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

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Background

Pseudomonas aeruginosa is the most common bacterial infection found to colonize the lungs of cystic fibrosis (CF) patients. These infections are associated with rapid lung function decline and higher mortality rates in pediatric CF patients. In literature and in practice, there is an observed 10-40% failure rate of eradication for first time *P. aeruginosa* infections in these pediatric CF patients. Reasons for this failure rate are not well understood. Some of the many factors that could lead to an explanation for treatment failure are phenotypic bacterial characteristics of the individual *P. aeruginosa* infections. A summer project was proposed to investigate any potential differences in the phenotypic bacterial characteristics of *P. aeruginosa* isolated from sputum cultures taken from CF pediatric patients who were successful vs unsuccessful in eradicating a first time infection. This project was completed over the course of 15 weeks at The Hospital for Sick Children in Toronto under the Division of Infectious Disease.

Objective

The main objective of the study was to determine phenotypic differences in first time infection *P. aeruginosa* isolates that were successfully eradicated compared to those that persisted in the CF lung. We also did some preliminary analyses on the pulmonary microbiome related to eradication in these pediatric patients.

Methods

This was a cross-sectional study from 2011-2014 of children with CF at the Hospital for Sick Children who had incident *P. aeruginosa* infection in the lung. *P. aeruginosa* cultures were isolated from frozen sputum samples taken from the patients. An incident infection was defined as a *P. aeruginosa* sputum culture preceded by a minimum of 4 negative cultures over the span of at least a year without inhaled tobramycin treatment. A persistent infection (unsuccessful eradication) was defined as a positive *P. aeruginosa* sputum obtained after the conclusion of antibiotic treatment. Different morphotypes were identified and recorded for each isolated sputum culture.

Phenotypic characteristics compared between the two groups included swimming motility, twitching motility, mucoid production, protease production, and crystal violet (CV) biofilm assays. Swimming, twitching, mucoid, and protease tests were done for each morphotype in triplicates using specialized plates. CV biofilm assays were done for each morphotype by growing free-floating cultures in 96-well plates and using CV stains to detect biofilm formation in the wells. An antimicrobial characteristic investigated was tobramycin MICs. The MICs were done for each

morphotype using tobramycin concentrations between 0 and 128 ug/mL. Genetic characteristics associated with biofilm production were investigated using PCR methods. The presence of *las* and *rhl* genes that code for synthease proteins in *P. aeruginosa* are thought to be associated with biofilm production. Amplification of these genes by PCR was preformed for each isolate to determine the presence or absence of the genes in the eradicated vs persistent isolates. A Mann-Whitney test of comparison was used for continuous variables and a two-tailed Fisher exact test of comparison was used for proportion values. A p-value of 0.05 was used as an indication of significance. In addition, sputum samples underwent 16S rRNAgene (V4 region, Illumina MiSeq) sequencing to characterize the bacterial community. The relative abundance of *P. aeruginosa* was determined for each sample and bacterial community metrics were compared.

Results

The study included sputum from a total of 43 patients, 10 (23%) of which had a persistent *P. aeruginosa* infection. Preliminary data suggests that *P. aeruginosa* infections that are more likely to persist in the CF lung display more chronic phenotypes akin to those of older established colonies. These phenotypes include less twitching motility, more mucoid, and higher average tobramycin MICs. CV biomass data suggests a trend toward increased biofilm production seen from persistent infections. Phenotypic studies involving biofilm production, genetic indicators for biofilm processes, and pulmonary microbiome are currently in progress. Initial studies of pediatric CF lung microbiome by 16S rRNA sequencing indicated equally diverse microbial populations between eradicated and persistent patient groups (Shannon Diversity Index 4.57 *vs* 4.58, p=0.36).

Table 1: Comparison of phenotypic characteristics of *P. aeruginosa* isolates.

	Eradicated PA n = 65 morphotypes	**Persistent PA n = 14 morphotypes**	**P-value**
No. of patients	43	10 (23%)	N/A
No. morphotypes/patient, mean (range)	1.86 (1-4)	1.56 (1-3)	0.4270
Protease production (mm), median (range)	13.33 (0-210	14.08 (0-19.5)	0.9461
Twitching motility (mm), median (range)	23.75 (0-51)	19.42 (0-28)	0.0223*
Swimming motility (mm), median (range)	12.83 (0-36)	13.92 (0-21)	0.9905
Mucoid, number of colonies (%)	44 (25%)	18 (46%)	0.0094*
Tobramycin MIC (ug/ml), median (range)	2 (2-128)	2 (2-128)	0.0403**

Conclusion

Results from this study suggest that first time *P. aeruginosa* isolates that persist in the CF lung display different phenotype characteristics from those that are eradicated. This preliminary data may be used in conjunction with further studies to develop treatment strategies that could potentially lower the eradication failure rate for *P. aeruginosa.* Next steps include completing data collection and analysis on genetic indicators and biofilm production. Phenotypic studies of *P. aeruginosa* isolates along with studies on CF lung microbiome will further reveal a full, integrated analysis of factors that may influence the treatment resistance of *P. aeruginosa* infections.