Activity of Macrolides and Fluoroquinolones in Models of Naive and Induced Biofilms of *Streptococcus pneumoniae*

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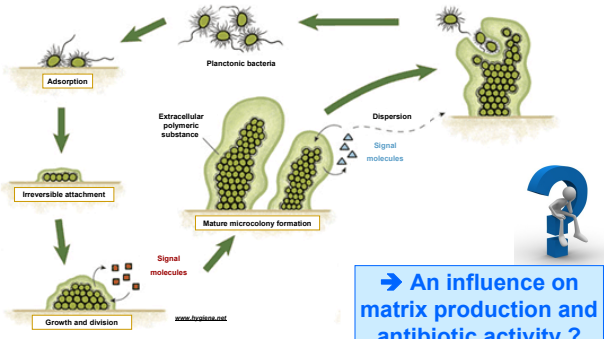
Introduction

1. **Chronic streptococcal infections 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

2. **During biofilm development, bacterial interactions mediated by quorum sensing take place, leading to behavior modifications**



➔ **An influence on matrix production and antibiotic activity ?**

Aim

Setting two *in vitro* biofilms models starting from
naive bacteria or **bacteria induced** for **matrix production**

➔ **to compare the rate of matrix production**

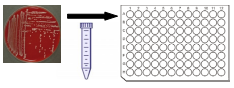
➔ **to study antibiotic activity on biofilm mass and bacterial survival**

Method

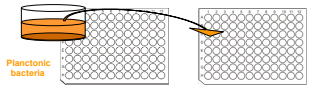
Culture: *S. pneumoniae* strains ATCC 49619 (capsulated) and R6 (non capsulated) were cultivated for 2, 4, 7 and 11 days in 96-well plates, using cation-ajusted Mueller Hinton broth supplemented with lysed horse blood (5%) and glucose (2%) as growth medium

Naive model

Bacterial suspension starting from bacterial colonies



Induced model

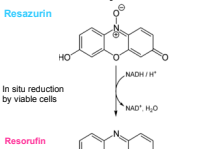


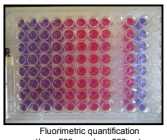
Exposure to antibiotics: 24h incubation
Macrolides/Ketolides/Quinolones at concentrations equal to 0.0001 to 1000-fold the MIC in broth

MIC in broth (µg/ml)	Macrolides		Ketolides		Fluoroquinolones		
	Clarithromycin	Azithromycin	Telithromycin	Solithromycin	Moxifloxacin	Levofloxacin	Gemifloxacin
ATCC 49619	0.032	0.064	0.016	0.008	0.125	1	0.032
R6	0.063	0.5	0.008	0.004	0.063	0.5	0.063

Evaluation of the antibiotic effect after 24 h

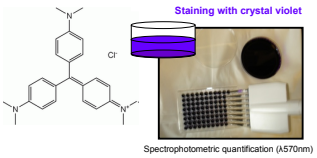
Bacterial viability within the biofilm





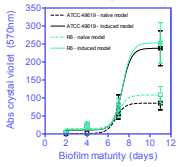
Fluorimetric quantification
(λexc 560nm - λem 590nm)

Total biofilm mass : matrix + bacteria



Spectrophotometric quantification (λ570nm)

Fig.1



Conclusions

- **Biofilm production is independent of the non-capsulated or capsulated phenotype and is accelerated when bacteria have been previously in contact with a biofilm. This suggests that a learning process, probably mediated by quorum sensing, occurs during the structure development, which may be worth of further exploration.**
- **Antibiotic activity seems to be strain-dependent and decreases upon biofilm aging for some molecules in the naive model.**
- **In the induced model, because of the large amount of matrix produced, it was not always possible to observe a difference of activity on survival between young and aged biofilms.**
- **For ketolides and azithromycin [against R6], and for azithromycin and solithromycin [against ATCC 49619], we noticed an increased efficacy towards viable counts upon biofilm aging, the mechanism of which remains to be further explored.**

Results

Figure 2: Concentration - effect studies: Moxifloxacin – Solithromycin

Antibiotic activity on bacterial survival within the biofilm and on matrix thickness

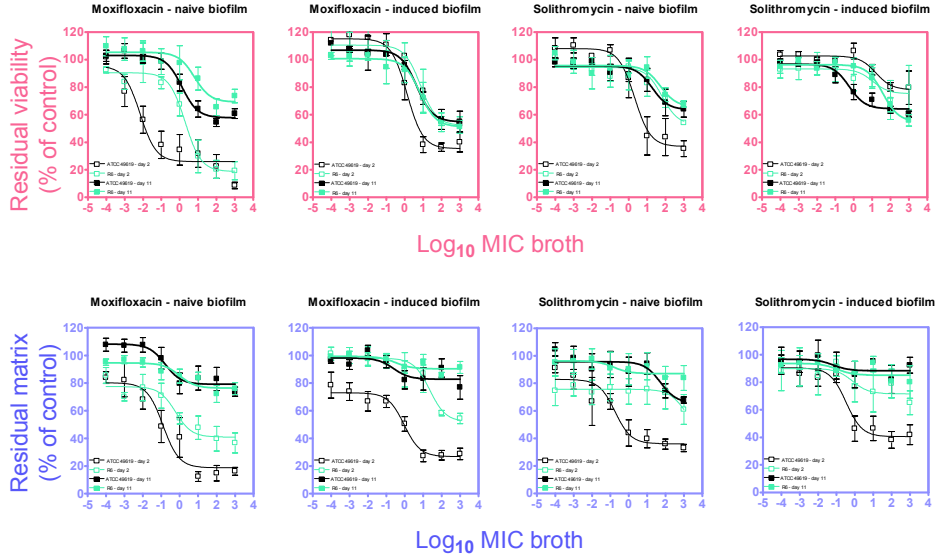
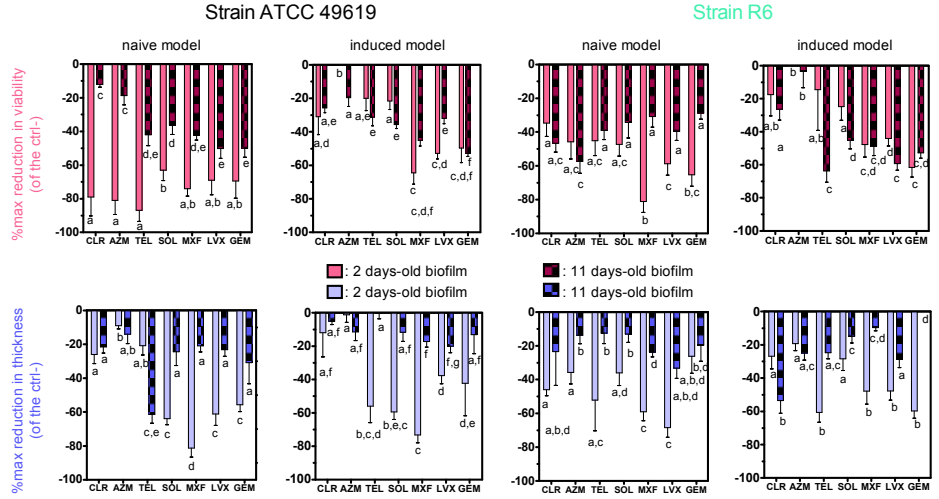


Figure 3: Maximal effect on bacterial survival within the biofilm and on matrix thickness



Bars with different lowercase letters are significantly different from each other in each panel (one-way ANOVA with Tukey post-test to compare antibiotic E_{max} values between molecules and maturity stages).

Figure 1 compares biofilm growth over time.
- In the naive model, CV OD increased from 0.2-1.55 to 40-130 between day 2 and day 11.
- In the induced model, CV OD was higher than for the naive model (between 2-11 at day 2 and 200-300 at day 11).

Figure 2 shows the activity of moxifloxacin and solithromycin as exemplative fluoroquinolone and ketolide against young and mature biofilms formed from naive bacteria or induced bacteria.
- For moxifloxacin, the relative potency and maximal efficacy towards viable bacteria were reduced over aging in both models for ATCC 49619, and in the naive model for R6. These parameters were in all cases affected by aging towards matrix.
- For solithromycin, aging did not markedly affect efficacy and potency against R6 (both towards viability and matrix) while it increased both potency and maximal efficacy towards ATCC 49619 viability in the induced model.

Figure 3 compares relative maximal efficacies.
- In the naive model, the increase of biofilm thickness over aging was accompanied by a reduction in antibiotic efficacy against bacterial survival for all molecules towards strain ATCC 49619 and for fluoroquinolones towards strain R6. Efficacy on the matrix over aging was globally decreased for fluoroquinolones against ATCC 49619 and for all drugs, against R6.
- In the induced model, aging caused a loss of efficacy of macrolides and ketolides against bacteria survival in young biofilms as compared to the naive model. The effects of antibiotics on the matrix were globally similar when tested against naive and induced biofilms.

References

del Prado *et al.*, Diagn Microbiol Infect Dis. 2010 Aug;67(4):311-8 ; Roveta *et al.*, Int J Antimicrob Agents. 2007 Nov;30(5):415-21; Tote *et al.*,Lett Appl Microbiol. 2008 Feb;46(2):249-54; Moscoso *et al.*, Int Microbiol. 2009 Jun;12(2):77-85 ; Simoes, Curr Med Chem. 2011;18(14):2129-45 ; Trappetti *et al.*, J Infect Dis.2009 May 15;199(10):1497-50 ; www.hygiena.net

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