



## Genome Note

# Whole-genome sequencing of an NDM-1- and OXA-58-producing *Acinetobacter towneri* isolate from hospital sewage in Sichuan Province, China



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## ABSTRACT

**Objectives:** *Acinetobacter* spp. isolates carrying the *bla*<sub>NDM-1</sub> gene are frequently reported. However, most reported *bla*<sub>NDM-1</sub> genes are carried by clinical strains. Here we report a carbapenem-resistant *Acinetobacter towneri* isolate from hospital sewage in China co-harboring *bla*<sub>NDM-1</sub> and *bla*<sub>OXA-58</sub> in the genome.

**Methods:** Whole-genome sequencing was performed using a single molecule, real-time (SMRT) sequencing platform with a Pacific Biosciences RS II Sequencer and MiSeq system. Reads were de novo assembled using Celera Assembler v.8.0. Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP), and the genome sequence was analysed by bioinformatics methods. **Results:** The 2 963 729-bp genome with a G+C content of 41.30% displayed 11 antimicrobial resistance genes, including *bla*<sub>NDM-1</sub> and *bla*<sub>OXA-58</sub>. Meanwhile, 2 plasmids and 19 genomic islands were predicted within the genome.

**Conclusion:** The whole-genome sequence reported here can be compared with other genomes of NDM-1-producing *Acinetobacter* spp. These data could facilitate further understanding of the specific genomic features of carbapenem-resistant *Acinetobacter* spp. in China.

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Carbapenems are recognised to be the most powerful drugs for the therapy of infections caused by multidrug-resistant Gram-negative bacteria [1]. However, increased use of carbapenems has resulted in the emergence of carbapenem resistance. Hospital sewage usually contains antimicrobial-resistant bacteria in high densities, providing a vital reservoir of resistance genes as well as being a hotspot for the transfer of resistance genes and mobile genetic elements [2]. Therefore, hospital sewage is considered to be a perfect place to search for powerful antimicrobial resistance genes [3].

In this study, hospital sewage was obtained in July 2017 from the influx of the main drainage system of the wastewater treatment plant of the Affiliated Hospital of Southwest Medical University located in Luzhou City (beside the River Yangtze), in

Sichuan Province, China. The sewage was diluted 1:10 and an aliquot (100 µL) was streaked onto CHROMagar™ Orientation medium (CHROMagar, Paris, France) agar plates containing 8 µg/mL meropenem and 32 µg/mL linezolid. The plates were incubated at 35 °C overnight. An *Acinetobacter* sp. isolate (SWBY1) with relatively high minimum inhibitory concentrations (MICs) to both imipenem and meropenem (16 µg/mL) was selected. Species identification of SWBY1 was performed by 16S rRNA and *rpoB* gene sequencing analysis, which revealed that the isolate was *Acinetobacter towneri*.

The whole genome of SWBY1 was sequenced using a single molecule, real-time (SMRT) sequencing platform with a Pacific Biosciences RS II sequencer (Pacific Biosciences, Menlo Park, CA) and MiSeq system (Illumina Inc., San Diego, CA). The reads were de novo assembled using Celera Assembler v.8.0. Annotation of the genome of SWBY1 was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). Antimicrobial resistance genes carried by SWBY1 were identified using ResFinder 3.0 (<https://cge.cbs.dtu.dk/services/ResFinder/>). Genomic islands (GIs)

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**Table 1**  
Antimicrobial resistance genes in *Acinetobacter towneri* strain SWBY1.

Antimicrobial class	Resistance gene	Genetic context	HSP length/query	Position	Predicted resistance phenotype	Accession no.
Aminoglycosides	<i>strA</i>	Chromosome	804/804	1 843 607–1844 410	Aminoglycosides	<a href="#">AF321551</a>
	<i>strB</i>	Chromosome	837/837	1 844 410–1845 246	Aminoglycosides	<a href="#">M96392</a>
β-Lactams	<i>aph(3')-VIa<sup>a</sup></i>	pSWBY1-1	779/780	7696–8475	Aminoglycosides	<a href="#">CP021210</a>
	<i>bla<sub>OXA-58</sub></i>	Chromosome	843/843	1 840 896–1841 738	β-Lactams	<a href="#">AY665723</a>
	<i>bla<sub>NDM-1</sub></i>	pSWBY1-1	813/813	9753–10565	β-Lactams	<a href="#">MF511773</a>
	<i>bla<sub>MCA</sub><sup>b</sup></i>	pSWBY1-1	1154/1174	37 340–38 161 1–340	β-Lactams	<a href="#">CP032277</a>
Tetracyclines	<i>tet(39)</i>	Chromosome	1122/1122	1 823 584–1824 705	Tetracyclines	<a href="#">KT346360</a>
Phenicolis	<i>floR</i>	Chromosome	1214/1214	1 852 878–1854 091	Phenicolis	<a href="#">AF118107</a>
Sulphonamides	<i>sul2</i>	Chromosome	816/816	1 847 594–1848 409	Sulphonamides	<a href="#">GQ421466</a>
Macrolides	<i>mph(E)</i>	pSWBY1-1	885/885	1327–2211	Macrolides	<a href="#">KY883660</a>
	<i>msr(E)</i>	pSWBY1-1	1476/1476	2267–3742	Macrolides	<a href="#">KX443408</a>

<sup>a</sup> This gene has one amino acid mutation (L84F) compared with *aph(3')-VIa*.

<sup>b</sup> The encoding genes for class C β-lactamases carried in the chromosome of non-*baumannii* *Acinetobacter* spp. have not been named officially, which were annotated by GeneMarkS+ 4.6 as *bla<sub>MCA</sub>*.

were predicted based on three different GI prediction tools (IslandPATH-DIMOB, IslandPick and SIGI-HMM) [4].

The total genome size of SWBY1 was 2 963 729 bp, consisting of a chromosome and two plasmids (pSWBY1-1 and pSWBY1-2). The G+C content was 41.30%. A total of 6474 high-quality filtered reads were generated with a >200× total coverage, including 21 rRNA operons, 77 tRNA, 19 GIs and 2772 predicted protein-coding sequences. The total genome of SWBY1 included genes conferring resistance to β-lactams, aminoglycosides, macrolides, sulphonamides, tetracyclines and phenicolis (Table 1).

The *bla<sub>NDM-1</sub>* gene was located on plasmid pSWBY1-1 (38 161 bp), inside a truncated Tn125 transposon, with complete *ISAbA125* and *ISAbA14* insertion sequences upstream, along with a complete *ble<sub>MBL</sub>* gene, a truncated *trpF* gene and *ISAbA3* downstream. Two pairs of genes encoding toxin–antitoxin (HigB–HigB) systems were located both upstream and downstream of the truncated Tn125 element. Besides, *mph(E)* and *msr(E)* were also located in pSWBY1-1, followed by XerC/XerD binding sites downstream (Supplementary Fig. S1 in the online version at DOI: [10.1016/j.jgar.2018.11.015](#)).

Nineteen GIs, designated SWBY1-GI-1 to SWBY1-GI-19 were identified. SWBY1-GI-15 (12 067 bp) carried *tet(39)*, whilst SWBY1-GI-16 (33 827 bp) carried *bla<sub>OXA-58</sub>*, *strA/B*, *sul2* and *floR*. SWBY1-GI-16 contained the site-specific resolvase-encoding gene *cinH*. An opposite pair of *IS1006 tnpA* was located upstream of the *cinH* gene, inside which was genes encoding aldehyde dehydrogenase and aryl-alcohol dehydrogenase, the Co/Zn/Cd-transporting ATPase-encoding gene *czcD*, the Cd(II)/Pb(II)-responsive transcriptional regulator-encoding gene *Cd(II)*, along with the toxin–antitoxin system-encoding genes *relE* and *relB*. Furthermore, SWBY1-GI-16 contained a 15 685-bp antimicrobial resistance-related sequence that involved four regions: region I ( $\Delta$ *ISAbA3-bla<sub>OXA-58</sub>-ΔISAbA3*); region II (*IS1008-strA-strB-ΔtraB-trbI-IS1008*), which was linked with region I by the partial *araC1* gene; region III (*ISAbA1-sul2-glmM-ΔISCR2*), which was part of Tn6450; and region IV ( $\Delta$ *ISCR2-lysR-floR-ΔICEAp12-virD2-ΔISCR2*), which was linked with region III by the *ISEc57* element (Supplementary Fig. S2 in the online version at DOI: [10.1016/j.jgar.2018.11.015](#)).

Here we report a carbapenem-resistant *A. towneri* isolate co-harboring *bla<sub>NDM-1</sub>* and *bla<sub>OXA-58</sub>* in China. *Acinetobacter towneri* clinical strains carrying *bla<sub>NDM-1</sub>*-harbouring plasmids were reported in China [5], however the *bla<sub>NDM-1</sub>* gene in SWBY1

was isolated from hospital sewage. To our knowledge, this is the first *A. towneri* carrying *bla<sub>NDM-1</sub>* identified in Sichuan Province, China. SWBY1 carried two plasmids and 19 GIs, which could offer great genetic flexibility to adapt to the antimicrobial selective pressure in sewage water. Surveillance of antimicrobial resistance genes in hospital sewage could provide complementary information to the monitoring data from clinical strains, which could be used to portray a panorama of antimicrobial resistance inside the hospital.

#### Nucleotide sequence accession no.

The complete genome sequences of *A. towneri* SWBY1 and its plasmids (pSWBY1-1 and pSWBY1-2) have been deposited in GenBank under the accession nos. [CP026616.1](#), [CP026617.1](#) and [MH999418](#), respectively.

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#### Competing interests

None declared.

#### Ethical approval

Not required.

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