Mechanisms of resistance to antibiotics

Novembre 2019

European Antibiotic Awareness Day (EAAD) will be marked on Monday 18 November 2019, in partnership with WHO's World Antibiotic Awareness Week (18 – 24 November 2019).

MEETING MULTIDISCIPLINARE
SUI MICRORGANISMI MULTIDRUG RESISTANT
E LE RELATIVE PROBLEMATICHE MICROBIOLOGICHE,
EPIDEMIOLOGICHE, DIAGNOSTICHE E CLINICHE

CEUB BERTINORO- 27 NOVEMBRE 2019

10.30 - 13.00 Welcome coffee

10.30 - 13.00 **I SESSIONE** — Microbiologia dei germi multiresistenti
Chairpersons: Claudio Farina, Vittorio Sambri
10.30 Meccanismi di resistenza ai farmaci antibatterici - Stefania Stefani
11.00 Storia e prospettiva della resistenza ai farmaci antibatterici - Paolo Fazii
11.30 Metodi microbiologici per la identificazione dei batteri multi resistenti - Edoardo Carretto
12.00 Metodologie molecolari per la identificazione dei batteri multi resistenti - Vittorio Sambri
12.30 Miceti e protozoi: AMR? Claudio Farina – Stefania Varani
13.10 Light Lunch

14.00 - 16.30 **II SESSIONE** — Epidemiologia, sorveglianza e rilevanza clinica dei germi MDR
Chairpersons: Francesco Cristini, Vanni Agnoletti
14.00 Epidemiologia dei germi MDR: colonizzazione ed infezione - M. Luisa Moro
14.30 Sorveglianza e controllo dei germi MDR in Romagna: il nucleo SPIAR - Carlo Biagetti
15.00 Le infezioni da germi MDR nel paziente critico - Maurizio Fusari
15.30 L'impatto della AMR nel paziente oncoematologico - Francesco Lanza
16.00 Le infezioni da germi MDR in chirurgia - Giorgio Ercolani
16.30 Conclusioni - Vittorio Sambri
Verifica dell'apprendimento

Provider & Segreteria Organizzativa: Proeventi — www.proeventi.it - info@proeventi.it

Informazioni Generali
L'evento è stato accreditato al Ministero della Salute con il n. 2773-277638 ed ha ottenuto 5 crediti ECM per n. 80 partecipanti:
Medici di Area Interdisciplinare, Biologi, Tecnici Sanitari di Laboratorio Biomedico
Obiettivo formativo: Documentazione clinica, Percorsi clinico-assistenziali diagnostici e riabilitativi, profili di assistenza - profili di cura
Per l'ottenimento dei crediti formativi è necessario:
Aver preso parte all'intero evento formativo - Aver compilato la documentazione in ogni sua parte - Aver superato la prova di apprendimento
L'iscrizione è gratuita, la scheda di adesione dovrà pervenire alla Segreteria Organizzativa oppure on line sul sito: www.proeventi.it entro il 25 Novembre 2019

si ringrazia per il Contributo Educazionale Non Condizionante — ARROW DIAGNOSTICS

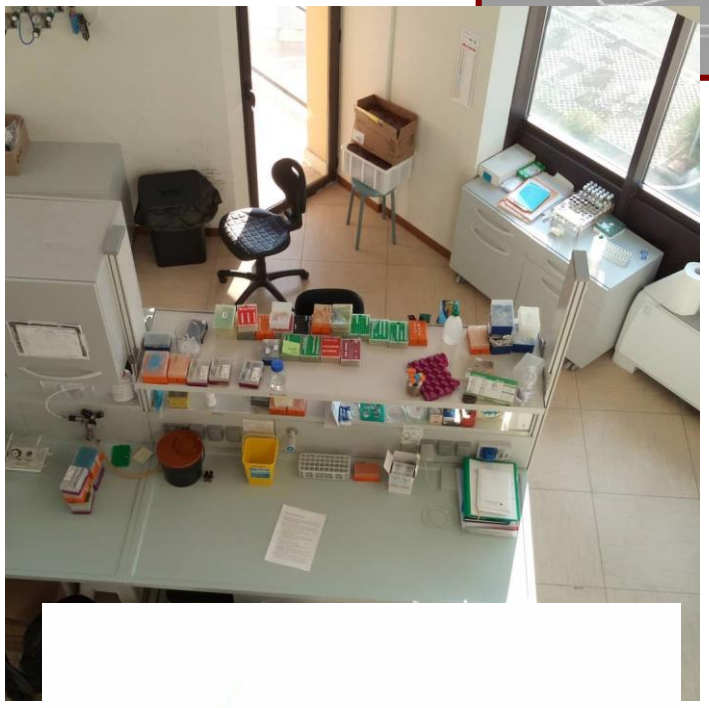
Stefania Stefani
Dipartimento di Scienze
Biomediche e Biotecnologiche
Policlinico – UNICT

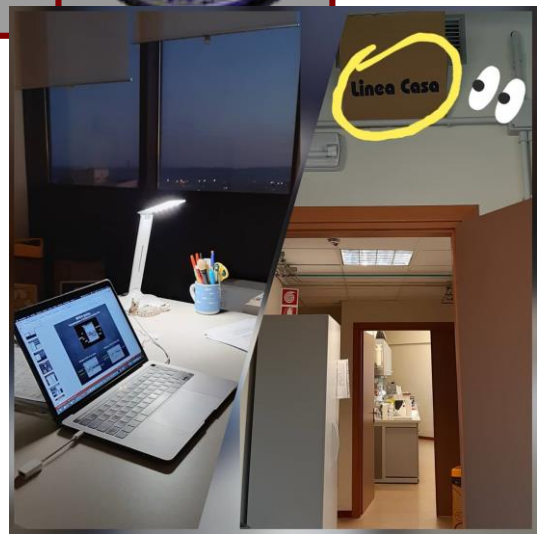
Rappresentante SIM presso Ministero salute per PNCAR
Gruppo di coordinamento progetto
Sepsi - REGIONE SICILIA
Responsabile Sorveglianze PNCAR SICILIA

Dichiarazione su potenziali conflitti di interesse
Consultant and board of speakers:
Pfizer Italy and Europe, MSD, Angelini, Nordic Pharma, Accelerate,Correvio Biomerieux, Cepheid
Research grants
DMG, Biotest, Zambon Italia, Basilea Pharma, Nordic Pharma, IHMA – Europe and USA, Liofilchem

MMAR

Microbiologia Medica Molecolare e Antibiotico Resistenza





World Health Organization

WHO PRIORITY PATHOGENS LIST FOR R&D OF NEW ANTIBIOTICS

Priority 1: CRITICAL#

Acinetobacter baumannii, carbapenem-resistant

Pseudomonas aeruginosa, carbapenem-resistant

Enterobacteriaceae*, carbapenem-resistant, 3rd generation cephalosporin-resistant

Priority 2: HIGH

Enterococcus faecium, vancomycin-resistant

Staphylococcus aureus, methicillin-resistant, vancomycin intermediate and resistant

Helicobacter pylori, clarithromycin-resistant

Best available evidence

1. **All-cause mortality**
2. **Healthcare and community burden**
3. **Prevalence of resistance**
4. **10-year trend of resistance**
5. **Transmissibility**
6. **Preventability in hospital and community settings**
7. **Treatability and current pipeline**

Ginevra, 25-27 Gennaio 2017

World Health Organization

11

GLOBAL PRIORITY LIST OF ANTIBIOTIC-RESISTANT BACTERIA TO GUIDE RESEARCH, DISCOVERY, AND DEVELOPMENT OF NEW ANTIBIOTICS

World Health Organization requested by **Member States** to develop **a global PP List of antibiotic-resistant bacteria**

Previous PPLs, issued by

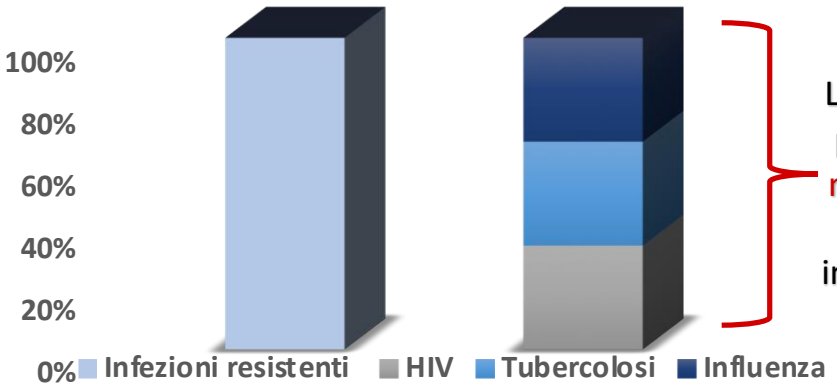
- CDC, Antibiotic Resistance Threats in the U. States, 2013
- Public Health Agency of Canada (PLoS One. 2015;10:1-11)

focused on **national public health priorities**
to increase scientific, political and public awareness
without including specific R&D criteria

Resistenza agli antibiotici: i numeri in Europa

671.689 infezioni resistenti alla cura con antibiotici riscontrate nel 2015 (dato mediano)

33.000 persone morte per infezioni resistenti

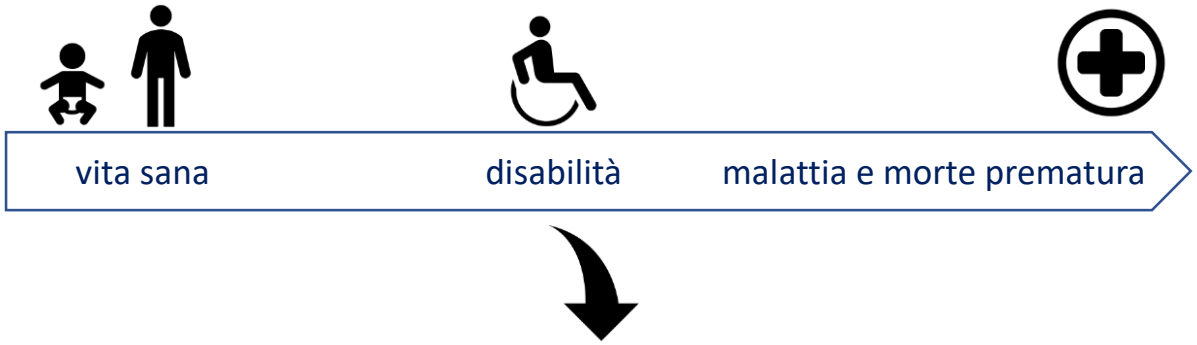


Le infezioni resistenti provocano lo stesso numero di morti e di DALY provocati da influenza, tubercolosi e HIV/AIDS messi insieme

I Paesi europei con un *burden* maggiore dovuto alle infezioni da batteri resistenti sono l'Italia e la Grecia

DALY o disabilità attribuibile

misura la gravità globale di una malattia espressa come il numero di anni persi a causa della malattia per disabilità o per morte prematura
1 DALY equivale ad 1 anno di vita persa



874.541 DALY solo nel 2015 da infezioni causate da batteri resistenti agli antibiotici

La proporzione di DALY dovuta a tutti i batteri resistenti ai carbapenemi, antibiotici di ultima generazione, è del 28%

La resistenza agli antibiotici è una minaccia sempre più grave per la salute pubblica globale e richiede l'intervento di tutti i settori governativi e della società in generale

UTILIZZA GLI ANTIBIOTICI IN MODO CONSAPEVOLE, FAI LA TUA PARTE

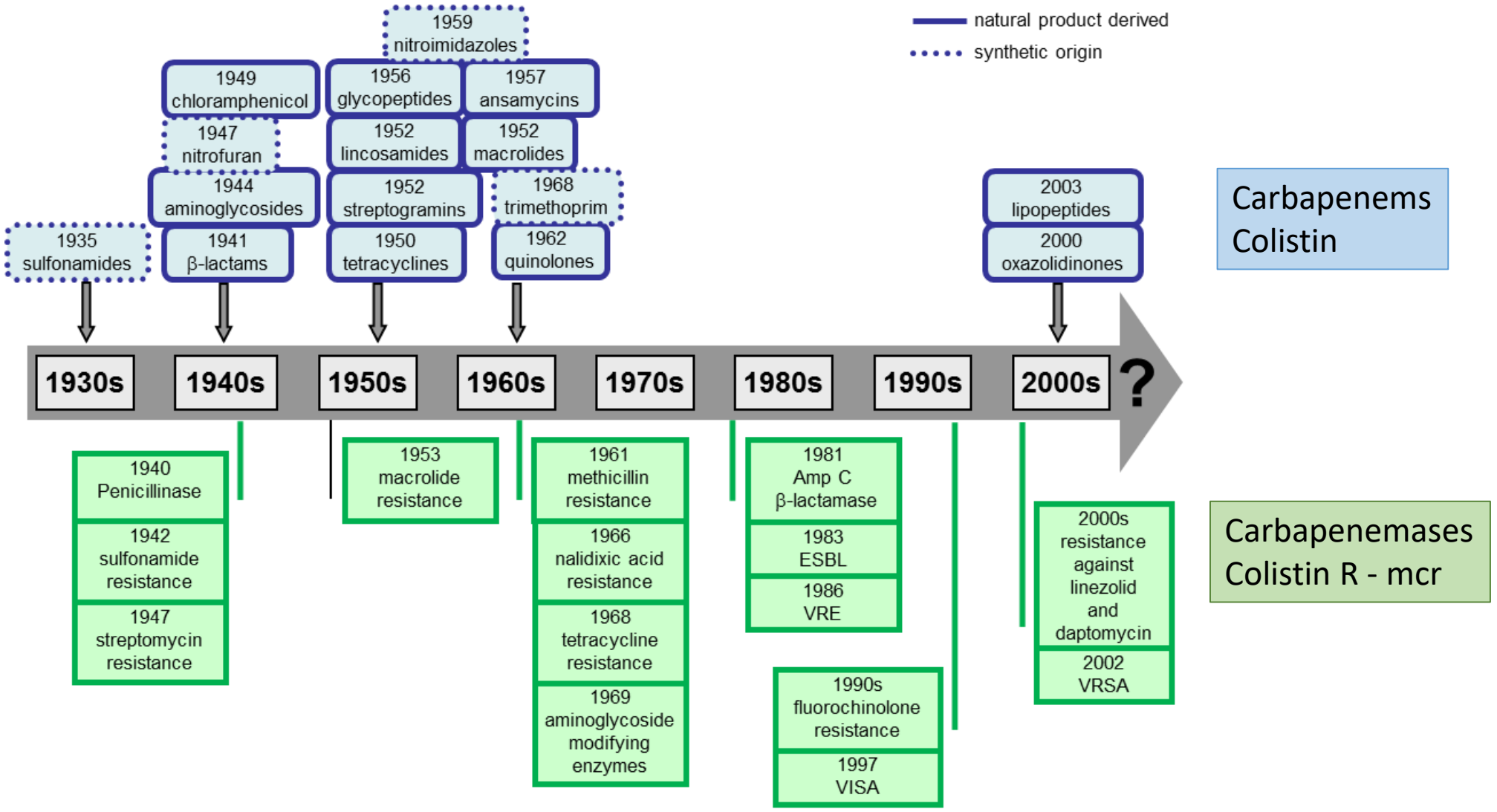
Resistance mechanisms - FADOI - 2019

Bibliografia. Cassini A et al. Lancet Infect Dis. 2018 Nov 5. pii: S1473-3099(18)30605-4.

How did we get to this point?

Just a flash back……

Introduction of new antibiotic classes

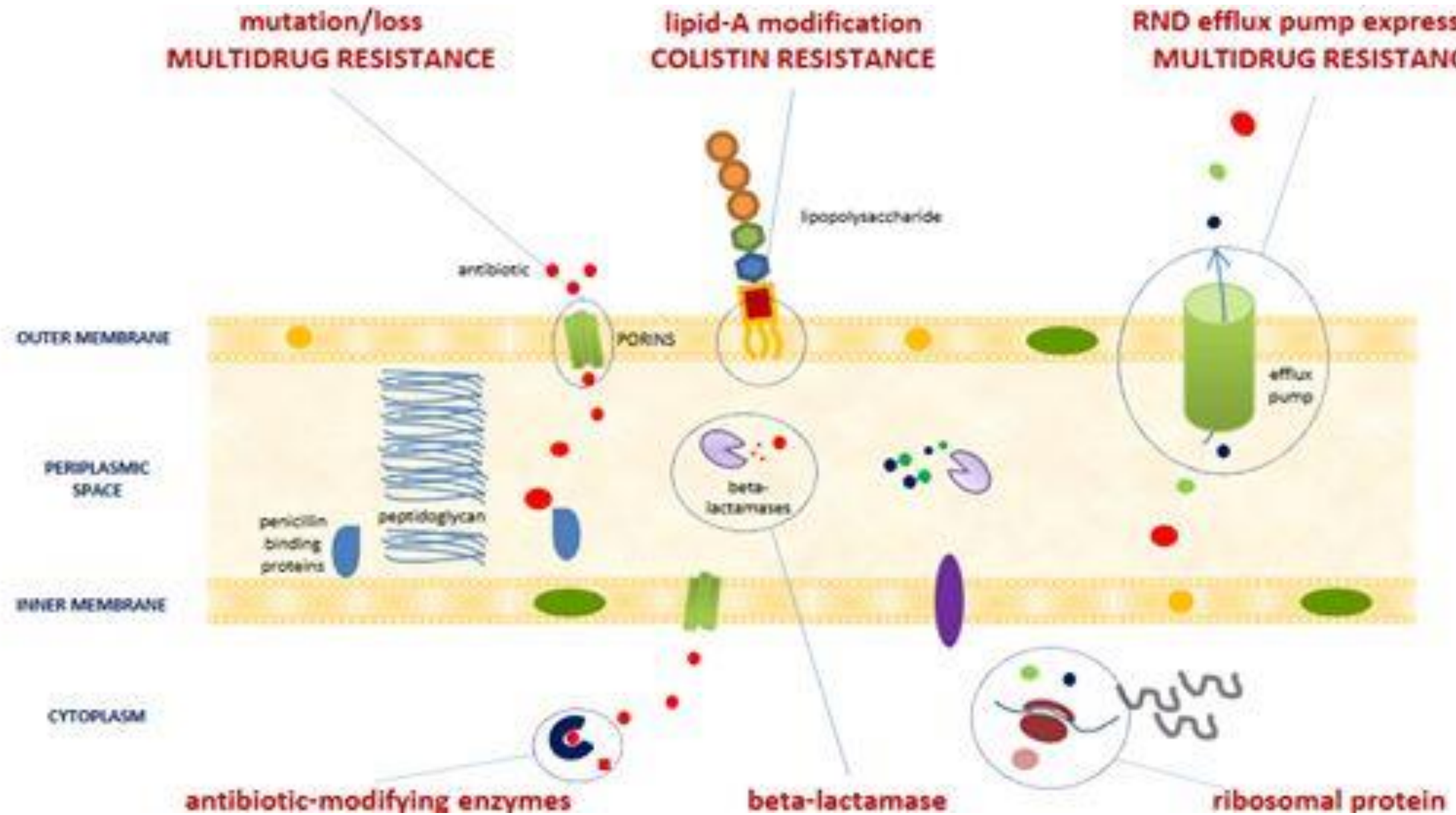


Development of bacterial resistance

Mechanisms of resistance

- **Altered permeability**
- **Altered target site**
- **Replacement of a sensitive pathway**
- **Inactivation of the antimicrobial agent**

- **A single microrganism can have multiple mechanisms of resistance**


mutation/loss
MULTIDRUG RESISTANCE
lipid-A modification
COLISTIN RESISTANCE
RND efflux pump expression
MULTIDRUG RESISTANCE
lipopolysaccharide
antibiotic
OUTER MEMBRANE
PORINS
efflux pump
PERIPLASMIC SPACE
beta-lactamases
penicillin binding proteins
peptidoglycan
INNER MEMBRANE
CYTOPLASM
antibiotic-modifying enzymes
beta-lactamase
ribosomal protein

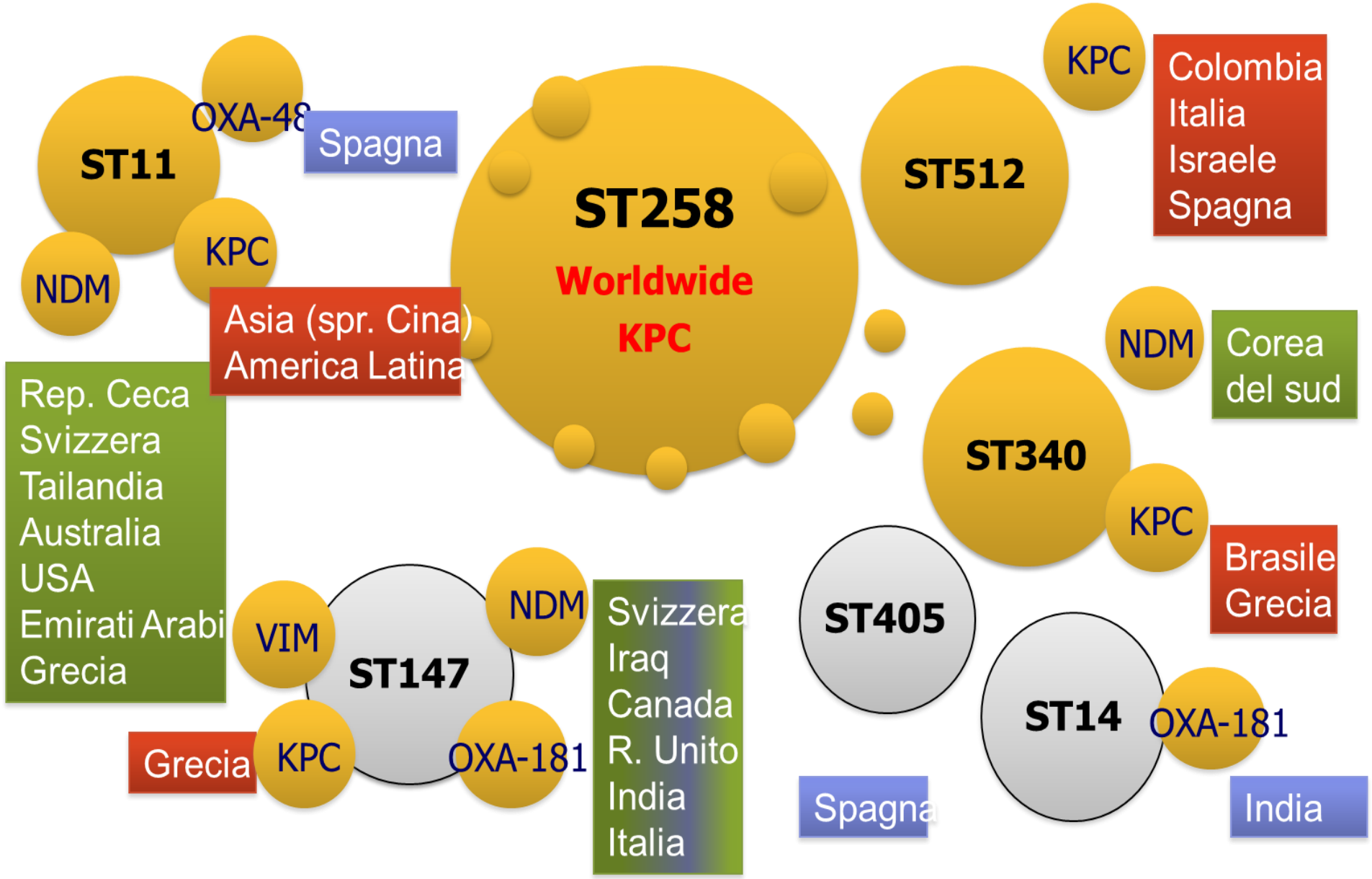
Mechanisms of transmission

Highly transmissible clone

LGT

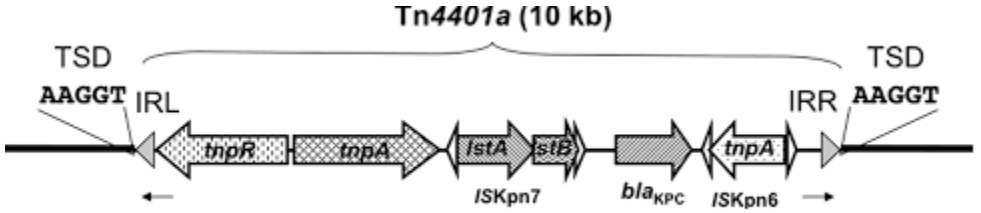
Explained with an example

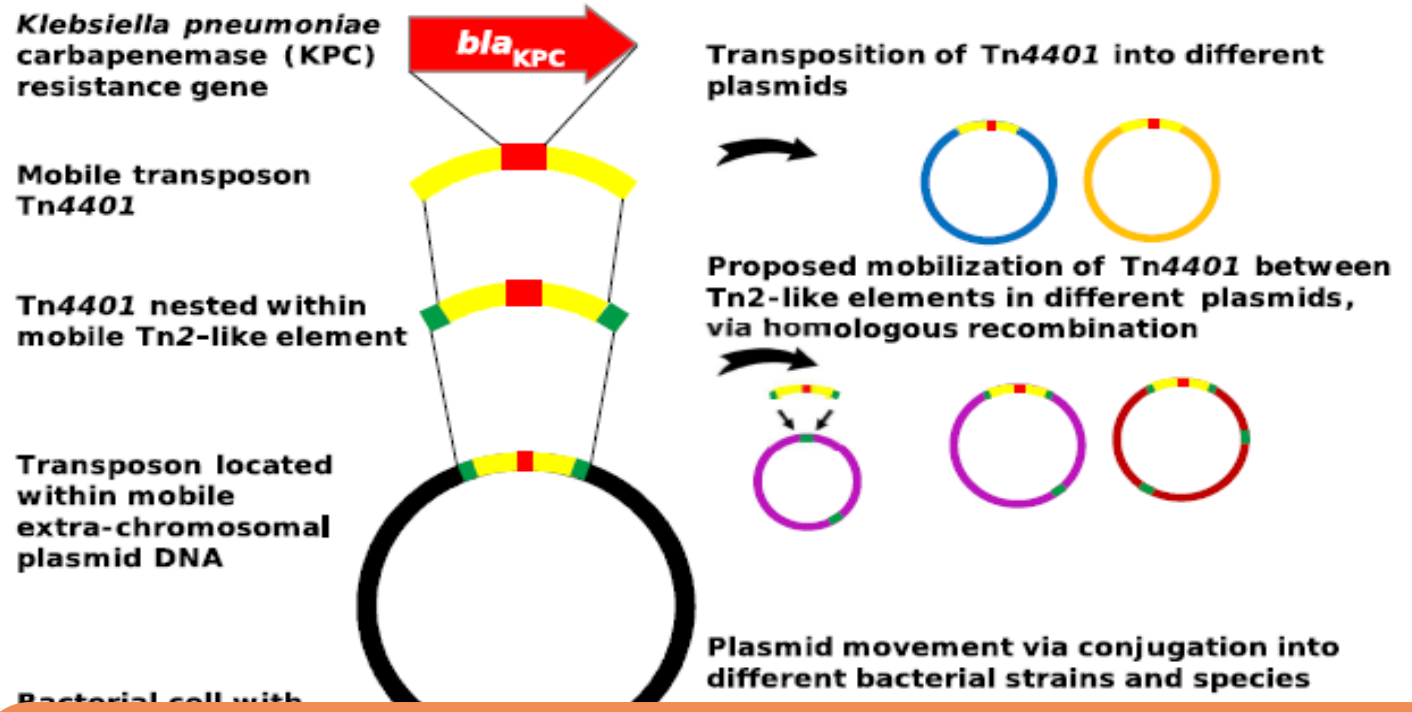
CLONI EPIDEMICI AD ALTO RICHIO (HiR)



How are *bla*$_{KPC}$ genes transferred?

KPC (*bla*$_{KPC-2\rightarrow24}$)





Il trasferimento di *bla*$_{KPC}$ tra i plasmidi è probabilmente rafforzato dalla ricombinazione omologa tra elementi Tn2, facilitando il movimento di Tn4401 da un plasmide ad un altro e la diffusione di questo anche ad altre specie batteriche.

Pitout J.D. et al. Antimicrob Agents Chemother 2015

Sheppard et al 2016 Antimicrob Agents Chemother. 60:3767–3778. doi:10.1128/AAC.00464-16

One of the more flexible mechanism of resistance

Beta-lactamase

***Table 2**. Overview of β-lactamases.*

Swedish ESBL-Terminology	Common name	Ambler class	Examples	Resistance phenotype[A] Pc./1GC	3GC	Carba-penem	Diagnostic inhibitor	Clinical inhibitor
	Penicillinase	A	Staphylococcal enzymes	Pc. only			-	CLA, TAZ, AVI, VAB
-	Oxacillinase	D	OXA-1	Oxacillin			-	AVI
	Broad-spectrum penicillinase	A	TEM-1[B], SHV-1[B]	x			-	CLA, TAZ, AVI, VAB
$ESBL_A$	Extended-spectrum β-lactamase	A	**CTX-M**, TEM[B], SHV[B]	x	x		CLA	CLA, TAZ, AVI, VAB
$ESBL_{AM}$	Plasmid AmpC cephalosporinase	C	pAmpC CIT, CMY, DHA	x	x		Cloxacillin	AVI, VAB
-	Chromosomal AmpC cephalosporinase	C	cAmpC	x	x		Cloxacillin	AVI, VAB
$ESBL_{CARBA}$	Serine carbapenemase	A	KPC, IMI, SME	x	x	x	Boronic acid	AVI, VAB
	Metallo-β-lactamase	B	**NDM**, IMP, VIM	x	x	x	EDTA	-
	Carbapenem-hydrolysing class D enzyme (Oxacillinase-derived)	D	OXA-48	x		x	-	AVI

[A]"x" means that the agent is a substrate for the enzyme. The level of resistance varies, some isolates appear susceptible despite presence of the enzyme.

[B]There are hundreds of different TEM- and SHV- enzymes. Some can hydrolyse 3GC ($ESBL_A$) and some (*e.g.* TEM-1 and SHV-1) only hydrolyse 1GC and are not classified as ESBLs.

1GC: 1st generation cephalosporin; 3GC: 3rd generation cephalosporin; Pc.: penicillin; CLA: clavulanic acid; TAZ: tazobactam; AVI: avibactam, VAB: vaborbactam

Table based on information in several reviews [15, 22-26].

Overview of antibiotics with effect on Gram-negative bacteria in clinical use

Legenda:
+ R uncommon <20%
(+) used in combination
+/- R common
(-) usually R

Antibiotic class Beta-lactams	Examples	Activity against ESBL PE	Activity against NDM	Target	Mode of resistance
Penicillins	Ampicillin	-	-		
3G –C	Cefotaxime Ceftriaxone Ceftazidime	(-) (-) (-)	- - -		
Carbapenems	Ertapenem Imipenem Meropenem	+ + +	- +/- +/-	Cell membrane Peptidoglycan synthesis	Hydrolisis Altered targets Porin loss Efflux
Monobactams	Aztreonam	-	+/-		
Beta-lactams +BLI	Piperacillin/tazobactam Ceftazidime/avibactam Ceftolozano/tazobactam Meropenem/vaborbactam	+ + + +	- - - +/-		

Modified by Morara et al Ann Rev Genet 2010

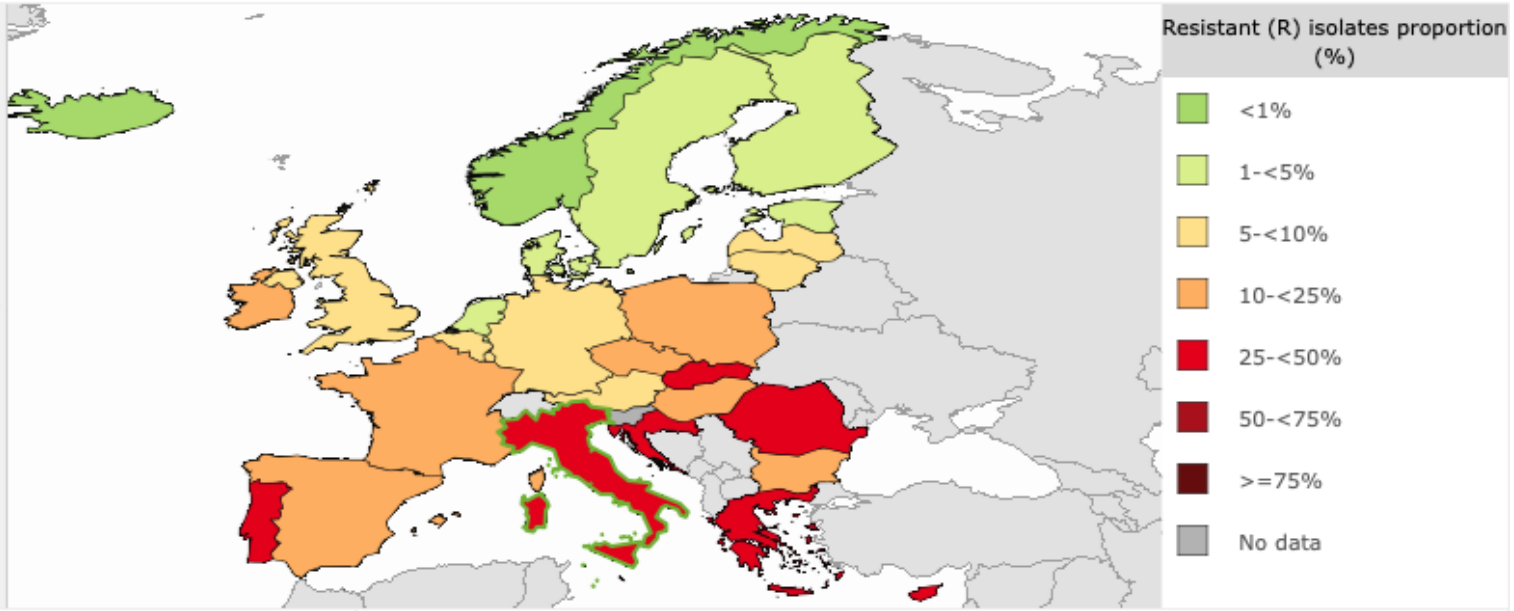
Antibiotic class others	Examples	Activity against ESBL PE	Activity against NDM	Target	Mode of resistance
Aminoglycosides	Amikacin Gentamicin Tobramycin	+ +/- +/-	- - -	Traslation	Phsphrylation, acetylation, nucleotydlation, efflux, altered target
Fluoroquinolones	Ciprofloxacin Levofloxacin	+/- +/-	(-) (-)	DNA replication	Acetylation efflux, altered target
Sulphonamides	Trimethoprim/sulfa	+/-	(-)	Nucleotide symthesis, C metabolism	Efflux, altered target
Cationic peptides	Colistin	+	+	Cell membrane, lipid A	Aletred target, efflux
Rifamycin	Rifampicin	(+)	(+)	Transcription	Altered target, efflux
Glycilcycline	Tigecycline Eravacycline	+ +	(-) (-)	Translation	Monooxygenation, efflux, altered target
Phosponic acid	Fosfomycin	+	(-)	Cell membrane, peptidoglycan synthesis	Decreased permeability, enzymatic modification

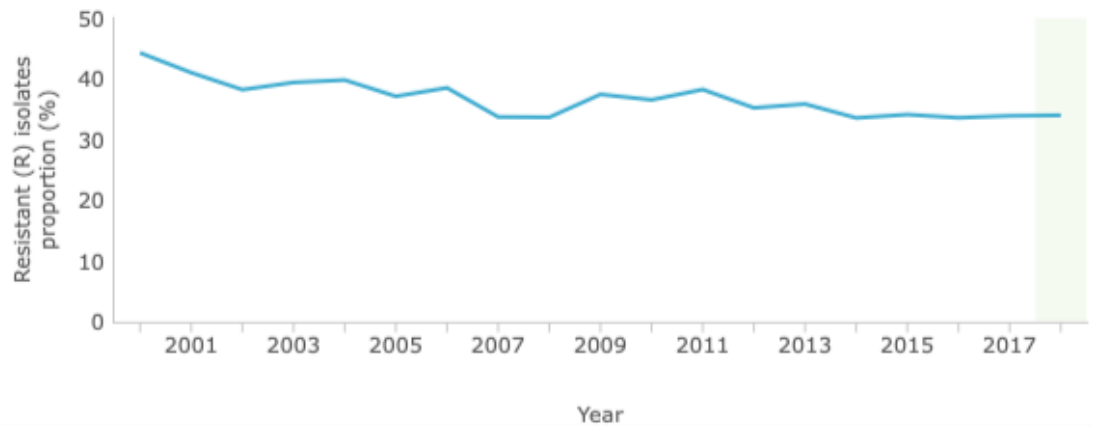
Modified by Morara et al Ann Rev Genet 2010

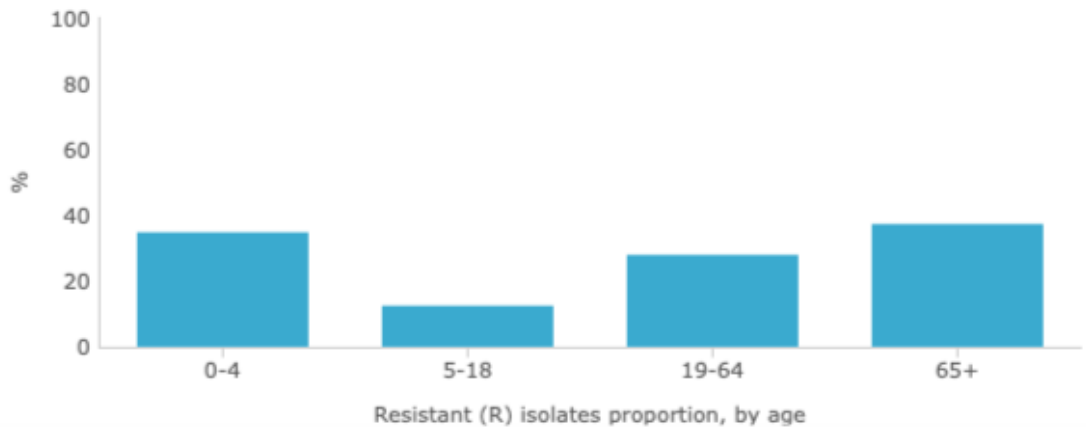
Surveillance Atlas of Infectious Diseases

Antimicrobial resistance | Staphylococcus aureus | Meticillin (MRSA) | Resistant (R) isolates proportion | 2018

Region	Resistant (R) isolates proportion (%)
Czech Republic	13.6
Denmark	1.7
Estonia	3.3
Finland	2.0
France	12.1
Germany	7.6
Greece	36.4
Hungary	23.1
Iceland	0.0
Ireland	12.4
Italy	34.0
Latvia	5.7







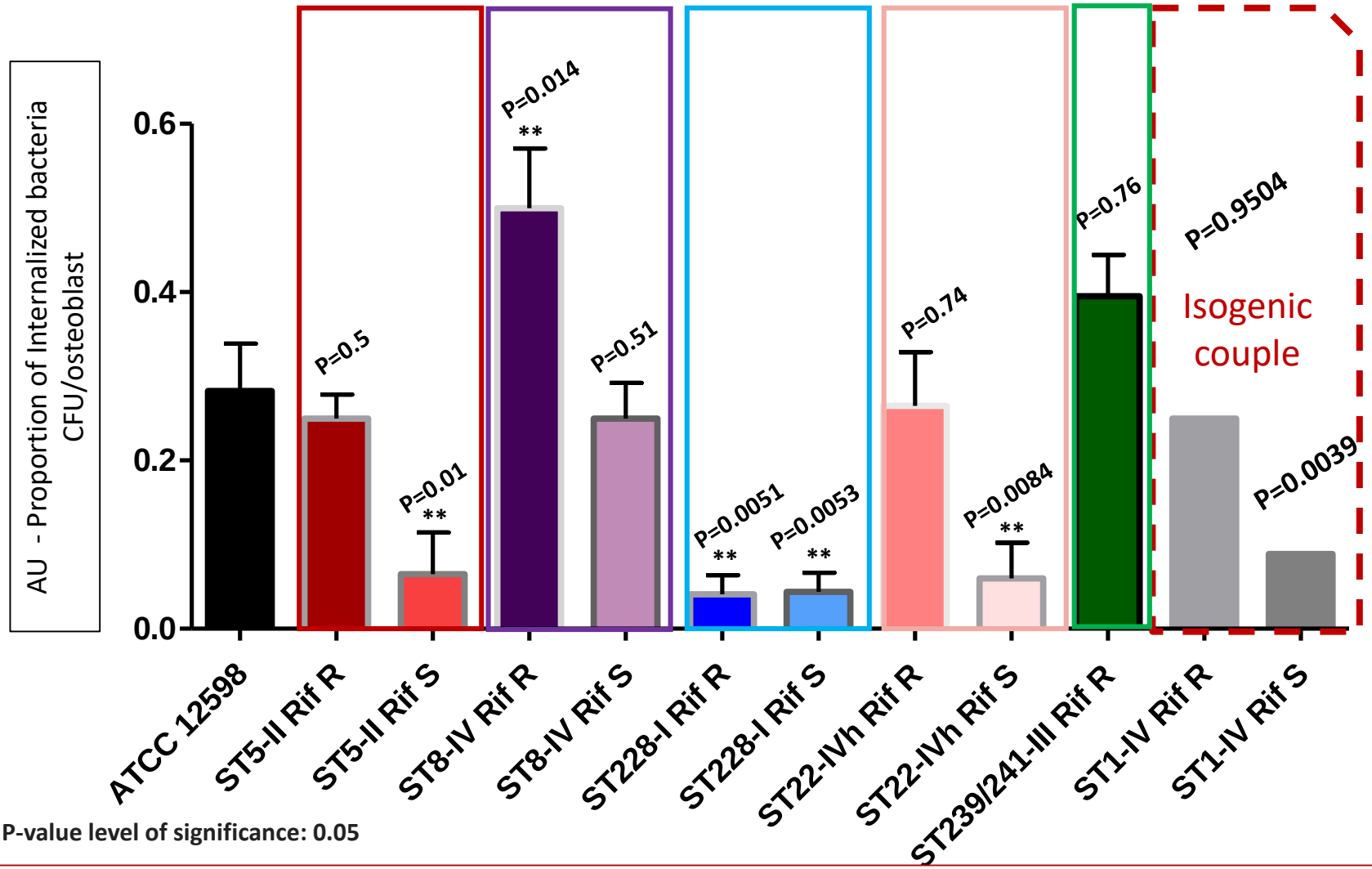
Italy

Staphylococcus aureus

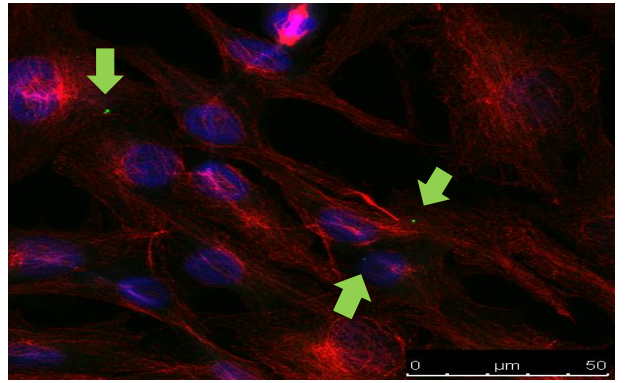
- One of the most problematic human pathogens[1]
- MSSA and MRSA rank as the second most common causes of hospital-associated bloodstream infections and are associated with increased mortality and longer hospital stay[2]
- Epidemiological settings: HA, CA, LA
- The S.aureus species population has been classified by molecular methods and belongs to more than ten dominant human lineages or clonal complexes (CC), with abundant minor lineages with different geographycal distribution[3]
- S.aureus is a persistent resident of the human nose in 20% of healthy population and is an intermittent colonizer in another 80%[4]
- S.aureus pathogenicity is very complex, and adaptation is the key point of its success
- MRSA infections: increased mortality rate

1) Levy FD – NEJM 1998; 339: 520; 2) Espisito S et al – JGAR 2013; 1: 71; 3) Chambers HF et al – Nat Rev Micorbiol 2009; 7: 629;
4) Cokfield JD et al – J med Microbiol 2007; 56: 814

PROPORTION OF INTERNALIZED BACTERIA USING EX-VIVO ASSAYS AND OBSERVATION BY CONFOCAL MICROSCOPY



P-value level of significance: 0.05



LEICA TCS SP3 CONFOCAL

- **Green: *S.aureus* cells**
  Vancomycin-Bodipy FL® (bacterial CW)
- **Red: MG63 cytoskeleton**
  Rodamin/palloidin (α-tubulin)
- **Blue: MG63 nucleus**
  DAPI (DNA)

Comparison of the proportion of internalized bacteria in MG63 osteoblastic cells for ATCC12598 invasive isolate and for selected MRSA clinical isolates. Data are represented as the means and standard deviations of three replicate cultures from all ex vivo assays. All RIF-R strains showed a percentage of SCVs ranging from 2-10% (ATCC12598: 0.28 CFU/osteoblast).

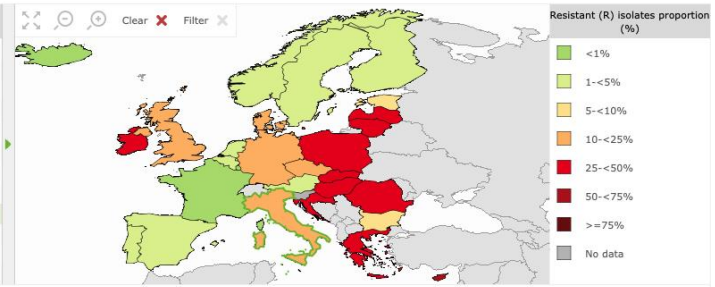
Bongiorno D et al. ECCMID 2018 P1256

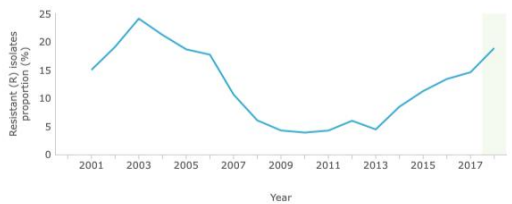
Enterococci

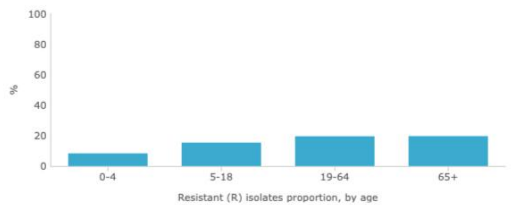
Surveillance Atlas of Infectious Diseases

Antimicrobial resistance | Enterococcus faecium | Vancomycin | Resistant (R) isolates proportion | 2018

Region	Resistant (R) isolates proportion (%)
Estonia	6.3
Finland	1.7
France	0.6
Germany	23.8
Greece	28.1
Hungary	39.5
Iceland	0.0
Ireland	40.2
Italy	18.9
Latvia	35.4
Lithuania	31.3
Luxembourg	0.0





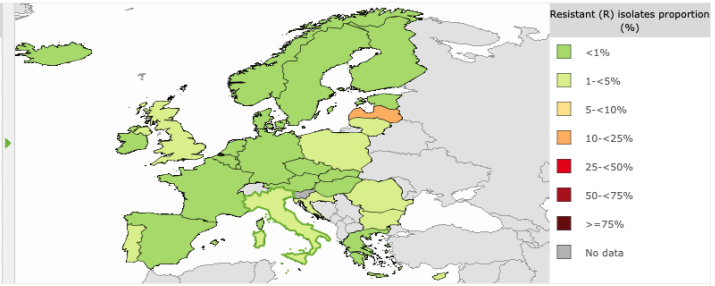


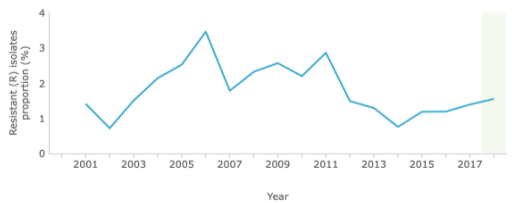
Italy

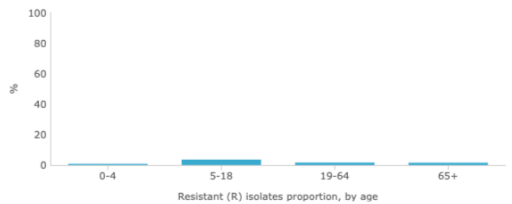
Surveillance Atlas of Infectious Diseases

Antimicrobial resistance | Enterococcus faecalis | Vancomycin | Resistant (R) isolates proportion | 2018

Region	Resistant (R) isolates proportion (%)
Austria	0.4
Belgium	0.3
Bulgaria	1.3
Croatia	2.1
Cyprus	1.1
Czech Republic	0.5
Denmark	0.2
Estonia	0.0
Finland	0.0
France	0.1
Germany	0.1
Greece	0.9





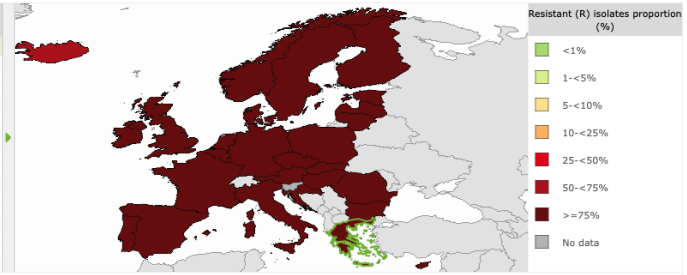


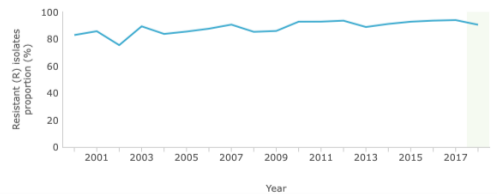
Italy

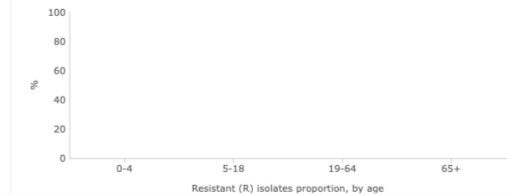
Surveillance Atlas of Infectious Diseases

Antimicrobial resistance | Enterococcus faecium | Aminopenicillins | Resistant (R) isolates proportion | 2018

Region	Resistant (R) isolates proportion (%)
Greece	90.5
Hungary	96.4
Iceland	62.5
Ireland	95.2
Italy	86.7
Latvia	94.1
Lithuania	89.9
Luxembourg	86.2
Malta	93.3
Netherlands	88.3
Norway	75.3
Poland	96.1





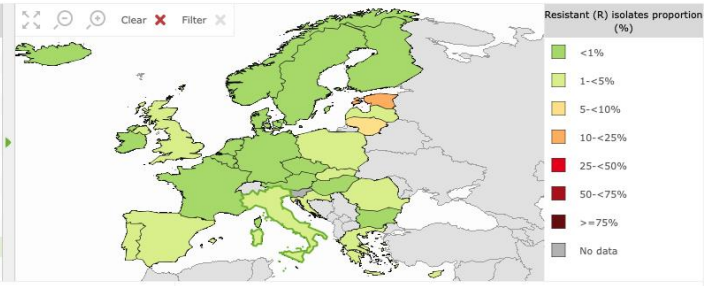


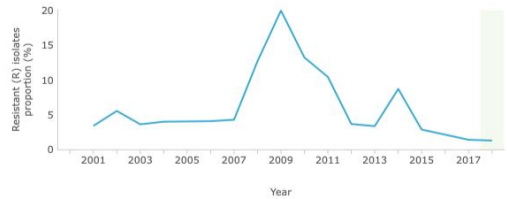
Greece

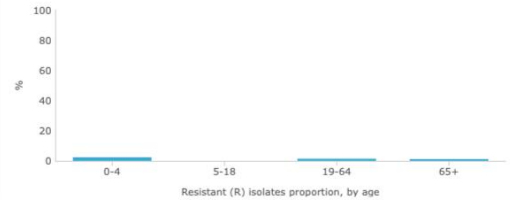
Surveillance Atlas of Infectious Diseases

Antimicrobial resistance | Enterococcus faecalis | Aminopenicillins | Resistant (R) isolates proportion | 2018

Region	Resistant (R) isolates proportion (%)
Czech Republic	0.7
Denmark	0.5
Estonia	10.2
Finland	0.0
France	0.3
Germany	0.2
Greece	1.0
Hungary	0.5
Iceland	0.0
Ireland	0.9
Italy	1.3
Latvia	2.8







Italy

Enterococci: something is happening in PBPs binding.....

Etest

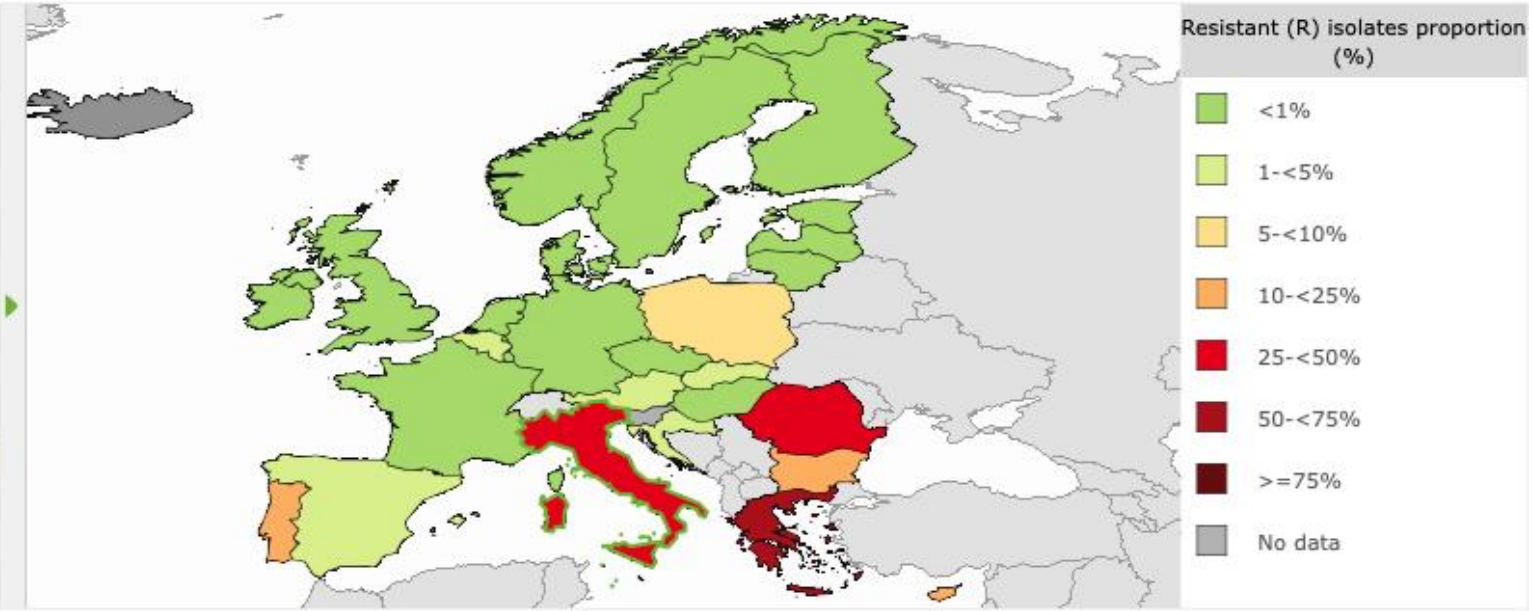
code	P	TZP	AMP	SAM	AML	AMC	IMP	CAZ	CTX	FEP	BPR
ATCC29212 E.fs	8	4	1	0.5	0.25	0.5	2	64	4	16	0.25
ATCC51299 E.fs vanB	2	2 (..*)	0.5	1	0.5	1	4	64	4	32	0.5
JH2-2 E.fs	4	4 (>256*)	1	0.5	0.5	0.5	2	32	2	16	0.25 (<32*)
6109525 E.fs	>32	12	2	2	0.5	1	4	>256	>32	>256	>32
6189258 E.fs	2	4	1	1	0.5	0.5	4	>256	>32	125	1
6108956 E.fs	1	4	2	2	1	1	4	>256	>32	32	0.25
6058833 E.fs	0.5	4	1	2	1	1	4	>256	>32	32	2
								ceftazidime	ceftriaxone	cefepime	Ceftobibrole
								III g	III g	IV g	V g

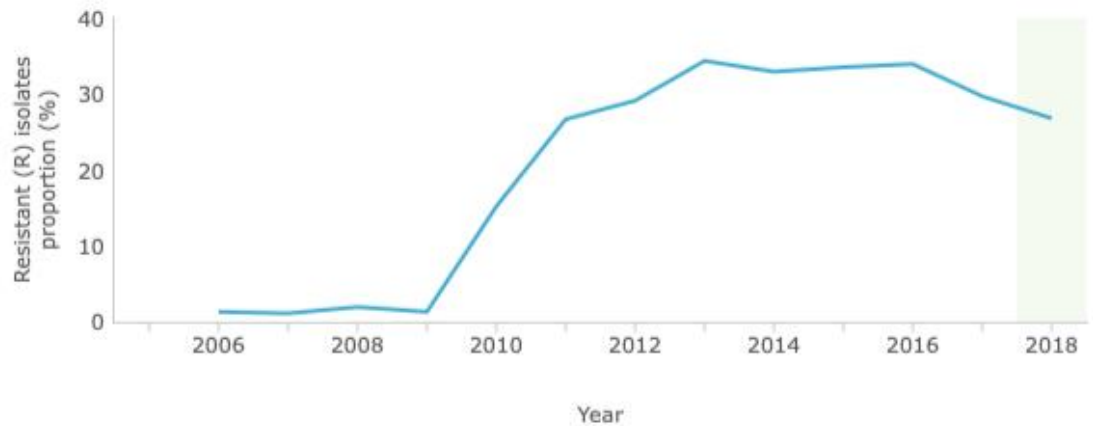
The MDR Gram negative storm

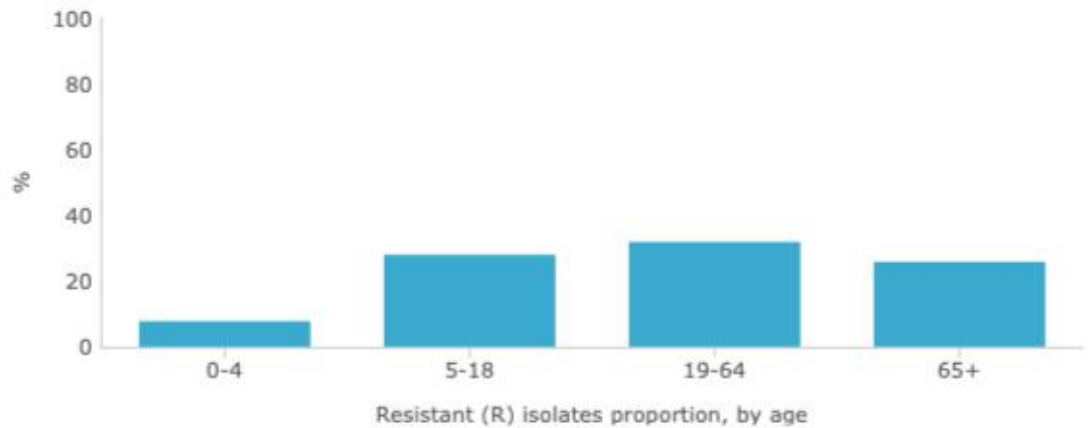
Surveillance Atlas of Infectious Diseases

Antimicrobial resistance | Klebsiella pneumoniae | Carbapenems | Resistant (R) isolates proportion | 2018

Region	Resistant (R) isolates proportion (%)
Estonia	0.6
Finland	0.6
France	0.5
Germany	0.4
Greece	63.9
Hungary	0.2
Iceland	-
Ireland	0.6
Italy	26.8
Latvia	0.5
Lithuania	0.3
Luxembourg	0.0





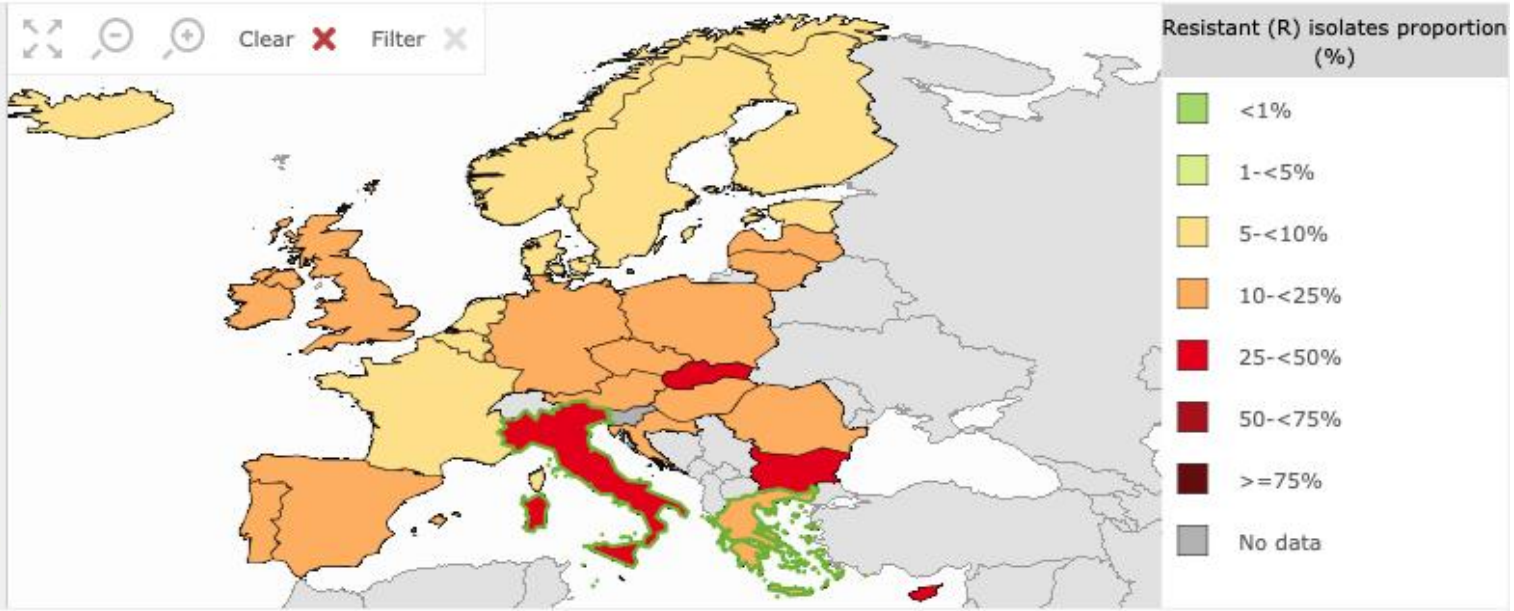


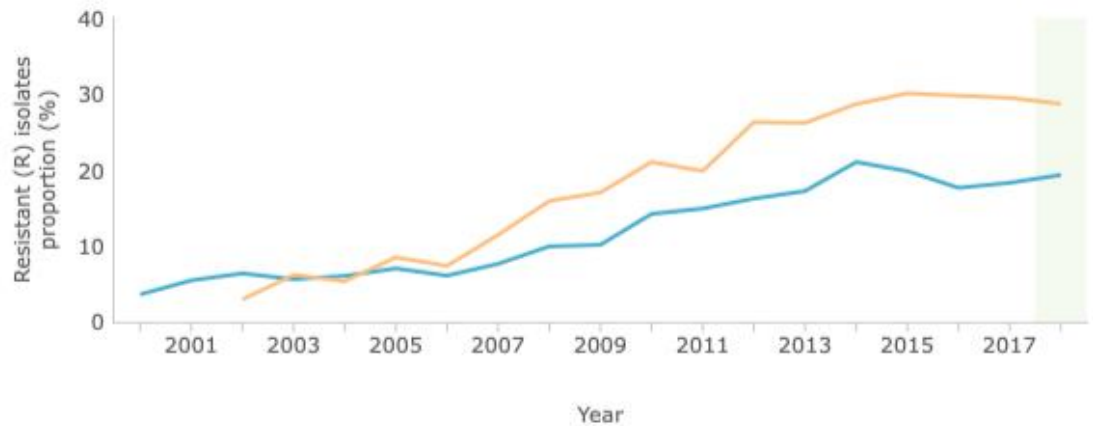
Italy

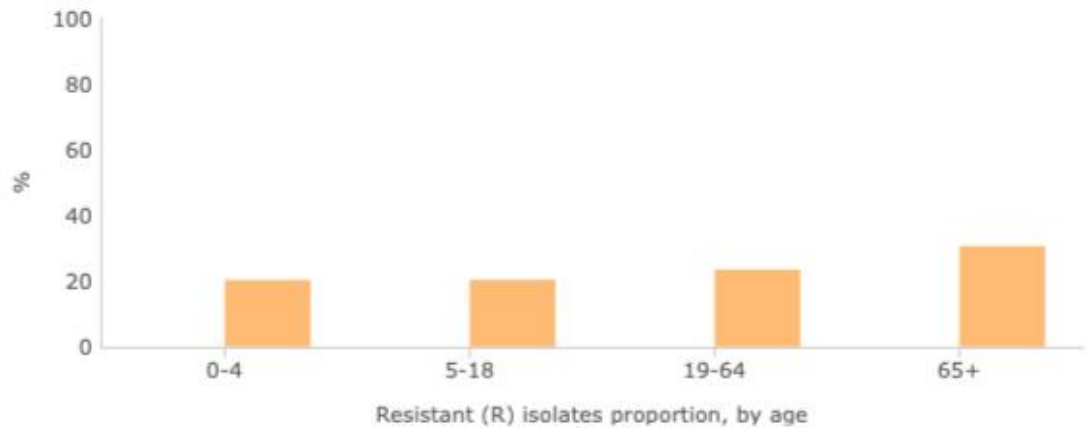
Surveillance Atlas of Infectious Diseases

Antimicrobial resistance | Escherichia coli | Third-generation cephalosporins | Resistant (R) isolates proportion | 2018

Region	Resistant (R) isolates proportion (%)
Czech Republic	15.2
Denmark	7.7
Estonia	9.8
Finland	7.6
France	9.6
Germany	12.2
Greece	19.3
Hungary	22.6
Iceland	8.1
Ireland	12.9
Italy	28.7
Latvia	20.4





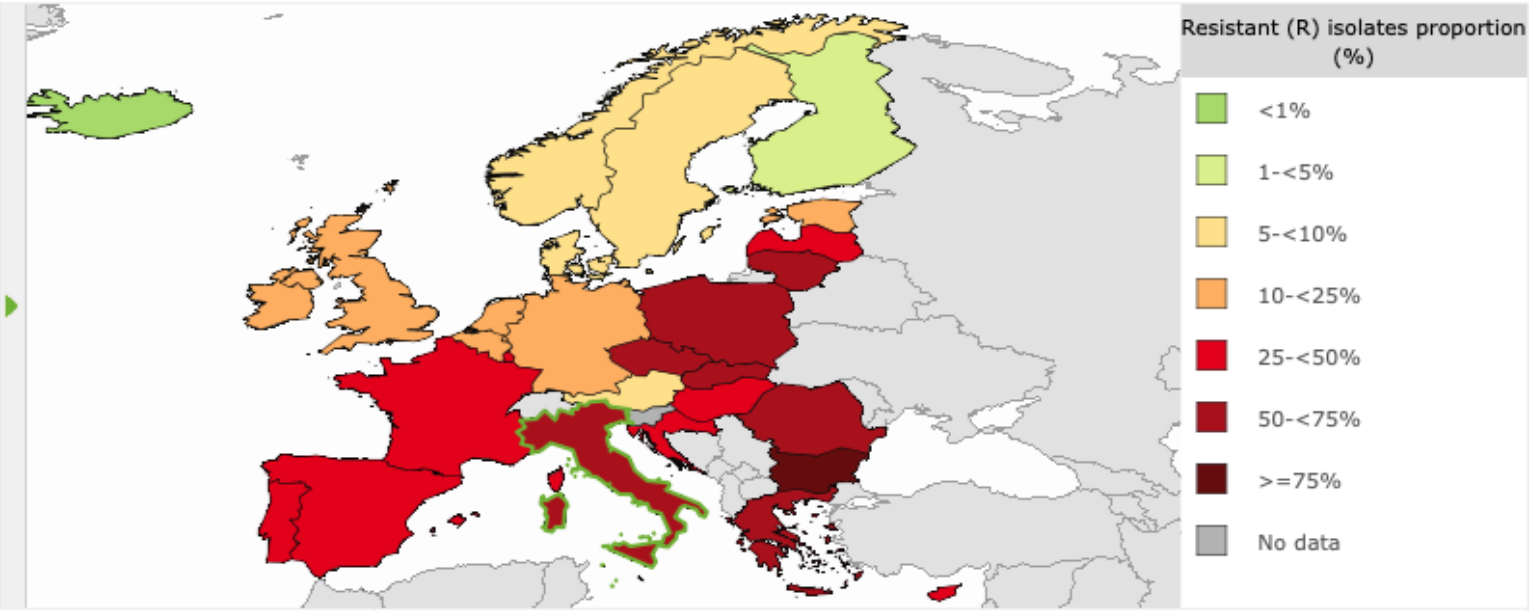


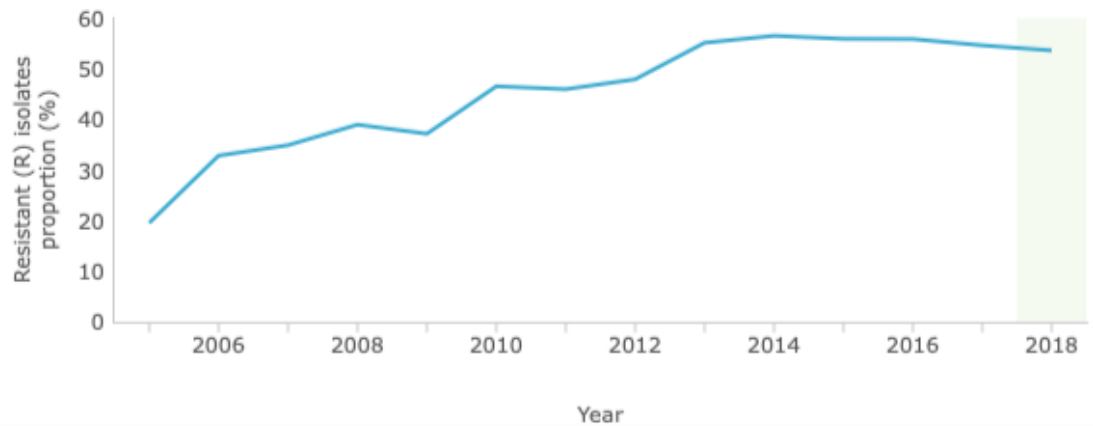
Greece Italy

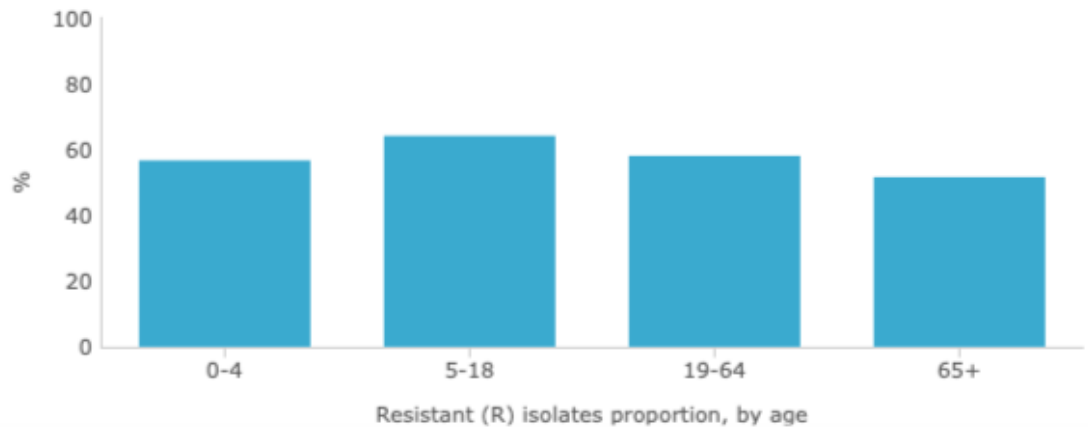
Surveillance Atlas of Infectious Diseases

Antimicrobial resistance | Klebsiella pneumoniae | Third-generation cephalosporins | Resistant (R) isolates proportion | 2018

Region	Resistant (R) isolates proportion (%)
Czech Republic	50.1
Denmark	6.5
Estonia	13.6
Finland	4.5
France	30.8
Germany	12.9
Greece	70.7
Hungary	40.2
Iceland	0.0
Ireland	14.5
Italy	53.6
Latvia	37.7







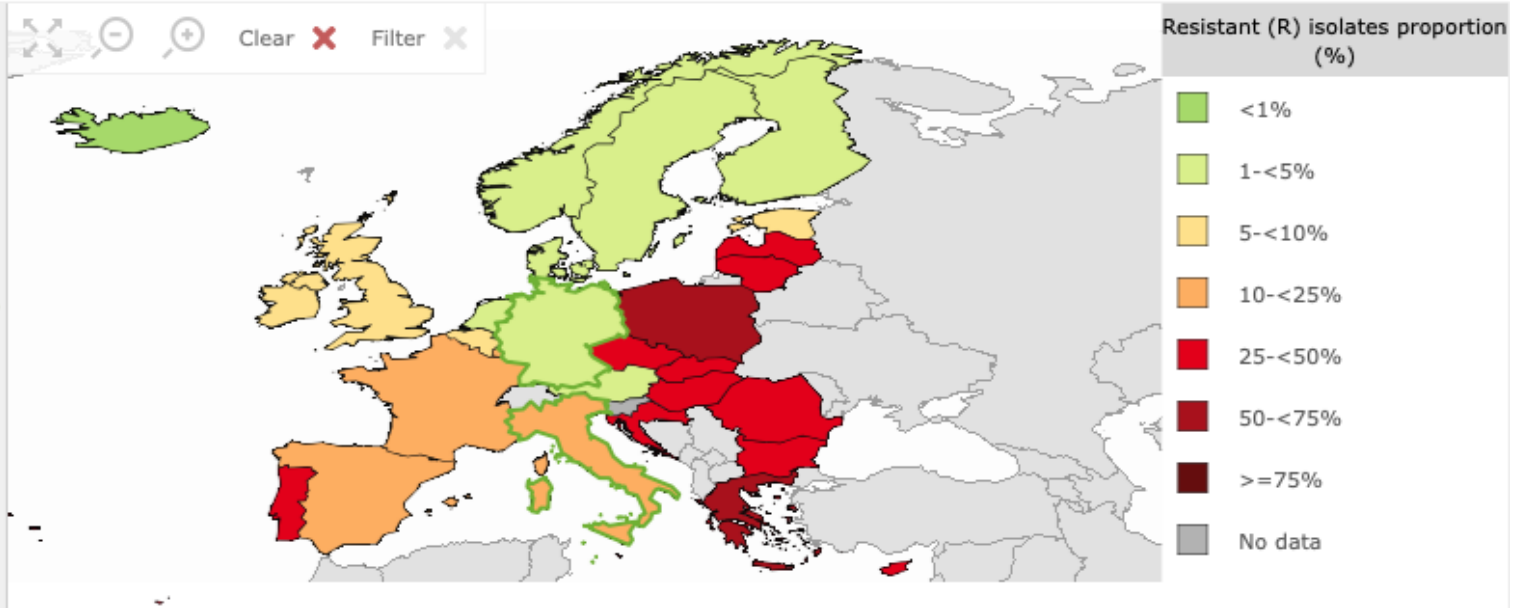
Italy

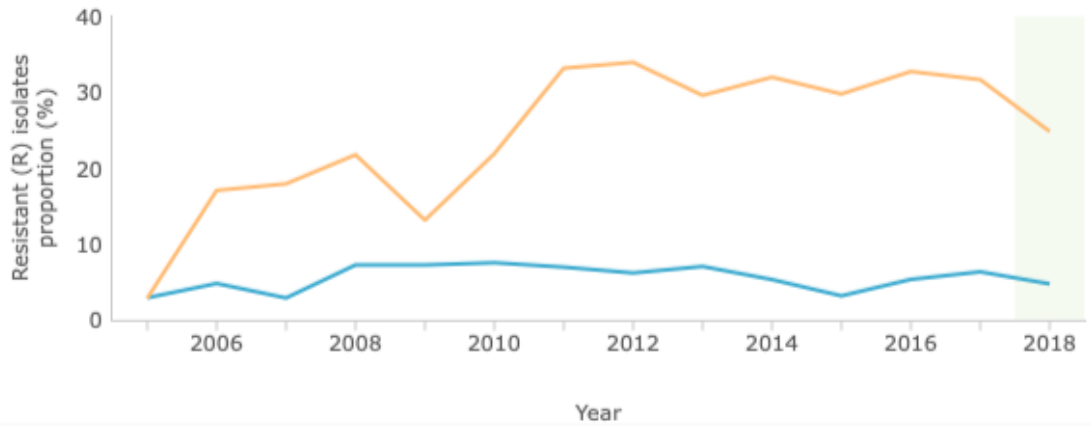
Surveillance Atlas of Infectious Diseases

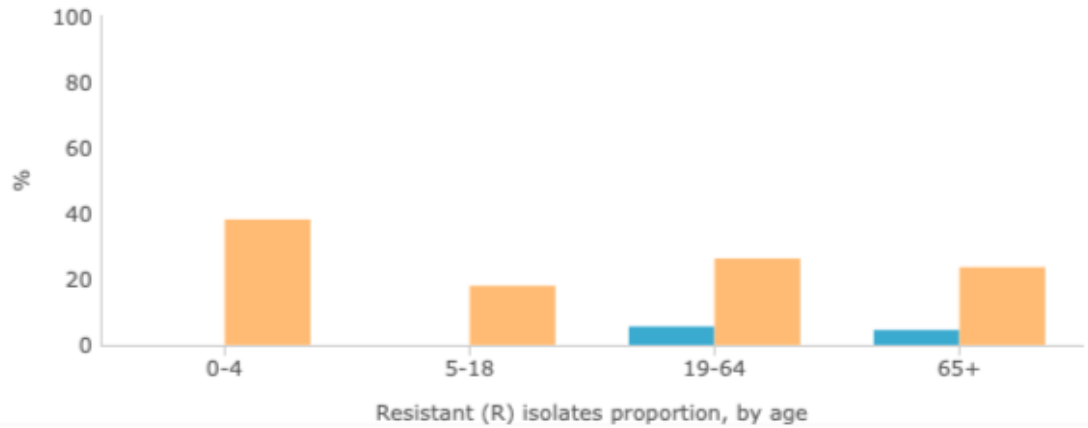
Antimicrobial resistance | Klebsiella pneumoniae | Combined resistance (third-generation cephalosporin, fluoroquinolones and aminoglycoside)

Resistant (R) isolates proportion | 2018

Region	Resistant (R) isolates proportion (%)
Iceland	0.0
Ireland	8.1
Italy	24.8
Latvia	27.6
Lithuania	45.1
Luxembourg	15.3
Malta	43.8
Netherlands	4.7
Norway	3.8
Poland	51.5
Portugal	26.7
Romania	46.3
Slovakia	49.5







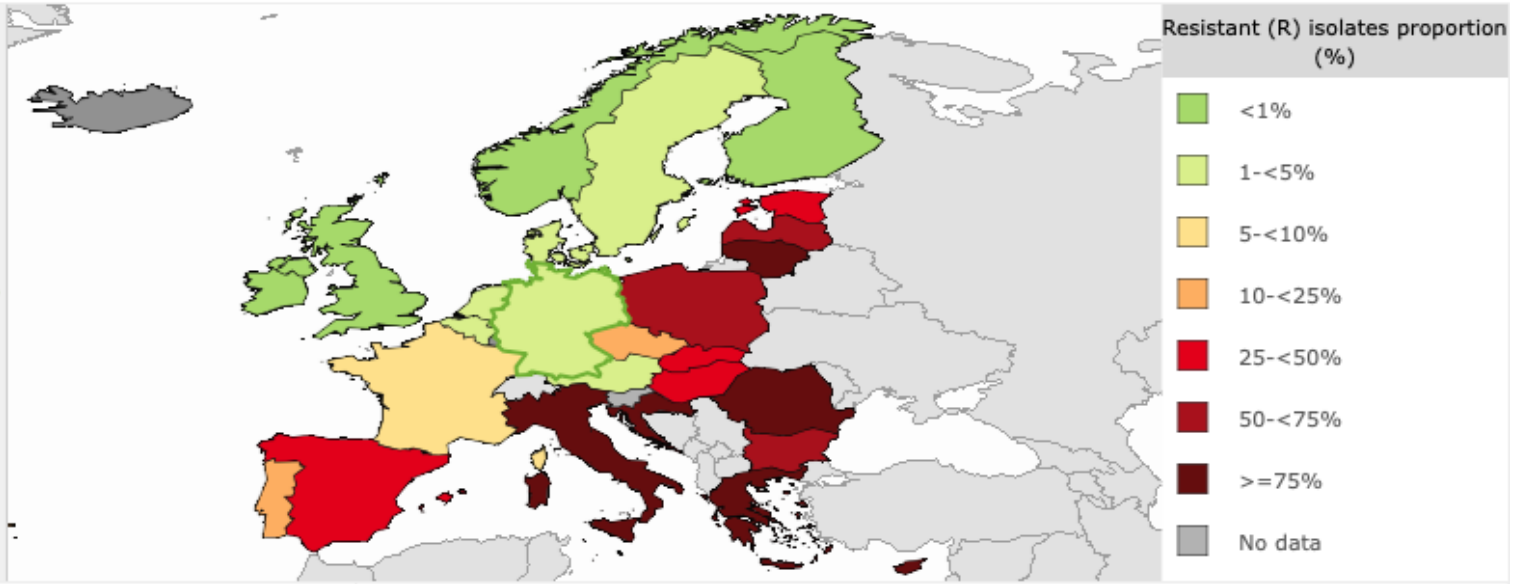
Germany | Italy

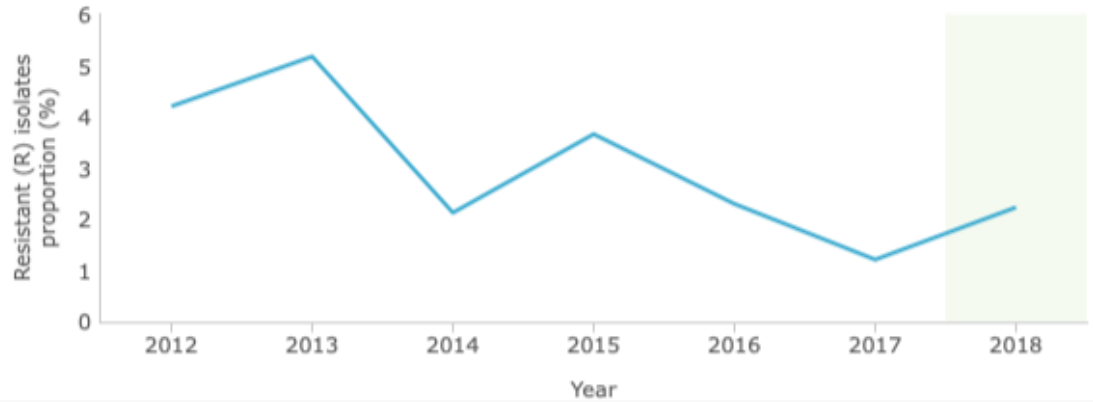
Surveillance Atlas of Infectious Diseases

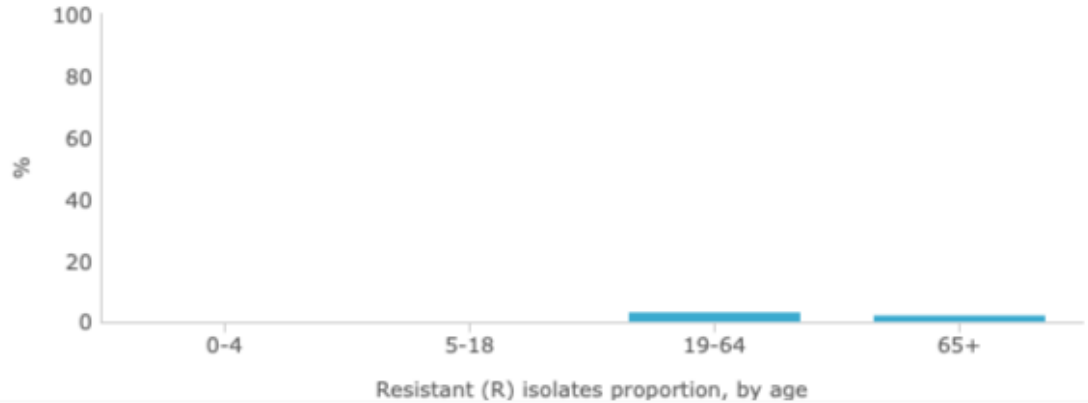
Antimicrobial resistance | Acinetobacter spp. | Combined resistance (fluoroquinolones, aminoglycosides and carbapenems) | Resistant (R) isolates proportion

2018

Region	Resistant (R) isolates proportion (%)
Estonia	36.4
Finland	0.0
France	5.5
Germany	2.2
Greece	81.3
Hungary	41.3
Iceland	-
Ireland	0.0
Italy	75.7
Latvia	56.8
Lithuania	85.1







Germany

Acinetobacter: a huge family of microrganisms

Comuni patogeni umani			
A. baumannii (genospecies 2) *A. nosocomialis* (genospecies 13TU) *A. pittii* (genospecies 3) *A. calcoaceticus* (genospecies 1)			
Specie meno comuni nelle infezioni umane			
A. baylyi *A. beijerinckii* *A. bereziniae* *A. boissieri* *A. bouvetii* *A. brisouii* *A. gerneri* *A. grimontii*[a]	*A. guillouiae* *A. gyllenbergii* *A. haemolyticus* *A. harbinensis* *A. indicus* *A. johnsonii* *A. junii* *A. kookii*	*A. lwoffii* *A. nectaris* *A. parvus* *A. puyangensis* *A. qingfengensis* *A. radioresistens* *A. rudis* *A. schindleri*	*A. soli* *A. tandoii* *A. tjernbergiae* *A. towneri* *A. ursingii* *A. venetianu*

[a]Synonym of *A. junii*.

Tab.3 MLST e PFGE

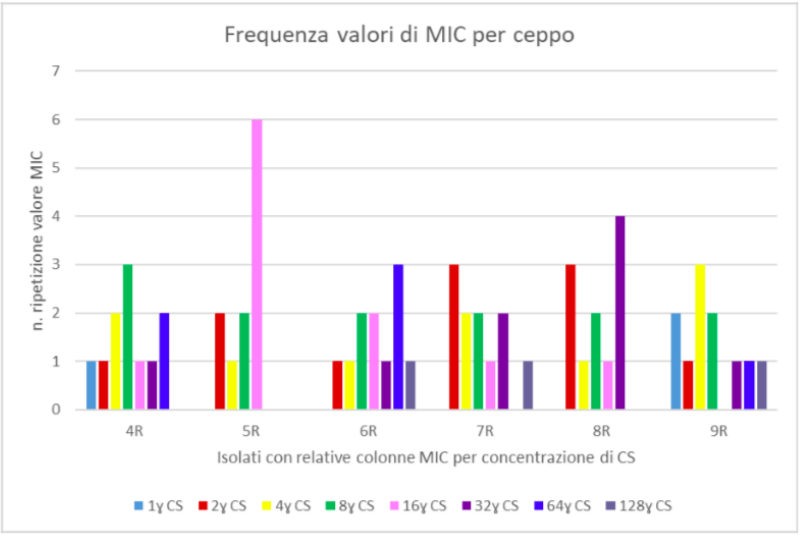
Ceppo	MLST *A. baumannii* Pasteur Institute	MLST *A. baumannii* Oxford University	PFGE Profilo Cloni
1 R	ST 187	ST 1839; 281	A
2 R	ST 2	ST 1839	A
3 R	ST 2	ST 1816; 195	A
4 R	ST 2	ST 218	A
5R	ST 2	ST 1808; 348	A
6R	ST 2	ST 1808; 348	A
7R	ST 2	ST 1808; 348	A
8R	ST 2	ST 1808; 348	A
9R	ST 2	ST 1808; 348	A

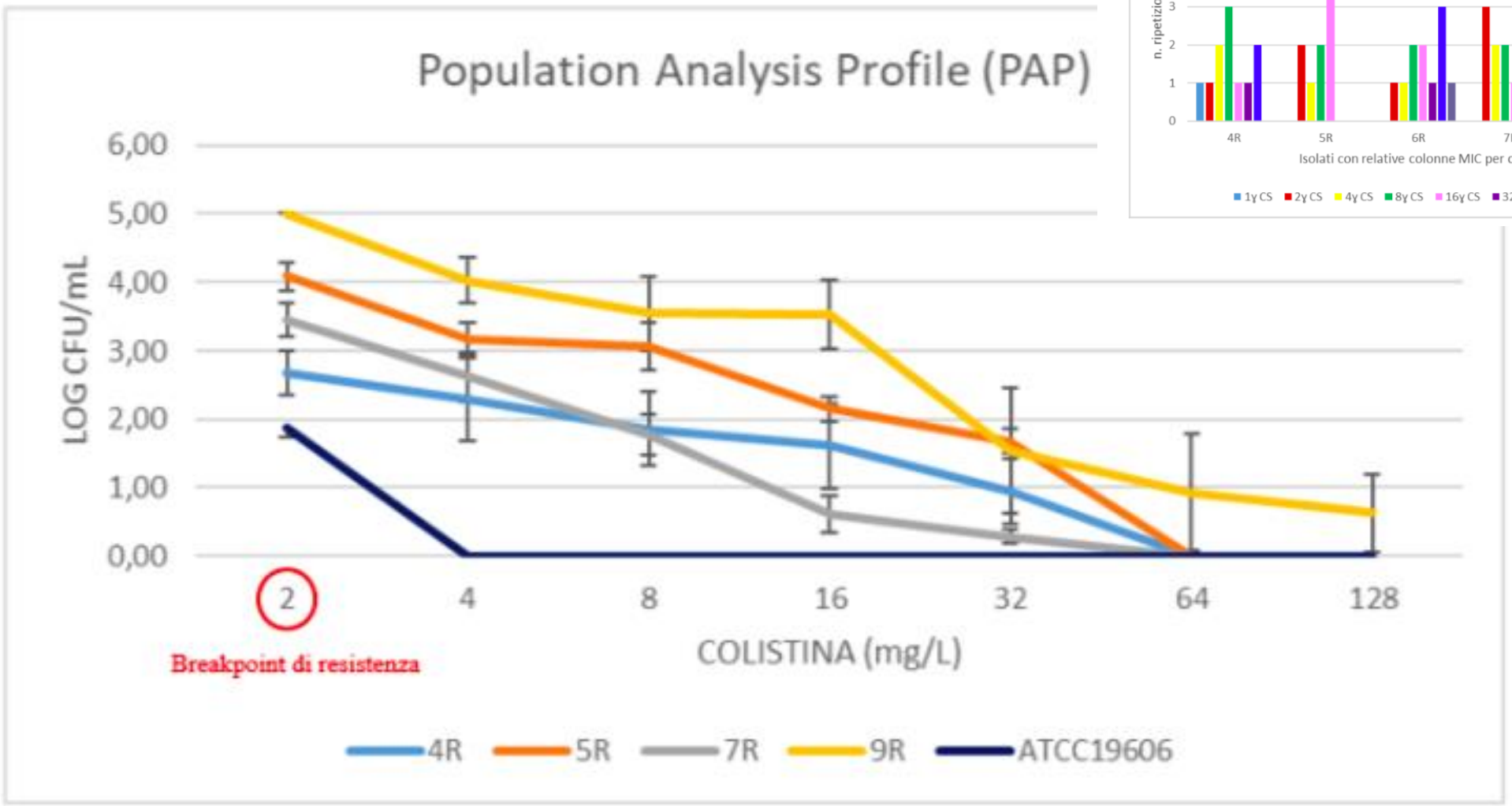
Col R

Very rare

Often heteroresistance

Heteroresistance: a diffused phenomenon





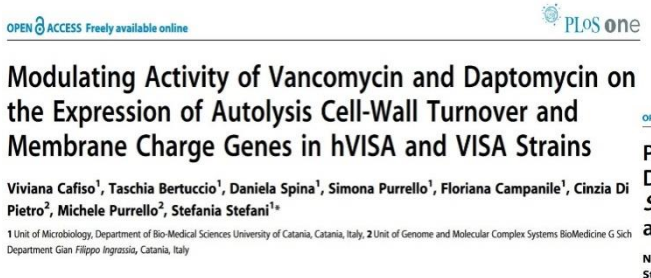
Dovere V., Cafiso V., Stefani S. PhD thesis 2019, unpublished

In-depth Resistome Analysis in Clinical Isolates

Our experience and contribution....

COL-R/Carbapenem-R *A.baumannii* represents a significant challenge for antibiotic treatment due to the **lack of new antimicrobials,** the development of new strategies and new antibiotics, effective against these resistant strains, is urgently needed.

Daptomycin (DAP) *Staphylococcus aureus* infections is one of the few **last-resort treatments** for low-level vancomycin resistant (hVISA/VISA). DAP-resistance onset, also linked to reduced vancomycin susceptibility, is a public health issue.



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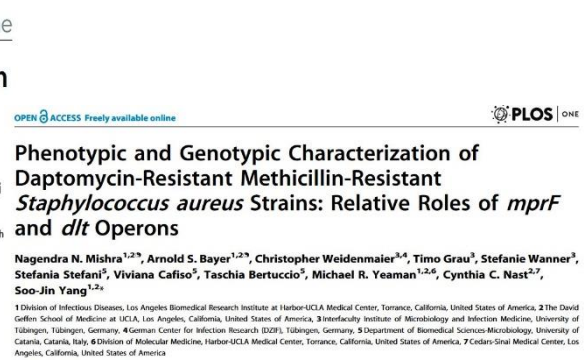
PLOS one

Modulating Activity of Vancomycin and Daptomycin on the Expression of Autolysis Cell-Wall Turnover and Membrane Charge Genes in hVISA and VISA Strains

Viviana Cafiso[1], Taschia Bertuccio[1], Daniela Spina[1], Simona Purrello[1], Floriana Campanile[1], Cinzia Di Pietro[2], Michele Purrello[2], Stefania Stefani[1]*

1 Unit of Microbiology, Department of Bio-Medical Sciences University of Catania, Catania, Italy, 2 Unit of Genome and Molecular Complex Systems BioMedicine G Sich Department Gian *Filippo Ingrassia*, Catania, Italy



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PLOS ONE

Phenotypic and Genotypic Characterization of Daptomycin-Resistant Methicillin-Resistant *Staphylococcus aureus* Strains: Relative Roles of *mprF* and *dlt* Operons

Nagendra N. Mishra[1,2¤], Arnold S. Bayer[1,2¤], Christopher Weidenmaier[3,4], Timo Grau[3], Stefanie Wanner[3], Stefania Stefani[5], Viviana Cafiso[5], Taschia Bertuccio[5], Michael R. Yeaman[1,2,6], Cynthia C. Nast[2,7], Soo-Jin Yang[1,2]*

1 Division of Infectious Diseases, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California, United States of America, 2 The David Geffen School of Medicine at UCLA, Los Angeles, California, United States of America, 3 Interfaculty Institute of Microbiology and Infection Medicine, University of Tübingen, Tübingen, Germany, 4 German Center for Infection Research (DZIF), Tübingen, Germany, 5 Department of Biomedical Sciences-Microbiology, University of Catania, Catania, Italy, 6 Division of Molecular Medicine, Harbor-UCLA Medical Center, Torrance, California, United States of America, 7 Cedars-Sinai Medical Center, Los Angeles, California, United States of America

International Journal of Antimicrobial Agents 43 (2014) 26–31

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International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

dltA overexpression: A strain-independent keystone of daptomycin resistance in methicillin-resistant *Staphylococcus aureus*

Viviana Cafiso[a], Taschia Bertuccio[a], Simona Purrello[a], Floriana Campanile[a], Caterina Mammina[b], Assunta Sartor[c], Annibale Raglio[d], Stefania Stefani[a,*]

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[b] Department of Sciences for Healthy Promotion 'G. D'Alessandro', University of Palermo, Palermo, Italy
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[d] UOC Microbiologia e Virologia, AO Ospedali Riuniti, Bergamo, Italy

Under revision in Scientific Report *(Nature)*

Eur J Clin Microbiol Infect Dis
DOI 10.1007/s10096-016-2581-4

ORIGINAL ARTICLE

In vivo development of daptomycin resistance in vancomycin-susceptible methicillin-resistant *Staphylococcus aureus* severe infections previously treated with glycopeptides

A. Capone[1] · V. Cafiso[2] · F. Campanile[2] · G. Parisi[3] · B. Mariani[3] · N. Petrosillo[1] · S. Stefani[2]

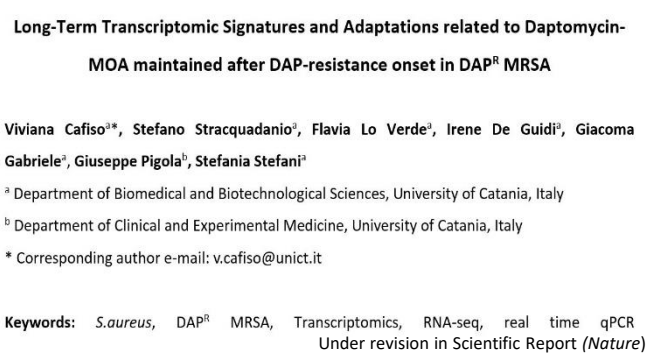
frontiers in Microbiology

ORIGINAL RESEARCH

Colistin Resistant *A. baumannii*: Genomic and Transcriptomic Traits Acquired Under Colistin Therapy

Viviana Cafiso[1], Stefano Stracquadanio[1], Flavia Lo Verde[1], Giacoma Gabriele[1], Maria Lina Mezzatesta[1], Carla Caio[1], Giuseppe Pigola[2], Alfredo Ferro[2] and Stefania Stefani[1]*

[1] Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy, [2] Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy



Long-Term Transcriptomic Signatures and Adaptations related to Daptomycin-MOA maintained after DAP-resistance onset in DAP^R MRSA

Viviana Cafiso[a]*, Stefano Stracquadanio[a], Flavia Lo Verde[a], Irene De Guidi[a], Giacoma Gabriele[a], Giuseppe Pigola[b], Stefania Stefani[a]

[a] Department of Biomedical and Biotechnological Sciences, University of Catania, Italy

[b] Department of Clinical and Experimental Medicine, University of Catania, Italy

* Corresponding author e-mail: v.cafiso@unict.it

Keywords: *S.aureus*, DAP^R MRSA, Transcriptomics, RNA-seq, real time qPCR

Under revision in Scientific Report *(Nature)*

The **understanding** of **Daptomycin** and **Glycopeptide** as well as **Colistin molecular resistance mechanisms** is a very important **challenge** to find **new strategies** to face the **ever-increasing spread** of these **resistances** in front of the **lack of new** therapeutic **options**.

Clinical description

- Patient 1: underwent a pulmonary lobectomy caused by a respiratory distress
- Patient 2 was a severely burned patient that had a cardiac arrest.
- Both patients were initially infected with COL- S *Ab*, consequently treated with colistin, and then infected with the COL-R *Ab.*
- The interval between the isolation of the COL-S and the COL-R *Ab* strain was 24 days for Patient 1 and 7 days for Patient 2.
- No reversion of colistin resistance upon colistin withdrawal was observed in both patients.

Colistin$^{R/S}$ *Acinetobacter baumannii* (*Ab*)

Mechanisms of Colistin Resistance in *A.baumannii*

In *A.baumannii* two colistin resistance mechanisms have been proposed:

i) The first involving mutations in the PmrAB two-component regulatory system, leading to lipid A modification (Adams et al., 2009, Arroyo et al., 2011 and Beceiro et al., 2011).

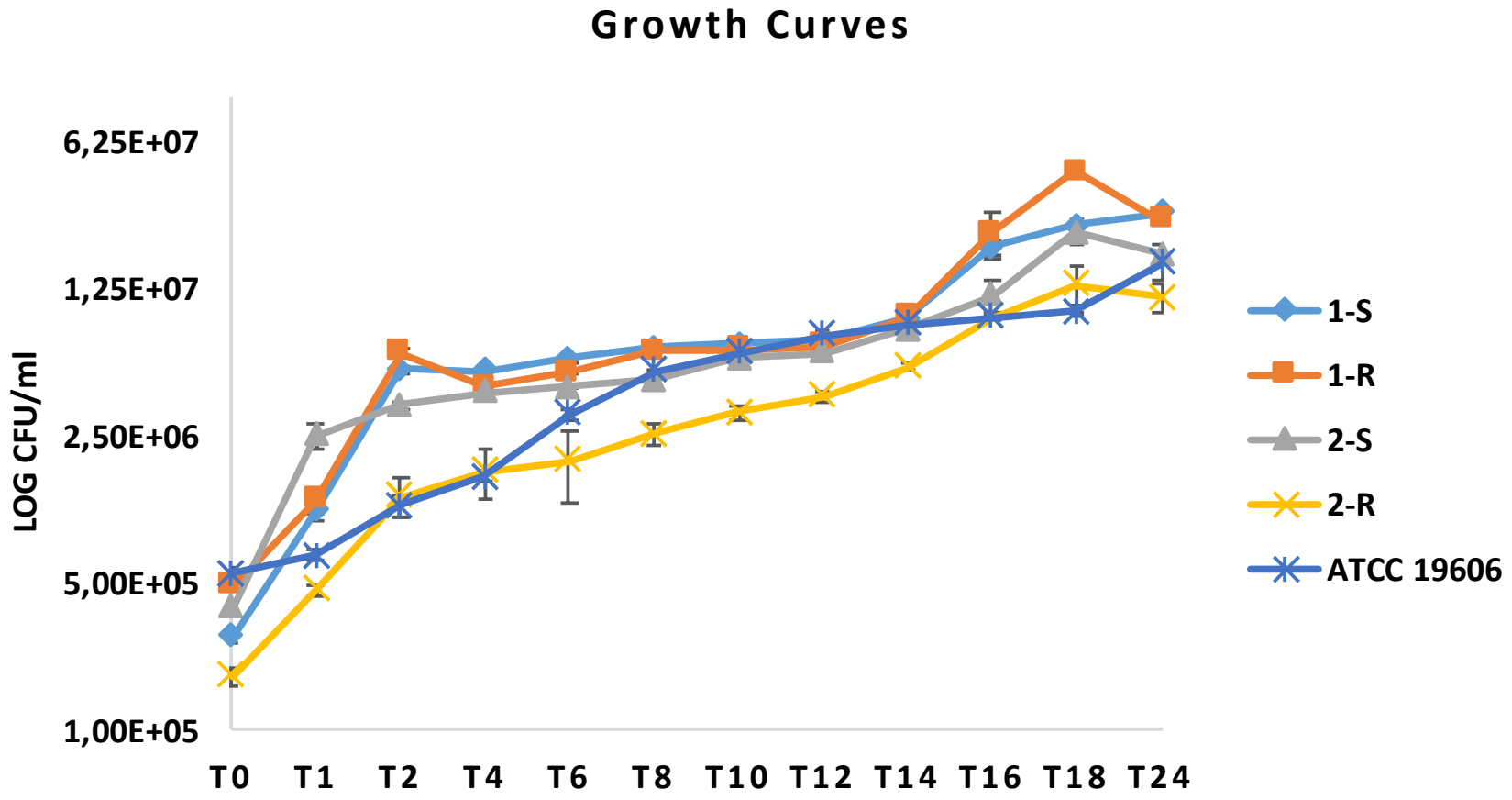
ii) The second evoking mutations or disruption of the genes encoding lipid A biosynthesis such as *lpxA*, *lpxC*, and *lpxD*, determinig a complete loss of LPS (Moffat et al., 2010 and Moffat etal., 2011).

Molecular and Genomic Characterization

The molecular characterization of our isolates showed that **all strains belonged to PFGE-A, ST-281, OXA-23 producers**, **global clone II**, and were resistant to imipenem, meropenem, ampicillin/sulbactam, ciprofloxacin, gentamicin, amikacin, trimethoprim/sulfamethoxazole, and susceptible to tigecycline.

STRAIN	OU MLST	PI MLST	PFGE	GC	MICs (mg/L)								
					COL	IPM	MEM	SAM	CIP	GEN	AK	TGC	SXT
1-S	281	2	A	II	0.125	16*	16*	>256*	>32*	8*	128*	2	8*
1-R	281	187	A	II	64*	16*	16*	>256*	>32*	16*	128*	2	8*
2-S	281	2	A	II	0.125	16*	16*	>256*	>32*	8*	64*	2	16*
2-R	281	2	A	II	256*	16*	16*	>256*	>32*	8*	32*	2	16*

Fitness experiments in ColR/S *A.baumannii*



Showed values are the means of three replicates.
1-S, 2-S COL-S XDR *A. baumannii*
1-R, 2-R COL-R XDR *A. baumannii*
ATCC 19606 antibiotic-susceptible *A. baumannii*

Cafiso V et al – Front Microbiol 2019; 9:3195

Surface attached polysaccharide (SP) (indirect) and LPS (direct) quantification

Strain	SP amount ± SD (µg/mL)	LPS amount ± SD (µg/mL)
1-S	144.84 ± 43.69	6298.40 ± 1267.04
1-R	71.770 ± 31.86	6002.95 ± 1178.74
2-S	178.38 ± 65.24	6003.80 ± 3717.05
2-R	124.54 ± 58.79	5184.77 ± 2526.66
COL-S *A. baumannii* ATCC 19606	170.69 ± 51.67	5843.86 ± 1102.01

1-S, 2-S COL-S XDR *A. baumannii*
1-R, 2-R COL-R XDR *A. baumannii*
ATCC 19606 antibiotic-susceptible *A. baumannii*

Cafiso et al. **Colistin Resistant A. baumannii: Genomic and Transcriptomic Traits Acquired Under Colistin Therapy.** *Front Microbiol.* 2019 Jan 7;9:3195. doi: 10.3389/fmicb.2018.03195. eCollection 2018.

Adaptive response in A.b COLS/R pairs

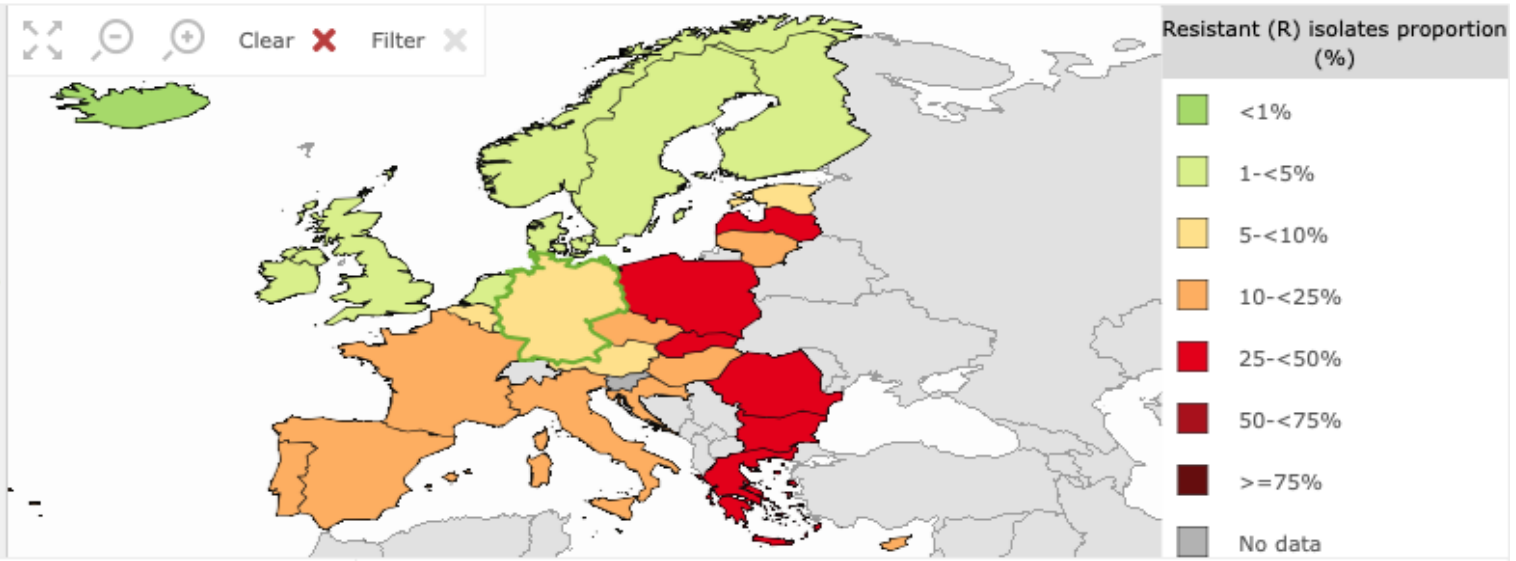
Over-expressed transcripts	Functions upregulated	Associated phenotype
pgaB lipoprotein	Increase primary amine content; biofilm matrix production regulation	Biofilm production Increase Glucosamine acetylation
diacylglycerol kinase	Lipid recycling	Integrity of cell membrane, putatively acting as colidtin resistance
membrane non-ribosomal peptide synthetase	Siderophore production	Protection from ROS and oxidative stress
Lipid A phosphoethanol aminotransferase PmrC	LPS modification	Associated with COL R
Hypothetical protein 1	Periplasmatic membrane protein	?
Hypothetical protein 2	Was described in a n hypothetical phage protein; DNA damage response	Acquisition of resistance increase mutagenesis
Hypothetical protein 3	Photreceptor protein Light response	Motility, biofilm, susceptibility to tigecycline, minocycline?

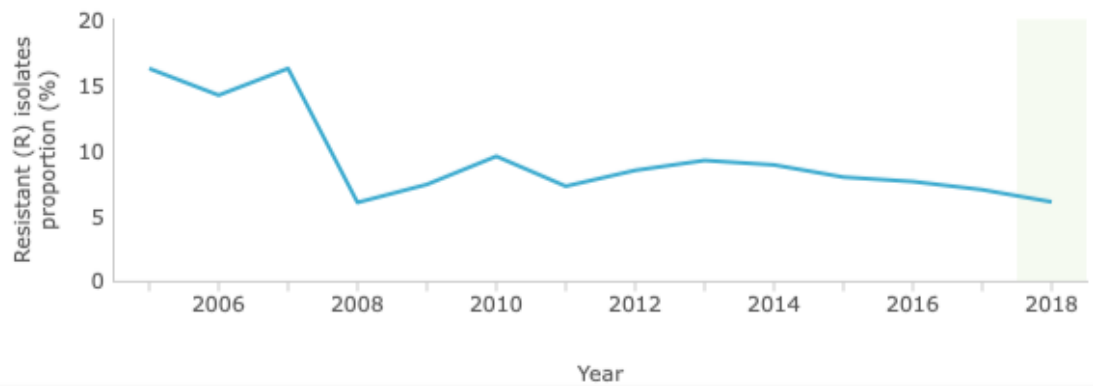
Surveillance Atlas of Infectious Diseases

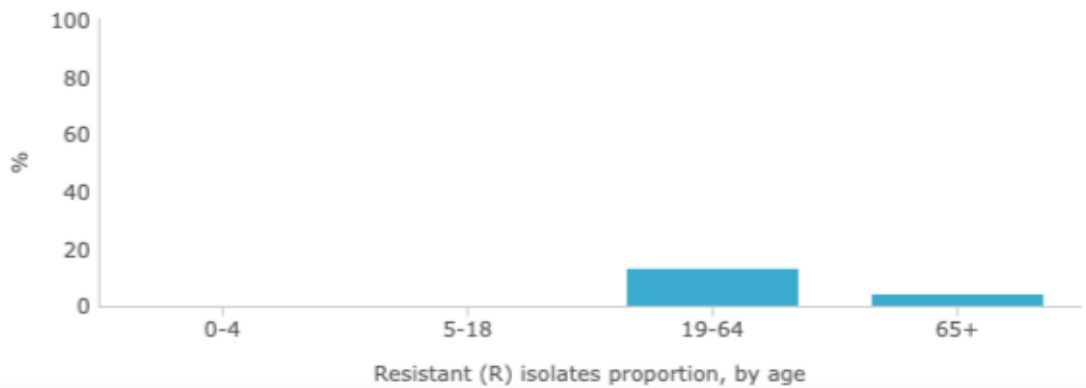
Antimicrobial resistance | Pseudomonas aeruginosa | Combined resistance (at least three of piperac. and tazob., fluoroq., ceftaz., aminogl. and carbapenems)

Resistant (R) isolates proportion | 2018

Region	Resistant (R) isolates proportion (%)
Iceland	0.0
Ireland	3.3
Italy	14.9
Latvia	30.8
Lithuania	11.9
Luxembourg	3.4
Malta	3.4
Netherlands	2.1
Norway	2.4
Poland	29.4
Portugal	15.3







Germany

P.aeruginosa: a flexible and opportunistic pathogen

- Naturally MDR: which explain its success in becoming one of the most important nosocomial pathogen
- Environmental isolates are commonly more susceptible than the HAI
- Since the 1980s the standard of care was Piperacillin or ceftazime plus an aminoglycoside
- Emergence of resistant organisms above all in intensive care, or chronic and persistence in vulnerable patient populations.
- Other strategies become necessary

Table 1. Chromosomally encoded or imported resistance mechanisms of *P. aeruginosa*.

Location	Resistance mechanisms	Targeted antibiotics	Type of resistance
Intrinsic (chromosomal)	AmpC–type cephalosporinase	β-lactams	Antibiotic inactivation
	Class D oxacillinase OXA-50	β-lactams	Antibiotic inactivation
	Aminoglycosides inactivating enzymes	Aminoglycosides	Antibiotic inactivation
	Efflux systems (overexpression)	Multiple antibiotic classes	Efflux systems
	Decreased membrane permeability	Multiple antibiotic classes	Membrane impermeability and purines
	DNA gyrase and topoisomerase IV	Fluoroquinolones	Target modification
	LPS modification	Colistin	Target modification
Imported (Mobile genetic elements)	Class A serine β-lactamases (PSE, CARB, TEM)	β-lactams	Antibiotic inactivation
	Class A serine ESBL (TEM, SHV, CTX-M, PER, VEB, GES, IBC)	β-lactams	Antibiotic inactivation
	Class D ESBL (OXA-types)	β-lactams	Antibiotic inactivation
	Class B Metallo-β-lactamase (IMP, VIM, SPM, GIM)	Carbapenems	Antibiotic inactivation
	Class A serine carbapenemase (KPC)	Carbapenems	Antibiotic inactivation
	Class D carbapenemase (OXA-types: OXA-40)	Carbapenems	Antibiotic inactivation
	Aminoglycosides inactivating enzymes	Aminoglycosides	Antibiotic inactivation
	Ribosomal methyltransferase enzymes	Aminoglycosides	Target modification

Table 4. New drugs and usual clinical dosage for new anti-*Pseudomonas* agents.

Drug	Current clinical indications	Usual clinical dosage for serious infections	Other comment
Cephalosporins			
Cefiderocol	Complicated UTI	2 g intravenous every 8 hours	-
Cephalosporin + β-lactamase inhibitor			
Ceftolozane-tazobactam	Complicated UTI and IAI	Loading dose 1.5 g or 3 g intravenous in 1 hour, followed by 1.5 g or 3 g intravenous every 8 hours	Extended infusion (over 3 h) 1.5 g or 3 g every 8 hours is recommended
Ceftazidime-avibactam	Complicated UTI and IAI, HAP and VAP and Gram-negative infections when other treatments might not work	Loading dose 2.5 g intravenous in 1 hour, followed by 2.5 g intravenous every 8 hours	Extended infusion (over 3 h) 2.5 g every 8 hours is recommended
Carbapenem + β-lactamase inhibitor			
Meropenem-vaborbactam	Complicated UTI	2 g/2 g intravenous every 8 hours	Not active against MDR strains
Imipenem-relebactam	Not yet approved by any regulatory authority	500 mg/250 mg intravenous every 6 hours	Not active against MDR strains
Aminoglycoside			
Plazomicin	Not yet approved by any regulatory authority	15 mg/kg every 24 hours	-

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Human metallo-β-lactamase enzymes degrade penicillin

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Nonribosomal peptides are assemblages, including antibiotics, of canonical amino acids and other molecules. β-lactam antibiotics act on bacterial cell walls and can be cleaved by β-lactamases. β-lactamase activity in humans has been neglected, even though eighteen enzymes have already been annotated such in human genome. Their hydrolysis activities on antibiotics have not been previously investigated. Here, we report that human cells were able to digest penicillin and this activity was inhibited by β-lactamase inhibitor, i.e. sulbactam. Penicillin degradation in human cells was microbiologically demonstrated on *Pneumococcus*. We expressed a MBLAC2 human β-lactamase, known as an exosome biogenesis enzyme. It cleaved penicillin and was inhibited by sulbactam. Finally, β-lactamases are widely distributed, archaic, and have wide spectrum, including digesting anticancer and β-lactams, that can be then used as nutriments. The evidence of the other MBLAC2 role as a *bona fide* β-lactamase allows for reassessment of β-lactams and β-lactamases role in humans.

There is no conclusion on this topic……only thoughts and ideas to change future behavior

- Resistance is ineluctable, being intrinsic in the microrganism's style of life
- Antibiotics act as selectors of gene/clone expansion
- Resistance is not only due to a gene acquisition and mutation of specific targets but also as a consequence of a global regulation/induction of general networks of the cells into selected environments (biofilm, chronicity, drug under-exposition etc)
- Antibiotics are extremely important but must be used under strict control and stewardship programs
- Stopping transmission and igiene
- Surveillances, detection of resistance mechanisms, rapid diagnostics and high throughput methods are indispensable tools for understanding future scenario