Corso Precongressuale C

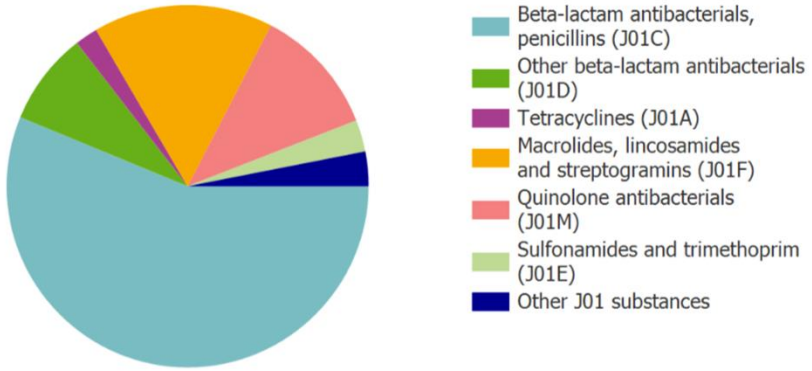
NUOVI E VECCHI ANTIBIOTICI PER NUOVI E VECCHI PATOGENI:
SPETTRO D'AZIONE E CRITICITÀ METODOLOGICHE DEI TEST D

L'antibiogramma per gli aminoglicosidi e i fluorochinoloni

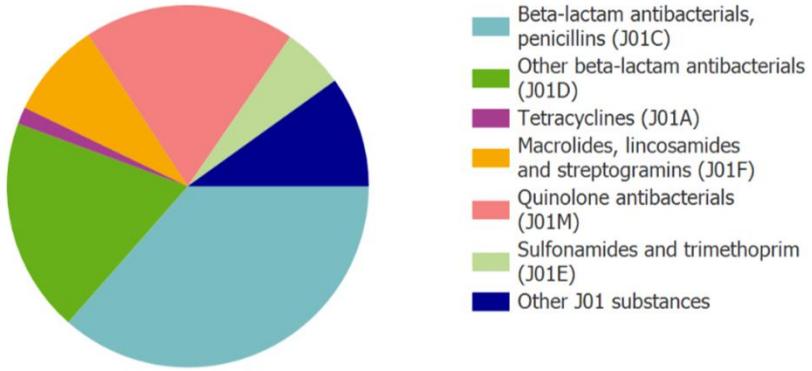
Giulia De Angelis

Antimicrobial consumption in Italy, 2017

Distribution of the consumption in the community (primary care sector) of ATC group J01



Distribution of the consumption in the hospital sector of ATC group J01



EUCAST EUROPEAN C
ON ANTIMICF
SUSCEPTIBIL
European Society of Clinical Microbiology and Infect

Redefining susceptibility testing categories **S**, **I** and **R**.

New definitions are valid from 2019-01-01 (EUCAST breakpoint table v.9.0)

Definitions 2002 – 2018
("the old definition")

Clinically Intermediate (I)

- a micro-organism is defined as intermediate by a level of antimicrobial agent activity associated with uncertain therapeutic effect. It implies that an infection due to the isolate may be appropriately treated in body sites where the drugs are physically concentrated or when a high dosage of drug can be used; it also indicates a buffer zone that should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations.
- a micro-organism is categorized as intermediate (I) by applying the appropriate breakpoints in a defined phenotypic test system
- these breakpoints may be altered with legitimate changes in circumstances

I – Susceptible, increased exposure: A microorganism is categorised as *Susceptible, Increased exposure** when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection.

* Exposure is a function of how the mode of administration, dose, dosing interval, infusion time, as well as distribution, metabolism and excretion of the antimicrobial agent will influence the infecting organism at the site of infection.

Aminoglicosidi

Table 1 Representative aminoglycoside antibiotics currently used in medical, animal husbandry and agricultural fields in G7 countries

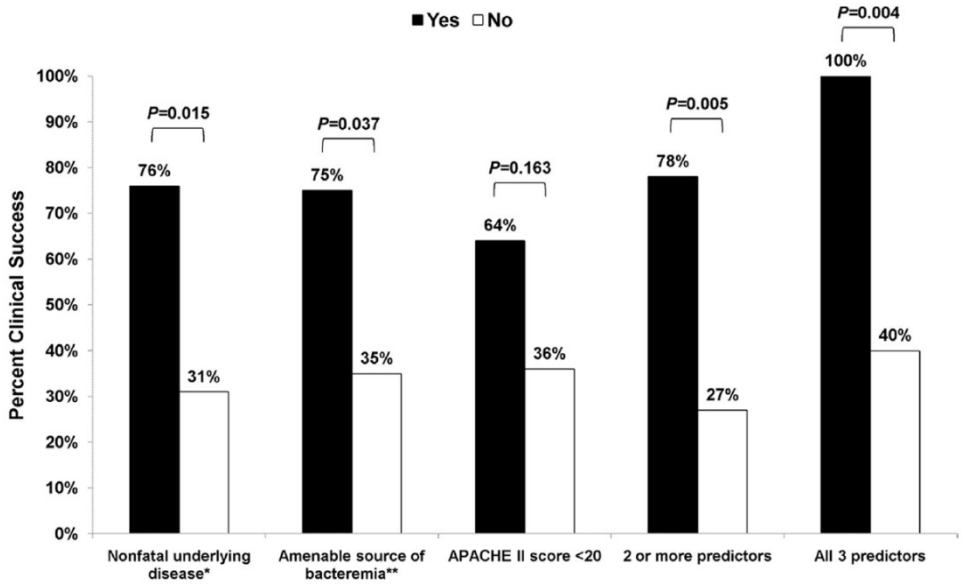
Antibiotic	*Origin*		*Discovered*	*Marketed*	*Target organism*	*Adaptive disease*	*Discoverer*	*Producing microorganism*
	Nature	*Semi-synthetic*						
Human medicines								
Streptomycin	○		1944	1946	Acid-fast bacteria	Tuberculosis	Waksman *et al.*	*Streptomyces griseus*
Neomycin	○		1949	1950	G+	Bacterial infection	Waksman *et al.*	*Streptomyces fradiae*
Kanamycin	○		1957	1958	G+, G−, Acid-fast bacteria	Bacterial infection, Tuberculosis	Umezawa *et al.*	*Streptomyces kanamyceticus*
Paromomycin	○		1959	1959	Intestinal protozoa	Intestinal amebiasis	Haskell *et al.*	*Streptomyces rimosus*
Spectinomycin	○		1961	1967	*N. gonorrhoeae*	Gonocide	Mason *et al.*	*Streptomyces spectabilis*
Gentamicin	○		1963	1967	G+, G−	Bacterial infection	Weinstein *et al.*	*Micromonospora purpureochromogenes*
Tobramycin	○		1967	1968	G−	Bacterial infection	Stark *et al.*	*Streptomyces tenebrarius*
Ribostamycin	○		1970	1975	G+, G−	Bacterial infection	Shomura *et al.*	*Streptomyces ribosidificus*
Dibekacin		○	1971	1975	G+, G−	Bacterial infection	Umezawa *et al.*	–
Amikacin		○	1972	1977	G+, G−, Acid-fast bacteria	Bacterial infection, Tuberculosis	Kawaguchi *et al.*	–
Arbekacin		○	1973	1990	MRSA	MRSA infection	Kondo and Umezawa *et al.*	–
Isepamicin		○	1975	1988	G−	Bacterial infection	Wright *et al.*	–
Veterinary and herbal medicines								
Dihydrotreptomycin	○	○	1946	1963	G+, G−	Bacterial infection	Bartz *et al.*	*Streptomyces griseus*
Apramycin	○		1976	1985	*E. coli*	Bacterial diarrhea	O'Connor *et al.*	*Streptoalloteichus hindustanus*
Kasugamycin	○		1965	1970	*Magnaporthe oryzae*	Rice blast	Umezawa *et al.*	*Streptomyces kasugaensis*
Validamycin A	○		1970	1972	*Rizoctinia sorani*	Rice sheath blight	Iwase *et al.*	*Streptomyces hygroscopicus*

Abbreviations: G+, Gram-positive bacteria; G−, Gram-negative bacteria.

Aminoglicosidi
Indicazioni

- Monoterapia (prima linea): tularemia, peste
- Monoterapia (seconda linea): gonorrea
- **Terapia empirica di combinazione con altri classi antibiotiche**
- Terapia mirata di combinazione (enterococchi)
- Terapia antitubercolare di seconda linea

Aminoglycosides for Treatment of Bacteremia Due to C Resistant *Klebsiella pneumoniae*

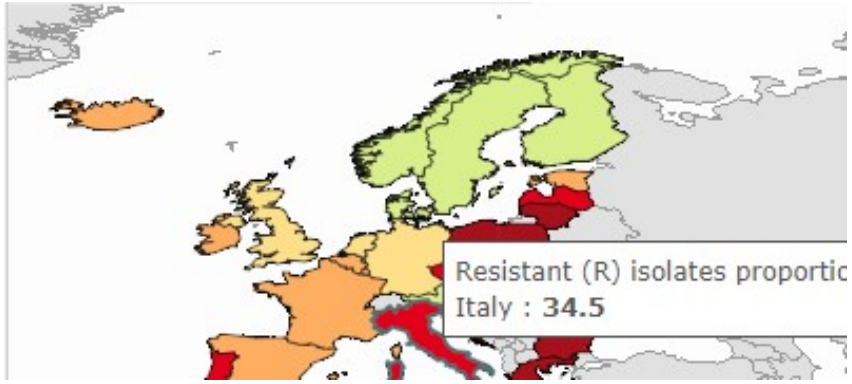


* As defined by the McCabe-Jackson classification criteria (16).

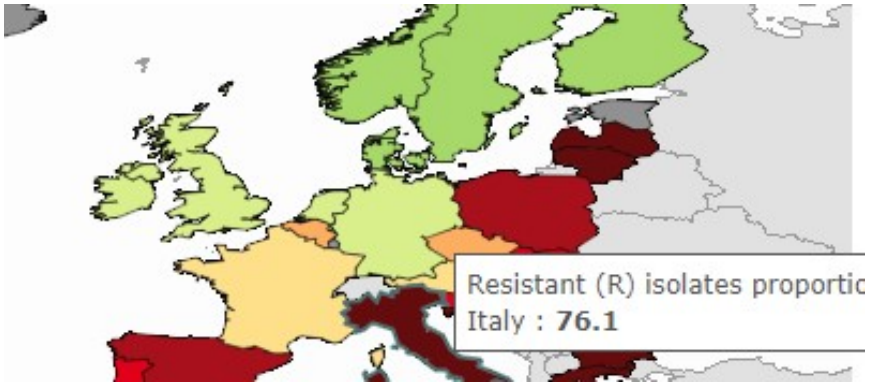
** Amenable sources of bacteremia include vascular catheters, soft tissues, and urinary tract.

FIG 1 Factors associated with clinical success following aminoglycoside therapy for CR *K. pneumoniae* (CR-Kp) bacteremia.

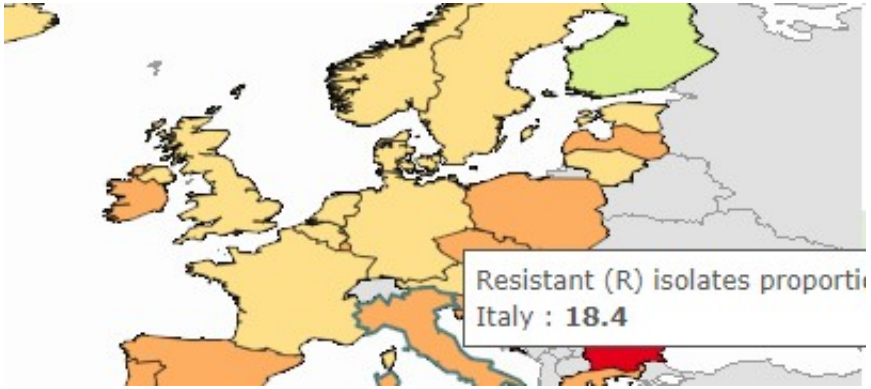
Klebsiella pneumoniae



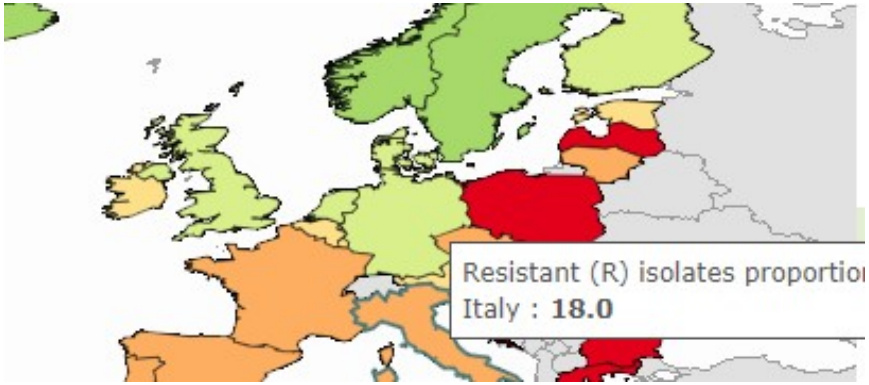
Acinetobacter baumannii



Escherichia coli

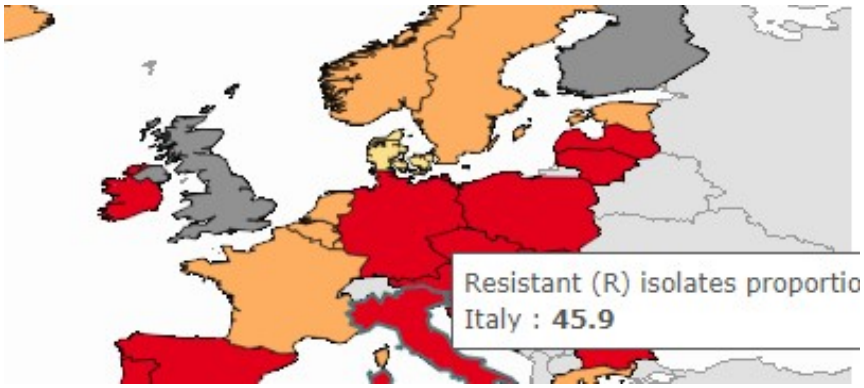


Pseudomonas aeruginosa

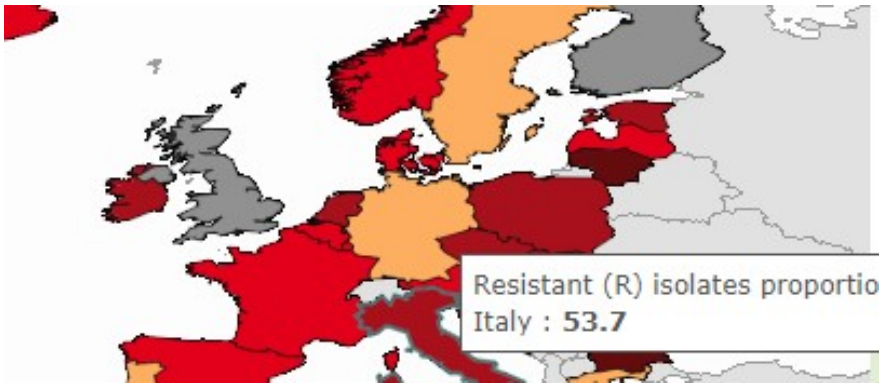


Data from the ECDC Surveillance
Antimicrobial resistance

Enterococcus faecalis

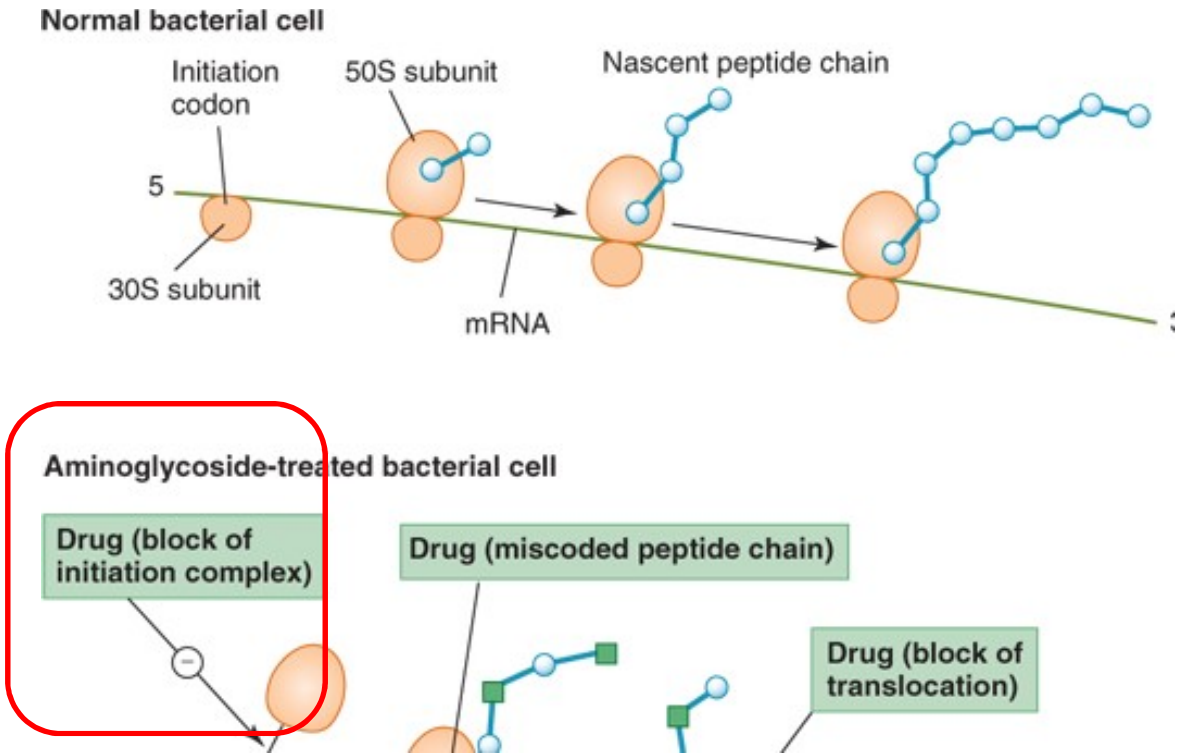


Enterococcus faecium



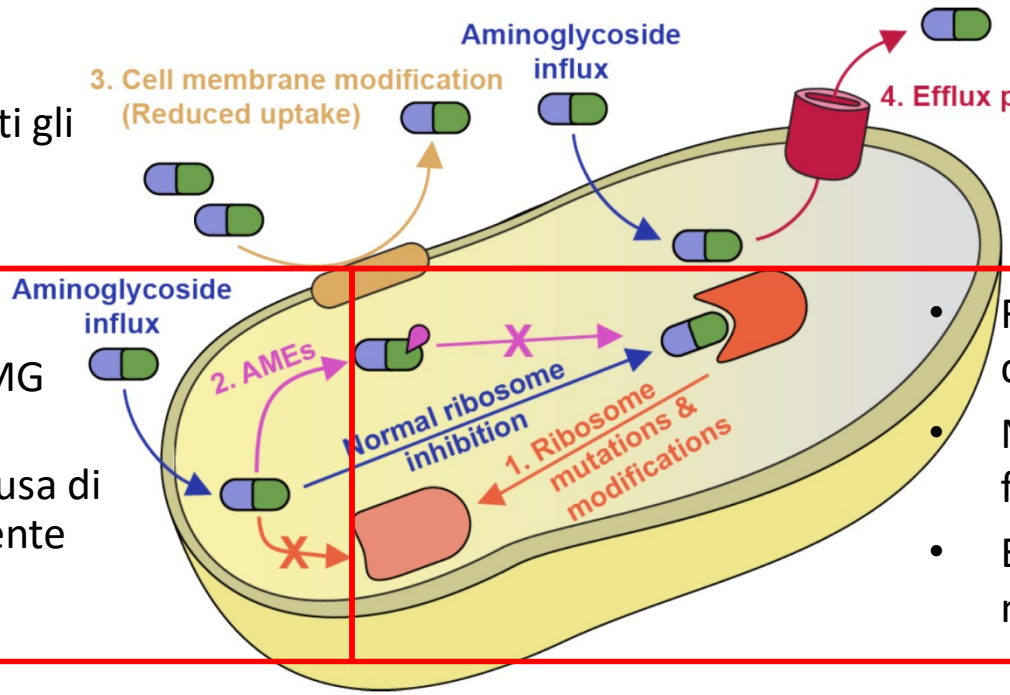
Data from the ECDC Surveillance
Antimicrobial resistance

Meccanismo d’azione



Meccanismi di resistenza & fenotipo

- Basso livello di resistenza a tutti gli AMG



- Resistenza a AMG diversi
- Non sempre causa di fenotipo resistente

- Resistenza a AMG diversi
- Non sempre causa di fenotipo resistente
- Eccezione: 16S RNA metilasi

GEN	NET	TOB	AMK	KAN	NEO	Inter
E. coli and other Enterobacteriaceae not shown						
S	S	S	S	S	S	classi
R	S	S	S	S	S	AAC
R	R	R	S	R	S	AAC
R	R	R	S	r	R	AAC
S/r	R	R	R	R	R	AAC
R	S	R	S	R	S	ANT
Serratia spp.						
S/r	R	R	R	R	R	AAC
Providencia stuartii						
R	r	R	S	S	R	AAC

GEN	NET	TOB	AMK	KAN	NEO	Inter
P. aeruginosa						
S	S	S	S	R	R	classi
R	S	S	S	R	R	AAC
R	S	R	S	R	R	AAC
S/r	R	R	R	R	R	AAC
R	R	R	S	R	R	AAC
R	S	R	S	R	R	ANT

GEN	NET	TOB	AMK	KAN	NEO	Inte
Staphylococci						
S	S	S	S	S	S	class
S	S	R	R	R	S	AN
R	r	R	r	R	r	APH AA
S	S	S	S	R	R	APH
S	S	S	R	R	R	APH
r/R	r/R	R/R	r/R	r/R	r/R	'imp
E. faecalis						
R	R	R	R	R	R	class
R	R	HLR	HLR	HLR	R	AN
HLR	R	HLR	R	HLR	R	APH AA
R	R	R	R	HLR	HLR	APH
R	R	R	HLR	HLR	HLR	APH
E. faecium						

Journal of Antimicrobial Chemotherapy (2001) **48**, *Suppl. S1*, 87–102

16S Ribosomal RNA Methylation: Emerging Mechanism against Aminoglycosides

Table 1. Aminoglycoside resistance pattern confer 16S rRNA methylase.

Drug	Suscep
4,6-disubstituted DOS	Highly resista
Gentamicin	Highly resista
Tobramycin	Resistant, hig
Amikacin	Highly resista
4,5-disubstituted DOS; neomycin	Susceptible
Monosubstituted DOS; apramycin	Susceptible

Table 2. Genetic association and geographic distribution of 16S rRNA methylase genes.

16S rRNA methylase gene	Guanine cytosine content, %	Molecular weight of product, kDa	IS or transposon	Associated β-lactamase genes	Bacterial species (co
rmtA	55.4	27.4	IS*6100*, κγ element, Tn*4051*		*Pseudomonas aeruginosa* (J)
rmtB	55.6	27.4	Tn*3*	bla_{TEM-1}, $bla_{CTX-M-14}$	*Serratia marcescens* (J), *Esche Klebsiella pneumoniae* (J, T, *Citrobacter freundii* (K)
rmtC	41.1	32.1	ISEcp*1*		*Proteus mirabilis* (J)
rmtD	59.3	27.7	ISCR	bla_{SPM-1}	*P. aeruginosa* (Br)
armA	30.4	30.2	IS*26*, Tn*1548*	$bla_{CTX-M-3}$	*S. marcescens* (J, F, K), *C. fre ter amalonaticus* (Be), *K. pn* Be), *K. oxytoca* (Bu), *E. coli*

12.7	All *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*	Tobramycin, gentamicin, and amikacin	Amikacin	IF intermediately resistant or resistant to tobramycin and susceptible to gentamicin and amikacin, THEN report amikacin as intermediate for *Enterobacteriaceae* or resistant for *Pseudomonas* spp. and *Acinetobacter* spp.	Production of acquired AAC(6′)-I enzyme may not confer phenotypic resistance despite modification of amikacin	C
12.8	All *Enterobacteriaceae*	Gentamicin and other aminoglycosides	Gentamicin	IF intermediately resistant to gentamicin and susceptible to other aminoglycosides, THEN report as resistant to gentamicin	Expression of AAC(3)-I enzyme may be low, and isolates may have decreased susceptibility to gentamicin	C
12.9	All *Enterobacteriaceae*	Tobramycin, gentamicin, and amikacin	Tobramycin	IF intermediately resistant to tobramycin, resistant to gentamicin and susceptible to amikacin, THEN report as resistant to tobramycin	Expression of the ANT(2″) enzyme may be low and isolates may have decreased susceptibility to tobramycin	C
12.10	All *Enterobacteriaceae*	Netilmicin and gentamicin	Netilmicin	IF intermediately resistant to netilmicin and intermediately resistant or resistant to gentamicin and tobramycin, THEN report as resistant to netilmicin	Expression of the AAC(3″)-II or AAC(3″)-IV enzyme may be low and isolates may appear with decreased susceptibility to netilmicin	C

Se tobramicina R: amicacina da S a R

Se gentamicina I ma S a tutti gli altri AMG: gentamicina da I a R

Se gentamicina R e amicacina S: tobramicina da I a R

Se gentamicina R e tobramicina R: netilmicina da I a R

Rule no.	Organisms	Agent tested	Agents affected	Rule	Exceptions, scientific basis, and c
12.1	*Staphylococcus* spp.	Kanamycin	Amikacin	IF kanamycin MIC is >8 mg/L, THEN report as resistant to amikacin	Resistance to kanamycin is generally c production of APH(3′)-I-3, ANT(4′)(bifunctional APH(2′)-AAC(6) enzym determine loss of synergism of kana amikacin with β-lactams and glycope irrespective of MIC values
12.2	*Staphylococcus* spp.	Tobramycin	Kanamycin and amikacin	IF resistant to tobramycin, THEN report as resistant to kanamycin and amikacin	Resistance to tobramycin is generally production of ANT(4′)(4″)-I or bifu APH(2′)-AAC(6) enzymes that deter synergism of kanamycin, tobramycin with β-lactams and glycopeptides irr of MIC values
12.3	*Staphylococcus* spp.	Gentamicin	All aminoglycosides	IF resistant to gentamicin, THEN report as resistant to all aminoglycosides	Resistance to gentamicin is generally production of a bifunctional APH(2′) that determines loss of synergism of aminoglycosides (except streptomyc with β-lactams and glycopeptides irr MIC values
12.4	*Enterococcus* spp. and *Streptococcus* spp.	Streptomycin	Streptomycin	IF high level-resistance to streptomycin is detected (MIC of >512 mg/L), THEN report as high-level resistant to streptomycin	High-level resistance reflects product of other enzymes or of ribosomal m no synergistic effect between strept β-lactam agents in enterococci with resistance to streptomycin
12.5	*Enterococcus* spp., *Streptococcus* spp.	Kanamycin	Amikacin	IF high-level resistance to kanamycin is detected (MIC of >512 mg/L), THEN report as having high-level resistance to amikacin	High-level resistance to kanamycin is by the production of APH(3′)-I-3 or APH(2′)-AAC(6) enzymes that deter synergism of kanamycin and amikaci

Se kanamicina R: amicacina da S a R

Se tobramicina R: amicacina e kanamicina da S a R

Se gentamicina R: tutti AMG da S a R

Se kanamicina HL-R: amicacina da R a HL-R

Se gentamicina HL-R: tutti AMG da R a HL-R

Fluorochinoloni

Chinoloni e fluorochinoloni

Table 1. Fluoroquinolones licensed for clinical use current status

Generation	Drug	Use in clin
First generation	Nalidixic acid	Generic fo
	Cinoxacin	Discontinu
Second generation	Norfloxacin	Available
	Ciprofloxacin	Available and gener
	Lomefloxacin	Discontinu
	Ofloxacin	Available and gener
	Levofloxacin	Available and gener
Third generation	Sparfloxacin	Discontinu
	Gatifloxacin	Discontinu

Spettro d'azione:

- Bacilli gram negativi
- Cocchi gram positivi
- Micobatteri

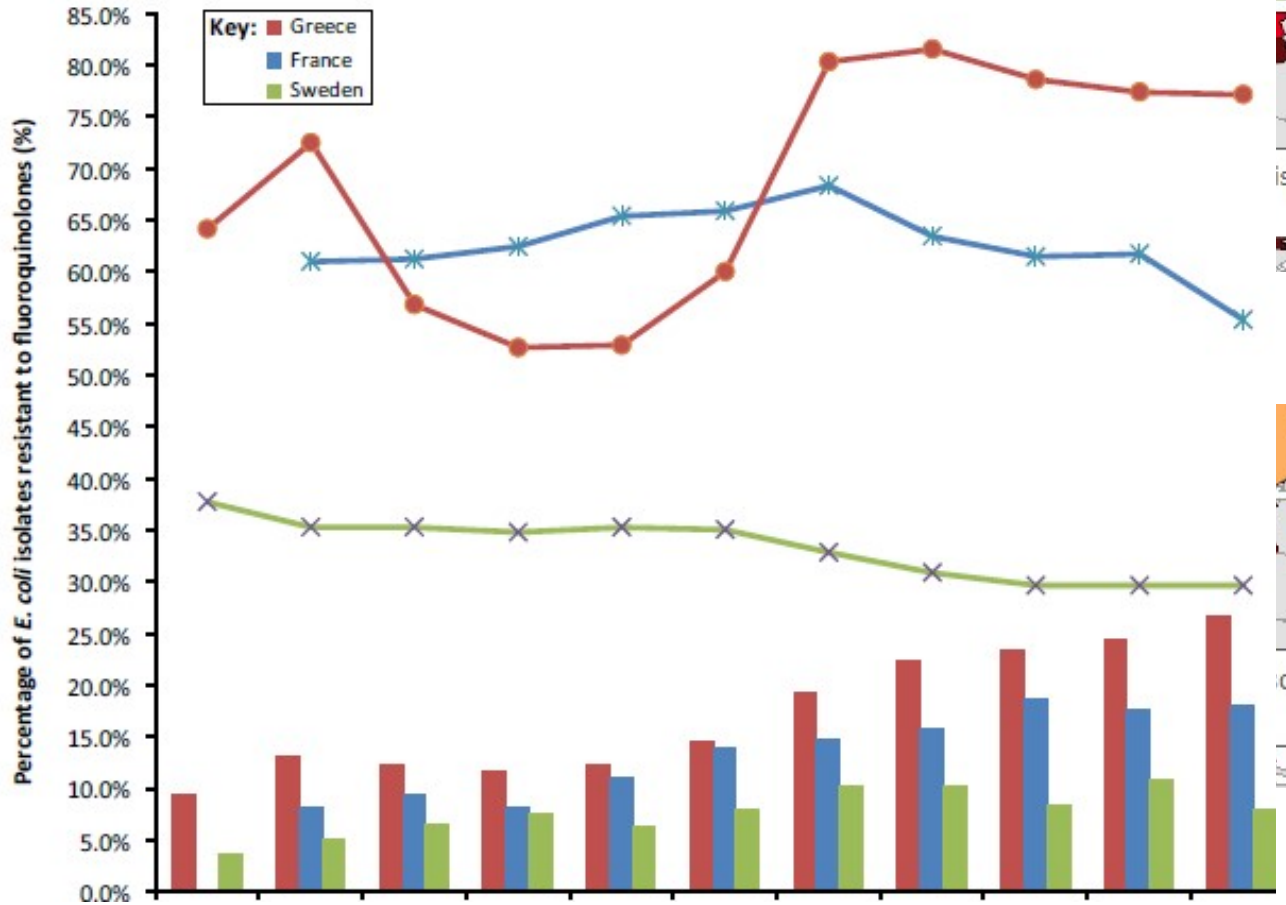
Trends in Microbiology, August 2014, Vol.

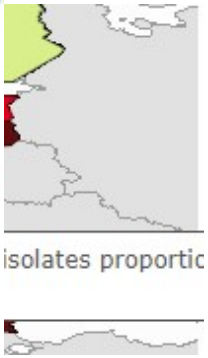
Klebsiella p

Escherichia

Data fro
Antimicr

Correlation of fluoroquinolone resistance and consumption in *E. coli* in Greece, Fra
Sweden





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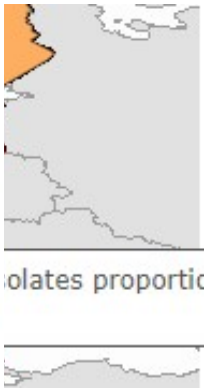
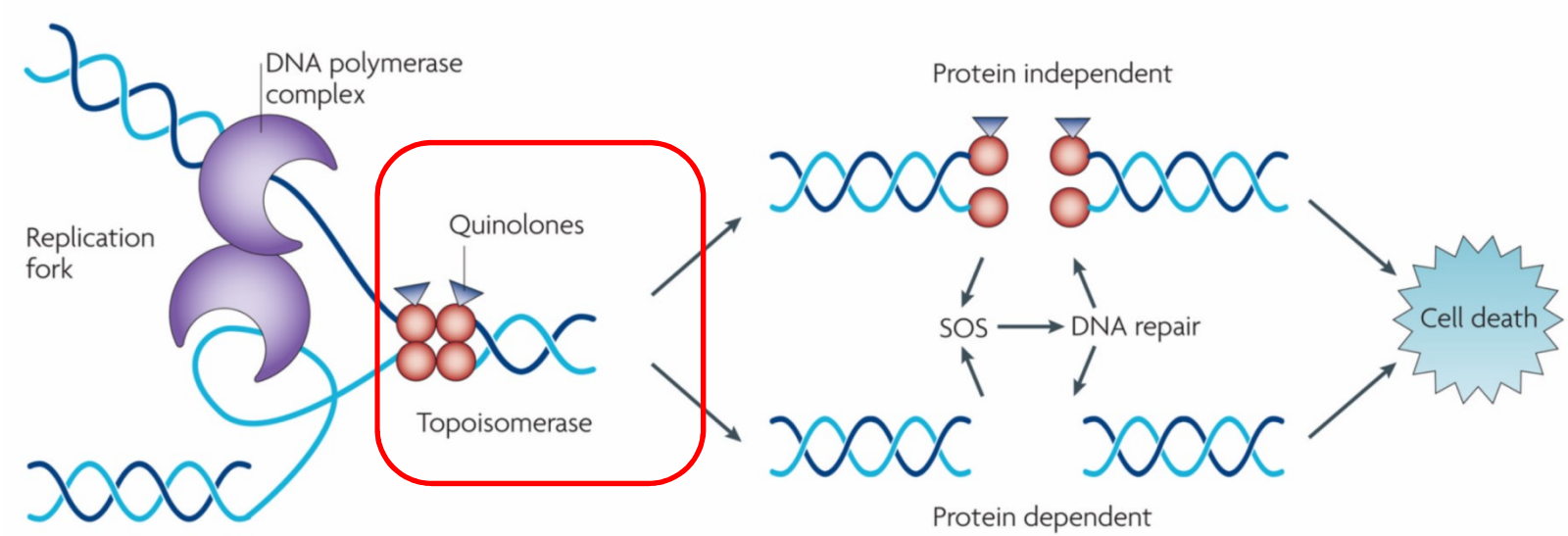
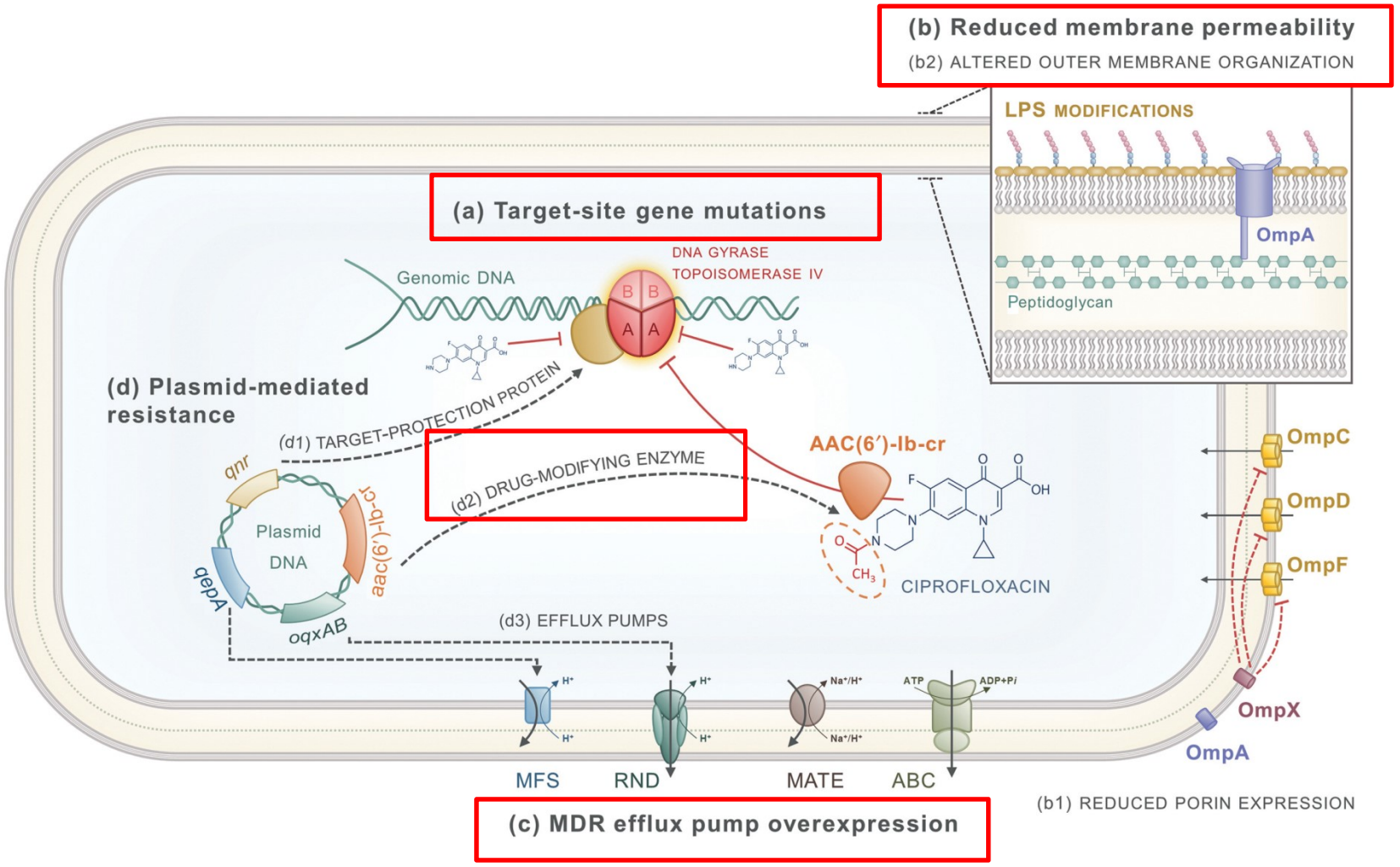


olates proportic

Meccanismo d’azione



Nat Rev Microbiol. 2010 June ; 8(6):



Correia *et al.*, *Journal of Medical Microbiology* 2017

Table 2. Summary of the impact of different resist mechanisms on susceptibility to ciprofloxacin

Resistance mechanism	Fold change in ciprofloxacin MIC	
Gram-negative species[a]		
Topoisomerase substitutions		
gyrA	10–16	
parC	0	
gyrA (× 2) + *parC*	60	
Permeability changes		
Efflux upregulation	4–8	
Porin loss	4	
PMQRs		
Carriage of *qnr* alleles	>30	
Carriage of *qepA*	32	
Carriage of *oxqAB*	16	
Carriage of *aac(6′)Ib-cr*	4	
Gram-positive species[b]		
Topoisomerase substitutions		
grlA	4–8	
grlB	4–8	
gyrA	0	

Trends in Microbiology, August 2014, Vol.

Table 2. Summary of the impact of different resis mechanisms on susceptibility to ciprofloxacin

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Gram-positive species[b]		
Topoisomerase substitutions		
grlA	4–8	
grlB	4–8	
gyrA	0	

Trends in Microbiology, August 2014, Vol.

TABLE 13. Interpretive rules for quinolones

Rule no.	Organism	Agents tested	Agents affected	Rule	Exceptions, scientific basis, and comments	Evidence grade	References
13.1	*Staphylococcus* spp.	Ofloxacin, ciprofloxacin, levofloxacin, and moxifloxacin	All fluoroquinolones	IF resistant to ofloxacin or ciprofloxacin, but not to levofloxacin or moxifloxacin, THEN report warning of risk for development of resistance during therapy with quinolones	Acquisition of at least one target mutation in *grlA*	C	[86,92]
13.2	*Staphylococcus* spp.	Levofloxacin and moxifloxacin	All fluoroquinolones	IF resistant to levofloxacin or moxifloxacin, THEN report as resistant to all fluoroquinolones	Acquisition of combined mutations in *grlA* and *gyrA* leads to complete or partial cross-resistance to all fluoroquinolones	C	[92,116,117]
13.3	*Streptococcus pneumoniae*	Ofloxacin, ciprofloxacin, levofloxacin, and moxifloxacin	All fluoroquinolones	IF resistant to ofloxacin or ciprofloxacin, but not to levofloxacin or moxifloxacin, THEN report warning that acquisition of a first-step mutation may lead to resistance development under therapy with other quinolones	Acquisition of at least one target mutation in, for example, *parC* (*parE*). First-step mutations can be more reliably detected in tests with norfloxacin	C	[94,118–120]
13.4	*Streptococcus pneumoniae*	Levofloxacin and moxifloxacin	All fluoroquinolones	IF resistant to levofloxacin or moxifloxacin, THEN report as resistant to all fluoroquinolones	Acquisition of combined mutations in, for example, *parC* and *gyrA* leads to complete or partial cross-resistance to all fluoroquinolones	B	[121]

In staphylococci and viridans group streptoco
tance to the less active, but not to the more act
oquinolones indicates that a first-step mutatior
present. In this case, a warning should be added t
ceptibility testing report, alerting clinicians to the
for selection of a higher-level resistance mechanism

TABLE 13. **Interpretive rules for quinolones**

Rule no.	Organism	Agents tested	Agents affected	Rule	Exceptions, scientific basis, and comments	Evidence grade	References
13.5	*Enterobacteriaceae*	Ciprofloxacin	All fluoroquinolones	IF resistant to ciprofloxacin, THEN report as resistant to all fluoroquinolones	Acquisition of at least two target mutations in either *gyrA* or *gyrA* plus *parC*. Exceptionally, production of the AAC(6′)-Ib-cr enzyme may affect ciprofloxacin but not levofloxacin	B	[93]

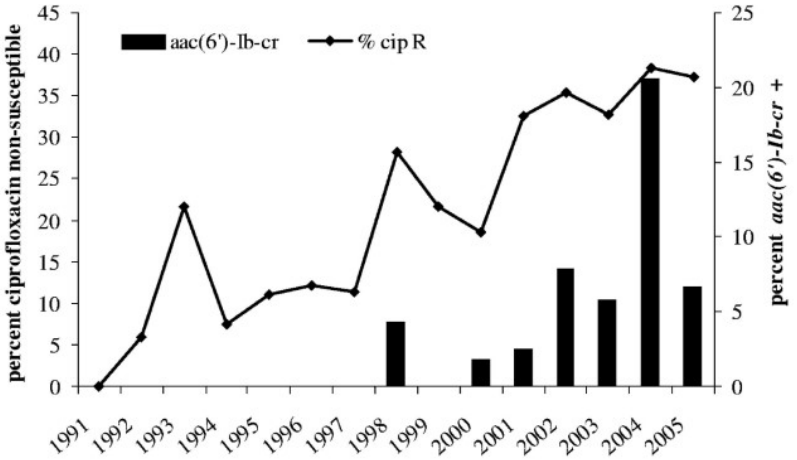


FIG. 2. Percentage of clinical *E. coli* isolates (n = 718) harboring resistance to ciprofloxacin (MIC ≥ 2 μg/ml) and harboring *aac(6′)-Ib-cr*. No *aac(6′)-Ib-cr* genes were found before 1998.

TABLE 13. Interpretive rules for quinolones

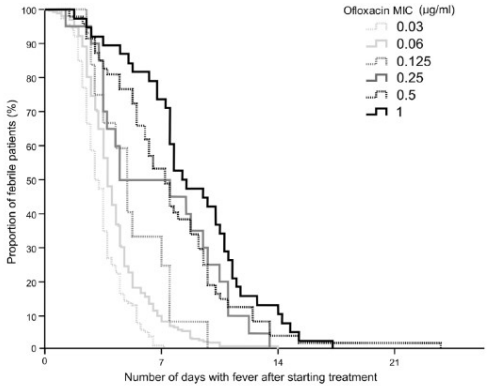
Rule no.	Organism	Agents tested	Agents affected	Rule	Exceptions, scientific basis, and comments	Evidence grade	References
13.6	*Salmonella* spp.	Ciprofloxacin	All fluoroquinolones	IF ciprofloxacin MIC is >0.06 mg/L, THEN report as resistant to all fluoroquinolones	Evidence for clinical failure of fluoroquinolones in cases of resistance caued by the acquisition of at least one target mutation in *gyrA*	A (*Salmonella typhi*), B (other *Salmonella* spp.)	[95,97,98]

Table 3. Documented Cases of Ciprofloxacin (CIP) Treatment Failures in Patients Infected with Decreased CIP Susceptibility Nontyphoidal *Salmonella*

Study	Underlying Condition	*Salmonella* Serovar	Infection	MIC Following CIP Treatment (μg/mL) CIP	NAL	Ciprofloxacin Treatment Dose	Outcome
Boswell et al [36]	Spherocytosis	*S.* Virchow	Gastroenteritis	0.75	Resistant	500 mg p.o. b.i.d., 14 d	*Salmonella* eradicated by 7 d course p.o. trimethoprim
Vasallo et al [37]	Diabetes	*S.* Enteritidis	Bacteremia	1.0	NA	200 mg i.v. b.i.d., 12 d	*Salmonella* eradicated by imipenem therapy
Vasallo et al [37]	AIDS	*S.* Enteritidis	Bacteremia + Septic Arthritis	0.5	NA	750 mg p.o. b.i.d., 12 d	Patient expired
Piddock et al [38, 39]	NA	*S.* Typhimurium	Upper Urinary Tract Infection	2.0	256	500 mg b.i.d., 14 d; formulation not reported	NA
Piddock et al [38, 39]	Aortic Aneurysm Surgery	*S.* Typhimurium	Bacteremia + Wound Infection	0.25	256	200 mg i.v. b.i.d., 10 d	Patient recovered following 12 w i.v. aztreonam 2 g b.i.d.
Chang et al [40]	Chronic Liver Disease	*S.* Choleraesuis	Bacteremia + Vertebral Osteomyelitis	0.19	>256	300 mg i.v. b.i.d., 14 d, followed by 750 mg p.o. b.i.d for 7 d	Patient recovered following CIP + cefotaxime therapy
de Toro et al [68]	NA	*S.* Typhimurium	Gastroenteritis	0.5	16	7 d (no dosage provided)	NA

Abbreviations: b.i.d., twice daily; CIP, ciprofloxacin; IV, intravenous; MIC, minimum inhibitory concentration; NA, not available; NAL, nalidixic acid; p.o., per os.

Clinical Infectious Diseases 2012;55(8):1107–13



PLoS Negl Trop Dis. (2011)

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (μg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Ciprofloxacin	0.25	0.5	5	26	24
Ciprofloxacin, *Salmonella* spp.[1]	0.06	0.06		Note[A]	Note[A]
Pefloxacin (screen), *Salmonella* spp.[1]	NA	NA	5	24[B]	24[B]
Levofloxacin	0.5	1	5	23	19
Moxifloxacin	0.25	0.25	5	22	22
Nalidixic acid (screen)	NA	NA		NA	NA

Notes

Numbered notes relate to general comments and/or MIC breakpoi
Lettered notes relate to the disk diffusion method.

1. There is clinical evidence for ciprofloxacin to indicate a poor response in syste low-level ciprofloxacin resistance (MIC >0.06 mg/L). The available data relate ma reports of poor response with other *Salmonella* species.

A. Tests with a ciprofloxacin 5 μg disk will not reliably detect low-level resistance resistance in *Salmonella* spp., use the pefloxacin 5 μg disk. **See Note B.**
B. Susceptibility of *Salmonella* spp. to ciprofloxacin can be inferred from pefloxac

EUCAST Clinical Breakpoint Tables v. 8.1, valid from 2018-05-15

Clin Microbiol Infect 2013; 19: 141–160

Test di sensibilità

Performance of Etest and Disk Diffusion for Detecti
Ciprofloxacin and Levofloxacin Resistance in *Salmo*

TABLE 3 Performances of ciprofloxacin and levofloxacin Etests and disk diffusion compared to that of BMD for testing S
($n = 135$)

Method and antibiotic	No. of isolates[a]				Performance (no. [%])[b]		
	Total	Susc	Int	Res	EA	CA	mE
Etest							
Ciprofloxacin	135	25	89	21	131 (97.0)	121 (89.6)	14 (10.
Levofloxacin	135	24	98	13	125 (92.6)	112 (83.7)	22 (16.
Disk diffusion							
Ciprofloxacin	135	25	89	21	NA	119 (88.2)	16 (11.
Levofloxacin	135	24	98	13	NA	127 (94.1)	8 (5.9)

Development of a Pefloxacin Disk Diffusion Method for Detection of Fluoroquinolone-Resistant *Salmonella enterica*

TABLE 2 Ranges of inhibition zone diameters for various quinolone disks versus ciprofloxacin susceptibility and fluoroquinolone resistance mechanisms for 126 isolates tested at three laboratories (756 readings per disk)

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (μg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Ciprofloxacin	0.25	0.5	5	26	24	**1.** There is clinical evidence for ciprofloxacin to indicate a poor response in systemic infections caused by *Salmonella* spp. with low-level ciprofloxacin resistance (MIC >0.06 mg/L). The available data relate mainly to *Salmonella* Typhi but there are also case reports of poor response with other *Salmonella* species.
Ciprofloxacin, *Salmonella* spp.[1]	0.06	0.06		Note[A]	Note[A]	
Pefloxacin (screen), *Salmonella* spp.[1]	NA	NA	5	24[B]	24[B]	
Levofloxacin	0.5	1	5	23	19	**A.** Tests with a ciprofloxacin 5 μg disk will not reliably detect low-level resistance in *Salmonella* spp. To screen for ciprofloxacin resistance in *Salmonella* spp., use the pefloxacin 5 μg disk. **See Note B.**
Moxifloxacin	0.25	0.25	5	22	22	**B.** Susceptibility of *Salmonella* spp. to ciprofloxacin can be inferred from pefloxacin disk diffusion susceptibility.
Nalidixic acid (screen)	NA	NA		NA	NA	
Norfloxacin (uncomplicated UTI only)	0.5	1	10	22	19	
Ofloxacin	0.25	0.5	5	24	22	

Ofloxacin	5	24–35	15–27	24–35	16–25	15–27	19–21	21
Pefloxacin	5	24–34	6–24	24–34	11–24	6–23	14–16	0.3

[a] Overlap in zone diameter between isolates without and with resistance mechanisms.

[b] One isolate with no identified FQ resistance mechanism and a ciprofloxacin MIC of ≤0.064 mg/liter had a 2-μg norfloxacin inhibition zone diameter of 21 mm. Including this isolate would result in a 14% overlap.

Challenges to accurate susceptibility testing and int of quinolone resistance in Enterobacteriaceae: res Spanish multicentre study

Table 5. Description of errors in the inferred resistance mechanisms

		Wrong inferred mechanisms
Strain (characteristics)	no. (%)	description of mistak
CC-00 (WT)	0 (0)	no mistakes
CC-01 [GyrA 1, *aac(6′)-Ib-cr*]	34 (54.8)	multiple QRDR modifications (12); PMQR not de
CC-02 (*qnrA1*)	15 (24.2)	single QRDR modification at GyrA and PMQR no
CC-03 [*qnrS2*, *aac(6′)-Ib-cr*]	7 (11.3)	single QRDR modification at GyrA and PMQR no efflux pump phenotype and PMQR not detec
CC-04 (*qnrB48*)	10 (16.1)	single QRDR modification at GyrA and PMQR no efflux pump phenotype and PMQR not detec
CC-05 [GyrA 2, ParC 1, *aac(6′)-Ib-cr*, *oqxAB*]	0 (0)	no mistakes
CC-06 (GyrA 2, ParC 1, *qepA1*)	0 (0)	no mistakes
CC-07 (GyrA 1, OmpK35^{-}, efflux)	6 (9.6)	PMQR (6)
CC-08 (GyrA 1, ParC 1)	4 (6.5)	PMQR (4)
CC-09 (GyrA 1, ParC 1, MarR^{-})	5 (8)	PMQR (5)

Table 3. Distribution of discrepancies and categorical error rates by antimicrobial agent tested

Quinolone (*n*)	Percentage of discrepancies in RCC/ICC[b,c]	Percentage of errors in RCC/ICC (*n*)[a]											
		all methods and devices (2305)			MicroScan WalkAway (919)			Wider (162)			Vitek 2 (520)		
		mE[c]	ME[d]	VME[e]	mE[c]	ME[d]	VME[c]	mE[c]	ME[d]	VME[e]	mE[c]	ME[d]	VME[e]
CIP (805)	19.1/42.0	14.4/41.7	7.4/0	0.3/0.6	17.9/43.1	12.4/0	0.7/1.4	8.3/48.5	4.3/0	0/0	7.5/38.5	1.9/0	0/0
LVX (243)	13.5/53.3	9.8/53.3	4.7/0	0/0	3.7/68.5	0/0	0/0	0/0	0/0	0/0	0/21.4	18.1/0	0/0
MXF (77)	25.9/36.7	22.1/35.7	3.4/0	6.6/2.9	15.4/28	0/0	0/0	0/35.3	0/0	0/0	0/0	0/0	0/0
OFX (354)	28.5/37.8	21.4/37.8	22.2/0	6.6/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
NOR (42)	27.4/43.1	20/43.1	8.8/0	1.8/0	23/46.3	9.2/0	1.2/0	31.5/57.8	18.2/0	0/0	0/0	0/0	0/0
All FQs (1521)	20.7/43.2	15.6/43.0	7.3/0	1.4/0.1	17.7/45.6	8.3/0	0.6/0.4	13.2/48.1	7.4/0	0/0	7.1/37.6	3.0/0	0/0
NAL (784)	7/10.8	1.1/2.2	19.1/27.1	0/0.4	0/0.6	9.2/20.4	0/0.8	0/1.4	4.3/4.3	0/0	0/1.9	34.2/40.7	0/0
All agents (2305)	16.1/31.5[f]	10.6/28.3[f]	9.6/18.1	0.7/0.5	11.3/29	9.7/13.8	0.4/1	7.4/29.3	6.5/2.8	0/0	3.6/19.8	12.8/26.7	0/0

CIP, ciprofloxacin; LVX, levofloxacin; MXF, moxifloxacin; OFX, ofloxacin; NOR, norfloxacin; FQs, fluoroquinolones; NAL, nalidixic acid.
[a]RCC and ICC results from participating laboratories were compared with reference centre values and discrepancies were classified as mE, ME and VME, following standard criteria.[33]
[b]The most favourable CC for the evaluated centre was chosen when the MIC value (obtained by the reference centres) gave rise to two different CCs according to EUCAST/CLSI breakpoints. In this case, the breakpoints chosen were those most in concordance with the CC issued by the evaluated centre.
[c]The denominator is the number of susceptibility-testing determinations per antibiotic.
[d]The denominator is the number of susceptible strains per antibiotic.
[e]The denominator is the number of resistant strains per antibiotic.
[f]The denominator is the total number of susceptibility determinations.

False Susceptibility to Amikacin by V
in *Acinetobacter baumannii* Harborin

Table 1. Results of AN susceptibility test of 3 *armA*-harboring isolates by VITEK 2/ disk diffusion tes

Strains	VITEK 2 / disk diffusion test	Agar dilution

Annals of Clinical & Laboratory Science, vol. 40, no. 2, 2010

Discrepant susceptibility to gentamicin despite amil
resistance in *Klebsiella pneumoniae* by VITEK 2 repr
susceptibility associated with the *armA* 16S rRNA m
gene

Patient no.	Specimen	VITEK 2		Broth microdilution		Antibiotic resistance genes			
		AMK	GEN	AMK	GEN	16S rRNA methylase	*AME*	ESBL*	Carbapenemase
1	Blood	≥64	4	32	2	–	*AAC(6′)-Ib*	Positive	–
2	Blood	≥64	4	>128	>256	*armA*	*AAC(6′)-Ib*	–	NDM-1
3	Blood	≥64	4	>128	>256	*armA*	*AAC(6′)-Ib*	–	NDM-1, OXA-232
4	Blood	≥64	8	>128	>256	*armA*	*AAC(6′)-Ib*	–	NDM-1
5	Blood	≥64	8	>128	>256	*armA*	*AAC(6′)-Ib*	–	–
6	Urine	≥64	4	>128	>256	*armA*	*AAC(6′)-Ib*	–	NDM-1, OXA-232
7	Urine	≥64	4	>128	>256	*armA*	*AAC(6′)-Ib*	–	NDM-1, OXA-232
8	Urine	≥64	4	>128	>256	*armA*	*AAC(6′)-Ib*	–	NDM-1, OXA-232
9	Urine	≥64	2	>128	>256	*armA*	*AAC(6′)-Ib*	–	NDM-1, OXA-232
10	Urine	≥64	4	>128	>256	*armA*	*AAC(6′)-Ib*	–	NDM-1, OXA-232
11	Urine	≥64	2	>128	>256	*armA*	*AAC(6′)-Ib*	–	NDM-1, OXA-232
12	Stool	≥64	8	>128	>256	*armA*	*AAC(6′)-Ib*	–	–

*Presented by the VITEK 2 system.

Ko *et al.*. *Journal of Medical Microbiology* 2017:66

Accuracy of different methods for susceptibility testing of gentamicin with KPC carbapenemase-producing *Klebsiella pneumoniae*

Fabio Arena [a], Tommaso Giani [a], Guendalina Vaggelli [b], Giovanni Terenzi [c], Patrizia Pecile [b], Gian Maria Rossolini [a,b,c,*]

Table 1
Results of gentamicin susceptibility testing with 57 KPC-KP using reference BMD, BMD with TREK panel, Vitek2, and Etest.

Method	MIC (μg/mL)				Category (%)			EA (%)	CA (%)	mD (%)	MD (%)	VMD (%)
	≤1	2	4	>4	S	I	R					
BMD reference	46	6	1	4	52	1	4	NA	NA	NA	NA	NA
BMD TREK panel	11	35	7	4	46	7	4	52 (91.2)	49 (86)	8 (14)		
Vitek2	10	5	42	0	15	42	0	18 (31.6)	12 (21)	41 (71.9)		4 (100)
Etest	4	23	26	4	27	26	4	35 (61.4)	30 (52.6)	27 (47.4)		

Interpretation of MIC results was according to EUCAST breakpoint table (EUCAST, 2014).

Aminoglycoside Resistance and Susceptibility Testing

Acinetobacter baumannii-calcoaceticus Comple

TABLE 3. Frequency of aminoglycoside-modifying enzyme genotypes, their predicted substrate specificity, numbe microdilution method, and correlation of PFGE types of 107 clinical isolates of *Acinetobacter baumannii-cal*

Genotype	No. (%) of isolates	Subtrate(s)[a]	No. of isolates susceptible to[a]:			PFGE[b]
			GEN	TOB	AMK	
aph(3′)-Ia + ant(2″)-Ia	38 (35.5)	GEN, TOB	0	1	2	1 (31), 8 (1),
ant(2″)-Ia	23 (21.5)	GEN, TOB	0	0	1	2 (15), 3 (5),
aac(6′)-Ih + aph(3′)-Ia	11 (10.3)	TOB, AMK	0	9	9	4 (4), 6 (1), 7
aac(3)-Ia	9 (8.4)	GEN	3	7	3	5 (6), 16 (1),
aac(6′)-Ih	6 (5.6)	TOB, AMK	0	5	0	5 (6)
aac(3)-IIa	4 (3.7)	GEN, TOB	0	0	0	14 (3), 20 (1)
aac(3)-Ia + aph(3′)-Ia	4 (3.7)	GEN	0	2	2	1 (1), 4 (2), 6
aac(3)-Ia + ant(2″)-Ia	2 (1.9)	GEN, TOB	0	0	0	9 (2)
aac(3)-Ia + aph(3′)-Ia + aph(3′)-VI	2 (1.9)	GEN	0	1	0	7 (2)
aac(3)-Ia + aph(3′)-VI	1 (0.9)	GEN, AMK	0	1	0	4 (1)
aac(6′)-Ib + ant(2″)-Ia	1 (0.9)	GEN, TOB, AMK	0	0	0	12 (1)
aac(6′)-Ih + aph(3′)-VI	1 (0.9)	TOB, AMK	0	1	0	15 (1)
aph(3′)-Ia + aph(3′)-VI	1 (0.9)		1	1	0	1 (1)
aph(3′)-VI + ant(2″)-Ia	1 (0.9)	GEN, TOB, AMK	0	0	0	2 (1)
aac(3)-Ia + aph(3′)-Ia + ant(2″)-Ia	1 (0.9)	GEN, TOB	0	0	0	1 (1)

TABLE 2. MICs and error rates obtained for 107 isol *Acinetobacter baumannii-calcoaceticus* complex test susceptibility to three aminoglycosides by six met

Drug and method	MIC (μg/ml)		% S[a]	VME[b] (%)	ME[c] (%
	50%	90%			
Amikacin					
DD[e]			16.8	0.9	0
ET[f]	128	≥256	16.8	0.9	0
MicroScan	≥32	≥32	15.9	0.9	0.9
Phoenix	≥32	≥32	21.5	5.6	0
Vitek 2	≥16	≥64	53.3	36.4	0
BMD[g]	128	≥256	16.8		
Gentamicin					
DD			4.7	0	0
ET	≥256	≥256	4.7	0	0
MicroScan	≥8	≥8	4.7	0	0
Phoenix	≥8	≥8	5.6	0	0.9
Vitek 2	≥16	≥16	4.8	0	0
BMD	≥32	≥32	4.7		
Tobramycin					
DD			27.1	2.8	1.9
ET	24	≥64	28.0	2.8	1.9
MicroScan	≥8	≥8	27.1	1.9	2.8
Phoenix	≥8	≥8	30.8	5.6	0.9
Vitek 2	8	≥16	43.8	13.1	0.0
BMD	≥32	≥32	27.1		

Nuove molecole

Plazomicina (ZEMDRI™)

- Approvazione FDA (Giugno 2018) per infezioni complicate delle vie urinarie, incluse pielonefriti, negli adulti
- Richiesta approvazione EMA (Ottobre 2018) per infezioni complicate delle vie urinarie, sepsi da Enterobacteriaceae, e altre infezioni da Enterobacteriaceae con limitate opzioni terapeutiche.
- 15 mg/kg die IV
- Tossicità:
  - Renale
  - Ototossicità
  - Neuromuscolare

Plazomicina (ZEMDRI™)

Vantaggi

- Non è inibito dalla maggior parte degli enzimi che modificano gli aminoglicosidi gentamicina, amicacina e tobramicina, inclusi acetiltransferasi, fofotransferasi e nucleotidiltransferasi.

Plazomicina (ZEMDRI™)

Vantaggi

Table 2 Activities of plazomicin against 300 MDR enterobacterial isolates with different resistance phenotypes

Species	Phenotype	No. of isolates	MIC (μg/ml)						
			0.25	0.5	1	2	4	8	16
Klebsiella pneumoniae	KPC	25		7	12	5	1		
	ESBL, KPC	113		15	69	26	3		
	VIM	32		4	14	10	4		
	ESBL, VIM	43		8	22	9	4		
	KPC, VIM	10			7	3			
	ESBL, KPC, VIM	4			3	1			
	ESBL	14			9	4	1		
	Total	241		34	136	58	13		
Escherichia coli	KPC	9		3	4	2			
	VIM	5		2	3				
	ESBL, VIM	4	1		3				
	ESBL	15		3	8	4			
	Total	33	1	8	18	6			
Enterobacter aerogenes	KPC	1			1				
	ESBL, KPC	1		1					
	VIM	5			4	1			
	Total	7		1	5	1			
Enterobacter cloacae	KPC	2		1	1				
	VIM	15		8	6	1			
	KPC, VIM	1							
	ESBL, VIM	1			1				
	Total	19		10	8	1			

L'attività di plazomicina è stata dimostrata *in vitro* verso Enterobacteriaceae in presenza di alcune beta-lattamasi, incluse cefalosporinasi (TEM, SHV, CTX-M, AmpC), carbapenemasi (KPC-2, KPC-3), e oxacillinasi (OXA-48).

Plazomicina (ZEMDRI™)

Limiti

- Inattivo contro isolati che producono la 16S rRNA metilasi.
- Ridotta attività verso Enterobacteriaceae che iperesprimono alcune pompe di efflusso (es. acrAB-tolC) o ipoesprimono porine di membrana (es, ompF or ompK36).
- Poco attivo verso streptococchi, enterococchi, anaerobi, *Stenotrophomonas maltophilia* e *Acinetobacter* spp e attività variabile verso *Pseudomonas aeruginosa*.

Delafloxacina (Baxdela™)

- Approvazione FDA (Giugno 2017) per infezioni cutanee e dei tessuti molli negli adulti
- Richiesta approvazione EMA (Marzo 2018) per infezioni cutanee e dei tessuti molli negli adulti (Quofenix™)
- Trial fase 3 per il trattamento di polmoniti comunitarie
- 300mg x2/die IV o 450mg x2/die OS
- Tossicità:
  - Tendinite e rottura tendinea
  - Neuropatia periferica

Delafloxacina (Baxdela™)

Vantaggi

- Aumentata penetrazione intracellulare e migliorata attività in microambiente acido (es, ascessi)
- Inibizione enzimatica bilanciata, quindi più basso rischio di mutazioni *in vitro*
- Attivo verso un'ampio spettro di patogeni sia gram-positivi che gram-negativi inclusi MRSA e ceppi resistenti agli altri fluorochinoloni
- Ben tollerato

Delafloxacina (Baxdela™)

Limiti

- Sviluppo di resistenza *in vitro* tramite mutazioni multiple cromosomiche sia nei gram-positivi che nei gram-negativi (multiple step mutations in the QRDRs of gram-positive and gram-negative bacteria)
- Cross-resistenza con altri fluorochinoloni

Take home messages

- L’antibiogramma per gli aminoglicosidi e fluorochinoloni è molto complesso
- I meccanismi di resistenza agli aminoglicosidi e ai fluorochinoloni non sono sempre responsabili di elevati livelli di resistenza
- Criticità metodologiche sprattutto nelle resistenze di basso livello