Antibiotic Resistance Acquisition

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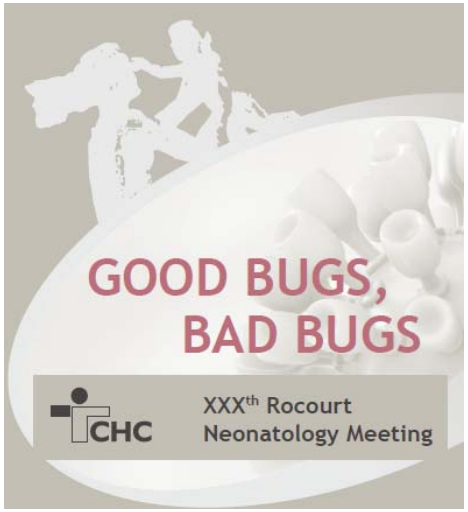

Saturday June 14th, 2014 - Cercle de Wallonie Liège - Esplanade du Val, Seraing

Disclosures

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- *Université catholique de Louvain* for past personal support
- Commercial Relationships:
  - AstraZeneca, GSK, Sanofi-Aventis, Bayer HealthCare, Cempra Pharmaceuticals, The Medicines Company, Northern Antibiotics, RibX, Cubist, Galapagos, …
- Other relationships in relation to this talk
  - Belgian Antibiotic Policy Coordination Committee,
  - European Medicines Agency (as expert for the agency and for Industry)
  - European Commmittee for Antibiotic Susceptibility Testing (EUCAST)

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

Do we have a problem ?

Obituary

J.-M. Ghuysen

This man discovered the mode of action of penicillin

Ann. Rev. Biochem. 1979. 48:73–101

USE OF MODEL ENZYMES IN THE DETERMINATION OF THE MODE OF ACTION OF PENICILLINS AND Δ^3-CEPHALOSPORINS[1]

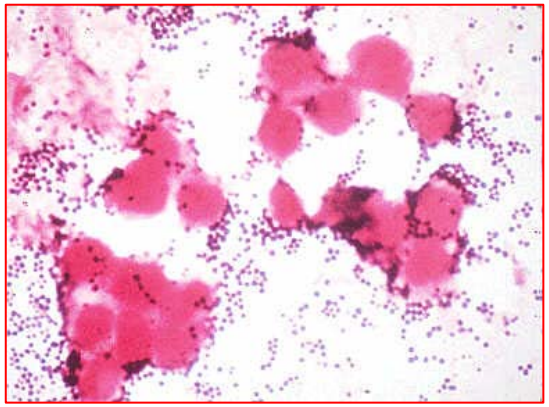
Jean-Marie Ghuysen, Jean-Marie Frère, Mélina Leyh-Bouille, Jacques Coyette, Jean Dusart, and Martine Nguyen-Distèche

Service de Microbiologie, Faculté de Médecine, Institut de Botanique, Université de Liège, 4000 Sart Tilman, Liège, Belgium

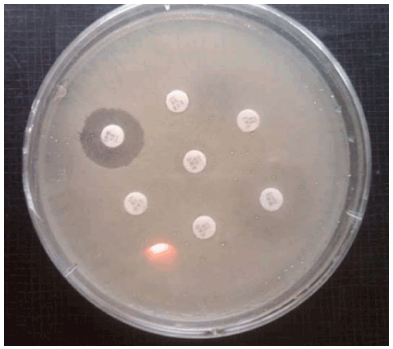
and died from invasive pneumococcal infection …

http://www.cip.ulg.ac.be/newsite/pdf/jmghuysen.pdf

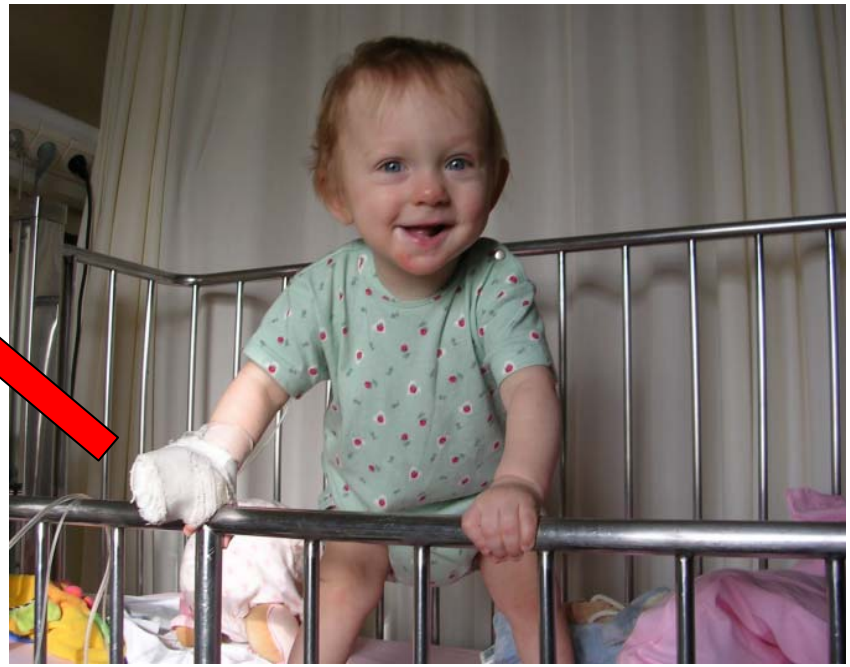
But what about this patient ?



Gram stain of *Staphylococcus aureus* in pustular exudate
http://www.tjclarkdirect.com/bacterial_diseases/staphylococcus.htm
Last accessed: 10/06/2014

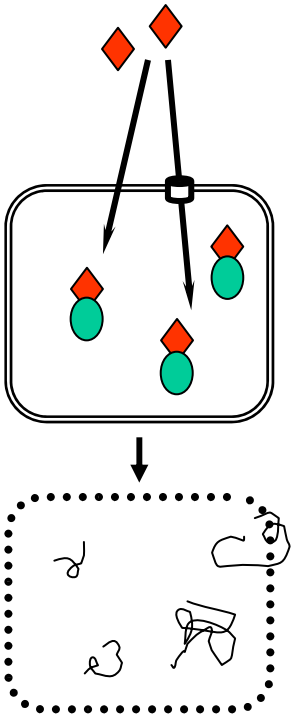


Etok et al. Aetiology and antimicrobial studies of surgical wound infections in University of Uyo Teaching Hospital (UUTH) Uyo, Akwa Ibom State, Nigeria. 1:341. doi:10.4172/scientificreports.341

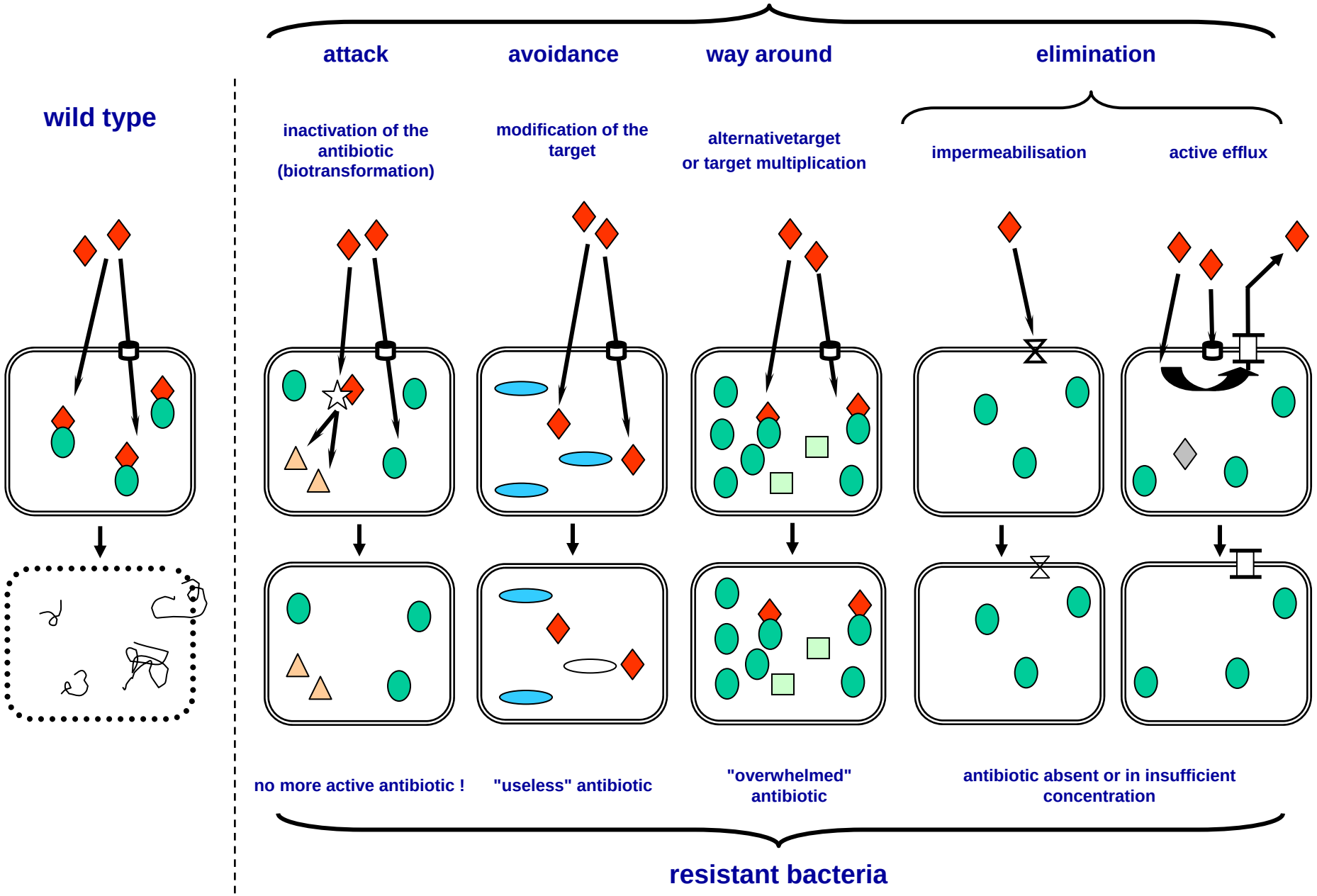


Which problem ?

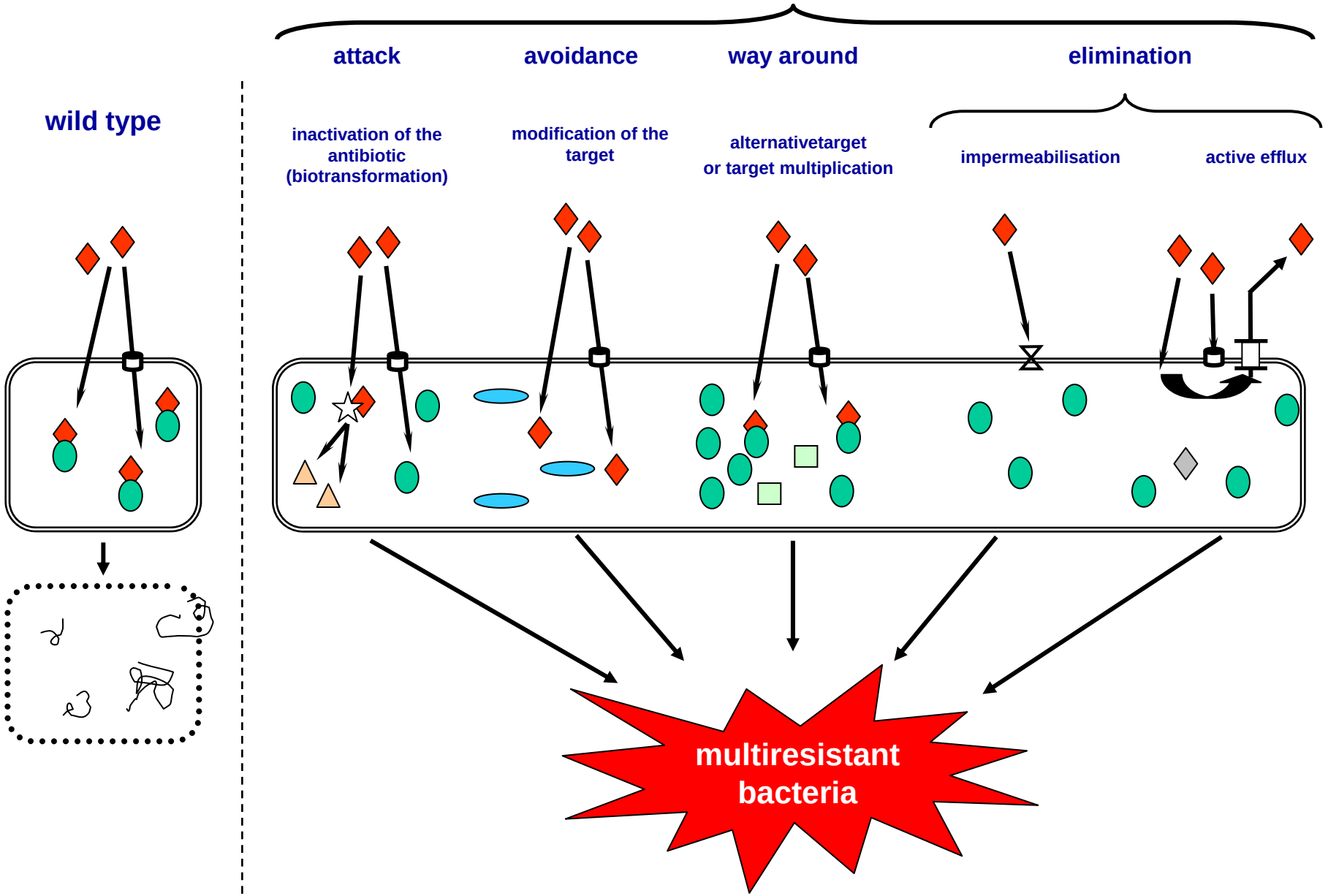
wild type



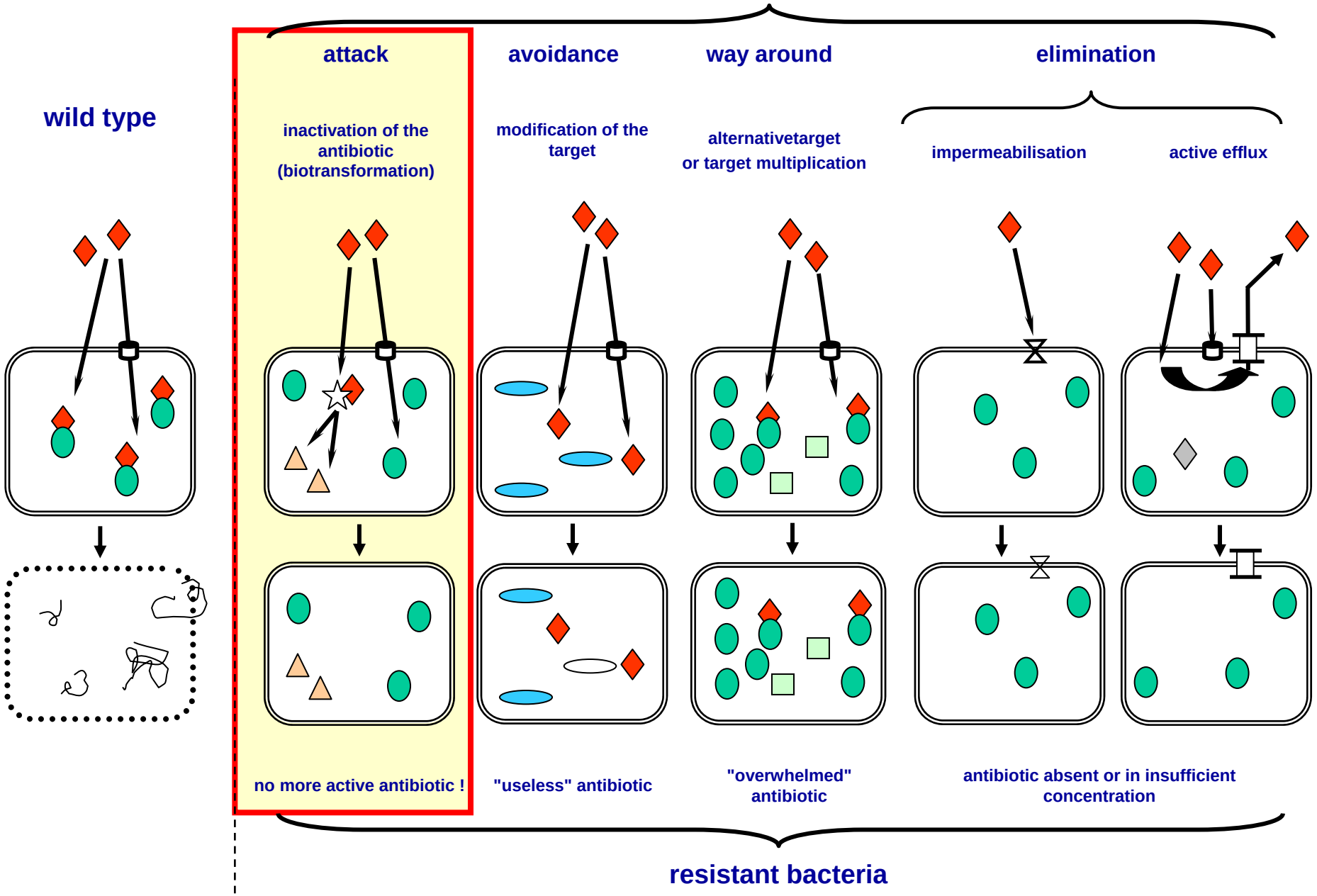
Which problem ?



But be aware ! Several mechanisms may coexist !



Which problem ?



Attack: the example of aminoglycosides

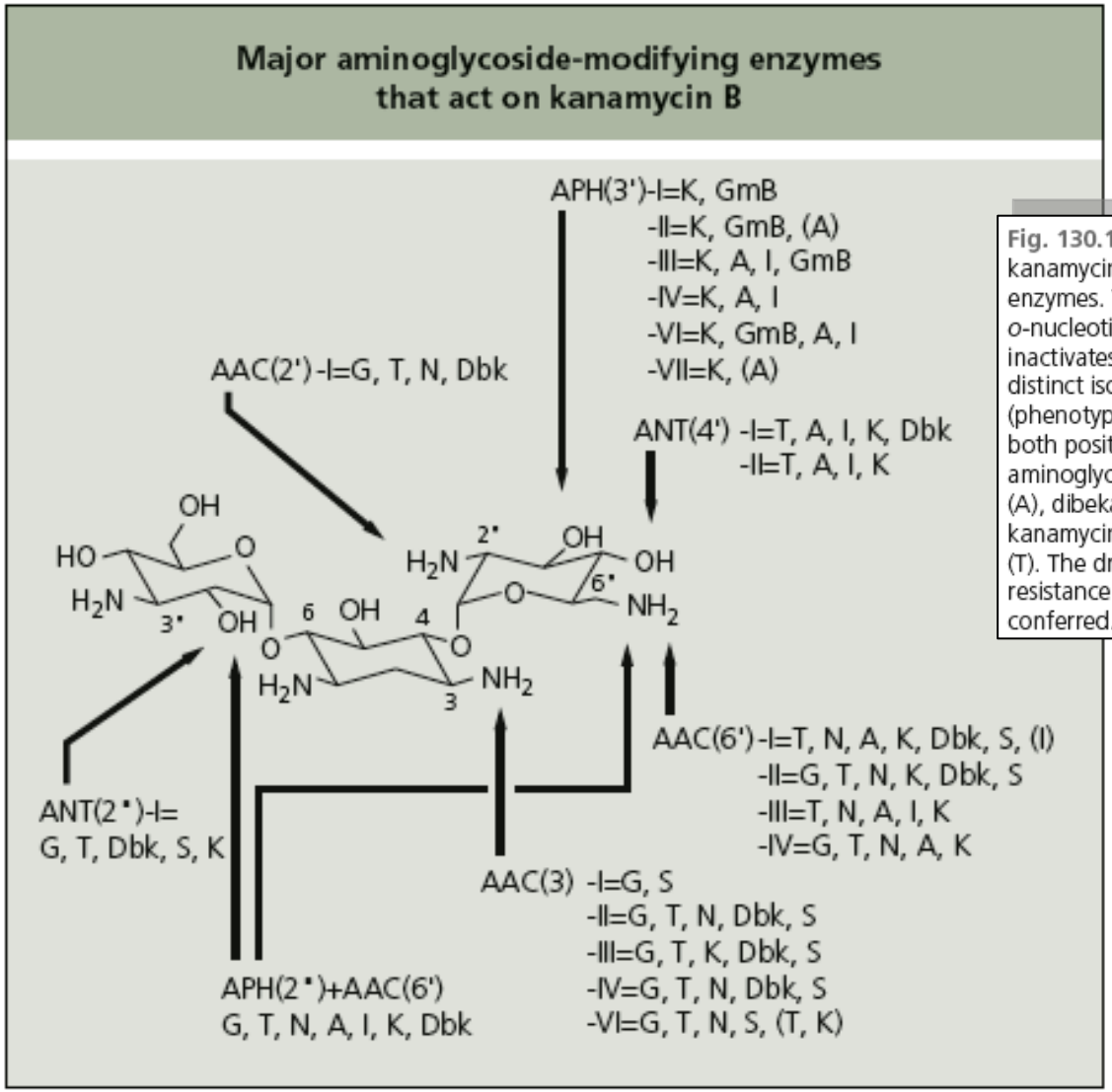
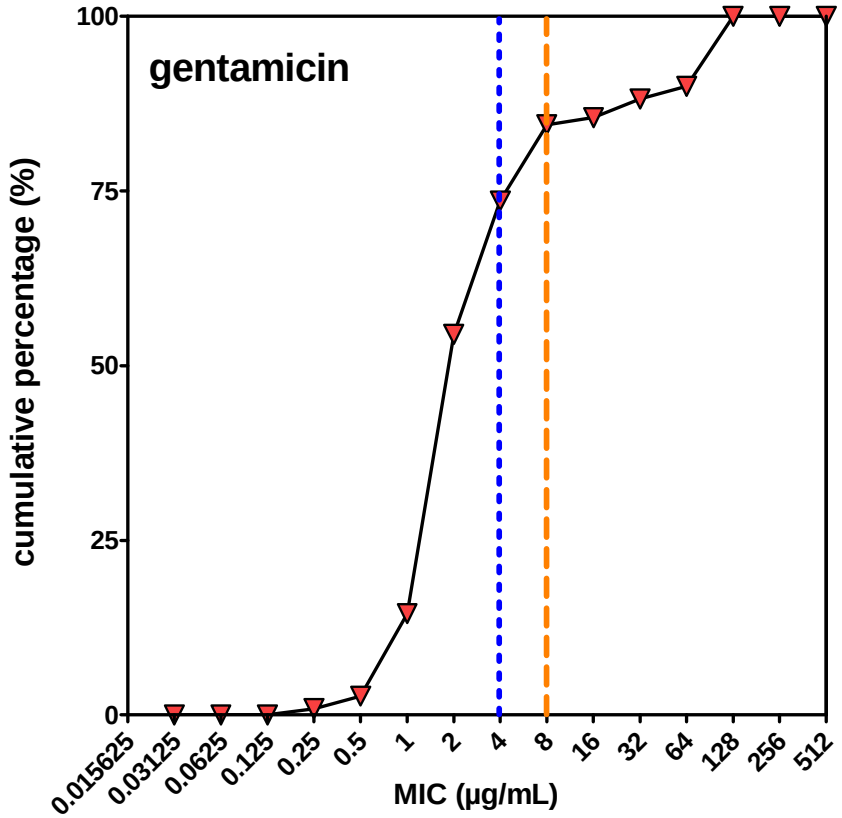
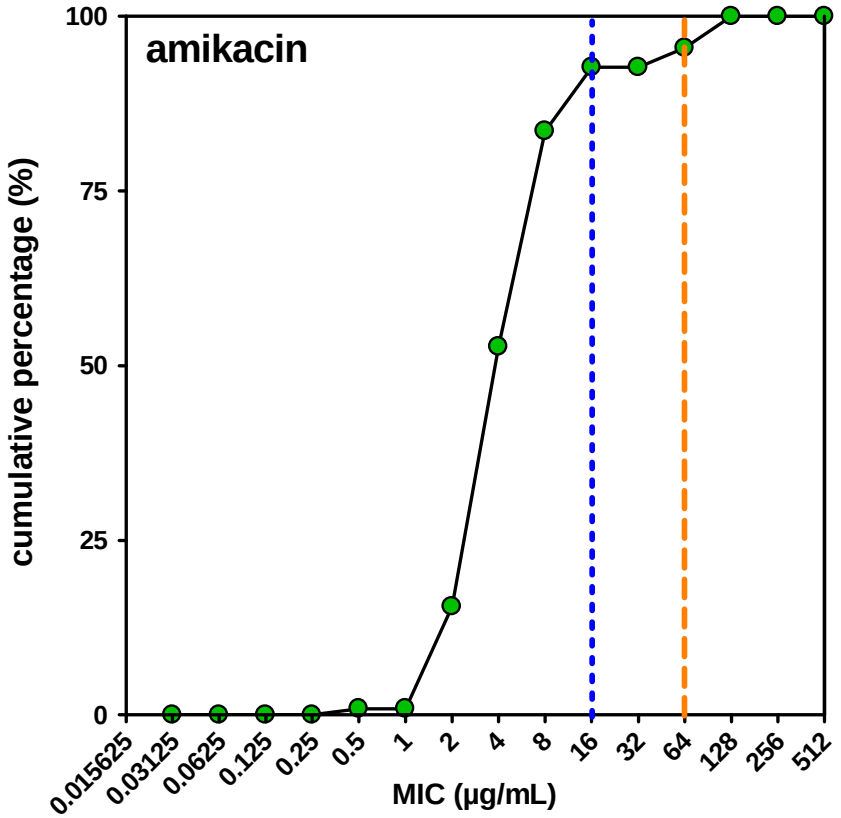


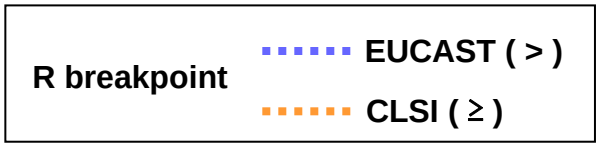
Fig. 130.10 Major aminoglycoside-modifying enzymes that act on kanamycin C. This aminoglycoside is susceptible to the largest number of enzymes. The *N*-acetyltransferases (AACs) affect amino functions and the *o*-nucleotidyltransferases affect hydroxyl functions. Each group of enzymes inactivates specific sites, but each of these sites can be acted upon by distinct isoenzymes (Roman numerals) with different substrate specificities (phenotypic classification). At least one enzyme is bifunctional and affects both positions 2'' (*o*-phosphorylation) and 6' (*N*-acetylation). The main aminoglycosides used clinically on which these enzymes act are amikacin (A), dibekacin (DbK), commercial gentamicin (G), gentamicin B (Gmb), kanamycin A (K), isepamicin (I), netilmicin (N), sisomicin (S) and tobramycin (T). The drug abbreviations that appear in parentheses are those for which resistance was detectable *in vitro* although clinical resistance was not conferred. Data from Shaw *et al.*[18]

Van Bambeke *et al*. Mechanisms of Action. In: Infectious Diseases (3d edition; J. Cohen, W. Powderly & S. Opal, eds), chapter 130, pp 1288-1307, Elsevier/Mosby, 2010

Aminoglycoside resistance in *Pseudomonas aeruginosa*:
an example in Belgium for patients with nososomial pneumonia

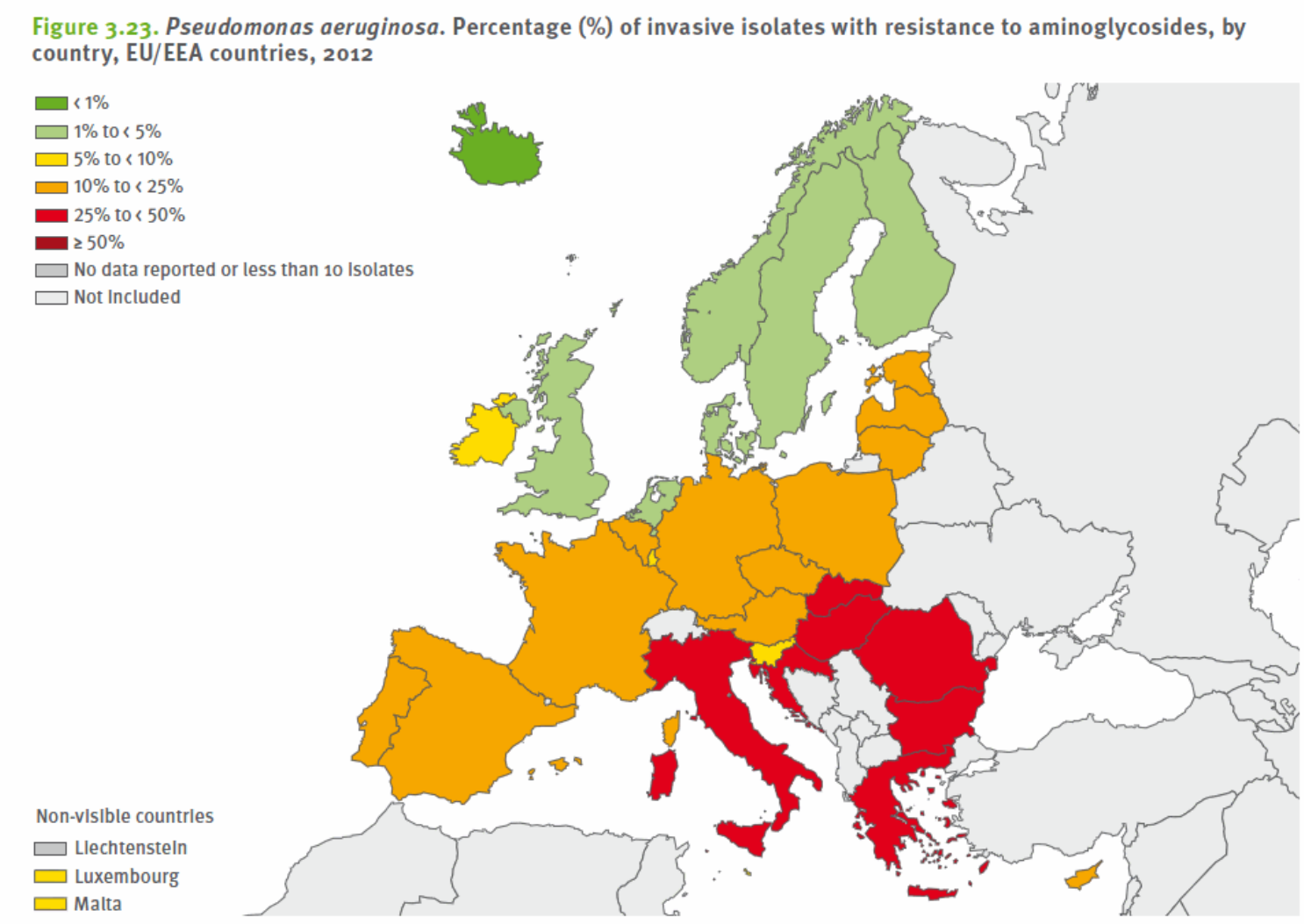






Data from Riou *et al*. Int J Antimicrob Agents. 2010 Dec;36(6):513-22

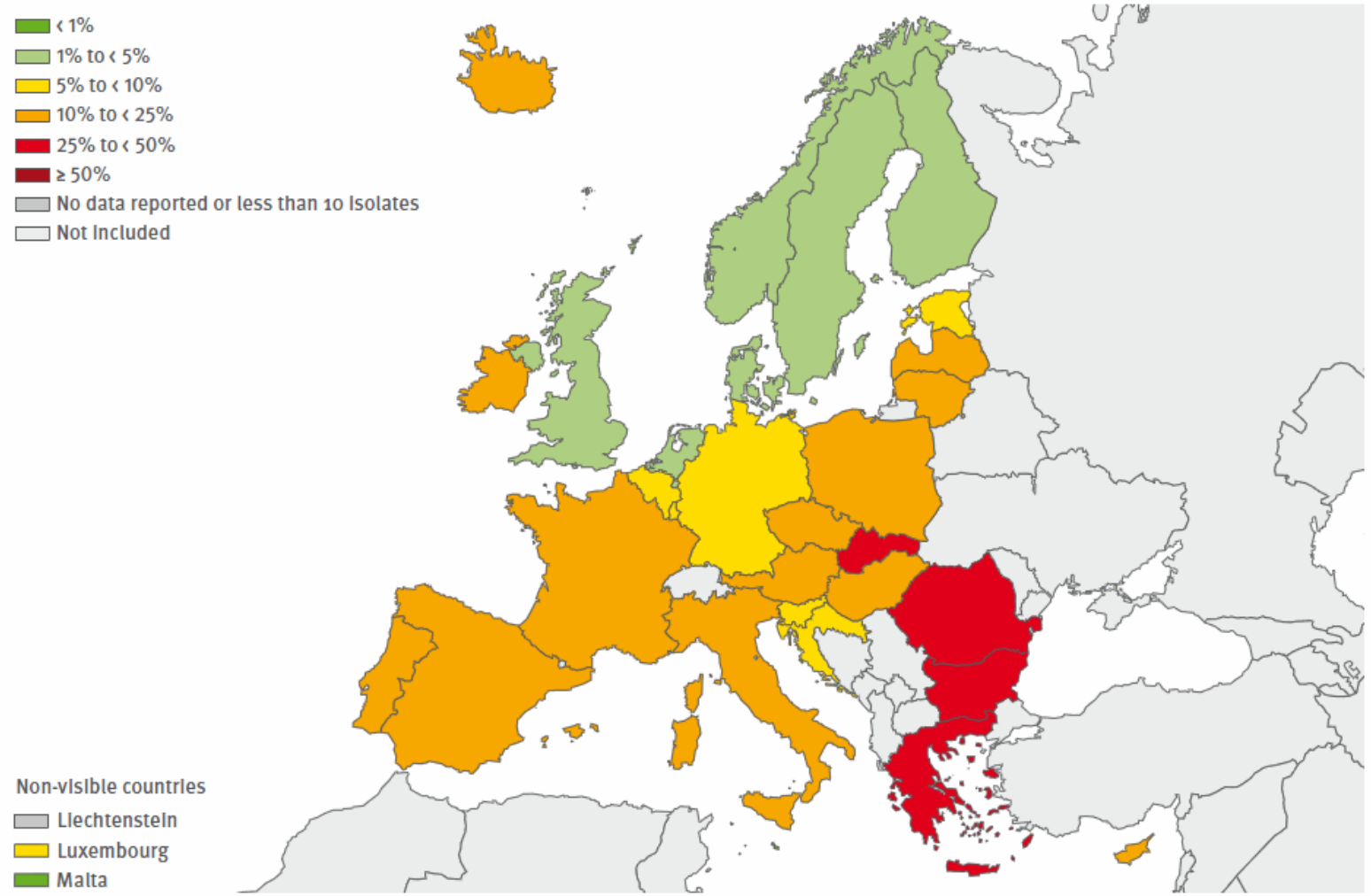
Aminoglycoside resistance in *Pseudomonas aeruginosa*: the situation may be worse elsewhere



European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2012. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: ECDC; 2013.

And co-resistance in *Pseudomonas aeruginosa* is frequent

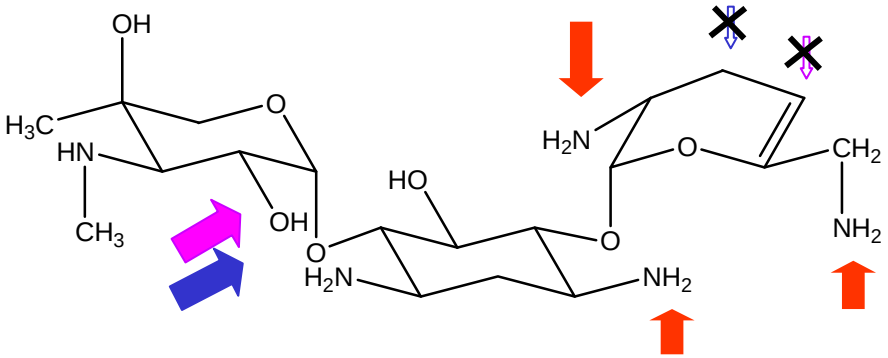
Figure 3.25. ***Pseudomonas aeruginosa*. Percentage (%) of invasive isolates with combined resistance (resistance to three or more antimicrobial classes among piperacillin (±tazobactam), ceftazidime, fluoroquinolones, aminoglycosides and carbapenems), by country, EU/EEA countries, 2012**



European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2012. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: ECDC; 2013.

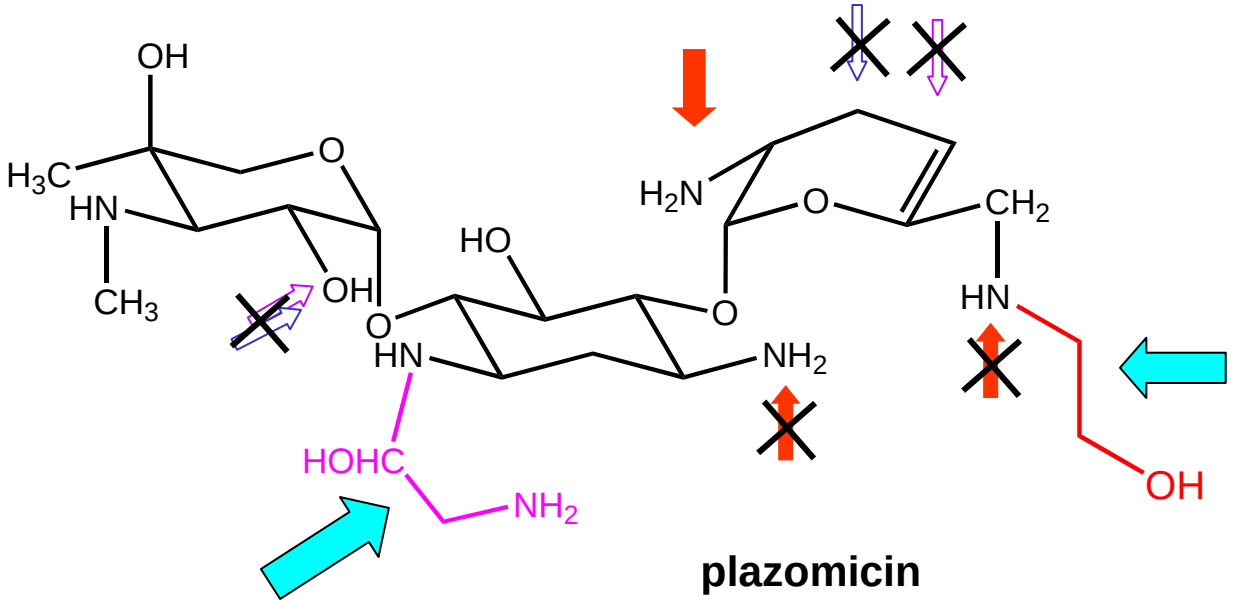
Aminoglycosides: can we do something ?

Plazomicin (ACHN-490): made from sisomicin



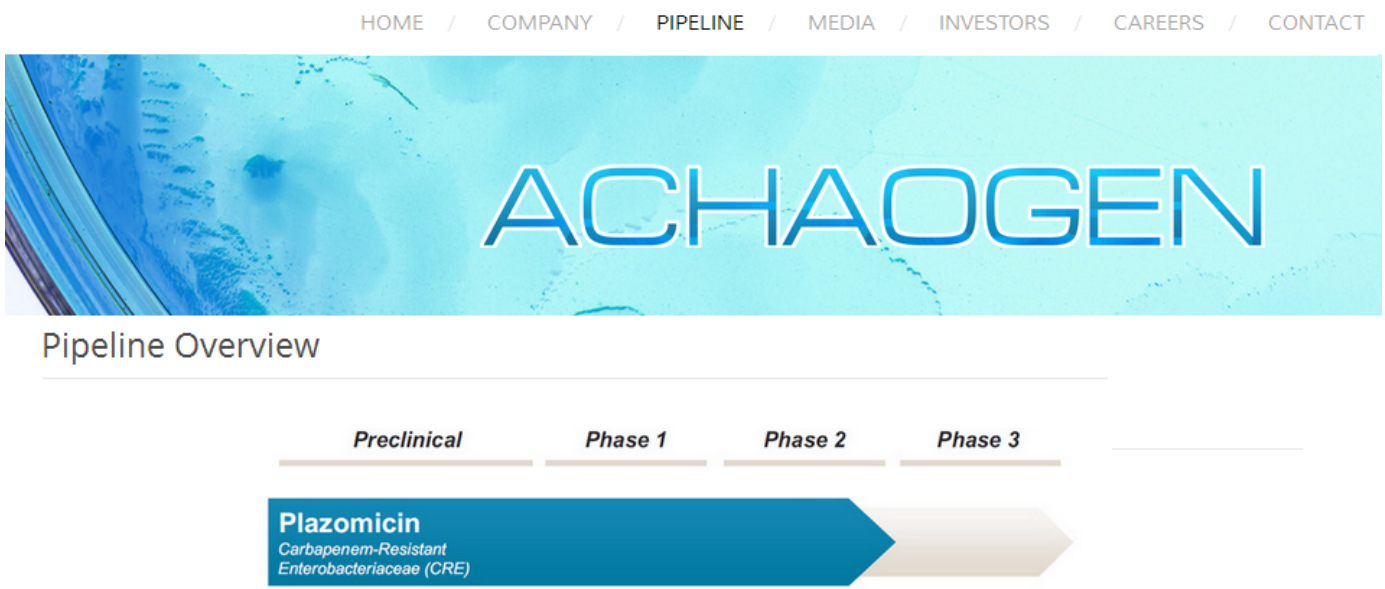
sisomicin

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



plazomicin

Plazomycin future ?



Potential to demonstrate a mortality benefit over currently available therapy in the treatment of life-threatening CRE infections.

***"*We have designed our pivotal Phase 3 trial for plazomicin as a superiority trial with a primary efficacy endpoint of all-cause mortality at 28 days. The trial will compare a plazomicin-based regimen versus a colistin-based regimen for the treatment of CRE bloodstream infections and pneumonia."**

http://www.achaogen.com/plazomicin/
Last visited: 10/06/2014

Attack: the example of the β-lactamases (part 1)

Table 130.1 Functional classification of β-lactamases

Group	Molecular class	Preferred substrates	Active β-lactams	Typical examples
Group 1: serine cephalosporinases not inhibited by clavulanic acid	C	Cephalosporins I, II, and III (>> cephalosporins IV, monobactams, penicillins)	Carbapenems Temocillin (cephalosporins III and IV; variable upon level of expression)	AmpC from Gram-negatives; variable upon the species
Group 2: serine β-lactamases				
2a: penicillinases inhibited by clavulanic acid	A	Penicillins (penicillin, ampicillin >> carbenicillin >> oxacillins)	Amoxicillin + clavulanic acid Cephalosporins Carbapenems	Penicillinases from Gram-positives
2b: broad-spectrum β-lactamases inhibited by clavulanic acid	A	Penicillins (penicillin, ampicillin >> carbenicillin >> oxacillins) Cephalosporins I and II	Cephalosporins III and IV Monobactams* Carbapenems Amoxicillin + clavulanic acid	TEM-1,TEM-2, SHV-1 from Enterobacteriaceae, *Haemophilus* spp., *Neisseria gonorrhoeae*
2be: extended spectrum β-lactamases inhibited by clavulanic acid (ESBL)	A	Penicillins Cephalosporins I II III (IV) Monobactams	Carbapenems Temocillin	TEM-3 to -29, 42, 43, 46–49, 52–58, 60, 61, 63, 65, 66, 72, 92 from Enterobacteriaceae SHV-2 to -9, 11–14, 18–22, 24 from *Klebsiella* spp. CTX-M-1 to CTX-M-54 (five phylogenetic groups) in Enterobacteriaceae K1-OXY from *Klebsiella oxytoca*
2br: broad spectrum β-lactamases with reduced binding to clavulanic acid	A	Penicillins	Most cephalosporins Monobactams* Carbapenems	TEM-30 to -41 (= IRT-1 to IRT-12), 44, 45, 50, 51, 59, 68, 73, 74, 76–79, 81–84 from *Escherichia coli* SHV-10 from *Klebsiella* spp.
2c: carbenicillin-hydrolyzing β-lactamases inhibited by clavulanic acid	A	Penicillins Carbenicillin (Cephalosporins I and II)	Piperacillin + tazobactam Cephalosporins III and IV Monobactams* Carbapenems	PSE-1, PSE-3, PSE-4 from *Pseudomonas aeruginosa*
2d: cloxacillin-hydrolyzing β-lactamases generally inhibited by clavulanic acid	D	Penicillins Cloxacillin Cephalosporins I and II	Carbapenems Cephalosporin III Monobactams* Piperacillin + tazobactam	OXA-1 to OXA-4 in Enterobacteriaceae OXA-2, OXA-10 (PSE-2) in *Pseudomonas aeruginosa* (pencillins, cefpirome, cefepime >> cephalosporins III) OXA-11 to -19, 28, 32, 45 are ESBLs in *P. aeruginosa* (R to Ceph 3, Ceph 4 and aztreonam) OXA-23, -24, -58 are carbapenemases in *Acinetobacter baumannii*

Van Bambeke *et al*. Mechanisms of Action. In: Infectious Diseases (3d edition; J. Cohen, W. Powderly & S. Opal, eds), chapter 130, pp 1288-1307, Elsevier/Mosby, 2010

Attack: the example of the β-lactamases (part 2)

Table 130.1 Functional classification of β-lactamases—cont'd

Group	Molecular class	Preferred substrates	Active β-lactams	Typical examples
2e: cephalosporinases inhibited by clavulanic acid	A	Cephalosporins I and II	Cephalosporins III and IV Monobactams* Penems	FPM-1 from *Proteus vulgaris* Cep-A from *Bacteroides fragilis*†
2f: carbapenem-hydrolyzing-nonmetallo β-lactamases	B	Most β-lactams, including carbapenems (low or high resistance level depending on enzyme, species and genetic environment)	Carbapenems Monobactams and β-lactam inhibitors (variable activity depending on type of enzyme, bacterial host and genetic environment)	SME-1 to -3 from *Serratia* spp. IMI-1/2 and NMC-A from *Enterobacter cloacae* KPC-1 to -4 from *Klebsiella* spp. other Enterobacteriaceae and *Pseudomonas* GES-1 to -11 in Enterobacteriaceae, *P. aeruginosa* and *A. baumannii*
Group 3: metallo-β-lactamases inhibited by EDTA	B	Most β-lactams, including carbapenems	Monobactams*‡	L-1, XM-A from *Stenotrophomonas maltophilia* CcrA from *B. fragilis* A2h, CphA from *Aeromonas hydrophila* IMP-1 to -23, VIM-1 to -18 in *Pseudomonas*, other Gram-negative nonfermenters and Enterobacteriaceae SPM-1, GIM-1, SIM-1, DIN-1 in *P. aeruginosa* and *A. baumannii*
Group 4: penicillinases not inhibited by clavulanic acid		Penicillins, including carbenicillin and oxacillins	Monobactams*‡ and generally carbapenems	SAR-2 from *B. cepacia*

*Monobactams are not active on Gram-positive bacteria.
†Penems are the only molecules active in this case.
‡Remain active for most of the rare published studies.
EDTA, ethylenediaminetetraacetic acid.
Compiled from [62, 63, 64, 65]. The number of enzymes as well as their spectrum of activity is continually evolving.

Van Bambeke *et al*. Mechanisms of Action. In: Infectious Diseases (3d edition; J. Cohen, W. Powderly & S. Opal, eds), chapter 130, pp 1288-1307, Elsevier/Mosby, 2010

β-lactamases: why so many ?

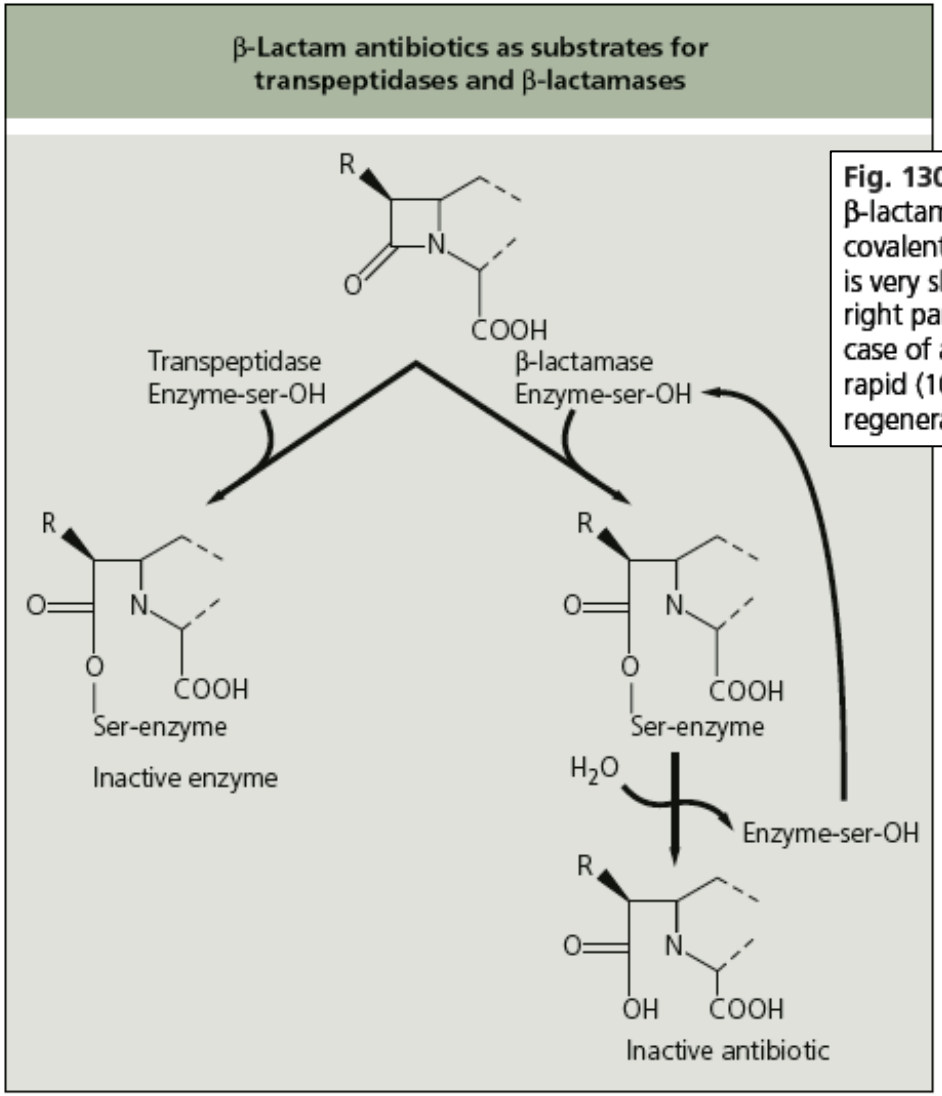


Fig. 130.3 β-Lactam antibiotics as substrates for transpeptidases and β-lactamases. The left part of the illustration shows how a β-lactam covalently binds to the transpeptidases. Hydrolysis of this acylated enzyme is very slow (one β-lactam per hour), making the enzyme inactive. The right part of the 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, Elsevier/Mosby, 2010

β-lactamases and PBPs may be very close

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

JMB

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

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]

β-lactamases and PBPs may be very close

doi:10.1016/j.jmb.2008.12.001

JMB

Avai

Structure of PBP-A
elongatus, a Penicil
to Class A β-Lactam

Carole Urbach[1]†, Christin
Jacques Fastrez[1], Patrice

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

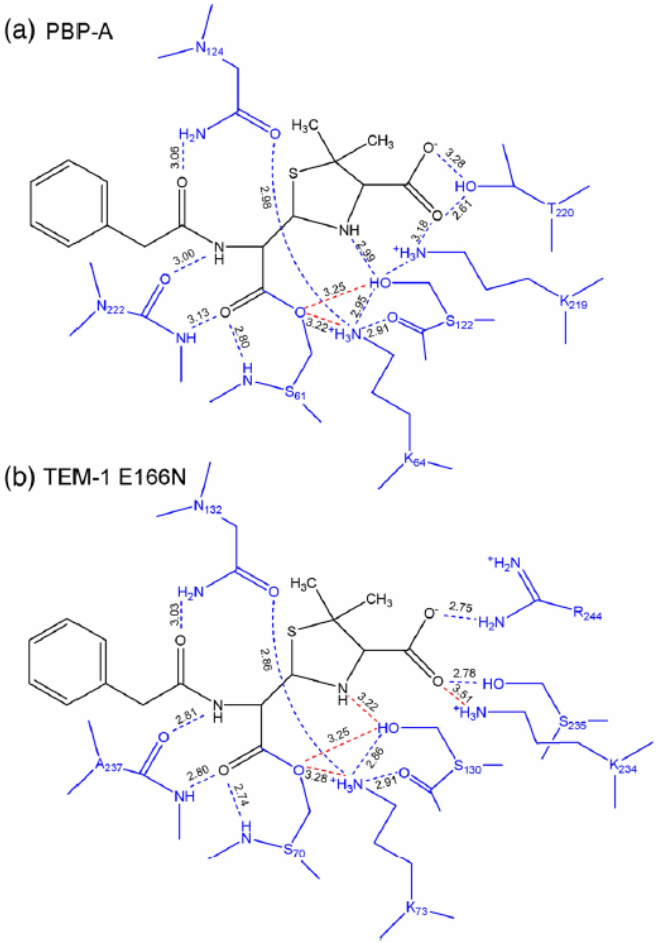


Fig. 5. Hydrogen bond network stabilizing the acyl–enzyme complex formed with benzylpenicillin and PBP-A (a) or TEM-1 E166N (b). Benzylpenicillin is black, residues from the enzymes are blue as well as hydrogen bonds, and red dot lines are weak hydrogen bonds.

and β-lactamases are often in mobile, higly transmissible genetic elements *(together with other resistance genes)*

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*

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

Review Article

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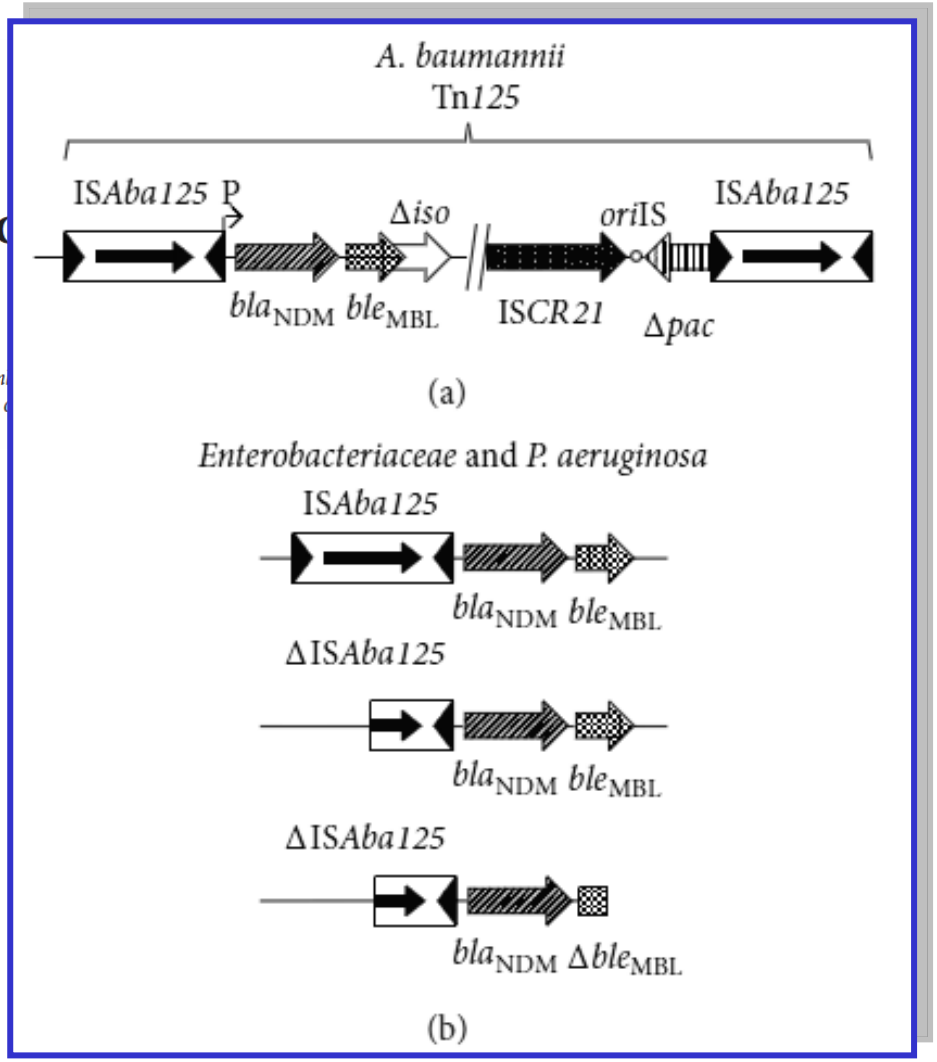
Gram-Negative Bacteria

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 Krem*
[2] *Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science, University*
3 Rue Albert Gockel, 1700 Fribourg, Switzerland



Schematic representation of *bla*$_{NDM}$-associated genetic structures identified among Gram-negative clinical isolates.

(a) Structure found in *A. baumannii (*part of the composite transposon Tn*125).*

(b) Structures found in Enterobacteriaceae and *P. aeruginosa* where IS*Aba125* is presented as full or truncated element with bleMBL gene also being present as full or truncated gene.

and β-lactamases are often in mobile, higly transmissible genetic elements *(together with other resistance genes)*

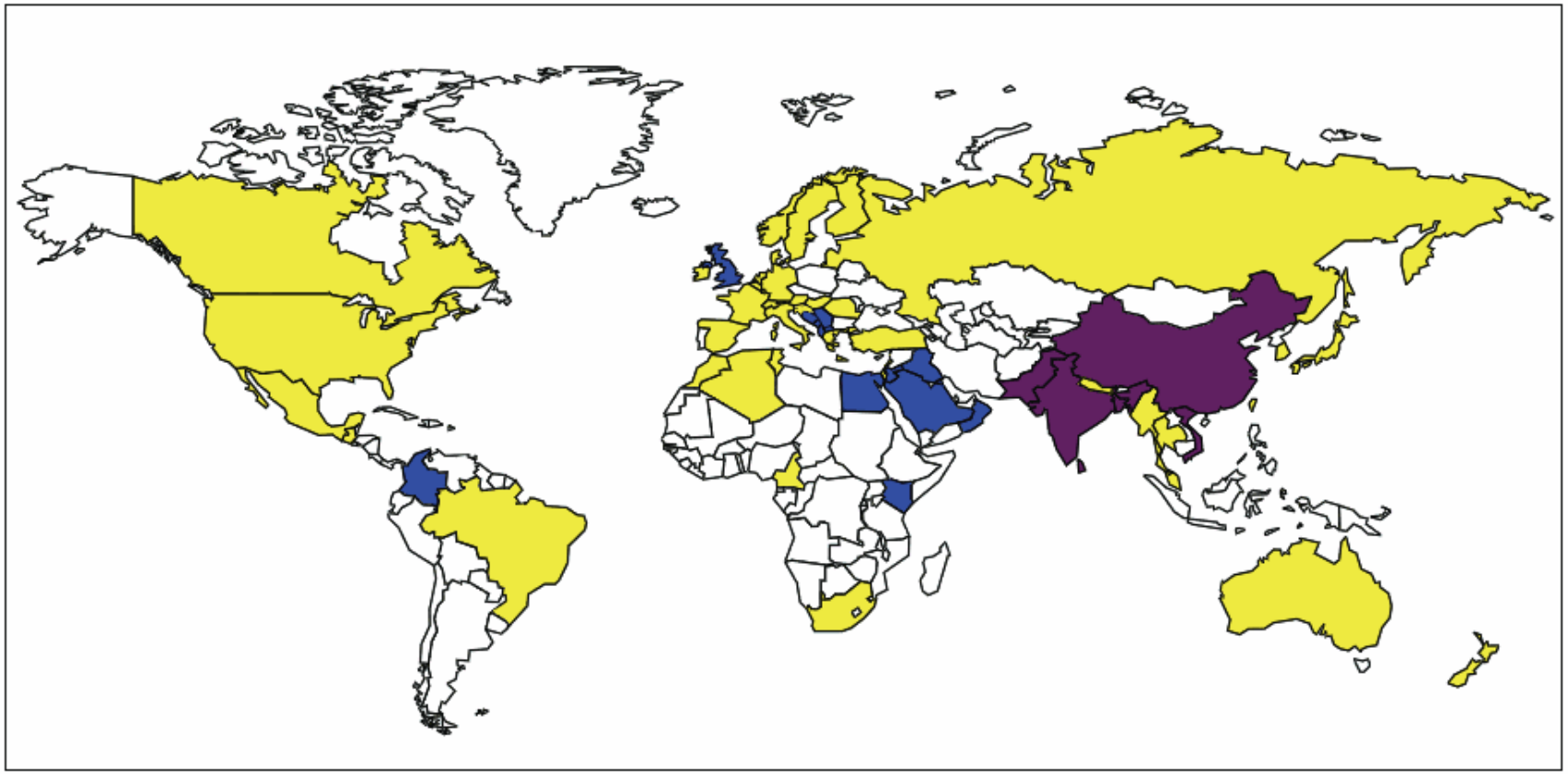


FIGURE 2: Geographical distribution of NDM producers.

and β-lactamases are often in mobile, higly transmissible genetic elements *(together with other resistance genes)*

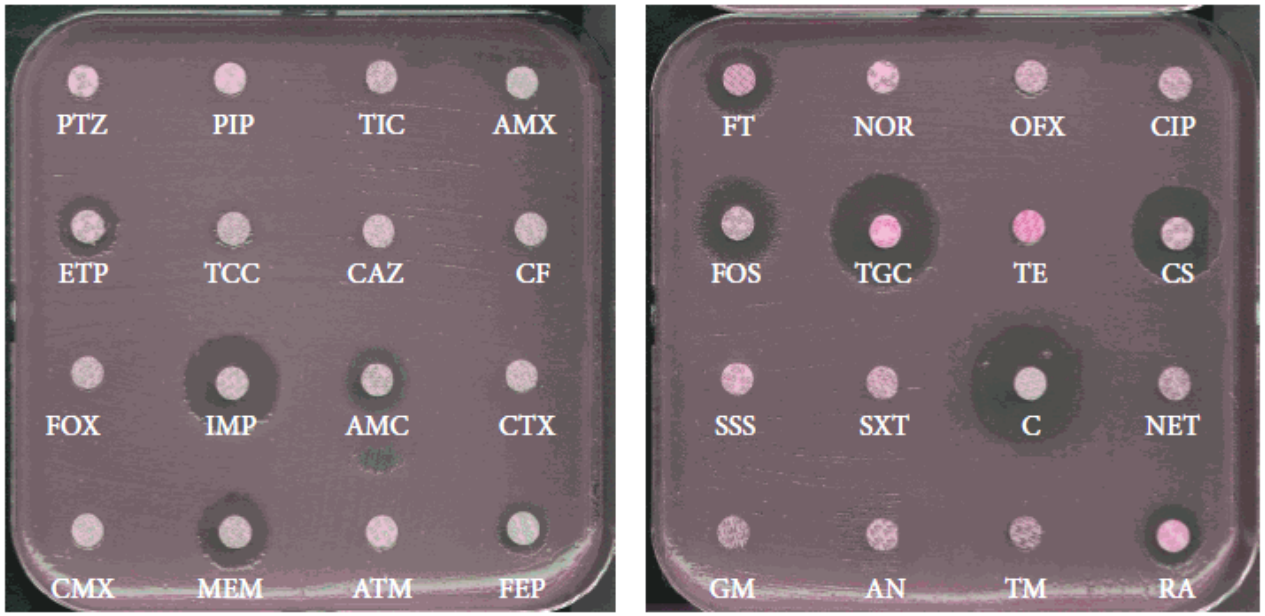
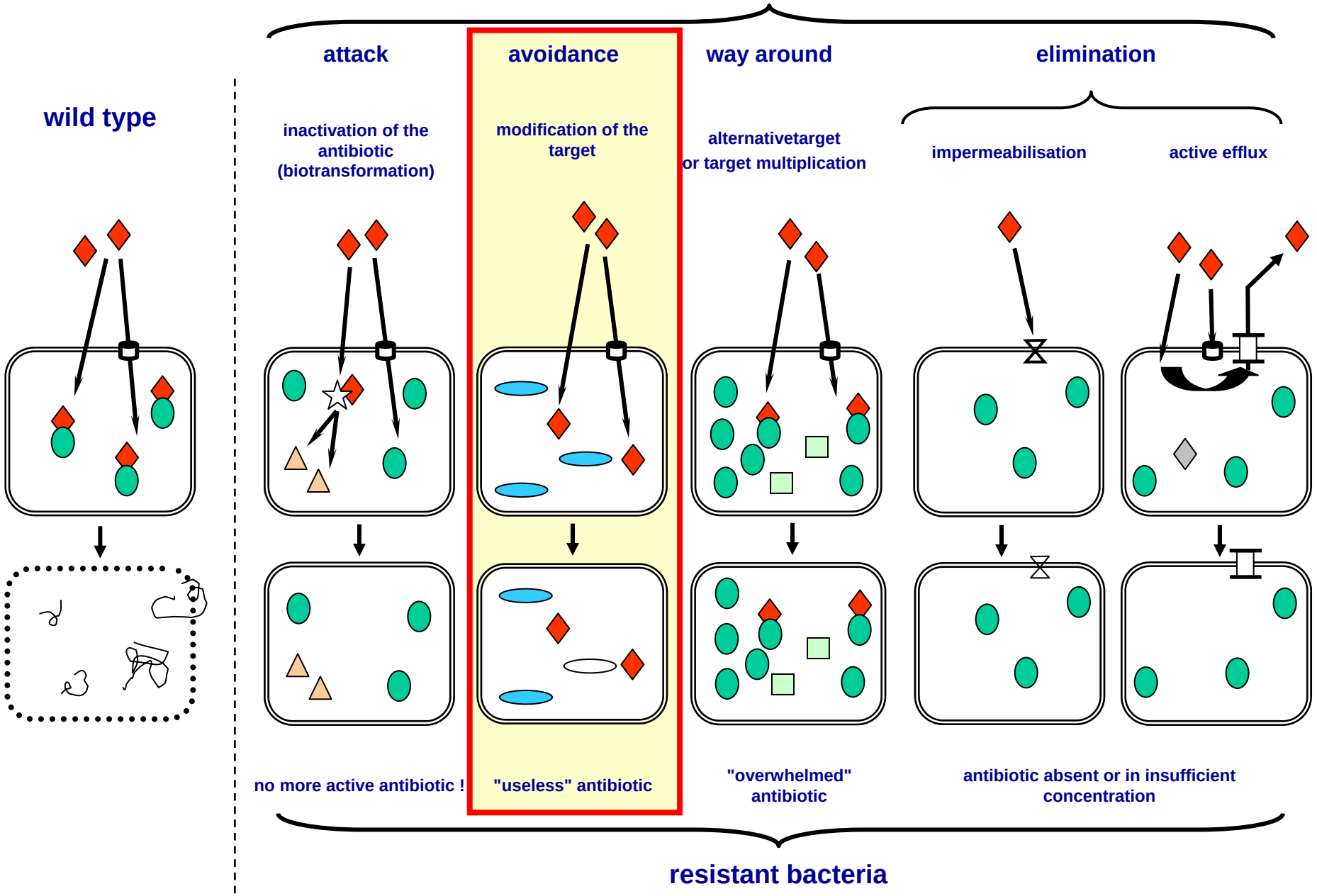
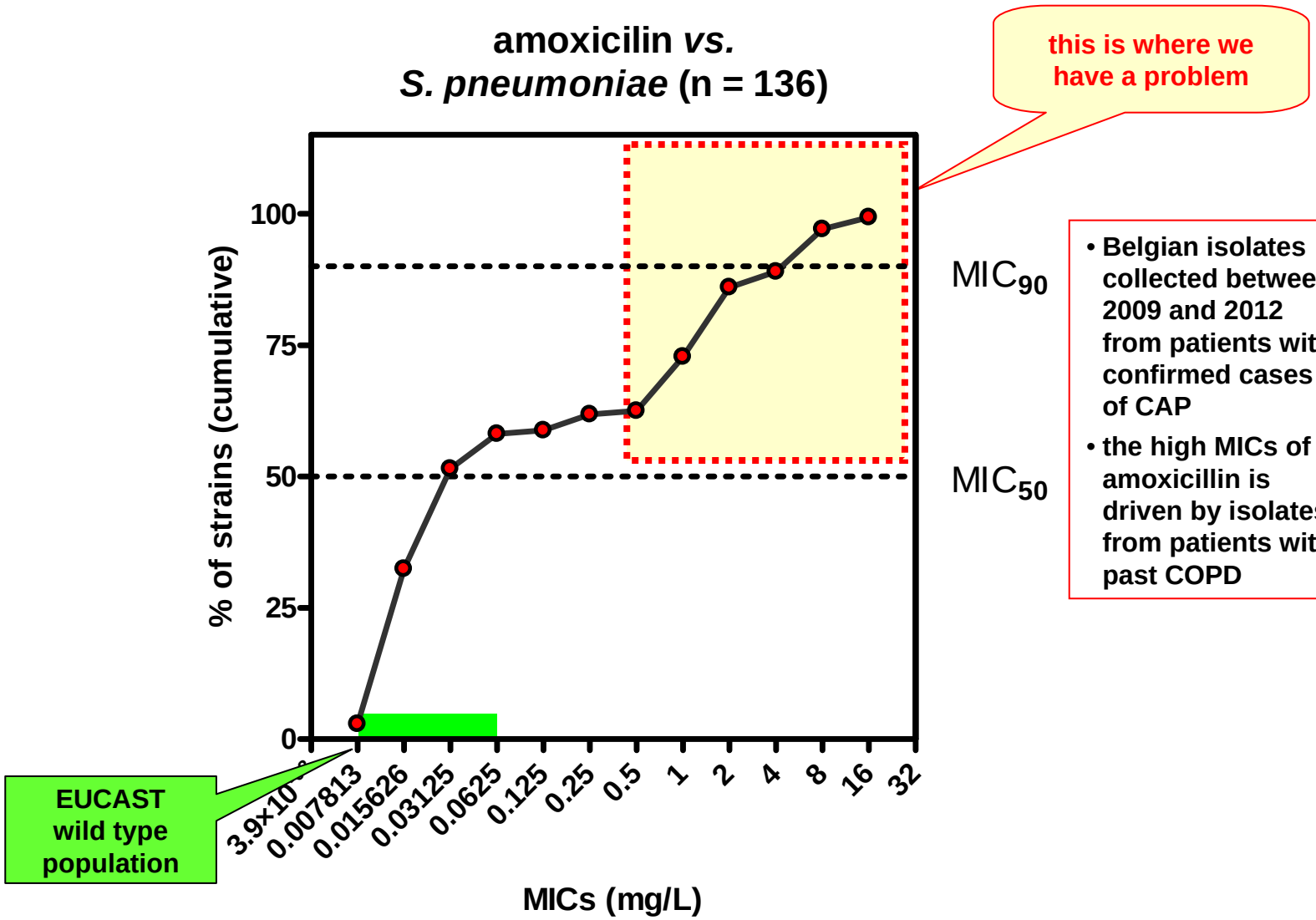


FIGURE 1: Antibiogram of a NDM-1-producing *K. pneumoniae* isolate. The $bla_{\text{NDM-1}}$ gene was located onto a IncHIIB plasmid of ca. ~200 kb in that strain that also harbored two additional β-lactamase genes ($bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-12}}$, $bla_{\text{OXA-1}}$) and an aminoglycoside methylase (*armA*) responsible for high-level resistance to all aminoglycosides. PTZ, piperacillin + tazobactam; PIP, piperacillin; TIC, ticarcillin; AMX, amoxicillin; ETP, ertapenem; TCC, ticarcillin + clavulanic acid; CAZ, ceftazidime; CF, cefalotin; FOX, cefoxitin; IMP, imipenem; AMC, amoxicillin + clavulanic acid; CTX, cefotaxime; CMX, cefuroxime; MEM, meropenem; ATM, aztreonam; FEP, cefepime; FT, nitrofurantoin; NOR, norfloxacin; OFX, ofloxacin; CIP, ciprofloxacin; FOS, fosfomycin; TGC, tigecycline; TE, tetracycline; CS, colistin; SSS, sulfonamide; SXT, sulfamethoxazole + trimethoprim; C, chloramphenicol; NET, netilmicin; GM, gentamicin; AN, amikacin; TM, tobramycin; RA, rifampicin.

Which problem ?



Amoxicllin and *Streptococcus pneumoniae*



- Belgian isolates collected between 2009 and 2012 from patients with confirmed cases of CAP
- the high MICs of amoxicillin is driven by isolates from patients with past COPD

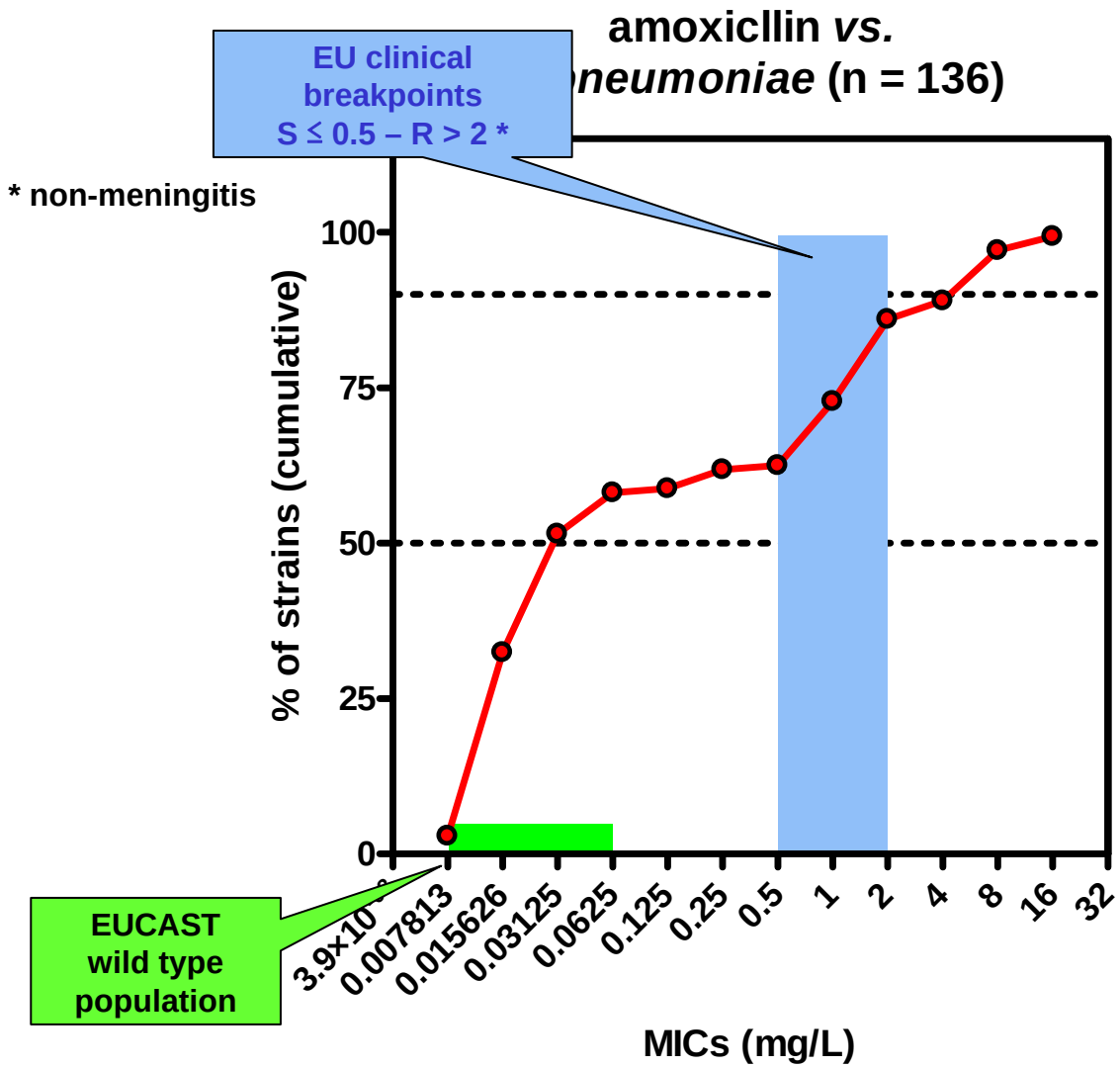
EUCAST: European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org)
MIC: minimum inhibitory concentration
CAP: community-acquired pneumonia
COPD: chronic obstructive pulmonary disease

Tulkens, unpublished

But which breakpoints do we need to use ?

To be honest, I always wondered ...

MIC distribution is a continuous variable…



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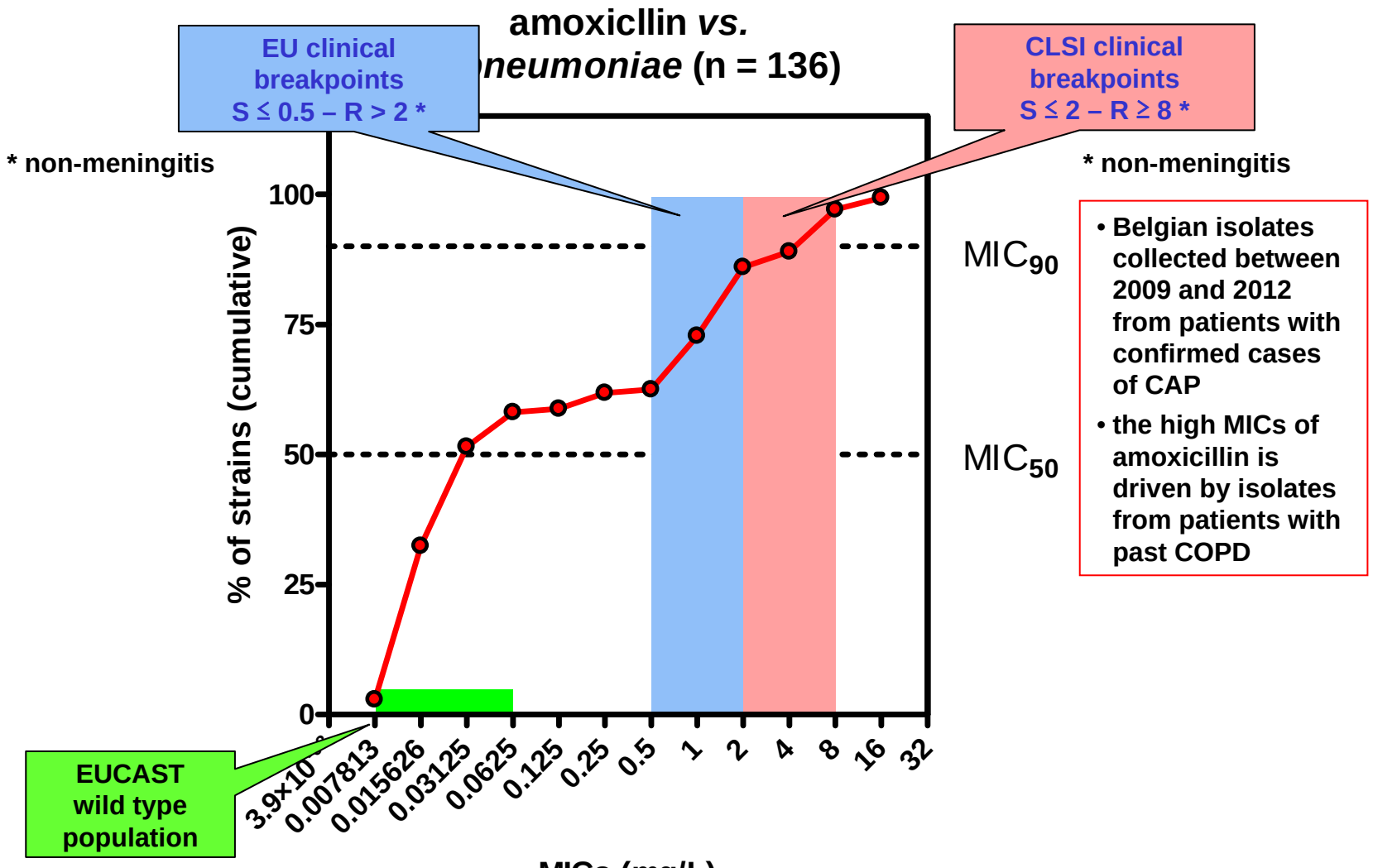
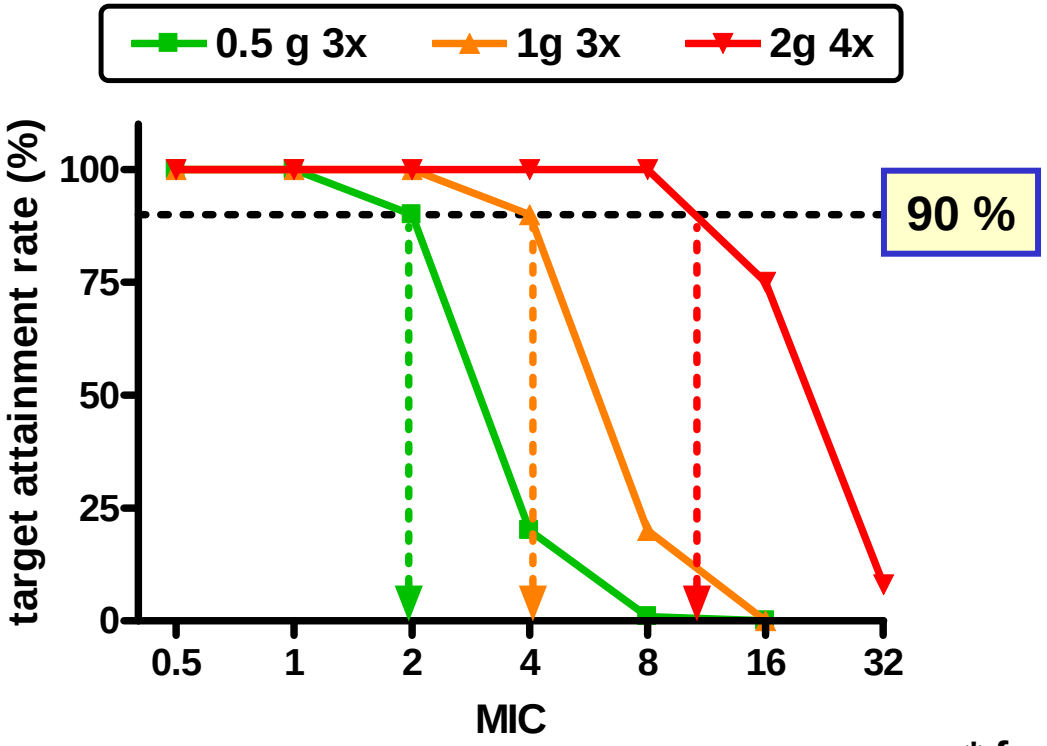


- Belgian isolates collected between 2009 and 2012 from patients with confirmed cases of CAP
- the high MICs of amoxicillin is driven by isolates from patients with past COPD

CLSI: Clinical and Laboratory Standards Institute (http://clsi.org)
EUCAST: European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org)
MIC: minimum inhibitory concentration
CAP: community-acquired pneumonia
COPD: chronic obstructive pulmonary disease

Tulkens, unpublished

EUCAST calculations of target attainment rate for amoxicillin against *S. pneumoniae*



*** for *f* T >MIC = 40%**

By increasing the dose and multiplying the number of daily administration, you may cover bacteria with MIC up to 8 mg/L… but the total daily dose will be very high…

Graph prepared from data in http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Rationale_documents/Amoxicillin_rationale_Nov2010_v_1.0.pdf

And chlidren may harbour more resistant organisms

Table 2
Antimicrobial susceptibility of all bloodstream isolates by age group, SENTRY 1997–2000

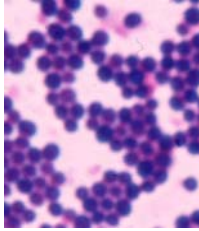
Organism	Antimicrobial	% Susceptible (number tested)						
		< 1 year	1–5 years	6–18 years	19–49 years	50–64 years	> 64 years	Overall
S. aureus	Oxacillin	80[a] (223)	85[b] (89)	87 (229)	74 (2181)	73 (1580)	65 (2200)	72 (6502)
Coagulase-negative staphylococci	Oxacillin	19[a] (400)	23 (99)	25 (122)	30 (947)	25 (661)	27 (1019)	26 (3248)
Enterococcus spp.	Vancomycin	100 (167)	90 (62)	87 (47)	86 (724)	83 (645)	85 (980)	86 (2625)
S. pneumoniae	Penicillin	62[a] (66)	64[a] (127)	73 (45)	77 (431)	78 (235)	75 (367)	74 (1271)
	Levofloxacin	100 (52)	100 (93)	100 (36)	100 (320)	99 (161)	99 (264)	> 99 (926)
E. coli	Ceftazidime	100 (193)	98 (41)	98 (100)	99 (1210)	99 (904)	99 (2013)	99 (4461)
	Cefepime	100 (193)	100 (41)	100 (100)	99 (1210)	99 (904)	99 (2012)	> 99 (4460)
	Ciprofloxacin	99 (192)	100 (41)	98 (100)	97 (1210)	96 (904)	97 (2012)	97 (4459)
Klebsiella spp.	Ceftazidime	98 (94)	95 (39)	89 (46)	96 (520)	96 (489)	97 (718)	96 (1906)
	Cefepime	100 (94)	97 (39)	96 (46)	99 (520)	99 (489)	99 (718)	99 (1906)
P. aeruginosa	Ceftazidime	83 (48)	90 (29)	85 (40)	81 (302)	88 (281)	87 (412)	85 (1112)
	Cefepime	94 (48)	97 (29)	83 (40)	84 (302)	90 (281)	89 (412)	88 (1112)
	Imipenem	96 (48)	83 (29)	93 (40)	89 (302)	90 (281)	94 (412)	90 (1112)
	Ciprofloxacin	100 (48)	97 (29)	95 (40)	81 (302)	87 (281)	86 (412)	86 (1112)
Enterobacter spp.	Ceftazidime	72 (90)	74 (31)	75 (40)	82 (305)	72 (208)	79 (243)	77 (917)
	Cefepime	100 (90)	97 (31)	98 (40)	99 (305)	100 (208)	99 (243)	> 99 (917)

[a] $P < 0.01$ for MIC distribution compared to 19–49, 50–64 and > 64 years age groups for same organism.
[b] $P = 0.005$ for MIC distribution compared to > 64 years age group for same organism.

* longitudinal surveillance program designed to track antimicrobial resistance trends nationally and internationally over a 5- to 10-year period and sponsored by Bristol-Myers Squibb

Diekema et al. Int. J. Antimicrob. Agents 2002; 412-418

Staphylococcus aureus



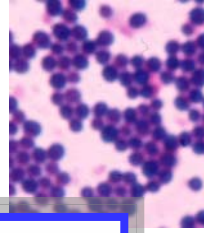
http://www.microbeworld.org/index.php?option=com_jlibrary&view=article&id=7611

- Nosocomial pneumonia involving hospital-acquired (HA) *S. aureus* is becoming increasingly frequent [1,2]
- In parallel, pneumonia caused by community-acquired (CA) MRSA while remaining rare in Europe[2] are becoming common in several other parts of the world including Asia [3]
- As many strains (even MSSA) produce toxins, they cause major tissue damage, and, hence a high mortality [3,4,5]

MRSA methicillin-resistant *Staphylococcus aureus*
MSSA methicillin-sensitive *Staphylococcus* aureus

1. Jones, *Clin Infect Dis.* 2010;51(suppl 1):S81-7
2. Valour, *et al Rev Pneumol Clin*. 2013;69:368-82
3. Karampela, *et al. Minerva Anestesiol*. 2012 Aug;78(8):930-40
   Kang & Song. *Infect Chemother.* 2013;45:22-31
4. Papazian & Donati. Nosocomial pneumonia. *In* Infectious Diseases, 3rd Edition, Cohen, Powderly & Opal, eds. Elsevier
   (available on line at http://www.expertconsultbook.com ; last visisted: 4 April 2014)
5. Catena, *et al Infez Med*. 2012;20:205-10 /.

S. aureus



- Nosocomial pneum
  S. aureus is becom
- In parallel, pneumo
- A
  major tissue dama

"***S. aureus* accounts for 2 to 5% of the etiologies of community-acquired pneumonia"**

"***S. aureus* represents 20 to 30% of cases of hospital-acquired pneumonia, including ventilator-associated pneumonia"**

1. Jones Cli S81-7
2.

Revue de Pneumologie clinique (2013) xxx, xxx–xxx

ELSEVIER MASSON

Disponible en ligne sur ScienceDirect www.sciencedirect.com

Elsevier Masson France EM|consulte www.em-consulte.com

SÉRIE : MICROBIOLOGIE ET APPAREIL RESPIRATOIRE

Infections broncho-pulmonaires à *Staphylococcus aureus*

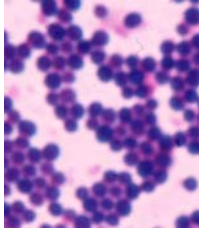
Staphylococcus aureus broncho-pulmonary infections

F. Valour[a,b,c,d], N. Chebib[a], Y. Gillet[b,c,d,e], P. Reix[b,f], F. Laurent[b,c,d,g], C. Chidiac[a,b,c,d], T. Ferry[a,*,b,c,d]

[a] *Service des maladies infectieuses et tropicales, hospices civils de Lyon, hôpital de la Croix-Rousse, groupement hospitalier Nord, 103, Grande-Rue-de-la-Croix-Rousse, 69004 Lyon, France*
[b] *Université Claude-Bernard Lyon 1, 69008 Lyon, France*
[c] *Inserm U1111, CNRS UMR5308, ENS de lyon, UCBL1, Centre international de recherche en infectiologie (CIRI), 69007 Lyon, France*
[d] *Centre national de référence des staphylocoques, hospices civils de Lyon, 69008 Lyon, France*
[e] *Service d'urgences pédiatriques, hospices civils de Lyon, hôpital Femme–Mère-Enfant, 69500 Bron, France*
[f] *Service de pneumologie, allergologie, mucoviscidose, hospices civils de Lyon, hôpital Femme–Mère-Enfant, 69500 Bron, France*
[g] *Laboratoire de bactériologie, hospices civils de Lyon, groupement hospitalier Nord, 69004 Lyon, France*

Valour, *et al Rev Pneumol Clin*. 2013;69:368-82

S. aureus



http://www.microbeworld.org/index.php?option=com_jlibrary&view=article&id=7611

- Nosocomial pne… HA)
  S. aureus is be…
- In parallel, pneu… ed
  (CA) MRSA wh… mon
  in several other…
- As many strains… ause
  major tissue da…

Table 4. Frequency of Bacterial Pathogens Associated with Hospital-Acquired Bacterial Pneumonia (HABP) and Ventilator-Associated Bacterial Pneumonia (VABP)

Organism	Percentage of isolates (no) HABP (n = 835)	VABP (n = 499)
MRSA	47.1 (48.6)	42.5 **(34.4)**
Pseudomonas species	18.4	21.2
Klebsiella species	7.1	8.4
Haemophilus species	5.6	**12.2**
Enterobacter species	4.3	5.6
Streptococcus pneumonaie	3.1	5.8
Acinetobacter species	2.0	3.0

NOTE. Data are from [2, 7]. Boldface indicates a significant change or difference in incidence compared with HABP. MRSA, methicillin-resistant *Staphylococcus aureus*.

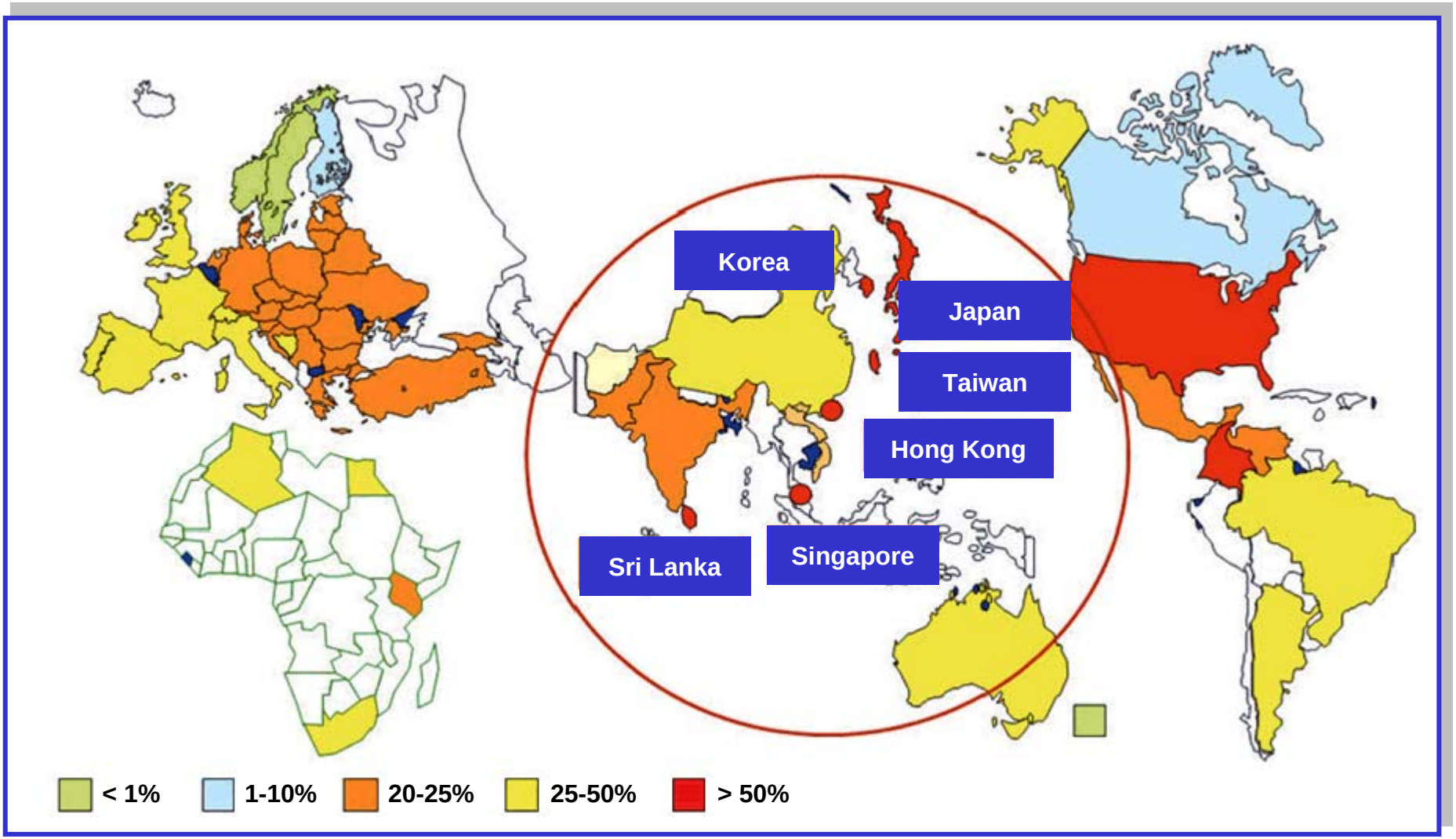
1. Jones Clin Infect Dis 2010;51(suppl 1…
2. Karampela et al Minerva Anestesiol. …
3. Papazian & Donati Nosocomial pneu… at
   http://www.expertconsultbook.com (L… 2013;69:368-82.

HABP: hospital-acquired bacterial pneumonia
VABP: ventilator-associated bacterial pneumonia
MRSA: methicillin resistant *Staphylococcus aureus*

Jones, *et al. Clin Infect Dis*. 2010;51(suppl 1):S81-7

MRSA in Asia

Prevalence of methicillin resistance among *S. aureus* isolates.
Some Asian countries have shown the highest prevalence rates of MRSA

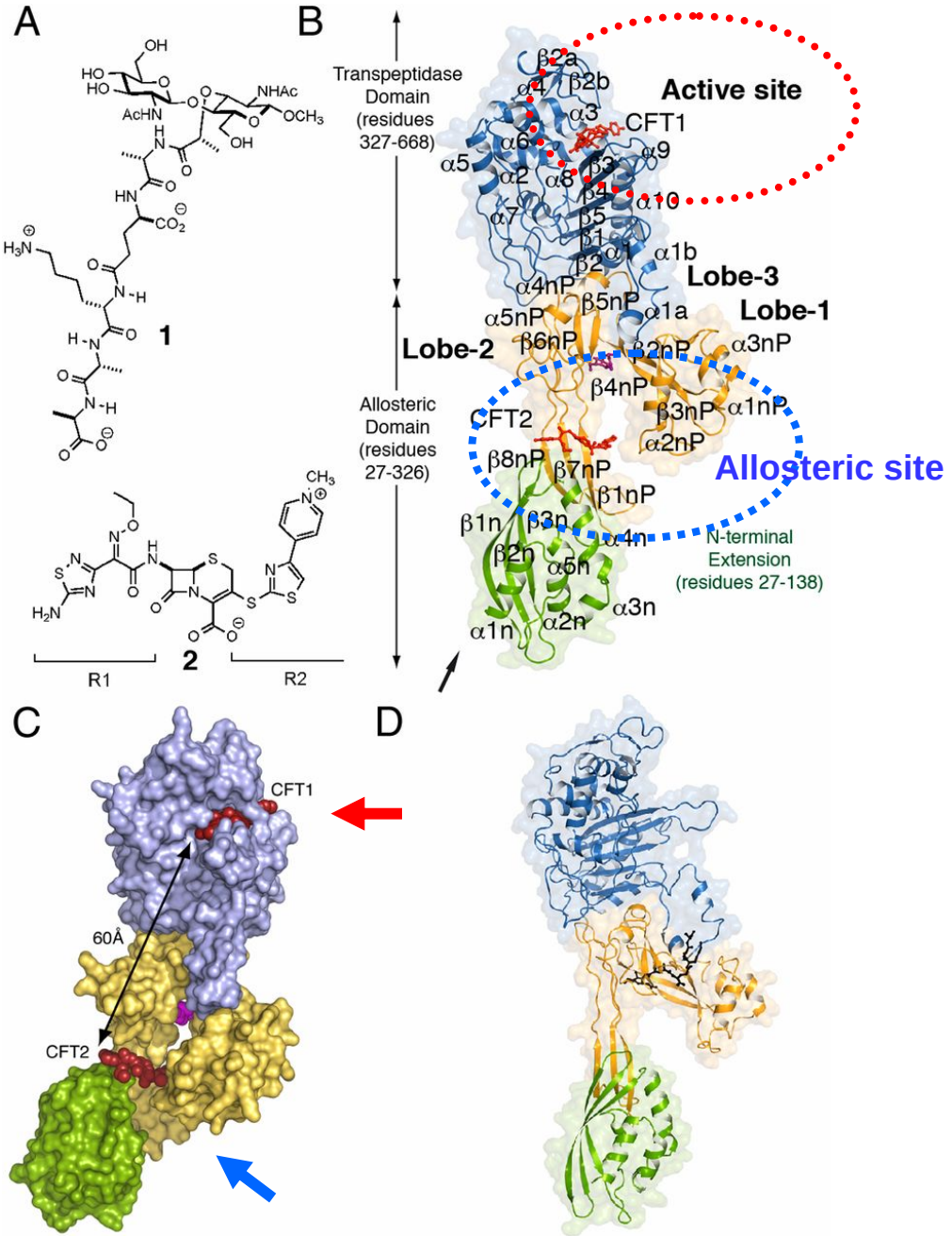


MRSA **methicillin restistant *Staphylococcus aureus***

Kang & Song. *Infect Chemother* 2013;45:22-31

Could ceftaroline (recently approved) be a solution ?

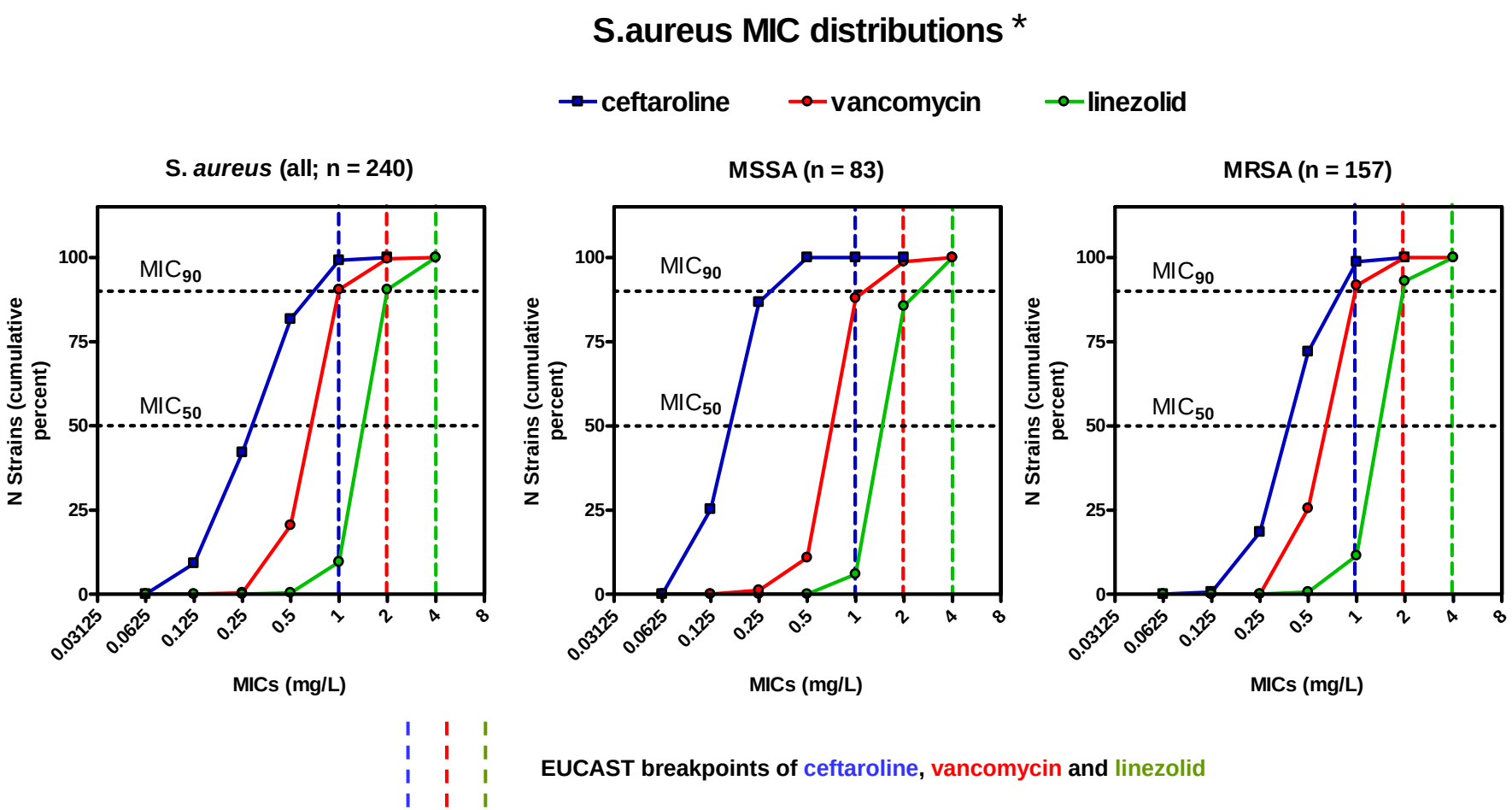
An allosteric mechanism !



Otero et al. Proc Natl Acad Sci USA. 2013 Oct 15;110(42):16808-13.

Fig. 1. Domains of PBP2a and key ligands. (*A*) The chemical structures of a synthetic NAG-NAM(pentapeptide) (1) and ceftaroline (2). The R1 and R2 groups of 2 are labeled. (*B*) Ribbon representation of PBP2a acylated by ceftaroline. The N-terminal extension is colored in green, the remaining allosteric domain is colored in gold, and the transpeptidase (TP) domain is colored in blue. These domain colors are retained in all other figures. Two molecules of ceftaroline (capped sticks in red) are found in complex with protein: one covalently bound as an acyl-enzyme in the TP domain (CFT1) and one intact at the allosteric domain (CFT2). A muramic acid saccharide (capped sticks in magenta) is found at the center of the allosteric domain. The arrow indicates the point of attachment of the membrane anchor. (*C*) The solvent-accessible surface representation for PBP2a is shown. The distance between the two ceftaroline molecules is 60 Å. (*D*) Ribbon representation of PBP2a in complex with 1 (black sticks). This view is rotated ~45° on the *y* axis compared with the view of *C*.

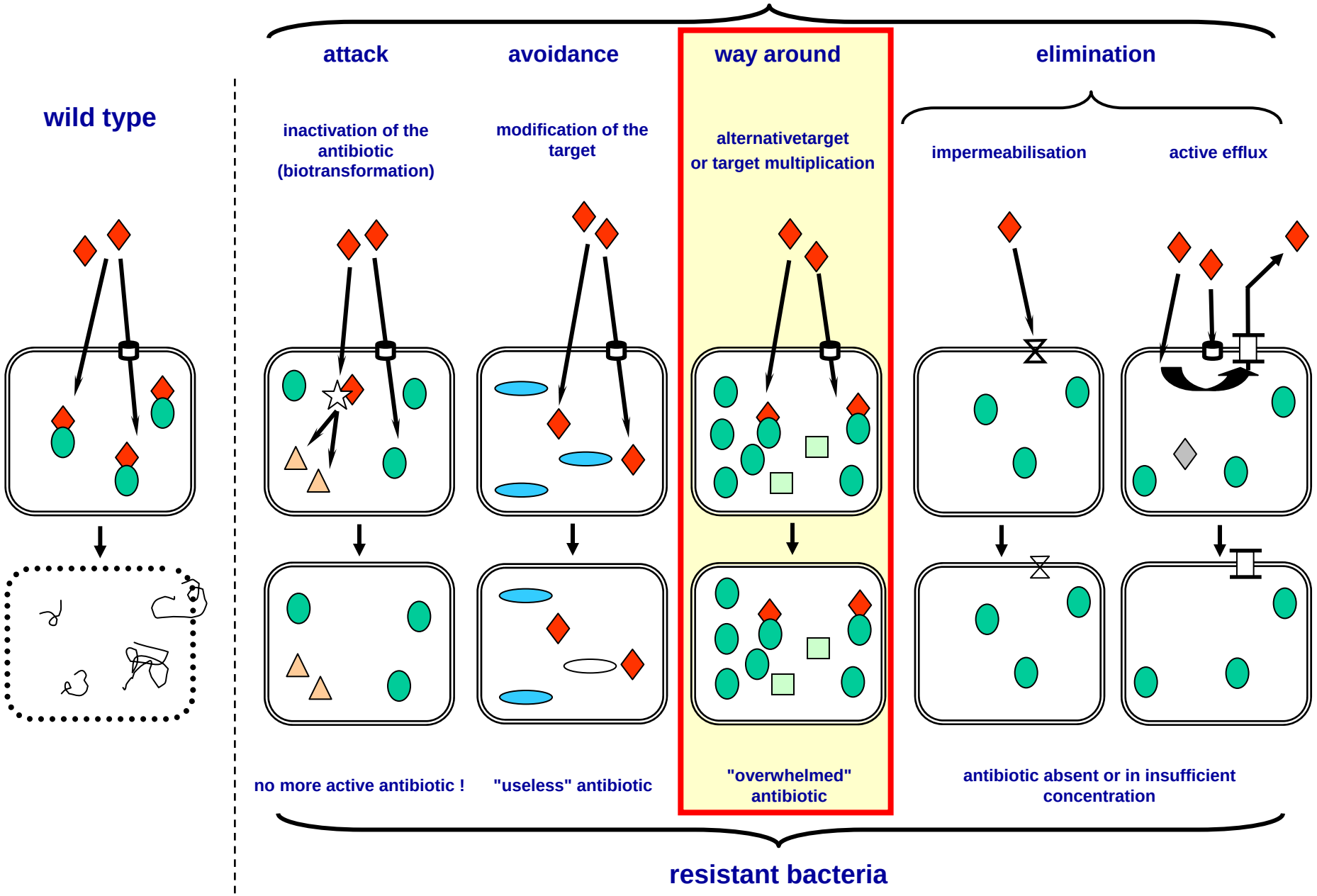
CEFTAROLINE: MICs



* isolates collected o, Belgium between 2011 and 2012 from patients suffering of wound infections in 3 hospitals (1 in South-East of Brussels; 1 in North of Brussels; 1 in Hainaut)

Tulkens et al. 26th ICC, 2013 and unpublished

Which problem ?



The VISA story…

- VISA stands for "**V**ancomycin **I**ntermediate **S**taphylococcus **A**ureus" (but also termed GISA (**G**lycopeptide-**I**ntermediate **S**taphylococcus **A**ureus) and denotes organisms with an increased MIC for vancomycin or teicoplanin
- First identified in Japan in 1997 but since then found in many other countries
- Resistance occurs by a tickening of the cell wall with increased amounts of free D-Ala-D-Ala termini that trap vancomycin (and glycopeptides).

The VISA story…

- VISA
  Staph
  (**G**lyc
  deno
  vanc
- First
  many
- Resis
  incre
  vanc

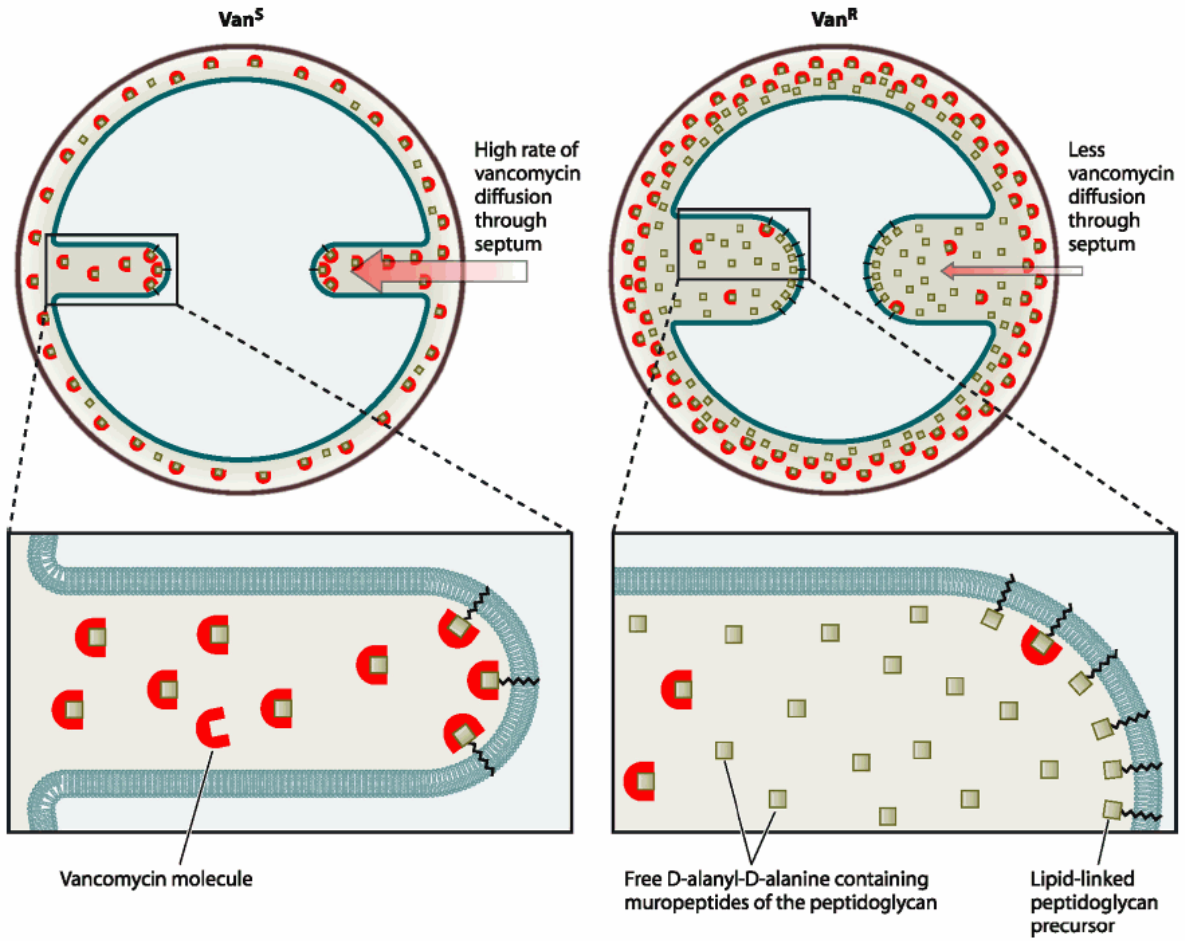


FIG. 2. Model depicting the site of vancomycin activity in the division septum and the changes associated with the VISA phenotype. The path of vancomycin to its lethal target (lipid II) should be through the division septum. In vancomycin-intermediate cells (Van^r), the rate of diffusion of vancomycin molecules to the septal tip is decreased, lowering the effective concentration of antibiotic that reaches the lipid-linked peptidoglycan precursor (lipid II) at the site of cell wall synthesis, per unit time, and therefore tilting the balance in favor of continued cell wall synthesis. This model implies that vancomycin efficiency varies during the cell cycle, as the path from the outside of the cell to the lethal targets is shorter when the septum starts to be formed and longer when septum synthesis approaches completion. (Adapted from reference 245 with permission.)

The VISA story…

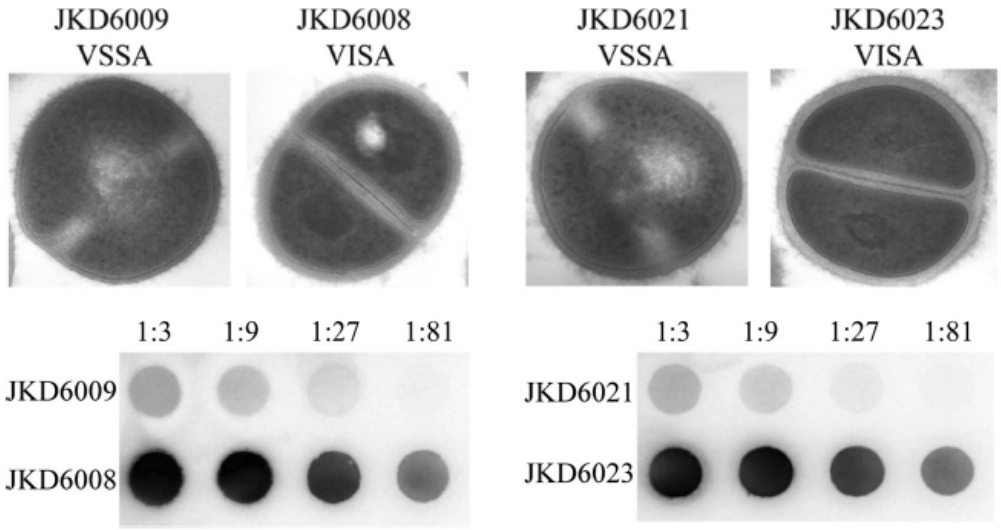


FIG. 5. Example of the cell wall and capsule changes that occur in hVISA and VISA strains in paired isolates from patients with persistent infections. The top panel demonstrates significant cell wall thickening in VISA strains compared to VSSA strains, while the bottom panel demonstrates significant increases in the expression of capsule by using an anticapsule type 8 immunoblot and serial dilutions of crude capsule extracts from paired VSSA and VISA strains. (Adapted from references 121 and 122, the latter of which was published under an open-access license agreement.)

Howden *et al*. Clin Microbiol Rev 2010;23:99–139.

- **The MICs of these strains are above the susceptibility breakpoint of EUCAST (2 mg/L) !**

Do you need to be afraid of VISA ?

J Antimicrob Chemother 2011; **66**: 1594–1599
doi:10.1093/jac/dkr169 Advance Access publication 26 April 2011

Journal of Antimicrobial Chemotherapy

Relevance of vancomycin-intermediate susceptibility and heteroresistance in methicillin-resistant *Staphylococcus aureus* bacteraemia

Riad Khatib*, Jinson Jose, Adina Musta, Mamta Sharma, Mohamad G. Fakih, Leonard B. Johnson, Kathleen Riederer and Stephen Shemes

Department of Medicine, St John Hospital and Medical Center, Grosse Pointe Woods, MI, USA

Do you need to be afraid of VISA ?

J Antimicrob Chemother 20
doi:10.1093/jac/dkr169 Ad

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heteroresist**

Riad Khatib*, Jins

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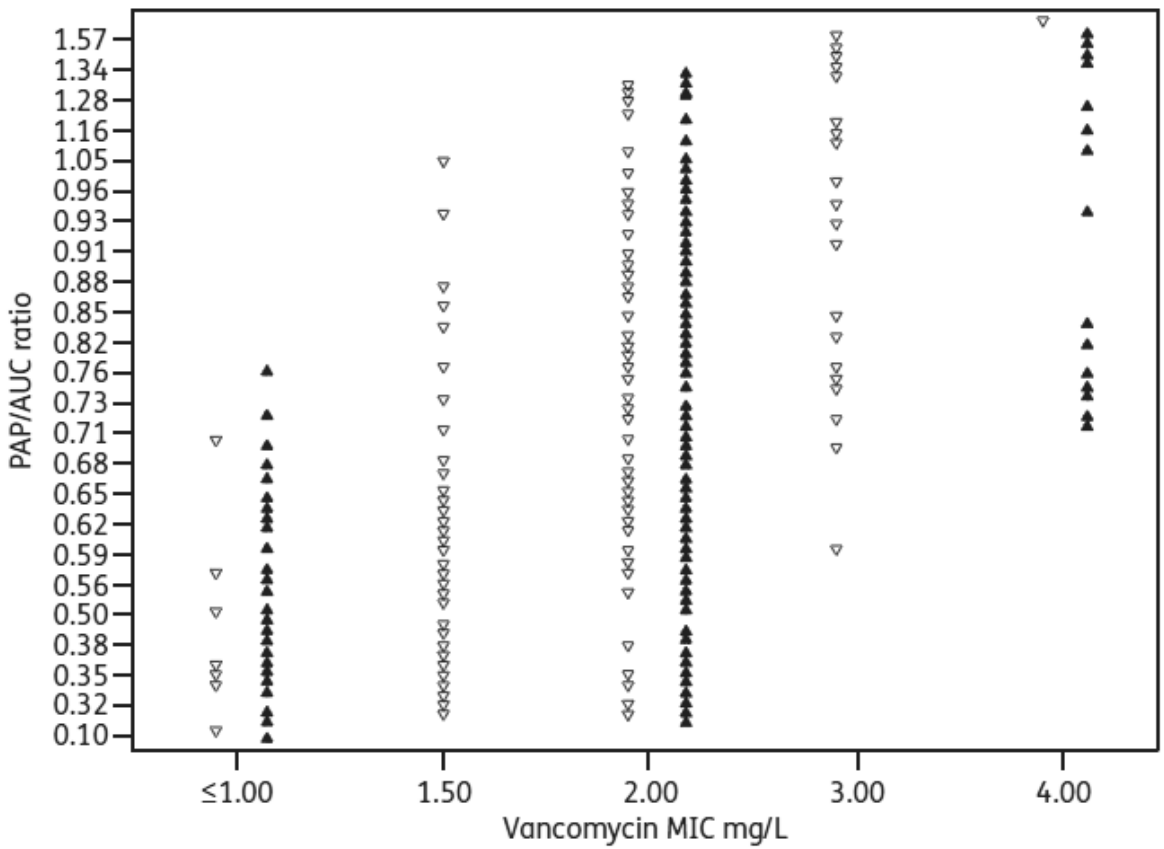

Figure 2. PAP/AUC ratios stratified according to vancomycin MIC by Etest (open triangles) and BMD (filled triangles).

Do you need to be afraid of VISA ?

J Antim
doi:10

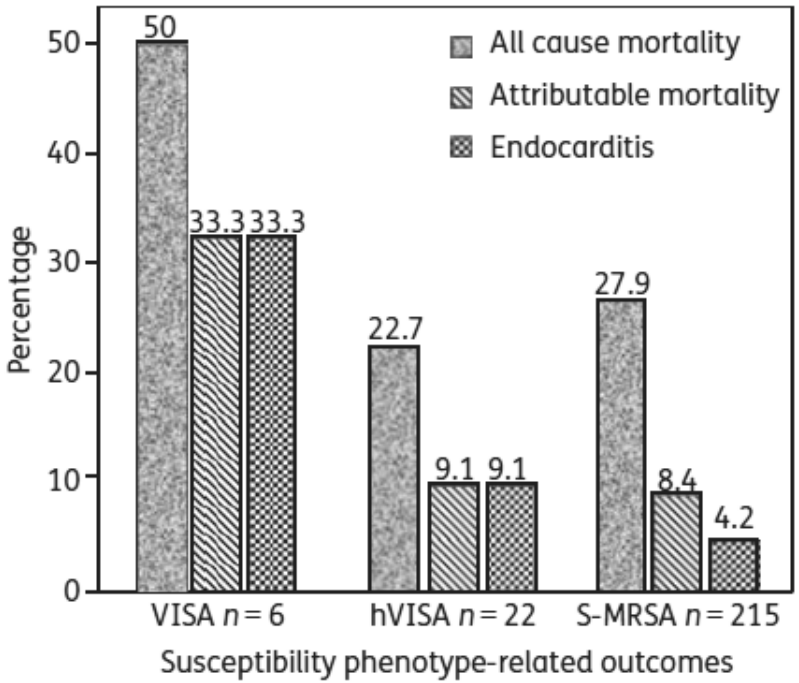

Figure 3. Comparative outcome measures stratified according to susceptibility status.

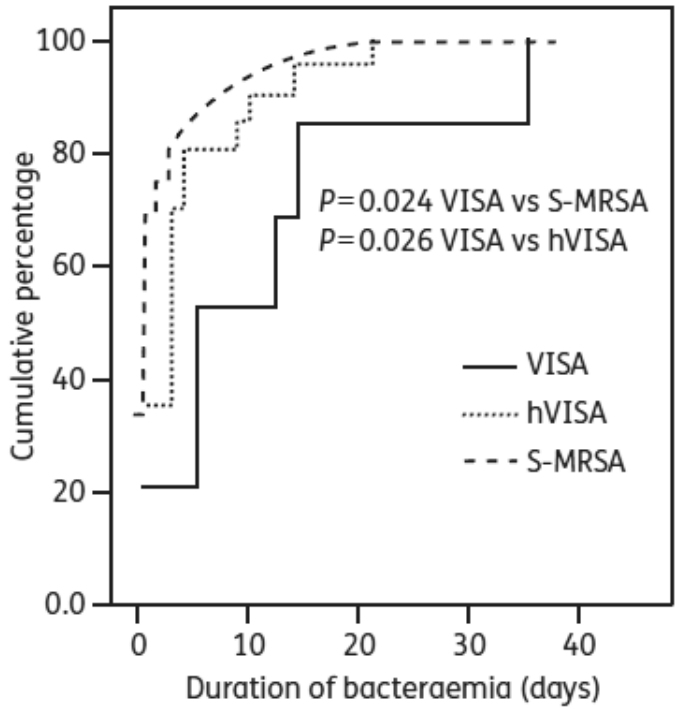

Figure 4. Duration of bacteraemia stratified according to susceptibility status. *P* values represent log rank statistics.

VISA and co-resistance: the daptomycin problem

J Antimicrob Chemother 2011; **66**: 1057–1060
doi:10.1093/jac/dkr066 Advance Access publication 2 March 2011

Journal of Antimicrobial Chemotherapy

Daptomycin non-susceptibility in vancomycin-intermediate *Staphylococcus aureus* (VISA) and heterogeneous-VISA (hVISA): implications for therapy after vancomycin treatment failure

Peter G. Kelley[1,2], Wei Gao[1,2], Peter B. Ward[1,2] and Benjamin P. Howden[1–3]*

[1]Department of Microbiology, Austin Health, Heidelberg, Victoria, Australia; [2]Austin Centre for Infection Research (ACIR), Infectious Diseases Department, Austin Health, Victoria, Australia; [3]Department of Microbiology and Immunology, University of Melbourne, Parkville, Victoria, Australia

*Corresponding author. Infectious Diseases Department, Austin Health, Studley Rd, Heidelberg, VIC, Australia 3084. Tel: +61-3-9496-6676; Fax: +61-3-9496-6677; E-mail: benjamin.howden@austin.org.au

VISA and co-resistance: the daptomycin problem

J Antimicrob Chemother 2011; **66**: 1057–1060
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Journal of Antimicrobial Chemotherapy

Daptomycin non-susceptibility in vancomycin-intermediate *Staphylococcus aureus* (VISA) and heterogeneous-VISA (hVISA): implications for therapy after vancomycin treatment failure

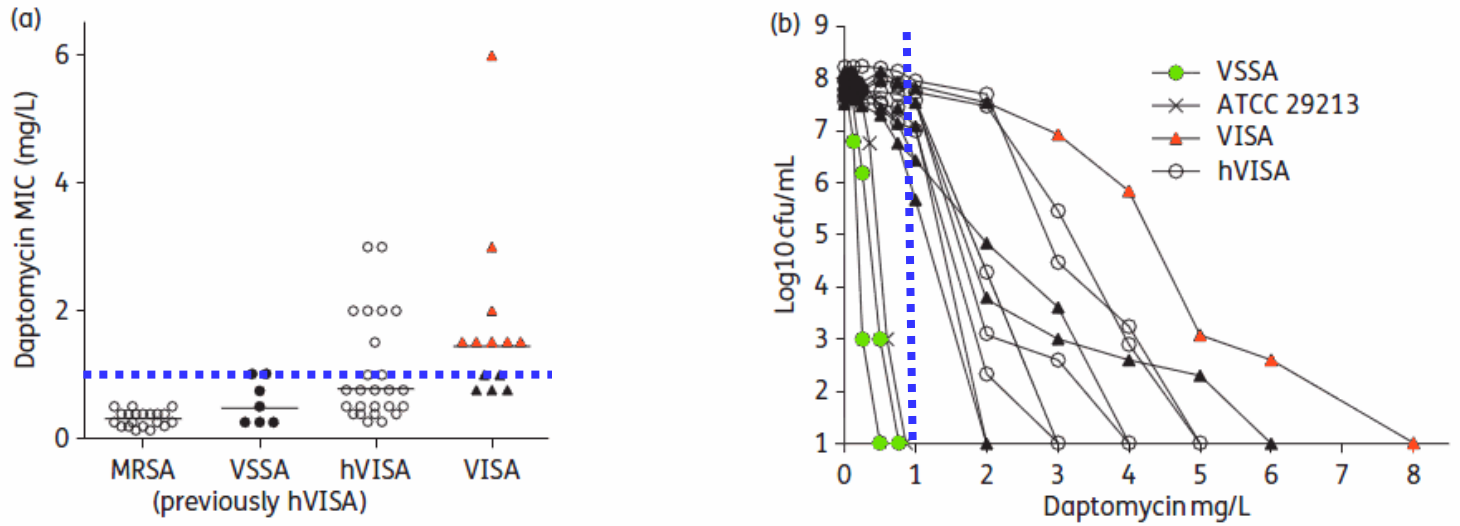
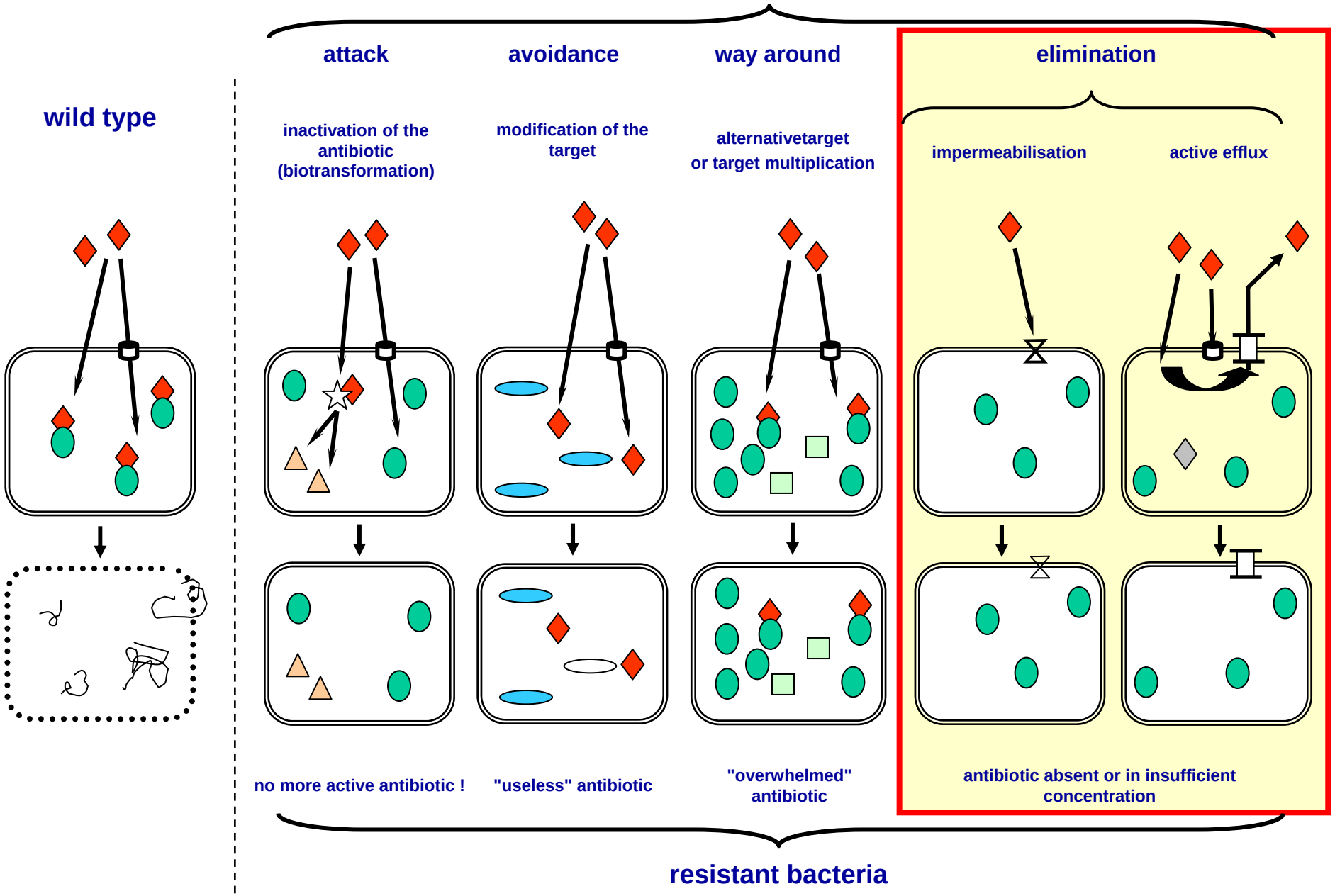


Figure 1. Analysis of daptomycin susceptibility by Etest MIC and PAP. (a) Daptomycin Etest MIC results and geometric means (continuous horizontal lines) for the three vancomycin susceptibility phenotypes for isolates recovered from storage and the ANZCOSS MRSA isolates. (b) Daptomycin PAPs for five hVISA isolates, five VISA isolates and two vancomycin-susceptible MRSA isolates. *S. aureus* ATCC 29213 was also included as a control.

daptomycin EUCAST breakpoint

Which problem ?



An original observation with cancer cells…

[CANCER RESEARCH 37, 4629–4634, December 1977]

Decreased Retention of Actinomycin D as the Basis for Cross-resistance in Anthracycline-resistant Sublines of P388 Leukemia

Makoto Inaba[1] and Randall K. Johnson[2]

Laboratory of Chemical Pharmacology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, Maryland 20014

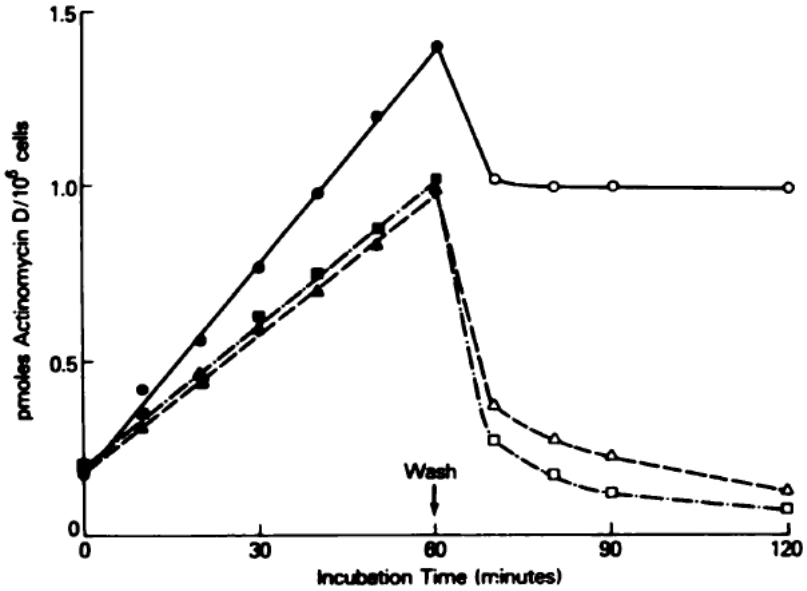


Chart 2. Time course of uptake and efflux of actinomycin D by P388/S (○, ●), P388/ADR (△, ▲) and P388/DAU (□, ■) cells. Cells were incubated in the presence of actinomycin D, 0.04 μg/ml, for 60 min, washed, and reincubated in drug-free medium for an additional 60 min. Each *point* represents the mean of 3 determinations. The coefficient of variation was less than 10%.

Historical observations on tetracyclines …

Proc. Natl. Acad. Sci. USA
Vol. 77, No. 7, pp. 3974–3977, July 1980
Biochemistry

Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants in *Escherichia coli*

(everted membrane vesicles/tetracycline transport/transposon Tn10/plasmids)

LAURA MCMURRY, RICHARD E. PETRUCCI, JR., AND STUART B. LEVY*

Department of Molecular Biology and Microbiology and Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts 02111

Communicated by Boris Magasanik, April 21, 1980

Historical observations on tetracyclines …

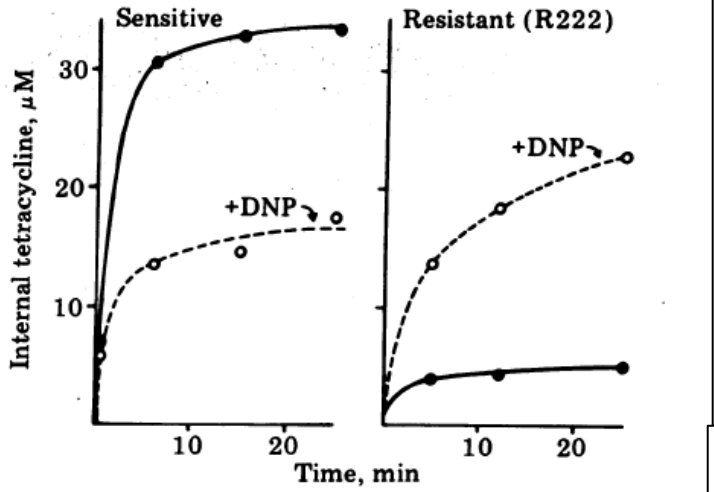


FIG. 1. Tetracycline uptake by *E. coli* ML308-225 (sensitive) and by R222-containing induced (resistant) cells with (○) and without (●) 1 mM DNP. Cells were grown overnight in medium A containing glucose and uptake was measured in the absence of added energy source.

Whole bacteria

Everted membranes

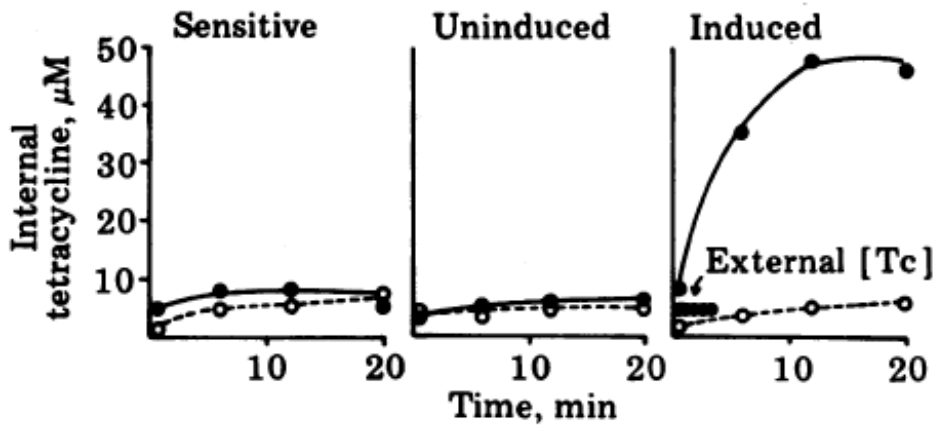
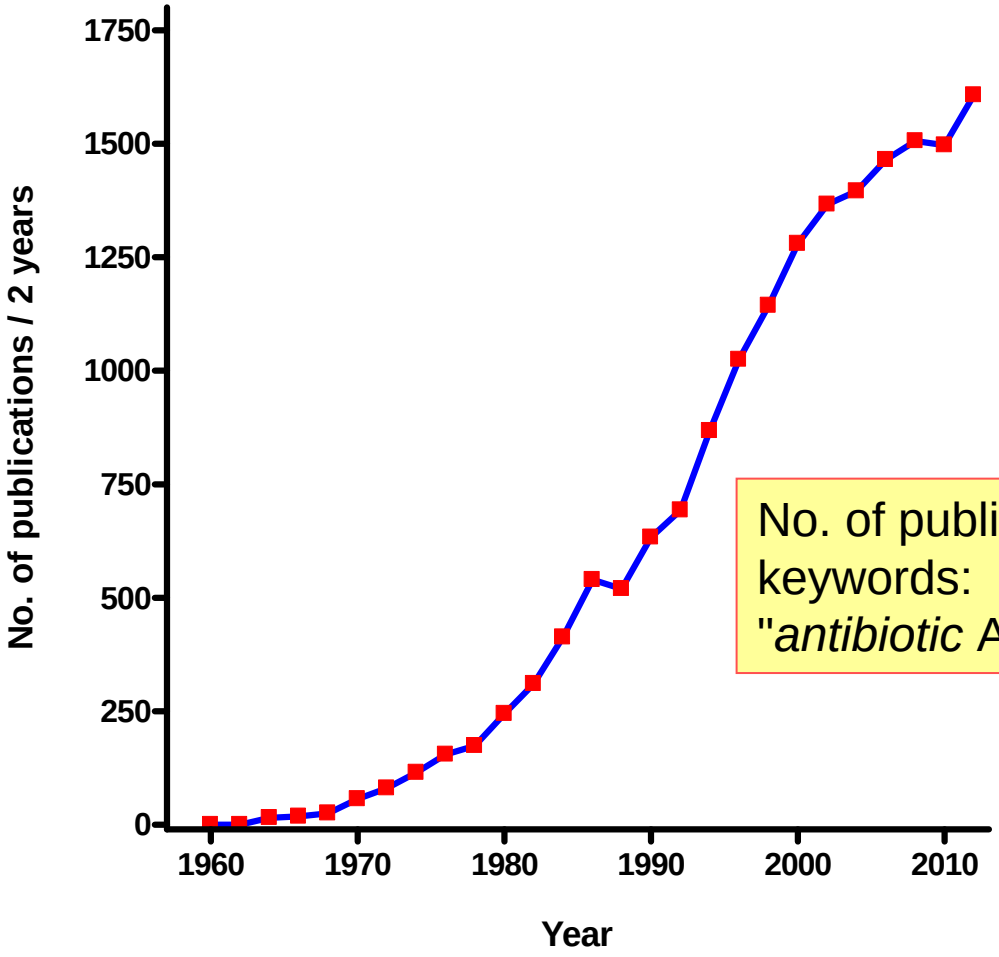


FIG. 2. Tetracycline (Tc) uptake by everted membrane vesicles made from sensitive ML308-225 cells and from uninduced and induced R222-containing cells. ○, No energy; ●, D-lactate. Cells were grown in glycerol and vesicles were frozen in 5 mM Tris·HCl, pH 7.2/70 mM KCl/0.25 mM dithiothreitol/50% glycerol. The assay was done at pH 6.6.

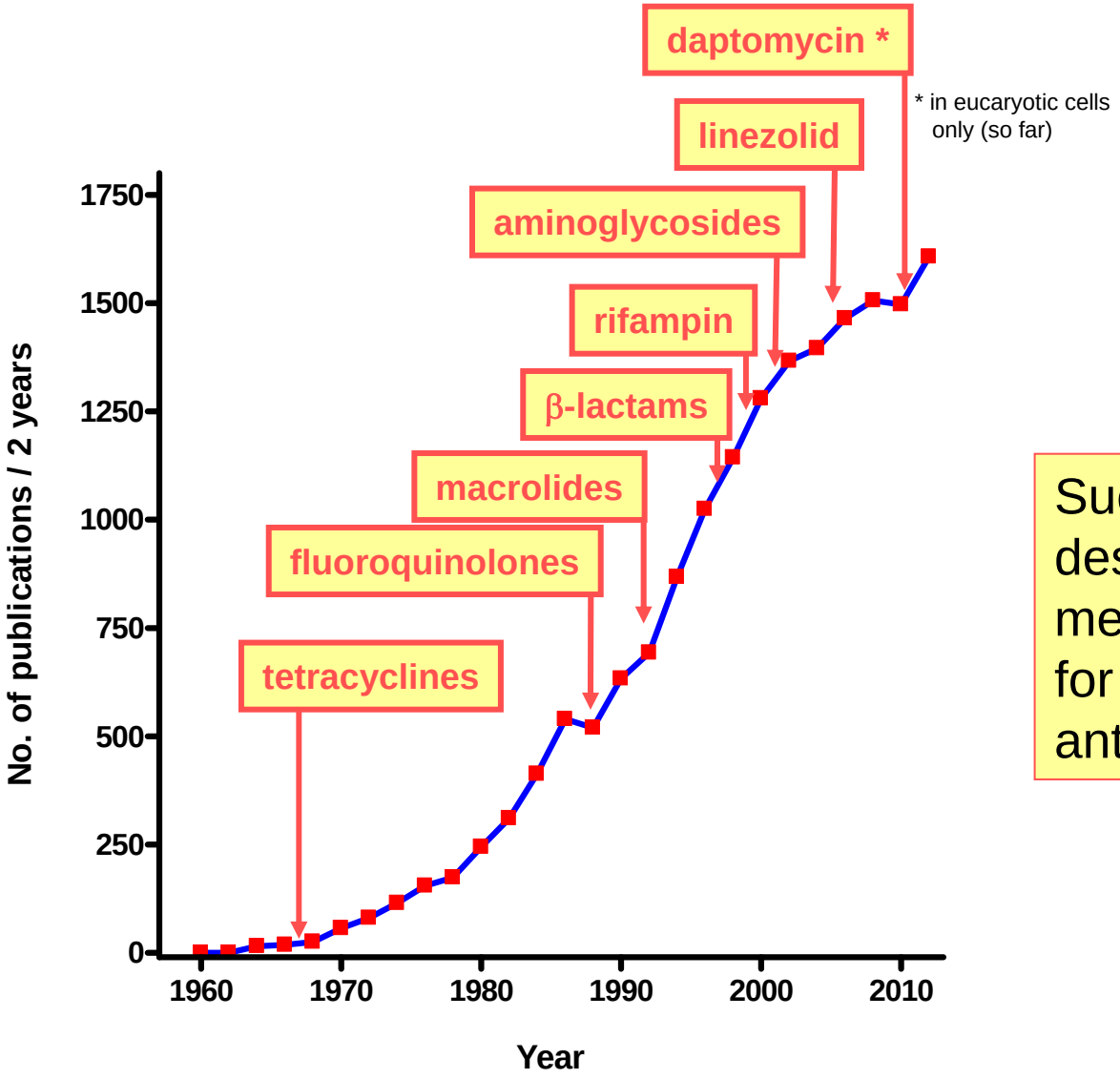
McMurry et al., PNAS 1980; 77:3974-3977

You said "antibiotic eflux"



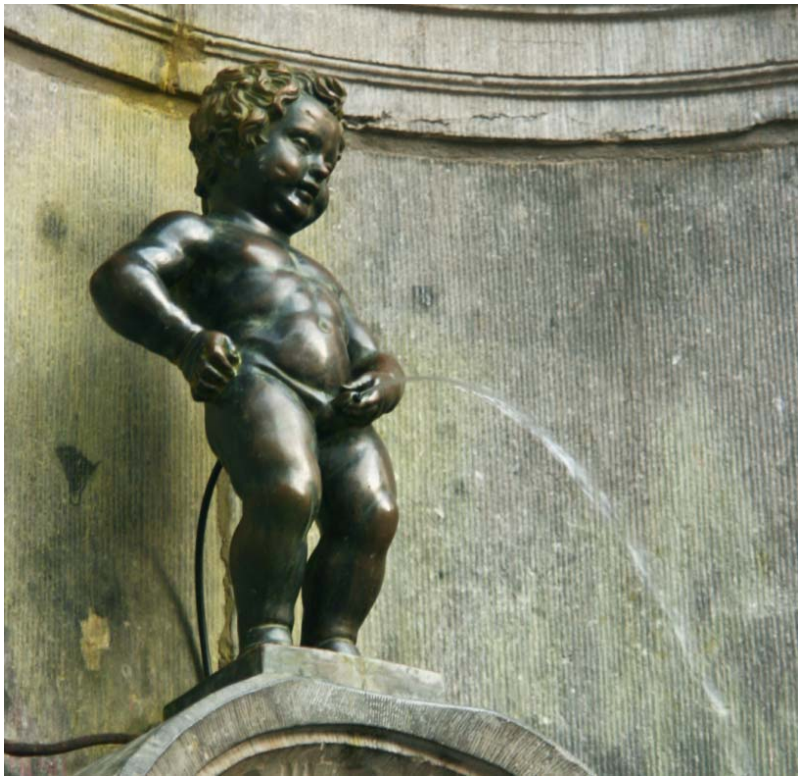
No. of publications in PubMed with keywords:
"*antibiotic* AND (*efflux* OR *transporter*)"

Historical landmarks …



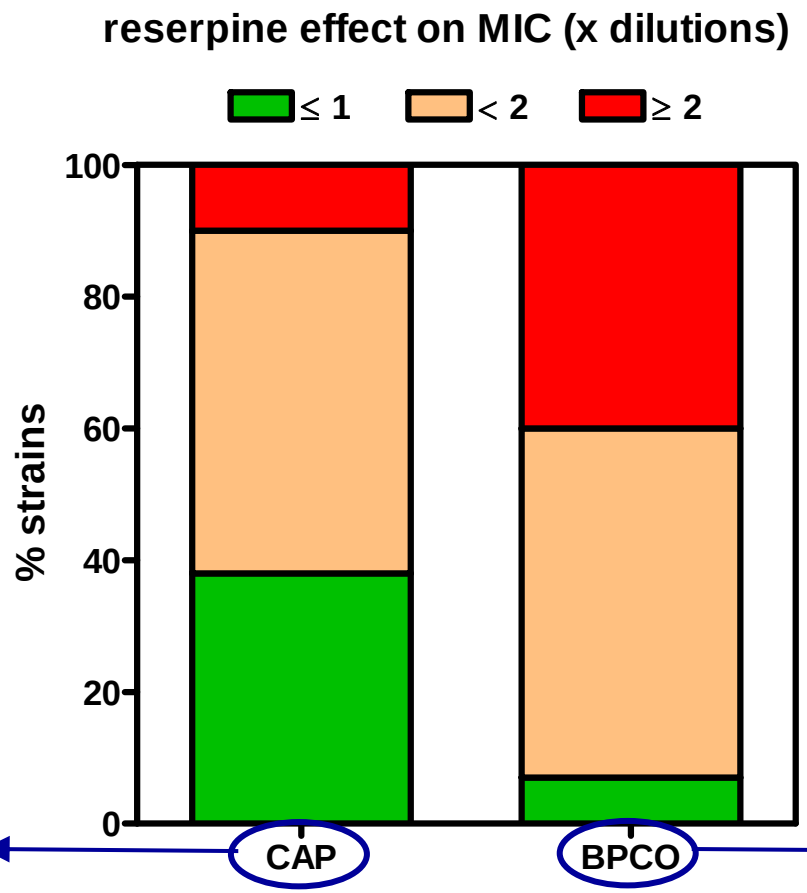
Successive description of efflux-mediated resistance for major classes of antibiotics

Role of efflux pumps in the clinics …



Efflux in *S. pneumoniae:* is it important in the clinics ?

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



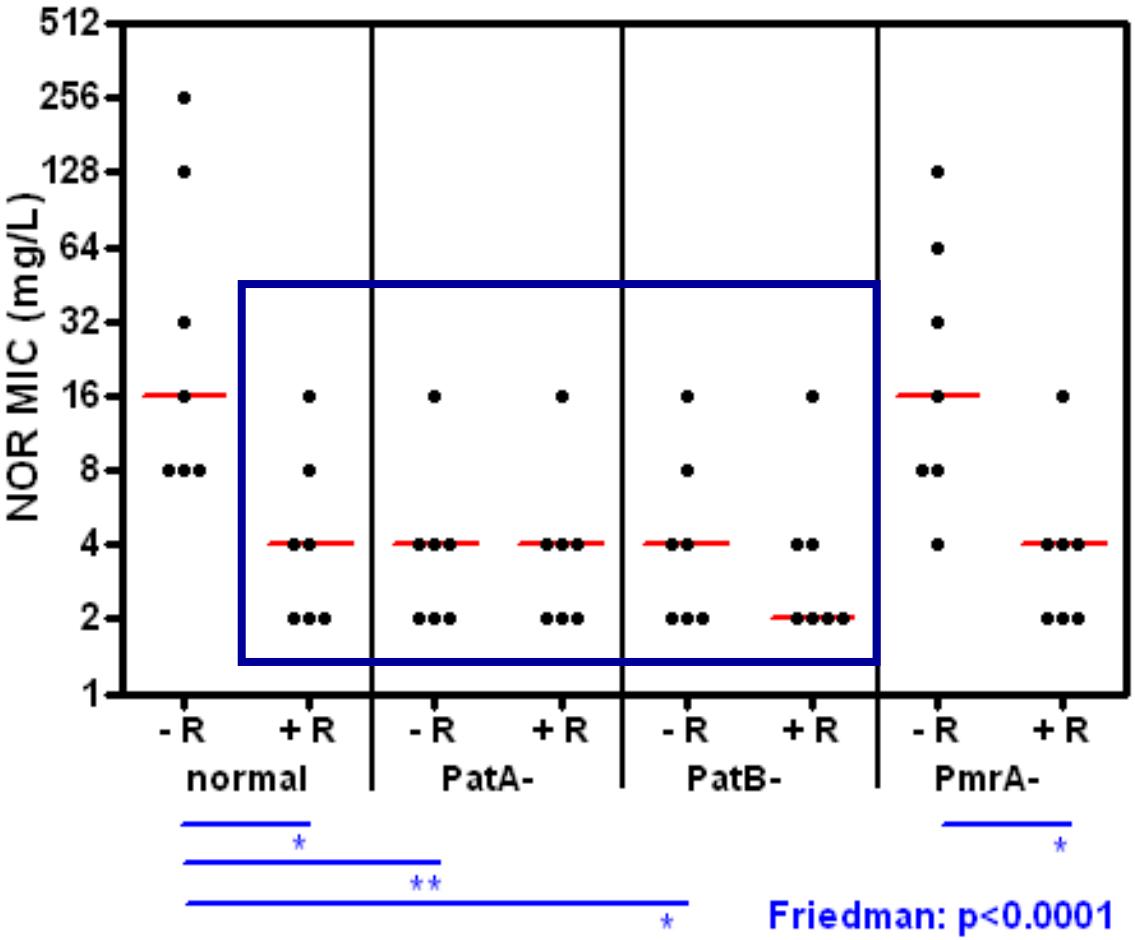
acute pathology
⬇
« one shot »
antibiotic exposure

chronic pathology
⬇
repetitive
antibiotic exposures

Lismond & Degives, unpublished

Efflux in *S. pneumoniae:* is it important in the clinics ?

Identification of FQ transporters in clinical isolates



Inactivation of *patA* or *patB* as efficient as reserpine to reduce MIC

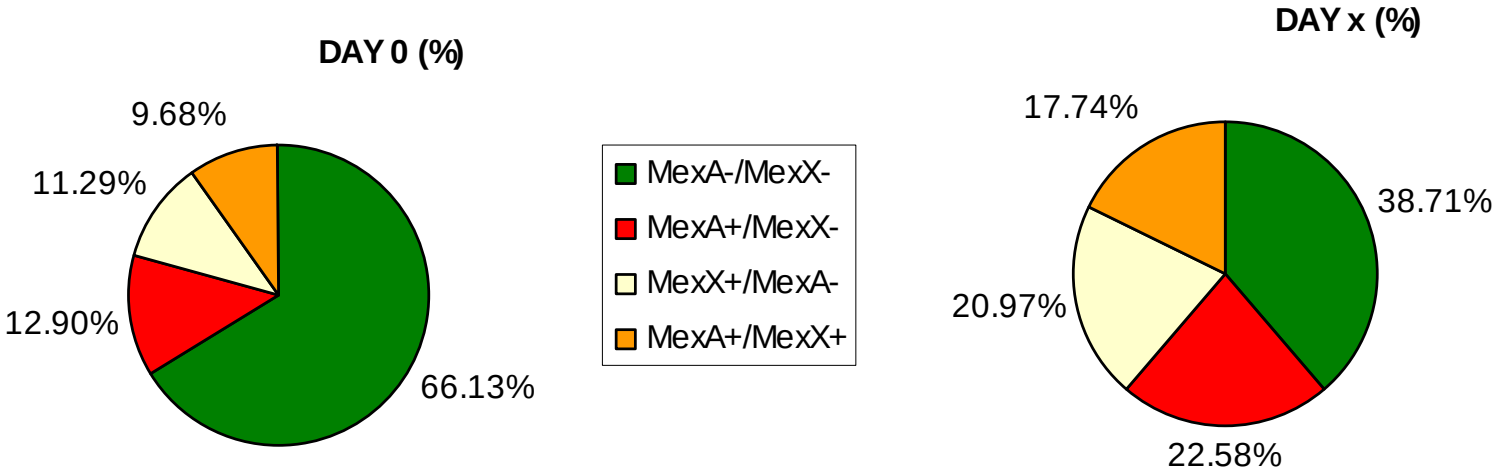
↓

- responsible for FQ efflux in clinical isolates
- work as heterodimers

Lismond et al, ECCMID 2010

Efflux in *P. aeruginosa:* is it important in the clinics ?

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



Riou et al, ECCMID 2010

Emergence of resistance during treatment

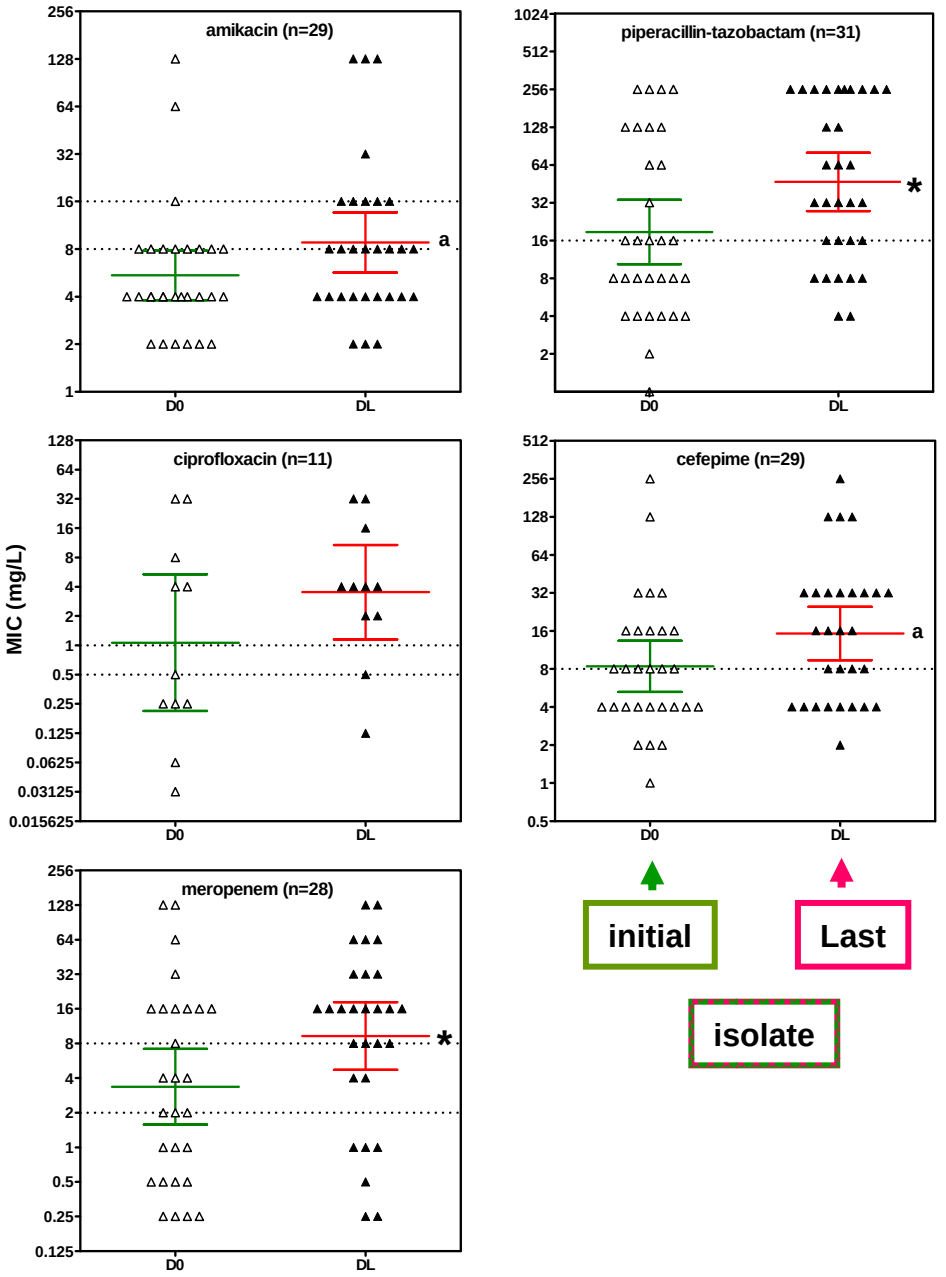
P. aeruginosa successive clonal isolates from the same patient (all patients treated with large doses of 1 to 3 antibiotics)

- 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

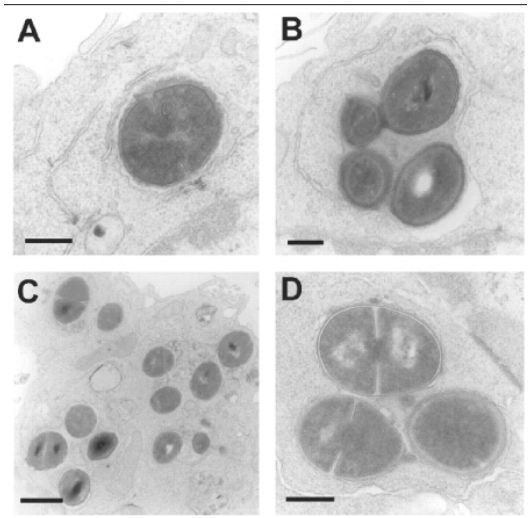
Note: stratification by time between D0 and DL gave no clue (too low numbers)



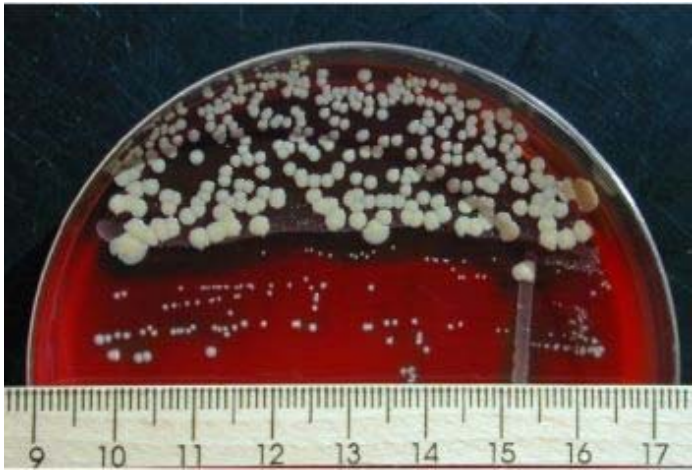
Riou, *et al. Int J Antimicrob Agents.* 2010;36:513-22

Is this all ?

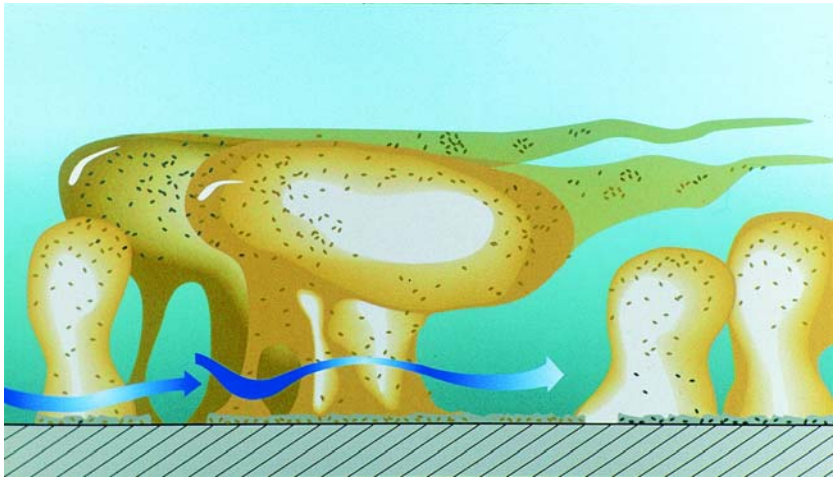
- Phenotypic "resistance"
  - small colony variants
  - dormant/persistent bacteria



http://www.facm.ucl.ac.be/intracellular_chemotherapy.htm
Last visited: 10/06/2014



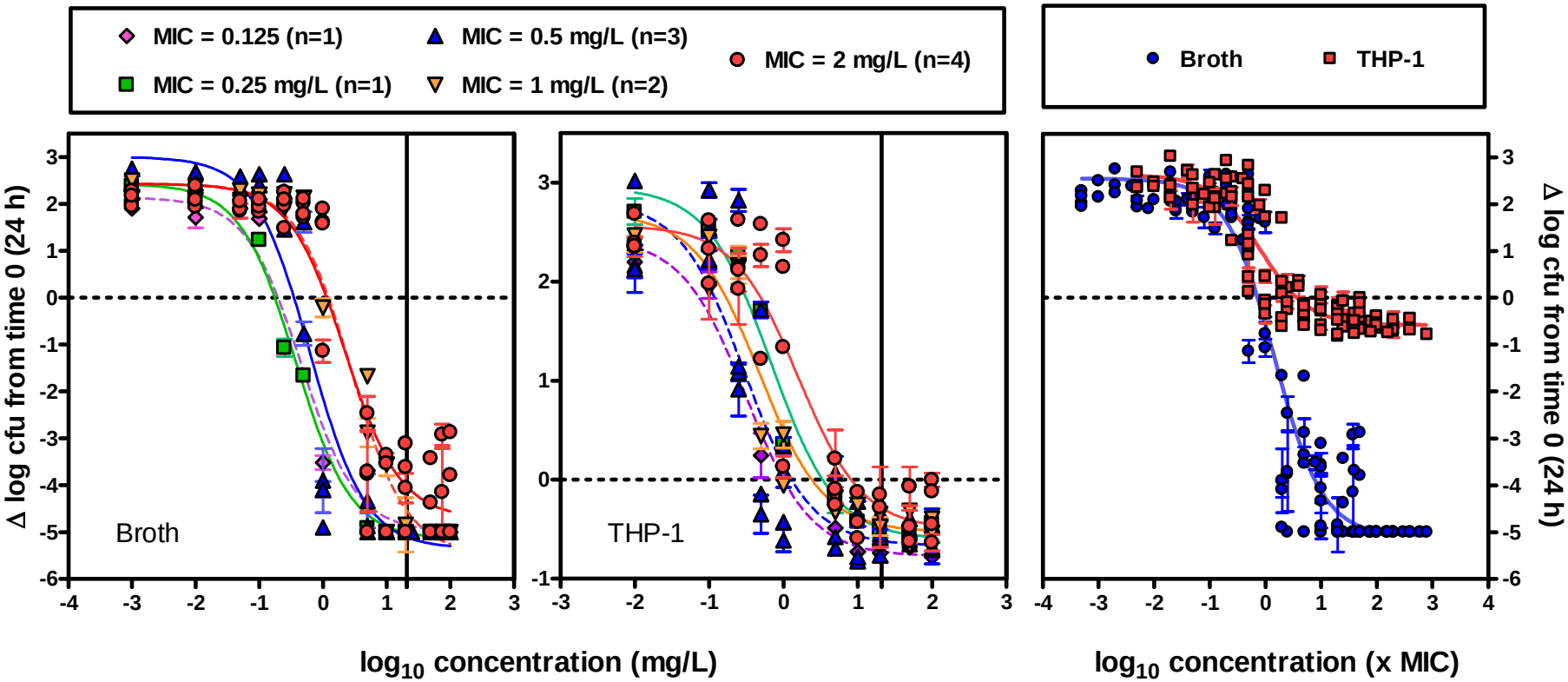
http://infekt.ch/2006/10/small-colony-variants-von-staphylococcus-aureus-schwierig-zu-behandelnde-infektionen/
Last visited 14/06/2014



http://cmr.asm.org/content/15/2/167.figures-only
Rodney & Costerton Clin. Microbiol. Rev. 2002, 15(2):167.

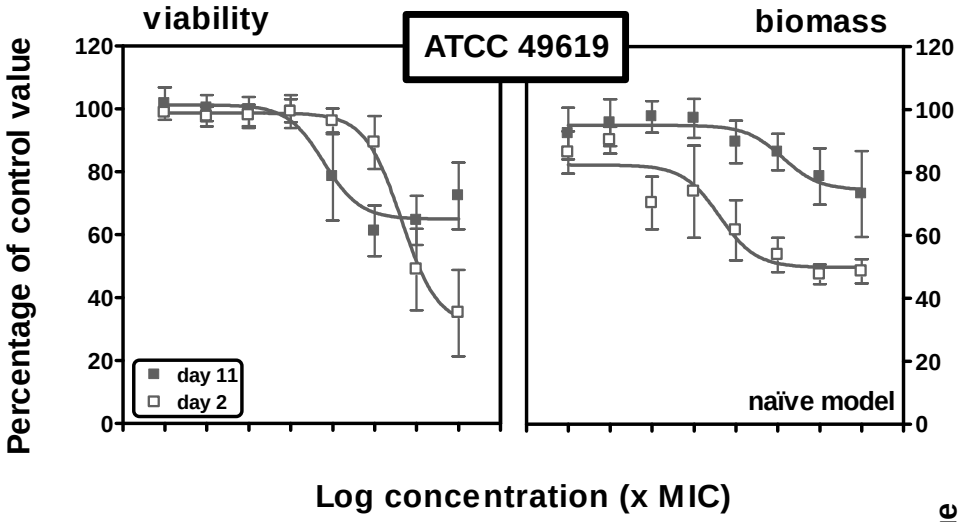
Intracellular infection and *S. aureus*

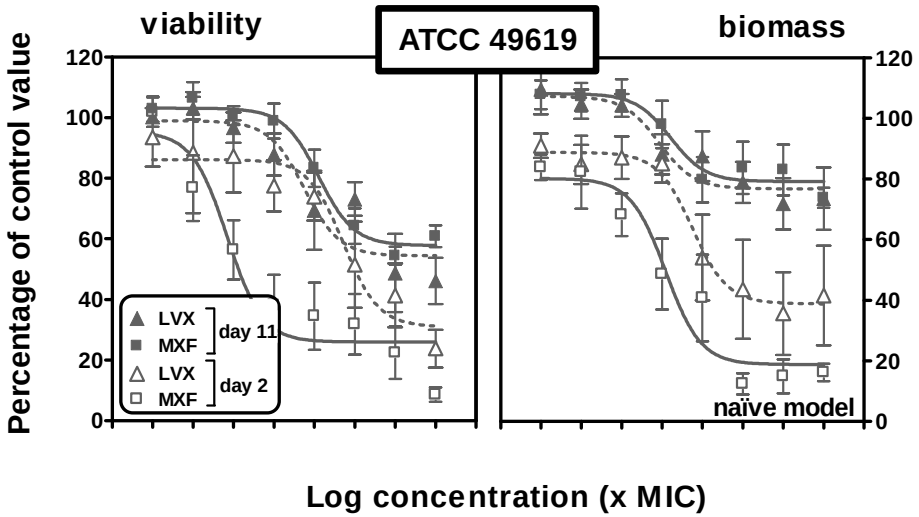
Activity of ceftaroline towards extracellular (broth) and intracellular forms of S. aureus with increasing MICs



Mélard *et al*. J Antimicrob Chemother. 2013;68:648-58

Biofilms and *S. pneumoniae*

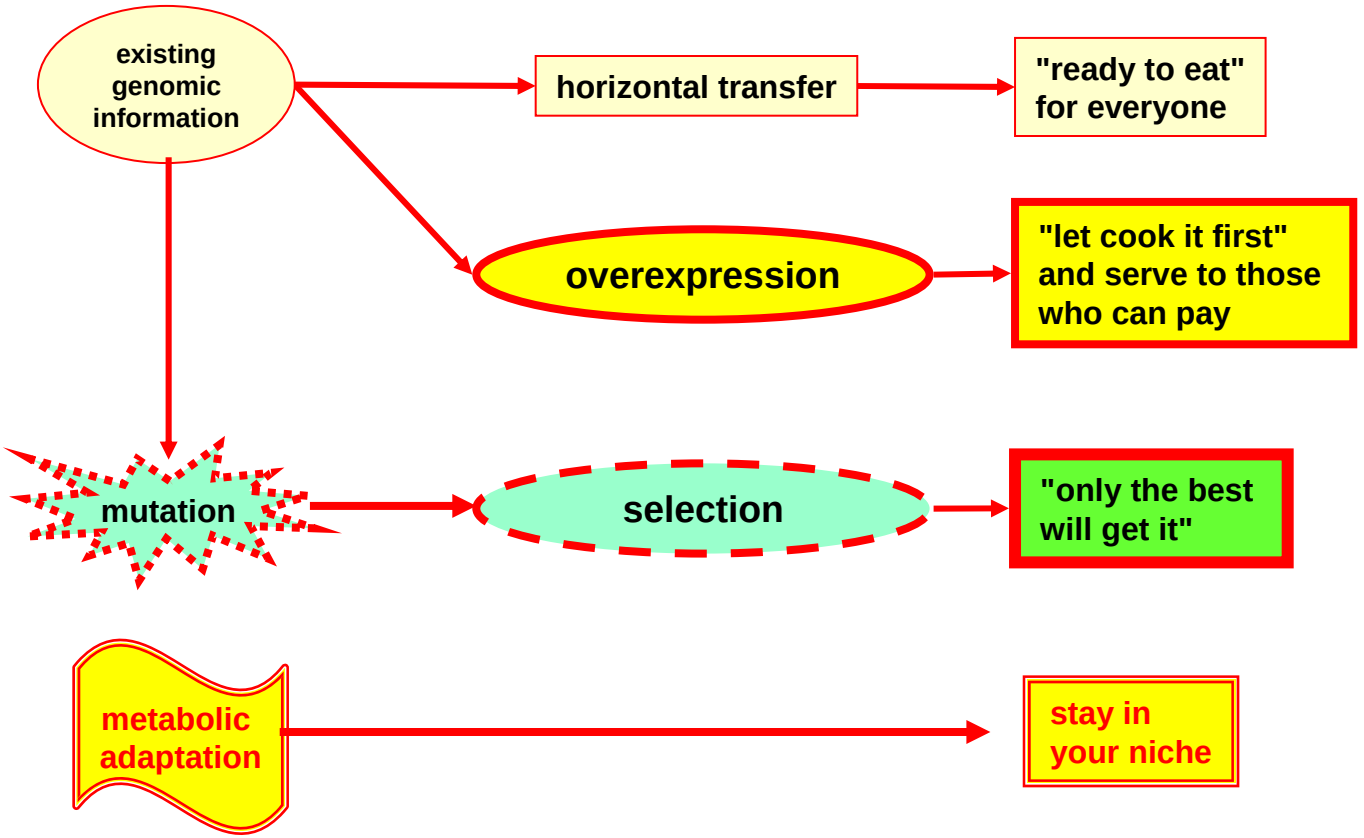




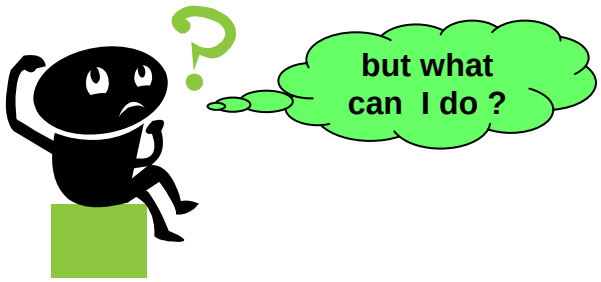
Vandevelde *et al.* Antimicrob Agents Chemother. 2014;58:1348-58.

The question that I should have addressed…

- How does all that is acquired and spread ?



The question that I should have addressed…



existing genomic information

horizontal transfer → "ready to eat" for everyone ← hygiene isolation

overexpression → "let cook it first" and serve to those who can pay ← reduce current antibiotic pressure

mutation → selection → "only the best will get it" ← don't give a chance to selection

metabolic adaptation → stay in your niche ← use "non-antibiotic" options

The real question …

What can do for him/her to have nice dreams … and to wake up in good health ?