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GENOME SEQUENCES

Complete Genome Sequence of *Providencia stuartii* CMC-4104, Isolated from a Human Splenic Abscess, Containing Multiple Copies of NDM-1 and PER-1 Carbapenem Resistance Genes

Jayasimha Rao,[a,b,c] Nicholas K. Stornelli,[b,d] Nathan A. Everson,[b,d] Lauren F. McDaniel,[b,d] Mariana Gomez De La Espriella,[a,b] Jason R. Faulhaber,[a,b,c] S. Michelle Todd,[e] Kevin K. Lahmers,[e] Roderick V. Jensen[f]

[a]Internal Medicine, Division of Infectious Disease, Carilion Medical Center, Roanoke, Virginia, USA
[b]Division of Infectious Disease, Virginia Tech Carilion School of Medicine, Roanoke, Virginia, USA
[c]Center for Emerging, Zoonotic, and Arthropod-Borne Pathogens, Virginia Tech, Blacksburg, Virginia, USA
[d]Department of Pharmacy, Carilion Roanoke Memorial Hospital, Roanoke, Virginia, USA
[e]Department of Biomedical Sciences and Pathobiology, VA-MD College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia, USA
[f]Department of Biological Sciences, Virginia Tech, Blacksburg, Virginia, USA

Jayasimha Rao and Nicholas K. Stornelli contributed equally to this article. Jayasimha Rao contributed the majority of the work, including writing the manuscript.

ABSTRACT We report the complete genome sequence of a clinical isolate of *Providencia stuartii* strain CMC-4104, isolated from a splenic abscess. Oxford Nanopore Technologies (ONT) and Illumina sequencing reads were assembled using Geneious to generate a 4,504,925-bp circular chromosome containing multiple copies of the NDM-1 and PER-1 genes in a genomic resistance island.

The spread of carbapenemase-producing *Enterobacterales* is increasing in health care facilities (1). We describe a multidrug-resistant (MDR) clinical isolate of *Providencia stuartii* recovered from an infected necrotizing pancreatitis patient, belonging to the class of difficult-to-treat resistance (2, 3) strains, panresistant to first-line antimicrobials, including newer β-lactam/β-lactamase inhibitor combinations (4–6).

P. stuartii CMC-4104, isolated from a patient with splenic abscess aspirate, was inoculated onto sheep blood agar and MacConkey agar plates and incubated at 35°C aerobically for 24 to 72 h. Antibiotic susceptibility testing for 17 antimicrobial agents based on the CLSI M100 standards (7) was carried out by Quest Diagnostics at the Carilion Clinic (Table 1).

A single colony of *P. stuartii* CMC-4104 was grown in 25 mL lysogeny broth at 37°C and 200 rpm for 18 h. The cell pellet was used for genomic DNA isolation with a Genomic-tip 20/G kit (8, 9). The unsheared DNA was sequenced without size selection using the ONT 9.4.1 MinION flow cell with the SQK-LSK109 ligation sequencing kit. MiSeq Illumina paired-end sequencing (350-bp insert size) was performed using the TruSeq DNA PCR-free library prep kit.

The ONT sequencing generated 19,109 reads with a maximum length of 170,418 bp and an average length of 15,700 bp for a total of 306 Mbp. Base calling was performed using Guppy 4.4.1, and Porechop 0.2.4 (10) was used for adaptor trimming. The resulting fastq files were initially assembled using Flye 2.8 (11) in Geneious Prime 2022.0.2 to produce a large, closed 4.59-Mbp draft genome with ~65× coverage. The Illumina sequencing generated 2,290,766 paired-end reads 150 bp long for a total of 344 Mbp. The "Map to Reference" tool in Geneious Prime was used (with low sensitivity settings) to align all of the Illumina reads to the draft genome sequence and correct ONT sequencing errors. Variations in the Illumina read coverage were used to identify three circular plasmid sequences and a highly repeated set of MDR gene cassettes in the

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Address correspondence to Jayasimha Rao, jrao@carilionclinic.org, or Roderick V. Jensen, rvjensen@vt.edu.

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TABLE 1 Antimicrobial susceptibility for *P. stuartii* strain CMC-4104

Antimicrobial	MIC[a]	Result
Trimethoprim sulfamethoxazole	≥320	R
Ampicillin+sulbactam	≥32	R
Ertapenem	≥8	R
Imipenem	≥16	R
Piperacillin+tazobactam	≥128	R
Cefazolin	≥64	R
Cefepime	≥32	R
Ceftazidime	≥64	R
Ceftriaxone	≥64	R
Ceftazidime+avibactam[b]	≥250	R
Imipenem+relebactam[b]	≥32	R
Meropenem+vaborbactam[b]	≥250	R
Tobramycin/gentamicin	4	R
Levofloxacin	≥16	R
Cefiderocol[c]	2	S

[a] MICs were determined (7) using the Vitek and Verigene testing systems at the Quest Diagnostics microbiology laboratory at Carilion. The carbapenemase-producing strain was confirmed by the Commonwealth of Virginia Consolidated Laboratory Services, VDH.
[b] Susceptibility testing was performed using the E-test for ceftazidime+avibactam, imipenem+relebactam, and meropenem+vaborbactam.
[c] Cefiderocol susceptibility testing was completed by Associated Regional and University Pathologists Laboratories in Salt Lake City, UT.

main chromosome. Finally, Geneious Prime was used to align the ONT reads to the plasmid and genome sequences (with medium sensitivity settings) to confirm closure of the circular plasmid and main chromosome sequences, as well as the repetition of the MDR gene cassettes. Default parameters were used for all software unless otherwise specified.

Final assembly of the *P. stuartii* CMC-4104 genome resulted in a main chromosome (GenBank accession number CP095443) of 4,504,925 bp with 41.4% GC content and an average Illumina coverage (AIC) of 65×; a large, low-copy, circular plasmid (CP095444) of 278,489 bp with 47.3% GC content and an AIC of 85×; a small, high-copy, circular plasmid (CP095445) of 2,683 bp with 41.8% GC content and an AIC of 4,616×; and a phage-like circular sequence (CP095442) of 51,458 bp with 41.7% GC content and an AIC of 105×. In the main chromosome (CP095443), the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) 6.1 (12) was used to identify 4,066 protein coding genes, 79 tRNAs, 22 rRNAs, and 4 CRISPR arrays. Of particular note was a large 215-kbp genomic resistance island (*Ps*GRI) integrated with a site-specific integrase into the 3′ end of a tRNA modification GTPase gene (*mnmE*), like the *Salmonella* genomic island (13, 14). Remarkably, the *Ps*GRI contained 15 copies of the MDR gene cassettes containing $bla_{\text{NDM-1}}$ and 2 copies of $bla_{\text{PER-1}}$ shown in Fig. 1. The large plasmid (CP095444) also contained one complete copy each of the $bla_{\text{NDM-1}}$ and $bla_{\text{PER-1}}$ MDR cassettes and an additional $bla_{\text{OXA-10}}$ MDR cassette (14–18). The small, high-copy plasmid (CP095445) also contained a fluoroquinolone resistance gene, *qnrD* (19). The assembly revealed a circular phage-like sequence (CP095442) that is identical to a prophage in the main chromosome and is very similar to a second prophage integrated into a CRISPR locus (20).

Data availability. The annotated complete genome assembly of strain *Providencia stuartii* CMC-4104 is available at GenBank under accession numbers CP095442.1, CP095443.1, CP095444.1, and CP095445.1, SRA accession numbers SRR18691816 and SRR18691817, BioProject accession number PRJNA824933, and BioSample accession number SAMN27484493.

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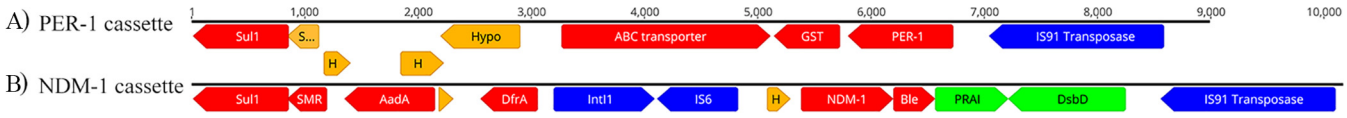

FIG 1 Gene organization in the repeated multidrug-resistant (MDR) cassettes. (A) The PER-1 cassette (8,986 bp) included genes for sulfonamide-resistant dihydropteroate synthase (*sul1*), small multidrug resistance (SMR) (*qacE*Δ1) (truncated), hypothetical proteins, ATP-binding protein/permease (ABC transporter), glutathione *S*-transferase (GST) family protein, class A extended-spectrum β-lactamase (bla_{PER-1}), and IS*CR1* family transposase (IS*91*). (B) The New Delhi metallo β-lactamase (bla_{NDM-1}) cassette (10,494 bp) included genes for sulfonamide-resistant dihydropteroate synthase (*sul1*), small multidrug resistance (SMR) *qacE*Δ1, aminoglycoside 3″-*O*-nucleotidyltransferase (*aadA*), DUF1010 domain-containing hypothetical protein, trimethoprim-resistant dihydrofolate reductase (*dfrA*12), class 1 integron integrase (*intI1*), IS*26* family transposase (IS*6*), IS*30* family transposase (truncated), subclass B1 β-lactamase (bla_{NDM-1}), bleomycin resistance protein (Ble), phosphoribosylanthranilate isomerase (PRAI), cytochrome c-type biogenesis protein/protein-disulfide reductase (*dsbD*), and IS*CR1* family transposase (IS*91*). Red represents drug resistance genes; blue, transposases; green, metabolic genes; and yellow, hypothetical or pseudogenes (truncated or overlapped). Annotated genes were used from NCBI GenBank, and the scale indicates the number of base pair residues.

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