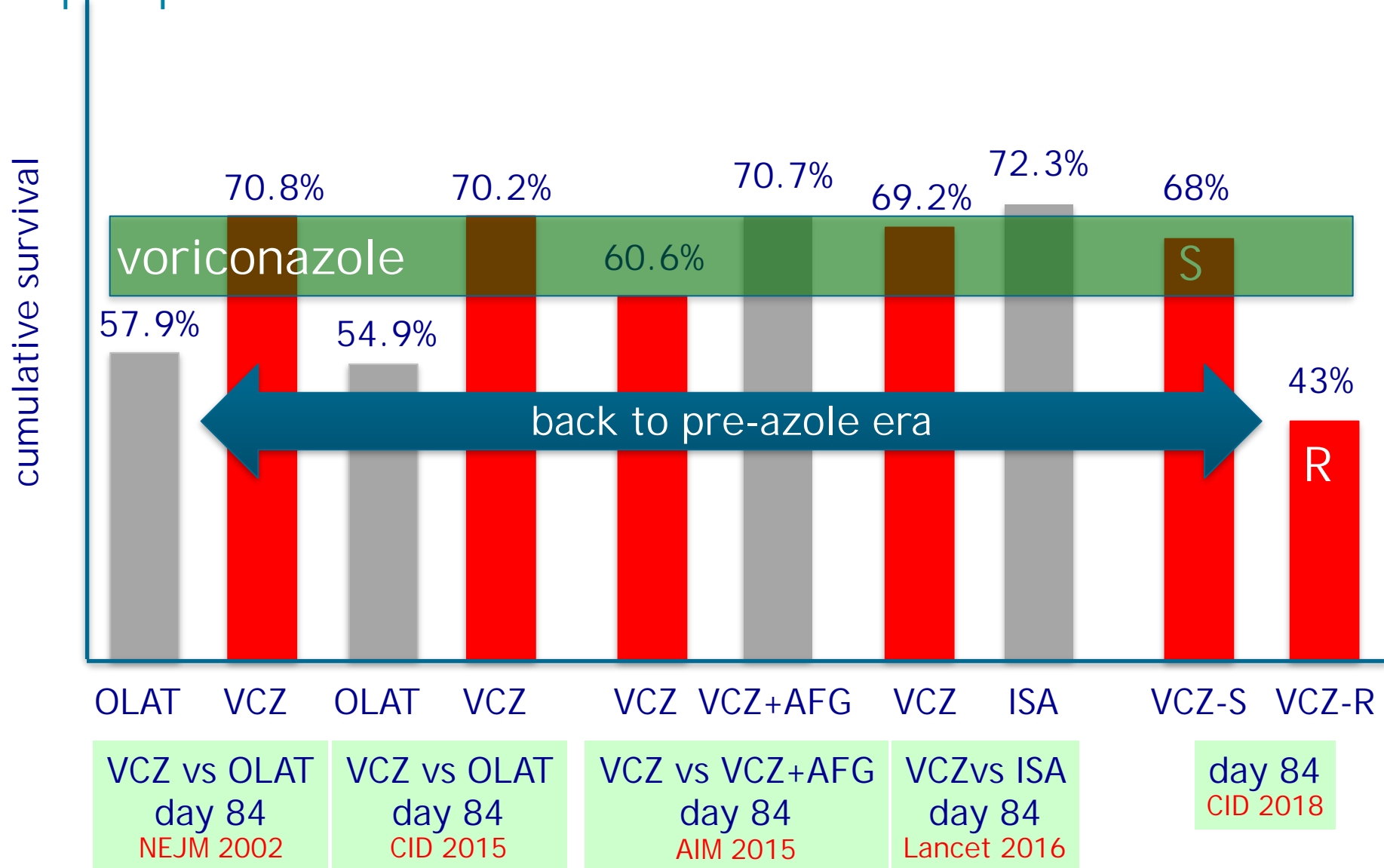
What to do - treatment? ICU Antimicrobial stewradship

KEEP CALM AND ESCALATE

first: VCZ or ISA

If resistance detected

L-AmB or combination

Resistance frequency

10%

KEEP CALM AND DE-ESCALATE

first: L-AmB or combination

If resistance excluded

VCZ or ISA

Dutch national guideline: invasive aspergillos

maximum effort to diagnose

MIC-testing / resistance PCR

Unknown	Confirmed S	Confirmed R	Mixed
VCZ / ISA + L-AmB VCZ / ISA + Ecand	VCZ / ISA	L-AmB	VCZ / ISA + L-AmB VCZ / ISA + Ecand
L-AmB	L-AmB / POS	Ecand	

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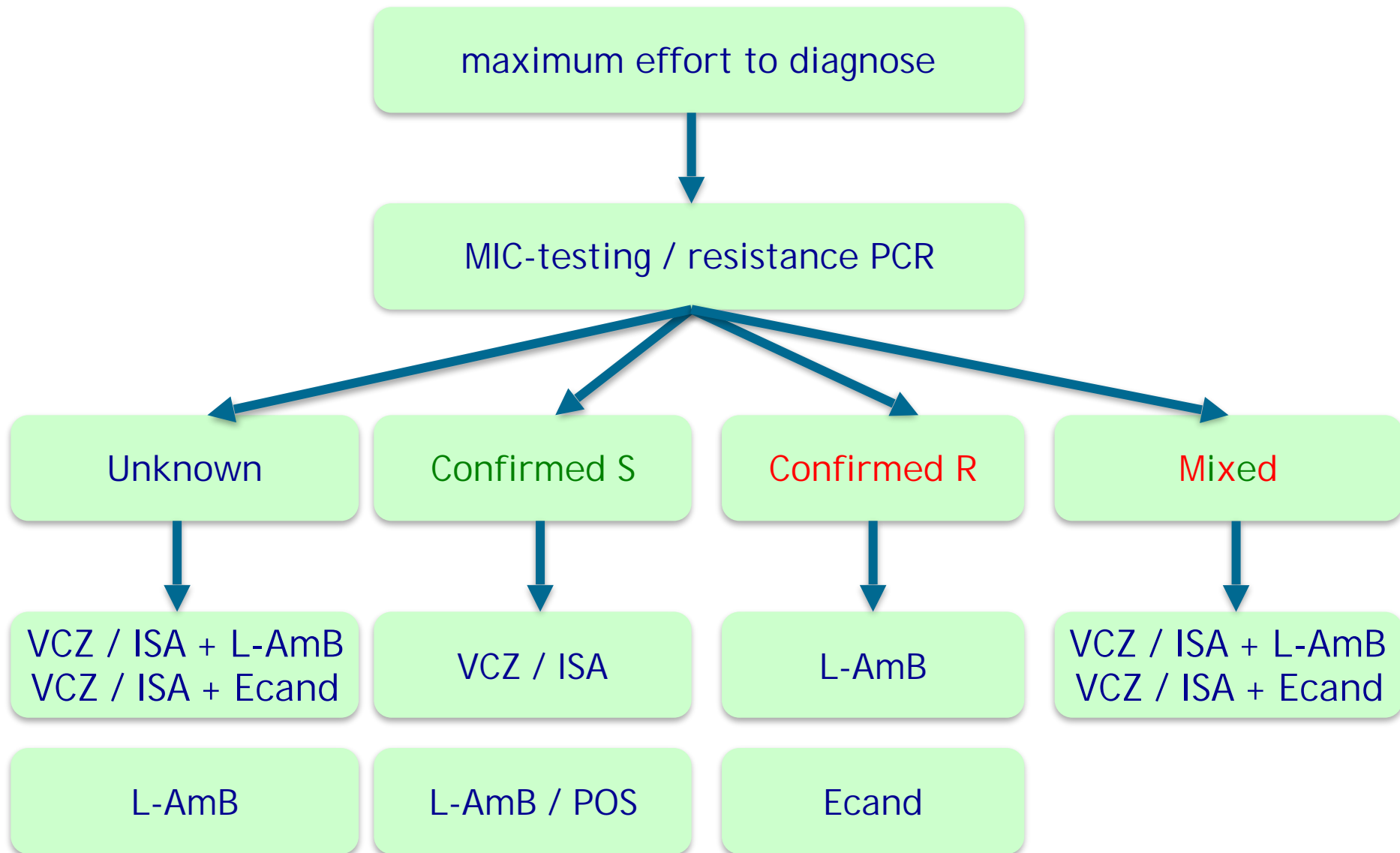
Conclusions

Resistance to VCZ was associated with excess mortality (21% to 33%) compared for VCZ susceptible infection

Inappropriate initial antifungal therapy was associated with increased mortality

These data support initial therapy that covers voriconazole resistant isolates with de-escalation once sensitivity becomes available

Most resistant isolates show an <u>environmental origin</u>: TR 34 and TR 46 mutations

The challenge of infection control in a small animal ICU

Joris H Robben PhD Dip ECVECC Dip ECVIM-CA

Universiteit Utrecht

Department of Clinical Sciences of Companion Animals
Faculty of Veterinary Medicine, Universiteit Utrecht

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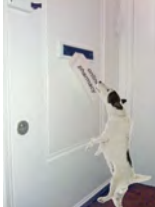
case

Kira

- dog ate 10 gram from a tube of 5% baclofen delivered by postal mail
- hypersalivation, ataxia ⇒ lateral recumbency, dysphoria/screaming, hypotonia, flaccid
- contact with National Poison Information Centre
- admitted to the ICU of the Dept. Clin. Sci. Comp. Anim. (DCSCA) of the Fac. Vet. Med. in Utrecht

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case

Tx of Kira

- oxygen Tx: oxygen cage
- sedation
- endotracheal intubation: secure airway + Tx O_2
- temperature control
- Intralipid® 20%
- mechanical ventilation for Tx of hypoventilation

Supportive care

- urinary catheter
- care of
  - mouth
  - pharynx
  - eyes
  - tracheal tube
- chlorhexidine based protocol

Acinetobacter Baumannii

Acinetobacter baumannii

The bacterium

- first isolation in the hospital of the DCSCA
- but not necessarily rare in veterinary medicine
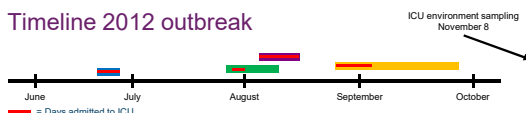
Acinetobacter baumannii

Timeline 2012 outbreak

ICU environment sampling November 8

June   July   August   September   October

= Days admitted to ICU

- Dog 1: 20/6 – 23/6: Jack Russell Terrier: baclofen poisoning → endotracheal tube

- Dog 2: 24/7 – 10/8: Dachshund: abscesses shoulder, renal failure, GI problems → urine

- Dog 3: 4/8 – 14/8: Labrador retriever: joint problems, renal failure → urine (in isolation ward of the ICU)

- Dog 4: 26/8 – 27/9: Chihuahua: bite trauma, abscessation → wound (end of Tx; with MRSP)

Leendertse M et al. 5th Symposium on Antimicrobial Resistance in Animals and the Environment ARAE 30 June – 3 July 2013, Ghent, Belgium, p 12

Conclusions

- Self limiting outbreak of MDR *A. Baumannii* at small animal ICU of the DCSCA.
- Four dogs with indistinguishable strains.
- Despite *OXA-51-like* + *ISAba1* gene combination: (intermediate) susceptible for meropenem + imipenem.
- Although, no advanced cleaning action took place, the strain could not be cultured from the ICU, which was in parallel with the fact that no additional clinical cultures were found positive for MDR *A. baumannii*.
- **The source of the outbreak was not identified**. The owner of the first dog was known to suffer from a diabetic foot with ulcers. The owner was not a known carrier of this *A. baumannii* strain.

Leendertse M et al. 5th Symposium on Antimicrobial Resistance in Animals and the Environment ARAE 30 June – 3 July 2013, Ghent, Belgium, p 12

Second outbreak

- 2014
- 3 dogs
- whole genome sequencing
  - two independent events
  - isolates diverged 30 years before

2012

2014
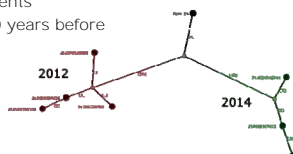
Zomer A et al. 6th Congress of European Microbiologists. Maastricht the Netherlands, June 7-11 2015, p 150.

ACVIM Consensus Statement on Therapeutic Antimicrobial Use in Animals and AMR

There are 3 main routes to reduce AMR development
- reduction of overall antimicrobial drug use
- improvement of antimicrobial drug use
- prevention of disease occurrence - use of infection control measures in veterinary hospitals

Weese JS et al. J Vet Intern Med 2015;29:487–498
ACVIM Consensus Statement on Therapeutic Antimicrobial Use in Animals and Antimicrobial Resistance

Slide 1

Antimicrobial (AM) stewardship program

- A-team
- education
- Tx guidelines
- discourage the use of AM for their non-antimicrobial effects

Working towards antimicrobial stewardship at the department of Clinical Sciences of Companion Animals at Utrecht University - defining the current baseline situation

F.P.J. van Bree*, E.M. Broens*, I.M. Gaijlevijk*, A.M. van Dongen*

Slide 2

Results baseline study

- Overall antimicrobial consumption (2013-2017): 4.44 defined daily doses animals (DDDAs) per year (2.60 DDDAs in general practice (2014)).
- AM use decreased from 2013 through 2016, but increased in 2017.
- Second-line drugs (54.0%), primarily amoxicillin/clavulanic acid (62.6%).
- 213 bacterial isolates (2013-2017) from 154 patients (4% of total samples) were labelled as multidrug resistant (MDR):
  - methicillin-resistant (MR) staphylococci (48.4%)
  - MDR Pseudomonas sp. (21.1%)
  - extended-spectrum beta-lactamase producing (ESBL) Enterobacteriaceae (18.8%).
- Eight different recommendations regarding the development of an antimicrobial stewardship program were formulated.

Slide 3

Recommendations

1. Optimize the on-site availability of the Companion Animal Formulary provided by the WVAB of the Dutch Royal Veterinary Association (KNMvD).
2. Optimize the on-site visibility of the AM classification according to Antibiotics Policy Working Group (WVAB).
3. Guidelines for focused AM prescription.
4. Guidelines for caring of patients carrying MDR bacteria.
5. Optimize multidisciplinary communication on AMs and MDR bacteria.
6. Optimize client and student education.
7. Optimize instructions on handling AMs and patients carrying MDR bacteria for para-veterinary staff members.
8. Education on AM administration and resistance for para-veterinary staff members.

Slide 1

Results baseline study - ICU

Working towards antimicrobial stewardship at the department of Clinical Sciences of Companion Animals at Utrecht University - defining the current baseline situation

- cascade (second-line) AM: amoxicillin/clavulanic acid
  - IV use
  - broad spectrum
- use in ICU often influences AM use in the rest of the clinic
  - result of discharge
  - educational effect: "what's good for ICU is good for us"

3.9%
12.7%
2.6%
80.8%

Intensive Care Unit (16.4%)

- first-line antimicrobial drug
- second-line antimicrobial drug
- third-line antimicrobial drug
- cascade antimicrobial drug

Slide 2
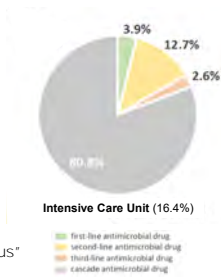
De-escalation: is it possible in ICU?

- short hospitalization period (<3 days) compared to human medicine
- limited availability narrow spectrum AM
- repeating diagnostic testing is expensive
- short treatment period

https://www.anythingpawsable.com/

Slide 3

Controlling disease without AMs

- not all sick animals have a bacterial infection
  - viral infections, immune-mediated conditions, pancreatitis, and neoplasia can cause fever and other signs attributed to bacterial infection → early and appropriate diagnostic testing
- not all bacterial infections need treatment with antimicrobials
  - treating underlying condition you may treat the (secondary) bacterial infection more effectively
  - consider other treatment modalities than AM
- worsening disease state in critically ill animals does not always necessitate escalation of Tx AM

Weese JS et al. J Vet Intern Med 2015;29:487–498

Canadian Committee on Antibiotic Resistance
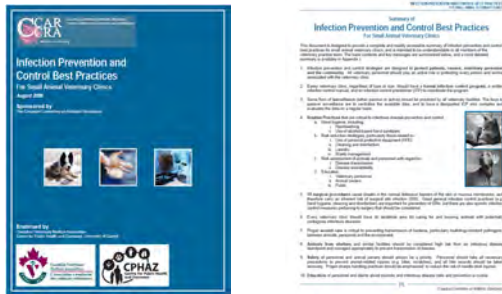
Basic Principles

- Decrease exposure
  - reduce sources
    - people, patients, environment, i.e. facility and equipment
  - decrease transmission
- Decrease susceptibility
- Increase resistance

Transmission routes

community

practice

admission

1

2

3

Antimicrobial resistance

JAMA | Original Investigation | CARING FOR THE CRITICALLY ILL PATIENT

Decontamination Strategies and Bloodstream Infections With Antibiotic-Resistant Microorganisms in Ventilated Patients
A Randomized Clinical Trial

Bastiaan H. Wittekamp, MD, PhD; Nienke L. Plantinga, MD, PhD; Ben S. Cooper, PhD; Joaquin López-Contreras, MD, PhD; Pere Coll, MD, PhD; Jordi Mancebo, MD, Matt P. Wise, MD, PhD; Matt P. S. Morgan, MD, PhD; Pieter Depuydt, MD, PhD; Jerina Boelens, MD, PhD; Thierry Dugernier, MD, PhD; Valérie Verhaegen, PhD; Philippe G. Jorens, MD, PhD; Walter Verbrugghe, MD; Surbhi Malhotra-Kumar, PhD; Pierre Damas, MD, PhD; Cécile Meex, PhD; Kris Leleu, MD; Anne-Marie van den Abeele, MD; Ana Olga Gomes Pimenta de Matos, MSc; Saïa Fernández Méndez, MD; Andrea Vergara Gómez, MSc; Viktorija Tomic, MD, PhD; Franc Sifrer, MD; Esther Villarreal Tello, MD; Jesus Ruiz Ramos, PhD; Irene Kruger, MD; Claudia Santos, MD; Roberta H. H. Sperning, Msc; Patricia Cappuccino, BSc; Giuseppe Nardi, MD; Christian Brun-Buisson, MD, PhD; Marc J. M. Bonten, MD, PhD

Conclusion

"Among patients receiving mechanical ventilation in ICUs with moderate to high antibiotic resistance prevalence, use of CHX mouthwash, SOD, or SDD was not associated with reductions in ICU-acquired bloodstream infections caused by MDRGNB compared with standard care."

Wittekamp BH et al. JAMA. 2018;320(20):2087-2098.

Hierarchy of infection control measures

- Personal control measures
- Administrative controls
- Engineering controls

Engineering controls

- planning and design of new and existing facilities
- less attention in veterinary medicine
- *very important* for staff compliance

facility design

ICU design

facility design

ICU design

Small Animal Critical Care Medicine (Second Edition)

2015, Pages 1073-1081

Chapter 209 - Intensive Care Unit Facility Design

Jens H. Robben DVM, PhD, DECVIM-CA

Show more

https://doi.org/10.1016/B978-1-4557-0306-7.00209-8
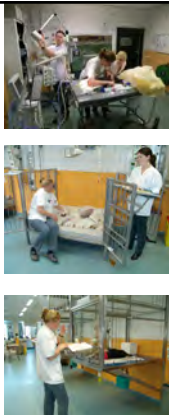
infection prevention and control

ICU Design

- have enough space: invest in "empty"
- have good lighting and open visual lines
- pay attention to ergonomics
- separate functions
- have enough storage facilities
- isolate the "uncleanable"

infection prevention and control

Floor

infection prevention and control

Hand hygiene

facility design

Hand hygiene

Serratia Marcescens

NEW

OLD

Outcome case

Day 1 after admission
- weaning of mechanical ventilator was unsuccessful

Day 2
- dexmedetomidine
  (1 µg/kg/hr) was added as sedative: recovery was successful but dog developed tremors that made her body temperature go up to 40°C
- Tx cooling with wet towels

Day 3
- discharge

What does veterinary medicine need?

- resources
  - finances
  - information
  - professionals (hygienists)
  - staff (nurses)
  - small spectrum AM
- awareness
  - professionals
  - lay people → owner
- veterinary medicine needs to be inventive

Is small animal Intensive Care in trouble?

- median length-of-stay (LOS): 2-3 days
- patients are less sick/debilitated.

Thank you for your attention!

Questions?

Antimicrobial Therapy Guidelines in Humans

J. M. Blondeau, M.Sc., Ph.D., RSM(CCM), SM(AAM), SM(ASCP), FCCP
Head, Clinical Microbiology
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Royal University Hospital & Saskatchewan Health Authority
Adjunct Professor of Microbiology and Immunology
Clinical Associate Professor of Pathology
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University of Saskatchewan
Saskatoon, Saskatchewan, Canada

Introduction

- **International practice (therapy) guidelines**
  - Arose out of a need to standardize treatment for common infections based on the "best" evidence available
  - Responsible antimicrobial use
  - Impact antimicrobial resistance (?)
    - Considers epidemiology of AMR in recommendations
  - Define "length of therapy"…including shorter durations of therapy…without compromising patient care

The Alarm….

- **AMR…currently…700,000 deaths/year (est)**
- **Unchecked**
  - **10 million deaths/year by 2050 (WHO)**
- **Strategies**
  - **"One Health" – reduce Abx use world wide**
    - **Humans, animals**
  - **Priority pathogens**
  - **New drug development**
  - **Therapeutic guidelines ensuring minimum standard of care (evidence based)**

World Health Organization

WHO PRIORITY PATHOGENS LIST
FOR R&D OF NEW ANTIBIOTICS

Priority 1: CRITICAL[#]

Acinetobacter baumannii, carbapenem-resistant

Pseudomonas aeruginosa, carbapenem-resistant

Enterobacteriaceae*, carbapenem-resistant, 3rd generation cephalosporin-resistant

Priority 2: HIGH

Enterococcus faecium, vancomycin-resistant

Staphylococcus aureus, methicillin-resistant, vancomycin intermediate and resistant

Helicobacter pylori, clarithromycin-resistant

Campylobacter, fluoroquinolone-resistant

Salmonella spp., fluoroquinolone-resistant

Neisseria gonorrhoeae, 3rd generation cephalosporin-resistant, fluoroquinolone-resistant

Priority 3: MEDIUM

Streptococcus pneumoniae, penicillin-non-susceptible

Haemophilus influenzae, ampicillin-resistant

Shigella spp., fluoroquinolone-resistant

[#] *Mycobacteria* (including *Mycobacterium tuberculosis*, the cause of human tuberculosis), was not subjected to review for inclusion in this prioritization exercise as it is already a globally established priority for which innovative new treatments are urgently needed.

* Enterobacteriaceae include: *Klebsiella pneumonia, Escherichia coli, Enterobacter* spp., *Serratia* spp., *Proteus* spp., and *Providencia* spp, *Morganella* spp.

High mortality
-decreasing ABX
-IPC issue

Antimicrobial Resistance

- **Global pandemic**

- **Multidrug-resistant strains**

- **Drugs of last resort**

- **Combination therapy being explored…not necessarily for clinical outcome but for resistance prevention**

- **Global Organizations/Societies/Governments**
  - **Statements regarding resistance and prevention strategies**

Government of Canada's response to antimicrobial resistance.
We are working to prevent and control the spread of antimicrobial resistance (AMR). Learn how the Government of Canada monitors AMR and supports appropriate antimicrobial (antibiotic) use (AMU) in both humans and animals.

Antimicrobial Resistance: 2005-2017+

Environment   Patients   Health Care Providers

Patient

MICU

SICU

Neurology

Oncology

Dialysis

Emergency

Resistant Organisms

Enterobacteriaece
P. aeruginosa
Afermentors
Staph CoN
S. aureus - MSSA
        - MRSA
        - GRSA
Enterococcus – VRE
ESBL+

Community

S. pneumoniae - PRSP
S. pneumoniae - FQR*
S. aureus – MRSA, FQR
H. influenzae - TMP/SMX R
E. coli - Amp R, TMP/SMX R; ESBL+

Long Term Care

Enterobacteriaece - ESBL+

Animal-Human Interactions

*Older agents, i.e. ciprofloxacin, levofloxacin

Blondeau, JM, 2017

EDITORIAL

Antimicrobial resistance & 'Man's best friend': what they give to us we might be giving right back

"Antimicrobial resistance follows antimicrobial use. . ."

Joseph M Blondeau*,1

"*direct contact is likely the quickest and easiest way by which bacteria are transferred in either direction between humans and animals......*"

Schwartz *et al*, Veterinary Dermatology 2017; 28(1):82-e19

Blondeau et al, ICOHAR, 2019 Utrecht…transmission of *S. pseudintermedius* from family pets (dogs) to oncology patients

The Superbug Challenge

Acronym	Definition	Screening	Bacteria	Significance
CRE	Carbapenemase resistant Entero	Carb resistant	Kleb, Pseud, Entero	R to carbapenems
ESBL	Extended spectrum beta-lactamase cephalosporins	R to 3rd gen cephalosporins*	*E. coli*, *Kleb*. Spp. *Enterobacteriaceae*	R to most
MRSA	methicillin R *S. aureus*	R to oxacillin PCR – *mec* A Chromo agar Cefoxitin R	*S. aureus*	R to all beta-lactams**
VRE	vancomycin R *Enterococcus* van genes	Van screen plate Chromo agar	*Enterococcus* spp.	R to vancomycin PCR-
VISA	Vancomycin inter *S. aureus*	reduced S to Van	*S. aureus*	reduced S to van
VRSA	Vancomycin R	resistance to Van	*S. aureus*	R to vancomycin

*cefotaxime, cefpodixime, ceftriaxone, ceftazidime
** penicillins, cephalosporins, carbapenems, monobactams

Blondeau, JM, 2013, STAT – Steps to Antimicrobial Therapy, Companion Animals, 2nd Edition: North American Compendium

8

Contributors to resistance

- **Overuse**
- **Non-clinical use**
- **Under dosing**
- **Prolonged therapy**
- **Incorrect therapy**
- **Ease of use (minimal side effects)**
- **Patient expectations**
  - **Vet Med-Owner expectations (?)**

- **Susceptibility testing – underestimates**
- **Breakpoints ?**
  - **Laboratory**
  - **clinical**
- **Prophylactic use without clear benefits**
- **Empiric use in non-critically ill patients**

Question…?

Q. **Is their a disconnect between human and veterinary medicine regarding the diagnosis and treatment of infectious diseases:**

R. **Perhaps**

- **Choice of initial empiric antibiotics**

- **Duration of therapy**

- **Rapid Diagnostics**

  - **Minutes to hours for organism identification and resistance genes detection**

  - **WGS not yet there for rapid diagnostics but will get there**

Human Medicine…and what about Veterinary Medicine!!!

Editorial

The 24-h clinical microbiology service is essential for patient management

Joseph M Blondeau*,[1,2] & Evgeny A Idelevich[3]

[1]Department of Clinical Microbiology, Royal University Hospital & Saskatchewan Health Authority; Saskatoon, Saskatchewan, Canada

[2]Departments of Microbiology & Immunology, Pathology & Ophthalmology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

[3]Institute of Medical Microbiology, University Hospital Münster, Münster, Germany

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"optimal patient care requires access to necessary laboratory testing including clinical microbiology. A rethinking of hours of operation is required to shorten time to accurate result reporting."

Guidelines for the diagnosis and antimicrobial therapy of canine superficial bacterial folliculitis (Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases)

Andrew Hillier*, David H. Lloyd†, J. Scott Weese‡, Joseph M. Blondeau§, Dawn Boothe¶, Edward Breitschwerdt**, Luca Guardabassi††, Mark G. Papich**, Shelley Rankin‡‡, John D. Turnidge§§ and Jane E. Sykes¶¶

infection. Most studies evaluating the efficacy of AMDs indicate that SBF infections are resolved after 3 weeks or more of systemic AMD treatment; rapid improvement over the first 1–2 weeks is typically observed, but resolution of all lesions and prevention of rapid recurrence of disease requires 3–6 weeks of treatment.[17–22,28] Although there is no significant difference in the likelihood of resolution of MSSP after 3–4 weeks of systemic AMD treatment compared with MRSP infections, it has been reported that MRSP infections took longer to treat compared with MSSP infections.[60]

Journal of Veterinary Internal Medicine

Antimicrobial use Guidelines for Treatment of Respiratory Tract Disease in Dogs and Cats: Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases

M.R. Lappin, J. Blondeau, D. Boothe, E.B. Breitschwerdt, L. Guardabassi, D.H. Lloyd, M.G. Papich, S.C. Rankin, J.E. Sykes, J. Turnidge, and J.S. Weese

Monitoring Treatment of Bacterial Pneumonia

The current recommendation in most veterinary textbooks is to treat bacterial pneumonia for 4–6 weeks, but evidence to support this duration of treatment in either cats or dogs is lacking. Although such lengthy courses of antimicrobial treatment might be necessary for some animals with severe pulmonary involvement or

Antimicrobial Use Guidelines for Treatment of Urinary Tract Disease in Dogs and Cats: Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases

J. Scott Weese,[1] Joseph M. Blondeau,[2] Dawn Boothe,[3] Edward B. Breitschwerdt,[4] Luca Guardabassi,[5] Andrew Hillier,[6] David H. Lloyd,[7] Mark G. Papich,[4] Shelley C. Rankin,[8] John D. Turnidge,[9, 10] and Jane E. Sykes[11]

Adequate evidence regarding duration of treatment is lacking, precluding the ability to make a specific recommendation for treatment duration. Typically, uncomplicated UTIs are treated for 7–14 days. However, the Working Group acknowledges the likelihood that a shorter treatment time (≤7 days) may be effective. Accordingly, in the absence of objective data, 7 days of appropriate antimicrobial treatment is reasonable. Clinical trials supporting shorter durations for treatment of UTIs in dogs and cats are strongly encouraged.

International Clinical Practice Guidelines for the Treatment of Acute Uncomplicated Cystitis and Pyelonephritis in Women: A 2010 Update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases

Kalpana Gupta,[1] Thomas M. Hooton,[2] Kurt G. Naber,[9] Björn Wullt,[10] Richard Colgan,[3] Loren G. Miller,[4] Gregory J. Moran,[5] Lindsay E. Nicolle,[8] Raul Raz,[11] Anthony J. Schaeffer,[6] and David E. Soper[7]

Table 1. Strength of Recommendations and Quality of Evidence

Category/grade	Definition
Strength of recommendation	
A	Good evidence to support a recommendation for or against use
B	Moderate evidence to support a recommendation for or against use
C	Poor evidence to support a recommendation
Quality of evidence	
I	Evidence from ⩾1 properly randomized, controlled trial
II	Evidence from ⩾1 well-designed clinical trial, without randomization; from cohort or case-controlled analytic studies (preferably from >1 center); from multiple time-series; or from dramatic results from uncontrolled experiments
III	Evidence from opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees

NOTE. Data are from the periodic health examination. Canadian Task Force on the Periodic Health Examination. Health Canada, 1979. Adapted and Reproduced with the permission of the Minister of Public Works and Government Services Canada, 2009 [32].

Woman with acute uncomplicated cystitis
- Absence of fever, flank pain, or other suspicion for pyelonephritis
- Able to take oral medication

→ No → Consider alternate diagnosis (such as pyelonephritis or complicated UTI) & treat accordingly (see text)

↓ Yes

Can one of the **recommended antimicrobials*** below be used considering:
Availability
Allergy history
Tolerance

Nitrofurantoin monohydrate/macrocrystals 100 mg bid X 5 days
(avoid if early pyelonephritis suspected)

OR

Trimethoprim-sulfamethoxazole 160/800 mg (one DS tablet) bid X 3 days
(avoid if resistance prevalence is known to exceed 20% or if used for UTI in previous 3 months)

OR

Fosfomycin trometamol 3 gm single dose
(lower efficacy than some other recommended agents; avoid if early pyelonephritis suspected)

OR

Pivmecillinam 400 mg bid x 5 days
(lower efficacy than some other recommended agents; avoid if early pyelonephritis suspected)

→ No → Fluoroquinolones
(resistance prevalence high in some areas)

OR

β-lactams
(avoid ampicillin or amoxicillin alone; lower efficacy than other available agents; requires close follow-up)

*The choice between these agents should be individualized and based on patient allergy and compliance history, local practice patterns, local community resistance prevalence, availability, cost, and patient and provider threshold for failure (see Table 4)

↓ Yes

Prescribe a recommended antimicrobial

Table 4. Treatment Regimens and Expected Early Efficacy Rates for Acute Uncomplicated Cystitis

Drug (dosage)	Mean percentage (range)		Common side effects	References
	Estimated clinical efficacy[a,b]	Estimated microbiological efficacy[b]		
Nitrofurantoin monohydrate/ macrocrystals (100 mg twice daily for 5–7 days)	93 (84–95)	88 (86–92)	Nausea, headache	[36, 37, 39]
Trimethoprim-sulfamethoxazole (160/800 mg twice daily for 3 days)	93 (90–100)	94 (91–100)	Rash, urticaria, nausea, vomiting, hematologic	[36, 37]
Fosfomycin trometamol (3 g single-dose sachet)	91	80 (78–83)	Diarrhea, nausea, headache	[39, 40]
Pivmecillinam (400 mg twice daily for 3–7 days)	73 (55–82)	79 (74–84)	Nausea, vomiting, diarrhea	[29, 43]
Fluoroquinolones (dose varies by agent; 3–day regimen)[c]	90 (85–98)	91 (81–98)	Nausea/vomiting, diarrhea, headache, drowsiness, insomnia	[35, 43, 44, 46–52]
β-lactams (dose varies by agent; 3–5 day regimen)[d]	89 (79–98)	82 (74–98)	Diarrhea, nausea, vomiting, rash, urticaria	[38, 52, 54]

[a] Efficacy rates refer to cure rates on the visit closest to a 5–9-day period following treatment, and are averages or ranges calculated from clinical trials discussed in the text.

[b] Estimated clinical efficacy and microbiological efficacy rates should not necessarily be compared across agents, because study design, efficacy definition, therapy duration, and other factors are heterogeneous. Studies represent clinical trials published since publication of the 1999 Infectious Disease Society of America guidelines so as to represent efficacy rates that account for contemporary prevalence of antibiotic-resistant uropathogens. Note that efficacy rates may vary geographically depending on local patterns of antimicrobial resistance among uropathogens. See text for details.

[c] Data on fluoroquinolones are compiled from regimens of ofloxacin, norfloxacin, and ciprofloxacin from the referenced clinical trials and not other fluoroquinolones that are no longer commercially available. See text for details.

[d] Data on βlactams data cited are derived from clinical trials examining second and third generation cephalosporins and amoxicillin-clavulanate. See text for details.

Optimal duration of antibiotic therapy for uncomplicated urinary tract infection in older women: a double-blind randomized controlled trial

Thomas Vogel, René Verreault, Marie Gourdeau, Michèle Morin, Lise Grenier-Gosselin, Louis Rochette

Results: The proportion of patients with bacterial eradication at 2 days after treatment was 98% (91/93) in the 3-day group and 93% (83/89) in the 7-day group ($p = 0.16$). The frequency of adverse events, including drowsiness, headache, nausea or vomiting, and loss of appetite, was significantly lower in the 3-day group.

Interpretation: These results suggest that a 3-day course of antibiotic therapy is not inferior to a 7-day course for treatment of uncomplicated symptomatic UTI in older women, and that the shorter course is better tolerated.

Table 2: Therapeutic efficacy at 2 days and 6 weeks after completion of treatment

Measure of efficacy	No. (and %) of subjects		p value
	3-day group	7-day group	
2 days after treatment			
Bacterial eradication	91/93 (98)	83/89 (93)	0.16
Symptom improvement*			
Nocturia (≥ 1/night)	64/73 (88)	57/69 (83)	0.86
Urgency	35/48 (73)	43/49 (88)	0.05
Frequency	24/33 (73)	27/35 (77)	0.44
Burning on micturation	31/31 (100)	33/34 (97)	0.99
Suprapubic pain	12/14 (86)	21/25 (84)	0.71
6 weeks after treatment			
Reinfection	13/93 (14)	16/89 (18)	0.54
Relapse	14/93 (15)	12/89 (13)	0.83

*Among subjects who presented the symptom at baseline (time of entry into the study) and who also provided information on symptom relief at follow-up.

Valutazione della velocità di guarigione clinica e batteriologica della pradofloxacina nei cani affetti da **infezioni delle vie urinarie non complicate**

Andrea Vercelli*, José M. Mottet**
*Ambulatorio Veterinario Associato, Corso Traiano 99/d, Torino
**Bayer Animal Health GmbH, Monheim (Germany)

Figura 1. Distribuzione degli uropatogeni isolati dai cani affetti da UTI non complicata il giorno 0
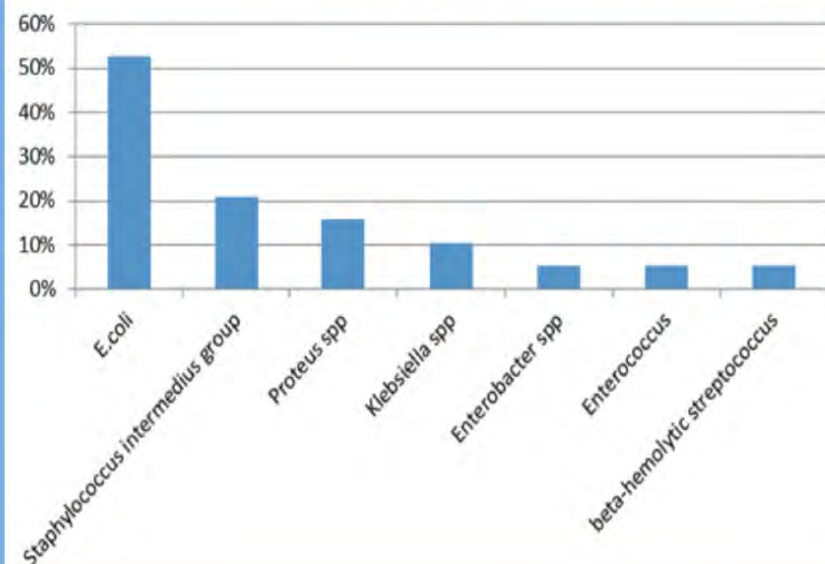
Figura 2. Tassi di cura clinica (colore blu) e batteriologica (colore rosso) dei cani trattati con pradofloxacina il giorno 3 ed il giorno 7

3 days not inferior to 7 days
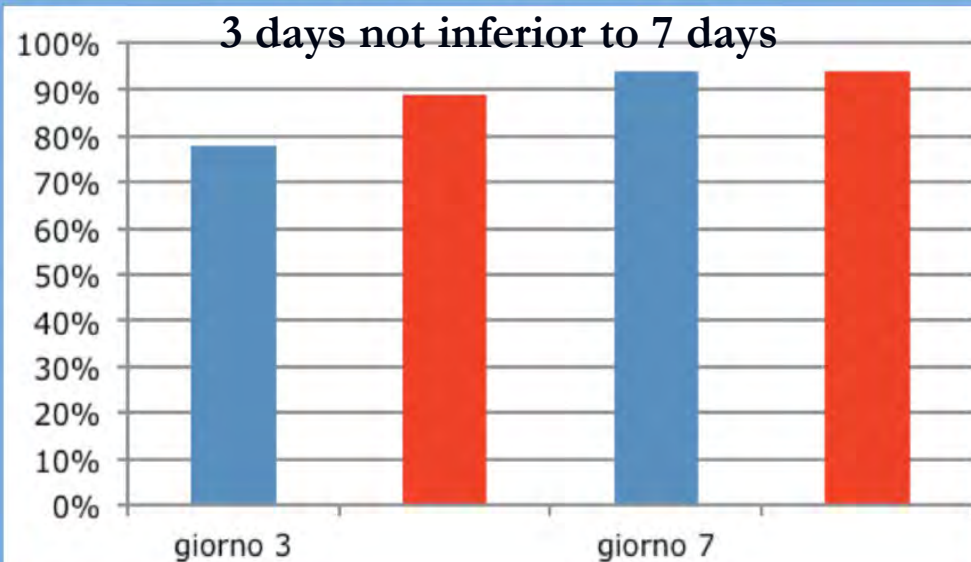
Infectious Diseases Society of America/American Thoracic Society Consensus Guidelines on the Management of Community-Acquired Pneumonia in Adults

Lionel A. Mandell,[1,a] Richard G. Wunderink,[2,a] Antonio Anzueto,[3,4] John G. Bartlett,[7] G. Douglas Campbell,[8] Nathan C. Dean,[9,10] Scott F. Dowell,[11] Thomas M. File, Jr.[12,13] Daniel M. Musher,[5,6] Michael S. Niederman,[14,15] Antonio Torres,[16] and Cynthia G. Whitney[11]

Table 1. Levels of evidence.

Evidence level	Definition
Level I (high)	Evidence from well-conducted, randomized controlled trials.
Level II (moderate)	Evidence from well-designed, controlled trials without randomization (including cohort, patient series, and case-control studies). Level II studies also include any large case series in which systematic analysis of disease patterns and/or microbial etiology was conducted, as well as reports of data on new therapies that were not collected in a randomized fashion.
Level III (low)	Evidence from case studies and expert opinion. In some instances, therapy recommendations come from antibiotic susceptibility data without clinical observations.

Table 5. Clinical indications for more extensive diagnostic testing.

Indication	Blood culture	Sputum culture	*Legionella* UAT	Pneumococcal UAT	Other
Intensive care unit admission	X	X	X	X	X[a]
Failure of outpatient antibiotic therapy		X	X	X	
Cavitary infiltrates	X	X			X[b]
Leukopenia	X			X	
Active alcohol abuse	X	X	X	X	
Chronic severe liver disease	X			X	
Severe obstructive/structural lung disease		X			
Asplenia (anatomic or functional)	X			X	
Recent travel (within past 2 weeks)			X		X[c]
Positive *Legionella* UAT result		X[d]	NA		
Positive pneumococcal UAT result	X	X		NA	
Pleural effusion	X	X	X	X	X[e]

NOTE. NA, not applicable; UAT, urinary antigen test.

[a] Endotracheal aspirate if intubated, possibly bronchoscopy or nonbronchoscopic bronchoalveolar lavage.
[b] Fungal and tuberculosis cultures.
[c] See table 8 for details.
[d] Special media for *Legionella*.
[e] Thoracentesis and pleural fluid cultures.

Table 6. Most common etiologies of community-acquired pneumonia.

Patient type	Etiology
Outpatient	*Streptococcus pneumoniae*
	Mycoplasma pneumoniae
	Haemophilus influenzae
	Chlamydophila pneumoniae
	Respiratory viruses[a]
Inpatient (non-ICU)	*S. pneumoniae*
	M. pneumoniae
	C. pneumoniae
	H. influenzae
	Legionella species
	Aspiration
	Respiratory viruses[a]
Inpatient (ICU)	*S. pneumoniae*
	Staphylococcus aureus
	Legionella species
	Gram-negative bacilli
	H. influenzae

NOTE. Based on collective data from recent studies [171]. ICU, intensive care unit.

[a] Influenza A and B, adenovirus, respiratory syncytial virus, and parainfluenza.

Table 7. Recommended empirical antibiotics for community-acquired pneumonia.

Outpatient treatment
1. Previously healthy and no use of antimicrobials within the previous 3 months

    A macrolide (strong recommendation; level I evidence)

    Doxycyline (weak recommendation; level III evidence)
2. Presence of comorbidities such as chronic heart, lung, liver or renal disease; diabetes mellitus; alcoholism; malignancies; asplenia; immunosuppressing conditions or use of immunosuppressing drugs; or use of antimicrobials within the previous 3 months (in which case an alternative from a different class should be selected)

    A respiratory fluoroquinolone (moxifloxacin, gemifloxacin, or levofloxacin [750 mg]) (strong recommendation; level I evidence)

    A β-lactam **plus** a macrolide (strong recommendation; level I evidence)
3. In regions with a high rate (>25%) of infection with high-level (MIC ≥16 μg/mL) macrolide-resistant *Streptococcus pneumoniae*, consider use of alternative agents listed above in (2) for patients without comorbidities (moderate recommendation; level III evidence)

Inpatients, non-ICU treatment

    A respiratory fluoroquinolone (strong recommendation; level I evidence)

    A β-lactam **plus** a macrolide (strong recommendation; level I evidence)

Inpatients, ICU treatment

    A β-lactam (cefotaxime, ceftriaxone, or ampicillin-sulbactam) **plus** either azithromycin (level II evidence) **or** a respiratory fluoroquinolone (level I evidence) (strong recommendation) (for penicillin-allergic patients, a respiratory fluoroquinolone and aztreonam are recommended)

Special concerns

    If *Pseudomonas* is a consideration

    An antipneumococcal, antipseudomonal β-lactam (piperacillin-tazobactam, cefepime, imipenem, or meropenem) plus either ciprofloxacin or levofloxacin (750 mg)

    or

    The above β-lactam plus an aminoglycoside and azithromycin

    or

    The above β-lactam plus an aminoglycoside and an antipneumococcal fluoroquinolone (for penicillin-allergic patients, substitute aztreonam for above β-lactam)

    (moderate recommendation; level III evidence)

    If CA-MRSA is a consideration, add vancomycin or linezolid (moderate recommendation; level III evidence)

NOTE. CA-MRSA, community-acquired methicillin-resistant *Staphylococcus aureus*; ICU, intensive care unit.

Table 8. Epidemiologic conditions and/or risk factors related to specific pathogens in community-acquired pneumonia.

Condition	Commonly encountered pathogen(s)
Alcoholism	*Streptococcus pneumoniae*, oral anaerobes, *Klebsiella pneumoniae*, *Acinetobacter* species, *Mycobacterium tuberculosis*
COPD and/or smoking	*Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Legionella* species, *S. pneumoniae*, *Moraxella cararrhalis*, *Chlamydophila pneumoniae*
Aspiration	Gram-negative enteric pathogens, oral anaerobes
Lung abscess	CA-MRSA, oral anaerobes, endemic fungal pneumonia, *M. tuberculosis*, atypical mycobacteria
Exposure to bat or bird droppings	*Histoplasma capsulatum*
Exposure to birds	*Chlamydophila psittaci* (if poultry: avian influenza)
Exposure to rabbits	*Francisella tularensis*
Exposure to farm animals or parturient cats	*Coxiella burnetti* (Q fever)
HIV infection (early)	*S. pneumoniae*, *H. influenzae*, *M. tuberculosis*
HIV infection (late)	The pathogens listed for early infection plus *Pneumocystis jirovecii*, *Cryptococcus*, *Histoplasma*, *Aspergillus*, atypical mycobacteria (especially *Mycobacterium kansasii*), *P. aeruginosa*, *H. influenzae*
Hotel or cruise ship stay in previous 2 weeks	*Legionella* species
Travel to or residence in southwestern United States	*Coccidioides* species, *Hantavirus*
Travel to or residence in Southeast and East Asia	*Burkholderia pseudomallei*, avian influenza, SARS
Influenza active in community	Influenza, *S. pneumoniae*, *Staphylococcus aureus*, *H. influenzae*
Cough >2 weeks with whoop or posttussive vomiting	*Bordetella pertussis*
Structural lung disease (e.g., bronchiectasis)	*Pseudomonas aeruginosa*, *Burkholderia cepacia*, *S. aureus*
Injection drug use	*S. aureus*, anaerobes, *M. tuberculosis*, *S. pneumoniae*
Endobronchial obstruction	Anaerobes, *S. pneumoniae*, *H. influenzae*, *S. aureus*
In context of bioterrorism	*Bacillus anthracis* (anthrax), *Yersinia pestis* (plague), *Francisella tularensis* (tularemia)

NOTE. CA-MRSA, community-acquired methicillin-resistant *Staphylococcus aureus*; COPD, chronic obstructive pulmonary disease; SARS, severe acute respiratory syndrome.

Table 9. Recommended antimicrobial therapy for specific pathogens.

Organism	Preferred antimicrobial(s)	Alternative antimicrobial(s)
Streptococcus pneumoniae		
Penicillin nonresistant; MIC <2 μg/mL	Penicillin G, amoxicillin	Macrolide, cephalosporins (oral [cefpodoxime, cefprozil, cefuroxime, cefdinir, cefditoren] or parenteral [cefuroxime, ceftriaxone, cefotaxime]), clindamycin, doxycycline, respiratory fluoroquinolone[a]
Penicillin resistant; MIC $\geqslant$2 μg/mL	Agents chosen on the basis of susceptibility, including cefotaxime, ceftriaxone, fluoroquinolone	Vancomycin, linezolid, high-dose amoxicillin (3 g/day with penicillin MIC $\leqslant$4 μg/mL)
Haemophilus influenzae		
Non–β-lactamase producing	Amoxicillin	Fluoroquinolone, doxycycline, azithromycin, clarithromycin[b]
β-Lactamase producing	Second- or third-generation cephalosporin, amoxicillin-clavulanate	Fluoroquinolone, doxycycline, azithromycin, clarithromycin[b]
Mycoplasma pneumoniae/Chlamydophila pneumoniae	Macrolide, a tetracycline	Fluoroquinolone
Legionella species	Fluoroquinolone, azithromycin	Doxycyline
Chlamydophila psittaci	A tetracycline	Macrolide
Coxiella burnetii	A tetracycline	Macrolide
Francisella tularensis	Doxycycline	Gentamicin, streptomycin
Yersinisa pestis	Streptomycin, gentamicin	Doxycyline, fluoroquinolone
Bacillus anthracis (inhalation)	Ciprofloxacin, levofloxacin, doxycycline (usually with second agent)	Other fluoroquinolones; β-lactam, if susceptible; rifampin; clindamycin; chloramphenicol
Enterobacteriaceae	Third-generation cephalosporin, carbapenem[c] (drug of choice if extended-spectrum β-lactamase producer)	β-Lactam/β-lactamase inhibitor,[d] fluoroquinolone
Pseudomonas aeruginosa	Antipseudomonal β-lactam[e] **plus** (ciprofloxacin or levofloxacin[f] or aminoglycoside)	Aminoglycoside **plus** (ciprofloxacin or levofloxacin[f])
Burkholderia pseudomallei	Carbapenem, ceftazadime	Fluoroquinolone, TMP-SMX
Acinetobacter species	Carbapenem	Cephalosporin-aminoglycoside, ampicillin-sulbactam, colistin
Staphylococcus aureus		
Methicillin susceptible	Antistaphylococcal penicillin[g]	Cefazolin, clindamycin
Methicillin resistant	Vancomycin or linezolid	TMP-SMX
Bordetella pertussis	Macrolide	TMP-SMX
Anaerobe (aspiration)	β-Lactam/β-lactamase inhibitor,[d] clindamycin	Carbapenem
Influenza virus	Oseltamivir or zanamivir	
Mycobacterium tuberculosis	Isoniazid plus rifampin plus ethambutol plus pyrazinamide	Refer to [243] for specific recommendations
Coccidioides species	For uncomplicated infection in a normal host, no therapy generally recommended; for therapy, itraconazole, fluconazole	Amphotericin B
Histoplasmosis	Itraconazole	Amphotericin B
Blastomycosis	Itraconazole	Amphotericin B

NOTE. Choices should be modified on the basis of susceptibility test results and advice from local specialists. Refer to local references for appropriate doses. ATS, American Thoracic Society; CDC, Centers for Disease Control and Prevention; IDSA, Infectious Diseases Society of America; TMP-SMX, trimethoprim-sulfamethoxazole.

[a] Levofloxacin, moxifloxacin, gemifloxacin (not a first-line choice for penicillin susceptible strains); ciprofloxacin is appropriate for *Legionella* and most gram-negative bacilli (including *H. influenza*).

[b] Azithromycin is more active in vitro than clarithromycin for *H. influenza*.

[c] Imipenem-cilastatin, meropenem, ertapenem.

[d] Piperacillin-tazobactam for gram-negative bacilli, ticarcillin-clavulanate, ampicillin-sulbactam or amoxicillin-clavulanate.

[e] Ticarcillin, piperacillin, ceftazidime, cefepime, aztreonam, imipenem, meropenem.

[f] 750 mg daily.

[g] Nafcillin, oxacillin flucloxacillin.
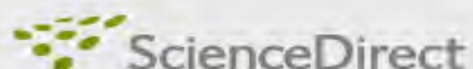
ELSEVIER

REVIEW

Short-course fluoroquinolone therapy in exacerbations of chronic bronchitis and COPD

Antonio Anzueto [a],*, Marc Miravitlles [b]

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[b] Fudació Clínic. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clínic, 08036 Barcelona, Catalonia, Spain
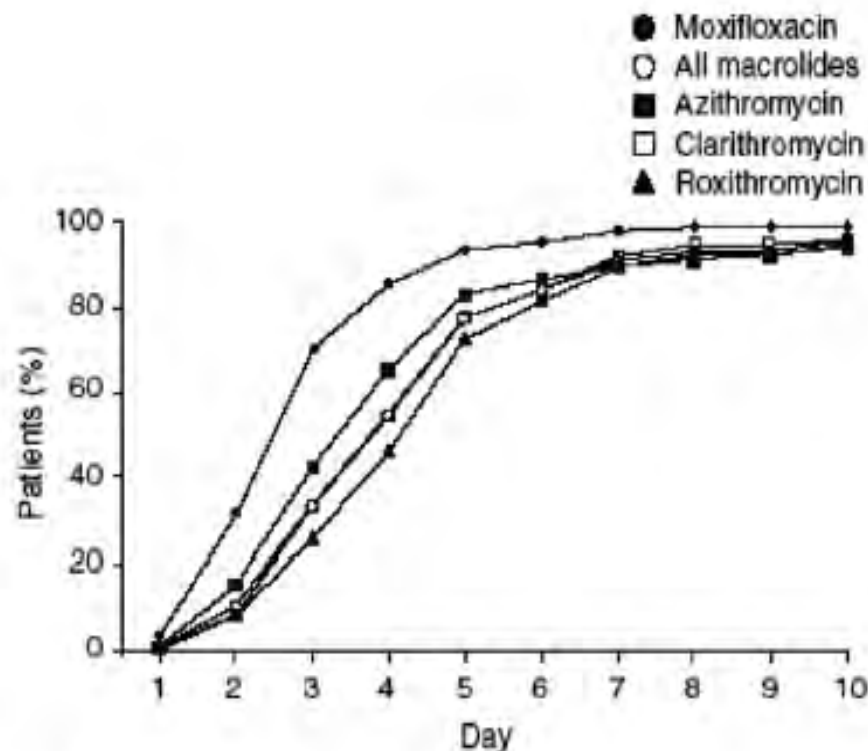
Figure 2 Improvement rate over the first 10 days of observation. Mean duration until improvement in moxifloxacin-treated patients was 3.2 days compared with 4.5 days in macrolide-treated patients. The difference of 1.2 days was statistically significant ($P < 0.0001$).[44] Reproduced with

greater clinical success. Evidence suggests that short-course antimicrobial therapy can be as effective as standard duration therapy (>7 days) in treating exacerbations. Randomized trials have shown that clinical and bacteriological success rates are comparable with both 5-day and standard antibiotic courses. Furthermore, 5-day fluoroquinolone therapy is associated with faster recovery, fewer relapses, prolonged duration between episodes, and less hospitalization when compared with standard therapy. Both moxifloxacin and gemifloxacin have received FDA-approval for 5-day therapy in AECB.

Short course antibiotics in community acquired pneumonia

▲ El Moussaoui R, de Borgie CA, van den Broek P, et al. Effectiveness of discontinuing antibiotic treatment after three days versus eight days in mild to moderate-severe community acquired pneumonia: randomised, double blind study. BMJ 2006;**332**;1355–8

This Dutch study, undertaken between November 2000 and July 2003, took adults with a pneumonia severity index score of ≤110 and randomly assigned those who substantially improved after 72 hours of intravenous amoxicillin to either 750 mg oral amoxicillin (n = 63) or placebo (n = 56) three times daily for 5 days thereafter.

Clinical, bacteriological and radiological outcomes were assessed. The clinical success rate at day 10 (per protocol analysis) was 93% in both groups (50/54 in the 3 day treatment group and 56/60 in the 8 day treatment group: difference 0.1% (95% CI −9 to 10)). At day 28 clinical success rates were 90% (47/52) in the 3 day treatment group and 88% (49/56) in the 8 day treatment group (difference 2% (95% CI −9 to 15)). There was therefore little difference between the two groups.

This study suggests that a short course of antibiotic therapy is not inferior to a longer course in patients with mild to moderate-severe uncomplicated community acquired pneumonia who show clinical improvement after 3 days of intravenous antibiotics.

Figure 1. Flow chart of guideline recommendations on empirical antibiotic treatment of CAP

Management of community-acquired pneumonia in adults: 2016 guideline update from the Dutch Working Party on Antibiotic Policy (SWAB) and Dutch Association of Chest Physicians (NVALT)

W.J. Wiersinga[1,2], M.J. Bonten[3], W.G. Boersma[3], R.E. Jonkers[4], R.M. Aleva[5], B.J. Kullberg[6],
J.A. Schouten[7], J.E. Degener[8], E.M.W. van de Garde[9], T.J. Verheij[10], A.P.E. Sachs[10], J.M. Prins[1]

- Primary care setting
- Pneumonia?
  - yes
- Home treatment possible
  - no → Referral to hospital for clinical assessment
  - yes → Start monotherapy with 1. amoxicillin or 2. doxycycline
- Clinical improval after 48 hours
  - no → 1. Reconsider diagnosis CAP 2. If started with doxycycline: refer 3. If started with β-lactam: refer or change to macrolide or doxycycline
  - refer

Category I — Mild CAP — Ambulatory: CURB-65 0-1 or PSI 1-2
→ Start monotherapy with 1. amoxicillin or 2. doxycycline
→ Clinical improval after 48 hours
- no → 1. If started with amoxicillin: change to doxycyline or macrolide 2. If started with doxycycline: consultation expert
- yes → Continue amoxicillin or doxycycline

Category II — Moderately severe CAP — Ward: CURB-65 2 or PSI 3-4
→ Risk factors for Legionella: 1. Recently abroad or 2. Legionella epidemic or 3. Failure of β-lactam
- yes → Amoxicillin iv or penicillin iv — Legionella antigen test < 24 h
  - Legionella pos → Levofloxacin or moxifloxacin
  - Legionella neg
- no → Amoxicillin iv or penicillin iv

Category III — Severe CAP — Ward: CURB-65 3-5 or PSI 5
→ 2nd/3rd generation cephalosporin
→ Legionella and pneumococcal antigen test (PUAT)
- PUAT pos → Penicillin or amoxicillin
- both neg → 2nd/3rd generation cephalosporin
- Legionella pos → Levofloxacin or moxifloxacin

Category IV — Severe CAP — ICU admission
→ Moxifloxacin or 2nd/3rd gen. cephalosporin + ciprofloxacin
→ Legionella and pneumococcal antigen test (PUAT)
- both neg → Continue emipirical therapy + further diagnostics (culture, PCR)
- PUAT pos → Continue empirical therapy and de-escalate when clinically improved or microbiological diagnosis
- Legionella pos → Levofloxacin or moxifloxacin

Are all antibiotics the same…

- **NO**
  - **Bactericidal vs bacteriostatic**
  - **Distribution**
  - **Serum versus tissue**
  - **Rate of kill**
  - **Protein binding >60%**

Tricorder
Starship-Enterprise

- **Could choice of antibiotic influence duration of therapy?**
  - **Faster kill…shorter durations of therapy?**
  - **Some differences captured in therapy guidelines**
  - **ISCAID…using best evidence available**

Blondeau 2018

"Antimicrobial use guidelines for urinary and respiratory tract infections in human and veterinary medicine: A One Health Perspective"

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Colorado State University

ESGVM

ESCMID
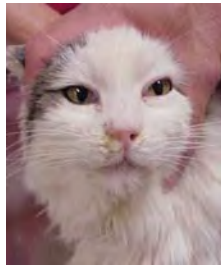
ICOHAR International Conference on One Health Antimicrobial Resistance

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The WSAVA One Health Committee Mission Statement:

To ensure the prominence of the small companion animal-human interface in the global One Health agenda

WSAVA One Health Committee

http://www.wsava.org/educational/one-health-committee

The WSAVA Therapeutics Guideline Group

The mission of the TGG is to ensure best practices for the selection and use of medicines including their quality, availability and responsible use in companion animals while engaging participation of stakeholders and the WSAVA Global Community under the concept of One Health

https://www.wsava.org/Committees/Therapeutics-Guideline-Group

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WSAVA Position Statement on Equitable Access to Veterinary Therapeutics for Veterinarians Globally

"Ready access by healthcare professionals to pharmaceuticals (e.g., medicines, anesthetics/analgesics, etc), biologicals (e.g., vaccines, etc), parasiticides, and antiseptics is one of the key pillars of appropriate patient care, whether in human or veterinary medicine. Inequities in availability and access exist between various regions of the world for a variety of reasons. We call upon key stakeholders (regulatory authorities, manufacturers, and healthcare professionals) to seek solutions that would broaden access while maintaining the sanctity of the veterinary-client-patient+/- pharmacist relationship, where warranted."

Endorsements for the WSAVA Position Statement on Equitable Access to Veterinary Therapeutics

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Companion Animal Antimicrobial Use in the USA

AVMA
American Veterinary Medical Association

www.avma.org/

Antimicrobial Use and Resistance: AVMA Policies

The AVMA has a wide range of policies related to the use of antimicrobials in veterinary medicine

- Aminoglycosides
- AVMA Strategy Regarding Antimicrobial-resistant Bacteria
- Antimicrobial Stewardship, Definition and Core Principles
- Antimicrobial Use Guidelines for Veterinary Practice
- Continuous Monitoring of Antimicrobial Use and Resistance, Joint AVMA-FVE-CVMA Statement
- Definitions of Antimicrobial Use for Treatment, Control and Prevention
- Drug Products, Withdrawal of FDA Approval
- Extralabel Use of Veterinary Feed Directive Drugs for Minor Species
- FARAD Program, Support for
- Food Safety
- Judicious Therapeutic Use
- Limited Prohibition on Extralabel Drug Use
- National Antimicrobial Resistance Monitoring System (NARMS)
- Pharmaceutical Disposal, Best Management Practices
- Residues in Foods of Animal Origin, Veterinarian Notification
- Responsible and Judicious Use, Joint AVMA-FVE-CVMA Statement

Species-Specific
- Antimicrobials in Aquatic Animals, Judicious Use
- Aquatic Animal Therapeutic Agents
- Antimicrobials in Cats, AAFP/AAHA Basic Guidelines for Judicious Therapeutic Use
- Antimicrobials in Cattle, AASRP-AVMA Judicious Therapeutic Use of
- Antimicrobials in Horses, AAEP/AVMA Prudent Drug Usage Guidelines
- Antimicrobials in Poultry, AAAP-AVMA Guidelines for Judicious Therapeutic Use
- Antimicrobials in Swine, AASV Guidelines for Judicious Therapeutic Use in Pork Production
- Antimicrobials in Food-Producing Animals, Approval and Availability

Role of the Veterinarian
- Antimicrobial Use, Role of the Veterinarian
- One Health, Joint AVMA-FVE-CVMA Statement on the Essential Role of Veterinarians
- Veterinary Foresight and Expertise in Antimicrobial Discussions
- Veterinarian-Client-Patient Relationship (VCPR)

Judicious Therapeutic Use of Antimicrobials

Position Statement
When the decision is reached to use antimicrobials for treatment, control, or prevention of disease, veterinarians should strive to optimize therapeutic efficacy and minimize resistance to antimicrobials to protect public and animal health and well-being.

Objectives
- Support development of a scientific knowledge base that provides the basis for judicious therapeutic antimicrobial use.
- Support educational efforts that promote science-based judicious antimicrobial use.
- Maintain efficacy of antimicrobials by minimizing potential for development and transmission of resistance.
- Foster an atmosphere within industry research and development programs and government regulatory bodies that facilitate current and future availability of veterinary antimicrobials.

AVMA
American Veterinary Medical Association

American Association of Feline Practitioners/American Animal Hospital Association Basic Guidelines of Judicious Therapeutic Use of Antimicrobials

Introduction
The Basic Guidelines of Judicious Therapeutic Use of Antimicrobials in cats and dogs are designed to provide information to aid practicing veterinarians in choosing appropriate antimicrobial therapy to best serve their patients and to help minimize the development of antimicrobial resistance. Presented below are the Principles of Judicious Therapeutic Use of Antimicrobials adopted as a framework document for the recommended guidelines developed for cats and dogs.

Position Statement
Veterinarians agree to protect animal and public health when they pledge the Veterinarian's Oath. It is the responsibility of veterinarians to maintain patient health by routine examinations, preventative strategies, and client education. When a medical condition exists it is important to obtain an accurate clinical diagnosis whenever possible. Once the decision is reached to use antimicrobial therapy, veterinarians strive to optimize therapeutic efficacy, minimize resistance to antimicrobials, and protect public and animal health.

Feline Lower Urinary Tract Signs

- #1 cause of signs is sterile interstitial cystitis (75%)
- Recent clinic survey in the USA
  - 19,123 cats with signs were administered antibiotics
  - Only 1,299 cats were cultured
    - 372 cats were culture positive
    - 927 cats were culture negative

Judicious?

Feline Upper Respiratory Infections

- Over 95% of these kittens have been exposed to feline herpesvirus 1
- Many veterinarians administer antibiotics

Judicious?

International Society for Companion Animal Infectious Diseases (ISCAID)

www.iscaid.org

ISCAID seeks to promote and improve the health of all species by encouraging collaboration between physicians, veterinarians, and other scientific health professionals.

Research Article

Antimicrobial Use Guidelines for Treatment of Urinary Tract Disease in Dogs and Cats: Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases

J. Scott Weese,[1] Joseph M. Blondeau,[2] Dawn Boothe,[3] Edward B. Breitschwerdt,[4] Luca Guardabassi,[5] Andrew Hillier,[6] David H. Lloyd,[7] Mark G. Papich,[4] Shelley C. Rankin,[8] John D. Turnidge,[9,10] and Jane E. Sykes[11]

Veterinary Medicine International
Volume 2011, Article ID 263768, 9 pages

Slide 1

International Society for Companion Animal Infectious Diseases (ISCAID) guidelines for the diagnosis and management of bacterial urinary tract infections in dogs and cats

J. Scott Weese[a,*], Joseph Blondeau[b,c], Dawn Boothe[d], Luca G. Guardabassi[c,j], Nigel Gumley[g], Mark Papich[h], Lisbeth Rem Jessen[i], Michael Lappin[j], Shelley Rankin[k], Jodi L. Westropp[l], Jane Sykes[l]

Outside reviewers

Dr. Autumn Davidson
Dr. Rosanne Jepson
Dr. Annette Litster
Dr. Lena Pelander
Dr. Gilad Segev
Dr. Shelly Rankin

Slide 2

- **Syndromes**
  - **Sporadic cystitis**
  - **Recurrent bacterial cystitis**
  - **Pyelonephritis**
  - **Bacterial prostatitis**
  - **Subclinical bacteriuria**
  - **Urinary catheters**
  - **Urological surgery**
  - **Medical Rx uroliths**

- **Sections**
  - **Introduction**
  - **Classification**
  - **Diagnosis**
  - **Treatment**
  - **Followup**
  - **Prevention**

International Society for Companion Animal Infectious Diseases (ISCAID) guidelines for the diagnosis and management of bacterial urinary tract infections in dogs and cats

J. Scott Weese[a,*], Joseph Blondeau[b,c], Dawn Boothe[d], Luca G. Guardabassi[c,j], Nigel Gumley[g], Mark Papich[h], Lisbeth Rem Jessen[i], Michael Lappin[j], Shelley Rankin[k], Jodi L. Westropp[l], Jane Sykes[l]

Slide 3

Table 3 — Drug table summarizing recommendations for the management of bacterial urinary tract infection in dogs and cats.

It is great to have a Table of drug doses for this body system that a group of experts agree upon as optimal!

Slide 4

Table 2 — Clinical studies evaluating treatment duration for sporadic bacterial cystitis in dogs.

We attempt to address treatment duration issues via peer reviewed literature

Slide 5

Antimicrobial use Guidelines for Treatment of Respiratory Tract Disease in Dogs and Cats: Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases

M.R. Lappin, J. Blondeau, D. Booth, E.B. Breitschwerdt, L. Guardabassi, D.H. Lloyd, M.G. Papich, S.C. Rankin, J.E. Sykes, J. Turnidge, and J.S. Weese

Respiratory tract disease can be associated with primary or secondary bacterial infections in dogs and cats and is a common reason for use and potential misuse, improper use, and overuse of antimicrobials. There is a lack of comprehensive treatment guidelines such as those that are available for human medicine. Accordingly, the International Society for Companion Animal Infectious Diseases convened a Working Group of clinical microbiologists, pharmacologists, and internists to share experiences, examine scientific data, review clinical trials, and develop these guidelines to assist veterinarians in making antimicrobial treatment choices for use in the management of bacterial respiratory diseases in dogs and cats.

Key words: Bronchitis; Pneumonia; Pyothorax; Rhinitis.

Slide 6

ISCAID Respiratory Guidelines

- **Committee members:**

Drs. Joseph Blondeau; Dawn Boothe; Ed Breitschwerdt; Luca Guardabassi; Michael Lappin; David Lloyd; Mark Papich; Shelly Rankin; Jane Sykes; John Turnidge; Scott Weese

- **Outside reviewers**

Drs. Leah Cohn, Joshua Daniels, Eleanor Hawkins, Steve Holloway, Lynelle Johnson, and Carol Reneiro

Guidelines rating

A draft document was developed over several years and an attempt to reach 100% agreement on each recommendation

100% agreement was not always reached and so the committee employed a modified Delhi rating system on the final draft.

Each guideline committee members and the outside reviewers were asked to independently select whether they agreed, were neutral, or disagreed with each recommendation."

For those recommendations that received any "disagree" votes from the 17 total reviewers (Working Group and outside reviewers), the percentage distribution of all reviewers and appropriate comments are presented.

Bacterial Respiratory Infections

- **Feline upper respiratory tract disease**
  - **Acute and chronic**
- **Canine infectious respiratory disease complex**
- **Bronchitis**
- **Pneumonia**
- **Pyothorax**

> Overall 40 recommendations
>
> 14 recommendations had 1 -3 disagree votes with comments

Bacterial Respiratory Infections

- **Summary of Recommendations**
  - **Diagnosis of the syndrome**
    - **Emphasis on documenting a bacterial (and less commonly protozoal) infection exists**
  - **Treatment of the syndrome**
  - **Monitoring treatment**

Table 1.	First line drugs
Table 2.	All drugs and doses with comment

Table 1. First line anti-microbial options for treatment of bacterial respiratory infections in dogs and cats

Infection Type	First-Line Drug Options
Acute bacterial upper respiratory infection (URI) in cats	Doxycycline[a] or amoxicillin per os (PO)
Chronic bacterial URI in cats	Doxycycline or amoxicillin PO Base the choice on C&S[b] if available
Canine infectious respiratory disease complex (bacterial component)	Doxycycline[a] or amoxicillin-clavulanate PO
Bacterial bronchitis (dogs or cats)	Doxycycline[a] PO Base changes if needed on clinical responses and C&S if available
Pneumonia in animals with extensive contact with other animals that have no systemic manifestations of disease (ie, fever, lethargy, dehydration)	Doxycycline[a] PO Base changes if needed on clinical responses and C&S if available
Pneumonia with or without clinical evidence of sepsis[c]	Parenteral administration of a fluoroquinolone[d] and a penicillin or clindamycin[e] initially Base oral drug choices to follow on clinical responses and C&S results if available
Pyothorax (dogs or cats)[b]	Parenteral administration of a fluoroquinolone[d] and a penicillin or clindamycin[e] initially combined with therapeutic lavage initially Base oral drug choices to follow on clinical responses and C&S results if available

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Feline Upper Respiratory Disease

Feline Upper Respiratory Disease

- **"Syndrome consisting of clinical signs that may include serous to mucopurulent ocular and nasal discharges, epistaxis, sneezing, and conjunctivitis."**
  - **Acute (≤ 10 days)**
  - **Chronic (> 10 days)**
- **The term "upper respiratory infection (URI)" is reserved for cats with clinical signs of URTD that are directly associated with one or more of the known pathogenic viral, bacterial, or fungal organisms.**

ISCAID Recommendation

"The Working Group recommends that antimicrobial therapy be considered within the 10-day observation period only if fever, lethargy, or anorexia are present concurrently with mucopurulent nasal discharge"

ISCAID Recommendation

"The Working Group recommends empirical administration of doxycycline (Table 1 and Table 2) for 7 – 10 days to cats with suspected acute bacterial URI as the first line antimicrobial option"

"Of the 17 reviewers, 16 (94.1%) agreed with this Working Group recommendation and one disagreed because there is no breakpoint data for this antimicrobial for B. bronchiseptica or other bacteria in cats and there are no pharmacokinetics, controlled clinical trials, susceptibility data, or pharmacodynamic data on which to base the recommendation"

Duration of therapy?

- Bacterial pneumonia in dogs and cats
  - Textbook recommendations for 4-6 weeks of treatment
- Committee discussion/recommendation

"The consensus opinion of the Working Group that shorter courses of appropriate treatment, such as those used to treat pneumonia in humans, might be effective in some situations. In the face of insufficient data supporting a shorter course of treatment, the Working Group recommends re-evaluation of animals with pneumonia no later than 10–14 days after starting treatment. At that point, decisions to extend treatment should be based on clinical, hematological, and radiographic findings. Additional studies evaluating durations of treatment that are shorter than 4–6 weeks are required"

Antimicrobial Use Guidelines

- Veterinary issues
  - Generally underpowered studies
  - Regional trends from susceptibility patterns not easily accessible
  - Tools to measure impact of guidelines development generally lacking
  - In USA, lack of legislative recommendation for use of specific guidelines

"Antimicrobial use guidelines for urinary and respiratory tract infections in human and veterinary medicine:
A One Health Perspective"

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ESGVM   ESCMID

ICOHAR International Conference
on One Health Antimicrobial Resistance

SENSITIVE PATHOGEN DETECTION AND RAPID AST IN THE ONE HEALTH FUTURE

Alex van Belkum

ICOHAR, 18 April, Utrecht

30 minutes

PIONEERING DIAGNOSTICS
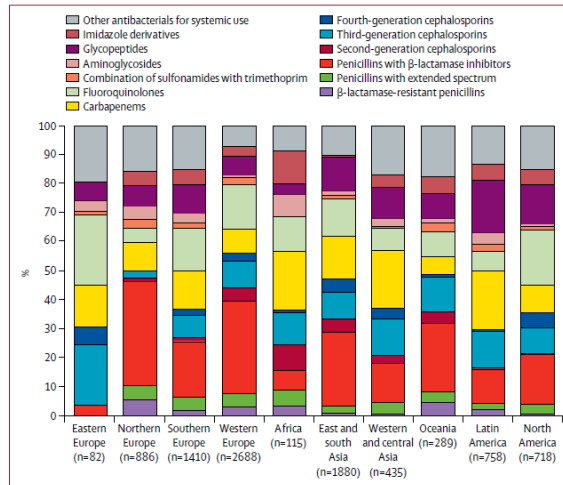
Figure 1: Proportion of prescribed antibiotics for systemic use for health-care-associated infections among adult inpatients, 2015 (n=9261)
East and south Asia includes south, east, and southeast Asia.
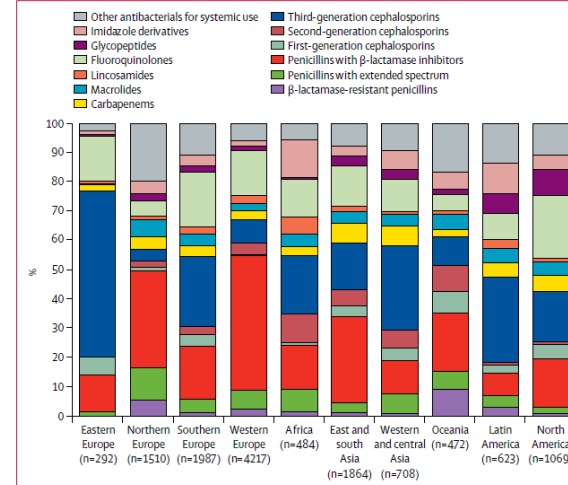
Figure 2: Proportion of prescribed antibiotics for systemic use for community-acquired infections among adult inpatients, 2015 (n=13 226)
East and south Asia includes south, east, and southeast Asia.

Ann Versporten et al

NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-019-08853-3

ARTICLE
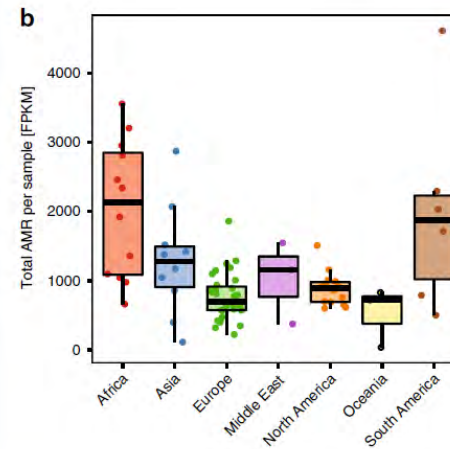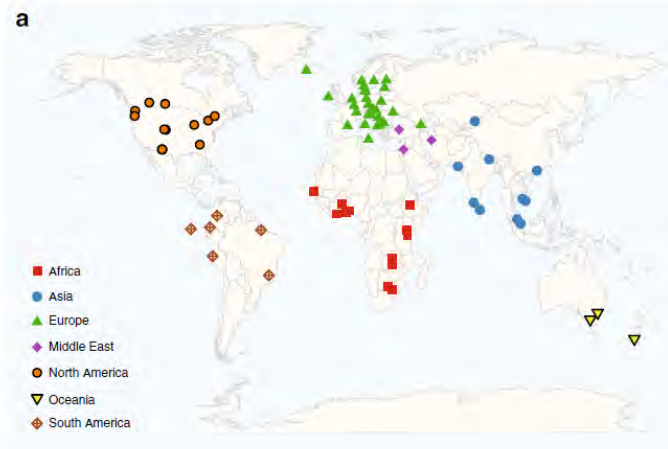
Hendriksen et al., 2019

USE OF ANTIBIOTICS DRIVES RESISTANCE UP

One example out of many: Meropenem at the hospital
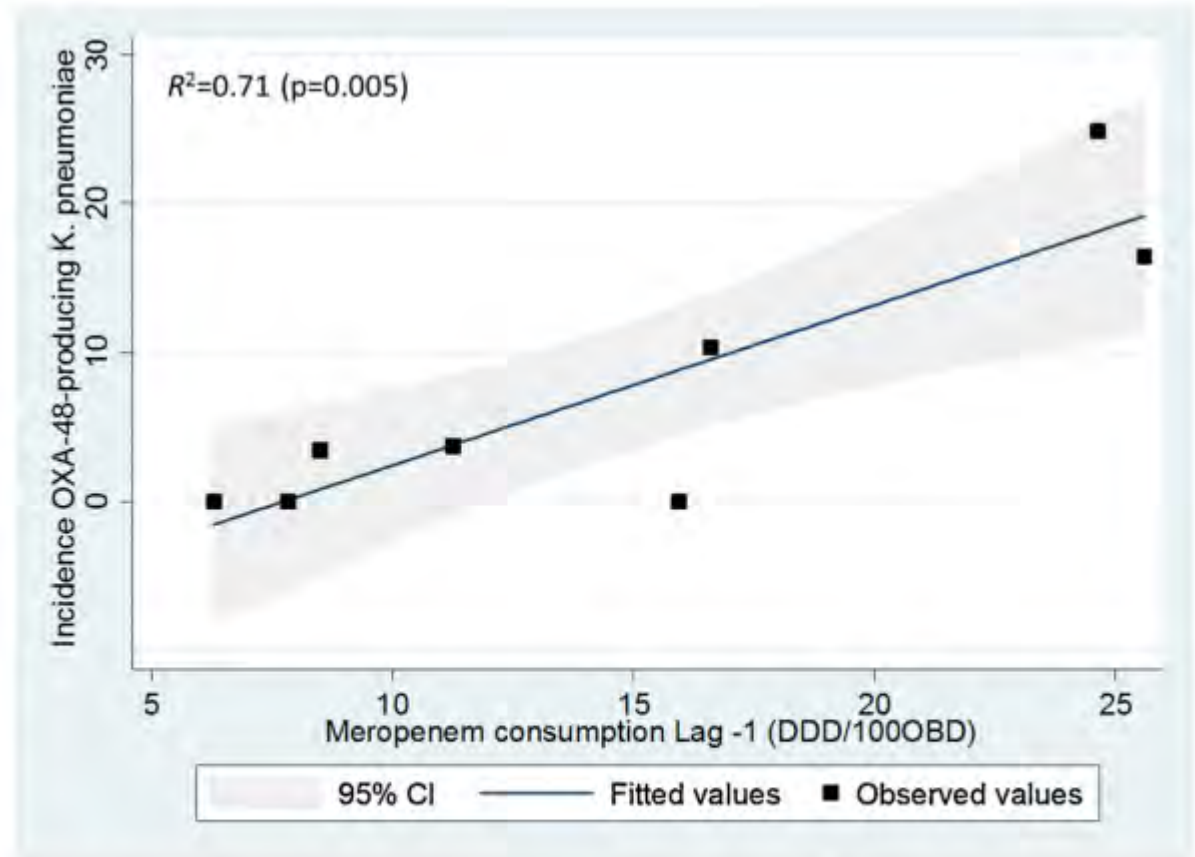
Fig. 1. Cross-correlation between meropenem consumption lag −1 (the preceding year) and the incidence rate of OXA-48-producing *Klebsiella pneumoniae* in a West London renal unit from 2008–2009 to 2013–2014.

Source : M. Gharbi et al. / International Journal of Antimicrobial Agents 46 (2015) 150–15

TRANSMISSION ROUTES ARE OMNIPRESENT: CAN WE TRACE THEM ALL?
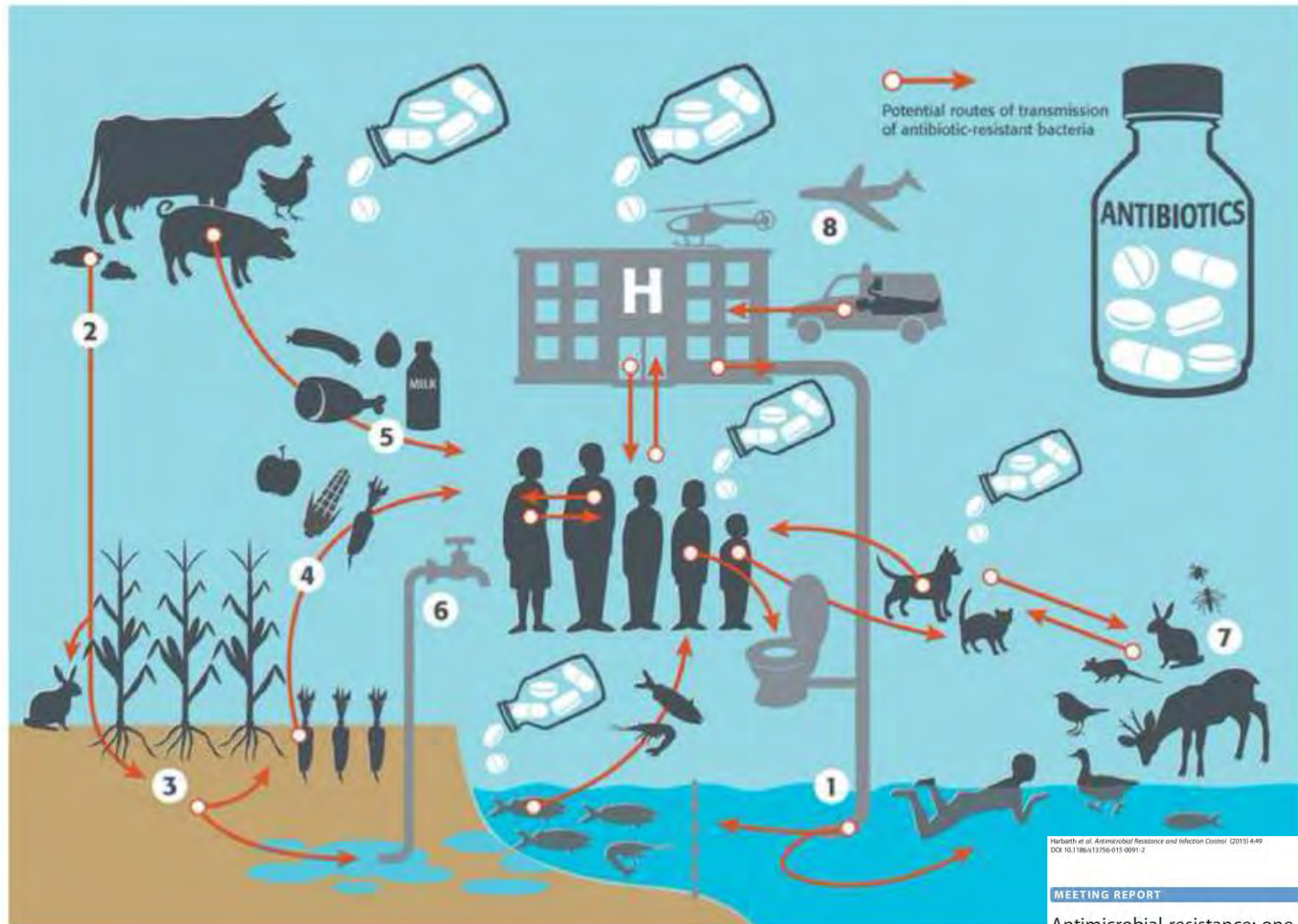
Harbarth et al. Antimicrobial Resistance and Infection Control (2015) 4:49
DOI 10.1186/s13756-015-0091-2

MEETING REPORT

Open Access

Antimicrobial resistance: one world, one fight!

Stephan Harbarth[1], Hanan H. Balkhy[2], Herman Goossens[3], Vincent Jarlier[4], Jan Kluytmans[5], Ramanan Laxminarayan[6], Mirko Saam[7], Alex Van Belkum[8], Didier Pittet[1] and for the World Healthcare-Associated Infections Resistance Forum participants

A RESISTANT INFECTION IS MORE LIKELY TO BE LETHAL AND CERTAINLY MORE COSTLY

An example in a US hopital

Mortality rate
11.5%

$16,000
to treat drug-
sensitive infection
(MSSA)

Mortality rate
24%

$35,000
to treat drug-resistant
infection (MRSA

Source : Filece GA ey al. 2010. Infection Control& Hospital epidemiology 31 (4à

WHO PRIORITY PATHOGEN LIST!
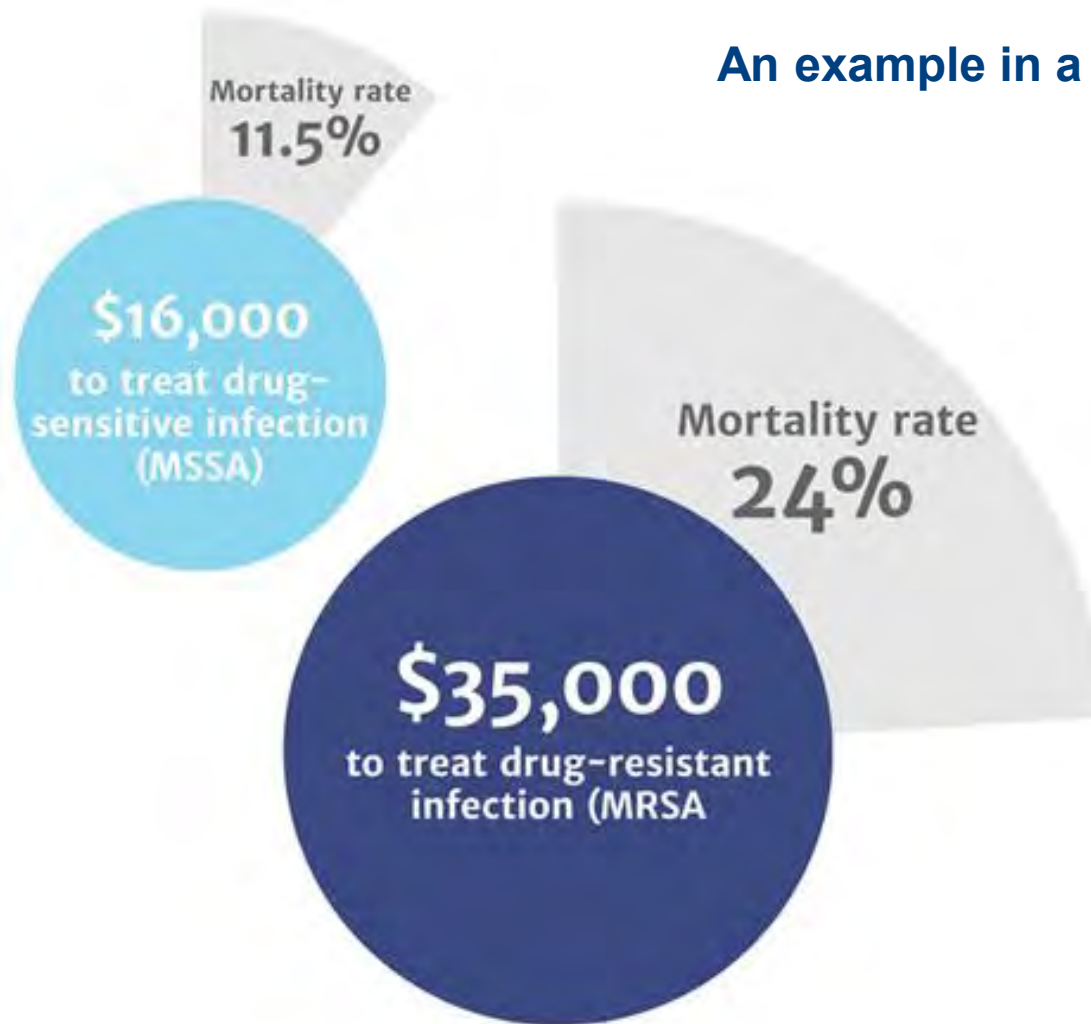
FROM BROAD SPECTRUM EMPIRIC THERAPY TO TARGETED-PERSONALIZED THERAPY

While minimizing Adverse drug events

WHO ASSURED CRITERIA!
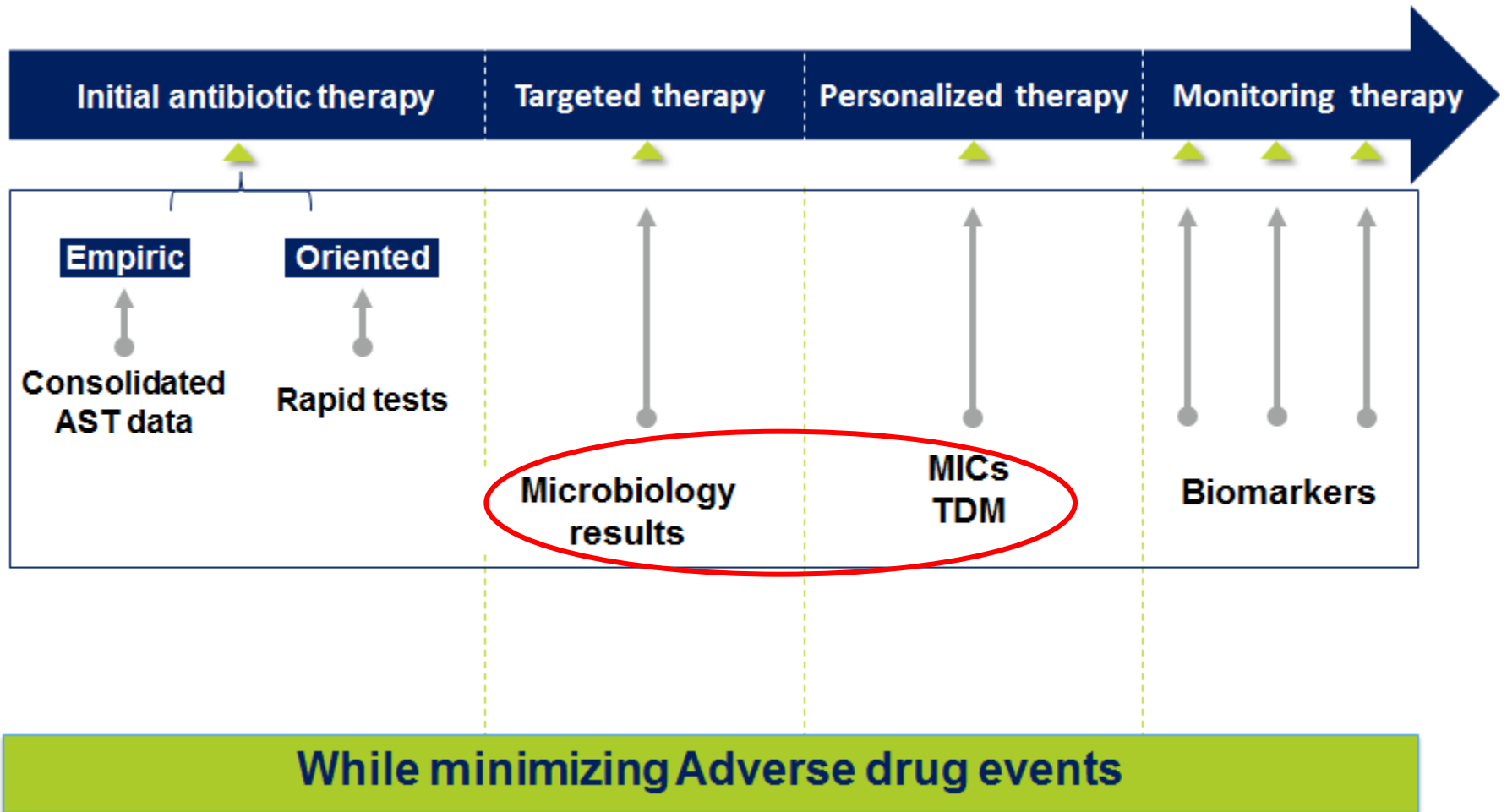
IS IT BACTERIAL OR VIRAL?

Objective

- Provide information within the span of a medical visit, that can help decide on antibiotic or antiviral therapy. Or nothing at all!

Rapid test (lateral flow):

- Confirm the bacterial or viral etiology of infection

Host response biomarkers (CRP, PCT)

- Help distinguish patients with bacterial versus viral infection or identify those without infection

Rapid multiplex PCR tests direct on specimen:

- Identify causative organisms (bacteria, viruses, fungi, parasites; syndromic approach).

MOLECULAR AMR DIAGNOSTICS

Stolen from Till Bachmann

FILMARRAY® RESPIRATORY (RP) PANELS

1 Test. 20 Respiratory Pathogens. All in about an hour.

Viruses
- Adenovirus
- Coronavirus HKU1
- Coronavirus NL63
- Coronavirus 229E
- Coronavirus OC43
- Human Metapneumovirus

- Human Rhinovirus/ Enterovirus
- Influenza A
- Influenza A/H1
- Influenza A/H1-2009
- Influenza A/H3

- Influenza B
- Parainfluenza 1
- Parainfluenza 2
- Parainfluenza 3
- Parainfluenza 4
- Respiratory Syncytial Virus

Bacteria
- *Bordetella pertussis*
- *Chlamydophila pneumoniae*
- *Mycoplasma pneumoniae*
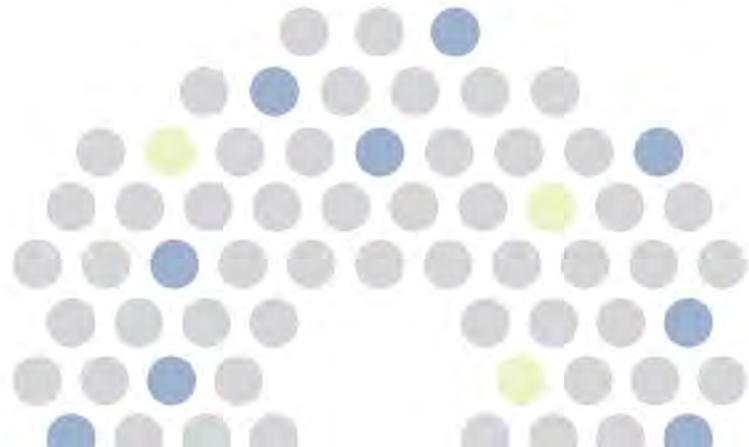
RP2 (US): Improved detection of 14 targets
Includes *B. parapertussis*

RP2 (OUS) and RP2Plus (US): Improved detection of 14 targets
Includes *B. parapertussis* and MERS CoV
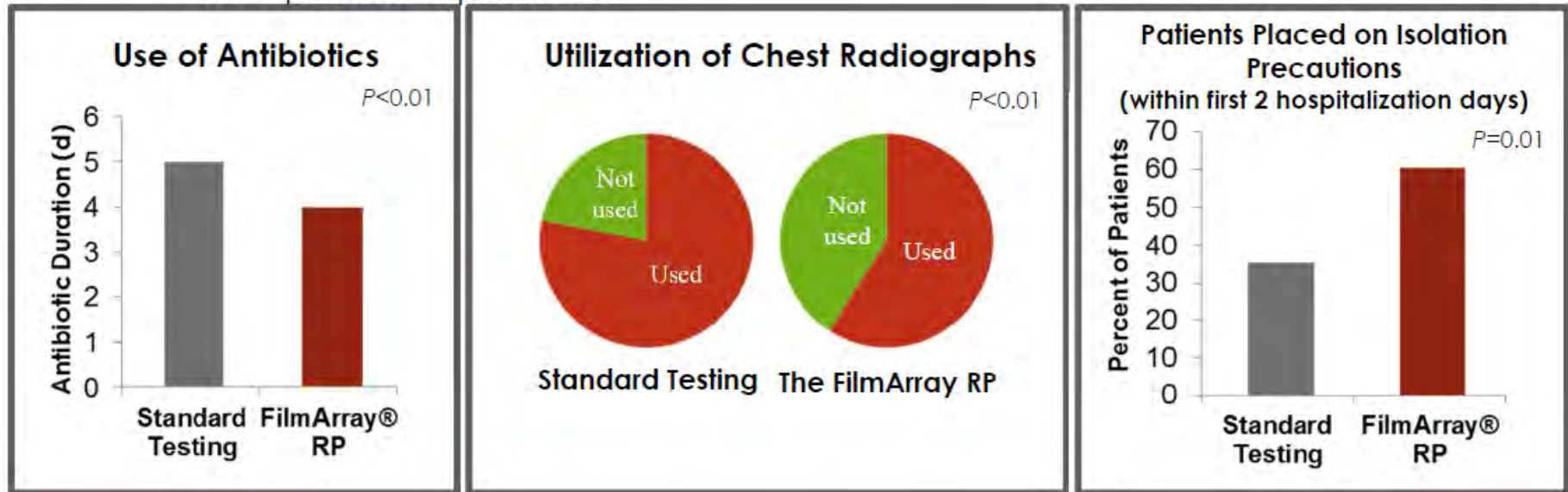
CULTURE: MAJOR PROBLEM OR IMPORTANT REQUIREMENT?
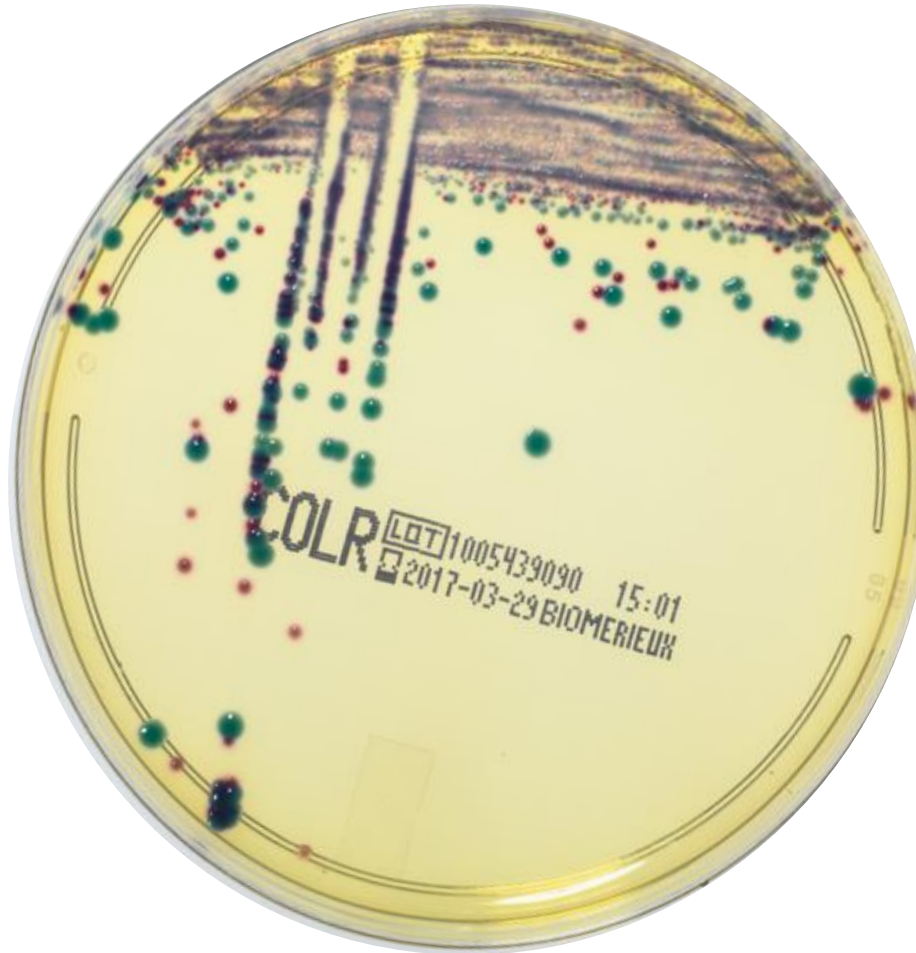
Screening of Colistin-resistant Enterobacteriaceae from rectal swabs and stools
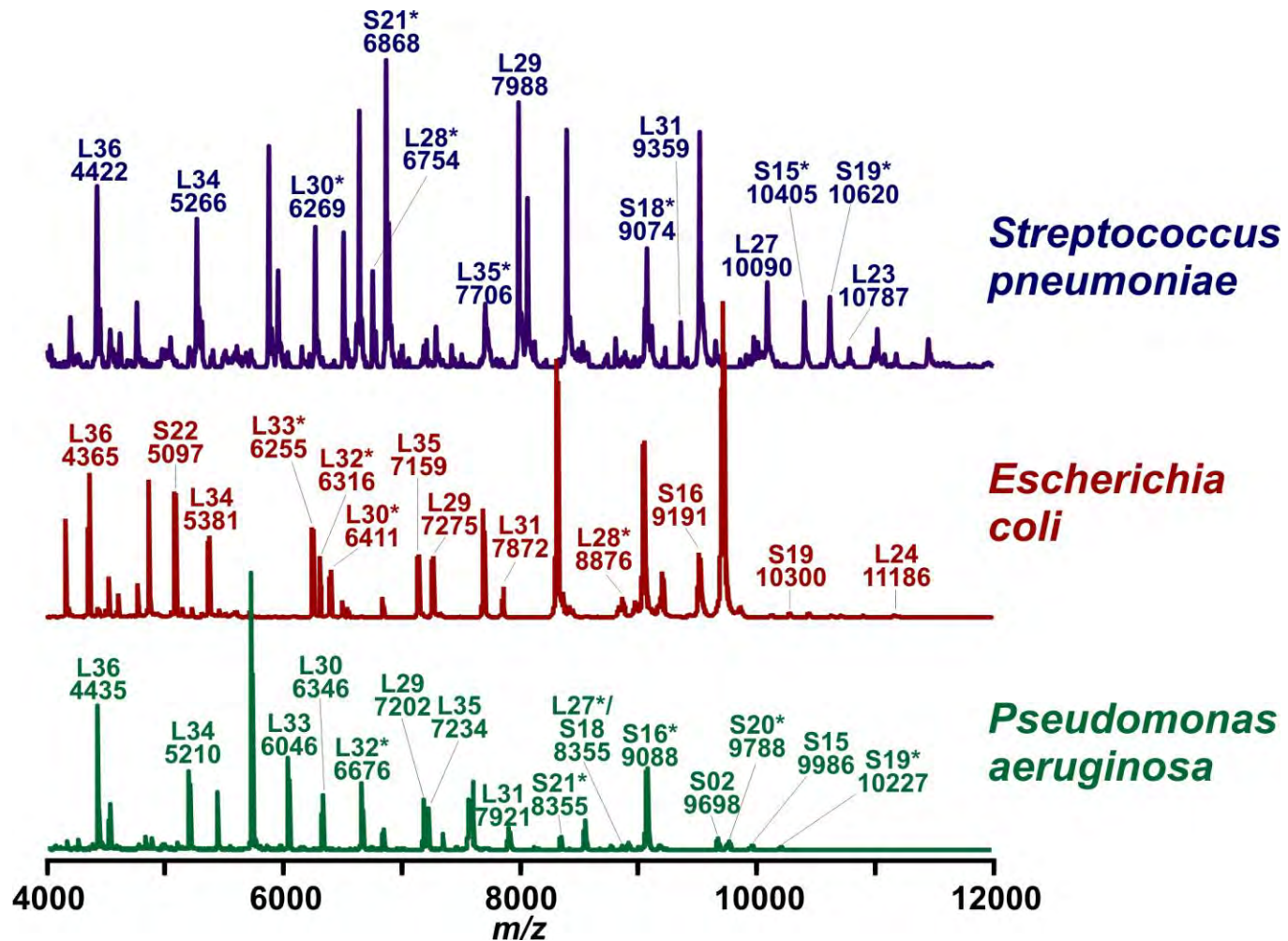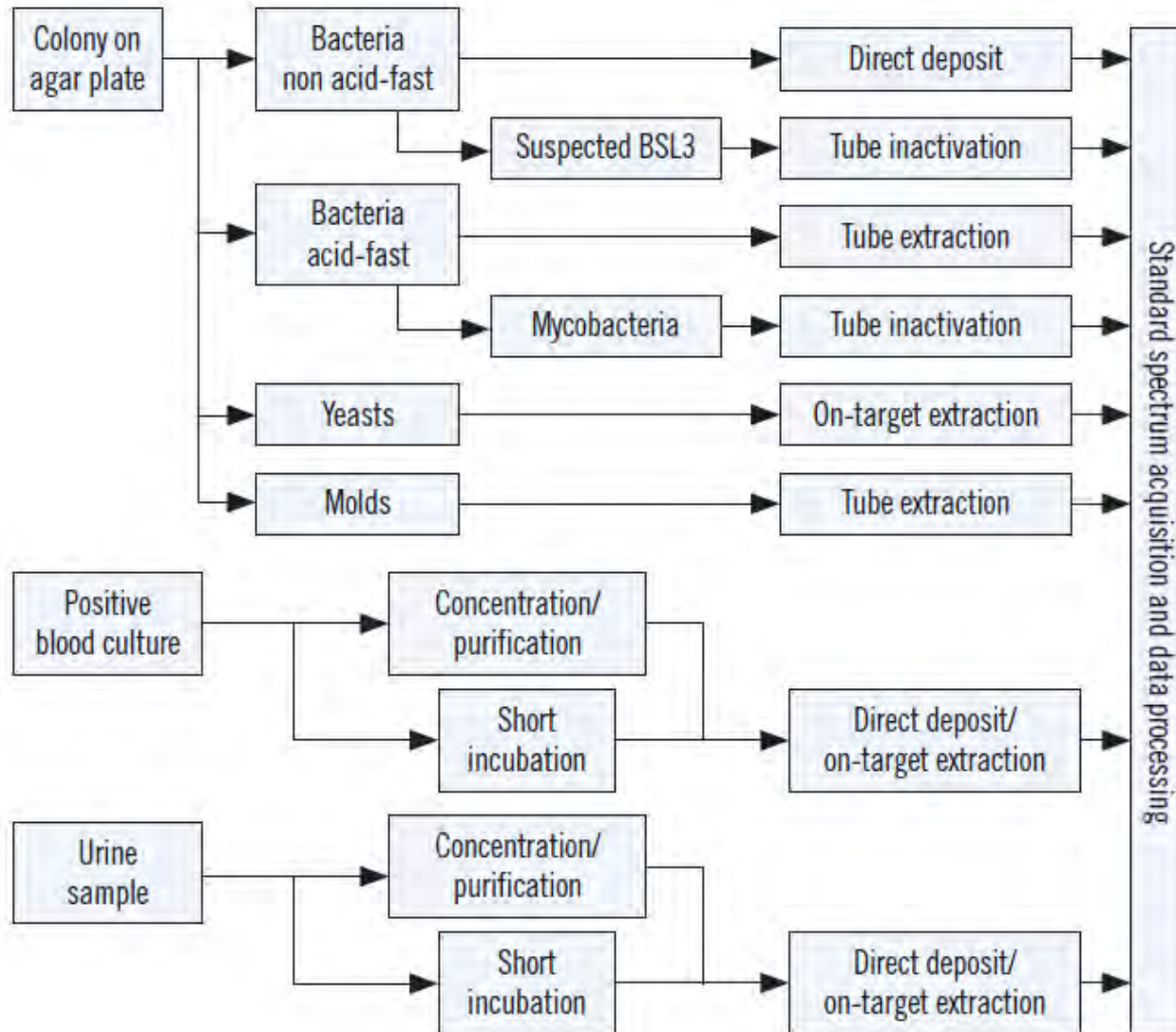
RESISTANCE DETECTION BY MALDI TOF MS

1. Detection of degradation or modification of antibiotics.
2. Use of incorporation of stable-isotope labeled amino acids.
3. MS mediated nucleic acid sequencing (Iridica, Abbott).
4. Direct detection of resistance (associated) factors (including next gen MS and pre-purification methods).
5. Changes in metabolic patterns.
6. Quantitation of MALDI detectable compounds.
7. Application of next gen MS methods

colistin-resistant *Klebsiella pneumoniae*
colistin-susceptible *Klebsiella pneumoniae*
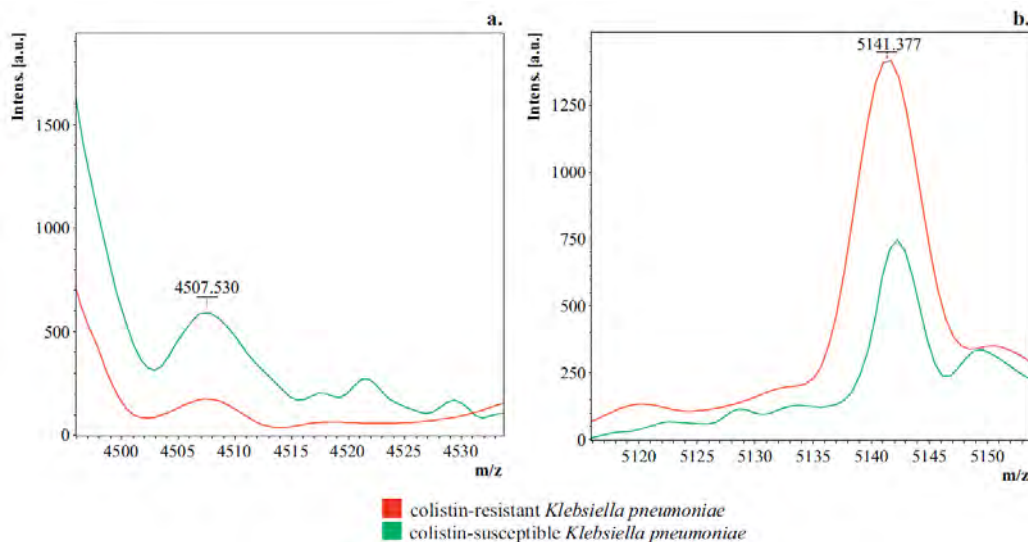
Giordano C, Barnini S. Rapid detection of colistin-resistant Klebsiella pneumoniae using MALDI-TOF MS peak-based assay. J Microbiol Methods. 2018 Dec;155:27-33.

BARRIERS

MICROBIOLOGY

- **SMALL NUMBERS OF CELLS**
- **SLOW GROWTH**
- **LAG TIME**
- **HETEROGENEITY OF ANTIBIOTIC RESISTANCE**
- **INDUCTION OF RESISTANCE**
- **LOW LEVEL RESISTANCE**
- **CIDAL VERSUS STATIC ANTIBIOTICS**
- **DETECTION OF NEW MECHANISMS**

TECHNOLOGY

- **NEED FOR ID AND AST AT THE SAME TIME (?)**
- **SPEED – PHENOTYPIC AST IN LESS THAN 4 H IS A CHALLENGE.**
- **MANDATORY DAILY QC TESTING (IRRESPECTIVE OF METHOD)**
- **RECURRING ISSUES AND DEVELOPMENT DELAYS IN SEMI AUTOMATED AST (MICROSCAN, PHOENIX, VITEK2)**
- **POOR QUALITY OF ASSAYS FROM SOME MANUFACTURERS**
- **RECURRING ISSUES WITH GRADIENT TEST QUALITY**
- **INFLUX OF NEW PHENOTYPIC METHODS – DIFFICULT TO ASSESS.**

MORE DRUGS ON A SINGLE CARD

VITEK® 2 OPUS AST CARD

Test more antibiotics

60% MORE ANTIBIOTICS

Save time Increase efficiency with less offline tests

Easy transition (Same set up and same VITEK®2)

REPORT APPROPRIATE ANTIBIOTICS FOR BETTER TREATMENT DECISIONS

BIOMÉRIEUX

MICROFLUIDICS AND SINGLE CELL HANDLING.
TRANSCRIPTOMICS.
NEXT GENERATION MASS SPECTROMETRY.
(FLOW) CYTOMETRY.
CANTILEVERS.
ISOTHERMAL MICRO-CALORIMETRICS.
MAGNETIC BEAD ROTATION.
MICRODROPLETS.
NMR.
MICROSOUND.
METABOLOMICS (ROS AND CELLULAR RESPIRATION).
RAMAN, IR AND OTHER SPECTROSCOPIES.
BACTERIOPHAGES.
REAL-TIME, VIDEO ENHANCED MICROSCOPY.
APOPTOSIS MARKERS.
ELECTRONIC NOSES.
IMPEDANCE MARKERS.
ETC ETC
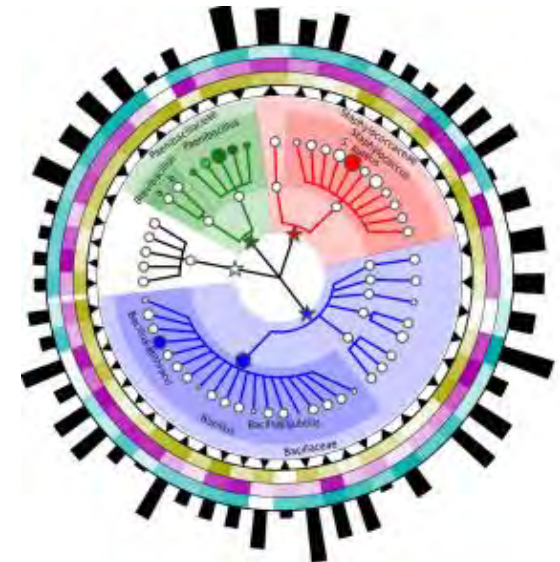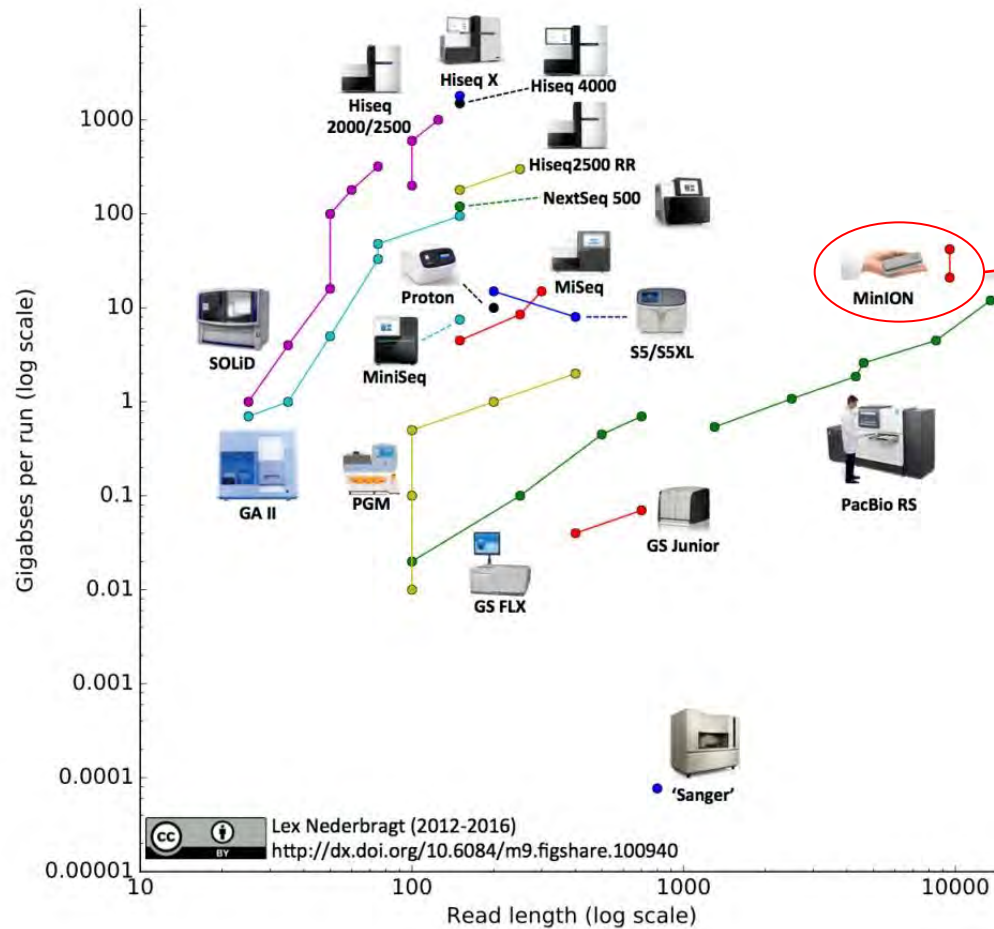
- **DIRECT DETECTION OF** ~~...~~ **M CLINICAL MATERIALS (BIOM** ~~...~~

- **EPIDEMIOLO** ~~...~~ **L WHOLE GENOM** ~~...~~

- **TRA** ~~...~~ **ES (ANTI** ~~...~~ **NG AND DETECT** ~~...~~ **MECHANISMS)**

CAN ALL OF THIS BE DONE IN A SINGLE DAY AT 100% SENSITIVITY AND SPECIFICITY, FOR MINIMAL COST AND AS SIMPLE AS BE??

21

Provided by Stéphane Schicklin

FIG 3 Comparison of genotype 4.3.1 (H58) isolates from Bangladesh, Pakistan, and Nepal in a wgSNP-derived MLT. No singleton was considered in the consensus SNP data. The tree is colored by country. Different data points, including lineage, sublineage (details), presence of different *gyrA*-83 mutations, MDR, cip resistance, presence of *qnr* genes, and cro resistance phenotypes are indicated (by colors) in different circles around the tree.

ONE HEALTH TECHNO ASSESSMENT

- Classical microbiology will not soon cease to be …..
- Automation and mobile applications (phone, drone, robots) are being developed but at a pace that is not as high as initially foreseen; mostly robotic replacement for standard human actions and manipulations.
- MALDI TOF MS is the new gold standard for bacterial identification and its rapidity has clinical impact.
- PCR testing is the single methodology to date allowing rapid and reliable direct-from-sample diagnostics.
- OMICS testing is entering the routine laboratory.
- Next generation sequencing, currently considered a panacea by many, will fill in many significant niches in the routine laboratory.
- The diagnostic use of "big clinical data" will become commonplace.
- Hence, diagnostic TAT will go down. Question is how much and whether this will be clinically actionable ….

ACKNOWLEDGMENTS

- Marie Françoise Gros and Claude Mabilat for (many) slides.

- The Point Prevalence Surveillance team.

- Marc van Nuenen for BioFire slides.

- Victoria Girard for any MALDI TOF MS stuff.

- Mike Dunne for (post-retirement) AST activities.

- François Vandenesch et al for *S. aureus* genomics.

- Fondation Mérieux and Tanmoy for *S. typhi* collaboration.

Antifungal use in veterinary practice and emergence of resistance

Amir Seyedmousavi, DVM, PhD, (F)ECMM

Senior Staff Clinical Microbiologist

Department of Laboratory Medicine,
National Institute of Health Clinical
Center, Bethesda, MD, USA

Is resistance in fungi a concern for you?

Medically relevant fungal groups

Aspergillus

Hypocreales

Glomerellales

Xylariales

83

Microascales

Lomentospora

...eria

...phiostom...

...is

...odiales

Aureo...

Onygenales

80

Coccidioides

Zoophilic dermatophytes

Anthropophilic dermatophytes

Geophilic dermatophytes

...ides

...phora

...cet...

...toc...

Mal...

...iob...

Entomophthorales

Conidiobolus

93

Mucorales — *Mucor, Rhizopus, Rhizomucor, Lichtheimia*

Rhizophidiales — *Batrachochytrium*

86

0.05
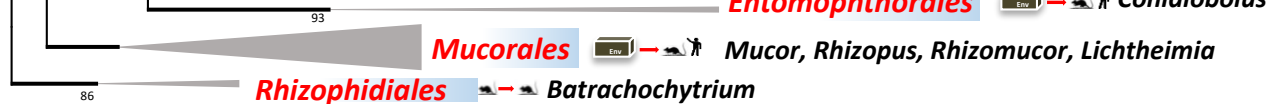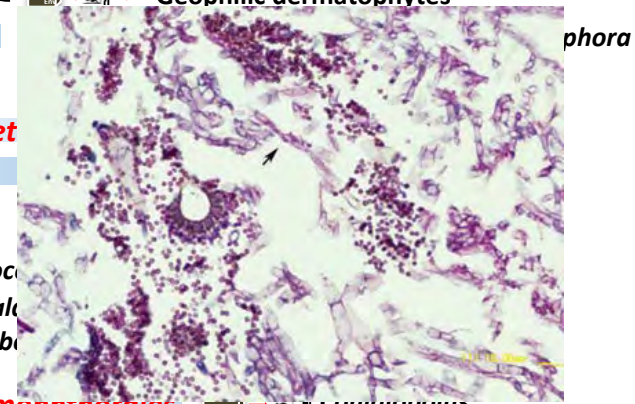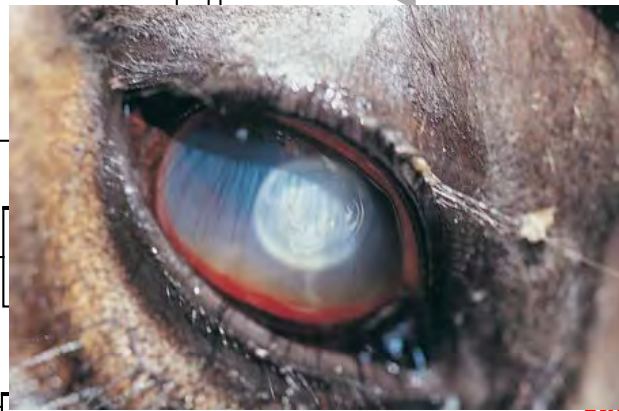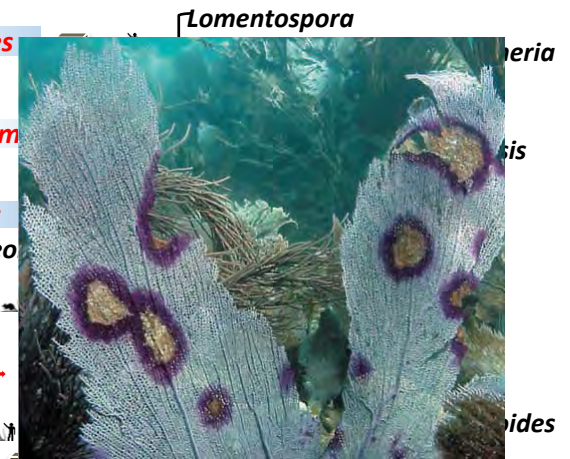
Medically relevant fungal groups

Dermatophytes
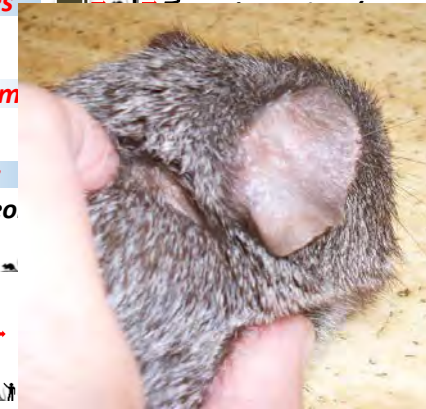
Hypocreales

merellales

Xylariales

83

Microascales

Lomentospora

alleseheria

ckii

asiliensis

iostom

iales

Aureo

occidioides

Coccidioides

Zoophilic dermatophytes

Anthropophilic dermatophytes

Geophilic dermatophytes

Onygenales

80

Chaetothyriales

97

Cladophialophora, Exophiala,Phialophora

Sacchar

ocystid

itirachiales

us

chochytrium

86

0.05

Medically relevant fungal groups

Chytridiomycosis
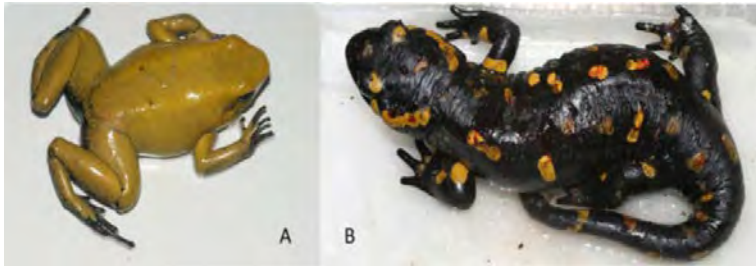
Sporothrix

Chaetothyriales

P. destructans

Cryptococcus gattii

0.05
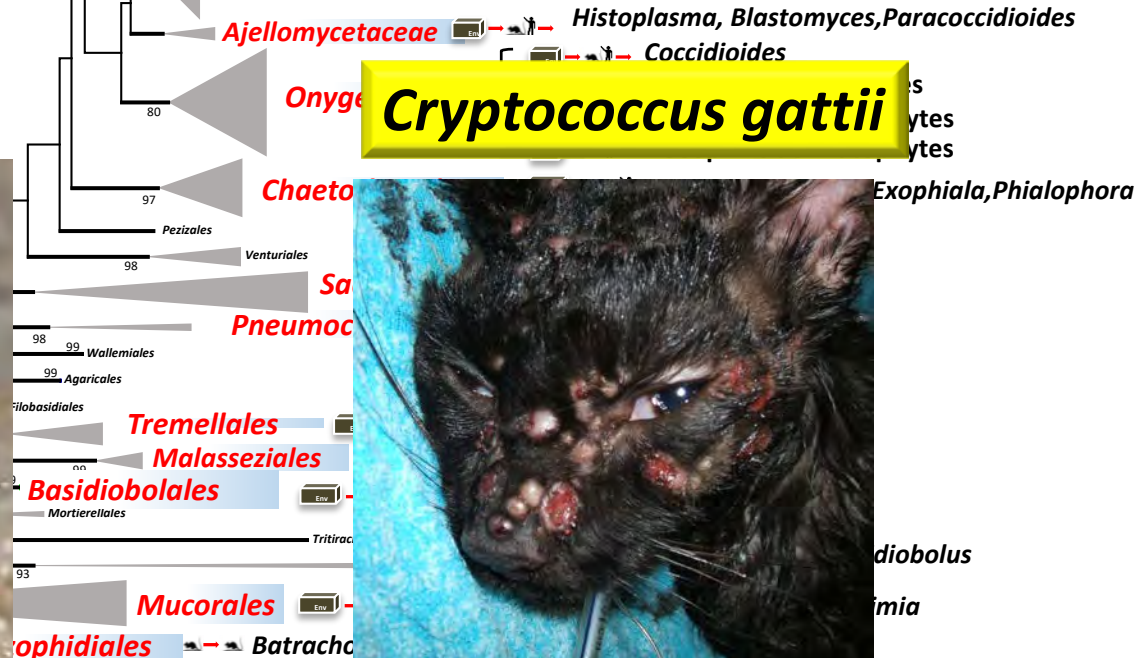
Antifungal therapy is a central component of protecting human and animals against fungal infections

Topical and/or systemic antifungal drugs can be used

The drug: principal antifungals in clinical practice from the 1950s to present
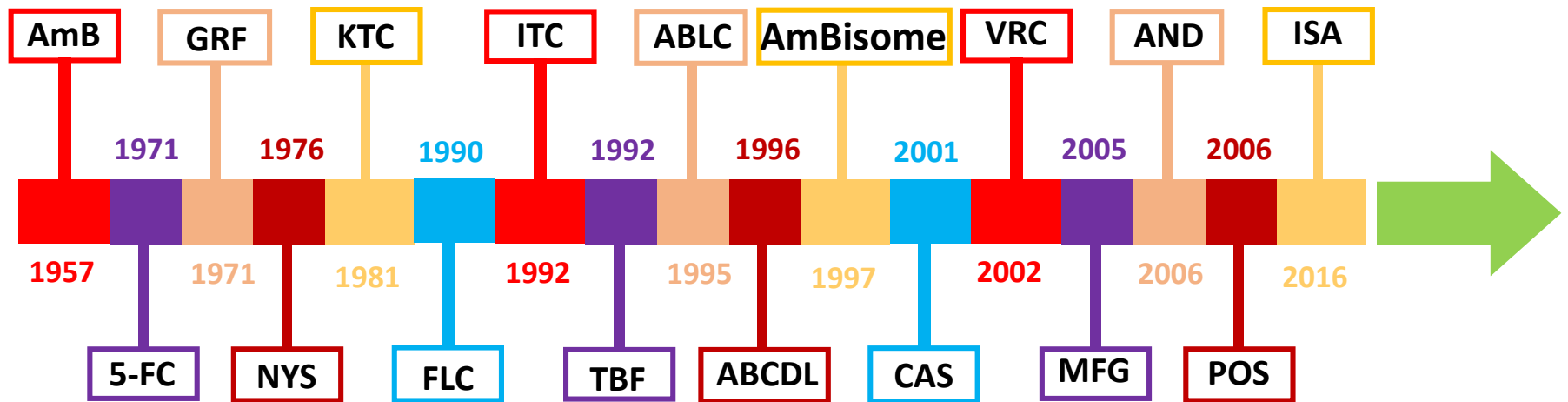
Antifungals in France

Griseofulvin	dermatophytosis
Nystatin	*Malassezia* otitis
Ketoconazole	dermatophytosis
Enilconazole	dermatophytosis
Parconazole	candidosis
Miconazole	*Malassezia* dermatitis
Itraconazole	dermatophytosis
Posaconazole	*Malassezia* otitis
Terbinafine	*Malassezia* otitis

Off-label use of human antifungals is quite common

Jacques Guillot. Ecole nationale vétérinaire d'Alfort, France

FDA / EMA Approved Animal Drug Products

Application#	Sponsor Name	Proprietary Name	Ingredients	Application Status
012-258	Zoetis Inc.	Panolog® Ointment	Neomycin Sulfate Nystatin Thiostrepton Triamcinolone	Approved
		Cream		Withdrawn
096-676	Zoetis Inc.	Panolog® Cream	Neomycin Sulfate Nystatin Thiostrepton Triamcinolone	Approved
140-810	Med-Pharmex, In		ate Nystatin riamcinolone	Approved
140-847	Fougera Pharmaceuticals, Inc.		ate Nystatin riamcinolone	Approved
140-879	Zoetis Inc.		ate Nystatin riamcinolone	Approved
140-889	Biocraft Laboratories, Inc.		ate Nystatin riamcinolone	Voluntarily Withdrawn
		Derm-Otic Ointment	Neomycin S Thiostrepto Acetonide	
		Derma-Vet Cream	Neomycin S Thiostrepto Acetonide	
		Animax® Cream	Neomycin S Thiostrepto Acetonide	

Only a few products are licensed for animals

MIC distributions for clinical *A. fumigatus* isolates
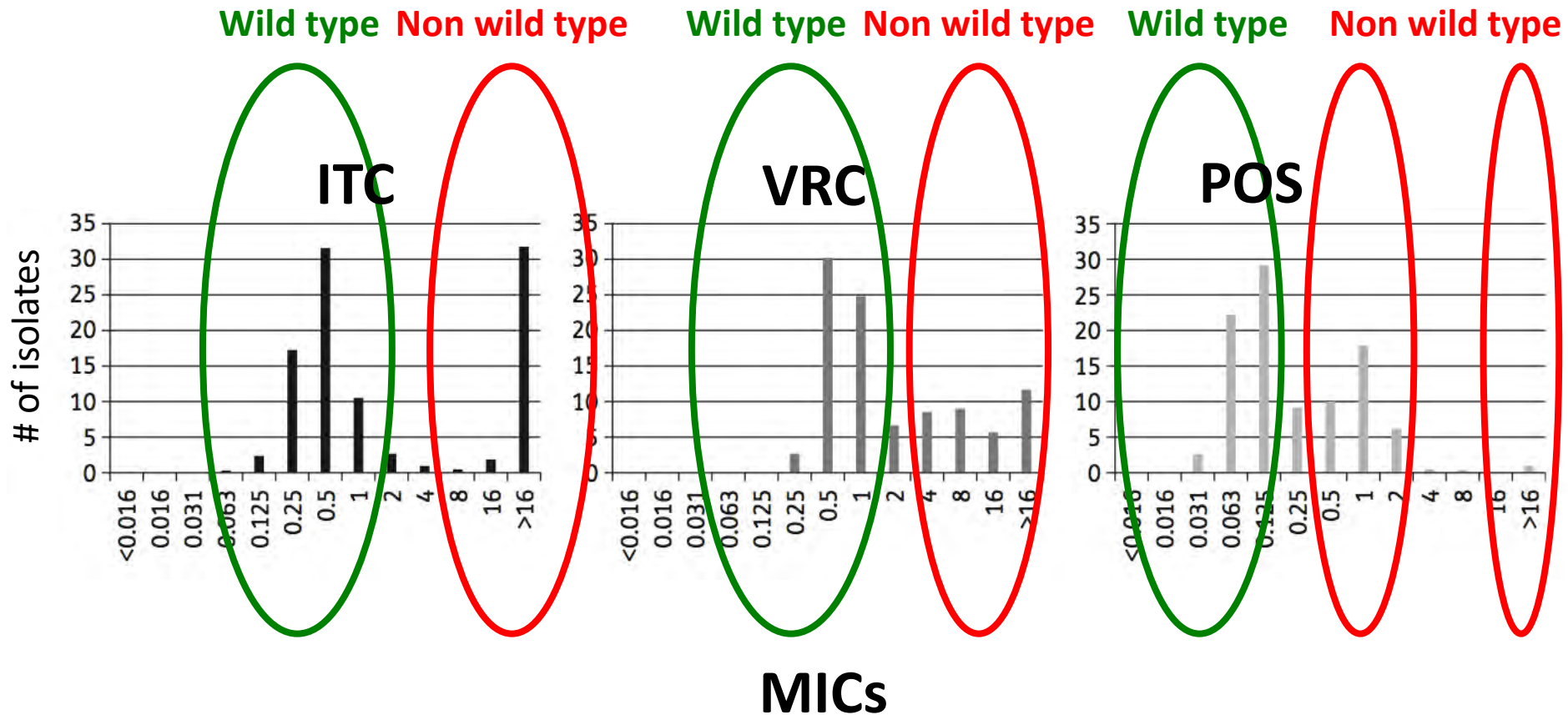
Most isolates were multi-azole resistant

Antifungal azole drug targets

Geographic spread: clinical and environmental

Lestrade et al. Clinical Microbiology and Infection. 2019. In press.
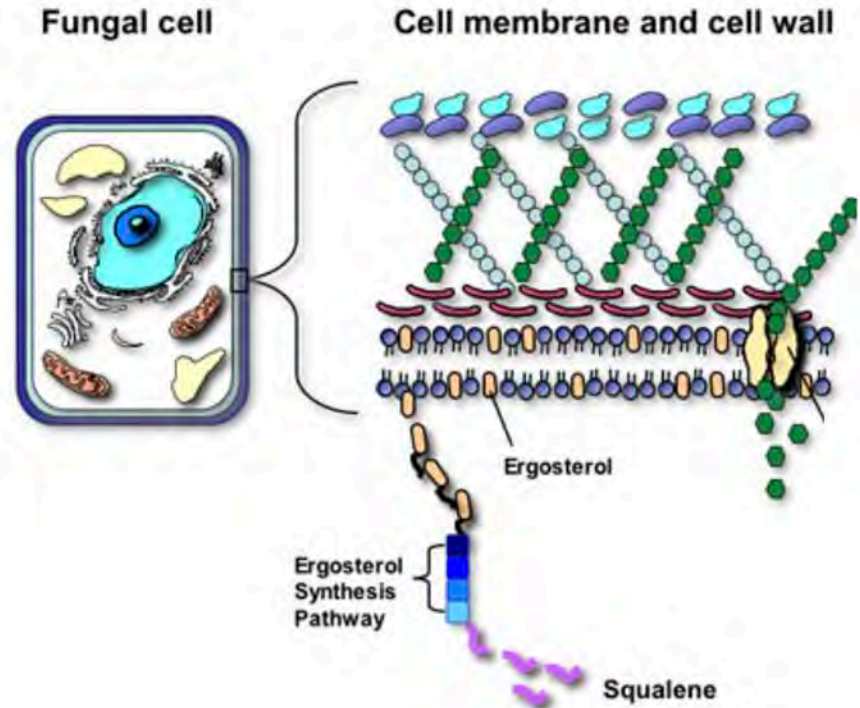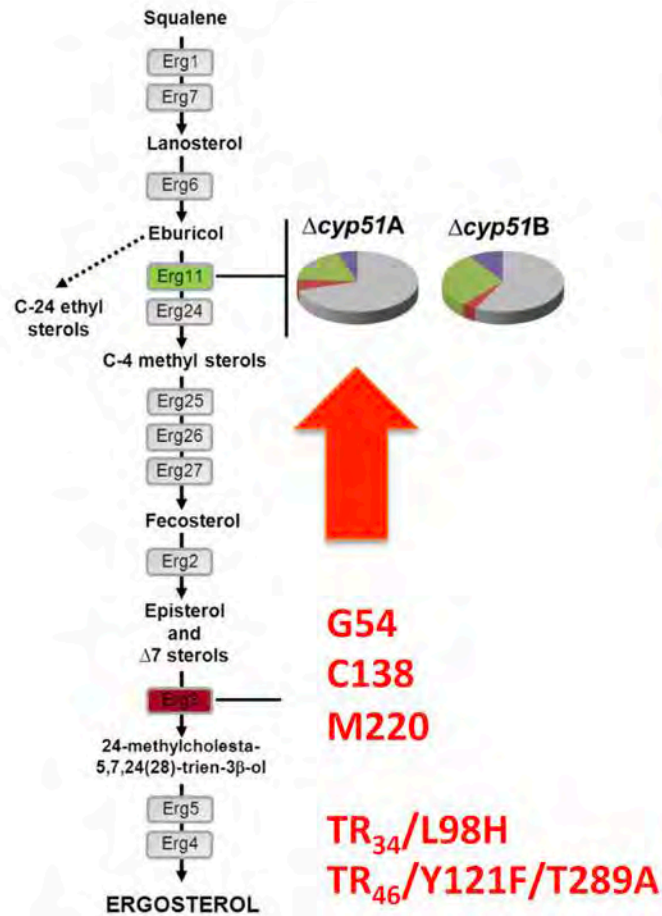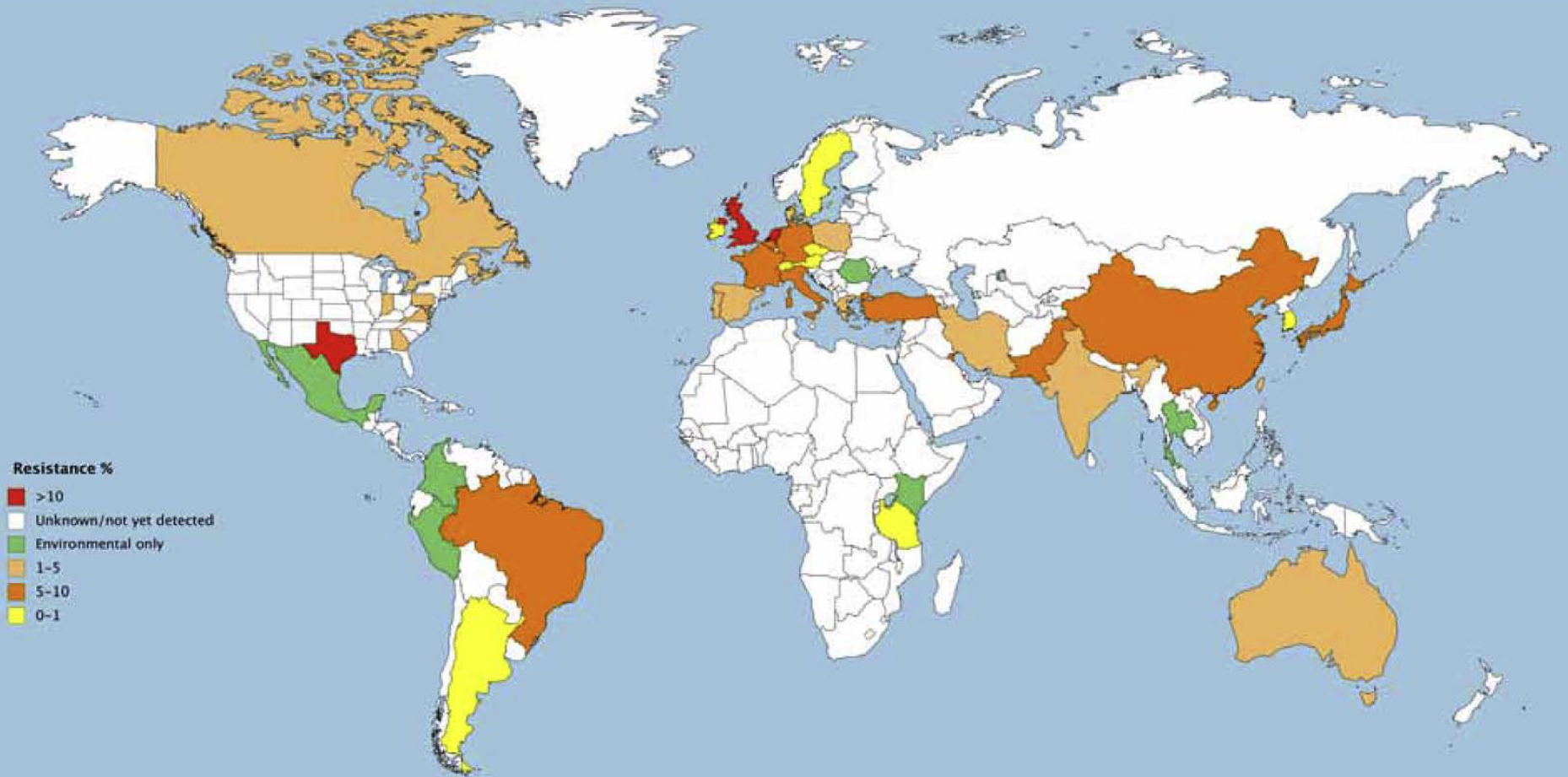
Veterinary cases of azole-R in *A. fumigatus*

Emerging *Aspergillus* species associated with CGD
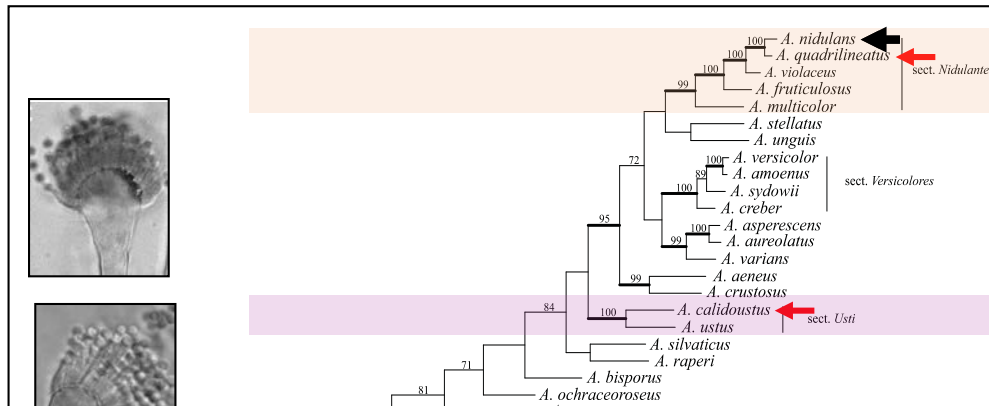
A. quadrilineatus

A. calidoustus

Extremely difficult to treat
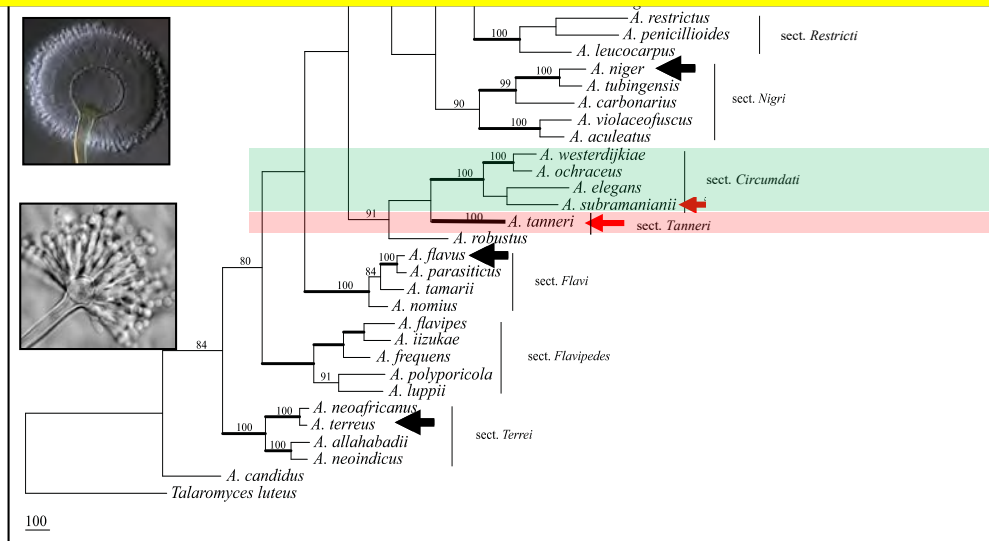
A. subramanianii
A. tanneri

Veterinary: *A. viridinutans* species complex

A. udagawae, A. felis clade species (*A. felis, A. pseudofelis, A. parafelis*), *A. pseudoviridinutans, A. wyomingensis*

ITZ - 20 (54.1 %) - (>1 mg/L)
VCZ - 31 (83.8 %) - (>1 mg/L)
ISA - 30 (81.1%) - (>1 mg/L)
POS – 1 isolate
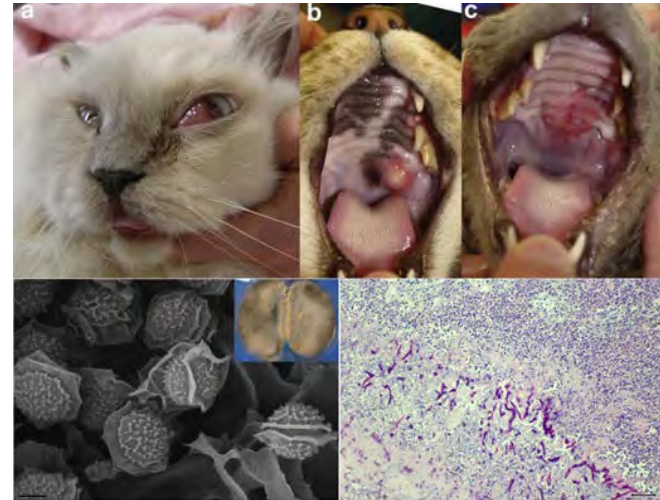
Resistance mutations detected!

G138C

Talbot et al. Medical Mycology, 56, 1, 2018, 1–12

inherently resistant to antifungals
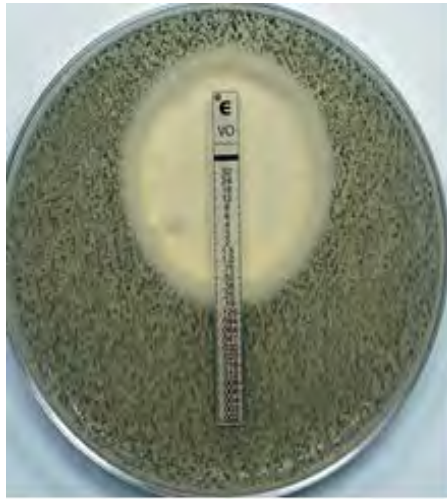
A. fumigatus

A. tanneri

➢ **No change in CYP51A**

A.f: A. fumigatus

A.fl: A. flavus

A.t: A. tanneri
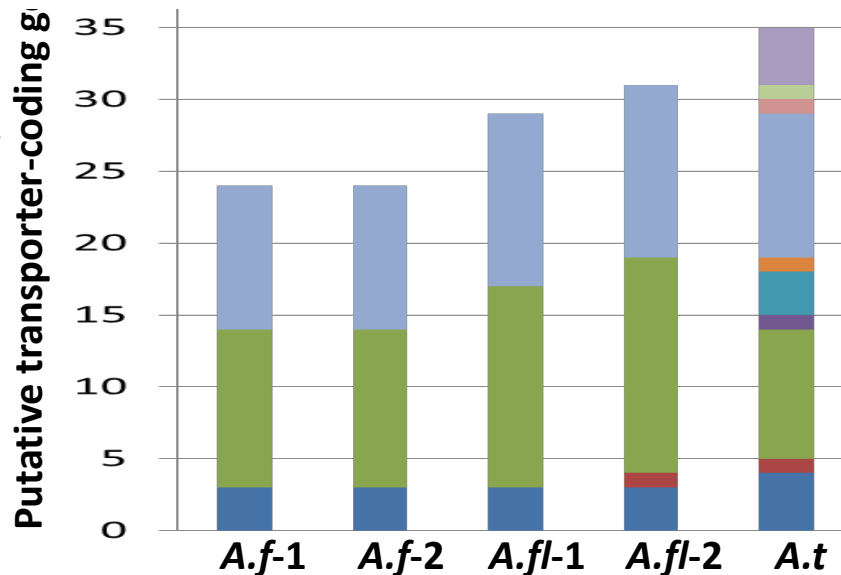
➢ **Various efflux pumps**

➢ **Total number of ABC transporters**

Pleiotropic drug resistance proteins (PR115), ABC superfamily protein

bile acid ABC transporter

ABC drug exporter AbcA

ABC bile acid transporter

Putative transporter-coding g[...]

Dolphin with invasive aspergillosis

A 10 year old female captive bottlenose dolphin (175 kg)

Culture: *Aspergillus fumigatus*

Treatment during 1 year with POS (a dose of 600 mg/day)

Progression of the infection

Treated with antibiotics for bacterial pneumonia caused by *Vibrio alginolyticus*

6 months into therapy: multiple white, raised lesions in the trachea and bronchi

Bunskoek and Seyedmousavi et al. Med Mycol Case Rep 2017;16:16-19
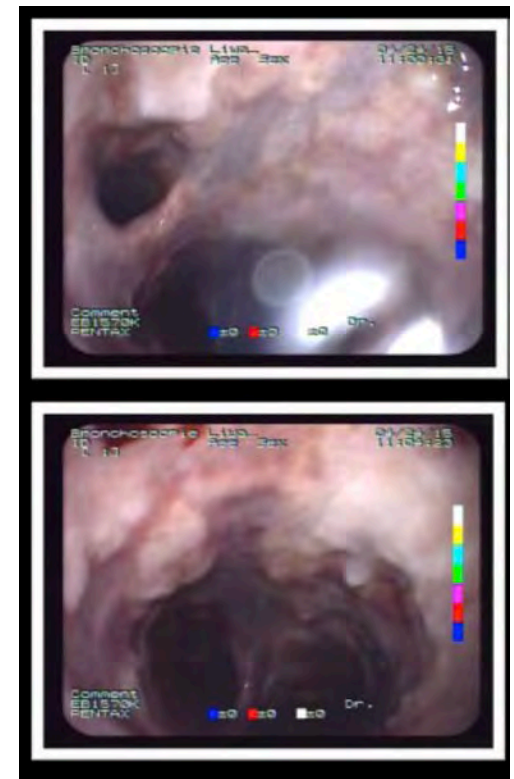
CT scan

Small granulomas and some infiltrates
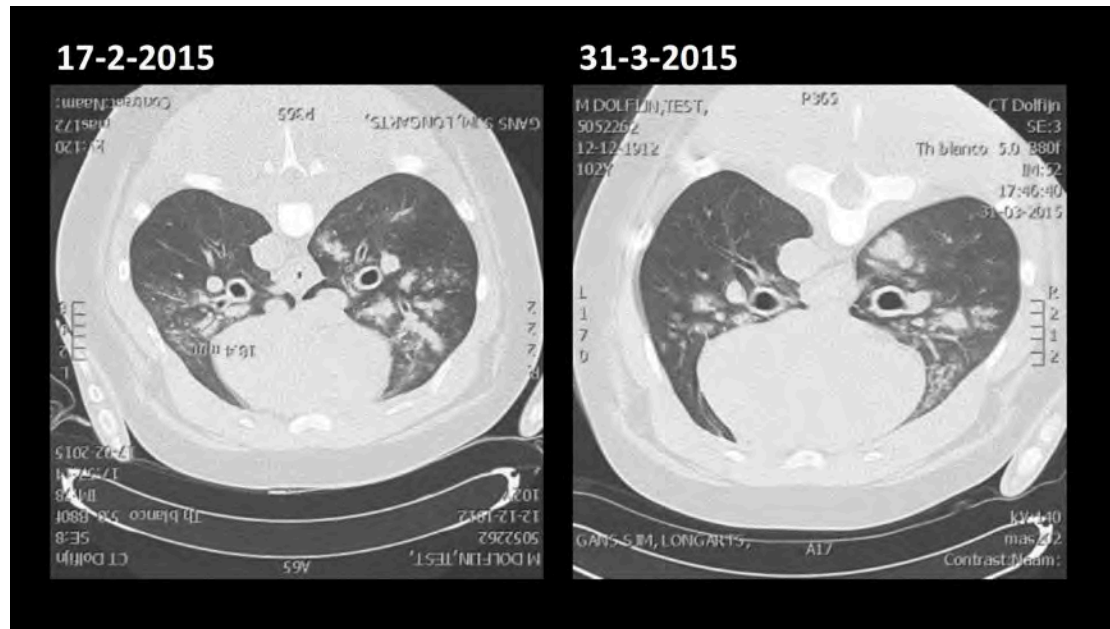
MIC test:

AmB 0.5 mg/l
ITC >16 mg/l   **R**
VRC 16 mg/l   **R**
POS 0.5 mg/l   **R (low)**
AFG 0.06 mg/l

The strain harbored the TR46/Y121F/T289A resistance mutation in the *cyp*51A-gene

How to treat azole R *Aspergillus* diseases?

In vivo efficacy of voriconazole

Efficacy of 2.5-20 mg/kg VRC-monotherapy against VRC-S *A.fumigatus* (MIC 0.25)
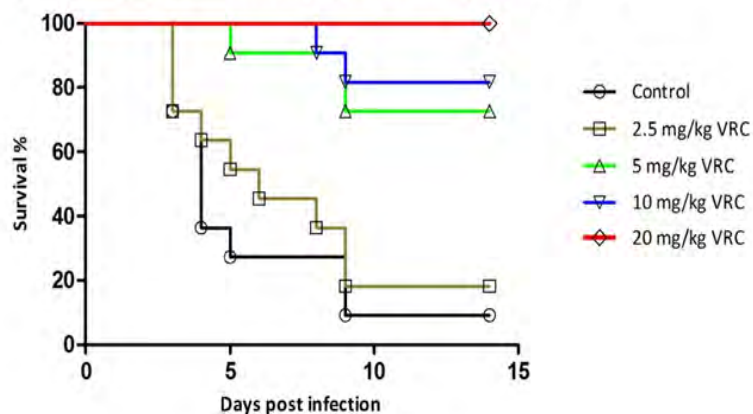
Efficacy of 2.5-20 mg/kg VRC-monotherapy against VRC-R *A.fumigatus* (MIC 4)

VRC-S

Max 100%

VRC-R

Max 72 %

Seyedmousavi et al. Journal of Antimicrobial Chemotherapy,2013, 68, 2, 385–393
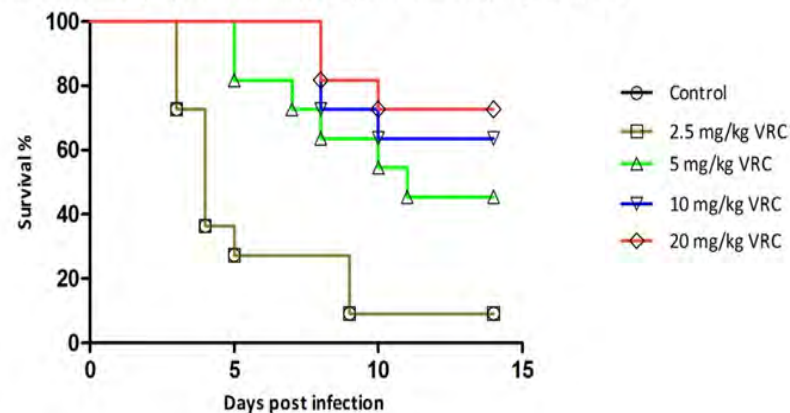
In vivo efficacy of anidulafungin
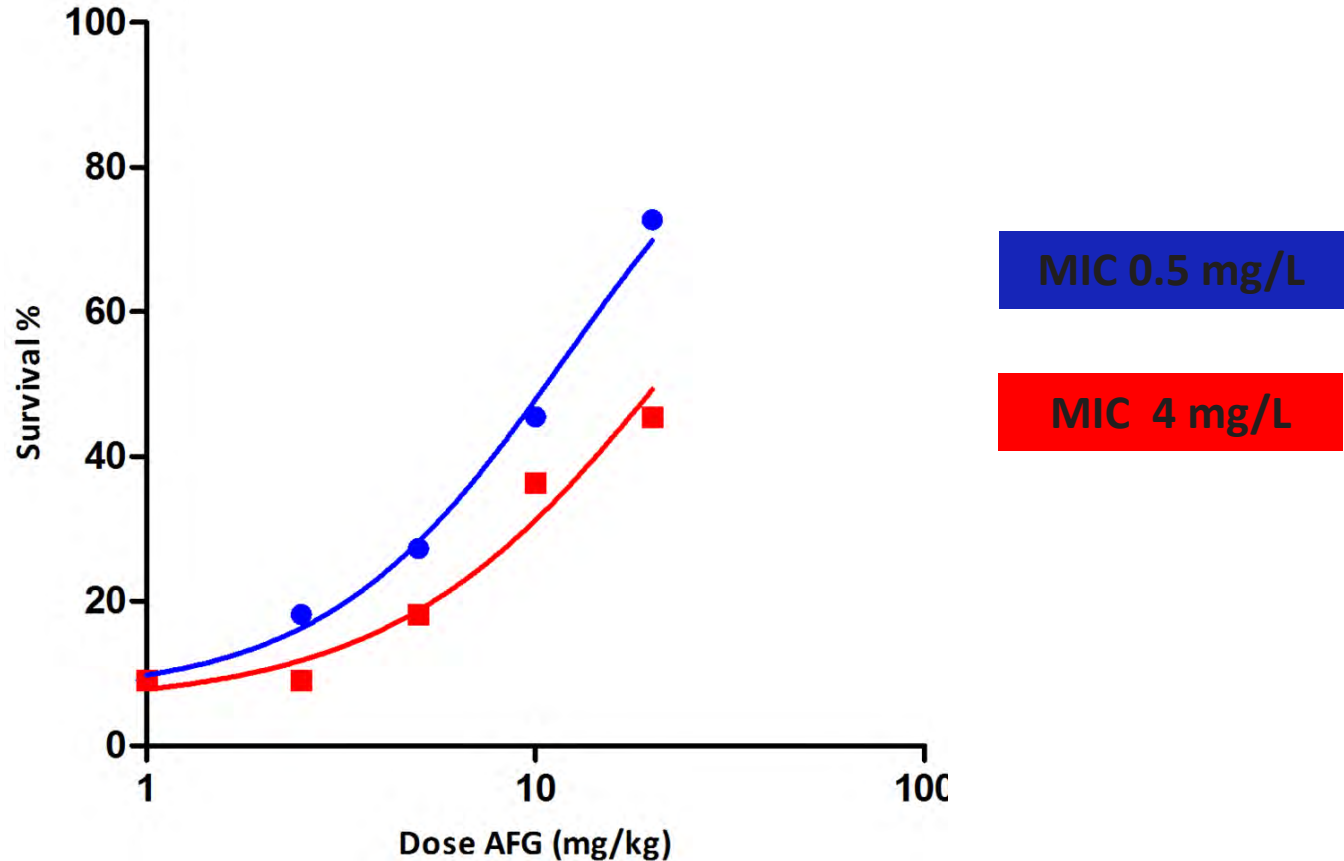
A maximal response was not achieved with either isolates

Seyedmousavi et al. Antimicrobial Agents and Chemotherapy,2013, 57, 1, 303-308

in vitro and *in vivo* combination: VRC+AFG

Bliss independence 3-dimensional plot: AZN 81-96 (MIC 0.25 mg/L VCZ)
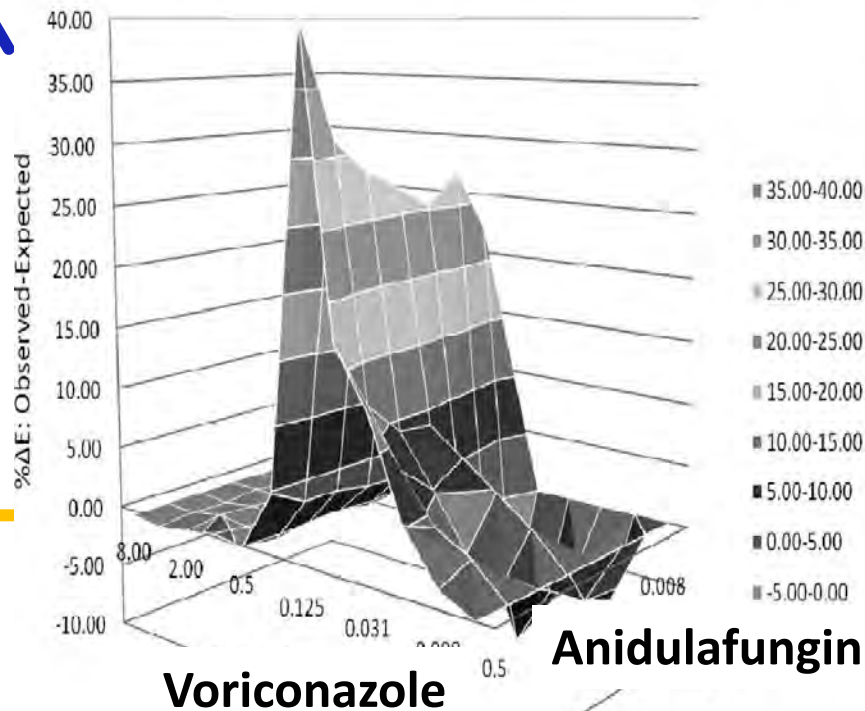
Bliss independence 3-dimensional plot: V 52-35 (MIC 4 mg/L VCZ)

VRC-S

VRC-R

Seyedmousavi et al. Antimicrobial Agents and Chemotherapy 2013; 57: 796–803
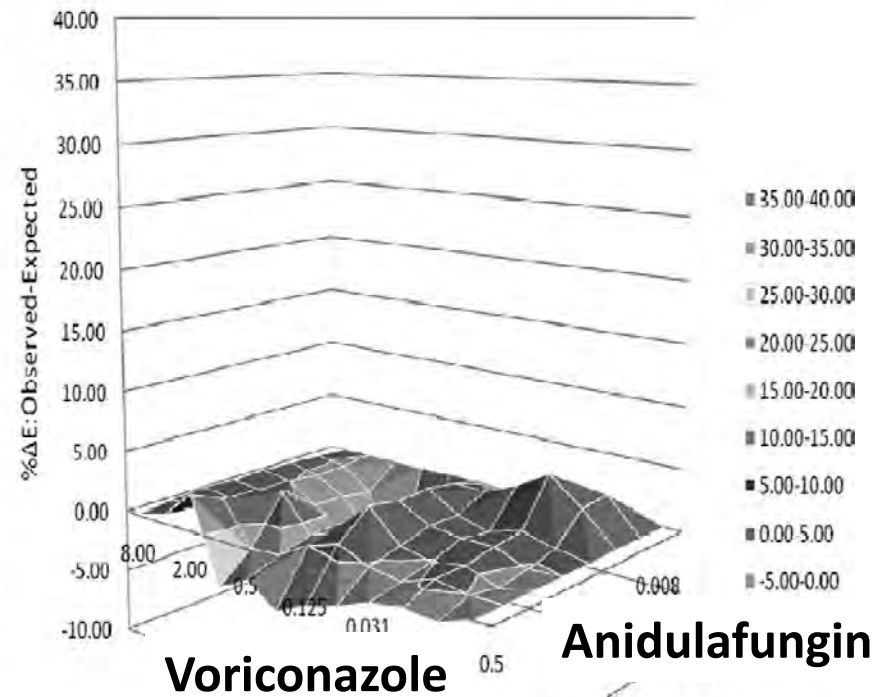
MIC based dosing

POS

Mutation in target gene

AUC/MIC

C trough (TDM)   > 3.09 required

Oral-fasted

Oral-non fasted

IV

Seyedmousavi et al. Drug Resist Updat. 2014;17(3):37-50.

What advice to give?

MIC test:

AmB 0.5 mg/l

ITC >16 mg/l   **R**

VRC 16 mg/l   **R**

POS 0.5 mg/l   **R (low)**

AFG 0.06 mg/l

The strain harbored the TR46/Y121F/T289A resistance mutation in the *cyp*51A-gene

Recommendation:
- ➢ **continue POS therapy**
- ➢ **try to achieve a drug level of > 3.09 mg/l**

Follow-up bronchoscopy and CT showed complete resolution of the lesions

Bunskoek and Seyedmousavi et al. Med Mycol Case Rep 2017;16:16-19
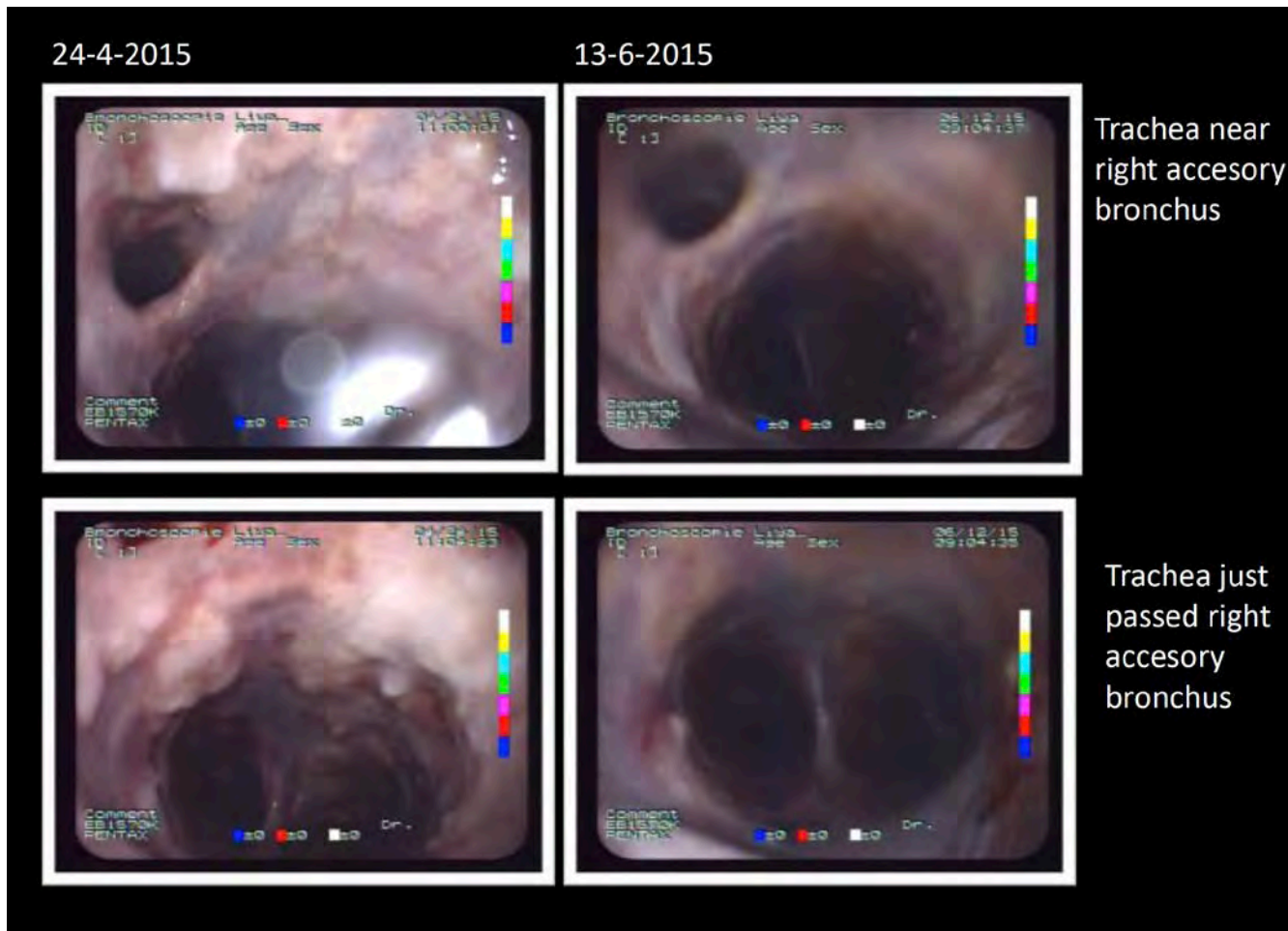
POS serum levels of 3-9.5 mg/l were achieved without significant side-effects ultimately leading to clinical cure

Bunskoek and Seyedmousavi et al. Med Mycol Case Rep 2017;16:16-19
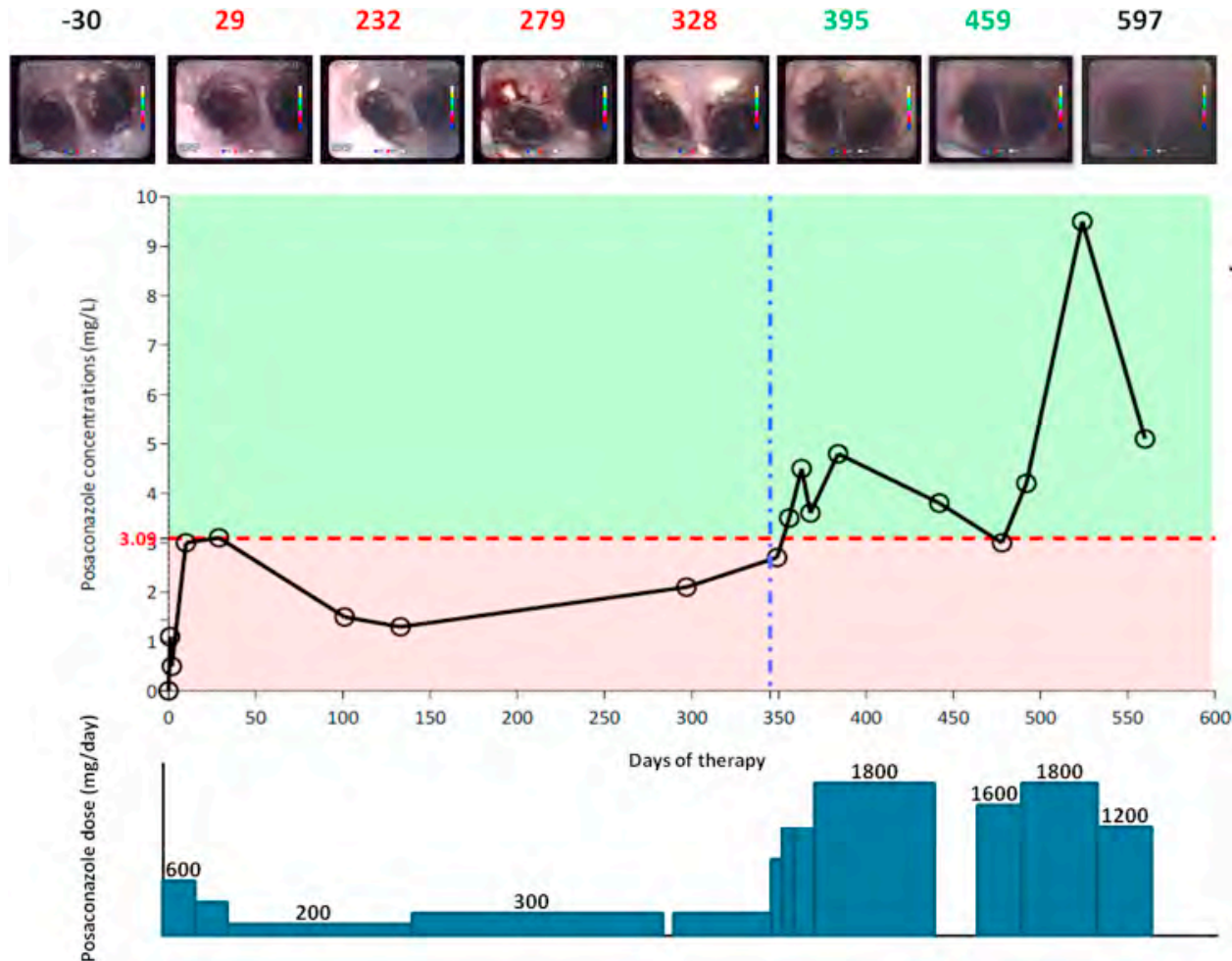
Conclusion

Azole resistance is an emerging concern in medically important fungi

Importance of environmental route of resistance selection

Focus on your local epidemiology

Acknowledgement

RadboudUMC, Nijmegen, NL
Prof. Paul Verweij
Dr. Willem Melchers
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Hein van der Lee

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Westerdijk Fungal Biodiversity Institute, Utrecht, NL
Prof. Sybren de Hoog

MMS, LCIM, NIAID, NIH
Dr. June Kwon-Chung

MMS, LCIM, NIAID, NIH
Intramural program

University of Tehran and Mazandaran UMC, Iran
Prof. Mohammad Hedayati

Pharmaceutical companies
Gilead, Pfizer, MSD, Astellas, Basilea

Veterinary Mycology Working Group
Aspergillus Resistance Surveillance Working Group

ESCMID Fungal Infection Study Group - EFISG

ASM Country Ambassador to Iran

Medical Mycology

ISHAM
INTERNATIONAL SOCIETY FOR
HUMAN AND ANIMAL MYCOLOGY

OXFORD
UNIVERSITY PRESS

Seyedmojtaba Seyedmousavi
G. Sybren de Hoog
Jacques Guillot
Paul E. Verweij
Editors

Emerging and Epizootic Fungal Infections in Animals

Springer

Veterinary Mycology Working Group
Aspergillus Resistance Surveillance Working Group

ESCMID Fungal Infection Study Group - EFISG

International Science Ambassador

Thank you for your attention

CBPs, ECOFFs, "I" and All that Jazz

John Turnidge

EUCAST Scientific Secretary

Joint Curator EUCAST MIC database

Terminology

- (Clinical) Breakpoints
  - Susceptible
  - Intermediate (CLSI)
  - Susceptible—Increased exposure (EUCAST)
  - Resistant
  - Non-susceptible (CLSI)

- Epidemiological Cutoff Values
  - Wild type
  - Non-wild type (above wild type)

Predicting treatment outcomes

Predicting resistance mechanisms

Understanding MIC distributions

1. What is an MIC, mathematically speaking?
   an <u>interval</u> measure,
   and the MIC is at the **UPPER** end of the interval
2. Why the 2-fold dilution series?
   a happy accident of history
3. How are wild-type MICs distributed?
   anything but wildly, they are **log-normally** distributed
4. Why are wild-type MICs distributed that way?
   because of variation**s**

Why are wild-type MICs distributed that way?

- **Variations!**
  - Assay variation
    - reagents
    - intra-laboratory (reading)
    - inter-laboratory (conditions)
  - Biological variation
    - strain-to-strain

CVs are typically 50-100%

Table 1. Media lot comparisons and inter- and intra-laboratory comparisons of finafloxacin MIC results versus *E. coli* ATCC 25922.

Same strain, same batches of reagents

MIC (µg/ml)	Occurrences By Lot			Laboratory Code (Occurrences)								Total N
	A	B	C	A	B	C	D	E	F	G	H	
0.002												
0.004												
0.008												
≤0.015	11	10	47	11			17		12	11	17	68
0.03	41	42	17	14	2	6	13	16	18	18	13	100
0.06	26	28	15	5	27	23		14				69
0.12	2				1	1						2
0.25												
0.5												
1												
2												
Total	80	80	79	30	30	30	30	30	30	29	30	239
Mode	0.03	0.03	0.015	0.03	0.06	0.06	0.015	0.03	0.03	0.03	0.015	0.03
GeoMean	0.035	0.035	0.023	0.026	0.059	0.053	0.020	0.041	0.023	0.023	0.020	0.030
Range	4	3	3	3	3	3	2	2	2	2	2	4

What is an MIC?

- The way the data are usually presented...
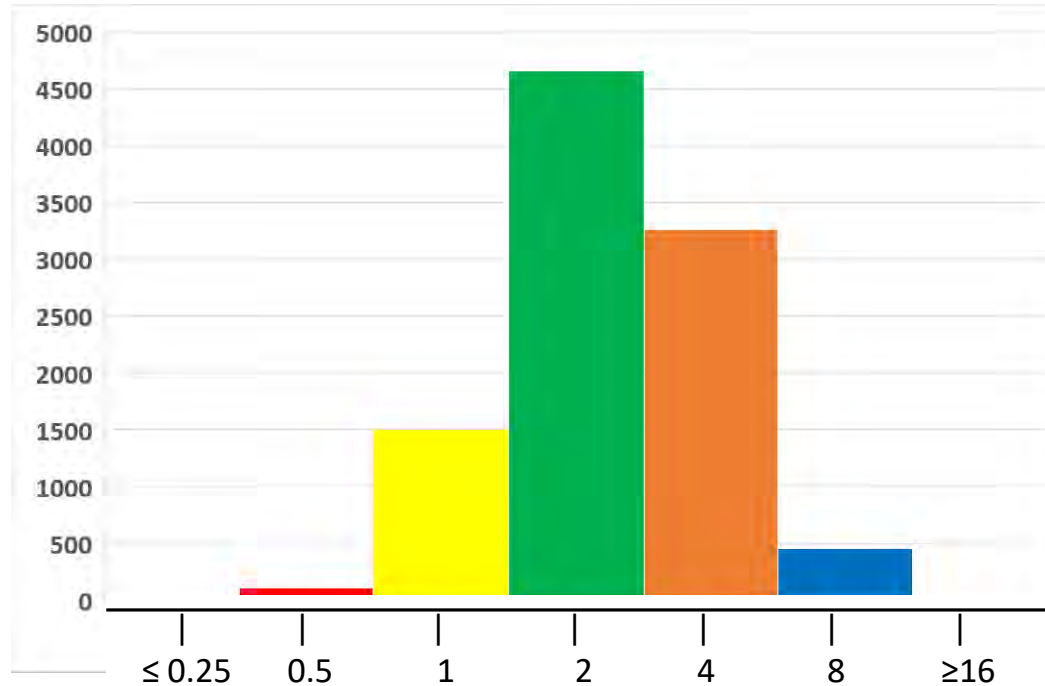
What is an MIC?

• The correct way to present the data…
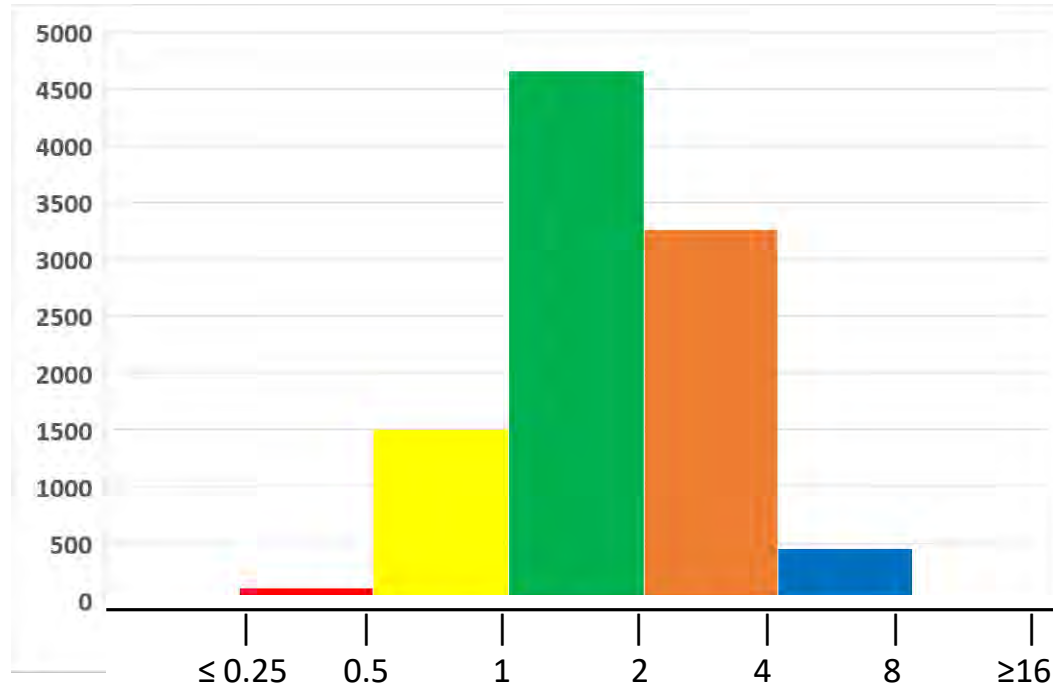
What is an MIC?

• Or mathematically, as a formal histogram <u>on a log$_2$ scale</u>…

"**(**" means down to but not including

"**]**" means up to and including
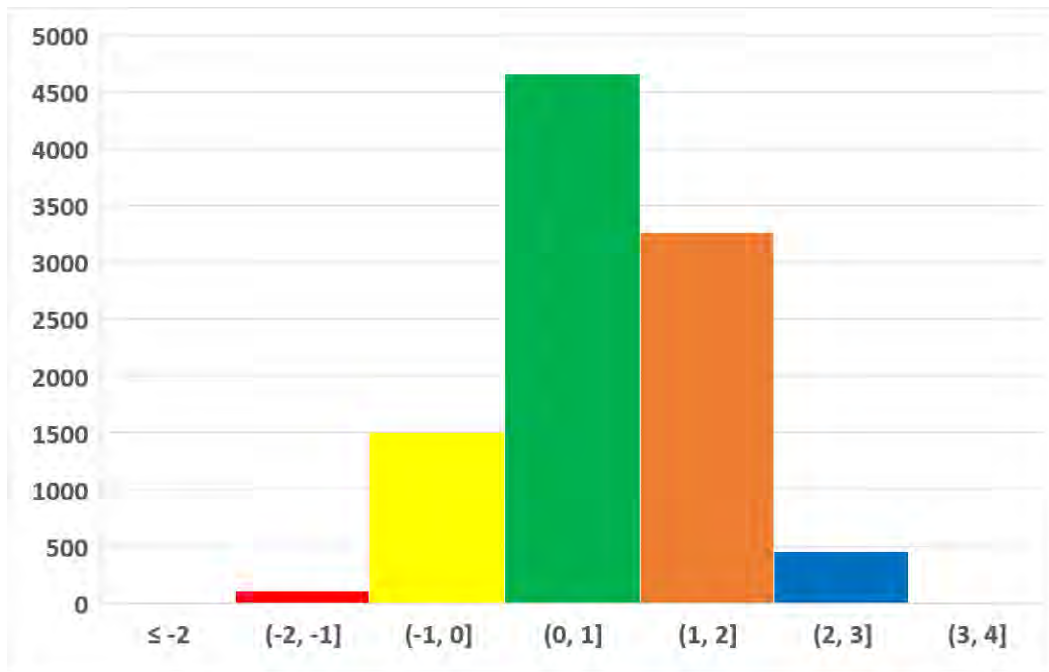
Interval labels

Setting ECOFFs

EUCAST controlled document	EUCAST SOP 10.0
Date of issue: 14 November 2017	Page 1 of 18

EUCAST
EUROPEAN COMMITTEE
ON ANTIMICROBIAL
SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Standard Operating Procedure

MIC distributions and the setting of epidemiological cut-off (ECOFF) values.

PS: Not everyone uses this approach

Epidemiological cutoff values

- Definition
  - The highest MIC in the wild-type distribution without **phenotypically-detectable** resistance
  - Note: the finding of a resistance gene does NOT change the ECOFF

Epidemiological cutoff values

- Methods for estimating ECOFFs
  - Visual symmetry ("eyeball")
  - Iterative statistical method (ECOFFinder)
  - NRI
  - Second derivative method

ECOFFinder XL 2010 v2.0 - Excel

Data Entry & Results — *S. pneumoniae* — Ciprofloxacin

No of Distributions Included in Data

MIC	Log₂MIC	Raw Count or %	Cum. Count ot %	Fitted Values	Fitted %
0.001	-10		0	0.0	
0.002	-9		0	0.0	
0.004	-8		0	0.0	
0.008	-7	8	8	0.0	
0.016	-6	13	21	0.0	
0.031	-5	26	47	0.0	
0.063	-4	31	78	0.0	
0.125	-3	104	182	1.1	
0.25	-2	1534	1716	378.8	0.6%
0.5	-1	10058	11774	11591.2	17.8%
1	0	38077	49851	37755.3	58.1%
2	1	14228	64079	14669.2	22.6%
4	2	1182	65261	618.5	1.0%
8	3	271	65532	2.4	
16	4	70	65602	0.0	
32	5	109	65711	0.0	
64	6	87	65798	0.0	
128	7	10	65808	0.0	
256	8	3	65811	0.0	
512	9	229	66040	0.0	
1024	10		66040		

Selected Subset	≤ 4	Dil Range	
Modal MIC	1	3	
Log₂MIC Mode	0		
Max Log₂MIC	9		
Selected Log₂ Mean	-0.4452	=0.734 µg/mL	
Selected Log₂ SD	0.6166		
CV	44.8%		

Selected Values	Exact	R'd-up	%Obs>	%@ECOFF
ECOFF 95.0%	1.4836	2	3.0%	22.6%
ECOFF 97.5%	1.6975	2	3.0%	22.6%
ECOFF 99.0%	1.9853	2	3.0%	22.6%
ECOFF 99.5%	2.2087	4	1.2%	1.0%
ECOFF 99.9%	2.7519	4	1.2%	1.0%

PARKED DATA (see Instructions)

MIC	Log₂MIC	Count
	1	
	1	
	1	

Clear Data | Clear Bug | Clear Drug

REVIEW AREA

Copy Numerical Results | Copy Results Image

Data Entry | Analysis | Demo data | About ECOFFinder | Changes from previous version | Instructions | Results summaries

The MIC Reference Standard

INTERNATIONAL STANDARD

ISO 20776-1

First edition 2006-11-15

Clinical laboratory testing and *in vitro* diagnostic test systems — Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices —

Part 1:
Reference method for testing the *in vitro* activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases

Systèmes d'essais en laboratoire et de diagnostic in vitro — Essais de réceptivité d'agents infectieux et évaluation des performances des dispositifs de réceptivité antimicrobienne —

Partie 1: Méthode de référence pour la détermination de la sensibilité in vitro aux agents microbiens des bactéries aérobies à croissance rapide impliquées dans les maladies infectieuses

Reference number
ISO 20776-1:2006(E)

© ISO 2006

ALL other methods are derivative, even if they generate an MIC

They are compared using this document →

INTERNATIONAL STANDARD

ISO 20776-2

First edition 2007-07-01

Clinical laboratory testing and *in vitro* diagnostic test systems — Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices —

Part 2:
Evaluation of performance of antimicrobial susceptibility test devices

Systèmes d'essais en laboratoire et de diagnostic in vitro — Sensibilité in vitro des agents infectieux et évaluation des performances des dispositifs pour antibiogrammes —

Partie 2: Evaluation des performances des dispositifs pour antibiogrammes

Référence number
ISO 20776-2:2007(E)

© ISO 2007

The 'How To' Documents

Breakpoints – CLSI approach

1. MIC distributions
2. ECOFFs
3. Resistance mechanisms and genes
4. Pre-clinical PK-PD
5. Clinical PK-PD (protein binding)
6. MIC versus clinical outcomes

Grey = nice to have, not required

Breakpoints – EUCAST approach

1. MIC distributions
2. ECOFFs
3. Resistance mechanisms and genes
4. Pre-clinical PK-PD
5. Clinical PK-PD (protein binding)
6. MIC versus clinical outcomes

Grey = nice to have, not required

Breakpoints – CLSI vs EUCAST Why can they differ?

- Test media may be different
  - e.g. for streptococci
- PK-PD targets (cutoffs) may differ
  - Stasis versus 1 $\log_{10}$ kill
- Acceptable target attainment rates in Monte Carlo simulation differ
  - CLSI, typically 90%, EUCAST typically 97.5%

Breakpoints – VAST approach

1. MIC distributions
2. ECOFFs → CO_{WT}
3. Resistance mechanisms and genes
4. Pre-clinical PK-PD → CO_{PD}
5. Clinical PK-PD (protein binding)
6. MIC versus clinical outcomes → CO_{CL}

Breakpoints -- VAST approach

VET02-A3

Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents; Approved Guideline—Third Edition

Table C9b: Suggested Decision Table with 3 Cut-off Values Available Including CO_{CL} as a Supportive Parameter

Ranking of Cutoffs	Suggested Breakpoint	Comments
WT > PD > CL	PD	Could accept CO_{WT} as breakpoint if CO_{WT} only 1 dilution higher than CO_{PD}
WT > CL > PD	CL	Could accept CO_{WT} as breakpoint if CO_{WT} only 1 dilution higher than CO_{CL}
PD > WT > CL	WT	CO_{WT} preferred. CO_{PD} gives a "confidence or safety factor" for breakpoint to be higher than the observed CO_{CL}
PD > CL > WT	CL	CO_{CL} is acceptable as it is below CO_{PD}
CL > WT > PD	WT	CO_{PD} being lower than the other two cut-off values raises some concerns. Therefore, CO_{WT} is preferred over CO_{CL}
CL > PD > WT	PD	CO_{PD} may be the preferred choice.
WT = PD > CL	WT = PD	$CO_{WT} = CO_{PD}$ increases confidence that CO_{WT} would be appropriate, even though it exceeds CO_{CL}
CL = WT > PD	CL = WT	$CO_{CL} = CO_{WT}$ is acceptable
PD = CL > WT	PD = CL	$CO_{PD} = CO_{CL}$ is acceptable
WT > PD = CL	PD = CL	Could accept CO_{WT} as breakpoint only if it is 1 dilution higher than $CO_{PD} = CO_{CL}$
CL > WT = PD	WT = PD	$CO_{WT} = CO_{PD}$ is acceptable
PD > WT = CL	WT = CL	$CO_{WT} = CO_{CL}$ is the more conservative approach. Might consider CO_{PD} if only one dilution higher than the other cutoffs.
WT = PD = CL	WT = PD = CL	All cut-offs the same

Abbreviations: CL, clinical cutoff value (CO_{CL}); PD, pharmacodynamic cutoff value (CO_{PD}); WT, wild type cutoff value (CO_{WT}).

Breakpoints – Veterinary issues

- So many species!
  - Dogs, cats, cattle, pigs, horses, chickens……….
- Lack of PK-PD targets
- Lack of PK-PD for
  - single dose products
  - medicated feeds
  - Intramammary preparations
- Lack of MIC vs outcome data for generic agents

Breakpoints -- VetCAST approach

Breakpoints -- VetCAST approach

FIGURE 2 | Clinical breakpoints (CBP) are the values of the MIC (mg/L) selected by an *ad hoc* results of AST as Susceptible (S), Intermediate (I), or Resistant (R). CBPs are determined by ta cut-off when available. CBP is established also by taking into account any aspects (scientific o countries.

The setting of a PK/PD breakpoint

Step 1
Selection of a PK/PD index predictive of clinical efficacy and/or prevention of resistance

Step 2
Determination of the critical value (size) of the selected PK/PD index

Step 3
Computation, for a given animal species and for all **possible** (not probable) **MICs** of the percentage (proportion) of animals able to achieve the critical value of the selected PK/PD index (computation of so-called Probability of Target Attainment (PTA))

FIGURE 4 | The three steps for the determination of a PK/PD breakpoint. The first step is to select one of the two PK/PD indices predictive of clinical efficacy, i.e., either the time for which plasma concentration remains above the MIC during the dosage interval ($fT > MIC$) or the ratio of area Under the plasma Concentration curve over the MIC ($fAUC/MIC$); the second step is to determine the size of the selected index required to ensure clinical and bacteriological efficacy. The third step is to determine, using Monte Carlo Simulation, the highest possible MIC for which a given percentage of animals in the target population (e.g., a prediction interval of 95%) is able to achieve the selected PK/PD index.

Breakpoints -- VetCAST approach

1. MIC distributions
2. ECOFFs $\longrightarrow$ CO_{WT}
3. Resistance mechanisms and genes
4. Pre-clinical PK-PD $\longrightarrow$ CO_{PD}
5. Clinical PK-PD (protein binding)
6. MIC versus clinical outcomes $\longrightarrow$ CO_{CL}

Susceptible -- standard dosing regimen (S)

S - Susceptible, standard dosing regimen: A microorganism is categorised as *Susceptible, standard dosing regimen*, when there is a high likelihood of therapeutic success using a standard dosing regimen of the agent.

* Exposure is a function of how the mode of administration, dose, dosing interval, infusion time, as well as distribution, metabolism and excretion of the antimicrobial agent will influence the infecting organism at the site of infection.

Susceptible -- Increased exposure (I)

I – Susceptible, increased exposure: A microorganism is categorised as *Susceptible, Increased exposure** when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection.

* Exposure is a function of how the mode of administration, dose, dosing interval, infusion time, as well as distribution, metabolism and excretion of the antimicrobial agent will influence the infecting organism at the site of infection.

Resistant (R)

R - Resistant: A microorganism is categorised as *Resistant* when there is a high likelihood of therapeutic failure even when there is increased exposure*.

* Exposure is a function of how the mode of administration, dose, dosing interval, infusion time, as well as distribution, metabolism and excretion of the antimicrobial agent will influence the infecting organism at the site of infection.

SIR – the old definitions

Susceptible

Intermediate
Uncertain effect.
Buffer zone for technical variation.
For a high dose.
Where concentrated for pharmacokinetic reasons.

Resistant

37642 observations (32 data sources)
Clinical breakpoints: S ≤ 0.064 mg/L, R > 2 mg/L

MIC
Epidemiological cut-off: WT ≤ 0.064 mg/L
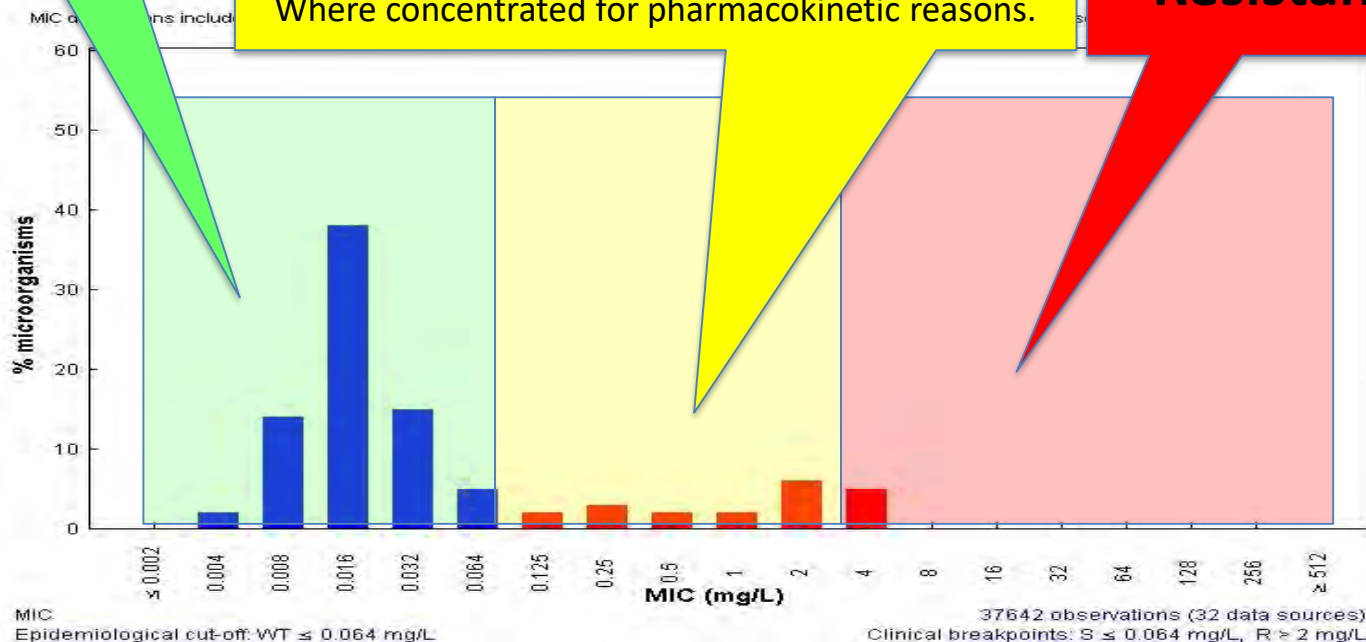
SIR - new definitions 2019

www.eucast.org
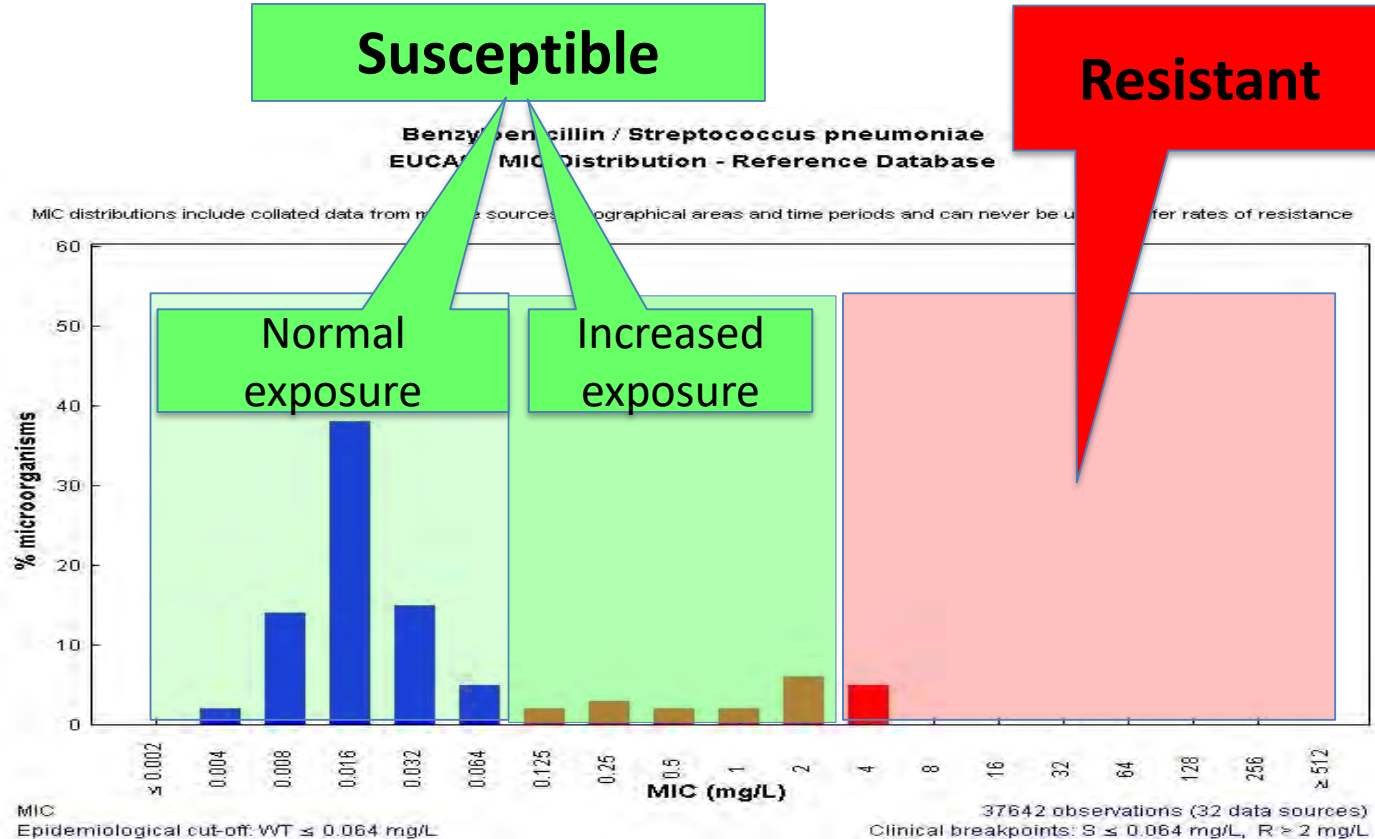
Area of Technical Uncertainty (ATU)

- EUCAST´s ability to detect areas where the technical uncertainty is such that it seriously affect the predictive value of antimicrobial susceptibility testing (AST) has improved.

- In 2019 we introduce the term "ATU" in susceptibility testing where a warning is needed to alert the laboratory to the uncertainty of the AST result.

- The warning affects the laboratory, not the clinician, and the laboratory needs a strategy to (1) ascertain the correctness or (2) to report the uncertainty of the result.

To ascertain correctness or uncertainty of AST results.

The warnings are typically in the form of a defined **MIC or inhibition zone interval** (overlap between susceptible and resistant organisms) where interpretation is uncertain. The warning is between the AST system and the laboratory and the laboratory needs to decide how to react to the warning.

Amoxicillin-clavulanic acid 20-10 μg vs MIC
Enterobacterales, 325 isolates

MIC with fixed concentration of clavulanic acid at 2 mg/L

Breakpoints (systemic infections)
MIC   S≤8, R>8 mg/L
Zone diameter   S≥19, R<19 mm

ATU 19-20 mm

MIC (mg/L)
- ≥128
- 64
- 32
- 16
- 8
- 4
- 2
- 1
- 0.5

No of observations

Inhibition zone diameter (mm)
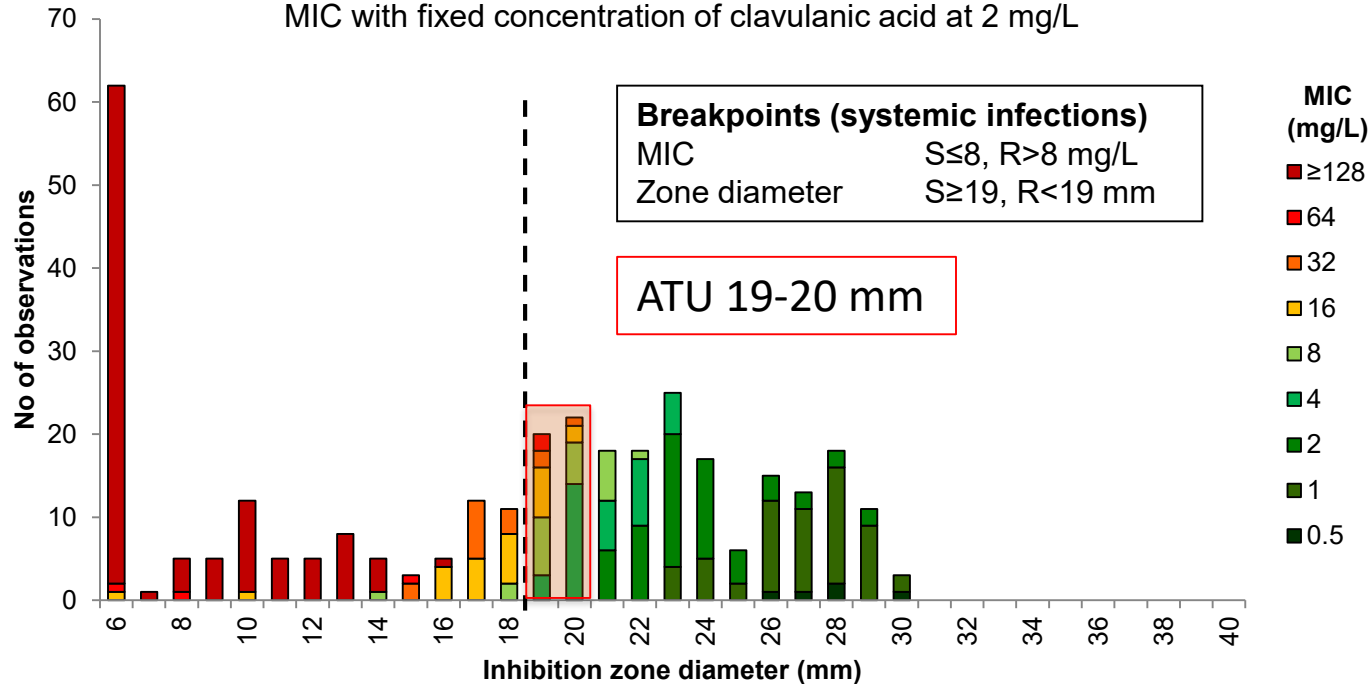
Take home message

Learn to love the numbers

Thanks to two keynote speakers at ICOHAR

Professor Lloyd Reeve-Johnson (AUS):

- ✓ **Complexity economics** vs. classical economic view on health provision
- ✓ **Integrate** intervention in human, animal and environment simultaneously
- ✓ Key attributes in successful OH initiatives: **inclusiveness**, **absence of hierarchy**, **adaptive** thinking, agreed measureable end-points etc.
- ✓ One Health **specialisation** to develop understanding of multiple aspects of health (clinics, microbiology, epidemiology, health economics.....)

Professor Paul Flowers (UK): AMR is a biopsychosocial problem

- ✓ **Psychosocial and sociocultural mechanisms spread** through societies just like genes coding for AMR or resistant microorganisms do
- ✓ AMR is a systemic, complex problem -> requires **systems thinking** to tackle
- ✓ Focus on the **intersections** between disciplines and sectors – allow **time**
- ✓ Professionalise **training** on systems approaches

Rather than (only) digging more and deeper to gain new knowledge....
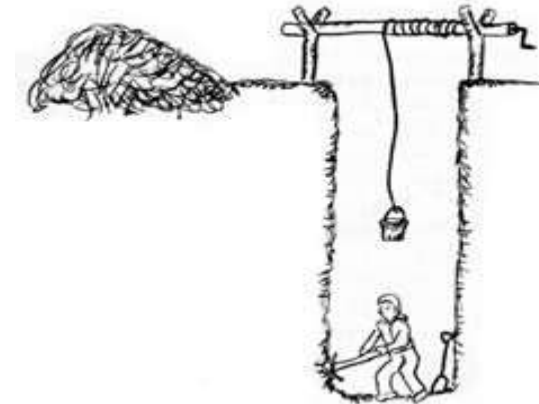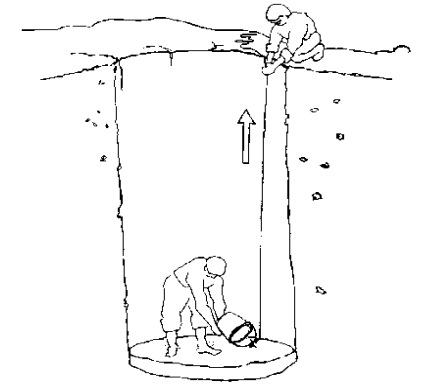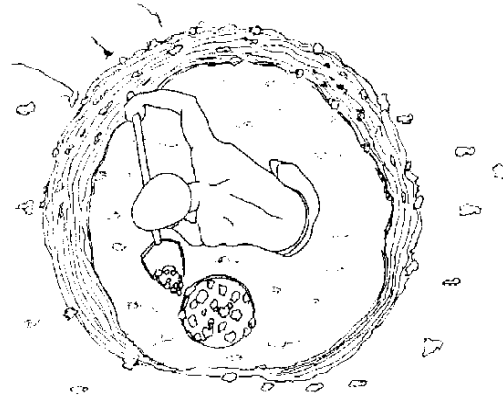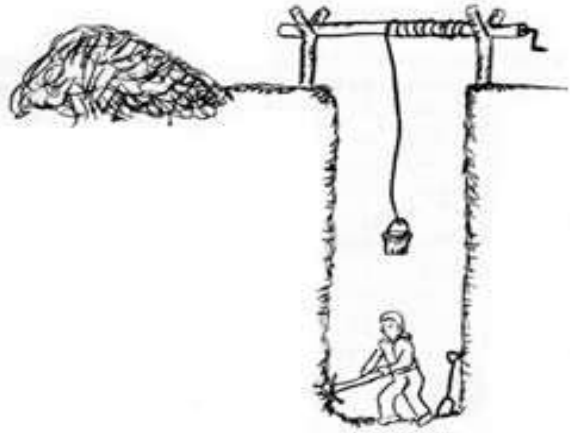
(Multi-) disciplinary thinking, planning and working

...... One Health initiatives need to (also) focus on (how to) bringing the knowledge into play
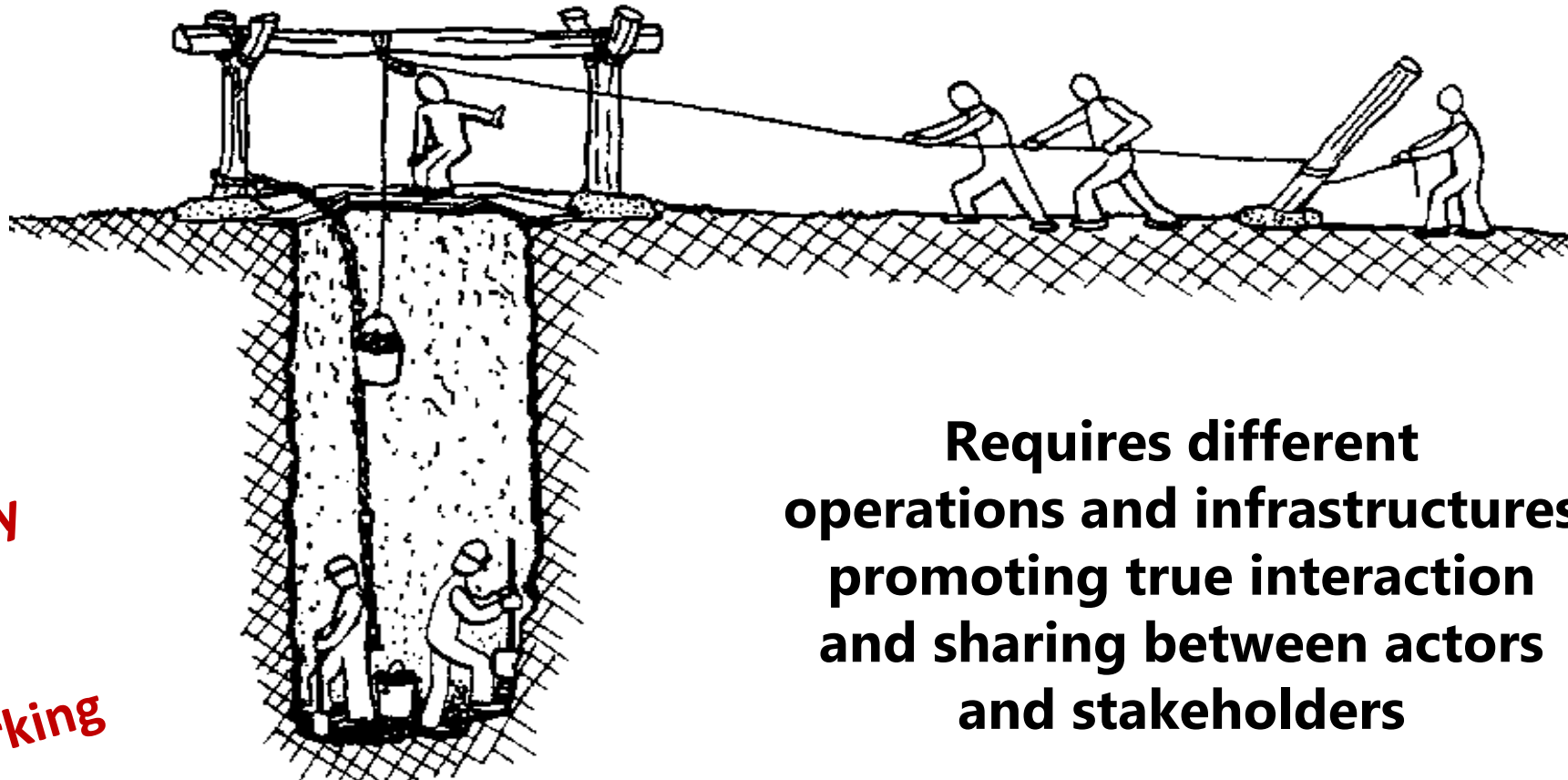
Trans-disciplinary thinking, planning and working

Requires different operations and infrastructures promoting true interaction and sharing between actors and stakeholders

What is NEOH?

Network One Health consortium
~250 people
~29 countries
4-year project, 2014-2018

Leader:
Barbara Häsler,
RVC, London

What has NEOH done?
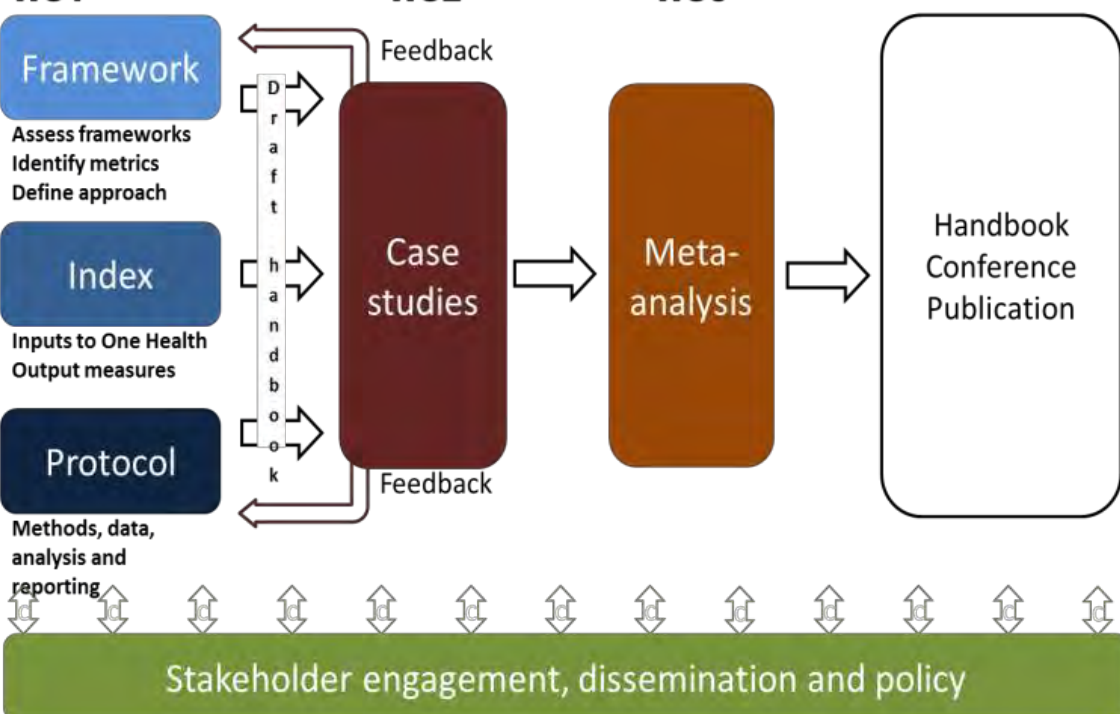
Activities

MC-meetings

WG-meetings

One training school/year

Short term scientific missions (grants)

Workshops

Stakeholder meetings

Final conference Sept'2018

Publications
(handbook, protocol, case studies, journal papers, conference talks and posters, flyers, films.....)
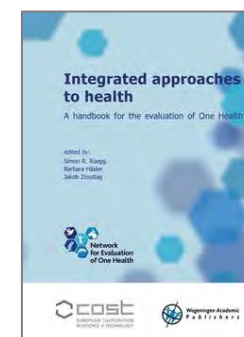
UC-Care: a One Health initiative - Research project
University of Copenhagen Research Centre for Control of Antibiotic Resistance
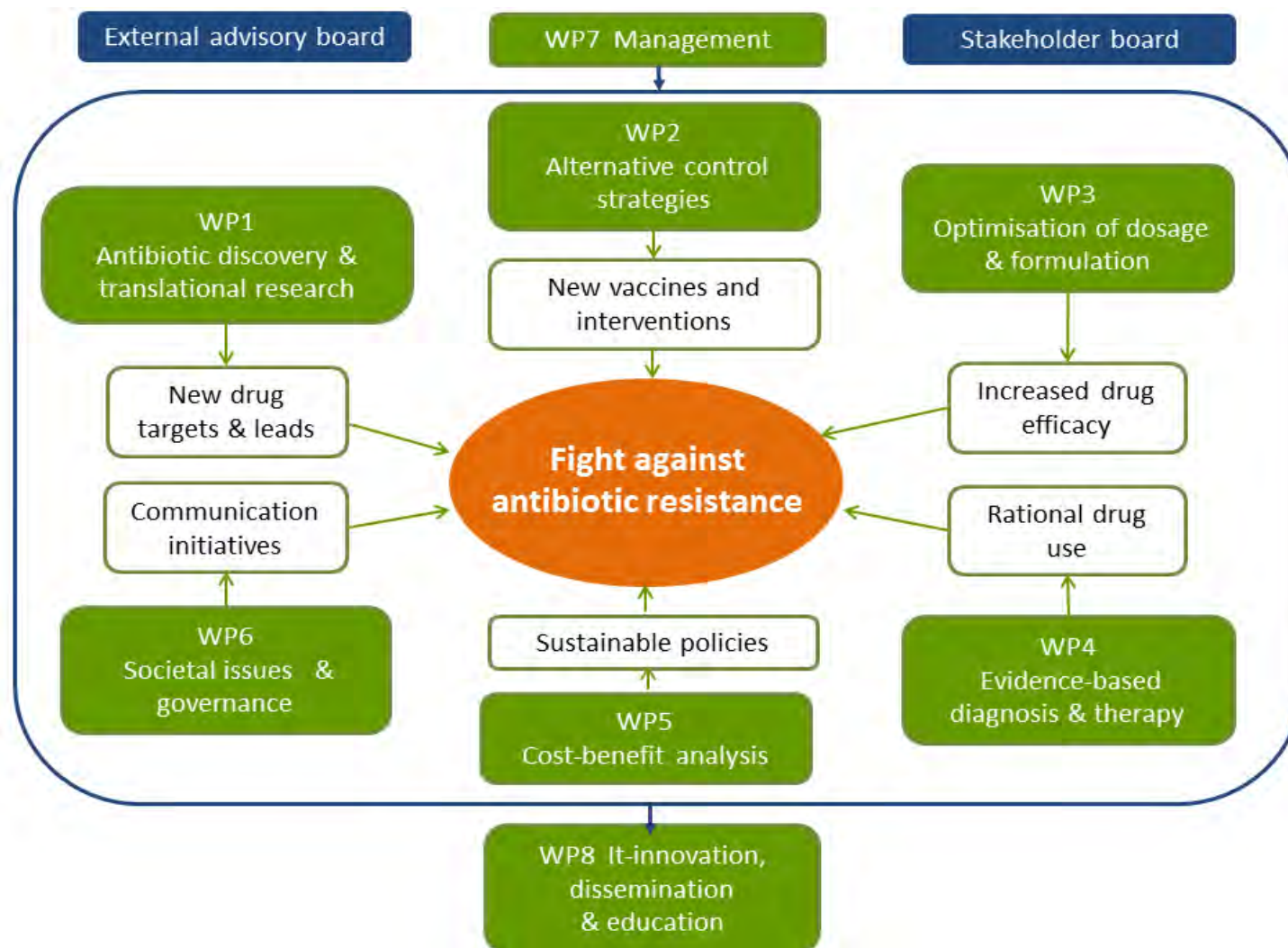
2012-2016
University funding
for ambitious
cross-faculty research
4 faculties, 14 departments

4.3 mill euro

Mainly PhDs and
postdocs funded

One annual
plenary seminar
with scientific
presentations

Evaluation of UC-Care – based on final report

UC-Care
generates knowledge and tools to combat antimicrobial resistance in humans and animals

- **Excellence** in research: scientific discoveries and achievements

- **Publications** (quantity, ranking) >120 peer-reviewed journal papers plus books, conference proceedings, presentations etc.

- **Capacity building:** Students (21 PhD's), 12 Postdocs, new PhD course, joint med-vet mandatory course module (2½ days)

- **New tools or products** (yes), **patents** (yes) plus continued research (33M+ euro)

- **Collaboration**: e.g. international (yes), industry (yes: pharma, farming)

- **Effect** on further research or other stakeholders: YES: professionals, legislation

- **Societal impact:** Ministerial Council members, guidelines on use of AM

- **Public outreach**: yes – Euro Science Open Forum stand, debate meetings and panels, public events, focus groups, farmer meetings, newspaper articles on AMR …….

IN CONCLUSION A VERY SUCCESSFUL RESEARCH PROJECT!

CAN WE LEARN MORE?

EVALUATION OF ONE HEALTH USING THE NEOH APPROACH

One Health initiatives

e.g. development projects, educational programmes
research projects , cross-sectorial and governance campaigns,
surveillance, control programmes ....

OH initiatives have common, identifiable
<u>operating principles</u> i.e. characteristic
thinking, planning and working

<u>supported by infra-structures</u>:
sharing, learning and systemic organisation

Full NEOH framework
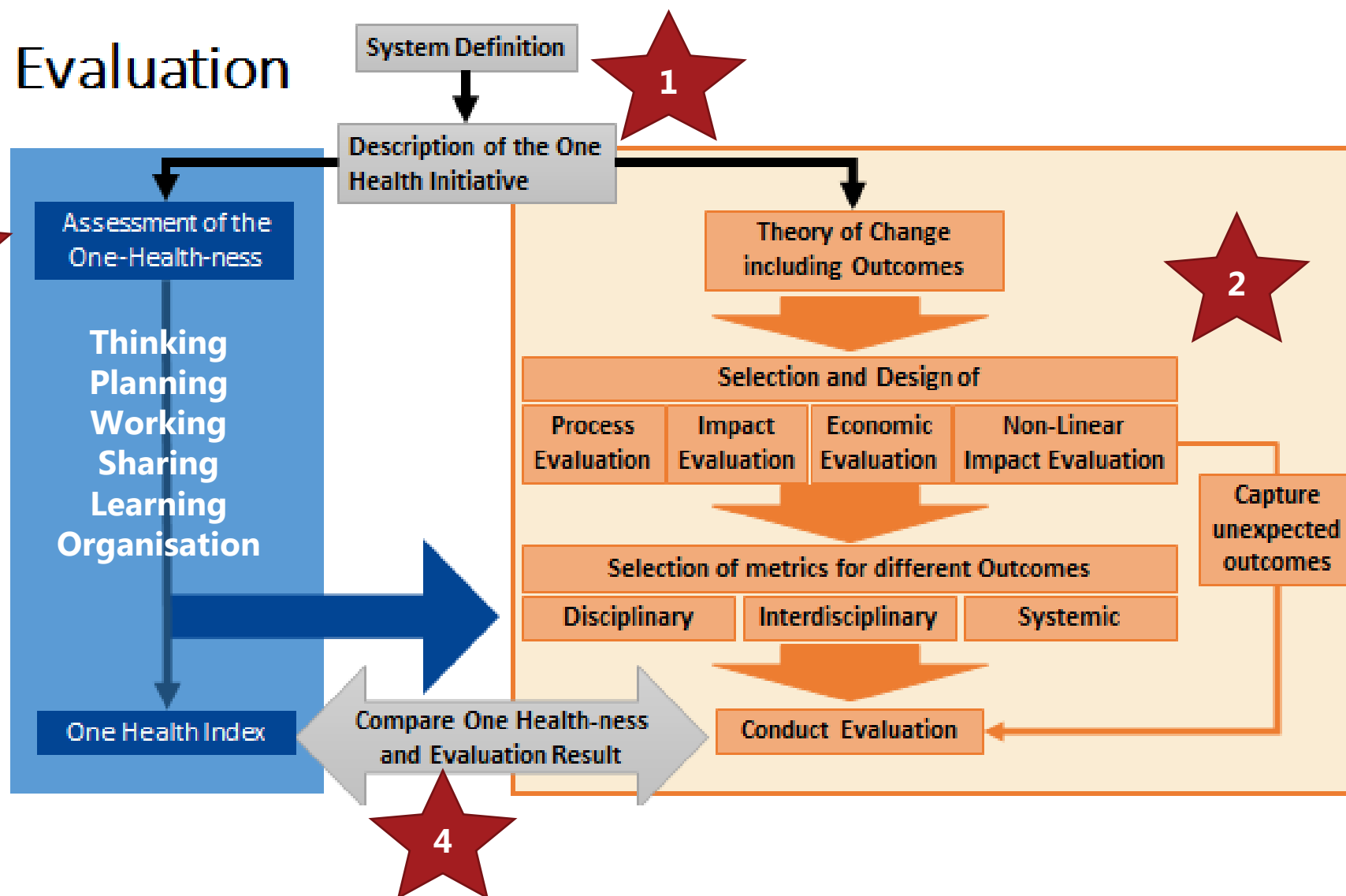
NEOH-tools for operations and infrastructure evaluation

Handbook
Excel-tool

Interviews
Study proposals
reports.....

External evaluation of UC-Care
by Anaïs Léger from SAFOSO + ECVPH
Trained for One Health evaluation in NEOH

Funding proposal, publications and internal documents, mid-term evaluation report

1 hour semi-structured interviews with key consortium members

Focus group meetings with PhDs and Postdocs in UC-Care

Online questionnaire for external participants and stakeholders

OH: Systems thinking

Must understand the system (context)

Network for Evaluation of One Health

CIPARS

Evolution of One Health

Drivers
- Social
- Economic
- Environmental

One Health Operations

Thinking
- Globally
- Multidisciplinary
- Multisectoral
- Multiple scales

Planning
- Common Aims, Problems & Financing

Working
- Transdisciplinary
- Transsectoral
- Teamwork
- Participation

Outcomes
- Sustainability
- Health & Welfare
- Interspecies Equity & Stewardship
- Effectiveness & Efficiency

Sharing
- Data
- Knowledge
- Resources
- Staff

Learning
- Knowledge Exchange
- Institutional Memory
- Feedback
- Self-Regulation

Systemic Organisation
- Polycentric
- High Connectivity
- Synchronisation
- Multidimensional

Supporting Infrastructures

The model – version 8

Ref: Jane Parmley
Public Health Agency
of Canada, 2018

Rüegg et al. (2017) A Blueprint to Evaluate One Health, Frontiers of Public Health

One Health thinking

Systems thinking – is there an understanding of the context?
What feature of the system is targeted by the initiative?
→ **Events, patterns or underlying structures**

Dimensions and scales in system and initiative – do they match?
→ Integrated approach to health?

Development over time, delayed effects, feedback loops considered?

The **three pillars of sustainability** considered?
→ Ecosystem/environmental, economic and social

Hierarchies in socio-ecological systems acknowledged and used?

Beliefs about evidence, values about health, cultural grounding considered?

All essential **stakeholders' perspectives** considered?

IT TAKES TIME TO DEVELOP SYSTEMS THINKING!

One Health thinking in UC-Care?
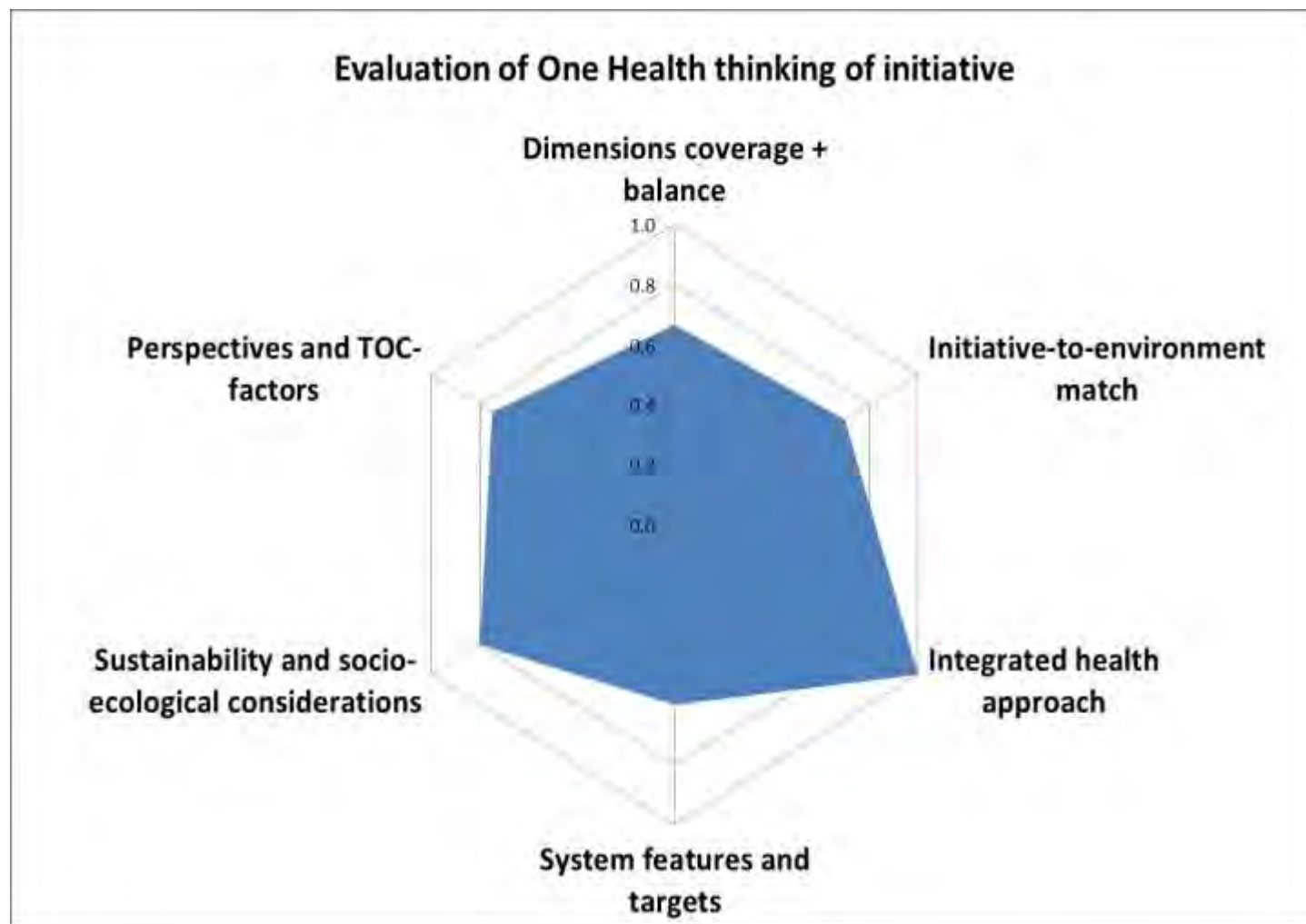
Qualitative assessment comments provided in Excel tool
and published with the paper

NEOH-evaluation of One Health operations - PLANNING

Evaluation of planning and ressource allocation towards One Health outcomes in the initiative

Center implementation and economic self-sustainability
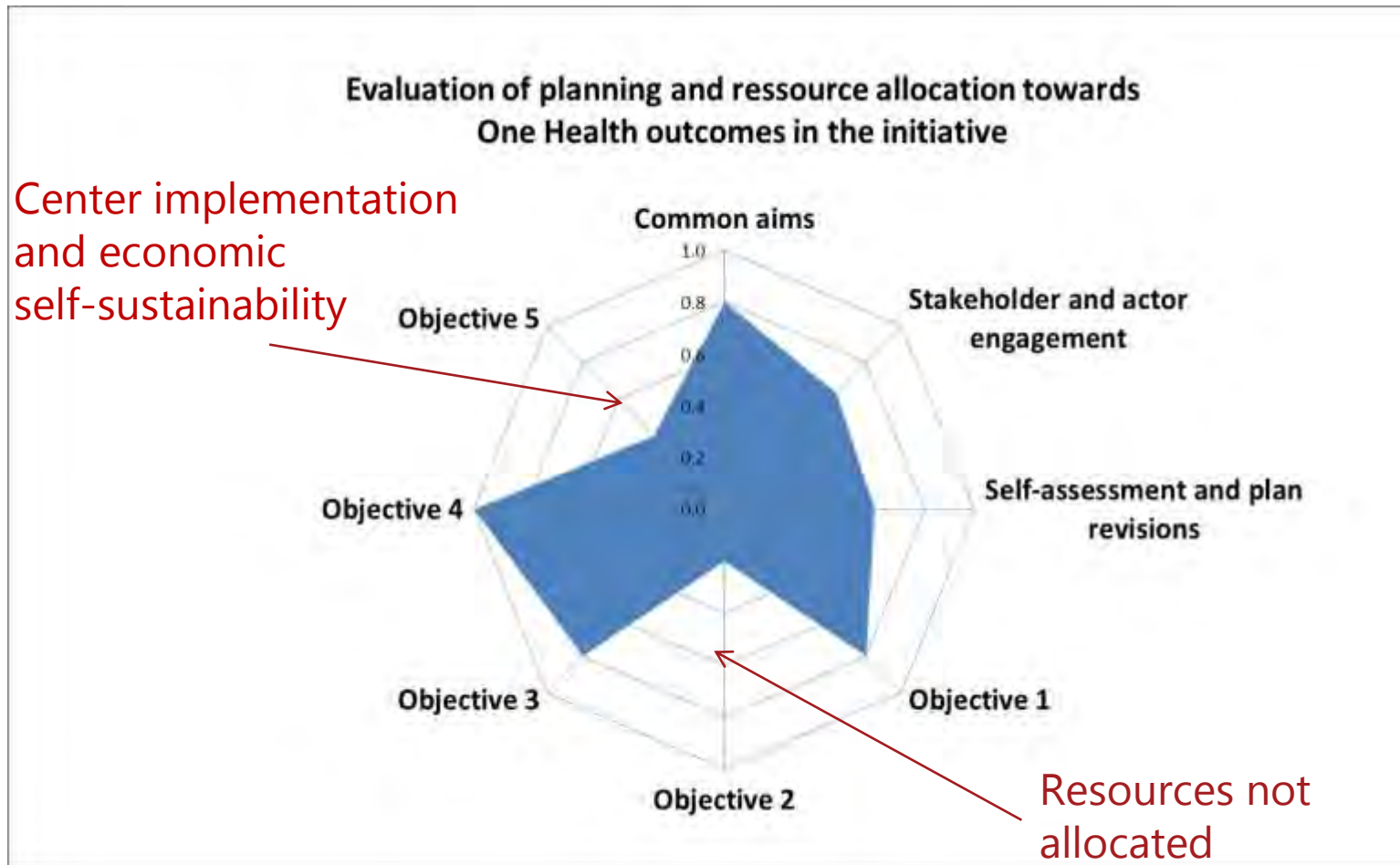
Resources not allocated to IT-tools development

NEOH-evaluation of One Health operations - WORKING

Evaluation of working towards One Health outcomes in the initiative
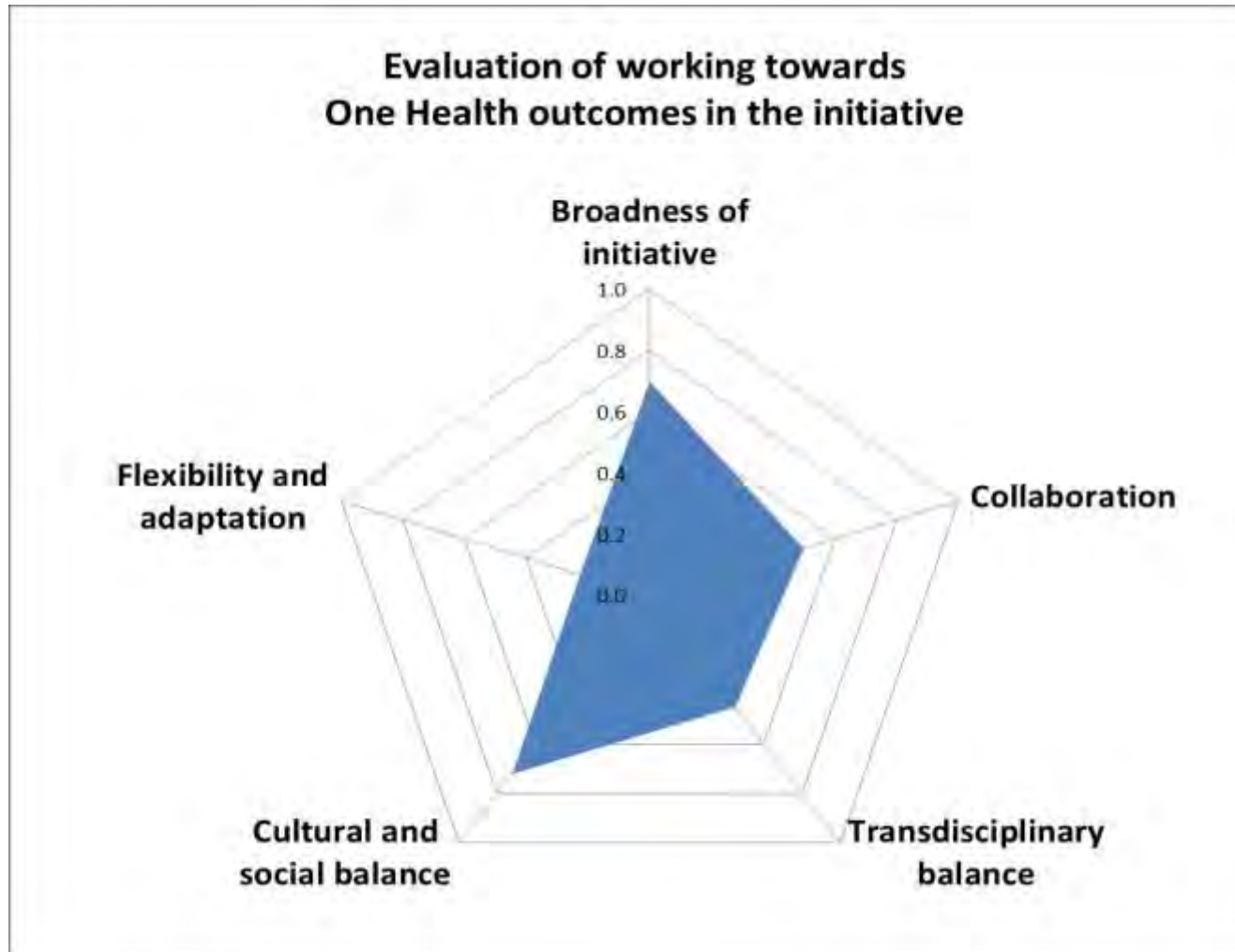
Most resources tied in PhD's and Postdocs with fixed objectives

No resources for adaptive activities + sharing and learning activities within the consortium – little collaboration between work packages E.g. staff exchange

Socioeconomic research and public outreach / implementation under-prioritised (lack of funding)

NEOH-evaluation of One Health infrastructure: SHARING
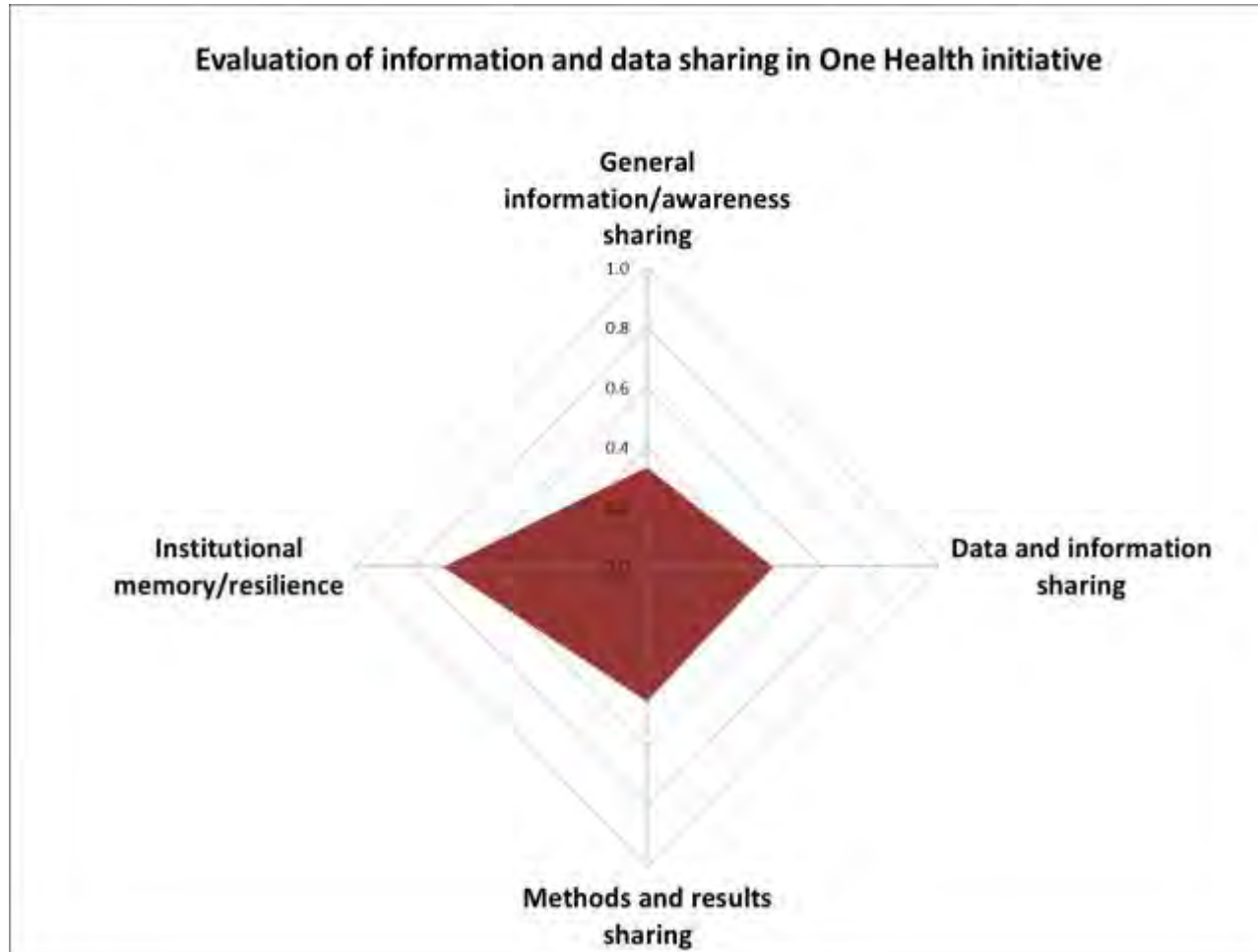
NEOH-evaluation of One Health infrastructure:
LEARNING

Evaluation of learning infrastructures in One Health initiative
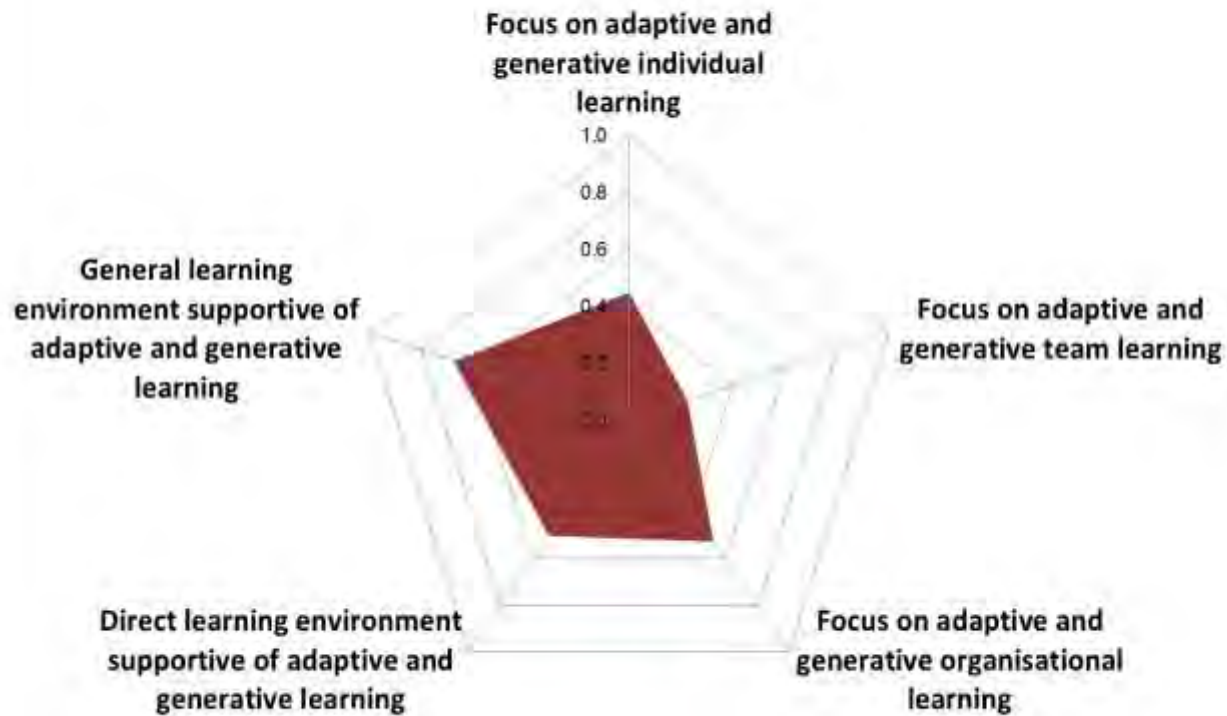
NEOH-evaluation of One Health infrastructure –
SYSTEMIC ORGANISATION (LEADERSHIP)

Evaluation of systemic organisation in UC-Care
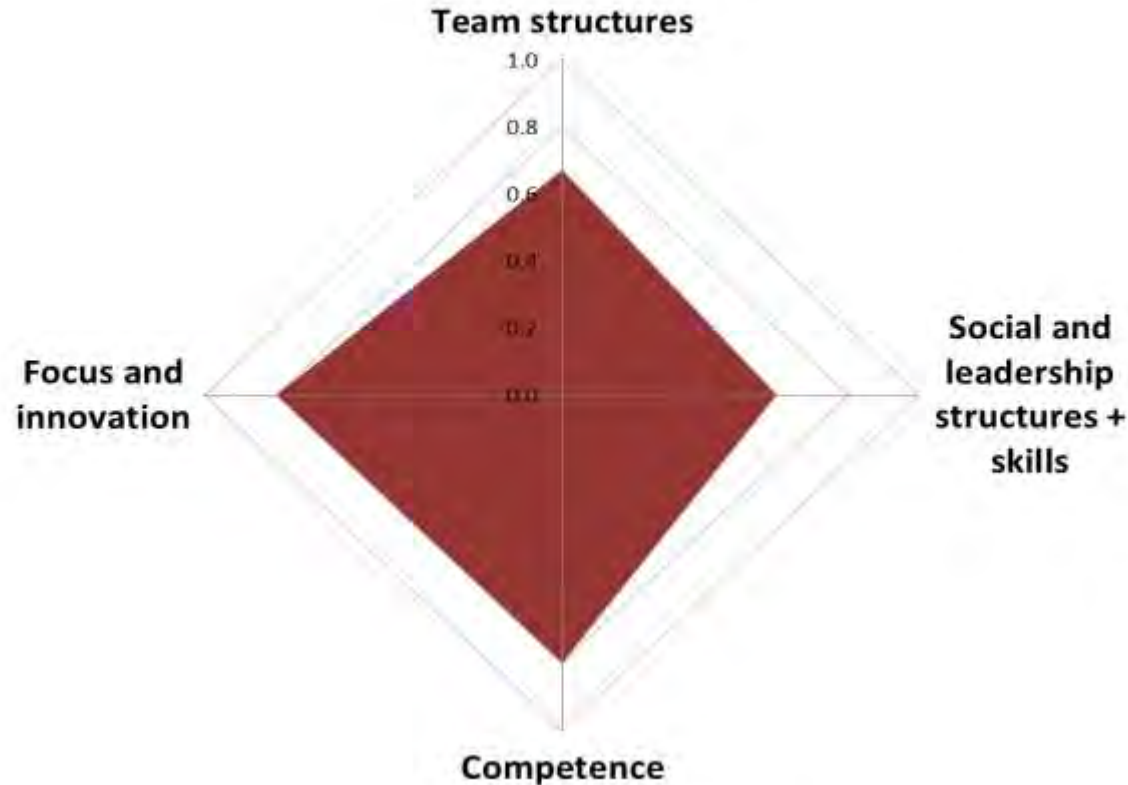
Gender imbalance
Teams ok within work packages
not between WPs / across consortium

Early career investigators not interacting
OH perceived as 'only for senior scientists'

NEOH-evaluation of One Health-ness: UC-CARE

One Health Index of UC-Care
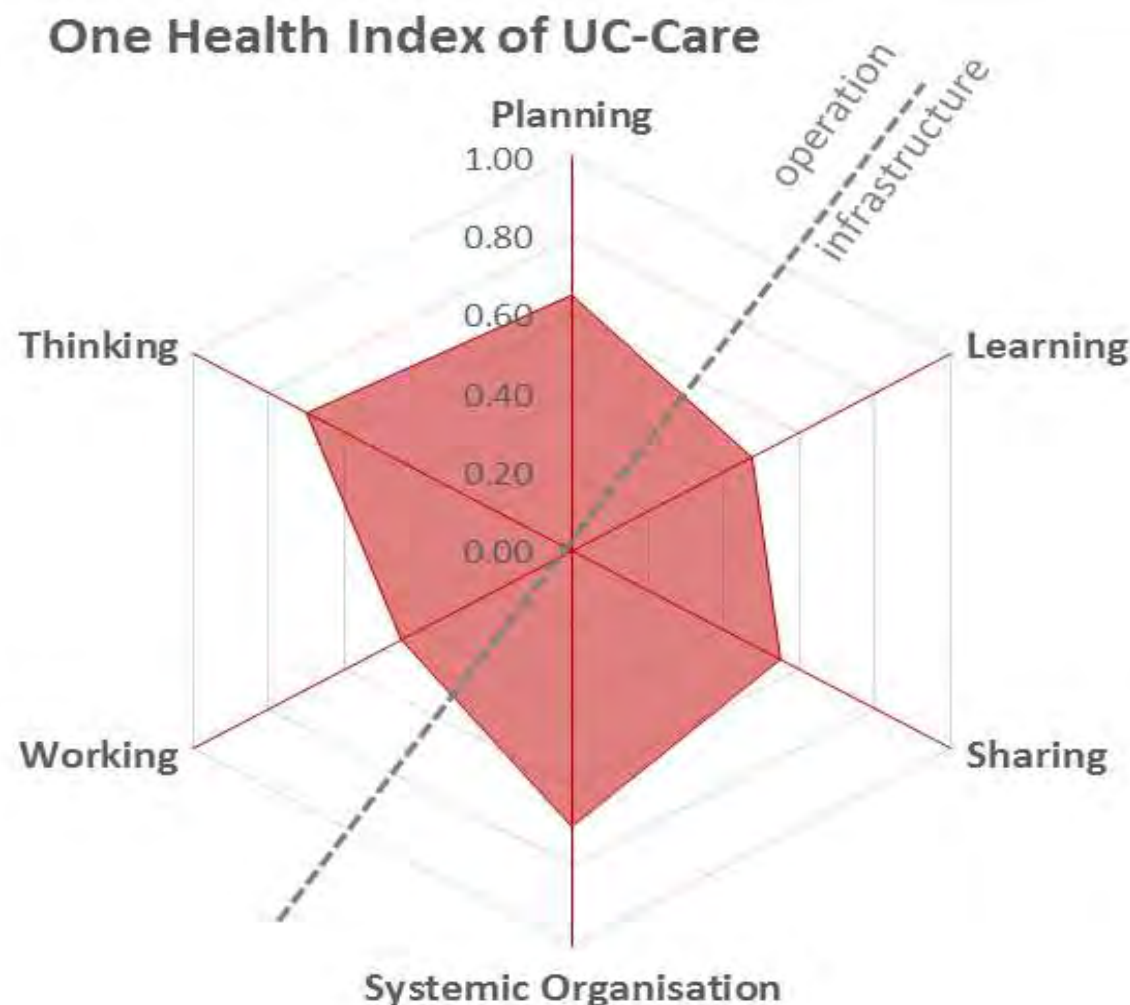
In conclusion:
UC-Care was a very successful interdisciplinary project

However, evaluating One Health operations and infrastructure led to insights not provided by conventional research evaluation.

Missed opportunities and potentials? Innovation? Impact in the long term?

One Health Index	0.34
One Health Ratio	1.1

What can we learn from OH evaluation of interdisciplinary research projects?

✓ **Sharing** essential for learning within and out of initiative – IMPACT

✓ **Adaptive** planning and shared leadership – CHALLENGE FOR ACADEMIA?

✓ **Reflection**: External evaluation vs. internal/self-evaluation – LEARNING

✓ **Evaluation is a learning** experience in itself – FUTURE PLANNING

✓ Systems thinking **takes time** to get used to and to do – RARELY FUNDED

✓ Easier to think about characteristics of the initiative than to think about how the initiative fits and targets elements in its' **context** -> ONE HEALTH!

✓ The NEOH framework and tools facilitate more **holistic evaluation** than more traditional research evaluation – ADAPT ACADEMIA?
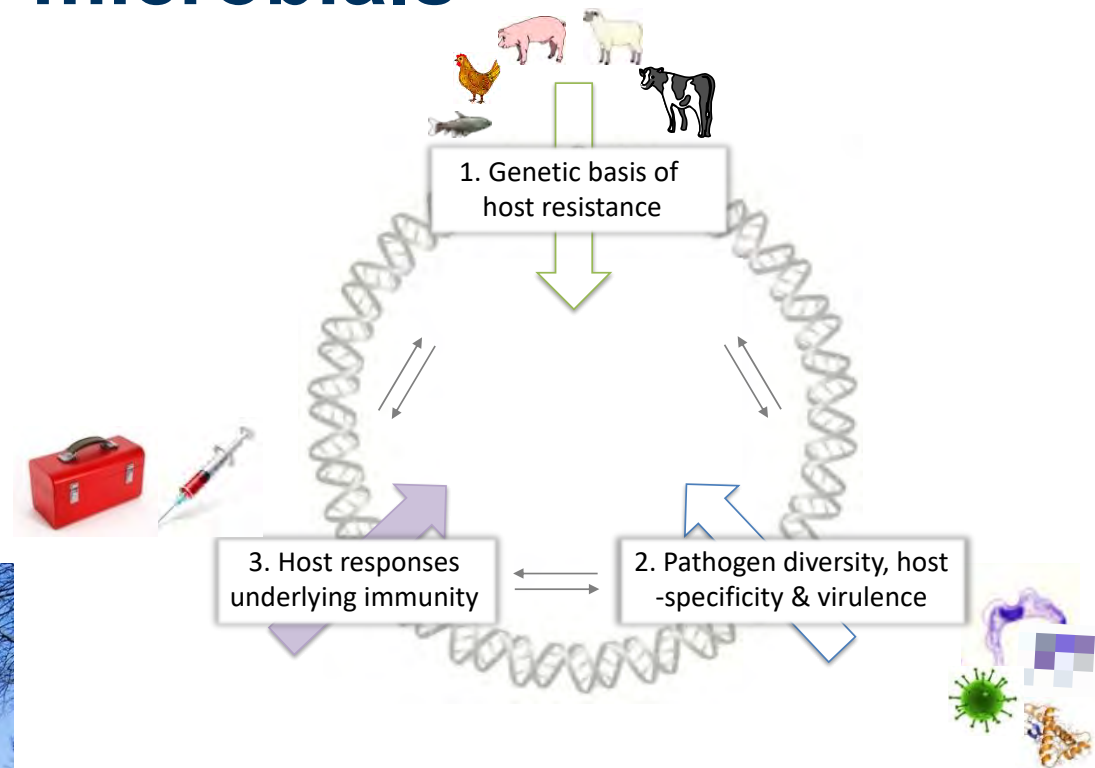
Alternatives to existing anti-microbials

Figure B: ermB, dfrA1, tetQ, 16S, tetB

Y-axis: Log 10 (Copies+1)/g DM
X-axis: Week of sampling (W1–W25)

Legend: Sows, None, Acidified water, Acidified water + chlortetracycline, Chlortetracycline, Tylosin

B

- **High levels; some AMR genes present at > 1 copy per bacterium**
- **Relatively stable despite microbiome changes during development**

Main issues

- Pressure to reduce or even eliminate antimicrobial use.

- In example system; reduce use > disease.

- The antibiotics still work.

- Break the pattern: High health status.

- Pathogens are still the driver for use:

  Biosecurity,

  Epidemiology,

  Accurate diagnosis

  Targeted treatments

Microbiome manipulation

- Altered diets: additives, pre- and pro-biotics to manipulate the microbiome

- Provide resilience to disease and yet improve feed conversion?

- Could a 'low AMR flora' be introduced after birth?

- Interactions with animal genetics and local environments

- Need the data – then interrogate it
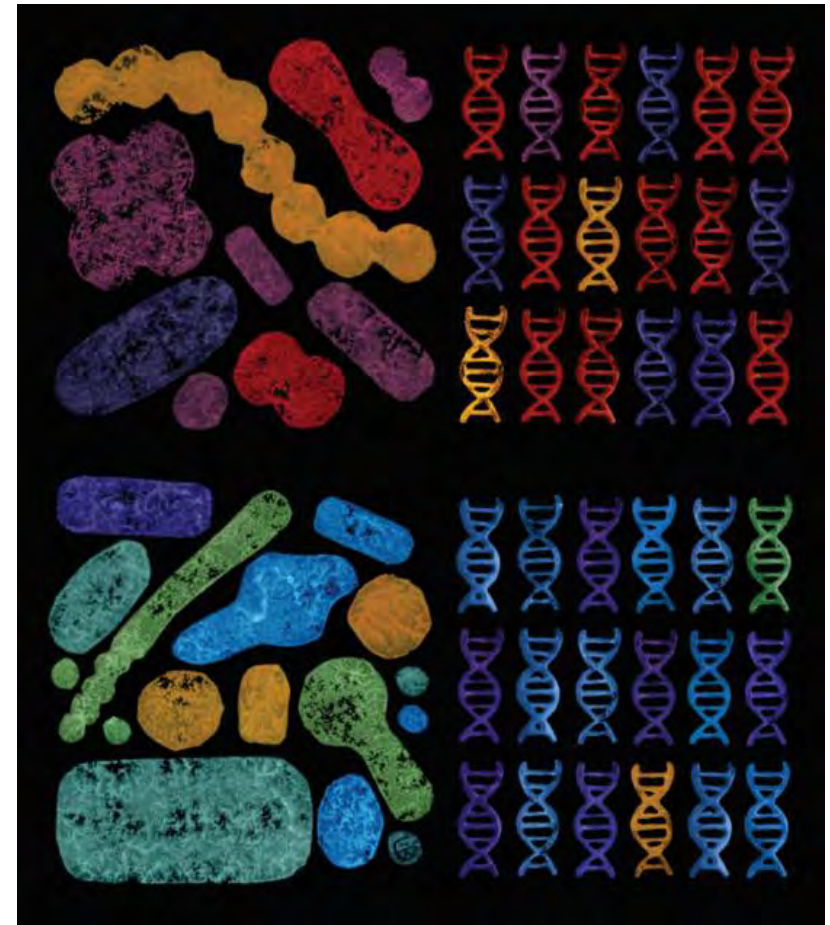
Vaccine development in food-producing animals

- Chickens: *E. coli* , IBDV, *C. perfringens*, *Eimeria*, IBV.

- Swine: *S. suis*, *H. parasuis*. *P. multocida*, *M. hyopneumoniae*, *A. pleuropneumoniae*, PRRSv, SIV. *E. coli*, *L. intracellularis*, *Brachyspira* spp, Rotaviruses

- Aquaculture: *Aeromonas* spp, *Pseudomonas* spp, *Streptococcus* spp, *Vibrio* spp.

- Cattle: Mastitis, respiratory disease complex organisms, lumpy skin disease virus

From Hoelzer *et al* 2018. Vet Res: 49:64

Vaccines: opportunities & hurdles

- Epidemiology and WGS.
- Autogenous vaccines.
- Live vectors to stimulate immunity
- Poor knowledge of immunity in some production species,
- Toolbox needs
- Species-specific adjuvants
- 'Marked' DIVA vaccines & diagnostics

Courtesy of Ross Fitzgerald, UoE
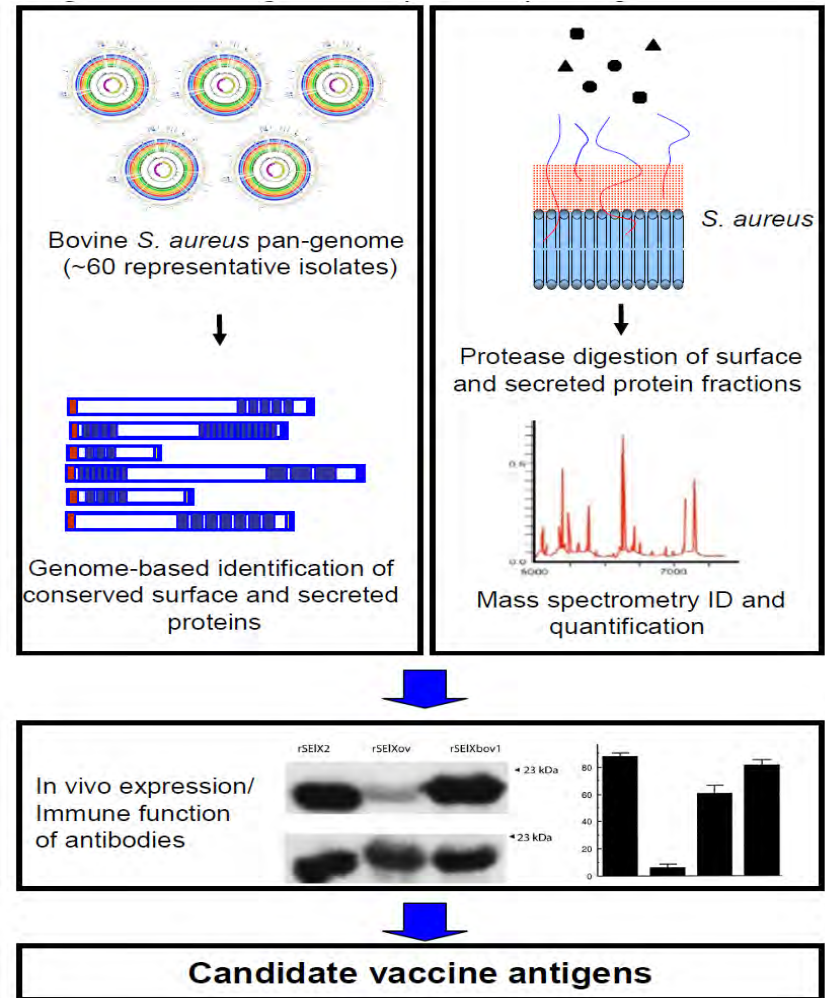
Immuno-modulation

- Enhance the hosts (innate) response

- Augment responses to aid pathogen clearance & reduce pathology.

- Enhance macrophage microbicidal mechanisms

- Restrict negative consequences of excessive microbicidal generation

- Repurposed drugs targeting specific pathways

Courtesy of David Dockrell, UoE
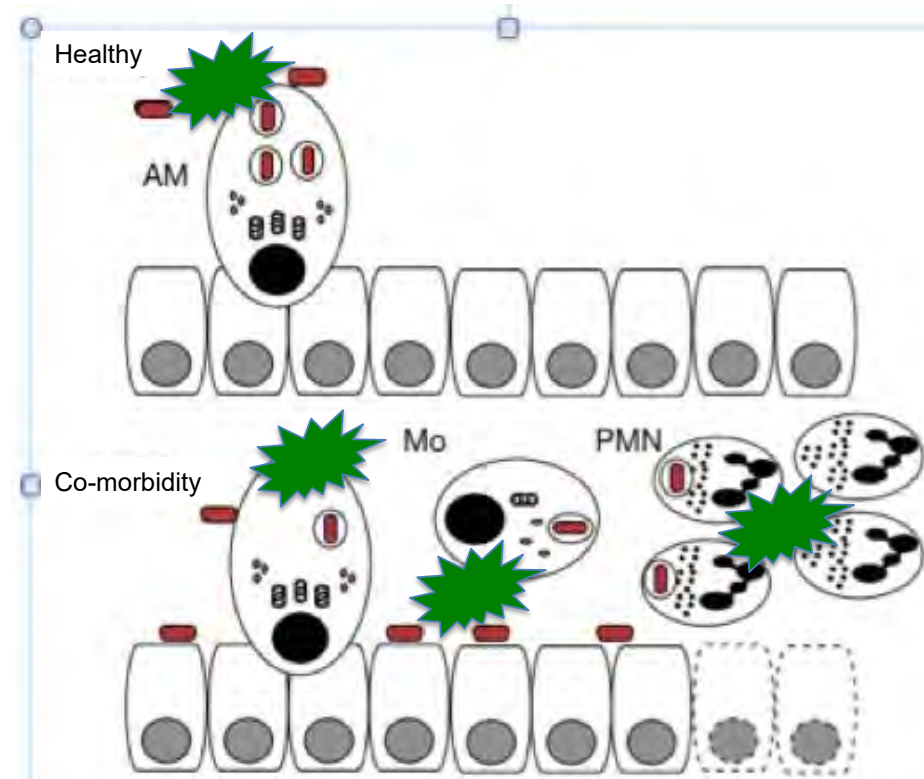
Breeding for resistance to infectious diseases

- BovineTB: Sire index used
- Swine resistance to Enterotoxigenic *E. coli* (adhesins)
- Avian resistance to *Campylobacter* and *Eimeria*
- Ovine resistance to parasites
- Extensive opportunities in aquaculture

GWAS: Holsteins in TB breakdown herds

Atlantic salmon
susceptible

Coho salmon
resistant

Farmed salmon is UK's largest farmed food export
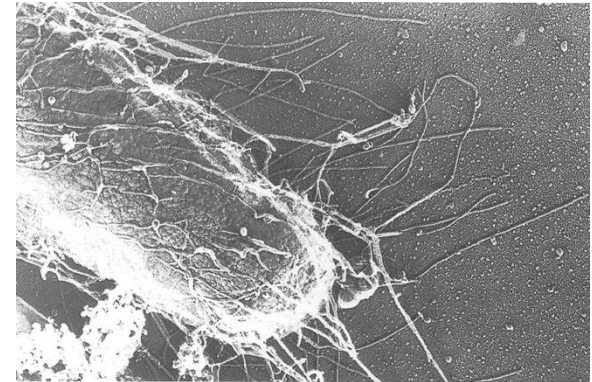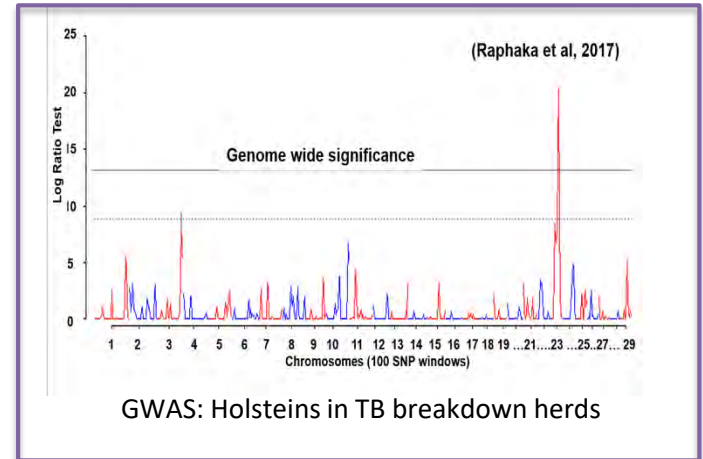
Host-pathogen Genome-Wide Screens

Genome wide Crispr KO libraries
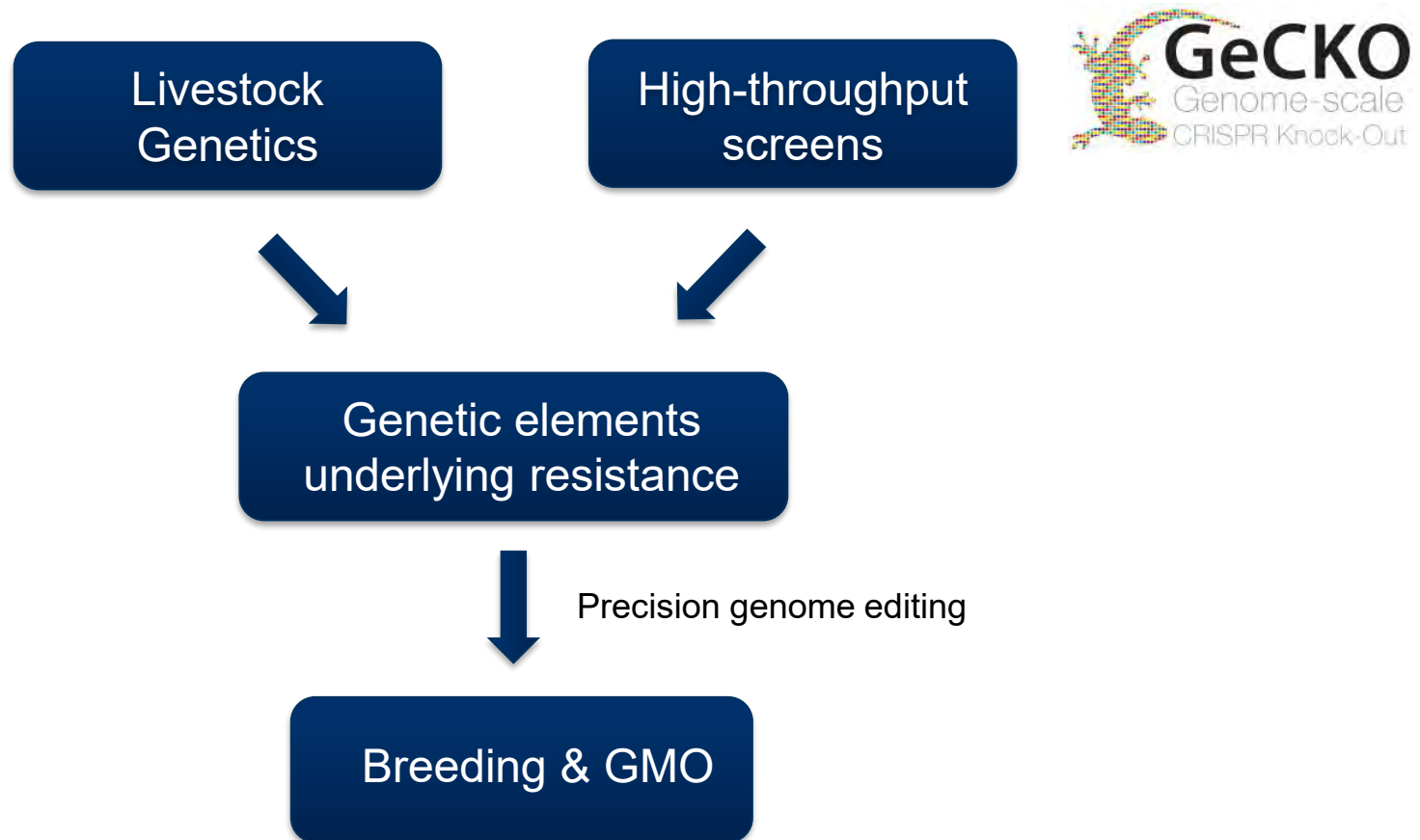
Editing for disease resistance in pigs
Porcine Reproductive and Respiratory Syndrome

- Symptoms:
inappetence, fever, lethargy, respiratory distress
→ **Decreased growth rate / efficiency**

- Mainly affects *pre-weaned piglets*
  - Diarrhea, severe respiratory distress
  - Fatality rate: 40-100% (strain dependent)
  and *pregnant sows*
  - Displacement of placenta
  - Complete abortion or death and mummification *in utero*
  → **Loss of animals / Food waste in the production chain**

- Strong immunomodulation during infection
  - Increased susceptibility to infection with other pathogens

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PRRSV Replication Cycle
In macrophage / monocyte lineage host cells
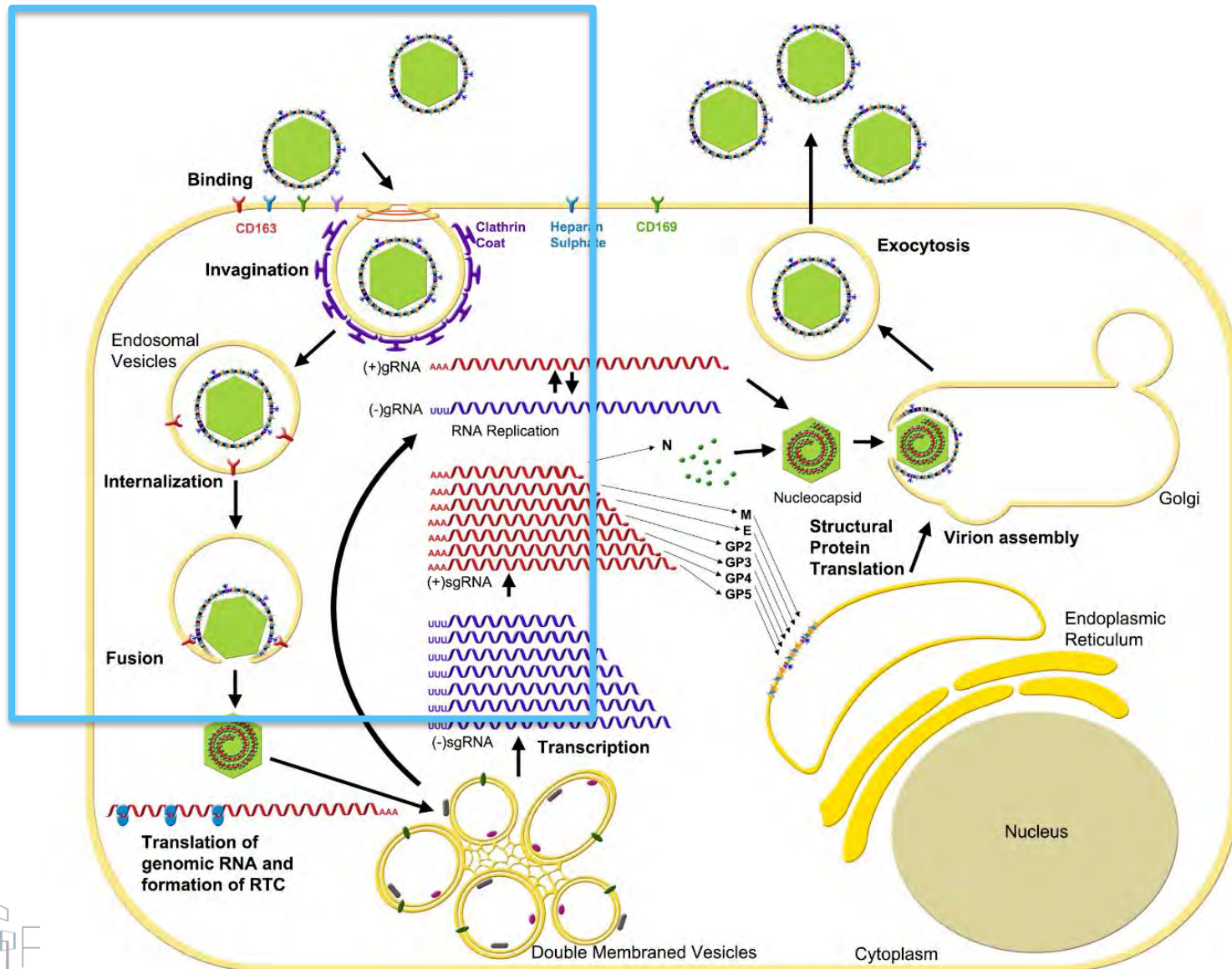
Interaction of PRRSV with CD163
and biological function of CD163
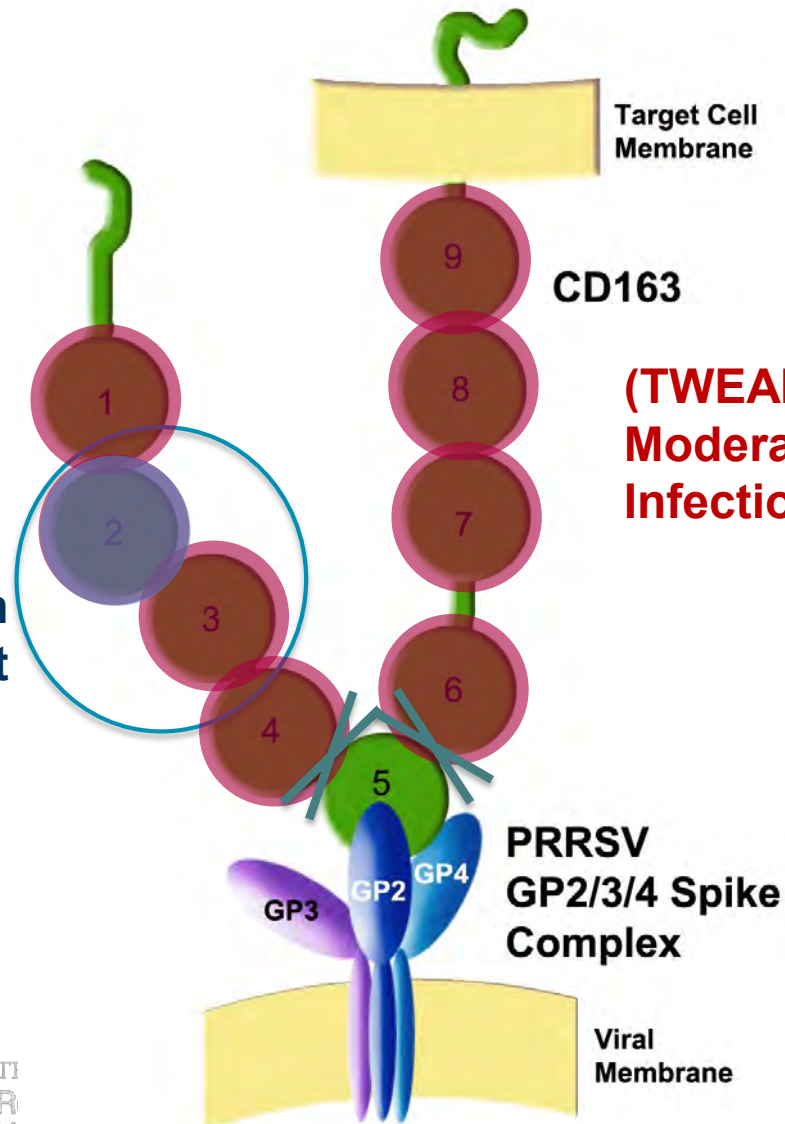
Excising domain 5 from the pig genome
using genome editors
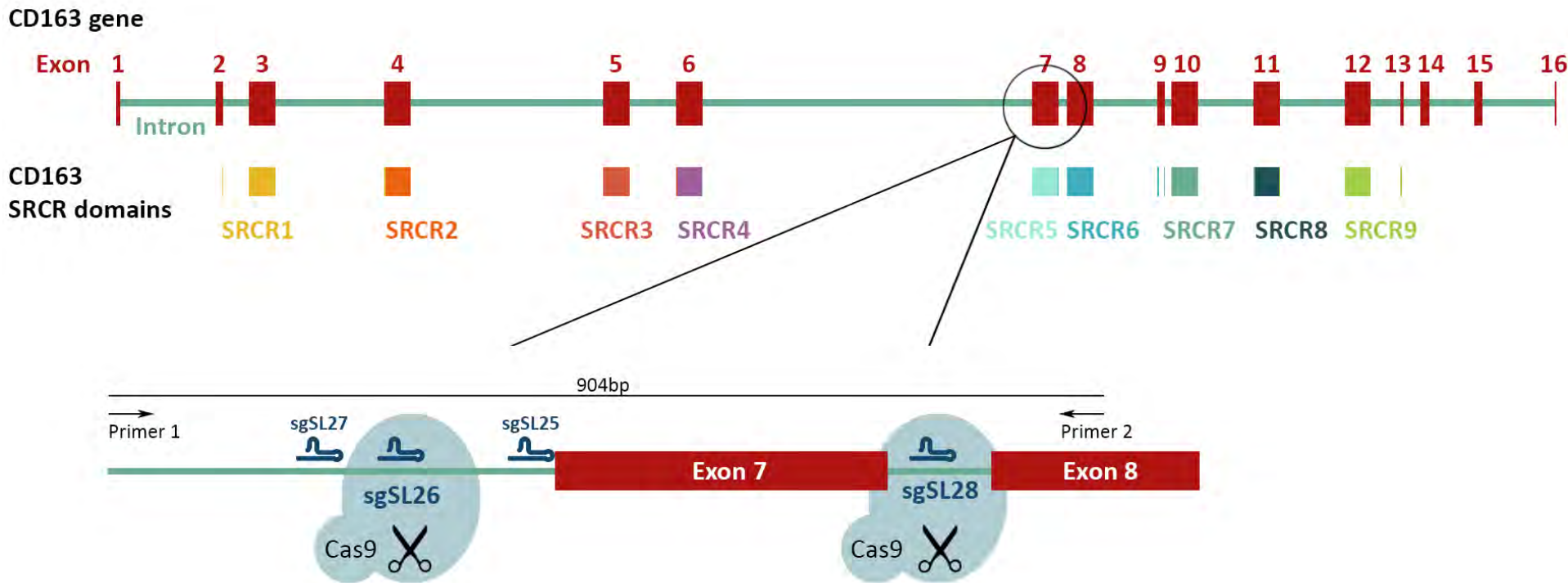
Editing pig zygotes
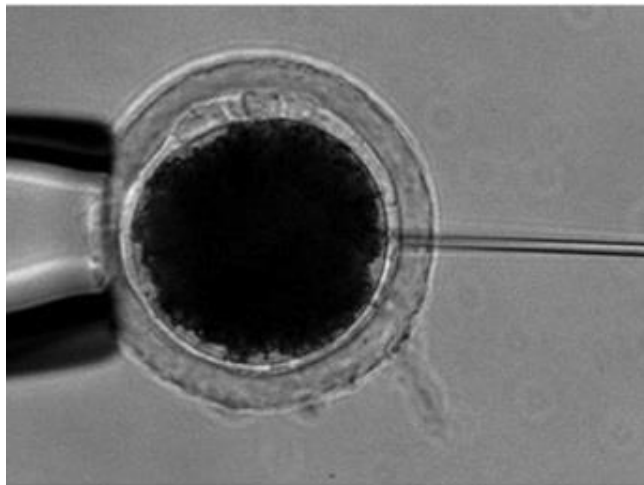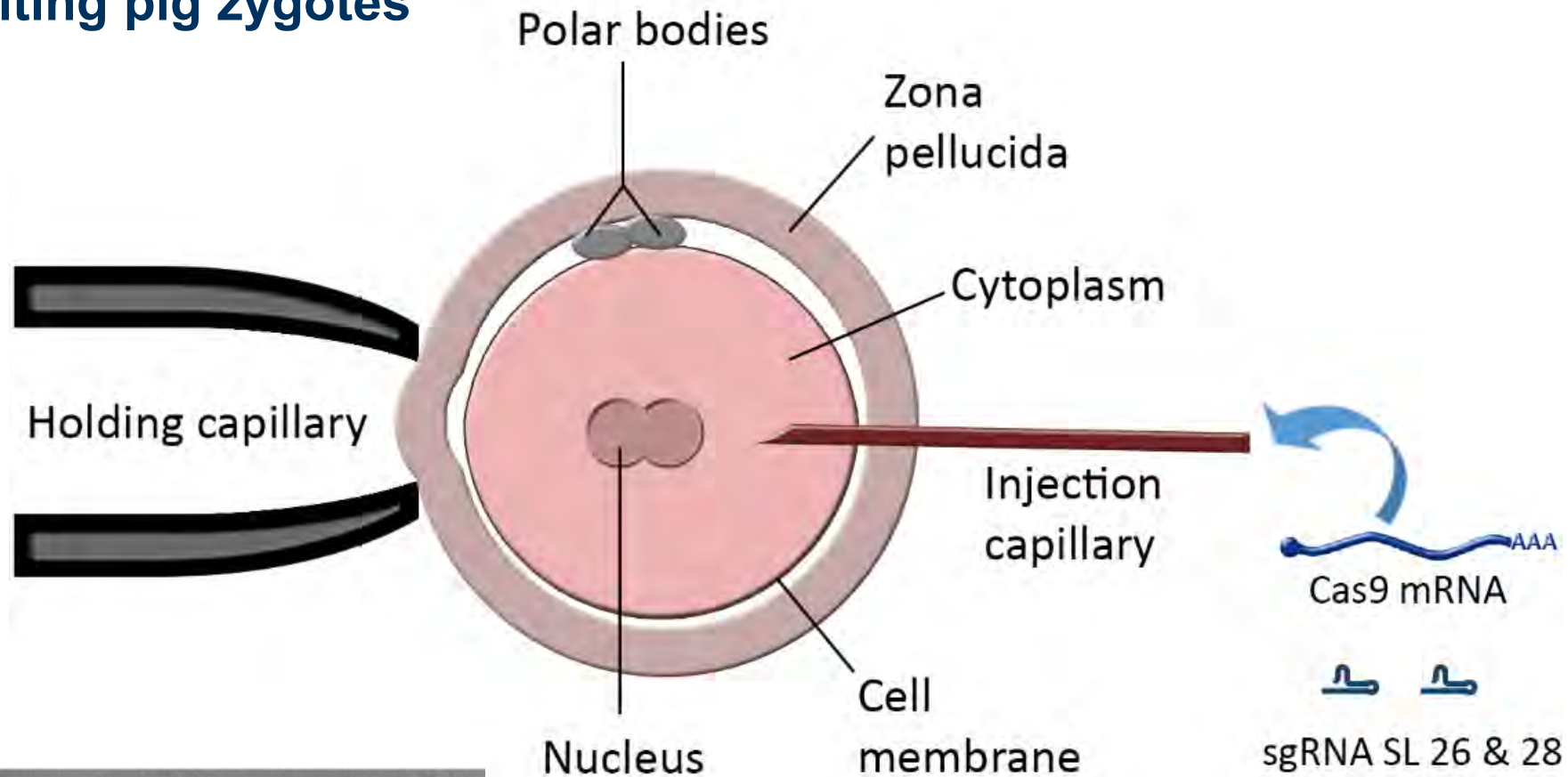
Microscope image of microinjection in pig zygote

In vitro resistance in pigs
replication of PRRSV-1 in macrophages
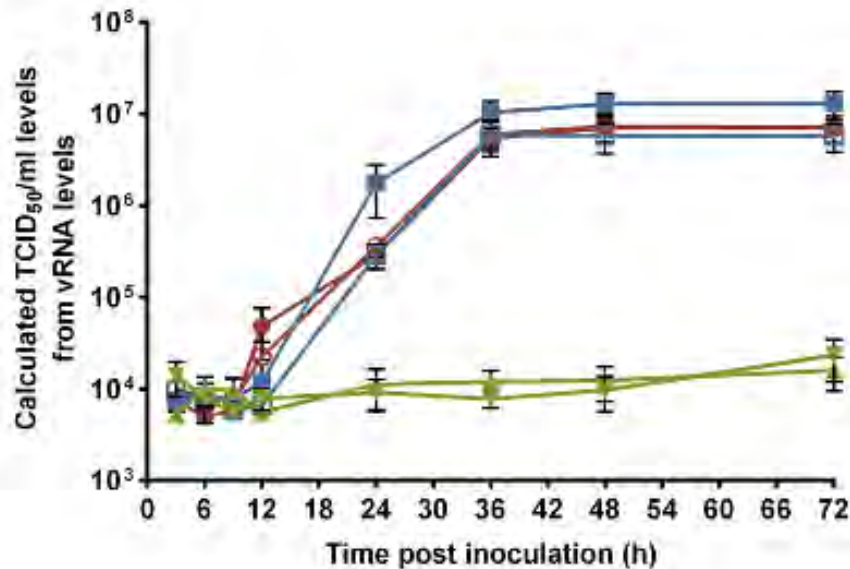
In vivo resistance in pigs
replication of PRRSV-1 in pigs: serum levels
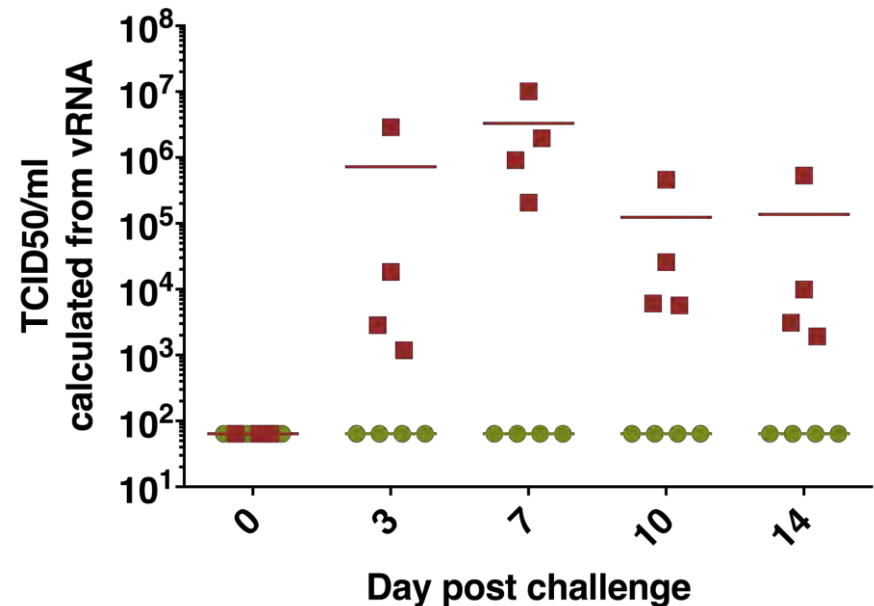
- **Pigs lacking domain 5 of CD163 are resistant to PRRSV infection**

- **CD163 is still expressed and maintains it's main biological functions**

- **Pigs lacking domain 5 grow and breed normally: the pigs are**

Targeted killing of pathogens

- Bespoke approaches that are enabled by accurate identification of the pathogen

- Real-time diagnostics informing different types of treatment

- Trojan horse anti-bacterials

- Anti-virulence/pacification strategies

- Phage therapy revisited based on prediction of phage activity from WGS
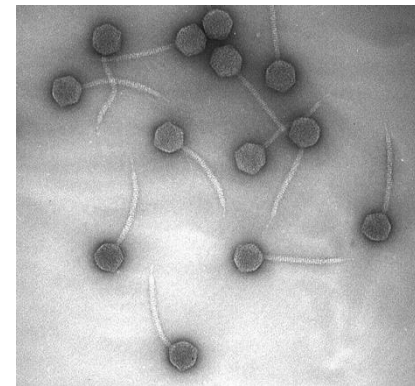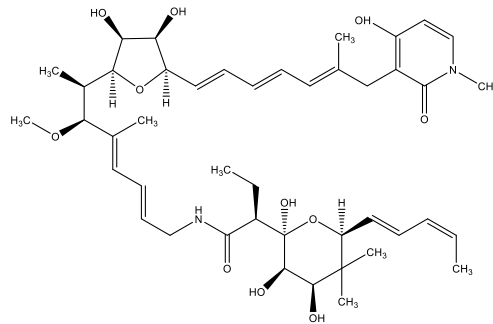
Trojan Horse Antibiotics: siderophore conjugates
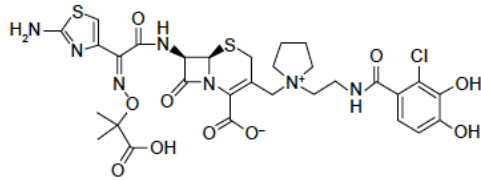
Cefiderocol – a novel siderophore cephalosporin antibiotic
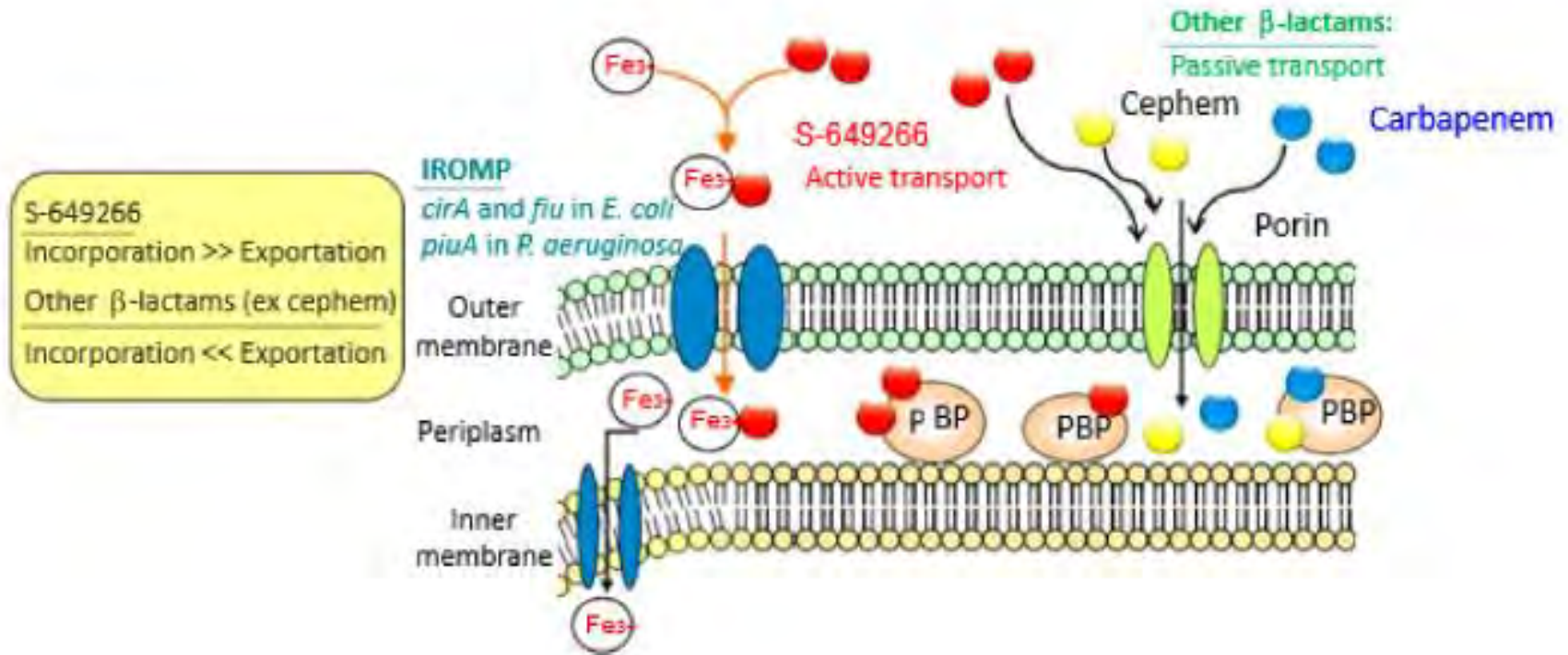
Tillotson. 2016 Infectious Diseases: Research and Treatment 2016:9 45–52.
Carvalho & Fernandes. *Front Microbiol*. 2014; 5:
290. PMID: 24971080

Anti-virulence compounds

Specific pathogen interaction

Defined virulence factor

Identified inhibitor e.g. Aurodox

Activity *in vitro* & *in vivo*
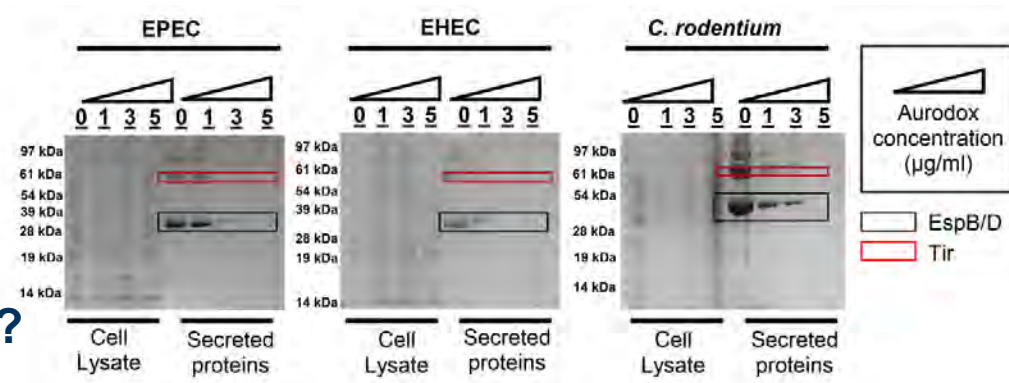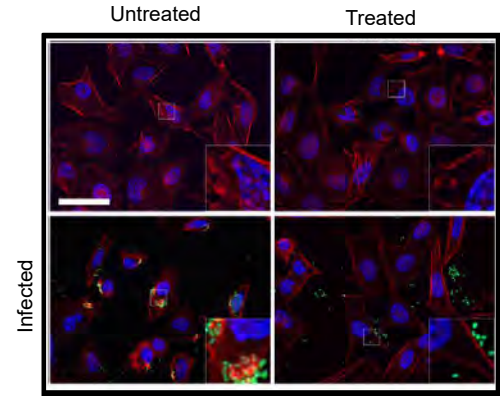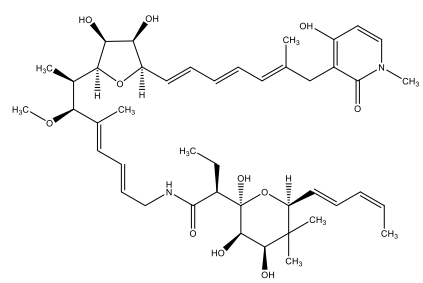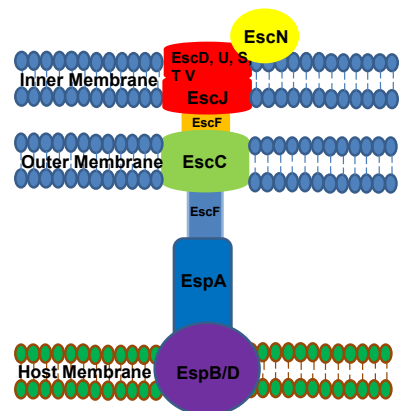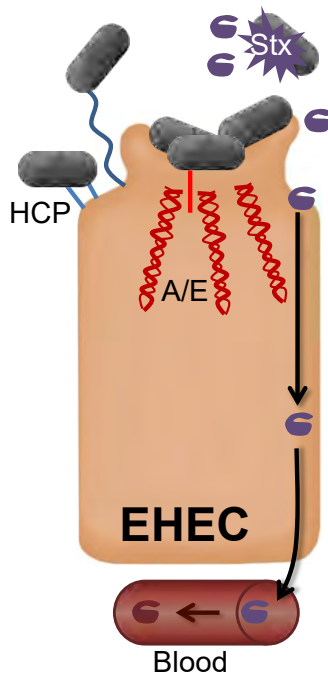
Different selection pressures to AM?

Thanks to Andrew Roe & Rebecca McHugh, University of Glasgow

Alternatives to conventional antibiotics

- Antibodies
- Probiotics
- Lysins
- Wild Bacteriophages
- Engineered phages
- Immune stimulation
- Vaccines
- Antimicrobial peptides
- Host/innate defense peptides
- Antibiofilm peptides
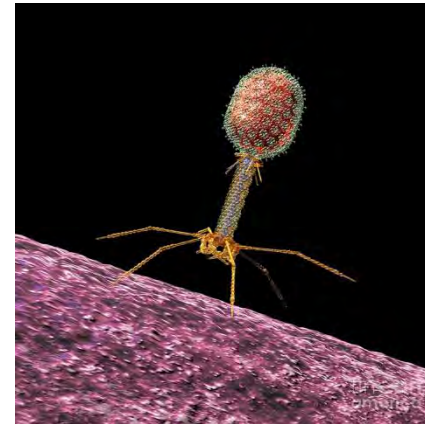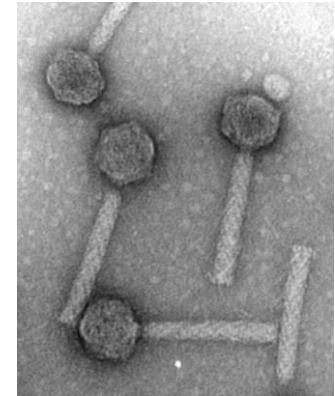
Czaplewski, Bax, Clokie, et al. (2016). Alternatives to antibiotics-a pipeline portfolio review.
Lancet Infectious Diseases

Phage treatment of bacterial infections
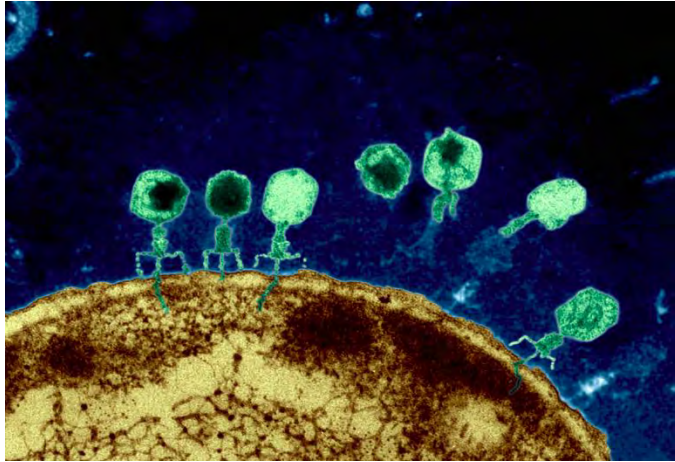
The **biology** - a complex and continually evolving predator-prey relationship between a potentially infinite number of phages and bacteria

The **economics** - an 'open source' product that can be easily copied with minimal know-how, tough to standardise & regulate

Predictive phage therapy workflow
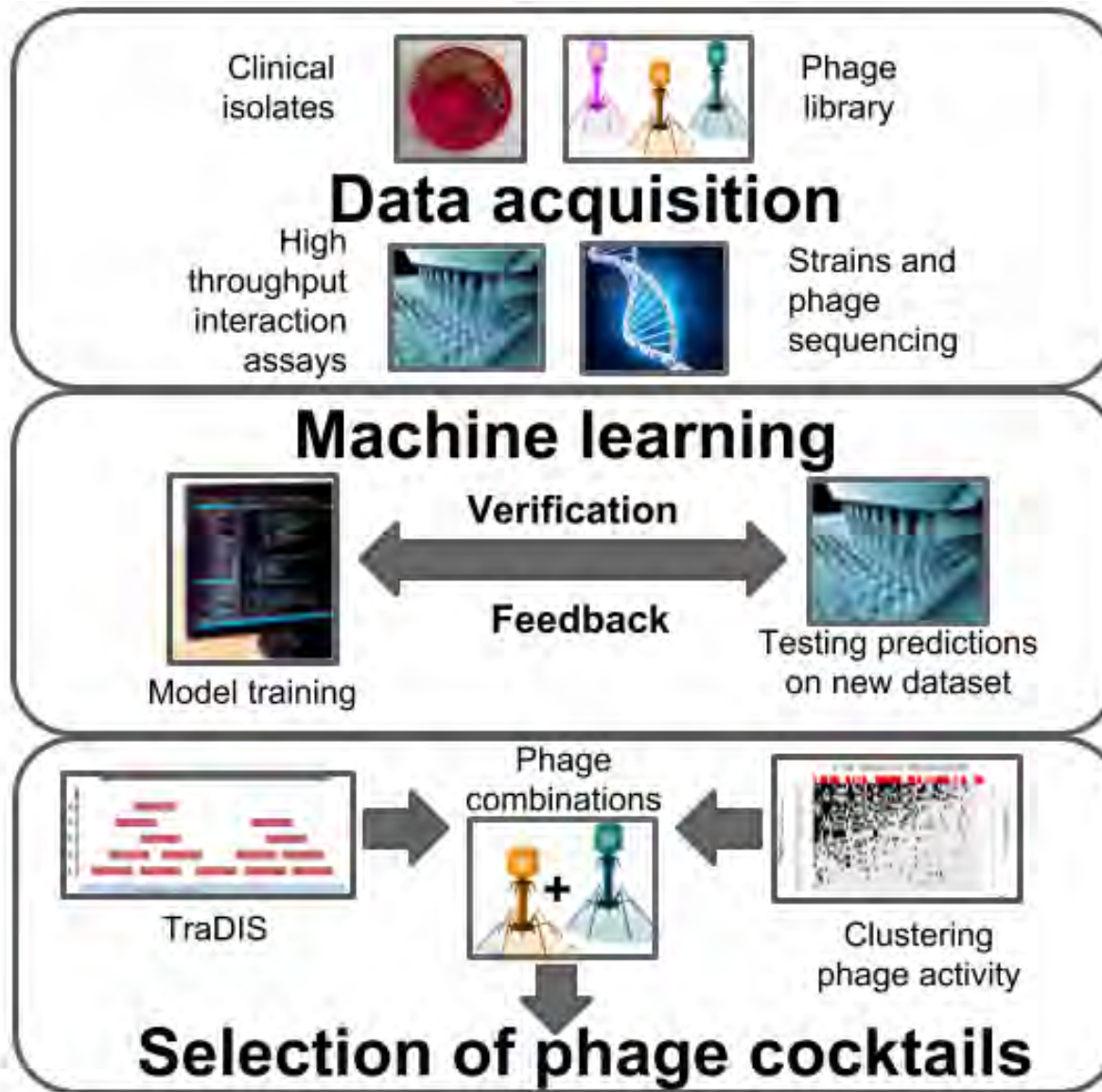
Accuracy of prediction: T4 phages on *E. coli* O157
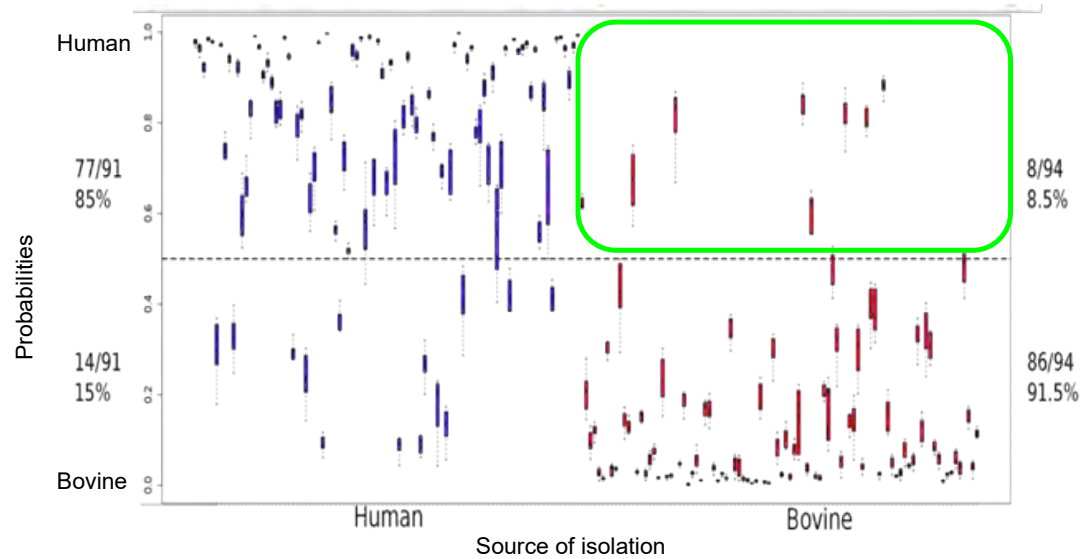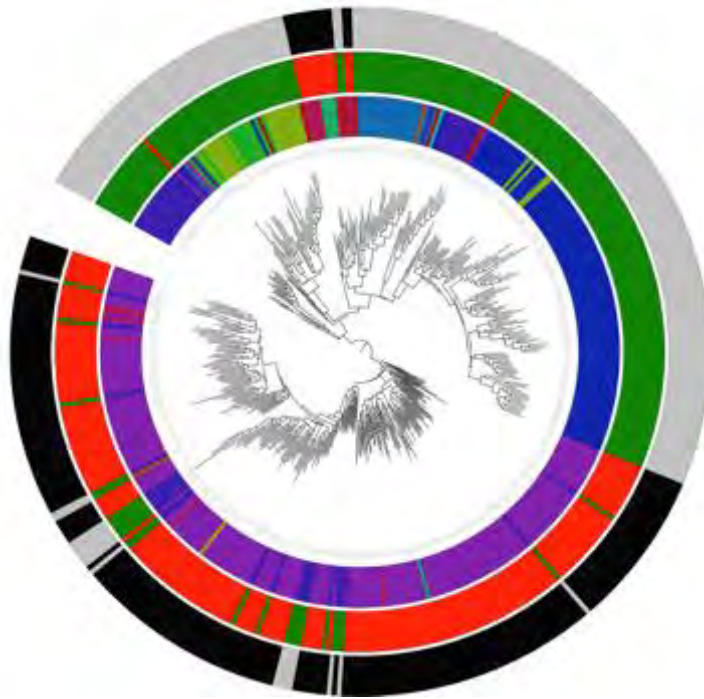
427 *E. coli* O157 isolates. Inner ring is phage type.

The middle ring phage resistance (red) or susceptibility (green)

The outer ring shows the SVM prediction: resistant- black;, susceptible-grey.

94-97% accurate for 6 phages

Canine chronic UTIs

Targeting MDR chronic UTIs associated with *E. coli*

Local epidemiology of isolates

TraDIS for mechanisms

Training set – ex vivo?

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BBSRC
bioscience for the future
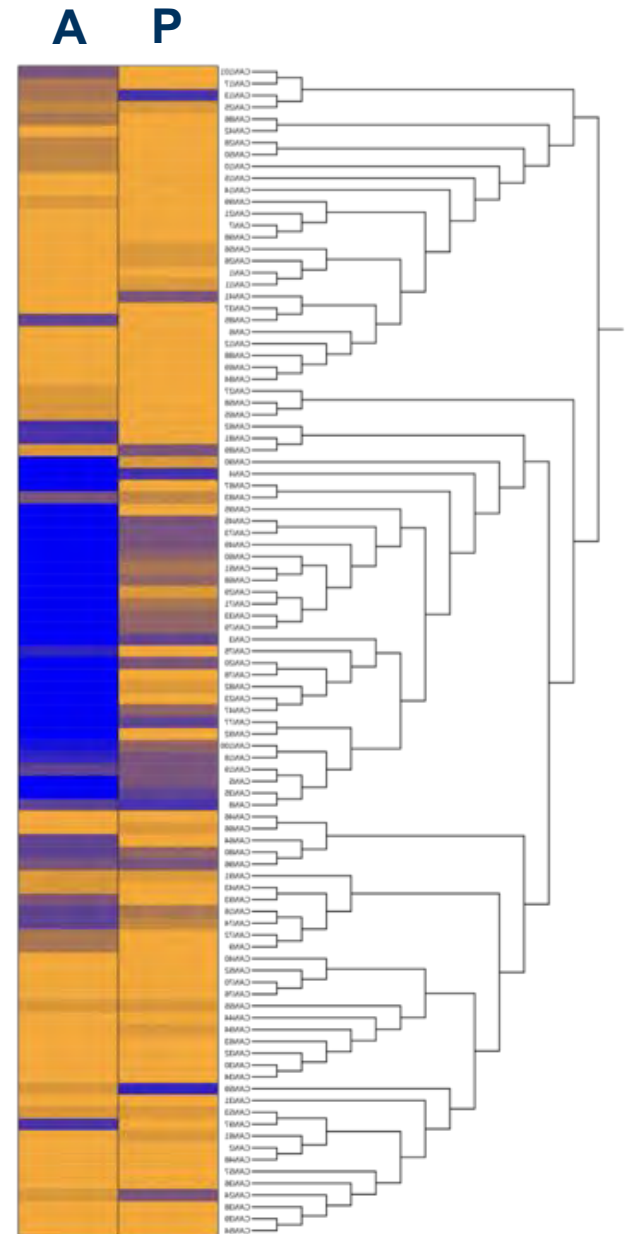
Modified Phage and targeted CRISPR

Phage tail switching
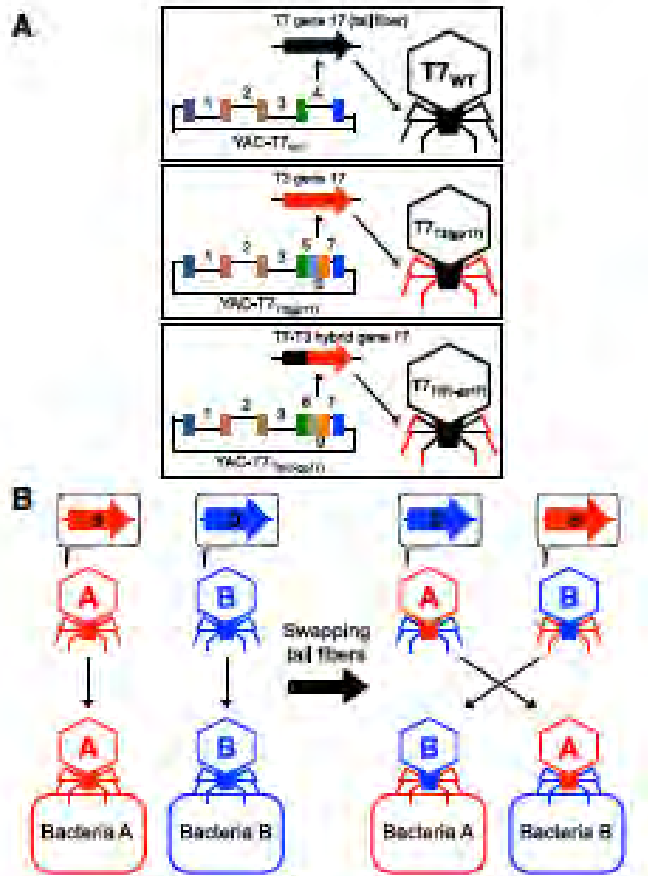
CRISPR-delivery into bacte
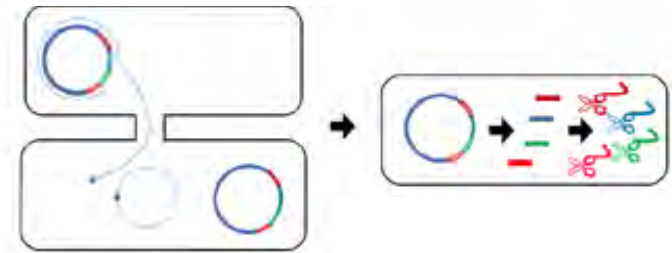
CRISPR gene + guides transferred into bacteria by conjugation or phage (phagemids)

Target AMR or virulence factors

Nemesis Bioscience Cambridge UK)

Summary

- Targeting infectious disease

- Major gains from system management

- Fundamental understanding of pathogen & host interactions, basic immunology & epidemiology

- Vaccines & adjuvants

- High throughput screens are powerful approaches for the identification of key pathogen and host determinants

- Breeding or manipulation for resistance/resilience

- Computational tools for complex datasets analysis

- Combined treatments to match accurate (genome-based) diagnostics

Acknowledgments

PRRSv resistance

Christine Tait-Burkard

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- Tahar Ait-Ali
- Tanja Opriessnig
- Chris Proudfoot
- Tim King
- David Davies
- Eddie Clutton

GECKO libraies

Finn Grey

Bruce Whitelaw

- Oliver Lin
- Stephen Chiweshe
- Abraham Lee
- Kenny Baillie
- Paul Digard
- Tim Regan
- Bob Dalziel
- Spring Tan

AMR pig farm

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- Nadejda Lupolova
- Geoffrey Mainda
- Barend Bronsvoort,
- Laura C. Duggan

Phage research

Alison Low (Tidswell)

Nadejda Lupolova

3

Transmission of CMY-2-producing *Escherichia coli* ST405 between humans and companion animals in South Korea

W. Song[1], J.S. Hong[2], H.-M. Park[3], J.Y. Oh[3], J.-C. Chae[4], J.-I. Han[5]
[1]Department of Laboratory Medicine, Hallym University College of Medicine, [2]Department of Laboratory Medicine, Yonsei University College of Medicine, [3]Department of Veterinary Internal Medicine, Konkuk University College of Veterinary Medicine, Seoul, [4]Division of Biotechnology, [5]College of Veterinary Medicine, Chonbuk National University, Iksan, Republic of Korea

Antimicrobial-resistant (AMR) Enterobacteriaceae are an emerging problem in human and veterinary medicine. We collected the microorganisms from rectal swab of 431 companion animals (361 dogs and 70 cats), 198 feces of humans (93 staffs and 105 guardians), and 384 environmental samples in 36 veterinary hospitals and 14 households for 2 years (2017 - 2018) in South Korea. The samples were enriched and selected on CHROMagar ESBL®. Bacterial identification and antimicrobial susceptibility testing were performed by MALDI-TOF MS and disk diffusion method, respectively. The presence of extended-spectrum beta-lactamase (ESBL) and AmpC genes was tested by PCR and DNA sequencing. The strains were assessed for their genetic relatedness by multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE). The total of 267 cefotaxime-nonsusceptible *Escherichia coli* was recovered. Of these organisms, the most common genotypes were CMY-2-like [29.6%, n=79/267 (57 dogs, 8 cats, 7 humans, and 7 environments)]. The eleven CMY-2-producing *E. coli* isolates were classified into sequence type (ST) 405 by MLST which were recovered from humans (n=4), companion animals (n=6), and environment (n=1) with more than 86% similarity on PFGE. Notably, when performed PFGE with four known CMY-2-producing *E. coli* ST405 collected from blood sample in human patients, they showed also close relationship with the 11 isolates. The results indicate active transmission and dissemination of AMR *E. coli* among humans and companion animals. Therefore, a One Health approach integrating human and companion animal surveillance data is essential to understand the root of antimicrobial resistance and develop effective prevention and control strategy.
Disclosure: Nothing to disclose

5
Evaluating Antimicrobial Stewardship Policy from a One Health Perspective: A Conceptual Framework for Quantitative Evaluation

N. Naylor, J. Lines, J. Waage, G. Knight
London School of Hygiene & Tropical Medicine, London, United Kingdom

Background: Antimicrobial resistance is an issue that requires urgent cross-disciplinary action. Evaluating the full impact of new control measures, such as antimicrobial stewardship (AMS), requires a One Health perspective with multiple angles to account for interacting complexities. To inform the design of future evaluations we performed a literature search to determine what quantitative evaluations for interventions related to issues that potentially impact human health, agriculture and the environment (such as climate change) have been utilised previously. Using this, we propose a new framework of quantitative evaluation of AMS.
Methods: WebofScience, EconLit and Google were searched with combinations of "one health", "economic", "evaluation", "health", "agriculture" and "climate change" in August 2018, to collate previous evaluations. Literature reviews on AMS impact within human health and agriculture, respectively, were consulted to extract relevant outcomes needed from future AMS evaluations.
Results: 1244 unique abstracts were retrieved from the structured literature search. After two rounds of review (title/abstract and full text), 36 previous evaluations were included. The most commonly utilised methods included general equilibrium or systems approaches. Proposed outcomes include epidemiological measures, human morbidity and mortality measures, intervention cost to individual sectors and productivity measures. The proposed framework links together mathematical epidemiological, microeconomic and macroeconomic impact models, to provide the impact of AMS interventions on the aforementioned outcomes. A long time-horizon (100 years) is recommended.
Conclusion: Quantitative evaluations of AMS policy, utilising the proposed framework, will help stakeholders across the One Health system have the information needed to efficiently tackle the issue of antimicrobial resistance.
Disclosure: Nothing to disclose

7

Comparison of antimicrobial susceptibility in staphylococci from first-time canine pyoderma cases versus staphylococci from cases submitted for routine diagnostics

E.M. Broens[1], M. Gonggrijp[2], M. Biesheuvel[2], J. van Hout[2], M.A.M. van Dijk[1]
[1]Department of Infectious Diseases and Immunology, Utrecht University, Utrecht, [2]GD Animal Health, Deventer, The Netherlands

Pyoderma is a common condition in dogs caused by staphylococci and often treated with antimicrobials. For a good empirical choice, data on antimicrobial resistance in staphylococci from pyoderma cases is needed. Most resistance data are obtained in a passive way from routine diagnostic laboratories. Submissions to these laboratories might be biased towards samples from recurrent cases possibly affecting antimicrobial resistance prevalence.

The aim of this study was to assess whether the prevalence of antimicrobial resistance in staphylococci from first-time canine pyoderma cases differs from the prevalence in staphylococci from canine pyoderma cases submitted to routine diagnostic laboratories.

From February till August 2018, companion animal veterinarians were requested to submit samples from first-time canine pyoderma cases before antimicrobial treatment ('active monitoring') to the Veterinary Microbiological Diagnostic Centre (VMDC) from Utrecht University for bacteriological examination and determination of minimal inhibitory concentrations (MICs). Samples from canine pyoderma cases submitted to the VMDC for routine diagnostics ('passive monitoring') during the study period were used for comparison.

Active monitoring resulted in 58 staphylococci isolates, passive monitoring in 148 staphylococci isolates. MICs of actively and passively obtained staphylococci showed significant differences in resistance prevalence for four of the nineteen antimicrobials tested: chloramphenicol (17.2% vs. 31.7%), clindamycin (15.5% vs. 33.1%), kanamycin (17.2% vs. 41.5%) and erythromycin (17.2% vs. 38.7%).

This study shows that the prevalence of resistance for clinically relevant antimicrobials (e.g. clindamycin is the first choice for the treatment of canine pyoderma according to Dutch guidelines) might be overestimated when data from routine diagnostics are used.

Disclosure: This pilot study was part of the VETMAP project funded by the Dutch Ministry of Agriculture, Nature and Food Quality.

10

Colistin susceptibility profiles of *E. coli* from intensive and natural pig farms in Thailand

P. Amavisit[1], P. Ketkhao[2], S. Thongratsakul[3], P. Poolperm[3], C. Poolkhet[3]

[1]*Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University Bangken Campus, Bangkok,* [2]*Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus,* [3]*Faculty of Veterinary Medicine, Kasetsart University, Kampangsaen Campus, Nakhon Pathom, Thailand*

In Thailand, pig farms have different farming systems for example the differences of health management programs, and the use of antimicrobials. Colistin was used in pig farms in several countries for more than two decades. Recently WHO was reclassified colistin as a very high important human medicine because it is a last resort treatment option for multidrug resistant bacterial infection. In this study *E.coli* collected from healthy pig feces and farm environmental samples of four pig farms that had different farming system, were tested for colistin minimum inhibitory concentration (MIC) and amplified for plasmid mediated colistin resistance genes, *mcr-1* and *mcr-2*. Comparison of the resistant rates, a common intensive farm (Farm A) had significantly higher rate than an intensive farm without using colistin (Farm N) ($P< 0.05$). However the resistant rate of Farm A was significantly lower than Farm L that was an intensive farm with low biosecurity system. Interestingly the rates of resistances between Farm A and Farm S (small scale natural farming without using vaccine and antimicrobial) were not different. Colistin resistant rates of *E. coli* from Farm A, N, L and S were 58.1%, 14.3%, 84.6% and 41.8% respectively. In each MIC levels, the detection of *mcr-1* were not significantly different ($P>0.05$).

Disclosure: Nothing to disclose

12
Detection and molecular characterization of SHV beta-lactamases-producing *E. coli* from German livestock and meat

G. Zhao[1], J.A. Hammerl[2], B.-A. Tenhagen[2], S. Schmoger[2], M. Grobbel[2], A. Käsbohrer[2,3], A. Irrgang[2]
[1]China Animal Health and Epidemiology Center (CAHEC), Qingdao, China, [2]Department Biological Safety, German Federal Institute for Risk Assessment, Berlin, Germany, [3]University of Veterinary Medicine/Institute for Veterinary Public Health, Vienna, Austria

Resistance of bacteria to 3rd generation cephalosporins mediated by beta-lactamases (ESBL, pAmpC) is a public health concern. In livestock, CTX-M-1 is the most common ESBL in Germany, but in poultry production SHV beta-lactamases are also widespread. In this study, *E. coli* isolates obtained within the German monitoring on zoonosis in 2016 and 2017 were screened for the presence of bla_{SHV} genes. The enzyme variants were determined by PCR sequencing. Further molecular characterization was conducted for selected isolates (PFGE, phylogenetic groups, plasmid characterization). Next generation sequencing was performed for the six bla_{SHV-2} isolates.

More than 1500 isolates were screened and the presence of a bla_{SHV}-variant was confirmed for 161 isolates. Of these 91% (n=147) were obtained from poultry production and meat, the other 9% originated from pigs and calves from livestock. The SHV-12 beta-lactamase was most frequent (155/161, 96%). Of these, 44 isolates were further investigated. A phylogenetic relationship of the isolates was not observed by PFGE analysis. Nevertheless, in S1 nuclease restriction revealed that the gene was harbored on a 40 kb plasmid in half of the isolates. In contrast, for 3 of the 6 SHV-2-isolates' PFGE, whole genome sequencing and SNP-analysis indicated a clonal spread. These belonged to sequence type 533. The bla_{SHV-2} gene in all six isolates was harbored on IncF plasmids.

In conclusion, the spread of bla_{SHV-12} producing *E. coli* within the German food chain is probably mainly based on plasmid transmission. SHV-2 was found only sporadically with a clonal spread of ST533 isolates.

Disclosure: Nothing to disclose

13

Engaging medical, pharmacy, and veterinary students in antimicrobial resistance communication research

C. Primeau[1,2], C. Carson[2], J. McWhirter[1], S. McEwen[1], J. Parmley[2]
[1]Population Medicine, University of Guelph, [2]Public Health Agency of Canada, Guelph, ON, Canada

Introduction: A significant driver of antimicrobial resistance (AMR) is antimicrobial use (AMU) in human and veterinary medicine. Therefore, education and awareness among antimicrobial prescribers and dispensers is critical. Both human and veterinary health professionals have important roles to play and studies have shown that engaging stakeholders prior to developing communication materials can increase relevance, awareness, and dissemination of research findings.

Objectives: To explore medical, pharmacy, and veterinary student perceptions and understanding of factors associated with emergence of AMR, and to identify key messages, knowledge translation and transfer (KTT) methods, and dissemination strategies for effective communication of AMR information to future antimicrobial prescribers.

Aims: To help inform messaging used in future KTT and communication activities to promote positive behavioural change and enhance awareness of AMR among medical and veterinary health professionals.

Methods: Beginning in November 2018, focus groups were conducted with medical, pharmacy, and veterinary students in Ontario, Canada. A semi-structured format using standardized open-ended questions and follow-up probes was followed. Thematic analysis was used to identify and analyze patterns within the data.

Results: Preliminary analyses showed that students believe AMR to be an important global issue, and the main drivers include prophylactic AMU in animals and treating without confirmation of diagnoses. Students felt that although infographics provide easily digestible information, KTT materials such as fact sheets are more effective at providing sufficient information without overwhelming target audiences.

Conclusions: This research may help inform future communication materials and develop tailored KTT tools for dissemination of important AMR information.
Disclosure: Nothing to disclose

17
The National Antimicrobial Resistance Monitoring System (NARMS): a one health system in the United States

G. Tyson, H. Tate, C. Kabera, P. McDermott
Center for Veterinary Medicine, U.S. Food and Drug Administration, Laurel, MD, United States

NARMS is a One Health collaborative program that tracks antimicrobial resistance in bacteria from food animals, retail meats, and human patients. Data from NARMS are combined to assess changes in resistance of foodborne pathogens resulting from antibiotic use in food animal production. These data are used in risk assessment for the approval of antimicrobials in food animals by the FDA, as well as to attribute human infections to their animal and food sources. NARMS comprises a large group of federal and state experts in microbiology, epidemiology, biostatistics, genetics, bioinformatics, veterinary medicine, and human medicine. NARMS also works with the Veterinary Laboratory Investigation and Response Network (Vet-LIRN) to monitor antimicrobial resistance in companion animal pathogens. Other future enhancements designed to meet the goals of a One Health approach are being planned. These include the addition of animal feed, seafood and other commodities, as well as resistance in food animal pathogens, the periodic testing of minor food animal species, and an environmental component. Whole-genome sequencing is now a routine process in NARMS, making it possible to monitor resistance at the allelic and nucleotide level. NARMS publishes interactive displays to make these complex datasets accessible to a variety of stakeholders, including food producers, academic researchers, other governmental agencies, and the public at large.

Disclosure: Nothing to disclose

18

Treatment outcomes of multi-drug resistant tuberculosis and associated factors among patients at Iganga and Mbale treatment centres, Uganda: a retrospective cohort study

D.R. Zemei[1], E. Buregyeya[2], S. Kisaka[3]
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Introduction: The emergence of multidrug resistant tuberculosis (MDR-TB) threatens the existing efforts to eliminate tuberculosis due to the complex treatment thereof. The study aimed to describe the treatment outcomes, determine the factors associated with unsuccessful treatment outcomes and explore facilitators and barriers of treatment success of MDR-TB patients in Iganga and Mbale treatment centres, Uganda.

Methods: The study was a retrospective cohort analysis of data from medical records of patients at Iganga and Mbale treatment centres for the period June 2013 to May 2018. This data was complemented by qualitative interviews with selected health workers and former patients; these were analysed using thematic analysis. Quantitatively, Modified Poisson regression and mortality risk differences were performed to determine associations between factors and the treatment outcomes of MDR-TB using Stata 13.

Results: Of the 95 patients, 74 (77.9%) had successful outcomes and 21 (22.1%) had unsuccessful outcomes. There were 62% males, 41% were between 30-44 years, 88% had history of tuberculosis treatment and 34% were HIV positive. Only HIV status was likely to be associated with unsuccessful outcomes at bivariate analysis CPR 3.35 (CI 1.4-8.09) and the mortality rate attributable to HIV infection was 60% for five year period. Facilitators of treatment success included good communication and coordination mechanisms, availability of adherence enablers, self motivation and family support whereas barriers included delayed treatment initiation, alcohol consumption and stigma.

Conclusion: There was high treatment success of MDR-TB patients however the prevalence of unsuccessful outcomes particularly mortality was high and associated with HIV infection.

Disclosure: Nothing to disclose

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Development of a low-cost field based molecular diagnostic device for the detection of poultry pathogens

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Chicken production accounts for 15% of the agricultural output of the Philippines and is growing at a rate of a few percent per annum. One of the key factors affecting the growth of this industry is an inability to rapidly detect disease and control outbreaks. The main poultry disease pathogens are Newcastle disease virus, Infectious Bursal Disease virus, avian Infectious Bronchitis virus, *Salmonella spp.*, Avian Pathogenic *E. coli* and *Mycoplasma gallisepticum* and *synoviae*. Currently, diagnosis relies on a drop in production performance, presence of clinical signs, pathological lesions and serological tests, which can be time-consuming. Therefore, the use of rapid field based molecular testing has the potential to reduce diagnosis time to approximately 1 hour, help to prevent disease spread, facilitate appropriate selection of treatments and thus potentially reduce antimicrobial resistance.

A consortium of researchers from the UK (Brunel University London, University of Surrey, and Lancaster University) and from the Philippines (University of the Philippines Los Banos, Cavite State University and University of Eastern Philippines) are currently working on the development of a low-cost handheld molecular diagnostic platform test. The system will consist of a sample preparation device and a small instrument running an isothermal DNA amplification process (LAMP) to rapidly amplify the DNA from faecal/respiratory swabs and tissue samples. DNA amplification will lead to a colorimetric detection integrated into a smartphone application. The application will run the assay, display the results and, enable diagnostic data to be sent to a central database for integrated disease tracking and management systems.

Disclosure: Nothing to disclose

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Increased Dissemination and Parallel Evolution of Antimicrobial Resistance in *Salmonella enterica* serovar Paratyphi B variant Java from Poultry in Latin America and Europe

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Background

Isolates of *Salmonella enterica* serovar Paratyphi B variant Java (here referred as Java) from poultry are known to carry AMR genes and belong to Multi Locus Sequence Type (ST) 28. The objective of this study was to investigate the evolutionary relatedness of Java-ST28 from multiple Latin American (LA) and European (EU) countries.

Methodology

Java-ST28 strains were selected from previous studies from Colombia, Costa Rica, Guatemala and the Netherlands and subjected to whole genome sequencing. Additional genomes were collected from Enterobase. Characterization of AMR was made with ResFinder. Time resolved phylogeny and effective population size (N_e) were inferred using Bayesian Evolutionary Analysis Sampling Trees (BEAST) and Bayesian skyline plot.

Results

A clear phylogenetic distinction was observed between EU and LA Java strains. EU strains exhibited gyrase mutations conferring resistance to fluoroquinolones. In turn, LA strains carried the *qnrB19* gene conferring reduced-susceptibility to quinolones. Resistance to β-lactams was mainly mediated by *bla*TEM-1B in EU and by *bla*CMY-2 in LA. Molecular clock was estimated at 1.7 single nucleotide polymorphisms/genome/year [Confidence Interval (CI):1.44-2.0]. Evolutionary separation was observed between strains from EU and LA and dated to 1987 (CI: 1978-1988) with BEAST. N_e in EU increased sharply in 1995 (CI: 1992-1998) and in LA in 2005 (CI: 2001-2007).

Conclusions

Java-ST28 from LA and EU form two distinct clades. The estimated years of N_e increase in EU are in accordance with literature reports. The EU and LA clades have acquired resistance to fluoroquinolones and β-lactams independently, indicating parallel evolution of AMR in both regions.

Disclosure: Nothing to disclose

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Methicillin resistant *Staphylococcus aureus spa*-types t003, t586 and t014 common cause of MRSA infection in Czech Republic

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of healthcare-associated infections world-wide. The aim of the study was to characterize epidemiological structure of MRSA strains currently circulating in the Czech Republic.

Material and methods: Between September 2017 and January 2018 non-duplicated (single patient) MRSA isolates were collected from 11 hospitals across the Czech Republic. Isolates causing infection or colonizing patients of both healthcare and community origin were characterized. Resistance to oxacillin and cefoxitin was confirmed by disk diffusion method. The presence of genes encoding Panton-Valentine Leucocidin (PVL) and *mecA* gene were detected by PCR. Isolates were assign to the known multilocus sequence type clonal complexes (CC) based on corresponding *spa*-types.

Results: Total of 441 MRSA isolates were characterized, 78% (n=343) of them belonged to a single clonal complex CC5 represented by *spa*-types t003 (n=136), t586 (n=92), t014 (n=81), t002 (n=20) and other (n=14). *spa*-types belonging to the CC5 were dominant (more than 50% of isolates) in all participating hospitals, with exception of one hospital where t008 (CC8) was one of the top three *spa*-types. Livestock-associated MRSA (CC398) was identified in 10 isolates (Figure 1). Except oxacillin and cefoxitin, the MRSA isolates were most frequently resistant to erythromycin (88.0%), clindamycin (84.8%), and ofloxacin (82.8%).

Conclusion: High prevalence of a limited number of *spa*-types, originating from healthcare-associated CC5 lineage (t003, t586, t014), was found in eleven Czech healthcare facilities suggesting spread and circulation of these strains within and between healthcare institutions in the Czech Republic.
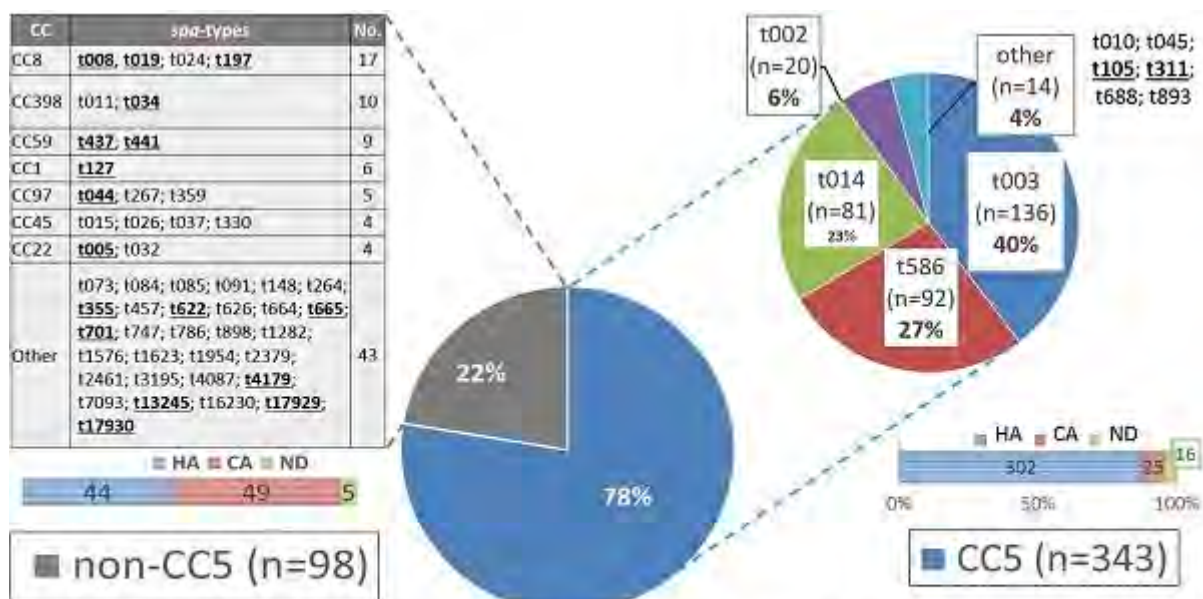
Figure 1. Distribution of MRSA isolates according their corresponding clonal complexes (CCs) and a origin of the infection (colonisation)

HA - Healthcare-associated, **CA** - Community-acquired, **ND** - unknown origin.

spa-types of PVL positive isolates (n=38) are underlined.

[Figure 1.]

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Characterization of new VIM-1 producing *Escherichia coli* from German pig production

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Carbapenems are critically important broad-spectrum beta-lactam antimicrobials. Resistance to carbapenems is often mediated by degrading enzymes (carbapenemases). As the genetic information for these enzymes is mostly encoded on mobile genetic elements, horizontal and vertical transmission between bacterial strains is possible.

Within an extended specific monitoring on CPE in food animal production according to Commission implementing decision 2013/652/EU, one isolate (17-AB01027), was detected in faeces of fattening pigs at farm. A second CPE (17-AB02384) was found in caecum content of a fattening pig at slaughter within the monitoring on ESBL/AmpC-producing *E. coli*. Genotype of both isolates was confirmed by PCR sequencing and characterized by PFGE, Southern Blot hybridization, MLST and NGS.

Isolate 17-AB01027 was a ST48 *E. coli* of phylogenetic group A, while isolate 17-AB02384 belonged to ST7593 and phylogenetic group B1. Both strains differed substantially from each other and previously described isolates by PFGE analysis and wgMLST. Plasmids from both isolates were conjugative and highly similar to the *Salmonella* Infantis VIM-1 plasmid pSE15-SA01028 (CP026661.1) and the *E. coli* plasmid pRH-R178 (HG530658.1). An additional bla_{SHV-12} gene was located on the plasmid of the strain 17-AB02384.

The results of the characterization of the isolates suggest horizontal spread of the VIM-1 carbapenemase within the German pig production and a high transmission potential via plasmid conjugation.

Disclosure: Nothing to disclose

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Isolation of a CC133 *Staphylococcus aureus* strain of ruminant origin in a patient with tracheobronchitis and previous consumption of farm cheese

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We report a case of a previously healthy 28-years-old male who presented with ventricular fibrillation out-of-hospital cardiac arrest. He was intubated and admitted to a coronary unit. At day 3, he presented with tracheobronchitis and was empirically treated with cefepime. Culture of endotracheal aspirate showed susceptible *Staphylococcus aureus* prompting a change to cloxacillin. *S.aureus* was still detected at days 9 and 13. The patient was discharged at day 22 with a final diagnosis of arrhythmogenic right ventricular dysplasia. An implantable cardioverter defibrillator was placed.

The sequential isolates were genotyped by microarray and assigned to Clonal Complex133. They were *mecA*-negative and did not carry resistance markers. They harboured leukocidin genes *lukM/lukF-P83* as well as superantigen genes. The beta-haemolysin gene was not truncated, and genes associated with *hlb*-converting phages were absent showing that the strain was not adapted to human hosts. Patient was interviewed afterwards and denied contact to animals but remembered consumption of farm cheese few days prior to admission. A nasal swab at follow-up showed *S.aureus*, but another strain, *mecA*-negative CC398. Isolates underwent whole genome sequencing that confirmed array results and revealed a low level of variation showing two non-synonymous variants of genes encoding DsbA family proteins and transcriptional repressor MraZ.
It is, to our knowledge, the first time that a strain known only from small ruminants was found in a clinically infected human, most likely after food-borne transmission. Molecular findings showed that the strain was not adapted to human hosts but was able to cause infection and to persist.
Disclosure: Nothing to disclose

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Putting away the muddy wellies: Taking a Behavioural Approach to Antimicrobial Stewardship with vets and farmers in a UK context as part of One Health

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Background

Antimicrobial resistance (AMR) is an increasing concern in human and animal health. Theoretically informed antimicrobial stewardship (AMS) interventions within veterinary medicine and farming may be influential in reducing the consequences of AMR for both animal and human health as part of a One Health approach to AMR.

Research aims

This research study set out to: identify AMS behaviours and the barriers and enablers of these behaviours; and to generate evidence based recommendations to support vets and farmers to develop AMS behaviours.

Methods

The research used qualitative methods. Eleven vets and 17 farmers took part in semi-structured interviews. AMS behaviours and their related barriers and enablers were identified using thematic analysis techniques. Psychological behavioural theories (Theoretical Domains Framework and the Behaviour Change Wheel) were used to generate recommendations.

Findings

The overall behavioural domain identified was vets' and farmers' roles in preventative veterinary medicine and farming. Vets talked about 'putting away the muddy wellies' to illustrate this shift in focus from treatment to health planning and prevention. Factors relating to professional identity, economics, and engagement were identified as enablers, while factors relating to resources, knowledge, skills, beliefs and motivations were identified as barriers to behaviour change.

Discussion

The intervention recommendations of the research included a focus on prevention in education for vets and farmers and working with vets to look at alternative business models based on preventative services rather than medicines sales. The recommendations have relevance for vets and farmers and wider stakeholders such as government, agricultural advisors, supermarkets, and consumers.
Disclosure: Nothing to disclose

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A systematic review and meta-analysis of the health and healthcare system burden due to resistant *Escherichia coli* infections in humans

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The objectives were to evaluate whether the measures of health or healthcare system burden increase in humans with *E. coli* infections that are resistant to third/fourth/fifth generation cephalosporins or quinolones, or are multidrug resistant when compared to those with susceptible infections.

The protocol was registered with PROSPERO (CRD42018111197). The population of interest was humans with confirmed *E. coli* infections. Resistance to third/fourth/fifth generation cephalosporins or quinolones, or multidrug resistance were the exposures of interest. Included studies had a comparator group without the exposure of interest. The outcomes of interest for health burden were mortality and treatment failure, and for healthcare system burden were length of hospital stay (LOS) and costs. Included studies were analytic observational study designs. Data related to the characteristics of the study and participants, and results for the health and healthcare system outcomes were extracted. Mortality and LOS outcomes will be synthesized by meta-analyses and sources of heterogeneity will be explored using subgroup meta-analyses.

The literature search retrieved 26,038 articles and after duplicates were removed there were 14,759 articles for primary screening. There were 543 articles for secondary screening and 83 articles were included in the systematic review: 65 articles addressed resistance to third/fourth/fifth generation cephalosporins, 21 articles addressed resistance to quinolones, and 11 articles addressed multidrug resistance. The complete results of the systematic review and meta-analysis will be presented.

The current evidence for the health and healthcare system burden from resistance in human *E. coli* infections will be synthesized by the systematic review and meta-analysis.

Disclosure: Nothing to disclose

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Moxifloxacin-resistant *Clostridium difficile* ribotypes 001 and 176 (027-like) drive hospital epidemiology of *Clostridium difficile* infections in Slovakia

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Background and aims
Recently, an increase in the incidence of *Clostridium difficile* infections (CDI) was reported in several Slovakian hospitals. In order to obtain valid data on current *C. difficile* epidemiology in Slovakia (a *C. difficile* typing service is unavailable in Slovakia) we aimed to perform a CDI surveillance study and to characterize *C. difficile* isolates.

Methods
Between April and December 2018, fourteen Slovakian hospitals (Figure 1) submitted stool samples and epidemiological data of patients with laboratory-confirmed CDI to the Motol University Hospital, Prague, Czech Republic. In *C. difficile* isolates, a capillary-electrophoresis ribotyping was performed according to the new consensus protocol (Fawley et al., PONE, 2015). The antibiotic susceptibility of the isolates to metronidazole, vancomycin and moxifloxacin was determined by agar dilution method.

Results
A total of 142 *C. difficile* isolates were cultured from 146 stool samples. The most prevalent PCR ribotypes (RTs) were 001 (n=66, 46.5%) and 176 (n=46, 32.4%). A total of 121 (85.2%) of isolates were resistant to moxifloxacin (>4 mg/L). Of them, 112 (92.6%) belonged to epidemic RTs 001 and 176 (027-like), (p=0.00001). A reduced susceptibility to metronidazole (>2 mg/L) was observed in 8 isolates (RTs 001 and 176).

Conclusions
We revealed a dramatic proportion of moxifloxacin-resistant *C. difficile* strains that were responsible for causing CDI in fourteen Slovakian hospitals. Our findings call for an urgent reduction in prescriptions of fluoroquinolones and the implementation of effective CDI surveillance in healthcare settings in Slovakia.

[Figure1: Geographical distribution of participating hospitals in Slovakia with number of isolates.]

Disclosure: Nothing to disclose

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Establishment of multi-sectoral joint project to One Health approach against Antimicrobial Resistance in Republic of Korea

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Introduction: In today´s international society, the persistent occurrence and spread of Antimicrobial resistance(AMR) is a public health problem that threatens and causes serious social and economic problems. WHO, OIE and FAO have emphasized a new approach to health policy by introducing the concept of "One Health", and strongly urged strategic cooperation in the field of AMR. Accordingly, advanced countries in the world are emphasizing AMR to the approach of One Health.
Objectives: Since antimicrobials are used not only in human but also in ecosystem such as agriculture, livestock, fisheries, food, environment, etc., a multi-disciplinary R&D studies are needed to reduce and to prevent the spread of AMR.
Aims: In addition, due to the spread of AMR throughout the whole field, integrated research and comprehensive management of ministries are required through cooperation among government ministries that manage each field.
Methods: In line with this, Republic of Korea has been planning the "Multi-sectoral joint project to One Health approach against AMR" since 2016, and conducting the "One Health AMR research project" as a pilot studies such as: companion animals-surroundings-guardians, livestock-barn-workers, and human-hospital-river environment from 2017 to 2019.
Results: Significant results have been derived and analyzed for the interrelationship researches among the fields through antimicrobial susceptibility tests and various genetic analyses.
Conclusions: It is expected to become an important R&D project for the management of AMR in Republic of Korea.
Disclosure: Nothing to disclose

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Risk factors for puerperal infection: a systematic review and meta-analysis

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OBJECTIVE To provide evidence for decision-making and further research on prevention of puerperal infection through identifying the risk factors of puerperal infection. **METHODS** The results from 19 selected studies on risk factors of puerperal infection were analyzed quantitatively by meta-analysis. **RESULTS** gestational hypertension (OR =3.54), gestational diabetes (OR =2.70), anemia (OR =2.13), multipara (OR =1.19), caesarean section (OR =2.12), prolonged labor (OR =2.62), premature rupture of membranes (OR =3.40), soft birth canal injury (OR =5.84), forceps or head pull (OR =2.41), placenta (OR =3.50) and postpartum hemorrhage (OR =2.49) have significant association with puerperal infection. **CONCLUSIONS** To prevent puerperal infection, we suggest that strengthen health education for pregnant women and treat basic diseases actively before the delivery, obey aseptic techniques strictly to avoid the soft birth canal injury during labor, and closely monitor the uterine contractions and the amount of postpartum bleeding after delivery.

Key words: Puerperal infection; Risk factors; Meta-analysis

Disclosure: Nothing to disclose

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Dose optimization of Enrofloxacin in Broiler Chicken against *Salmonella* Enteritidis by Integrating Pharmacokinetic and Pharmacodynamic Profiles

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It is crucial to optimize the dose of fluoroquinolones for controlling their resistance and attaining clinical success. It was intended in this study to optimize the dose of enrofloxacin against *Salmonella* Enteritidis in chicken by assessing its pharmacokinetic/pharmacodynamic (PK/PD) indices. The antibacterial activities of enrofloxacin against *S*. Enteritidis were evaluated. After administering 10 mg/kg body weight (b.w.) of enrofloxacin to broiler chickens of both sexes by intra-venous (IV) and per-oral (PO) routes, blood samples of different periods were drawn and enrofloxacin concentrations in serum were quantified. The integration of PK and PD data was done to calculate PK/PD indices. The elimination half-lives ($t_{1/2}$), time required to reach peak concentration (T_{max}), peak concentration (C_{max}) and area under curve (AUC) after administering enrofloxacin by PO and IV routes were 25.84±1.40 h, 0.65±0.12 h, 3.82±0.59 µg/mL and 20.84±5.0 µg.h/mL, and 12.84±1.4 h, 0.22±0.1 h, 6.74±0.03 µg/mL and 21.13±0.9 µg.h/mL, respectively. Enrofloxacin's bioavailability was 98.6±8.9% after administering by PO route. The MICs of enrofloxacin were (0.0625?1) µg/mL against *S*. Enteritidis strains, and the MIC_{50} was 0.50 µg/mL. The C_{max}/MIC_{50} were 7.64±0.2 and 13.48±0.7, and the 24 h AUC, (AUC_{0-24})/MIC_{50} were 41.68±0.1 and 42.26±0.3 correspondingly, after administering the drug through PO and IV routes. The data of this study indicate that the application of 50 mg/kg b.w. of enrofloxacin to chicken through PO and IV routes with a dosing interval of 24 h can effectively cure S. Enteritidis infection, which demonstrated the 5-times increase of the recommended-dosage of enrofloxacin in chicken.
Disclosure: Nothing to disclose

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Antimicrobial susceptibility monitoring of veterinary pathogens and zoonotic and commensal organisms throughout Europe - The CEESA programs

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Antimicrobial resistance is a global concern for both animal and human health. Programs to monitor antimicrobial susceptibility among veterinary pathogens as well as zoonotic and commensal bacteria are therefore essential.

The antibiotic susceptibility monitoring programs of the Executive Animal Health Study Center (CEESA) are an ongoing collaboration among veterinary pharmaceutical companies for over two decades. CEESA conducts two types of monitoring: the European Antimicrobial Susceptibility Surveillance in Animals (EASSA) program collects zoonotic and commensal bacteria at slaughter from healthy food-producing animals, and the target pathogen programs (VetPath, MycoPath and ComPath) collect bacterial isolates from diseased animals prior to antibiotic treatment. The latter programs are the only longstanding pan-European projects in veterinary medicine where antibiotic susceptibility data for a large variety of target pathogens are generated.

All CEESA projects apply uniform sample collection and bacterial isolation and identification to species level in various European countries. A single central laboratory for each subprogram conducts quantitative antibiotic susceptibility testing to determine the Minimal Inhibitory Concentrations to a range of commonly used, licensed antibiotic compounds. Data are primarily used by member companies in registration and renewal dossiers but the programs also contribute to scientific research such as characterisation of ESBL/AmpC, and also quinolone, colistin and meticillin resistance determinants (*qnr, mcr* and *mecA*).

The standardised methodology of the CEESA programs makes these robust and valuable tools to address food safety concerns and to support responsible use of antibiotics in the field by giving the veterinarian information on resistance patterns in target pathogens.

Disclosure: Several of the authors are full-time employees of veterinary pharmaceutical companies.

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Monitoring of antimicrobial susceptibility of respiratory tract pathogens isolated from diseased cattle across Europe, 2015-2016: VetPath results

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VetPath is a pan-European antimicrobial susceptibility monitoring programme collecting pathogens from diseased cattle, pigs and poultry before antibiotic-treatment initiation.

In total, 281 isolates from cattle with respiratory disease were tested. Lung samples or nasopharyngeal/nasal swabs were collected from animals with acute clinical signs in eight countries during 2015-2016. *Pasteurella multocida, Mannheimia haemolytica* and *Histophilus somni* were isolated by standard methods and MIC values of 21 antibiotics were determined in a central laboratory by broth micro-dilution as per CLSI standards. $MIC_{50/90}$ values are reported; results were interpreted using CLSI clinical breakpoints (VET08, 2018) where available.

P. multocida (n=155) isolates were fully susceptible to ceftiofur (100%) and susceptibility was >95% for enrofloxacin (98.1%), penicillin (98.1%), tulathromycin (98.1%), danofloxacin (97.4%), gamithromycin (97.4%) and florfenicol (96.8%). Spectinomycin and tetracycline susceptibility was 91.6% and 88.4%, respectively. Susceptibility of *M. haemolytica* (n=91) was as follows: ceftiofur 98.9%, spectinomycin 96.7%, florfenicol 94.5%, tulathromycin 93.4%, gamithromycin 89.0%, penicillin 89.0%, enrofloxacin 87.9%, tilmicosin 85.7%, danofloxacin 81.3% and tetracycline 78.0%. *H. somni* (n=35) were fully susceptible (100%) to ceftiofur, enrofloxacin, florfenicol, gamithromycin, penicillin, spectinomycin, tetracycline, and tulathromycin. For antibiotics without CLSI breakpoints such as amoxicillin, cefquinome, colistin, doxycycline, marbofloxacin and trimethoprim/sulfamethoxazole, MIC_{90} ranged from 0.008 to 2 mg/L for all three pathogens. For tiamulin, tylosin and lincomycin MIC_{90} ranged from 32-128 mg/L with broad but unimodal MIC distributions, except for *H. somni* where MIC_{90} was 0.5-4 mg/L.

In conclusion, for those antibiotics where the results could be interpreted using clinical breakpoints, the antimicrobial susceptibility generally remains around 90% or higher.
Disclosure: Several of the authors are full-time employees of veterinary pharmaceutical companies

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Susceptibility to florfenicol and oxytetracycline of the intracellular pathogen *Piscirickettsia salmonis* isolated from the Chilean salmon industry

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Intensive salmon farming in Chile favours the development of infectious diseases and consequently the use of antimicrobial agents for the treatment of bacterial infections has increased. The most important bacterial pathology in Chilean salmon farms is caused by the intracellular pathogenic species *Piscirickettsia salmonis*, responsible for 95% of administered antimicrobials. The main aim of the study was to determine the susceptibility of Chilean isolates of *P. salmonis* to the antimicrobials mostly used in the Chilean salmon industry. A number of 35 isolates of *P. salmonis* were recovered from Chilean salmon farms with confirmed outbreaks of Piscirickettsiosis occurred from December 2016 to May 2018. Species identity of isolates was confirmed by PCR, and their Minimum Inhibitory Concentration (MIC) values for florfenicol and oxytetracycline were determined using a standardized broth micro-dilution method. Isolates were categorized as fully susceptible wild type (WT) or non-fully susceptible non-wild type (NWT) to the assayed antibacterials, using previously stated epidemiological cut-off values. An important percentage of reduced susceptibility to florfenicol was detected, observing that 42.9% of the total assayed isolates were categorized as non-wild type (NWT). Otherwise, most of the assayed isolates were highly susceptible to oxytetracycline, of which only 5.7% were categorized as NWT. The increase in the number of florfenicol non-wild type isolates of *P. salmonis* prompts the urgent need of the implementation of a continuous surveillance program of antimicrobial resistance of this pathogen in the Chilean salmon farming industry.

Disclosure: Nothing to disclose

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Antimicrobial Resistance Patterns of *Salmonella* Isolated from Chickens at slaughterhouses in NE, Thailand

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The objective of this study is to determine the prevalence and antimicrobial resistance pattern of *Salmonella* spp. isolated from chickens at slaughterhouses in northeast of Thailand. During 2015-2016, all samples were isolated and identified by ISO 6579:2002. A total of 604 rectal swab samples were collected and isolated for the presence of *Salmonella* spp. *Salmonella* spp. was detected in 109 of 604 (18.05%) samples. The most prevalent serovars were *Salmonella* Kentucky (22.94%), Give (20.18%) and Typhimurium (7.34%). In this study, 66.97% of the isolates were resistant to at least one antimicrobial drug and 38.39% were multidrug resistant. The highest resistances were found in Nalidixic acid (49.54%), ampicillin (30.28%), tetracycline (27.52%), amoxicillin (26.61%), ciprofloxacin (23.85) and norfloxacin (19.27%). The results showed high prevalence of *Salmonella* spp. in chickens and antimicrobial resistance patterns. Prevention and control of *Salmonella* contamination in chickens impact on health and wellness of both chickens and consumers.
Disclosure: Nothing to disclose

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Prevalence and antimicrobial resistance of *Salmonella* spp. isolated from pigs at NE, Thailand

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The objective of this study is to determine prevalence and antimicrobial resistance pattern of *Salmonella* spp. isolated from pigs in slaughterhouses in northeast of Thailand. During 2015-2016, all samples were isolated and identified by ISO 6579:2002. A total of 699 samples of rectal swab were collected and isolated for the presence of *Salmonella* spp. *Salmonella* spp. was detected in 275 of 699 (39.34%) samples. 24 serovars were identified in the 275 isolates. The most prevalent serovars were Rissen (36.97%), *S. enterica* ser.4,5,12:i: (25.35%) and Typhimurium (21.33%). In this study, 76.30% of the isolates were resistant to at least one antimicrobial drug, and 38.39% were multidrug resistant. The highest resistances were found in ampicillin (69.20%), tetracycline (66.35%), sulfamethoxazole/trimethoprim (35.55%) and chloramphenicol (9.00%) The results showed high prevalence of *Salmonella* spp. in pigs and high antimicrobial resistance among the isolates, and indicated the need for monitoring program to control *Salmonella* contamination and reduce the dissemination of antimicrobial resistance in pig supply chain.

Disclosure: Nothing to disclose

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Low-level β-lactam resistance in Dutch and Danish methicillin-resistant *Staphylococcus pseudintermedius* is associated with clonal complexes

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Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is increasingly isolated from dogs and incidentally causes infections in humans. Screening for methicillin resistance is done by oxacillin (OXA) MIC testing. Applying EUCAST's MRSA expert rule, OXA-resistant isolates must be reported resistant to all β-lactams. This study aims to identify possible associations between β-lactam resistance levels and MRSP clones.

MICs of OXA, amoxicillin/clavulanic acid (AMC) and cephalothin (CEP) were determined in 92 canine *mec*A-positive *S. pseudintermedius* isolates from the Netherlands (n=50) and Denmark (n=42) by broth microdilution. Clonal complexes (CC) and SCC*mec* type were determined, using standard MLST and PCR methods for Danish isolates and whole genome sequencing for Dutch isolates.

The dominant clones were CC71 SCC*mec* II-III (n=37), CC258 *SCCmec* IV (n=36), followed by CC45 (n=11), and independant sequence types (n=8). CC71 displayed higher MICs for all β-lactams tested. OXA MICs were ≥2 µg/ml in all CC71 isolates, whereas other isolates mostly (80%) displayed MICs of 0.5 or 1 µg/ml. Similarly, most CC71 isolates had AMC MIC > 1 (84%) and CEP MIC > 2 (68%), whereas high MIC values were rare among other isolates.

Non-CC71 isolates containing SCC*mec* IV, V and non-typeable STs were associated with low-level β-lactam resistance. This finding may be of clinical relevance since MICs for CEP and AMC are usually below the clinical breakpoint. Clinical studies are warranted to evaluate whether some MRSP infections can be cured using these first line agents, avoiding second line antimicrobials with higher toxicity (e.g. rifampicin; chloramphenicol) or last resort human drugs (e.g. vancomycin).

Disclosure: Nothing to disclose

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Mobilome and resistome analysis of multidrug-resistant *Escherichia coli* isolates from human urinary tract infections

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Introduction: Urinary tract infections (UTIs) are one of the most common clinical presentations in health care facilities worldwide. The most frequent aetiology of UTIs is *Escherichia coli*, a widespread bacterium often carrying multiple genes responsible for resistance to antibiotic treatment. These genes are commonly encoded by mobile elements that can be transferred to a wide range of pathogenic and commensal bacteria.

Objectives: The main objective was to study the most prevalent genetic elements involved in antimicrobial resistance (AMR) transmission in uropathogenic *E. coli* (UPEC) isolates.

Aims: The ultimate aim was to understand how AMR genes are transmitted in order to help treat bacterial infections.

Methods: A collection of 245 UPEC strains were isolated from three different hospitals in the South England area and genotypically characterised by multiplex PCR for the presence of genes conferring resistance to β-lactams (*blaTEM/SHV/OXA/CTX-M/AmpC*) and colistin (*mcr-1/-2*). A panel of 94 isolates was sequenced to further analyse the presence of mobile AMR determinants.

Results: The panel of isolates was mainly composed of multidrug-resistant isolates and particularly resistant to extended-spectrum β-lactam antibiotics. Most of the isolates (78%) were positive for one or more β-lactam resistance genes, while all of them were found to be negative for the colistin resistance genes. The bioinformatics analysis of the 94 sequenced *E. coli* isolates will provide additional information, including phylotype, serotype, virulence traits, metal resistance genes, mobility genes, and plasmid content.

Conclusions: Detailed molecular analysis of multidrug-resistant isolates is essential to understand the genetic basis of AMR transmission.
Disclosure: Nothing to disclose

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Antimicrobial resistance in selected respiratory pathogens of veal calves: a pilot study towards a nationwide representative monitoring system

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Introduction:
A pilot study was conducted with the aim to setup a representative monitoring system of antimicrobial resistance (AMR) in *Mannheimia haemolytica* (MHA) and *Mycoplasma* species (MYC) isolates from veal calves in the Netherlands.

Methods:
Veterinarians were requested to submit nasal swabs from veal calves meeting inclusion criteria of the study (2-8 weeks of age and clinical signs of respiratory disease). In total 3-5 calves per farm, originating from 35 different farms were sampled ('active' monitoring). In addition, samples were collected from respiratory tracts of veal calves with relevant pathological findings that were submitted for post-mortem examination to GD Animal Health ('passive' monitoring). Samples of active and passive monitoring were submitted for bacteriological examination and AMR testing by broth microdilution. The results of the AMR of the passive monitoring were compared with the results of the active monitoring.

Results:
A high response in actively submitted nasal swabs was observed. Furthermore it was found that with 3-5 calves sampled per farm median three MYC isolates and one MHA isolate were obtained per farm (and on 13 farms no MHA isolates). Comparing results from actively and passively obtained samples, it was concluded that for some antimicrobials the prevalence of resistant MYC isolates in the passive monitoring was overestimated. However, when these overestimations are well described, the use of samples of both active and passive monitoring have an added value because the increase in number of samples results in a more precise estimation of the AMR of the majority of tested antimicrobials-pathogen combinations.

Disclosure: Nothing to disclose

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Keeping pace with selection pressure: the ever-evolving antimicrobial resistance in zoonotic pathogen *Campylobacter*

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Campylobacter is a zoonotic pathogen and a significant concern for One Health. In response to antibiotics used for animal production and human medicine, *Campylobacter* constantly evolves by acquiring new antibiotic resistance mutations and determinants. Our recent work revealed the rapid rise of fluoroquinolone resistance in ruminant (bovine and sheep) *Campylobacter* in the United States, which is mediated by clonal expansion of dominant genotypes and is possibly driven by fluoroquinolone usage. As a result of response to the selection pressure from florfenicol, Campylobacter has acquired a novel cfr variant [*cfr(C)*] that confers resistance to five different classes of antibiotics. Comparative genomics suggested that *Campylobacter* acquired *cfr(C)* from a Gram-positive source. Interestingly, the spread of cfr(C) is also driven by expansion of a predominant *Campylobacter* genotype. Historically, macrolide resistance in *Campylobacter* is mediated by mutations in 23S rRNA and has been relatively low in prevalence. However, *erm(B),* which mediates high-level resistance to macrolides, has recently emerged in *Campylobacter*. The *erm(B)* gene is horizontally transferable between *Campylobacter* species and threatens the utility of macrolide antibiotics. Additionally, a potent multidrug efflux pump variant, named RE-CmeABC, has been recently discovered in *Campylobacter*. RE-CmeABC is much more potent than the typical efflux pump in conferring resistance to multiple antibiotics and mediates exceedingly high-level resistance to fluoroquinolones .These examples illustrate the extraordinary ability of *Campylobacter* to evolve in response to antibiotic selection pressure and underscore the need for innovative measures to curb the development and spread of antibiotic-resistant *Campylobacter*.

Disclosure: Nothing to disclose

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A multicentre survey on ESBL-producing *Escherichia coli* provides no evidence of the human pandemic ST131 clone circulating in food producing animals in Italy, in 2016-2018

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Between 2016 and 2018 508 ESBL-producer *Escherichia coli* from human urine and blood samples were selected by systematic sampling from all isolates of the microbiology laboratories of 12 hospitals in six Italian Regions. These strains were compared to 445 ESBL-producer *E. coli* isolates from bovines, swine and poultry of industrial herds of the same geographical area. ESBL-producer *E. coli* isolates from clinical animals represent an at-risk sampling thus it has to be considered the worst scenario for antimicrobial resistance. The research question was whether food producing animals intensively reared are colonised with ESBL-producer *E. coli* clones affecting humans and circulating in human care units. We compared the phylogenetic group, the MLST type, the beta-lactamases genes and the colistin resistance attributable to *mcr*-1 and *mcr*-2. Results supported current knowledge of human ESBL-producer *E. coli* mostly (393, 77.4%) belonging to B2 phylogenetic group and largely (321, 87.5%) classified within the successfully pandemic ST131 clone, whereas fewer (19, 4.3%) animal isolates were classified in this group. Four B2 ST131 human isolates were *mcr*-1 and bla_{CTX-M1} group carriers. One B2 ST131 poultry isolate carried both *mcr*-1 and bla_{SHV-12}, yet not bla_{CTX-M}. The remaining 36 animal isolates *mcr*-1-carryers belonged to clones other than ST131. So far, in our reference population there is no evidence of food producing animals being colonised by the human ST131 pandemic clone.
Disclosure: Nothing to disclose

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Acuitas Resistome - a rapid molecular typing tool for detection of multidrug resistant Gram-negative bacteria and infection control in Veterinary Hospitals.

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Background: Hospital-acquired infections associated with multidrug-resistant Gram-negative (MDR-GN) bacteria are an emerging concern in veterinary healthcare settings, especially in intensive care units (ICUs).

Methods: To understand the molecular epidemiology of MDR-GN isolates in two veterinary hospitals (Equine and Small Animal Hospitals), we performed a six month pilot study during which faecal and environmental samples were obtained from selected patients admitted to our ICUs, during the first and after 48 hours from admission. In total, 317 MDR-GN were collected and analysed using the Acuitas Resistome Test (OpGen, Denmark) a PCR-based microfluidic array assay which screens for 50 antimicrobial resistance genes, including those encoding production of extended spectrum beta-lactamase (ESBLs), TEM/SHV/OXA or AmpC beta-lactamases and carbapenemases. Combining organism identification and antimicrobial susceptibility data to genotyping results, unique 'Acuitas Lighthouse Profiles' were generated that can be used for typing the isolates and tracking transmission events.

Results: The most prevalent MDR-GNs isolates circulating in both the Small animal and the Equine Hospital consist of *Pseudomonas aeruginosa* and *Enterobacter cloacae* (21.8% each), *Klebsiella pneumoniae* (15%), *Acinetobacter baumannii* (14%) and *Escherichia coli* (12%), all harbouring a combination of genes encoding for beta-lactamases and ESBLs. One important finding is the identification of isolates carrying transmissible resistance to last resort antimicrobials (i.e. carbapenems) within the hospital environments, represented by three *Acinetobacter* spp harbouring bla_{OXA-23} and *E. coli* with bla_{OXA-48}.

Conclusion: The findings from this project will rapidly inform infection control policies in our hospitals to prevent transmission of nosocomial infections associated with these pathogens.

Disclosure: Nothing to disclose

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In vitro horizontal gene transfer in staphylococci: Transduction of *tet(M)* but not *fusB* and *fusC* from MRSP into MSSP

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The emergence of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) as a multidrug-resistant canine pathogen with zoonotic potential has renewed concern over transmission of resistance genes amongst staphylococci.

This study investigated whether the antibiotic resistance genes *tet(M), fusB, fusC*, located on small mobile genetic elements (plasmids or transposons), can be transduced by phage between different *S. pseudintermedius* and *S. aureus* (SA) isolates.

Phage were induced from each donor using UV light. Transduction experiments were performed in triplicate for 101 donor-recipient combinations (Table 1) using sub-inhibitory bottom agar (0.3mg/L tetracycline; 0.03mg/L fusidic acid) and selective top agar (30mg/L tetracycline; 16mg/L fusidic acid). Up to nine transductant colonies were selected from any plate. Successful transduction was confirmed through PCR of transductants (for staphylococcal species-specific *nuc, mecA* and *fusB/fusC/tet(M)*).

In 3/96 *fusB,* 12/153 *fusC* and 6/54 *tet(M)* experiments at least one transductant was recovered and confirmed for the expected species and methicillin-resistance. For *tet(M),* all transductants carried the desired gene, confirming transfer between the MRSP donor and three different MSSP recipients. Transduction was also confirmed for *tet(M)* into the hyper-recipient control isolate MSSA RN4220 from MRSA (COL) as the donor but not from any of the potential *S. pseudintermedius* donors. No transductants were found to carry *fusB* or *fusC.* Growth of *fusB* and *fusC*-negative colonies after transduction experiments may be due to spontaneous chromosomal mutation, as described in SA.

This is the first description of resistance gene transduction between MRSP and MSSP. Although found at low frequency *in vitro*, more frequent occurrence is likely *in vivo*.

Table 1: *Staphylococcus pseudintermedius* and *S. aureus* isolates used for attempting transduction of *fusB, fusC* and *tet(M)*.

Isolates used as	Gene of interest	Bacterial type	Species originally derived from	Number isolates used
Donor	*fusB*	MRSP	Canine	2
	fusC	MRSP	Canine	3
	tet(M)	MRSP	Canine	1
		MRSA	Human	1 (COL)^
Recipient	*fusB*	MSSP	Canine	4
	fusC	MSSP	Canine	5
	tet(M)	MSSP	Canine	7
	fusB/fusC	MRSA	Human	2
	fusB / fusC / tet(M)	MSSA	Canine	3
		MRSA	Human	5
		Restriction-deficient MSSA	Human	1 (RN4220)
		Restriction-deficient MSSA	Human	1 (NE667)

MRSP: methicillin-resistant *Staphylococcus pseudintermedius*; MSSP: methicillin-sensitive *S. pseudintermedius*; MSSA: methicillin-sensitive *S. aureus*; MRSA: methicillin-resistant *S. aureus*.
^Only used for transfer into restriction-deficient MSSA RN4220.

[Table 1]

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Antibiotic residues in drinking water and vegetables-Potential human health risk

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Objective: To assess a primary human health risk of exposure to antibiotic residues via drinking water and vegetables consumption. **Methods:** Environmental samples (drinking water, and edible parts of vegetables) were collected in twelve villages in Shandong province in eastern China. High performance liquid chromatography-tandem mass spectrometry was used to determine the concentration of antibiotic residues. Threshold of Toxicological Concern (TTC) decision tree approach based on Cramer classification will be applied for prior screening and primary risk assessment. Hazard quotients (HQ) for each mixture component $HQ_{i\ toxicological}$ = $Exposure_{individualsubstance}$ (mg/kg body weight per day)/ TTC $value_{individual\ substance}$ (mg/kg body weight per day). Exposure = C * IngR/ BW, TTC values will be obtained by dividing the respective TTC values for the appropriate Cramer class for classes I, II and III by 60 (adult body weight in kilograms). $HI_{mixturedw}$ = HQ_A + HQ_B + HQ_C + HQ_D …+HQ_J. $HI_{mixture\ v}$ = HQ_A + HQ_B + HQ_C + HQ_D …+HQ_J. HI $\geq$ 1 is identifies as a potential risk and higher-tier assessment would be needed. A hazard index ($HI_{mixturedw+v}$) for the mixture in drinking water and vegetables as sum of multi-pathway $HI_{mixturedw+v}$ = $HI_{mixturedw}$ + $HI_{mixturev}$. For the risk of gut microbiota disruption, the methodology will be based on microbiology ADI by using this equation: $ADI_{microbiology}$ = No Observed Adverse Effect Concentration (NOAEC) * Mass of Colon Content (220 g/day)/ Fraction of oral dose x 60 kg person. $Exposure_{dw}$ = C * IngR*EF*ED/ BW*AT. $HQ_{i\ resistance\ development}$ = $Exposure_{dw}$ / $ADI_{microbiology}$..
Disclosure: Nothing to disclose

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Emergence of carriage of CTX-M-15 in faecal *Escherichia coli* in horses at an equine hospital in the UK; increasing prevalence over a decade (2007-2017)

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Background: The global spread of the $bla_{CTX-M-15}$ gene amongst human and animal isolates is widely reported but there are limited reports of $bla_{CTX-M-15}$ in horses. The aims of the study were to investigate the changes over time in the epidemiology of extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* within a single equine referral hospital in the UK. **Methods:** Faecal samples were collected from hospitalised horses in 2007 and 2017 and processed using selective media and standard susceptibility laboratory methods. A novel real-time PCR (RT-PCR) with melt curve analysis was used to distinguish between $bla_{CTX-M-1}$ and $bla_{CTX-M-15}$ within CTX-M-1 group. **Results:** In 2007, 457 faecal samples from 103 horses were collected, with ESBL-producing *E. coli* identified in 131 samples (28.7%, 95% CI 24.6-33.1). In 2017, 314 faecal samples were collected from 74 horses with ESBL-producing *E. coli* identified in 157 samples (50.0%, 95% CI 44.5-55.5) There were 135 and 187 non-duplicate ESBL-producing isolates from 2007 and 2017, respectively. In 2007 only 12.6% of isolates belonged to CTX-M-1 group, all carrying $bla_{CTX-M-1}$, whilst in 2017, 94.1% of isolates were CTX-M-1 group positive and of these 39.2% and 60.8% of isolates carried $bla_{CTX-M-1}$ and $bla_{CTX-M-15}$ respectively. In addition, doxycycline resistance amongst isolates increased from 39.3% in 2007 to 92.0% in 2017. **Conclusions:** RT-PCR proved reliable and cost-effective to distinguish between $bla_{CTX-M-1}$ and $bla_{CTX-M-15}$. Furthermore, its use in this study demonstrated the emergence of faecal carriage of CTX-M-15 in hospitalised horses, with an increase in prevalence of ESBL-producing *E. coli* as well as increased antimicrobial resistance to doxycycline.
Disclosure: Nothing to disclose

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Extended Spectrum β Lactamase -producing *Enterobacteriaceae* (ESBL-E) colonization in hospitalized and healthy farm horses

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Background: Animals, including horses, may serve as zoonotic reservoir for multidrug resistance. We aimed to investigate prevalence, molecular characteristics and risk factors of ESBL-producing *Enterobacteriaceae* (ESBL-E) colonization in different equine cohorts.

Methods: A prospective study (Oct 2015-Sep 2018) was performed sampling three cohorts: (i) on-admission horses in the Koret-School of Veterinary Medicine- Veterinary Teaching Hospital (n=168); (ii) farm horses, originating from 13 different farms (n=192); (iii) Horses hospitalized for ≥72 hours re-sampled from cohort (i) (n=86). Enriched rectal swabs were plated onto CHROMagarESBL plates and ESBL-production was confirmed (EUCAST). Identification and antibiotic susceptibility were determined (Vitek-2). CTX-M ESBL genes were identified (PCR). Medical records and owners' questioners were reviewed for risk factor analysis (SPSS).

Results: ESBL-E colonization rate increased from 20% (n=34/168, 95% CI 14-27%) on admission, to 78% (n=67/86, 95% CI 68-86%) during hospitalization (p< 0.0001). Overall, 145 bacteria were isolated. The main species were *E.coli* (51%, 74/145), *Enterobacter* sp. (19%, 28/145) and *Klebsiella pneumoniae* (15%, 22/145). The main gene was CTX-M-1 (75%). Resistance rates were: Trimethoprim-sulfa-89%, quinolones-25%, gentamicin-75%, amikacin-8%, with no resistance to carbapenems. Within farm horses, colonization rate was 21% (n=40/192, 95% CI 15-27%), with 48 bacteria isolated. The major species was *E. coli* (79%, 38/45) and the major gene- CTX-M-1 (95%). Resistance rates: Trimethoprim-sulfa-90%, quinolones-6%, gentamicin-75%, and 100% susceptibility to amikacin and carbapenems. Risk factors for ESBL-E colonization in farms: Sex (Stallion, OR=4.18), younger-age (OR=0.899), previous hospitalization (OR=1.752) and antibiotic treatment (OR=10.624).

Conclusions: We demonstrated the potential zoonotic reservoir of ESBL-E in equine clinics and farms.

Disclosure: Nothing to disclose

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A comparative exposure assessment of antimicrobial resistance arising from food-producing animals in Canada

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Introduction: Antimicrobial resistance (AMR) transmission routes are complex, and evidence suggests the transfer of resistant bacteria or resistance genes from animals can contribute to AMR in humans. Although it is challenging to determine the exact contribution of AMR from the food chain to human health impacts, policy makers and other stakeholders are interested in identifying the food-producing species that significantly contribute to resistant human infections.

Objectives: To generate a semi-quantitative assessment comparing human exposure to resistant *Salmonella, E. coli*, *Enterococcus*, and *Campylobacter* spp. from 13 domestically produced food animals in Canada.

Aims: To provide evidence-based recommendations to prioritize areas for future surveillance, research and mitigation efforts in Canada.

Methods: Food animals were selected based on the quantity consumed and produced in Canada. Scientific and social criteria were developed, weighted, and scored to rank the species. Scientific criteria included antimicrobial use, frequency of recovery and resistance of the bacterial species, human consumption rates, and projections for AMR and consumption trends. The social criteria included public perception, and stakeholder perception and will. The results provide a summary of the weighted scientific and social criteria for each of the selected food-producing species.

Results: Preliminary results indicate chicken, turkeys, and pigs were ranked highest in terms of human exposure to resistant bacteria. These species also had the fewest data gaps, suggesting that research is lacking in other food-producing species.

Conclusions: This assessment provides rapid and transparent comparisons for prioritizing future public health efforts, and also highlights important data gaps in the AMR literature.
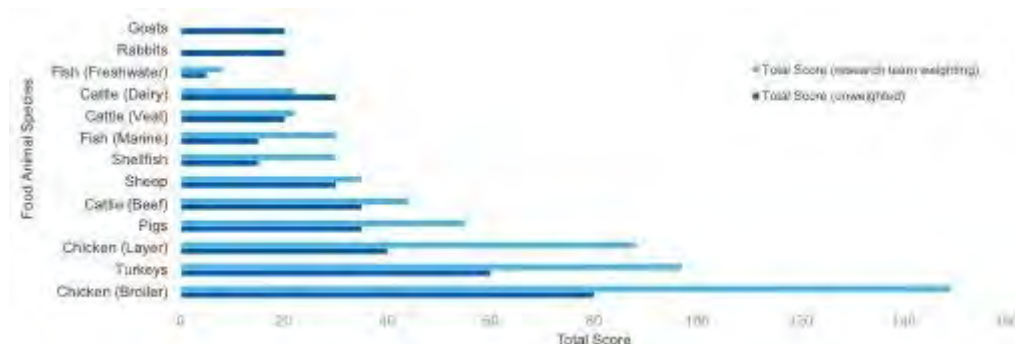
[Figure 1: The preliminary ranking of food animal species' contribution to AMR in humans in Canada.]

Disclosure: Nothing to disclose

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SODAPOP: A Mnemonic Tool for the Selection of Antimicrobial Therapy

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Antimicrobial resistance in bacterial pathogens has dictated a need for veterinarians to practice "antimicrobial stewardship." Veterinary educators lack effective tools to teach stewardship but mnemonic tools have been shown to be effective tools to teach complex processes. SODAPOP is a tool designed to integrate case-based learning of antimicrobial selection into the veterinary curriculum. SODAPOP suggests that students consider the **s**ource and **o**rganism before they **d**ecide to treat. Susceptible **a**ntimicrobials are considered with regard to **p**atient contraindications. The **o**ptions are weighed and a **p**lan formulated. To evaluate the efficacy of SODAPOP, an intervention study was performed with veterinary students during a clinical rotation. A pre-survey evaluated (1) perceived barriers to antimicrobial selection, (2) confidence in antimicrobial selection and (3) quality of antimicrobial choice and plan. Participants then viewed an instructional video on the concept of SODAPOP. The post-survey re-evaluated (1) confidence in antimicrobial selection, (2) views on SODAPOP as a tool and (3) quality of antimicrobial choice and plan. The top factor identified as a barrier to antimicrobial selection was lack of knowledge about distribution and tissue penetration of drugs (86%, 26/30). Pre-intervention the weighted (0= no confidence, 4= very confident), average confidence levels were 1.73, 2.16 and 1.5 for urinary tract, skin and respiratory infections respectively. Post-intervention these levels increased 2.23, 2.63 and 2.13 respectively (p< .001). 28/30 (93%) of students agreed or strongly agreed that SODAPOP was a useful tool. This study showed that SODAPOP is a valuable tool and proposes an approach for integration into the veterinary curriculum.
Disclosure: Nothing to disclose

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Plasmid similarities indicate a genetic link between ESBL in livestock and the general Dutch population: A Whole Genome Sequencing story

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The prevalence of carriage of Extended Spectrum β-Lactamase and AmpC β-lactamase producing *Escherichia coli* (ESBL-E) in the general Dutch population is approximately 5%. Over the past years, transmission of ESBL-E between animals and humans via direct contact has been reported. Moreover, transmission via the food chain was suggested. The contribution of livestock to ESBL-E carriership and human infections, however, remains unclear. To get a better understanding of this contribution 310 ESBL-E isolates obtained from livestock (broilers, laying hens, pigs, and goats), farmers and individuals from the general Dutch population were sequenced and analysed. Clonal as well as horizontal gene transfer through plasmids was studied.

Isolates selected had either a $bla_{CTX-M-1}$ (189), bla_{CMY-2} (96), bla_{SHV-12} (19), or bla_{TEM-52} (6) gene. Whole genome sequencing (WGS) was performed. Plasmids were reconstructed and assigned to a plasmid (sub)type. Genes of plasmids with the same (sub)type were compared to establish plasmid similarities. More than 60% of the $bla_{CTX-M-1}$ carrying plasmids belonged to IncI1-ST3 obtained from different hosts (all hosts except goats), which confirms the important role of ST3 in the dissemination of this ESBL gene. Furthermore, the analysis suggests that ESBL-gene carrying plasmids were highly similar between isolates obtained from different farms, different farm animals and farmers, but also between farmers and individuals in the population at large, and more importantly, livestock and humans in the community at large. When investigating possible transmission events of ESBL-E it is important to study horizontal plasmid transmission in addition to *E. coli* clonal transmission.

Disclosure: Nothing to disclose

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Correlation Between Antimicrobial Use and the Isolation of Antimicrobial Resistant Non-Uropathogenic *Escherichia coli* Strains from Companion Animals

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The importance of antimicrobial resistant (AR) *E. coli* urinary tract infections (UTIs) in dogs and cats cannot be understated. We previously described the correlation between phylogroup, virulence gene association and patient clinical characteristics of *E. coli* isolates from UTIs and the majority (66.6%) were not classified as uropathogenic *E. coli* (UPEC). The aim of this study was to understand the association between clinical characteristics, *E. coli* pathotype and AR. AR was compared to the measured clinical characteristics and was positively associated with current antimicrobial therapy ($p < 0.0005$); previous antimicrobial therapy ($p < 0.0005$) or previous hospitalization ($p = 0.034$) within the three months prior to the positive culture. AR was compared to *E. coli* pathotype and UPEC was negatively associated with the presence of AR ($p = 0.034$) and the presence of one or more AR genes ($p = 0.001$). A lack of pathotype identification was positively associated with the presence of AR ($p < 0.0005$) and with the presence of one or more AR genes ($p < 0.0005$). The results identified some preliminary associations between pathotype, AR and certain clinical characteristics, and include some associations of concern between administration of antimicrobials, hospitalization, and the presence of AR genes. UPEC isolates were negatively associated with previous antimicrobial therapy and the presence AR, yet current and previous antimicrobial therapy were both positively associated with the presence of AR. This was not necessarily unanticipated, but strongly emphasizes the association between the use of antimicrobials and the isolation of antimicrobial resistant ExPEC, non-UPEC, strains from animals currently, or recently, exposed to antimicrobials.

Disclosure: Nothing to disclose

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Comparison of genotypic characteristics of ESBL-producing E. coli from animals and patients

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Introduction: The main issue of current antimicrobial resistance(AMR) management is surveillance and management of "One Health" concept. ESBL producing E. coli is frequently found in animals and humans, and is a threat to public health due to limited selection of therapeutic antimicrobials.

Objectives: In this study, we analyzed the share and distribution of AMR genes of ESBL E. coli from patients and animals to present the scientific basis of AMR bacteria control.

Aims: Genetic analysis of ESBL E. coli isolated from humans and animals has been used to compare the share of AMR genes and host-specific genes.

Methods: We compared E. coli with ESBL related genes from animals and patients. Specimens of livestock and companion animals were analyzed by comparing 140 pig-derived strains, 97 dogs and cats-derived strains, and 627 strains isolated from patient's blood specimens.

Results: The CTX-M-1 group and the CTX-M-9 group were analyzed 123 strains and 113 strains, respectively, of 236 livestock and companion animals. Subtypes of major bla genes were distributed in the order of CTX-M-55, CTX-M-14, CTX-M-15. These were analyzed as 36.7%, 29.7% and 12.3%, respectively. The subtype of the major bla genes in the patient-derived strains were CTX-M-15, CTX-M-14 and CTX-M-27. These were analyzed as 35.2%, 20.4% and 11.1%, respectively.

Conclusions: This analysis confirmed the distribution pattern of AMR gene and host-specific AMR genes between livestock and companion animals. These results indicate the importance of public health surveillance of ESBL E. coli and require continued surveillance and research.

Disclosure: Nothing to disclose

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Antibacterial resistant bacteria associated to larval culture of the red cusk eel *Genypterus chilensis* fed with untreated and treated live feed

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The use of antibiotics to treat live feed used in fish larval culture of red cusk eel *Genypterus chilensis* is frequent due to low larval survival rates mainly because of bacterial infections.

The main aim of the study was to evaluate the occurrence of antimicrobial resistant bacteria in a commercial culture of *G. chilensis* larvae. Samples of red cusk eel larvae fed with untreated and florfenicol-treated (20 µg/mL during 2 h) rotifer and *Artemia* cultures, currently used as live feed in a commercial hatchery located in northern Chile were collected at 6, 18 and 32 d of culture. Total and antibacterial-resistant culturable counts of reared larvae were performed by a spread plate method using Plate count agar added with 2% NaCl alone and containing 30 µg/mL of florfenicol or oxytetracycline, respectively. Larval cultures exhibited high levels of total culturable counts and *Vibrio* spp. along the sampled period ($7.70 \times 10^5 \pm 4.92 \times 10^5$ CFU/g to $2.17 \times 10^7 \pm 1.19 \, 10^7 \times$CFU/g, and $5.08 \times 10^5 \pm 2.28 \times 10^5$ CFU/g to $5.46 \times 10^6 \pm 1.84 \times 10^6$ CFU/g, respectively). Percentages of resistance to florfenicol and oxytetracycline ranged from 5.48% to 16.27% and 11.18% to 20.23%, respectively. The high prevalence of antibiotic resistant bacteria in reared fish larvae prompts the need of developing proper management strategies to prevent future drug therapy failures, as well as results suggest that administered antibiotic therapy is not efficient to significantly reduce the levels of vibrios after 32 d of larval culture (Study supported by grant 1171772 of CONICYT, Chile).

Disclosure: Nothing to disclose

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Conceptualising Antimicrobial Resistance Surveillance Systems

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Introduction: Antimicrobial resistance (AMR) surveillance is the keystone of efforts to identify, monitor, and respond to emerging AMR threats. Worldwide, innumerable surveillance systems are in use, ranging from local to global. Many systems focus solely on humans whereas others integrate One Health attributes. With their widespread and increasing use, there is value in assessing the core concepts that underpin AMR surveillance systems.

Objectives: The objective of this assessment was to examine a broad range of approaches to AMR surveillance, and identify common philosophical elements that underscore their application.

Methods: Approaches towards AMR surveillance were considered with visits to numerous centres involved in AMR surveillance located across North America and Europe. Centres visited included intergovernmental organizations, government organizations, and non-profit organizations. AMR surveillance was considered across the local through global context. A One Health approach was utilised that considered the human, animal, agricultural and environmental components of surveillance. Approaches utilised in resource wealthy versus resource constrained locations were also considered.

Results: Descriptors that capture key philosophical elements of AMR surveillance include being: accessible, actionable, adaptable, archivable, attainable, comparable, contextual, defined, descriptive, distributed, equitable, ethical, evaluable, fail-safe, harmonic, independent, integrated, interconnected, interoperable, maintainable, manageable, measurable, perceptive, positive-sum, predictive, prescriptive, regulated, representative, responsive, scalable, secure, structured, sustainable, tangible, transparent, unbiased, and, in summation, useful.

Conclusions: This analysis establishes a broad-based framework for conceptualising elements underpinning AMR surveillance systems. There is value in further work exploring how these theoretical underpinnings structurally translate into the practicalities of ideal and functioning surveillance systems.

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Surveillance of antibiotic prescribing by informal healthcare providers: A "missing link" in one-health approach for antibiotic stewardship in rural India

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Introduction: In low and high middle income countries, areas with weak healthcare system are predominantly served by the informal healthcare providers (IHCPs). IHCPs constitute the majority of active healthcare providers in rural India; prescribe allopathic medicines, including antibiotics, without formal training. Surveillance of antibiotic prescription practices of IHCPs can provide crucial information in one-health approach.

Aim: To explore the antibiotic prescription patterns of IHCPs for common illnesses in rural Ujjain, India, by repeated follow-ups.

Method: A repeated cross-sectional study. Prescriptions to outpatients by IHCPs were collected over 18 months (April 2014-September 2016) for six seasons (2-pre-monsoons, 2-monsoons, 1-post-monsoon, 1-winter), in customized prescription pads provided to them.

Results: A total 15322 prescriptions for 323 different combinations of presenting complaints were analysed. Overall, 74% prescriptions contained antibiotics. Total 11336 prescriptions contained 15472 antibiotics prescribed either singly or upto five antibiotics. Antibiotics were prescribed more frequently to children-81% than to adults-71% (odds ratio-2.20, 95% confidence interval-1.95 to 2.49; p< 0.001) and during the monsoon (76%). Antibiotic prescribing for presenting complaints analysed was: injuries-89%, oral and dental problems-88%, fever-87%, upper respiratory tract infections-81%, skin infection-79%, gastro-intestinal disorders-60% and unspecified pain-30%. Fluoroquinolones (ofloxacin) and third-generation cephalosporin (cefotaxime) were the commonly prescribed antibiotic class.

Conclusions: Study results reveal high, unindicated and inappropriate antibiotic prescribing for common illnesses in children and adults, mostly broad-spectrum antibiotic prescribing, that warrants immediate and coordinated efforts to reduce unnecessary antibiotic prescriptions by IHCPs and therefore forms an essential missing link in one-health approach for antibiotic stewardship.
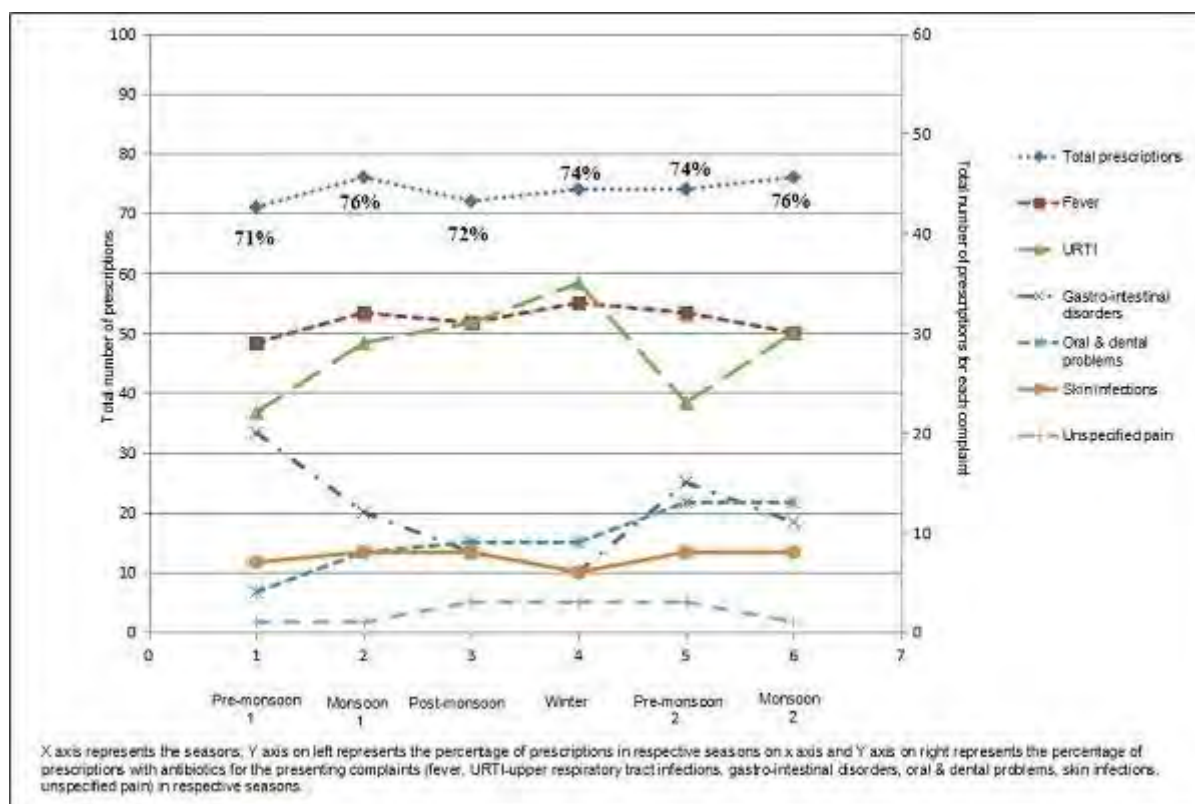
[Relative distribution of % of total antibiotic prescriptions by season & presenting complaints]

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Temperature-associated trend of the antibiotic resistance of bacteria
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Seasonality was demonstrated for gram-negatives infections in bloodstream diseases, peaking in the summer correlated with increasing temperatures. On the other hand, it has been reported that resistance rate of gram-positive infection is lowered when the temperature increases. To date there has been no investigation whether it is seasonality of antibiotic resistance in both community and hospital-associated cases. In this study, we analyzed the trend of antimicrobial resistance of bacteria (*S. aureus*, *E. coli* and *K. pneumoniae*) isolated from bloodstream on a temperatures.
From May 2016 to December 2017, we collected blood isolates from the eight general hospitals participating in the Korea-Global Antimicrobial Resistance Surveillance System. Antimicrobial susceptibility test was performed according to CLSI guideline. We confirmed Class A, B, D ß-lactamase and Extended-spectrum ß-lactamase (ESBL) by PCR and sequencing.
A total of 5,109 blood isolates (1,106 *S. aureus*, 2,884 of *E. coli* and 1,119 of *K. pneumoniae*) was collected and community-acquired (CA) were more than twice as much as hospital-associated (HA). In this study no significant seasonal variations were observed for the isolation rates of bacteria in HA. In the case of cefoxitin, HA-*S. aureus* showed no difference in temperature, but in CA, the resistance rate was decreased with increasing temperature. Gram-negative bacteria were increased in both HA and CA as the temperature increased. ESBLs producing gram-negative bacteria were also increased with increasing temperature.
Although there are some other mechanisms that support the association between resistance and temperature, differences in temperature-associated resistance rates in CA suggest a link between them.
Disclosure: Nothing to disclose

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Patients with sore throat look for advice rather than antibiotics: a survey across 13 countries

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Introduction: Sore throat (pharyngitis) is typically a self-limiting condition where antibiotics can be prescribed inappropriately due to uncertainty about patient need, perceived patient pressure, and diagnostic uncertainty.

Objectives: To evaluate the behaviour, experience, and needs of people with sore throat and their reasons for visiting a healthcare professional (HCP).

Aims: This insight from multiple countries can inform doctors and could help improve their prescribing decisions.

Methods: 5196 adults from 13 countries (~400 per country) who had experienced sore throat in the previous year voluntarily completed a questionnaire on their experience of sore throat, contact with HCPs, and treatment practices.

Results: A notable proportion of patients sought HCP advice for sore throat with 30% contacting a general practitioner and 14% a specialist doctor/consultant. The most common reasons for visiting a doctor were to learn about treatment options (90%), gain an explanation of how serious the problem was (87%), identify the cause (85%), get pain relief (83%), and learn likely recovery times (83%). 55% of patients visited the doctor to seek antibiotics, but this varied widely between countries. Antibiotics were the third most common main treatment for sore throat (12% overall), ranging from 5% in the UK to 21% in Saudi Arabia.

Conclusions: The main reasons for patients with sore throat consulting doctors relate to the cause of sore throat, advice on treatments, pain relief, and reassurance, whereas desire for antibiotics was rated much lower. Doctors could use this information to educate on appropriate symptomatic relief and improve their antibiotic prescribing behaviour.

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Genomic signatures associated with methicillin-resistant *Staphylococcus pseudintermedius* lineage ST-71

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Staphylococcus pseudintermedius is a common cause of opportunistic canine skin and mucosal infections. Multidrug resistant (MDR) and methicillin-resistant *S. pseudintermedius* (MRSP) lineages, such as ST-71, have disseminated globally in the last decade and present significant treatment challenges. Furthermore, the treatment of MRSP infections in animals carrying methicillin-resistant *Staphylococcus aureus* (MRSA) may give rise to additional transferable resistances that compromise treatment efficacy of MRSA in humans. The aim of this study was to elucidate the genetic basis for the success of ST-71 as an opportunistic pathogenic lineage, beyond the acquisition of antimicrobial resistance (AMR) genes.

Preliminary analysis of *S. pseudintermedius* isolates from a UK referral vet practice demonstrated that the majority of isolates belonged to ST-71 clonal complex. Analysis of the accessory genomes of these isolates and 138 publically available *S. pseudintermedius* genome sequences using t-distributed stochastic neighbour modelling revealed a distinct cluster of genomes that show considerable divergence in accessory genome content (**Figure 1**). This cluster comprised almost exclusively of ST-71 genomes. Using a genome-wide association study we have identified genetic features associated with this ST-71 cluster, that potentially engender the success of this lineage.

These findings will enable the development of a rapid test that could be used for the detection of MDR-MRSP genotypes from clinical samples. This will facilitate more pragmatic treatment of canine soft tissue infections, thereby reducing a significant reservoir of AMR genes with potential to disseminate into *S. aureus* lineages causing human disease.

PANINI plot of the accessory genome content of 137 *S. pseudintermedius* genomes based on a tSNE plot and constructed using Microreact (https://microreact.org/project/rkZW3HE1V).

ST-71

The plot is a 2D diagrammatical representation of the relatedness of each strain based on presence/absence of accessory genes.

This dendrogram is created using the binary presence and absence of accessory genes file generated by Roary (https://sanger-pathogens.github.io/Roary/).

ST-71

Figure 1: Divergence in accessory gene content of *S. pseudintermedius* ST-71 visualised using T-distributed Stochastic Neighbour Embedding generated using PANINI.

[Figure 1: Divergence in accessory genome content of ST-71]

Disclosure: Nothing to disclose

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Survey on ESBL-producing *Escherichia coli* in Italian dogs

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Colistin-resistant *Escherichia coli* carrying extended-spectrum β-lactamases (ESBL-producing *E. coli*) pose a serious risk of therapy failure in humans and strict surveillance of animal ESBL-producing *E. coli* reservoirs is of importance for risk mitigation. Close cohabitation of humans with pets favors transmission of *E. coli* from dogs to humans and vice versa.

Aim of our study was to describe the occurrence of β-lactamases encoding genes (*bla*) and mobile colistin resistance genes (*mcr*) in *E.coli* isolates from dogs. In 2016-2017, 303 dogs were selected by systematic sampling among carcasses or faecal samples submitted to the Istituto Zooprofilattico Sperimentale delle Venezie's laboratories. Presumptive ESBL-producing *E. coli* were detected by cefotaxime selective media, identified by MALDI-TOF MS and ESBL-production was confirmed by antimicrobial susceptibility testing (broth microdilution).

One isolate per dog was further investigated for ESBL, AmpC and colistin resistance genes and classified according to the phylogenetic group and multilocus sequence type (ST). Among selected dogs 15% (45/303) harbored ESBL-producing *E. coli*. No carbapenem-resistant isolates were detected. 28% of *E. coli* isolates displayed several associations of *bla* types (Table 1). Almost 20% of dog isolates belonged to B2 and D phylogenetic groups, which are the most represented in human ESBL-producing *E. coli*. One isolate carrying $bla_{CTX-M-15}$ and displaying fluoroquinolones resistance, yet colistin susceptible, belonged to the human pandemic clone ST131 O25:H4. Three isolates carried *mcr-1*. 14/45 strains (31.1%) were multidrug resistant, the extended-spectrum cephalosporins/quinolones/sulfonamides/trimethoprim pattern mostly detected (five isolates). Our results highlight companion dog harboring *E. coli* clones that may threaten human health.

Phylogenetic group	Number of isolates (%)	Sequence type	β-lactamases types (number of isolates)
A	15 (33.3)		CTX-M-15/TEM-1 (7) TEM-1/CMY-2 (3) TEM-1 (2) SHV-12 (1) CMY-2 (1) CTX-M-1 (1)
B1	15 (33.3)		CTX-M-15 (6) CMY-2 (3) SHV-12 (2) TEM-1 (1) CTX-M-15/TEM-1 (1) SHV-12/TEM-1 (1) CTX-M-1/SHV-12 (1)
B2	2 (4.4)	28, 131	CTX-M-15 (2)
B2	1 (2.2)	12	CMY-2 (1)
B2	1 (2.2)	429	TEM-1/CMY-2 (1)
C	2 (4.4)		CTX-M-15 (1) CMY-2 (1)
D	5 (11.1)		CTX-M-15 (4) TEM-1(1)
E	2 (4.4)		CTX-M-15 (1) TEM-1/CMY-2 (1)
F	2 (4.4)		CTX-M-15 (1) CTX-M-15/TEM-1 (1)

[Table 1: Phylogenetic groups, sequence types (ST), β-lactamases types detected in the 45 ESBL-producing Escherichia coli isolated from Italian dogs]

Disclosure: Nothing to disclose

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Using a One Health approach to unravel *Mycobacterium leprae* transmission

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Despite decades of availability of adequate treatment for leprosy, *Mycobacterium leprae* transmission is unabated as shown by the stable number of new cases over the last decade. To investigate potential routes of transmission, we determined the presence of *M. leprae* in: i) leprosy patients, ii) their household contacts (HC), iii) soil samples, iv) red squirrels, v) armadillos.
M. leprae DNA was isolated from slit skin smears (SSS) and nasal swabs (NS) of index cases and their associated HC. For all isolation sources, presence of *M. leprae* DNA was determined by RLEP PCR/qPCR. Genotype and antimicrobial resistance (AMR) was determined by Sanger sequencing or whole genome sequencing (WGS).
M. leprae was identified in SSS and NS of leprosy patients as well as healthy HC. Presence of *M. leprae* in HC is higher in NS (~29% positivity) than SSS (~19% positivity) whilst in patients the percentage of RLEP PCR positivity is higher in SSS. *M. leprae* was also present in 16.0% of soil from houses of leprosy patients (Bangladesh), in 10.7% of soil from armadillos' holes (Suriname) and in 5% of soil from the habitat of lepromatous red squirrels (British Isles). In tissue form squirrels from Belgium and The Netherlands as well as armadillos from Suriname, *M. leprae* was not detected. Importantly, a new subtype of *M. leprae* was identified in patients from Bangladesh which is related to subtype 1A. Genotype 1D was also identified in Bangladesh. Currently, in our sample set AMR to dapsone, rifampicin or ofloxacin has not been identified.
Disclosure: Nothing to disclose

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Effect of sub-minimal inhibitory concentrations of fluoroquinolones on bacterial antibiotic resistance development and mutagenesis: a systematic literature review

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Background: Fluoroquinolones are among the most widely used classes of antibiotics in both the human and veterinary sector. Studies show that while fluoroquinolone resistance is increasing, its drivers are not fully understood. Substandard fluoroquinolones (poor quality drugs often containing sub-therapeutic doses) are likely to contribute to antimicrobial resistance (AMR) acquisition and its spread; however, this relationship remains poorly understood.
Aim: To understand the potential effects of medicine quality on AMR, we performed a systematic literature review to synthesize evidence on AMR development and mutagenesis in bacteria following exposure to sub-standard and sub-minimal inhibitory concentrations of fluoroquinolones.
Methods: Following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), we searched primary literature and reviews in English between 1975 and 2018 using PubMed and established keywords. Study selection was performed independently by two reviewers. Data extracted included bacteria analyzed, drug and concentrations, treatment conditions, key findings and study bias risk.
Results: Forty-six papers were relevant. The most studied fluoroquinolone and bacteria were ciprofloxacin and *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus,* respectively. Over half (27/46) of the publications reported an experimental link between sub-lethal drug levels and AMR acquisition among clinical and environmental bacterial isolates.
Conclusions: The data implicates changes in selective pressure, mutation rate and different stress responses as drivers of AMR. The data also identifies gaps in knowledge regarding medicine quality and AMR. As the prevalence of substandard medicines and AMR increases, future studies which directly test the impacts of different aspects of medicine quality on AMR are needed.
Disclosure: Nothing to disclose

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Antimicrobial resistance prevalence in young harbour seals (*Phoca vitulina*) stranded in the Netherlands and antibiotic treatment effect on their gut microbiome during subsequent rehabilitation

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Every year young sick harbour seals (*Phoca vitulina*) with critical health status are admitted for rehabilitation at the Sealcentre Pieterburen.
In this study, we aimed to investigate the prevalence of antimicrobial resistance (AMR) in bacteria isolated from the rectum of harbour seals admitted to the Sealcentre. Moreover, we analyzed the gut microbiome composition of the seals before and during rehabilitation to reveal the influence of antibiotic (AB) therapy and the rehabilitation process on their commensal gut flora.
During summer 2015 and winter 2015-2016, rectal swabs were collected from 200 harbour seals at admission, during rehabilitation and before release. If the seal received AB treatment, samples were taken before and after treatment. The swabs collected at admission were streaked onto different selective media to screen for clinically relevant AMR bacteria. From all swabs collected, DNA was isolated and amplicon sequencing was performed using Illumina Miseq 2x300bp on 450 bp of the 16S V3-V4 region. Reads were analyzed using Mothur. α- and β-diversity were determined using Shannon and Unifrac, respectively. Whole genome sequencing was applied to AMR bacteria isolated from seals to compare their genetic relatedness with those found in humans and to identify AMR genes.
We observed a low prevalence of ESBL-producing *E. coli* (2%) and MRSA (0.5%) in stranded young harbor seals. The isolated bacterial strains are closely related to those found in humans and livestock and contain the same resistance genes. The effect of AB treatment on the seal gut microbiome is extensive, however it is transient.
Disclosure: Nothing to disclose

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Understanding the perception and knowledge on antimicrobial usage and antimicrobial resistance among pet owners in Selangor, Malaysia

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The emergence of antimicrobial-resistant organisms isolated from companion animals (i.e., dogs, cats, and "pocket pets") has resulted to subsequent implications for public health and use of veterinary drugs is suspected to be one of the major drive in the development of antimicrobial resistance (AMR). This survey involving pet owners in Selangor, Malaysia was conducted to assess their level of knowledge and perception towards antimicrobial usage in pets and AMR. A questionnaire comprised of five sections: the demographic characteristics, general management, assessment on knowledge and perception on antimicrobial usage and AMR was developed and distributed to a total of 90 pet owners. This study revealed that majority of the respondents had sufficient knowledge on certain aspects of antimicrobial usage in pets with 99% (34/90) of them adhered to veterinarian's direction following antimicrobial prescription. Although statistically insignificant, higher proportion of respondents (48%; 43/90) stopped antimicrobial treatment when the pet's condition improves. They believed proper explanation on antimicrobial usage by veterinarian is important (84%; 76/90) and more than 70% of them agreed that AMR occurred when antimicrobials were no longer work in treating sick animals. There was a significant association for both age and education status ($p < 0.05$) with their level of perception towards AMR. Though majority of pet owners had fair knowledge and perception on the issue, continuous education and awareness should be provided to increase their knowledge and understand the importance of their roles as pet owners in order to reduce the occurrence of AMR amongst pets and pet owners.

Disclosure: Nothing to disclose

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Genetic basis for tetracycline resistance in *Campylobacter jejuni* in broilers and turkeys in Italy

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Campylobacteriosis has been the most reported zoonosis causing bacterial gastroenteritis in Europe since 2005. The main reservoir of *Campylobacter jejuni* are poultry. The prevalence of *C. jejuni* resistant to tetracycline in Italy in 2016 was 72.1% and 68.7% in broiler chicken and turkeys, respectively, according to the EU Harmonised AMR Monitoring. The aim of this study was to determine the genetic basis of tetracycline resistance in *C. jejuni* in Italy.

53 *C. jejuni,* phenotypically resistant to tetracycline (MIC> 1mg/L), isolated in the frame of National Monitoring activities (Decision 2013/652/EU) in 2016 from caecal content samples from different epidemiological units of poultry flocks (n=34 broiler chickens, n=19 fattening turkeys), were Whole Genome Sequenced using Illumina technology. "*De novo*" assembly was performed using *SPAdes. Accessory genes and chromosomal mutations conferring resistance were determined using ResFinder 3.0, and MLST and cgMLST, using pubMLST.org.*

Two different genes encoding resistance to tetracycline were found: 84.9% (45/53) presented *tet*(O) (10 different variants, according to the non-silent combinations of mutations, which ranged from 5 to 9), and 15% (8/53) harboured a mosaic gene, designated *tet*(O/32/O).

Preliminary results indicated the presence of a rather homogeneous *C. jejuni* population in Italian broilers and turkeys, since 60.3% (32/53) of them belonged to the same ST (ST-2863) and to four cgSTs (cgST-3066; cgST-11302; cgST-19645; cgST-19772). All of them presented *tet*(O), but with different mutations.

This study is the first description of the mosaic *tet*(O/32/O) gene and of potential important variants of *tet*(O) in *C. jejuni*.

Disclosure: Nothing to disclose

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Monitoring antimicrobial resistance: understanding the mechanisms and drivers of antimicrobial resistance as an integrated One Health approach

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Antimicrobial resistance is an important health problem in both hospital and community acquired infections. However, resistant organisms exist in water, soil, plants and animals, and can be either part of the normal microbial populations or they can be the result of contamination by anthropogenic sources. Antibiotics are released into the environment and, consequently, increased concentration of antibiotics raises the diversity and the abundance of resistance genes. Research has been conducted in order to understand how the environmental resistome intersects with the resistome of nosocomial bacteria. Major efforts have been made in order to better understand the development, dissemination and persistence of antimicrobial drug resistance. Addressing the rising threat of antimicrobial resistance requires a holistic and multisectoral approach because antimicrobials used to treat various infectious diseases in animals may be the same or similar to those used for humans. Therefore, the assessment of antimicrobial resistance needs to take into account the roles of people, animals and the environment in the emergence, spread and persistence of antimicrobial resistance genes. Monitoring the prevalence of resistance in indicator bacteria in different populations makes it possible to compare the prevalence of resistance and to detect the transference of resistance genes from animals to humans and vice-versa. The frequency of antibiotic resistant bacteria has been shown to be directly proportional to the use of antibiotics. Therefore, integrated approaches to reduce selection pressure and stop antimicrobial resistance transmission on a global scale must be in accordance with the One Health principles and also based on economic evidence.

Disclosure: Nothing to disclose

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Occurrence antimicrobial resistance (AMR) of *Salmonella* and *Campylobacter* in humans and broiler chicken

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Introduction: Foodborne infections represent a significant public health burden. Moreover, antimicrobial resistance (AMR) in *Salmonella* and *Campylobacter* is a growing problem, which is linked to antimicrobial use in food animals. We aimed to get insight on the occurrence and AMR of *Salmonella* and *Campylobacter* isolated from humans with diarrhoea and broiler chicken in Albania as such data can help inform national policymaking on food safety and AMR.
Methods: We conducted a survey during October - February 2016. We included a total of 200 intestinal samples from healthy broiler chicken and 200 samples from human patients with acute diarrhoea.
Results: *Salmonella* and *Campylobacter* were isolated from the diarrhoeal disease cases, and were the etiological agents in 7% and 5% of the cases, respectively. Of the broiler chicken samples, 13% were positive for *Salmonella* and 45% for *Campylobacter*. We observed a high level of multiresistance among the *Salmonella* isolates: 58% of isolates from broiler chicken were resistant to four antimicrobial classes. The *Campylobacter* isolates from both humans and broiler chicken 30% and 23.3%, respectively were resistant to fluoroquinolones. Antibiograms for the *Campylobacter* isolates from humans and broiler chicken showed comparable patterns.
Conclusion: Both *Salmonella* and *Campylobacter* seem to be important causes of diarrhoeal disease among humans in Albania, and broiler chicken seems to be a contributing source of infection. The level of AMR seems high among *Campylobacter* and *Salmonella* from both broiler chicken and humans, which may partly reflect the use of antimicrobial agents in the poultry industry in Albania.
Disclosure: No conflict of interest

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Methicillin-Resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* Circulating in dogs, Bangladesh

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We conducted a cross-sectional study to determine the prevalence and risk factor (s) of *S. aureus, S. pseudintermedius,* MRSA*,* and MRSP in dogs. Total 358 swab samples were collected from different body sites of 150 dogs admitted to a university teaching hospital. Standard bacteriological methods were followed for the isolation and identification of *S. aureus* and *S. pseudintermedius,* which was further verified through characterizing *nuc* and *pse* genes, respectively. The isolates were tested for the susceptibility to a panel of 14 antimicrobials. Isolates displayed resistance to cefoxitin and oxacillin were screened for the presence of the *mecA* gene to identify MRSA and MRSP. The prevalence of *S. aureus* and *S. psudintermedius* in dogs were 16% (95% confidence interval (CI), 11-23%) and 49% (95% CI, 41-57%), respectively. Notably, all staphylococcal isolates showed resistance to ≥3 classes of antimicrobials (multi-drug resistant; MDR). The prevalence of MRSA and MRSP was 46% (95% CI, 30-64%) and 8% (95% CI, 4-15%), respectively. Dogs with dermatitis (odds ratio [OR], 12.24; 95% CI, 3.12 to 57.33; P < 0.001) and with the history of antibiotic use (OR 8.73; 95% CI, 2.23 to 43.10; P < 0.001) more frequently carried MRSA while the presence of otitis (OR 14.22; 95% CI, 1.64 to 103.58; P < 0.008) and oral lesions (OR 9.48; 95% CI, 1.14 to 64.82; P< 0.002) were identified as the significant risk factors for the carriage of MRSP in dogs. To our knowledge, this is the first report of MRSA and MRSP in dogs in Bangladesh.
Disclosure: Nothing to disclose

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Transmission of *Staphylococcus pseudintermedius* (SP) to human oncology patients from family pets.

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Background: SP is a well known dog pathogen and colonizes dog skin and mucosal surfaces. SP has been recovered from human patients but a clear link to the source has not always been established. We report on 3 human oncology patients with SP infections and compare strains to SP collected from family pets.

Materials and **Methods:** SP isolates identified in the clinical diagnostic laboratory were used to identify patients following which permission and ethics approval was received to collect specimens from family pets. Human specimens were blood, catheter tip, abdominal drainage and hemocath exit site. Specimens from pets (dogs and cats) were collected from the oral cavity and rectum. SP was identified by Matrix assisted laser desorption ionization -time of flight (MALDI-TOF) and Vitek® II. Susceptibility testing was by Vitek II and confirmed by broth micro dilution. Strain comparisons were by antibiograms, MALDI-TOF spectrogram, pulsed field gel electrophoresis and 16s ribosomal sequencing.

Results: One pediatric patient was bacteremic and two adult patients had cathether associated infections - one with positive cultures 16 days apart and the other 33 and 48 days apart. All strains were methicillin-susceptible. Isolates from humans and their pets had identical antibiograms and MALDI-TOF spectrograms. The human-pet paired isolates analyzed by PFGE and 16 s ribosomal sequencing were identical.

Conclusions: We reported on SP infections in human oncology patients including bacteremia, catheter-associated and persistent infections with human SP strains identical to family pet strains. Caution with family pets may be warranted for oncology patients.

Disclosure: Nothing to disclose

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Veterinary antimicrobial resistance containment in Bangladesh: A scoping review of policy, regulatory and practice gaps

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Introduction: The World Health Organization's Global Action Plan (GAP) on Antimicrobial Resistance (AMR) provides guidance to countries on AMR containment using a One-health approach encompassing humans, animals, plants, and the environment. With 164 million people and 404 million terrestrial animals on a landmass of 147 000 square kilometers subject to flooding, Bangladesh has a high communicable disease burden with AMR being a major and growing concern. The aim of this scoping review is to identify current policies and regulations outlined in the Bangladesh's 2017 National Action Plan (BNAP) on AMR containment and corresponding evidence on their implementation.

Methodology: The BNAP was benchmarked against GAP and those of the USA, Bhutan, Pakistan, and Thailand; this was followed by a scoping review to identify evidence on their implementation by review of peer-reviewed and grey literature.

Results: The BNAP aligns strongly with GAP's strategic objectives, particularly infection control and prevention. However, policy and regulatory gaps were identified in the areas of veterinary antimicrobial stewardship including surveillance, veterinary antimicrobial use, veterinary vaccinations, and specifications of targets for the reduction of non-therapeutic antibiotic use. Evidence on extent of inclusion of AMR contents in veterinarian professional curriculum and establishment of reference microbiology laboratories was lacking.

Conclusion: Closing current policy and regulatory gaps can strengthen the BNAP. Findings from this review can support the development of a research agenda for action in containment of AMR in Bangladesh and have implications for other low and middle-income countries with similar veterinary sector.
Disclosure: Nothing to disclose

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Antimicrobial use in 44 Dutch companion animal clinics: time trends and seasonality expressed in number of Defined Daily Doses (2012-2015)

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Use of antimicrobials in humans and animals promotes selection and dissemination of antimicrobial resistance (AMR). To reduce AMR, responsible use of antimicrobials is needed and should therefore be promoted both in human and veterinary medicine. To optimise antimicrobial prescribing behaviour in Dutch companion animal clinics, insight in current antimicrobial use (AMU) is pivotal.

The objective of this cross-sectional study was to describe systemic AMU during a 3-year time period (July 2012-June 2015) using monthly prescription data from 44 Dutch companion animal clinics. Number of Defined Daily Doses Animal (DDDAs) were calculated from prescription data for total, 1st, 2nd and 3rd choice AMU (classification based on Dutch Policy on Veterinary AMU). Aminopenicillins (with and without clavulanic acid) accounted for the majority of DDDAs (38.7% of total AMU (first year) and 39.3% (third year)). Total AMU decreased in the same period from 1.82 to 1.56 DDDA/year. A similar decrease was seen for 2nd and 3rd choice AMU, whereas 1st choice AMU increased over the study period.

Time trends and seasonality in total, 1st, 2nd and 3rd choice AMU were explored using statistical modelling. These models confirmed the decreasing trends in use of 2nd and 3rd choice AMU and the shift towards more 1st choice AMU, which was apparent in the raw data. Strong seasonal patterns were observed in AMU, with highest use between August and October and lowest use in February. Although AMU is changing and decreasing, there is still room for improvement, especially with regards to the classes of antimicrobials used.

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Systematic review of beta-lactamase resistance genes from Nigeria reveals shared genes between animals, humans and environment

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This systematic review was carried out to identify different beta-lactamase resistance genes reported in published literature from Nigeria and to determine the distribution of the resistance genes between animals, humans and environment. Fifty-six (56) articles were included in this review based on the eligibility criteria. All the beta-lactamases reported were detected from the Gram-negative bacteria, most especially from bacteria of family *Enterobacteriaceae* (n=53), while others includes *Acinetobacter baumannii* (n=2) and *Vibrio* spp. (n=1).Thirty-four (34) different beta-lactamase genes have been detected and reported from Nigeria. Sixteen (16) genes have been detected from animals, 28 genes from humans and 11 genes from the environment. These genes belongs to the narrow-spectrum (bla_{OXA-1}, bla_{OXA-2}, bla_{SHV-1}, bla_{SHV-11}, bla_{TEM-1}, bla_{TEM-2} and bla_Z), AmpC (bla_{AmpC}, bla_{ACT}, bla_{CMY}, bla_{ACC}, bla_{FOX-1}, and bla_{DHA},), extended-spectrum (bla_{CTXM-1}, bla_{CTXM-2}, $bla_{CTXM-14}$, $bla_{CTXM-15}$, $bla_{CTXM-27}$ and $bla_{CTXM-55}$), and carbapenemase (bla_{OXA-23}, bla_{OXA-48}, $bla_{OXA-181}$, bla_{NDM-1}, bla_{VIM-1}, bla_{VIM-2}, bla_{VIM-5} and bla_{KPC}) beta-lactamase resistance genes. Eight (8) genes (bla_{CMY}, bla_{ACT}, bla_{DHA}, bla_{CTXM-1}, $bla_{CTXM-14}$, bla_{GES}, bla_{OXA-1}, and bla_{OXA-2}) were shared between animals and humans, 6 genes (bla_{SHV-1}, bla_{SHV-2}, bla_{SHV-11}, bla_{SHV-12}, bla_{VEB-1}, and bla_{NDM-1}) were common to both humans and environment while none of the genes was unique to both animals and environment. Three genes including bla_{TEM-1}, bla_{AmpC} and internationally pandemic $bla_{CTXM-15}$ genes were unique to animals, humans and environment. This study has provided information on the beta-lactamases distribution in Nigeria. This is necessary for better understanding of one health and molecular epidemiology of clinically important beta-lactamases especially the extended-spectrum beta-lactamases and carbapenemases both in Nigeria and globally.
Disclosure: Nothing to disclose

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Genomic epidemiology of interconnected human and livestock resistomes in a developing country urban landscape

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Livestock have been proposed as a reservoir for antibiotic resistant (AMR) bacteria and AMR genetic determinants that may infect humans, yet quantitative evidence regarding their epidemiological role remains lacking. We used a combination of genomics, epidemiology and ecology to investigate patterns of AMR carriage in *Escherichia coli*, regarded as a sentinel organism. We conducted a structured epidemiological survey of 99 households across Nairobi, Kenya and cultured *E. coli* from 315 human and 594 livestock faecal samples.

We detected high rates of AMR gene carriage, 60 different acquired genes and 14 point mutations, and found that 10/74 of the genes were significantly more common in human than in livestock isolates. Further, AMR genes were not associated with host type or household location, and AMR genes frequently co-occurred, potentially enabling the acquisition of multi-drug resistance in a single step. We found that, whilst AMR gene carriage in humans was not directly associated with the presence of livestock in the household (figure 1), the impact of keeping livestock on human AMR gene carriage was instead influenced by livestock-keeping practices, in particular the presence or absence of animal manure in the household.

In conclusion, we did not find any evidence to support the hypothesis that the keeping of livestock is a risk factor for emergence and dissemination of AMR genes to humans in this setting. Our characterisation of AMR patterns in which co-habiting human and livestock populations were systematically sampled provides insight into the broader epidemiology of AMR in complex and interconnected urban environments.
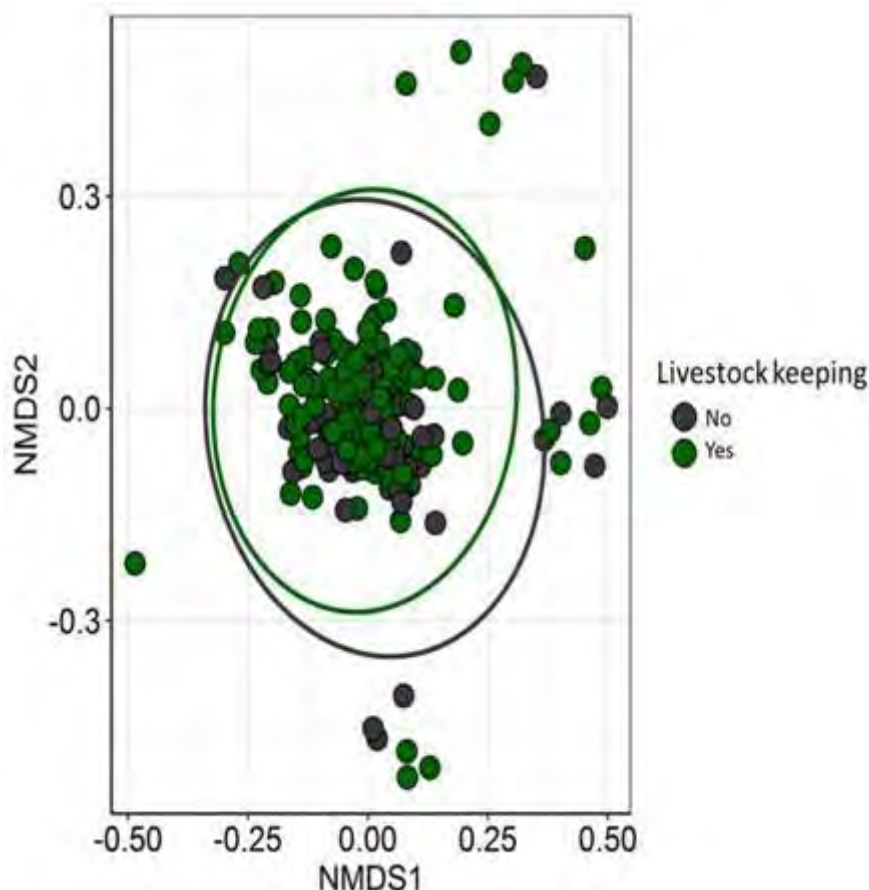
[Human AMR gene assemblage by livestock keeping]

Disclosure: Nothing to disclose

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Antimicrobial resistance profiles of *Escherichia coli* isolates from canine and feline urinary tract infections

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Introduction: an increasing number of scientific reports underline the elevated level of antimicrobial resistance in both human and veterinary pathogens with *Escherichia coli* as one of the most relevant prototypes of multidrug resistant bacteria.

Objectives: the research evaluated and characterized the antimicrobial resistance profiles of *Escherichia coli* isolates from dogs and cats with urinary tract infections (UTI) referred to the veterinary medical teaching hospital and local clinics.

Aims: this retrospective study was aimed to investigate the percentage and patterns of antimicrobial resistance.

Methods: Urine samples collected during the period 2016 - 2018 from a total number of 150 animals with confirmed UTI were subjected to microbiological processing involving culture on enrichment and selective media, and biochemical properties evaluation and *in vitro* antimicrobial susceptibility testing using Vitek 2 system.

Results: Overall, the determined rates of antimicrobial susceptibility towards the antimicrobials most commonly used in small animals practice were relatively low, and resistance to at least two antimicrobial agents was demonstrated in both canine and feline isolates. Several distinct resistance patterns were established for feline and canine isolates (11 and 9, respectively), notably involving multidrug resistance (MDR) and pandrug-resistance. The highest rates of resistance were recorded towards β-lactams, tetracyclines, and aminoglycosides for cats and tetracyclines, aminoglycosides and fluoroquinolones for dogs.

Conclusions: these results emphasize the importance of monitoring antibiotic usage and resistance patterns in the management of *E. coli* associated UTI.

Disclosure: Nothing to disclose

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Optimization of lung infection model with carbapenemase producing *Klebsiella pneumoniae* ST258 (KPC-2) for intranasal treatment in neutropenic NMRI mice

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When testing *in vivo* efficacy of new antimicrobial compounds it is important to use clinically relevant models. Various models are available, however the treatment of pneumonia with intranasally administered compounds is challenging considering clinical progression and animal welfare constraints.

We tested a lung infection model in naturally immunodeficient DBA/2 mice and compared that to NMRI mice rendered neutropenic. The clinical scores progressed rapidly during the first 8 hours in DBA/2 mice and then stabilized at 20-24h, while neutropenic NMRI mice had a slower progression of clinical scores

Treatment with gentamicin (70 mg/kg) was performed at 20 or 24 h using intranasal administration under anesthesia , comparing two methods for anesthetizing the mice (Zoletil® mix sc or isoflurane). Fixed anesthesia with Zoletil® mix was found to cause respiratory failure in DBA/2 and low weight NMRI mice, but tolerance was improved using larger NMRI mice. In NMRI mice a reduction of 4 log cfu compared to vehicle treatment was observed, whereas in DBA/2 mice only a 1 log reduction was observed. Isofluorane anesthesia reduced the efficacy of gentamicin in DBA/2 but not in NMRI mice.

By optimizing the pneumonia model a better efficacy of the treatment with gentamicin was obtained as well as better control of the disease progression and welfare of the mice. We suggest that this optimized model may be a useful tool to evaluate the effect of novel antimicrobial compounds via the intranasal route.

Disclosure: Nothing to disclose

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Global overview on antibiotic use and resistance in *E. coli* in poultry

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Poultry is one of the world´s fastest growing sources of meat production. The objective of this study was to identify the type and amount of antibiotics used in poultry production and the level of antibiotic resistance in *E. coli* isolated from broilers. Isolate information was obtained from national monitoring programs and research studies published since 2000 and conducted in large poultry producing regions: US, China, Brazil, Poland, United Kingdom, Germany, France and Spain.

Qualitative data of registered antibiotics from every country were evaluated. The fluoroquinolones, 3rd generation cephalosporins, macrolides and polymyxins ("highest priority critically important" antibiotics for human medicine according to WHO) are approved for use in large poultry-producing regions, with the exception of fluoroquinolones in US and cephalosporins in the EU. Data on antibiotic resistant *E. coli* is available for most regions but detection of resistance and number of isolates in each study differs among regions. The global harmonized approach in the monitoring of antibiotic use and evaluation of resistances using the same methodology is needed.

Tetracyclines, aminoglycosides, sulfonamides and penicillins are registered for use in poultry in all evaluated countries. The average resistance rates in *E. coli* to representatives of these antibiotic classes are higher than 40% in all countries, with the exception of ampicillin in the US. The resistance rates to fluoroquinolones in the US, where fluoroquinolones are not registered for use, are below 5%, while the average of resistant *E. coli* is above 40% in Brazil, China and EU, where use of fluoroquinolones is legalized.

Disclosure: Nothing to disclose

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Do vegetarians less frequently carry ESBL/pAmpC-producing *E.coli/K. pneumoniae* compared to non-vegetarians?

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Introduction: Extended-spectrum β-lactamase (ESBL) and plasmid-mediated AmpC (pAmpC)-producing *Escherichia coli/Klebsiella pneumoniae* (ESBL-E/K) are frequently found on meat products in Dutch retail, especially on poultry.

Aim: To investigated if vegetarians are at lower risk to carry ESBL-E/K compared to persons who consume meat.

Methods: Vegetarians, pescatarians (vegetarians who eat fish) and non-vegetarians (persons who eat meat ≥3 times per week) were asked to send in a faecal sample and a questionnaire to collect information about their diet and risk factors for ESBL-E/K carriage. ESBL-E/K was cultured and multilocus sequence types (MLSTs) were determined. ESBL/pAmpC-genes were analyzed using polymerase chain reaction (PCR) and sequencing. The risk of ESBL-E/K carriage in the three study groups were analysed using multivariable logistic regression.

Results: Prevalence of ESBL-E/K carriage was 8.0% in vegetarians (63/785; 95%CI 6.3-10.1), 6.9% in pescatarians (27/392; 95%CI 4.8-9.8) and 3.8% in non-vegetarians (14/365; 95%CI 2.3-6.3). Multivariable analysis showed an OR for ESBL-E/K carriage of 1.8 for vegetarians (95%CI 0.9-3.8) and 1.3 for pescatarians (95%CI 0.6-3.0) compared to non-vegetarians. The predominant MLST was *E. coli* ST 131 and most common ESBL genes were $bla_{CTX-M-15}$, $bla_{CTX-M-27}$, $bla_{CTX-M-14}$ and $bla_{CTX-M-1}$ in all diet groups. Independent risk factors for ESBL-E/K carriage were travel to Africa/Latin America/Asia (OR 4.4) or South/East Europe (OR 1.7) and rarely/never washing of hands before food preparation (OR 2.2).

Conclusions: Vegetarians and pescatarians do not have a lower risk of ESBL-E/K carriage compared to non-vegetarians, indicating that eating meat is not an important risk factor for ESBL-E/K carriage.

Disclosure: Nothing to disclose

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Prevalence and antimicrobial resistance patterns of *Staphylococcus* spp. isolated from canine pets and their owners in Trinidad

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Introduction: Staphylococci are opportunistic pathogens of which some species have been shown to be able to colonize both pets and humans. Moreover, several staphylococcal species have been shown to be frequently resistant to multiple antimicrobial agents.
Objectives: We investigated the various species of staphylococci present on humans and their canine pets and determined their antimicrobial resistance against 9 antimicrobials.
Aims: We aimed to estimate a possible exchange of these bacteria between humans and animals.
Methods: A total of 440 strains were isolated from 112 humans and their dogs. Of these, 43.2% were from the owners and 53.9% from dogs. The isolates were identified using the MALDI Biotyper (Bruker). Their antimicrobial susceptibility was determined using the Kirby Bauer disk diffusion method.
Results: Of the 24 *Staphylococcus* spp. identified, 39.1% were coagulase positive (30.5% belonging to the *Staphylococcus intermedius* group (SIG), *S. aureus* (8.2%) and *S. lutrae* (0.2%)) and 54.3% of isolates were coagulase negative of which the most abundant species were *S. sciuri* (24%) *S. simulans* (10.7%) and *S. epidermidis* (9.1%). *S. schleiferi* (6.0%) and *S. agnetis* (0.7%) were the coagulase variable species. Resistance to at least one antimicrobial was found in 68.2% of the isolates. Of all *S. aureus* isolates 70% (25/36) were methicillin resistant while 8.5% (8/94) of the *S. pseudintermedius* isolates were methicillin resistant.
Conclusion: Further studies using whole genome sequencing will enable us to determine the risks of these findings.
Disclosure: Nothing to disclose

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Quantifying transfer dynamics of ESBL/pAmpC *E. coli* across the broiler production pyramid

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Introduction: Extended-spectrum β-lactamase (ESBL)- and pAmpC-producing *E. coli* (ESBL/pAmpC-EC) in food producing animals is a major public health concern.

Objectives: To establish baseline prevalence data for ESBL/pAmpC-EC and quantify their stepwise transfer in Italy's broiler production pyramid.

Aim: To provide quantitative data to inform ESBL/pAmpC-EC risk-mitigating strategies.

Methods: Three production chains of an integrated broiler company were investigated. Cloacal swabs were taken from Parent Stock (PS) chickens and offspring broiler flocks in 4 fattening farms per chain. Carcasses from sampled broiler flocks were collected at slaughterhouse. Samples were processed on selective media and growing *E. coli* were screened for ESBL/pAmpC production. ESBL/pAmpC genes were detected by PCR and sequencing. Average pairwise overlap of ESBL/pAmpC-EC gene occurrences between subsequent production stages was estimated using the proportional similarity index, modelling both uncertainty and variability in a Monte Carlo simulation.

Results: 820 samples were processed from which 513 ESBL/pAmpC-EC were obtained. We found a high prevalence (92.5%, 95%CI 78.9-97.6%) in one-day-old PS chicks, where bla_{CMY} genes predominated, dropped significantly to 20% (11.7-32.1%) at laying period. In fattening broilers, prevalence was 69.2% (57.5-78.7%) at the start of production, 54.2% (35.1-72.1%) before slaughter, and 61.2% (55.6-66.6%) in carcasses. Significantly decreasing and increasing trends for bla_{CMY} and $bla_{CTX-M-group-1}$ gene occurrences were respectively found across subsequent production stages. The estimated average stepwise transfer of ESBL/pAmpC-EC genes between subsequent production stages was 47.7% (42.3-53.4%).

Conclusions: ESBL/pAmpC-EC persists in broiler production and its stepwise transfer contributes significantly to broiler colonisation in subsequent production levels.
Disclosure: Nothing to disclose

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Antibiotic resistance in *Escherichia coli* from diseased pigs in the Netherlands from 2015 - 2017: reliability and representativeness of passively acquired data

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To further reduce and refine the use of antibiotics in livestock, monitoring of antibiotic resistance (ABR) of veterinary pathogens is of utmost importance. A project was run to develop a nationwide, representative, reliable system for ABR monitoring in livestock pathogens. As part of this project, reliability and representativeness of passively acquired *Escherichia coli* (ECO) isolates from diseased pigs were evaluated.

Antibiotic susceptibility testing results of enteropathogenic ECO from pigs were obtained from the LIMS of GD Animal Health. Data were analysed using Stata.

972 ECO isolates from 616 unique, commercial pig farms were available from 2015 - 2017 for further analysis. 752 isolates originated from post-mortem examinations and 220 isolates were cultured from faecal samples. For 575 isolates the age category (suckling, weaned, grow/finish) was known.

The 972 isolates provide a reliable estimation of ABR levels of ECO for different antibiotics and allow for detection of changes in ABR of 5% or more. Considering province and farm size of origin, collected ECO isolates are a fairly representative sample. Several ABR levels were significantly affected by age category (lower ages showing higher ABR levels) and by farm of origin.

The passively acquired data on ECO resistance in pigs can well be used within a national framework monitoring ABR in livestock pathogens. It is recommended to collect additional data per isolate (antibiotic treatment history, age of the pigs) to further evaluate whether these factors impact ABR levels and whether, for example, treatment advices for ECO should be further differentiated regarding the age.

Disclosure: Nothing to disclose

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Antimicrobial prescription behaviour among veterinary practitioners in the Netherlands; a cultural theory on attitudes and trade-off decision making

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Background:

To curb the increasing threat of antimicrobial resistance, antimicrobial use in farm animals should be minimized. Veterinary antimicrobial prescription behaviour is influenced by trade-off decision making. Trade-offs are value decisions, which derive from a hierarchy of attitudes. In this study we explore and theorize on changes and differences in professional values and attitudes, affecting trade-off decision making, using a cultural anthropological methodology

Materials/methods:

The theory has been formed by deduction from findings of recent qualitative and quantitative research concerning antimicrobial prescription behaviour of farm animal veterinarians in the Netherlands and literature review.

Results:
For the veterinary profession, four fundamental attitudes have been identified as constituting work values, job satisfaction and as underpinning trade-off decision making. These are:
1. 'intrinsic to the work' attitude
2. 'intellectually challenging' attitude
3. 'accountable to society' attitude
4. 'economic efficiency' attitude

All four can be present, although seldom equally strong, in the individual veterinarian, While making a trade-off decision, one of the four attitudes dominates the other three. In case of an antimicrobial prescription decision, especially the third and fourth attitude can cause a dilemma. This may result in veterinarians deciding differently in comparable prescribing situations, depending on the dominant attitude. Submersion in a context (sector subset) in which economic efficiency values prevail, is likely to induce a bias in trading off towards the economic efficiency attitude.

Conclusion:
The scope of antimicrobial reduction policy interventions should broaden from farm level to sector and market level, because economic efficiency values may counteract further antimicrobial reduction.
Disclosure: Nothing to disclose

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Social influences in antibiotic prescription behaviour among veterinary practitioners in the Netherlands

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Background:

Insights in the key factors that drive antibiotic use and prescription by veterinarians can serve in strategically influencing veterinary antibiotic prescription behaviour and thereby counteract the increasing threat of antimicrobial resistance. In this study, we elicit how social influences, as one of the key factors, affect antibiotic prescribing of farm animal veterinarians.

Methods:

Semi-structured interviews were held with 11 farm animal veterinarians and subsequently a questionnaire was developed and analysed. 135 Veterinarians working in the Netherlands responded.

Results:

Farmers, nutritionists and their immediate colleagues were regarded to belong to the veterinary practitioners' direct social environment. According to the respondents, this narrow social distance to their clients helps them in their advisory role. They did not perceive this narrow relationship as influencing their prescribing practices. Nevertheless, they indicated to sometimes be afraid of liability issues when not prescribing antibiotics and the majority did not perceive much support from their direct social environment to (further) alter their antibiotic prescription behaviour. In contrast, they did perceive an urge from the indirect social environment (general public, policy makers, scientists) to alter their prescription behaviour. This leads to conflicts of interests towards the direct and indirect social environment of veterinarians.

Conclusions:

Socially, practitioners are deeply invested in their farmers and amidst a web of regularly conflicting interests. Depending on the situation, social influence plays a role in their decision making regarding the prescription of antibiotics. Further investigation is needed to enhance social reference and support for actively reducing antibiotic prescription.

Disclosure: Nothing to disclose

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Antimicrobial susceptibility of non-typhoidal Salmonella isolated from bacteremic children in western Kenya

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Introduction: Invasive non-typhoidal Salmonella (NTS) infections cause a significant disease burden in sub-Saharan Africa. NTS is associated with pediatric co-infections such as malaria and HIV; mortality is up to 45%. NTS is an important zoonotic pathogen and may be maintained in animal reservoirs. Antimicrobial resistance (AMR) further complicates treatment of NTS infections as multidrug resistant NTS is prevalent in many parts of Africa. Unrestricted use of antimicrobials in both livestock and human populations can exert selection pressure on shared pathogens such as NTS.
Objective: To assess changes in NTS AMR to commonly used antimicrobials between 2004-2012.
Methods: From 1,654 blood cultures from febrile children less than three years of age in western Kenya (2004-2006 and 2009-2012), 71 NTS isolates were analyzed along with patient history and AMR patterns.
Results: We observed that 17% of the children with NTS bacteremia had a history of antimicrobial administration in the seven days prior to blood culture. The most frequent antimicrobial was trimethoprim/sulfamethoxazole (SXT). Resistance rates for SXT were high (85.7% non-susceptible). However, a history of SXT administration in the past week was not associated with SXT resistant infections. High rates of AMR to other commonly used antimicrobials was also observed: chloramphenicol (70.1% non-susceptible) and amoxicillin-clavulanic acid (73.2% non-susceptible). AMR rates were not significantly different between the 2005-2006 isolates and the 2009-2012 isolates.
Conclusion: There is a consistently high prevalence of AMR in NTS to commonly used antimicrobials in western Kenya since the early 2000s.
Disclosure: Nothing to disclose

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Detection of antimicrobial resistance (AMR) in broiler chickens supplying urban areas in Western Java: A pilot AMR surveillance programme in Indonesia

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Approximately 80% of commercial broiler farms in Indonesia are sector-3 farms, i.e. farms with poor biosecurity. This leads to substantial usage of antimicrobials for therapeutic and non-therapeutic purposes. A pilot antimicrobial resistance survey in broiler chickens was conducted in 15 districts of the Subang Disease Investigation Centre catchment area from September to November 2017. The aim was to detect the resistance of zoonotic (*Salmonella* spp) and commensal (*Escherichia coli*) bacteria and to identify factors influencing the recovery rate of bacteria to inform AMR Surveillance guidelines. A total of 623 chicken caecal samples were randomly collected from 209 poultry abattoirs. The target bacteria were isolated and identified using Indonesian National Standard Bacterial Analytical Manual methods (SNI 2897:2008I). Seventy-nine *Salmonella spp* (13%) and 352 *E. coli* (57%) isolates were recovered. The antimicrobial susceptibility was determined for 61 *Salmonella spp* isolates and 61 *E.coli* isolates by the National Animal Product Quality Testing and Certification Laboratory. Analysis was done using the agar dilution method according to the CLSI VET01A4E and VET01S2E guidelines. Resistance levels of *Salmonella spp* were 87%, 80% 55%, 38%, 17%, and 3% for tetracycline, ciprofloxacin, trimethoprim, gentamicin, ampicillin, and chloramphenicol respectively, and *E. coli* were 88%, 72%, 70%, 53%, 51%, and 23% for ampicillin, ciprofloxacin, trimethoprim, tetracycline, gentamicin and chloramphenicol respectively. The relatively low recovery rate of *E. coli* and other improvements from this pilot initiative in the Subang area were all noted and will be addressed in the future routine national AMR surveillance work in broiler poultry in Indonesia

Disclosure: Nothing to disclose

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Antibiotic-induced, increased conjugative transfer is common to several naturally occurring ESBL plasmids in *Escherichia coli*

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Conjugative plasmid transfer (PT) is the main mechanism for spread of Extended-Spectrum Beta-Lactamase (ESBL)-encoding genes in *Enterobacteriaceae*. Previously, we showed that cefotaxime (CTX) exposure increases transfer of an IncI1/pST49/CTX-M-1 plasmid in *Escherichia coli*. This study aimed at investigating whether that observation was unique for that plasmid/strain combination or whether it is a general phenomenon.

Each of 25 *E. coli* harboring different conjugative ESBL plasmids was exposed separately to CTX, ampicillin (AMP) and ciprofloxacin (CIP) at therapeutically relevant concentrations in a LB broth culture at 37°C until OD_{600}=0.5. After antimicrobial removal, each strain was used for filter-mating conjugation on LB plates with rifampicin (RIF)-resistant *E. coli* J53-2 as recipient, in a 1:1 donor to recipient ratio. The frequency of PT was compared with that of donors not exposed to antibiotics. RT-qPCR was used to measure mRNA levels of PT genes *traF, traI, traL, traM* and *pilS*, and SOS response genes *recA* and *sfiA* in the transconjugants.

Eight (30.7%) plasmids, namely IncI1/pST7/CTX-M-1, IncI1/pST49/CTX-M-1, IncI1/pST3/CTX-M-1, IncI1/pST293/CTX-M-1, IncI1/pST295/CTX-M-1, IncI1/pST16/CTX-M-55, IncFII/CTX-M-14 (n=2) were affected by antibiotics. CTX, AMP and CIP increased PT in all, six and three strains, respectively. RT-qPCR showed that all target plasmid genes were upregulated in presence of the different antimicrobials, whereas SOS-response genes were upregulated following CIP exposure only.

Our findings reveal that AMP, CTX and CIP increase conjugation frequency of different plasmids in different *E. coli*. Thus, antibiotic-induced conjugation transfer of ESBL plasmids appears to be a general phenomenon in *E. coli*. The mechanisms underlying these observations are under investigation.

Disclosure: Nothing to disclose

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Monitoring of antimicrobial susceptibility of *Streptococcus suis* in the Netherlands, 2013-2018
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Streptococcus suis is an important pig pathogen, and additionally, an emerging zoonotic bacterium. The objective of this study was to analyse the in-vitro antimicrobial susceptibility (AMS) of *S. suis* from samples from diseased pigs in the Netherlands. *S. suis* isolates, over 3000, originated from diagnostic submissions of pigs sent to GD, from April 2013 until December 2018. Minimal inhibitory concentrations (MICs) of 14 antimicrobials were assessed by broth microdilution following CLSI recommendations. MIC_{50} and MIC_{90} values were determined and MICs were interpreted as susceptible, intermediate and resistant using CLSI veterinary breakpoints (when available). Emergence of resistance among *S. suis* from diseased pigs appeared to be limited. Percentage of resistance to ampicillin, ceftiofur, enrofloxacin, florfenicol, penicillin, and trimethoprim/sulfamethoxazole was low, to clindamycin medium, and to tetracycline high. Cross-resistance between penicillin and ampicillin appeared to be incomplete. For several antimicrobials, an effect of age category on MIC values and percentages of resistance was found.

It has to be kept in mind that the results represent only part of the Dutch pig population. However, given the high number of isolates, this passive monitoring is considered to provide a reliable and representative picture of the AMS of *S. suis* isolates in the Netherlands. Interpretation of MICs of (clinically relevant) antimicrobials tested for treatment of *S. suis* infection is strongly hampered by the lack of clinical breakpoints that are animal species- and body-site-specific. Therefore, and to conduct a clinically reliable monitoring of AMS of veterinary pathogens, more species- and body-site-specific veterinary breakpoints are urgently needed.
Disclosure: Nothing to disclose

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AMR at human -animal interface,results from the largest one health surveillance study India

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India is currently facing a huge challenge of multi-drug and extremely drug resistant bacteria. Foodborne infections are very common in India. Increased demands of livestock have resulted in intensive farming where antibiotic usage is rampant. Residues of antibiotics remain active which may alter human intestinal microflora and cause resistance gene transfer. We conducted the largest surveillance study of AMR at the human-animal interface in north India as a part of WHO-AGISAR India grant. The study included surveillance of foodborne pathogens and commensals at the human - animal interface across agriculture-intensive areas of Chandigarh, Panjab, Haryana, Uttarahkand and Himachal Pradesh . Human stool samples included community acquired diarrhoea and healthy children. Animal intestinal contents were collected from poultry, goat/sheep and pigs along with corresponding meat samples .Antibiotic usage data from farms was collected. Whole genome sequencing (WGS) was performed for Nontypoidal *Salmonella* (NTS) and MLST for *E.coli* pathotypes. A high burden of *Campylobacter*, NTS, and various pathotypes of diarrhoeaggenic E coli was found at human-animal interface.CTXM-15, NDM and CMY genes were the predominant genes coding for AMR. Overall very high level of resistance was observed towards fluroquinolones, tetracycline, aminoglycosides corresponding to their usage in animal farms. Colistin resistance is emerging. MLST revealed circulation of highly drug resistance clones like ST 131 and avian pathogenic/extra intestinal clones ST117 . Extensive use of antibiotics in the food animals, particularly in poultry was noted. Food chain is an important route for spread of food-borne pathogens and spread of drug resistant bacteria as evidenced by WGS.
Disclosure: Authors declare no conflict

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Antimicrobial resistance: A call for education

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Introduction: Antimicrobial resistance has been a global public concern over the years. Although the World Health Organization has issued the strategies for containment of antimicrobial resistance, the effectiveness of implementation is controversial. Since antimicrobial resistance can affect the health of human well-being that the infectious diseases are not treatable with the available antimicrobial agents, there is an emergent need to call for attention on the strategies to minimize the use of antimicrobial agents from educational perspectives.

Objectives: The objective of this study is to explore the strategies to minimize the use of antimicrobial agents from educational perspectives.

Methods: A systematic review of current literature was conducted using multiple database such as Medline, PubMed, Embase. Related articles within 2013-2018 were reviewed systematically and the results were presented by thematic analysis.

Results: Results supported that education, both for clinicians and public, were positively associated with minimizing the use of antimicrobial agents. For the clinician's perspective, active clinician education, provide reminders, implement audit and feedback were reported. For the public's perspective, educational programmes centered on the consumer drug use were significant.

Conclusion: Antimicrobial resistance is a global challenge in public health context. This study concluded that education is one of the key strategies to minimize the use of antimicrobial agents. Thus, intervention programmes focusing on minimizing antimicrobial agent use should consider including education elements in programme design.

Disclosure: Nothing to disclose

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Pharmacokinetics of sulfadiazine-trimethoprim in mink

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Introduction: Antibiotics are extensively used off-label for mink. In order to rationalize antimicrobial use in this species, PK data are needed to determine optimal antimicrobial dosages and clinical breakpoints targeting mink pathogens. PK studies in mink are hampered by the difficulties in obtaining venous blood over an extended time period. Therefore, nail blood could serve as an alternative to venous blood.

Objectives: To investigate PK parameters of sulfadiazine and trimethoprim in fasted and fed mink, and to compare results obtained from parallel nail and venous blood samples.

Methods: Ten male adult minks were divided into groups of fed (n=5) and 12-hour fasted (n=5). Sulfadiazine/trimethoprim (30 mg/kg) was injected IV, and blood was withdrawn from nail and vein before injection and at nine time points until 24h. Injections and aspirations were under sevoflurane anesthesia. Serum concentrations were measured by LC-QTOF. Data were analyzed using non-linear regression models by WinNonlin 8.0 and non-linear mixed effect models by NLME 8.0.

Results: Both antibiotics were best described by a mono-compartment model with CL, V, k10 value of 46.83 (ml/h/kg), 706 (ml/kg), 0.07 (h^{-1}), respectively for sulfadiazine and 496.89 (ml/h/kg), 5130.9 (ml/kg), 0.177 (h^{-1}), respectively for trimethoprim. Based on NLME models, there were no significant difference ($p < 0.05$) between nail and vein blood and fed and fasted groups.

Conclusion: Pending MIC data for mink pathogens and bioavailability data will be used with the obtained PK data for dose optimization and CBP development. Nail samples can be representative of venous blood for these antimicrobials.

Disclosure: Nothing to disclose

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Occurrence of multiple drug resistant *Escherichia coli* and *Enterococcus* species in village chickens from four farms in Selangor, Malaysia.

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Intensification of poultry farming is associated with the use of antimicrobial agents either as growth promoters, feed additives or prophylaxis, leading to antimicrobial resistant bacteria in chickens which may serve as a risk to human health via the food chain. In Malaysia, village chickens has built its reputation among the locals as an alternative source of protein, perceived to be free from hazards associated with commercial poultry farms including drug residues and poor animal welfare. Nevertheless hazards including antimicrobial resistant bacteria may still occur in village chickens since birds are reared outdoors and fed with food- or agro-based wastes. Therefore, this study aimed to determine if antimicrobial resistance is prevalent in 2 commensal bacterial species in the poultry intestinal tract; *Escherichia coli* and *Enterococcus* spp. Cloacal swabs were acquired from 60 village chickens on 4 farms in the Hulu Langat district, Selangor, Malaysia. *Escherichia coli* and *Enterococcus* spp. were isolated from the samples with the overall prevalence of 89.3% and 37.5%, respectively. *E. coli* isolates were resistant to tetracycline (78%), ampicillin (56%), amoxicillin (52%), streptomycin (38%), chloramphenicol (34%), cephalexin (28%), enrofloxacin (24%), gentamicin (8%) and aztreonam (2%), but susceptible to meropenem. *Enterococcus* spp. isolates were resistant to tetracycline (100%), norfloxacin (72%), enrofloxacin (52%) gentamicin (33%), ciprofloxacin (33%), ampicillin (29%) and amoxicillin (10%), but susceptible to vancomycin. Overall, multiple drug resistance in the village chicken isolates were determined as high *E. coli* (59%) and *Enterococcus* spp. (67%), considering the nature of the farming system adopted.

Disclosure: Nothing to disclose

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Evaluation of CHROMID® Colistin R agar, new chromogenic medium for screening of Enterobacteriaceae with acquired resistance to colistin in veterinary caecal samples

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Introduction
o Since 2015, eight different *mcr*-genes and 53 variants of Colistin plasmid-mediated resistance were reported. The *mcr*-genes have been identified worldwide from human, animal and environmental samples mostly but not exclusively in *Escherichia coli*. As colistin remains as a last resort antibiotic for multidrug resistant infections in human, why tremendous importance to be able to detect strains with acquired resistance to colistin.
Materials and Methods
o A total of 105 caecal samples, from poultry, veal calf, and swine were collected among Danish slaughterhouses and included in this study.
o Samples were tested using CHROMID® Colistin R agar before (specificity study) and after (sensitivity study) artificial contamination at ≈1.5×10^5 CFU/ml with characterized strains with acquired resistance to colistin. The strains set included nine *Salmonella enterica* and 26 *Escherichia coli* harbouring *mcr*-1, -2, -3, -4 or -5 genes. The tests were performed following agar's instruction for use.
Results
o Specificity was 96% using CHROMID® Colistin R plate (screening intended use). The colony of interest growing on those plates were subcultured with an inoculum of 10^4 CFU on a second CHROMID® Colistin R plate (resistance detection intended use). Subsequently, specificity raised to 100%.
o Among the 105 artificially contaminated samples, all the characterized strains were recovered highlighting a sensitivity of 100%.
Discussion and Conclusion
o Combining easy to read chromogenic properties, high sensitivity and specificity, CHROMID® Colistin R is an well adapted for large screening trials and confirmation of colistin resistance in Enterobacteriaceae among dominant or subdominant flora.
Disclosure: Nothing to disclose

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Emergence of fluoroquinolone resistant Salmonella Kentucky ST198 from India

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Introduction: Non-Typhoidal *Salmonella*(NTS) has huge reservoirs in animals and generally induce a self-limiting gastroenteritis in humans. S. Kentucky has appeared on global NTS landscape in animal husbandry but had a low prevalence in human infections. Kentucky infections have been largely associated with single sequence type(ST198) exhibiting resistance to ciprofloxacin. India is likely a key location for the emergence and international transmission of diarrhoeal pathogens, but there are limited concise data.

Objectives: To characterize NTS from humans and food-animals to access potential role of food-animals in infection and spread of antimicrobial resistance.

Methods: Stool and meat samples from food-animals and diarrhoeagenic stool samples from humans were collected from five states of North India. Salmonella were isolated by microbiological culture methods and whole genome sequencing was performed. Bioinformatic analysis was done to identify the MLST sequence types and antibiotic resistance genes. Minimum Inhibitory Concentration were also estimated to common antibiotics.

Results: A total of 889 animal samples and 1726 human stool samples were collected and a total of 117 NTS were identified. Among all the NTS, S. Kentucky was the most common serovar (23/117). All Kentucky isolates belonged to a single type ST198 and were resistant to ciprofloxacin. On phylogenetic analysis it was found that these isolates had similar mutation profile as that of previously described epidemic clone from international travelers.

Conclusions: We conclude that Ciprofloxacin resistant S. Kentucky ST198 is endemic in India with huge reservoirs in food animals and there is an utmost need for surveillance systems in all sectors

Disclosure: Nothing to disclose

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Development of a Novel Antimicrobial for the Treatment Of Bovine Mastitis

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The occurrence of antibiotic-resistant bacteria is an increasingly prevalent societal issue globally. The fact that many antibiotics are no longer effective against bacteria is of particular concern in the veterinary sector, the largest consumer of antibiotics. Bovine mastitis is the most critical infectious disease affecting the dairy industry, leading to recurrent treatment failures, poor milk quality, loss of income to farmers, and the premature culling of animals.

Westway Health is currently developing LARS (Long Acting Reactive Species), a novel treatment for bovine mastitis in lactating cows, based on the reaction between peroxide and iodide. The antimicrobial activity of LARS was tested, *in vitro*, against a panel of mastitis isolates (including *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus* species). Minimum Inhibitory Concentrations (MICs) were determined to be comparable to many antibiotic treatments; and when delivered in a specially manufactured excipient, time-kill assays demonstrated an equivalent kill profile to a product currently on the market. Induction of resistance was attempted over 12 days. Resistance developed against all antibiotics tested within the experimental time frame, yet no resistance developed to LARS.

Clinical trials were conducted on a multitude of farms across Europe, where Somatic Cell Counts (SCCs) and iodine residues have been measured over time, and bacterial analysis has been carried out. The treatment was well tolerated by the cows and individual SCCs decreased in response to treatment, associated with clinical and bacteriological cures. Long-term follow-up of the animals indicated no adverse effects. We're now working towards regulatory approval of a novel mastitis treatment.

Disclosure: Nothing to disclose

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Threats by *Acinetobacter*spp. in domestic and exotic animals - occurrence and resistance trends in the United States, 2013-2018

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Acinetobacter has emerged as an important opportunistic pathogen due to its remarkable ability to acquire antimicrobial resistance. While its relevance as a human pathogen is unquestionable, knowledge of *Acinetobacter* in Veterinary Medicine is scarce. We used statistical modelling analysis to answer questions regarding the distribution of different species of *Acinetobacter* in domestic and exotic animals, their antimicrobialresistance profiles, multidrug-resistance rates, and resistance trends in the past years. Isolates were identified as *A. baumannii*, *A. lwoffii*, *A. jonhsonii*, *A. radioresistens*, *A. ursingii* and *A. junii*. *Acinetobacter* spp. were isolated from domestic and exotic animals, but commonly isolated from domestic pets, which cohabit with humans, acting as potential vectors of bacteria and resistance genes.

MICs demonstrated resistance profiles that were intrinsically associated with different *Acinetobacter* species.This emphasizes the importance of species identification and the necessity to implement MIC breakpoints for different species of *Acinetobacter* from animal origin. We propose that veterinary laboratories must cautiously interpret the intrinsic resistance guidelines when reporting antimicrobial susceptibility testing of *Acinetobacter*.There was a significant multidrug resistance percentage of *A. baumannii* (72.1%) and *A. haemolyticus* (80%). Importantly, any of the isolates were resistantto Imipenem, which is the treatment of choiceagainst multi-drug resistant *Acinetobacter*. There was no significant increase or decrease of resistance to any of the tested antimicrobial classes between 2013 and 2018. In summary, *Acinetobacter*is emerging as an important pathogen in veterinary medicine. Our findings stress that *A. baumannii* is the species that requires special attention at veterinary clinics due to high prevalence and multi-drug resistant phenotypes.

Disclosure: Nothing to disclose

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Molecular characterization of multi-drug resistant gram-negative strains isolated during a prevalence survey of rectal colonization in Verona (Italy) long-term care facilities

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Introduction: Few data are available on characterization and prevalence of multidrug-resistant (MDR) Gram-negative in long-term care facility (LTCF) residents.
Aim: The study characterized the multi-drug resistant strains isolated among residents in LTCFs during a point prevalence survey (PPS).
Methods: A PPS was conducted in seven LTCFs situated in Verona area (Italy). A rectal swab was collected to identify ESBL and carbapenemase-producing isolates, using ChromoID ESBL and MCconkey media, with added ertapenem and meropenem disk respectively. Carbapenemase and ESBL productions were checked by CarbaNP test and multiplex PCR for major carbapenemases and ESBL groups were carried out. Clonal relationship was evaluated by PFGE, MLVA and phylogenetic group determination.
Results: A total of 453 residents were enrolled. 241 (53,2%) residents resulted colonized at least by one MDR gram-negative. 275 gram-negative cephalosporin-resistant were isolated. 14 residents harbor a *K. pneumoniae* KPC-producer and all resulted clonal by PFGE. Four harbors OXA-23 producer *A. baumannii*, 116 out of 140 *E. coli* resistant to cephalosporins isolated. were ESBL producers. Molecular analysis showed 79% harbor a CTX-M-1 group enzyme, !8,2% a CTX-M-9 group, TEM-1 (32.7%) and SHV (4,5%) were always co-harbored with a CTX-M enzyme. Phylogenetic group analysis of E. coli ESBL-producer strains showed a great prevalence of B2 group (77,2%).
Conclusions: We reported a high rate of colonization of *E, coli* ESBL-producing among LTCF residents. All of them carried a CTX-M-1 or CTX-M-9 group enzyme and the 77,2% show the same phylogenetic group B2. KPC was the only carbapenemase detected in *Enterobacteriaceae* (5%).
Disclosure: Nothing to disclose

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Efficacy enhancement of antibacterials against *Escherichia coli* by Histamine H_1 receptor antagonists

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Introduction

The aim of this study was to find out if antihistaminic compounds like mepyramine, which is used for the treatment of allergic diseases, have the ability to influence the activity of antibacterials. Therefore, the checkerboard method was chosen to detect these possible effects in vitro.

Materials/Methods

Checkerboard tests were carried out in 96 well plates. The H_1 antagonist mepyramine was serially diluted along the rows (0-1000 µg/ml), various antibacterial agents along the columns starting at zero and ending at two times MIC. Two different *Escherichia coli* (*E. coli)* strains were used. *E. coli* ATCC® 25922™, a reference strain for antimicrobial susceptibility testing and *E. coli* PIG 01 isolated from pigs in own experiments and resistant to enrofloxacin. Bacterial growth was compared measuring the absorbance after 24 hours. A dose reduction index (DRI) was calculated based on the compounds concentration which inhibited the bacterial growth.

Results

The effect of amoxicillin, sulfadiazine/trimethoprim, colistin, enrofloxacin, tetracycline and florfenicol was enhanced by mepyramine in vitro. In contrast, mepyramine had no influence on the antibacterial effect of gentamicin and kanamycin.

Conclusion

The combined use of antihistamines and antibacterials might be a potential option to treat infectious diseases in future. In vivo studies are in progress to confirm the in vitro results.

Disclosure: Nothing to disclose

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Occurrence of *Gallibacterium anatis* in chicken production systems in Iran and evaluation of the genetic diversity of the Gtx-A toxin, a potential vaccine candidate

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Gallibacterium anatis is a common cause of reproductive tract infection in egg-laying hens which calls for development of new prophylactic measures. The purpose of this study is to investigate the occurrence of *G. anatis* in chicken production system in Iran and to evaluate the diversity of the toxin, which has been suggested as a promising vaccine antigen. The occurrence of *G. anatis* was assessed in battery-cage layer and broiler flocks with two biosecurity levels. The 12 flocks originated from three different climatic zones. Ten chickens from each flock were sampled by tracheal swabs utilized for identification of *G. anatis* by PCR and MALDI-TOF. A total of 30 broilers and 54 battery-cage layers sampled positive for *G. anatis* indicating that *G. anatis* is highly prevalent in Iranian commercial poultry. However, the prevalence proportions were influenced by the biosecurity level, climatic conditions and health status of the chickens. Genotyping of the 71 isolates using PFGE suggested that the Iranian isolates grouped in 24 clusters with 88% similarity revealing a considerable genotypic variability. Yet, we also found clones that were present in different flocks and production systems. *gtxA* toxin gene sequencing was performed on 23 strains to allow evaluation of the genetic diversity of the gene. Comparison of the 23 Iranian strains and 26 strains from other parts of the world showed at least 92% *gtxA* sequence similarity. This indicated that *gtxA* has a conserved sequence across a range of epidemiologically independent strains, which favors the use GtxA in vaccine development activities.

Disclosure: Nothing to disclose

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Detection by real-time PCR of colistin resistance genes (*mcr*-1 to *mcr*-3) in animal manure and agricultural soil in Northern Italy

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Introduction: The presence of antimicrobials, such as colistin, in animal manure may promote the dissemination of plasmid-mediated colistin resistance (*mcr* genes) into the environment.

Objectives: To assess resistance to colistin mediated by *mcr* genes in soil fertilized with manure from intensive animal farms.

Aim: To provide qualitative data for *mcr*-1 to *mcr*-3 genes in manure and soil using a real-time PCR approach.

Methods: A total of 84 samples (1 sample of manure/slurry; 1 sample of soil before fertilization, T0; and 1 sample of soil about 30 days after fertilization, T30 per farm) collected from dairy ($n = 11$), swine ($n = 10$) and chicken ($n = 7$) farms were analysed by real-time PCR.

Results: *Mcr*-1, *mcr*-2 and *mcr*-3 genes were detected in all three farms and samples. In swine farms, all 3 *mcr* genes (*i.e.* 5 slurry, 2 T0 and 3 T30 for *mcr*-1; 3 slurry for *mcr*-2; 3 slurry, 2 T0 and 2 T30 for *mcr*-3) were detected. In chicken and dairy farms, only *mcr*-1 (*i.e.* 3 manure in dairy, and 3 T0 and 1 T30 in chicken) and *mcr*-3 genes (*i.e.* 2 manure in dairy, and 1 manure and 1 T0 in chicken) were found. *Mcr*-1 was the most prevalent gene (20%), followed by *mcr*-3 (13%) and *mcr*-2 (3.5%).

Conclusions: The presence of *mcr* genes into the environment, following fertilization with animal manure, may be of concern for public health since colistin is considered as last therapeutic option for treating severe human infections.

Disclosure: Nothing to disclose

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Plasmid-mediated gentamicin resistance in MDR, ESBL/AmpC-producing *E. coli* isolated in turkey meat industry

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Aminoglycosides have been extensively used in food producing animals. In Italy, the EU Harmonised AMR Monitoring (Decision 2013/652/EU), has detected high levels of gentamicin resistance in commensal *E. coli* and *Salmonella* since 2014 (21.8% and 30.4% respectively). The aim of this study was to determine the genetic basis of gentamicin resistance in MDR, ESBL/AmpC-producing *E. coli* isolated from fattening turkeys and meats thereof in Italy in 2018. Thirteen phenotypically MDR, ESBL/AmpC-producing *E. coli* from 7 caecal contents of fattening turkeys and from 6 turkey meat samples, displaying gentamicin MIC values >2 mg/L, were in-depth characterized by Whole Genome Sequencing. MLST, accessory genes, chromosomal mutations conferring antimicrobial resistance and plasmid replicons were determined using bioinformatic tools. Preliminary results indicated that 9/13 isolates belonged to different Sequence Types. All isolates presented multiple plasmid replicons, ESBL/AmpC, aminoglycoside and sulphonamide resistance genes. All but one also presented tetracycline resistance genes and the majority (10/13) also chromosomal point mutations on *gyr*A and *par*C conferring fluoroquinolone resistance. 10/13 isolates harboured at least one gene (*aac*(3)-IIa, *aac*(3)-IVa, *aac*(3)-VIa, *aac*(3)-IId, *ant*(2´´)-Ia) associated with gentamicin resistance. Interestingly, in one isolate *aac*(3)-IIa and *aac*(3)-VIa were located in the contigs containing IncQ and IncA/C plasmid replicons, respectively. These findings are of growing concern, since demonstrate that ESBL/AmpC-producing *E. coli* from the Italian turkey industry often acquire additional extra-chromosomal transferable resistance to HPCIAs like aminoglycosides. This occurrence is likely due to the extensive administration, especially by oral route, of aminosidine (paromomycin) and gentamicin/apramycin in the turkey industry.
Disclosure: Nothing to disclose

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Dynamics of antimicrobial prescription behaviour of farm animal veterinarians in the Netherlands

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Background:
Insights in determinants that drive antimicrobial prescription behaviour of farm animal veterinarians might be supportive in developing new policies to further reduce antimicrobial use in agriculture. In this study we investigated dynamics of antimicrobial prescribing behaviour of Dutch veterinarians over time and possible factors influencing prescribing practices of individual veterinarians.

Methods:
Antimicrobial prescription data of individual veterinarians over the years 2013-2017 were obtained from different data sources and subsequently anonymized by an independent third party. Additional data as affiliation (to a veterinary practice), characteristics of farms they served and other demographic data were collected and handled likewise. Mixed models were used to explore which determinants influence the level of antimicrobials prescribed. Examined determinants include farm size, number of farms served and year. Structural high prescribing veterinarians were defined as veterinarians with a level of antimicrobials prescribed that exceeded the 65th percentile for three consecutive years. Similarly, structural low prescribers prescribed less than the 35th percentile for three consecutive years.

Results:
Preliminary results indicate that a substantial part of farm animal veterinarians have a relatively constant antimicrobial prescription behaviour over subsequent years. This results in a relatively stable group of 'structural high' and 'structural low' prescribers. Several determinants are influencing the level of antimicrobial prescribed and they differ between animal species. These determinants are currently being analysed and will be presented.

Implications:
Knowledge of prescription determinants might unravel possibilities how veterinarians can (further) lower their antimicrobial prescription level and will inform policy makers how to support veterinarians in doing so.
Disclosure: Nothing to disclose

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Use of Whole Genome Sequencing to Evaluate Phenotypic Carbapenem Resistance from a Cluster of Companion Animal *Escherichia coli* Isolates

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Carbapenem-resistant Enterobacteriaceae (CRE) are important agents of human nosocomial infections but have been rarely isolated from companion animals. Resistance to carbapenems is typically due to an acquired beta-lactamase gene or mutation of outer membrane proteins (OMP) due to deletion. This study evaluated a cluster of 7 isolates from 6 animals housed in an intensive care unit over 30 days. Bacteria were isolated from specimens from 5 dogs (4 respiratory and 2 urine) and 1 cat (1 respiratory) in a clinical microbiology lab using standard methods. Identification and antimicrobial susceptibility testing were performed on the Vitek2 system (Biomerieux) and the imipenem resistant phenotype was confirmed by E-test. Whole genome sequencing (WGS) was performed on an Illumina MiSeq and contigs were evaluated with PlasmidFinder and CARD to identify plasmids and antibiotic resistance genes respectively. Translated amino acid sequences for the OmpC and OmpF proteins were compared to *E. coli* k12. All 7 isolates had an imipenem MIC of 4-8 ug/ml by E-test. The first two isolates had same MLST type (ST69), all other isolates were all unique. IncF plasmids were detected in 6/7 isolates. No carbapenamase genes were identified. OmpC amino acid sequences were more divergent from K12 than OmpF, but no premature stop codons were detected in either protein. It is still unclear what the mechanism of resistance is in these isolates following WGS analysis. This study highlights the continued importance of phenotypic analyses when evaluating antimicrobial susceptibility of clinical isolates.

Disclosure: Nothing to disclose

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Resistome of Wight Toothed Shrews and Wood Mice strongly correlates with host specific gut microbiota composition

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Increased antibiotic resistance of bacteria threatens human health and food security. Here we investigated the faecal resistome of White Toothed Shrews (*Crocidura russula*, WTS) and Wood Mice (*Apodemus sylvaticus*, WM) captured at six different pig farms across The Netherlands with different levels of veterinary antibiotic use. 120 faecal samples were collected from WTS(n=70) and WM(n=50). DNA was extracted from individual samples. For microbial composition profiling the V3-V4 region of 16S rRNA genes was PCR-amplified, HiSeq-sequenced and data was processed using NG-tax. For resistome assessment we used shot-gun metagenomics (HiSeq 2500) on DNA-pools per area and species (n=12). Numbers of reads containing antibiotic resistance gene (ARG) [NvdB1] motives (ARGM) and ARG-classes were assessed using DeepARG. Statistical analysis and visualization were performed using R. Reads with ARGM accounted for 0.26%-0.66% (95%, CI[0.38, 0.5]) of all reads. WTS and WM showed near-significant (p=0.07) differences in relative abundance of reads with ARGM, and hierarchical clustering based on log-transformed weighted ARG composition showed separation by animal species. Comparison of Principal Coordinates ordinations of gut microbiota and ARG composition showed a high level of symmetry using Procrustes rotation (0.83, p < 0.0001). Correlation of ARG abundance and antibiotic use was less obvious and requires further investigation, however, preliminary results suggested species dependent correlation of the abundance of certain ARG classes and veterinary use of antibiotics. In conclusion this work for the first time provided an overview of free living WTS and WM resistomes, as well as showed a clear correlation between gut microbiota and resistome.

Disclosure: Nothing to disclose

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Harmonised Antimicrobial Resistance and Use indicators - how useful are they?

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Background:
Harmonised surveillance indicators for antimicrobial resistance (AMR) and use (AMU) have been developed by the European Centre for Disease Prevention and Control (ECDC), European Food Safety Authority (EFSA) and European Medicines Agency (EMA) to assist countries in assessing progress in reducing AMR and AMU in humans and food-producing animals. We present outcomes for harmonised AMR and AMU indicators for the United Kingdom.

Materials/methods:
National data captured by UK surveillance or monitoring programmes from the human sector and animal sector between 2013 and 2017 were used to produce AMR and AMU indicators in humans and food-producing animals.

Results:
Primary AMR indicators showed 50% reduction in methicillin-resistant *Staphylococcus aureus* (humans), 29% decrease in *Escherichia coli* resistant to 3rd generation cephalosporins (humans) and 30% increase in fully susceptible *E. coli* (food-producing animals) between 2013 and 2017. Secondary AMR indicators (humans) demonstrated reduced resistance to key antibiotics for *Klebsiella pneumoniae* and *Streptococcus pneumoniae* but increased carbapenem (*K. pneumoniae*) and penicillin *(S. pneumoniae*) resistance. Resistance to key antibiotics and proportion of ESBL-/AmpC-producing isolates decreased in indicator *E. coli* (food-producing animals).
For AMU, primary indicators demonstrated 5% reduction of total consumption of systemic antibiotics (humans) and 41% reduction of sales of veterinary antibiotics. Secondary AMU indicators showed increased use of broad-spectrum antibiotics in hospitals (humans) and decreased sales of quinolones, 3rd/4th generation cephalosporins and colistin (veterinary sector).

Conclusions:
Harmonised AMR and AMU surveillance indicators are valid tools for monitoring progress and areas where increased effort is needed to tackle AMR and reduce antimicrobial usage.

Disclosure: Nothing to disclose

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The Results of Interlaboratory Proficiency Testing for One Health Research Laboratory

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For one health consortium initiated by Korean Center for Disease Control, five research teams participated in it. They collected specific pathogens from pet, livestock, river, food and human. The pathogens were *E. coli, K. pneumoniae, P. aeruginosa, A. baumannii, A. nosocomialis, A. pittii, S. aureus, S. epidermidis, S. pseudintermedius, E. faecalis, E. faecium, Salmonella* spp. and *Shigella* spp. They were tested for antimicrobial susceptibility. About 700 isolates were collected every month, in which 5% isolates were selected and transfered to quality assurance center which conducted interlaboratory proficiency testing. If susceptable results were reported as resistant results and vice versa, it was decided as major error. If intermediate results were reported as resistant or susceptible results and vice versa, it was decided as minor error. For one year, major error rate was 2.6% and minor error rate was 3.7%. The major causes of error were the errorneous reading of inhibition zone by autoscanner and e-test reading error of specific antimicrobials.

Disclosure: Nothing to disclose