Differential recognition of quinolones by efflux pumps in Gram-positive (*Staphylococcus aureus*, *Streptococcus pneumoniae*) and Gram-negative (*Pseudomonas aeruginosa*) bacteria.

C. Vallet, J. Buyck, F. El Garch, P.M. Tulkens, F. Van Bambeke

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

Mailing address : F. Van Bambeke, UCL 73.70, av. E. Mounier, 73, 1200 Brussels, Belgium, francoise.vanbambeke@uclouvain.be

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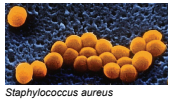

Staphylococcus aureus

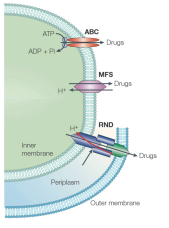

Streptococcus pneumoniae


Pseudomonas aeruginosa

INTRODUCTION AND AIM

Fluoroquinolones are widely used antibiotics, showing favorable properties such as broad spectrum, intense bactericidal activity and excellent bioavailability. Active efflux, however, may confer resistance to quinolones in both Gram (+) and Gram (-) bacteria. The drug structural features determining the recognition by transporters remain largely unknown.

The aim of this study was to examine to what extent typical representatives of superfamilies of transporters present in 3 important pathogens affect the activity of quinolones, namely



Adapted from Krulwich et al., Nature Rev Microbiol 2005; 3: 566-572

- **PatA/B** (ATP Binding Cassette [ABC] superfamily) and **PmrA**, (Major Facilitator Superfamily [MFS]) in *Streptococcus pneumoniae*
- **NorA** (MFS) in *Staphylococcus aureus*
- **MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexJK-OprM, and MexXY-OprM** (resistance-nodulation-cell-division superfamily [RND]) in *Pseudomonas aeruginosa)*

MATERIALS AND METHODS

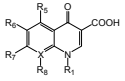
Bacterial strains:

- *Staphylococcus aureus* (*S.a.*) : ATCC25923, and its derivative SA-1 overexpressing the efflux pump NorA.[1]
- *Streptococcus pneumoniae* (*S.p.*) : SP335, which overexpresses PatA/PatB and PmrA, and its 3 derivatives where *patA*, *patB* or *pmrA* were inactivated.[3]
- *Pseudomonas aeruginosa* (*P.a.*) : PAO1 with basal expression of efflux systems and its mutidisruptant derivative PAO509 (PAO1 Δ(*mexAB-oprM*) Δ(*mexCD-oprJ*) Δ(*mexEF-oprN*) Δ(*mexJK*) Δ (*mexXY*)).[2]

MIC: microdilution method, using CLSI recommendations, using Mueller-Hinton broth for *S.a.*, Mueller-Hinton broth cation adjusted (MHBCa) supplemented with 5% lysis horse blood for *S.p.*, and MHBCa for *P.a.*

Quinolones antibiotics: 14 quinolones with structures shown in the Table.

NOR, ENX and PEF were purchased from Sigma-Aldrich. The other molecules were received from their manufacturers: CIP and MXF from Bayer HealthCare AG (Leverkusen, Germany), DIF and SAR, from Abbott Laboratories (Abbott Park, Illinois), LOM from G.D. Searle and Co., FIN from MerLion Pharmaceuticals GmbH (Berlin, Germany), GAR from Bristol-Myers Squibb (New Brunswick, CT), SPX from Rhône-Poulenc Rorer (Antony, France), GMF from LG Life Sciences Ltd (Seoul, Korea), LVX from Aventis Pharma (Antony, France), and MAR from Vetoquinol (Lure, France).



Quinolones	X	R_8	R_1	R_5	R_6	R_7
Ciprofloxacin **(CIP)**	C	H		H	F	
Norfloxacin **(NOR)**	C	H	CH_2-CH_3	H	F	
Enoxacin **(ENX)**	N	-	CH_2-CH_3	H	F	
Pefloxacin **(PEF)**	C	H	CH_2-CH_3	H	F	
Lomefloxacin **(LOM)**	C	F	CH_2-CH_3	H	F	
Moxifloxacin **(MXF)**	C	O-CH_3		H	F	
Finafloxacin **(FIN)**	C	CN		H	F	
Garenoxacin **(GAR)**	C	O-CHF_2		H	H	
Sparfloxacin **(SPX)**	C	F		NH_2	F	
Gemifloxacin **(GMF)**	N	-		H	F	
Difloxacin **(DIF)**	C	H		H	F	
Sarafloxacin **(SAR)**	C	H		H	F	
Levofloxacin **(LVX)**				H	F	
Marbofloxacin **(MAR)**				H	F	

RESULTS

A. MICs of 14 quinolones on *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*

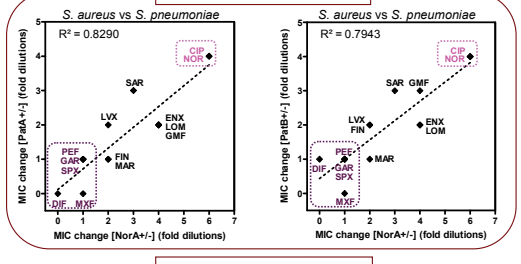
quinolones	*Staphylococcus aureus*				*Streptococcus pneumoniae*					*Pseudomonas aeruginosa*	
	ATCC25923 (WT)		SA-1 (+ NorA)		SP335					PAO1 (WT)	PAO509 (ΔMex-Opr)
	-R	+R	-R	+R	-R	+R	ΔPatA	ΔPatB	ΔPmrA		
CIP	**0.25**	**0.06**	**4**	**0.5**	**16**	**1**	**1**	**1**	16	**0.125-0.25**	**0.008**
NOR	**0.5**	**0.25**	**16**	**2**	**64**	**4**	**4**	**4**	64	**0.5**	**0.03-0.06**
ENX	0.5-1	0.5	8	2	64	16	16	16	64	**1**	**0.03**
PEF	**0.25-0.5**	**0.25**	**0.5**	**0.5**	**16**	**16**	**8**	**8**	16	**2**	**0.03**
LOM	0.5	0.25	4	2	32	8	8	8	32	**1**	**0.03**
MXF	**0.03**	**0.03**	**0.06**	**0.03-0.06**	**0.25**	**0.25**	**0.25**	**0.25**	0.25	**2**	**0.03**
FIN	0.125	0.125	0.5	0.25	4	2	2	1	8	**4**	**0.06**
GAR	**0.016-0.03**	**0.016**	**0.03**	**0.03**	**0.06**	**0.03**	**0.03**	**0.03**	0.06	**2**	**0.03**
SPX	**0.06**	**0.06**	**0.125**	**0.06**	**0.5**	**1**	**0.25**	**0.25**	0.5	**1**	**0.008-0.016**
GMF	0.016	0.008	0.125	0.03	0.125	0.03	0.03	0.016	0.125	**0.25**	**0.016**
DIF	**0.125-0.25**	**0.25**	**0.25-0.5**	**0.25**	**4**	**4**	**4**	**2**	4	**2**	**0.03**
SAR	0.125	0.125	1	0.25	8	1	1	1	8	**0.25**	**0.016**
LVX	0.125	0.125	0.5	0.25	4	1	1	1	4	**0.5**	**0.016**
MAR	0.25	0.25	1	0.5-1	4	2	2	2	4	**0.5**	**0.016**

Legend :

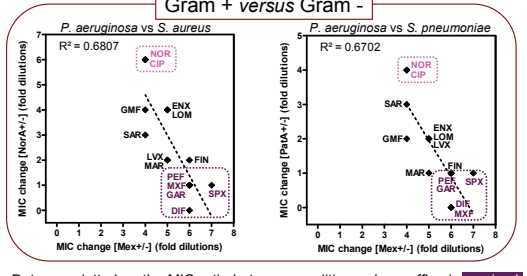
- +R / -R : + or without reserpine (10 mg/L; inhibitor of Gram (+) efflux pumps).
- Δ : strains disrupted for PatA, or PatB, or PmrA, or the Mex-Opr efflux system (=MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexJK-OprM, and MexXY-OprM).
- MICs on a pink background : conditions where efflux is minimal (lowest MICs)
- MICs on a purple background: conditions where efflux is maximal (highest MICs)

B. Correlations

Gram + *versus* Gram +



Gram + *versus* Gram -



Data are plotted as the MIC ratio between conditions where efflux is maximal and efflux is minimal for each strain.

CONCLUSIONS

- There is a high degree of correlation between efflux mediated by NorA (MFS) in *S.a.* and by PatA or PatB (ABC) in *S.p.* In contrast, PmrA (MFS) has no influence on MICs in *S.p.*

  This suggests that the same molecular determinants govern recognition by efflux systems in Gram-positive bacteria, whatever their phylogenic classification.

- On the contrary, all quinolones are substrates for the RND efflux systems in *P.a.*, which makes correlations between NorA or PatA/B and Mex largely irrelevant.
- CIP and NOR are affected by efflux pumps in all three bacteria tested, whereas DIF, PEF, GAR, SPX and MXF are only substrates of the broad spectrum efflux systems of the Gram-negative bacterium.

REFERENCES

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