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Ciprofoxacin Selects for PatA/PatB Over-expression in a wild-type and a PmrA-overproducer *S. pneumoniae* Exposed to Half-MIC Concentrations

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ABSTRACT

Background: Resistance to quinolones in *S. pneumoniae* is mediated by target site mutation or by increased expression of efflux pumps (PmrA or PatA/PatB). We previously showed that exposure to sub-inhibitory MIC concentration selects for a reserpine-sensitive resistance mechanism when exposed to CIP, and for target site mutations when MXF is used (ICAAC 2006; C1-0041). This study aims at identifying the efflux mechanism involved in CIP-selected resistance and to examine potential cross-resistance with other quinolones.
Methods: *S. pneumoniae* ATCC49619 (wild-type) and clinical isolate SP32 (I460V in ParE) were both exposed for up to 13 days to CIP at half its MIC, with daily readjustment. Efflux was detected by the reversal of MIC increase in the presence of reserpine arithmetic dilution) and by real time PCR of *pmrA* or c-RT PCR of *patA/patB*. Mutations in *parC, parE and gyrA* QRDRs were detected by sequencing.
Results : The table shows the MICs of 5 quinolones and the expression of *pmrA* and *patA/patB* at day 0 and day 13. No additional mutations were detected.

	ATCC49619				SP32			
	Day 0		Day 13		Day 0		Day 13	
MIC (mg/L) -/+ reserpine	-	+	-	+	-	+	-	+
CIP	0.5	0.5	2.5	**1**	1.5	**0.5**	4	**1**
LVX	0.5	0.5	1.5	1.5	0.75	0.75	1.5	**1**
MXF	0.15	0.15	0.25	0.25	0.15	0.15	0.2	0.2
GRN	0.05	0.05	0.1	0.1	0.05	0.05	0.1	0.1
GMF	0.05	0.05	0.08	**0.05**	0.03	0.03	0.06	**0.04**
***pmrA* expression**	1 (a) *		1.7 ± 0.54 (a)		11.4 ±3.20 (b)		3.4 ± 1.00 (c)	
***patA/patB* expression**	1 / 1 (a) *		1.88 ± 0.37 / 1.35 ± 0.29 (b)		0.86 ± 0.34 / 1.04 ± 0.19 (a)		2.61 ± 0.65 / 1.67 ± 0.44 (b)	

* normalized value; MIC increases reversed by reserpine are highlighted in bold

Statistical analysis for gene expression levels: (a) not different from the expression level of the corresponding gene in ATCC49619 at day 0; (b) and (c) significantly different from (a) and from one another (ANOVA; $p < 0.05$)

Conclusions: Before CIP exposure, SP32 showed over-expression of pmrA associated with an increased MIC of CIP. Exposure to sub-inhibitory concentrations of CIP selected over-expression of patA/patB both in ATCC 49619 and SP32, which was associated with an increase in the MIC of CIP (~2-3 fold) and GMF (~1.5-2 fold), the latter increase causing only a non-clinically significant change in MIC.

INTRODUCTION

Two quinolone efflux sytems have been described so far in *S. pneumoniae,* namely PmrA, a member of the MFS transporter superfamilly (1), and PatA/PatB, belonging to the ABC transporter superfamilly (2).

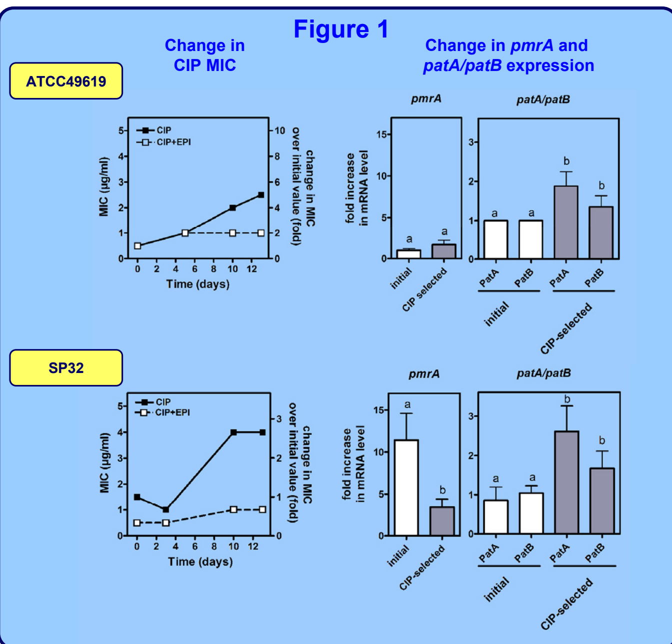
Resistance in *S. pneumoniae* through efflux following exposure to sub-inhibitory concentrations of quinolones as already been observed (3), but the expression level of the corresponding transporters was not quantified.

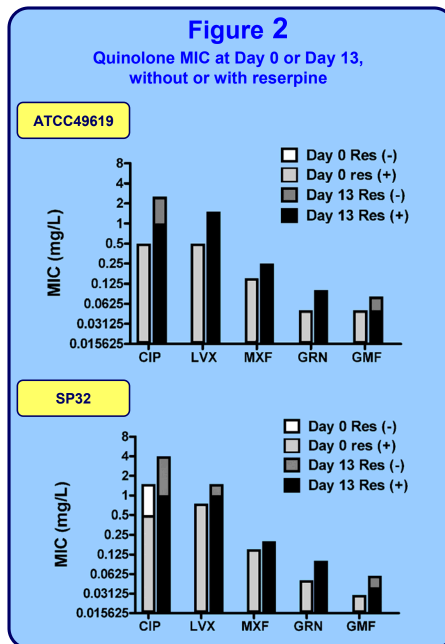
AIM OF THE STUDY

- **To identify the efflux mechanism involved in CIP-selected resistance in *S. pneumoniae* exposed to sub-MIC concentrations of ciprofloxacin;**
- **To examine for cross-resistance with other quinolones.**

RESULTS

- Before the experiment, at day 0 SP32 (clinical strain) had reduced susceptibility to CIP compared with ATCC49619 (wild strain), resulting from efflux (reversed by reserpine; increased *pmrA* expression);
- After 13 days of antibiotic pressure the MIC of CIP increased for ATCC49619 and SP32; these were partially reversed by reserpine (left panels of Figure 1);
- *patA/patB* genes were significantly over-expressed after 13 days of CIP-selection, both in ATCC49619 and SP32 (which, for this strain, was additional to the resistance PmrA-mediated efflux) (right panels of Figure 1). Notably, however, the level of expression of *pmrA* in SP32 was decreased upon CIP exposure but remained higher than in the ATCC49619 strain.
- CIP selection caused a modest increase in the MIC of CIP and LVX and marginal increases in the MIC of MXF, GRN, and GMF. MIC increases were partially reversed by reserpine for CIP, GMF, and for LVX in SP32 (Figure 2).
- No additional mutations in the QRDR of topoisomerase genes were detected over the observation period.
- The PFGE profiles were different for ATCC 49619 and SP32. However, they were unchanged for each strain and mutants thereof throughout the experiment.





METHODS

Strains: ATCC49619 (wild type) and SP32 (clinical isolate, mutation I460V in parE, CIP efflux positive).
Selection of resistance: bacteria were exposed for 13 days at half-MIC concentrations of CIP, with daily readjustments.
Minimal Inhibitory Concentrations (MICs) were determined by agar dilution method, in the absence or the presence of reserpine (EPI) as inhibitor of efflux (10 µg/mL) for CIP, LVX, MXF, GRN and GMF.
***pmrA* and *patA/paB* gene expression** were respectively quantified by Real Time PCR (syber Green method; *hexA* gene as housekeeping gene) and c-RT PCR (4) (16S rRNA as housekeeping gene).
Mutations in *parC, parE* and *gyrA* genes were detected by DNA sequencing.
Strain characterization was performed by PFGE (5).

CONCLUSION

- **Exposure to sub-MIC concentrations of CIP selects for increased expression of the PatA/PatB pump in a wild-type strain as well as in a PmrA-overproducer.**
- **This change is associated with**
  - **a marked increase in the MIC of CIP**
  - **a small and partially reserpine-reversible increase in the MIC of LVX and GMF**
  - **a marginal, reserpine-insensitive change in the MIC of MXF and GRN.**

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