



# Complete Genome Sequence of *Pseudomonas aeruginosa* CMC-097, Isolated from a Ventilator-Associated Pneumonia Patient, Containing a Novel Carbapenem Resistance Class 1 Integron

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**ABSTRACT** We report the complete genome of a clinical strain of *Pseudomonas aeruginosa* CMC-097, which was isolated from a ventilator-associated pneumonia patient with a chronic infection. Illumina sequence reads were assembled using Geneious to yield a 7,044,064-bp circular chromosome containing a carbapenem resistance integron, In2020.

Chronic and multidrug-resistant (MDR) *Pseudomonas aeruginosa* is a threat to ventilator patients, with increased mortality rates of up to 30% in intensive care units (1–3). A World Health Organization (WHO) survey reported that carbapenem-resistant (CR) *P. aeruginosa* ranked as the second most critical priority bacterium among 20 antimicrobial-resistant bacterial species (4). In the United States, CR *P. aeruginosa* was reported in 2004 for an isolate containing the Verona integron encoding a carbapenemase (5) associated with mobile insertional sequence (IS) elements that play a major role in global dissemination (6–8).

A prospective study was approved by the Carilion Clinic institutional review board and conducted from 2010 to 2012 (9). In this study, *P. aeruginosa* CMC-097 was obtained from the Quest Diagnostics microbiology laboratory at Carilion Clinic. The strain was isolated from a tracheal aspirate specimen from a chronic ventilator-associated pneumonia patient and was confirmed by antimicrobial tests to be CR (10). The isolate was grown on a blood agar plate and transported to the Carilion basic science research laboratory; glycerol stocks were made and stored at  $-80^{\circ}\text{C}$ .

A single colony of CMC-097 was grown in 25 ml lysogeny broth at  $37^{\circ}\text{C}$  at 200 rpm for 18 h. The cell pellet was used for genomic DNA isolation by Genomic-tip 20/G (9, 11). The genome was sequenced on the Illumina NextSeq platform at the Virginia Tech Genomics Resource Center with a library constructed using the Illumina TruSeq DNA preparation kit. Sequencing generated 72,739,130 paired-end (PE) reads of 150-bp length, which were assembled with the Geneious v11.0.4 *de novo* assembly algorithm with the default low sensitivity/fastest settings, which allow at most 10% base mismatches (9). No additional read quality filtering was necessary. This resulted in 77 contigs larger than 1,000 bp, with a maximum length of 597,066 bp and an  $N_{50}$  value of 214,826 bp. Then the Geneious algorithm map to reference was used to map the PE reads to these 77 contigs, with fine tuning set to iterate up to 10 times and custom sensitivity settings set to 0% mismatch and 0% gaps, to iteratively extend the ends of the 77 contigs until the ends overlapped and all of the gaps were closed into a circular genome (9). Synteny with highly similar *Pseudomonas* genomes (strains W60856 and

**Citation** Rao J, Adenikinju A, Kerkering TM, Garner DC, Jensen RV. 2021. Complete genome sequence of *Pseudomonas aeruginosa* CMC-097, isolated from a ventilator-associated pneumonia patient, containing a novel carbapenem resistance class 1 integron. Microbiol Resour Annuounc 10:e00774-21. <https://doi.org/10.1128/MRA.00774-21>.

**Editor** Julia A. Maresca, University of Delaware

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**Received** 30 July 2021

**Accepted** 6 August 2021

**Published** 2 September 2021

**TABLE 1** Genetic composition of the antibiotic resistance island containing the class 1 integron In2020

IS/integron element <sup>a</sup>	Gene identifier	Gene name	Direct/inverted repeat(s) <sup>b</sup>	Gene function <sup>c</sup>
		Hypothetical protein <sup>d</sup> 5'-PA0981 <sup>d</sup>	DR: AATAAgggct	
	IAU57_09040	Hypothetical protein		
	IAU57_09045	Hypothetical protein		
	IAU57_09050	Hypothetical protein		
	IAU57_09055	Conserved protein		DNA recombination protein RmuC
ISPa85	IAU57_09060	<i>istA</i>	IRL: tgcggattccacgctgactcggacacccattccac gcacatccgg	IS21 family transposase
	IAU57_09065	<i>istB</i>	IRR: tgcggattccacgcccattcggacactcagccca cgctgatccgga	IS21-like element ISUnCu3 family helper ATPase
	IAU57_09070	<i>tonB</i>		TonB C-terminal domain- containing protein
	IAU57_09075	Integrase		Tyrosine-type, site-specific recombinase/integrase
IS6100	IAU57_09080	<i>tnpA2</i>	IRL: ggctctgttgcaagattggcggcagtcagagg; IRR: ggctctgttgcaaaaatcgtgaagcttgagcat	IS6-like element
In2020	IAU57_09085	<i>N</i> -Acetyltransferase		GNAT family protein
	IAU57_09090	<i>sul1</i>		Sulfonamide-resistant dihydropteroate synthase
	IAU57_09095	<i>qacEΔ1</i>		Quaternary ammonium compound efflux SMR (truncated) transporter
	IAU57_09100	<i>bla</i> <sub>OXA-2</sub>		Oxacillin-hydrolyzing class D $\beta$ -lactamase
	IAU57_09105	<i>aacA27</i>		Aminoglycoside <i>N</i> - acetyltransferase AAC(6')-IIc
ISPsy42	IAU57_09110	<i>intl1</i> <sup>e</sup>		Class 1 integron integrase Intl1
	IAU57_09115	<i>yafQ</i>	IRR: aatgatgacctcaagccggttctgctcg	Type II toxin-antitoxin system
	IAU57_09120	Invertase		Recombinase family protein; DNA invertase Pin-like protein
	IAU57_09125	<i>tnpR</i>	IRL: aatgttctccgtgccccttccggccg DR: accccAATAA	TnpR resolvase protein
	IAU57_09130	Hypothetical protein		

<sup>a</sup> A novel integron was identified in this study.

<sup>b</sup> DR, direct repeat; IRL, left inverted repeat; IRR, right inverted repeat. Lowercase letters are used for gene sequences and capital letters are used for direct repeat sequences.

<sup>c</sup> Putative functions of the gene were identified from an NCBI protein BLAST search. GNAT, GCN5-related *N*-acetyltransferase; SMR, small multidrug resistance.

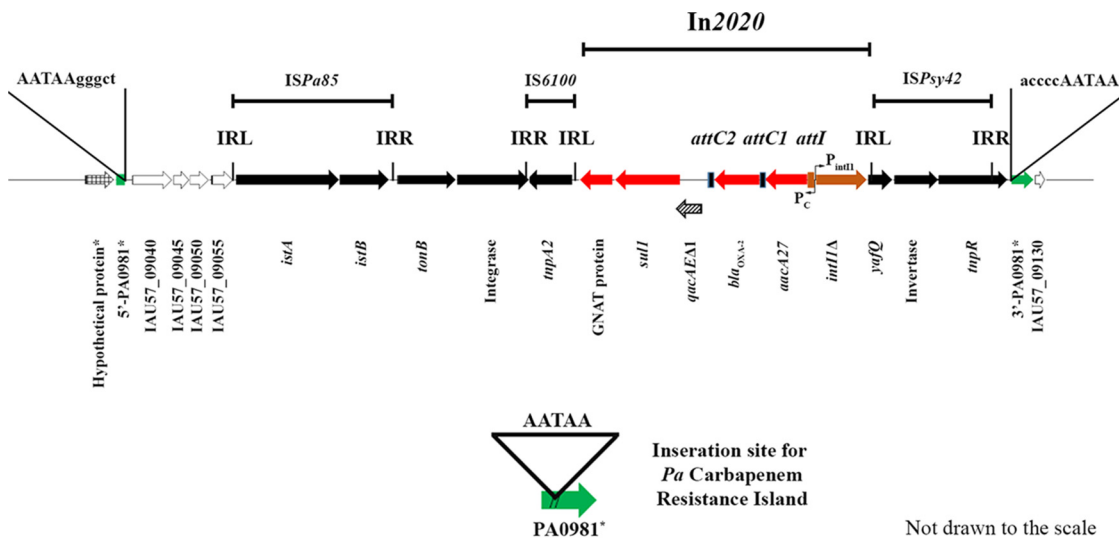
<sup>d</sup> Gene was not annotated in CMC-097.

<sup>e</sup> Gene was truncated and overlapped.

PABCH45) was used to assist in joining contigs separated by repeated sequences of IS elements and rRNA operons. Finally, Geneious was used to map 96% of the PE reads to this complete genome with a uniform average coverage of 1,487 $\times$ .

The complete assembly of CMC-097 resulted in a circular genome of 7,044,064 bp, with a G+C content of 66.4%. The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v5.0 (6, 7) identified 6,632 genes (IAU57\_00005 to IAU57\_33160), including 6,467 protein-coding genes, 82 RNA genes (65 tRNAs, 12 rRNAs, and 5 noncoding RNAs), 83 pseudogenes, and 1 CRISPR array.

BLAST analysis of the resulting genome found that it was highly similar to *P. aeruginosa* strain W60856 (GenBank accession number [CP008864.2](#)) and *P. aeruginosa* strain PABCH45 (GenBank accession number [CP056101.1](#)), with differences of <1/1,000 bp over genome stretches as long as 400,000 bp. However, the genome sequence was interrupted by many different IS elements, including 13 copies of an IS21 family transposon, consisting of a transposase (*istA*) gene and a transposition helper (*istB*) gene (e.g., IAU57\_01410 to IAU57\_01415), and 9 copies of an IS3 family transposase (e.g., IAU57\_01325). *P. aeruginosa* CMC-097 also contains a CR class 1 integron, called In2020, which was defined and named by INTEGRALL (12) and is detailed in Table 1 and shown in Fig. 1.



**FIG 1** Antibiotic resistance island containing In2020 in *P. aeruginosa* CMC-097 (nucleotide positions 1944662 to 1961187). The IS, inverted repeat, and transposon elements (*tnp*) were identified using ISfinder with their previously described names (13). The solid arrows represent the annotated open reading frames (ORFs) and their orientations, including the novel *P. aeruginosa* In2020 containing the GCN5-related *N*-acetyltransferase (GNAT), *sulI*, *qacAEM*, *bla*<sub>OXA-2</sub>, and *accA27* resistance genes in red. Open arrows indicate ORFs for hypothetical proteins. The terminal direct repeats belonging to the insertion sites in the PA0981 gene are in green. The P<sub>C</sub> (−10, nucleotide positions 1956769 to 1956774; −35, nucleotide positions 1956792 to 1956797) is the common promoter present in the integron, and P<sub>int1</sub> (−35, nucleotide positions 1956627 to 1956632; −10, nucleotide positions 1956650 to 1956655) is the promoter for the truncated integrase gene *IntI1*<sub>R32\_H39Δ</sub> (nucleotide positions 1956682 to 1957845). The recombination sites for *IntI1Δ*, *attI* (nucleotide positions 1956539 to 1956601), *attC1* (nucleotide positions 1955802 to 1955871), and *attC2* (nucleotide positions 19549326 to 1954995) are marked with vertical bars (12). Notably, insertion elements ISPa85 and IS6100 were found upstream and downstream of *tonB* and another integrase. Also, downstream of In2020 the ISPsy42 element is inserted into the broken segment of PA0981. The inverted repeats at the 5' and 3' ends (left and right ends), i.e., the left inverted repeat (IRL) and the right inverted repeat (IRR), for the IS elements and transposons are marked.

**Data availability.** The annotated complete genome assembly of *Pseudomonas aeruginosa* strain CMC-097 is available in GenBank under the accession numbers [CP065848](https://doi.org/10.1093/genbank/CP065848), [SRR14783931](https://doi.org/10.1093/genbank/SRR14783931), [PRJNA660482](https://doi.org/10.1093/genbank/PRJNA660482), and [SAMN15950776](https://doi.org/10.1093/genbank/SAMN15950776). The novel In2020 sequence was registered at INTEGRALL, a web-based platform dedicated to integron identification, under the accession number CP065848 (<http://integrall.bio.ua.pt/?acc=CP065848>).

## ACKNOWLEDGMENTS

We acknowledge the faculty members of the Division of Infectious Disease, Carilion Clinic, and the Carilion clinical microbiology laboratory for their assistance in collecting patient samples in the Carilion Clinic institutional review board-approved study. Experiments were carried out at the Carilion basic science research laboratory, Carilion Roanoke Community Hospital. We thank Sarah Cox, Radford University Carilion, for her valuable support in manuscript reading and suggestions.

This study was supported by the Carilion Clinic (D.C.G. and J.R.), a graduate student research supply grant to A.A. was approved by Virginia Tech, and the Illumina sequencing was completed with the support of the Fralin Life Science Institute (R.V.J.).

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