The Major Mechanisms of Resistance to Aminoglycosides, Macrolides, Fluoroquinolones

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2014 ISF Stephen F. Lowry Colloquium

Intersection between Virulence and Antimicrobial Resistance in the ICU

Paris, France -- 1-3 December 2014

Disclosures and slides availability

- Research grants
  - Theravance, Astellas, Targanta, Cerexa/Forest, AstraZeneca, Bayer, GSK, Trius, Rib-X, Eumedica
  - Belgian Science Foundation (*F.R.S.-FNRS*), Ministry of Health (*SPF*), and Walloon and Brussels Regions
- Speaking fees
  - Bayer, GSK, Sanofi, Johnson & Johnson, OM-Pharma, Cubist
- Decision-making and consultation bodies
  - General Assembly and steering committee (2008-2010) of EUCAST
  - European Medicines Agency (external expert)
  - US National Institutes of Health (grant reviewer)

Slides: http://www.facm.ucl.ac.be → Lectures

The aminoglycosides in short

- The first class of wide-spectrum antibiotics
  (as the result of a first systematic screening of natural sources)
- Concentration-dependent and highly bactericidal
- Active of most aerobic Gram-negative bacteria including *P. aeruginosa*
- For some of them, also active against Mycobacterium tuberculosis and other *Mycobacteriae*
- Synergy with cell-wall acting agents
- Resistance variable between regions and settings but usually low … because of limited use

Discovery by screening

streptomyces grisaeus

Waksman and Fleming …

From the point of view of human benefit, never was a Nobel prize so justifiably awarded as was the award to Selman Waksman for the discovery of streptomycin and other antibiotics produced from *Streptomyces spp*. Waksman and his talented team (many of whom went on to make important antibiotic discoveries in their own right) developed the concept of **systematic screening** of microbial culture products for biological activity, a technology which has provided the foundation of the antibiotic industry, and for this alone his name should rank high in any pantheon of microbiology.

J. Davies: In Praise of Antibiotics, ASM News
http://www.asm.org/memonly/asmnews/may99/feature6.html

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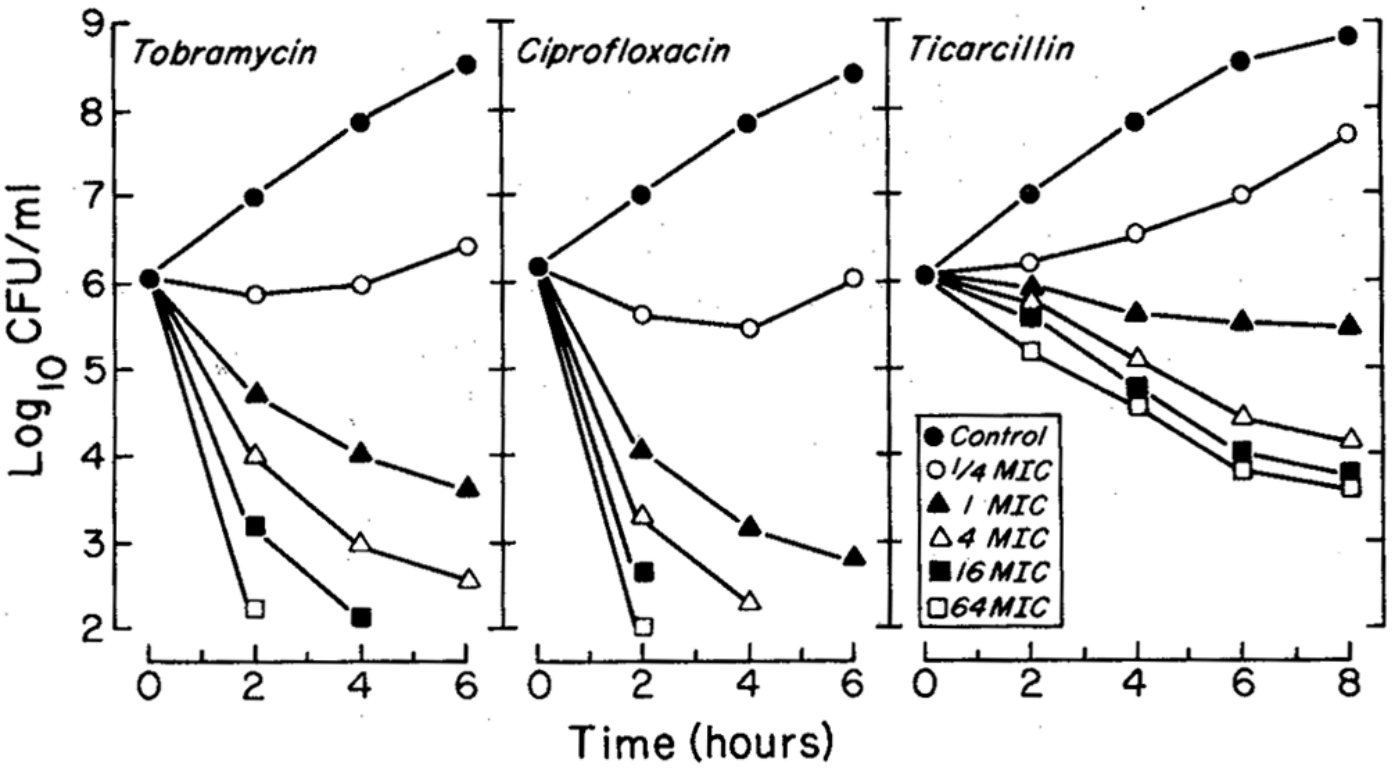
Concentration-dependence



Craig & Ebert SC. Killing and regrowth of bacteria in vitro: A review. Scand J Infect Dis. 1990;74:63-70.

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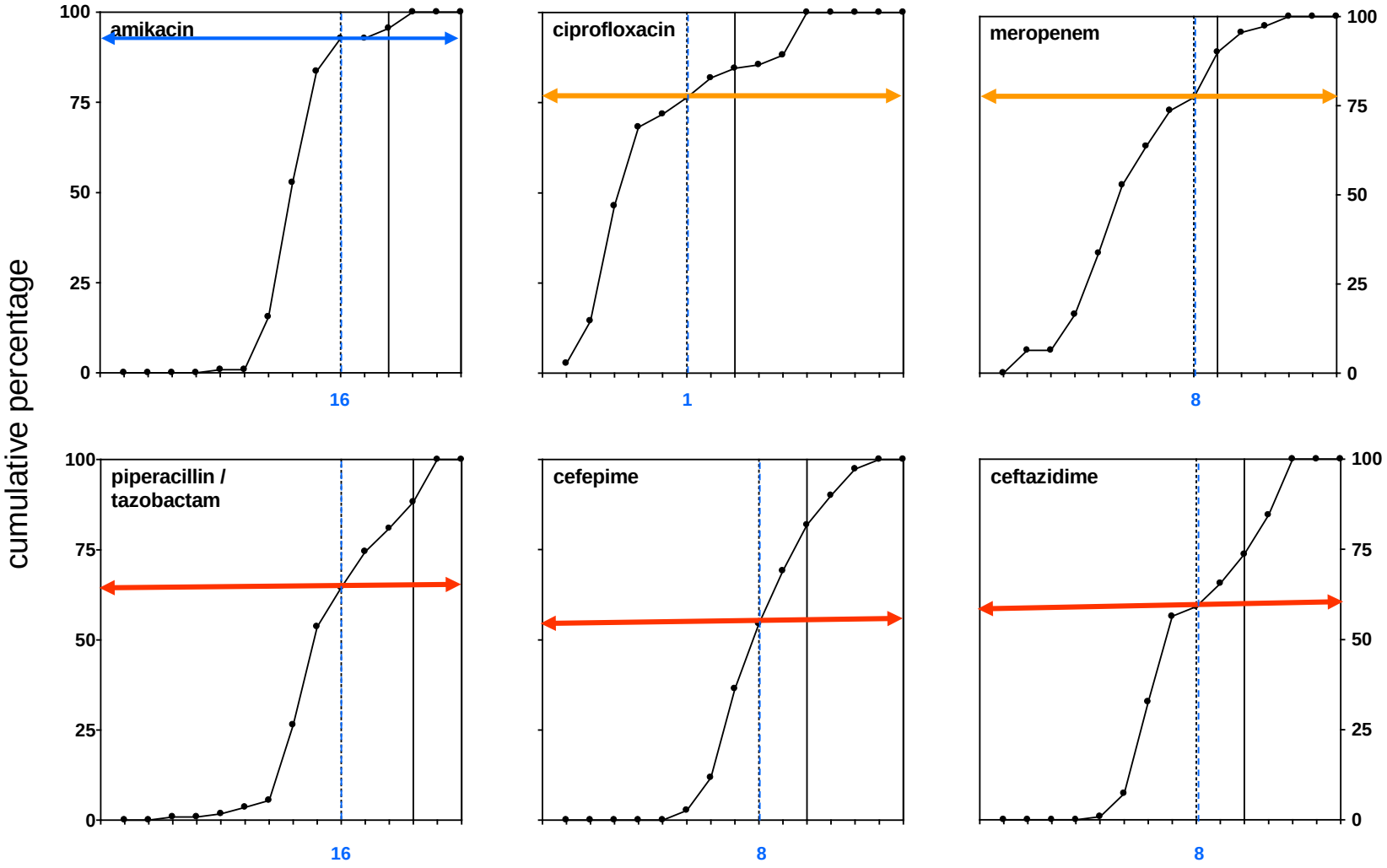
Resistance of *P. aeruginosa* to antibiotics in ICU (VAP patients; Belgian hospitals; 2007-2009)



Aminoglycosides: main mechanisms of resistance

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  - Enterococci (facultative anaerobics)
  - Bacteria with mutations at 16S rRNA (resistant *M. tuberculosis*)
    (includ. point mutations: *M. abcessens*, *M. chloanae*)

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  - Enzymatic modification
  - Methylation of target
  - Small colony variants
  - Biofilms

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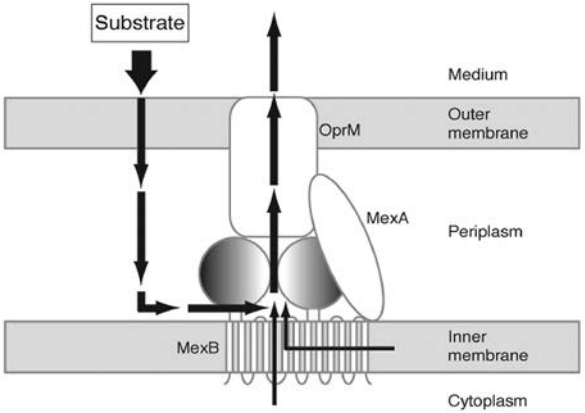
Aminoglycosides: efflux

- Mostly related to the presence tripartite pumps
  (MexXY-OprM in P. aeruginosa; AcrA/B-TolC in *Enterobacteriaceae*; AmrAB in *Burkholderia*…)

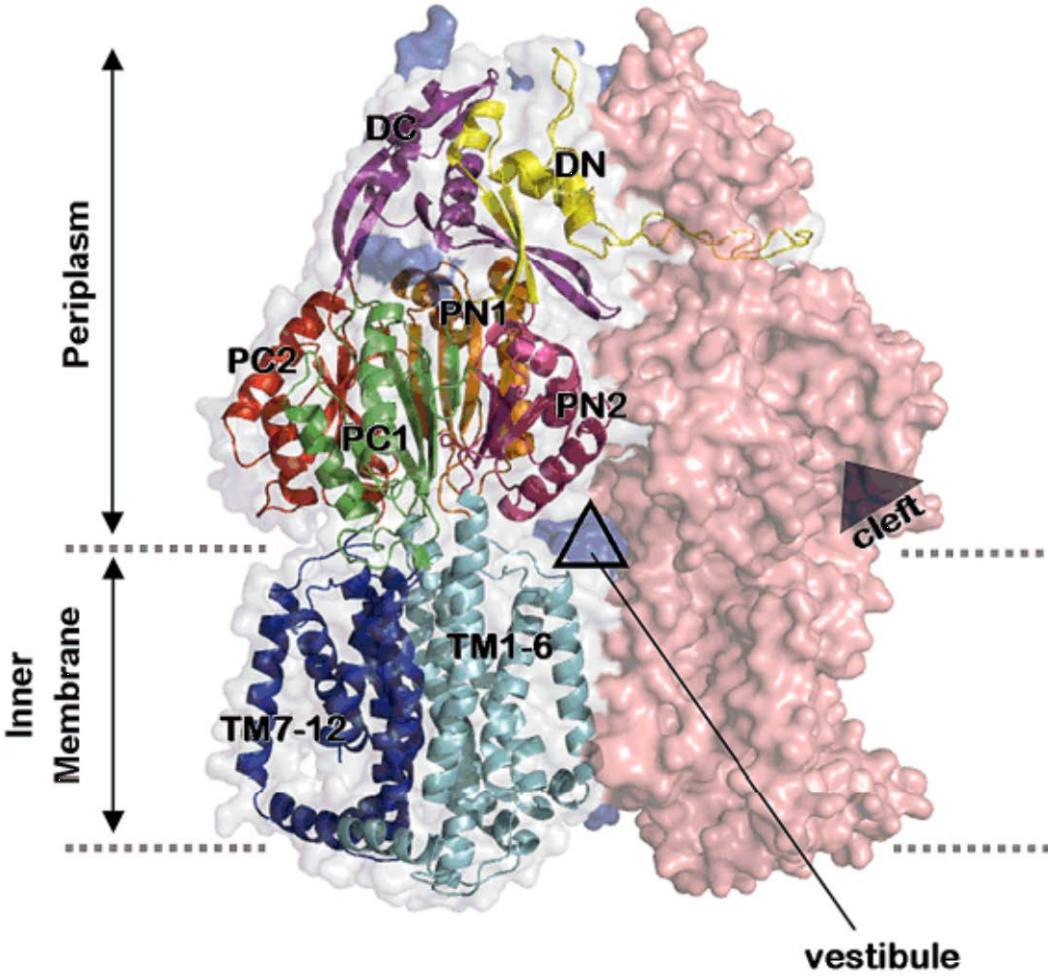
- Recognition may be after association/binding to phopsholipids (amphiphilic complex) but may also involve specific recognition sites

- Probably responsible for
  - Decreased susceptibility during treatment and/or exposure to sub-inhibitory concentrations (overexpression)
  - Adaptative resistance (↘ susceptibility after high peak)

Asymmetric trimer model of MexY
(homology modeling on the crystal structure of *E. coli* AcrB)

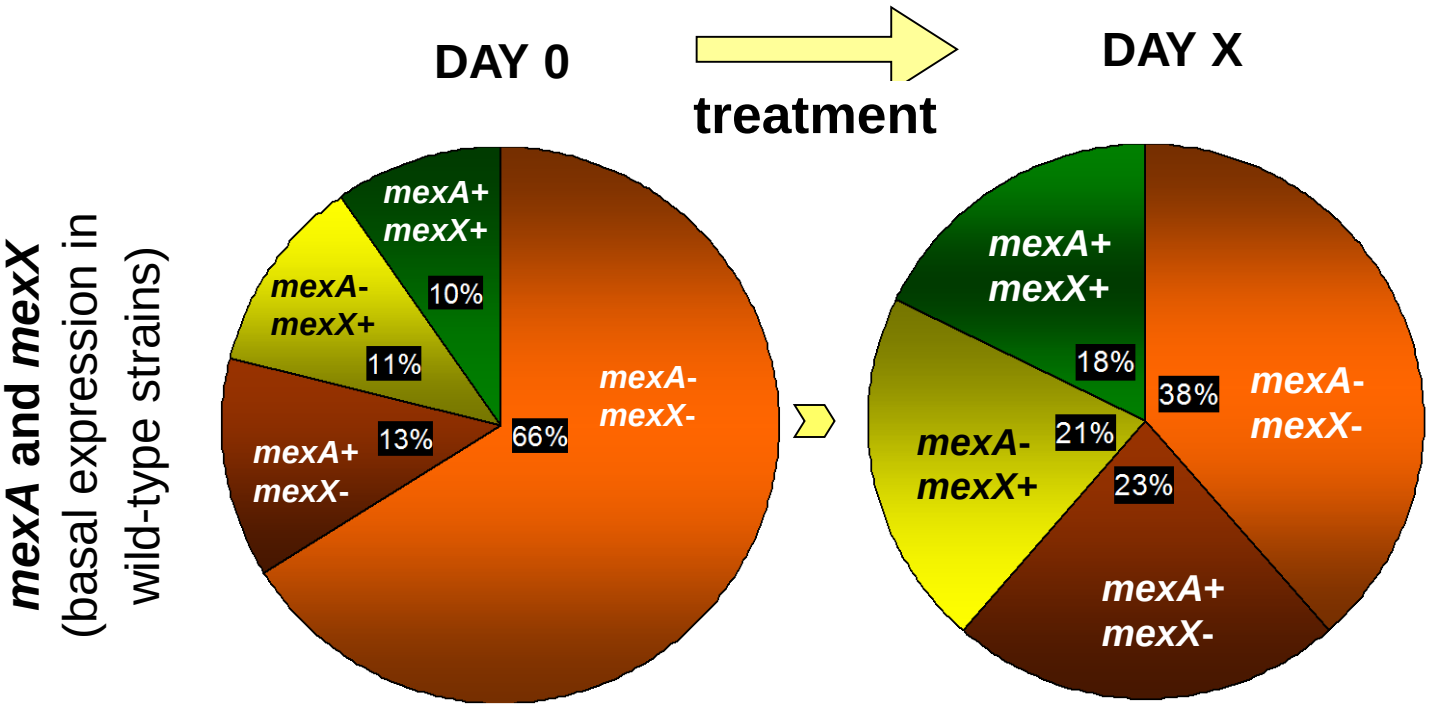


General structure of Mex pumps
Eda S et al. J. Biol. Chem. 2003;278:2085-2088



Lau et al. MBio. 2014 Apr 22;5(2):e01068.

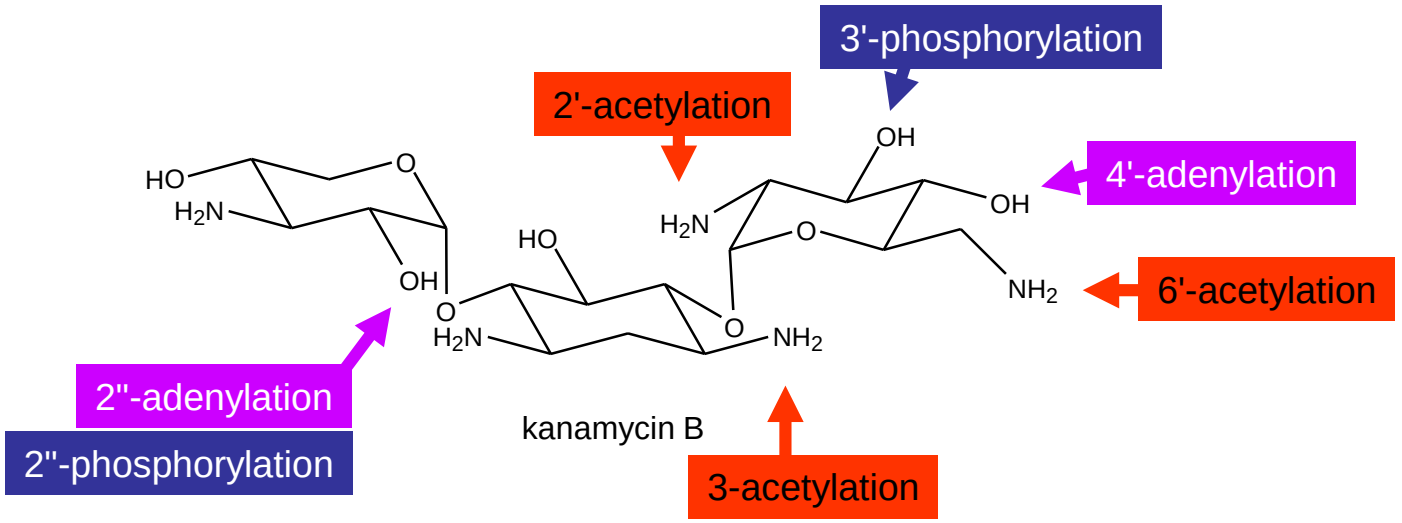
Prevalence of efflux pumps in 62 pairs of *Pseudomonas aeruginosa* collected from ICU patients

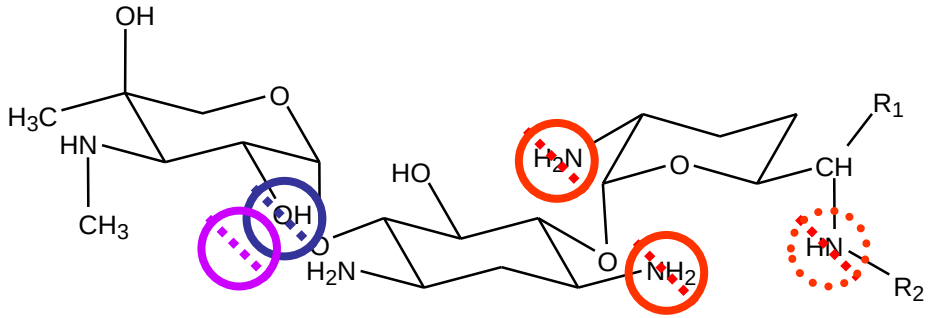


- The prevalence of *mexA*$^+$ and *mexX*$^+$ was already high in first isolates.
- For all genes tested, the number of isolates with overexpression increased during treatment ($P < 0.05$, Fisher's Exact Test 2-sided).

Riou *et al.* 20th ECCMID, 2010, Poster 780
Riou *et al.*, in preparation

Aminoglycosides: enzymatic modification

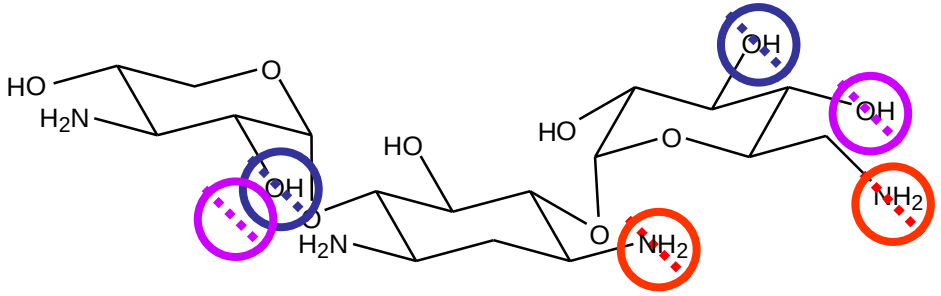




gentamicin C1 : $R_1 = CH_3$; $R_2 = CH_3$

gentamicin C1a: $R_1 = H$; $R_2 = H$

gentamicin C2: $R_1 = CH_3$; $R_2 = H$



kanamycin A

Enzymatic modifications: the situation in the late 90's

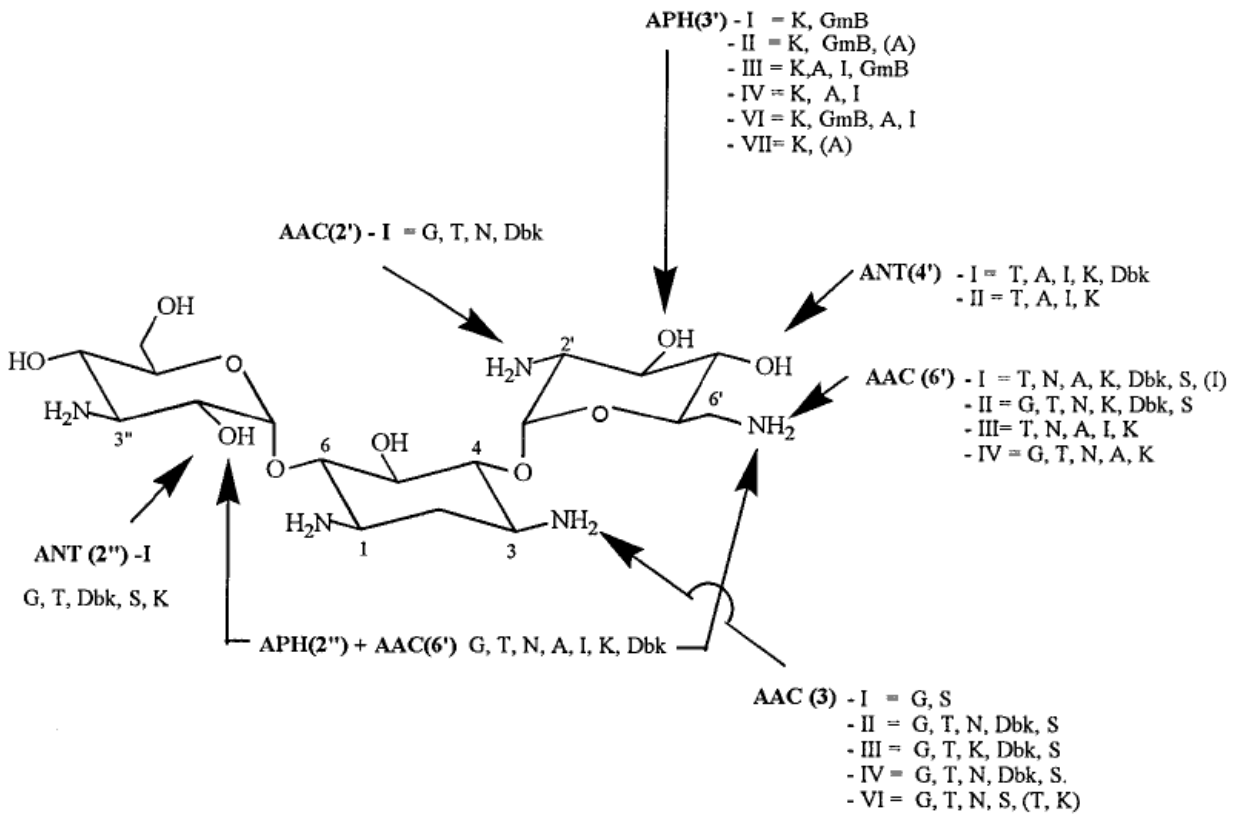


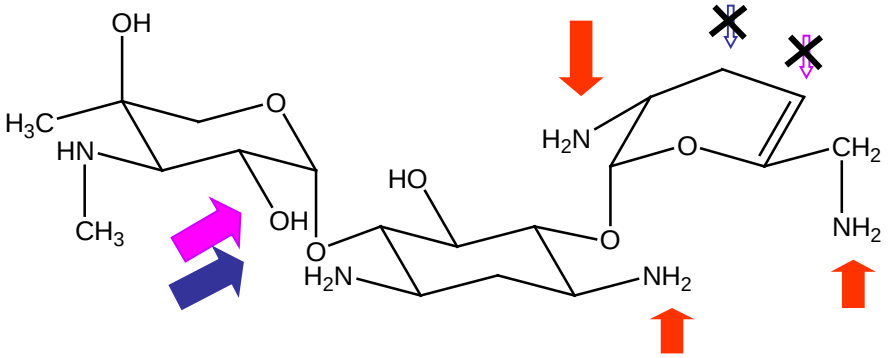
FIG. 3. Major aminoglycoside-modifying enzymes acting on kanamycin B (this aminoglycoside is susceptible to the largest number of enzymes). Each group of enzymes inactivates specific sites, but each of these sites can be acted upon by distinct isoenzymes (roman numerals) with different substrate specificities (phenotypic classification; each phenotype comprises several distinct gene products [denoted by lowercase letters after the roman numeral in the text]); at least one enzyme is bifunctional and affects both positions 2″ (*O*-phosphorylation) and 6′ (*N*-acetylation)). The main clinically used aminoglycosides on which these enzymes act are as follows: amikacin (A), dibekacin (Dbk), commercial gentamicin (G) (see text), gentamicin B (GmB), kanamycin A (K), isepamicin (I), netilmicin (N), sisomicin (S), and tobramycin (T) (see text for discussion of arbekacin, sagamicin, and dactimicin). The drug abbreviations which appear in parentheses are those for which resistance was detectable in vitro even though clinical resistance was not conferred. Based on the data of Shaw et al. (89).

Aminoglycosides: the family
(based on susceptibility to enzymatic resistance mechanisms)

group	typical approved or *in development* antibiotics			
	most susceptible	**less susceptible**	**lesser susceptible**	**least susceptible**
Streptomycins	streptomycin			
Neomycins				
Kanamycins	kanamycin A		→ amikacin	
	kanamycin B	→ tobramycin		
		→ dibekacin		
Gentamicin	"gentamicin" *			
gentamicin B	→	→	→ isepamicin	
Sisomicin	sisomicin	→	→ netilmicin	→ *plazomycin*
Spectinomycin				

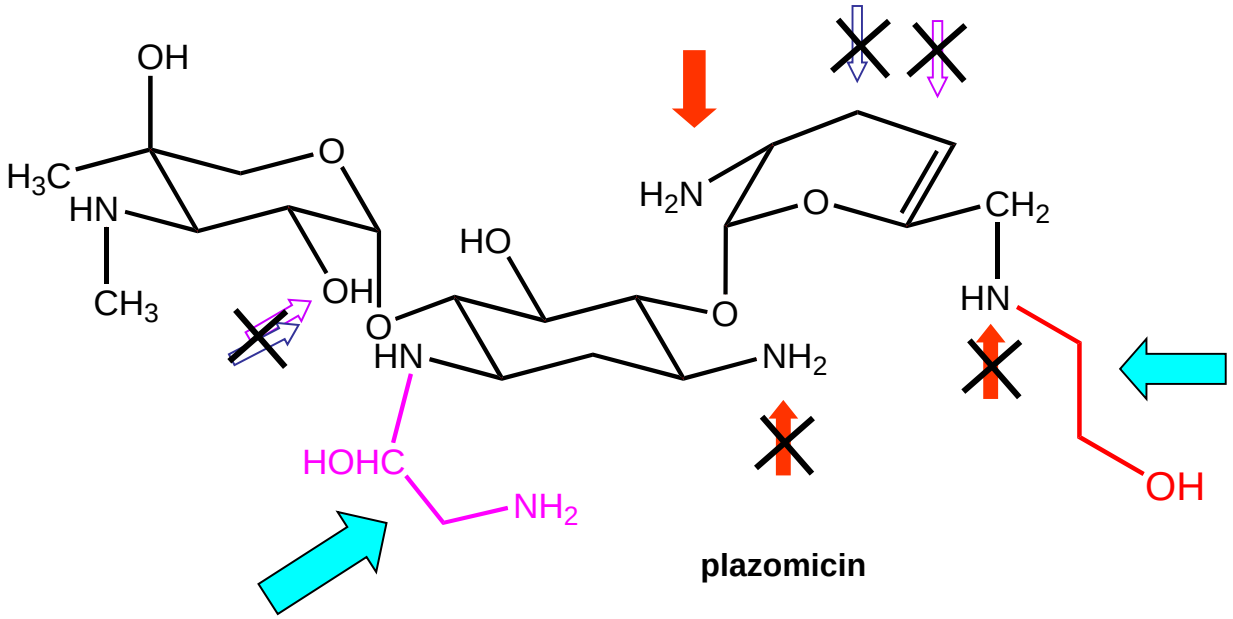
* Mixture of gentamicins C1, C1a, C2 and C2a

Plazomycin…



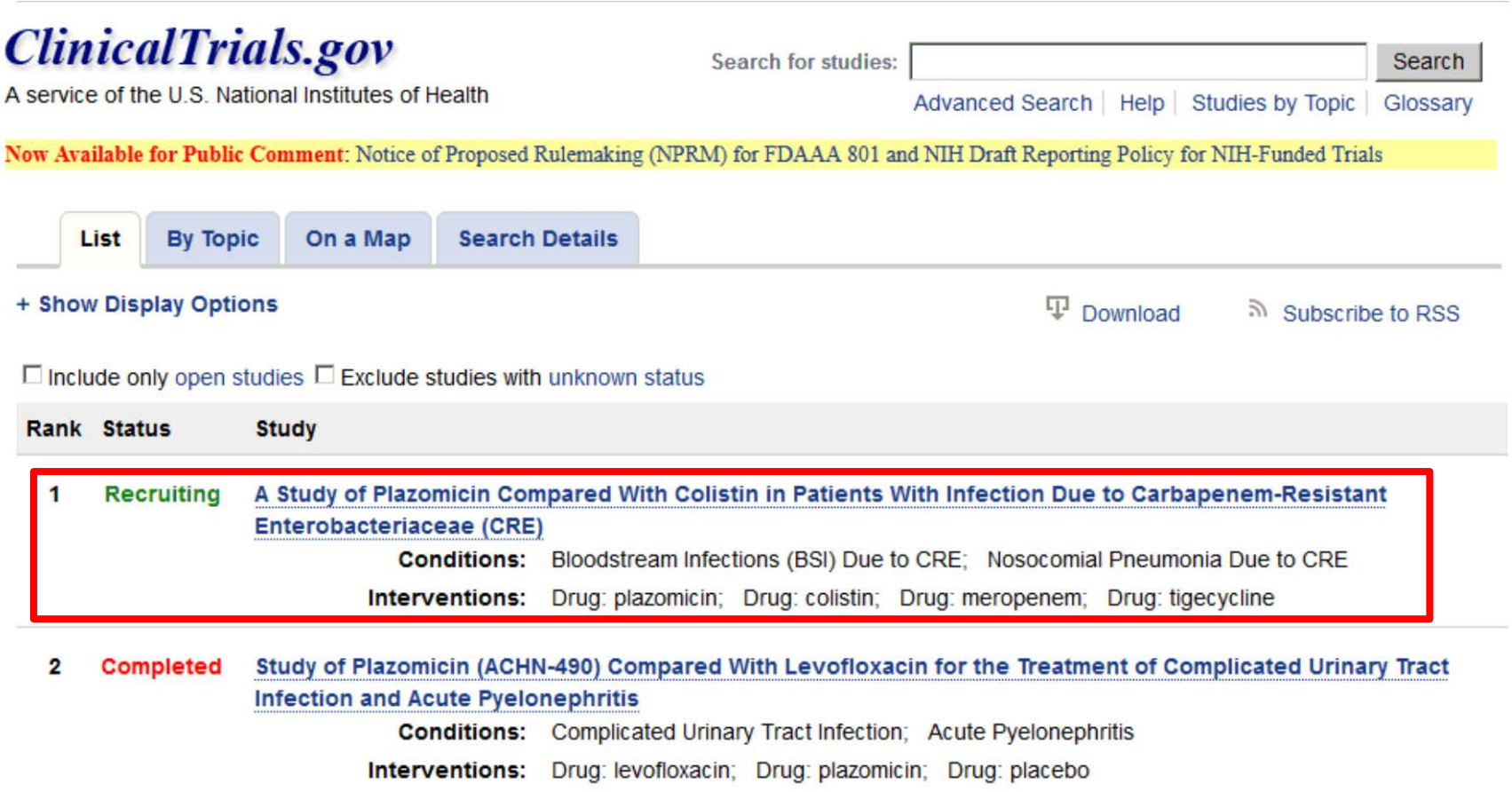
sisomicin

still susceptible
to AAC(2')
(but rare enzyme)



plazomicin

Plazomycin…



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Rank	Status	Study
1	Recruiting	A Study of Plazomicin Compared With Colistin in Patients With Infection Due to Carbapenem-Resistant Enterobacteriaceae (CRE) **Conditions:** Bloodstream Infections (BSI) Due to CRE; Nosocomial Pneumonia Due to CRE **Interventions:** Drug: plazomicin; Drug: colistin; Drug: meropenem; Drug: tigecycline
2	Completed	Study of Plazomicin (ACHN-490) Compared With Levofloxacin for the Treatment of Complicated Urinary Tract Infection and Acute Pyelonephritis **Conditions:** Complicated Urinary Tract Infection; Acute Pyelonephritis **Interventions:** Drug: levofloxacin; Drug: plazomicin; Drug: placebo

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Exogenously acquired 16S rRNA methyltransferase (16S-RMTase) genes responsible for a very high level of resistance against various aminoglycosides have been widely distributed among *Enterobacteriaceae* and glucose-nonfermentative microbes recovered from human and animal.

Wachino & Y. Arakawa, Drug Resist. Updat. 15 (2012) 133–148.

Methylases may be part of the normal bacterial “equipment”

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

Biochimica et Biophysica Acta 1844 (2014) 1648–1655

journal homepage: www.elsevier.com/locate/bbapap

Expansion of the aminoglycoside-resistance 16S rRNA (m^1A1408) methyltransferase family: Expression and functional characterization of four hypothetical enzymes of diverse bacterial origin

Marta A. Witek, Graeme L. Conn *

Department of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322, USA

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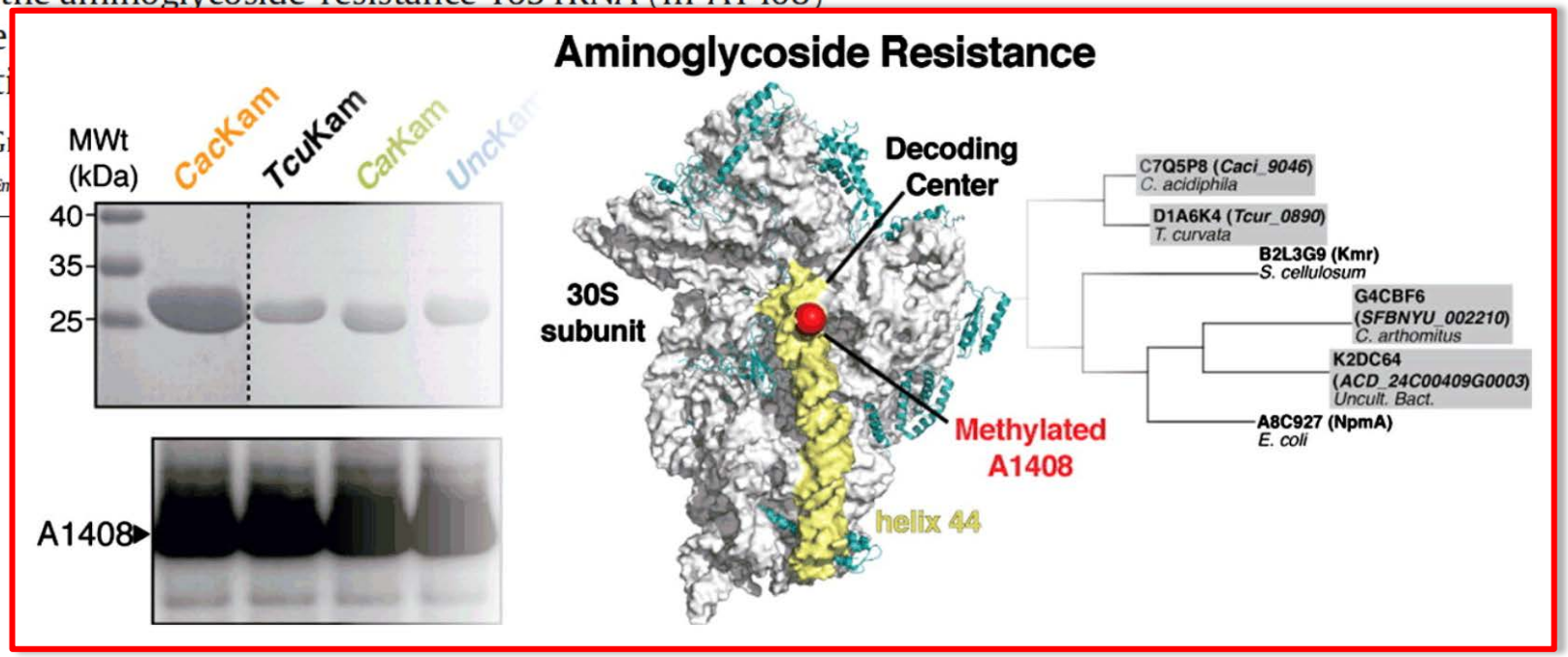
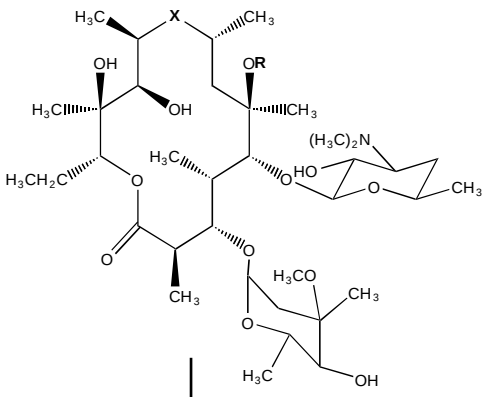


Macrolides

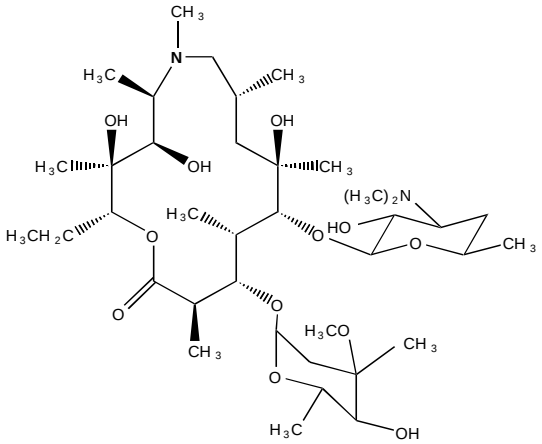
« OLD » MACROLIDES

14-membered

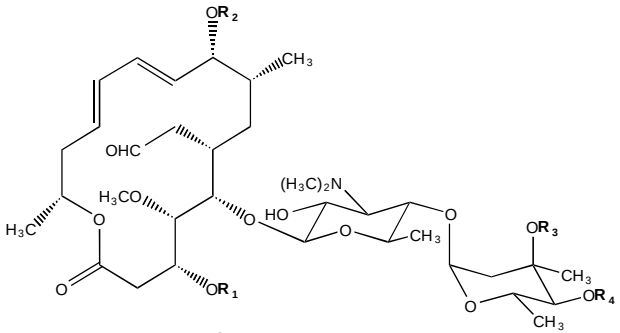


	X	R
Erythromycin	C=O	H
Roxithromycin	**C=N-O-CH_2-O-CH_2-CH_2-O-CH_3**	H
Clarithromycin	C=O	**CH_3**
Erythromycylamine	**C-NH_2**	H

15-membered



16-membered



	R_1	R_2	R_3	R_4
Spiramycin	H	Forosamine	H	H
Josamycin	$COCH_3$	H	H	$COCH_2CH(CH_3)_2$
Miocamycin	$COCH_2CH_3$	$COCH_3$	$COCH_3$	$COCH_2CH_3$
Rokitamycin	H	H	$COCH_2CH_3$	$CO(CH_2)_2CH_3$

Van Bambeke F. In Fundamentals off Antimicrobial Pharmacokinetics and Pharmacodynamics (201, p 257-278 Springer, ISBN 978-0-387-75612-7 - ISBN 978-0-387-75613-4 (eBook)

Macrolides main mechanisms of resistance

- Intrinsic resistance
  - Gram-negative bacteria: impermeability
    (with exceptions and possible modulation by the medium)

- Acquired
  - Efflux
    - *msrA* in Staphylococci (MS_B phenotype)
    - *mefA* in Streptococci and Enterococci
      (dissociation between clindamycin and macrolides)
  - Target mutations
    - dimethylation of the A2058 residue within a conserved region of domain V of the 23S rRNA
      (several genes and cross-resistance with clindamycin)
  - Drug inactivation (phosphotransferases, esterases…)

Can macrolides be active against *P. aeruginosa* ?

Increased Susceptibility of *Pseudomonas aeruginosa* to Macrolides and Ketolides in Eukaryotic Cell Culture Media and Biological Fluids Due to Decreased Expression of *oprM* and Increased Outer-Membrane Permeability

Julien M. Buyck,[1] Patrick Plésiat,[2] H. Traore,[3] F. Vanderbist,[3] Paul M. Tulkens,[1] and Françoise Van Bambeke[1]

[1]Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain, and [3]Laboratoires SMB, Brussels, Belgium; and [2]Laboratoire de Bactériologie, Hôpital Jean Minjoz, Besançon, France

Clinical Infectious Diseases (2012) 55:534-542

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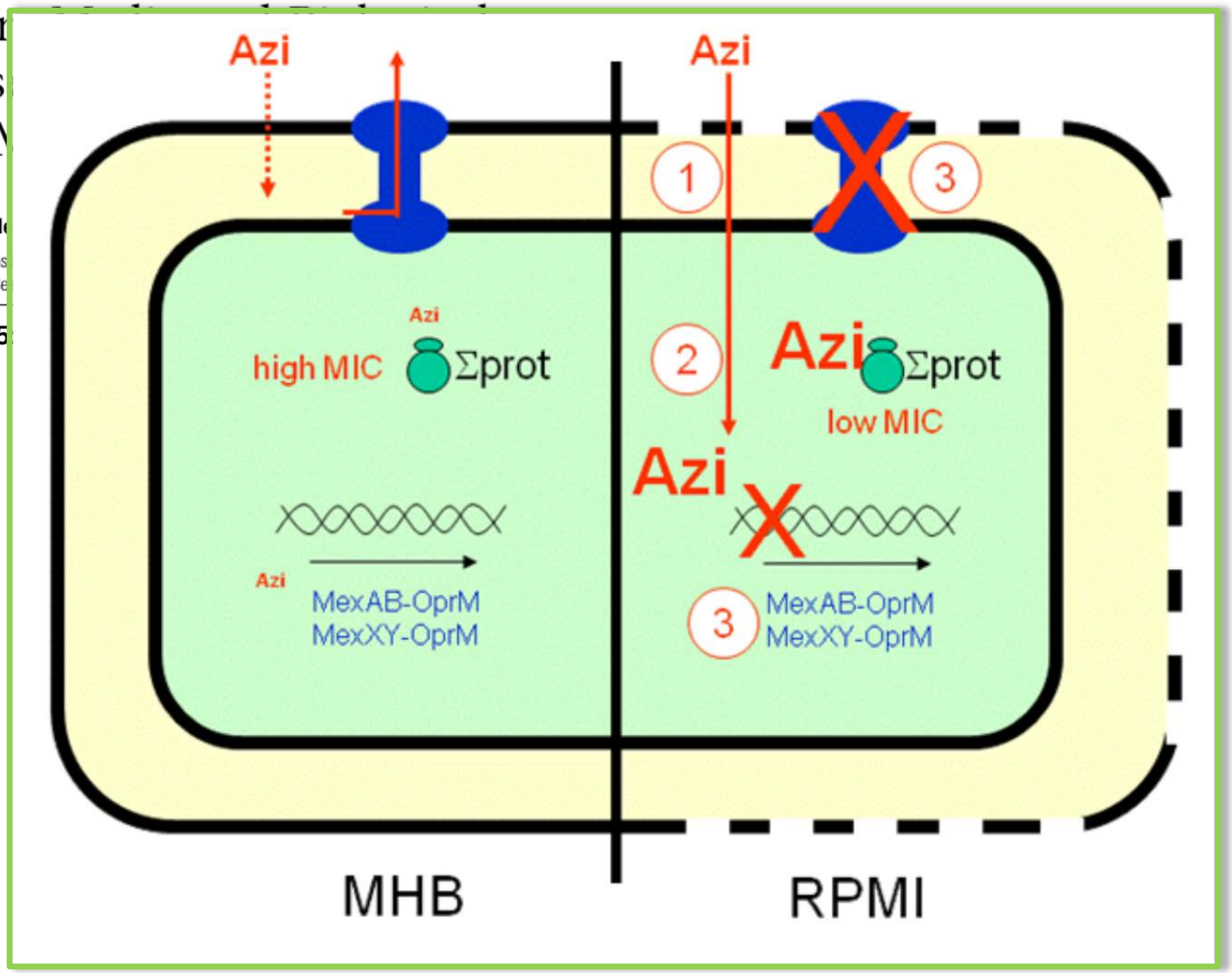
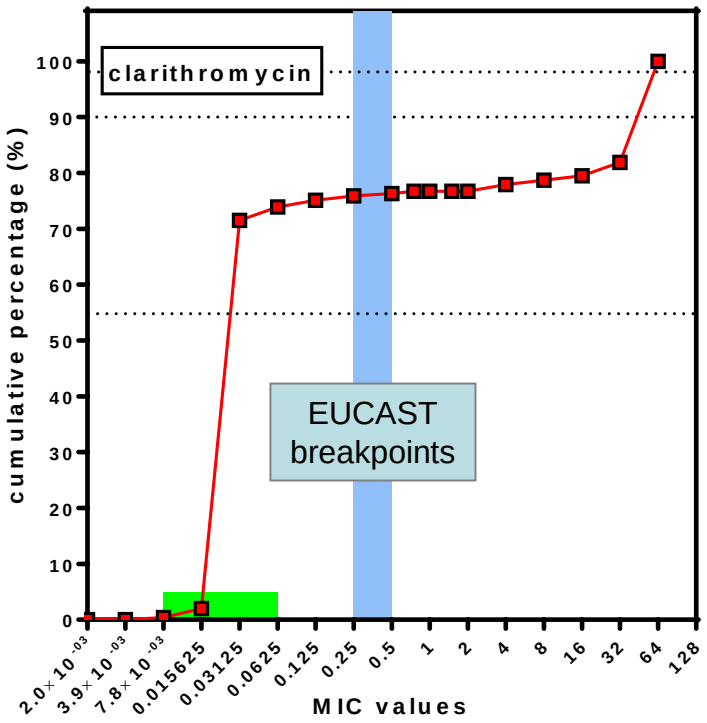
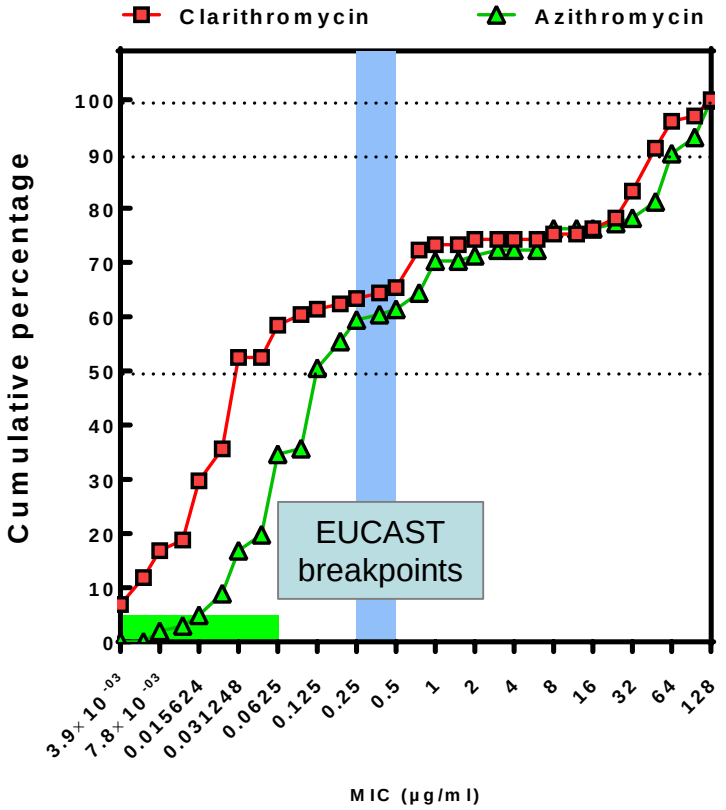


“Old” macrolides are no longer usable for “common” respiratory infections due to *S. pneumoniae*

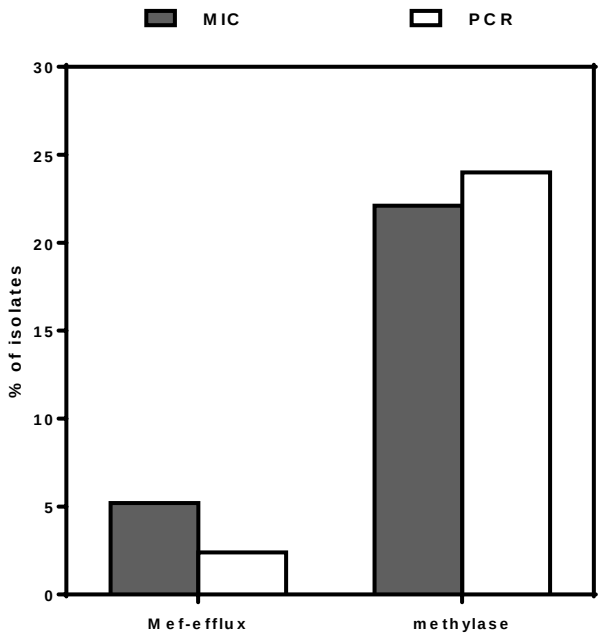


Community-acquired pneumonia 2006-2009
Lismond *et al.* Int J Antimicrob Agents (2012) 39:208– 216

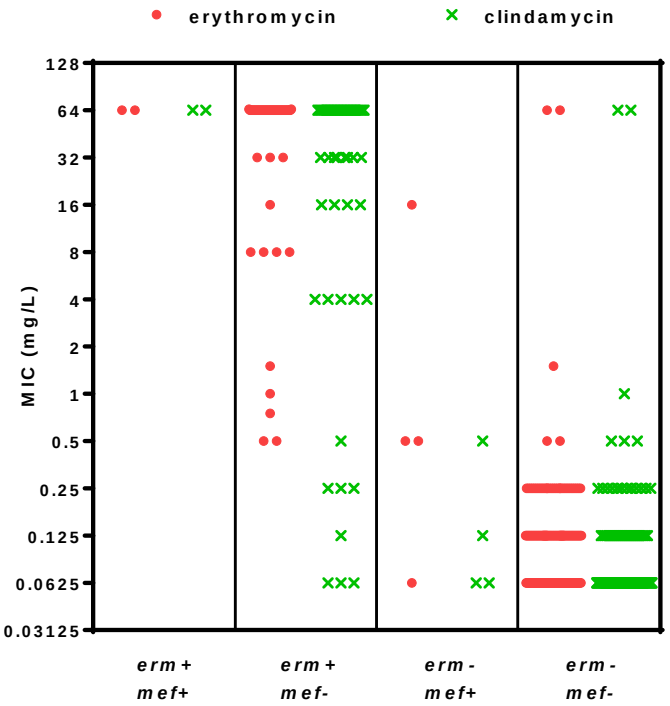


Chronic obstructive pulmonary disease 2010-2013
(bacterial exacerbations)
Vandevelde et al. Int J Antimicrob Agents (2014) 44:209–217

Macrolide resistance: mechanisms…



Most of the resistant isolates in Europe are “high level” and “methylase” positive



But clinical isolates may have both mechanisms… or additional ones

Lismond *et al.* Int J Antimicrob Agents (2012) 39:208– 216

A possible future for macrolides…

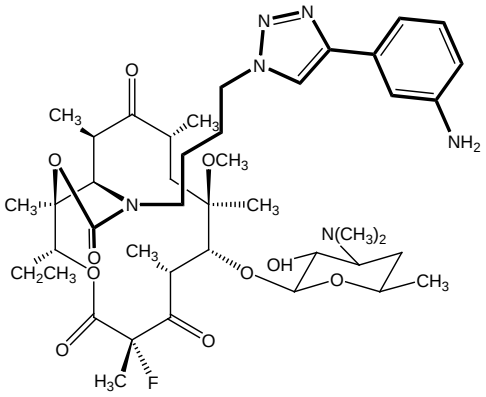
KETOLIDES

11-N-ketolides

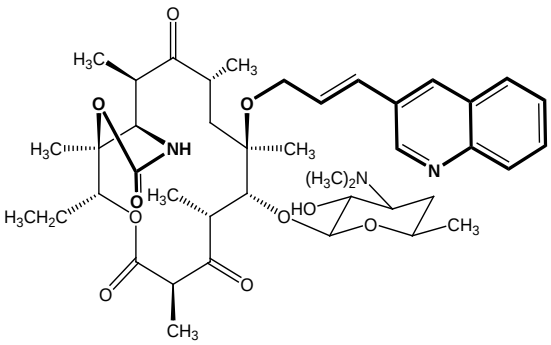
fluoroketolides

6-O-ketolides

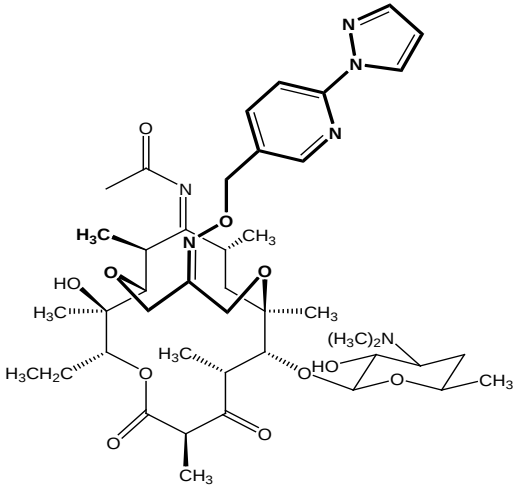
bridged bicyclic ketolides



Solithromycin

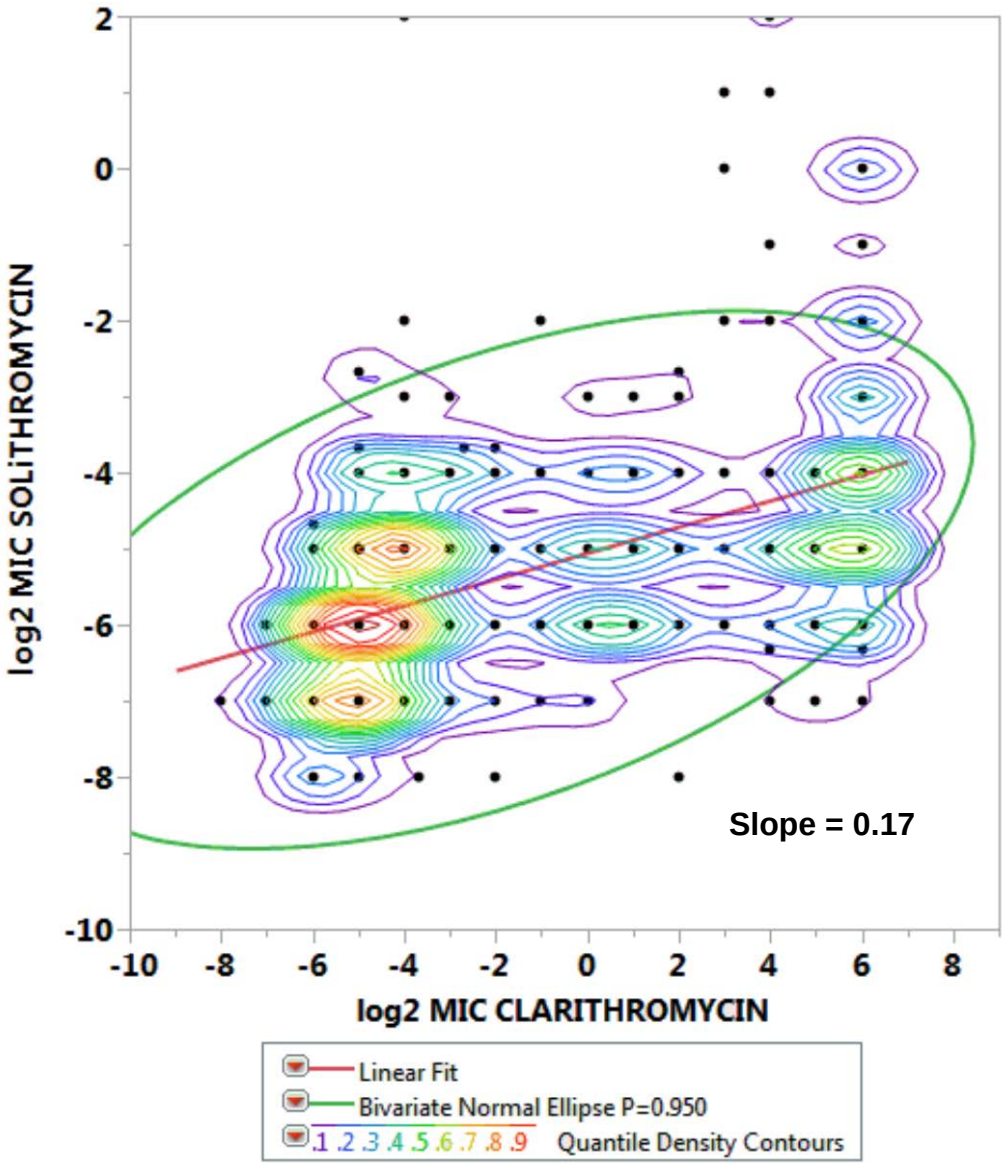


Cethromycin



Modithromycin

Solithromycin and resistance to “old” macrolides …



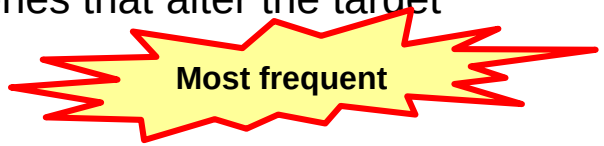
S. pneumoniae **isolates from Belgium and Germany (n=741)**

submitted

Fluoroquinolones

- The first major class of antibiotics of synthetic origin
  (no obvious equivalent in natural products)
  → resistance was though to be unlikely…

- But…
  - spontaneously occurring mutations in chromosomal genes that alter the target enzymes (DNA gyrase and topoisomerase IV)
    → **high frequency: 10^{-7} / 10^{-8} !!**



  - Efflux-mediated reduction of intrabacterial concentration in both Gram-positive and Gram-negative bacteria, even if not pre-exposed to the same molecule
    → **favours the selection of resistant mutants**

  - Acquisition of plasmid-encoded *qnr* genes protecting DNA gyrase and topoisomerase IV from quinolone action
    → **spreading…**

  - modification of a plasmid-encoded *aminoglycoside* acetylating enzyme (AAC(6′)-Ib-cr) that cetylates the C7 aminofunction of piperazinyl-substituted fluoroquinolones (ciprofloxacin, norfloxacin).

Fluoroquinolones: prevalence of resistance

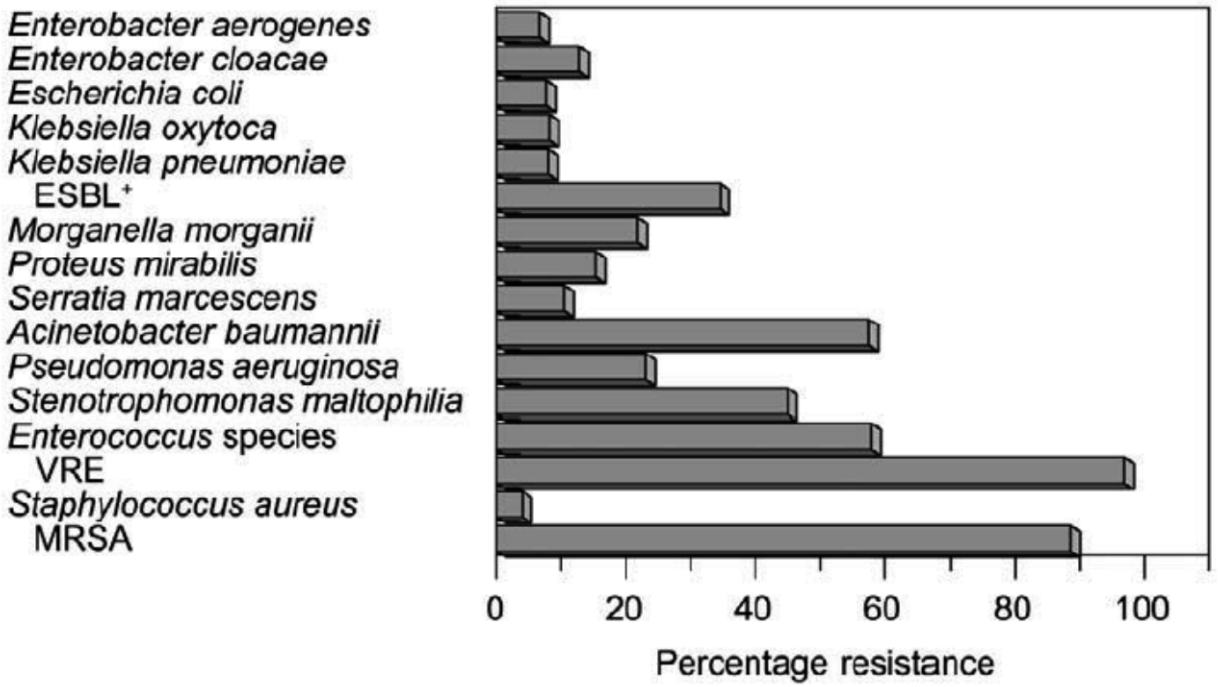
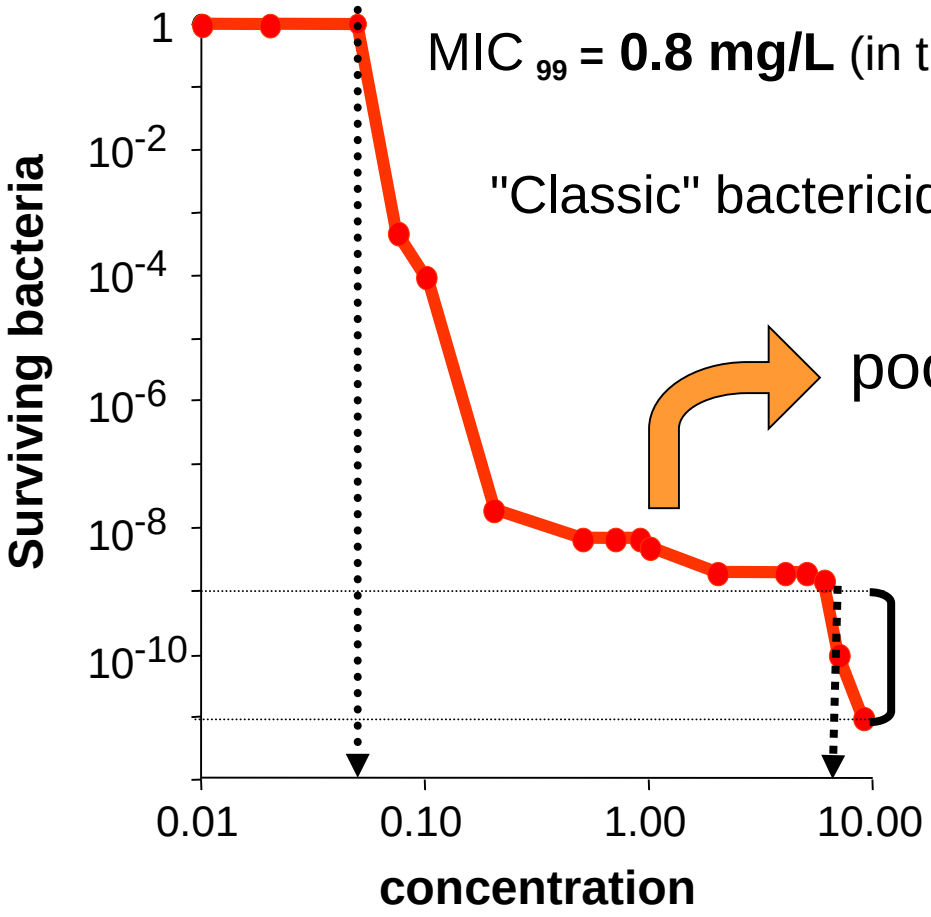


Figure 1. Current estimates of resistance to ciprofloxacin among isolates recovered from hospitals in the United States [2–5]. ESBL+, extended-spectrum β-lactamase producing; MRSA, methicillin (or oxacillin)–resistant *Staphylococcus aureus;* VRE, vancomycin-resistant enterococci.

Jacoby G. Clin Infect Dis. 2005 Jul 15;41 Suppl 2:S120-6

Fluoroquinolones: Mutant Prevention Concentration (MPC)



MIC $_{99}$ = **0.8 mg/L** (in this example)

"Classic" bactericidal effect

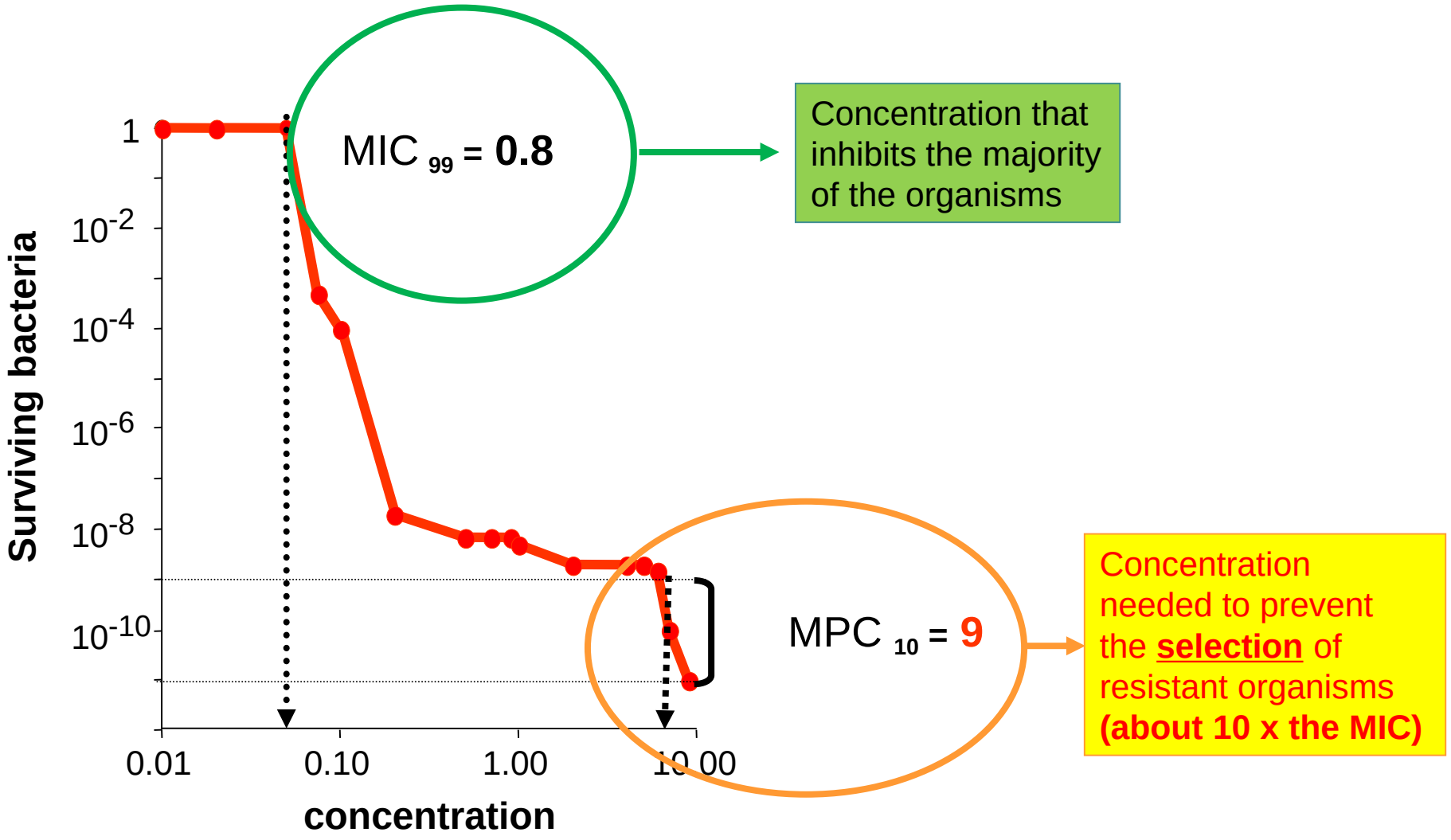
poorly susceptible organisms…

Elimination of resistant organisms

MPC_{10} = **9**

Dong et al: AAC 1999; 43:1756-1758

Fluoroquinolones: Mutant Prevention Concentration (MPC)



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Fluoroquinolones: a high peak may be necessary

Journal of Antimicrobial Chemotherapy Advance Access published November 6, 2014

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Journal of Antimicrobial Chemotherapy

Shape does matter: short high-concentration exposure minimizes resistance emergence for fluoroquinolones in *Pseudomonas aeruginosa*

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New York, USA; [3]*IBMP—Institute for Biomedic*
[4]*Institute of Pharmacolog*

- Delivering the same *f*AUC/MIC over short durations of exposure (i.e. 1, 4 or 10h) achieved more rapid killing with no or very limited emergence of resistance, whereas longer durations of exposure over 16 and 24h led to a dramatic (5 $\log_{10}$) increase in the concentration of resistant bacteria.

- Clinical ciprofloxacin regimens with high intensity, short-exposure durations may provide extensive and rapid bacterial killing with no or limited resistance.

Fluoroquinolones: dissociated resistance and low MICs

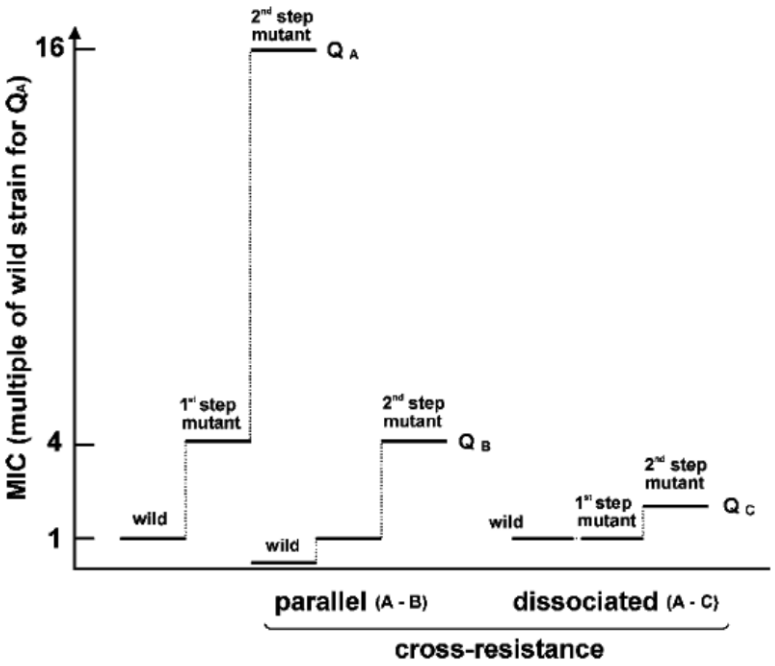
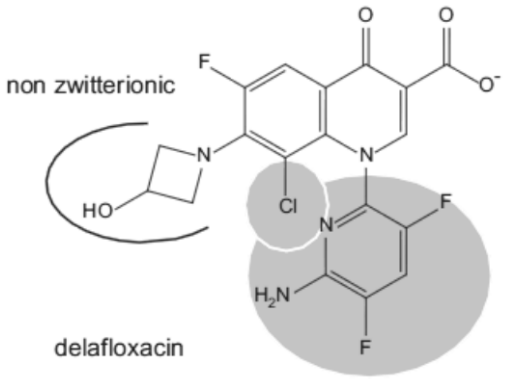


Fig. 4. Cross-resistance and dissociated resistance in quinolones. Q_A and Q_B illustrate a situation of cross-resistance: although the initial susceptibility of the strain may be different for molecules A and B, mutations in the target enzymes lead to similar changes in the susceptibility to both drugs. Q_C illustrates a situation of dissociated resistance: the susceptibility to molecule C does not change in spite of the acquisition of a first mutation, and will increase only upon acquisition of a second mutation.



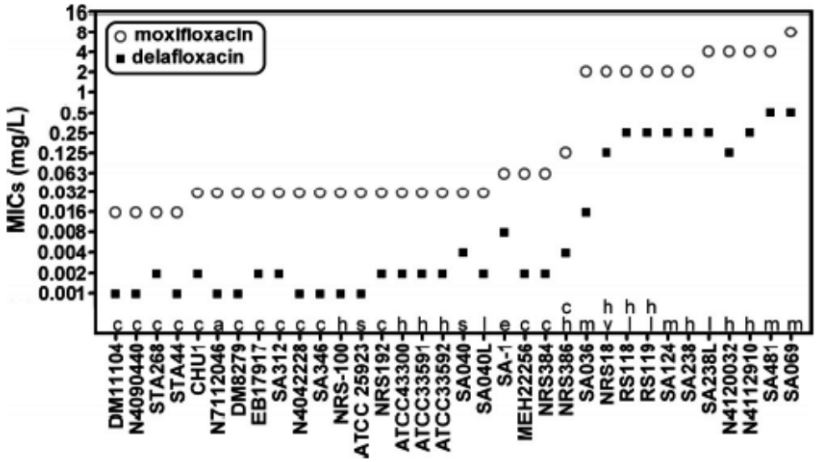
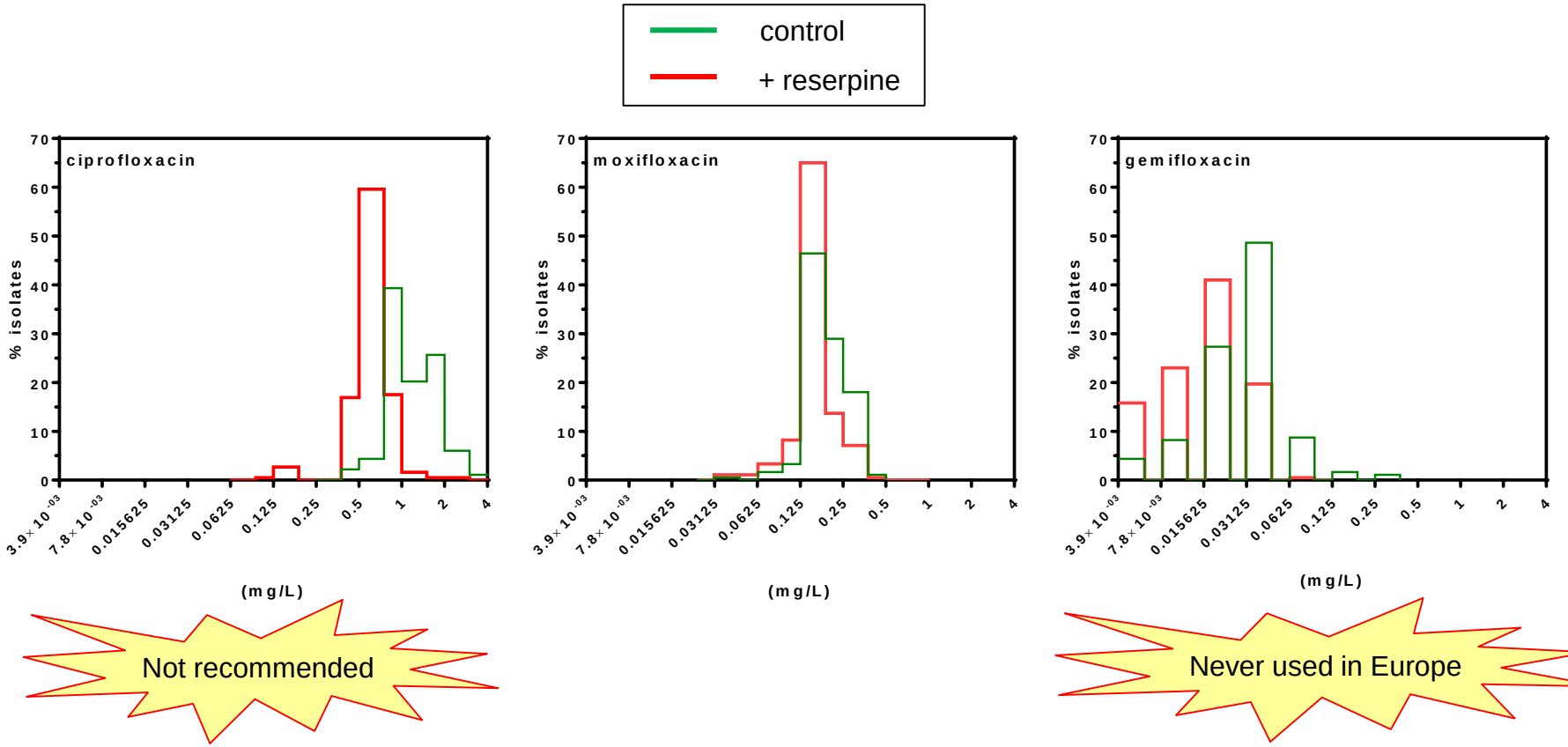


FIG. 2. Comparative susceptibilities of various *S. aureus* isolates to moxifloxacin (circles) or delafloxacin (squares). MICs were measured at pH 7.4, and strains are ranked based on their susceptibility to moxifloxacin. Resistance phenotypes and/or strain source are designated by lowercase letters along the *x* axis: a, animal MRSA; c, CA-MRSA; e, efflux (NorA); h, HA-MRSA; l, linezolid-resistant; m, characterized mutations in fluoroquinolone targets; s, MSSA.

Van Bambeke *et al.* Clinical Microbiology and Infection (2005) 11:256-280
Van Bambeke F Annals of Medicine (2014) 46(7):512-29.
Lemaire *et al.* Antimicrobial Agents and Chemotherapy (2011) 55:649-58.

Fluoroquinolones: preexisting efflux
(in *S. pneumonia* [Belgian CAP isolates])

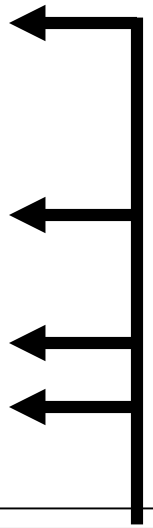


Lismond *et al.* J Antimicrob Chemother (2011) 66:948-951

Efflux and selection of resistance

Change in MICs of Levofloxacin in
***Pseudomonas aeruginosa* if deleting the efflux pump operons**

Pump status	LVX MIC	Frequency of LVX-resistant mutants
WT	0.25	2×10^7 - 4×10^7
Δ mexAB-oprM	0.015	
Δ mexCD-oprJ	0.25	
Δ mexEF-oprN	0.25	
Δ mexAB-oprM;Δ mexEF-oprN	0.015	
Δ mexCD-oprJ; Δ mexEF-oprN	0.25	
Δ mexAB-oprM; Δ mexCD-oprJ	0.015	
Δ mexAB-oprM; Δ mexCD-oprJ; Δ mexEF-oprN	0.015	



The MIC falls to low values ...

Lomovskaya *et al,*
AAC (1999: 43:1340-1346

Efflux and selection of resistance

Frequency of Levofloxacin-resistant mutants in *Pseudomonas aeruginosa* if deleting the efflux pump operons

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WT	0.25	2×10^7 - 4×10^7
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Δ mexCD-oprJ	0.25	2×10^7 - 4×10^7
Δ mexEF-oprN	0.25	2×10^7 - 4×10^7
Δ mexAB-oprM;Δ mexEF-oprN	0.015	2×10^7 - 10^7
Δ mexCD-oprJ; Δ mexEF-oprN	0.25	2×10^6
Δ mexAB-oprM; Δ mexCD-oprJ	0.015	1×10^9
Δ mexAB-oprM; Δ mexCD-oprJ; Δ mexEF-oprN	0.015	**$<1 \times 10^{11}$**

Lomovskaya *et al,*
AAC (1999) 43:1340-1346

AND the selection of mutants in FQ target becomes undetectable when ALL pumps are disrupted

Thank you for your attention!

And ask questions