Activity of macrolides, ketolides, and fluoroquinolones against *S. pneumoniae* in an *in vitro* pharmacodynamic model of biofilm

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Introduction

Persistent infections by *S. pneumoniae* are associated with in situ formation of biofilms.

➡ Therapeutic eradication becomes difficult due to the protective role of the matrix in which bacteria are embedded.

Aim

Development of an *in vitro* model
of young and aged biofilms of *S. pneumoniae* to study
the effect of antibiotics on biofilm mass and intra-matrix bacterial survival.

Method

1 Culture

2, 4, 7 and 11 days

S. pneumoniae
non capsulated strain R6
capsulated strain ATCC 49619

2 Exposure to antibiotics

24h incubation

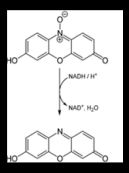
Macrolides/Ketolides/Quinolones
Concentrations:
0.0001 to 1000-fold the MIC in broth

3 Quantification of antibiotic activity (24h)

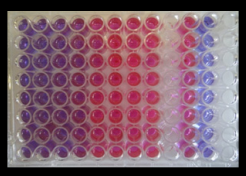
Bacterial viability within the biofilm

RESAZURIN

In situ reduction by viable cells



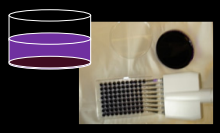
RESORUFIN
λexc 560nm - λem 590nm

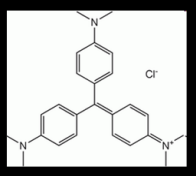


Fluorimetric quantification

Total biofilm mass : matrix + bacteria

Staining with crystal violet (λabs 570nm)





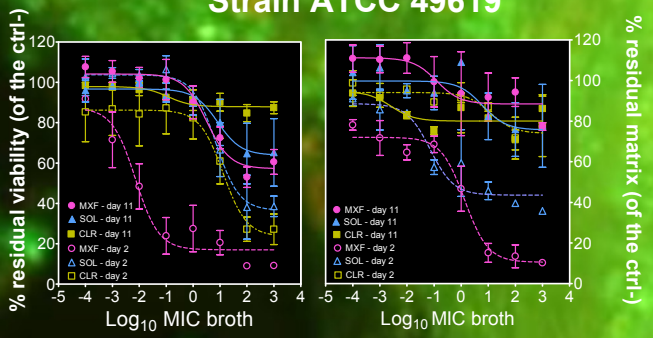
Spectrophotometric quantification

Experiments were performed 3 to 6 times independently

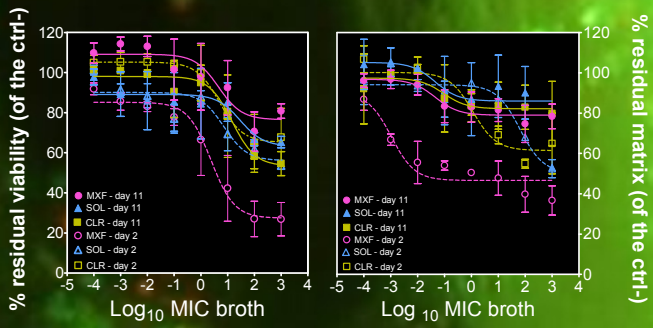
1. Concentration-effect curves (CLR - SOL – MXF)

Viability within the biofilm | Biofilm thickness (matrix)

Strain ATCC 49619



Strain R6



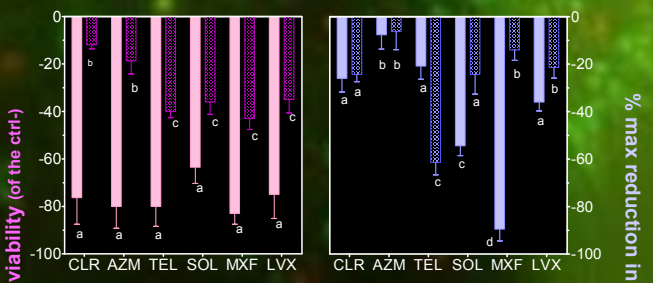
ATCC 49619	CLR	AZM	TEL	SOL	MXF	LEV
MIC broth (µg/ml)	0.032	0.064	0.016	0.008	0.125	1

R6	CLR	AZM	TEL	SOL	MXF	LEV
MIC broth (µg/ml)	0.063	0.5	0.008	0.004	0.063	0.5

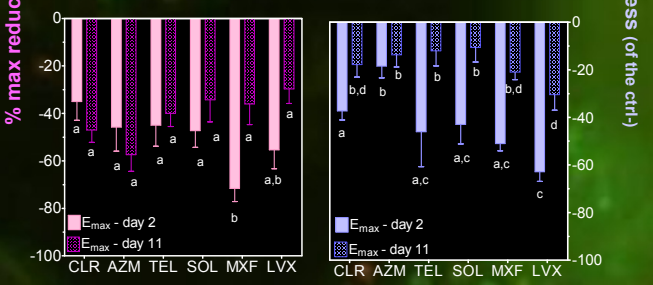
2. Maximal effect

on viability within the biofilm | on biofilm thickness

Strain ATCC 49619



Strain R6



Bars with different lowercase letters (a,b,c,d) are significantly different from each other in each panel (One-way ANOVA with Tukey post test to compare AB Emax between AB and unpaired two tailed t test to compare AB Emax between D2 and 11).

Results

- In the absence of antibiotic, the resorufin fluorescence signal increased from 3000 5000 (intensity values) to 5000-9000 between day 2 and 11, and the optical density of CV increased from 0.6-0.9 to 32-33 between day 2 and day 11 for both strains.
- Concentration-effect curves (for selected antibiotics; upper graphs) and maximal effects (for all antibiotics; lower graphs) show that
  - activity is markedly lower against old than young biofilms with respect to both viability and matrix;
  - for young biofilms, MXF is the most potent antibiotic (lowest EC_{50}), but all antibiotics achieve a similar E_{max} on viability, whereas ketolides and fluoroquinolones (only for R6) show higher efficacy on matrix
  - for old biofilms, all antibiotics show similar activity when expressed in multiples of their MIC (note that the MIC of solithromycin is lower than that of all other antibiotics including telithromycin).

Conclusion

- The amount of biofilm produced over time is independent of the non capsulated or capsulated phenotype and is accompanied over aging by a global decrease of antibiotic activity.
- Taking also into account MIC values in broth, the most effcient antibiotics (on a weight basis) are solithromycin amongst macrolides/ketolides, and moxifloxacin amongst fluoroquinolones.
- The combined high potency and efficacy of moxifloxacin may result from a combination of its bactericidal character (including cell lysis) and its high intrinsic activity.

References

del Prado *et al.*, 2010 *Diagn Microbiol Infect Dis* 67: 311-318 ; Roveta *et al.*,2007 *Int J Antimicrob Agents* 30: 415-421 ; Tote *et al.*, 2008 *Lett Appl Microbiol* 46: 249-254 ; Moscoso *et al.*, 2009 *Int Microbiol* 12: 77-85 ; Simoes,2011*Curr Med Chem* 18: 2129-2145 ; Trappetti *et al.*, 2009*J Infect Dis* 199: 1497-1505

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