Mechanisms of resistance and susceptibility in CF pathogens

Françoise Van Bambeke, PharmD, PhD
Pharmacologie cellulaire et moléculaire
Louvain Drug Research Institute
Université catholique de Louvain, Brussels, Belgium

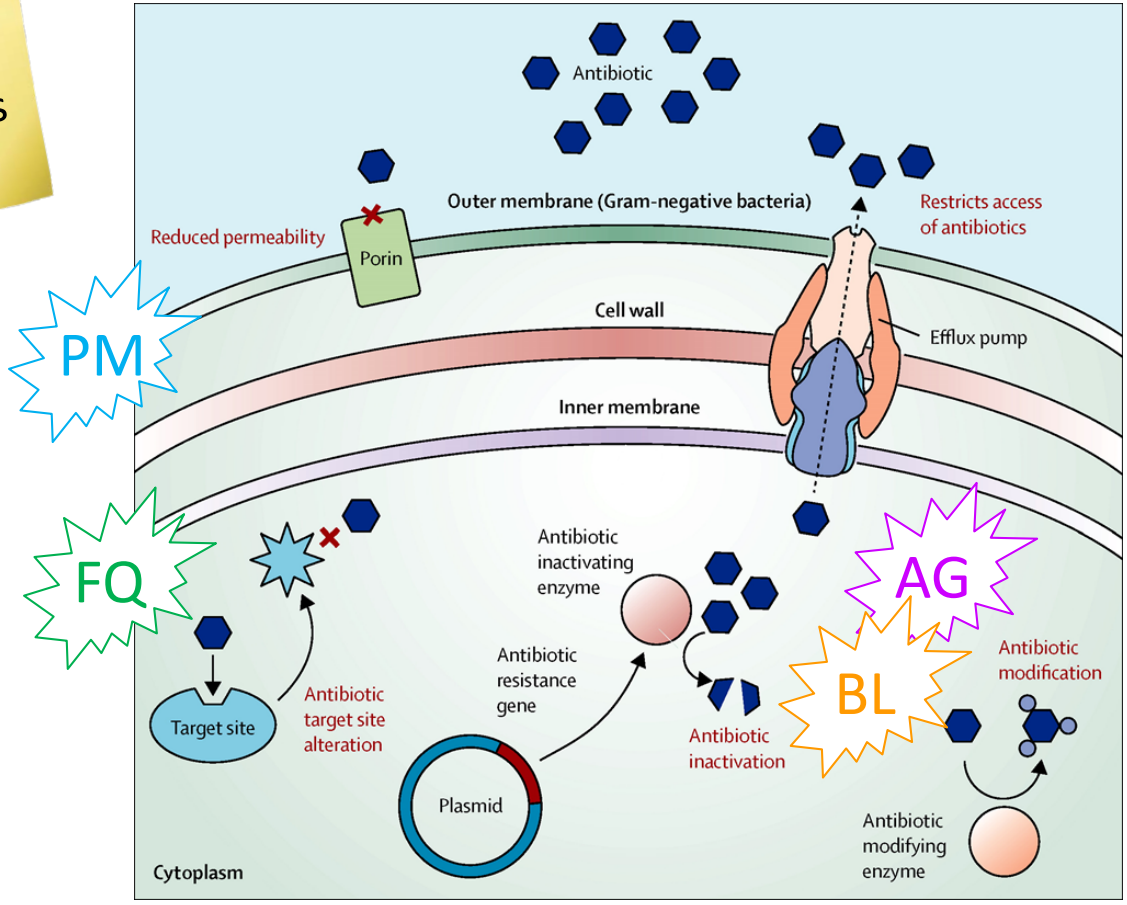
www.facm.ucl.ac.be

Main mechanisms of antibiotic resistance in Gram-(-) bacteria

RESISTANCE

- acquired: gene acquisition; mutations; change in expression
- intrinsic: poor penetration < constitutive expression of efflux pumps < low porin permeability

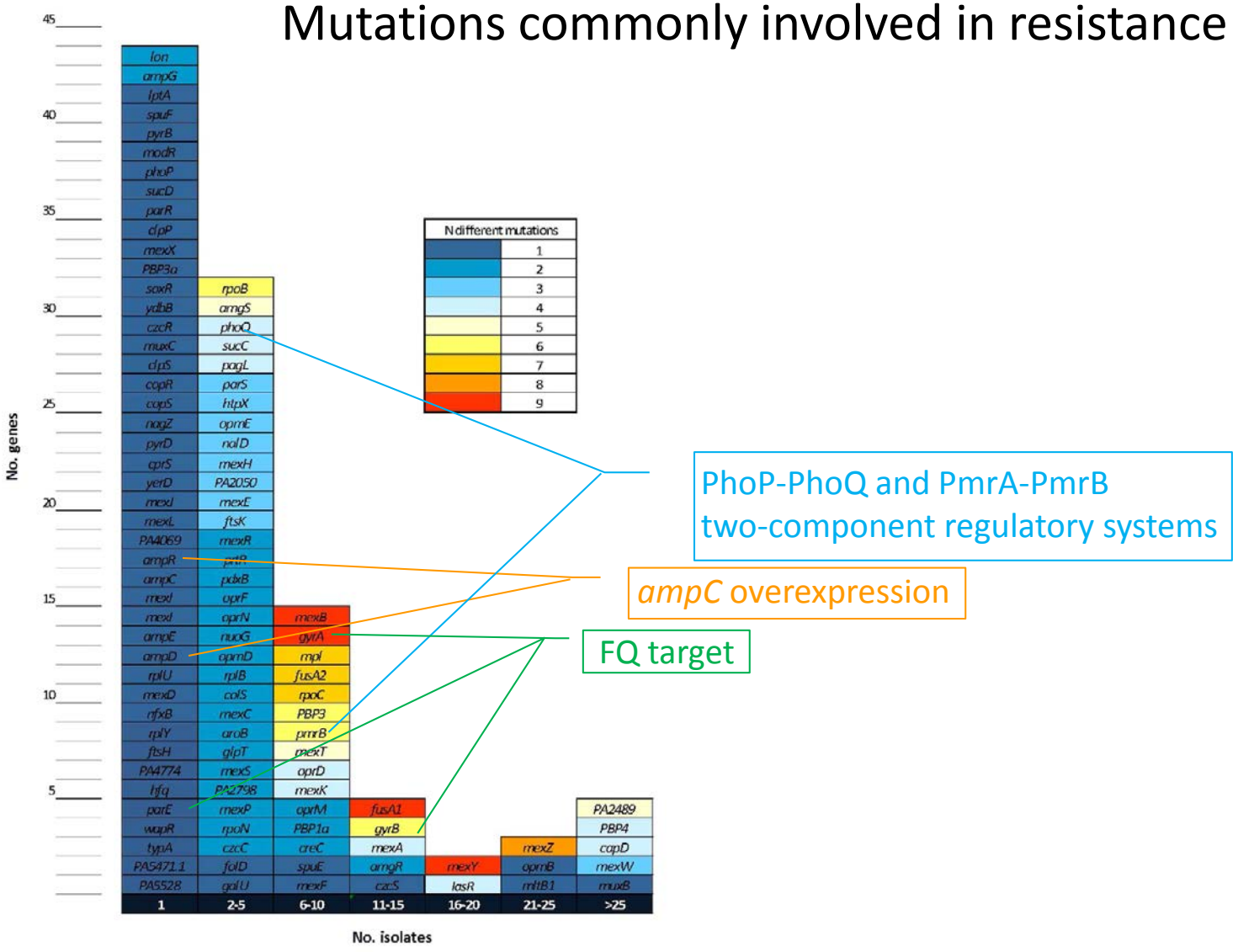
In most textbooks …



Sherrard et al, Lancet 2014; 384:703-713

Gene mutations in the CC274 *P. aeruginosa* clone

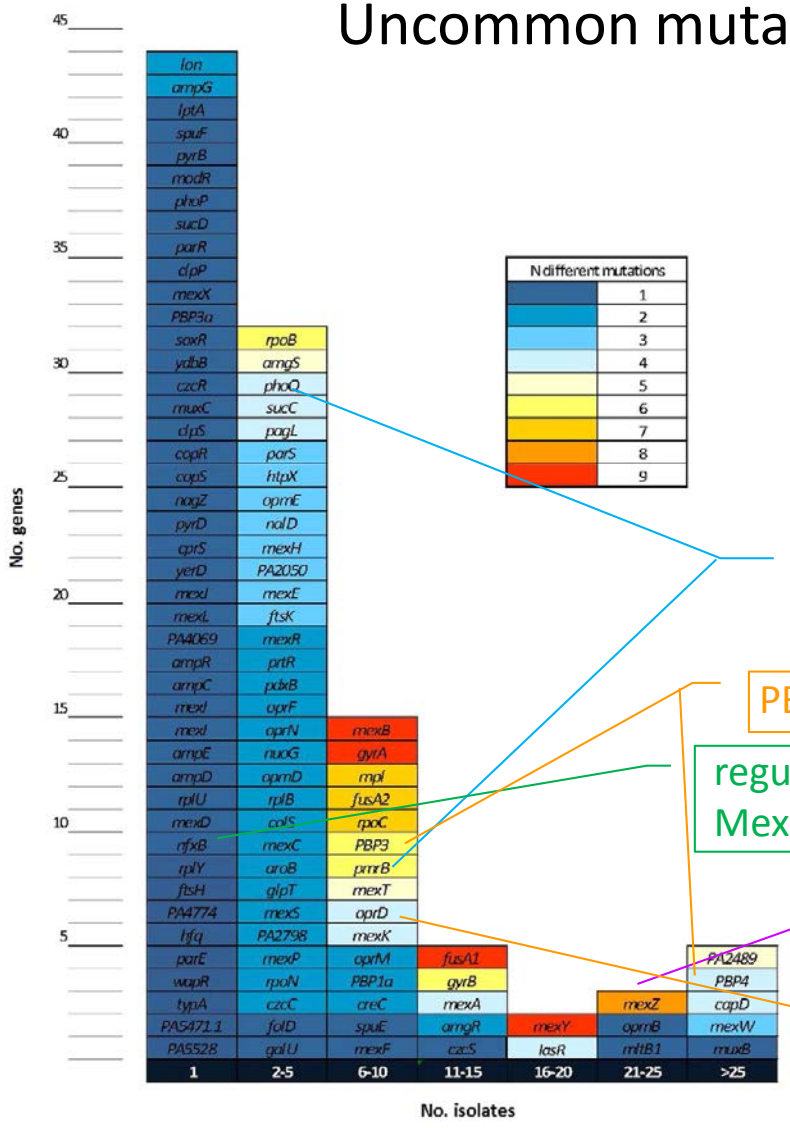
Mutations commonly involved in resistance ….



Lopez-Causapé et al, Sci Rep 2017; 7, 5555

Gene mutations in the CC274 *P. aeruginosa* clone

Uncommon mutations frequent in CF clones ….



PhoP-PhoQ and PmrA-PmrB two-component regulatory systems → specific mutations In CF isolates

PBP3-4 mutations → carbapenem resistance ?

regulator of MexCD-OprN → fluoroquinolone efflux; advantage in CF ?

regulator of MexXY-OprM → aminoglycoside / cefepime efflux

porin mutations → carbapenem resistance

Lopez-Causapé et al, Sci Rep 2017; 7, 5555

Main mechanisms of antibiotic resistance in *Pseudomonas*

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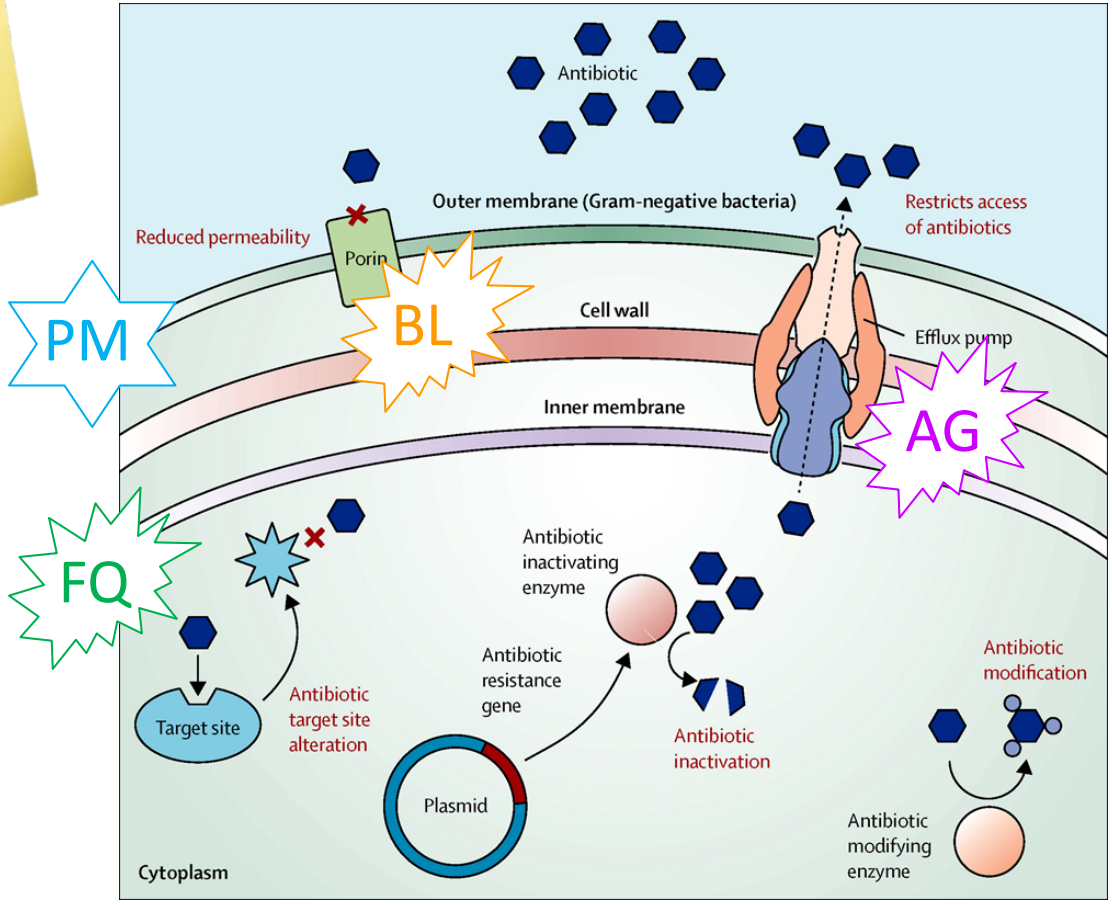
In CF ?



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Main mechanisms of antibiotic resistance in Gram-(-) bacteria

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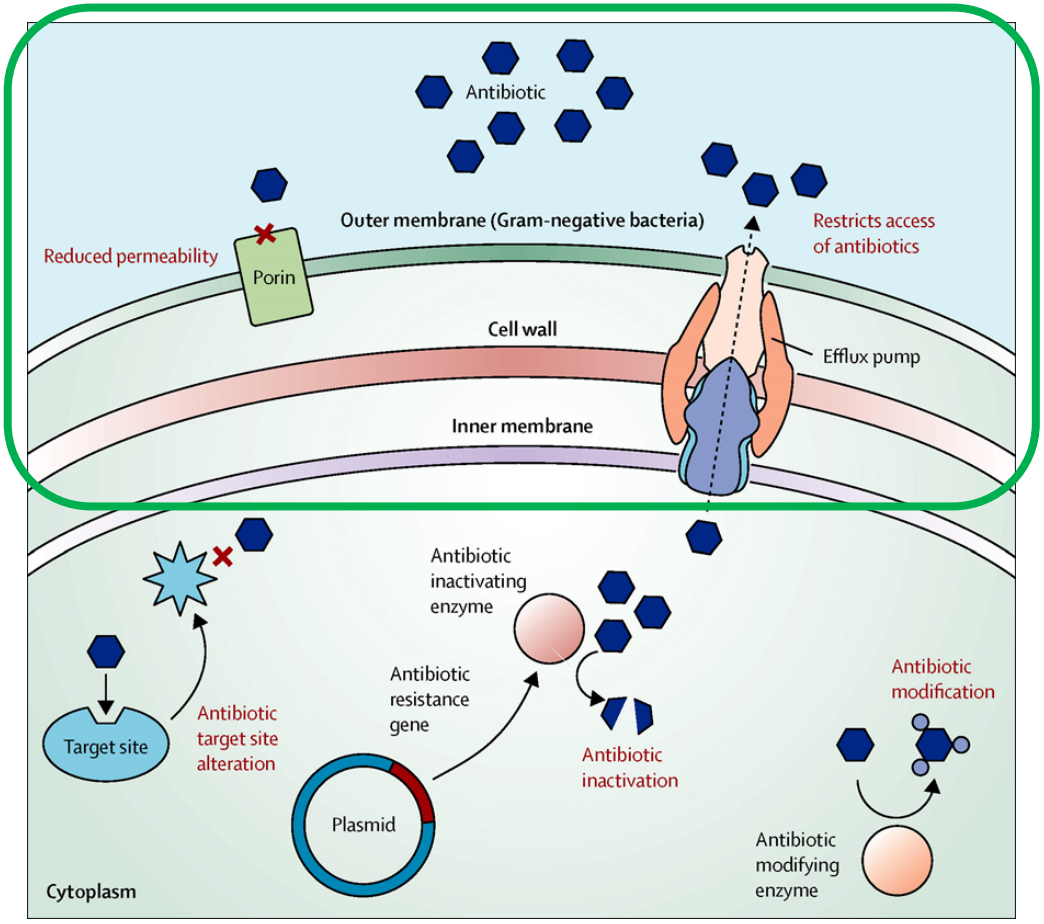

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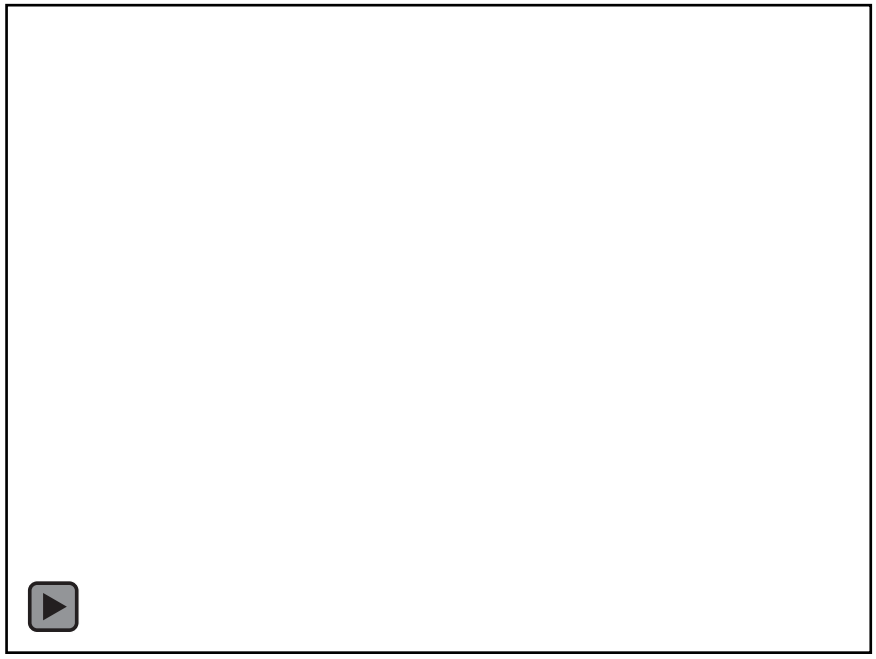
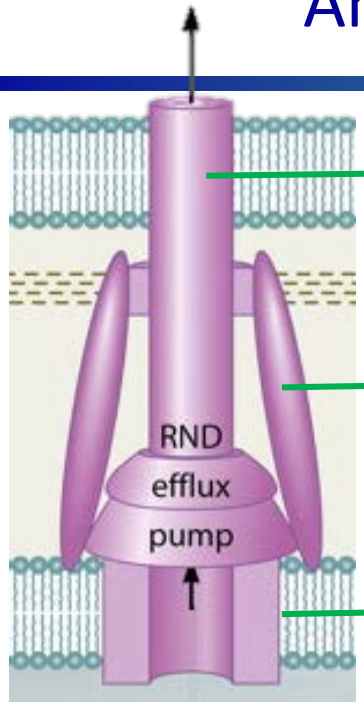
Role of efflux in intrinsic/acquired resistance



Old Faithful Geyser, Yellowstone

Antibiotic efflux pumps in *P. aeruginosa*



Outer Membrane protein: OprM – J – N - M

Membrane Fusion protein: MexA – C – E - X

Efflux transporter: MexB – D – F - Y

In most textbooks, low resistance level …

Table 1. RND major multidrug efflux systems of *Pseudomonas aeruginosa* and their substrates.[†]

System	Regulatory gene	Cytoplasmic membrane transporter protein	Membrane fusion protein	Outer membrane efflux protein	Mutation causing upregulation	Substrates
MexAB-OprM	*mexR*	MexB	MexA	OprM	nalB (affects *mexR*) and *nalC* (lies outside *mexR*)	BL, FQ, CM, TC, NV, TP, SM, ML, EB, AC, CV, SDS, AH, HL, CL, TL, IR, TS
MexCD-OprJ	*nfxB*	MexD	MexC	OprJ	*nfxB*	BL, FQ, CM, TC, NV, TP, ML, CV EB, AC, SDS, AH, CL, TS
MexEF-OprN	*mexT*	MexF	MexE	OprN	*nfxC*	FQ, CM, TP, AH, TS
MexXY-OprM	*mexZ*	MexY	MexX	OprM	ParRS[§]	FQ, AG, TC, ER

In instances where only one member of a class of antimicrobial agents has been tested or is known to be a substrate for a given efflux pump, that member is identified. Where several members of an antimicrobial class are known to be substrates, the class is identified rather than the actual compounds tested.

[†]Data taken from [8,131,132–145].

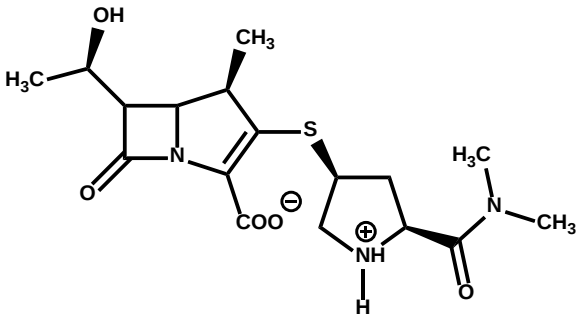
[§]Data taken from [146].. Mutations in the response regulator ParR or the sensor kinase ParS of the two-component regulatory system ParRS lead to MexXY overexpression.

AC: Acriflavine; AG: Aminoglycosides; AH: Aromatic hydrocarbons; BL: β-lactams; CL: Cerulenin; CM: Chloramphenicol; CV: Crystal violet; EB: Ethidium bromide; ER: Erythromycin; FQ: Fluoroquinolones; HL: Homoserine lactones; IR: Irgasan; ML: Macrolides; NV: Novobiocin; SDS: Sodium dodecyl sulphate; SM: Sulphonamides; TC: Tetracycline; TL: Thiolactomycin; TO: Toluene; TP: Trimethoprim; TS: Triclosan.

El Zowalaty et al, Future Microbiol. 2015; 10:1683-1706.

Efflux and high level resistance to carbapenems ?

Meropenem, a broad spectrum antibiotic



- resistant to most ESBLs; susceptible to carbapenemases
- spectrum XXL
- often last resort

International Journal of Antimicrobial Agents 48 (2016) 740–743

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journal homepage: www.elsevier.com/locate/ijantimicag

Short Communication

High-level resistance to meropenem in clinical isolates of *Pseudomonas aeruginosa* in the absence of carbapenemases:

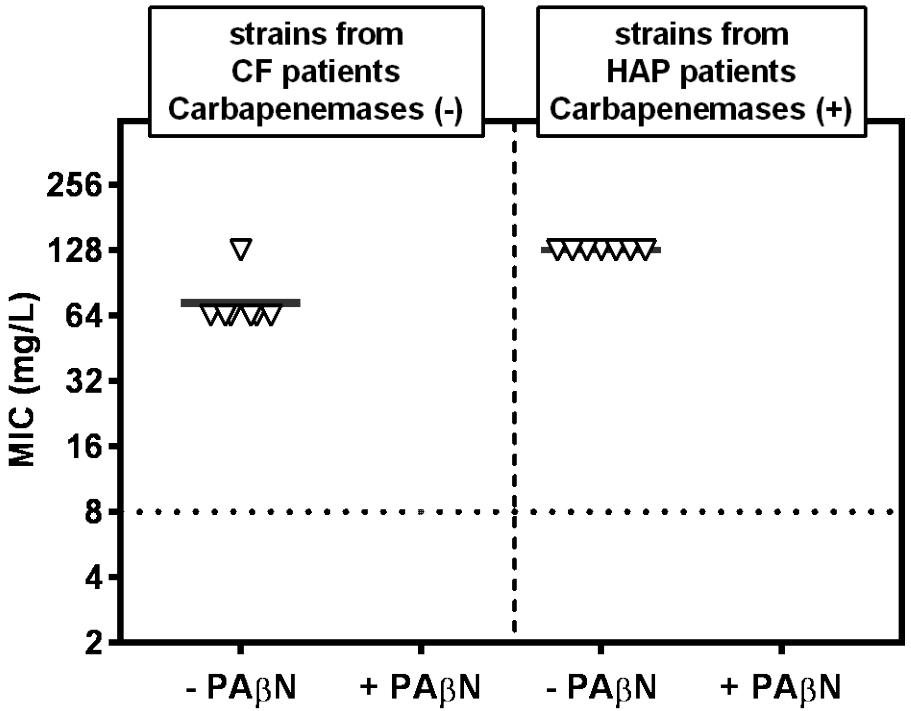
WHY ?

Hussein Chalhoub [a], Yolanda Sáenz [b], Hector Rodriguez-Villalobos [c], Olivier Denis [d], Barbara C. Kahl [e], Paul M. Tulkens [a], Françoise Van Bambeke [a,*]

[a] *Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium*
[b] *Centro de Investigación Biomédica de La Rioja (CIBIR), Logroño, Spain*
[c] *Laboratoire de microbiologie, Cliniques universitaires Saint-Luc, Brussels, Belgium*
[d] *Laboratoire de microbiologie, Hôpital Erasme, Université libre de Bruxelles, Brussels, Belgium*
[e] *University Hospital Münster, Münster, Germany*

Efflux and high level resistance to carbapenems ?

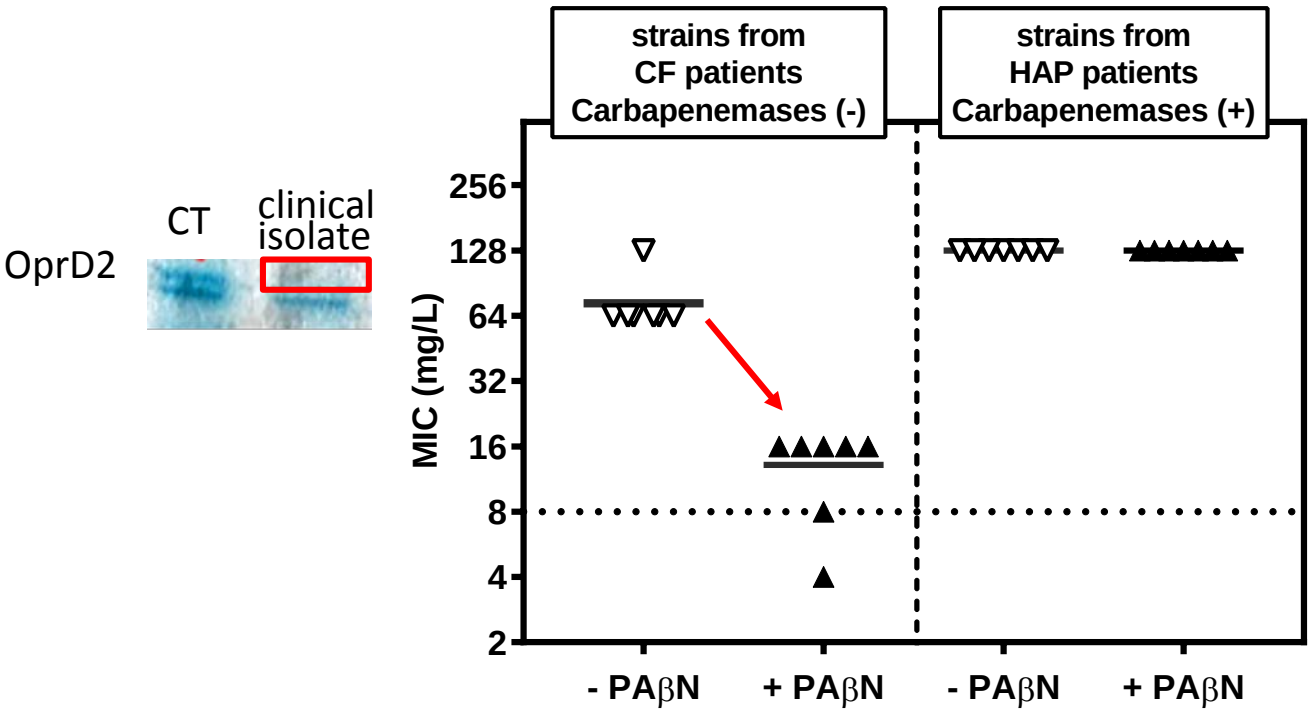
Elevated MICs to meropenem in CF isolates as in ICU isolates ….



High MIC in the absence of carbapenemases in CF isolates

Efflux and high level resistance to carbapenems ?

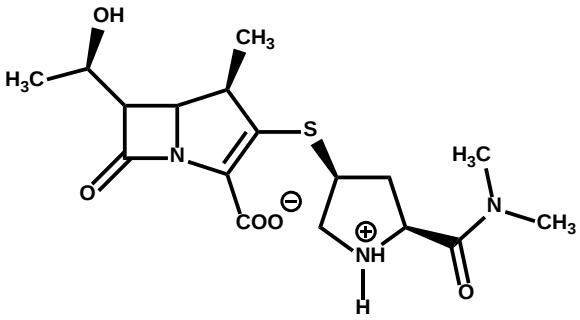
Influence of a broad-efflux pump inhibitor



Active efflux + porin defect also confer high level resistance

Efflux and high level resistance to carbapenems ?

Meropenem, a broad spectrum antibiotic



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Hussein Chalhoub [a], Yolanda Sáenz [b], Hector Rodriguez-Villalobos [c], Olivier Denis [d], Barbara C. Kahl [e], Paul M. Tulkens [a], Françoise Van Bambeke [a,*]

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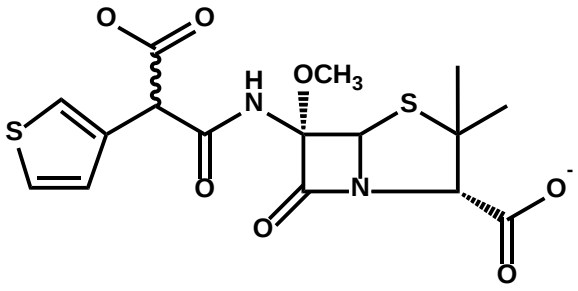
[e] *University Hospital Münster, Münster, Germany*

Efflux and intrinsic resistance to temocillin ?

Temocillin, an 'old-revived' antibiotic



- resistant to most ESBLs
- inactive on *P. aeruginosa*, but …

J Antimicrob Chemother 2012
doi:10.1093/jac/dkr543
Advance Access publication 3 January 2012

Role of MexAB-OprM in intrinsic resistance of *Pseudomonas aeruginosa* to temocillin and impact on the susceptibility of strains isolated from patients suffering from cystic fibrosis

Julien M. Buyck[1], Sophie Guénard[2], Patrick Plésiat[2], Paul M. Tulkens[1] and Françoise Van Bambeke[1]*

SCIENTIFIC REPORTS

OPEN

Mechanisms of intrinsic resistance and acquired susceptibility of *Pseudomonas aeruginosa* isolated from cystic fibrosis patients to temocillin, a revived antibiotic

Received: 13 September 2016
Accepted: 01 December 2016
Published: 16 January 2017

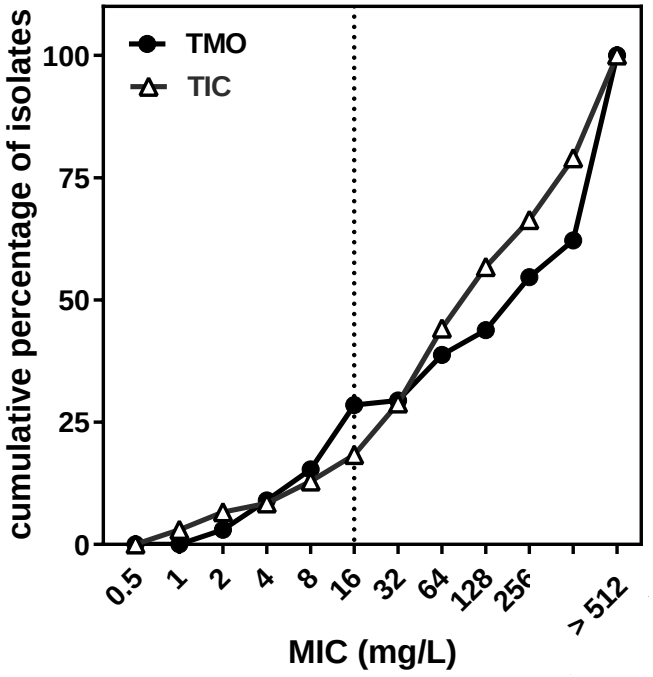
Hussein Chalhoub[1], Daniel Pletzer[2,+], Helge Weingart[2], Yvonne Braun[2], Michael M. Tunney[3], J. Stuart Elborn[3], Hector Rodriguez-Villalobos[4], Patrick Plésiat[5], Barbara C. Kahl[6], Olivier Denis[7], Mathias Winterhalter[2], Paul M. Tulkens[1] & Françoise Van Bambeke[1]

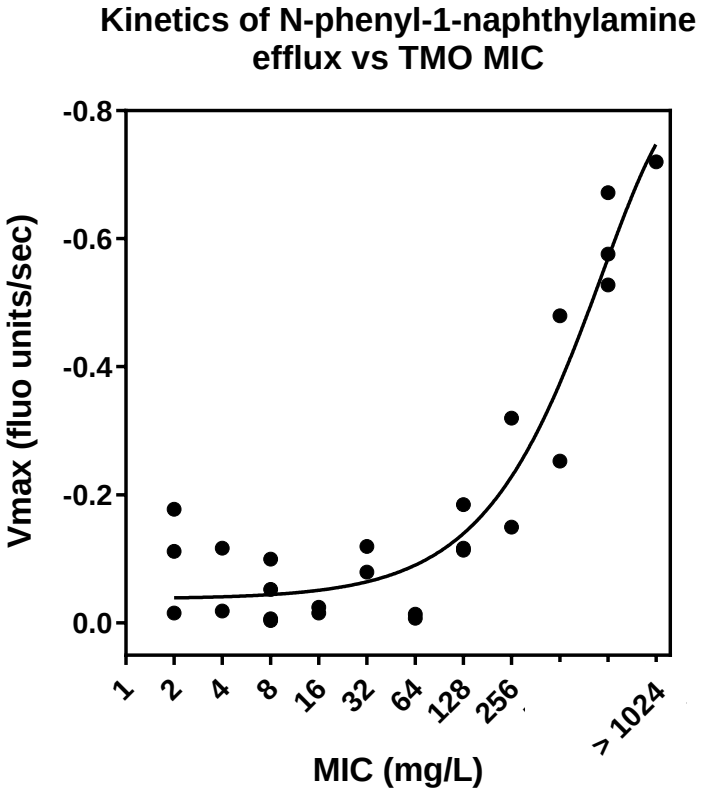
Efflux and acquired susceptibility to temocillin

Strain/genotype	MIC (mg/L)
PAO1	256
PAO1 Δ*mexAB-oprM*	4

Correlation between TMO MICs and efflux speed of a MexAB substrate

Clinical relevance ?



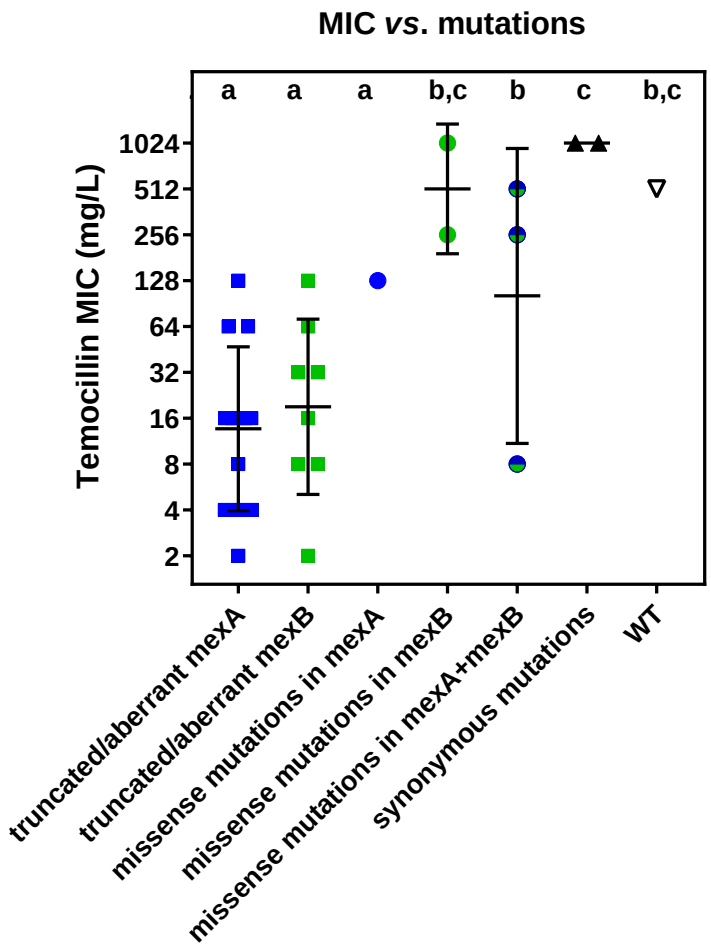


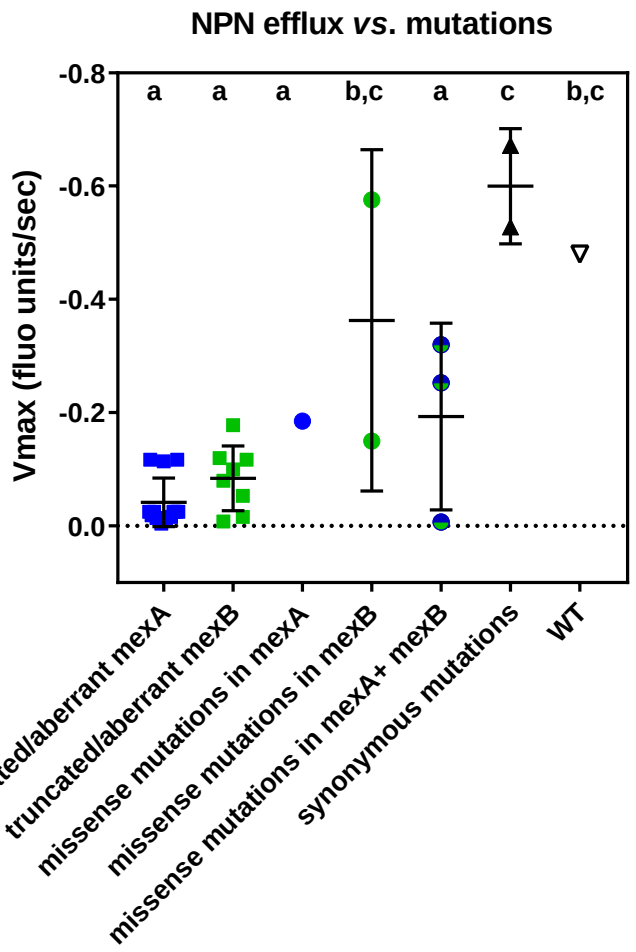
Buyck et al, JAC 2012; 67:771-5; Chalhoub et al, Sci Reports 2017; 7:40208

Efflux and acquired susceptibility to temocillin

Reason for altered efflux?

A non negligible proportion of CF isolates have natural mutations in efflux pumps restoring TMO activity

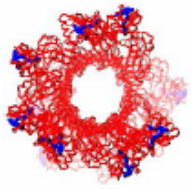
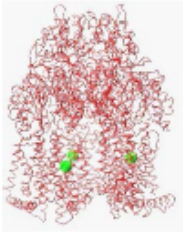




Chalhoub et al, Sci Reports 2017; 7:40208

Efflux and acquired susceptibility to temocillin

Isogenic isolates collected from patients in different countries

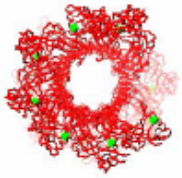
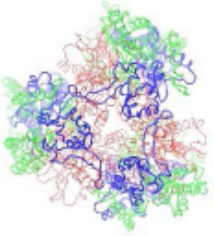
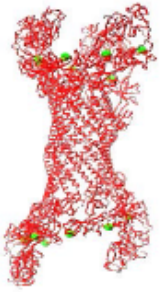
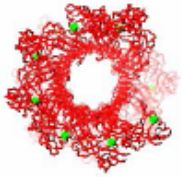
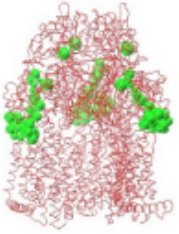
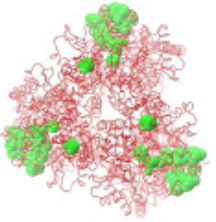
Isolates	Patient ID, country and date of collection	TMO MIC (mg/L)	Protein length (AA)		Encoded MexA		Encoded MexB	
			MexA	MexB	Side view	Top view	Side view	Top view
AG3	JP, UK (May 2006)	8	372	1046			Synonymous mutations	
128	DAF69, Belgium (Oct. 2010)	1024	383	1046	Synonymous mutations			
129-6	129, Germany (July 2012)	1024	383	1046	Synonymous mutations		Synonymous mutations	

Diversity of mutations in isogenic isolates …

Chalhoub et al, Sci Reports 2017; 7:40208

Efflux and acquired susceptibility to temocillin

Isogenic isolates collected from different patients in a single country

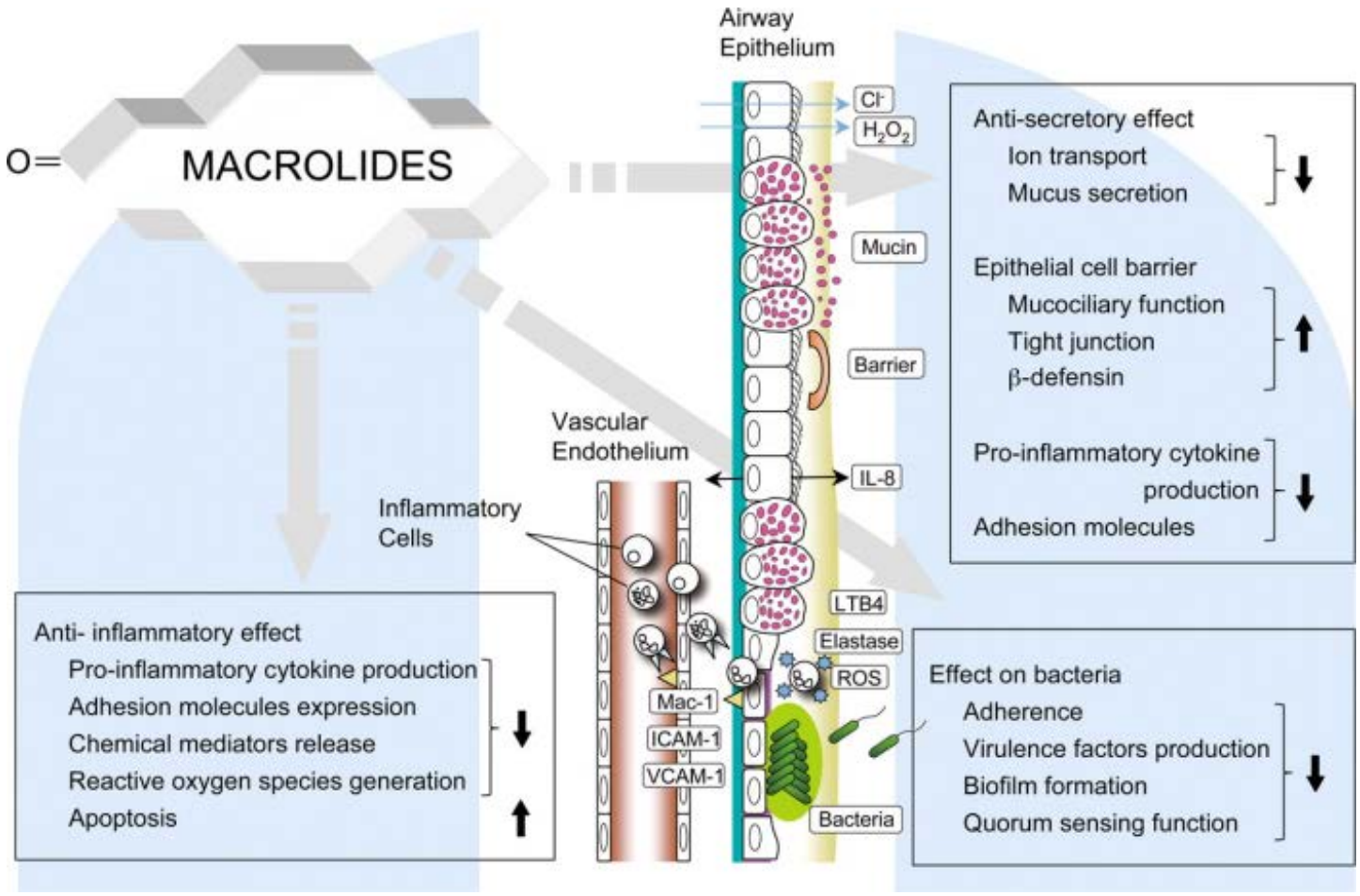
Isolates	Patient ID, country and date of collection	TMO MIC (mg/L)	Protein length (AA)		Encoded MexA		Encoded MexB	
			MexA	MexB	Side view	Top view	Side view	Top view
135-1	135, Germany (July 2012)	8	383	719				
208-3	208, Germany (Aug. 2012)	256	383	1045				

Diversity of mutations in isogenic isolates …

Chalhoub et al, Sci Reports 2017; 7:40208

Efflux and intrinsic / acquired resistance to macrolides

Macrolides are widely used in CF patients, although inactive on *P. aeruginosa*



Kanoh & Rubin; Clin. Microbiol. Rev. (2010) 23:590-615

Efflux and intrinsic / acquired resistance to macrolides

Macrolides are widely used in CF patients, although inactive on *P. aeruginosa*… *BUT*…

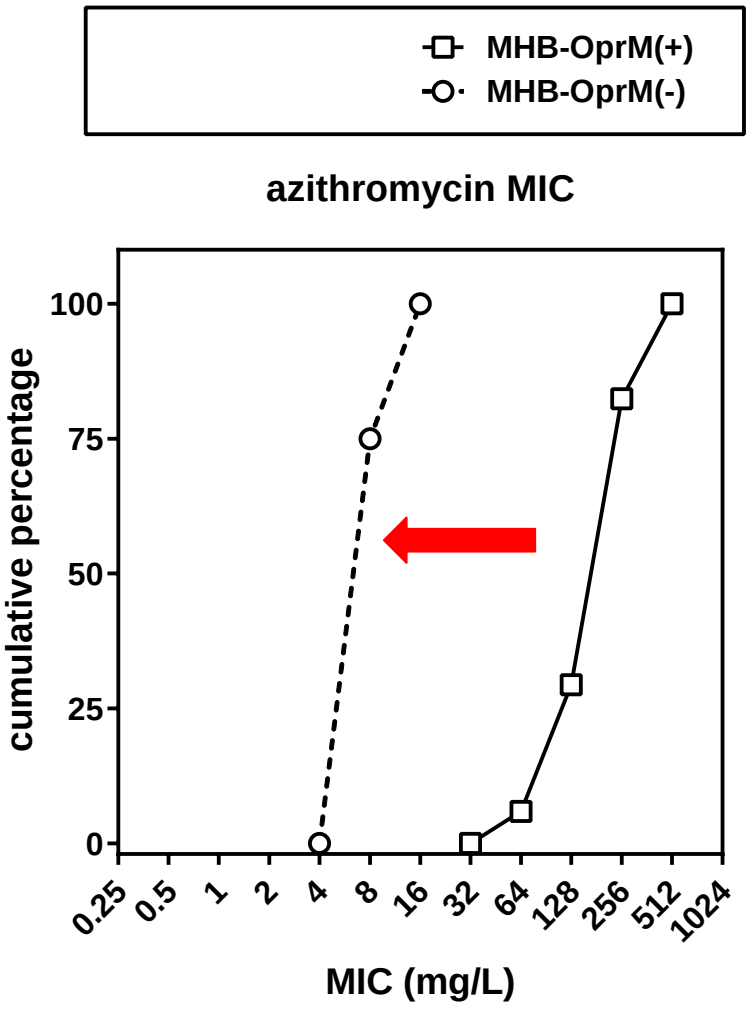
Eur Respir J 2017

ORIGINAL ARTICLE
RESPIRATORY INFECTIONS

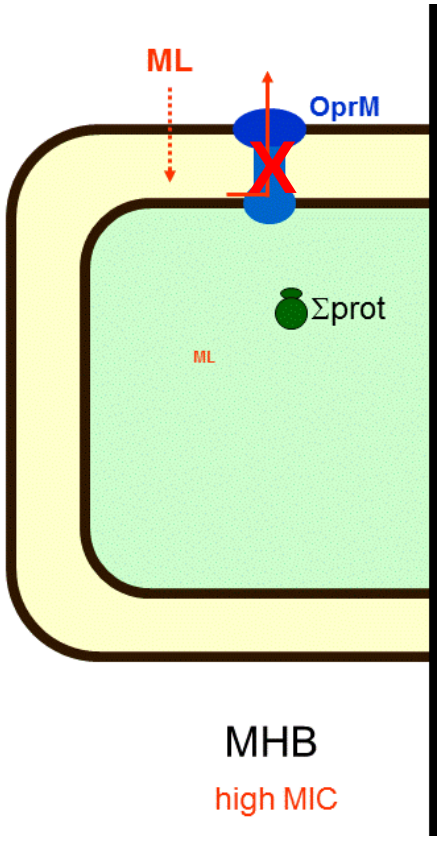
Acquired resistance to macrolides in *Pseudomonas aeruginosa* from cystic fibrosis patients

Muhammad-Hariri Mustafa[1,2], Shaunak Khandekar[1], Michael M. Tunney[3], J. Stuart Elborn[3], Barbara C. Kahl[4], Olivier Denis[5], Patrick Plésiat[6], Hamidou Traore[2], Paul M. Tulkens[1], Francis Vanderbist[2] and Françoise Van Bambeke[1]

Efflux and intrinsic / acquired resistance to macrolides

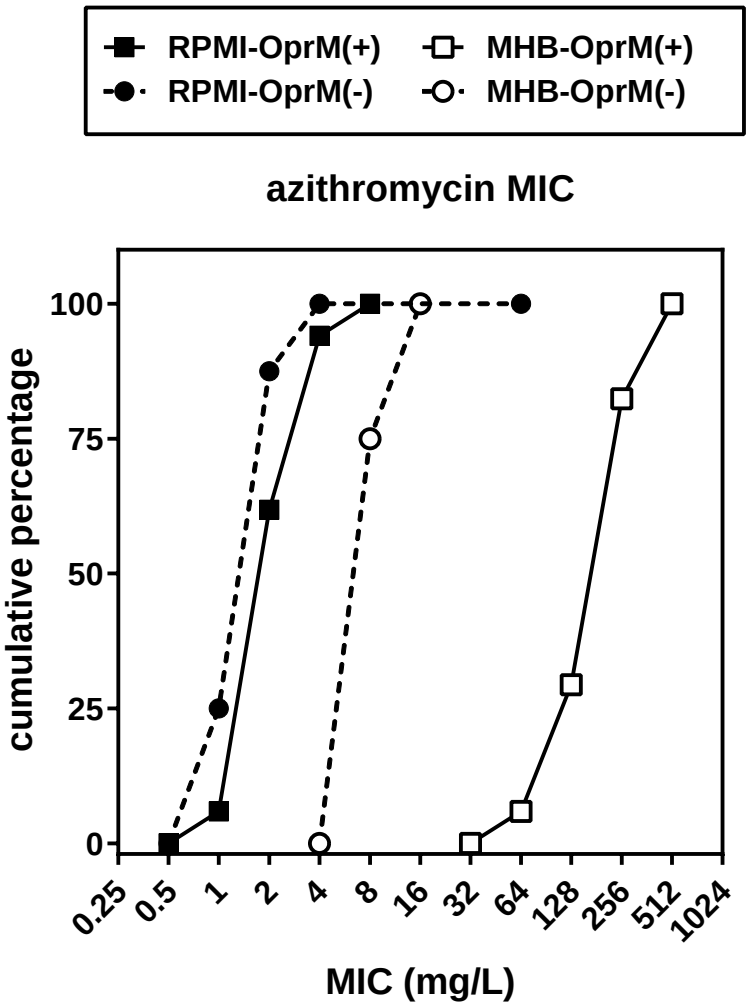


Macrolides inactive on *Pa* due to active efflux

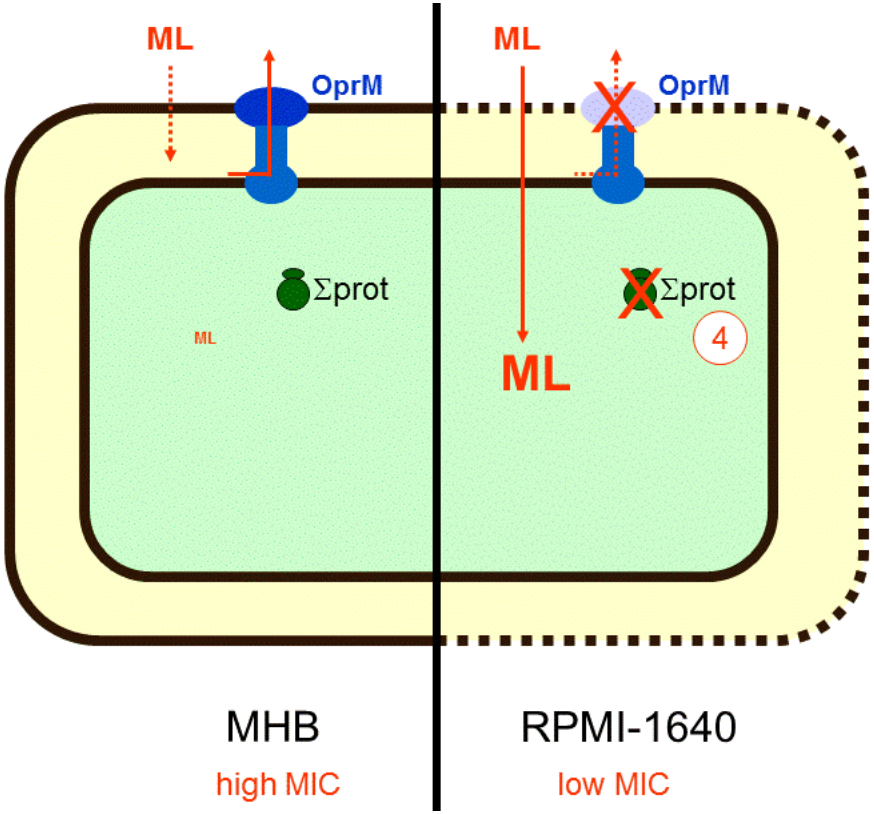


Buyck et al. Clin Infect. Dis. 2012; 55:534-542

Efflux and intrinsic / acquired resistance to macrolides



Activity recovered in RPMI-1640 (eukaryotic cell culture medium)



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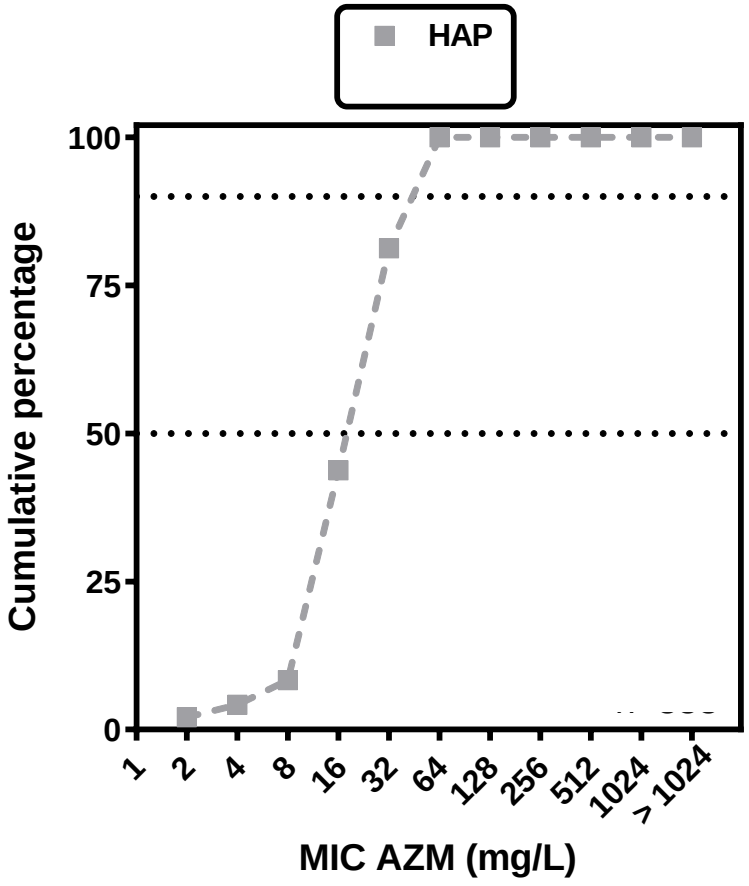
Efflux and intrinsic / acquired resistance to macrolides

Clinical relevance ?



Mustafa et al. Eur. Respir. J. 2017; 49:1601847

Efflux and intrinsic / acquired resistance to macrolides

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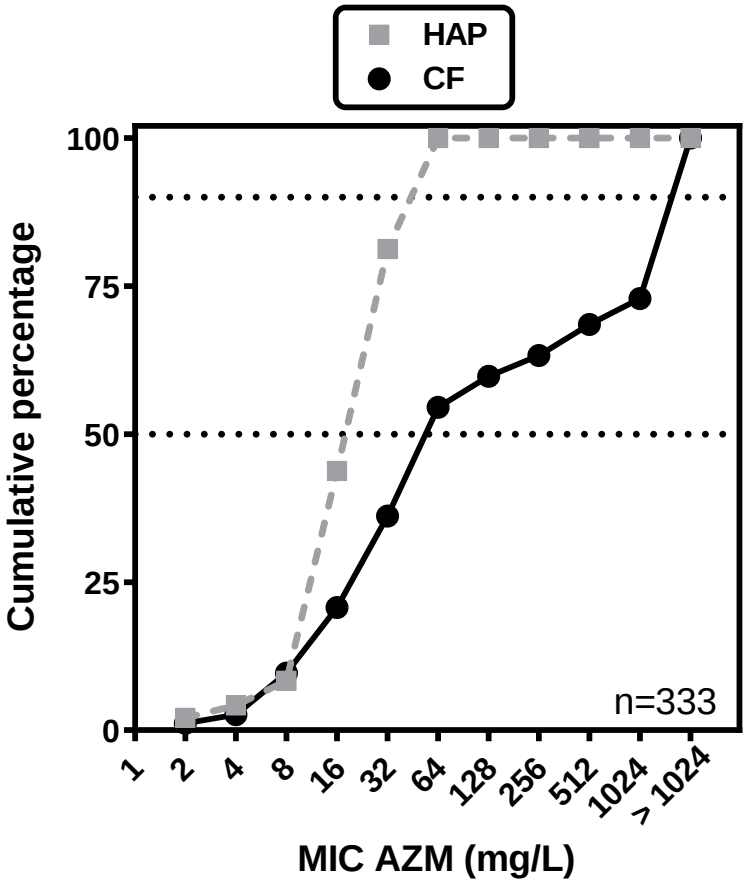


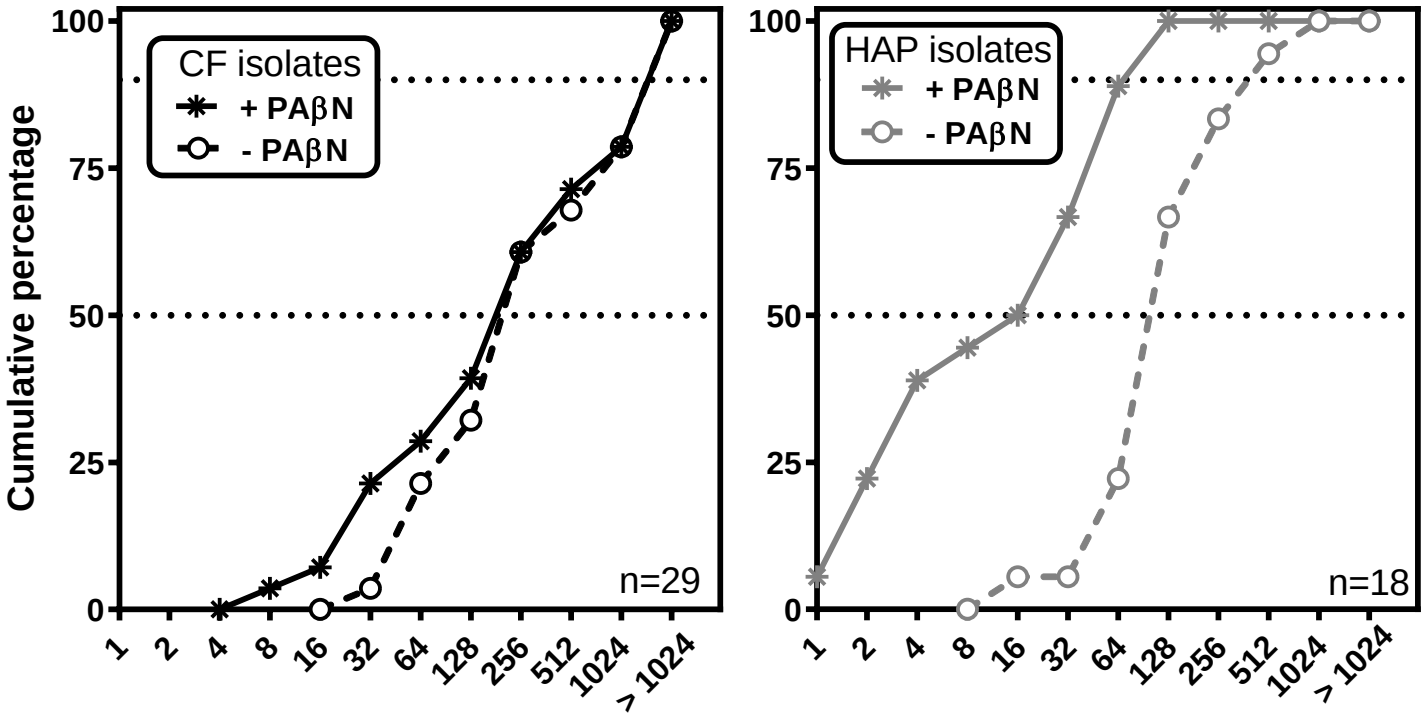
CF isolates more resistant to macrolides than HAP isolates…

MIC measured in RPMI-1640 + 10 % serum

Mustafa et al. Eur. Respir. J. 2017; 49:1601847

Efflux and intrinsic / acquired resistance to macrolides

Why this difference between CF and HAP isolates ?



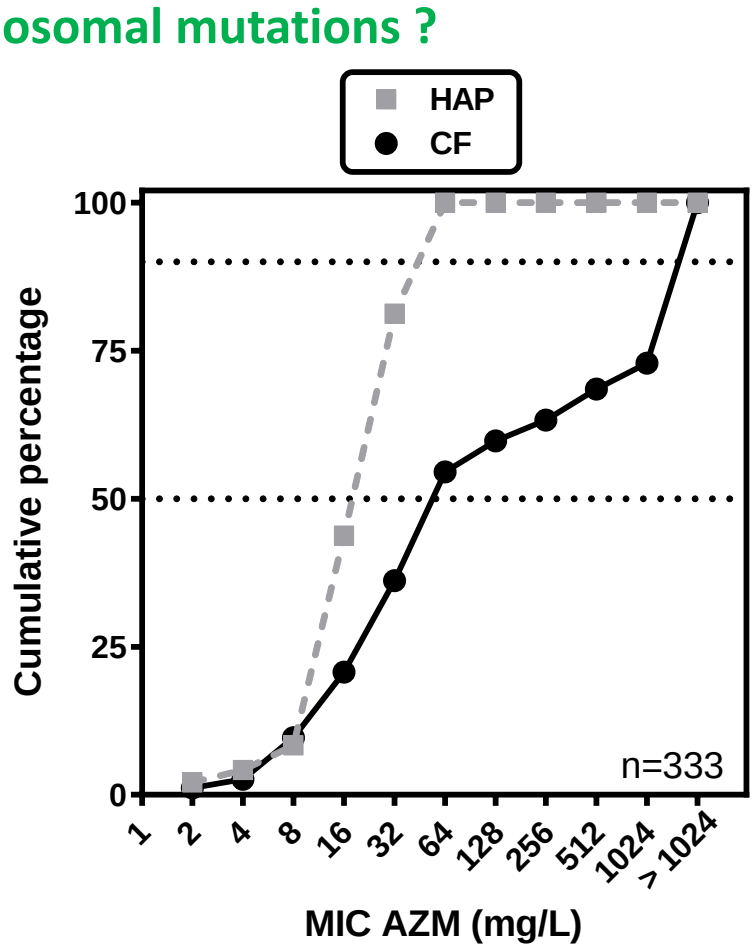
MIC reduced by efflux pump inhibitor in HAP isolates only

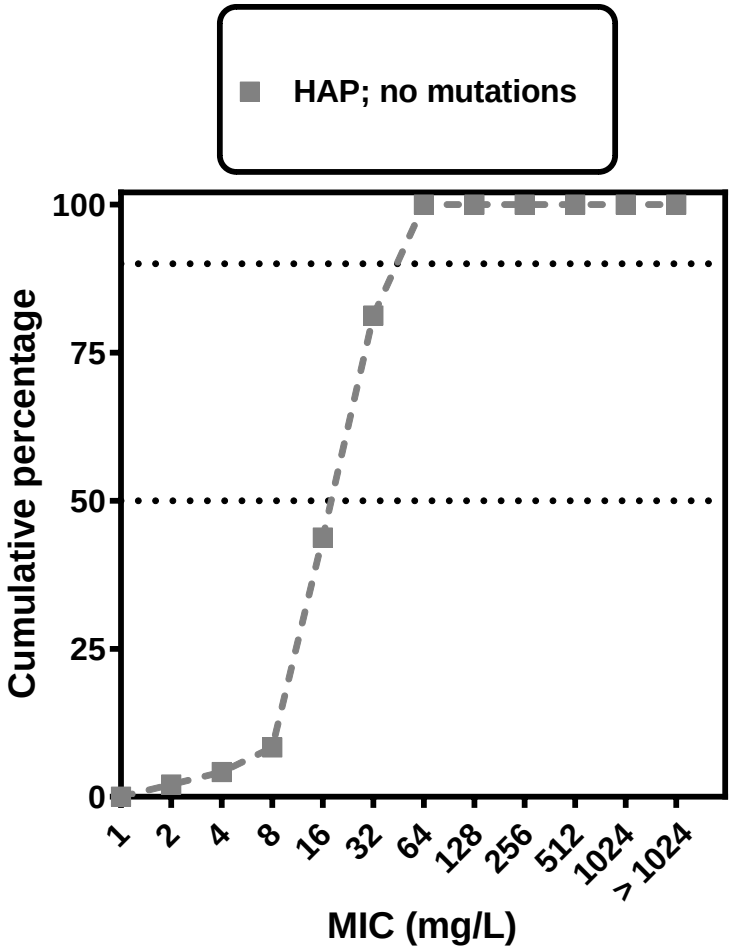
MIC measured in MHB-CA

Mustafa et al. Eur. Respir. J. 2017; 49:1601847

Efflux and intrinsic / acquired resistance to macrolides

Ribosomal mutations ?





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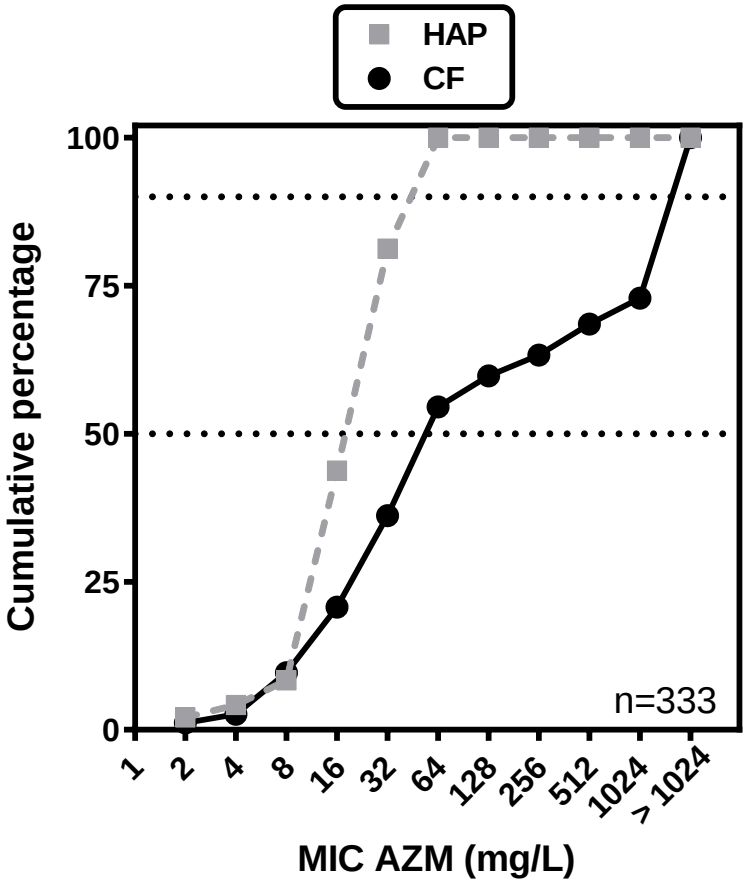

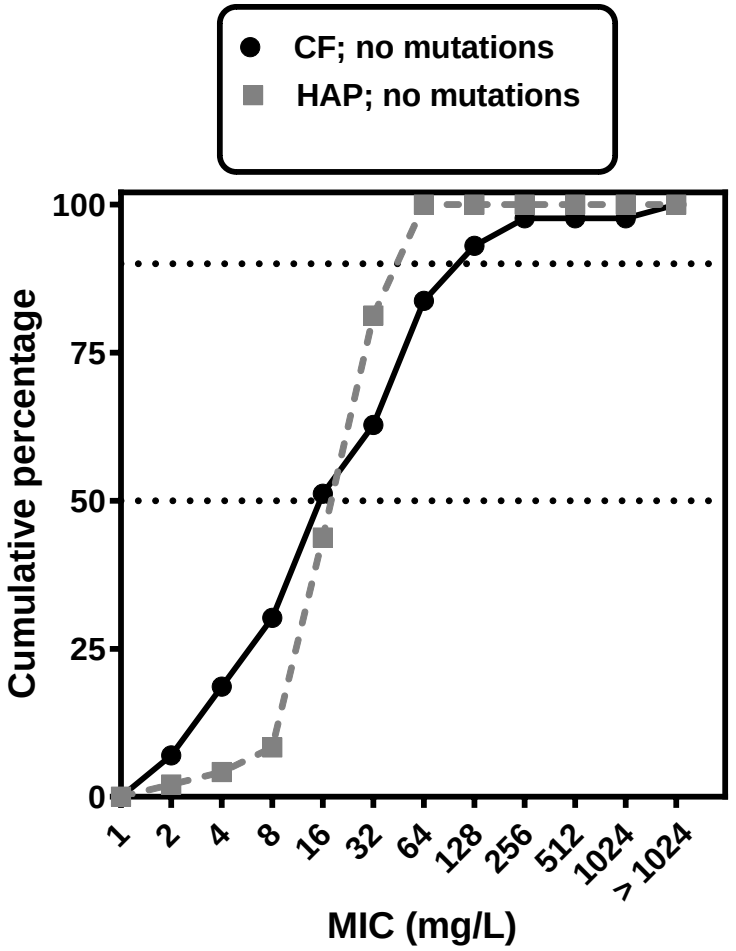

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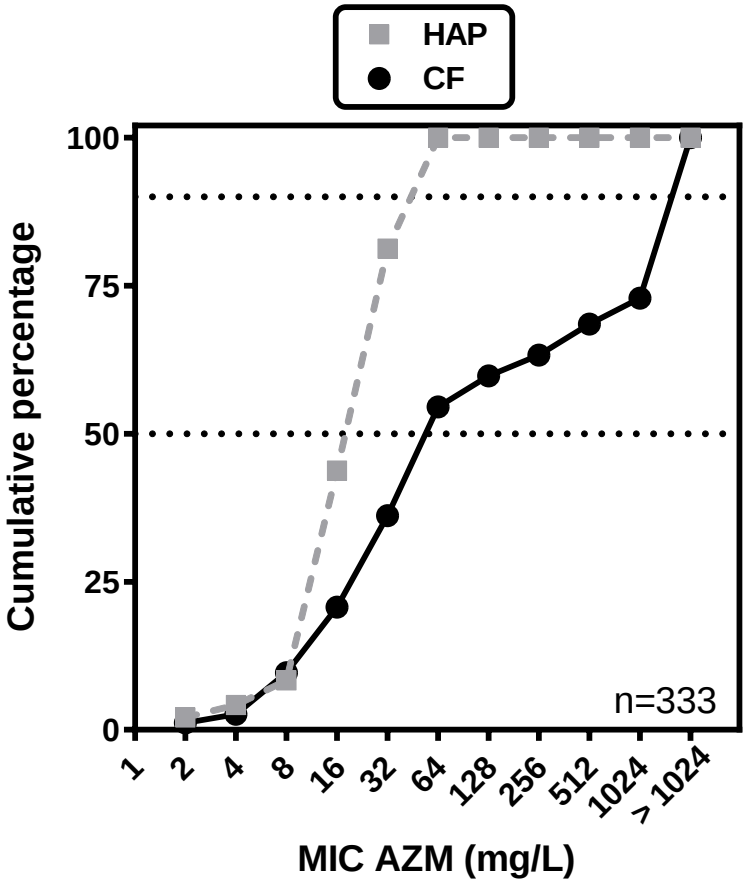
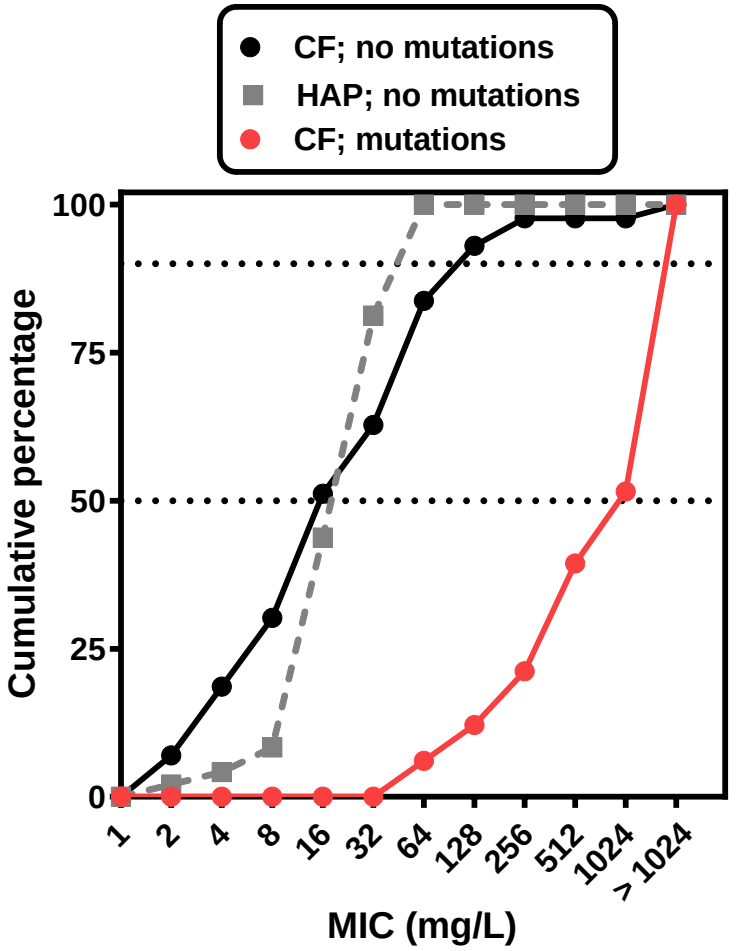
Efflux and intrinsic / acquired resistance to macrolides

Ribosomal mutations ?





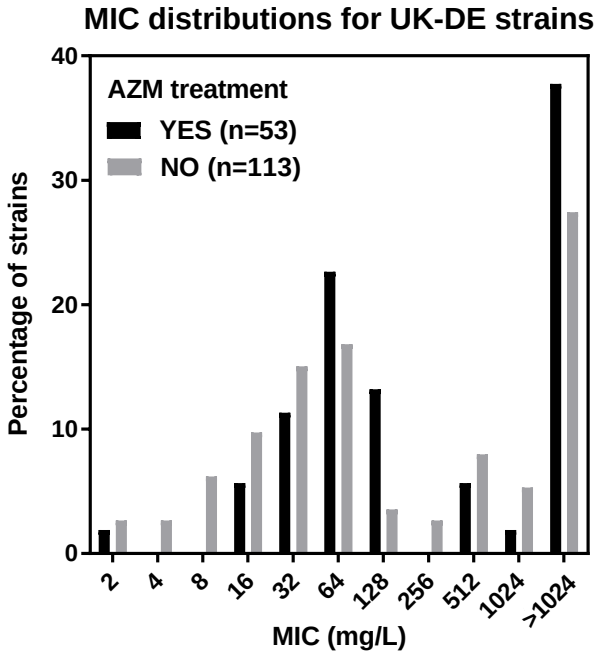
Active efflux may have selected for ribosomal mutations in patients exposed to macrolides …

MIC measured in RPMI-1640 + 10 % serum

Mustafa et al. Eur. Respir. J. 2017; 49:1601847

Efflux and intrinsic / acquired resistance to macrolides

Link with macrolide use ?



χ^2 tests			
	AZM treatment		χ^2
Parameter	YES	NO	p value
MIC ≤ 32 mg/L	10/53	41/113	0.01
MIC > 1024 mg/L	20/53	31/113	0.08

Lower MICs in non AZM-treated patients
Higher MICs in AZM-treated patients

MIC measured in RPMI-1640 + 10 % serum

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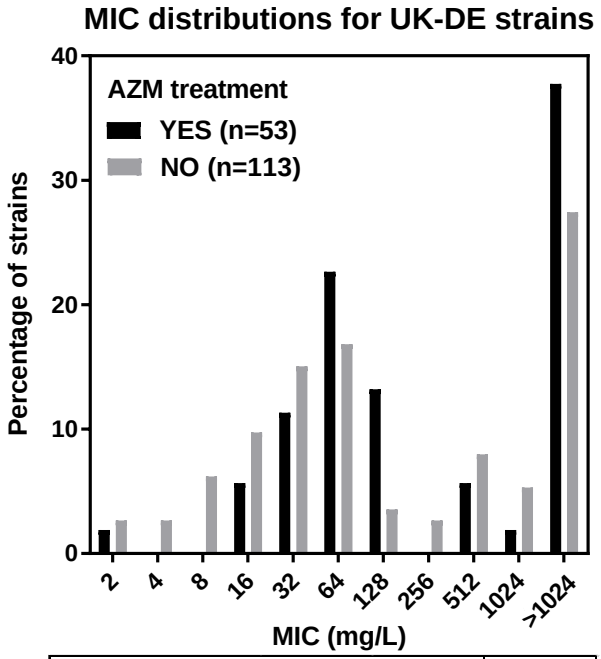
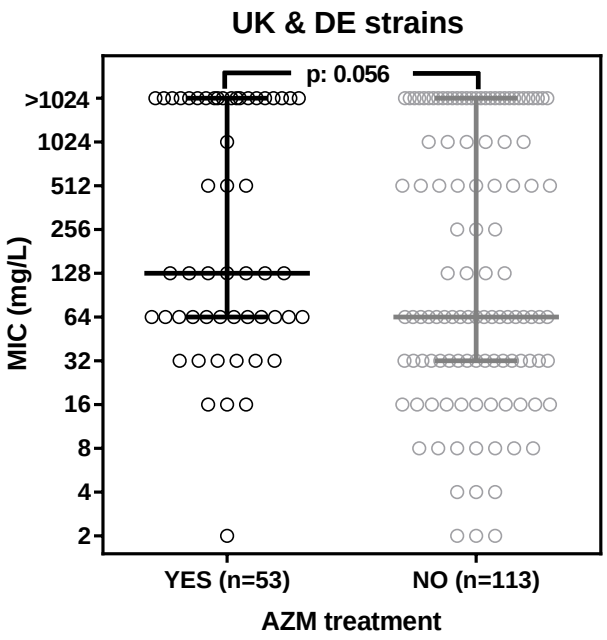
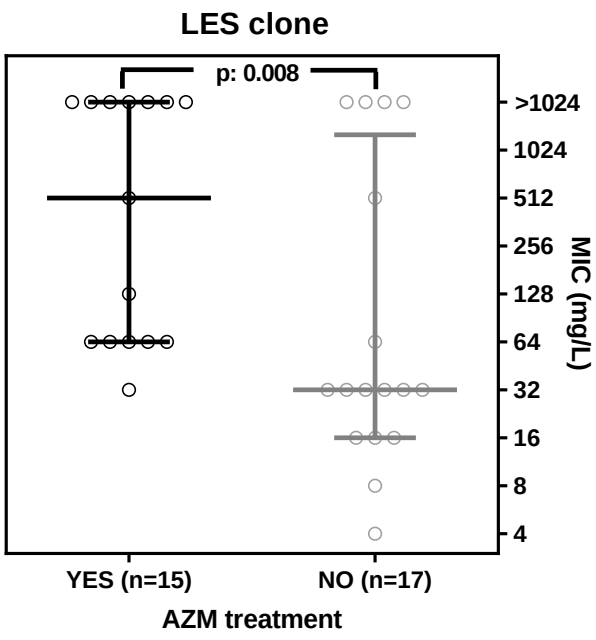
Efflux and intrinsic / acquired resistance to macrolides

Link with macrolide use ?







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Parameter	YES	NO	p value
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statistics on MIC (mg/L) values (95% CI)		
	AZM treatment	
Parameter	YES	NO
Median	128 (64-2048)	64 (64-512)
Geom. mean	246 (146-416)	141 (95-210)

statistics on MIC (mg/L) values (95% CI)		
	AZM treatment	
Parameter	YES	NO
Median	512 (64-2048)	32 (16-512)
Geom. mean	370 (140-979)	74 (25-225)

AZM treatment associated with resistance (significant in clonal isolates)

MIC measured in RPMI-1640 + 10 % serum

Mustafa et al. Eur. Respir. J. 2017; 49:1601847

Conclusions

- Resistance in CF pathogens may proceed from other mechanisms than in isolates from other origins:
  other clones, other evolution ….
- Efflux can play a preponderant role in acquired/intrinsic resistance to antibiotics and help to select for other resistance mechanisms (target mutations)
- Mutations in efflux systems may also confer “acquired susceptibility” !

- Resistance in CF is complex
- Adaptability plays a key role !

New ways of thinking are needed…

Acknowledgements

Cartoons: L. Desloover