Tolerance and resistance to antibiotics in *Pseudomonas aeruginosa*, or how do bacteria play with their environment

Françoise Van Bambeke, PharmD, PhD

Pharmacologie cellulaire et moléculaire

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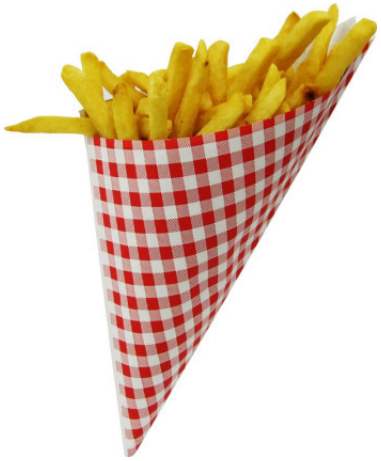
Université catholique de Louvain, Brussels, Belgium

www.facm.ucl.ac.be

Where do I come from ?

Gastronomic quizz: which one is typical from Belgium?

all of them !



Artistic quizz: which one is from a Belgian artist ?

all of them !

Geographic quizz: which one is located in Brussels ?

all of them !

Campus of UCL
Health Sciences Sector

Université catholique de Louvain

- 29 933 students of 127 different nationalities,
- 5 836 staff members,
- 150 000 alumni world wide.

- 5 campus
- 14 faculties
- 21 research Institutes

Louvain Drug Research Institute

- 15 PI
- 20 post-doc
- 56 PhD students
- 20 technicians

Bridging Sciences for better health

Pharmacologie cellulaire et moléculaire

- 1 emeritus prof.
- 2 PIs
- 7 post-docs
- 14 PhD students
- 5 technicians
- 8 undergraduate students

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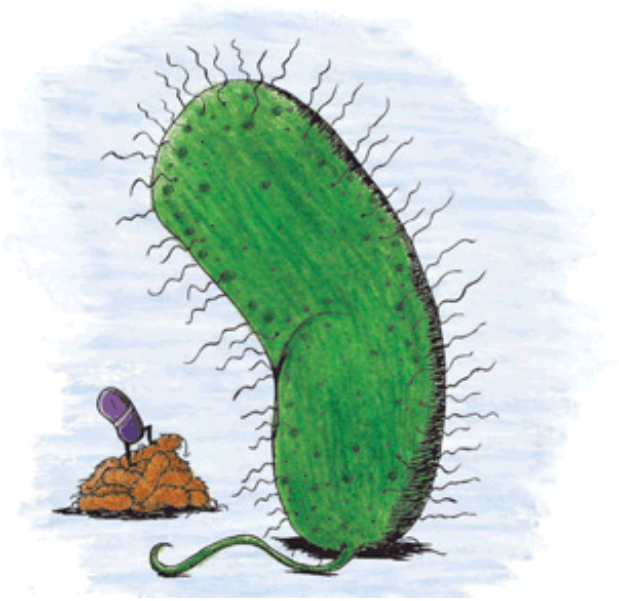
Infectious diseases: No ESKAPE !

***E**. faecium*

***E.** aerogenes*



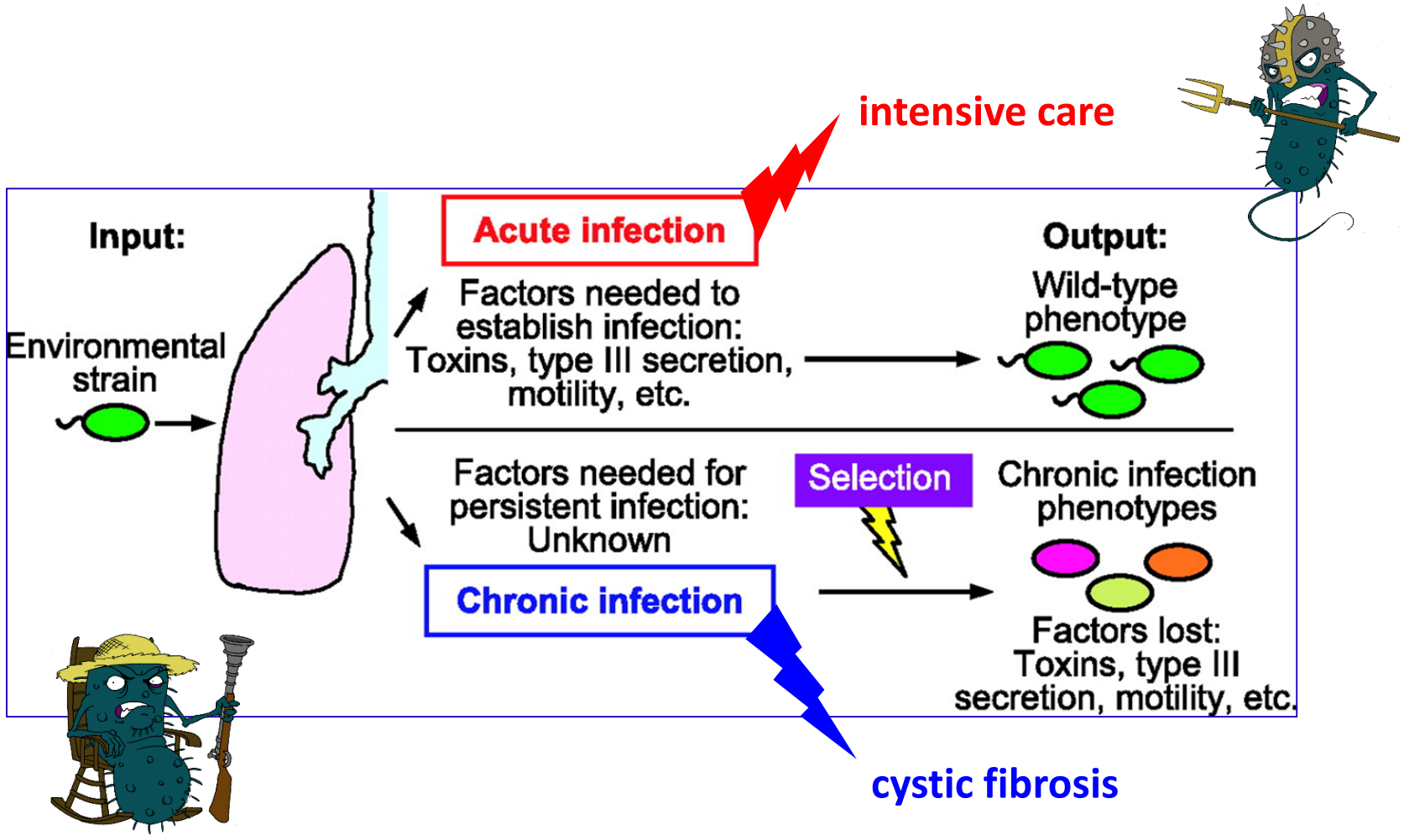
***S**. aureus*

***P**. aeruginosa*

***K**. pneumoniae*

***A.** baumannii*

Pseudomonas aeruginosa, an opportunistic pathogen

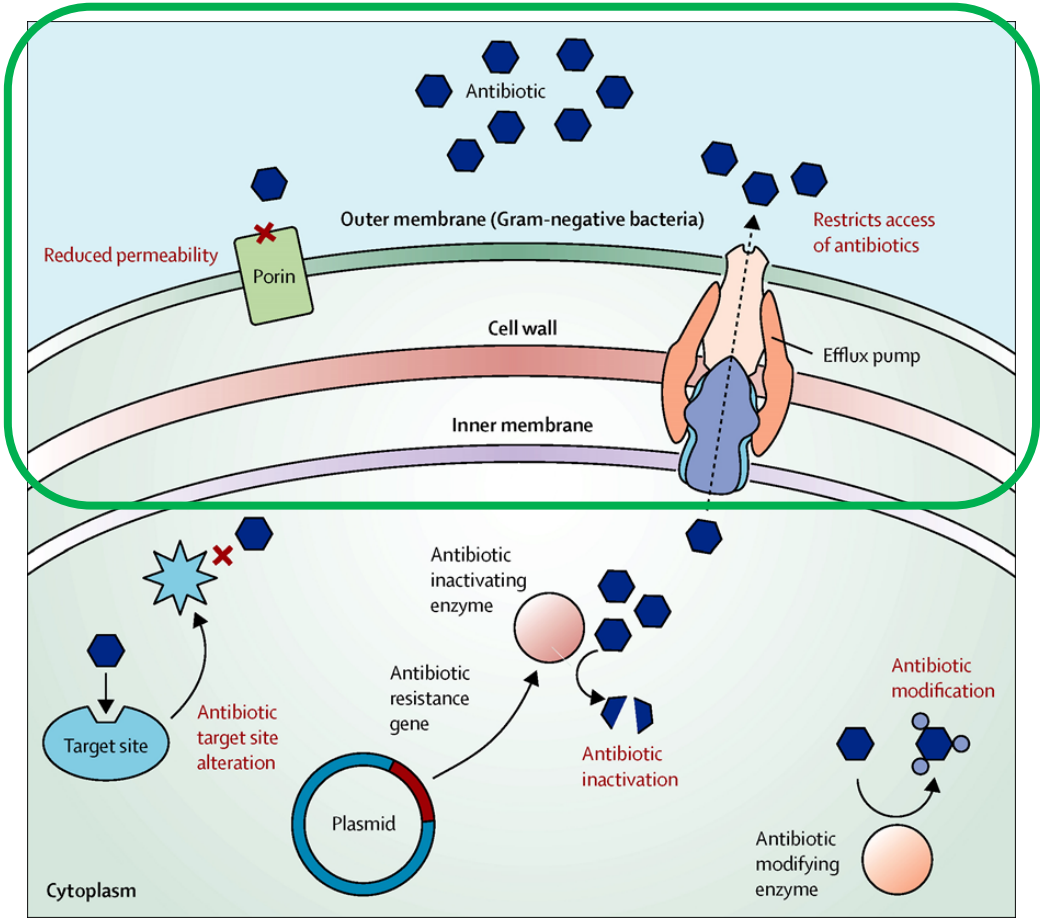


Nguyen & Singh, PNAS 2006;103:8305-8306

Poor response to antibiotics: resistance vs tolerance

RESISTANCE

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

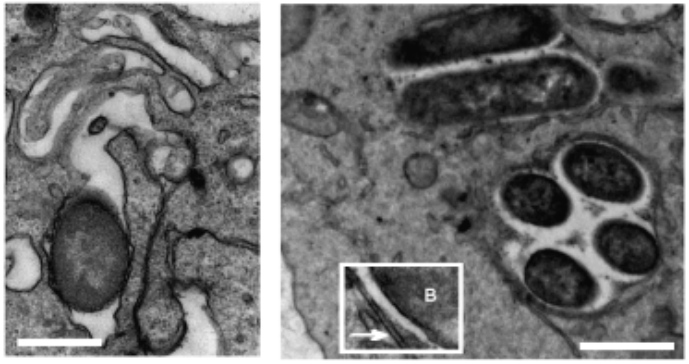


Sherrard et al, Lancet 2014; 384:703-713

Poor response to antibiotics: resistance vs tolerance

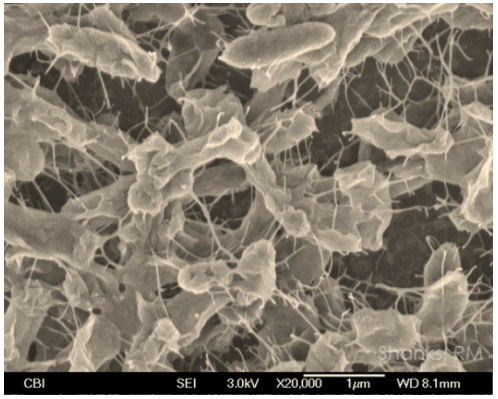
TOLERANCE : dormant modes of life

Intracellular survival



Buyck & Van Bambeke

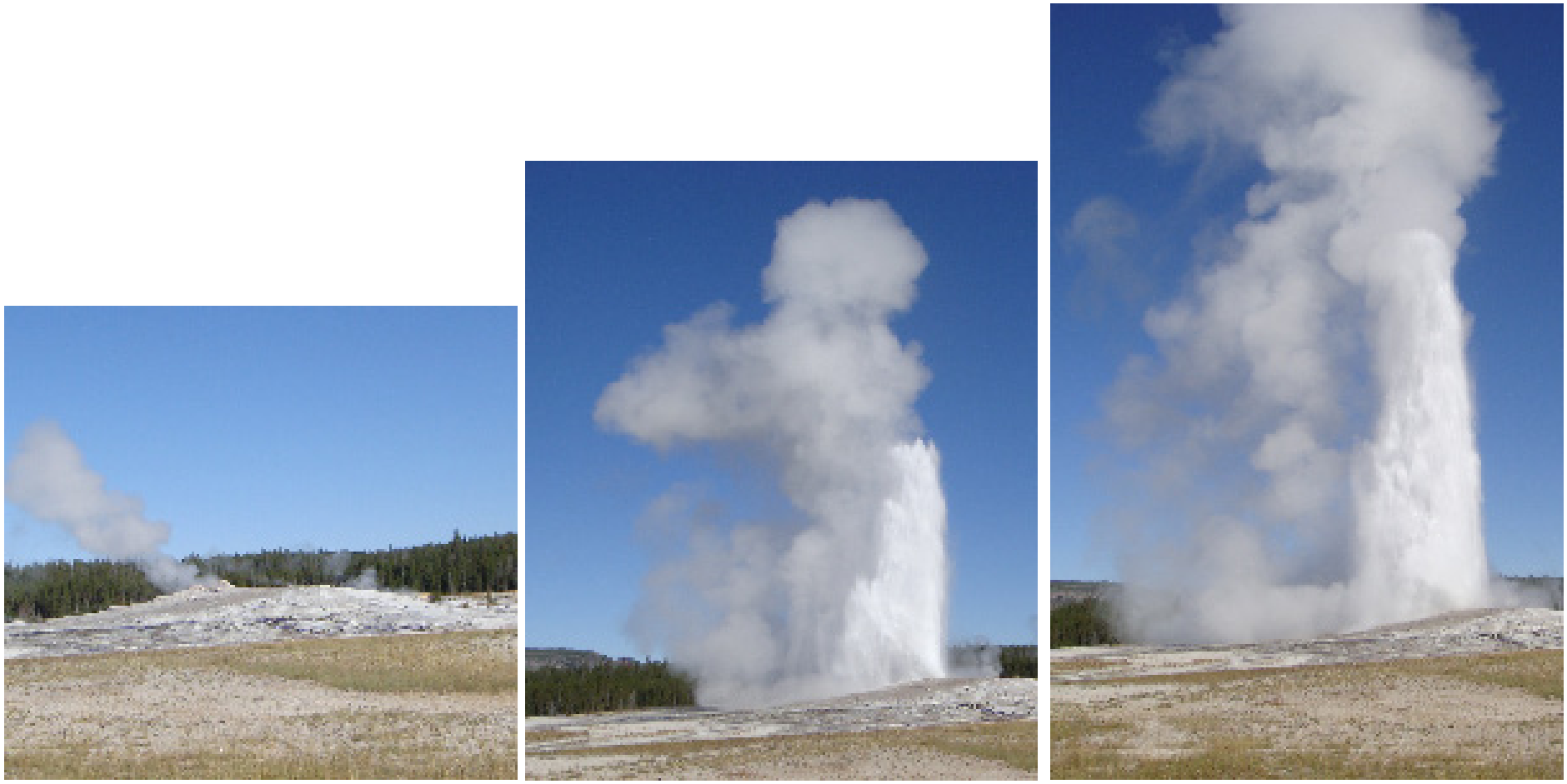
Biofilms



http://eyemicrobiology.upmc.com

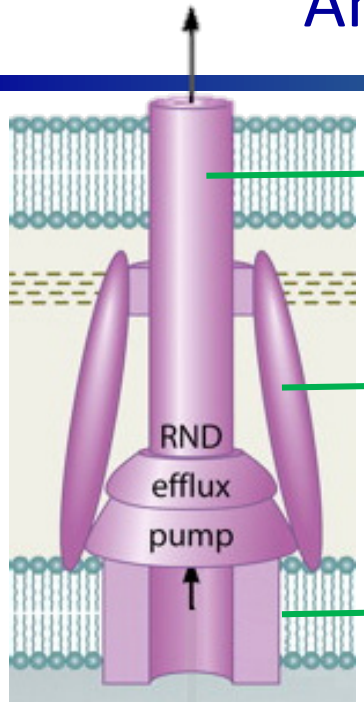
→ Protection against host defenses and antibiotics

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

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.

Role of efflux in intrinsic/acquired resistance

Acquired resistance in ICU patients

- Patients occasionally exposed to antibiotics
- Nosocomial clones, possibly MDR
- Expression of virulence mechanisms → high cytotoxicity

Role of efflux in intrinsic/acquired resistance

Acquired resistance in ICU patients

International Journal of Antimicrobial Agents 36 (2010) 513–522

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

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

In vivo development of antimicrobial resistance in *Pseudomonas aeruginosa* strains isolated from the lower respiratory tract of Intensive Care Unit patients with nosocomial pneumonia and receiving antipseudomonal therapy

Mickaël Riou[a,1], Sylviane Carbonnelle[a,2], Laëtitia Avrain[a,b], Narcisa Mesaros[a,3], Jean-Paul Pirnay[c], Florence Bilocq[c], Daniel De Vos[c,d], Anne Simon[e], Denis Piérard[f], Frédérique Jacobs[g], Anne Dediste[h], Paul M. Tulkens[a,*], Françoise Van Bambeke[a], Youri Glupczynski[i]

International Journal of Antimicrobial Agents 47 (2016) 77–83

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

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

Increase of efflux-mediated resistance in *Pseudomonas aeruginosa* during antibiotic treatment in patients suffering from nosocomial pneumonia☆

Mickaël Riou[a,1,4], Laëtitia Avrain[b,4], Sylviane Carbonnelle[a,2], Farid El Garch[a,c,3], Jean-Paul Pirnay[d], Daniel De Vos[d], Patrick Plésiat[e], Paul M. Tulkens[a], Françoise Van Bambeke[a,*]

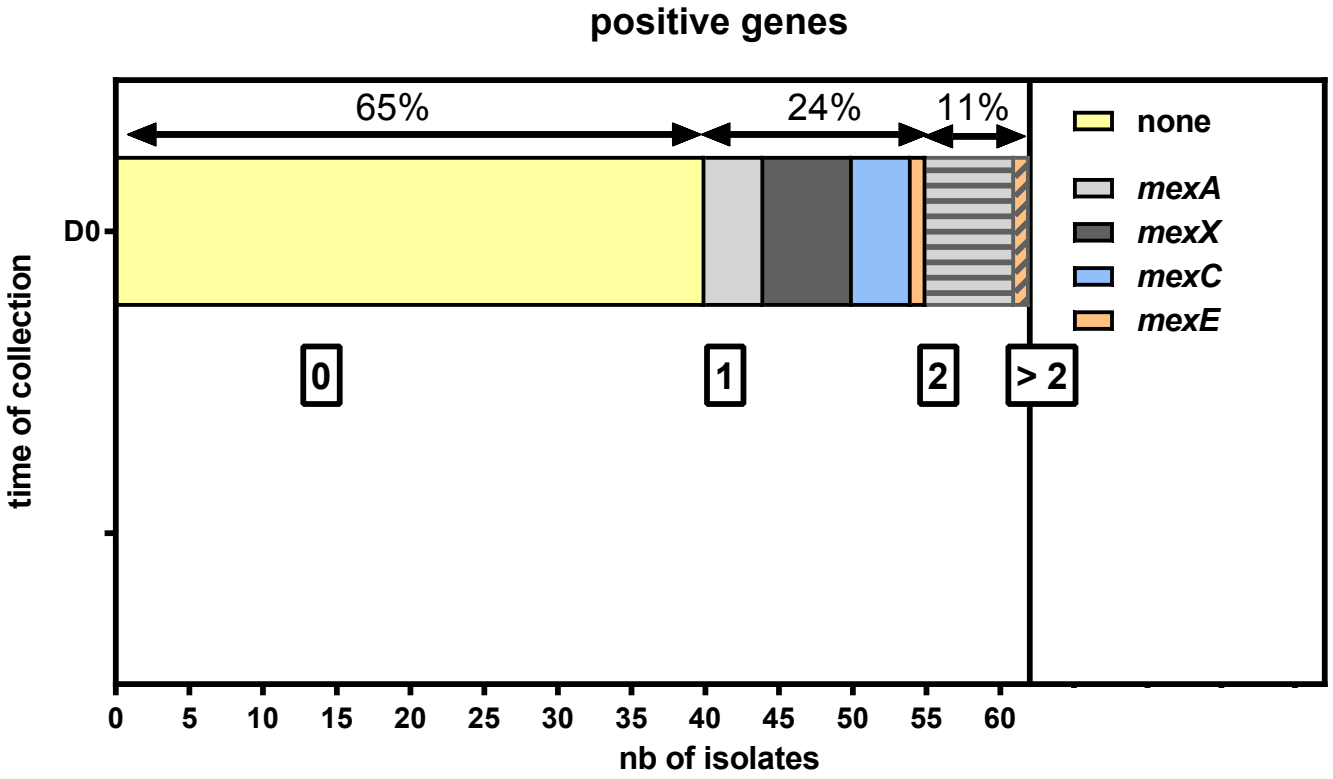
Acquired resistance by efflux in ICU patients

- diagnosis of pneumonia
- sampling

DAY 0 → AB treatment → DAY L

sampling (1-123 days)
mean: 23; median 17

isogenic clones (n=62)



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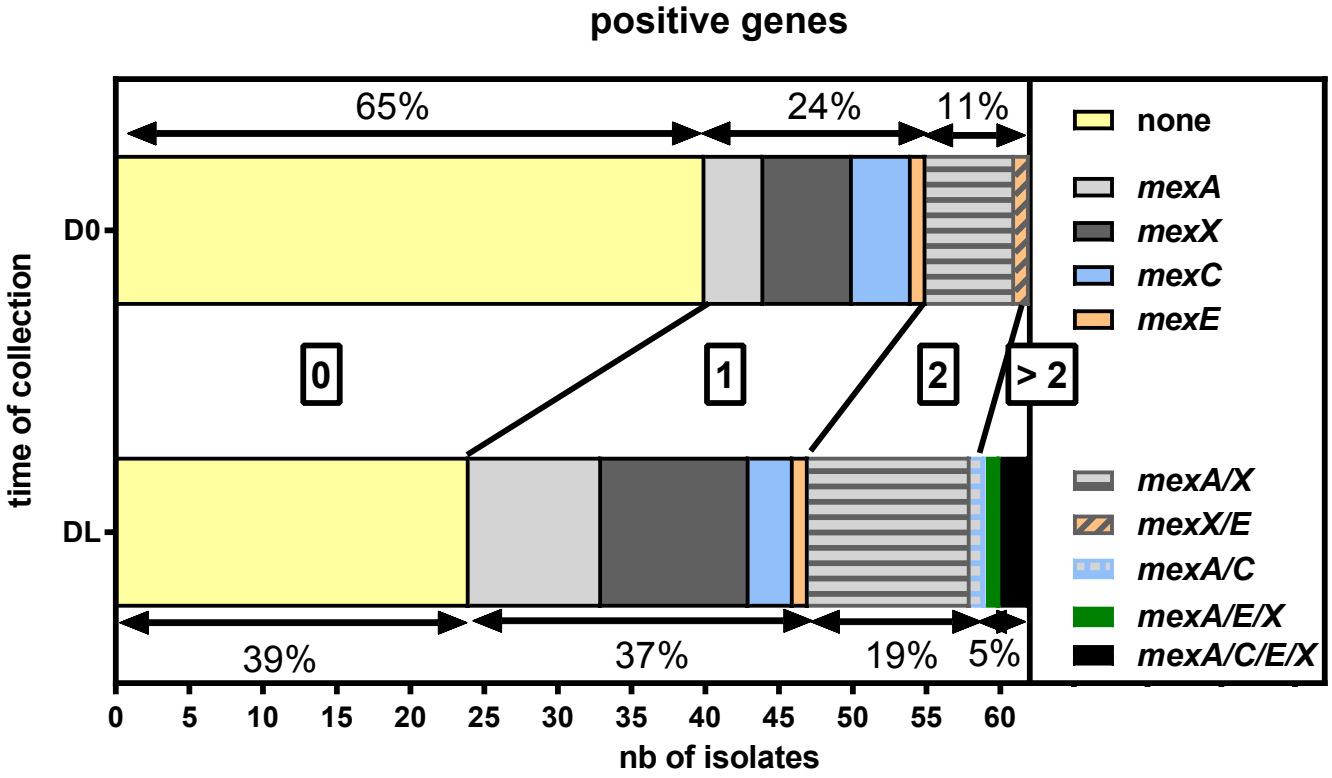
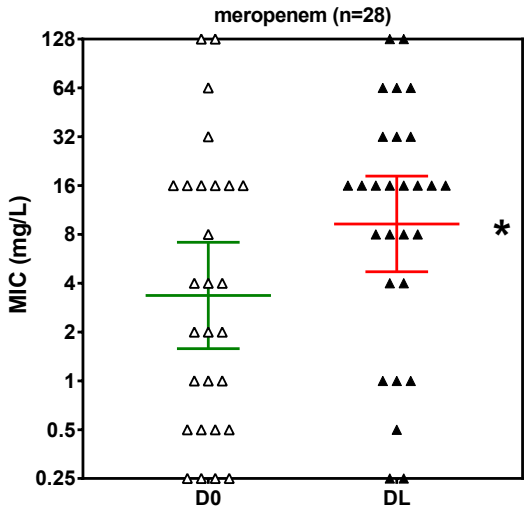
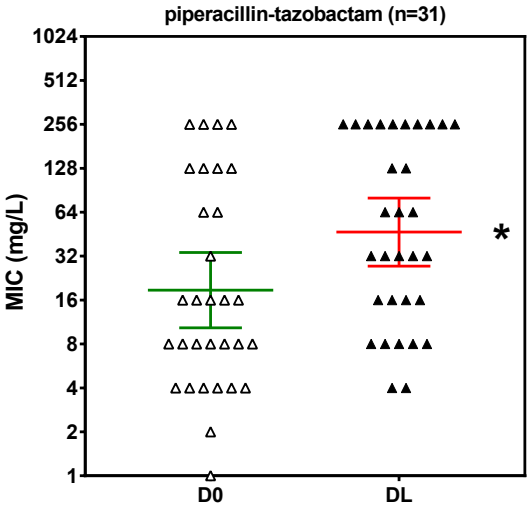


overexpression of efflux during treatment

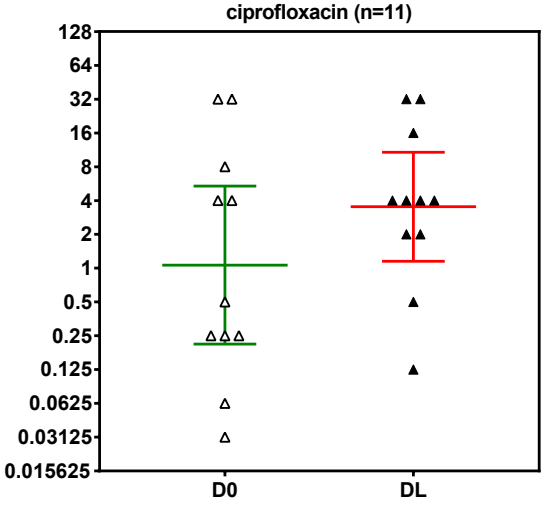
Impact on MICs and resistance profile

MexAB substrates

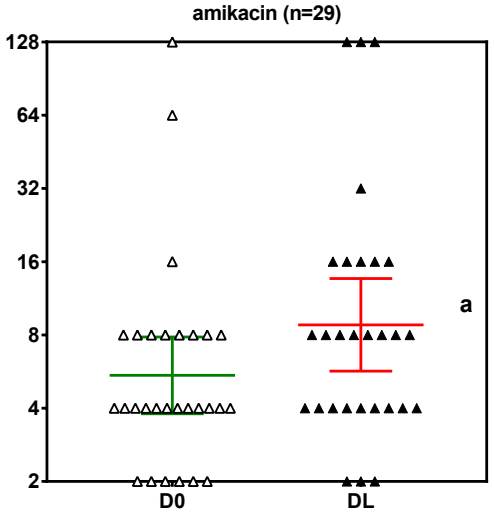


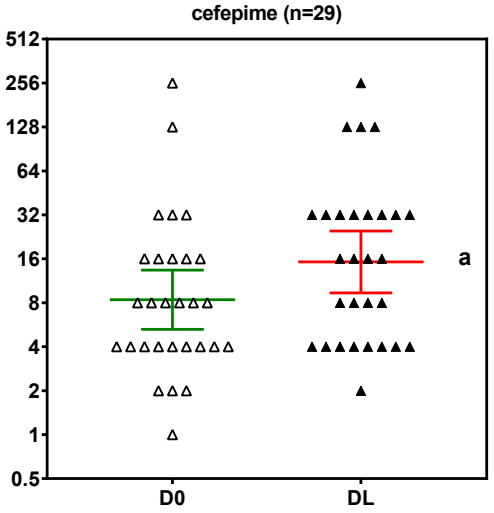


MexAB/XY substrate



MexXY substrates

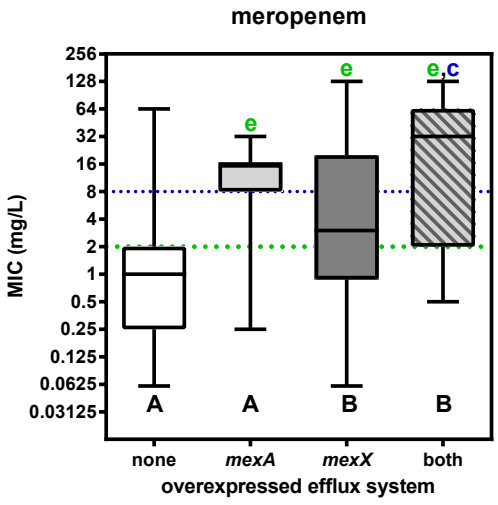


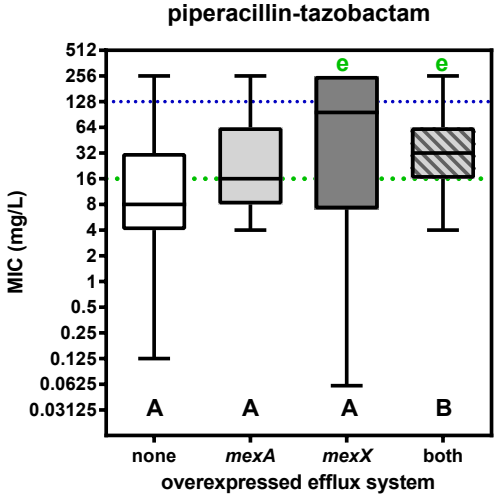


Increase in MIC during treatment

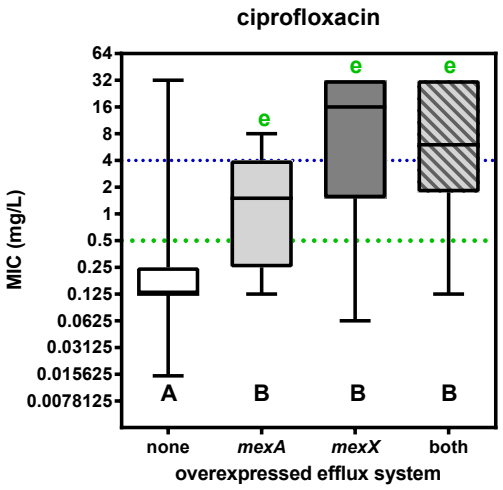
Impact on MICs and resistance profile

MexAB substrates

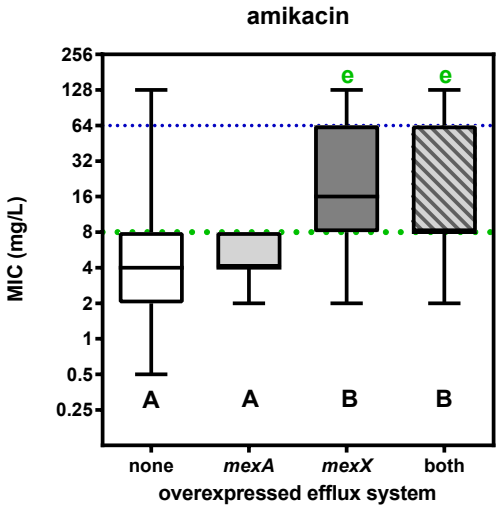


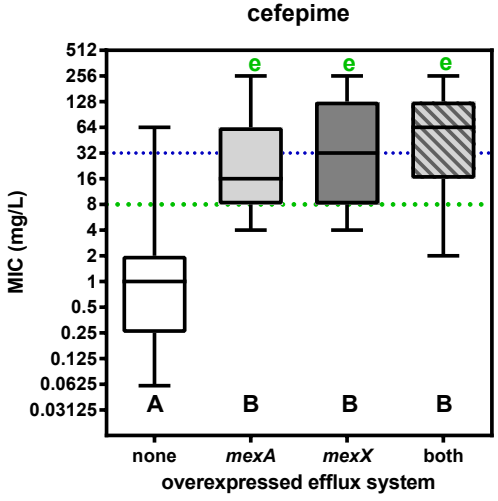


MexAB/XY substrate



MexXY substrates



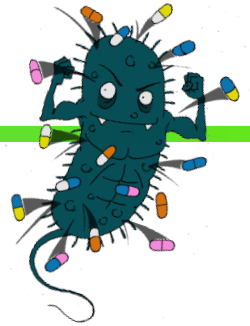


CLINICAL AND LABORATORY STANDARDS INSTITUTE

EUCAST

MICs above EUCAST breakpoints in efflux overexpressers

Efflux and resistance: conclusion (1)

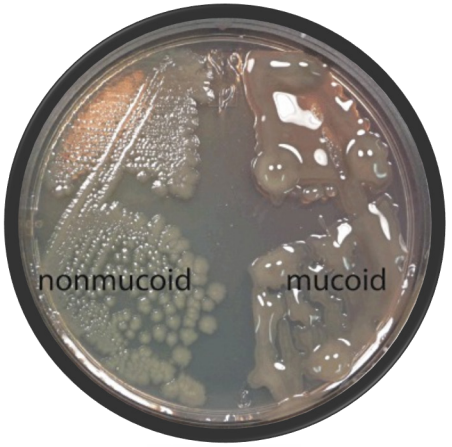


- **Efflux overexpression is selected by antibiotic exposure during therapy**
  - Reversibility ?
  - MDR phenotypes ?
  - Risk of selection of target mutations ? $\rightarrow$ Higher level of resistance

Role of efflux in intrinsic/acquired resistance

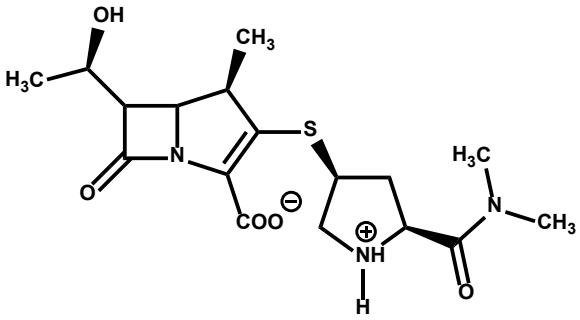
Intrinsic/acquired resistance in CF patients

- Patients frequently / chronically exposed to antibiotics
- Environmental clones → specifically adapted to the CF lung environment ?
  - hypermutable clones
  - Loss of virulence character
  - Alginate producers → biofilm



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

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

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:

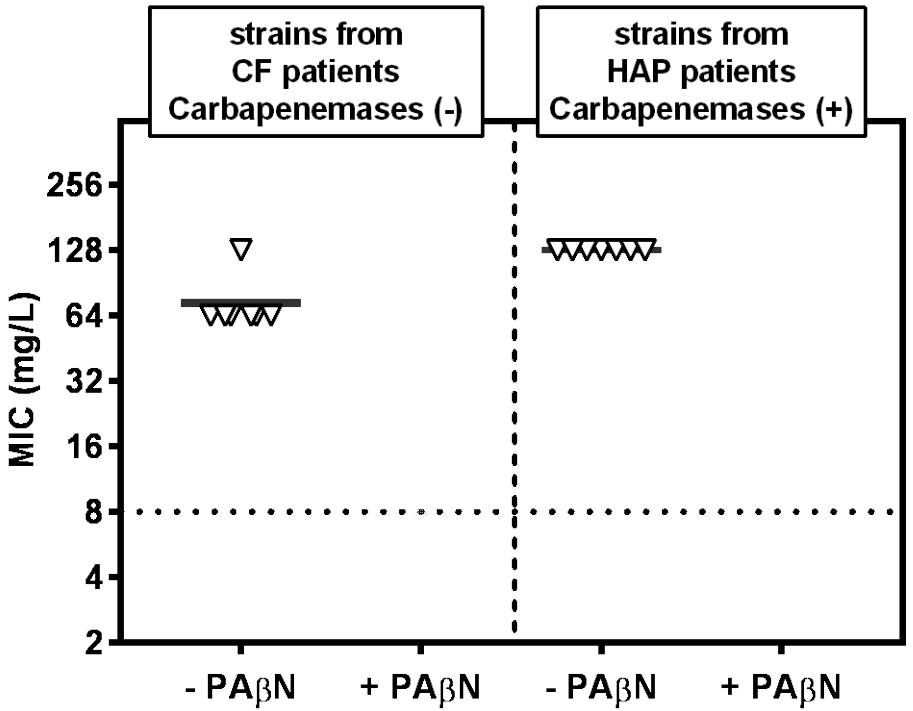
WHY ?

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 ?

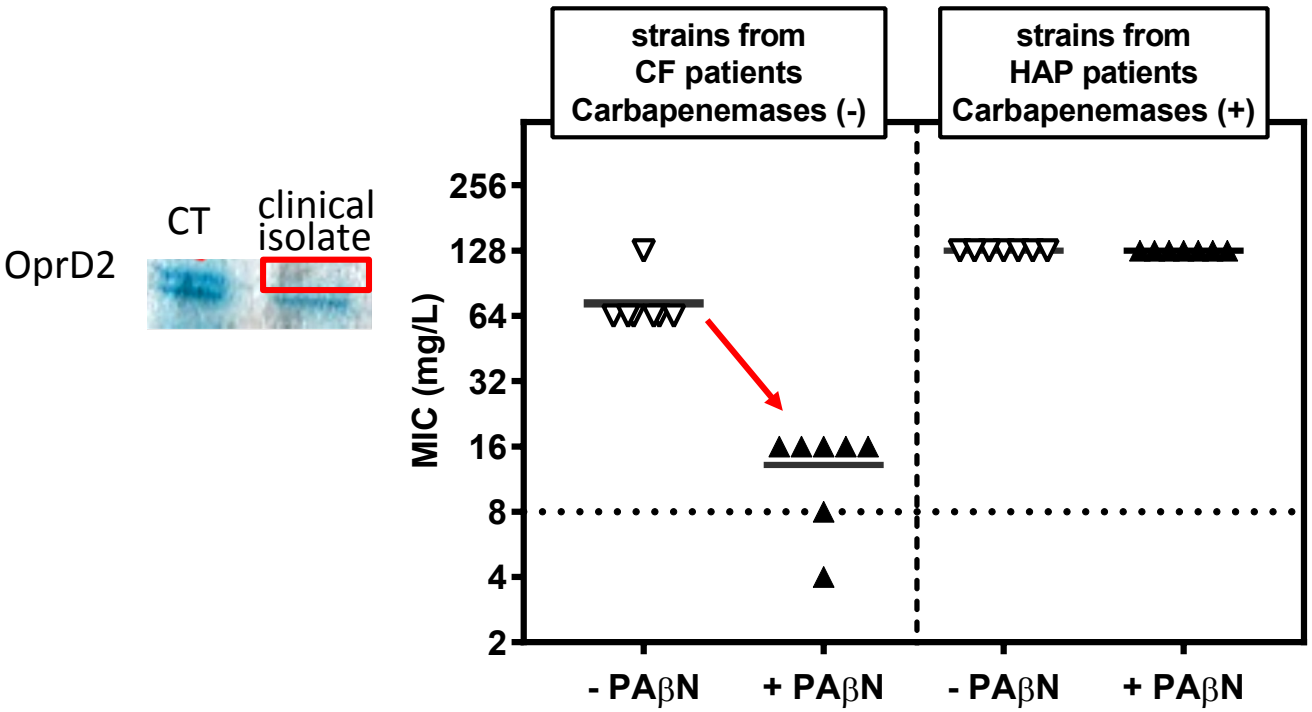
Elevated MICs to meropenem in CF isolates as in ICU isolates ….



High MIC in the absence of carbapenemases in CF isolates

Efflux and high level resistance to carbapenems ?

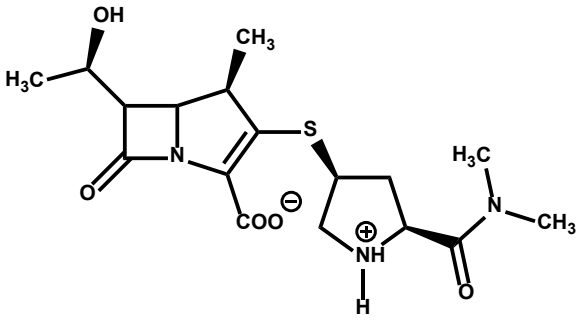
Influence of a broad-efflux pump inhibitor



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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Short Communication

High-level resistance to meropenem in clinical isolates of *Pseudomonas aeruginosa* in the absence of carbapenemases: role of active efflux and porin alterations ☆

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*
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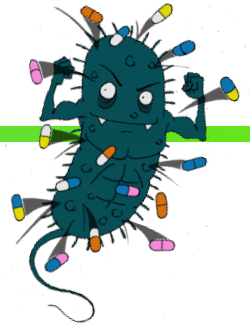
[d] *Laboratoire de microbiologie, Hôpital Erasme, Université libre de Bruxelles, Brussels, Belgium*
[e] *University Hospital Münster, Münster, Germany*

Efflux and resistance: conclusion (2)

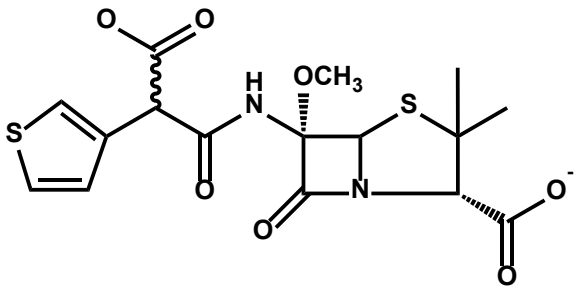


- **Efflux overexpression is selected by antibiotic exposure during therapy**
  - Reversibility ?
  - MDR phenotypes ?
  - Risk of selection of target mutations ? → Higher level of resistance
- **Efflux overexpression can confer high, clinically-significant resistance**
  - Underdiagnosed mechanism

    → Importance of setting-up appropriate diagnostic tests

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

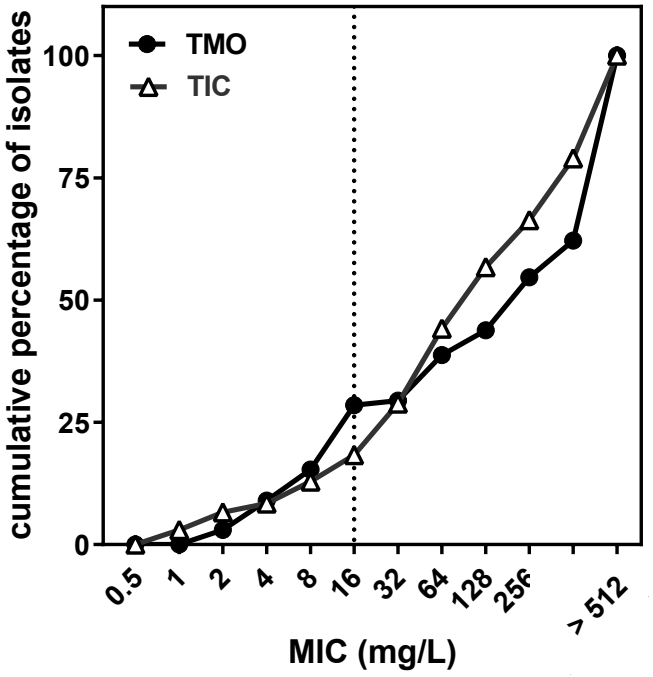
Hussein Chalhoub[1], Daniel Pletzer[2,†], Helge Weingart[2], Yvonne Braun[2], Michael M. Tunney[3], J. Stuart Elborn[3], Hector Rodriguez-Villalobos[4], Patrick Plésiat[5], Barbara C. Kahl[6], Olivier Denis[7], Mathias Winterhalter[2], Paul M. Tulkens[1] & Françoise Van Bambeke[1]

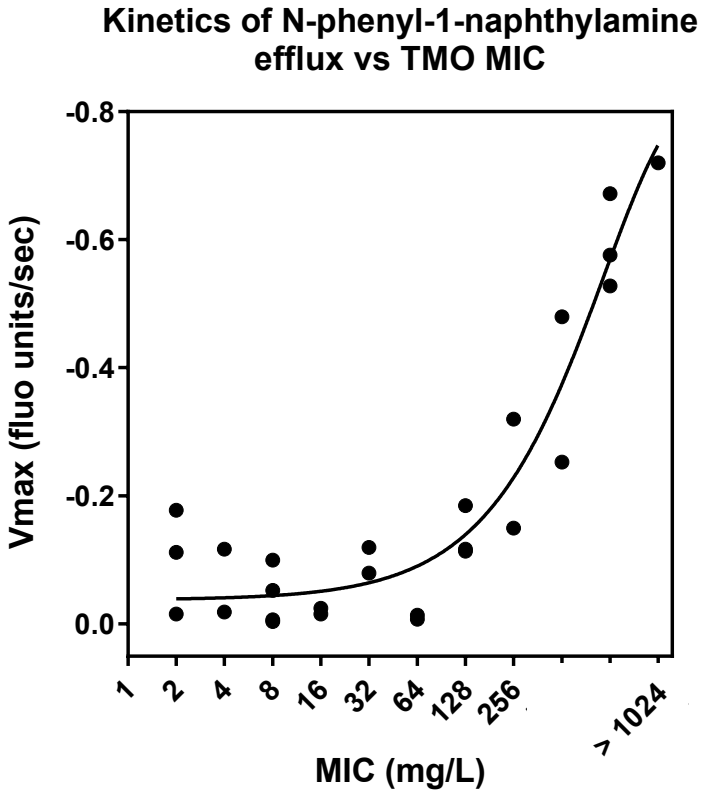
Efflux and acquired susceptibility to temocillin

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 ?



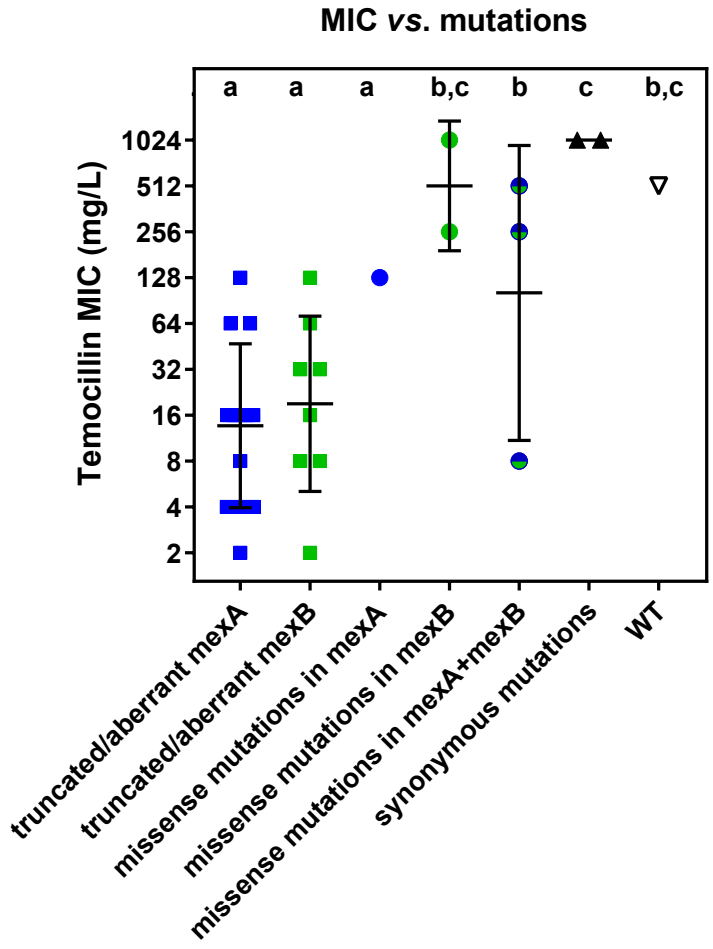


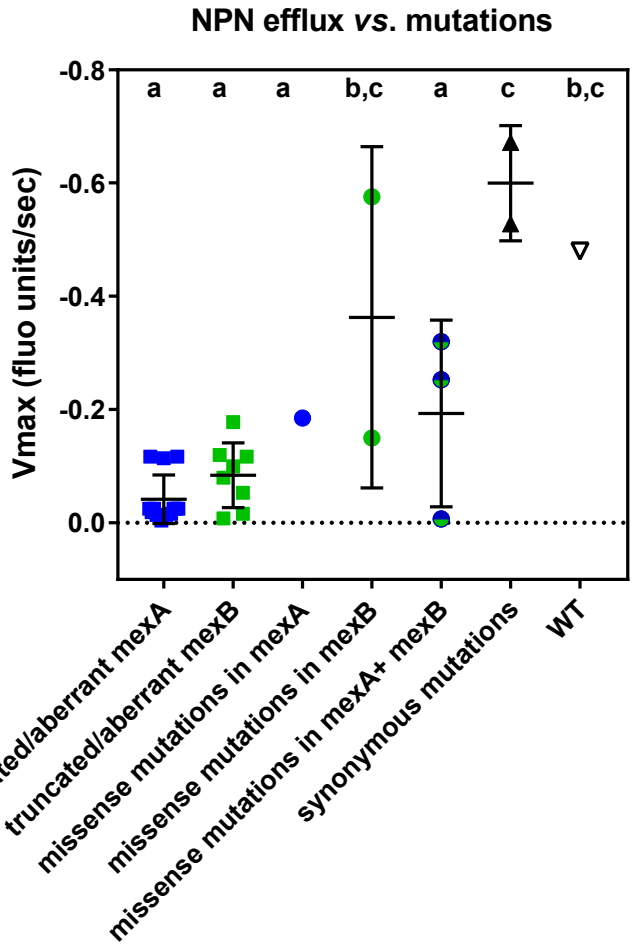
Buyck et al, JAC 2012; 67:771-5; Chalhoub et al, Sci Reports 2017; 7:40208

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

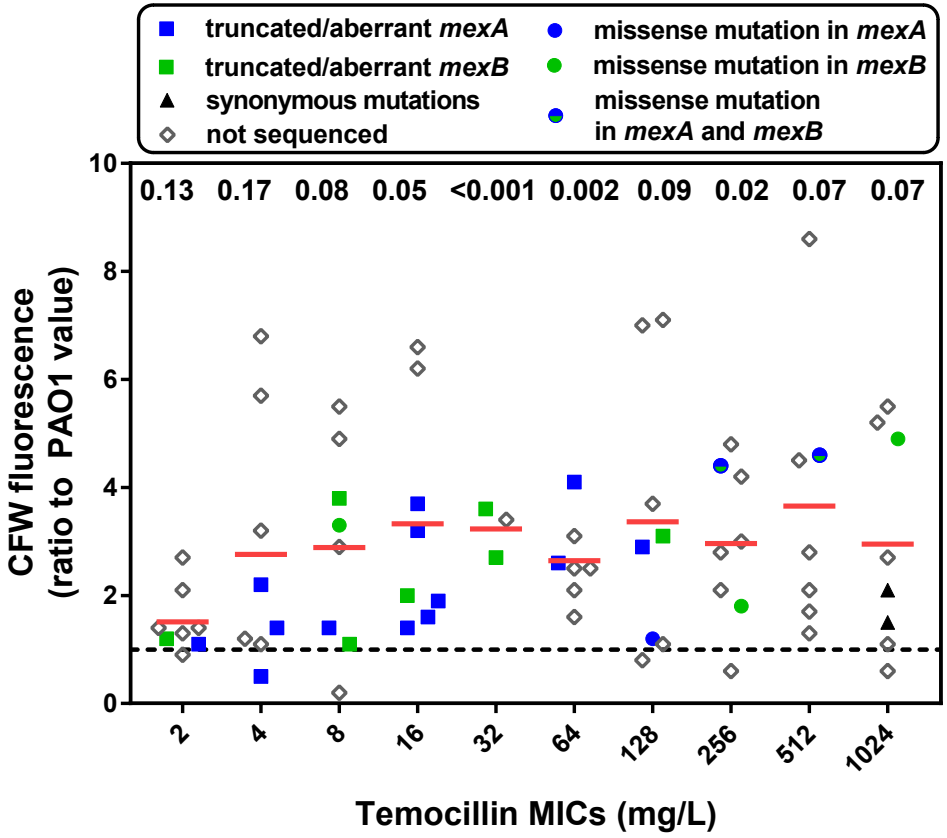




Chalhoub et al, Sci Reports 2017; 7:40208

Efflux and acquired susceptibility to temocillin

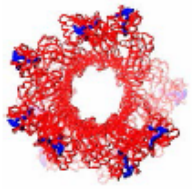
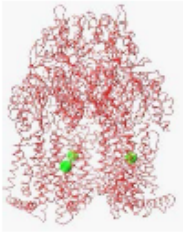
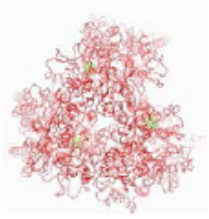
Alginate production: impact on MICs



Alginate production also contributes to resistance …

Efflux and acquired susceptibility to temocillin

Isogenic strains isolated from patients in different countries

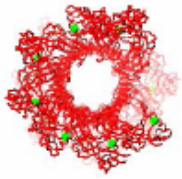
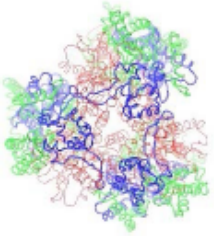
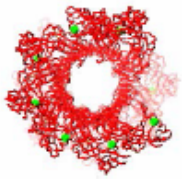
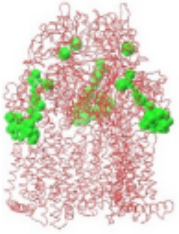
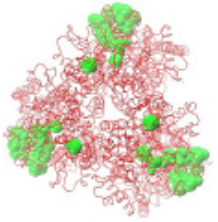
Isolates	Patient ID, country and date of collection	TMO MIC (mg/L)	Protein length (AA)		Encoded MexA		Encoded MexB	
			MexA	MexB	Side view	Top view	Side view	Top view
AG3	JP, UK (May 2006)	8	372	1046			Synonymous mutations	
128	DAF69, Belgium (Oct. 2010)	1024	383	1046	Synonymous mutations			
129-6	129,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

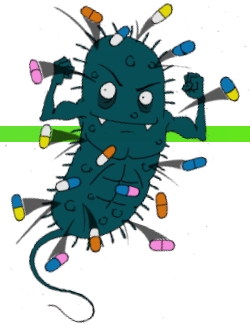
Isogenic strains isolated from different patients in a single country

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

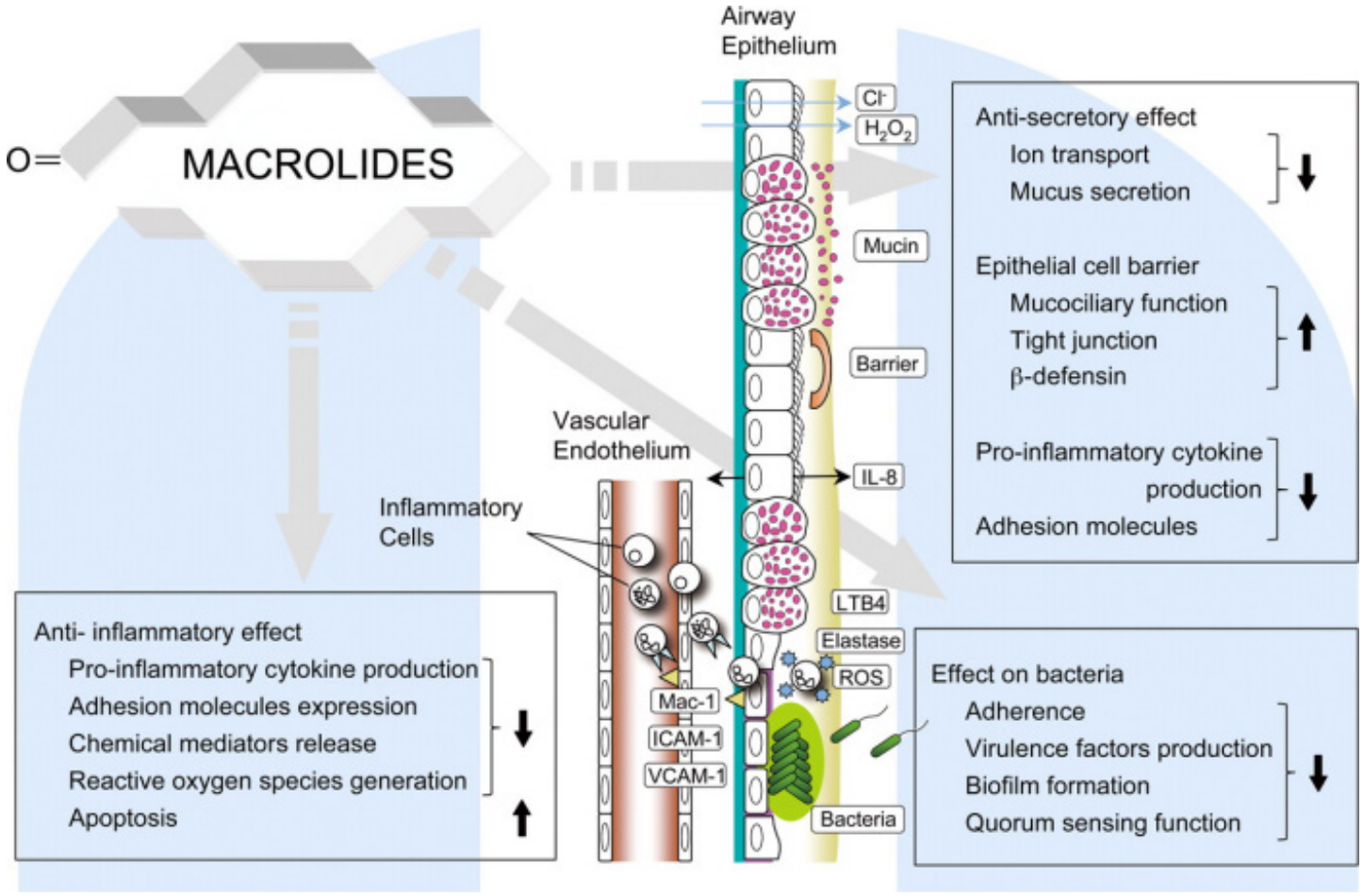
Efflux and resistance: conclusion (3)



- **Efflux overexpression is selected by antibiotic exposure during therapy**
  - Reversibility ?
  - MDR phenotypes ?
  - Risk of selection of target mutations ? → Higher level of resistance
- **Efflux overexpression can confer high, clinically-significant resistance**
  - Underdiagnosed mechanism
    → Importance of setting-up appropriate diagnostic tests
- **Natural mutations in efflux systems → acquired susceptibility**
  - Temocillin revived against *P. aeruginosa* ? → testing in routine ?
  - Roles of mutations in adaptation to CF lung ?

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*…

MAJOR ARTICLE

534 • CID 2012:55 (15 August)

Increased Susceptibility of *Pseudomonas aeruginosa* to Macrolides and Ketolides in Eukaryotic Cell Culture Media and Biological Fluids Due to Decreased Expression of *oprM* and Increased Outer-Membrane Permeability

Julien M. Buyck,[1] Patrick Plésiat,[2] H. Traore,[3] F. Vanderbist,[3] Paul M. Tulkens,[1] and Françoise Van Bambeke[1]

[1]Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain, and [3]Laboratoires SMB, Brussels, Belgium; and [2]Laboratoire de Bactériologie, Hôpital Jean Minjoz, Besançon, France

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]

An intriguing observation …

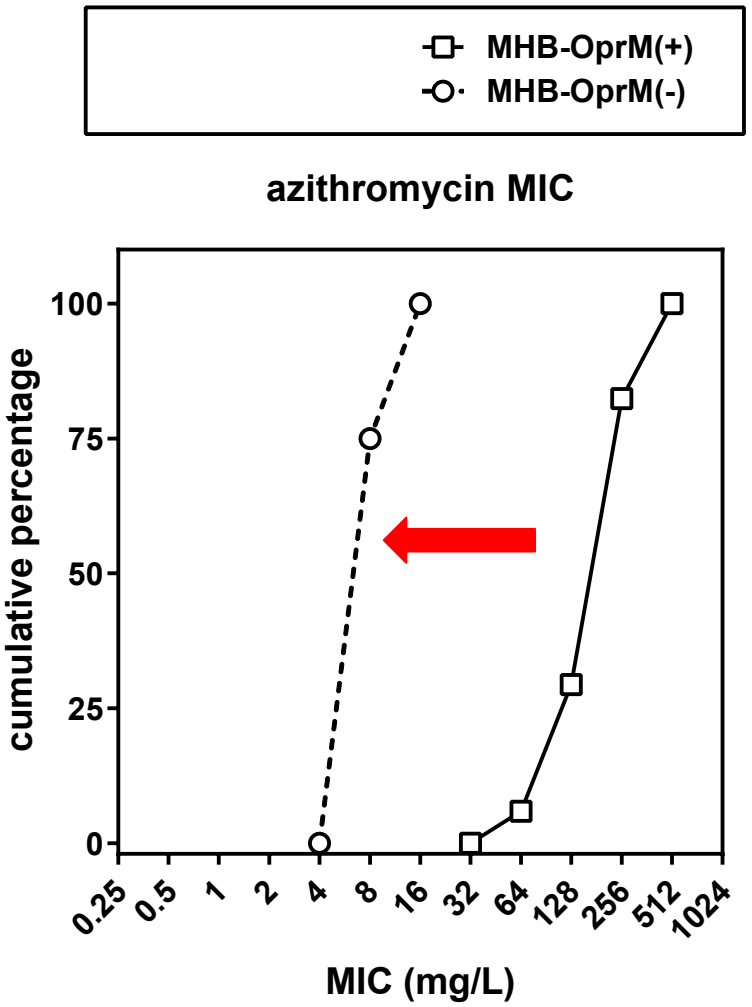
Antibiotic	MIC (mg/L)		
	CA-MHB		RPMI-1640
	pH 7.4	pH 5.5	
Aminoglycosides			
Gentamicin	**2**	**8**	**4**
Amikacin	**4**	**64**	**4**
Tobramycin	**1**	**8**	**1**
β-lactams			
Piperacillin/Tazobactam	**16**	**16**	**16**
Cefepime	**4**	**8**	**4**
Ceftazidime	**2**	**4**	**2**
Aztreonam	**8**	**16**	**8**
Meropenem	**1**	**1**	**2**
Fluoroquinolones			
Ciprofloxacin	**0.125**	**0.25**	**0.125**
Polymyxins			
Colistin	**1**	**2**	**2**
Azithromycin	128	>512	16

Macrolides regain activity against *P. aeruginosa* in « eukaryotic » media

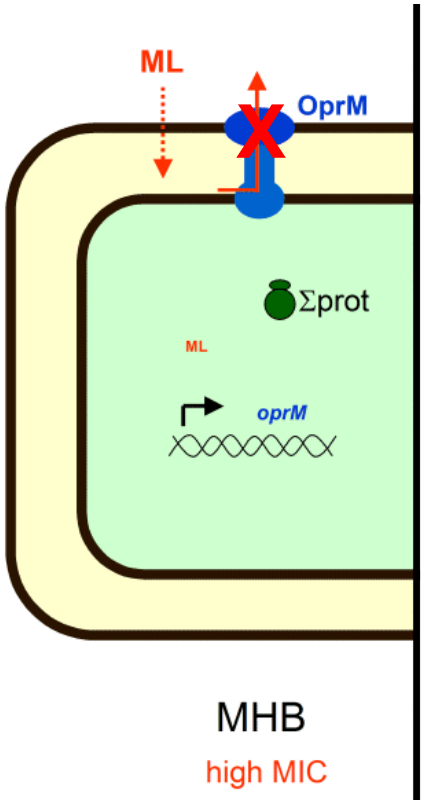
Also observed in serum or in bronchoalveolar lavage fluid

Buyck et al. Clin Infect Dis. 2012; 55:534-42

Efflux and intrinsic / acquired resistance to macrolides

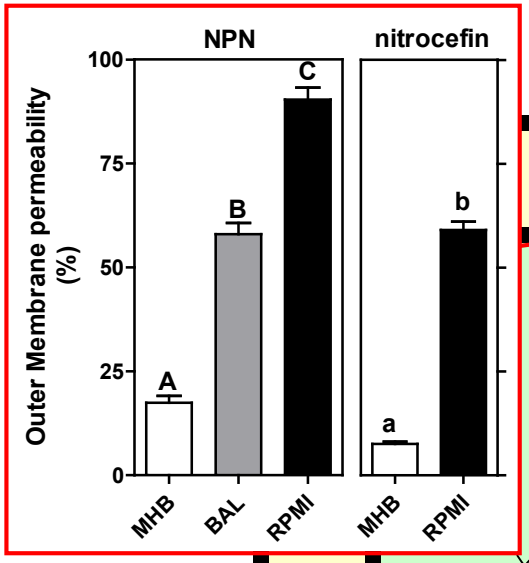


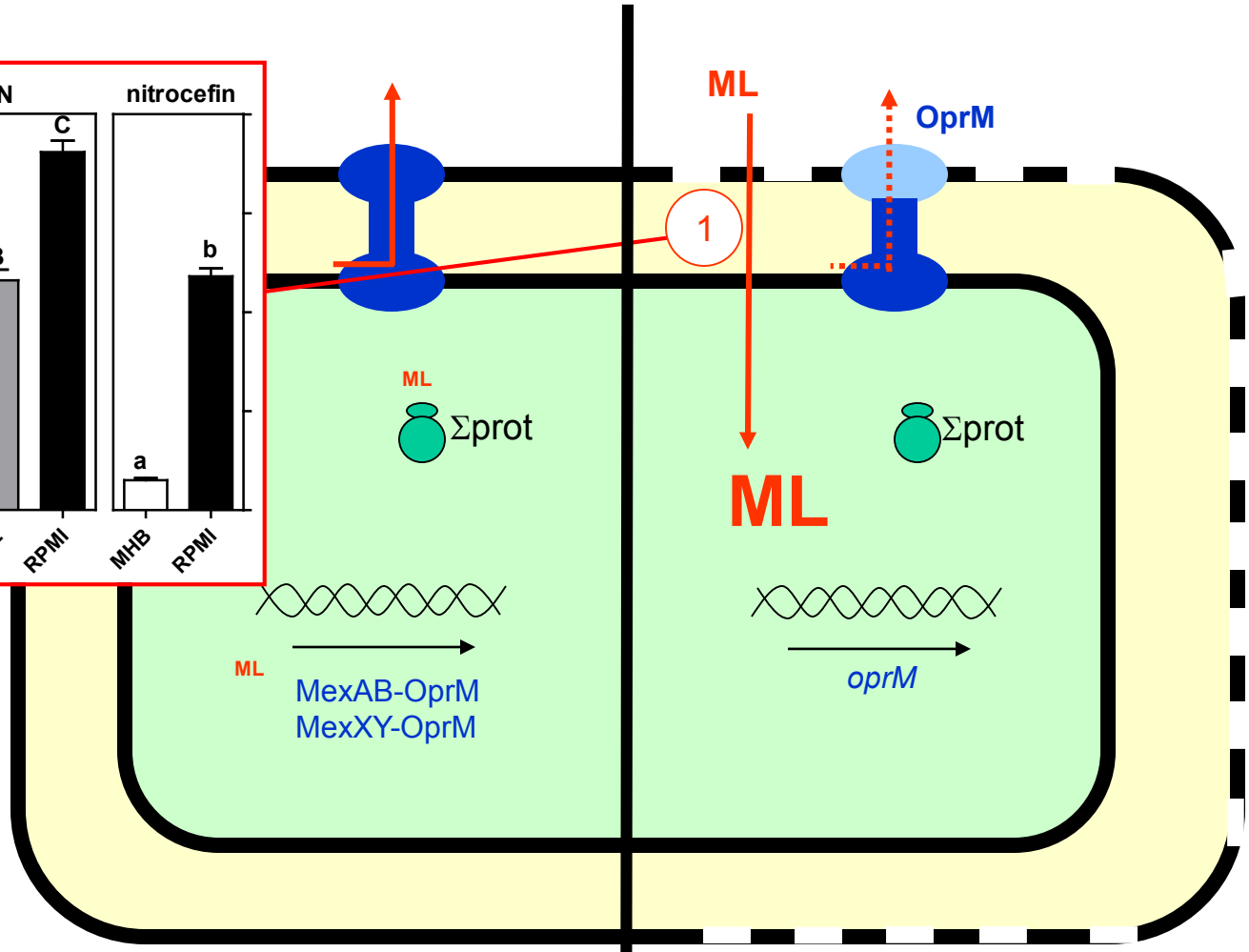
Macrolides inactive on *Pa* due to active efflux



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

Why do macrolides express their activity in « eukaryotic » media ?

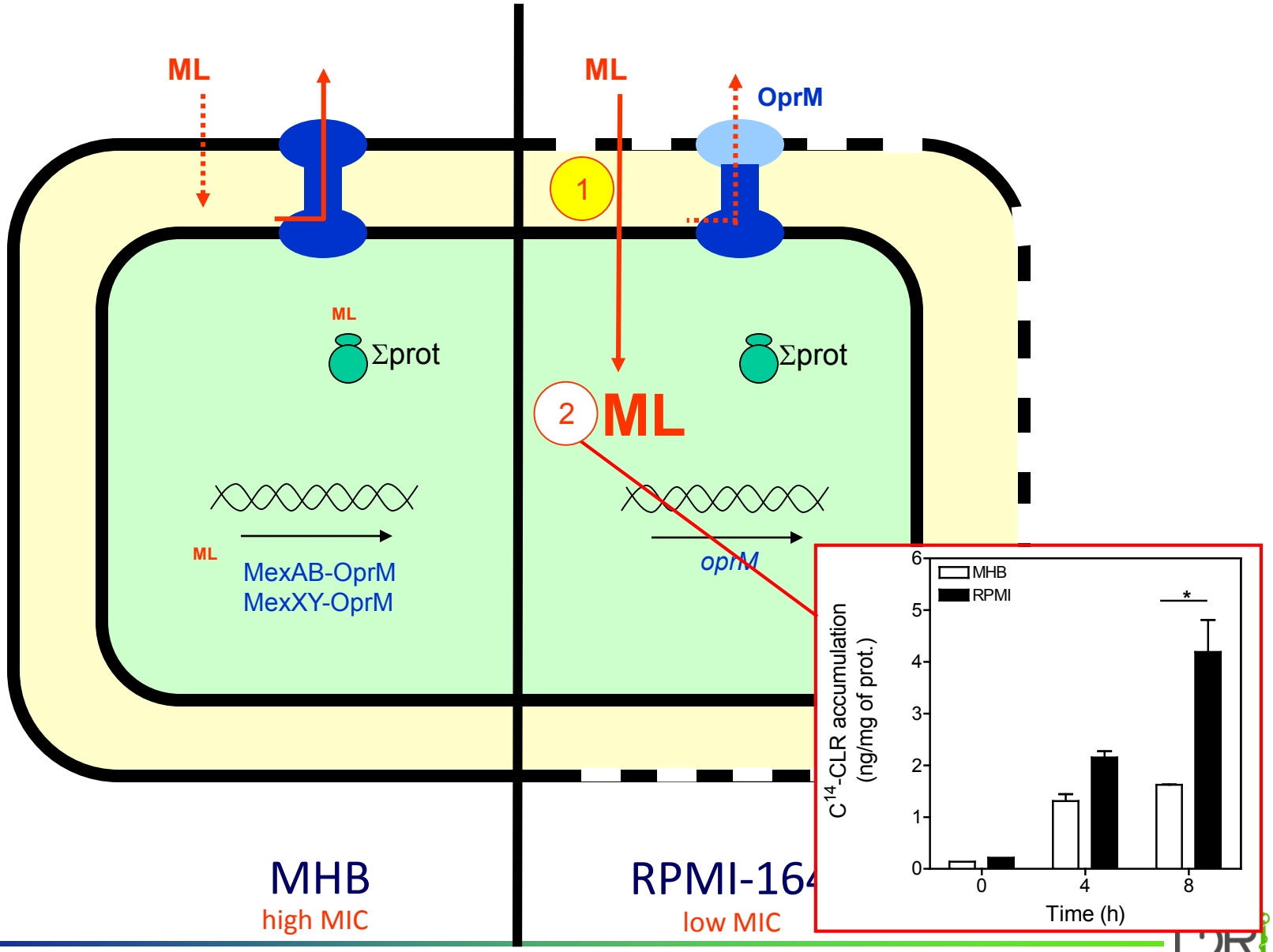




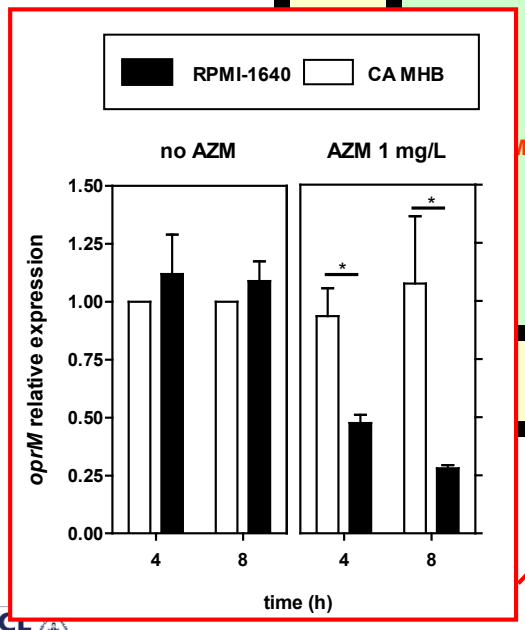
MHB
high MIC

RPMI-1640
low MIC

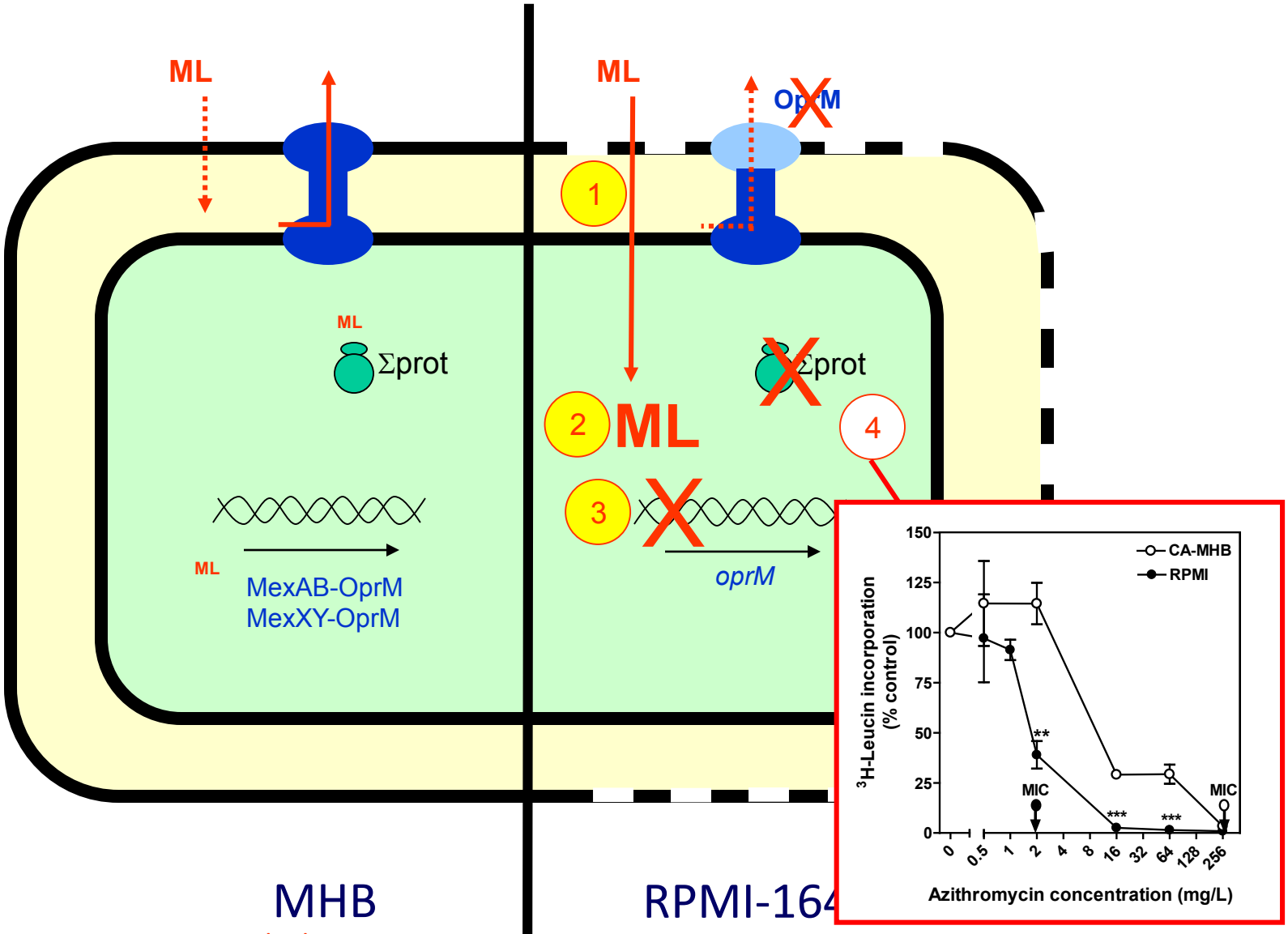
Why do macrolides express their activity in « eukaryotic » media ?



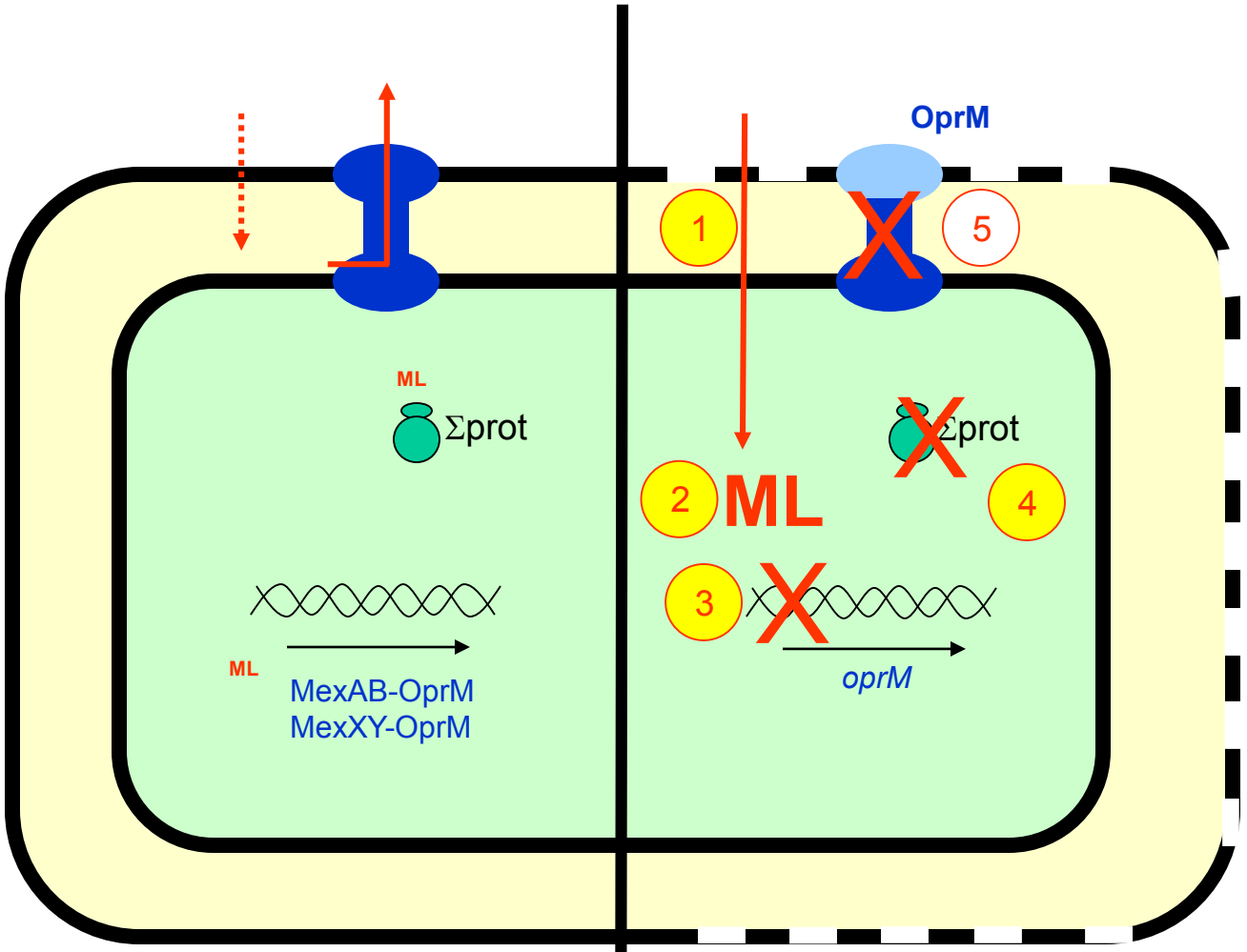
Why do macrolides express their activity in « eukaryotic » media ?



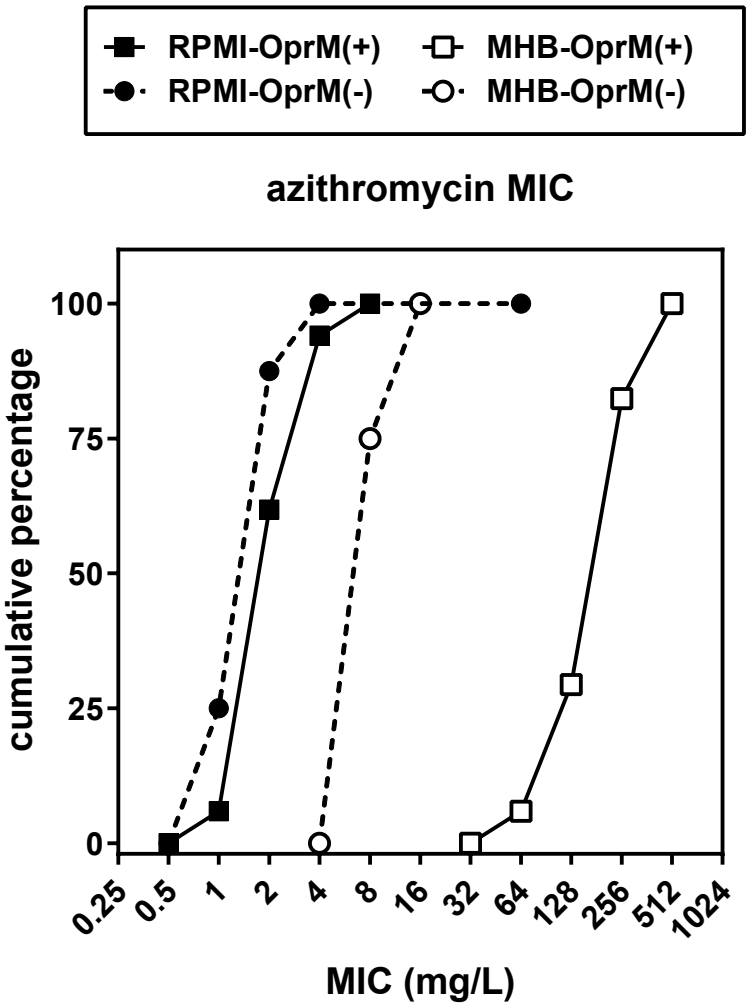
Why do macrolides express their activity in « eukaryotic » media ?



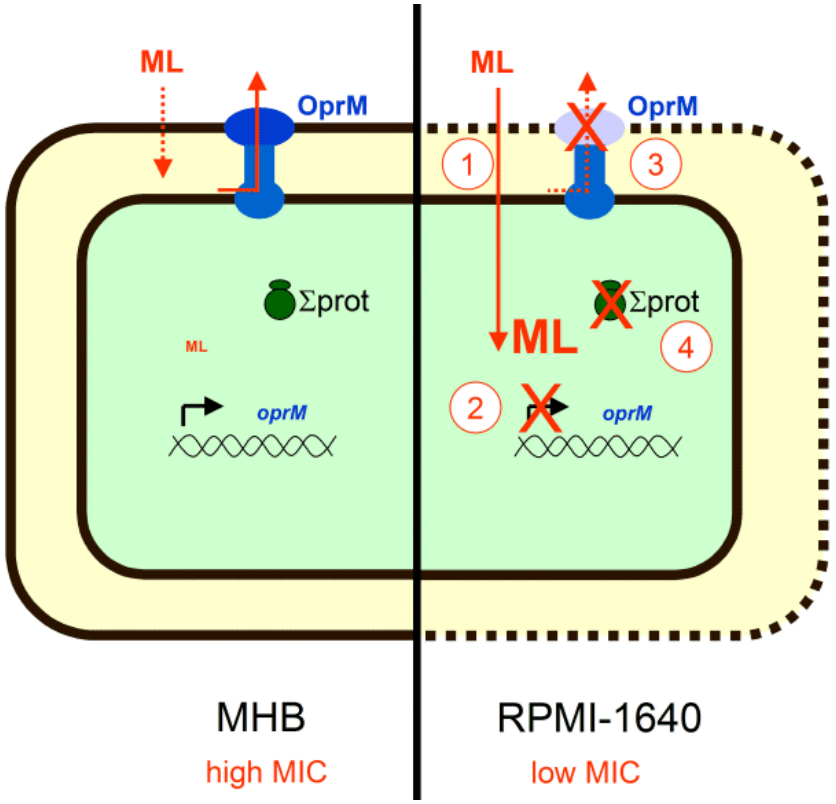
Why do macrolides express their activity in « eukaryotic » media ?



Efflux and intrinsic / acquired resistance to macrolides



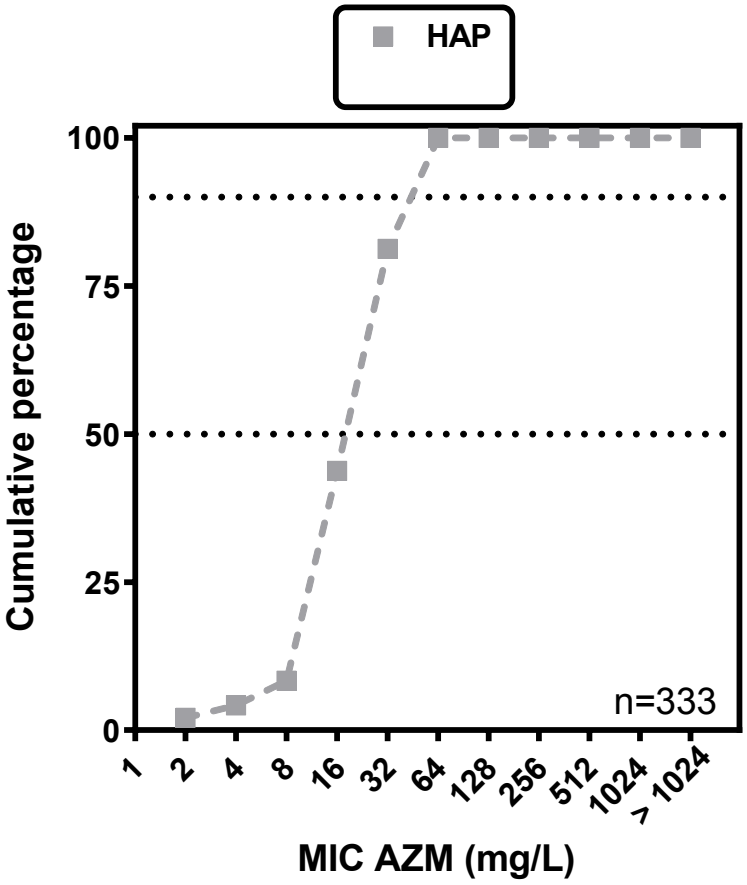
Activity recovered in 'biological' media due to increased OM permeability



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

Efflux and intrinsic / acquired resistance to macrolides

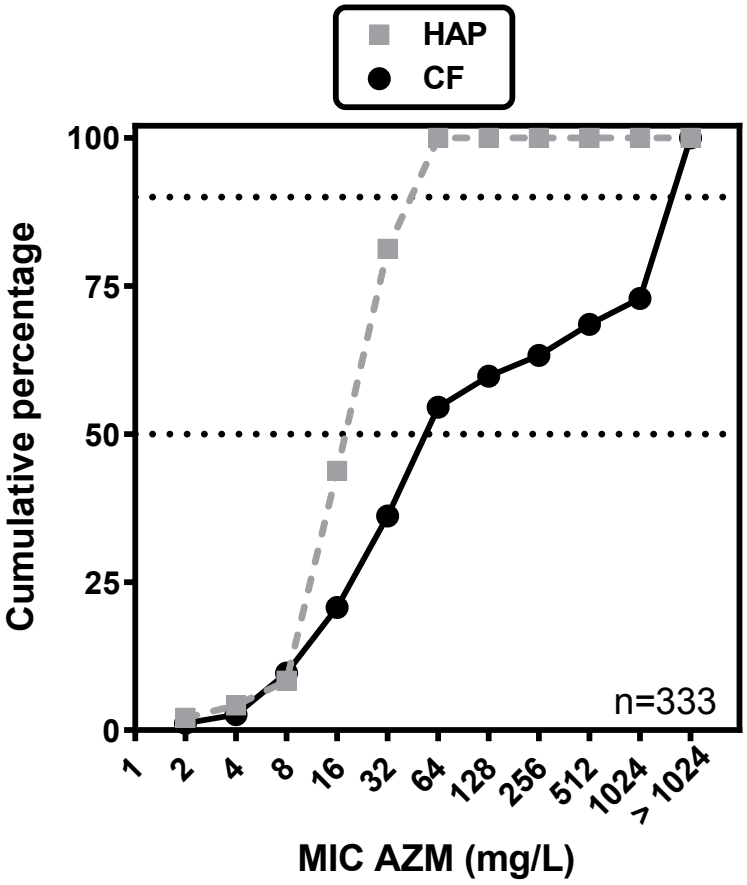
Clinical relevance ?



Mustafa et al. Eur. Respir. J. 2017; in press

Efflux and intrinsic / acquired resistance to macrolides

Clinical relevance ?



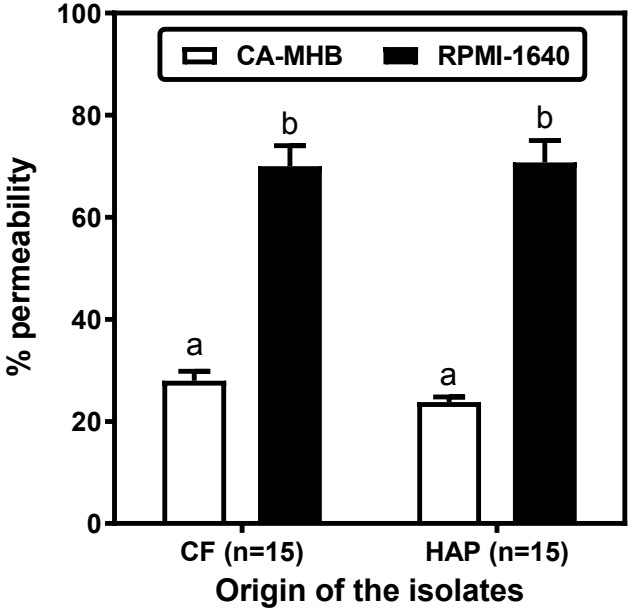
CF isolates more resistant to macrolides than HAP isolates…

Mustafa et al. Eur. Respir. J. 2017; in press

Efflux and intrinsic / acquired resistance to macrolides

Why this difference between CF and HAP isolates ?

Same OM permeability



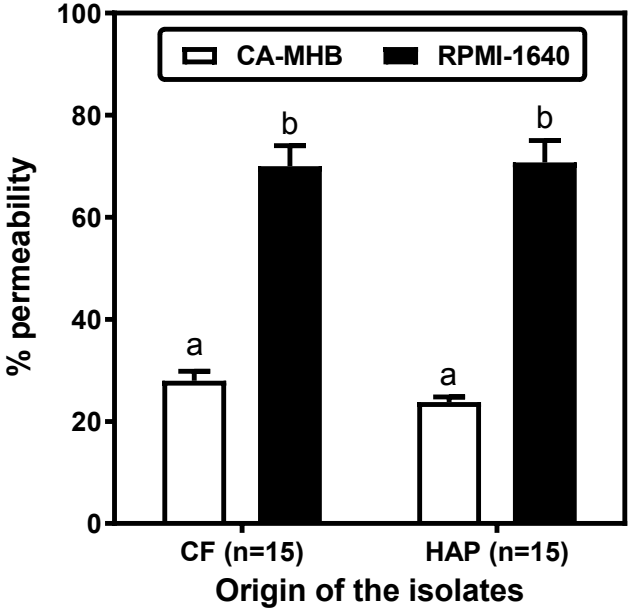
Mustafa et al. Eur. Respir. J. 2017; in press

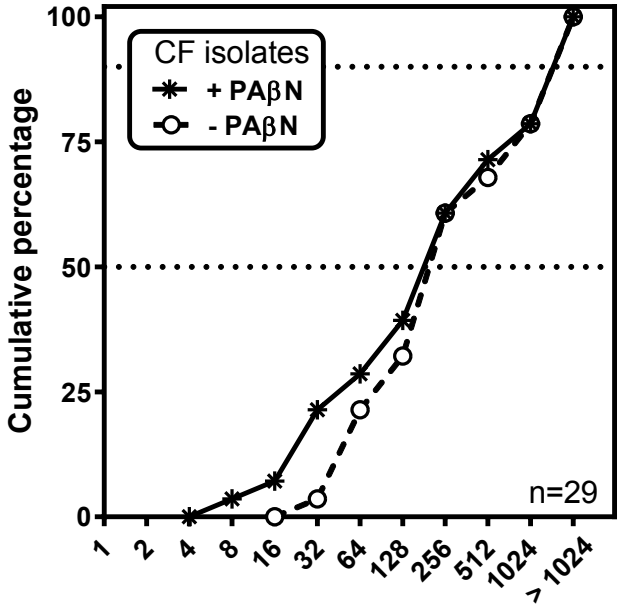
Efflux and intrinsic / acquired resistance to macrolides

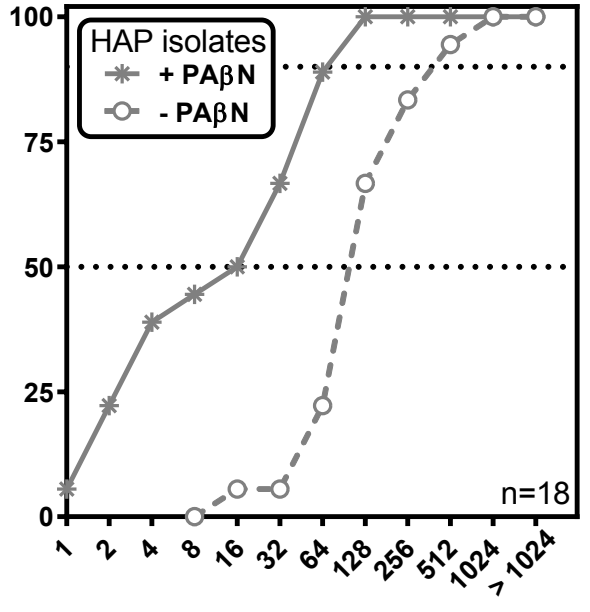
Why this difference between CF and HAP isolates ?

Same OM permeability

MIC reduced by efflux pump inhibitor in HAP isolates only



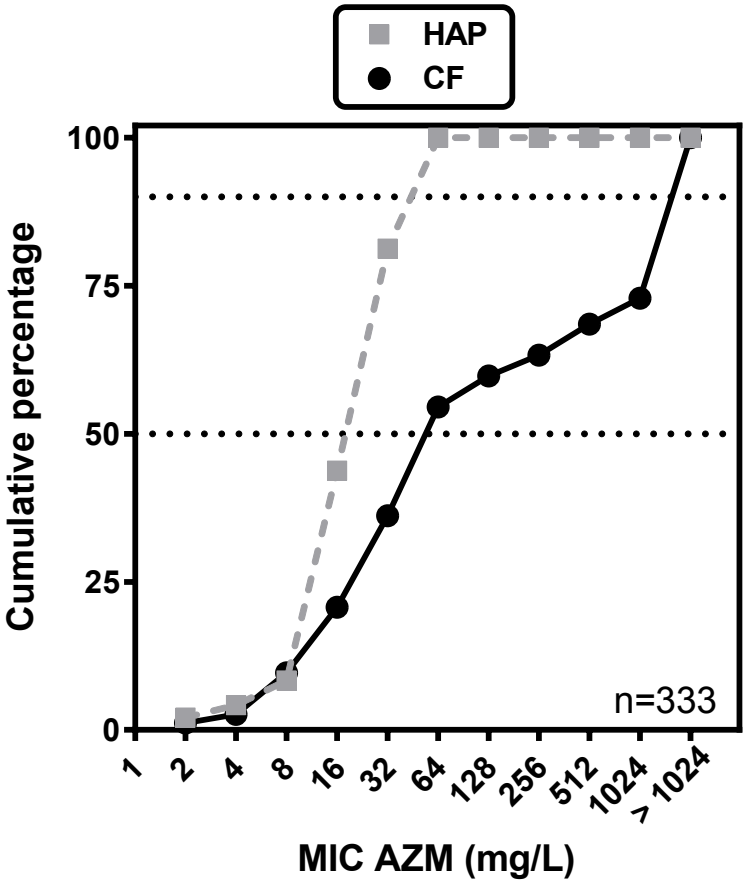


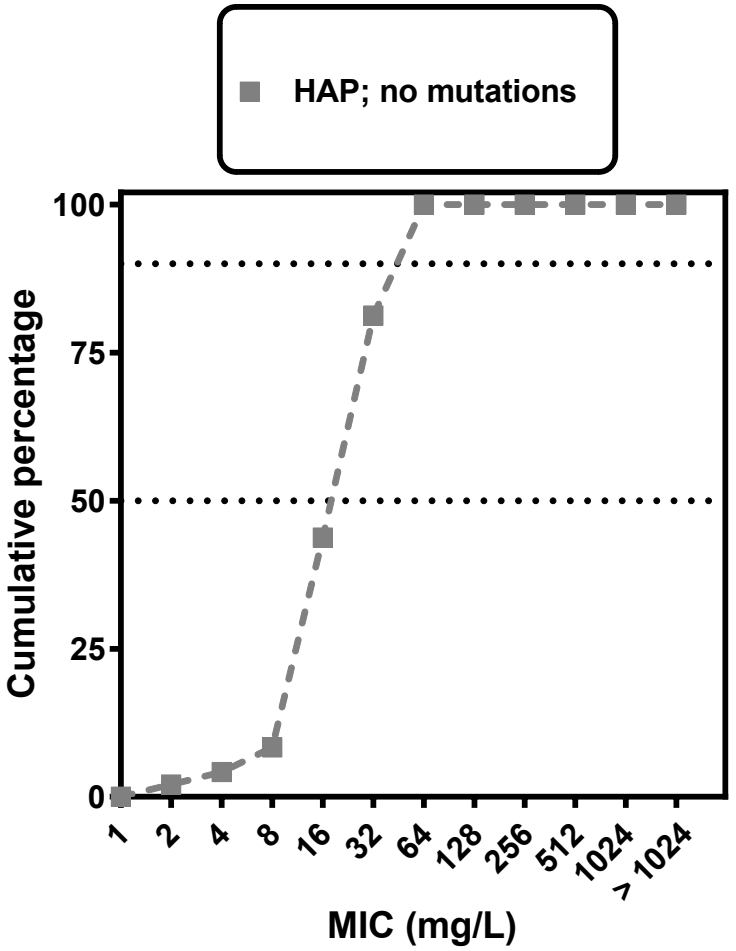


Mustafa et al. Eur. Respir. J. 2017; in press

Efflux and intrinsic / acquired resistance to macrolides

Ribosomal mutations ?

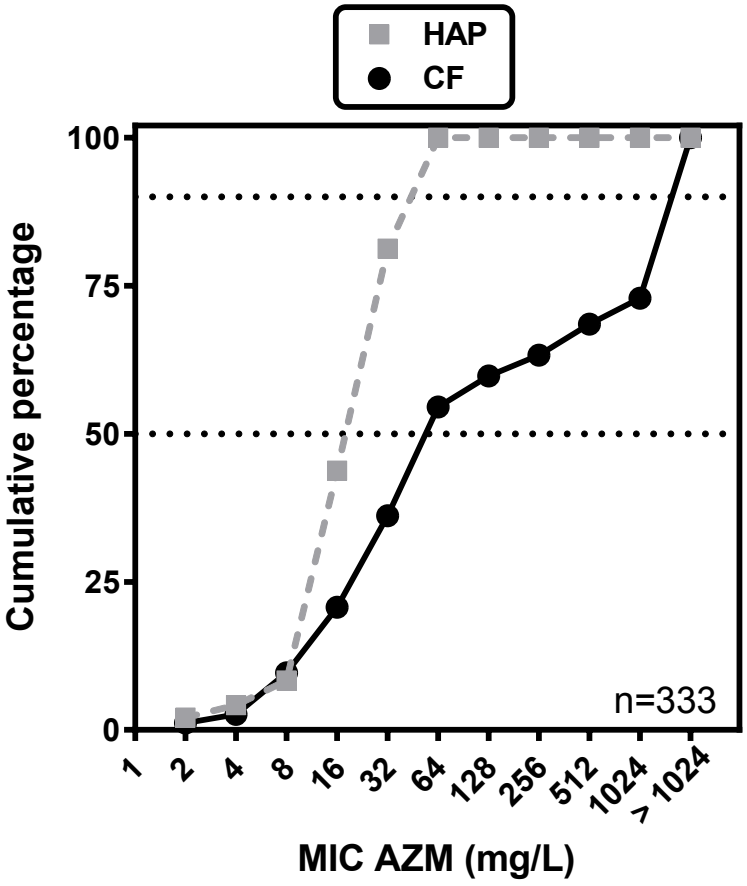


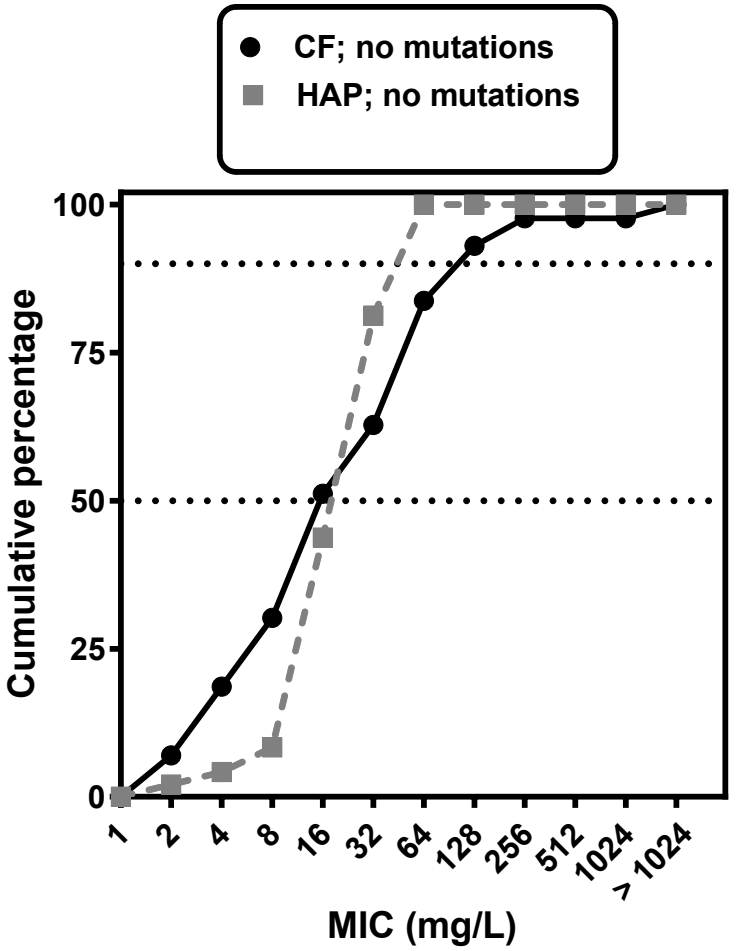


Mustafa et al. Eur. Respir. J. 2017; in press

Efflux and intrinsic / acquired resistance to macrolides

Ribosomal mutations ?

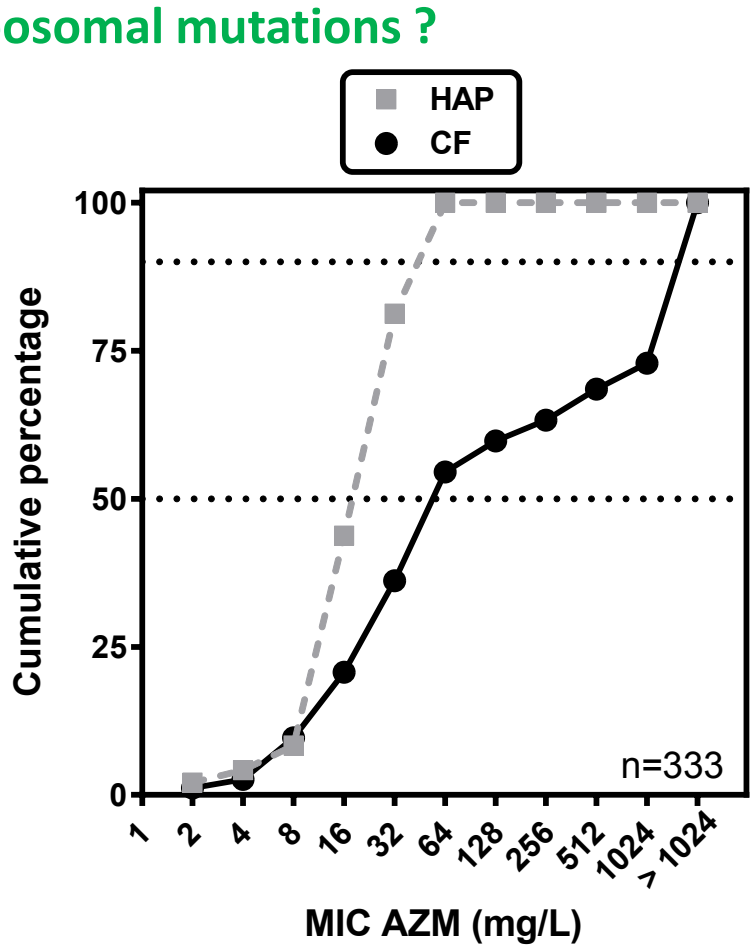


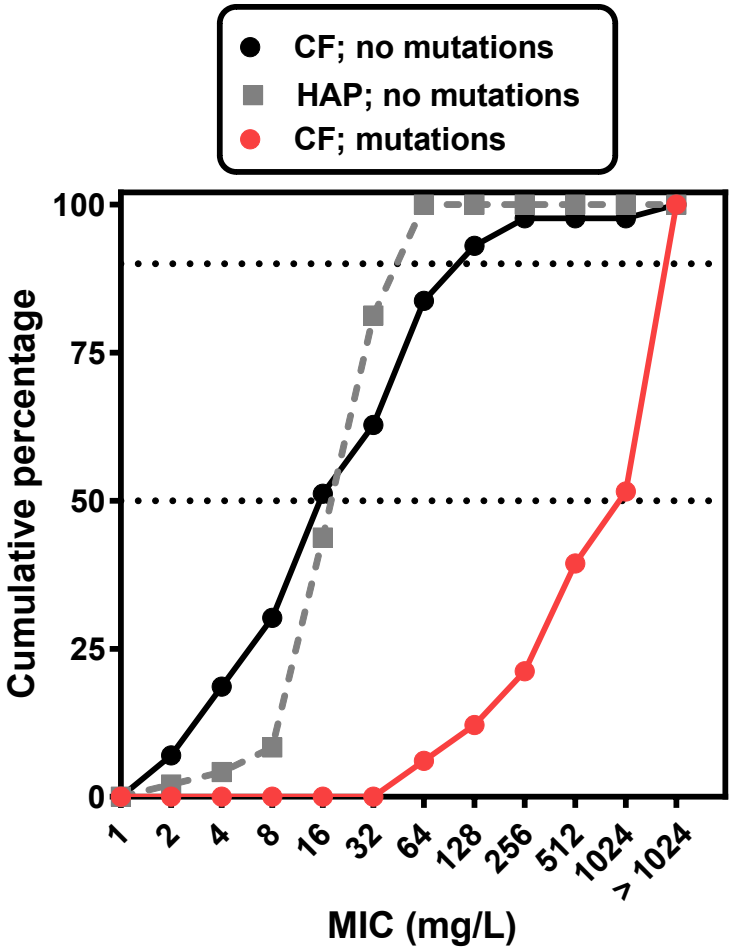


Mustafa et al. Eur. Respir. J. 2017; in press

Efflux and intrinsic / acquired resistance to macrolides

Ribosomal mutations ?



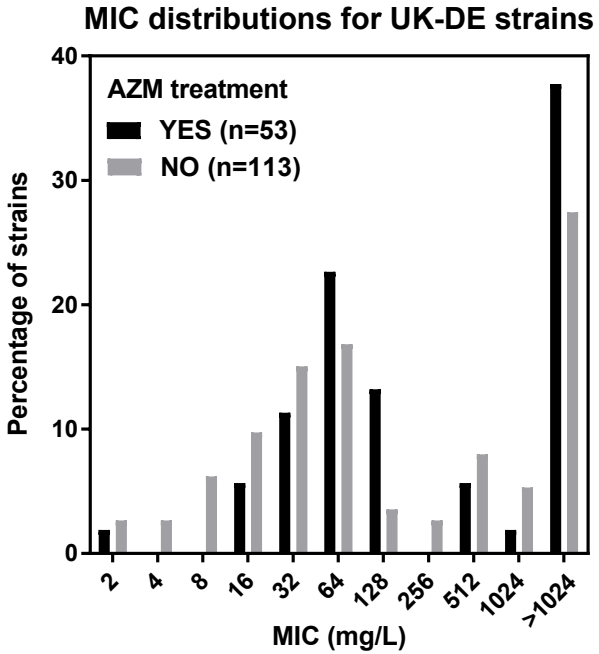


Active efflux may have selected for ribosomal mutations in patients exposed to macrolides …

Mustafa et al. Eur. Respir. J. 2017; in press

Efflux and intrinsic / acquired resistance to macrolides

Link with macrolide use ?



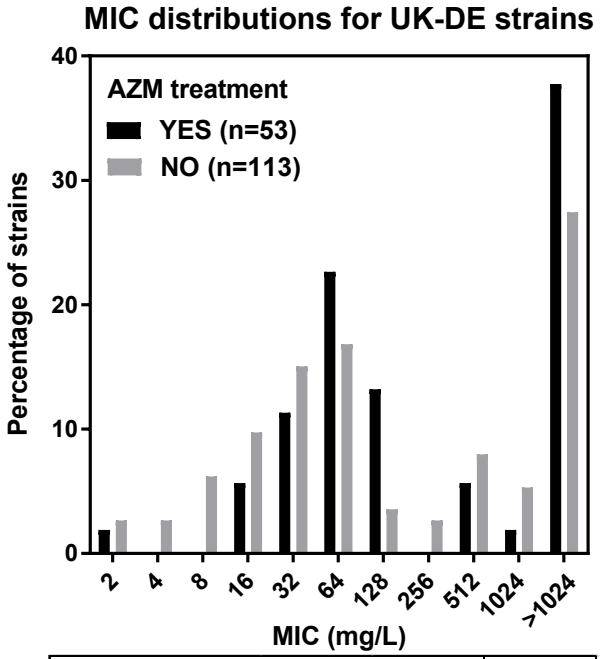
χ^2 tests			χ^2
	AZM treatment		
Parameter	YES	NO	p value
MIC ≤ 32 mg/L	10/53	41/113	0.01
MIC > 1024 mg/L	20/53	31/113	0.08

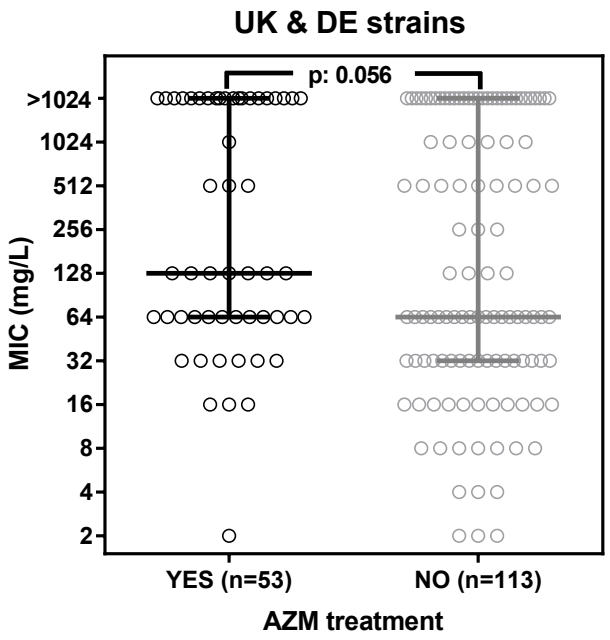
Lower MICs in non AZM-treated patients
Higher MICs in AZM-treated patients

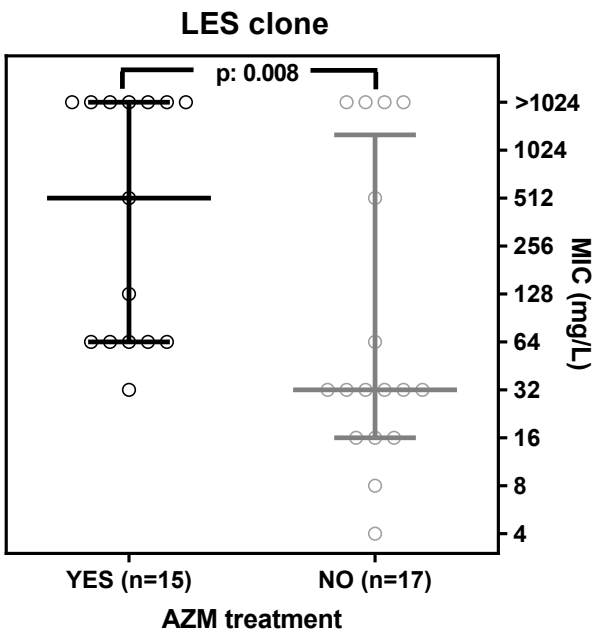
Mustafa et al. Eur. Respir. J. 2017; in press

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

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)

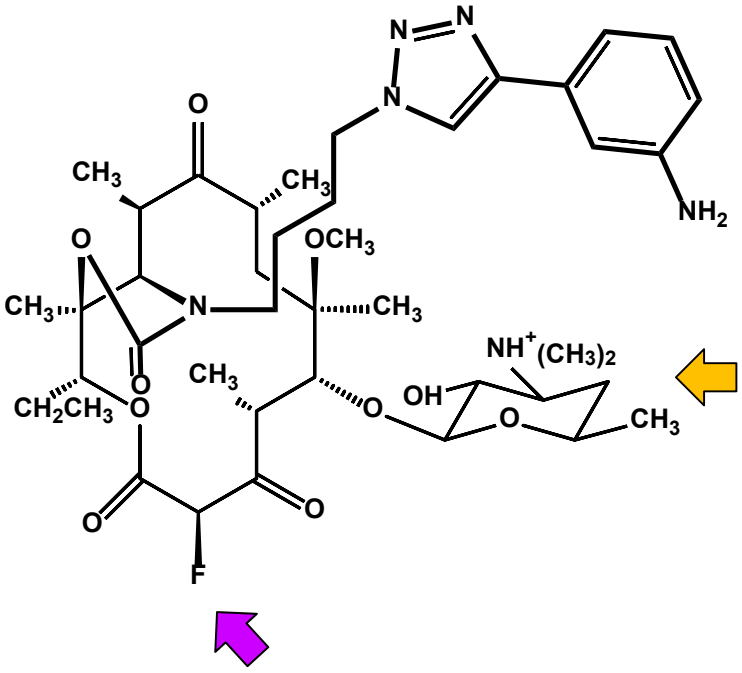
AZM treatment associated with resistance (significant in clonal isolates)

Mustafa et al. Eur. Respir. J. 2017; in press

Efflux and intrinsic / acquired resistance to macrolides

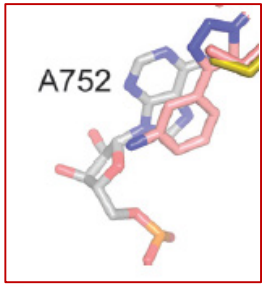
And ketolides ?

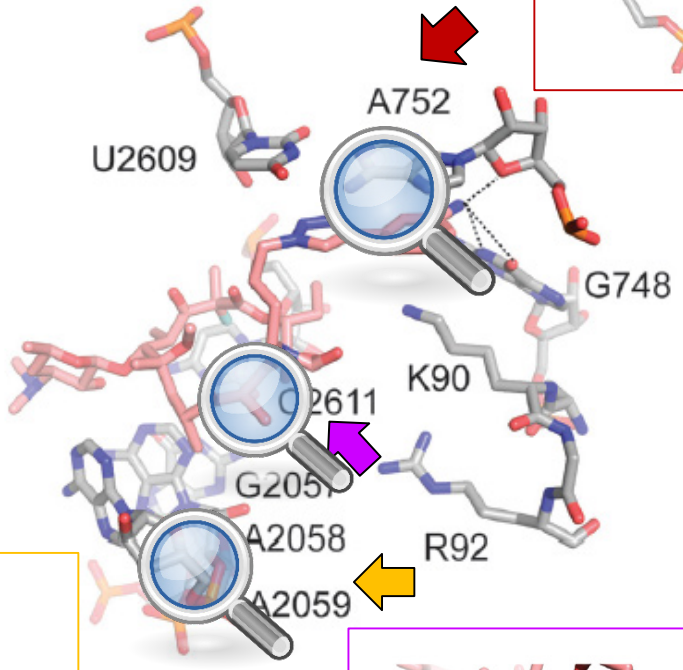
domain II

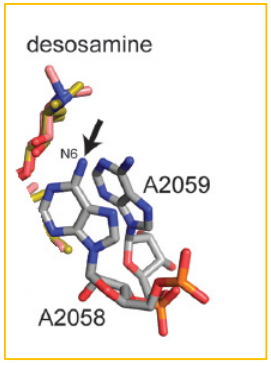


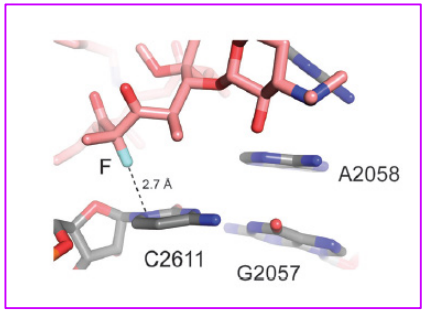
solithromycin

domain V





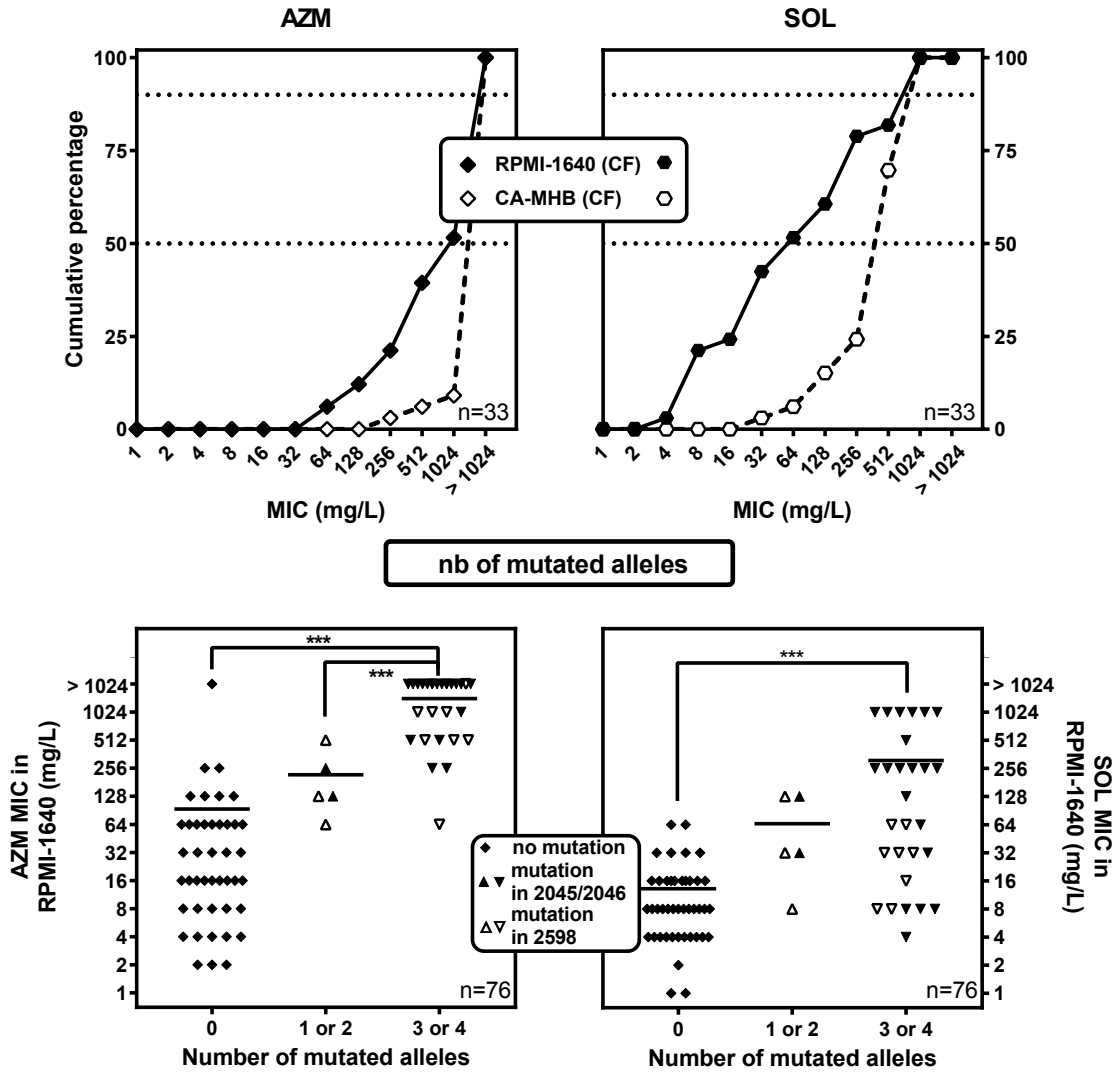




Llano-Sotelo et al. Antimicrob Agents Chemother. 2010;54:4961-70

Efflux and intrinsic / acquired resistance to macrolides

And ketolides ?



Some isolates still resistant to SOL

3-4 alleles mutated in position 2045-2046

Mustafa et al. Eur. Respir. J. 2017; in press

Efflux and intrinsic / acquired resistance to macrolides

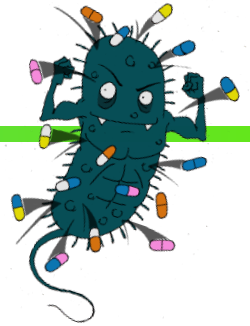
And ketolides ?

Biofilmogenic isolates …

Strain	Mucoidy¶	Solithromycin MIC in RPMI-1640 mg·L^{-1} #				
		Control	+20 mg·L^{-1} PAβN and 1 mM $MgSO_4$	+20 U·mL^{-1} alginate lyase	+0.02% DNase and 20 mM $MgCl_2$	+100 mg·L^{-1} Proteinase K
PAO1	–	*16*	*16*	*16*	*16*	*16*
Mutation: A2045G						
2162	+	256	512	*16*	256	512
2964	+	512	512	512	*32*	512
3066	++	256	256	256	64	*16*
154-1	+	64	64	*8*	64	256
195-2	++	256	*16*	*16*	*32*	>512
CF5	++	>512	>512	256	256	256
BM6	++	256	256	256	256	>512
CF12	+	128	*16*	256	*32*	*32*
Mutation: A2045T						
BF4	++	>512	>512	>512	>512	>512
Mutation: A2046G						
2751	++	128	128	128	*32*	128
2801	++	256	256	256	*16*	>512
127-2	++	>512	>512	>512	>512	>512
134-2	+	256	256	256	*16*	*16*
205	++	>512	>512	>512	>512	>512
CF15	SCV$^+$	>512	>512	>512	*8*	>512
CF45	+	>512	>512	>512	>512	>512
Mutation: C2598T						
196-3	++	64	128	*16*	64	128
116	++	128	64	*16*	128	512
Double mutation: A2046G and C2598G						
129-8	–	64	64	*16*	512	>512

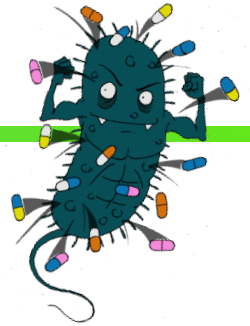
Mustafa et al. Eur. Respir. J. 2017; in press

Efflux and resistance: conclusion (4)



- **Efflux overexpression is selected by antibiotic exposure during therapy**
  - Reversibility ?
  - MDR phenotypes ?
  - Risk of selection of target mutations ? → Higher level of resistance
- **Efflux overexpression can confer high, clinically-significant resistance**
  - Underdiagnosed mechanism
    → Importance of setting-up appropriate diagnostic tests
- **Natural mutations in efflux systems → acquired susceptibility**
  - Temocillin revived against *P. aeruginosa* ? → testing in routine ?
  - Roles of mutations in adaptation to CF lung ?
- **Expression of resistance highly dependent on the environment**
  - Which medium to use in the lab ?
  - Do macrolides express their activity in vivo ?

Efflux and resistance: conclusion (5)



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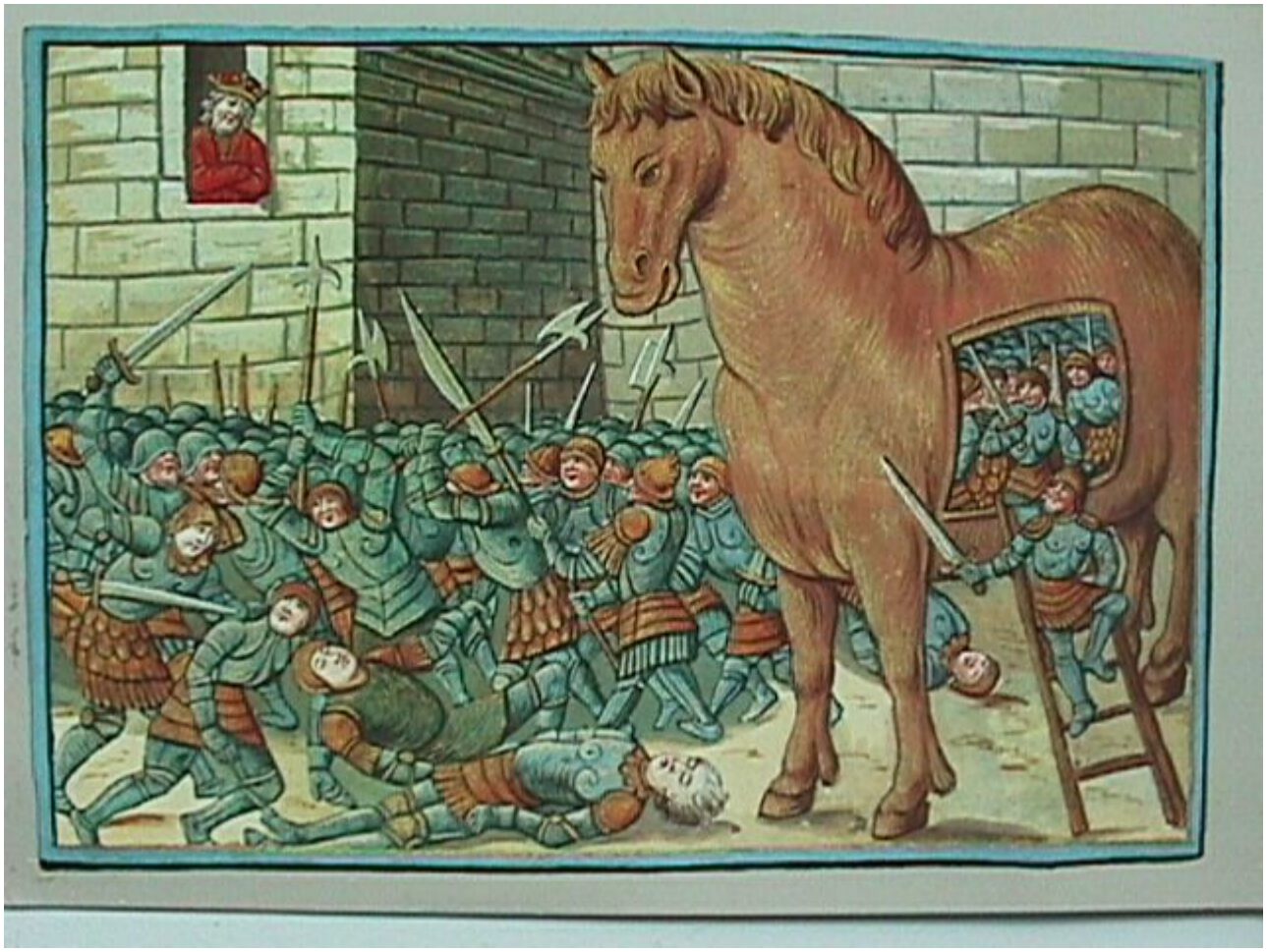
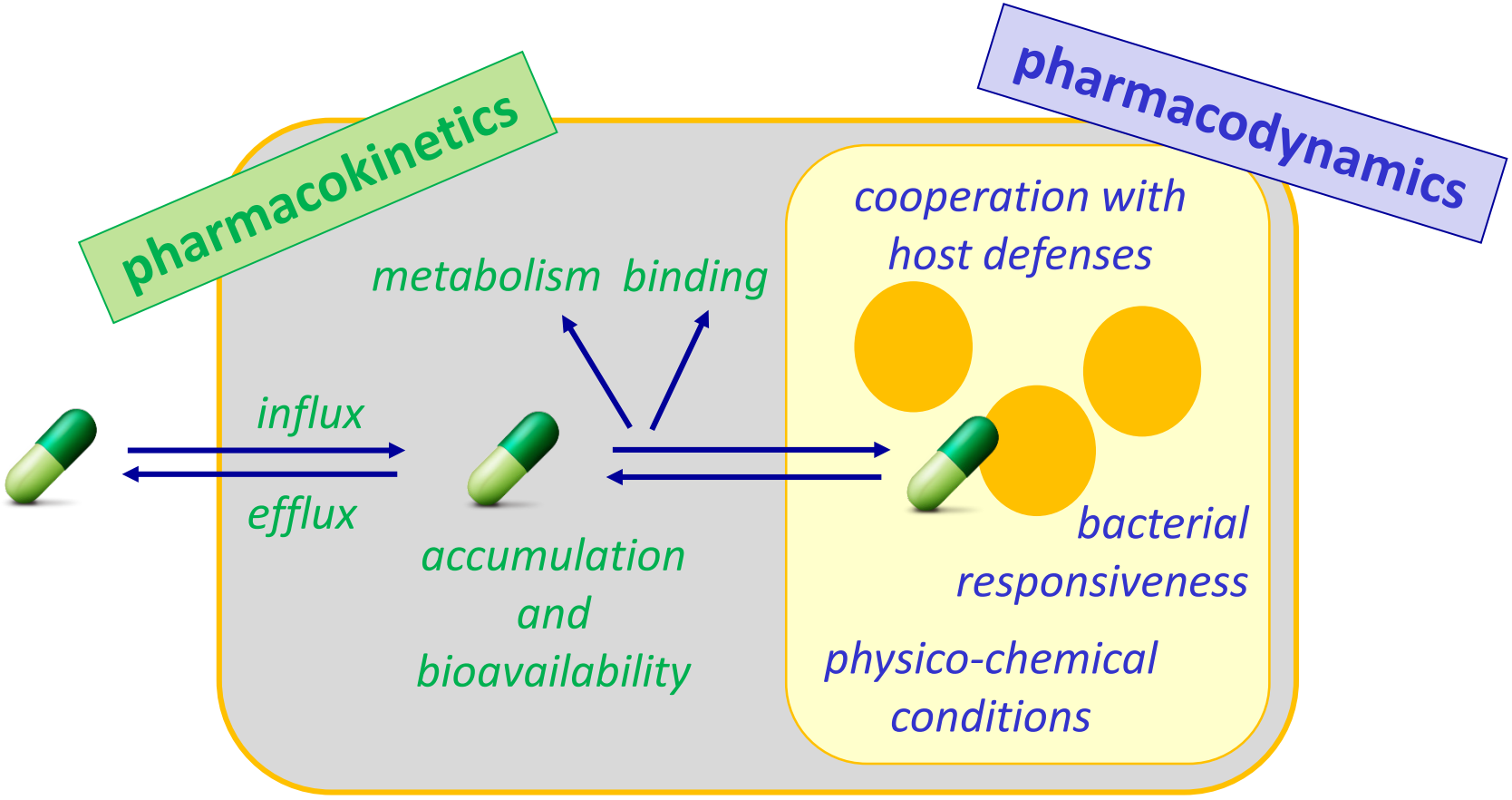
  - Temocillin revived against *P. aeruginosa* ? → testing in routine ?
  - Roles of mutations in adaptation to CF lung ?
- **Expression of resistance highly dependent on the environment**
  - Which medium to use in the lab ?
  - Do macrolides express their activity in vivo ?
- **Long term risk of macrolide resistance in CF isolates ?**
  - Anti-virulence effects depending on protein synthesis ?
  - Ribosome as a lost target for future drugs ?

Persistent infections and tolerance to antibiotics



PK/PD parameters against intracellular infections



Carryn et al, Infect Dis Clin North Am (2003) 17:615-34

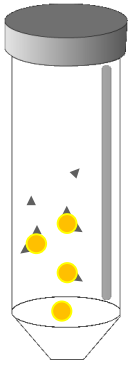
In vitro model of intracellular infection

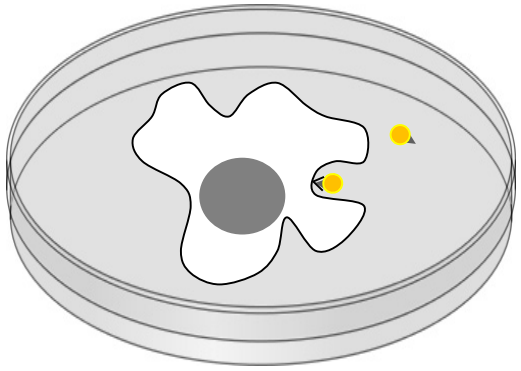
Opsonization
Incubation
with 10 % human serum
in culture medium

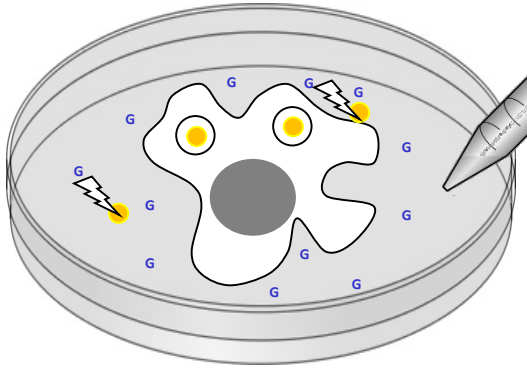
Phagocytosis
Incubation of bacteria with
cells at an appropriate MOI

Extracellular wash
Elimination of non phagocytosed bacteria
by incubation with 50-100 X MIC gentamicin;
elimination of gentamicin by washing

Gentamicin
(G)

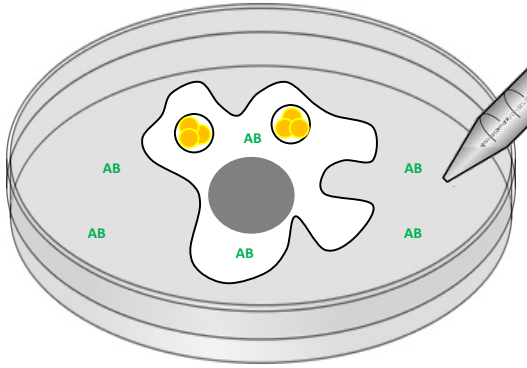






Intracellular infection
Incubation over time
in control conditions or with antibiotics

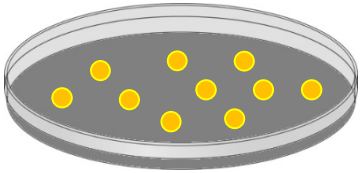
Antibiotic
(AB)

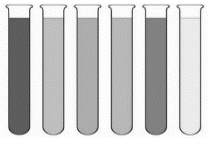


Determination of residual intracellular inoculum
Determination of cfu and protein content in cell lysates

cfu

proteins





Antibiotics against intracellular *P. aeruginosa*

AAC Journals.ASM.org

May 2013 Volume 57 Number 5 p. 2310–2318

Pharmacodynamic Evaluation of the Intracellular Activity of Antibiotics towards *Pseudomonas aeruginosa* PAO1 in a Model of THP-1 Human Monocytes

Julien M. Buyck, Paul M. Tulkens, Françoise Van Bambeke

AAC Journals.ASM.org

January 2015 Volume 59 Number 1

Activities of Antibiotic Combinations against Resistant Strains of *Pseudomonas aeruginosa* in a Model of Infected THP-1 Monocytes

Julien M. Buyck,* Paul M. Tulkens, Françoise Van Bambeke

J Antimicrob Chemother
doi:10.1093/jac/dkw587

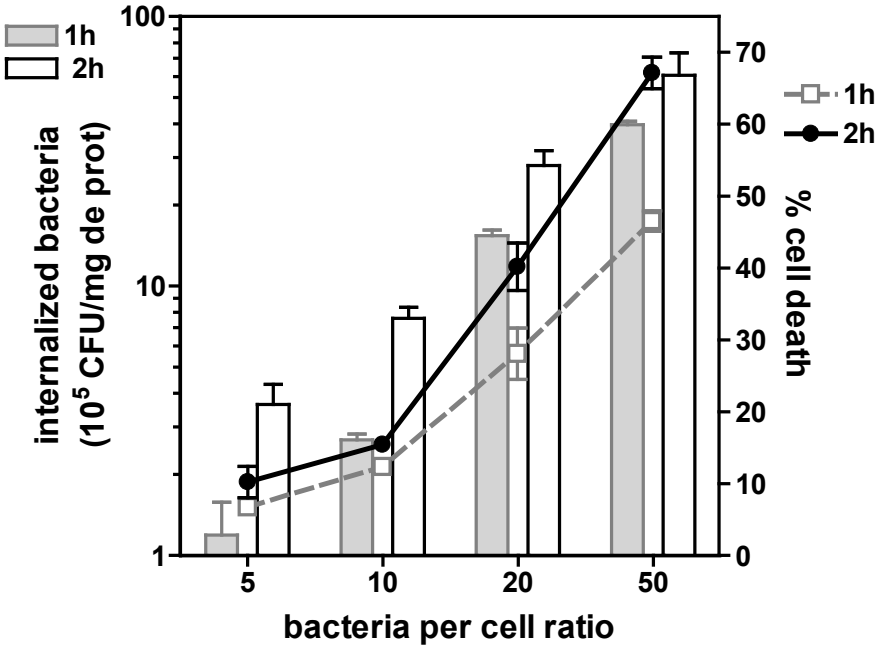
Journal of Antimicrobial Chemotherapy

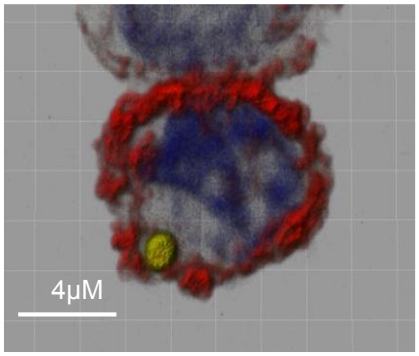
Pharmacodynamics of ceftazidime/avibactam against extracellular and intracellular forms of *Pseudomonas aeruginosa*

J. M. Buyck[1]†, C. Luyckx[1], G. G. Muccioli[2,3], K. M. Krause[4]‡, W. W. Nichols[5], P. M. Tulkens[1] and F. Van Bambeke[1]*

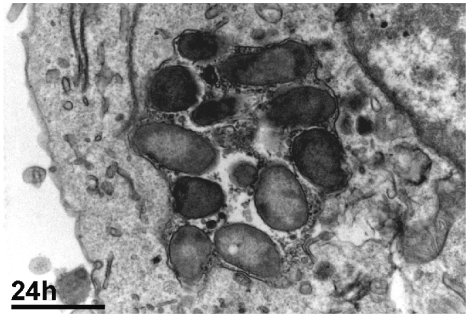
Intracellular infection by *P. aeruginosa*

Setting up the model





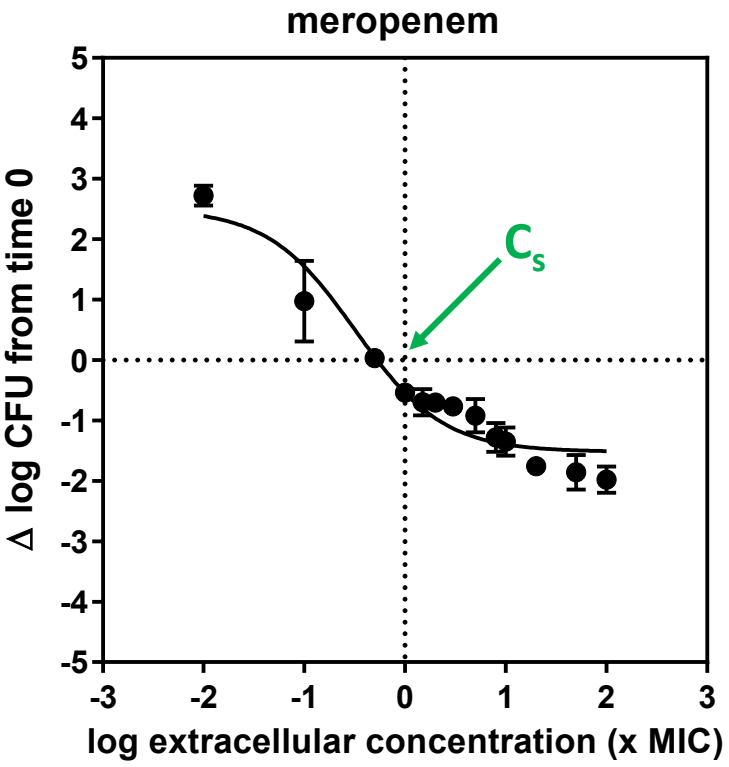
Blue: nucleus
Red: Actin
Green: Bacteria



Buyck et al, AAC 2013; 57:2310-18

A typical pharmacodynamic experiment in vitro …

C_s (static concentration): relative potency



- Estimation of the concentration needed to reach a specified effect
- Measure of the « intracellular MIC »

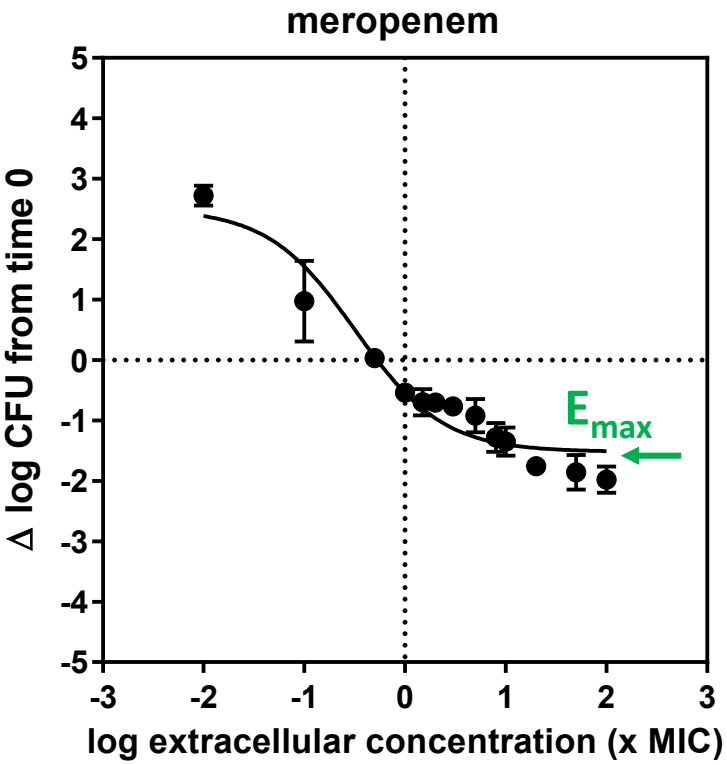
  ⇨ « PK-related » parameter:
  - accumulation in the infected compartment
  - intracellular bioavailability

  ⇨ influence of local environment on intrinsic activity
  - pH
  - oxidant species

Buyck et al, AAC 2013; 57:2310-18

A typical pharmacodynamic experiment in vitro …

E_{max} : maximal efficacy



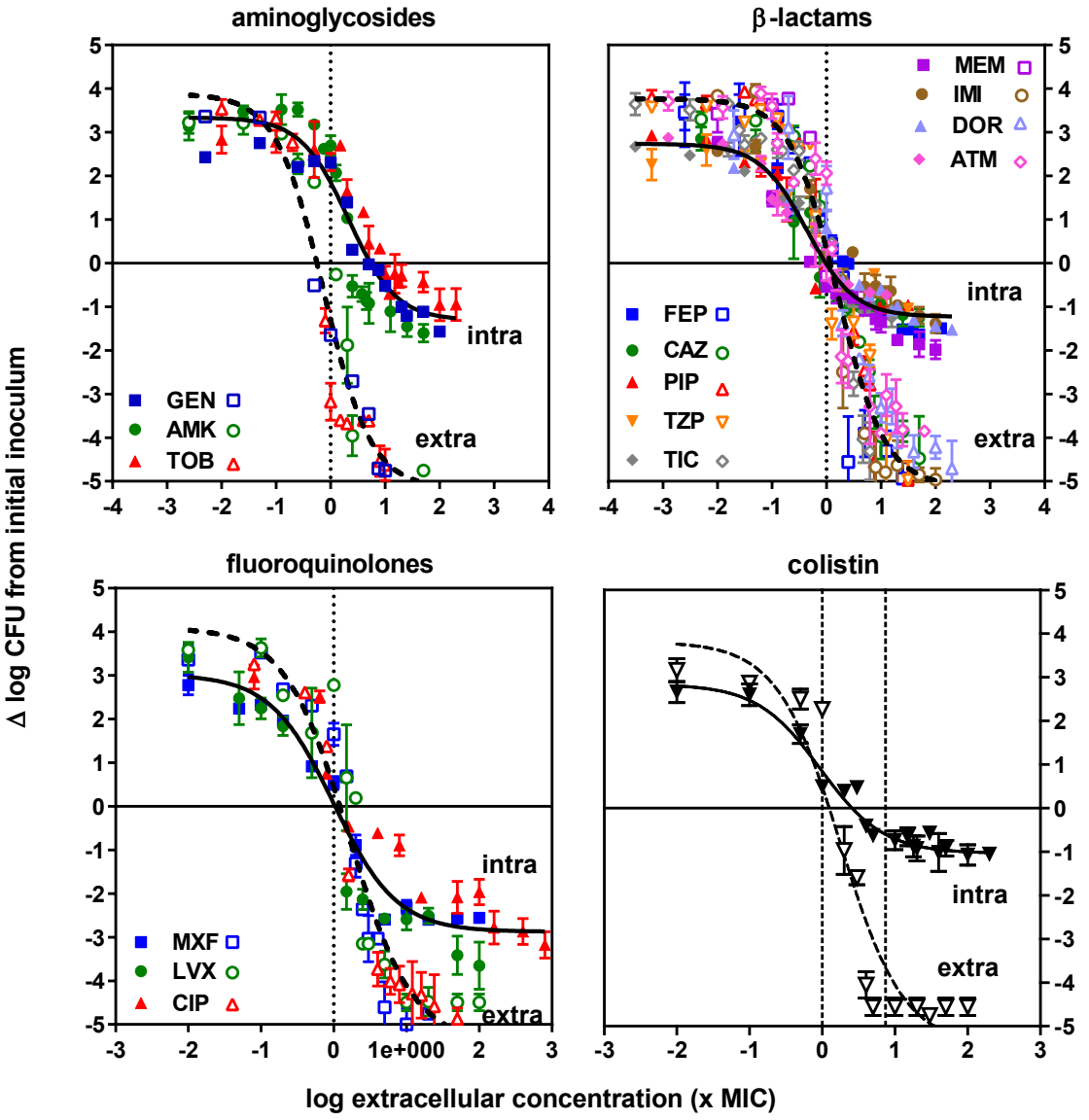
- Estimation of the maximal reduction in inoculum for an infinitely large concentration
- Measure of the killing capacity

⇨ « PD-related » parameter

- mode of action of the drug
- bacterial responsiveness
- cooperation with host defenses

Buyck et al, AAC 2013; 57:2310-18

Activity against extra- and intracellular *P. aeruginosa*

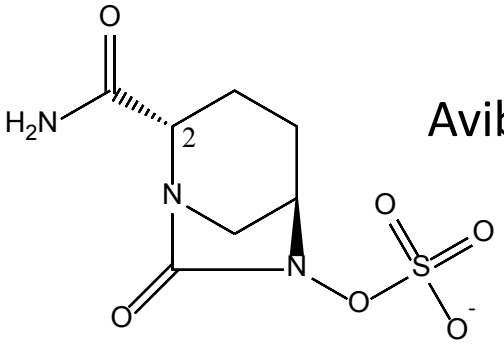


- E_{max} intra << extra
- C_s intra ≥ extra
- FQ more efficient
- All ABS in a single class behave the same

Buyck et al, AAC 2013; 57:2310-18

Playing with C_s : Can we increase intracellular concentration ?

- **Inhibitors of efflux**
- **Inhibitors of antibiotic degradation**

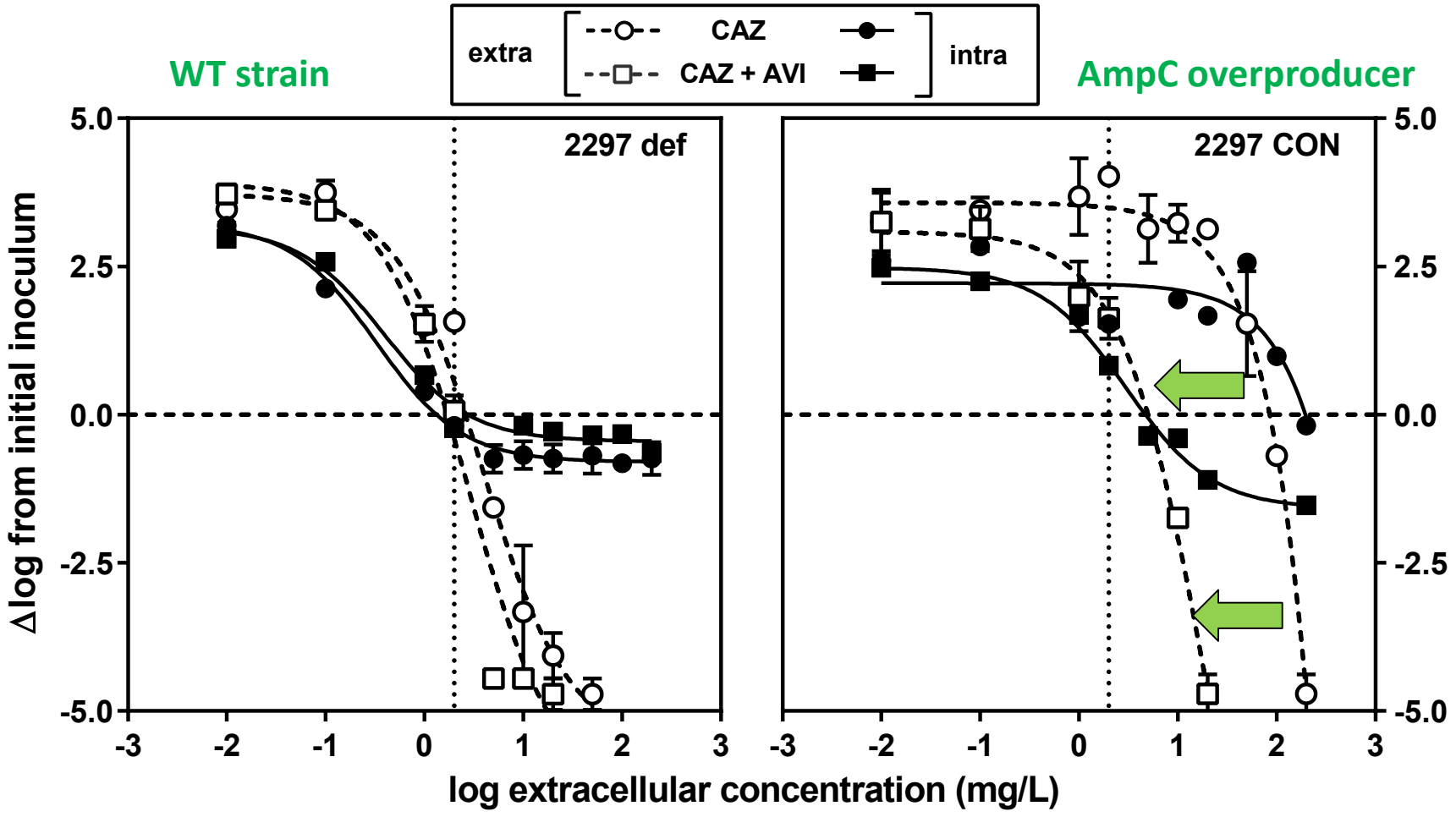


Avibactam, inhibitor of ESBLs including AmpC

Strains	MIC (mg/L)		β-lactamase
	CAZ	CAZ-AVI	
2297 def	2	2	AmpC-negative derivative of 2297
2297 CON	128	8	AmpC-positive derivative of 2297
M1405 def	4	4	AmpC-negative derivative of M1405
M1405 CON	128	8	AmpC-positive derivative of M1405

Buyck et al, JAC 2017 doi: 10.1093/jac/dkw587

Playing with C_s : Can we increase intracellular concentration ?

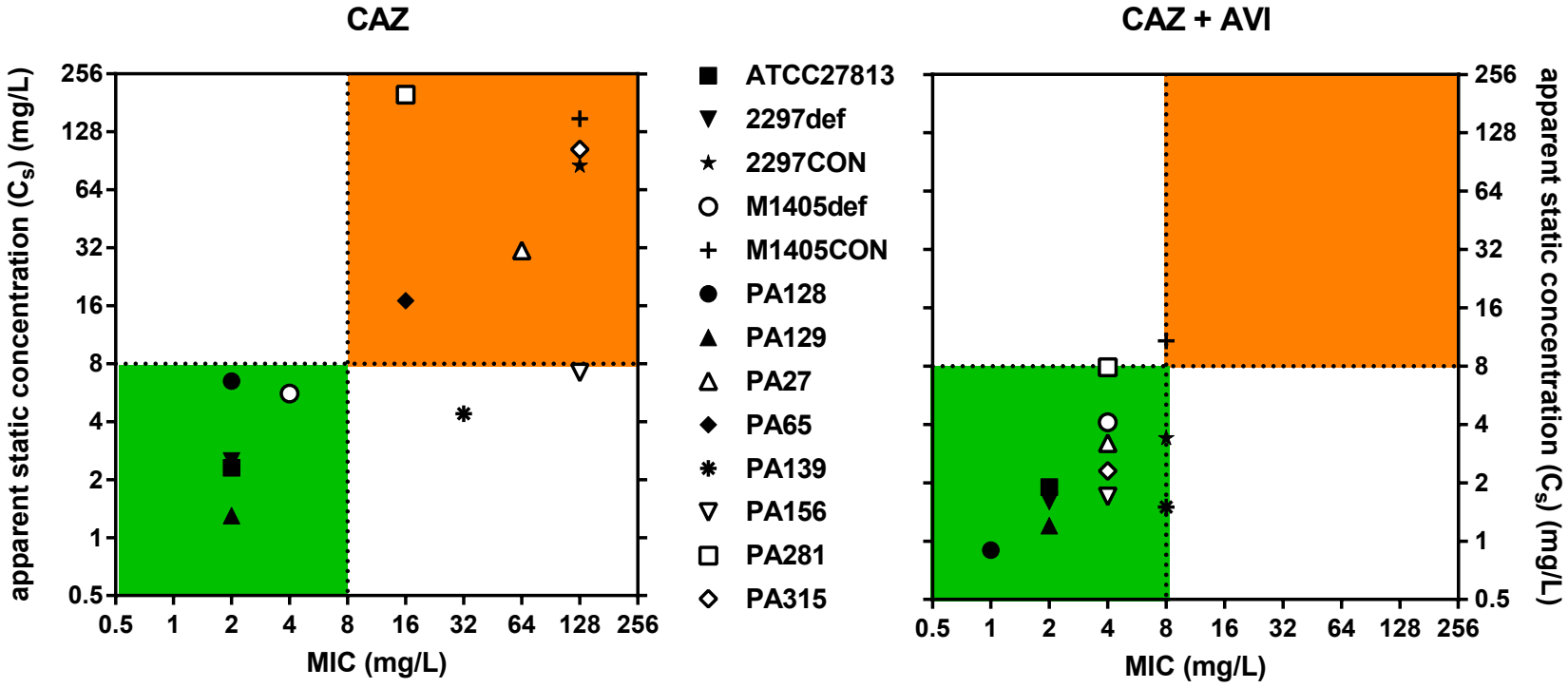


Avibactam makes ceftazidime more potent against both extracellular and intracellular *P. aeruginosa*

Buyck et al, JAC 2017 doi: 10.1093/jac/dkw587

Playing with C_s : Can we increase intracellular concentration ?

C_s vs MIC

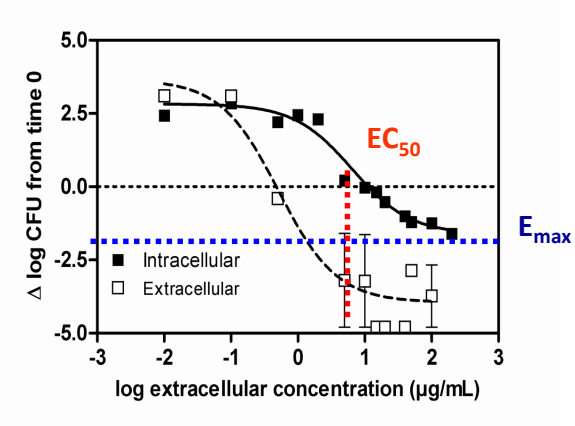


Avibactam reduces ceftazidime MIC and intracellular Cs to the same extend…

Buyck et al, JAC 2017 doi: 10.1093/jac/dkw587

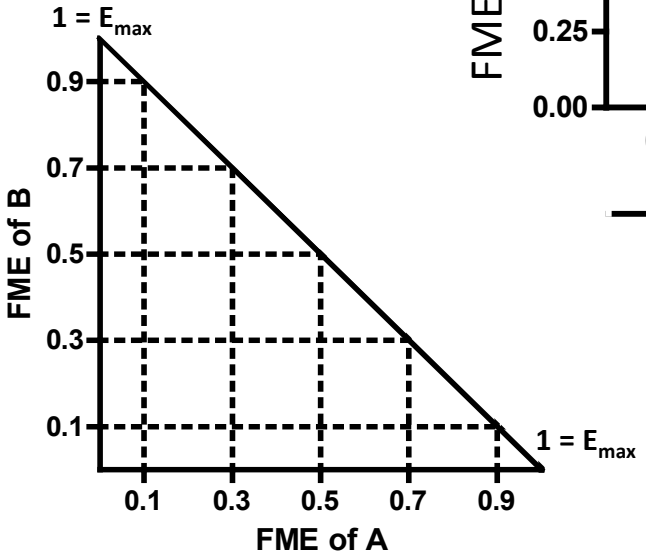
Playing with E_{max}: Can we do better with combinations ?

Fractional Maximal Effect method (FME)

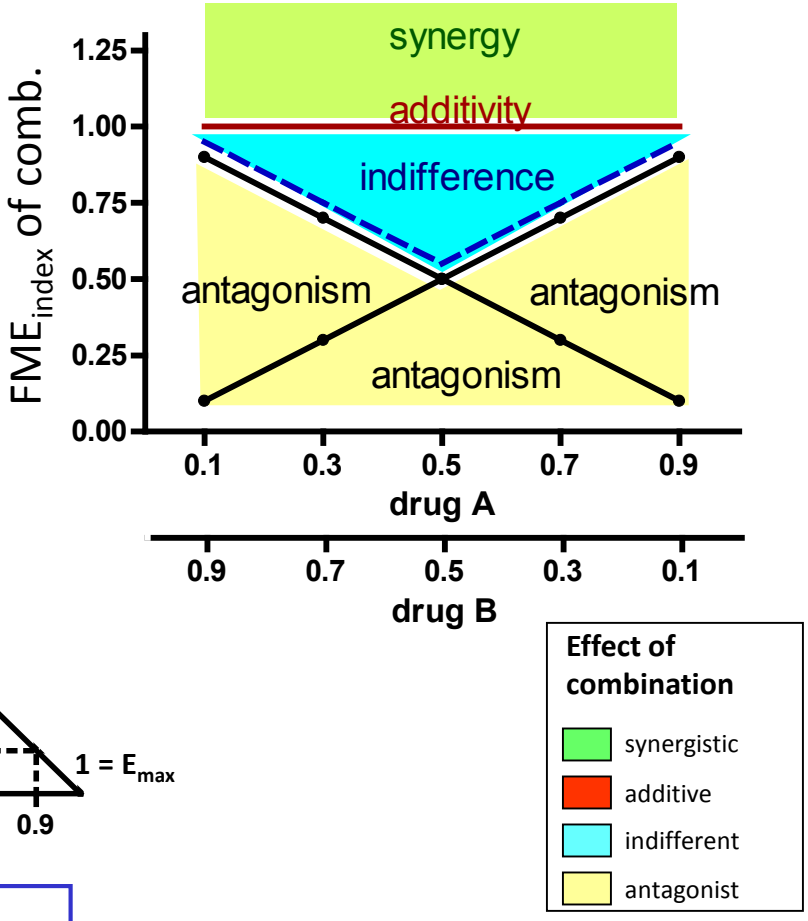


$$C_A = \frac{FME_A.EC_{50A}}{1-FME_A}$$

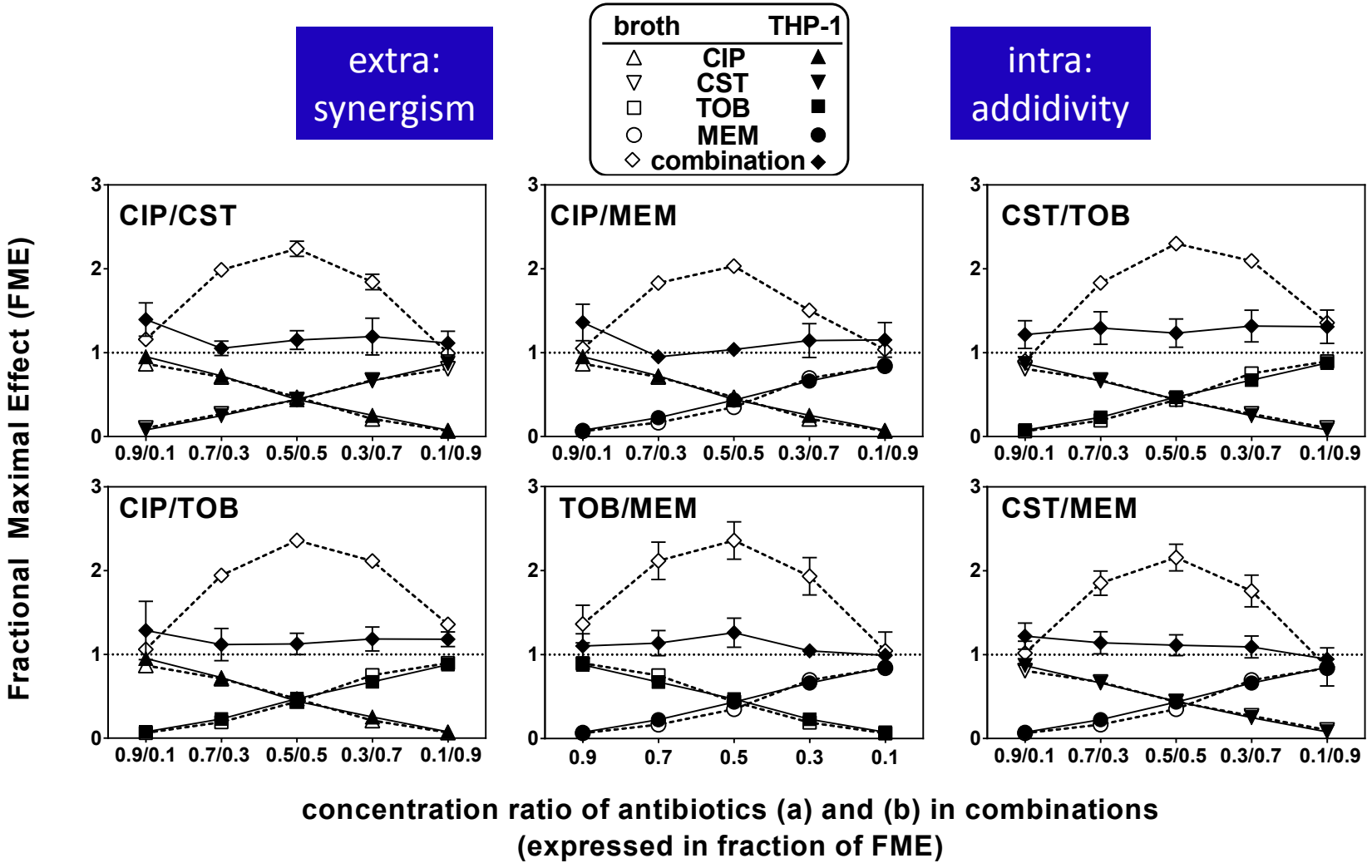
$$C_B = \frac{FME_B.EC_{50B}}{1-FME_B}$$



$FME_{comb.} = FME_A + FME_B = 1$

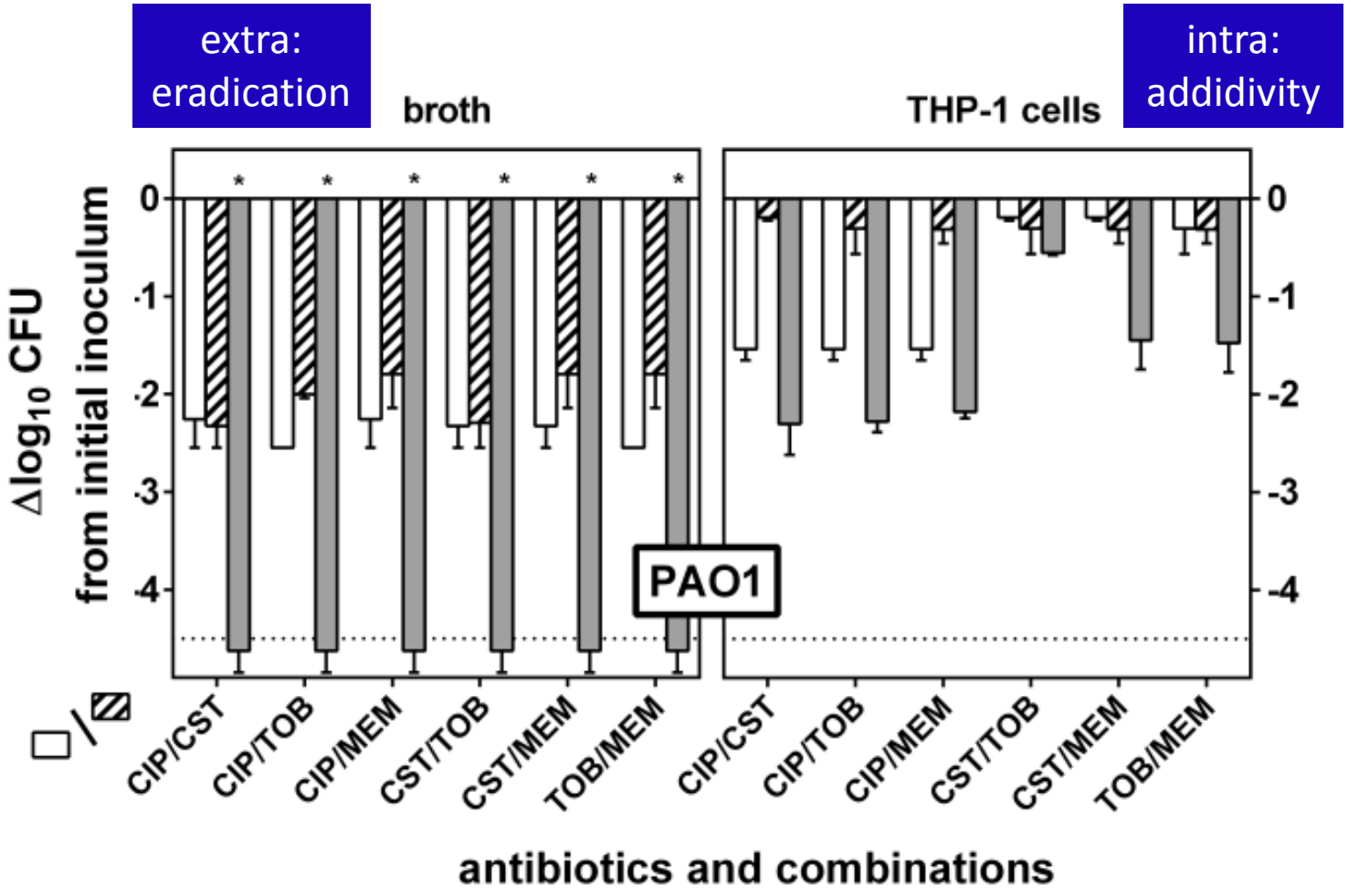


Playing with E_{max}: Can we do better with combinations ?



Buyck et al, AAC 2015; 59:258-268

Playing with E_{max}: Can we do better with combinations ?



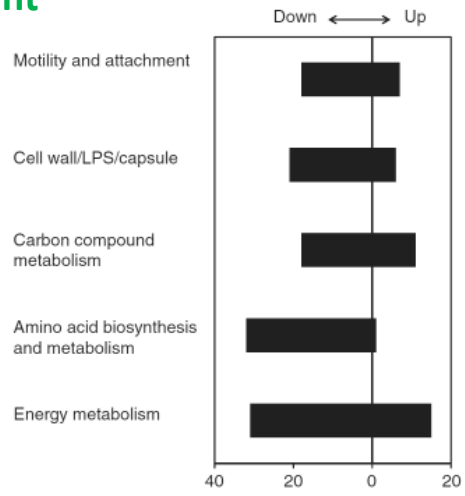
Buyck et al, AAC 2015; 59:258-268

Playing with E_{max}: importance of bacterial responsiveness

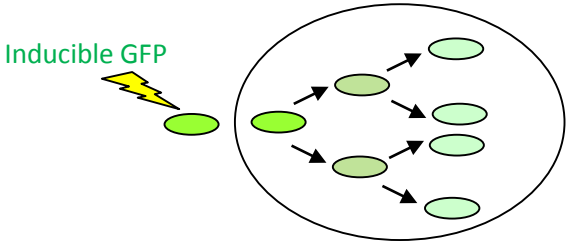
DnpA, a putative de-*N*-acetylase; no substrate identified

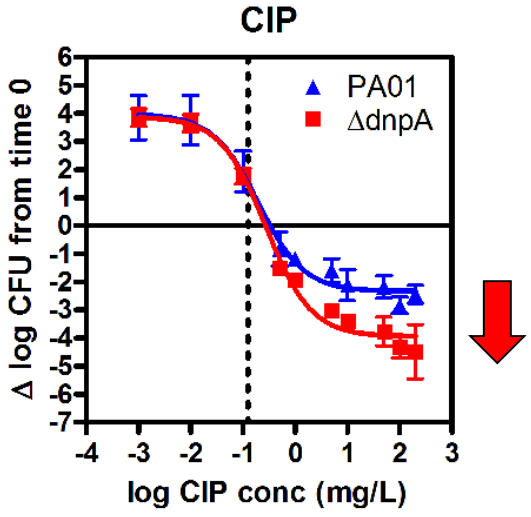
Gene expression in *ΔdnpA* mutant

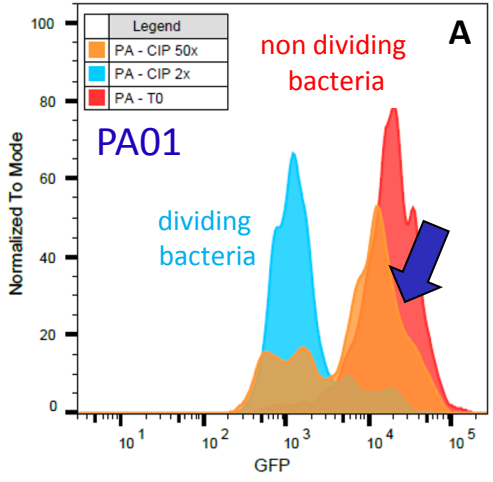
Liebens et al, Path Dis (2014), 71, 39–54

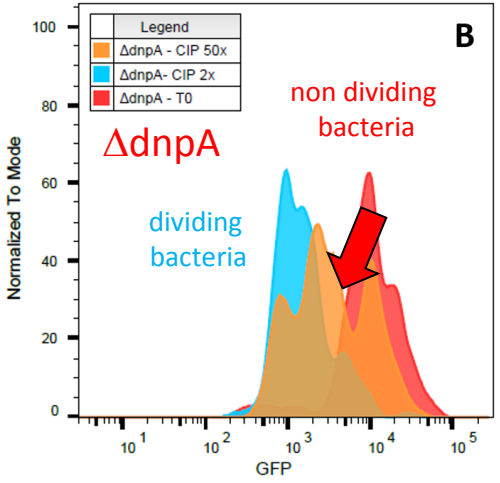


- FQ more effective on the *dnpA* mutant
- Intracellular *dnpA* mutants still dividing

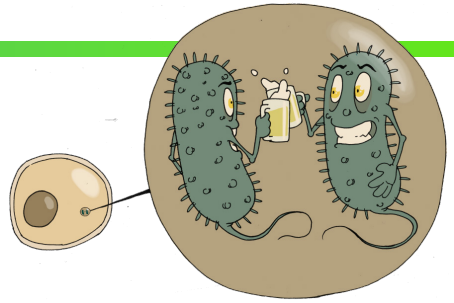








Intracellular infection & tolerance: conclusions

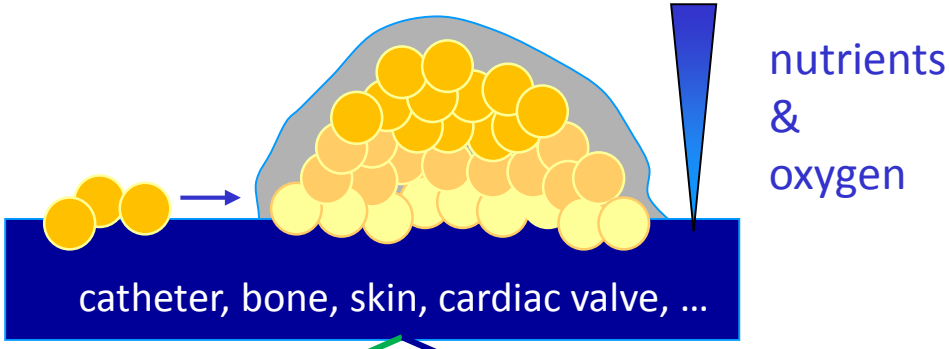


- **Relative potency (Cs) ~ MIC**
  - No influence of drug accumulation
    → intracellular bioavailability
  - Within a drug class, proportional to cellular concentration

- **Intracellular efficacy << extracellular efficacy**
  - Intracellular expression of activity of antibiotics ?
  - Intracellular responsiveness of bacteria ?

- **Innovative strategies needed !**

PK/PD parameters and activity against biofilms



pharmacokinetics

- diffusibility through the matrix
- bioavailability within the biofilm
- access to bacteria
- efflux out of bacteria

pharmacodynamics

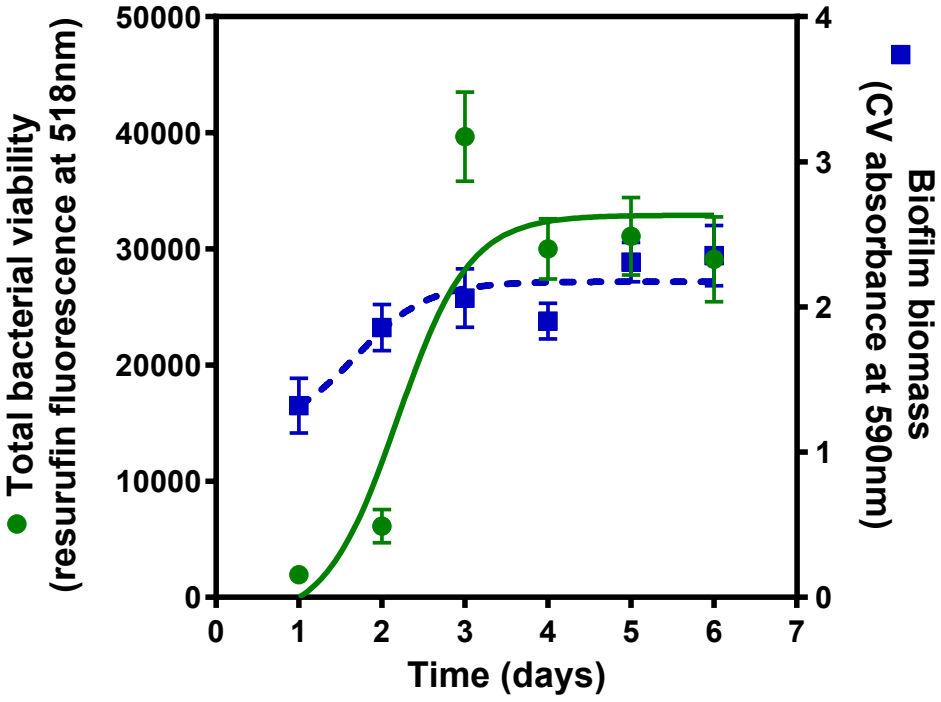
- bacterial responsiveness (metabolic activity of bacteria)
- antibiotic expression of activity (local environment [O_2, pH, ..])

In vitro biofilm model with *P. aeruginosa*

Setting up the model

Mucin
DNA
Lecithin source
Iron chelator
Salts
Amino acids

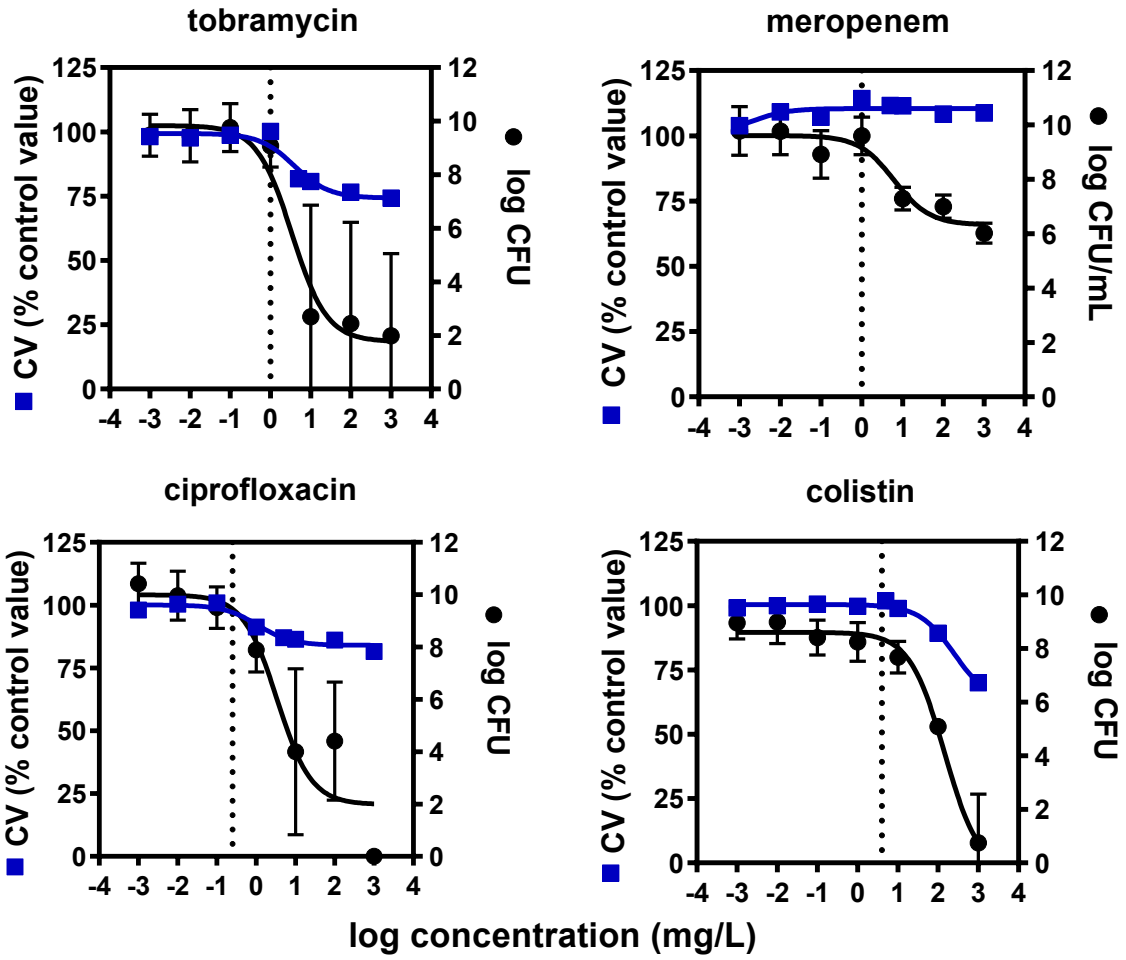
Protocol Exchange DOI:10.1038/protex.2010.212



Maturity reached after 4 days of culture

Mustafa et al, in preparation

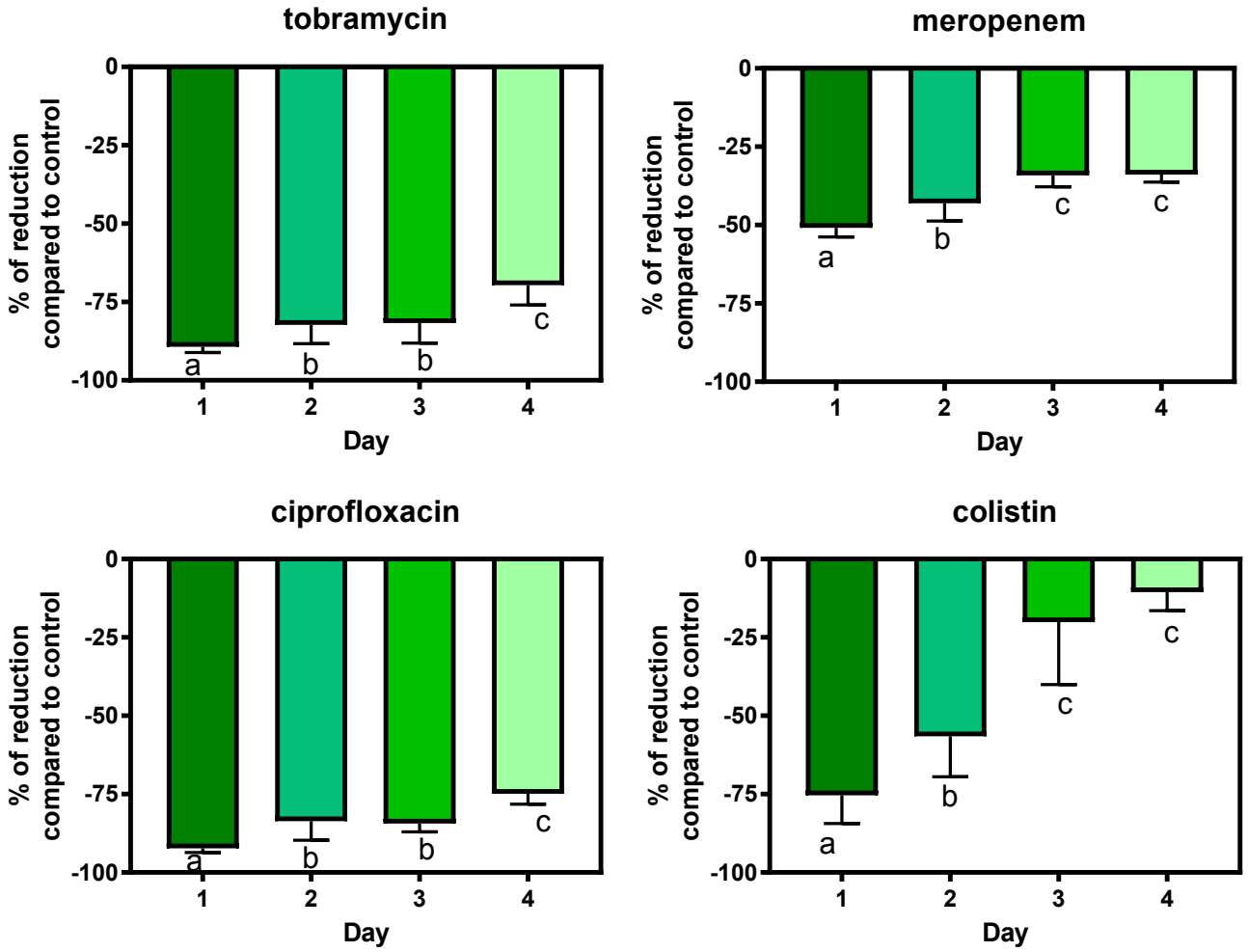
Activity against *P. aeruginosa* in biofilms



No activity against biomass;
Limited activity against viability; β-lactams less effective

Mustafa et al, in preparation

Influence of biofilm maturity



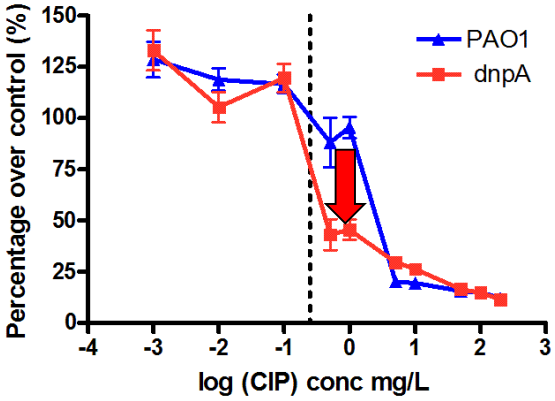
Efficacy decreases as biofilm matures (barrier effect)

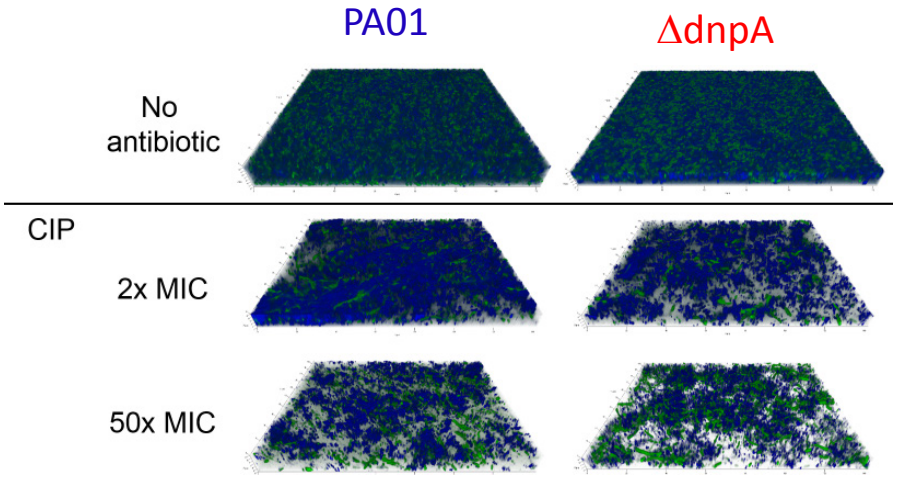
Mustafa et al, in preparation

Playing with E_{max}: importance of bacterial responsiveness

DnpA, a putative de-*N*-acetylase; no substrate identified

Gene expression in *ΔdnpA* mutant

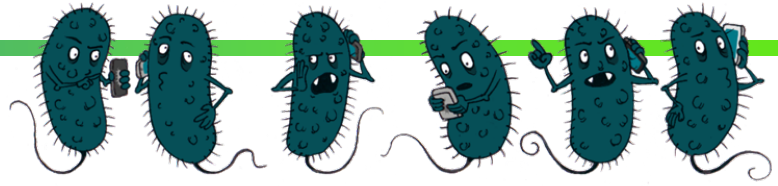




CFW (polysaccharides)/ GFP (bacteria)

FQ effective at lower conc. on the *dnpA* mutant

Biofilms & tolerance: conclusions



- **Relative potency (Cs)**
  - Activity at concentrations > MIC
    → bioavailability within the biofilm

- **Efficacy (Emax) reduced**
  - Expression of activity of antibiotics ?
  - Responsiveness of bacteria ?

- **Innovative strategies needed !**

Take home message: No ESKAPE ?

Resistance ?

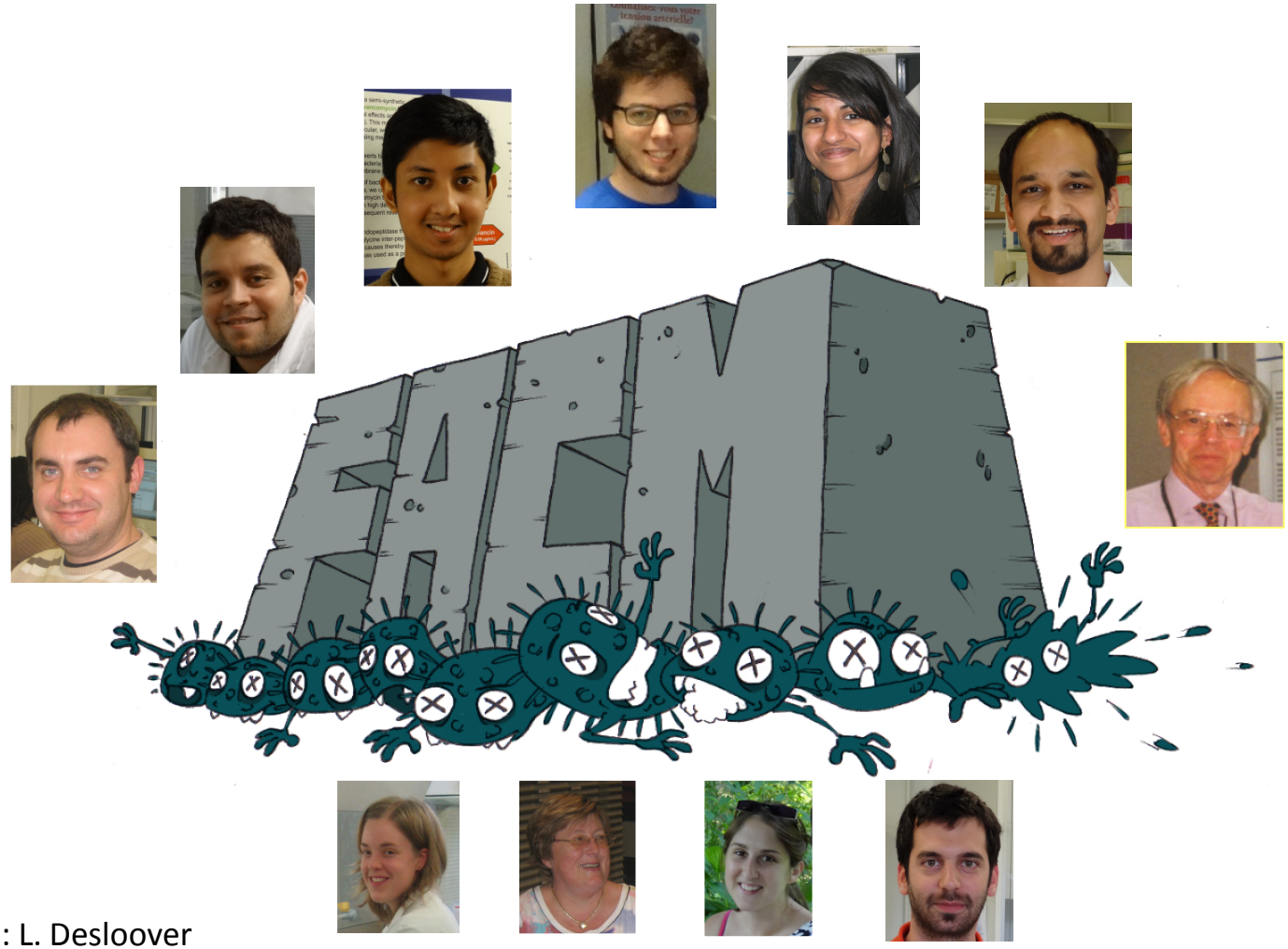
- ↘ antibiotic potency
- Can be overcome (to some extend) by ↗ concentrations

Take home message: No ESKAPE ?

Tolerance ?

- ↘ antibiotic efficacy
- Need of new strategies targeting bacterial responsiveness

Acknowledgements



Cartoons: L. Desloover