Resistance to colistin in *Pseudomonas aeruginosa* biofilms is modulated by the *arn* operon and pH

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Colistin is a cyclic antimicrobial peptide that interacts electrostatically with the lipid A moiety of LPS leading disruption of the outer membrane (OM) and, ultimately, cell death. In *Pseudomonas aeruginosa,* remodelling of the OM through aminoarabinosylation of lipid A leads to the emergence of colistin-resistant strains. Additionally, we observed that biofilms formed by colistin-sensitive strains were resistant to this drug. Considering that *P. aeruginosa* lives in a mild acidic environment in the airways of patients with cystic fibrosis, we observed greater biofilm resistance at pH 6 compared to pH 7, with a 2- to 4-fold increase in the colistin MBIC (Minimal Biofilm Inhibition Concentration). Biofilm resistance to colistin would suggest an upregulation of the *arn* operon. Accordingly, we observed that arnT, the last enzyme involved in aminoarabinosylation, was markedly upregulated in biofilms compared to planktonic cells in both reference and clinical *P. aeruginosa* Col^S strains. Furthermore, biofilm resistance to colistin was markedly lower in in *Δarn* mutant respect to the parental wt strains, with an average 8-fold reduction in MBIC. Similarly, inhibition of ArnT activity by FDO and FDO-H (doi:10.1093/jac/dkaa200; 10.1021/acs.joc.0c01459) significantly reduced MBIC values in all tested clinical isolates. Overall, our results demonstrate that upregulation of the *arn* operon contributes to intrinsic colistin resistance in *P. aeruginosa* biofilms, irrespective of the development of resistance in planktonic cells. Targeting the ArnT enzyme appears to be a promising strategy for restoring the efficacy of colistin in clinical settings where biofilm-associated infections are prevalent, such as chronic pulmonary colonization in cystic fibrosis.