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Detection of a matrix disassembly phenomenon improving the antibiotic killing activity towards very mature *S. pneumoniae* (Sp) *in vitro* biofilms.

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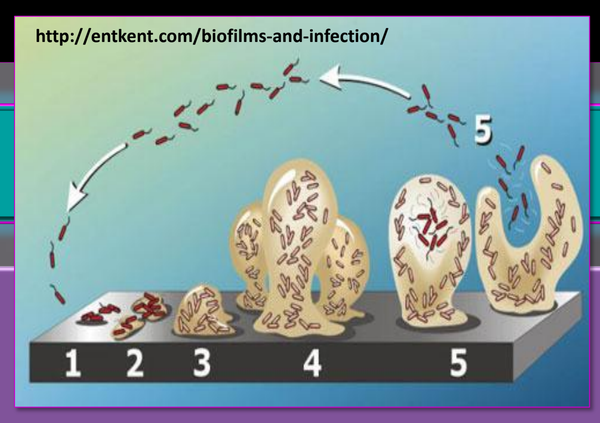
INTRODUCTION

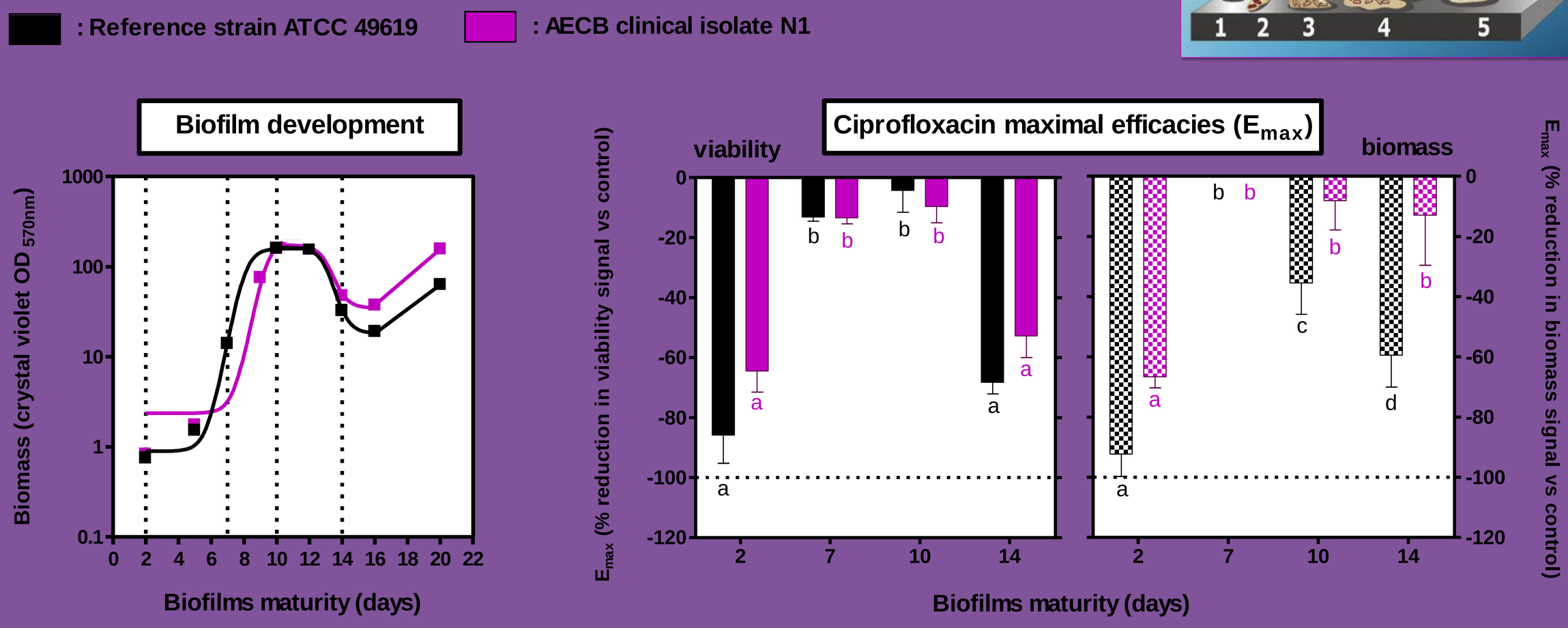
- Up to 80% of chronic bacterial infections, such as those occurring during Acute Exacerbations of Chronic Bronchitis (AECB), involve bacterial growth within biofilm [1].
- Biofilm development leads to infection recurrence, partly because of the protective role played by the matrix in which bacteria are embedded and which reduces antibiotic (AB) diffusion [2].
- However, spontaneous matrix disassembly has already been described for *S. aureus* biofilms [3] and promoting this phenomenon may be an appealing strategy for improving AB activity towards sessile bacteria.
- In this study, we investigated the occurrence of matrix disassembly in *in vitro S. pneumoniae* biofilms and its impact on ciprofloxacin activity.

METHODS

- Strains: Sp reference strain ATCC 49619 and AECB clinical isolate N1 were grown on blood agar plates.
- Biofilms: bacterial suspension (25µL; OD_{620nm}= 0.1) + culture medium (175 µL; caMHB + 5% lysed horse blood + 2% glucose) added in 96-well plates and incubated for up to 20 days ; growth medium refreshed every 2 days.
- Biomass and disassembly quantification: crystal violet OD_{570nm} measures.
- Killing activities on 2-, 7-, 10- and 14 days old biofilms : evaluated after 24h of incubation with a ciprofloxacin gradient (10^{-4} to 10^{3} x MIC broth) using the reduction, by viable cells, of resazurin into fluorescent resorufin[4]. A Hill equation was fitted to data (dose-response curves) to calculate E_{max}.



RESULTS



Detection of a matrix disassembly phenomenon improving the antibiotic killing activity towards very mature *S.pneumoniae* (*Sp*) biofilms

Left panel: Evolution of biofilm production (as evaluated by crystal violet [CV] absorbance) overtime in a naïve model and by the capsulated reference strain ATCC49619 (black) and AECB clinical isolate N1 (magenta). All values are means ± standard deviations (SD) of 2-8 independent determinations. When not visible, the SD bars are smaller than the size of the symbols. Middle and right panels: Maximal ciprofloxacin efficacies on pneumococcal survival (resorufin fluorescence; middle) and biomass thickness (crystal violet absorbance; right panel) as compared to controls (no antibiotic added) for 2-, 7-, 10 and 14-days old naive biofilms of strain ATCC49619 and N1. Results are expressed in percentage of residual viability and biomass after 24h of incubation in the presence of a ciprofloxacin gradient with values calculated as means ± SEM of 2-8 independent experiments performed in quadruplicates (when not visible, the bars are smaller than the size of the symbols), using the Hill equation of the concentration-response curves. Statistical analyses: one-way ANOVA with Tukey post-test for multiple comparisons between different maturity stages; values with different letters are significantly different from each other ($p<0.05$).

- **Biofilm development : For both strains, biomass firstly increased from day 2 (CV_{OD} = 0.8 ± 0.04) until reaching a plateau of maximal thickness at day 10 to 12 (CV_{OD} = 172.8 ± 24.9). From day 12 to 14, matrix loss occurred with a second plateau phase with lower biomass obtained between days 14 to 16 (CV_{OD} = 34.2 ± 10.2). From day 16 to 20, biofilm growth resumed until CV_{OD} = 110 ± 46.7 (day 20).**
- **Ciprofloxacin activity : Maximal efficacy of ciprofloxacin (E_{max}) towards bacterial survival and biomass decreased from 65% reduction (against 2-days old biofilms) to 7% against 10-days old biofilms, which also showed the highest biomass. Antibiotic activity was partly restored at day 14 with respectively 66 and 68% reduction in bacterial viability or in biomass.**

CONCLUSIONS

Biomass amount strongly affects ciprofloxacin killing activity against mature *Streptococcus pneumoniae* biofilms. However, spontaneous matrix loss, probably related to disassembly, allows a significant restoration of ciprofloxacin activity towards bacterial survival within old biofilms.

Agents promoting matrix disassembly are therefore promising to deal with the problem of infections recurrence and changes in matrix composition may be worth further exploration.

REFERENCES

[1]Moscoso et al, 2009 Int. Microb. 12 (2): 77-85; [2]Simoes, 2011 Curr. Med. Chem. 18 (14): 2129-45; [3]Boles et al, 2011 Trends Microbiol. 19 (9): 449-55; [4]Toté et al, Lett. Appl. Microbiol. 46 (2): 249-54;

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