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Genome note

Genomic features of a carbapenem-resistant OXA-219-positive *Acinetobacter baumannii* of international ST15 (CC15) from a patient with community-onset urinary tract infection in Chilean Patagonia

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ABSTRACT

Objective: Carbapenemase-producing Acinetobacter baumannii has been recognized as a critical priority pathogen by the World Health Organization. We hereby report the identification and the draft genome sequence of a carbapenem-resistant *A. baumannii* isolated from a patient with community-onset urinary tract infection, in a Chilean Patagonian city.

Methods: The whole genome was sequenced on an Illumina NextSeq platform and *de novo* assembled using Unicycler v.0.4. Resistome analysis and epidemiological investigation (based on MLST data and Pasteur scheme) were performed using bioinformatics tools available from the Center for Genomic Epidemiology.

Results: The genome size was calculated at 3 890 824 bp, with a GC content of 39.1%, comprising 3864 total genes, 30 tRNAs, 3 rRNAs, 4 ncRNAs, and 109 pseudogenes. Carbapenem-resistant *A. baumannii* Ab3_Ch strain belonged to the international sequence type ST15 (clonal complex, CC15), and harboured the IS*Aba-1*-$bla_{OXA-219}$ gene array, along to bla_{TEM-1B} and bla_{ADC-6} β-lactamase genes, and *aac(3)-IIa* and *aph(3′)-VIa* aminoglycoside resistance genes. Additionally, efflux pump encoding genes (*abaF*, *abaQ*, *abeS*, *adeI*, *adeK*, *adeL*, *adeN*, *adeR*, *adeS*, and *amvA*) were identified, and mutations in the quinolone resistance-determining region of *gyrA* (Ser81Leu) and *parC* (Ser84Leu) were considered responsible for fluoroquinolone resistance.

Conclusion: This genome sequence data could be used for comparative genomic studies of critical priority *A. baumannii* strains, as well as to understand the specific features of hospital-associated *A. baumannii* lineages of international clonal complexes emerging in community settings.

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) has been listed as critical priority pathogen by the World Health Organization, since it has become one of the most difficult bacteria to treat clinically. In this regard, *A. baumannii* possesses an intrinsic class D β-lactamase (OXA-51-like) that can confer carbapenem resistance [1]. However, the contribution of OXA-51 enzymes to carbapenem resistance depends on several factors, such as the variant of OXA-51 and presence and location of mobile promoters carried on IS*Aba* elements. In this regard, IS must be located upstream in the opposite transcriptional orientation to the bla_{OXA} gene, in order to provide a strong promoter that drives bla_{OXA} gene expression [2]. Additionally, acquired carbapenem-hydrolysing class D β-lactamases (CHDLs) OXA-23, OXA-24/40, OXA-58,

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OXA-143 and OXA-235 have been globally described. Specifically, in Chile, whereas OXA-23 and OXA-58 CHDLs have been prevalent, a novel variant (L167→V) of OXA-51 (designated OXA-219) with carbapenemase activity has been identified, being restricted so far to *A. baumannii* from hospital settings [3,4]. In this study, we report the identification and draft genome sequence of a CRAB carrying the IS*Aba-1-bla*$_{OXA-219}$ gene array, from a community-onset infection in an outpatient living in a Chilean Patagonian city, highlighting that the spread of this critical priority pathogen beyond the hospital is ongoing.

A 63-year-old paraplegic male outpatient, with history of type 2 insulin-dependent diabetes, Parkinson disease, and using an indwelling urinary catheter was attended by a municipal mobile healthcare unit (MHU) that provides medical care to community patients in their home, in Osorno city, a territory of northern Patagonia in Chile. Retrospective analysis of medical record data has shown that in the past 5 years the patient has not been admitted to public or private hospitals, and since the patient was confined to a wheelchair, medical care has only been provided by the MHU.

The patient presented signs and symptoms of urinary tract infection, such as cloudy and foul-smelling urine, dysuria, and body temperature ranging from 36.9 to 37.5 °C. Therefore, a urine sample was collected aseptically and sent to the community healthcare system laboratory. The urine culture was positive for *A. baumannii* (isolate Ab3_Ch). The strain was submitted to antimicrobial susceptibility tests by E-test (bioMérieuxSA, Marcyl'Etoile, France), MicroScan WalkAway (Beckman Coulter), and/or broth microdilution methods, following Clinical and Laboratory Standards Institute, and the European Committee on Antimicrobial Susceptibility Testing guidelines (EUCAST version 8.1). In this regard, Ab3_Ch displayed a multidrug-resistant profile to cefepime (≥256 μg/mL), cefotaxime (≥32 μg/mL), ceftazidime (≥256 μg/mL), amikacin (≥32 μg/mL), ciprofloxacin (≥32 μg/mL), imipenem (≥32 μg/mL), meropenem (≥256 μg/mL); remaining susceptible to ampicillin-sulbactam (16/8 μg/mL), colistin (0.125 μg/mL), gentamicin (4 μg/mL), tobramycin (≤4 μg/mL) and trimethoprim-sulfamethoxazole (≤2/38 μg/mL). Tigecycline MIC was 0.03 μg/mL.

Ab3_Ch isolate was submitted to whole genome sequencing, using the Illumina NextSeq platform (2 × 75 bp). A total of 7 759 284 reads were generated with 299× coverage, which were assembled *de novo* in 256 contigs, using Unicycler v. 0.4. The draft genome was automatically annotated by the NCBI Prokaryotic Annotation Pipeline (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). The genome size was calculated at 3 890 824 bp, with a GC content of 39.1%, comprising 3864 total genes, 30 tRNAs, 3 rRNAs, 4 ncRNAs, and 109 pseudogenes.

Resistome analysis, by using the Resfinder 3.2 tool (https://cge.cbs.dtu.dk/services/ResFinder/), the Comprehensive Antibiotic Resistance Database (https://card.mcmaster.ca/), and *in silico* prediction, revealed the presence of the IS*Aba-1-bla*$_{OXA-219}$ gene array, which has been associated with carbapenem resistance in nosocomial *A. baumanni* isolates [3,4]. Additionally, presence of *bla*$_{TEM-1B}$ and *bla*$_{ADC-6}$ (99.74% identity) β-lactamase genes, and aminoglycoside resistance genes *aac(3)-IIa* and *aph(3′)-VIa* was confirmed, as well as efflux pump encoding genes (*abaF*, *abaQ*, *abeS*, *adeI*, *adeK*, *adeL*, *adeN*, *adeR*, *adeS*, and *amvA*). Other efflux pump genes, such as *adeA*, *adeB*, *adeC*, *adeF*, and *adeJ*, and the porin-encoding genes, *occAB1 (oprD*-like*)* and *omp33-36*, were inquired by blastn, but were not detected. On the other hand, no mutation or insertion was detected in the porin-encoding gene *carO*, whereas mutations in the quinolone resistance-determining region of *gyrA* (Ser81Leu) and *parC* (Ser84Leu) were considered responsible for fluoroquinolone resistance.

Multilocus sequence typing (MLST), assigned by the MLST 2.0 web server (https://cge.cbs.dtu.dk/services/MLST/), revealed that the CRAB strain belonged to the international sequence type ST15 and clonal complex CC15 (Pasteur MLST scheme), which has been identified in European and Latin American countries, including Argentina, Brazil, Chile, and Ecuador [4–6]. Worryingly, *A. baumanni* of ST15 has experienced evolutionary success, being often associated with multidrug-resistant profiles and carbapenemase (mostly OXA-23-type oxacillinase) production, which has facilitated their rapid clonal expansion during recent years, mainly in South America [4–6].

Although *A. baumannii* is a leading cause of severe nosocomial infections in critically ill patients, in the last years CRABs have begun to be identified in community-acquired infections, causing predominantly pneumonia, bacteraemia and urinary tract infection in individuals presenting risk factors such as diabetes mellitus, renal and chronic lung diseases, alcoholism, and smoking [1]. In this regard, our study provides evidence that carbapenem-resistant *A. baumannii* ST15 has spread beyond the hospital setting, in a country with endemic occurrence of nosocomial infections caused by OXA producers [4]. On the other hand, although OXA-219 could be contributing to intrinsic carbapenem resistance in *A. baumannii*, as well to the persistence and dissemination of ST15/CC15, in Chile [4,5], a limitation of this study is the absence of assays directed to cloning the *bla*$_{OXA-219}$ gene without and with IS*AbaI* in a recipient strain with further kinetic studies, in order to verify if such genetic structure is capable or not of increasing carbapenem MICs.

In summary, this draft genome could be used for comparative genomic studies of critical priority *A. baumannii* strains, as well as to monitor and understand the specific features of hospital-associated *A. baumannii* lineages of international clonal complexes, emerging in community settings.

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession QFLH00000000. The version described in this paper is version QFLH00000000.1

Competing interest declaration: None declared.

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