Antibiotic resistance in a One Health approach

Université Paris Cité

Inserm

AP-HP. Nord Université de Paris

iame RESEARCH CENTER ON INFECTIOUS DISEASES

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Disclosures

Ancien consultant pour **DaVolterra**

Ancien membre du Conseil Scientifique de Pathoquest and **MaaT Pharma**

Fonds de recherche de la part de bioMérieux

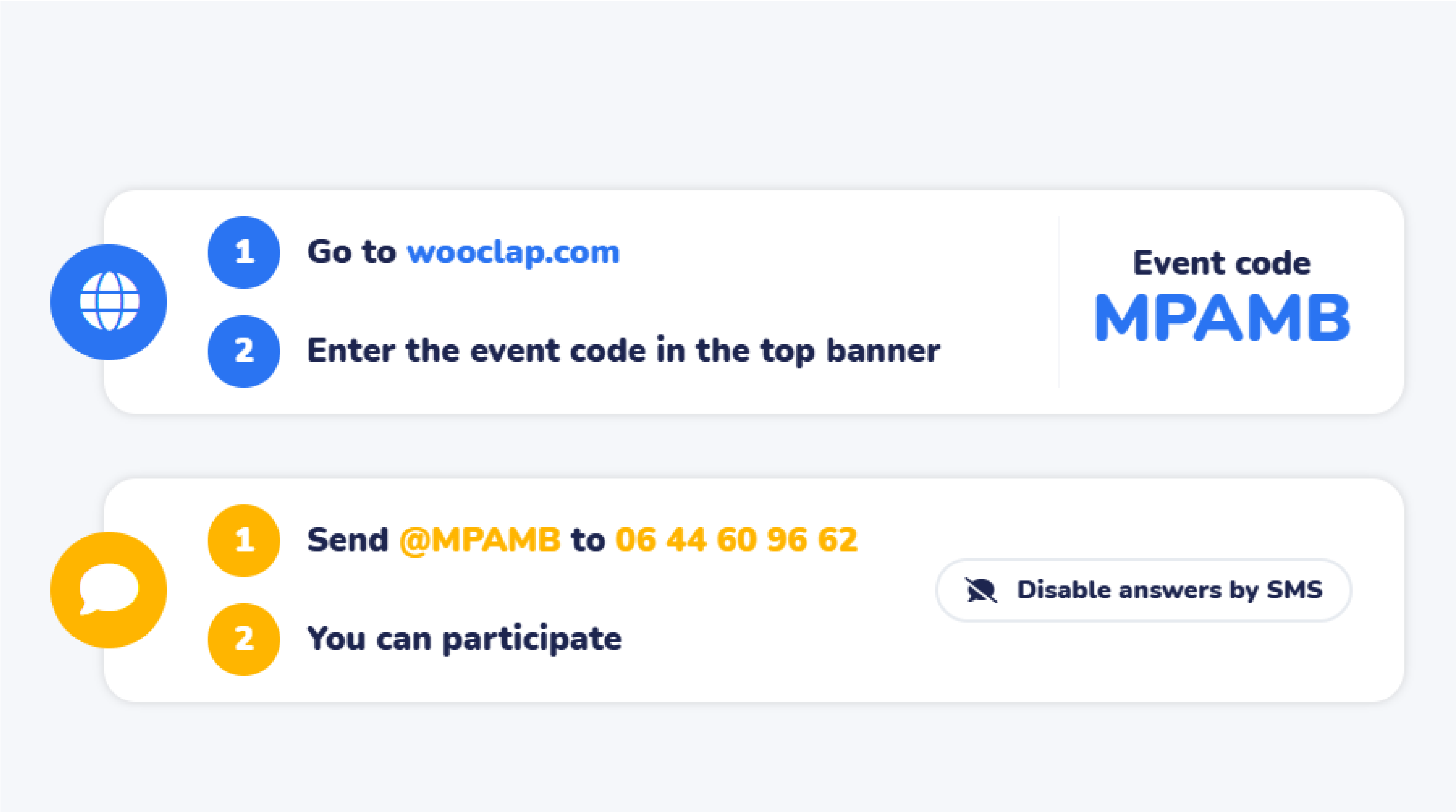
Interventions pour Mobidiag, Correvio, MSD, EUMEDICA, Shionogi

Frais de déplacements de la part Novartis, Sanofi

Plan

- Antibiotics: history, consumption
- Antibiotic resistance
- Making of a superbug: example of Mcr
- Do we share multidrug-resistant bacteria with animals?
- Role of travels
- Role of the microbiota
- Making of a superbug: example of Mcr
- Actions
- Conclusions and reading suggestions

Interactivity



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Antibiotics

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Question

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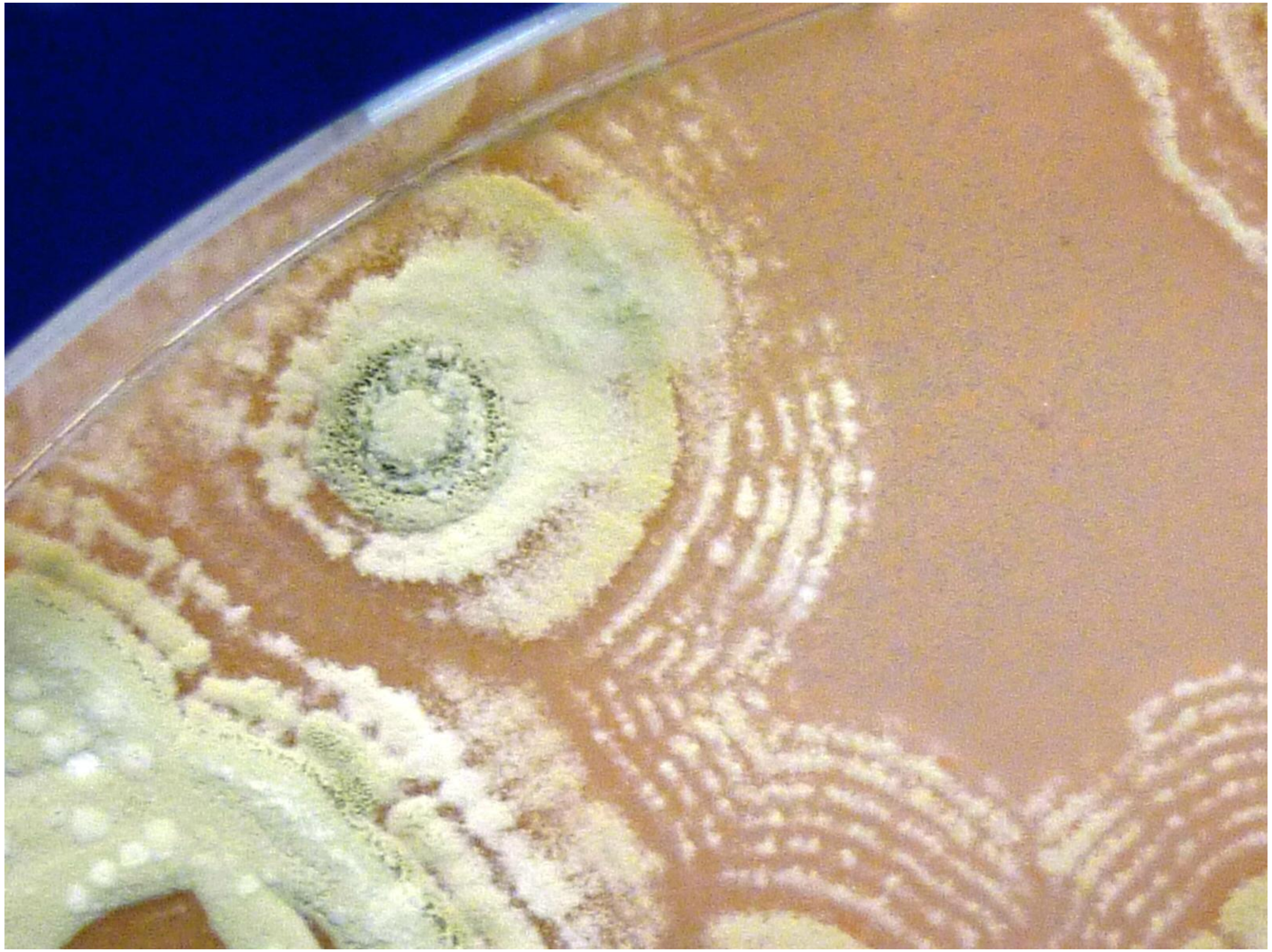
Antibiotics are ancient! Example of *Streptomyces*



Many antibiotics are produced by ***Streptomyces***: aminoglycosides, glycopeptides (vancomycin), macrolides, tetracyclines, rifamycins, chloramphenicol, fosfomycin, daptomycin and clavulanic acid!

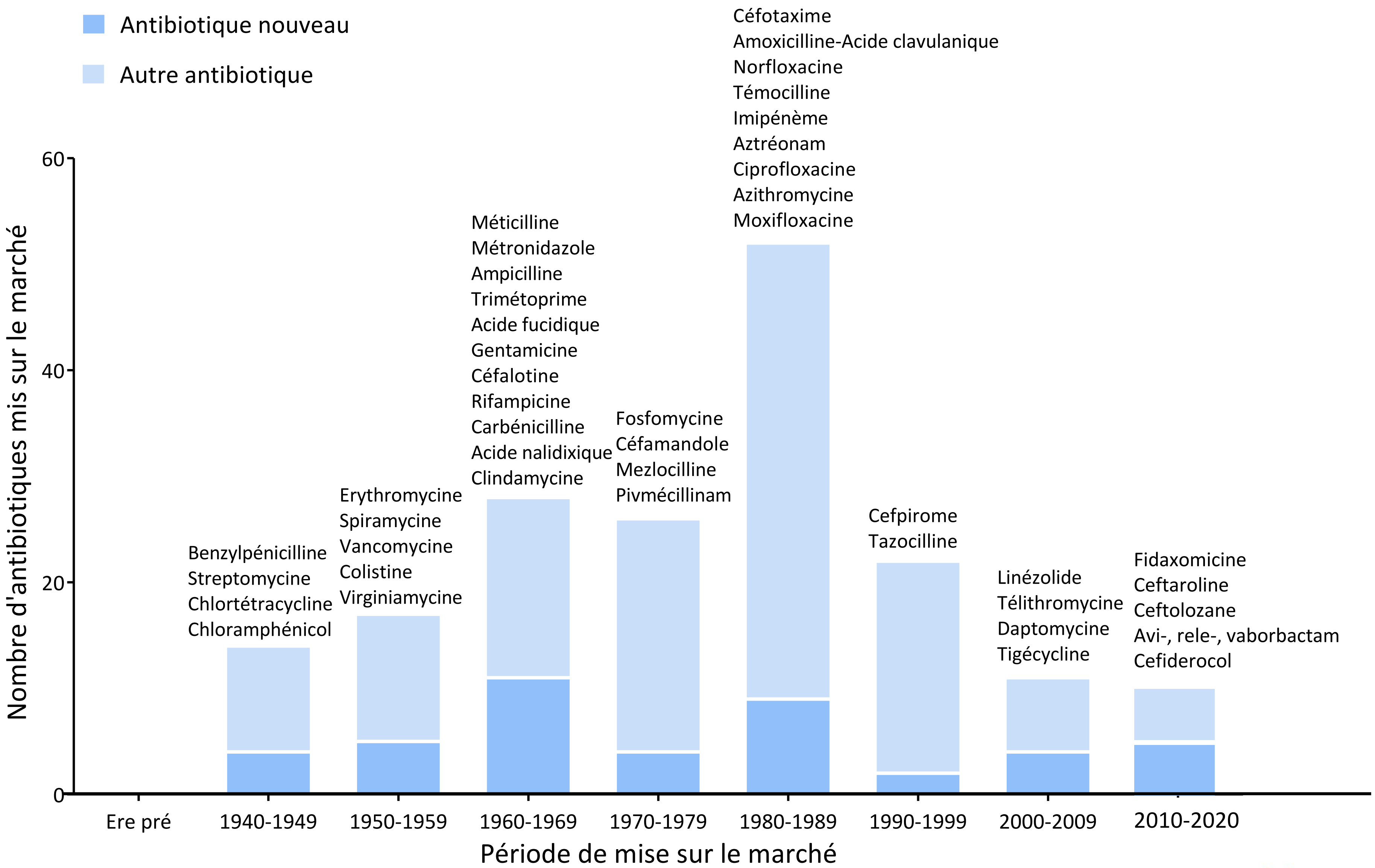
Other environmental microorganisms produce antibiotics.

Credit: Wikipedia

A brief history of antibiotics

- Antibiotics are recent medicines!
- 1928: discovery of penicillin by Alexander Fleming in England
- Beginning of massive use after the Second World War
- Golden age of antibiotics: 1960s to 1980s
- Then, the market dried up: few new molecules were put on the market
- Failing economic model for antibiotics
- At the same time, there is a continuous increase in antibiotic resistance

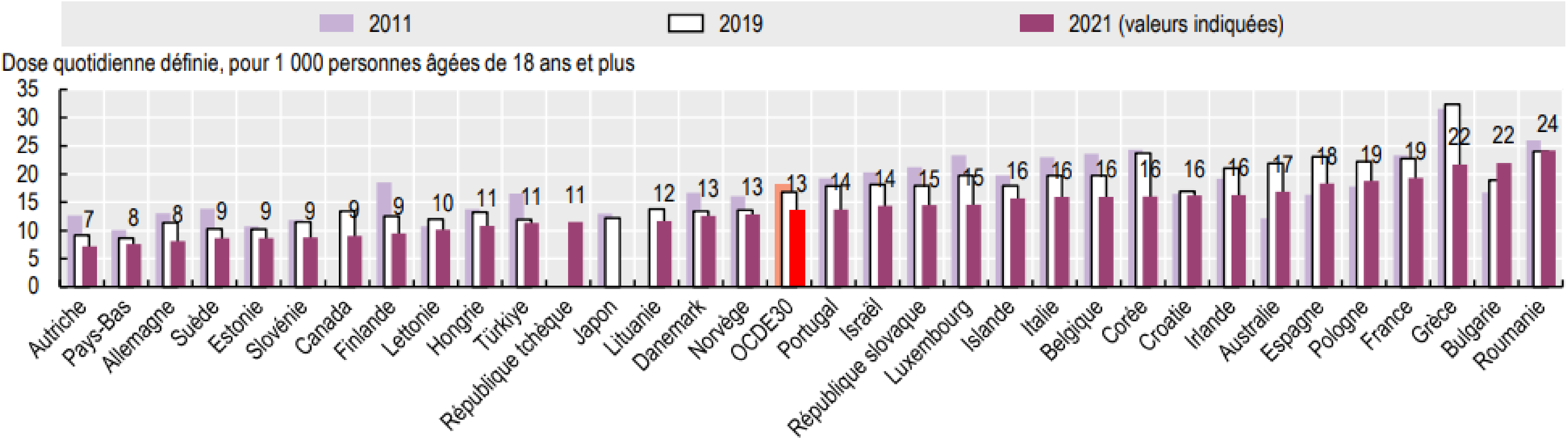
But their use by humans is recent



Source: Wikipedia

Consumption of antibiotics

Volume of antibiotics prescribed in 2021



High consumption of antibiotics in France (above the OECD average)

Source : Panorama Santé 2023, OCDE

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Consumption of antibiotics

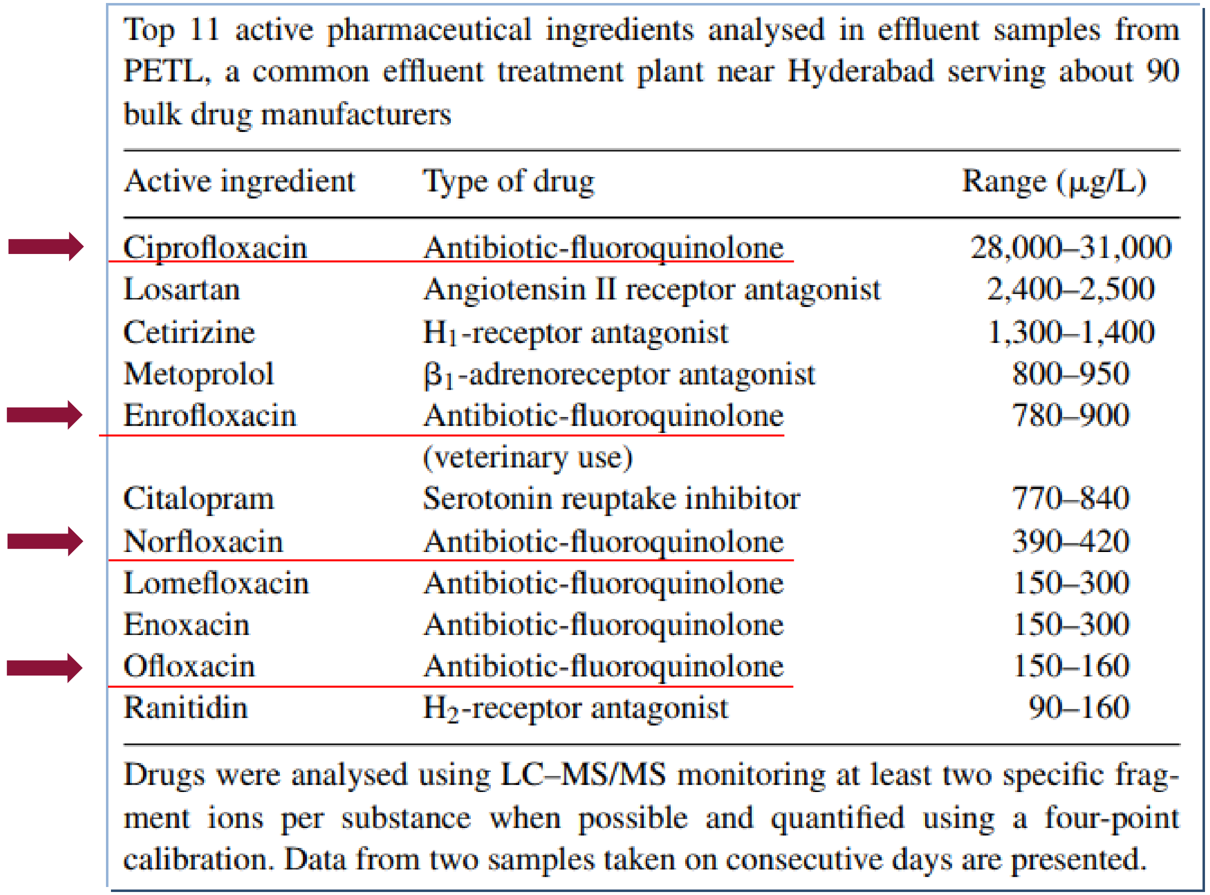
Consumption by animals :
Treatment of infections
Prophylaxis
Growth promoters (banned in Europe since 2006)

Classes of antibiotics used in veterinary medicine are mostly **common** with those used in human medicine.

AMR Review, O'Neill 2016

Antibiotics in the environment

Analysis of effluents from a wastewater treatment plant serving a large generic drug production site (Hyderabad, India)



Top 11 active pharmaceutical ingredients analysed in effluent samples from PETL, a common effluent treatment plant near Hyderabad serving about 90 bulk drug manufacturers

Active ingredient	Type of drug	Range (μg/L)
Ciprofloxacin	Antibiotic-fluoroquinolone	28,000–31,000
Losartan	Angiotensin II receptor antagonist	2,400–2,500
Cetirizine	H_1-receptor antagonist	1,300–1,400
Metoprolol	β_1-adrenoreceptor antagonist	800–950
Enrofloxacin	Antibiotic-fluoroquinolone (veterinary use)	780–900
Citalopram	Serotonin reuptake inhibitor	770–840
Norfloxacin	Antibiotic-fluoroquinolone	390–420
Lomefloxacin	Antibiotic-fluoroquinolone	150–300
Enoxacin	Antibiotic-fluoroquinolone	150–300
Ofloxacin	Antibiotic-fluoroquinolone	150–160
Ranitidin	H_2-receptor antagonist	90–160

Drugs were analysed using LC–MS/MS monitoring at least two specific fragment ions per substance when possible and quantified using a four-point calibration. Data from two samples taken on consecutive days are presented.

Larsson, D.G.J., et al. *J Hazard Mater* **148**, 751–755 (2007). Credit: Google

Antibiotic resistance

Question

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Antibiotic resistance is not a recent phenomenon

LETTER

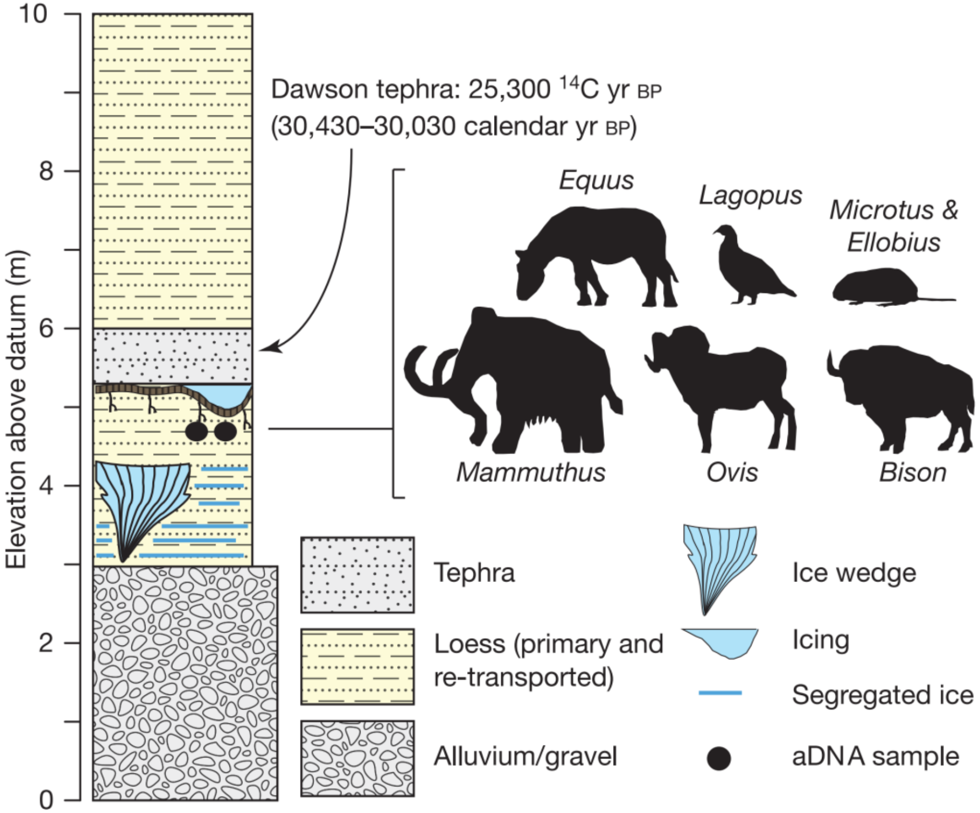
doi:10.1038/nature10388

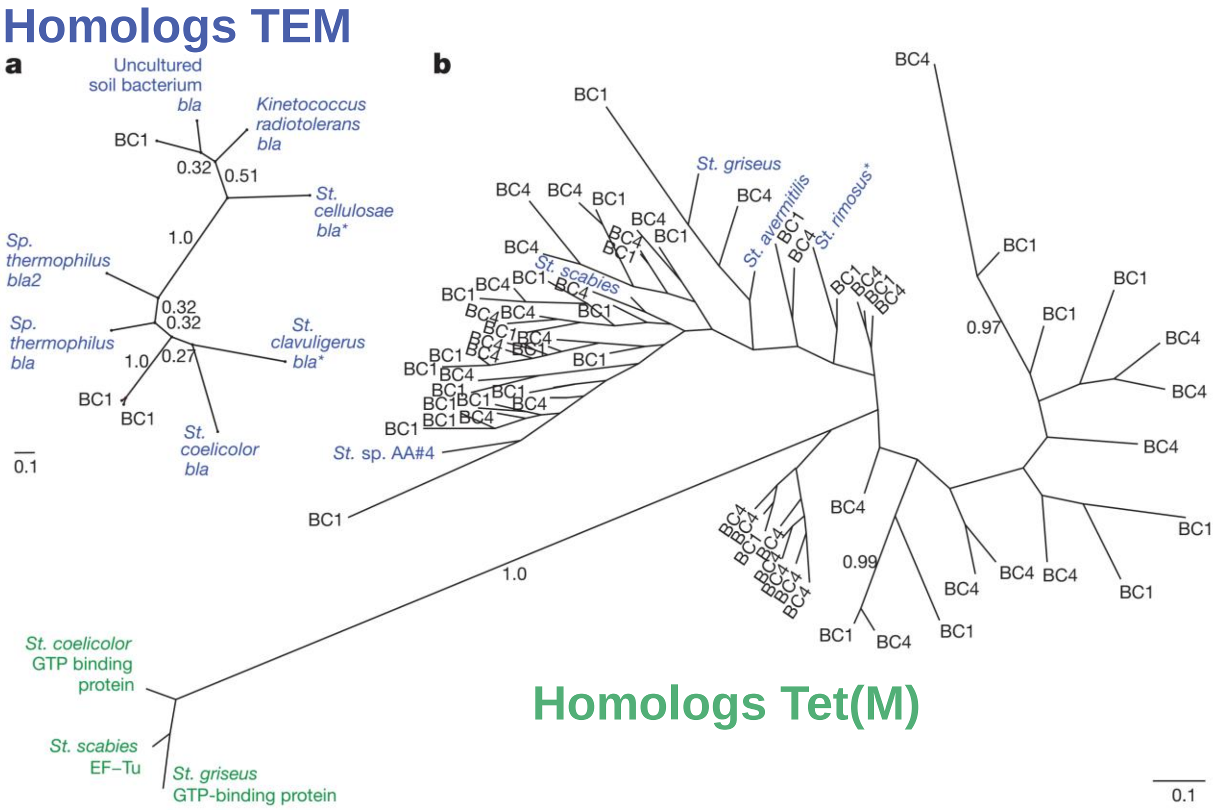
Antibiotic resistance is ancient

Vanessa M. D'Costa[1,2]*, Christine E. King[3,4]*, Lindsay Kalan[1,2], Mariya Morar[1,2], Wilson W. L. Sung[4], Carsten Schwarz[3], Duane Froese[5], Grant Zazula[6], Fabrice Calmels[5], Regis Debruyne[7], G. Brian Golding[4], Hendrik N. Poinar[1,3,4] & Gerard D. Wright[1,2]

D'Costa VM et al. Nature. 2011;477:457-61.

Antibiotic resistance is not a recent phenomenon

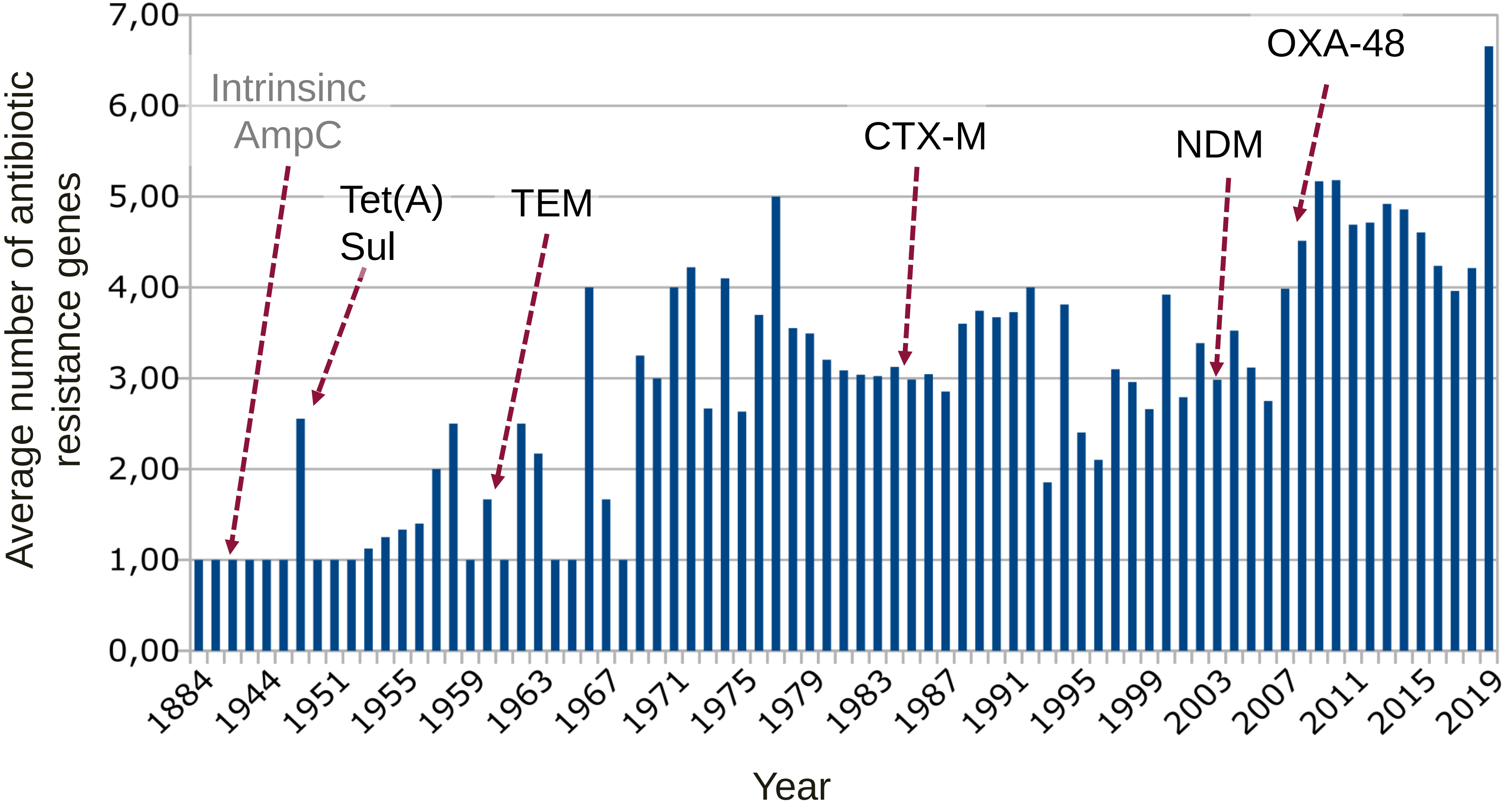




Presence of TEM (beta-lactamase), Tet(M) (tetracycline resistance) and also VanA (glycopeptide resistance) homologs (*not showed*)

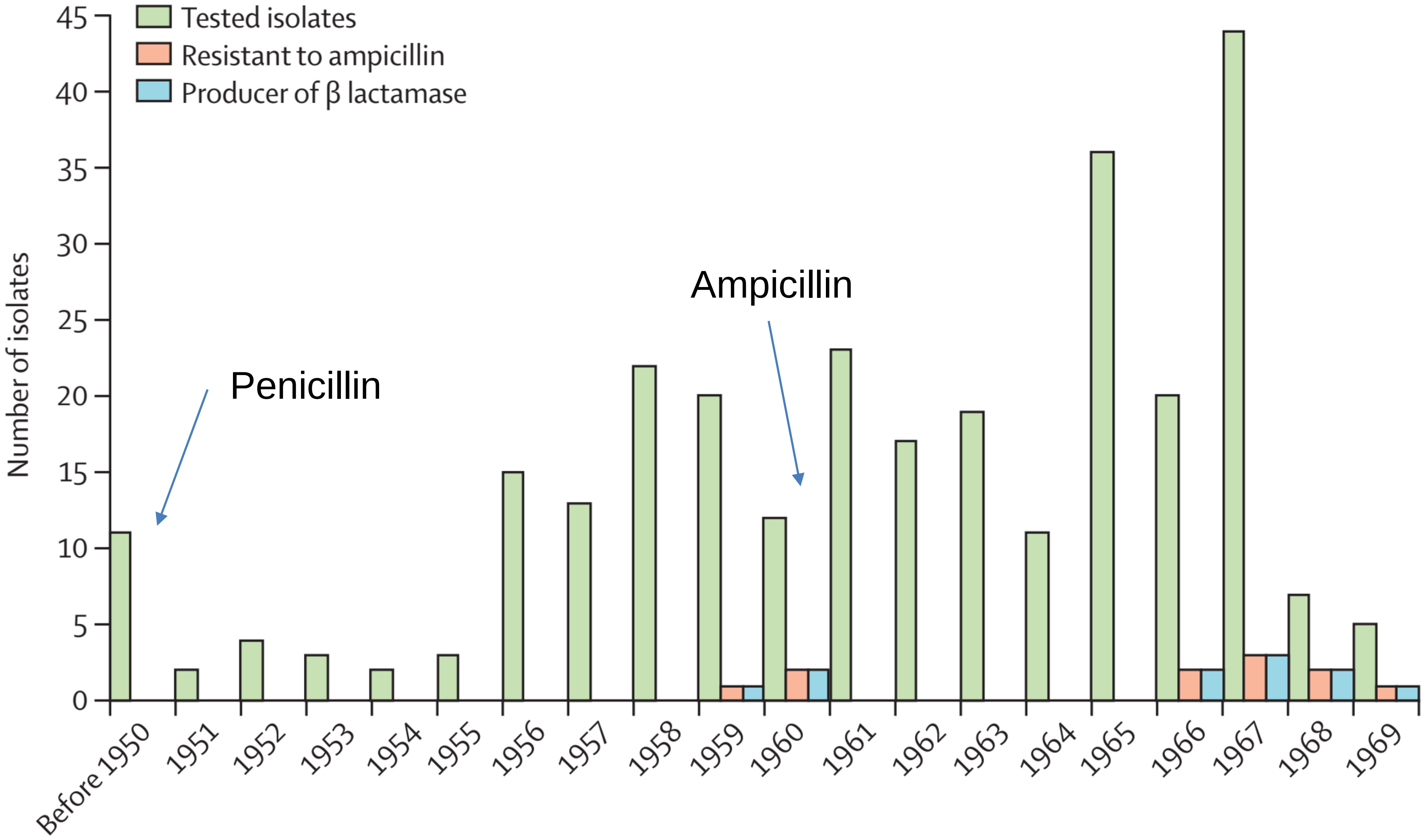
D'Costa VM et al. Nature. 2011;477:457-61.

Resistance in pathogens is more recent: example of *E. coli*



Petitjean and Ruppé. Unpublished data.

Resistance in pathogens is more recent: example of *Salmonella*



Tran-Dien A et al. Lancet Infect Dis. 2018;18:207-14.

How to define antibiotic resistance? Different point of views!

- Bacteria: Resistance to naturally occurring antibiotics
- Ecologists: Resistance based on MIC distribution
- Humans: Clinical resistance based on MICs and non-toxic concentrations achievable in the body.

Martínez JL et al. Nat Rev Microbiol. 2015;13:116-23.

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Question

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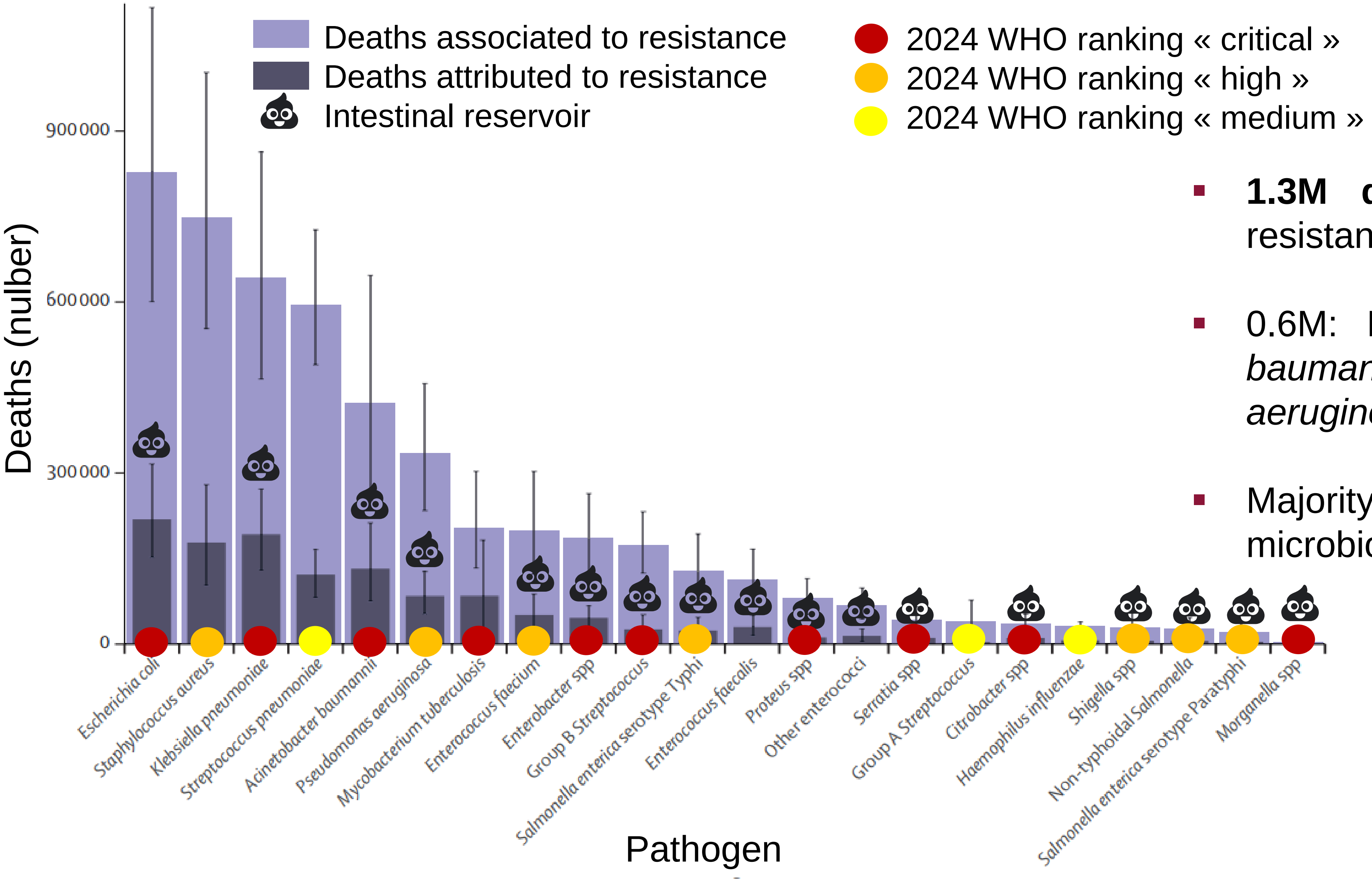
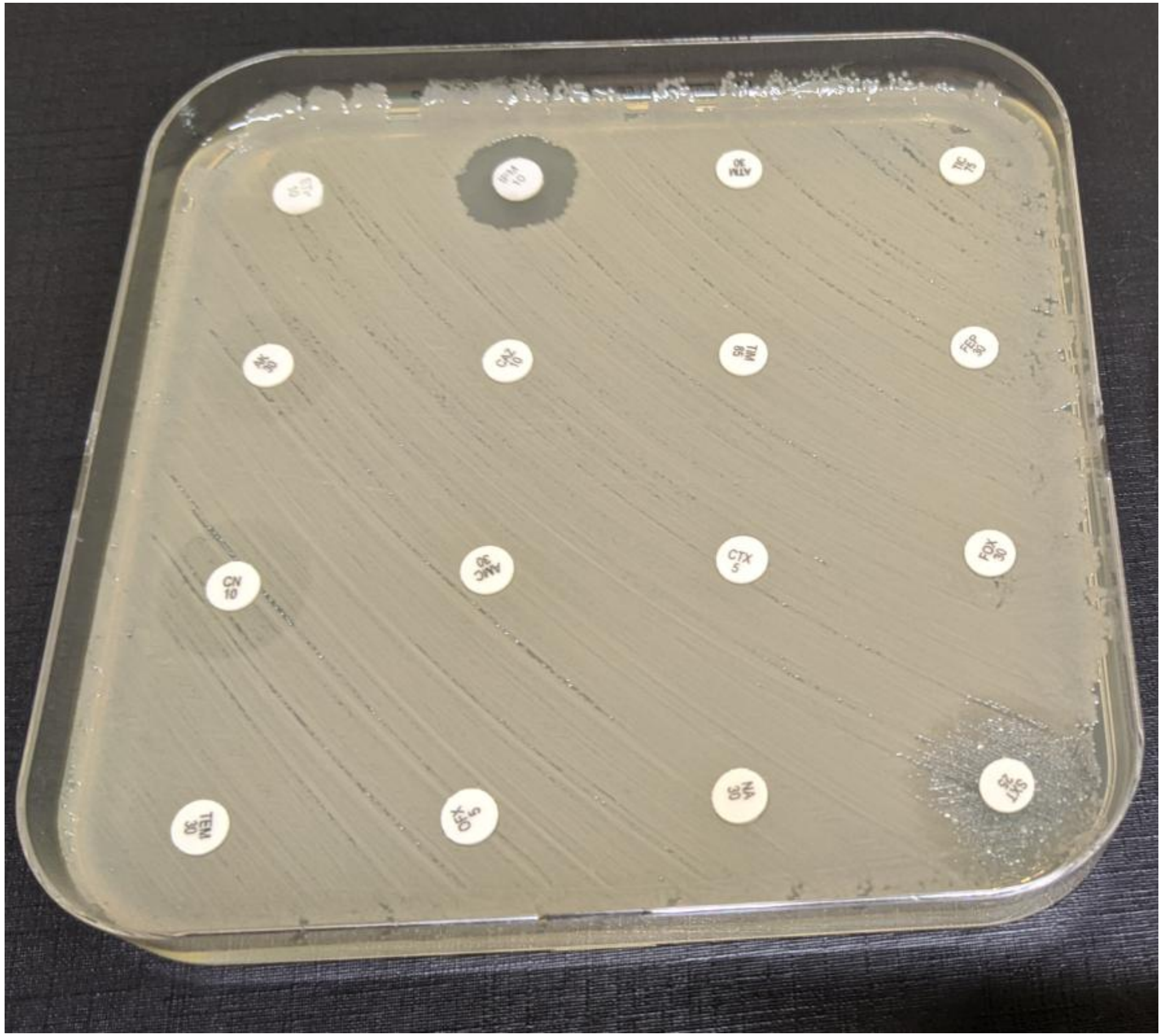
The threat of antibiotic resistance lies in the gut



- **1.3M deaths** attributed to antibiotic resistance (estimated 40M by 2050)
- 0.6M: Enterobacterales, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Proteobacteria)
- Majority of bacteria **present in the gut** microbiota before causing infections

Adapted from **Antimicrobial Resistance Collaborators**. et al. *The Lancet* **399**, 629–655 (2022).

Extensively resistant bacteria



There are bacteria **resistant to all available antibiotics**, both in France and abroad.

In case of infection: how to treat? In low-resources settings?

These bacteria are isolated in hospitals but also in the **community**

Question

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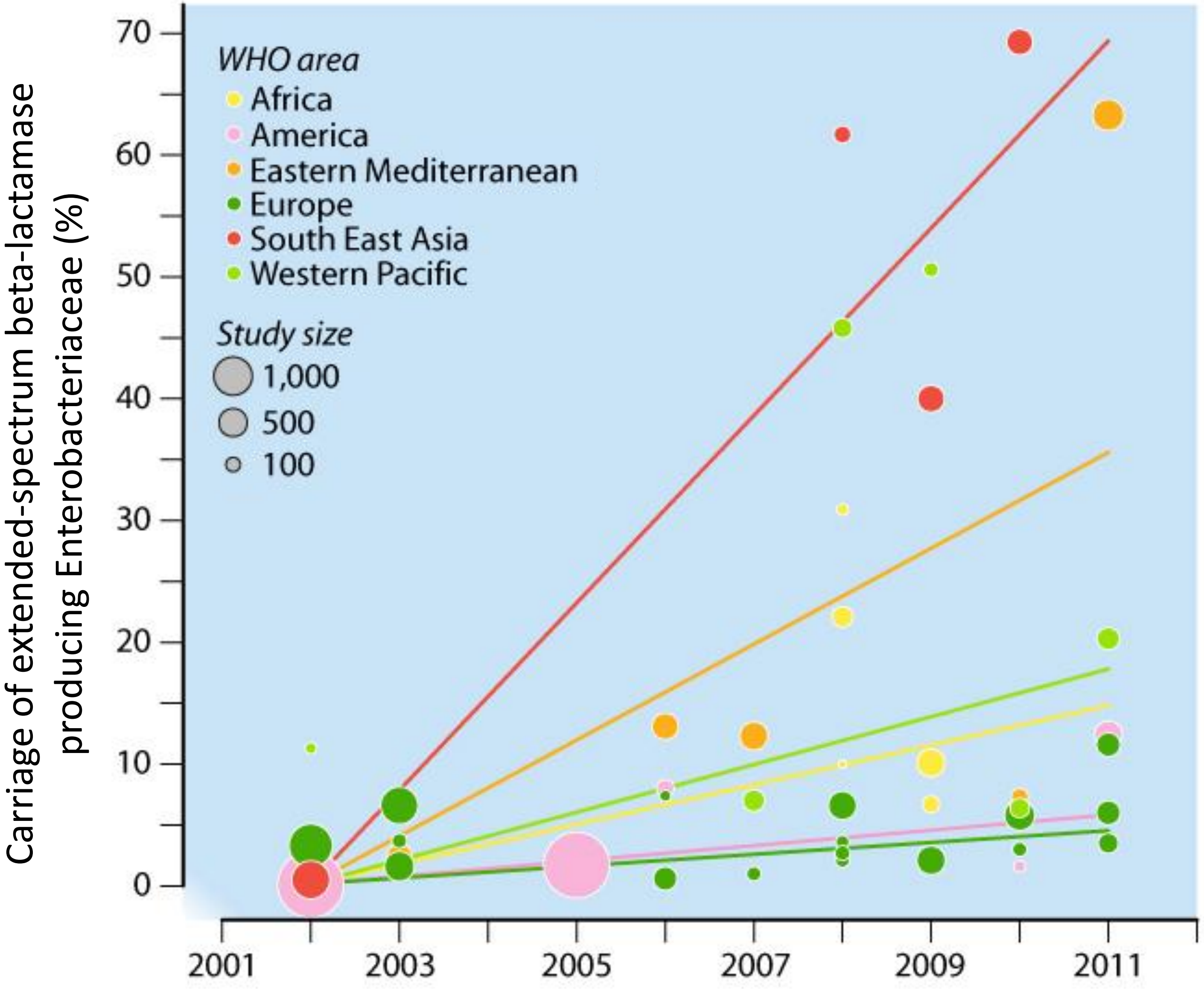
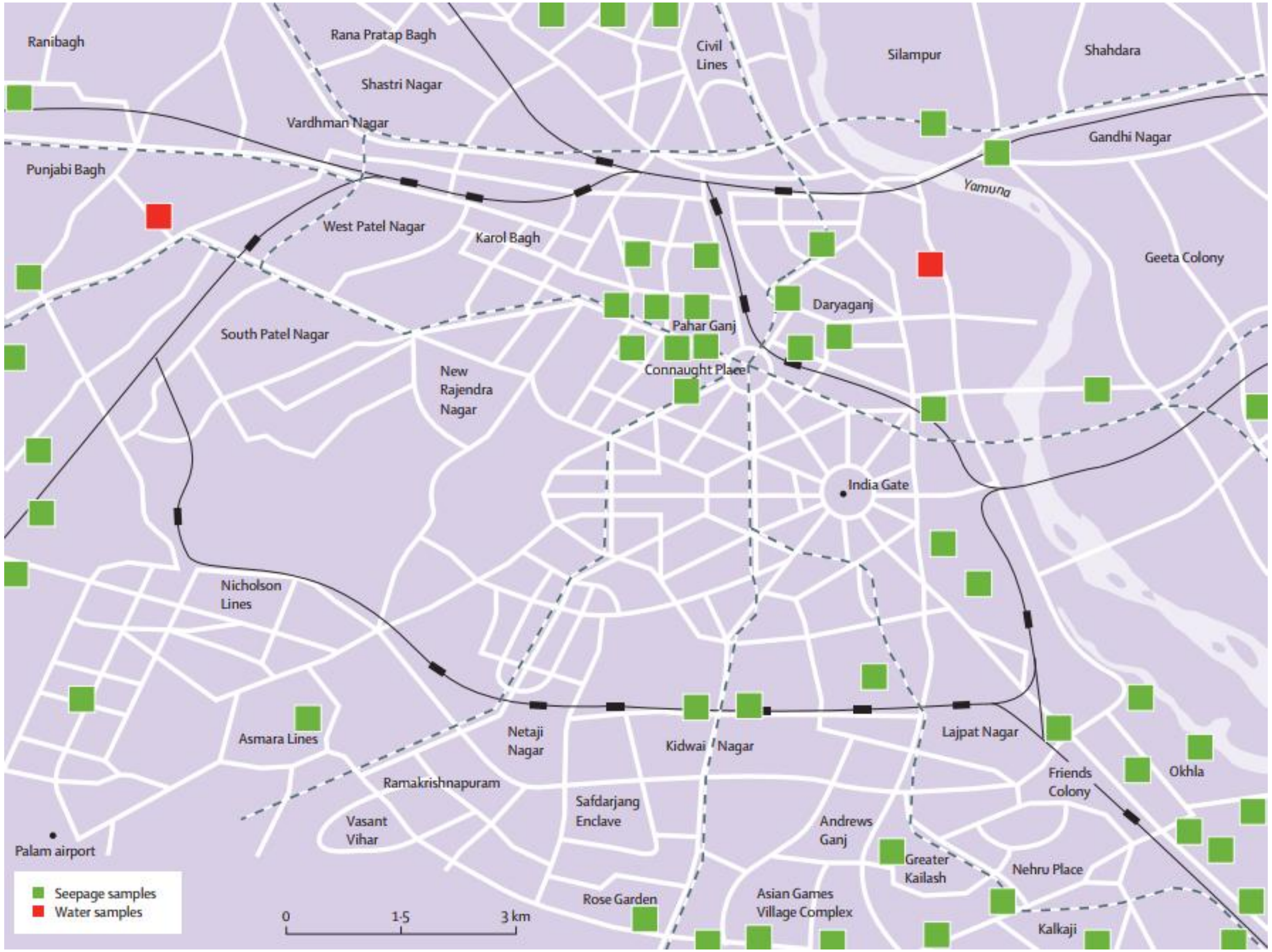
Multidrug-resistant Enterobacterales have hit developing countries



Woerther, P.-L., *et al. Clin. Microbiol. Rev.* **26,** 744–758 (2013).

Multidrug resistant bacteria in the environment



New Delhi (India): detection of carbapenemase producing bacteria (NDM gene)

12/171 (7%) water points positive
2/50 (4%) public taps positive

Bacterial species: *E. coli*, *Shigella boydii* and *Vibrio cholerae*.

Walsh, T.R., et al. *Lancet Infect Dis* **11**, 355–362 (2011).

Do we share the resistant bacteria with animals?

Question

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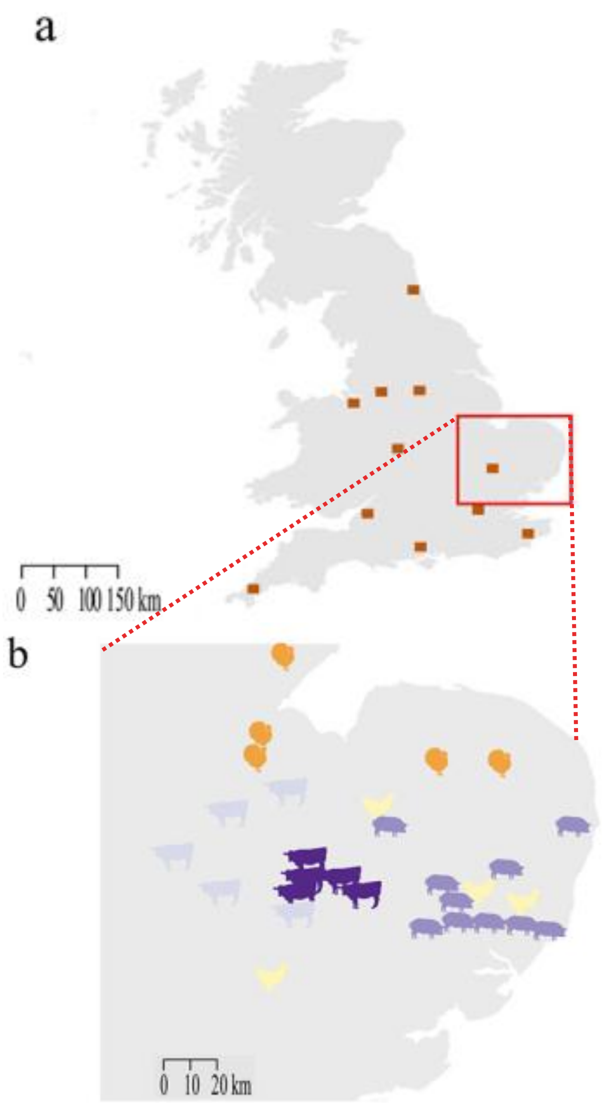
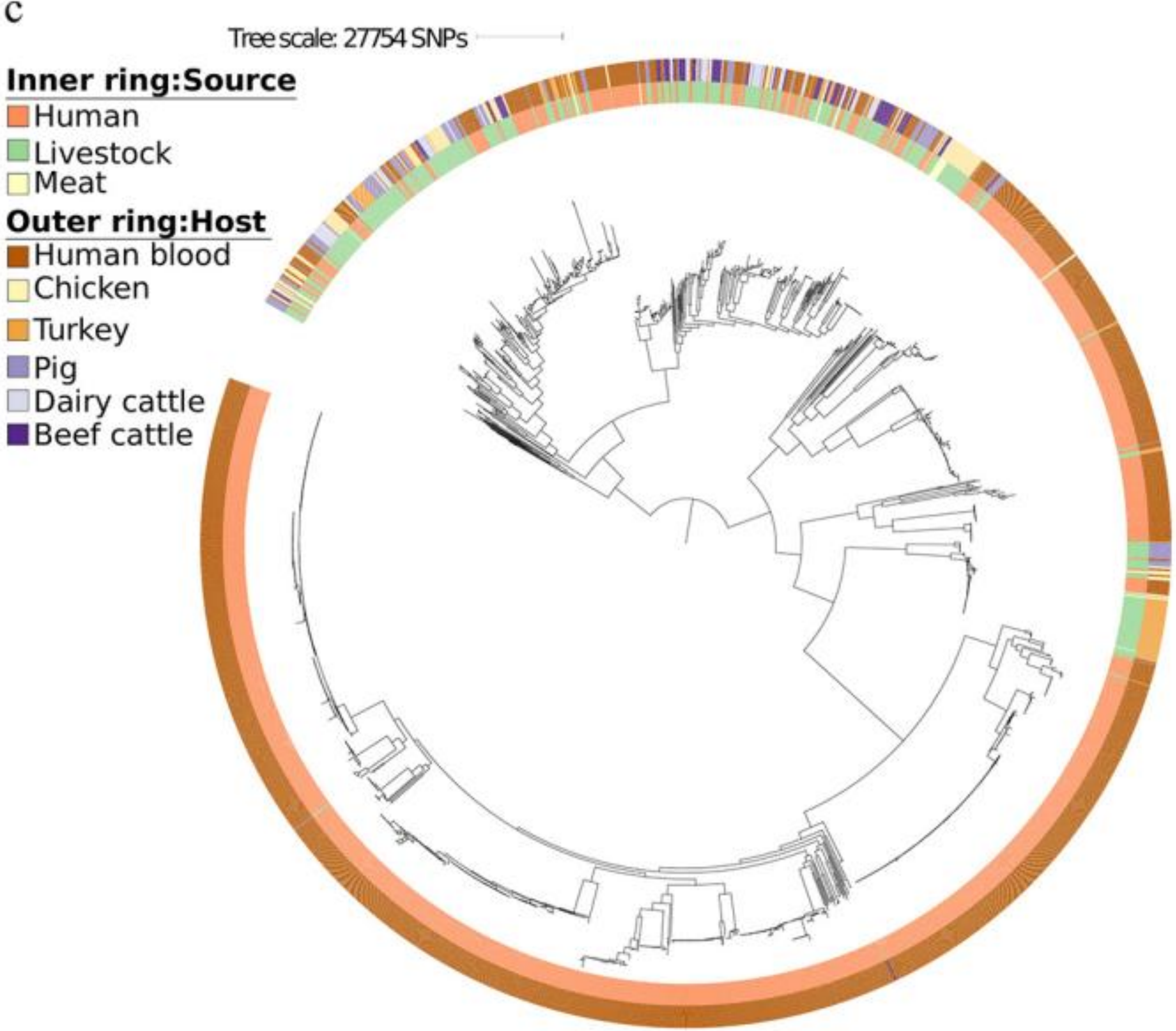
Comparison of human and animal *E. coli* in England, 2014-2015



- One Health genomic surveillance of *E. coli* shows distinct lineage and mobile genetic elements between humans and livestock
- Sequencing of 431 *E. coli* including 155 ESBL producers from cattle and 1517 *E. coli* associated with bacteremia
- All from the UK and isolated between 2014 and 2015

Ludden, C. et al. *mBio* (2019).

Human and animal *E. coli* are indeed distinct!



Median distance in number of SNPs 41.658

- 2 human strains with <15 SNPs compared to animal strains
- Significant frequency of strains with <5 SNPs within the animal sector

Ludden, C. et al. *mBio* (2019).

Travels and antibiotic resistance

Question

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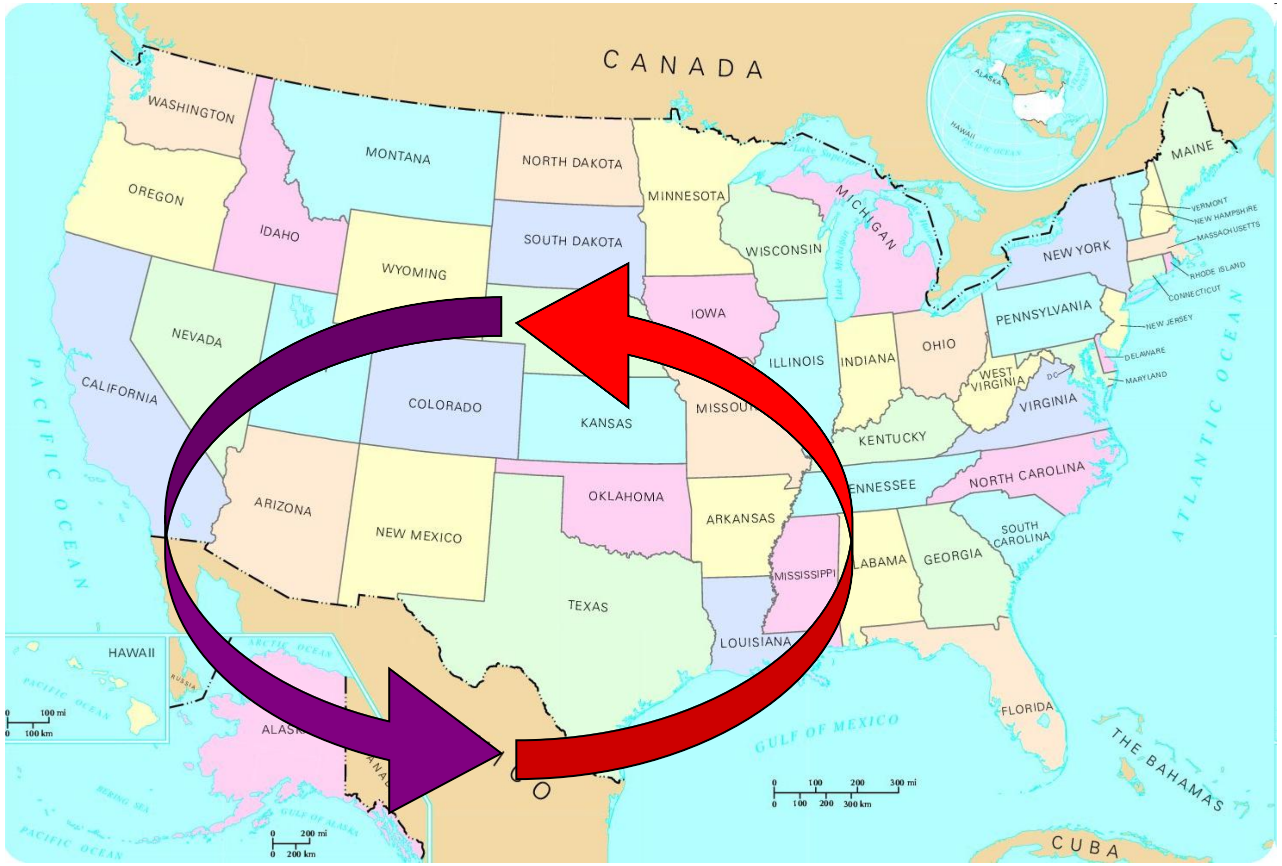
Travelers convey antibiotic resistant bacteria



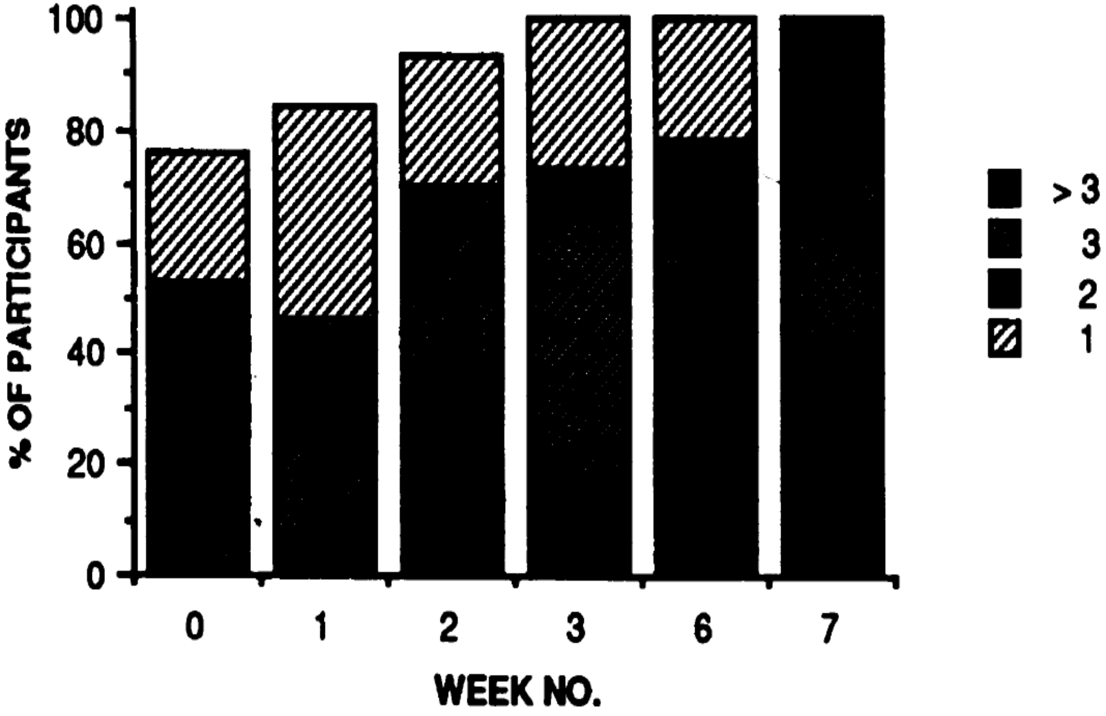
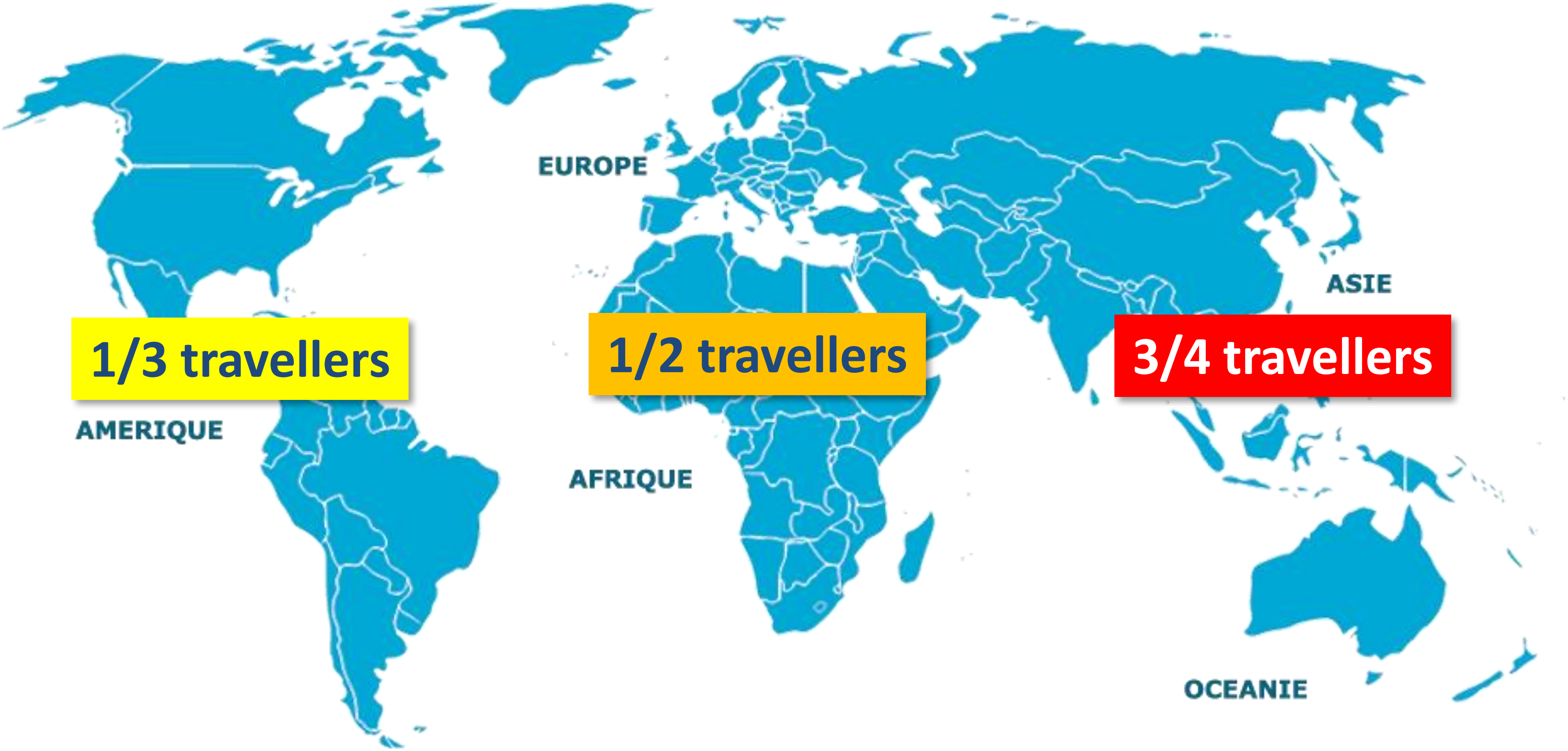


FIG. 1. Resistance to multiple antimicrobial agents among fecal *E. coli* strains. Percentages of participants with *E. coli* resistant to multiple antimicrobial agents increased with time. Bars indicate the presence of *E. coli* strains that are singly or collectively resistant to 1, 2, 3, or >3 agents.

Murray, B.E., et al. *Antimicrob. Agents Chemother.* **34**, 515–518 (1990).

Bad bugs travel with travellers

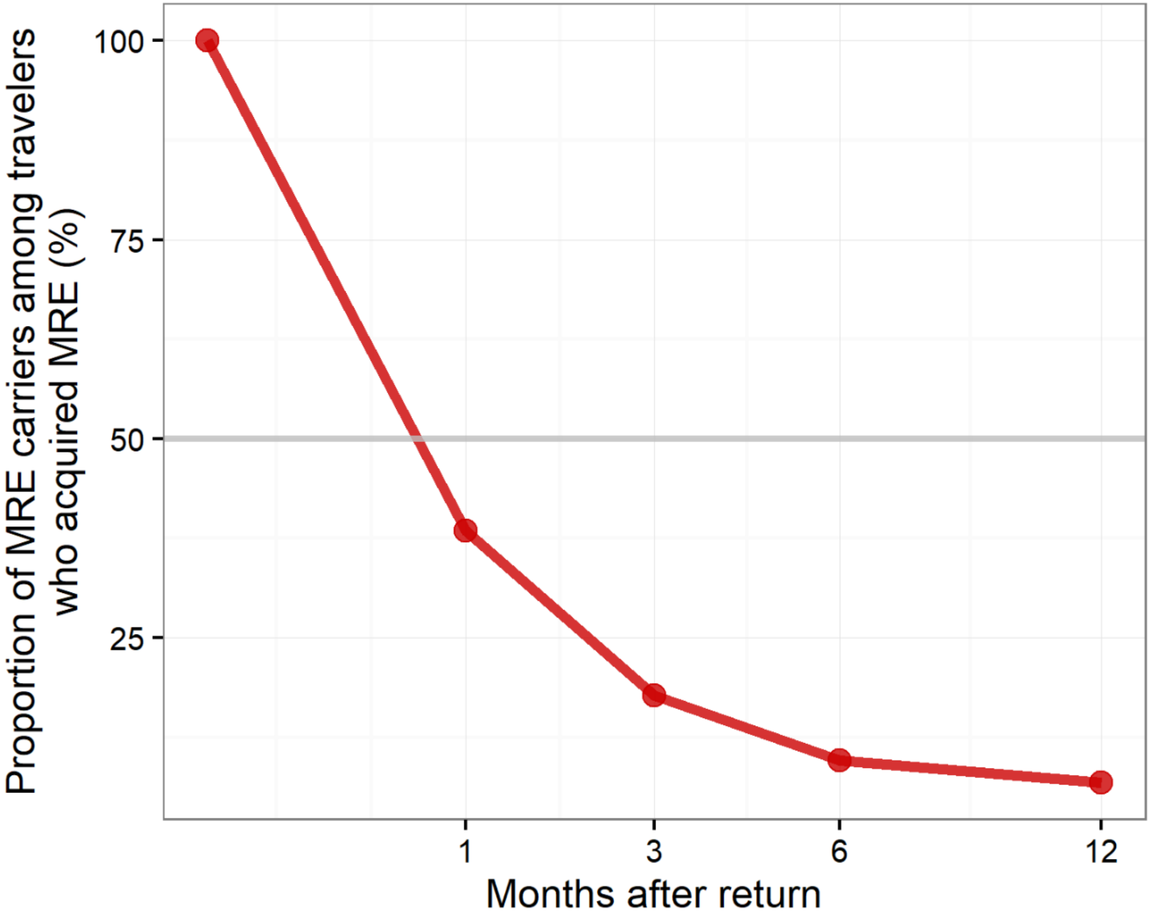
574 travellers to tropical regions
(screened negative for multidrug-resistant Enterobacteriaceae before departure)
MRE acquisition rate 51% (n=293)



Ruppé, E. *et al. Clin Infect Dis.* **61,** 593–600 (2015).

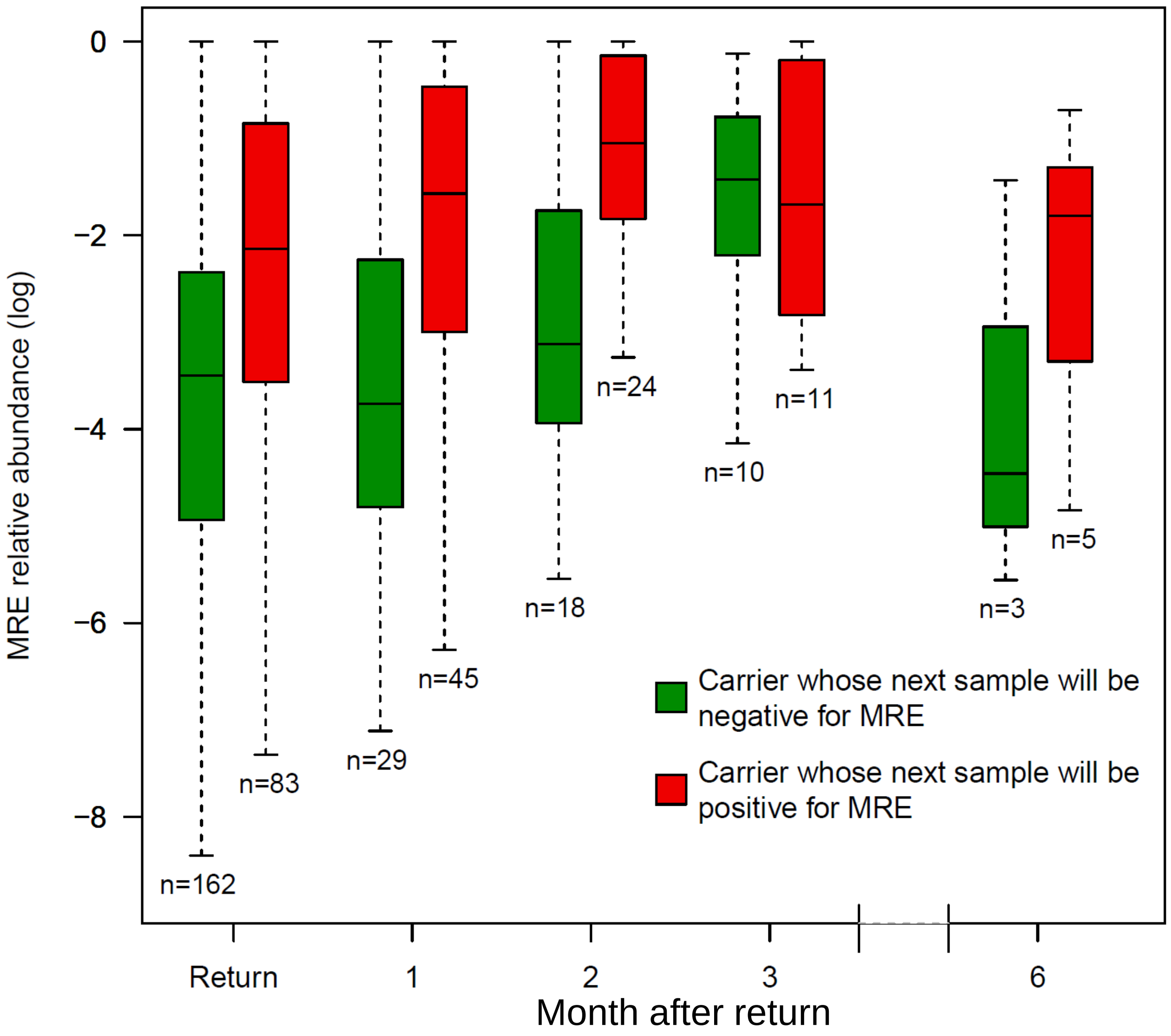
Duration of carriage

Most travellers are quickly cleared of ESBL-E carriage
But some remains long term carriers. Role of the intestinal microbiota?



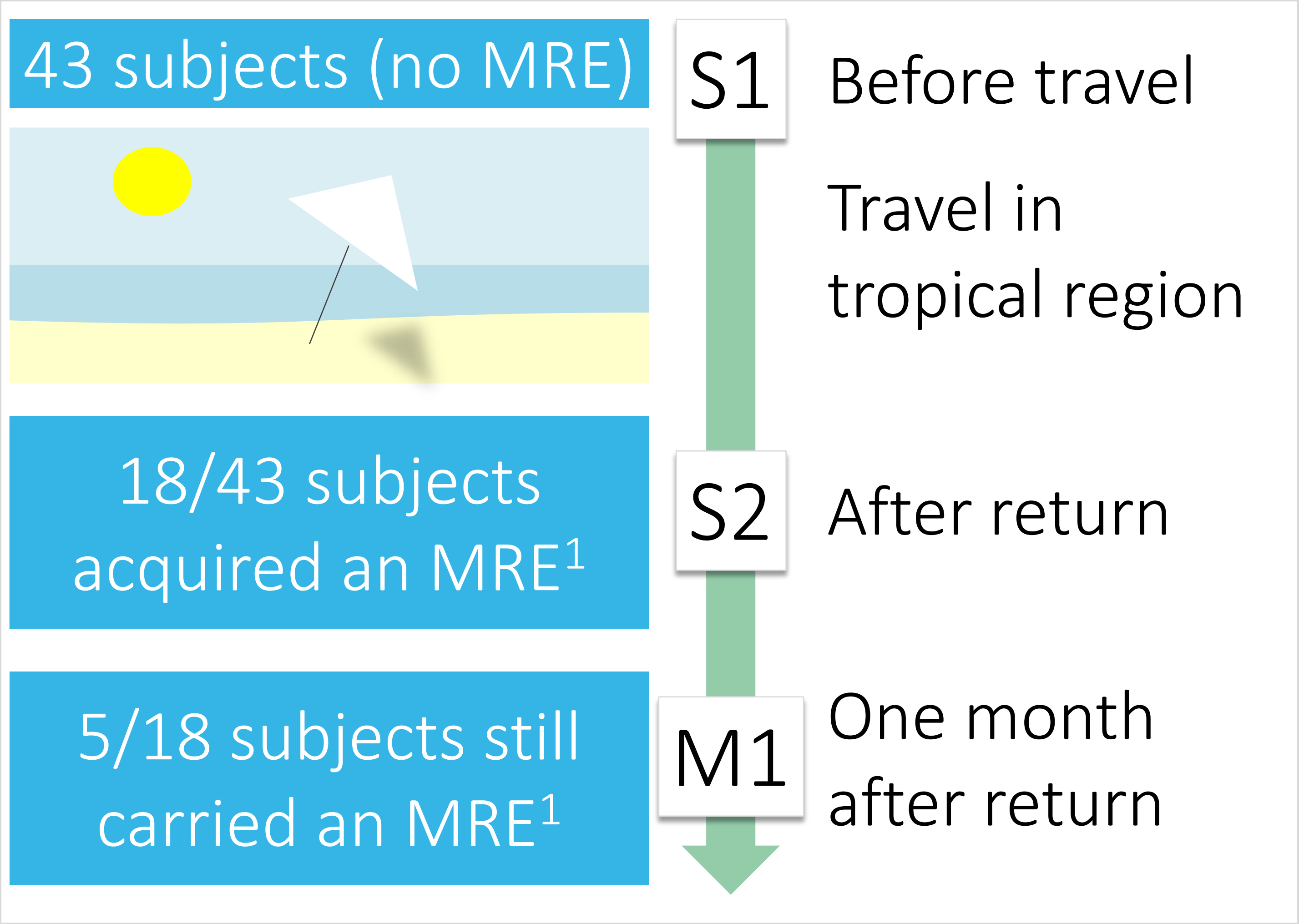
Arcilla, M. S. *et al. The Lancet Infectious Diseases* (2016); **Ruppé, E**. *et al. Clin Infect Dis.* **61,** 593–600 (2015).

The more abundant the MRE, the longer it takes to be cleared



Ruppé, E. *et al. Clin Infect Dis.* **61,** 593–600 (2015).

Duration of carriage, role of the microbiota?



43 subjects (no MRE)

S1 Before travel

Travel in tropical region

18/43 subjects acquired an MRE[1]

S2 After return

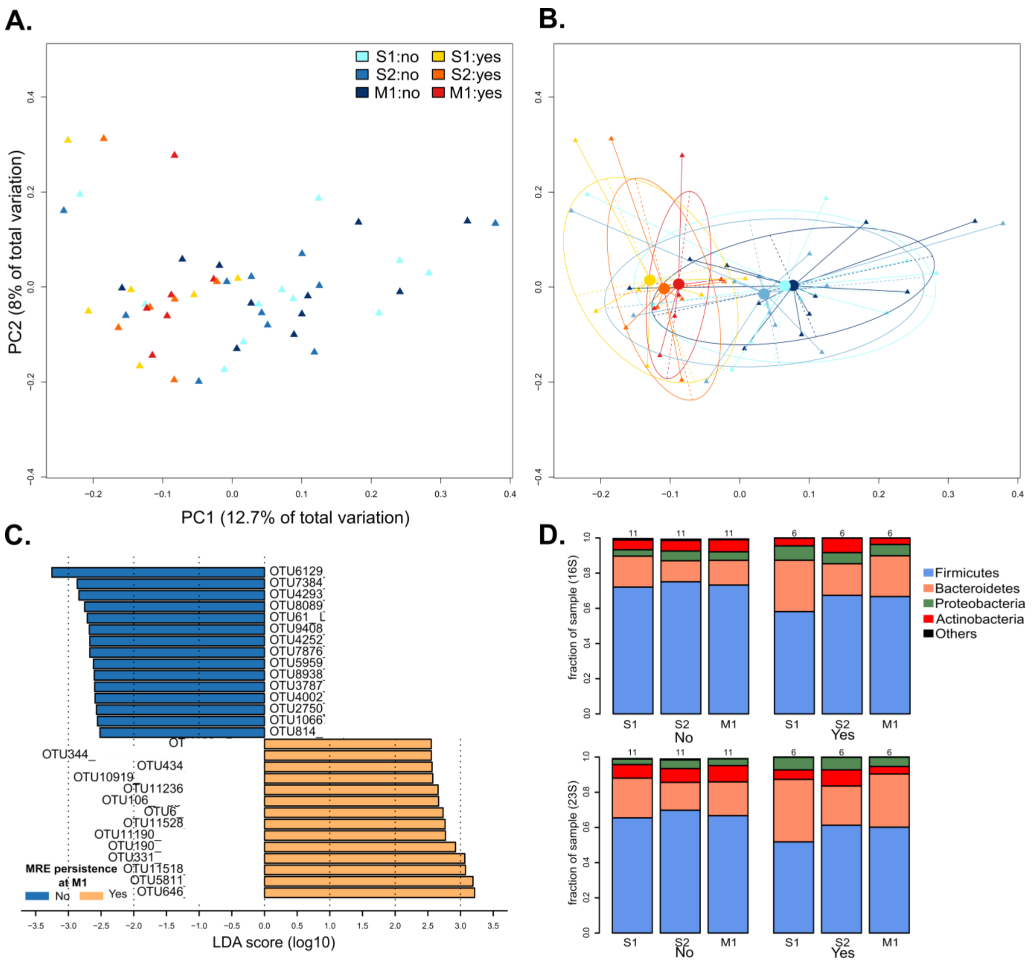
5/18 subjects still carried an MRE[1]

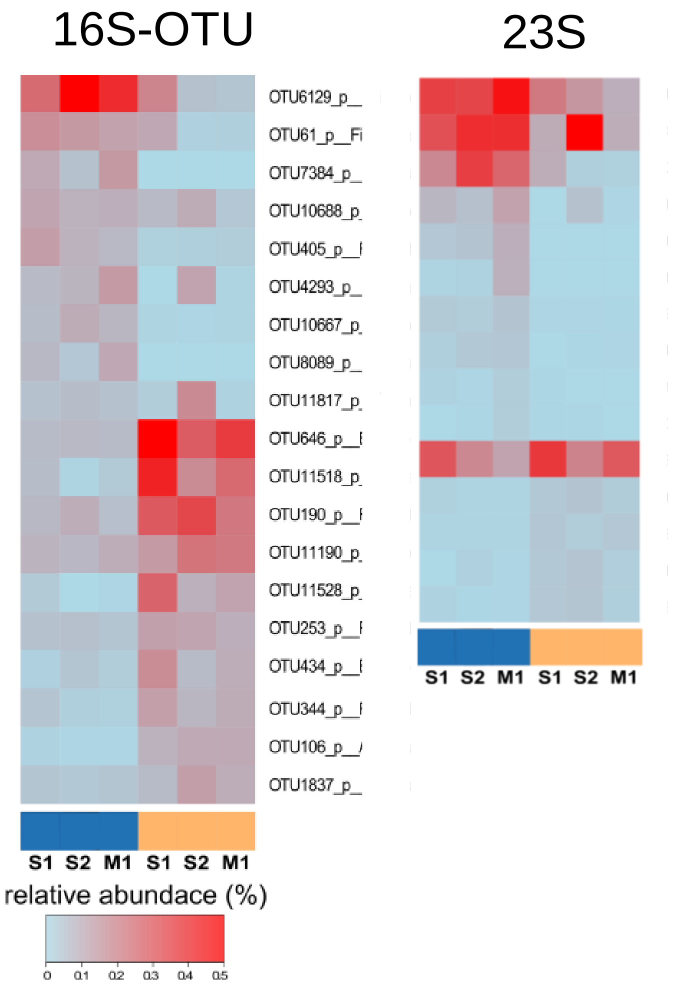
M1 One month after return

Ruppé, E. *et al. Clin Infect Dis.* **61,** 593–600 (2015).

Duration of carriage, role of the microbiota?

- Some bacteria seem to be associated with the intestinal clearance of MRE one month after travel
- The same pattern is found before travel

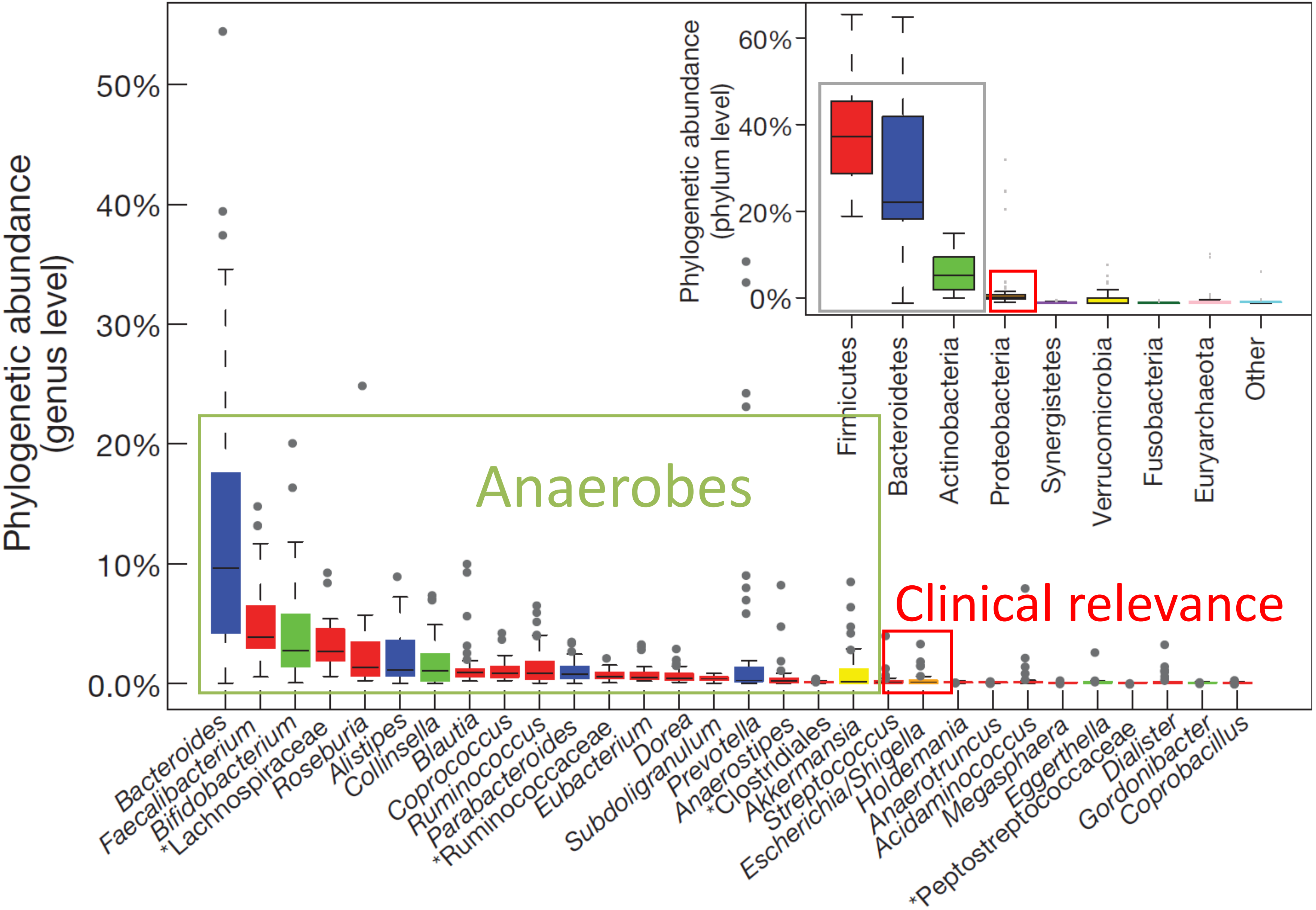




Leo, S. et al. *Gut Microbes* 1–11 (2019).

Role of the microbiota

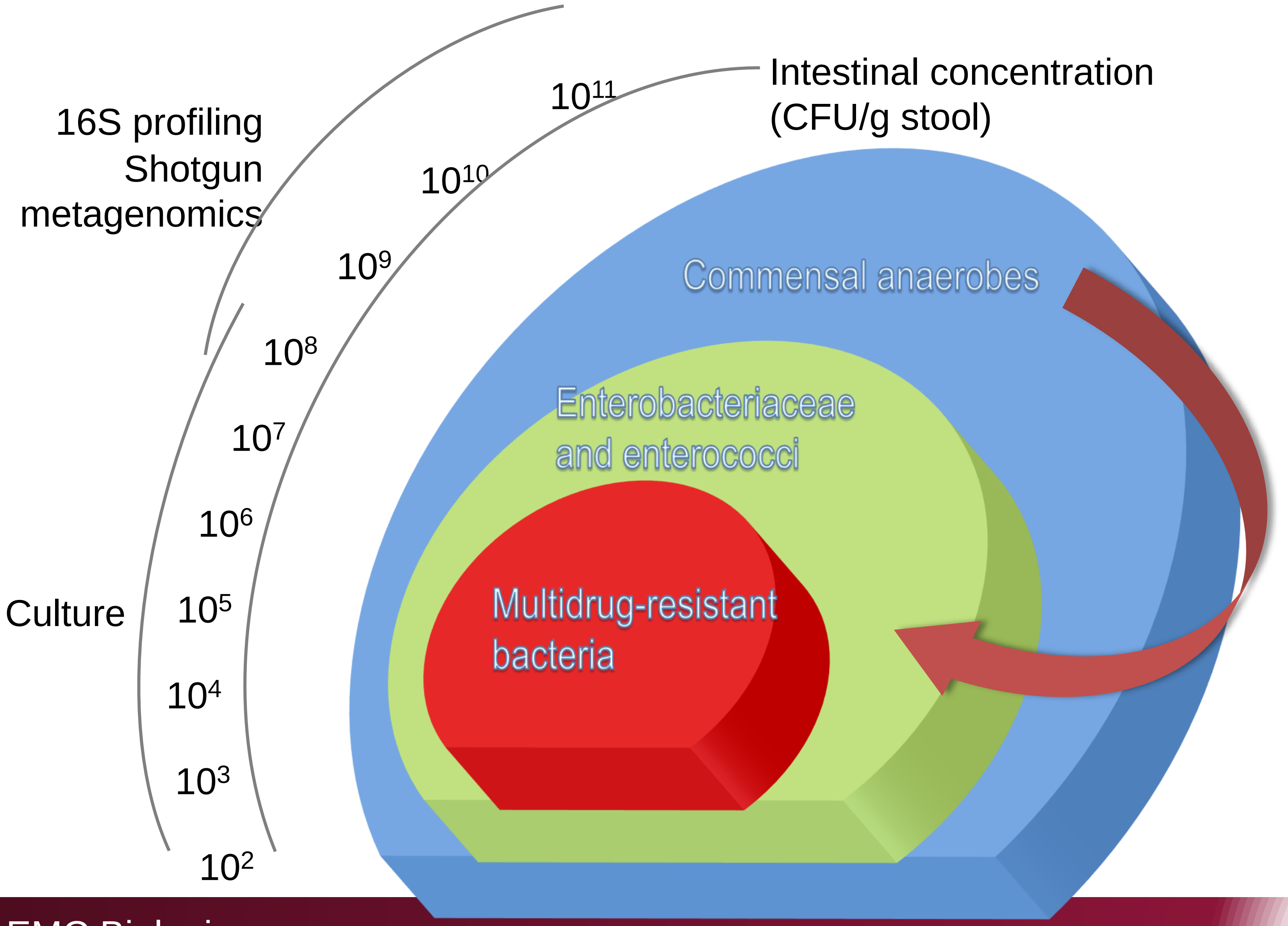
Composition of the intestinal microbiota



- **High number of bacteria nombre** ($3x10^{13}$)[1]
- **High diversity** (hundreds of species)
- Most bacteria are harldy culturable
- Pathogenic and antibiotic resistant bacteria are **sub-dominant : need for culture**

Figure from **Arumugam M**. Nature. (2011). [1]**Sender, R.** et al. *PLoS Biol* (2016);

Bad bugs are kept at low concentrations in the gut



16S profiling
Shotgun
metagenomics

Culture

10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}

Intestinal concentration
(CFU/g stool)

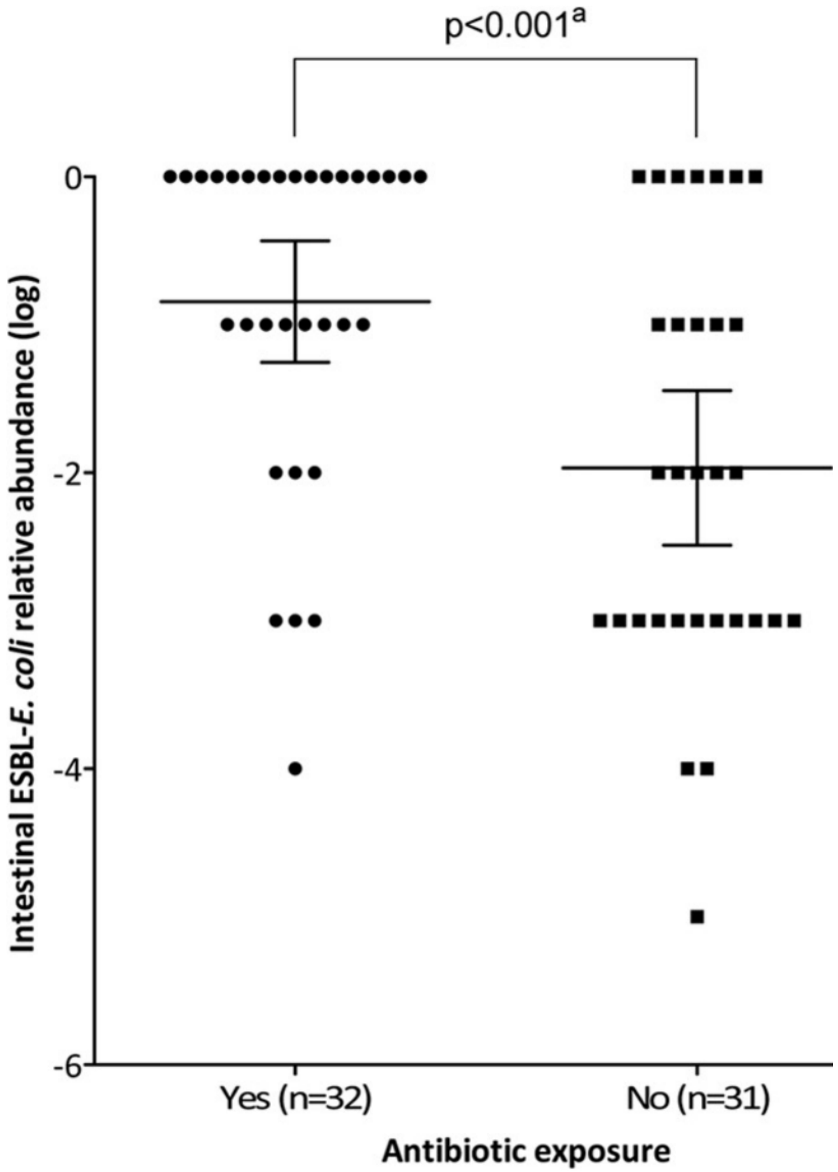
Commensal anaerobes

Enterobacteriaceae
and enterococci

Multidrug-resistant
bacteria

Grall N., *et al*. EMC Biologie.

Antibiotics increase the relative abundance of ESBL-*E. coli*

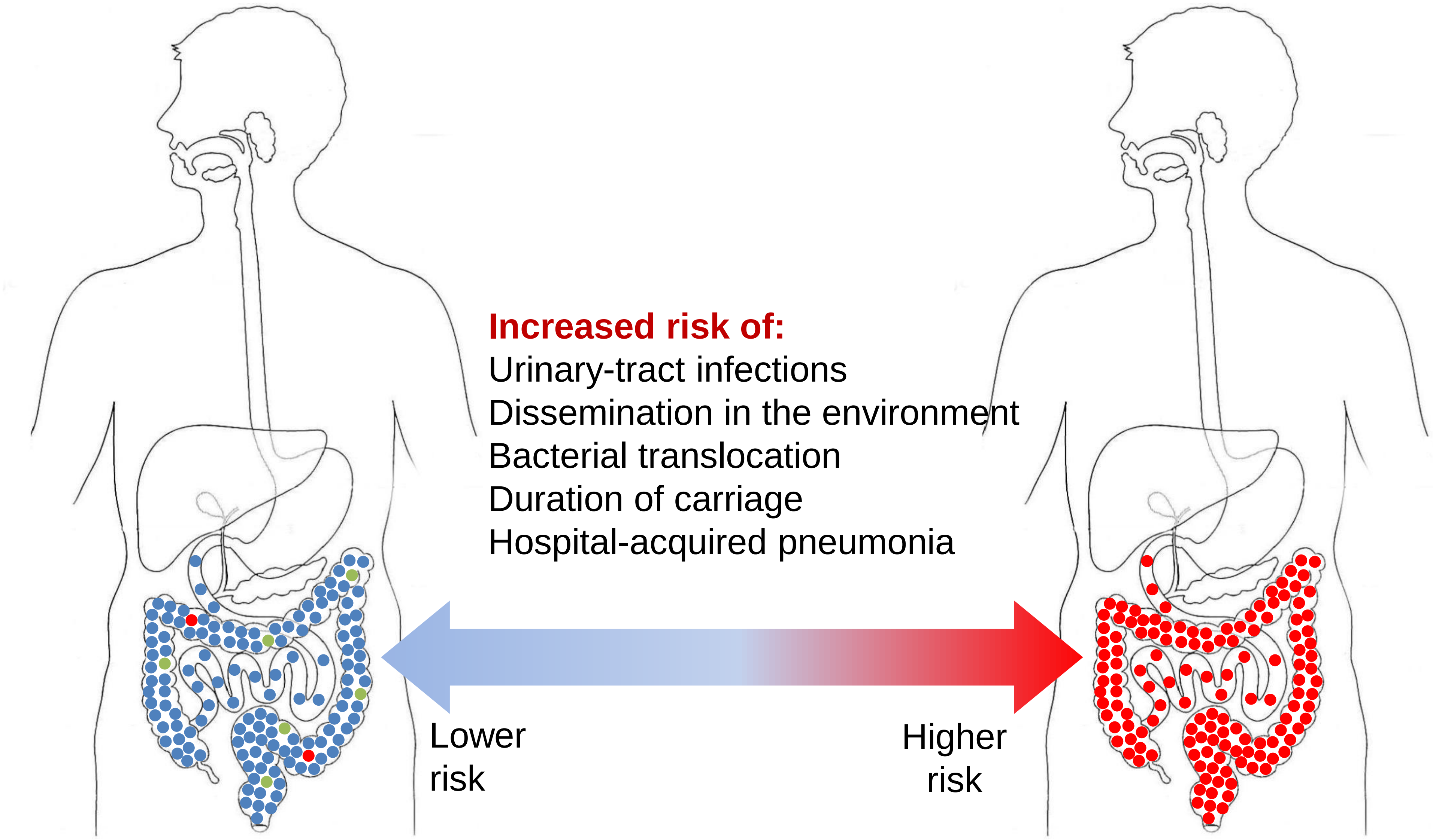


ESBL-*E. coli* relative abundance $= \dfrac{[\textit{ESBL-E. coli}]}{[\text{Total Gram-negative bacilli}]}$

The intestinal relative abundance of ESBL-*E. coli* increases after antibiotic exposure

Ruppé, E. et al. *Antimicrob. Agents Chemother.* **57**, 4512–4517 (2013). ESBL: extended-spectrum beta-lactamase

Quantification of multidrug-resistant bacteria in the gut: it matters!



Increased risk of:

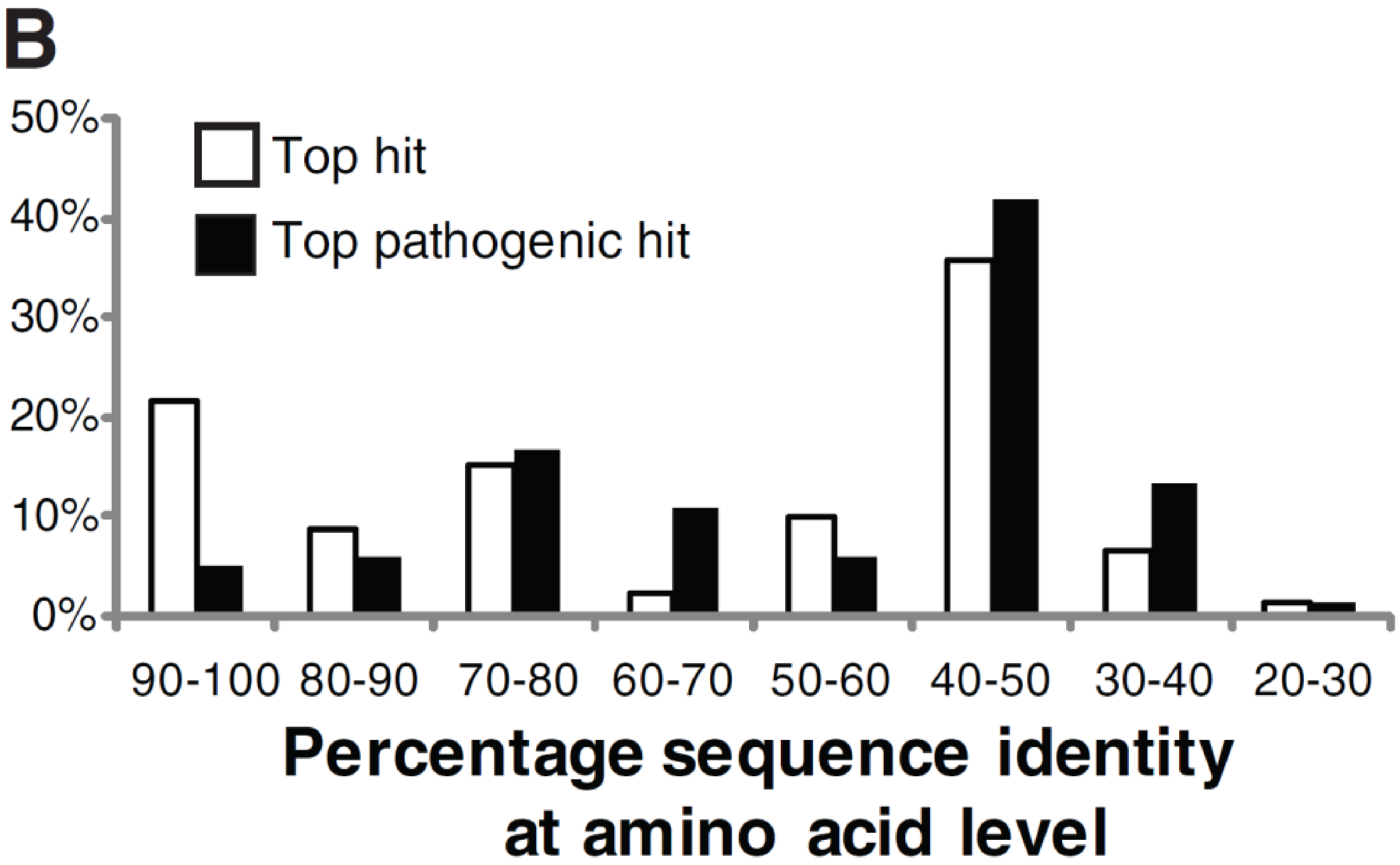
Urinary-tract infections
Dissemination in the environment
Bacterial translocation
Duration of carriage
Hospital-acquired pneumonia

Lower risk

Higher risk

Ruppé, E. & Andremont, A. *Front Microbiol* **4**, 129 (2013).

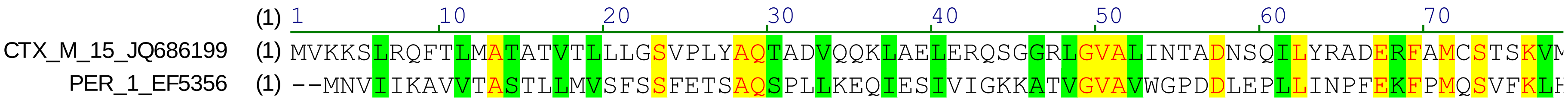
ARG from commensal bacteria differ from what we know



Sommer, M.O.A., et al. *Science* **325**, 1128–1131 (2009).

Leveraging the structure of proteins to characterize the intestinal resistome

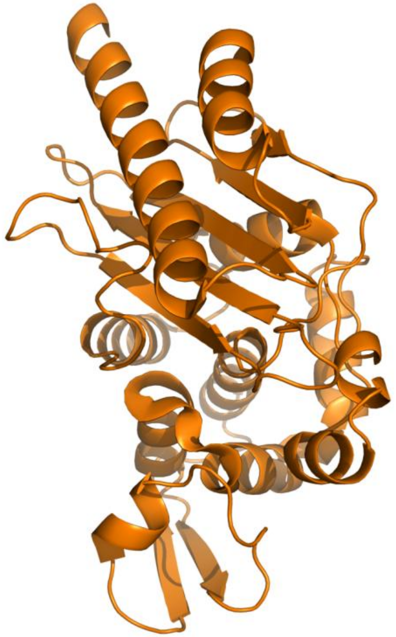
PER-1 and CTX-M-15 do not share more than 21% identity in aminoacid (1-dimension)



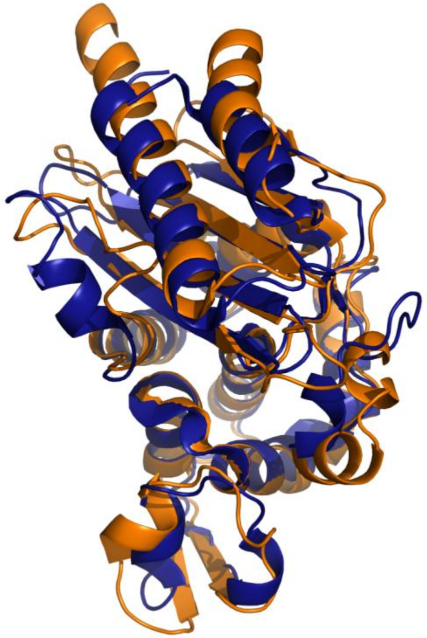
```
                   (1) 1       10        20        30        40        50        60        70
CTX_M_15_JQ686199  (1) MVKKSLRQFTLMATATVTLLLGSVPLYAQTADVQQKLAELERQSGGRLGVALINTADNSQILYRADERFAMCSTSKV
     PER_1_EF5356  (1) --MNVIIKAVVTASTLLMVSFSSFETSAQSPLLKEQIESIVIGKKATVGVAVWGPDDLEPLLINPFEKFPMQSVFKL
```

But they do share a high homology in 3-dimensions!

CTX-M-15



PER-1

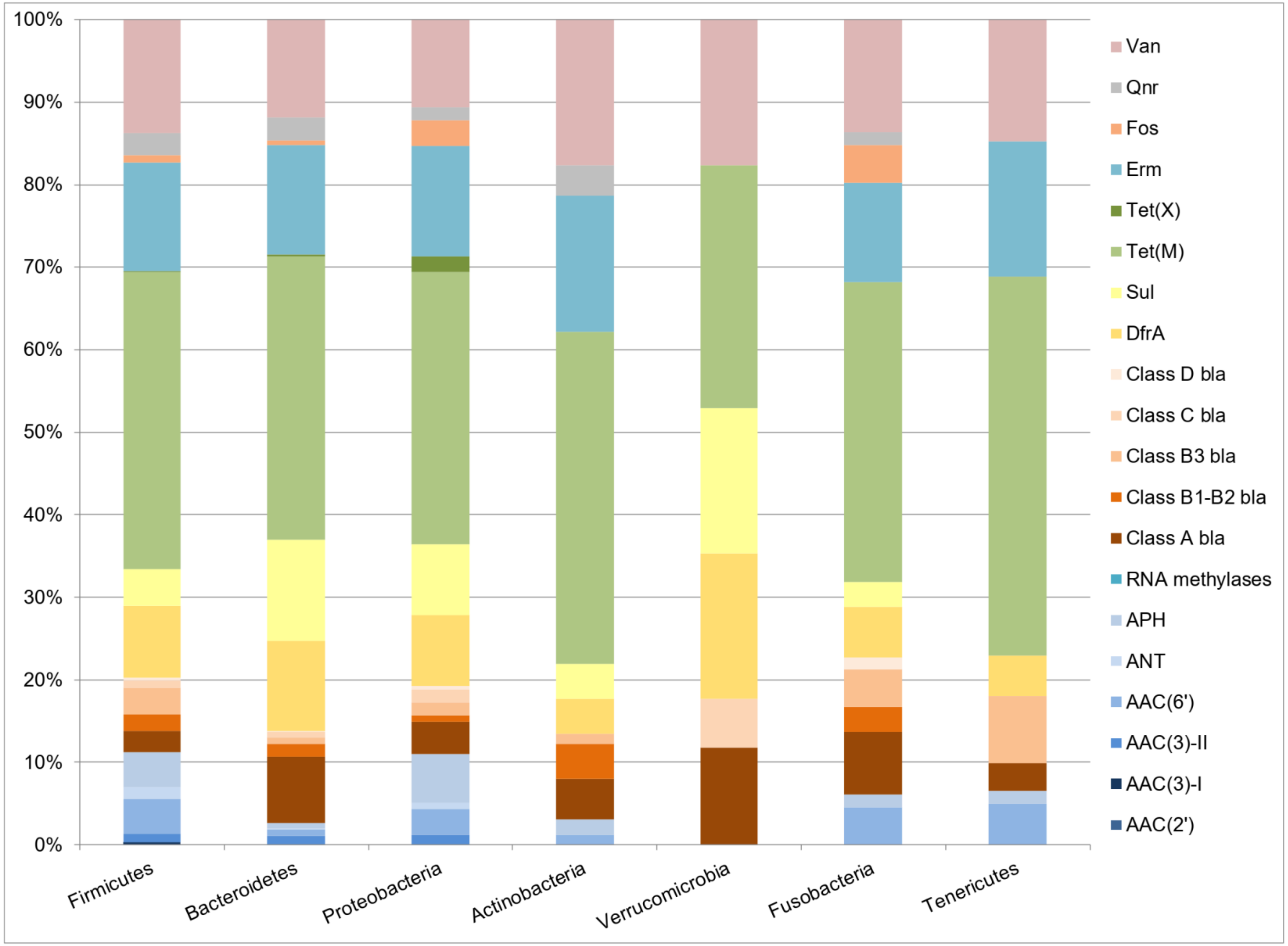


Alignement of CTX-M-15 and PER-1 (TMscore > 0.9)

Amine Ghozlane

Julien Tap

Intestinal bacteria harbor a vast diversity of antibiotic resistance genes (ARG)



- N=6095 ARG predicted (0.2% of 3,9 million gene catalogue)
- Average 1377 ARG per subject (min. 258, max. 2367)
- Low identity with known ARG (mean 29,8% amino-acid identity)
- Present in the main gut phyla
- Mostly chromosomal
- Interphyla transfers?


Amine Ghozlane


Julien Tap

Ruppé, Ghozlane, Tap et al. *Nat Microbiol* **4**, 112–123 (2019).

Is the phylum barrier insurmountable for ARG?

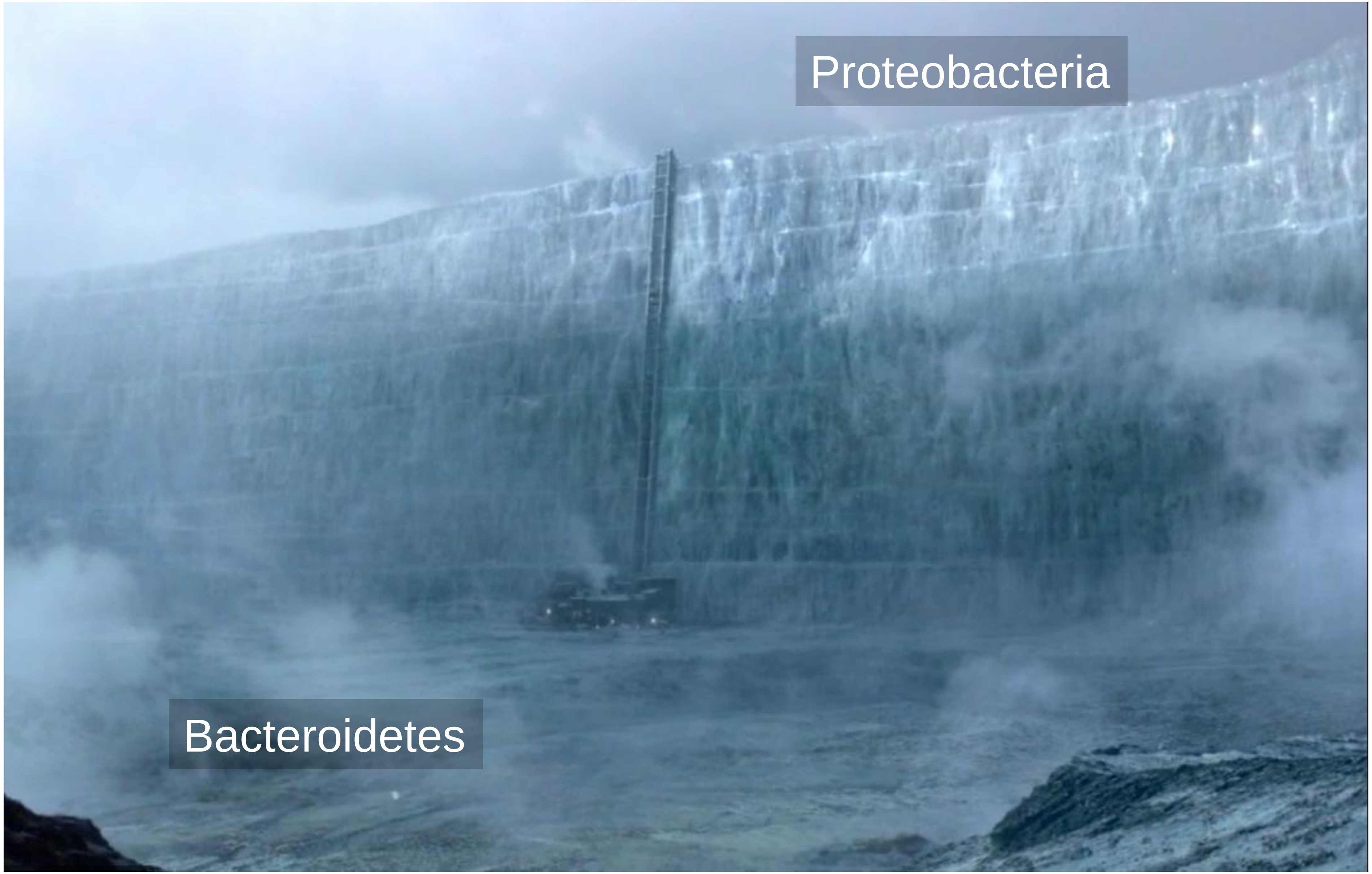
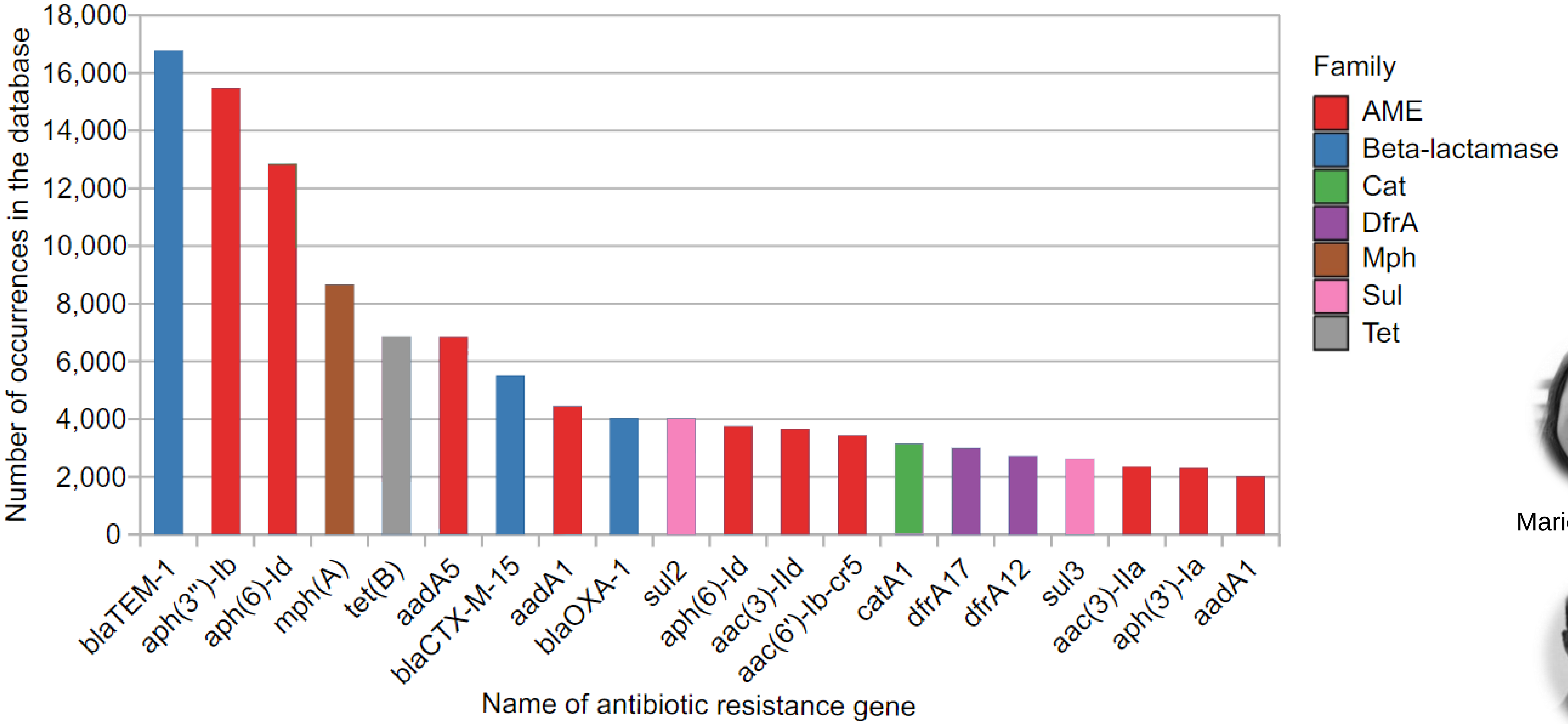


Image: Games of Throne/HBO

Le résistome d'*Escherichia coli*



Marie Petitjean

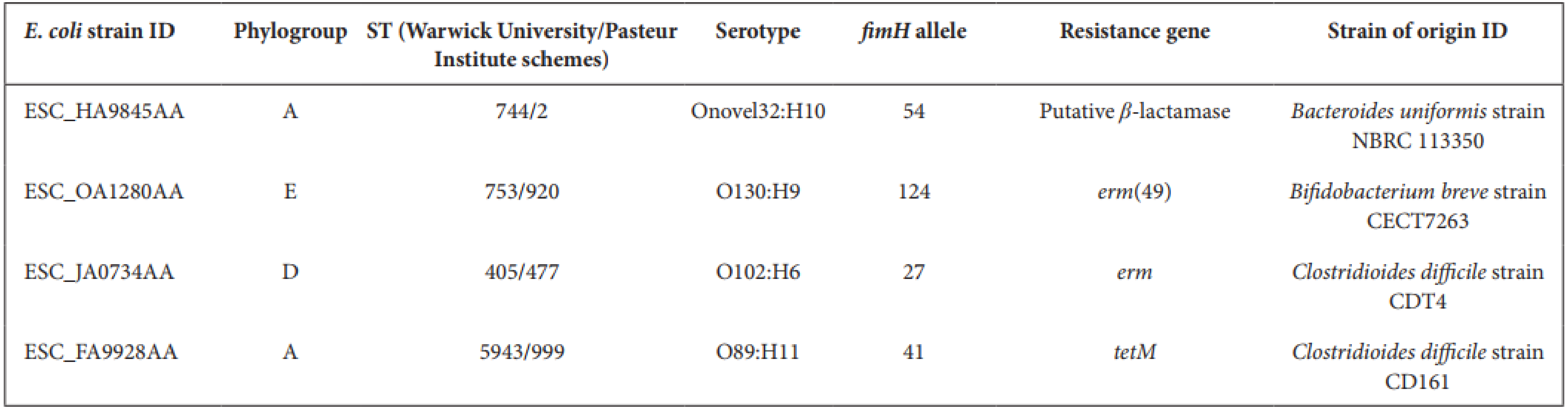
Rémi Gschwind

Presence d'ARG d'autres phyla ?

Petitjean, M., *Microb Genom* 7, (2021). **Gschwind, R.** et al (en preparation)

The intestinal resistome of *Escherichia coli*: digging into the Enterobase

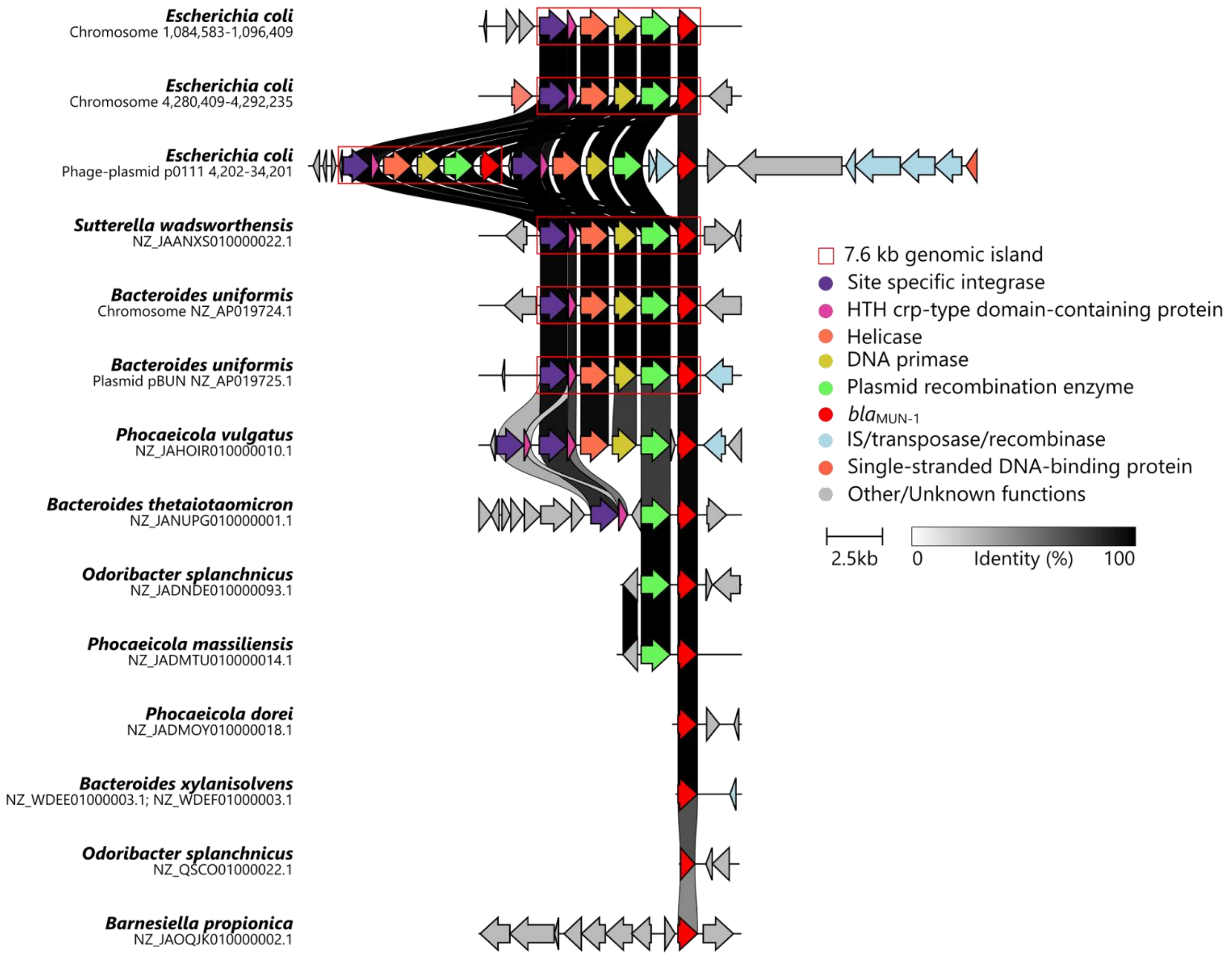
ARGs shared between *E. coli* and non-*Proteobacteria* strains: 4 candidate ARG



E. coli strain ID	Phylogroup	ST (Warwick University/Pasteur Institute schemes)	Serotype	*fimH* allele	Resistance gene	Strain of origin ID
ESC_HA9845AA	A	744/2	Onovel32:H10	54	Putative β-lactamase	*Bacteroides uniformis* strain NBRC 113350
ESC_OA1280AA	E	753/920	O130:H9	124	*erm*(49)	*Bifidobacterium breve* strain CECT7263
ESC_JA0734AA	D	405/477	O102:H6	27	*erm*	*Clostridioides difficile* strain CDT4
ESC_FA9928AA	A	5943/999	O89:H11	41	*tetM*	*Clostridioides difficile* strain CD161

Petitjean, M., *Microb Genom* **7**, (2021).

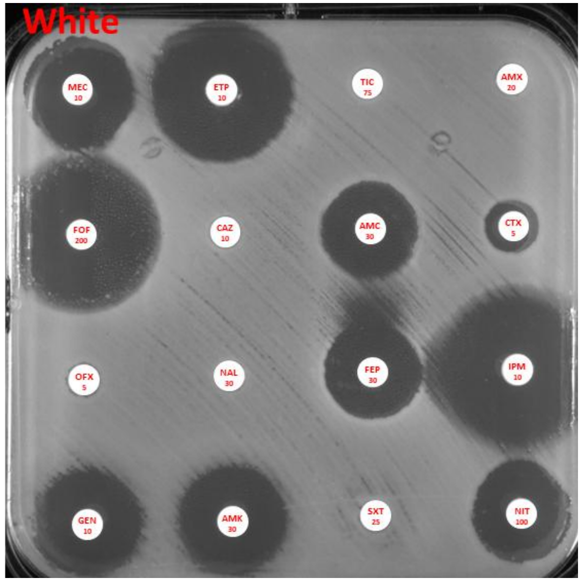
Genomic characterization of a beta-lactamase shared by 2 phyla



- Identification of a beta-lactamase – encoding gene see in strict anaerobic bacteria: 1st (?) identification of *bla* from intestinal Bacteroidota to Pseudomonadata (*E. coli*)

- Location on a 7,600b mobile genetic element, with 2 copies integrated in the genome

- ESBL phenotype



Marie Petitjean

Rémi Gschwind

Gschwind, R. et al.2023.11.10.566511 (2023).doi:10.1101/2023.11.10.566511

The smoking gun: example of *mcr*

Question

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The recipe to cook a resistant bacterium

Human presence

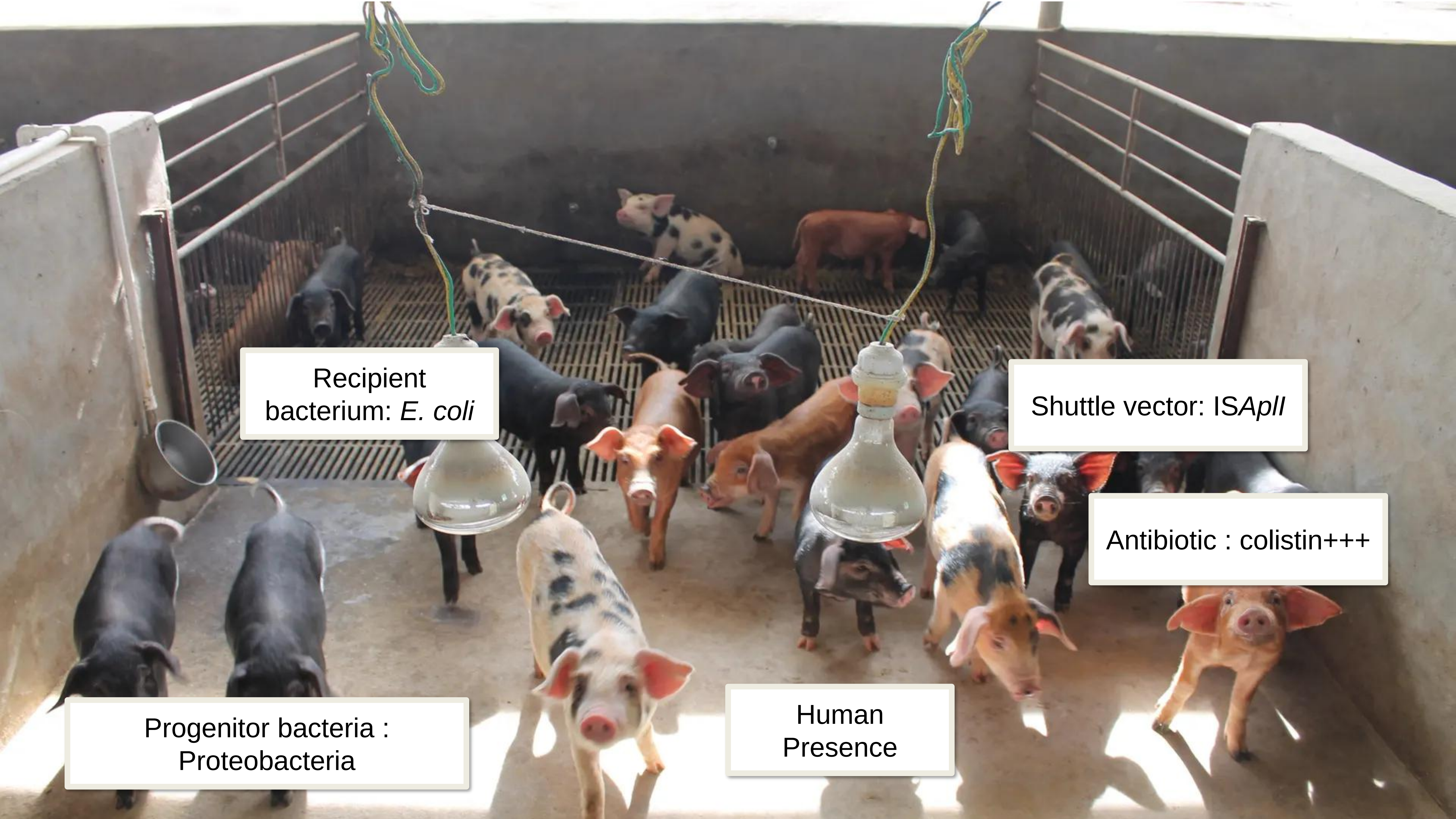
Recipient bacteria

Shuttle vector (plasmid, transposon)

Progenitor bacteria

Antibiotic pressure

- 1. Crédits INA



Recipient bacterium: *E. coli*

Shuttle vector: IS*AplI*

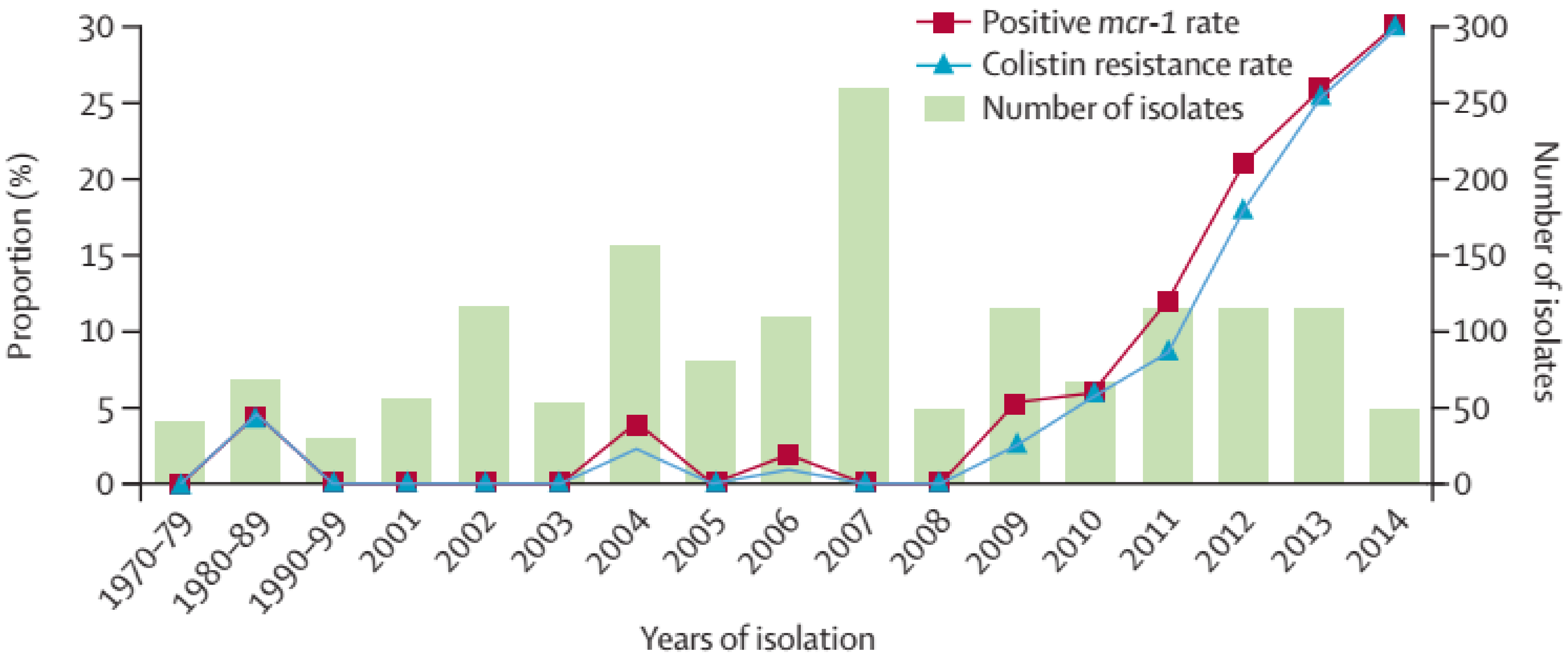
Antibiotic : colistin+++

Progenitor bacteria : Proteobacteria

Human Presence

Mcr has been with us for long

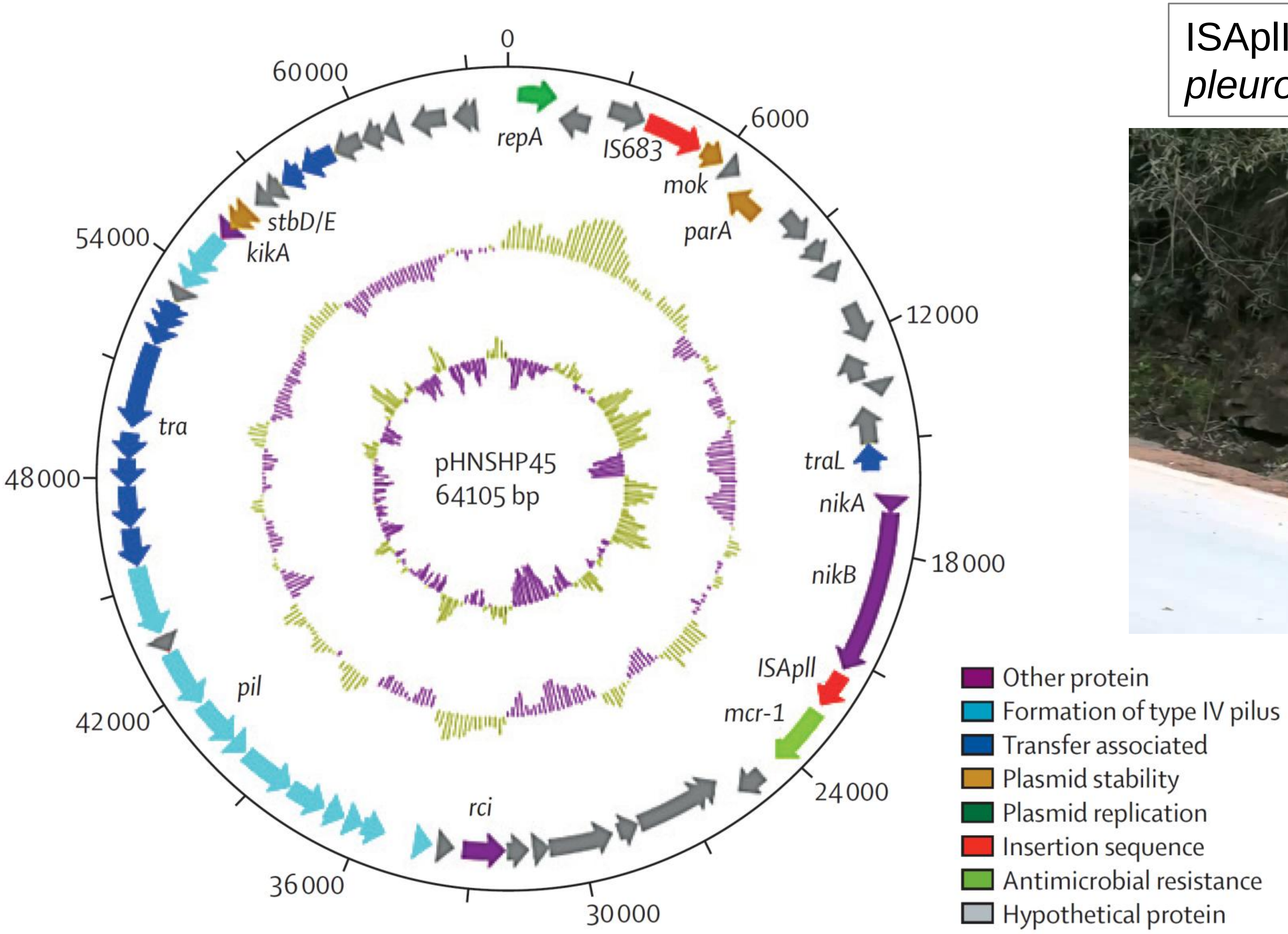
China: high consumption of colistin in pig farming (2500-2800 tons/year)



Shen, Z. et al. *Lancet Infect Dis* **16**, 293 (2016).

Origin of Mcr

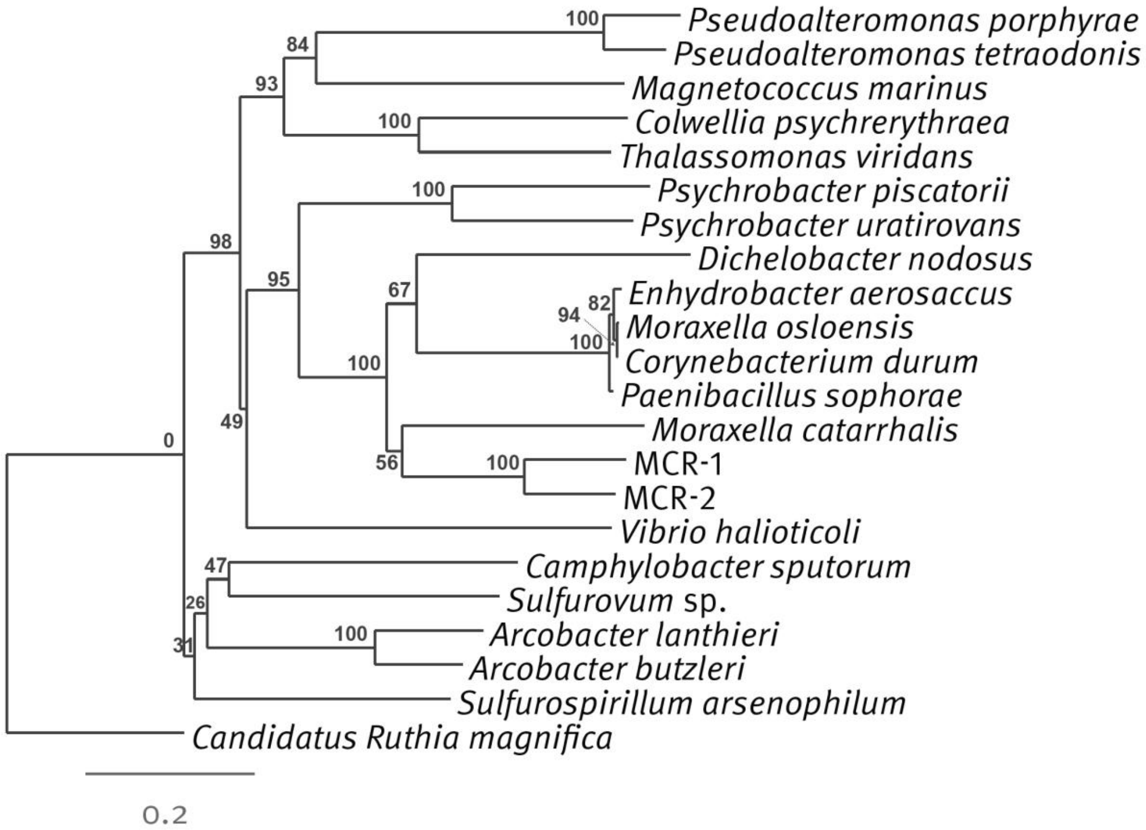
ISApII: from *Actinobacillus pleuropneumoniae* (porcine pathogen)



Liu, Y.-Y. *et al.* *Lancet Infect Dis* **16,** 161–168 (2016).

Origin of Mcr

- Reservoir: obviously major reservoir in livestock (pigs, calve, chicken…)
- More frequently isolated in livestock samples than in human samples (so far).
- 12M tons of colistin used in agriculture, mostly in China.
- Bacterial host: Mcr-1 has 63% aminoacid identity with a EptA from *Paenibacillus sophorae*.
- No Mcr in the pig intestinal metagenome.



Xavier, B. B. ***et al***. *Euro Surveill.* **21,** (2016); **Xiao, L.** ***et al***. *Nat Microbiol* 16161 (2016)

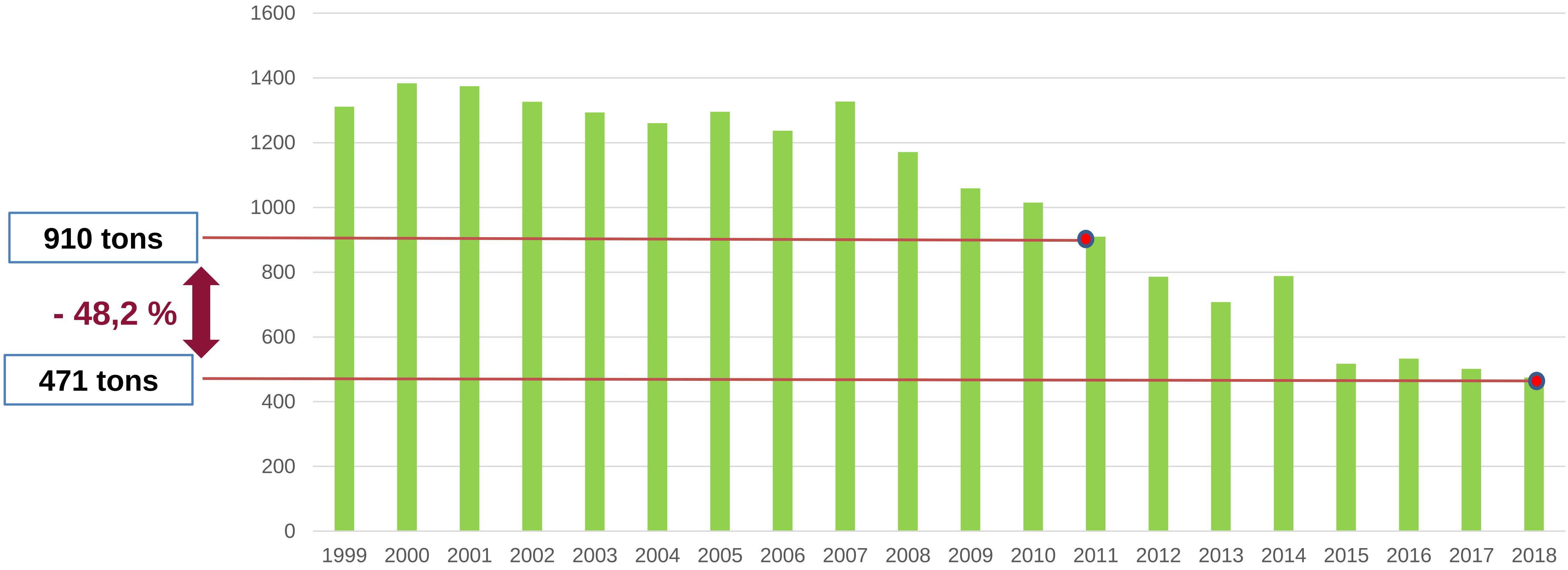
Actions!

Question

www.wooclap.com/MPAMB

Decreasing antibiotic consumption

Evolution of antibiotic tonnage in veterinary medicine in France



910 tons

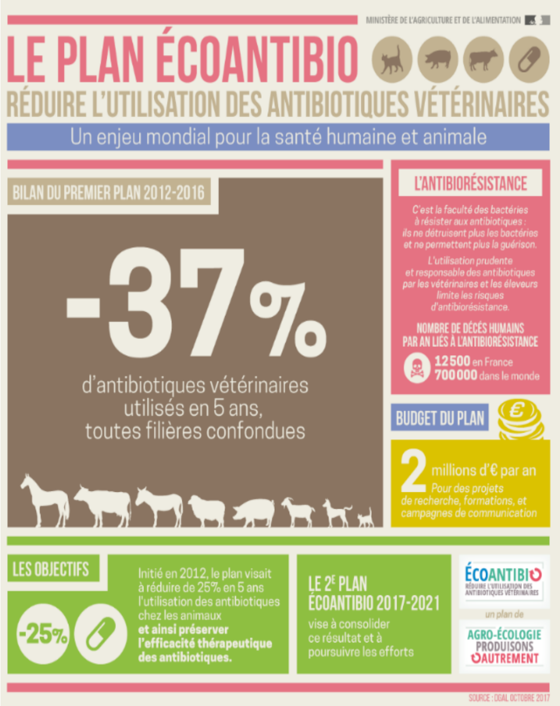
- 48,2 %

471 tons

Courtesy of Jean-Yves Madec (ANSES)

Exposure of animals to antibiotics

- 36.6% for all antibiotics in 5 years
- 81.3% for 3rd/4th generation cephalosporins in 3 years
- 74.9% for fluoroquinolones in 3 years



MINISTÈRE DE L'AGRICULTURE ET DE L'ALIMENTATION

LE PLAN ÉCOANTIBIO

RÉDUIRE L'UTILISATION DES ANTIBIOTIQUES VÉTÉRINAIRES

Un enjeu mondial pour la santé humaine et animale

BILAN DU PREMIER PLAN 2012-2016

-37%

d'antibiotiques vétérinaires
utilisés en 5 ans,
toutes filières confondues

L'ANTIBIORÉSISTANCE

C'est la faculté des bactéries à résister aux antibiotiques : ils ne détruisent plus les bactéries et ne permettent plus la guérison.

L'utilisation prudente et responsable des antibiotiques par les vétérinaires et les éleveurs limite les risques d'antibiorésistance.

NOMBRE DE DÉCÈS HUMAINS PAR AN LIÉS À L'ANTIBIORÉSISTANCE

12500 en France
700000 dans le monde

BUDGET DU PLAN

2 millions d'€ par an
Pour des projets de recherche, formations, et campagnes de communication

LES OBJECTIFS

-25%

Initié en 2012, le plan visait à réduire de 25% en 5 ans l'utilisation des antibiotiques chez les animaux **et ainsi préserver l'efficacité thérapeutique des antibiotiques.**

LE 2E PLAN ÉCOANTIBIO 2017-2021

vise à consolider ce résultat et à poursuivre les efforts

ÉCOANTIBIO
RÉDUIRE L'UTILISATION DES ANTIBIOTIQUES VÉTÉRINAIRES

un plan de

AGRO-ÉCOLOGIE
PRODUISONS AUTREMENT

SOURCE : DGAL OCTOBRE 2017

anses
agence nationale de sécurité sanitaire
alimentation, environnement, travail

Connaître, évaluer, protéger

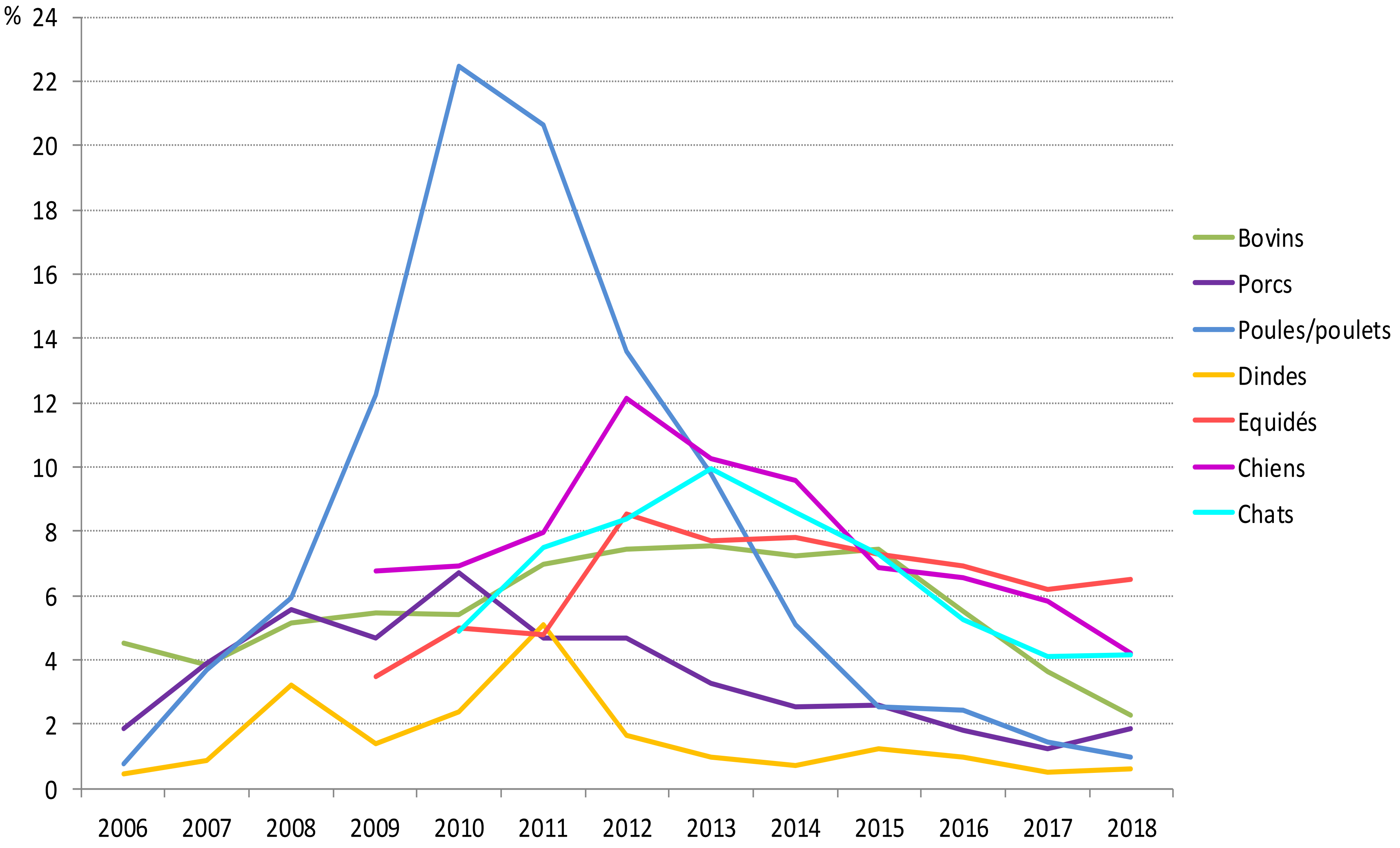
Suivi des ventes
de médicaments
vétérinaires
contenant
des antibiotiques
en France en 2016

Rapport annuel

Octobre 2017 Édition scientifique

Courtesy of Jean-Yves Madec (ANSES)

Resistance to cephalosporins



anses

Connaître, évaluer, protéger

Résapath

Réseau d'épidémiosurveillance de l'antibiorésistance des bactéries pathogènes animales

Bilan 2018

Novembre 2019 - Édition scientifique

1. Crédits: Jean-Yves Madec (ANSES)

Resistance to fluoroquinolones

Résapath

Réseau d'épidémiosurveillance de l'antibiorésistance des bactéries pathogènes animales

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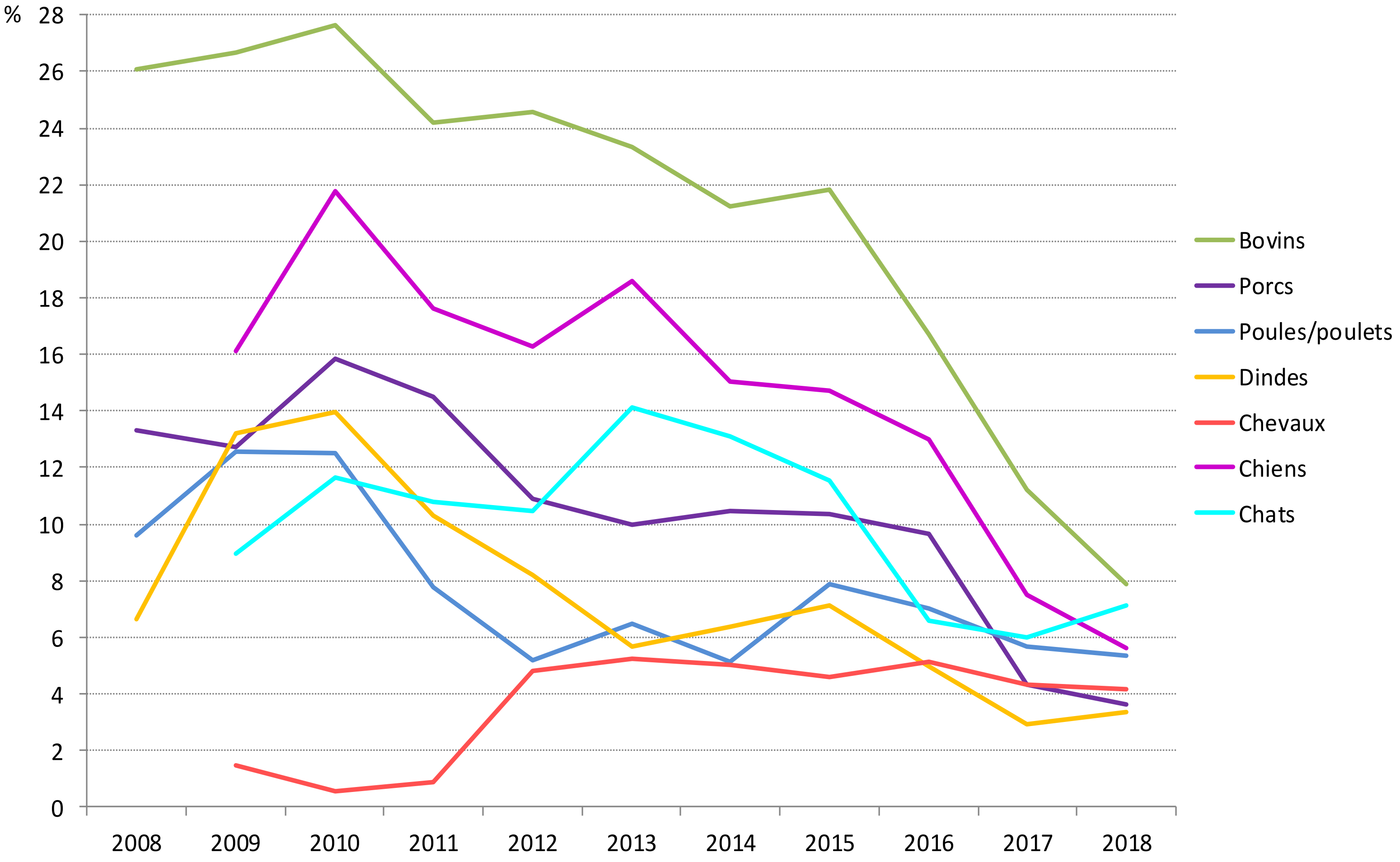
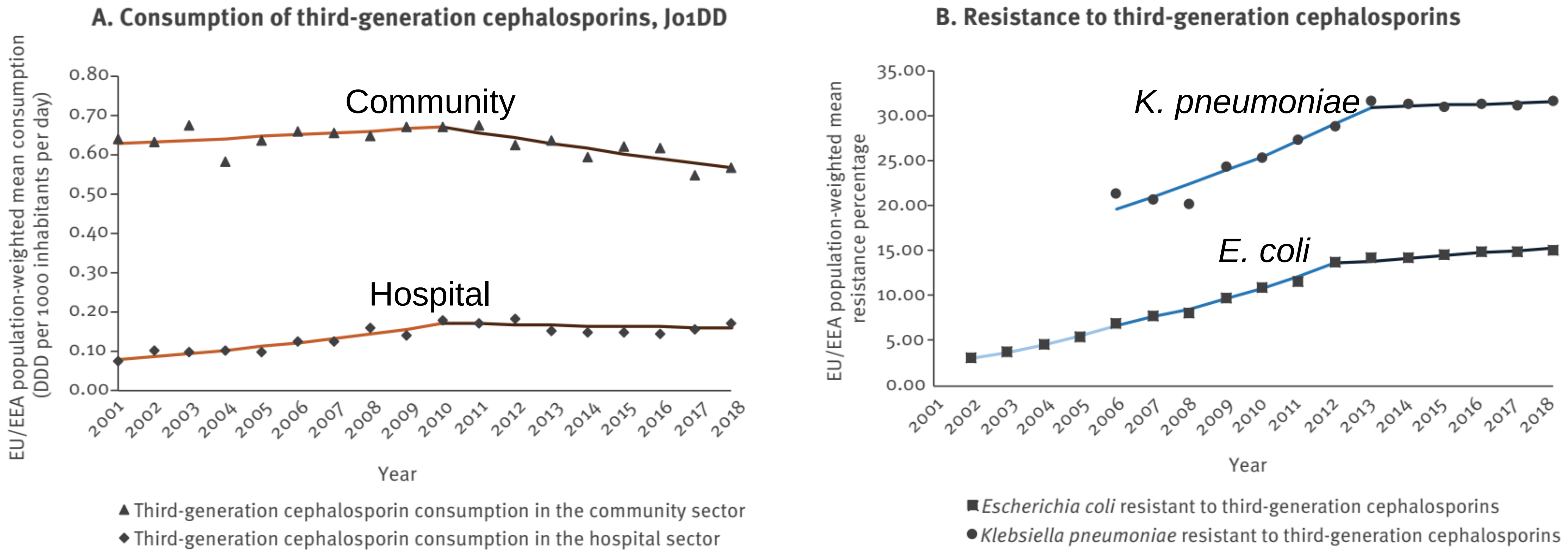


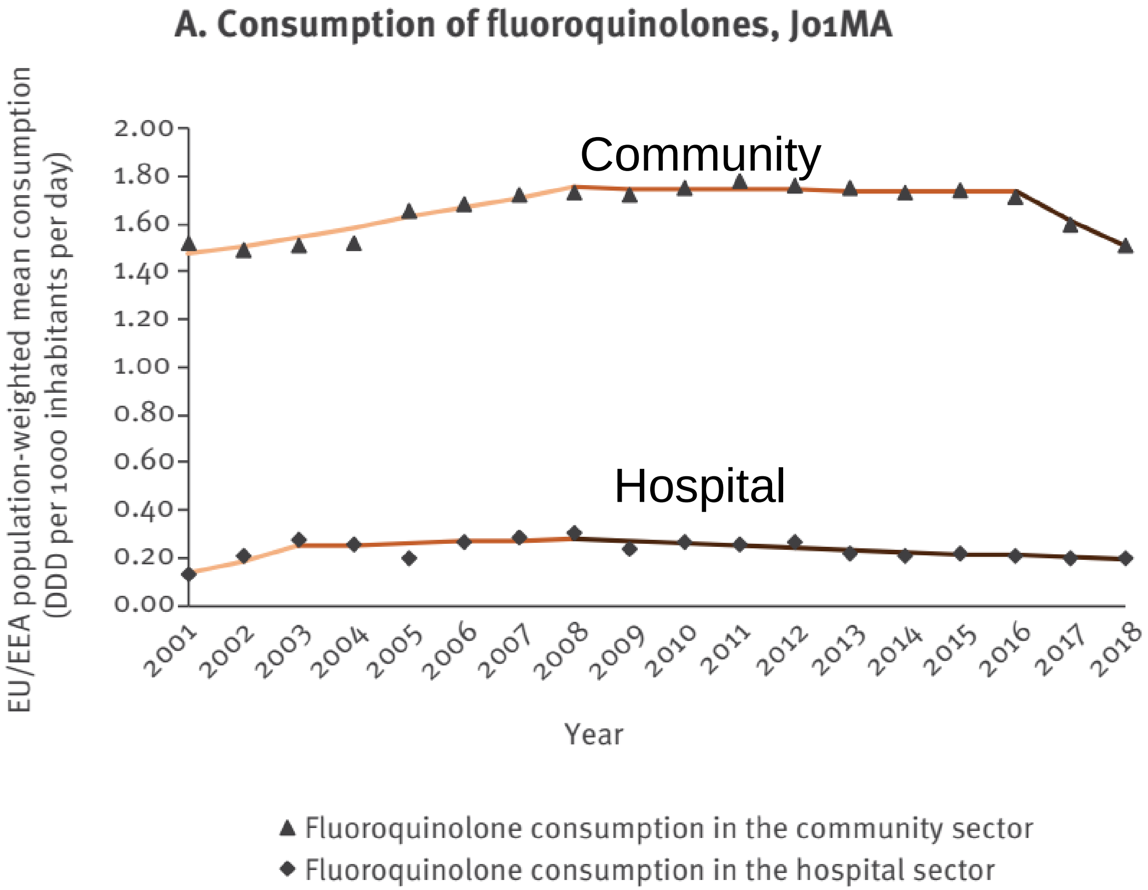
1. Crédits: Jean-Yves Madec (ANSES)

What about in humans?

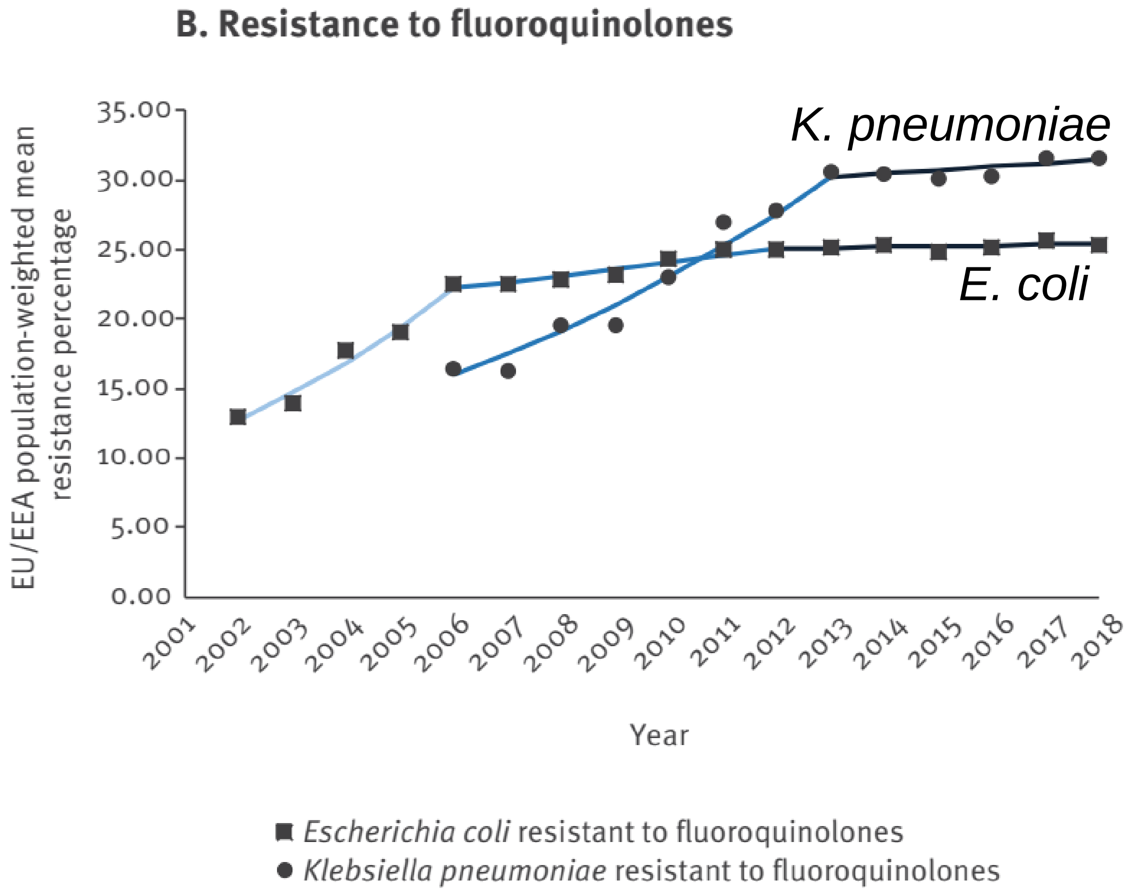


Penalva G. et al. Euro Surveill. 2019;24(46)

What about in humans?



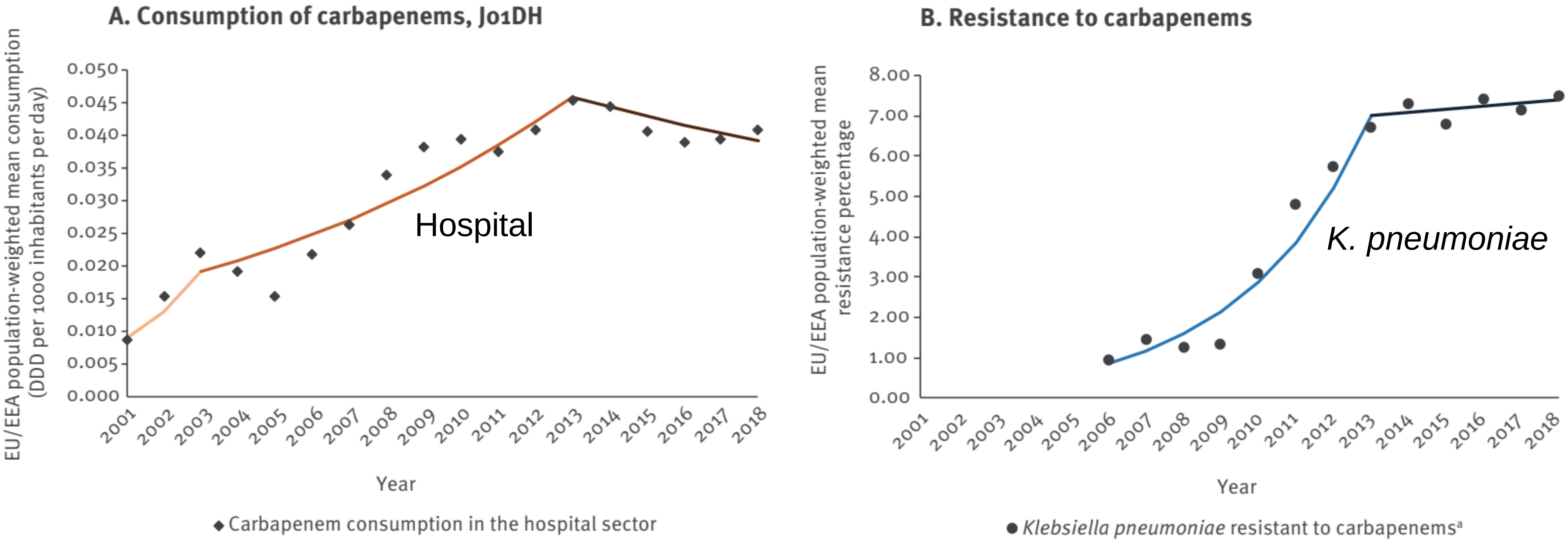
A. Consumption of fluoroquinolones, J01MA



B. Resistance to fluoroquinolones

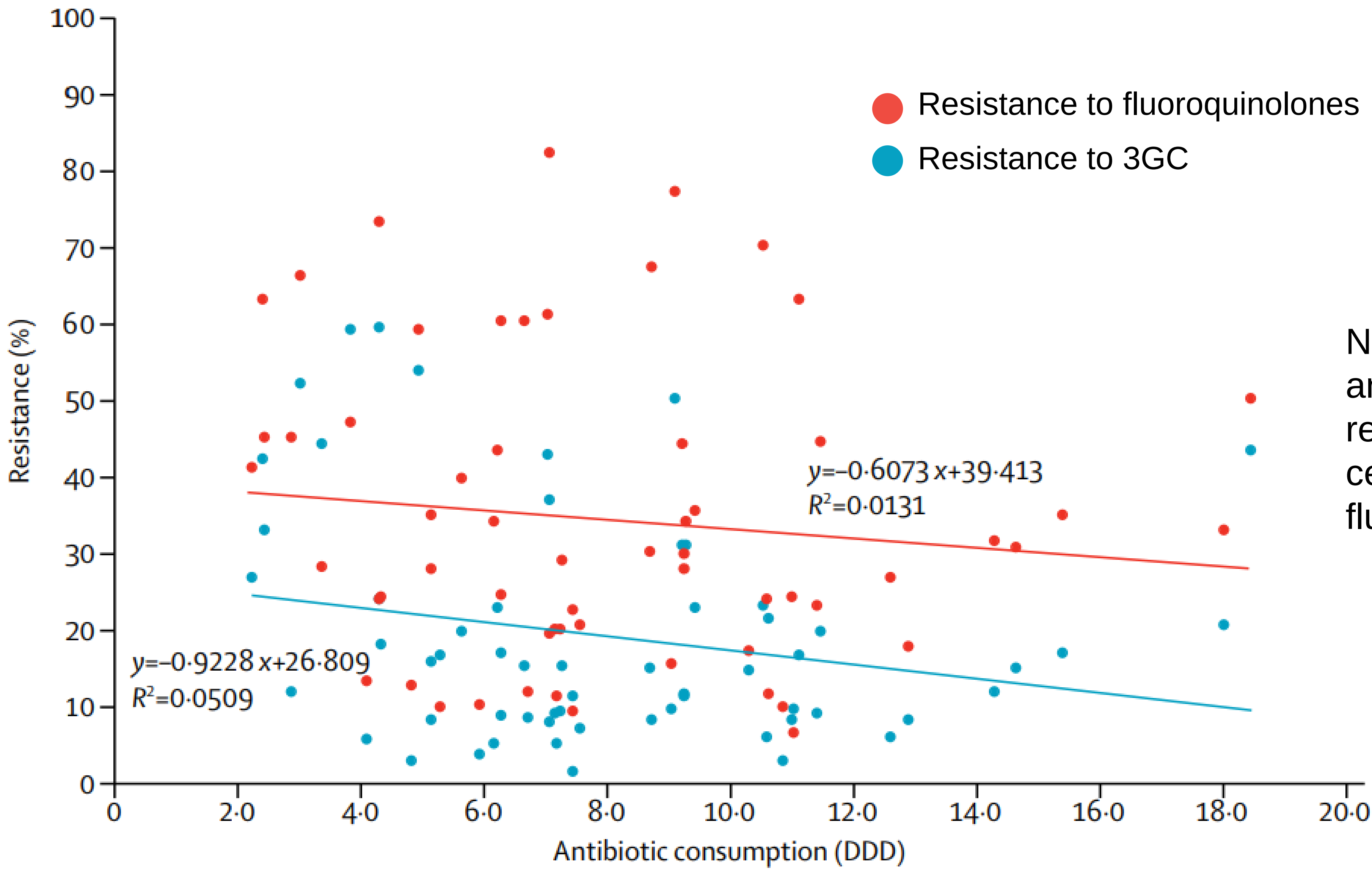
Penalva G. et al. Euro Surveill. 2019;24(46)

What about in humans?



Penalva G. et al. Euro Surveill. 2019;24(46)

Not only an antibiotic consumption issue



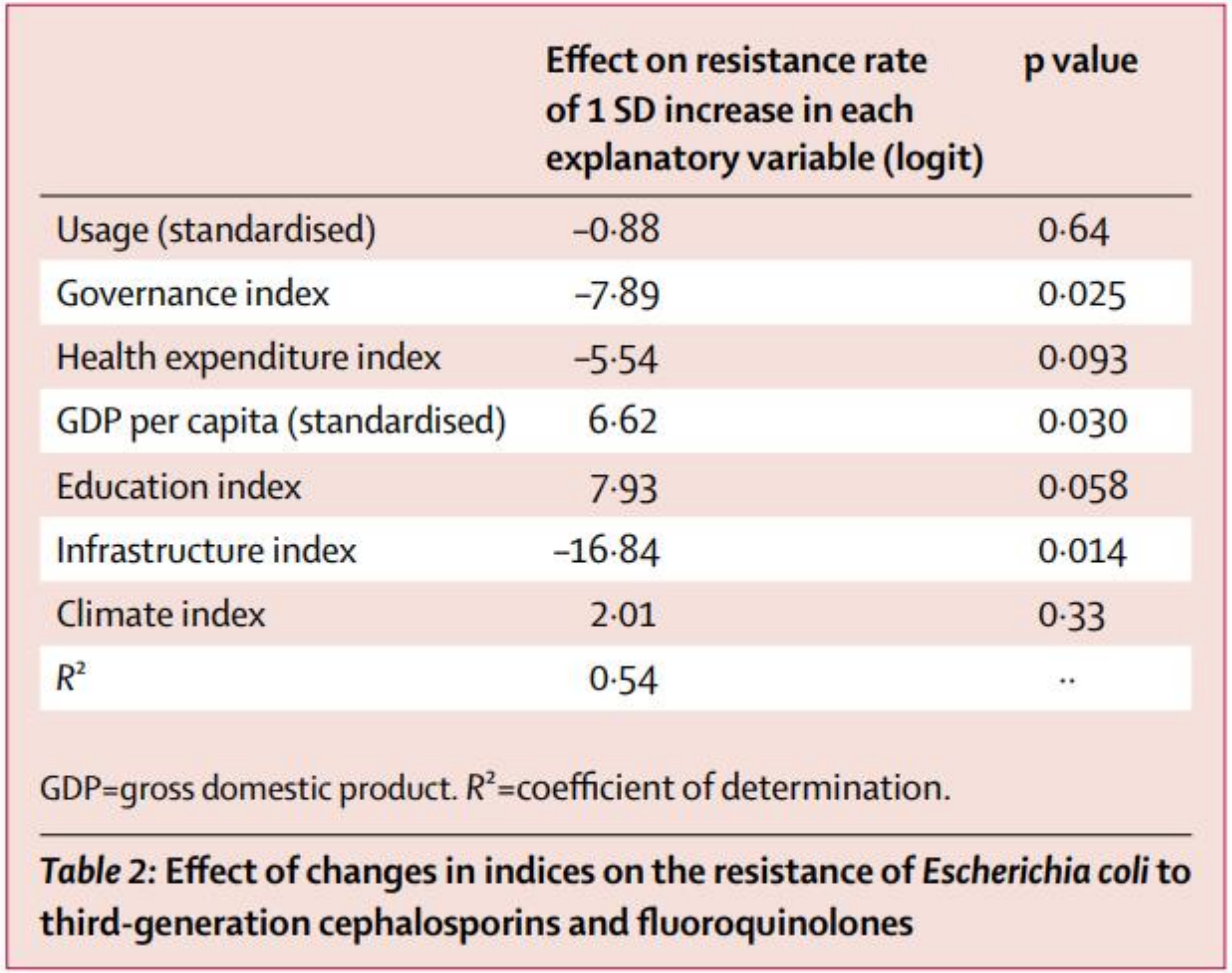
No connection between antibiotic consumption and resistance to 3rd generation cephalosporins (3GC) or fluoroquinolones (FQ)?

Collignon, P., et al. *The Lancet Planetary Health* **2**, e398–e405 (2018).

Not only an antibiotic consumption issue

Credit: financialtribune.com

Also a structural issue



	Effect on resistance rate of 1 SD increase in each explanatory variable (logit)	p value
Usage (standardised)	–0·88	0·64
Governance index	–7·89	0·025
Health expenditure index	–5·54	0·093
GDP per capita (standardised)	6·62	0·030
Education index	7·93	0·058
Infrastructure index	–16·84	0·014
Climate index	2·01	0·33
R^2	0·54	··

GDP=gross domestic product. R^2=coefficient of determination.

Table 2: **Effect of changes in indices on the resistance of *Escherichia coli* to third-generation cephalosporins and fluoroquinolones**

Multivariate analysis: **infrastructure** (p=0.014 and p=0.0052) and **better governance** (p=0.025) were associated with lower antimicrobial resistance indices

No association with antibiotic consumption

Collignon, P., et al. *The Lancet Planetary Health* **2**, e398–e405 (2018).

Take-home messages

Antibiotic resistance has continuously developed and increased among bacteria

Antibiotic consumption has promote the emergence and diffusion of resistance

Resistant bacteria are met in all sectors, but transmission between sectors seems limited in high resources settings

The microbiota plays a pivotal role in being at the interplay of antibiotic exposure, infections and carriage of reistant bacteria

Hygiene living conditions also play a major role in AMR

Combatting AMR is multifaceted

IAME Research Unit
Paris, France
https://www.iame-research.center