Phenotypic Analysis of Multi-Drug Resistant Cystic Fibrosis Clinical Isolates of *Pseudomonas aeruginosa* strains

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Abstract

Pseudomonas aeruginosa is a Gram negative opportunistic pathogen and a leading cause of lung infection in cystic fibrosis (CF) patients. This study was focused on characterizing two multi drug resistant (MDR) cystic fibrosis clinical isolates of *P. aeruginosa.* These clinical isolates were taken from patients in the Sick Children's Hospital, Ontario. Genomic analysis and phenotypic assays were done to assess the multi-drug resistant and virulence phenotype between these isolates compared to wild type PA01. The strains exhibit very similar resistance profiles apart from meropenem, however a difference is observed in biofilm formation, virulence, and growth in minimal media.

Introduction

P. aeruginosa is the leading Gram negative infective agent in CF patients (*1).* CF is a genetic disorder characterized by mucous accumulation in the lungs, which facilitates bacterial colonization *(2)*. Many strains of this bacterium exhibit MDR phenotypes, limiting the number of antibiotics that can be used for treatment. One of the resistance mechanisms used by *P. aeruginosa* is through chromosomally encoded genes (use of efflux pump or hydrolyzing enzymes). There are four clinically relevant Resistance-Nodulation-Division (RND) efflux pumps (MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY) present in *P. aeruginosa* and these pumps have broad antibiotic specificity *(1)*. With antibiotic resistance increasing rapidly in many strains, this study aims to understand mechanisms of resistance in MDR strains in the presence and absence of RND efflux pump overexpression.

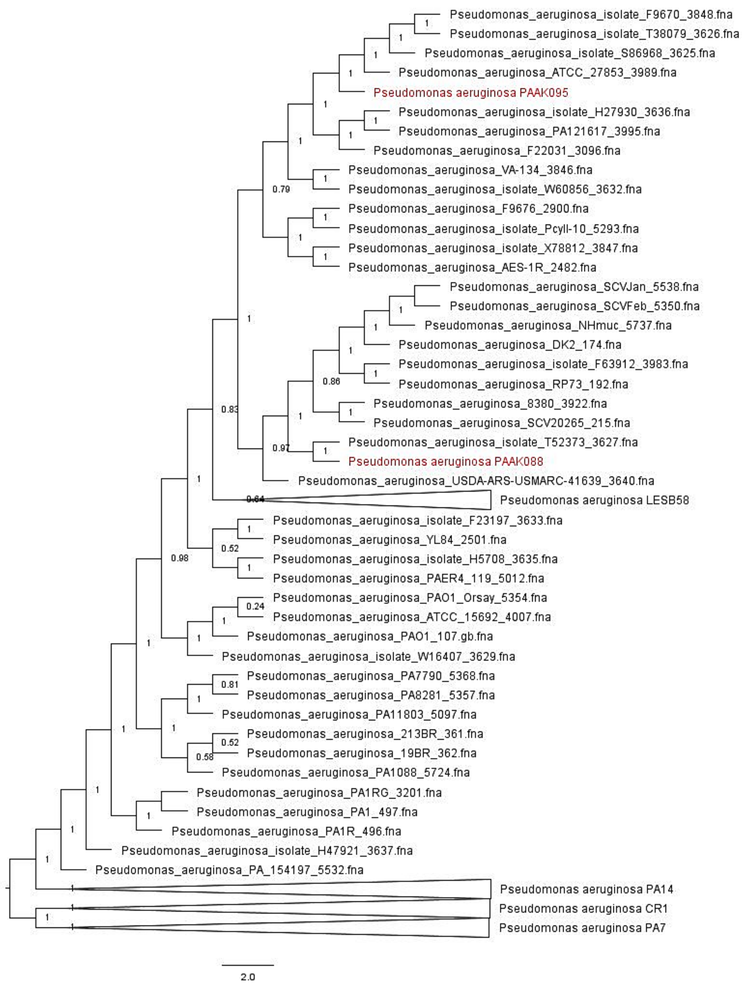

Figure 1. Phylogenetic tree for *P. aeruginosa* genomes *(2).* Whole genome SNPs based phylogeny was created using Harvest tools, allowing genetic recombination. Both of the isolates formed clusters with nosocomial clinical isolates but in two different clades.

Antibiotic Susceptibility Profiles

Table 1. Minimum Inhibitory Concentrations determined by two fold dilution method in MHB broth or disc diffusion assay. *(1)*

Antibiotic	PAAK088	PAAK095	PA01
Amikacin	≥128	≥128	≤8
Aztreonam	≥64	≥64	≤8
Cefepime	≥64	≥64	≤4
Ceftazidime	≥64	≥64	≤1
Ciprofloxacin	≥8	4	≤0.5
Gentamicin	≥32	≥32	≤2
Meropenem	≤1	≥16	≤0.5
Piperacillin	128	≥256	≤16
Piperacillin/Tazobactam	≥8	≥256/4	≤8/4
Tobramycin	≥16	≥16	≤2
Colistin#	S	S	S
Chloramphenicol#	I	I	I

*Concentrations are given in µg/mL. S=sensitive, I=intermediate.
#Carried out by disc diffusion.

qRT-PCR

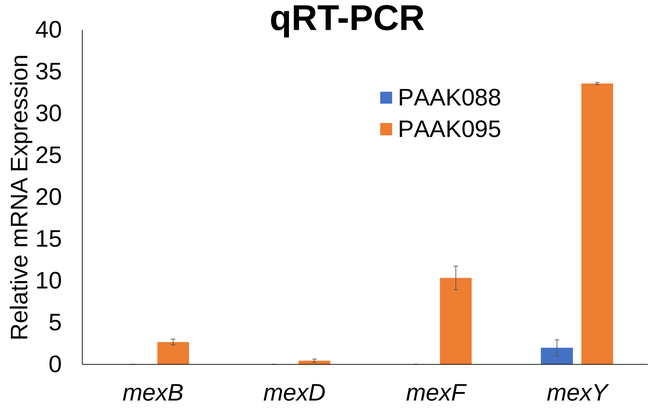

Figure 2.Relative detection of mRNA expression for RND efflux pumps in *P. aeruginosa* PAAK088 and PAAK095 in comparison to PA01 *(1).* PAAK095 shows overexpression *of mexB, mex F*, and *mexY* while PAAK088 does not overexpress any RND efflux pump genes.

Growth Curve

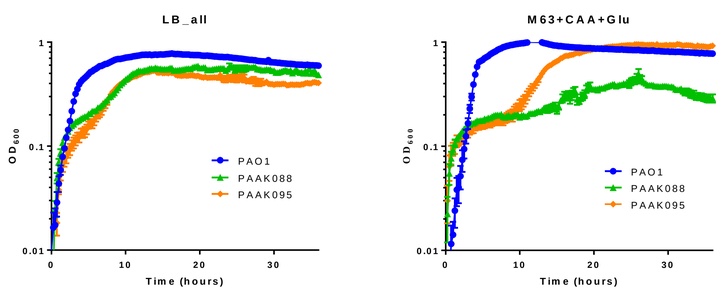

Figure 3. Growth curves in two types of media. LB broth and M63 media with 0.4% Glucose, 1mM MgSO4, and 0.5% CAS amino acid. The experiment was done with 2 biological and 5 technical replicates. PAAK088 and PAAK095 do not exhibit growth in M63+ Arg+ $MgSO_4$ media so we chose to study their growth in a different type of minimal media. Both of these strains showed reduced growth compared to that of PA01, in both minimal and complex media.

References

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Biofilm Formation

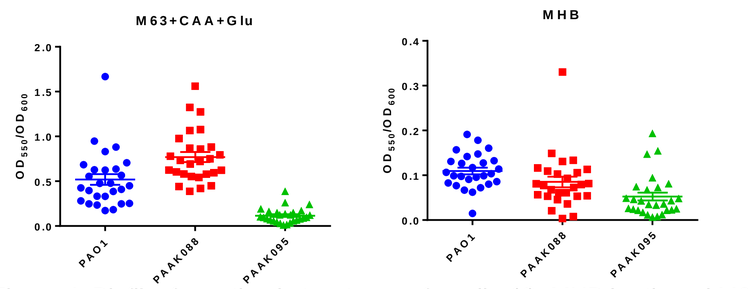

Figure 4. Biofilm formation in two types of media *(4)*. MHB broth and M63 media with 0.4% Glucose, 1mM $MgSO_4$, and 0.5% CAS amino acid. Biofilms were done using crystal violet staining method and normalized with A_{600} after 18 hours of static growth. PAAk088 showed more biofilm formation than PAAK095 in both minimal and complex media. In minimal media, PAAK088 also showed greater biofilm formation than wild type PA01.

Virulence Assays

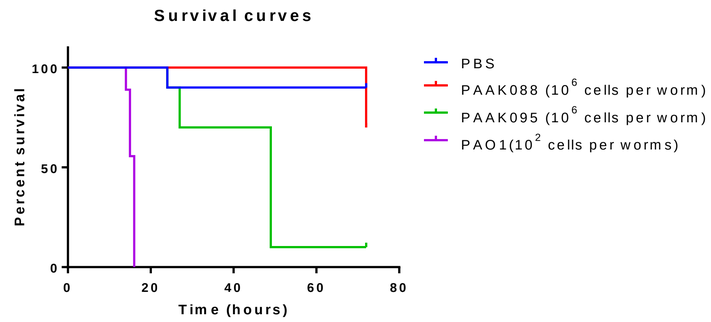

Figure 5. Virulence assay in *Galleria mellonella (*wax worms). Wax worms were infected with a standardized amount of cells in 10 µL volume and number of worms dead were counted every hour. Worms were determined dead when unresponsive and discolored. *(5)* 10 worms were used per experimental condition with PBS as injection control. We found that PAAK095 was more virulent than PAAK088 but both strains were less virulent in comparison to PA01.

Discussion

Both of the CF clinical isolates showed MDR phenotype, but only PAAK095 exhibited overexpression of RND efflux pump genes. This suggests other resistance mechanisms present in PAAK088 that confers resistance to different classes of antibiotic. Both of the isolates show a growth defect in comparison to PA01 which could be an adaptation property of clinical isolates. Interestingly, PAAK088 showed high biofilm formation although this strain shows the slowest growth and least virulence. Numerous surface associated motility assays were carried out but no conclusive data was obtained. It appears that both strains do not exhibit significant motility on the media used..

Conclusion

Since both isolates showed similar antibiotic resistance phenotypes but different virulence and growth phenotypes, further study is required to understand the resistance and virulence mechanisms that differ in these two isolates. Comparative genomics on these isolates will be helpful in explaining the phenotypic differences and resistance profiles observed. Understanding the mechanisms of resistance and virulence in CF isolates will help in developing therapeutic options for treating infections in CF patients.

Acknowledgements

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