



P-2176

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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

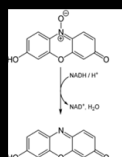
3

### Quantification of antibiotic activity (24h)

#### Bacterial viability within the biofilm

#### RESAZURIN

In situ reduction by viable cells



#### RESORUFIN

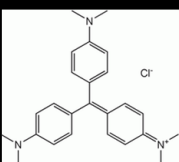
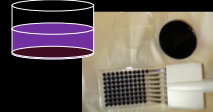
$\lambda_{exc}$  560nm -  $\lambda_{em}$  590nm



Fluorimetric quantification

#### Total biofilm mass : matrix + bacteria

Staining with crystal violet ( $\lambda_{abs}$  570nm)



Spectrophotometric quantification

Experiments were performed 3 to 6 times independently

## 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 efficient 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

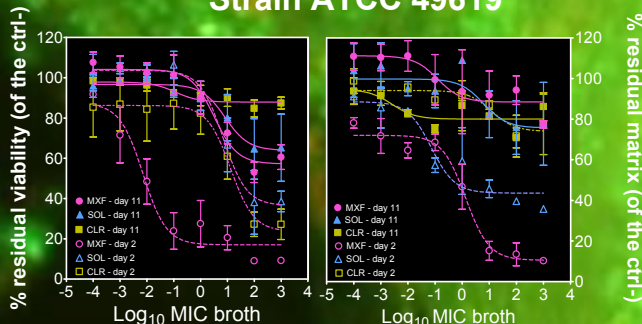
del Prado et al., 2010 *Diagn Microbiol Infect Dis* 67: 311-318; Roveta et al., 2007 *Int J Antimicrob Agents* 30: 415-421; Tote et al., 2008 *Lett Appl Microbiol* 46: 243-254; Moscoso et al., 2009 *Int Microbiol* 12: 77-85; Simoes, 2011 *Curr Med Chem* 18: 2129-2145; Trappetti et al., 2009 *J Infect Dis* 199: 1497-1505

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

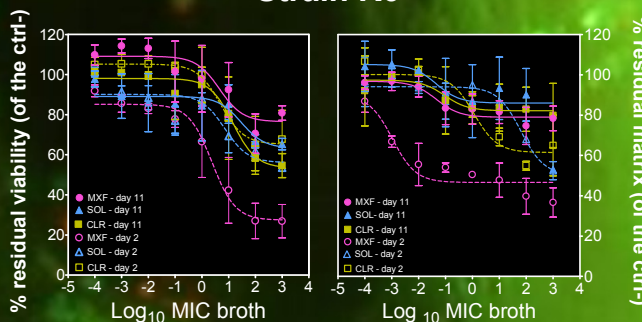
Viability within the biofilm

Biofilm thickness (matrix)

### Strain ATCC 49619



### Strain R6



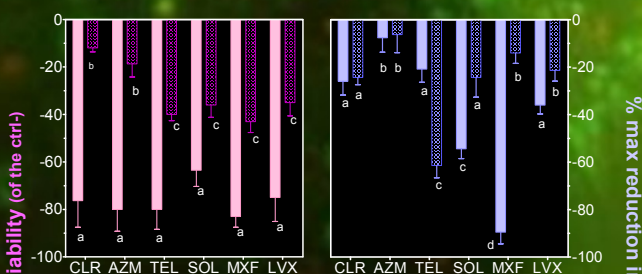
ATCC 49619	CLR	AZM	TEL	SOL	MXF	LEV	R6	CLR	AZM	TEL	SOL	MXF	LEV
MIC broth (μg/ml)	0.032	0.064	0.016	0.008	0.125	1	0.063	0.5	0.008	0.004	0.063	0.5	

## 2. Maximal effect

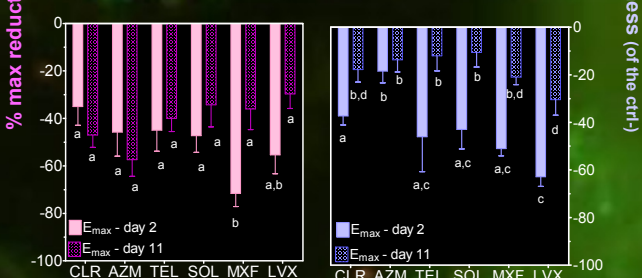
on viability within the biofilm

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  $E_{max}$  between AB and unpaired two tailed t test to compare AB  $E_{max}$  between D2 and 11).