Case report

Early dissemination of OXA-72-producing Acinetobacter baumannii strain in Colombia: a case report

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\textbf{A B S T R A C T}

Nosocomial infections caused by carbapenem-resistant Acinetobacter baumannii isolates have reached epidemic levels in past decades. Currently this microorganism is responsible for outbreaks of difficult eradication and with high mortality rates worldwide. We herein report a rare case of an OXA-72-producing A. baumannii isolate colonizing a 47-year-old male patient with peritonitis due to abdominal stab wound, four years earlier than the first report of this carbapenemase in Acinetobacter pittii in Colombia. Although OXA-72 presents a low prevalence compared with OXA-23, our study demonstrated that A. baumannii isolates carrying the bla\textsubscript{OXA-72} gene were present in the hospital environment in Colombia and could act as a reservoir for further spread to other Acinetobacter species, like A. pittii, causing carbapenem-resistance.

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\underline{Introduction}

Carbapenem-resistant Acinetobacter baumannii became a global health concern, especially in intensive care units (ICUs). In Colombia, A. baumannii was the fifth most frequent pathogen causing bloodstream infections between the years 2001 and 2008 at ICUs, with an increased of 40% in the carbapenem resistance. This fact could partially be explained by the spread of OXA-23-producing clones in Colombian hospitals. Montealegre and colleagues reported, for the first time in Colombia, an OXA-72-producing Acinetobacter pittii isolated in 2010 from a catheter tip culture. Here, we describe a case of OXA-72-producing A. baumannii strain colonizing a patient with peritonitis in Colombia, four years earlier than the first reported case.
**Case presentation**

On January 20, 2006, a 47-year-old male patient was hospitalized at a tertiary teaching hospital localized in Bogota, Colombia, due to an abdominal stab wound. The patient was submitted to exploratory laparotomy and a segmental small bowel resection was performed. Antimicrobial therapy with clindamycin (600 mg iv q8h) and amikacin (1 g iv q24h) was administered for seven days, and the patient was discharged in good clinical conditions eight days later. On February 2, 2006, the patient was readmitted due to severe abdominal pain and a new exploratory laparotomy was carried out. Peritonitis was diagnosed secondary to a small bowel perforation. Peritoneal fluid culture was not performed at that time. Antimicrobial therapy with ampicillin/sublactam (3 g iv q6 h) was empirically prescribed and maintained for eight days with clinical improvement. However, on February 9, 2006, the patient presented with vomit, severe abdominal pain, fever and leukocytosis. Another exploratory laparotomy was carried out and the presence of multiple intestinal perforations was diagnosed. The antimicrobial therapy was replaced by meropenem (2 g iv q8h) and the patient was then transferred to the ICU, where he remained for the next six days. The abdominal incision had been left completely open and subsequently intra-abdominal lavages were performed. In the two previous peritoneal lavages, the cultures were negative. On February 16, 2006, after the third peritoneal lavage, the peritoneal cavity was closed and the patient was transferred to the internal medicine ward. At this moment, a non-fermenting Gram-negative coccobacillus isolate (Acb7-31 strain) was cultured from the peritoneal fluid. It was initially identified as a carbapenem-resistant A. baumannii by VITEK 2 automated system (bioMérieux SA, Marcy l’Etoile, France). The patient was discharged at 23rd day of hospitalization in good clinical conditions.

Species identification by sequencing analysis of RNA polymerase β subunit (rpoB) gene, as previously published, confirmed the identification of Acb7-31 strain as A. baumannii. Antimicrobial susceptibility was evaluated by CLSI broth microdilution, except for amikacin and colistin MICs that were determined by Etest strips, according to the manufacturer’s recommendations (AB Biodisk, Solna, Sweden). According to CLSI breakpoints, the Acb7-31 isolate was susceptible to minocycline (MIC, ≤0.03 mg/L), ciprofloxacin (MIC, ≤0.125 mg/L), colistin (MIC, 0.5 mg/L), polymyxin B (MIC, 0.5 mg/L), gentamicin (MIC, 4 mg/L), ceftazidime (MIC, 8 mg/L), cefotaxime (MIC, 8 mg/L), intermediate to levofloxacin (MIC, 4 mg/L) and resistant to imipenem (MIC, 32 mg/L), meropenem (MIC, 32 mg/L), ampicillin/sublactam (MIC, 32/16 mg/L) and cefepime (MIC, 64 mg/L). In order to evaluate the contribution of overexpression of efflux pumps in the resistance to carbapenems, the MICs for imipenem and meropenem were also determined in the presence of 15 mg/L of the efflux pump inhibitor, Phe-Arg-β-naphthylamide (PAβN). Although a 4-fold decrease in the MICs for meropenem was observed in the presence of PAβN (MICs, 32 to 8 mg/L), the MICs for imipenem did not change significantly (MICs, 32 to 16 mg/L).

Multiplex-PCR assays targeting carbapenem-hydrolyzing class D β-lactamases (CHDLs) and metallo-β-lactamase (MβLs) encoding genes were performed, as previously published. The presence of blaoXA-51-like and blaoXA-24/40-like was confirmed by PCR. DNA sequencing identified the blaoXA-24/40-like amplicon as blaoXA-72 and revealed that it was flanked by XerC/XerD-binding sites, a structure implicated with its mobilization. Genomic DNA digested with the endonuclease I-Ceu-I and plasmid DNA from Acb7-31 strain were separated by PFGE, and subsequent Southern blot and hybridization with blaoXA-72-specific probe showed that the blaoXA-72 gene was located on a plasmid of ~20 kb. It seems that this is a non-conjugative plasmid since it was not successfully transferred by conjugation. Similar results were observed by Bonnin and colleagues who described three French A. pittii isolates carrying the blaoXA-72 gene mediated by a non-conjugative 20-kb plasmid. Interestingly, the sizes of plasmids carrying the blaoXA-72 gene in Acinetobacter spp. isolates varies considerably in South America, ranging from 83 kb to 163 kb, as previously reported.

**Conclusion**

Despite its lower prevalence, OXA-72 had been present in Colombia longer than we thought, since the Acb7-31 strain was isolated in 2006 in Bogota, located 462.4 km from Cali, where an OXA-72-producing A. pittii strain was isolated in 2010. Although the Acb7-31 strain was considered as a colonizer strain, our study documented that blaoXA-72 was present in the hospital environment and could act as a reservoir for further spread to other Acinetobacter species, like A. pittii. In addition, the overexpression of efflux pumps seems to contribute for increasing meropenem MICs for inhibiting the Acb7-31 strain.

**Funding**

This study was financed by Colciencias CODE: 11010416355, Agreement 444 and Universidad Nacional de Colombia CODE: 20201009713.

**Conflicts of interests**

A.C.G. recently received research funding and/or consultation fees from AstraZeneca and Thermo Fisher Scientific. Other authors have nothing to declare.

**Acknowledgements**

GREBO (Andrés González), Clínica de Occidente (Elkin Lemus, Norma Montoya, Marta Salinas, Fancelina González, Edgar Sánchez), Clínica Jorge Piñeros Corpas (Geny Díaz, Carlos Barrios), Clínica Reina Sofía (Edilma Torrado, Diego Garzón), Clínica San Pedro Claver (Carlos Alquiriche, Marta Ruiz, Gladis Ceballos, Pilar Hurtado, Jose Guillermo Ruiz Rodríguez), Fundación Cardioinfantil (Álvaro Arango, Patricia Bravo), Hospital Occidente de Kennedy (Romelia Villa, Nubia Escobar, Piedad Giraldo, Álvaro Jiménez), Hospital El Tunal (Marta Isabel...
Garzón, Julia Quijano, Rafael Pérez Yepes), Hospital Militar Central (Carlos Pérez, Matilde Méndez, María Nilsé González, Diana Ferrucho, Juan Pablo Velásquez), Hospital Universitario San Ignacio (Carlos Álvarez, Beatriz Ariza), Hospital Simón Bolívar (Constanza Correa, Luz Janeth Márquez, William Clavijo), Hospital Santa Clara (José Roberto Tamara, Gloria Inés Gallo, Guillermo Ortiz), Hospital Universitario Clínica San Rafael (Marta Pulido, Rabel Lobelo), Hospital Universitario La Samaritana (Johana Osorio, Elsa Marina Zubieta, Emilio Rey), Instituto Nacional de Cancerología (Jorge Cortes, Claudia Patricia Arroyo, Luz Marina Martínez, Elizabeth Rodríguez, Clara Inés Gómez), Policlinico del Olaya (Johana Carol Estrada, Ana Isabel Sánchez, Carlos Hurtado Hurtado).

REFERENCES