SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Unità Sanitaria Locale di Reggio Emilia
IRCCS Istituto in tecnologie avanzate e modelli assistenziali in oncologia

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Unità Sanitaria Locale di Modena

RegioneEmilia-Romagna

Convegno nazionale

L'antibioticoresistenza in Regione Emilia-Romagna

Giovedì 11 Ottobre 2018
ore 8.30-17.30

Hotel Classic
Via L. Pasteur 121/C, Reggio Emilia

13.00 Proposta di studio per la valutazione di Enterobacteriaceae produttrici di beta-lattamasi a spettro esteso negli operatori della filiera suina **Mario Sarti, Edoardo Carretto**

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Hot Topic

Extended-spectrum β-lactamases, carbapenemases and the *mcr-1* gene: is there a historical link?

A B S T R A C T

The plasmid mediated *mcr-1* gene encoding for *Enterobacteriaceae* colistin resistance has been recently identified across five continents. The objective of the present study was to trace historical events concerning the discovery and emergence of the *mcr-1* gene along with ESBL and *carbapenemase* genes since several studies have reported identifying *mcr-1* genes among Extended-Spectrum β-Lactamases (ESBL) and/or carbapenemase producing *Escherichia coli*. A retrospective study reported the identification of the *mcr-1* gene in *E. coli* strains isolated in the 1980s, and this seems to correspond to the first identification of ESBL enzymes. The first discovery of the New Delhi metallo-beta-lactamase-1 (NDM-1) in 2009 was associated with a significant increase in *mcr-1* gene prevalence in *E. coli* strains obtained from food producing animals. We noticed that a historical link has existed between *mcr-1*, ESBL and carbapenemase genes since the 1980s, and we believe that the re-evaluation of colistin use in livestock needs an overall approach that includes not only colistin use reduction but also the reduction of all antibiotic use.

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Hot Topic

Extended-spectrum β-lactamases, carbapenemases and the *mcr-1* gene: is there a historical link?

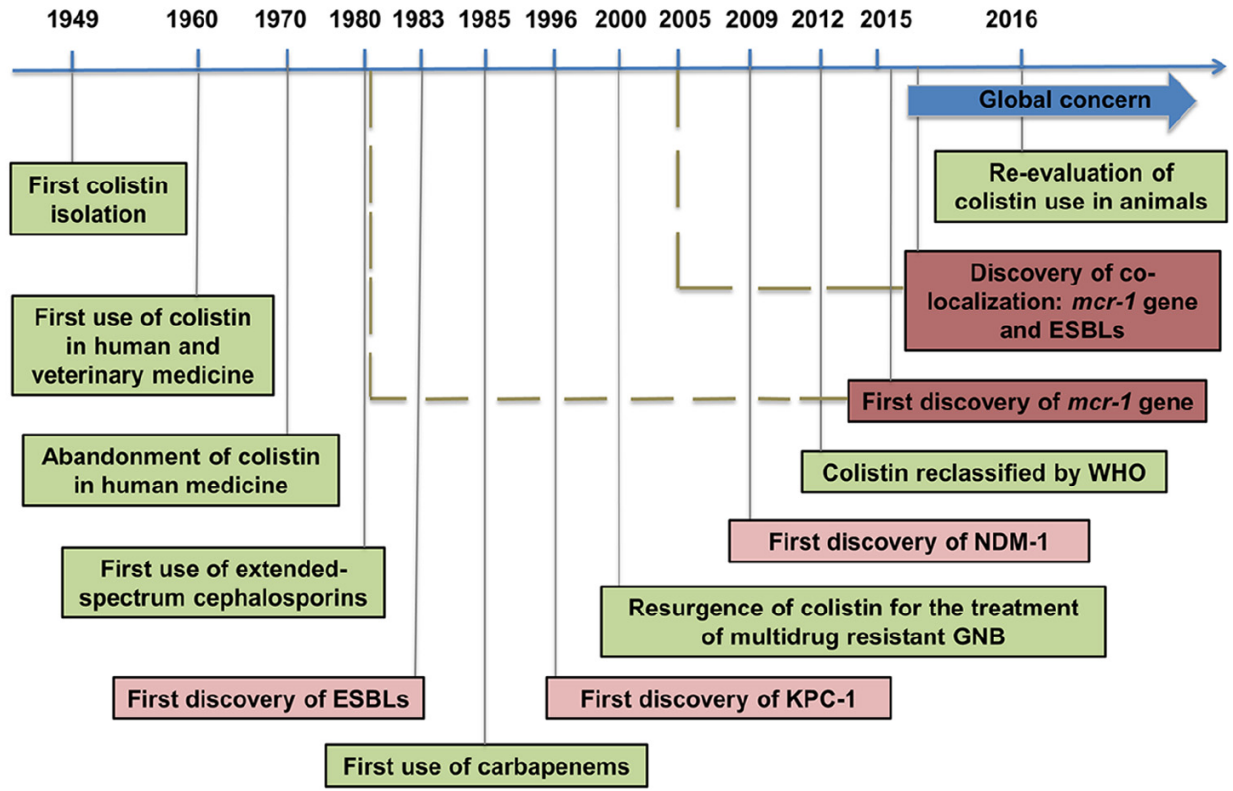

Fig. 1. Schematic illustration of some historical events that combine extended-spectrum β-lactamase (ESBL) and carbapenemase enzyme identification with colistin resistance *mcr-1* gene emergence. Dashed lines indicate a retrospective study. KPC-1, *Klebsiella pneumoniae* carbapenemase-1; GNB, Gram-negative bacteria; NDM-1, New Delhi metallo-β-lactamase-1; WHO, World Health Organization.

Colistina

- Antibiotico appartenente alla classe delle polimixine, derivato da ceppi di *Bacillus polymyxa.*
- Più che un antibiotico, è un detergente: è una molecola che presenta domini idrofili e lipofili. Tali siti interagiscono con la parete batterica, alterandone la struttura attraverso l'interazione con il lipopolisaccaride. Le regioni idrofobiche e idrofiliche interagiscono con la membrana citoplasmatica come un detergente, solubilizzandola.
- Questo particolare meccanismo d'azione spiega come la resistenza alla colistina sia incredibilmente difficile da esprimersi. I ceppi tendono inoltre a ritornare a condizioni di sensibilità.

Resistance mechanism	Genes involved	Bacteria	References
Modification of the lipid A or Kdo with aminoarabinose	*arnBCADTEF operon and pmrE*	*Salmonella enterica, Klebsiella pneumoniae, Escherichia coli, Proteeae* bacteria, *Serratia marcescens, Pseudomonas aeruginosa* and *Burkholderia cepacia* complex	Vaara et al., 1981; Boll et al., 1994; Nummila et al., 1995; Helander et al., 1996; Rozalski et al., 1997; Trent et al., 2001b; Moskowitz et al., 2004; Yan et al., 2007; Loutet and Valvano, 2011; Lin et al., 2014
Modification of the lipid A with phosphoethanolamine	*pmrC*	*S. enterica, K. pneumoniae, E. coli* and *Acinetobacter baumannii*	Zhou et al., 2001; Lee et al., 2004; Kim et al., 2008; Arroyo et al., 2011; Beceiro et al., 2011; Jayol et al., 2014
Activation of LPS-modifying operon by mutations in TCSs	*pmrA/pmrB* and *or phoP/phoQ*	*S. enterica, K. pneumoniae, P. aeruginosa* and *A. baumannii*	Roland et al., 1993; Sun et al., 2009; Arroyo et al., 2011; Owusu-Anim and Kwon, 2012; Cannatelli et al., 2014b; Jayol et al., 2014
Inactivation of *phoP/phoQ* negative feedback regulator	*mgrB*	*K. pneumoniae*	Cannatelli et al., 2013; López-Camacho et al., 2013; Gaibani et al., 2014; Olaitan et al., 2014b
Modification of the Kdo with phosphoethanolamine	*eptB, phoP/phoQ* and *mgrR*	*E. coli*	Reynolds et al., 2005; Moon and Gottesman, 2009
Increased acylation of lipid A enhancing its modification with aminoarabinose	*lpxM*	*S. enterica, K. pneumoniae* and *E. coli*	Tran et al., 2005; Clements et al., 2007; Murray et al., 2007; Velkov et al., 2013b
Trapping of polymyxins by capsule		*K. pneumoniae* and *P. aeruginosa*	Campos et al., 2004; Llobet et al., 2008
Efflux pump	*acrAB* and *kpnEF*	*K. pneumoniae*	Padilla et al., 2010; Srinivasan and Rajamohan, 2013
Loss of LPS	*lpxA, lpxC* and *lpxD*	*A. baumannii*	Moffatt et al., 2010, 2011
Glycosylation of lipid A with hexosamine		*A. baumannii*	Pelletier et al., 2013
Acquired/adaptive resistance to polymyxins through LPS modification with aminoarabinose	*colR/colS, cprR/cprS* and *parR/parS*	*P. aeruginosa*	Fernández et al., 2010; Muller et al., 2011; Fernandez et al., 2012; Gutu et al., 2013
Overexpression of outer membrane protein OprH	*oprH*	*P. aeruginosa*	Young et al., 1992

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- 2014: in Cina utilizzate circa 16.500 tonnellate di antibiotici, la maggior parte delle quali come growth promoters.
- 12.000 tonnellate di tali antibiotici erano colistina, farmaco abitualmente preservato in diversi paesi.
- Nell'estate 2015 riscontro di resistenza alla colistina in un ceppo di *Escherichia coli*. La resistenza era trasferibile *in vitro* ad altri isolati batterici.

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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*

Research in context

Evidence before this study

On Aug 15, 2015, we searched PubMed with the terms "*E coli* and colistin resistance", "*Klebsiella pneumoniae* and colistin resistance", "*Klebsiella* and colistin resistance", "China and colistin", and "plasmid mediated colistin resistance" for reports published between Jan 1, 2000, and Aug 15, 2015, with no language restrictions. Our search identified no results of relevance to this study. We also searched with the terms "*E coli* and colistin resistance" and "*Klebsiella* and colistin resistance" and found no reports of plasmid-mediated colistin resistance, novel mechanisms of colistin resistance, and in-vivo resistance.

We monitored the prevalence of antimicrobial resistance of *Escherichia coli* from food animals annually and found an increase of colistin resistance in recent years. From the published literature, we know that no plasmid-mediated colistin resistance mechanism has been reported.

Added value of this study

This study reports data for the following: the first report of plasmid-mediated colistin resistance (designated *mcr-1*), the proportion of *mcr-1*-positive samples in animals and human beings, rapid dissemination of *mcr-1* between Gram-negative strains, in-vivo colistin resistance mediated by *mcr-1*, MCR-1 modification of lipid A and mediating colistin resistance, structural modelling on MCR-1, and sequencing of a *mcr-1*-positive plasmid.

Implications of all the available evidence

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 spread further. Further surveillance and molecular epidemiological studies on the distribution and dissemination of *mcr-1* are urgently required, along with the re-evaluation of the use of polymyxins in animals. Our findings highlight the urgent need for coordinated global action in the fight against extensively-resistant and pan-resistant Gram-negative bacteria.

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Table 1. Isolation of bacteria carrying *mcr-1*

Bacteria	Host	Country	Year(s) of isolation	Reference(s)
E. coli	chicken meat, pork, pigs, humans	China	2011–14	5
K. pneumoniae	human	China	2014	5
Unknown	human microbiome	China	before 2011	8
E. coli, *K. pneumoniae*	humans	China	2014–15	9,10
E. coli	chicken meat	China	2014	11
Unknown	human microbiome	China	before 2011	12
E. coli	humans	China	2015	13
E. coli	chickens	China	1980–89, 2004, 2006, 2009–14	14
E. aerogenes, *E. cloacae*	humans	China	2014	15
E. coli	humans	China	2015	16
E. coli	human	China	2015	17
E. coli	dogs, cats	China	2016	17
E. coli	humans	China	2015	18
E. coli	human	Cambodia	2012	19
E. coli	cattle, pig	Japan	2012–13	20
E. coli	humans, pigs	Laos	2012	21
E. coli	pigs, chickens	Malaysia	2013	8
E. coli	chickens, pig, water	Malaysia	2013	22
E. coli	chickens, pig, human, chicken feed, water	Malaysia	2013	23
E. coli	humans	Taiwan	2010, 2012, 2014	24
E. coli	retail meat (beef, chicken, pork)	Taiwan	2012–13, 2015	24
E. coli	humans	Thailand	2012	21
E. coli	pigs and slaughterhouse environment	Vietnam	2014–15	25
E. coli	chicken, pigs	Vietnam	2013–14	26
S. somnei	human	Vietnam	2008	27
E. coli	calves, piglets	Belgium	2011–12	28
E. coli	pigs	Belgium	2011–12	29
E. coli	human patient, imported chicken meat	Denmark	2012–15	30
E. coli	humans	France	2012	21
Salmonella Derby	sausage	France	2013	31
Salmonella Paratyphi B	food of poultry origin	France	2012	31
Salmonella 1,4,[5],12:i:−	boot swab from broiler farm	France	2013	31
E. coli	veal calves	France	2005–14	32
E. coli	pigs	France	2011, 2013	33
E. coli	broilers	France	2013–14	33
E. coli	turkeys	France	2014	33
E. coli	pigs	Germany	2010–11	34
E. coli	human	Germany	2014	34
E. coli	humans	Great Britain	2013–14	35
Salmonella 1,4,[5],12:i:−	humans	Great Britain	2012, 2014–15	35
Salmonella Typhimurium	humans	Great Britain	2015	35
Salmonella Virchow	human	Great Britain	2014	35
Salmonella Paratyphi B	poultry meat, human	Great Britain	2014–15	35
E. coli	pigs	Great Britain	2015	36
Salmonella Typhimurium	pig	Great Britain	2015	36
E. coli	humans	Italy	2013–15	37
E. coli	humans	Italy	2015	38
E. coli	European herring gull	Lithuania	2016	39
E. coli	human	Poland	2015	40
Salmonella Typhimurium	food sample	Portugal	2011	8
Salmonella Typhimurium	retail meat (chicken, beef, pork)	Portugal	2011–12	41
E. coli	turkeys	Spain	2011, 2013–14	42
Salmonella Typhimurium	pigs	Spain	2009–11	42
Salmonella Rissen	pigs	Spain	2010	42
E. coli	humans	Spain	2012–15	43
E. coli	river water	Switzerland	2012	44
E. coli	vegetable from Asia	Switzerland	2014	44
E. coli	humans (travellers)	The Netherlands	2012–13	45
E. coli	retail chicken meat	The Netherlands	2009, 2014	46
E. coli	broilers, turkeys, veal calves	The Netherlands	2010–13	47
Salmonella Java	chicken meat	The Netherlands	2010–15	47
Salmonella Anatum	turkey meat	The Netherlands	2013	47
Salmonella Schwartzengrund	turkey meat	The Netherlands	2015	47
E. coli	chickens	Algeria	2015	21
E. coli	human	Egypt	2015	48
E. coli	dairy cow	Egypt	2014	49
E. coli	human	Nigeria	2012	21
E. coli	humans	South Africa	2014–16	50
E. coli	humans	South Africa	2014–15	51
E. coli	chickens	South Africa	2015	52
E. coli	chickens	Tunisia	2015	53
E. coli	humans	Argentina	2012–13, 2015–16	54
E. coli	kelp gulls	Argentina	2012	55
E. coli	chickens, pigs	Brazil	2012–13	56
E. coli	human	Canada	2011	57
E. coli	ground beef	Canada	2010	57
E. coli	human	USA	2016	58

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Journal of Antimicrobial Chemotherapy

Transferable resistance to colistin: a new but old threat

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Wang et al. *Emerging Microbes & Infections* (2018)7:122
DOI 10.1038/s41426-018-0124-z

Emerging Microbes & Infections
www.nature.com/emi

ARTICLE Open Access

Emergence of a novel mobile colistin resistance gene, *mcr-8*, in NDM-producing *Klebsiella pneumoniae*

Xiaoming Wang[1], Yao Wang[1], Ying Zhou[1], Jiyun Li[2], Wenjuan Yin[2], Shaolin Wang[1], Suxia Zhang[2], Jianzhong Shen[1], Zhangqi Shen[1] and Yang Wang[2]

Abstract

The rapid increase in carbapenem resistance among gram-negative bacteria has renewed focus on the importance of polymyxin antibiotics (colistin or polymyxin E). However, the recent emergence of plasmid-mediated colistin resistance determinants (*mcr-1*, *-2*, *-3*, *-4*, *-5*, *-6*, and *-7*), especially *mcr-1*, in carbapenem-resistant *Enterobacteriaceae* is a serious threat to global health. Here, we characterized a novel mobile colistin resistance gene, *mcr-8*, located on a transferrable 95,983-bp IncFII-type plasmid in *Klebsiella pneumoniae*. The deduced amino-acid sequence of MCR-8 showed 31.08%, 30.26%, 39.96%, 37.85%, 33.51%, 30.43%, and 37.46% identity to MCR-1, MCR-2, MCR-3, MCR-4, MCR-5, MCR-6, and MCR-7, respectively. Functional cloning indicated that the acquisition of the single *mcr-8* gene significantly increased resistance to colistin in both *Escherichia coli* and *K. pneumoniae*. Notably, the coexistence of *mcr-8* and the carbapenemase-encoding gene bla_{NDM} was confirmed in *K. pneumoniae* isolates of livestock origin. Moreover, BLASTn analysis of *mcr-8* revealed that this gene was present in a colistin- and carbapenem-resistant *K. pneumoniae* strain isolated from the sputum of a patient with pneumonia syndrome in the respiratory intensive care unit of a Chinese hospital in 2016. These findings indicated that *mcr-8* has existed for some time and has disseminated among *K. pneumoniae* of both animal and human origin, further increasing the public health burden of antimicrobial resistance.

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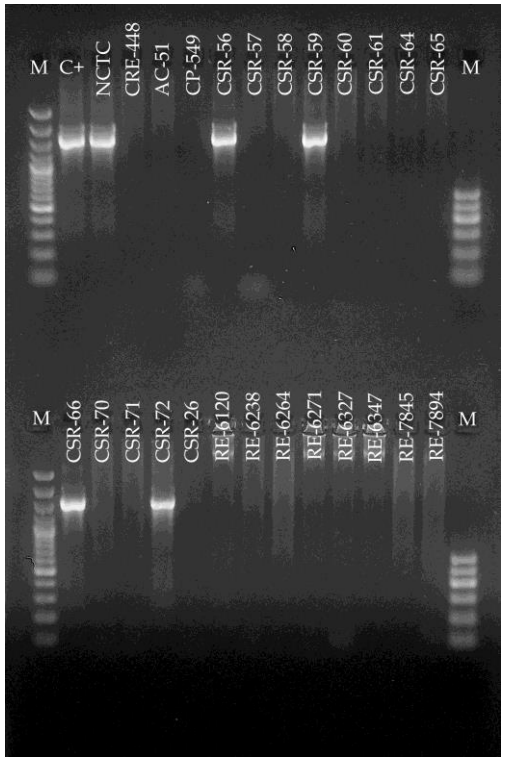
	Reggio Emilia			Baggiovara			Complessivo			%
	totale	*mcr* -1	mcr-2/5	totale	*mcr* -1	mcr-2/5	totale	*mcr* -1	mcr-2/5	
Escherichia coli	48	10	0	49	19	0	97	29	0	29,00
Klebsiella pneumoniae	33	0	0	25	3	0	58	3	0	3,00
Altri	15	0	0	4	0	2	19	0	2	10,53

Mcr -1 - Tre isolati da emocolture; 29 da campioni urinari, per lo più territoriali



RAPID COMMUNICATIONS

Detection of *mcr-4* positive *Salmonella enterica* serovar Typhimurium in clinical isolates of human origin, Italy, October to November 2016

Edoardo Carretto[1], Flavia Brovarone[1], Paola Nardini[1], Giuseppe Russello[1], Daniela Barbarini[2], Stefano Pongolini[3], Carlo Gagliotti[4], Alessandra Carattoli[5], Mario Sarti[6]

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Resistenza ai carbapenemici

CLASSE A	CLASSE B - MBL metallo-β-lattamasi	CLASSE D	Combinazione TR + deficit porine
KPC (*K. pneumoniae carbapenemases*)	IMP (imipenemasi)	OXA	ESBL + porin loss
SME (*Serratia marcescens* enzyme)	VIM (*Verona integron-encoded* MBL)		AMPc + porin loss
NCM-A / IMI (not metallo enzyme carbapenemase / imipenem hydrolysing beta-lactamase)	NDM-1 (New Dehli MBL)		
GES (Guiana extended spectrum)			

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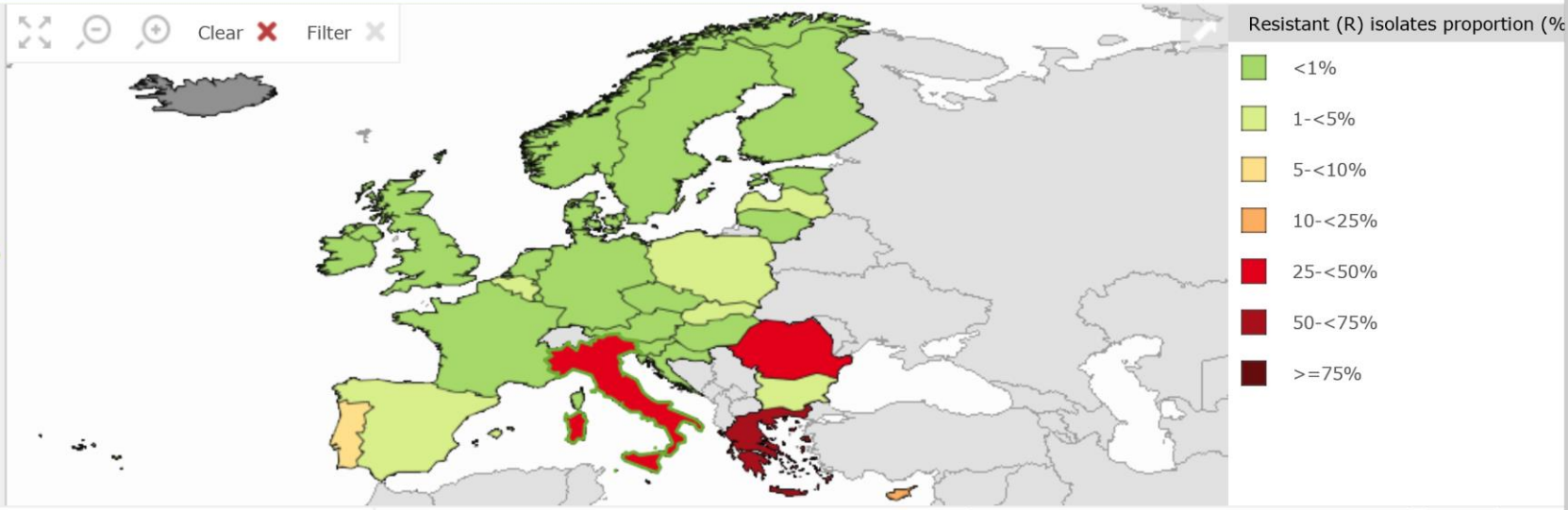
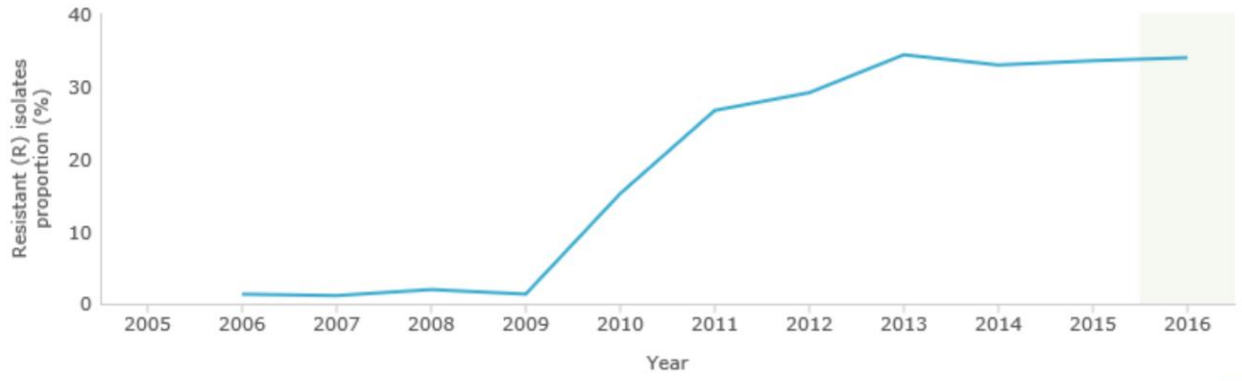
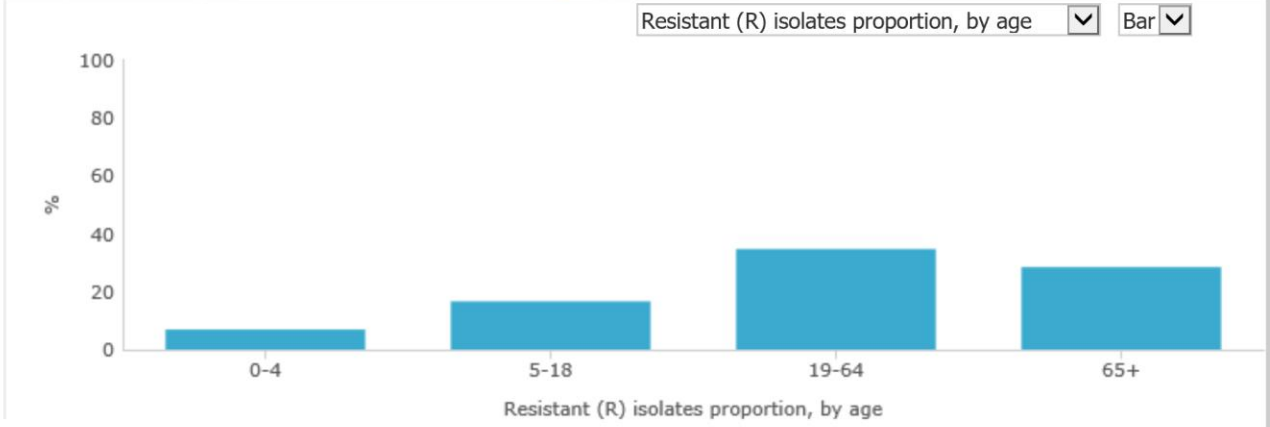
Surveillance Atlas of Infectious Diseases

Antimicrobial resistance | Klebsiella pneumoniae | Carbapenems | Resistant (R) isolates proportion | 2016

Region	Resistant (R) isolates proportion (%)
Estonia	0.0
Finland	0.3
France	0.4
Germany	0.5
Greece	66.9
Hungary	0.4
Iceland	-
Ireland	0.7
Italy	33.9
Latvia	2.2
Lithuania	0.0
Luxembourg	0.0







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REVIEWS OF INFECTIOUS DISEASES • VOL. 10, NO. 4 • JULY-AUGUST 1988
 0886-0162/88/1004-0033$02.00

Extended Broad-Spectrum β-Lactamases Conferring Transferable Resistance to Newer β-Lactam Agents in Enterobacteriaceae: Hospital Prevalence and Susceptibility Patterns

Vincent Jarlier, Marie-Hélène Nicolas, Geneviève Fournier, and Alain Philippon

From the Service de Bactériologie, Faculté de Médecine Pitié-Salpêtrière; and the Service de Bactériologie, Faculté de Médecine Cochin, Paris, France

Before 1985 at the Pitié-Salpêtrière Hospital in Paris (2,400 beds), resistance to cefotaxime in clinical isolates of Enterobacteriaceae involved only species producing inducible class 1 β-lactamase. Between November 1985 and April 1987, however, 62 isolates (57 of *Klebsiella pneumoniae* and five of *Escherichia coli*) showed decreased susceptibility to cefotaxime (mean MIC, 8–16 μg/mL). The transferability of cefotaxime resistance in *E. coli* K12 was demonstrated for 15 of 16 selected isolates. By isoelectric focusing using iodometric detection with 20 mg of ceftriaxone/100 mL and determination of substrate and inhibition profiles, three β-lactamases mediating cefotaxime resistance were identified as SHV-2 (isoelectric point [pI] 7.6), CTX-1 (pI 6.3), and "SHV-2-type" or SHV-3 (pI 6.98). The three β-lactamases hydrolyzed penicillins and cephalosporins (including cefotaxime and ceftriaxone) and were therefore designated "extended broad-spectrum β-lactamases" (EBS-Bla). The enzymes conferred to derivatives a high level of resistance to amoxicillin, ticarcillin, piperacillin, and cephalothin and a decreased degree of susceptibility (i.e., MICs increased by 10- to 800-fold) to cefotaxime, ceftriaxone, ceftazidime, and aztreonam. These β-lactamases did not affect the activity of cephamycins (cefoxitin, cefotetan, moxalactam) or imipenem. Synergy between clavulanate or sulbactam (2 μg/mL) and amoxicillin was greater against derivatives producing EBS-Bla than against those producing TEM-1, TEM-2, or SHV-1; this synergy was greater with clavulanate than with sulbactam against derivatives producing SHV-2 and the SHV-2-type enzyme but was similar with clavulanate and sulbactam against those producing CTX-1. A double-disk synergy test performed with cefotaxime and Augmentin disks (placed 30 mm apart, center to center) seemed a useful and specific test for the detection of strains producing EBS-Bla.

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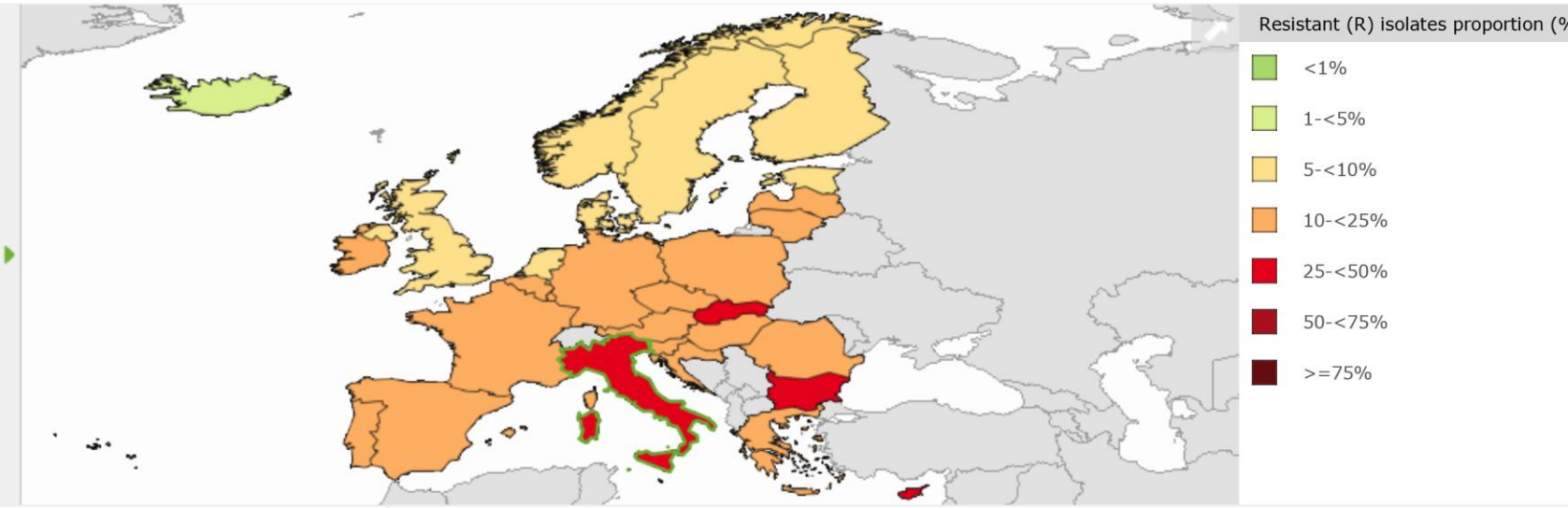
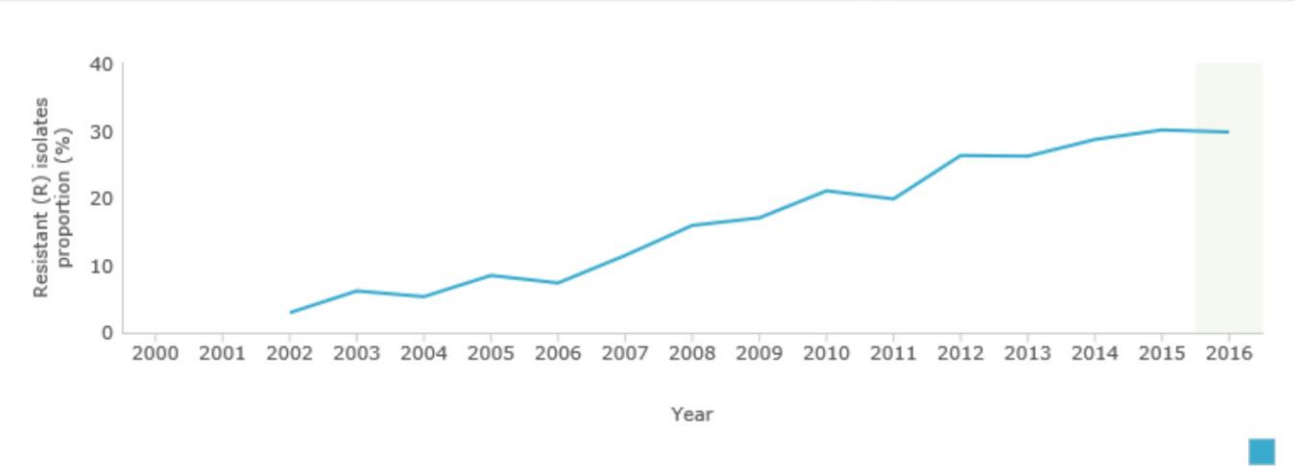
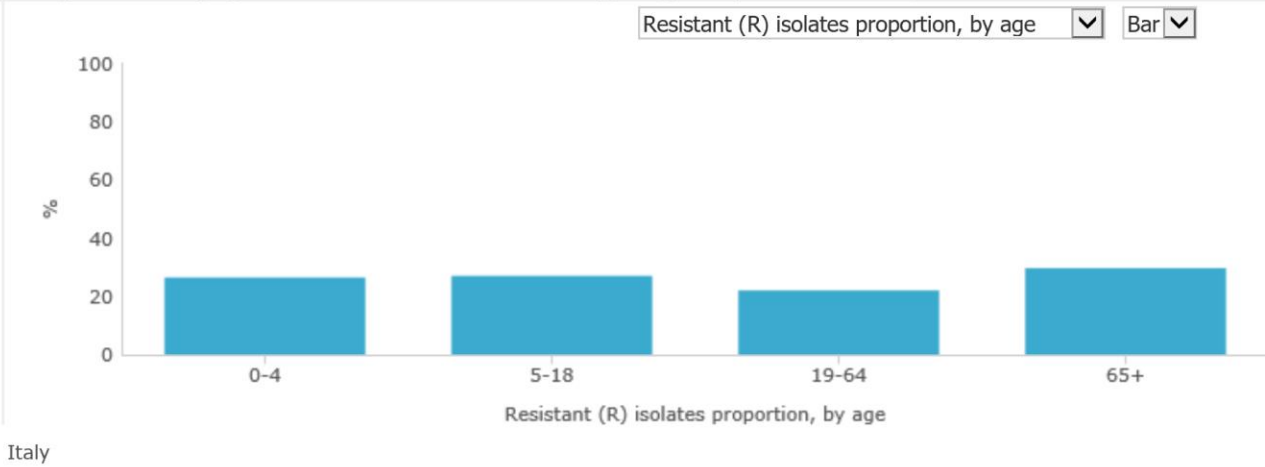
Surveillance Atlas of Infectious Diseases

Antimicrobial resistance | Escherichia coli | Third-generation cephalosporins | Resistant (R) isolates proportion | 2016

Region	Resistant (R) isolates proportion (%)
Finland	6.9
France	11.2
Germany	11.5
Greece	17.6
Hungary	16.7
Iceland	4.2
Ireland	11.4
Italy	29.8
Latvia	24.1
Lithuania	14.7
Luxembourg	13.6







Italy

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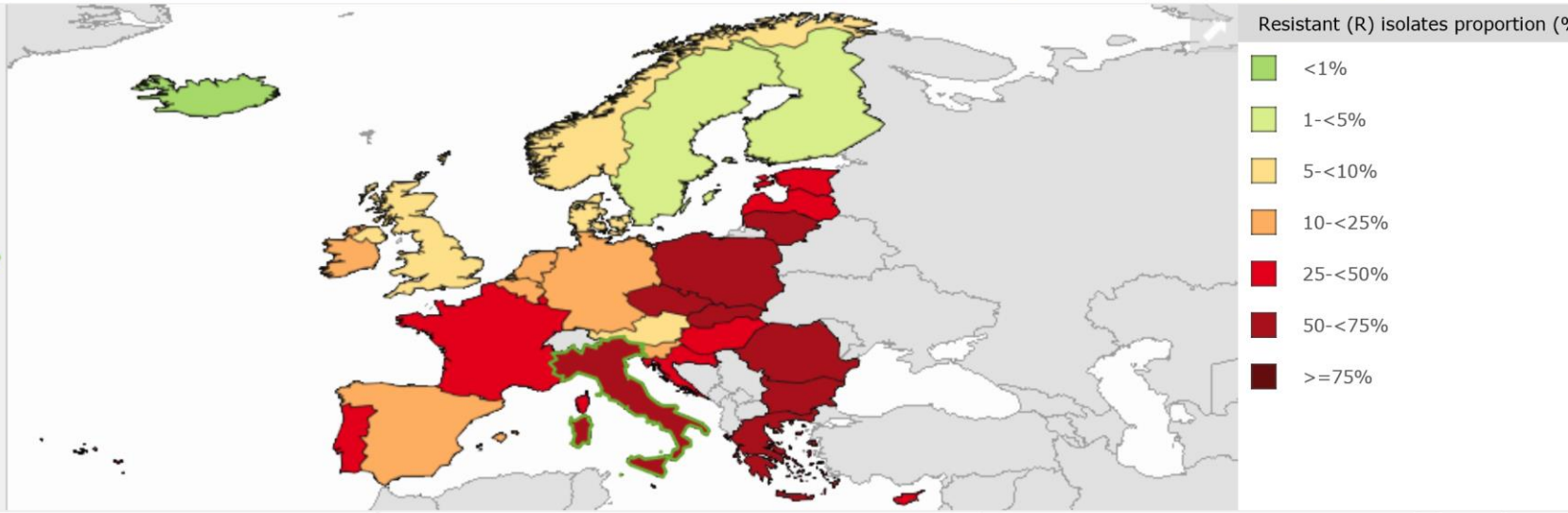
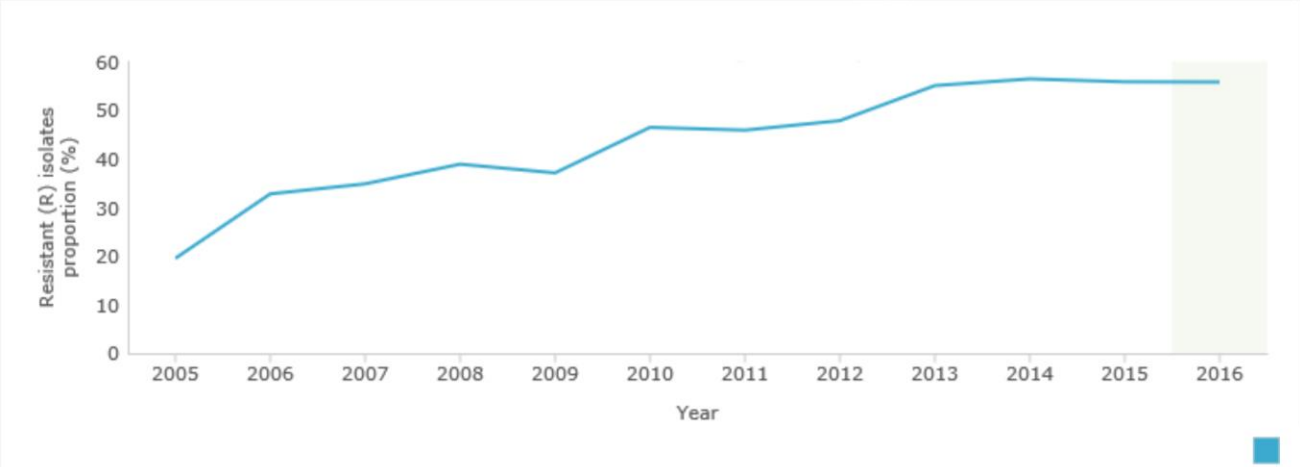
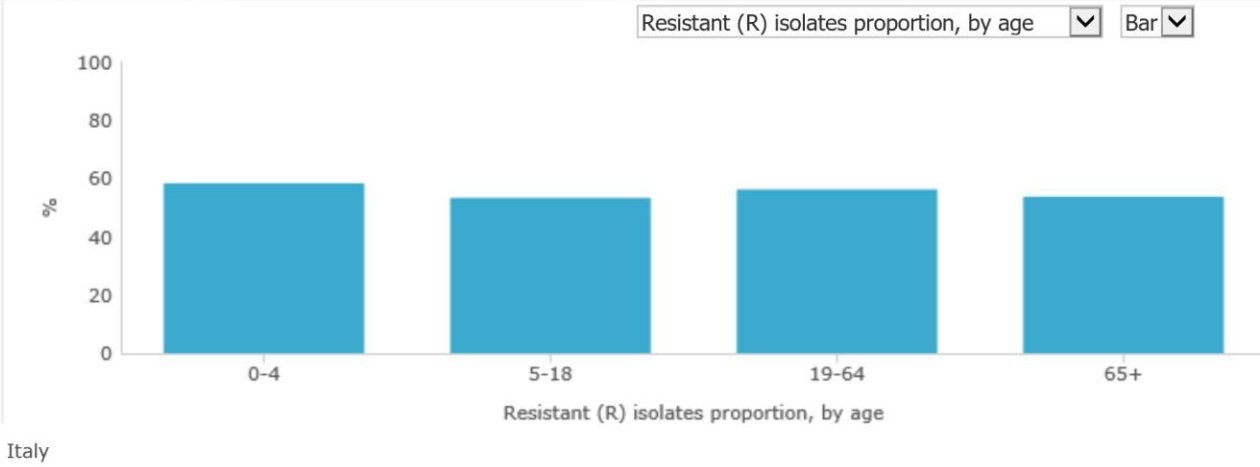
Surveillance Atlas of Infectious Diseases

Antimicrobial resistance | Klebsiella pneumoniae | Third-generation cephalosporins | Resistant (R) isolates proportion | 2016

Region	Resistant (R) isolates proportion (%)
Finland	4.1
France	28.9
Germany	13.7
Greece	72.5
Hungary	37.5
Iceland	0.0
Ireland	13.5
Italy	55.8
Latvia	47.4
Lithuania	56.7
Luxembourg	35.9
Malta	21.6







Italy

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Enterobatteri ESBL+: evoluzione

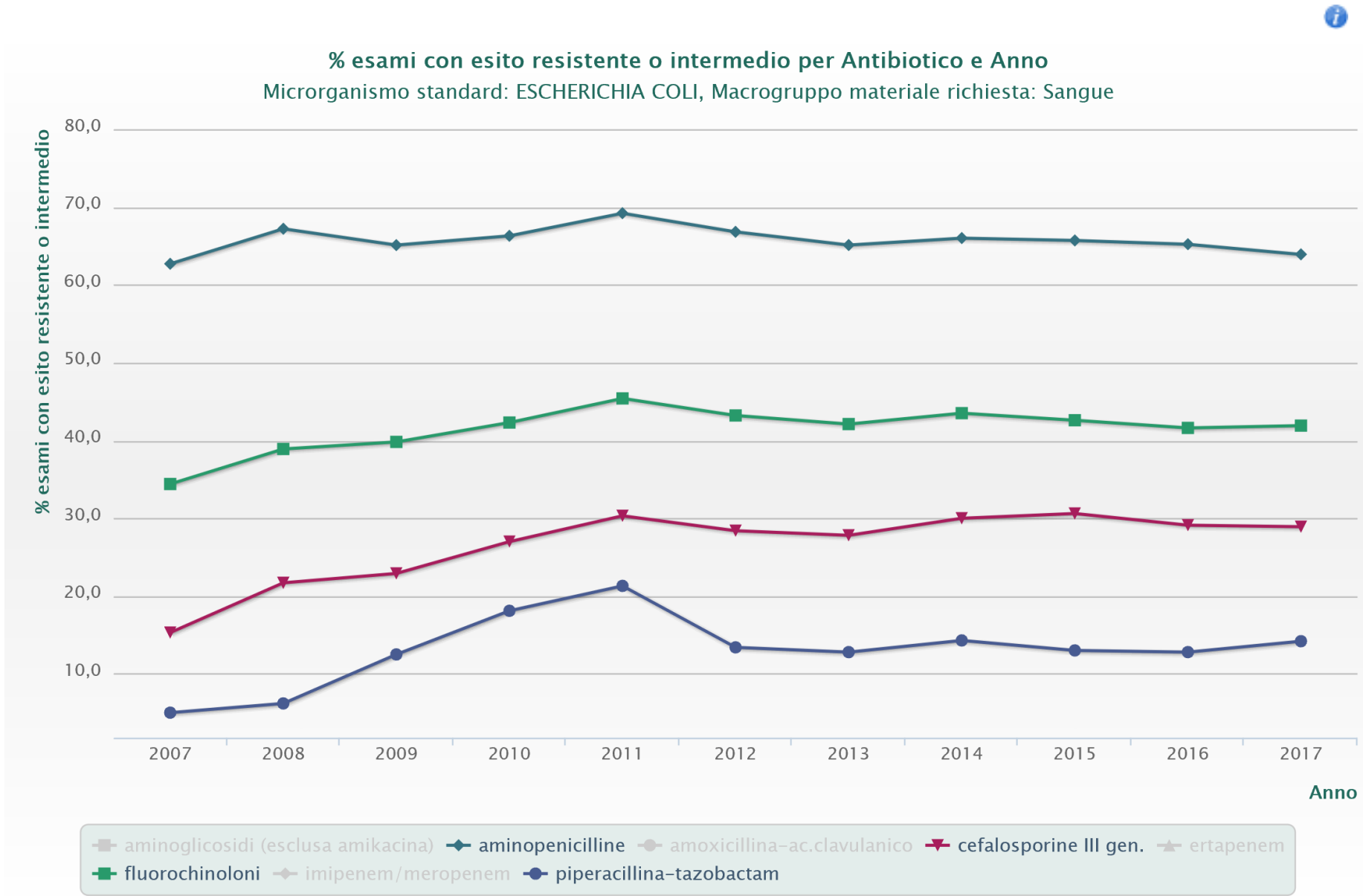
- Trasmissione prevalentemente interumana, sempre più frequente descrizione di serbatoi animali o alimentari
- Progressivo aumento di ceppi comunitari
- Diversità enzimi, ieri SHV (***s**ulf**h**ydryl **v**ariable)* e TEM (Temoneira), oggi CTX (Toho beta-lactamases, da *Kluyvera georgiana*), con aumento di *Escherichia coli* CTX-M
- Ampia circolazione di *E. coli* $bla_{\text{CTX-M15}}$ dall'India.
- Diffusione clonale di ST131 e diffusione orizzontale di plasmidi tipo IncFII correlati a multiresistenza.
- Diffusione di ceppi con plasmidi di resistenza tipo ceppi bla_{CMY} (ampC).

Pitout et al., AAC 2007, 51:1281 - Pitout et al., AAC 2009, 53:2846 –
Canton and Coque, Curr. Opinion Microbiol 2006, 9:466 - Rodriguez-Bano et al., CID 2010, 50:40
Coque et al., EID 2008, 14:195 – Yong et al., AAC 2009, 53:5046

E.C. 2018

LAB - Percentuali di resistenza

Percentuale resistenza



E.C. 2018

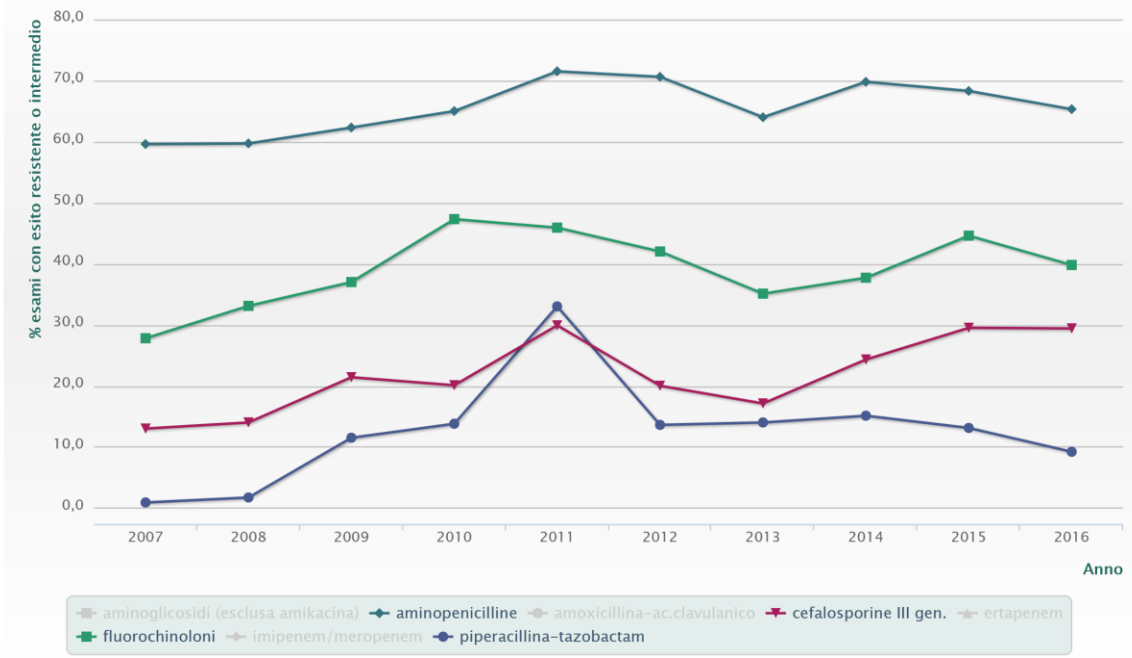
E-R | Salute > SISEPS > ReportER #OpenData > Area Sanità > LAB > Reportistica predefinita > Selezione report

LAB - Percentuali di resistenza

Percentuale resistenza

% esami con esito resistente o intermedio per Antibiotico e Anno

Microrganismo standard: ESCHERICHIA COLI, Macrogruppo materiale richiesta: Sangue, Azienda richiedente: AOSP REGGIO EMILIA



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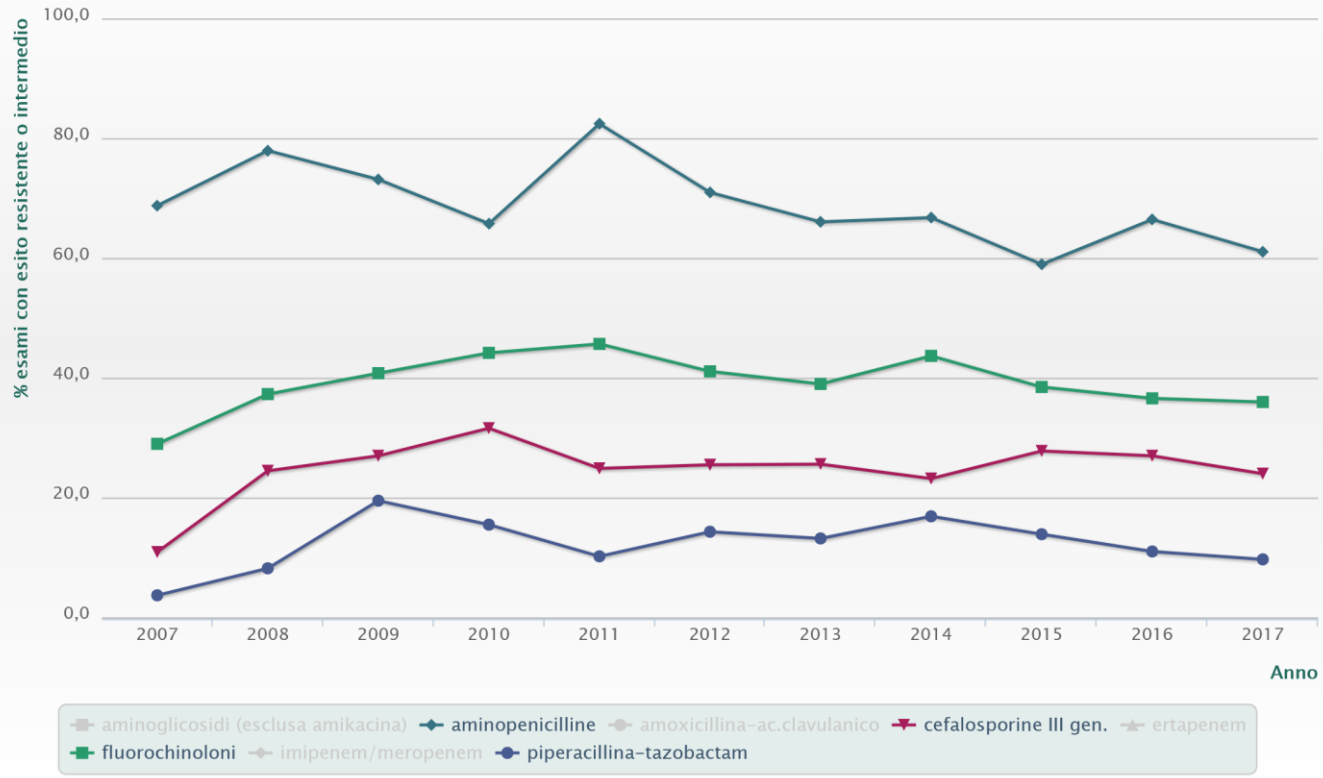
LAB - Percentuali di resistenza

Percentuale resistenza

% esami con esito resistente o intermedio per Antibiotico e Anno

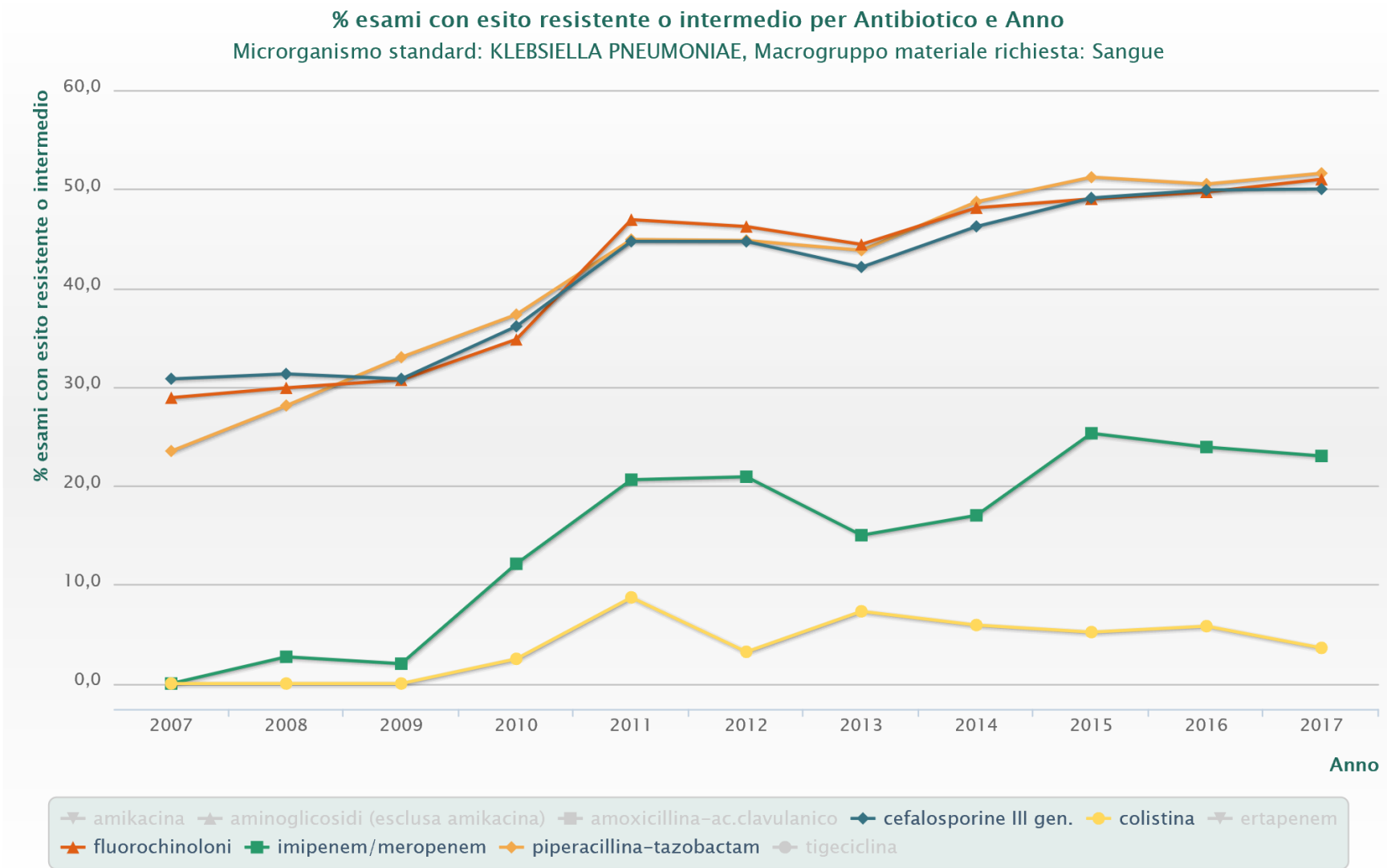
Microrganismo standard: ESCHERICHIA COLI, Macrogruppo materiale richiesta: Sangue, Azienda richiedente: AOSPU MODENA



E.C. 2018

LAB - Percentuali di resistenza

Percentuale resistenza



E.C. 2018

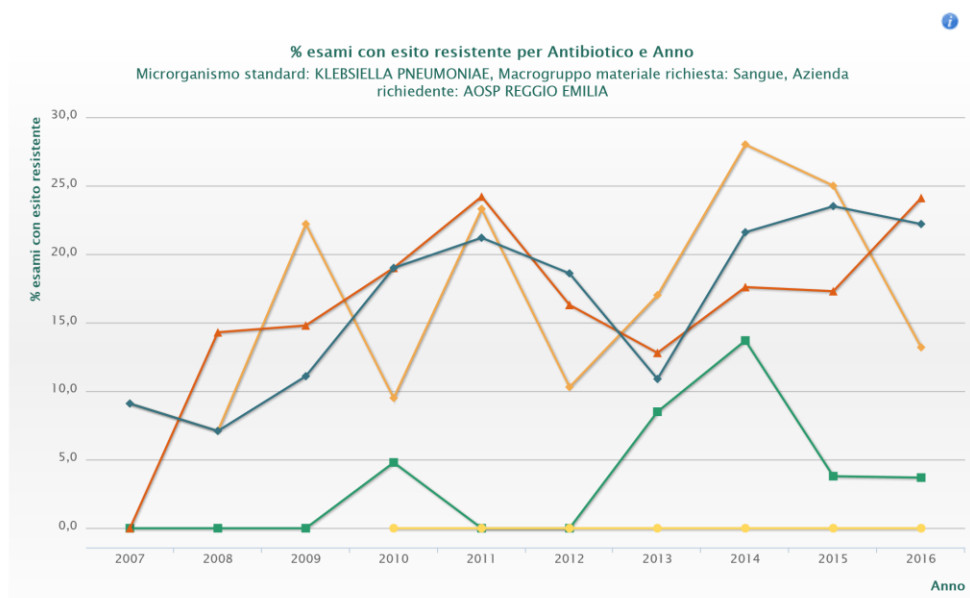
E-R | Salute > SISEPS > ReportER #OpenData > Area Sanità > LAB > Reportistica predefinita > Selezione report

LAB - Percentuali di resistenza

Percentuale resistenza

% esami con esito resistente per Antibiotico e Anno

Microrganismo standard: KLEBSIELLA PNEUMONIAE, Macrogruppo materiale richiesta: Sangue, Azienda richiedente: AOSP REGGIO EMILIA



Valore minimo dell'asse y: Automatico Zero

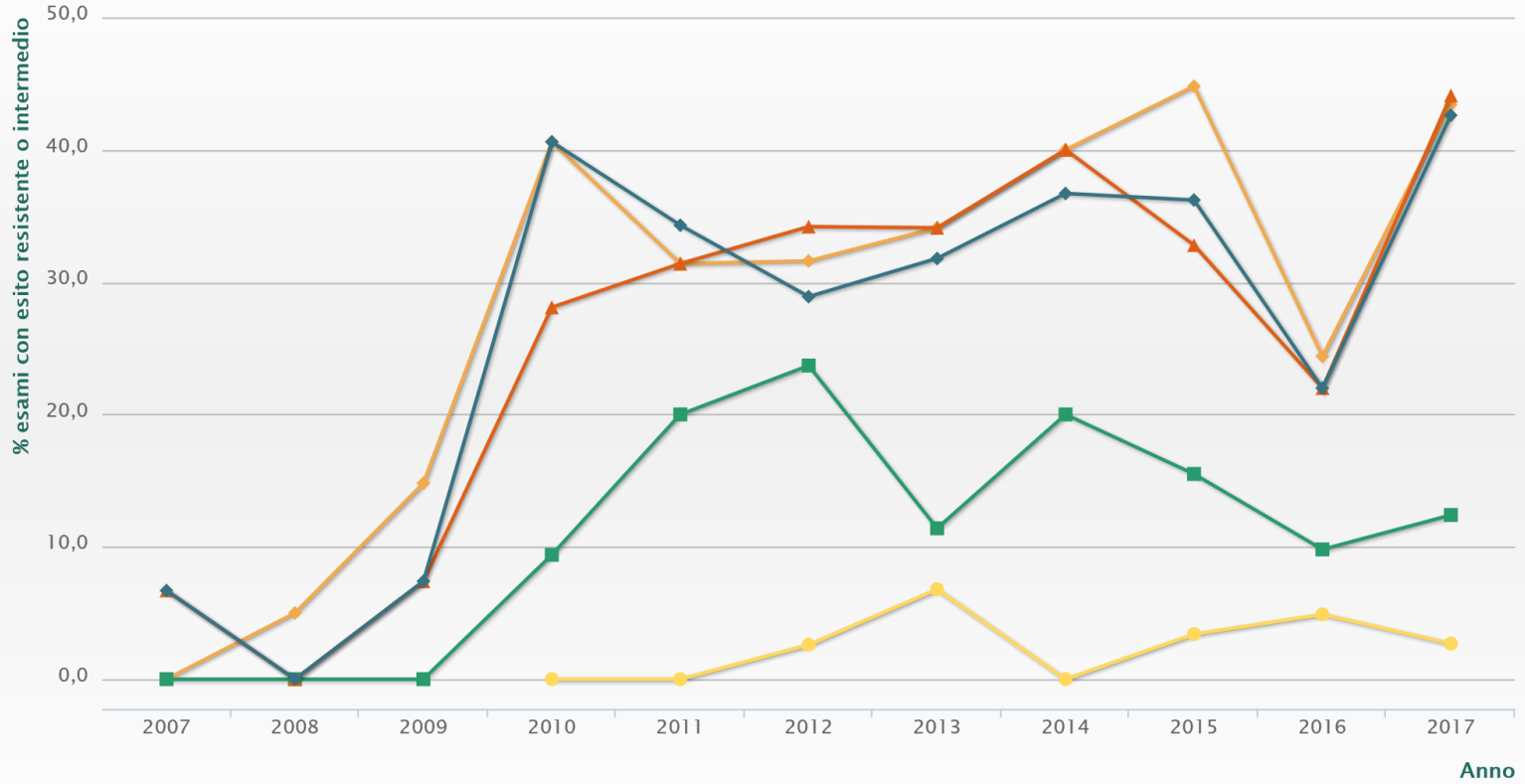
E-R | Salute > SISEPS > ReportER #OpenData > Area Sanità > LAB > Reportistica predefinita > Selezione report

LAB - Percentuali di resistenza

Percentuale resistenza

% esami con esito resistente o intermedio per Antibiotico e Anno

Microrganismo standard: KLEBSIELLA PNEUMONIAE, Macrogruppo materiale richiesta: Sangue, Azienda richiedente: AOSPU MODENA



Valore minimo dell'asse y: Automatico Zero