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Respective contribution of PatA/PatB and PmrA in fluoroquinolone resistance in clinical isolates of *Streptococcus pneumoniae*

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Background

Two efflux systems have been identified so far for fluoroquinolones (FQ) in *Streptococcus pneumoniae* (SP), namely PmrA (MFS superfamily),[1] and PatA / PatB (ABC transporters superfamily).[2] Previous studies have suggested a predominant role of PatA/PatB in FQ resistance of laboratory strains.[3,4] The aim of the present study was to determine which of these two systems could be primarily involved in the resistance of clinical SP isolates to FQ.

Methods

Clinical strains showing a phenotype suggestive of efflux were selected from a large collection of SP isolates obtained from CAP patients, and compared to ATCC49619 and to PatA/PatB-positive controls. MICs were measured in Mueller Hinton II agar supplemented with 5% defibrinated horse blood (± 20mg/L reserpine [efflux inhibitor]).

Expression levels of *patA, patB,* and *pmrA* were evaluated by real-time PCR. Gene inactivation was obtained by transformation using genomic DNA of ATCC49619 disrupted in one of the genes under study (spectinomycin resistance cassette inserted in the middle of the gene of interest).

Statistical analysis was made using Friedman test (non-parametric paired one-way ANOVA) and Dunn's post-test of selected pairs on strains showing efflux (control & clinical).

A copy of this poster will be made available after the meeting at *http://www.facm.ucl.ac.be/posters.htm*

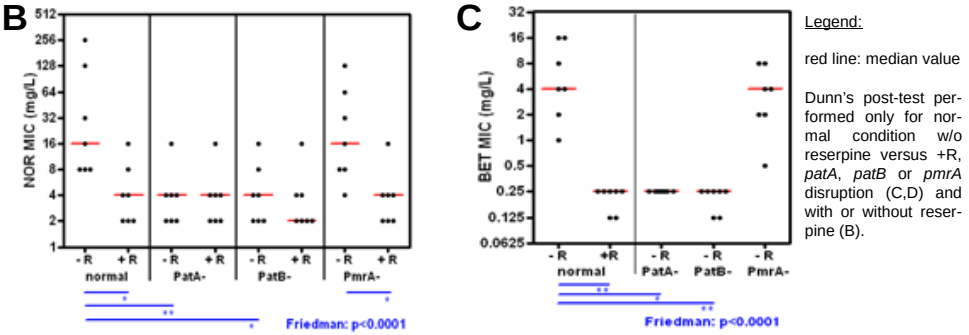
A

MICs of CIP and NOR for each strain measured without or with reserpine and in disruptants for *patA*, *patB*, or *pmrA*

strains	gene expression[a]			CIP MIC (mg/L)[b,c]					NOR MIC (mg/L)[b,c]				
	patA	*patB*	*pmrA*	w/o R	with R	*patA*-	*patB*-	*pmrA*-	w/o R	with R	*patA*-	*patB*-	*pmrA*-
ATCC 49619	basal	basal	basal	0.5	0.5	**0.5**	**0.5**	**0.5**	4	2	**4**	**2**	**4**
SP13	+	+	+	16	4	**2**	**2**	16	256	16	**16**	**16**	128
SP207	+	+	+	4	0.5	1	**0.5**	-[d]	32	2	**4**	**2**	no[d]
SP295	+	+	basal	2	0.5	**0.5**	**0.5**	1	16	2	**2**	**2**	8
SP257	basal	basal	+	1	0.5	**0.5**	**0.5**	1	8	2	**2**	**2**	**4**
SP298	basal	basal	+	2	0.5	**0.5**	**1**	2	8	2	**4**	**4**	16

[a] + denotes a value > 2-fold that measured in ATCC49619 (basal level)
[b] in control conditions (without reserpine ([w/o R]); + reserpine 20 mg/L [with R]; in strain disrupted for *patA* [*patA*-], *patB* [*patB*-], or *pmrA* ([*pmrA*-])
[c] figures in bold denote MICs brought back to those measured with reserpine (+/- 1 dilution)
[d] disruptant non obtained so far

NOR (B) and BET (C) MICs for strains showing efflux (3 ctrl + 4 clinical isolates) and their respective disruptants (for *patA*, *patB* or *pmrA*) measured without (- R) or with (+ R) reserpine



Legend:

red line: median value

Dunn's post-test performed only for normal condition w/o reserpine versus +R, *patA*, *patB* or *pmrA* disruption (C,D) and with or without reserpine (B).

D

	Friedman	Dunn's post-test			
		+R	patA-	patB-	prmA-
CIP	p<0.0001	p<0.01	p<0.01	p<0.01	NS
NOR	p<0.0001	p<0.01	p<0.01	p<0.01	NS
LVX	p=0.0163	p<0.05	NS	NS	NS
MXF	p=0.0724				
GEM	p=0.0032	NS	NS	NS	NS
ACR	p<0.0001	p<0.05	p<0.01	p<0.01	NS
BET	p<0.0001	p<0.01	p<0.05	p<0.01	NS

Statistical analysis: Friedman's test & Dunn's post-test (only comparison to normal MICs w/o reserpine) for graph C (BET tested) and other FQ or substrate of the pumps.

CIP= ciprofloxacin, NOR= norfloxacin, LVX= levofloxacin, MXF= moxifloxacin, GEM= gemifloxacin, ACR= acriflavin, BET= ethidium bromide, NS= non significant.

Results

A. - Reserpine or gene disruption had no effect in ATCC49619.
- Disruption of *patA* or *patB* was as effective as reserpine to decrease CIP and NOR MICs in clinical isolates, irrespective of the gene(s) overexpressed (SP13 did not revert to wild-type MIC because of the presence of target mutations).
- Disruption of *pmrA* had only a modest effect on NOR MIC in SP257.

B. For strains showing efflux of CIP and NOR (discounting SP207 [no *pmrA* disruptant]) and disrupted for *patA* or *patB*, addition of reserpine had no effect on NOR or CIP [not shown] MICs (in contrast with what was observed for *pmrA* disruptants).

C. For BET, *pmrA* disruption did not cause MIC decrease while reserpine was effective.

D. Similar experiments made with CIP, NOR, LVX, MXF or GEM showed that only CIP and NOR are affected by disruption of *patA*/*patB* (change in MIC for LVX in the presence of reserpine is 1 $\log_2$ dilution only).

Conclusions

- Disruption of *patA* or *patB* is sufficient to reduce NOR and CIP MIC to the value measured in the presence of reserpine.
- PatA/PatB, even when expressed at a basal level, contribute to resistance to these two FQ in the clinical isolates analyzed.
- PmrA seems to have almost no impact on resistance of the clinical isolates analyzed.

References

1. Gill *et al.* Identification of an Efflux Pump Gene, pmrA, Associated with Fluoroquinolone Resistance in Streptococcus pneumoniae. Antimicrob Agents Chemother. 1999; 43:187-189.
2. Marrer *et al.* Involvement of the Putative ATP-Dependent Efflux Proteins PatA and PatB in Fluoroquinolone Resistance of a Multidrug-Resistant Mutant of *Streptococcus pneumoniae*. Antimicrob Agents Chemother. 2006; 50: 685–693
3. Avrain *et al.* Selection of quinolone resistance in *Streptococcus pneumoniae* exposed in vitro to subinhibitory drug concentrations. Journal of Antimicrobial Chemotherapy. 2007; 60:, 965–972.
4. Garvey and Piddock. The Efflux Pump Inhibitor Reserpine Selects Multidrug-Resistant *Streptococcus pneumoniae* Strains that Overexpress the ABC Transporters PatA and PatB. Antimicrob Agents Chemother. 2008; 52: 1677–1685.