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.
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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 degrading 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.

Antibiotic Susceptibility Profiles

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<thead>
<tr>
<th>Antibiotic</th>
<th>PA01</th>
<th>PA0K88</th>
<th>PA0K95</th>
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*Concentrations are given in µg/mL. S=sensitive, I=intermediate.
#Carried out by disc diffusion.

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 discarded. (5) 10 worms were used per experimental condition with PBS as injection control. We found that PA0K95 was more virulent than PA0K88 but both strains were less virulent in comparison to PA01.

Discussion

Both of the CF clinical isolates showed MDR phenotype, but only PA0K95 exhibited overexpression of RND efflux pump genes. This suggests other resistance mechanisms present in PA0K88 that confer 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, PA0K88 showed high biofilm formation although strain shows the lowest 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.

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

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Figure 4. Biofilm formation in two types of media (a). MHB broth and M3 media with 0.4% Glucose, 1ml MgSO4, and 0.5% CAS amino acid. Biofilms were done using crystal violet staining method and normalized with AOD after 18 hours of static growth. PA0K88 showed more biofilm formation than PA0K95 in both minimal and complex media. In minimal media, PA0K88 also showed greater biofilm formation than wild type PA01.

Figure 3. Growth curves in two types of media. LB broth and M3 media with 0.4% Glucose, 1ml MgSO4, and 0.5% CAS amino acid. The experiment was done with 2 biological and 5 technical replicates. PA0K88 and PA0K95 do not exhibit growth in M3+ Arg+ M63+ 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.

Figure 2. Relative detection of mRNA expression for RND efflux pumps in P aeruginosa PA0K88 and PA0K95 in comparison to PA01 (1). PA0K88 shows overexpression of mexB, mexF, and mexY while PA0K95 does not overexpress any RND efflux pump genes.

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.

References

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 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.

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