The Role of Psl in Failure to Eradicate *Pseudonomas aeruginosa* in Children with Cystic Fibrosis

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Background

Cystic fibrosis (CF) is a genetically inherited, multisystem disorder, resulting in chronic bacterial infection, inflammation, and premature respiratory failure.[1–3] *Pseudomonas aeruginosa* (PA) is one of the most prevalent bacteria in CF pulmonary infections, and it produces an exopolysaccharide Psl which is important in biofilm formation and cell-to-cell interactions.[4–7] Psl production is also associated with resistance to antibiotics such as colistin, ciprofloxacin, and tobramycin.[8] Potential mechanisms through which it confers resistance include protective barrier effect, prevention of complement deposition and opsonization, and inhibition of phagocytosis by neutrophils.[8–10] It was previously demonstrated that increased anti-Psl antibody binding by Psl0096, a class I epitome binding monoclonal antibody (mAb), was associated with greater PA aggregation and tobramycin tolerance.[11] To validate these previous findings, the objective of this study was to examine PA isolates from sputum samples of patients with CF who either failed or successfully completed antibiotic eradication therapy. The isolates were examined in the *in vitro* chamber slide model for PA biofilm formation and Psl binding and assessed for tobramycin tolerance.

Methods

Children with CF confirmed for new onset PA infections at SickKids Hospital were enrolled in this study. New onset infection was defined as a PA positive sputum culture with at least three preceding negative cultures in the last 12 months. Their sputum was taken 28 days prior to starting antibiotic eradication therapy, and PA isolates were recovered on MacConkey agar with crystal violet which is selective for PA. If the patient's sputum culture was positive for PA six months after the initial positive culture, the isolate was defined as persistent. If it was negative for PA, the isolate was defined as eradicated. In total, seven eradicated and four persistent PA isolates were recovered then grown as biofilms in 8-well slide chambers. After exposure to SYTO9 and fluorescent labelled anti-Psl mAb Psl0096, biofilms were imaged via confocal microscopy and fluorescent labels were quantified using the Volocity software. To examine tobramycin tolerance, PA isolates grown as biofilms were exposed to tobramycin with and without 5% sputum supernatant (SS). Following tobramycin treatment, biofilms were stained with SYTO9, and confocal images were captured. Change in metabolic activity of the isolates was also measured using an ATP assay.

Results

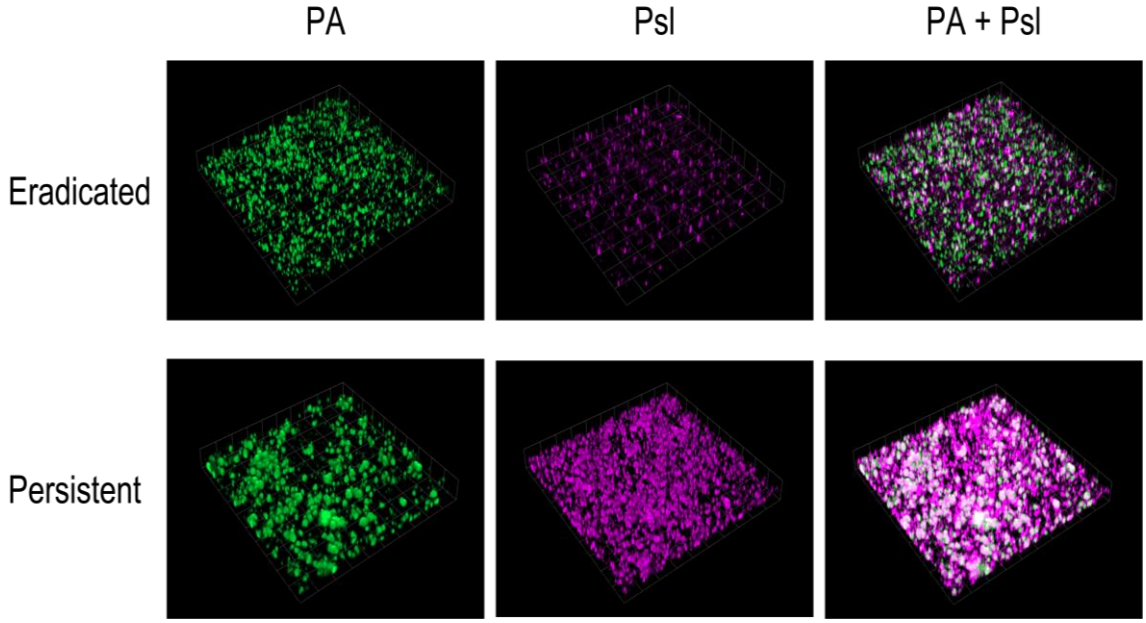

Figure 1. **Representative confocal images of persistent and eradicated Pseudomonas aeruginosa (PA) isolates grown as biofilms in a slide chamber model.** PA is labelled with SYTO9 (green) and anti-Psl mAb Psl0096 is labelled as magenta. Green and magenta channels are shown separately and together. Colocalized areas between green and magenta are seen as white. Images were acquired with a Quorum Spinning Disk Confocal Microscope under a 20X air lens.

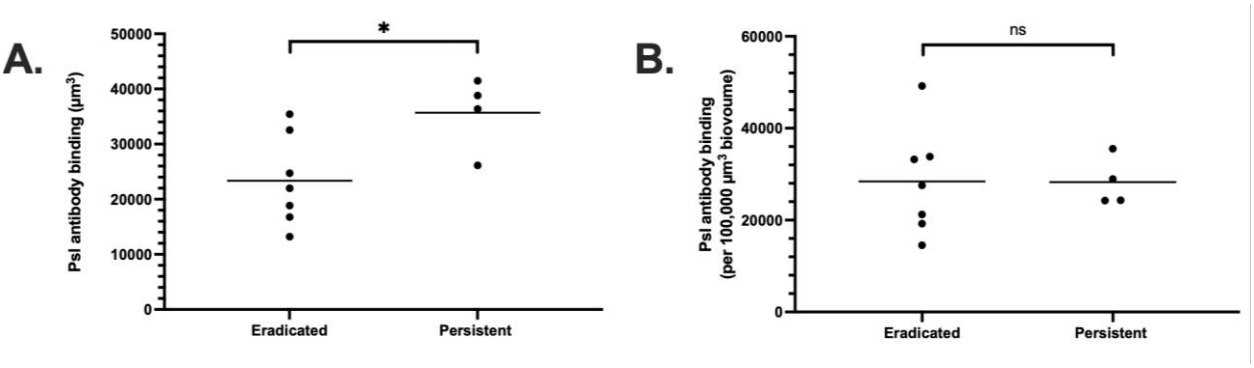

Figure 2. **Psl binding in persistent versus eradicated *Pseudomonas aeruginosa* (PA) isolates.** (A) Biofilms of persistent PA isolates had significantly greater Psl antibody binding than eradicated isolates. However, (B) once normalized per 100,000 µm^3 of PA biovolume, the amount of anti-Psl antibody binding did not differ between the two types of isolates. (A, B) Each data point represents a mean of three biological replicates. Statistical analysis by Mann-Whitney U-test. *p<0.05, ns: not significant

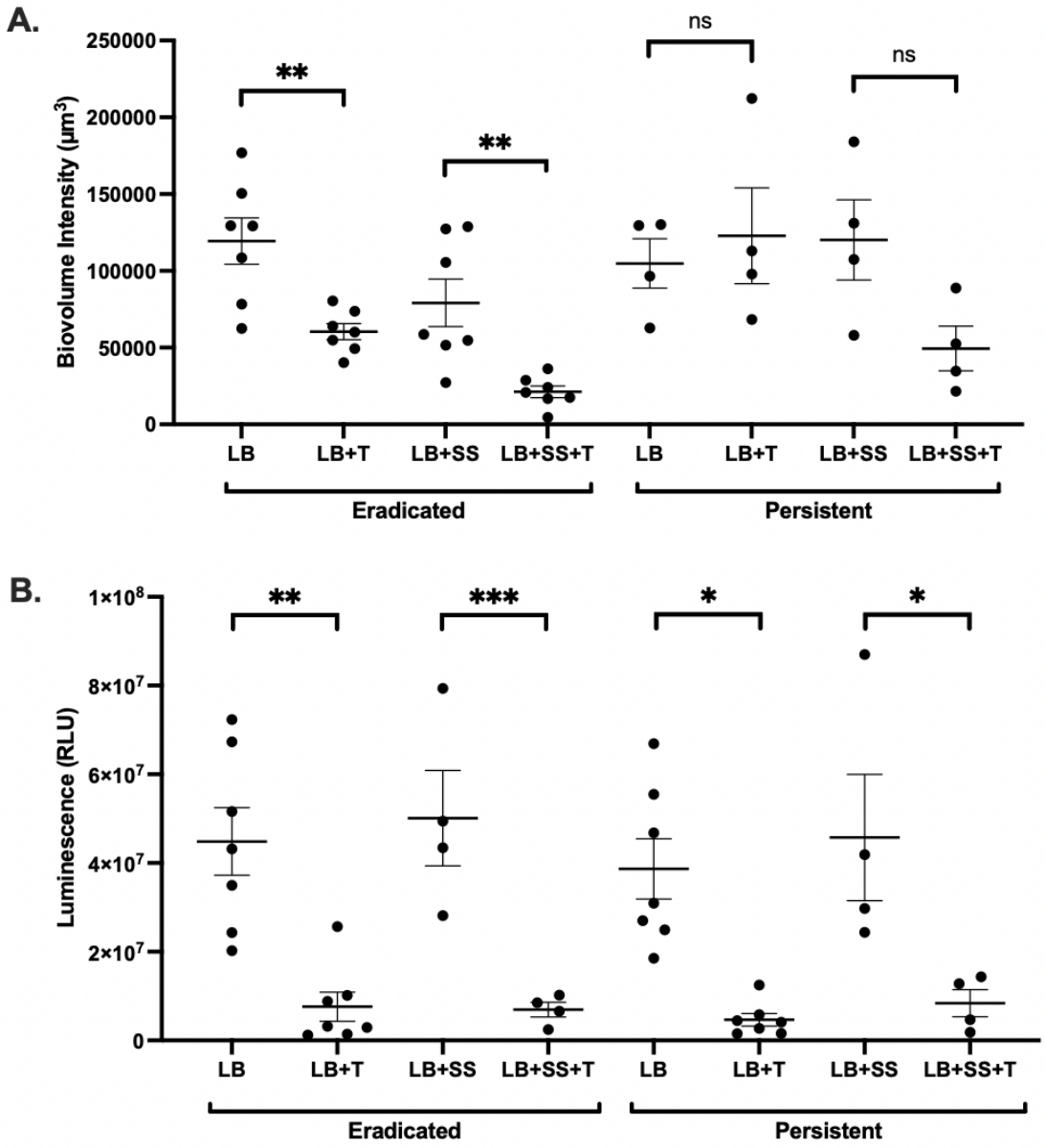

Figure 3. **The effect of tobramycin on *Pseudomonas aeruginosa* (PA) isolates**. (A) PA biovolume was measured after treatment with tobramycin (T) (1000 µg/ml) in the absence or presence of 5% sputum supernatant (SS). SS was pooled from all the CF sputum samples. Means are plotted with standard error of the mean. (B) ATP luminescence in relative light units (RLU). Statistical analysis by Mann-Whitney U test. *p<0.05, **p<0.01, ***p<0.001, ns: not significant

Discussions

This study examined differences in Psl antibody binding in persistent versus eradicated PA isolates and the effect of tobramycin on these isolates. Anti-Psl antibody binding was greater in persistent isolates, however, once normalized per 100,000 µm^3 of PA biovolume, it was similar between the

eradicated and persistent isolates. This contrasted our previous findings which showed greater anti-Psl antibody binding in persistent PA isolates even after adjusting for biovolume.[11] The change in biovolume after tobramycin exposure was only significant in the eradicated isolates. However, ATP assay showed that tobramycin significantly reduced the metabolic activity in both groups grown with or without SS, thus, we could not consistently demonstrate tobramycin tolerance. An area of weakness of this study is limited sample size (n=11). Additionally, a live/dead ratio to determine the proportion killed by tobramycin would have been useful in interpreting the experiment results and the effect of the antibiotic on the PA isolates.

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