In-vitro model of *Pseudomonas aeruginosa* pulmonary biofilm to evaluate the efficacy of cationic antibiotics

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Introduction

- *Pseudomonas aeruginosa* (*PA*) develop as biofilms in chronic pulmonary infections [1]
- Biofilms are aggregates of *PA* (50-100 µm) entrapped in a self-produced matrix of anionic polymers (alginate, DNA), often surrounded by patient mucus [2]
- The activity of cationic antibiotics "ATB" such as tobramycin (TOB) and colistin (COL) against these biofilms is low *in-vivo* because of their interactions with the matrix

Purpose

- Develop an *in-vitro* model composed of anionic polymers found *in-vivo* in pulmonary *PA* biofilms to evaluate the efficacy of inhaled ATB used to treat chronic pulmonary infections

Material & Methods

- A bioluminescent strain of *PA* (*PAO1-LUXCDEBA*) was incorporated into large calcium alginate beads (⌀ = 1200 µm).[2,3] These beads were dispersed in artificial sputum medium[4] (ASM) to produce an *in-vitro PA* pulmonary biofilm model
- The effectiveness of ATB (TOB and COL) was tested on the *in vitro PA* pulmonary biofilm by measuring :
  - *PA* bioluminescence kinetics during 40H
  - Bacterial concentrations (log10 CFU / ml) after 40 h of exposure to ATB. These values were plotted as a function of ATB concentrations and modelled using the following inhibitor Emax model.[5]

$$CFU(t) = CFU_0 \times \left(1 - \frac{C^{\gamma}}{C_{50}^{\gamma} + C^{\gamma}}\right)$$

CFU_0: CFU in the absence of ATB

C : concentration of ATB X MIC

C_{50} : [ATB] needed to achieve 50% of CFU_0

ɣ: Hill factor

- ✓ **The index with the best fit and low value of C_{50} is more effective**
- The development of resistance to these ATBs among surviving *PA*s was evaluated by measuring the MIC

Results

Evaluate the efficacy of ATB on the *in-vitro* model of *PA* pulmonary biofilm

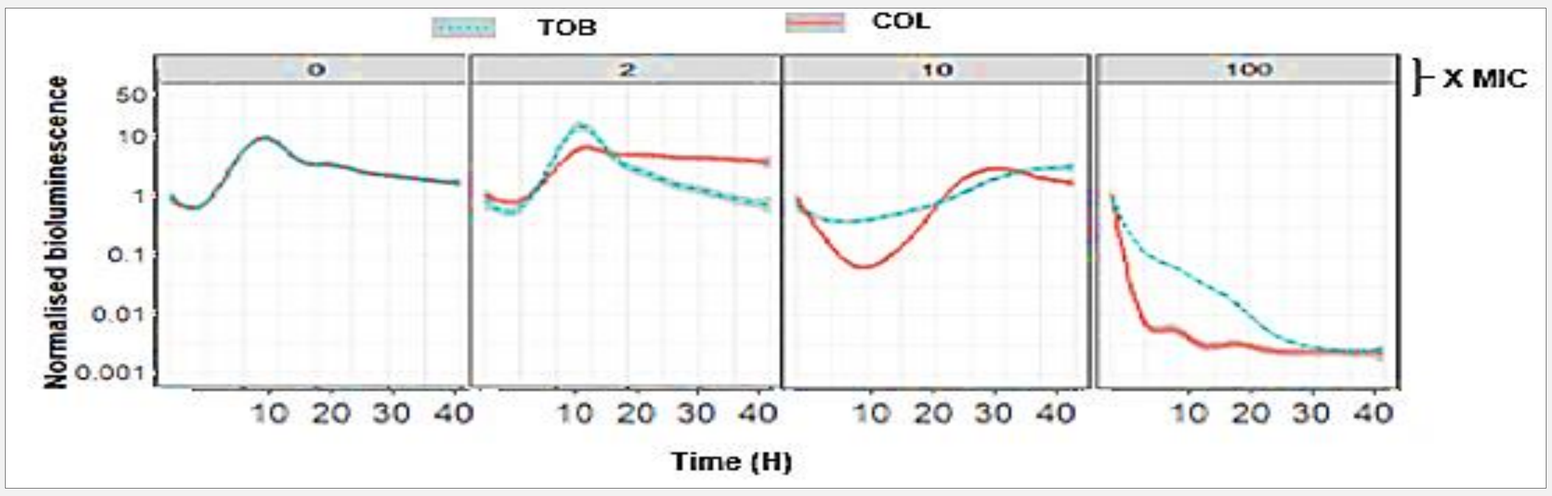
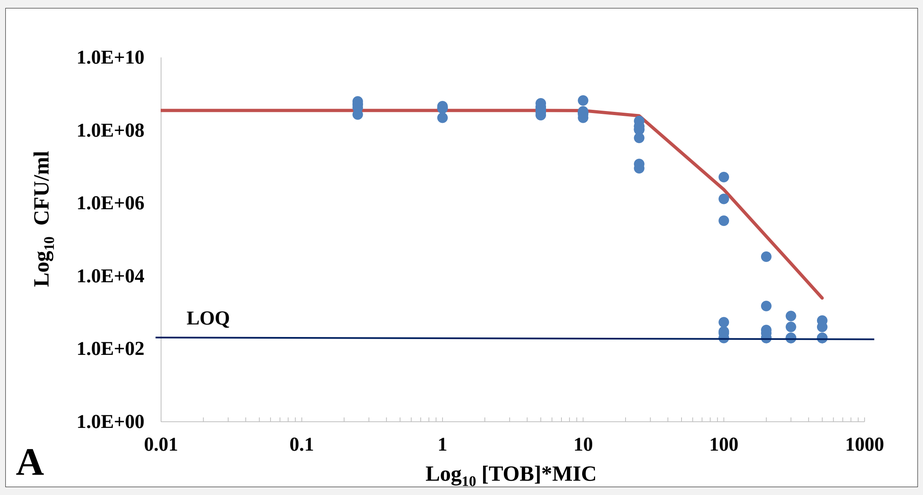


Fig.1: Bioluminescence kinetics of *PA* trapped in calcium alginate beads dispersed in ASM exposed to various COL or TOB concentrations times MIC (*MIC_{TOB} = 0.5 µg/ml , *MIC_{COL} = 1 µg/ml)

- **Above 10 times the MIC, the decrease in bioluminescence was greater and faster with COL compared to TOB**



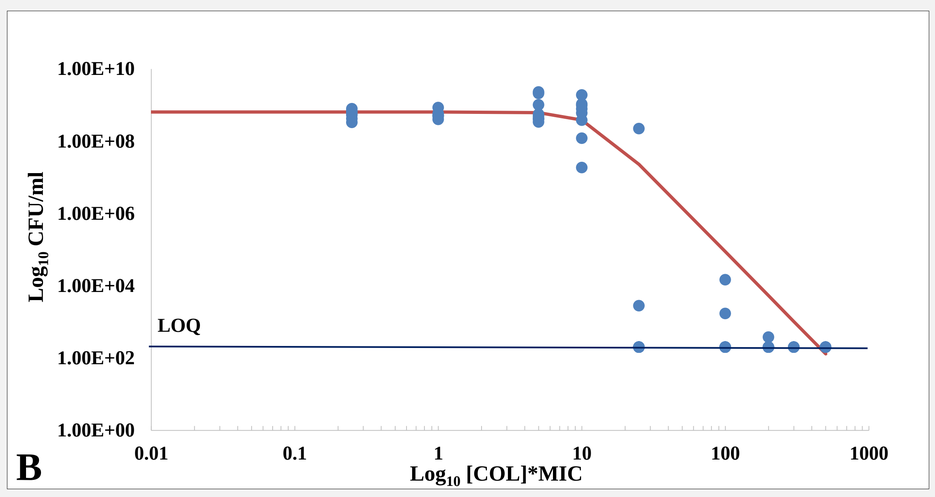


Fig. 2: *PA* concentrations loaded in calcium alginate beads dispersed in ASM after exposure during 40 hours to various TOB (A) or (COL) concentrations expressed in number of times the MIC (*LOQ: Limite of quantification = 200 CFU/ml). The estimated values of the Emax model parameters are shown in Table 1

Table 1 : Parameter estimates for the Emax model plotted with different ATB tested on the in vitro model of PA pulmonary biofilm. Data were expressed as mean ± SD

Parameters	TOB	COL
CFU_0 (*PA* colonies/ml)	3.52E+08 ± 4.4E+07	6.44E+08± 1.59E+08
C_{50} (µg/ml)	30.88 ± 3.26	11.07 ± 1.61
ɣ	4.26 ± 0.18	4.04± 0.18

- **The C_{50} (30.88 ± 3.26 µg / ml) obtained for the TOB was higher than that obtained for the COL (11.07 ± 1.61 µg / ml) suggesting a higher potency of the COL to reduce the bacterial burden in the *PA* pulmonary biofilm model.**
- **After 40H of exposure to ATB , *PA* showed similar MIC value compared to the bacteria before being treated with these ATBs**

Adaptation of *PA* to the presence of TOB

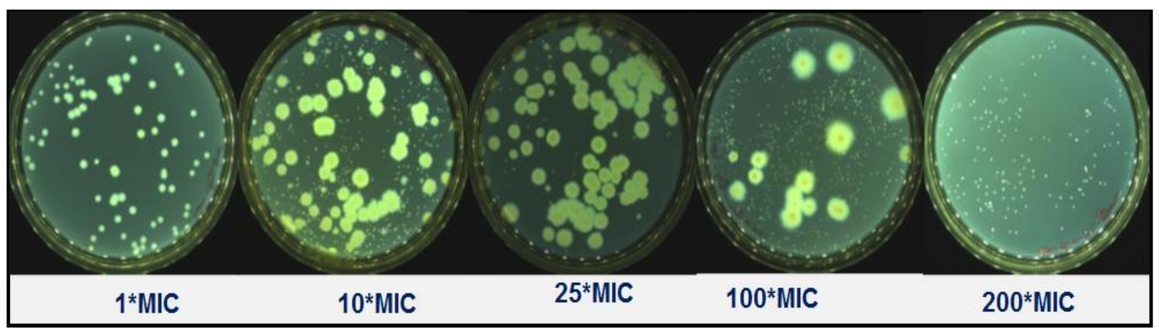


Fig. 3: Morphology of 72-hour PA colonies on Müller-Hinton agar obtained from PA extracted from the *in-vitro* pulmonary biofilm model exposed 40 h to TOB.

- **Two phenotypes of colonies were observed**
- ✓ **Normal and Small colony variant** "SCV" (Described in CF patients with chronic pulmonary infections [6])
- ✓ **The bacteria of both types of colonies were bioluminescent**
- ✓ **SCV appeared only after 48 h of plating on Müller-Hinton agars**
- ✓ **SCV had similar MIC value compared to the MIC of bacteria before treatment with TOB**
- ✓ **Percentage of small colonies relative to total number of colonies increased with TOB concentration**

Conclusion & Perspectives

- **Bioluminescence measurements and colony counts show that COL was more effective than TOB on an *in-vitro model* of *PA* pulmonary biofilm , suggesting a better clinical efficacy of COL than TOB when treating these biofilms**
- ***PA* may persist in biofilms even when exposed to high concentration of TOB without developing resistance to this antibiotic.**

References

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