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First Identification of OXA-72 Carbapenemase from *Acinetobacter pittii* in Colombia

Maria Camila Montealegre,[a] Juan José Maya,[a] Adriana Correa,[a] Paula Espinal,[b] Maria F. Mojica,[a] Sory J. Ruiz,[a] Fernando Rosso,[c] Jordi Vila,[b] John P. Quinn,[d]* and Maria Virginia Villegas[a]

International Center for Medical Research and Training (CIDEIM), Cali, Colombia[a]; Department of Microbiology, Hospital Clínic, School of Medicine, University of Barcelona, CRESIB/IDIBAPS, Barcelona, Spain[b]; Fundación Clínica Valle del Lili, Cali, Colombia[c]; and Pfizer Global Research and Development, Groton, Connecticut, USA[d]

OXA-72 has been reported in few countries around the world. We report the first case in Colombia in an *Acinetobacter pittii* clinical isolate. The arrival of a new OXA, into a country with high endemic resistance, poses a significant threat, especially because the potential for widespread dissemination is considerable.

The *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex comprises four genomic species, from which *A. baumannii*, *Acinetobacter pittii*, and *Acinetobacter nosocomialis* (14) are the most clinically relevant, being frequently associated with nosocomial infections and outbreaks (15). Resistance rates to carbapenems among *Acinetobacter* spp., caused by carbapenem-hydrolyzing class D β-lactamases (CHDLs), have increased dramatically in the last decade. Three subgroups of CHDLs, OXA-23-like, OXA-58-like, and OXA24/40-like, are frequently encountered (16); among them, OXA-23-like is the most ubiquitous of these enzymes worldwide (15). The OXA-24/40 subgroup consists of five variants, OXA-24/40, OXA-25, OXA-26, OXA-72 (16), and OXA-160 (19), with OXA-24/40 being the most prevalent variant within this group, particularly in the Iberian Peninsula where it is endemic (17). On the other hand, OXA-58 shares less than 50% amino acid identity with OXA-23 and OXA24/40, and OXA-58-like enzymes, as well as the other subgroups, are widely distributed (16).

In Colombia, dissemination of *A. baumannii* clones harboring $bla_{\text{OXA-23}}$ was reported in 2005 (21); since then, surveillance of carbapenem-resistant *A. baumannii* in the hospitals of the Colombian Nosocomial Resistance Study Group network has shown OXA-23 and OXA-51 as the only carbapenemases detected. We now document the first case in the country of OXA-72, identified in an *A. pittii* isolate.

OXA-72 was identified in a clinical isolate from a 70-year-old female patient with past medical history of diabetes mellitus, hypertension, renal failure, and cirrhosis secondary to hepatitis C. The patient underwent a hepatorenal transplant, for which she was taking immunosuppressive drugs, in May 2009. In March 2010, she developed an abdominal non-Hodgkin's lymphoma with extrinsic obstruction of the bile duct and was taken to surgery. In June 2010, she presented with fever with no clear source and was treated empirically with meropenem and vancomycin. In July 2010, she presented with fever, and cultures showed a positive catheter tip culture for *Acinetobacter* spp. (isolate 2688), identified by the Vitek 2 automatic system (bioMérieux, Marcy l'Etoile, France) as *A. calcoaceticus-A. baumannii* complex. In August 2010, she developed a soft tissue infection and sepsis with an extended-spectrum β-lactamase (ESBL)-positive *Escherichia coli* and was restarted on meropenem. Eventually, she developed ischemic hepatitis and multiorgan failure and died on 25 August 2010.

Isolate 2688 was sent to CIDEIM as part of the carbapenemase surveillance study. Antibiotic susceptibility testing was performed using the broth microdilution method (BMD) (Sensititre panels; TREK Diagnostic Systems, Westlake, OH), and MICs were interpreted according to the CLSI guidelines except where indicated (5). The isolate was resistant to carbapenems, piperacillin-tazobactam, and aztreonam, had reduced susceptibility to cefotaxime and ceftriaxone, and was susceptible to cefepime, ceftazidime, amikacin, polymyxin B, and ciprofloxacin (Table 1). We screened for carbapenemases in the cell extract using the three-dimensional test (3D) (18), obtaining a positive result. PCR was then performed using primers for the β-lactamase genes bla_{KPC}, bla_{IMP}, bla_{VIM}, $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV}, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24/40}}$, $bla_{\text{OXA-51}}$, and $bla_{\text{OXA-58}}$. As isolate 2688 was PCR negative for $bla_{\text{OXA-51}}$, a gene that has been suggested to be intrinsic to *A. baumannii* (20), amplified 16S rRNA gene restriction analysis (ARDRA) and matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry were used for the identification at the species level. These analyses, performed at the University of Barcelona, identified the isolate as belonging to *A. pittii*. The $bla_{\text{OXA-24/40}}$-like gene was the only resistance determinant identified by PCR, and sequencing of its entire coding sequence revealed the presence of $bla_{\text{OXA-72}}$. Localization of this gene was investigated using S1 nuclease digestion, followed by pulsed-field gel electrophoresis (PFGE) (2) and hybridization with a $bla_{\text{OXA-72}}$ probe. Results indicated that the isolate carried two plasmids of approximately 45 kb and 163 kb, and the specific $bla_{\text{OXA-72}}$ probe hybridized with the plasmid band of 163 kb. Following the protocol described by Johnson and Nola (9) for plasmid typing, these plasmids were shown to belong to FIA and P-I Alpha incompatibility groups. Further hybridization with corresponding probes is needed to define the large plasmid's *rep* group.

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Address correspondence to Maria Virginia Villegas, mariavirginia.villegas@gmail.com.

* Present address: John P. Quinn, AstraZeneca R&D, Waltham, MA.

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TABLE 1 MICs of selected antibiotics[a] for isolate 2688 (*Acinetobacter pitti*), *E. coli* Top10 plus pBSCK, and *E. coli* Top10 plus pBSCK-OXA-72

Strain	MIC (μg/ml)[f]													
	IPM	MEM	DOR[b]	FEP	CAZ	CTX	CRO	ATM[c]	TZP	CSL[d]	AMK	TGC[e]	PMB	CIP
A. pittii 2688	32 (R)	>64 (R)	>64 (R)	4 (S)	4 (S)	16 (I)	16 (I)	32 (R)	128/4 (R)	≤8/4 (S)	≤8 (S)	≤0.12 (S)	1 (S)	≤0.5 (S)
E. coli Top10 plus pBSCK[h]	0.125[g]	0.012[g]	0.012[g]	0.032[g]	≤1	≤1	≤1	≤2	≤8/4	≤8/4	≤8	≤0.5	≤0.5	≤0.5
E. coli Top10 plus pBSCK-OXA-72[h]	0.75[g]	0.032[g]	0.047[g]	0.094[g]	≤1	≤1	≤1	≤2	≤8/4	≤8/4	≤8	≤0.5	≤0.5	≤0.5

[a] IPM, imipenem; MEM, meropenem; DOR, doripenem; FEP, cefepime; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone; ATM, aztreonam; TZP, piperacillin-tazobactam; CSL, cefoperazone-sulbactam; AMK, amikacin; TGC, tigecycline; PMB, polymyxin B; CIP, ciprofloxacin.
[b] MICs according to EUCAST breakpoints (7).
[c] MICs according to CLSI guidelines for *Pseudomonas aeruginosa* (5).
[d] MICs according to Jones et al. (10).
[e] MICs according to BSAC criteria (3).
[f] Letters in parentheses indicate interpretation of MICs: R, resistant; I, intermediate; S, susceptible.
[g] MIC values determined by Etest.
[h] Organism was susceptible to all antibiotics tested.

In order to determine the genetic environment of the bla_{OXA-72} gene, PCRs targeting the insertion sequences ISA*ba*1, ISA*ba*2, and ISA*ba*3 were performed, with negative results. However, positive results were obtained with custom primers designed to the XerC/XerD-binding sites, both upstream and downstream from bla_{OXA-72}, suggesting that Xer-mediated recombination may be the mechanism responsible for the mobilization of this gene, as previously proposed (13).

Attempts to transfer a bla_{OXA-72}-carrying plasmid by conjugation using *Escherichia coli* J53 as the recipient strain, together with rifampin (256 μg/ml) and imipenem (1 μg/ml) as the selection markers, were unsuccessful. Therefore, in order to evaluate if expression of the bla_{OXA-72} gene in *E. coli* TOP10 conferred resistance or reduced susceptibility to β-lactams, cloning and subsequent MIC evaluations were performed. Transformants showed MIC increases of 6-, 2.7-, 3.9- and 2.9-fold for imipenem, meropenem, doripenem, and cefepime, respectively, compared to the recipient strain alone (Table 1).

The arrival of OXA-72 to Colombia led us to investigate the possible source of the isolate. According to the family, the patient had never traveled outside the country; however, she was visited by her nephews from Spain during her hospitalization. In order to study this possible link, repetitive sequence-based PCR (rep-PCR) was performed with a Spanish collection of *A. pittii* isolates, but no relation was encountered.

OXA-72 was first identified in 2004 in an *A. baumannii* isolate from Thailand (GenBank accession no. AY739646.1). Since then, *Acinetobacter* spp. carrying this carbapenemase have been reported in several countries in the Asiatic region (11, 12, 22), South Europe (1, 4, 6), Croatia (8), Brazil (23), and the United States (19). Colombia is now the second country in South America to report this enzyme, joining the brief but expanding list of nations where OXA-72 strains have caused disease. Given that dissemination of resistance genes via Xer recombination in different plasmids has been demonstrated, the arrival of OXA-72 to a country with high endemic resistance rates is a cause of concern. Surveillance is warranted considering the threat that this mechanism represents for the spread of carbapenemase genes among *Acinetobacter* species.

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