



Drug Resistance in *Pseudomonas aeruginosa* : Active Efflux and Membrane Impermeability

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Natural resistance of *P. aeruginosa*

amoxicillin
1stGC, 2ndGC
cefotaxime...

kanamycin
neomycin
spectinomycin

chloramphenicol



tetracyclines

trimethoprim
sulfamides

nitrofurans

older quinolones
pefloxacin...

macrolides
lincosamides
synergistines

Antipseudomonal antibiotics

β -lactams

- **ticarcillin** \pm clavu
- **piperacillin** \pm tazo
- aztreonam
- cefsulodin
- cefoperazone
- **ceftazidime**
- cefpirome
- **cefepime**
- **imipenem**
- meropenem

Aminoglycosides

- gentamicin
- netilmicin
- **tobramycin**
- **amikacin**
- isepamicin

Fluoroquinolones

- ofloxacin
- **ciprofloxacin**
- **levofloxacin**
- sitafloxacin

Others

- **colistin**
- polymyxin B
- rifampicin
- fosfomycin

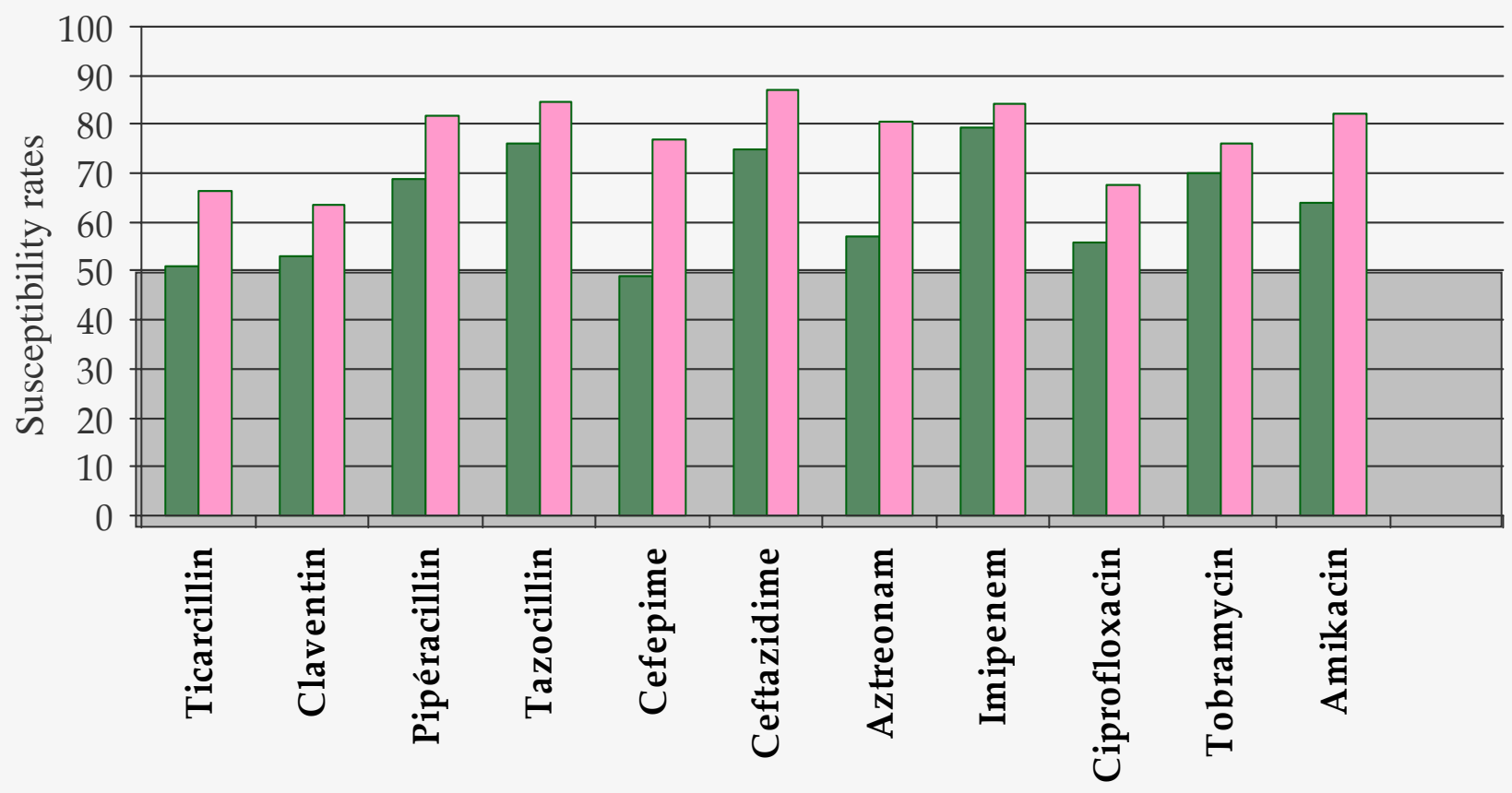
Natural resistance of *P. aeruginosa*

1. Inducible chromosomally-encoded β -lactamase AmpC
2. Aminoglycoside modifying enzyme APH(3')-II
3. Poor outer membrane permeability
4. Constitutive expression of efflux system MexAB-OprM
5. Inducible expression of efflux proteins MexXY



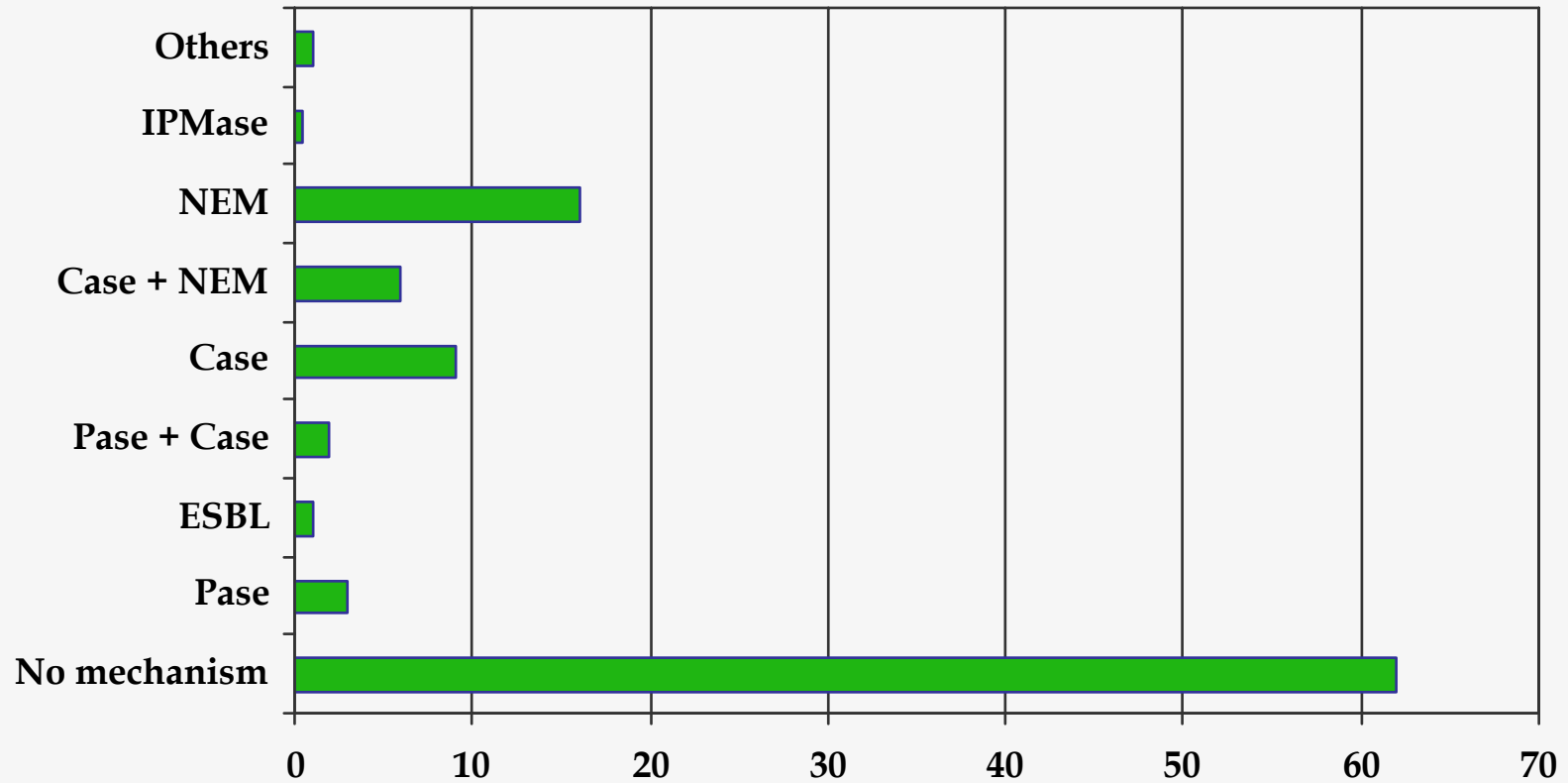
Additive or Synergistic Effects

Drug resistance of *P. aeruginosa* in France (1994-2004)



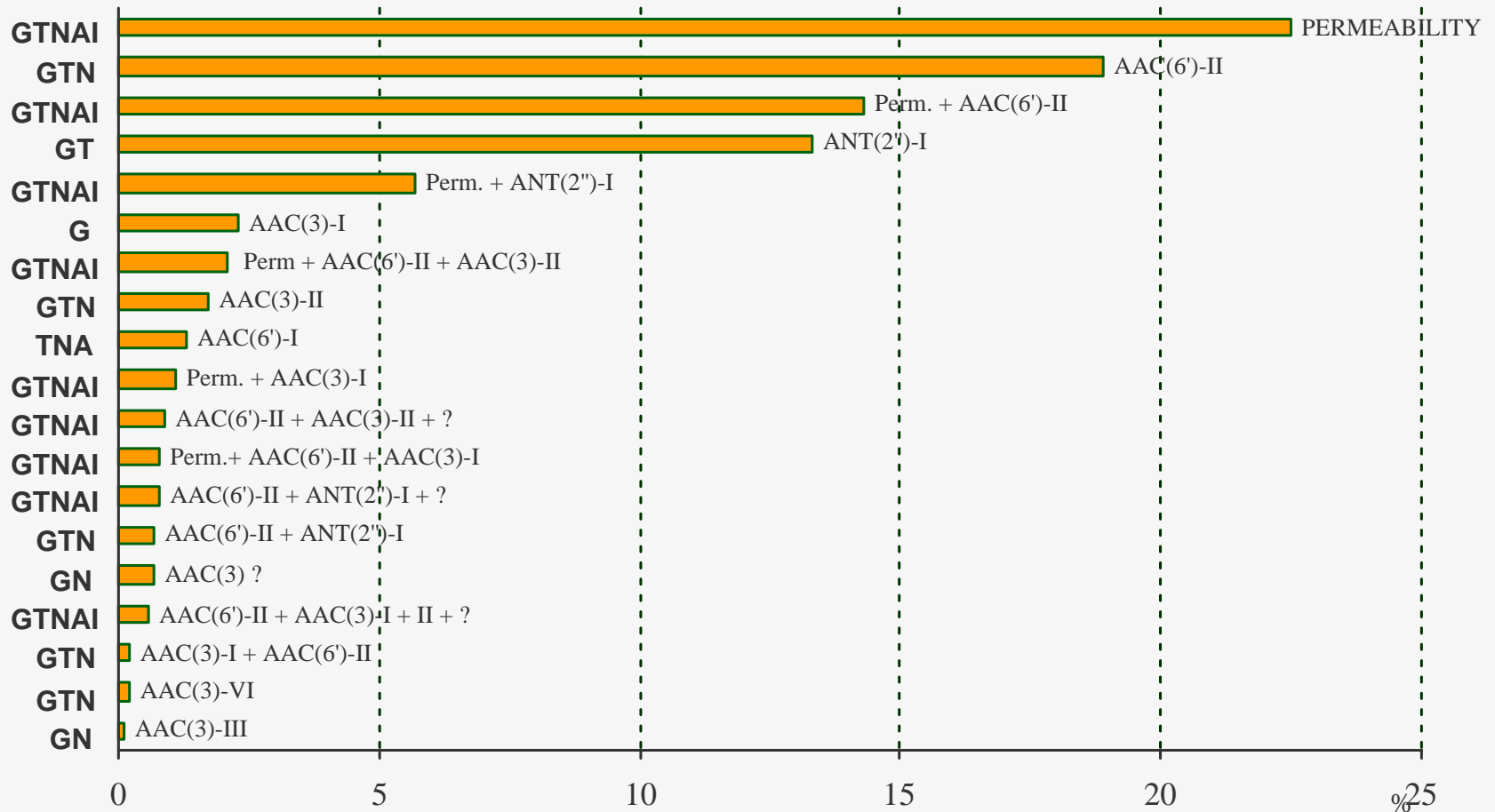
Resistance mechanisms to β -lactams

(GERPA 2004)



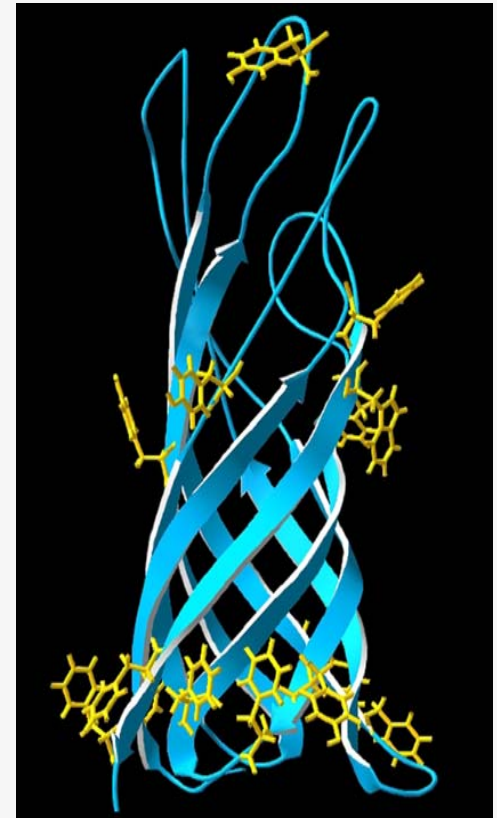
Resistance mechanisms to aminoglycosides

(Europe 1988-1993, n = 1,680)



Porin OprF

- ❖ Structure and function
 - major porin of *P. aeruginosa*
 - OmpA-like structure
 - non-specific uptake pathway
 - only 5% OprF molecules form open, functional channels : slow diffusion
 - structural role (o.m. integrity)
- ❖ Role in resistance
 - loss of OprF is deleterious to the cell
 - rarely documented (CF isolates)



Specific porins

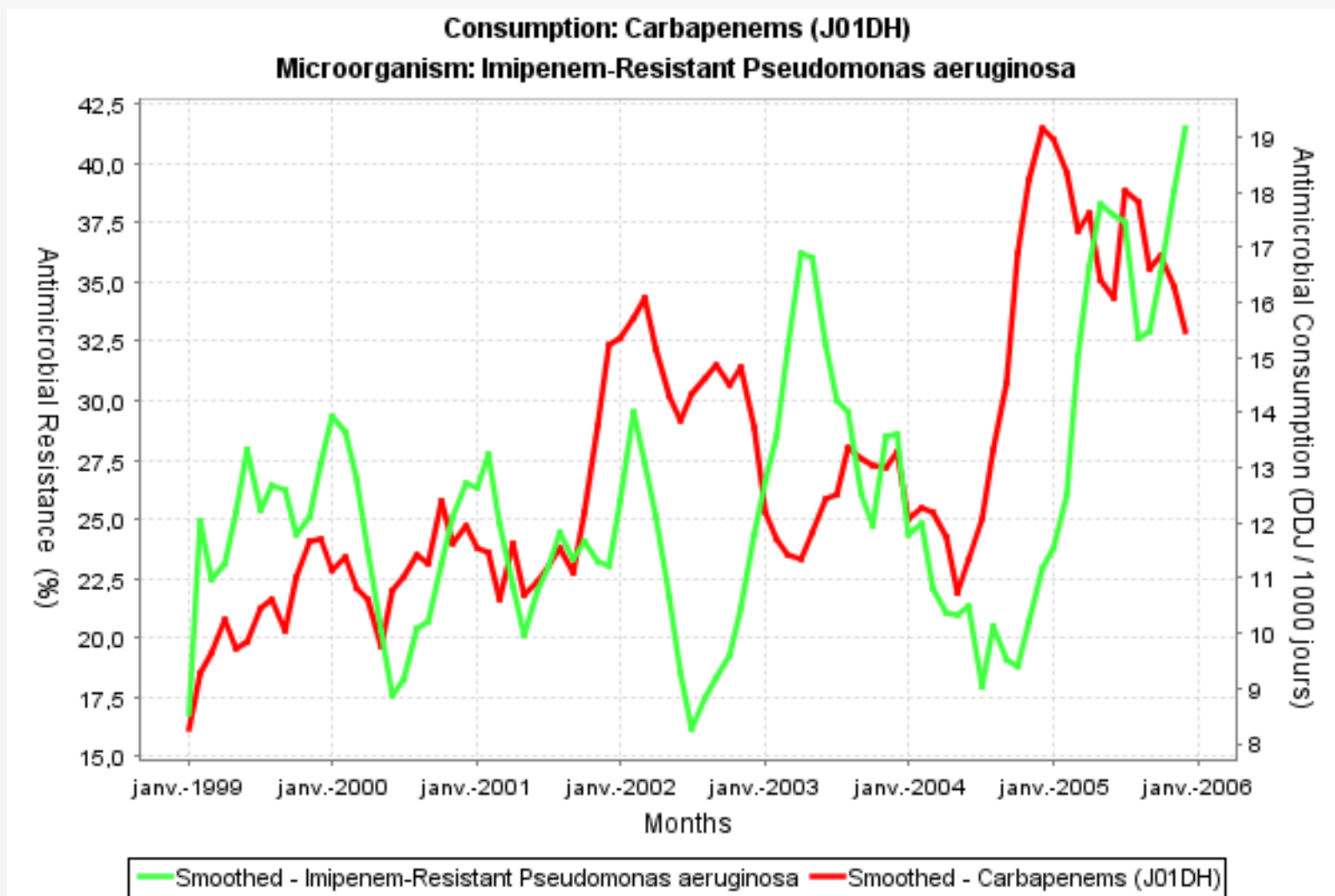
❖ Porins for specific substrates

- OprD for basic aa, gluconate... and carbapenems
- OprB for glucose uptake
- OprC, OprR, OprO, OprP
- and many OprD-like proteins...!

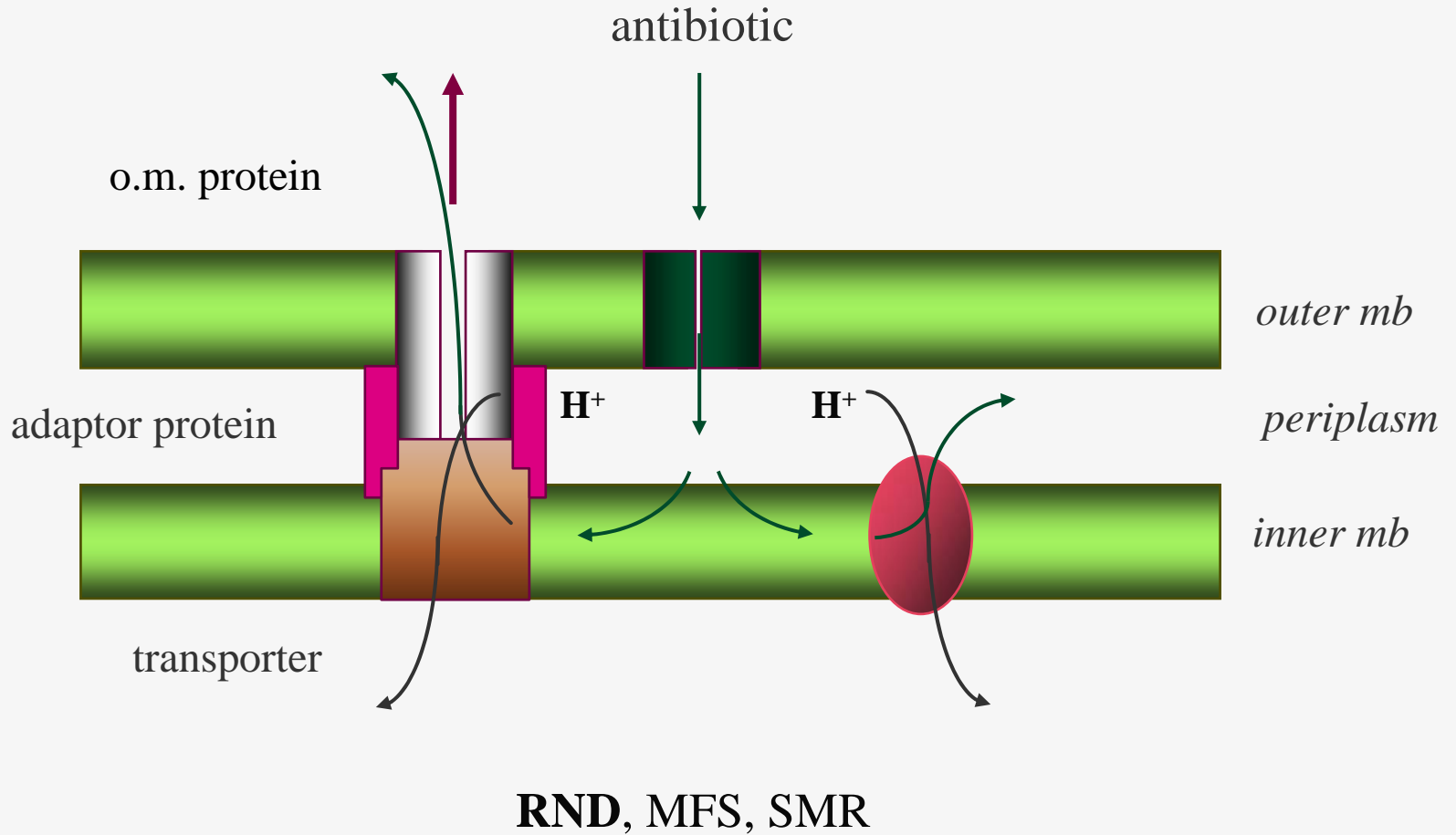
❖ Loss of OprD

- facilitated uptake pathway of imipenem and meropenem
- spontaneous OprD⁻ mutants: 10^{-6} à 10^{-7}
- resistance limited to carbapenems (4 - 16 µg/ml)
- OprD⁻ + stable overexpression of AmpC
- OprD⁻ + production of carbapenemase I MP-1

Imipenem : consumption and resistance



Structure of efflux systems



Substrates of RND efflux systems in *P. aeruginosa*

System	Operon	Substrates
MexAB-OprM	<i>mexAB, oprM</i>	FQ, β -lactam, Tmp, Cmp, Tet, Nov, Ery...
MexXY (OprM)	<i>mexXY</i>	FQ, <u>AG</u> , Fep, Cpo, Tet, Ery...
MexCD-OprJ	<i>mexCD, oprJ</i>	FQ, Cpo, Fep, Tmp, Cmp, Tet, Ery...
MexEF-OprN	<i>mexEF, oprN</i>	FQ, (Ipm), Tmp, Cmp...
MexGHI-OpmD	<i>mexGHI, opmD</i>	FQ...
MexJK (OprM)	<i>mexJK</i>	Tet, Ery...
MexVW (OprM)	<i>mexVW</i>	FQ, Cmp, Tet, Ery...

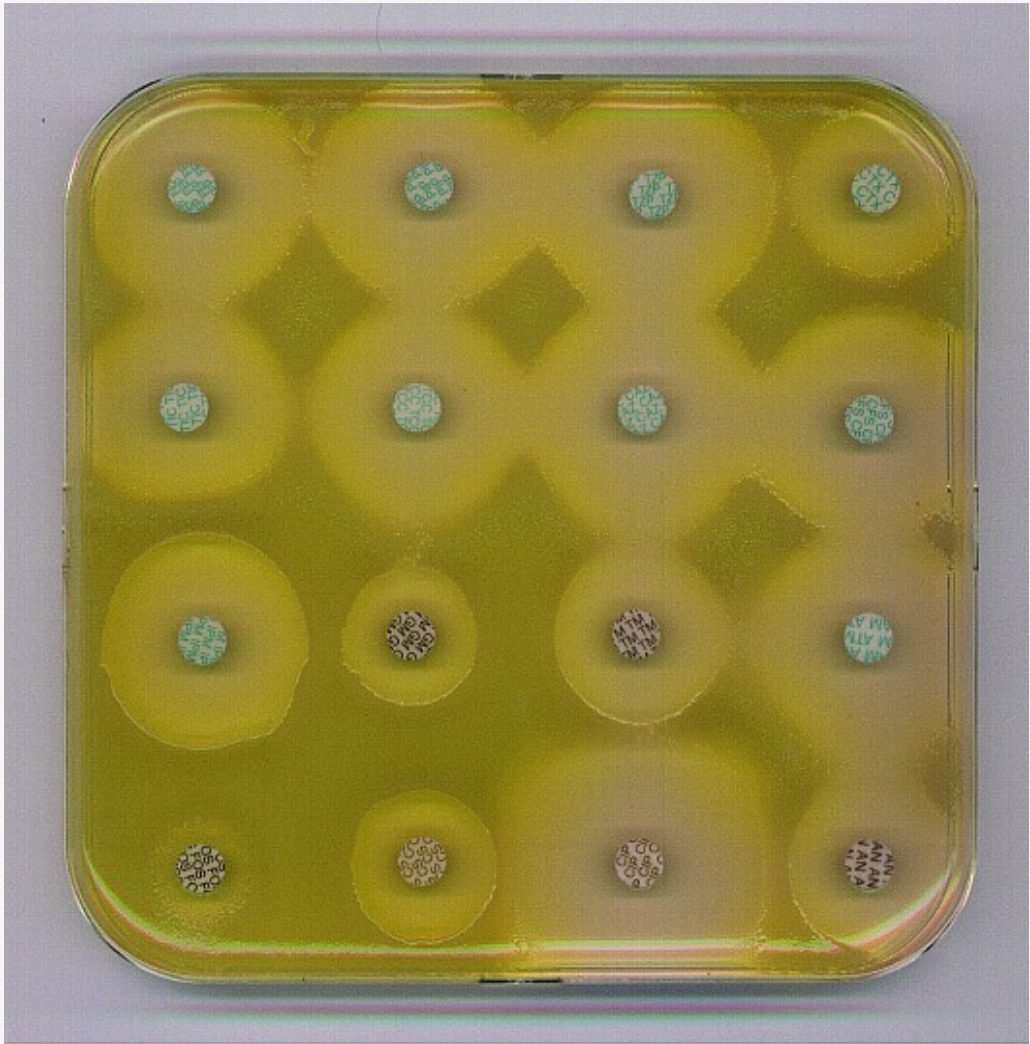
Fq (fluoroquinolones), β -lactam (except imipenem), Tmp (trimethoprim), Cmp (chloramphenicol), Tet (tetracycline), Nov (novobiocin), Ery (erythromycin), AG (aminoglycosides), Fep (cefepime), Cpo (ceftazidime), Ipm (imipenem).

Intrinsic mechanisms of resistance

Mechanisms	Rates	Tic	Tzp	Caz	Fep	Ipm	Mpm	Tob	Amk	Ofx	Cip
<i>Beta-lactamase</i>											
AmpC ↑	++	I-R	I-R	I-R	S-I-R						
<i>Active efflux</i>											
MexABM ↑	++	I-R								I-R	
MexXY ↑	++				I				S-I	I-R	
<i>Impermeability</i>											
Porine OprD ↓	++					I-R	S-I-R				
<i>Targets</i>											
GyrA/B, ParC/E	++									R	I-R

Tic (ticarcillin), Tzp (tazocillin), Caz (ceftazidime), Fep (cefepime), Ipm (imipenem), Mpm (meropenem), Tob (tobramycin), Amk (amikacin), Ofx (ofloxacin), Cip (ciprofloxacin)

Wild-type drug susceptibility (PAO1)



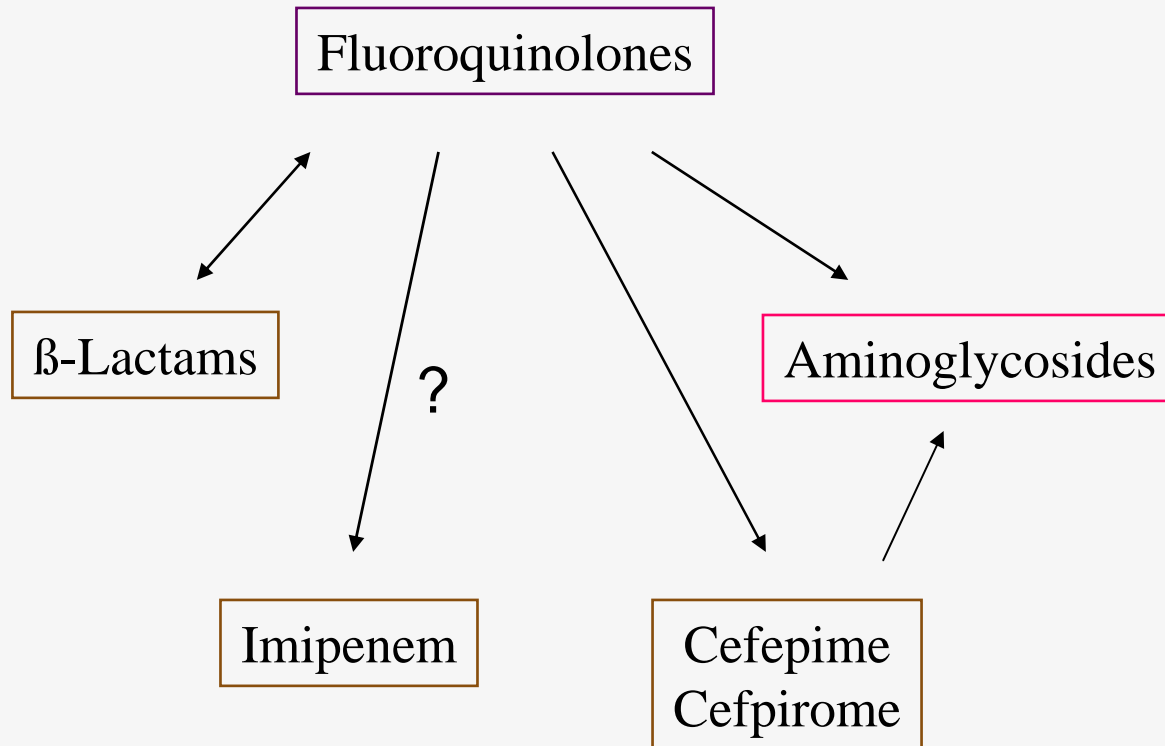
Mutant MexAB-OprM



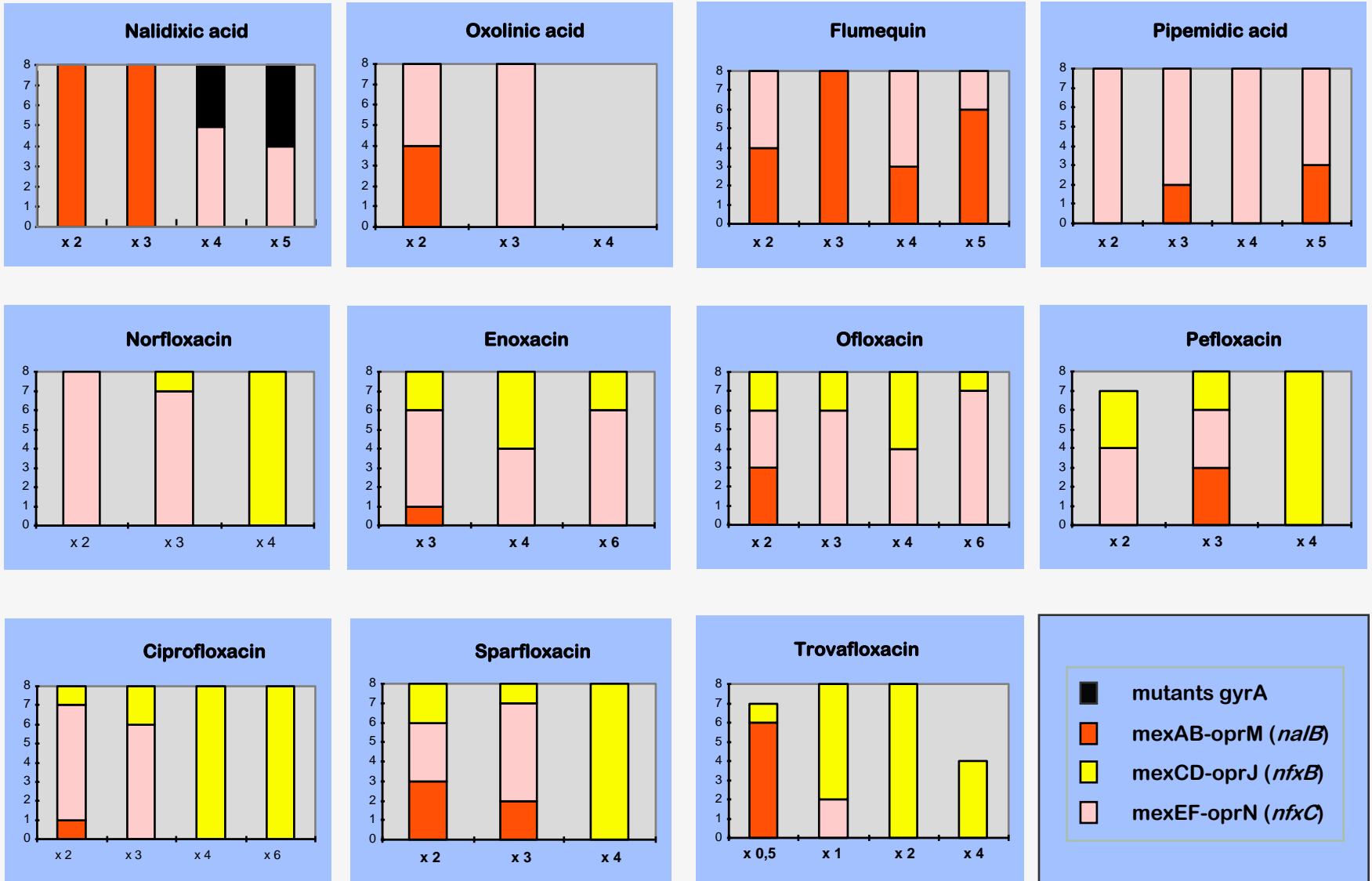
Mutant MexXY-OprM



Selection of multidrug resistance



In vitro selection of efflux mutants



Emergence and loss of resistance in *P. aeruginosa* in single patients

- ❖ Acquisition of resistance (n = 18)
 - Overproduction of AmpC βlase: 4 patients
 - Penicillinase: 1 patient
 - **MexAB-OprM overexpression: 8 patients**
 - Specific resistance to Ipm: 5 patients
- ❖ Loss of resistance (n = 7)
 - Penicillinase: 2 patients
 - **MexAB-OprM down-regulation: 4 patients**
 - Specific resistance to Ipm: 1 patient

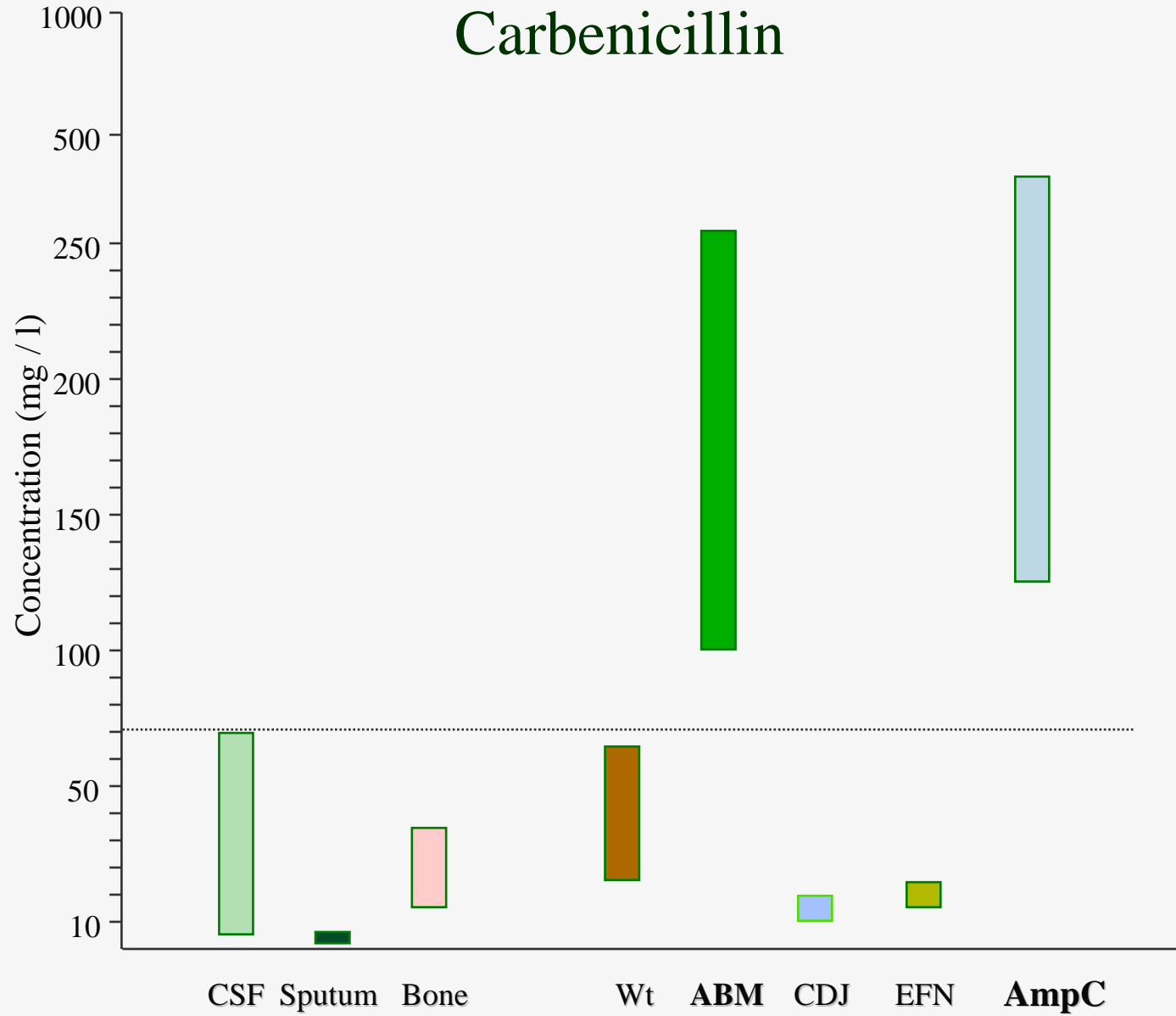
Emergence of mdr due to MexAB-OprM up-regulation

Patient	Site	Relevant therapy	Possible cause
#6	Resp. tract	Caz 6g / cip 0.8g	FQ
#7	Urine	Akn 1g / pef 0.8g	<u>FQ alone</u> for 13 days
#8	Resp.+ urine	Tcc 12g	<u>Tic alone</u> for 9 days
#9	Skin	Amc 3g / pef 0.8g	<u>Inadequate β-lactam</u> + FQ
#10	Skin	Pip 12g / net 0.4g	<u>Pip alone</u> for 10 days
#11	Resp. tract	Tcc 15g / akn 1g	<u>Tic alone</u> 7 days
#12	Faeces + resp	Amc 5g / oflo 0.8g	<u>Inadequate β-lactam</u> + FQ
#13	Urine	Amc 3g / cip 0.8g	<u>Inadequate β-lactam</u> + FQ

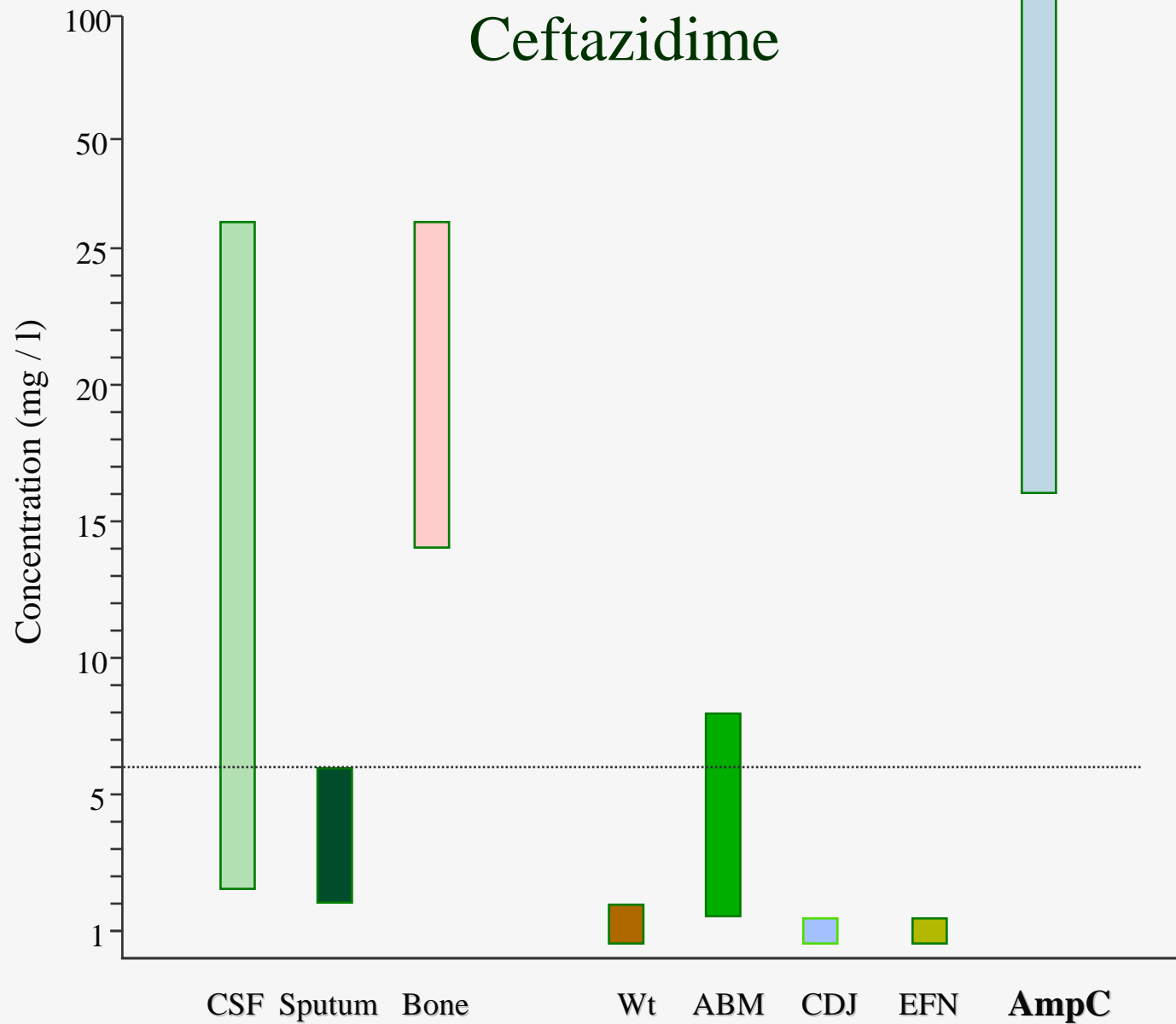
$$\text{EFFLUX} = \text{MIC} \times 2-16$$

Is it clinically significant ?!

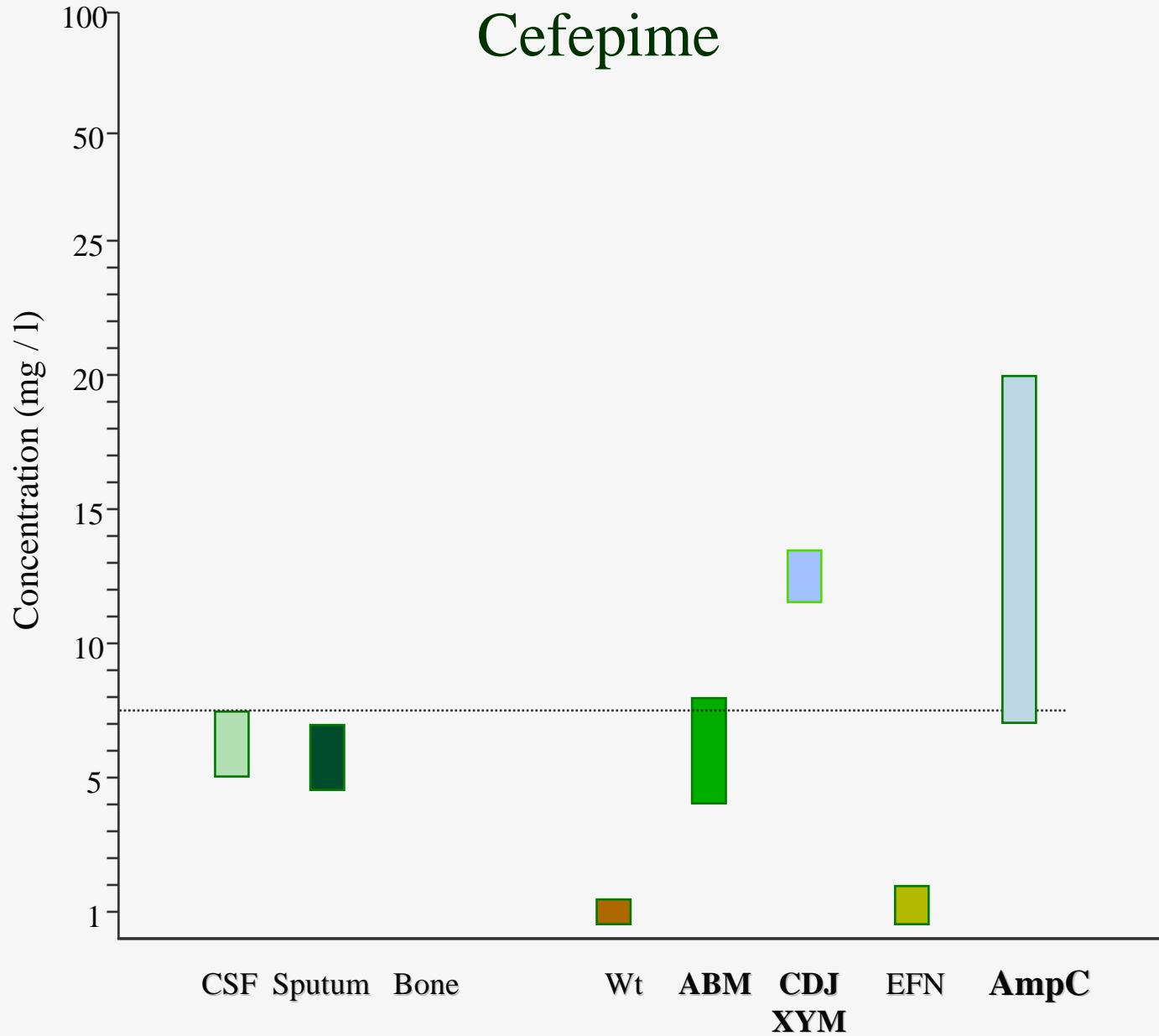
Carbenicillin



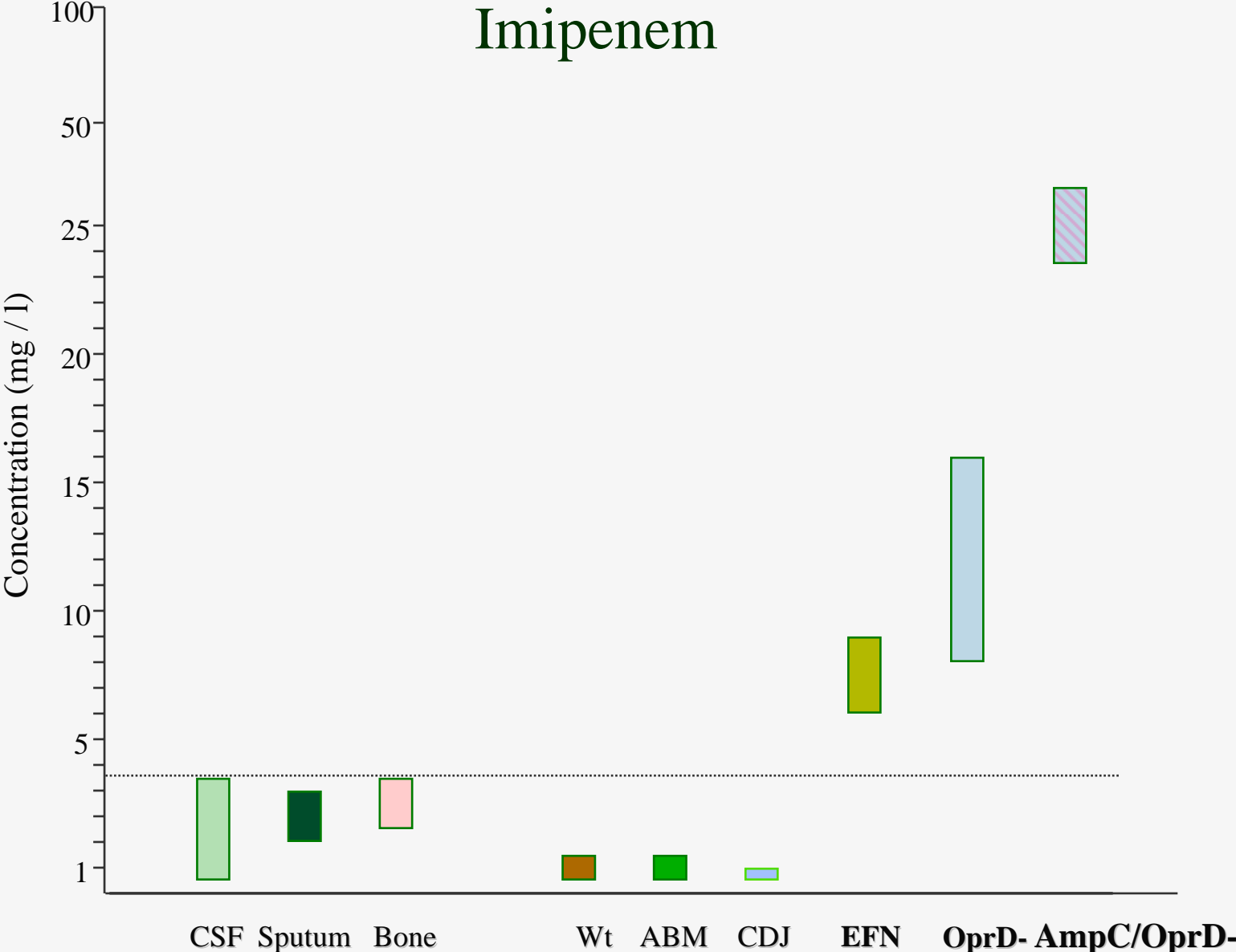
Ceftazidime



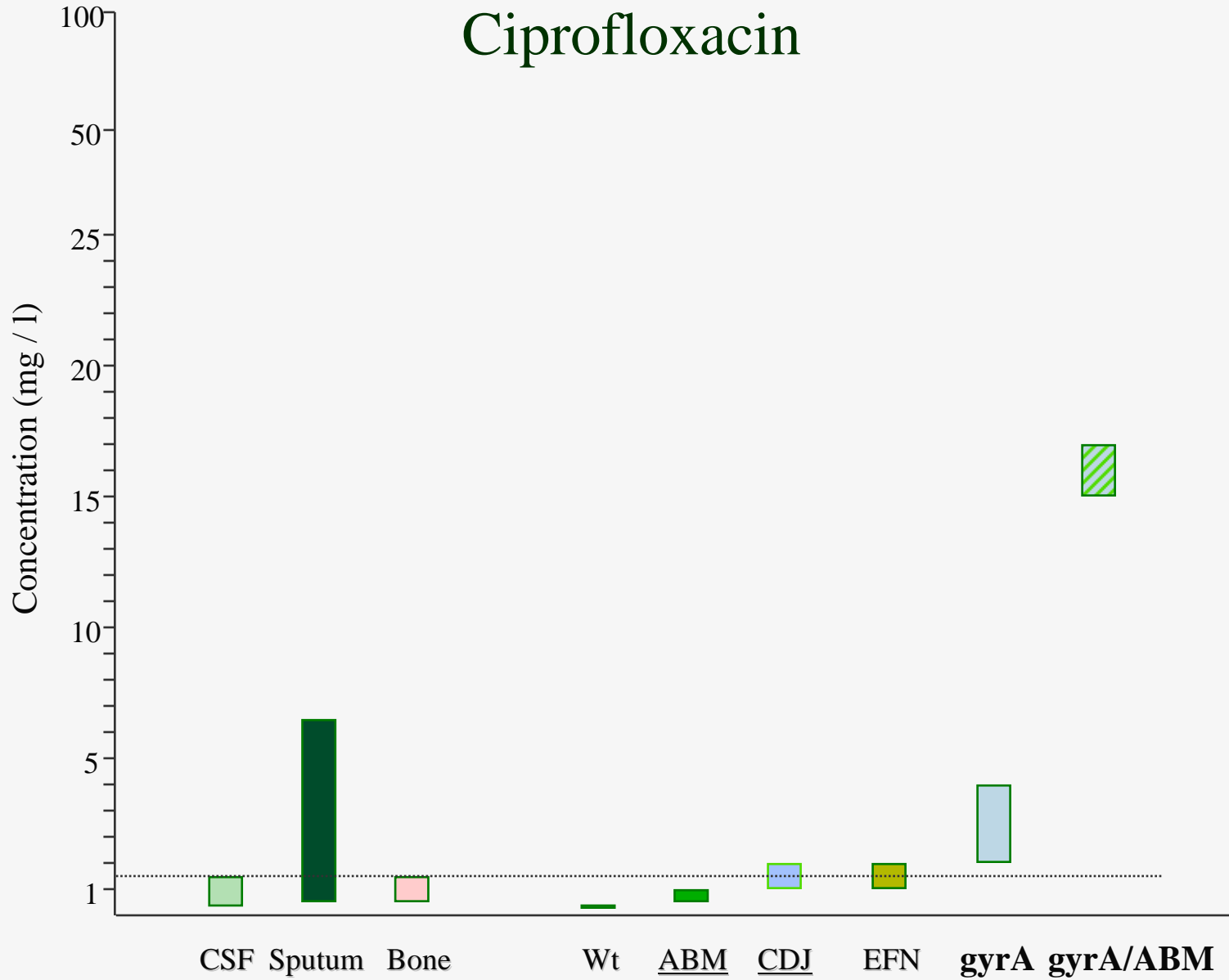
Cefepime



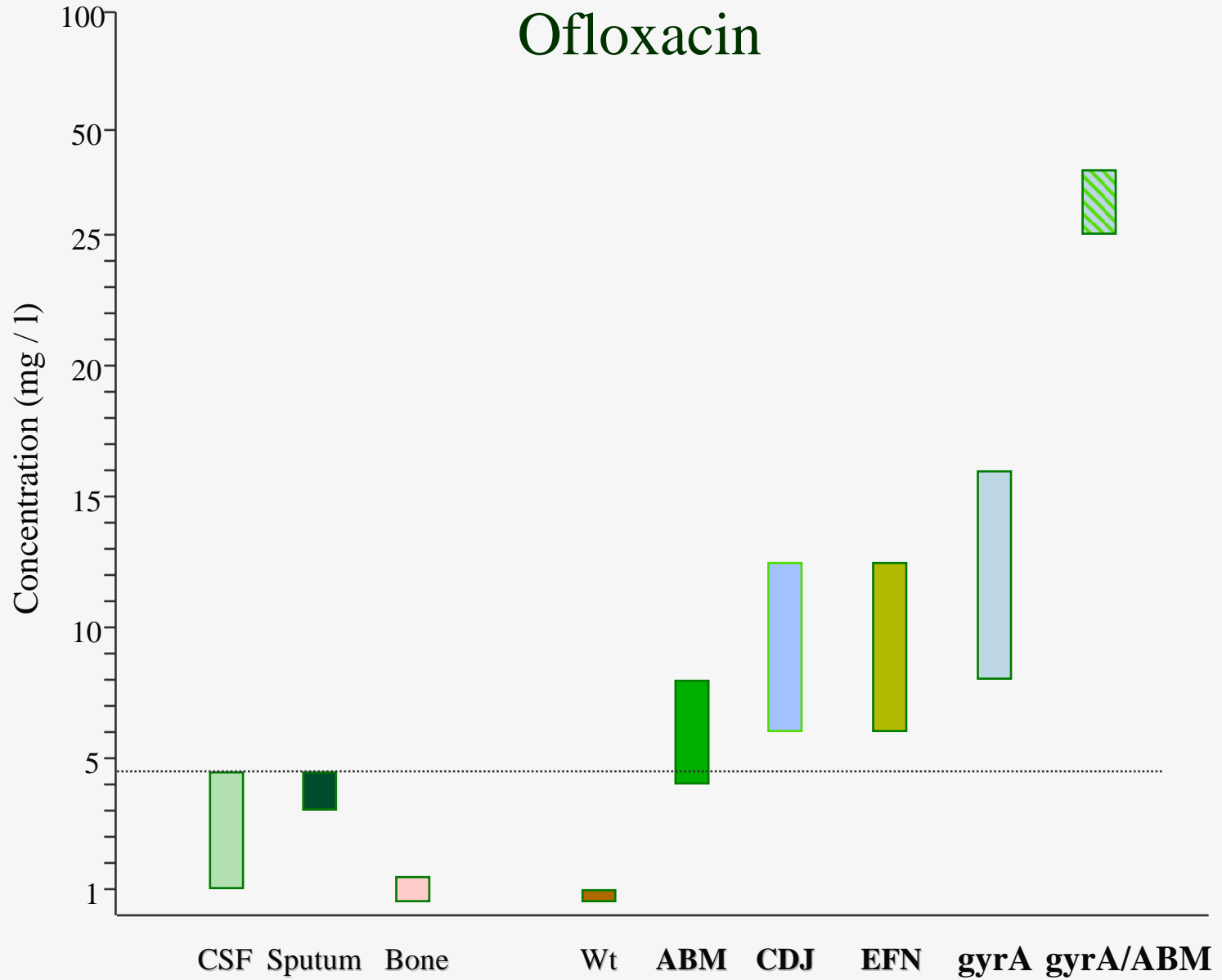
Imipenem



Ciprofloxacin



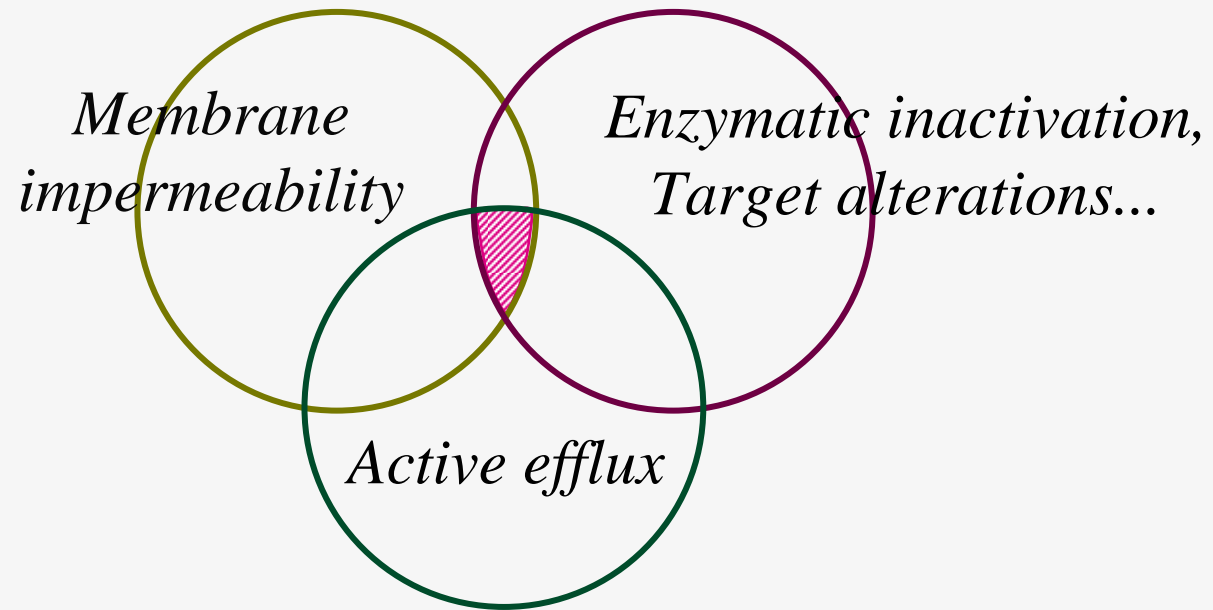
Ofloxacin



PK/PD Monte Carlo

Drug	Treatment		MIC (mg/L)	Target Attainment Rate (%)	
	total daily dosage (mg)	unitary dose interval (hours)		C _{max} /MIC > 10	AUC/MIC > 125
Ciproflox.	1200	8	0.12	66	87
			0.25	6	7
	1600	6	0.12	66	90
			0.25	5	12
	2400	8	0.12	98	100
			0.25	60	85
0.5	4.2	3.7			
Levoflox.	500	24	0.5	70	40
			1	4	3
	1000	12	0.5	72	72
			1	4	5

Multidrug resistance



Multidrug resistant phenotype



Additive (A) or multiplicative (M) effects

Other mechanisms	MexAB-OprM	MexXY (OprM)
β -lactamases	-	-
OprD loss	-	-
LPS alterations	?	A
Active uptake	-	A
Ribosome alteration	-	A
PLP alteration	?	-
GyrA, GyrB, ParC	A/M	A
MexAB-OprM		A
MexXY (OprM)	A	

C. Llanes *et al.* Antimicrob. Agents Chemother. 2004, 48: 1797

I. Le Thomas *et al.* J. Antimicrob. Chemother. 2001, 48: 553

Target/efflux mutants

MIC levofloxacin (mg/l)

Target mutations	Wt	ABM ⁺⁺	ABM ⁺⁺ + inh. 10 mg/l
Aucune	0.25	2	0.03
<i>gyrA</i> (Thr83->Ile)	2	8	0.5
<i>gyrA</i> (Thr83->Ile) + <i>parC</i> (Ser87->Leu)	4	32	2
<i>gyrA</i> (Thr83->Ile + Asp87->Tyr) + <i>parC</i> (Ser87->Leu)	16	128	8

Multiple mechanisms

(69 bacteremic resistant strains GESPA 1999)

MexXY : 16

MexXY + EnzMod : 5

MexXY + AmpC : 2

MexXY + EnzMod + AmpC : 2

MexXY + EnzMod + Pase : 3

MexAB : 4

MexAB + AmpC : 2

MexXY + MexAB : 3

MexXY + MexAB + EnzMod : 4

MexXY + MexAB + AmpC : 2

MexXY + MexAB + EnzMod + AmpC : 2

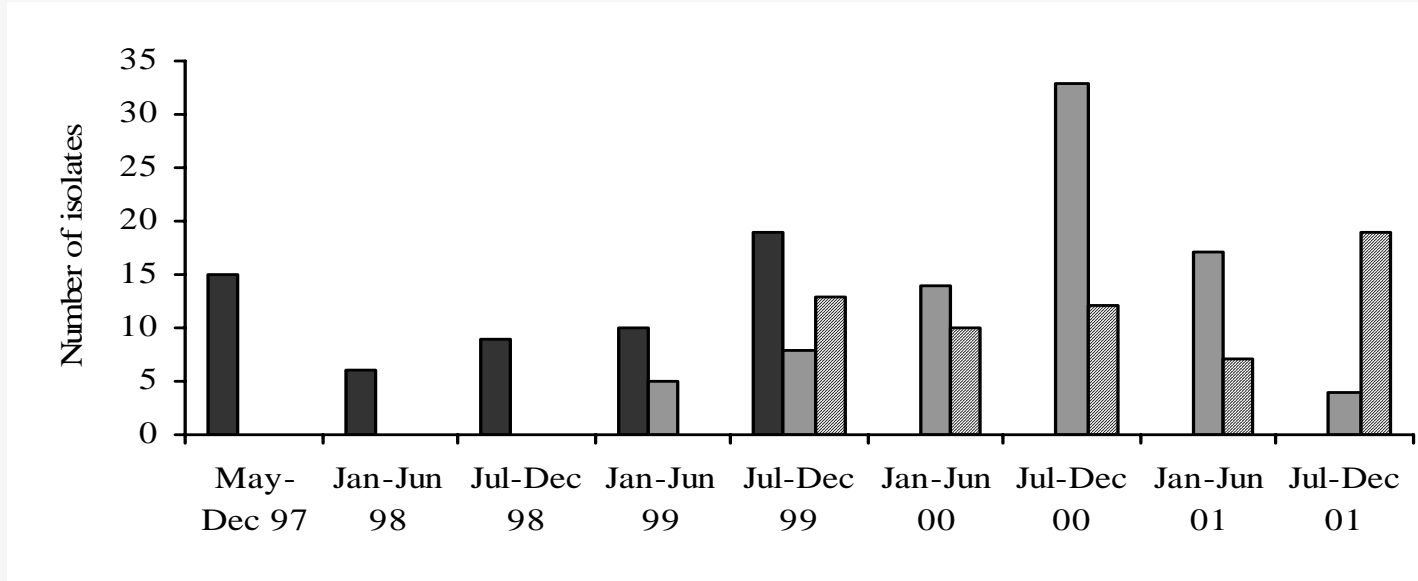
MexXY + MexAB + EnzMod + Pase : 5

MexXY + MexAB + EnzMod + AmpC + Pase : 1

Unknown mechanisms of resistance to aminoglycosides : 18

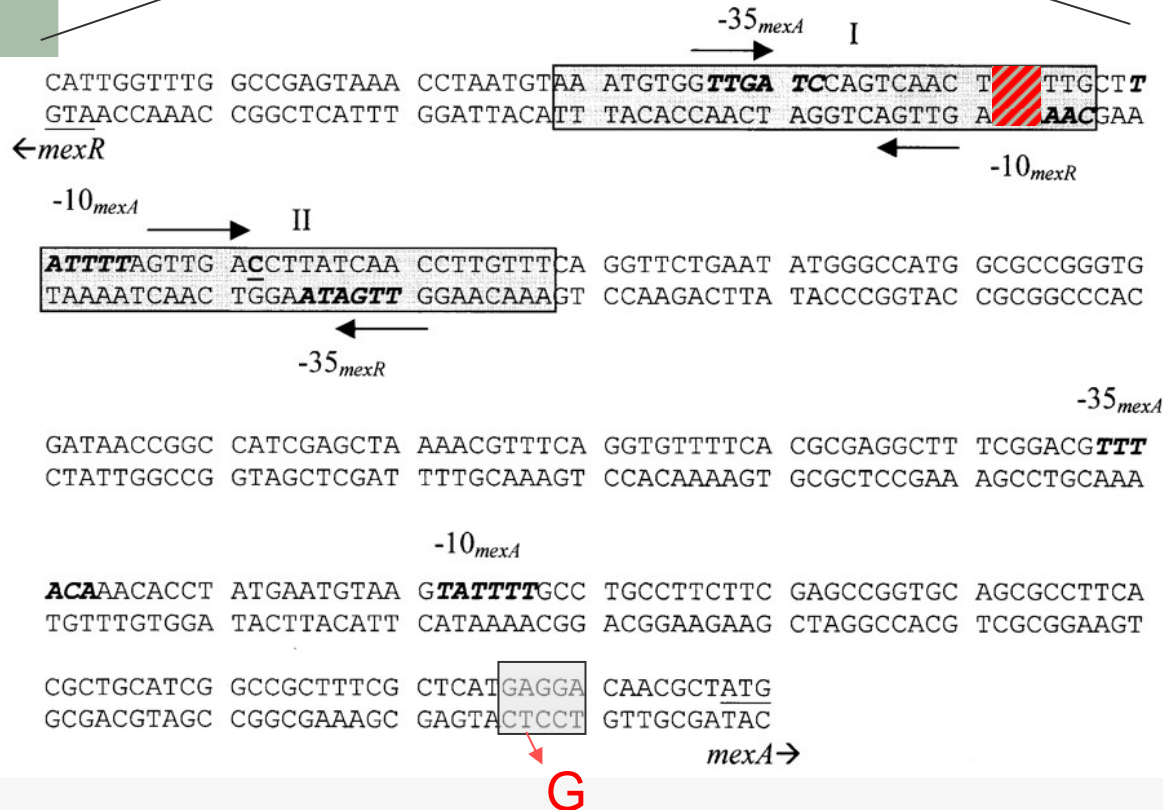
Target alterations (*gyrA*, *gyrB*, *parC*), loss of porins (OprD)...

An epidemic clone overexpressing MexAB-OprM



■ surgical ICU
■ medical ICU
▨ other wards

Reversion to wild-type susceptibility



mexA : 1.6-3.5

mexA : 0.5-0.8

Summary

❖ Loss of porin OprD

- High prevalence of OprD deficient mutants
- Moderate resistance to carbapenems (only)
- Mutants readily selected *in vivo* by carbapenems

❖ Derepression of active efflux pumps

- High prevalence of MexAB-OprM and MexXY/OprM overproducing mutants
- Moderate therapeutic impact regarding β -lactams (Tic, 4thGC) and aminoglycosides (Gm, Akn)
- Significant therapeutic impact regarding fluoroquinolones with poor antipseudomonal activities !
- Contributive factors to pandrug resistance



Cyril Amstutz

Didier Hocquet

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Katy Jeannot

Farid El'Garch

Catherine Llanes

BACTERIO-WEB



Accueil

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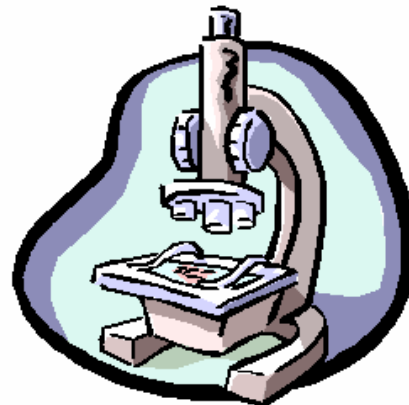
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Outil pédagogique multimédia - Mis à jour en novembre 2004

Cette base de donnée en Bactériologie est destinée aux étudiants en médecine de DCEM-1, aux biologistes en cours de formations dans le cadre du DES de Biologie Médicale, et aux technicien(ne)s des services de bactériologie.

Elle a été réalisée à l'initiative des enseignants du service de Bactériologie du CHU Jean Minjot et de la Faculté de Médecine-Pharmacie de Besançon. Posez vos questions à l'équipe [l'équipe d'enseignants](#).

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