# Ciprofloxacin/amikacin combination therapy: evaluation in biofilms of Pseudomonas aeruginosa overproducting efflux pumps

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## Introduction

- **Eradicating biofilm-related infections without mechanical biofilm dispersal remains a challenge**
- **The contribution to tolerance of low-level resistance related to the over-expression of efflux systems has not been evaluated in biofilms**
- **Combination therapies are still considered as an approach to enhance killing of biofilm-embedded cells and minimize the emergence of resistance**

### ❖ Objectives:

**Efficacy of ciprofloxacin and amikacin used separately and in combination, against *P. aeruginosa* planktonic and biofilm cultures**

**Impact of efflux systems on antibiotic (ATB) efficacy in planktonic and biofilm cultures**

## Methods

❖ **PAO1, a wild type clinical strain (WT), and 3 clinical strains overexpressing the efflux MexAB-OprM (AB), MexXY-OprM (XY) and MexCD-OprJ (CD)**

❖ **Planktonic and 2-day-old biofilm (6-wells plate)** cultures of *P. aeruginosa*

→ challenged with **ciprofloxacin** (4 mg/L) and **amikacin** (40 mg/L) separately, in combination, and successively

❖ Number of viable and resistant cells determined on MH2 without ATB and supplemented with 4-fold MIC of ciprofloxacin or amikacin

❖ **Characterization of resistant mutants** = ATB susceptibility, QRDR sequencing, and efflux pumps gene expression

❖ Determination of ciprofloxacin and amikacin **group's MICs** (by recovering surviving cells from biofilms on MH2 plates after 72 h of ATB exposure)

❖ Measurement of ciprofloxacin and amikacin concentrations in the biofilm at the end of the ciprofloxacin and amikacin exposure

## Results

### ❖ Strains

**Table 1.** Main characteristics of the strains used.

| | MIC (mg/L) | | | | Mutation frequency | Mean relative gene expression | | |
|---|---|---|---|---|---|---|---|---|
| | TIM (S≤16 -R>16) | CIP (S≤0.5 -R>0.5) | LVX (S≤1 -R>1) | AMK (S≤8 -R>16) | | *mexB* | *mexY* | *mexC* |
| PAO1 | 16 | 0.125 | 0.25 | 2 | $1.8 \times 10^{-8}$ | 1.0 | 1.0 | 1.0 |
| WT | 16 | 0.06 | 0.25 | 2 | $3.4 \times 10^{-9}$ | 1.7 | 1.1 | 0.3 |
| AB | >256 | 0.06 | 1 | 2 | $8.1 \times 10^{-9}$ | 3.6 | 5.3 | 0.7 |
| CD | 4 | 0.5 | 4 | 0.5 | $3.5 \times 10^{-8}$ | 1.3 | 1.7 | 14.6 |
| XY | 32 | 0.125 | 1 | 16 | $2.9 \times 10^{-9}$ | 1.3 | 10.1 | 4.1 |

S, susceptible; R, resistant; TIM, ticarcillin/clavulanate; CIP, ciprofloxacin; LVX, levofloxacin; AMK, amikacin

- **PAO1, WT, AB, and CD were susceptible to ciprofloxacin and amikacin**
- **XY strain was intermediate to amikacin (CMI = 16 mg/L)**
- No mutation in the QRDR
- No hypermutable strains
- AB, CD and XY respectively overexpressed MexAB-OprM, MexCD-OprJ and MexXY-OprM

### ❖ Planktonic time-kill assay

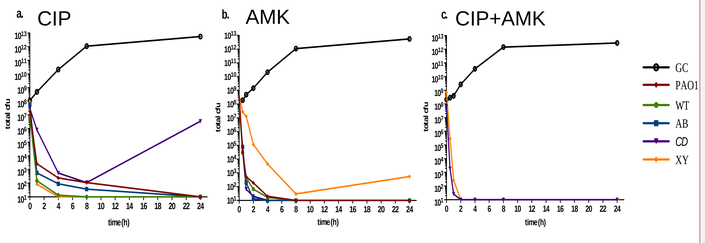


**Figure 1.** Planktonic time-kill assays with ciprofloxacin (a), amikacin (b) and the combination of ciprofloxacin and amikacin (c).
The total cfu numbers are the mean value for three different experiments.
GC: growth control

- ➢ **≥3 $\log_{10}$ cfu/mL reduction** after 4 h of ciprofloxacin or amikacin in all strains
- ➢ Regrowth of high-level resistant mutants:

**- when CD was exposed to ciprofloxacin** (mutations in the QRDR of GyrA [Thr-83->Ile] and ParC [Ser-80->Leu ])

**- when XY was exposed to amikacin**

- ➢ **Eradication with a ciprofloxacin + amikacin combination in XY and CD strains**
- ➢ Eradication with ciprofloxacin or amikacin in all the other strains

### ❖ Biofilm time-kill assay

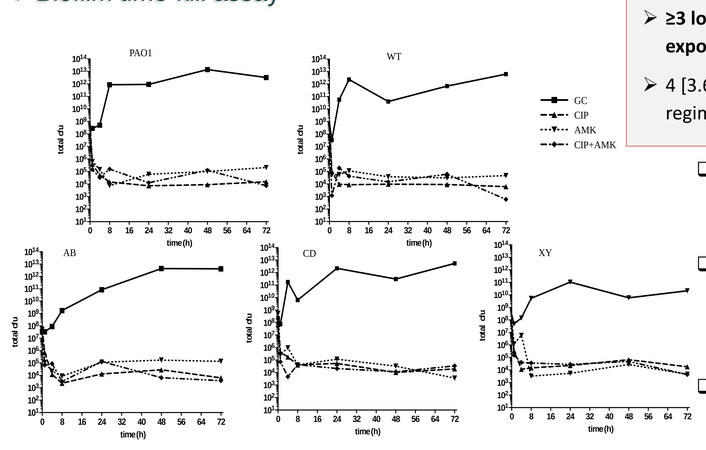


**Figure 2.** Biofilm time-kill assays with ciprofloxacin, amikacin and the combination of ciprofloxacin and amikacin.
The total cfu numbers are the mean value for three different experiments.
GC, growth control; CIP, ciprofloxacin; AMK, amikacin

- ➢ **≥3 $\log_{10}$ cfu/mL reduction** in all strains **after 8 h of ATB exposure** (similar killing rates for all regimens [p>0.05])
- ➢ 4 [3.62 – 4.17] $\log_{10}$ cfu/mL **plateau** in all strains and for all regimens after 8 h and until 72 h of ATB exposure

- ❑ **No MIC creep : group's MICs** for the surviving cells after 72 h of ATB exposure **unchanged compared to pre-exposure MIC**
- ❑ Erratically, for all regimens including combination therapy, 4-fold MIC ciprofloxacin or amikacin resistant mutants isolated from detached biofilm: low density, no mutation in the QRDR, and **no bacterial regrowth**
- ❑ Sequential ATB exposure: bacterial reduction after the first ATB exposure significantly higher than after the second ATB exposure ($p<0.05$)
- ❑ **Stable ATB concentrations along time:** no ATB degradation according ciprofloxacin and amikacin concentration measurements

## Conclusion

❖ **The ciprofloxacin-amikacin combination enhances killing and prevents emergence of resistance for low-level resistant strains in planktonic cultures, but not in biofilm.**

❖ The low-level resistance conferred by MexCD-OprJ and MexXY-OprM efflux pumps may reduce intracellular antibiotic concentrations and hence facilitate the selection of high-level resistant mutants by target site mutations ,

→ In planktonic conditions, we suggest that the **MIC value of ciprofloxacin** should be determined, and **levofloxacin evaluated**, in order to detect MexCD-OprJ overexpression.

❖**In biofilm, the lack of bacterial eradication was related to an antibiotic-recalcitrant population composed of persister cells, refractory to antibiotics used alone or in combination.** No regrowth due to high-level resistant mutant was detected whatever the antibiotic/strain pair studied.