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P1525 The putative de-N-acetylase DnpA increases intracellular and biofilm-associated persistence upon fluoroquinolone exposure in Pseudomonas aeruginosa

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Background: Persisters are antibiotic-treated bacteria that are transiently refractory to antibiotic killing, associated with dormant lifestyles and causing treatment failures. The putative de-*N*-acetylase DnpA has been shown to increase persister levels in *P. aeruginosa* exposed to fluoroquinolones in broth (Liebens *et al*., Pathog Dis 2014;71:39-54). This study assesses the possible role of DnpA in the poor efficacy of antibiotics against *P. aeruginosa* intracellularly and in biofilms.

Materials/methods: PAO1 and its *dnpA* deletion mutant (Δ*dnpA*) were used. Extracellular persistence assay: bacteria were exposed to antibiotics at 100xMIC for 5h; the persister fraction was calculated as the ratio of cfu for antibiotic-treated bacteria to the untreated control. Intracellular activity: infected human THP-1 monocytes were incubated for 24h with antibiotics (0.001-200 mg/L) to obtain a full concentration-response curve, allowing the calculation of E_{max} (Buyck *et al.*, AAC 2013;57:2310-8). Activity against biofilms: 24h biofilms were exposed to the same range of antibiotic concentrations and residual viability assessed by metabolic assay (fluorescein diacetate hydrolysis). Gene expression: quantitative reverse transcription PCR.

Results: See Table. When exposed to ciprofloxacin (but not meropenem or amikacin), the Δ*dnpA* mutant showed a decreased persister fraction in broth. In parallel, ciprofloxacin (but not the other drugs) was more effective intracellularly, more potent in biofilms, and caused lower overexpression of *gyrB* in the Δ*dnpA* mutant than in PAO1.

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.

Antibiotic	Extracellular Persister fraction Δ*dnpA*/PAO1	Intracellular efficacy Emax (log cfu)[a]		Potency in biofilm C_{50} (xMIC)[b]		Expression of *gyrB*[c]	
		Δ*dnpA*	PAO1	Δ*dnpA*	PAO1	Δ*dnpA*	PAO1
ciprofloxacin	0.37±0.17*	-4.0±0.2*	-2.3±0.1	1.4±0.2*	5.4±0.0	3.0±0.4*	4.5±0.6
meropenem	1.21±0.17	-1.3±0.3	-0.7±0.3	1.3±0.1	1.3±0.1	0.9±0.2	1.4±0.3
amikacin	1.02±0.57	-1.3±0.7	-1.2±0.4	0.6±0.1	0.4±0.1	2.1±0.5	2.3±0.6

[a] maximal cfu decrease for an infinitely large antibiotic concentration
[b] concentration causing 50% reduction in viability
(both calculated from Hill functions fitted to concentration-response data)
[c] ratio to control culture
* $p<0.05$ when comparing Δ*dnpA* to PAO1 (value set to 1 for extracellular assay)