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The putative de-*N*-acetylase DnpA increases intracellular and biofilm-associated persistence upon fluoroquinolone exposure in *Pseudomonas aeruginosa*

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Introduction & Purpose

Persisters are antibiotic-treated bacteria that are transiently refractory to antibiotic killing (1). They are associated with dormant lifestyles and cause treatment failures.

The putative de-N-acetylase DnpA (unknown substrate) has been shown to increase persister levels in *P. aeruginosa* exposed to fluoroquinolones in broth (2).

This study assesses the possible role of DnpA in the poor efficacy of antibiotics against *P. aeruginosa* in two models of persistent infections (intracellular infection & biofilms).

Materials & Methods

Bacterial strains: PAO1 and its *dnpA* deletion mutant (1).

Extracellular persistence assay: bacteria exposed to antibiotics at 50xMIC for 5 h; persister fraction calculated as the ratio of cfu for antibiotic-exposed bacteria to controls (2).

Intracellular activity: 24 h incubation of infected human THP-1 monocytes with antibiotics (0.001-200 mg/L) to obtain a full concentration-response curves, allowing to calculate E_{max} (3).

Activity against biofilms: 24h incubation of biofilms with antibiotics (same conc. range); residual viability assessed by metabolic assay (fluorescein diacetate hydrolysis [4]).

Confocal microscopy: biofilms with GFP-expressing strains.

Gene expression: quantitative reverse transcription PCR.

Acknowledgments

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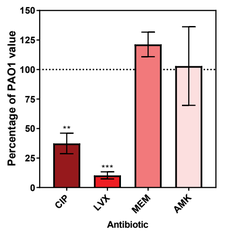
This poster will be made available after the meeting at http://www.facm.ucl.ac.be/posters.htm

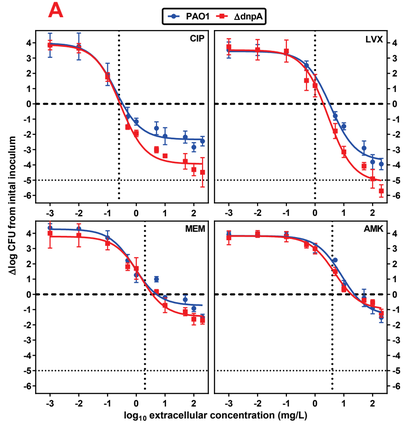
Results

1. Persisters in broth



Relative persister fraction for extracellular ΔdnpA expressed as percentage of the value measured for PAO1. Bacteria were exposed during 5 h to antibiotics at a concentration of 50x MIC).

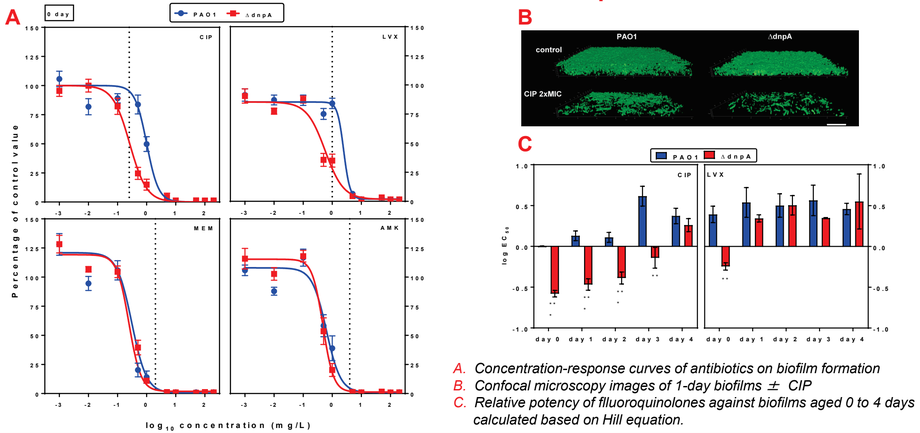
2. Intracellular activity of antibiotics



A. Concentration-response curves of antibiotics against intracellular P. aeruginosa. Vertical dotted lines: MIC

B. Emax calculated based on Hill equation.

3. Antibiotic effect on biofilm formation and preformed biofilm

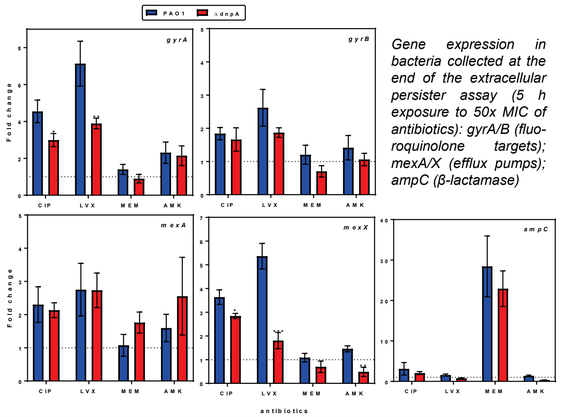


A. Concentration-response curves of antibiotics on biofilm formation

B. Confocal microscopy images of 1-day biofilms ± CIP

C. Relative potency of fluoroquinolones against biofilms aged 0 to 4 days calculated based on Hill equation.

4. Influence of antibiotics on gene expression



Gene expression in bacteria collected at the end of the extracellular persister assay (5 h exposure to 50x MIC of antibiotics): gyrA/B (fluoroquinolone targets); mexA/X (efflux pumps); ampC (β-lactamase)

1. **Extracellularly,** less persisters in Δ*dnpA* than PAO1 specifically after exposure to fluoroquinolones
2. **Intracellularly,** higher efficacy (more negative E_{max}) against Δ*dnpA* than POA1 when exposed to fluoroquinolones only
3. **In biofilms,** higher potency (lower EC_{50}) against young biofilms from Δ*dnpA* than from POA1 when exposed to fluoroquinolones only
4. **Gene expression**: lower induction of *gyrA* (fluoorquinolone target) in Δ*dnpA* than in POA1 when exposed to fluoroquinolones

Conclusions

DnpA contributes to persistence of *P. aeruginosa* exposed to ciprofloxacin intracellularly or in biofilms. The underlying mechanism could involve the overexpression of the fluoroquinolone target.

Inhibiting DnpA is an attractive strategy to improve fluoroquinolone activity in persistent infections.

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