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Identifying Environmental Reservoirs of a Cystic Fibrosis Epidemic Strain of *Pseudomonas aeruginosa*

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INTRODUCTION

Pseudomonas aeruginosa is an important cause of infection, particularly amongst Cystic Fibrosis (CF) patients. While a rare opportunistic pathogen, it is commonly isolated from CF patients as it infects 70% of these individuals [1]. The dogma that was held for many years was that each patient was independently infected by locally acquired non-clonal strains of *P. aeruginosa* from their own environment. However, it has been increasingly recognized that many patients attending the same clinic may be infected with genetically related strains, suggesting that infection of these strains may also be achieved through patient-to-patient transmission, such as the Prarie Epidemic Strain concentrated in Southern Alberta [1]. While several strains of *P. aeruginosa* are 'transmissible', the majority of patients acquire infection with unique isolates [2]. There is a need to understand the relationship between patients living in a particular local and the likelihood of infection with organisms endemic in that local. Determining these pockets is essential as *P. aeruginosa* is a leading cause of morbidity and mortality in patients with CF. Here we report the results of 309 water samples collected from heavily populated CF regions in Southern Alberta. The data confirms that pockets of *P. aeruginosa* exist in this region.

METHODS

Riverine isolates were cultured from surface water grabs, plant decay and rocks with biofilms. Geographical regions included industrial and rural. 50mL of each sample was vortexed to dislodge biofilms, and then passed through a PFTE membrane filter. For sensitivity, each filter was plated on BBL M-PA-C agar, a *P. aeruginosa* selective media, and DIFCO Pseudomonas isolation agar (PIA). Samples were incubated at 37°C and 42°C for 48 hours. Phenotypic colony morphology, oxidase strip tests and Polymerase Chain Reactions targeting housekeeping genes common to *P. aeruginosa* – gyraseB and ECF - were used as specific methods to narrow the number of potential colonies.

RESULTS

Overall, 390 water samples were collected from 14 different communities across Southern Alberta, and 1492 unique colonies were phenotypically identified. 84 colonies were sent for sequencing. Of these, 8.3% (n=7) were identified as *P. aeruginosa* as shown in Table 1.

Table 1. Environmental *P. aeruginosa* colonies agar, location & sample type.

Plate	Colony Morphology	Location	Sample Type
PIA	Small, round, white, blue tint in media	Red Deer River	Surface grab + rock with biofilm
PIA	Large, round, white tipped	Red Deer River	Surface grab + rock with biofilm
PIA	White, blue tint in media	Red Deer River	Surface grab + rock with biofilm
M-PA-C	Tiny, white + black dot	Glenmore Reservoir-Drain 13	Moss/plant decay
M-PA-C	Tiny, white + black dot	Bow River - Drain 34	Grass/plant decay
M-PA-C	Small, round, black	Glenmore Reservoir-Drain 13	Moss/plant decay
M-PA-C	Tiny, white + black dot	Bow River - Drain 34	Surface grab + rock with biofilms
PIA	Cream, medium, round	Raymond Hutterite Colony	Plant decay + waste

DISCUSSION AND CONCLUSIONS

The majority of the samples plated on the M-PA-C agar produced round white colonies with black tips that, in theory, are *P. aeruginosa.* Strikingly however, less than 1% of these colonies were *P. aeruginosa* even though ~60% of the samples produced this phenotype.

Using the extensively optimized methodology, more environmental strains of *P. aeruginosa* will be identified. Upon completion, genotypes of environmental *P. aeruginosa* will be compared to the Prarie Epidemic Strain and sequenced isolates from patients at the Calgary Adults Cystic Fibrosis Clinic. A positive match will strengthen the notion that a link exists between *P. aeruginosa* found in the environment and patients. This will further serve to delineate whether certain characteristics of a strain are required to result in chronic infection. Furthermore, this project may provide more information regarding a potential site of origin for the Prarie Epidemic Strain.

REFERENCES

1. Workentine M, et al. *PLOS One.* **8**(4): 2013.
2. Kidd T, et al. *PLOS One.* ***8****(4)*: 2012.