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Genome Note

Genomic characterisation of a clinical *Acinetobacter baumannii* ST1928 isolate carrying a new *ampC* allelic variant $bla_{ADC-196}$ gene from China

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ABSTRACT

Objectives: The prevalence of multidrug-resistant *Acinetobacter baumannii* is of serious concern in hospital settings. Here we report the genome sequence and genomic characterisation of a clinical *A. baumannii* isolate from China belonging to a novel sequence type (ST) harbouring $bla_{OXA-383}$ and a new *ampC* allelic variant $bla_{ADC-196}$ simultaneously.

Methods: Whole genomic DNA from *A. baumannii* A42 was extracted and sequenced using an Illumina HiSeq X10 platform. *De novo* genome assembly was performed using Unicycler, and the draft genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline. Genomic analyses were performed through various bioinformatics web servers from the Center for Genomic Epidemiology as well as BacWGSTdb.

Results: The genome size was calculated as 3 800 237 bp, with 3610 protein-coding sequences and a GC content of 38.9%. *A. baumannii* A42 belongs to a rare sporadic clone ST1928. The resistome contains genes encoding resistance to β-lactams ($bla_{OXA-383}$ and a new *ampC* allelic variant $bla_{ADC-196}$). Virulence factor genes encoding biofilm-associated protein (*bap*), acinetobactin biosynthesis protein (*basA–J*), penicillin-binding protein (*pbpG*) and biofilm synthesis *N*-glycosyltransferase (*pgaA–D*) as well as 16 genomic islands and multiple insertion sequences were also identified in the genome of *A. baumannii* A42.

Conclusion: This is the first report of the genome sequence of an *A. baumannii* ST1928 clinical isolate carrying a novel class C β-lactamase gene from China. The genome sequence data can be used as a reference sequence for comparative studies and would facilitate further understanding of the antimicrobial resistance mechanisms of *A. baumannii* at the genomic level.

Acinetobacter baumannii has emerged as a prevalent multidrug-resistant nosocomial pathogen worldwide with a strong capacity for clonal transmission and acquisition of antimicrobial resistance determinants [1,2]. Multidrug-resistant *A. baumannii* is considered a major public-health threat and has been identified by the World Health Organization (WHO) as the top priority organism requiring new antimicrobials in the year 2017 [3]. Recently, a growing number of chromosomal β-lactamases conferring resistance to extended-spectrum cephalosporins have been found in *A. baumannii*. Overexpression of the intrinsic *ampC* gene, also named bla_{ADC}, owing to the acquisition of a strong promoter located on an insertion sequence (IS) element is the main mechanism of resistance to third-generation cephalosporins in *A. baumannii*. Despite increasing research efforts on the clinical epidemiology of *A. baumannii*, large gaps remain in our understanding of the genomic features that contribute to its antimicrobial resistance and clonal dissemination. Here we report the genomic characteristics of a clinical *A. baumannii* isolate recovered from China belonging to a new sequence type (ST1928) and carrying a novel class C β-lactamase gene.

A 42-year-old male patient was hospitalised with symptoms of pneumonia and fever. *A. baumannii* isolate A42 was cultured from a sputum sample of the patient within 24 h after admission. The patient resided in the countryside, without a history of recent travel or hospitalisation. The purified isolate was grown overnight at 37 °C in Mueller–Hinton broth (Oxoid Ltd., Basingstoke, UK). The bacterial species was identified using a MALDI Biotyper (Bruker Daltonics, Billerica, MA, USA) and *rpoB* gene sequencing.

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Antimicrobial susceptibility testing was performed using an automated VITEK®2 system (bioMérieux, Marcy-l'Étoile, France) and was interpreted according to Clinical and Laboratory Standards Institute (CLSI) 2018 guidelines (M100-S28).

Genomic DNA was extracted using a QIAamp® DNA Mini Kit (QIAGEN, Valencia, CA, USA) according to the protocol recommended by the manufacturer. The quality of the extracted DNA was examined using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and a Qubit v.2.0 fluorometer (Life Technologies, Carlsbad, CA, USA). A DNA library was subsequently prepared using a Nextera™ DNA Sample Preparation Kit (Illumina Inc., San Diego, CA, USA), and whole-genome sequencing of *A. baumannii* A42 was performed using a HiSeq X10 platform (Illumina Inc.) with the 150-bp paired-end protocol. Following sequencing, all sequence reads were pre-processed to remove low-quality or artefactual bases. FastQC 0.11.8 was used to assess the quality of the raw data, and Trimmomatic 0.39 was used to trim the raw sequence reads. The trimmed reads were *de novo* assembled using Unicycler v.0.4.7 with the Pilon v.1.23 option for modification of the assembled reads [4].

The genome sequence was automatically annotated by the NCBI Prokaryotic Genomes Annotation Pipeline (PGAP). The multiple online web servers ResFinder 3.2, Comprehensive Antibiotic Resistance Database (CARD) 2019, Virulence Factors Database (VFDB) 2019 and PlasmidFinder 2.0 were used to identify the acquired antimicrobial resistance genes, virulence genes and plasmid replicons using the assembled genome. *In silico* multilocus sequence typing (MLST) and bacterial source tracking using a core genome multilocus sequence typing (cgMLST) strategy were performed by BacWGSTdb server (http://bacdb.org/BacWGSTdb) [5]. Further bioinformatics analyses, such as identification of genomic islands, IS elements, prophage sequences, clustered regularly interspaced short palindromic repeat (CRISPR) sequences and secondary metabolite gene clusters, were predicted by application of IslandViewer 4, ISfinder 1.0, PHASTER 2016, CRISPRCasFinder 1.0 and antiSMASH 5.0.0 tools, respectively, with default parameters. GrapeTree was used to construct and visualise the minimal spanning tree generated using cgMLST allelic profiles of 34 closely-related *A. baumannii* isolates retrieved from the NCBI GenBank database.

The draft genome sequence of *A. baumannii* A42 consisted of 54 contigs comprising 3 800 237 bases, and the PGAP server predicted a total of 3610 protein-coding sequences. The overall G + C content of this strain amounted to 38.9%. In total, 63 tRNA genes and 6 rRNA operons were identified. The genome contained two β-lactam resistance genes ($bla_{OXA-383}$ and $bla_{ADC-196}$) that were not preceded by an IS element. In addition, this strain lacked the *A. baumannii* antibiotic resistance island that confers resistance to multiple antibiotics. The $bla_{ADC-196}$ gene is a novel variant allele of the gene bla_{ADC}, first reported in this study and submitted to the GenBank database (GenBank accession no. **MN249721.2**). The allele of $bla_{ADC-196}$ gene is a variant (1109/1152 nucleotide identities) of the intrinsic bla_{ADC-25} gene of *A. baumannii* strain 17368 (class C β-lactamase ADC-25, complete CDS, NCBI reference sequence **EF016355.1**). It is located on the chromosome between *folE*, encoding a GTP cyclohydrolase 1 enzyme, and an open reading frame encoding a hypothetical protein (locus tag **FQK04_05215**). Previous studies have confirmed that particular *ampC* alleles could link to certain clinically important clones of *A. baumannii*. Therefore, analysis of the *ampC* locus may serve as an alternative approach for exploring the epidemiology of *A. baumannii*. The genome also contains at least 16 genomic islands and several IS elements, the majority belonging to the IS*3*, IS*5* and IS*110* families. Similarly, one prophage sequence and three CRISPR sequences can be predicted in the genome. The presence of five putative secondary metabolite gene clusters, including the acinetobactin, acinetoferrin, *N*-tetradecanoyl tyrosine, fengycin and berninamycin biosynthetic gene clusters, can also be predicted. Several virulence factors were found in the genome, including biofilm-associated protein (*bap*), acinetobactin biosynthesis protein (*basA–J*), penicillin-binding protein (*pbpG*) and biofilm synthesis *N*-glycosyltransferase (*pgaA–D*). *A. baumannii* A42 can be classified into a novel sequence type (ST1928) based on a new combination of the known alleles (*gltA*-1, *gyrB*-35, *gdhB*-67, *recA*-6, *cpn60*-69, *gpi*-140 and *rpoD*-147) according to the MLST scheme of *A. baumannii* developed by the University of Oxford. The phylogenetic relationship between *A. baumannii* A42 and a total of 4067 *A. baumannii* strains currently deposited in the NCBI GenBank database was analysed. Data from the current study suggest that *A. baumannii* A42 belongs to a rare sporadic clone, and the closest relative was an isolate recovered from ear pus of a patient in the USA that differed by >1700 cgMLST loci (Fig. 1). However, the patient in the current study had no history of recent overseas travel or direct contact with foreigners. Hence, there was no epidemiological link identified between this Chinese patient and the previously reported case. One possible explanation is that the patient acquired this strain from the community, however this hypothesis requires further verification to acquire direct evidence.

In summary, here we report the genomic characteristics of a clinical *A. baumannii* ST1928 isolate from China carrying $bla_{OXA-383}$ and a novel *ampC* allelic variant $bla_{ADC-196}$. These data can be used as a reference sequence for future comparative genomic analysis, including acquisition and mobilisation of antimicrobial resistance genes. To the best of our knowledge, this is the first report of the genome sequence of an *A. baumannii* ST1928 clinical isolate from China.

Nucleotide sequence accession number

The genome sequence of *A. baumannii* A42 (BioSample ID **SAMN12368606**) can be accessed at DDBJ/ENA/GenBank under the accession no. **VNWR00000000**. The version described in this paper is the first version (**VNWR01000000**).

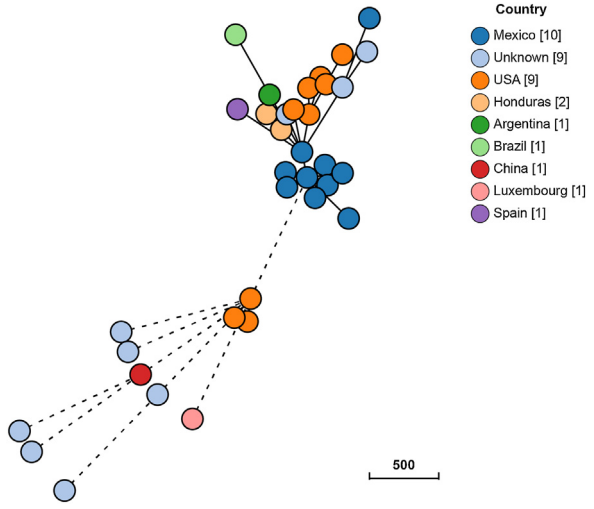

Fig. 1. Phylogenetic relationship between *Acinetobacter baumannii* A42 and closely-related *A. baumannii* strains currently deposited in the NCBI GenBank database. The lines connecting the circles indicate the clonal relationship between different isolates. The scale bar represents a pairwise allelic difference of 500 core genome multilocus sequence typing (cgMLST) loci. The number of isolates from each country is given in square brackets.

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Competing interests

None declared.

Ethical approval

Not required.

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