Antibiotic resistance

- why ?
- mechanisms
- Belgian situation (as an example)

With the support of *Wallonie-Bruxelles-International*

Antibiotic resistance: why ?

A simple application of Darwin's concepts ...

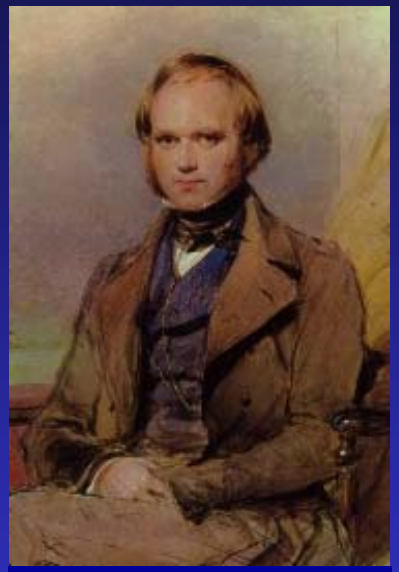
gene

↓

enzyme / nucleoprotein

↓

function



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

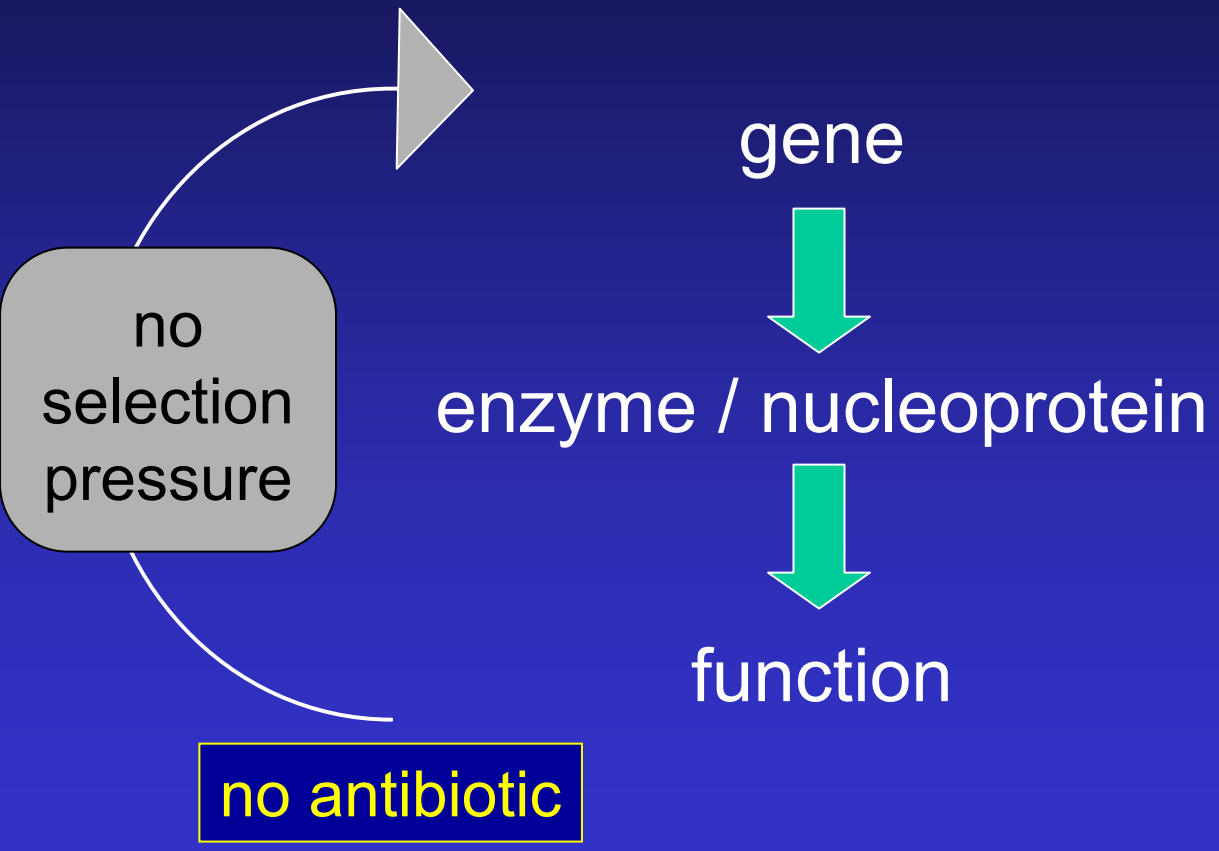
Antibiotic resistance: why ?

A simple application of Darwin's concepts …
to a highly changeable material

- typical infectious foci contain as much as 10^6 - 10^9 organisms
- most bacteria are VERY quickly (20 min…) multiplying with a high level of errors (10^{-6} – 10^{-8})
- pathogenic bacteria easily exchange genetic material

Rapid acquisition and dissemination of resistance determinants

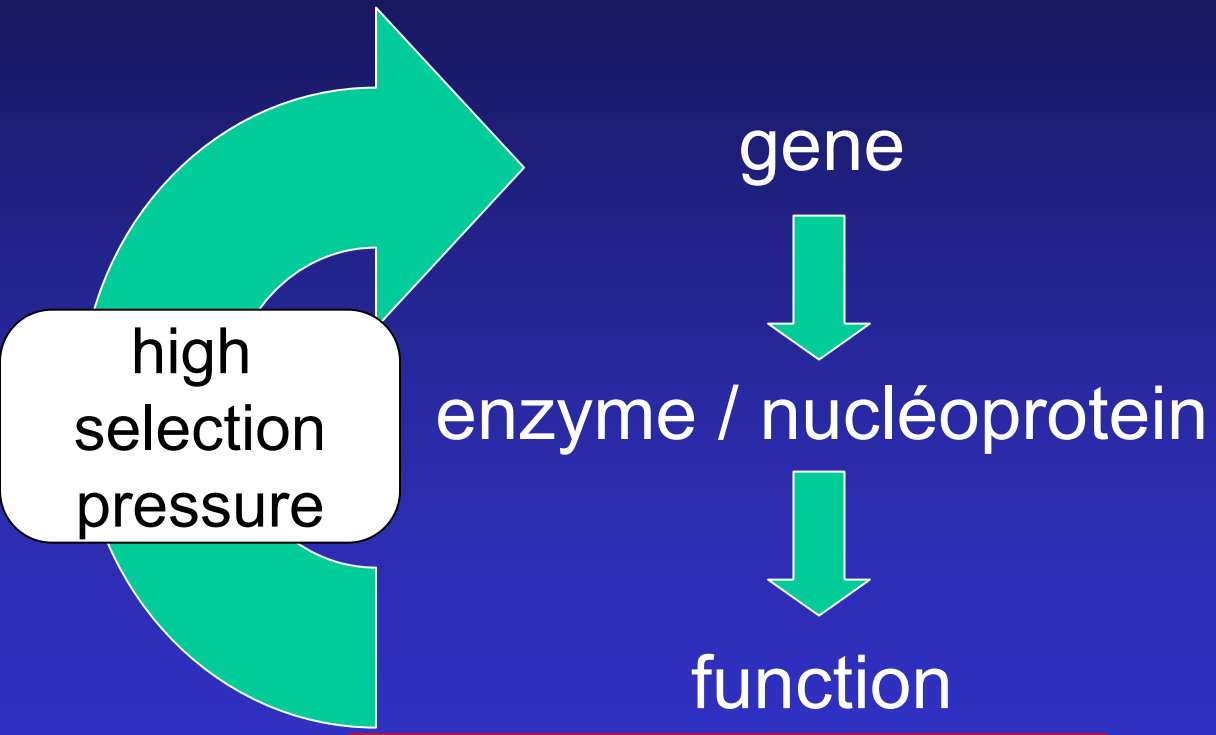
Antibiotic resistance: why ?



Resistance ↗ if

- High consumption **and**
- Inappropriate use

Antibiotic resistance: why ?



High and inappropriate antibiotic consumption;
A lot of surviving bacteria

Resistance ↗ if

- High consumption **and**
- Inappropriate use

A simple experiment …

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

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

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

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

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Nguyen et al., presented at the 8th ISAAR, Seoul, Korea, 6 April 2011

sub-MIC concentrations create resistance !

Thus, you need to do something …

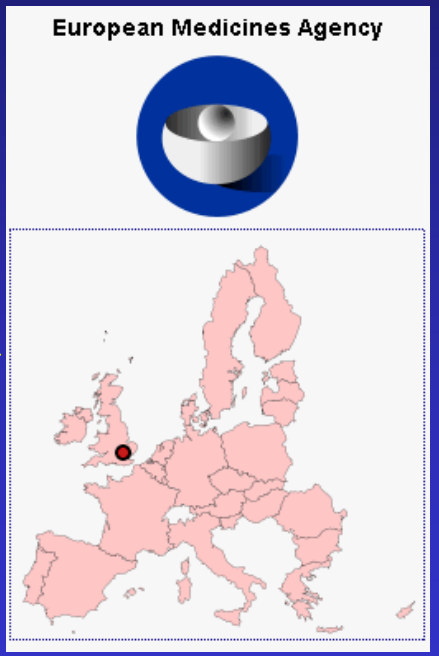
- "HIT HARD & HIT FAST ?"

Paul Ehrlich:

‚Frapper fort et frapper vite' (Hit hard and early) –

Address to the 17th International Congress of Medicine, 1913

Ehrlich P, Lancet 1913; 2:445–51.



PK /PD and resistance in Europe in 1999

" Inadequate dosing of antibiotics is probably an important reason for misuse and subsequent risk of resistance.

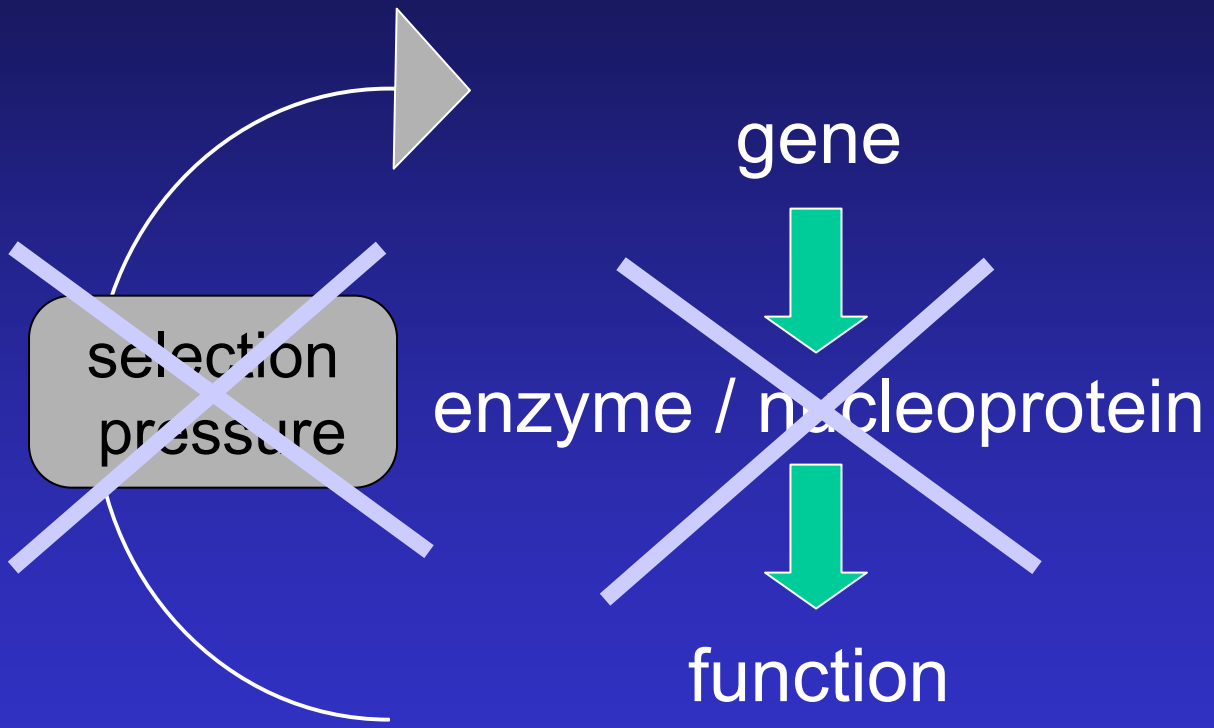
A recommendation on proper dosing regimens for different infections would be an important part of a comprehensive strategy.

The possibility of approving a dose recommendation based on pharmacokinetic and pharmacodynamic considerations will be further investigated in one of the CPMP* working parties… "

---

* Committee for Proprietary Medicinal Products – European Medicines Agency

Antibiotic resistance: the PK/PD way



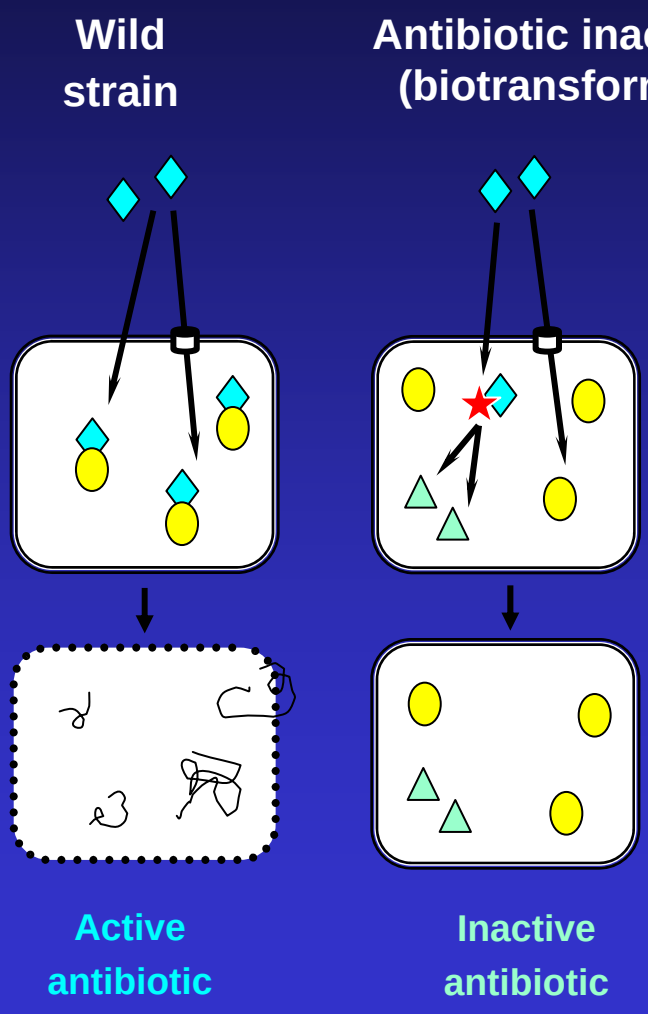
Resistance ↗ if

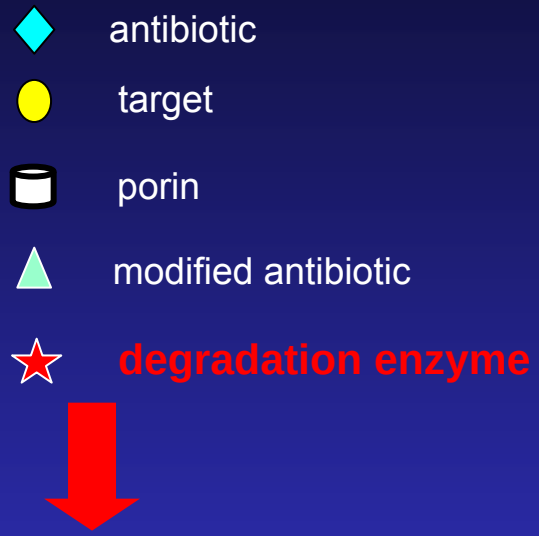
- High consumption **and**
- Inappropriate use

Appropriate dose of antibiotic;
No surviving bacteria

Antibiotic resistance: mechanisms

1. « fighting » strategy

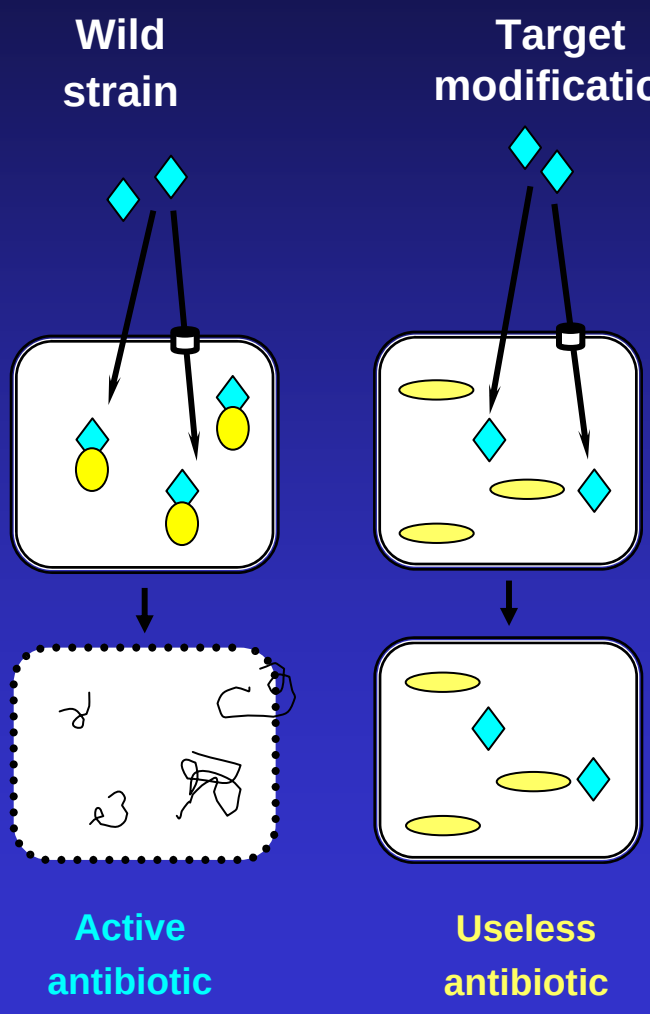




- **β-lactamases**
  (*S. aureus, H. influenzae, E. coli, P. aeruginosa, …*)
- **aminoglycoside-inactivating enzymes**
  (enterobacteriaceae)
- **macrolide-inactivating enzymes**
  (*E. coli*)

Antibiotic resistance: mechanisms

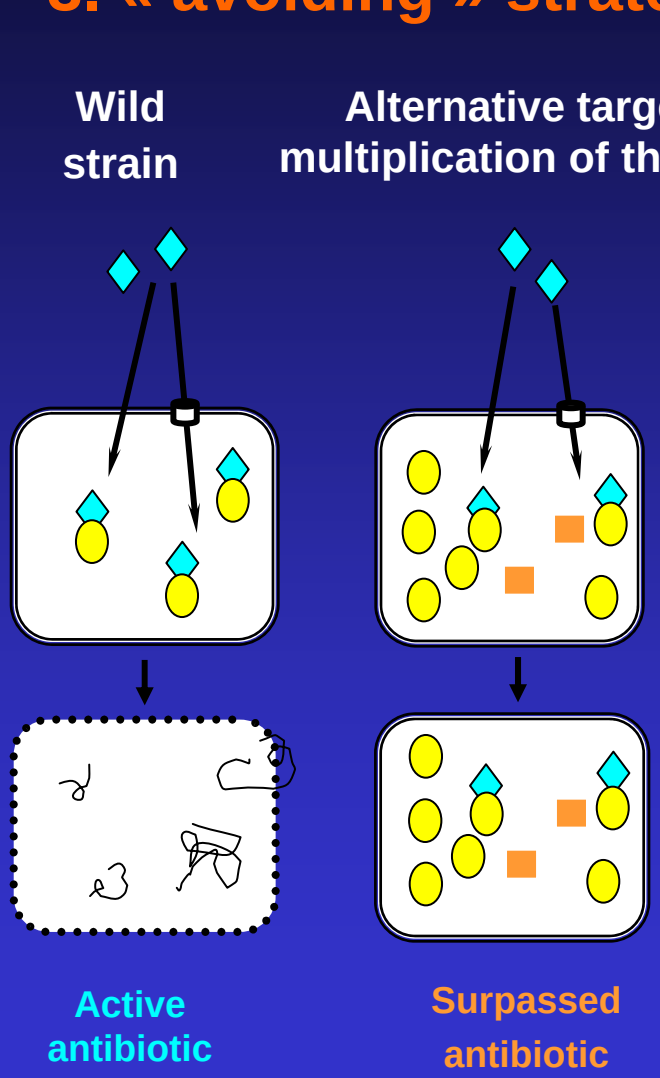
2. « escaping » strategy

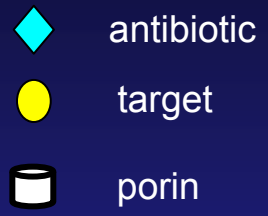


- **quinolone target mutation (GyrA et ParC subunits of the enzymes responsible for ADN supercoiling/decoiling)**
  (*S. aureus, S. pneumoniae, P. aeruginosa, …*)
- **ribosome methylation at the site of macrolides binding**
  (*S. aureus, S. pneumoniae)*
- **mutation of PBP (target for β-lactams)**
  (*S. aureus* [= MRSA !], *S. pneumoniae)*

Antibiotic resistance: mechanisms

3. « avoiding » strategy

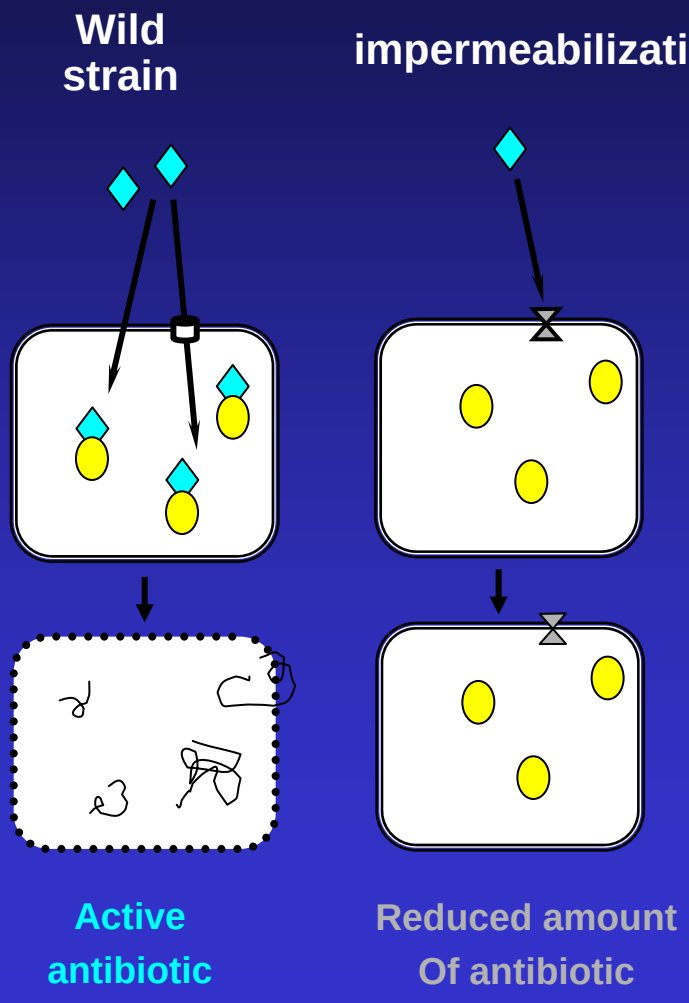


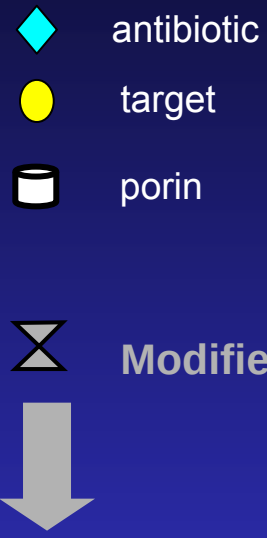


- **production of an altered peptidoglycan not recognized by glycopeptides**
  (enterococci, …)
- **production of a thicker cell wall, saturating glycopeptide binding**
  (*S. aureus* [VISA])

Antibiotic resistance: mechanisms

4. « elimination » strategy

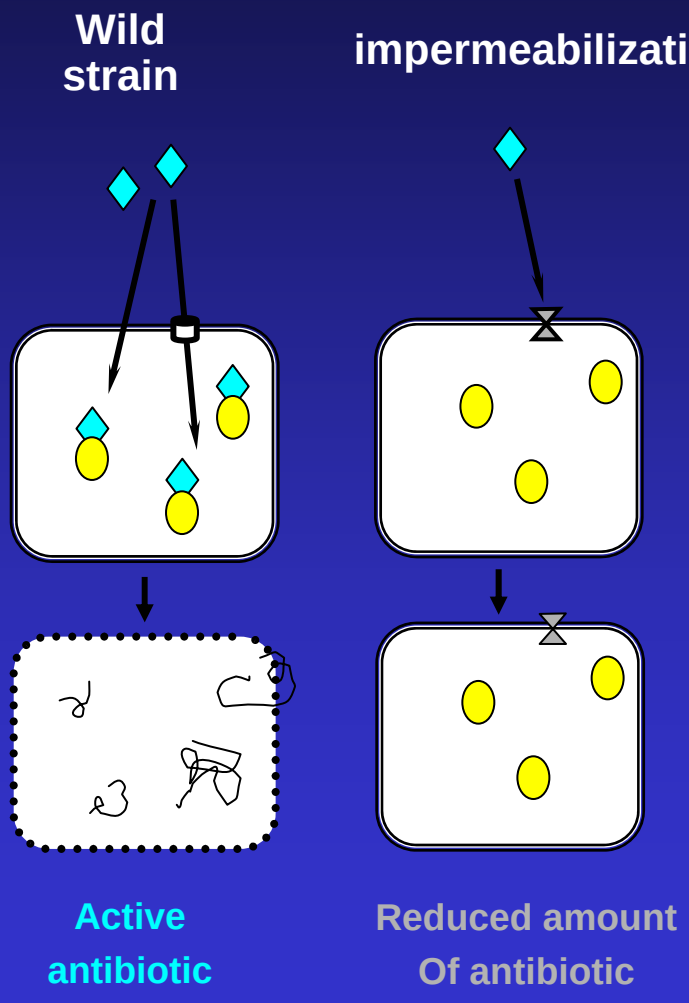


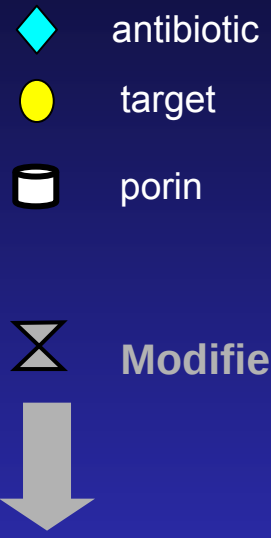


- **mutation of the OprD porine reducing the penetration of various antibiotics in *Pseudomonas aeruginosa***

Antibiotic resistance: mechanisms

4. « elimination » strategy



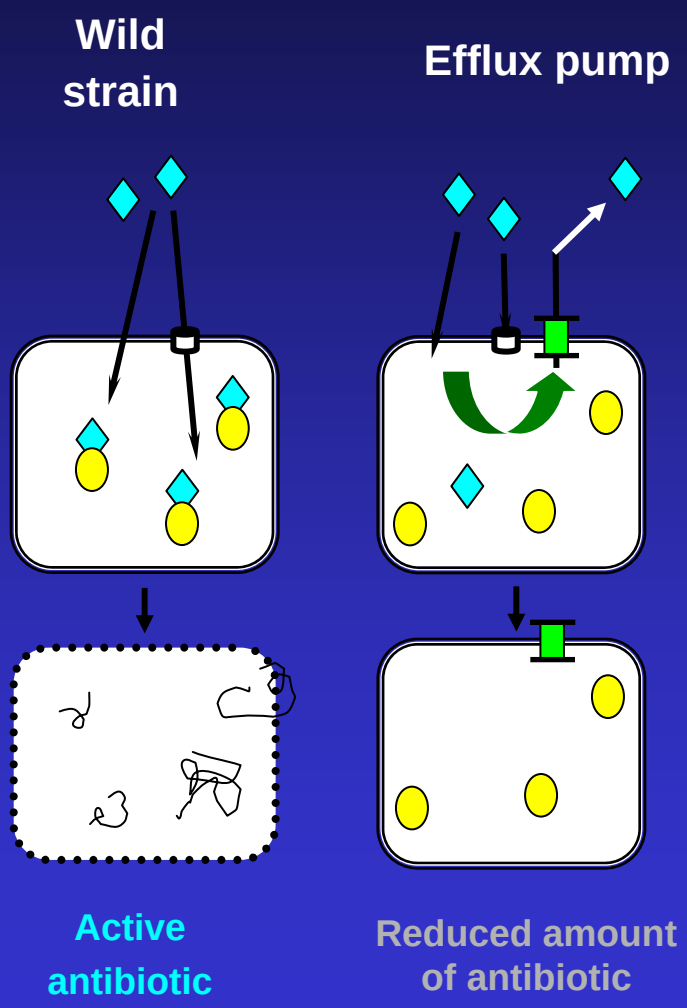


- **mutation of the OprD porine reducing the penetration of various antibiotics in *Pseudomonas aeruginosa***

responsible for « intrinsic » resistance of *P.aeruginosa* to a large number of antibiotics

Antibiotic resistance: mechanisms

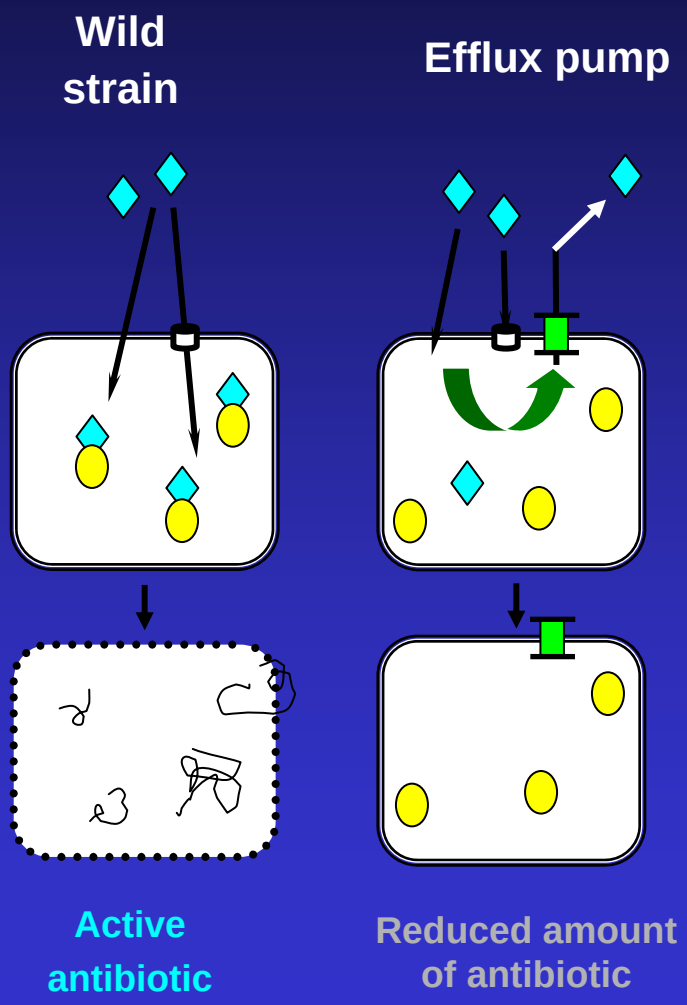
4. « elimination » strategy



- overexpression of wide spectrum efflux pumps conferring cross-resistance to a large number of antibiotics in *Pseudomonas aeruginosa and E. coli*
- overexpression of narrow spectrum pumps conferring resistance to a given class of antibiotics in *S. aureus* and *S. pneumoniae*

Antibiotic resistance: mechanisms

4. « elimination » strategy

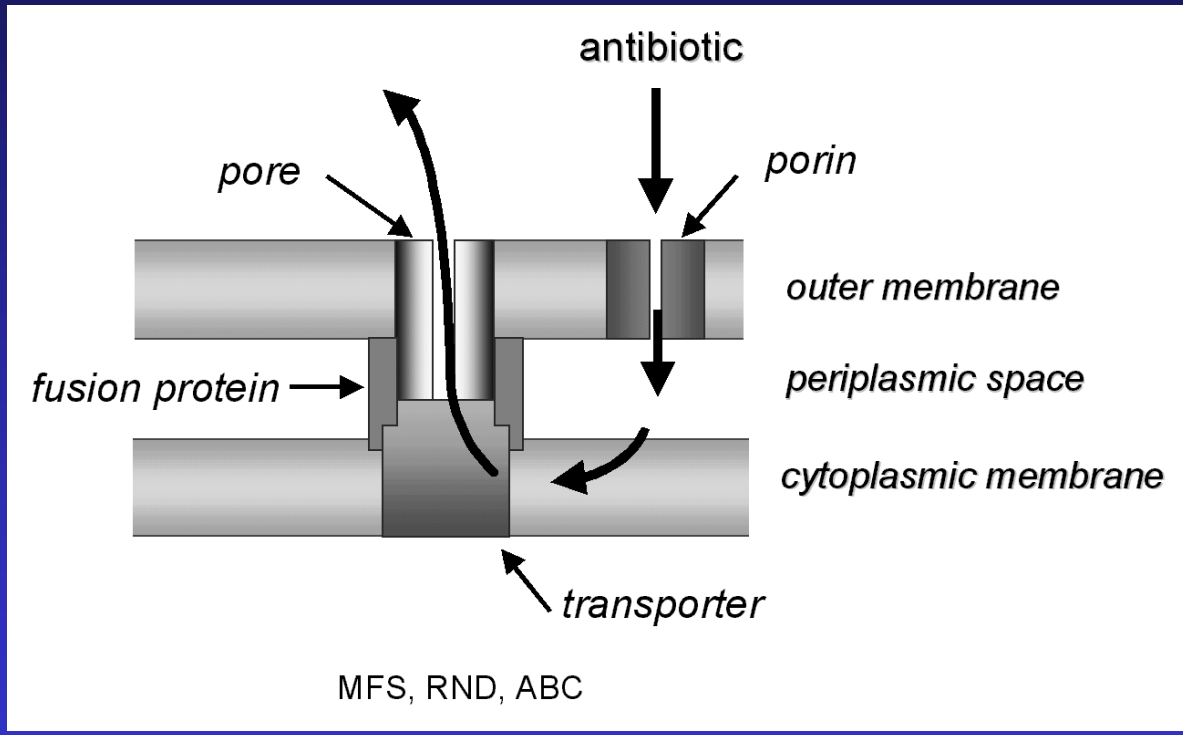


- overexpression of … spectrum efflux pumps conferring … to a large number of … *Pseudomonas* …
- overexpression of narrow … conferring resistance to a given class of antibiotics in *S. aureus* and *S. pneumoniae*

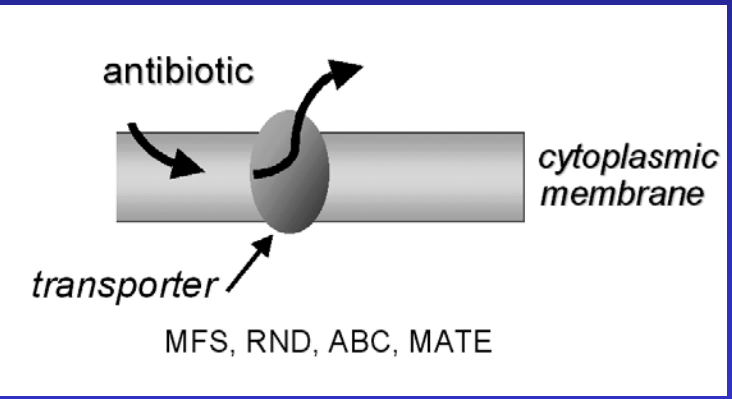
Responsible for « intrinsic » resistance of *P.aeruginosa* to a large number of antibiotics

Antibiotic transport through bacterial membranes

Gram(-)



Gram(+)



Van Bambeke *et al* JAC (2003) 51:1067-1077

Antibiotic efflux in Gram (+)

organism	family	pump	antibiotic						
			β-lactams	Aminoglycosides	Fluoroquinolones	Macrolides	Tetracyclines	Trimetoprim	Sulfamides
S. aureus	ABC	MsrA				■			
	MFS	MdeA		■					
		NorA			■				
		TetK-L					■		
S. pneumoniae	MSF	MefA				■			
		MefE				■			
		PmrA			■				
		TetK-L					■		

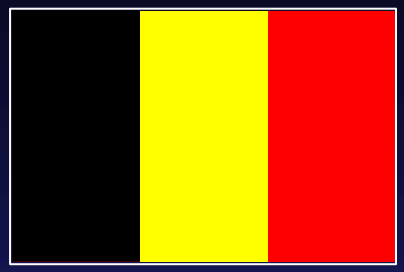
Antibiotic efflux in Gram (-)

organism	famiy	pump	antibiotic						
			β-lactams	Aminoglycosides	Fluoroquinolones	Macrolides	Tetracyclines	Trimetoprim	Sulfamides
E. coli	ABC	MacAB-TolC				■			
	MFS	ErmAB-TolC			■		■		
		TetA-E					■		
	RND	AcrAB-TolC	■		■	■	■	■	
		AcrCD-TolC		■					
		AcrEF-TolC	■		■	■	■	■	
	SMR	ErmE				■	■		■

…and the list is much longer

Antibiotic efflux in Gram (-)

organism	family	pump	antibiotic						
			β-lactams	Aminoglycosides	Fluoroquinolones	Macrolides	etracyclines	Trimetoprim	Sulfamides
P. aeruginosa	MFS	TetA,C,E					■		
	RND	MexAB-OprM	■		■	■	■	■	■
		MexCD-OprJ	■		■		■	■	
		MexEF-OprN			■			■	
		MexJK-OprM				■	■		
		MexXY-OprM		■		■	■		

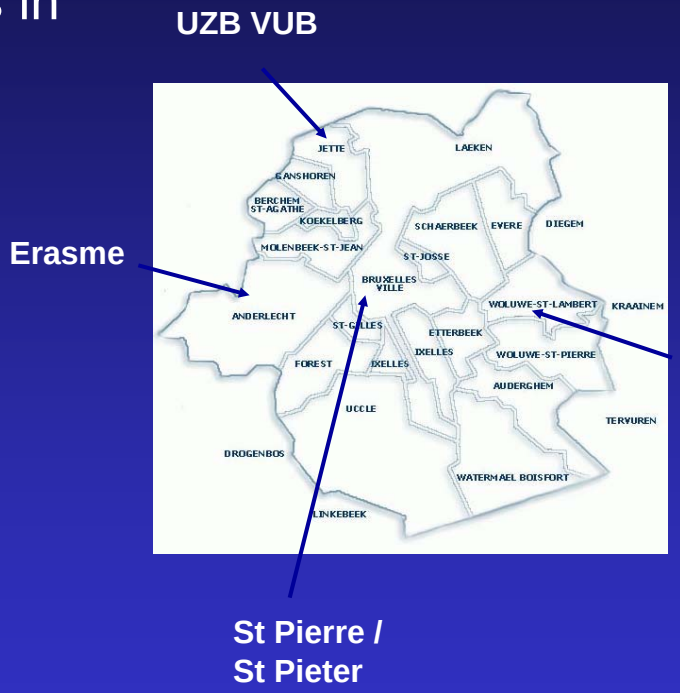


Antibiotic resistance in bacteria responsible for respiratory tract infections : how is doing Belgium at the beginning of the XXI century ?

A recent study on pneumococci …

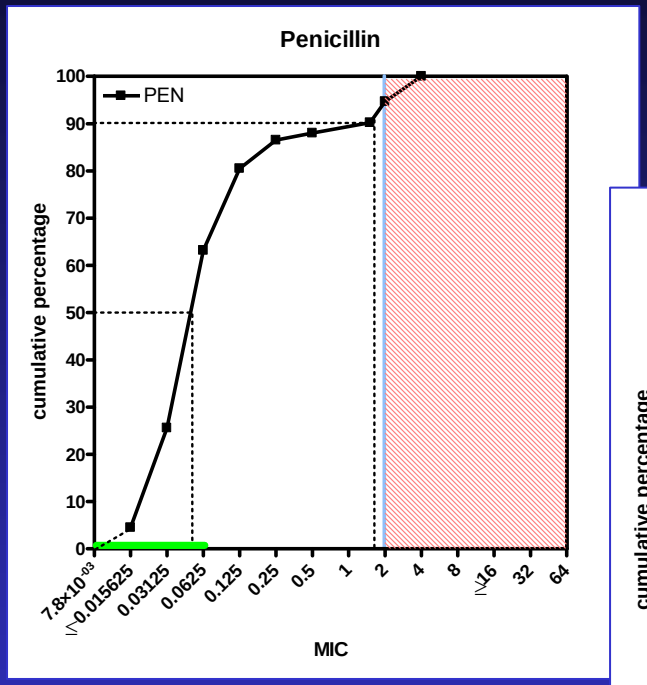
- **Bacteria:**
  146 samples of *S. pneumoniae* isolated in 2004-2007 from patients in 4 large hospitals in the Region of Brussels with a diagnostic of community acquired pneumonia

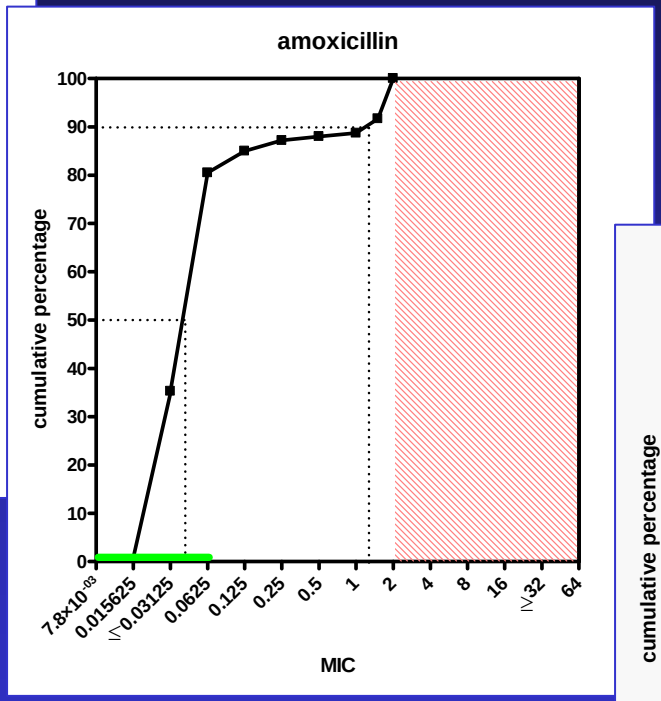
- **Susceptibility testing:**
  - MICs (microdilution)
  - Resistentance throuh active efflux
    - for macrolides: comparison between erythromycin and clindamycin
    - for quinolones: addition of reserpine

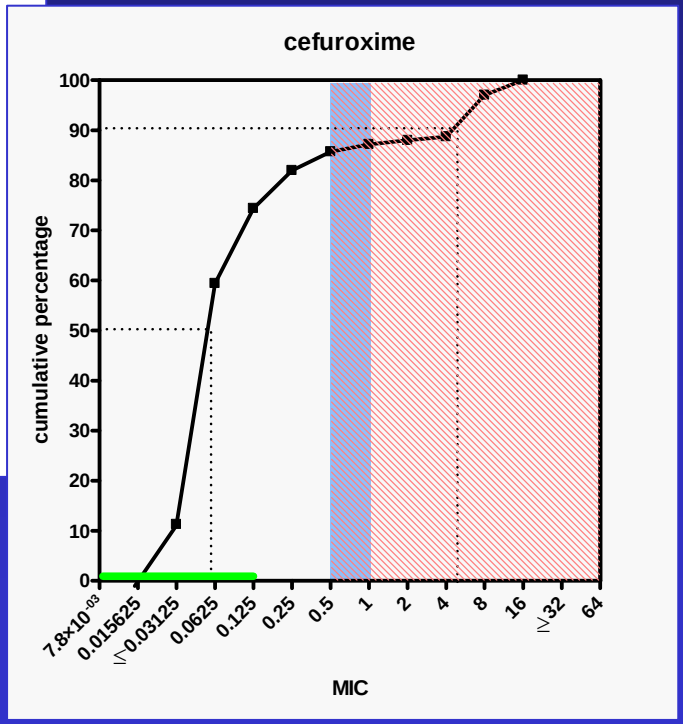


Epidemiological survey of antibiotic resistance in a Belgian collection of CAP isolates of *Streptococcus pneumoniae* (SP)
A. Lismond, F. Van Bambeke, S. Carbonnelle, F. Jacobs, M. Struelens, J. Gigi, A. Simon, . Van Laethem, A. Dediste, D. Pierard, A. De Bel, & P.M. Tulkens, RICAI, Paris, 2007 / ECCMID, Barcelona, 2008 (in voorbereiding)

S. pneumoniae susceptibility for patients with CAP





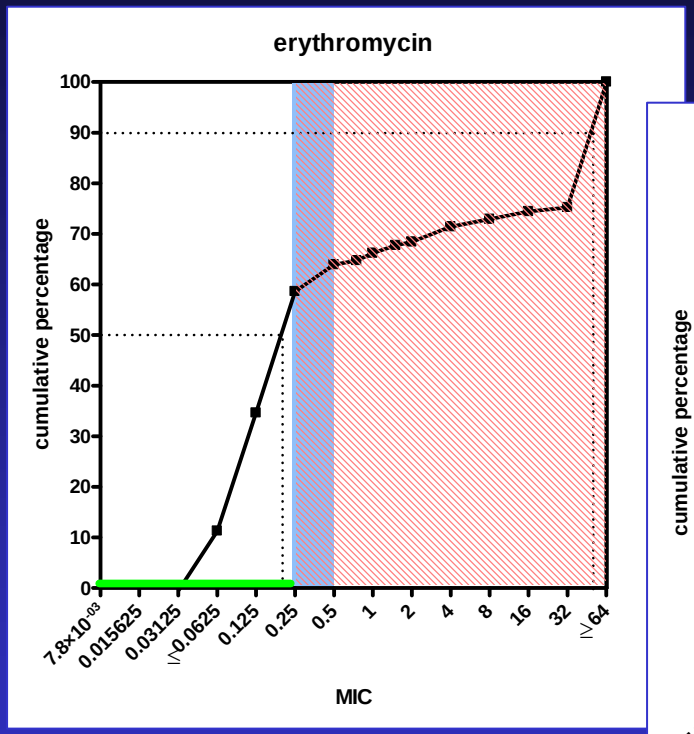


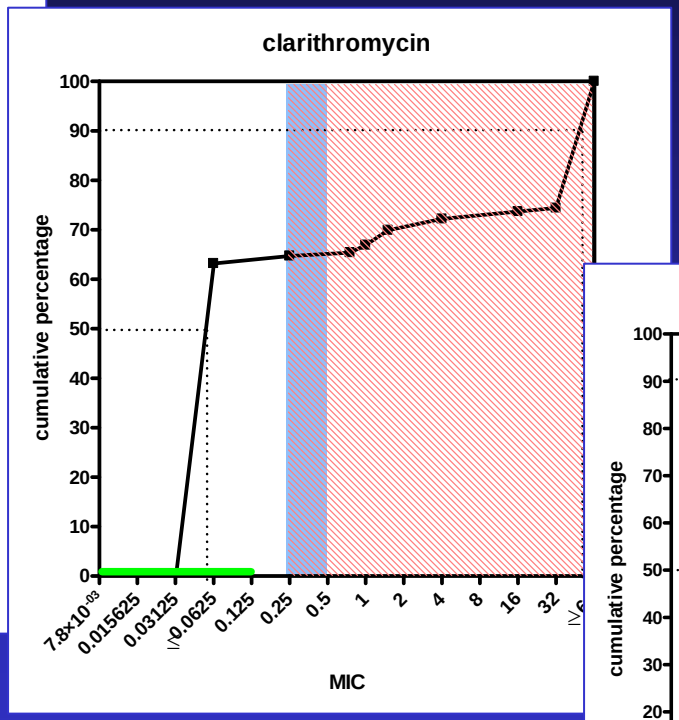
susceptible

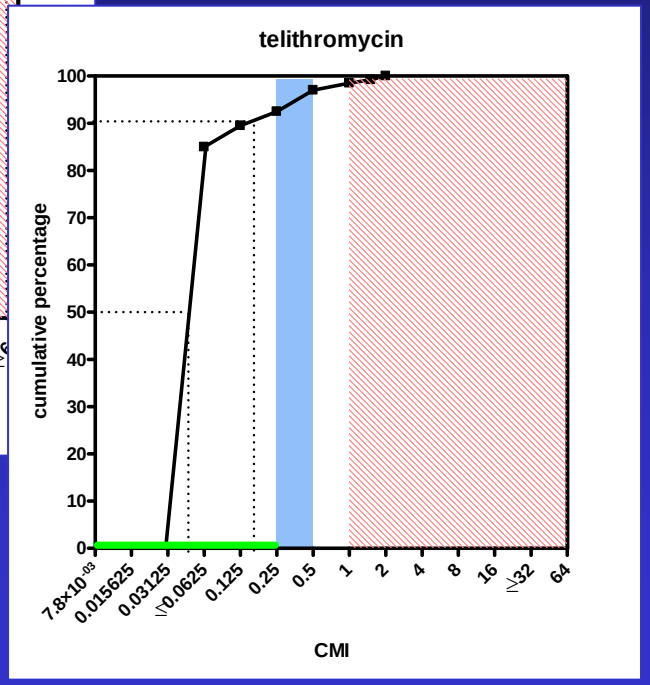
decreased sucseptibility (EUCAST)

resistant (CLSI)

S. pneumoniae susceptibility for patients with CAP





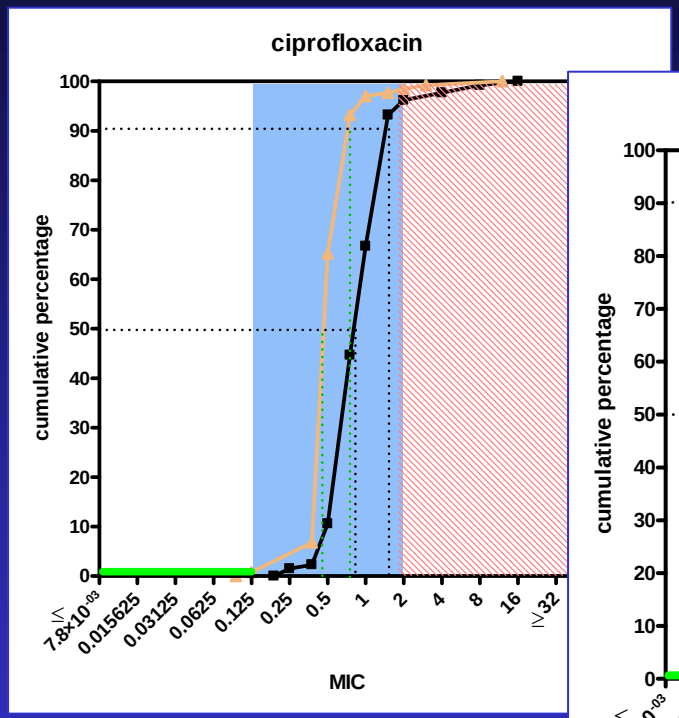


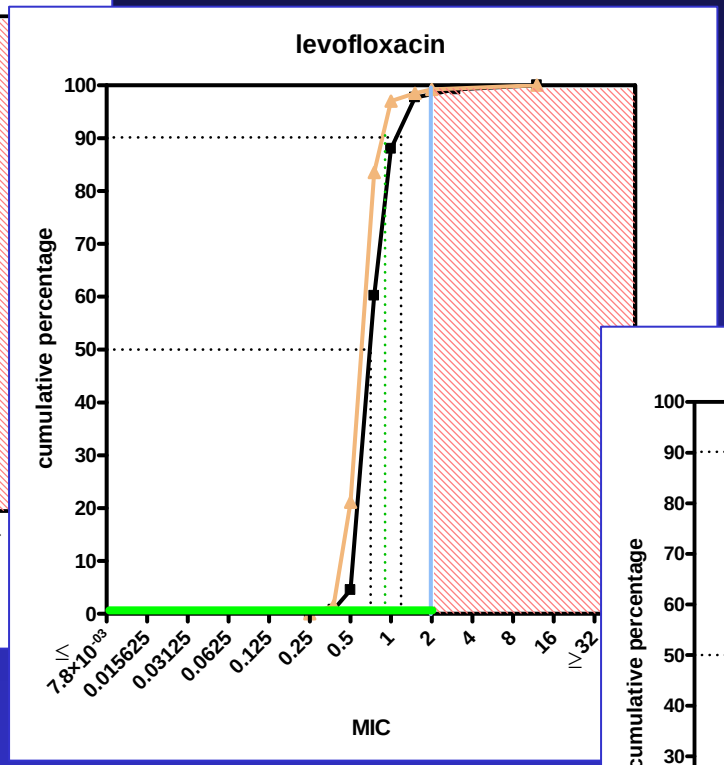
susceptible

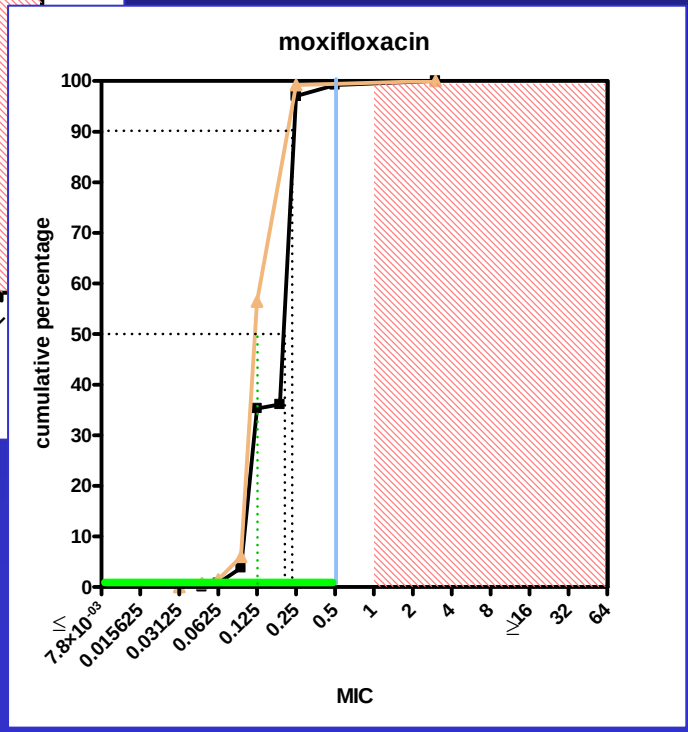
decreased sucseptibility (EUCAST)

resistant (CLSI)

S. pneumoniae susceptibility for patients with CAP







susceptible

decreased sucseptibility (EUCAST)

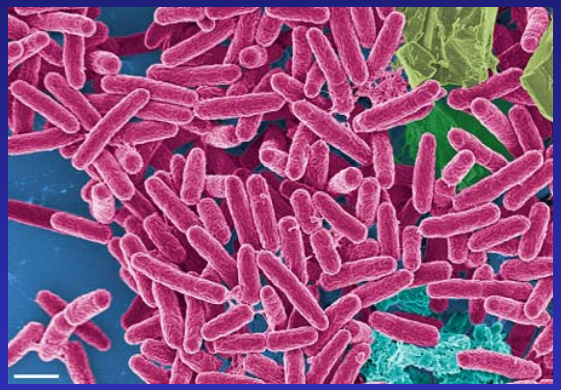
resistant (CLSI)

with efflux (reserpine)

S. pneumoniae : clinical attitude to cope with the increase of resistance

Antibiotic class	Resistance mechanism	Clinical attitude
β-lactams	• Target modification causing a progressive reduction in susceptibilities	• increase the dose (« I » strains) • change AB class (« R » strains)
macrolides	• Target modification causing a marked change in susceptibility • efflux	• Prefer ketolide (higher affinity for the mutated target; less subjected to efflux) or 16-membered macrolides (miocamycine; less susceptibles to efflux) • change AB class
fluoroquinolones	• target modification • efflux	• Select the molecule with highest intrinsic activity (ciprofloxacine <<< levofloxacine < moxifloxacine) • Change AB class
tetracyclines	• modification de la cible • efflux	• change antibiotic class

Other useful local data useful for the next steps of our journey …



Focus on *Pseudomonas aeruginosa*

What is the problem ?

Pseudomonas aeruginosa: resistance and therapeutic options at the turn of the new millennium

N. Mesaros[1], P. Nordmann[2], P. Plésiat[3], M. Roussel-Delvallez[4], J. Van Eldere[5], Y. Glupczynski[6], Y. Van Laethem[7], F. Jacobs[8], P. Lebecque[9], A. Malfroot[10], P. M. Tulkens[1] and F. Van Bambeke[1]

ABSTRACT (summarized)

Pseudomonas aeruginosa is a major cause of nosocomial infections.

It resists to many antibiotics, either intrinsically (because of constitutive expression of β-lactamases and efflux pumps, combined with low permeability of the outer-membrane) or following acquisition of resistance genes (e.g., genes for β-lactamases, or enzymes inactivating aminoglycosides or modifying their target), over-expression of efflux pumps, decreased expression of porins, or mutations in quinolone targets.

Susceptibility testing is therefore crucial in clinical practice.

Empirical treatment usually involves combination therapy, selected on the basis of known local epidemiology.

Innovative therapeutic options for the future remain scarce.

Accepted: 24 November 2006

Clin Microbiol Infect 2007; **13**: 560–578

What can you do ?

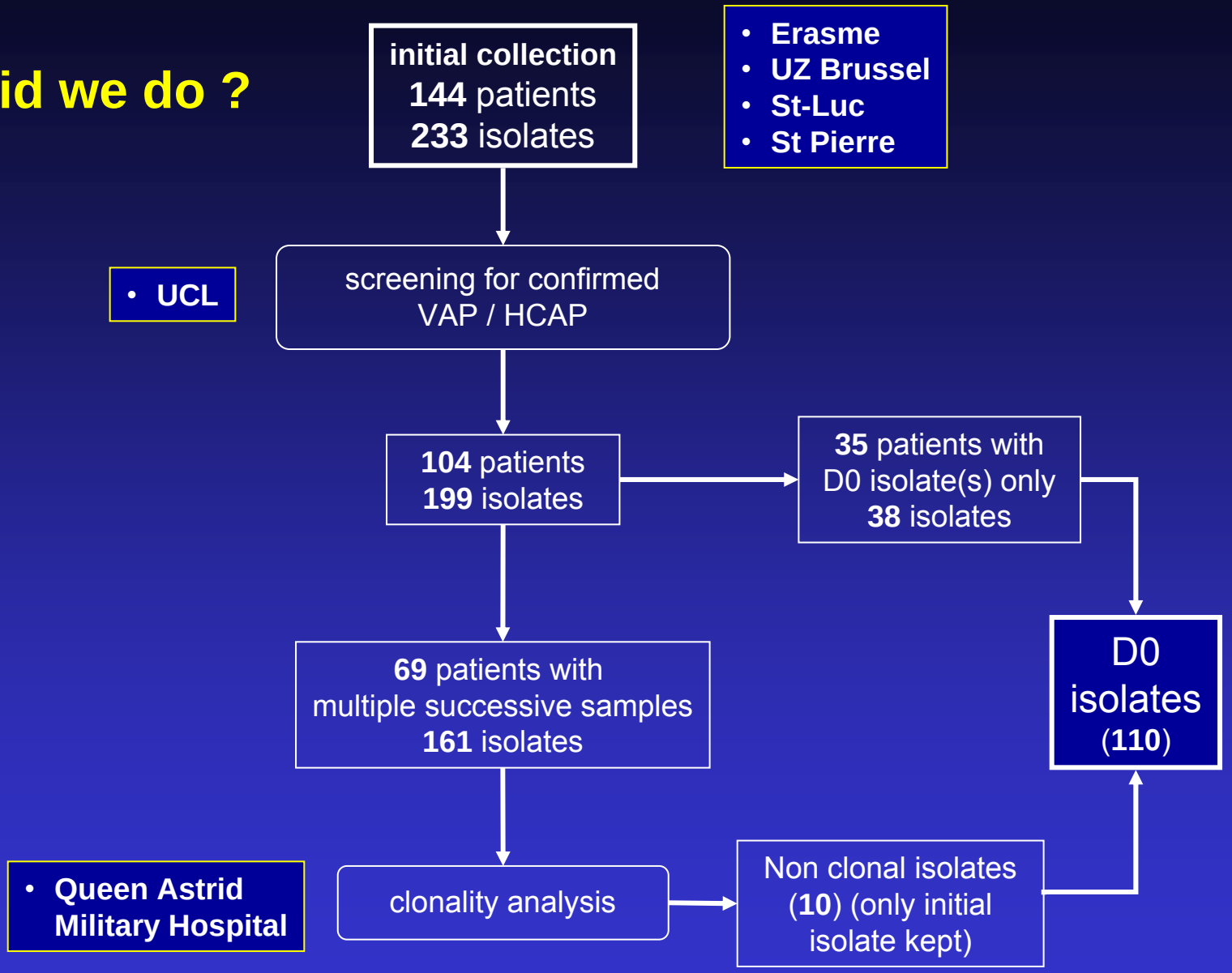
- Survey the level of resistance in Brussels Hospitals and relate it to therapy
- Examine the mechanisms of resistance acquisition (with special reference to efflux pumps)
- Assess new antibiotics and novel approaches (immunotherapy)
- Examine the susceptibility to biocides

Study #1

Impact of therapy on the development of in vitro antimicrobial resistance in *Pseudomonas aeruginosa* strains isolated from lower respiratory tract of Intensive Care Units (ICU) patients with nosocomial pneumonia

Supported by the
- "Région Bruxelloise/Brusselse Gewest" (Research in Brussels)
- FNRS (post-doctoral fellowships)
- FRSM

What did we do ?



Characteristics of the patients

Total population (n=104)

Age	**lowest**	**geom. mean**	**mean±SD**	**median**	**highest**
years	1.2	54.1	60.0 ± 19.3	63.1	85.0

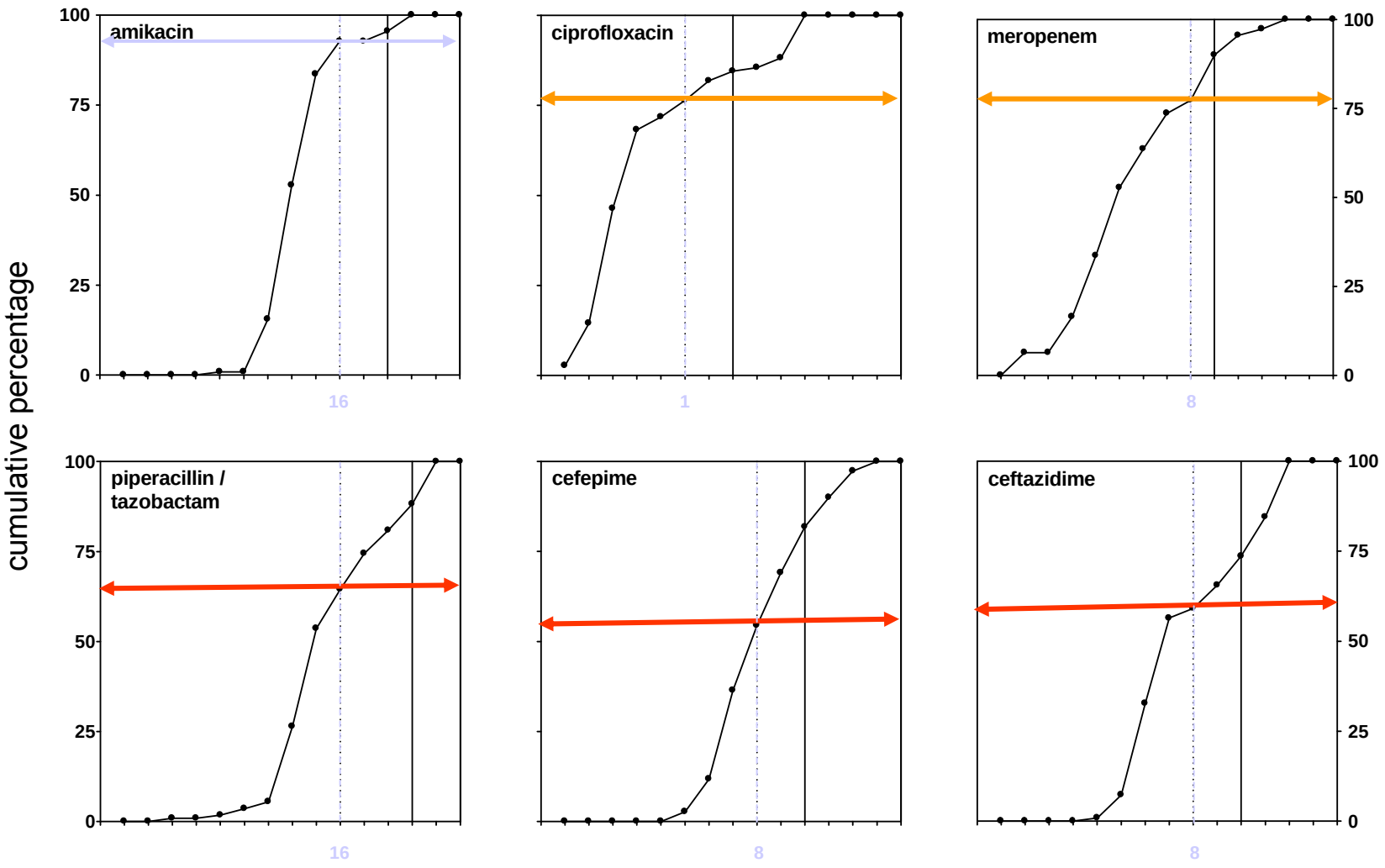
Ventilated	**yes**	**no**
no. of patients	74	30

Enrolment based upon

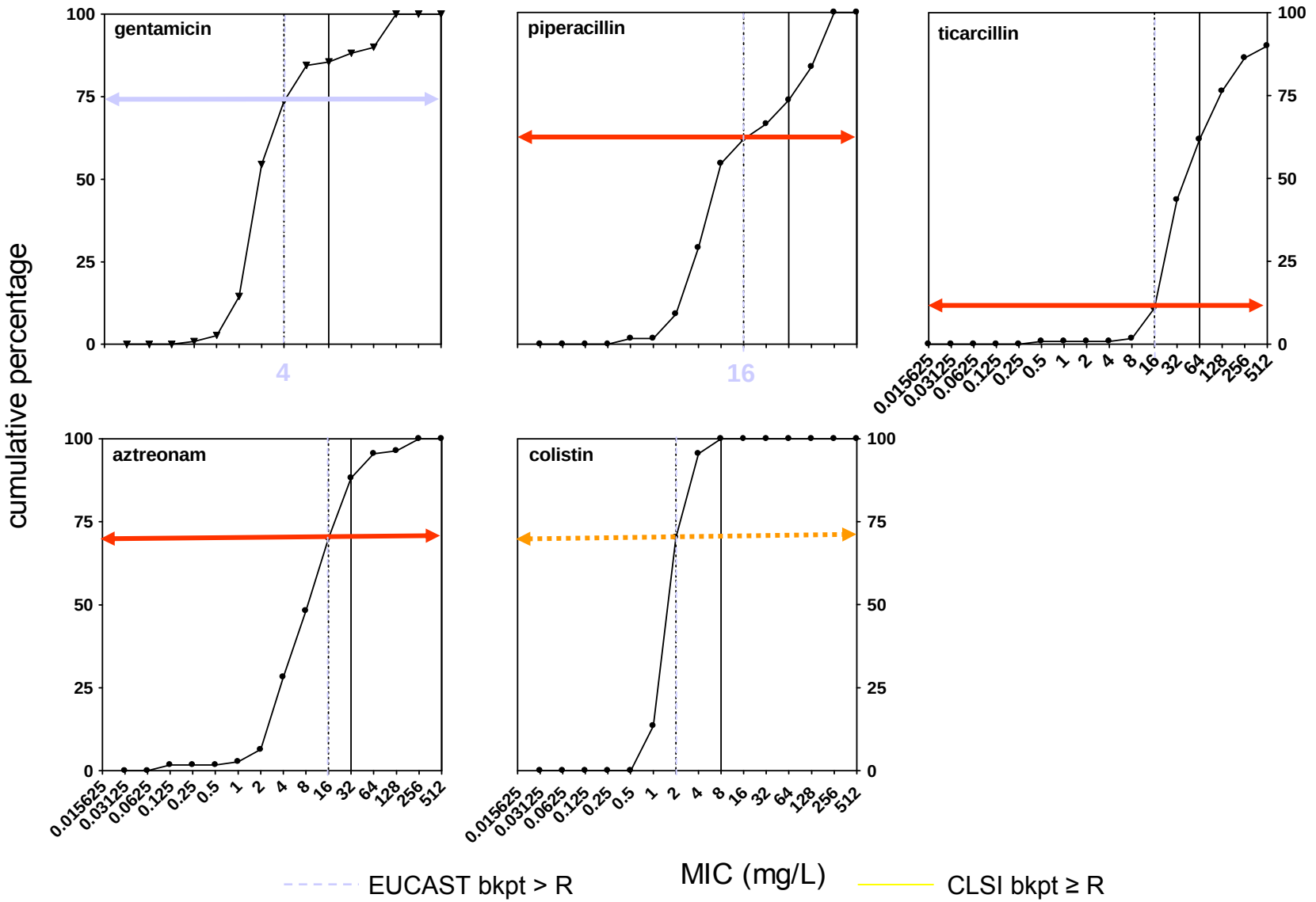
- report of the isolation of *P. aeruginosa* as single or predominant microorganism from the lower respiratory tract [endotracheal or bronchial aspirates, broncho-alveolar lavages] and/or from pleural fluid, and
- radiological confirmation of the pneumonia (presence of infiltrates).

Cystic fibrosis patients systematically excluded.

What is the situation at day 0 ?



What is the situation at day 0 ?



What is the situation at day 0 ?

antibiotic	$MIC_{50/90}$ (mg/L)	% non-susceptible isolates according to			
		EUCAST		CLSI	
		breakpoint [a] (≤ S / R >) mg/L	isolates I / R	breakpoint [b] (≤ S / R ≥) mg/L	isolates I / R
AMK	4 / 16	8 / 16	9 / 8	16 / 64	1 / 7
CIP	0.25 / 8	0.5 / 1	7 / 23	1 / 4	4 / 18
MEM	1 / 16	2 / 8	12 / 24	4 / 16	3 / 24
TZP	8 / 128	16 / 16	**34** [c]	64 / 128	7 / 12
FEP	8 / 64	8 / 8	**46** [c]	8 / 32	**17 / 30**
CAZ	4 / 64	8 / 8	**39** [c]	8 / 32	**6 / 33**
GEN	2 / 64	4 /4	**26** [c]	4 / 16	10 / 15
PIP	8 / 128	16 / 16	**36** [c]	64 [d] / 128	0 / **26**
TIC	64 / 512	16 / 16	**86** [c]	64 / 128	0 / **39**
ATM	8 / 32	1 / 16	**68 / 30**	8 / 32	**20 / 30**
CST	2 / 4	2 / 2	**33** [c]	2 / 8	26 / 0

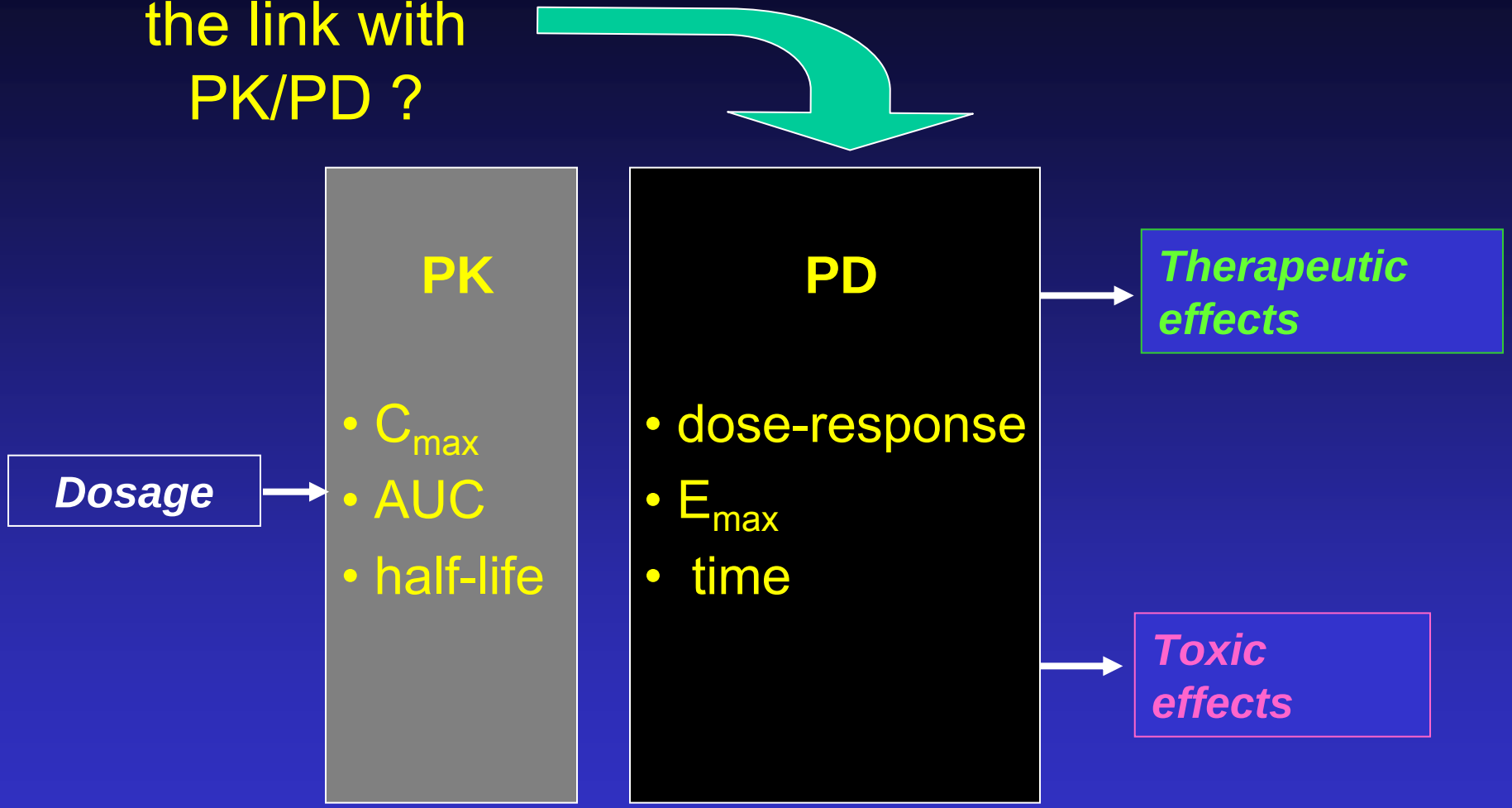
Are they cross-resistances at day 0 ?

	AMK	CIP	MEM	TZP	FEP	CAZ	*GEN*	*PIP*	*TIC*	*ATM*	*CST*
AMK	18 / 8	14 / 8	12 / 5	16 / 7	17 / 4	17 / 5	*14 / 8*	*16 / 6*	*18 / 8*	*18 / 8*	*4 / 0*
	CIP	**31 /** 26	21 / 16	22 / 8	27 / 24	23 / 21	*21 / 20*	*23 / 13*	***29** / 21*	***31** / 24*	*11 / 0*
		MEM	**40 / 29**	23 / 7	***28** / 22*	25 / 20	*18 / 13*	*23 / 12*	***37** / 20*	***40** / 22*	*11 / 0*
			TZP	**39** / 21	***37** / 20*	***39** / 21*	*22 / 11*	***38** / 21*	***33** / 17*	***39** / 20*	*8 /0*
				FEP	**50 / 50**	***39 / 39***	*28 / 28*	***38** / 26*	***42** / 26*	***50 / 44***	*14 / 0*
					CAZ	**45 / 45**	*24 / 24*	***42 / 29***	***45 / 32***	***45 / 40***	*11 / 0*
						GEN	***29 / 29***	*24 / 17*	*29 / 24*	***29 / 29***	*7 / 0*
							PIP	***42 / 29***	*21 / 12*	***42 / 28***	*9 / 0*
								TIC	***98 / 42***	***98 / 38***	*27 / 0*
									ATM	***107 / 57***	***32** / 0*
										CST	***33 / 0***

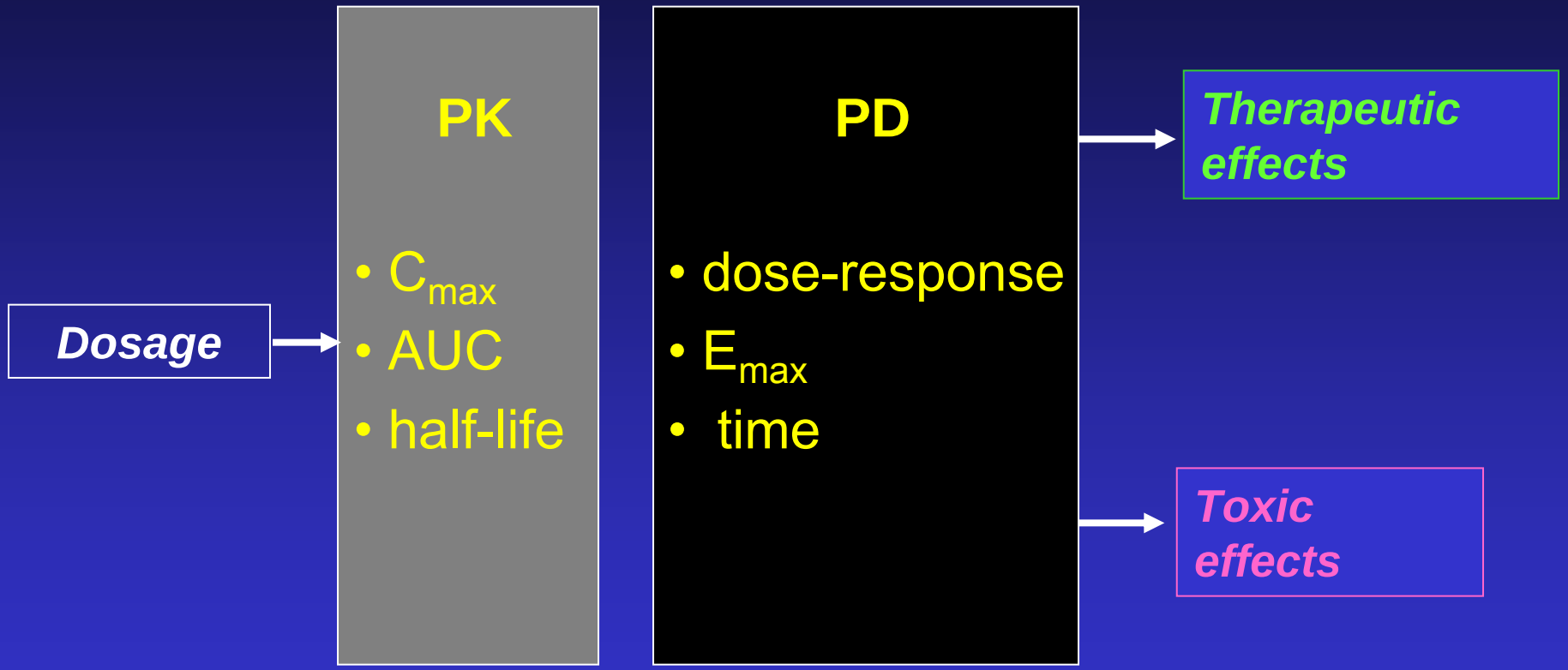
Number of isolates (out of 110 initial isolates [D0]) categorized as resistant to the two antibiotics (row – column) using the criteria of EUCAST (first figure) or CLSI (last figure).

- **red-bold**: combinations for which cross-resistance > 25% of isolates
- EUCAST only -- EUCAST and CLSI

But what is the link with PK/PD ?



But what is the link with PK/PD ?



Let's go and see in the section:
PK/PD to fight resistance …

Section 4 B