Infection and Drug Resistance

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ORIGINAL RESEARCH

Whole-Genome Analysis of Two Copies of bla_{NDM-1} Gene Carrying *Acinetobacter johnsonii* Strain Acsw19 Isolated from Sichuan, China

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

Methods: A carbapenem-resistant *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 [*cmlB1*-like, bla_{NDM-1}, bla_{OXA-58}, *aph (3')-VIa, msr(E)* and *mph(E)*] in the plasmid pAcsw19-2 and 6 ($bla_{OXA-211}$-like, bla_{NDM-1}, *aph(3")-Ib, aph(6)-Id, sul2*, and *floR*) in the chromosome genome. Specifically, the *cmlB1*-like gene shared 86.33%, 71.7% and 71.9% similarities with the *cmlB1, cmlA4* and *cmlA8* gene, and the $bla_{OXA-211}$-like gene shared 94.4%, 95.39% and 96.36% similarities with $bla_{OXA-211}$, $bla_{OXA-643}$ and $bla_{OXA-652}$, at the nucleotide level, respectively. Phylogenetic analysis showed that the $bla_{OXA-211}$-like gene and *cmlB1*-like gene had the closest evolutionary relationship with $bla_{OXA-643}$ and *cmlB1*, respectively. These results indicated that the $bla_{OXA-211}$-like and *cmlB1*-like genes identified in the current study should be the novel variant resistance genes.

Conclusion: Carrying of two copies of bla_{NDM-1}, *cmlB1*-like, $bla_{OXA-211}$-like and along with other 8 antimicrobial resistance genes, 3 plasmids, 15 genomic islands and 8 prophages *Acinetobacter johnsonii* strain might increase the possibility of spreading of resistance genes.

Keywords: *Acinetobacter johnsonii*, bla_{NDM-1}, bla_{OXA}, genomic island

Introduction

Producing of carbapenemases, including the β-lactamases of Ambler classes A, B (metallo-β-lactamases) and D, are the most common mechanism of bacterial carbapenems resistance.[1–3] Especially, the New Delhi Metallo-β-lactamase (NDM), Klebsiella pneumoniae carbapenemase (KPC) and some Class D β-lactamases (CHDLs) have been identified worldwide in gram-negative bacterial isolates from clinical, environmental samples, and food animals, especially in *Enterobacteriaceae*,[1,4–9] and also in *Pseudomonas aeruginosa* and *Acinetobacter species*.[10–12] Carbapenem-resistant

Acinetobacter species are mainly associated with the carbapenem-hydrolyzing NDM and CHDLs, such as bla_{OXA-23}-like, $bla_{OXA-24/40}$-like, bla_{OXA-51}-like, $bla_{OXA-134}$-like and $bla_{OXA-211}$-like gene.[13–18] Especially, co-carrying of the bla_{NDM} and bla_{OXA} in clinical, food, environmental derived isolates of *Acinetobacter* species were prevalent in the world.[11,19-21] In addition, the genes encoding bla_{NDM} and bla_{OXA} are 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.[11,22–25]

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_{NDM-1}, *cmlB1-like* gene, and $bla_{OXA-211}$-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), 16srRNA 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_{NDM} and bla_{KPC}) as previously reference.[7]

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/).[26] 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.[27] The resistome was identified using ResFinder 2.1 (https://cge.cbs.dtu.dk/services/ResFinder/)[28] (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)[29–31] and the Prophage was predicted by using phiSpy.[32]

Results and Discussion

Bacterial Isolate, Identification and Resistance Gene Detection

A gram-stain-negative, bla_{NDM-1} and bla_{OXA} producing *Acinetobacter johnsonii* Acsw19 was isolated and identified by the Vitek2 system (BioMérieux, France), 16srRNA 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.[33,34]

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 [(bla_{NDM-1}, $bla_{OXA-211}$-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),* bla_{OXA-58}, bla_{NDM-1} and *aph(3')-VIa*] in plasmid pAcsw19-2 (Table 2). None of the drug-resistance gene was detected in plasmid pAcsw19-1 and pAcsw19-3. The full lengthen 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 lengthen of $bla_{OXA-211}$-like gene consisted of 825 nucleotides encoding a protein with 275 amino acids. Sequence analysis showed that the $bla_{OXA-211}$-like gene shared 94.4%, 95.39%, 95.52%, 96% and 96.36% identity with the known $bla_{OXA-211}$, $bla_{OXA-643}$, $bla_{OXA-281}$, $bla_{OXA-645}$ and $bla_{OXA-652}$ 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 $bla_{OXA-211}$-like gene and *cmlB1*-like gene had the closest evolutionary relationship with $bla_{OXA-643}$ (Figure 1) and *cmlB1* (Figure 2). These results indicated that the $bla_{OXA-211}$-like and *cmlB1*-like genes maybe two new allelic variant of the gene bla_{OXA} 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]

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

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 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	bla_{NDM-1}	100	813/813	3,091,502.3092314	Beta-lactam resistance	FN396876
	$bla_{OXA-211-like}$	95.64	826/825	16,507.17331	Beta-lactam resistance	HG931732
	aph(3")-Ib(*strB*)	99.88	804/804	2,803,602.2804405	Aminoglycoside resistance	AF321551
	aph(6)-Id (strA)	100	837/837	2,802,766.2803602	Aminoglycoside resistance	M28829
	sul2	100	816/816	2,797,798.2798613	Sulphonamide resistance	AY034138
	floR	98.35	1214/1215	2,801,117.2802330	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 Streptogramin B resistance	FR751518
	aph(3')-*VIa*	100	780/780	256,925.257704	Aminoglycoside resistance	X07753
	bla_{NDM-1}	100	813/813	250,228.251040	Beta-lactam resistance	FN396876
	bla_{OXA-58}	100	843/843	57,613.58455	Beta-lactam resistance	AY665723
	cmlB1-like	86.51	1268/1266	28,217.29482	Phenicol resistance	AM296481

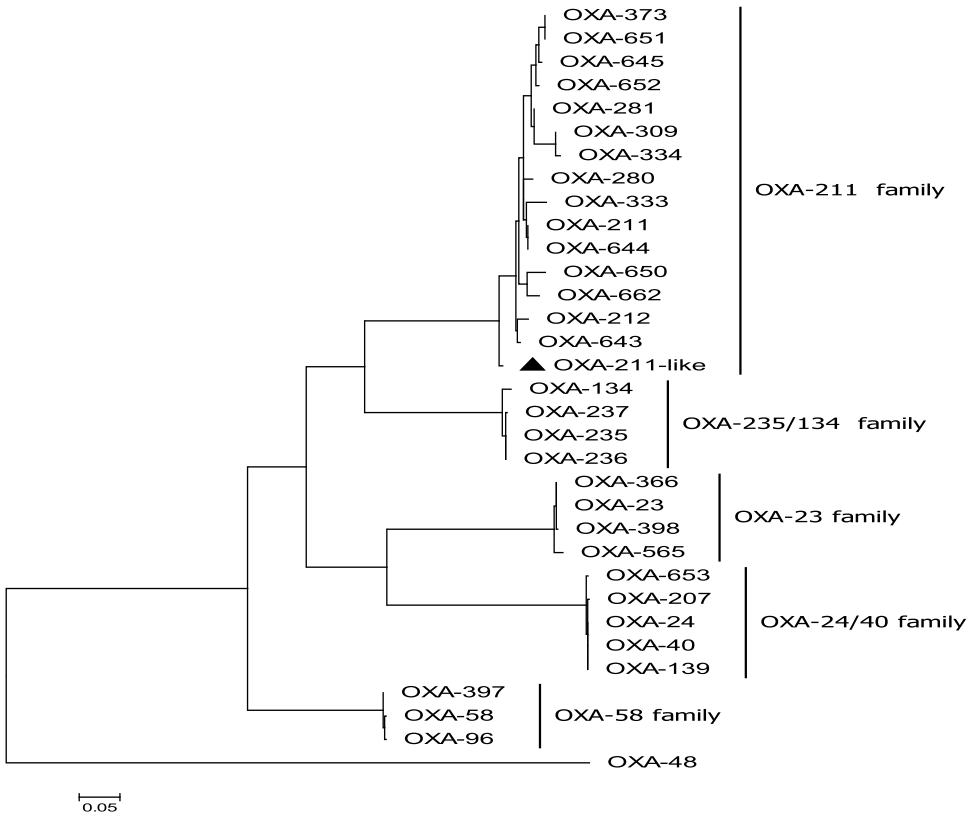

Figure 1 Molecular phylogenetic analysis by maximum likelihood method with 1000 bootstraps of OXA-211 like (a novel variant of OXA-211 family) with the six Class D β-lactamases (CHDLs) OXA family (OXA-23, OXA-24/40, OXA-235/134, OXA-58, OXA-48, OXA-211) representative sequences retrieved from GenBank database. The black triangle was indicated the gene which was found in this study.

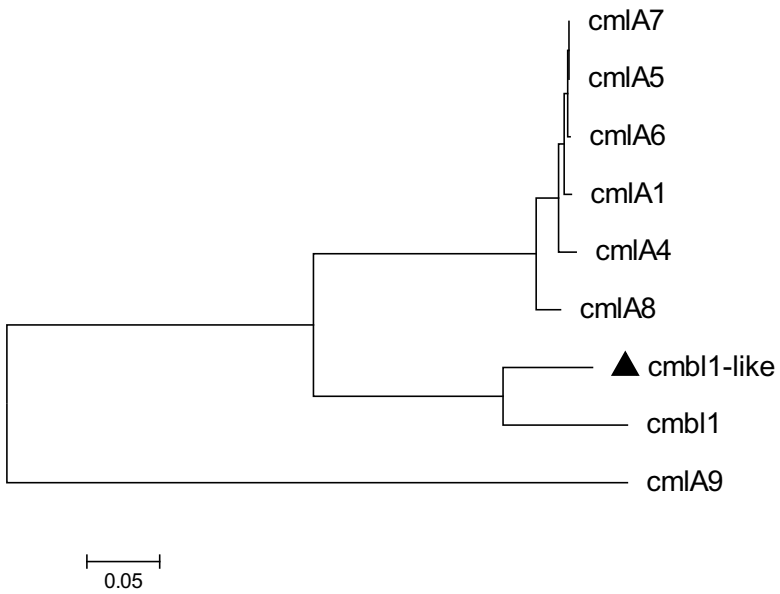

Figure 2 Molecular phylogenetic analysis by maximum likelihood method with 1000 bootstraps of CmlA/FloR family chloramphenicol efflux MFS (*cmlB1*-like) with the eight CmlA/FloR family chloramphenicol efflux MFS representative sequences retrieved from GenBank database. The black triangle was indicated the gene which was found in this study.

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)[37] and pM131-2 (JX101647) at nucleotide level, respectively.[11]

Plasmid pAcsw19-2 carried 6 resistance genes [*cmlB1*-like, *mph(E), msr(E),* bla_{OXA-58}, bla_{NDM-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 *IS*L3-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 bla_{OXA-58}]. The first two resistance gene *mph(E)* and *msr(E)* were carried by the genetic context which was constituted by *IS*30 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), *IS*Aba1, and AraC transcriptional regulator gene. The AraC was linked to the third resistance gene bla_{OXA-58} carrying area which was constituted by *IS*Aba3-bla_{OXA58}-*IS*Aba3 (Figure 3). The context of bla_{OXA-58} carrying area (*IS*Aba3-bla_{OXA58}-*IS*Aba3) was same to the previous reports.[11,38-40]

The third region carried 2 resistance genes [plasmid borne bla_{NDM-1} and *aph* (3′)-*VIa*]. Nucleotide sequence analysis revealed that the bla_{NDM-1} gene was flanked in the upstream region of *IS*5 transposase-*dmc*-unknown ORF-*umuD*-Y-family DNA polymerase-unknown ORF-*trpF*-ble_{MBL} and downstream by the *IS*30 family transposase and 6 unknown ORFs carried region, which linked to the resistance gene *aph(3')-Via* carrying genetic context [*IS*Aba125-*aph(3')-Via* -*IS*Aba125]. This resistance area (*IS*Aba125-*aph(3')-Via-IS*Aba125) 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

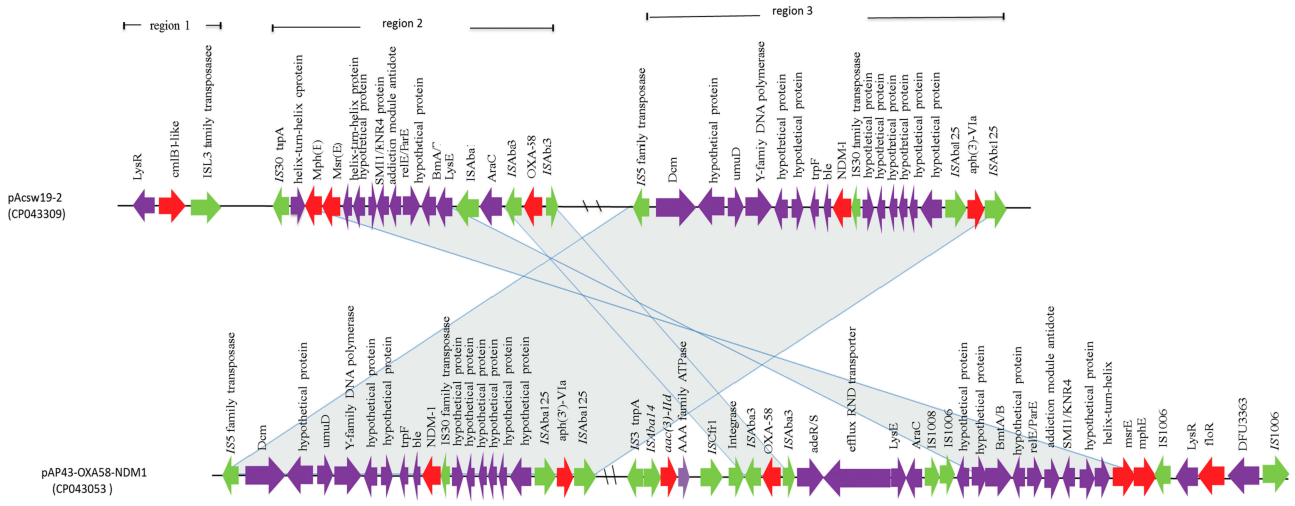

Figure 3 Schematic map of the genetic context of resistance gene regions in pAcSW19-2. The resistance genes are indicated by red arrows, the mobile genes are indicated by the green arrows and other function genes are indicated by the purple arrows. Gray areas between open reading frames (ORFs) denote nucleotide identities with the similarity context.

Table 3 Overall Features of the *Acinetobacter johnsonii* Strain Acsw19 Genomic Islands

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%)	*IS*3 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 (1,816,879–1,835,718)	18,840	39.44	*Acinetobacter sp.* WCHA45 plasmid pNDM1_010045 (34%, 90.83%)	*IS*1, *IS*3 and three *IS*5 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%)	*IS*Aha1 family transposase
GI_Acsw19-6	Chromosome (1,919,728–1,925,691)	5964	35.78	*Acinetobacter haemolyticus* strain AN54 chromosome gemome (35%, 98.66%)	Two *IS*5 family transposases
GI_Acsw19-7	Chromosome (2,033,708–2,038,927)	5220	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%)	IS5 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, *IS*Aha1 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%)	*IS*1 family transposase, Integrase, *IS*Aba1, *sul2, IS*91-like transposase, *aph(3")-Ib*, *aph(6)-Id*, *IS*Aba, *floR*
GI_Acsw19-12	Chromosome (3,082,734–3,095,042)	12,309	54.18	*Acinetobacter baumannii* strain IOMTU 433 complete genome (96%, 100%)	*IS*91 family transposase, NDM-1 and Two *IS*Aba125
GI_Acsw19-13	Chromosome (3,233,129–3,327,995)	94,867	43.12	*Acinetobacter nosocomialis* strain KAN01 chromosome genome (64%, 87.11%)	*IS*5 family transposase, Integrase
GI_Acsw19-14	pAcsw19-2 (59,672–67,958)	8287	32.59	*Acinetobacter sp.* WCHA55 pOXA58_010055 (84%, 99.98%)	*IS*6-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 *strA* and *strB*, sulphonamides resistance gene *sul2* and phenicol resistance gene *floR*. Genomic island GI_Acsw19-12 (12,309 bp) carried the chromosome borne 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 *A. baumannii* MRSN15313 chromosome genome (CP033869), *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/integrase gene (1137 bp) and an *IS1* family *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 *feoAB*, Cd(II)/Pb(II)-responsive transcriptional regulator encoding gene *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 *Acinetobacter* species.[41] The 4 resistance genes (*strA* and *strB, sul2* and *floR)* carried region B was 8980 bp in length (Figure 3). These four resistance genes genetic context is *IS*Aba1-*sul2*-*glmM*-*IS*Vsa3-LysR-*floR*-DUF3363-*strB*-*strA*-*IS*Aba1.

The *sul2 and floR* carrying area (6814 bp) was similar to the corresponding region of plasmid pOXA58_100004, while the *strB* and *strA* carrying area (2832 bp) was similar to corresponding region of plasmid pNDM-AP_882 harbored by the *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 *A. baumannii* AR_0083 genome (CP027528),

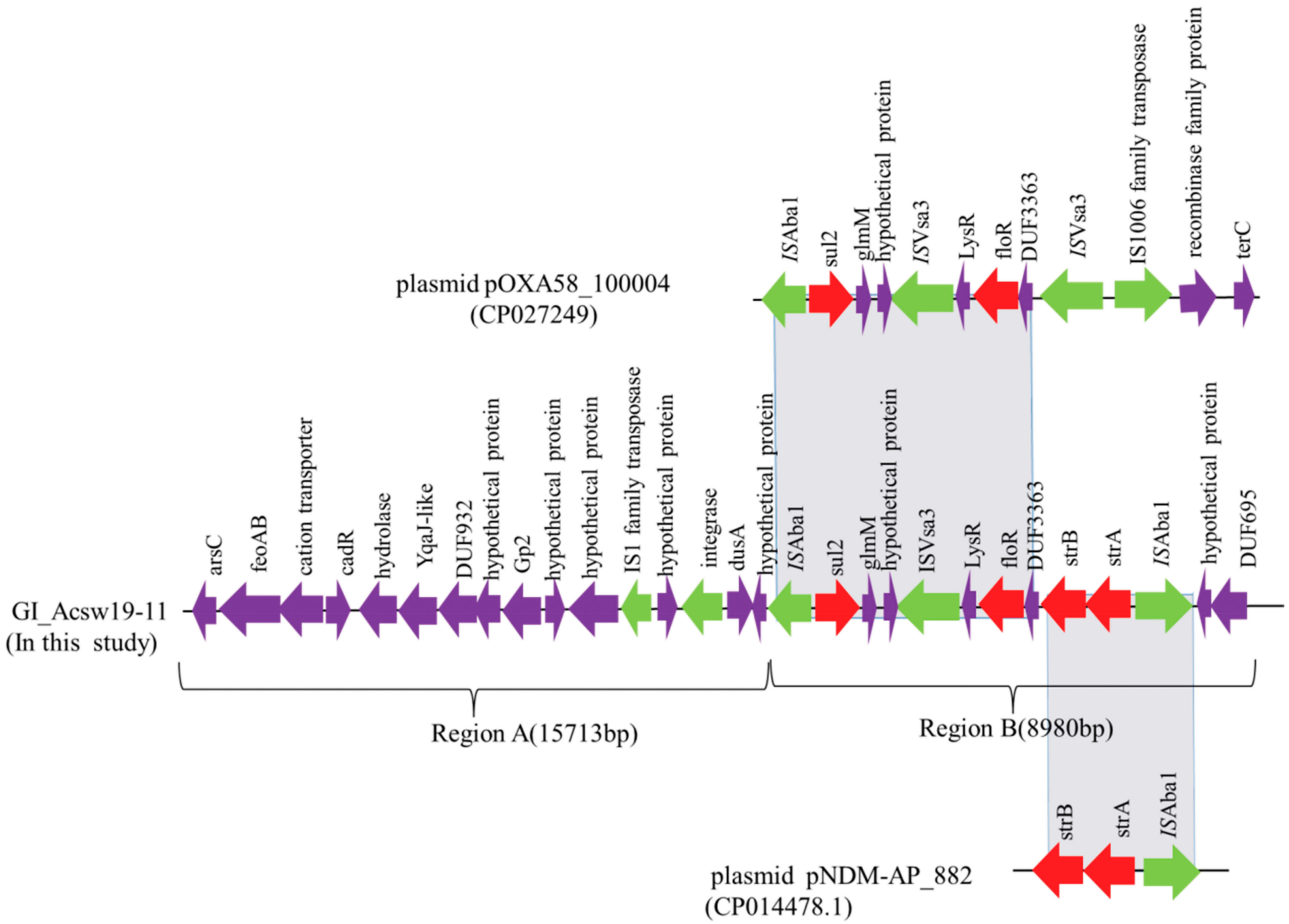

Figure 4 Schematic map of the genetic context of the GI_Acsw19-11. The resistance genes are indicated by red arrows, the mobile genes are indicated by the green arrows and other function genes are indicated by the purple arrows. Gray areas between open reading frames (ORFs) denote nucleotide identities with the similarity context.

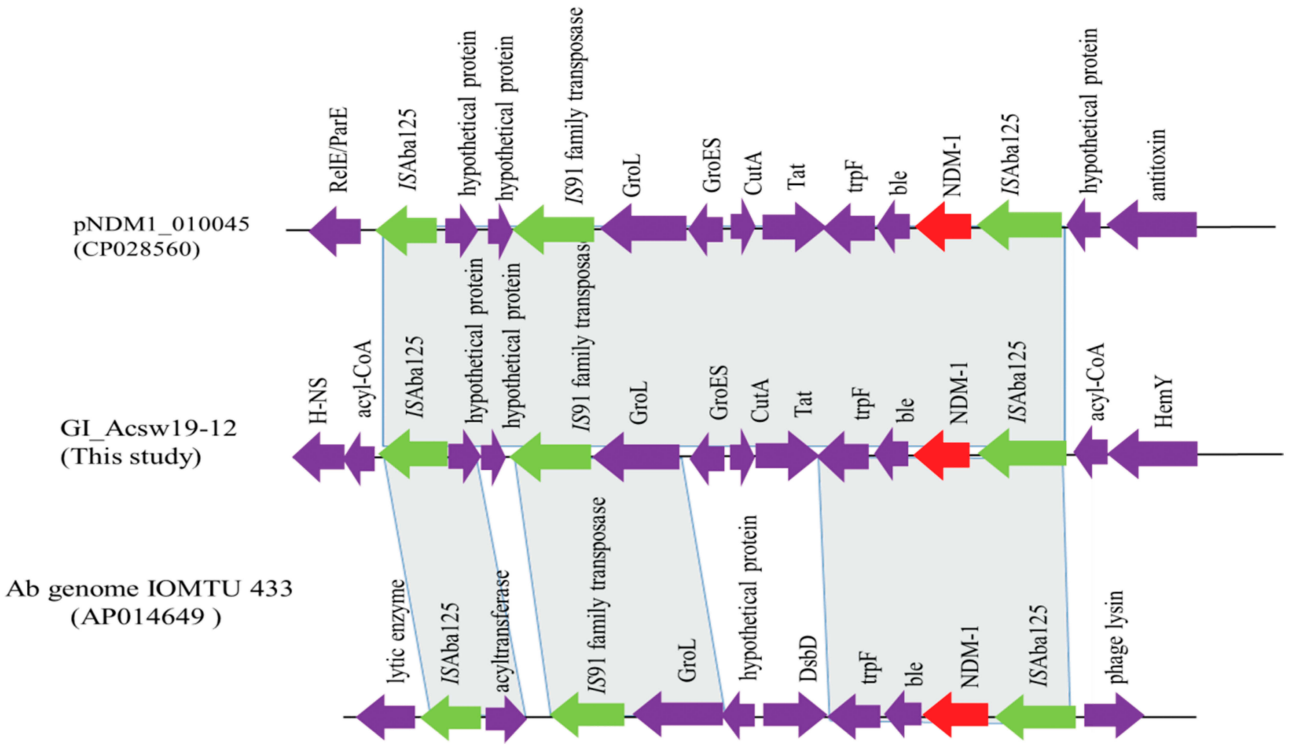

Figure 5 Schematic map of the genetic context of the GI_Acsw19-2. The resistance genes are indicated by red arrows, the mobile genes are indicated by the green arrows and other function genes are indicated by the purple arrows. Gray areas between open reading frames (ORFs) denote nucleotide identities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

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.

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

Prophage_ID	Location (Start- End)	Length (bp)	G+C %	Closest Match in Genbank (Covery % 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%)	*cmlB*1-like, Integrase, *IS*3, *IS*L3, *IS*1006 and two *IS*6 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, *IS*1 family transposase, *IS*5 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 *IS*5 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%)	*IS*91 family transposase, bla_{NDM-1} and two *IS*Aba125
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 *bla*$_{NDM}$ was located in the genetic element (10,023 bp) *IS*Aba125-*IS*91-family tnpA-*groL-groES-cutA-tat-trpF-ble-bla*$_{NDM-1}$-*IS*Aba125 (Figure 5). This *bla*$_{NDM}$ 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 lengthen 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 *bla*$_{NDM}$-carrying genomic islands GI_ Acsw19-12 was located in the prophage Pp_Acsw19-7 and the *cmlB1*-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 *bla*$_{NDM-1}$, *cmlB1*-like gene, *bla*$_{OXA-211}$-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).

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Disclosure

The authors declare no conflict of interest.

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