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Comparative genomic analyses of two novel *qnrVC6* carrying multidrug-resistant *Pseudomonas. spp* strains

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ABSTRACT

Objectives: This study focused on the comparative genomic analyses of two *qnrVC6* carrying *Pseudomonas* spp. strains which might give us insights on the similarity and difference in the genomic contexts of *qnrVC6* gene.
Methods: Comparative genomic analyses of the novel *qnrVC6* carrying *Pseudomonas* spp. genomes with emphasis on their antimicrobial resistance genes and virulence factors were performed.
Results: Most Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, Clusters of Orthologous Groups of proteins (COG) categories, and (Gene Ontology) GO terms are shared by both genomes. Although *qnrVC6* gene is responsible for the increase of quinolone resistance in both strains, but it duplicated in *P. putida* strain Guangzhou-Ppu420. And the resistance to β-lactams and aminoglycosides are dependent on different genes. Sharing some adherence, antiphagocytosis, and iron uptake related genes with *P. putida* strain Guangzhou-Ppu420, *P. aeruginosa* strain Guangzhou-Pae617 specifically acquires biosurfactant, pigment, protease, regulation, secretion system, and toxin related virulence factors.
Conclusions: Sharing most KEGG pathways, COG categories, and GO terms, *P. putida* strain Guangzhou-Ppu420 and *P. aeruginosa* strain Guangzhou-Pae617 differ in antimicrobial resistance genes and virulence factors.

1. Introduction

Members of the genus *Pseudomonas* contain clinically important pathogens including *P. aeruginosa* and *P. putida*. *P. aeruginosa* is a major opportunistic human pathogen notable for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious hospital-acquired infections. *P. putida* has been considered to be a safe bacterial species. However, nosocomial infections caused by multidrug-resistant *P. putida* have also been incidentally reported [1,2]. Quinolones are considered to be effective in treating several nosocomial infections including *Pseudomonas*. However, resistance to quinolones has been a problem since nalidixic acid was introduced in the clinical practice in 1964 [3,45]. Quinolone-resistance determinants in the *qnr* family including *qnrVC* genes have been reported to possess an additive effect and may facilitate the acquisition of full quinolone resistance [4,46]. As an emerging quinolone resistance gene in *Pseudomonas*, *qnrVC6* has only been identified in *Acinetobacter baumannii* and occasional detection in *Vibrio parahaemolyticus*, *Pseudomonas putida*, and *Citrobacter freundii* besides the two *Pseudomonas. spp* strains in this study [5,6,47]. The *qnrVC6* carrying multidrug resistant *P. aeruginosa* strain Guangzhou-Pae617 is a clinical isolate from the sputum of a patient who was suffering from respiratory disease in Guangzhou, China, in 2012 [7,8]. Another *qnrVC6* carrying multidrug resistant strain *P. putida* Guangzhou-Ppu420 was isolated from urine of a patient suffering from urinary tract infection in Guangzhou, China, in 2011 [9]. The complete genomes of *P. putida* strain Guangzhou-Ppu420 and *P. aeruginosa* strain Guangzhou-Pae617 had been sequenced and annotated in our previous study [7–9].

This study focused on the comparative genome analyses of the two *qnrVC6* carrying *Pseudomonas spp.* strains which might give us insights on the similarity and difference in the genomic contexts of *qnrVC6* gene.

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2. Material and methods

2.1. Nucleotide accession numbers

The complete sequences of *P. putida* strain Guangzhou-Ppu420 genome and *P. aeruginosa* strain Guangzhou-Pae617 genome and plasmid are available in GenBank Database under accession number CP017073, CP016214, and CP016215, respectively.

2.2. Genome alignment and functional analysis

The genome of *P. putida* strain Guangzhou-Ppu420 was aligned against *P. aeruginosa* strain Guangzhou-Pae617 genome by determining local collinear blocks (LCBs) using the progressiveMauve algorithm in Mauve [10] and BLAST Ring Image Generator (BRIG) 0.95 [11]. Enrichment analyses and comparison of GO terms, COG categories, KEGG pathways annotated to the chromosome and plasmid of *P. aeruginosa* strain Guangzhou-Pae617 and the chromosome of *P. putida* strain Guangzhou-Ppu420 were performed [7–9]. The multidrug-resistance genes and virulence factors acquired by both *Pseudomonas* spp. strains were also compared [7–9].

3. Results and discussion

3.1. General genomic features

The genome of *P. aeruginosa* strain Guangzhou-Pae617 composes of one chromosome sequence and one plasmid with the length of 6,430,493 bp and 423,017 bp, respectively and GC content of 66.43% (Table 1). No plasmid was identified in the genome of *P. putida* strain Guangzhou-Ppu420 which has 6,031,212 in length and 62.01% in GC content (Table 1). The chromosome of *P. aeruginosa* strain Guangzhou-Pae617 showed 51% coverage and 85% identity with that of *P. putida* strain Guangzhou-Ppu420 (Figs. 1 and 2). The two strains were isolated from the same region (Guangzhou, China) which suggested a regional prevalence.

3.2. Antimicrobial resistant genes

According to our previous antimicrobial resistance data [8,9], both strains acquired resistance to β-lactams, aminoglycosides, and quinolones, with *P. aeruginosa* strain Guangzhou-Pae617 showing stronger resistance. The bla_{IMP-45} and bla_{OXA-1} located in the class 1 integron of plasmid and bla_{OXA-50} and bla_{PDC} located in the chromosome contribute to the β-lactam resistance of *P. aeruginosa* strain Guangzhou-Pae617 [7,8]. Only bla_{VIM-2} gene is responsible for β-lactam resistance of *P. putida* strain Guangzhou-Ppu420 which might explain its lower β-lactam resistance [9]. Concerning the genes confer resistance to aminoglycosides, *armA*, *aacA4*, *aphA7* are located in the plasmid and *aph (3′)-IIb* in the chromosome of *P. aeruginosa* strain Guangzhou-Pae617 [7,8], and *strA*, *strB*, and *aacA4* are located in the genome of *P. putida* strain Guangzhou-Ppu420 [9]. Although sharing similar antibiotic resistance pattern, the antimicrobial resistance genes acquired by *P. aeruginosa* strain Guangzhou-Pae617 and *P. putida* strain Guangzhou-Ppu420 differ. However, the novel *qnrVC6* gene, which duplicated in *P. putida* strain Guangzhou-Ppu420, contributes to the quinolone resistance of both strains [8,9]. The *qnrVC6* genes in both strains are surrounded by IS*CR1* elements which might contribute to the acquisition of the *qnrVC6* genes. However, the *qnrVC6* gene in *P. aeruginosa* strain Guangzhou-Pae617 is located in the plasmid [8], while the two copies of *qnrVC6* gene in *P. putida* strain Guangzhou-Ppu420 are located in the chromosome [9]. *QnrVC6* gene has been reported to in association with mobile genetic elements [12], which is in accordance with the genetic context of *qnrVC6* in *P. aeruginosa* strain Guangzhou-Pae617. However, the two copies of *qnrVC6* gene are not located in mobile genetic elements such as plasmid, SXT, and class 1 integron. It is possible that the *qnrVC6* gene with the surrounding IS*CR1* elements in the plasmid of *P. aeruginosa* strain Guangzhou-Pae617 was inserted within the chromosome of the *P. putida* strain Guangzhou-Ppu420 with a duplication.

Table 1
General genome features of the two novel *qnrVC6* carrying *Pseudomonas spp.* trains [7–9].

	Pseudomonas aeruginosa Guangzhou-Pae617	*Pseudomonas putida* Guangzhou-Ppu420
GenBank accession number	CP016214, CP016215	CP017073
Location of isolation	Guangzhou, China	Guangzhou, China
Isolation source	Sputum	Urine
Isolation year	2012	2011
Genome size (bp)	6,430,493	6,031,212
Plasmid size (bp)	423,017	N/A
GC content (%)	66.43	62.01
Protein coding genes	6226	5256
rRNAs	4, 4, 4 (5 S, 16 S, 23 S)	8, 7, 7 (5 S, 16 S, 23 S)
tRNAs	62	77
Other ncRNAs	4	4
Pseudogenes	46	57

3.3. Virulence factors

A total of 335 and 134 virulence factors were identified in the genome of *P. aeruginosa* strain Guangzhou-Pae617 and *P. putida* strain Guangzhou-Ppu420 [7]. The major virulence factors in *Pseudomonas* include genes in association with adherence (flagella, lipopolysaccharide (LPS), and type IV pili), antiphagocytosis (alginate), biosurfactant (rhamnolipid), iron uptake (pyochelin and pyoverdine), pigment (pyocyanin), protease (alkaline protease, lasA, and lasB), regulation (quorum sensing), secretion system (HcpI secretion island I (HSI-I), type III secretion system (TTSS), and xcp secretion system), and toxin (exoA, exoS, exoT, exoU, exoY, and PLC) (VFDB database, http://www.mgc.ac.cn/cgi-bin/VFs/genus.cgi?Genus=Pseudomonas).

All flagella related genes which contribute to the swimming motility and play roles in biofilm formation and other pathogenic adaptations [13–15] were identified in the genome of *P. aeruginosa* strain Guangzhou-Pae617, with *flgD, flgL, fliD,* and *fliO* missing in the *P. putida* strain Guangzhou-Ppu420 genome. LPS related genes including *waaA, waaC, waaF, waaG*, and *waaP* were found in both genomes. LPS mediates biological effects including resistance to serum killing and phagocytosis, and its binding to normal cystic fibrosis transmembrane conductance regulator (CFTR) and invasion of host cells may contribute to virulence in human [16–18]. All the type IV pili related genes involved in biogenesis and mechanical function of pili, transcriptional regulation and chemosensory pathways that control the expression or activity of the twitching motility of the pili [19,20] were identified in the genome of *P. aeruginosa* strain Guangzhou-Pae617. However, only *fimV, pilA, pilD, pilG, pilH, pilI, pilJ, pilQ, pilR*, and *pilT* appear in the *P. putida* strain Guangzhou-Ppu420 genome. Type IV pili attaches to host cells, but not to mucin, causing a twitching motility that allows the bacteria to move along the cell surface and contribute to biofilm formation [20–22]. It indicated the weaker adherence and twitching motility of *P. putida* strain Guangzhou-Ppu420 than *P. aeruginosa* strain Guangzhou-Pae617.

P. aeruginosa chronic lung infections in cystic fibrosis (CF) patients are the leading cause of morbidity and mortality [23]. Alginate (mucoid exopolysaccharide) acts as an adhesin, preventing the bacteria from being expelled from the lung, and its slime layer makes it more difficult for phagocytes to ingest and kill the bacteria, thus contributes to the persistence of the bacteria in the CF lung [24,25]. It also allows the bacteria to form biofilm [25]. Alginate related genes were identified in both genomes which indicated the antiphagocytosis of the strains.