Pharmacokinetic/pharmacodynamic modelling of the quickly occurring and reversible adaptive resistance of *Pseudomonas aeruginosa* to colistin using a bioluminescent strain *in vitro*.

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Introduction

The pharmacodynamics (PD) of antibiotics is usually studied by observing changes in viable counts of colony forming units (CFU) over time as a function of concentrations.

Bioluminescence is a non-destructive, real-time reporter of bacterial metabolism that can be used for instantaneous bacteria counting [1].

The aim of this study was to investigate the PD of colistin on a bioluminescent strain of *P. aeruginosa* with a special focus on the quickly occurring changes in colistin PD.

Methods

Time-kill curves experiments:

- A strain of *P.aeruginosa* rendered bioluminescent by the luxCDABE operon was used (CNRS GDR3171, Besançon, France).
- The *P. aeruginosa* inoculum was prepared by suspension of the bacteria from a 18-h logarithmic-growth-phase culture in Muller-Hinton broth, adjusted to a final concentration of $5x10^6$ CFU/mL. Colistin was added at conc. of 0, 0.5, 2, 4, 16, 32 and 64 µg/ml. Three replicates were performed.
- At 0, 2, 5, 8, 24 and 30h, bioluminescence was measured by a luminometer (IVIS, Caliper Life Sciences, Hopkinton, MA) and CFUs were counted on Muller-Hinton agar. At 30h the bacteria exposed to 2 µg/mL of colistin were harvested by centrifugation (5000 t/min, 10min) and washed 2 times with NaCL solution. The resulting bacteria were plated at 37°C on free-drug Muller-Hinton agar for a washout period of 0 (immediate re-exposure to colistin), 18, 42 or 66 h.
- After the washout period on free-drug agar, a new bacterial suspension was prepared. Colistin was added to obtain concentration of 32, 64, 128, 256, 512 µg/mL. Bioluminescence was measured at 0, 2, 5, 8, 24 and 30h.
- Mechanism of resistance was explored by sequencing the *pmr*B gene of bacteria exposed 30h to colistin 2 µg/mL.

Data Analysis and model building:

- Relationship between bioluminescence and CFU counts was modelled with a power model.
- Bioluminescence data were transformed into CFU counts (CFU*eq*) and fitted with the PD model presented in Figure 1 [2]. Bacteria were initially in a susceptible state (AR_{off}) and when exposed to colistin switched to a resistant state (AR_{on}). All data were log-transformed and fitted using NONMEM7 with LAPLACIAN and M3 method for handling data below the limit of quantification.

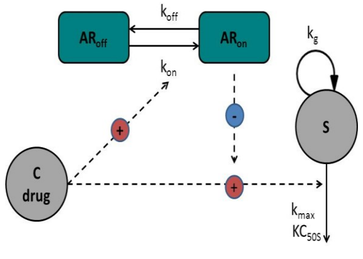

Figure 1. Pharmacodynamic model

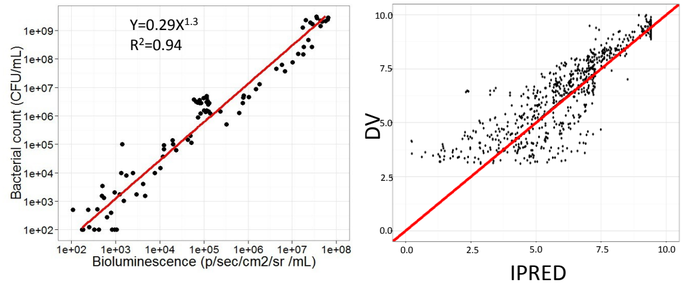

Figure 4. Diagnostic plots

Results

- Without prior exposure to colistin, CFU*eq* decayed rapidly but then regrew in a 0.5-16 µg/mL range of concentrations (Fig. 2 left).
- The *pmr*B gene was mutated in bacteria isolated 30h after the initiation of colistin exposure.
- After exposure for 30h to colistin 2 µg/mL and without wash-out, an initial decay was observed when colistin concentrations were at least equal to 128 µg/mL and a regrowth were observed for colistin concentrations up to 256 µg/mL (Fig.2 right).
- Washout periods allowed bacteria to progressively and partially recover their initial sensitivity (Fig. 3). The half-life of resistance reversal was estimated at 17h by modelling approach.
- A PK-PD model was selected based on modeling criterion and provided a satisfactory fit of the experimental data (Fig.4).
- However this model does not reflect the complex mechanism involving stable mutation with reversible loss of sensitivity.

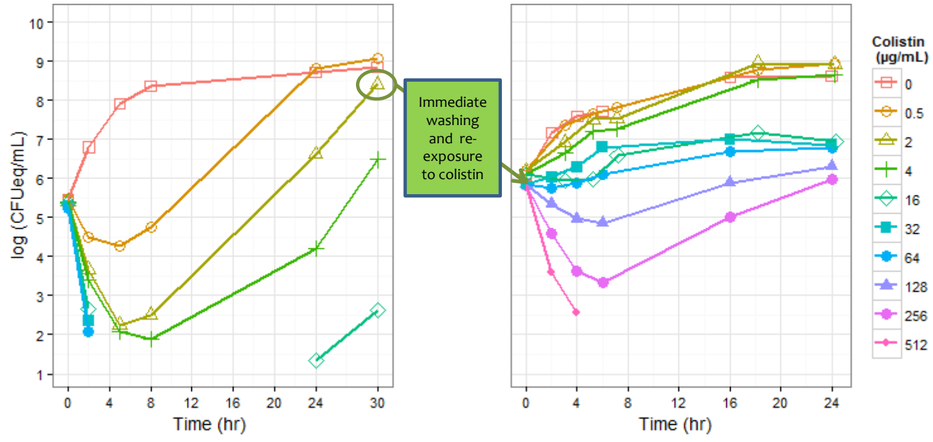

Figure 2. Kill curves for *P.aeruginosa* non pre-exposed to colistin (left) and pre-exposed for 30h to 2 µg/mL of colistin (right)

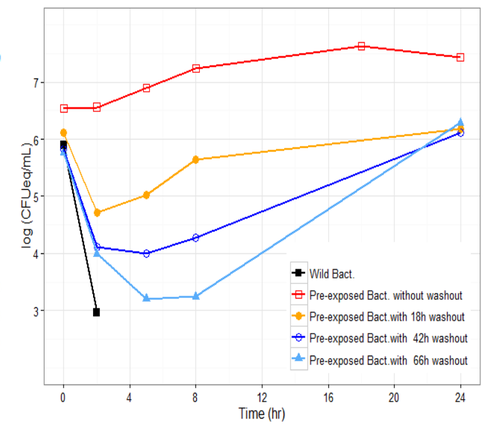

Figure 3. Kill curves obtained at 32 mg/L of colistin for *P.aeruginosa* pre-exposed for 30h to 2 µgm/L of colistin and without or after 18, 42 or 66h of washout

Conclusions

- Using bioluminescence bacteria constitute an effective approach to assess the rapid and reversible changes in antibiotics PD.
- This study has demonstrated that in the presence of colistin (0.5 – 16 µg/mL) a mutation of the *pmr*B gene occurred within 30h, leading to a stable mutant with considerably reduced sensitivity to colistin. However this phenomenon was reversible since the initial sensitivity was progressively recovered after colistin was retrieved from the medium. A PK-PD model was successfully used to describe this reversible phenomenon, although the underlying mechanism has not been fully characterized yet.

References

[1] Greer L.F. et al. Luminescence. 2002 17(1):43-74
[2] Mohamed AF et al. Antimicrob Agents Chemother .2012; 56, 179-188.