



## Letter to the Editor

**Detection of OXA-58-producing *Acinetobacter bereziniae* in Brazil**

Sir,

*Acinetobacter bereziniae*, formerly *Acinetobacter* genospecies 10, has gained attention due to its emergence as a causative agent of healthcare-associated infection. Importantly, *A. bereziniae* isolates harbouring genes encoding resistance to multiple antimicrobial agents, including carbapenems, located mainly on mobile genetic elements, have been increasingly reported [1].

The widespread mechanism of carbapenem resistance in *Acinetobacter* spp. is mediated by the production of carbapenem-hydrolysing class D  $\beta$ -lactamases (CHDLs). In particular, carbapenem resistance in *A. bereziniae* has been previously associated with the expression of metallo- $\beta$ -lactamases (IMP, VIM, SIM and NDM types) or overexpression of variants of the intrinsic OXA-228-like [1]. To date, the *bla*<sub>OXA-58</sub> gene has only been detected in one *A. bereziniae* isolate each in Ireland and Portugal [1,2]. In Brazil, the *bla*<sub>OXA-58</sub> gene has been detected sporadically in *A. baumannii* and *A. seifertii*. Herein, we report the first detection of an OXA-58-producing *A. bereziniae* (Ac374/14) in Brazil.

Strain Ac374/14 was isolated from an endotracheal aspirate sample ( $10^6$  CFU/mL) obtained from an 83-year-old female patient diagnosed with ventilator-associated pneumonia in August 2014. Species identification was confirmed by *rpoB* gene sequencing [3]. Antimicrobial susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) M100-S28 and M07-A9 guidelines. The antimicrobial susceptibility profile is reported in Table 1, demonstrating a multidrug-resistant phenotype. Carbapenemase production was confirmed by the CarbAcinet NP test. Multiplex PCR assay revealed that the isolate possessed a *bla*<sub>OXA-58-like</sub> gene but was negative for other CHDL genes analysed [4]. Nucleotide analysis of this amplicon showed 100% identity with a known *bla*<sub>OXA-58</sub> sequence (GenBank accession no. CP028560.1/*Acinetobacter* sp.).

Whole-genome sequencing of *A. bereziniae* Ac374/14 was performed on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) using a 300-bp paired-end library, which generated 4 663 604 reads. These reads were subjected to trimming and filtering using CLC Genomics Workbench software v.11.0.1, in which reads with an average Phred quality of <30 and with one or more ambiguities were removed. Finally, reads with a size of <50 bp as well as the last 10 nucleotides of the 3' end of each read were removed. After trimming, 4 660 646 reads were used in the assembly, which generated a genome coverage corresponding to ca. 233-fold based on the reference genome size of 4 509 124 bp for *A. bereziniae* strain XH901 (accession no. CP018259.1). The genome of

Ac374/14 was assembled using CLC Genomics Workbench software v.11.0.1 and a total of 242 contigs were generated, comprising a total length of 4 837 458 bp with coverage depth of 160, an  $N_{50}$  value of 45.186, a maximum length of 154 002 bp, a minimum length of 579 bp and a G + C content of 38.1%. Annotation of the draft assembly was performed using Rapid Annotation using Subsystem Technology (RAST) v.2.0 (available from [rast.nmpdr.org/](http://rast.nmpdr.org/)), which included 4572 coding genes and 77 RNA genes.

The acquired antimicrobial resistance genes were identified using ResFinder 3.0 (<https://cge.cbs.dtu.dk/services/ResFinder/>) available from the Center for Genomic Epidemiology. Resistome analysis revealed the presence of genes encoding resistance to  $\beta$ -lactams (*bla*<sub>OXA-58</sub>, *bla*<sub>OXA-257</sub> and *bla*<sub>CARB-49</sub>), aminoglycosides [*aph* (3')-VIa, *ant*(2'')-Ia], phenicols (*floR*) and sulfonamides (*sulII*). Furthermore, according to RAST, point mutations in the *parC*, *parE*, *gyrA* and *gyrB* genes and the presence of *bla*<sub>AmpC</sub> gene responsible for fluoroquinolone and intrinsic  $\beta$ -lactam resistance, respectively, were observed. Moreover, efflux pumps families [resistance-nodulation-cell division (RND), major facilitator superfamily (MFS), multidrug and toxic compound extrusion (MATE) and ATP-binding cassette (ABC) superfamily] were identified.

Based on in silico analysis, the *bla*<sub>OXA-58</sub> gene was found to be flanked on either side by insertion sequence IS<sub>Aba3</sub>. However, the upstream IS<sub>Aba3</sub> sequence was interrupted by IS<sub>Aba125</sub>, resembling that previously reported in *Acinetobacter* genospecies 3 [5]. The downstream IS<sub>Aba3</sub> sequence was followed *ampC* and *rep* sequences; the presence of a *rep* sequence indicates that the *bla*<sub>OXA-58</sub> gene may be located in a plasmid (Supplementary Fig. S1).

A cluster of genes associated with colicin E (*crbC*-like, *creB*, *creC* and *creD*), colicin V and bacteriocin production (*dedA*, *dedE*, R1, R3, R4, R5, R8 and *purF*) as well as genes associated with invasion and intercellular resistance, including active virulence operons involved in protein synthesis (rv0682, rv0683, rv0684, rv0685, rv1641, rv1642 and rv1643), DNA transcription (rv0667 and rv0668), quinolinate biosynthesis (rv1594, rv1595 and rv1596) and internalin-like protein were found. The virulence factors detected in a manual inspection of the 242 contigs are included in Table 1. In addition, the presence of adhesin genes *ompA* and *bap* may be associated with the strong biofilm-producer phenotype of *A. bereziniae* strain Ac374/14. Biofilm formation was evaluated using the crystal violet staining method in polystyrene plates following 24 h of incubation at 37 °C, using *A. baumannii* ATCC 19606 and brain-heart infusion broth as positive and negative controls, respectively.

The draft genome sequence of *A. bereziniae* Ac374/14 has been deposited in DDBJ/EMBL/GenBank under accession no. QWFFV000000000. The version described in this paper is the first version (QWFFV010000000).

**Table 1**Phenotypic and molecular characteristics of extensively drug-resistant *Acinetobacter bereziniae* strain Ac374/14.

|                            |                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|----------------------------|--------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Phenotypic characteristics | Antimicrobial susceptibility profile | R: TZP, SXT, IPM, MEM, CRO, FEP, GEN, AMK, CIP<br>I: CAZ, LVX<br>S: SAM, COL, PMB, TGC<br>Positive                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Molecular characteristics  | Carbapenemase production             | Strong biofilm producer                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                            | Biofilm formation capability         | β-Lactams: <i>bla</i> <sub>OXA-58</sub> , <i>bla</i> <sub>OXA-257</sub> , <i>bla</i> <sub>CARB-49</sub> , <i>ampC</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                            | Resistance genes                     | Aminoglycosides: <i>aph(3')-VIa</i> , <i>ant(2'')-Ia</i><br>Phenicol: <i>floR</i><br>Sulfonamides: <i>sullI</i><br>GyrA: Ser84Phe<br>GyrB: Ile171Val<br>ParC: Ser84Phe<br>ParE: Asn532Ala                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                            | Mutations in topoisomerases          | ABC-type efflux pump (MacAB–TolC tripartite efflux pump)<br>RND-type efflux pumps (CmeABC, AdeABC and AdeJJK)<br>AcrB multidrug efflux pump<br>MATE family efflux pumps<br>MFS transporters                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|                            | Efflux systems                       | Adhesin<br>Alginate biosynthesis protein<br>Alkaline phosphatase family protein<br>BapA prefix-like domain-containing protein<br>BrnT family toxin<br>DotU family type IV/VI secretion system protein<br>Haemolysin III family protein<br>OmpA family protein<br>Patatin-like phospholipase family protein<br>Phospholipase (C and D)<br>ShlB/FhaC/HecB family haemolysin secretion/activation protein<br>Type I secretion C-terminal target domain-containing protein<br>Type II/IV secretion system protein<br>Type II secretion system F family protein<br>Type II secretion system protein (GspD, GspE, GspF, GspG, GspI, GspJ)<br>Type II toxin–antitoxin system (HipA family toxin, RelB/DinJ family antitoxin, VapC family toxin, YafQ family toxin)<br>Type VI secretion system-associated protein<br>(Hcp, TagF, TssA, TssE, TssF, TssG, TssH, TssK, TssM, VgrG)<br>Zeta toxin |
|                            | Virulence factors                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
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R, resistant; I, intermediate; S, susceptible; TZP, piperacillin/tazobactam; SXT, trimethoprim/sulfamethoxazole; IPM, imipenem; MEM, meropenem; CRO, ceftriaxone; FEP, cefepime; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin; CAZ, ceftazidime; LVX, levofloxacin; SAM, ampicillin/sulbactam; COL, colistin; PMB, polymyxin B; TGC, tigecycline; ABC, ATP-binding cassette; RND, resistance–nodulation–cell division; MATE, multidrug and toxic compound extrusion; MFS, major facilitator superfamily.

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## Competing interests

None declared.

## Ethical approval

Not required.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jgar.2019.08.011>.

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