Impact of the antibiotic treatment on resistance of Pseudomonas aeruginosa in nosocomial pneumonia, or

***Pseudomonas* in Brussels in 2010**

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SEMINAIRES DE MICROBIOLOGIE ET MALADIES INFECTIEUSES - 24 mars 2010

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 ?

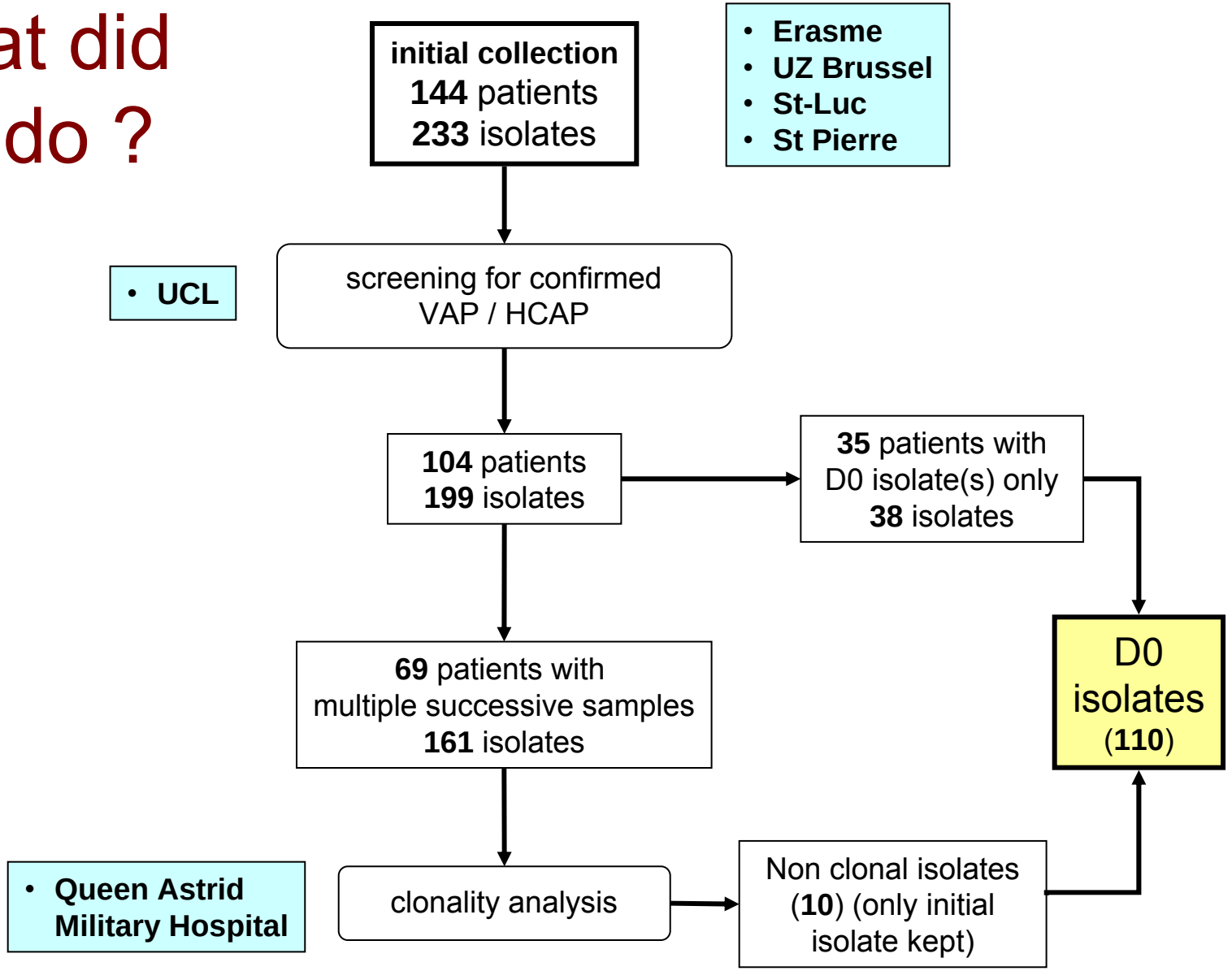
- Survey the level of resistance in Brussels Hospitals and relate it to therapy
- Examine the mechanisms of resistance acquisition (with special reference to efflux pumps)
- Assess new antibiotics and novel approaches (immunotherapy)
- Examine the susceptibility to biocides

Study #1

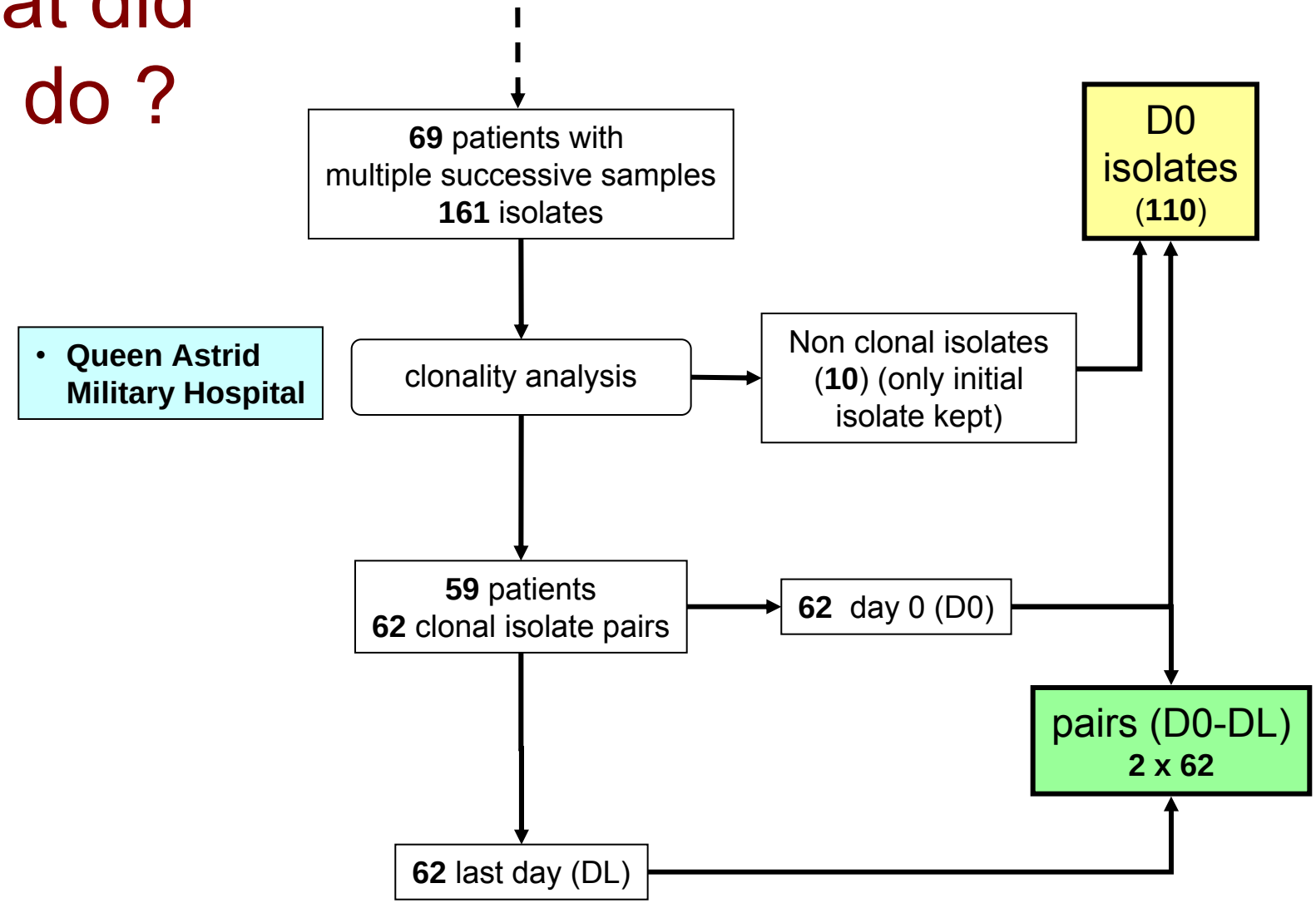
Impact of therapy on the development of in vitro antimicrobial resistance in *Pseudomonas aeruginosa* strains isolated from lower respiratory tract of Intensive Care Units (ICU) patients with nosocomial pneumonia

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

What did we do ?



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

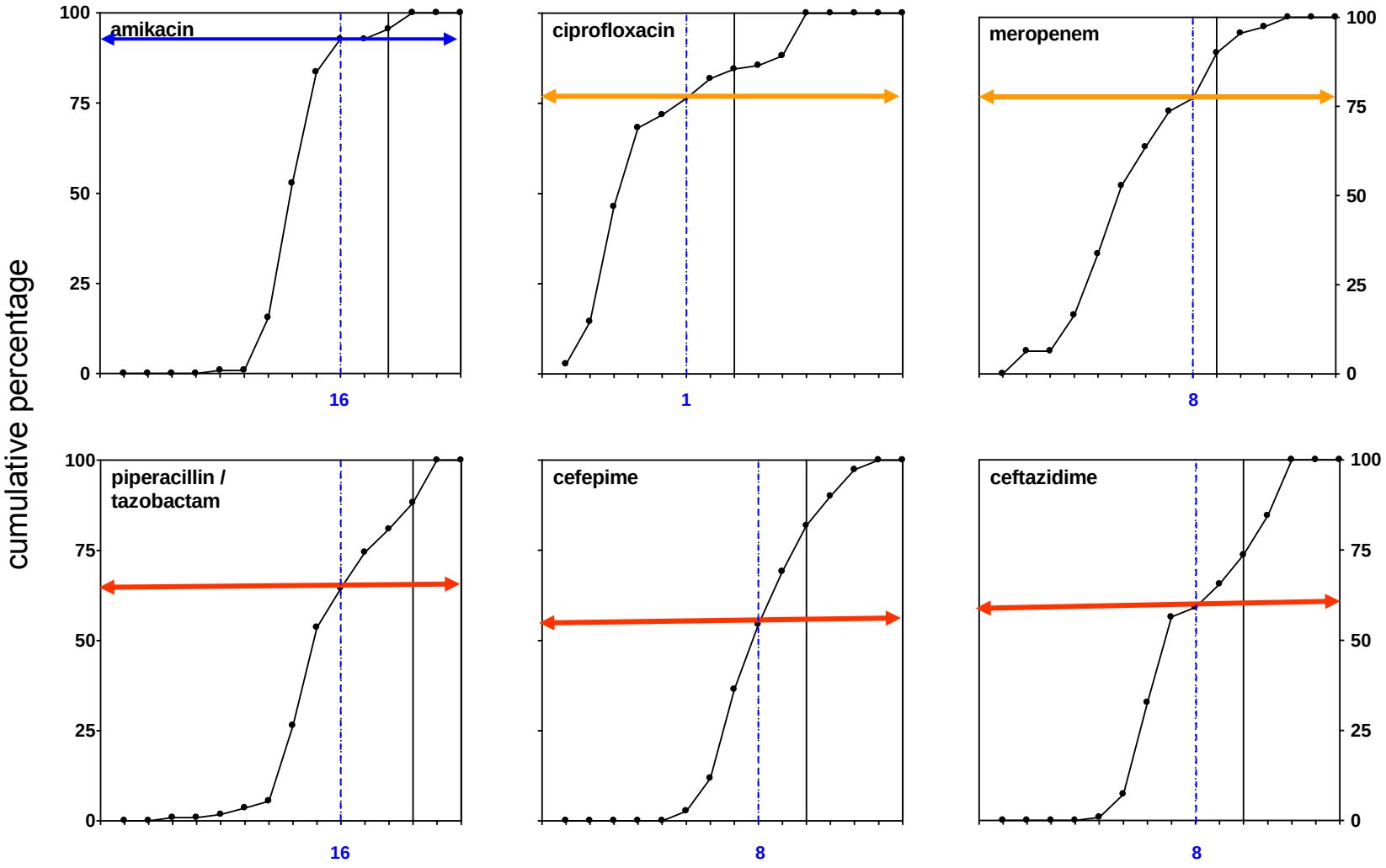
Ventilated	**yes**	**no**
no. of patients	74	30

Enrolment based upon

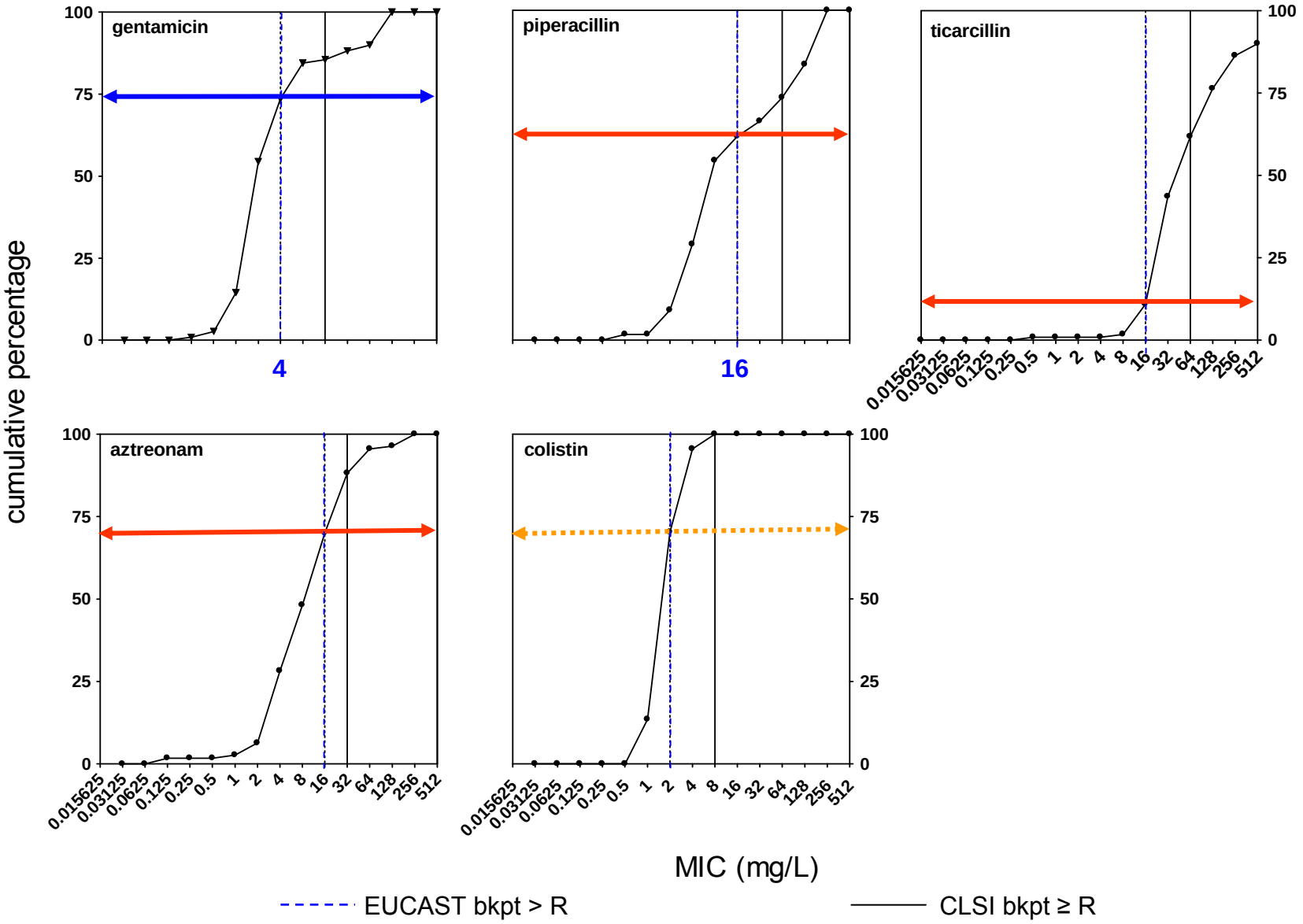
- report of the isolation of *P. aeruginosa* as single or predominant microorganism from the lower respiratory tract [endotracheal or bronchial aspirates, broncho-alveolar lavages] and/or from pleural fluid, and
- radiological confirmation of the pneumonia (presence of infiltrates).

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***

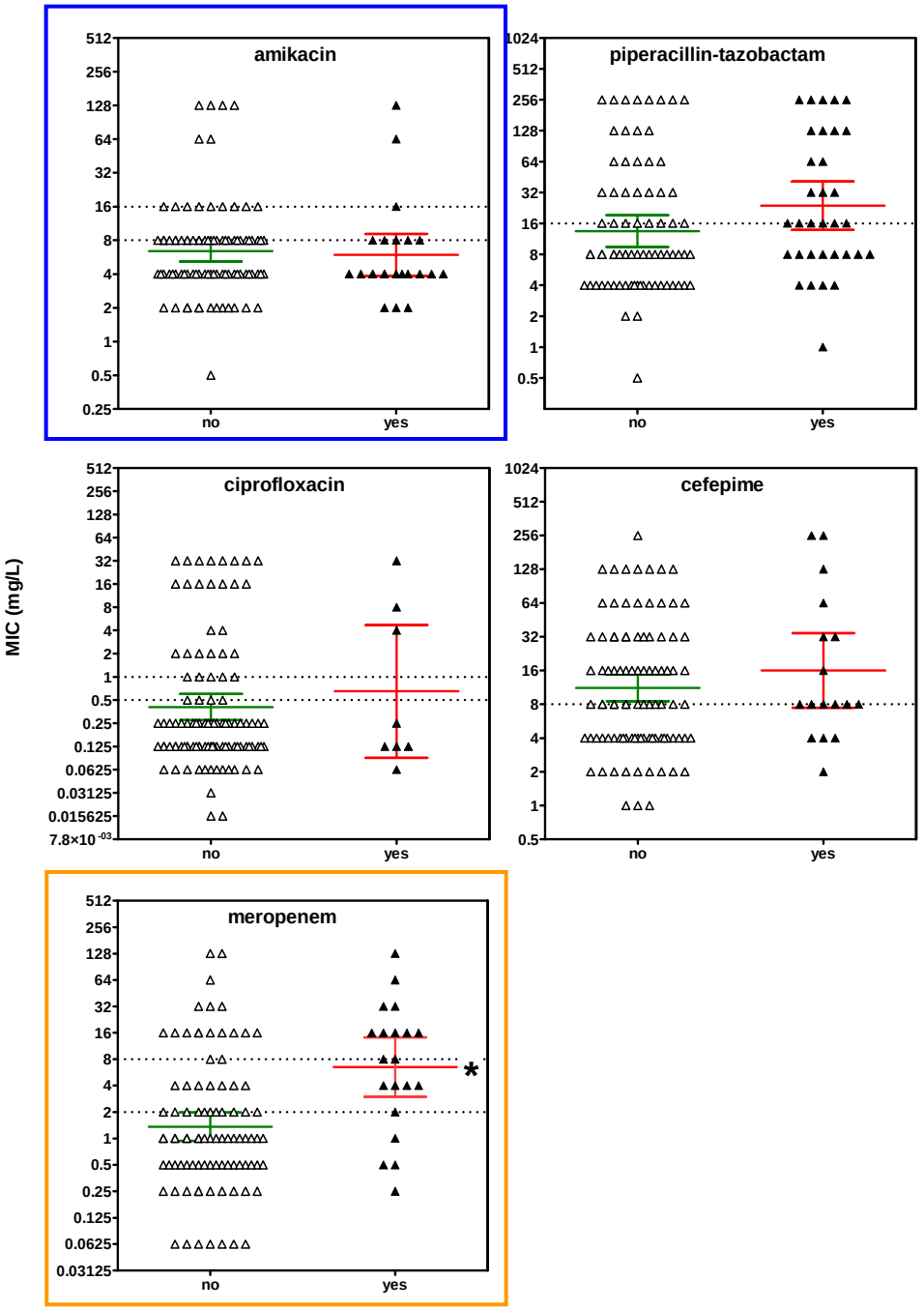
Number of isolates (out of 110 initial isolates [D0]) categorized as resistant to the two antibiotics (row – column) using the criteria of EUCAST (first figure) or CLSI (last figure).

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

What are the susceptibilities at day 0 if you have received (or not) the same antibiotic up to 1 month before ?

- individual values with geometric mean (95 % CI)
- S (lowest line) and R (highest line) EUCAST breakpoints

* $p < 0.05$ by unpaired t-test (two-tailed) and Mann-Whitney non-parametric test



Was the initial treatment microbiologically adequate ?

% of adequate initial therapies **(n; total = 54)**

	no. of patients	no. of adequate antibiotics	*based on breakpoints of*	
			EUCAST	**CLSI**
monotherapy	**26**	1/1	57.7 (15)	73.1 (19)
bitherapy	**14**	2/2	71.4 (10)	85.7 (12)
		1/2	28.6 (4)	14.3 (2)
tritherapy	**13**	3/3	41.7 (5)	46.2 (6)
		2/3	33.3 (4)	30.8 (4)
		1/3	25.0 (3)	23.1 (3)
quadritherapy	**1**	4/4	0	0
		3/4	100 (1)	100 (1)

Antipseudomonal antibiotics only
Based on MIC of the initial isolate(s) and using EUCAST or CLSI criteria for non-resistant organisms (S or I)

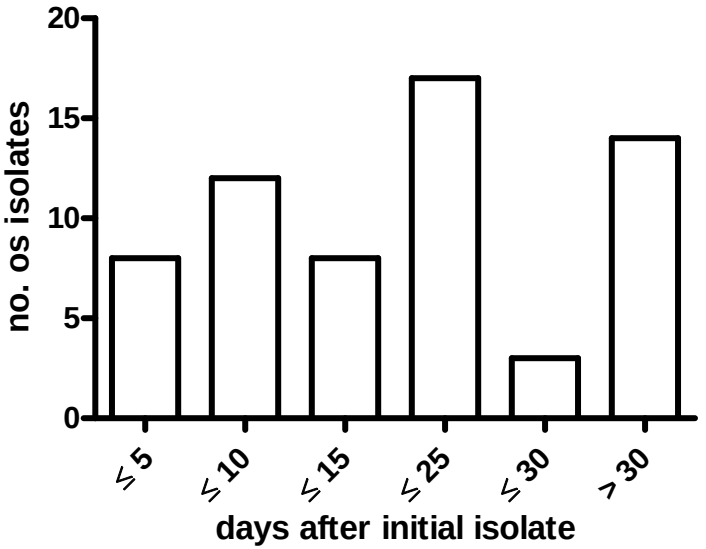
What happens after the initial day ?

Patients with clonal pairs (n=59)

antibiotics with antipseudomonal potential (initial treatment [a]; no. of patients)

drug	AMK	CIP	MEM	TZP	FEP	CAZ
no. of patients	29	11	28	31	29	4

Pairs were obtained
from day 1 to > day 30



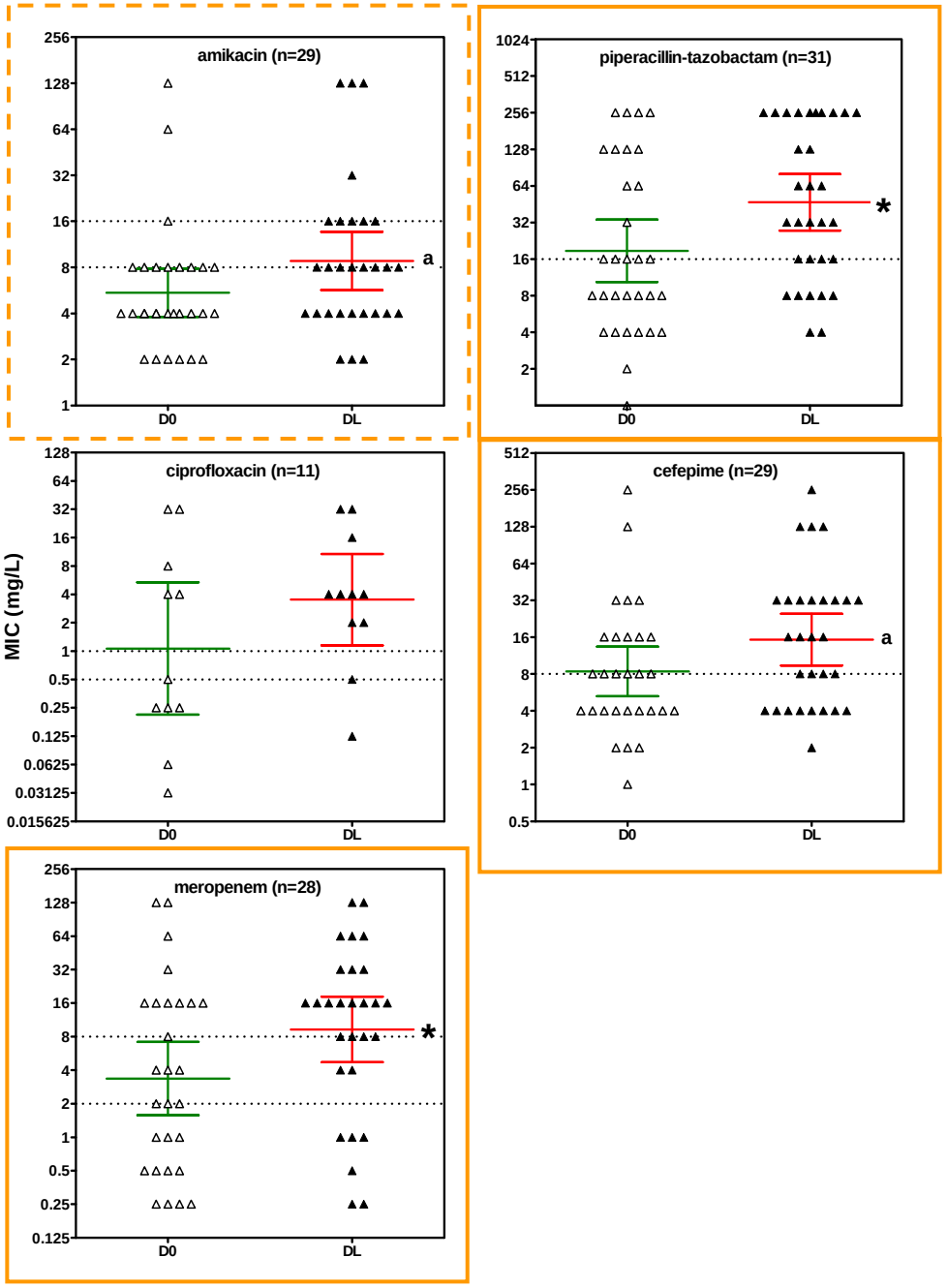
What happens during treatment ?

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

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

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

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



Are these real clonal pairs ?

Diversilab™ assessment of clonality

A : DNA genotyping (repetitive-element-based polymerase chain reaction assay; score set at ≥ 95% similarity [red line])

* typical example of a clonal pair;

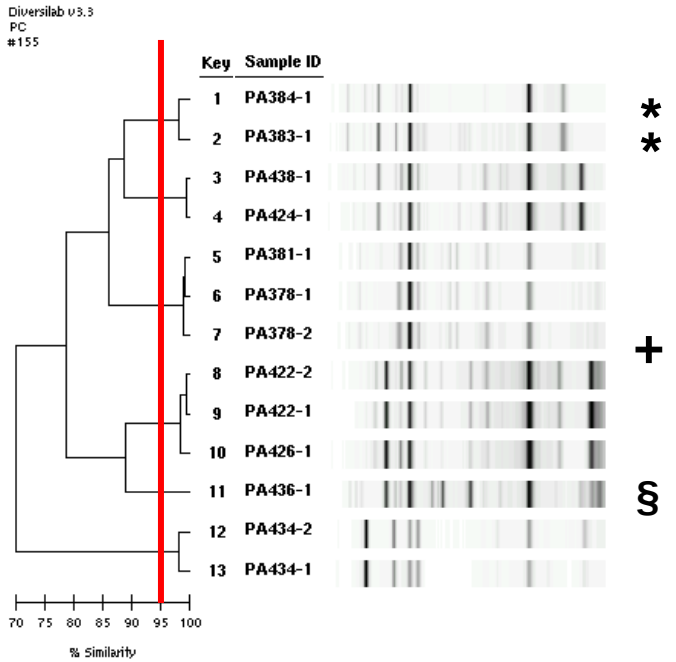
\+ quality control strain

§ non-clonal or independent isolate

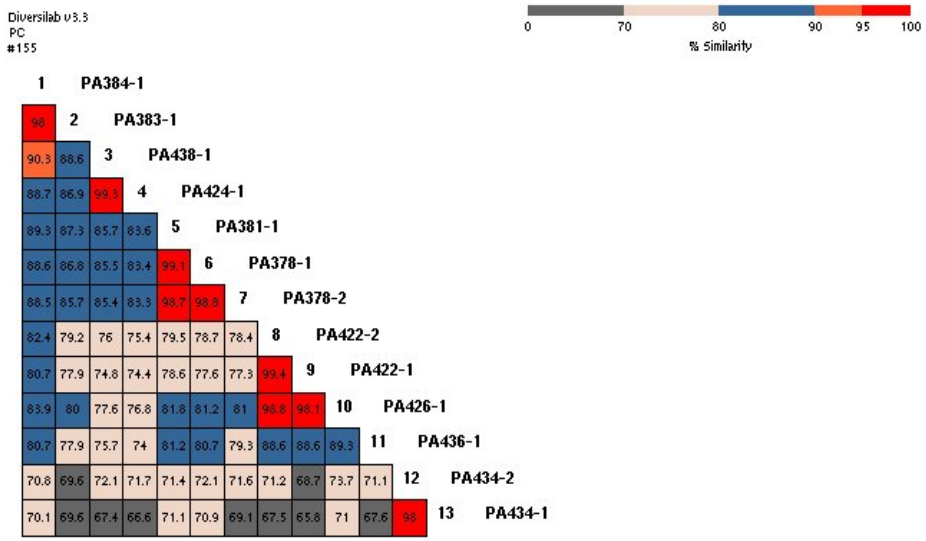
B: interpretation of the results

Only isolates with ≥ 95% similarity were considered as clonal.

A



B



Are the antibiotics the cause of the problem ?

antibiotic	use (%)	non susceptible isolates according to EUCAST (% I / R) [a] D0	non susceptible isolates according to EUCAST (% I / R) [a] DL	non susceptible isolates according to CLSI (% I / R) [a] D0	non susceptible isolates according to CLSI (% I / R) [a] DL	loss of susceptibility (%) during treatment [b] and correlation with antibiotic use: EUCAST	loss of susceptibility (%) during treatment [b] and correlation with antibiotic use: CLSI
AMK	22.0	1.6 / 11.3	11.3 / 16.1	0.0 / 11.3	4.8 / 11.3	14.5	4.8
CIP	8.3	4.8 / **25.8**	4.8 / **35.5**	3.2 / 22.6	6.5 / **29.0**	9.7	9.7
MEM	21.2	12.9 / 22.6	14.5 / **35.5**	1.6 / 22.6	6.5 / **35.5**	14.5	17.7
TZP	23.5	**33.9** [d]	**53.2** [d]	0.0 / 17.7	0.0 / **32.3**	19.5	14.6
FEP	22.0	**40.3** [d]	**53.2** [d]	12.9 / **27.4**	8.1 / **45.2**	14.5	12.9
CAZ	3.0	**35.5** [d]	**46.8** [d]	8.1 / **27.4**	8.1 / **38.7**	11.3	11.3
						r=0.89 [c] **(p=0.03)**	r=0.27 [c] (p=0.66)

[a] **red bold**: resistance in > 25 % of all isolates

[b] % of isolates moving from S to I or R between day 0 and day ≥ 3

[c] non parametric correlation (Spearman rank) between the % of use of each antibiotic (% of all antibiotic prescriptions) in the whole population (AMK, 24.0; CIP, 9.6; MEM, 20.2; FEP, 15.4; CAZ, 3.8) and the increase in % of isolates with change in susceptibility (moving from S to I, I to R, or S to R) for the corresponding antibiotic

But what happened with the patients ?

Clinical outcome

	alive	death from	
		pneumonia	other cause
no. of patients	41	9	9

assessed after 90 days following the collection date of the first isolate except for 2 patients (alive) for whom the observation period was extended to 202 and 213 days.

Main points and tentative conclusions of part #1

- Treatment of pneumonia caused by *P. aeruginosa* is often unsatisfactory, with overall mortality rates reaching 40 % or higher.
  The present mortality rate related to the infection was 21%...
  ➔ patients are in good hands in Brussels…
- Although not designed nor powered enough to provide a true epidemiological estimate, the present study largely confirms that initial resistance levels are high …
  ➔ clinicians have a hard time for choosing the "good antibiotic" in Brussels
- Several multiple therapies were, actually, non- or less-multiple than thought

✓ **First conclusion:**

Efforts are needed to accelerate the early assessment of bacterial susceptibility and to improve the communication of data that are directly usable by the clinicians.

Reasons:

- decrease the risk of therapeutic failure
- Lower the incidence of undesired side effects (even if microbiologically ineffective, antibiotics, like any other drug, remain potentially toxic). reducing the clinician's choice for active antibiotics becomes increasingly narrow in the present environment.

Main points and tentative conclusions of part #1

- Emergence of resistance during therapy of pseudomonal infection is not novel finding … but this is the first time **clonal analysis** was systematically applied to all isolates
  ➔ resistance is very likely to have developed from the initial isolate (as opposed to re-infection)…
- Susceptibility changes, although visible, were not always statistically significant…
  ➔ clinicians have nearly always used optimized treatments (based on recorded doses and schedules)
  ➔ we may need to expand the study … but to what extent and where ?

✓ **Second conclusion:**

We do need new antibiotics with (i) strong bactericidal activity, and (ii) less propensity to select for resistance within the initial population …

We may also foster the development of novel, non-antibiotic approaches (alone or in combination with antibiotics)

Reason:

– decrease the initial inoculum as fast and as effectively as possible …

But what happened ?

- Study #2 : "classical" resistance
- Study #3 : efflux-mediated resistance

Classical resistance (study #2)

- Antibiogram (with interpretation) at high and low density inocula
- Direct genomic determination for suspected mechanisms

Hard work still in progress …

Classical resistance (study #2)

- provisional results (genomic analysis)
  - aminoglycosides
    - amikacin (n=65)
      - AAC (6')-Ib: 16
      - APH (3)-VI : 9 (5 in common with AAC (6')-Ib
    - gentamicin (n=89)
      - AAC (3)-Ia: 2
      - ANT (2')-Ia: 19
      - APH (3)-VI: 13 (1 in common with ANT(2')-Ia

30% one enzyme
6 % two enzymes

  - fluoroquinolones (n=100)
    - GyrA
      - 81 Gly → Asp: 1
      - 83 Thr → Ile: 55
      - 87 Asp → Asn, Gly, or Tyr: 15
      - 2 mutations: 3
    - ParC (all in common with GyrA)
      - 87 Ser → Leu: 30 (+ 10 ambiguous)
      - 87 Ser → Trp: 3
    - GyrB: 5 (none in common with GyrA or ParC)

43 % single mutation
30 % double mutation

Classical resistance (study #2)

- provisional results (genomic analysis)
  - β-lactames (n=70)
    - AmpC: 8 (1 with efflux; 7 with porin OprD/efflux)
    - bel-1: 3 (1 with porin OprD/efflux)
    - oxa2-like: 4 (2 with efflux)
    - oxa 3,9,18,20 4
    - per1: 3 (1 with oxa1)
    - vim2: 6

40 %

AmpC: 3d generation cephalosporins
oxa: penicillins, cephalosporins I and II
bel-1*(Roeselare, Belgium)*: ticarcillin (high level) and ceftazidime (intermediate)
per (*Pseudomonas extended resistance)*: ESBL (ceftazidime high level resistance)
vim *(Verona IMiPénémase)*: carbapenemase

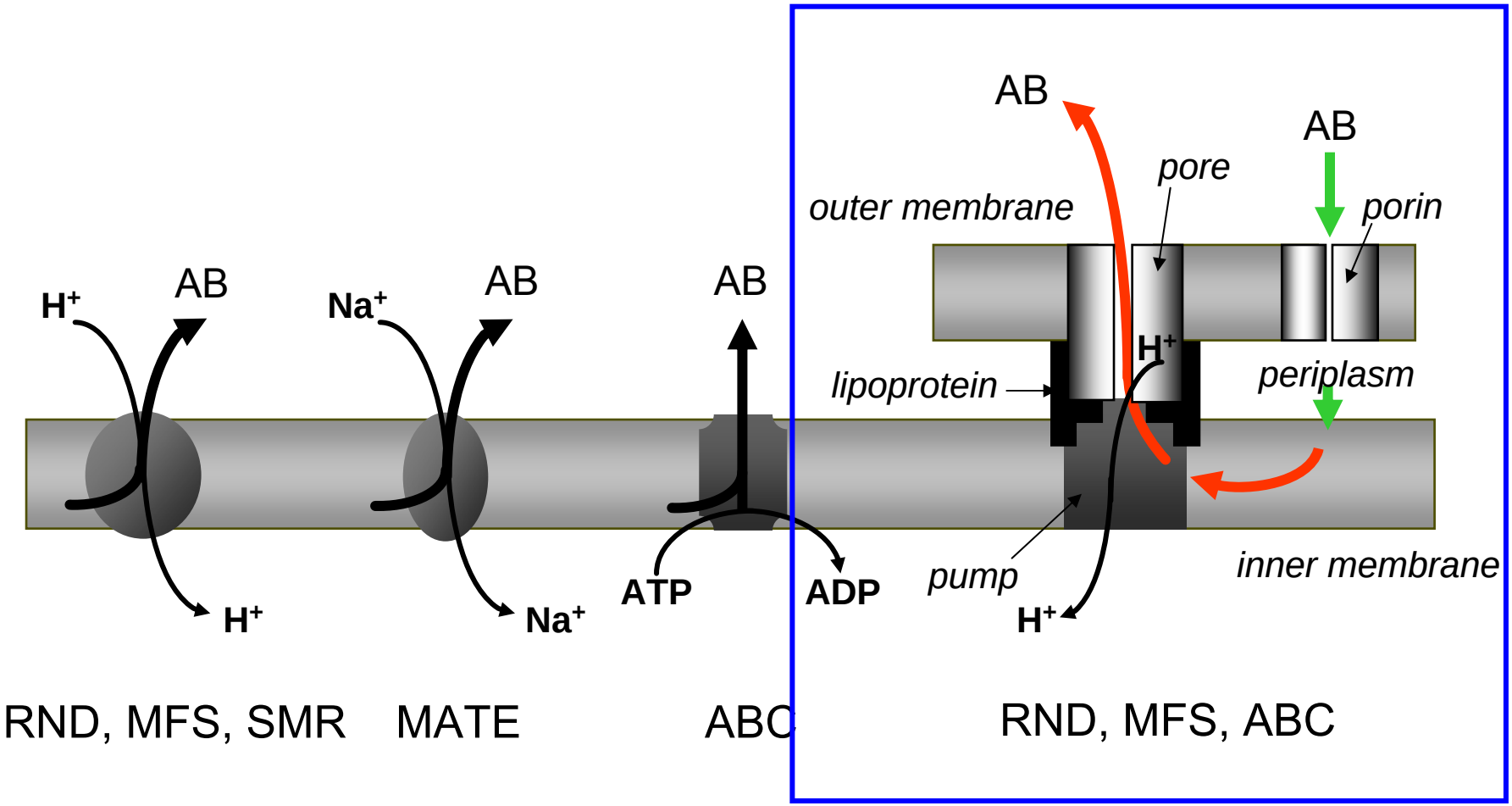
Study #3 Efflux

Antibiotic efflux in *P. aeruginosa,* a major nosocomial pathogen, isolated from Brussels Hospitals:

- Evidencing and characterizing transporters in clinical isolates
- Analyzing the relation to environmental conditions and antibiotic pressure

Project "Research in Brussels" 2007-2008
Post-doctoral fellowship FNRS 2008-2009

What is efflux ?



Van Bambeke et al., JAC 2003; (2003) 51:1055-1065

Efflux pumps overexpression *

Type of PCR	Genetic status	Day 0 (%)	Day X (%)
Real time PCR (constitutive genes)	**MexA- / MexX-**	66.13	38.71
	MexA+ / MexX-	19.90	22.58
	MexA- / MexX+	11.29	20.97
	MexA+ / MexX+	9.68	17.74
Classical PCR (inductive genes)	**MexC- / MexE-**	90.50	87.00
	MexC+ / MexE-	6.50	11.00
	MexC- / MexE+	3.00	6.50
	MexC+ / MexE+	0.00	5.00

* Gene expression evaluated by Real Time PCR (mex Q-Test Kit, Coris BioConcept) for mexA (constitutively expressed) and mexX (inducible with low expression level in WT strains), and by PCR on cDNA for mexC and mexE (repressed in WT strains).

Riou et al., ECCMID 2010 (accepted abstract)

But what happened ?

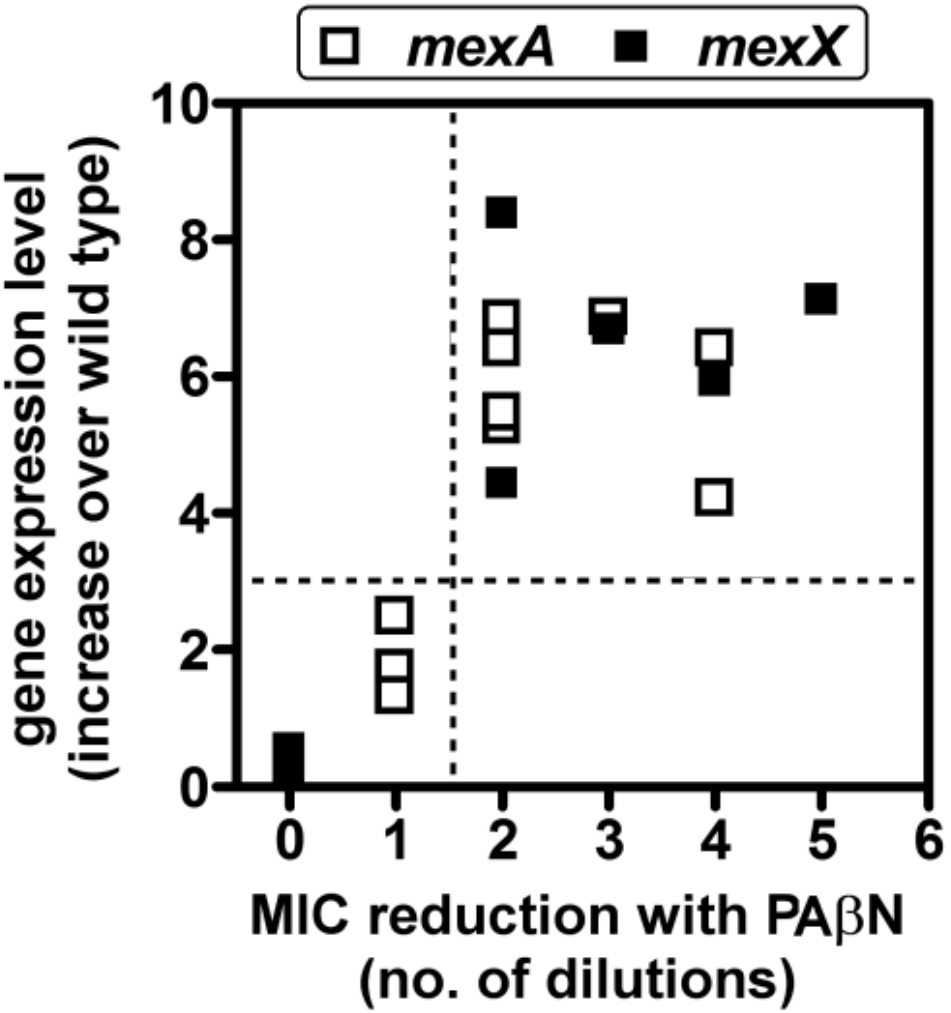
- Which pumps pump what ?
  - MexAB-OprM: β-lactams and fluoroquinolones;
  - MexXY-OprM: aminoglycosides, fluoroquinolones, cefepime

Patients with clonal pairs (n=59)

antibiotics with antipseudomonal potential (initial treatment [a]; no. of patients)

drug	AMK	CIP	MEM	TZP	FEP	CAZ
no. of patients	29	11	28	31	29	4

What happens if you overexpress MexA and/or Mex X ?



➔You increase your MIC by 2 to 5 dilutions… and you cross the S/R breakpoint …

Mesaros et al., JAC (2007) 59:378-386

Main points and tentative conclusions of part #3

- There was a large prevalence of genes coding for efflux mechanisms towards the main antipseudomonal antibiotics in initial *Pseudomonas aeruginosa* isolates …
  but this prevalence further increased during treatment.
  ➔ resistance is partly due to this overexpression (to be confirmed)
- As efflux pumps have broad substrate specificities, resistance may affect simultaneously several classes of drugs, even those not used in the study …
  ➔ clinicians may be surprized by novel, unusual combinations of resistance and the culprit may not be the one you were thought at school …

✓ **Conclusions:**

An early detection of the genomic and functional overexpression of these efflux transporters may be useful for both epidemiological and therapeutic purposes.

New "non-substrate" antibiotics are needed…

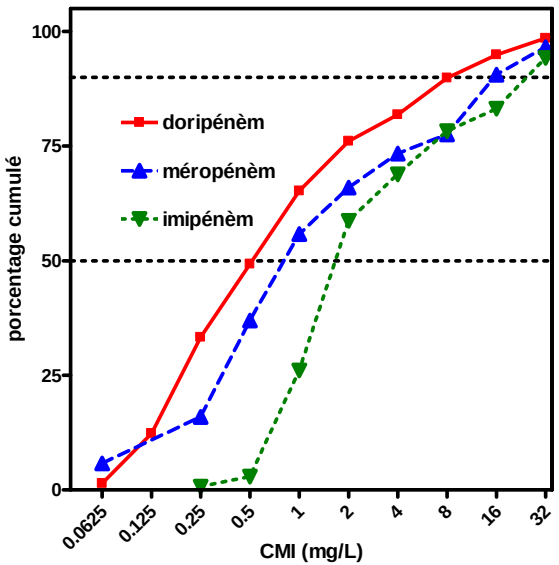
What can you do ?

- 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

Novel antibiotics ?

Suggested in our 2007 review…

- ceftobiprole: on hold … for some time (rejected by FDA and EMEA for cSSSI)
- sitafloxacine: no progress…
- doripenem: available but somewhat similar to meropenem…



but with dosages and PK

		DOR	MEM	IMI
EUCAST [a]	**S**	**65.2**	**65.9**	**68.8**
	I	**16.7**	**11.6**	**9.4**
	R	**18.1**	**22.5**	**21.7**
CLSI / FDA [b]	**S**	**76.1**	**75.4**	**68.8**
	I		2.1	9.4
	R		22.5	21.7

[a] **Eu**ropean **C**ommittee for **A**ntibiotic **S**usceptibility **T**esting
≤ S / R >: DOR: 1 / 4; MEM: 2 / 8; IMI: 4 / 8

[b] **C**linical and **L**aboratory **S**tandard **I**nstitute / **F**ood and **D**rug **A**dministration
≤ S / R ≥: DOR: 2 (pas de catég. I ou R); MEM: 4 / 16; IMI: 4 / 16

Novel antibiotics ?

Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America

Helen W. Boucher,[1] George H. Talbot,[2] John S. Bradley,[3,4] John E. Edwards, Jr,[5,6,7] David Gilbert,[8] Louis B. Rice,[9,10] Michael Scheld,[11] Brad Spellberg,[5,6,7] and John Bartlett[12]

[1]Division of Geographic Medicine and Infectious Diseases, Tufts University and Tufts Medical Center, Boston, Massachusetts; [2]Talbot Advisors, Wayne, Pennsylvania; [3]Division of Infectious Diseases, Rady Children's Hospital San Diego, and [4]University of California at San Diego, San Diego, [5]Division of Infectious Diseases, Harbor–University of California at Los Angeles (UCLA) Medical Center, and [6]Los Angeles Biomedical Research Institute, Torrance, and [7]The David Geffen School of Medicine at UCLA, Los Angeles, California; [8]Division of Infectious Diseases, Providence Portland Medical Center and Oregon Health Sciences University, Portland; [9]Medical Service, Louis Stokes Cleveland Veterans Administration Medical Center, and [10]Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, Ohio; [11]Department of Medicine, University of Virginia School of Medicine, Charlottesville; and [12]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland

Clinical Infectious Diseases 2009; 48:000–000

1058-4838/2009/4801-00XX$15.00
DOI: 10.1086/595011

Help IDSA Find Patients with Gram-Negative Resistant Infections and MRSA

Bad Bugs Need Drugs
10 x '20
Ten new ANTIBIOTICS by 2020

IDSA needs your help in putting a human face on the problem of antimicrobial resistance and in advancing the Society's 10 x '20 initiative, which calls for a global commitment to develop 10 new antibiotics by 2020 (see related *IDSA News* article). To educate the public, policymakers, and the media about the threat posed by drug-resistant infections and the lack of new drugs to treat them, IDSA, with help from its members, is trying to identify patients who are willing to share their experiences, particularly those involving multi-drug resistant (MDR) gram-negative bacteria and methicillin-resistant *staphylococcal aureus* (MRSA).

Immunotherapy ?

- vaccines
- therapeutic antibodies

Vaccine. 2009 May 11;27(21):2755-9. Epub 2009 Mar 13.

Immune responses in the airways by nasal vaccination with systemic boosting against Pseudomonas aeruginosa in chronic lung disease.

Sorichter S, Baumann U, Baumgart A, Walterspacher S, von Specht BU.

Department of Pneumology, University of Freiburg, Freiburg, Germany.

RATIONALE: Pneumonia caused by Pseudomonas (P.) aeruginosa is a leading cause of morbidity and mortality in patients with chronic lung diseases. Systemic vaccination in patients with cystic fibrosis has been only successful in part. Mucosal vaccination could lead to enhanced airway immunogenicity. Pathogen specific secretory IgA antibodies could prevent bacterial invasion into the lung mucosa. OBJECTIVES: A phase 1-2 mucosal vaccination trial with an intranasal P. aeruginosa vaccine was performed. METHODS: 12 patients with chronic lung diseases (8 COPD, 2 cystic fibrosis, 1 bronchiectasis, 1 histiocytosis X) were vaccinated three times intranasally followed by a systemic booster vaccination with a recombinant hybrid protein encompassing the main protective epitopes of two outer membrane proteins of P. aeruginosa. Mucosal and systemic antibody responses were measured after boosting and after a half-year follow-up compared to a representative control cohort. MEASUREMENTS: Specific IgG and IgA antibodies in the patient's sera, saliva and sputum were determined by enzyme-linked immunosorbent assay (ELISA) and IgG subclass distributions were defined with monoclonal mouse antibodies. RESULTS: Both forms of vaccination were well tolerated. Significant elevated IgA and IgG antibodies could be measured in sputum, saliva and in the sera of 11/12 patients. CONCLUSIONS: Mucosal vaccination followed by systemic boost with an outer membrane protein vaccine against P. aeruginosa leads to airway immunogenicity against the pathogen. Further clinical trials should elucidate the protective efficacy of this vaccination method.

PMID: 19366571 [PubMed - indexed for MEDLINE]

At least one therapeutic monoclonal antibody will soon enter Phase IIb …

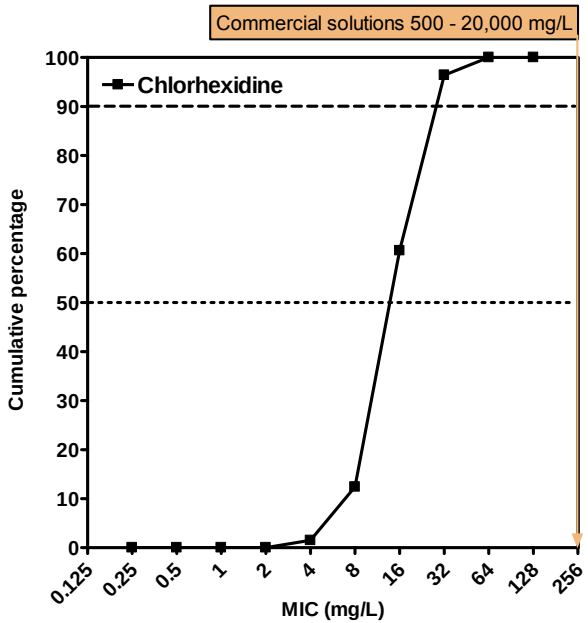
Susceptibility to biocides

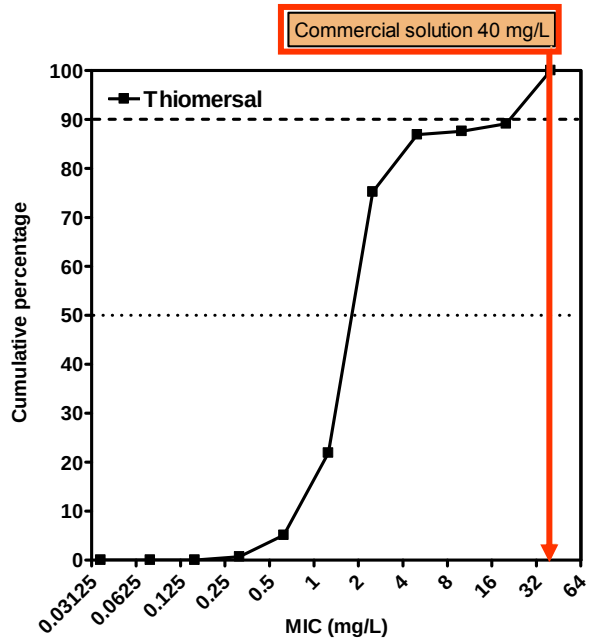
Biocides and antibiotic efflux in *P. aeruginosa*:
prevalence and significance in the hospitals of the Brussels Region:

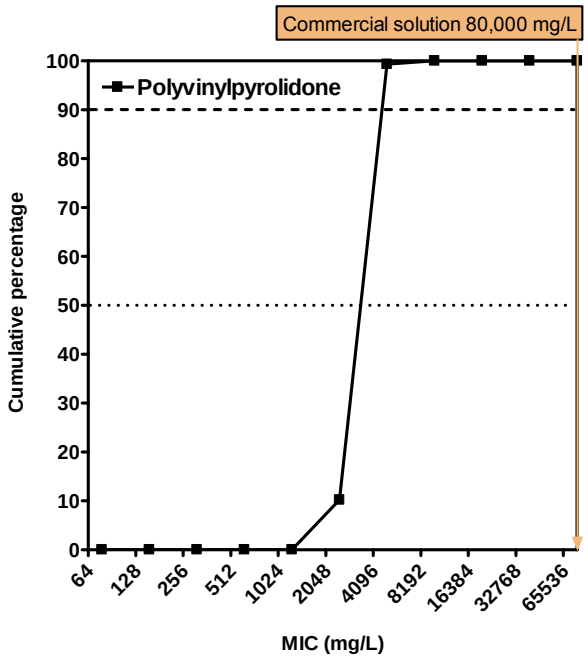
Implications for biocide and antibiotic policy at the Regional and National level

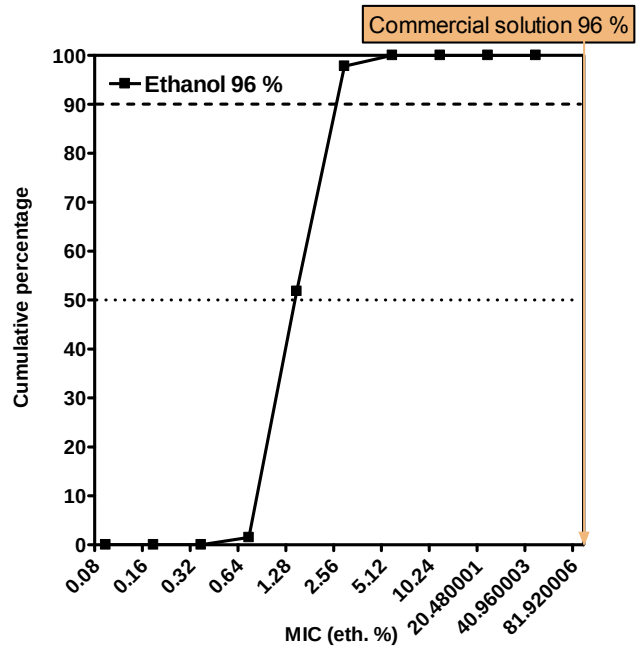
Ongoing project with the support of the Region

Susceptibility to biocides



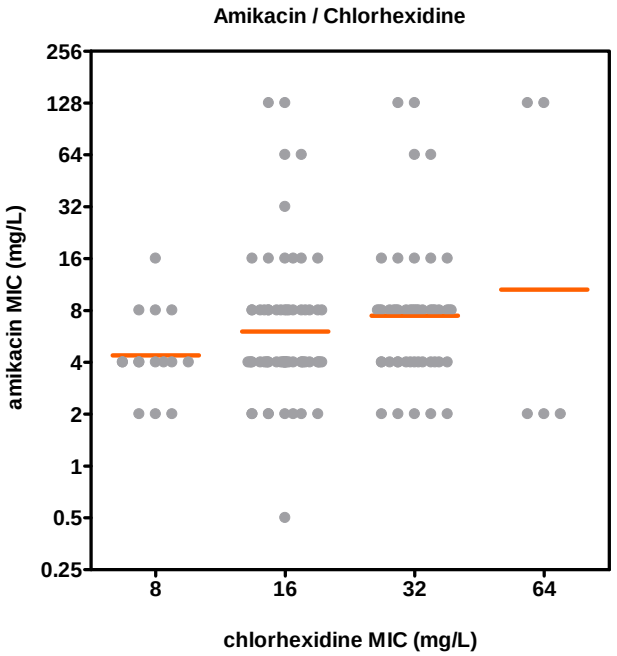


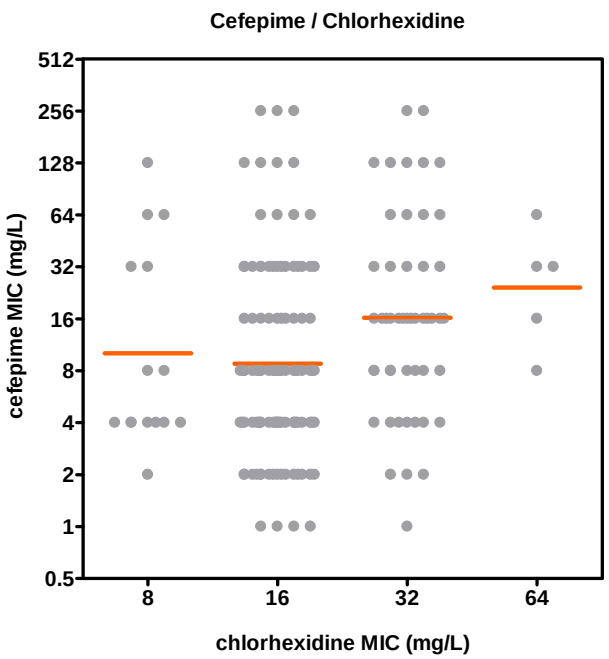


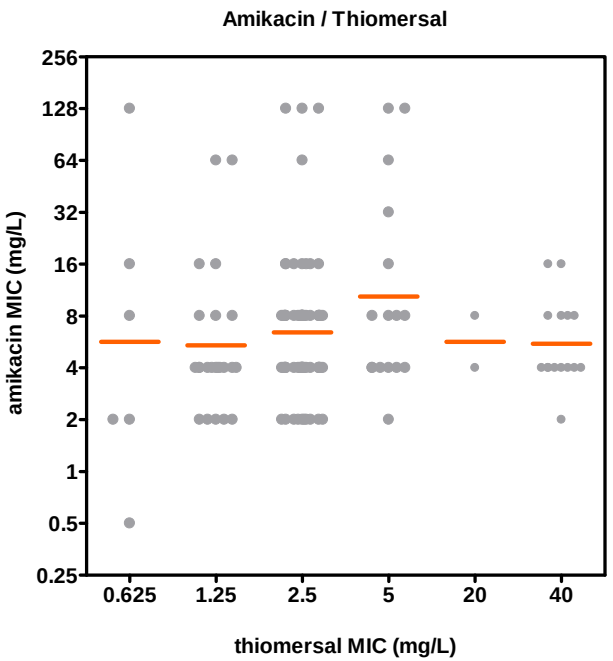


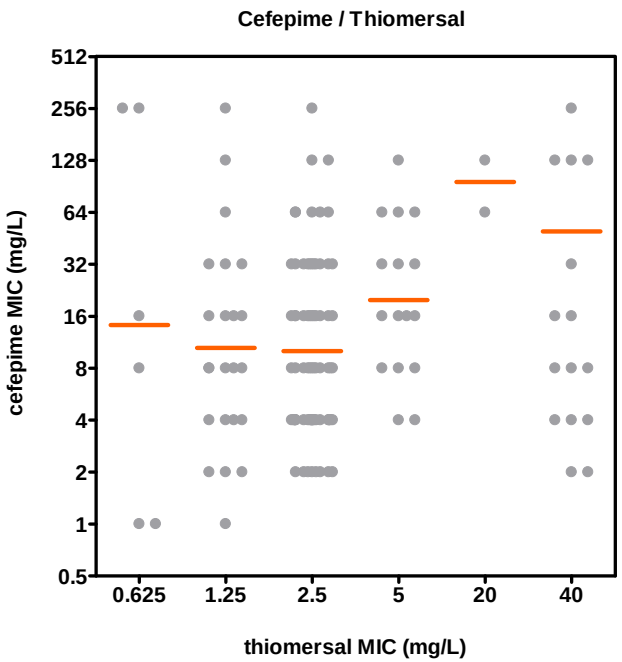
Riou et al., ICAAC 2009

Cross-resistance ?









Riou et al., ICAAC 2009

General conclusions

- We have problems with
  - resistance: certainly
  - novel therapies: probably for some time
- We could..
  - improve early diagnostics
    (for more directed therapies)
- We may be better than we thought for biocides
(but keep both eyes open…)

and thank you for
this nice collaboration