Discovery of new antibiotics: Why and some approaches… *

Paul M. Tulkens, MD, PhD *

Cellular and Molecular Pharmacology &
Centre for Clinical Pharmacy
Louvain Drug Research Institute
Université catholique de Louvain, Brussels, Belgium

http://www.facm.ucl.ac.be

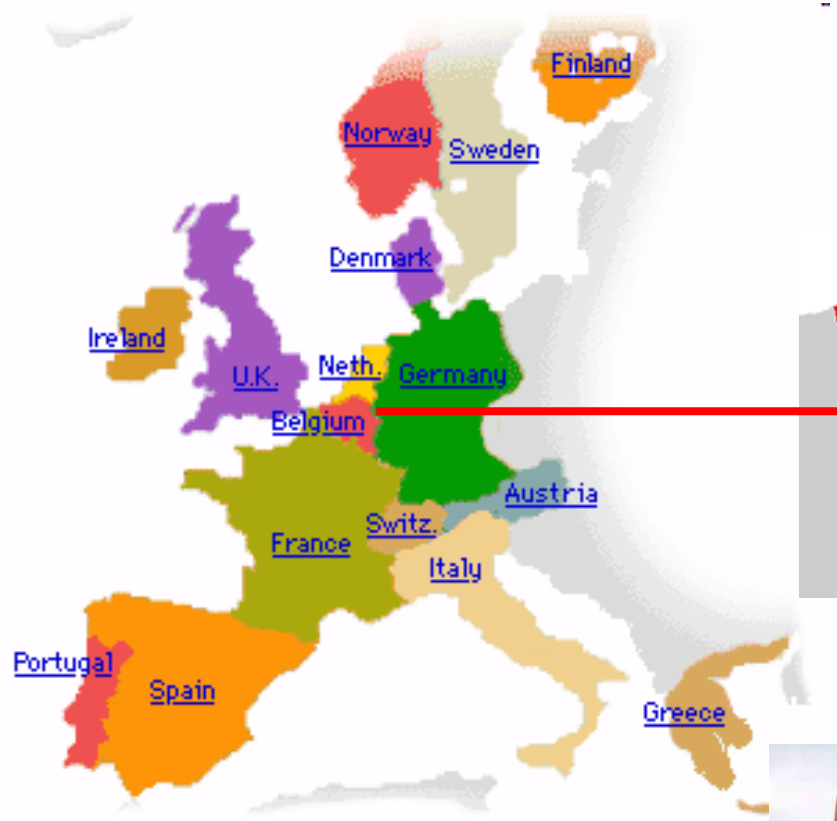
International University - Vietnam National University, Ho Chi Minh City, Vietnam

* with a short presentation of Belgium and the *Université catholique de Louvain* (slides 3-9)

Contents of the presentation

- Belgium: where is it ? (slide 3)
- The Catholic University of Louvain (slides 4-9)
- The antibiotic crisis: a pictorial view (slides 10-18)
- Public Health Approaches (very brief) (slides 19-25)
- Actions based on knowledge of resistance / toxicity patterns
- Examples of discovery of novel molecules (a personal selection)
  - ill-exploited targets: D-Ala-D-Ala ligase inhibitors
  - refurbishing old antibiotics: novel aminoglycosides
  - restarting from old compounds: novel polymyxins
  - expanding the spectrum of old antibiotics: temocillin
  - fine-tuning existing drugs: finafloxacin - delafloxacin

Belgium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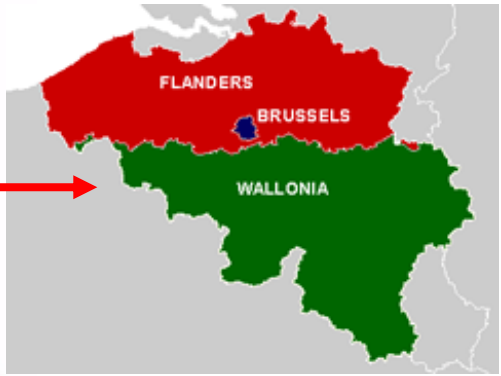
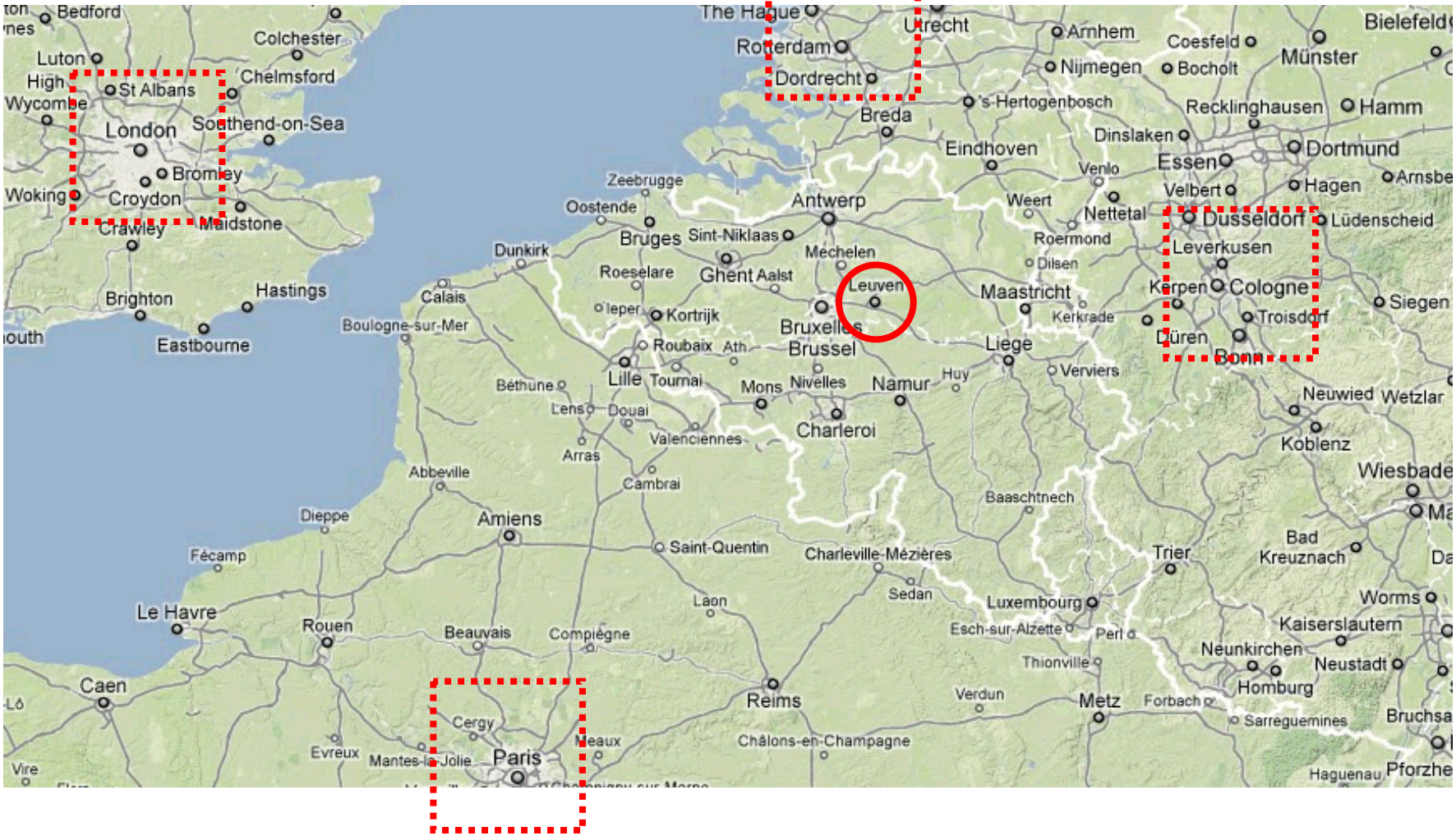
Spain
Greece


FLANDERS
BRUSSELS
WALLONIA

The Catholic University of Louvain in brief (1 of 5)

- originally founded in 1425 in the city of **Louvain** (in French; known as **Leuven** in Flemish)

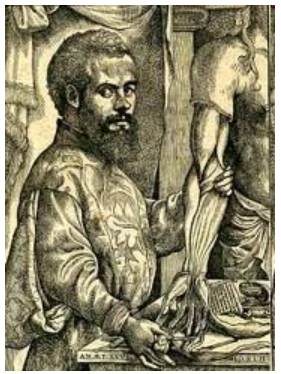


The Catholic University of Louvain in brief (2 of 5)

- It was one of the major University of the so-called "Low Countries" in the 1500 – 1800 period, with famous scholars and discoverers (Vesalius for anatomy, Erasmus for philosophy, …). Teaching was in Latin, Greek, and Hebrew (College of the 3 languages…)

The University in the 1500's

Erasmus



Vesalius

The Catholic University of Louvain in brief (3 of 5)

- The University was closed by the French when invading the "Low Countries" in 1791 but re-opened in 1831, always in Louvain
- Teaching was in French until the early 1900's, when a Flemish-speaking section was opened and courses were given in both languages, attracting many students and celebrities…

Albert Einstein in conversation with Prof. G. Lemaître, professor at the *Université catholique de Louvain,* who, in 1927, published a paper * in which he showed that the Universe had to be unstable and expanding from a primeval atom…

*Un Univers homogène de masse constante et de rayon croissant rendant compte de la vitesse radiale des nébuleuses extra-galactiques". Annales de la Société Scientifique de Bruxelles 47: 49. Bibcode 1927ASSB...47...49L.
In French – In English

- The University was later divided into a French-speaking **Université catholique de Louvain** and a Flemish-speaking **Katholieke Universiteit Leuven…**

The Catholic University of Louvain in brief (4 of 5)

- Because Louvain is a Flemish-speaking city, the French-speaking ***Université catholique de Louvain*** moved in 1968-1976 from Louvain to
  - Brussels for the Faculty of Medicine and associated schools (Pharmacy, Dentistry, and so on …)
  - Louvain-la-Neuve for all other Faculties

  while keeping the name of "Louvain" in both French and English (thus, **Catholic University of Louvain**)

Since 2010, the University has 2 main campuses and 4 smaller ones, with a total of about 30,000 students

http://www.uclouvain.be

The Catholic University of Louvain in brief (5 of 5)

- The Flemish-speaking ***Katholieke Universiteit Leuven*** has remained in Louvain (Leuven) and is named in English "**Catholic Universiteit Leuven**".

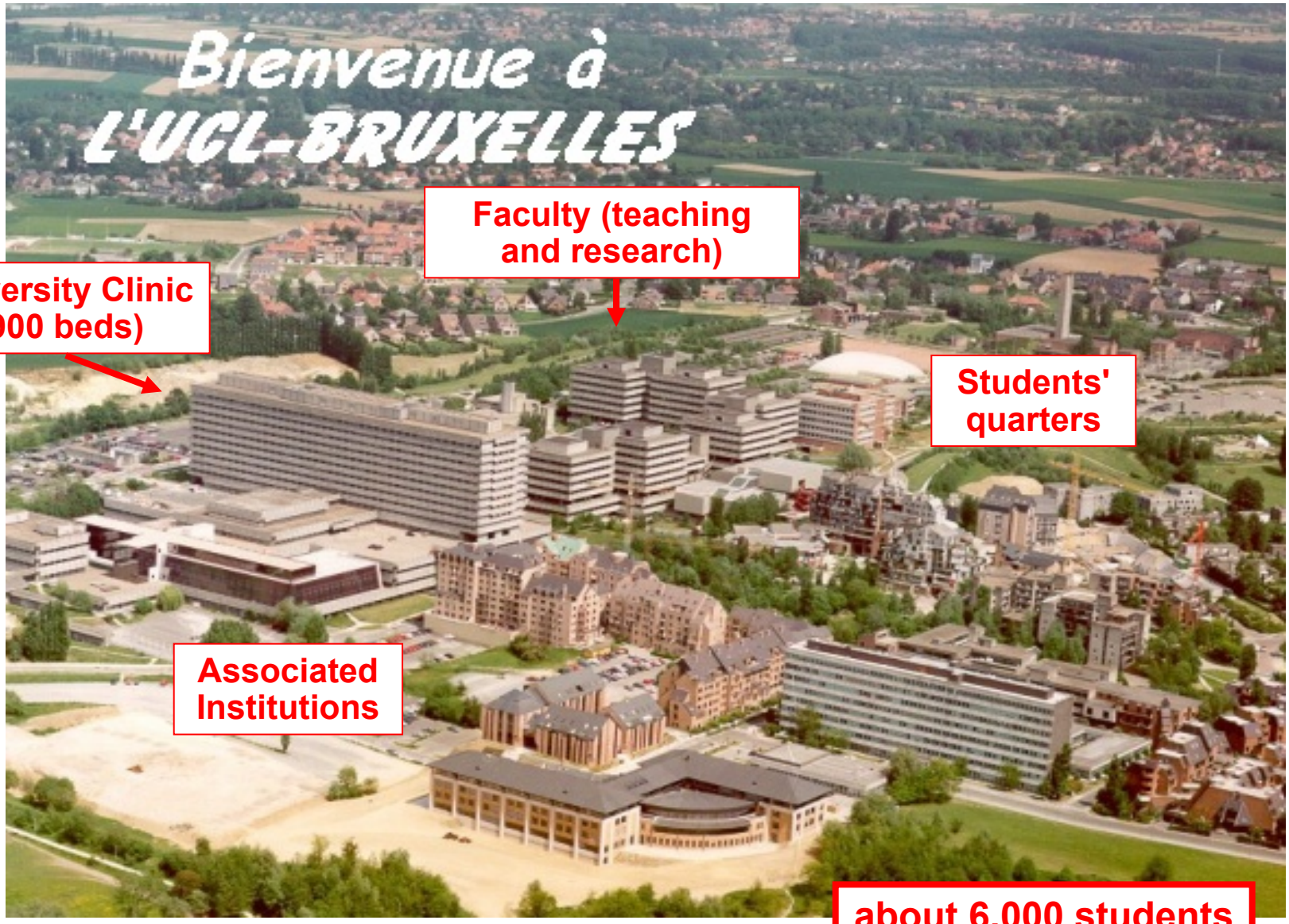
  The distance between Universities is only 25 km…

Université catholique de Louvain
http://www.uclouvain.be

Katholieke Universiteit Leuven
http://www.kuleuven.be

- Together, the two Universities have about 55,000), which is ~ 2/5 of all University population in Belgium (~10^6 inhabitants)

Our campus in Brussels (Health Sciences)…



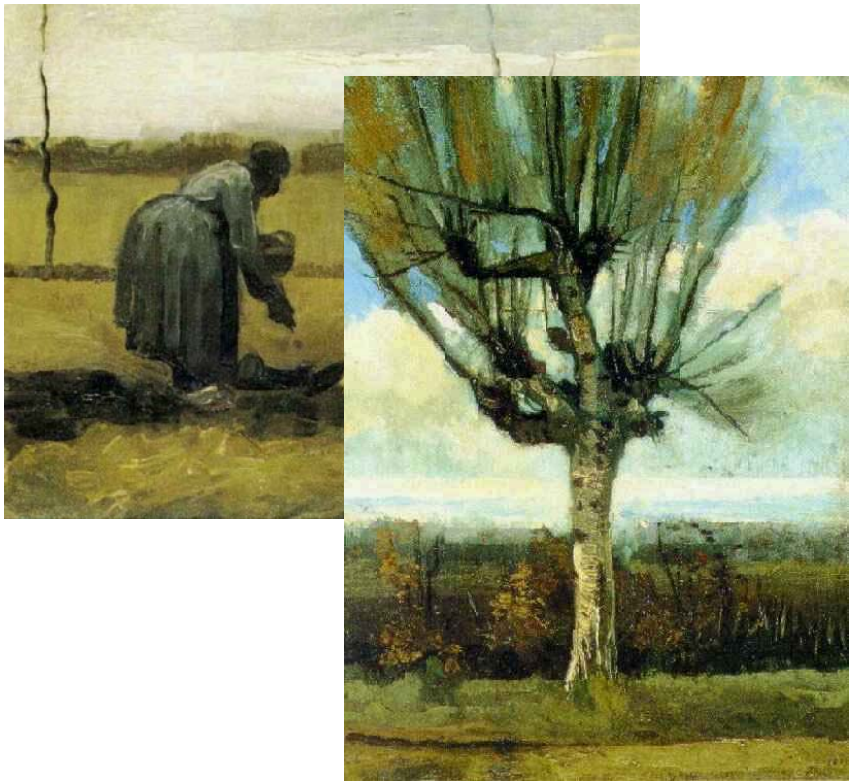
The antibiotic crisis *

* A pictorial view using 4 paintings of Van Goch (who stayed briefly in Belgium when moving from Holand to France) and selected Belgian and International data

Are antibiotics following a path to madness ?

discovery in soil bacteria and fungi

Are antibiotics following a path to madness ?



and then we all saw the blooming tree of semi-synthetic and totally synthetic antibiotics

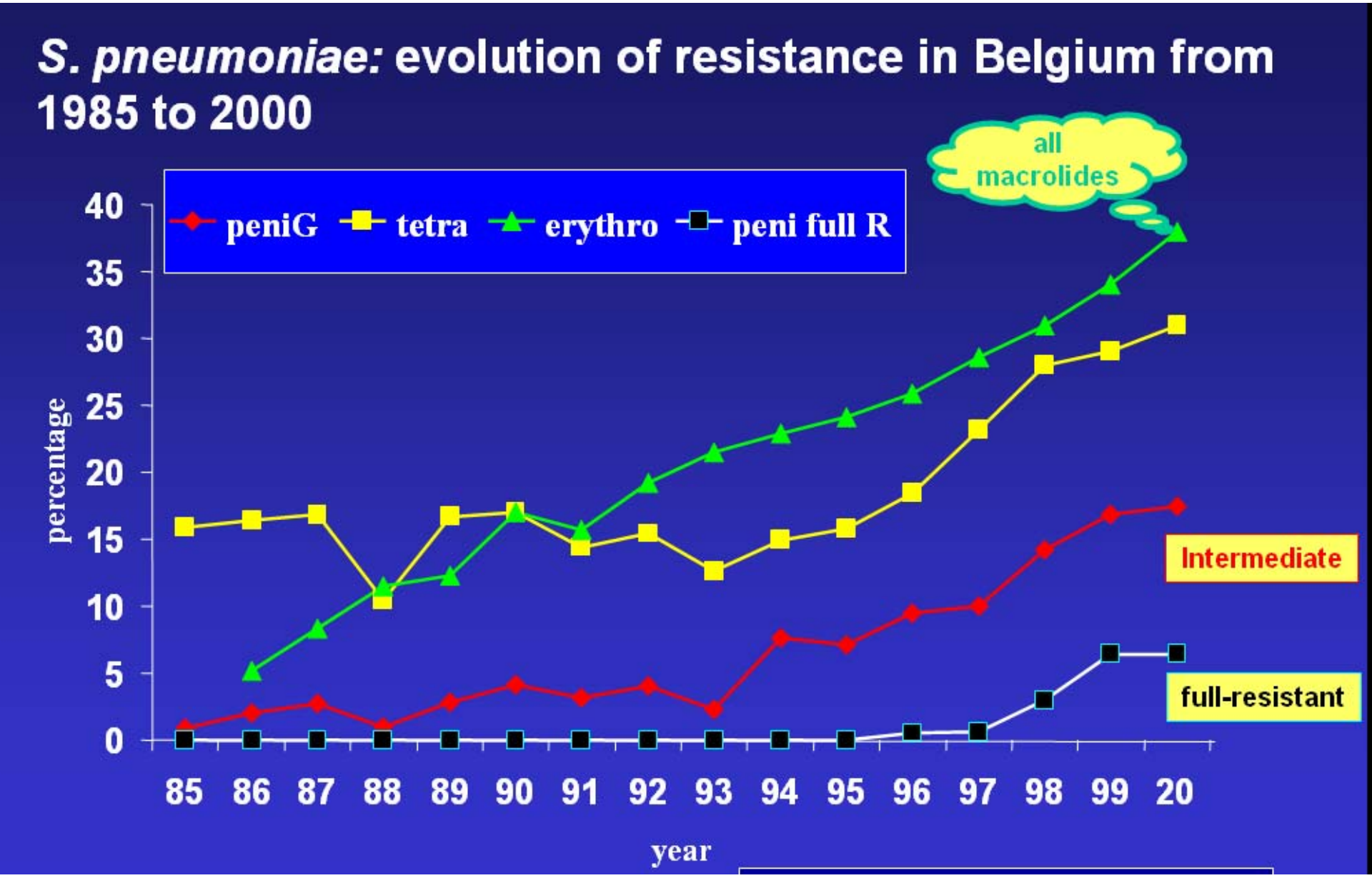
Are antibiotics following a path to madness ?

and the General Surgeon told us
that the fight was over

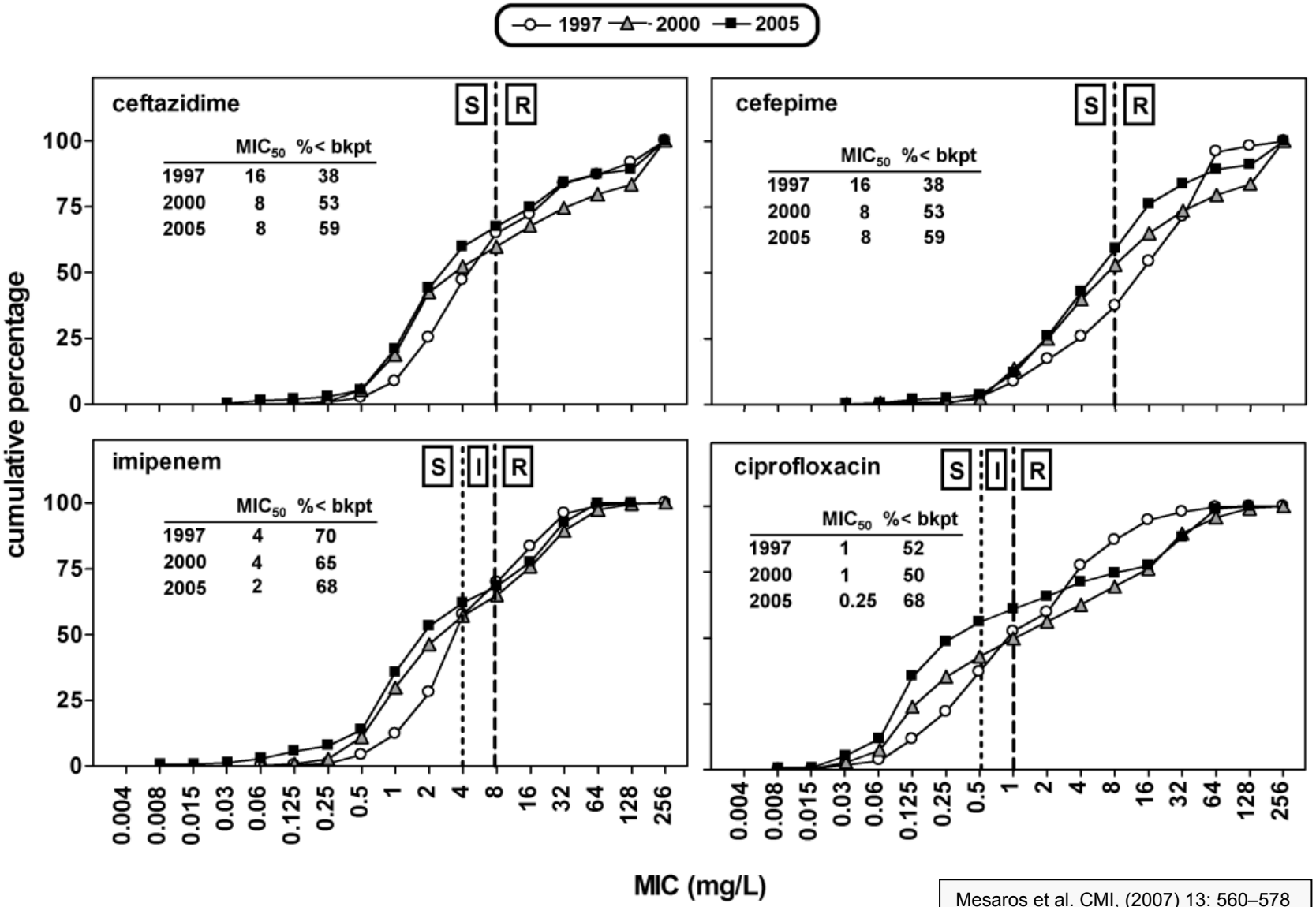
Are antibiotics following a path to madness ?

But…

Example #1: longitudinal studies with S. *pneumoniae*



Example #2: Extent of resistance of *P. aeruginosa*



Mesaros et al. CMI, (2007) 13: 560–578

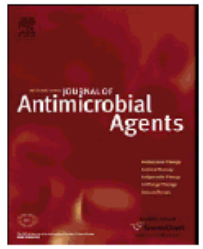
Example #3 : the hidden risk of therapy

International Journal of Antimicrobial Agents 36 (2010) 513–522

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

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



In vivo development of antimicrobial resistance in *Pseudomonas aeruginosa* strains isolated from the lower respiratory tract of Intensive Care Unit patients with nosocomial pneumonia and receiving antipseudomonal therapy

Mickaël Riou[a,1], Sylviane Carbonnelle[a,2], Laëtitia Avrain[a,b], Narcisa Mesaros[a,3], Jean-Paul Pirnay[c], Florence Bilocq[c], Daniel De Vos[c,d], Anne Simon[e], Denis Piérard[f], Frédérique Jacobs[g], Anne Dediste[h], Paul M. Tulkens[a,*], Françoise Van Bambeke[a], Youri Glupczynski[i]

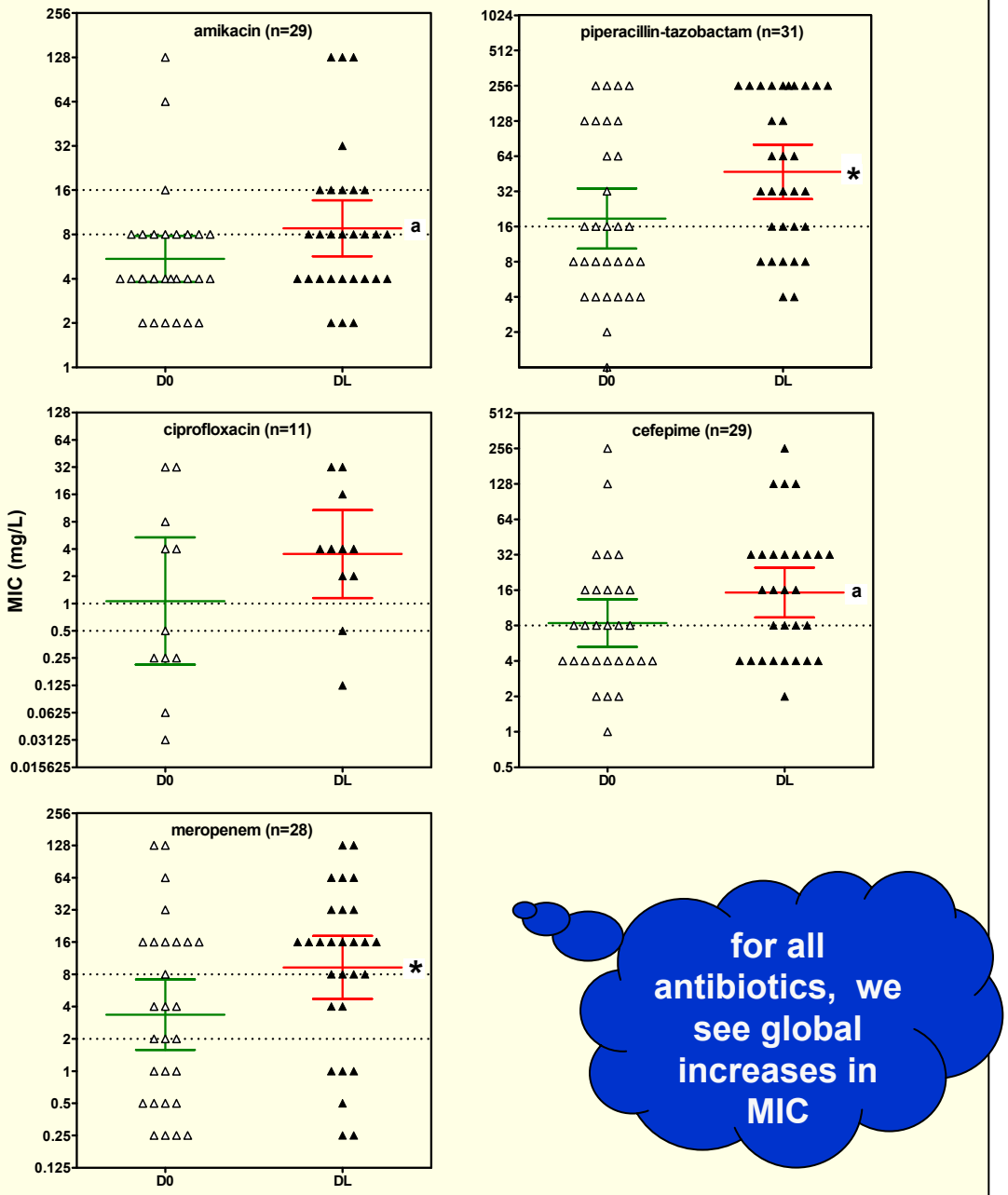
What happens during treatment ?

- D0: initial isolate
  DL: last isolate obtained
- individual values with geometric mean (95 % CI)
- S (lowest line) and R (highest line) EUCAST breakpoints

* $p < 0.05$ by paired t-test (two-tailed) and Wilcoxon non-parametric test

[a] $p < 0.05$ by Wilcoxon non-parametric test only

Note: stratification by time between D0 and DL gave no clue (too low numbers)

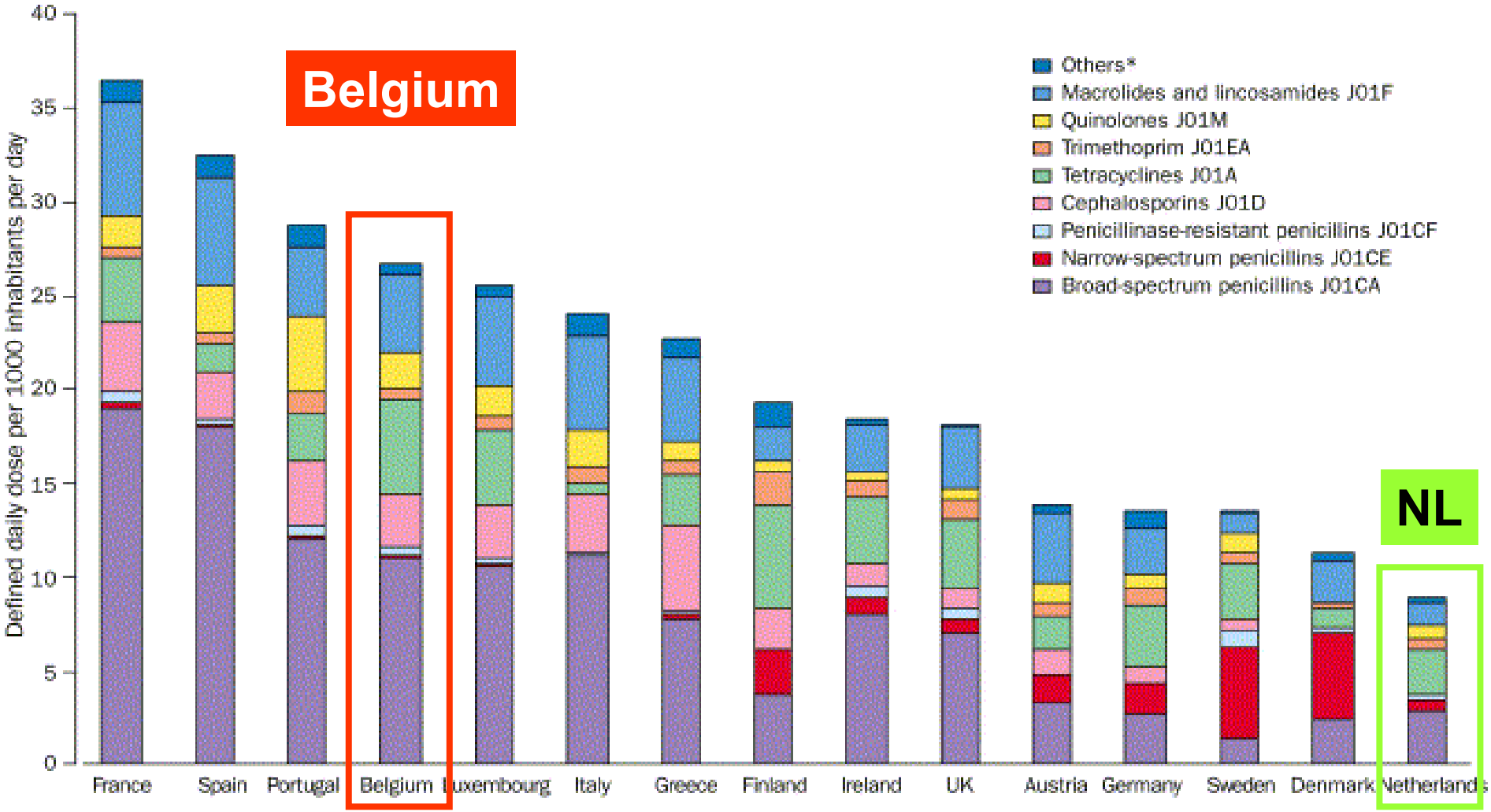


for all antibiotics, we see global increases in MIC

Public Health Approaches

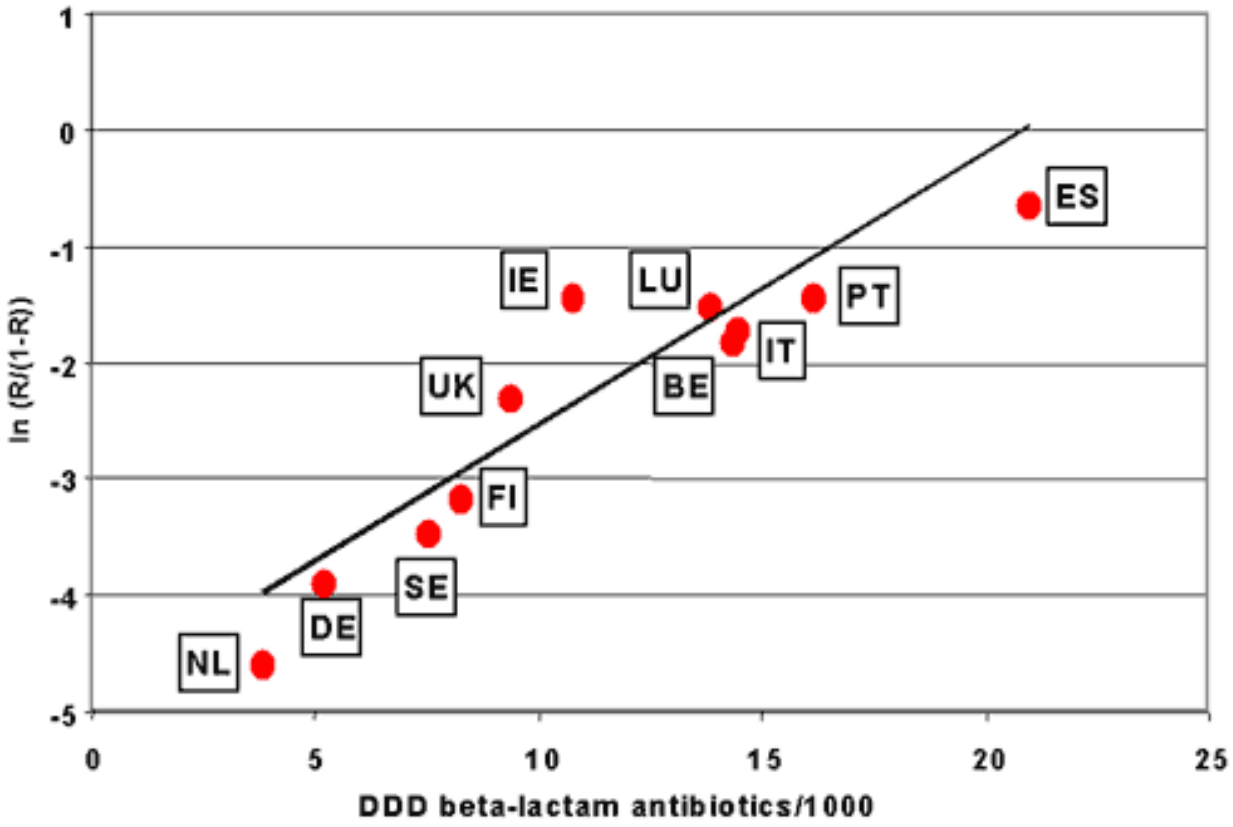
Antibiotic consumption is highly variable between countries (for no good reason)

Step 1 : knowing what you do



Cars et al., Lancet. 2001 Jun 9;357(9271):1851-3.

Overuse is part of the problem …



Risk of resistance to β-lactams among invasive isolates of *Streptoccus pneumoniae* regressed against outpatient sales of beta-lactam antibiotics in 11 European countries

- **resistance data are from 1998 to 1999; antibiotic sales data 1997.**
- **DDD = defined daily doses**

Bronzwaer SL, Cars O, et al. Emerg Infect Dis 2002 Mar;8(3):278-82

Public campaigns in Belgium

Step 2 : Informing and counseling

less…

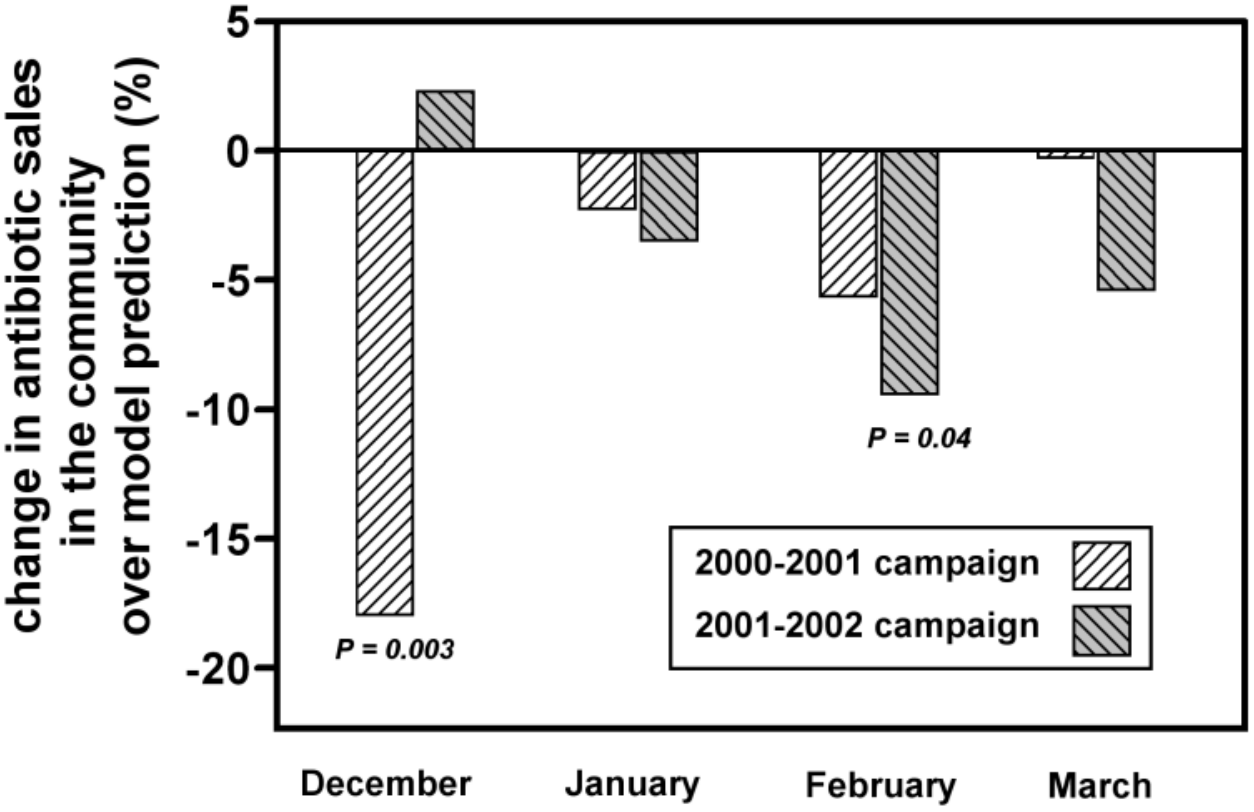
Public campaigns in Belgium

Step 2 : Informing and counseling

and better …

Public campaigns in Belgium

Step 3 : Monitoring usage



from Bauraind *et al.*, JAMA 2004; 292:2468-70;
more details on
http://www.antibiotiques.org/english/

- significant reduction of AB prescriptions (sales = prescriptions in Belgium) during the influenza epidemic periods
- no significant-side effect detected
- cost-effective for public health

What should public authorities do ?

- promote better and cheap diagnostic approaches
- support the collection of resistance data in the community and in the hospital
- support the setting of guidelines adapted to the reality of the country
- reinforce the role of the pharmacists and doctors-pharmacists relationships for quality of care and enforcement of guidelines
- decreasing the pressure on parents and family towards doctors for a "fast cure" in face of minor diseases (socio-economical factors)
- control Industry pressure (and find new economic structures)

This is what I'm doing in Hanoi
http://www.facm.ucl.ac.be/advance-courses.htm

Actions based on knowledge of resistance / toxicity patterns

Resistance: mechanisms

It was six men of Hindustan
To learning much inclined,
Who went to see the Elephant
(Though all of them were blind),
That each by observation
Might satisfy his mind

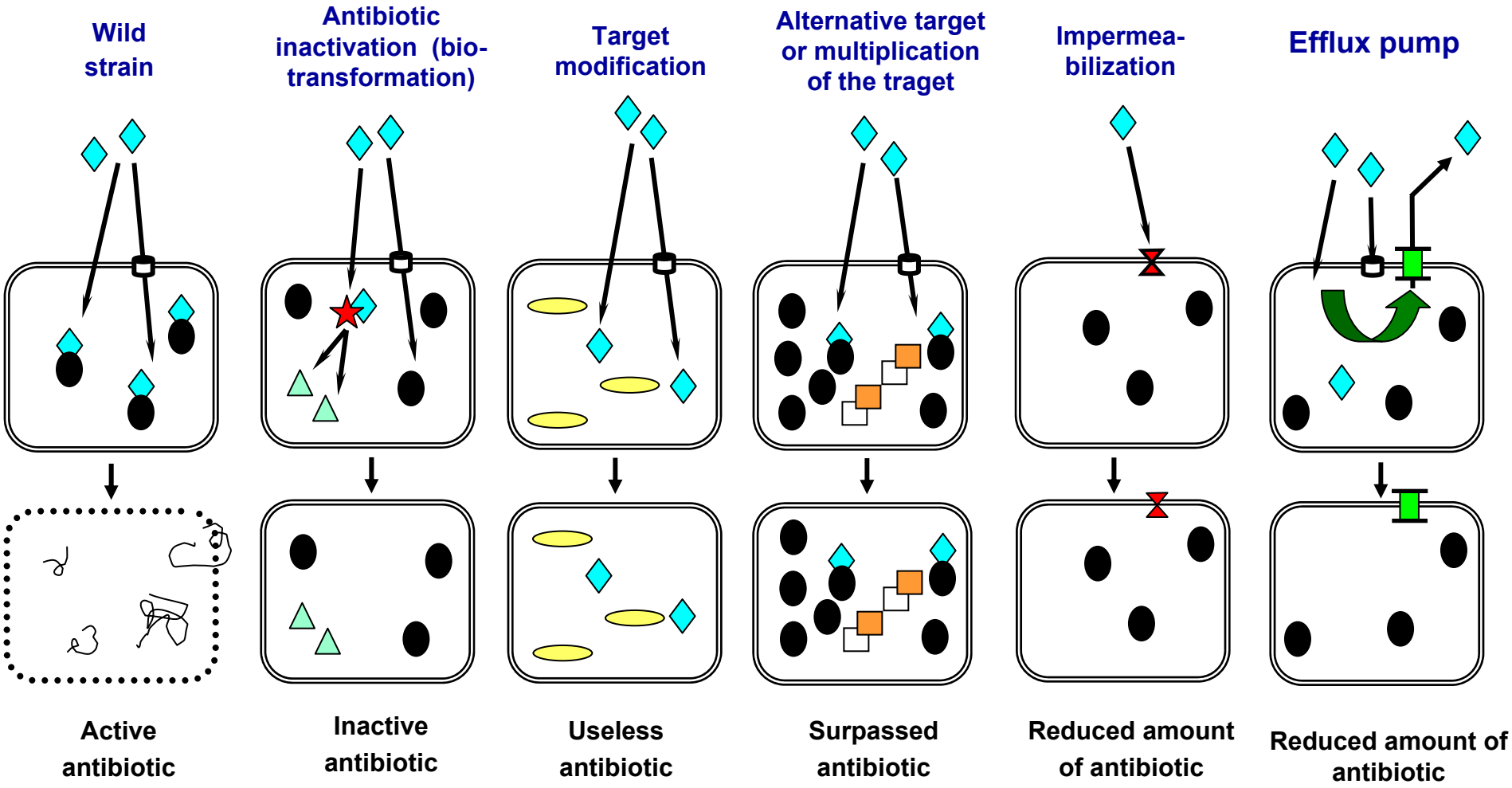
They conclude that the elephant is like a wall, snake, spear, tree, fan or rope, depending upon where they touch.

Sri Ramakrishna Paramahamsa (February 18, 1836 - August 16, 1886), a famous mystic, used this parable to discourage dogmatism

In Saxe's version, the conflict is never resolved.

John Godfrey Saxe (June 2, 1816 – March 31, 1887), American poet, best known for his re-telling of the Indian parable "The blind men and the Elephant".

Antibiotic resistance: short overview of main mechanisms



Actually, triggering resistance is easy…

Exposure of *E. aerogenes* to anrti-Gram (-) penicillin (temocillin) to 0.25 MIC for 14 days with daily readjustment of the concentration based on MIC détermination

strains	Initial			TEM-exposed			Revertant		
	MIC (mg/L) [a]			MIC (mg/L)			MIC (mg/L)		
	TEM	FEP	MEM	TEM	FEP	MEM	TEM	FEP	MEM
2114/2 [c]	8	2	0.25	**2048**	**> 128**	**16**	**32**	4	0.5
2502/4 [c]	8	2	0.125	**8192**	4	0.25	**4096**	1	0.125
3511/1 [c]	**32**	2	0.125	**4096**	**32**	0.125	**4096**	**8**	0.5
7102/10 [d]	**512**	**32**	1	**16384**	**> 128**	4 [e]	**8192**	**64**	1

[a] figures in bold indicate values > the R breakpoint for Enterobacteriaceae (EUCAST for MEM [8] and FEP [4]; BSAC and Belgium for TEM [16])
[b] dotblot applied with antiOmp36 antibody; signal quantified for grey value after subtraction of the signal of a porin-negative strain (ImageJ software); negative values indicate a signal lower than the background
[c] ESBL TEM 24 (+) ; [d] ESBL (-) and AmpC (+) [high level] ; [e] Intermediate (I) according to EUCAST

Nguyen et al., presented at the 8th ISAAR, Seoul, Korea, 8 April 2011

A simple experiment …

Exposure of *E. aerogenes* to anrti-Gram (-) penicillin (temocillin) to 0.25 MIC for 14 days with daily readjustment of the concentration based on MIC détermination

strains	Initial			TEM-exposed			Revertant		
	MIC (mg/L) [a]			MIC (mg/L)			MIC (mg/L)		
	TEM	FEP	MEM	TEM	FEP	MEM	TEM	FEP	MEM
2114/2 [c]	8	2	0.25	**2048**	**> 128**	**16**	**32**	4	0.5
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Nguyen et al., presented at the 8th ISAAR, Seoul, Korea, 6 April 2011

sub-MIC concentrations create resistance !

Novel targets

Design of novel inhibitors of D-Ala-D-Ala ligase

Patents

1. Poupaert J, Prevost M., Vandevuer S, Van Bambeke F, Colacino E, Tytgat I, Tulkens PM.
   Inibitors of D-Ala-D-Ala-ligase as antibacterial agents.
   Patent number: WO2009080788 (A2); classification: international: C07D263/56; C07D263/00; european: C07D263/56
   Application number: WO2008EP68100 20081219; Priority number(s): EP20070150355 20071221
   Full text

Full Papers

QSAR & Combinatorial Science QCS

Structure-Based Design of Benzoxazoles as new Inhibitors for D-Alanyl – D-Alanine Ligase

Isabelle Tytgat,[a] Stéphane Vandevuer,[b] Isabelle Ortmans,[b+] Finton Sirockin,[c‡] Evelina Colacino,[d§] Françoise Van Bambeke,[a] Colette Duez,[e] Jacques H. Poupaert,[d] Paul M. Tulkens,[a] Annick Dejaegere,[c] and Martine Prévost[b*]

 Current Medicinal Chemistry, 2009, *16*, 2566-2580

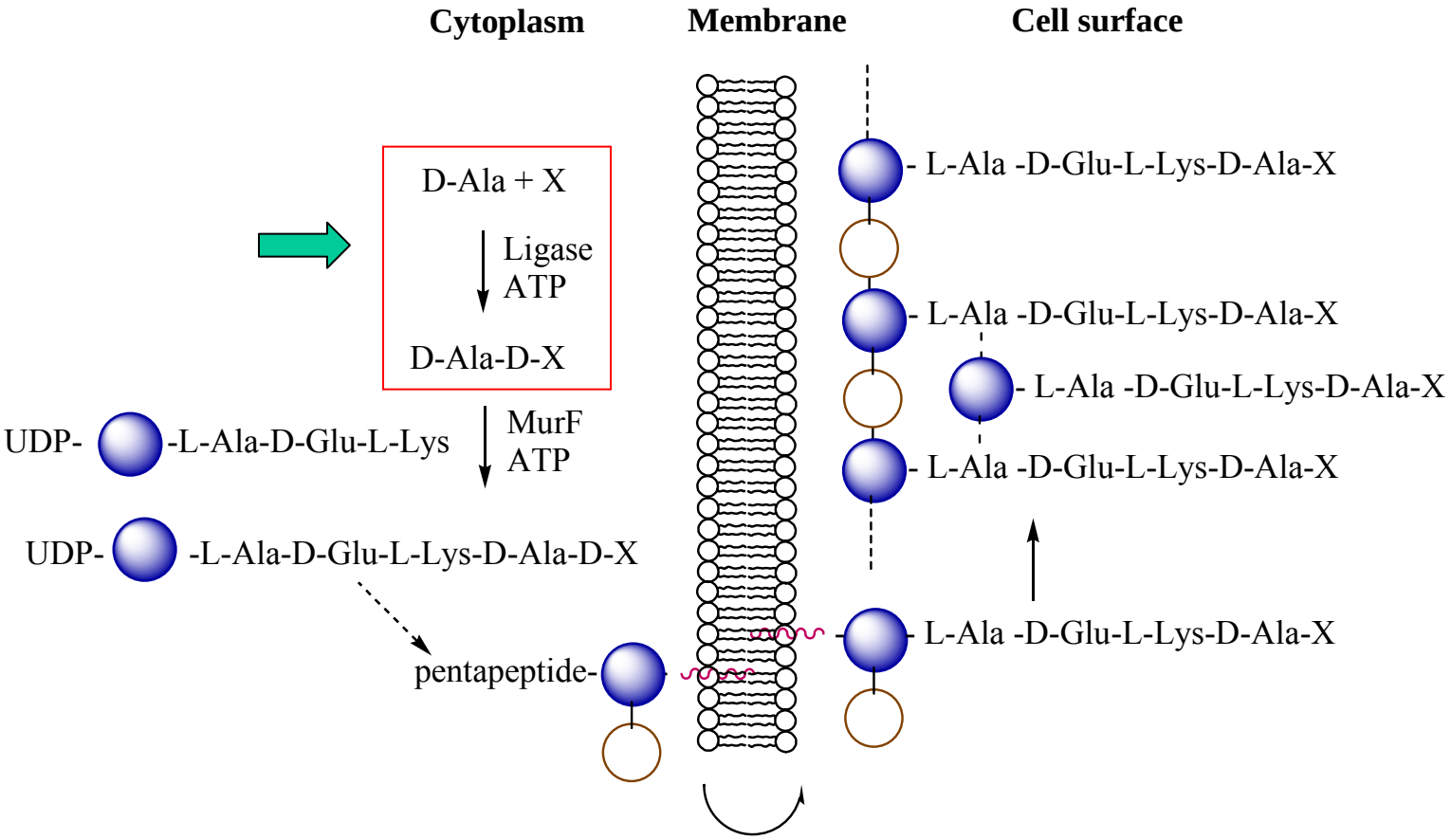
DD-Ligases as a Potential Target for Antibiotics: Past, Present and Future

I. Tytgat[1], E. Colacino[$,2], P.M. Tulkens[1], J.H. Poupaert[2], M. Prévost[3] and F. Van Bambeke*[,1]

[1]*Unité de Pharmacologie cellulaire et moléculaire,* [2]*Unité de Chimie pharmaceutique et radiopharmacie, Louvain Drug Research Institute, Université catholique de Louvain;* [3]*Structure et Fonction des Membranes Biologiques, Université Libre de Bruxelles, Brussels, Belgium*

Background

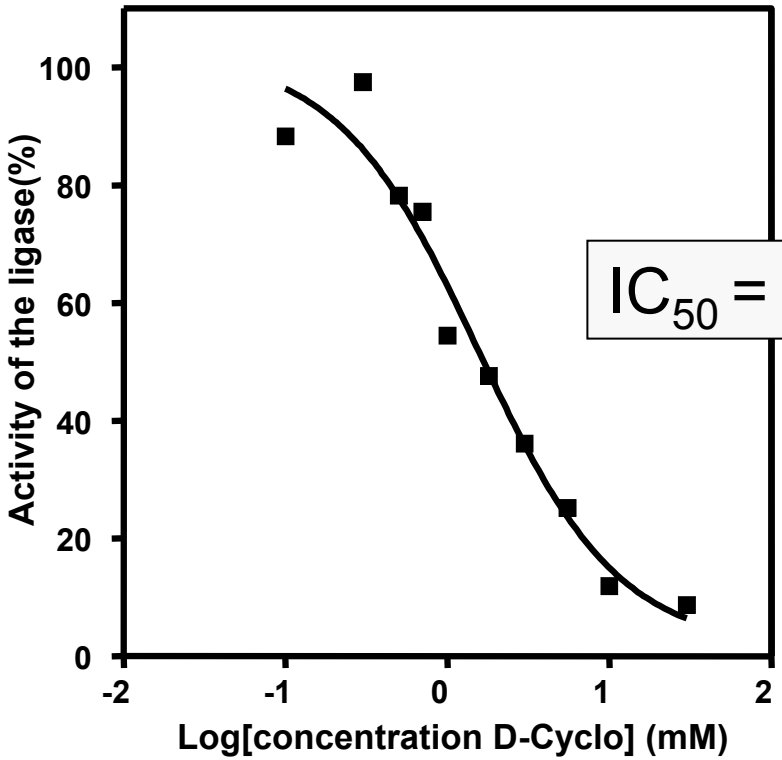
D-Ala-D-X ligases act in the very early steps of peptidoglycan synthesis and are essential enzymes for bacterial growth



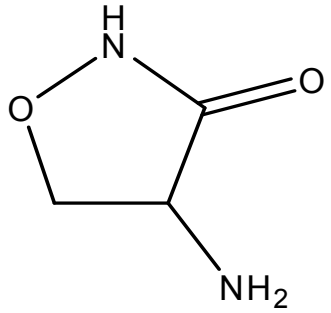
Rationale

- D-Ala-D-Ala ligases are essential enzymes
- This target has been only poorly explored
  - cycloserine: poor inhibitor and toxic)
  - Phosphinates: active on the enzyme but do not penetrate in the bacteria (too polar)
- Two approaches:
  - through conventional pharmacochemical approaches (modeling around know substrate)
  - de novo modeling from analysis of the protein conformation
  - BUT always using compounds that will enter the bacteria

Inhibition of purified His-tagged D-Ala – D-Ala ligase by D-cycloserine *



IC_{50} = 1.55 mM

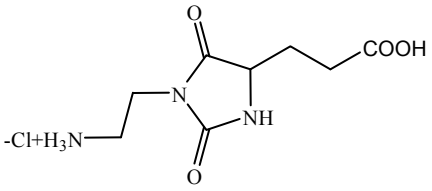


Literature data:
- 2 mM (Feng et al., AAC 2003;47:283-291)
- 0.8 mM (Park et al., PNAS 1997;94:10040-10044)

* broad but weak antimicrobial activity (through inhibition of D-Ala-racemase and, to a lesser extent, D-Ala-D-Ala ligase); used as 2d/3d line drug against M. tuberculosis (MIC: 5-20 mg/L; 50-200 µM); usage limited because of CNS toxicity

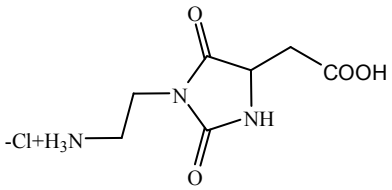
First evaluation of molecules in the family of hydantoïns
(phamacochemical design starting from D-Ala-D-Ala)

Hyd A



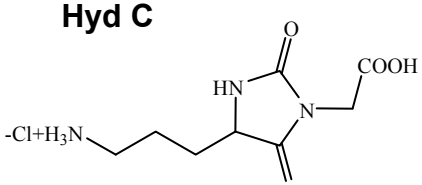
2-[4-(2-Carboxy-ethyl)-2,5-dioxo-imidazolidin
-1-yl]-ethyl-ammonium; chloride
Mw : 251
$C_8H_{14}ClN_3O_4$

Hyd B



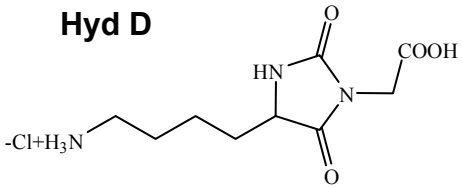
2-(4-Carboxymethyl-2,5-dioxo-imidazolidin
-1-yl)-ethyl-ammonium chloride
Mw : 237
$C_7H_{12}ClN_3O_4$

Hyd C



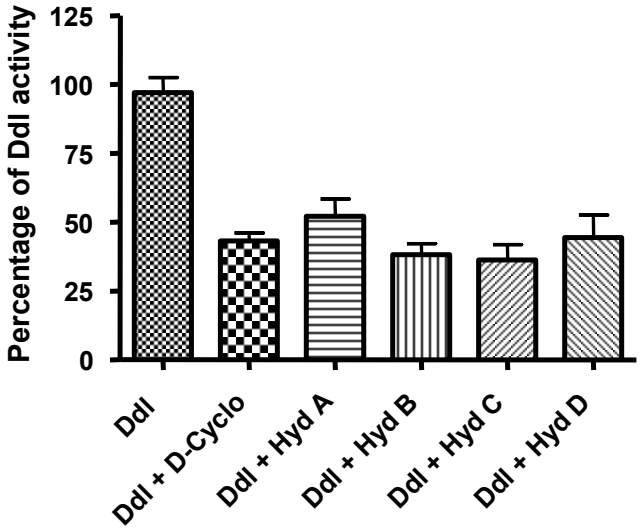
3-(1-Carboxymethyl-2,5-dioxo-imidazolidin
-4-yl)-propyl-ammonium chloride
Mw : 251
$C_8H_{14}ClN_3O_4$

Hyd D



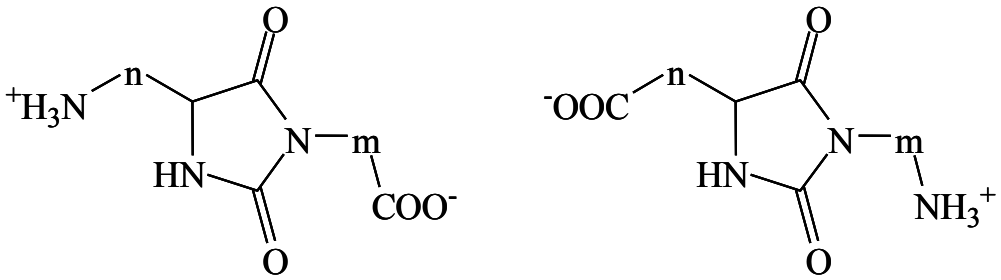
2-[4-(2-Carboxy-ethyl)-2,5-dioxo-imidazolidin
-1-yl]-ethyl-ammonium; chloride
Mw : 251
$C_8H_{14}ClN_3O_4$

Activity of Ddl in the presence of inhibitors (1.5 mM) with 5 min of preincubation



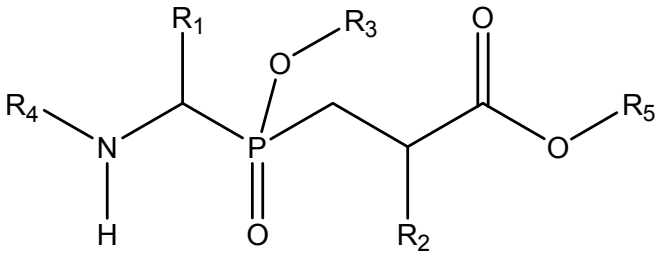
From hydantoines to Benzoxazoles

Hydantoïnes (UCL)



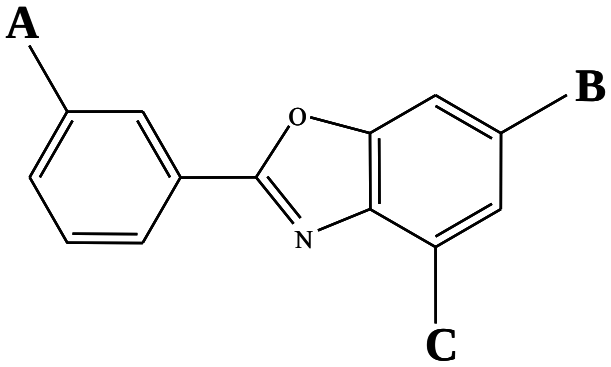
n et m sont des chaînes alkyles de longueur variable

Phosphinate analogues (UCL)



R_1 = H, CH_3, Ph.
R_2 = H, CH_3, Ph.
X= O, N, S, CH_2, CF_2.

Benzoxazoles
(design de novo [*Unité de Bioinformatique Génomique et Structurale, ULB*])

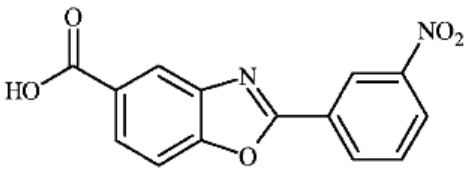


A) Groupe basique
B) Groupe Hydrophile
C) Groupe Hydrophobe

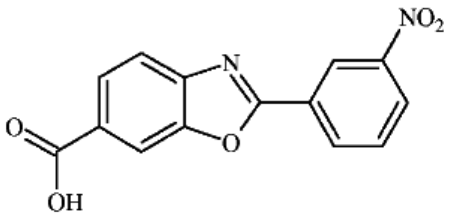
Benzoxazoles

CLAIMS

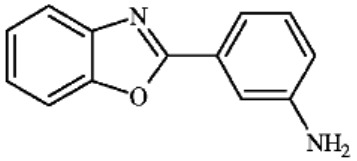
1. A compound of any of formulas (I-a), (II-a), (III-a), (IV-a) or (V-a):



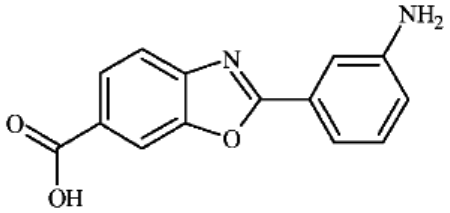
(I-a)



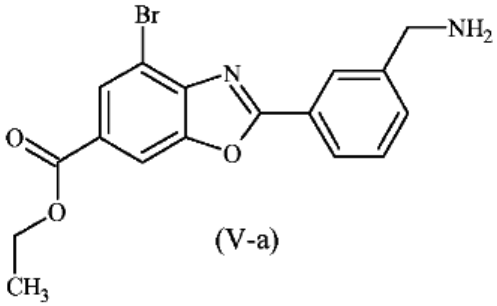
(II-a)



(III-a)



(IV-a)

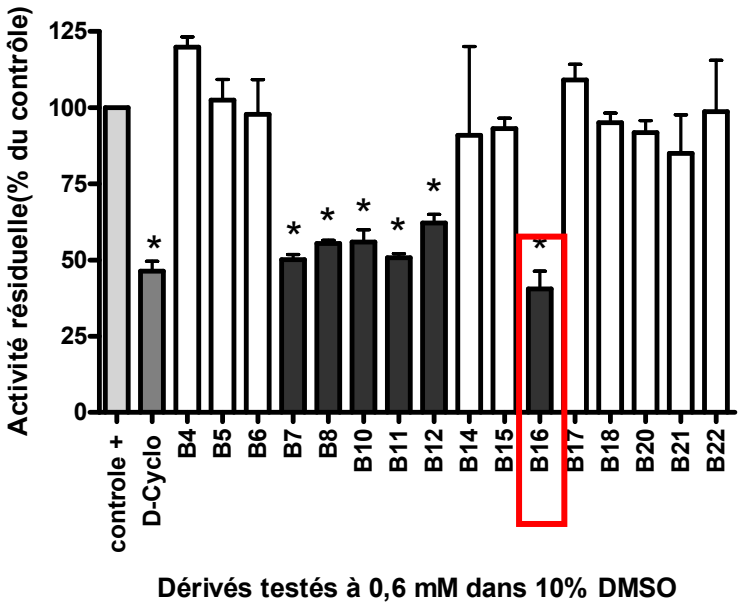


(V-a)

or a pharmaceutically acceptable *N*-oxide form, addition salt, prodrug or solvate thereof, for use in the treatment of a bacterial infection.

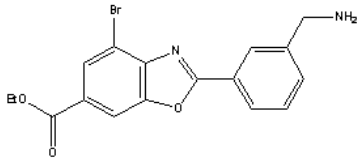
Benzoxazoles

Inhibition of D-Ala-D-Ala-ligase

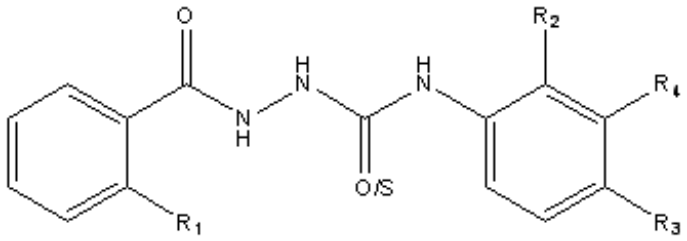
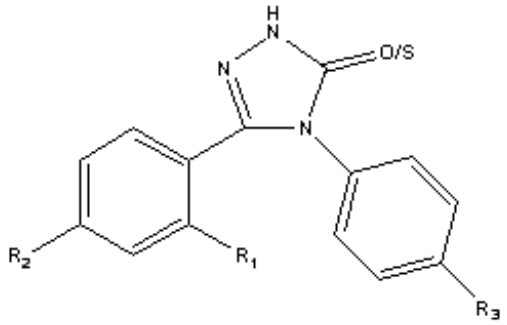
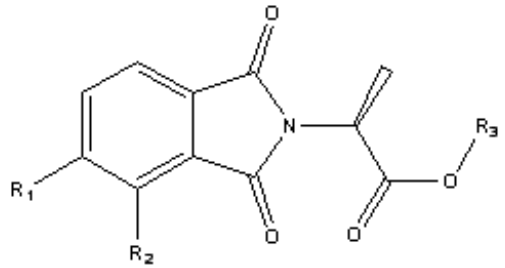


Dérivés testés à 0,6 mM dans 10% DMSO

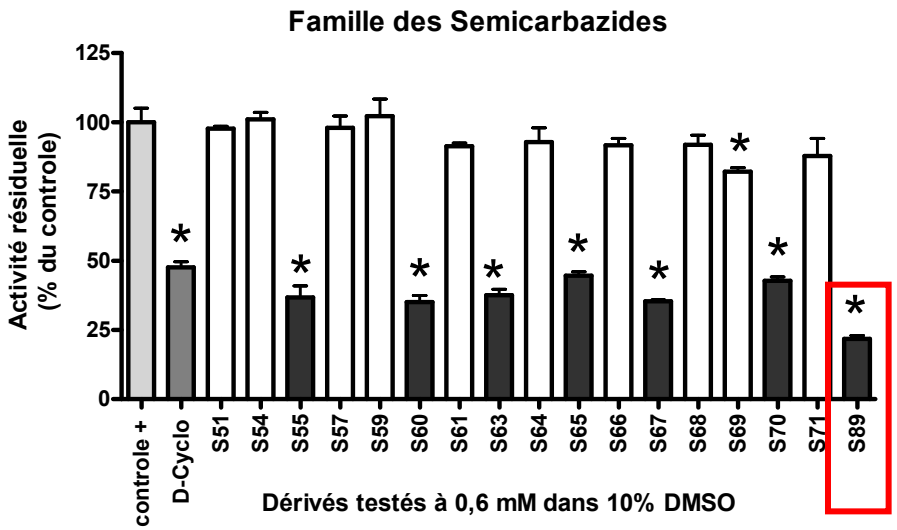
Antibacterial activity

Dérivés	Structure	EC_{50} [a] (mM)	Affinité théorique (kcal/mol)	CMI (µg/ml)		
				E. coli ATCC 25922	*S. aureus* ATCC 25923	*E. faecalis* ATCC 29212
B16		0,432 (0,2676 à 0,6987)	-9,2	128	32	32

Other molecules…

Semicarbazides 17 molécules	
Triazoles 8 molécules	
Dioxo-Indolines 7 molécules	

Semi-carbazides…



S89 is fairly active

Souche	Caractéristique et référence	CMI (µg/ml)	
		S89	D-cyclosérine
Enterococcus faecalis ATCC29212	Souche sensible	16	128
E. faecalis V583	VRE (type VanB)	32	128
E. faecalis BM4405	VRE (type VanE)	32	>128
E. faecium BM4147	VRE (type VanA)	32	32
Staphylococcus aureus ATCC25923	Souche sensible	8	32
S. aureus ATCC33591	HA-MRSA (Lemaire *et al.*, 2008)	16	/
S. aureus NRS192	CA-MRSA (PV+) (Lemaire *et al.*, 2008)	8	/
S. aureus NRS126	HA/MRSA/VISA (Lemaire *et al.*, 2008)	16	/
S. aureus VRS-1	HA-MRSA/VRSA (type VanA) (Lemaire *et al.*, 2008)	16	/
Streptococcus pneumoniae ATCC49619	Souche sensible	32	/
Listeria monocytogenes EGD	Souche sensible (Ouadrhiri *et al.*, 1999)	16	/

Refurbishing old antibiotics: novel aminoglycosides *

* using proprietary data of Achaogen Inc., South San Francisco, Cal.

Refurbishing aminoglycosides: the challenges and the advantages

- Advantages
  - wide spectrum and highly bactericidal
  - no metabolism and linear pharmacokinetics
  - extensive knowledge of their therapeutic and toxicological properties (leading to simple "once-daily dosing")

- Challenges
  - extensive development of resistance (mostly enzyme-mediated → aminoglycoside-modifying enzymes [AME])
  - nephrotoxicity and ototoxicity remain of concern and seem linked to activity

Clinically Relevant AMEs

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1999, p. 727–737 Vol. 43, No. 4
0066-4804/99/$04.00+0

MINIREVIEW

Aminoglycosides: Activity and Resistance

MARIE-PAULE MINGEOT-LECLERCQ,[1]* YOURI GLUPCZYNSKI,[2] AND PAUL M. TULKENS[1]

Unité de Pharmacologie Cellulaire et Moléculaire, Université Catholique de Louvain, Brussels,[1] and Service de Microbiologie, Cliniques Universitaires UCL de Mont-Godinne, Yvoir,[2] Belgium

Clinically Relevant AMEs

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1999, p. 727–737 Vol. 43, No. 4
0066-4804/99/$04.00+0

MA
Unité

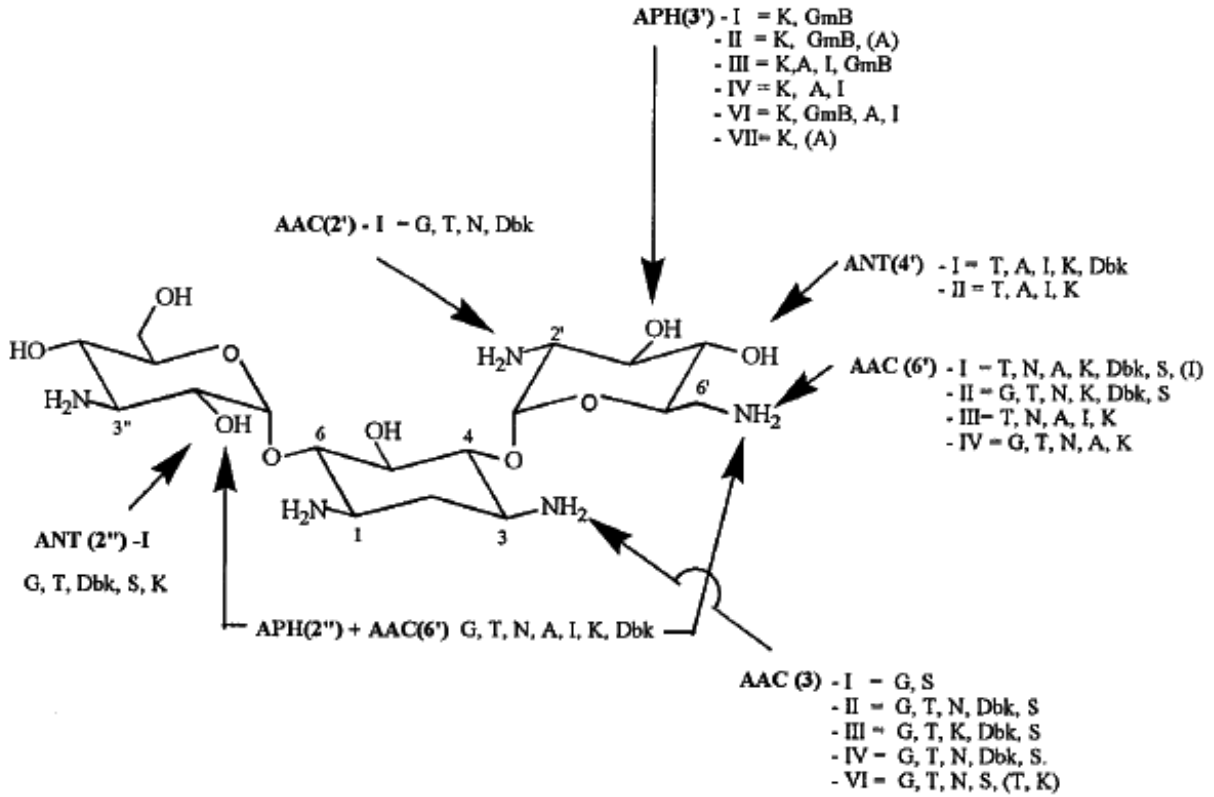


FIG. 3. Major aminoglycoside-modifying enzymes acting on kanamycin B (this aminoglycoside is susceptible to the largest number of enzymes). Each group of enzymes inactivates specific sites, but each of these sites can be acted upon by distinct isoenzymes (roman numerals) with different substrate specificities (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).

Aminoglycoside nephtotoxicity

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1999, p. 1003–1012 Vol. 43, No. 5
0066-4804/99/$04.00+0

Aminoglycosides: Nephrotoxicity

MARIE-PAULE MINGEOT-LECLERCQ* AND PAUL M. TULKENS
Unité de Pharmacologie Cellulaire et Moléculaire, Université Catholique de Louvain, Brussels, Belgium

Aminoglycoside nephtotoxicity

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1999, p. 1003–1012 Vol. 43, No. 5
0066-4804/99/$04.00+0

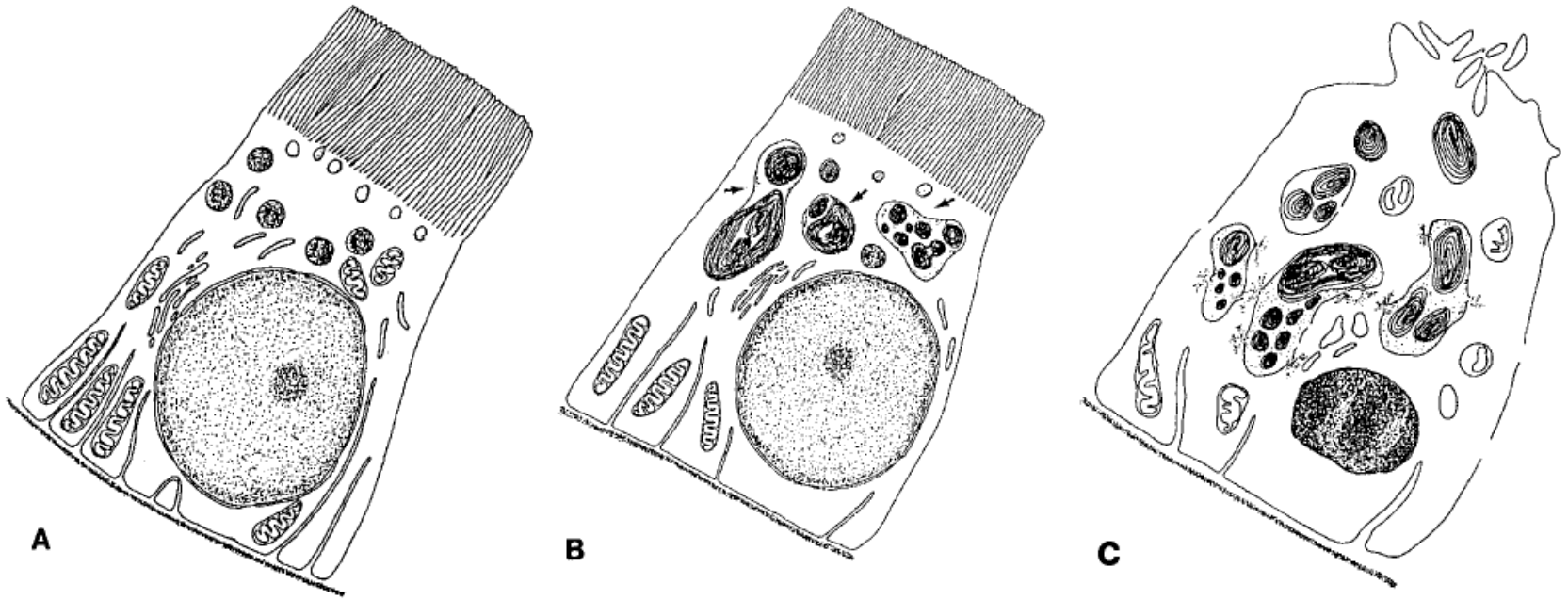
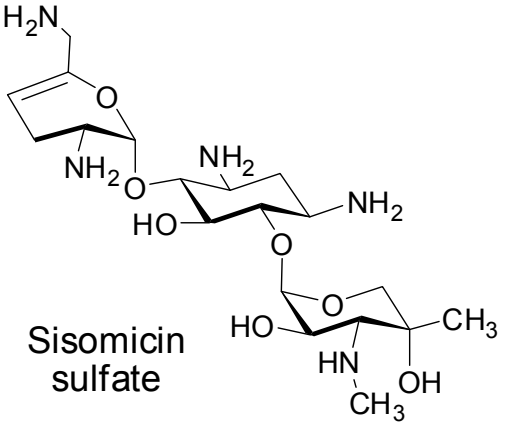


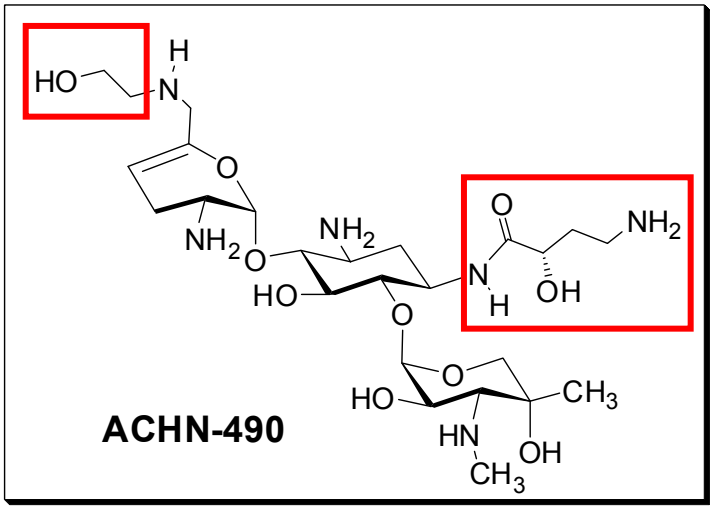
FIG. 1. Ultrastructural alterations induced in proximal tubular cells during aminoglycoside treatment. (A) Control. Changes detected early on and at low doses (B) consist mainly of the enlargement of lysosomes, which most likely occurs by fusion of preexisting structures and which is caused by the progressive deposition of polar lipids which adopt a concentric lamellar disposition (myelin-like structures, most commonly referred to as *myeloid bodies*); the other subcellular structures are usually well preserved. Later changes or changes observed with high doses (C) include the apparent rupture of lysosomes (with the release of myeloid bodies in the cytosol), extensive mitochondrial swelling and damage, dilatation of the endoplasmic reticulum cisternae, shedding of the apical brush-border villi, pericellular membrane discontinuities, and the occurrence of apoptotic nuclei. These alterations do not necessarily coexist in all cells. The figure is adapted from reference 76 and is based on the typical descriptions given in references 38, 40, 71, 76, 77, 127, and 138.

Synthesis and Structure of the novel aminoglycoside ACHN-490

- ACHN-490 is a derivative of sisomycin (known to be highly active but toxic)
- The modifications made provide protection against most pevalent AMEs
- Equally active against gentamicin-S and gentamicin *Enterobacteriaceae* and *Staphylococci*
- Indications being considered include cUTI, HAP, cIAI, and blood stream infections



8 Steps



Aggen J, et al, ICAAC 2009 Poster F1-840

Activity of ACHN-490 against Contemporary Gram-Negative Clinical Isolates from Brooklyn, NY Hospitals

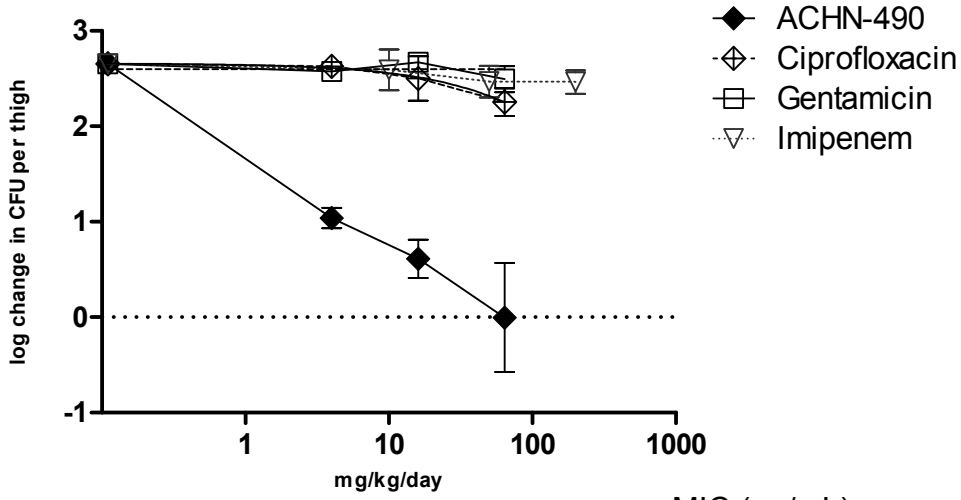
Organism	Agent	MIC_{50}	MIC_{90}	%S
K. pneumoniae **(n=71)**	ACHN-490	0.5	1	
	Amikacin	16	64	58%
	Gentamicin	1	>64	59%
	Imipenem	0.25	>8	79%
	Ceftazidime	>16	>16	37%
	Ciprofloxacin	8	>8	47%
E. coli **(n=32)**	ACHN-490	1	2	
	Amikacin	4	16	91%
	Gentamicin	1	64	72%
	Imipenem	0.12	8	82%
	Ceftazidime	1	4	69%
	Ciprofloxacin	>8	>8	31%
Enterobacter **spp. (n=30)**	ACHN-490	1	4	
	Amikacin	4	16	94%
	Gentamicin	1	4	70%
	Imipenem	0.5	2	94%
	Ceftazidime	>16	>16	27%
	Ciprofloxacin	0.12	1	74%

Landman D, et al, ICAAC 2009 Poster F1-842

In Vivo Efficacy of ACHN-490 against Enterobacteriaceae and MRSA

- Murine neutropenic thigh model
  - MRSA
  - Susceptible and MDR Enterobacteriaceae
    - *E. coli (+/- KPC)*
    - *K. pneumoniae (wt, + KPC, CIP-R/GEN-R)*
    - *S. marcescens (+KPC)*
- ED_{50} and ED_{50} /MIC values lower for ACHN-490 compared to gentamicin
  - Gentamicin ineffective against GEN-R strains
- Bacterial titers reduced to or below the initial bacterial load (static level) for each strain
- Confirmatory of in vitro susceptibilities

Activity against KPC-expressing *K. pneumoniae* AKPN1109 in the murine neutropenic thigh model
All mice dosed SC twice daily at 2 and 14 hours



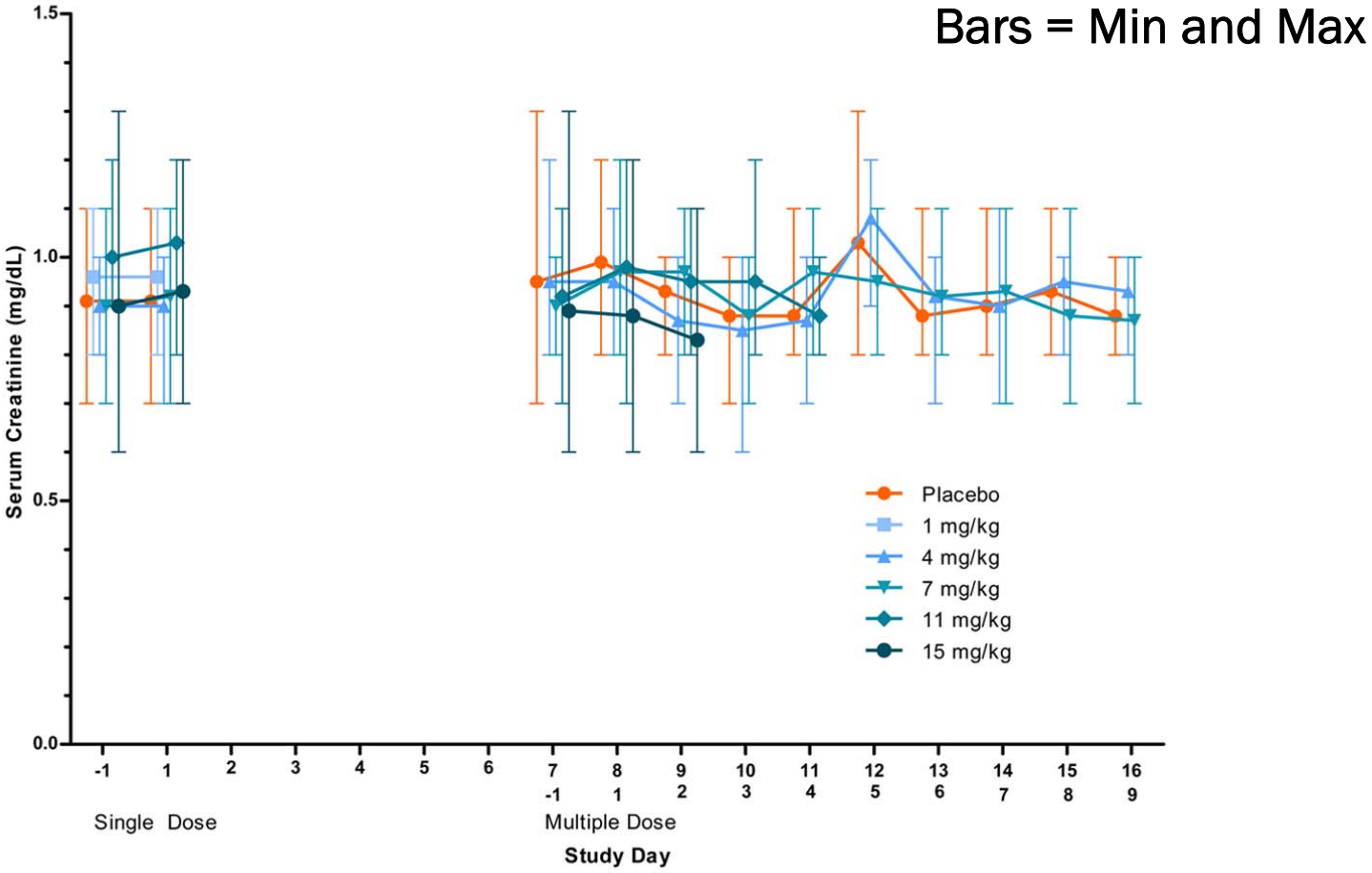
MIC (μg/mL)
ACHN-490 = 0.5
Cipro = 2
Gent = 64
Imi = 32

Reyes N, et al, ICAAC 2009 Poster F1-845

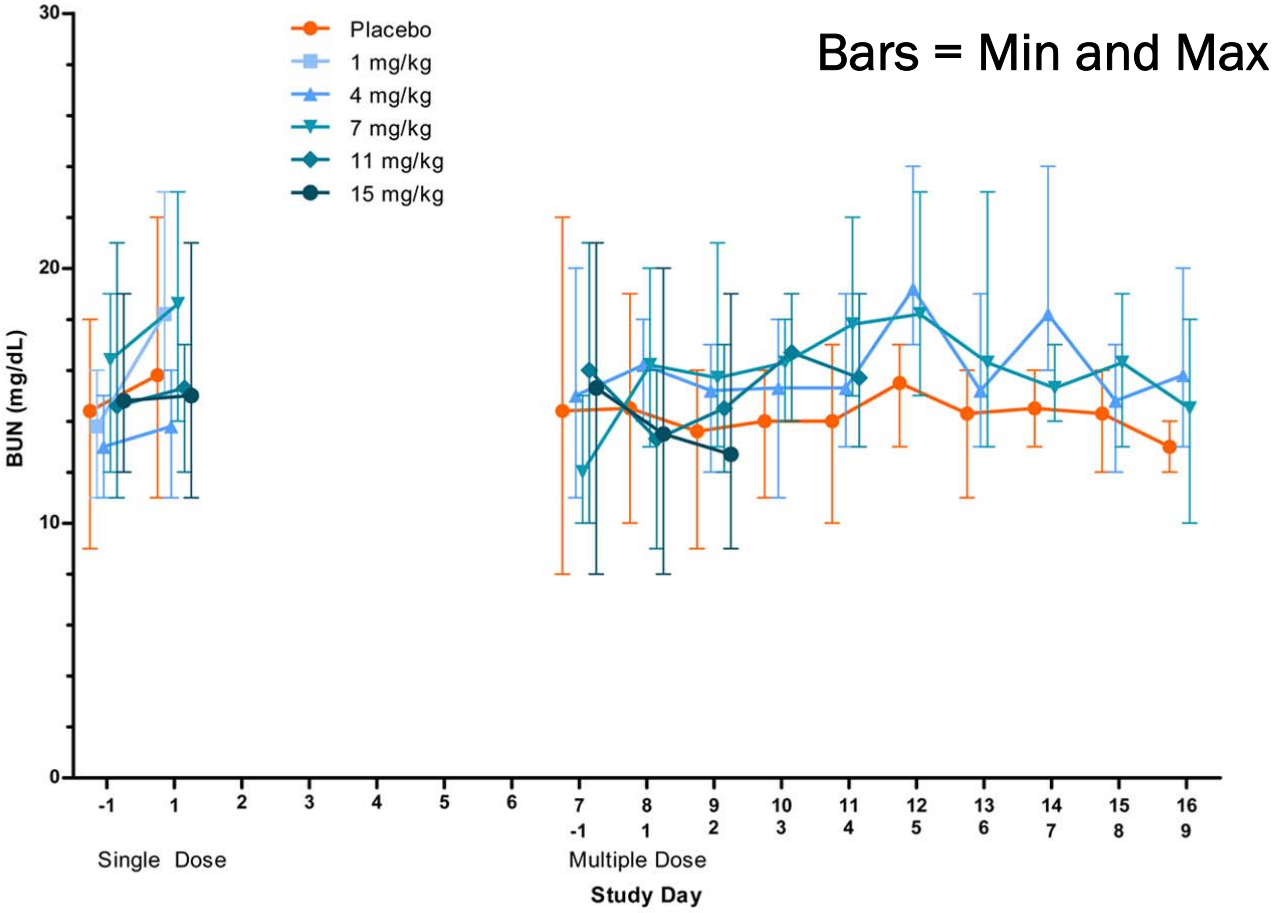
Extensive Safety Monitoring Focused on Nephrotoxicity and Ototoxicity

- Adverse Event monitoring
- Routine safety laboratory assessments
- Renal
  - Daily BUN & Cr during dosing
  - Calculated Creatinine clearance using Cockroft-Gault formula
  - Measured Creatinine clearance based on 24-hour urine collection
  - Additional GFR monitoring through Iothalamate clearance
- Cochlear
  - Full Audiograms with bone conduction
    - Test range 2 to 20 kHz (normal hearing range 2 to 8 kHz)
  - Daily Otoacoustic Emission (OAE) testing during multiple dose period
- Vestibular
  - Full Electronystagmography (ENG) with calorics
    - Tests: Unilateral Weakness, Directional Preponderance, Pendulum Tracking, Fixation
  - Daily Dynamic Visual Acuity (DVA) tests during multiple dose period

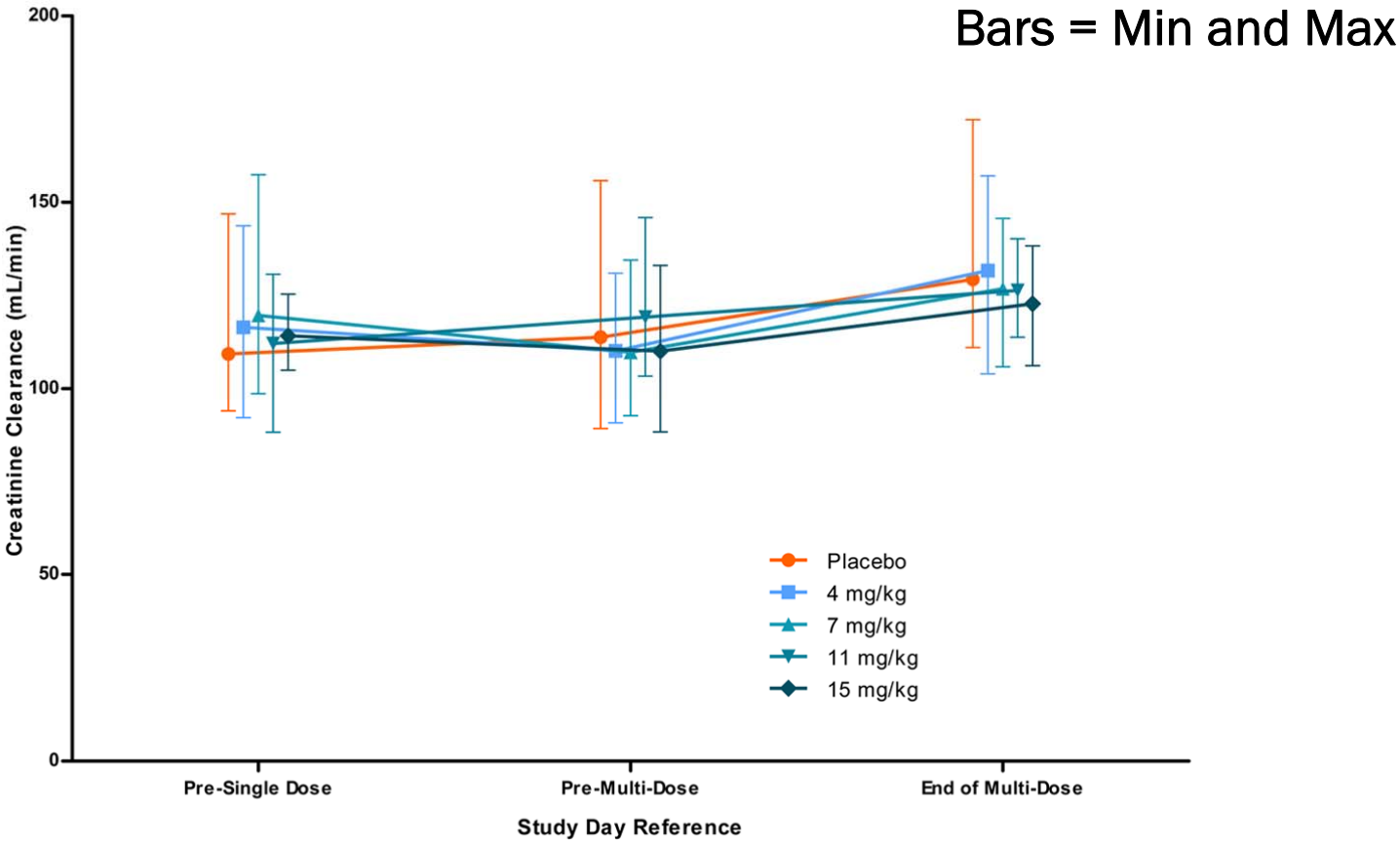
No Evidence of Nephrotoxicity Based on Daily Serum Creatinine



No Evidence of Nephrotoxicity Based on Daily BUN Measurements



No Evidence of Nephrotoxicity Based on Measured Creatinine Clearance



Novel polymyxins

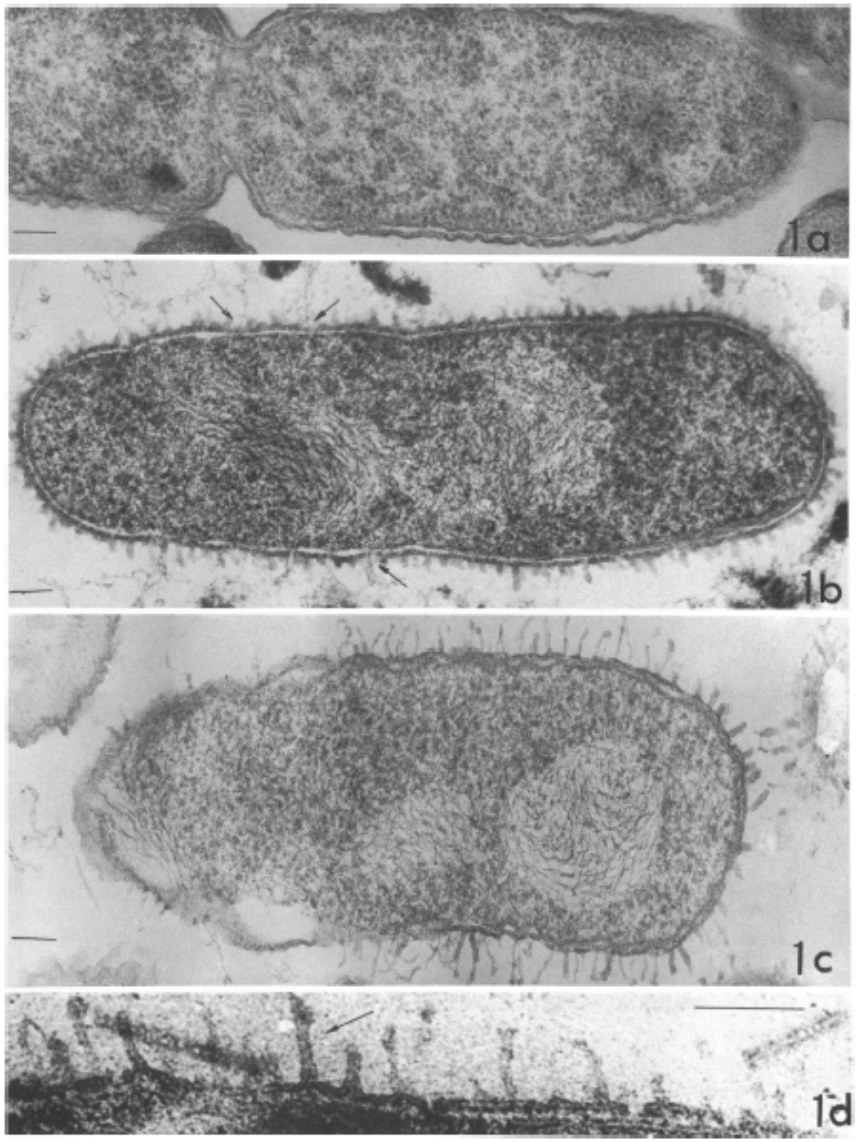
Why novel polmyxins ?

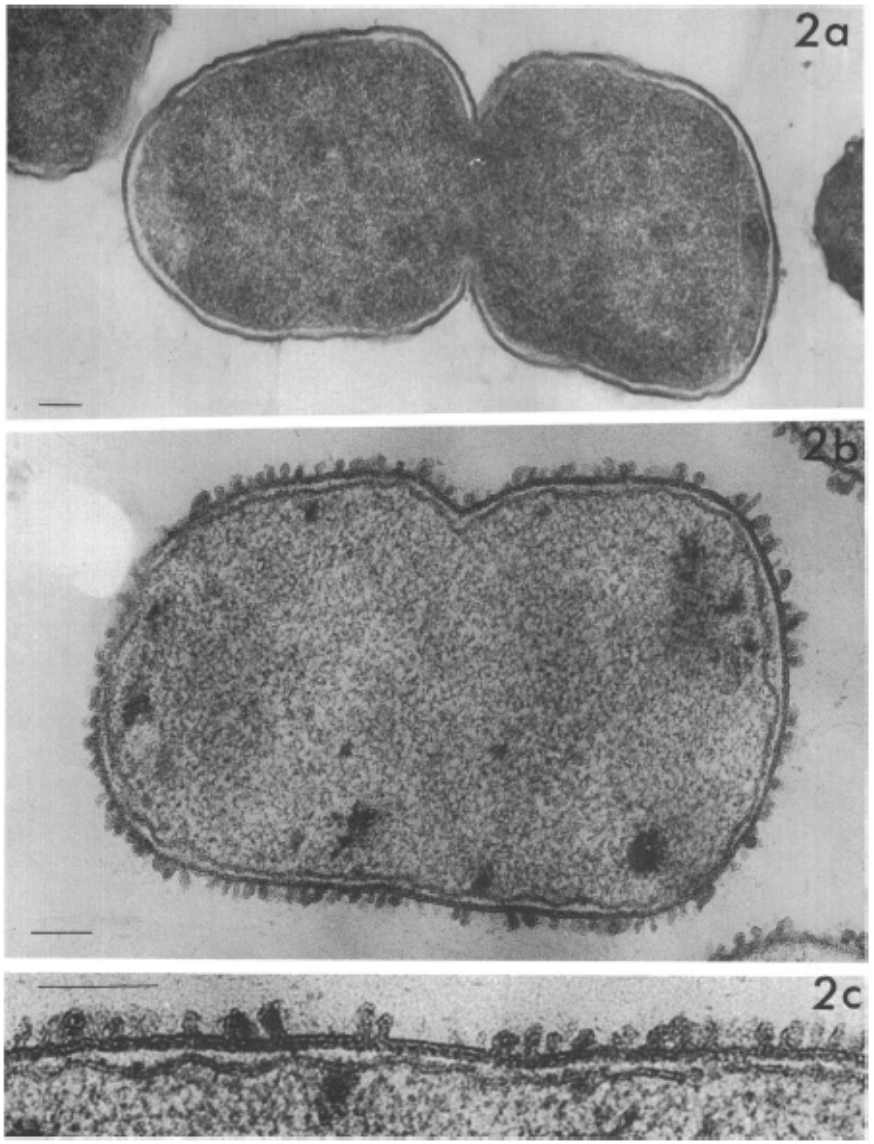
- Colistin (Polymyxin E; discovered in 1949 and without clinical use for long) has now become the "last resource" antibiotics in the treatment of infecions caused by multi-resistant organisms…

Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections.
Falagas ME, Kasiakou SK.
Clin Infect Dis. 2005 May 1;40(9):1333-41. Epub 2005 Mar 22. Review. Erratum in: Clin Infect Dis. 2006 Jun 15;42(12):1819. Dosage error in article text.
PMID: 15825037 [PubMed - indexed for MEDLINE] **Free Article**
Related citations

- But colistin is a fairly toxic antibiotic (nephrotoxicity), which limits the concentrations that can be safely used, and therefore, limits its activity).
- Polmyxin B is more active but more toxic …
- Better compounds are badly needed, but the mode of action of colistin (membrane permabilization)should be retained because it ensures a fast bactericidal effect AND synergy with other antibiotics

Colistin Microbiology: morphological aspects

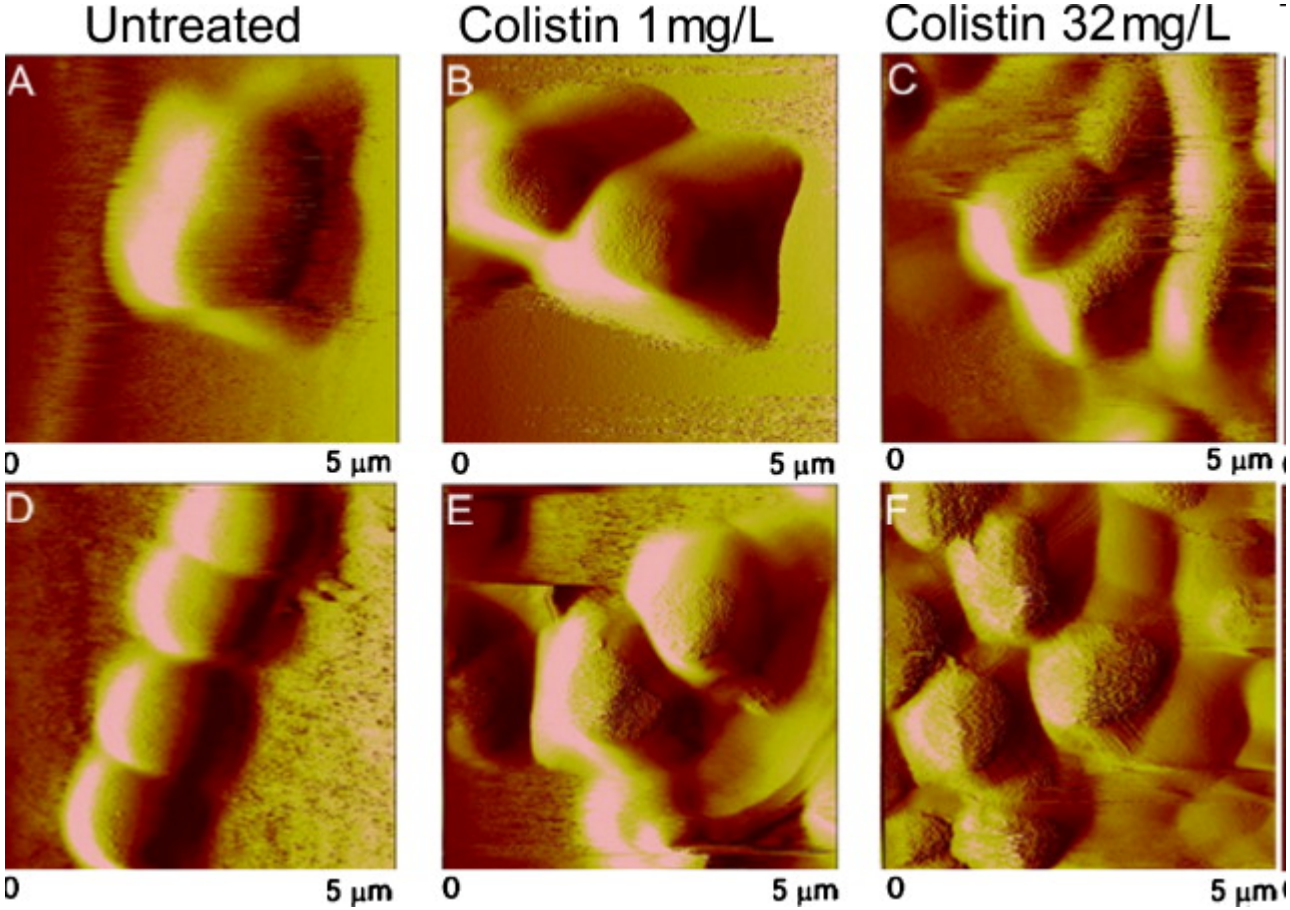




Koike et al. J. Bacteriol. 1969; 97:448-452

Colistin Microbiology: morphological aspects

Live *Acinetobacter baumanii* as seen in Atomic Force Microscopy (AFM°

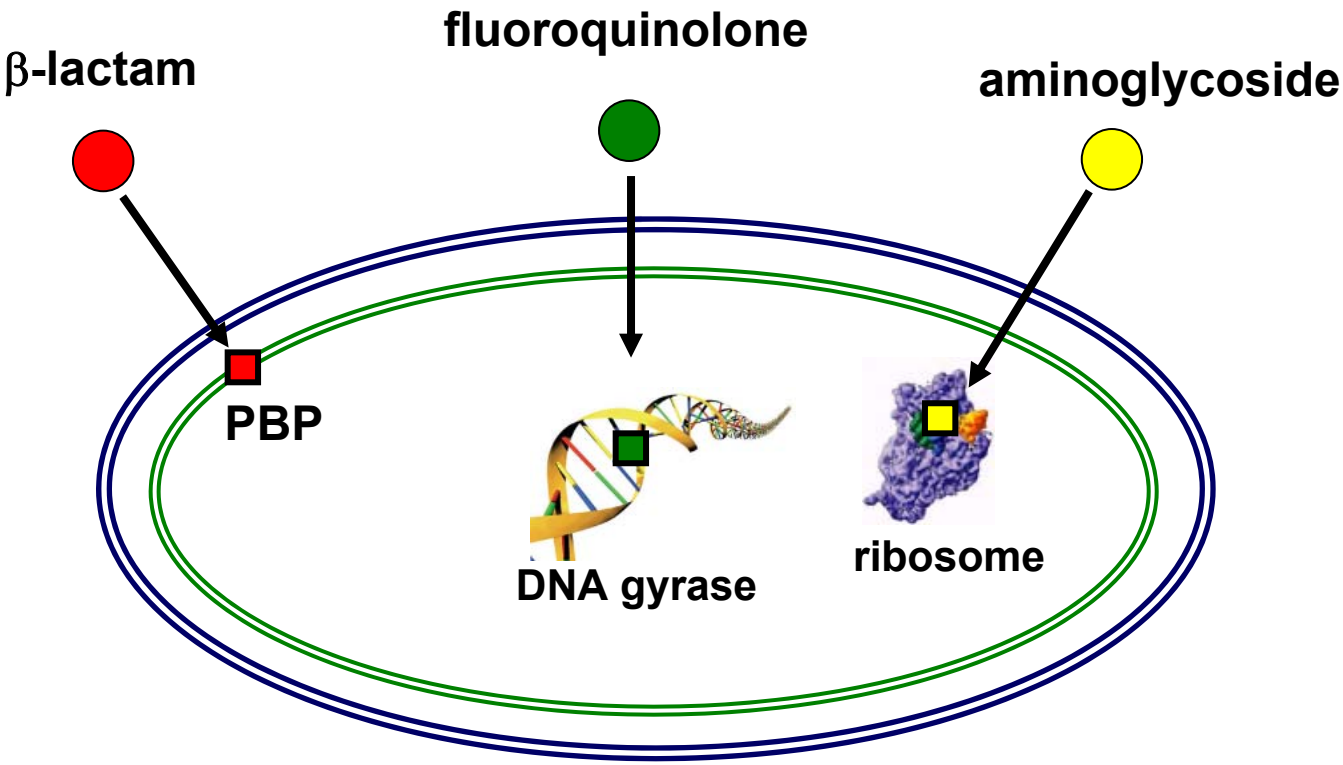


susceptible

resistant

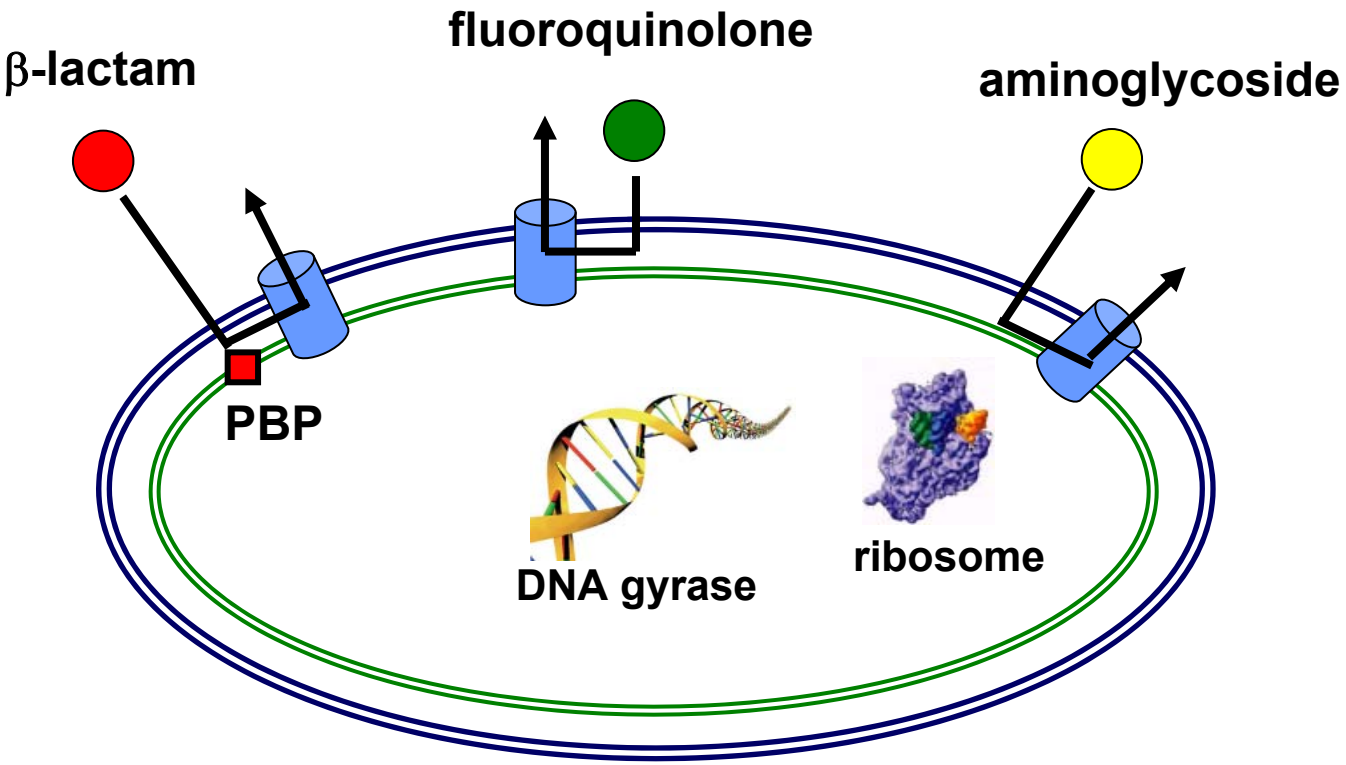
Soon et al. Int J Antimicrob Agents. 2011 Sep 16. [Epub ahead of print]

Polymyxins synergy: the rationale (1 of 3)



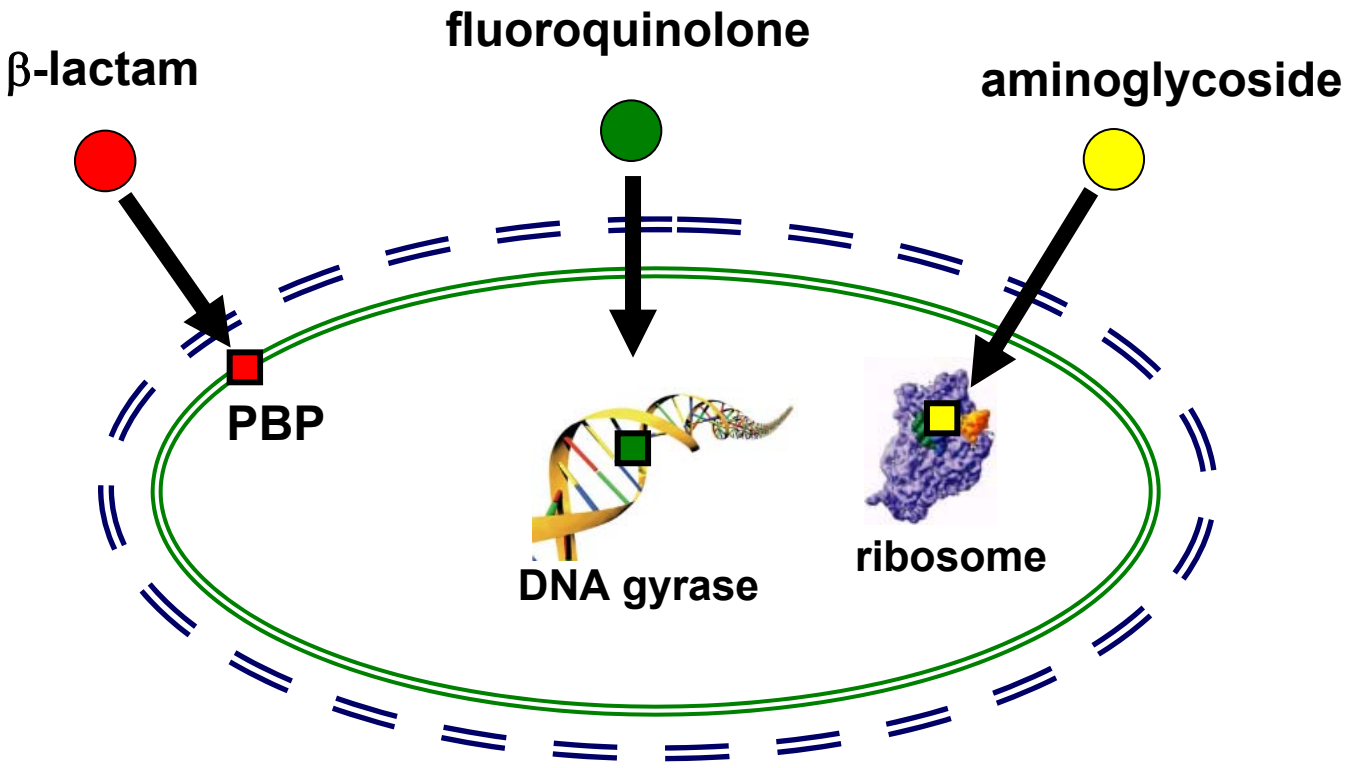
- Gram-negative bacteria have two membanes (OM and IM)
- Antibiotic targets are most often located in the IM or intracellularly
- Most antibiotics must at least pass across the OM to reach their target, which may represent a limiting step

Polymyxins synergy: the rationale (2 of 3)



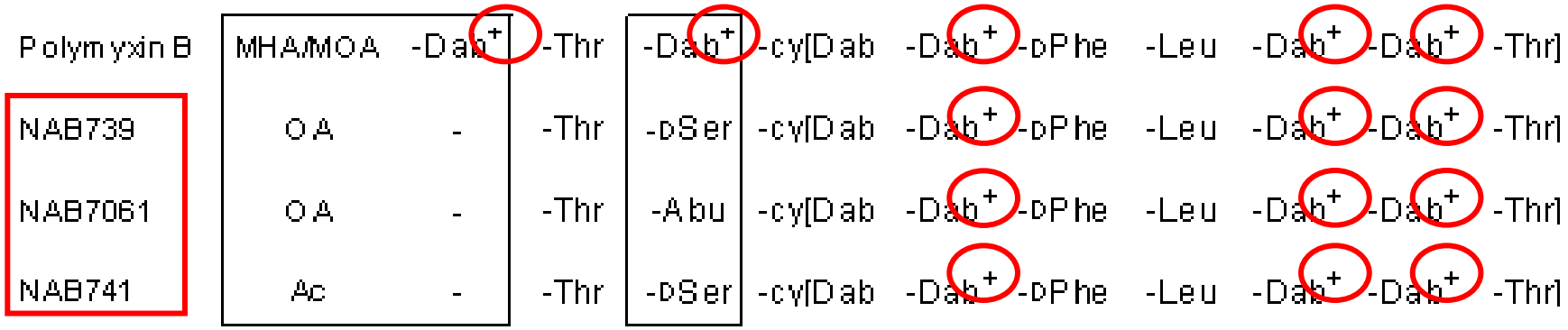
- Gram-negative bacteria have also efflux systems defeating the passage of drugs across the OM and explaining the low activity of many antibiotics (intrinsic resistance) and the so-called "adaptative" resistance (aminglycosides)

Polymyxins synergy: the rationale (3 of 3)



- **Disrupting the OM (as colistin does) will facilitate access of the other antibiotics to their targets**
- **This may apply EVEN to antibiotics for which the bateria are resistant (if due to OM impermeability/efflux phenomenon)**

Novel polymyxin B derivatives

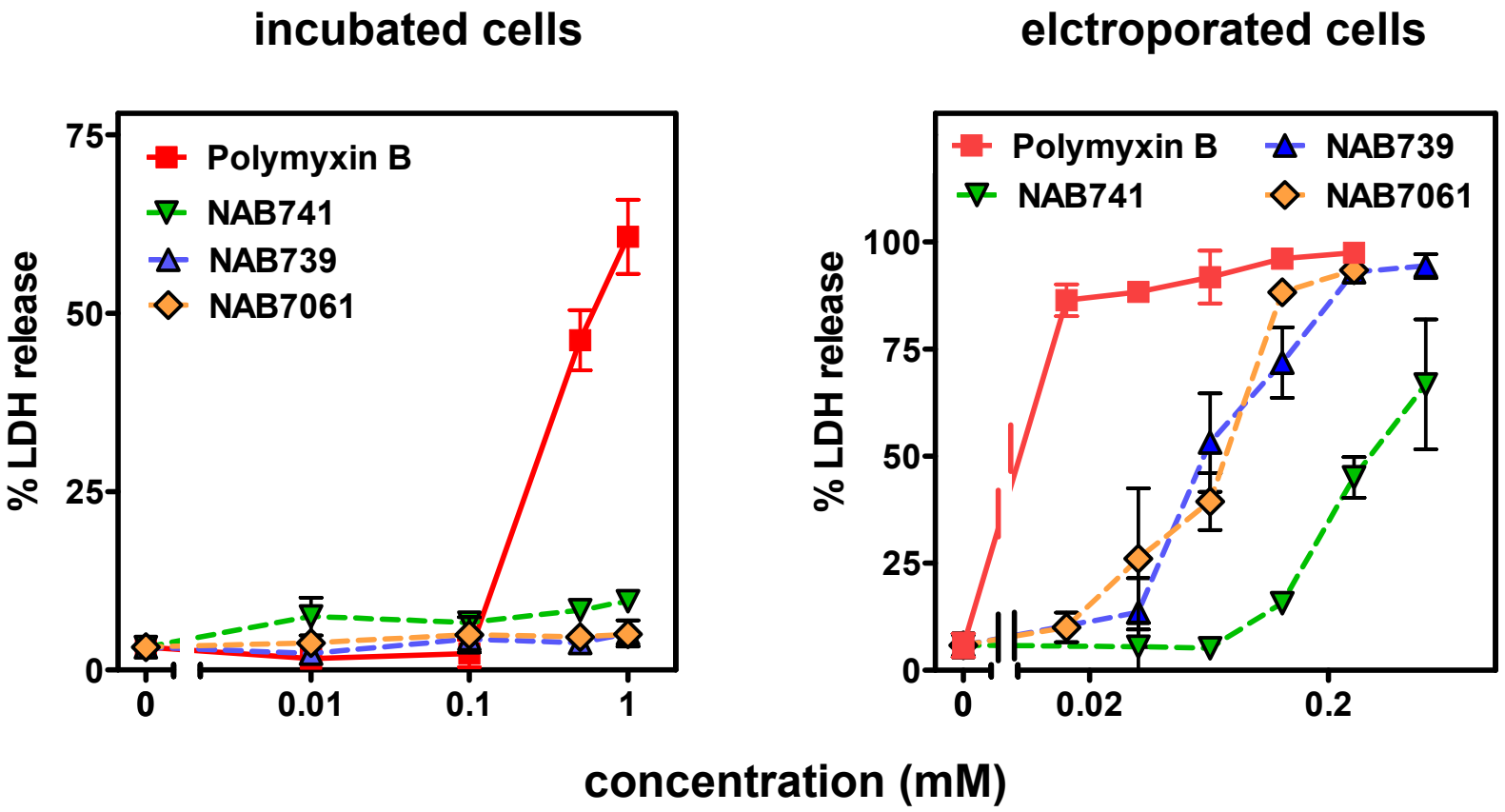


- **The MIC90 of NAB739 for *E. coli* and Enterobacteriaceae are similar to those of polymyxin B (1-2 mg/L).**
- **NAB739 is also active against *Acinetobacter baumannii*, and *Pseudomonas aeruginosa.***
- **NAB7061 and NAB741 strongly synergize the activity of antibiotics (including rifampicin, macrolides, fusidic acid and vancomycin) towards Gram (-) pathogens**

Vaara et al. 2008, Antimicrob. Agents Chemother. 52:3229-3236 - Vaara et al. 2010a, Antimicrob. Agents Chemother. 54, 3341-3346 - Vaara et al. 2010b, J. Antimicrob. Chemother. 65, 942-945.

Novel polymyxin B derivatives: toxicity

LDH release (cytotoxicity) in cultures renal cells (LLC-PK1)



Mingeot-Leclercq et al. 51st Interscience Conference on Antmicrobial Agents and Chemotherapy, Chicago, IL, 2011

Expanding the spectrum of an old β-lactam with unusual resistance to β-lactamases : Temocillin

Temocillin in a nutshell

Journal of Antimicrobial Chemotherapy (2009) **63**, 243–245
doi:10.1093/jac/dkn511
Advance Access publication 18 December 2008

JAC

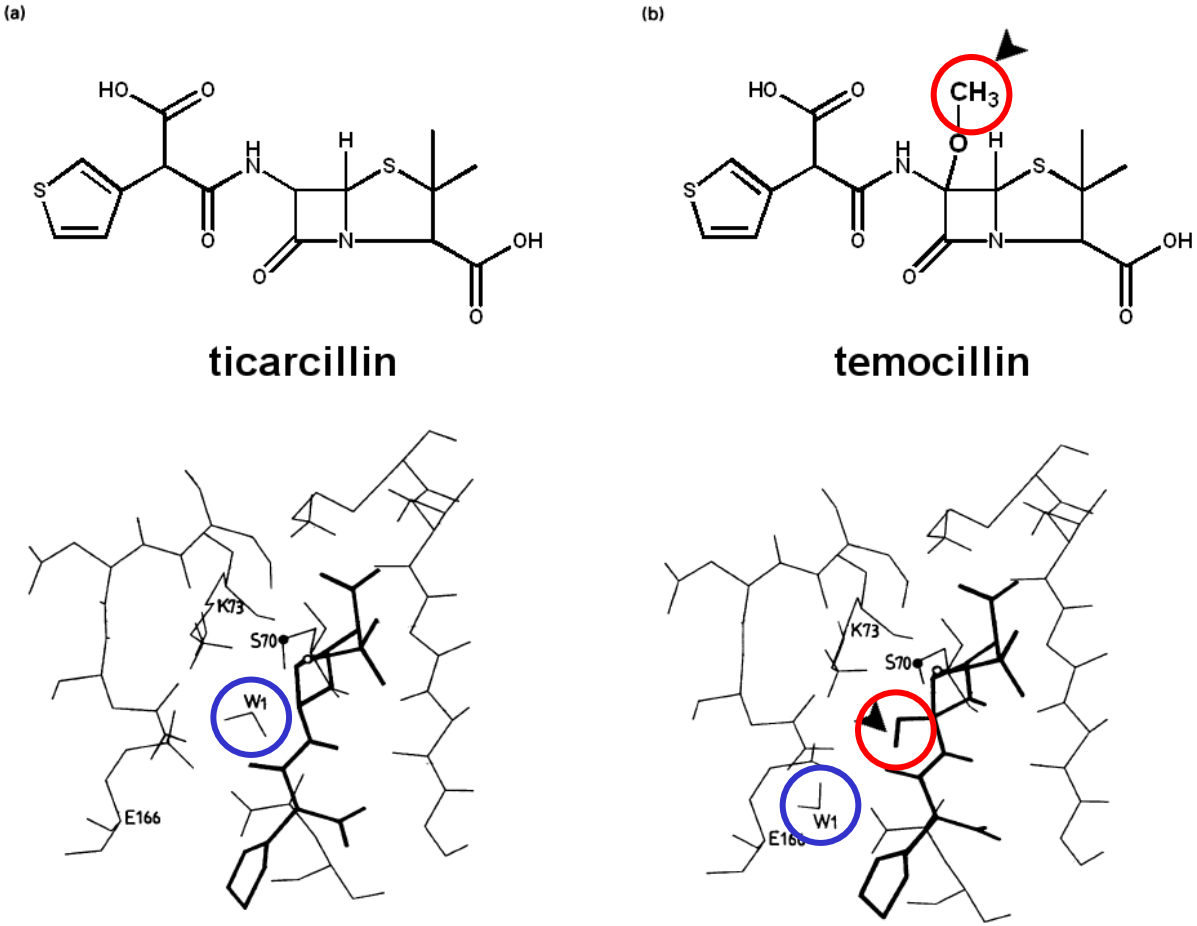
Temocillin revived

David M. Livermore[1]* and Paul M. Tulkens[2]

[1]*Antibiotic Resistance Monitoring and Reference Laboratory, Health Protection Agency Centre for Infections, 61 Colindale Avenue, London NW9 5EQ, UK;* [2]*Unité de Pharmacologie Cellulaire et Moléculaire & Centre de Pharmacie Clinique, Université Catholique de Louvain, Bruxelles, Belgium*

Resistance in Gram-negative pathogens is an increasing concern, with carbapenems often appearing as the only acceptable treatment option in serious infections. Reviving older compounds that have fallen into disuse may help to alleviate this burden. Temocillin (6-α-methoxy-ticarcillin) is resistant to most if not all classical and extended-spectrum β-lactamases and to AmpC enzymes. It is also chemically stable, allowing administration by continuous infusion. Pharmacokinetic/pharmacodynamic analysis, aided by Monte-Carlo simulations, suggests a breakpoint of 8 mg/L for the registered maximum dosage of 4 g daily. Temocillin's weaknesses, explaining its limited previous use, are a lack of activity against Gram-positive organisms, anaerobes and *Pseudomonas*. In settings where these are unlikely or are covered by other agents, temocillin may be useful, potentially 'sparing' carbapenems and having little apparent potential to select for *Clostridium difficile*.

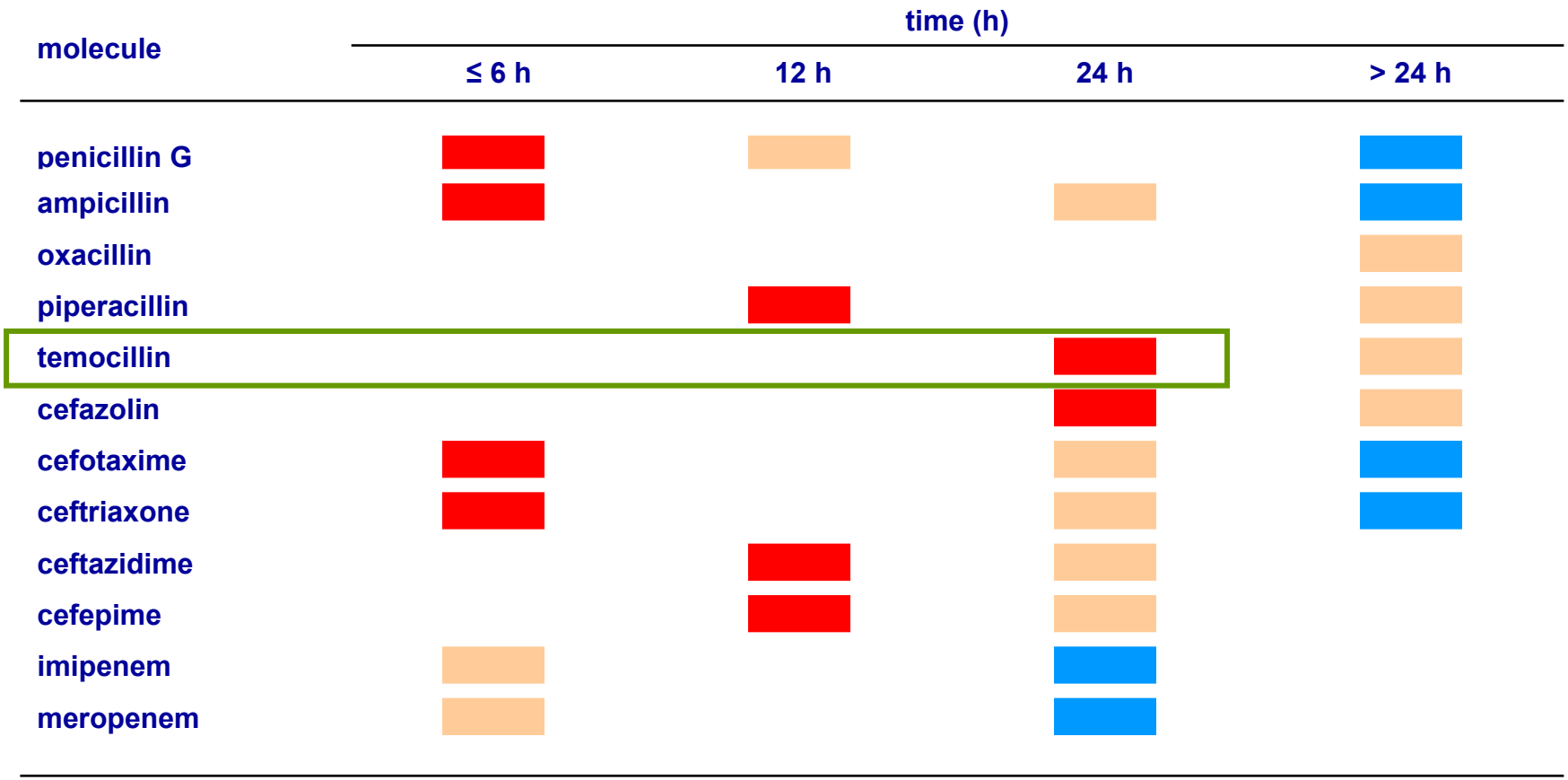
Temocillin in a nutshell



Structural formulae of ticarcillin and temocillin emphasizing the importance of the α-methoxy group (arrow) in temocillin for blocking access of water (W1) to the active serine (S70) of β-lactamase, thereby blocking the chain of molecular events leading to hydroysis of the β-lactam ring (and inactivation of the antibiotic) and regeneration of an active enzyme.10 (Matagne *et al.* 1993Biochem J 1993; 293:607-11)

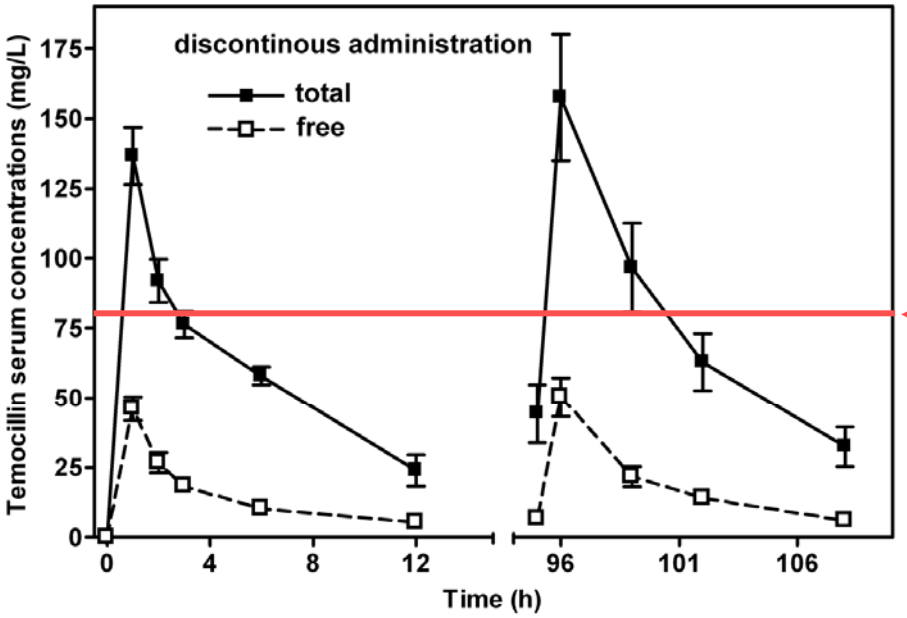
Temocillin is also very thermostable

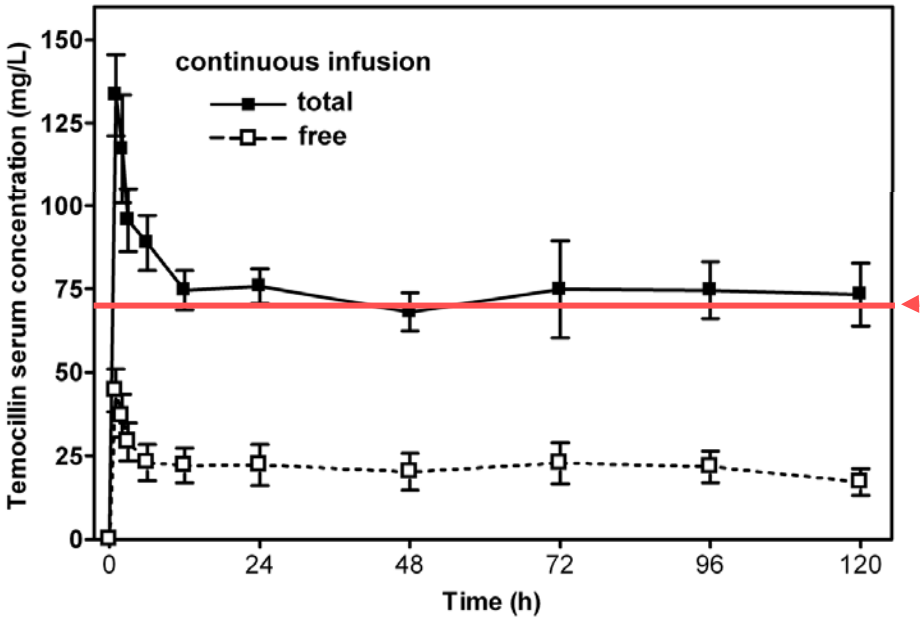
- **Definition: > 90% intact product (Pharmacopeia)**
- **Conditions: mimicking the total daily dose (commercial product) in 48 mL (motor operated syringe) water without pH adjustment and maintained at a fixed temperature (*)**
- **key:** 37°C 25°C 4°C



* Servais & Tulkens, AAC 2001;45:2643-7 – Viaene et al. AAC 2002;46:2327-32 - Baririan et al. JAC 2003;51:651
other references for indvual drugs in in Berthoin et al. (in preparation).

Temocillin can easily be used by continuous infusion





Concentration at equilibrium (total): 73 ± 3 (40 - 142)

J. Antimicrob. Chemother. 2008 Feb;61(2):382-8

Why is temocillin not active against *P. aeruginosa* ?

Table 1. MICs of temocillin and ticarcillin against *P. aeruginosa* strains with known expression of the efflux Mex components in Mueller-Hinton broth (MHB) and in MHB supplemented with the broad spectrum efflux transporter inhibitor Phe-Arg-β-naphthylamide (PAβN; 50 µg/mL)

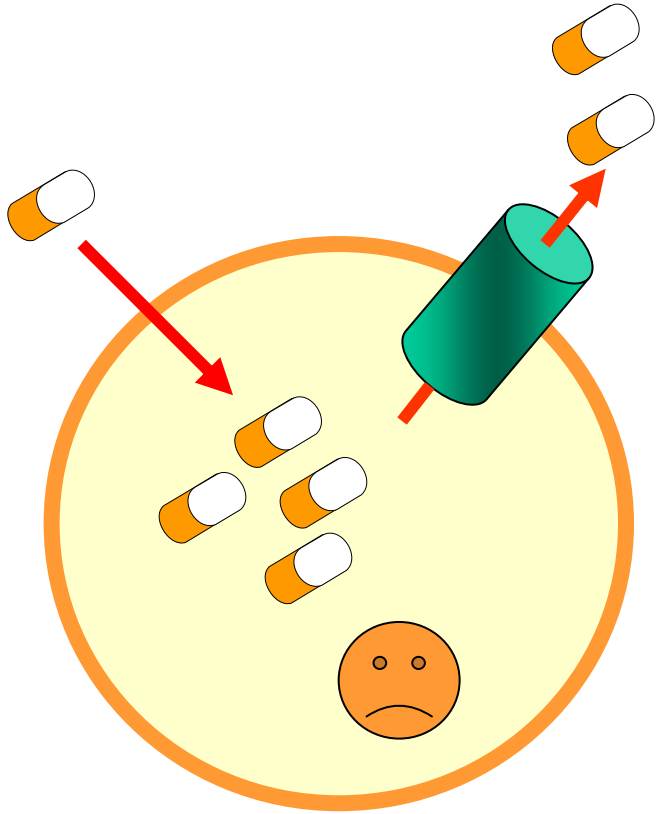
Strains	Origin or Ref.	Description	Expression of Efflux system					MIC (mg/L)	
			AB [a]	XY [a]	OprM [a]	CD [b]	EF [b]	Temocillin (+PAβN)	Ticarcillin (+PAβN)
Reference strain									
PAO1	ATCC		1	1	1	-	-	256 (64)	32 (16)
Clinical isolates									
12	d		**3.97**	9.04	ND	+	+	**512** (128)	64 (64)
11	d		**3.56**	5.68	ND	-	-	**>512** (64)	32 (32)
156	d		0.33	0.95	ND	-	+	512 (64)	256 (32)
68	d		0.87	44.94	ND	-	-	512 (64)	32 (16)
333A	d		**2.17**	2.29	ND	-	-	**>1024** (1024)	128 (128)
34	d		**6.86**	1.26	ND	-	-	**>1024** (512)	256 (128)
168B	d		1.15	0.89	ND	-	-	256 (32)	16 (16)
Engineered strains									
FB1	3	PAO1Δ(*mexB::FRT*)	ND	ND	ND	ND	ND	**2**	0.5
PAO1 *mexAB*	4	PAO1Δ(*mexAB::FRT*)	**0**[e]	1.08	ND	-	+	**4** (2)	2 (2)
PAO200	4	PAO1Δ(*mexAB-oprM*)	**0**[e]	1.26	ND	-	-	**4** (0.5)	2 (0.5)
CB536	5	PAO1Δ(*mexCD-oprJ*)	1.09	1.65	ND	-	+	128 (16)	8 (1)
CB603	5	PAO1Δ(*mexEF-oprN*)	1.21	1.06	0.51	-	-	128 (32)	16 (16)
CB602	5	PAO1Δ(*mexXY-oprM*)	1.10	0.06	0.55	-	+	64 (16)	16 (16)
PAO1Δ(oprM)		PAO1 Δ(*oprM*)	ND	ND	ND	ND	ND	**2**	0.5
4098	6	Clinical strain	1.26	1.62	0.33	-	-	256 (128)	32 (32)
4098E	6	4098 overproducing OprM	**5.41**	1.31	**3.19**	-	-	**1024** (512)	64 (32)
4098ET	6	4098E Δ(*oprM*)	2.18	0.04	**0.02**	-	-	**2** ([f])	2 ([f])

[a] Real-time PCR (threshold ratio compared to PAO1; values of ≥ 2 and 5 are considered to denote highly significant overexpression of *mexAB* and *mexXY*, respectively. [b] RT-PCR (qualitative detection [+ / -]). [c] Phe-Arg-β-naphthylamide (broad spectrum efflux inhibitor) used at 50 mg/L. [d] isolated from Intensive Care patients with a clinical diagnostic of health care-associated pneumonia. [e] complete absence of detection. [f] No growth, PAβN MIC = 25 mg/L.

Buyck et al. 51th ICCAC, Chicago, IL, 2011

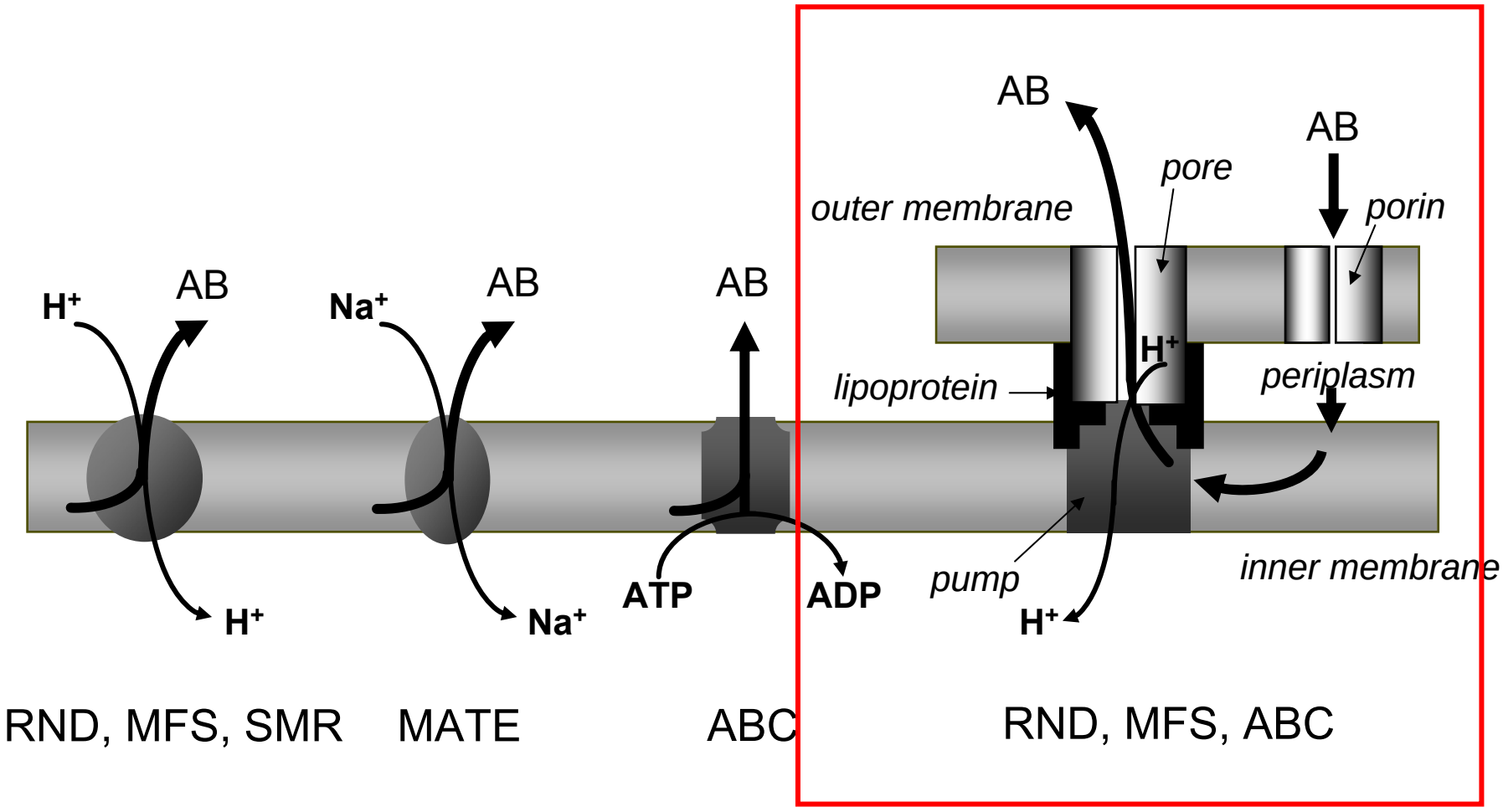
Efflux and resistance

- efflux is a universal mechanism for cell protection against "toxic" membrane-diffusing agents
- many drugs diffuse though membranes because we made them amphiphilic to favor their diffusibility …and become opportunistic substrates for efflux pumps
- for AB, efflux decreases the amount of drug in bacteria and impairs activity, increasing the MIC …
- insufficient drug exposure favors the selection of less sensitive organisms



Van Bambeke et al.
J Antimicrob Chemother. 2003;51:1055-65.

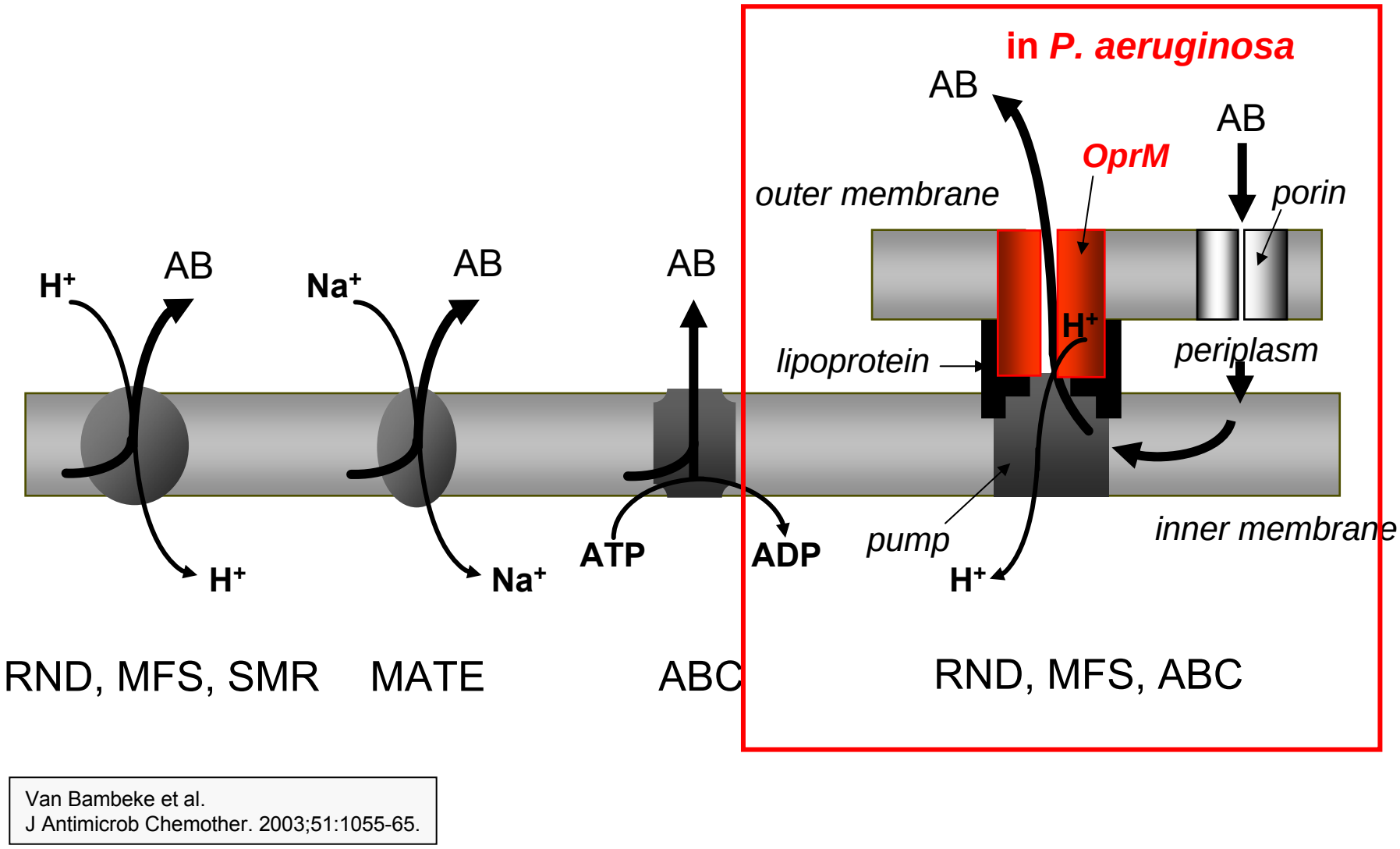
Structure of antibiotic efflux transporters



Van Bambeke et al.
J Antimicrob Chemother. 2003;51:1055-65.

Gram (-) transporters (incl. mex pumps)

Structure of antibiotic efflux transporters



Van Bambeke et al.
J Antimicrob Chemother. 2003;51:1055-65.

Design of efflux pump inhibitors

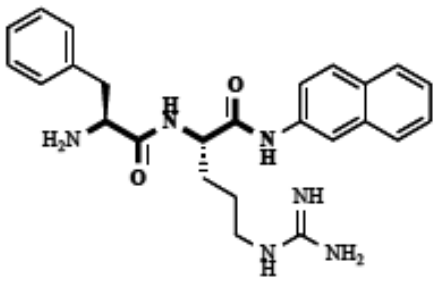
138 *Frontiers in Anti-Infective Drug Discovery*, 2010, *1*, 138-175

Inhibitors of Bacterial Efflux Pumps as Adjuvants in Antibacterial Therapy and Diagnostic Tools for Detection of Resistance by Efflux

Françoise Van Bambeke[*,1], Jean-Marie Pagès[2] and Ving J. Lee[3, 4]

[1]*Unité de Pharmacologie Cellulaire et Moleculaire, Université Catholique de Louvain, Brussels, Belgium;* [2]*EA2197 Enveloppe Bactérienne, Perméabilité et Antibiotiques, Faculté de Médecine, Université de la Méditerranée, Marseille, France;* [3]*Adesis, Inc., New Castle, DE 19720, USA;* [4]*Limerick BioPharma, Inc., S*

wide spectrum inhibitor active on efflux of penicillins in *P. aeruginosa*

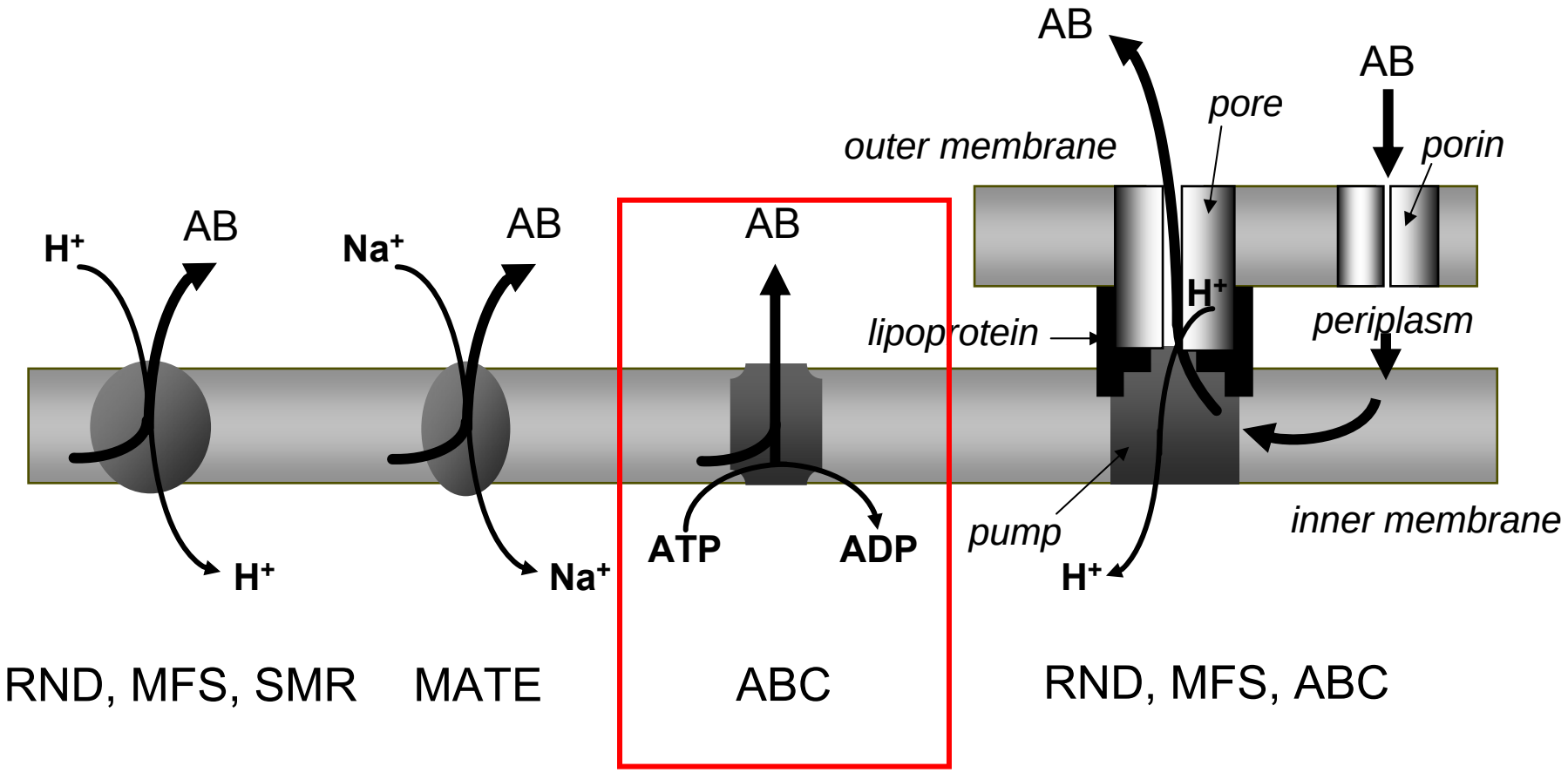


MC 207, 110 (28)

	Fluoroquinolones chloramphenicol erythromycin carbenicillin tetracycline ethidium bromide	*P. aeruginosa*	10 µg/ml
	spectinomycin clarithromycin nalidixic acid	*B.pseudomallei* *A. baumannii* *S. maltophilia* *Y.enterocolitica* *S. enterica*	
	fluoroquinolones quinolones chloramphenicol	*E. aerogenes* *E. coli*	

Structure-based approach

Example with an ABC transporter



Van Bambeke et al.
J Antimicrob Chemother. 2003;51:1055-65.

Structure-based approach

J. Phys. Chem. B **2010,** *114,* 15948–15957

Dynamics and Structural Changes Induced by ATP Binding in SAV1866, a Bacterial ABC Exporter

Jean-Paul Becker,[†] Françoise Van Bambeke,[‡] Paul M. Tulkens,[‡] and Martine Prévost*[,†]

Structure et Fonction des Membranes Biologiques, Université Libre de Bruxelles, Boulevard du Triomphe CP 206/2, B-1050 Brussels, Belgium, and Unité de Pharmacologie cellulaire et moléculaire, Université catholique de Louvain, Avenue E. Mounier 73, B-1200 Brussels, Belgium

Received: April 28, 2010; Revised Manuscript Received: September 6, 2010

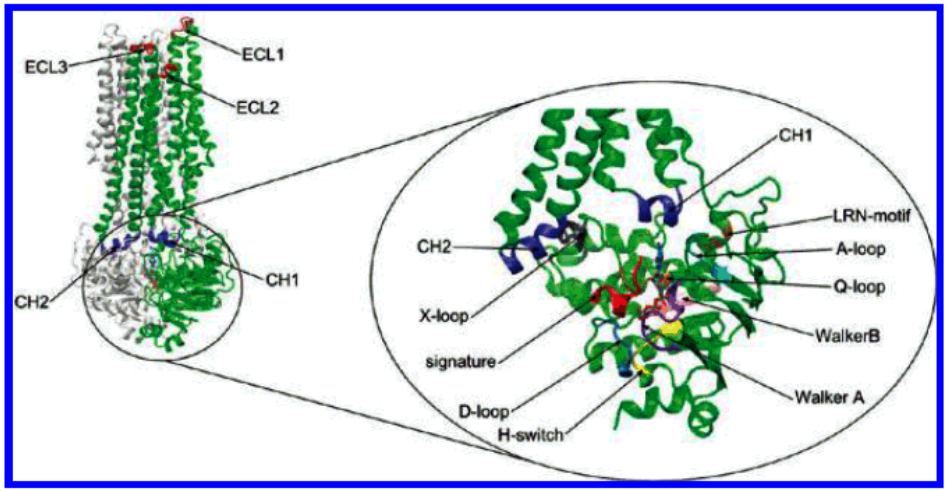


Figure 1. Ribbon representation depicting the crystal structure of the SAV1866 dimer (PDB code: 2ONJ). One monomer is green, the second monomer is white. The ATP molecules located in the NBDs are represented as sticks and are colored according to the atom represented (carbon in cyan, nitrogen in blue, oxygen in red, and phosphorus in bronze). ECL and CH stand for the extracellular loops and the intracellular coupling helices. The enlarged view shows a close-up illustration of one of the NBDs and of the main motifs common to members of the ABC exporter family.

Structure-based approach: the substrate trajectory

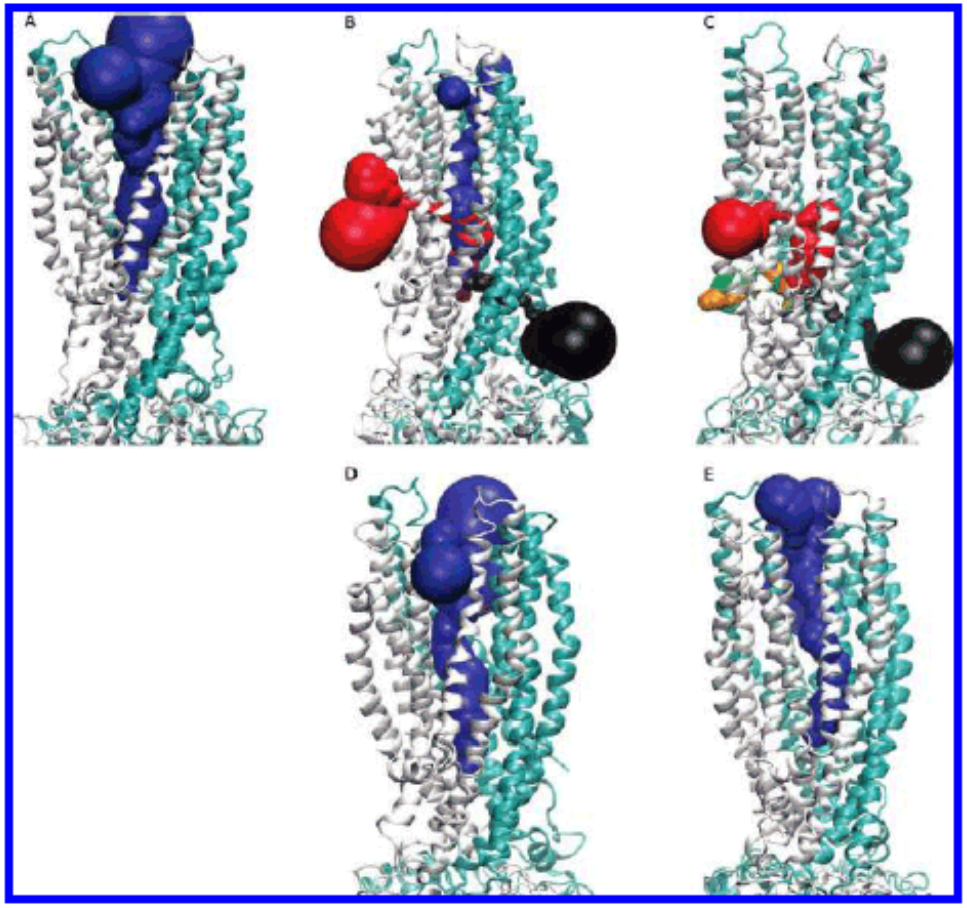


Figure 3. Dynamics of the internal TMD chamber in the nucleotide-free and the nucleotide-bound trajectories. Access paths originating at the bottom center of the TMD identified with the MOLE program[57] at 0, 40, and 80 ns of the simulation time. Upper: In the nucleotide-free trajectory. (A) At 0 ns a central path is identified that eventually splits at its extracellular extremity. (B) At 40 ns a central path persists, albeit with a much smaller radius. (C) At 80 ns, no central cavity exiting to the extracellular medium is identified anymore. Other, narrower paths exit to the cytosol and inner membrane leaflet. Lower: In the nucleotide-bound trajectory. (D) and (E) At 40 and 80 ns a central tunnel is observed. The 0 ns conformation is not shown as it is identical to that in the nucleotide-bound trajectory.

Enhancing fluoroquinolone activity: finfloxacin * - delafloxacin **

* under development at Merlion Pharmaceuticals, Berlin, Germany & Singapore, Malaysia and at RibX Pharmaceuticals, New Haven, Conn.

Fluroquinolones: some of the challenges

- Activity against resistant strains
  - if target mutations: get lower MIC (to cope for 1 or 2 mutations)
  - if efflux: modify stucture to avoid recognitoin by efflux transporters

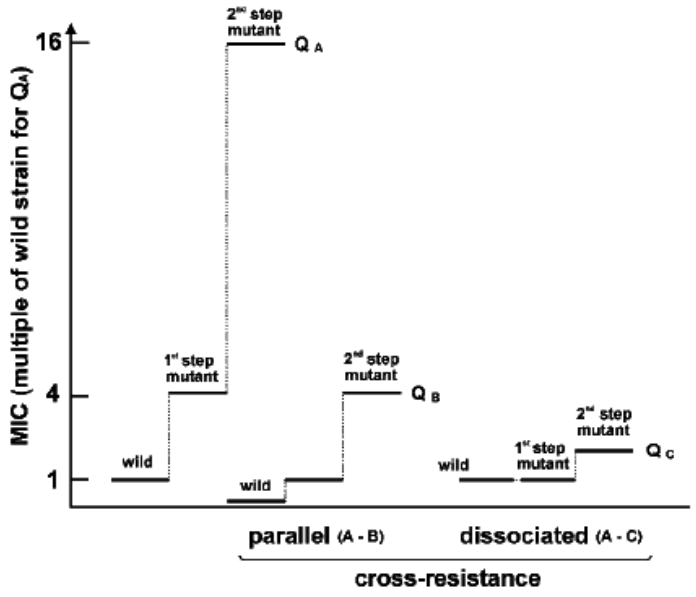
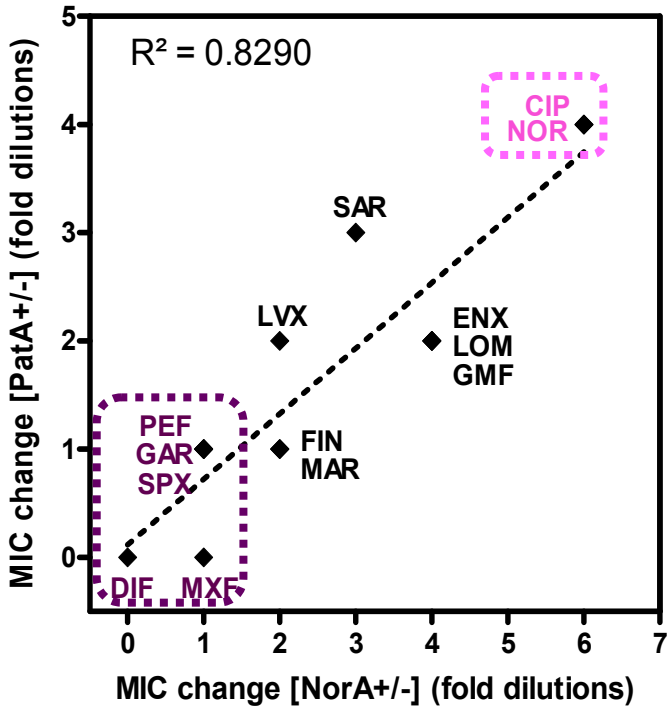


Fig. 4. Cross-resistance and dissociated resistance in quinolones. Q_A and Q_B illustrate a situation of cross-resistance: although the initial susceptibility of the strain may be different for molecules A and B, mutations in the target enzymes lead to similar changes in the susceptibility to both drugs. 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.

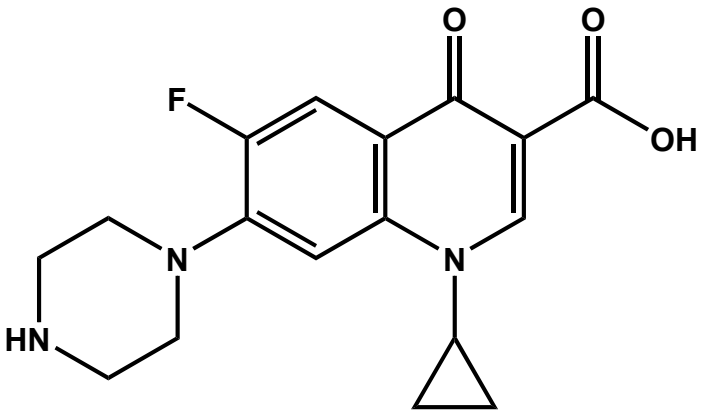
Van Bambeke et al. Clin. Microbiol. Infect. 2005; 11: 256–280



Comparative efflux (measured by MIC change between isogenic strains + and – for NorA (*S. aureus*) and PatA (*S. pneumoniae*) transporters

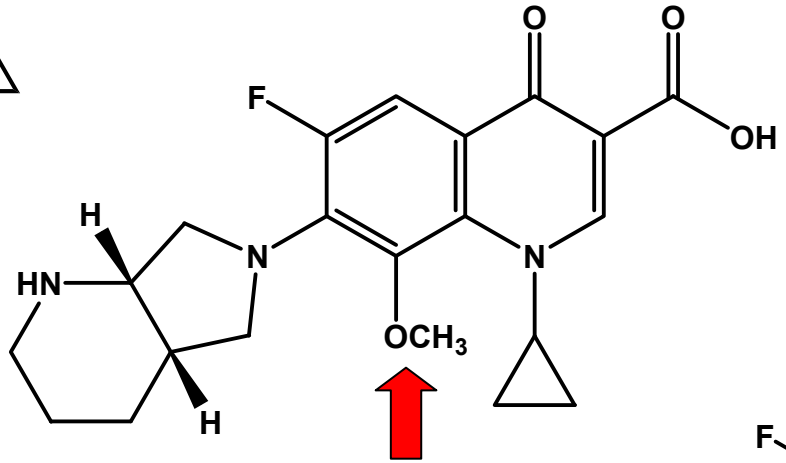
Vallet et al. 21st ECCMID & 27th ICC,Milan, Italy, 2001

From ciprofloxacin to finafloxacin…



ciprofloxacin

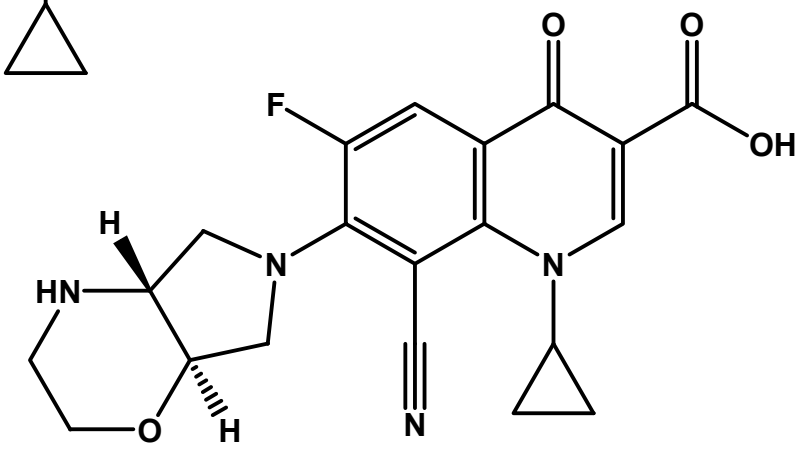
avoids efflux



moxifloxacin

(Bayer HealthCare)

lowers MIC



finafloxacin

Finafloxacin: activity…

Table 1. Susceptibility testing of *S. aureus* strains with various resistance phenotypes and laboratory strains of *L. monocytogenes* and *L. pneumophila* against finafloxacin and ciprofloxacin. For ciprofloxacin and *S. aureus*, figures in bold for ciprofloxacin indicate MIC values exceeding the susceptible clinical breakpoint of EUCAST (http://www.eucast.org).

Species and phenotype	collection no.	origin	MIC (mg/L)	
			finafloxacin	ciprofloxacin
S. aureus				
MSSA	ATCC 25923	Laboratory strain [a]	0.06	0.125
	SA-1	NorA overexpressing strain (derived from ATCC 25923) [b]	0.25-0.5	**4**
CA-MRSA	N4042228	Belgian clinical isolate [d]	0.25	0.25
	NRS192	US clinical isolate [c]	0.25	0.5
	CHU1	Asian clinical isolate [f]	0.125	0.5
	MEH22256	Asian clinical isolate [g]	0.25	1
	N7112046	Animal MRSA (food-animal caregiver) [d]	0.25	0.25
HA-MRSA	COL (NRS100)	Laboratory strain [c]	0.125	0.125
	ATCC 33591	Laboratory strain [a]	0.125	0.25
	N4112910	Belgian clinical isolate [d]	16	**128**
	N4120032	Belgian clinical isolate [d]	4	**128**
HA-MRSA / VISA	NRS18b	US clinical isolate [c]	4	**32**

Vallet et al. Int. J. Antimicrob. Agents 2011; 38:249– 256

Finafloxacin: activity… in acid media

Table 2: Influence of pH on the MIC of wild type and efflux-resistant *S. aureus*

	MIC (mg/L)			
pH	finafloxaxin		ciprofloxacin	
	SA [a]	SA-1 [b]	SA [a]	SA-1 [b]
7.4	0.0.625	0.25	0.125	4
7.0	0.0625	0.25	0.125	4
6.7	0.0625	0.25	0.125	4
6.5	0.03125	0.25	0.125	4
6.0	0.03125	0.125	0.25	8
5.7	0.015625	0.0625	0.5	8
5.5	0.015625	0.0625	1	8

[a] *S. aureus* isogenic strain of SA1 (originally ATCC 25923)

[b] *S. aureus* overexpressing NorA (from C. Quentin (University Victor Segalen Bordeaux, France [57-Ba et al. 2006])

Vallet et al. Int. J. Antimicrob. Agents 2011; 38:249– 256

Finafloxacin: activity… in acid media

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6.7	0.0625	0.25	0.125	4
6.5	0.03125	0.25	0.125	4
6.0	0.03125	0.125	0.25	8
5.7	0.015625	0.0625	0.5	8
5.5	0.015625	0.0625	1	8

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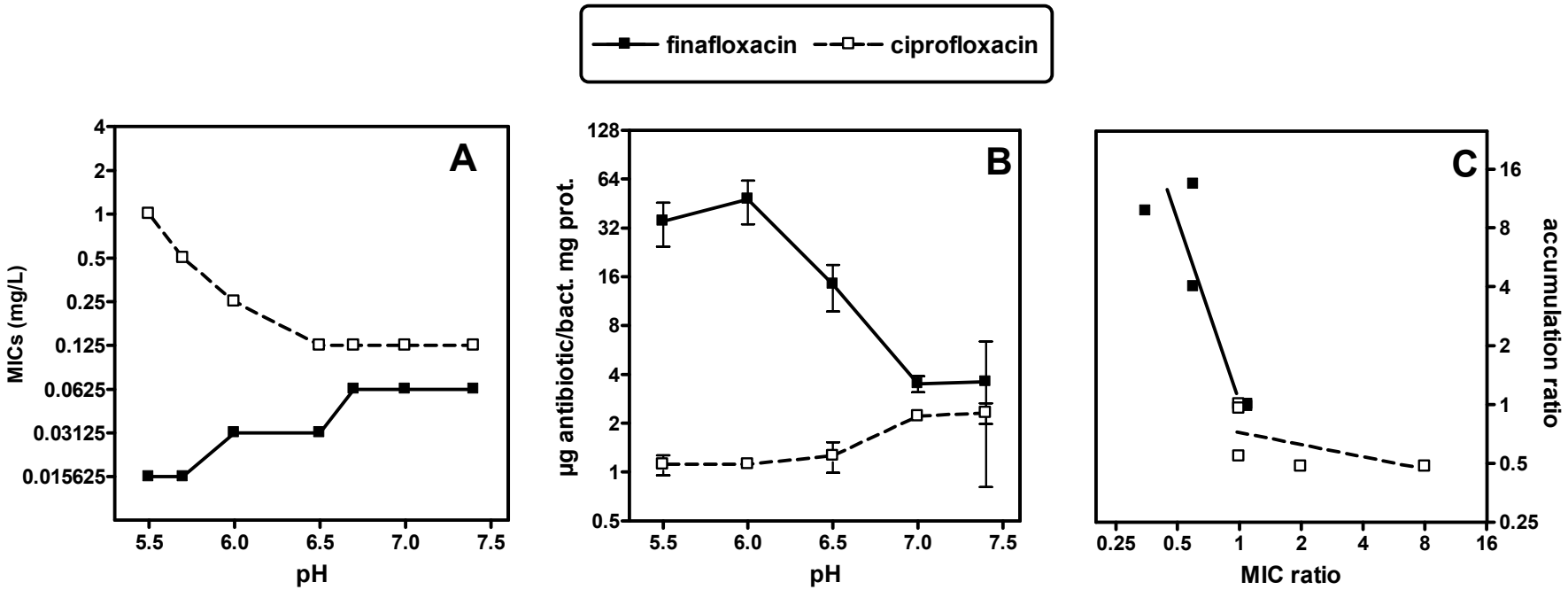
[b] *S. aureus* overexpressing NorA (from C. Quentin (University Victor Segalen Bordeaux, France [57-Ba et al. 2006])

Vallet et al. Int. J. Antimicrob. Agents 2011; 38:249– 256

Finafloxacin: why more active at acid pH…



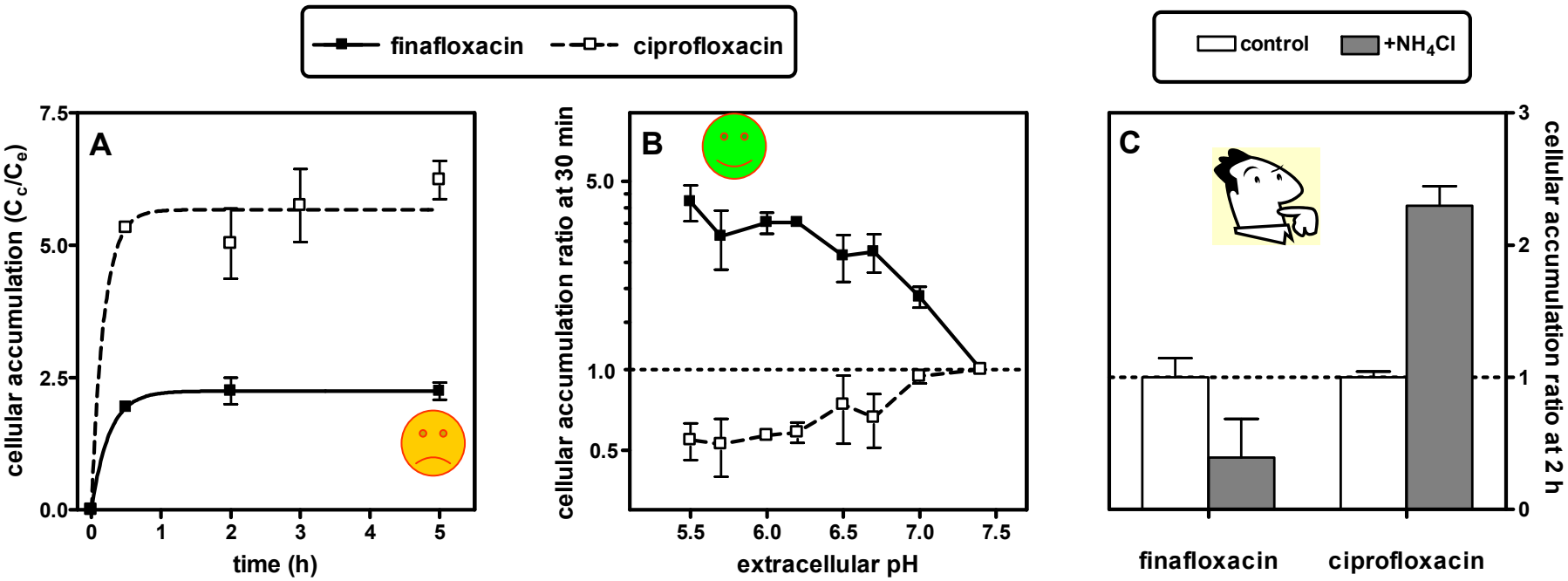
Change in MIC

Change in accumulation (bacteria)

correlation

Vallet et al. Int. J. Antimicrob. Agents 2011; 38:249– 256

Finafloxacin: what about acumulation in cells ?



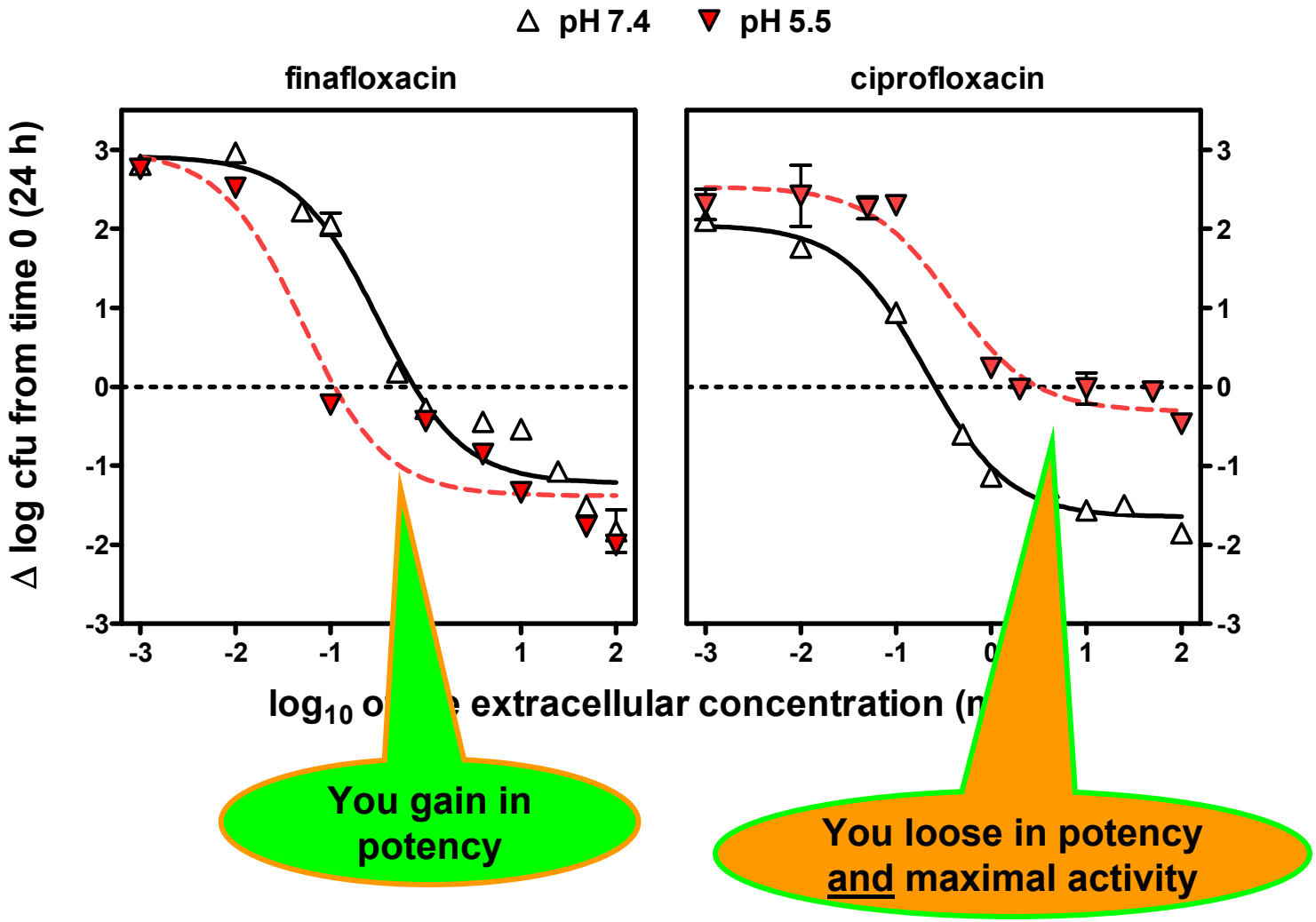
Kinetics of uptake at pH 7

2 h accumulation at different pH

Effect of NH_4Cl (neutralizes lysosomes)

Vallet et al. Int. J. Antimicrob. Agents 2011; 38:249– 256

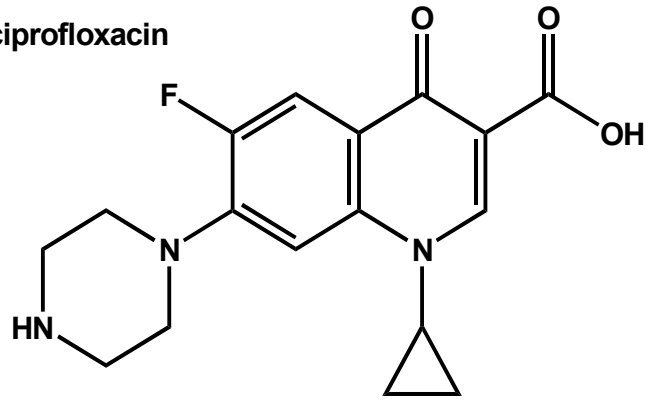
Finafloxacin: what about intracellular *S. aureus* ?



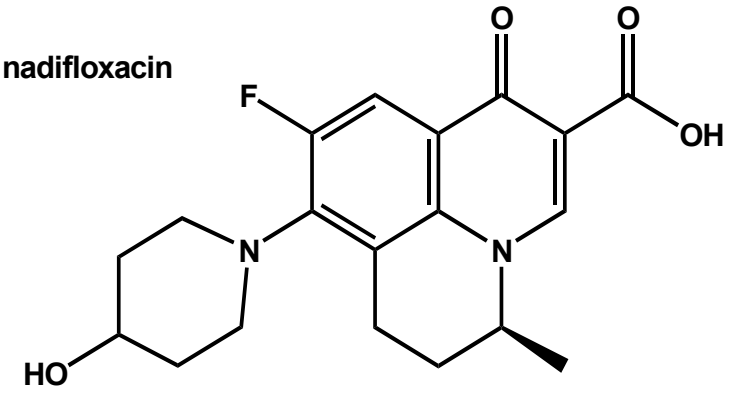
Vallet et al. Int. J. Antimicrob. Agents 2011; 38:249– 256

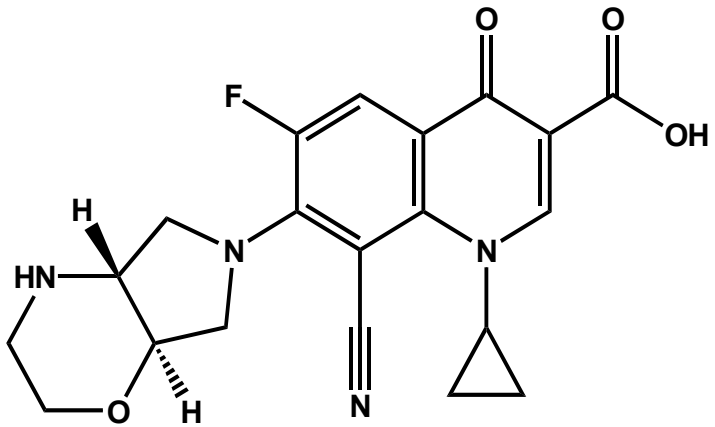
Other fluroquinolones with enhanced acid pH activity

ciprofloxacin

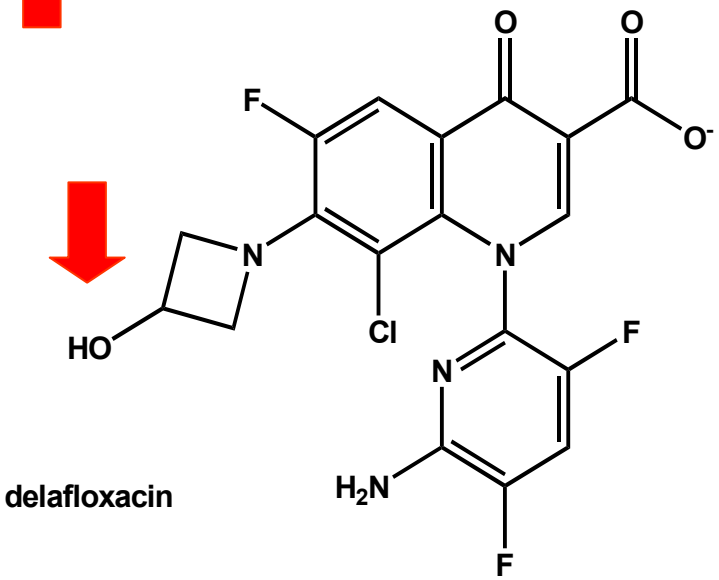


nadifloxacin



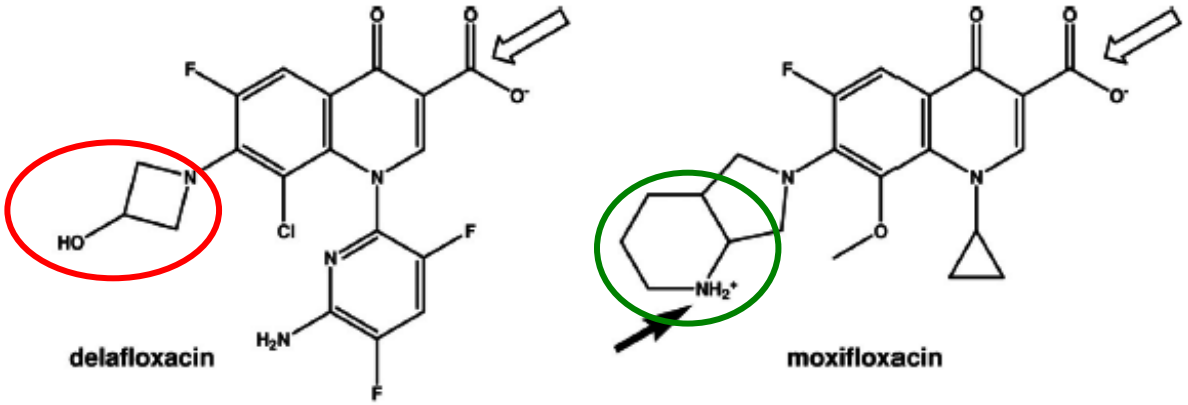


finafloxacin



delafloxacin

Delafloxacin



Drug	pKa_1 [a]	pKa_2 [a]	species in solution (calculated ratio) [b] (%; pH 7.4)	species in solution (calculated ratio) [b] (%; pH 5.4)	calculated logP [a]	calculated logD (pH 7.4) [a]
moxifloxacin	6.3	9.3			1.90	-0.63
$^{+}H_2N....COOH$			7	**89**		
$^{+}H_2N....COO^{-}$			**92**	11		
$HN....COO^{-}$			1	0		
delafloxacin	5.4	-			0.94	-0.58
N....COOH			1	**50**		
$N....COO^{-}$			**99**	50		

FIG. 1. Structural formula and physicochemical properties of moxifloxacin and delafloxacin. The open arrows point to the acidic function of fluoroquinolones and the plain arrow to the basic function of moxifloxacin, which is protonated at physiological pH. The table shows the calculated pK_as, log *P* (partition coefficient), and log *D* (distribution coefficient) at neutral pH together with the ratio of species in solution at neutral and acidic pHs. In columns labeled with a superscript a, sources of values are as follows. pK_a, reference 22 for moxifloxacin; data are on file from Abbott Laboratories for delafloxacin. Log *P* and log *D*, Scifinder Scholar software program, version 2007. Values in the column labeled with a superscript b were calculated as follows. Moxifloxacin, % fully protonated form ($^{+}H_2N....COOH$) = $100/(1 + 10^{pH - pKa_1} + 10^{2 \cdot pH - pKa_1 - pKa_2})$; % zwitterion ($^{+}H_2N....COO^{-}$) = $100/(1 + 10^{pKa_1 - pH} + 10^{pH - pKa_2})$; % fully deprotonated form ($HN....COO^{-}$) = 100 − % ($^{+}H_2N....COO^{-}$) − % ($^{+}H_2N....COOH$). Delafloxacin, % uncharged form (N....COOH) = $100/(1 + 10^{pH - pKa_1})$; % deprotonated form ($N....COO^{-}$) = 100 − % (N....COOH). Each value in bold is the most abundant form for each drug at the considered pH.

Lemaire et al. Antmicrob. Agents Chemother. 2001; 55:649-658

Delafloxacin

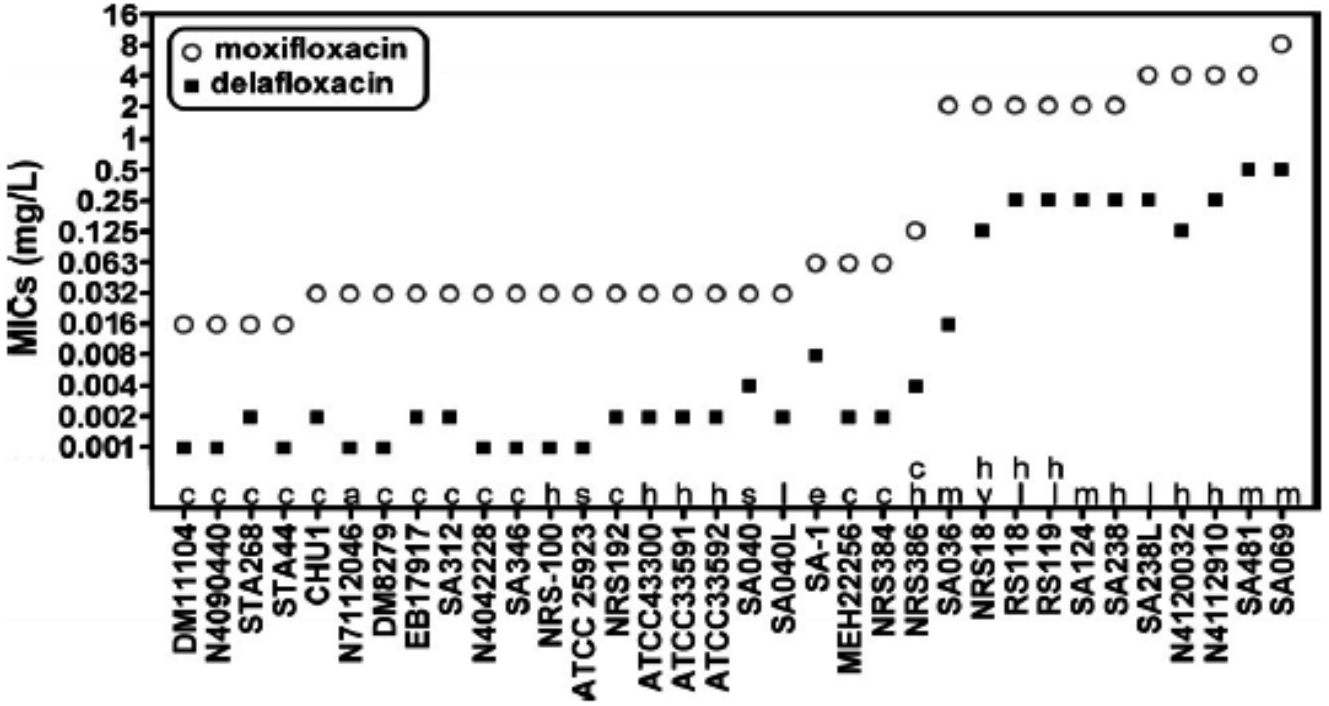


FIG. 2. Comparative susceptibilities of various *S. aureus* isolates to moxifloxacin (circles) or delafloxacin (squares). MICs were measured at pH 7.4, and strains are ranked based on their susceptibility to moxifloxacin. Resistance phenotypes and/or strain source are designated by lowercase letters 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.

Lemaire et al. Antmicrob. Agents Chemother. 2001; 55:649-658

Delafloxacin and activity at acid pH

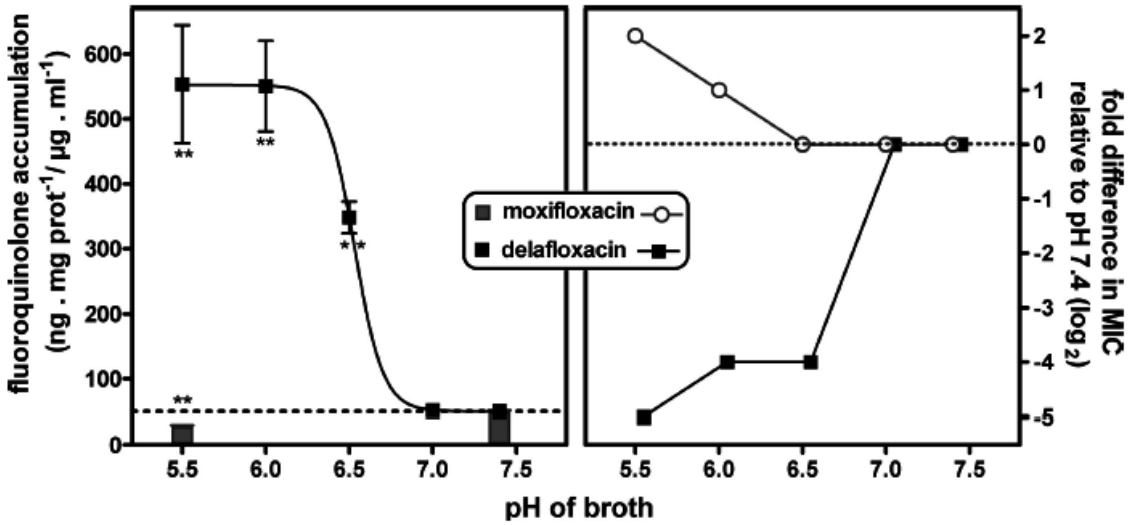


FIG. 3. Accumulation in *S. aureus* ATCC 25923 (left) or MIC (right) of fluoroquinolones in broth at different pHs. Left: growing bacteria were incubated for 30 min in pH-adjusted broth with delafloxacin (100 mg/liter) or moxifloxacin (50 mg/liter). Values are expressed as the cellular-to-extracellular concentration ratio and are shown as means ± SD for three independent samples. The dotted line shows the value measured for both drugs at pH 7.4. For statistical analysis, data with asterisks are significantly different from those for controls (pH 7.4; $P < 0.01$), as determined by one-way analysis of variance (ANOVA) (Dunnett multiple-comparison test). Right: MICs of delafloxacin and moxifloxacin (expressed as the change [in $\log_2$ units] from the MIC determined at pH 7.4) in pH-adjusted broth. Values are means for three independent samples (yielding 3 identical values).

Lemaire et al. Antmicrob. Agents Chemother. 2001; 55:649-658

Other examples…

- forcing the opening of PBP2a

  → recover activity against MRSA

Supplemental Material can be found at:
http://www.jbc.org/cgi/content/full/M800079200/DC1

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 283, NO. 19, pp. 12769–12776, May 9, 2008

Restoration of Susceptibility of Methicillin-resistant *Staphylococcus aureus* to β-Lactam Antibiotics by Acidic pH

ROLE OF PENICILLIN-BINDING PROTEIN PBP 2a[*][S]

Received for publication, January 4, 2008, and in revised form, March 11, 2008 Published, JBC Papers in Press, March 12, 2008, DOI 10.1074/jbc.M800079200

Sandrine Lemaire[‡1], **Cosimo Fuda**[§], **Françoise Van Bambeke**[‡2], **Paul M. Tulkens**[‡], **and Shahriar Mobashery**[§]

From the [‡]*Unité de Pharmacologie Cellulaire et Moléculaire, Université Catholique de Louvain, B-1200 Brussels, Belgium and the* [§]*Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556*

Other examples…

- Proteomic Analaysis of intracellular L. monocytogenes reveal a deficit in D-Ala-DAla ligase

  → combine ampicillin with D-Ala-D-Ala ligase inhibitors

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RESEARCH ARTICLE

Isolation and 2-D-DIGE proteomic analysis of intracellular and extracellular forms of *Listeria monocytogenes*

Sébastien Van de Velde[1], Edouard Delaive[2], Marc Dieu[2], Stéphane Carryn[1,3], Françoise Van Bambeke[1], Bart Devreese[4], Martine Raes[2] and Paul M. Tulkens[1]

[1] Unité de pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium
[2] Unité de recherche en biologie cellulaire, Facultés Universitaires Notre-Dame de la Paix, Namur, Belgium
[3] Eumedica Pharmaceuticals, Manage, Belgium
[4] Laboratorium voor Eiwitbiochemie en Biomoleculaire Engineering, Universiteit Gent, Ghent, Belgium

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