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Predicting Carbapenem Resistance in *Pseudomonas aeruginosa* through Whole Genome Sequencing Analysis

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Background

Rapidly and efficiently determining a pathogen's antibiotic resistance is crucial for patient treatment and minimizing antibiotic overuse. Whole genome sequencing (WGS) has been increasingly accessible to clinical labs and used to predict antibiotic resistance of pathogens. *Pseudomonas aeruginosa* have developed three mechanisms to confer resistance to carbapenems, potent "last-line" antibiotics: acquisition of carbapenem degradation enzyme genes; mutations of porin genes (e.g. oprD) to decrease outer membrane permeability; and overexpression of efflux pump systems. In this study, we analyzed the relationship of the relevant genes in *P. aeruginosa* with resistance to meropenem, a carbapenem

Methods and Materials

Data acquirement: WGS assemblies and phenotype data describing meropenem-resistance for 399 *P. aeruginosa* isolates were acquired from public databases.

Determination of the resistance genes variants: To determine the variants of antibiotic resistance genes, the whole genome sequences were used to query antibiotic resistance gene databases created at OpGen (Gaithersburg, MD, USA). The carbapenem related resistance genes were extracted for analysis in this study.

Detection of the mutations in chromosomal encoded genes: The whole genome sequences were compared with the reference genes in the *P. aeruginosa* strain PAO1 (NCBI: NC_002516.2). Three types of loss of function (LOF) mutations (truncation, gain of stop codon and frameshift) were identified in chromosomal genes encoding porins and regulator of efflux pumps.

Results

Carbapenem degradation enzyme: Six families of carbapenem resistance related enzymes are present in 87 out of 399 isolates: GES, IMP, KP, OXA, SPM and VIM. Most prevalent enzyme is OXAs and VIM. Interestingly, fifteen isolates harboring one of these genes are susceptible to meropenem (**Table 1**). GES is the weakest resistance gene to meropenem. Among 62 isolates harboring OXAs, ten of them are susceptible to meropenem. The distribution of these susceptible isolates harboring different subtypes of OXAs are listed in **Table 2**. Due to low number of isolates harboring OXA-9, -11, and -129, we could not determine the role of these genes in meropenem resistance. More data and further investigation are needed to determine the cause of the different phenotypes of the isolates harboring the same carbapenem resistance genes.

Table 1. Phenotype of the Isolates Harboring Carbapenem Resistance Genes

Gene Family	Resistant	Susceptible
GES	7	3
IMP	7	1
KPC	4	0
OXA	52	10
SPM	5	0
VIM	30	1

Table 2. Phenotype of the Isolates Harboring Different OXA Subtypes

Subtype	Resistant	Susceptible
OXA-2	18	2
OXA-4	9	3
OXA-9	0	1
OXA-10	10	1
OXA-11	0	1
OXA-129	0	2

Regulation of efflux pump systems: The overexpression of the efflux pump systems will reduce the antibiotic concentration within the bacterium cell and increase the tolerance to the corresponding antibiotic. These efflux pump systems are usually organized as the operons and the common mechanism of the regulation is the mutation of the transcription repressors. LOF mutations of these repressors within the surveyed isolates are listed in **Table 3**. These data suggest: (1) The efflux pump systems MexCD-OprJ and MexEF-OprN are not involved in the transportation of meropenem. (2) The efflux pump system MexAB-OprM participates in the transportation of the meropenem. Interestingly, MexR has lower effect than NalD. (3) The role of the efflux pump system MexJK-OprM isn't clear because all three isolates harboring MexL LOF mutation with resistance to meropenem also have NalD LOF mutation. (4) The efflux pump system MexXY-OprM might have a minor role in the transportation of meropenem.

Table 3. Phenotype of the isolates harboring LOF mutation within transcription repressor of efflux pump systems

Regulator	Targeted Efflux System	Resistant	Susceptible
NalD	MexAB-OprM	26	7
MexR		10	7
NfxB	MexCD-OprJ	3	3
MexS	MexEF-OprN	5	11
MexZ	MexXY-OprM	44	32
MexL	MexJK-OprM	3	1

The loss of function mutation within porins: Porins form pores on the membrane to facilitate the antibiotic dispersion into bacterium cell. Therefore, the mutations within porin protein will affect the resistance to relevant antibiotic. It is well known that mutations within OrpD confer resistance to carbapenem. After surveying the mutations within other porins in these 399 isolates and their resistance phenotype data against meropenem, we found mutations within opdD also confer resistance to meropenem (**Figure 1**).

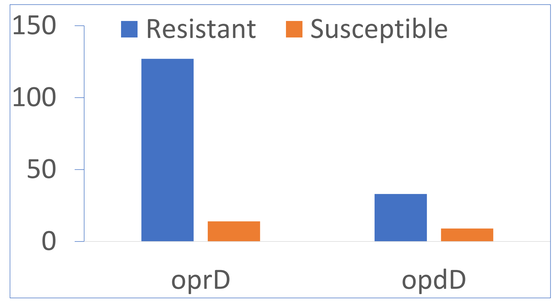

Figure 1: Phenotype of the Isolates Harboring Loss of Function Mutation within Porin OprD and OpdD.

Prediction of meropenem resistance phenotype: 198 isolates harbor at least one enzyme or LOF mutation within the three most relevant repressors of efflux pump systems or porins: *nalD*, *oprD* and *opdD*; of these, 162 isolates were resistant to meropenem and 36 were susceptible (**Table 4**). Using these as indicators of the resistance to meropenem, the prediction accuracy is calculated at 86.2%, sensitivity at 89.5%, specificity at 83.5%, positive predictive value at 81.8% and negative prediction value at 90.5%. Moreover, isolates with a loss of function mutation in *oprD* or with carbapenem degradation enzyme genes have a positive predictive rate at 90.1% and 83.9%, respectively.

Table 4. The correlation of genotype and phenotype of isolates

	Resistant	Susceptible
enzyme	73	14
nalD	26	7
oprD	127	14
opdD	33	9
Total_mut_Enzyme	162	36
No_mut_enzyme	19	182

Conclusion

P. aeruginosa can become resistant to meropenem through acquisition of carbapenem degradation enzymes, mutations in porin genes and efflux pump systems. Using WGS data we can predict resistance to meropenem**.** The accuracy, specificity and sensitivity can be further increased if we can accumulate more data to eliminate non-functional subtype of enzyme and identify more resistance mechanism.