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Complete genome of IMP-6-producing *Pseudomonas aeruginosa* strain HPA0118 isolated from the urine of a patient with a urinary tract infection

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The IMP-6 metallo-β-lactamase-producing *Pseudomonas aeruginosa* strain HPA0118 was isolated from the urine of a human patient with a urinary tract infection. The genome of strain HPA0118 consisted of a chromosome (6,926,878 bp, 66.1% G + C content) with one plasmid (49,266 bp, 59.2% G + C content) containing 6,654 protein-coding sequences, 12 rRNA genes, and 61 tRNA genes. The carbapenemase gene, bla_{IMP-6}, was found in two copies at different chromosomal locations.

Keywords: *Pseudomonas aeruginosa*, bla_{IMP-6}, human patient

Pseudomonas aeruginosa resistant to carbapenems has been reported as a representative opportunistic pathogen that can cause various acute infections in clinical practice (Buehrle et al., 2016). Among carbapenemases, imipenemase (IMP)- or Verona integron-encoded metallo-beta-lactamase (VIM)-type metallo-β-lactamase is the most predominant in *P. aeruginosa* worldwide (Tenover et al., 2022). *P. aeruginosa* sequence type 235 (ST235) harboring IMP-6 and VIM-2, and ST773 carrying New Delhi metallo-β-lactamase 1 (NDM-1) are highly prevalent in the Republic of Korea (Hong et al., 2015). Particularly, IMP-6, a variant of IMP-1 with Ser196Gly substitution, was first reported in *Serratia marcescens* isolated from the urine of a patient with a urinary tract infection in Japan (Yano et al., 2001) and showed relatively enhanced hydrolysis activity on meropenem compared to imipenem.

P. aeruginosa strain HPA0118 carrying bla_{IMP-6} was isolated from a urine sample obtained from an 85-year-old male patient with a urinary tract infection at the general hospital in Gyeonggi province, the Republic of Korea. Clinical samples were streaked on cetrimide agar (BD Difco, USA), a selective medium for *Pseudomonas* spp., and incubated aerobically at 37°C for 18–24 h. Bluish-green pigmented colonies were selected and further subcultured in tryptic soy broth for identification.

The 16S rRNA gene sequence (1,541 bp) of strain HPA 0118 exhibited 100% identity to that of *P. aeruginosa* strain H02 (accession number CP093032). The sequence type (ST) of the strain was classified as ST4399 by multilocus sequence typing (MLST) of seven housekeeping genes (*acsA*, *aroE*, *guaA*, *mutL*, *nuoD*, *ppsA*, and *trpE*) on the PubMLST web portal (https://pubmlst.org/organisms/pseudomonas-aeruginosa). Antibiotic susceptibility was investigated by disk diffusion and broth microdilution methods according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2022). The strain HPA0118 exhibited resistance to cephalosporins (ceftazidime and cefepime), monobactam (aztreonam), carbapenems (meropenem and imipenem), aminoglycosides (gentamicin, tobramycin, and amikacin), and fluoroquinolone

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(ciprofloxacin), while it was intermediate susceptible to β-lactam/β-lactamase inhibitor combinations (piperacillin and piperacillin–tazobactam) and susceptible to colistin. The corresponding resistance genes were identified in the genome as described below.

For whole-genome sequencing, genomic DNA was extracted with the LaboPass^{TM} Bacteria Mini DNA purification kit (CosmoGenetech, Republic of Korea). The genome sequence was determined using the Illumina iSeq 100 (Illumina, USA) and the MinION (Oxford Nanopore Technologies, UK) devices for short- and long-read sequencing, respectively. Low-quality, adapter, and primer sequences were trimmed from the resulting short-read raw sequences with Trimmomatic (v.0.39) (Bolger et al., 2014). The long-read raw sequences were processed with Filtlong (v.0.2.0) and Porechop (v.0.2.4) to remove low-quality sequences and adapter sequences, respectively. De novo hybrid assembly of the obtained sequences was performed using Unicycler (v.0.4.9b) (Wick et al., 2017). Genome annotation was conducted on the bacterial and viral bioinformatics resource center (BV-BRC) portal (Olson et al., 2023).

The genome of HPA0118 consisted of one chromosome (6,926,878 bp with a G + C content of 66.1%) and one plasmid (49,266 bp with a G + C content of 59.2%) which encoded 6,654 protein-coding sequences, 12 rRNA genes, and 61 tRNA genes as shown in Table 1. Of the 6,654 predicted proteins, 5,042 were assigned to functional groups based on the Clusters of Orthologous Groups (COG) database. The most common COG categories were COG-E (amino acid transport and metabolism; 481 genes; 9.5%), COG-K (transcription; 476 genes; 9.4%), and COG-R (general function prediction only; 320 genes; 6.3%) (data not shown).

Table 1. Genome features of the bla_{IMP-6}-producing *Pseudomonas aeruginosa* HPA0118

Genomic features	Chromosome	Plasmid
Genome size (bp)	6,926,878	49,266
G + C content (%)	66.1	59.2
No. of CDS	6,577	77
No. of rRNA	12	ND
No. of tRNA	61	ND

CDS, protein coding sequences; ND, not detected

The carbapenemase gene bla_{IMP-6}, which was 741 bp in size, was duplicated on two different regions A (position between 1,696,097 bp and 1,696,837 bp) and B (position between 5,358,952 bp and 5,359,692 bp) of the chromosome based on the linear chromosomal sequence in GenBank database (Fig. 1). The bla_{IMP-6} and various other antibiotic resistance genes were found within the gene cassettes of a class 1 integron, which are known to play a central role in the spread of antibiotic resistance genes (Gillings et al., 2008). Each gene cassette in regions A and B commonly contained bla_{IMP-6} (carbapenems), bla_{OXA-1} (amoxicillin/clavulanic acid), *aph(3')-IIa* (gentamicin and kanamycin), and *sul1* (sulfamethoxazole) resistant genes with the same consecutive gene arrangement. The class 1 integron gene cassette contained *tnpA* and *tnpR* which belonged to the transposon Tn*3* family (Nicolas et al., 2015), and located upstream of bla_{IMP-6} in the reverse orientation. These genes were arranged downstream of *intI1* (the class 1 integron integrase gene) in the same direction. However, a difference in gene organization between regions A and B was found downstream of *sul1*. In region A, the *cmx* gene responsible for resistance to

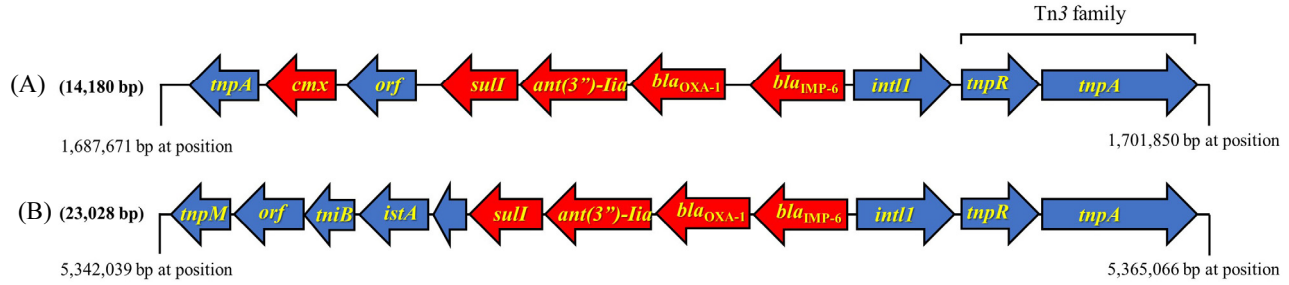

Fig. 1. The class 1 integron gene cassettes located on two different positions (region A and B) of chromosome in *Pseudomonas aeruginosa* HPA0118. The sky-blue arrows are mobile element genetic elements involving in integration and excision, while the red arrows indicate antibiotic resistance genes. *orf*, unknown protein.

phenicols was located between *tnpA* and unknown mobile element protein gene in the same direction. In contrast, *istA*, *tniB*, and *tnpM* with the other two unknown mobile element protein genes were located downstream of *sul1* in region B.

In addition, other antibiotic resistance genes were found on the chromosome including bla_{PAO} (amoxicillin), *aadA1* (streptomycin and spectinomycin), *aac(6')-Ib* (tobramycin and amikacin), *catB7* (chloramphenicol), *tmexD3* (tetracycline), and *fos* (fosfomycin). However, no resistance genes were present on the plasmid.

Carbapenem-resistant *P. aeruginosa* has raised significant public health concerns and is considered one of the most cautionary pathogenic microorganisms. This study provides genomic information that can support further research into understanding antimicrobial resistance and the epidemiological relatedness of carbapenemase-producing *P. aeruginosa*.

Nucleotide sequence accession numbers

Pseudomonas aeruginosa strain HPA0118 has been deposited in the Korean Collection for Type Cultures (KCTC) under deposition number BP1918134. The genome sequences have been deposited in GenBank/EMBL/DDBJ under accession numbers CP137522 and CP137523.

Acknowledgments

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Conflict of Interest

The authors have no conflicts of interest to declare. Jong-Chan Chae is an editor of KJM. He was not involved in the review process of this article.

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