Title: Targeted inhibition of small RNA00203 disrupts biofilm-mediated antibiotic resistance in *Acinetobacter baumannii*

Authors: Abebe Mekuria SHENKUTIE[a,b] , Daniel Gebrelibanos [a,] , Pao Ho lai [a,] Polly H.M. Leung [a]

a Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong SAR, China

b Department of Microbiology, Immunology, and Parasitology, St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

E-mail; abebe.shenkutie@polyu.edu.hk

Phone: +85256140696

Background: Biofilm-mediated antibiotic resistance in *A. baumannii* complicates treatment of infections. This study aimed to investigate biofilm-mediated antibiotic resistance & assess the therapeutic potential of antisense oligomers targeting sRNA00203.

Methods: A total of 104 *A. baumannii were* evaluated for biofilm-forming ability & its susceptibility. Comparative transcriptome analysis of untreated & treated biofilm cells of *A. baumannii* was performed. The sRNA00203 & its predicted mRNA target were identified & characterized, & the gene encoding sRNA00203 was deleted to evaluate its impact on biofilm-mediated antibiotic resistance. The five sets of antisense oligomers for genes encoding sRNA00203 were designed, synthesized & evaluated for their effect on biofilm-mediated antibiotic resistance.

Results*:* Analysis 104 *A. baumannii* revealed 59.6% formed biofilms; of these, 66.1% were non-multidrug resistant (non-MDR). The minimum biofilm eradication concentrations for colistin, ciprofloxacin, & imipenem were 44-, 407-, & 364-fold higher than planktonic minimum bactericidal concentrations (MBC), respectively. The reversibility test for antibiotic susceptibility showed biofilm formation induced reversible antibiotic tolerance in non-MDR strains but a higher level of irreversible resistance in extensively drug-resistant strains. The RNA sequencing data showed 5 of 138 small RNAs (sRNAs) were differentially expressed in biofilm. Of these, sRNA00203 exhibited the highest expression levels in biofilm. Impairment of sRNA00203 reduced biofilm biomass by 85%. & minimum biofilm inhibitory concentrations for imipenem & ciprofloxacin by 1024- & 128-fold, respectively. Deletion of sRNA00203 downregulated genes involved in biofilm matrix synthesis (*pgaB*), efflux pump production (*novel00738*), lipopolysaccharide biosynthesis (*novel00626*), preprotein translocase subunit (secA), & CRP transcriptional regulator. Among the five sets of antisense oligomers synthesized, an oligomer complementary to the 9-23 nt sequence of sRNA00203 inhibited biofilm formation at 2.5 μM compared with scrambled controls (>20 μM) in multidrug-resistant strains.**Conclusion**: The findings identified sRNA00203 as an essential regulator of biofilm-mediated resistance and showed the potential for CPP-PNA-based strategies to disrupt the biofilm-mediated antibiotic resistance pathway. Further *in vivo* models & clinical trials are needed to validate the use of antisense oligomers complementary to 9-23 nt of the sRNA00203 sequence to treat biofilm mediated infection.

Keywords: Acinetobacter baumannii, biofilm, antibiotic resistance, sRNA00203, CPP-PNA, gene-targeted therapy