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Genome Note

Draft genome sequence of an OXA-23, OXA-66, ADC-25 and TEM-1D co-producing *Acinetobacter baumannii* ST195 isolated from a patient with neonatal pneumonia in China

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ABSTRACT

Objectives: The rapid spread of multidrug-resistant (MDR) *Acinetobacter baumannii* poses a substantial threat for morbidity and mortality worldwide. In particular, carbapenem-resistant *A. baumannii* has caused a severe challenge to human health. Here we reported the draft genome sequence of *A. baumannii* S131434, an OXA-23, OXA-66, ADC-25 and TEM-1D co-producing strain recovered from a patient with neonatal pneumonia in China and belonging to the globally disseminated sequence type 195 (ST195) of clonal complex 92 (CC92).
Methods: Genomic DNA was sequenced using an Illumina HiSeq platform and the reads were de novo assembled into contigs using CLC Genomics Workbench. The assembled contigs were annotated and bioinformatics analysis was performed.
Results: The genome comprised a circular chromosome of 3 898 344 bp. The presence of the *bla*$_{OXA-23}$, *bla*$_{OXA-66}$, *bla*$_{ADC-25}$ and *bla*$_{TEM-1D}$ genes was detected. In addition, genes conferring resistance to aminoglycosides, macrolides and tetracycline were also identified. Antimicrobial susceptibility testing revealed that the isolate was resistant to all of the tested antibiotics except for polymyxin B, piperacillin/sulbactam and trimethoprim/sulfamethoxazole.
Conclusion: To our knowledge, this is the first report of a clinical *A. baumannii* ST195 (CC92) isolate producing OXA-23, OXA-66, ADC-25 and TEM-1D in southern China. This draft genome will facilitate further our understanding of the antimicrobial resistance and pathogenic mechanisms in this strain and provides valuable information regarding the colonisation and adaptation of MDR pathogens.

Antimicrobial resistance is a growing public-health crisis [1]. Carbapenem-resistant *Acinetobacter baumannii* strains, which belong to the highest-risk group for antimicrobial resistance, are a global concern [2]. The most common mechanism responsible for carbapenem resistance in *A. baumannii* is the production of carbapenemases, of which OXA-23 has been most frequently reported worldwide. In addition, clonal complex 92 (CC92) is the most widely distributed clonal complex of carbapenem-resistant *A. baumannii* in most Asian locales, including China [3]. Although *A. baumannii* strains carrying *bla*$_{OXA-23}$ and *bla*$_{TEM-1}$ have been sporadically reported worldwide, the coexistence of *bla*$_{OXA-23}$ and *bla*$_{TEM-1}$ with other carbapenemase genes in *A. baumannii* is rarely reported. Here we reported the draft genome sequence of an *A. baumannii* isolate co-harbouring *bla*$_{OXA-23}$, *bla*$_{OXA-66}$, *bla*$_{ADC-25}$

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and *bla*$_{TEM-1D}$ and belonging to CC92, isolated from a sputum sample of a patient with neonatal pneumonia in China.

Acinetobacter baumannii strain S131434 was isolated from a 19-day-old female with neonatal pneumonia admitted to a tertiary hospital of Shunde First People's Hospital in 2017. A sputum sample was streaked onto blood agar and *A. baumannii* S131434 was tested using a BD Phoenix^{TM} 100 Automated Identification and Susceptibility Testing system (BD, USA). Strain S131434 displayed an extremely drug-resistant profile, being resistant to all of the tested antibiotics except for polymyxin B, piperacillin/sulbactam and trimethoprim/sulfamethoxazole. The patient received piperacillin/sulbactam for 9 days and recovered. The isolate was further identified as producing OXA-23 and TEM-1D by PCR and sequencing.

Bacterial DNA was extracted using a HiPure Bacterial DNA Kit (MAGEN) according to the manufacturer's instructions. The VAHTS^{TM} Universal DNA Library Prep Kit for Illumina was used to create libraries for genome sequencing. Whole-genome sequencing was performed using an Illumina HiSeq system (Illumina Inc.) generating 2 × 150-bp paired-end reads. The draft genome sequence was de novo assembled into contigs using CLC Genomics Workbench 10.1 (CLC Bio, Aarhus, Denmark) and was annotated by NCBI Prokaryotic Annotation Pipeline (PGAP) (http://www.ncbi.nlm.nih.gov/genome/annotation_prok/) and RAST (Rapid Annotations using Subsystems Technology) server [4]. Multilocus sequence typing (MLST), acquired antimicrobial resistance genes, virulence genes, plasmid replicons and pathogenicity were identified using MLST 2.0, ResFinder 3.0, VirulenceFinder 1.5, PlasmidFinder 1.3 and PathogenFinder 1.1, respectively, available from the Center for Genomic Epidemiology (http://genomicepidemiology.org/). In addition, insertion sequence (IS) elements, clustered regularly interspaced short palindromic repeat (CRISPR) sequences and secondary metabolite gene clusters were predicted by ISfinder, CRISPRFinder and antiSMASH tools, respectively.

A total of 62 contigs were assembled with 525× coverage and an N_{50} contig size of 167 847 bp. The genome size was calculated as 3 898 344 bp with a G + C content of 39.0%. Using PGAP analysis, 3771 total genes, 3633 coding sequences, 80 pseudogenes, 58 rRNAs, 51 tRNAs and 4 ncRNAs were obtained in strain S131434; whilst the RAST server predicted 3671 protein-coding sequences and 55 RNA genes. The distribution of subsystems was also described by RAST server. Proteins responsible for amino acids and derivatives (435 ORFs), carbohydrates (305 ORFs) and cofactors, vitamins, prosthetic groups and pigments (259 ORFs) were abundant among the subsystem categories.

MLST analysis showed that strain S131434 belonged to sequence type 195 (ST195) of CC92, which is a globally disseminated clonal complex. Resistance genes to aminoglycosides [*aph(3′)-Ia*, *armA*, *strA* and *aph(6)-Id*], β-lactams (*bla*$_{OXA-66}$, *bla*$_{OXA-23}$, *bla*$_{ADC-25}$ and *bla*$_{TEM-1D}$), macrolide–lincosamide–streptogramin B antibiotics [*msr*(E)], macrolides [*mph*(E)] and tetracycline [*tet*(B)] were identified (Table 1). In addition, the genome had no plasmid replicons or virulence genes, whereas the presence of 1377 pathogenic family proteins was predicted. Similarly, there were several IS elements in the genome, with the majority belonging to the IS*4* and IS*3* families. Six multidrug resistance efflux pump families were also identified.

Acinetobacter baumannii isolates have been found to exhibit an extremely worrying level of antimicrobial resistance, especially with combined carbapenemase genes. The *bla*$_{OXA-23}$ gene is the most prevalent carbapenemase-encoding gene in *A. baumannii* worldwide as well as co-existing with other carbapenemase genes including *bla*$_{OXA-66}$ and *bla*$_{NDM-1}$. Recently, studies have reported the frequency of extended-spectrum β-lactamase (ESBL) genes (such as *bla*$_{TEM}$, *bla*$_{CTX}$ and *bla*$_{SHV}$) in *A. baumannii* ranging from 25% to 93.5%. *bla*$_{TEM-1/TEM-1D}$ is the most frequently detected ESBL gene in several countries as well as the prevalent factor involved in sulbactam resistance of *A. baumannii* isolates. Outbreaks caused by OXA-23-producing *A. baumannii* ST195 have been reported in north China [5]. However, the coexistence of *bla*$_{OXA-23}$, *bla*$_{OXA-66}$, *bla*$_{ADC-25}$ and *bla*$_{TEM-1D}$ in *A. baumannii* ST195 has never been identified in China. In summary, this is the first report of the coexistence of OXA-23, OXA-66, ADC-25 and TEM-1D in clinical isolates of *A. baumannii* ST195 (CC92) in southern China. In addition, the strain also carried genes conferring resistance to aminoglycosides, macrolides and tetracycline. This finding is in agreement with previous observations reporting that the majority of OXA-23-producing *A. baumannii* isolates also carry resistance genes to aminoglycosides and/or tetracycline. These important resistance determinants coexisting with other resistance genes, including efflux pump genes, would culminate in difficult-to-treat infections. Therefore, monitoring infected patients is of crucial importance to control the spread of bacteria carrying clinically significant antimicrobial resistance genes.

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession **QVQG00000000**. The version described in this paper is the first version QVQG01000000.

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Table 1
Antimicrobial resistance genes in *Acinetobacter baumannii* strain S131434.

Gene	% identity	HSP length/query	Contig	Position in contig	Predicted phenotype	Accession no
Aminoglycoside resistance						
aph(3′)-Ia	99.39	816/814	contig_54	130..943	Aminoglycoside resistance	**V00359**
armA	100	774/774	contig_14	162095..162868	Aminoglycoside resistance	**AY220558**
strA	100	804/804	contig_15	59221..60024	Aminoglycoside resistance	**M96392**
aph(6)-Id	100	837/837	contig_15	60024..60860	Aminoglycoside resistance	**M28829**
β-Lactam resistance						
bla$_{OXA-66}$	100	825/825	contig_23	133328..134152	β-Lactam resistance	**FJ360530**
bla$_{OXA-23}$	100	822/822	contig_42	1695..2516	β-Lactam resistance	**HQ700358**
bla$_{ADC-25}$	99.83	1152/1152	contig_27	69..1220	β-Lactam resistance	**EF016355**
bla$_{TEM-1D}$	100	861/861	contig_55	842..1702	β-Lactam resistance	**AF188200**
Macrolide–lincosamide–streptogramin B (MLS_B) resistance						
msr(E)	100	1476/1476	contig_14	165167..166642	MLS_B resistance	**EU294228**
mph(E)	100	885/885	contig_14	166698..167582	Macrolide resistance	**EU294228**
Tetracycline resistance						
tet(B)	100	1206/1200	contig_15	64233..65432	Tetracycline resistance	**AP000342**

Competing interests

None declared.

Ethical approval

Not required.

References

[1] Brown ED, Wright GD. Antibacterial drug discovery in the resistance era. Nature 2016;529:336–43.

[2] Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 2018;18:318–27.

[3] Ruan Z, Chen Y, Jiang Y, Zhou H, Zhou Z, Fu Y, et al. Wide distribution of CC92 carbapenem-resistant and OXA-23-producing *Acinetobacter baumannii* in multiple provinces of China. Int J Antimicrob Agents 2013;42:322–8.

[4] Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 2008;9:75.

[5] Qu JY, Du Y, Yu RJ, Lv XJ. The first outbreak caused by *Acinetobacter baumannii* ST208 and ST195 in China. Biomed Res Int 2016;2016: 9254907.