Efflux as a new challenge in antibacterial chemotherapy

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Unité de pharmacologie cellulaire et moléculaire

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Transport across membranes: Multiple drug resistance, mechanisms and new tools
Bremen, Germany, July 9th, 2007, … in the evening

VolkswagenStiftung

Influenced in large part by my active participation to the European Committee for Antibiotic Susceptibility Testing (EUCAST) and to the International Society of Antiinfective Pharmacology (ISAP)

You said "Challenge" ?

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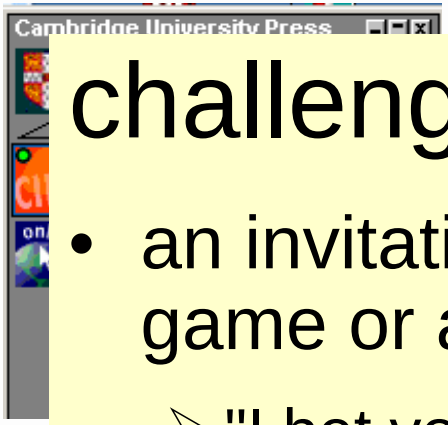

challenge (INVITATION) /"tS{l.IndZ/ noun [C]

- an invitation to compete or take part, esp. in a game or argument
  - "I bet you can't eat all that food that you've got on your plate." "Is that a challenge?"
  - Is there going to be a challenge for the position of chairperson when the next election for the committee is held?
  - She issued a challenge to her rival candidates to take part in a public debate, but they did not accept it.

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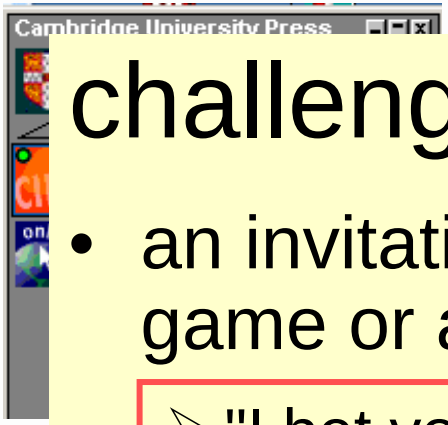

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the hungry microbiologist

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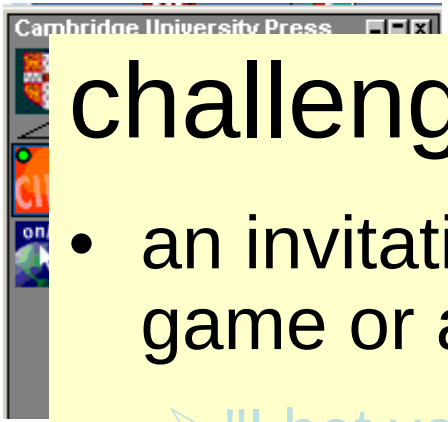

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for the next conference on efflux

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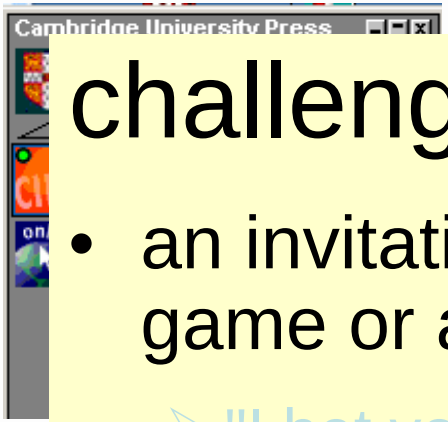

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Are you talking about a political person here ?

Challenges of efflux in antibacterial chemotherapy

- **recognizing its existence:** is it a major and general means of resistance ?
- **its role:** does it need to change our vision on (and decisions about) existing antibiotics ?
- **the way we find it:** how can we detect it (and do we need to do this ?)
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  - and what about efflux inhibitors ?
  - is efflux important in pharmacokinetics/drug interactions ?

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Historical observations on tetracyclines …

1: Nature. 1963 Oct 26;200:384-5.

DISAPPEARANCE OF OXYTETRACYCLINE ACCUMULATION IN THE CELLS OF MULTIPLE DRUG-RESISTANT ESCHERICHIA COLI.

IZAKI K, ARIMA K.

PMID: 14087909 [PubMed - indexed for MEDLINE]

Who remembers that car ?

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Who remembers that graph ?

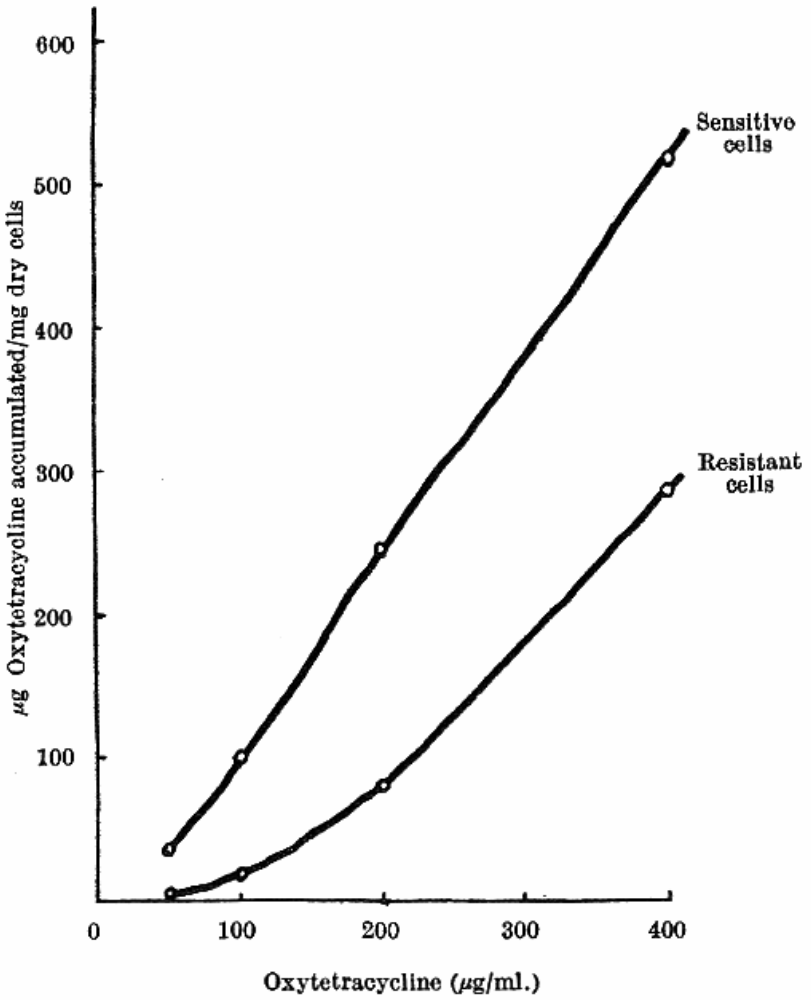

Fig. 1. Accumulation of oxytetracycline in *E. coli* K-12 at various concentrations of oxytetracycline added. The reaction mixture contains 1 ml. suspension (0·7 mg dry weight) oxytetracycline hydrochloride, 1 ml. (0·5–4·0 mg/ml.) and 1 ml. of 10 per cent (w/v) glucose, 2 per cent K_2HPO_4 and 0·1 per cent $MgSO_4.7H_2O$ respectively in a total volume of 10 ml. Incubation was carried out aerobically at 30° C for 90 min

Historical observations on tetracyclines …

Biochem. J. (1965) **94**, 54

Resistance of *Escherichia coli* to Tetracyclines

BY T. J. FRANKLIN AND A. GODFREY
Imperial Chemical Industries Ltd. (Pharmaceuticals Division), Alderley Park, Macclesfield, Cheshire

(*Received* 23 *March* 1964)

1. A strain of *Escherichia coli* highly resistant to chlortetracycline and partially cross-resistant to tetracycline has been isolated. 2. The nitro-reductase system of the resistant cells was inhibited to a smaller extent by chlortetracycline than was the corresponding enzyme of sensitive cells. 3. The incorporation of leucine *in vitro* into the ribosomal protein of cell-free preparations from sensitive and resistant cells was equally inhibited by chlortetracycline. 4. Resistant cells accumulated much less chlortetracycline and tetracycline than did sensitive cells when both were cultured in the presence of these drugs. 5. The uptake of tetracycline by both sensitive and resistant *E. coli* was dependent on the presence of glucose in the medium. 6. Fractionation of cells cultured in medium containing [^{14}C]chlortetracycline indicated that the largest proportion of radioactivity in sensitive cells was in the fraction consisting mainly of cell-wall material. There was no concentration of radioactivity in any one fraction of the resistant cells. 7. No evidence could be obtained for a specific tetracycline-excretion system in the resistant cells. 8. The significance of these results in relation to current theories of the antibiotic action of and resistance to the tetracycline drugs is discussed.

Historical observations on tetracyclines …

Table 1. *Binding of* [^{14}C]*chlortetracycline and* [^{3}H]*tetracycline to sensitive and resistant* E. coli *cells*

Cells were cultured for 1 hr. in the presence of the drugs and harvested (about 6×10^8 sensitive cells and 9×10^8 resistant cells/ml. of medium), and the radioactivities of disrupted unfractionated preparations were determined. [^{14}C]Chlortetracycline was undiluted with unlabelled drug. [^{3}H]Tetracycline (0·02 μc/ml. of medium) was diluted with unlabelled drug to give a final concentration of 10 μg./ml. of medium.

Drug	Organism	Concn. of drug in medium (μg./ml.)	Radioactivity bound by cells (disintegrations/min./mg. of protein)	Fraction of total drug bound by cells (%)	Drug bound by cells (μg./mg. of protein)
Chlortetracycline	Sensitive	1·0	446	13·0	1·01
	Resistant	1·0	50	2·5	0·11
	Sensitive	10·0	5183	15·0	11·80
	Resistant	10·0	172	0·8	0·39
Tetracycline	Sensitive	10·0	12808	4·2	2·90
	Resistant	10·0	2156	1·3	0·48

Franklin & Godfrey, Biochem. J. 1965; 94:54

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Historical observations on tetracyclines …

15 years later...

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 …

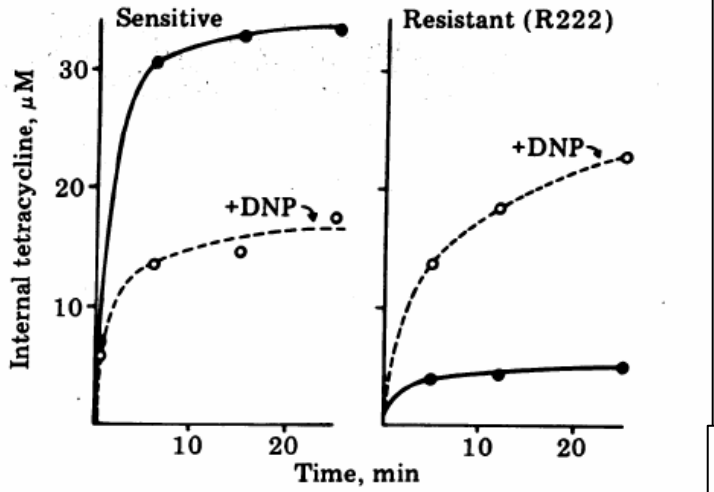


FIG. 1. Tetracycline uptake by *E. coli* ML308-225 (sensitive) and by R222-containing induced (resistant) cells with (○) and without (●) 1 mM DNP. Cells were grown overnight in medium A containing glucose and uptake was measured in the absence of added energy source.

Whole bacteria

Everted membranes

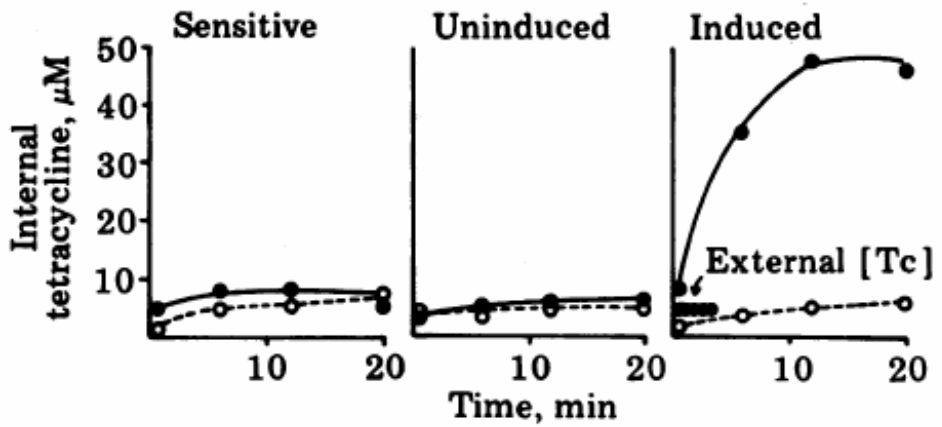
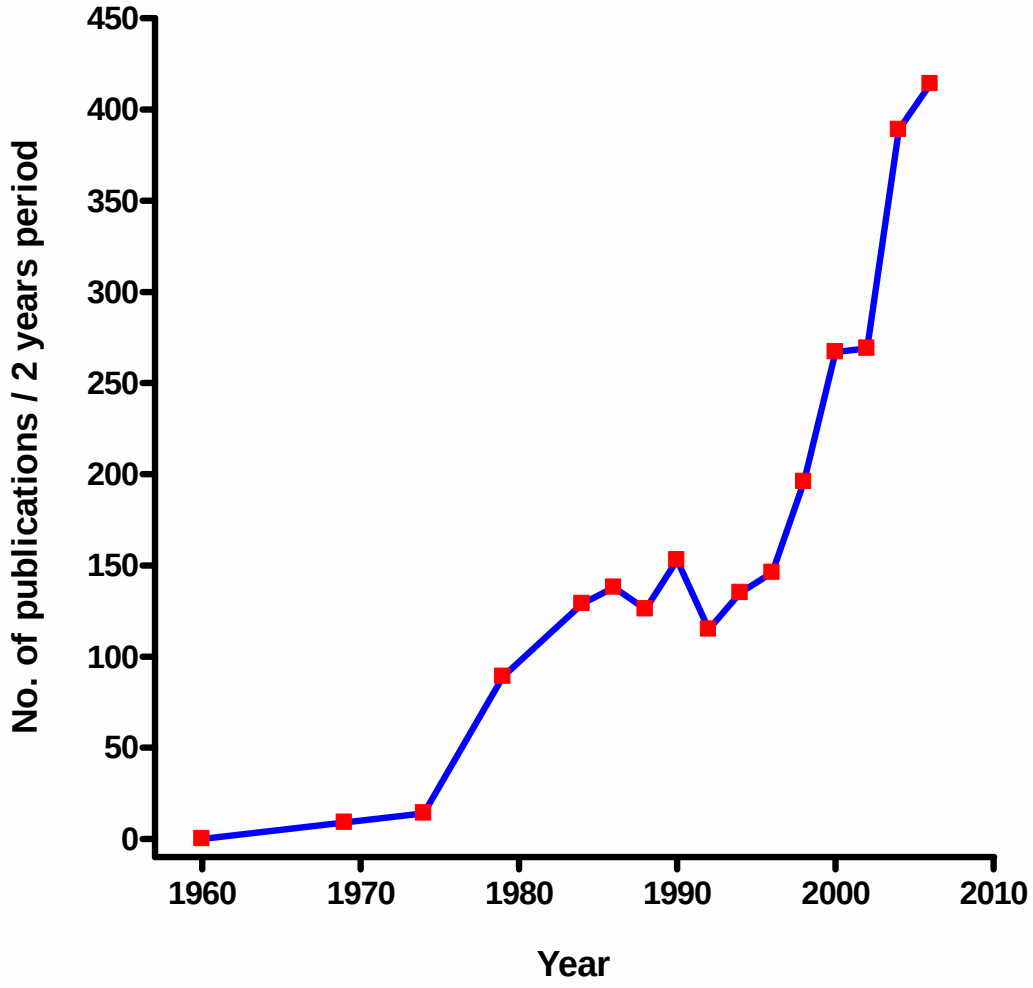


FIG. 2. Tetracycline (Tc) uptake by everted membrane vesicles made from sensitive ML308-225 cells and from uninduced and induced R222-containing cells. ○, No energy; ●, D-lactate. Cells were grown in glycerol and vesicles were frozen in 5 mM Tris·HCl, pH 7.2/70 mM KCl/0.25 mM dithiothreitol/50% glycerol.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

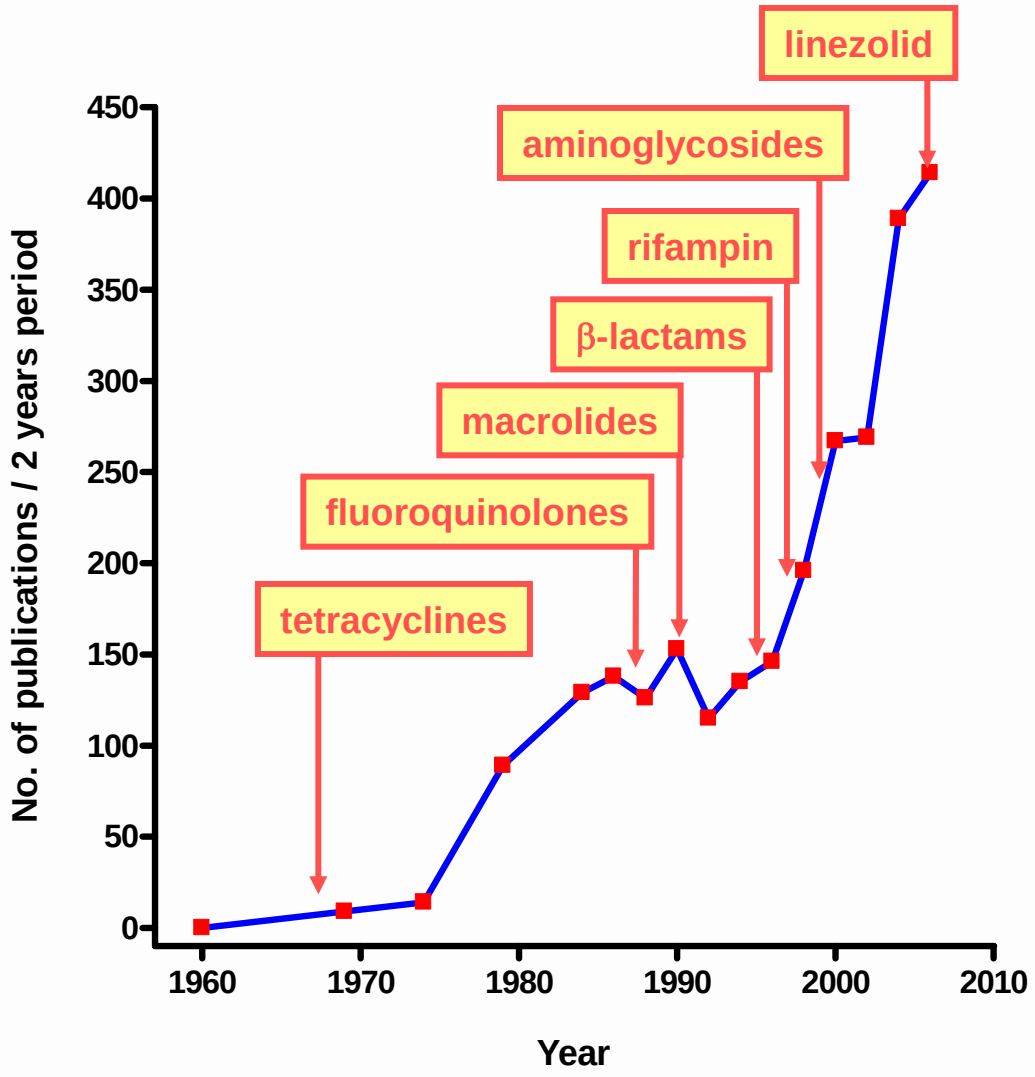
McMurry et al., PNAS 1980; 77:3974-3977

Historical trends …



No. of publications in PubMed with keywords: antibiotic & efflux

Historical landmarks …



Successive description of efflux-mediated resistance for major antibiotics

Challenges …

- Efflux has, slowly but surely, been shown to affect most if not all major antibiotic classes …

  ? Are they classes that will never show efflux-mediated resistance (glycopeptides [vancomycin…], lipoglycopeptides [telavancin], lipopeptides [daptomycin], …)

  ? Is efflux taken in full consideration in present teaching not only of microbiology but in everyday clinical practice (including in clinical microbiology reports) and is understood by clinicians ?

Challenges of efflux in antibacterial chemotherapy

- **recognizing its existence:** is it a major and general means of resistance ?
- **its role:** does it need to change our vision on (and decisions about) existing antibiotics ?
- **the way we find it:** how can we detect it (and do we need to do this ?)
- **the way we treat patients:**
  - can we make non-effluxed drugs ?
  - and what about efflux inhibitors ?
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Challenges …

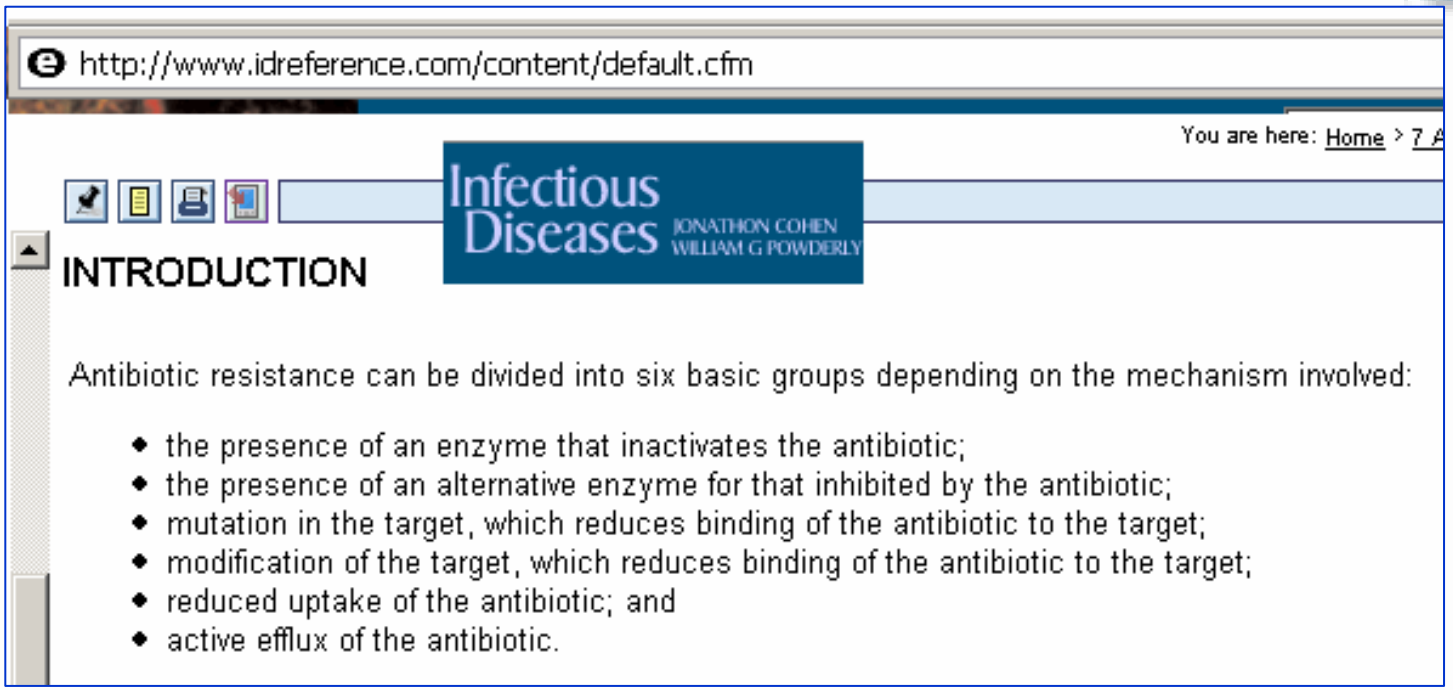
- Why is efflux still so poorly taken into account in teaching, in textbooks, and, in general, in medical education and practice ?
  - ➢ efflux most often causes only low levels of resistance, which tends to make it "clinically insignificant" …
    (see more about that later on)

- 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.

Challenges …

- Why is efflux so poorly taken into account in teaching, in textbooks, and, in general, in medical education ?
  - ➢ the multiplicity of structurally and pharmacologically unrelated substrates make efflux hard to grasp and difficult to enter in specific chapters or sections



Challenges …

- Why is efflux so poorly taken into account in teaching, in textbooks, and, in general, in medical education ?
  - ➢ the multiplicity of pumps with "hard to understand names" does not help…

Tableau I. Principales classes de pompes à efflux reconnues capables de transporter les antibiotiques au niveau des bactéries. Chaque classe contient un nombre élevé de transporteurs différents, dont les propriétés et la distribution dans le monde bactérien peuvent varier considérablement.

Famille (dénomination*)	Acronyme	Source d'énergie
ATP-Binding Cassette	ABC	ATP
Major Facilitator Superfamily	MFS	Gradients d'ions
Resistance-Nodulation-cell Division	RND	
Small Multidrug Resistance	SMR	

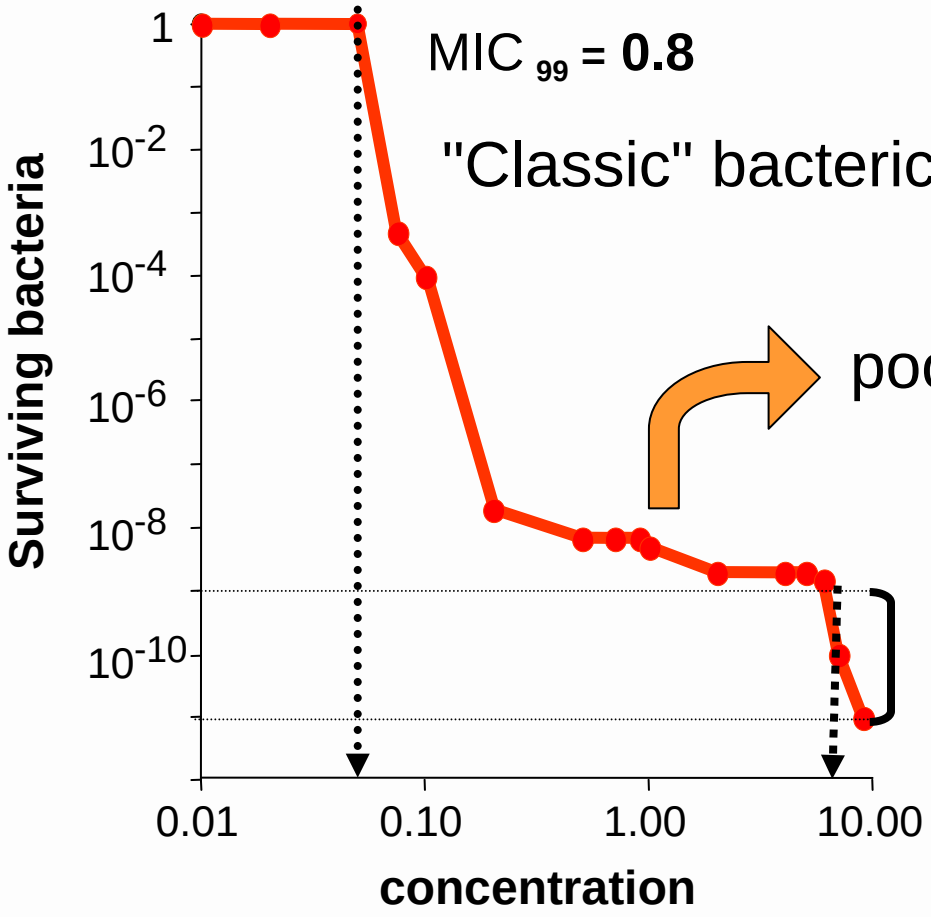
* Les dénominations sont celles données lors de la découverte des transporteurs, et correspondent souvent à des éléments contingents liés à cette découverte, ce qui explique qu'ils ne soient pas nécessairement en rapport avec les fonctions établies sur la base des travaux ultérieurs.

La Lettre de l'Infectiologue - Tome XX - n° 4 - juillet-août 2005

Challenges …

- The real challenges are about educating microbiologists, clinicians and responsible persons about the real impacts of efflux
  - efflux cooperates with other resistance mechanisms to move towards high resistance phenotypes
  - efflux favours the emergence of "first-mutants" by subjecting bacterial targets to suboptimal concentrations
  - efflux causes an overall decrease in antibiotic efficacy

Emergence of first mutants : the MPC concept…



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

"Classic" bactericidal effect

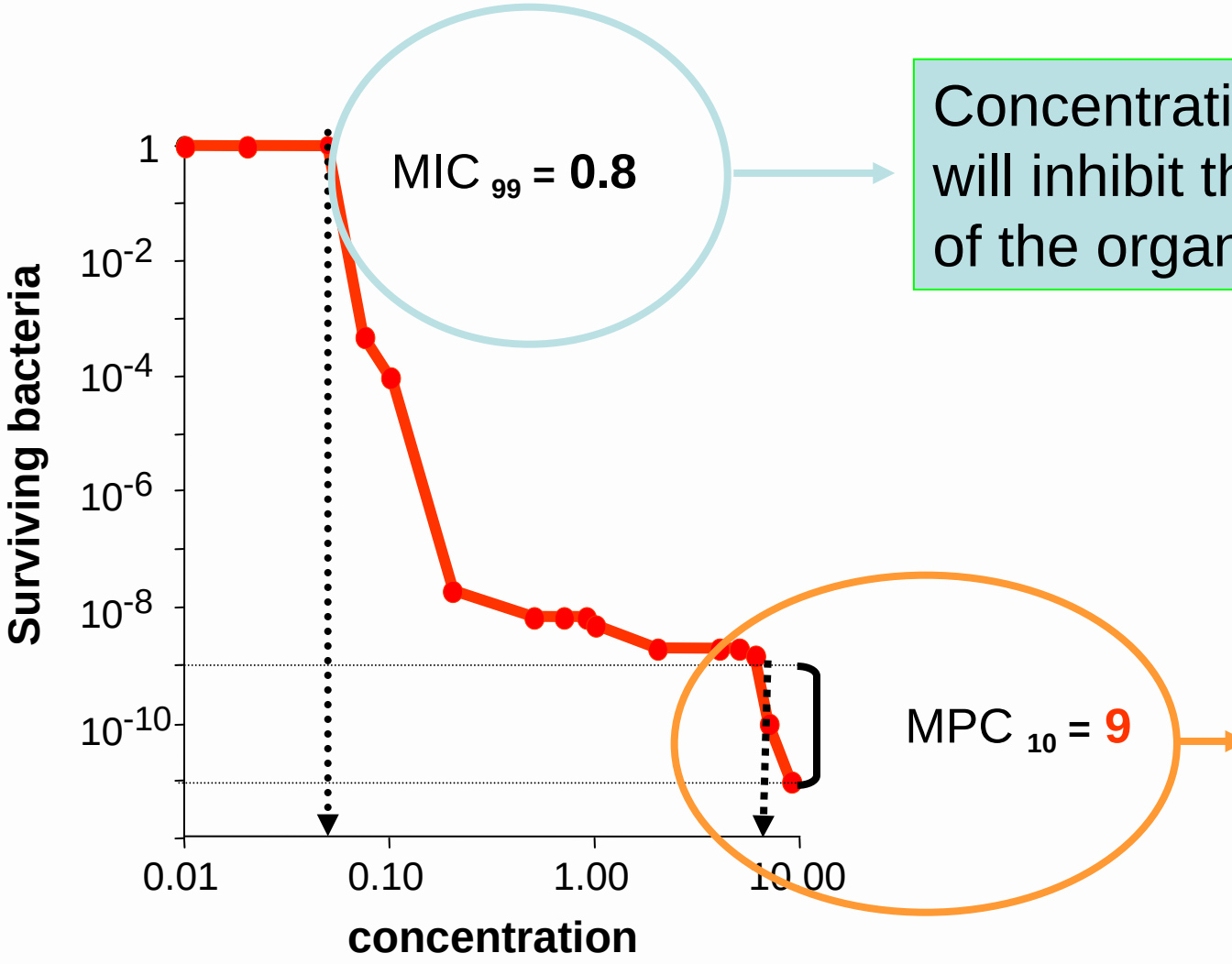
poorly sensitive organisms…

Elimination of resistant organisms

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

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

Mutant Prevention Concentration …

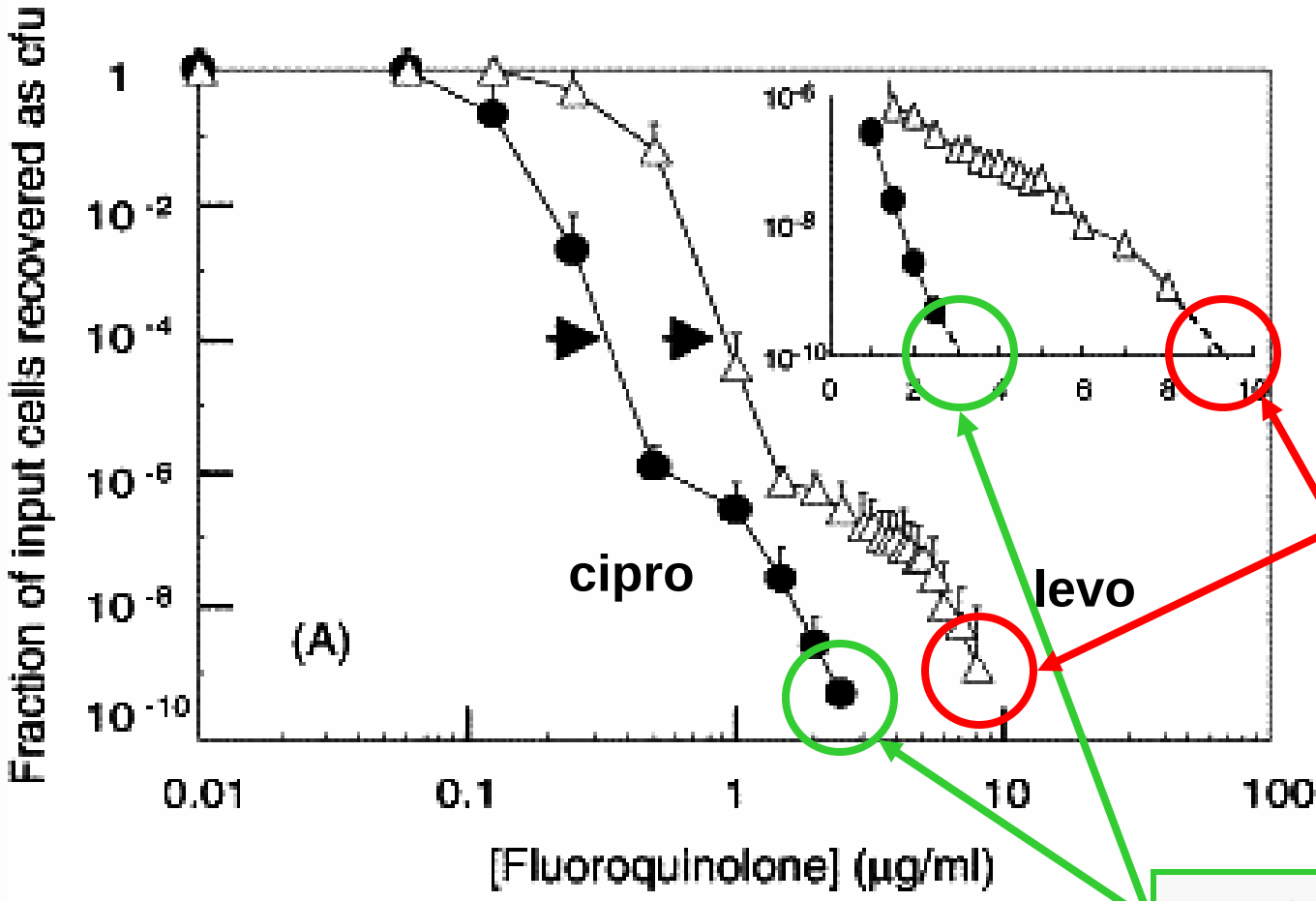


Concentration which will inhibit the majority of the organisms

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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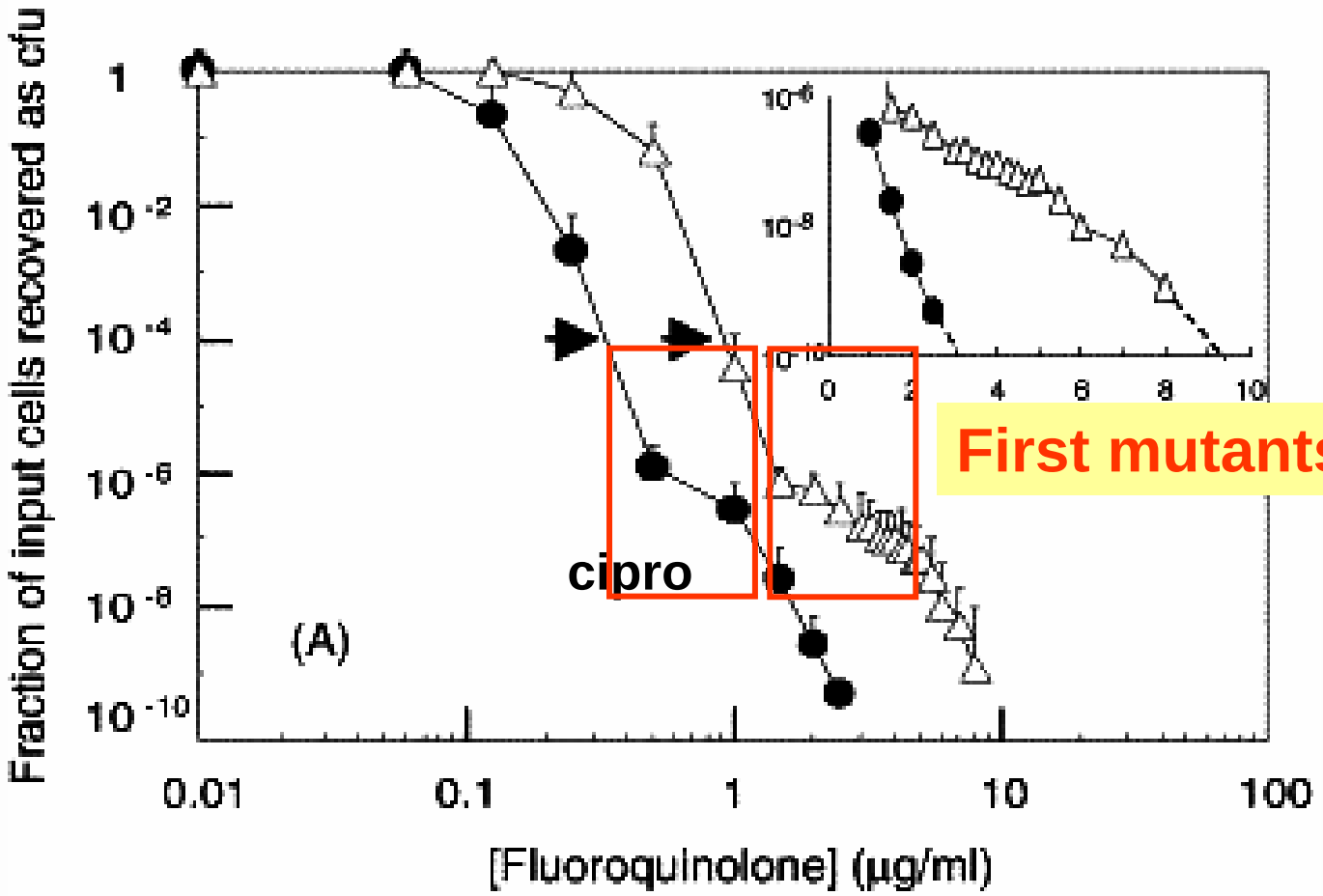
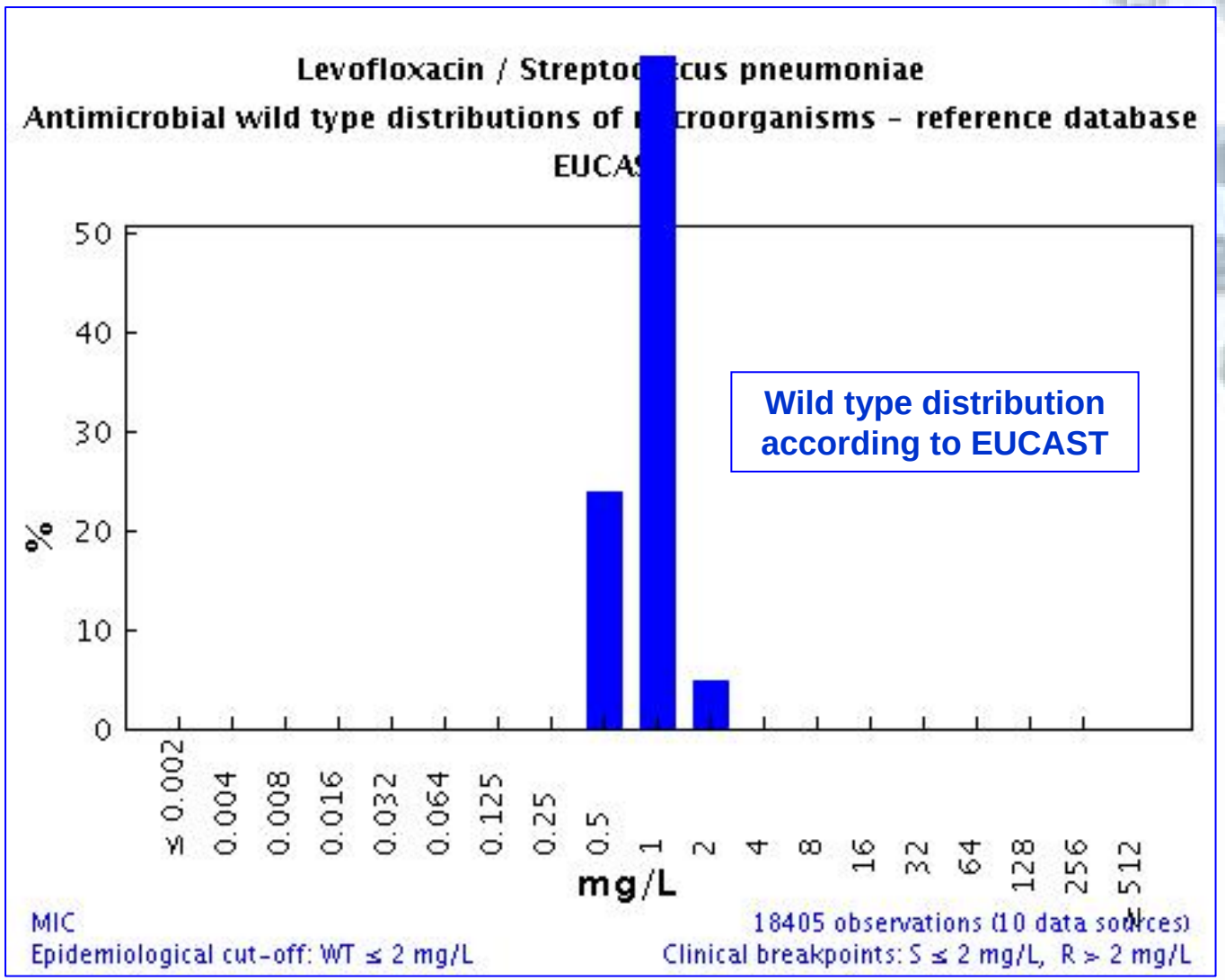


First mutants or efflux ?

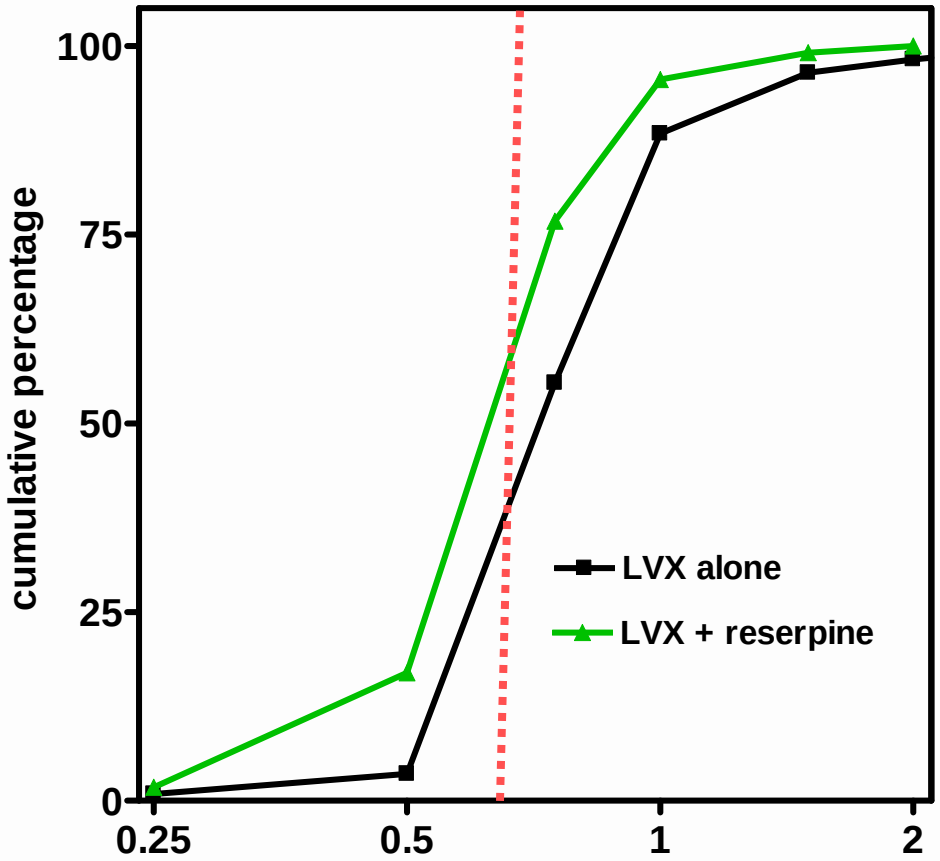
Hansen et al. I.J.Antimicrob. Agents 2006;27:120-124

Levofloxacin and pneumococci in the World



Levofloxacin and pneumococci in Belgium

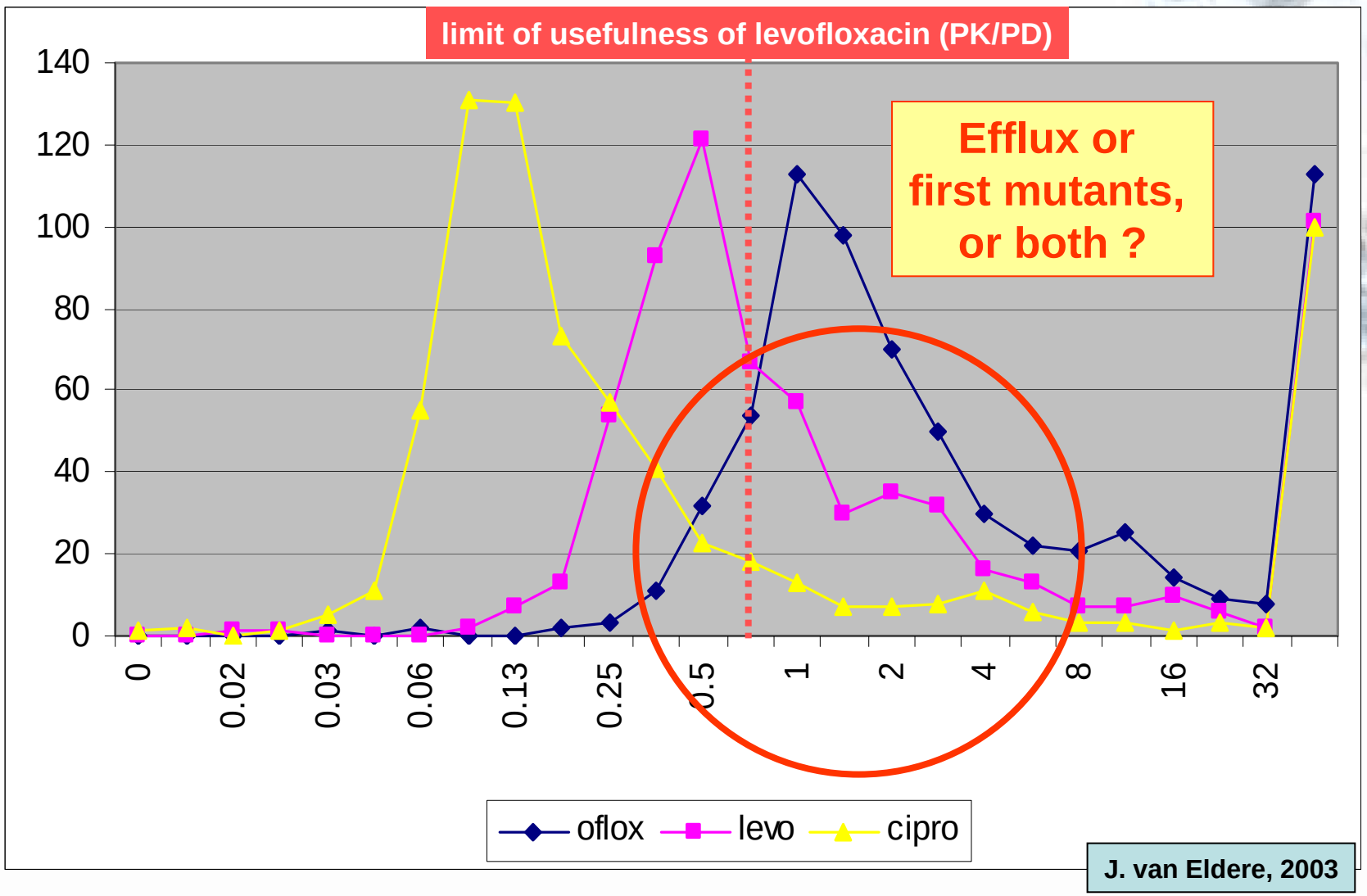
Levofloxacin MIC distributions in Belgian isolates



A. Lismond et al., unpublished

- **In original surveys of levofloxacin susceptibility in the mid 90's, most MICs were ≤ 0.25 mg/L**
- **Most Belgian isolates with MIC > 0.25 mg/L are carrying weak (in terms of MIC change) but clearly detectable reserpine-sensitive transporter(s)**
- **the limit of efficacy for the most commonly used dosage (500 mg) is 0.7 mg /L**
- **the presence of an efflux does contributes in reducing levofloxacin usefulness…**

Fluoroquinolones and *P. aeruginosa* at an Academic Hospital in Belgium



Challenges

- Efflux is like QnR for quinolones or arm for aminoglycosides: it is "before our nose" and "known by experts" but not much beyond that…
- the challenge is, therefore to clearly and unambiguouly detect efflux in poorly sensitive isolates…

Challenges in Diagnostic

- **Thesis:**

  Efflux will continue to be largely ignored (and go undetected) as long as clinical microbiologists swear only (sorry, report susceptibility) by breakpoints only, derived from "classical" antibiograms made with the antibiotics they use in their hospital...

- **Action:**

  Since breakpoints will continue to exist and to be used, efflux needs to be detected by additional techniques to provide added-on warning…

You said "breakpoints" ?

- a magic number derived from ***in vitro*** susceptibility testing, and used by the clinical microbiologists use to tell the clinician whether the antibiotic will work, could work, or will fail with his/her **patient**.
- this number is usually derived from the measurement of a diameter [1] of growth inhibition in an agar plate around a disk loaded with a standard amount of antibiotic;
- while what is measured is *per definition* a continuous variable (i.e. a diameter of any size [from 0 mm to the limit of the dish…), microbiologists and authorities like to cut the results it in 3 discrete categories
  - less than x mm ➔ RESISTANT
  - larger than y mm ➔ SUSCEPTIBLE
  - between x and y ➔ INTERMEDIATE

which is what the clinician will get…

[1] may be converted into an MIC (see later); automatic machines use growth rates…

Why do we use breakpoints ?

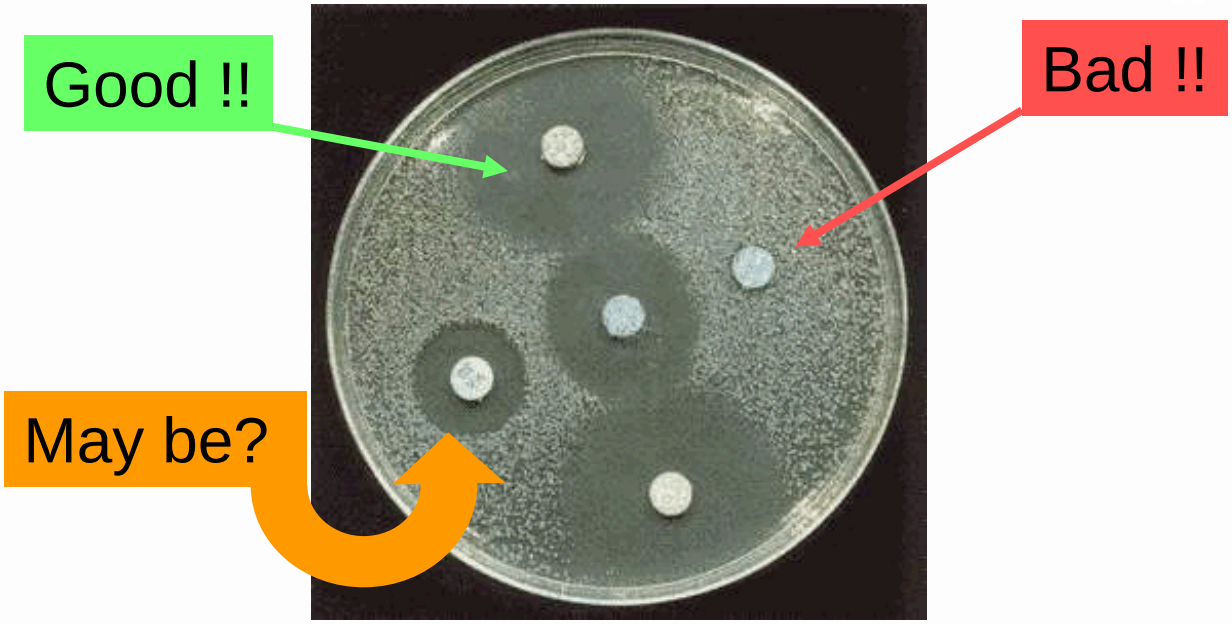
To be honest, I always wondered …

Why do we need breakpoints ?

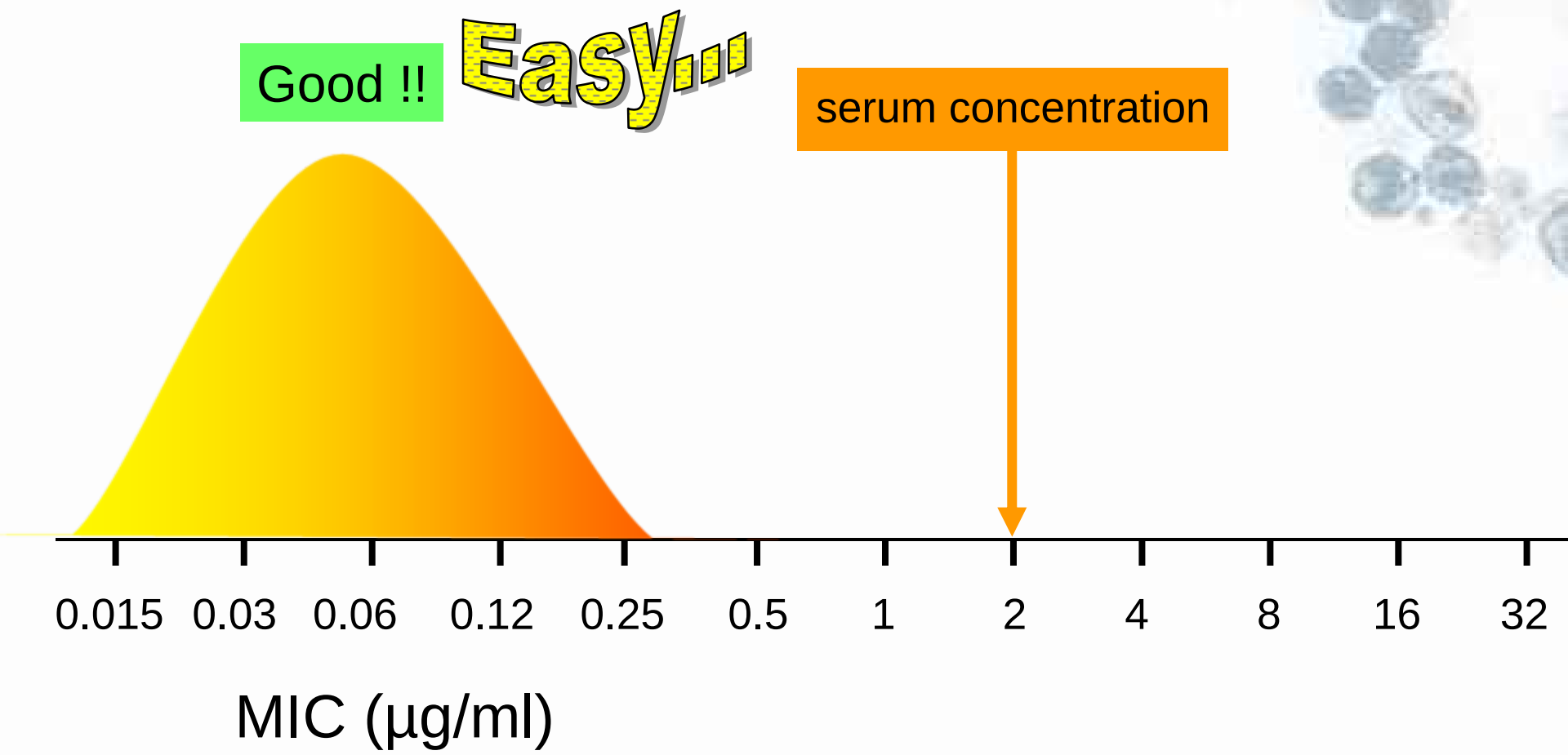
but perhaps…

1. Doctors like to know if the bug is "**good**" or "**bad**" …
2. Regulators like to tell people "**DO**" or "**Don't**"
3. Industry likes to know "**When can I**" and "**When I cannot**"
4. Lawyers like you to be "**guilty**" or "**innocent**" …
5. Microbiologists wish to give them all **simple answers…**

Simple answers …

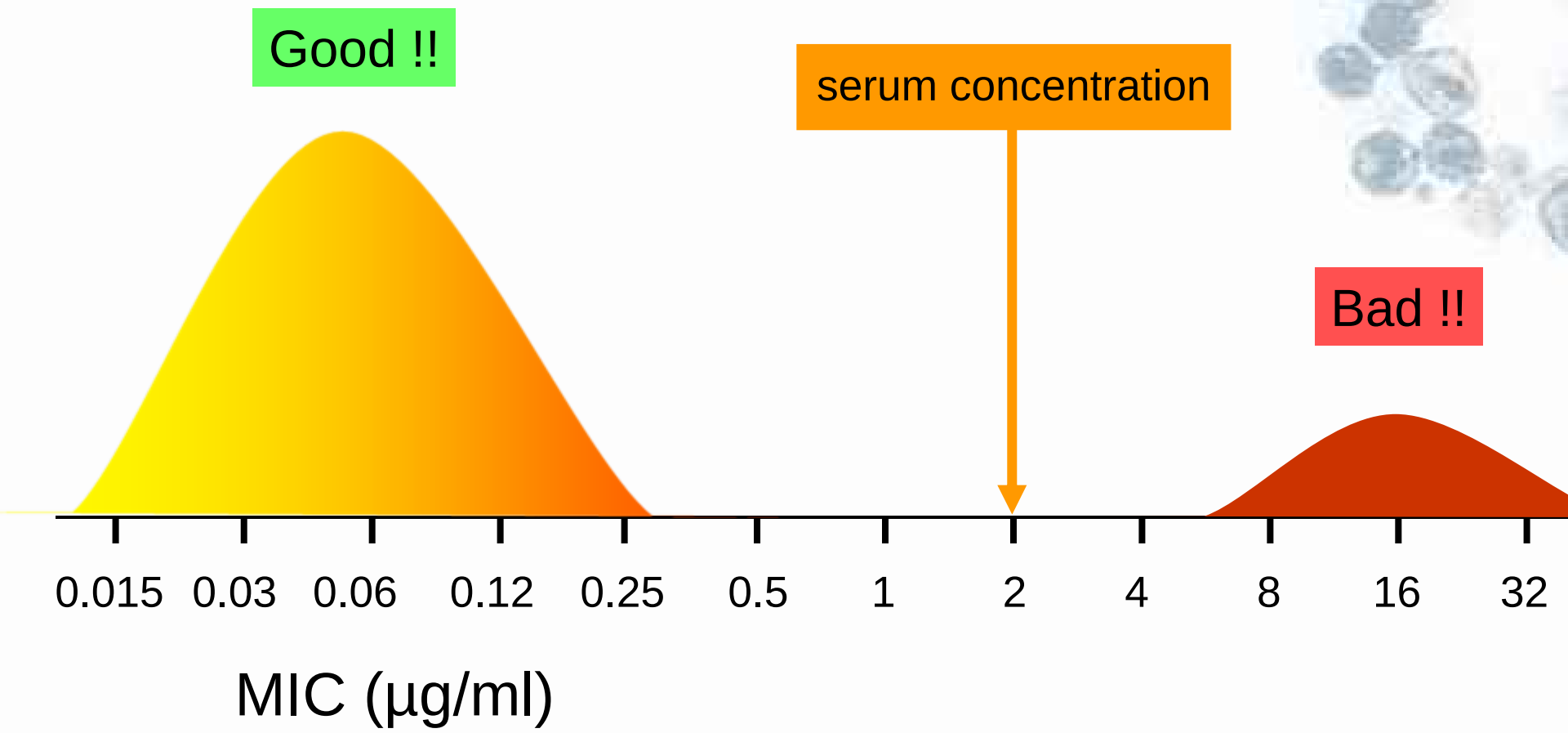


But, what is good ?

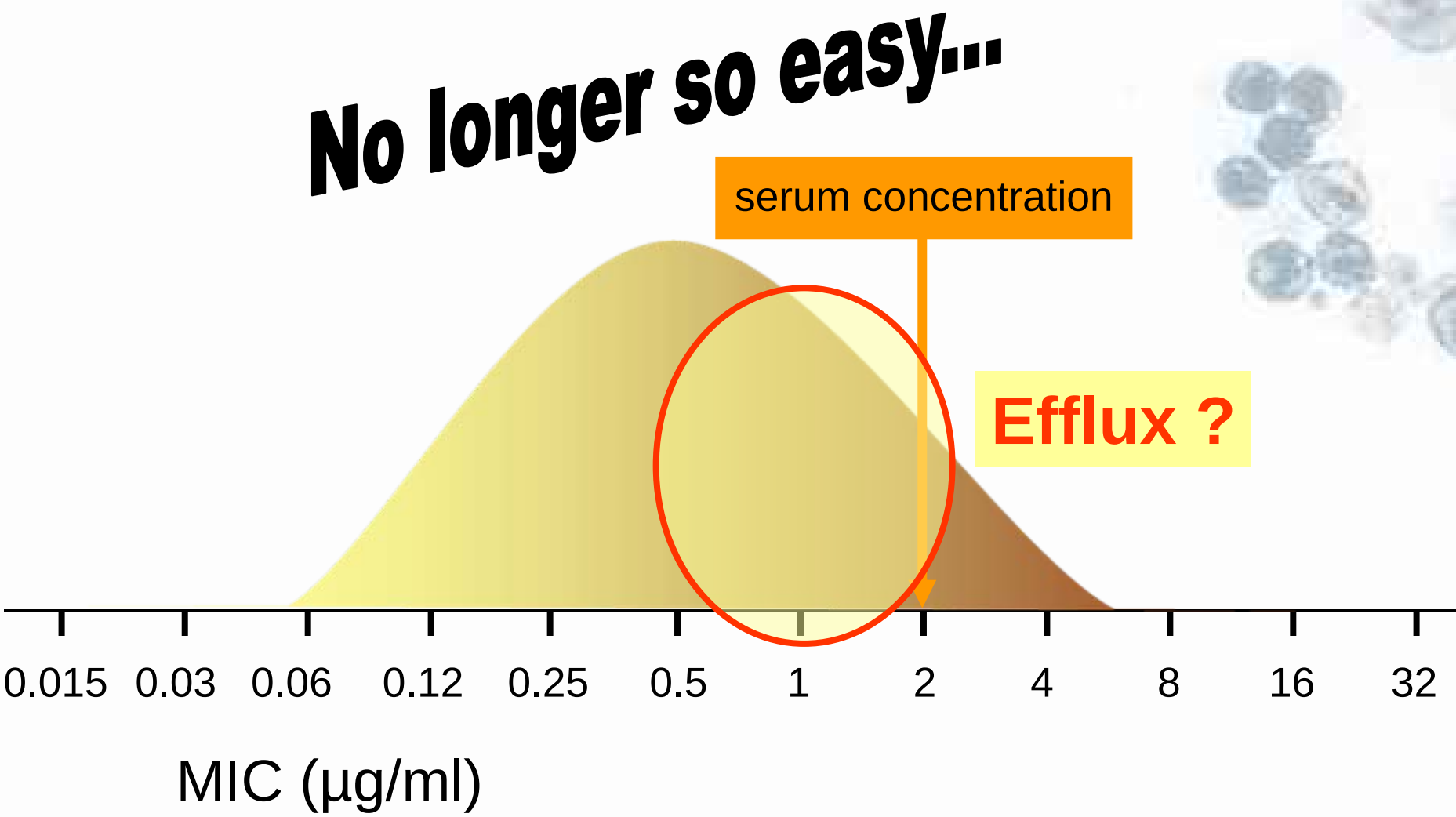


But, what is bad ?

Still Easy...

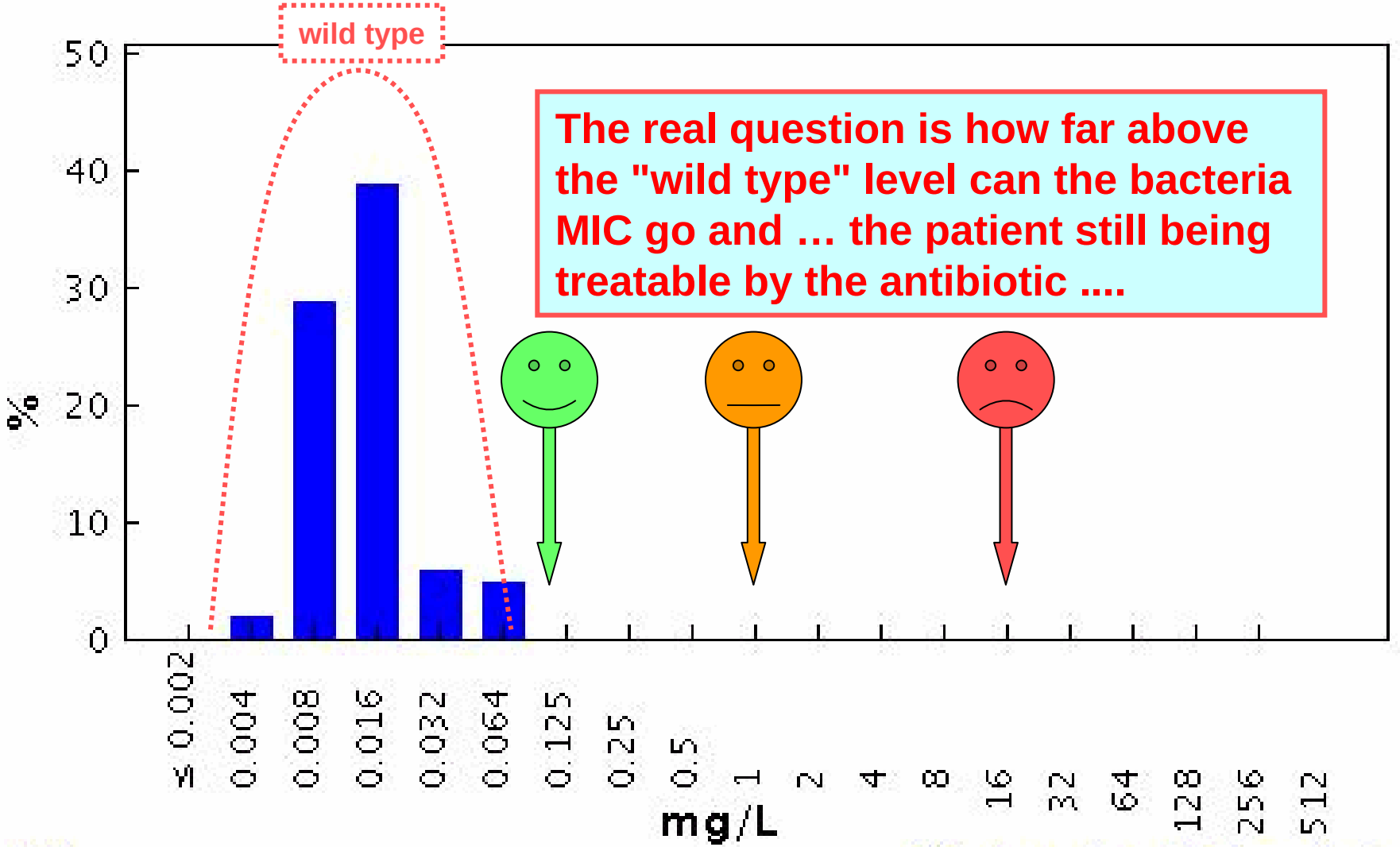


How…

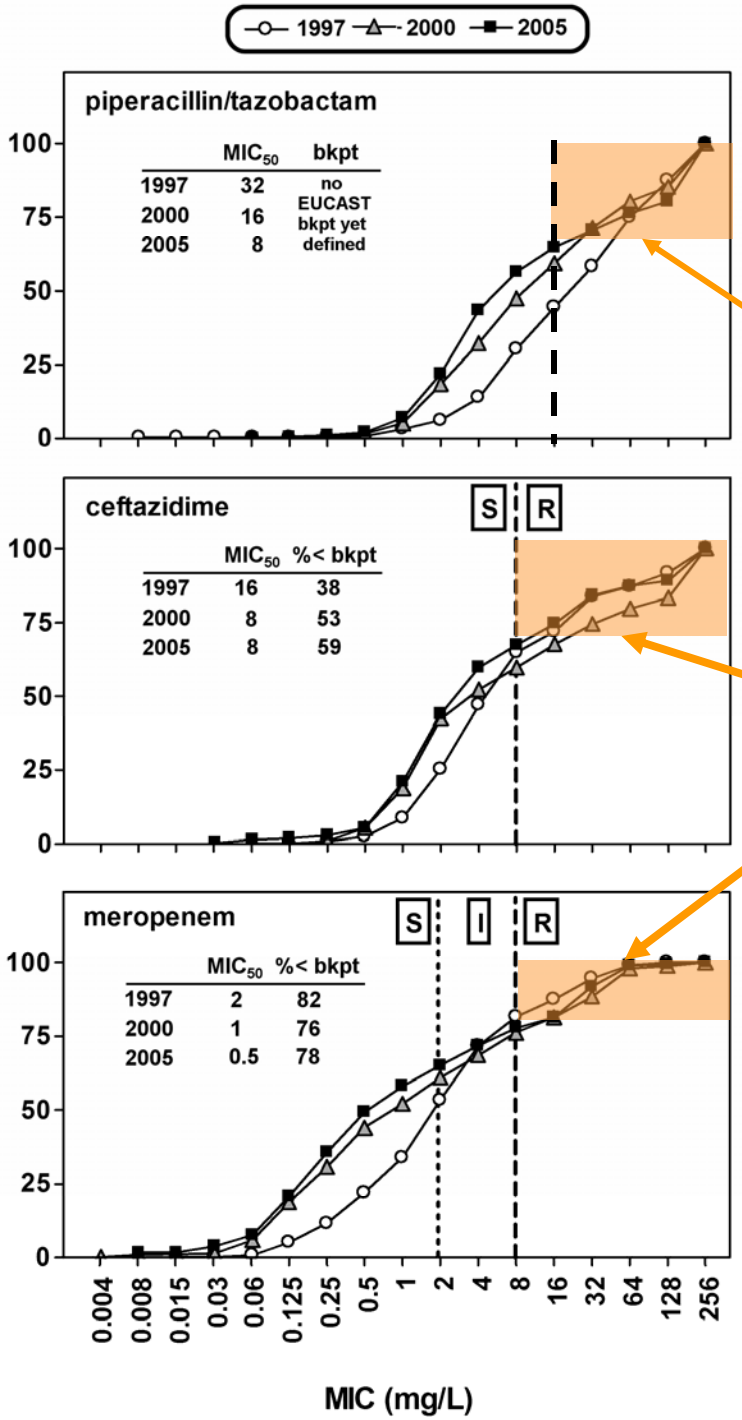

No longer so easy...
serum concentration
Efflux ?
0.015
0.03
0.06
0.12
0.25
0.5
1
2
4
8
16
32
MIC (µg/ml)

Ciprofloxacin / Escherichia coli

Antimicrobial wild type distributions of microorganisms - reference database EUCAST



Application for Pseudomonas…



high risk zone (above the <u>unofficial</u> R breakpoint)

high risk zone (above the 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)

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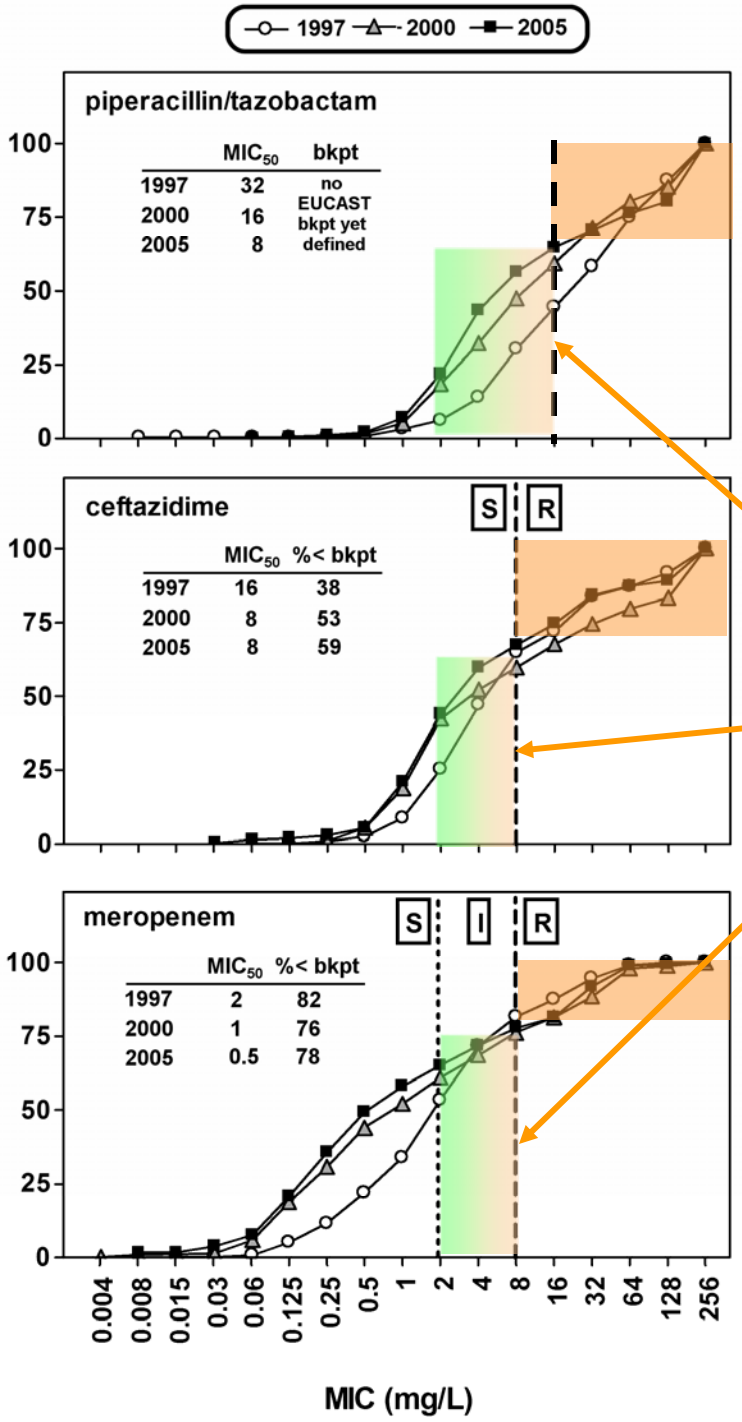

but this is also a risky zone…

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)

Diagnostic approaches …

Journal of Antimicrobial Chemotherapy (2007) **59**, 378–386
doi:10.1093/jac/dkl504
Advance Access publication 8 February 2007

JAC

A combined phenotypic and genotypic method for the detection of Mex efflux pumps in *Pseudomonas aeruginosa*

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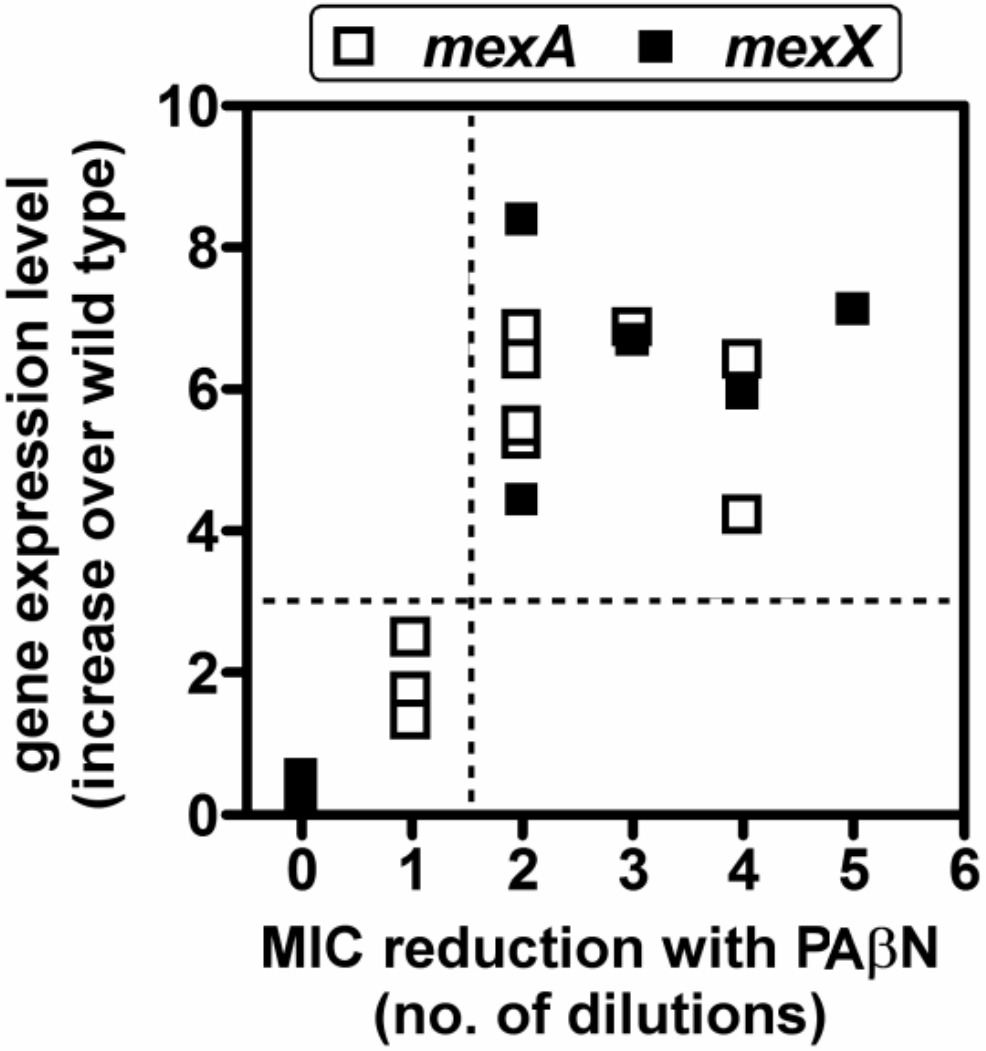
Diagnostic approaches …



Correlation between the level of expression (PCR) of constitutive Mex pumps and the effect of PAβN on the MIC of reporter antibiotics (carbenicillin for mexA and gentamicin for mexX).

Data are grouped in two quadrants of potentially different diagnostic significance

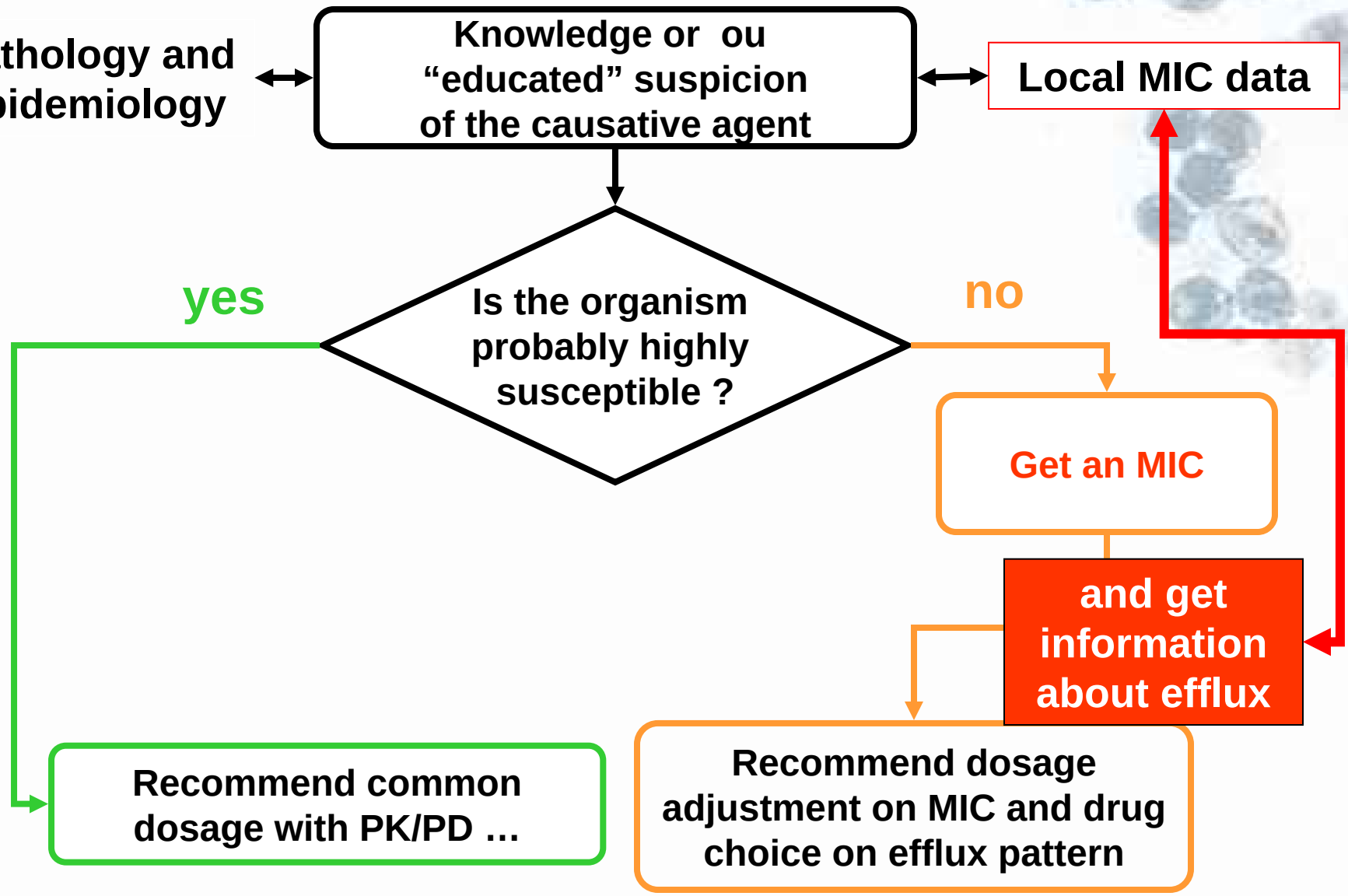
- **lower left, no or minimally meaningful efflux-mediated decrease of susceptibility**
- **upper right, efflux is likely to be the cause of the decreased susceptibility).**

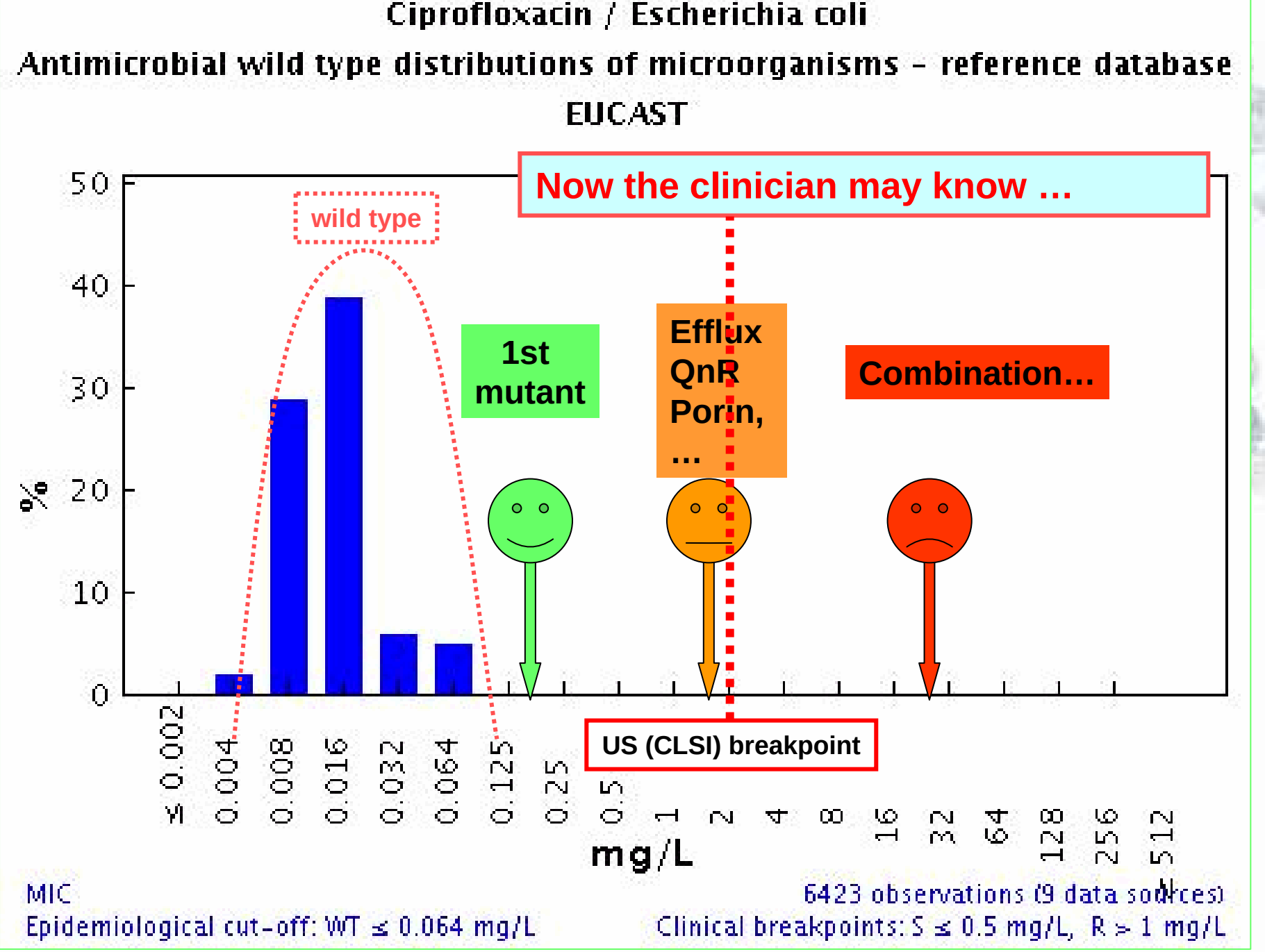
Mesaros et al., J Antimicrob Chemother. 2007; 59:378-86.

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 could be combined (E-test combined with mRNA detection)
  - Proteomic tests (using antibody-based detection techniques) could be added also.

A key to success ...




Ciprofloxacin / Escherichia coli
Antimicrobial wild type distributions of microorganisms – reference database EUCAST
Now the clinician may know …
wild type
1st mutant
Efflux
QnR
Porin,
…
Combination…
US (CLSI) breakpoint
%
50
40
30
20
10
0
≤ 0.002
0.004
0.008
0.016
0.032
0.064
0.125
0.25
0.5
1
2
4
8
16
32
64
128
256
512
mg/L
MIC
Epidemiological cut-off: WT ≤ 0.064 mg/L
6423 observations (9 data sources)
Clinical breakpoints: S ≤ 0.5 mg/L, R > 1 mg/L

Better breakpoints in Europe

Fluoroquinolones - EUCAST clinical MIC breakpoints 2006-06-20 (v 2.2)

Fluoroquinolone[2] Click on antibiotic name to see wild type MIC distributions		Species-related breakpoints (S≤/R>)											Non-species related breakpoints[1] S≤/R>
		Entero-bacteriaceae[3]	*Pseudo-monas/*	*Acineto-bacter*	*Staphylo-coccus*	*Entero-coccus*	*Strepto-coccus A,B,C,G*	*S.pneu-moniae*[5]	*H.influenzae M.catarrhalis*	*N.gonorr-hoeae*	*N.menin-gitidis*[8]	*Gram-negative anaerobes*	
Ciprofloxacin	RD	0.5/1	0.5/1	1/1[4]	1/1[5]	--	--	0.125/2	0.5/0.5[7]	0.03/0.06	0.03/0.06	--	0.5/1
Levofloxacin	RD	1/2	1/2	1/2	1/2	--	1/2	2/2	1/1[7]	IE	IE	--	1/2
Moxifloxacin	RD	0.5/1	--	--	0.5/1	--	0.5/1	0.5/0.5	0.5/0.5[7]	IE	IE	IE	0.5/1
Norfloxacin	RD	0.5/1	--	--	--	--	--	--	--	IE	--	--	0.5/1
Ofloxacin	RD	0.5/1	--	--	1/1[3]	--	--	0.125/4	0.5/0.5[7]	0.12/0.25	IE	--	0.5/1

1. Non-species related breakpoints have been determined mainly on the basis of PK/PD data and are independent of MIC distributions of specific species. They are for use only for species that have not been given a species-specific breakpoint and not for those species where susceptibility testing is not recommended (marked with -- or **IE** in the table).
2. For breakpoints for other fluoroquinolones (eg. **pefloxacin** and **enoxacin**) - refer to breakpoints determined by national breakpoint committees.
3. *Salmonella* spp - there is clinical evidence for ciprofloxacin to indicate a poor response in systemic infections caused by *Salmonella* spp with low-level fluoroquinolone resistance (MIC>0.064 mg/L). The available data relate mainly to *S.typhi* but there are also case reports of poor response with other *Salmonella* species.
4. The S/I breakpoint has been increased from 0.5 to1 mg/L to avoid dividing the wild type MIC distribution. Thus there is no intermediate category for *Acinetobacter* species
5. *Staphylococcus* spp - breakpoints for ciprofloxacin and ofloxacin relate to high dose therapy.
6. *Streptococcus pneumoniae* - wild type *S.pneumoniae* are not considered susceptible to ciprofloxacin or ofloxacin and are therefore categorized as intermediate. For ofloxacin the I/R breakpoint was increased from 1.0 to 4.0 mg/L and for levofloxacin the S/I-breakpoint from 1.0 to 2.0 to avoid dividing the wild type MIC distribution. The breakpoints for levofloxacin relate to high dose therapy.
7. Strains with MIC values above the S/I breakpoint are very rare or not yet reported. The identification and antimicrobial susceptibility tests on any such isolate must be repeated and if the result is confirmed the isolate sent to a reference laboratory. Until there is evidence regarding clinical response for confirmed isolates with MIC above the current resistant breakpoint (in italics) they should be reported resistant. *Haemophilus/Moraxella* - fluoroquinolone low-level resistance (ciprofloxacin MIC:s of 0.125 - 0.5 mg/L) may occur in *H.influenzae*. There is no evidence that low-level resistance is of clinical importance in respiratory tract infections with *H.influenzae*.
8. *Neisseria meningitidis* - breakpoints apply to the use of ciprofloxacin in the prophylaxis of meningococcal disease.

-- = Susceptibility testing not recommended as the species is a poor target for therapy with the drug.
IE = There is insufficient evidence that the species in question is a good target for therapy with the drug.
RD =Rationale document listing data used for setting EUCAST breakpoints.

http://www.eucast.org

But we may do better: defining “safety” breakpoints

Drug	Typical daily dosage[a]	Typical PK values		Proposed PK/PD upper limit of sensitivity (μg/ml) for	
		C_{max} in mg/L total/free (dose)	$AUC_{24\ h}$ (mg × h/L) total/free	Efficacy[b]	Prevention of resistance[c]
Norfloxacin	800 mg	1.4/1.1 (400 mg PO)	14/11	0.1–0.4	0.1
Ciprofloxacin	1000 mg	2.5/1.75 (500 mg PO)	24/18	0.2–0.8	0.2
Ofloxacin	400 mg	4/3 (400 mg PO)	40/30	0.3–0.9	0.4
Levofloxacin	500 mg	4/2.8 (500 mg PO)	40/28	0.3–0.9	0.3
Moxifloxacin	400 mg	3.1/1.8 (400 mg PO)	35/21	0.2–0.7	0.2

Van Bambeke F, Michot JM, Van Eldere J, Tulkens PM.
Quinolones in 2005: an update. Clin Microbiol Infect. 2005 Apr;11(4):256-80. PMID: 15760423

Challenges…

Will diagnostic solve everything ?

- Level of efflux expression *in vivo* ?
- How can dosage adjustment help ?
- Combination of antibiotics ?
- Tissue accumulation of antibiotics ?
- Influence of other drugs that may share the same transporter(s)

Challenges of efflux in antibacterial chemotherapy

- **recognizing its existence:** is it a major and general means of resistance ?
- **its role:** does it need to change our vision on (and decisions about) existing antibiotics ?
- **the way we find it:** how can we detect it (and do we need to do this ?)
- **the way we treat patients:**
  - can we make non-effluxed drugs ?
  - and what about efflux inhibitors ?
  - is efflux important in pharmacokinetics/drug interactions ?

Challenges of efflux in antibacterial chemotherapy

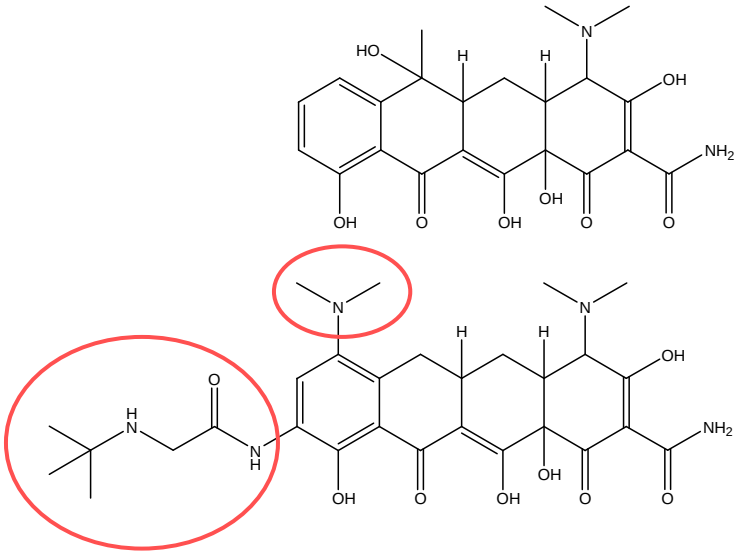
- **the way we treat patients:**
  - can we make non-effluxed drugs ?

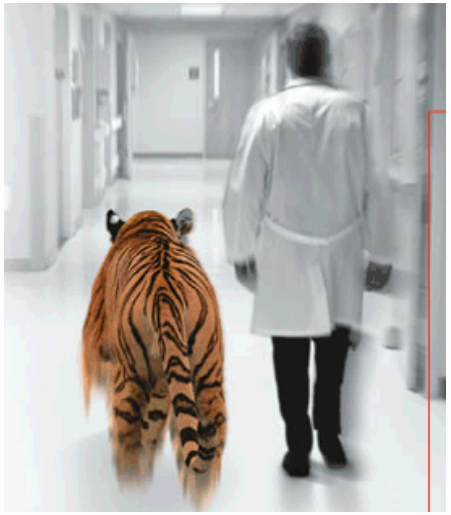
Thesis:

Yes you can if you specifically aim at OR if you exploit what good nature (or good chemists) give you, but be prepared for partial successes …

Tigecycline…

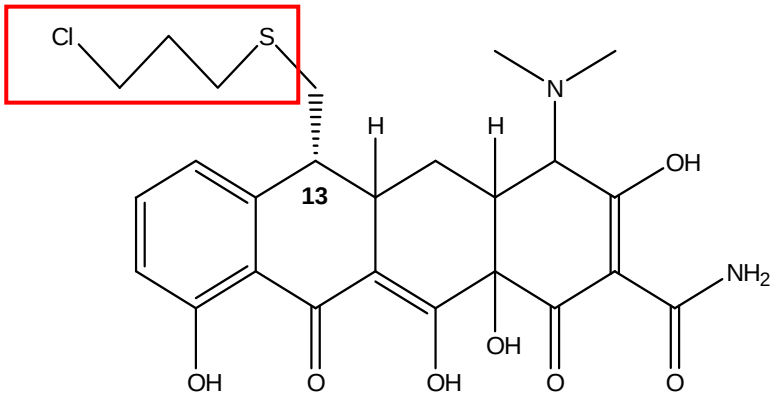
- truly <u>made</u> to resist efflux-mediated resistance in Gram(-) bacteria
- broad spectrum including MRSA (MIC < 2 mg/L) and VISA
- tet(M) [ribosomal protection] or tet(K) [efflux] have no discernible effect on MICs (AAC 2006 Feb;50(2):505-10).
- approved by the FDA in 2005 and by the EMEA in 2006 for use in patients with complicated skin infections, skin-structure infections and intra-abdominal infections



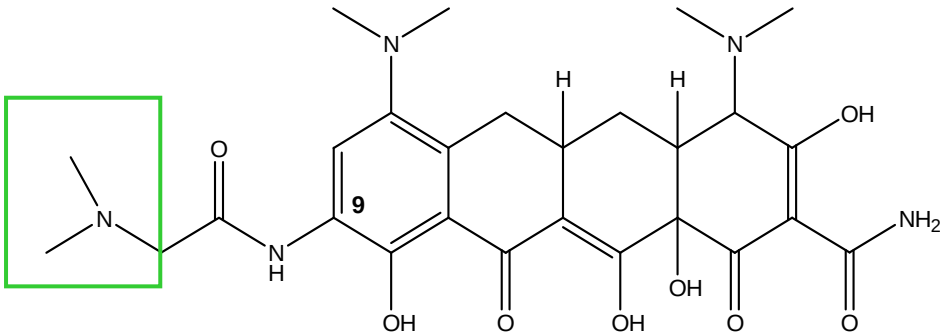


Origin of tigecyclin

Compound **2**
(13-((3-chloropropyl)thio)-5-hydroxy-6-deoxytetracycline),
a strong inhibitor of efflux but with reduced antibacterial activity



Compound **4**
(9-N,N-dimethylglycylamido-minocyclin),
a minocycline with enhanced activity



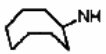
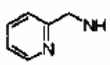
Two critical steps towards the discovery to efflux-resistant antibiotics maintaining antibacterial efficacy

- Synthesis of the **hydrophobic** compound (**2**) from **doxycycline** by Levy and co-workers at the Center for Adaptation Genetics and Drug Resistance (Tufts University, School of Medicine, Boston, Mass.) revealed an an inbitory effect on tetracycline efflux (Nelson et al., 1994; J Med Chem 37: 1355-1361)
- Synthesis of compound (**4**) from **minocycline** (**additional aminogroup** slightly away from position 9) by Sum and colleagues at Chemical Sciences Laboratories (Wyeth-Ayerst Research, Pearl River, New York) showed that the activity could be improved (Sum et al. 1994; J Med Chem 37: 184-188).

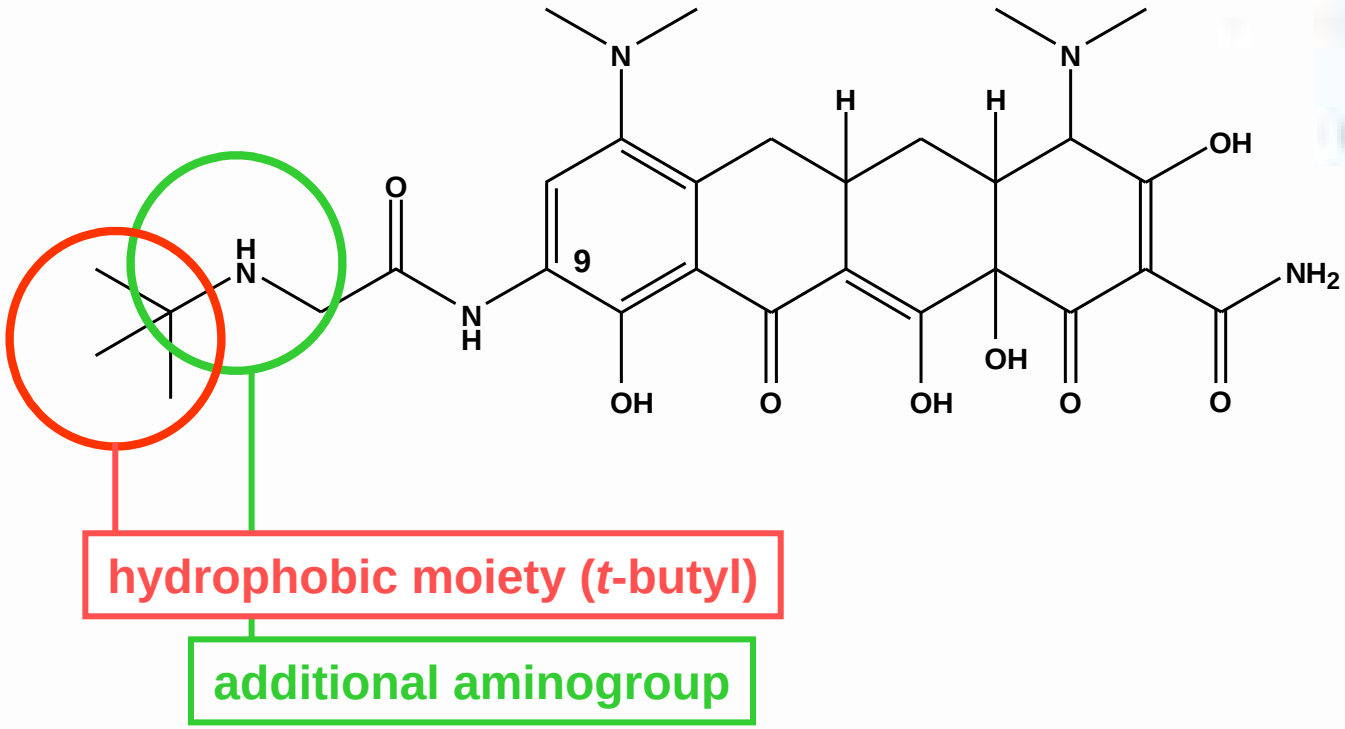
The final selection of tigecycline was the result of a systematic research to combine the hydrophobic moiety AND the additional aminogroup in a substituent attached to position 9

tigecyline

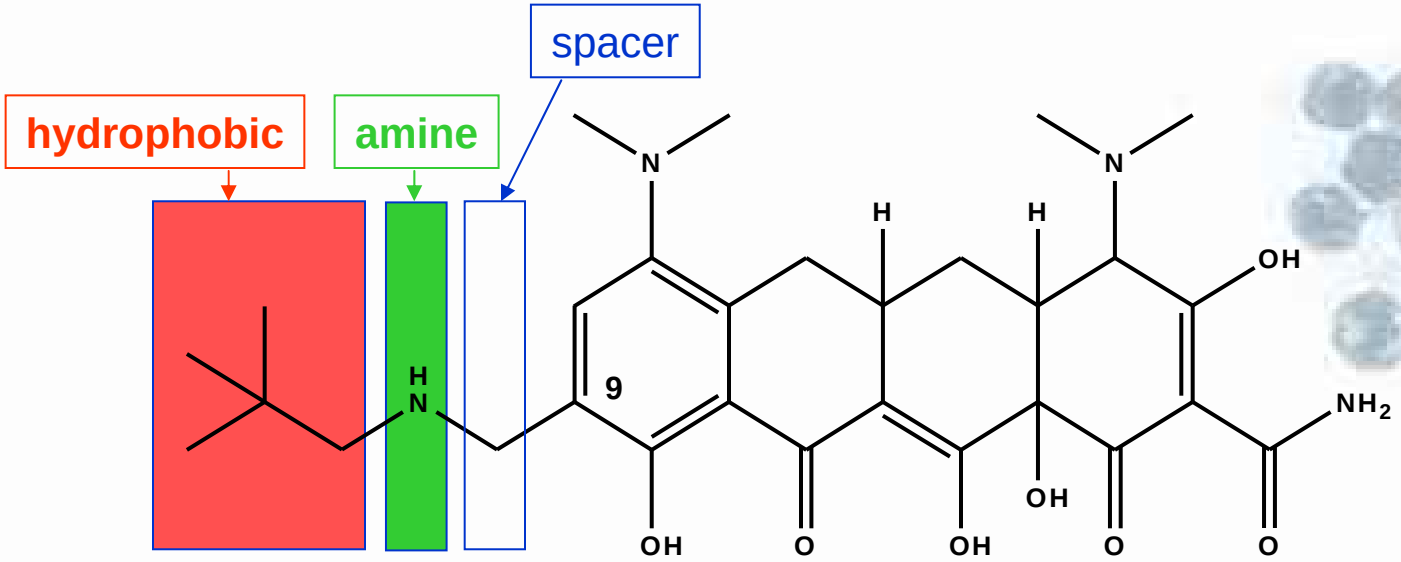
Table 2. In Vitro Antibacterial Activity of Compounds **13–25**.

		Organism; minimum inhibitory concentration (MIC) μg/mg										
	R_1	*E. coli* UBMS 88-1 Tet B	*E. coli* PRP1 Tet A	*E. coli* J3272 Tet C	*E. coli* J3272 Tet D	*E. coli* UBMS 90-4 Tet M	*E. coli* UBMS 90-5 sensitive	*S. aureus* UBMS 88-7 Tet K	*S. aureus* UBMS 90-1 Tet M	*S. aureus* UBMS 90-3 sensitive	*S. aureus* Smith sensitive	Entero-coccus ATCC 29212
13	MeNH	1	16	8	0.5	NT	1	16	1	0.5	0.5	0.5
14	*n*-PrNH	0.5	2	0.5	0.12	0.25	0.5	2	0.5	0.25	0.25	0.25
15	*n*-BuNH	0.5	1	0.5	0.25	0.25	0.5	2	0.5	0.25	0.12	0.12
16	*t*-BuNH	0.5	0.25	0.25	0.12	0.12	0.25	0.5	0.12	0.25	0.25	0.12
17	*n*-HexylNH	0.5	0.5	0.5	0.12	0.25	0.25	2	0.25	0.06	0.12	0.12
18	UndecylNH	32	32	32	32	32	16	2	16	0.5	0.5	2
19		4	32	8	2	2	2	4	0.5	0.5	0.25	0.25
20		0.25	1	0.25	0.12	0.25	0.25	2	0.25	0.12	0.25	0.12
21		4	2	2	0.5	2	4	0.5	1	0.25	0.5	0.25
22		0.5	1	0.5	0.25	0.5	0.5	0.5	0.5	0.25	0.25	0.12
23		0.5	4	0.5	0.25	0.5	0.5	4	1	0.5	0.5	0.25
24	$PhCH_2NH$	2	4	2	0.5	0.5	0.5	2	0.5	0.25	0.25	0.25
25		16	32	16	8	8	16	32	8	4	4	2

Tigecycline is significantly modified compared to minocycline



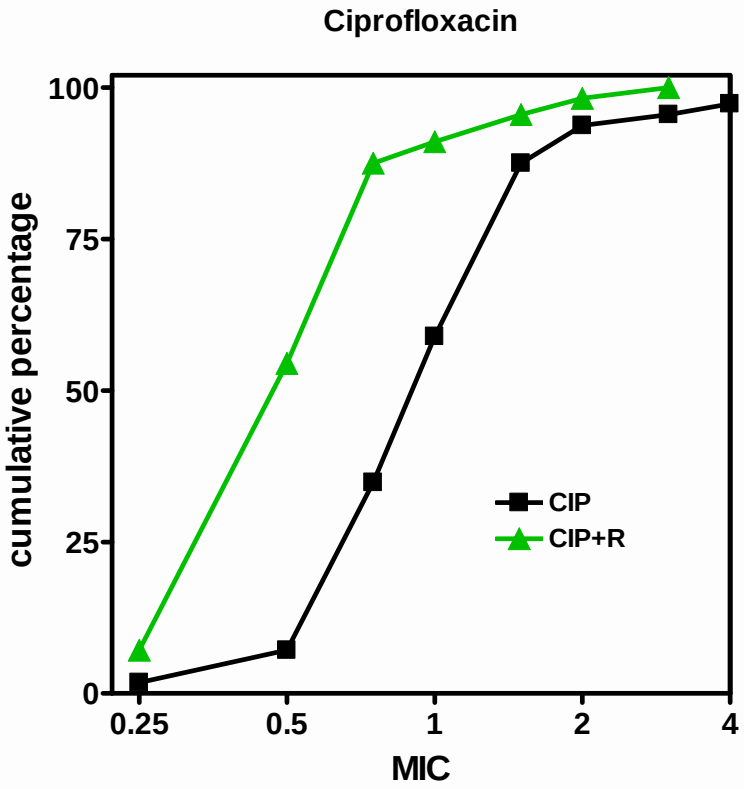
Here is the "next in the pipeline" of the tetracylines "insensitive to efflux-mediated resistance"

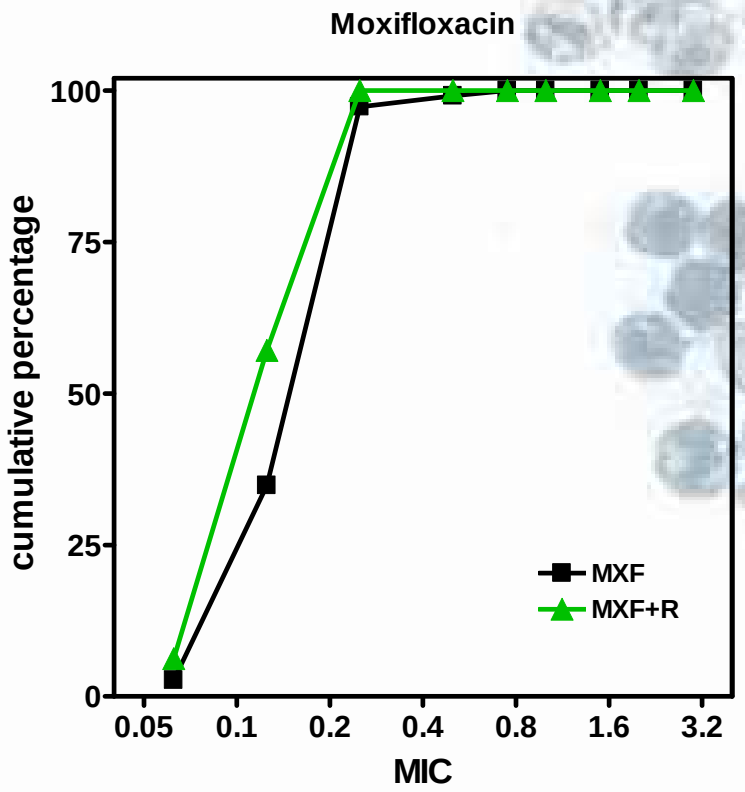


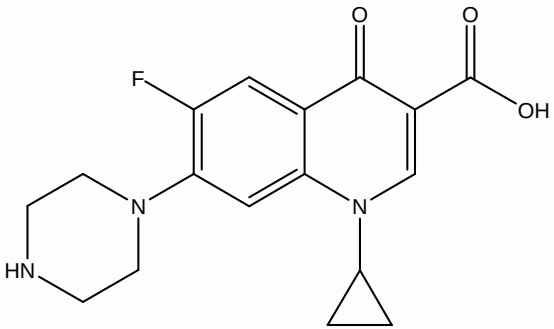
9-[[(2,2-**dimethylpropyl**)**amino**]methyl]-minocycline

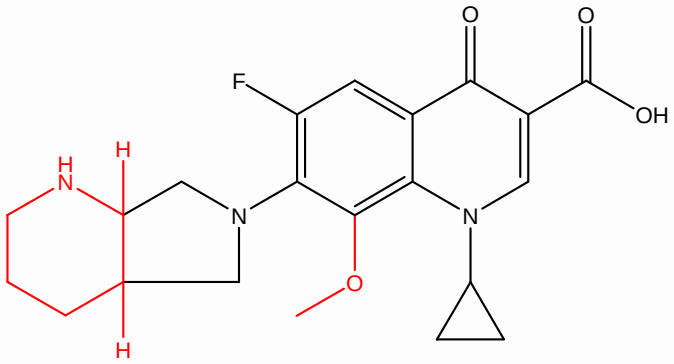
MK-2764/PTK 0796 - the most advanced compound from a new class called the **aminomethylcyclines** (AMC) were derived from the tetracyclines. … an oral and IV once-daily antibiotic antibiotic agent with activity against resistant and susceptible gram-positive, gram-negative, atypical and anaerobic bacteria… target community infections of the skin and respiratory tract as well as complicated skin, pneumonias and other infections requiring hospitalization.
(from: http://www.paratekpharm.com/pt_tet_inhib.html)

Moxifloxacin vs. ciprofloxacin for *S. pneumoniae*…









A. Lismond et al., unpublished

Challenges of efflux in antibacterial chemotherapy

- **the way we treat patients:**
  - and what about efflux inhibitors ?

Thesis:

This will be very difficult because of

- complete changes in the basic rules of drug-design (no link to specific chemical structure) and (porbable) multiplicity of ligand-recognition sites in transporters;
- potential for unanticipated toxicities (related to unknown functions of eucaryotic homologues of the target efflux transporter(s)

Challenges of efflux in antibacterial chemotherapy

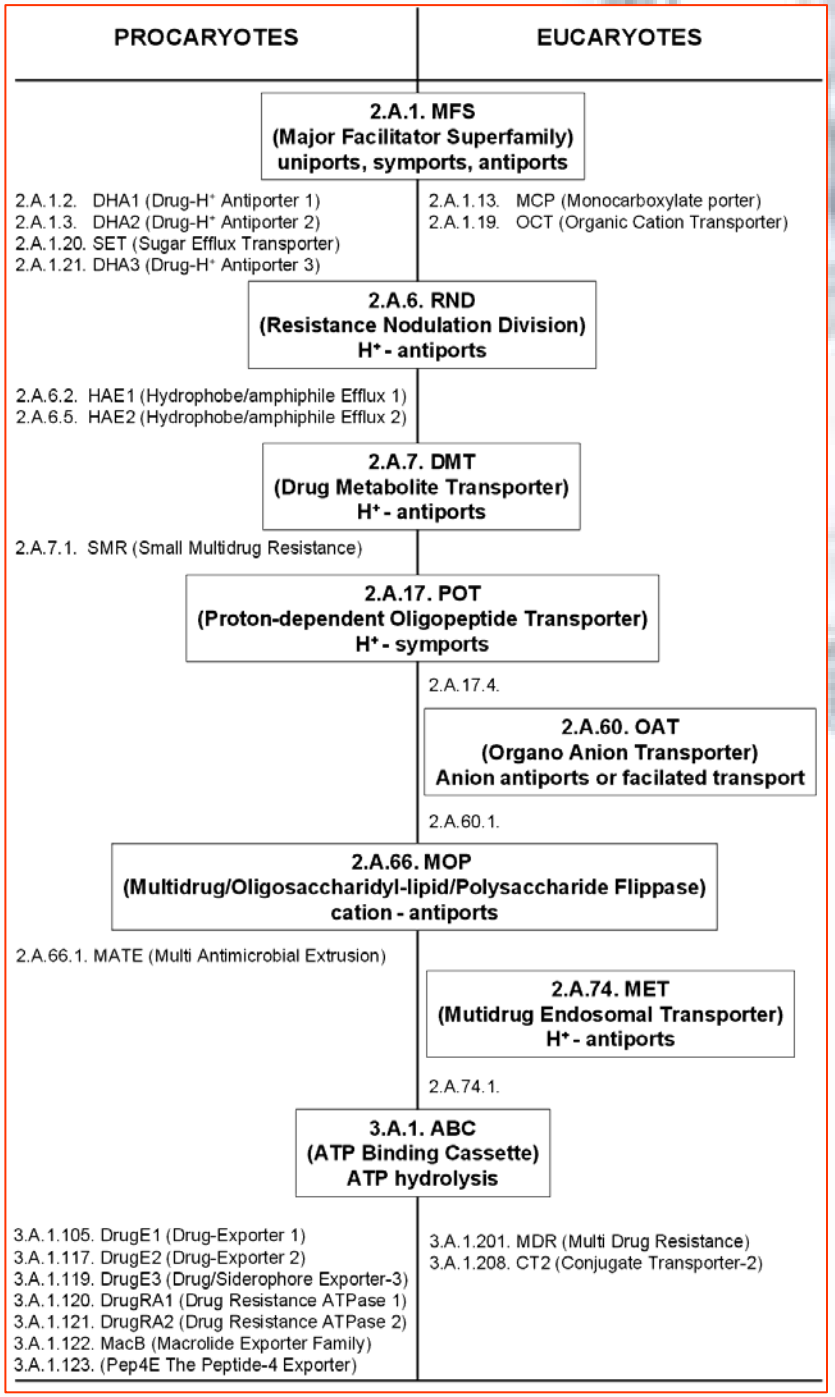
- **the way we treat patients:**
  - is efflux important in pharmacokinetics/drug interactions ?

Thesis:

Many procaryotic transporters have eucaryotic homologues, and antibiotics are often (for that reason or another …) substrate to them… (sometimes in an unanticipated fashion)

Efflux is widespread because it is a common mechanism of protection of both procaryotic and eucaryotic cells against membrane-permeant toxins…

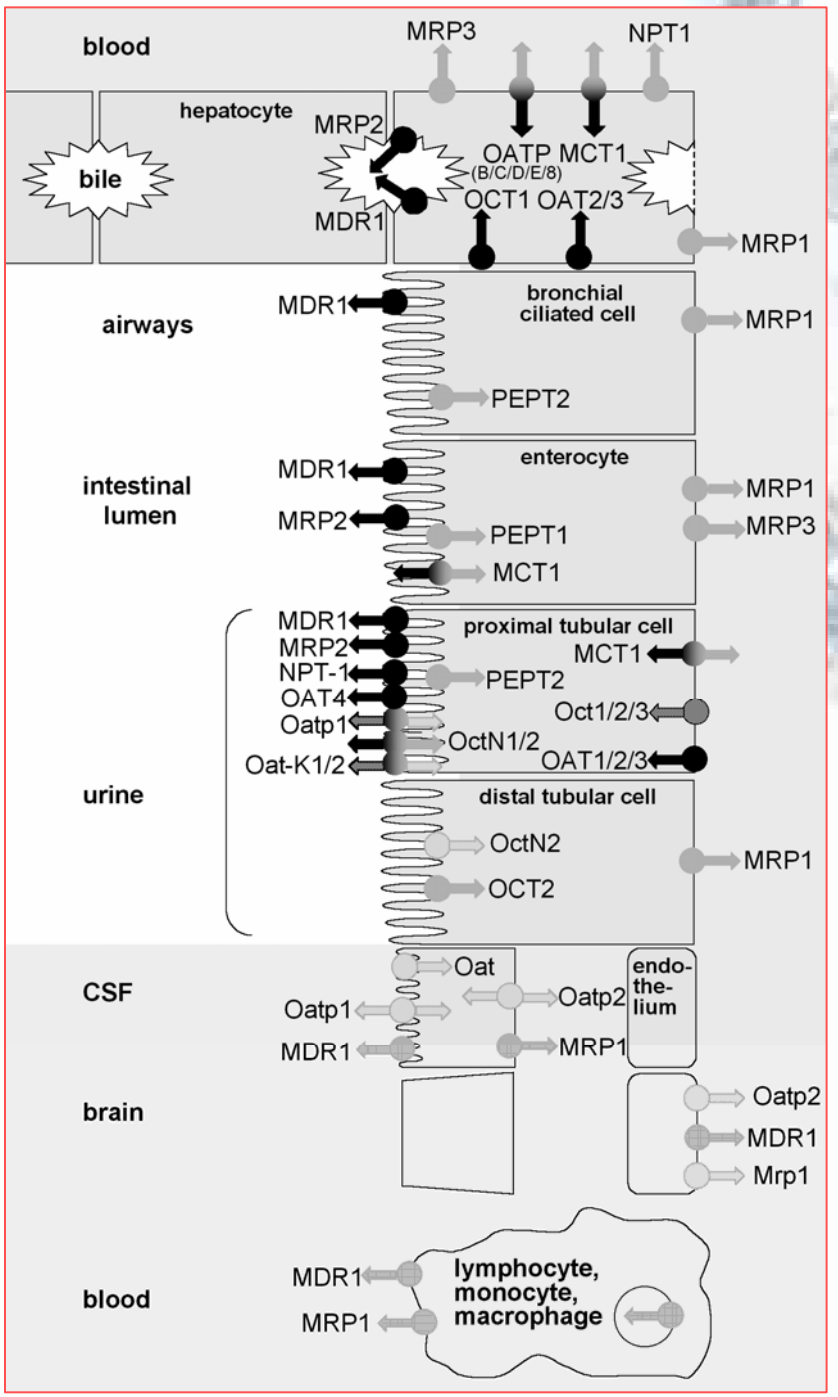
and antibiotics are simply opportunistic substrates (in both types of cells !) …



Van Bambeke et al. (2003)
J Antimicrob Chemother 51: 1055-1065.

Challenges in Chemotherapy:

a new approach (and new problems) in pharmacokinetics (and the potential modulation of antibiotic intracellular activity)



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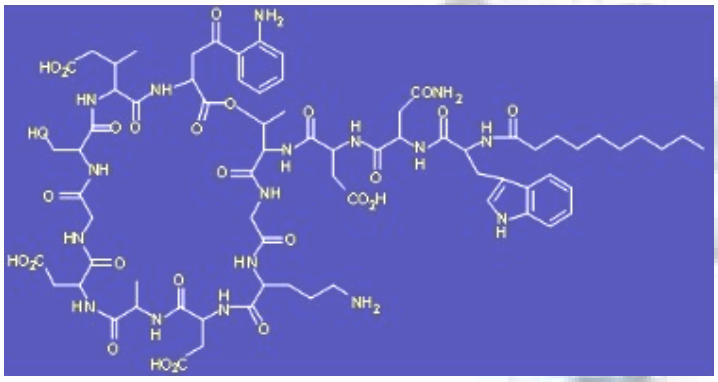
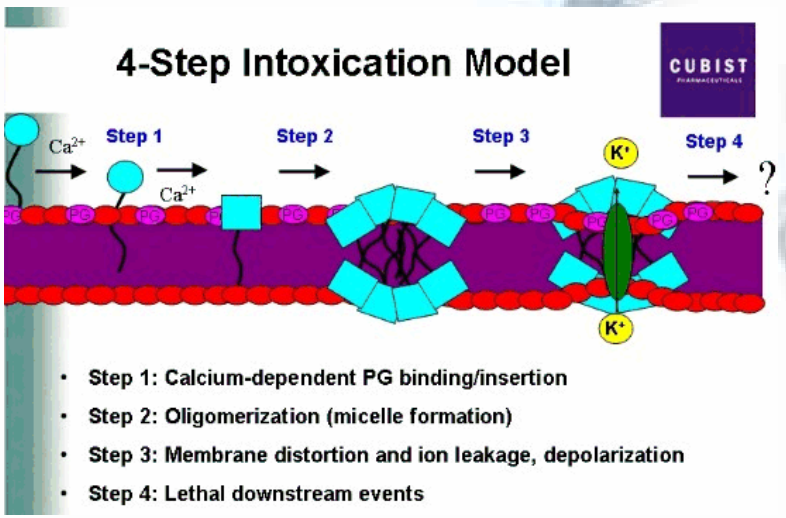
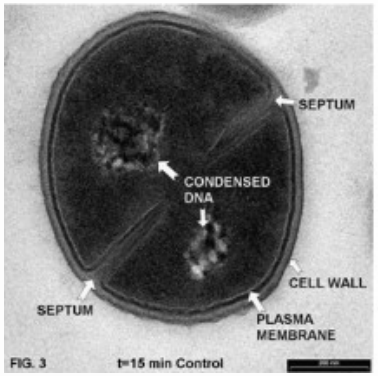
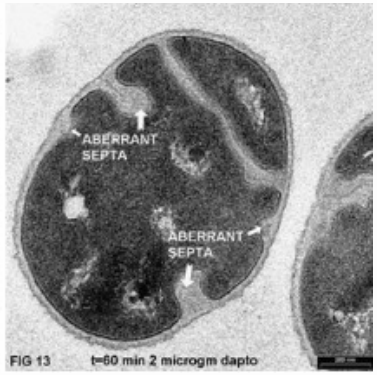
Modulation of cellular pharmacokinetics and intracellular activity: the case of daptomycin

- very bactericidal towards Gram (+) organisms through membrane destabilization (no need of proteinaceous receptor!)
- BUT intrinsically inactive against Gram(-) due to LPS protection
- spare mammalian cells because they lack phosphatidylglycerol (critical for binding to Gram(+) membranes
- got a fast track registration in the US because of activity against vancomycin-resistant enterococci (VRE)



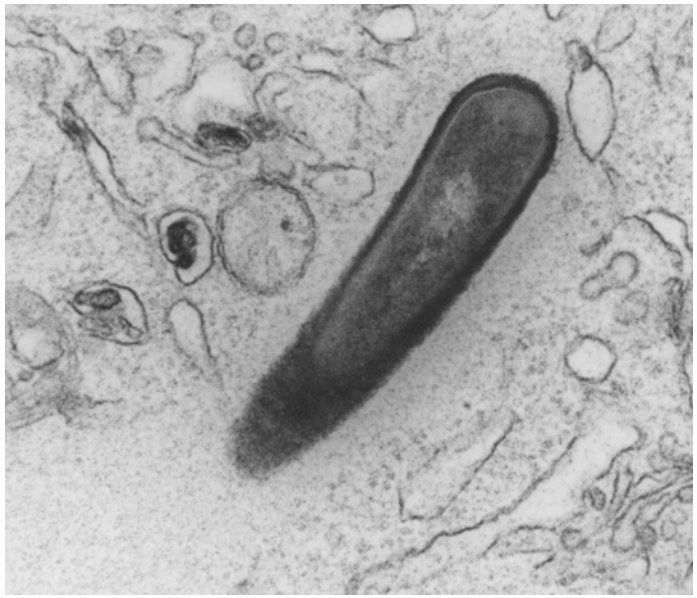






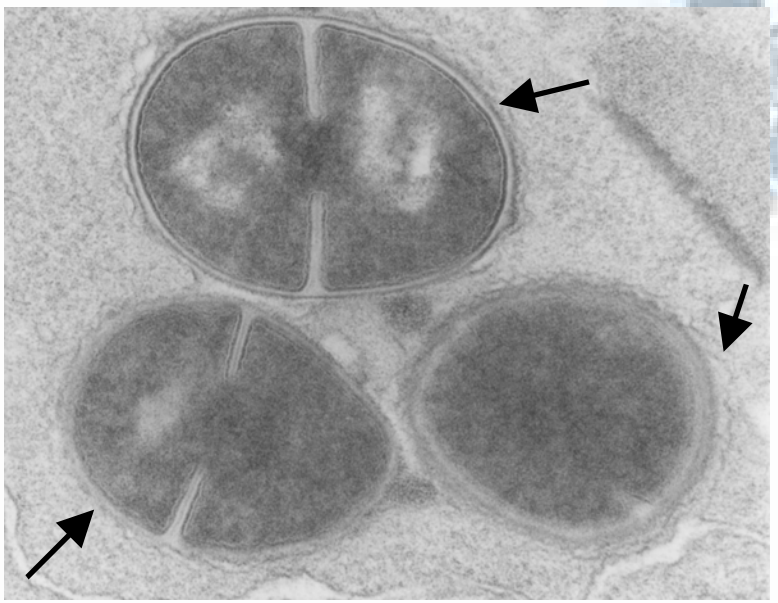
Models of intracellular infection

L. monocytogenes



cytosol

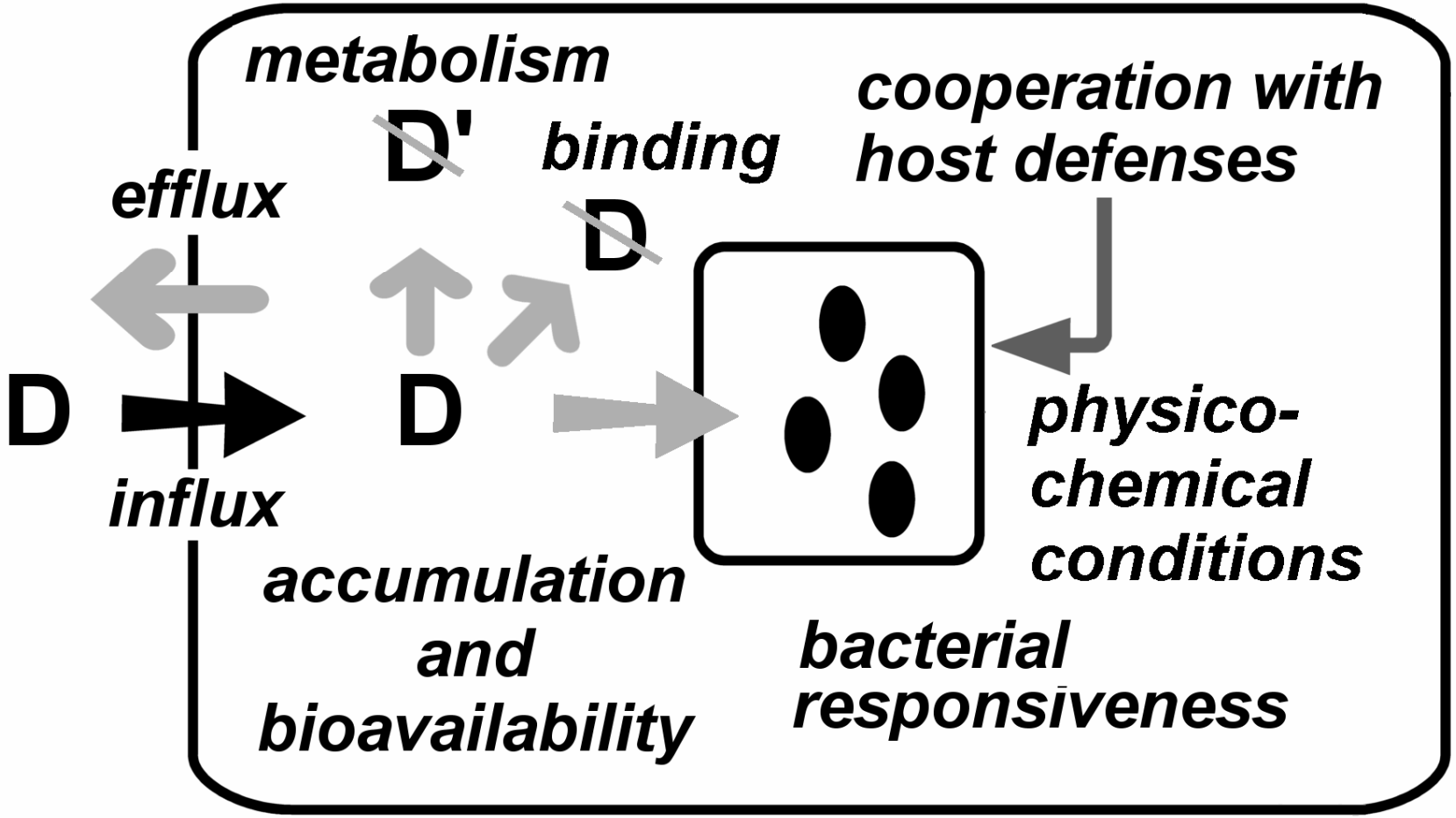
S. aureus



phagolysosomes

Intraphagoytic *S. aureus*

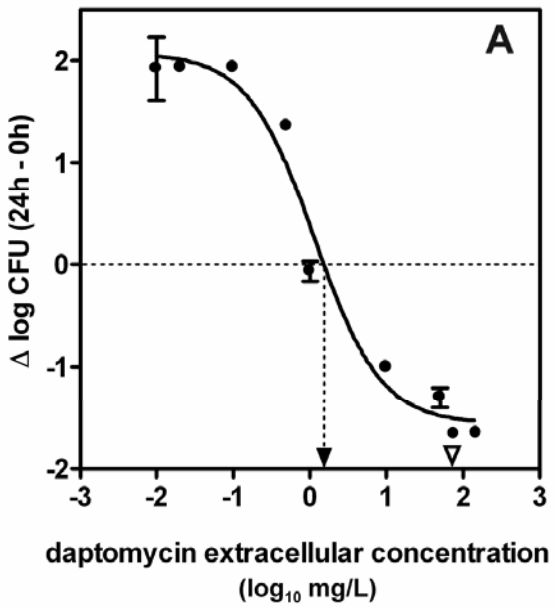
A simple scheme …



Carryn *et al.* Infect Dis Clin North Am. 2003 Sep;17(3):615-34.

Modulation of cellular pharmacokinetics and intracellular activity: the case of daptomycin

Daptomycin activity against <u>intracellular</u> *S. aureus* (THP-1 macrophages)



Lemaire et al., Antimicrob Agents Chemother. 2007 Jun 4; [Epub ahead of print]

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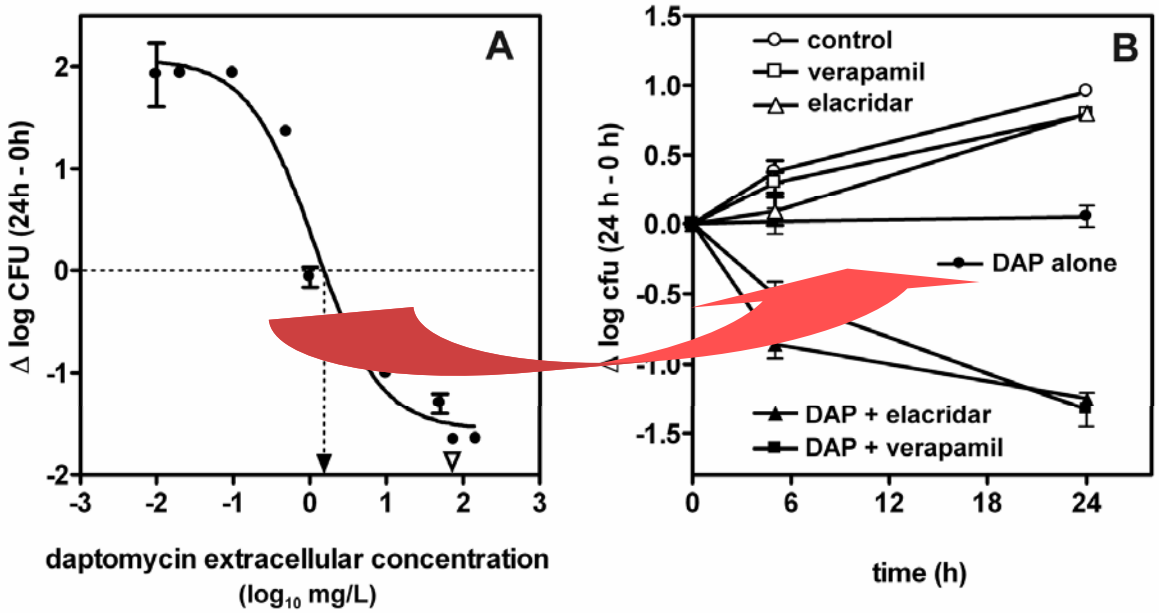

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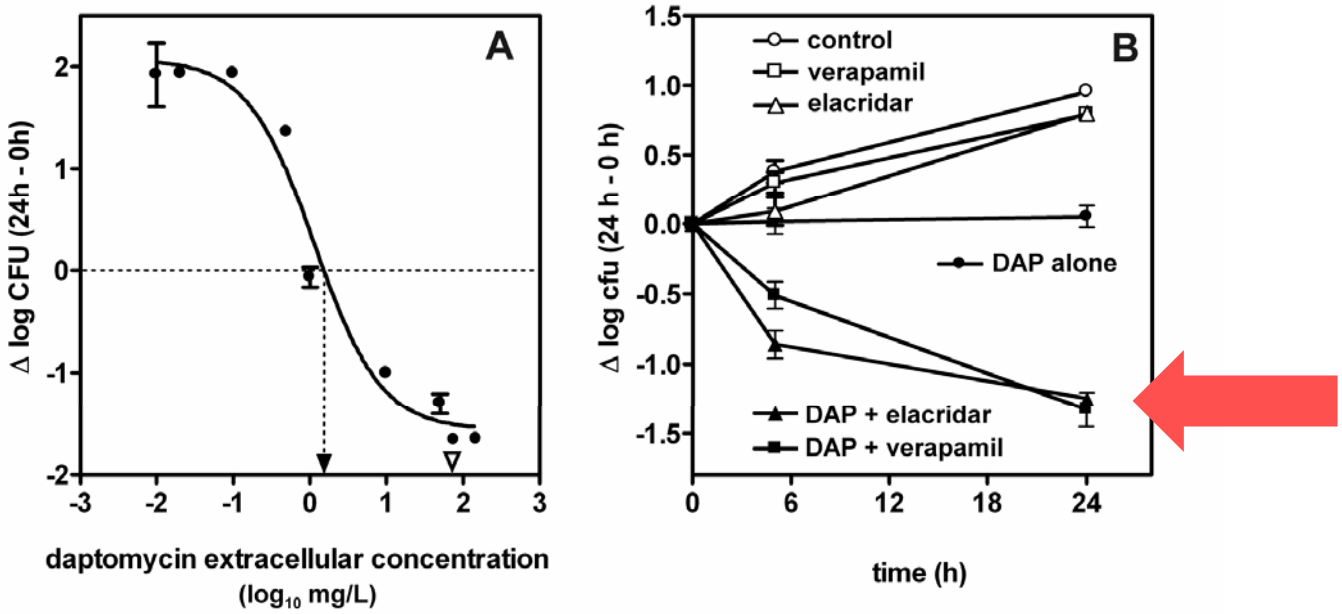

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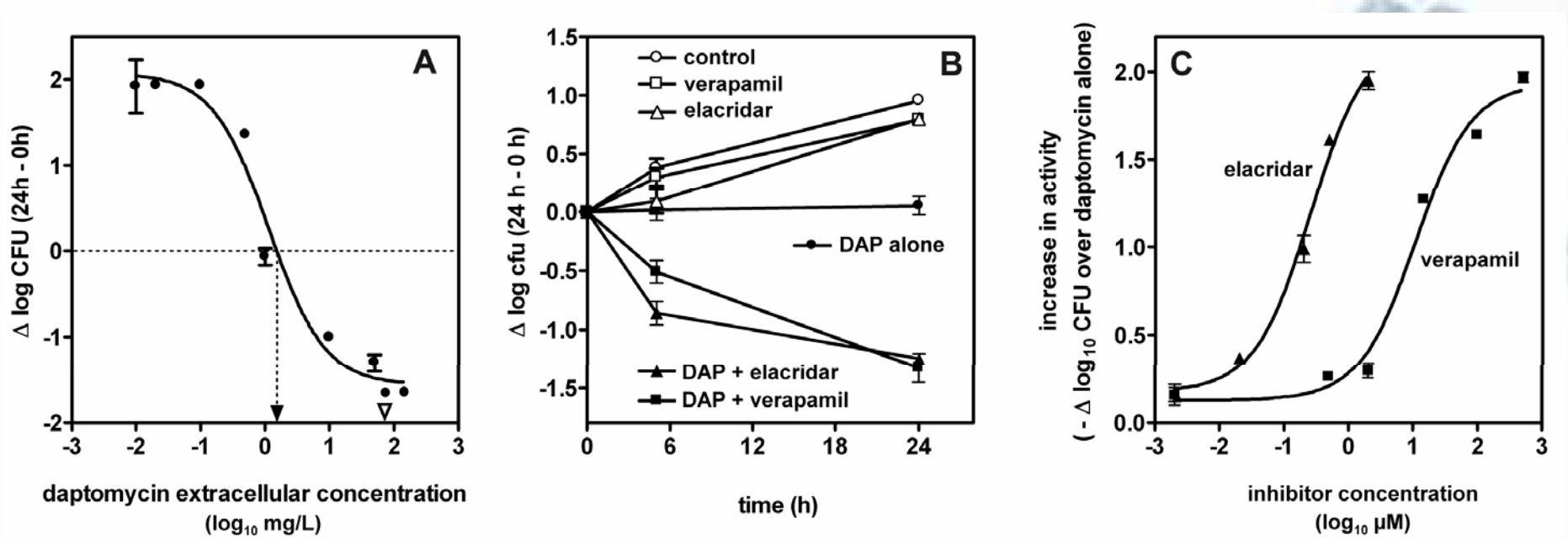
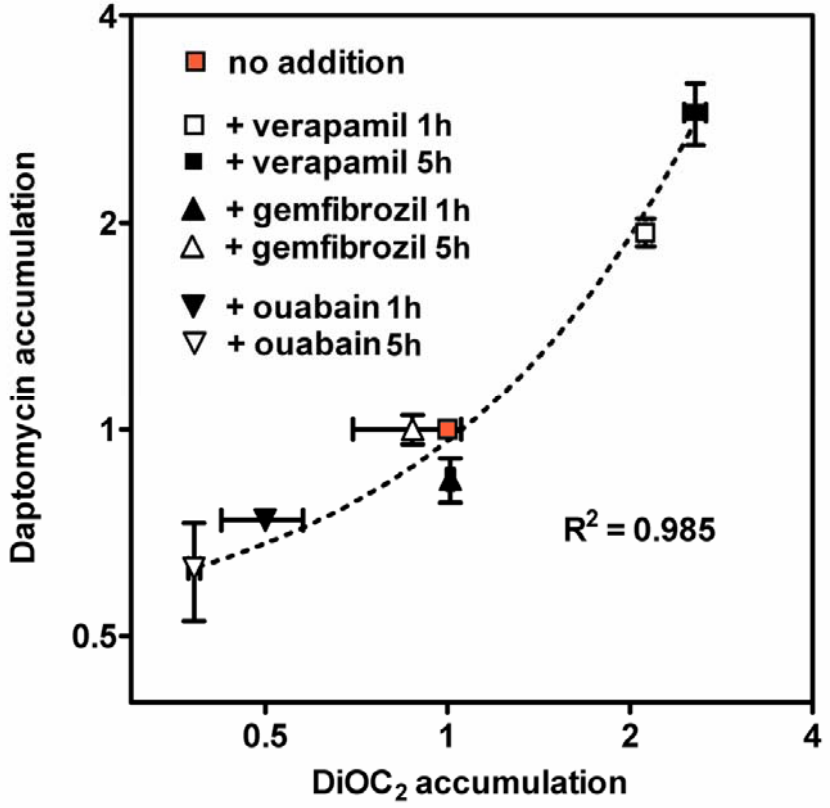
Daptomycin activity against intracellular S. aureus (THP-1 macrophages)



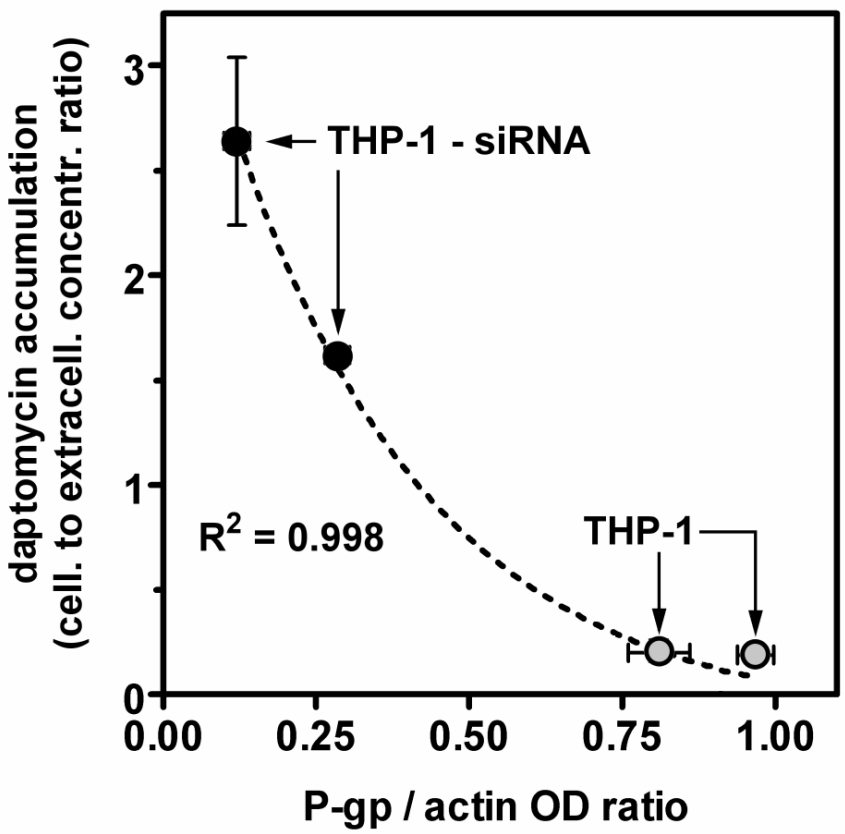
Lemaire et al., Antimicrob Agents Chemother. 2007 Jun 4; [Epub ahead of print]

Modulation of cellular pharmacokinetics and intracellular activity: the case of daptomycin

P-gp inhibitors and daptomycin accumulation



mdr-1 silencing and daptomycin accumulation



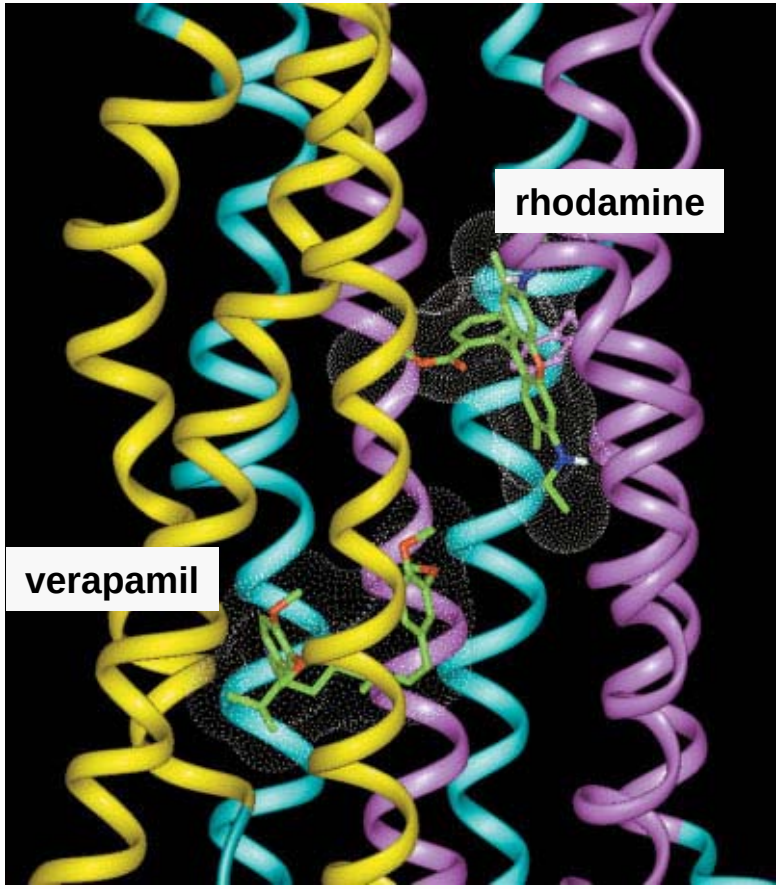
Lemaire et al., Antimicrob Agents Chemother. 2007 Jun 4; [Epub ahead of print]

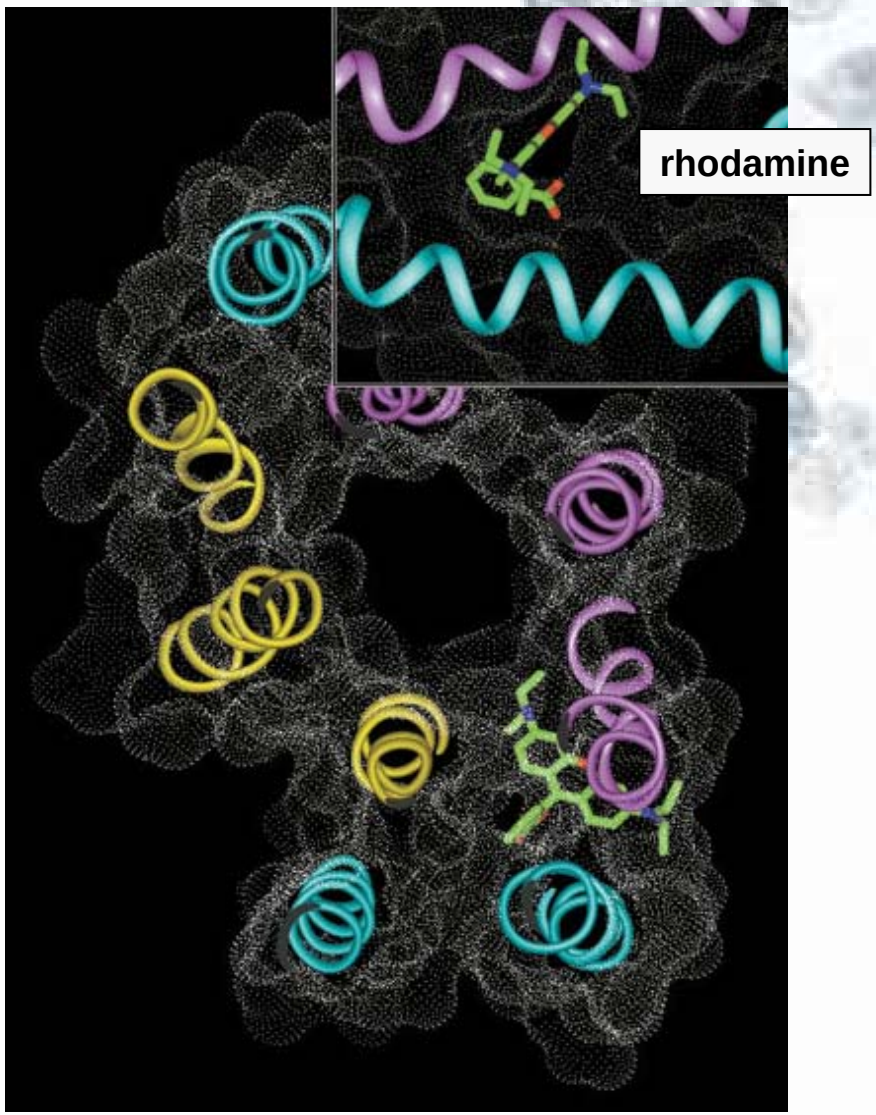
Challenges in pharmacokinetics

- efflux will tend to make intracellular activities suboptimal (fluoroquinolones, macrolides, daptomycin…)…
- but will it not make antibiotics more toxic ?
- and, incidentally, how can we explain/avoid recognition by eucaryotic efflux pumps ?

(quiz: daptomycin has a log P of -4.07 and a log D of -9.6 at pH 7… How can it be a substrate of the P-gp ?)

Modeling of P-glycoprotein 3d structure





Vandevuer et al. Proteins (Proteins-structure function and bioinformatics) (2006) 63:466-478

Challenges of efflux in antibacterial chemotherapy

- **recognizing its existence:** is it a major and general means of resistance ?
- **its role:** does it need to change our vision on (and decisions about) existing antibiotics ?
- **the way we find it:** how can we detect it (and do we need to do this ?)
- **the way we treat patients:**
  - can we make non-effluxed drugs ?
  - and what about efflux inhibitors ?
  - is efflux important in pharmacokinetics/drug interactions ?

Let us believe in pumps…
(each has its own set of challenges)

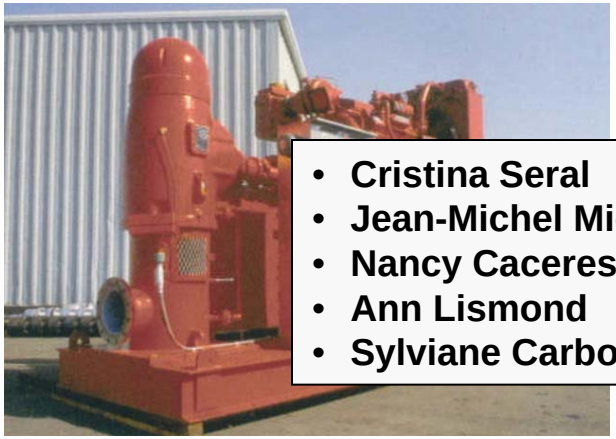
**the fire pump…
(save your life)**

**the gas pump…
(keeps you moving)**

**the pump for everything …
(white product)**

**the little amateur one…
but with great expectations …**

Let us also believe in pumps operators… (each met her/his own set of challenges)



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And thank your for the invitation in Bremen …

Herzlich willkommen

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ALPHA PUMPEN-TECHNIK

Pumpen

Armaturen

Reparatur

Kunden-
und Notdienst

Anlagenbau

Dichtungstechnik

Zubehör

Angebot
des Monats

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