Whole-Genome Analysis of Two Copies of \( \text{bla}_{\text{NDM-1}} \) Gene Carrying \textit{Acinetobacter johnsonii} Strain Acsw19 Isolated from Sichuan, China

Lingtong Tang\textsuperscript{1,2}, Wei Shen\textsuperscript{2,3}, Zhikun Zhang\textsuperscript{2}, Jingping Zhang\textsuperscript{2}, Guangxi Wang\textsuperscript{2}, Li Xiang\textsuperscript{2}, Junping She\textsuperscript{2}, Xiaoyan Hu\textsuperscript{2}, Guoyuan Zou\textsuperscript{4}, Baoli Zhu\textsuperscript{5}, Yingshun Zhou\textsuperscript{2}

\textsuperscript{1}Department of Clinical Laboratory, The People’s Hospital of Gao County, Sichuan 644000, People’s Republic of China; \textsuperscript{2}Department of Pathogenic Biology, School of Basic Medicine, Southwest Medical University, Luzhou, Sichuan, People’s Republic of China; \textsuperscript{3}Department of Clinical Laboratory, The First People’s Hospital of Yibin, Yibin 644000, Sichuan, People’s Republic of China; \textsuperscript{4}Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Science, Beijing, People’s Republic of China; \textsuperscript{5}Key Laboratory of Pathogenic Microbiology & Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, People’s Republic of China

Correspondence: Yingshun Zhou
Department of Pathogenic Biology, School of Basic Medicine, Southwest Medical University, No. 319, Zhongshan Road, Luzhou 646000, Sichuan, People’s Republic of China
Tel +86-0830-3160073
Email yingshunzhou@swmu.edu.cn

Guoyuan Zou
Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Science, Beijing, People’s Republic of China
Email gyzou@163.com

Purpose: To characterize the genetic feature of the carbapenems resistant \textit{Acinetobacter johnsonii} strain Acsw19 isolated from municipal sludge. This strain was found to carry two copies of \( \text{bla}_{\text{NDM-1}} \), \textit{cmlB1}-like gene, and \( \text{bla}_{\text{OXA-211}} \)-like gene along with other 8 antimicrobial resistance genes, 3 plasmids, 15 genomic islands and 8 prophages.

Methods: A carbapenem-resistant \textit{Acinetobacter johnsonii} strain Acsw19 isolated from municipal sludge was subjected to whole-genome sequencing (WGS) via the PacBio and Illumina MiSeq platforms. Thereafter, the characteristic was analyzed by a series of bioinformatics software.

Results: The results showed that the genome of Acsw19 was consisted of a 3,433,749 bp circular chromosome and 3 circular plasmids, pAcsw19-1 (11,161 bp), pAcsw19-2 (351,885 bp) and pAcsw19-3 (38,391bp), respectively. Resistome analysis showed that Acsw19 carried 12 antimicrobial resistance genes, including 6 \{\textit{cmlB1}-like, \( \text{bla}_{\text{NDM-1}} \), \( \text{bla}_{\text{OXA-58}} \), \( \text{aph}(3’)-\text{Vfa} \), \( \text{msr(E)} \) and \( \text{mph(E)} \}\) in the plasmid pAcsw19-2 and 6 \{\( \text{bla}_{\text{OXA-211}} \)-like, \( \text{bla}_{\text{NDM-1}} \), \( \text{aph}(3’)-\text{Ib} \), \( \text{aph}(6’)-\text{Id} \), \( \text{sul2} \) and \( \text{floR} \}\) in the chromosome genome. Specifically, the \( \text{cmlB1}\)-like gene shared 86.33%, 71.7% and 71.9% similarities with the \( \text{cmlB1} \), \( \text{cmlA4} \) and \( \text{cmlA8} \) gene, and the \( \text{bla}_{\text{OXA-211}} \)-like gene shared 94.4%, 95.39% and 96.36% similarities with \( \text{bla}_{\text{OXA-211}} \), \( \text{bla}_{\text{OXA-643}} \) and \( \text{bla}_{\text{OXA-652}} \) at the nucleotide level, respectively. Phylogenetic analysis showed that the \( \text{bla}_{\text{OXA-211}} \)-like gene and \( \text{cmlB1}\)-like gene had the closest evolutionary relationship with \( \text{bla}_{\text{OXA-643}} \) and \( \text{cmlB1} \), respectively. These results indicated that the \( \text{bla}_{\text{OXA-211}} \)-like and \( \text{cmlB1}\)-like genes identified in the current study should be the novel variant resistance genes.

Conclusion: Carrying of two copies of \( \text{bla}_{\text{NDM-1}} \), \( \text{cmlB1}\)-like, \( \text{bla}_{\text{OXA-211}}\)-like and along with other 8 antimicrobial resistance genes, 3 plasmids, 15 genomic islands and 8 prophages \textit{Acinetobacter johnsonii} strain might increase the possibility of spreading of resistance genes.

Keywords: \textit{Acinetobacter johnsonii}, \( \text{bla}_{\text{NDM-1}} \), \( \text{bla}_{\text{OXA-211}} \), genomic island

Introduction

Producing of carbapenemases, including the \( \beta \)-lactamases of Ambler classes A, B (metallo-\( \beta \)-lactamases) and D, are the most common mechanism of bacterial carbapenems resistance.\textsuperscript{1,3} Especially, the New Delhi Metallo-\( \beta \)-lactamase (NDM), Klebsiella pneumoniae carbapenemase (KPC) and some Class D \( \beta \)-lactamases (CHDLs) have been identified worldwide in gram-negative bacterial isolates from clinical, environmental samples, and food animals, especially in \textit{Enterobacteriaceae}.\textsuperscript{1,4–9} and also in \textit{Pseudomonas aeruginosa} and \textit{Acinetobacter} species.\textsuperscript{10,12} Carbapenem-resistant
*Acinetobacter* species are mainly associated with the carbapenem-hydrolyzing NDM and CHDLs, such as *bla*<sub>NDM</sub>-like, *bla*<sub>OXA-23</sub>-like, *bla*<sub>OXA-24/40</sub>-like, *bla*<sub>OXA-51</sub>-like, *bla*<sub>OXA-134</sub>-like and *bla*<sub>OXA-211</sub>-like gene. Especially, co-carrying of the *bla*<sub>NDM</sub> and *bla*<sub>OXA</sub> in clinical, food, environmental derived isolates of *Acinetobacter* species were prevalent in the world. In addition, the genes encoding *bla*<sub>NDM</sub> and *bla*<sub>OXA</sub> is known to be carried on some mobile genetic elements that inserted into the chromosome or plasmids, and it is suspected the mechanism of horizontal gene transfer (HGT) promotes the exchange of resistance genes among pathogenic microorganisms isolated from the clinical, environmental samples, and food animals.

In this study, we mainly characterized the genetic feature of a carbapenems resistant strain *Acinetobacter johnsonii* Acsw19 isolated from municipal sludge. This strain was found to carry two copies of *bla*<sub>NDM-1</sub>, *cmlB1* -like gene, and *bla*<sub>OXA-211</sub>-like gene along with other 8 antimicrobial resistance genes, three plasmids, 15 genomic islands and 8 prophages.

### Materials and Methods

#### Bacterial Isolate, Identification and Antimicrobial Susceptibility Testing

The sample of municipal sewage was obtained from the influx of wastewater-related plant in Luzhou City (Sichuan Province, China) in March 2019. The sewage was 1:10 diluted and an aliquot (10 μL) was streaked onto a CHROM Agar Orientation (CHROMagar, Paris, France) agar plate containing 2 mg/L meropenem (Solarbio, China) and then incubated at 37 °C overnight. Bacterial species identification was carried out by the Vitek2 system (BioMérieux, France), 16sRNA sequencing and matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry. The minimal inhibitory concentrations (MICs) of 15 antimicrobial agents (Solarbio, China) including meropenem, imipenem, cefepime, cefotaxime, ceftazidime, piperacillin-tazobactam, amoxicillin-clavulanic acid, gentamicin, amikacin, aztreonam, erythromycin, chloramphenicol, sulfadiazine, colistin and ciprofloxacin were determined by broth microdilution method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI 2013, M100-S23). *Escherichia coli* strain ATCC 25,922 was used as quality control. Polymerase chain reaction (PCR) amplification and DNA sequencing were performed to identify the key carbapenemase-encoding genes (*bla*<sub>NDM</sub> and *bla*<sub>KPC</sub>) as previously reference.

Whole-Genome Sequencing and Analysis

Genomic DNA of the strain *A. johnsonii* Acsw19 was extracted using the DNA Kit (QIAGEN, Germany). The 10kb sequencing library and a 300 bp paired-end library were constructed using the standard PacBio RS sample and Illumina DNA sample preparation instructions, and then sequenced on Pacific Biosciences RS II and MiSeq systems sequencing platforms (Novogene, China). The reads were de novo assembled using the software Celera Assembler (version 8.0). Gene prediction was performed for the whole genome with Glimmer 3.02 (http://www.cbcb.umd.edu/software/glimmer). And the annotation of the Acsw19 genome was achieved using the NCBI Prokaryotic Genome Annotation Pipeline. Pairwise alignment was performed by BLASTn search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The average nucleotide identity (ANI) analysis was performed by the computer. The resistome was identified using ResFinder 2.1 (https://cge.cbs.dtu.dk/services/ResFinder) (minimum threshold for identity, 85%; minimum coverage, 60%) and Comprehensive Antibiotic Resistance Database (CARD) (https://card.mcmaster.ca/).

The genomic island sequences were predicted based on three different genomic islands (GIs) prediction software (IslandPATH-DIMOB, IslandPick, and SIGI-HMM) and the Prophage was predicted by using phiSpy.

### Results and Discussion

#### Bacterial Isolate, Identification and Resistance Gene Detection

A gram-stain-negative, *bla*<sub>NDM-1</sub> and *bla*<sub>OXA</sub> producing *Acinetobacter johnsonii* Acsw19 was isolated and identified by the Vitek2 system (BioMérieux, France), 16sRNA sequencing and matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry in Luzhou City, Southwestern China. *Acinetobacter johnsonii* strain Acsw19 was resistant to meropenem, imipenem, cefepime, cefotaxime, ceftazidime, piperacillin-tazobactam, amoxicillin-clavulanic acid, gentamicin, amikacin, aztreonam, erythromycin, chloramphenicol and sulfadiazine and it was susceptible to colistin and ciprofloxacin according to CLSI breakpoints (M100-S23) (Supplementary Table 1). To the best of our knowledge, the occurrence of multiple antibiotic genes in the multidrug resistant bacterial isolates from sewage has been evidenced in numerous studies, with the involvement of numerous species.
Characterization of the Whole Genome of Acinetobacter johnsonii Strain Acsw 19

We got 1,876,876,663 bp data of the whole genome by the WGS. The genome of Acinetobacter johnsonii Acsw19 consisted of a 3,433,749bp circular chromosome and three circular plasmids, pAcsw19-1, pAcsw19-2 and pAcsw19-3 in the size of 11,161bp, 351,885bp and 38,391 with the 35.66%, 38.79% and 33.76% G+C content, respectively. The chromosome has a 41.78% G+C content, 21 rRNA operons, 88 tRNAs, 44ncRNAs and 3413 predicted protein coding sequences (CDSs) (Table 1).

Resistome Analysis

A total of 12 drug-resistance genes was detected in the whole genome of Acsw19. Specifically, 6 resistance genes [(blaNDM-1, blaOXA-211-like, aph(3")-Ib (strB), aph(6)-Id (strA), sul2, and floR)] were located in the chromosome genome and 6 [cmlB1-like, mph(E), msr(E), blaOXA-58, blaoXA-645 and blaoXA-652] in plasmid pAcsw19-2 (Table 2). None of the drug-resistance gene was detected in plasmid pAcsw19-1 and pAcsw19-3. The full length of cmlB1-like gene consisted of 1266 nucleotides encoding a protein with 422 amino acids. Sequence analysis showed that the cmlB1-like gene shared 86.33%, 71.7% and 71.9% sequence similarities with the known cmlB1, cmlA4, and cmlA8 genes at nucleotide level and 67.8%, 44.8% and 44.5% at amino acid level, respectively. The full length of blaOXA-211-like gene consisted of 825 nucleotides encoding a protein with 275 amino acids. Sequence analysis showed that the blaOXA-211-like gene shared 94.4%, 95.39%, 95.52%, 96% and 96.36% identity with the known blaOXA-211, blaoXA-645, blaoXA-281, blaoXA-645 and blaoXA-652 gene at nucleotide level and 94.4%, 95.4%, 95.5%, 96% and 96.4% at amino acid level, respectively. Phylogenetic analysis showed that the blaOXA-211-like gene and cmlB1-like gene had the closest evolutionary relationship with blaoXA-645 (Figure 1) and cmlB1 (Figure 2). These results indicated that the blaOXA-211-like and cmlB1-like genes maybe two new allelic variant of the gene blaoXA and cmlB1. To our the knowledge, there are more new allelic variant of the resistance genes have been found from the sewage derived isolates.33,35,36

Characterization of Three Plasmids

Plasmid pAcsw19-1 contained 18 putative coding open reading frames (ORFs). Sequence analysis showed that pAcsw19-1 had 100%, 92%, 68% and 68% query cover and 96.55%, 91.5%, 87.8% and 87.8% sequence similarities with the plasmids p2_010062 (CP033122), p4_010055 (CP032283), p3_010030 (CP029391) and pALWEK1.4 (CP032107) at nucleotide level, and these reported plasmids

---

**Table 1** Characteristic of the Whole Genome of Acinetobacter johnsonii Acsw19

| Context       | Size (bp) | G+C (%) | No. of Predicted ORFs |
|---------------|-----------|---------|-----------------------|
| Chromosome    | 3,433,749 | 41.78%  | 3413                  |
| Plasmid pAcsw19-1 | 11,161     | 35.66%  | 18                    |
| Plasmid pAcsw19-2 | 351,885   | 38.79%  | 383                   |
| Plasmid pAcsw19-3 | 38,391    | 33.76%  | 58                    |

---

**Table 2** Distribution of the Resistance Genes in Acinetobacter johnsonii Strain Acsw19

| Resistance Gene | Identity % | Query/Template Length | Position in Context | Predicted Phenotype               | Accession Number |
|-----------------|------------|-----------------------|---------------------|-----------------------------------|------------------|
| Chromosome      |            |                       |                     |                                   |                  |
| blaoXA-211-like | 95.64      | 813/813               | 3,091,502.309231    | Beta-lactam resistance            | FN396876         |
| blaoXA-211      | 99.88      | 826/825               | 16,307.17331        | Beta-lactam resistance            | HGG91732         |
| aph(3")-Ib      | 100        | 804/804               | 2,803,602.2804405   | Aminoglycoside resistance         | AF321551         |
| aph(6)-Id       | 100        | 837/837               | 2,802,766.2803602   | Aminoglycoside resistance         | M28829           |
| su2             | 100        | 816/816               | 2,797,792.2798613   | Sulphonamide resistance           | AF031438         |
| floR            | 98.35      | 1214/1215             | 2,801,117.280233    | Phenicol resistance               | AF118107         |
| pAcsw19-2       |            |                       |                     |                                   |                  |
| mph(E)          | 100        | 885/885               | 47,669.48553        | Macrolide resistance              | DQ839391         |
| msr(E)          | 100        | 1476/1476             | 48,609.50084        | Macrolide, Lincosamide and        | FR751518         |
| aph(3")-Vla     | 100        | 780/780               | 256,925.257704      | Streptogramin B resistance        | X07753           |
| blaoXA-58       | 100        | 813/813               | 250,228.251040      | Aminoglycoside resistance         | FN396876         |
| cmlB1-like      | 86.51      | 843/843               | 57,613.58455        | Phenicol resistance               | FY665723         |
| cmlB1-1-like    |            |                       |                     |                                   |                  |
were all harbored by the *Acinetobacter* species. None of antimicrobial resistance gene was determined in plasmid pAcsw19-1. Plasmid pAcsw19-1 carried two copies of plasmid replicons, two mobilization proteins (mobL-like), and several hypothetical ORFs. Complete sequence analysis showed that the 351,885 bp plasmid pAcsw19-2 contained...
383 putative coding ORFs. Plasmid pAcsw19-2 had 87.34%, 14.76% and 7.06% query cover and 99.28%, 99.78% and 100% sequence similarities with the plasmids pXBB1-9 (CP010350), pACI-df08 (CP026426) and pM131-2 (JX101647) at nucleotide level, respectively.  

Plasmid pAcsw19-2 carried 6 resistance genes [cmlB1-like, mph(E), msr(E), blaOXA-58, blaNDM-1, and aph(3’)-Via] which were distributed in three regions. The first region carried the cmlB1-like gene. The genetic context of this region was ISL3-like transposase-cmlB1-like-LysR (Figure 2). This region was similar to the corresponding area of plasmid pOXA58_010030 (CP029396), pOXA58_010055 (CP032285), and pXBB1-9 (CP010351). The second region carried three resistance genes [mph(E), msr(E), and blaOXA-58]. The first two resistance gene mph(E) and msr(E) were carried by the genetic context which was constituted by IS30 family transposase, helix-turn-helix-ORF, mph(E), msr(E), helix-turn-helix-ORF, plasmid stability associated protein-coding ORFs (addiction module antidote protein, RelE/ParE, brnA/T, and LysE family), ISaba1, and AraC transcriptional regulator gene. The AraC was linked to the third resistance gene blaOXA-58 carrying area which was constituted by ISaba3-blaOXA58-ISaba3 (Figure 3). The context of blaOXA-58 carrying area (ISaba3-blaOXA58-ISaba3) was same to the previous reports.  

The third region carried 2 resistance genes [plasmid borne blaNDM-1 and aph (3’)-Via]. Nucleotide sequence analysis revealed that the blaNDM-1 gene was flanked in the upstream region of IS5 transposase-dmc-unknown ORF-umuD-Y-family DNA polymerase-unknown ORF-trpF-bleMBL and downstream by the IS30 family transposase and 6 unknown ORFs carried region, which linked to the resistance gene aph(3’)-Via carrying genetic context [ISaba125-aph(3’)-Via -ISaba125]. This resistance area (ISaba125-aph(3’)-Via-ISaba125) was similar to the corresponding region of the plasmid pAP43-OXA58-NDM1 (CP043053), which was harbored by Acinetobacter pittii.  

The plasmid pAcsw19-3 contained 58 putative coding ORFs. Sequence analysis showed that pAcsw19-3 had 72%, 76%, and 83% query cover and 99.90%, 99.96% and 99.84% sequence similarities with the plasmids p3_010055 (CP032282.1), p2_010030 (CP029390), and p4_010060 (CP031712) at the nucleotide level, and these plasmids were all harbored by the Acinetobacter species strains. None of the antimicrobial resistance gene was determined in plasmid pAcsw19-3, either.

Characterization of the Genomic Islands (GI)  
Fifteen genomic islands, named GI_Acsw19-1 to GI_Acsw19-15, were identified by the software IslandPATH-DIMOB, IslandPick and SIGI-HMM. Sequence analysis showed that the length of the 15 genomic islands were ranged from ~5.1 kb to ~94.86 kb with the average G+C context of 32.59% to 54.18%, respectively. Moreover, 13 genomic islands (GI_Acsw19-1 to GI_Acsw19-13) were located in the chromosome and 2 genomic islands (GI_Acsw19-14 and GI_Acsw19-15) in the plasmid pAcsw19-2. Among the 15 GIs, two (GI_Acsw19-11 and GI_Acsw19-12) were the resistant
| GIs_id        | Location (Start-End)   | Length (bp) | G+C %  | Closest Match in Genbank (Query Cover and Identity) | Resistance Genes and Mobile Genes Carried |
|--------------|------------------------|-------------|--------|-----------------------------------------------------|-----------------------------------------|
| GI_Acsw19-1  | Chromosome (1,005,689–1,014,205) | 8,517       | 38.56  | Acinetobacter johnsonii strain M19 chromosome genome (99%, 98.97%) | None                                     |
| GI_Acsw19-2  | Chromosome (1,533,242–1,543,640) | 10,399      | 39.81  | Acinetobacter johnsonii strain IC001 chromosome genome (96%, 98.97%) | IS3 family transposase                  |
| GI_Acsw19-3  | Chromosome (1,804,441–1,809,505) | 5,065       | 36.92  | Acinetobacter haemolyticus strain TJS01 chromosome genome (58%, 98.68%) | Integrase                               |
| GI_Acsw19-4  | Chromosome (1,816,879–1,833,718) | 18,840      | 39.44  | Acinetobacter sp. WCHA45 plasmid pNDM1_010045 (34%, 90.83%) | IS1, IS3 and three IS5 family transposase |
| GI_Acsw19-5  | Chromosome (1,886,561–1,897,546) | 10,986      | 34.84  | Acinetobacter johnsonii strain XBB1 chromosome genome (88%, 96.43%) | ISAha1 family transposase              |
| GI_Acsw19-6  | Chromosome (1,919,728–1,925,691) | 5,964       | 35.78  | Acinetobacter haemolyticus strain AN54 chromosome genome (35%, 98.66%) | Two IS3 family transposases            |
| GI_Acsw19-7  | Chromosome (2,033,708–2,038,927) | 5,220       | 37.09  | Acinetobacter johnsonii strain XBB1 chromosome genome (26%, 85%) | None                                     |
| GI_Acsw19-8  | Chromosome (2,531,192–2,545,937) | 1,4746      | 38.74  | Acinetobacter johnsonii strain M19 chromosome genome (99%, 96.27%) | ISS family transposase                 |
| GI_Acsw19-9  | Chromosome (2,555,415–2,582,484) | 27,070      | 36.29  | Acinetobacter johnsonii strain LXL_C1 chromosome genome (79%, 99.09%) | Two IS3 family transposases, ISAha1 family transposase |
| GI_Acsw19-10 | Chromosome (2,586,932–2,600,597) | 13,666      | 39.02  | Acinetobacter sp. LoGeW2-3 chromosome, genome (70%, 92.78%) | Integrase                               |
| GI_Acsw19-11 | Chromosome (2,780,884–2,807,016) | 26,133      | 42.83  | Acinetobacter baumannii MRSN15313 chromosome genome (47%, 100%) | IS1 family transposase, Integrase, ISABA1, sul2, IS91-like transposase, aph(3")-Ib, aph(6)-Id, ISABA, floR |
| GI_Acsw19-12 | Chromosome (3,082,734–3,095,042) | 12,309      | 54.18  | Acinetobacter baumannii strain IOMTU 433 complete genome (96%, 100%) | IS91 family transposase, NDM-1 and Two ISABA125 |
| GI_Acsw19-13 | Chromosome (3,233,129–3,327,995) | 94,867      | 43.12  | Acinetobacter nosocomialis strain KAN01 chromosome genome (64%, 87.11%) | ISS family transposase, Integrase       |
| GI_Acsw19-14 | pAcsw19-2 (59,672–67,958)       | 8287        | 32.59  | Acinetobacter sp. WCHA55 pOXA58_010055 (84%, 99.98%) | IS6-like transposase                    |
| GI_Acsw19-15 | pAcsw19-2 (293,855–301,112)       | 7258        | 42.37  | Acinetobacter sp. WCHA55 pOXA58_010055 (99%, 100%) | IS4 family transposase                 |
genomic islands. Genomic island GI_Acsw19-11 (26,133 bp) carried the aminoglycosides resistance genes \textit{strA} and \textit{strB}, sulphonamides resistance gene \textit{sul2} and phenicol resistance gene \textit{floR}. Genomic island GI_Acsw19-12 (12,309 bp) carried the chromosome borne \textit{bla}_{NDM-1} (Table 3). Sequence analysis showed that GI_Acsw19-11 had 47%, 37%, and 34% query cover and 100% sequence similarities with the DNA sequence of \textit{A. baumannii} MRSN15313 chromosome genome (CP033869), \textit{A. pittii} WCHAP100004 plasmid pOXA58_100004 (CP027249), and uncultured bacterium HHV216 plasmid pHHV216 (FJ012880). Based on the specific genetic content, GI_Acsw19-11 could be divided into two regions (regions A and regions B) (Figure 4). The region A was 15,713bp in length which served as the backbone of GI_Acsw19-11. It mainly carried the tyrosine-type recombinase/integrate gene (1137 bp) and an \textit{IS1} family \textit{tnpA}, which might be responsible for encoding the site-specific resolvase and transposition. Additionally, the other genes of region A, including ferrous iron transporter gene \textit{feoAB}, Cd(II)/Pb(II)-responsive transcriptional regulator encoding gene \textit{cadR}, hydrolase encoding gene, some of hypothetical protein-encoding genes, were found to be located in the backbone. Sequence of region A was high similar to the corresponding region of other various \textit{Acinetobacter} species.\textsuperscript{41} The 4 resistance genes (\textit{strA} and \textit{strB}, \textit{sul2} and \textit{floR}) carried region B was 8980 bp in length (Figure 3). These four resistance genes genetic context is \textit{ISAba1-sul2-glmM-ISVsa3-LysR-floR-DUF3363-strB-strA-ISAba1.}

The \textit{sul2} and \textit{floR} carrying area (6814 bp) was similar to the corresponding region of plasmid pOXA58_100004, while the \textit{strB} and \textit{strA} carrying area (2832 bp) was similar to corresponding region of plasmid pNDM-AP_882 harbored by the \textit{A. pittii} AP_882 (CP014478.1).

Sequence analysis showed that GI_Acsw19-12 had 96%, 96%, and 96% query cover and 100%, 99.9%, and 99.9% sequence similarities with the corresponding region of sequences of \textit{A. baumannii} AR_0083 genome (CP027528).

![Figure 4 Schematic map of the genetic context of the GI_Acsw19-11. The resistance genes are indicated by red arrows, the mobile genes are indicated by the green arrows and other function genes are indicated by the purple arrows. Gray areas between open reading frames (ORFs) denote nucleotide identities with the similarity context.](image-url)
A. pittii ST220 genome (CP029610), and A. baumannii AR_0083 genome (CP027528). GI_Acsw19-12 had 82%, 82%, and 84% query cover and 100%, 100%, and 99.9% sequence similarities with the plasmids including the pNDM1_060092 (CP035935), pNDM-GJ01 (KT965092)\(^{42}\) and pNDM-JVAP01(KM923969)\(^{43}\) at nucleotide level.

**Figure 5** Schematic map of the genetic context of the GI_Acsw19-2. The resistance genes are indicated by red arrows, the mobile genes are indicated by the green arrows and other function genes are indicated by the purple arrows. Gray areas between open reading frames (ORFs) denote nucleotide identities with the similarity context.

**Table 4** Overall Features of the *Acinetobacter johnsonii* Strain Acsw19 Prophage

| Prophage_ID | Location (Start- End) | Length (bp) | G+C % | Closest Match in Genbank (Coverage % and Identity %) | Resistance Genes and Mobile Genes Carried |
|-------------|------------------------|-------------|--------|-----------------------------------------------------|------------------------------------------|
| Pp_Acsw19-1 | Plasmid pAcsw19-2 (4463–36,078) | 31,616 | 42.82 | *Acinetobacter defluvii* WCHA30 plasmid pOXA58_010030 (99%, 99.98%) | cmlB1-like, Integrase, IS3, IS6, IS1006 and two IS6 family transposases |
| Pp_Acsw19-2 | Chromosome (1,557,755–171,680) | 159,049 | 40.78 | *Acinetobacter johnsonii* strain M19 chromosome genome (97%, 97.04%) | None |
| Pp_Acsw19-3 | Chromosome (1,757,515–182,333) | 65,817 | 40.03 | *Acinetobacter johnsonii* strain M19 chromosome genome (64%, 95.15%) | Integrase, IS1 family transposase, IS5 family transposase |
| Pp_Acsw19-4 | Chromosome (1,889,367–1,972,910) | 83,544 | 40.3 | *Acinetobacter johnsonii* strain M19 chromosome genome (92%, 97.11%) | 4 IS5 family transposases |
| Pp_Acsw19-5 | Chromosome (2,031,361–2,061,501) | 30,141 | 40.65 | *Acinetobacter johnsonii* strain M19 chromosome genome (67%, 95.92%) | Site-specific integrase |
| Pp_Acsw19-6 | Chromosome (2,957,108–297,432) | 17,222 | 43.01 | *Acinetobacter johnsonii* XBB1 genome (99%, 97.34%) | None |
| Pp_Acsw19-7 | Chromosome (3,085,102–3,130,818) | 45,717 | 47.12 | *Acinetobacter sp.* WCHA55 chromosome genome (99%, 93.93%) | IS91 family transposase, *blaNDM-1* and two ISAb125 |
| Pp_Acsw19-8 | Chromosome (3,156,419–3,188,118) | 31,700 | 42.5 | *Acinetobacter johnsonii* strain M19 chromosome genome (99%, 96.24%) | None |
Moreover, the chromosome borne blaNDM was located in the genetic element (10,023 bp) ISAb125-ISJ91-family tpnA-groL-groES-cutA-tat-trpF-ble-blaNDM−1-ISAb125 (Figure 5). This blaNDM carrying element was similar equal to the corresponding region of the pNDM1_010045.

Characterization of Prophages
A total of eight prophages, named Pp_Acsw19-1 to Pp_Acsw19-8, was identified by phiSpy (Table 4). Sequence analysis showed that one prophage (Pp_Acsw19-1) was located in the plasmid pAcsw19-2 and 7 prophages (Pp_Acsw19-2 to Pp_Acsw19-8) were located in the chromosome genome. The length of eight prophages was ranged from ~17.22 kb to ~159 kb with average G+C context of 40.03% to 47.12%, respectively. Interestingly, the blaNDM− carrying genomic islands GI_Acsw19-12 was located in the prophage Pp_Acsw19-7 and the cmlBI-like gene carrying prophage Pp_Acsw19-1 was located in the plasmid pAcsw19-2. These findings suggested that some mobile genetic elements can be excised from chromosome or mobile genetic elements and then integrated into the chromosome or other mobile genetic elements.44-47 These situations may promote the exchange of resistance genes among pathogenic microorganisms.

Conclusions
The current study characterize the complete genome sequence of Acinetobacter Acsw19, which carried two copies of blaNDM−1, cmlBI-like gene, blaOXA−21-like gene along with other 8 antimicrobial resistance genes in 3 plasmids, 15 genomic islands and 8 prophages, which could provide great genetic plasticity for the host dissemination of antimicrobial resistance. The occurrence of multiple antibiotic genes in bacterial isolates from sewage has been evidenced in numerous studies, with the involvement of numerous species, it may be serving as an important reservoir of resistance genes and a hot spot for the transfer of resistance genes and mobile genetic elements. We should be vigilant these isolates or resistance genes transfer to the clinical bacterial.

Nucleotide Sequence Accession Numbers
The genome sequence of Acinetobacter johnsonii Acsw19 is deposited in the NCBI database under accession numbers CP043307 (chromosome) and CP043308 to CP043310 (plasmid pAcsw19-1 to pAcsw19-3).

Funding
This work was supported by the National Natural Science Foundation of China (31500114), and United Funds of Luzhou and Southwest Medical University [2018LZXNYD-ZK51].

Disclosure
The authors declare no conflict of interest.

References
1. Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamas. Clin Microbiol Rev. 2007;20(3):440–58, table of contents. doi:10.1128/CMR.00001-07
2. Fu L, Huang M, Zhang X, et al. Frequency of virulence factors in high biofilm formation blaKPC-2 producing Klebsiella pneumoniae strains from hospitals. Microb Pathog. 2018;116:168–172. doi:10.1016/j.micpath.2018.01.030
3. Poirel L, Nordmann P. Carbapenem resistance in Acinetobacter baumannii: mechanisms and epidemiology. Clin Microbiol Infect. 2006.
4. Liu Y, Zhang H, Zhang X, et al. Characterization of an NDM-19-producing Klebsiella pneumoniae strain harboring 2 resistance plasmids from China. Diagn Microbiol Infect Dis. 2019;93(4):355–361. doi:10.1016/j.diagmicrobio.2018.11.007
5. Fu L, Ang GY, Yu CY, et al. Co-carrying of KPC-2, NDM-5, CTX-M-3 and CTX-M-65 in three plasmids with serotype O89; H10 Escherichia coli strain belonging to the ST2 clone in China. Microb Pathog. 2019;128:1–6. doi:10.1016/j.micp.2018.12.033
6. Zmarlicka MT, Nairl MD, Nicolau DP. Impact of the New Delhi metallo-beta-lactamase on beta-lactam antibiotics. Infect Drug Resist. 2015;8:297–309. doi:10.2147/IDR.S39186
7. Liu X, Zhang J, Li Y, et al. Diversity and frequency of resistance and virulence genes in blaKPC and blaNDM co-producing Klebsiella pneumoniae strains from China. Infect Drug Resist. 2019;12:2819–2826. doi:10.2147/IDR
8. Kiaei S, Moradi M, Hosseini-nave H, et al. Endemic dissemination of different sequence types of carbapenem-resistant Klebsiella pneumoniae strains harboring blaNDM and 16S rRNA methylase genes in Kerman hospitals, Iran, from 2015 to 2017. Infect Drug Resist. 2019;12:45–54. doi:10.2147/IDR
9. Papagiannis CC, Bitar I, Mali E, et al. IncC blaKPC−2-positive plasmid characterised from ST648 Escherichia coli. J Glob Antimicrob Resist. 2019;19:73–77. doi:10.1016/j.jgar.2019.05.001
10. Hu Y, Feng Y, Qin J, Zhang X, Zong Z. Acinetobacter chinensis, a novel Acinetobacter species, carrying bladNDM−1, recovered from hospital sewages. J Microbiol. 2019;57(5):350–355. doi:10.1007/s12275-019-8485-0
11. Feng Y, Yang P, Wang X, et al. Characterization of Acinetobacter johnsonii isolate XBB1 carrying nine plasmids and encoding NDM-1, OXA-58 and PER-1 by genome sequencing. J Antimicrob Chemother. 2015;71(1):71. doi:10.1093/jac/dkv324
12. Liu WJ, et al. Frequency of antiseptic resistance genes and reduced susceptibility to biocides in carbapenem-resistant Acinetobacter baumannii. J Med Microbiol. 2017;66(1).
13. Waltherrasmussen J, Haiby N. OXA-type carbapenemases. J Antimicrob Chemother. 2006;57(3):373. doi:10.1093/jac/dki482
14. Hammoudi D, Moubareck CA, Hakime N, et al. Spread of imipenem-resistant Acinetobacter baumannii co-expressing OXA-23 and GES-11 carbapenemases in Lebanon. Int J Infect Dis Off Publ Int Soc Infect Dis. 2015;36(C):56–61. doi:10.1016/j.ijid.2015.05.015
15. Higgins PG, Poirel L, Lehmann M, et al. OXA-143, a novel carbapenem-hydrolyzing class D β-lactamase in Acinetobacter baumannii. *Antimicrob Agents Chemother*. 2009;53(12):5035–5038. doi:10.1128/AAC.00856-09

16. Nuno TA, Lamoureux TL, Toth M, et al. Class D β-lactamases: are they all carbapenemases? *Antimicrob Agents Chemother*. 2014;58(4):2119–2125. doi:10.1128/AAC.02522-13

17. Yang Q, Rui Y. Two multiplex real-time PCR assays to detect and differentiate acinetobacter baumannii and non- baumannii acinetobacter spp. carrying blaNDM, blaOXA-23-like, blaOXA-40-like, blaOXA-51-like, and blaOXA-58-like genes. *PLoS One*. 2016;11(7):e0159858. doi:10.1371/journal.pone.0159858

18. Khorsí K, et al. ISAba36 inserted into the outer membrane protein gene carO and associated with the carbapenemase gene blaOXA-24-like in Acinetobacter baumannii. *J Glob Antimicrob Resist*. 2018;15:107–108. doi:10.1016/j.jgar.2018.08.020

19. Nishida S, Ono Y. Comparative analysis of the pathogenicity between multidrug-resistant Acinetobacter baumannii clinical isolates: isolation of highly pathogenic multidrug-resistant A. baumannii and experimental therapeutics with fourth-generation cephalosporin cefozopran. *Infect Drug Resist*. 2018;11:1715–1722. doi:10.2147/IDR.S166154

20. Zenati K, Touati A, Bakour S, et al. Characterization of NDM-1- and OXA-23-producing Acinetobacter baumannii isolates from inanimate surfaces in a hospital environment in Algeria. *J Hosp Infect*. 2016;92(1):19–26. doi:10.1016/j.jhin.2015.09.020

21. Guerra B, Fischer J, Helmuth R. An emerging public health problem: acquired carbapenemase-producing microorganisms are present in food-producing animals, their environment, companion animals and wild birds. *Vet Microbiol*. 2014;173(3–4):290–297. doi:10.1016/j.vetmic.2014.02.001

22. Sørensen SJ, Bailey M, Hansen LH, et al. Studying plasmid horizontal transfer in situ: a critical review. *Nat Rev Microbiol*. 2005;3(9):700–710. doi:10.1038/nrmicro1232

23. Peter JG, Townsend, JP. Horizontal gene transfer, genome innovation and evolution. *Nat Rev Microbiol*. 2005;3(9):679–687. doi:10.1038/nrmicro1204

24. Brown-jaque M, Calero-cáceres W, Muniesa M. Transfer of antibiotic-resistance genes via phage-related mobile elements. *Plasmid*. 2015;79(3):1–7. doi:10.1016/j.plasmid.2015.01.001

25. Zhou S, Chen X, Meng X, et al. “Roar” of blaNDM-1 and “silence” of blaOXA-58 co-exist in Acinetobacter pittii. *Sci Rep*. 2015;5(1):8976. doi:10.1038/srep08976

26. Delcher AL, Bratke KA, Powers EC, et al. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics*. 2007;23(6):673–679. doi:10.1093/bioinformatics/btm009

27. Goris J, Goris J, Vandamme P, et al. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Evid Microbiol*. 2007;57(3):81–91. doi:10.1099/ijem.0.64483-0

28. de Z, Hasman H, Cosentino S, et al. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother*. 2012;67(11):2640–2644. doi:10.1093/jac/dks261

29. Bertelli C, Brinkman F. Improved genomic island predictions with IslandPath-DIMOB. *Bioinformatics*. 2018;34(13):2161–2167. doi:10.1093/bioinformatics/bty095

30. Langille MG, Hisao WW, Brinkman FS. Evaluation of genomic island predictors using a comparative genomics approach. *BMC Bioinformatics*. 2008;9(1):329. doi:10.1186/1471-2105-9-329

31. Waack S, Keller O, Asper R, et al. Score-based prediction of genomic islands in prokaryotic genomes using hidden Markov models. *BMC Bioinformatics*. 2006;7(1):1–12. doi:10.1186/1471-2105-7-142
