Pharmacological approaches to the discovery and optimized development of novel antibiotics

Paul M. Tulkens, MD, PhD

Cellular and Molecular Pharmacology Group
Louvain Drug Research Institute
Université catholique de Louvain
Brussels, Belgium

Vietnam National University HCMC
INTERNATIONAL UNIVERSITY

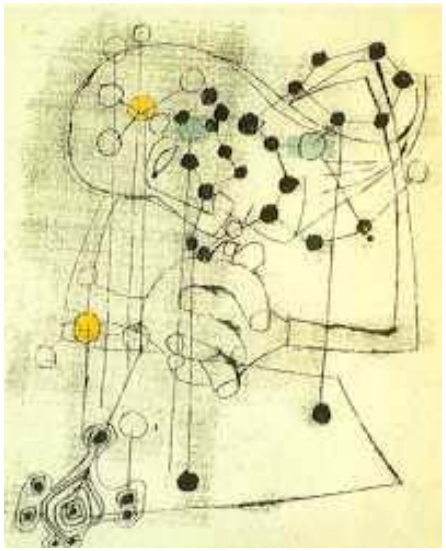
The approach in a nutshell

pharmacodynamics

pharmacokinetics

antibiotic toxicity

resistance



novel bacterial targets

clinical applications

antibiotics: from molecules to man

What will it be all about ?

- **The antibiotic crisis …**
  - are antibiotics following a **path of madness** ?
    (the reality in hospitals and in the community…)
  - the **"resistome"** (or why do we will always have resistance…)
  - the "**selectome**" (or why do we favor emergence of resistance)
  - the "**connectome"** (or why we loose several antibiotics at the same time)

- **The main lines of action** (for research)
  - the **7 pillars of wisdom ?**

- **Laboratory and translational studies at LDRI** (examples)
  - poorly exploited targets (D-Ala-D-Ala ligase)
  - refurbishing old antibiotics (aminogycosides, polymyxins, temocillin)
  - better antibiotic use (PK/PD, intracellular bacteria
  - PK/PD approaches to mitigate the emergence of resistance (β-lactams and fluoroquinolones)

Are antibiotics following a path to madness ?

discovery in soil bacteria and fungi

1928 - …

Are antibiotics following a path to madness ?

1950 – 1980 …

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

Are antibiotics following a path to madness ?

and the US General Surgeon told us that the fight was over

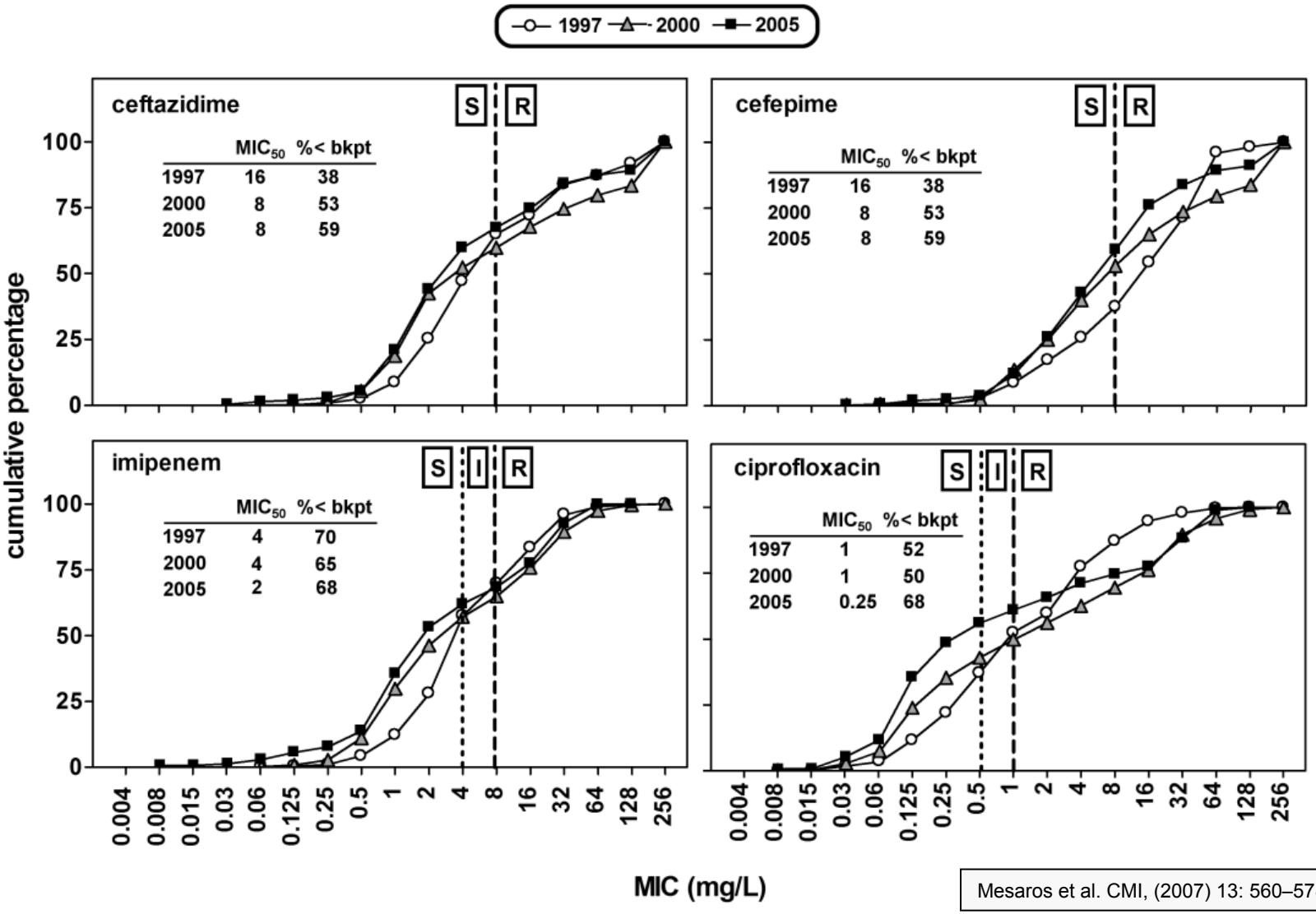
1970 …

Are antibiotics following a path to madness ?

But…

2012 …

Resistance of *P. aeruginosa in hospitals*
(International data – EUCAST breakpoints)



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

Spreading of NDM-1 in the community …

Outbreak of Carbapenem-Resistant Enterobacteriaceae Containing *bla*$_{NDM-1}$, Ontario, Canada

Sergio Borgia,[1,3,8] Olga Lastovetska,[4,5] David Richardson,[1,2,9] Alireza Eshaghi,[4] Jianhui Xiong,[4] Catherine Chung,[4] Mahin Baqi,[1,3] Allison McGeer,[5,6,7] Gloria Ricci,[2] Rachael Sawicki,[3] Rajni Pantelidis,[3] Donald E. Low,[4,5,6] Samir N. Patel,[4,5,a] and Roberto G. Melano[4,5,6,a]

[1]Division of Infectious Diseases, [2]Department of Laboratory Medicine, and [3]Infection Prevention and Control, William Osler Health System, Brampton, [4]Public Health Ontario, Public Health Laboratories, [5]Department of Laboratory Medicine and Pathobiology, University of Toronto, [6]Department of Microbiology and [7]Infection Prevention and Control, Mount Sinai Hospital, Toronto, and Departments of [8]Medicine and [9]Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada

Clin Infect Dis. 2012; 55:e109-17

AAC Journals.ASM.org

Antimicrob Agents Chemother. 2012; 56:3432–3434.

NDM-1-Producing *Klebsiella pneumoniae* Resistant to Colistin in a French Community Patient without History of Foreign Travel

Corinne Arpin,[a] Patrick Noury,[b] Delphine Boraud,[b] Laure Coulange,[a] Alain Manetti,[c] Catherine André,[a] Fatima M'Zali,[a] and Claudine Quentin[a]

Université de Bordeaux, Microbiologie Fondamentale et Pathogénicité UMR 5234, Bordeaux, France[a]; Laboratoire de Biologie Médicale EXALAB, Site de Villenave d'Ornon, Villenave d'Ornon, France[b]; and Agence Régionale de Santé, Espace Rodesse, Bordeaux,[c] France

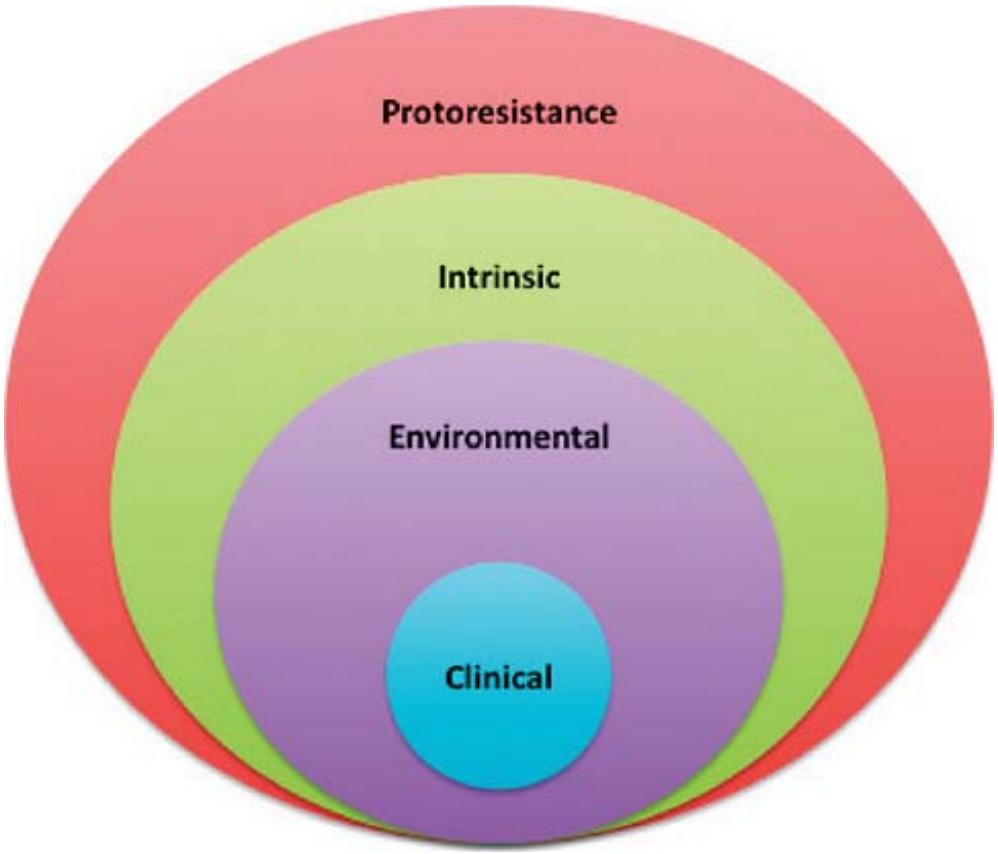
A carbapenem-resistant *Klebsiella pneumoniae* strain, Kp5196, was responsible for an uncomplicated cystitis in a patient living at home and without history of foreign travel. This isolate produced the metallocarbapenemase NDM-1 and was resistant to all antibiotics except tetracyclines and colistin. The *K. pneumoniae* strain belonged to sequence type ST15, and *bla*$_{NDM-1}$ was carried by a nontypeable conjugative plasmid. Two months later, a similar ST15 isolate, Kp5241, was present in the patient but was additionally colistin resistant.

The resistome …

- Resistance emergence is a natural process that has gone on for time immemorial.
  - **Example:** Parts of the operon mediating **vancomycin resistance** have been found in the permafrost layer, demonstrating the ancient nature of the problem…
    (many other examples of “resistance” in pre-antibiotic era)
  - **Significance:** resistance was with us since ever and we will never get rid of it …

- Horizontal gene transfer has long been considered as the main mechanism by which the resistome has been built over years
  - β-actamases, MRSA (PBP2a), Penicillin-resistant *S. pneumoniae* (mosaic genes),aminoglycoside-inactivating enzymes, QnR (fluoroquinolones-target protecting protein) …

The resistome …



The antibiotic resistome.

- all the genes and their products that contribute to antibiotic resistance.
- highly redundant and interlocked system
- clinical resistance under represents the resistance capacity of bacteria.
- existing biochemical mechanisms (protoresistome) serve as a deep reservoir of precursors that can be co-opted and evolved to

Antibiotic Resistance:Implications for Global Health and Novel Intervention Strategies: Workshop Summary
http://www.nap.edu/openbook.php?record_id=12925

Clinical resistance: the tip of the iceberg ?

- **“Clinical” resistance genes** are found on **pathogenic bacteria**. These are the fewest but also the most problematic ones at present.
- “**Father resistance genes**” found on antibiotic producers. (microorganisms that naturally produce antibiotics have their own protection mechanisms to avoid the adverse effects of the antibiotics on themselves).
  - ➢**These genes are a strong source for the pathogenic bacteria.**
- **Cryptic resistance genes**.
  (genes are embedded in the bacterial chromosome that may be overexpressed when “needed”)
- **Precursor genes**.
  (encode proteins with basal level activity against antibiotics but may evolve to a “full resistance genes” given the appropriate selection pressure.

"Father resistance genes": an original example with aminoglycosides

Proc. Nat. Acad. Sci. USA
Vol. 70, No. 8, pp. 2276–2280, August 1973

Aminoglycoside Antibiotic-Inactivating Enzymes in Actinomycetes Similar to Those Present in Clinical Isolates of Antibiotic-Resistant Bacteria

(streptomyces/origin of R-factors/gentamicin-acetate)

RAOUL BENVENISTE* AND JULIAN DAVIES†

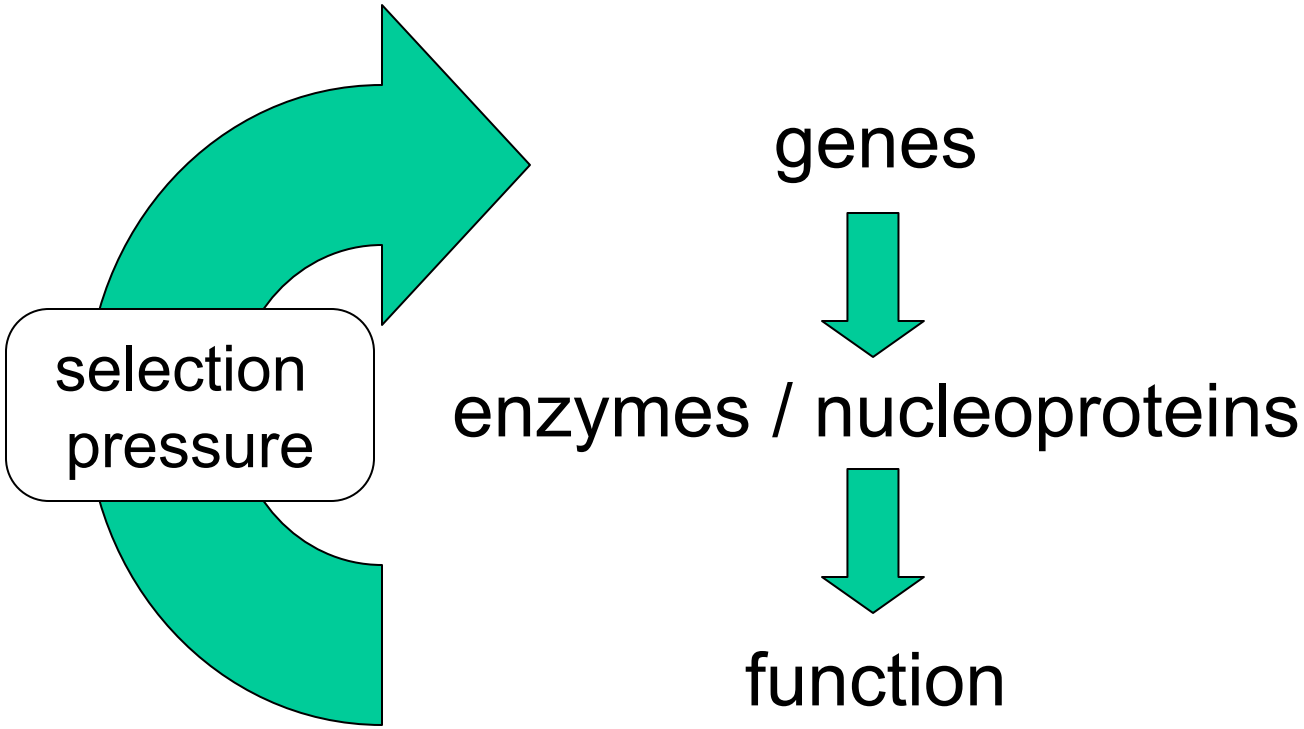
Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin—Madison, Madison, Wis. 53706

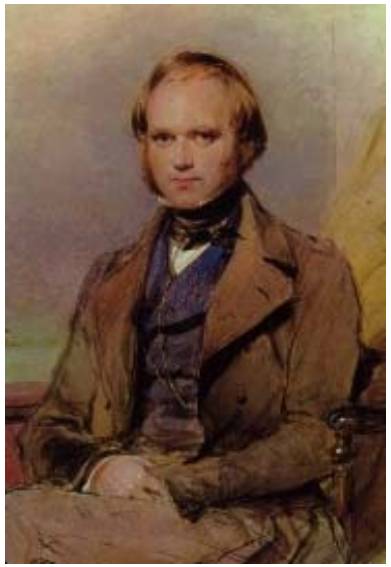
Communicated by Henry Lardy, May 11, 1973

One of the most striking properties of the actinomycetes is the extent to which they produce antibiotics; most of the aminoglycoside antibiotics (streptomycin, neomycin, kanamycin, gentamicin, tobramycin, and lividomycin) are produced by them.

The selectome

A simple application of Darwin's principles ...



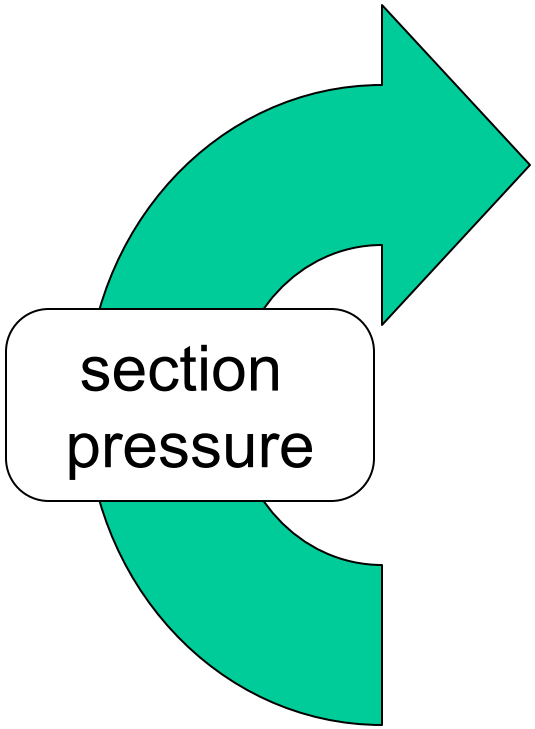


Detail of watercolor by
George Richmond, 1840.
Darwin Museum at Down House

How and why can you select so easily ?

A simple application of Darwin's principle…

to a highly plastic material…



- an infectious focus typicaly contains more than 10^6 - 10^9 organisms
- most bacteria multiply VERY quickly (20 min…) and do mistake …
- they are not innocent or useless mistakes

fast selection of the fitest !

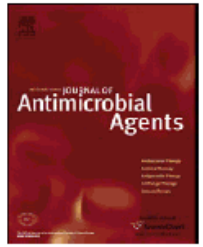
The hidden risk of therapy (in our hospitals …)

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]

[a] *Unité de Pharmacologie Cellulaire et Moléculaire & Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium*
[b] *Coris BioConcept, Gembloux, Belgium*
[c] *Laboratory for Molecular & Cellular Technology, Queen Astrid Military Hospital, Neder-over-Heembeek, Brussels, Belgium*
[d] *Department of Molecular and Cellular Interactions, Vrije Universiteit Brussel, Brussels, Belgium*
[e] *Laboratoire de Microbiologie, Cliniques Universitaires St-Luc, Brussels, Belgium*
[f] *Laboratorium voor Microbiologie, Universitair Ziekenhuis Brussel, Brussels, Belgium*
[g] *Clinique des Maladies Infectieuses, Hôpital Erasme, Brussels, Belgium*
[h] *Laboratoire de Microbiologie, Centre Hospitalier Universitaire Saint-Pierre, Brussels, Belgium*
[i] *Laboratoire de Microbiologie, Cliniques Universitaires UCL de Mont-Godinne, Yvoir, Belgium*

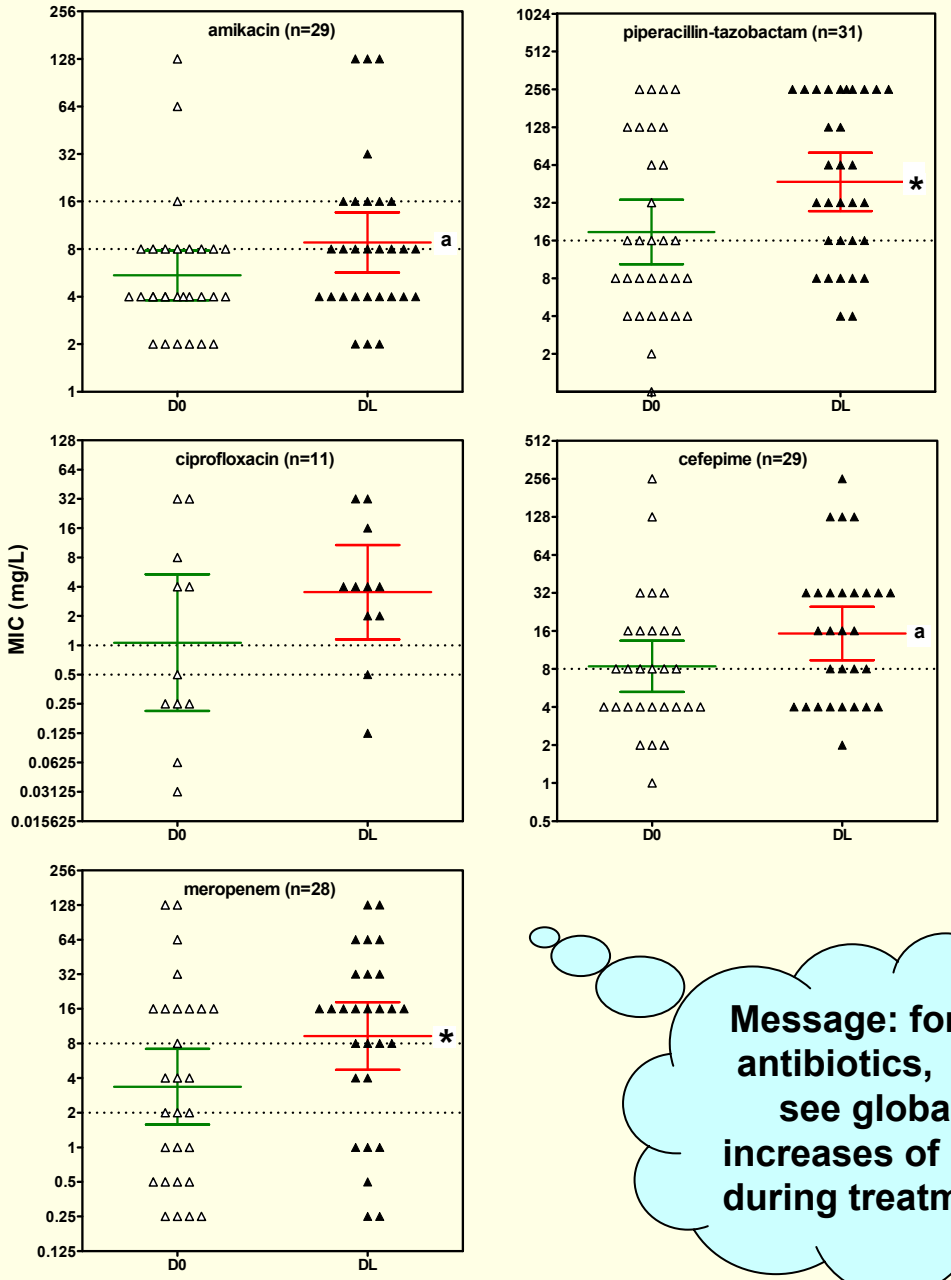
Do you remain effective while treating ?

- 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)



Message: for all antibiotics, we see global increases of MIC during treatment

Actually, selecting for resistance is easy even in a closed system…

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

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*. (post-doc at LDRI)
presented at the 8th ISAAR, Seoul, Korea, 8 April 2011 and additional work in progress

A simple experiment …

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

strains	Initial			TEM-exposed			Revertant		
	MIC (mg/L) [a]			MIC (mg/L)			MIC (mg/L)		
	TEM	FEP	MEM	TEM	FEP	MEM	TEM	FEP	MEM
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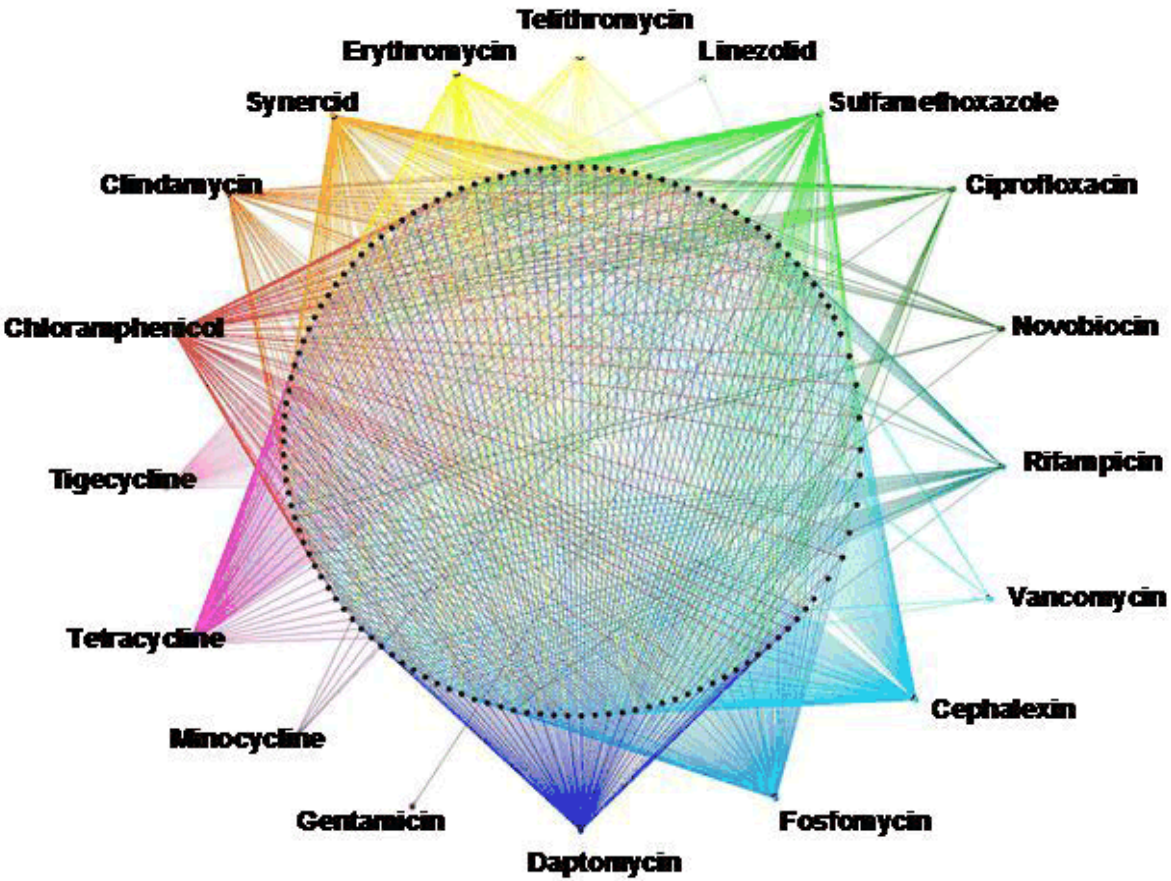
Nguyen *et al.* (post-doc at IDRI)
presented at the 8th ICAAR, Seoul, Korea, 9th April 2012 and in progress

sub-MIC concentrations selects for resistance !

The connectome….
(cross-resistance)



http://wrightlab.mcmasteriidr.ca/

Michael G. DeGroote INSTITUTE FOR INFECTIOUS DISEASE RESEARCH — McMaster University

Potential lines of action

ESSAY

Tackling antibiotic resistance

Karen Bush, Patrice Courvalin, Gautam Dantas, Julian Davies, Barry Eisenstein, Pentti Huovinen, George A. Jacoby, Roy Kishony, Barry N. Kreiswirth, Elizabeth Kutter, Stephen A. Lerner, Stuart Levy, Kim Lewis, Olga Lomovskaya, Jeffrey H. Miller, Shahriar Mobashery, Laura J. V. Piddock, Steven Projan, Christopher M. Thomas, Alexander Tomasz, Paul M. Tulkens, Timothy R. Walsh, James D. Watson, Jan Witkowski, Wolfgang Witte, Gerry Wright, Pamela Yeh and Helen I. Zgurskaya

Nature Reviews Microbiology 9, 894-896 (December 2011)

7 pillars of wisdom ?

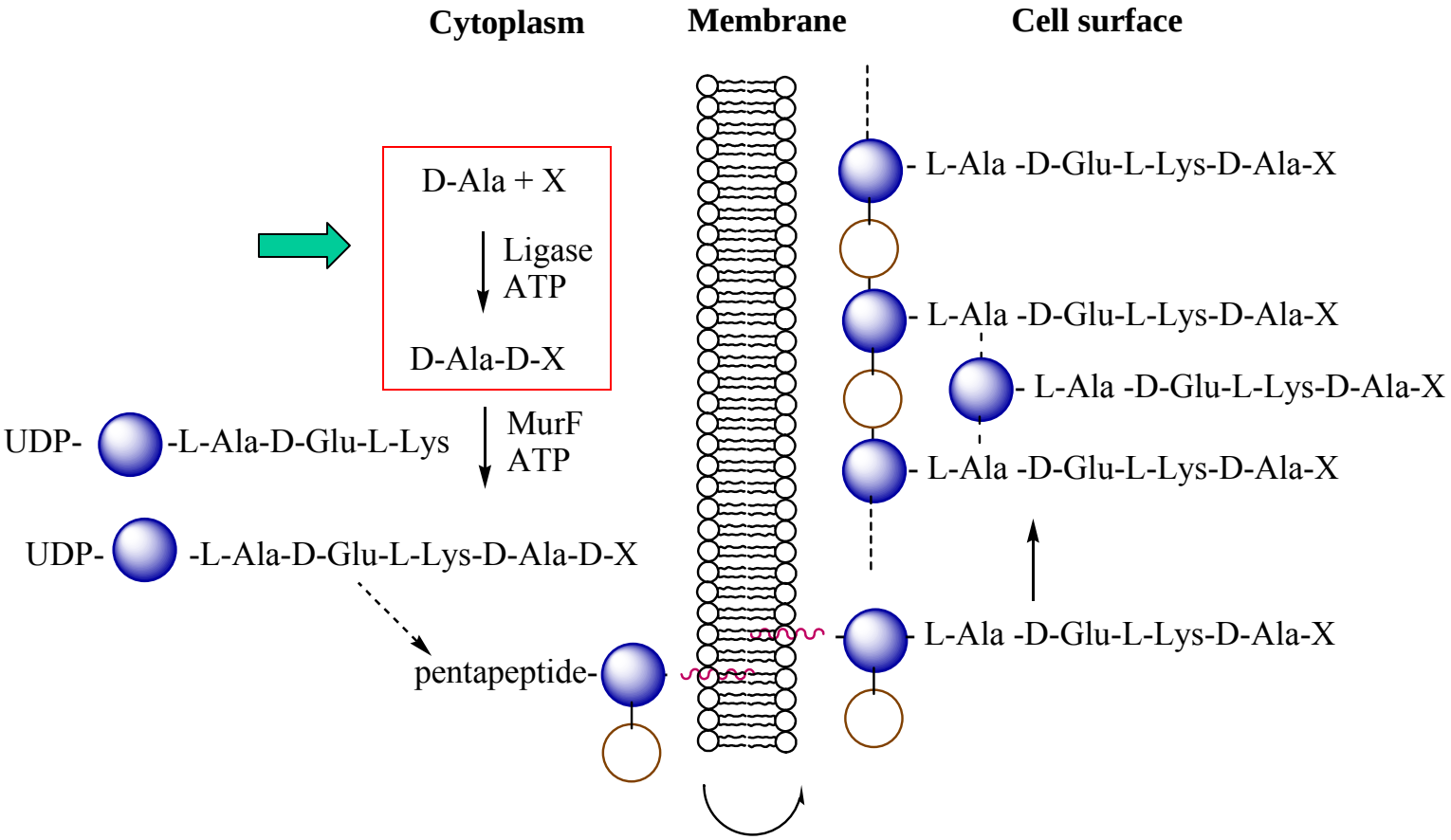
1. Public education
2. Public health, sanitation and quality of life
3. New antibiotics → new / poorly exploited targets
4. Old antibiotics
5. Better antibiotic use
6. Alternatives to antibiotics
7. Collaborative approach

Bush et al. Nature Reviews Microbiology 9, 894-896 (December 2011)

Poorly exploited targets: D-Ala-D-Ala ligase

D-Ala-D-X ligases

- **act in the very early steps of peptidoglycan synthesis**
- **are essential enzymes for bacterial growth**



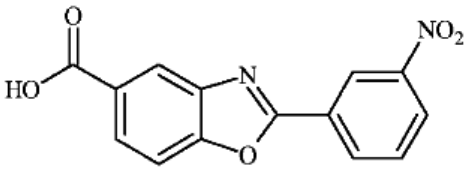
Rationale for a valid target …

- 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

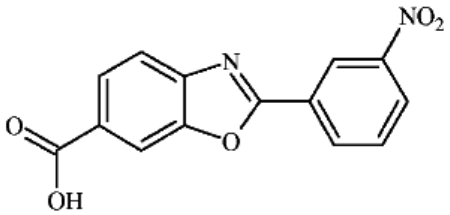
Benzoxazoles

CLAIMS

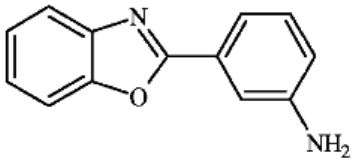
1. A compound of any of formulas (I-a), (II-a), (III-a), (IV-a) or (V-a):



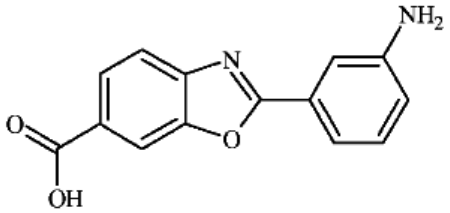
(I-a)



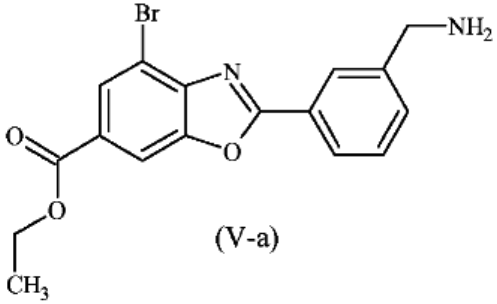
(II-a)



(III-a)



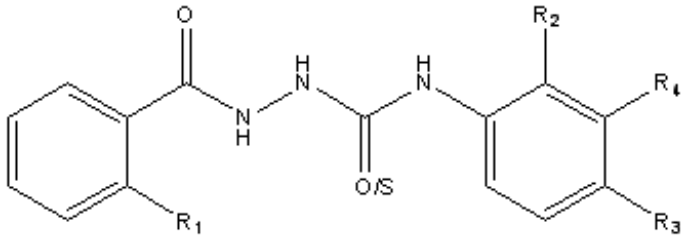
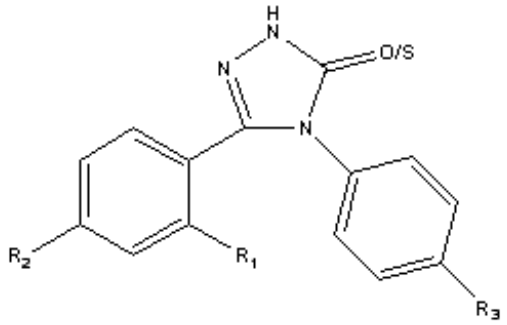
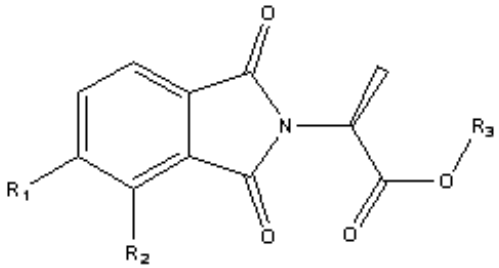
(IV-a)



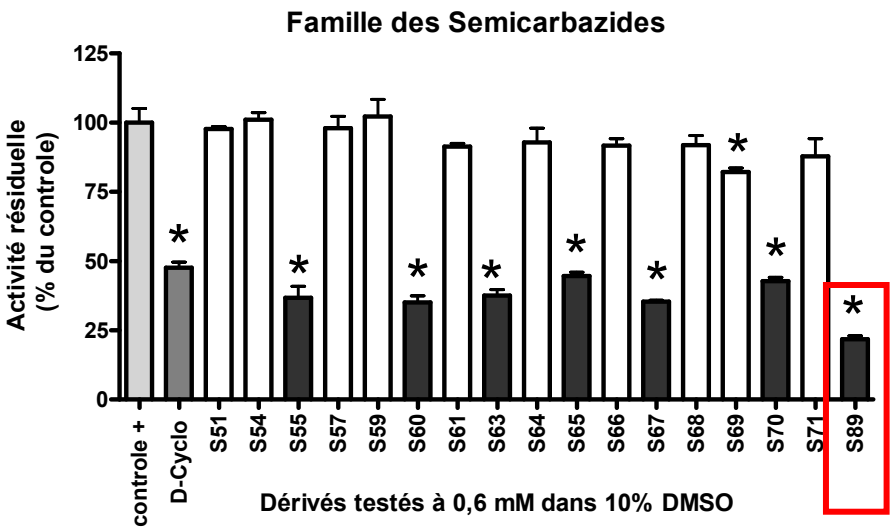
(V-a)

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

Other molecules…

Semicarbazides 17 molécules	
Triazoles 8 molécules	
Dioxo-Indolines 7 molécules	

Semi-carbazides are better …



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	/

7 pillars of wisdom….

1. Public education
2. Public health, sanitation and quality of life
3. New antibiotics → new / poorly exploited targets
4. Old antibiotics
   → aminoglycosides – polymyxins - temocillin
5. Better antibiotic use
6. Alternatives to antibiotics
7. Collaborative approach

Novel aminoglycosides *

- 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

* using proprietary data of Achaogen Inc., South San Francisco, Cal. and example of collaborative approach

Aminoglycosides:
starting from academic expertise in resistance

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

Main aminoglycoside-degrading enzymes…

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

MA
Unité

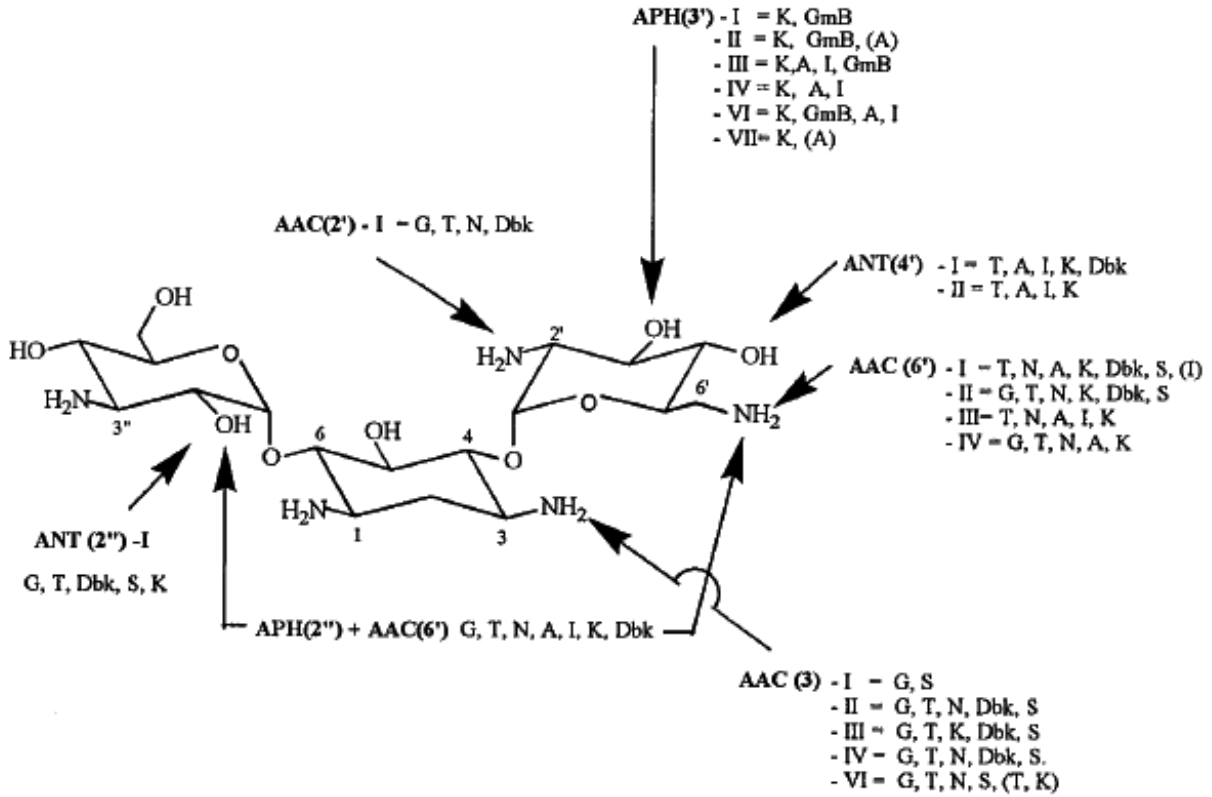


FIG. 3. Major aminoglycoside-modifying enzymes acting on kanamycin B (this aminoglycoside is susceptible to the largest number of enzymes). Each group of enzymes inactivates specific sites, but each of these sites can be acted upon by distinct isoenzymes (roman numerals) with different substrate specificities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

Academic expertise in nephrotoxicity

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 nephrotoxicity

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

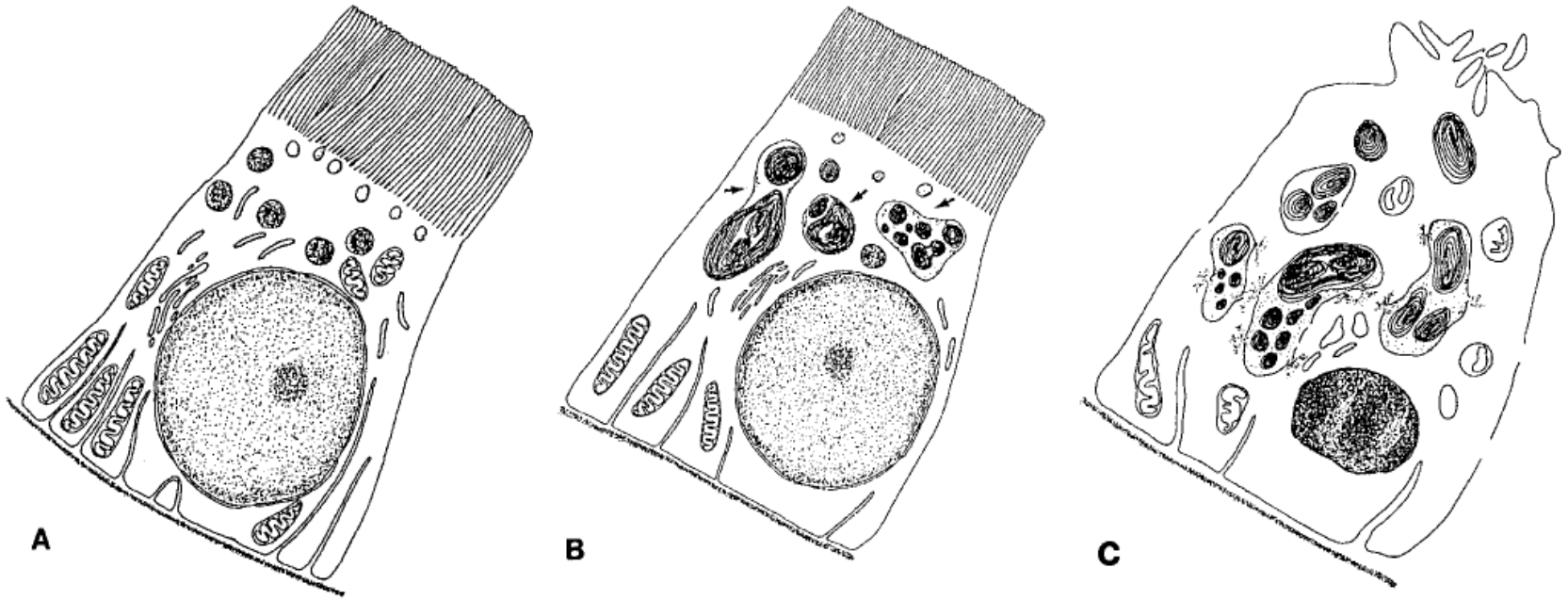
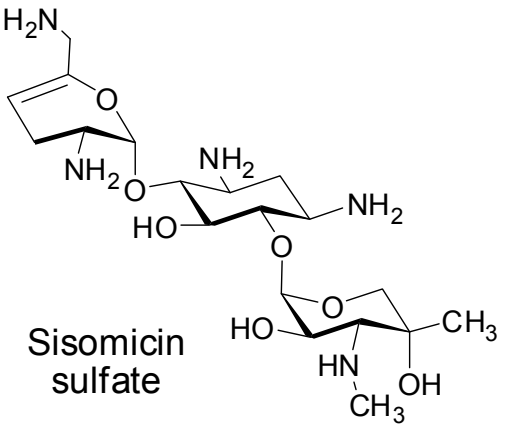


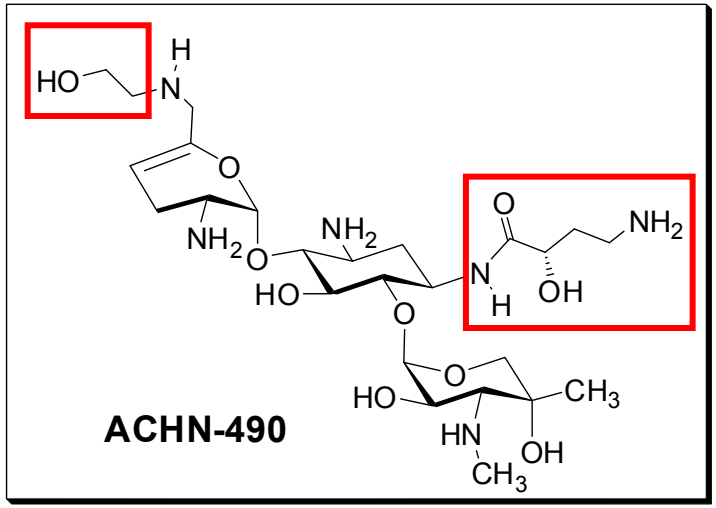
FIG. 1. Ultrastructural alterations induced in proximal tubular cells during aminoglycoside treatment. (A) Control. Changes detected early on and at low doses (B) consist mainly of the enlargement of lysosomes, which most likely occurs by fusion of preexisting structures and which is caused by the progressive 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*
- less toxic than gentamicin in *in vitro* and animal studies
- Indications currently tested 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%

but the weakness is *Pseudomonas* (efflux)

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

Extensive Safety Monitoring
Focused on Nephrotoxicity and Ototoxicity showed no major effect

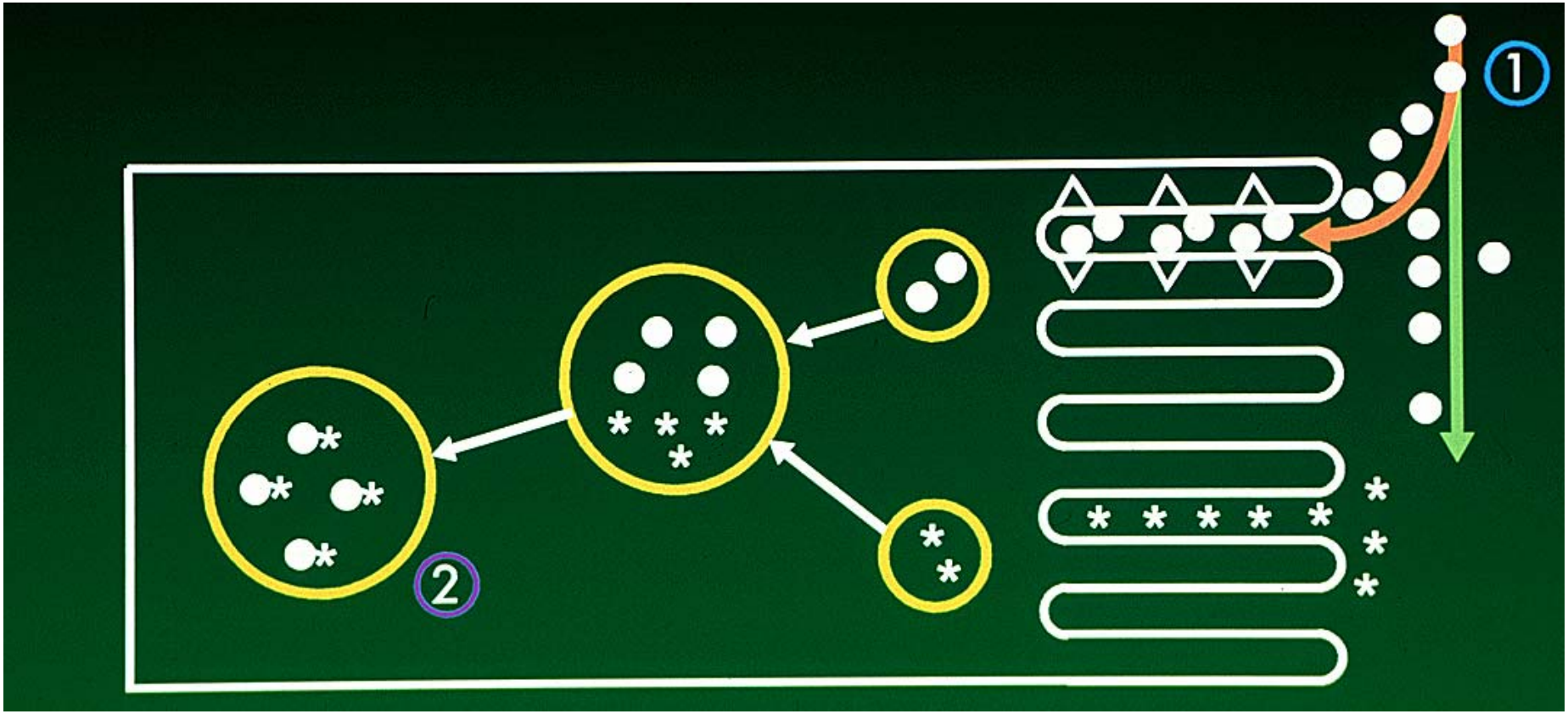
- Adverse Event monitoring
- Routine safety laboratory assessments
- Renal
  - Daily BUN & Cr during dosing
  - Calculated Creatinine clearance using Cockroft-Gault formula
  - Measured Creatinine clearance based on 24-hour urine collection
  - Additional GFR monitoring through Iothalamate clearance
- 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

with once-daily dosing

- phase I
- phase II

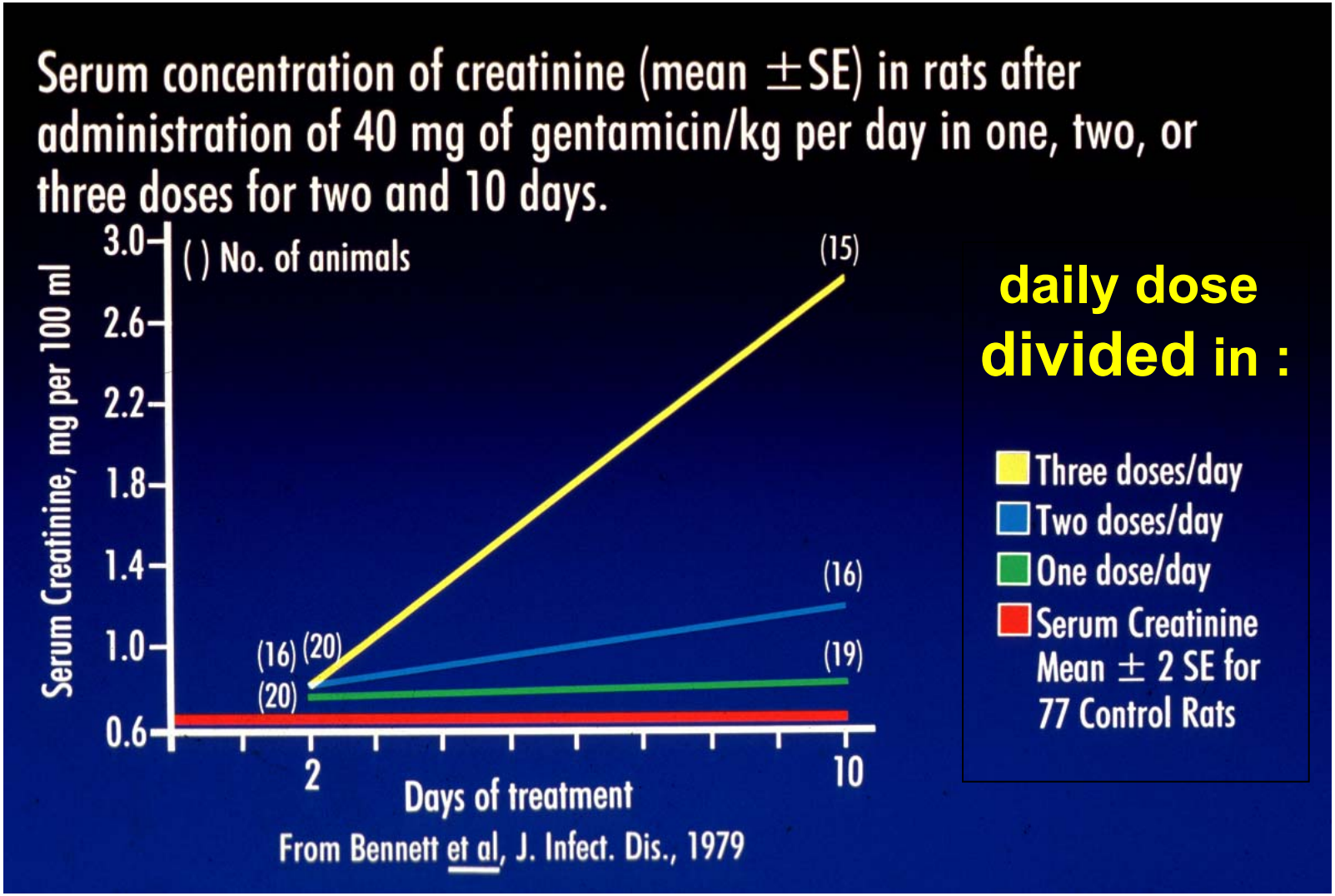
phase III ongoing !

Why are aminoglycosides nephrotoxic ?

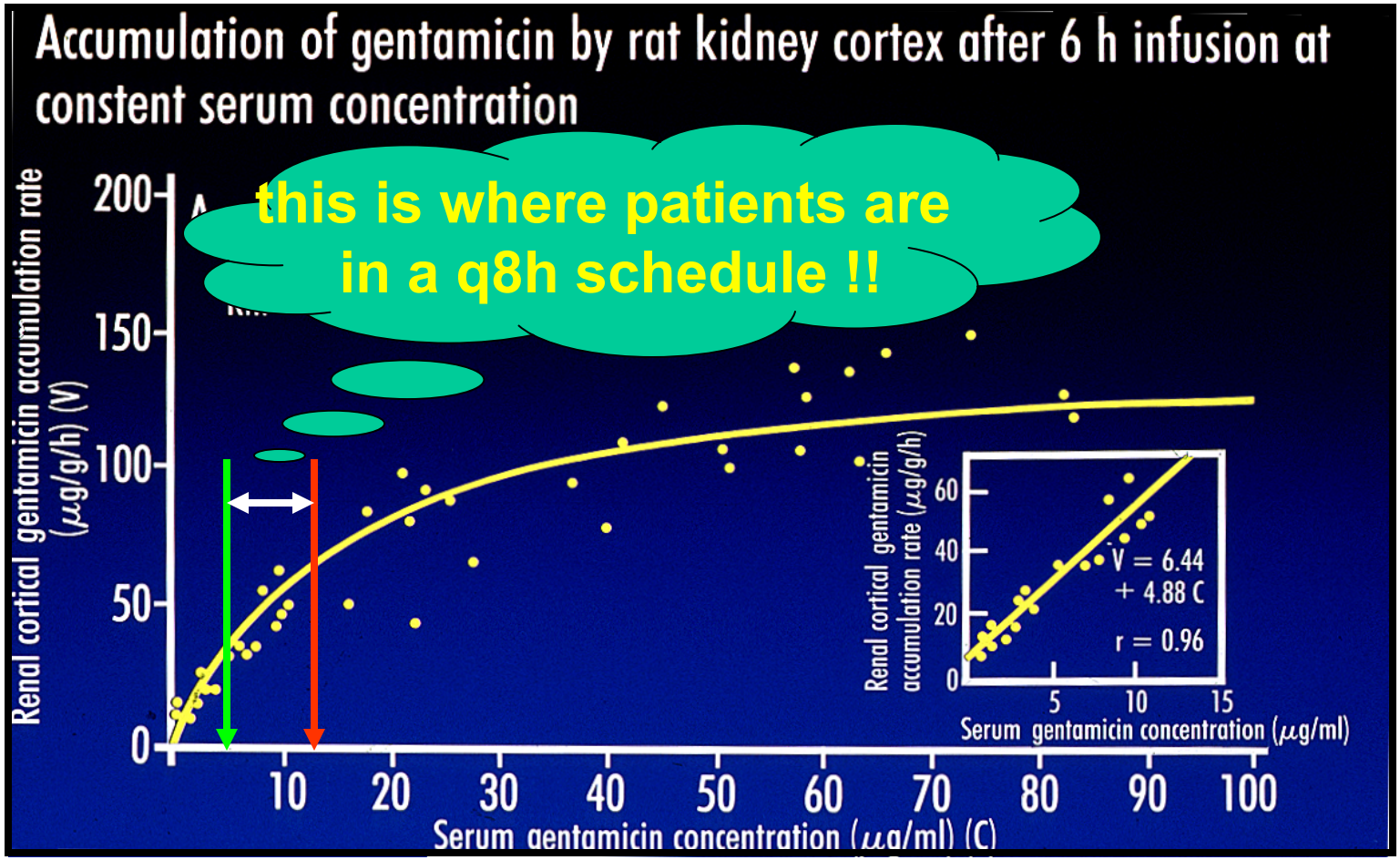


1. **binding to brush border**
2. **accumulation in lysosomes**

Observation: aminoglycoside toxicity is not linked to peak ...

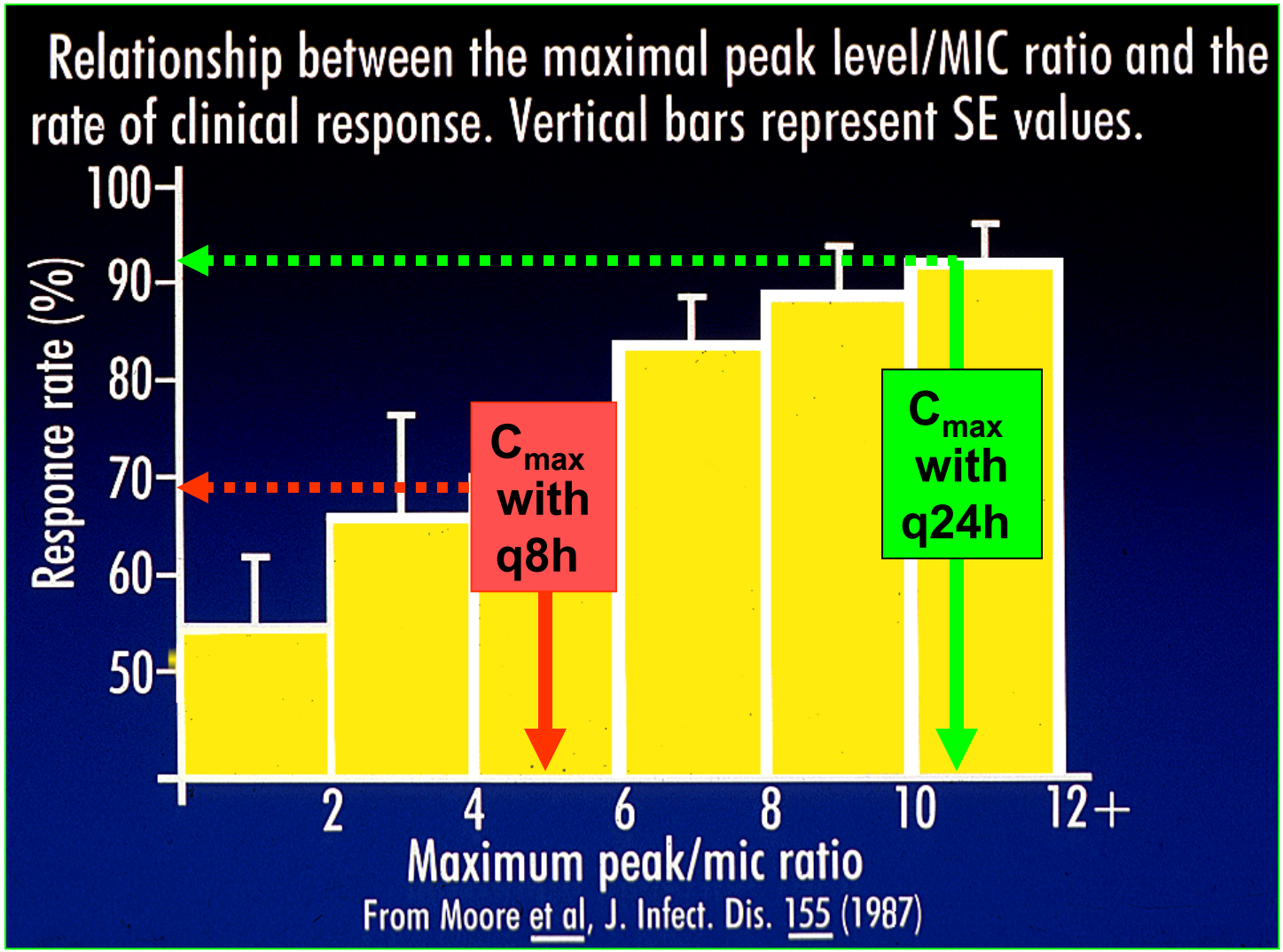


Aminoglycoside accumulation is kidney is saturable at clinically meaningful concentrations * ...

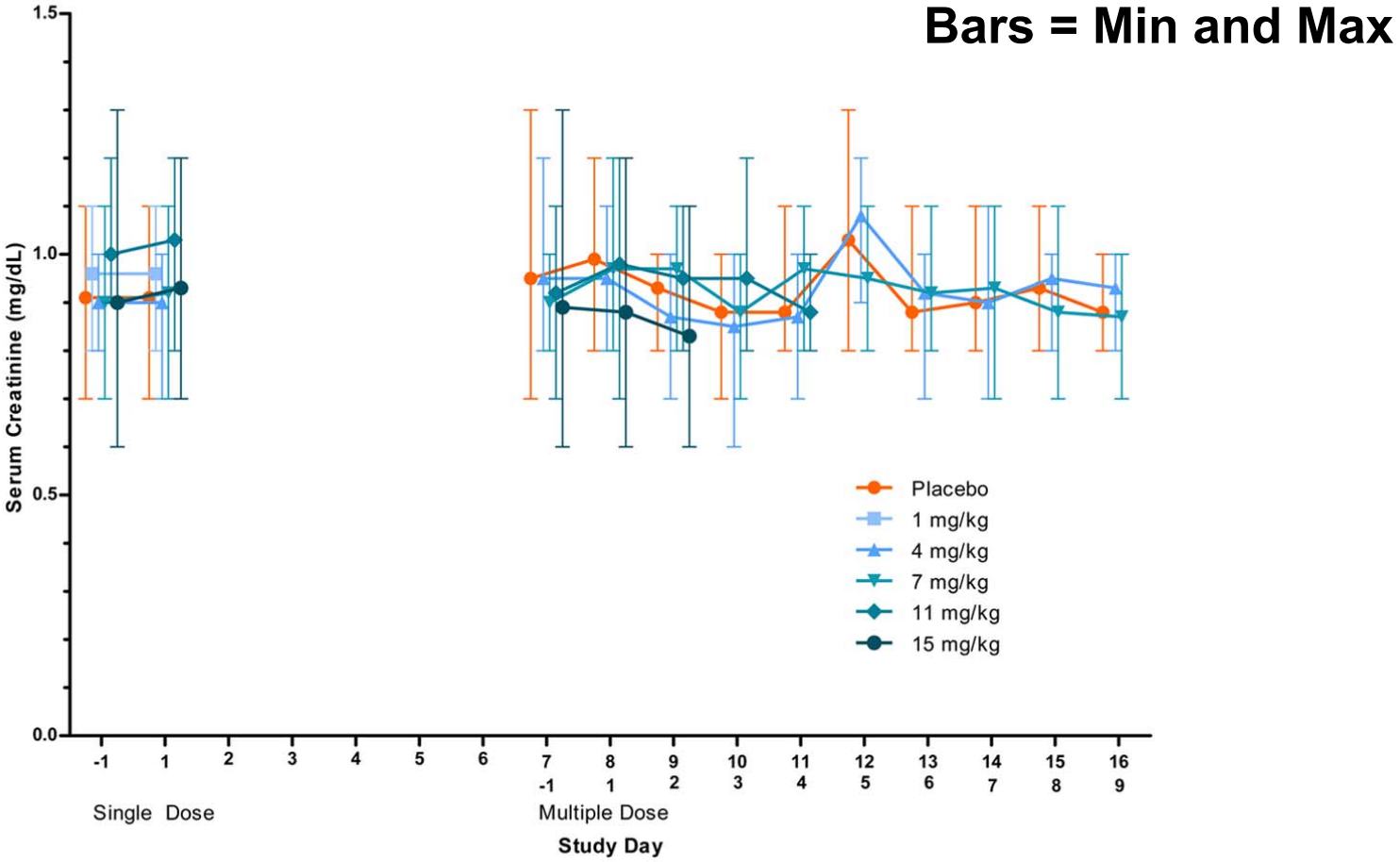


* Giuliano *et al.*, J. Pharm. Exp. Ther., 1986

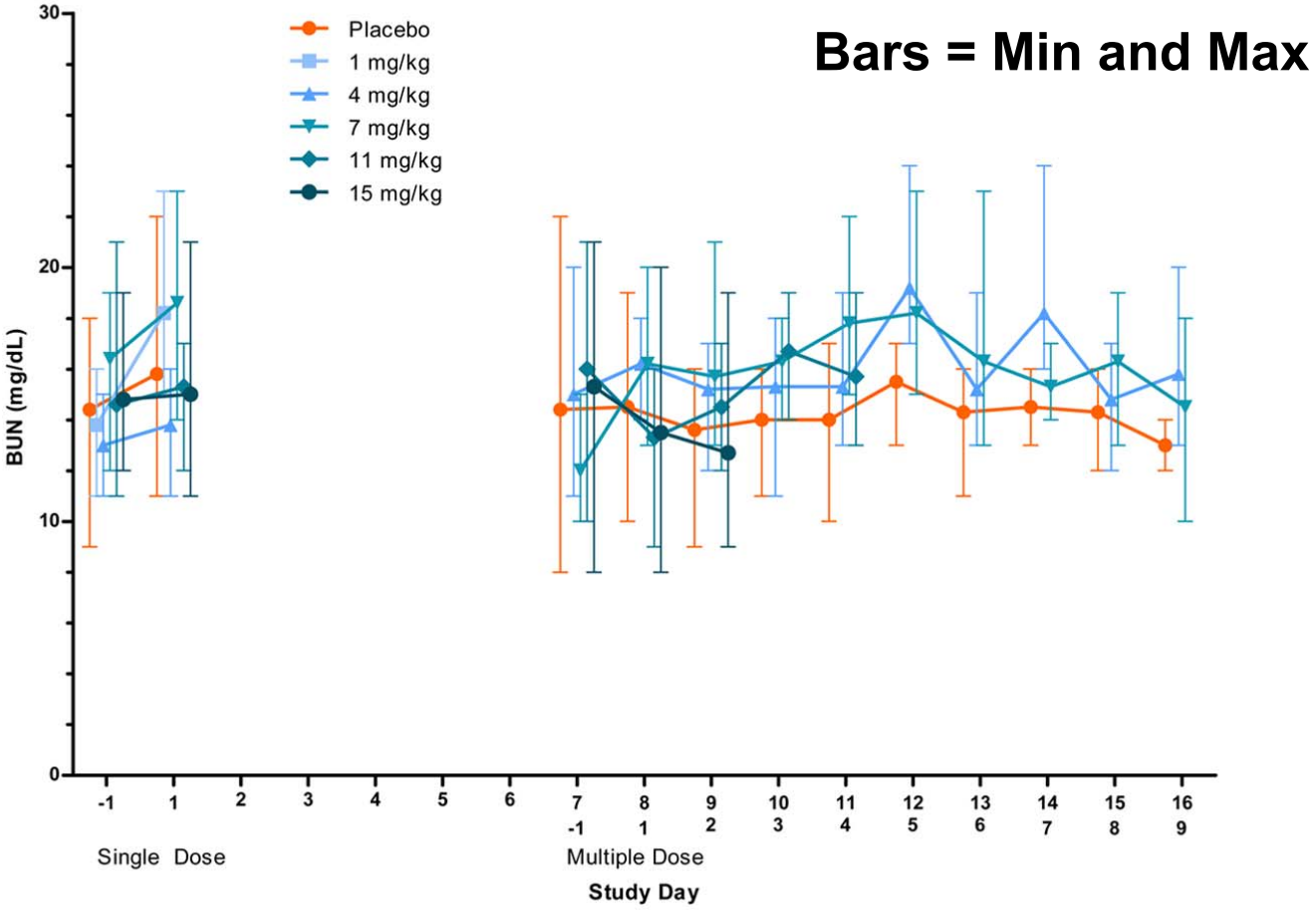
Aminoglycoside peak / MIC ratio is predictive of clinical efficacy



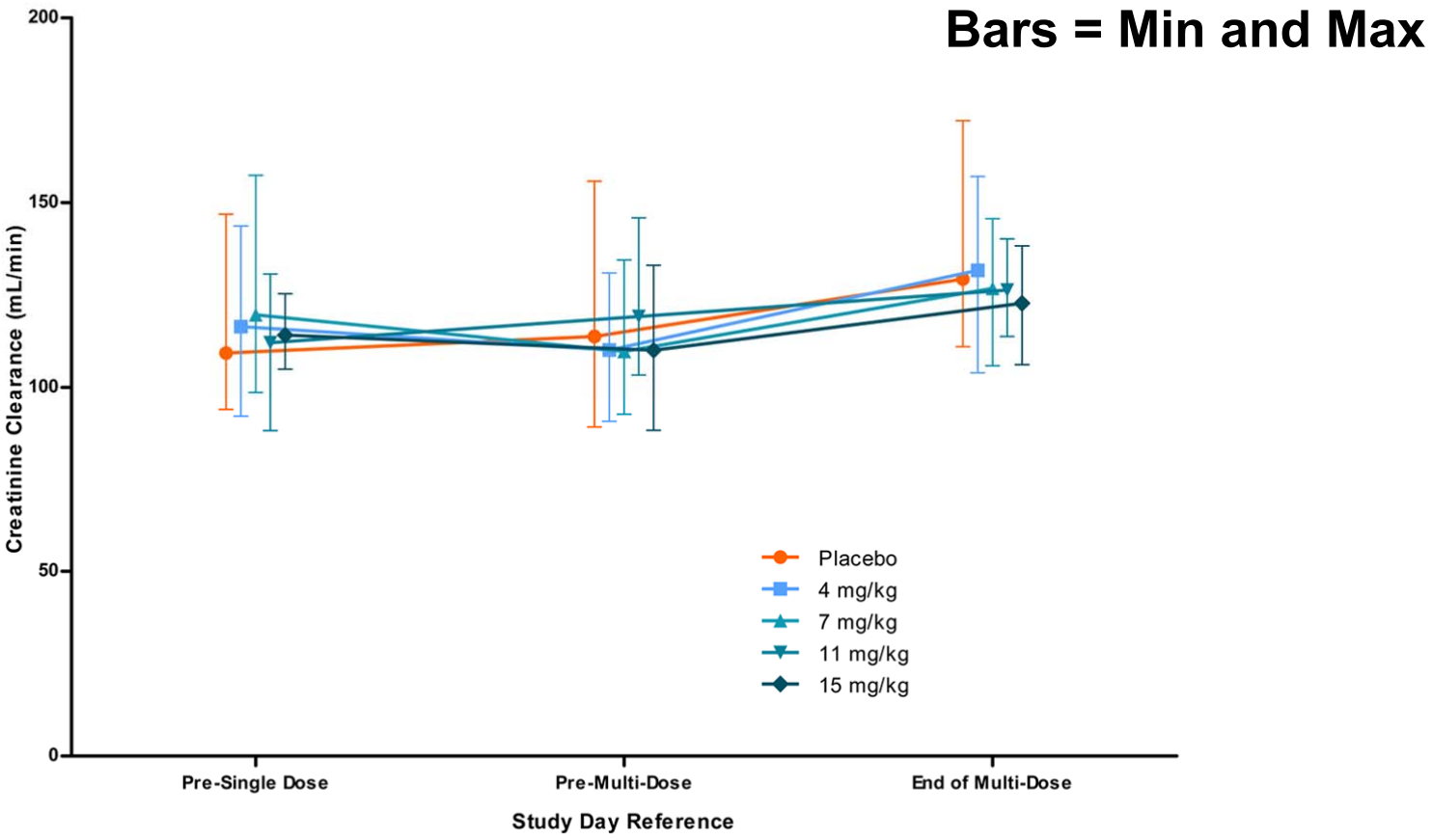
ACHN-490: No Evidence of Nephrotoxicity Based on Daily Serum Creatinine



ACHN-490: No Evidence of Nephrotoxicity Based on Daily BUN Measurements



ACHN-490: No Evidence of Nephrotoxicity Based on Measured Creatinine Clearance



Refurbishing old antibiotic:
2. Novel polymyxins * ?

- 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

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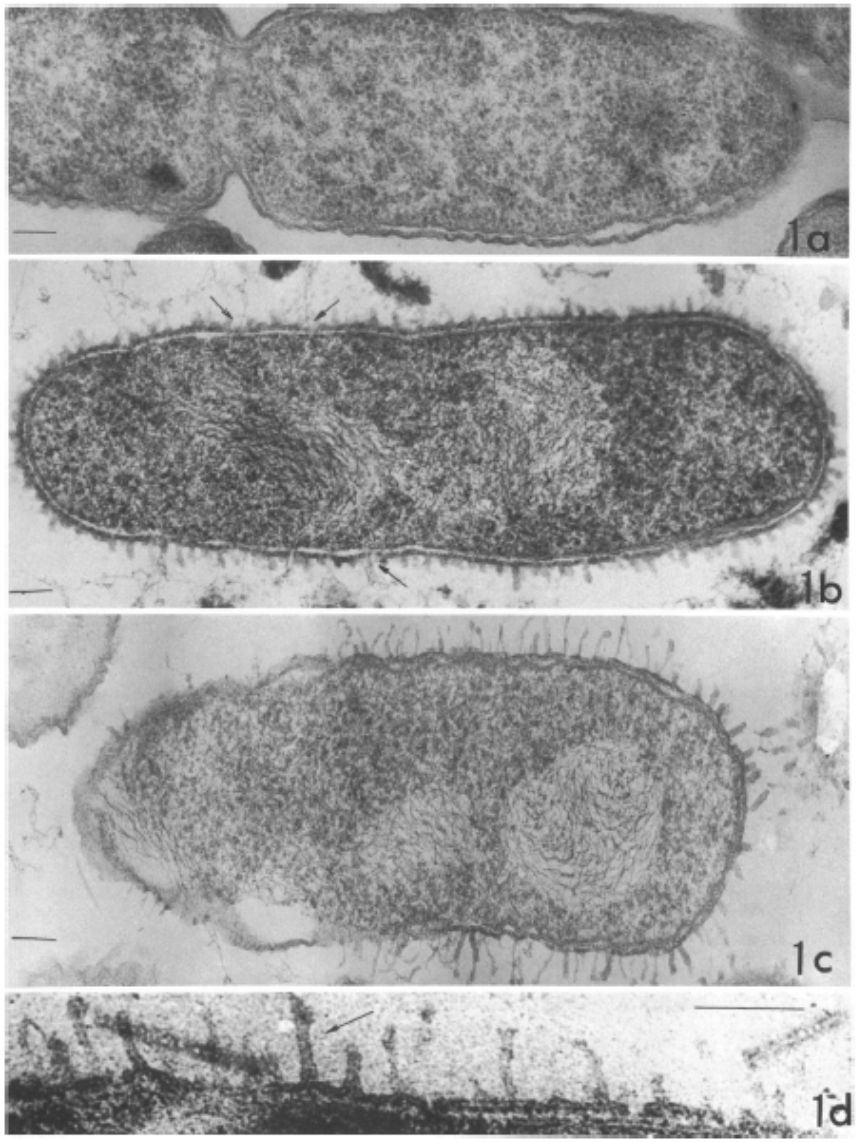
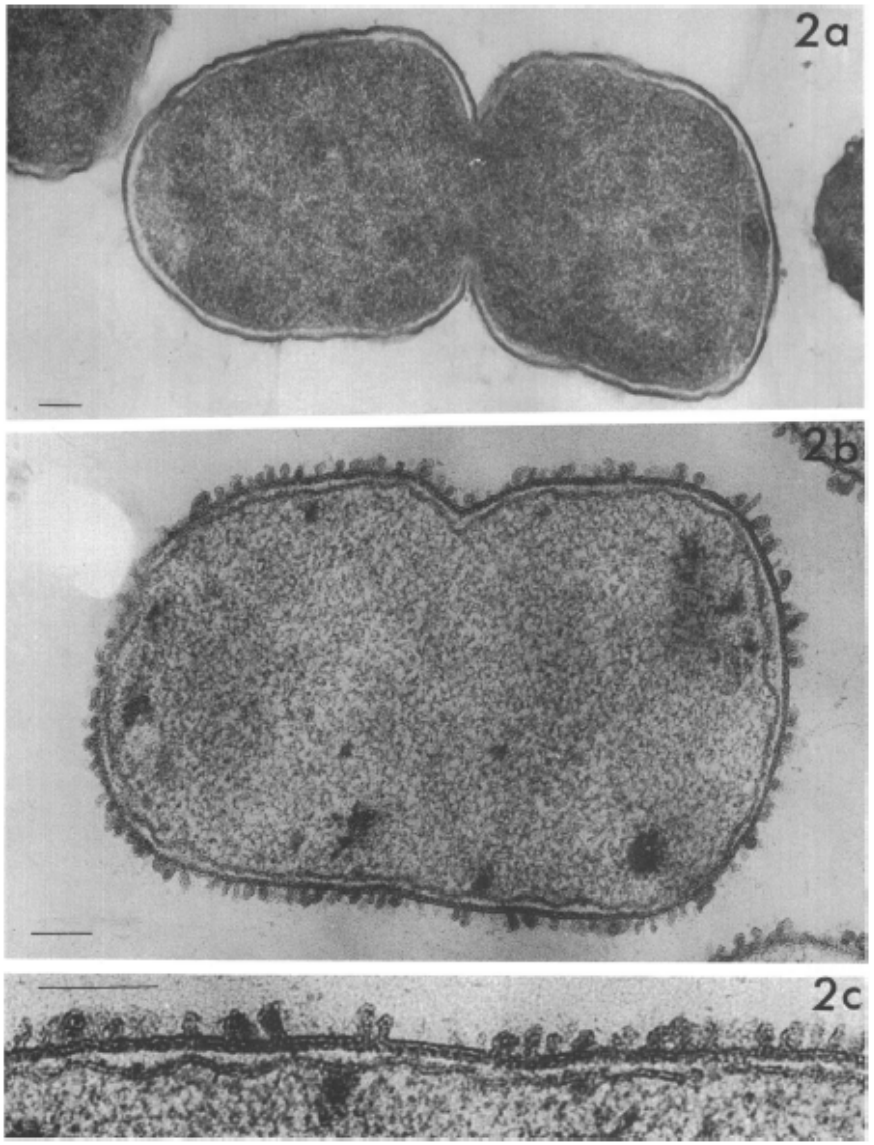
* in collaboration with Northern Antibiotics, Finland

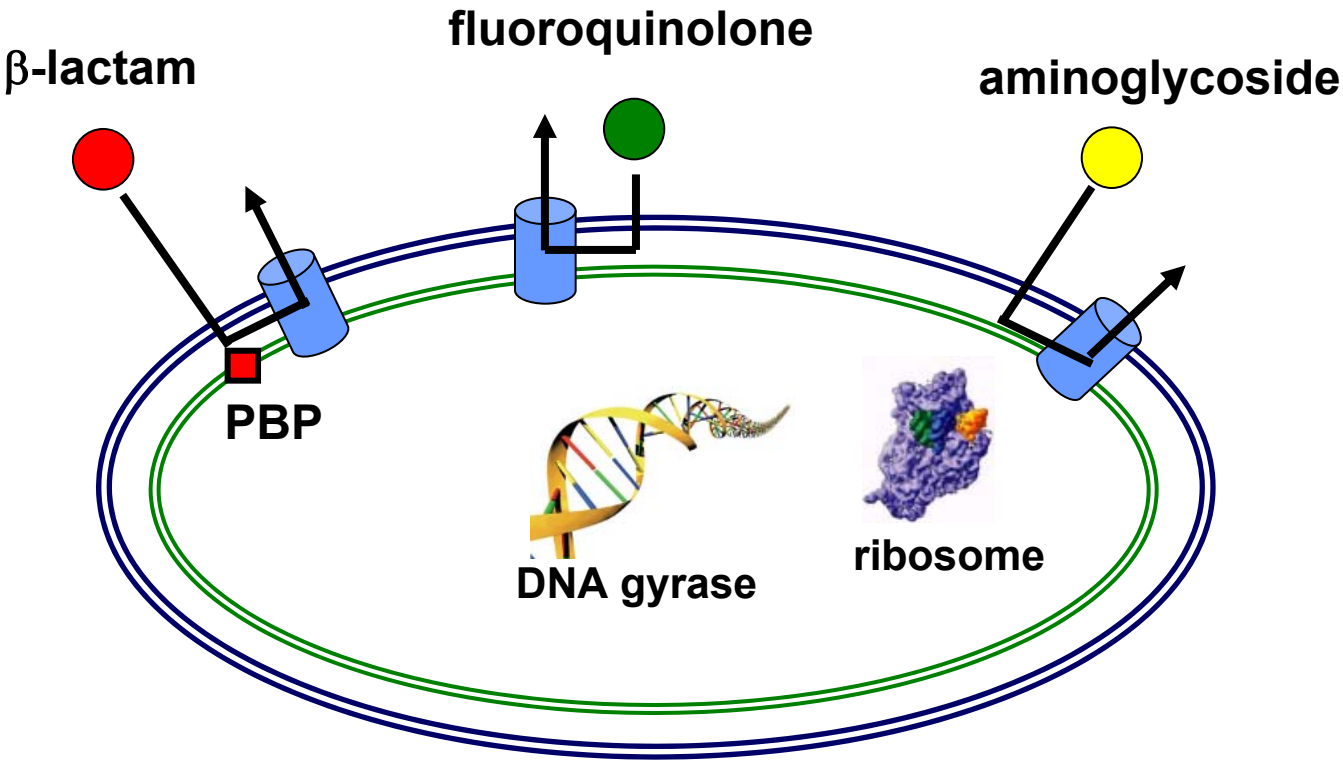
Colistin Microbiology: morphological aspects





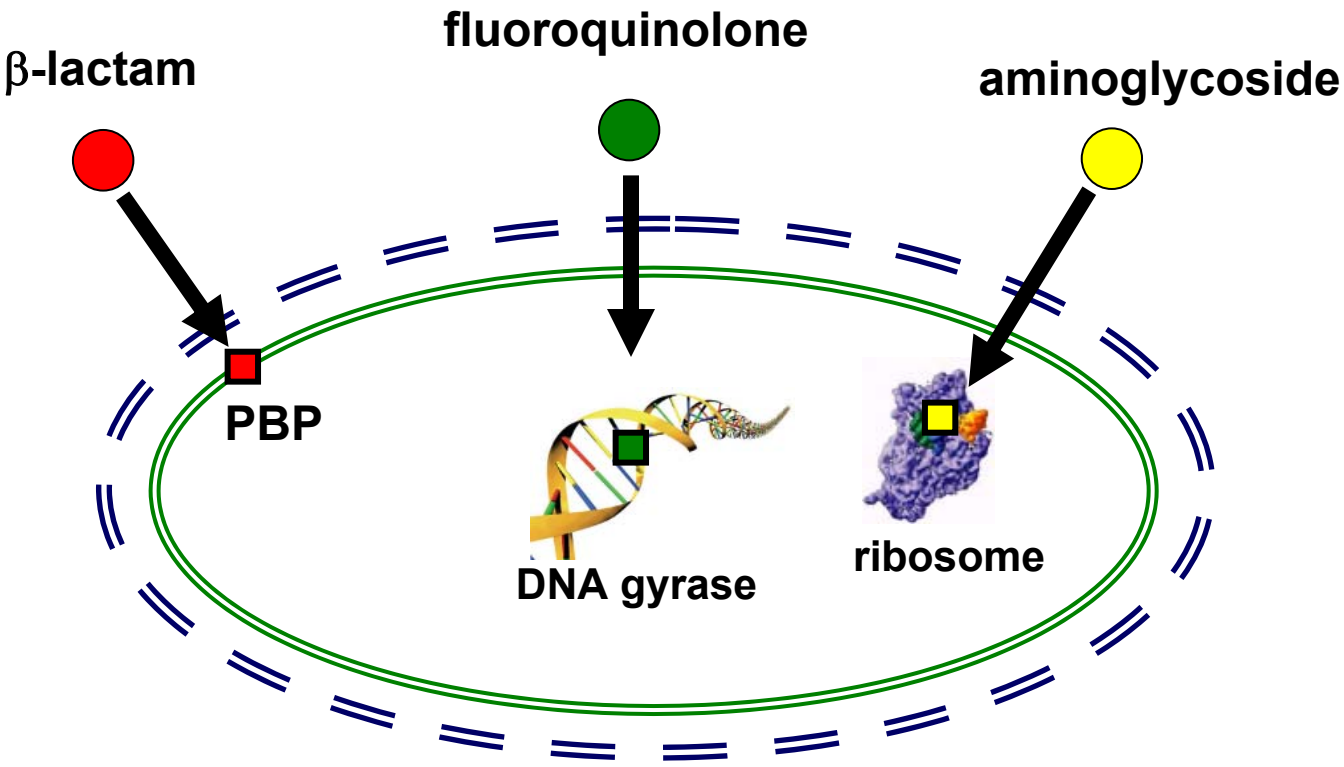
Koike et al. J. Bacteriol. 1969; 97:448-452

Polymyxins synergy: the rationale (1)



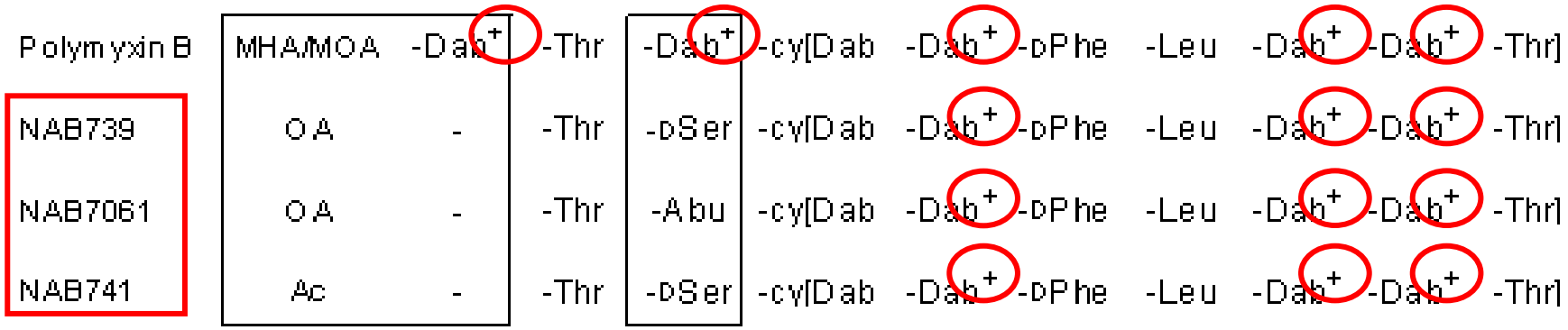
- **Gram-negative bacteria have also efflux systems defeating the passage of drugs across the OM and explaining the low activity of many antibiotics (intrinsic resistance) and the so-called "adaptative" resistance (aminglycosides)**

Polymyxins synergy: the rationale (2)



- **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

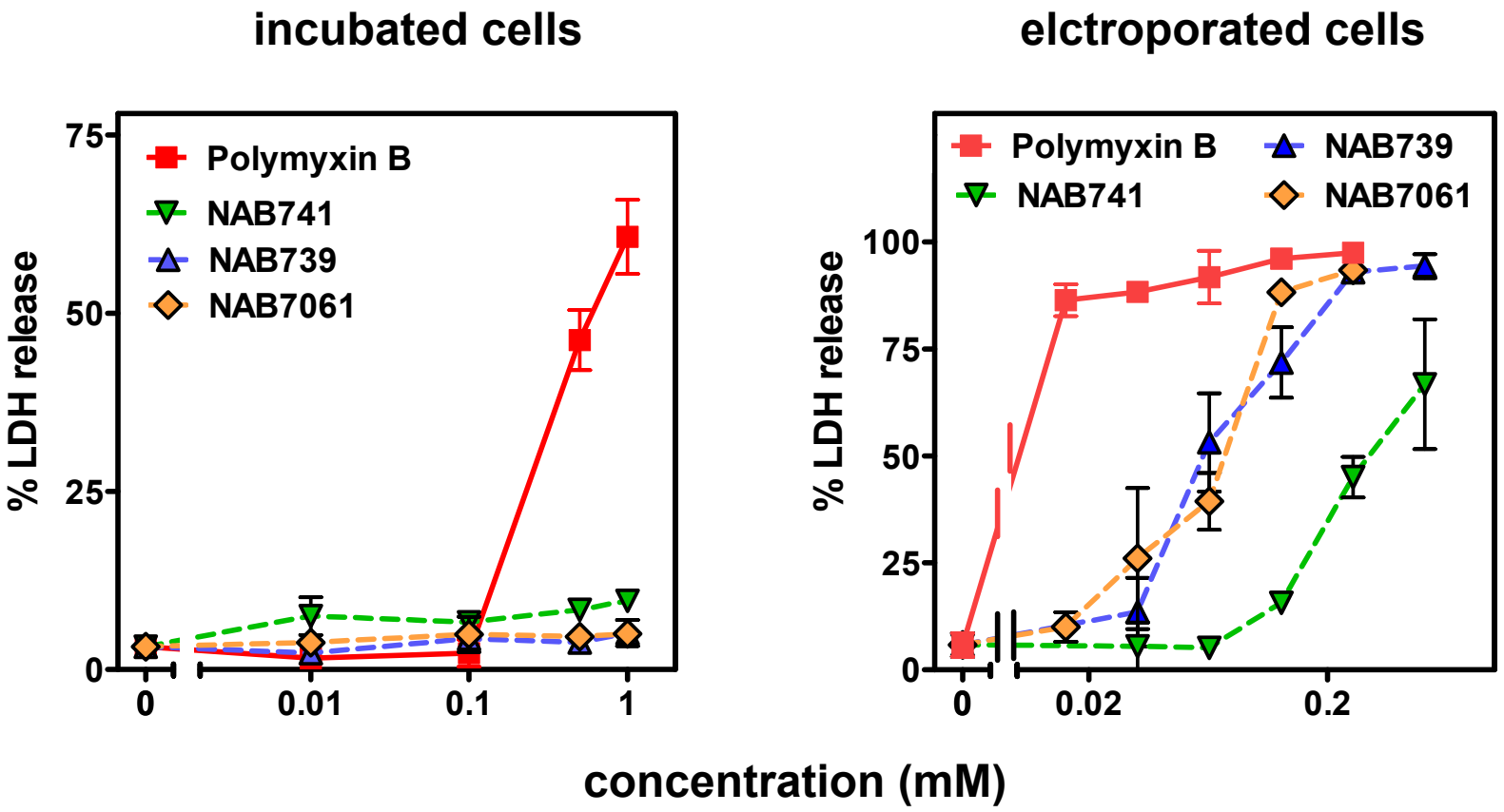


- **The MIC90 of NAB739 for *E. coli* and Enterobacteriaceae are similar to those of polymyxin B (1-2 mg/L).**
- **NAB739 is also active against *Acinetobacter baumannii*, and *Pseudomonas aeruginosa.***
- **NAB7061 and NAB741 strongly synergize the activity of antibiotics (including rifampicin, macrolides,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

NAB compounds are less cytotoxic than polymyxin B

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



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

Refurbishing old antibiotics
3. Temocillin *

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*.

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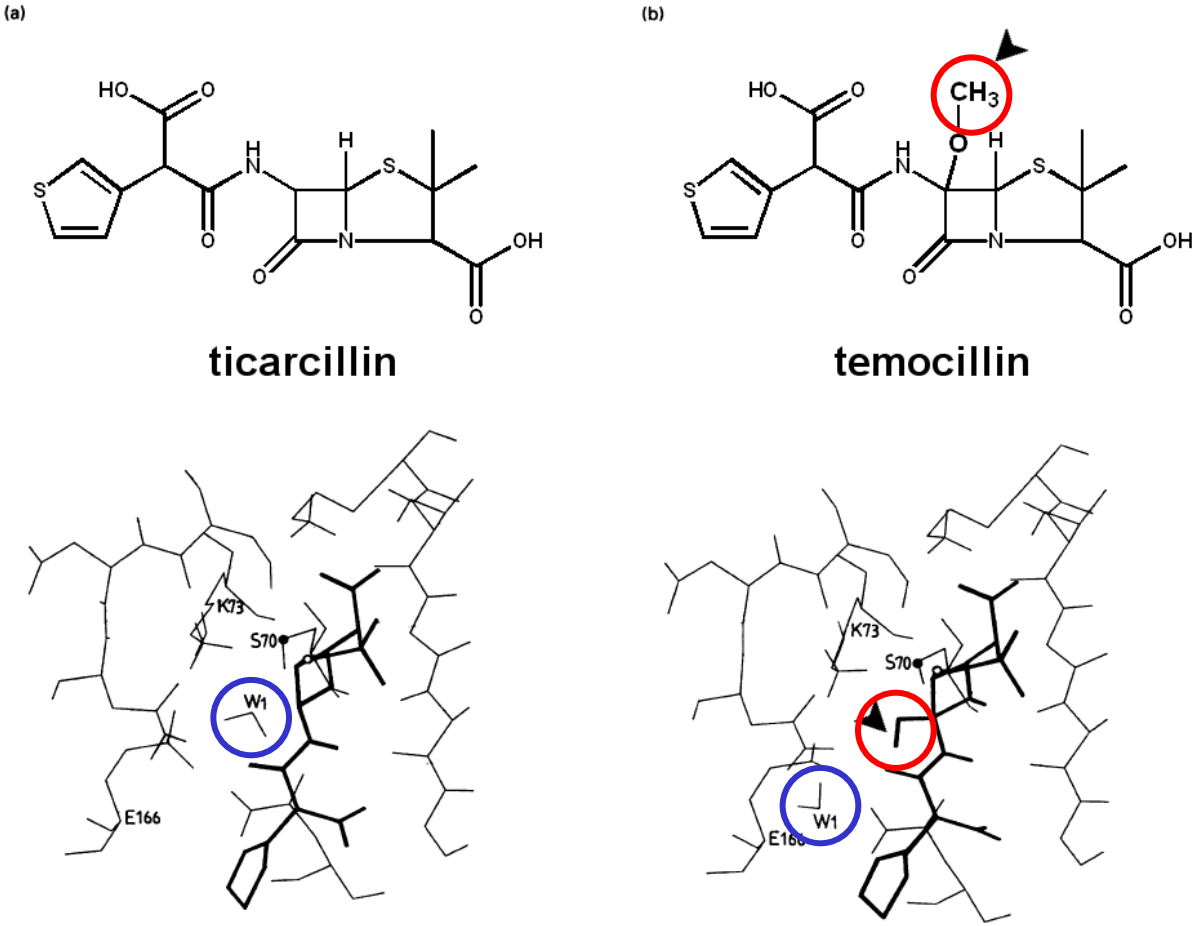
* in collaboration with Eumedica (Belgian SME)

Temocillin in a nutshell

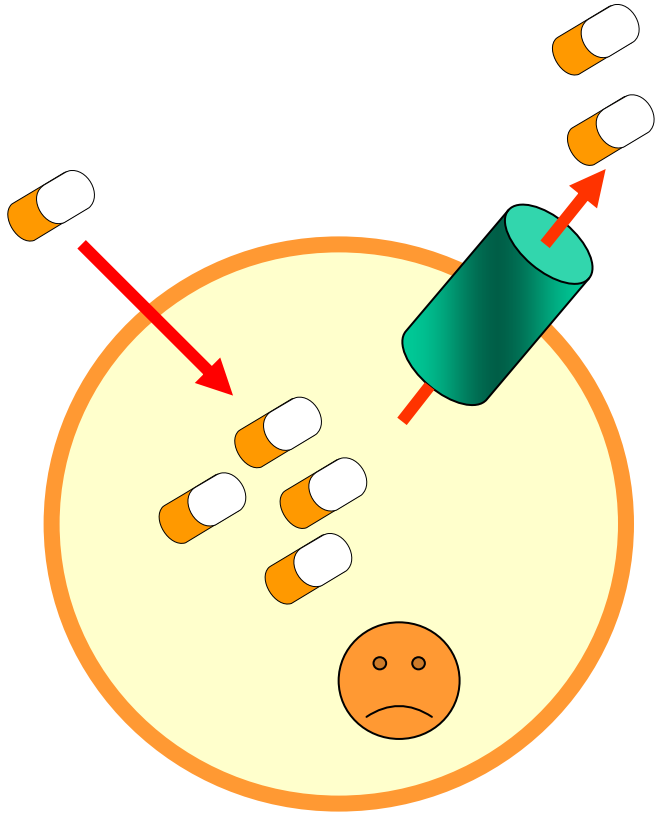


The α-methoxy group (arrow) in temocillin blocks access of water (W1) to the active serine (S70) of β-lactamase, thereby blocking the chain of molecular events leading to hydroysis

Matagne *et al.* Biochem J 1993; 293:607-11

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.

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

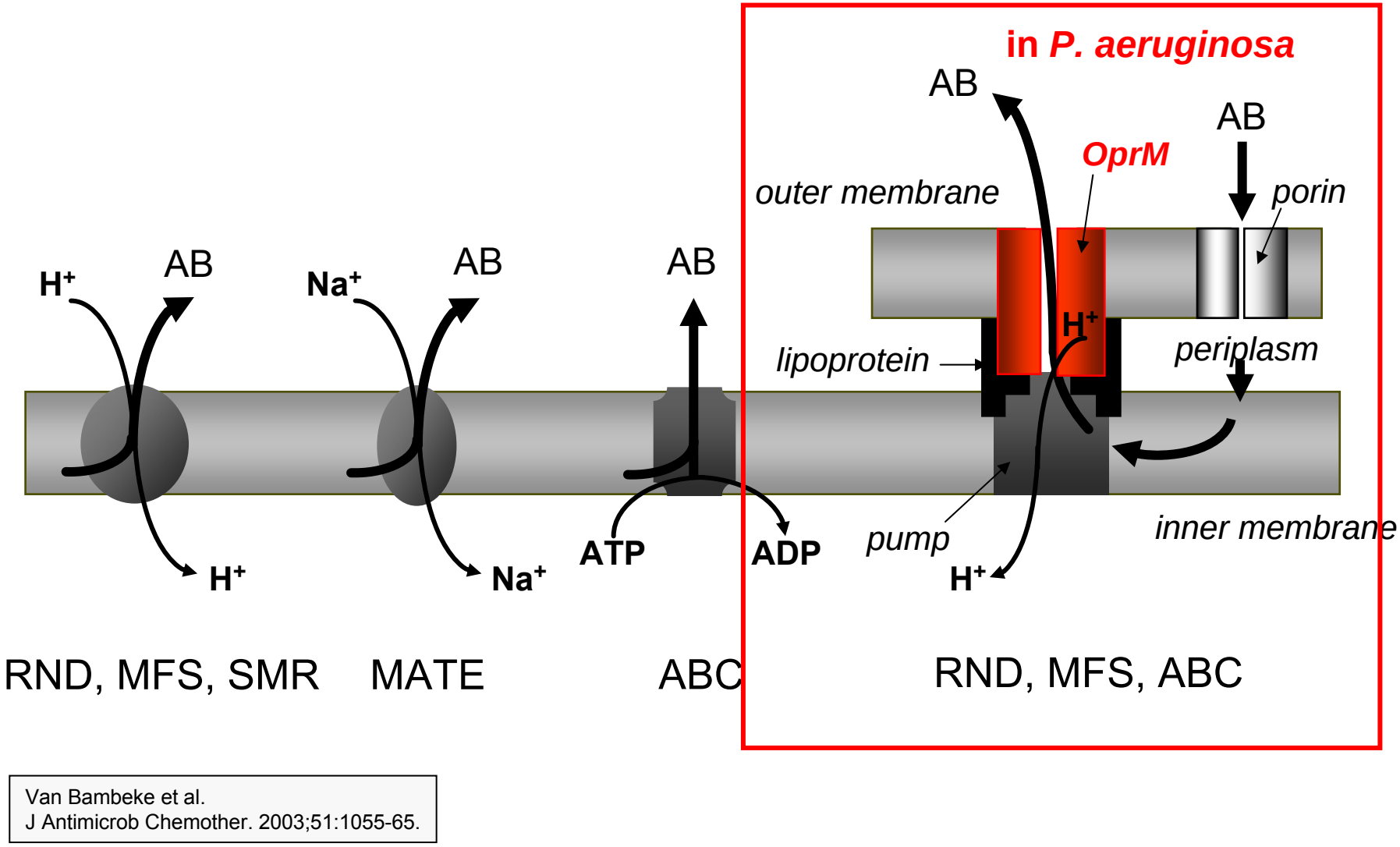
Table 1. MICs of temocillin and ticarcillin against *P. aeruginosa* strains with known expression of the efflux Mex components in Mueller-Hinton broth (MHB) and in MHB supplemented with the broad spectrum efflux transporter inhibitor Phe-Arg-β-naphthylamide (PAβN; 50 µg/mL)

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.

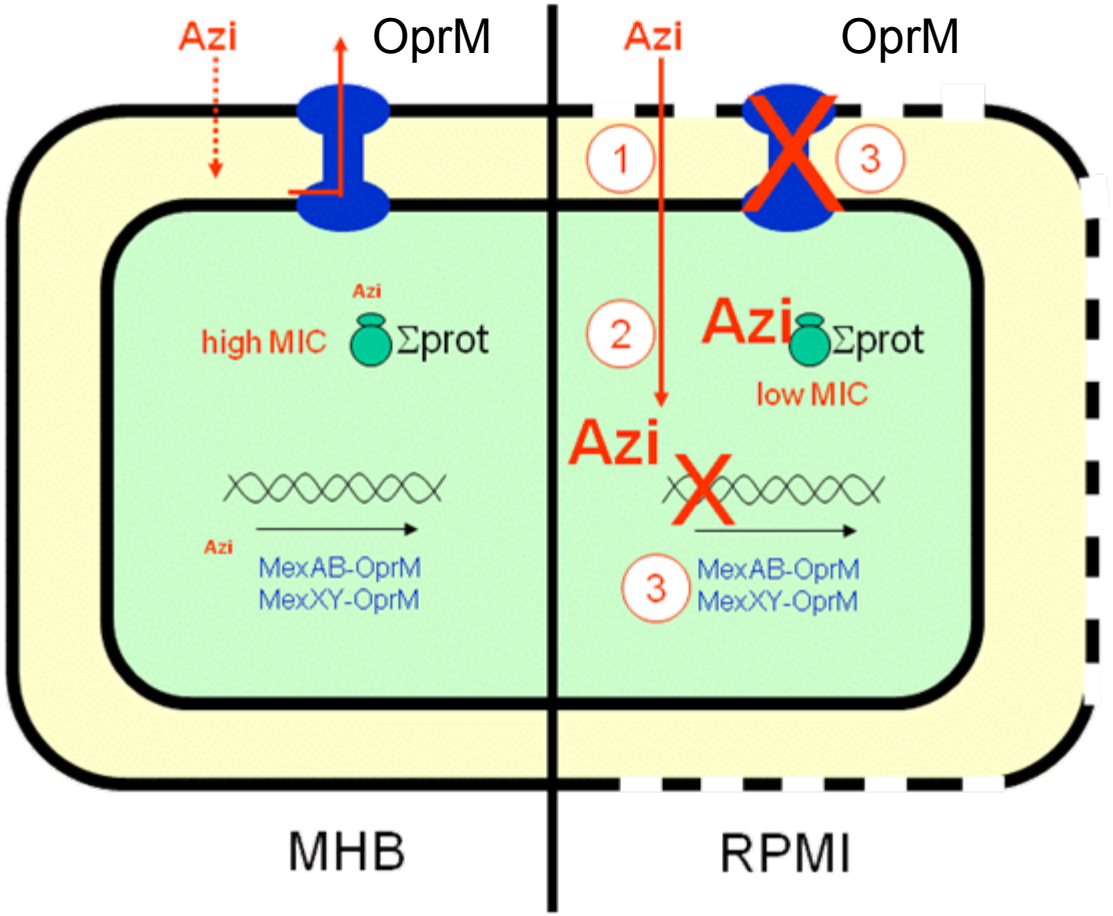
Buyck et al. 51th ICCAC, Chicago, IL, 2011

Structure of antibiotic efflux transporters



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

Using macrolides to block the synthesis of OprM in *P. aeruginosa*

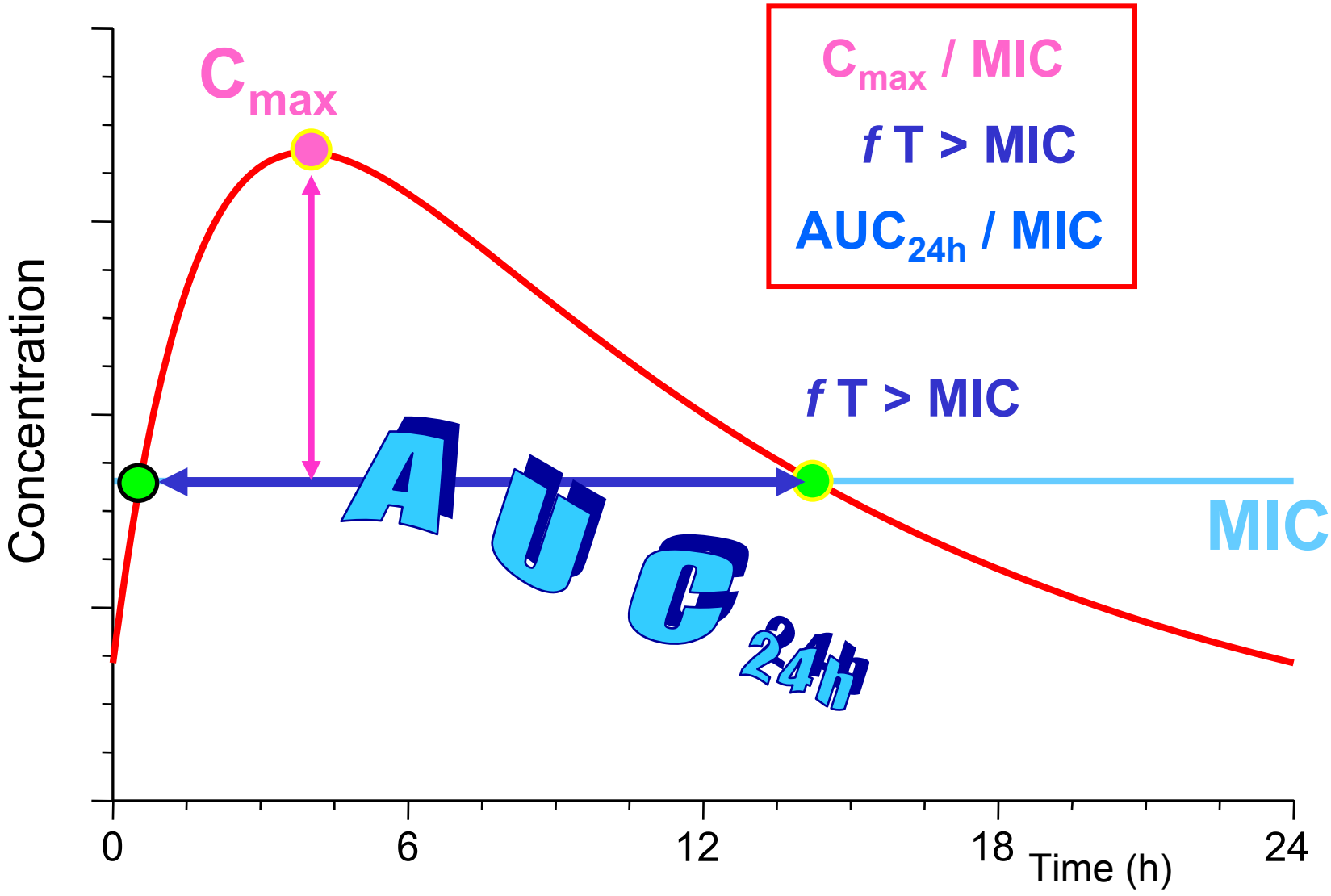


Buyck et al. Clin. Infect. Dis. 2012; 55:534-542

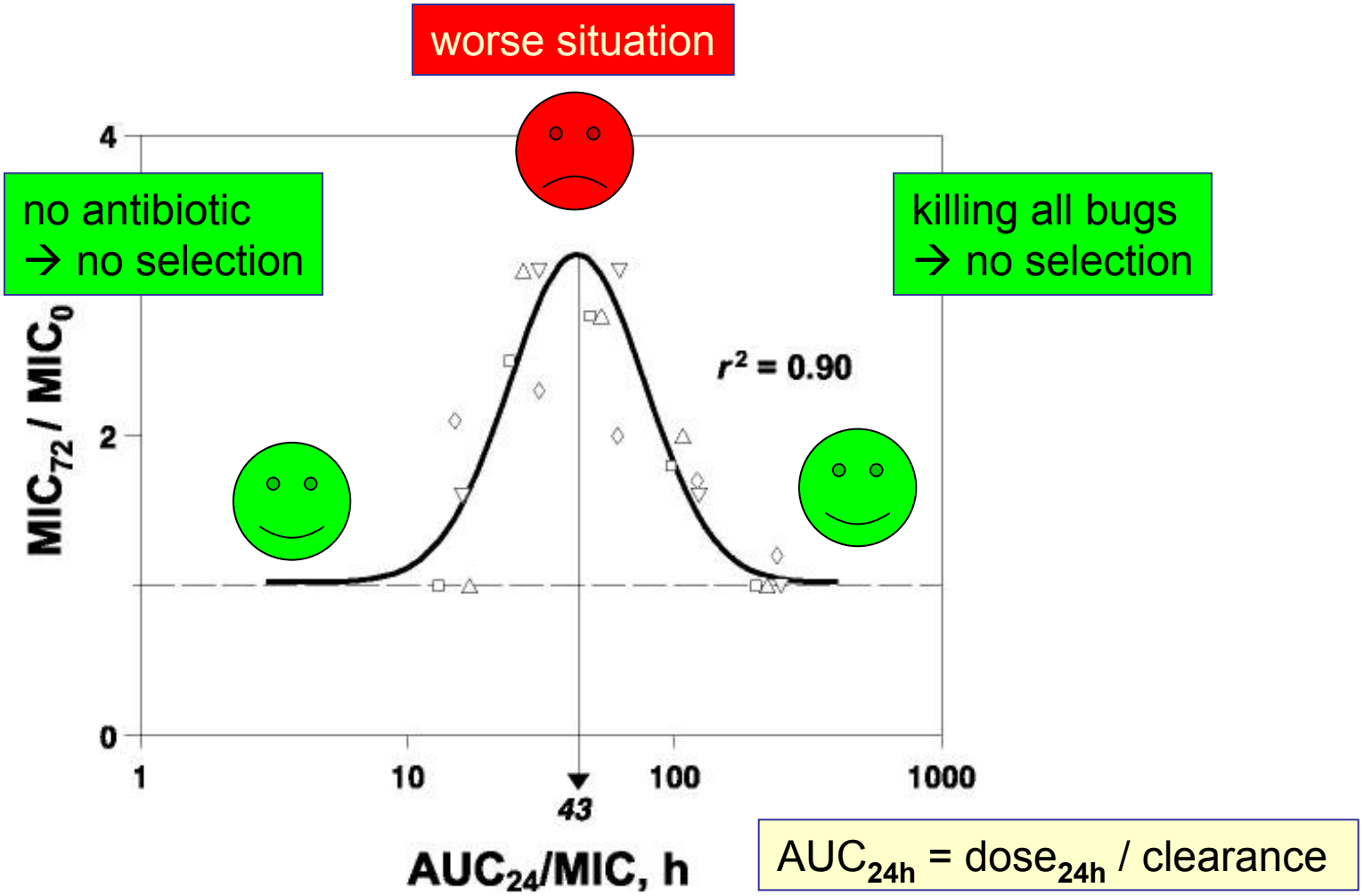
7 pillars of wisdom….

1. Public education
2. Public health, sanitation and quality of life
3. New antibiotics → new / poorly exploited targets
4. Old antibiotics
   → aminoglycosides – polymyxins - temocillin
5. **Better use of antibiotics**
   → PK/PD approaches against resistance
   → Intracellular bacteria
6. Alternatives to antibiotics
7. Collaborative approach

Pharmacokinetics/Pharmacodynamics of antibiotics

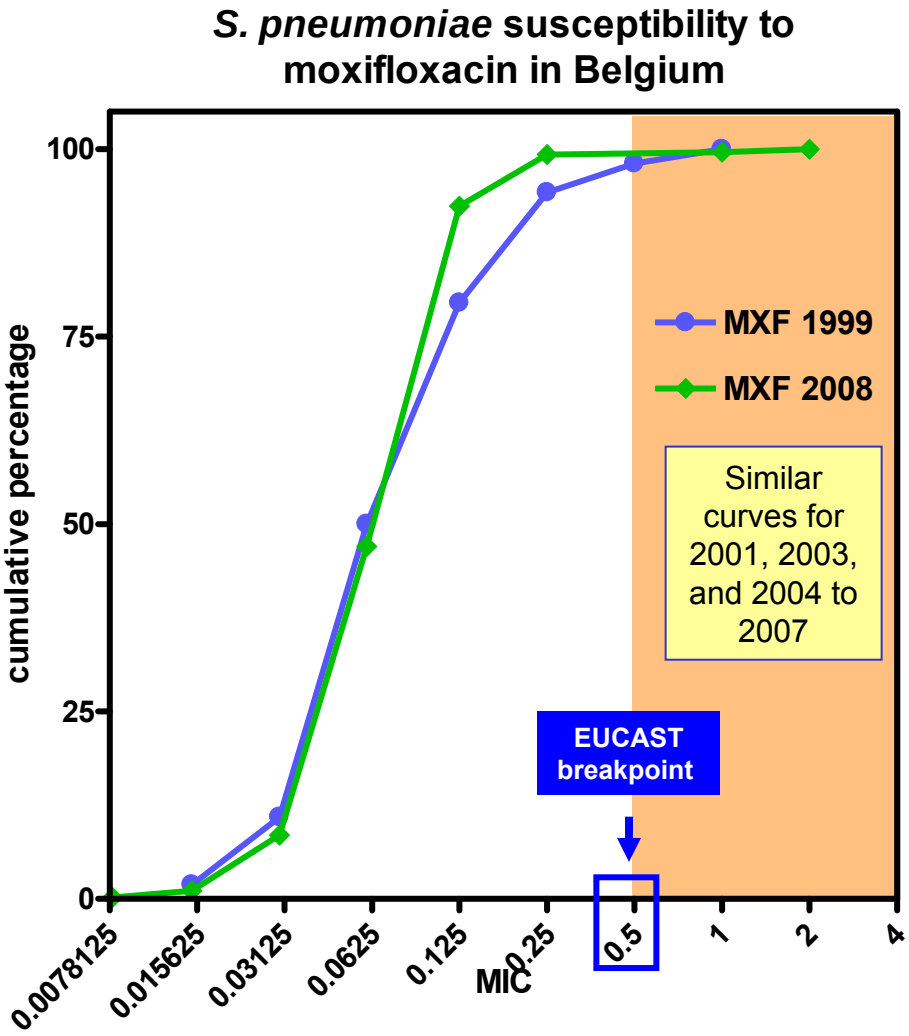


Avoiding selection of resistant mutants during treatment: an example with fluoroquinolones



Firsov et al. In vitro pharmacodynamic evaluation of the mutant selection window hypothesis using four fluoroquinolones against *Staphylococcus aureus*. Antimicrob Agents Chemother. 2003 May;47(5):1604-13.

Lack of resistance of *S. pneumoniae* to moxifloxacin over 10 years of large use in the community in Belgium



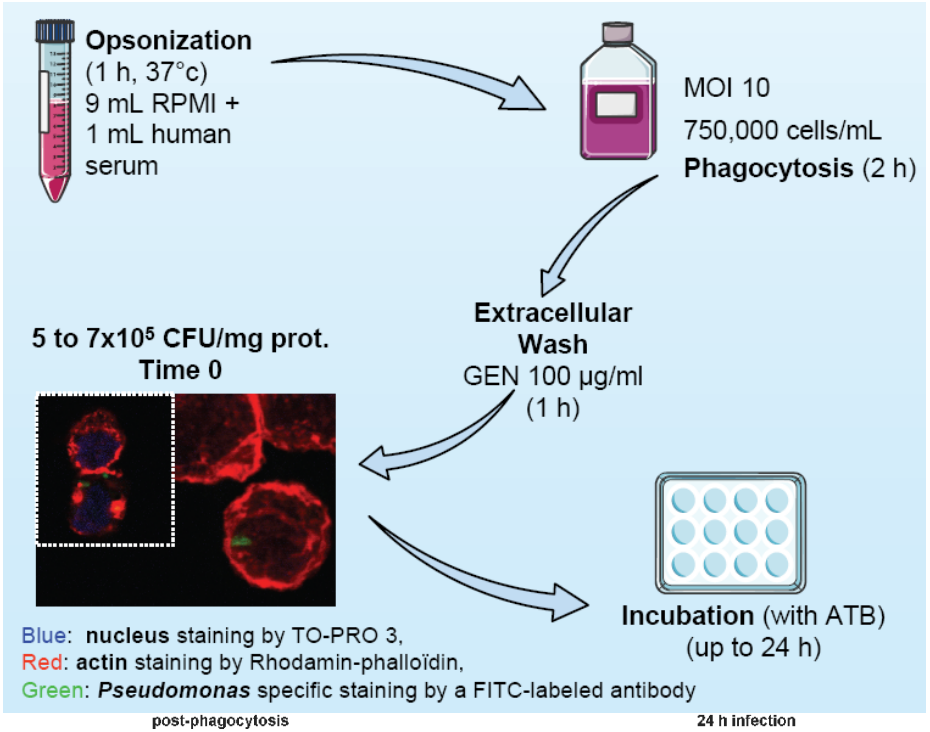
From data of a national collection

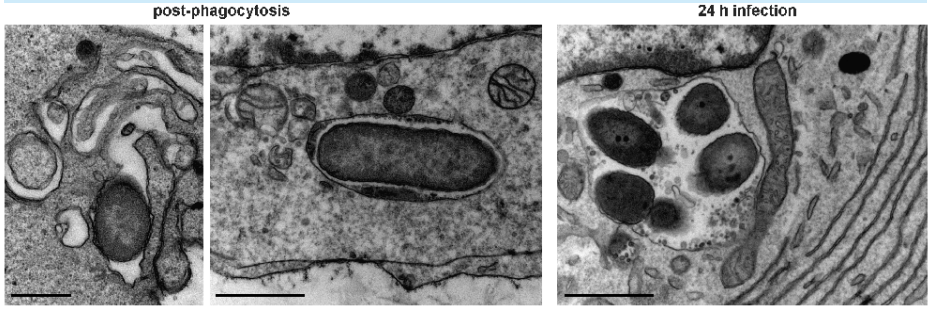
- Non invasive respiratory tract infections
- similar results in 2008 for a collection of S.pneumoniae from clinically-confirmed CAP)

- Surveys from the Belgian Scientific Institute for Public Health for *S. pneumoniae* from community isolates (n=156 in 1999 and 448 in 2008)
- Data available yearly for 1999 through 2008
- http://www.iph.fgov.be

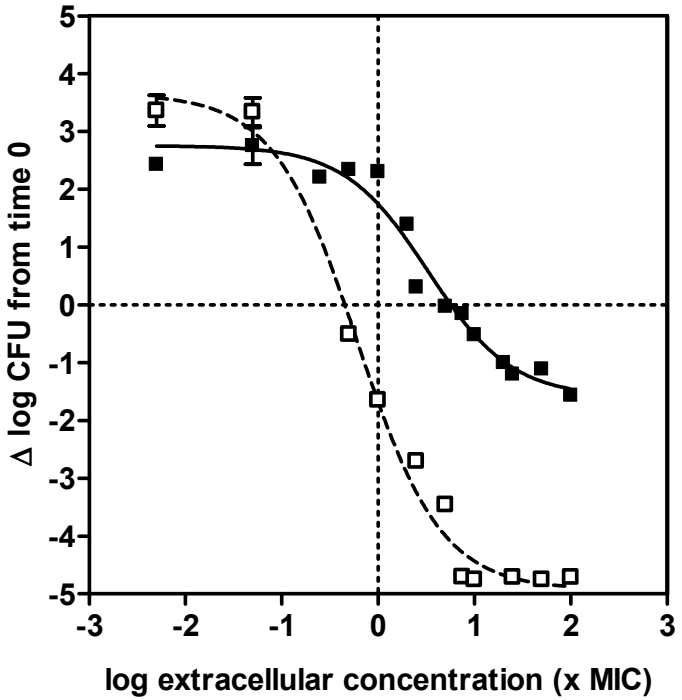
Vanhoof RLM, et al. 19th European Congress of Clinical Microbiology and Infectious Diseases. May, 16-19 2009, Helsinki.
Lismond et al. Antimicrobial susceptibility of Streptococcus pneumoniae isolates from vaccinated and non-vaccinated patients with a clinically confirmed diagnosis of community-acquired pneumonia in Belgium. Int J Antimicrob Agents. 2012; ;39:208-16.

Intracellular bacteria: setting up a model



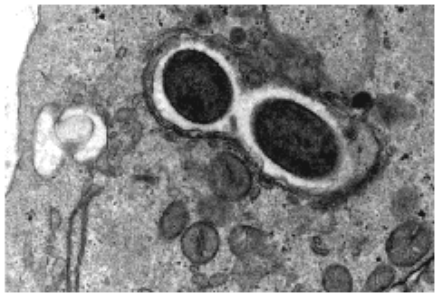


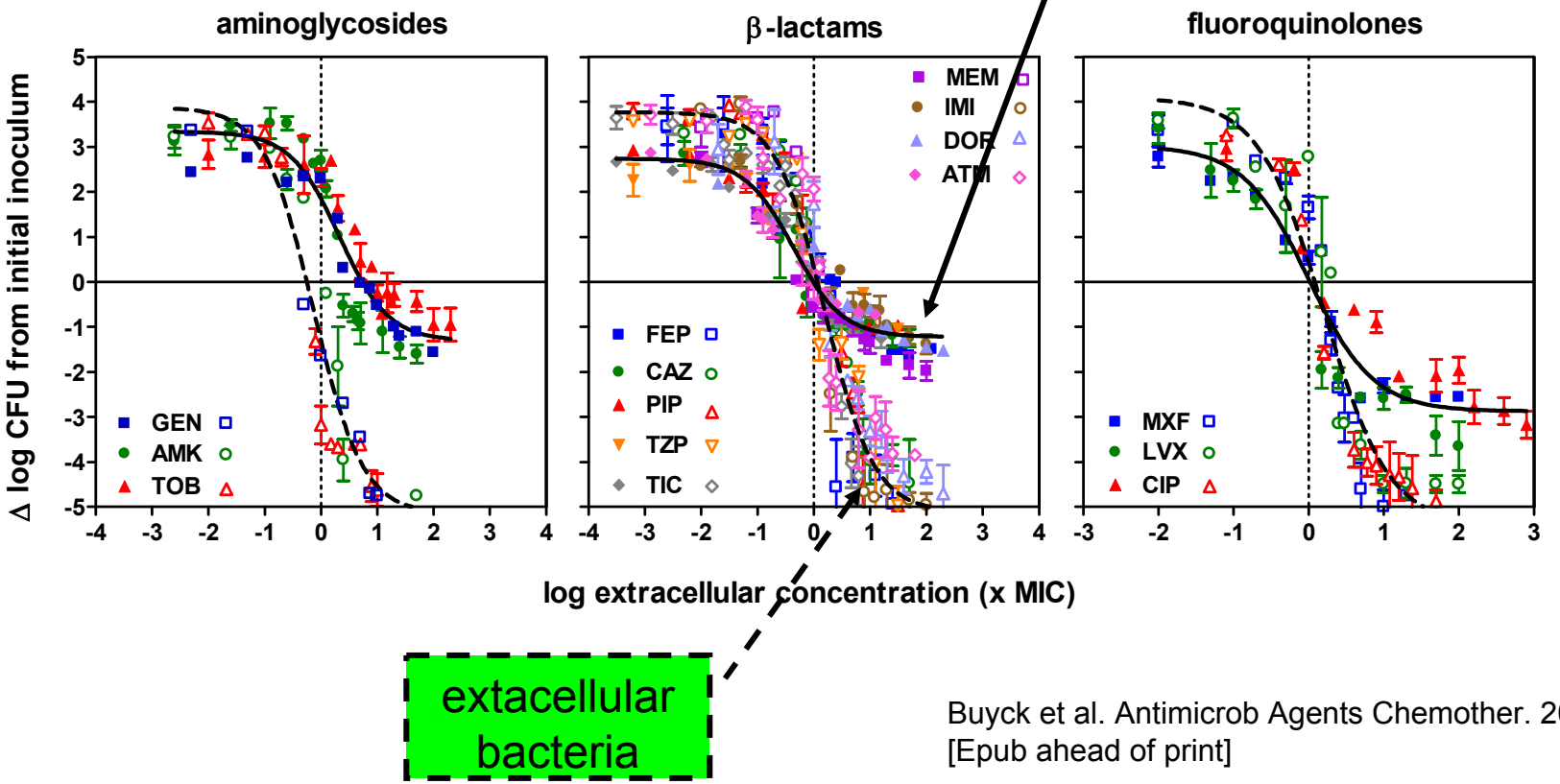
pharmacological concentration-response curves



- Buyck et al. Antimicrob Agents Chemother. 2013 Mar 11 [Epub ahead of print]
- Van Bambeke, unpublished

The difficulties in eradicating intracellular (hidden) bacteria:
an example with *P. aeruginosa*



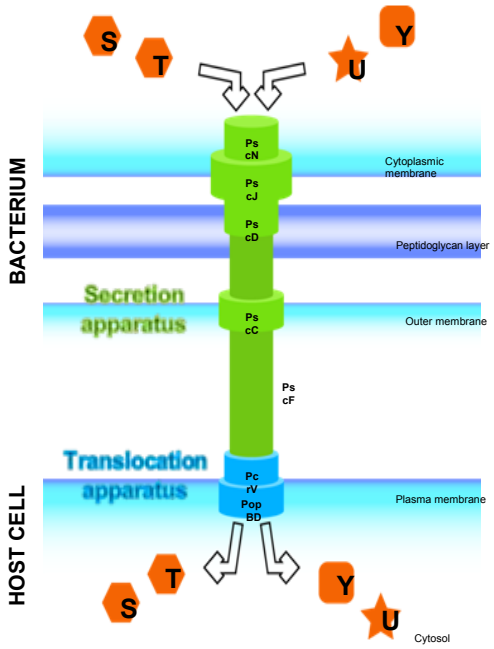


Buyck et al. Antimicrob Agents Chemother. 2013 Mar 11 [Epub ahead of print]

7 pillars of wisdom….

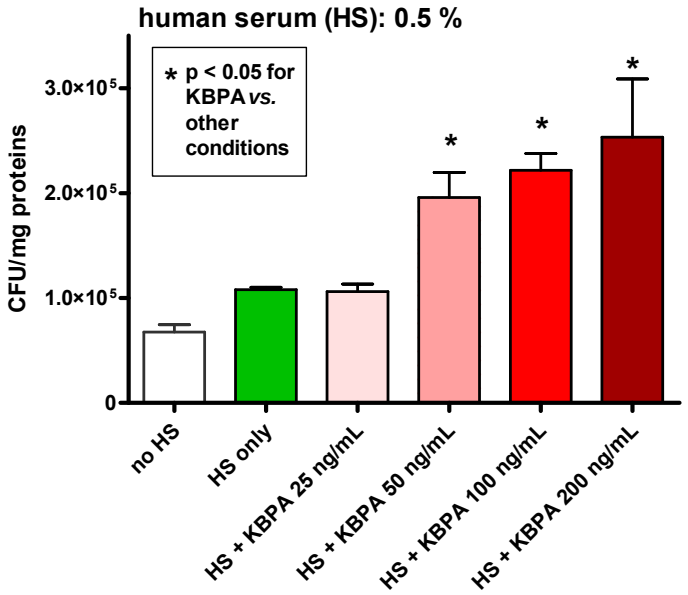
1. Public education
2. Public health, sanitation and quality of life
3. New antibiotics → new / poorly exploited targets
4. Old antibiotics
   → aminoglycosides – polymyxins - temocillin
5. Better use of antibiotics
6. **Alternatives to antibiotics**
7. Collaborative approach

Alternatives to antibiotics



Inhibitors of type III secretion systems in *P. aeruginosa*

Anantharajah *et al.* ICAR 2012
(in collaboration with Creative Antibiotics, Umea, Sweden')



Stmulation of phagocytosis of P. aeruginosa by fully-human monoclonal antibody (panomacuab)

Jacqmin et al. ICAAC 2012
(in collaboration with Kenta Biotech, Zurich, Switzerland)

Towards medicine … and success ?

Healing Buddha

The last Judgment
Hieronymus Bosch (c1450-1516)
Vienna Art Academy

Academic partnerships

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Université Joseph Fourier Grenoble

de Duve Institute

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UNIVERSITEIT GENT

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Main Industrial partnerships for common projects *

*** most having led to peer-reviewed publications on novel compounds or concepts**

Collaborative approach to bring discovery to the clinics

Who made that all possible ?

Who made that all possible ?

Disclosures

Financial support from

- the Belgian *Fonds de la Recherche Scientifique* (and other federal and regional funding agencies) for basic research on pharmacology and toxicology of antibiotics and related topics and for support to a PhD fellow (D. Das)
- The *Université catholique de Louvain* for personal support
- the Belgian Public Federal Service "Public Health" for "Appropriate antibiotic use" studies in General Practice
- The *Pôles d'Attraction Interuniversitaire/ Interuniversitair Attractie Polen* programme of the Belgian Federal Governement, the *Région Bruxelloise/Brusselse Gewest* and the *Région Wallonne* for support to post-doctoral fellows
- Collaborations with Achaogen, Northern Antibiotics, RibX, Merlion, Trius, Cerexa, Bayer, AstraZeneca, and GSK.