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

Problematiche legate all'uso della colistina: la necessità di pensare alle possibili conseguenze per la salute degli uomini, degli animali e dell'ambiente

Stefano Pongolini

Ozzano 28 aprile 2017


Inchiesta

Antibiotici
il rischio nel piatto

Alcuni batteri possono sviluppare un pericoloso meccanismo di resistenza ai farmaci. Li abbiamo cercati nella carne di pollo. E nell'84% dei casi li abbiamo trovati.

L'INCHIESTA IN QUATTRO PASSI

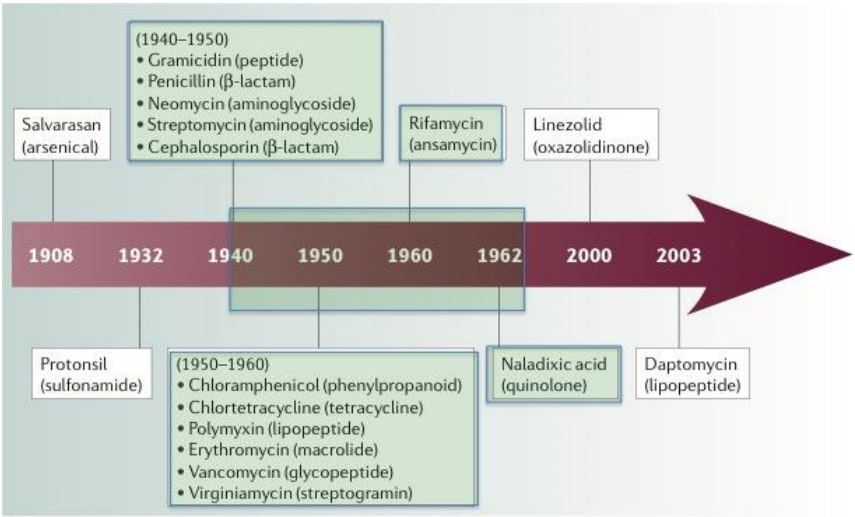
84% Italia
74% Portogallo
73% Belgio
72% Spagna

QUANTI BATTERI RESISTONO?

LA NOSTRA INCHIESTA

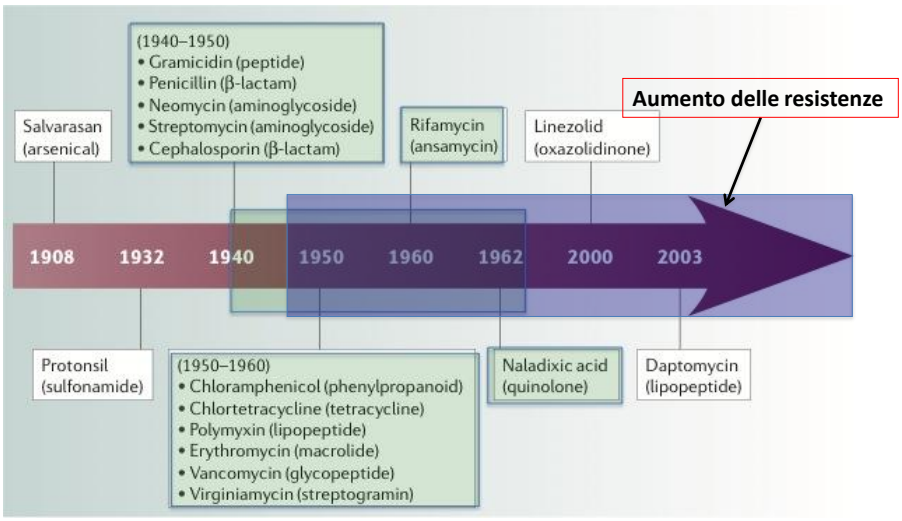
OCCHIO AI BATTERI

Antibiotico-Resistenza: storia



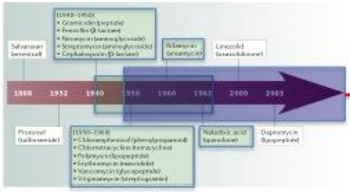
Nature Reviews Microbiology 5, 175-186 (March 2007)

Antibiotico-Resistenza: evoluzione



Nature Reviews Microbiology 5, 175-186 (March 2007)

Antibiotico-Resistenza: presente

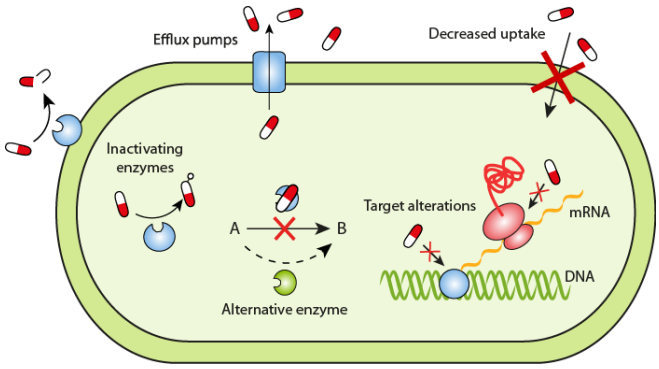


Aumento delle resistenze

Negli ultimi 50 anni non siamo piu' riusciti a scoprire nuove molecole!

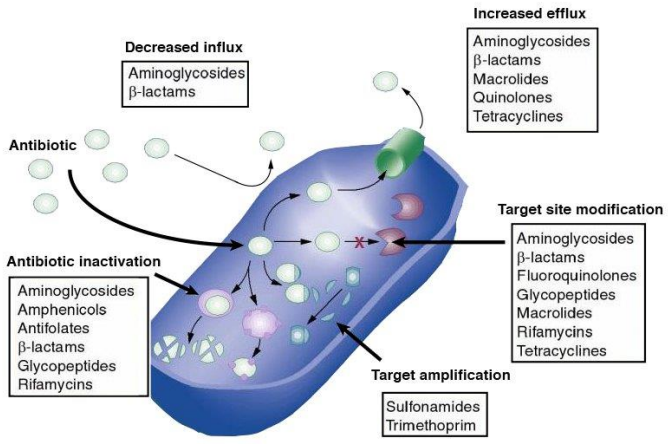
ALLARME MONDIALE!

La resistenza gli antibiotici



ReAct – Action on Antibiotic Resistance

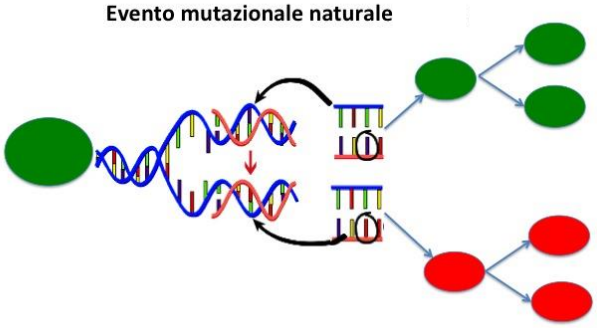
La resistenza gli antibiotici



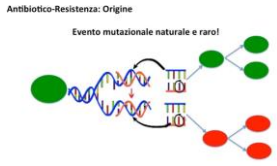
A Daily Deed - WordPress.com

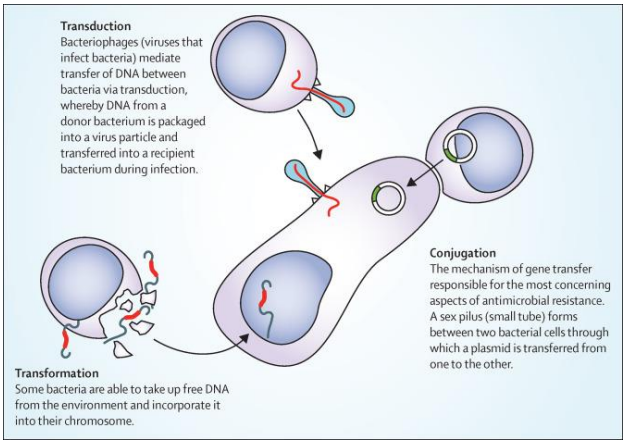
La resistenza gli antibiotici - genetica

Antibiotico-Resistenza: Origine



La resistenza gli antibiotici - genetica





The Lancet

Antibiotico-Resistenza: evoluzione

- la resistenza e' un fenomeno naturale
- esisteva ancora prima della scoperta degli antibiotici

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

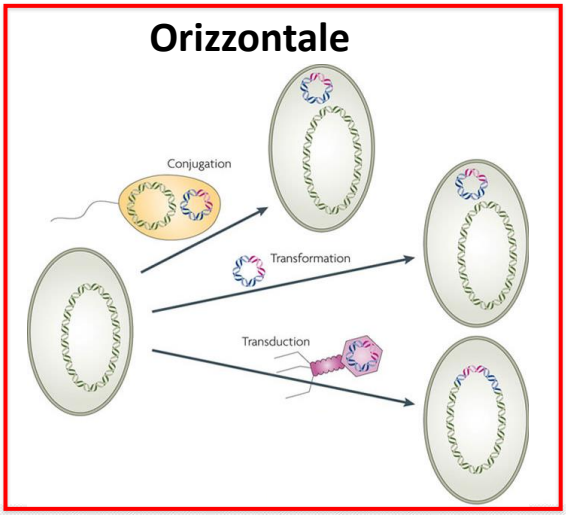
Julian Davies* and Dorothy Davies
Department of Microbiology and Immunology, Life Sciences Institute, University of British Columbia, 2350 Health Sciences Mall, Vancouver, British Columbia V6T 1Z3, Canada

- In natura esistono *MOLTI* piu' geni di resistenza rispetto a quelli che solitamente si cercano nei patogeni (~34.000)

La resistenza gli antibiotici - propagazione

Verticale

Orizzontale



Antibiotico-Resistenza: evoluzione

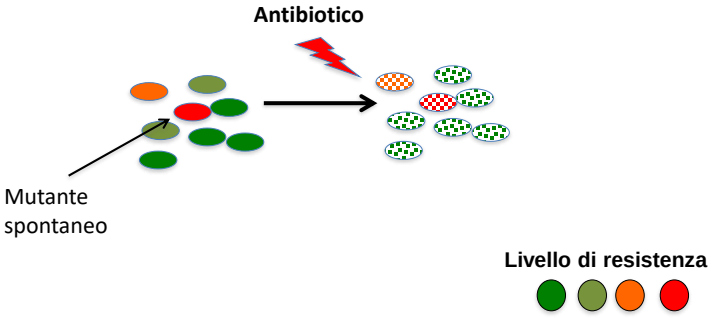
La resistenza e' un fenomeno naturale!

impossibile da eliminare

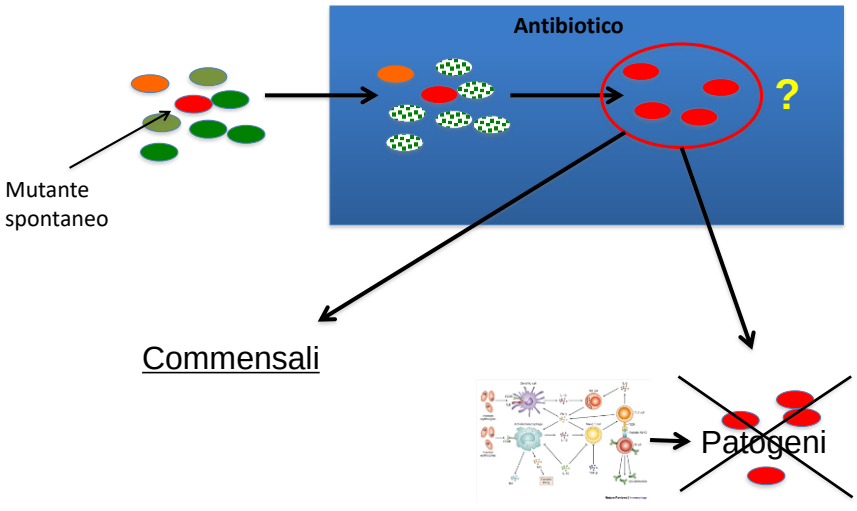
possibile contenerla?

è questione di pressione selettiva

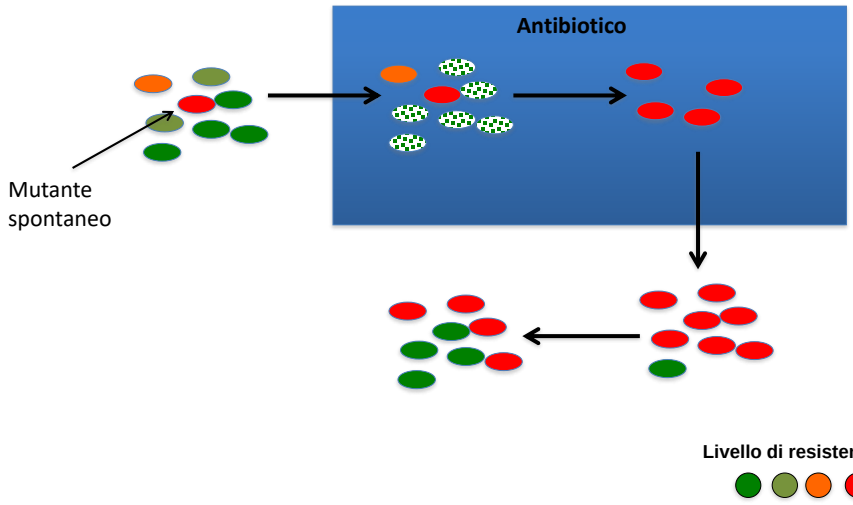
Pressione di selezione: Il vecchio credo…



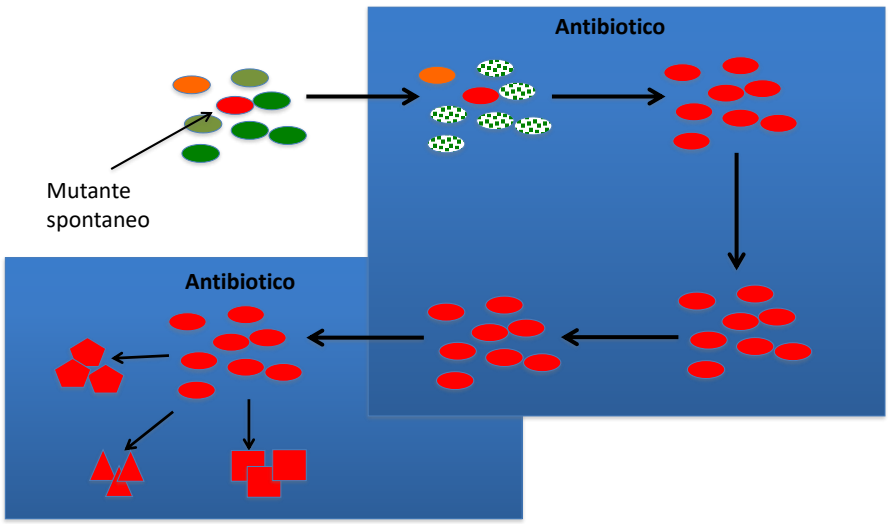
Pressione di selezione!



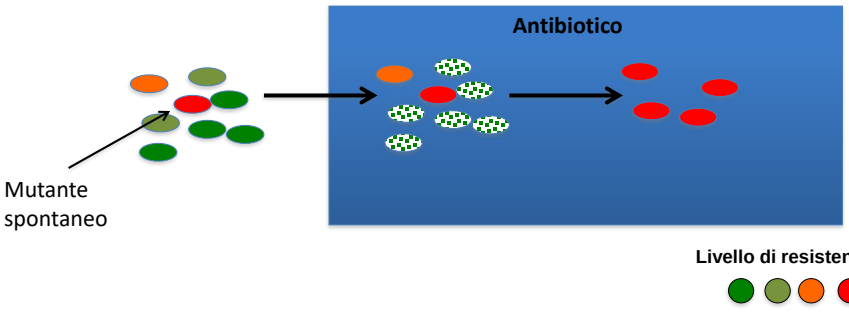
Pressione di selezione!



Pressione di selezione: permanenza antibiotico

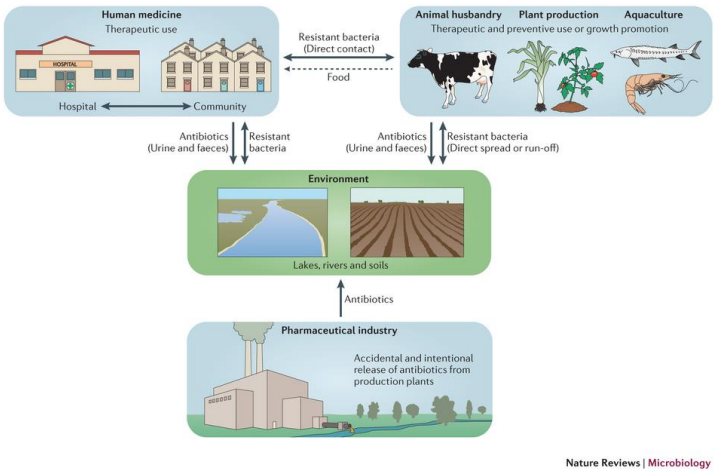


Pressione di selezione!



1) La presenza di antibiotico seleziona i batteri piu' resistenti

2) La presenza continua di antibiotico consolida la resistenza



- AMR si propaga con il propagarsi di geni di resistenza, non solo di batteri resistenti
- I geni di resistenza persistono nell'ambiente per anni

- C'è un maggior responsabile?
- E' stimabile il "peso" di ciascun comparto

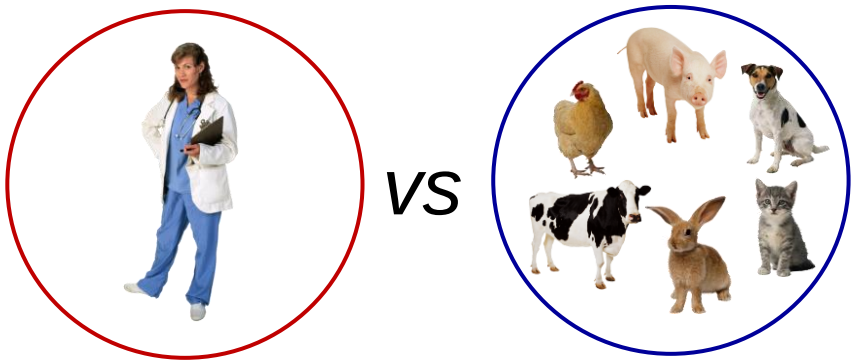
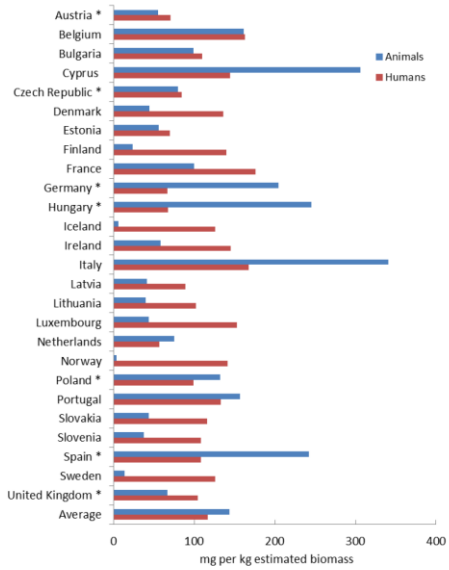


Figure 3. Comparison of biomass-corrected consumption of antimicrobials (milligrams per kilogram estimated biomass) in humans and food-producing animals by country in 26 EU/EEA countries in 2012[18,19,20]



Colistina – una storia controversa (*prima del nov. 2015*)

- Famiglia: polymixine (polimixina E)
- Prodotta da *Bacillus polymyxa* var. colistinus (1950)

- Naturalmente resistenti
  - Gram positivi
  - Gen. *Proteus*
  - *Morganella morganii*
  - *Serratia marcescens*
  - *Yersinia pseudotuberculosis*

- Attiva contro (Resistenza rarissima!)
  - E. coli
  - Salmonella enterica
  - Shigella spp.
  - Klebsiella pneumoniae
  - Klebsiella oxytoca
  - Enterobacter cloacae
  - Enterobacter aerogenes
  - Citrobacter spp.

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Colistina – una storia controversa (*prima del nov. 2015*)

- Introdotta in terapia negli anni 1960
- Riconosciuta come tossica nei ’70 e bandita dall’uso umano
- 1990 - primo paziente con setticemia da Gram- MDR – uso della colistina viene riconsiderato
- 2000 - *Klebsielle* e *E. coli* XDR

- 2012 OMS dichiara colistina farmaco critico

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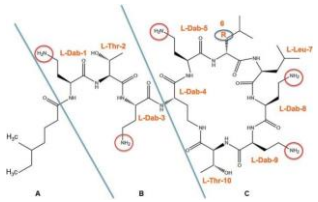
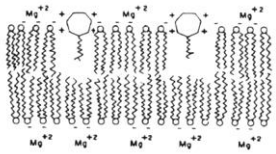
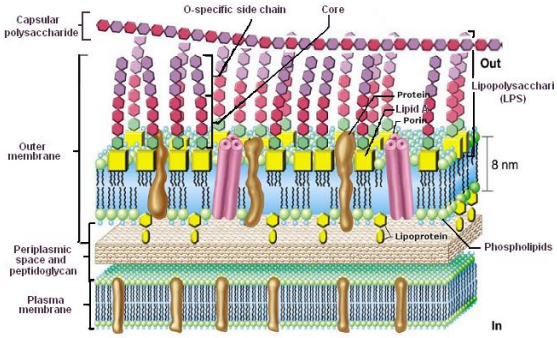
Colistina – struttura e funzione



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

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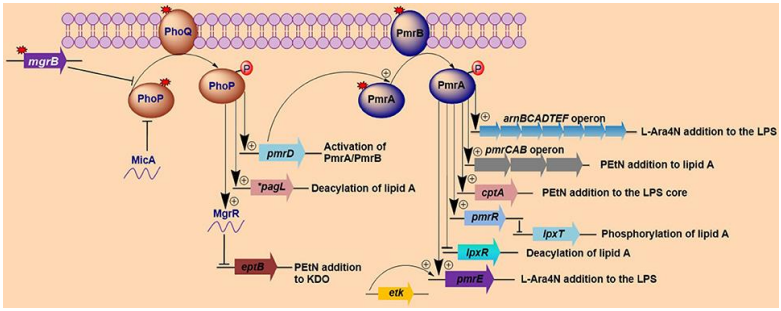
Colistina – resistenza (*prima del nov. 2015*)



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

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THE LANCET Infectious Diseases

Articles

Published: 18 November 2015

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*

Summary

Background Until now, polymyxin resistance has involved chromosomal mutations but has never been reported via horizontal gene transfer. During a routine surveillance project on antimicrobial resistance in commensal *Escherichia coli* from food animals in China, a major increase of colistin resistance was observed. When an *E coli* strain, SHP45, possessing colistin resistance that could be transferred to another strain, was isolated from a pig, we conducted further analysis of possible plasmid-mediated polymyxin resistance. Herein, we report the emergence of the first plasmid-mediated polymyxin resistance mechanism, MCR-1, in Enterobacteriaceae.

Methods The *mcr-1* gene in *E coli* strain SHP45 was identified by whole plasmid sequencing and subcloning. MCR-1 mechanistic studies were done with sequence comparisons, homology modelling, and electrospray ionisation mass spectrometry. The prevalence of *mcr-1* was investigated in *E coli* and *Klebsiella pneumoniae* strains collected from five provinces between April, 2011, and November, 2014. The ability of MCR-1 to confer polymyxin resistance in vivo was examined in a murine thigh model.

Findings Polymyxin resistance was shown to be singularly due to the plasmid-mediated *mcr-1* gene. The plasmid carrying *mcr-1* was mobilised to an *E coli* recipient at a frequency of 10^{-1} to 10^{-3} cells per recipient cell by conjugation, and maintained in *K pneumoniae* and *Pseudomonas aeruginosa*. In an in-vivo model, production of MCR-1 negated the efficacy of colistin. MCR-1 is a member of the phosphoethanolamine transferase enzyme family, with expression in *E coli* resulting in the addition of phosphoethanolamine to lipid A. We observed *mcr-1* carriage in *E coli* isolates collected from 78 (15%) of 523 samples of raw meat and 166 (21%) of 804 animals during 2011–14, and 16 (1%) of 1322 samples from inpatients with infection.

Interpretation The emergence of MCR-1 heralds the breach of the last group of antibiotics, polymyxins, by plasmid-mediated resistance. Although currently confined to China, MCR-1 is likely to emulate other global resistance mechanisms such as NDM-1. Our findings emphasise the urgent need for coordinated global action in the fight against pan-drug-resistant Gram-negative bacteria.

Lancet Infect Dis 2015

Published Online November 18, 2015 http://dx.doi.org/10.1016/S1473-3099(15)00424-7

See Online/Articles http://dx.doi.org/10.1016/S1473-3099(15)00463-6

*Contributed equally

College of Veterinary Medicine, National Risk Assessment Laboratory for Antimicrobial Resistance of Microorganisms in Animals, South China Agricultural University, Guangzhou, China (Y-Y Liu BS, L-X Yi BS, X Huang PhD, L-F Yu BS, X Chen MS, L Lv MS, D He MS, Prof Z Liang MS, Prof J-H Liu PhD); Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Veterinary Medicine, China Agricultural University, Beijing, China (Y Wang PhD, B Dong BS, H Ren BS, Prof J Shen PhD); Department of Medical Microbiology and Infectious Disease, Institute of Infection

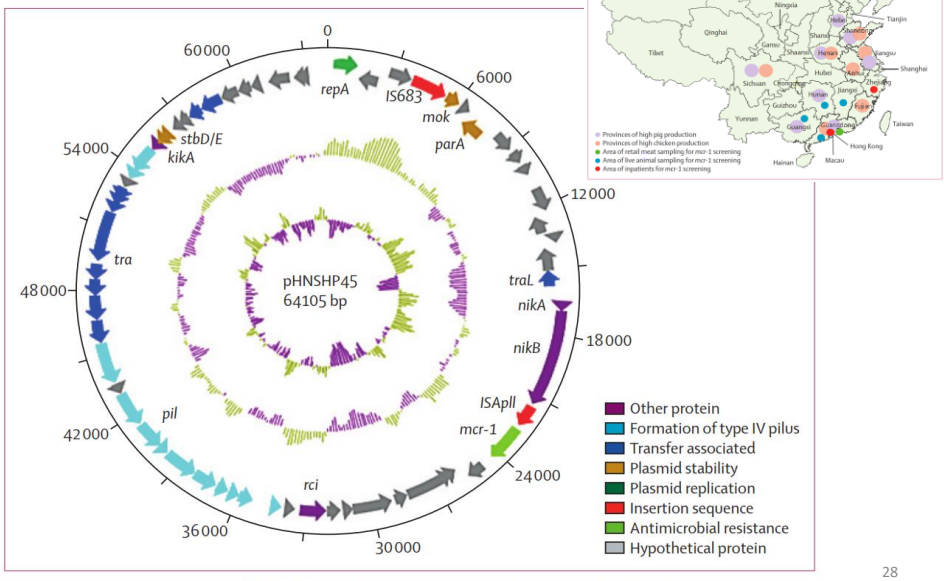


Figure 2: **Structure of plasmid pHNSHP45 carrying *mcr*-1 from *Escherichia coli* strain SHP45**

	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?

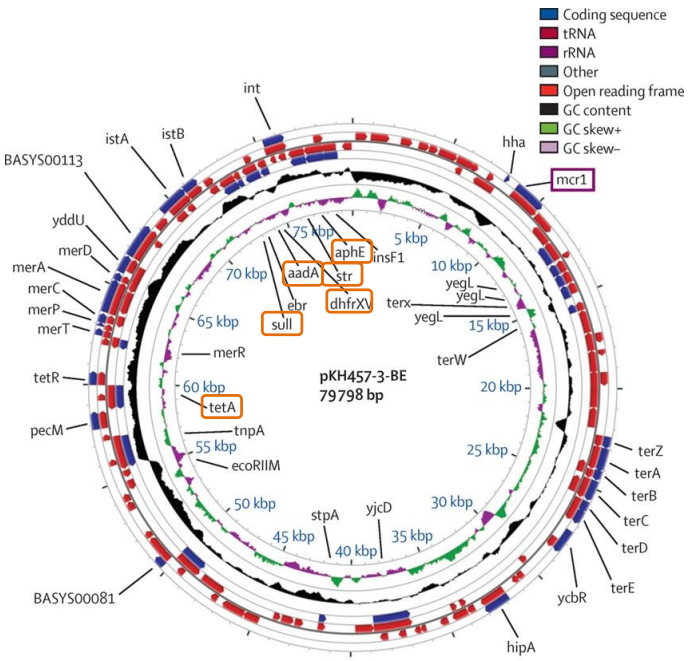
Colistin resistance gene mcr-1 harboured on a multidrug resistant plasmid

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

Belgio

- 105 E. coli (52 vitelli – 53 suinetti) colistina resistenti
- 13 positivi per *mcr-1*
- 1 plasmide di uno dei coli multiresistente analizzato



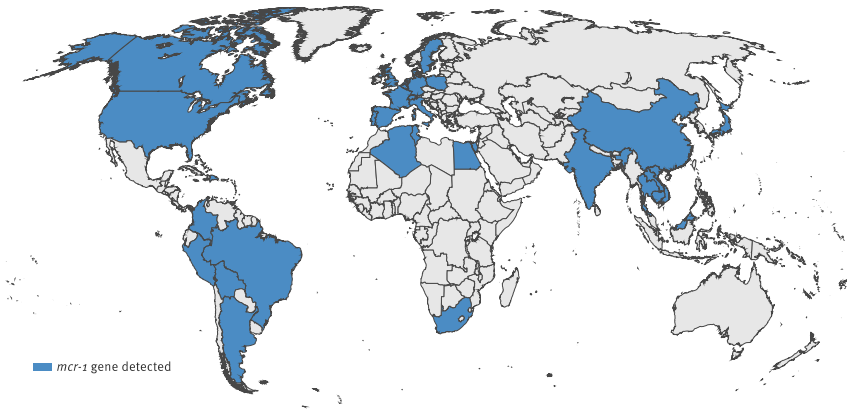
Blast comparison with pHNSHP45 showed 100% similarity only in a short, 2604 bp region that included *mcr-1* (1626 bp) and a truncated ISA*pl1* mobile element that did not include the transposase encoding *tnpA* gene.

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

EDITORIAL

Plasmid-mediated colistin resistance (*mcr-1* gene): three months later, the story unfolds

RL Skov [1], **DL Monnet** [2]
1. Department of Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark
2. Office of Chief Scientist, European Centre for Disease Prevention and Control, Stockholm, Sweden

Correspondence: Robert L. Skov (rsk@ssi.dk)

Source	Year	Country	Type of specimen/ animal /infection	Origin/ travelled region	Isolates n (%)	Species	Extended-spectrum beta-lactamase (ESBL)	Carbapenemase	Reference
Food animals	1980s–2014	China	Chickens	a	104	*E. coli*	NA	NA	[21]
	2005–2014	France	Veal calves	a	106	*E. coli*	CTX-M-1 (n = 7)	No	[10]
	2008–10	Japan	Pigs	a	2	*E. coli*	NA	NA	[23]
	2010–2011	Germany	Pigs	a	3	*E. coli*	CTX-M-1 (n = 3)	No	[7]
	2010-2015	The Netherlands	Chickens, veal calves, turkeys	a	4 (< 1%)	*E. coli*	NA	NA	[5]
	2011	France	Pigs	a	1 (< 1%)	*E. coli*	NA	NA	[16]
	2011–12	Belgium	Pigs	a	6	*E. coli*	No	No	[13]
	2011–12	Belgium	Veal calves	a	7	*E. coli*	No	No	[13]
	2012	Laos	Pigs	a	3	*E. coli*	NA	NA	[30]
	2012	China	Pigs	a	31 (14%)	*E. coli*	NA	NA	[1]
	2012–13	Japan	Cattle	a	4	*E. coli*	CTX-M-27	No	[23]
	2013	Japan	Pigs	a	1	*Salmonella* Typhimurium	NA	NA	[23]
	2013	China	Pigs	a	68 (25%)	*E. coli*	NA	NA	[1]
	2013	Malaysia	Chickens	a	3	*E. coli*	NA	NA	[17]
	2013	Malaysia	Pigs	a	1	*E. coli*	NA	NA	[17]
	2013	France	Pigs	a	1 (< 1%)	*E. coli*	No	No	[16]
	2013	France	Chickens	a	3 (2%)	*E. coli*	No	No	[16]
	2013	France	Chickens (farm)	a	1	*Salmonella* 1,4 [5],12:i:-	NA	NA	[26]
	2014	France	Broilers	a	4 (2%)	*E. coli*	No	No	[16]
	2014	France	Turkeys	a	14 (6%)	*E. coli*	CMY-2	No	[16]
	2014	Italy	Turkeys	a	1	*E. coli*	No	No	[4]

Complessivamente il quadro che emerge dice che:

- *mcr-1* è estremamamente conservato
- Il contesto genetico in cui si trova (plasmidi) è molto vario

Multi-drug Resistance

↓ e.g. NDM-1 (resistance to carbapenems)

Extensively-drug Resistance

↓ e.g. MCR-1 (resistance to polymixins/colistin)

Pan-drug Resistance

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

27 July 2016
EMA/CVMP/CHMP/231573/2016
Committee for Medicinal Products for Veterinary use (CVMP)
Committee for Medicinal Products for Human Use (CHMP)

Updated advice on the use of colistin products in animals within the European Union: development of resistance and possible impact on human and animal health

1. Executive summary

Colistin is an antibacterial agent of the polymyxin class. Following the discovery of a new colistin horizontally transferable resistance mechanism (MCR-1), the European Commission (EC) requested the European Medicines Agency (EMA) to update the previous advice on the impact of and need for colistin use for human and animal health (EMA, 2013). This updated advice provides an analysis of the colistin toxicity, susceptibility testing, activity and resistance mechanisms, risk profile (based upon the consumption patterns and epidemiology), and risk management options.

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Figure 5. Spatial distribution of sales of polymyxins in veterinary medicine, in mg/kg biomass, in 26 EU/EEA countries, for 2013. No sales reported in Finland, Iceland and Norway. (EMA/ESVAC, 2015)

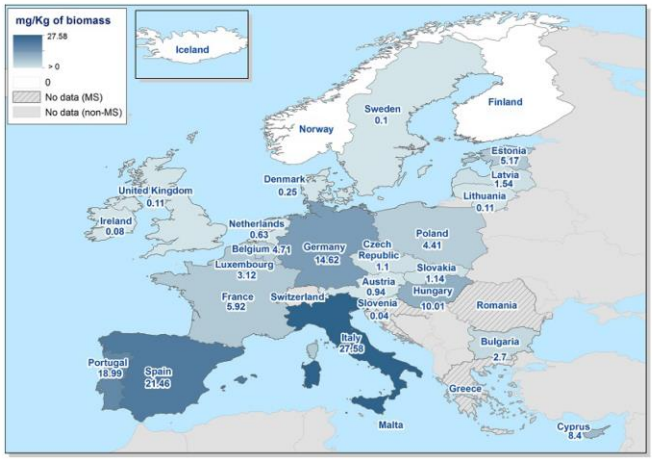
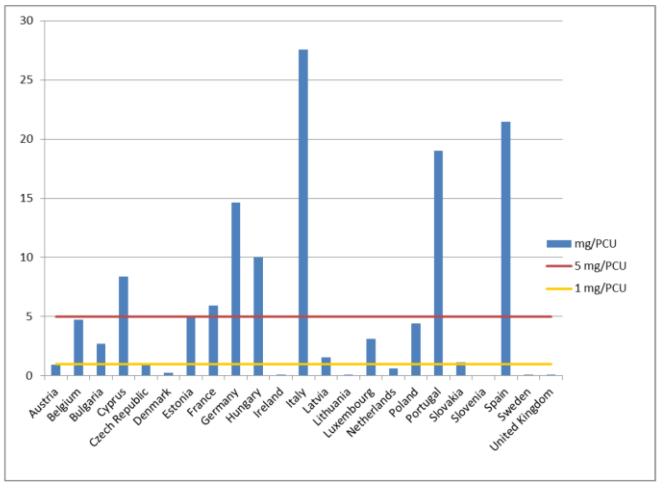
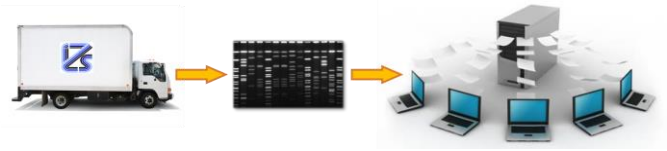


Figure 4. Sales of colistin in for use in animals in mg/PCU in 2013 (ESVAC data), including the 5 and 1 mg/PCU levels. No sales reported in Finland, Iceland and Norway.



La sorveglianza Enternet in Emilia-Romagna (da 6/2012):

- 10 laboratori medici coinvolti
- 1 laboratorio di riferimento regionale (IZSLER - Parma)
- isolati medici di Salmonella: ~ 800-900/anno
- isolati veterinari di Salmonella: ~ 1300/anno
- 1 solo database condiviso in IZSLER (one-health)
- opera in tempo reale
- genotipizza in modo sistematico



Dati (2012/2015)

Isolati: **5811**

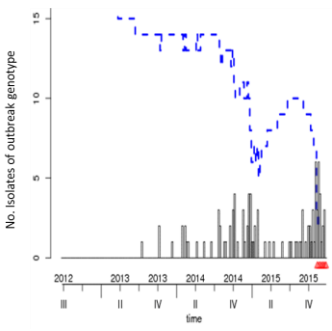
umani: **3745**

veterinari: **2066**

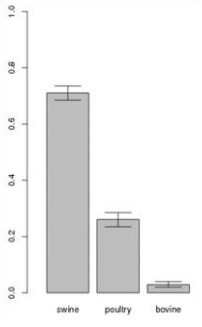
n. profili PFGE : **1279**

n. focolai: **14**

Analisi di trend

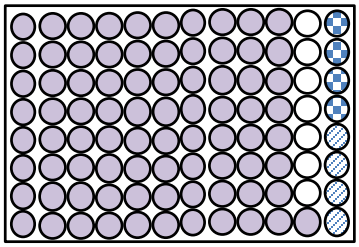


Attribuzione dei casi alle fonti



progressiva integrazione medico-veterinaria

Screening fenotipico su *Salmonella*



Testati 5.305 isolati

1) 3.865 clinici
2) 1.440 veterinari

↓

totale: 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 2012-2015 di 5.305 isolati (4,473 per colistina)

AMP = AMPICILLINA
FFN = FLORFENICOLO
CIP = CIPROFLOXACINA

CTX=CEFOTAXIMA
CHL = CLORAMFENICOLO
COL=COLISTINA

MEM=MEROPENE

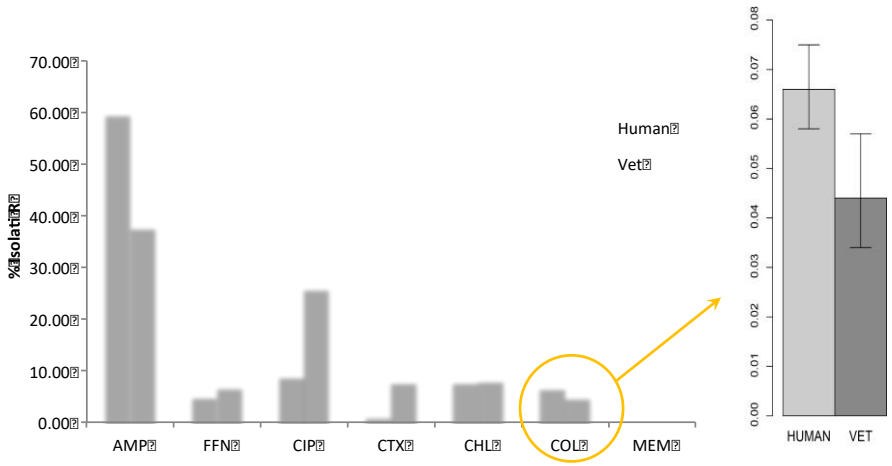
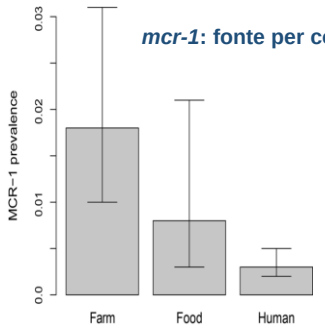


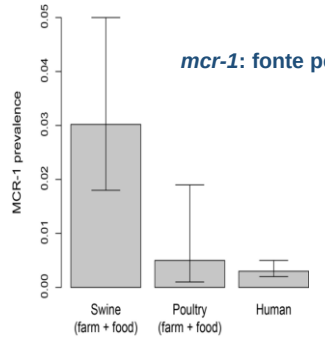
TABLE 1 Results of screening for colistin resistance and presence of the *mcr-1* gene in 4,473 *Salmonella* isolates collected from different sources between 2012 and 2015 in Emilia-Romagna, Italy

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)

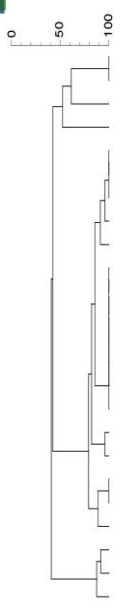
***mcr-1*: fonte per comparto**



***mcr-1*: fonte per specie**



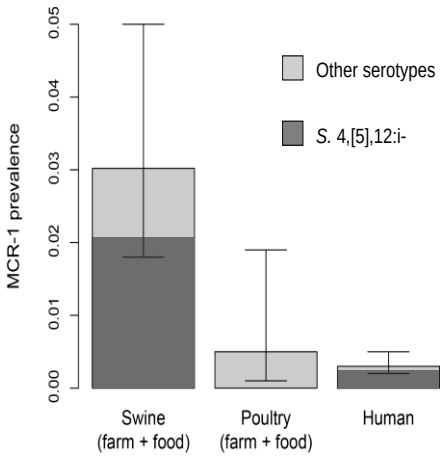
I campioni *mcr*-1 positivi



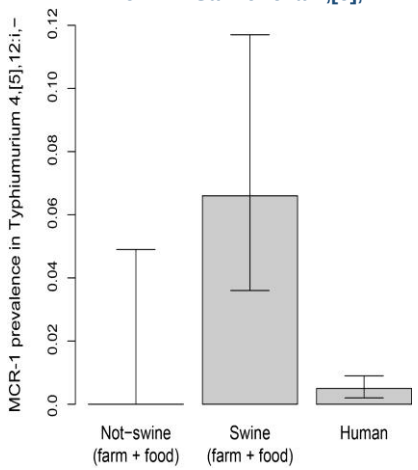
PFGE profile	Serotype	Source	Colistin MIC (mg/L)	AMR pattern
SXB_PR.1095	Bovismorbificans	Pork	16	AMP-CIP-CHL-STR-TET
SXB_PR.1095	Bovismorbificans	Human	16	AMP-CHL-STR-TET
SXB_PR.0926	Schwarzengrund	Poultry	16	AMP-CIP-STR-TET
SXB_PR.1135	Newport	Human	16	AMP-TET
STYMXB_PR.0004	4,[5],12:i:-	Human	16	AMP-STR-TET
STYMXB_PR.0004	4,[5],12:i:-	Human	8	AMP-CHL-FFN-STR-TET
STYMXB_PR.0004	4,[5],12:i:-	Human	8	CHL-FFN
STYMXB_PR.0003	4,[5],12:i:-	Pig	8	AMP-STR-TET
STYMXB_PR.1141	4,[5],12:i:-	Human	16	AMP-STR-TET
STYMXB.0131	4,[5],12:i:-	Pig	8	AMP-STR-TET
STYMXB.0131	4,[5],12:i:-	Pig	8	AMP-STR-TET
STYMXB.0131	4,[5],12:i:-	Pork	16	AMP-STR-TET
STYMXB.0131	4,[5],12:i:-	Pork	16	AMP-STR-TET
STYMXB.0131	4,[5],12:i:-	Human	8	AMP-CHL-FFN-STR-TET
STYMXB.0131	4,[5],12:i:-	Human	16	AMP-STR-TET
STYMXB.0131	4,[5],12:i:-	Human	16	AMP-STR-TET
STYMXB_PR.0715	4,[5],12:i:-	Pork	16	AMP-CHL-FFN-STR-TET
STYMXB_PR.1537	4,[5],12:i:-	Pig	16	AMP-CHL-FFN-STR-TET
STYMXB_PR.0498	4,[5],12:i:-	Pig	16	AMP-CIP-STR-TET
STYMXB_PR.0498	4,[5],12:i:-	Pig	16	AMP-STR-TET
STYMXB_PR.1347	4,[5],12:i:-	Human	16	AMP-STR-TET
SXB_PR.0621	Derby	Pig	8	AMP-STR-TET
SXB_PR.0451	Derby	Pig	8	STR-TET
SXB_PR.0249	Derby	Pig	4	-
DNA autodegradation	Saint Paul	Poultry	8	AMP-TET

mcr-1 per comparto e sierotipo

***mcr-1* in *Salmonella* spp.**



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



Domande aperte:

- E' un'introduzione recente in attivo trasferimento orizzontale?
- La prevalenza di mcr-1 tra gli isolati animali e' piu' alta perche':
  a) di recente introduzione negli animali > in diffusione agli umani
  b) c'è instabilita' nel compartimento umano dovuta a bassa pressione di selezione?

Grazie a tutti!

- Servizio di Sanità Pubblica Emilia-Romagna
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