Proposal: The role of *Pseudomonas aeruginosa* and the lung microbiome in *Pseudomonas* eradication in cystic fibrosis

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Research Objectives:

1. To compare the photyic characteristics of persistent vs. eradicated *Pseudomonas aeruginosa* in cystic fibrosis
2. To compare the pulmonary microbiome in CF patients in whom *Pseudomonas aeruginosa* is eradicated vs. in CF patients in whom *Pseudomonas aeruginosa* persists following tobramycin inhalation therapy

Background: The most common cause of death in cystic fibrosis (CF) patients is respiratory failure due to chronic bacterial pulmonary infection(1). The majority of CF patients are ultimately chronically infected with *P. aeruginosa* (PA) in their lungs, which is associated with more rapid lung function decline and increased mortality (2-4). As children with CF age, an increasing number will acquire PA infection. The current standard of care is to try to eradicate new PA acquisition, usually with inhaled tobramycin treatment, in order to prevent its long-terms deleterious effects(5-6). However, typically, in 10-40% of patients, eradication treatment fails(7-8). There is little known about whether there are specific bacterial characteristics of the original infecting PA organism or properties of the co-infecting microbial community (microbiome), that contribute to the persistence of PA in the lung and eradication failure (9). We need to understand these factors in order to learn how to better eradicate PA from children with CF and prevent its harmful effects.

Materials and Methods: From 2011-2013, 38 CF patients had new (incident) PA infection cultured from sputum samples; 31 subsequently cleared their PA infection following inhaled tobramycin therapy whereas in 7 patients, PA infection persisted (18% eradication failure rate). The original sputum samples from these 38 patients have been saved as part of the CF Sputum Biobank (established by the PI). The applicant L.S. recovered PA from frozen sputum samples for 33 of them (PA did not grow in 5 patients). A maximum of 3 colonies from each morphotype were sampled (total 128 colonies from 53 morphotypes). She tested each colony in triplicate for the following phenotypic characteristics: antimicrobial susceptibility, mucoidy status, protease production, swimming and twitching motility. The average result for all colonies for each morphotype is summarized in **Table 1**:

	Eradicated PA n=41 morphotypes	Persistent PA n=12 morphotypes	P-value
No. of patients	28	5	N/A
No. morphotypes/patient, mean (range)	2.13 (1-3)	2.25 (1-3)	0.86
Protease production (mm), median (range)	12.67 (0-20.5)	11.39 (0-21)	0.47
Twitching motility (mm), median (range)	27.94 (0-52)	14.17 (0-29.5)	0.02
Swimming motility (mm), median (range)	12.46 (0-23)	9.86 (0-24.5)	0.61
Mucoid, n (%)	3 (7%)	4 (33%)	0.04
Tobramycin MIC (µg/ml), mean (range)	2 (2-8)	2 (2-128)	0.09

Mann-Whitney test of comparison for continuous variables; Fisher's exact test for proportions

These preliminary analyses suggest that compared to eradicated PA, persistent PA have less twitching motility and are more mucoid (possibly with higher tobramycin MICs), characteristics of a late infection phenotype. For Objective 1, L.S. will determine the biofilm (and mucoidy) production both in the presence and absence of subinhibitory concentrations of tobramycin and confirm these phenotypic findings in a larger collection of isolates (2013-2014).

The corresponding sputum samples have undergone metagenomic profiling using Serial Illumina sequencing (SI-Seq) as part of a CIHR funded CF Microbiome project. We will examine the bacterial diversity within each sample using the Shannon index and compare the Shannon indices between patients with eradicated vs persistent PA. In addition, we will examine operational taxonomic units (OUT) occurrence and relative abundance by calculating weighted and un-weighted UniFrac and Bray-Curtis (BC) distances for each sample. These distances will then be visualized by PCoA and statistically interrogated via Procrustes analysis to determine whether the communities in patients with eradicated PA differ from those with persistent PA(10).

Summary and Future Directions: The ultimate goal of this study is to determine the microbiome and associated bacterial characteristics that account for the persistence of PA in the lung in order to identify more successful eradication treatments of this harmful pathogen in children with CF.

References

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