

Workshop Annuale del Laboratorio Nazionale di Riferimento per l'Antibioticoresistenza e del Centro di Referenza Nazionale per l'Antibioticoresistenza 2021

Antimicrobicoresistenza (AMR) - L'approccio ONE HEALTH al tempo della pandemia da COVID-19

Caratterizzazione dell'AMR: carbapenemasi nell'uomo prospettiva One Health

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WHO priority pathogens list for R&D of new antibiotics

Priority 1: CRITICAL

- *Acinetobacter baumannii*, carbapenem-resistant
- *Pseudomonas aeruginosa*, carbapenem-resistant
- *Enterobacteriaceae*, carbapenem-resistant, ESBL-producing

Priority 2: HIGH

- *Enterococcus faecium*, vancomycin-resistant
- *Staphylococcus aureus*, methicillin-resistant, vancomycin-intermediate and resistant
- *Helicobacter pylori*, clarithromycin-resistant
- *Campylobacter* spp., fluoroquinolone-resistant
- *Salmonellae*, fluoroquinolone-resistant
- *Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant

Priority 3: MEDIUM

- *Streptococcus pneumoniae*, penicillin-non-susceptible
- *Haemophilus influenzae*, ampicillin-resistant
- *Shigella* spp., fluoroquinolone-resistant

Patogeni MDR/XDR frequentemente isolati nella pratica clinica

Antibiotico	MIC mg/L (S/I/R)
Pip/Tazo	>128 R
Ceftazidime	64 R
Cefepime	>32 R
Aztreonam	>32 R
Imipenem	>16 R
Meropenem	16 R
Amikacina	16 I
Gentamicina	>8 R
Ciprofloxacina	>2 R
Colistina	4 R
Fosfomicina	>128
Ceftolozano-tazobactam	>32 R

Antibiotico	MIC mg/L (S/I/R)
Imipenem	>32 R
Meropenem	64 R
Amikacin	32 R
Gentamicin	>16 R
Ciprofloxacin	>32 R
TMP/SMZ	>320 R
Colistin	1 S

**CRAB (Carbapenem-R
Acinetobacter)**

Antibiotico	MIC mg/L (S/I/R)
Amoxi/Clav	>64 R
Pip/Tazo	>256 R
Ceftriaxone	>64 R
Ceftazidime	>64 R
Cefepime	>64 R
Ertapenem	>32 R
Imipenem	>32 R
Meropenem	>32 R
Amikacin	>64 R
Gentamicin	>8 R
Ciprofloxacin	>4 R
Tigecycline	4 R
Colistin	>8 R
Fosfomicin	>64 R
Chloramphenicol	>16 R
Ceftazidime/avibactam	1 S
Meropenem/vaborbactam	0.5 S

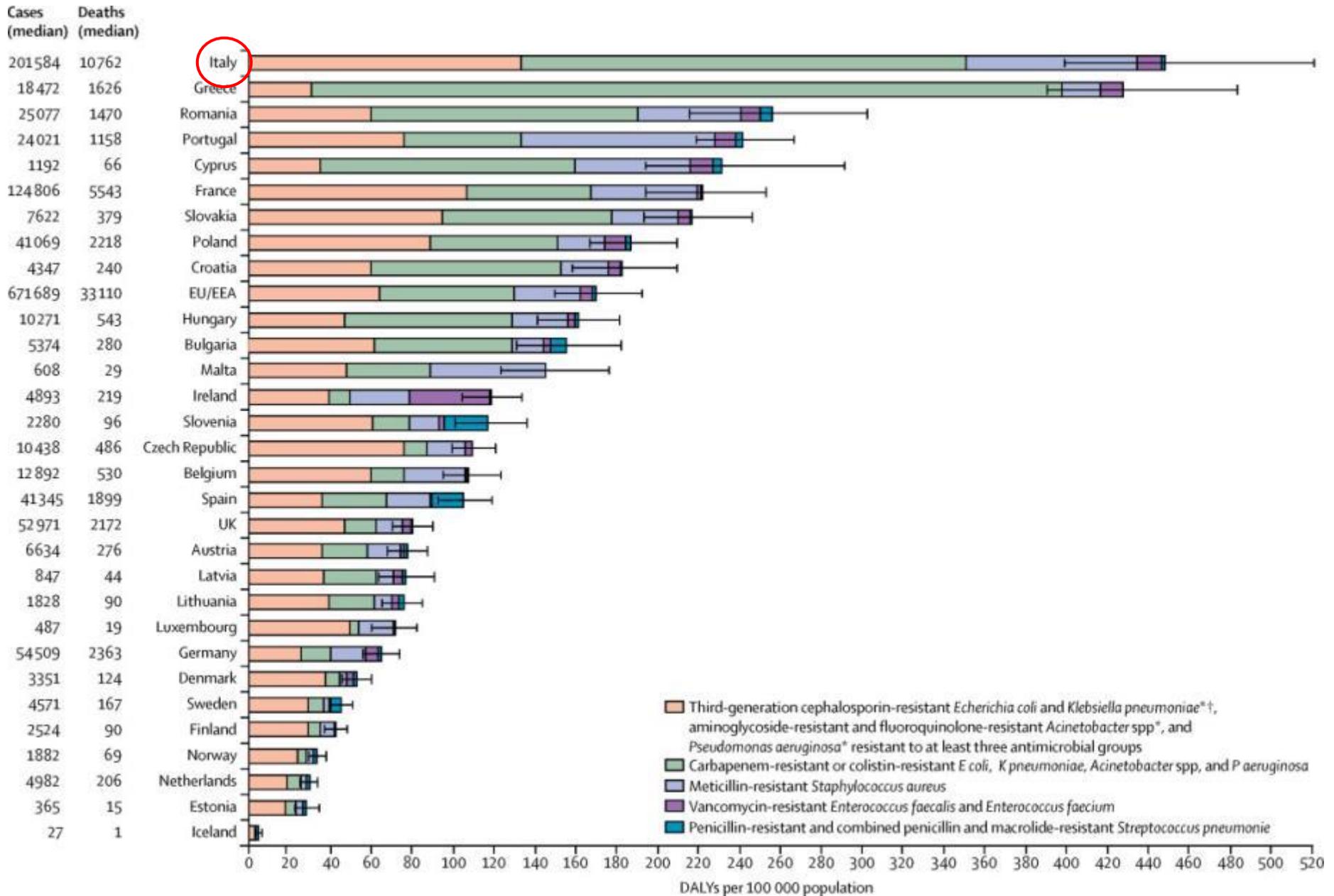
**CRE (Carbapenem-R
Klebsiella pneumoniae)**

XDR *Pseudomonas aeruginosa*

Fenotipi di resistenza complessi:

- MDR: multi-drug resistant
- XDR: extensively-drug resistant
- PDR: pan-drug resistant

Cassini et al. The Lancet Infectious Diseases 2019



Meccanismi di resistenza ai carbapenemi

Mutazioni cromosomiche
che causano
l'impermeabilità della
membrana esterna
(modificazione/perdita di
porine)
+
ESBL/AmpC β -lattamasi
overproduzione



- Resistenza a basso livello
- Casi sporadici/ outbreaks

Produzione di carbapenemasi

Class A (serine)

KPC
GES

Class D (serine)

OXA-48
OXA-23
OXA-24/40
OXA-58

Class B (metallo)

VIM
NDM
IMP

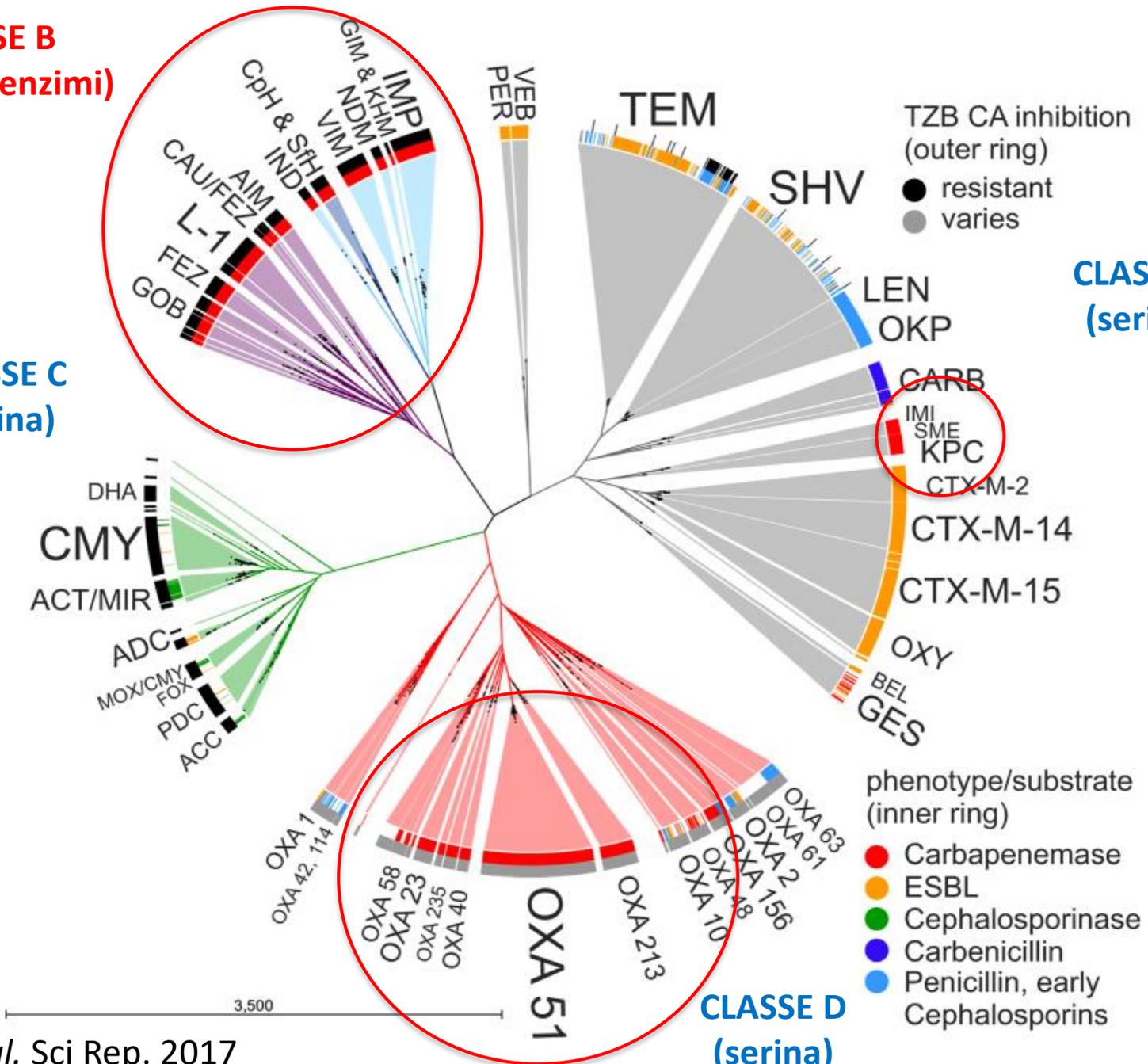
- Trasferibili (spesso su plasmidi)
- Associazione con cloni ad alto rischio
- Outbreaks, diffusione epidemica

CLASSE B
(metallo-enzimi)

CLASSE C
(serina)

CLASSE A
(serina)

CLASSE D
(serina)



Caratteristiche funzionali diverse delle carbapenemasi acquisite

	KPC-type	OXA-48-like	Metallo-enzymes (MBLs)
CARBAPEN. ACTIVITY	Strong	Weak	Strong
SPECTRUM	Extended (most β -lactams)	Narrow (penicillins, NS cepheids)	Extended (most β -lactams exc. monobactams)
INHIBITION	<ul style="list-style-type: none">• Avibactam• Relebactam• Vaborbactam	<ul style="list-style-type: none">• Avibactam	

Adapted from: Naas T, et al. *Current Drug Targets* 2016;17:1006–1028. Drawz SM, et al. *Antimicrob Agents Chemother* 2014;58:1835–1846.

Adapted from: Oueslati S, et al. *J Antimicrob Chemother* 2015;70:1059–1063. Lahiri SD, et al. *ACS Chem Biol* 2015;10:591–600.

Adapted from: Bush K, Bradford PA. *Cold Spring Harb Perspect Med* 2016 ;6; pii: a025247.

Opzioni terapeutiche limitate per le carbapenemase-producing *Enterobacterales* (CPE)

K. pneumoniae KPC+

Antibiotic	MIC mg/L (S/I/R)
Amp/Sulb	>32 R
Pip/Tazo	>128 R
Ceftriaxone	>64 R
Ceftazidime	>64 R
Ceftazidime-avibactam	2 S
Cefepime	>64 R
Ertapenem	>32 R
Imipenem	>32 R
Meropenem	>32 R
Meropenem-vaborbactam	2 S
Amikacin	>64 R
Gentamicin	2 S
Ciprofloxacin	>4 R
Colistin	0.25 S

K. pneumoniae OXA-48+CTX-M

Antibiotic	MIC mg/L (S/I/R)
Amp/Sulb	>256 R
Pip/Tazo	>128 R
Cefotaxime	>64 R
Ceftazidime	64 R
Ceftazidime-avibactam	2 S
Cefepime	16 R
Ertapenem	32 R
Imipenem	2 S
Meropenem	4 I
Amikacin	4 I
Gentamicin	1 S
Ciprofloxacin	>32 R
Colistin	0.5 S

E. coli NDM+

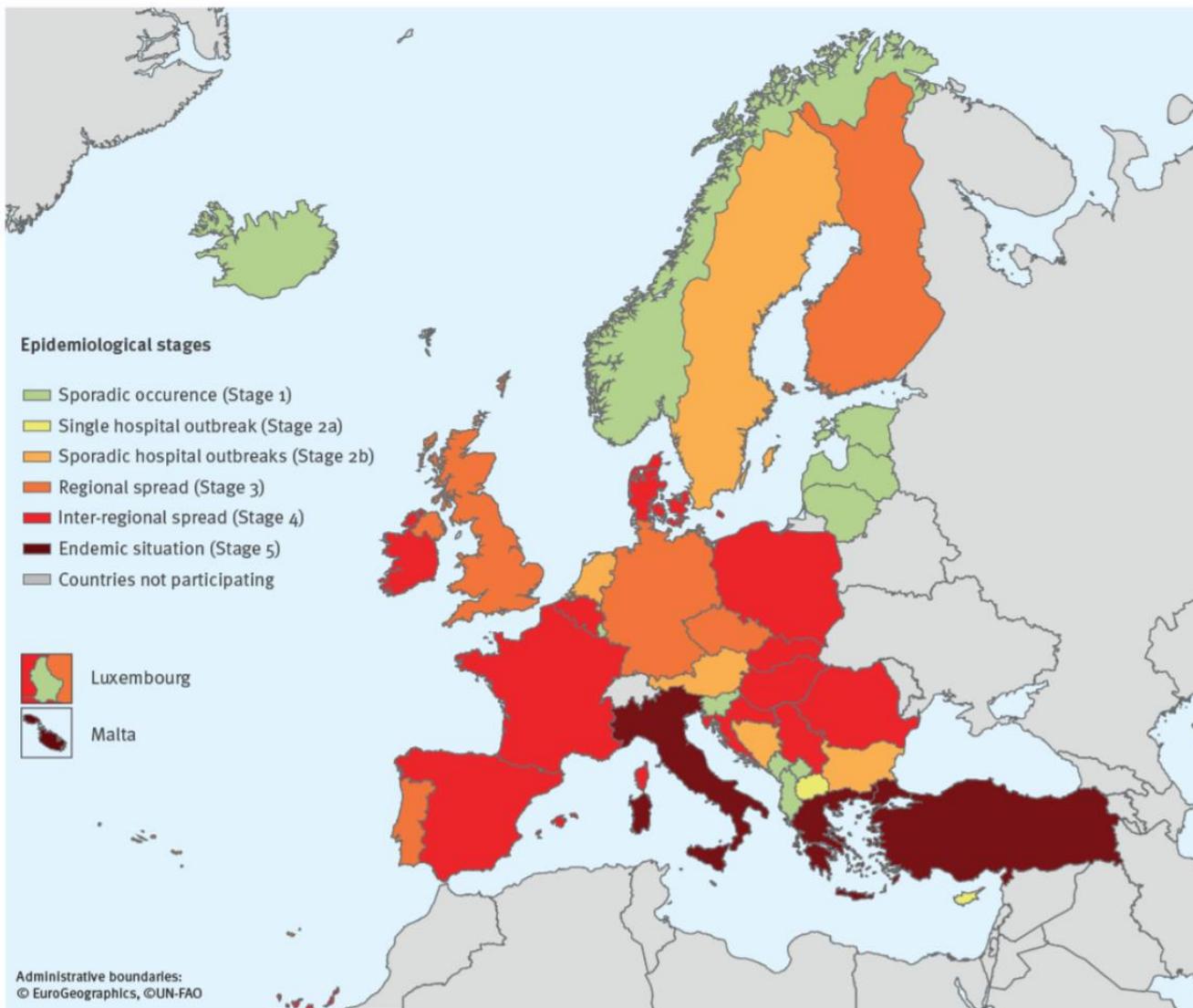
Antibiotic	MIC mg/L (S/I/R)
Amoxi/Clav	>64 R
Pip/Tazo	>128 R
Cefotaxime	>64 R
Ceftazidime	>64 R
Cefepime	>64 R
Ceftazidime-avibactam	>64 R
Ertapenem	>32 R
Imipenem	8 I
Meropenem	16 R
Meropenem-vaborbactam	16 R
Amikacin	>64 R
Gentamicin	>16 R
Levofloxacin	>8 R
Tigecycline	0.5 S
Colistin	0.5 S

Giani T, et al. J Clin Microbiol 2009;47:3793–3794.

Giani T, et al. J Clin Microbiol 2014;52:2702–2705.

D'Andrea MM, et al. J Clin Microbiol 2011;49:2755–2758.

Figure 2. Epidemiological situation of carbapenemase-producing Enterobacteriaceae, assessment by national experts in European countries, July 2018 (n=37) [2]



ecdc
EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

RAPID RISK ASSESSMENT

Carbapenem-resistant Enterobacteriaceae – second update

26 September 2019

Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study

Hajo Grundmann*, Corinna Glasner*, Barbara Albiger, David M Aanensen, Chris T Tomlinson, Arjana Tambić Andrasević, Rafael Cantón, Yehuda Carmeli, Alexander W Friedrich, Christian G Giske, Youri Glupczynski, Marek Gniadkowski, David M Livermore, Patrice Nordmann, Laurent Poirel, Gian M Rossolini, Harald Seifert, Alkiviadis Vatopoulos, Timothy Walsh, Neil Woodford, Dominique L Monnet, and the European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) Working Group†

Nov 2013, Apr 2014 **455 ospedali, 36 nazioni**

Incidenza enterobatteri produttori di carbapenemasi su 10000 ricoveri:

Italia: 5,96

Grecia: 5,78

Spagna 4,1

Germania 0,56

UK 0,19

Institute for Medical Microbiology, Immunology and Hygiene, Cologne University, Cologne, Germany (Prof H Seifert MD); German Center for Infection Research (DZIF), Braunschweig, Germany (Prof H Seifert); Department of Microbiology, National School of Public Health, Athens, Greece (Prof A Vatopoulos MD); and Section of Medical Microbiology IIB, School of Medical Sciences, Cardiff University, Heath Park Hospital, Cardiff, UK (Prof T Walsh PhD)

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See Online for appendix



Figure: Locations of participating sentinel hospitals

	Hospitals submitting carbapenem non-susceptible <i>K pneumoniae</i> isolates (n)	Number of submitted carbapenem non-susceptible <i>K pneumoniae</i> isolates	Confirmed carbapenemase-producing <i>K pneumoniae</i> isolates					Other (n, %)*
			KPC (n, %)	NDM (n, %)	OXA-48-like (n, %)	VIM (n, %)	Total (n, %)	
Albania	3	8	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	8 (100)
Austria	6	15	6 (40.0)	2 (13.3)	2 (13.3)	0 (0)	10 (66.7)	5 (33.3)
Belgium	11	48	13 (27.1)	2 (4.2)	18 (37.5)	0 (0)	33 (68.8)	15 (31.3)
Bulgaria	3	4	0 (0)	2 (50.0)	0 (0)	0 (0)	2 (50.0)	2 (50.0)
Croatia	14	48	1 (2.1)	0 (0)	1 (2.1)	5 (10.4)	7 (14.6)	41 (85.4)
Cyprus	1	1	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)
Czech Republic	5	26	0 (0)	1 (3.8)	1 (3.8)	0 (0)	2 (7.7)	24 (92.3)
Denmark	3	8	1 (12.5)	3 (37.5)	2 (25.0)	0 (0)	6 (75.0)	2 (25.0)
Estonia	1	9	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	9 (100)
Finland	1	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
France	11	27	1 (3.7)	0 (0)	10 (37.0)	0 (0)	11 (40.7)	16 (59.3)
Germany	13	36	8 (22.2)	2 (5.6)	12 (33.3)	0 (0)	22 (61.1)	14 (38.9)
Greece	10	86	56 (65.1)	12 (14.0)	2 (2.3)	9 (10.5)	79 (91.9)	7 (8.1)
Hungary	7	36	0 (0)	0 (0)	0 (0)	26 (72.2)	26 (72.2)	10 (27.8)
Ireland	7	12	2 (16.7)	2 (16.7)	2 (16.7)	0 (0)	6 (50.0)	6 (50.0)
Israel	7	39	31 (79.5)	2 (5.1)	1 (2.6)	0 (0)	34 (87.2)	5 (12.8)
Italy	22	195	187 (95.9)	1 (0.5)	1 (0.5)	3 (1.5)	192 (98.5)	3 (1.5)
Latvia	1	4	0 (0)	0 (0)	0 (0)	2 (50.0)	2 (50.0)	2 (50.0)
Lithuania	4	4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (100)
Luxembourg	3	10	4 (40.0)	0 (0)	2 (20.0)	2 (20.0)	8 (80.0)	2 (20.0)
Malta	1	9	0 (0)	0 (0)	9 (100)	0 (0)	9 (100)	0 (0)
Montenegro	1	10	0 (0)	10 (100)	0 (0)	0 (0)	10 (100)	0 (0)
Norway	4	5	0 (0)	1 (20.0)	0 (0)	0 (0)	1 (20.0)	4 (80.0)
Poland	10	34	2 (5.9)	2 (5.9)	0 (0)	0 (0)	4 (11.8)	30 (88.2)
Portugal	17	61	36 (59.0)	0 (0)	0 (0)	0 (0)	36 (59.0)	25 (40.9)
Romania	8	68	4 (5.9)	5 (7.4)	50 (73.5)	2 (2.9)	61 (89.7)	7 (10.3)
Serbia	11	67	1 (1.5)	33 (49.3)	9 (13.4)	0 (0)	43 (64.2)	24 (35.8)
Slovakia	5	22	1 (4.5)	0 (0)	0 (0)	0 (0)	1 (4.5)	21 (95.5)
Slovenia	4	12	0 (0)	1 (8.3)	1 (8.3)	1 (8.3)	3 (25.0)	9 (75.0)
Spain	20	116	9 (7.8)	0 (0)	81 (69.8)	12 (10.3)	102 (87.9)	14 (12.1)
Macedonia	1	3	2 (66.7)	0 (0)	0 (0)	0 (0)	2 (66.7)	1 (33.3)
Turkey	17	124	0 (0)	9 (7.3)	98 (79.0)	5 (4.0)	112 (90.3)	12 (9.7)
UK-England and Northern Ireland	15	47	14 (29.8)	3 (6.4)	7 (14.9)	1 (2.1)	25 (53.2)	22 (46.8)
UK-Scotland	4	8	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	8 (100)
Total	251	1203	379 (31.5)	93 (7.7)	310 (25.8)	68 (5.7)	850 (70.7)	353 (29.3)

Iceland, Kosovo, and Sweden did not find any *K pneumoniae* isolates that were suspected non-susceptible to carbapenems during the study period. *Other mechanism of carbapenem non-susceptibility, since none of the genes coding for the four major types of carbapenemases (KPC, NDM, OXA-48-like and VIM) were detected. All data are n, except where otherwise indicated.

Table 2: *Klebsiella pneumoniae* clinical isolates submitted as non-susceptible to carbapenems, confirmed as producing a carbapenemase and type of carbapenemase, by country

Italia: 95,9% KPC, 1,5% VIM, 0,5% NDM, 0,5% OXA-48

Grecia: 65,1% KPC, 14% NDM, 10,5% VIM, 2,3% OXA-48

UK: 29,8% KPC, 14,9% OXA-48, 6,4% NDM, 2,1% VIM,

Francia: 37% OXA-48, 3,7% KPC

Spagna: 69,8% OXA-48, 10,3% VIM, 7,8% KPC,

Portogallo: 59% KPC

Germania: 33,3% OXA-48, 22,2% KPC, 5,6% NDM

Epidemiologia di *Klebsiella pneumoniae* resistente ai carbapenemi

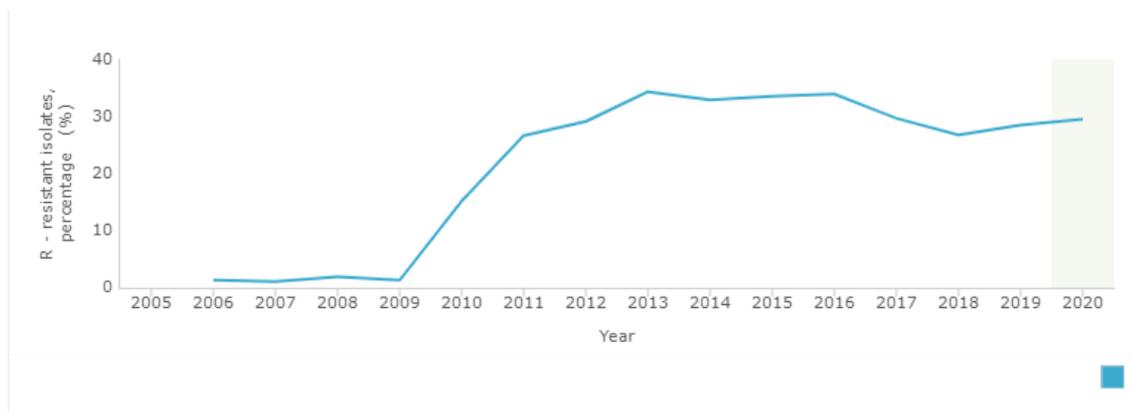
Surveillance
Atlas of
Infectious
Diseases, 2020

Infezioni
invasive in
Europa



29,5%

Incremento dal 2009



KPC+ *K. pneumoniae*: scenario Italiano



fine 2008

2009



inizio 2011



fine 2018

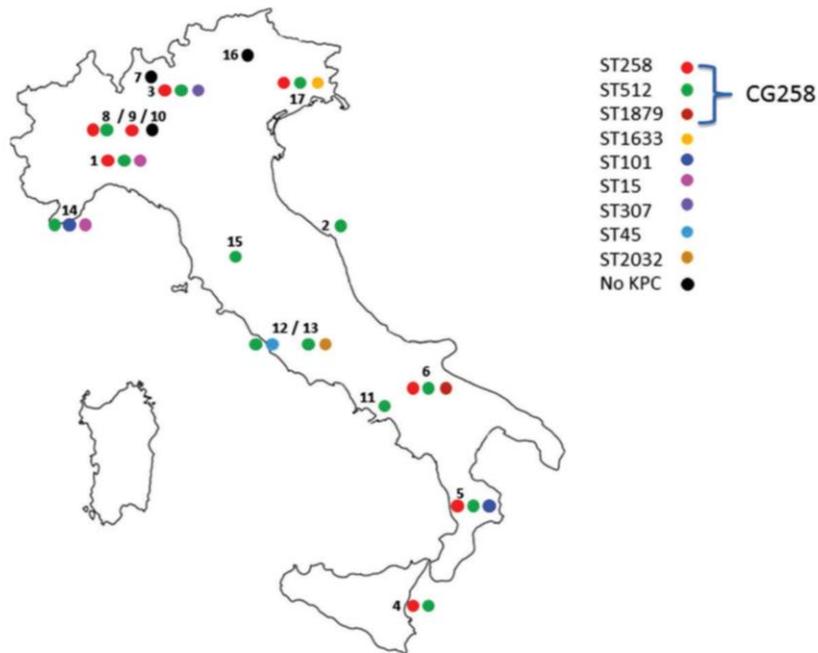
Giani *et al* – JCM 2009
Santoriello *et al* – unpublished
Mammaia *et al* – JCM 2010

Fontana *et al* – BMC Res Notes 2010
Marchese *et al* – J Chemother 2010
Ambretti *et al* – New Microb 2010
Gaibani *et al* – Eurosurv 2011
Mezzatesta *et al* – CMI 2011
Agodi *et al* – JCM 2011
Richter *et al* – JCM 2011
Di Carlo *et al* – BMC Gastroenterol 2011

Giani *et al* – Eurosurveillance 2013
Aschbacher *et al* – IJAA 2013
Migliavacca *et al* – New Microb 2013
Pulcrano *et al* – APMIS 2013
Giuffrè *et al* – JHI 2013
Di Carlo *et al* – BMC Anesth 2013
ARISS – CoSA survey 2012-2013
Antonelli *et al* – ECCMID 2015

Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* from invasive infections in Italy: increasing diversity with predominance of the ST512 clade II sublineage

Viola Conte¹, Monica Monaco², Tommaso Giani¹, Fortunato D'Ancona³, Maria Luisa Moro⁴, Fabio Arena¹, Marco Maria D'Andrea¹, Gian Maria Rossolini^{1,5-7*} and Annalisa Pantosti² on behalf of the AR-ISS Study Group on Carbapenemase-Producing *K. pneumoniae*†



17 Ospedali, anni **2012-2013**.

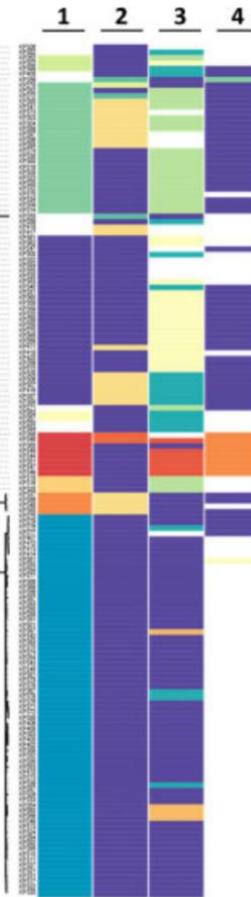
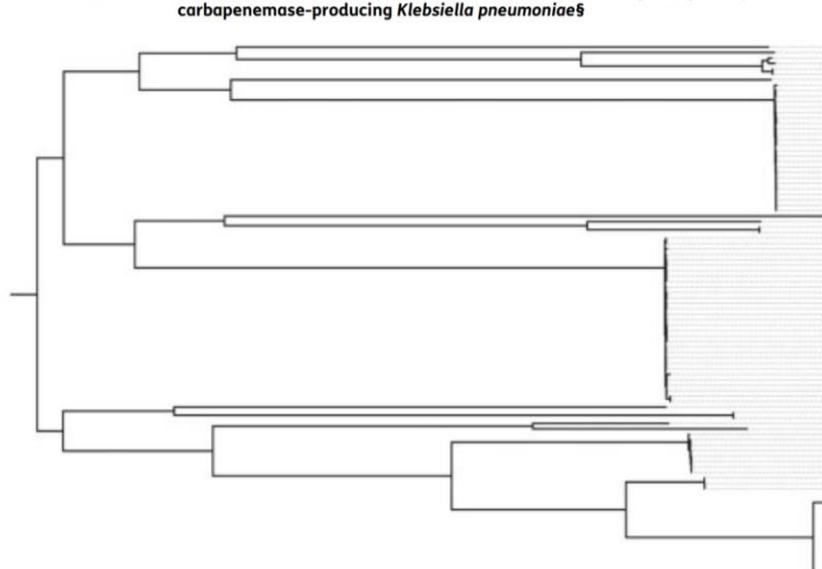
Tra le infezioni invasive da *K. pneumoniae* il **36,2%** erano resistenti ai carbapenemi.

97% produttrici di **KPC**,
94% appartenenti al clonal group
(CG) 258.

Figure 1. Geographical location of the 17 laboratories participating in the survey. The KPC-KP STs detected in different laboratories are indicated by different colours. Cities were as follows: 1, Alessandria; 2, Ancona; 3, Bergamo; 4, Catania; 5, Cosenza; 6, Foggia; 7, Lecco; 8–10, Milan; 11, Naples; 12 and 13, Rome; 14, San Remo; 15, Siena; 16, Trento; and 17, Venice. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

The changing epidemiology of carbapenemase-producing *Klebsiella pneumoniae* in Italy: toward polyclonal evolution with emergence of high-risk lineages

Vincenzo Di Pilato^{1†}, Giulia Errico^{2,3†}, Monica Monaco², Tommaso Gianì^{1,4}, Maria Del Grosso², Alberto Antonelli¹, Sophia David⁵, Erika Lindh^{2,3}, Romina Camilli², David M. Aanensen^{5,6}, Gian Maria Rossolini^{1,4} and Annalisa Pantosti^{2*} on behalf of the AR-ISS Laboratory Study Group on carbapenemase-producing *Klebsiella pneumoniae*s



1 – ST	512	307	101	395	258	15	11	147	others
2 – Carbapenemase	KPC-3	KPC-2	VIM-1	OXA-48	NDM-1				
3 – Aminoglycoside resistance	AAC(6')-Ib	ArmA	AAC(3')-IIa	ANT(2'')-Ia	AAC(6')-Ib*	others			
4 – Virulence factors	Ybt	luc+Ybt	luc	luc+Ybt+Iro+RmpA					

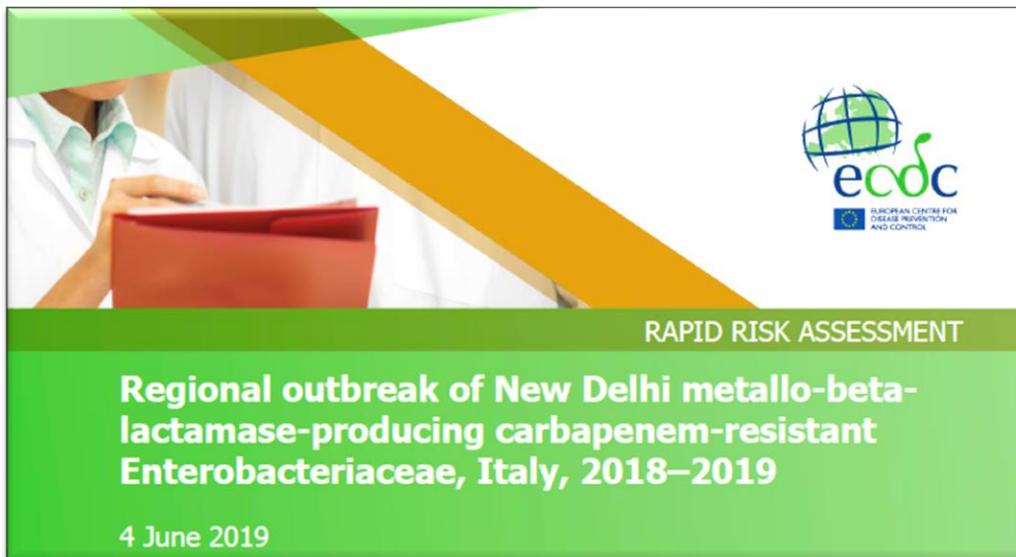
Marzo-Luglio 2016 24 centri, isolati da emocoltura



96% KPC. Diffusione poli-clonale,
47,7% CC258 e aumento
prevalenza di ST307, ST101, ST395,
ST147

NDM outbreak in Toscana

- **2009-18**: casi sporadici di NDM in Italia (in gran parte correlati a viaggiatori)
- **Da fine 2018**: outbreak di *K. pneumoniae* NDM



Open Forum Infectious Diseases

BRIEF REPORT

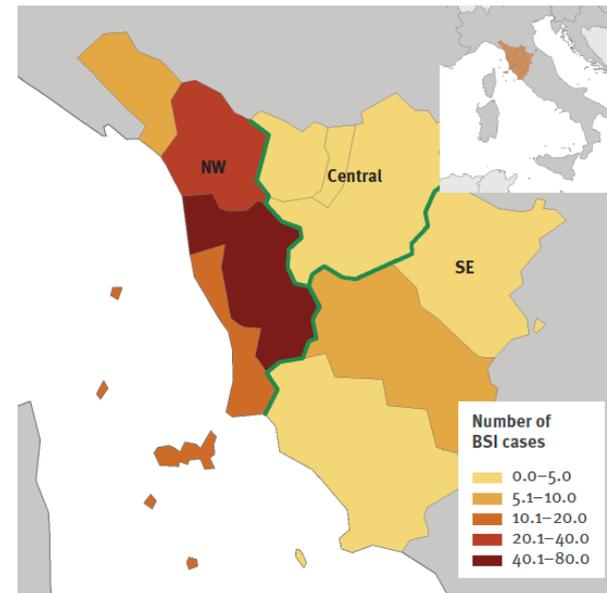
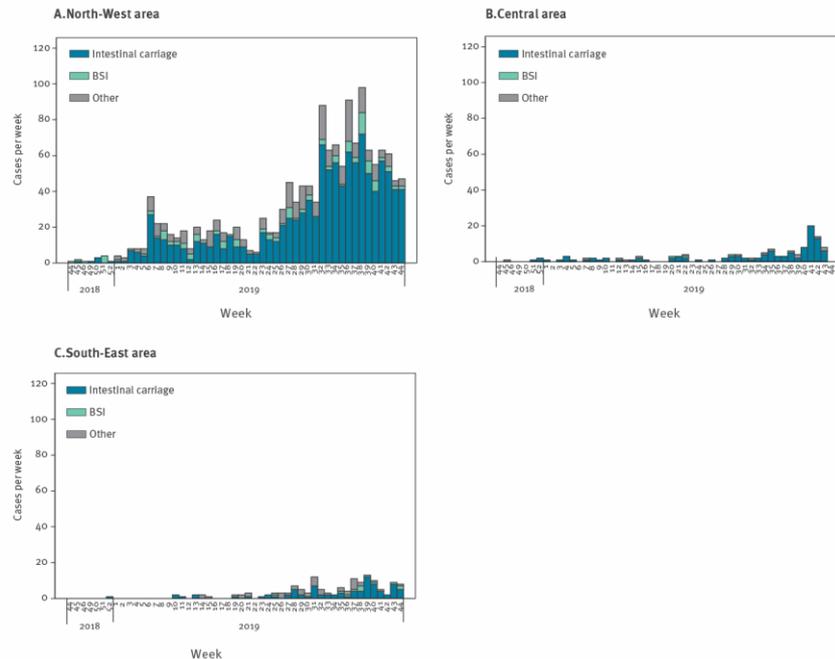
Clinical Features and Outcomes of Bloodstream Infections Caused by New Delhi Metallo- β -Lactamase-Producing *Enterobacteriales* During a Regional Outbreak

Marco Falcone,¹ Giusy Tiseo,¹ Alberto Antonelli,^{2*} Cesira Giordano,³ Vincenzo Di Pilato,² Pietro Bertolucci,⁴ Eva Maria Parisio,⁵ Alessandro Leonildi,⁴ Noemi Aiezza,² Ilaria Baccani,² Enrico Tagliaferri,⁶ Lorenzo Righi,⁷ Silvia Forni,⁸ Spartaco Sani,⁹ Maria Teresa Mechi,⁷ Filippo Pieralli,¹⁰ Simona Barnini,³ Gian Maria Rossolini,^{2,11} and Francesco Menichetti¹

¹Infectious Disease Unit, Azienda Ospedaliera Universitaria Pisana, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy, ²Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy, ³Microbiology Unit, Azienda Ospedaliera Universitaria Pisana, Pisa, Italy, ⁴Faculty of Medicine, University of Pisa, Pisa, Italy, ⁵Operative Unit of Chemical-Clinical and Microbiological Analysis, San Luca Hospital, Lucca, Italy, ⁶Infectious Diseases Unit, Azienda Ospedaliera Universitaria Pisana, Pisa, Italy, ⁷Quality of Care and Clinical Networks, Tuscany Region, Italy, ⁸Agenzia Regionale di Sanità della Toscana, Florence, Italy, ⁹Infectious Disease Unit, Livorno Hospital, Livorno, Italy, ¹⁰Intermediate Care Unit, Florence Careggi University Hospital, Florence, Italy, and ¹¹Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italy

Prolonged outbreak of New Delhi metallo-beta-lactamase-producing carbapenem-resistant Enterobacterales (NDM-CRE), Tuscany, Italy, 2018 to 2019

Lara Tavoschi¹, Silvia Forni², Andrea Porretta^{4,5}, Lorenzo Righi³, Filippo Pieralli⁴, Francesco Menichetti⁵, Marco Falcone⁵, Giulia Gemignani⁵, Spartaco Sani⁶, Paola Viviani⁷, Tommaso Bellandi⁸, Danilo Tacconi⁹, Lucia Turini¹⁰, Giulio Toccafondi¹³, Gaetano Privitera^{4,5}, Pierluigi Lopalco^{4,5}, Angelo Baggiani^{4,5}, Fabrizio Gemmi², Grazia Luchini⁵, Maurizio Petrillo¹¹, Lorenzo Roti¹⁰, Patrizio Pezzotti¹², Annalisa Pantosti¹², Stefania Iannazzo¹³, Maria Teresa Mechi³, Gian Maria Rossolini^{4,14}, on behalf of the Tuscan Clinical Microbiology Laboratory Network¹⁵



Consequente implementazione di metodologie di screening fenotipico e molecolare per CRE in tutta la regione

Epidemiologia di *Pseudomonas aeruginosa* resistenti ai carbapenemi

Surveillance Atlas of Infectious Diseases, 2020

Infezioni invasive in Europa, 2020

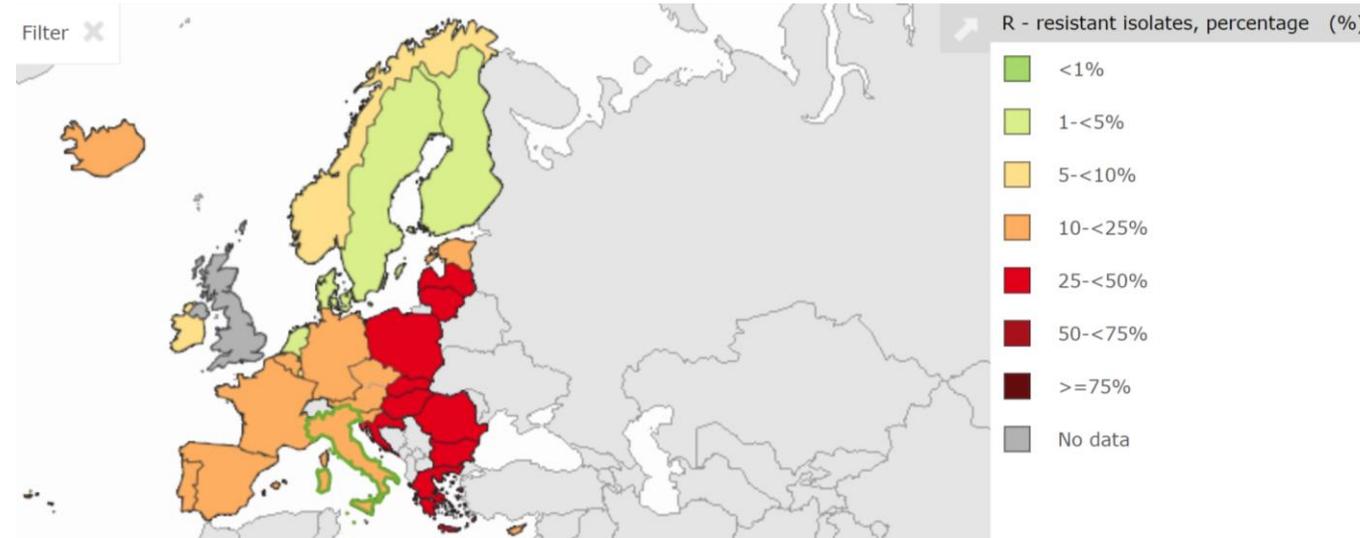
Meccanismi principali:

- Overespressione pompe di efflusso (MexAB-OprM)
- perdita di porine (OprD)
- Overespressione AmpC cromosomica

Principali carbapenemasi:

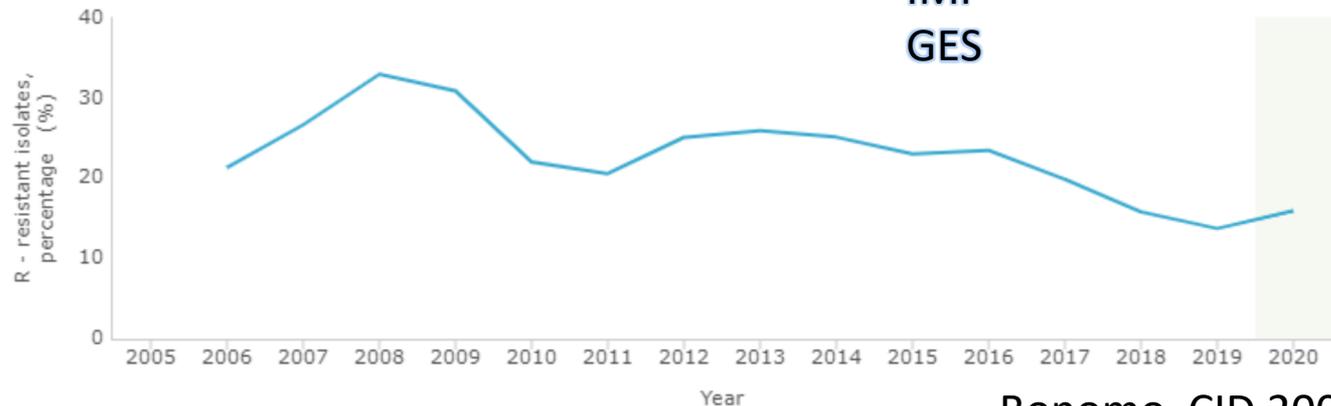
VIM
IMP
GES

Bonomo, CID 2007



15,9%

Lieve decremento della percentuale di isolati resistenti in Italia nel corso degli anni



Article

Retrospective Data Insight into the Global Distribution of Carbapenemase-Producing *Pseudomonas aeruginosa*

Min-Ge Wang ^{1,2}, Zhi-Yong Liu ³, Xiao-Ping Liao ^{1,2,4}, Ruan-Yang Sun ^{1,2}, Run-Bo Li ^{1,2}, Yan Liu ⁵, Liang-Xing Fang ^{1,2}, Jian Sun ^{1,2,4}, Ya-Hong Liu ^{1,2,4} and Rong-Min Zhang ^{1,2,*}

328 produttori di carbapenemasi su 2953 isolati clinici fino a Ottobre 2019

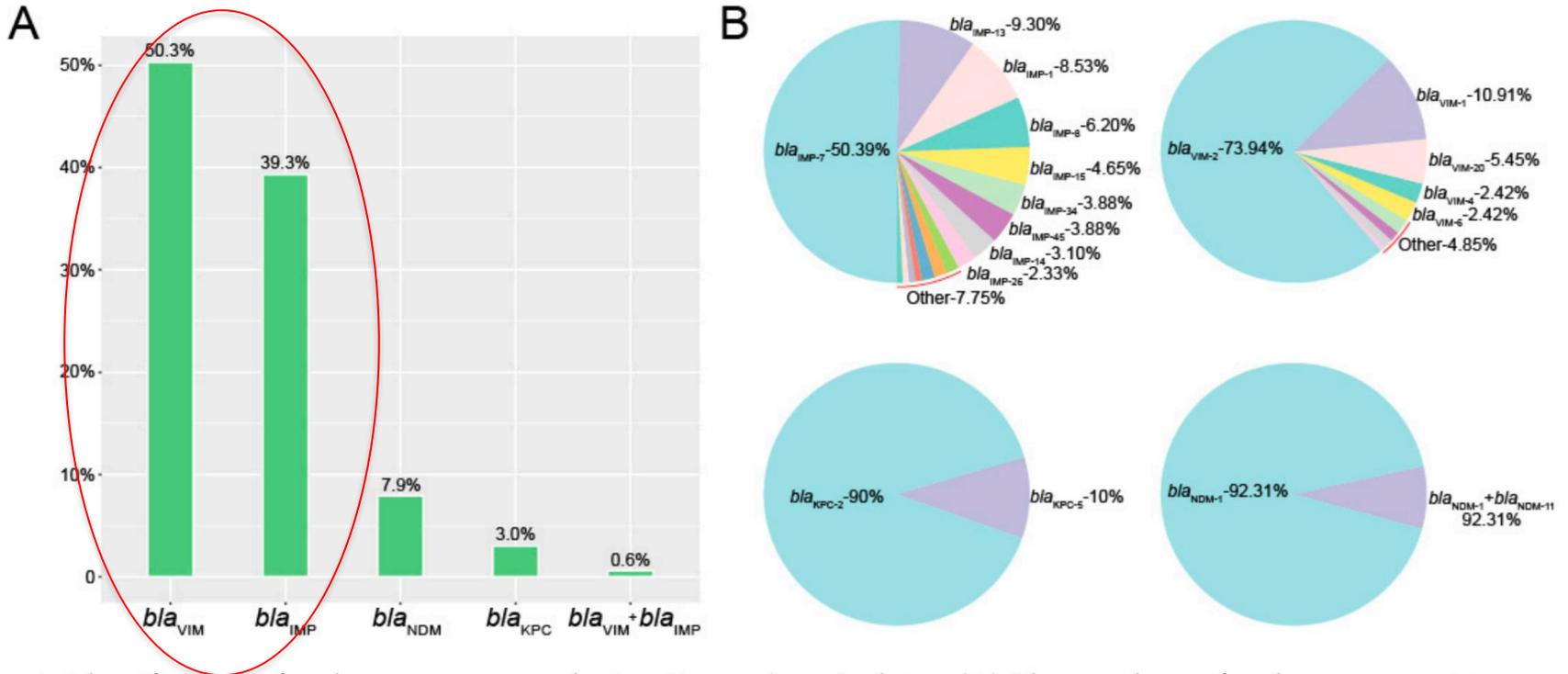


Figure 1. Identification of carbapenemase-producing *P. aeruginosa* isolates. (A) The numbers of carbapenem resistance genes in *P. aeruginosa* isolates. (B) The rates and numbers of variants in carbapenem resistance genes.

Presenza di cloni ad alto rischio di *P. aeruginosa* diffusi a livello globale

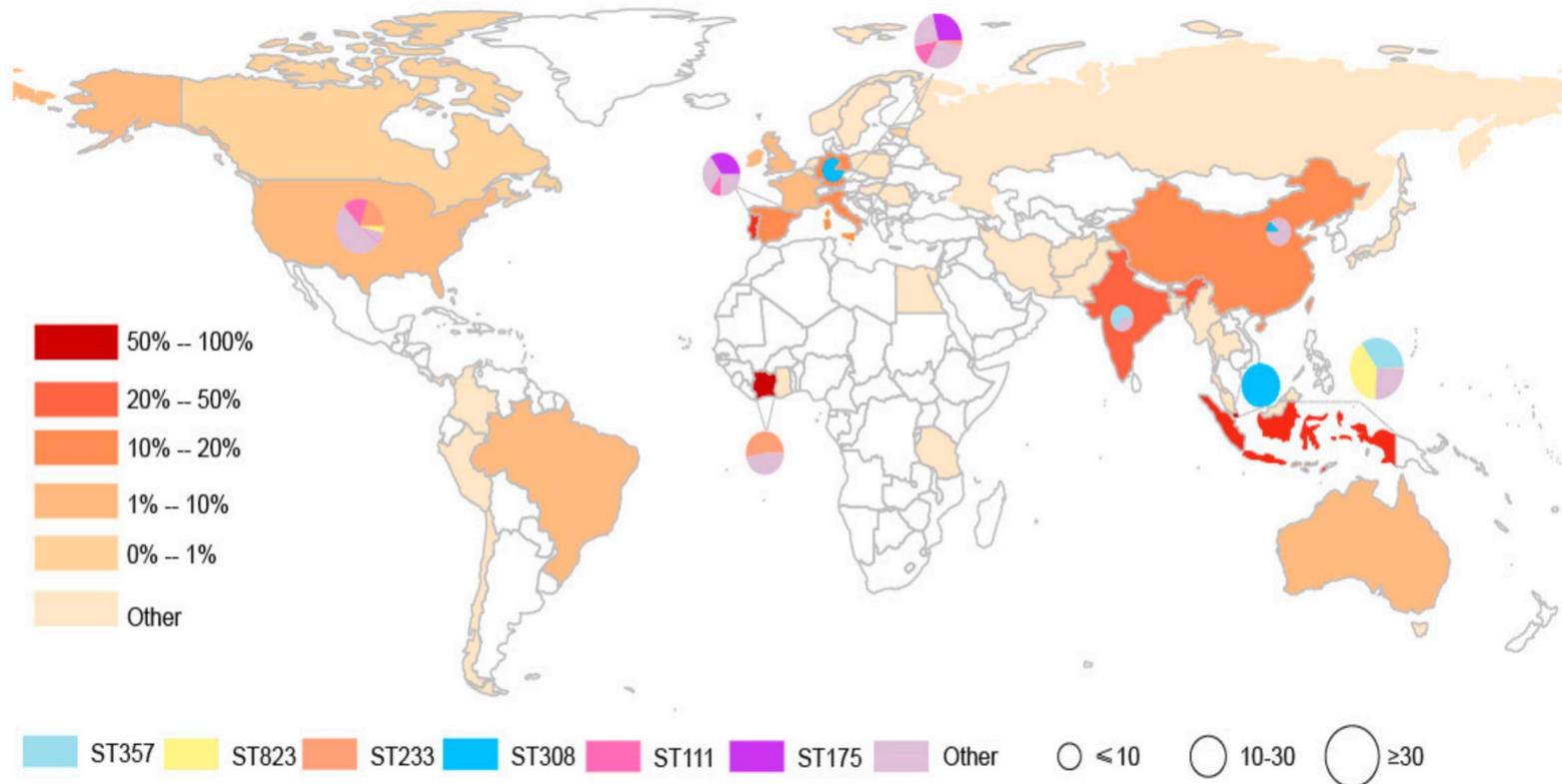
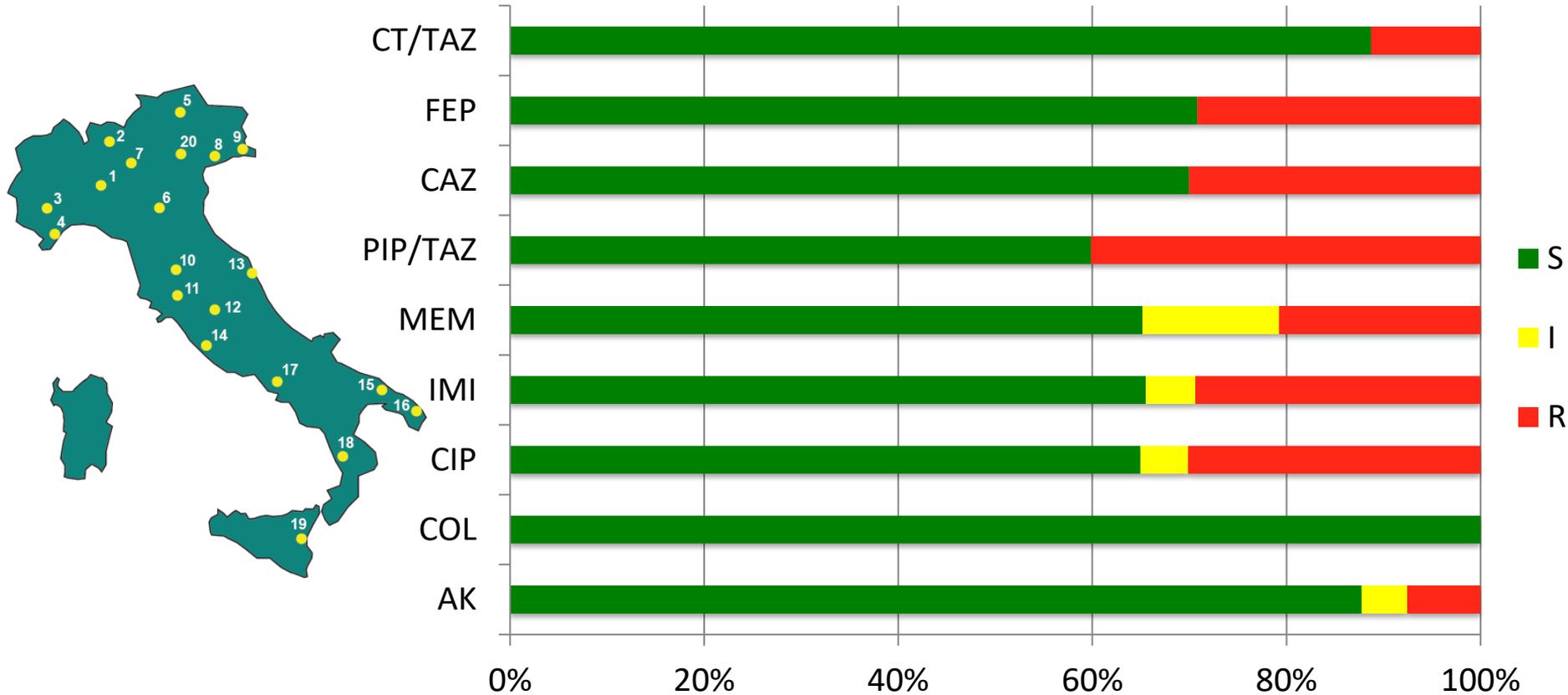


Figure 2. Geographic distribution and MLST diversity of carbapenemase-producing *P. aeruginosa* isolates. The presence of the carbapenemase-producing *P. aeruginosa* isolates is indicated by brown; the pie chart represents MLST diversity.

Italian nationwide survey on *Pseudomonas aeruginosa* from invasive infections: activity of ceftolozane/tazobactam and comparators, and molecular epidemiology of carbapenemase producers

Tommaso Giani, Fabio Arena, Simona Pollini, Vincenzo Di Pilato, Marco Maria D'Andrea, Lucia Henrici De Angelis, Matteo Bassetti, Gian Maria Rossolini, *Pseudomonas aeruginosa* Working Group JAC 2018

N = 939 isolati non-replicati da BSI o HAP/VAP

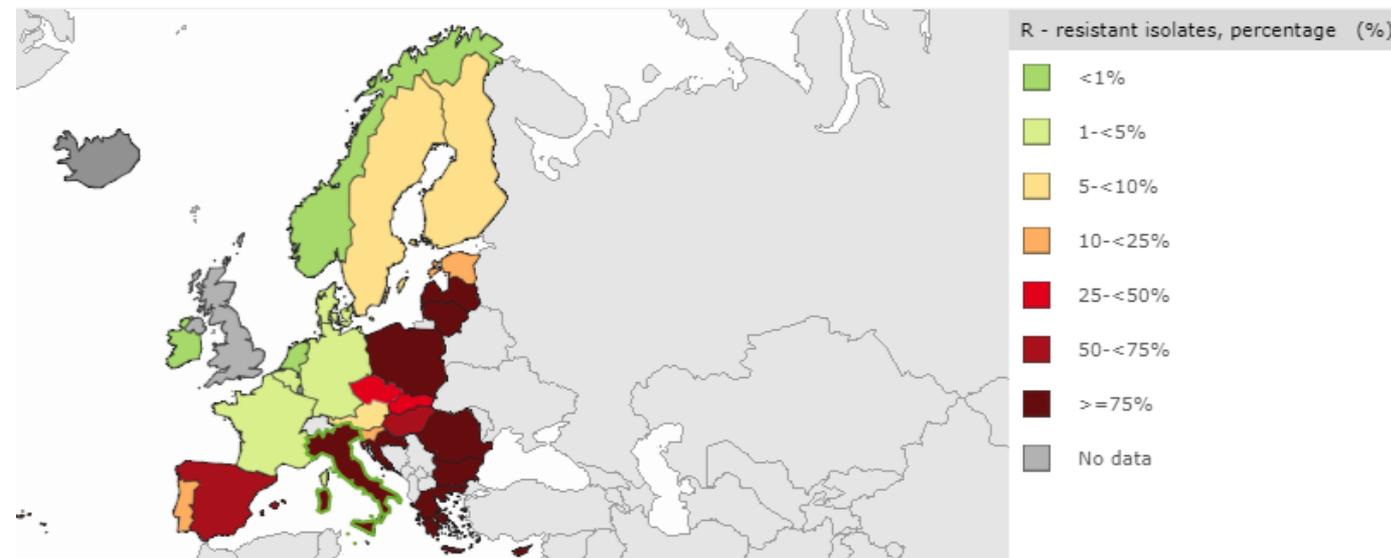


20 centri **2013-2014** Resistenti a meropenem **21%**, produttori di carbapenemasi **5,1%**

Epidemiologia di *Acinetobacter baumannii* resistenti ai carbapenemi

Surveillance Atlas of Infectious Diseases, 2020

Infezioni invasive in
Europa, 2020



Principali carbapenemasi

OXA-23

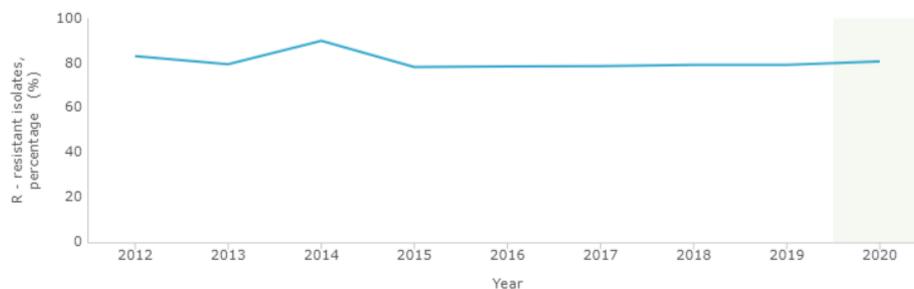
OXA-24/40

OXA-58

(NDM)

80,8%

Percentuale elevata
costante di isolati
resistenti in Italia nel
corso degli anni



Epidemiological characterization and distribution of carbapenem-resistant *Acinetobacter baumannii* clinical isolates in Italy

M. L. Mezzatesta¹, M. M. D'Andrea², R. Migliavacca³, T. Giani², F. Gona¹, E. Nucleo³, G. Fugazza³, L. Pagani³, G. M. Rossolini^{2,3,4} and S. Stefani¹

1) Department of Microbiology, University of Catania, Catania, 2) Department of Molecular Biology, Section of Microbiology, University of Siena, Siena, 3) Department of Microbiology, University of Pavia, Pavia and 4) Azienda Ospedaliera Universitaria Senese, Dipartimento dei Servizi, UOC di Microbiologia e Virologia, Siena, Italy

CMI 2012

TABLE 2. Genotypic and epidemiological analysis of strains isolated during: (a) 2004–2005; and (b) 2008–2009

No. of strains	PFGE type	PFGE subtype	ST	Hospitals	OXA content		
					58	58 + 23	
(a) 110	A	A1, A2, A3	2	Bari, Naples Federico II, Naples Cardarelli, Naples Tufano, Rome S. Camillo, Avellino, Pavia S. Matteo, Pavia Maugeri, Reggio Calabria, Florence, Turin, Foggia and Varese	110	0	
4	B		1	Bari	4	0	
6	C	C1, C2	20	Palermo	0	6	
120					114	6	
Clone II (RUH 134)	A	–	2				
Clone I (RUH 875)	B	–	1				
(b)					OXA content		
					58	58 + 23	23
66	A	A1, A2, A3	2	Bari, Naples Federico II, Catania OVE, Rome Gemelli, Rome Umberto I, Palermo Chiarini, Milan and Pavia S. Matteo	27	12	27
5	B		1	Novara, Rome S. Pertini and Bari	2	3	
11	D		78	Naples Federico II, Novara and Catania Policlinico	11	0	
82					40	15	27

PFGE, pulsed-field gel electrophoresis; ST, sequence type.

2004-2005 e 2009-2010

202 isolati resistenti ai carbapenemi

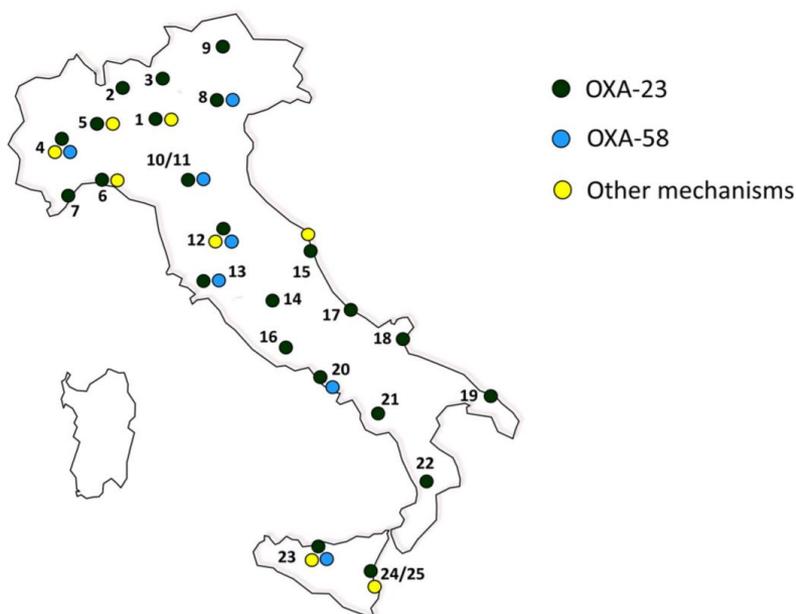
22 ospedali in tutta Italia

154 di 202 (76,23%) sono risultati essere produttori di **OXA-58**, 21 anche produttori di OXA-23, e 27 (13,3%) produttori solamente di **OXA-23**

Epidemic Diffusion of OXA-23-Producing *Acinetobacter baumannii* Isolates in Italy: Results of the First Cross-Sectional Countrywide Survey

Luigi Principe,^a Aurora Piazza,^b Tommaso Gianni,^c Silvia Bracco,^a Maria Sofia Caltagirone,^b Fabio Arena,^c Elisabetta Nucleo,^b Federica Tammaro,^c Gian Maria Rossolini,^{c,d,e} Laura Pagani,^b Francesco Luzzaro,^a AMCLI-CRAB Survey participants

Microbiology and Virology Unit, Department of Laboratory Medicine, A. Manzoni Hospital, Lecco, Italy^a; Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, Section of Microbiology, University of Pavia, Pavia, Italy^b; Department of Medical Biotechnologies, University of Siena, Siena, Italy^c; Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy^d; Clinical Microbiology and Virology Unit, Department of Laboratory Medicine, Careggi University Hospital, Florence, Italy^e



25 centri

2011

OXA-23 81,7%

OXA-58 4,5%

OXA-23+OXA-58 2,4%

ST2 clone prevalente

FIG 1 Location of the laboratories participating in the survey ($n = 25$). 1, Milan; 2, Varese; 3, Lecco; 4, Turin; 5, Novara; 6, Genoa; 7, Sanremo; 8, Verona; 9, Bolzano; 10 to 11, Modena; 12, Florence; 13, Siena; 14, Perugia; 15, Ancona; 16, Rome; 17, Pescara; 18, San Giovanni Rotondo; 19, Lecce; 20, Naples; 21, Avellino; 22, Cosenza; 23, Palermo; and 24 and 25, Catania. The presence of OXA-23 and/or OXA-58 determinants (as well as other mechanisms) is also indicated. The map was generated using Magic Maps version 1.4.6.

SMART: rete di sorveglianza microbiologica e della resistenza agli antibiotici in Toscana

Dati di sorveglianza per isolati batterici invasivi (emocolture)

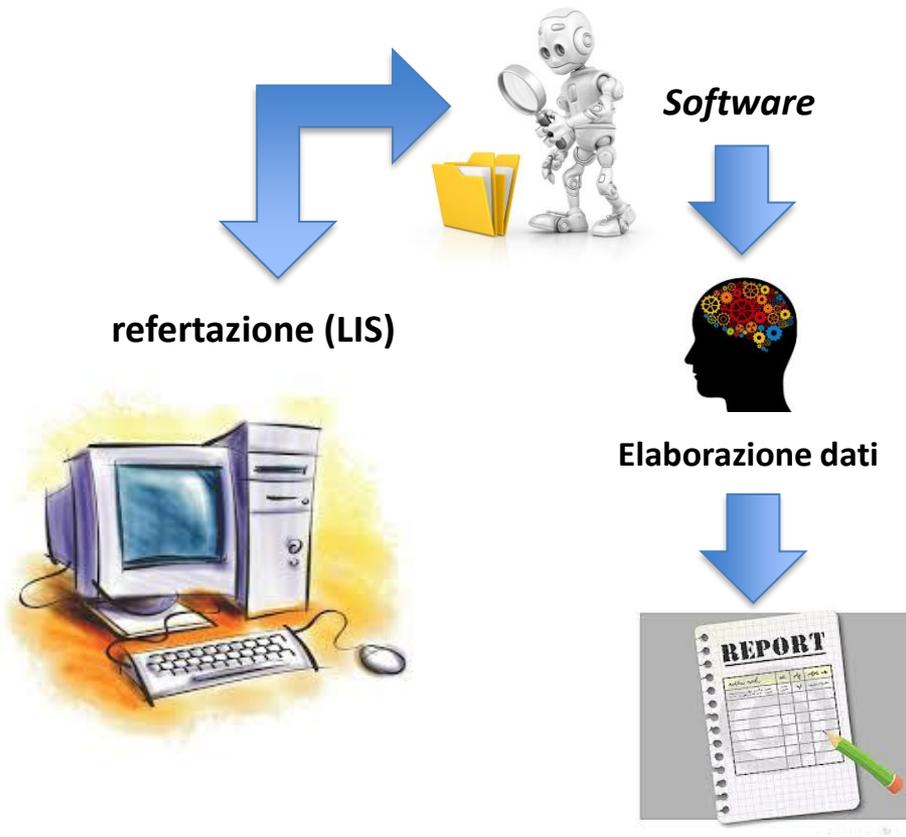
14 laboratori

8 specie sorvegliate:

- *Staphylococcus aureus*
- *Enterococcus (faecalis e faecium)*
- *Streptococcus pneumoniae*
- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Pseudomonas aeruginosa*
- *Acinetobacter spp.*
- *Candida spp.*



Sorveglianza epidemiologica locale (Laboratorio di Microbiologia e Virologia)



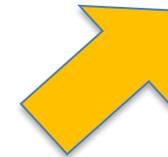
**report semestrale
consultabile in intranet**

La storia naturale delle infezioni ospedaliere da CRE

Exposure



Source of
contamination



I colonizzati rappresentano la maggiore fonte di contaminazione

Il rischio di infezione è maggiore nei colonizzati

Ruolo della sorveglianza nel prevenire e controllare la diffusione di patogeni Gram-negativi MDR

ESCMID PUBLICATIONS

10.1111/1469-0691.12427

ESCMID guidelines for the management of the infection control measures to reduce transmission of multidrug-resistant Gram-negative bacteria in hospitalized patients

E. Tacconelli¹, M. A. Cataldo², S. J. Dancer³, G. De Angelis⁴, M. Falcone⁵, U. Frank⁶, G. Kahlmeter⁷, A. Pan^{8,9}, N. Petrosillo², J. Rodríguez-Baño^{10,11,12}, N. Singh¹³, M. Venditti⁵, D. S. Yokoe¹⁴ and B. Cookson¹⁵

CMI, 2014

CRAB (Carbapenem-R *Acinetobacter*) e CRPsA (Carbapenem-R *Pseudomonas*) : evidenze non sufficienti su impatto sorveglianza pro-attiva

CRE: forte raccomandazione a fare sorveglianza pro-attiva



Guidelines for the prevention and control of carbapenem-resistant Enterobacteriaceae, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in health care facilities



WHO, novembre 2017

Screening della colonizzazione da CRE presso AOUC



- **Tampone rettale di screening all'ammissione**
 - terapie intensive, oncoematologia, medicine, altri reparti a rischio
- **Controllo settimanale per i negativi**
- **~30,000 tamponi rettali/anno**



Tamponi in fase liquida



**Terreno selettivo
per CRE**

**Controllo di crescita
(agar sangue)**



**ID con
MALDI-ToF**



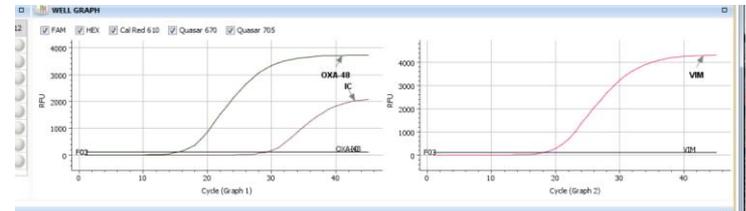
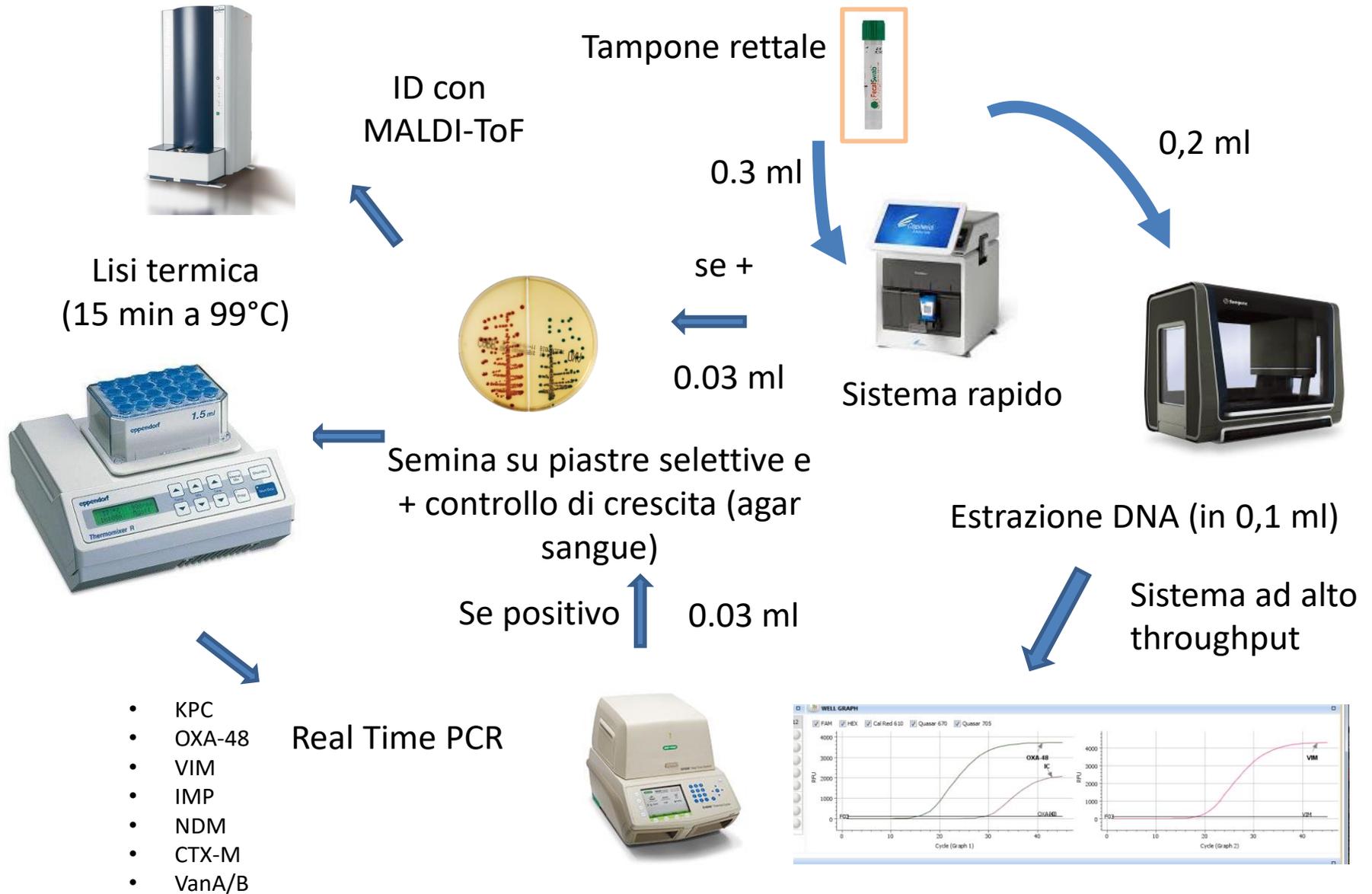
**Antibiogramma
(casi selezionati)**



**R mechanism:
multiplex
RT PCR**

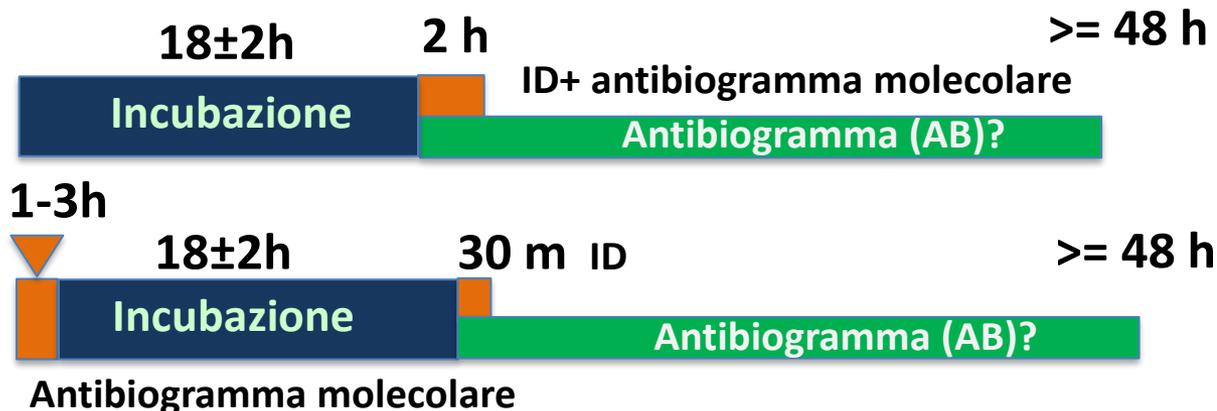


Screening molecolare diretto dei CPE pre-COVID-19



Impatto dello screening molecolare diretto

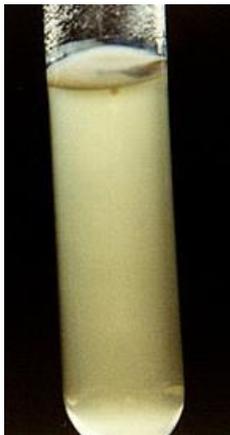
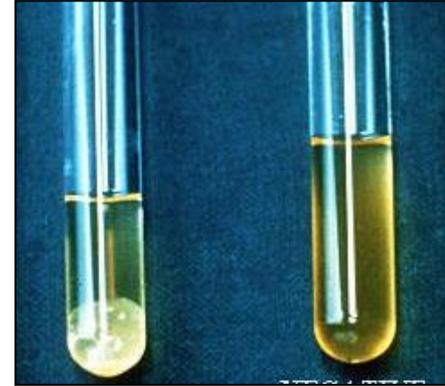
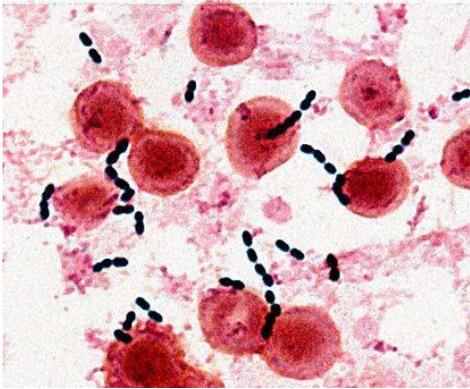
*Tampone rettale
all'ammissione*



Isolamento preventivo **Se positivo per CPE isolamento definitivo**

Isolamento preventivo/definitivo in giornata

Le tecnologie convenzionali in microbiologia clinica diagnostica



Nuove tecnologie in diagnostica microbiologica

UTILIZZO DIRETTO DA CAMPIONE CLINICO

Sistemi Fenotipici Rapidi
(*emocoltura positiva*)



Sistemi Molecolari
(*emocoltura positiva*)



Sistemi Molecolari
(*sangue intero*)



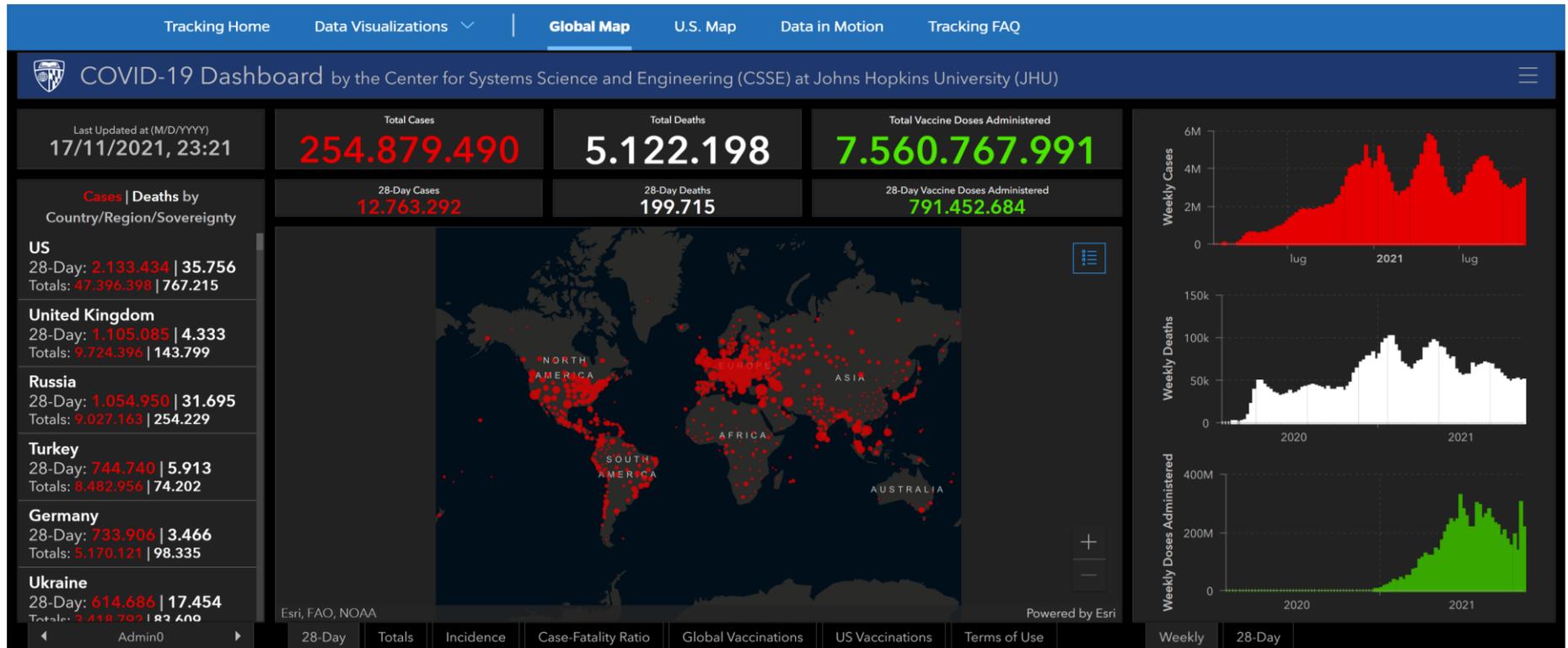
Sequenziamento
genomico



**Nuove tecnologie
diagnostiche**

- + Maggiore rapidità
- + Maggiore sensibilità
- + Maggiore accuratezza
- **Maggiore costo**

Impatto globale della pandemia da SARS-CoV-2



➤ Riuscirà la vaccinazione di massa a contenere la pandemia da SARS-CoV-2?



Key considerations on the potential impacts of the COVID-19 pandemic on antimicrobial resistance research and surveillance

Jesús Rodríguez-Baño^{a,b,c}, Gian Maria Rossolini^{d,e}, Constance Schultz^f, Evelina Tacconelli^g, Srinivas Murthy^h, Norio Ohmagariⁱ, Alison Holmes^j, Till Bachmann^k, Herman Goossens^l, Rafael Canton^{m,n}, Adam P. Roberts^o, Birgitta Henriques-Normark^{p,q}, Cornelius J. Clancy^r, Benedikt Huttner^s, Patriq Fagerstedt^t, Shawon Lahiri^u, Charu Kaushic^{u,v}, Steven J. Hoffman^w, Margo Warren^x, Ghada Zoubiane^y, Sabiha Essack^{y,z}, Ramanan Laxminarayan^{aa}, and Laura Plant^{u,e}

Major consequences of COVID-19 pandemic on antimicrobial resistance

Table 1. Interventions implemented for COVID-19 likely to have an impact on antimicrobial resistance (AMR) in the future

	Patient-related factors	COVID-19 management-related factors	Health-system related factors
Positive impact	<ul style="list-style-type: none"> • Personal hygiene/hand and respiratory hygiene • Environmental cleaning • Physical distancing • Altered health-seeking behaviour • Decreased travel 	<ul style="list-style-type: none"> • Hand hygiene by HCW + • Use of PPE + • Physical distancing + • Environmental cleaning + • Universal masking + 	<ul style="list-style-type: none"> • Implementation of IPC policies • Implementation of AMS policies • Microbiology and pathology laboratory infrastructure with EQA • Isolation wards • Training of personnel on IPC measures
Negative impact	<ul style="list-style-type: none"> • Increased susceptibility to bacterial and fungal infections 	<ul style="list-style-type: none"> • Increased antibiotic exposure, specifically broad-spectrum drugs - • Increased risk of HAI due to invasive interventions and use of immunosuppressive agents - • Reuse of PPE - • Lack of isolation wards - • Biocide use - 	<ul style="list-style-type: none"> • Non-compliance/breakdown of IPC and AMS policies • Deprioritisation of antimicrobial use and resistance surveillance • Overcrowding of patients • Absence of clear guidelines • Increase in telemedicine • Decreased laboratory capacity on AMR (antimicrobial susceptibility testing, surveillance cultures...) • Excess stress of healthcare providers

Abbreviations: AMS, antimicrobial stewardship; EQA, external quality assessment; HAI, healthcare-associated infections; HCW, healthcare workers; IPC, infection prevention control; PPE, personal protective equipment.

Key recommendations for the future

The COVID-19 pandemic has magnified weaknesses in fighting AMR and there are many lessons that can be learnt from the current situation that will affect the ways in which AMR can be addressed. Knight et al. recently published an overview of the impact of the COVID-19 pandemic on AMR and made a call for action for the AMR community to consider the global perspective when dealing with this global health challenge.⁴³ Below, we describe recommendations to define a strong research agenda to facilitate this knowledge and enhance the understanding of beneficial and detrimental practices that drive or limit the spread of AMR. These recommendations are summarised in Figure 1.

Microbiological diagnosis of coinfections and secondary infections

To study the impact of the COVID-19 pandemic on AMR, it is critical to **maintain existing screening and diagnostic systems** and to ensure appropriate collection of microbiological samples for guiding the individual management of patients. Research and innovation focused on developing diagnostic tests differentiating between bacterial infection and SARS-CoV-2 infection, or the use of multiplex diagnostic tests targeting viruses and bacterial pathogens, would prevent the overuse of antibiotics.

Collection of microbiological samples should be guided by clinical presentation and microbiological diagnostics. Practices to collect samples should guarantee the safety of healthcare

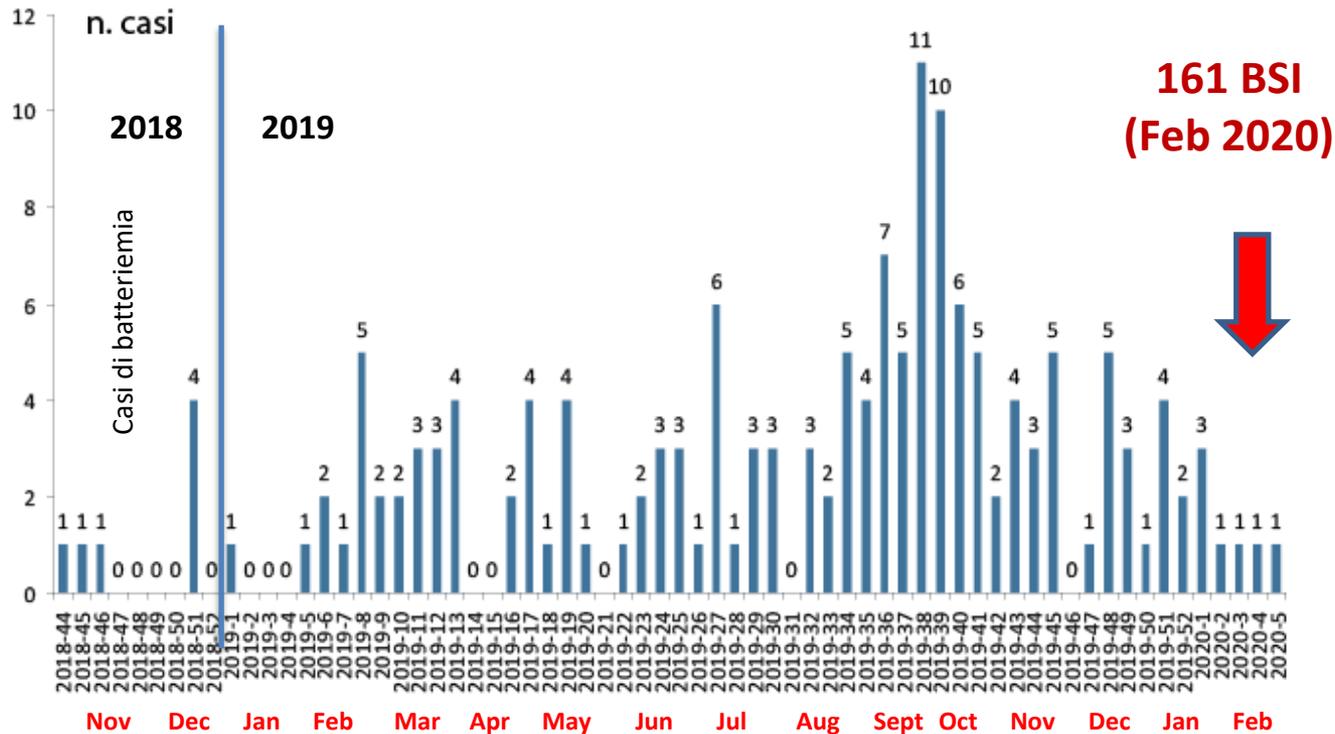
Difficoltà principali durante la pandemia da COVID-19

La maggior parte dei reattivi e strumenti sono stati dedicati alla rilevazione di SARS-CoV-2 a livello globale

Mancanza di reagenti e personale per la rilevazione molecolare dei geni di resistenza agli antimicrobici!



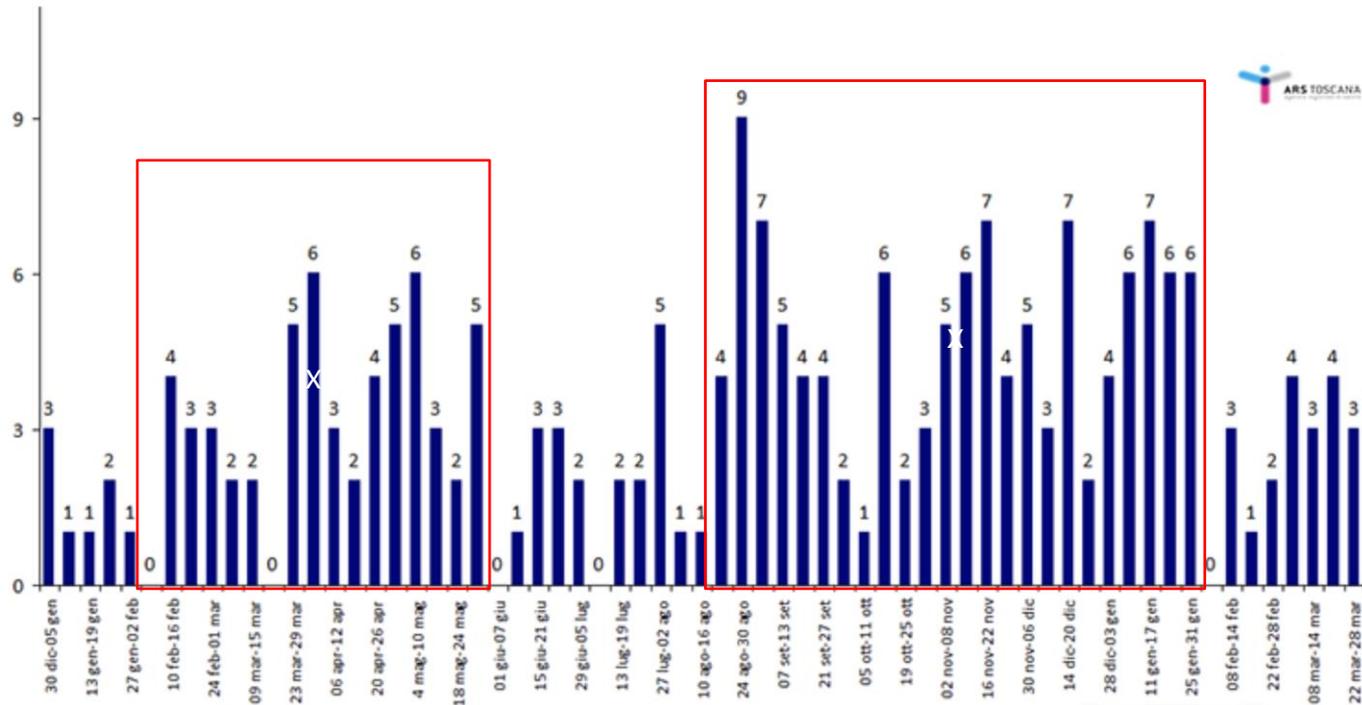
Trend of BSI da NDM-CRE in Toscana: Nov 2018 – Feb 2020



www.ars.toscana.it

Iniziale trend di riduzione a fine 2019 e inizio 2020

Trend di BSI causate da NDM-CRE in Toscana: Gen 2020 – Mar 2021



www.ars.toscana.it

Nuovo incremento durante la prima e seconda ondata COVID-19

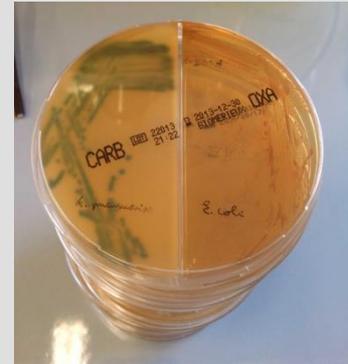
Possibile impatto della ridotta attenzione a sorveglianza ed infection control derivata dall'emergenza COVID-19?

E per quanto riguarda le acque reflue ospedaliere?

Campioni di acque reflue sono state seminate su terreni selettivi per la crescita di enterobatteri produttori di carbapenemasi

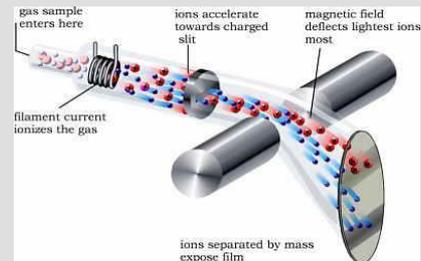


Semina su piastre selettive



Isolamento delle colonie e identificazione tramite spettrometria di massa

MALDI-TOF MS



Identificazione del meccanismo di resistenza: tipologia di carbapenemasi

Test fenotipici

Meropenem+EDTA



Meropenem+Boronic acid



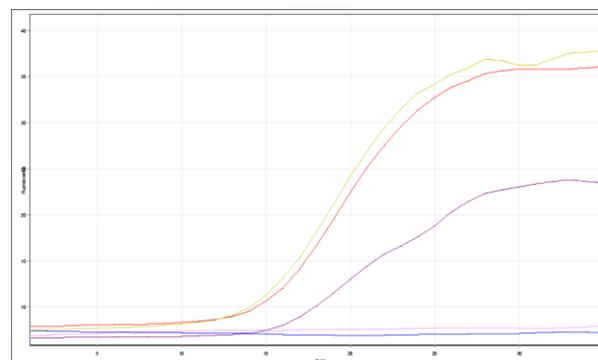
Screening molecolare

Rilevazione ed amplificazione carbapenemasi



K. pneumoniae and *E. coli* KPC

K. pneumoniae VIM



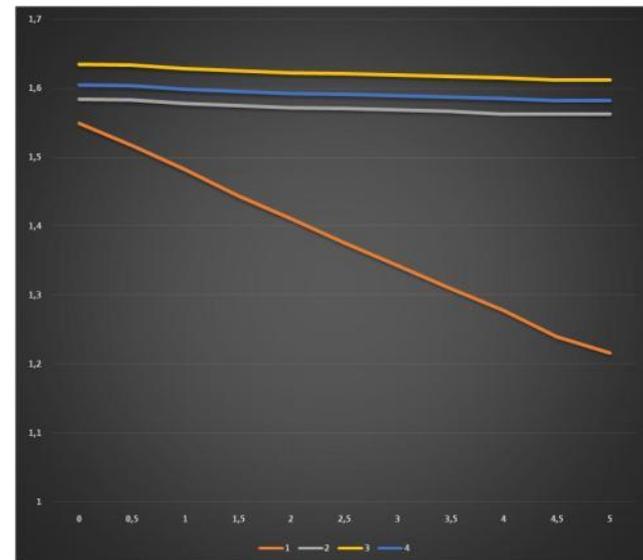
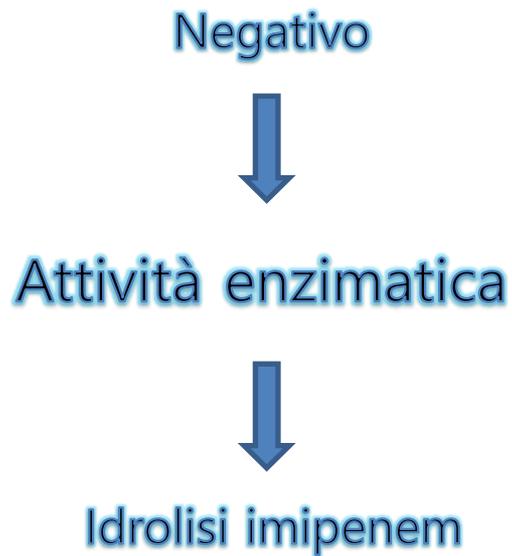
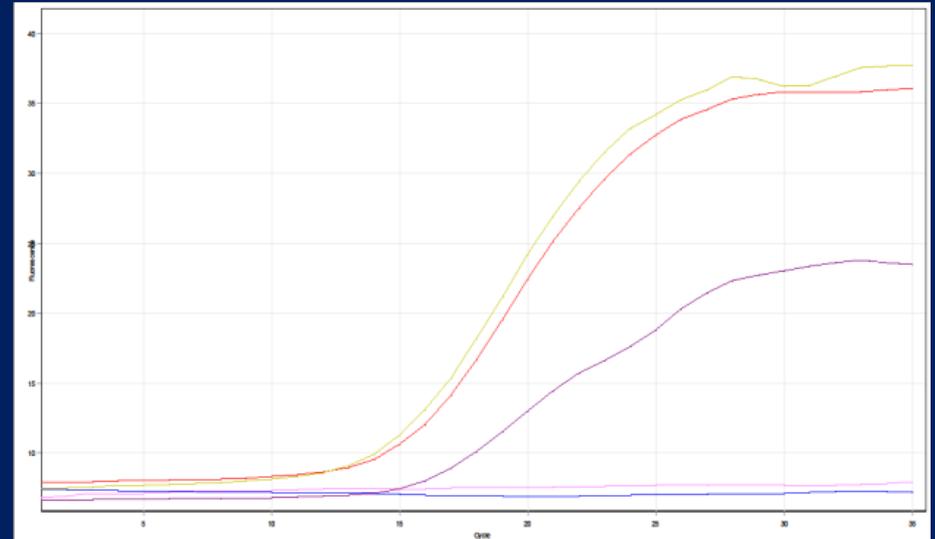
Real Time PCR

*bla*_{KPC} genes GREEN

*bla*_{OXA-48-like} genes YELLOW

*bla*_{VIM} genes ORANGE

*bla*_{NDM} genes RED



Isolamento di un *Citrobacter freundii*

Crescita su terreno selettivo



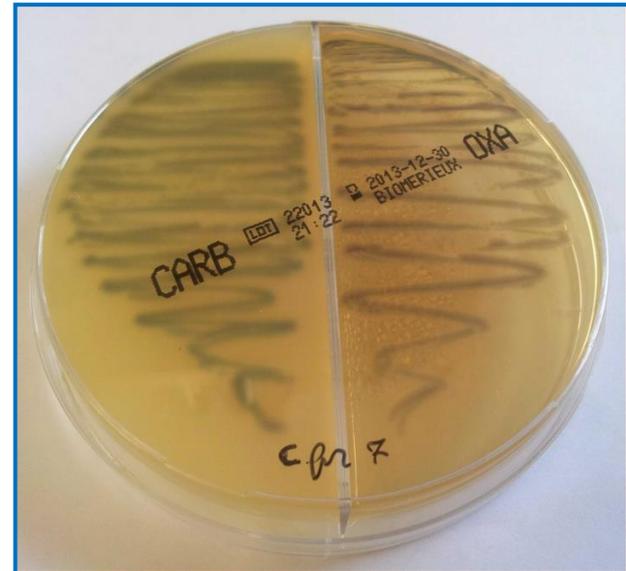
Negativo ai test
fenotipici di inibizione
acido boronico ed
EDTA



Negativo alla PCR
per VIM, NDM,
KPC e OXA-48

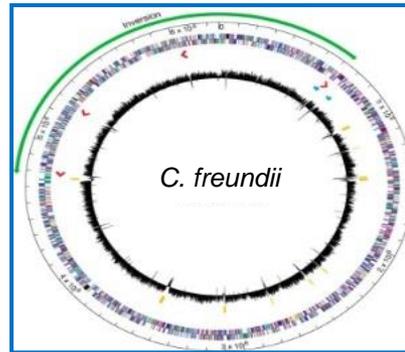


Presenza di attività idrolitica nei confronti
dell'imipenem
(8.8 nmol/min mg)



DRAFT WHOLE GENOME SEQUENCING

Illumina Miseq sequencing (2x250bp paired end)



Assemblaggio: ABySS 5,3 Mbp 1732 contigs

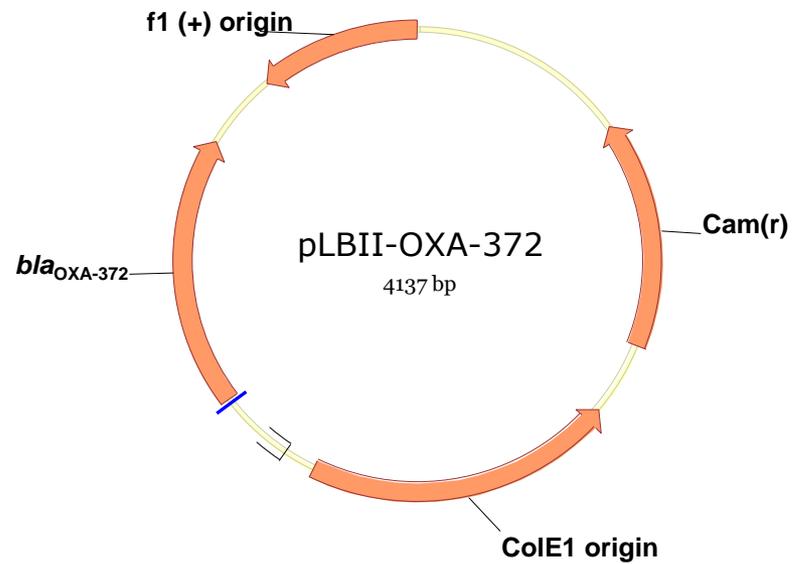
RAST (Rapid annotation using subsystem technology)

4 β -lattamasi: AmpC, $bla_{\text{OXA-10}}$, una nuova classe C ($bla_{\text{MOX-9}}$) and...

Una nuova β -lattamasi di classe D OXA-372

*bla*_{OXA-372} clonaggio su pLBII

Cloning in DH5α pLBII



Hydrolysis on imipenem (49.4 nmol/min mg)

56% di identità con OXA-198

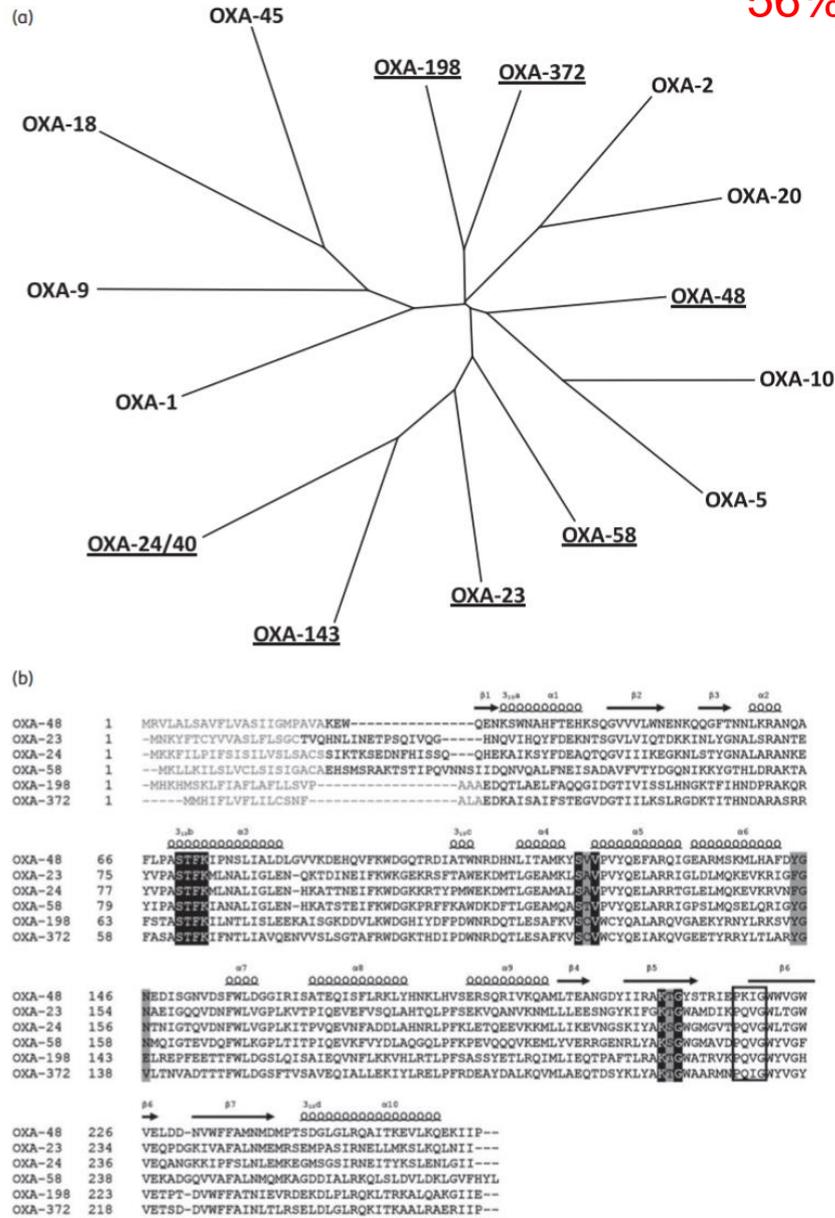


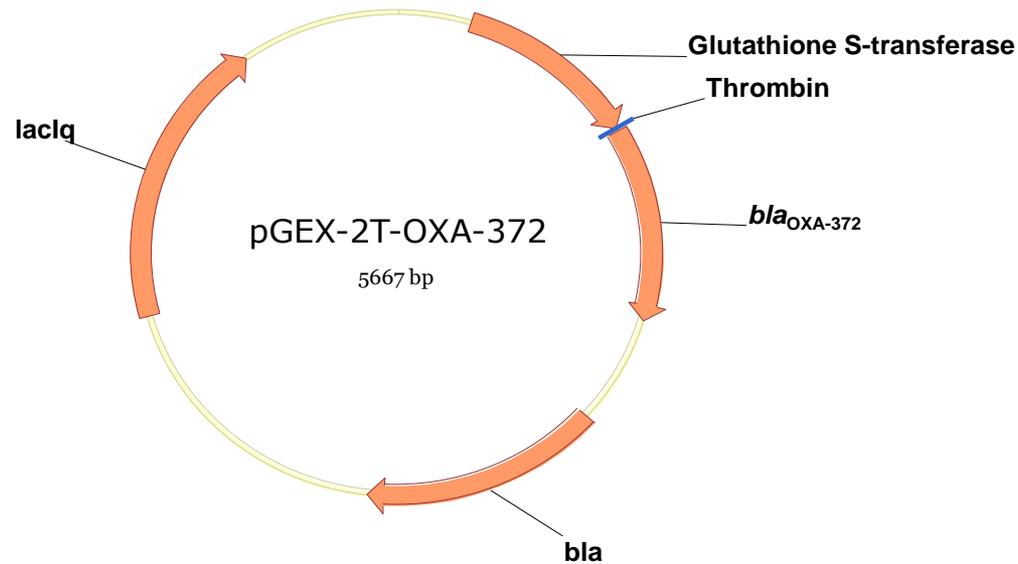
Figure 1. (a) Phylogenetic tree showing the relationship of OXA-372 with other acquired class D β -lactamases (CHDLs are underlined), whose sequences were retrieved from the Lahey Clinic web site (<http://www.lahey.org/Studies>). (b) Amino acid sequence alignment of OXA-372 with selected CHDLs, in which the conserved motifs (black) and other relevant regions, such as the poorly conserved YGN motif (shaded in grey) and the PxxG motif (boxed) found in all CHDLs, are highlighted; the secondary structure elements of OXA-48 are indicated above the sequences.

Impatto della *bla*_{OXA-372} sul fenotipo di Cfr-FI-07: attività antimicrobica

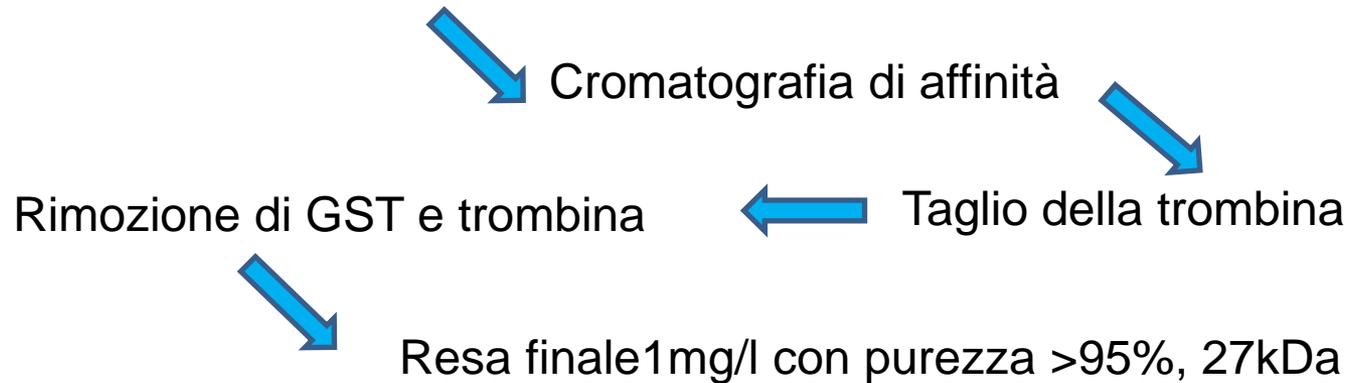
Table 2. Antimicrobial susceptibility of *C. freundii* Cfr-FI-07, *E. coli* DH5 α (pLBII-OXA-372) (producing the OXA-372 enzyme) and *E. coli* DH10B(pCfr-FI-07) to different β -lactams; susceptibilities of *E. coli* DH5 α carrying the empty vector (pBC-SK) and of *E. coli* DH10B are also shown for comparison

Antibiotic	MIC (mg/L)				
	Cfr-FI-07	DH5 α (pLBII-OXA-372)	DH5 α (pBC-SK)	DH10B(pCfr-FI-07)	DH10B
Penicillin G	>256	>256	64	>256	64
Ticarcillin	>256	256	4	>256	4
Ampicillin	>256	128	4	>256	4
Oxacillin	>256	>256	256	>256	256
Temocillin	>256	128	8	>256	8
Cefalotin	>256	8	8	8	4
Aztreonam	8	0.25	0.25	4	0.5
Imipenem	>32	2	0.125	2	0.25
Meropenem	16	0.06	≤ 0.015	0.25	0.03
Ertapenem	16	0.03	≤ 0.015	1	≤ 0.015
Cefotaxime	>32	0.06	0.06	0.25	0.06
Ceftazidime	>32	0.25	0.125	4	0.25
Cefepime	4	0.125	0.06	0.5	0.125
Ceftriaxone	>32	0.03	0.03	0.125	0.03

OXA-372: pGEX-2T clonaggio e purificazione



Strategia di diffusione con GST in DH5 α pGEX-2T



OXA-372, a novel carbapenem-hydrolysing class D β -lactamase from a *Citrobacter freundii* isolated from a hospital wastewater plant

Alberto Antonelli^{1,2}, Marco Maria D'Andrea¹, Guendalina Vaggelli³, Jean-Denis Docquier¹ and Gian Maria Rossolini^{1,3*}

¹Department of Medical Biotechnology, University of Siena, Siena, Italy; ²Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy; ³Clinical Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italy

Table 3. Kinetic parameters measured for the purified OXA-372 β -lactamase

Substrate	k_{cat} (s^{-1})			K_M (μM)			k_{cat}/K_M ($\mu M^{-1} \cdot s^{-1}$)		
	OXA-372	OXA-48 ^a	OXA-198 ^b	OXA-372	OXA-48 ^a	OXA-198 ^b	OXA-372	OXA-48 ^a	OXA-198 ^b
Penicillin G	40	446	15	110	79	14	2.8	5.6	1.1
Oxacillin	145	130	25	125	95	30	1.2	1.4	0.83
Ampicillin	85	955	37	85	395	216	1.0	2.4	0.17
Ticarcillin	110	45	— ^c	190	55	—	0.58	0.82	—
Temocillin	3	0.3	—	35	45	—	0.086	0.0066	—
Cefalotin	0.17	44	0.19	57	195	12	0.003	0.23	0.016
Cefotaxime	NH ^d	>9	NH	—	>900	—	—	0.01	—
Ceftriaxone	NH	—	—	—	—	—	—	—	—
Ceftazidime	NH	NH	NH	—	—	—	—	—	—
Cefepime	NH	>0.6	NH	—	>550	—	—	0.0011	—
Imipenem	5.8	4.8	0.1	26	13	0.15	0.22	0.37	0.67
Meropenem	0.13	0.07	0.01	0.7 ^e	11	0.006	0.52	0.0062	1.7
Ertapenem	0.49	0.13	—	0.25 ^e	100	—	0.7	0.0013	—
Aztreonam	NH	NH	NH	—	—	—	—	—	—

Data are the means of three independent measurements. Standard deviations were always within 10% of the mean values. Kinetic parameters for OXA-48 and OXA-198 are also shown for comparison.

^aKinetic parameters for all antibiotics, except benzylpenicillin (determined in this study) and ticarcillin and aztreonam (Poirel et al. 2004), are from Docquier *et al.* 2009

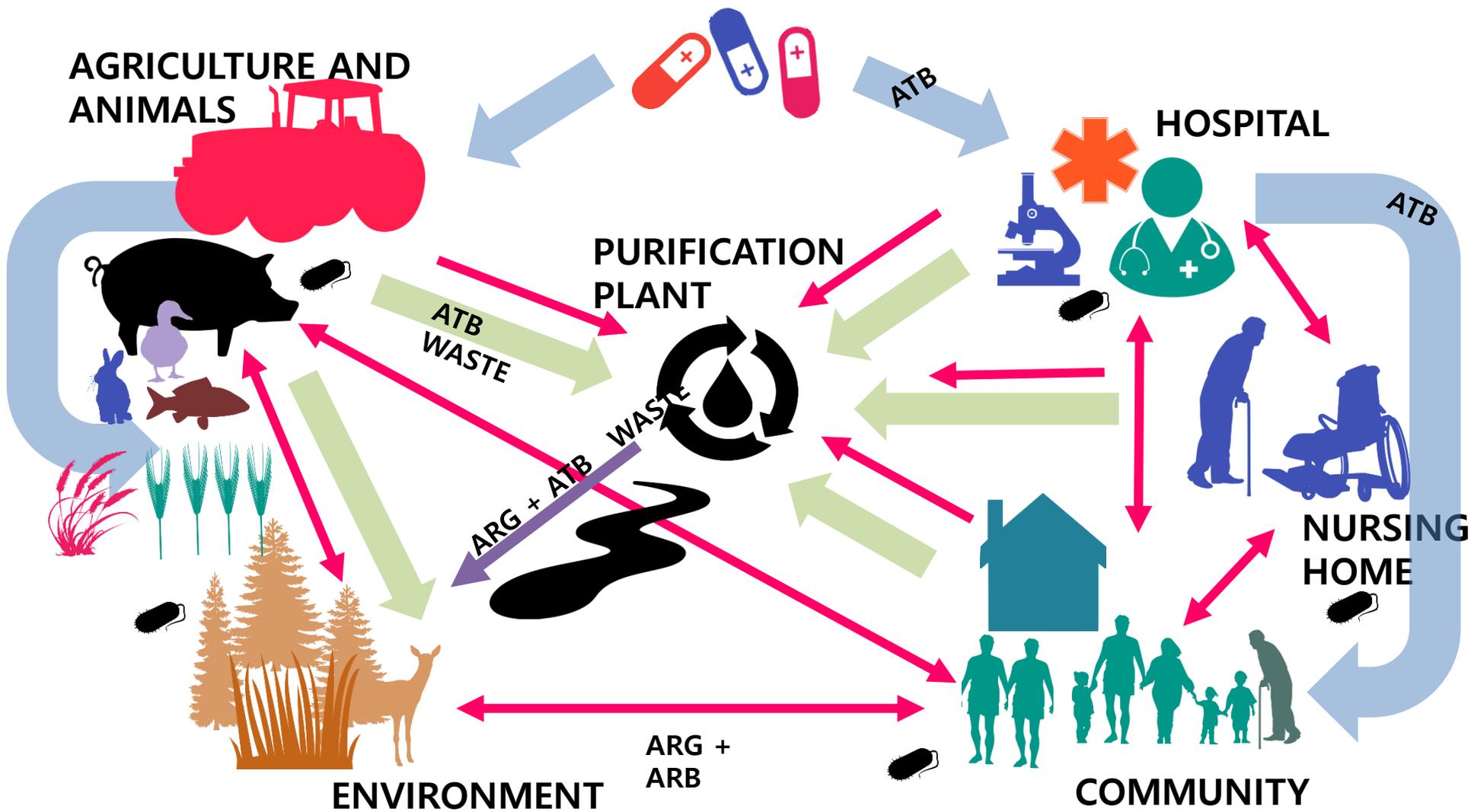
^bKinetic parameters for all antibiotics are from El Garch *et al.*

^c—, No data available.

^dNH, no hydrolysis could be detected with concentrations of enzyme up to 340 nM.

^eDetermined as K_i with benzylpenicillin as the substrate.

IMPACT to antimicrobial resistance (AMR)



“One Health Approach” WHO, 2015



**27000 vite
potrebbero
essere salvate
ogni anno**

What can EU/EAA countries do next?

Public health actions to tackle AMR have a positive impact on population health...

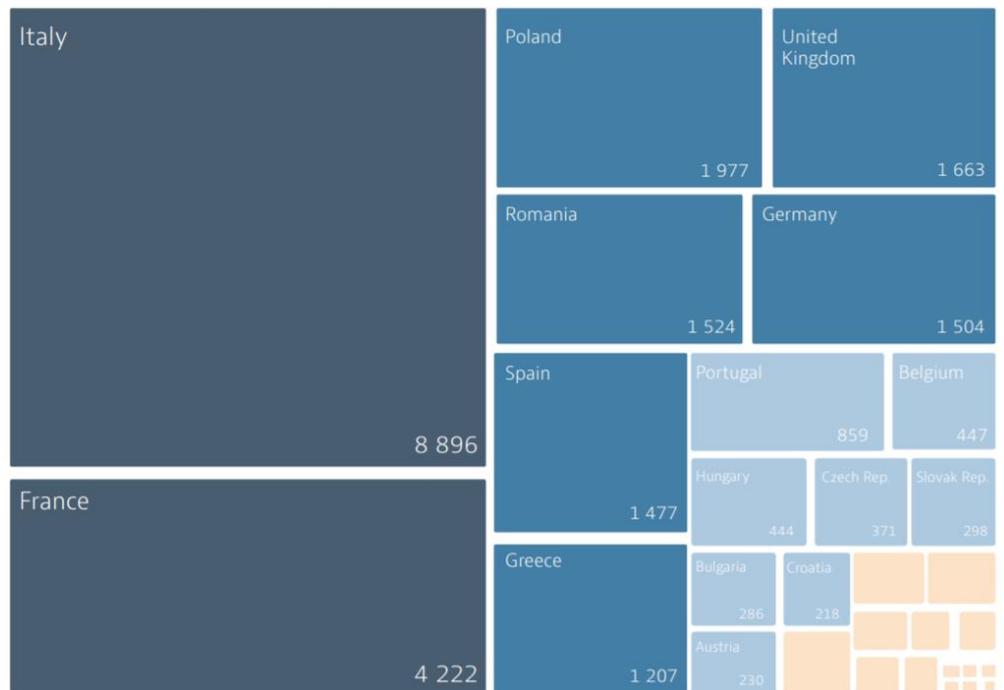
The OECD has identified interventions that, for their impact on population health and heavy costs avoided, could be defined as 'best buys' to tackle AMR. The set of policies assessed is aligned with the WHO Global Action Plan on AMR and encompasses:

- improving hygiene in health care facilities, including promotion of hand hygiene and better hospital hygiene (e.g. disinfection of surfaces and equipment in hospitals);
- stewardship programmes promoting more prudent use of antibiotics to end decades of over-prescription;
- use of rapid diagnostic tests in primary care to detect whether an infection is bacterial or viral;

- delayed prescriptions; and
- public awareness campaigns.

Simple measures, such as promoting hand hygiene and better hygiene in health care facilities more than halve the risk of death and decrease the health burden of AMR – measured in DALYs – by about 40%. Antibiotic stewardship programmes are similarly effective. Outside of hospitals, interventions designed to tackle AMR, such as the use of rapid diagnostic tests, delayed prescriptions and mass media campaigns would have a more limited health impact but remain important policies to address a multifaceted and complex phenomenon.

Figure 9. A mixed intervention package would save about 27 000 lives per year across EU/EEA countries



Note: The countries shown in orange are the following, by descending order: **The Netherlands** (193), **Ireland** (170), **Sweden** (149), **Denmark** (102), **Lithuania** (79), **Slovenia** (77), **Finland** (74), **Cyprus** (63), **Norway** (54), **Latvia** (33), **Malta** (25), **Luxembourg** (15), **Estonia** (14) and **Iceland** (1).

Source: OECD (2018), Stemming the Superbug Tide: Just a Few Dollars More. Available at [oecd/amr-2018](https://www.oecd.org/health/amr/).

Conclusioni

- **Le infezioni da patogeni produttori di carbapenemasi rappresentano un problema di sanità pubblica crescente**
- **Screening per CRE: sempre più importante anche durante la pandemia di COVID-19**

Azioni da intraprendere

- **Necessità di promuovere nuovi studi di sorveglianza nazionale per la caratterizzazione dei meccanismi di resistenza emergenti in patogeni Gram-negativi**
- **Implementazione di misure integrate tra microbiologi clinici, ambientali e veterinari per limitare la diffusione di patogeni multiresistenti produttori di carbapenemasi applicando un approccio One Health**
- **Utilizzo combinato di metodiche molecolari, fenotipiche e genotipiche per la caratterizzazione dei meccanismi di resistenza e della clonalità degli isolati multiresistenti**



Grazie per l'attenzione

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