Phenotypic characteristics of eradicated vs persistent *Pseudomonas aeruginosa* isolates from children with cystic fibrosis

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Background

Pseudomonas aeruginosa (*P. aeruginosa*) is the most common bacterial pathogen infecting the lungs of cystic fibrosis (CF) patients. Infections are associated with rapid lung function decline and earlier death for these patients. The eradication of first time *P. aeruginosa* infections in pediatric CF patients appears to have a 10-40% failure rate and reasons for this are not well understood.

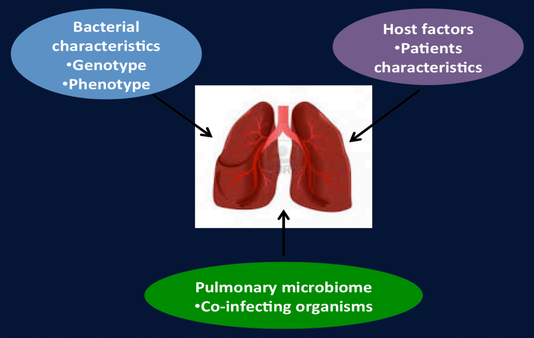


Figure 1: Failure of eradication of first time *P. aeruginosa* may be due to a variety of factors, shown schematically above.

Objective

The goal of this study was to determine if there were differences in the phenotypes of *P. aeruginosa* isolated from first time infection that were successfully eradicated compared to those that persisted in the CF lung

Methods

This was a cross-sectional study from 2011-2014 of children with CF at Sickkids who had incident *P. aeruginosa* infection. Isolates were recovered from frozen sputum samples (CF sputum biobank).

Incident infection: defined as a positive *P. aeruginosa* sputum culture preceded by a minimum of 4 negative cultures over the span of at least a year without inhaled tobramycin treatment.

Persistent infection: defined as positive *P. aeruginosa* sputum culture obtained after conclusion of antibiotic treatment.

Phenotypic	Antimicrobial	Genetic
Swimming, twitching, mucoid, protease, CV biofilm	Tobramycin MICs	PCR for biofilm

- 1 to 3 morphotypes of *P. aeruginosa* were isolated from each sputum
- 3 colonies per morphotype were assessed for phenotypic assays
- Experiments for phenotypic characteristics were performed in triplicate
- Antimicrobial and genetic assays done for each morphotype

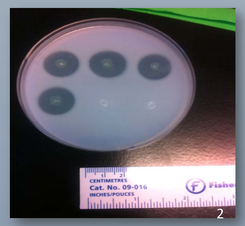


Figure 2 : Protease production – diameters of clear halo around inoculum point measured in millimeters.

Figure 3: Swim motility – cloudy halo around inoculum points measured in millimeters.

Figure 4: Twitch motility - crystal violet stain used to visualize and measure twitch halo after agar is removed.

Figure 5: Mucoid status – mucoid (top) and non-mucoid (bottom) strains on a YEM plate.

Results

Figure 6: Tobramycin (80mg/2ml) and Tobramycin Inhalation Solution (TIS 300mg/5ml) failure rates

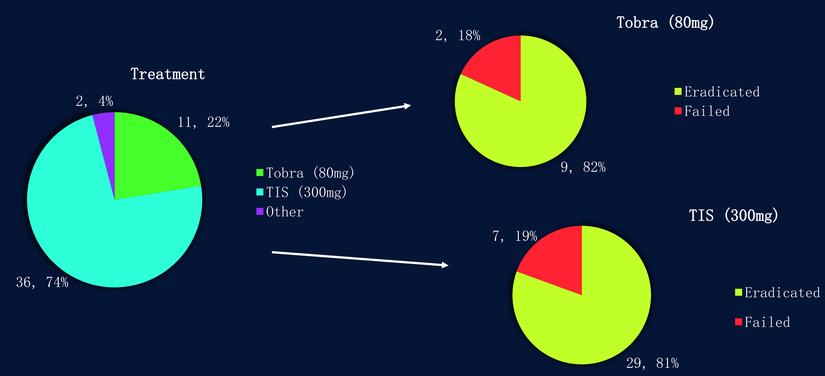
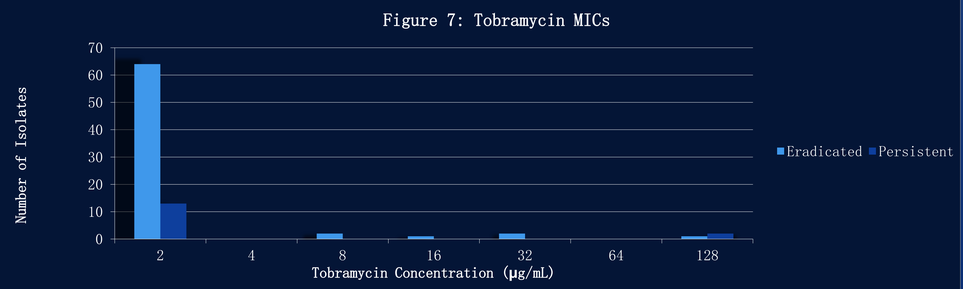


Table 1: Comparison of phenotypic characteristics of eradicated vs persistent PA isolates.

	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 (0-21)	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 (µg/ml), median (range)	2 (2-128)	2 (2-128)	0.0403 **

Mann-Whitney test of comparison for continuous variables
Two-tailed Fisher exact test of comparison for proportion variables

**Note this result may appear to be significant mainly due to the differences in group sample sizes.



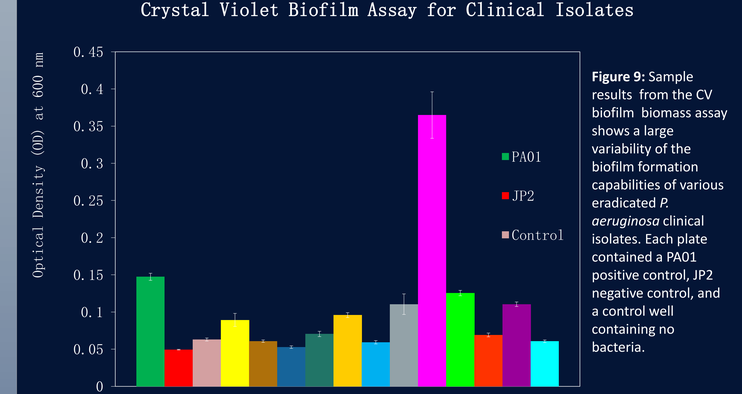


Figure 9: Sample results from the CV biofilm biomass assay shows a large variability of the biofilm formation capabilities of various eradicated *P. aeruginosa* clinical isolates. Each plate contained a PA01 positive control, JP2 negative control, and a control well containing no bacteria.

- *las* and *rhl* are genes that code for synthetase proteins related to quorum sensing systems in *P. aeruginosa*
- Presence of these genes are thought to be associated with biofilm production
- Amplification of *las* and *rhl* by PCR was performed *on P. aeruginosa* isolates to determine the presence or absence of QS genes in eradicated vs persistent isolates

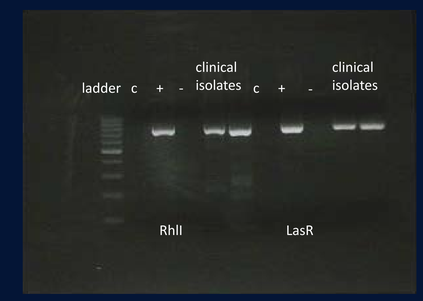


Figure 8: Agarose gel of PCR Amplification of inducer *rhl* gene and the regulatory protein *las* gene on 2 clinical *P. aeruginosa* isolates. Each gel included PA01 as a positive control (+), a negative clinical isolate as a negative control (-), and a water control containing no DNA (c).

Conclusions

•Preliminary data suggests *P. aeruginosa* infections that are more likely to persist in the CF lung, display more chronic phenotypes akin to those of older established colonies, due to:

- •Less twitching motility
- •More mucoid
- •Higher average tobramycin MICs

•CV biofilm biomass data suggests increased biofilm production in persistent infections

•Phenotypic studies involving biofilm production and pulmonary microbiome are currently in progress

Acknowledgments

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