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GENOME SEQUENCES

# Whole-Genome Sequences of Two NDM-1-Producing *Pseudomonas aeruginosa* Strains Isolated in a Clinical Setting in Albania in 2018

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**ABSTRACT** Isolation of metallo-β-lactamase-producing, carbapenem-resistant, *Pseudomonas aeruginosa* strains is increasingly being documented worldwide; their presence constitutes a public health threat. Here, we report draft genome sequences of two New Delhi metallo-β-lactamase-1-producing, multidrug-resistant, *P. aeruginosa* strains of sequence type 235 that were isolated from the surgical wound of two patients hospitalized in the same ward.

*Pseudomonas aeruginosa* isolates belonging to sequence type 235 (ST235), an international high-risk clone that has the potential to cause nosocomial outbreaks with poor clinical outcomes, are a cause of serious concern. A recent study (1) estimated that the ST235 sublineage emerged in Europe around 1984 and has successfully spread worldwide since then. Antibiotic inactivation through metallo-β-lactamase (MBL) possession is one of the resistance mechanisms. New Delhi MBL-1 (NDM-1)-producing *P. aeruginosa* strains have been reported in Serbia, Romania, (2, 3), and Italy (4) but not in Albania. The presence of this enzyme in Albania was first documented in 2018 in a *Klebsiella pneumoniae* isolate from a digestive carrier (5). Little is known regarding the spread of MBLs in Albania. A case of a *K. pneumoniae* carbapenemase 3 (KPC-3)-producing *K. pneumoniae* isolate was described in 2015 (6). Here, we report the genome sequences of two NDM-1-producing *P. aeruginosa* strains of ST235 (PA4 and PA5) that were isolated from the surgical wound of two patients hospitalized in the same ward.

Species identification was performed with the BBL Crystal enteric/nonfermenter identification kit (Becton, Dickinson, Sparks, MD), and results were confirmed by matrix-assisted laser desorption ionization–time of flight (MALDI–TOF) mass spectrometry on a MALDI Biotyper system (Bruker Daltonics, Germany).

Bacterial cultures were purified for DNA extraction by two successive single-colony selections after streaking on blood agar medium (Becton, Dickinson) and incubation overnight at 37°C. DNA was extracted from a liquid suspension of the purified cultures by using the Maxwell SEV 16-cell DNA purification kit, in combination with a Maxwell 16 instrument, to perform automated isolation of genomic DNA.

All strains were sequenced at the San Raffaele Hospital (Milan, Italy) on the NextSeq 500 platform (Illumina, Inc., San Diego, CA), with a paired-end run of 300 cycles, after Nextera XT library preparation, targeting a minimum coverage of 50-fold. Output raw reads were trimmed using Trimmomatic v.0.33 software to

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**TABLE 1** RGI results for PA4 and PA5

| Strain | ARO[a] term | RGI criteria |
|---|---|---|
| PA4 | AAC(6′)-Il | Perfect |
| | *adeF* | Strict |
| | ANT(2″)-Ia | Perfect |
| | APH(3′)-IIb | Strict |
| | *arnA* | Strict |
| | *basR* | Strict |
| | *basS* | Strict |
| | *bcr-1* | Strict |
| | FosA | Strict |
| | MexA | Perfect |
| | MexB | Strict |
| | MexC | Strict |
| | MexD | Strict |
| | MexE | Strict |
| | MexF | Perfect |
| | MexG | Perfect |
| | MexH | Strict |
| | MexI | Strict |
| | MexJ | Strict |
| | MexK | Perfect |
| | MexL | Perfect |
| | *mexM* | Strict |
| | *mexN* | Strict |
| | *mexP* | Strict |
| | *mexQ* | Strict |
| | MexR | Strict |
| | MexS | Strict |
| | MexT | Strict |
| | MexV | Strict |
| | MexW | Strict |
| | MexZ | Strict |
| | MuxA | Strict |
| | MuxB | Perfect |
| | MuxC | Perfect |
| | *nalC* | Strict |
| | *nalD* | Strict |
| | NDM-1 | Perfect |
| | OpmB | Perfect |
| | OpmD | Strict |
| | *opmE* | Strict |
| | OpmH | Perfect |
| | OprJ | Strict |
| | OprM | Perfect |
| | OprN | Strict |
| | OXA-488 | Perfect |
| | PDC-2 | Strict |
| | PmpM | Strict |
| | *Pseudomonas aeruginosa* catB7 | Strict |
| | *Pseudomonas aeruginosa* CpxR | Perfect |
| | *Pseudomonas aeruginosa emrE* | Perfect |
| | *Pseudomonas aeruginosa gyrA* conferring resistance to fluoroquinolones | Strict |
| | *Pseudomonas aeruginosa soxR* | Perfect |
| | *qacH* | Strict |
| | *sul1* | Perfect |
| | TriA | Strict |
| | TriB | Perfect |
| | TriC | Strict |
| | Type A NfxB | Strict |
| PA5 | AAC(6′)-Il | Perfect |
| | *adeF* | Strict |
| | ANT(2″)-Ia | Perfect |
| | APH(3′)-IIb | Strict |
| | *arnA* | Strict |
| | *basR* | Strict |
| | *basS* | Strict |
| | *bcr-1* | Strict |

(Continued on next page)

**TABLE 1** (Continued)

| Strain | ARO[a] term | RGI criteria |
|---|---|---|
| | FosA | Strict |
| | MexA | Perfect |
| | MexB | Strict |
| | MexC | Strict |
| | MexD | Strict |
| | MexE | Strict |
| | MexF | Perfect |
| | MexG | Perfect |
| | MexH | Strict |
| | MexI | Strict |
| | MexJ | Strict |
| | MexK | Perfect |
| | MexL | Perfect |
| | *mexM* | Strict |
| | *mexN* | Strict |
| | *mexP* | Strict |
| | *mexQ* | Strict |
| | MexR | Strict |
| | MexS | Strict |
| | MexT | Strict |
| | MexV | Strict |
| | MexW | Strict |
| | *mexY* | Strict |
| | MexZ | Strict |
| | MuxA | Strict |
| | MuxB | Perfect |
| | MuxC | Perfect |
| | *nalC* | Strict |
| | *nalD* | Strict |
| | NDM-1 | Perfect |
| | OpmB | Perfect |
| | OpmD | Strict |
| | *opmE* | Strict |
| | OpmH | Perfect |
| | OprJ | Strict |
| | OprM | Perfect |
| | OprN | Strict |
| | OXA-488 | Perfect |
| | PDC-2 | Strict |
| | PmpM | Strict |
| | *Pseudomonas aeruginosa catB7* | Strict |
| | *Pseudomonas aeruginosa* CpxR | Perfect |
| | *Pseudomonas aeruginosa emrE* | Perfect |
| | *Pseudomonas aeruginosa gyrA* conferring resistance to fluoroquinolones | Strict |
| | *Pseudomonas aeruginosa soxR* | Perfect |
| | *qacH* | Strict |
| | *sul1* | Perfect |
| | TriA | Strict |
| | TriB | Perfect |
| | TriC | Strict |
| | Type A NfxB | Strict |

[a] ARO, Antibiotic Resistance Ontology.

remove the adapters. Cleaned reads were used for *de novo* assembly with SPAdes v.3.6.1 (7) using the following parameters: PHRED quality offset for the input reads of 33, "careful mode" (which reduces the number of mismatches and short indels and also runs Mismatch Corrector, a postprocessing tool that uses the BWA tool), and default *k*-mer length settings to set *k*-mer lengths of 21, 33, 55, and 77. The quality of the assemblies was checked using a quality control tool for high-throughput sequence data, FastQC v.0.11.8 (https://www.bioinformatics.babraham.ac.uk/projects/download.html#fastqc).

The assembled contigs were evaluated with ResFinder v.3.0 (8), which is available from the Center for Genomic Epidemiology (http://www.genomicepidemiology.org), and Resis-

tance Gene Identifier (RGI) v.5.1.0 from the Comprehensive Antibiotic Resistance Database (CARD), v.3.0.5 (9) (http://arpcard.mcmaster.ca). ResFinder was used for the specific identification of acquired resistance genes, while RGI was used to complement the data for resistome prediction, including not only acquired resistance but also intrinsic and mutation-driven resistance. The following parameters were used with RGI: selection of perfect and strict hits only, exclusion of the nudge of loose hits with ≥95% identity to strict hits, and high sequence quality and coverage. Multilocus sequence typing (MLST) was performed using the *P. aeruginosa* PubMLST database (10) (https://pubmlst.org/paeruginosa). Core-genome MLST (cgMLST) and whole-genome MLST (wgMLST) were performed using SeqSphere+ v.5.1.1 (Ridom, Muenster, Germany).

The read length was 300 cycles, and the numbers of total reads for each strain were 2,323,831 for PA4 and 1,837,472 for PA5. The assembly of PA4 resulted in 480 contigs ($N_{50}$, 37,820 bp) comprising 6,941,401 bp, with a GC content of 66.1%. The assembly of PA5 resulted in 507 contigs ($N_{50}$, 37,045 bp) comprising 6,887,548 bp, with a GC content of 66.3%.

Through the CARD, a total of 58 antibiotic resistance genes were identified in PA4 (19 perfect hits and 39 strict hits), including genes conferring resistance to β-lactams, aminoglycosides, fluoroquinolones, macrolides, and tetracyclines through different mechanisms, such as antibiotic efflux ($n = 37$), antibiotic efflux and antibiotic target alteration ($n = 3$), antibiotic inactivation ($n = 11$), antibiotic target alteration ($n = 6$), and antibiotic target replacement ($n = 1$). PA5 expressed all 58 antibiotic resistance genes of PA4 plus the antibiotic efflux pump gene *mexY* (19 perfect hits and 40 strict hits). RGI results for PA4 and PA5 are summarized in Table 1.

ResFinder identified genes responsible for acquired resistance to aminoglycosides [*aph(3′)-IIb*, *ant(2″)-Ia*, and *aac(6′)-Il*], β-lactams ($bla_{PAO}$, $bla_{NDM-1}$, and $bla_{OXA-488}$), fluoroquinolones (*crpP*), fosfomycin (*fosA*), phenicols (*catB7*), and sulfonamides (*sul1*). cgMLST showed 8 of 4,283 allele differences, whereas only 10 of 5,188 allele differences were found using wgMLST.

**Data availability.** The whole-genome shotgun project has been deposited in GenBank under BioProject accession number PRJNA522042. The BioSample accession numbers are SAMN10923322 for PA4 and SAMN10923323 for PA5.

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