Antibiotic transporters in *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*: impact on resistance and clinical significance

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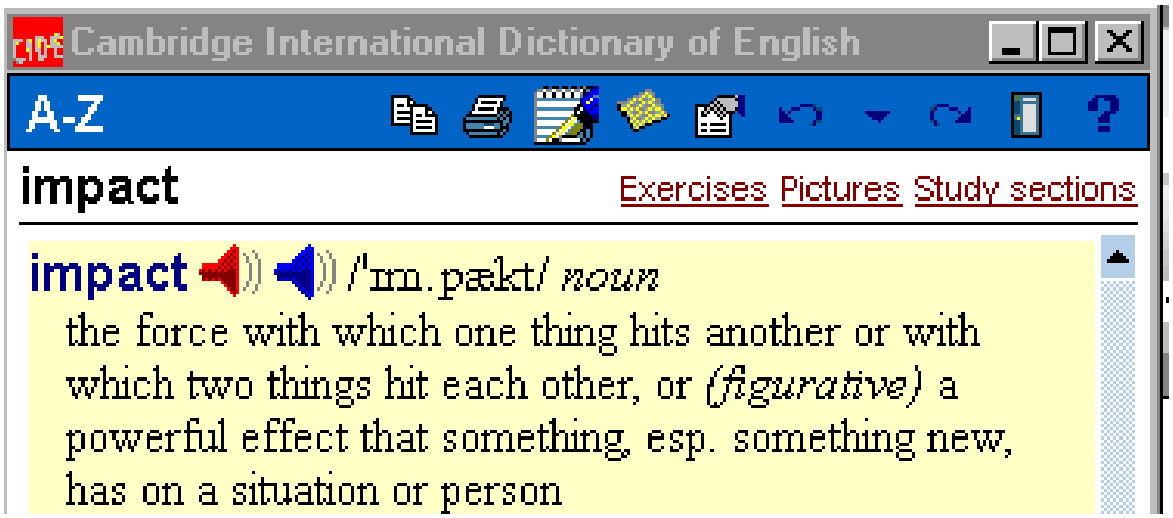
462nd WE Heraeus Seminar
Jacobs University Bremen, 4. - 10. July 2010

Transport across membranes:
Multiple drug resistance, mechanisms and new tools

You said "Impact" ?



(figurative) The anti-smoking campaign had ***had/made*** *quite an impact* ***on*** *young people.* [U]
(figurative) The new proposals were intended to ***soften*** *the impact of the reformed tax system.* [U]

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Bad Bugs, No Drugs... 10 New Antibiotics by 2020

IDSA Testimony on Antibiotic Resistance, delivered before the House Energy and Commerce Health Subcommittee (pdf) June 9, 2010
Brad Spellberg, MD, FIDSA, presented IDSA's statement at a hearing on "Promoting the Development of Antibiotics and Ensuring Judicious Use in Humans." FDA's and other statements and the hearing video are located here.

The 10 x '20 Initiative Inaugural Statement (pdf) April 2010
IDSA and other organizations address the dry antibiotic pipeline and call for 10 new antibiotics by 2020.

10 x '20 Endorsing Organizations (pdf) June 29, 2010

What are (some) companies thinking ?

- **Antibiotic Market**
  - With annual sales of over $26 billion, antibiotics represent one of the largest therapeutic categories from a revenue perspective.
- **What should we do ?**
  - We believe that the growing problem of drug-resistant bacteria will continue to drive growth in new and expanding market opportunities. While much attention has been focused on resistance in Gram-positive pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), increasing antibiotic resistance in Gram-negative organisms such as *Pseudomonas aeruginosa* represents a significant threat, with far fewer treatment options available.

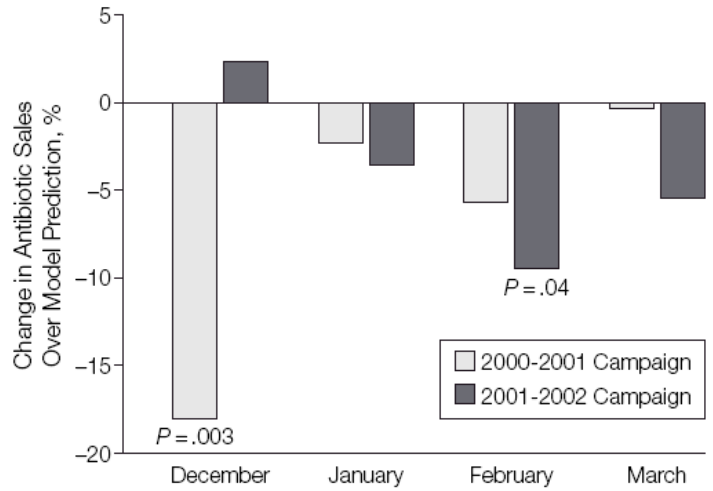
But at the same time …

- **Public authorities launch campaigns for reducing antibiotic use…**

RESEARCH LETTER

Association Between Antibiotic Sales and Public Campaigns for Their Appropriate Use

JAMA, November 24, 2004—Vol 292, No. 20 **2469**



Residual seasonal autoregressive terms: lag period, 12 months; estimated coefficient: 0.83 [SE, 0.06]; constant: 7 459 075 (SD, 431 387) defined daily doses/mo. The *P* values are indicated for the months and campaigns for which the changes were statistically significant.

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And "Big Pharma" is leaving the antibiotic area…

- **Roche, Bayer, E. Lilly, Sanofi-Aventis … Why ?**
  - difficulties in finding truly novel molecules …
  - too low ROI because antibiotics are (i) cheap; (ii) used only for short periods of time… (10 days average)
  - regulatory hurdles for novel compounds (ceftobiprole, oritavancin, iclaprim, telavancin [so far in EU], faropenem) making development uncertain
  - safety issues (over)emphasized for (some) existing compounds by fear of overuse (telithromycin, moxifloxacin) and/or because of inappropriate initial positioning (trovafloxacin)
  - restricted use of novel compounds in several countries (daptomycin, tigecycline) and/or difficulties in positioning when facing generic (or soon generic) equivalents (doripenem)

And "Big Pharma" is leaving the antibiotic area…

- **Roche, Bayer, E. Lilly, Sanofi-Aventis …**

All these were made for resistant strains !

  - [illegible]
  - too low ROI because antibiotics (i) are cheap; (ii) are used only for short periods of time
  - regulatory hurdles for novel compounds (ceftobiprole, oritavancin, iclaprim, telavancin [so far in EU], faropenem) making development uncertain
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A simple price comparison…

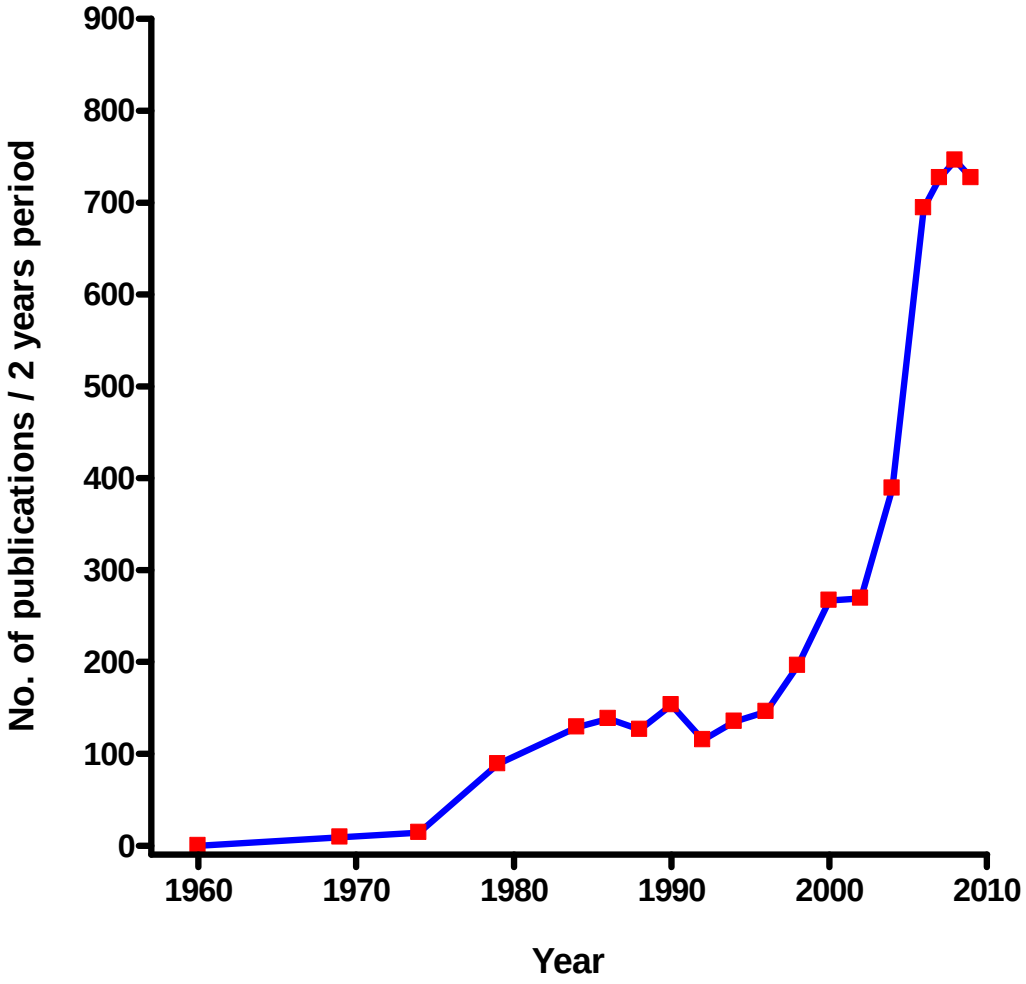
- **Treating a community-acquired pneumonia**
  - 5-30 % mortality if left untreated
  - almost 100 % chances of success if appropriate antibiotic
  - average price in Europe for a full treatment (based on guidelines):
    - from 7.88 € (generic amoxicillin, low dose, 7 days)
    - to **127.5 €** for levofloxacin (non-generic, high dose, 10 days)

- **Treating cancer with antibody** (bevacizumab as an example)
  - mean survival: 20.3 *vs.* 15.6 mo (placebo)
  - response rate: 45 *vs.* 35 % (placebo)
  - average price for a 1 year treatment (based on official indications for metastatic colorectal-cancer):
    - **63,000 US$** (US prices)

Challenges in the development of an antibiotic acting against resistant strains

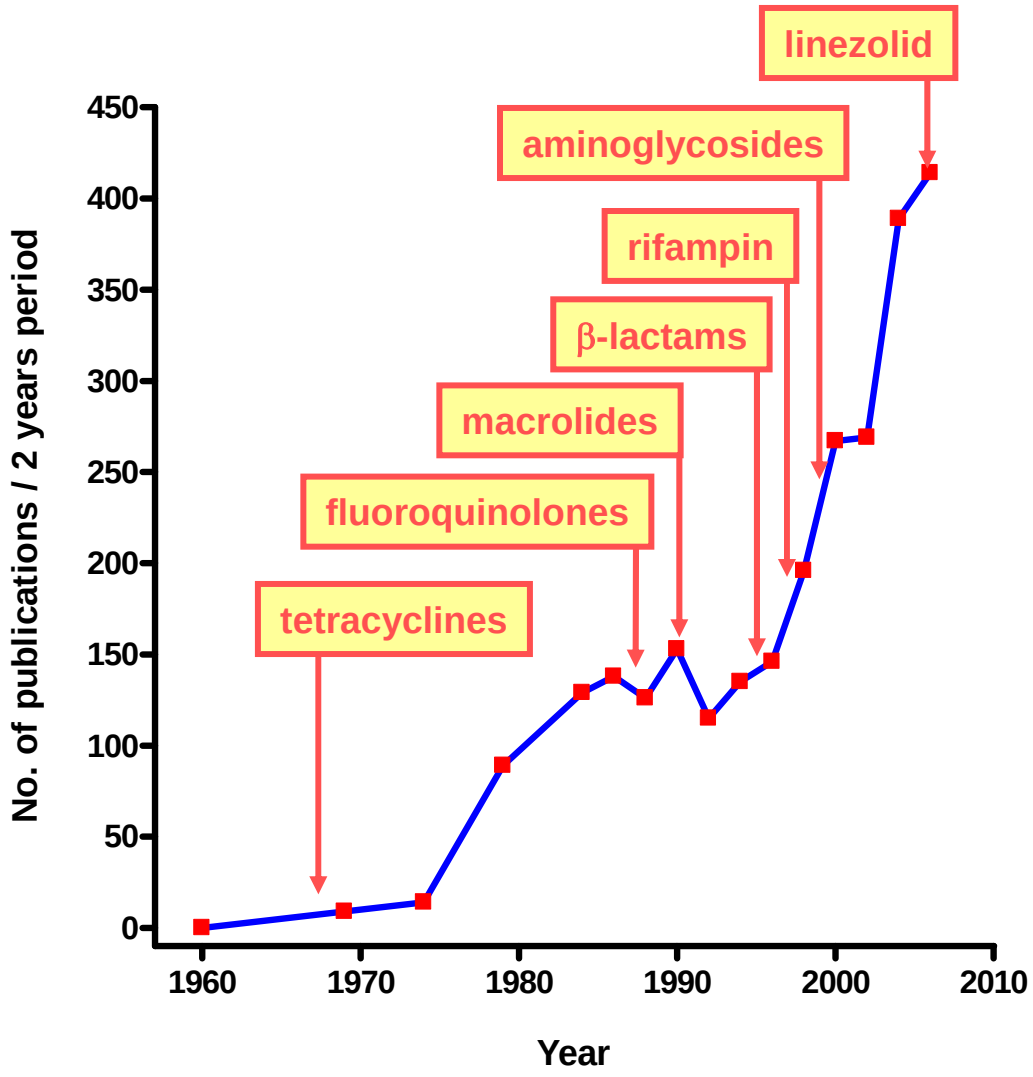
- **defining the indications for which it must be developed**
  - ? often "niche" or "semi-niche" indications
- **defining the level of acceptable risk to the patient**
  - ? stay away from indications where safer compounds are (still) available
- **finding it:**
  - ? solving the discovery bottlenecks
- **developing and selling it:**
  - ? chemical development
  - ? preclinical development
  - ? clinical development
  - ? pricing …

Is efflux an important mechanism of resistance ?



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

Historical landmarks …



Successive description of efflux-mediated resistance for major antibiotics

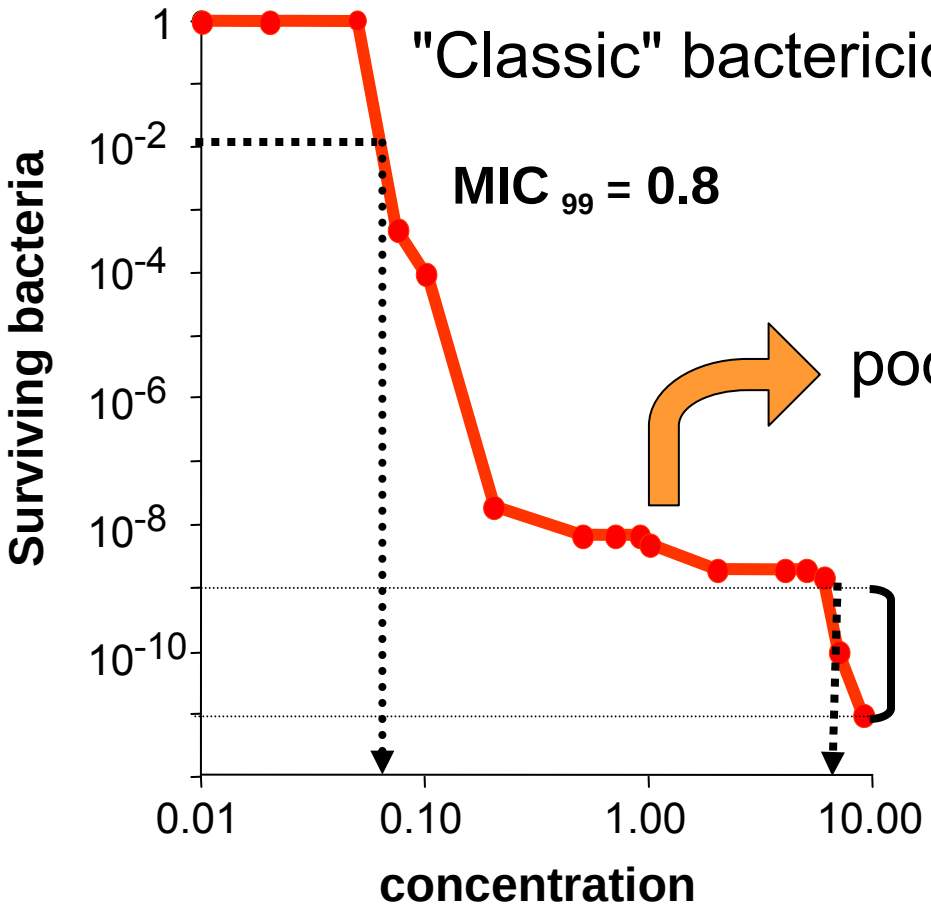
But is efflux really important ?

- Efflux has long been taken lightly in clinical practice …
  - ➢ because it most often causes only low levels of resistance, which have long been considered as "clinically insignificant" …

- Bacteria carrying the gene encoding macrolide efflux (i.e. the mefE gene) display relatively low-level resistance. Azithromycin, because of its ability to achieve concentrations at sites of infections, is capable of eradicating mefE-carrying strains.

  (Int. J. Antimicrob. Agents 2001;18 Suppl 1:S25-8.

Things are changing : the MPC concept…



"Classic" bactericidal effect

MIC $_{99}$ = 0.8

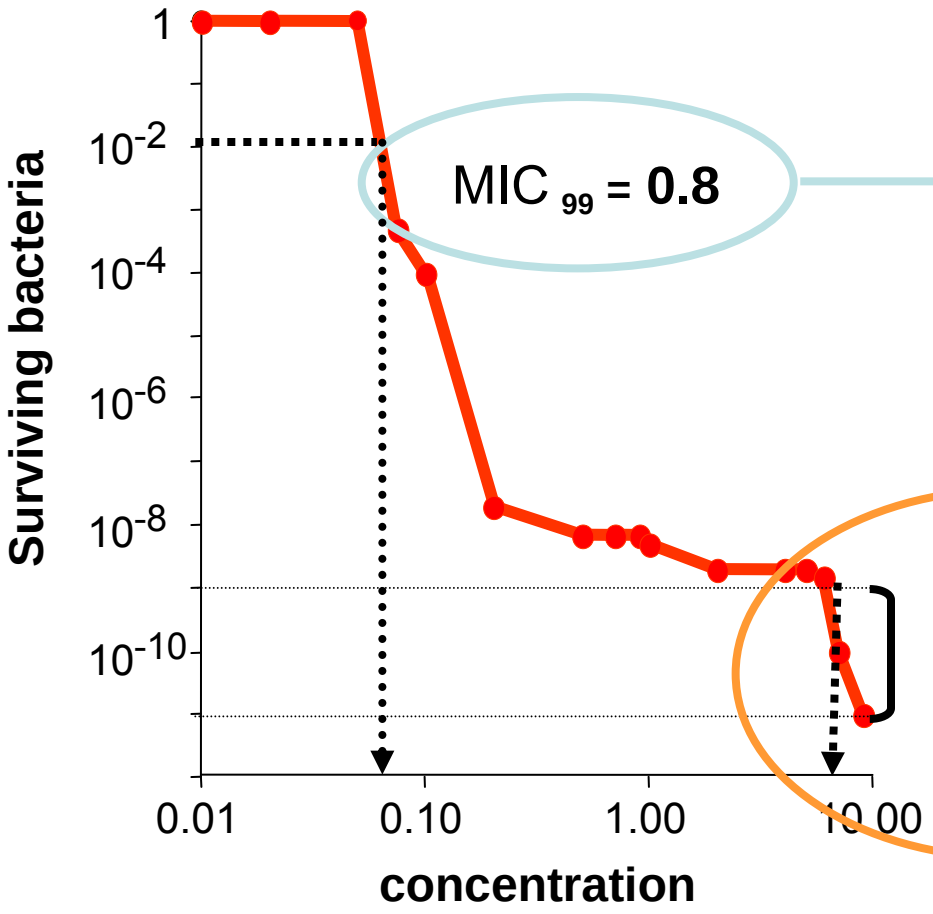
poorly sensitive organisms…

Elimination of resistant organisms

MPC $_{10}$ = 9

Dong *et al*; AAC 43:1756-1758

Mutant Prevention Concentration …



MIC $_{99}$ = **0.8**

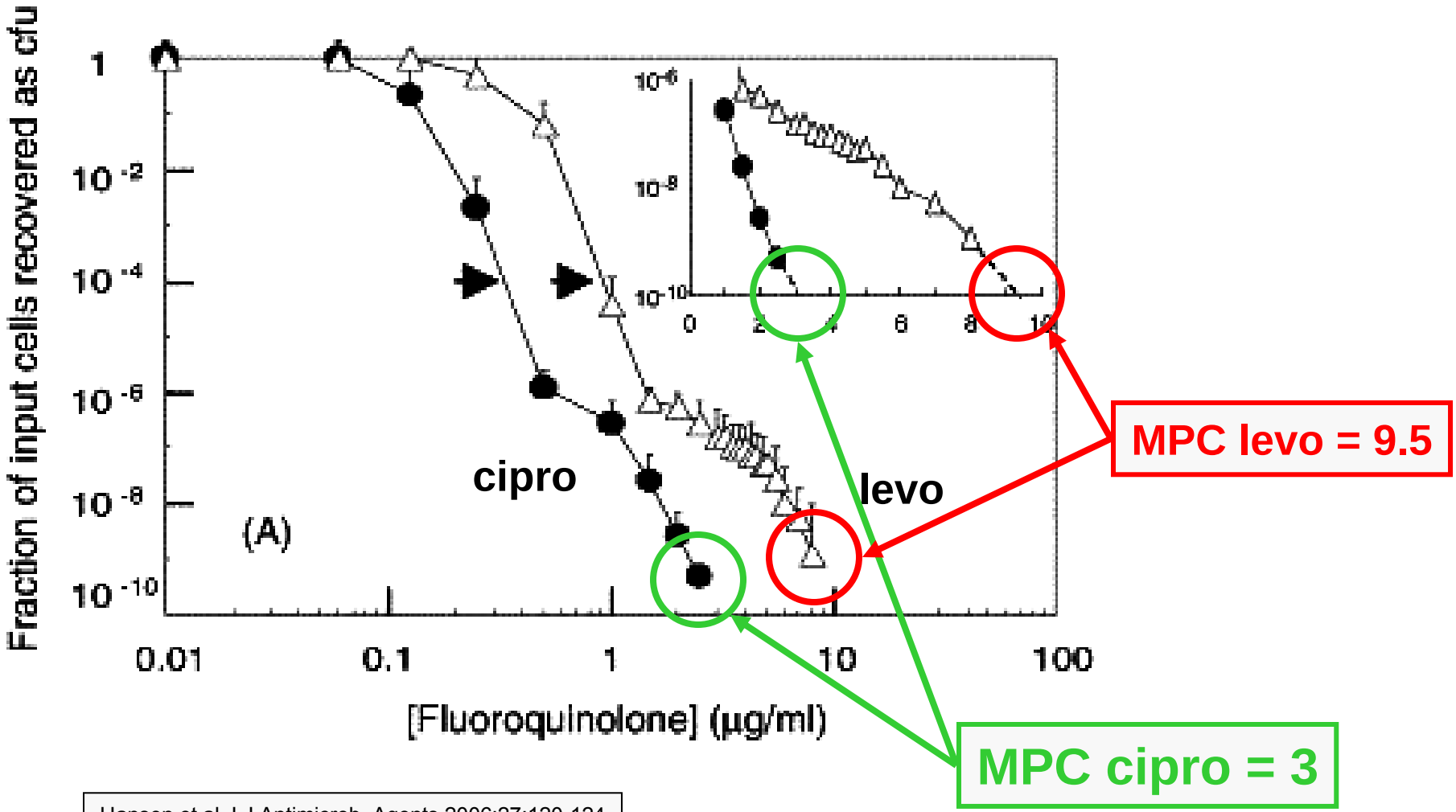
Concentration which will inhibit the majority of the organisms

MPC $_{10}$ = **9**

Concentration needed to prevent the selection of resistant organisms

Dong *et al*; AAC 43:1756-1758

Mutant Prevention Concentration of ciprofloxacin and levofloxacin in *P. aeruginosa* (clinical isolates) with "normal" susceptibility (MIC = 0.33 and 0.9 mg/L) …



Hansen et al. I.J.Antimicrob. Agents 2006;27:120-124

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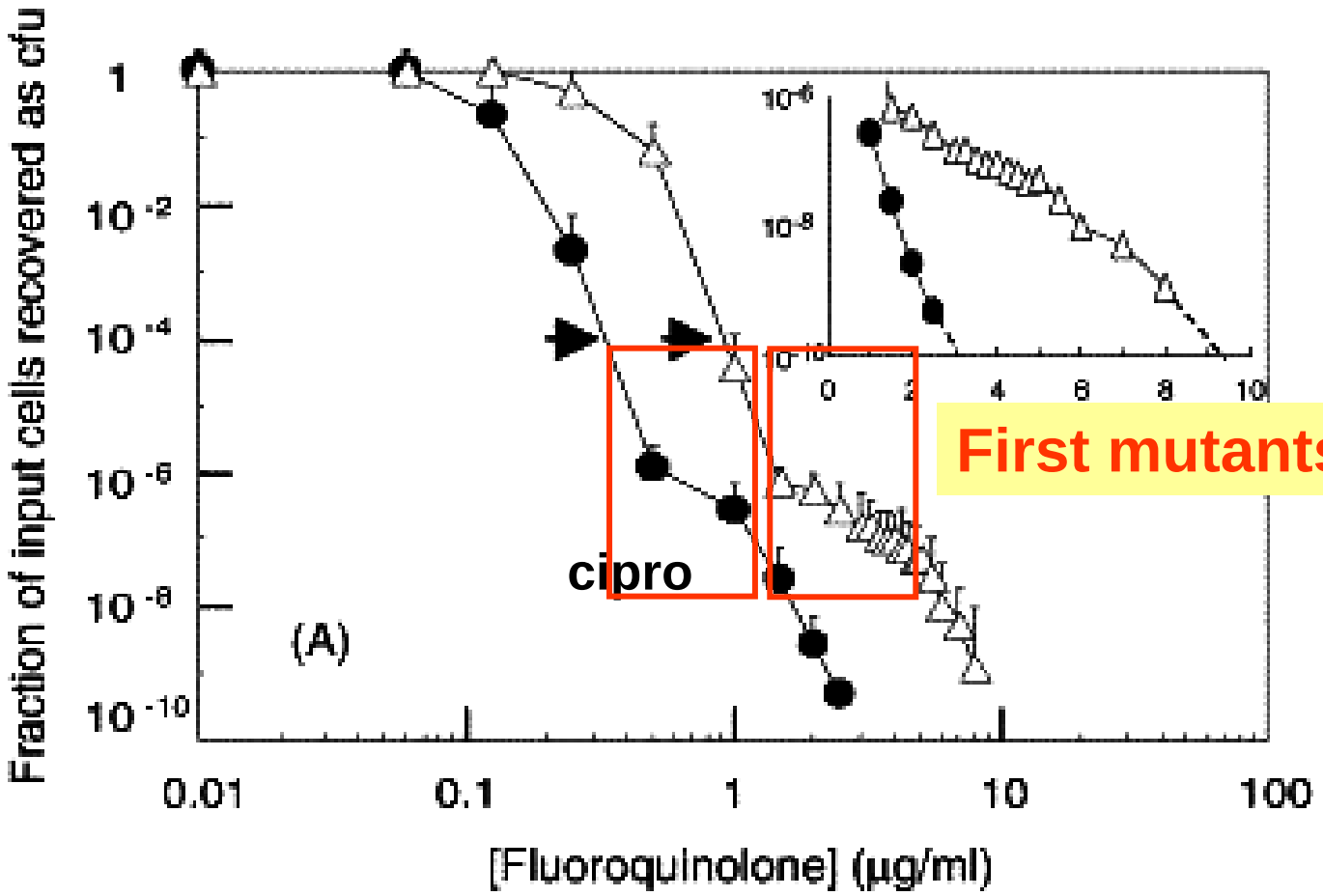


First mutants or efflux ?

Hansen et al. I.J.Antimicrob. Agents 2006;27:120-124

Things are changing: the new breakpoints of EUCAST …

Before EUCAST …

cefotaxime vs. *E.coli*		S≤ / R
BSAC	United Kingdom	2 / ≥4
CA-SFM	France	4 / >32
CRG	The Netherlands	4 / >16
DIN	Germany	2 / ≥16
NWGA	Norway	1 / ≥32
SRGA	Sweden	0.5 / ≥2

Yet, these breakpoints were used everyday by clinical microbiology laboratories to advise clinicians about which antibiotic(s) they could sucessfully use against the bacteria they were supposed to fight …

A simple decision …

cefotaxime vs. *E.coli*		**S≤ / R**
BSAC	United Kingdom	2 / ≥4
CA-SFM	France	4 / >32
CRG	The Netherlands	4 / >16
DIN	Germany	2 / ≥16
NWGA	Norway	1 / ≥32
SRGA	Sweden	0.5 / ≥2
NCCLS	U.S.A.	8 / ≥64

Would this not be a smart decision ?

A simple decision …

The US clinician can treat all patients !

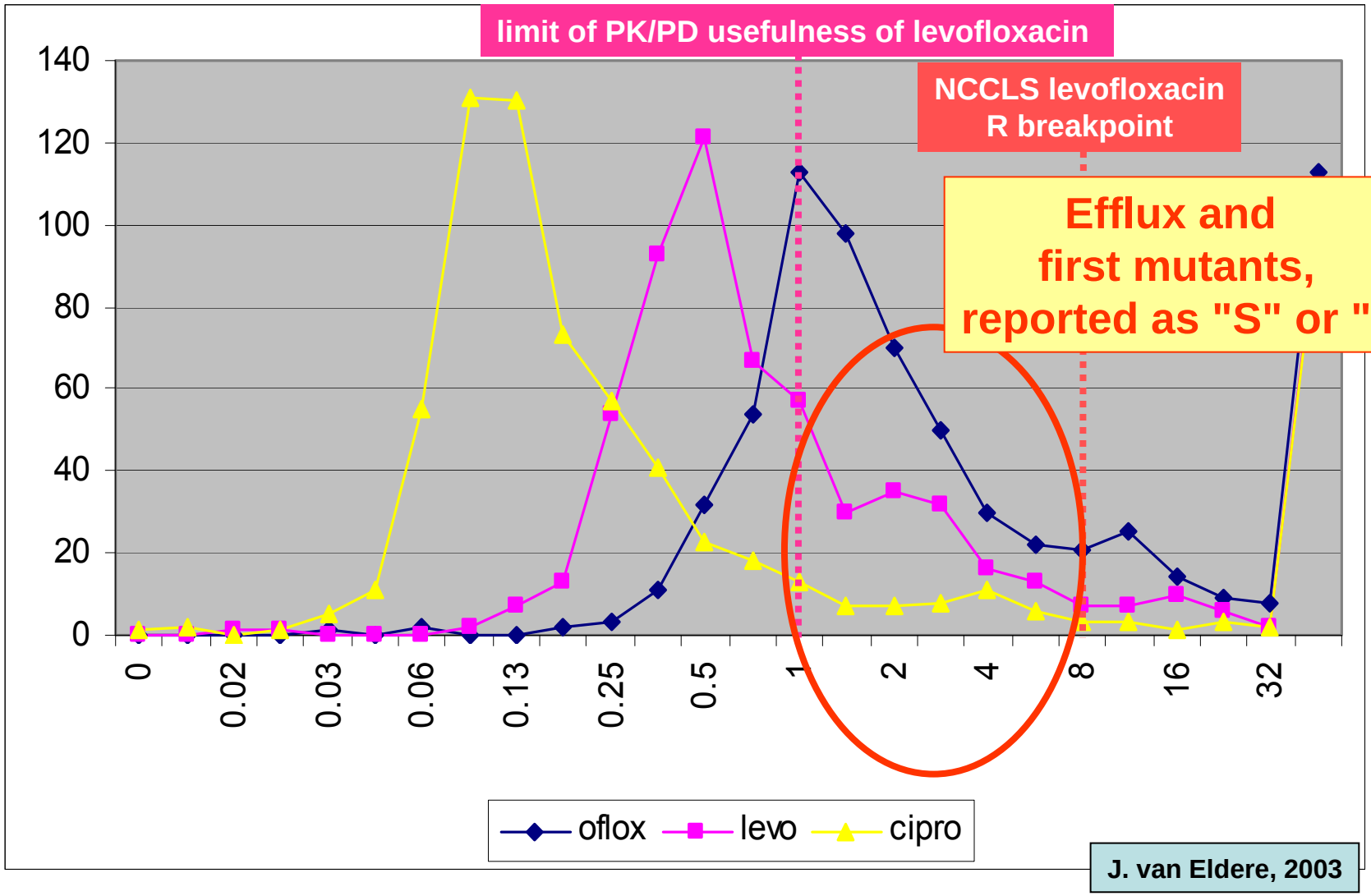
NCCLS	U.S.A.	8 / ≥64

EUCAST breakpoints in a nutshell

- based on PK/PD considerations with efficacy in the clinical set-up as the first and most important element of decision
- most often considerably lower than all former (and many current) NCCLS (presently CLSI) breakpoints
- put many isolates with low "resistance mechanisms" in the intermediate or resistant category

more at http://www.eucast.org

An example for *P. aeruginosa* in an Academic Hospital
(Leuven, Belgium)



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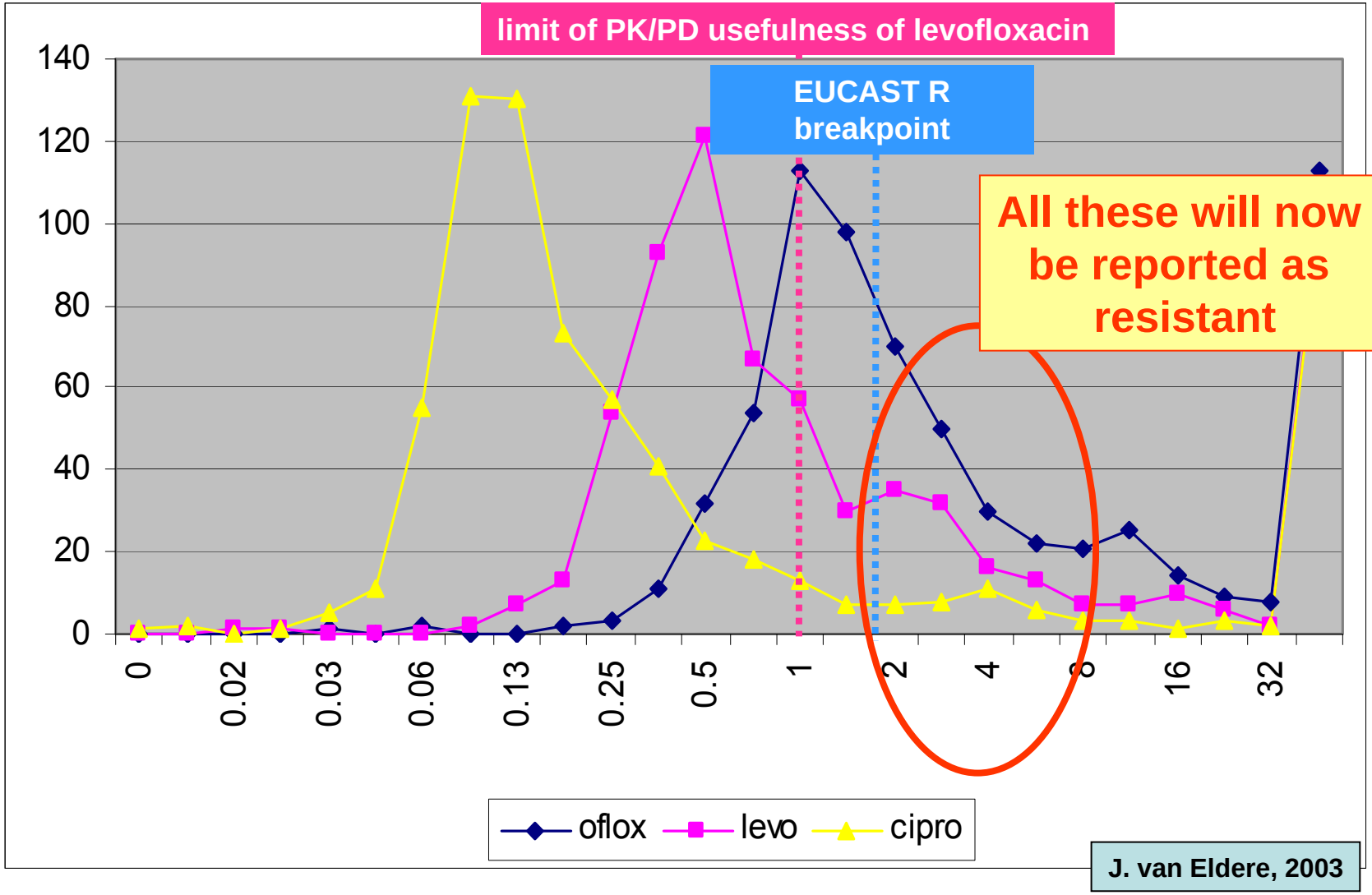
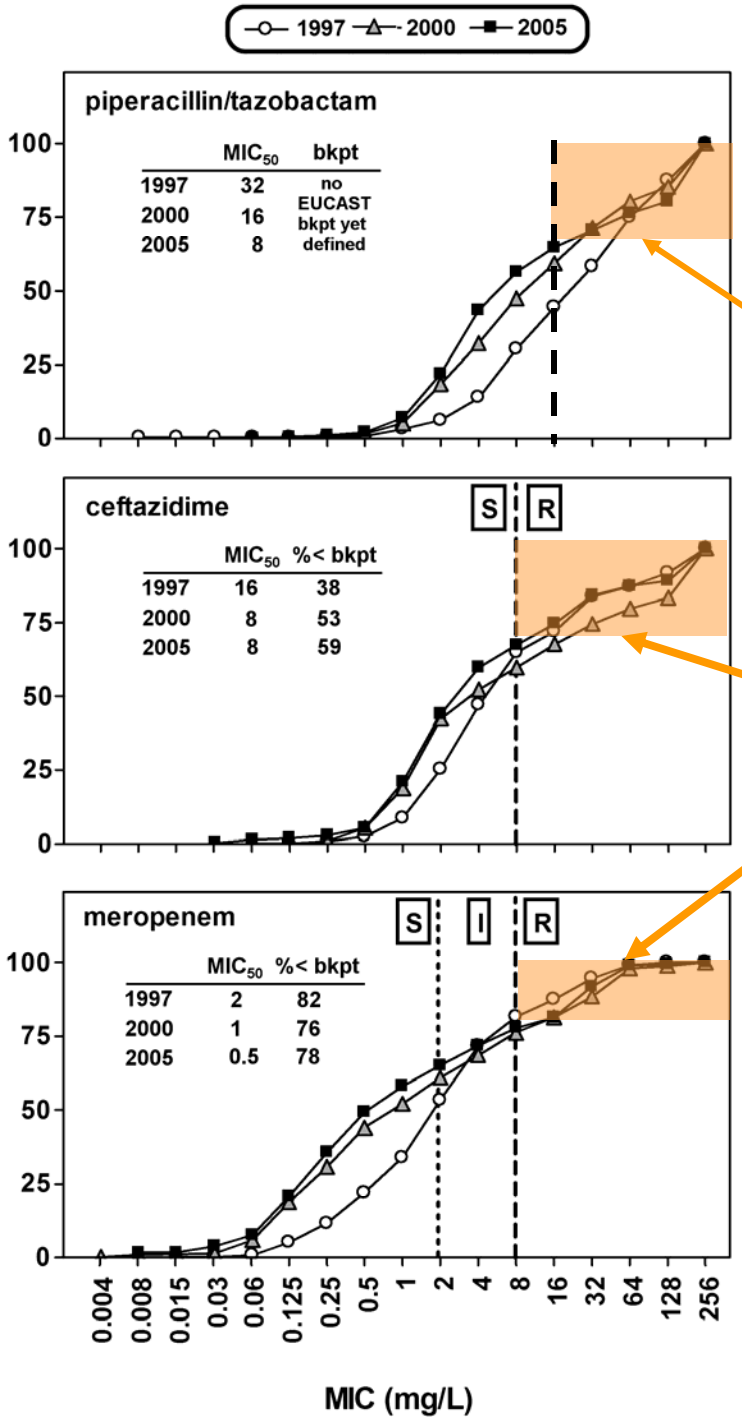


Application for Pseudomonas…

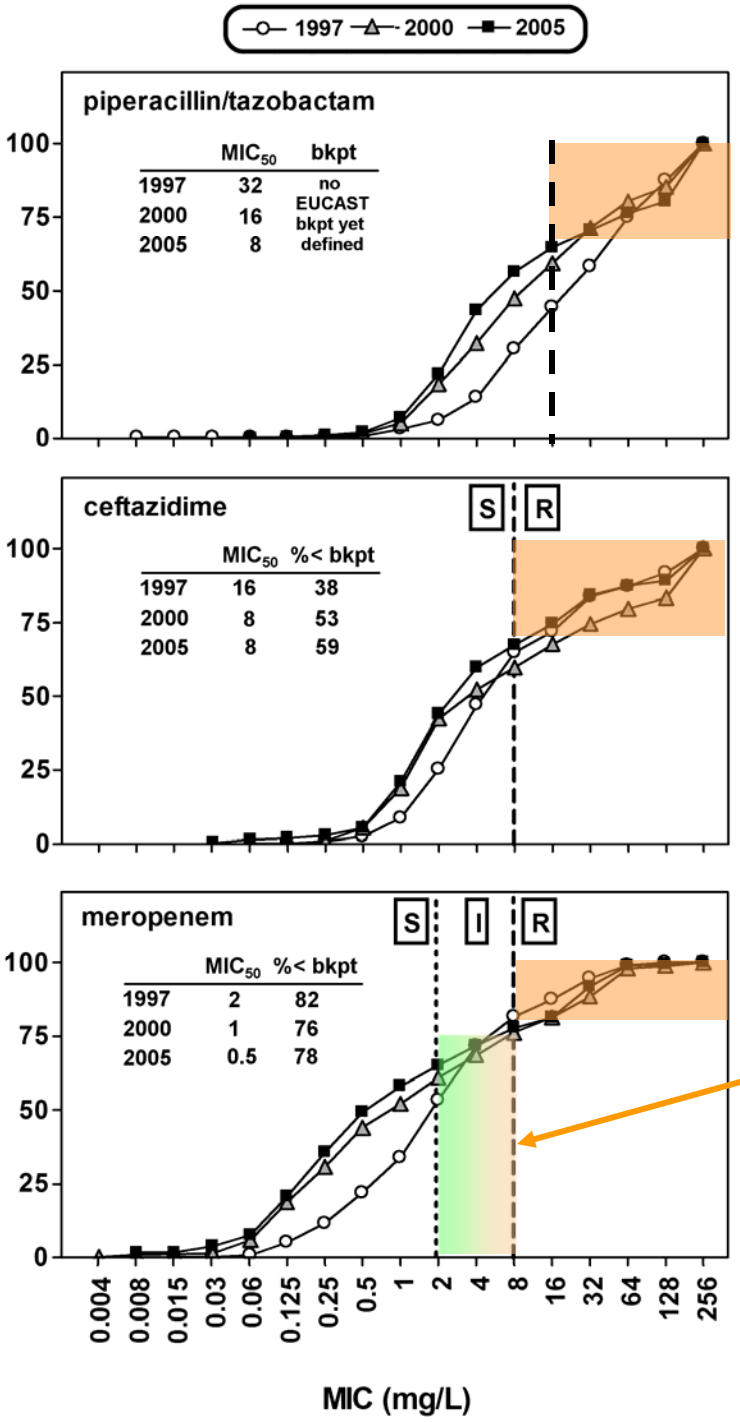


high risk zone (above the EUCASTR breakpoint)

high risk zone (above the EUCAST R breakpoint)

MIC data extracted from the MYSTIC database (http://www.mystic-data.org/) but limited to European countries; breakpoints are from EUCAST (http://www.eucast.org)

Application for Pseudomonas…



but this is also a risky zone…
(clinical outcome uncertain)

MIC data extracted from the MYSTIC database (http://www.mystic-data.org/) but limited to European countries; breakpoints are from EUCAST (http://www.eucast.org)

Study #1: *Pseudomonas* in Brussels in 2010

- **Mickaël Riou, Sylviane Carbonnelle, Laëtitia Avrain, Narcisa Mesaros, Qing Tan, Françoise Van Bambeke, Youri Glupczynski**
- **Jean-Paul Pirnay, Daniel De Vos**
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- Laboratory for Molecular & Cellular Technology, Queen Astrid Military Hospital, Neder-over-Hembeek
- Department of Molecular and Cellular Interactions, Vrije Universiteit Brussel, Brussels;
- Laboratoire de microbiologie, Cliniques universitaires Saint-Luc, Brussels; Laboratorium voor microbiologie, Universitair Ziekenhuis Brussel, Brussels, Service d'infectiologie, Hôpital Erasme, Brussels; 8Laboratoire de microbiologie, Centre hospitalo-universitaire Saint-Pierre, Brussels; Laboratoire de microbiologie, Cliniques universitaires UCL de Mont-Godinne, Yvoir Belgium.

Riou *et al*., submitted

What was 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

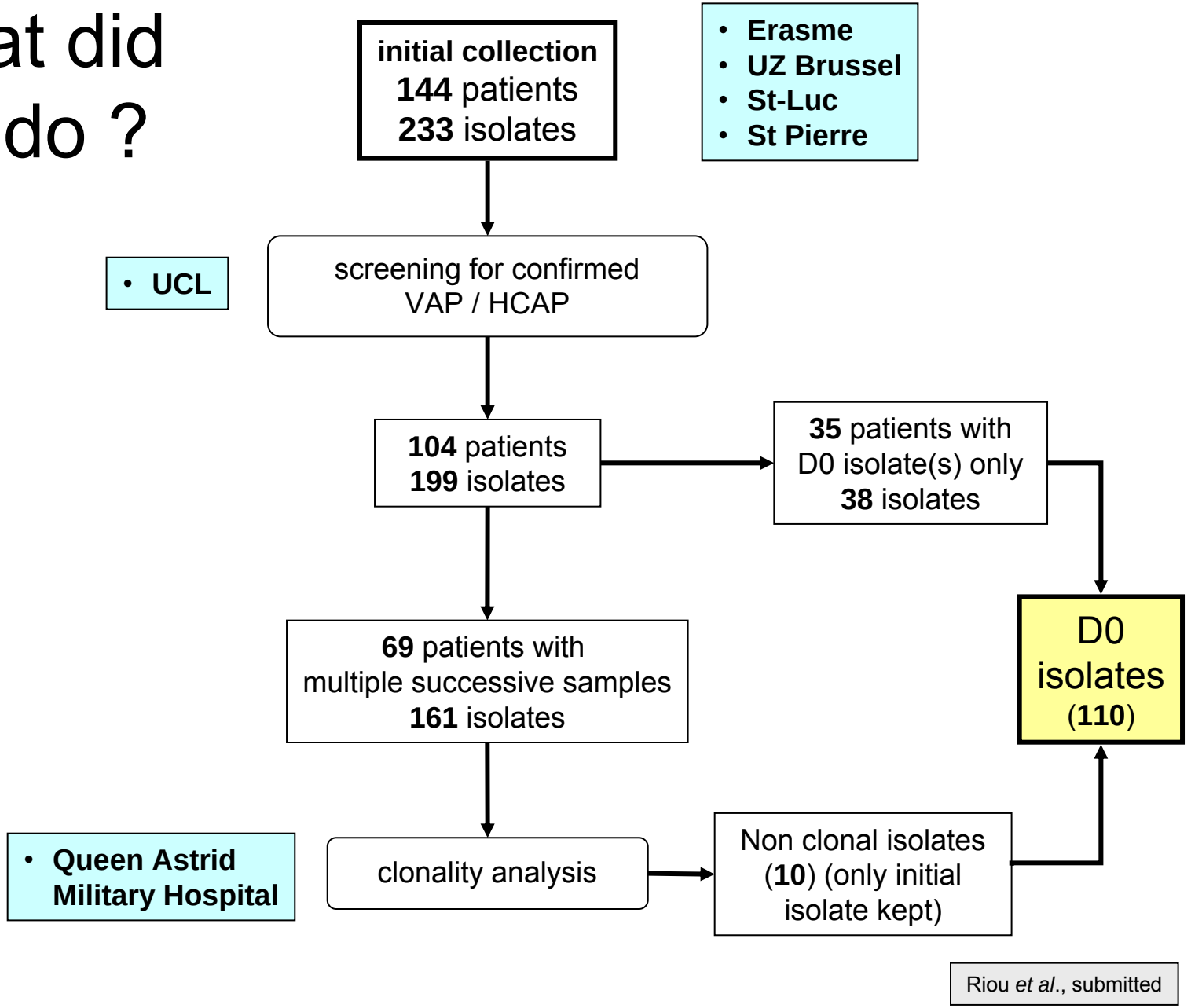
Clin Microbiol Infect 2007; **13**: 560–578

Epidemiological study

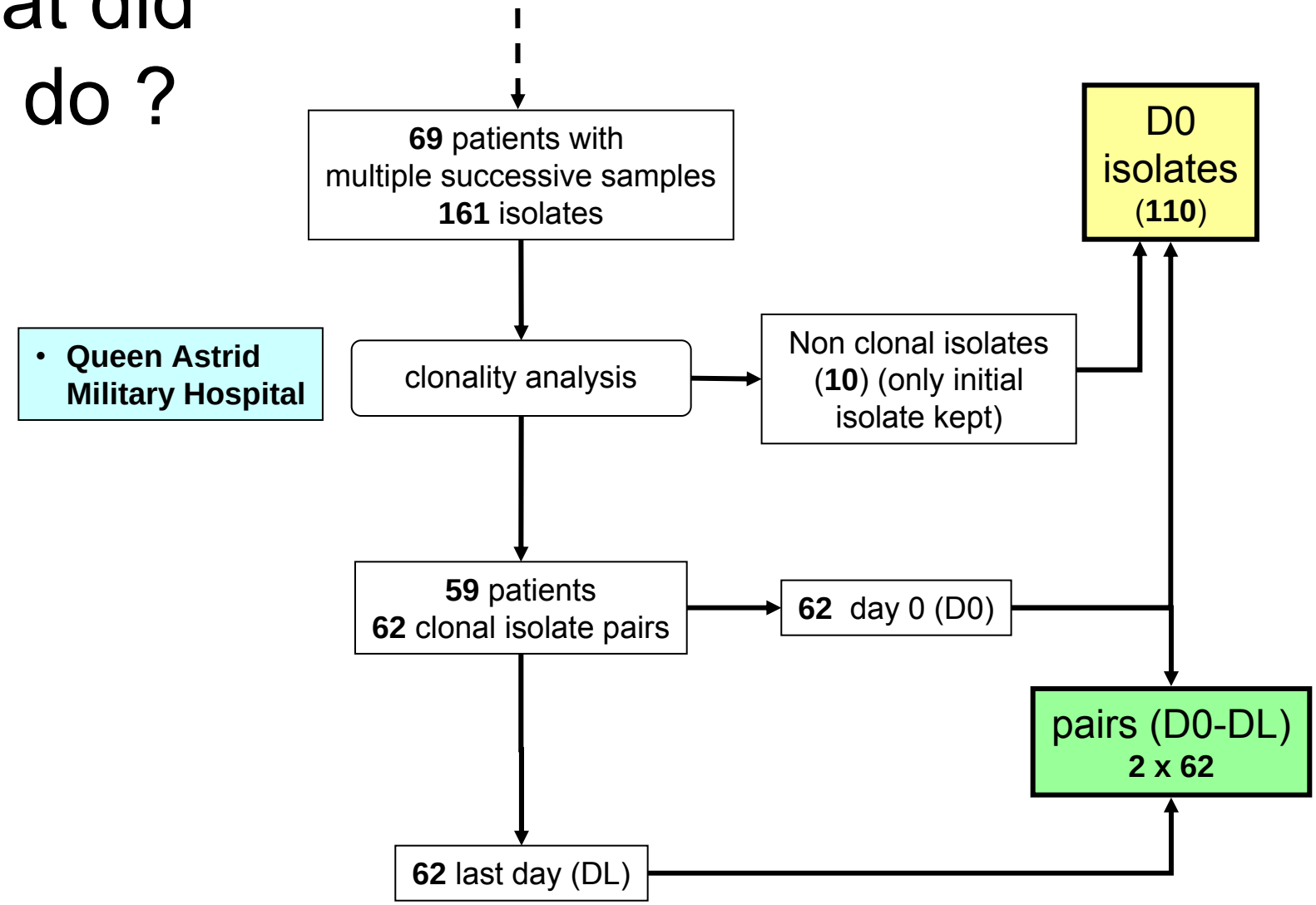
Impact of therapy on the development of in vitro antimicrobial resistance in *Pseudomonas aeruginosa* strains isolated from lower respiratory tract of Intensive Care Units (ICU) patients with nosocomial pneumonia

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

What did we do ?

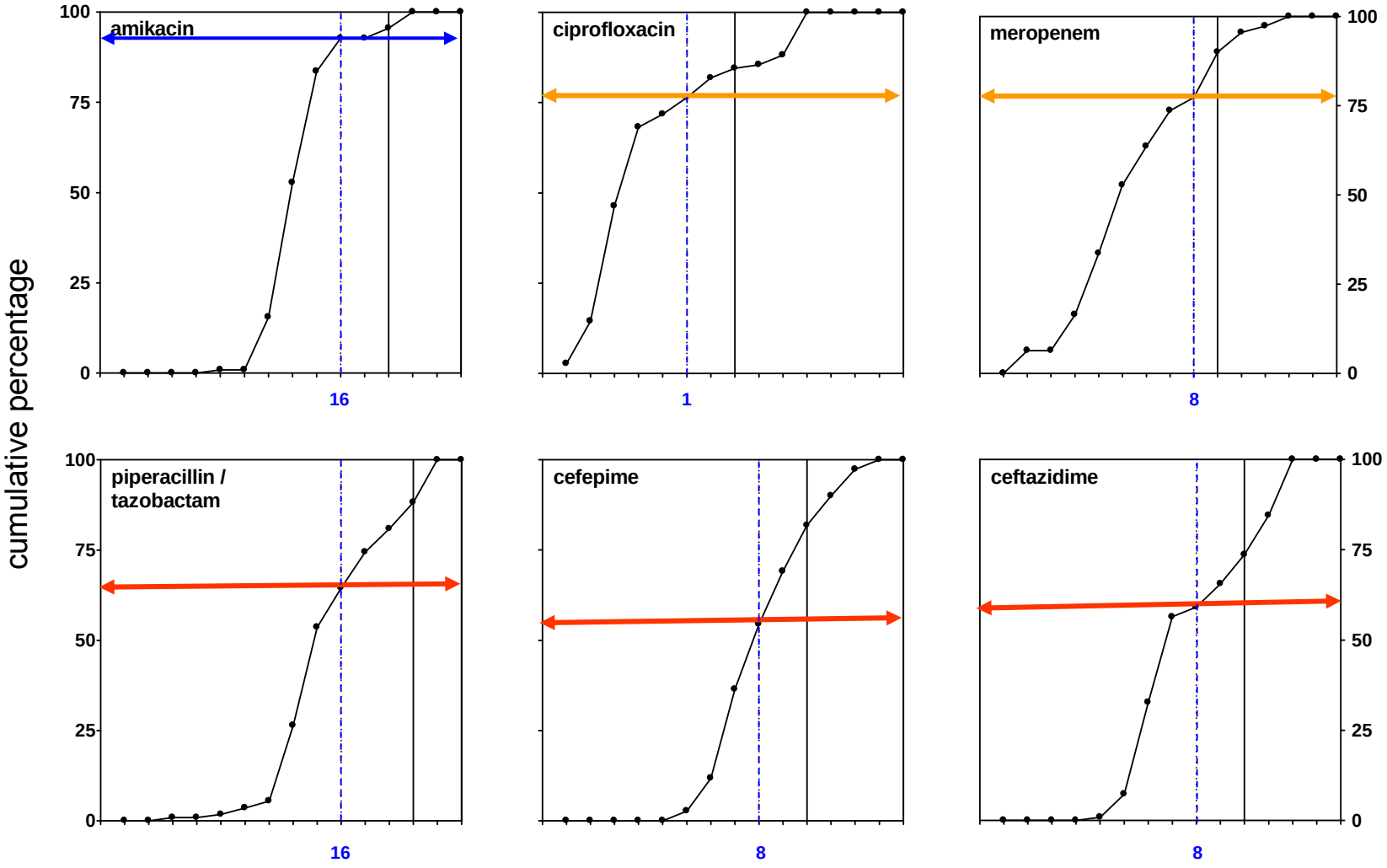


What did we do ?



Riou *et al*., submitted

What is the situation at day 0 ?



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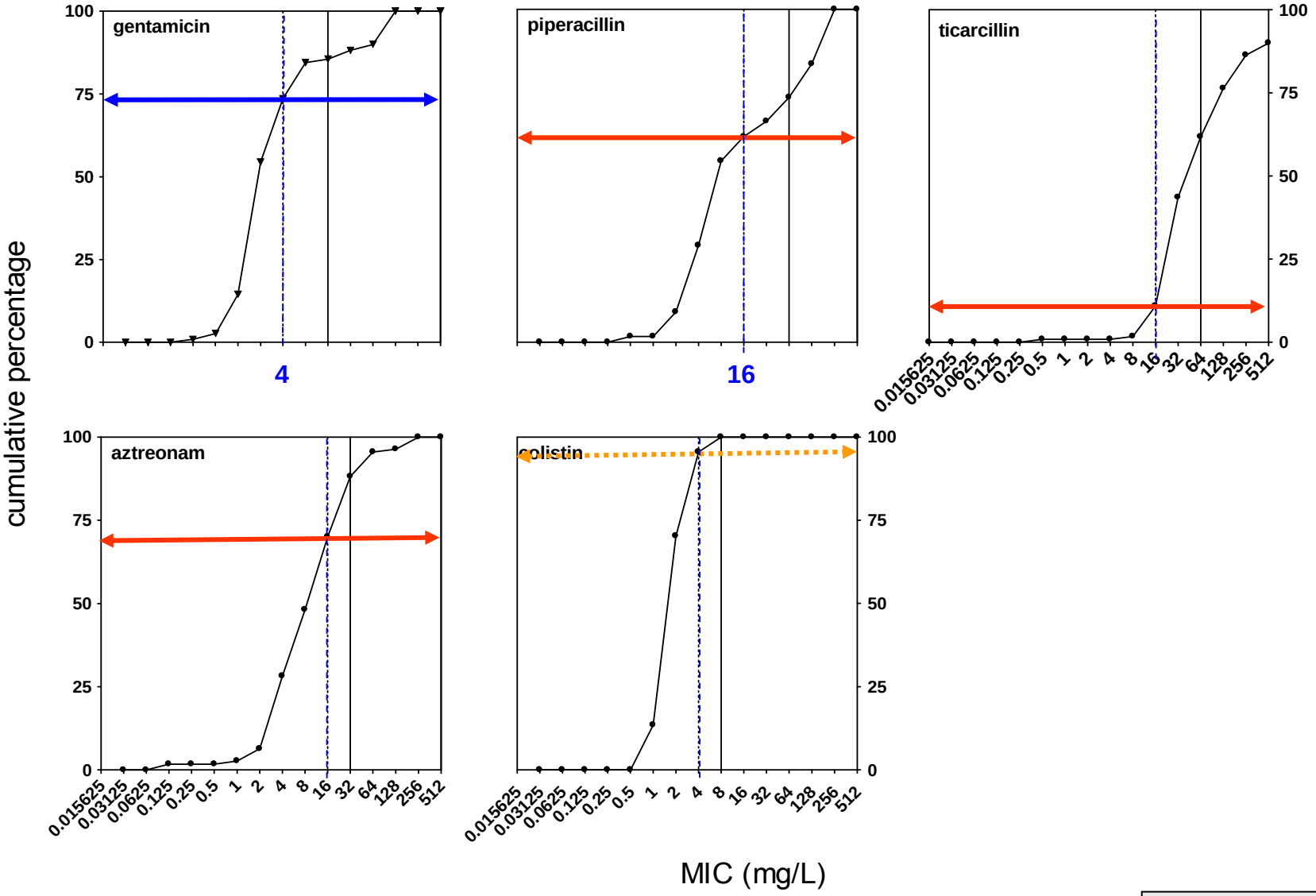
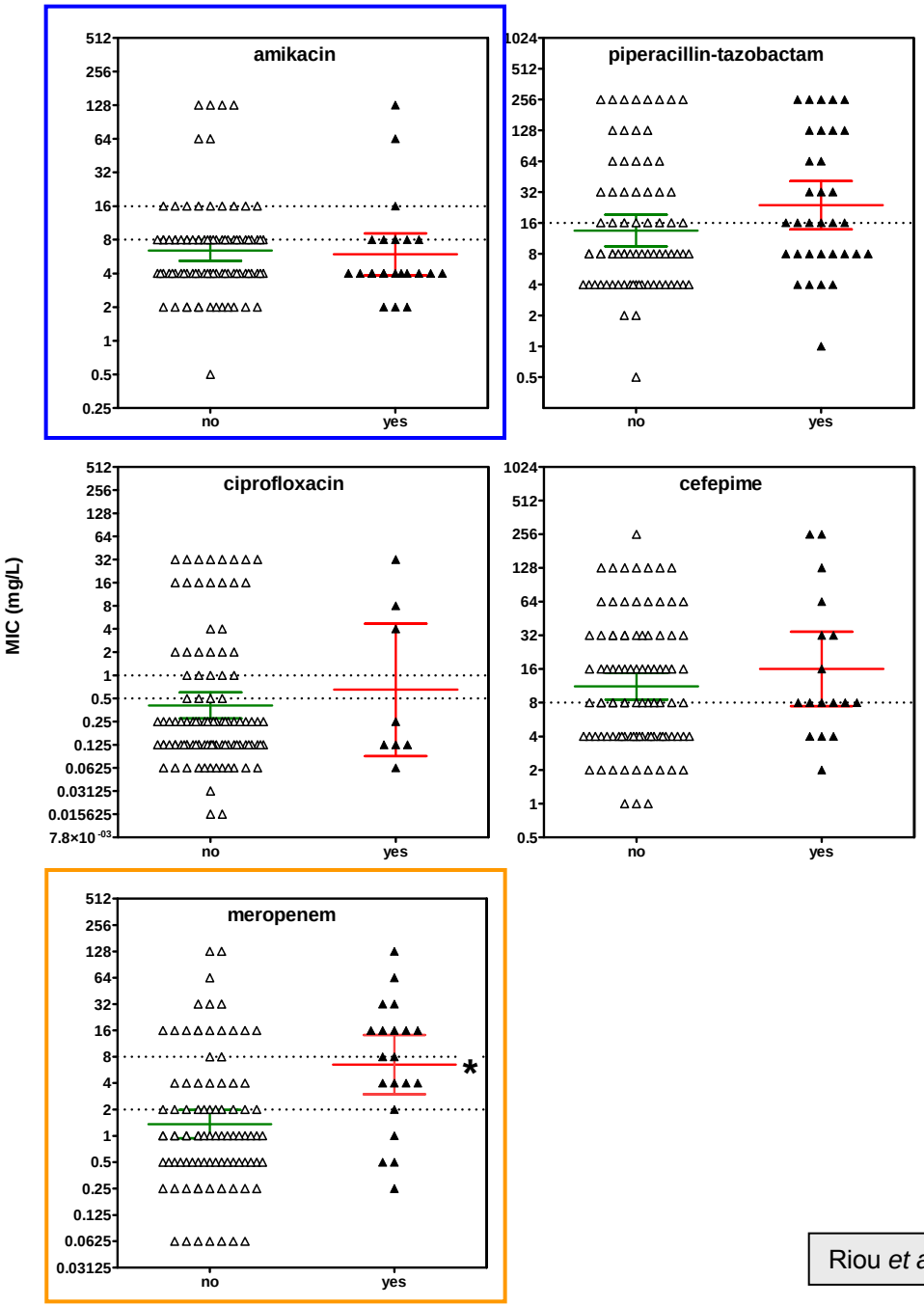


Riou *et al.*, submitted

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



Riou *et al.*, submitted

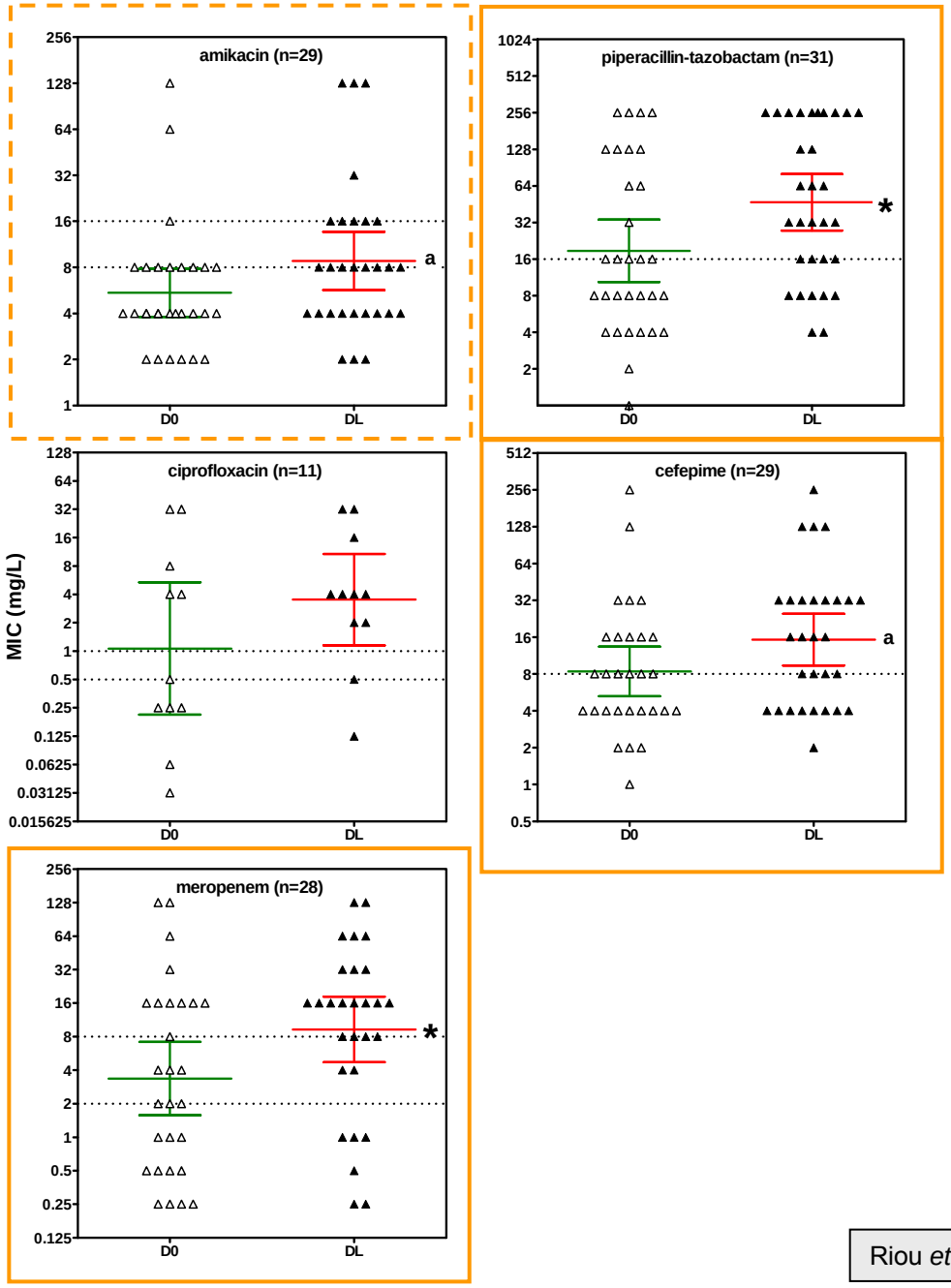
What happens during treatment ?

- 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)



Riou *et al.*, submitted

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

[d] no intermediate catgeory for EUCAST

Riou *et al*., submitted

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.

Riou *et al*., submitted

But what happened to the bacteria ?

- "classical" resistance
- efflux-mediated resistance

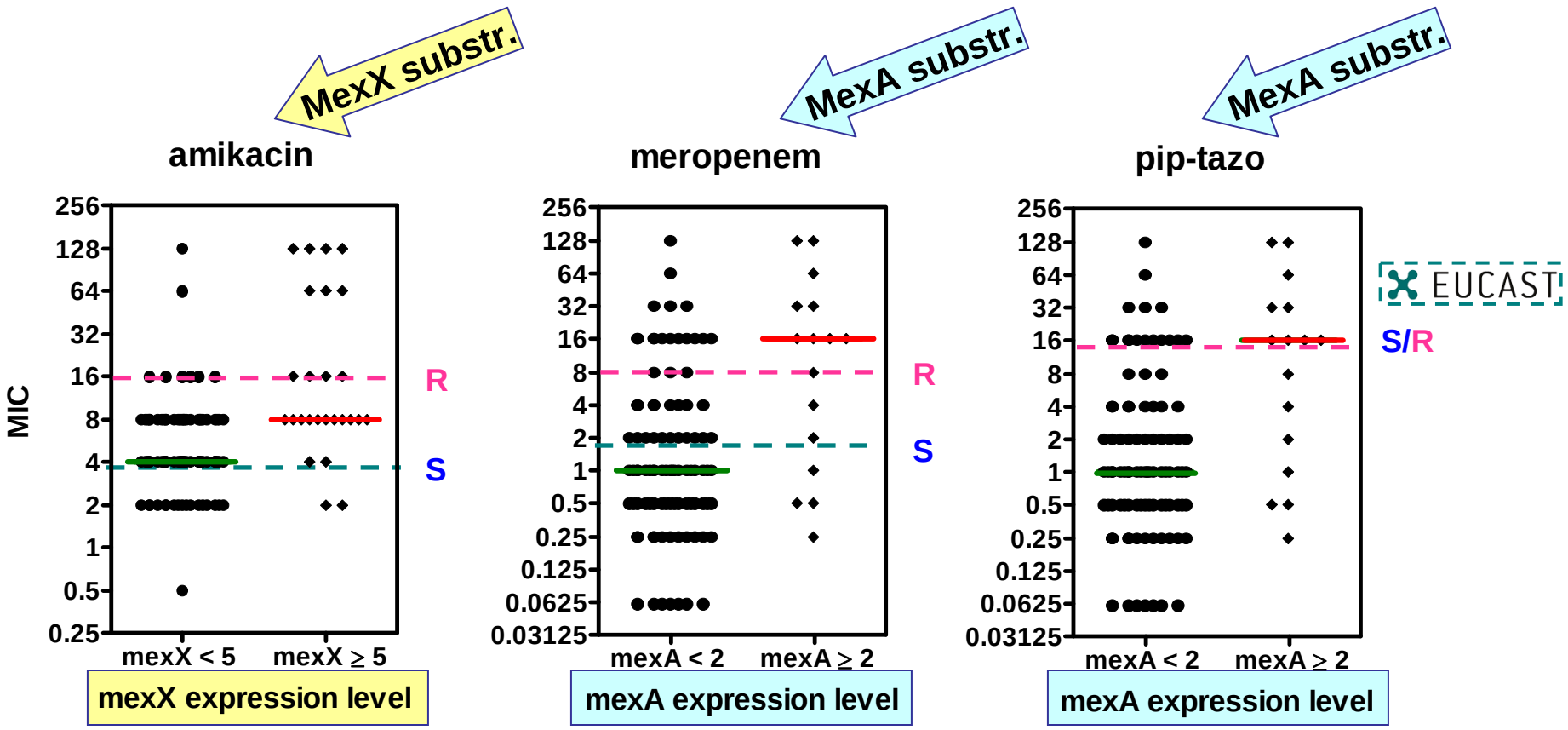
Classical resistance…

- Antibiogram (with interpretation) at high and low density inocula
- Direct genomic determination for suspected mechanisms (enzymes, porins …)
  - ➢ **Multiple mechanisms …**

Hard work still in progress …

Efflux and clinical resistance in *Pseudomonas aeruginosa* at the onset of treatment (day 0)

MICs *vs* EUCAST breakpoints for 109 *P. aeruginosa* without or with efflux mechanisms, isolated from ICU patients (VAP)



Riou et al., ECCMID 2010

Efflux pumps overexpression *

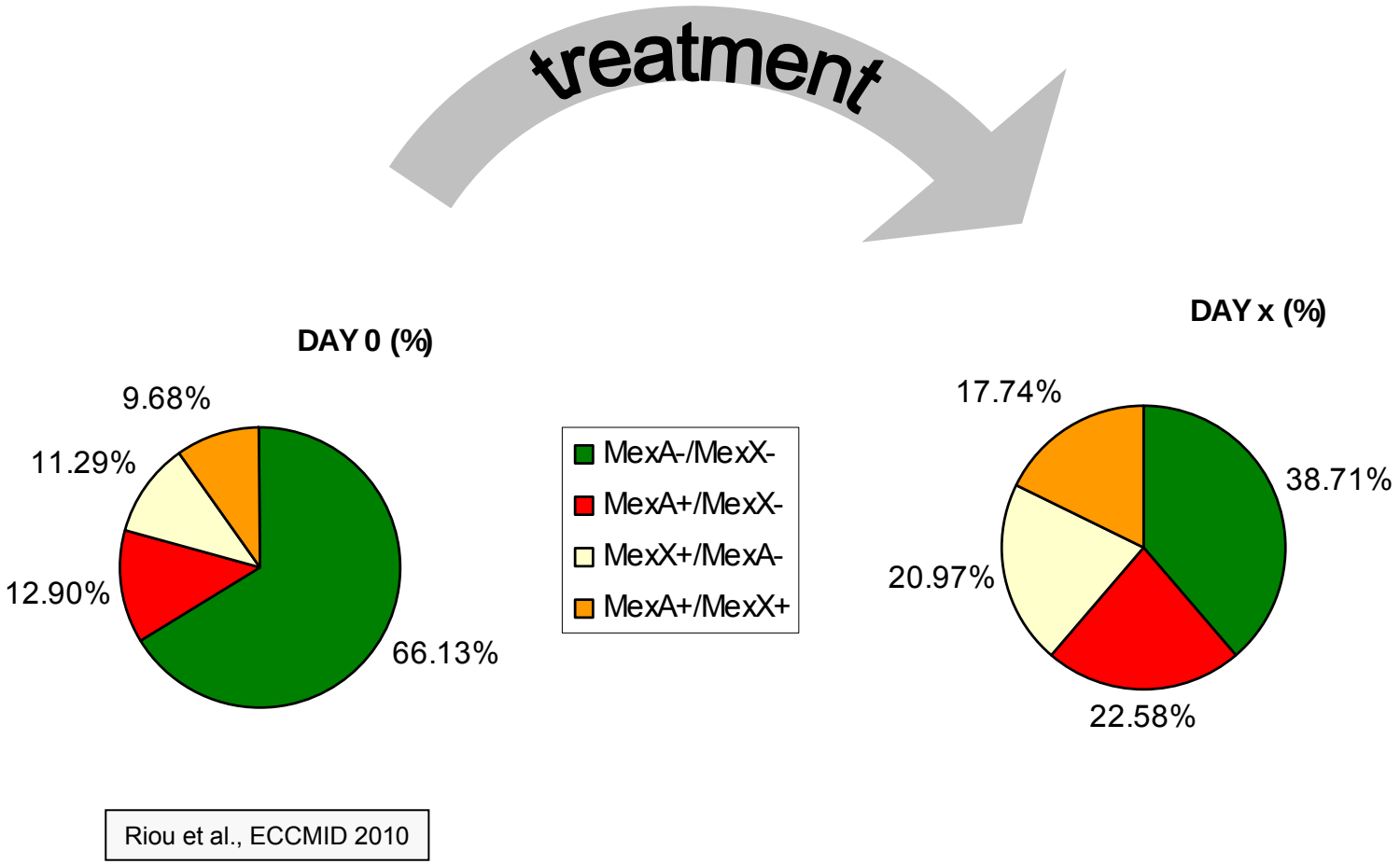
Type of PCR	Genetic status	Day 0 (% of isolates)	Day X (% of isolates)
Real time PCR (constitutive genes)	**MexA- / MexX-**	66.13	38.71
	MexA+ / MexX-	19.90	22.58
	MexA- / MexX+	11.29	20.97
	MexA+ / MexX+	9.68	17.74
Classical PCR (inductive genes)	**MexC- / MexE-**	90.50	87.00
	MexC+ / MexE-	6.50	11.00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

Efflux selection during treatment

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



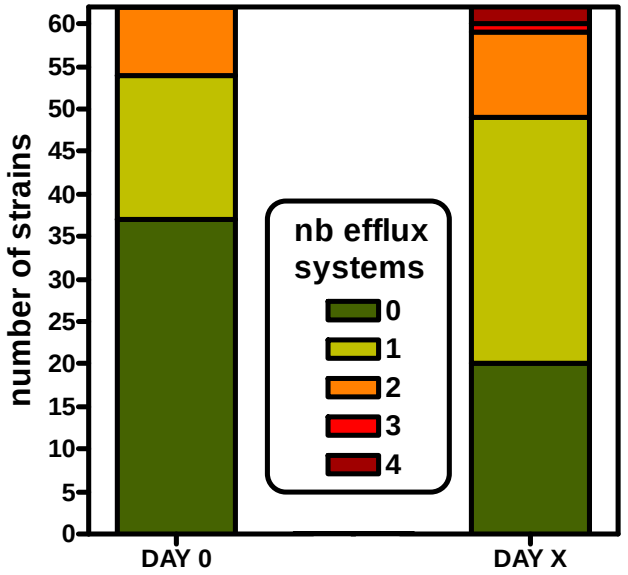
Riou et al., ECCMID 2010

Efflux selection during treatment

Antipseudomonal antibiotics received by the patients during treatment

Antibiotic	no. patients	
Piperacillin-tazobactam	26	69% combinations
Amikacin	22	
Meropenem	20	
Cefepime	19	
Ciprofloxacin	6	

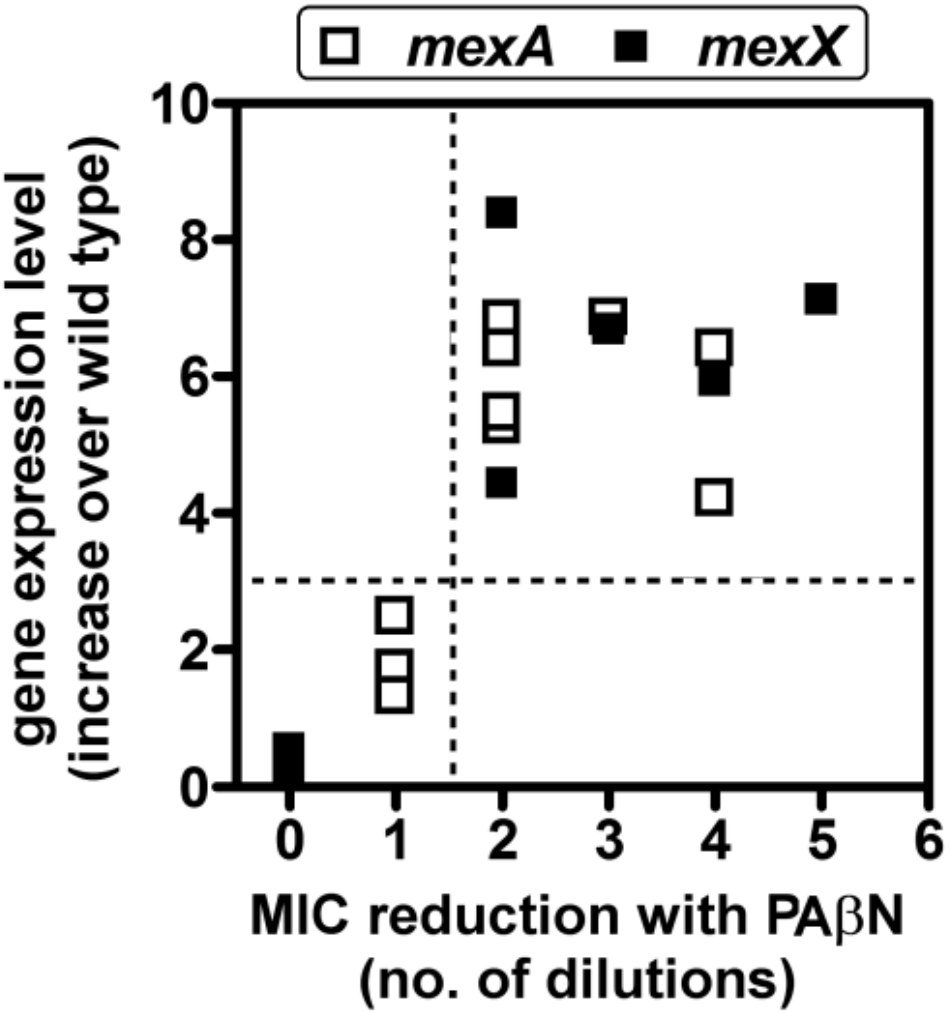
global influence of treatment



number of efflux systems detected at day 0 and day X

Riou et al., ECCMID 2010

What happens if you overexpress MexA and/or MexX ?



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

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

Diagnostic approaches …

- Tests must be simple but also as accurate as possible…
  - Genomic techniques are being rapidly introduced in the clinical laboratory and can either be automated (PCR) or made into fast-test assays
  - Accurate phenotypic and genotypic tests need to be combined (E-test with mRNA detection)
  - Proteomic tests (using antibody-based detection techniques) could be added also.

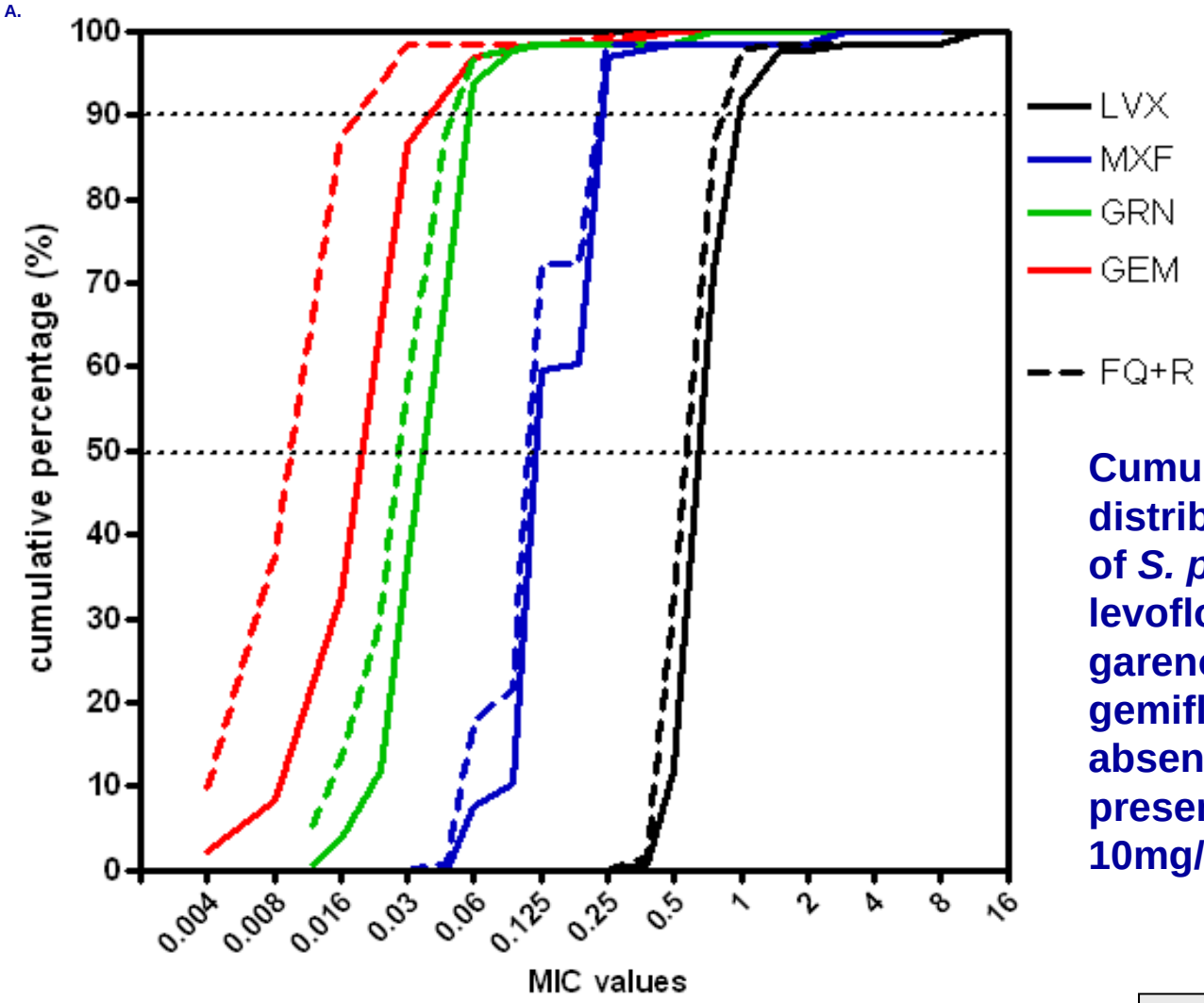
http://www.corisbio.com

Study #2: *Streptococcus pneumoniae* in Belgium in 2010

- **Ann Lismond, Farid El Garch, Sibille Delvigne, Sylviane Carbonnelle, Françoise Van Bambeke**
- **Mark Garvey, Laura Piddock, Jean-Paul Pirnay, Daniel De Vos**
- **Frank Verschuren, Fréderique Jacobs, Denis Pierard, Paul Jordens**

- Unité de pharmacologie cellulaire et moléculaire, Université catholique de Louvain, Bruxelles
- Antimicrobial Research Group, University of Bimingham, Birmingham
- Cliniques universitaires Saint-Luc, Brussels; Hôpital Erasme, Brussels, Universitair Ziekenhuis Brussel, Brussels, O.LV. Ziekenhuis, Aalst.

What do we have for fluoroquinolones (CAP patients) ?

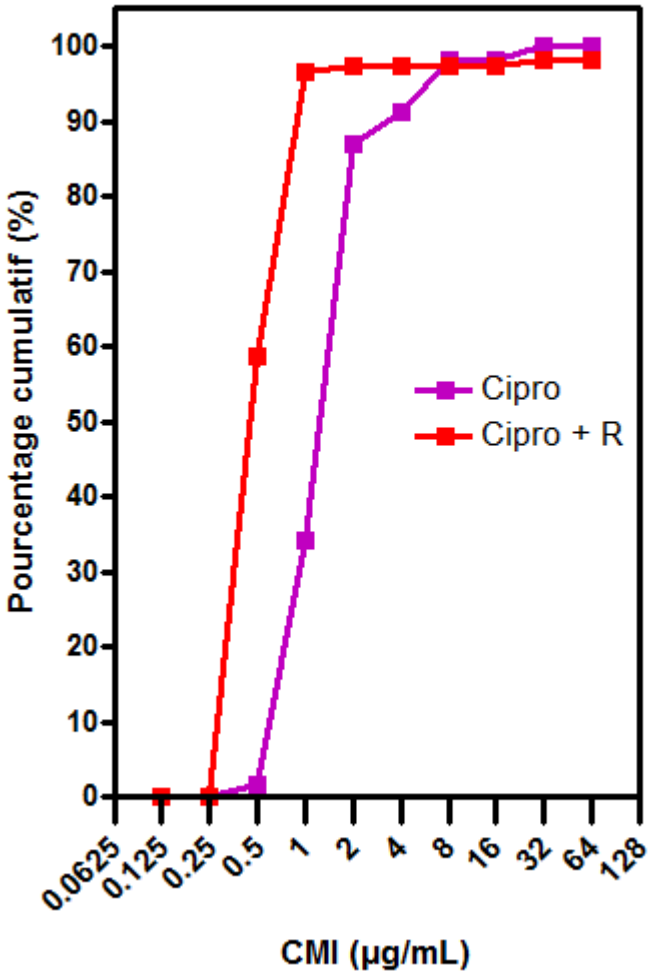


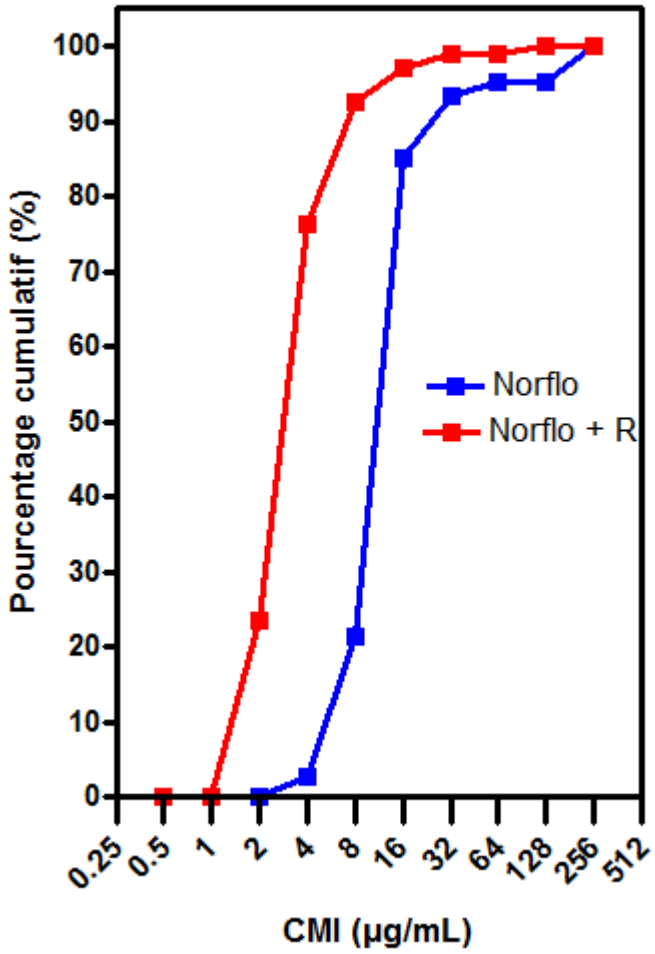
Cumulative MIC distribution for 134 isolates of *S. pneumoniae* towards levofloxacin, moxifloxacin, garenoxacin and gemifloxacin in the absence (solid lines) or presence (dotted lines) of 10mg/L reserpine.

Lismond *et al.* ECCMID 2009

And with "reporter" fluoroquinolones" (COPD patients; n=107) ?

Effect of reserpine on MIC distributions of ciprofloxacin and norfloxacin

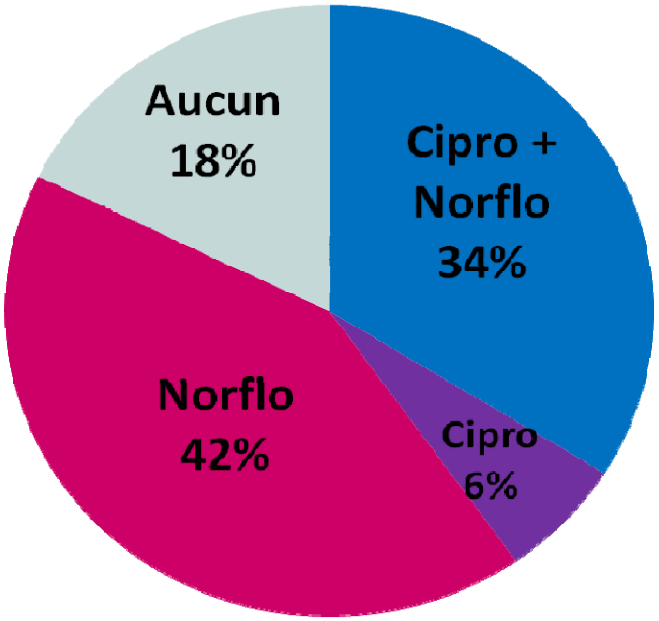




Lismond *et al.* ECCMID 2010

Does this represent efflux ?

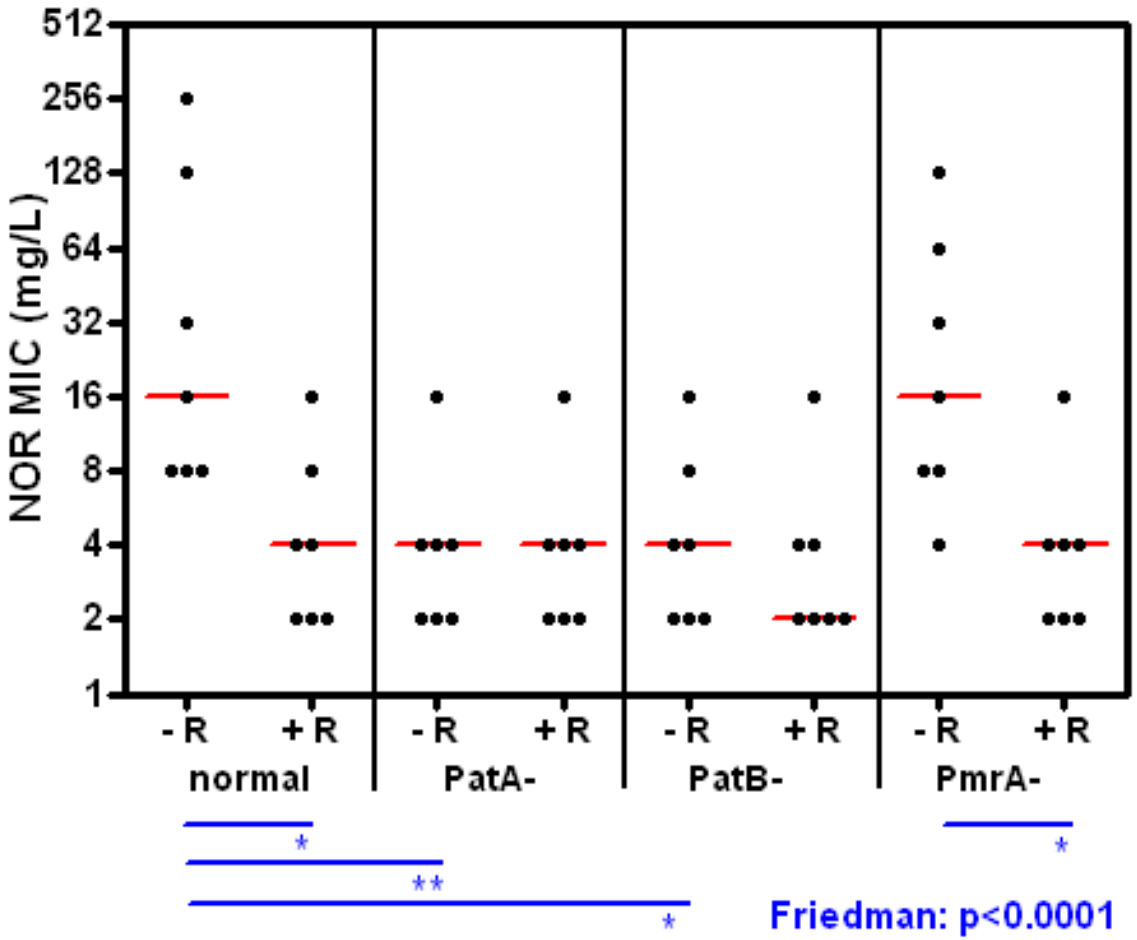
Suspected efflux based on phenotypic analysis



Lismond *et al.* ECCMID 2010

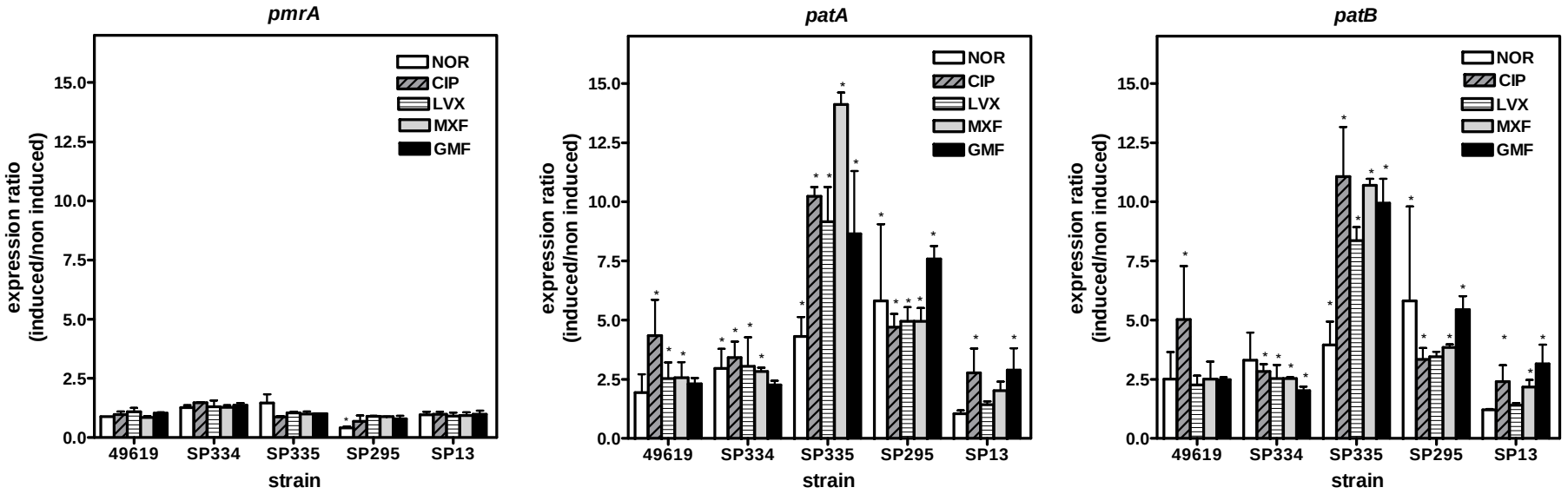
Does this represent efflux ?

Suspected efflux based on genotypic analysis



Lismond *et al.* ECCMID 2010

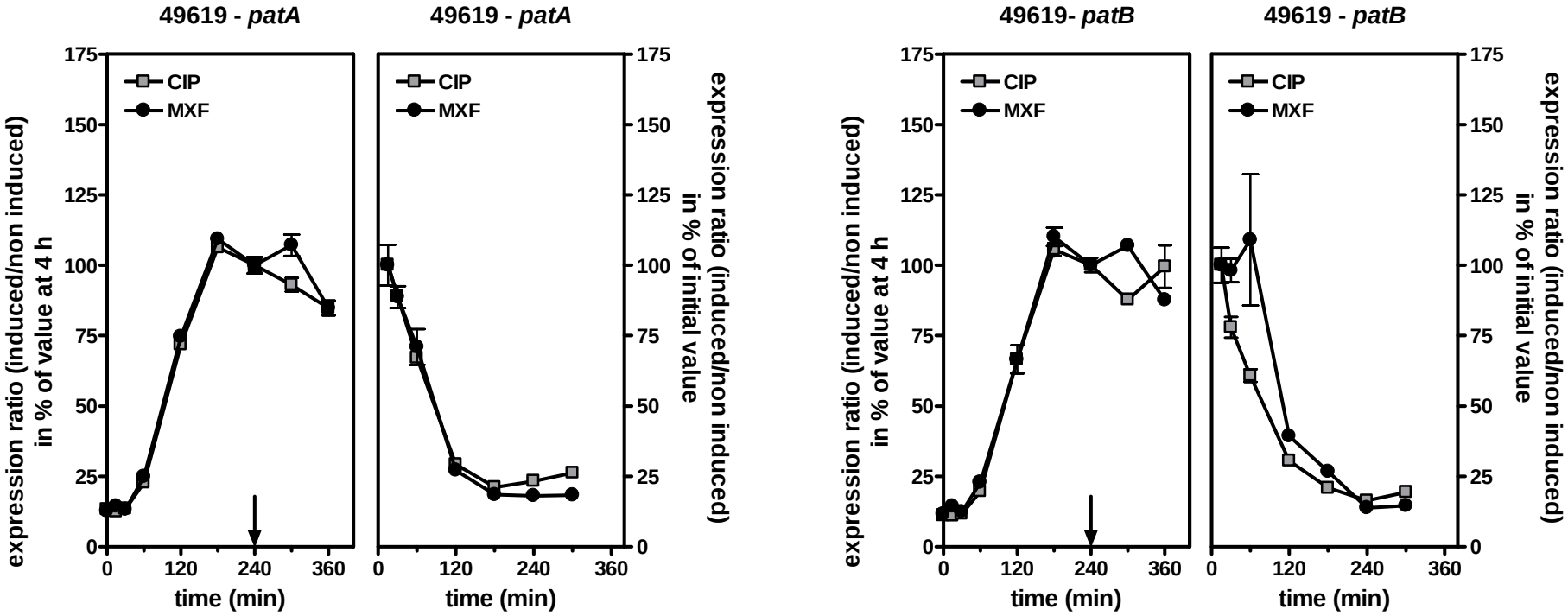
Can *pmrA* and *patA*/*patB* be induced ?



Induction of *pmrA*, *patA,* and *patB* expression in *S. pneumoniae* exposed for 4 h to half MIC of various fluoroquinolones.
Data are presented as the ratios of expression measured in induced and non-induced conditions.
Values are the mean ± SEM of duplicates from 2 independent experiments.
Statistical analysis: *: $p < 0.05$ (one-way ANOVA with Dunnett's post-hoc test for comparison with non-induced condition).

El Garch *et al.*, JAC, in press

Is patA/patB induced overexpression fast … and reversible ?



- Kinetics of induction and desinduction of *patA* and *patB* expression by ciprofloxacin and moxifloxacin in *S. pneumoniae* ATCC49619
- Exposure to half MIC of ciprofloxacin or moxifloxacin during 6 h.
- For reversion, bacteria induced for 4 h were harvested and regrown in broth without antibiotic for 5 h.

El Garch *et al.*, JAC, in press

What should we conclude ?

- Efflux is a reality and cause detectable, clinically-meaningful resistance … which will now be reported more and more by clinical microbiologists (study #1 - *Pseudomonas*)
- Antibiotics induce efflux, even those which are not (apparent) substrate… (study #2 – *Pneumococci*)
- This affects several classes of antibiotics (cross-resistance)

- So, the impact is at two levels:
  - decreased choice for the clinician
    - ➔ **novel antibiotics are a must …**
  - new challenge for the drug designer
    - ➔ **novel antibiotics should not only be non-substrate but should also not induce efflux … of other drugs…)**

And thank your for the invitation in Bremen …

Herzlich willkommen

0421/48589-0
0421/48589-99

ALPHA PUMPEN-TECHNIK

Pumpen

Armaturen

Reparatur

Kunden-
und Notdienst

Anlagenbau

Dichtungstechnik

Zubehör

Angebot
des Monats

Kontakt

Alpha-Pumpen-Technik GmbH - Thalenhorststraße 9 - 28307 Bremen

And here is (part of) the "pump team

Welcome to all of you

00-32-2-764-7378

00-32-2-764-7373

http://www.facm.ucl.ac.be

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