

## Letter to the Editor

# First detection of the *bla*<sub>OXA-23</sub> gene in a multidrug-resistant *A. baumannii* clinical isolate from Cochabamba, Bolivia

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Dear Editor,

*Acinetobacter baumannii* is an opportunistic pathogen responsible for severe infections and is associated with nosocomial outbreaks worldwide [1,2]. Carbapenems are the agents of choice to treat these infections, but multidrug-resistant isolates are being reported in hospitals worldwide [1]. One of the most important mechanisms of resistance to carbapenems is the production of carbapenem-hydrolyzing class D  $\beta$ -lactamases encoded by *bla*<sub>OXA-23</sub>-like, *bla*<sub>OXA-40</sub>-like, *bla*<sub>OXA-51</sub>-like, *bla*<sub>OXA-58</sub>-like, and *bla*<sub>OXA-143</sub>-like genes [1]. The increase of the resistance in *Acinetobacter baumannii* makes the treatment due to these last-resort drugs challenging, as life-threatening infections due to carbapenem-resistant isolates are on the rise in developing countries such as Bolivia.

The aim of this work was to analyze an especially virulent strain of *Acinetobacter baumannii*, named Bo995, obtained from a 51-year-old woman who was admitted to Viedma Hospital, a tertiary care university hospital in Cochabamba, Bolivia, with a diagnosis of odontogenic abscess; this process rapidly evolved to a severe sepsis and mediastinitis, which required surgery, exploratory thoracotomy, and drainage. One day after the procedure, the patient was admitted to the intensive care unit (ICU), where *Acinetobacter baumannii* with resistance to different antibiotics, including imipenem, was isolated from a tracheal aspirate. She died 10 days later due to general sepsis. In this work, we present the

characterization of this virulent strain with regard to its antibiotic resistance and the identification of carbapenemases as a mechanism of carbapenem resistance.

## The study

In order to analyze the antibiotic resistance profile, minimum inhibitory concentrations (MICs) were determined by broth microdilution method following the recommendations of the Clinical and Laboratory Standards Institute (CLSI) of the most common antibiotics used in treatment. The results revealed that this isolate was resistant to all the antibiotics tested, showing the following MIC values: imipenem (MSD, Madrid, Spain), 64  $\mu$ g/mL; meropenem (AstraZeneca, Wilmington, USA), 64  $\mu$ g/mL; ceftazidime (GlaxoWellcome, Madrid, Spain), > 128  $\mu$ g/mL; cefotaxime (Aventis, Madrid, Spain), > 128  $\mu$ g/mL; cefepime (BMS, Sermoneta, Italy), 64  $\mu$ g/mL; gentamicin (MSD, Madrid, Spain), >128  $\mu$ g/mL; amikacin (BMS, Sermoneta, Italy), 32  $\mu$ g/mL; and norfloxacin, (MSD, Madrid, Spain), > 128  $\mu$ g/mL.

This isolate, resistant to all antibiotics tested except colistin, could represent the “tip of the iceberg” of the population of *A. baumannii* isolates obtained from the Viedma Hospital, where the rates among the total population during 2014 were reported to be very high and the antibiogram data showed that 72.9% of the isolates had the same resistant phenotype of this strain.

Moreover, an important increase in the resistance rates occurred from 2008 to 2014, increasing from 35% to 82.8% for imipenem and from 0% to 19.1% for colistin [3,4]. This increasing trend has also been observed in previous Pan American Health Organization (PAHO) and nationwide reports [5,6]. The results obtained in this hospital are comparable to those found in other countries of Central and South America, such as Mexico (76%), Argentina (78%), Panama (77%), Guatemala (64%), Colombia (> 60%), and Brazil (56%), and higher than the values described in Honduras, El Salvador, Nicaragua, and Chile (20%) [5,6].

As the detection of carbapenem-resistant *A. baumannii* isolates is a worrying event that requires rapid control measures, we decided to thoroughly investigate the presence of carbapenem-hydrolyzing enzymes, focusing on OXA-type carbapenemases, which are the most prevalent in this species [2]. Multiplex polymerase chain reaction (PCR) to search for OXA-type families (-23 like, -40 like, -51 like, -58 like, and -143 like) was performed, showing the presence of the *bla*<sub>OXA-51</sub>-like and *bla*<sub>OXA-23</sub>-like carbapenemase genes. Further sequencing experiments showed 100% homology with the *bla*<sub>OXA-23</sub> gene as previously described [2]. This isolate represented the first description of the OXA-23 carbapenemase in isolates of *A. baumannii* from Cochabamba, Bolivia [7]. In addition, we found that both *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-51</sub> genes were related to IS*Aba1* insertion sequences upstream that leads to the overexpression of the enzymes [1,2]. PCR experiments for detecting other metallo-β-lactamases (VIM, IMP, GIM, SIM, and NDM) were negative.

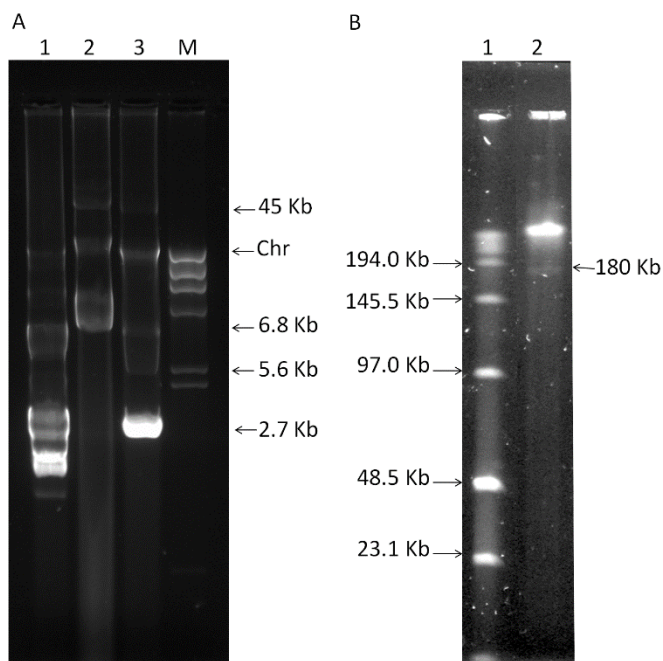
In our group's previous study, carried out in the same hospital in 2008, an outbreak of 15 multidrug-resistant *Acinetobacter baumannii* isolates belonging to a unique clone and producing the OXA-51 and OXA-58 carbapenemases was reported, and represented the first description of OXA-type enzymes in Bolivia [4]. At present, strain Bo995 represents the first isolate harboring the *bla*<sub>OXA-23</sub> carbapenemase gene in Cochabamba, Bolivia [7]. Detection of new carbapenemase families in this hospital is of great concern, as it confirms the spread of carbapenem-resistant isolates in the same hospital environment, in the country (where an OXA-23 carbapenemase-producing isolate was recently reported [8]), and along the continent [6]. The *bla*<sub>OXA-23</sub> carbapenemase gene was first detected in Edinburgh [9] and has been identified worldwide. In Latin America, Brazil was, in 2003, the first country to report OXA-23-producing

isolates, followed some years later by Colombia, Argentina, and México [10-15].

The *bla*<sub>OXA-51</sub> gene is usually located on the chromosome, but the *bla*<sub>OXA-23</sub> gene has been reported to be encoded also by plasmids of different sizes (from 25 to 200 kb) or on the chromosome. To analyze the presence of these mobile structures and locate the genes, plasmid DNA was extracted with a commercial midi plasmid extraction kit (Qiagen, Hilden, Germany) and by pulsed-field gel electrophoresis (PFGE) with S1 enzyme (Takara, Otsu, Japan). Plasmid size was determined by comparison with those from the standard strains *Escherichia coli* NCTC 50193 and NCTC 59192 (National Collection of Type Cultures, Salisbury, UK), containing plasmids ranging in size from 2 to 163.3 kb. Plasmids of 2.7, 5.7, 6.8, and 34 kb were detected by the commercial kit, and a plasmid of 180 kb was detected by PFGE. (Figure 1).

To locate the *bla*<sub>OXA-51</sub>-like and the *bla*<sub>OXA-23</sub> genes, gels were transferred to a nylon membrane and Southern blot was performed with specific PCR-generated probes labeled with dUTP-digoxigenin (Roche, Mannheim, Germany). Detection of hybrids

**Figure 1.** Plasmid profile of isolate Bo995.



**A.** Plasmid extraction with a commercial kit. Lane 1: control strain *E. coli* NCTC 50193 containing plasmids ranging in size from 2 to 53.3 kb; lane 2: control strain *E. coli* NCTC 59192 containing plasmids ranging in size from 7.6 to 163.3 kb; lane 3: plasmid profile of isolate Bo995 with structures of 2.7, 5.7, 6.8, and 34 kb; chr: chromosome; M: molecular weight marker dig-labelled. **B.** Plasmid detection by PFGE with S1. Lane 1: low range PFG marker (Biolabs); lane 2: isolate Bo995.

was done using an anti-digoxigenin antibody coupled with alkaline phosphatase, following the manufacturer's instructions (Roche, Mannheim, Germany). Hybridization experiments located both the *bla*<sub>OXA-51</sub> and the *bla*<sub>OXA-23</sub> gene on the chromosome.

Typing experiments to determine clonal relationship with other worldwide genotypes revealed that Bo995 did not belong to international clones I, II, or III. Moreover, no homology was found with isolates that had been previously identified in the same hospital [3,4]. The dissemination of the *bla*<sub>OXA-23</sub> gene through South America has been related to both monoclonal and polyclonal spread [6,14], so further genotypic analysis is needed to demonstrate the epidemiological origin of Bo995, and in-depth surveillance studies must be carried out in order to prevent the spread of this strain.

### Conclusions

This is the first description of an OXA-23 carbapenemase-producing *A. baumannii* isolate in Cochabamba, Bolivia. It is the second acquired OXA-type enzyme described in the country, as a multidrug-resistant *Acinetobacter baumannii* clone producing OXA-58 were previously identified in the same hospital during 2008.

Both *bla*<sub>OXA-51</sub> and *bla*<sub>OXA-23</sub> genes were located on the chromosome and related to an *ISAbal1* upstream, which contributes to the overexpression of the enzymes.

The detection of new carbapenemase-producing strains in this hospital of Cochabamba, Bolivia, highlights the need to implement control measures to stop the spread of this isolate among the nosocomial environment.

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