Presentation
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Mechanisms of Resistance in *S. pneumoniae* Exposed to Half MICs of CIP and MXF

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REVISED ABSTRACT

Background: Efflux is now increasingly recognized as an important resistance mechanism for *S. pneumoniae*. Little is however known about its emergence in bacteria exposed to subtherapeutic concentrations.
Methods: *S. pneumoniae* ATCC 49619 (fully sensitive, no mechanism of resistance detected) and SP32 (I460V in *parE*; *pmrA* over producer) were exposed for up to 13 days to CIP or MXF at 0.5X their MIC, with daily readjustment to meet the increase in MIC (measured with arithmetic dilutions). Efflux was detected by the reversal of MIC increase in the presence of reserpine (R) and by real time PCR of *pmrA*. Mutations in *parC*, *parE* and *gyrA* genes were detected by sequencing.
Results: The table shows the changes in MIC and the expression of *pmrA* at day 0 and day 13, and the additional mutations detected at day 13.

		CIP MIC (mg/L)				pmrA expression (average)		Additional mutation
		D0		D13		D0	D13	D13
Strain	Inducer	R (-)	R (+)	R (-)	R (+)			
ATCC 49619	CIP	0.5	0.5	2.5	1	1*	1.51	-
	MXF			5	5		0.93	S79Y (parC); S81F (gyrA)
SP 32	CIP	1.5	0.5	4	1	8.83	7.13	-
	MXF			2	1		7.70	R447C (parE)

*Arbitrarily set to this value (basal expression)

Conclusion: CIP easily induces efflux-mediated (reserpine-sensitive) resistance, which, however, is not correlated with the level of *pmrA* over expression. In contrast, MXF, which is not susceptible to efflux, causes mutation-mediated resistance. Both mechanisms, however, may lead to similar levels of resistance (MIC = 8-10X the value of wild type).

INTRODUCTION

Efflux mechanisms are now increasingly recognized as a potential risk of low to medium resistance and are suggested to favor the selection of other resistance mechanisms like target mutations (Bast *et al.*, 2000; Van Bambeke *et al.*, 2003).

In *S. pneumoniae*, different quinolone efflux pumps have been described (Piddock et *al*., 2002, Brenwald et *al*., 2003),among which pmrA is the best characterized (Brenwald *et al.*, 1998).

AIM OF THE STUDY

- **To evaluate whether exposure of *S. pneumoniae* to sub-MIC concentrations of ciprofloxacin or moxifloxacin triggers the development of efflux-mediated resistance and/or selects for target mutations.**

METHODS

Induction of resistance: *S.pneumoniae* ATCC49619 (fully sensitive to quinolones; no mutation and efflux detected) and SP32 (mutation in *parE*; pmrA over producer) strains were exposed to CIP and MXF at half their MIC for 13 days, with daily readjustment to meet MIC increases.

Minimal Inhibitory Concentrations (MICs) were determined by agar dilution method, in the absence or the presence of reserpine as inhibitor of efflux (10 µg/mL).

***pmrA* gene expression** was quantified by Real Time PCR using Sybr Green method, using *hexA* gene as house keeping gene.

Mutations in *parC*, *parE* and *gyrA* genes were detected by sequencing.

Strain characterization was performed by PFGE (McEllistrem et *al*., 2000).

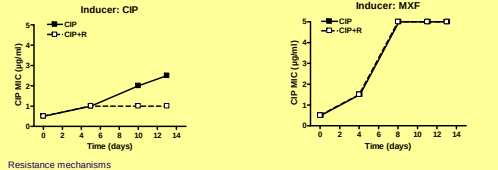
RESULTS

- **Strains characterization** by PFGE shows that the restriction patterns of DNA from both ATCC 49619 and SP32 are different from one other, and remained unmodified throughout the experiment.
- **Before induction,** SP 32 strain shows a reserpine-sensitive resistance to CIP, associated to an elevated expression of pmrA (~9X the basal expression level measured in ATCC 49619)
- **Exposure of both strains to both quinolones causes an elevation of CIP MIC**
  - when induced by CIP, CIP resistance is reversed by reserpine, but this increase in not associated with the level of pmrA over expression.
  - when induced by MXF, CIP resistance is not reversed by reserpine and associated to target mutations.

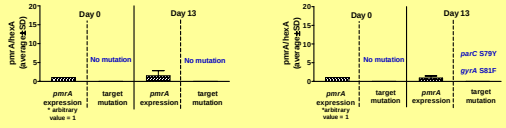
Influence of a 13 days exposure of *S. pneumoniae* to half MIC of CIP and MXF on CIP MIC (measured in the absence or in presence of reserpine) and associated resistance mechanisms

ATCC 49619 strain

Phenotypic characterization

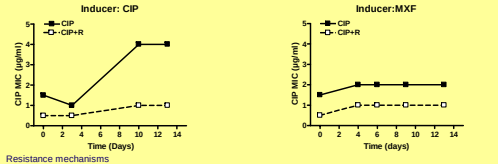


Resistance mechanisms

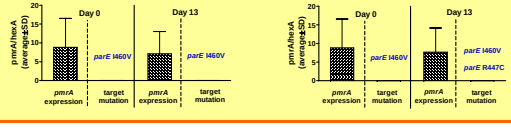


SP 32 strain

Phenotypic characterization



Resistance mechanisms



CONCLUSION

- **CIP easily induces efflux-mediated (reserpine-sensitive) resistance, which, however, is not correlated with the level of *pmrA* over expression.**
- **In contrast, MXF, which is not susceptible to efflux, selects for resistance by target mutation.**
- **Both mechanisms, however, may lead to similar levels of resistance (MIC = 8-10X increase in MIC values).**

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