From Microbial Pathogenesis to the Discovery of Antivirulence Drugs
Les Diablerets, Switzerland, 4-8 October 2009

Efflux pump inhibitors to restore antibiotic efficacy

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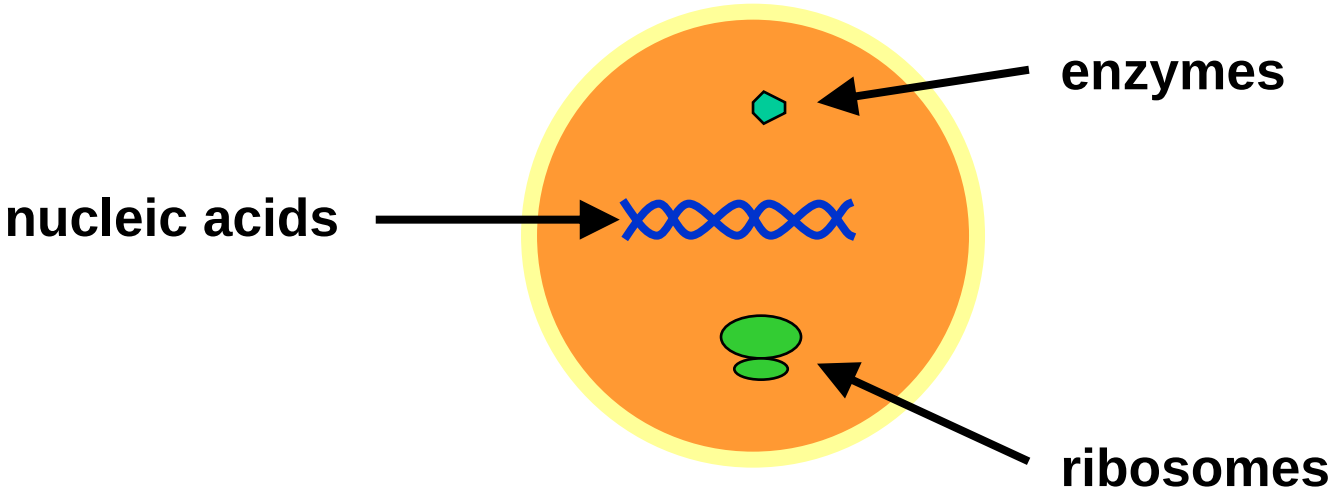
Why active efflux ?

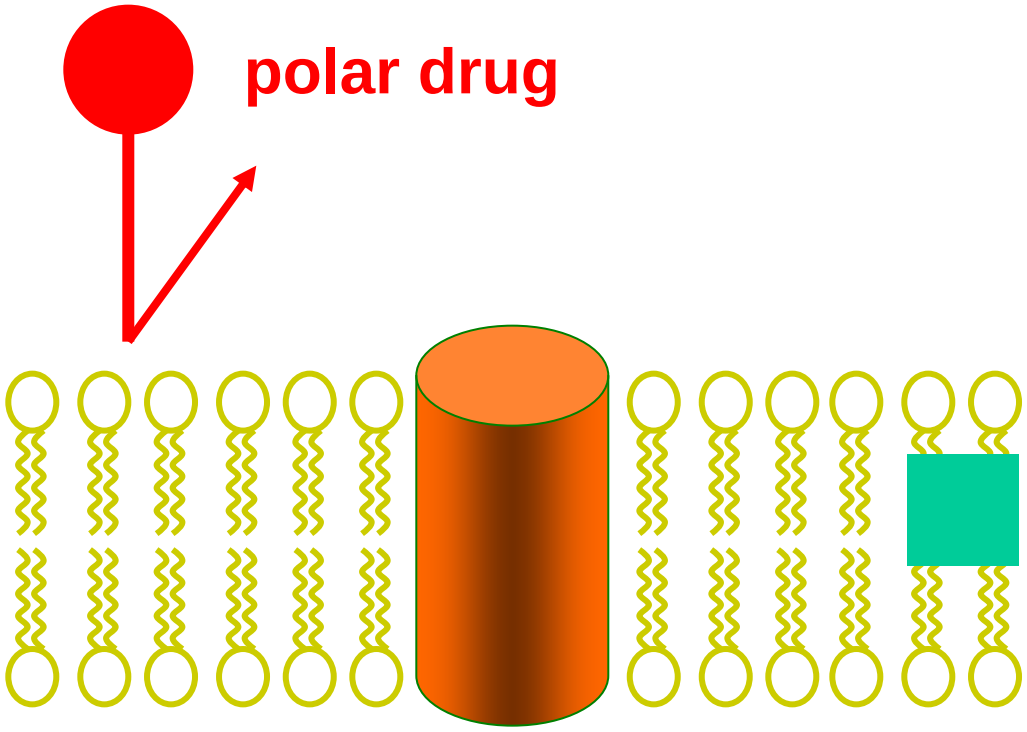
Manneken Pis, who saved Brussels from fire

Chemotherapeutic agents exert toxic effects on specific targets



How can these drugs reach their target inside the cells ?

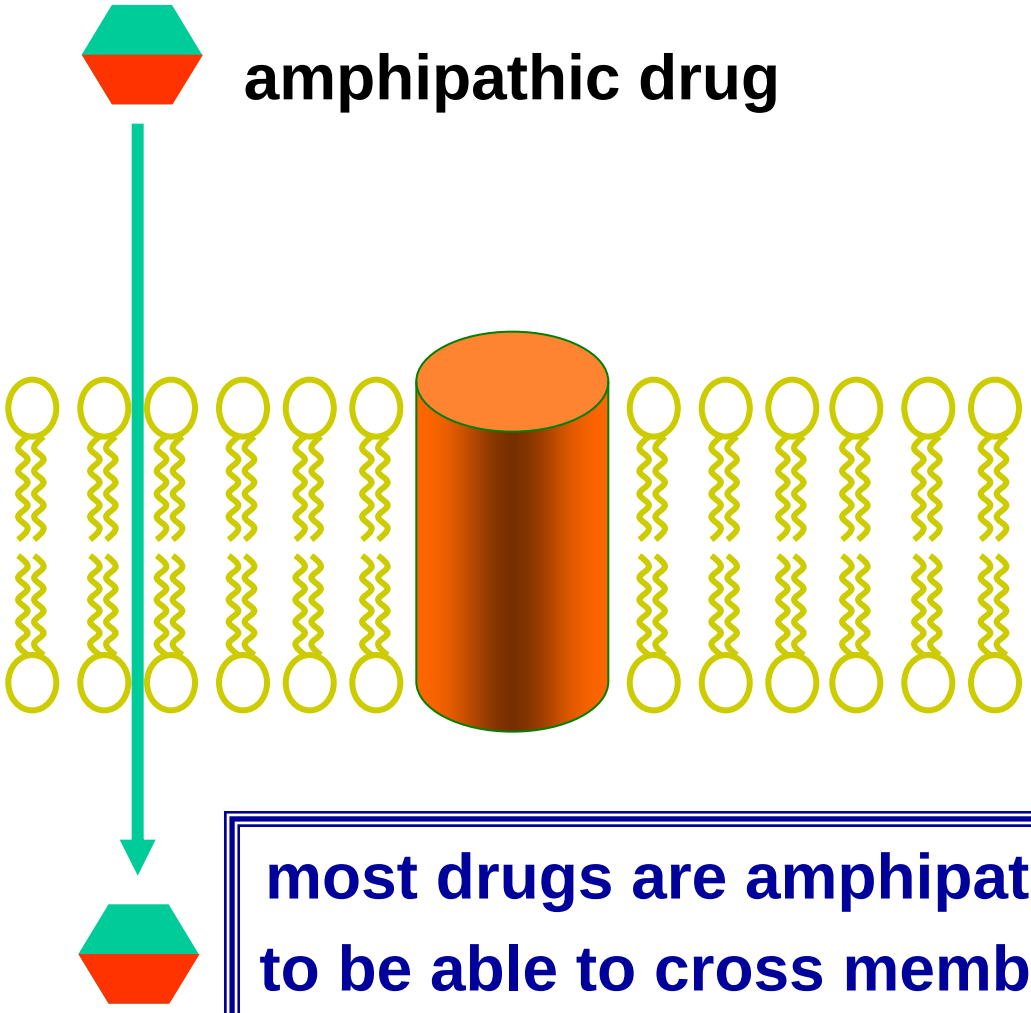
Reaching an intracellular target …



physico-chemical properties are inadequate for reaching an intracellular target !

Van Bambeke et al., Biochem. Pharmacol (2000) 60:457-70

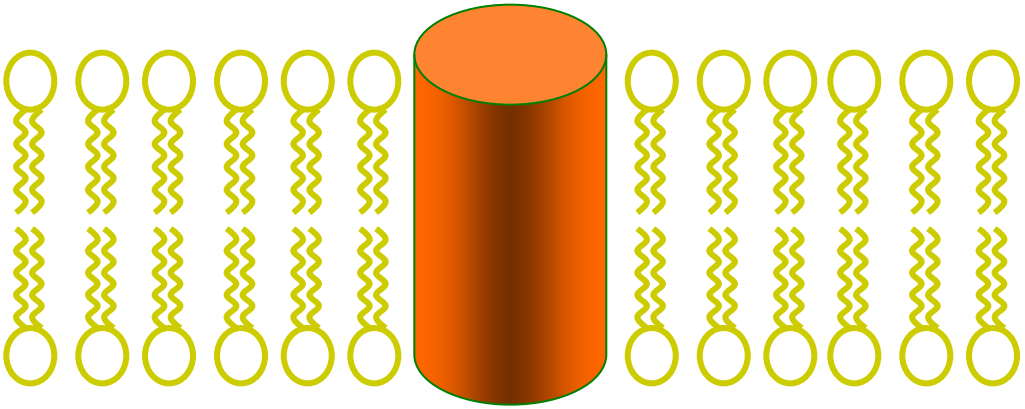
Reaching an intracellular target …

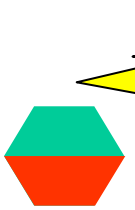


most drugs are amphipathic by design, to be able to cross membrane barriers !

Van Bambeke et al., Biochem. Pharmacol (2000) 60:457-70

Intracellular chemotherapeutic agents



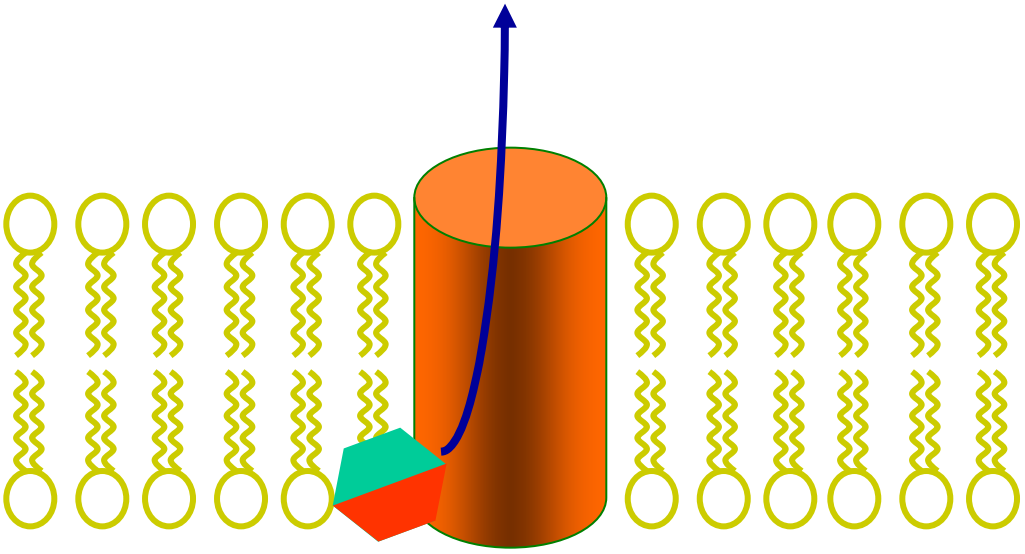


But a diffusible compound may have potentially harmful effects !

Van Bambeke et al., Biochem. Pharmacol (2000) 60:457-70

Why efflux transporters ?

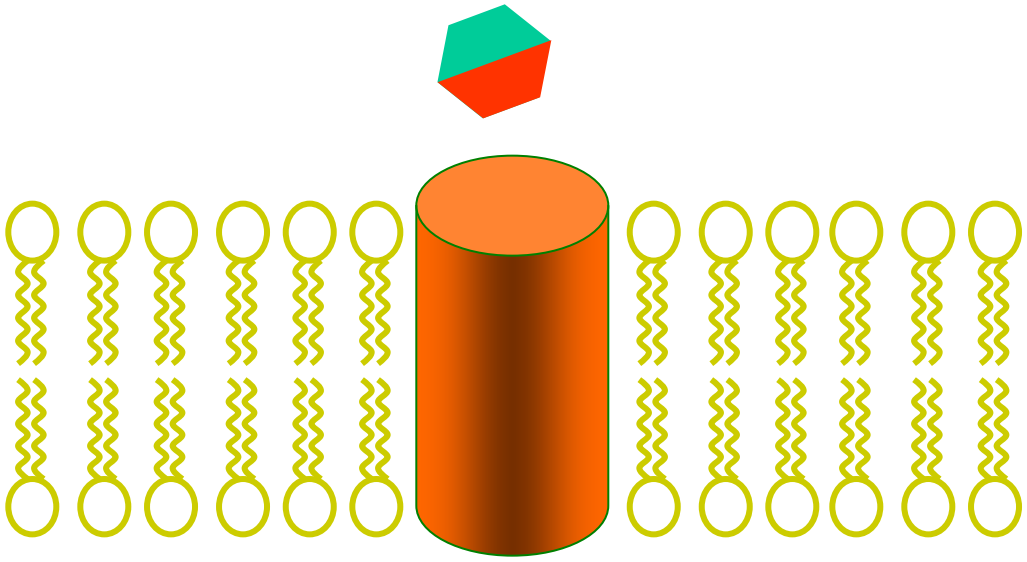
Extrusion by efflux pumps



Van Bambeke et al., Biochem. Pharmacol (2000) 60:457-70

Why efflux transporters ?

Extrusion by efflux pumps

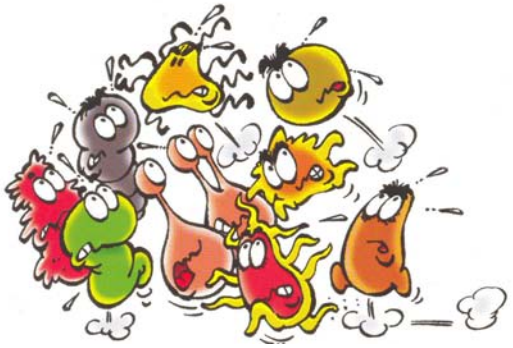


general mean of protection
against cell invasion by diffusible molecules

Van Bambeke et al., Biochem. Pharmacol (2000) 60:457-70

Typical ‘toxic’ diffusible substances as substrates for efflux pumps

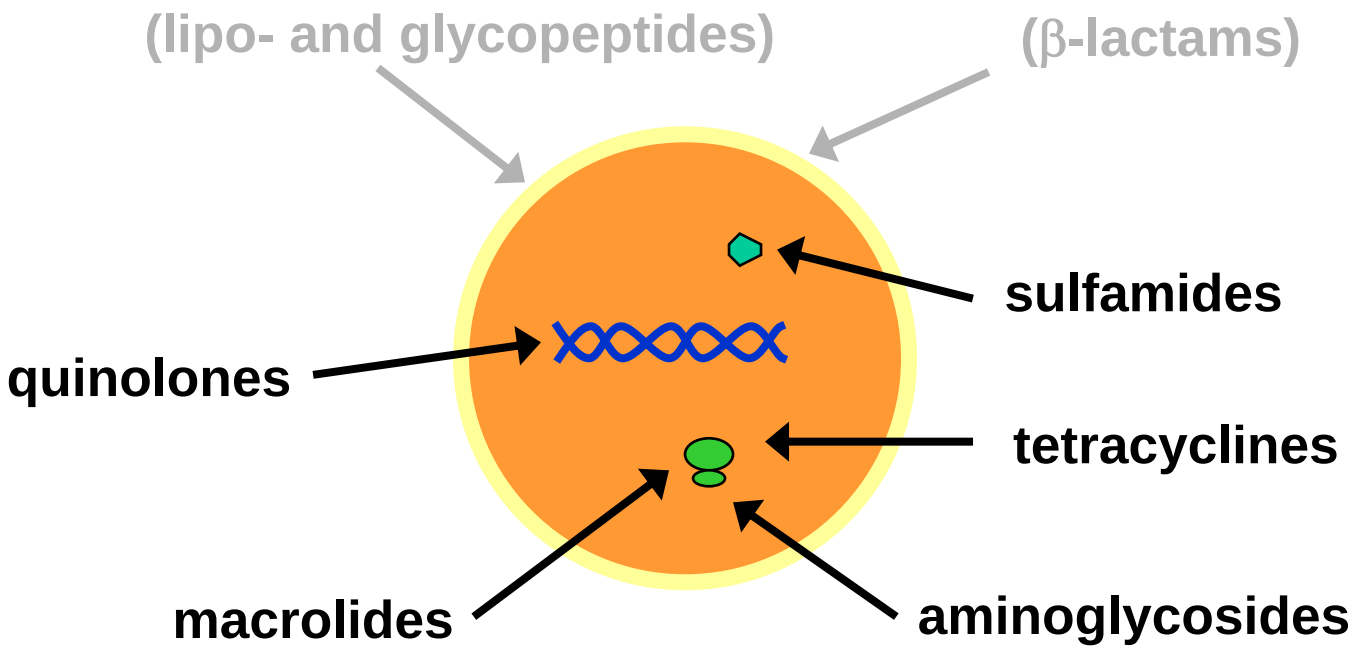
antibiotics



antifungals

anticancer agents

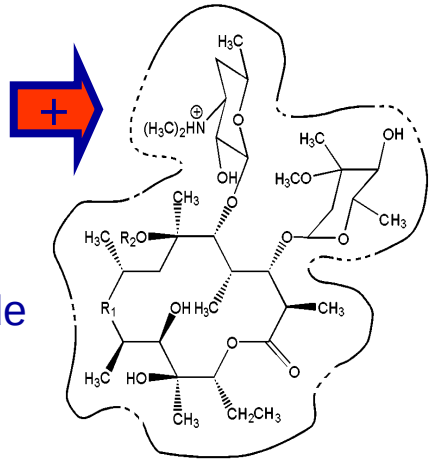
Most antibiotics do act on intracellular targets



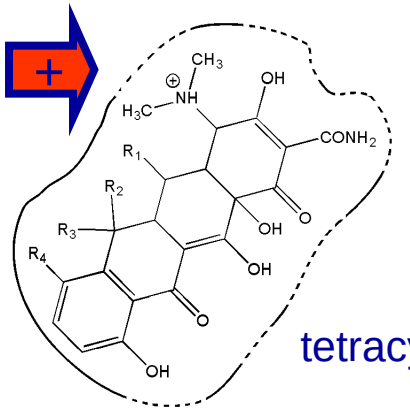
Efflux as a mechanism of resistance
by reducing antibiotic concentration inside the bacteria

Most antibiotics are amphiphilic !

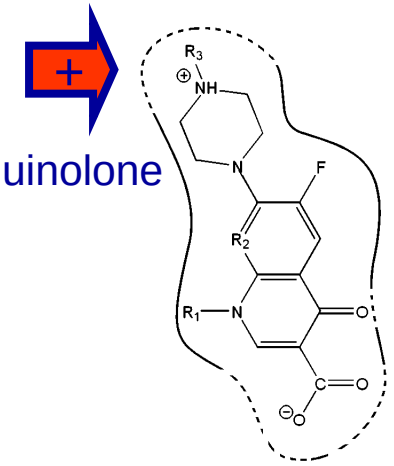
cationic amphiphiles



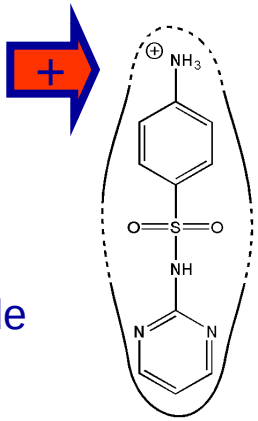
macrolide



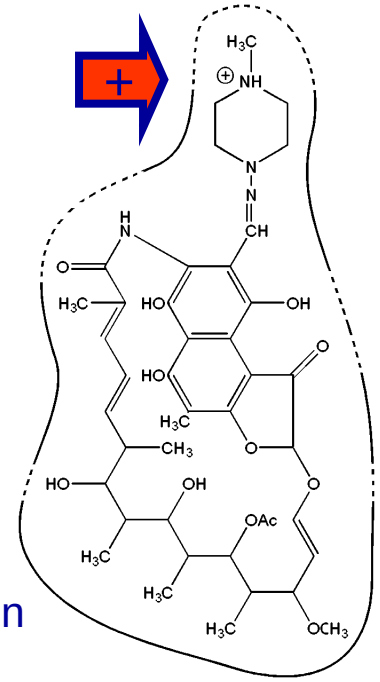
tetracycline



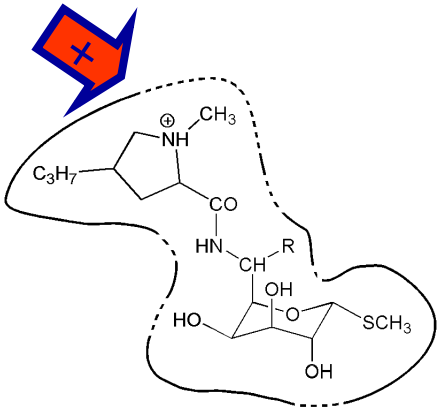
fluoroquinolone



sulfamide



rifampicin

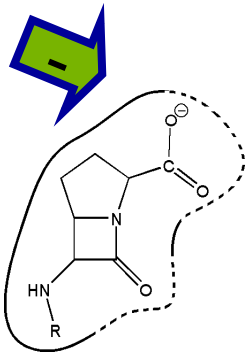


lincosamide

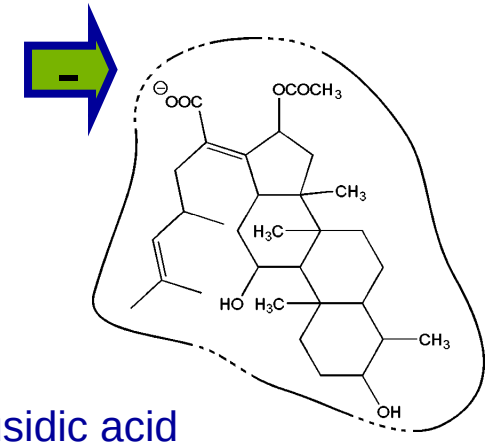
Van Bambeke et al., Biochem. Pharmacol (2000) 60:457-70

Most antibiotics are amphiphilic !

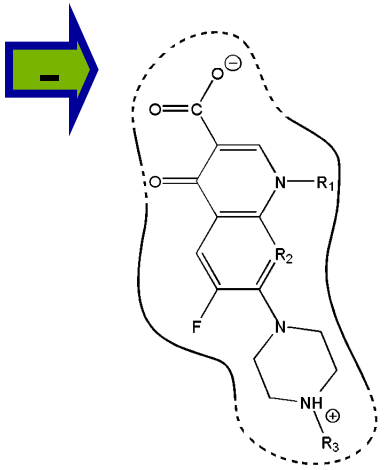
anionic amphiphiles



β-lactam



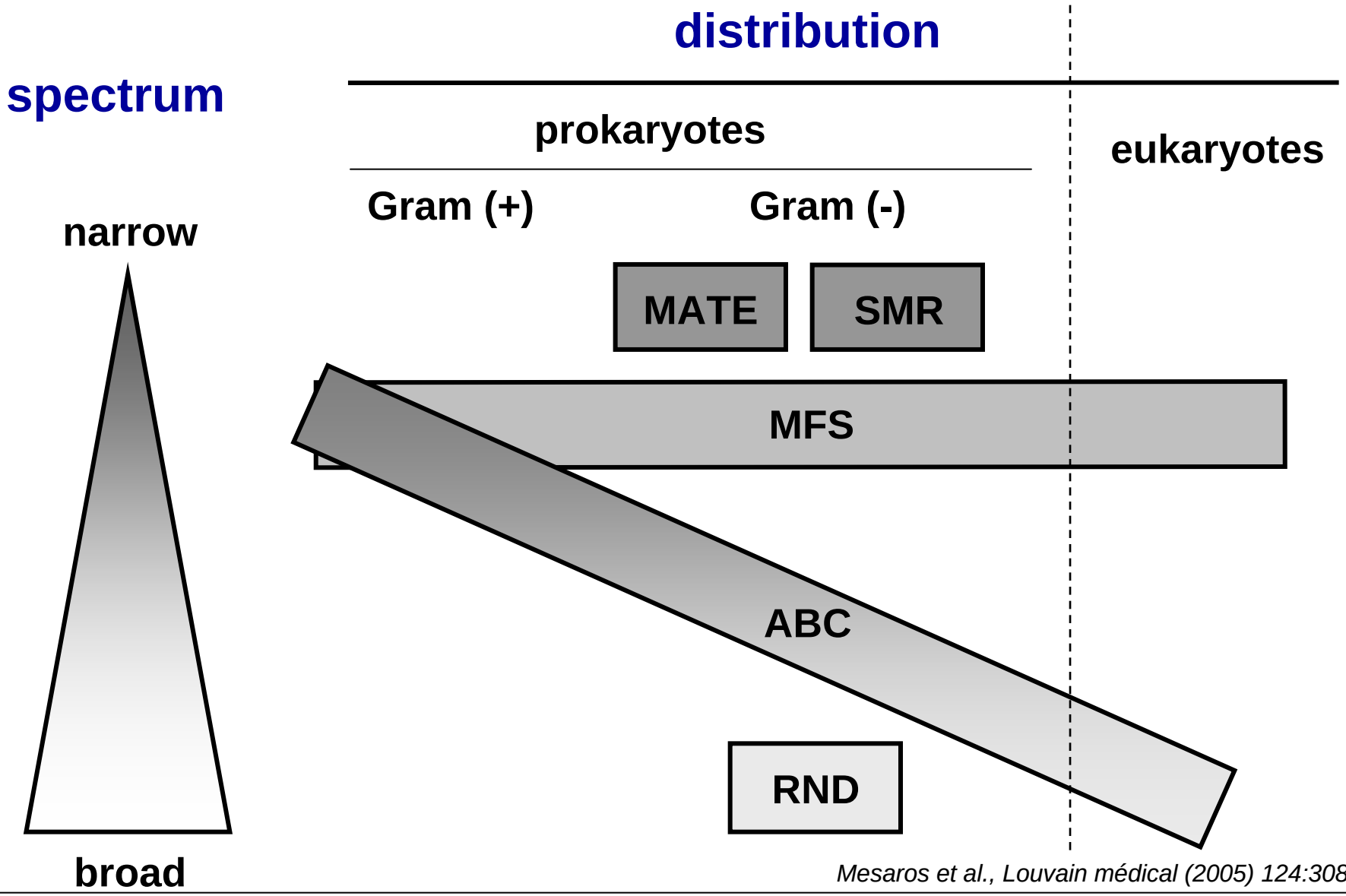
fusidic acid



fluoroquinolone

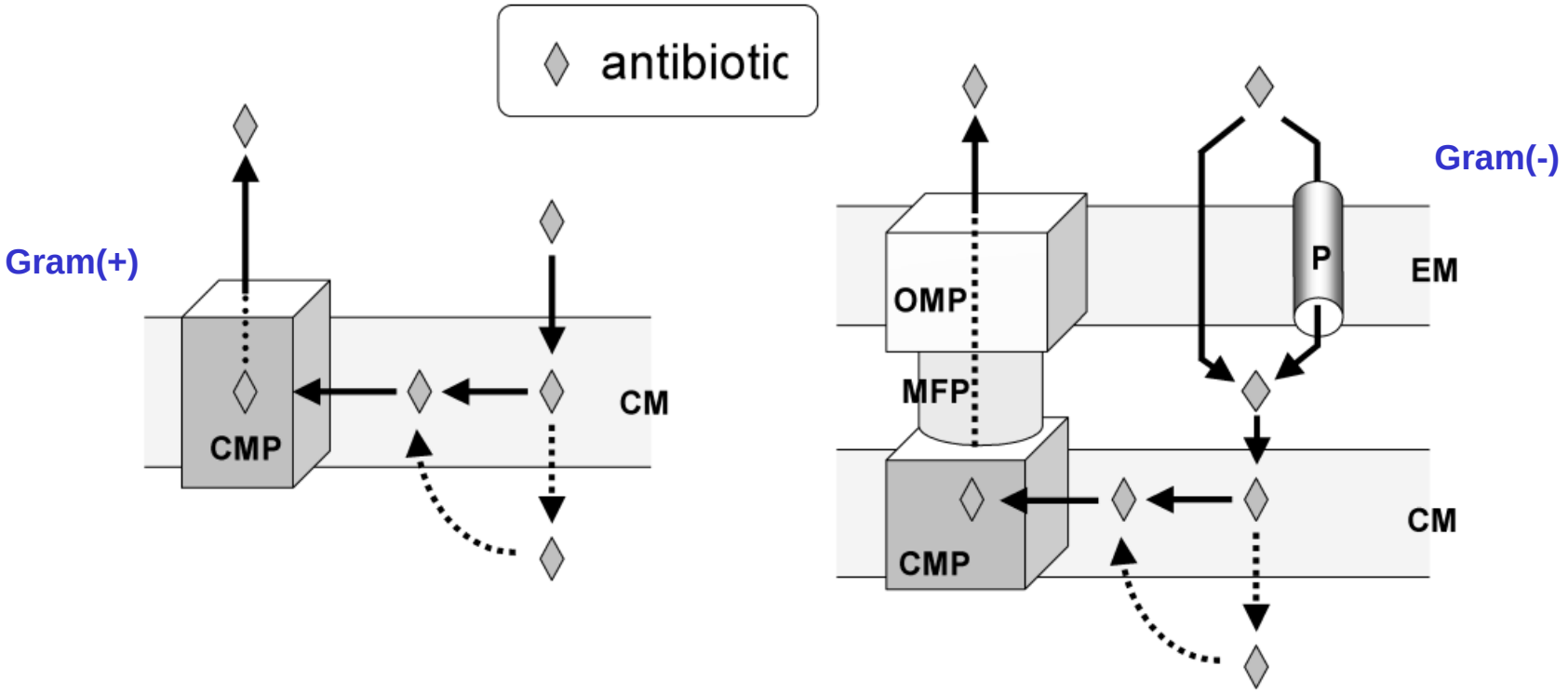
Van Bambeke et al., Biochem. Pharmacol (2000) 60:457-70

Antibiotic efflux transporters are ubiquitous



Mesaros et al., Louvain médical (2005) 124:308-20

General structure of efflux pumps in bacteria



CM: cytoplasmic membrane
EM: external membrane
P: porin
CMP: cytoplasmic membrane protein
MFP: membrane fusion protein
OMP: outer membrane protein

Mesaros et al., Louvain médical (2005) 124:308-20

Efflux and resistance in pathogenic bacteria

bacteria	efflux pump	super family	β-lactams					FA	AG	Tet	OX	ML	LM	CP	Rif	Q		SM	TM
			penams	cephems	penems	mbact.	inhib β-ase									NA	FQ		
S.aureus	NorA	MFS												●			●		
	TetK-L	MFS								●									
S. pneumoniae	PatA/B	ABC															●		
	MefE	MFS										●							
	pmrA	MFS															●		
	TetK-L	MFS								●									
S. pyogenes	MefA	MFS										●							
E. coli	EmrE	SMR								●		●						●	
	SetA	MFS							●										
	EmrB	MFS								●				●		●	●		
	TetA-E	MFS								●				●		●			
	Bcr	MFS																●	
	MdfA	MFS							●	●		●		●	●		●		
	AcrAB	RND	●					●		●	●	●		●	●	●	●		
	AcrD	RND							●										
P. aeruginosa	CmlA	MFS												●					
	TetA,C,E	MFS								●									
	MexAB OprM	RND	●	●	●	●	●	●		●		●	●	●	●		●	●	●
	MexCD OprJ	RND	●	●	●	●				●		●	●	●			●		●
	MexEF OprN	RND	●	●	●	●	●							●			●		●
	MexXY	RND	●	●		●			●	●		●	●	●			●		

Van Bambeke et al., J. Antimicrob. Chemother (2003) 51:1055-65

Efflux and resistance in pathogenic bacteria

1 bacteria → **several pumps** → **multi-resistance**

1 pump → **several classes of antibiotics** → **cross-resistance**

1 class of antibiotics → **several pumps** → **efficacy of inhibitors ?**

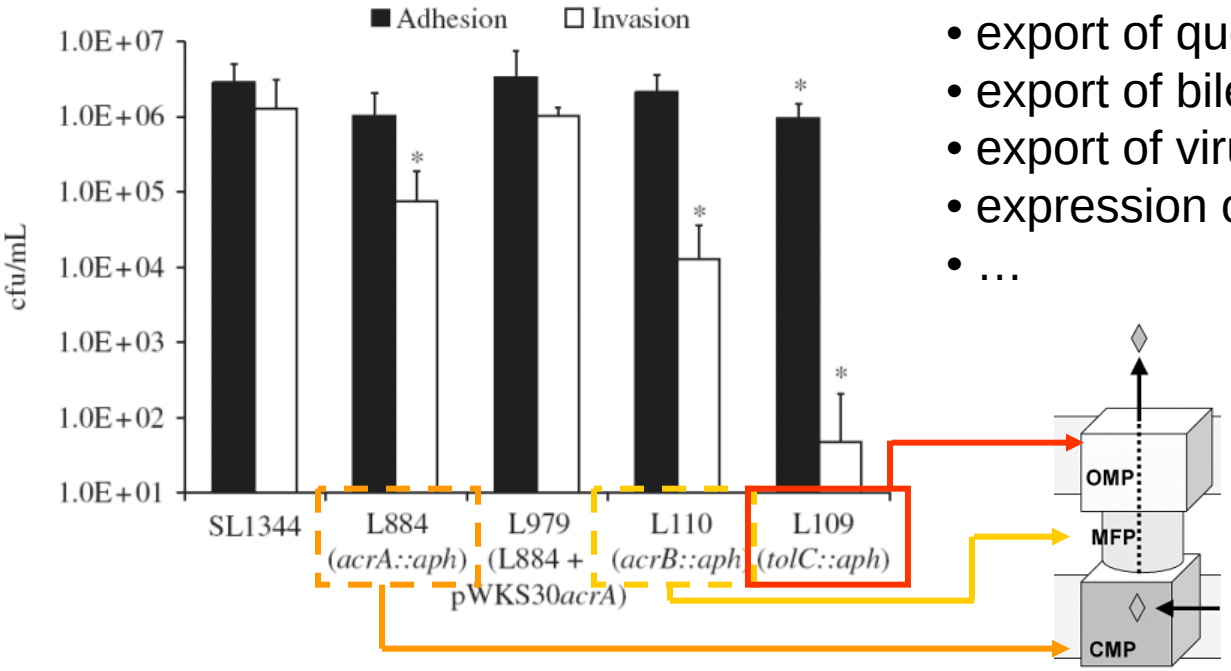
Role of efflux in pathogenicity

Belgian beer

Colonization

Efflux pumps contribute to invasion in enteric pathogens

Disruption of efflux pump expression reduces adhesion and invasion of *Salmonella* in cultured cells and *in vivo*



- export of quorum sensing molecules ?
- export of bile salts ?
- export of virulence factors ?
- expression of virulence factors ?
- …

Blair et al, JAC (2009) Epub [PMID: 19744979]; Buckley et al, Cell Microbiol (2006) 8:847-56

Colonization

Gene expression alterations in efflux deficient strains

Disruption of efflux pump expression reduces the expression of genes involved in motility and anaerobic metabolism

Gene/operon or function of encoded protein	Change (fold) in expression relative to SL1344		
	L884 (*acrA*::*aph*)	L110 (*acrB*::*aph*)	L109 (*tolC*::*aph*)
Multidrug transport/ regulation			
acrB	3.22	0.01	0.15
ompC	—	—	—
ompF	—	0.25	0.33
ompR	—	1.55	1.54
ompX	—	0.69	0.68
ramA	—	1,226.41	25.41
rob	—	0.53	—
Anaerobic metabolism			
napA	2.77	0.09	—
napB	—	0.13	—
napC	—	0.14	—
napF	—	0.09	—
narG	2.38	0.01	—
narH	4.69	0.01	—
narI	3.95	0.04	—
narJ	—	0.01	—
narK	3.39	0.01	—
nirB	—	0.01	—
nirC	1.98	0.01	—
nirD	2.62	0.01	—

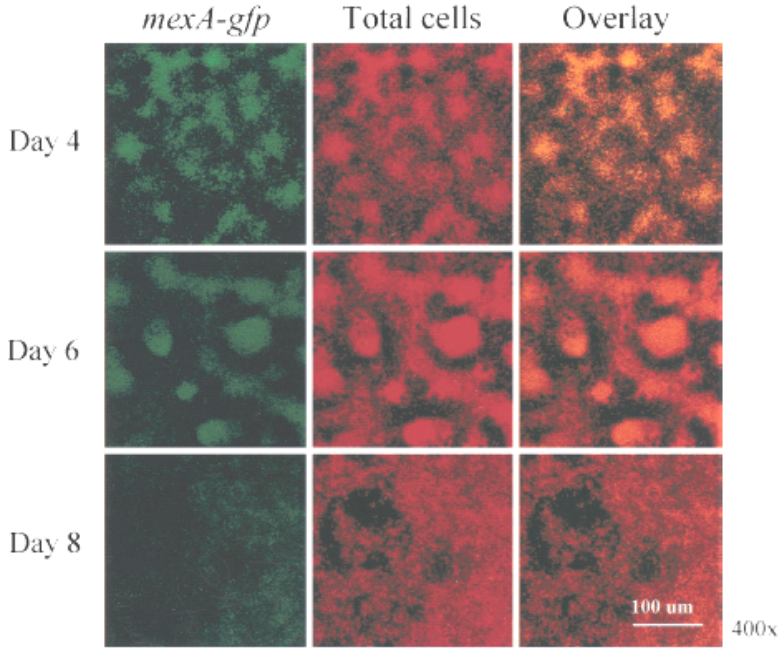
Gene/operon or function of encoded protein	Change (fold) in expression relative to SL1344		
	L884 (*acrA*::*aph*)	L110 (*acrB*::*aph*)	L109 (*tolC*::*aph*)
Motility/chemotaxis			
cheA	2.20	0.07	—
cheM	3.66	0.02	0.39
cheR	—	0.04	—
cheW	2.15	0.01	0.07
cheY	1.96	0.02	0.05
flgC	1.49	0.01	0.13
flgD	1.97	0.01	—
flgE	2.12	0.01	0.11
flgF	2.07	0.05	—
flgG	2.35	0.01	0.13
flgJ	1.83	0.04	—
flgK	1.93	0.01	0.11
flgL	2.10	0.01	0.05
flgM	—	0.01	0.07
flgN	2.12	0.01	0.09
flhD	—	0.61	—
fliA	—	0.01	—
fliD	—	0.13	—
fliS	—	0.07	—
tar	—	0.03	0.09

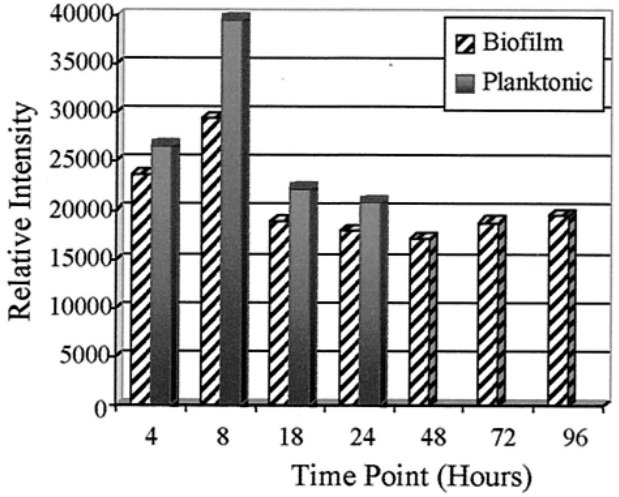
Webber et al, J. Bacteriol. (2009) 191:4276-85

Biofilm formation

Efflux pumps expression in biofilms

Higher expression of MexAB-OprM in *P. aeruginosa* growing in biofilms



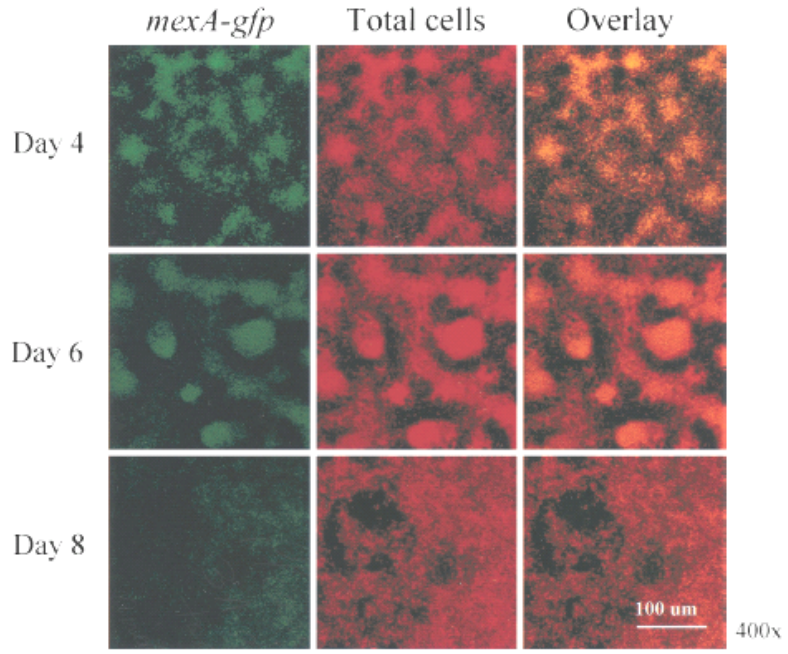


De Kievit et al, Antimicrob Agents Chemother. (2001) 45:1761-70

Biofilm formation

Efflux pumps expression in biofilms

Reduced susceptibility to antibiotics in *P. aeruginosa* growing in biofilms



Contribution of the MexAB-OprM efflux operon to antimicrobial susceptibility of *P. aeruginosa* biofilms

Antibiotic	K767 (wild type)		K1119 (Δ*mexAB-oprM*)		OCR1 (K767 *nalB*; hyperexpresses *mexAB-oprM*)	
	MBEC (μg/ml)	MIC (μg/ml)	MBEC (μg/ml)	MIC (μg/ml)	MBEC (μg/ml)	MIC (μg/ml)
Aztreonam	1,024	32	512	64	>1,024	32
Chloramphenicol	1,024	128	1,024	64	>1,024	1,024
Ciprofloxacin	16	<2	<2	<2	8	<2
Erythromycin	1,024	32	1,024	8	>1,024	256
Gentamicin	16	4	4	<2	32	4
Piperacillin	1,024	32	1,024	32	>1,024	128
Tetracycline	512	64	128	64	>1,024	256
Tobramycin	64	<2	16	<2	512	<2

reduced susceptibility to AB in bioflims

role of MexAB-OprM in biofilm resistance

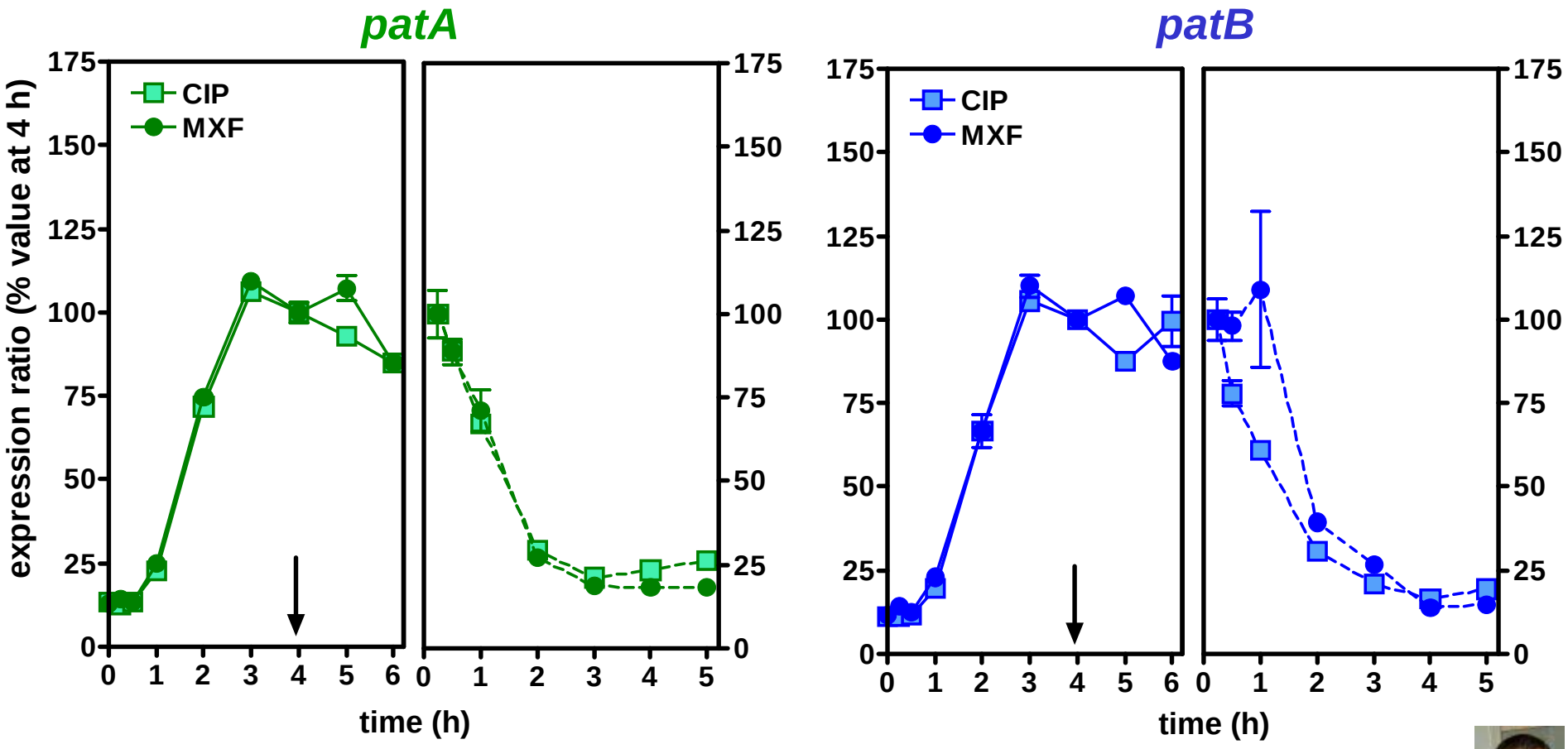
De Kievit et al, Antimicrob Agents Chemother. (2001) 45:1761-70

Role of efflux in resistance

Niagara Falls

Antibiotic exposure can induce efflux pump expression

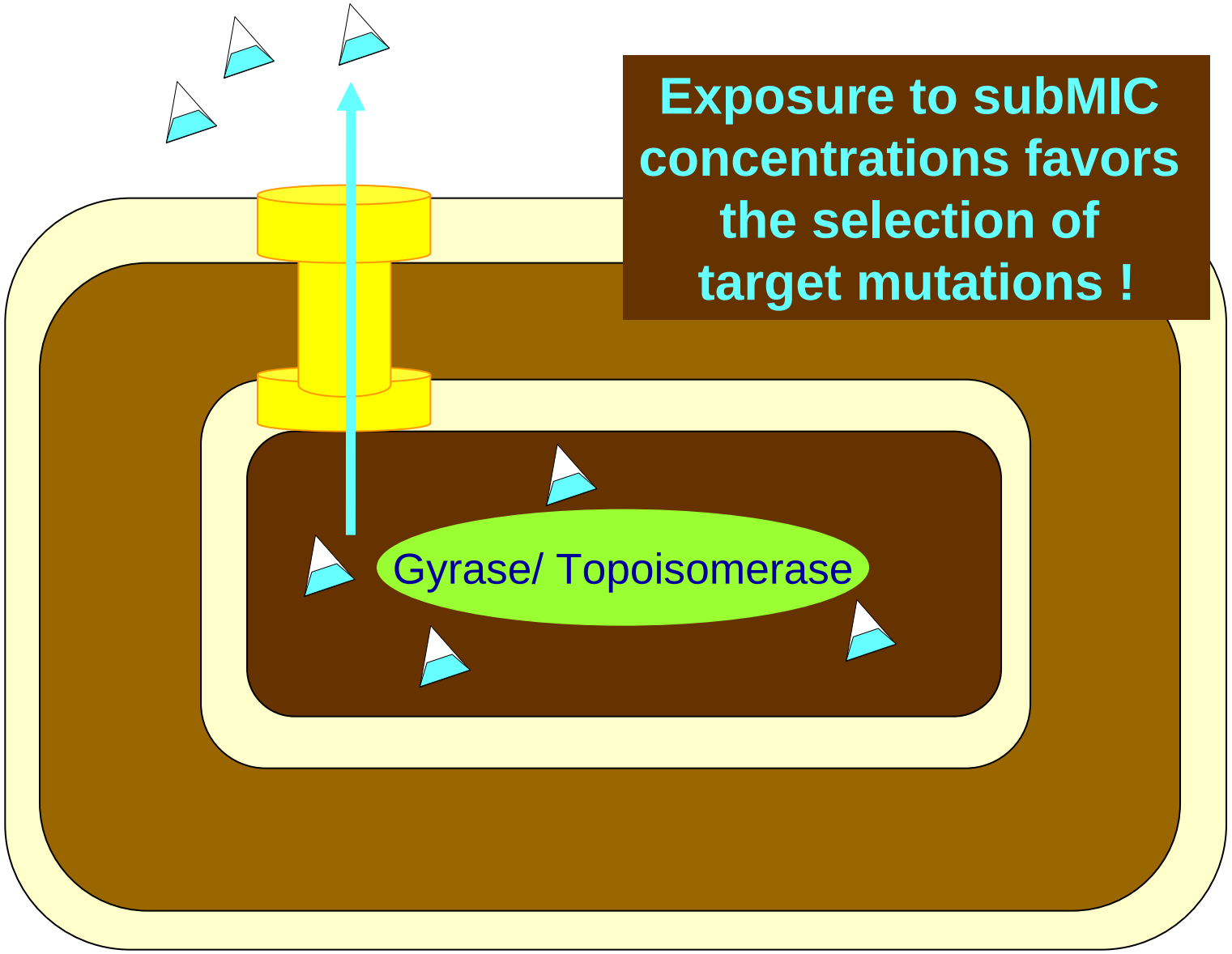
FQ, whether substrates or not, induce the expression of *patA/patB in S. pneumoniae*



4 h with ½ MIC; up to 5 h without FQ

El Garch et al, ECCMID (2009) O495

Efflux and selection of resistance to FQ



Efflux and selection of resistance to FQ

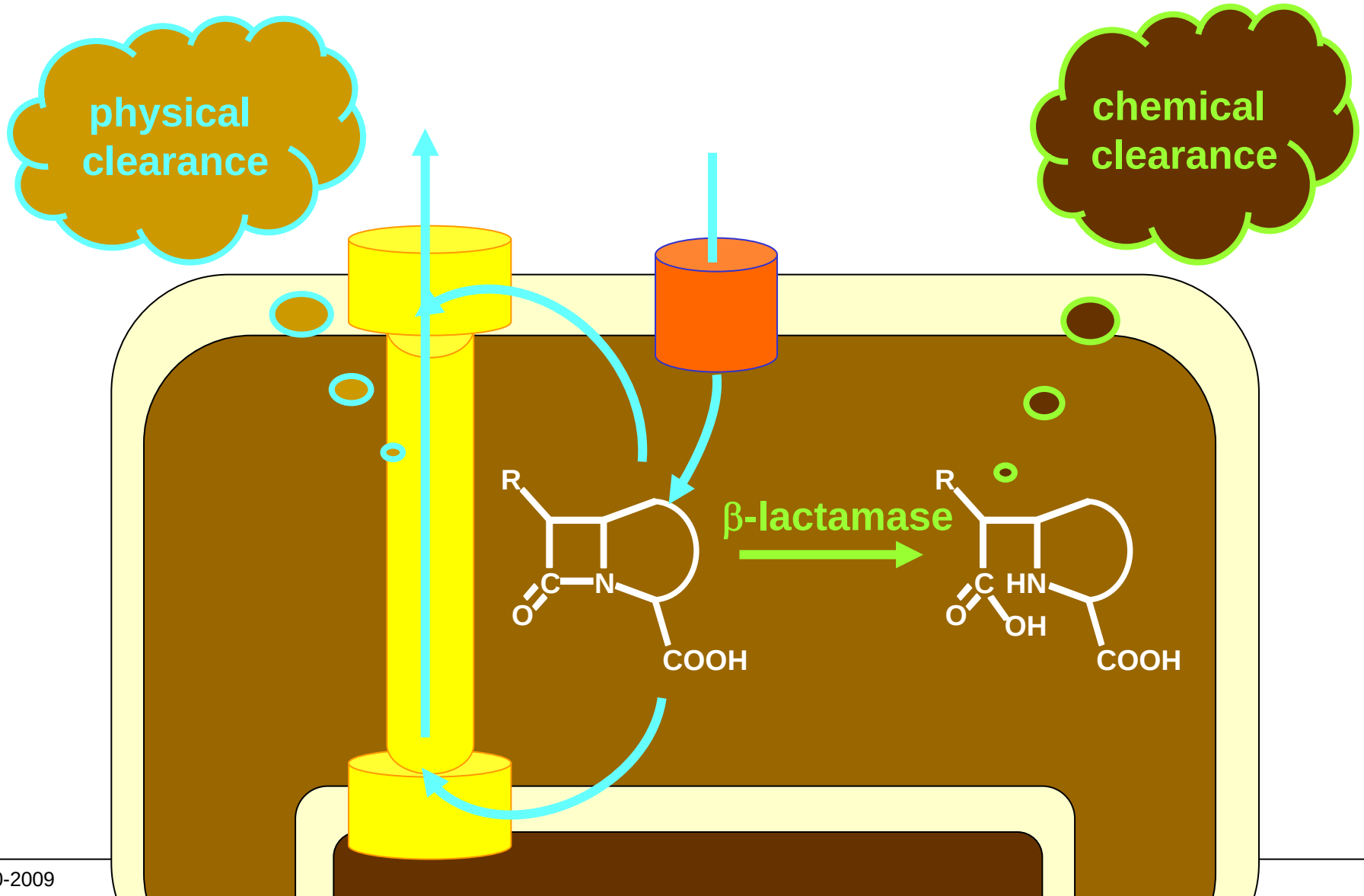
Frequency of Levofloxacin-resistant mutants in *Pseudomonas aeruginosa* with deletions of the efflux pump operons

Pump status	LVX MIC	Frequency of LVX-resistant mutants
WT	0.25	2×10^7 - 4×10^7
Δ mexAB-oprM	0.015	2×10^7 - 4×10^7
Δ mexCD-oprJ	0.25	2×10^7 - 4×10^7
Δ mexEF-oprN	0.25	2×10^7 - 4×10^7
Δ mexAB-oprM;Δ mexEF-oprN	0.015	2×10^7 - 10^7
Δ mexCD-oprJ; Δ mexEF-oprN	0.25	2×10^6
Δ mexAB-oprM; Δ mexCD-oprJ	0.015	1×10^9
Δ mexAB-oprM; Δ mexCD-oprJ; Δ mexEF-oprN	0.015	**$<1 \times 10^{11}$**

Selection of mutants in FQ target undetectable if ALL pumps are disrupted

Lomovskaya et al, AAC (1999) 43:1340-1346

Efflux cooperates with other mechanisms of resistance



Efflux cooperates with other mechanisms of resistance

Contributions of the AmpC β-lactamase and the AcrAB Multidrug Efflux System in intrinsic resistance of *E. coli* to β -lactams

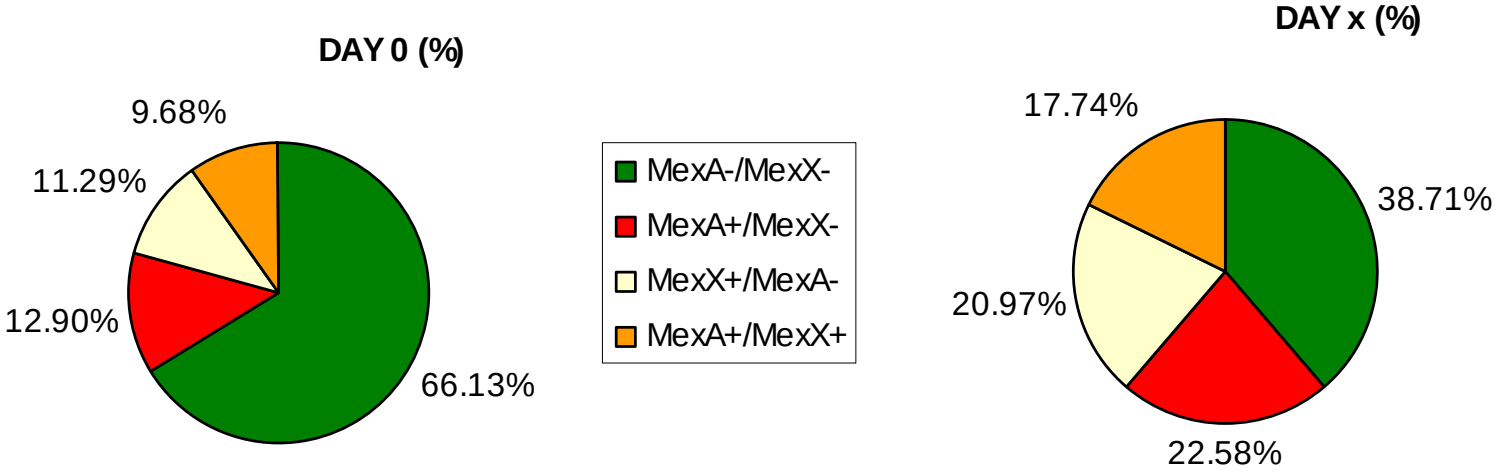
Efflux	β-lactamase	MIC carbenicillin	MIC ofloxacin
-	-	0.2	0.05
+	**-**	**12.5**	**0.2**
+++	-	50	1.56
-	+	100	0.05
+	+	200	0.39
+++	+	400	1.56

WT: intrinsic resistance !

Mazzariol et al, AAC (2000) 44:1387-1390

Efflux selection during treatment

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





Riou, Avrain, Van Bambeke, Tulkens, et al; unpublished data

Efflux and therapeutic failure

Rabbit Endocarditis – *P. aeruginosa*; mimicking human dosages

Antibacterial activity of drugs in the rabbit endocarditis model after 48 h of treatment.

Antibiotic regimen	Log_{10} CFU/g of vegetation (mean ± S.D. [no. of rabbits])	
	PAO4098E **(MexAB++)**	PAO4098ET **(WT)**
Control	7.17 ± 0.2 [10]	6.6 ± 0.8 [10]
Ticarcillin 15 g/day CI	6.2 ± 0.4 [6]	Sterile [6]
Ticarcillin 15 g/day ID	6.4 ± 0.5 [6]	Sterile [6]
Ticarcillin 18 g/day CI	6.1 ± 1.2 [6]	Sterile [6]
PIP/TAZ 12 g/day CI	6.0 ± 1.2 [6]	Sterile [6]
PIP/TAZ 16 g/day CI	6.0 ± 1.2 [6]	Sterile [6]
PIP/TAZ 16 g/day ID	6.2 ± 1.2 [6]	Sterile [6]
Ceftazidime 3 g/day CI	5.9 ± 0.8 [6]	Sterile [6]
Ceftazidime 6 g/day CI	2.7 ± 0.4 [6]*,**	Sterile [6]
Ceftazidime 6 g/day ID	4.8 ± 0.7 [6]*	Sterile [6]

CFU, colony-forming units; S.D., standard deviation; CI, continuous infusion; ID, intermittent bolus administration; PIP/TAZ, piperacillin/tazobactam.

* $P < 0.01$ vs. control group.

** $P < 0.01$ vs. ID.

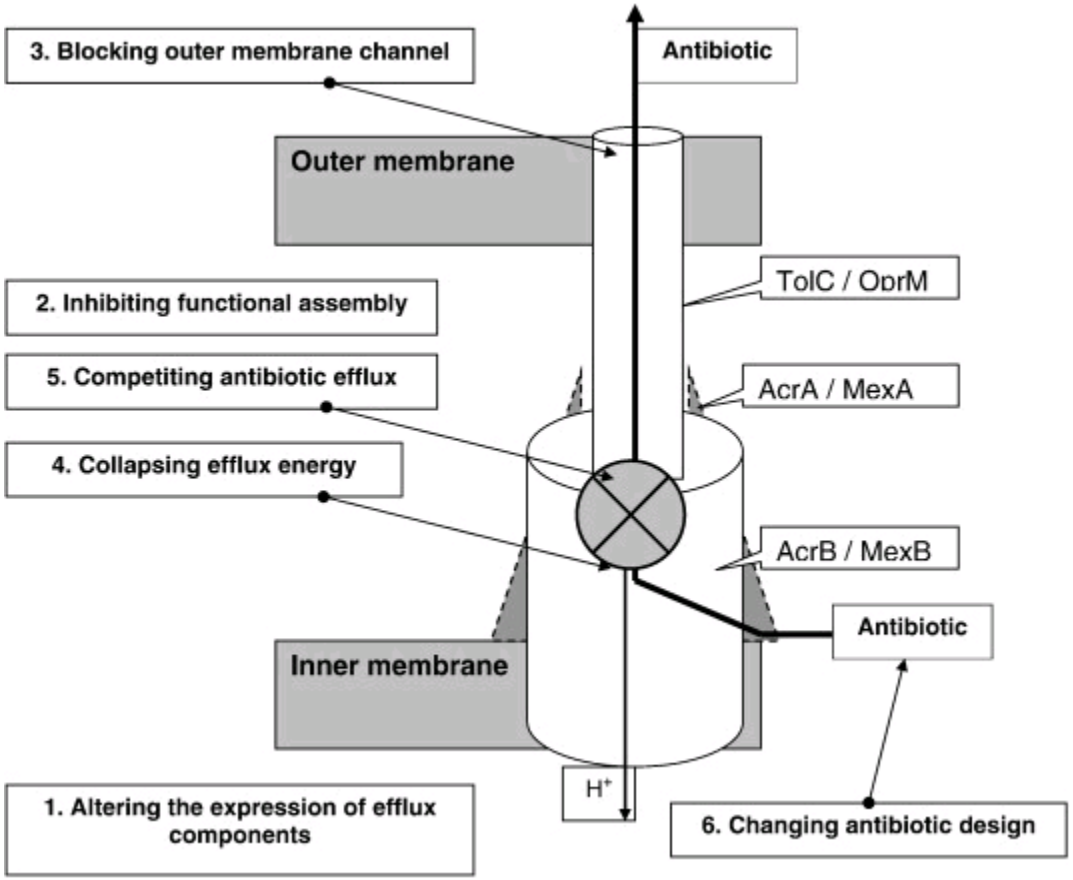
Boutouille et al, Int J Antimicrob Agents (2009) 33:417-20

How to prevent resistance by efflux ?

J.M. Folon, La Hulpe, Belgium

How to prevent resistance by efflux ?

general strategies

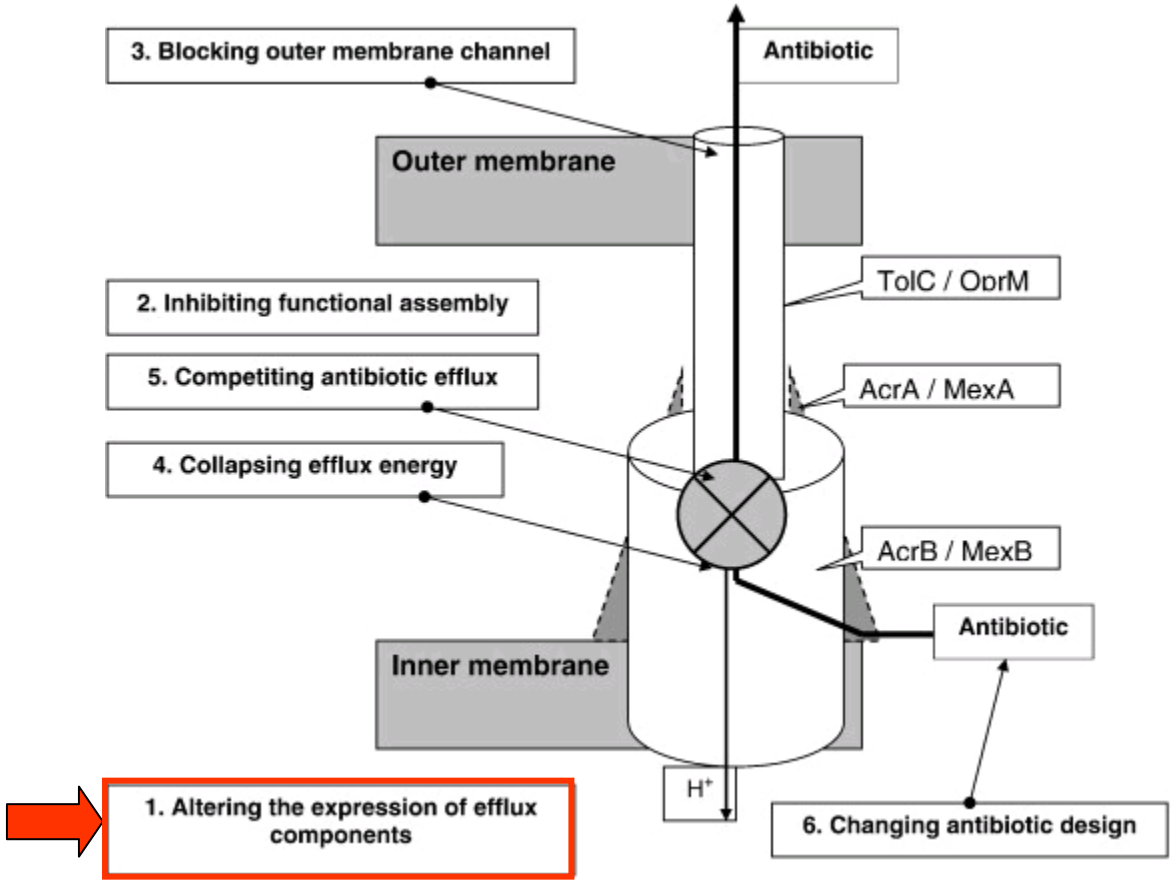


Pages & Amaral, Biochim. Biophys. Acta (2009) 1794:826-33

What is the way to go ?

How to prevent resistance by efflux ?

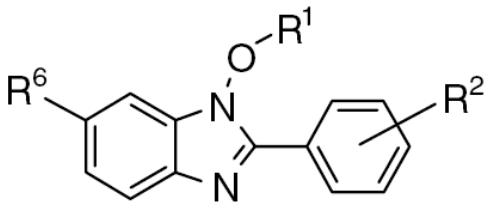
general strategies



Pages & Amaral, Biochim. Biophys. Acta (2009) 1794:826-33

Altering the expression of pump components

1-hydroxybenzimidazoles



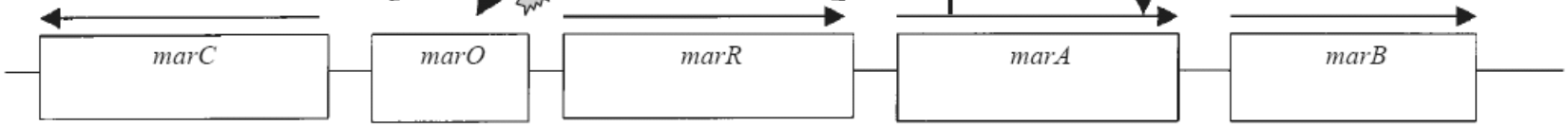
inhibitors of MarA

Once the repressor *marR* is inhibited from binding to the operator *marO* (e.g. due to salicylate binding to the repressor), the transcriptional activator marA is transcribed.

Once the transcriptional activator MarA is transcribed it up-regulates its own production[a].

MarA up-regulates a total of 47 genes[a] including AcrA and TolC, both components of the AcrAB efflux pump, which pumps out unrelated antibiotics, dyes and disinfectants[b].

MarA down-regulates 15 genes[a] including OmpF, an outer membrane protein through which hydrophilic substances enter the cell[c].



T. E. Bowser et al, Bioorg. Med. Chem. Lett. (2007) 17: 5652–5655
Randall and Woodward, Res Vet Sci. (2002) 72:87-93

Altering the expression of pump components

genetic strategies

A first strategy to inhibit efflux pump activity could consist of repressing the expression of corresponding genes.

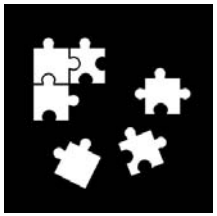
This approach may employ antisense oligonucleotides or small interfering RNA (which selectively prevent the transcription of the gene coding for the pump), or other non-traditional antisense molecules, which can interfere with the transcription or the translation of that gene of that RNA.

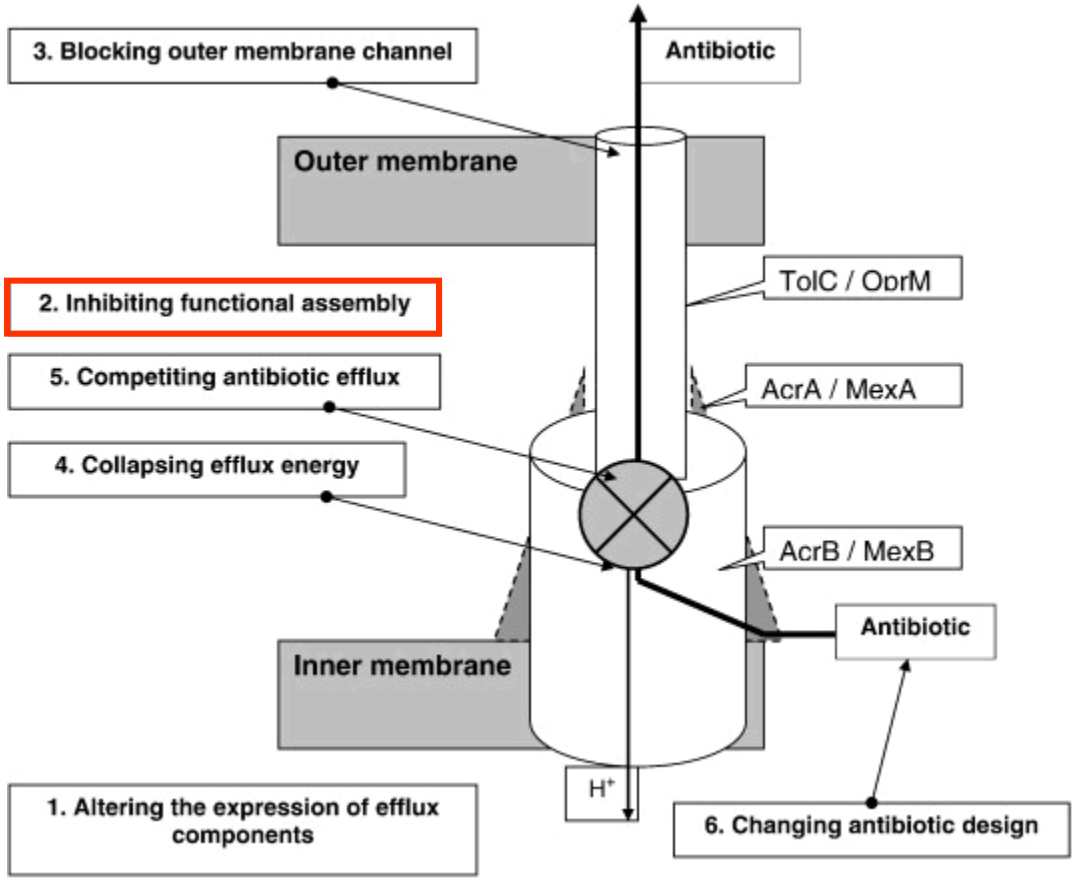
This patented* strategy was exemplified for the inhibition of the AcrAB efflux pump in *E. coli*

** Oethinger & Levy (2004) US6677133*

How to prevent resistance by efflux ?

general strategies

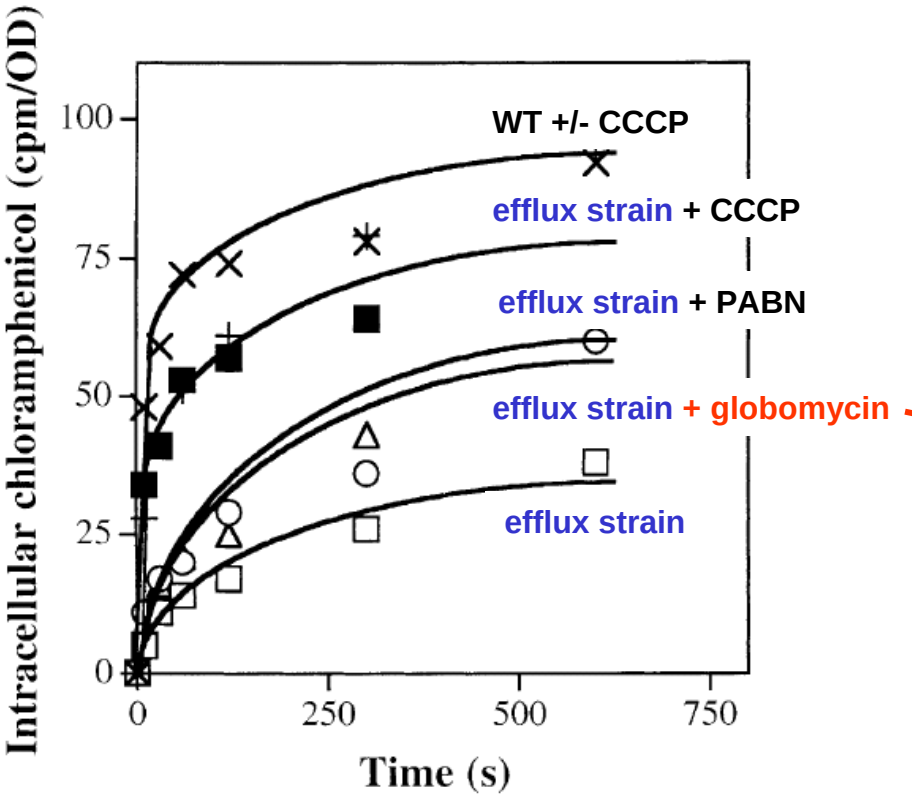


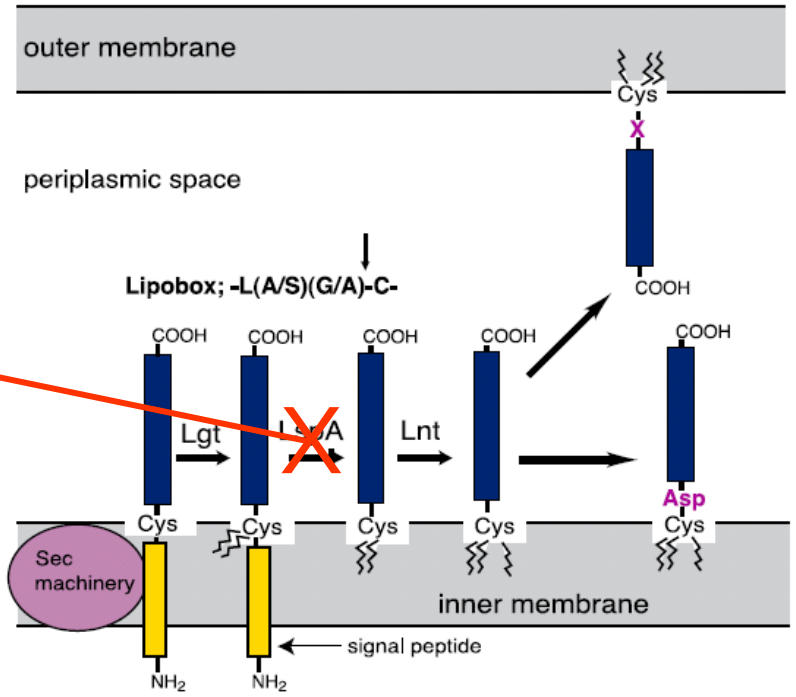


Pages & Amaral, Biochim. Biophys. Acta (2009) 1794:826-33

Inhibiting functional assembly

Alteration of addressing of OM proteins



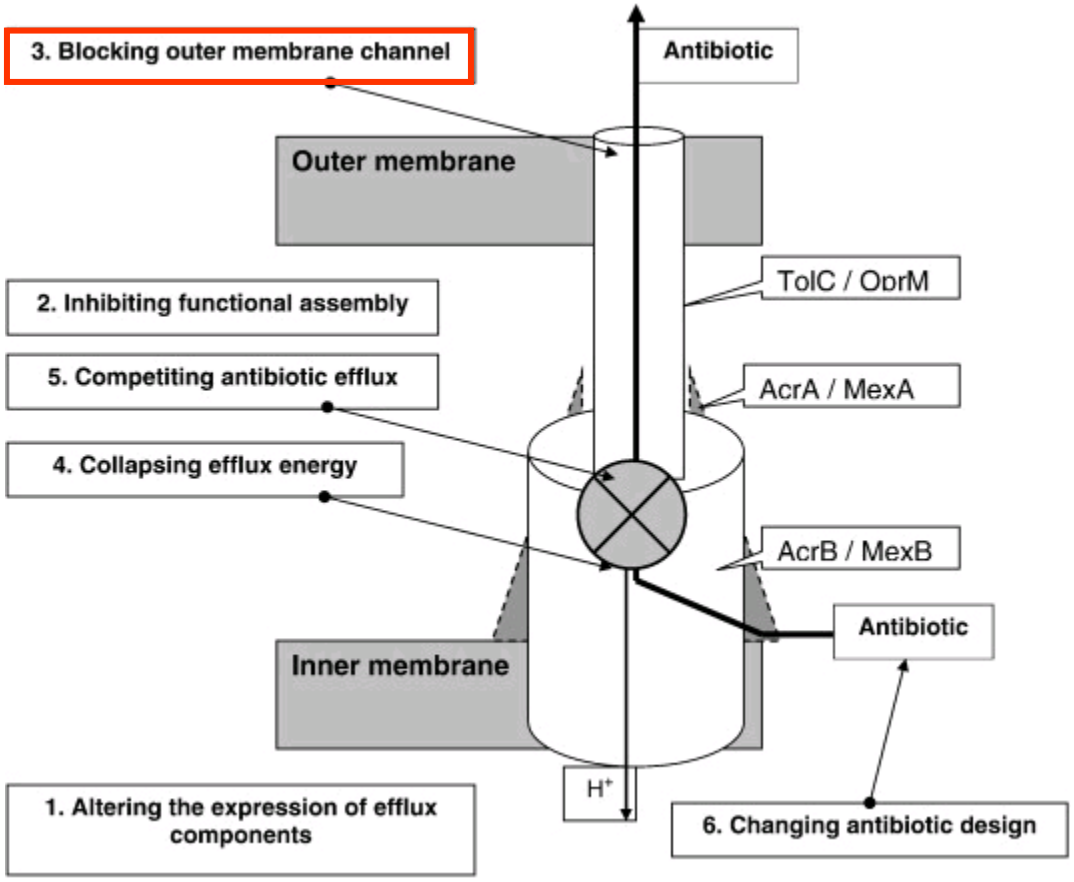


active only on growing cells !

Malléa et al, Biochem. Biophys. Res. Comm. (2002) 293:1370–1373
Tokudaa & Matsuyama, Biochim. Biophys. Acta (2004) 1693:5 – 13

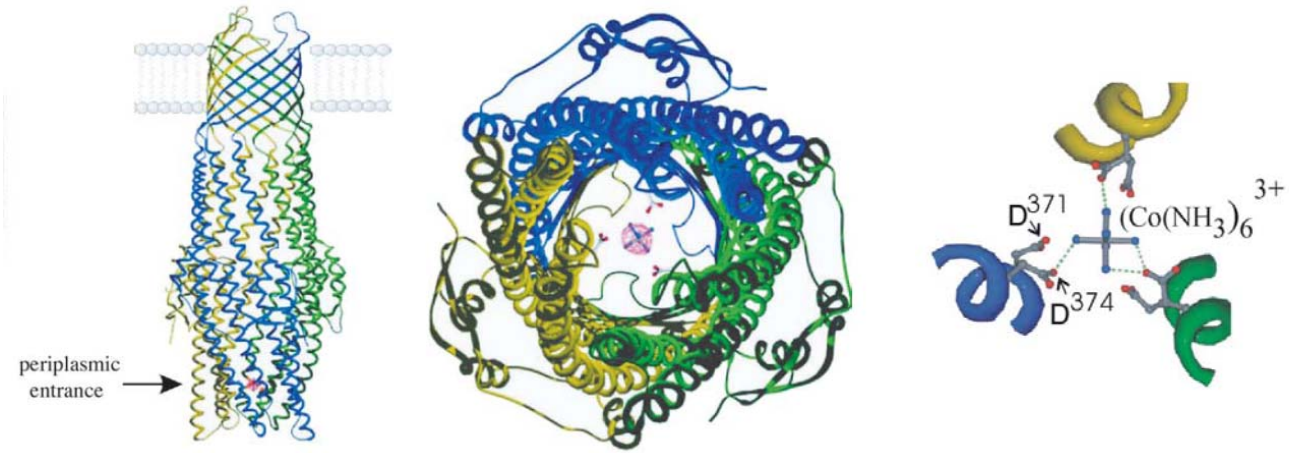
How to prevent resistance by efflux ?

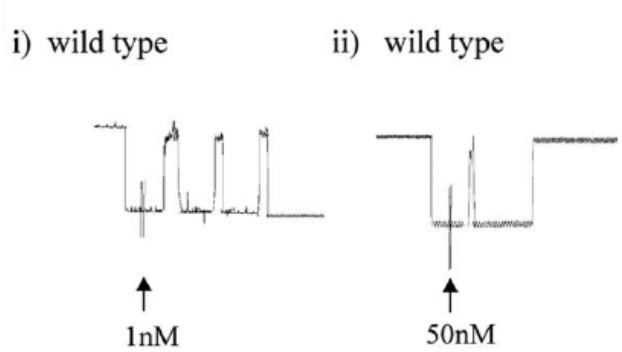
general strategies



Pages & Amaral, Biochim. Biophys. Acta (2009) 1794:826-33

Blocking outer membrane channel



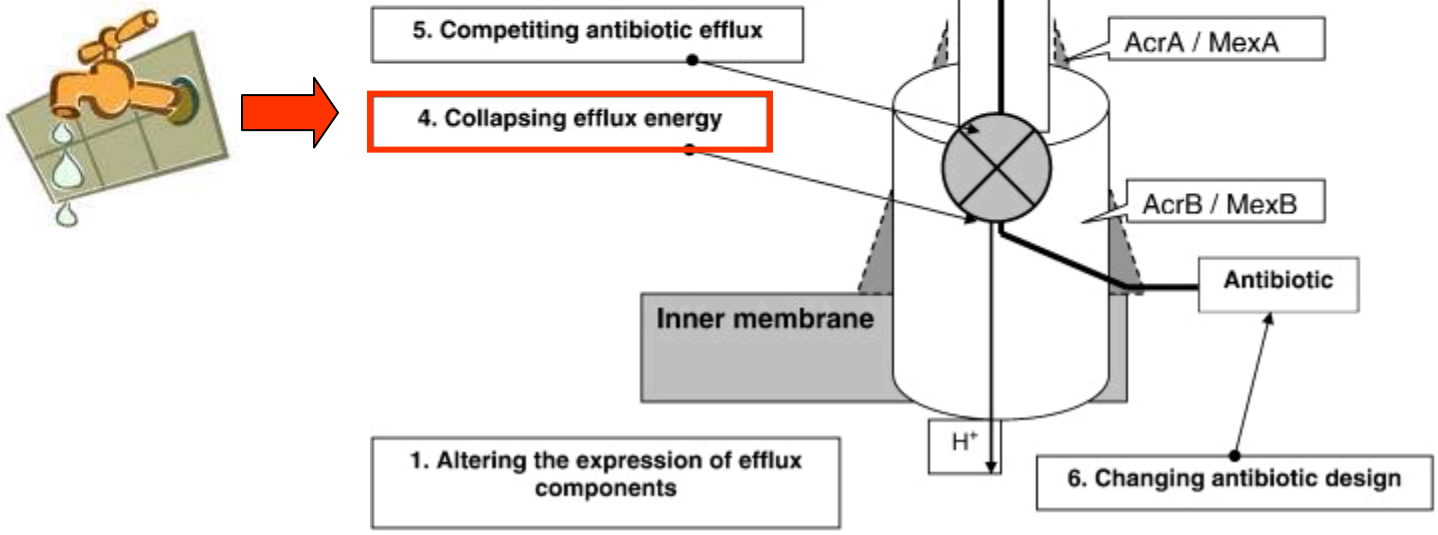


blocking of the channel activity of TolC by $Co(NH_3)_6$

Higgins et al, J Mol Biol. (2004) 342:697-702

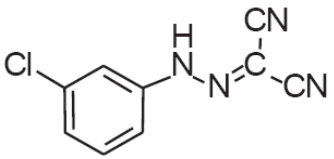
How to prevent resistance by efflux ?

general strategies



Pages & Amaral, Biochim. Biophys. Acta (2009) 1794:826-33

Collapsing energy source



Carbonyl cyanide
m-chlorophenylhydrazone
(CCCP)

proton ionophore

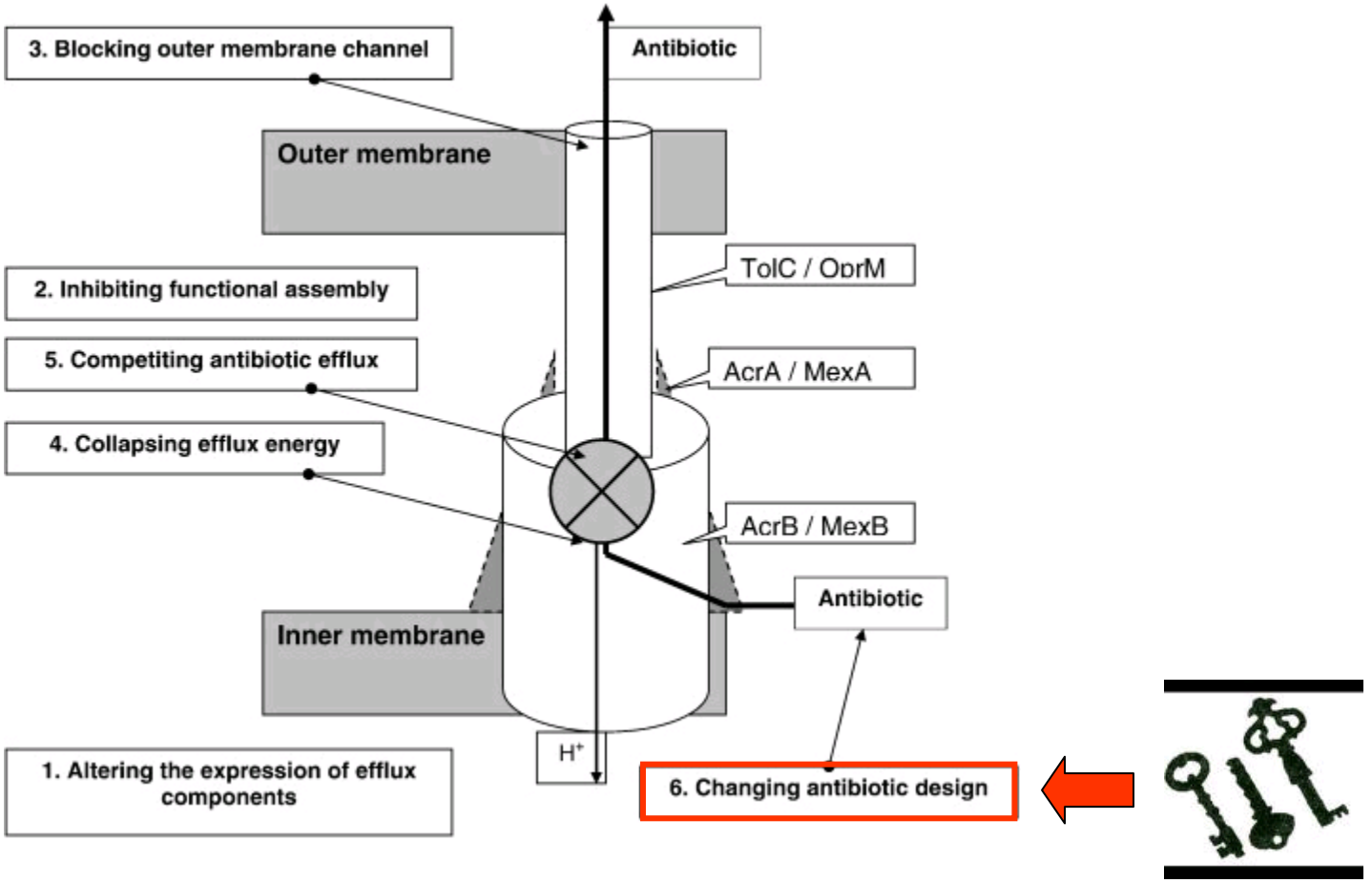
uncoupler of mitochondrial respiration

energy source for most efflux pumps

very useful in vitro!

How to prevent resistance by efflux ?

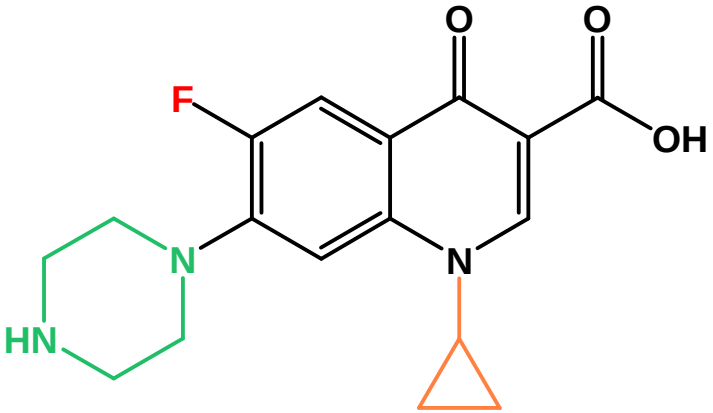
general strategies



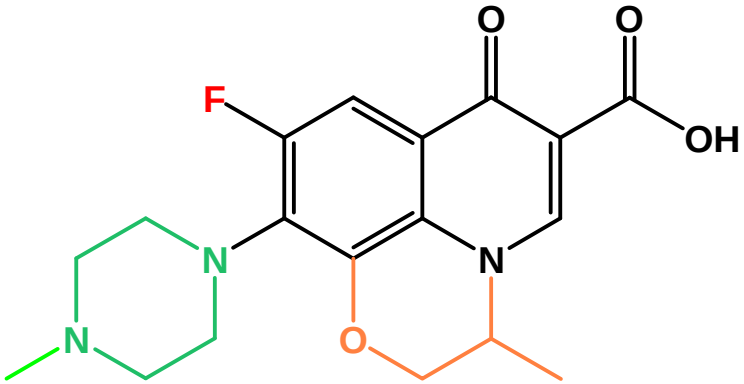
Pages & Amaral, Biochim. Biophys. Acta (2009) 1794:826-33

ciprofloxacin vs levofloxacin

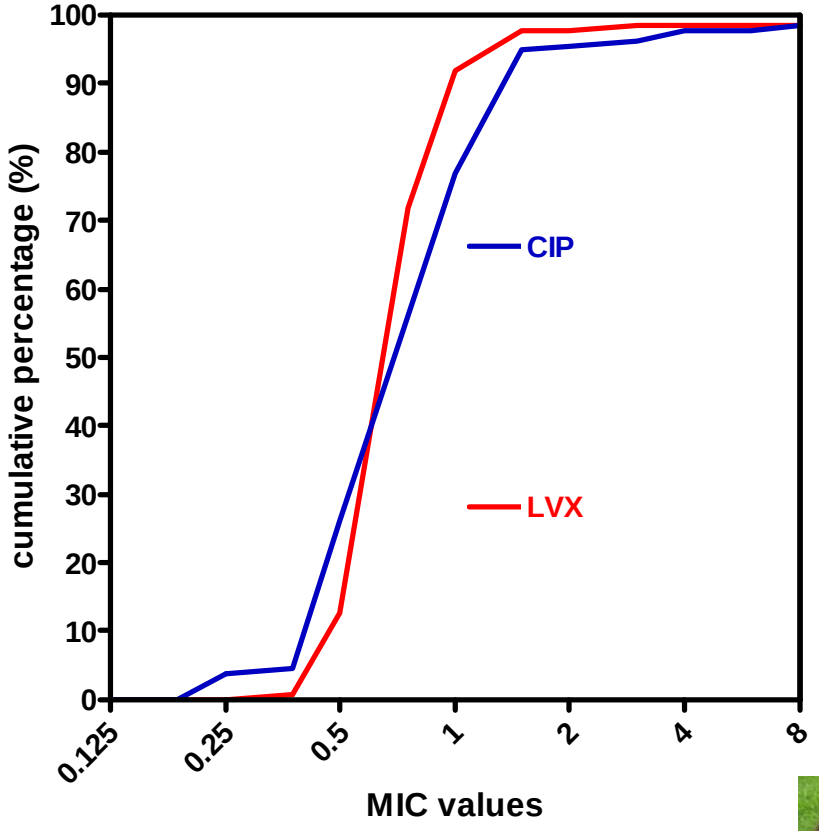
MIC distribution in *S. pneumoniae* isolated from CAP patients



ciprofloxacin



levofloxacin



adapted from Lismond et al, ECCMID 2009-P1068

ciprofloxacin vs levofloxacin

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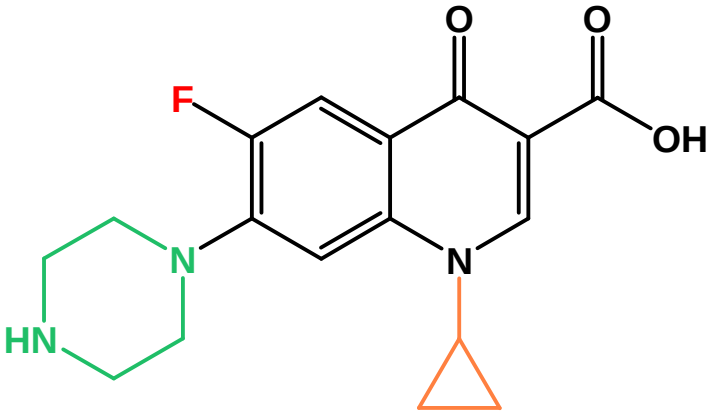
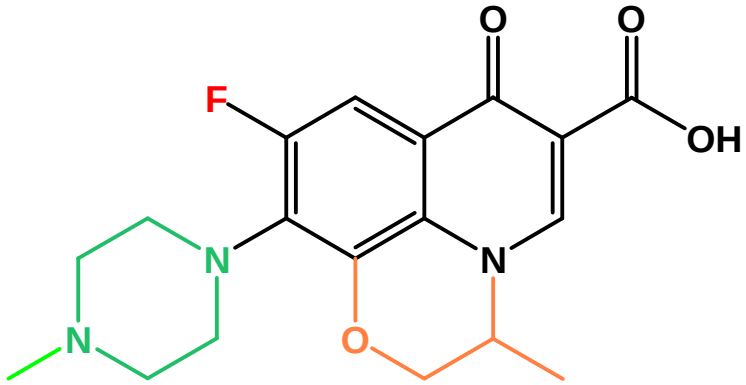
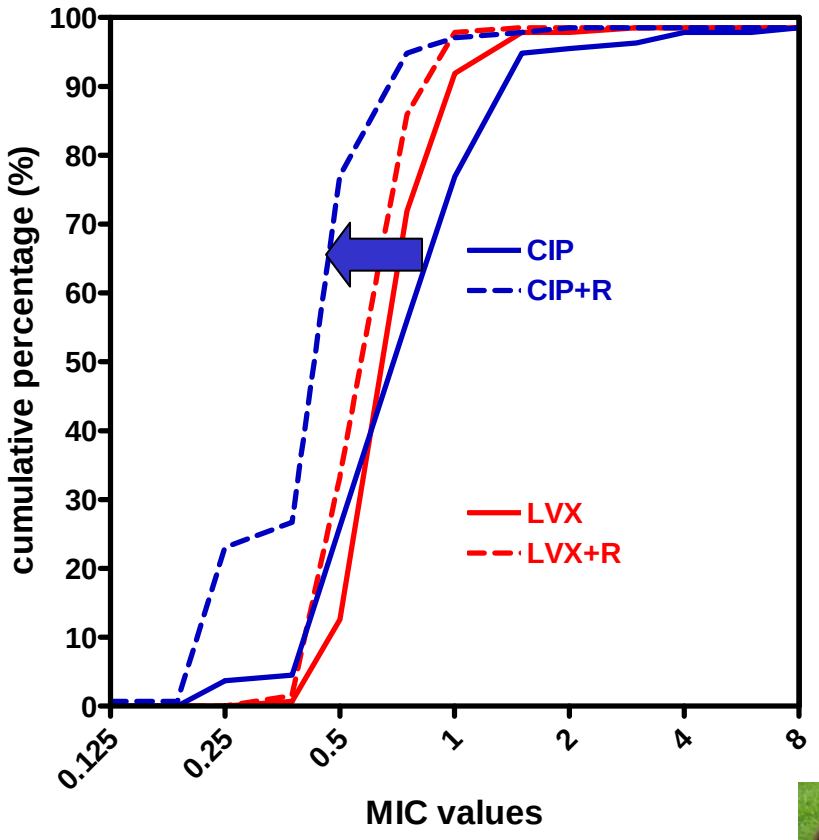


ciprofloxacin

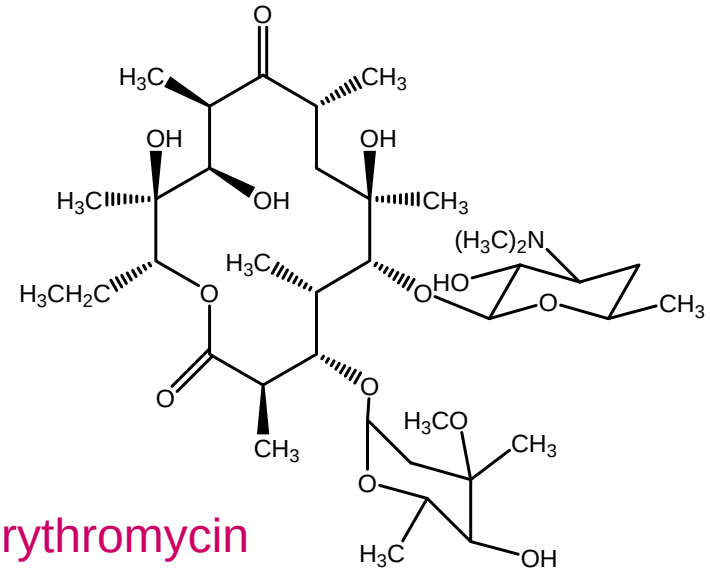


levofloxacin

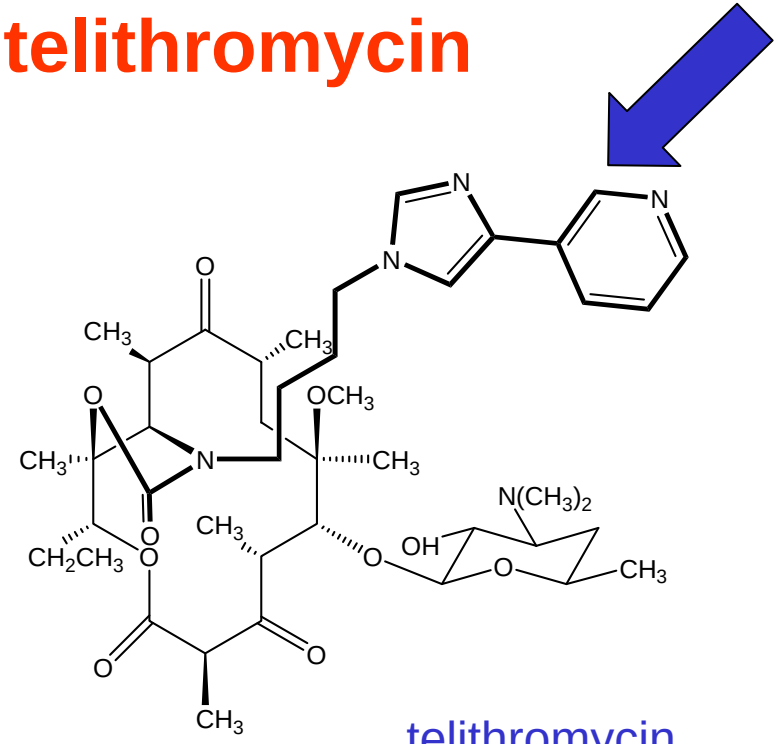


adapted from Lismond et al, ECCMID 2009-P1068

erythromycin vs telithromycin



erythromycin

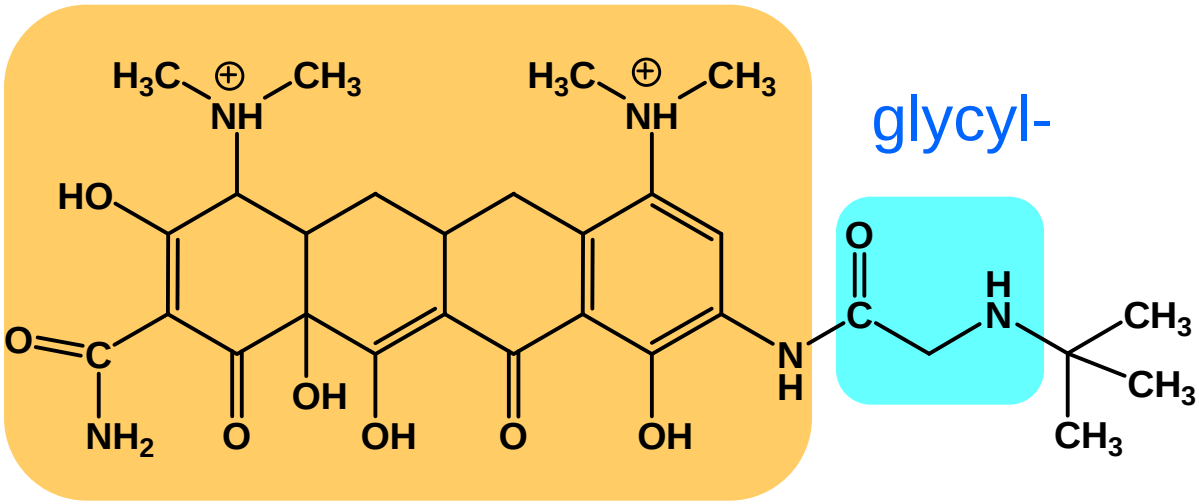


telithromycin

Species		Erythromycin	Telithromycin
S. pyogenes	(WT)	0.03	0.08
	(*mef*)	8	0.5
S. pneumoniae	(WT)	0.03	0.008
	(*mef*)	2	0.125

minocycline vs tigecycline

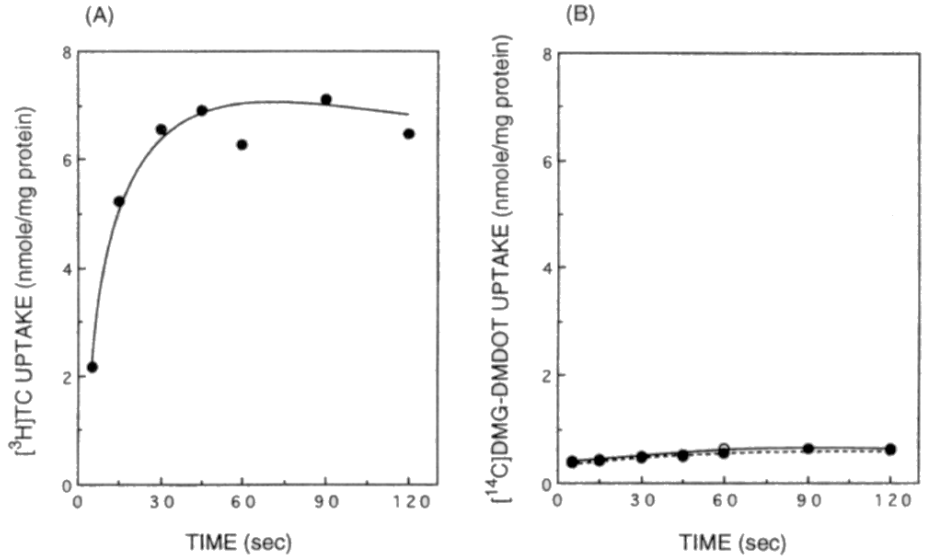
minocycline



glycyl-

tigecycline
[tertbutyl-glycyl-minocycline]

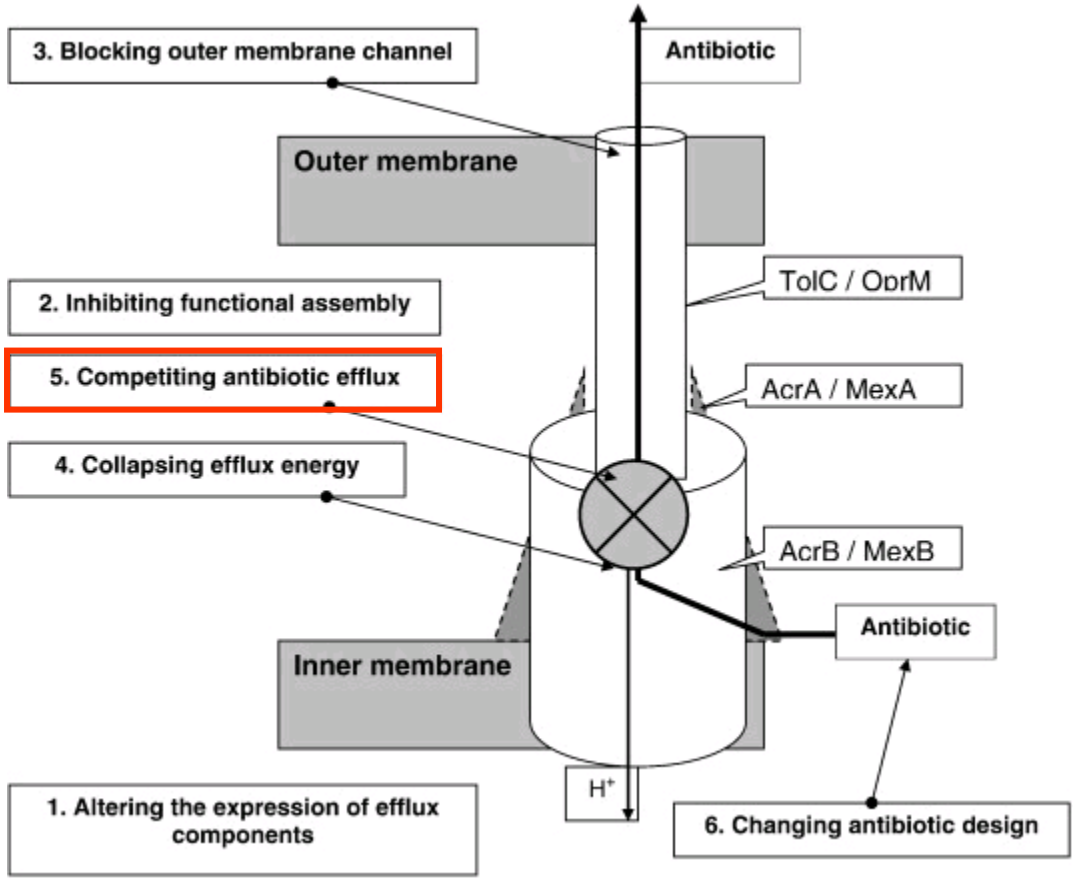
Influx of tetracycline (left)
and glycylcycline (right)
in inverted vesicles



Someya et al, AAC (1995) 39:247-249

How to prevent resistance by efflux ?

general strategies



Pages & Amaral, Biochim. Biophys. Acta (2009) 1794:826-33

Characteristics of the ideal EPI

« to do » list for a winning molecule :

- ✓ Enhance activity of AB in efflux pumps overproducers by inhibiting efflux

- ✓ Not affect AB activity in strains lacking efflux pumps
- ✓ Not potentiate activity of AB that are not effluxed
- ✓ Not affect proton gradients across the inner membrane (Gram-negative bacteria)

- ✓ Not affect eucaryotic efflux pumps

Lomovskaya et al. AAC (2001) 45:105-116

Different types of EPI

- Pharmacological agents fortuitously found to inhibit efflux
  - BUT active at supra-therapeutic concentrations !
  - unusable in vivo because of toxicity !
- Analogs of antibiotic substrates
  - narrow spectrum efflux pumps ?
- Natural molecules and semi-synthetic derivatives thereof
  - standardisation
- New chemical entities
  - often partial characterization against a small number of bacteria and for a few antibiotics
  - potential interest under-estimated ?

Van Bambeke et al. Rec. Patents Antiinf. Drug Discov. (2006) 1:157-175

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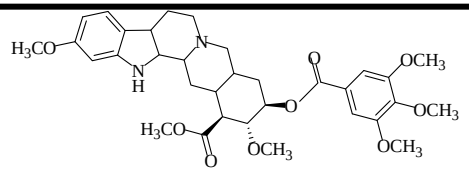
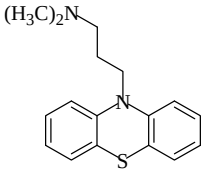
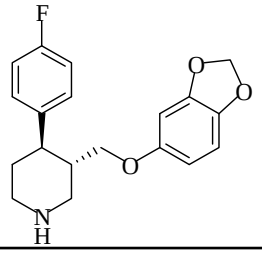
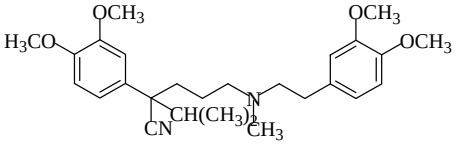
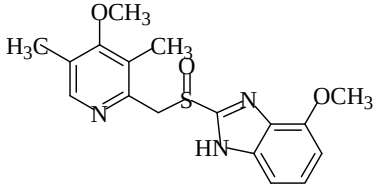
Pharmacological agents

Type of inhibitor	structure	activity		
		ABs	bacteria	µg/ml
alkaloids (reserpine)		FQ	*S. pneumo* *S. aureus*	20
phenothiazines (chlorpromazine)		TET	*E. coli*	45
selective serotonin reuptake inhibitors (paroxetine)		TET isoniazid	*E. coli* *M. smegmatis*	120 25
Ca $^{2+}$ antagonists (verapamil)		FQ TET	*S. aureus* *E. coli*	20
proton pump inhibitors (omeprazole)		FQ	*S. aureus*	100

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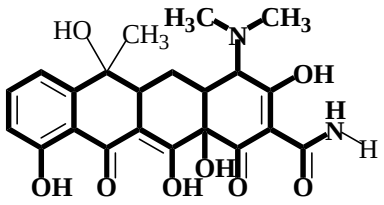
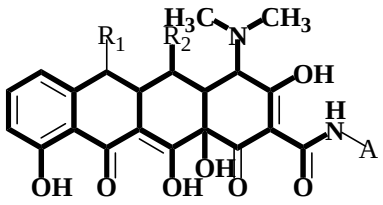
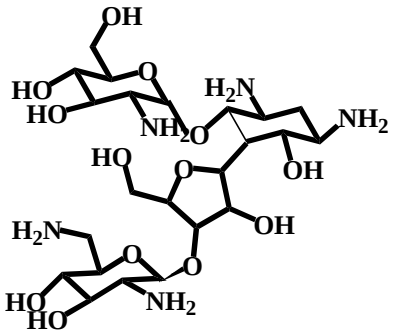
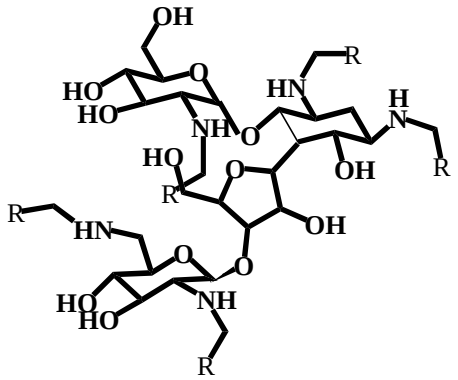
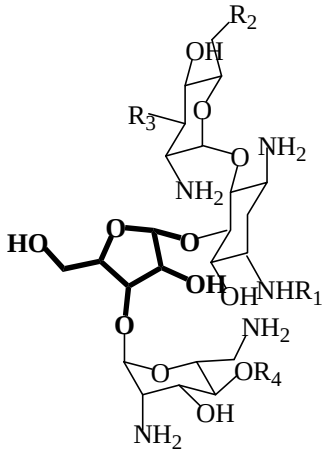
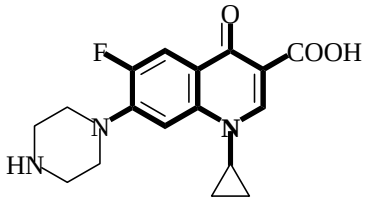
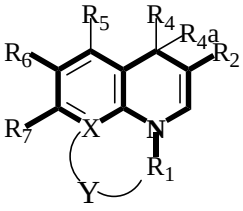
Van Bambeke et al. Rec. Patents Antiinf. Drug Discov. (2006) 1:157-175

Analogs of antibiotic substrates

	antibiotic	inhibitors	
TET	 tetracycline	 (1-16 µg/ml)	
AGs	 paromomycin		
FQ	 ciprofloxacin	 (4-20 µg/ml)	

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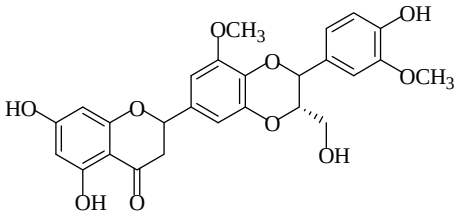
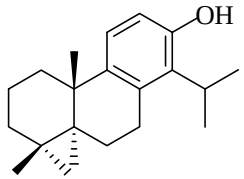
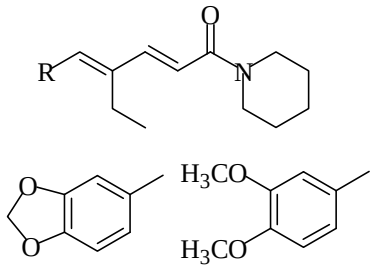
Van Bambeke et al. Rec. Patents Antiinf. Drug Discov. (2006) 1:157-175

Natural products

Type of inhibitor	structure	activity		
		ABs	bacteria	µg/ml
flavonolignans		FQ	*S. aureus*	10
phenolic diterpenes		ML FQ TET	*S. aureus*	1
piperine analogues		FQ	*S. aureus*	6.25

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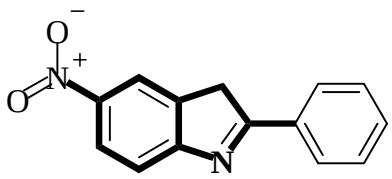
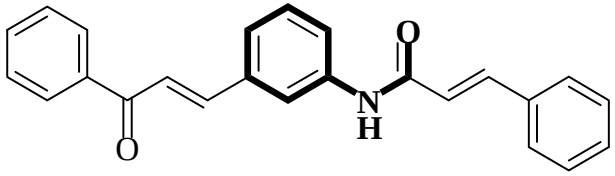
Van Bambeke et al. Rec. Patents Antiinf. Drug Discov. (2006) 1:157-175

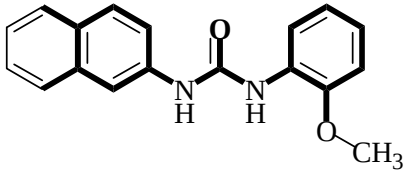
New chemical entities ~ Gram(+) efflux pumps



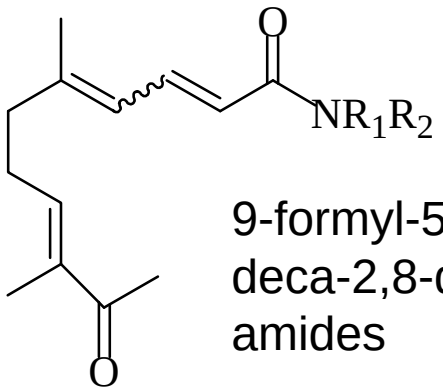
indoles



aromatic amides

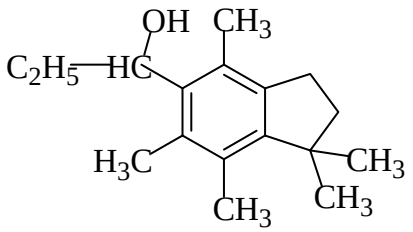


ureas



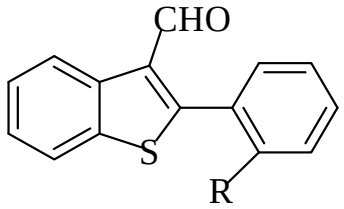
9-formyl-5-methyl-deca-2,8-dienoic acid amides

FQ



indans

TET

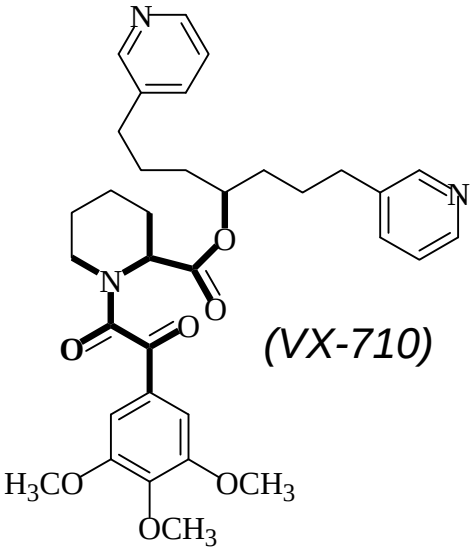


thiophenes

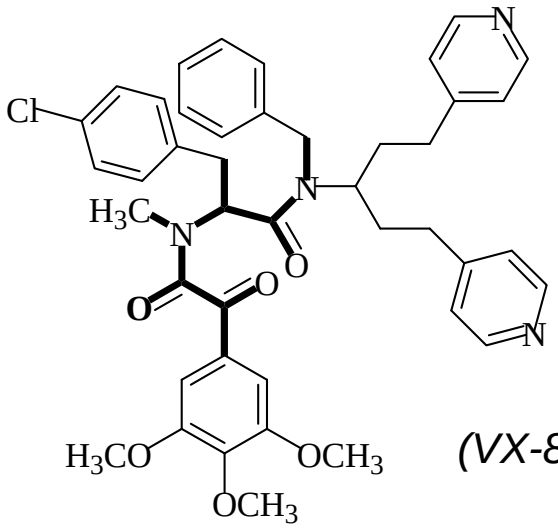
ML, FQ

New chemical entities ~ Gram(+) efflux pumps

piperidines-carboxylic acid derivatives



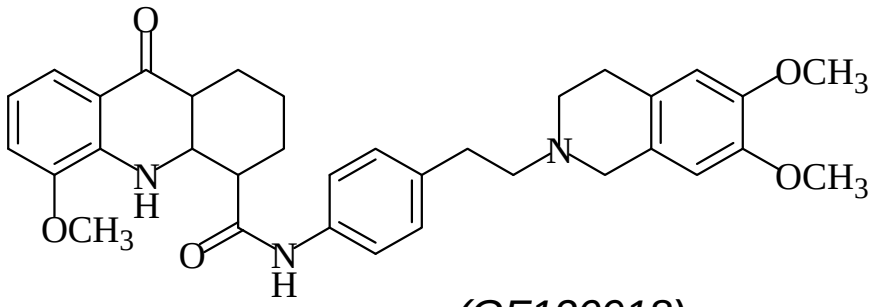
(VX-710)



(VX-853)

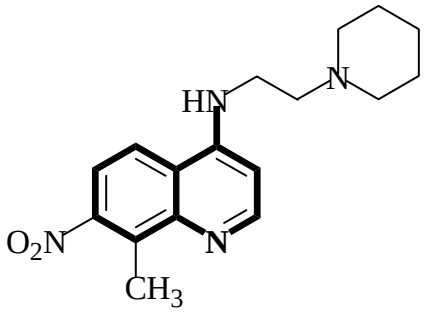
also inhibitors of human P-gp

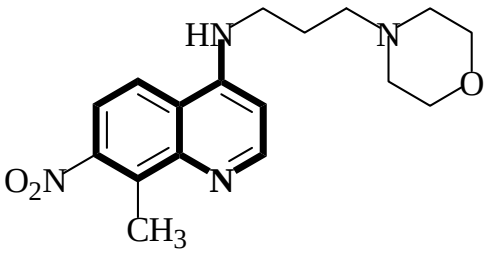
acridine carboxamides



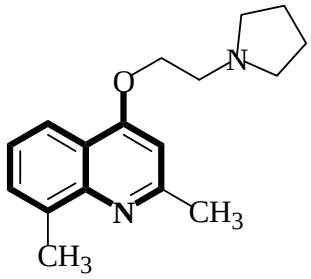
(GF120918)

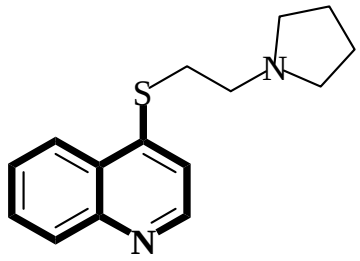
New chemical entities ~ Gram(-) efflux pumps



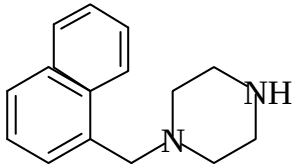


alkylaminoquinolines

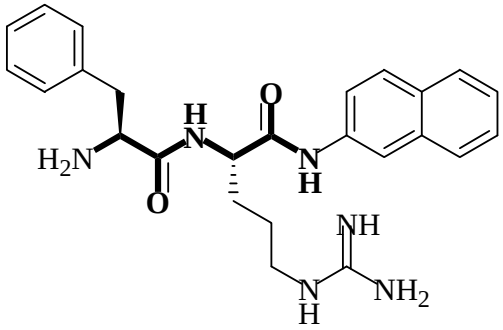




alkoxy- and thioalkoxy-quinolines



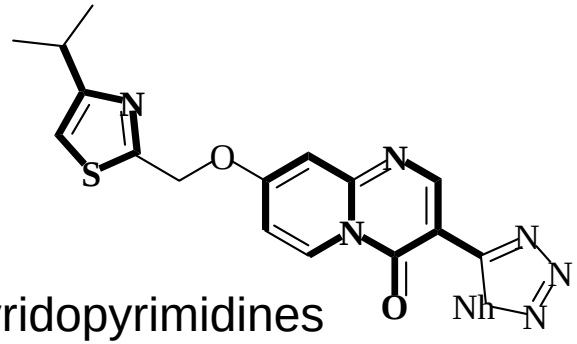
arylpiperazines



peptidomimetics

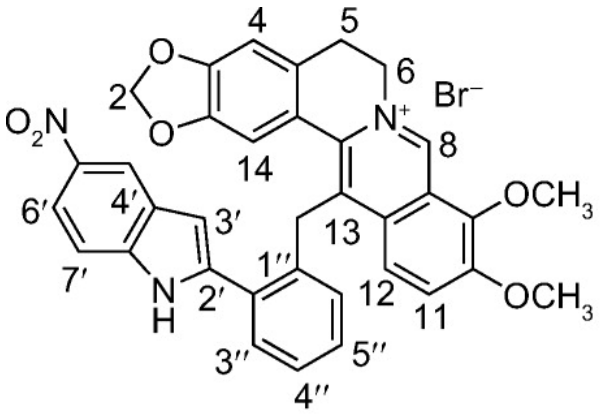
broad spectrum
→ do not bind to the « substrate » site!

specific of MexAB-OprM in *P. aeruginosa*

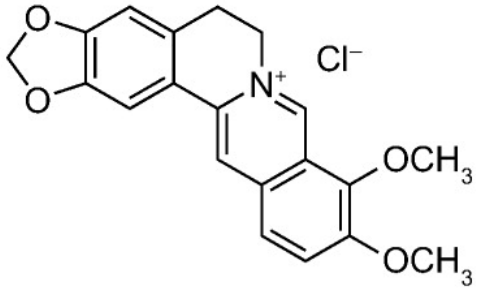


pyridopyrimidines

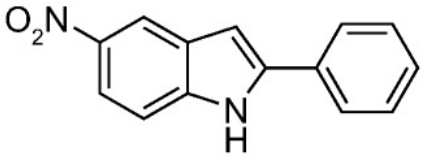
New chemical entities : hybrids (AB+inhibitor)



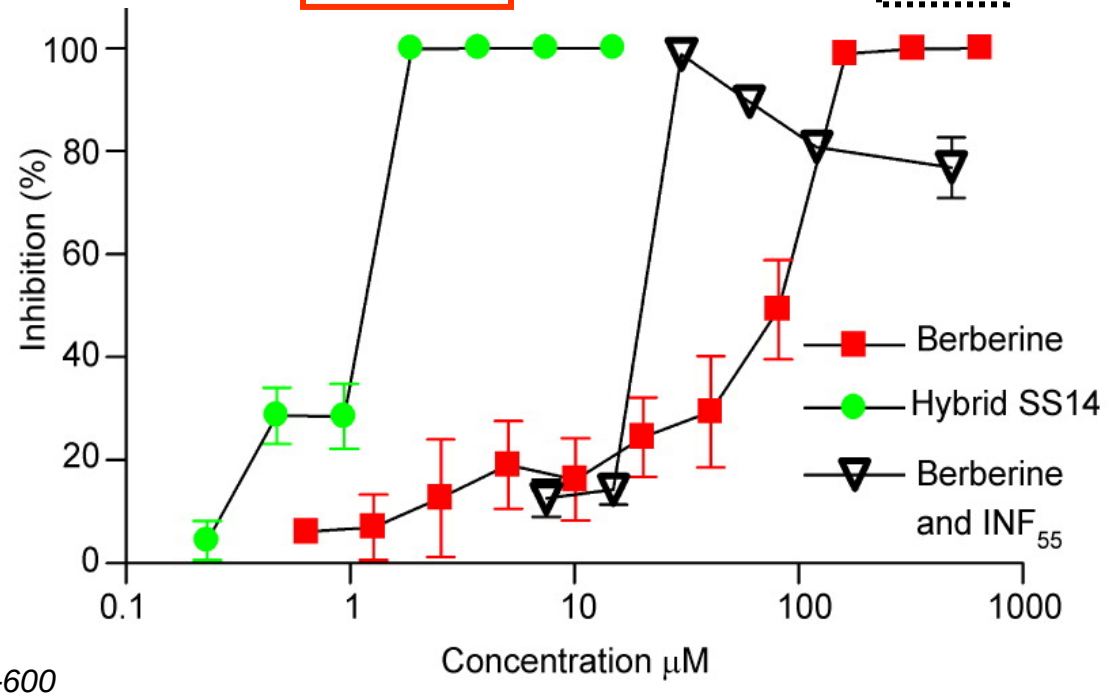
SS14



Berberine



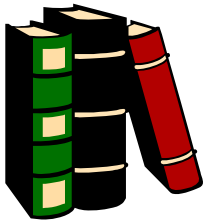
INF$_{55}$



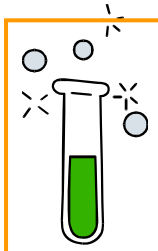
Ball et al, ACS Chem Biol. (2006) 1:594-600

An example of development : peptidomimetics from Mpex Pharmaceuticals….

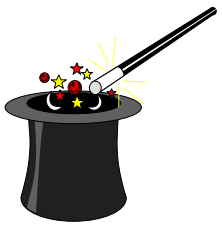
An example of development : peptidomimetics from Mpex Pharmaceuticals….

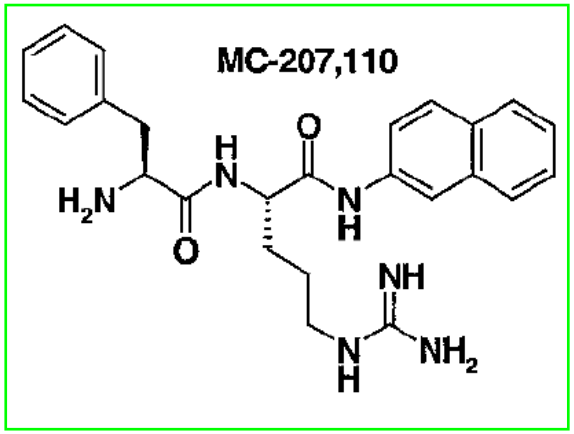


librairy of 200,000 synthetic and natural compounds



in vitro screening in combination with levofloxacin against *P. aeruginosa* overexpressing Mex pumps





Renau et al. J. Med. Chem. (1999) 42: 4928-31; Lomovskaya et al. JMMB (2001) 3: 225-36

Demonstration of in vitro activity

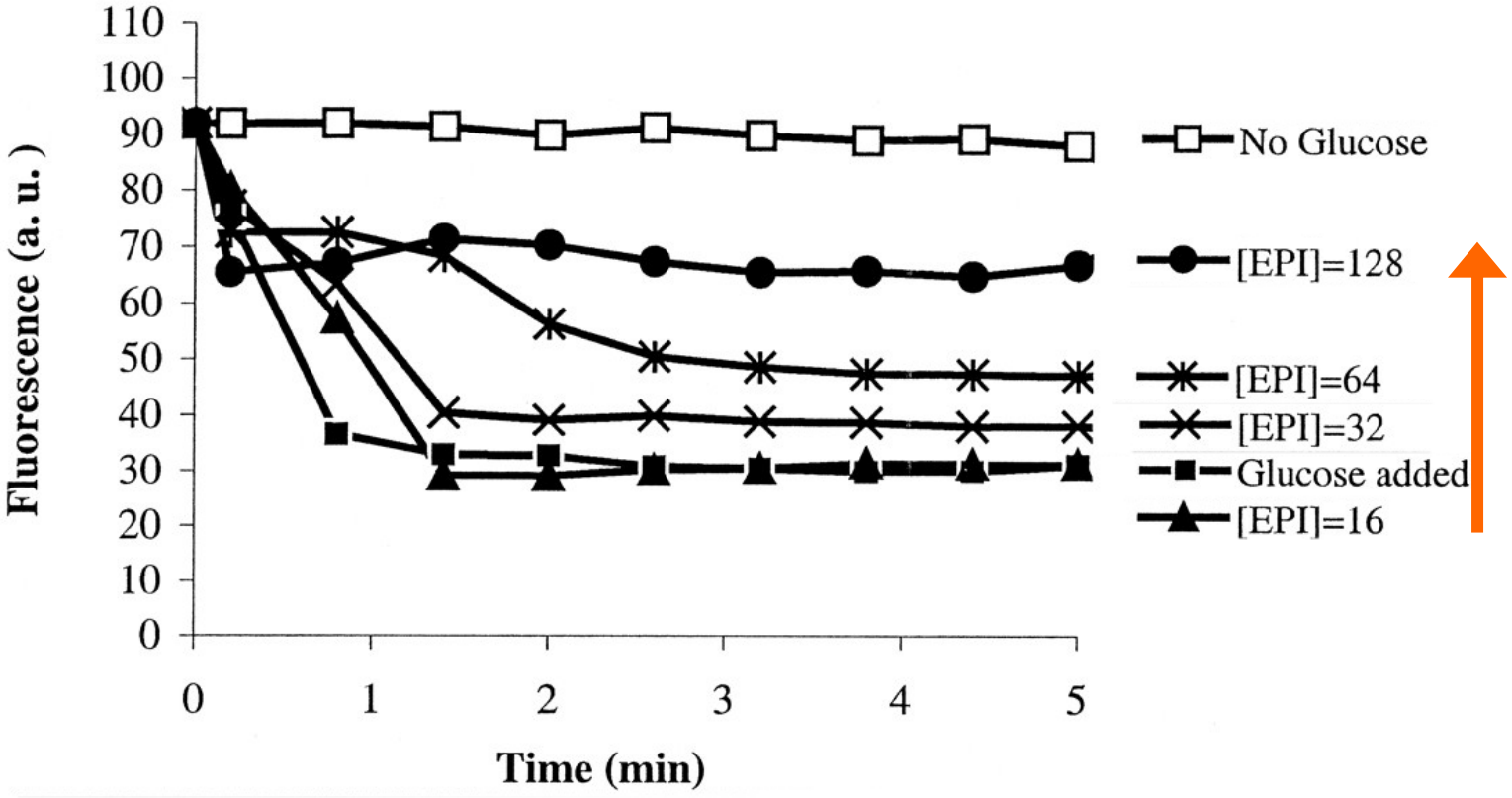
EPI are as effective as disruption of pump genes to restore antibiotic efficacy !

	MIC ratio	
antibiotic	**WT strain / Δ MexAB-OprM**	**AB / AB + MC-207,110**
levofloxacin	64	32
sparfloxacin	32	128
erythromycin	32	32
chloramphenicol	512	128

Lomovskaya et al, AAC (2001) 45:105-116

Demonstration of the mode of action

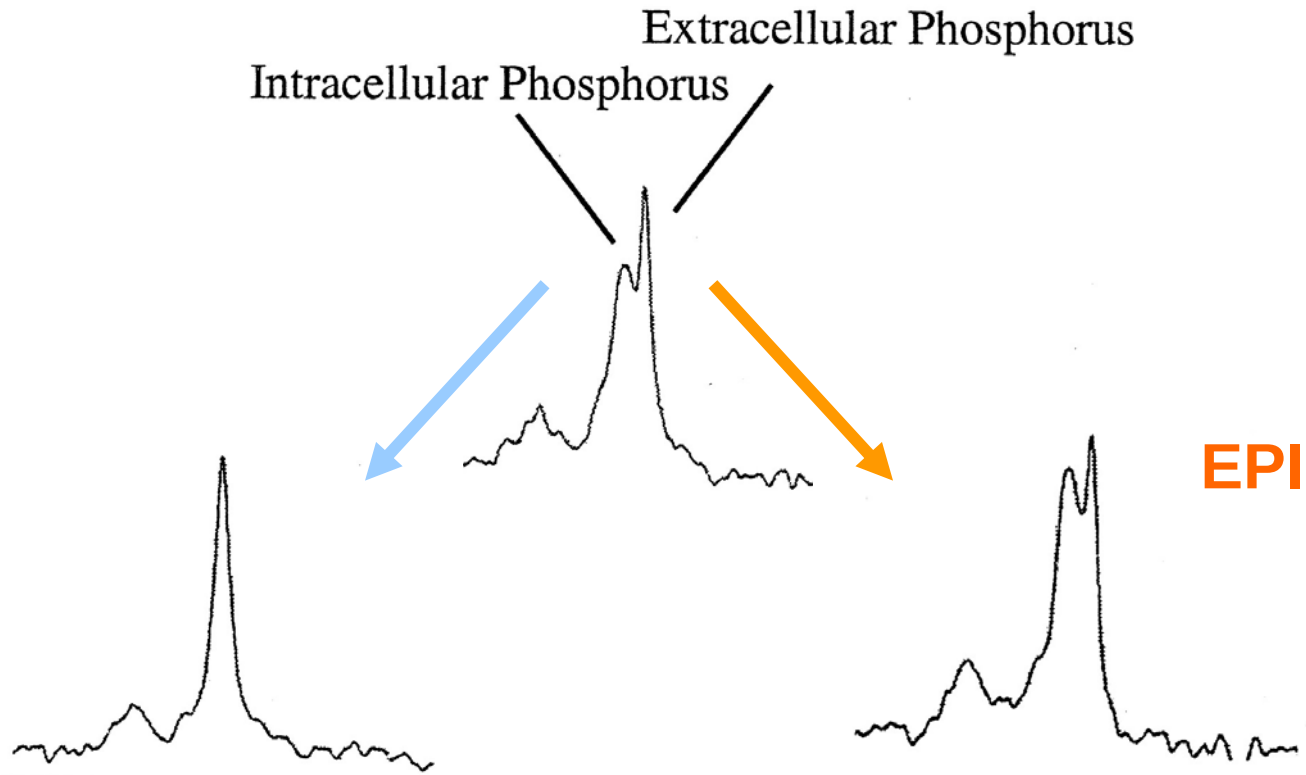
Concentration-dependent inhibition of *N*-phenyl-1-naphtylamine efflux



Ocaktan et al. JBC (1997) 272: 21964-69; Lomovskaya et al. AAC (2001) 45:105-116

EPI does not affect proton gradients across the IM

NMR spectra of ^{31}P to detect pH gradients

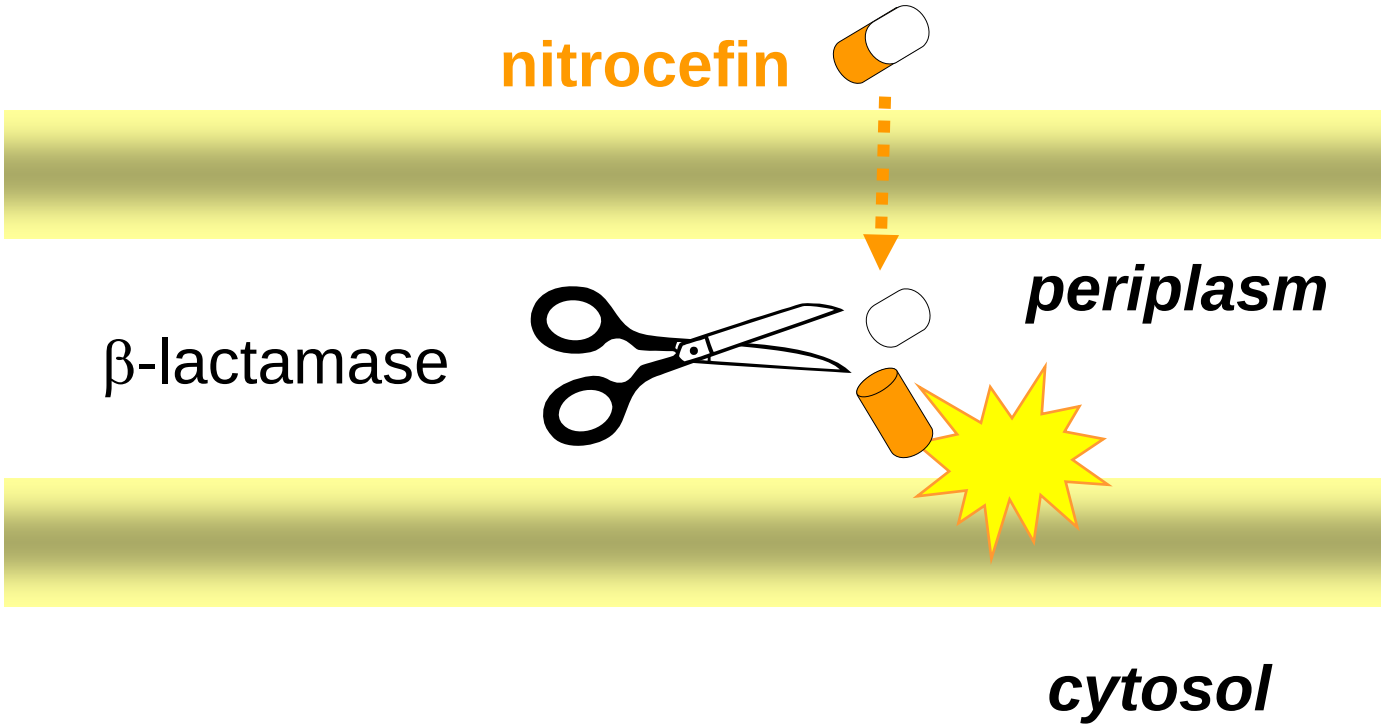


other mode of action ?

Lomovskaya et al. AAC (2001) 45:105-116

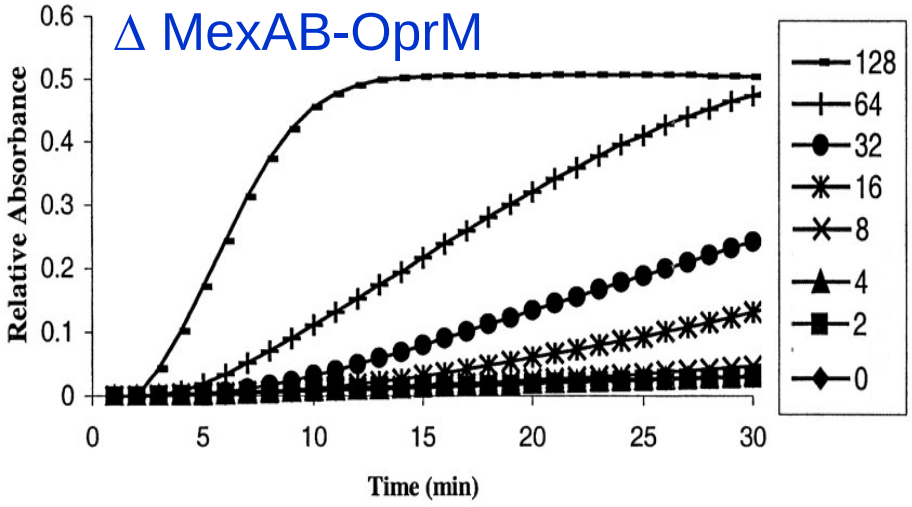
EPI as permeabilizing agents in strains lacking efflux pumps ?

Testing the hydrolysis rate of a non permeant β-lactam

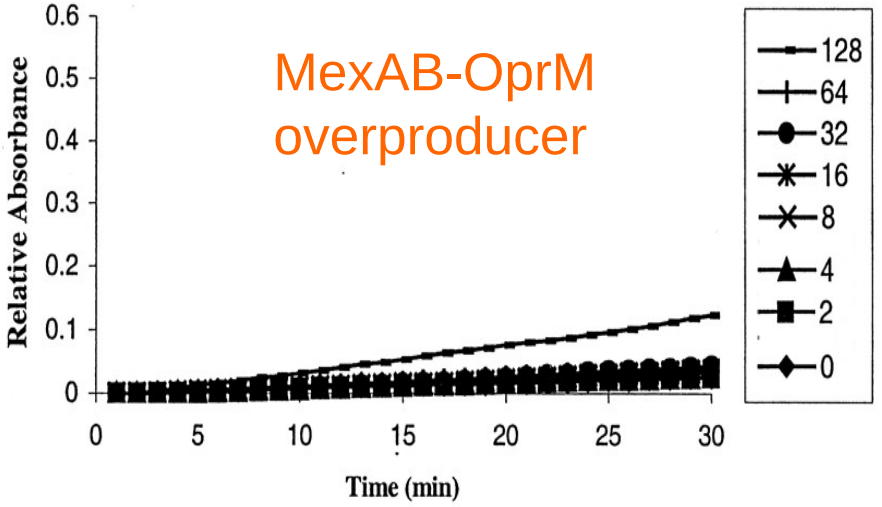


Lomovskaya et al. AAC (2001) 45:105-116

EPI as permeabilizing agents in strains lacking efflux pumps ?



EPI increases OM permeability when MexAB-OprM not functional

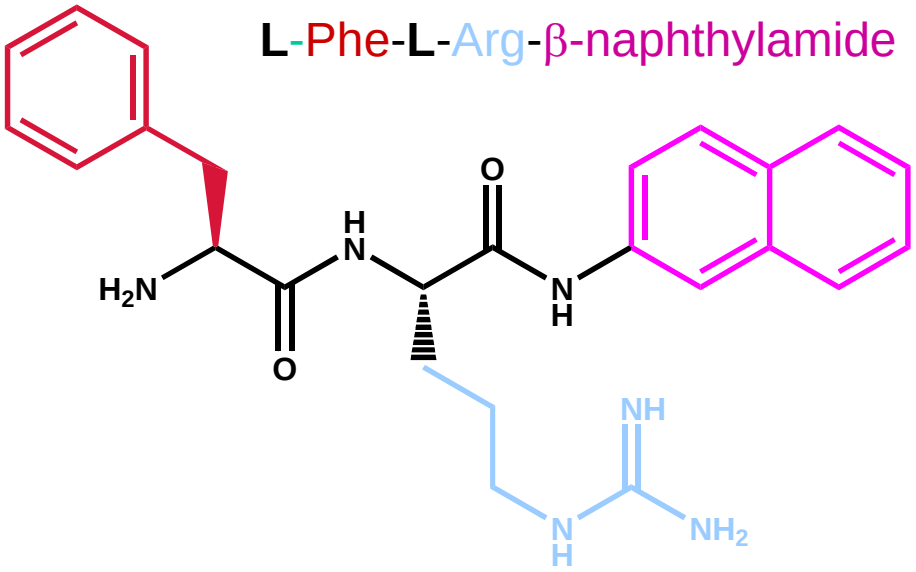


EPI as substrates of efflux pumps ?

Lomovskaya et al. AAC (2001) 45:105-116

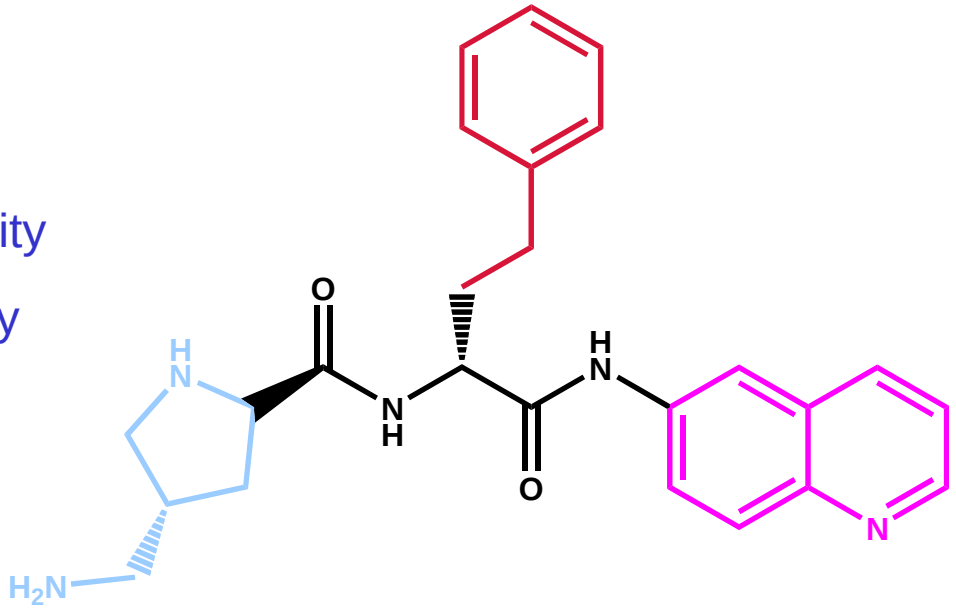
On the way to a « drug-able » molecule ….

L-Phe-L-Arg-β-naphthylamide



- switching amino-acids position keeps activity
- using D-series amino-acids confers stability
- conformationally restricted analogues avoid toxicity

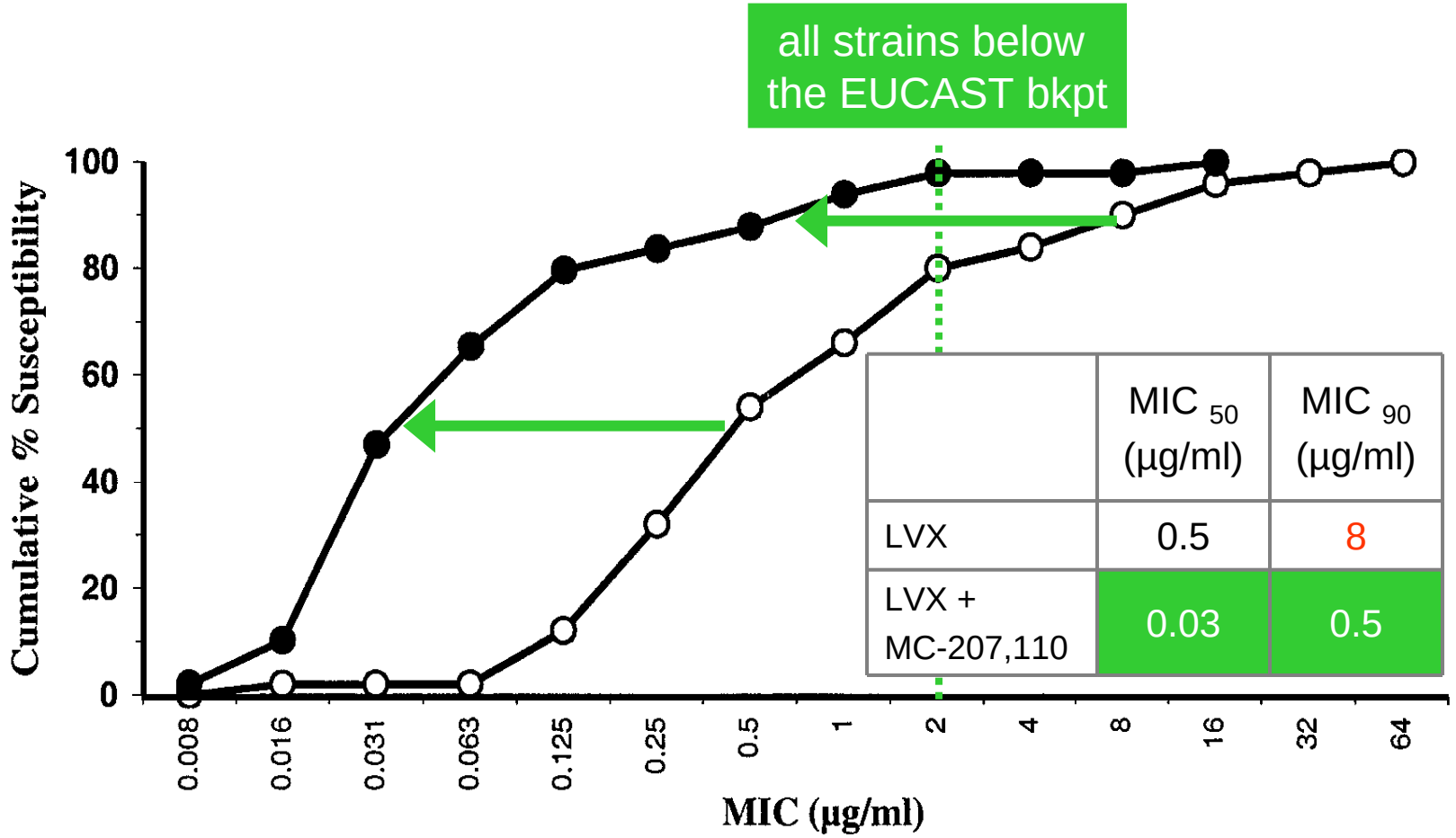
4-aminomethyl-
2-pyrrolidinecarboxamide
-D-hPhe-6-aminoquinoline



Renau et al. Bioorg. Med. Chem Lett. (2001) 11:663-7; (2003) 13:2755-58

EPI increases susceptibility of clinical isolates

MIC distribution for levofloxacin in clinical isolates of *P. aeruginosa*



	MIC $_{50}$ (µg/ml)	MIC $_{90}$ (µg/ml)
LVX	0.5	8
LVX + MC-207,110	0.03	0.5

Lomovskaya et al. JMMB (2001) 3: 225-36

EPI as adjuvant therapy

EPI (MC-04,124) potentiates levofloxacin activity in *P. aeruginosa* mouse thigh model

regimen	LVX MIC (mg/L)	effective regrowth time (h)	max Δ log CFU
LVX (30 mg/kg)	2	3	0.1
LVX (30 mg/kg) + MC-04,124 (25 mg/kg)	0.125	13	3.6

Griffith et al. ICAAC (2001) F-340

The story is not yet finished …..

Mpex Pharmaceuticals

Home : Product Development Programs : Team : News : Employment : Contact

Product Development Programs:

Efflux Pump Inhibitor Program

Mpex's lead EPI compounds are now being optimized in anticipation of selecting development candidates. In 2008, Mpex entered into an alliance with GlaxoSmithKline on this program to develop multiple fixed-combination drug products consisting of an antibiotic and an EPI for systemic treatment of serious infections due to multi-drug resistant (MDR) gram-negative bacterial pathogens.

Potential interests of efflux pumps inhibitors

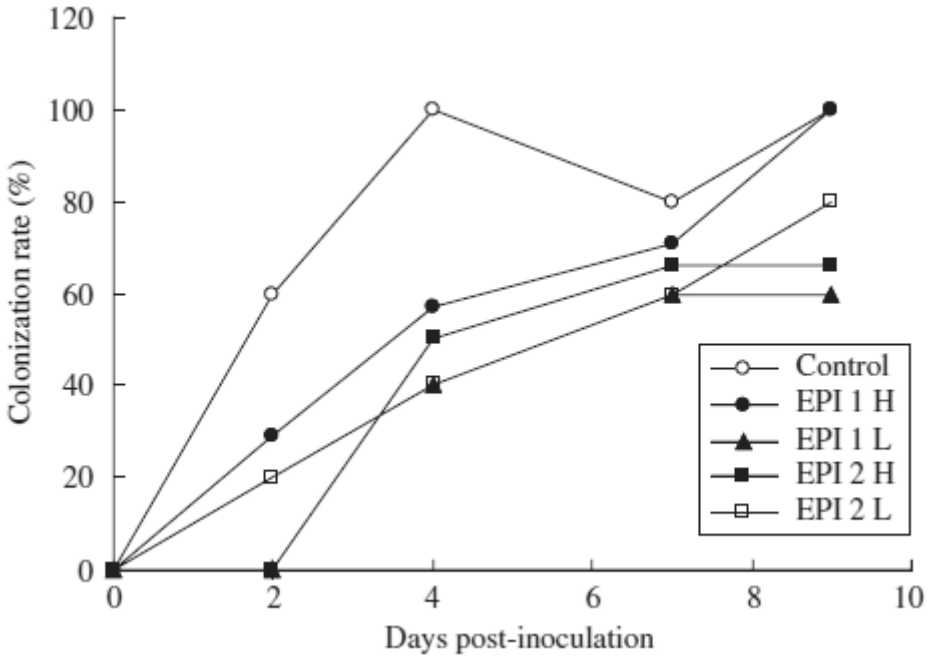
Old Faithful Geyser

Prevention of colonization

Efflux pumps contribute to resistance to bile salts in enteric pathogens

EPI prevent colonization of chicken by *Campylobacter* by increasing susceptibility to bile salts

Antimicrobial	MIC (mg/L) MH	MH + MC[a]
Sodium dodecyl sulphate	256	4 (64)
Cholate	8000	250 (32)
Taurocholate	64 000	500 (128)
Chenodeoxy cholate	8000	125 (64)
Glycocholate	32 000	250 (128)

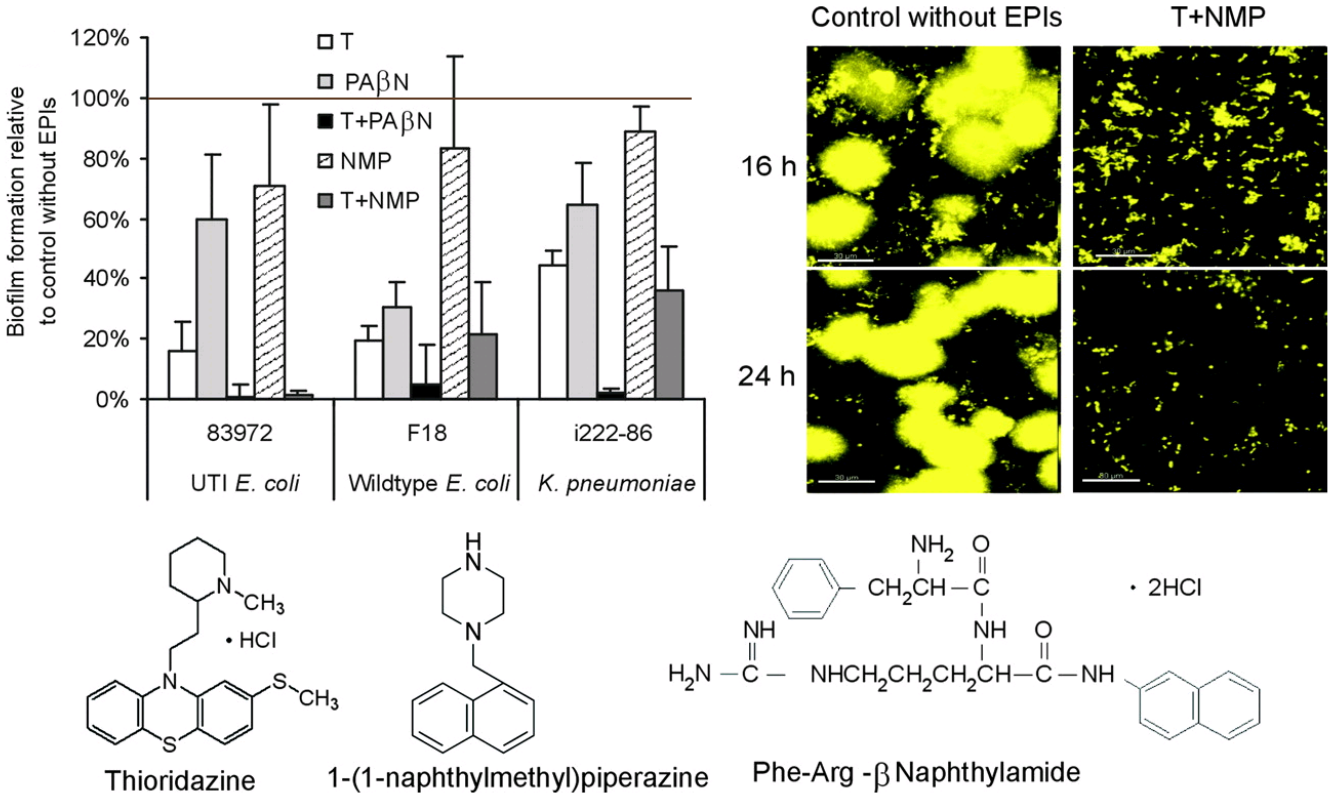


Lin & Martinez, JAC (2006) 58:966-72

Inhibition of biofilm formation

Efflux pumps contribute to biofilm formation

EPI decrease biofilm formation



Kvist et al, Appl Environ Microbiol. (2008) 74:7376-82

Increased activity against intracellular bacteria

L. monocytogenes in broth

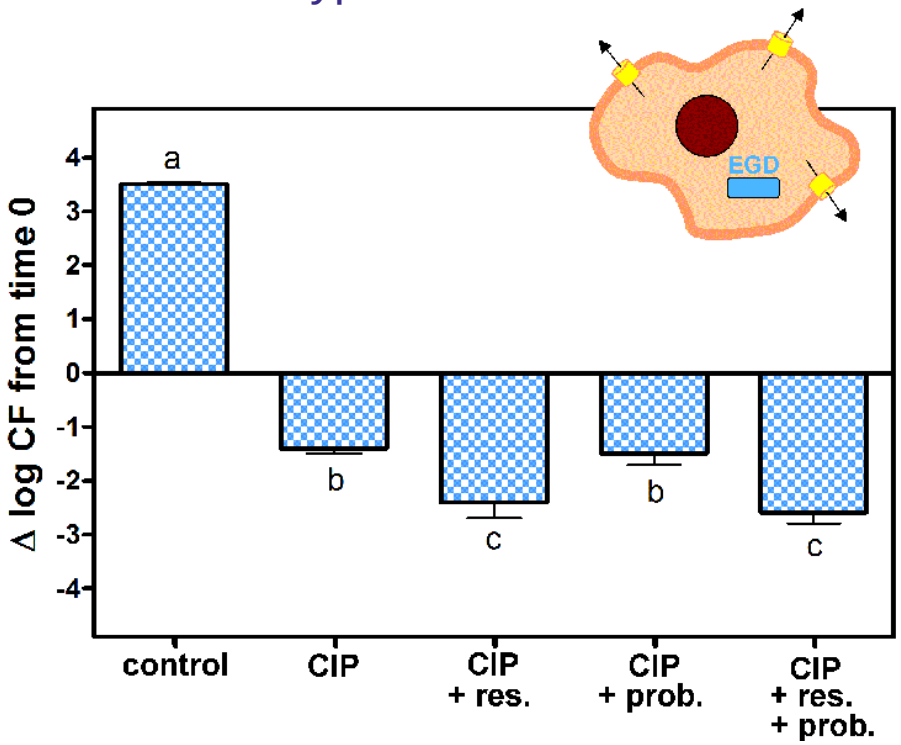
quinolone	MIC (mg/L)			
	EGD		CLIP	
	Res. (-)	Res. (+)	Res. (-)	Res. (+)
CIP	1.2	1.0	**5.0**	1.0
MXF	0.6	0.6	0.5	0.25

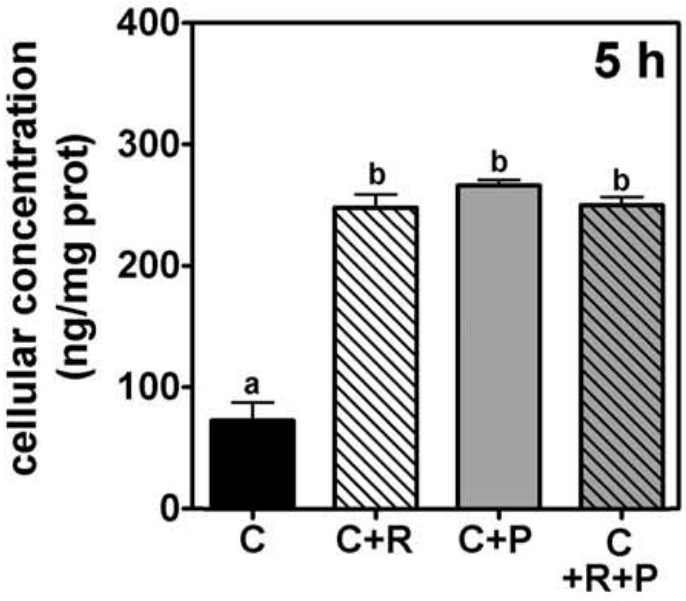
Lismond et al., Antimicrob. Ag. Chemother. (2008) 52:3040-46

Increased activity against intracellular bacteria

Ciprofloxacin and *Listeria* inside macrophages

Wild-type cells and bacteria



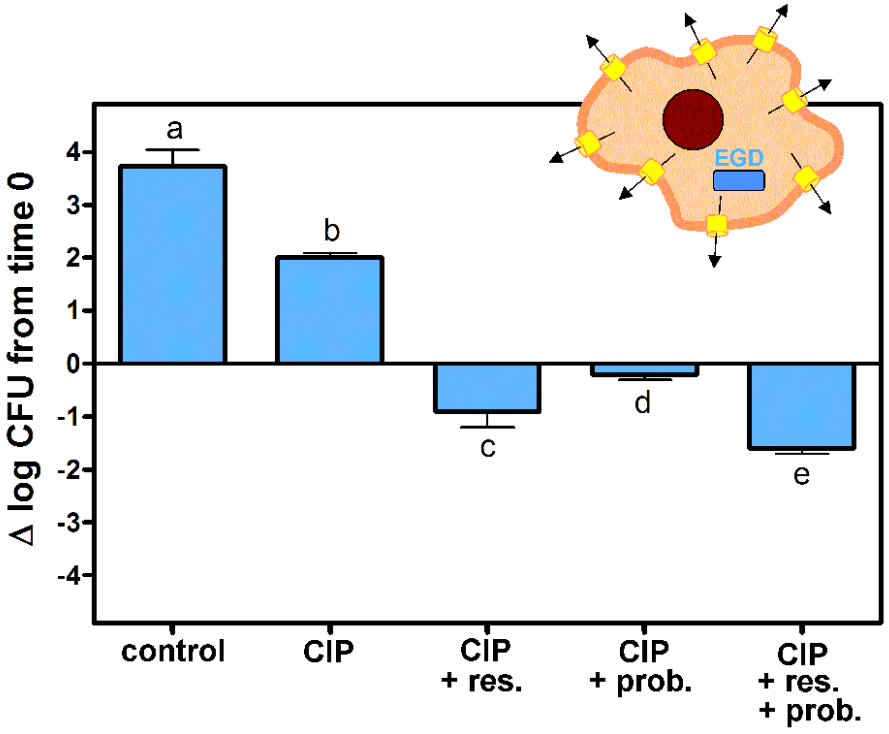


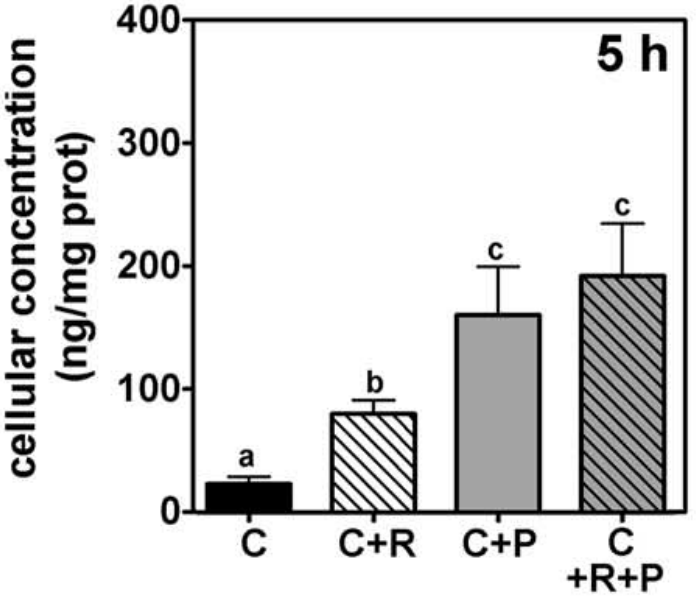
Lismond et al., Antimicrob. Ag. Chemother. (2008) 52:3040-46

Increased activity against intracellular bacteria

Ciprofloxacin and *Listeria* inside « resistant » macrophages

cells and overproducing
efflux pumps for ciprofloxacin



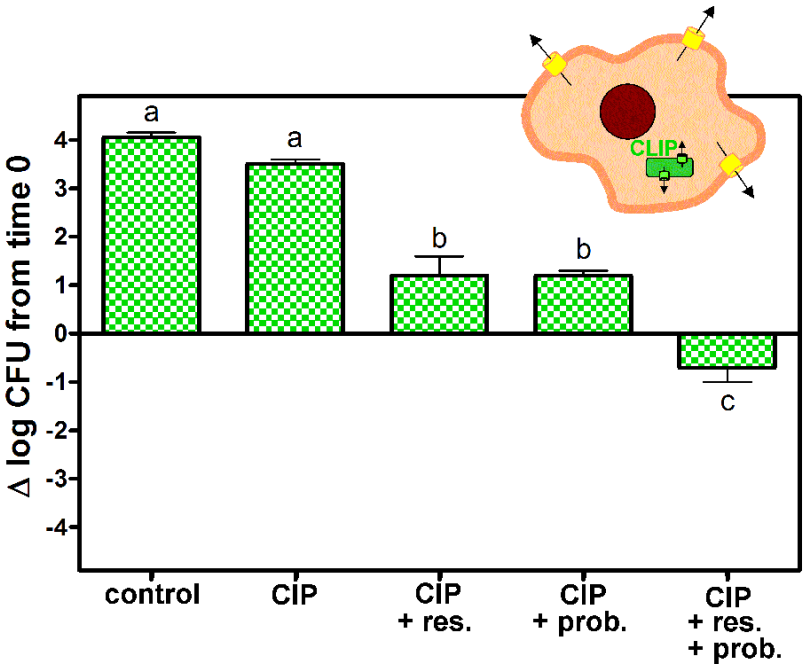


Lismond et al., Antimicrob. Ag. Chemother. (2008) 52:3040-46

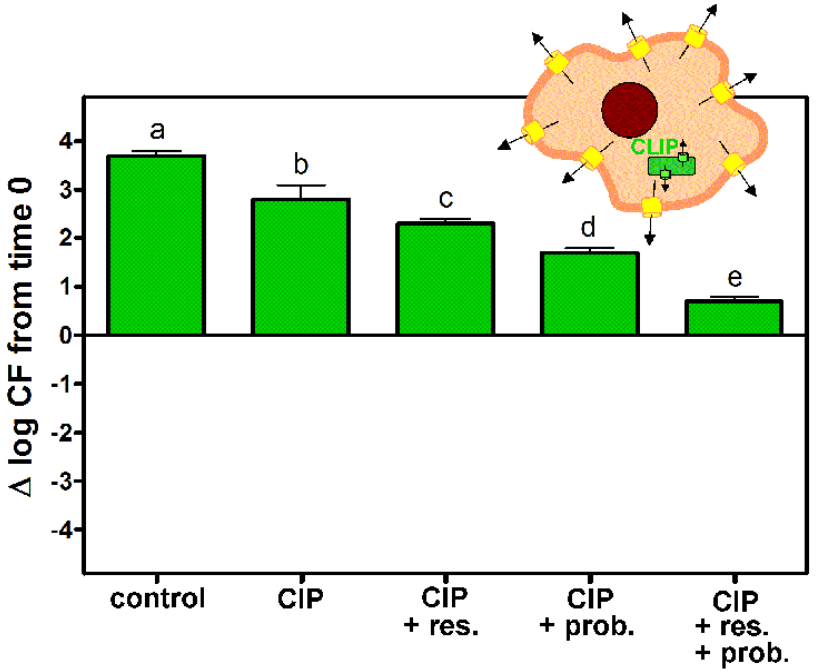
Increased activity against intracellular bacteria

Ciprofloxacin and resistant *Listeria* inside macrophages

bacteria overproducing efflux pumps for ciprofloxacin



bacteria and cells overproducing efflux pumps for ciprofloxacin

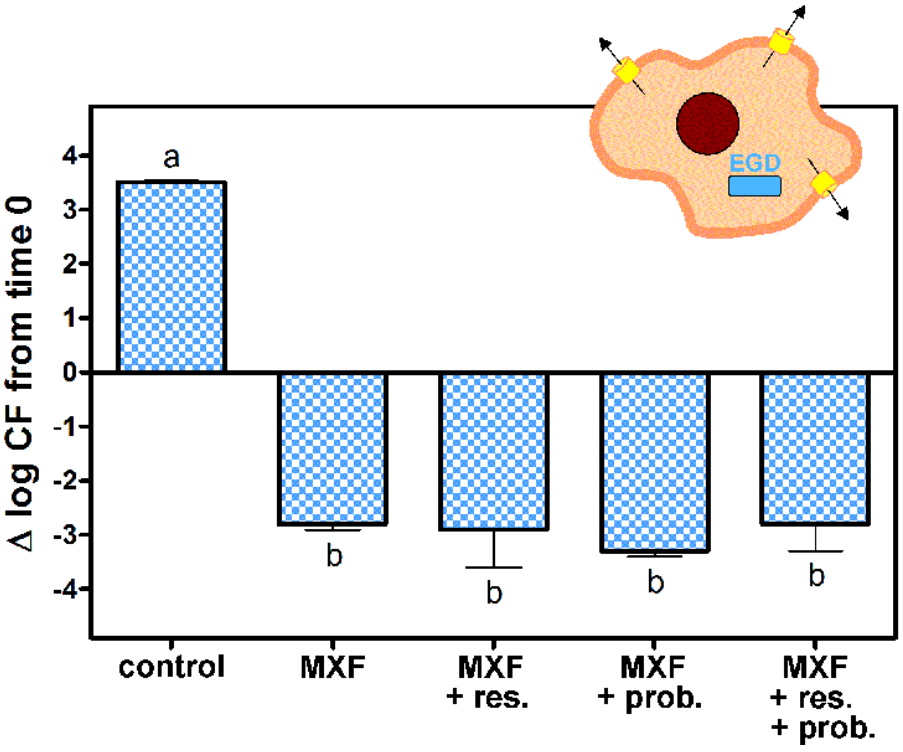


Lismond et al., Antimicrob. Ag. Chemother. (2008) 52:3040-46

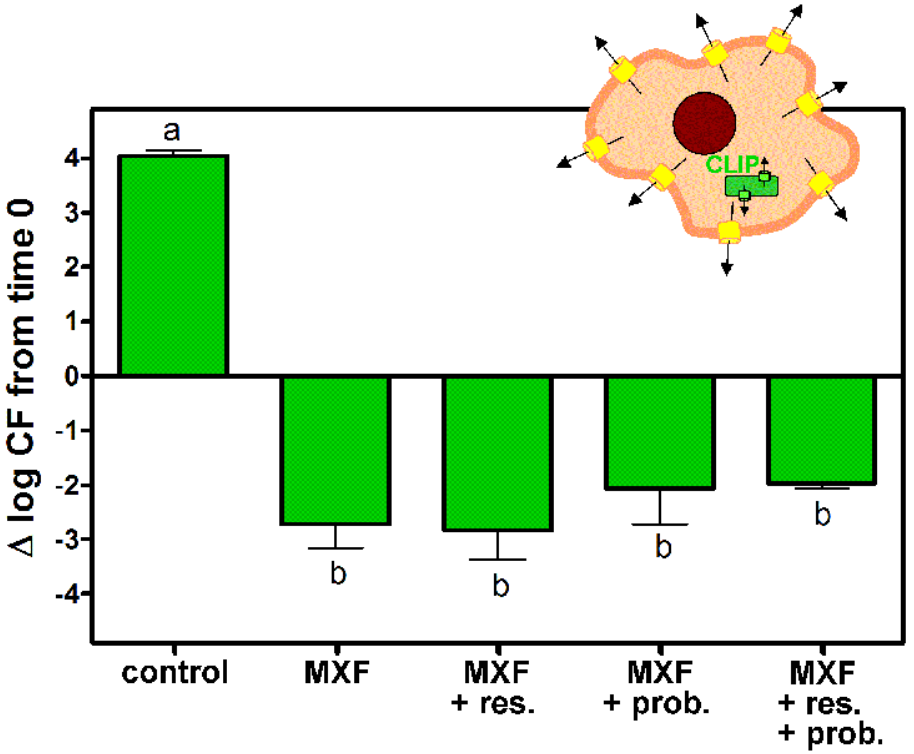
Increased activity against intracellular bacteria

Moxifloxacin and *Listeria* inside macrophages

Wild-type cells and bacteria



cells and bacteria overproducing efflux pumps for ciprofloxacin



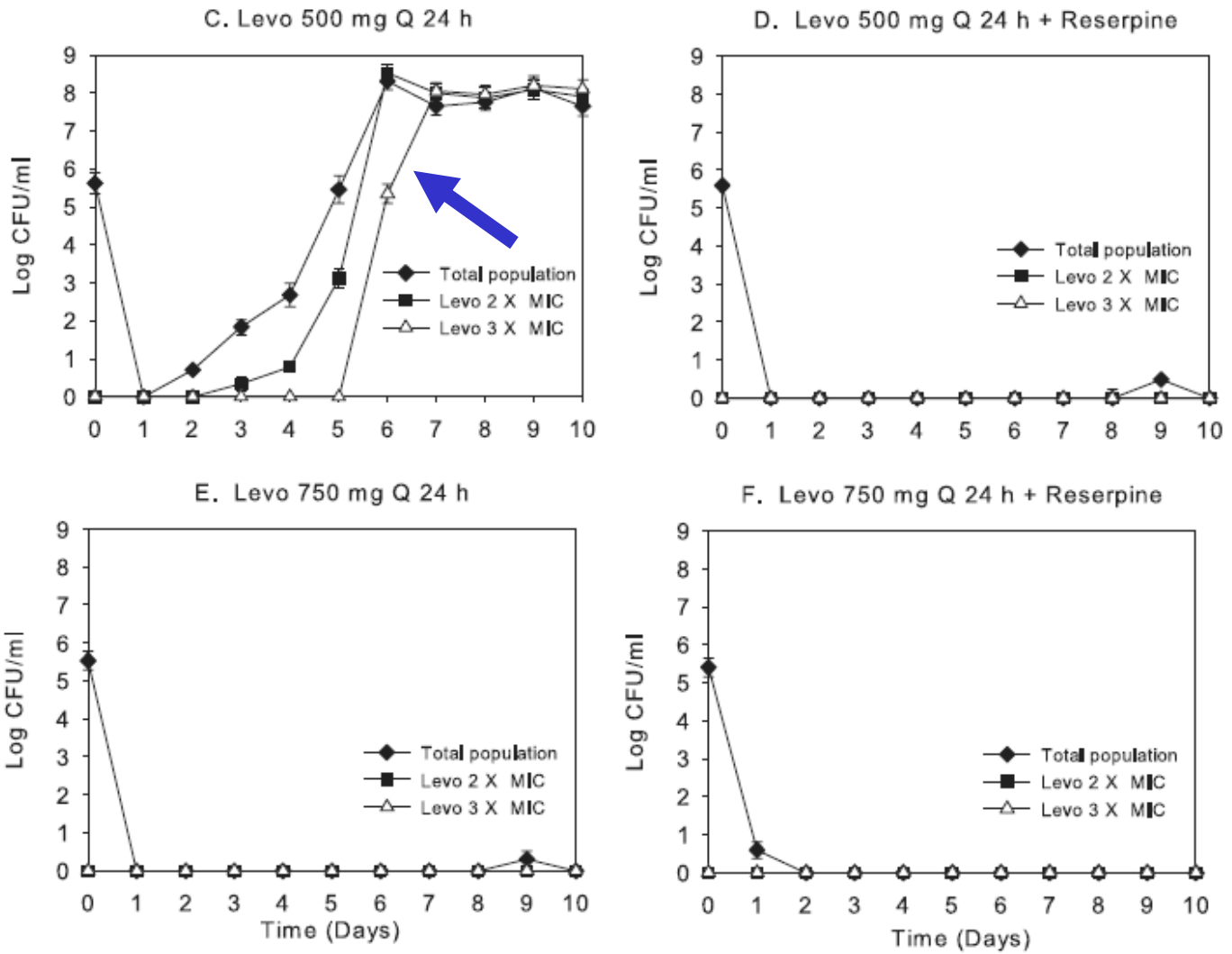
Lismond et al., Antimicrob. Ag. Chemother. (2008) 52:3040-46

Reduction of selection of resistance

In vitro PD model (mimicking human treatment), WT *S. pneumoniae*

Selection of mutants growing at 2-3 X MIC if :

- low AB dosage
- no efflux pump inhibitor



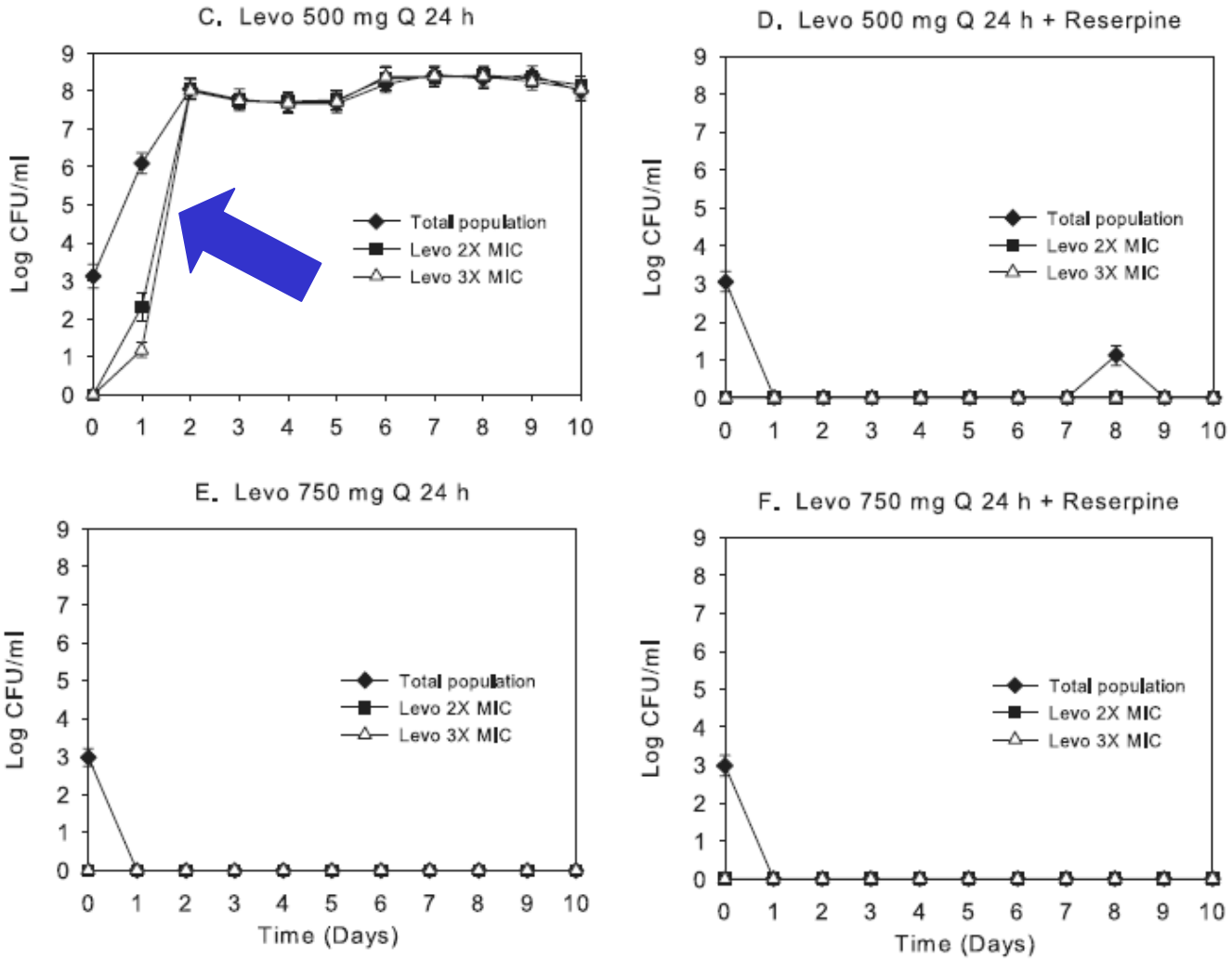
Louie et al, AAC (2007) 51: 3988–4000

Reduction of selection of resistance

In vitro PD model (mimicking human treatment), efflux (+) *S. pneumoniae*

Much more rapid selection of mutants growing at 2-3 X MIC if :

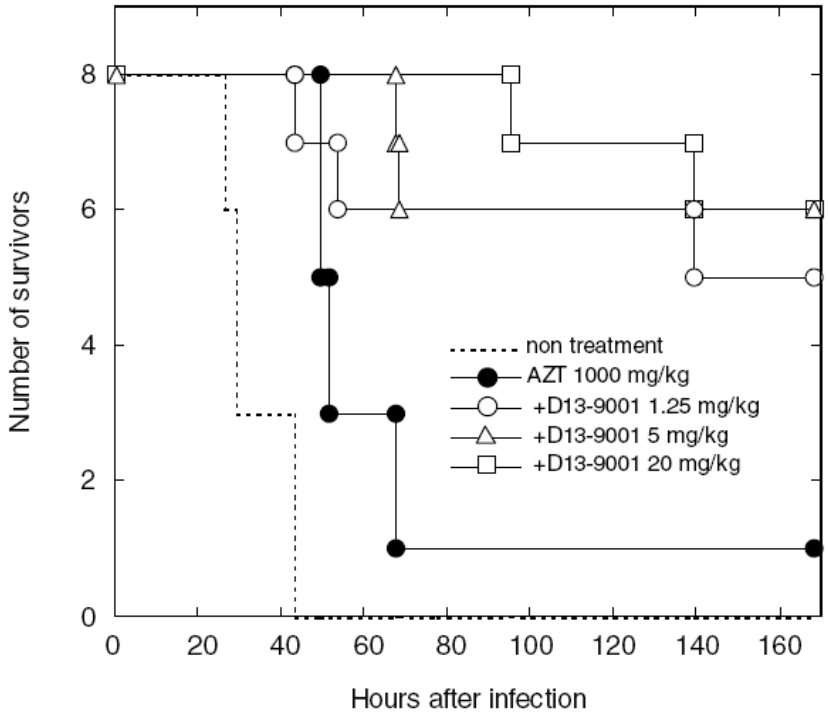
- low AB dosage
- no efflux pump inhibitor

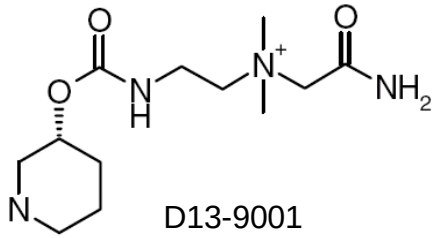


Louie et al, AAC (2007) 51: 3988–4000

Increased efficacy of antibiotic treatments

model of *P. aeruginosa* pneumonia in the rat





D13-9001
MexAB-OprM inhibitor

improves efficacy of
aztreonam (and levofloxacin)

Yoshida et al., Bioorg Med Chem. (2007) 15:7087-97.

Efflux pump inhibitors as diagnostic tools

would require a universal inhibitor !

Gene expression analyses in bloodstream isolates of *Staphylococcus aureus* as determined by quantitative real-time

Gene	Screen-positive ($N = 114$)[a]		Screen-negative ($N = 118$)[a]	
	No. (%) with increased expression	Fold increase (±S.D.)	No. with increased expression (%)	Fold increase (±S.D.)
mepA	5 (4.4)	9.32 ± 2.46	8 (6.8)	10.32 ± 5.58
mdeA	13 (11.4)	10.47 ± 5.3	25 (21.2)	8.47 ± 6.75
norA	28 (24.6)	14.69 ± 10.38	21 (17.8)	20.41 ± 16.88
norB	29 (25.4)	13.67 ± 14.73	36 (30.5)	23.86 ± 32.85
norC	19 (16.7)	10.06 ± 7.85	19 (16.1)	7.85 ± 4.14
qacA/B present	0 (0)	N/D	4 (3.4)	N/D

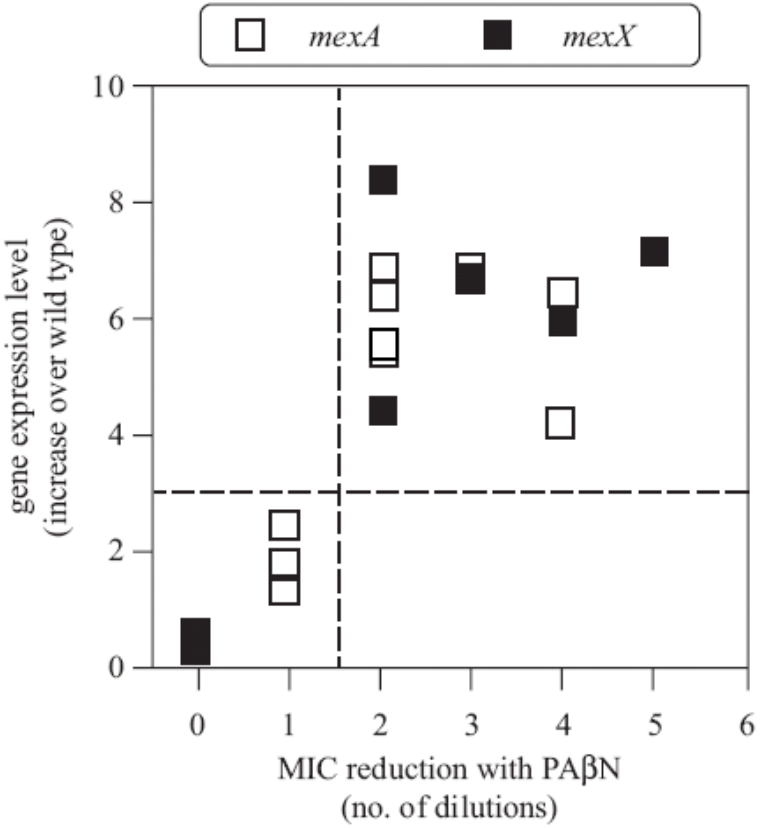
S.D., standard deviation; N/D, not done.

[a] A four-fold reduction in the minimum inhibitory concentration of at least three test compounds, or two if reserpine was considered a positive screen. Screen-negative strains did not meet these criteria.

Frempong-Manso et al, Int J Antimicrob Agents (2009) 33:360-3

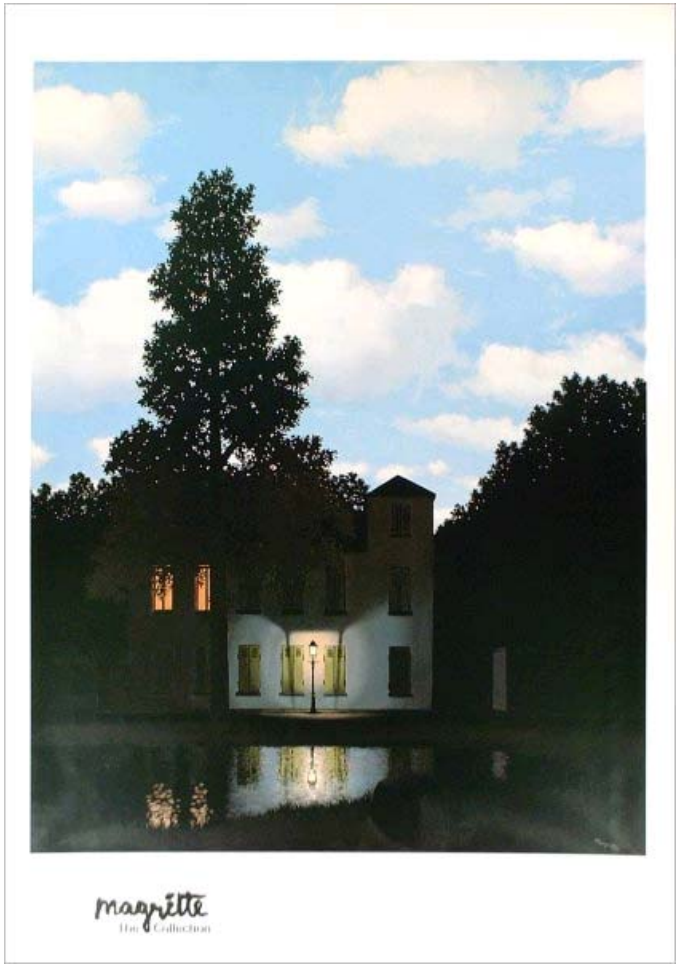
Efflux pump inhibitors as diagnostic tools

or need to be combined with genotypic approaches !



Mesaros et al, J. Antimicrob. Chemother. (2007) 59:378-386

A still uncertain future for EPI ….

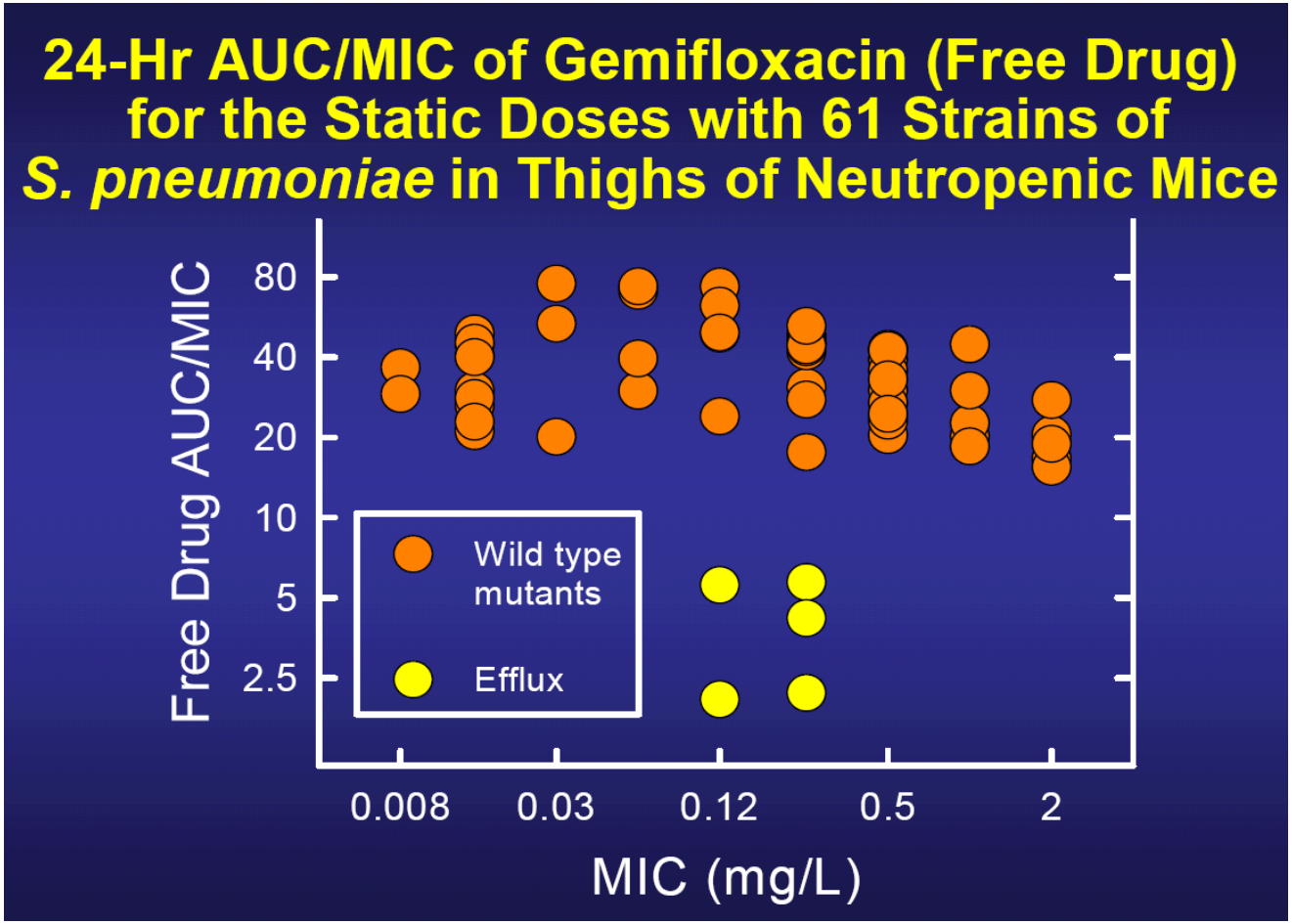


Magritte, Belgian surrealist

Lack of published clinical data …..

- **lack of in vivo relevance of efflux ?**

Efflux-positive strains easier to treat !



Craig, ICAAC 2004

Lack of published clinical data …..

- **toxicity ?**

« At least one class of broad-spectrum bacterial efflux pump inhibitors (EPIs) has been previously reported and extensively characterized both in vitro and in vivo.

While these efforts demonstrated a significant potential for developing small molecule inhibitors of efflux pumps with acceptable serum pharmacokinetics and efficacy and no mechanism-based toxicities, they could not overcome unfavorable tissue accumulation and concomitant local organ toxicity of these compounds.

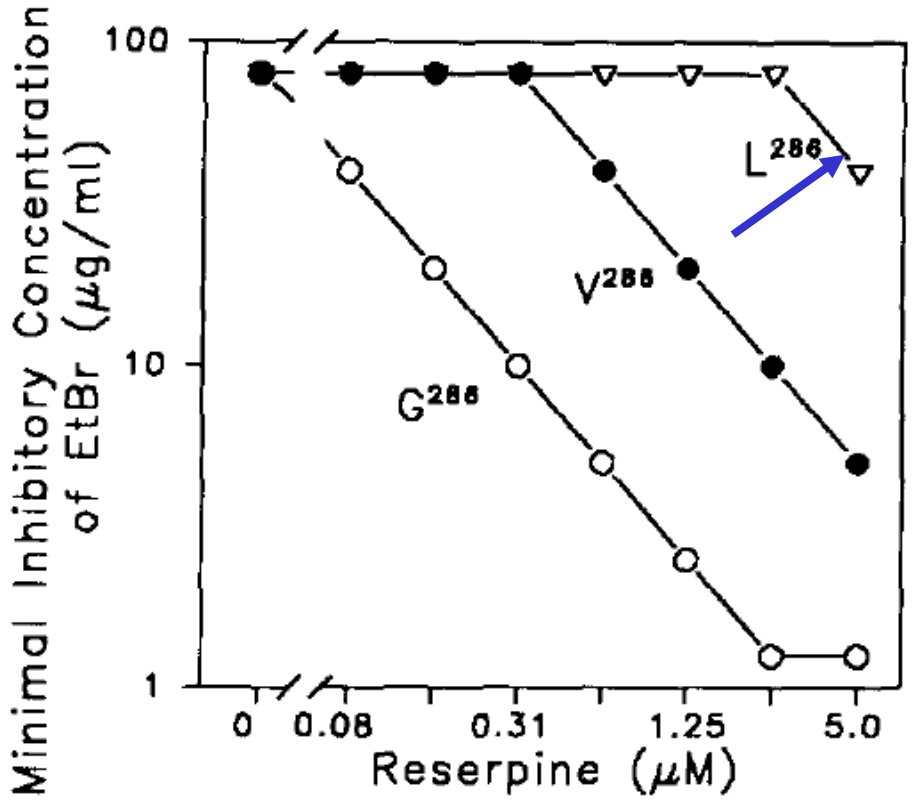
We have initiated an EPI discovery program and conceived of the approach to avoid the tissue accumulation toxicity. This resulted in synthesis of MP-01,003, which demonstrated a persistent serum level but due to specific enzymatic instability, rapidly degraded in tissues, thus avoiding tissue accumulation and concomitant local toxicity. »

O. Lomovskaya, Mpex Pharmaceuticals, in
OPTIMIZING POSITIVE "HITS" FOR POTENCY AND SAFETY,
National Institute for Allergy and Infectious Diseases
February 7-8, 2007

Lack of published clinical data …..

- **resistance ?**

Mutation (Val to Leu) in Bmr pump of *B. subtilis* confers resistance to reserpine



Reserpine no more capable of reverting resistance to EtBr

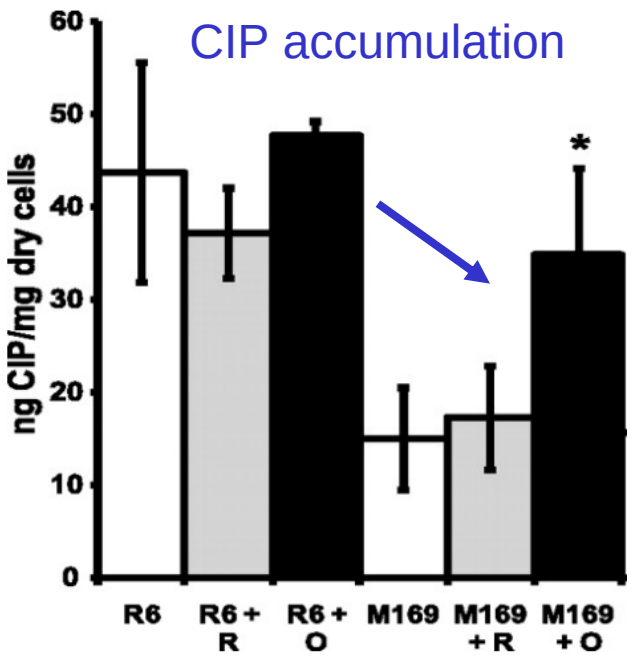
Ahmed et al, JBC (1993) 268:11086-9

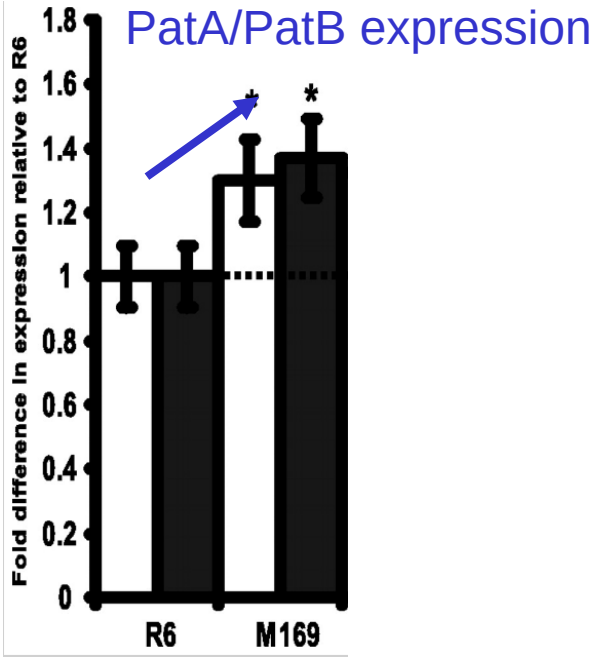
Lack of published clinical data …..

- **resistance ?**

Reserpine induces PatA/PatB expression in *S. pneumoniae*

strain	description	MIC (µg/ml)			
		CIP	+ RES	+ O-vanadate	RES
R6	WT	1	0.5	1	64
M169	R6 *resR* mutant	>4	2	2	256





Garvey & Piddock, AAC (2008) 52:1677-85

Conclusions

- ✓ efflux mechanisms largely spread and contributing to
  - increased pathogenesis
  - reduced susceptibility to antibiotics
- ✓ strategies of potential interest :
  - selection of « poor substrates » antibiotics
  - co-administration of EPI
- ✓ EPI usefulness demonstrated for :
  - reducing pathogenesis
  - reducing MICs and selection of resistance
  - increasing efficacy in vitro / in vivo

➢ human data critically lacking so far !

Still a lot of work ahead ….

Thank you for your attention and have a safe trip back home!