Antibiotic efflux: from discovery to epidemiological, clinical, and veterinary impact

Françoise Van Bambeke & Paul M. Tulkens

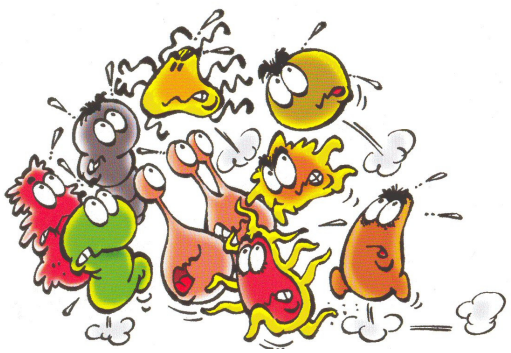
Pharmacologie cellulaire et moléculaire
Louvain Drug Research Institute
Université catholique de Louvain, Brussels, Belgium

<www.facm.ucl.ac.be>

Discovery and significance ….

Chemotherapeutic agents exert toxic effects on specific target cells

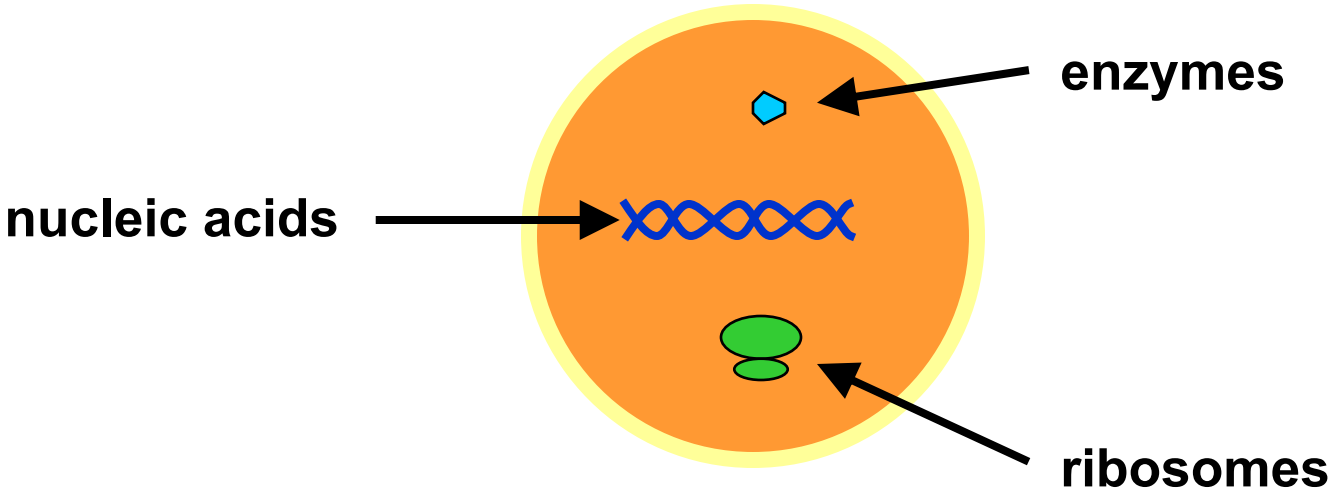
antibiotics



antifungals

anticancer agents

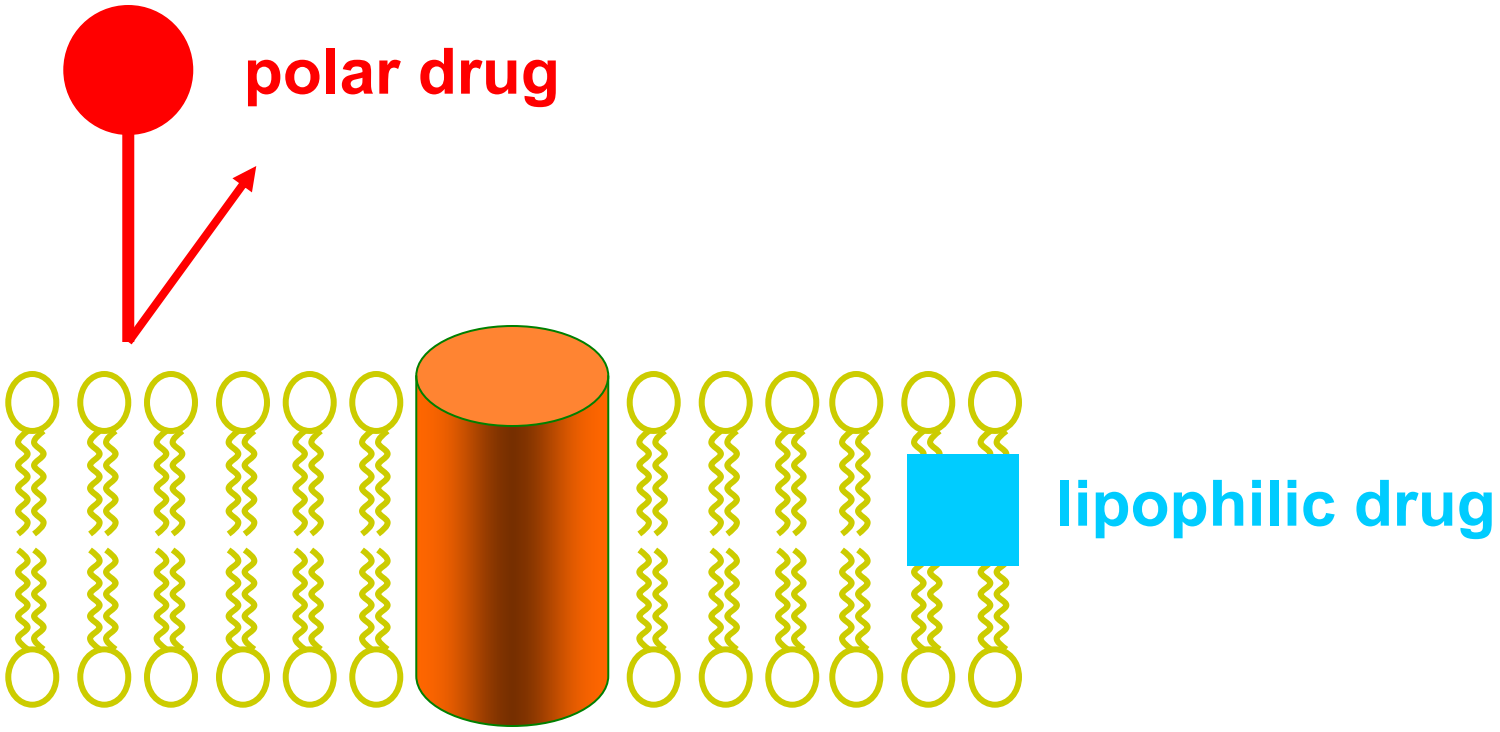
Chemotherapeutic agents exert toxic effects on specific target cells



How can these drugs reach their target inside the cells ?

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

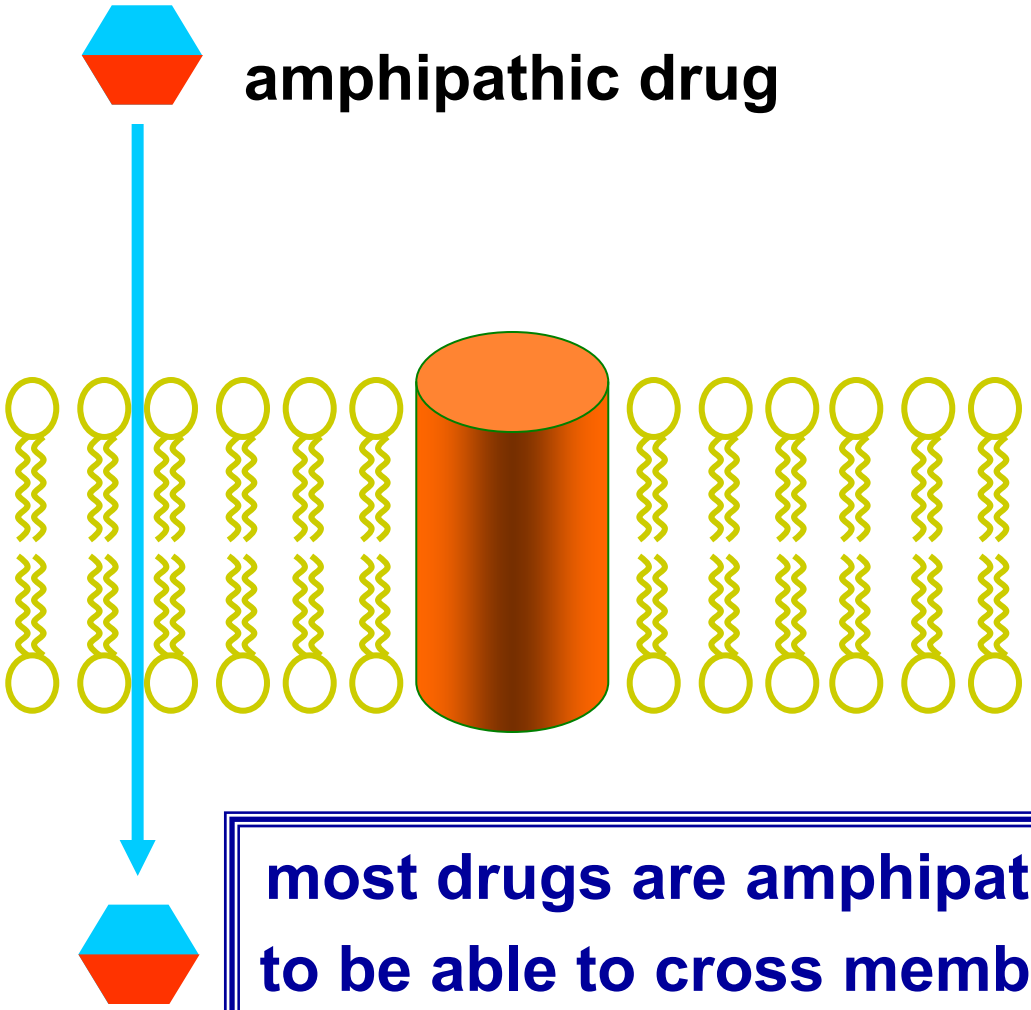
Reaching an intracellular target …



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

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

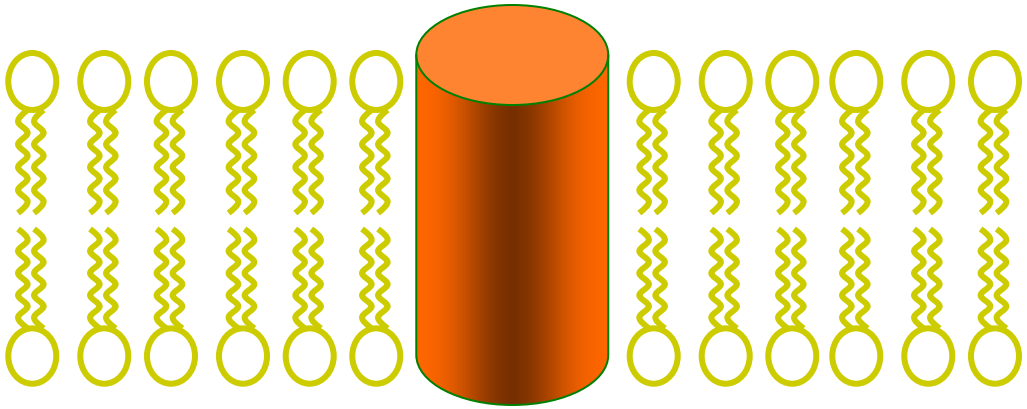
Reaching an intracellular target …

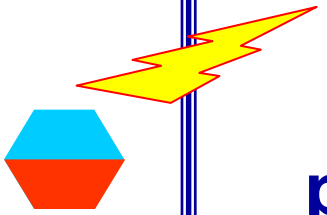


most drugs are amphipathic by design, to be able to cross membrane barriers !

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

Intracellular chemotherapeutic agents



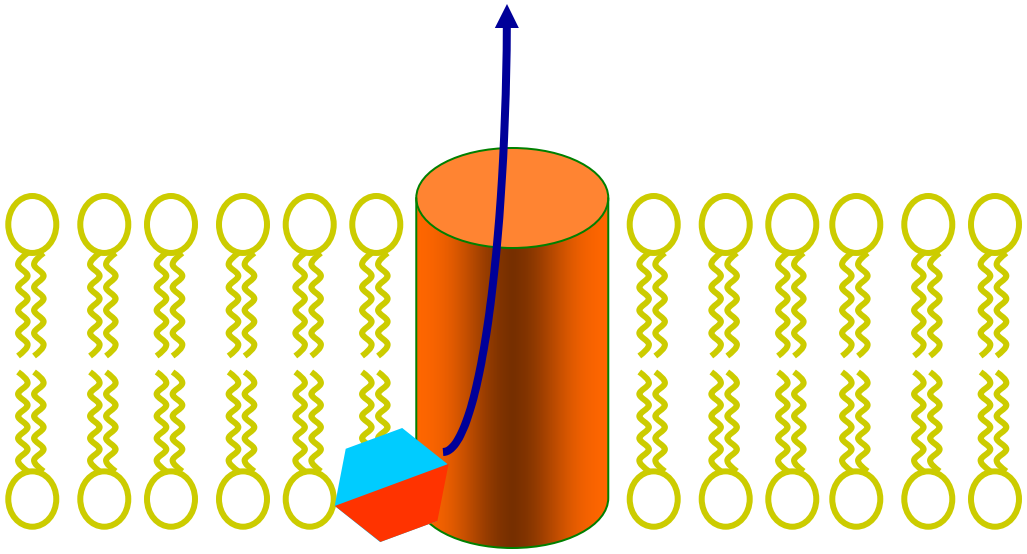


But a diffusible compound may have potentially harmful effects !

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

Why efflux transporters ?

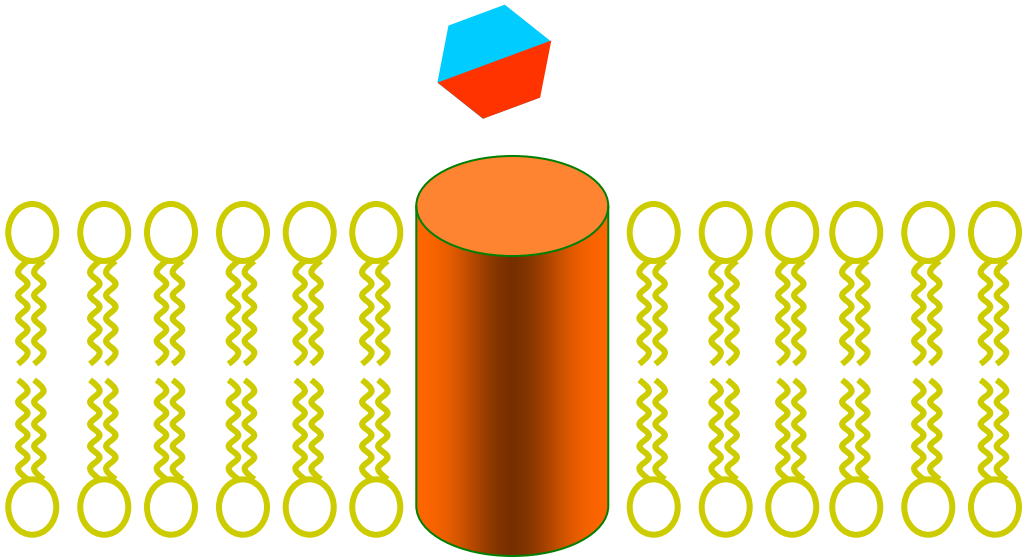
Extrusion by efflux pumps



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

Why efflux transporters ?

Extrusion by efflux pumps



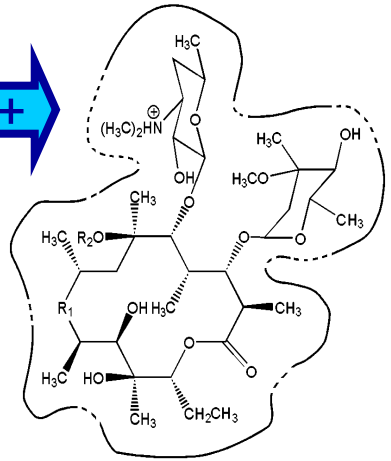
general mean of protection
against cell invasion by diffusible molecules

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

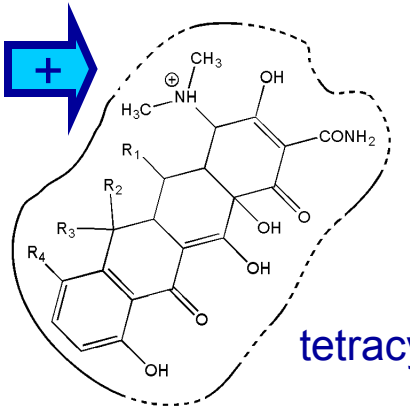
Most antibiotics are amphiphilic !

cationic amphiphiles

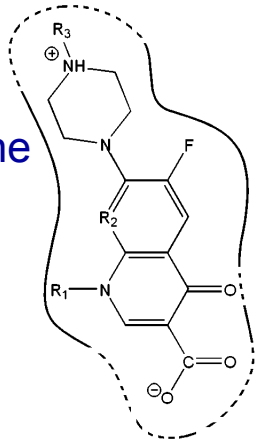
macrolide



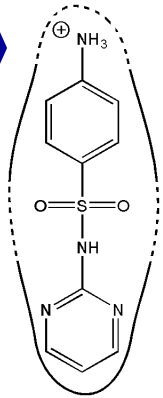
tetracycline



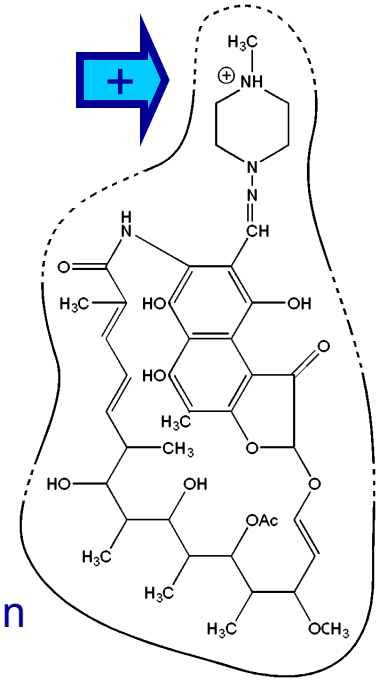
fluoroquinolone



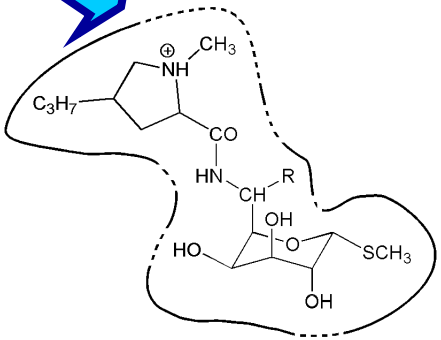
sulfamide



rifampicin



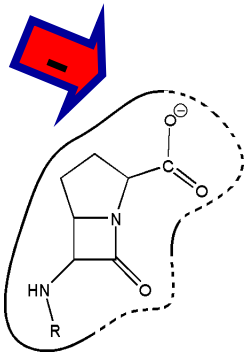
lincosamide



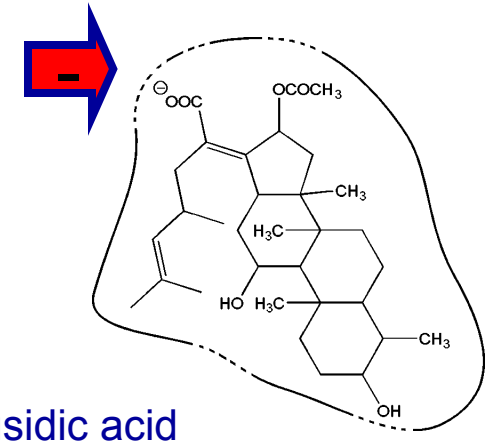
Van Bambeke et al. (2000) Biochem. Pharmacol. 60:457-70

Most antibiotics are amphiphilic !

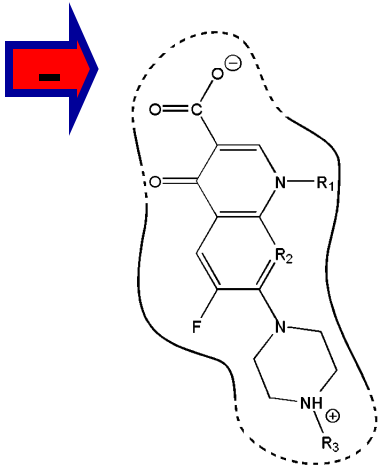
anionic amphiphiles



β-lactam



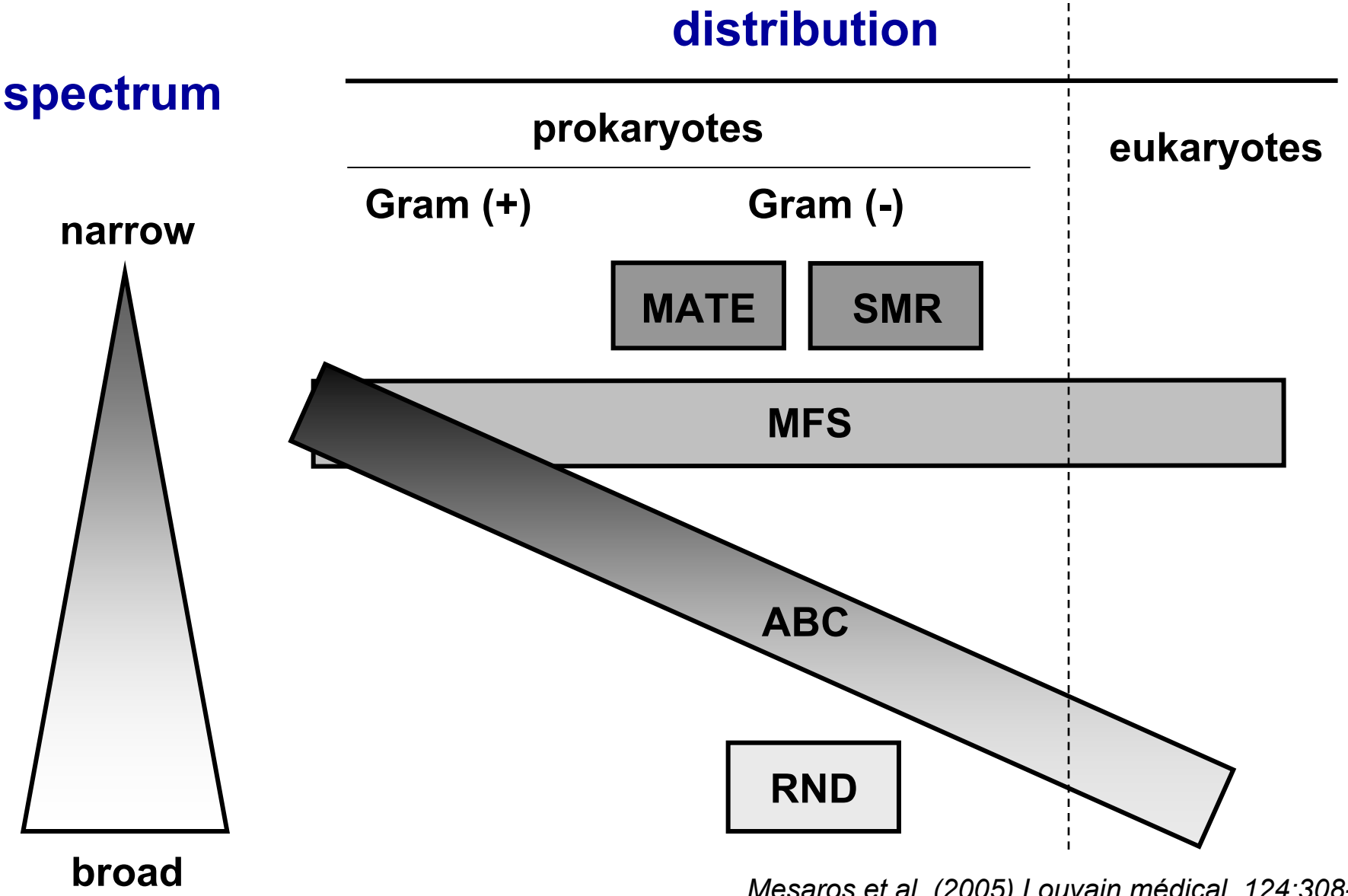
fusidic acid



fluoroquinolone

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

Antibiotic efflux transporters are ubiquituous



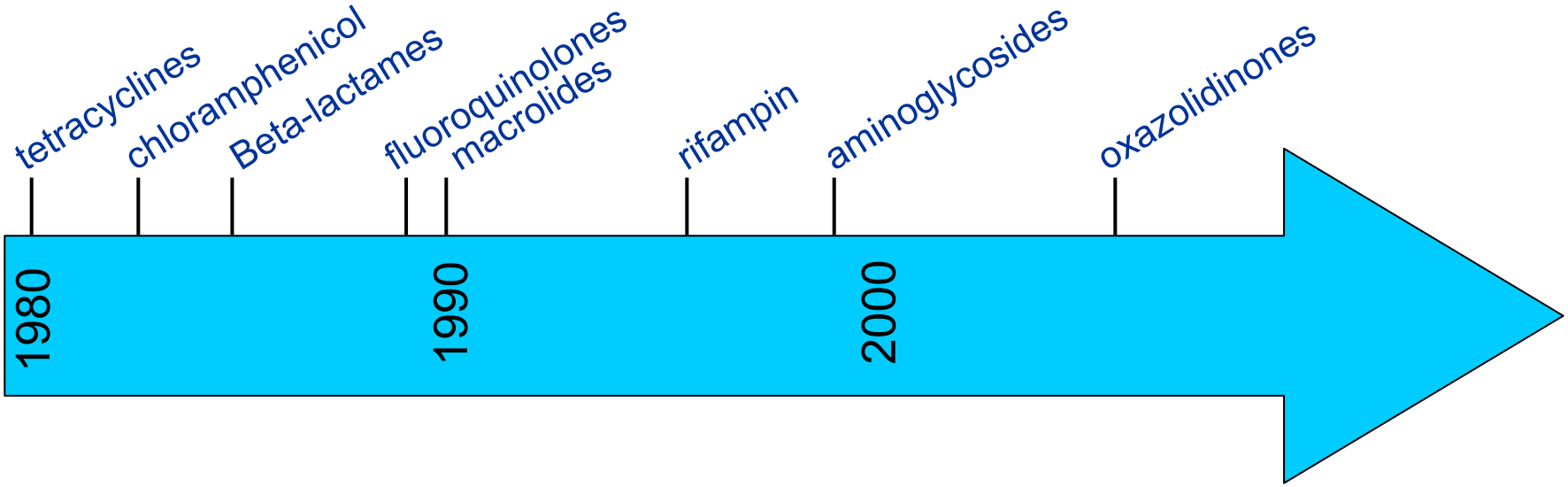
Mesaros et al. (2005) Louvain médical. 124:308-20

Antibiotics as substrates of efflux pumps

Antibiotic class	bacteria Gram (+)	bacteria Gram(-)	fungi	superior eucaryotes
β-lactams	●	●	●	●
fusidic acid		●		
macrolides	●	●	●	●
streptogramins	●			●
tetracyclines	●	●	●	●
aminoglycosides		●	●	
chloramphenicol	●	●	●	
rifamycins				●
sulfamides			●	
trimethoprim		●		
fluoroquinolones	●	●		●

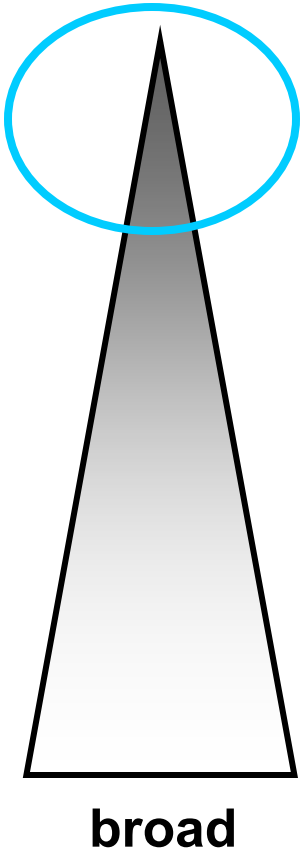
Van Bambeke et al. (2000) Biochem. Pharmacol. 60:457-70

Antibiotic efflux and resistance: time line …



Efflux as a mechanism of export in antibiotic producers

spectrum



specific for the produced antibiotic

ABC

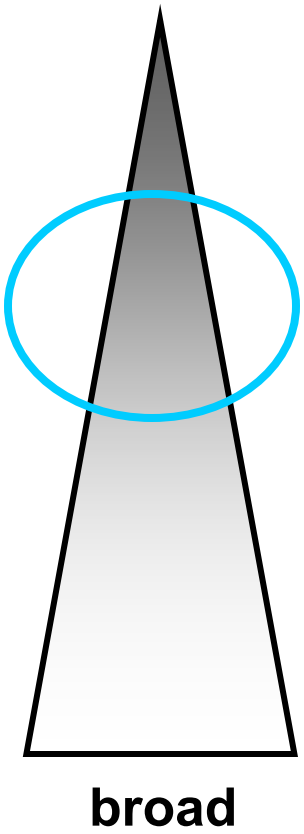
OleB or C of *S. antibioticus*
→ oleandomycin
SmB of *S. ambofaciens*
→ macrolides
TIrc of *S. fradiae*
→ tylosin

MFS

Ptr of *S. pristinaespiralis*
→ pristinamycin
LmrA of *S. lincolnensis*
→ lincomycin
RifP of *A. mediterranei*
→ rifampicin

Efflux as a mechanism of resistance in Gram-positive bacteria

spectrum



specific for one (or a few) families of drugs

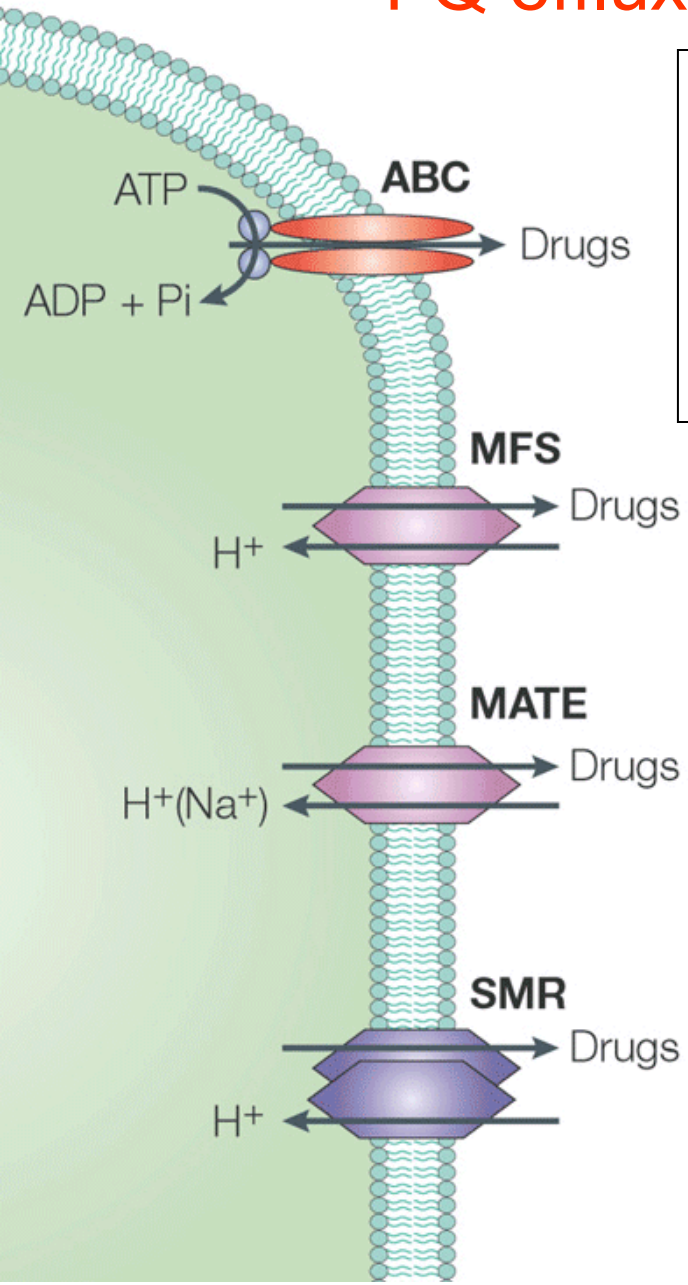
ABC

PatA/PatB of *S. pneumoniae*
→ FQ, chl
MsrA of *S. epidermidis*
→ erythromycin

MFS

NorA of *S. aureus*
→ FQ,Tet, chl
MefE of *S. pneumoniae*
→ ML
PmrA of *S. pneumoniae*
→ FQ
MefA of *S. pyogenes*
→ ML

FQ efflux pumps in *S. pneumoniae*



Primary transporters
« **A**TP-**B**inding **C**assette »

PatA/PatB
Marrer et al, AAC 2006; 50:685-93

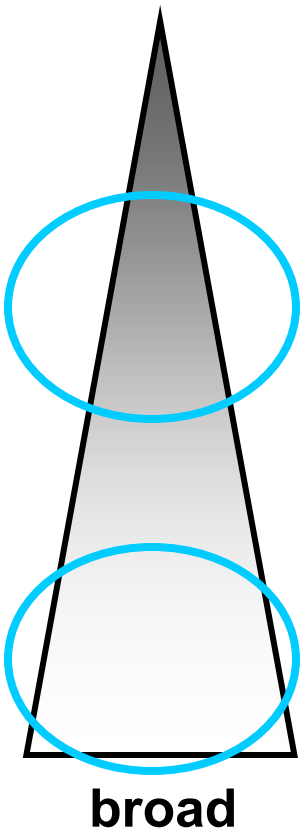
Secondary transporters
(Proton motive force)

PmrA
Gill et al, AAC 1999; 43:187-9

Terry et al., Nature Reviews Microbiology 2005; 3: 566-572

Efflux as a mechanism of resistance in Gram-negative bacteria

spectrum



specific for one (or a few) families of drugs

MFS

TetA of *E. coli*
→Tet
MsrA of *S. epidermidis*
→ erythromycin

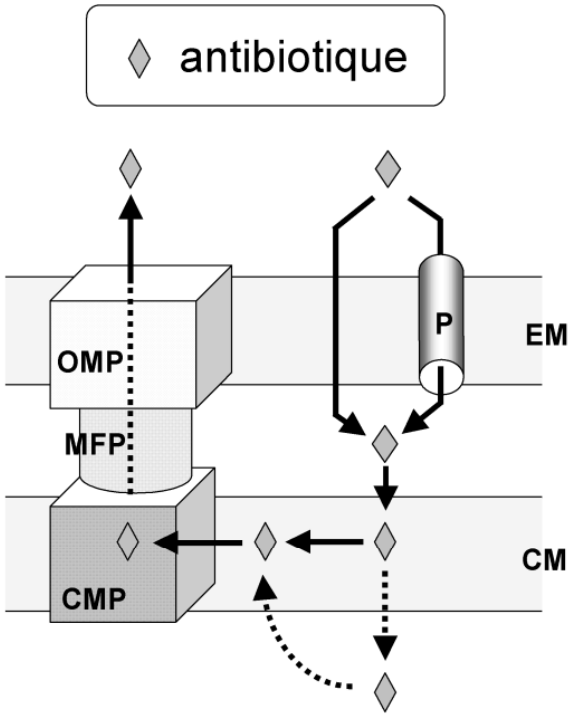
broad spectrum, conferring cross-resistance

RND

MexAB-OprM of *P. aeruginosa*
→ β-lac, FQ,Tet, ML, chl, rif, sulf
AcrAB-TolC of *E. coli*
→ β-lac, FQ,Tet, ML, chl, rif, sulf

Efflux and resistance in *P. aeruginosa*

Constitutive basal expression overexpressed upon induction		No basal expression; expression upon induction	
MexB	MexY	MexD	MexF
MexA	MexX	MexC	MexE
OprM	OprM	OprJ	OprN



CM: cytoplasmic membrane
(membrane cytoplasmique)

CMP: cytoplasmic membrane protein
(protéine de la membrane cytoplasmique)

EM: external membrane
(membrane externe)

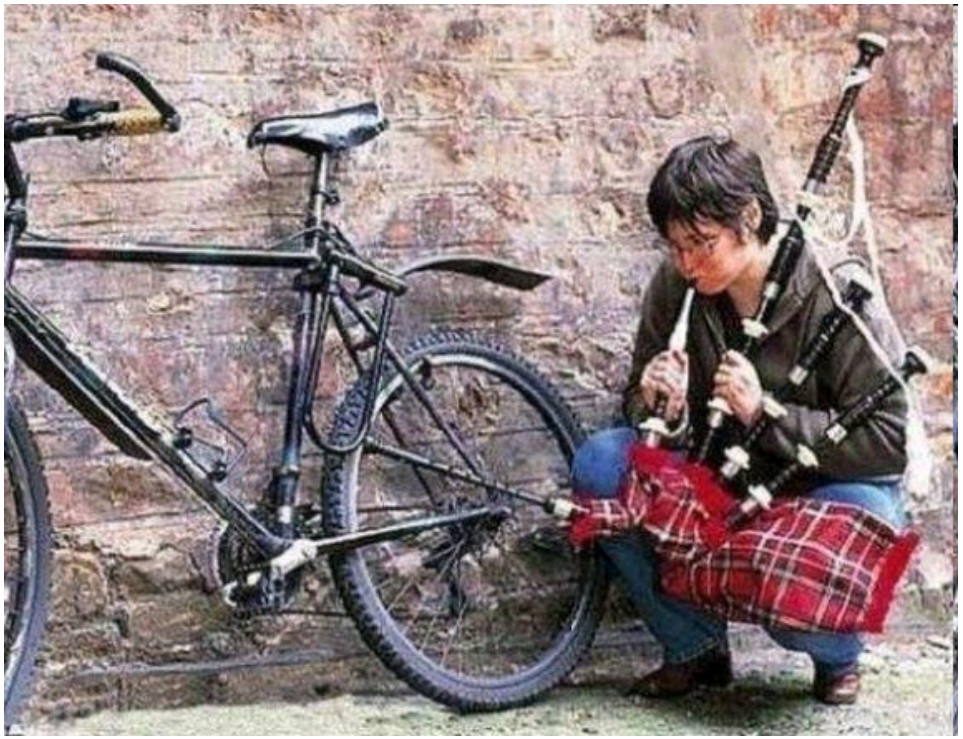
MFP: membrane fusion protein
(protéine de fusion [entre membranes])

P: porin
(porine)

OMP: outer membrane protein
(protéine de membrane externe)

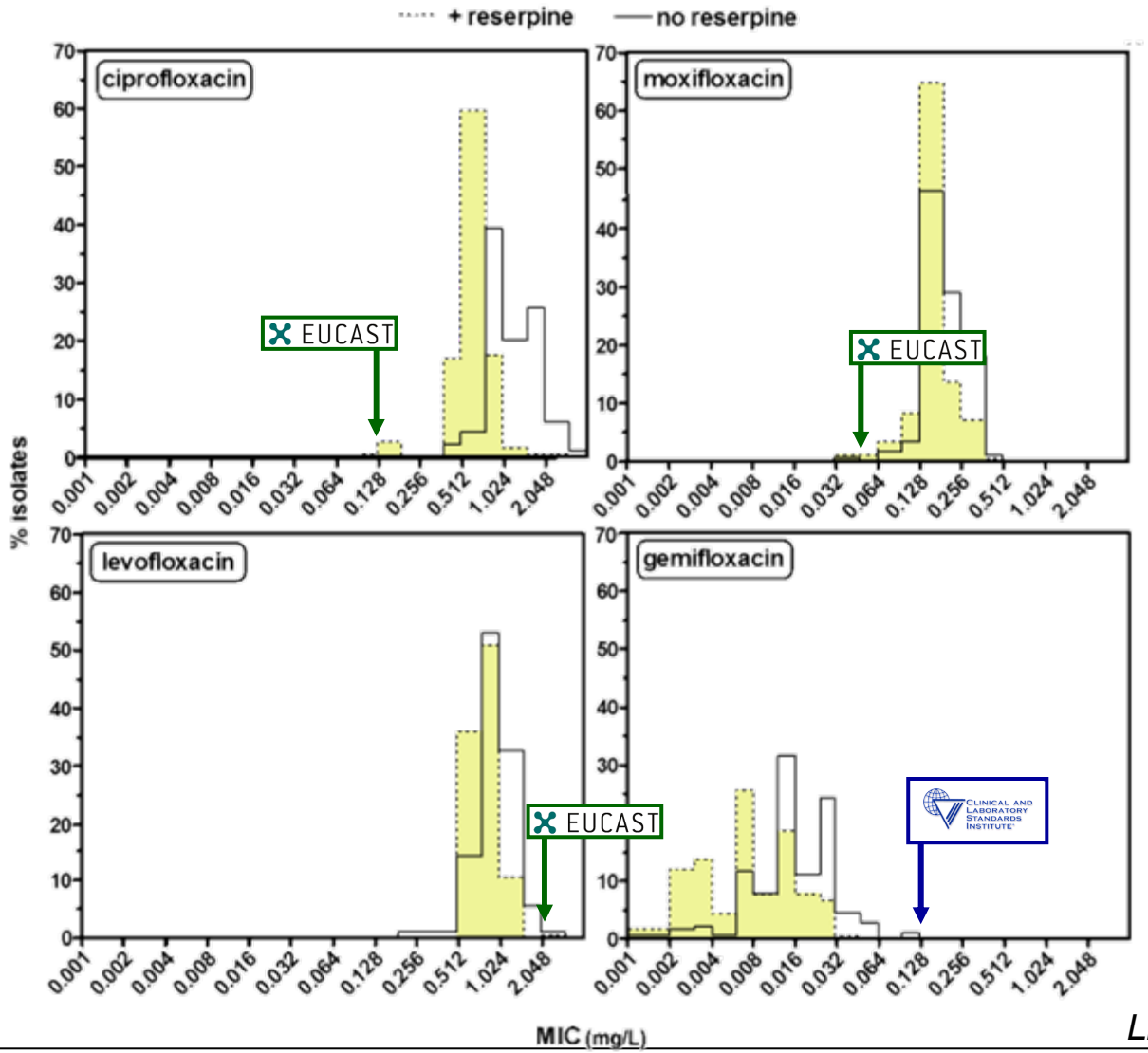
Mesaros et al. (2005) Louvain médical. 124:308-20

Role of antibiotic efflux in epidemiology and resistance ….



Does efflux mean « resistance » vs. epidemiological breakpoints ?

MICs vs breakpoints for 183 *S. pneumoniae* isolated from CAP



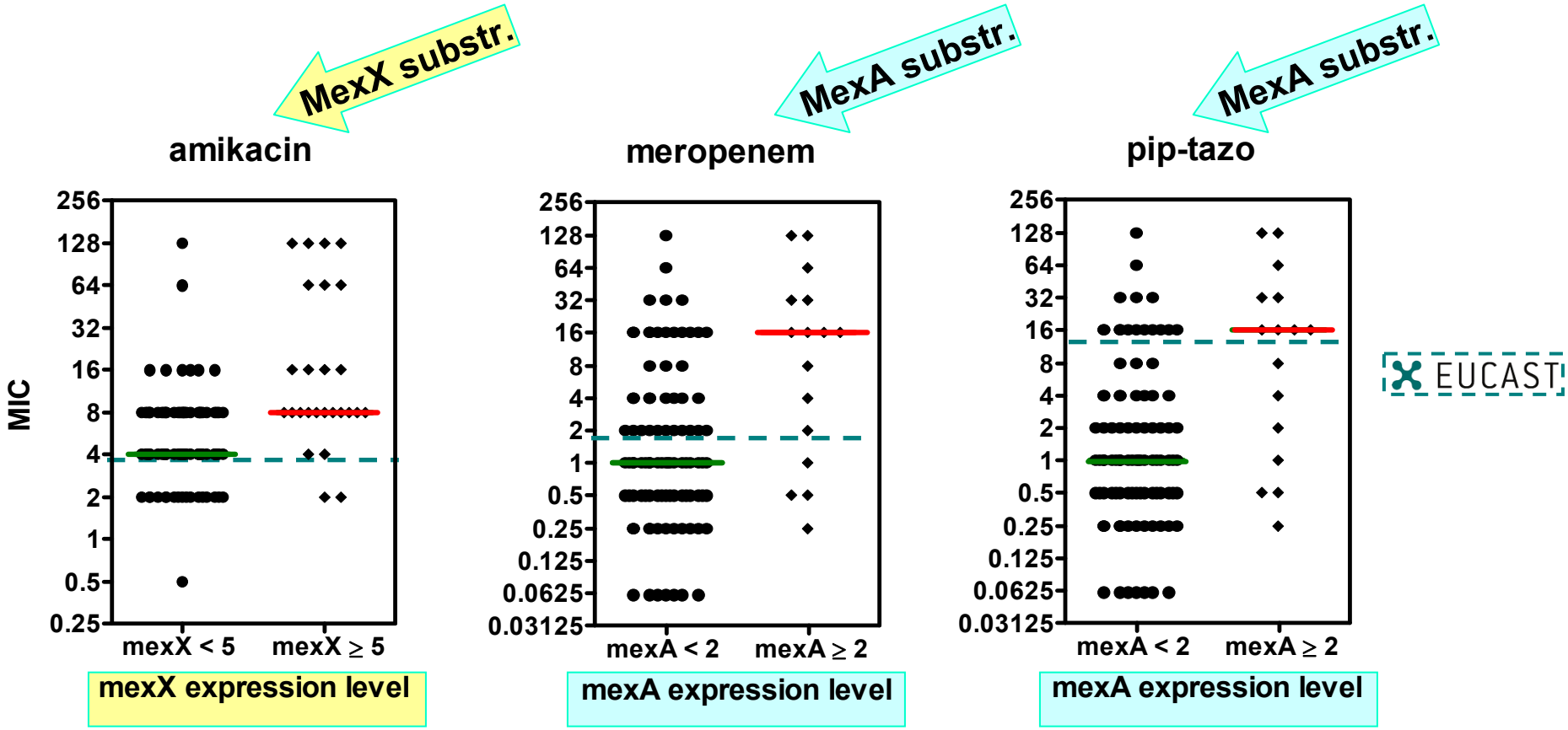
- Efflux (+) strains considered as susceptible

- FQ with high intrinsic activity can be substrates for efflux !

Lismond et al., JAC (2011) 66:948-951

Does efflux mean « resistance » vs. epidemiological breakpoints ?

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



Riou et al, ECCMID 2010

Efflux and intrinsic resistance

Is *Pseudomonas* « intrinsically » resistant to temocillin ?

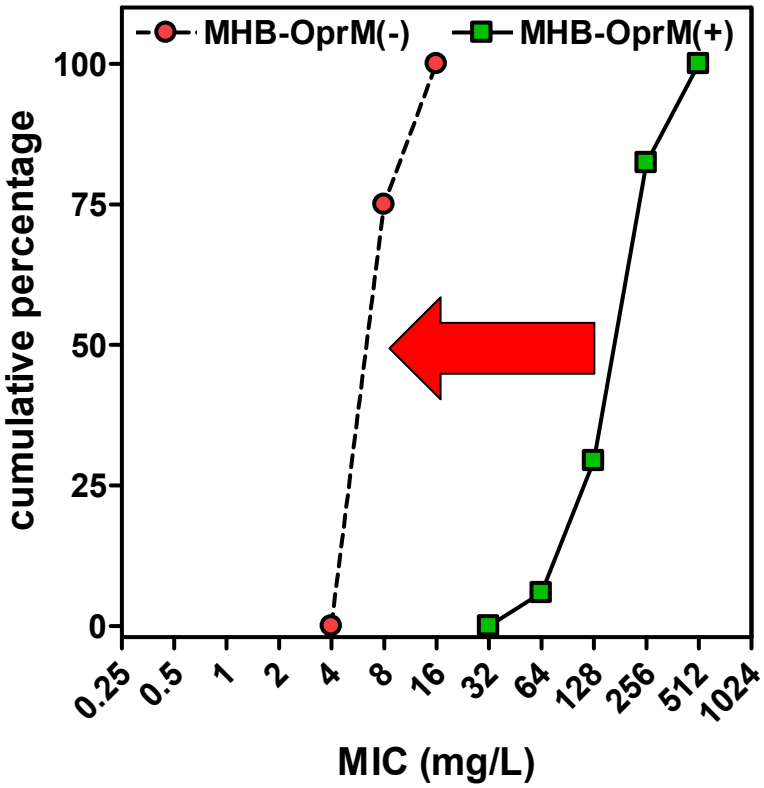
Strain	Description	Efflux characteristics					MIC (mg/L)	
		Gene expression level						
		mexA [a]	*mexX* [a]	*oprM* [a]	*mexC* [b]	*mexE* [b]	temocillin (+ PAβN [c])	ticarcillin (+ PAβN [c])
Reference strain								
PAO1		1	1	1	-	-	256-512 (64)	32 (16)
Engineered strains								
CB 536	PAO1 Δ*mexCD-oprJ*	1.09	1.65	ND	-	+	128 (16)	8 (1)
CB603	PAO1 Δ*mexEF-oprN*	1.21	1.02	0.51	-	-	128 (32)	16 (16)
CB602	PAO1 *mexXY::FRT*	1.10	*0.06*	0.55	-	+	64 (16)	16 (16)
PAO1 mexAB	PAO1 *mexAB::FRT*	*0* [m]	1.08	ND	-	+	4 (2)	2 (2)

MexAB-OprM mutants are highly susceptible!

Buyck et al, J. Antimicrob. Chemother. (2012) 67(3):771-5

Efflux and intrinsic resistance

Is *Pseudomonas* « intrinsically » resistant to macrolides ?



Major role
of constitutively-expressed
transporters!

Buyck et al, ICAAC (2011)

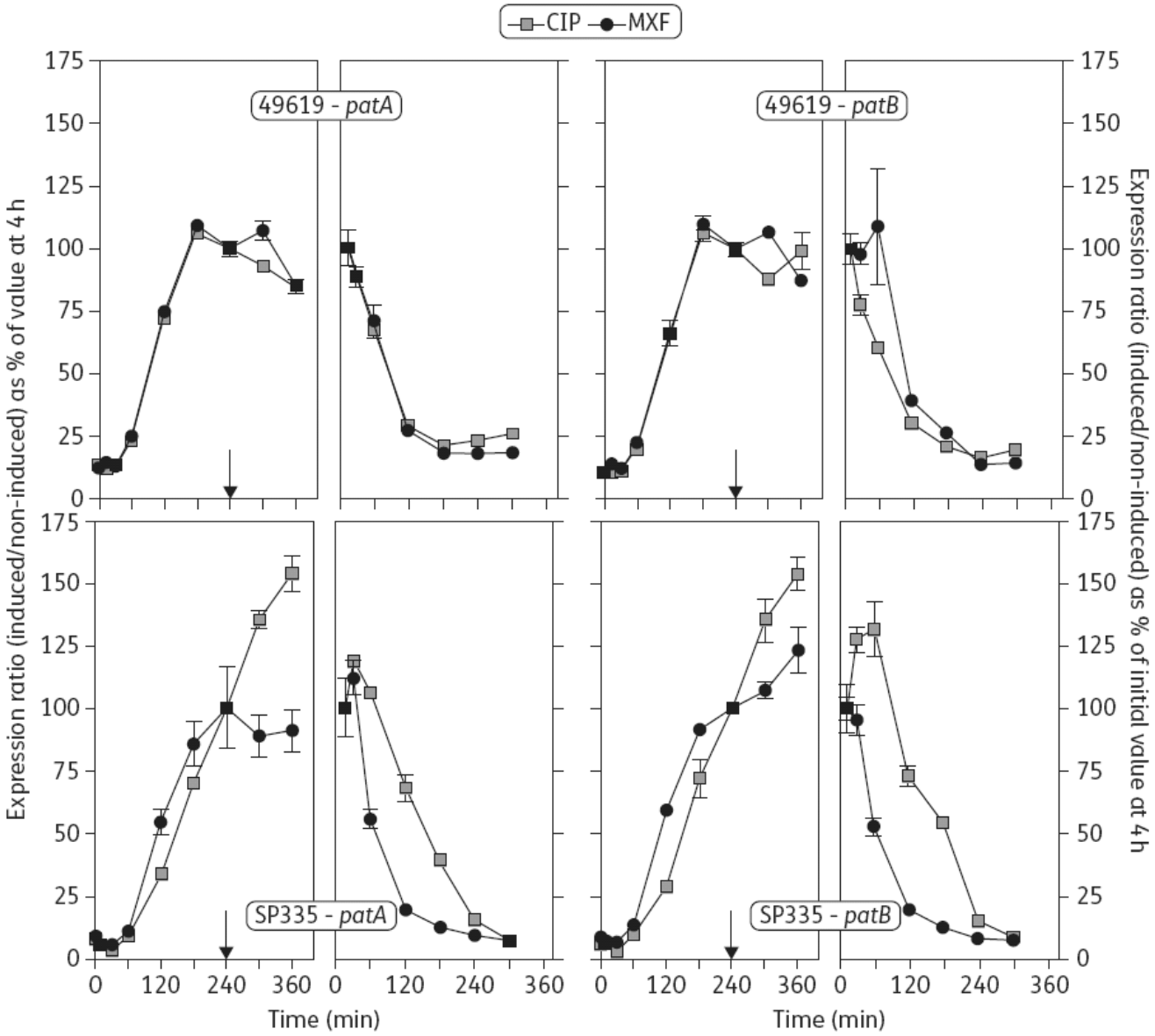
Role of efflux pumps in the clinics …

FQ resistance by efflux in *S. pneumoniae* is mediated by PatA/PatB

FQ / strains	CIP		MXF	
	-Res	+Res	-R	+R
49619	**1**	**0.5**	**0.125**	**0.125**
Δ ***patA***	**0.5**	**0.5**	**0.125**	**0.125**
Δ ***patB***	**0.5**	**0.5**	**0.125**	**0.125**
Δ ***pmrA***	**1**	**0.5**	**0.125**	**0.125**
SP335	32	**2**	**0.5**	**0.25**
Δ ***patA***	**1**	**0.5**	**0.125**	**0.125**
Δ ***patB***	**1**	**0.5**	**0.125**	**0.125**
Δ ***pmrA***	8	**0.5**	**0.25**	**0.125**

CIP affected but not MXF !

FQ induce efflux, whether substrates or not

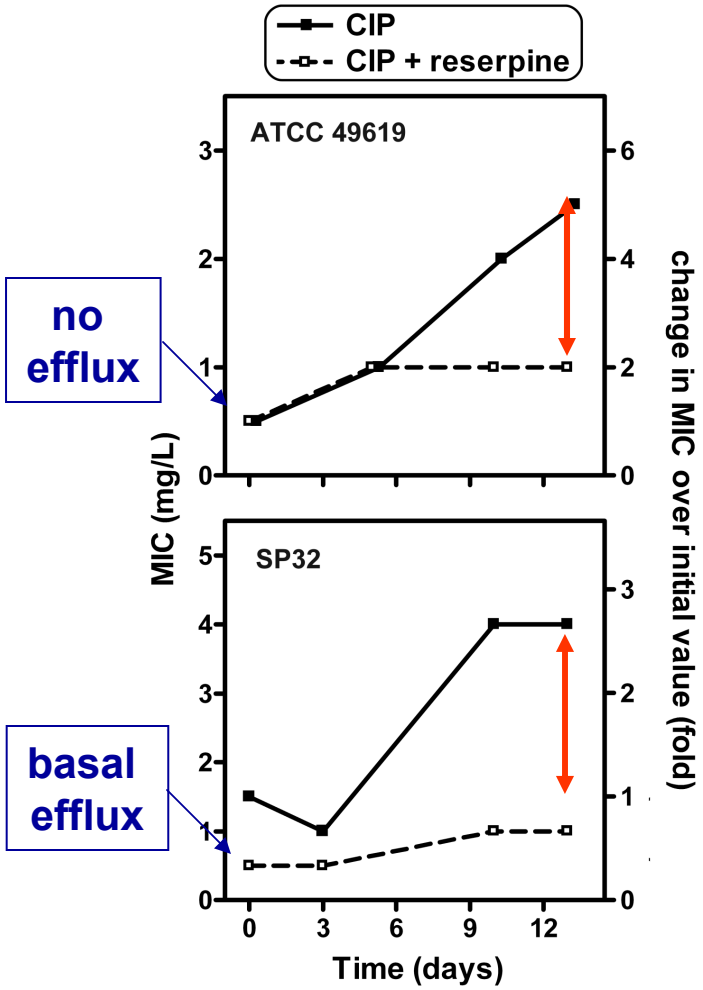


Both
CIP and MXF
are inducers !

El Garch et al., JAC (2010) 65:2076-82

Selection of resistance in *S. pneumoniae* by subMIC concentrations of FQ

ciprofloxacin



Ciprofloxacin selects for resistance by efflux

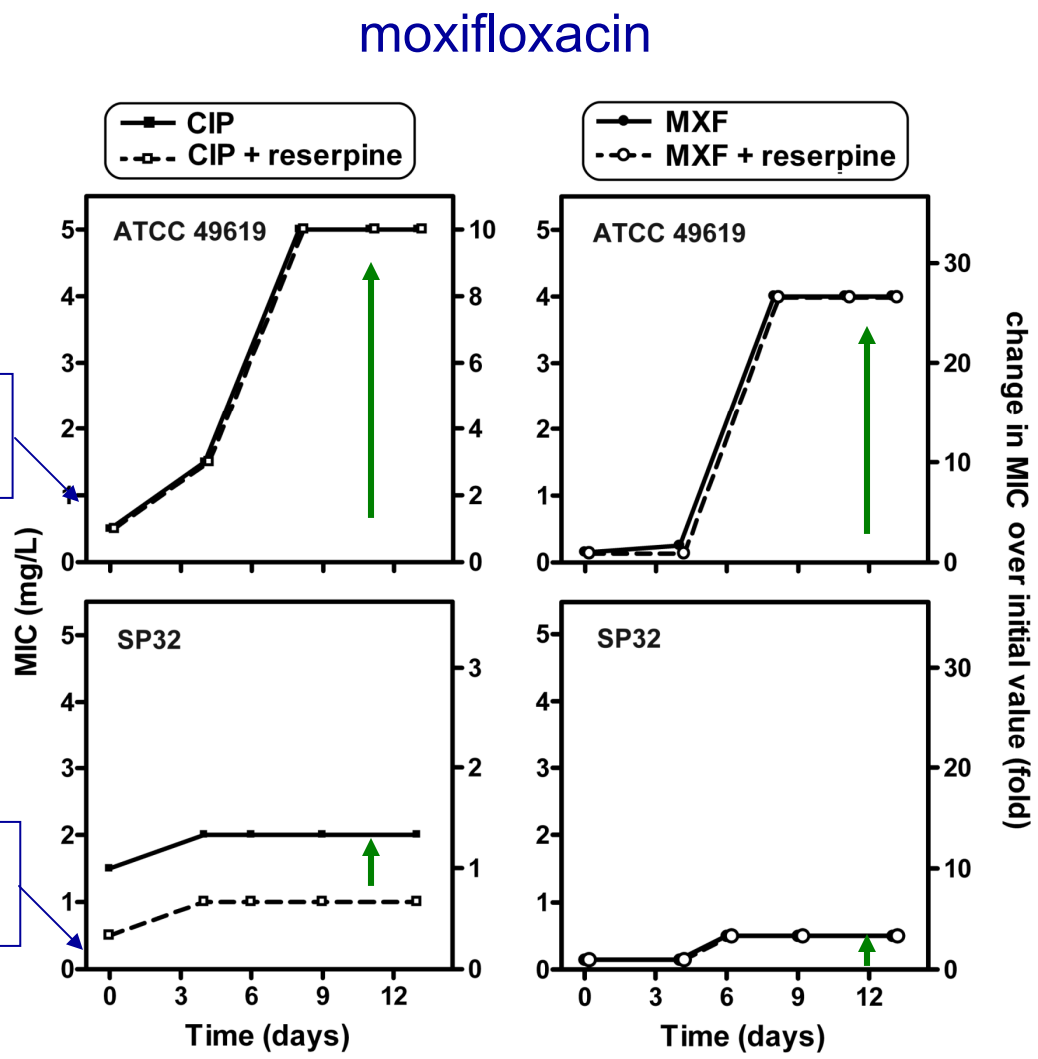
Avrain et al, JAC (2007) 60:965-972

Selection of resistance in *S. pneumoniae* by subMIC concentrations of FQ

GyrA (Ser81Phe)
ParC (Ser79Tyr)

Moxifloxacin selects for resistance by target mutation (topoisomerases)

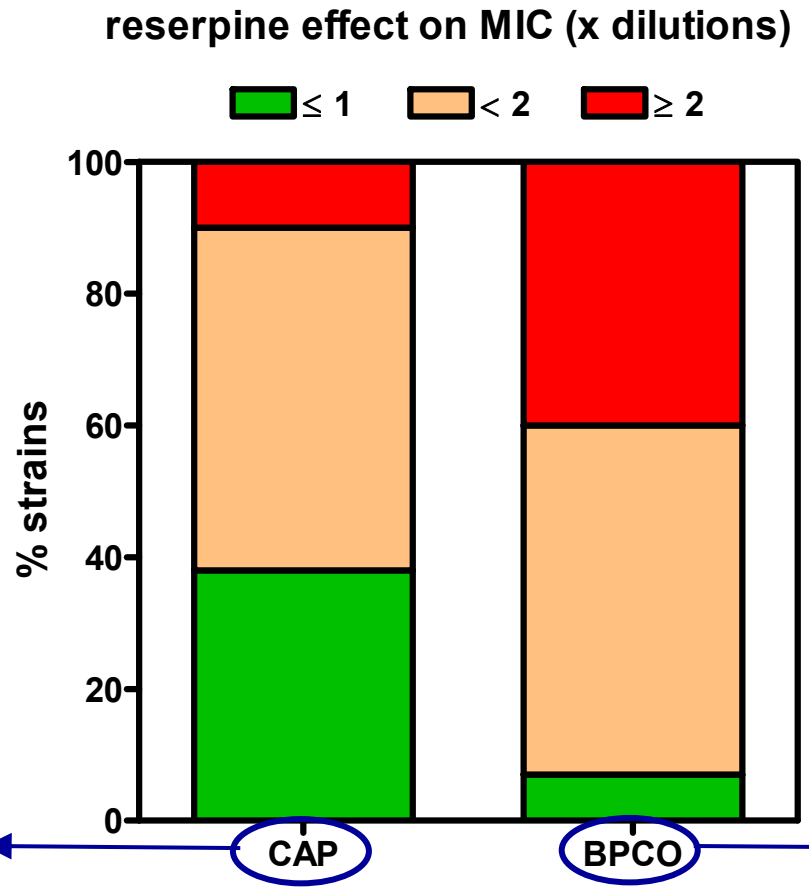
ParE (Ile460Val)
ParE (Arg447Cys)



Avrain et al, JAC (2007) 60:965-972

Efflux in *S. pneumoniae:* is it important in the clinics ?

Suspected efflux based on phenotypic analysis (CIP MIC +/- reserpine)



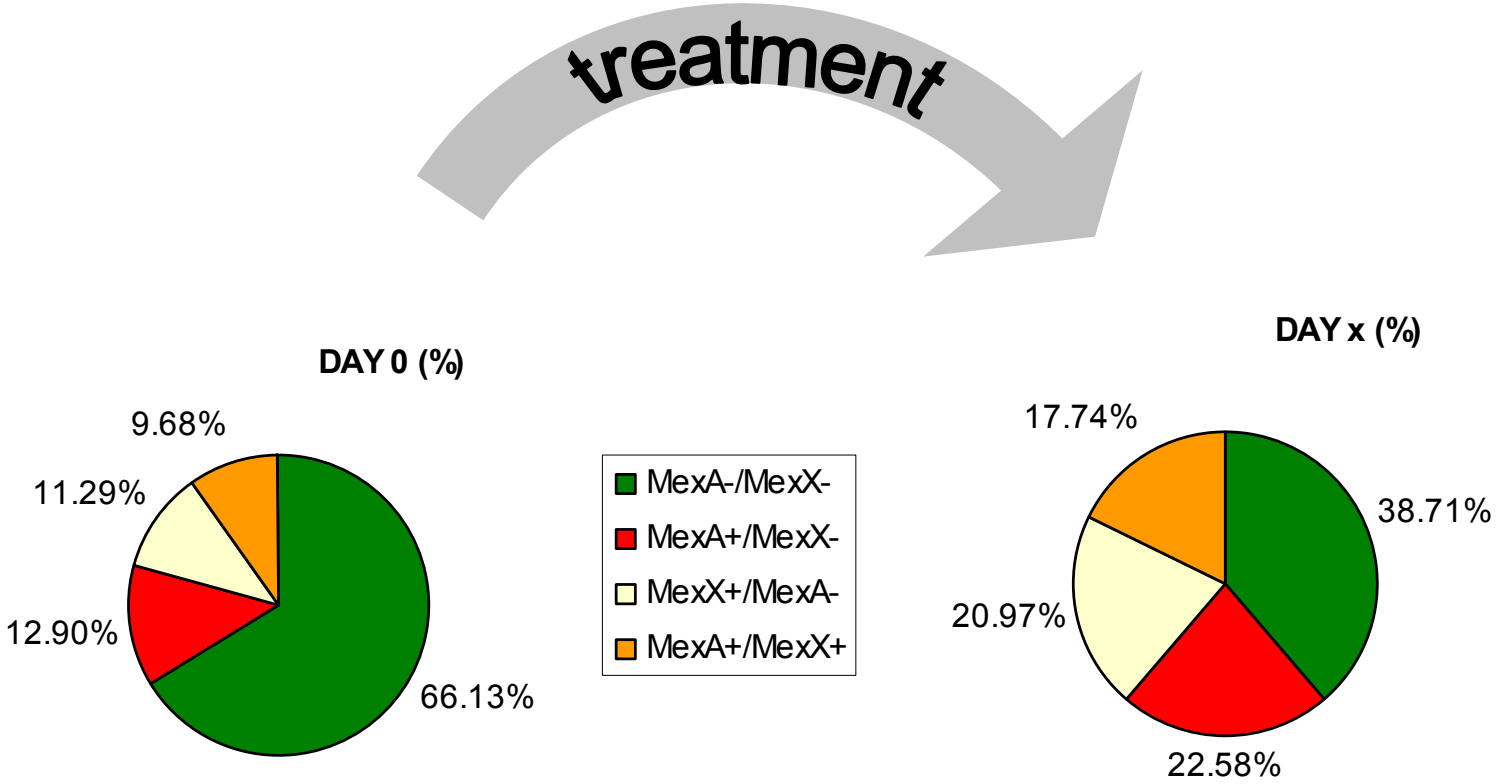
acute pathology
⬇
« one shot »
antibiotic exposure

chronic pathology
⬇
repetitive
antibiotic exposures

Lismond & Degives, unpublished

Efflux in *P. aeruginosa:* is it important in the clinics ?

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



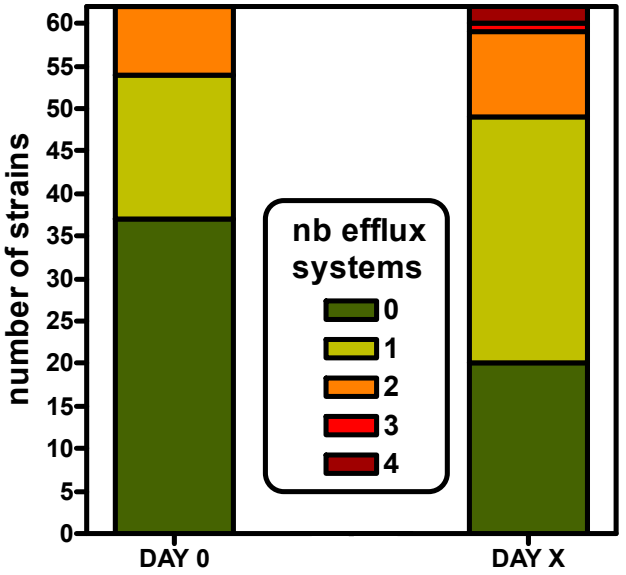
Riou et al, ECCMID 2010

Efflux selection in *P. aeruginosa* during treatment

Antipseudomonal antibiotics received by the patients during treatment

Antibiotic	no. patients	
Piperacillin-tazobactam (TZP)	26	69% combinations
Amikacin (AMK)	22	
Meropenem (MEM)	20	
Cefepime (CEF)	19	
Ciprofloxacin (CIP)	6	

global influence of treatment



number of efflux systems detected at day 0 and day X

Riou et al, ECCMID 2010

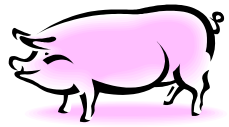
And in veterinary practice ?

Efflux and resistance

Relative contribution of target gene mutation and efflux to fluoroquinolone and erythromycin resistance, in French poultry and pig isolates of *Campylobacter coli*

Sophie Payot [a,*], Laetitia Avrain [b], Catherine Magras [c], Karine Praud [a], Axel Cloeckaert [a], Elisabeth Chaslus-Dancla [a]

International Journal of Antimicrobial Agents 23 (2004) 468–472



Efflux involved
in intrinsic resistance
and low level resistance

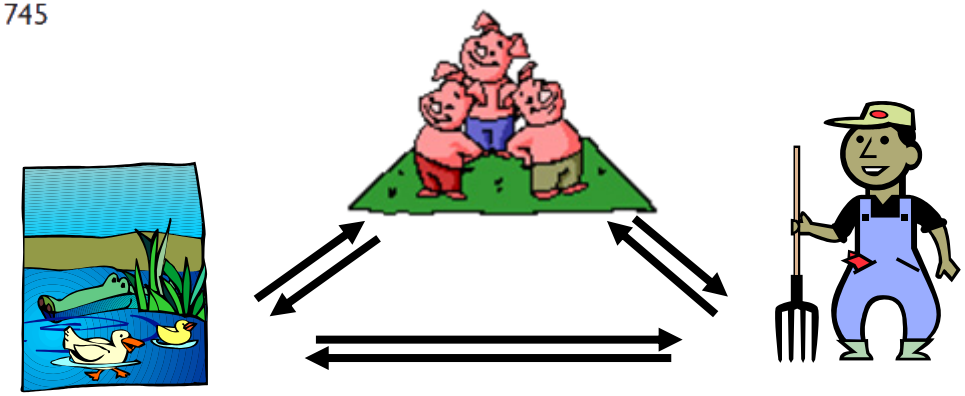
Number	Source	MIC (mg/l)			
		Erythromycin		Enrofloxacin	
		−EPI[a]	+EPI[b]	−EPI	+EPI
C308	Poultry	0.5	0.03	0.03	≤0.03
C161	Poultry	0.5	0.06	0.03	≤0.03
A7542	Pig	0.5	0.125	0.06	≤0.03
A1541	Pig	2	0.125	0.06	≤0.03
C1318	Poultry	8	0.25	4	2
C268, C606	Poultry	8	0.5	8	4
C340	Poultry	8	0.25	8	8
C342	Poultry	8	0.125	8	4
C782	Poultry	8	0.25	8	1
C1568	Poultry	8	0.125	8	2
A11321, O1511	Pig	8	0.25	8	2
O2121	Pig	8	0.5	8	4
O3911	Pig	8	0.5	16	4
A101E5, A10221, A11521	Pig	16	0.5	8	1
A10111	Pig	16	0.5	8	2
A10112, A10122, A10322, A10323, A10211	Pig	16	0.5	16	1
A10141, A10212, A10311	Pig	16	0.5	16	2
C850	Poultry	16	0.5	16	2
C1665, C1667	Poultry	16	0.5	32	8
C288	Poultry	256	128	4	2
C1332, C774	Poultry	512	128	4	1
C1578	Poultry	512	128	8	2
C1362	Poultry	>512	128	4	0.5
C1201, C776	Poultry	>512	128	4	1
C1208, C1564	Poultry	>512	256	4	2
C1256	Poultry	>512	128	4	2
C455	Poultry	>512	256	8	1
O1611	Pig	>256	256	16	8

Risks of cross-contamination!

Dissemination of IncFII plasmids carrying *rmtB* and *qepA* in *Escherichia coli* from pigs, farm workers and the environment

Y. Deng[1], Z. Zeng[1], S. Chen[2], L. He[1], Y. Liu[1], C. Wu[1,3], Z. Chen[1], Q. Yao[1], J. Hou[1], T. Yang[1] and J.-H. Liu[1]

Clin Microbiol Infect 2011; **17:** 1740–1745



Efflux system

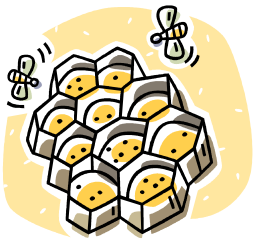
Strain	Origin	PLG type	PFGE	MLST	MIC (mg/L) AMP	GEN	AMI	NAL	NOR	CIP	NaN_3	Other resistance profiles	Plasmid incompatibility group	Plasmid RFLP	QRDR mutation[a] *gyrA*	*parC*	Other PMQR gene(s)
6-4	Weaner 2	A	UT	ST160	>256	>512	>1024	>128	>32	>64	128	TET, STR, CHL, SXT			L83, N87	I80, A84	*oqxAB*
6-4-T					>256	>512	>1024	4	1	0.125	1024		FII	Ic			
23-2	Boar	A	UT	ST461	>256	>512	>1024	>128	>32	64	128	TET, STR, CHL, SXT			L83, N87	I80	*oqxAB*
18-1-T					>256	>512	>1024	8	0.5	0.06	>512		FII	Ib			
13-5	Sow 2	A	UT	ST160	>256	>512	>1024	>128	>32	>64	128	TET, STR, CHL, SXT			L83, N87	I80, A84	*oqxAB*
13-5-T					>256	512	>1024	8	0.25	0.03	>512		FII	Ib			
39-4	Pond water	A	UT	ST160	>256	>512	>1024	>128	>32	>64	128	TET, STR, CHL, SXT			L83, N87	I80, A84	*oqxAB*
3-4-T					>256	512	>1024	8	1	0.125	>512		FII	Ib			
38-4	Worker 2	A	UT	ST160	>256	>512	>1024	>128	>32	>64	128	TET, STR, CHL, SXT			L83, N87	I80, A84	*oqxAB*

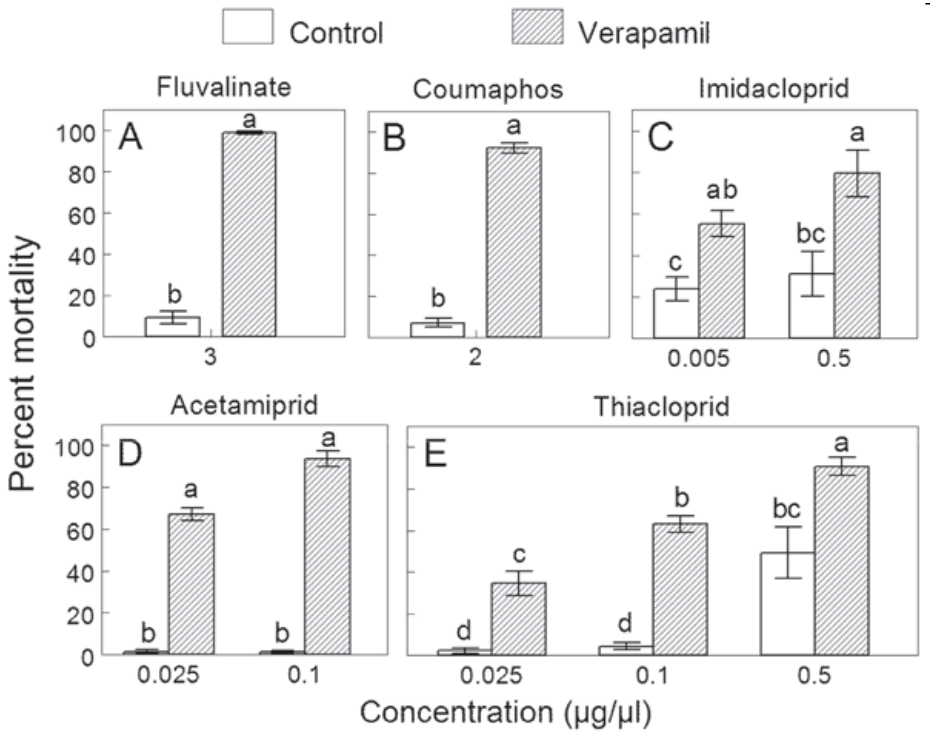
Antibiotics as inhibitors of efflux

Killing Them with Kindness? In-Hive Medications May Inhibit Xenobiotic Efflux Transporters and Endanger Honey Bees

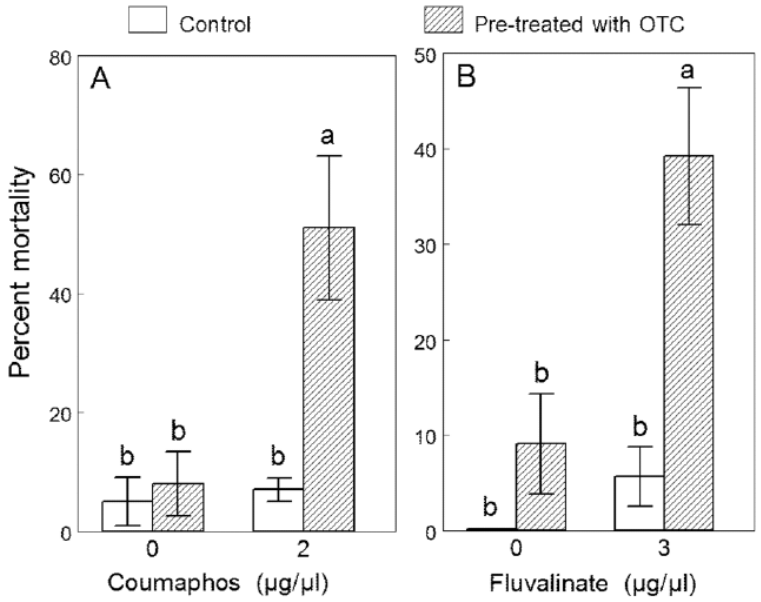
David J. Hawthorne*, Galen P. Dively

Citation: Hawthorne DJ, Dively GP (2011) Killing Them with Kindness? In-Hive Medications May Inhibit Xenobiotic Efflux Transporters and Endanger Honey Bees. PLoS ONE 6(11): e26796. doi:10.1371/journal.pone.0026796

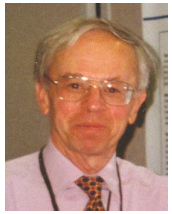




Tetracyclines as inhibitors of efflux



Efflux and bacteria in our team …



L. Avrain

M. Riou

GRAM(-)

J. Buyck

A. Anantharajah

V. Mohymont

GRAM(+)

F. el Garch

C. Vallet

A. Lismond

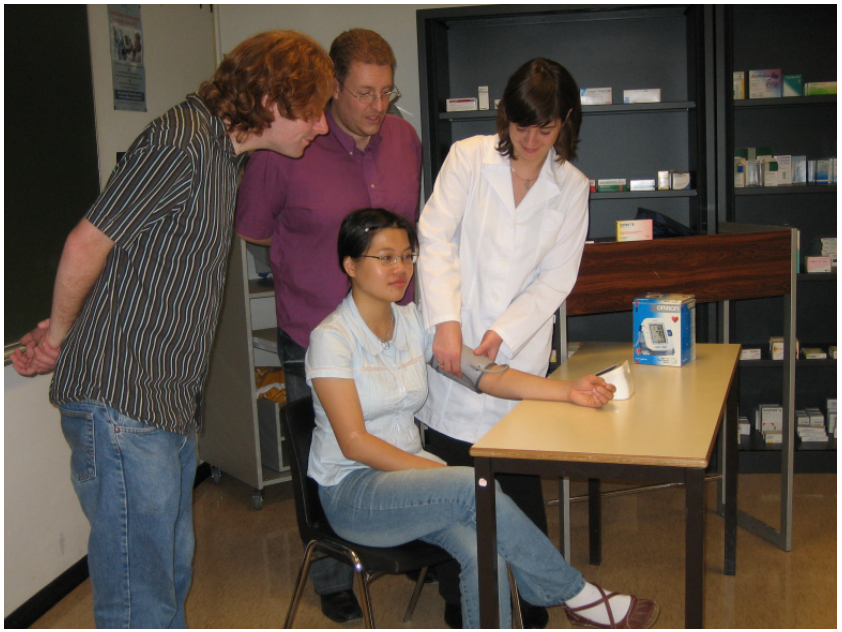
F. Degives

UCL Université catholique de Louvain

www.facm.ucl.ac.be

Les aventures d'un Tourangeau à Bruxelles …

Appliqué à suivre les travaux pratiques des étudiants …



… et à tester la gastronomie locale !