ISTITUTO ZOOPROFILATTICO SPERIMENTALE
DELLA LOMBARDIA E DELL'EMILIA ROMAGNA
"BRUNO UBERTINI"
ENTE SANITARIO DI DIRITTO PUBBLICO

LA NOSTRA ESPERIENZA, LA VOSTRA **SICUREZZA.**

Identifying early signs: the example of antimicrobial resistance

Stefano Pongolini

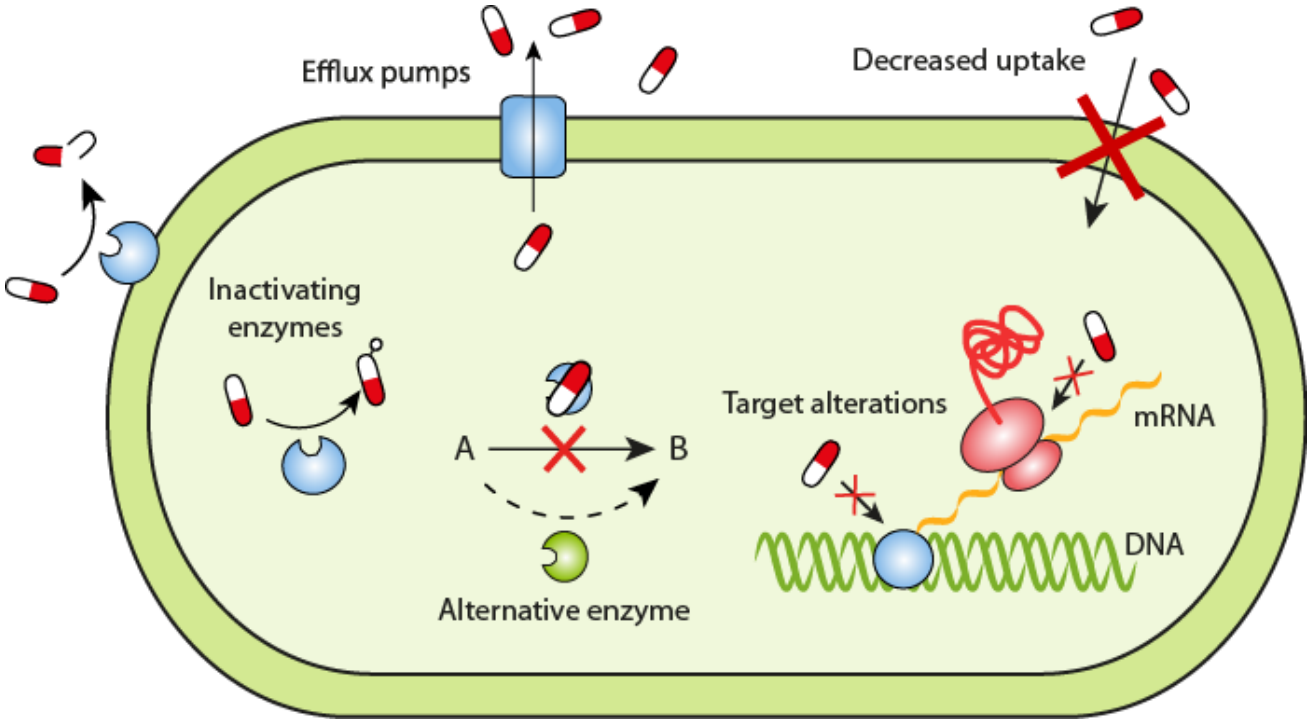
Parma, 15 May 2018

Antibiotici

il rischio nel piatto

Alcuni batteri possono sviluppare un pericoloso meccanismo di

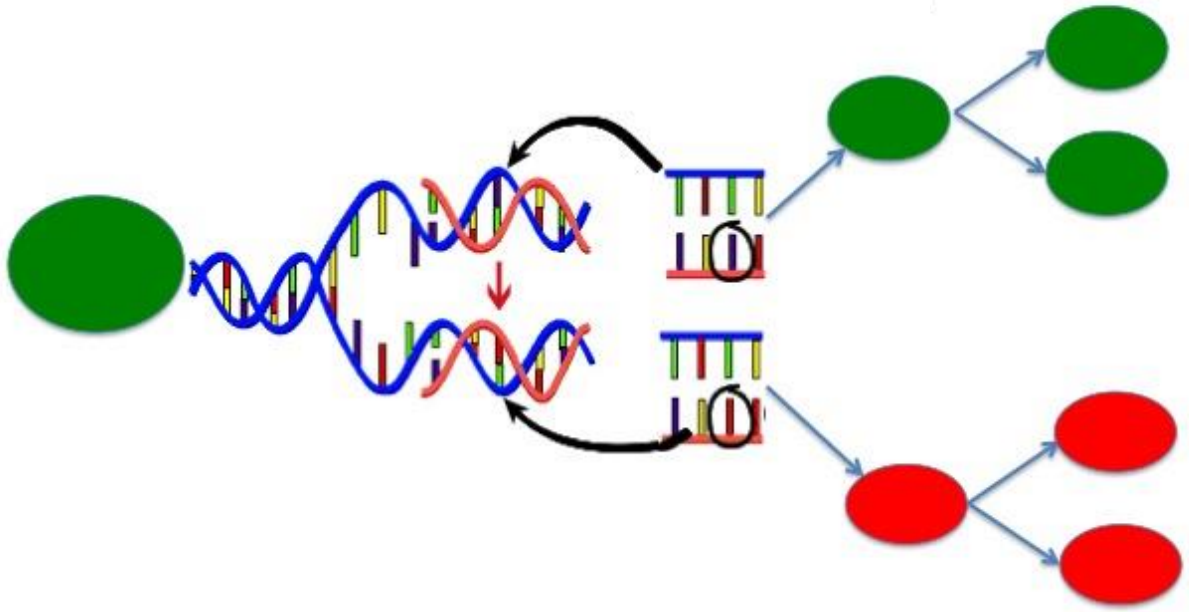
Resistance to antimicrobials - mechanisms



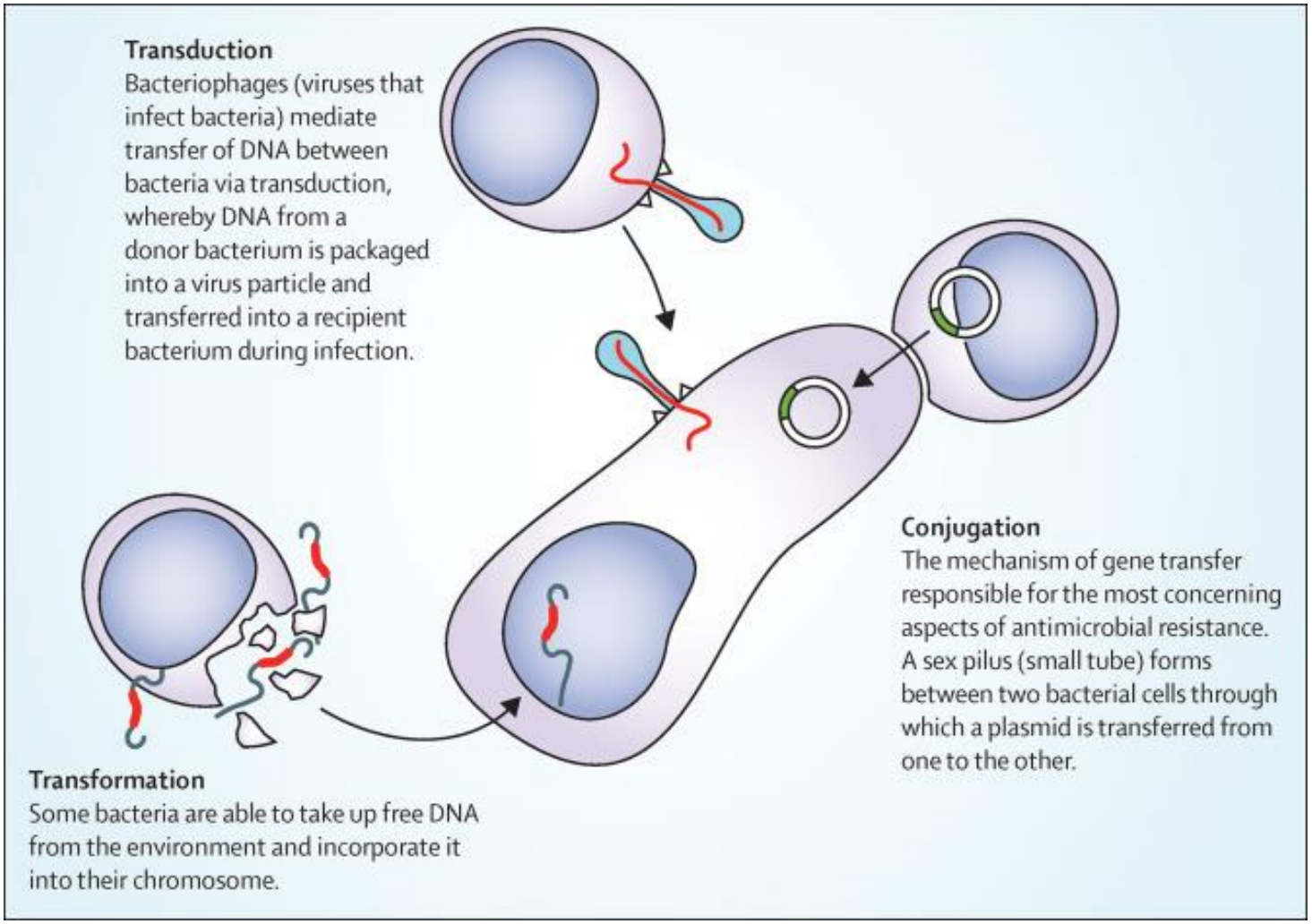
[ReAct – Action on Antibiotic Resistance]

Resistance to antimicrobials - genetics

spontaneous DNA mutations



Resistance to antimicrobials - genetics



The Lancet

Antimicrobial resistance: origin and evolution

- resistance existed *before* the discovery of antimicrobials
- it is a natural phenomenon

MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, Sept. 2010, p. 417–433 Vol. 74, No. 3
1092-2172/10/$12.00 doi:10.1128/MMBR.00016-10

Origins and Evolution of Antibiotic Resistance

Julian Davies* and Dorothy Davies

Department of Microbiology and Immunology, Life Sciences Institute, University of British Columbia, 2350 Health Sciences Mall, Vancouver, British Columbia V6T 1Z3, Canada

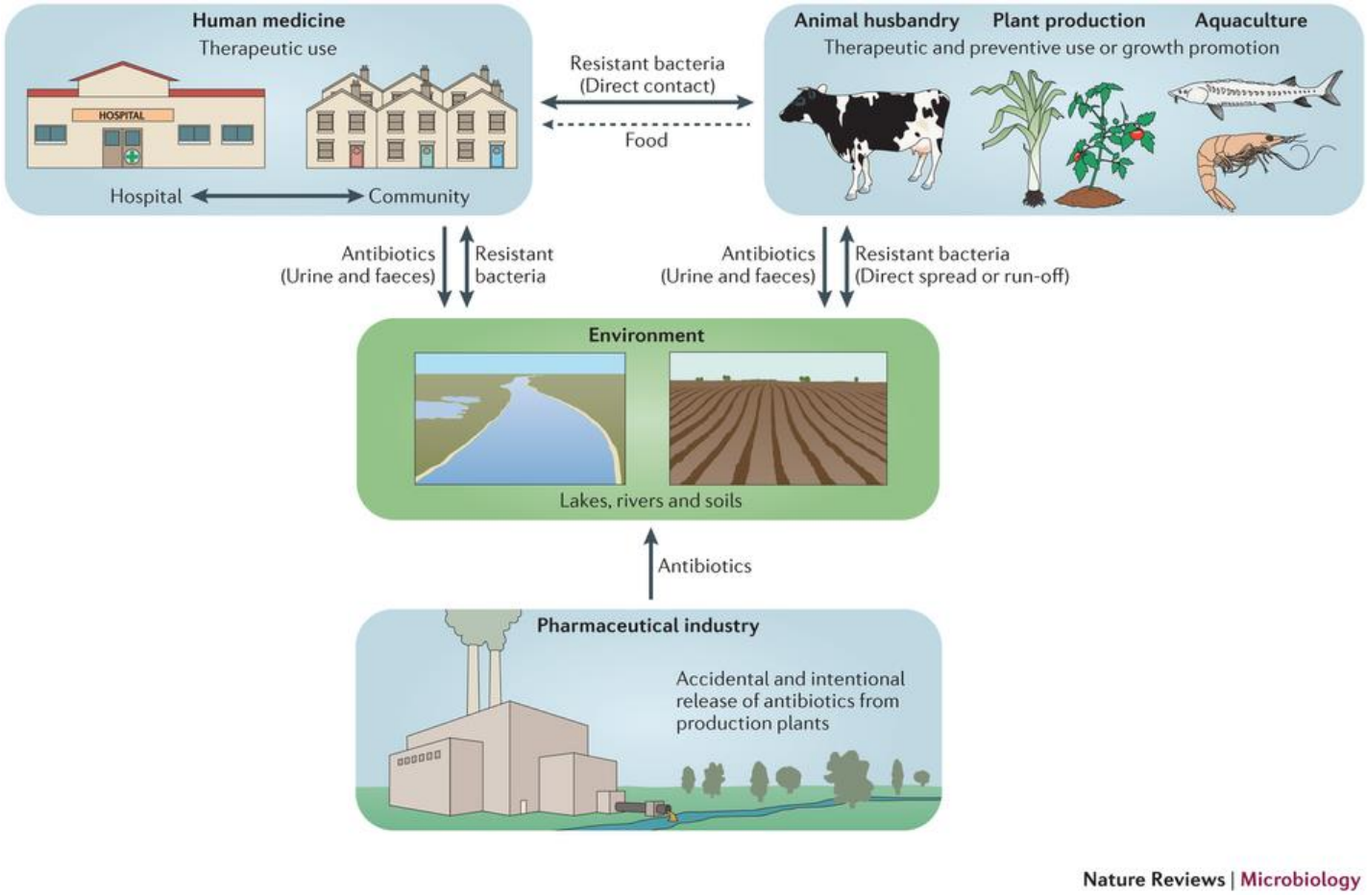
- many more resistance genes exist in nature than we commonly look for (~34.000)

Antimicrobial resistance: origin and evolution

- resistance is a natural phenomenon

- impossible to eradicate
- possible to control?

- it is a matter of selective pressure



- AMR spreads through resistant bacteria and resistance genes
- AMR can be seen as a complex ecological issue

AMR – critical questions

- what is the contribution of humans, animals and environment to AMR in human pathogens?
- what is the correlation between drug consumption and AMR in human and vet compartments (strong, week, cross-compartment, stable, unstable)?
- What is the transmission dynamics across compartments (how intense? Bidirectional? clonal vs horizontal)?

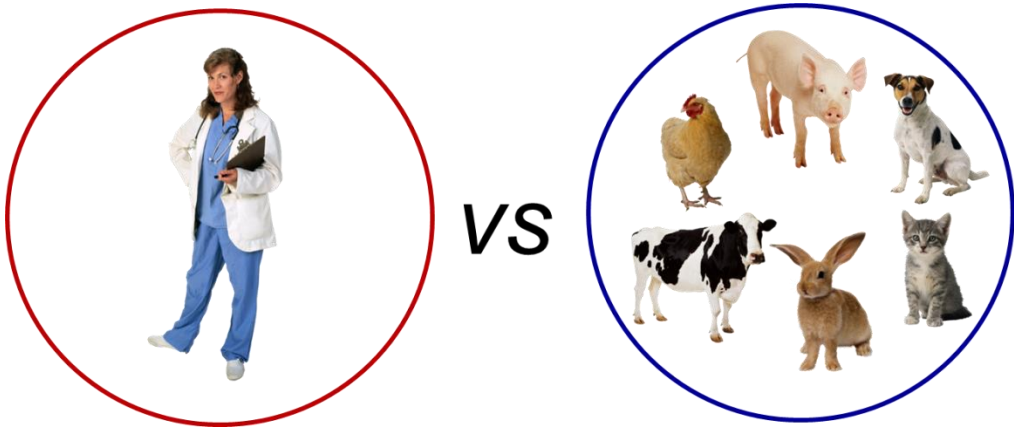


Figure 21. Spatial distribution of overall sales of all antimicrobials for food-producing animals, in mg/PCU, for 29 countries, for 2014

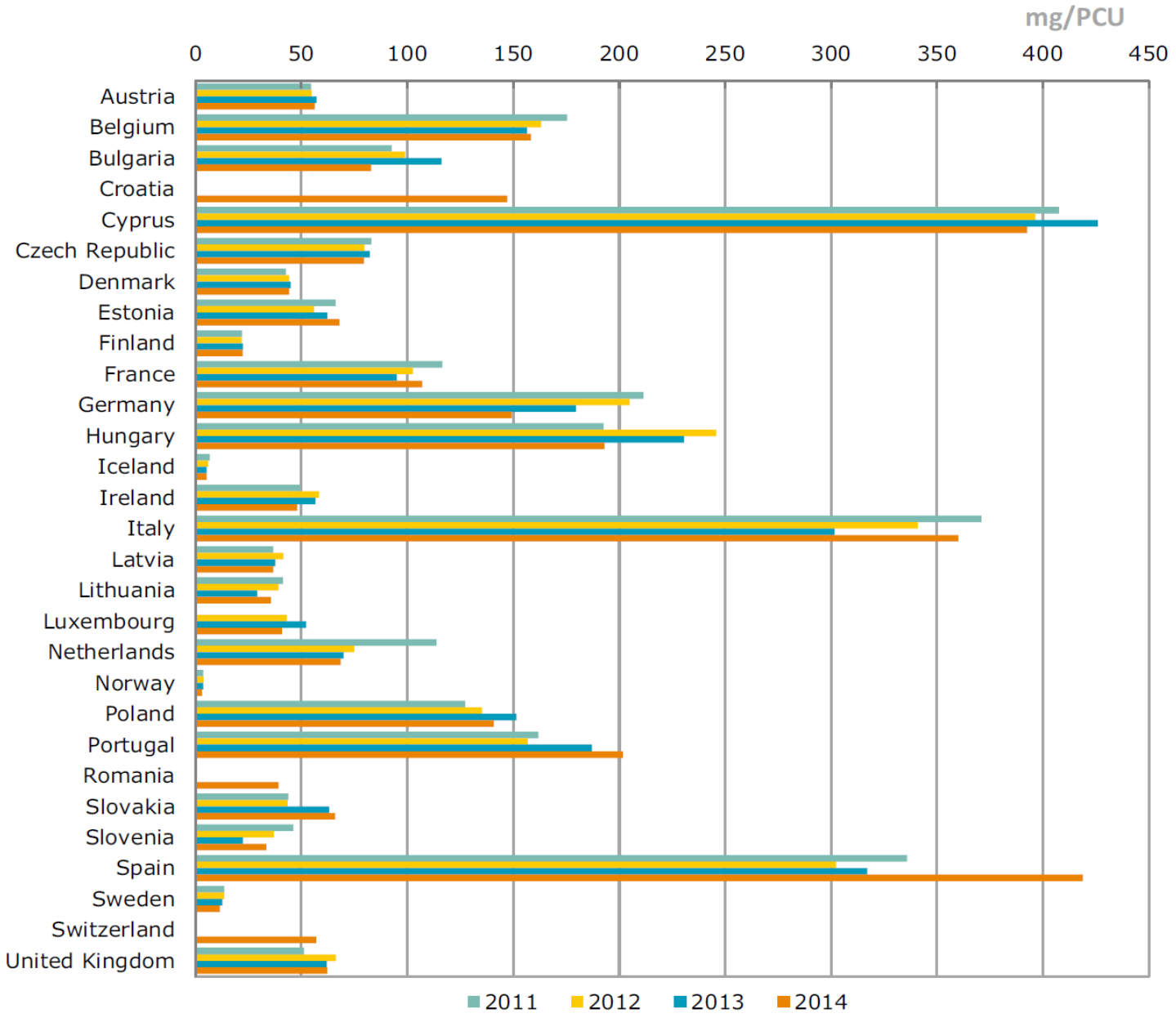
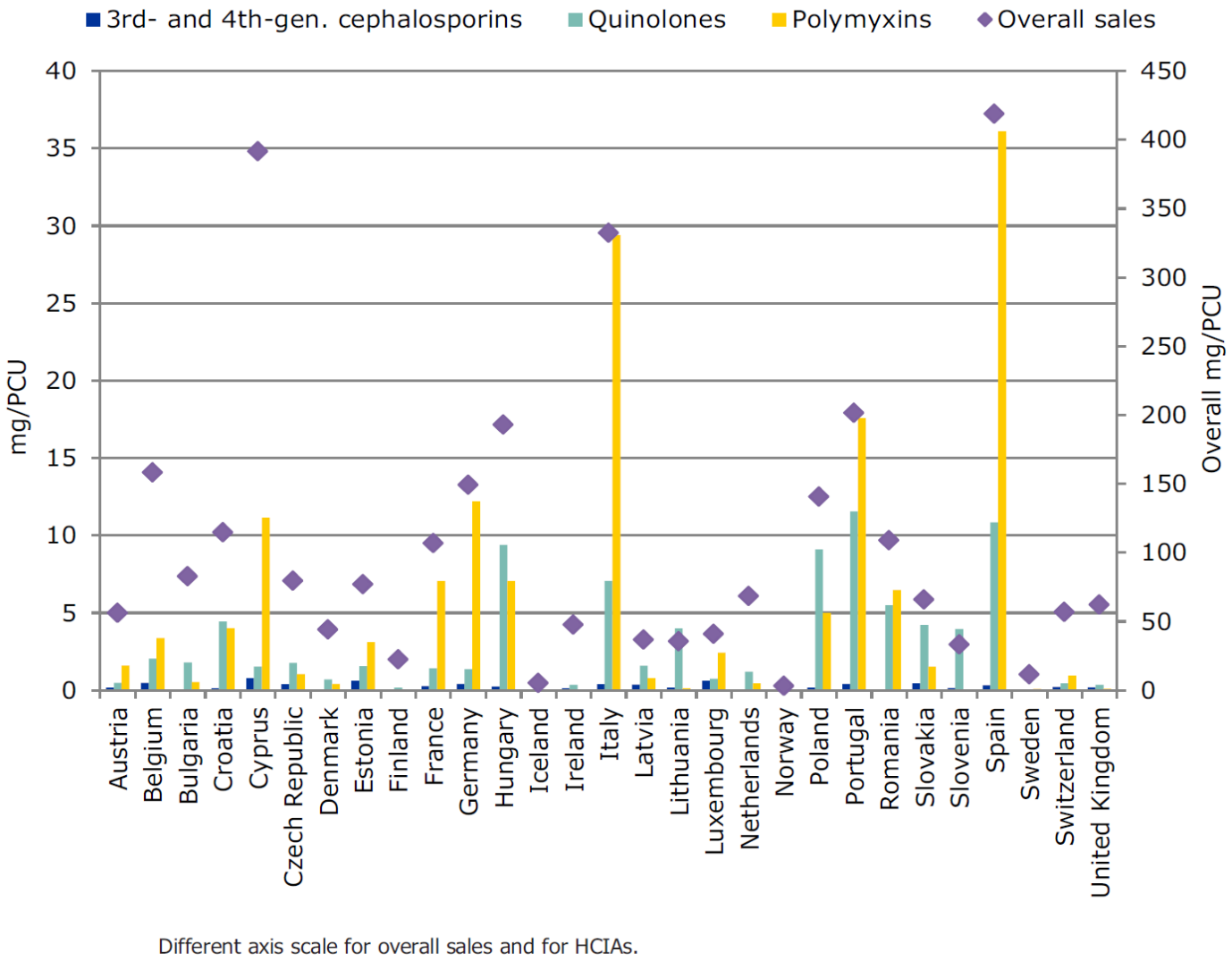


Figure E.1: Overall sales of veterinary antimicrobial agents for food-producing species, in mg/PCU, from 2011 to 2014, for 29 European countries



Different axis scale for overall sales and for HCIAs.

Figure E.2: Sales of veterinary antimicrobial agents for food-producing species, in mg/PCU, overall (right Y-axis) and of 3rd- and 4th-generation cephalosporins, quinolones and polymyxins (left Y-axis), for 2014, for 29 European countries

Colistin – a controversial history (*before Nov. 2015*)

- Family: polymixine (polimixin E)
- Produced by *Bacillus polymyxa* var. colistinus (1950)

- Naturally resistant
  - Gram positives
  - Gen. *Proteus*
  - *Morganella morganii*
  - *Serratia marcescens*
  - *Yersinia pseudotuberculosis*

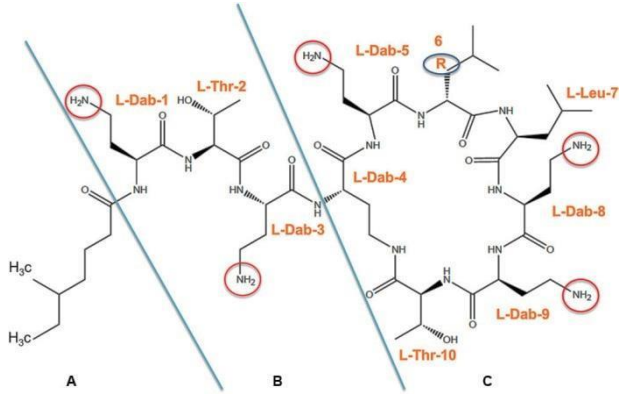
- Active against
  - *E. coli*
  - *Salmonella enterica*
  - *Shigella spp.*
  - *Klebsiella pneumoniae*
  - *Klebsiella oxytoca*
  - *Enterobacter cloacae*
  - *Enterobacter aerogenes*
  - *Citrobacter* spp.

resistance extremely rare!

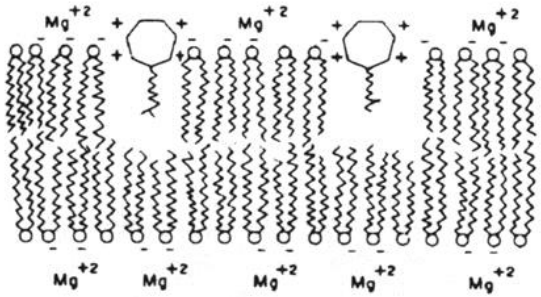
Colistin – a controversial history (*before Nov. 2015*)

- Introduced in therapy in the1960s
- Recognized as toxic in the 1970s and banned for human use
- 1990 – first case of septicaemia by MDR Gram-, use of colistin reconsidered
- 2000 - *Klebsiella* and *E. coli* XDR
- 2012 WHO identifies colistin as a critical antimicrobial

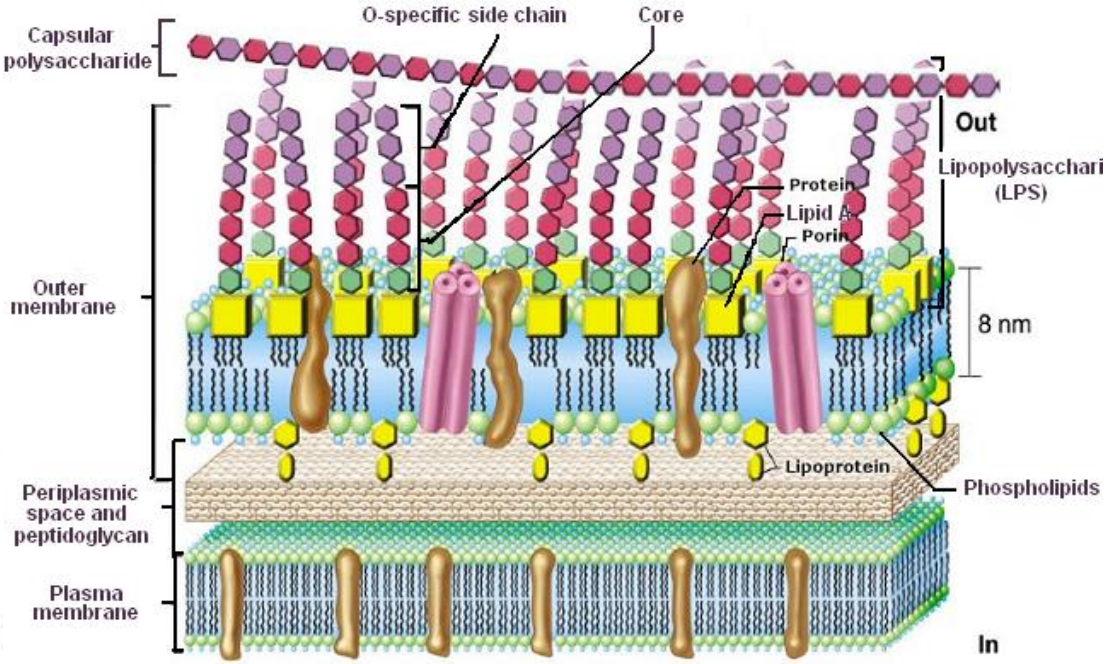
Colistin – structure and function



Front. Microbiol., 11 November 2016 | https://doi.org/10.3389/fmicb.2016.01789

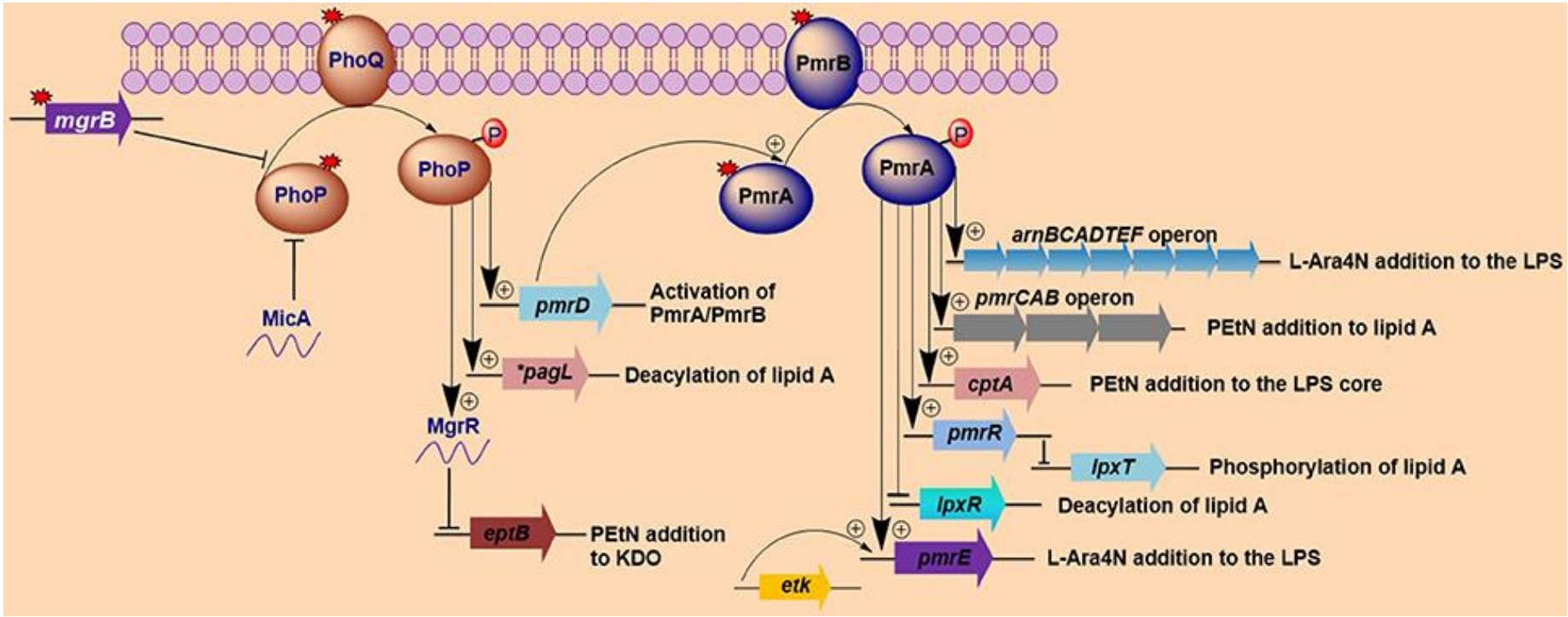


Hypothetical model for interaction of polymyxin with a phospholipid bilayer. It is proposed that the fatty acid tail of the peptide penetrates the hydrophobic domain of the bilayer, with the peptide amino groups interacting electrostatically with phospholipid phosphates. (*From Storm et al: Annu Rev Biochem 46:723, 1977.*)



www.intechopen.com

Colistin – resitance (*before Nov. 2015*)



Front. Microbiol., 26 November 2014 | https://doi.org/10.3389/fmicb.2014.00643

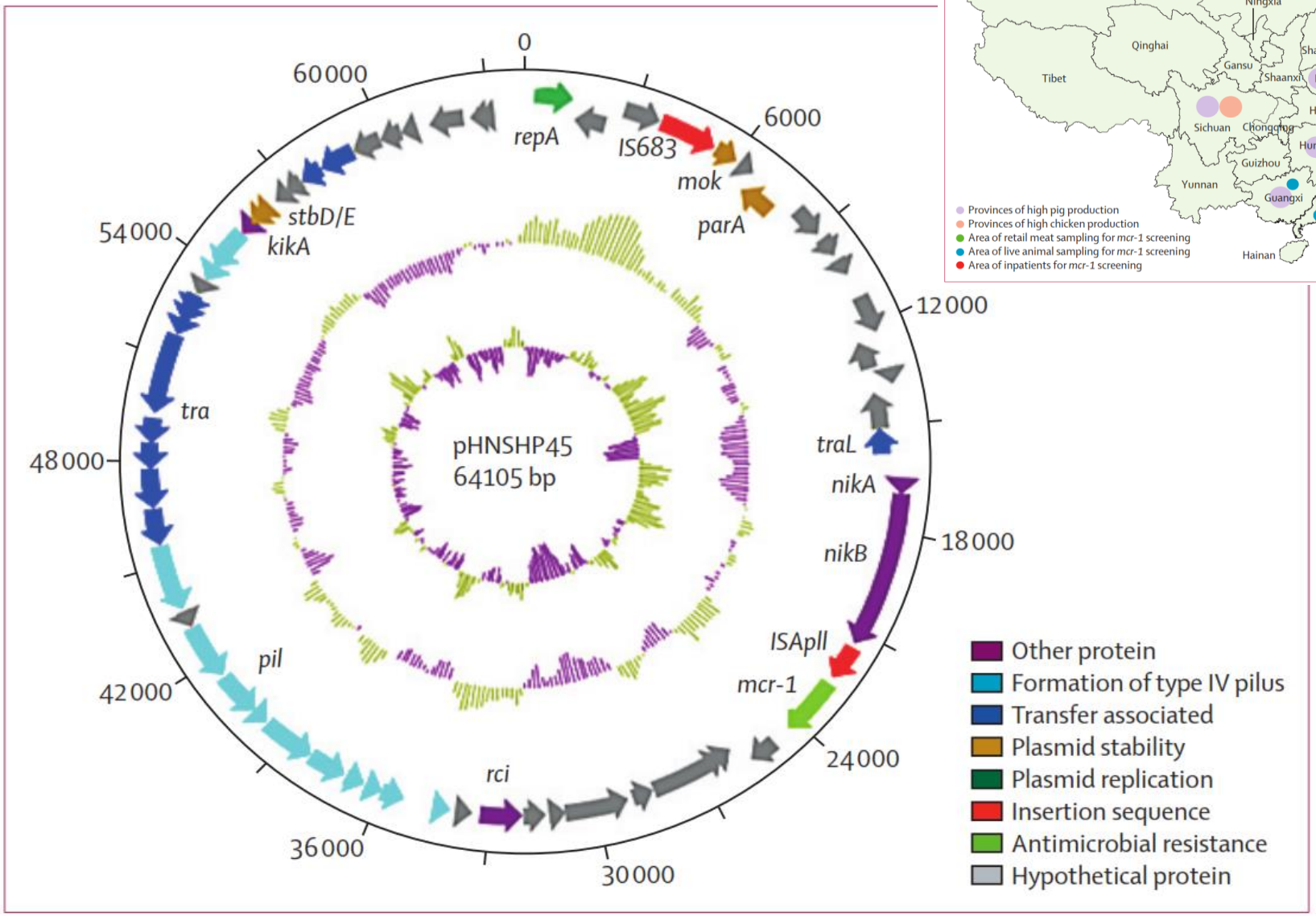


***Figure 2*: Structure of plasmid pHNSHP45 carrying *mcr-1* from *Escherichia coli* strain SHP45**

	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**

Is plasmid-mediated colistin resistance a purely Chinese phenomenon?

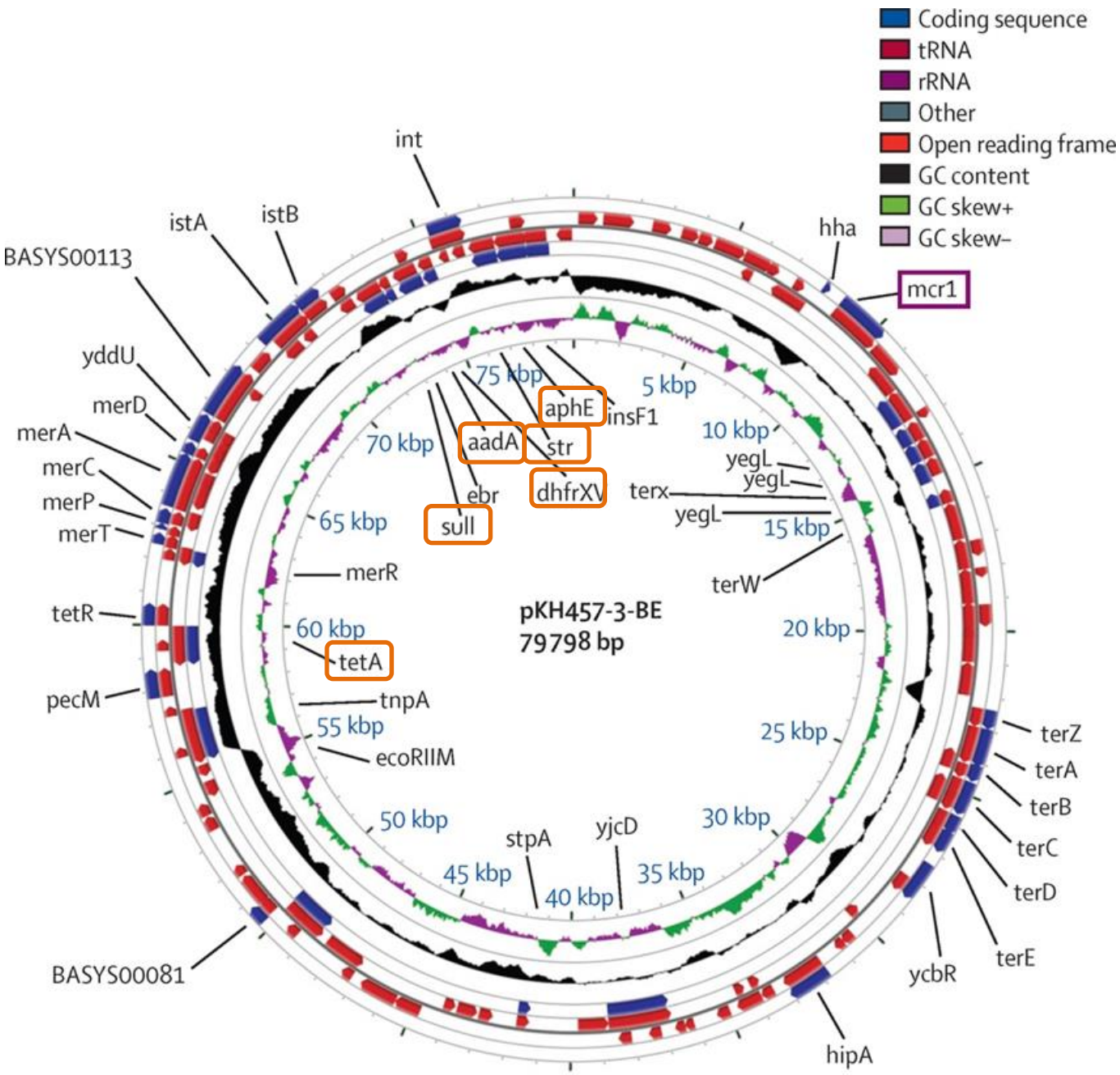
Colistin resistance gene mcr-1 harboured on a multidrug resistant plasmid

Surbhi Malhotra-Kumar, Basil Britto Xavier, Anupam J Das, Christine Lammens, Patrick Butaye, Herman Goossens

The Lancet Infectious Diseases
Volume 16, Issue 3, Pages 283-284 (March 2016)
DOI: 10.1016/S1473-3099(16)00012-8

Belgium

- 105 colistin resistant E. coli (52 calves – 53 piglets)
- 13 *mcr-1* positives
- 1 plasmid of a multiresitant E. coli analized



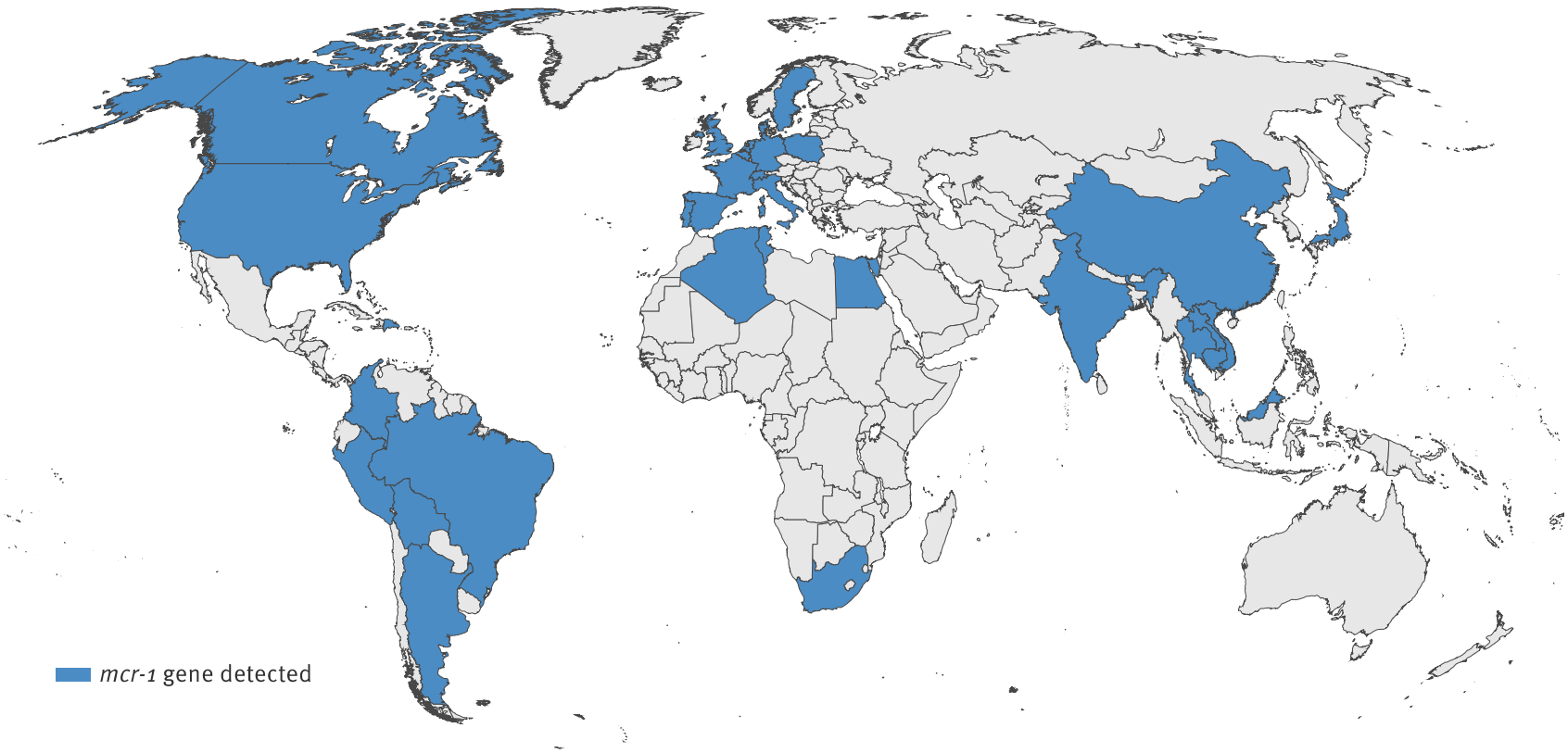
Blast comparison with pHNSHP45 showed 100% similarity only in a short, 2604 bp region that included *mcr-1* (1626 bp) and a truncated ISA*pI1* mobile element that did not include the transposase encoding *tnpA* gene.

pKH-457-3-BE showed 99% similarity (73% query coverage) to plasmid pHXY0908 (GenBank access number KM877269) identified in *Salmonella enterica* serotypeTyphimurium isolated from chicken stool in China.

By contrast withpHNSHP45, pKH-457-3-BE harboured several resistance-encoding genes to trimethoprim (*dfrA1*), tetracycline (*tetA*), aminoglycoside (*aadA1, aph(6)-Id* or *strA,*and *aph(3")-Ib/strB*), and sulphonamide(*sul1*) antibiotics.

The Lancet Infectious Diseases 2016 16, 283-284DOI: (10.1016/S1473-3099(16)00012-8)

Countries (n = 30) reporting presence of *mcr-1* in samples of animal, environmental or human origin (data collected till 27 June 2016)

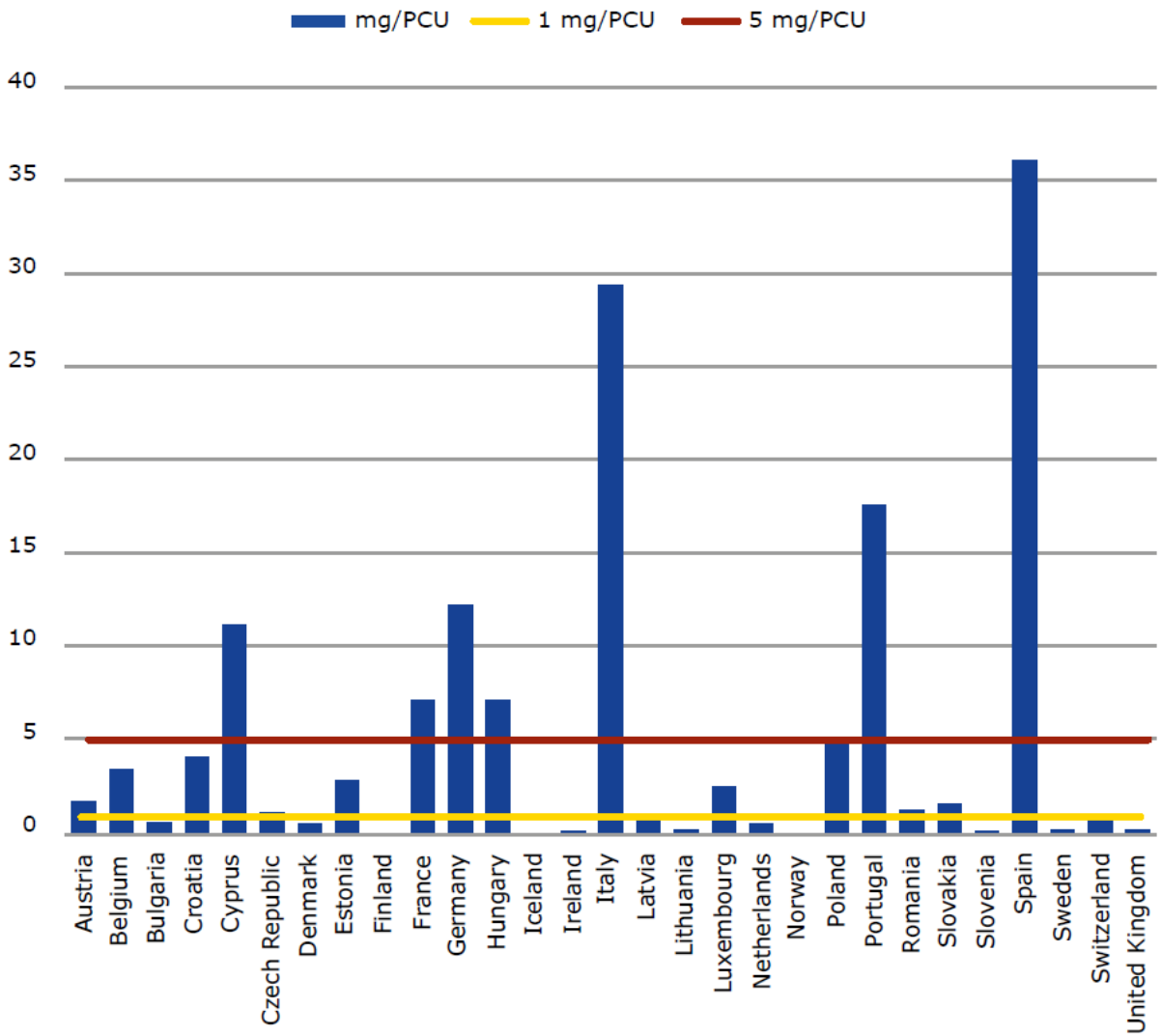


Citation style for this article: Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, Malhotra-Kumar S. Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in Escherichia coli, Belgium, June 2016. Euro Surveill. 2016;21(27):pii=30280. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.27.30280

Figure 46. Spatial distribution of sales of polymyxins, in mg/PCU, by country, for 2014[1]

[1] No sales in Finland, Iceland and Norway.

Figure A1. Sales of colistin for use in food-producing animals, in mg/PCU, in 2014, including the 5 and 1 mg/PCU levels[1]



[1] No sales in Finland, Iceland and Norway.

Regional surveillance system of Emilia-Romagna:

- 8 medical laboratories
- 1 regional reference laboratory
- human isolates of Salmonella: 15-25/week ~ 800-900/year
- animal-food isolates of Salmonella: 20-30/week ~ 1000-1500/year
- unique database for human and animal-food isolates (PFGE/MLVA)
- WGS progressively integrated into the system
- typing in “real-time” for reporting of potential outbreaks
- source attribution of human cases

Data (2012-2017)

Isolates: **9306**

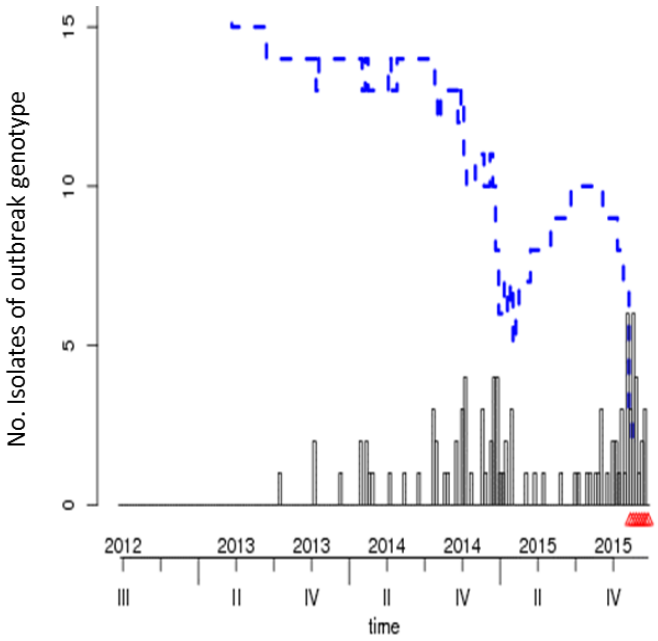
human: **5376**

veterinary: **3930**

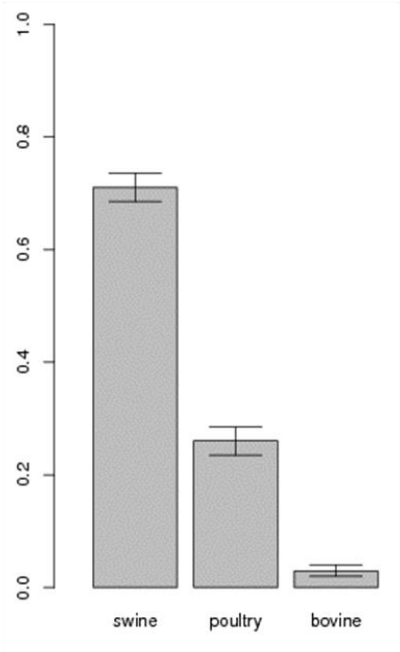
n. PFGE profiles: 2567

n. outbreaks: **21**

Trend analysis

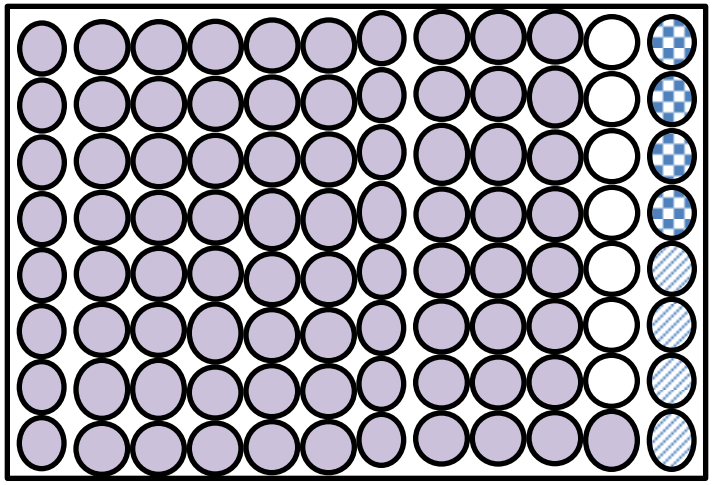


Source attribution



progressive medical-veterinary integration

Salmonella phenotypic screening



5.305 isolates tested

1) 3.865 clinical
2) 1.440 veterinary

↓

total: 37.135

Antibiotico	Concentrazione*
Ampicillina	8 mg/l
Cloramfenicolo	16 mg/l
Colistina	2 mg/l
Ciprofloxacina	0,064 mg/l
Meropenem	0,125 mg/l
Florfenicol	16 mg/l
Cofetaxime	2 mg/l

AMR screening 2012-15 of 5.305 isolates

AMP = AMPICILLIN
FFN = FLORFENICOL
CIP = CIPROFLOXACIN

CTX=CEFOTAXIME
CHL = CLORAMFENICOL
COL=COLISTIN

MEM=MEROPENEM

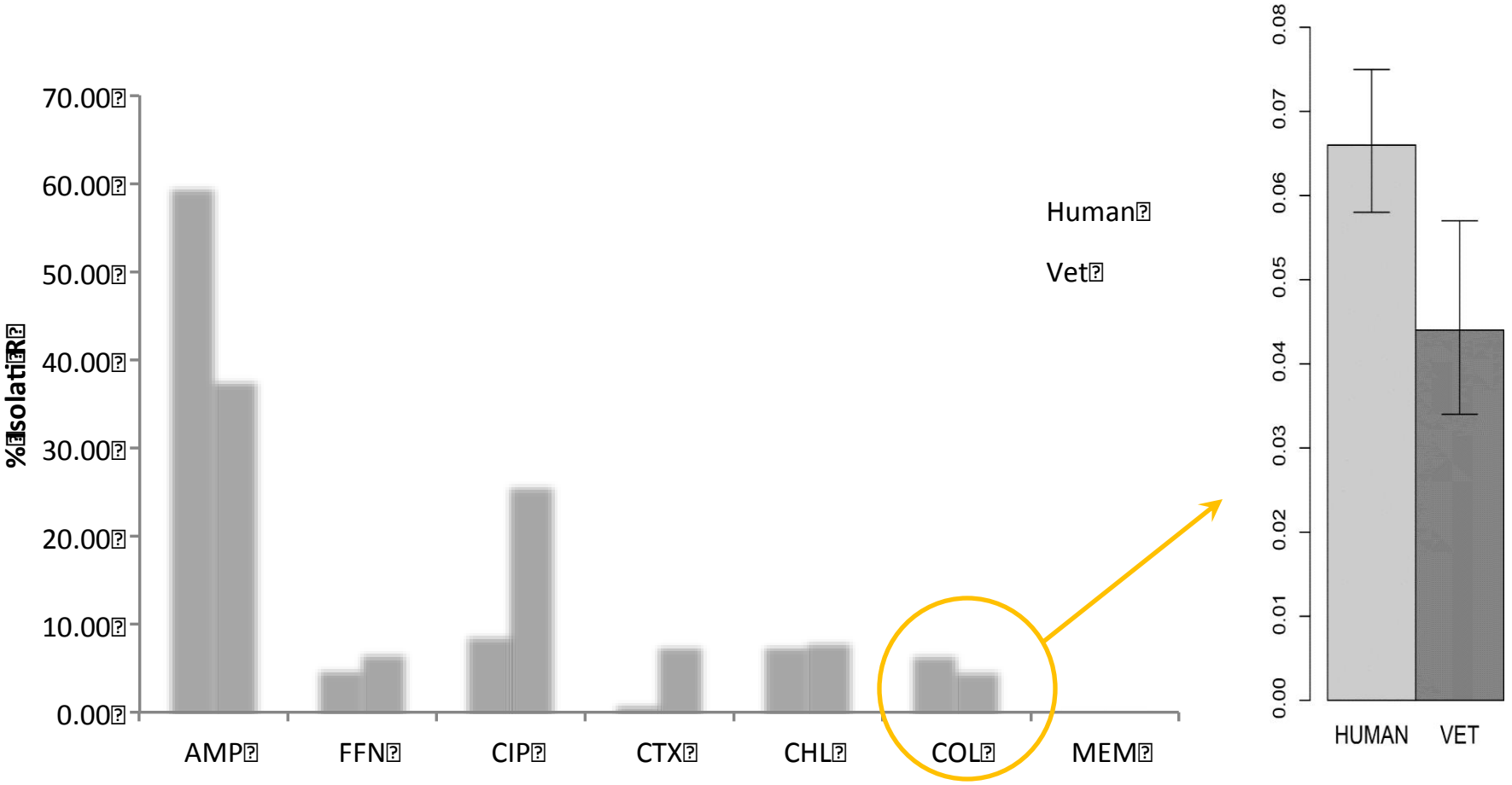
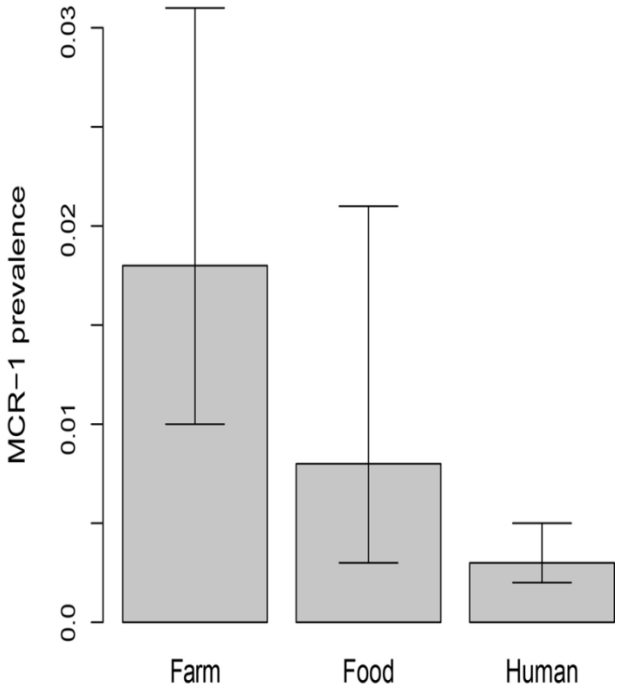


TABLE 1 Results of screening for colistin resistance and presence of the *mcr-1* gene in 4,473 *Salmonella* isolates collected from different sources between 2012 and 2015 in Emilia-Romagna, Italy

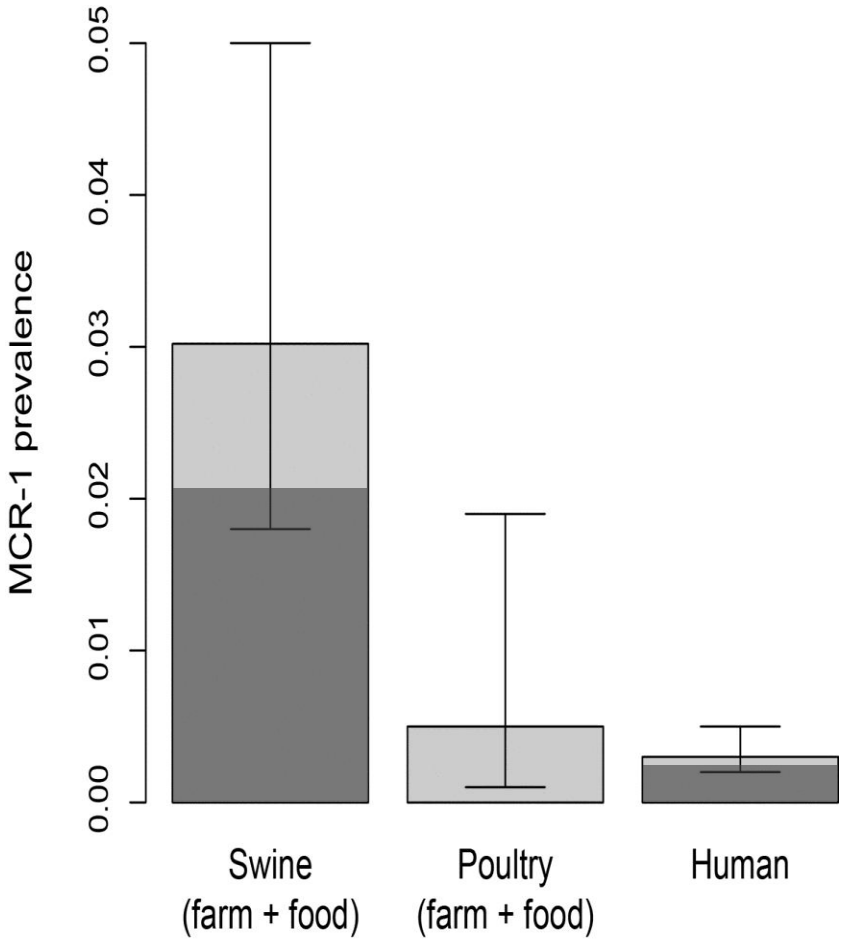
Source	Yr of isolation	No. of isolates		No. of *mcr-1*-positive isolates (% of those tested)[c]
		Tested[a]	Growing on screening plates[b]	
Farmed animals				
Poultry	2012–2015	243	13	2 (0.8)
Swine	2012–2015	222	16	9 (4.1)
Bovines	2013–2015	30	3	0 (0)
Mussels	2013–2015	21	1	0 (0)
Horses	2014–2015	19	1	0 (0)
Goats	2014	1	0	0 (0)
Feed	2013–2015	28	0	0 (0)
Pets				
Cats	2013–2015	6	1	0 (0)
Dogs	2012, 2014	2	0	0 (0)
Nonpets				
Mammals	2012–2015	48	0	0 (0)
Birds	2013–2015	39	3	0 (0)
Reptiles	2013–2015	7	0	0 (0)
Humans	2012–2015	3,294	217	10 (0.3)
Food				
Pork	2013–2015	223	6	4 (1.8)
Poultry meat	2013–2015	93	1	0 (0)
Beef	2013–2015	7	0	0 (0)
Eggs	2013–2014	2	0	0 (0)
Other	2012–2015	152	6	0 (0)
Environment (seawater)	2013–2015	36	1	0 (0)
Total		4,473	269	25 (0.6)

mcr-1: source by sub-group

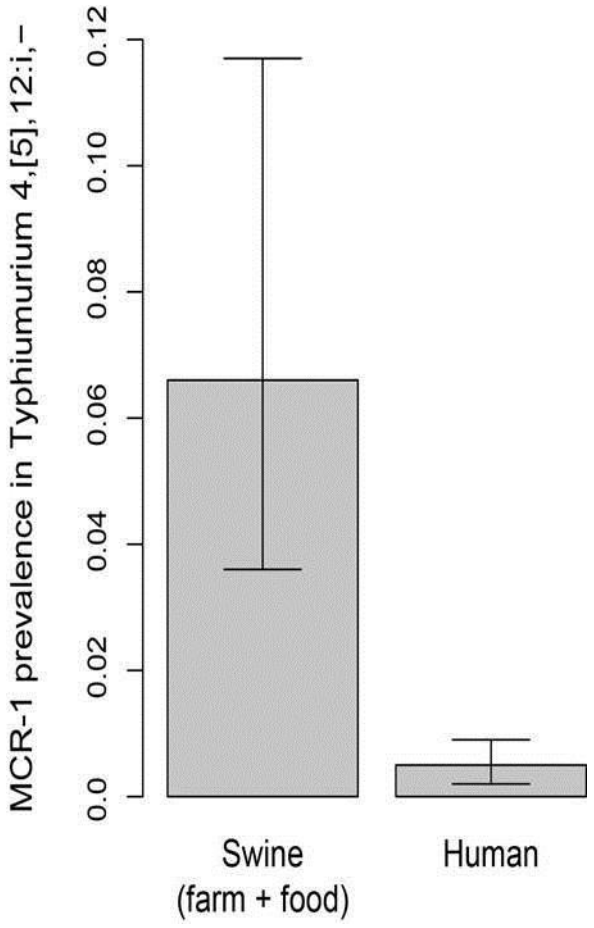


mcr-1 by source and serotype

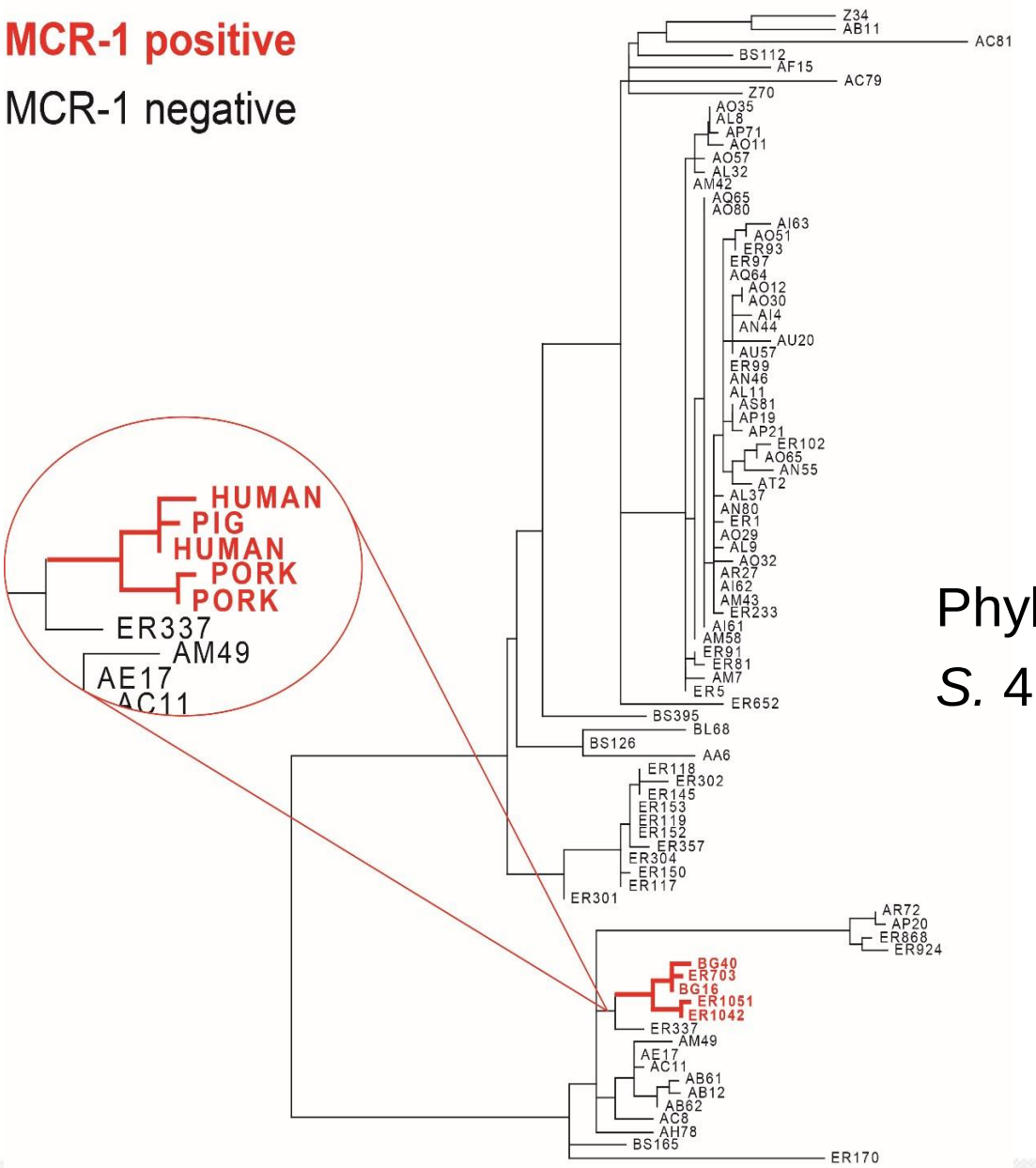
mcr-1 in *Salmonella* spp. by source



mcr-1 in *Salmonella* 4,[5],12:i:-



mcr-1: swine to human transmission?



Phylogenetic tree of

S. 4,[5],12:i- genotype

Open questions:

- Is it more prevalent in animal than human isolates because of
  - dynamic disequilibrium due to recent introduction in animals?
  - instability in the human compartment due to low selective pressure?

Thank you for attention

- Regional Public Health Service of Emilia-Romagna
- Regional Hospital and Health Authorities Network
- IZSLER Laboratories
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- Paola Bellotti
- Erika Scaltriti
- Gabriele Casadei
- Luca Bolzoni

JAMA. 2012;308(18):1934-1934