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Genome Note

Draft genome sequence of a multidrug-resistant bla$_{OXA-69}$-producing *Acinetobacter baumannii* L13 isolated from Tarim River sample in China

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ABSTRACT

Objectives: Acinetobacter baumannii (*A. baumannii*) is the world's most significant opportunistic pathogen that causes nosocomial infections; there is widespread health concern due to its ability to acquire multidrug resistance. This study reports a new variety of *A. baumannii* L13 isolated from Tarim River in Xinjiang Uygur Autonomous Region of China, with *β*-lactamase resistance gene *OXA-69* in the genome.
Methods: Genomic DNA was extracted using an E.Z.N.A.® Bacterial DNA Kit according to the manufacturer's recommended protocol. The genome of *A. baumannii* L13 was sequenced using an Illumina HiSeq™ 2000 sequencing system, assembled with SOAPdenovo and optimised with GapCloser. The rRNAs were predicted by barrnap, and tRNAs were predicted by tRNA-scan-SE. Resistance genes were analysed using CARD databases.
Results: The whole genome size of *A. baumannii* L13 was calculated at 3 969 074 base pairs (bp), which was assembled into 60 contigs and 56 scaffolds (>1000 bp length), with a G + C content of 38.9% and N rate of 0.001%. The number of CDSs was 3815, with length accounting for approximately 85.61% of the whole genome, 7.6% and 8.2% of which were related to virulence genes and multidrug-resistance genes, respectively.
Conclusions: The genome sequence of *A. baumannii* L13 reported here will benefit comparative analysis of the *Acinetobacter* genus, and promote further understanding of the specific genomic feature in terms of multidrug resistance in *A. baumannii*.

1. Introduction

Acinetobacter baumannii (*A. baumannii*) appears as a globally-prevalent, opportunistic, nosocomial, Gram-negative bacterial pathogen. It can cause a large amount of infections – including respiratory, bloodstream, meningitis, endocarditis and genitourinary tract – which has become a major public and clinical health concern [1,2]. The 2016 China CHINET monitoring data showed that the resistance rate of *A. baumannii* to cefoperazone-sulbactam, minocycline, panipenem, imipenem and meropenem were 43%, 44.9%, 66.9%, 67.4% and 71%, respectively [3]. Therefore, the World Health Organization (WHO) has recently labelled *A. baumannii* as the top-priority pathogen for developing new antibiotics [4].

Recently, the current laboratory isolated a new variety of *A. baumannii* from Tarim River samples in Xinjiang Uygur Autonomous Region of China based on the analysis of 16S DNA sequence and cell morphology, which was named *A. baumannii* L13. Antimicrobial susceptibility testing of strain L13 was conducted using Etest and the results were analysed based on the Clinical and Laboratory Standards Institute (CLSI) 2017 guidelines [5]. The results showed that L13 strain was resistant to numerous antimicrobials, including: carbapenem, β-lactamase antibiotic, polymyxin, streptomycin, fosfomycin, chloramphenicol, tetracycline, lipopeptide antibiotic, peptide antibiotic, macrolide antibiotic, fluoroquinolone, rifampin, mupirocin, linezolid and trimethoprim.

Genomic DNA was extracted from *A. baumannii* L13 using the E.Z.N.A.® Bacterial DNA Kit (Omega Bio-Tek, Inc., Norcross, GA) according to the manufacturer's recommended protocol. The

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genome of strain L13 was sequenced using an Illumina HiSeq™ 2000 sequencing system (Shanghai Majorbio Bio-pharm Technology Co., Ltd.). The NCBI-NR, COG, GO, Pfam, Swiss-prot and KEGG databases were used to annotate the function of strain L13. The sequencing data were assembled using SOAPdenovo (Version 2.04) and optimised using GapCloser (Version 1.12). Gene Iland and CRISPR-Cas were analysed and predicted by using IslandPath-DIMOB (Version 0.2) and Minced, respectively. The rRNAs were predicted by barrnap (Version 0.8) and tRNAs were predicted by tRNA-scan-SE (Version 2.0). Resistance and virulence genes were analysed using CARD databases (Version 1.1.3) and VFDB databases (Version 4.4), respectively.

The strain L13 draft genome was calculated at 3 969 074 base pairs (bp), which was assembled into 60 contigs and 56 scaffolds (>1000 bp length), with a G + C content of 38.9% and N rate of 0.001%. The IslandPath-DIOMB was performed to dig out at least six genomic islands. Similarly, the genome of *A. baumannii* L13 was found to contain seven possible clustered regularly interspaced short palindromic repeats (CRISPRs) for further genome editing, as predicted in seven scaffolds by CRISPRFinder. Identified through tRNA-scan-SE and Barrnap, the genome comprised 73 tRNA gene regions and three rRNA gene regions, including one 16S rRNA region, one 5S rRNA region and one 23S rRNA region. There were 3815 candidate protein-coding sequences (CDSs), of which ca. 7.6% and 8.2% were related to virulence genes and multidrug-resistance genes, respectively, the length of which was accounted for by approximately 85.61% of the whole-genome.

Based on genome analysis by the CARD database, 314 resistance genes were found in *A. baumannii* L13. Some of the predicted resistance genes with an identity of >99% are further presented in Table 1. Most strains of this species develop resistance to carbapenems by mechanisms associated with carbapenem-hydrolysing class D β-lactamases (CHDLs), especially with *OXA*. β-lactamase *OXA-69* in *A. baumannii* L13 has been found to share 97% amino acid identity with the recently described *OXA-51* enzyme of *A. baumannii* and 62 and 56% amino acid identity with the carbapenem-hydrolysing oxacillinases *OXA-24* and *OXA-23*, respectively [6,7]. In addition, detection of the cephalosporin-hydrolysing class C-β-lactamase gene (*ADC-2*) in *A. baumannii* L13, which mediates cephalosporin resistance, has increased in recent years in *A. baumannii* clinical isolates [8]. Furthermore, the polymyxin resistance genes, glycopeptide resistance genes, and Na^+-driven multidrug efflux pump were also identified. The information on both pathogenic bacterial virulence factors and the virulence gene contained in the strain L13 was obtained through VFDB database annotation. The results showed that there were 290 virulence genes annotated in the strain L13, including regulation of virulence-associated genes, offensive virulence factors, nonspecific virulence factors and defensive virulence factors. In brief, the number of candidate protein-coding sequences was 3815, of which ca. 7.6% and 8.2% were related to virulence genes and predicted resistance genes, respectively. These results will provide sufficient information for further research in terms of drug and multidrug-resistance mechanisms.

The current study reports the draft genome sequence of a multidrug-resistant *A. baumannii* L13 strain harbouring the bla_{OXA-69} gene in China. The draft genome sequencing of *A. baumannii* L13 was aimed at finding correlative multidrug-resistant genes for further

Table 1
Resistance genes in Acinetobacter baumannii L13.

ARO name	Nucleotide length	Protein length	Location	Pfam	ARO description	Accession no.	Identity (%)
OXA-69	759bp	252aa	Scaffold4	PF00905.21-Transpeptidase	*OXA-69* is an *OXA* family carbapenem-hydrolysing class D-beta-lactamase.	KY126239.1	99.6
ADC-2	1152bp	383aa	Scaffold1	PF00144.23-Beta-lactamase	*ADC-2* is an *ADC* family cephalosporin -hydrolysing class C-beta-lactamase.	NG055285.1	98.2
abeS	330bp	109aa	Scaffold1	PF00893.18-Multi_Drug_Res	*AbeS* in an efflux pump of the *SMR* family of transporters found in *Acinetobacter baumannii*.	CP021347.1	100
abeM	1296bp	431aa	Scaffold5	PF01554.17-MatE	*AbeM* is a multidrug efflux pump found in *Acinetobacter baumannii*.	CP033768.1	99.3
adeK	1455bp	484aa	Scaffold12	PF02321.17-OEP	*AdeK* is the outer membrane factor protein in the *adeIJK* multidrug efflux complex.	CP026125.1	100
adeI	1248bp	415aa	Scaffold12	PF16576.4-HlyD_D23; PF13533.5-Biotin_lipoyl_2	*AdeI* is the membrane fusion protein of the *adeIJK* multidrug efflux complex.	CP023029.1	100
adeJ	3120bp	1039aa	Scaffold12	PF00873.18-ACR_tran; PF03176.14-MMPL	*AdeJ* is an *RND* efflux protein that acts as the inner membrane transporter of the *adeIJK* efflux complex. It has 57% identity with *E. coli acrB*.	CP033768.1	99.9
adeH	1410bp	469aa	Scaffold1	PF02321.17-OEP	*AdeH* is the outer membrane channel protein of the *adeFGH* multidrug efflux complex.	CP034092.1	99.6
adeR	744bp	247aa	Scaffold25	PF00072.23-Response_reg; PF00486.27-Trans_reg_C	*AdeR* is a positive regulator of *adeABC* efflux system. *AdeR* inactivation leads to susceptibility to aminoglycoside antibiotics.	FJ495118.1	99.6
adeG	3180bp	1059aa	Scaffold1	PF00873.18-ACR_tran; PF03176.14-MMPL	*AdeG* is the inner membrane transporter of the *adeFGH* multidrug efflux complex.	CP020588.1	99.4
adeB	3108bp	1035aa	Scaffold25	PF00873.18-ACR_tran; PF03176.14-MMPL	*AdeB* is the multidrug transporter of the *adeABC* efflux system.	CP018332.1	99.4
adeF	1218bp	405aa	Scaffold1	PF13533.5-Biotin_lipoyl_2; PF16576.4-HlyD_D23	*AdeF* is the membrane fusion protein of the multidrug efflux complex *adeFGH*.	CP027530.1	99.3
adeN	546bp	181aa	Scaffold3	PF00440.22-TetR_N	*AdeN* is a repressor of *adeIJK*.	CP033869.1	98.3
adeA	1191bp	396aa	Scaffold25	PF16576.4-HlyD_D23; PF13533.5-Biotin_lipoyl_2	*AdeA* is the membrane fusion protein of the multidrug efflux complex *adeABC*.	CP033858.1	98.2
adeS	1086bp	361aa	Scaffold25	PF02518.25-HATPase_c; PF00512.24-HisKA	*AdeS* is a sensor kinase in the *adeRS* regulatory system of *adeABC*. It is essential for *adeABC* expression.	FJ600366.1	96.7

study of the antimicrobial resistance mechanisms. The sequencing results will help to elucidate the genetic mechanisms contributing towards antimicrobial resistance linked to clinical infection and to protect humans against the diseases caused by drug resistance. Furthermore, the above data will deepen understanding of the genomic features and facilitate greater understanding of the global public health concern caused by this nosocomial pathogen.

Nucleotide sequence accession no

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession RIBX00000000. The version described in this paper is version RIBX01000000. The BioProject ID in GenBank is PRJNA503756.

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Competing interests

None declared.

Ethical approval

Not required.

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