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# Long-read nanopore sequencing-based draft genome of a carbapenem-resistant *Pseudomonas aeruginosa* isolate

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ABSTRACT

*Objectives:* *Pseudomonas aeruginosa* is a common Gram-negative bacterium causing various serious infections, such as lower respiratory tract infection and urinary tract infection in catheterised patients. Here we report the draft genome sequence of a carbapenem-resistant *P. aeruginosa* (CRPA) isolate.
*Methods:* The genome of the CRPA isolate was sequenced using a combination of short, highly accurate Illumina reads and additional coverage in very long Oxford Nanopore reads.
*Results:* The resulting assembly was highly contiguous, containing a total of 6 624 003 bp with a GC content of 66.21%. Annotation identified 6389 protein-coding genes. Mutations in the *oprD* and *mexR* genes conferred resistance to carbapenems in the CRPA isolate.
*Conclusion:* The draft genome sequence of this CRPA isolate could provide a solid basis for further research on the resistance mechanisms and the development of drug therapy for drug resistance genes.


## 1. Introduction

*Pseudomonas aeruginosa* is a ubiquitous organism that is the focus of intense research because of its important role in infectious diseases [1]. It is an opportunistic pathogen in burn victims, chronic wounds, chronic obstructive pulmonary disease, urinary tract infections in catheterised patients and surface growth on implanted biomaterials, where it poses a host of threats to vulnerable patients [2]. *P. aeruginosa* is hard to eradicate and is associated with increased morbidity [3]. With the widespread application of antibacterial drugs, bacterial resistance is becoming more and more serious, especially carbapenem resistance in *P. aeruginosa*, which causes great difficulties in clinical treatment. Here we report the genome sequence of a carbapenem-resistant *P. aeruginosa* (CRPA) isolate obtained from a patient in China.

## 2. Materials and methods

The CRPA isolate in this study was obtained from a blood specimen of an inpatient with lower respiratory tract infection and liver failure. The isolate was extracted according to an aseptic operating procedure and was cultured in bacterial culture medium with Columbia agar plus 5% sheep blood. The *P. aeruginosa* isolate was confirmed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (bioMérieux SA, Marcy-l'Étoile, France). Antimicrobial resistance of the CRPA isolate was determined using a VITEK®2 Compact Automatic Microbial Identification & Drug Sensitivity Analysis System (bioMérieux) with AST-GN09 Gram-negative identification card (bioMérieux). The results of antimicrobial susceptibility testing were interpreted based on Clinical and Laboratory Standards Institute (CLSI) guidelines [4]. The reference strain *P. aeruginosa* ATCC 27853 was used for quality control.

DNA samples were quantified using a Qubit fluorometer (Thermo Fisher Scientific). A total of 1 ng of purified DNA was used to generate Nextera XT transposase-based libraries. End repair was conducted by adding a phosphate to the 5′ end and 'A' to the 3′ end. DNA fragments (~300–600 bp) were retained from

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**Table 1**
Antimicrobial susceptibility profile of the *Pseudomonas aeruginosa* isolate, given as the minimum inhibitory concentration (MIC, in (μg/mL) and breakpoint interpretation or epidemiological cut-off value.

| Ampicillin | SAM | TZP | Cefazolin | Cefotetan | Ceftazidime | Ceftriaxone | Cefepime |
|---|---|---|---|---|---|---|---|
| ≥32 (R) | ≥32 (R) | ≥128 (R) | ≥64 (R) | ≥64 (R) | ≥64 (R) | ≥64 (R) | ≥32 (R) |
| Imipenem | Amikacin | Gentamicin | Tobramycin | Ciprofloxacin | Levofloxacin | Nitrofurantoin | SXT |
| ≥16 (R) | ≥64 (R) | ≥16 (R) | ≥16 (R) | ≥4 (R) | ≥8 (R) | ≥512 (R) | ≥320 (R) |

SAM, ampicillin/sulbactam; TZP, piperacillin/tazobactam; SXT, sulfamethoxazole/trimethoprim; R, resistant.

barcoded adapter ligation by PCR. The library was then purified using AMPure XP (Beckman Coulter). Following purification, library preparations were sequenced on a MiSeq platform (Illumina Inc., San Diego, CA, USA) generating 3.94 million reads (2 × 300-bp) with a mean fragment size of 500 bp, for a total of 1.18 Gb of data. A total of 1.5 μg of purified DNA was transferred into the LSK-108 Oxford Nanopore Technologies ligation protocol (Xiamen Cingene Science and Technology Co. Ltd.). The Oxford Nanopore MinION run produced approximately 0.59 million ultralong reads for a total of 1.06 Gb of data.

The long subreads were used to assemble the genome of *P. aeruginosa* using Canu v.1.7 [5] and also to correct base errors in the preliminary assembled genome using Illumina sequenced data. Glimmer v.3.02 and ORFfinder were employed to predict open-reading frames (ORFs) in *P. aeruginosa*. The predicted ORFs were annotated in NCBI nr and Swiss-Prot databases with blastX program, Clusters of Orthologous Groups (COG), Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway using eggNOG. Multilocus sequence typing (MLST) was performed using MLST 2.0 and antimicrobial resistance genes were

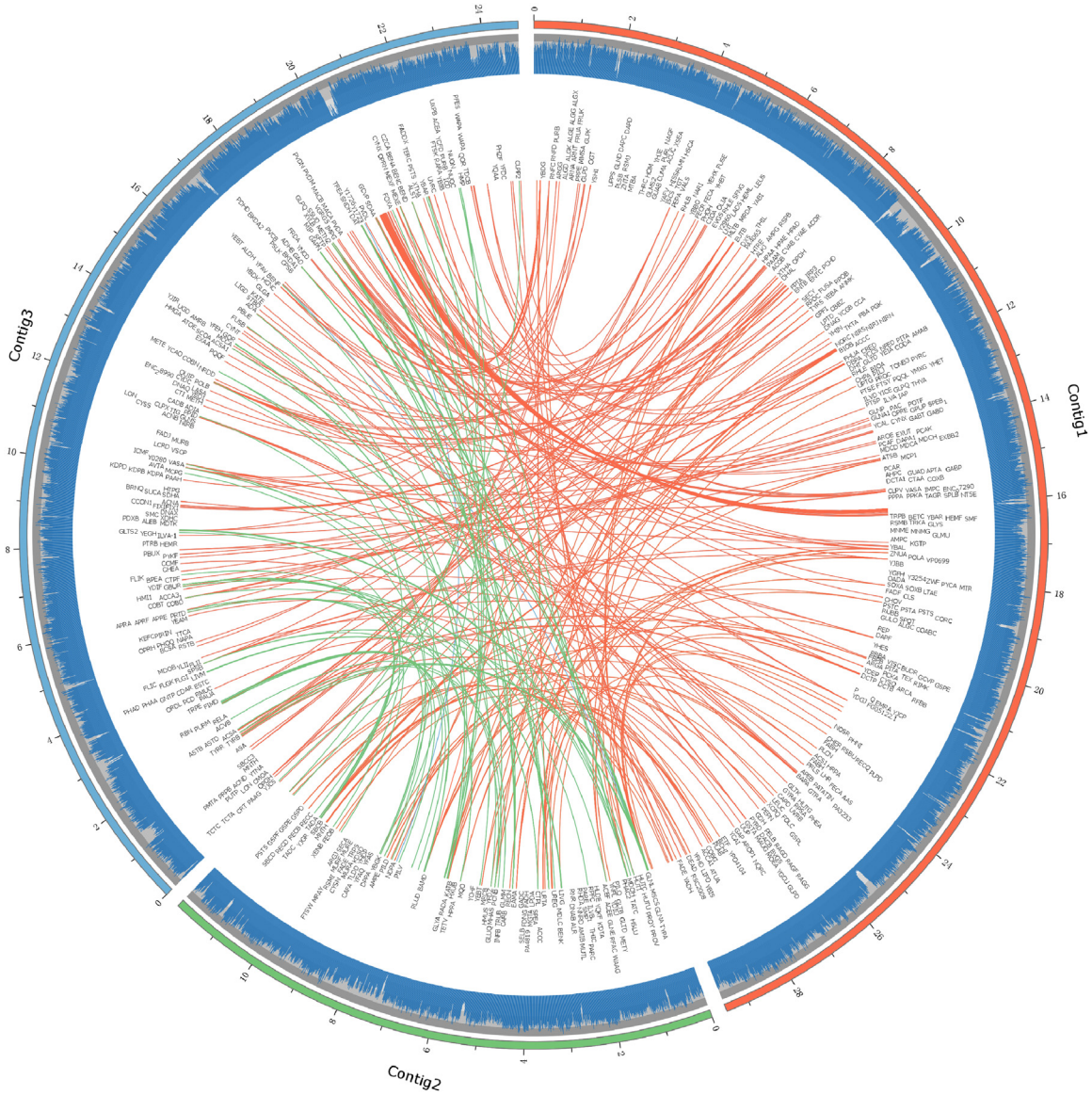


**Fig. 1.** Circular representation of the carbapenem-resistant *Pseudomonas aeruginosa* genome. The outermost circle indicates the chromosomal location in base pairs (each tick is 100 kb). The blue bar is percentage G + C content plotted as the average for non-overlapping 1-kb windows spanning one strand for the entire *P. aeruginosa* genome. Genes >1 kb in length are represented along with contigs. Curves in the centre denote links between different contigs.

**Table 2**
Carbapenem resistance genes in the *Pseudomonas aeruginosa* isolate.

| ORF | Protein ID | Gene | Gene length (bp) |
|---|---|---|---|
| 4336 | **NP_249649.1** | *oprD* | 1332 |
| 1094 | **NP_249115.1** | *mexR* | 444 |

ORF, open-reading frame.

detected using ResFinder 3.0, both available from the Center of Genomic Epidemiology (http://genomicepidemiology.org/).

## 3. Results and discussion

Antimicrobial susceptibility testing (Table 1) revealed that the *P. aeruginosa* strain was resistant to all antibiotics tested, including cephalosporins, penicillins, quinolones, aminoglycosides and carbapenems [imipenem minimum inhibitory concentration (MIC) $\geq$16 μg/mL].

A total of 1.01 Gb of long reads were generated after removing low-quality and short reads, with a mean and $N_{50}$ length of 2223 bp and 4058 bp, respectively. After polishing with long reads and high-quality short reads, the genome of the CRPA isolate was assembled into 6.62 Mb. The genome was composed of three contigs with a 66.21% GC content, and the contig $N_{50}$ reached up to 2.47 Mb (Fig. 1). In addition, a total of 6389 protein-coding genes were obtained with an $N_{50}$ length of 906 bp, and 6241 genes (97.7%) were annotated in various protein databases.

It is necessary to pay more significant attention to the research of resistance mechanisms in CRPA in order to develop new therapeutic strategies. The most frequent mutation conferring carbapenem resistance in *P. aeruginosa* is in the outer membrane porin D (*oprD*) gene [6]. Mutation in the *oprD* gene was found in the isolate in the current study. The *oprD* gene in the genome had an internal stop codon, suggesting that it was this stop codon in *oprD* that conferred carbapenem resistance to the *P. aeruginosa* isolate. Meanwhile, another important mechanism of carbapenem resistance in *P. aeruginosa* is conferred by mutation in MexR [7], and a V126E amino acid mutation and an internal stop codon were found in the current study, leading to a change in function (Table 2).

Moreover, several genes encoding resistance to aminoglycosides [*aph(3′)-IIb*], β-lactams ($bla_{\text{OXA-50}}$ and $bla_{\text{PAO}}$), fosfomycin (*fosA*) and chloramphenicol (*catB7*) were also predicted, and such identified resistance genes were in accordance with the results of antimicrobial susceptibility testing. The CRPA isolate was identified as being closest to sequence type 730 (ST730).

The genome shotgun sequencing data and long reads of Oxford Nanopore data have been deposited at NCBI/GenBank as BioProject **PRJNA484660**. The draft genome sequence of the carbapenem-resistant *P. aeruginosa* has been deposited at DDBJ/ENA/GenBank under accession no. **PRJEB32155**.

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## Competing interests

None declared.

## Ethical approval

This study was performed in accordance with the Institutional Ethical Committee of the Faculty of Medicine, Mengchao Hepatobiliary Hospital of Fujian Medical University (Fuzhou, China) (2018-05-01).

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