Emerging antibiotic resistance

Paul M. Tulkens, MD, PhD

Cellular and Molecular Pharmacology Group
& Center for Clinical Pharmacy

Louvain Drug Research Institute

Université catholique de Louvain

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Emerging Infections

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Disclosures

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- 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 (until 2010)
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- Collaborations with Achaogen, Northern Antibiotics, RibX, Merlion, Trius, Cerexa, Bayer, AstraZeneca, and GSK.

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

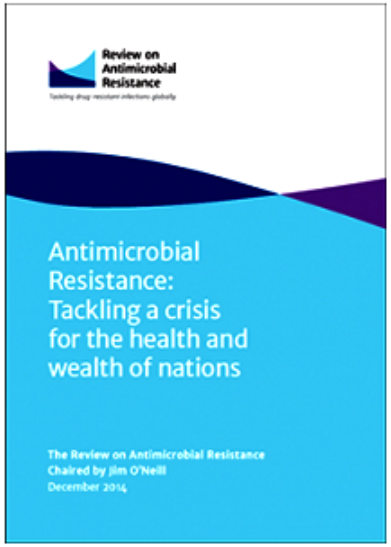
1970 …

Are antibiotics following a path to madness ?

But…

2012 …

An now, even Economists …
…. and Prime Ministers are concerned



December 11, 2014

The Review on Antimicrobial Resistance publishes its first paper

The Review has published its first paper, *Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations* which sets out the global threat of not tackling AMR. The paper was published on December 11 at a launch event hosted by its Chair, Jim O'Neill, in London. In this report we explain why failing to tackle drug-resistant infections will cause 10 million deaths a year and cost up to US$ 100 trillion by 2050.

Jim O'Neill, Chairman of the Review on AMR, said:

> *"Drug-resistant infections already kill hundreds of thousands a year globally, and by 2050 that figure could be more than 10 million. The economic cost will also be significant, with the world economy being hit by up to $100 trillion by 2050 if we do not take action."*

> *If we fail to act, we are looking at an almost unthinkable scenario where antibiotics no longer work and we are cast back into the dark ages of medicine" – David Cameron, UK Prime Minister*

Review on Antimicrobial Resistance : http://amr-review.org/
(last visited: 06/06/2015)

You said “emerging”…?

Sir Alexander Fleming Discovers Penicillin

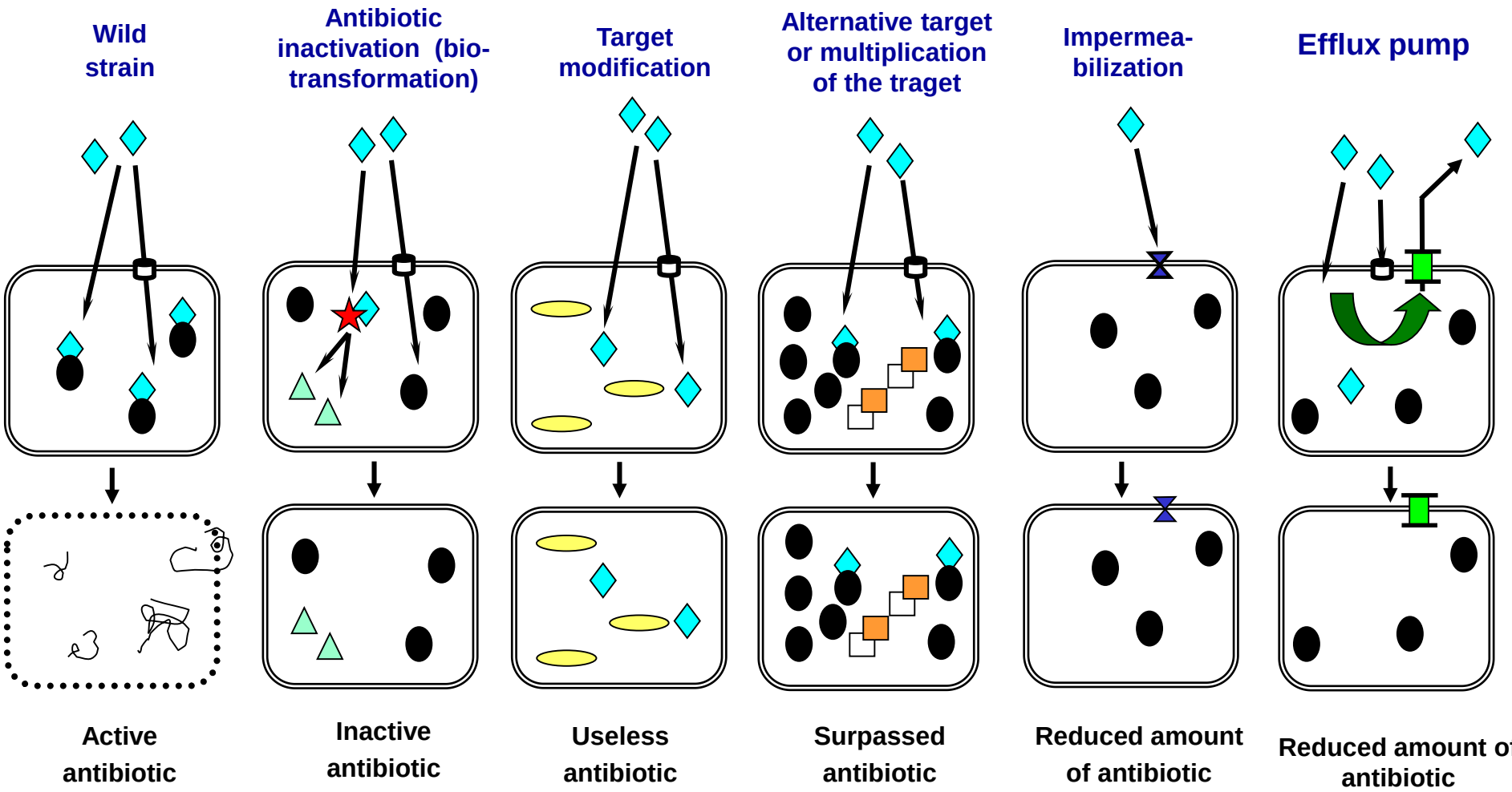
- 1928: A mold on a petri dish was observed to inhibit growth of *Staphylococcus* bacteria.
- The active ingredient isolated from this mold was found to be a safe and effective bacteria-killing agent of enormous potency.
- 1945 Nobel Prize Acceptance Speech: Sir Alexander Fleming warns of the danger of resistance:

> *“It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body…”*

Fleming, Nobel Lecture http://www.nobelprize.org/nobel_prizes/medicine/laureates/1945/fleming-lecture.pdf (last visited: 06/06/2015)

Castano et al. Antibiotic resistance: challenges and solutions https://www.cugh.org/sites/default/files/96_Antibiotic_Resistance_FINAL.pdf (last visited: 06/06/2015)

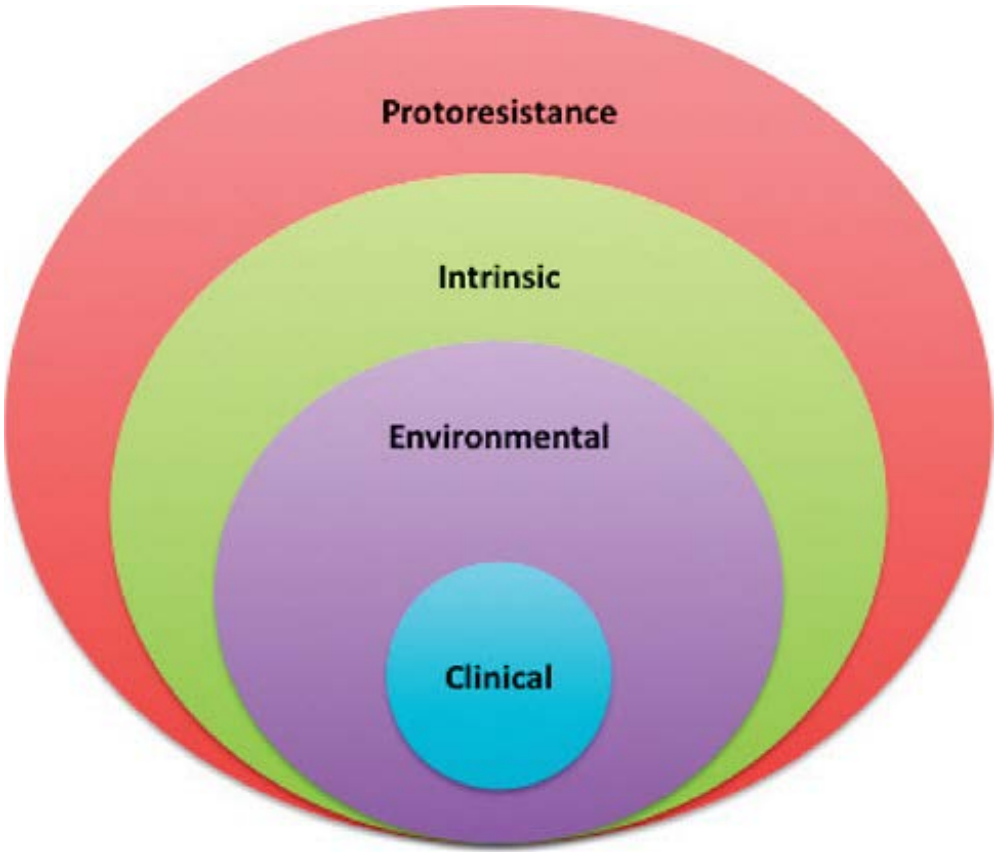
Antibiotic resistance: short overview of main mechanisms



Why is it so ?

- **The Resistome …**
  - **what it (probably) is …**
  - **how does it translate into clinically meaningful resistance ?**
  - **how does it spread ?**
- **The Selectome**
- **The results… (selected examples)**

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 usually found first 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.

Clinical resistance: the tip of the iceberg ?

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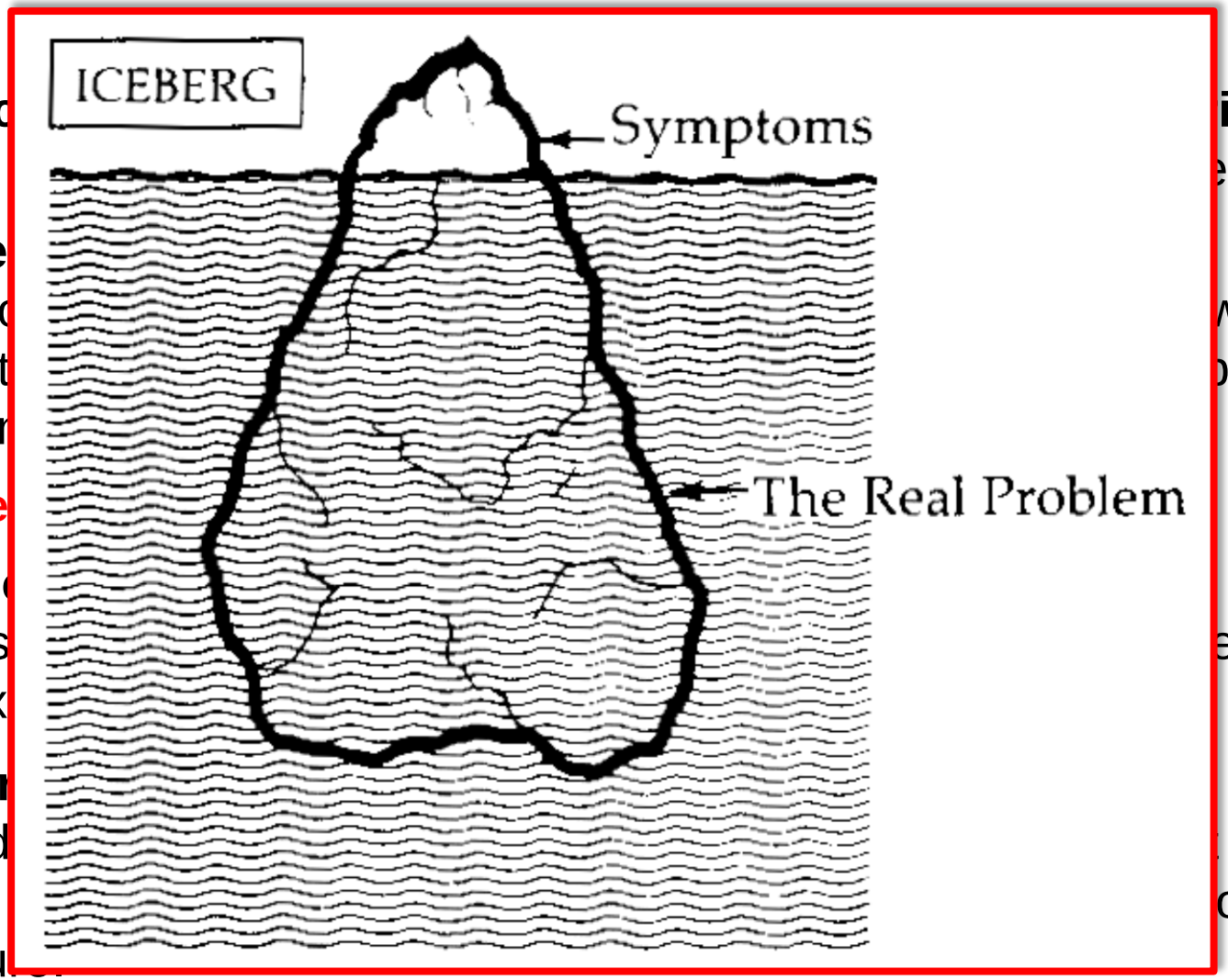

“Father resistance genes”: an original example with aminoglycosides

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.

“Father resistance genes”: an original example with aminoglycosides

Proc. Nat. Acad. Sci. USA
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Department of Biochemistry, College of A

Communicated by Henry Lardy, May 11

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mycin, and lividomy

TABLE 3. *Aminoglycoside acetylating, phosphorylating, and adenylylating enzymes in actinomycetes**

Actinomycete	Antibiotic produced	Gentamicin C_{1a} acetylation†	Neomycin phosphorylation	Streptomycin phosphorylation	Streptomycin or gentamicin C_{1a} adenylylation
S. kanamyceticus	Kanamycins	+	−	−	−
M. purpurea	Gentamicins	−	−	−	−
S. coelicolor	None	+	−	+	−
S. spectabilis	Spectinomycin	+	+	+	−
S. fradiae	Neomycins	−	+	−	−

* (+) means activity was detected with the phosphocellulose paper binding assay. (−) = no enzymatic activity detected.

† All extracts that acetylated gentamicin C_{1a} also acetylated neomycin B and paromomycin, the other antibiotics tested.

Nature contains pre-resistance genes

The Genomic Enzymology of Antibiotic Resistance

Mariya Morar and Gerard D. Wright

M.G. DeGroote Institute for Infectious Disease Research and the Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario, L8N 3Z5, Canada; email: wrightge@mcmaster.ca

Morar & Wright Annu Rev Genet. 2010;44:25-51 - PMID: 20822442.

Nature contains pre-

The Genomic Enzymology of Antibiotic Resistance

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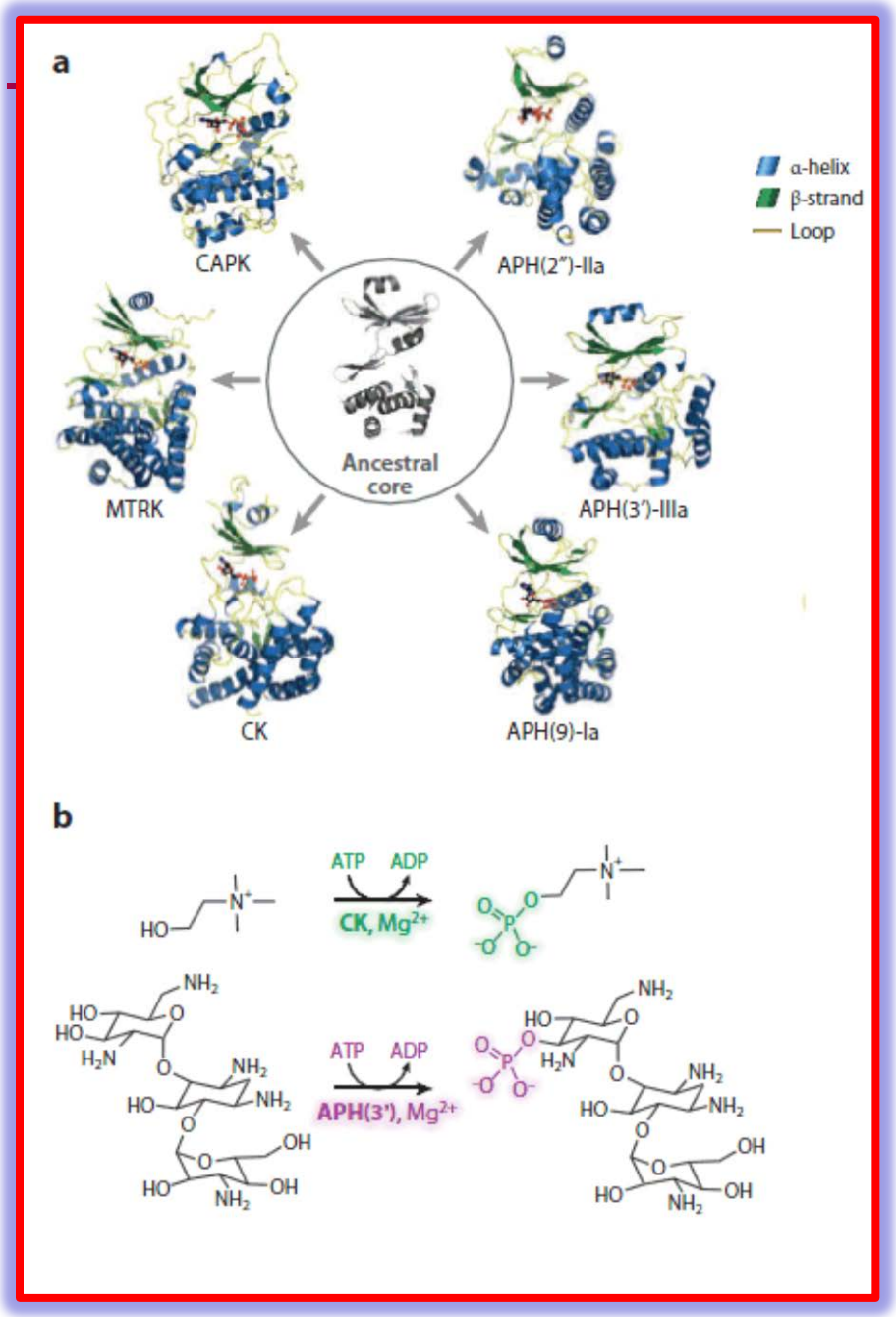


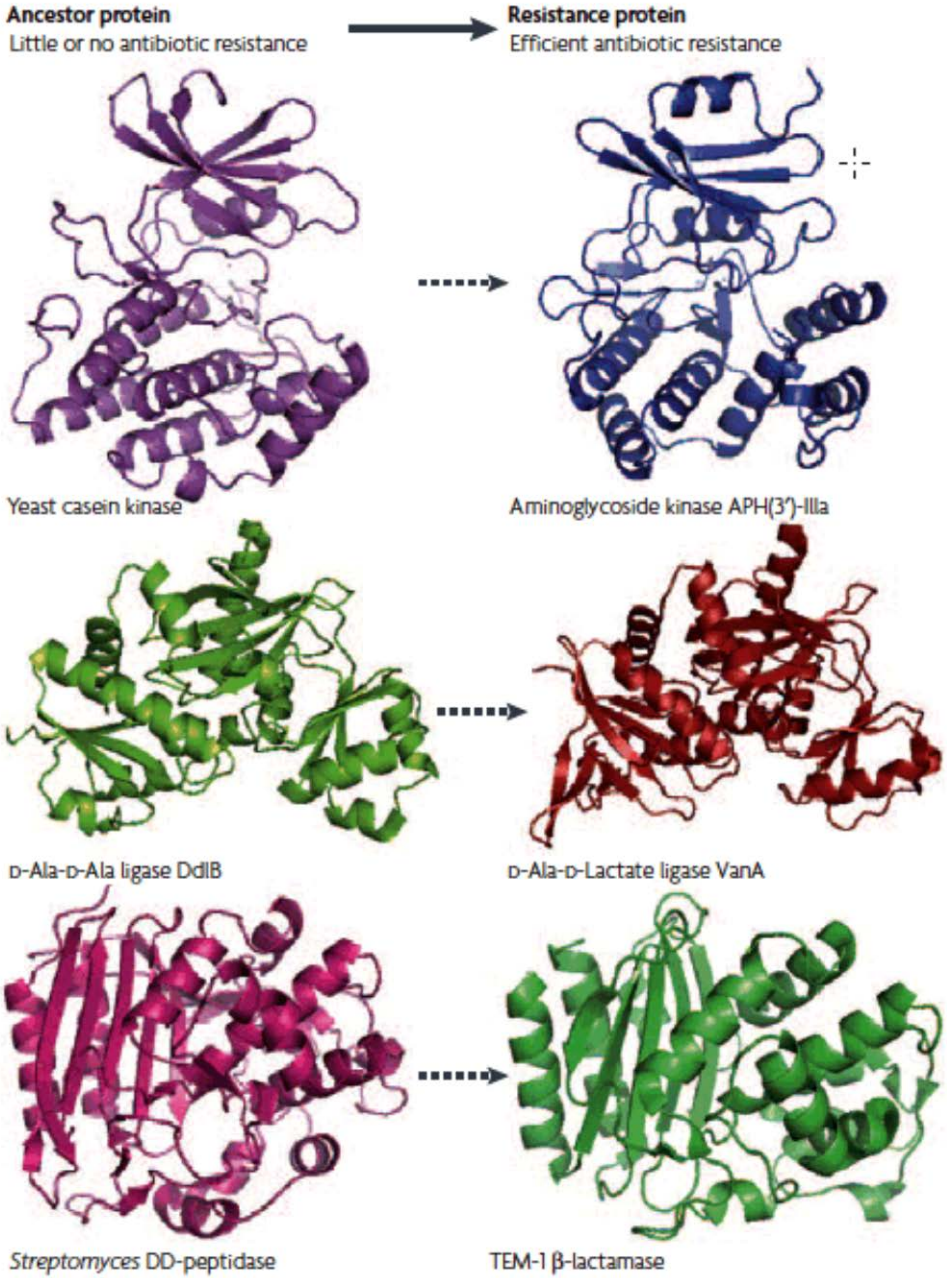
Antibiotic phosphotransferases:

a) Conservation in structure between aminoglycoside phosphotransferases APH(2”-IIa (pdb id:3HAV), APH(3’-IIIa (pdb id: 2B0Q), APH(9)-Ia (pdb id: 3I0O), choline kinase (CK pdb id: 2CKP), 5-methylthioribose kinase (MTRK; pdb id: 2OLC), and cAMP-dependent protein kinase (CAPK; pdb id: 1ATP)

b) Reaction catalyzed by CK versus APH(3”-IIIa, with similarities in the transformation highlighted in green for the housekeeping protein and in purple for the resistance protein.

Morar & Wright Annu Rev Genet. 2010;44:25-51 - PMID: 20822442.

More about "adaptation"



Evolution of antibiotic resistance proteins.

Protein structure and mechanism studies reveal that antibiotic resistance proteins are related to proteins with little or no antibiotic affinity.

The dotted arrows indicate that proteins might either be the immediate precursors of resistance proteins, or that they might share common ancestry with resistance proteins.

Wright GD. Nat Rev Microbiol. 2007;5:175-186 - PMID 17277795.

The source of clinical "emerging resistance" may be the environmental bacteria

Table 2 | Examples of resistance mechanisms in clinical isolates that evolved from natural functions in environmental bacteria.

Antimicrobial group	Mechanisms	Related natural protein	Natural reservoirs
Aminoglycosides	AcetylationPhosphorylation	Histone-acetylasesProtein kinases	*Streptomyces*
Tetracyclines	Efflux (mar)	Major facilitator superfamily EF-Tu, EF-G	*Streptomyces*
Chloramphenicol	AcetylationEfflux (mar)	AcetylasesMajor facilitatorsuperfamily EF-Tu, EF-G	*Streptomyces*
Macrolides	Target mutation	50S ribosomal subunit	*Streptomyces*
β-lactams (methicillin)	PBP2a	Homologous PBP2a	*Staphylococcus sciuri*
β-lactams (carbapenems)	OXA-48 inactivating enzyme	Proteins participating in peptidoglycan synthesis	*Shewanella xiamenensis*
	OXA-23 inactivating enzyme	Proteins participating in peptidoglycan synthesis	*Acinetobacter radioresistens*
Fluoroquinolones	Topoisomerase protection	Qnr-like protein	*Shewanella algae*

Galán et al. Front Microbiol. 2013;4:9 - PMID: 23404545

The soil may actually be the source of many resistance genes of clinical interest

The Shared Antibiotic Resistome of Soil Bacteria and Human Pathogens

Kevin J. Forsberg,[1*] Alejandro Reyes,[1*] Bin Wang,[1,2] Elizabeth M. Selleck,[3] Morten O. A. Sommer,[4,5]† Gautam Dantas[1,2]†

Soil microbiota represent one of the ancient evolutionary origins of antibiotic resistance and have been proposed as a reservoir of resistance genes available for exchange with clinical pathogens. Using a high-throughput functional metagenomic approach in conjunction with a pipeline for the de novo assembly of short-read sequence data from functional selections (termed PARFuMS), we provide evidence for recent exchange of antibiotic resistance genes between environmental bacteria and clinical pathogens. We describe multidrug-resistant soil bacteria containing resistance cassettes against five classes of antibiotics (β-lactams, aminoglycosides, amphenicols, sulfonamides, and tetracyclines) that have perfect nucleotide identity to genes from diverse human pathogens. This identity encompasses noncoding regions as well as multiple mobilization sequences, offering not only evidence of lateral exchange but also a mechanism by which antibiotic resistance disseminates.

Forsberg et al. Science 2012;337:1107-1111 - PMID 22936781

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The Shared Antibiotic Resist
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Table 1. Nonredundant antibiotic resistance genes with 100% identity to known human pathogens.

Gene name	GenBank ID	Number of selections*	Antibiotic class	Annotation [mechanism]	Pathogens hit (GI number)
AB95_PI_68.1	JX009363	4	β-lactam	blaP1 [enzymatic degradation]	*A. baumannii* (94960156), *K. pneumoniae* (114147191), *P. aeruginosa* (117321883), *S. typhimurium* (12719011), *P. mirabilis* (157674381)†
AB95_CH_13.1	JX009364	1	Amphenicol	Chloramphenicol efflux [efflux]	*A. baumannii* (169147133), *P. aeruginosa* (260677483)
AB95_TE_2.2	JX009366	3	Tetracycline	tetA(G) [efflux]	*A. baumannii* (169147133), *S. typhimurium* (12719011)
AB95_TE_1.1	JX009365	3	Tetracycline	tetA [efflux]	A. baumannii (169147133), *E. coli* (312949035), *K. pneumoniae* (290792160), *S. typhimurium* (37962716)†
AB95_GE_3.3	JX009367 JX009373	2	Aminoglycoside	aadB [covalent modification]	*E. cloacae* (71361871), *K. pneumoniae* (206731403), *P. aeruginosa* (37955767), *S. typhimurium* (17383994)†
AB95_GE_3.1	JX009368 JX009374	2	Sulfonamide	sul1 [target modification]	*C. diptheriae* (323714042) *E. cloacae* (71361871), *K. pneumoniae* (206731403), *P. aeruginosa* (37955767), *S. typhimurium* (17383994), *Yersinia pestis* (165913934)†
AB95_CH_21.1	JX009369	1	Aminoglycoside	aacA4 [covalent modification]	*A. baumannii* (164449567), *K. pneumoniae* (238865601), *P. aeruginosa* (219872982), *S. typhi* (34014739)†

*Number of selections in which the entirety of a given gene was captured. †More pathogens exist for which 100% nucleotide identity was observed than listed

Forsberg et al. Science 2012;337:1107-1111 - PMID 22936781

Genes for resistance are easily transmitted

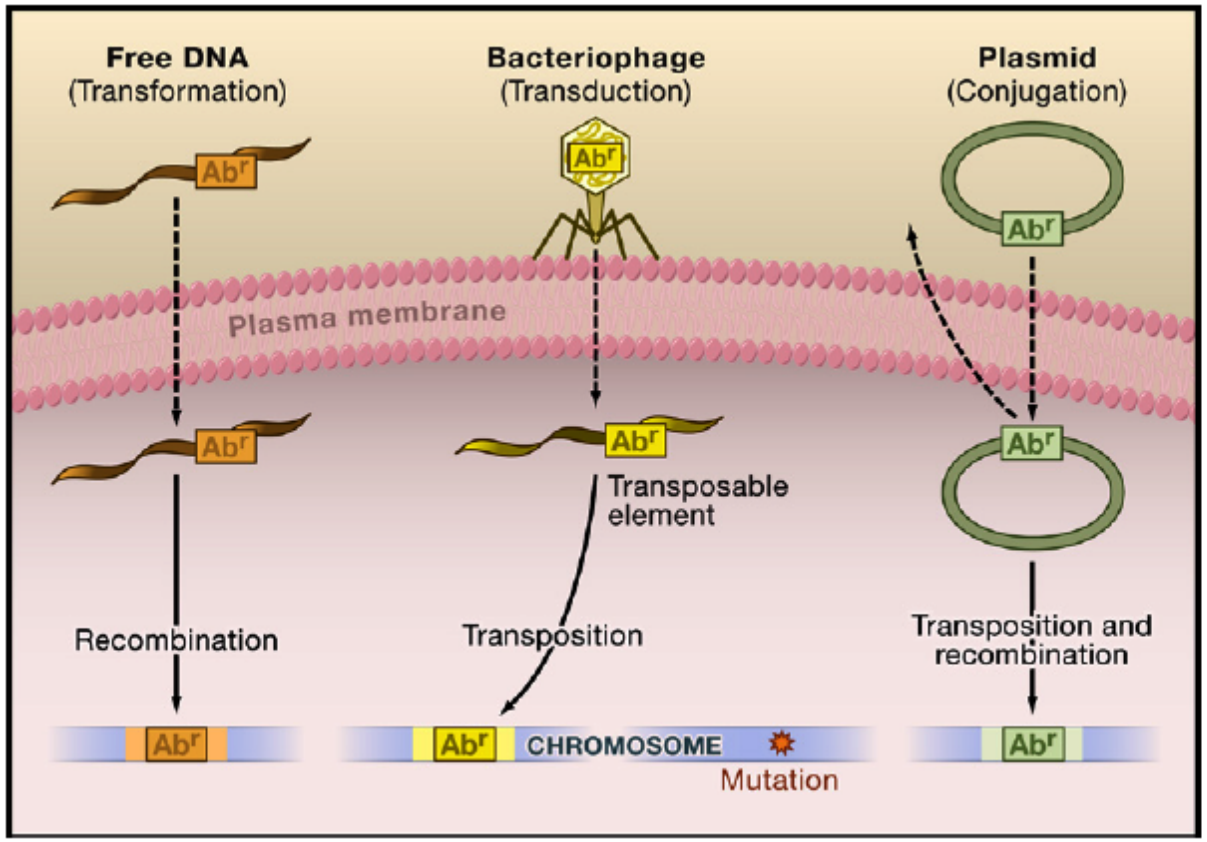

Figure 1. Acquisition of Antibiotic Resistance Bacteria can become antibiotic resistant (Ab^r) by mutation of the target gene in the chromosome. They can acquire foreign genetic material by incorporating free DNA segments into their chromosome (transformation). Genes are also transferred following infection by bacteriophage (transduction) and through plasmids and conjugative transposons during conjugation. The general term transposable element has been used to designate (1) an insertion sequence, (2) composite (compound), complex, and conjugative transposon, (3) transposing bacteriophage, or (4) integron.

Alekshun & Levy: Cell 2007;128:1037-50 - PMID 17382878.

Multiple mobile elements favour transmission

Table 2. Mobile Genetic Elements

Genetic Element	General Characteristics	Resistance Determinant(s) Specified/Examples[a]
Plasmid	Variable size (1- >100 kb), conjugative, and mobilizable	R factor: multiple resistances
Insertion sequence	Small (<2.5 kb), contains terminal inverted repeats, and specifies a transposase	IS1, IS3, IS4, etc.
Composite (compound) transposon	Flanked by insertion sequences and/or inverted repeats	Tn5: Kan, Bleo, and Str
Complex transposon	Large (>5 kb), flanked by short terminal inverted repeats, and specifies a transposase and recombinase	Tn1 and Tn3: β-lactamase Tn7: Tmp, Str, Spc Tn1546: glycopeptides
Conjugative transposon	Promotes self-transfer	Tn916: Tet and Mino Tn1545: Tet, Mino, Ery, and Kan
Transposable bacteriophage	A bacterial virus that can insert into the chromosome	Mu
Other transposable elements	Other than composite, complex, and conjugative transposons	Tn4: Amp, Str, Sul, and Hg Tn1691: Gen, Str, Sul, Cm, and Hg
Integron	Facilitates acquisition and dissemination of gene cassettes; specifies an integrase, attachments sites, and transcriptional elements to drive expression of multiple resistance genes	Class 1: Multiple single determinants and MDR efflux pump (Qac)[b] Class 2: Tmp, Strp, Str, and Spc (Tn7) Class 3: carbapenems Class 4: *Vibrio* spp. super-integron

See Figure 1.

[a]Abbreviations: Amp, ampicillin; Bleo, bleomycin; Cm, chloramphenicol; Ery, erythromycin; Fus, fusidic acid; Gen, gentamicin; Hg, mercury; Kan, kanamycin; Mino, minocycline; Spc, spectinomycin; Str, streptomycin; Strp, streptothricin; Sul, sulfonamide; Tet, tetracycline; Tmp, trimethoprim; Van, vancomycin.

[b]See Figure 2.

Alekshun & Levy: Cell 2007;128:1037-50 - PMID 17382878.

Mobile elements may contain multiple genes acting together: the vancomycin story

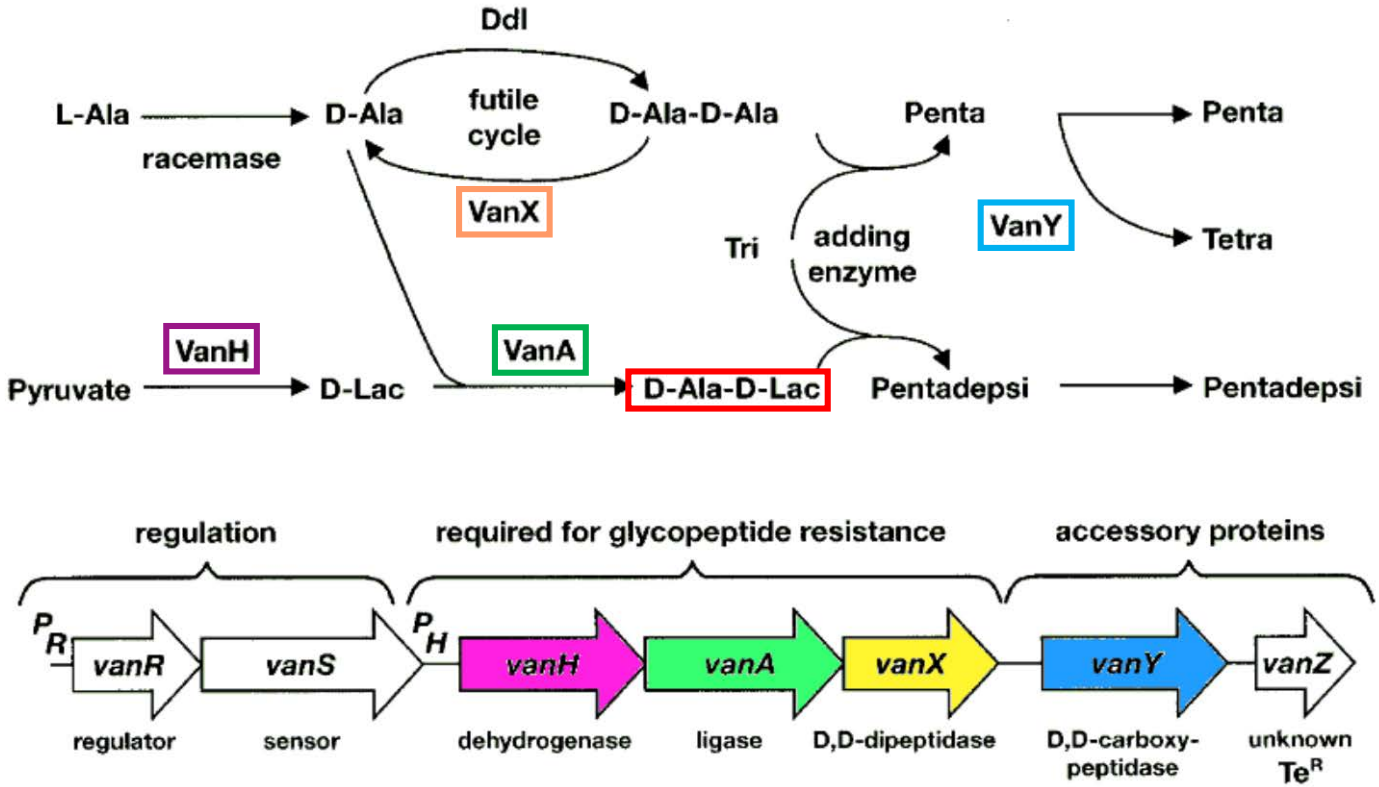

Figure 2. VanA-type glycopeptide resistance. *Top,* Synthesis of peptidoglycan precursors in a VanA-type resistant strain. Ddl, D-Ala:D-Ala ligase; penta, L-Ala-γ-D-Glu-L-Lys-D-Ala-D-Ala; Pentadepsi, L-Ala-γ-D-Glu-L-Lys-D-Ala-D-Lac; Tetra, L-Ala-γ-D-Glu-L-Lys-D-Ala; Tri, L-Ala-γ-D-Glu-L-Lys. *Bottom,* Organization of the *vanA* operon. Open arrows represent coding sequences and indicate the direction of transcription. The regulatory and resistance genes are cotranscribed from promoters P_R and P_H, respectively.

Courvalin Clin Infect Dis. 2006;42 Suppl 1:S25-34 - PMID 16323116.

Vancomycin-resistance genes (or homologues) are actually widespread in the environment

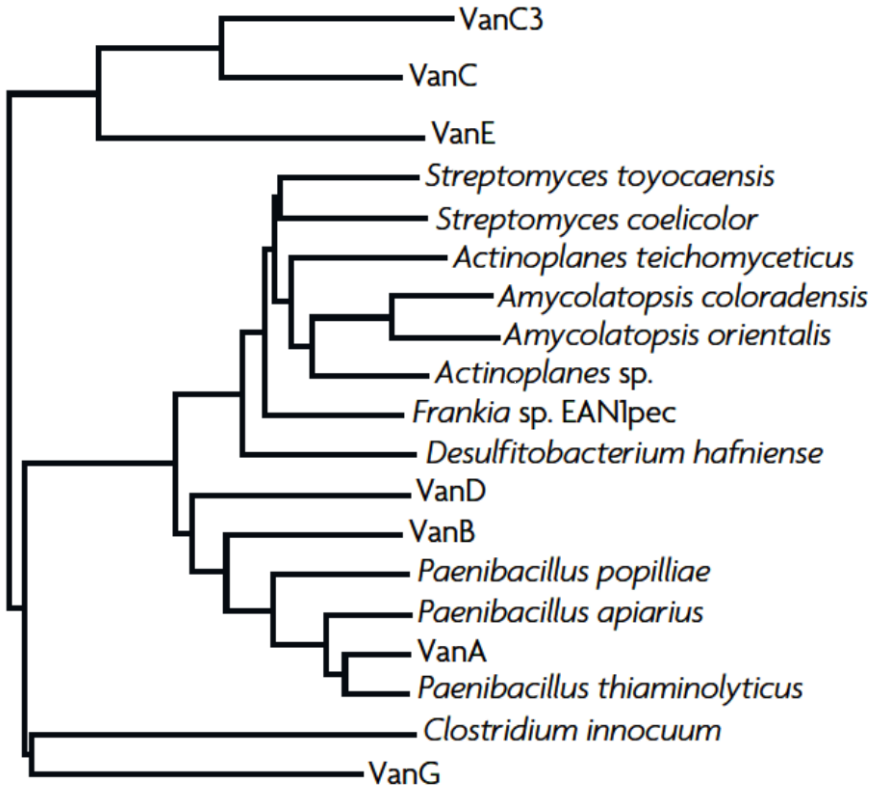

Figure 5 | **Vancomycin-resistance genes are widespread in the environment.** Homologues of VanA were identified by a BLAST search, the alignment was constructed with ClustalW[98] and the tree was drawn with NJplot[99]. Known D-Ala-D-Ala ligases were omitted from the analysis. Note that the trees are not a rigorous phylogenetic analysis, but rather are an attempt to convey sequence relationship among these enzymes.

Wright GD. Nat Rev Microbiol. 2007;5:175-186 - PMID 17277795.

Mobile elements do contain multiple genes conferring co-resistance: a sour recent story

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

International Journal of Antimicrobial Agents 46 (2015) 111–116

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

Short communication

Molecular epidemiology of carbapenemase-producing *Klebsiella pneumoniae* in a hospital in Madrid: Successful establishment of an OXA-48 ST11 clone

Patricia Brañas[a,1], Jennifer Villa[a,1], Esther Viedma[a,b], Jesús Mingorance[b,c], M. Angeles Orellana[a,b], Fernando Chaves[a,b,*]

[a] Servicio de Microbiología Clínica, Hospital Universitario 12 de Octubre, Madrid, Spain
[b] Spanish Network for the Research in Infectious Diseases (REIPI RD12/0015), Instituto de Salud Carlos III, Madrid, Spain
[c] Hospital Universitario la Paz, IdiPAZ, Madrid, Spain

Branas et al. Int J Antimicrob Ag 2015;46:111-116 - PMID 25914088

Mobile elements do contain multiple genes conferring co-resistance: a sour recent story

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Short commu

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pneumoni
OXA-48 S

Patricia Brai
M. Angeles

[a] Servicio de Microbi
[b] Spanish Network fo
[c] Hospital Universita

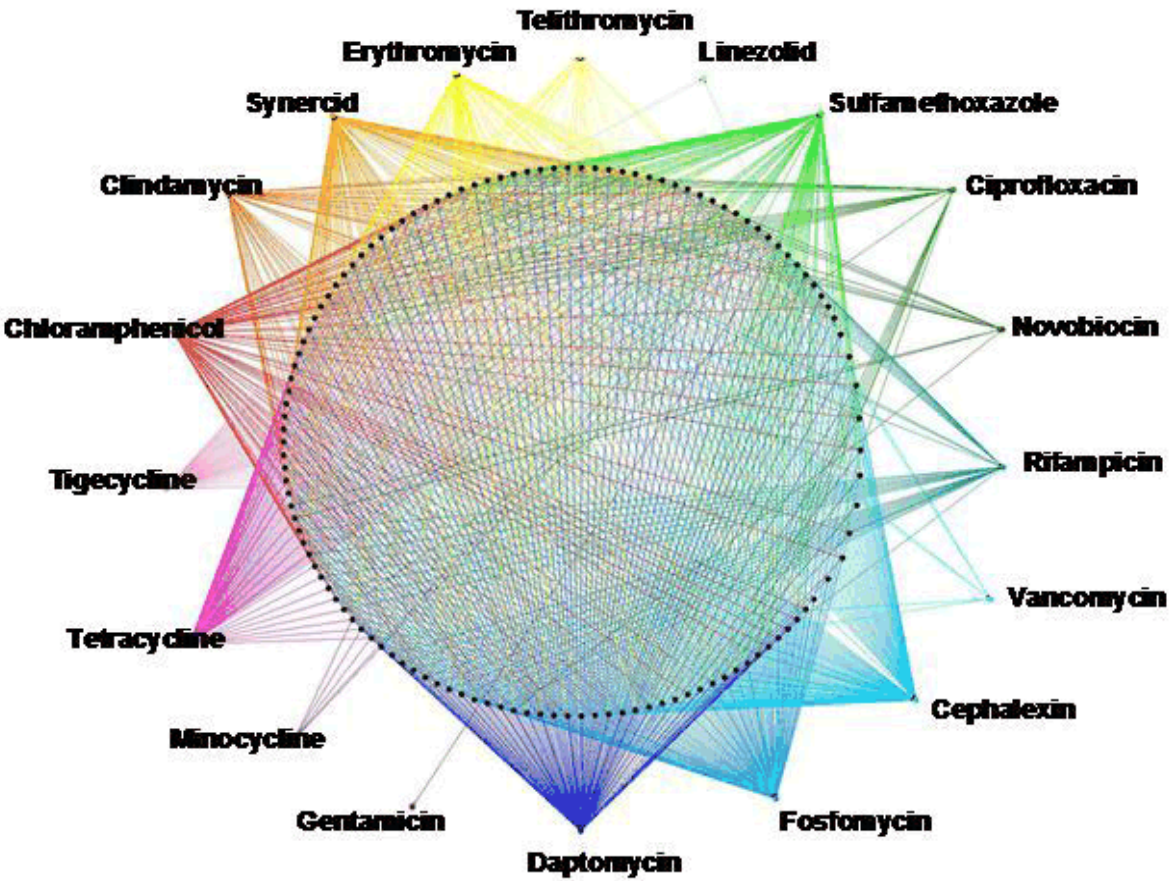
Supplementary Table S1

Antibiotic resistance genes identified in isolate KP_ST11_OXA-48

Antibiotic	**Gene(s)**
β-Lactams	bla_{SHV-11}, bla_{OXA-1}, $bla_{CTX-M-15}$, bla_{OXA-48}
Aminoglycosides	*aac(6')Ib*-cr, *aac(3)-IIa*, *aadA2*, *aph(3')-Ia*
Fluoroquinolones	*qnrB1*
Macrolides	*mph*-A
Phenicol	*catA1*, *catB2*
Sulphonamide/trimethoprim	*sul1*, *dfrA12*
Carbapenems	*ompK35* and *ompK36* [a]

Branas et al. Int J Antimicrob Ag 2015;46:111-116 - PMID 25914088
See also: Yang et al. Antimicrob Agents Chemother. 2014;58:6328-6330 – PMID 25114139

The connectome….
(cross-resistance)



http://wrightlab.mcmasteriidr.ca/
(last accessed: 2/3/2013 - no longer available)

The origin and spontaneous spreading of resistance

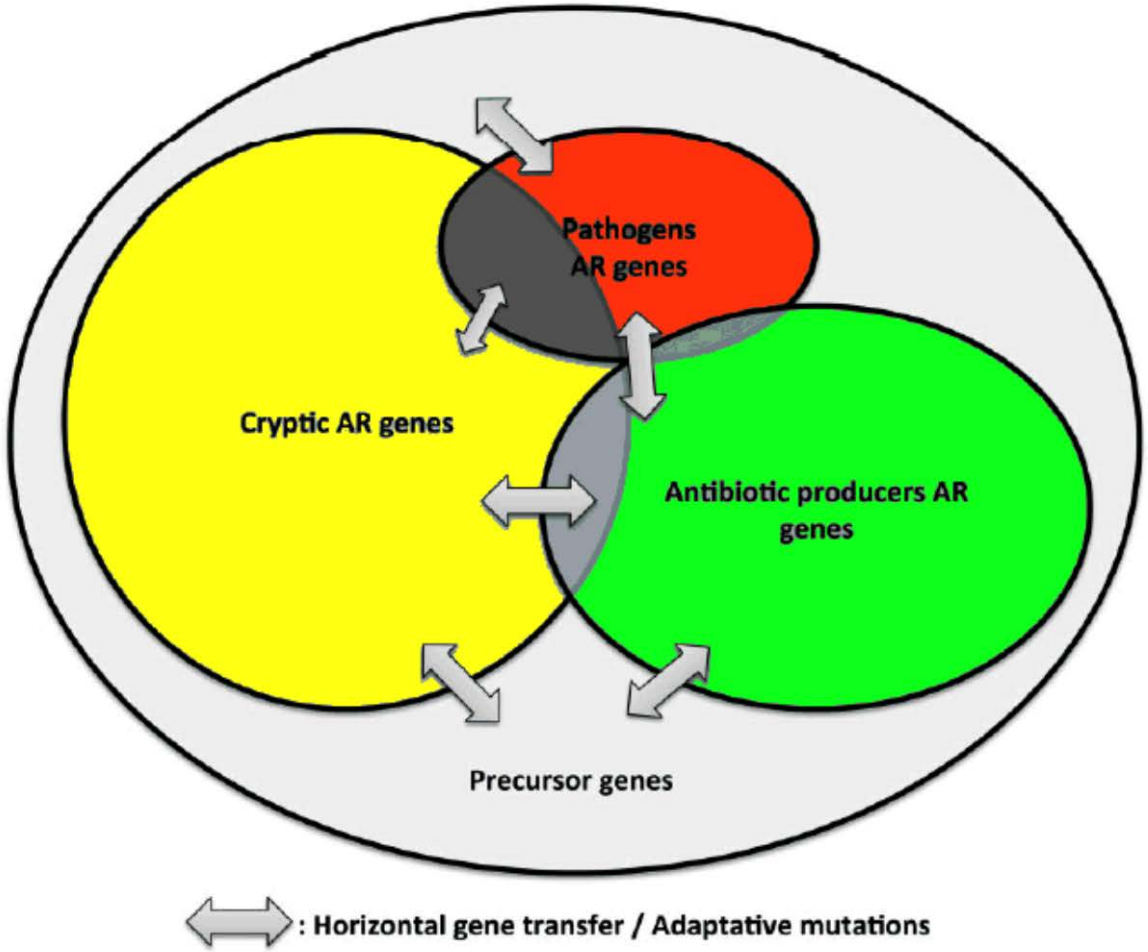


FIg. 2. The resistome: potential for antibiotic resistance. The resistome is all genes coding antibiotic resistance (AR)-related proteins or similar protein likely to evolve toward potent antibiotic resistance. This encompass problematic AR gene identified in pathogenic bacteria, AR gene from antibiotic producers, cryptic AR genes (i.e. a resistance gene with no obvious association to antibiotic resistance), which may be or not expressed but could confer resistance in another genetic context for example and AR precursor genes coding protein with low level of affinity or resistance to antibiotic molecules. A significant part of those different genes subset overlaps due to sequence similarities and are most likely sharing a common evolutionary history. Genes can of course switch from one state to another through either horizontal gene transfer (HGT) or point mutation or recombination. Adapted from Wright, 2007.

Nesme et al. Environ Microbiol. 2015;17:913-30 - PMID 25286745.
See also: Perry et al. Curr Opin Microbiol 2014;21:45-50 - PMID 25280222.

But nature (soil) can be re-injected …

mBio 5(2):e01017-13 - PMID 24757214

RESEARCH ARTICLE

Diverse Antibiotic Resistance Genes in Dairy Cow Manure

Fabienne Wichmann,[a] Nikolina Udikovic-Kolic,[a]* Sheila Andrew,[b] Jo Handelsman[a]

Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut, USA[a]; Department of Animal Sciences, University of Connecticut, Storrs, Connecticut, USA[b]

* Present address: Nikolina Udikovic-Kolic, Division for Marine and Environmental Research, Rudjer Boskovic Institute, Zagreb, Croatia.

Wichmann et al. mBio 5(2):e01017-13 - PMID 24757214 .

But nature (soil) can be re-injected …

mBio 5(2):e01017-13 - PMID 24757214

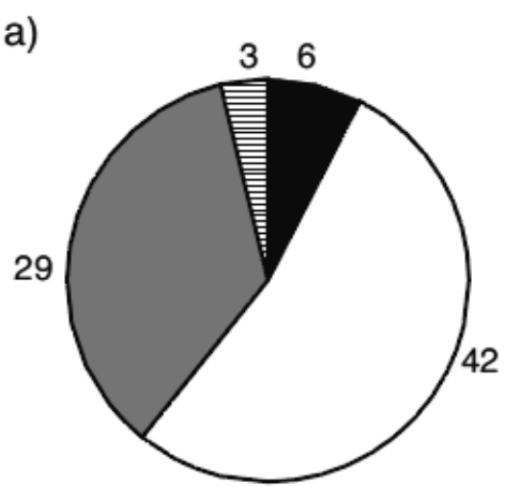
RESEARCH ARTIC

Diverse A

Fabienne Wichma

Department of Molecu
Storrs, Connecticut, US

* Present address: Nikolin



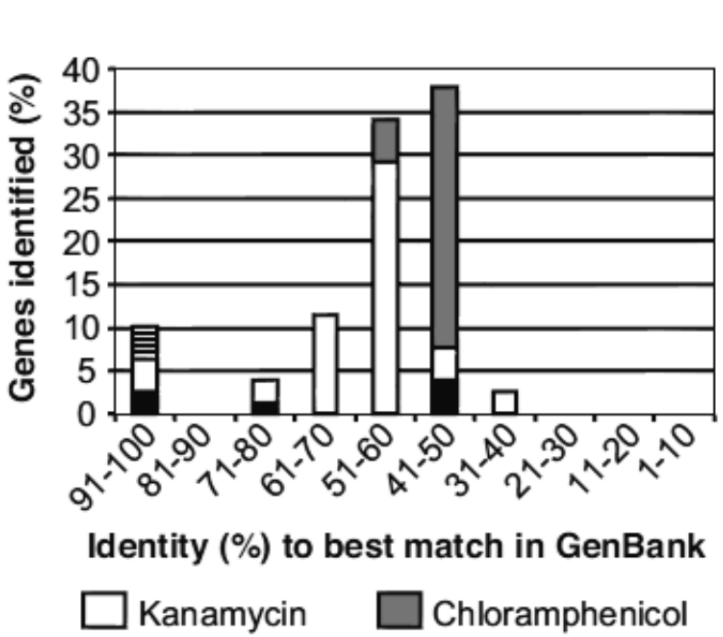


FIG 1 Antibiotic resistance genes from dairy cow manure.
(a) Distribution of 80 unique antibiotic resistance (AR) genes among four classes of antibiotics.
(b) Distribution of similarity of 80 unique AR genes from manure compared to homologues in GenBank.

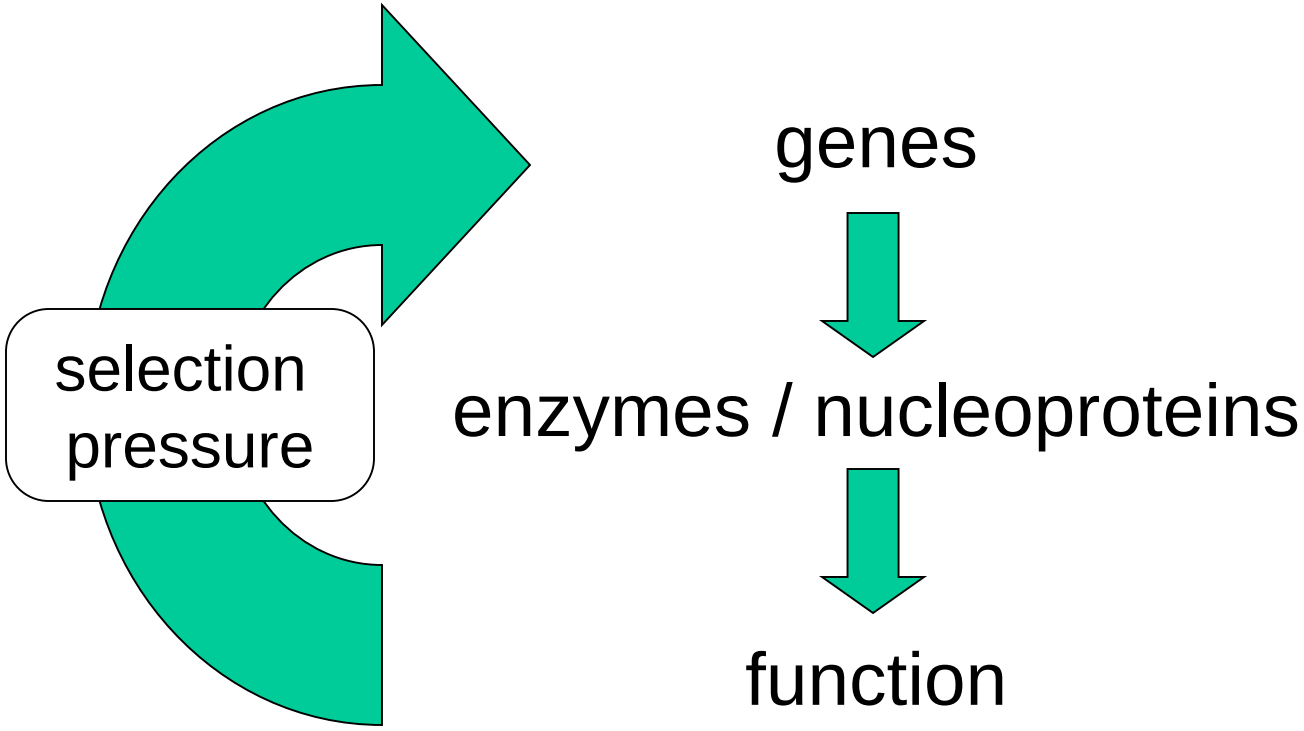
Wichmann et al. mBio 5(2):e01017-13 - PMID 24757214 .

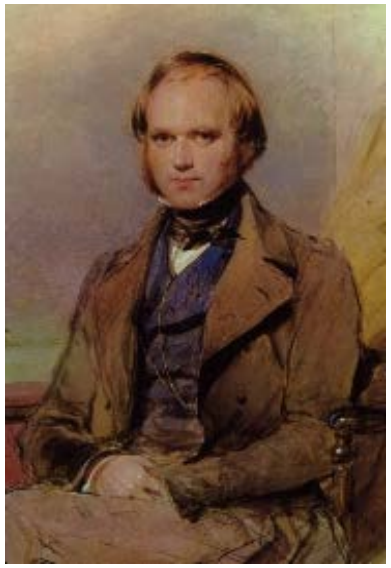
Why is it so ?

- The Resistome …
- **The Selectome**
  - **bacteria are in large numbers in infected foci …**
  - **Fleming was right: suboptimal treatments select for less susceptible organisms…**
  - **adaptation can be faster than you thought…**
- The results… (selected examples)

The selectome

A simple application of Darwin's principles ...



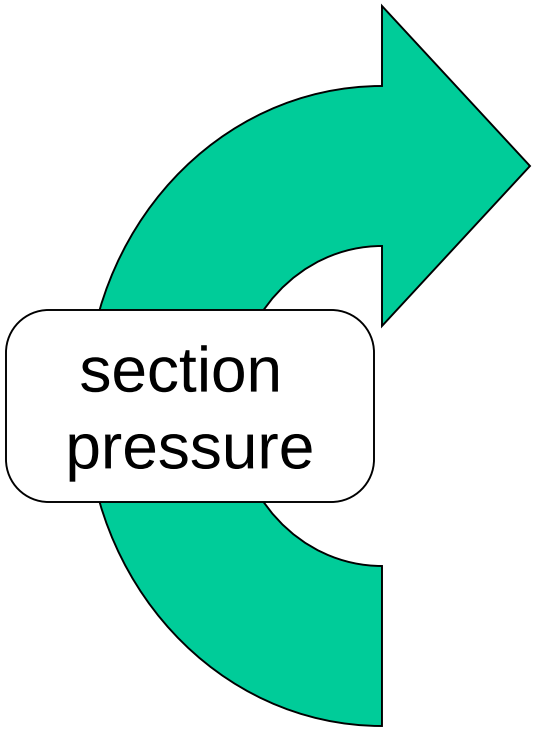


Detail of watercolor by
George Richmond, 1840.
Darwin Museum at Down House

How and why can you select so easily ?

A simple application of Darwin's principle…

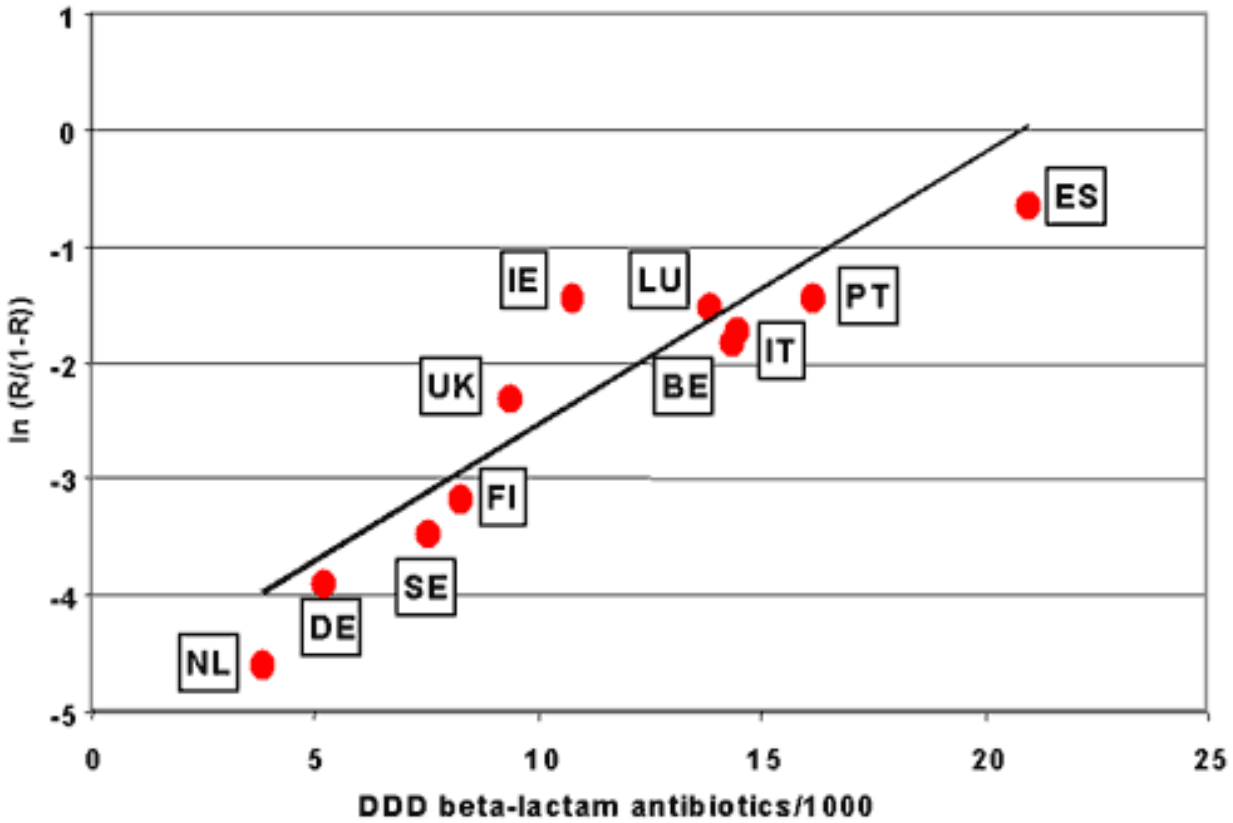
to a highly plastic material…



- 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: an example in the community in relation to antibiotic consumption



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

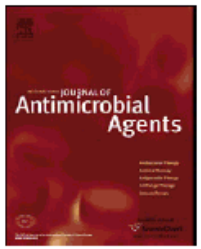
The hidden risk of therapy in the hospital

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*

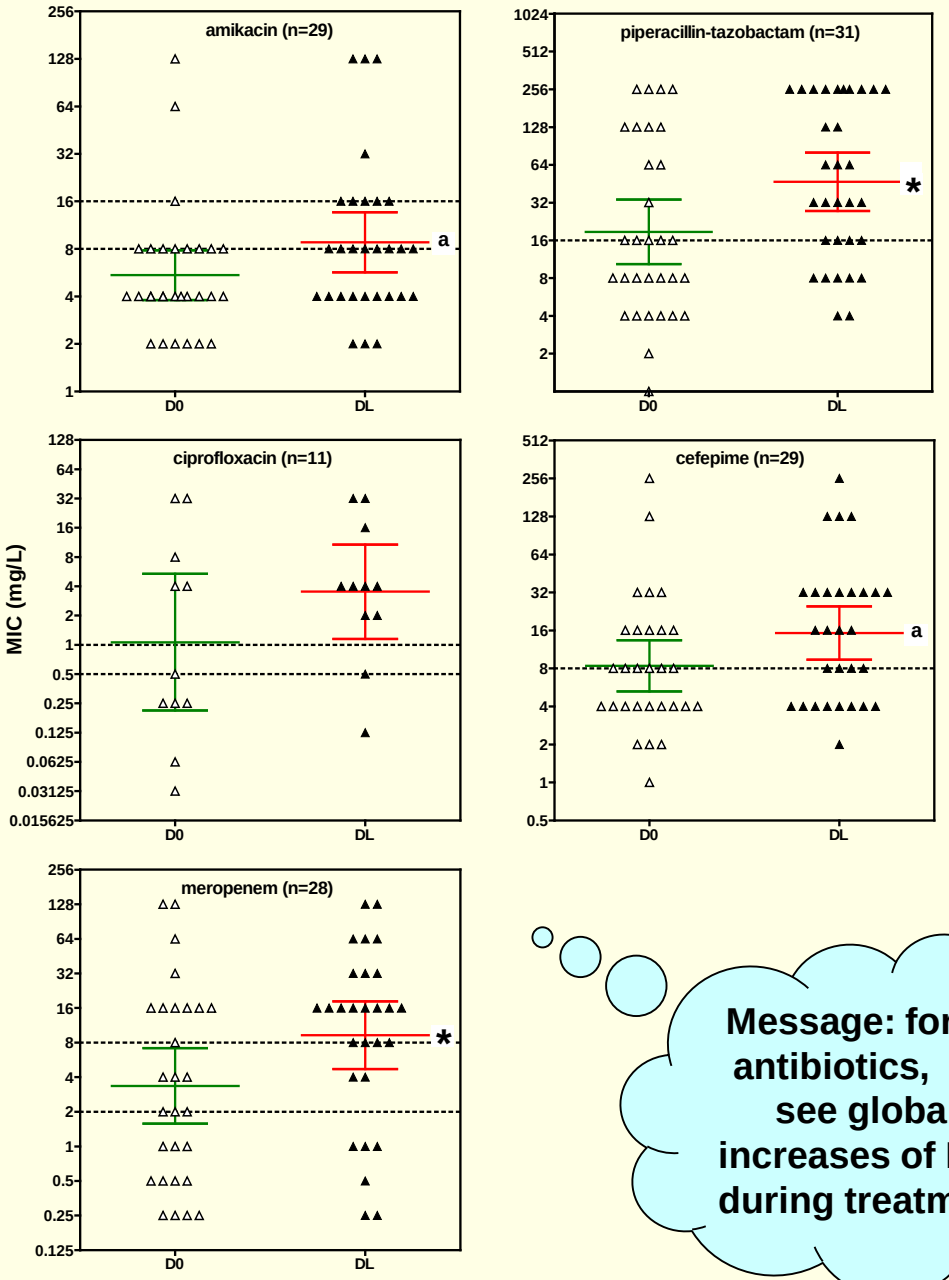
Do you remain effective while treating ?

- 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

The pressure of antibiotics is critical …

frontiers in MICROBIOLOGY

REVIEW ARTICLE
published: 08 February 2013
doi: 10.3389/fmicb.2013.00009

Antibiotics as selectors and accelerators of diversity in the mechanisms of resistance: from the resistome to genetic plasticity in the β-lactamases world

Juan-Carlos Galán[1,2,3], Fernando González-Candelas[4,5], Jean-Marc Rolain[6,7] and Rafael Cantón[1,3]*

Galán et al. Front Microbiol. 2013;4:9 - PMID: 23404545

And may exert it-self even at low concentrations

frontiers in
MICROBIOLO

Antibiotic
mechanis
plasticity

Juan-Carlos Galá

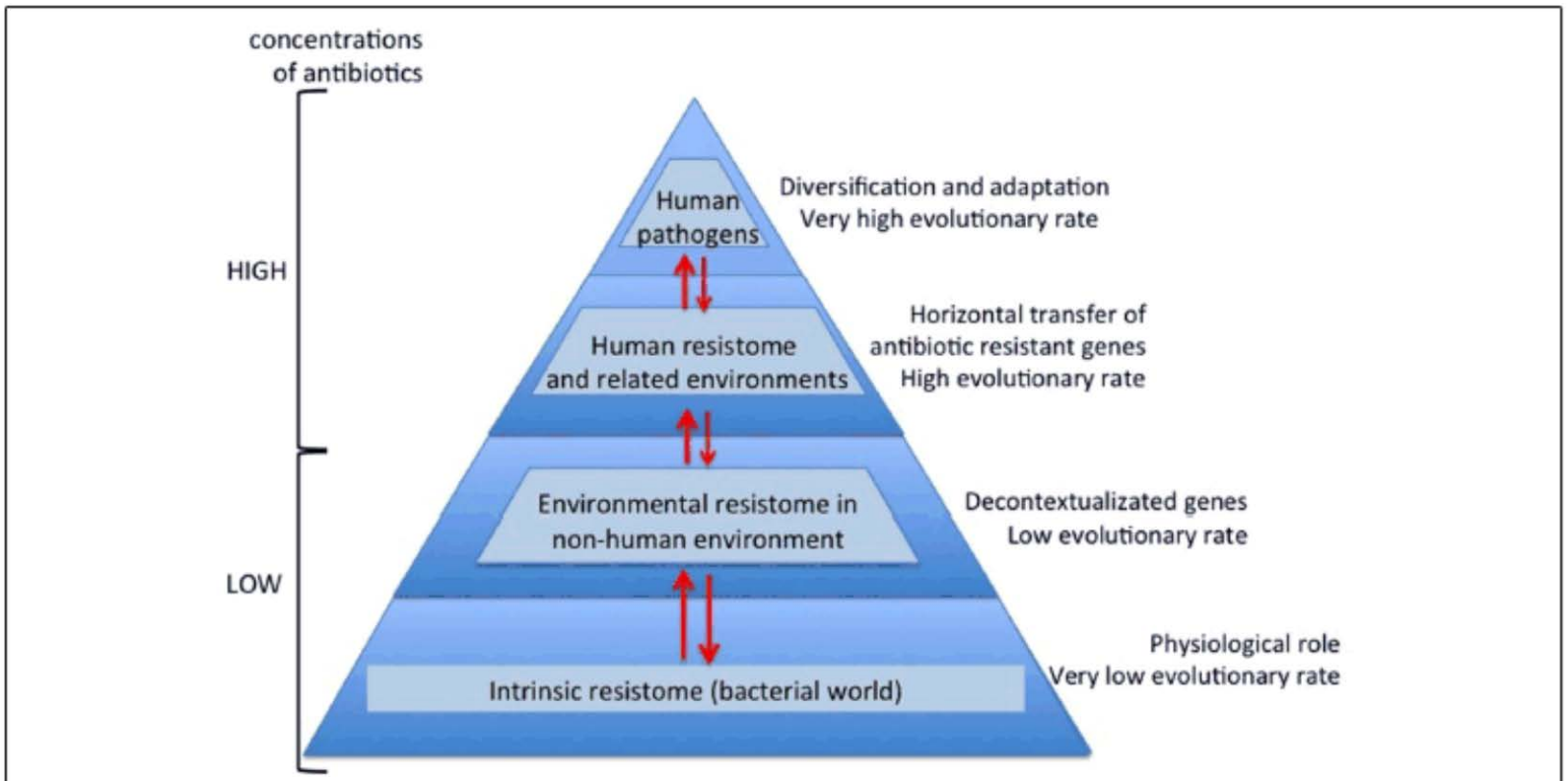
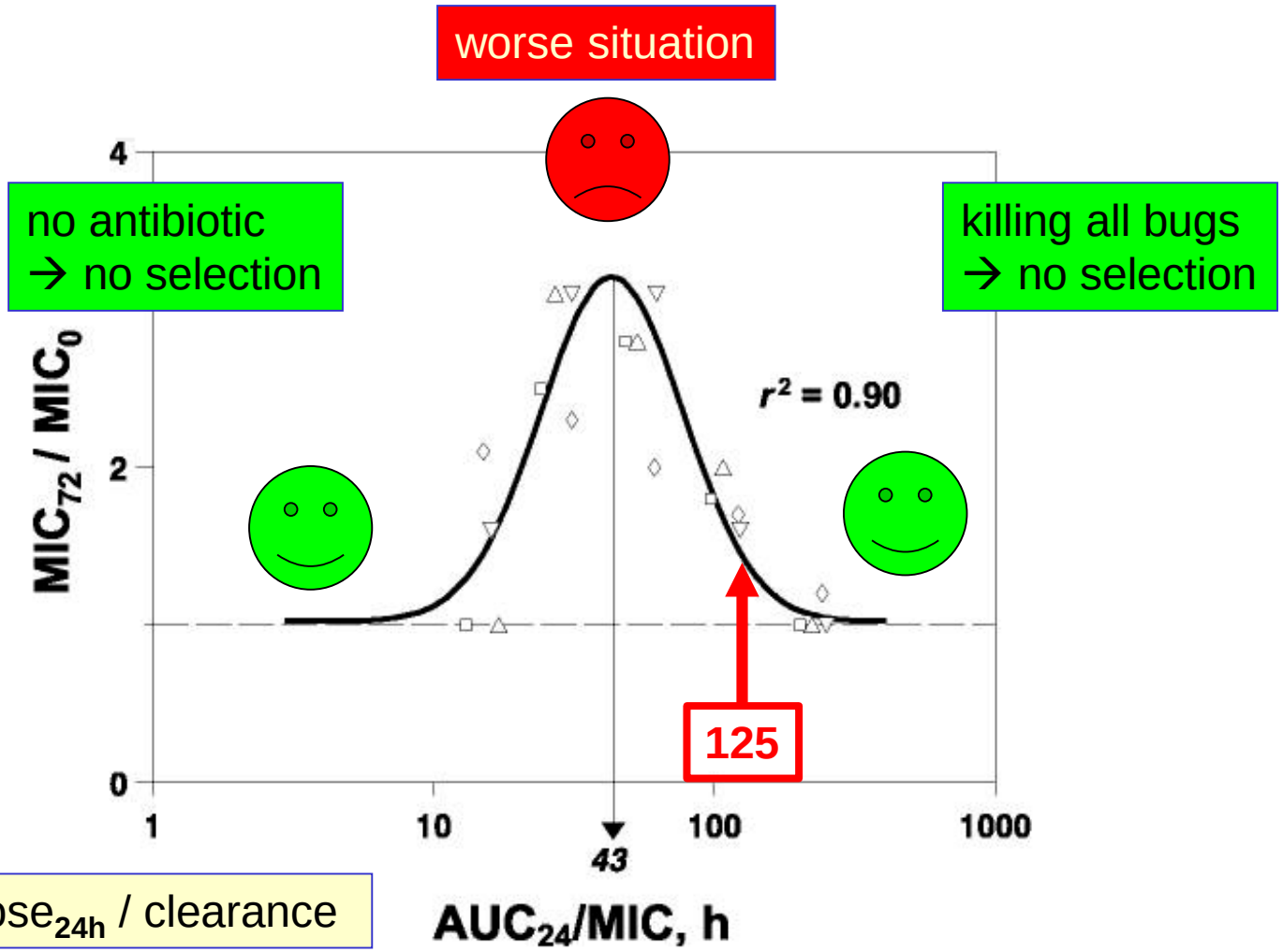


FIGURE 1 | Overview evolutionary processes of the antibiotic resistance. During millions of years antibiotics and antibiotic resistance genes have co-evolved slowly. In this long period the first transition was the acquisition of pre-resistance genes by different bacteria (exaptation). This genetic transference allowed the evolution toward true and more efficient antibiotic resistance genes. However, the great evolutionary transition was the discovery, mass production and consumption of antibiotics. Antibiotics accelerated dramatically the diversification of resistance genes and selection for reaching extraordinary efficient variants.

Galán et al. Front Microbiol. 2013;4:9 - PMID: 23404545

Selection by suboptimal treatments: an example with fluoroquinolones



AUC_{24h} = $dose_{24h}$ / clearance

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.

The trouble is that "clinical breakpoints" are /have been too high …

Drug	Typical daily dosage[a]	$AUC_{24\ h}$ (mg × h/L) total/free	Proposed PK/PD upper limit (mg/L)	
			Efficacy[b]	Prevention of resistance[c]
Norfloxacin	800 mg	14/11	0.1–0.4	0.1
Ciprofloxacin	1000 mg	24/18	0.2–0.8	0.2
Ofloxacin	400 mg	40/30	0.3–0.9	0.4
Levofloxacin	500 mg	40/28	0.3–0.9	0.3
Moxifloxacin	400 mg	35/21	0.2–0.7	0.2

EUCAST, European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org)
NCCLS, National Committee for Clinical Laboratory Standards (http://www.nccls.org).

a typical dosages (as per PI data)

b based on AUC_{24h}/MIC of 30 (Gram-positive) to 125 (Gram-negative)

c based on AUC_{24h}/MIC of 125 (and C_{max}/MIC > 10 mg/L)

Adapted from Michot *et al.* Clinical Microbiology and Infection (2005) 11:256-280 - Erratum published: 11:513, 2005

The trouble is that "clinical breakpoints" are /have been too high …

Drug	Typical daily dosage[a]	$AUC_{24\ h}$ (mg × h/L) total/free	Proposed PK/PD upper limit (mg/L)		Breakpoints (mg/L)	
			Efficacy[b]	Prevention of resistance[c]	EUCAST R	NCCLS R
Norfloxacin	800 mg	14/11	0.1–0.4	0.1	>1	16
Ciprofloxacin	1000 mg	24/18	0.2–0.8	0.2	>1	4
Ofloxacin	400 mg	40/30	0.3–0.9	0.4	>1	8
Levofloxacin	500 mg	40/28	0.3–0.9	0.3	>2	8
Moxifloxacin	400 mg	35/21	0.2–0.7	0.2	>1	4

EUCAST, European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org)
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a typical dosages (as per PI data)

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c based on AUC_{24h}/MIC of 125 (and C_{max}/MIC > 10 mg/L)

Adapted from Michot *et al.* Clinical Microbiology and Infection (2005) 11:256-280 - Erratum published: 11:513, 2005

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

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

strains	Initial		
	MIC (mg/L) [a]		
	TEM	FEP	MEM
2114/2 [c]	8	2	0.25
2502/4 [c]	8	2	0.125
3511/1 [c]	**32**	2	0.125
7102/10 [d]	**512**	**32**	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*. ISAAR, Seoul, Korea, 8 April 2011

A simple experiment …

Exposure of *E. aerogenes* to anti-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

sub-MIC concentrations selects for resistance !

Nguyen *et al*. ISAAR, Seoul, Korea, 8 April 2011

Why is it so ?

- The Resistome …
- The Selectome
- **The results… (selected examples)**
  - **β-lactamases**
  - **Target modifications (in very short and with an escape…)**
  - **Efflux**

Application #1: β-lactamases

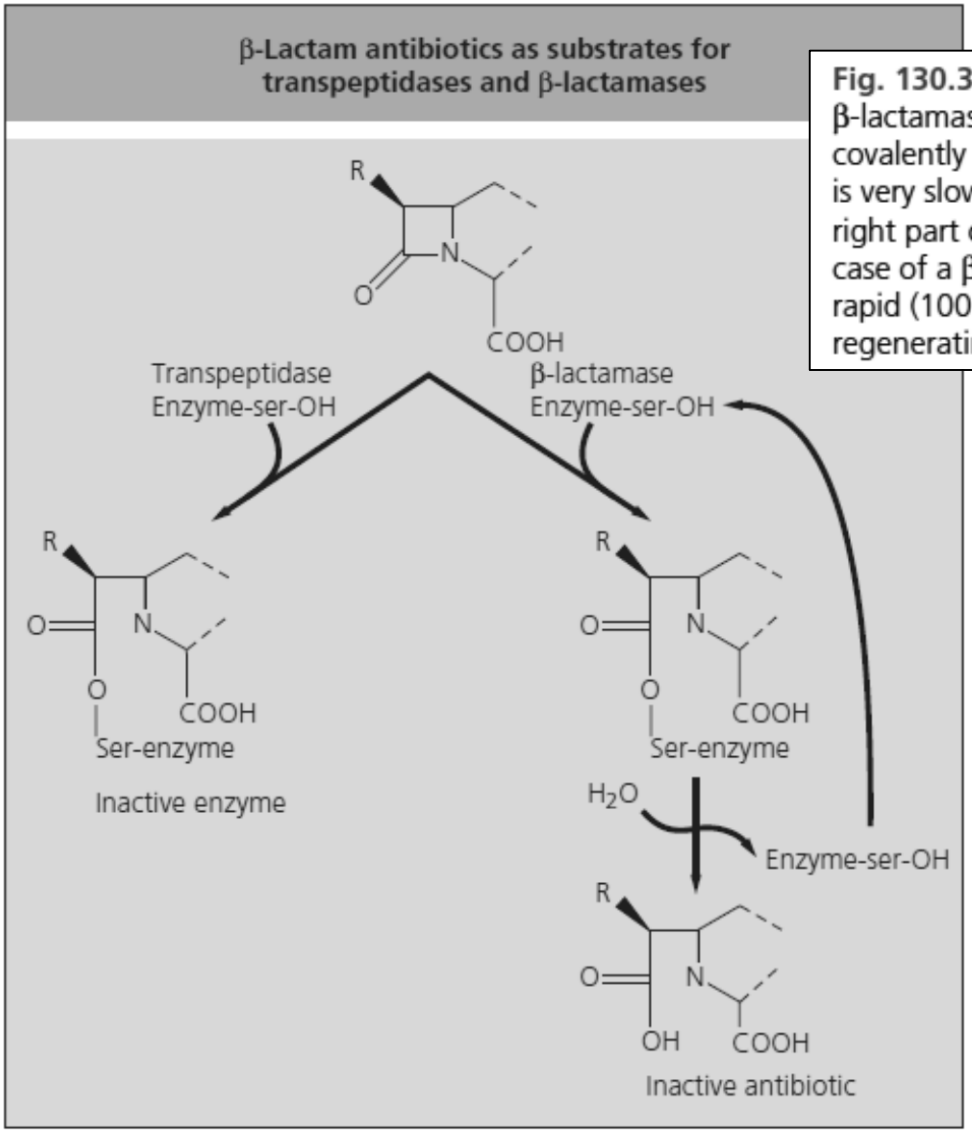


Fig. 130.3 β-Lactam antibiotics as substrates for transpeptidases and β-lactamases. The left part of the illustration shows how a β-lactam covalently binds to the transpeptidases. Hydrolysis of this acylated enzyme is very slow (one β-lactam per hour), making the enzyme inactive. The right part of the illustration shows that the same reaction occurs in the case of a β-lactamase. Hydrolysis of the acylated enzyme is, however, very rapid (1000 β-lactams per second), making the antibiotic inactive and regenerating the enzyme for a new cycle of hydrolysis.

Van Bambeke et al. Mechanisms of Action. In: Infectious Diseases (3d edition; J. Cohen, W. Powderly & S. Opal, eds), chapter 130, pp 1288-1307

Relationship with DD-peptidases (Penicillin Binding Proteins)

Annu. Rev. Microbiol. 1991. 45:37–67

SERINE β-LACTAMASES AND PENICILLIN-BINDING PROTEINS

Jean-Marie Ghuysen

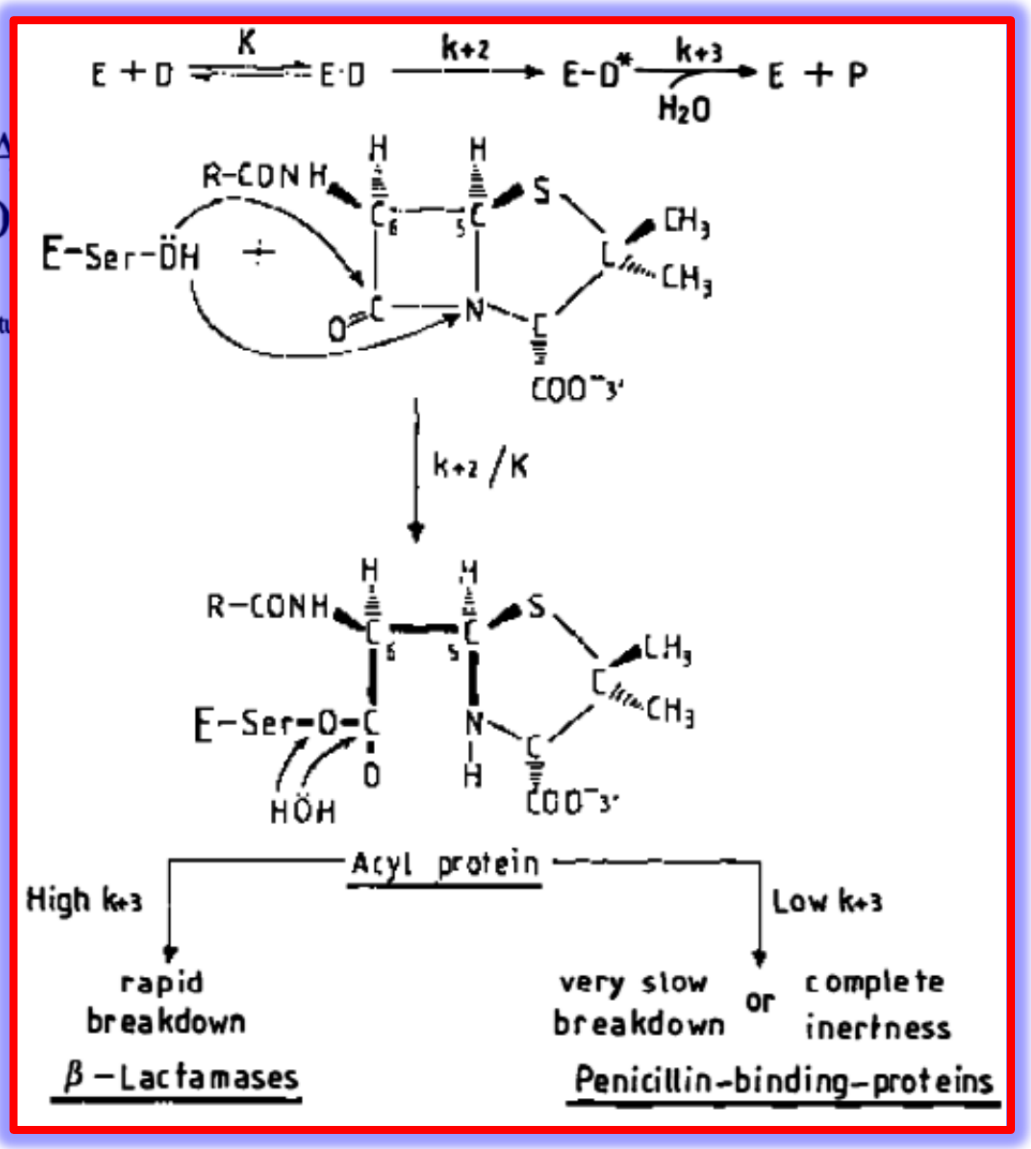
Centre d'Ingénierie des Protéines, Université de Liège, Institut de Chimie, B6, B-4000 Sart Tilman (Liège 1), Belgium

Relationship with DD-peptidases (Penicillin Binding Proteins)

Annu. Rev. Microbiol. 1991. 45:37–67

SERINE β-LACTAMASES A
PENICILLIN-BINDING PRO

Jean-Marie Ghuysen
Centre d'Ingénierie des Protéines, Université de Liège, Institu
Sart Tilman (Liège 1), Belgium



Relationship with DD-peptidases (Penicillin Binding Proteins)

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SERINE β-LACTAMASES AND
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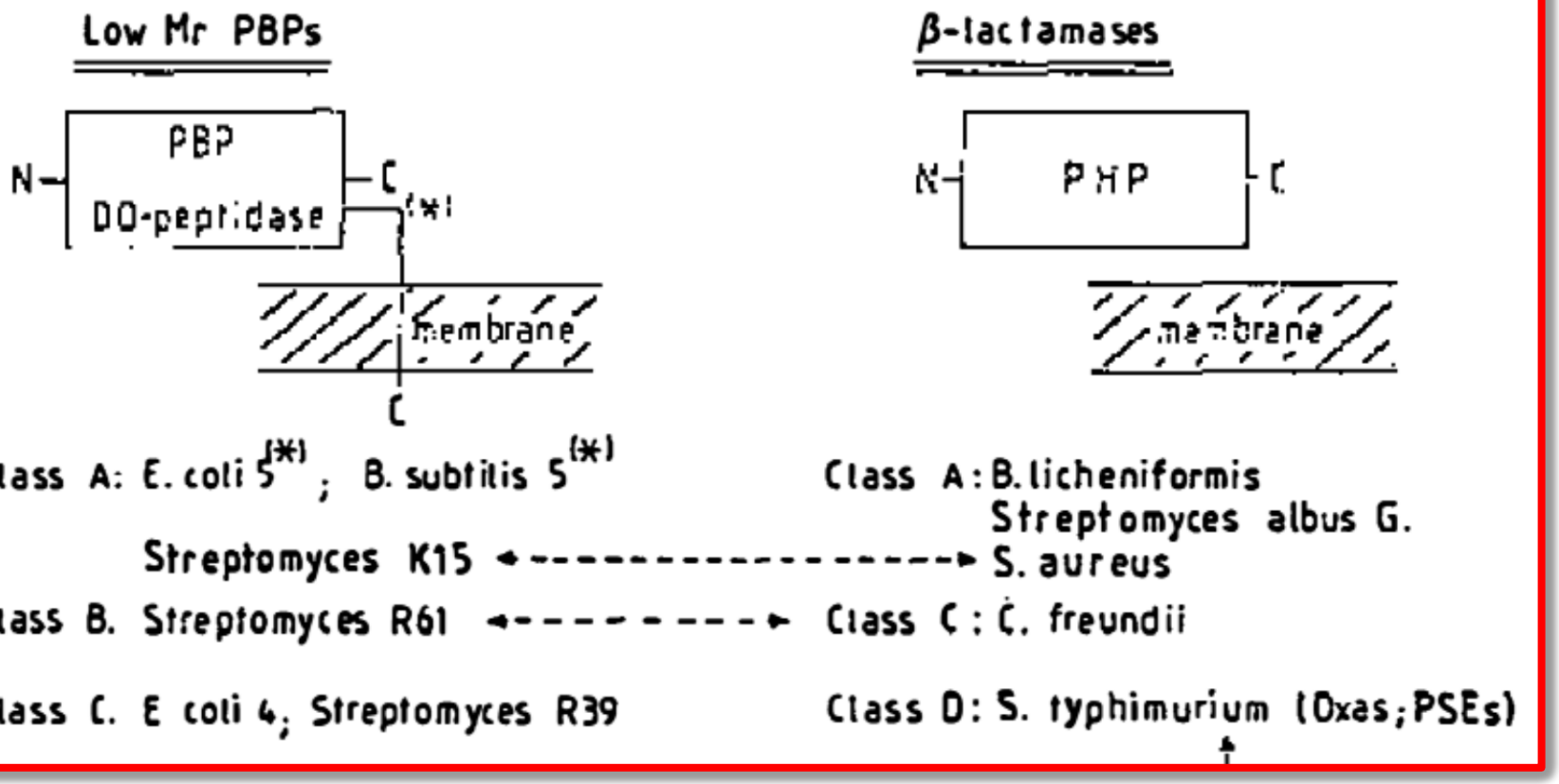
Jean
Centre
Sart T



DD-peptidases and serine β-lactamases: biochemical similarities and differences

Table 1. The three equivalent functional elements of active-site serine β-lactamases and penicillin-sensitive DD-peptidases.

Class	Element 1	Element 2	Element 3
	70	130	234
Class A	S*–X–X–K	S–D–N S–D–S	K–T–G K–S–G R–S–G R–T–G
	64	150	314
Class C	S*–X–X–K	Y–A–N	K–T–G
	70	144	214
Class D	S*–X–X–K	Y–G–N	K–T–G
	62	159	298
S. R61 DD-peptidase	S*–X–X–K	Y–S–N	H–T–G
Other known PBPs	S*–X–X–K	S–X–N S–X–C Y–G–N	K–T–G K–S–G

Frère JM Molecular Microbiology 1995;**16:**385-395

β-lactamases could originate from DD-peptidases (PBPs)

Critical Reviews in Microbiology, 26(4):205–220 (2000)

Penicillin Binding Proteins, β-Lactams, and Lactamases: Offensives, Attacks, and Defensive Countermeasures

Arthur L. Koch

Biology Department, Indiana University, Bloomington, IN 47405-6801 USA

β-lactamases could originate from DD-peptidases (PBPs)

Critical Reviews in Microbiology, 26(4):205–22

Penicillin Binding P
Lactamases: Offens
Defensive Counterm

Arthur L. Koch
Biology Department, Indiana University

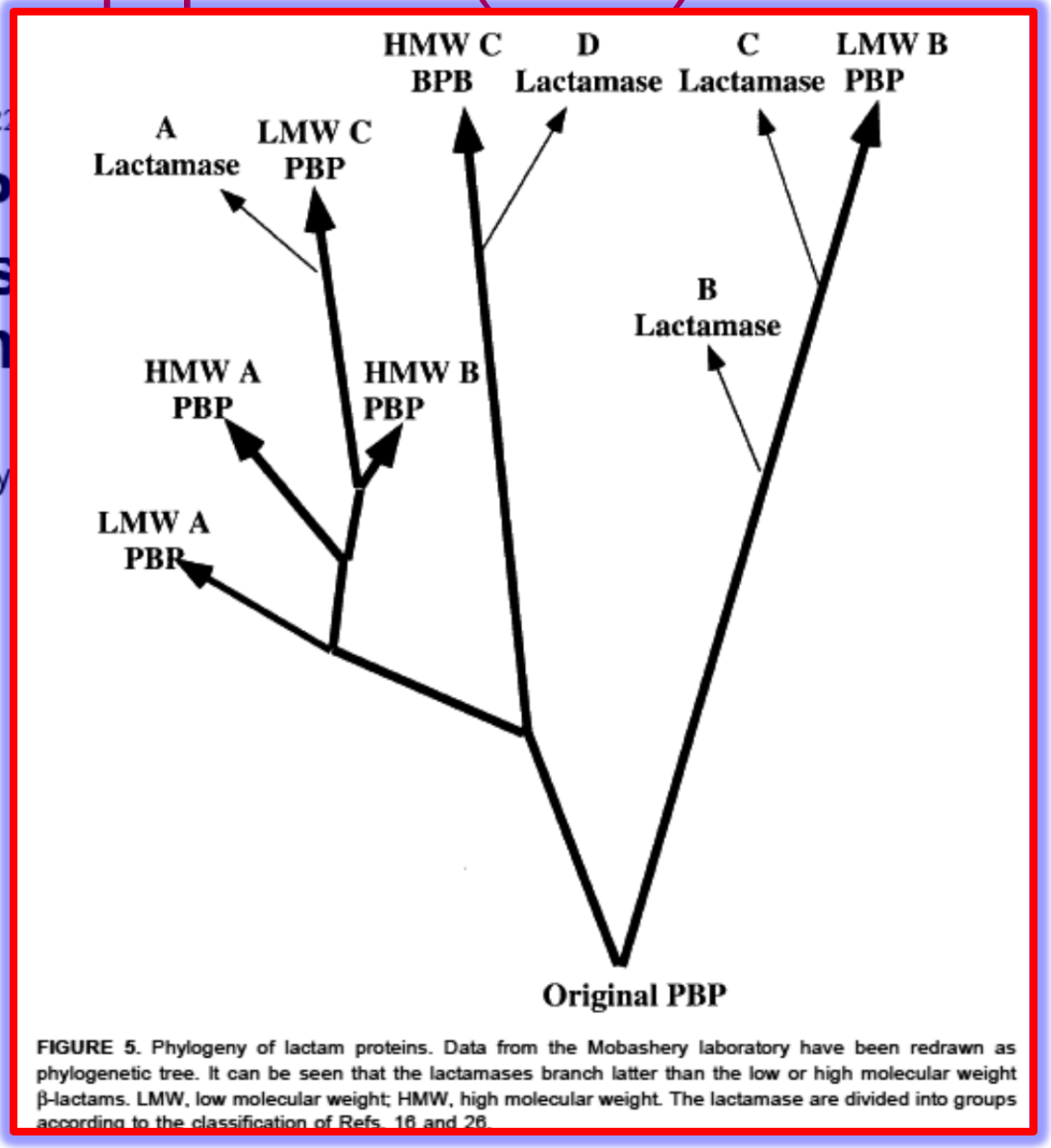


FIGURE 5. Phylogeny of lactam proteins. Data from the Mobashery laboratory have been redrawn as phylogenetic tree. It can be seen that the lactamases branch latter than the low or high molecular weight β-lactams. LMW, low molecular weight; HMW, high molecular weight. The lactamase are divided into groups according to the classification of Refs. 16 and 26.

DD-peptidases and serine β-lactamases: biochemical similarities and differences

doi:10.1016/j.jmb.2008.12.001 *J. Mol. Biol.* (2009) **386**, 109–120

JMB

Available online at www.sciencedirect.com

ScienceDirect

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Structure of PBP-A from *Thermosynechococcus elongatus*, a Penicillin-Binding Protein Closely Related to Class A β-Lactamases

Carole Urbach[1]†, Christine Evrard[2]†, Vaidas Pudzaitis[1], Jacques Fastrez[1], Patrice Soumillion[1]* and Jean-Paul Declercq[2]

DD-peptidases and serine β-lactamases: biochemical similarities and differences

doi:10.1016/j.jmb.2008.12.001

JMB

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ScienceDirect

**Structure of PBP-A from *Thermosynec*
elongatus, a Penicillin-Binding Protein
to Class A β-Lactamases**

**Carole Urbach[1]†, Christine Evrard[2]†, Vaidas Pudz
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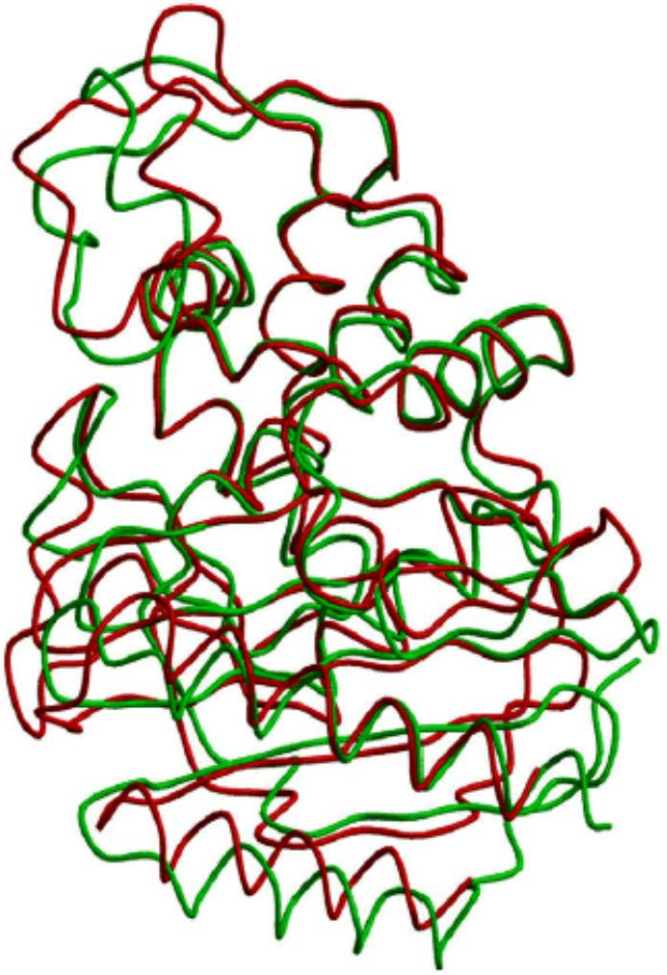

Fig. 1. Superposition of PBP-A molecule A (green) and TEM-1 β-lactamase (red).

DD-peptidases and serine β-lactamases: biochemical similarities and differences

doi:10.1016/j.jmb.2008.12.001 *J. Mol. Biol.* (2009) **386**, 109–120

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Available online at www.sciencedirect.com

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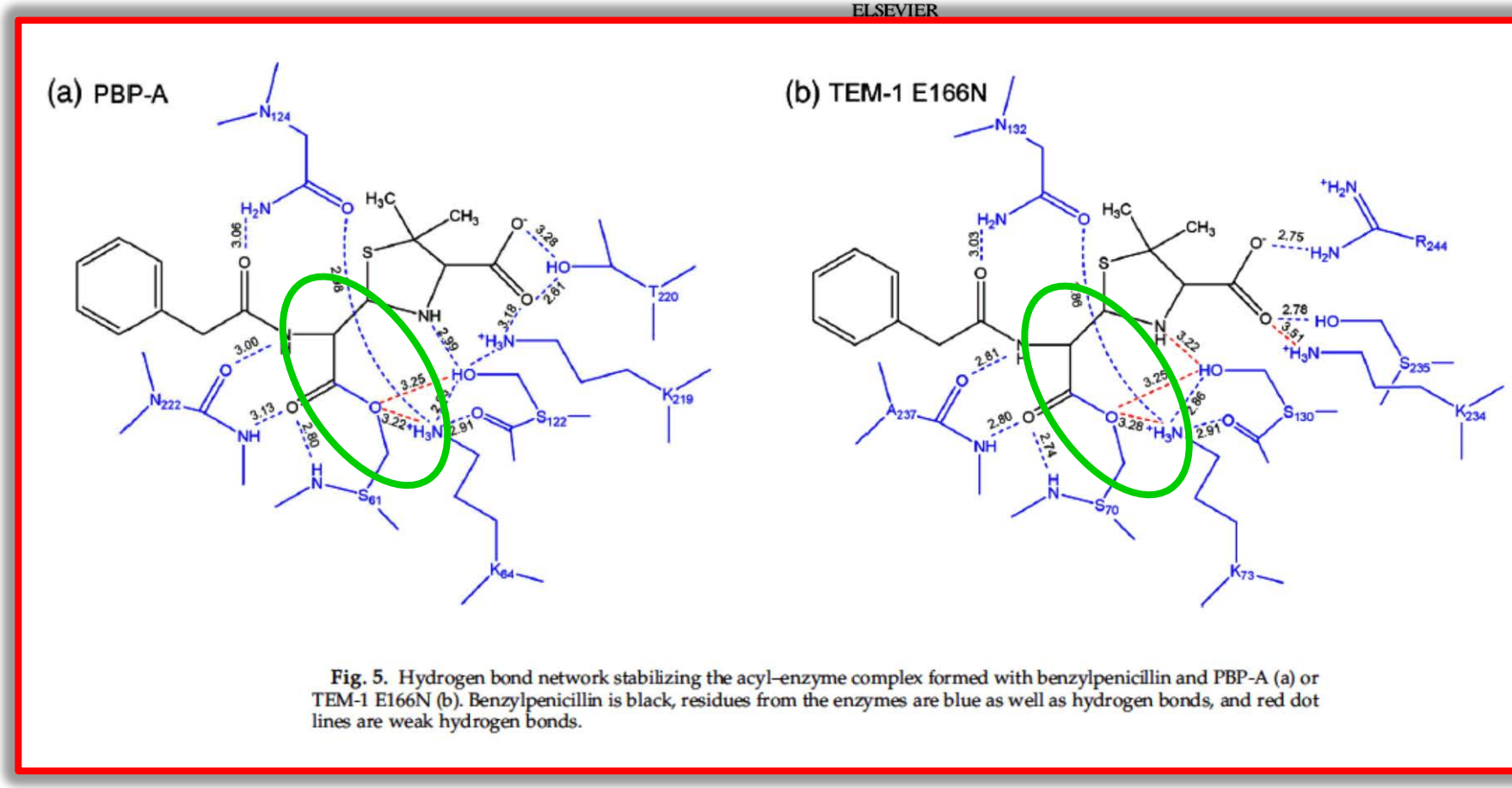
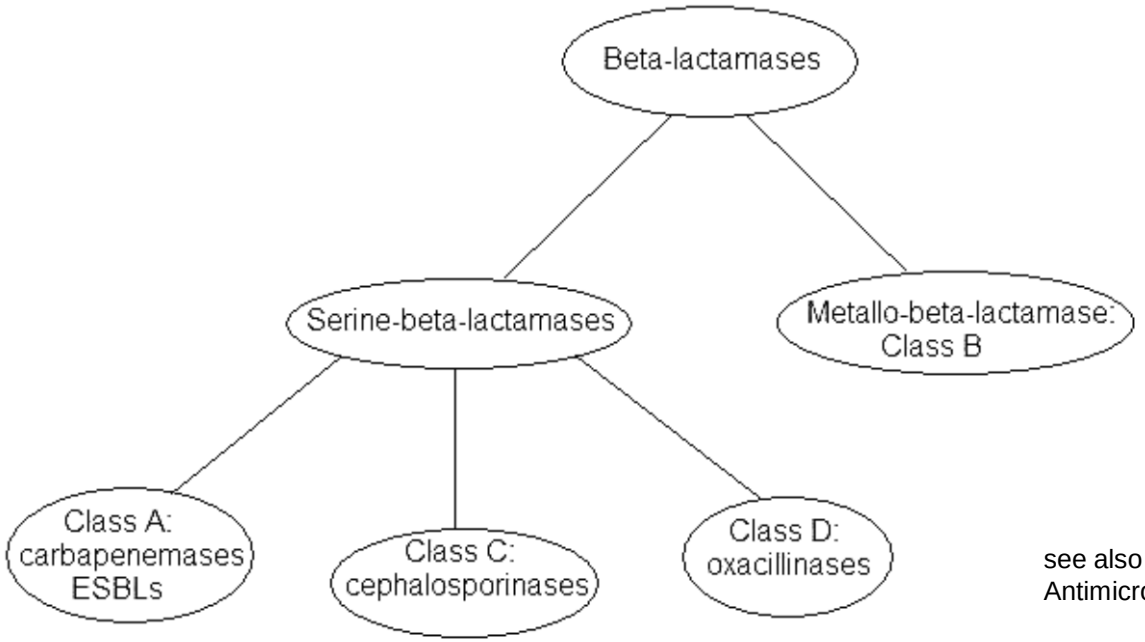


Fig. 5. Hydrogen bond network stabilizing the acyl–enzyme complex formed with benzylpenicillin and PBP-A (a) or TEM-1 E166N (b). Benzylpenicillin is black, residues from the enzymes are blue as well as hydrogen bonds, and red dot lines are weak hydrogen bonds.

From Biochemistry to current clinical situation…

Figure 2. Relationship of β-lactamases as Described by Ambler.



see also the functional classification: Bush et al. Antimicrob Agents Chemother, 1995;39(6):1211-33

A	**penicillins → narrow and broad spectrum cephalosporins → extended-spectrum β-lactamases (ESBLs) → serine carbapenemases**
C	**penicillins and cephalosporins (usually chromosomally-mediated)**
D	**penicillins, cephalosporins, extended-spectrum cephalosporins, and carbapenems.**
B	**Metallo-β-lactamases (including NDM-1) -->everything except aztreonam (often co-resistance)**

From Biochemistry to current clinical situation…

Epidemiological Expansion, Structural Studies, and Clinical Challenges of New β-Lactamases from Gram-Negative Bacteria

Annual Review of Microbiology
Vol. 65: 455-478 (Volume publication date October 2011)
DOI: 10.1146/annurev-micro-090110-102911

Karen Bush[1] and Jed F. Fisher[2]

[1]Biology Department, Indiana University, Bloomington, Indiana 47401; email: karbush@indiana.edu

[2]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556; email: jed.f.fisher.57@nd.edu

From Biochemistry to current clinical situation…

Epidemiological Expansion, Structur
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Karen Bush[1] and Jed F. Fisher[2]
[1]Biology Department, Indiana University, Blo
karbush@indiana.edu
[2]Department of Chemistry and Biochemistry
Dame, Indiana 46556; email: jed.f.fisher.57@

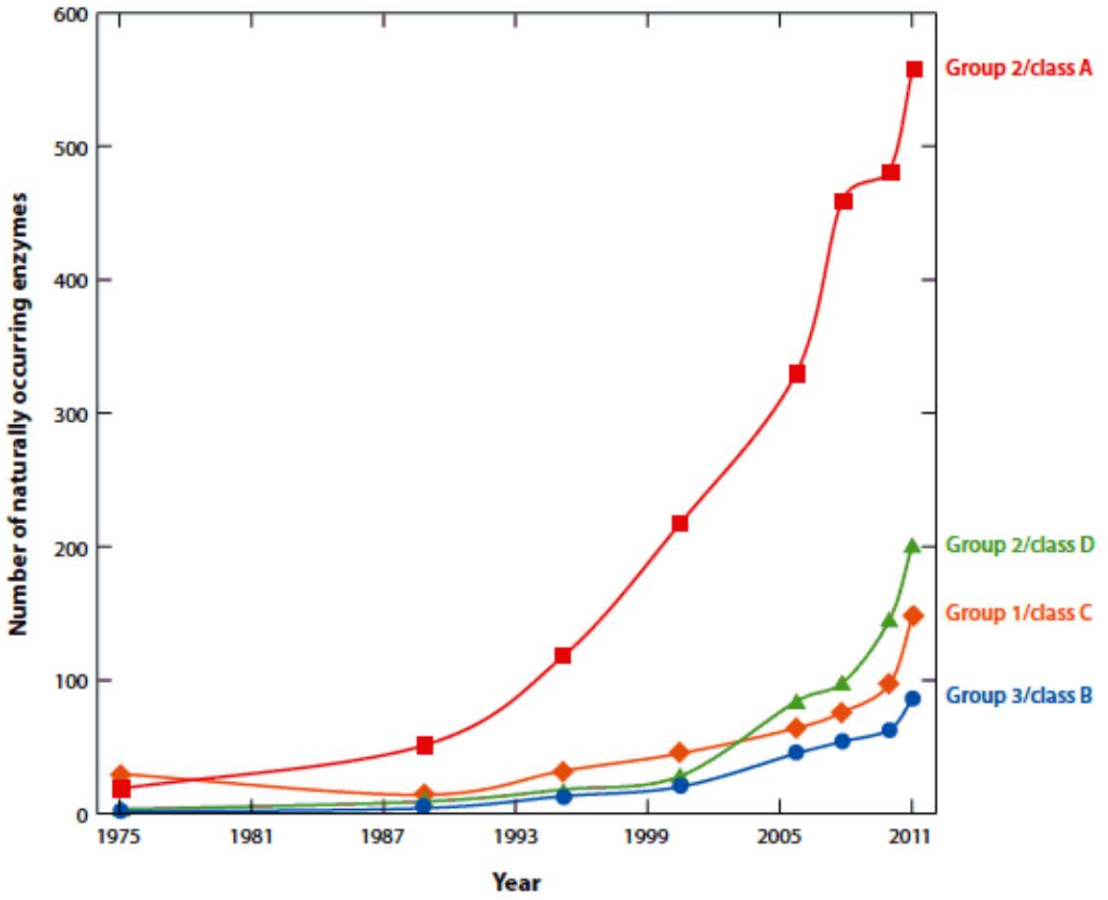

Figure 2

The number of naturally occurring β-lactamases identified from the main functional groups and molecular classes as indicated by the year in which the structures were provided to the curators of the β-lactamase Web site (46), or by the year in which the enzymes were reported in the literature (12), adapted with permission (copyright © American Society for Microbiology, 2010).

Worldwide spreading: the case of the NDM-1

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 249856, 12 pages
http://dx.doi.org/10.1155/2014/249856

Review Article

Worldwide Dissemination of the NDM-Type Carbapenemases in Gram-Negative Bacteria

Laurent Dortet,[1] Laurent Poirel,[1,2] and Patrice Nordmann[1,2]

[1] *INSERM U914 "Emerging Resistance to Antibiotics", 78 Avenue du Général Leclerc, 94270 Le Kremlin-Bicêtre, France*
[2] *Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science, University of Fribourg, 3 Rue Albert Gockel, 1700 Fribourg, Switzerland*

- originally observed as an isolated case in Sweden in a patient coming from India
- more cases observed in England … and related to India
- clusters observed in India and Pakistan and related to community and poor hygiene conditions
- spreading since then

NDM-1 and co-resistance

β-lactam antibiotics non-β-lactam antibiotics

	UK (n=37)		Chennai (n=44)		Haryana (n=26)	
	MIC_{50}; MIC_{90} (mg/L)	Proportion susceptible*	MIC_{50}; MIC_{90} (mg/L)	Proportion susceptible*	MIC_{50}; MIC_{90} (mg/L)	Proportion susceptible*
Imipenem	32; 128	0%	64; 128	0%	32; 128	0%
Meropenem	32; 32	3%	32; >32	3%	>32; >32	3%
Piperacillin-tazobactam	>64; >64	0%	>64; >64	0%	>64; >64	0%
Cefotaxime	>256; >256	0%	>256; >256	0%	>256; >256	0%
Ceftazidime	>256; >256	0%	>256; >256	0%	>256; >256	0%
Cefpirome	>64; >64	0%	>64; >64	0%	>64; >64	0%
Aztreonam	>64; >64	11%	>64; >64	0%	>64; >64	8%
Ciprofloxacin	>8; >8	8%	>8; >8	8%	>8; >8	8%
Gentamicin	>32; >32	3%	>32; >32	3%	>32; >32	3%
Tobramycin	>32; >32	0%	>32; >32	0%	>32; >32	0%
Amikacin	>64; >64	0%	>64; >64	0%	>64; >64	0%
Minocycline	16; >32	0%	32; >32	0%	8; 16	0%
Tigecycline	1; 4	64%	4; 8	56%	1; 2	67%
Colistin	0·5; 8	89%†	1; 32	94%†	1; 2	100%†

MIC=minimum inhibitory concentration. *Susceptibility defined by British Society for Antimicrobial Chemotherapy and European Committee on Antimicrobial Susceptibility Testing breakpoints; doxycycline breakpoints were used for minocycline. †Colistin-resistant UK isolates were one isolate of *Morganella morganii* and one *Providencia* sp (both intrinsically-resistant species), also one *Klebsiella pneumoniae* and one *Enterobacter* sp.

Table: **Antibiotic susceptibilities for NDM-1-positive Enterobacteriaceae isolated in the UK and north (Chennai) and south India (Haryana)**

Kumarasamy et al. Lancet Infect Dis 2010;10: 597–602

Worldwide spreading

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 249856, 12 pages
http://dx.doi.org

Review

World
Gram-

Laurent

[1] *INSERM U*
[2] *Medical ar*
3 Rue Albe

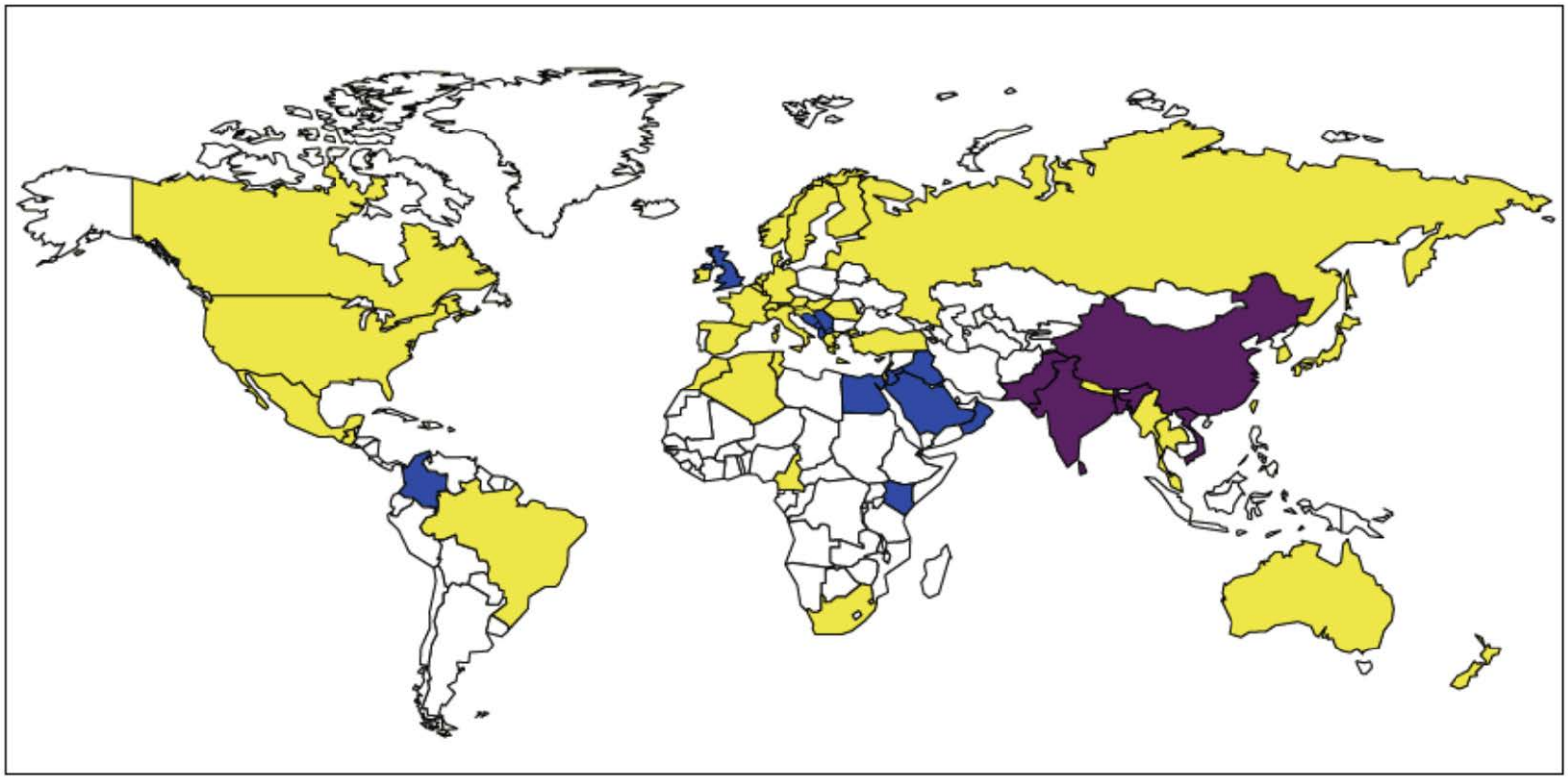

FIGURE 2: Geographical distribution of NDM producers.

Spreading or new local emergence ?

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

International Journal of Infectious Diseases 34 (2015) 53–54

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

Short Communication

First case of NDM-1 producing *Klebsiella pneumoniae* in Caribbean islands

Sylvaine Bastian [a,*], Patrice Nordmann [c,d,e,f], Elodie Creton [c,d], Edith Malpote [a], Guillaume Thiery [a,b], Frederic Martino [a], Sebastien Breurec [a,b], Laurent Dortet [c,d,g,h]

[a] University Hospital of Pointe-à-Pitre/Abymes, Pointe-à-Pitre, Guadeloupe, France
[b] University of Antilles, Pointe-à-Pitre, Guadeloupe, France
[c] INSERM U914 « Emerging Resistance to Antibiotic », Le Kremlin-Bicêtre, France
[d] Associated National Reference Center to Antibiotic Resistance, Le Kremlin-Bicêtre, France
[e] University of Fribourg, Fribourg, Switzerland
[f] Hôpital Fribourgeois - Hôpital Cantonal, Fribourg, Switzerland
[g] South Paris University, Faculty of Medecine, Le Kremlin-Bicêtre, France
[h] Assistance Publique des Hôpitaux de Paris, Bicêtre Hospital, Le Kremlin-Bicêtre, France

Patient hospitalized at la Guadeloupe after a stay in Cuba

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Patient hospitalized at la Guadeloupe after a stay in Cuba

MICs of β-lactams for *K. pneumoniae* KHU clinical isolate and the transconjugant *E. coli* J53 harboring the natural plasmid from *K. pneumoniae* KHU

β-Lactam (s)[a]	*K. pneumoniae* KHU	Tc[b] *E. coli* J53
Amoxicillin	> 256	> 256
Amoxicillin +CLA	> 256	> 256
Ticarcillin	> 256	> 256
Ticarcillin + CLA	> 256	> 256
Piperacillin	> 256	128
Piperacillin + TZB	> 256	128
Ceftazidime	> 256	> 256
Cefotaxime	> 256	> 256
Cefepime	> 256	64
Cefoxitin	> 512	> 512
Aztreonam	0.12	0.12
Imipenem	16	6
Meropenem	32	6
Ertapenem	32	4
Doripenem	12	6

[a] CLA, clavulanic acid; TZB, tazobactam, at 4 μg/ml.
[b] transconjugant.

Current geographical dispersion of the most "annoying" β-lactamases

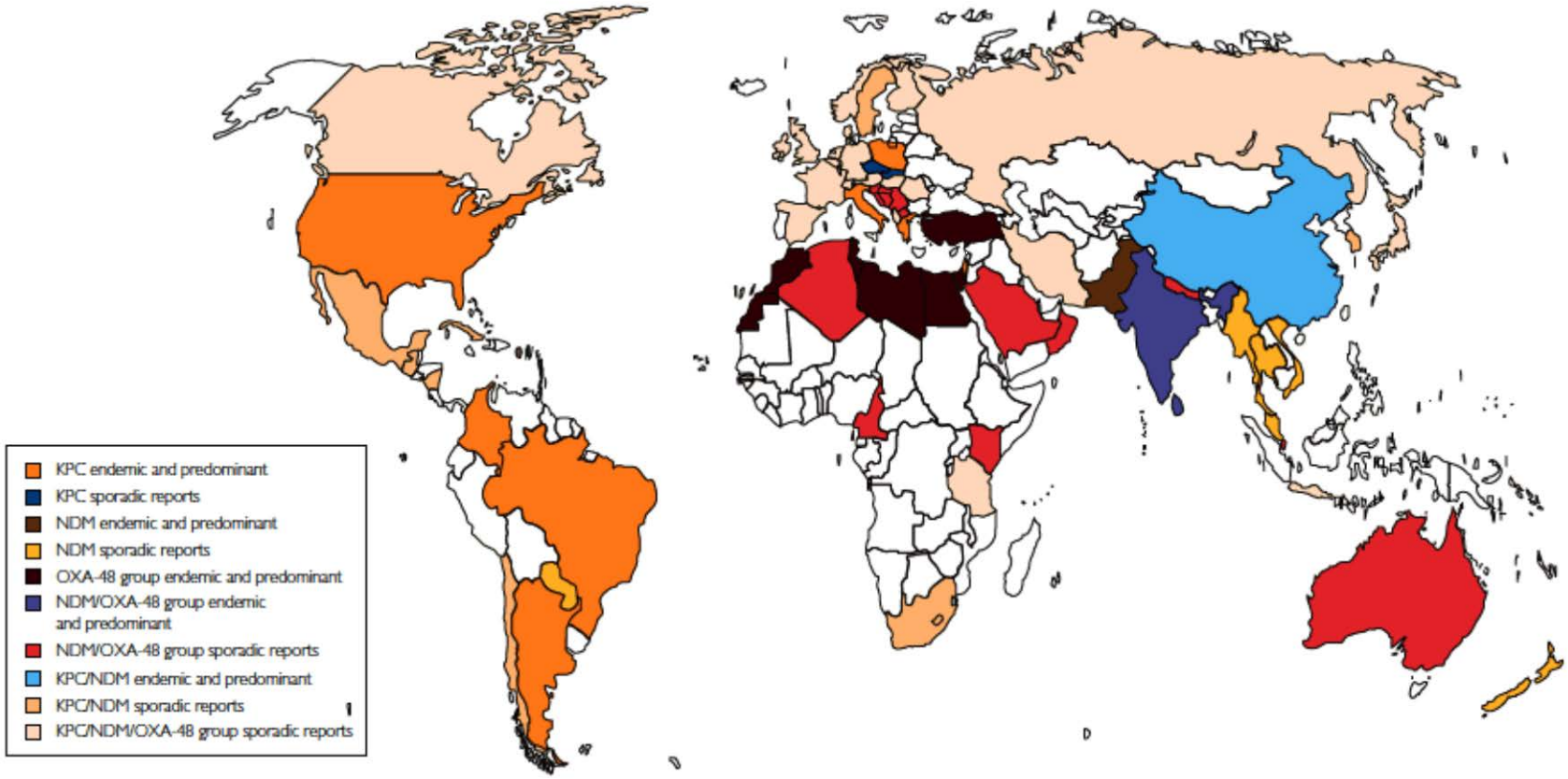

FIGURE. Distribution of *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), and OXA-48 group carbapenemases worldwide.

Vasoo et al. Mayo Clin Proc. 2015;90:395-403

Fast phenotypic screening is necessary

Contents lists available at SciVerse ScienceDirect

International Journal of Antimicrobial Agents

International Journal of Antimicrobial Agents 41 (2013) 99–109

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

Review

Towards a phenotypic screening strategy for emerging β-lactamases in Gram-negative bacilli

Elise Willems[a], Jan Verhaegen[a], Koen Magerman[b], Sita Nys[b], Reinoud Cartuyvels[b,*]

[a] *Department of Clinical Microbiology, University Hospital Leuven, Herestraat 49, 3000 Leuven, Belgium*
[b] *Department of Clinical Microbiology, Jessa Hospital, Stadsomvaart 11, 3500 Hasselt, Belgium*

Fast phenotypic screening is necessary

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Review

Towards a phenotypic
Gram-negative bacilli

Elise Willems[a], Jan Verhaeg

[a] Department of Clinical Microbiology, Universit
[b] Department of Clinical Microbiology, Jessa Hos

Reliable detection of emerging β-lactamases in GNB is crucial in minimising their spread and optimising antimicrobial therapy.

In the past two decades, different phenotypic screening and confirmation methods compatible for use in routine clinical microbiology laboratories have been developed and evaluated for the detection and identification of the emerging β-lactamases in GNB.

In previous sections, we reviewed approved guidelines on this topic, incorporated newly available literature and discussed controversies.

In this section, recommendations on the best and/or acceptable methods are formulated that could be helpful for clinical microbiology laboratories in developing efficient strategies for optimising the detection of emerging β-lactamase-producing GNB.

β-lactamases: where are we ?

SUMMARY POINTS

1. More than 1,000 naturally occurring β-lactamases have been identified, frequently as the result of the facile transfer of mobile elements from one gram-negative organism to another; yet, only a few of these β-lactam-hydrolyzing enzymes have become prominent resistance determinants worldwide. The most common acquired β-lactamases are the AmpC cephalosporinases (CMY family), the ESBL CTX-M-14 and CTX-M-15 enzymes, the serine carbapenemases (KPC enzymes), and the metallo-β-lactamases (NDM-1, VIM, and IMP families).
2. Gram-negative bacteria that produce acquired carbapenemases are often resistant to most, if not all, β-lactams. In addition, they are resistant to other antibiotic classes (such as aminoglycosides, tetracyclines, and fluoroquinolones) because of the concerted movement of mobile resistance elements.
3. Environmental β-lactamases are a source of some of the newer β-lactamases that are appearing in both commensal and nosocomial isolates.
4. Many organisms now produce multiple β-lactamases from different functional groups, making it more difficult to synthesize new β-lactams to counteract the evolving challenges to these agents.
5. Our ability to interpret structural and mechanistic experiments on these β-lactamases in terms of resistance development is primitive. Nonetheless, as we better understand the genetic development of the β-lactamases, i.e., where are they coming from and what next may they present us with, we secure better guidance for the empirical development of β-lactamase-resistant structures.

Bush & Fisher, Annu Rev Microbiol 2011;65:455-478

Target modification: the case of linezolid and tedizolid

- Linezolid is a totally synthetic anti-Gram-positive antibiotic
- Last novel class of antibiotics registered since 2000… with a largely novel mode of action (inhibitor of the formation of the initiation complex in protein synthesis)
  → **no cross resistance with most clinically used anti-Gram-positive drugs**

Chloramphenicol-florfenicol resistance (Cfr)

- First identified in several staphylococcal species (cattle, swine) (Schwarz 2000; Kehrenberg 2006)
- CM05 (Colombia) - first clinical isolate documented to carry the *cfr* gene (Toh 2007)
- C-8 methylation of ribosome target at A2503 (Kehrenberg 2005; Giessing 2009)
- $PhLOPS_A$ phenotype leads to cross resistance to 6 drug classes!
  - Phenicols, Lincosamides, Oxazolidinones, Pleuromutilins, Streptogramin A and 16 membered macrolides (Long, 2006; Smith & Mankin 2008)
- Tedizolid retains potency against *cfr* strains and demonstrates 8-fold better activity than linezolid (Shaw 2008, Jones 2009, Livermore 2009, Locke 2009)

full

to 16

Activity against Cfr$^+$ resistant strains … (*cfr*$^+$ bacteria)

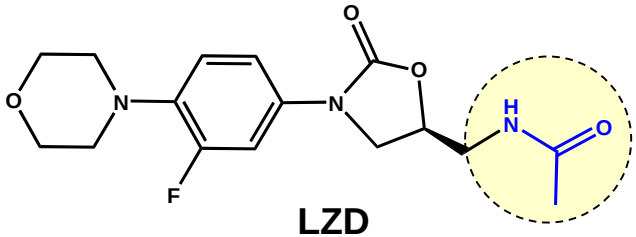
Oxazolidinone MICs for *S. aureus cfr* strains

Strain	Reference	Presence of *cfr*	MIC (μg/ml)[a]	
			LZD	TR-700
RN4220(pLI50)	68	−	2	0.5
RN4220(pLXM1)[b]	68	+	8	0.5
CM05Δ[c]	44	−	2	0.5
CM05[c]	68	+	8	0.5
29213	ATCC	−	2	0.5
29213(p42262)[d]	45	+	16	0.5
42262[e]	51	+	16	0.5

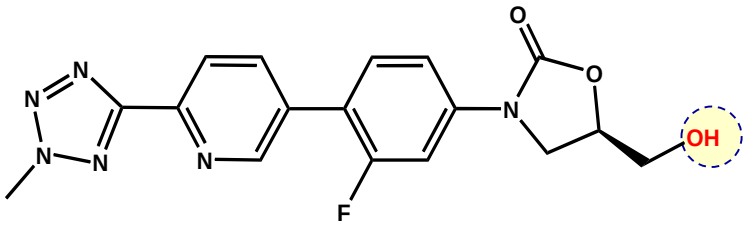
[a] MICs (broth microdilution: CLSI)
[b] The pLXM1 *cfr*-containing plasmid is isogenic to the empty pLI50 vector.
[c] CM05Δ is isogenic to the CM05 clinical *cfr*-positive strain but lacks *cfr* and one copy of *ermB*.
[d] 29213(p42262) was generated through transformation of ATCC 29213
[e] 42262 is a clinical *cfr*-positive isolate from a 2008 hospital outbreak in Madrid, Spain.

Locke et al. AAC 2010;54:5337-5343

Why is tedizolid active against LZD^R strains (*cfr*) ?



LZD



TR700

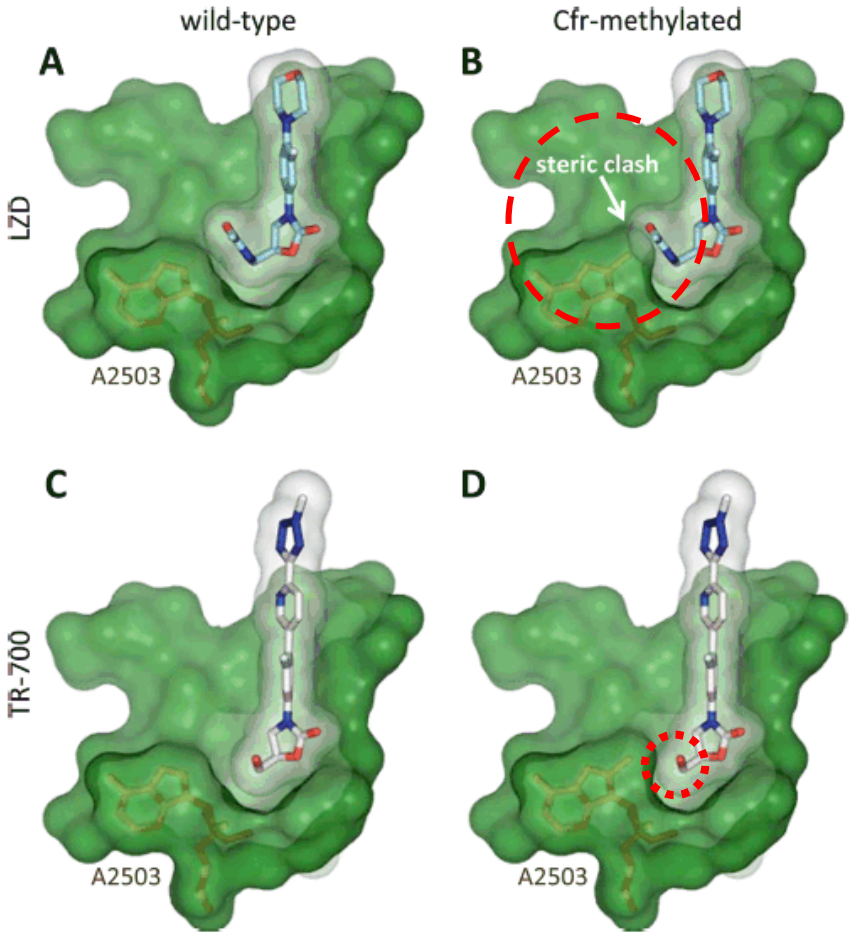


FIG. 2. Structural analysis of oxazolidinone binding in the presence of Cfr methylation. (A) Crystal structure of LZD-bound *H. marismortui* 50S ribosome (30). (B) Model of LZD binding in the Cfr-methylated state. (C and D) Proposed models of TR-700 bound to wild-type (C) or Cfr-methylated (D) ribosome. Substantial steric hindrance between the LZD C-5 acetamide group and the 23S rRNA base A2503 carbon-8 methyl (bonds shown in brown) likely contributes to reduced binding affinity (B). As modeled, the TR-700 hydroxymethyl substituent does not display this steric clash with the A2503 methyl group (D), explaining its retained activity against *cfr* strains. A group of PTC bases were removed from the images to improve clarity. Images were generated with PyMOL (16).

Locke et al. AAC 2010;54:5337-5343

But (very) recently efflux comes in …

J Antimicrob Chemother
doi:10.1093/jac/dkv116

Advance Access published May 14, 2015

Journal of Antimicrobial Chemotherapy

A novel gene, *optrA*, that confers transferable resistance to oxazolidinones and phenicols and its presence in *Enterococcus faecalis* and *Enterococcus faecium* of human and animal origin

Yang Wang[1]†, Yuan Lv[2]†, Jiachang Cai[3]†, Stefan Schwarz[4]†, Lanqing Cui[2], Zhidong Hu[5], Rong Zhang[3], Jun Li[1], Qin Zhao[1], Tao He[1], Dacheng Wang[6], Zheng Wang[1], Yingbo Shen[1], Yun Li[2], Andrea T. Feßler[4], Congming Wu[1], Hao Yu[6], Xuming Deng[6], Xi Xia[7] and Jianzhong Shen[1]*

[1]*Department of Veterinary Pharmacology, College of Veterinary Medicine, China Agricultural University, Beijing, China;* [2]*Institute of Clinical Pharmacology, Peking University First Hospital, Beijing, China;* [3]*The Second Affiliated Hospital of Zhejiang University, Zhejiang University, Hangzhou, China;* [4]*Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Neustadt-Mariensee, Germany;* [5]*Tianjin Medical University General Hospital, Tianjin, China;* [6]*Institute of Zoonosis, College of Veterinary Medicine, Jilin University, Changchun, China;* [7]*Beijing Key Laboratory of Detection Technology for Animal Food Safety, China Agricultural University, Beijing, China*

- novel plasmid-borne ABC **transporter** gene optrA from *E. faecalis*
- combined resistance linezolid and tedizolid, and chloramphenicol and florfenicol
- functionally expressed in *E. faecalis*, *E. faecium* and *Staphylococcus aureus*.
- detected in food-producing animals humans

But (very) recently efflux comes in …

J Antimicr
doi:10.10

oxazo

Yang War
Tao

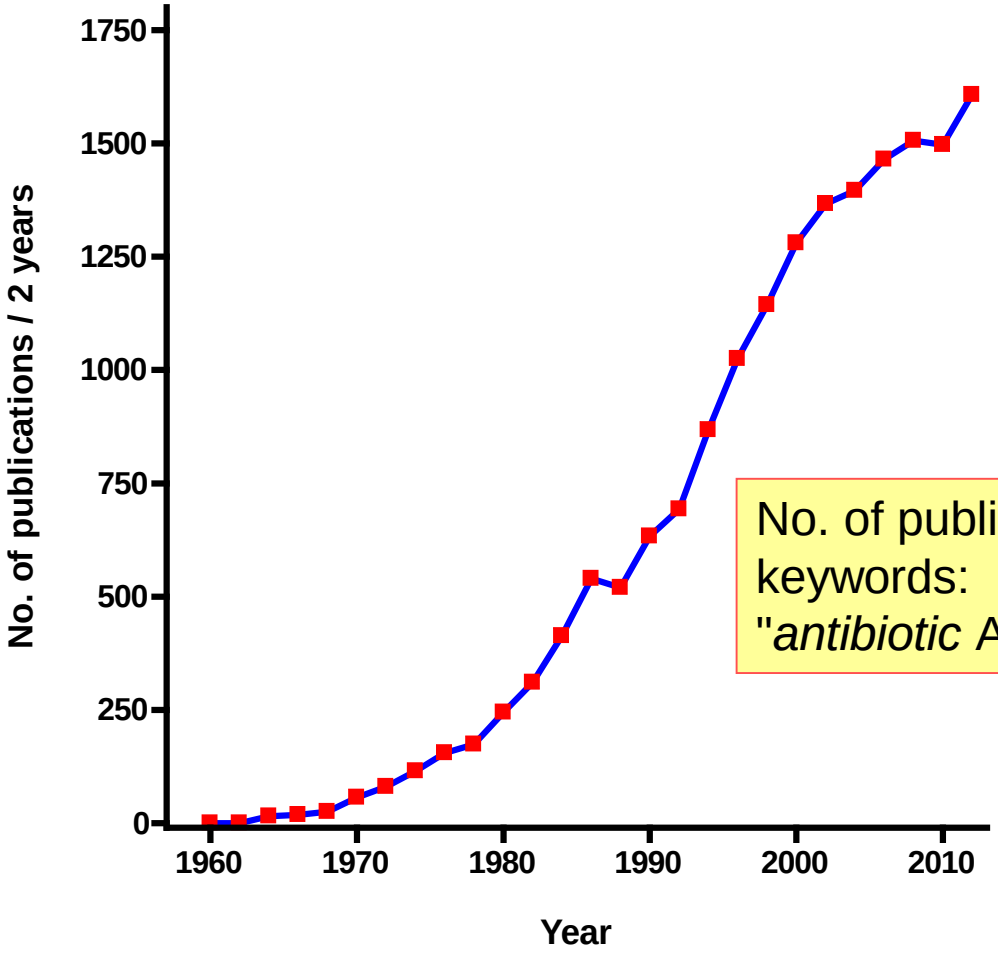
[1]Departme
Pharmacol
Hangzhou
University

Table 1. MICs for *E. faecalis* E349, *E. faecium* A4, their transformants and transconjugants, and the recipient strains

Bacterial isolate	MIC (mg/L)				
	CHL	FFC	LZD	TZD	VAN
Clinical *E. faecalis* E349 (with *optrA*-carrying pE349)	64	64	8	2	1
E. faecalis FA2-2	4	2	2	0.5	1
Transconjugant *E. faecalis* FA2-2-E349	32	64	8	2	1
E. faecalis JH2-2	4	4	2	0.5	1
Transformant *E. faecalis* JH2-2/pE349	64	64	16	2	1
Transformant *E. faecalis* JH2-2/pAM401	≥128[a]	4	2	0.5	1
Transformant *E. faecalis* JH2-2/pAM401 + *optrA*	≥128[a]	64	16	2	1
S. aureus RN4220	8	4	2	0.25	1
Transformant *S. aureus* RN4220/pAM401	≥128[a]	4	2	0.25	1
Transformant *S. aureus* RN4220/pAM401 + *optrA*	≥128[a]	64	8	1	1
Clinical *E. faecium* A4 (with *vanA*-carrying pA4)	4	2	2	0.25	>128
Transconjugant *E. faecium* A4-E349	32	128	8	2	>128
Transconjugant *E. faecalis* E349-A4	64	64	8	2	128

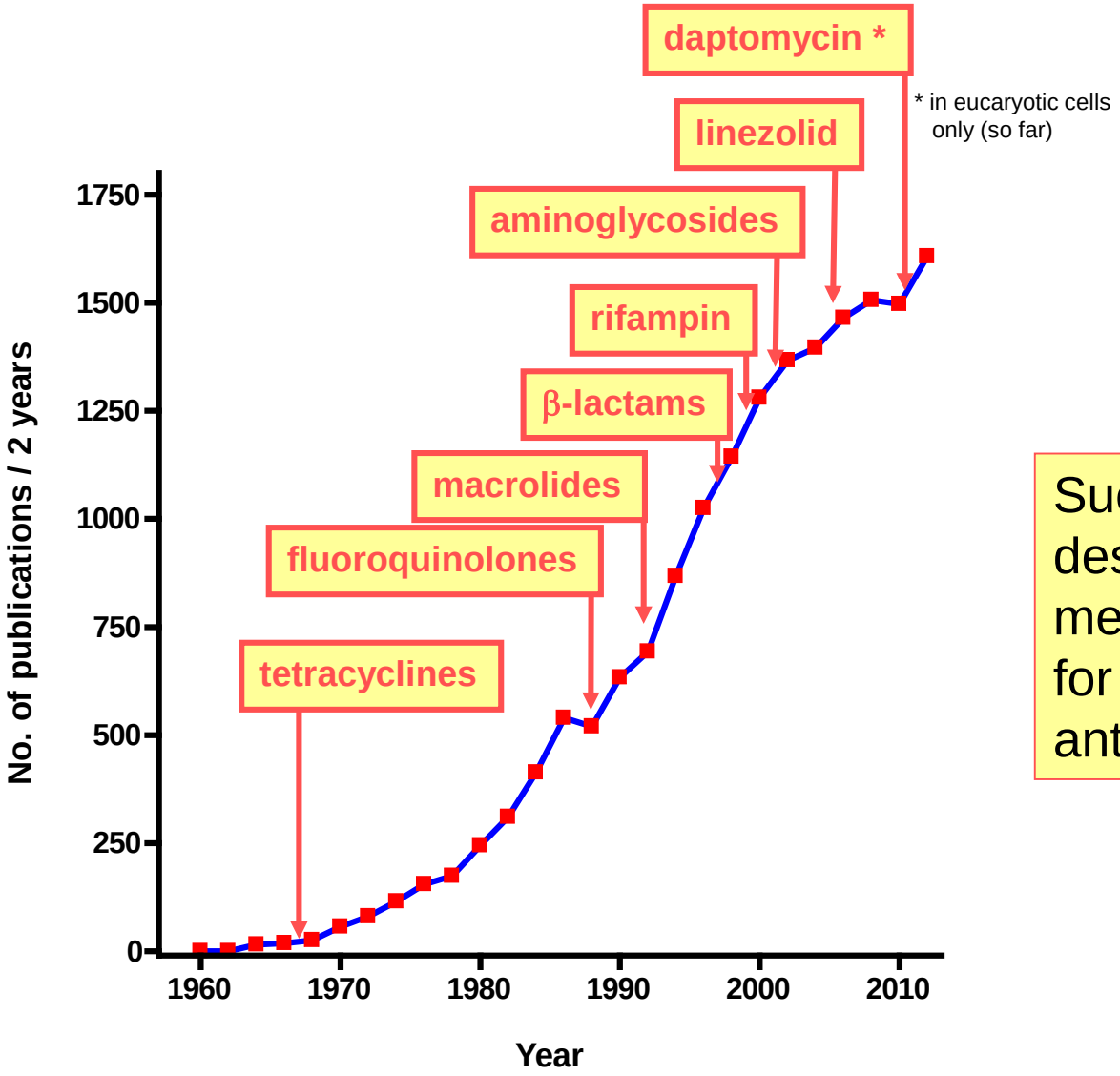
CHL, chloramphenicol; FFC, florfenicol; LZD, linezolid; TZD, tedizolid; VAN, vancomycin.
[a]The shuttle vector pAM401 carries a pIP501-analogous *cat* gene that confers high-level resistance to chloramphenicol, but not to florfenicol.

You said "antibiotic eflux"



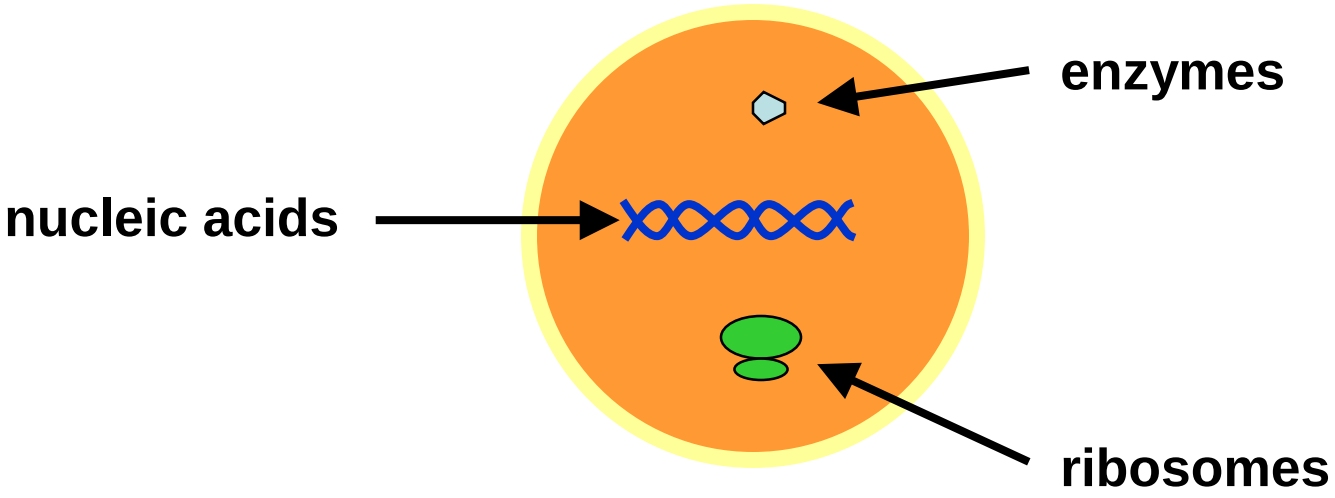
No. of publications in PubMed with keywords:
"*antibiotic* AND (*efflux* OR *transporter*)"

Historical landmarks …



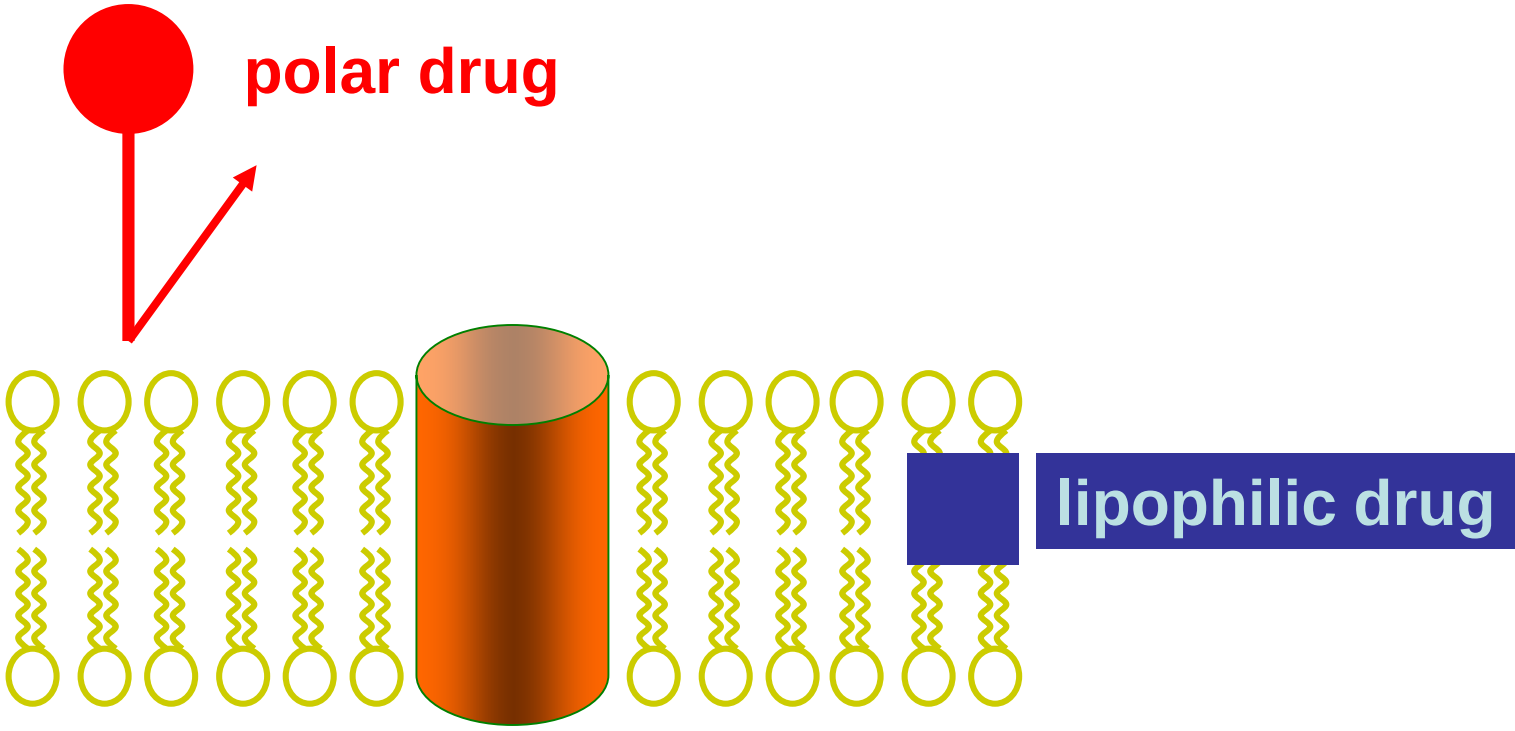
Successive description of efflux-mediated resistance for major classes of antibiotics

Most chemotherapeutic agents must reach an **intracellular** target…



How can these drugs reach their target inside the cells ?

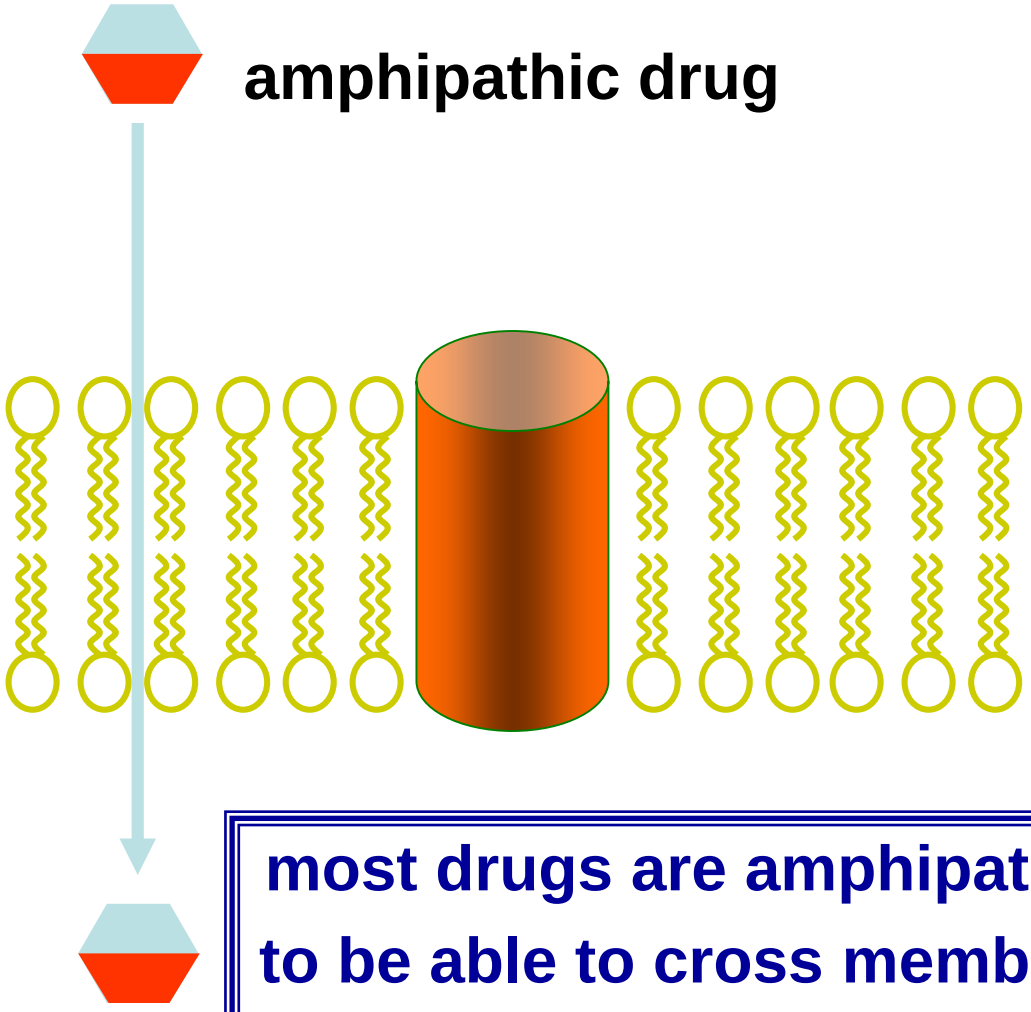
Reaching an intracellular target …



physico-chemical properties are inadequate for reaching an intracellular target !

Van Bambeke et al., Biochem. Pharmacol (2000) 60:457-70

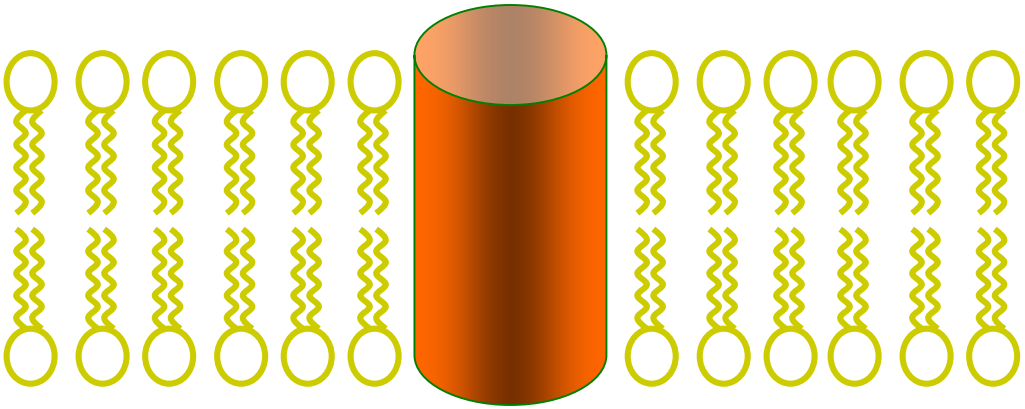
Reaching an intracellular target …



most drugs are amphipathic by design, to be able to cross membrane barriers !

Van Bambeke et al., Biochem. Pharmacol (2000) 60:457-70

Intracellular chemotherapeutic agents



But a diffusible compound

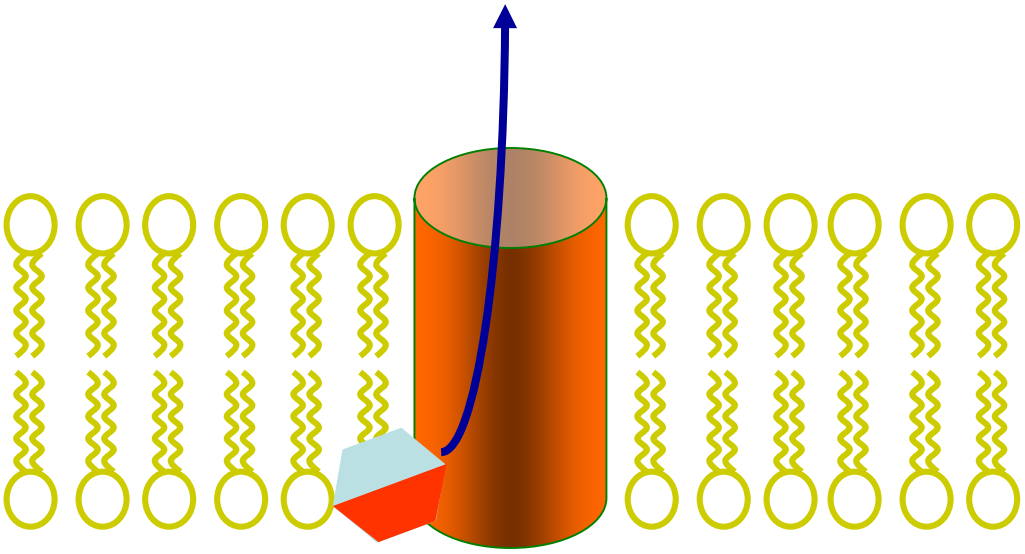
may have

potentially harmful effects !

Van Bambeke et al., Biochem. Pharmacol (2000) 60:457-70

Why efflux transporters ?

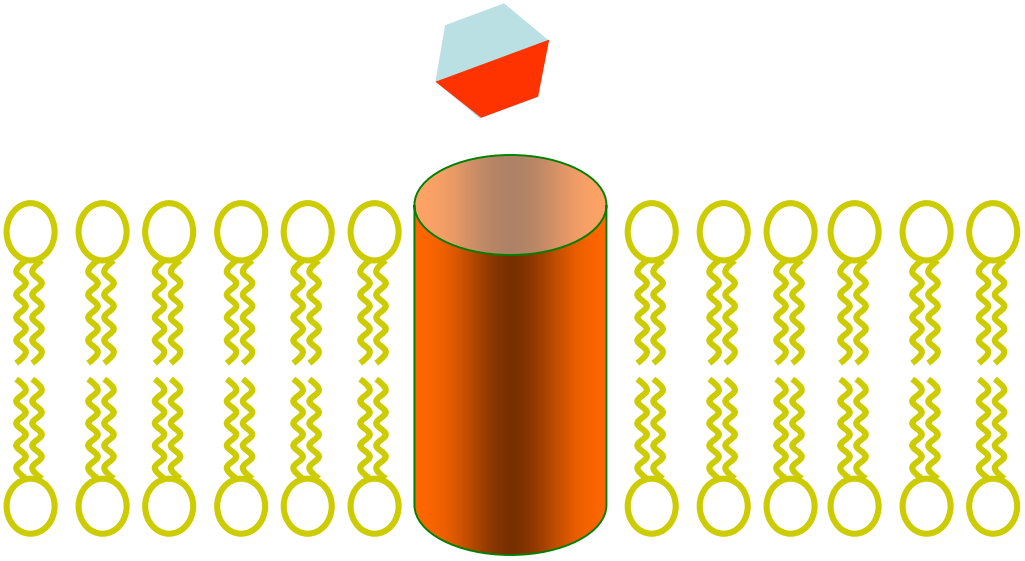
Extrusion by efflux pumps



Van Bambeke et al., Biochem. Pharmacol (2000) 60:457-70

Why efflux transporters ?

Extrusion by efflux pumps

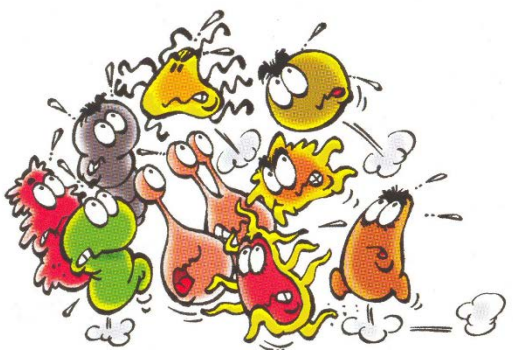


general mean of protection
against cell invasion by diffusible molecules

Van Bambeke et al., Biochem. Pharmacol (2000) 60:457-70

Typical ‘toxic’ diffusible substances as substrates for efflux pumps

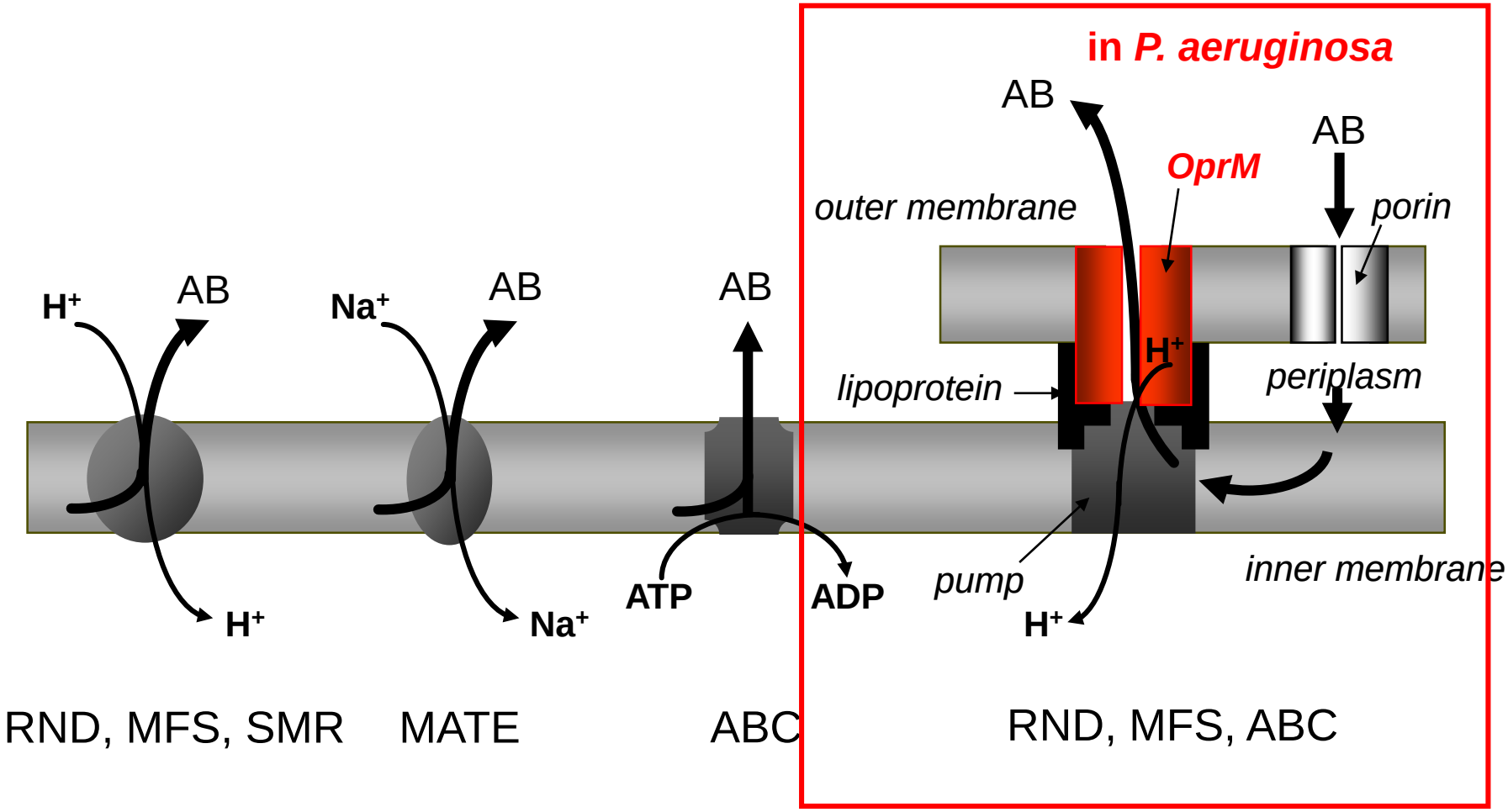
antibiotics



antifungals

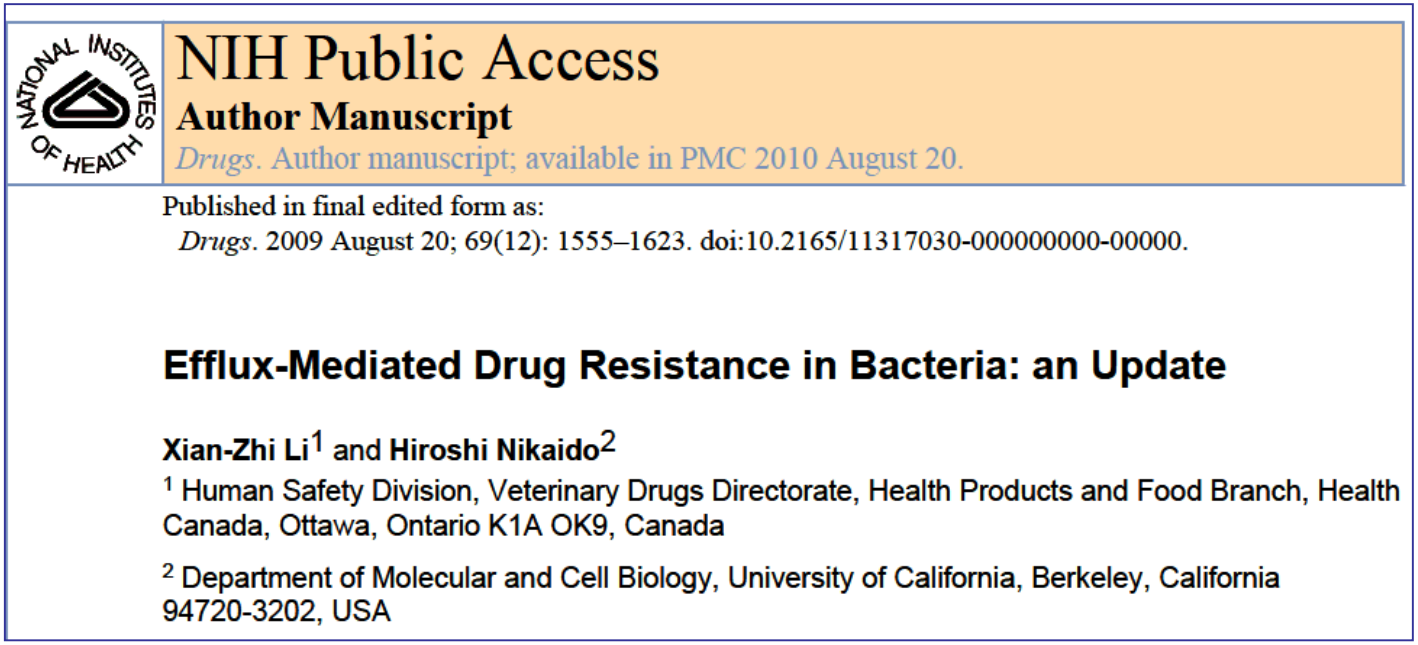
anticancer agents

Structure of antibiotic efflux transporters in bacteria



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

A brief survey of the many transporters (2009)



Published in final edited form as:
Drugs. 2009 August 20; 69(12): 1555–1623. doi:10.2165/11317030-000000000-00000.

Efflux-Mediated Drug Resistance in Bacteria: an Update

Xian-Zhi Li[1] and **Hiroshi Nikaido**[2]

[1] Human Safety Division, Veterinary Drugs Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario K1A OK9, Canada

[2] Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-3202, USA

809 references

A brief survey of the many transporters: *S. aureus*

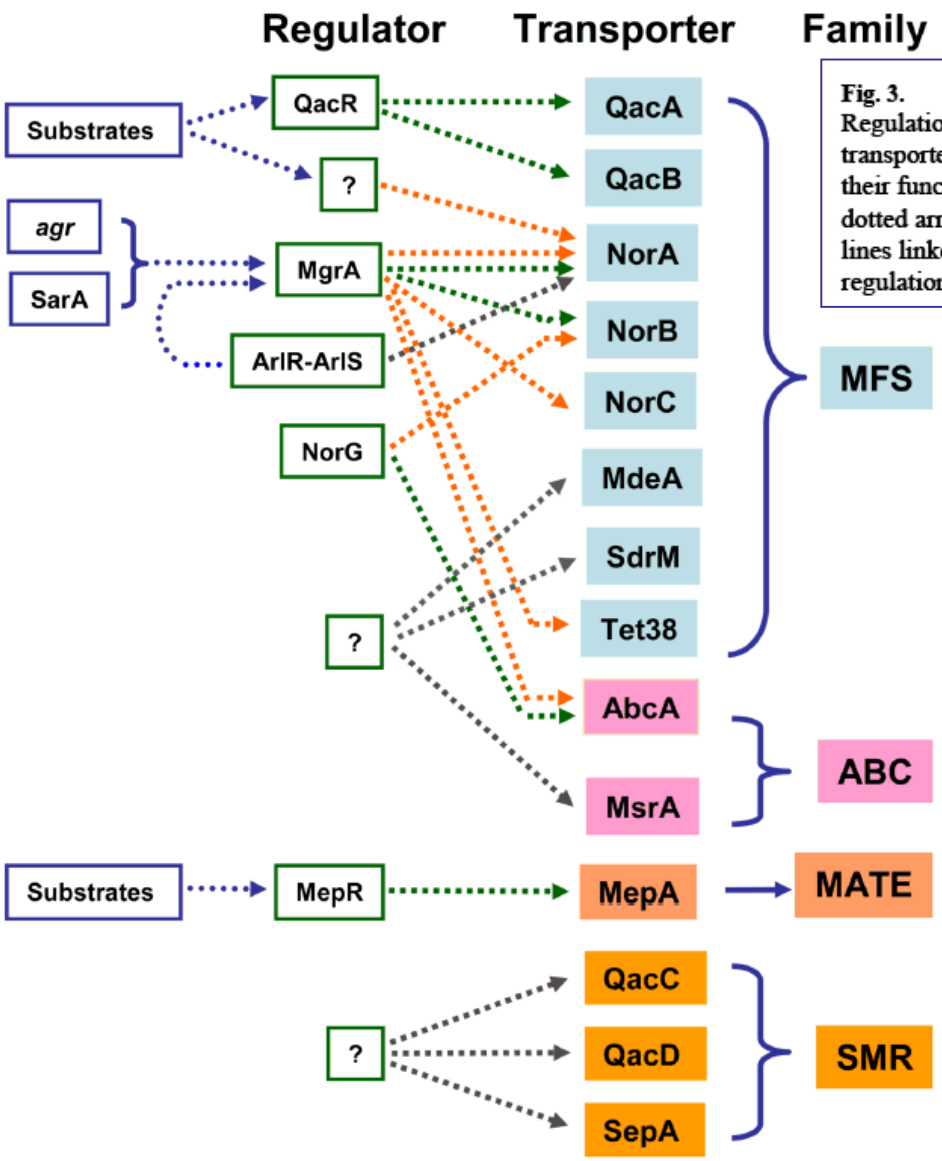
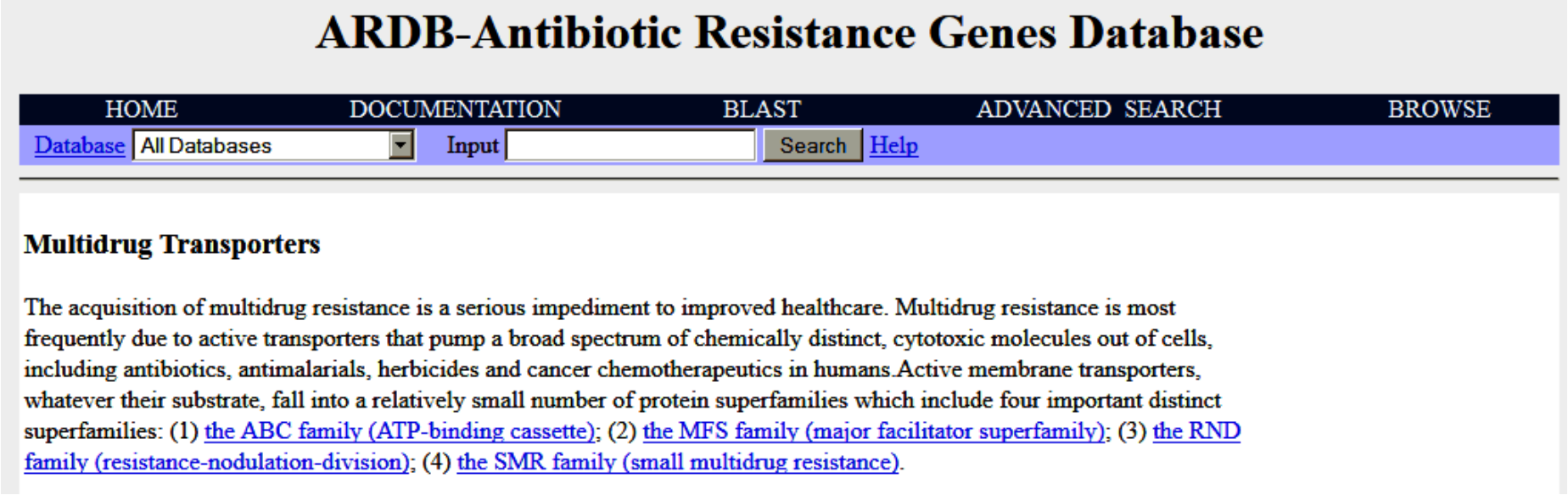


Fig. 3.
Regulation of multidrug or drug-specific efflux transporters of *S. aureus*. The efflux transporters are shown in colour blocks. All regulators are presented in the green boxes, and their functions as repressor or activator are indicated, respectively, by the green or orange dotted arrows. Unknown regulators are marked with a question mark (?) with the dotted grey lines linked to the relevant transporters. See text and relevant references for details of the regulation.

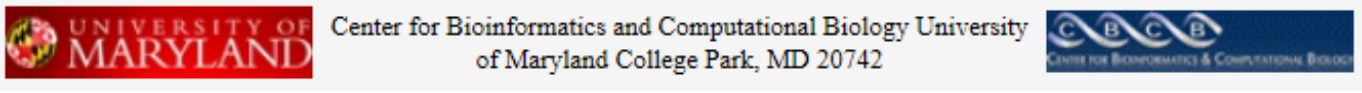
14 distinct transporters for S. aureus (only) in 2009 *vs.* 4 in 2003

What do you wish to know ?

- Specific information about antibiotic transporters in procaryotes

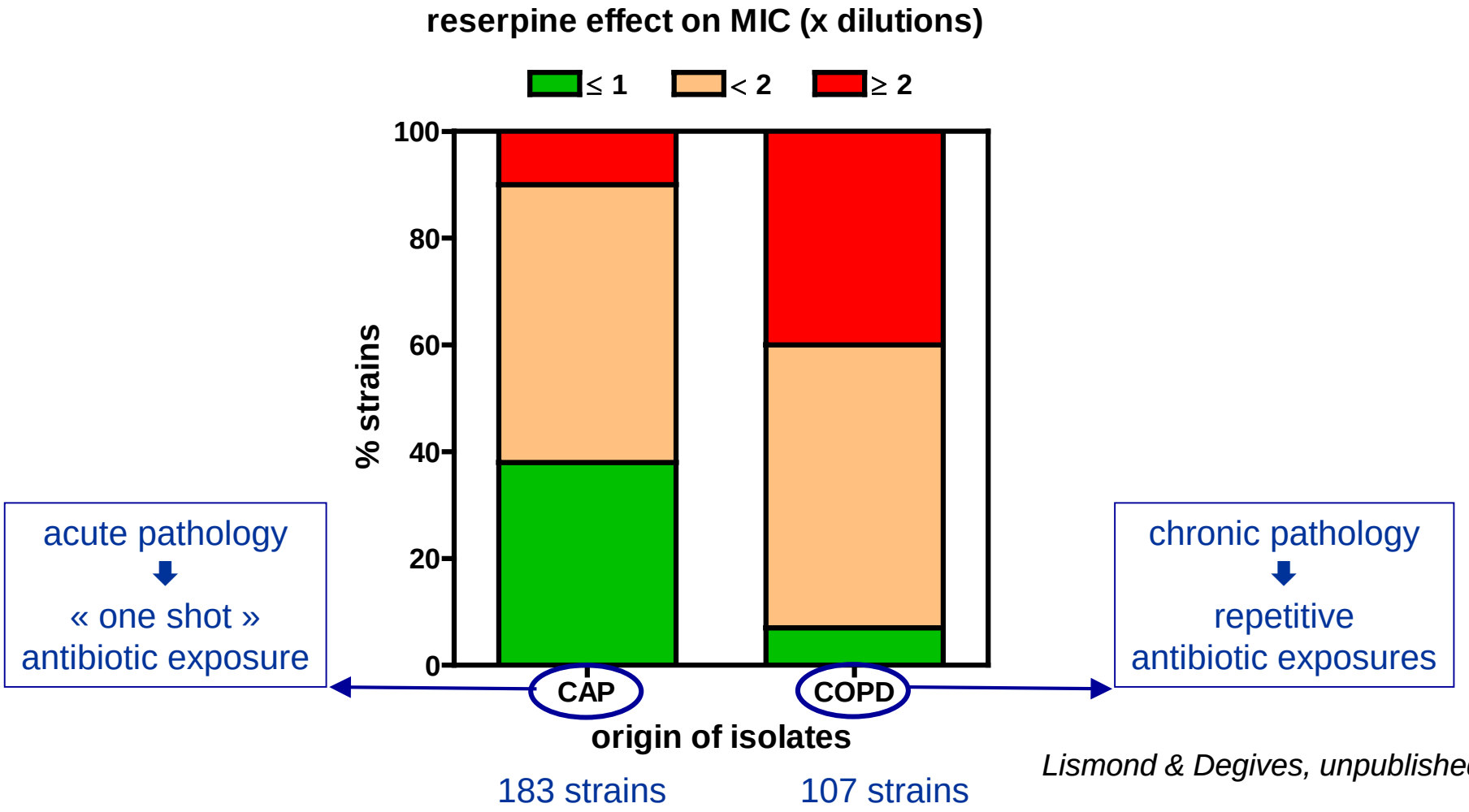


http://ardb.cbcb.umd.edu/browse/multidrug.shtml



Efflux can be triggered by antibiotic treatments: the case of *S. pneumonia* in patients with **community-acquired pneumonia (CAP)** *vs* **chronic obstructive pulmonary disease (COPD)**

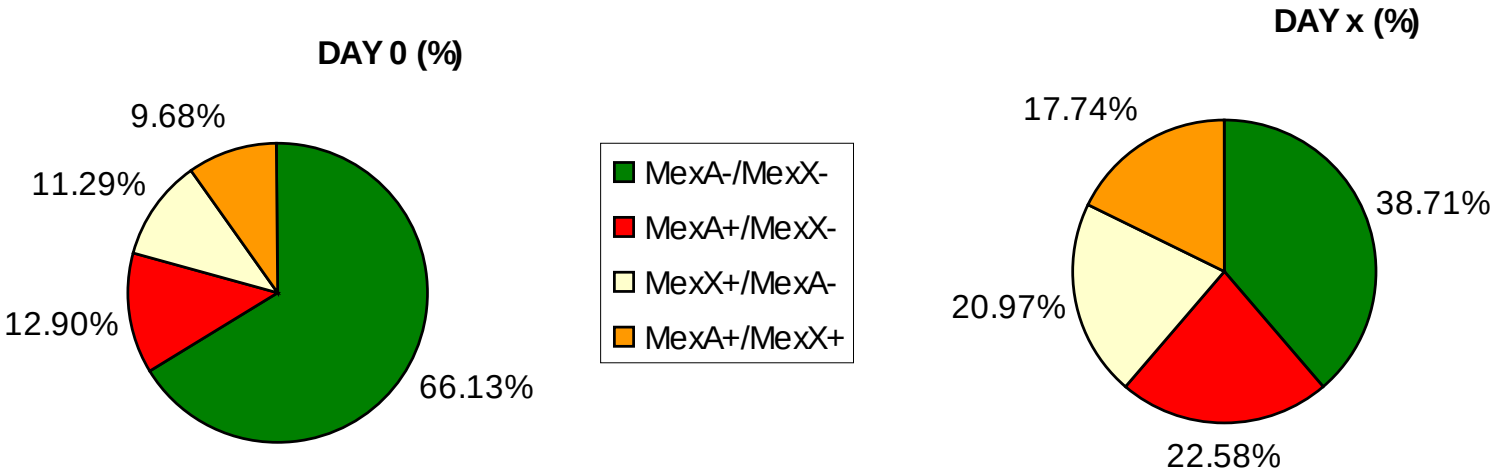
Suspected efflux based on phenotypic analysis (CIP MIC +/- reserpine)



Lismond & Degives, unpublished

Increase of *P.* aeruginosa during treatment: is efflux involved ?

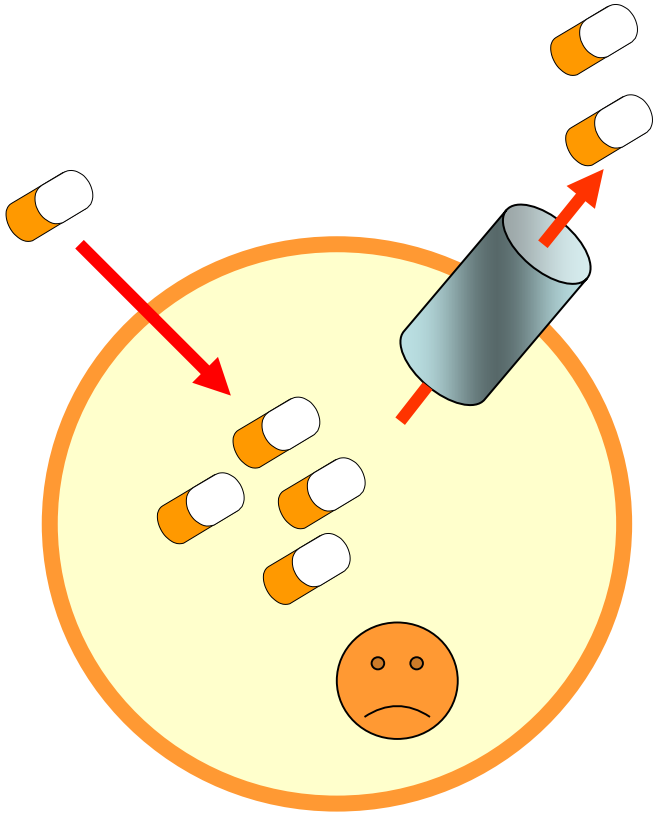
Prevalence of MexA and MexX overexpressers in 62 phylogentically-related pairs of *P. aeruginosa* isolated from ICU patients (VAP)



Riou et al, ECCMID 2010
Riou et al. submitted for publication

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



In a nutshell …

- Bacterial resistance emergence is a natural process that has gone on for immemorial times but is accelerated by use of antibiotics…
  - **evidence**: all these resistance genes existing in the environment
  - **significance:** resistance was with us since ever and we will never get rid of it …
- Bacterial resistance is transmissible and adaptable
  - **evidence:** β-actamases, MRSA (PBP2a), Penicillin-resistant *S. pneumoniae* (mosaic genes), aminoglycoside-inactivating enzymes, QnR (fluoroquinolones-target protecting protein) …
  - **significance:** isolation / Hygiene is of critical importance
- Bacterial resistance is selectable and this is greatly accelerated by use of antibiotics…
  - **evidence:** emergence of resistance during treatment
  - **significance:** chemotherapy has intrinsic limitations and all you can do is slow down the process … and rely on new therapies at regular intervals…

Who made that all possible ?

slides: www.facm.ucl.ac.be → Lectures

Back-up slides