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Activity of antibiotics against *P. aeruginosa* and *S. aureus* biofilms

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Introduction & Purpose

- *Pseudomonas aeruginosa* and *Staphylococcus aureus* are major causes of chronic pulmonary infections in Cystic Fibrosis patients due to their capacity to grow as biofilms, which are refractive to the action of antibiotics. As these 2 bacteria are frequently isolated in patients[1], appropriate models to evaluate antibiotic activity against biofilms are warranted.
- The aim of the study is to evaluate the activity of different relevant antibiotics used to treat chronic infections caused by *Staphylococcus aureus* and *Pseudomonas aeruginosa* in CF patients in an *in vitro* model of single species biofilm.

Methods

- One reference strain was used for each species :
  - *S. aureus* ATCC 25923
  - *P. aeruginosa* PAO1
- Biofilms were grown in TGN (Tryptic soy broth + 1% Glucose + 2% NaCl) using 96 well cell culture microplates (clear, TC surface treatment) for cell culture.
- Kinetics of biofilm formation were followed over 4 days (Fig. 1).
- Mature biofilms were exposed during 24h to antibiotics selected among broad spectrum agents (tobramycin and meropenem), an anti Gram-positive agent (linezolid) and a broad spectrum agent indicated for infections by Gram-negative bacteria (ciprofloxacin).
- Antibiotic concentrations ranged from 0.001 to 1000 mg/L in order to obtain full concentration-response curves.
- After 24h of incubation, plates were harvested and used to evaluate biofilm biomass by crystal violet staining and total bacterial viability within biofilm by the fluorescein diacetate assay (FDA) for *P. aeruginosa* and by the resazurin assay (RF) for *S. aureus*[2] (Fig. 2).
- Two pharmacodynamic parameters (Fig. 3) were calculated based on the equation of the sigmoidal regression fitted of the data:
  - E_{max} (maximal efficacy): reduction in viability/biomass for an infinitely large concentration
  - C_{25} or C_{50} (relative potency): concentration causing a 25% or 50% in viability/biomass

Conclusions

- Antibiotics are more effective against *S. aureus than against P. aeruginosa* in biofilms but only show modest effects on the matrix.
- The more effective drugs are linezolid and meropenem against *S. aureus* and ciprofloxacin against *P. aeruginosa.*
- Further studies are needed to determine whether these dissimilarities are related to differences in antibiotic bioavailability within biofilms or in bacterial responsiveness.

References

1. Fugère A. *et al.* Interspecific small molecule interactions between clinical isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus* from adult cystic fibrosis patients. PLoS One (2014) 9(1): e86706 PMID : 24466207
2. Peeters E. *et al.* Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. Journal of microbiological methods (2008) 72:157-65 PMID : 18155789

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Results

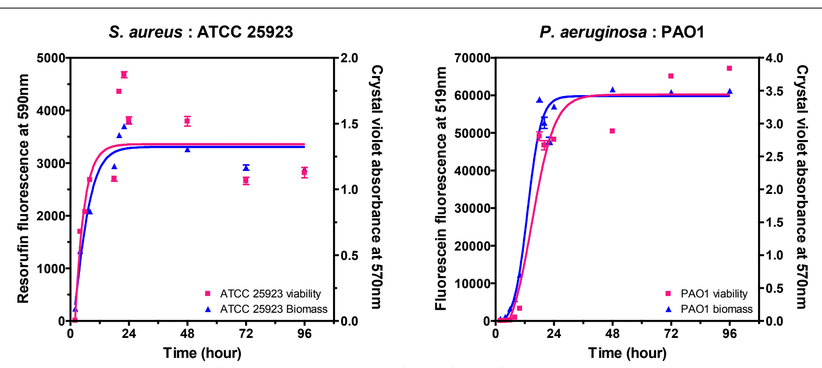

Fig. 1 : Kinetics of biofilm formation

- Against both bacterial species, a plateau value was obtained for both viability and biomass after 24 h of incubation.
- 24h–old biofilmq were therefore considered as mature and exposed to antibiotics for an additional 24 h period.

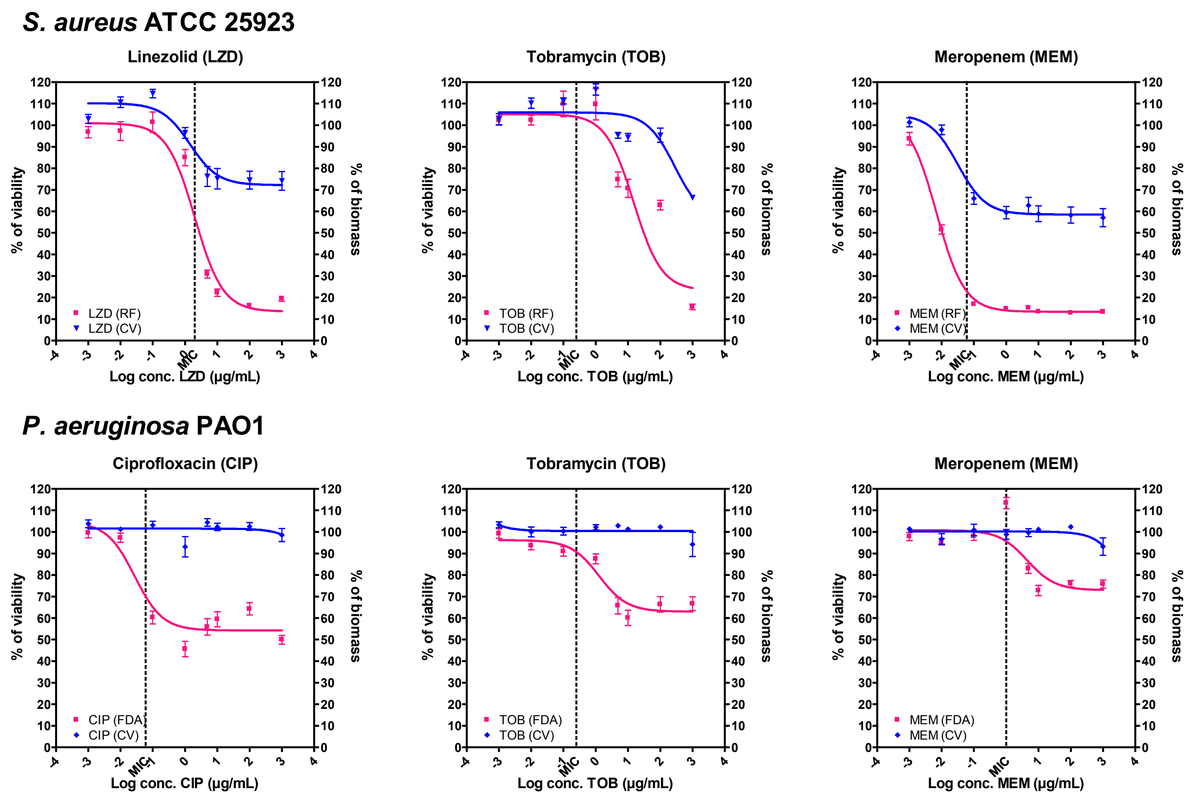

Fig. 2 : Activity of antibiotics against mature biofilms. Up : *S. aureus* biofilms exposed during 24 h to linezolid, tobramycin and meropenem. Down : *P. aeruginosa* biofilms exposed during 24 h to ciprofloxacin, tobramycin and meropenem.

- Against *S. aureus*, antibiotic activity was concentration-dependent towards both viability and biomass, with higher efficacy and potency observed towards viability as compared to biomass.
- Against *P. aeruginosa*, antibiotic activity was also concentration-dependent against viability, but no activity was observed against biomass.
- TOB and MEM were more effective (higher E_{max}) to reduce viability in *S. aureus* biofilms than in *P. aeruginosa* biofilms.

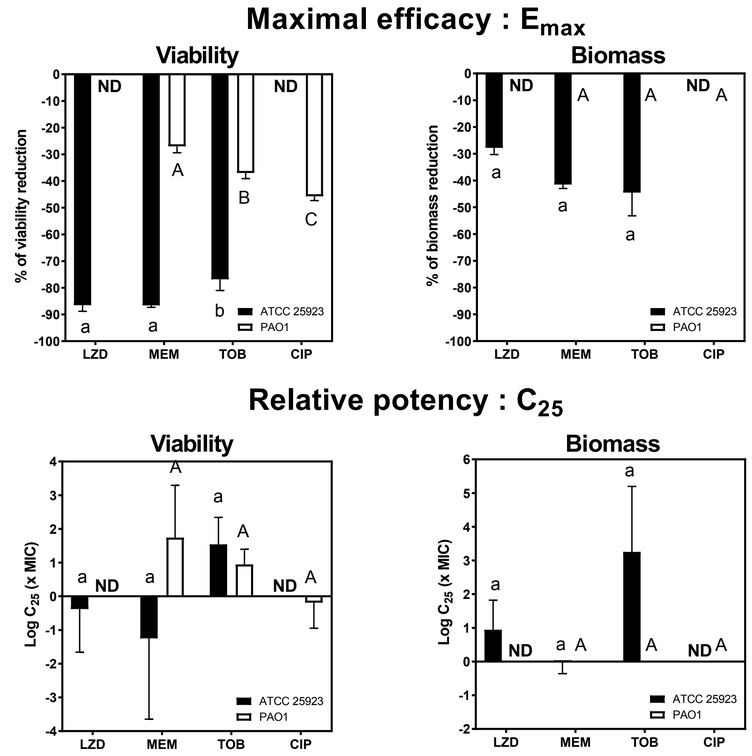

Fig. 3 : Pharmacodynamic parameters. Up : maximal efficacy (E_{max}). Down : Relative potency (C_{25})