Comprehensive Analysis of Fluoroquinolone Resistance in *Pseudomonas aeruginosa*

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Background

With decreased cost of NGS, analyses of whole genome sequences (WGS) have been used to predict antibiotic resistance of pathogens. Fluoroquinolones target DNA topoisomerases and bacteria have developed three mechanisms to confer resistance to these antibiotics: Mutations of DNA topoisomerases to alter the binding of the antibiotic; Overexpression of efflux pump systems; Acquisition of Qnr genes. In this study, we analyzed the relationship of the relevant genes with resistance to two fluoroquinolone: levofloxacin and ciprofloxacin.

Material and Method

524 *Pseudomonas aeruginosa* isolates with available WGS assemblies and phenotype data describing levofloxacin-resistance were acquired from public databases. Of these, 234 isolates were resistant to levofloxacin and 290 isolates were susceptible. All known Qnr genes and chromosomal gene mutations conferring resistance to fluoroquinolones were analyzed by comparison to reference Qnr genes and the reference strain *Pseudomonas aeruginosa* PAO1 (NCBI: NC_002516.2). The results were further validated with pheonotype and sequencing data of *P. aeruginosa* 92 isolates from OpGen repository.

Results

All three mechanisms were analyzed. Among 524 isolates from public databases, 137 isolates have loss of function mutations in four efflux system transcription repressors which theoretically increase the expression of efflux pump systems; 189 isolates have crucial site mutations in gyrase A and/or parC. In all, 253 isolates harbor at least one mutation described above; of these, 217 isolates were resistant to levofloxacin and 36 were susceptible(**Table. 1**). Using these mutations as indicators of the resistance to levofloxacin, the prediction accuracy is calculated at 89.89%, sensitivity at 92.74%, specificity at 87.59%, positive prediction value at 85.77% and negative prediction value at 93.73%. Isolates with any two mutations across these genes have a positive predictive rate at 98.86%.

Table 1. The correlation of genotype and phenotype of public data

Genotype	R	S
> 2 Repressor Null	17	0
Single repressor Null + gyrA/parC mut	71	2
Efflux pump system repressor Single Null	19	28
>2 gyrA/parC mut	86	0
Single Gyr/parC mut	24	6
Total isolates with mutation	217	36
Total isolates without mutation	17	254
Total	234	290

Although 100% of isolates with loss of function mutations in at least two of four efflux systems transcription repressors are resistant to levofloxacin, a single loss of function mutation is not a good indicator of levofloxacin resistance. The ability to predict resistance in such cases might be improved by adding an analysis of promoter and coding regions of genes from efflux pump systems. There were no Qnr genes detected in these 524 *P. aeruginosa* isolates.

Table 2. The correlation of genotype and phenotype of isolates from OpGen repository

Genotype	Levofloxacin		Ciprofloxacin	
	R	S	R	S
Qnr Enzyme only	1	0	1	0
Efflux Pump System Repressor Only	4	0	4	0
Efflux pump repressor + Qnr Enzyme	1	0	1	0
Gyr/parC mut	62	1	62	1
Total isolates with mutation or Qnr Enzyme	68	1	68	1
Total Isolates without mutation or Qnr Enzyme	7	16	5	18
Total	75	17	73	19

The results were further validated with 92 *P. aeruginosa* isolates in OpGen repository. Among them, two isolates have Qnr enzyme; 22 isolates have loss of function mutation in at least one of four transcription repressor of efflux systems and 62 isolates have crucial site mutations in gyraseA and/or parC. In all, 69 isolates harbor at least one mutation or Qnr enzyme; of these 68 isolates were resistance to levofloxacin and ciprofloxacin, and one was susceptible to levofloxacin and ciprofloxacin (**Table 2**). Using these mutation and Qnr enzymes as indications of the resistance to levofloxacin and ciprofloxacin, the prediction accuracies are calculated at 91.30% and 93.48%, sensitivities at 90.67% and 93.15%, specificities at 94.12% and 94.72%, positive prediction value at 98.55 % and 98.55%, negative prediction value at 69.57% and 78.26%, respectively (**Table 3**).

Table 3. The phenotype prediction stats of isolates from OpGen repository

	Levofloxacin	Ciprofloxacin
Accuracy	91.30%	93.48%
Sensitivity	90.67%	93.15%
Specificity	94.12%	94.74%
PPV	98.55%	98.55%
NPV	69.57%	78.26%

Discussion

Our prediction accuracy, sensitivity, specificity and PPV using in-house data are better than the prediction results using data in the public databases because of the difference of data quality: the quality of in-house data are well controlled and the quality of the public data vary a lot depending on the data submitters, platform used and the age of data, some old data were generated with previous versions of NGS equipment.

In the current analysis, we only checked the lost function mutation (e.g. gain of the stop codon and frameshift) of the regulatory genes of the efflux pump systems. However, the expression of these efflux pump systems can also be impacted by mutation the promoter and coding region of the their transcription regulators and these efflux pimps system genes them selves. The prediction power can be further enhanced if these mutations are included in the analysis.

Conclusion

Pseudomonas aeruginosa can become resistant to fluoroquinolones through mutations in DNA topoisomerases and efflux pump systems. With WGS data we can accurately predict resistance to fluoroquinolone such as levofloxacin and Ciprofloxacin.