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Emergence of carbapenem-resistant *Acinetobacter pittii* carrying the bla_{OXA-72} gene in the Amazon region, Brazil

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ABSTRACT

We sought to characterize the genetic context of bla_{OXA-72} gene in a carbapenem-resistant *Acinetobacter pittii* strain recovered from a hospitalized patient from Belém, North Brazil, in the Amazon region. We found that the bla_{OXA-72} gene was carried by a small plasmid, pIEC338SCox, that is 10,498 bp. The gene is flanked by XerC/XerD-like recombinase sites, which suggests that this gene was acquired onto this plasmid by recombination.

Acinetobacter spp. is a threatening nosocomial pathogen that has been reported worldwide. Different mechanisms may confer carbapenem resistance in *Acinetobacter* spp., but production of carbapenem-hydrolyzing class D β-lactamases (CHDLs) is considered the most important one. The metallo-β-lactamases can also be found among *Acinetobacter* spp., although less frequently than CHDLs (Poirel and Nordmann, 2006).

In the last decade, high rates of resistance to carbapenems have been observed in *A. baumannii* in Brazilian hospitals, mainly related to the emergence and clonal dissemination of OXA-23-production *A. baumannii* (Chagas et al., 2014). On the other hand, recent studies also provide evidence for the emergence of OXA-72 in *A. baumannii* isolates from Brazil and other Latin American countries such as Colombia, Ecuador, and Mexico (Gonzalez-Villoria and Valverde-Garduno, 2016). These findings raise a concern about the spreading potential of this carbapenemase. The OXA-72 β-lactamase, a variant of OXA-24/40-like, was first identified in *A. baumannii* isolates from Thailand in 2004 and has since been described in several countries (Pfeifer et al., 2016).

Although *A. baumannii* is the most clinically significant species of the *Acinetobacter* genus, non-*baumannii Acinetobacter* have also emerged as agents of infection recently throughout the world (Liu et al., 2017). In Brazil, *A. pittii* and *A. nosocomialis* have rarely been associated with hospital-acquired infections, possibly because it is difficult to accurately distinguish some species of this genus by automated biochemical methods (Chusri et al., 2014).

In this work, we report the isolation of a carbapenem-resistant OXA-72–producing *A. pittii* from a patient admitted to the intensive care unit (ICU) of a hospital in the city of Belém, North Brazil. Little information is available on the susceptibility profiles and resistance mechanisms of the bacterial species from this region of Brazil. Indeed, *A. pittii* strains producing OXA-72 have only been reported in clinical isolates from few countries such as China; Colombia; France; and, most recently, Brazil (Chagas et al., 2017). Thus, to shed light on the genetic context surrounding the dissemination of OXA-72 in *A. pittii,* we hereby present the complete circularized sequences of all the replicons in the *A. pittii* strain IEC338SC. The replicon architecture and the genetic context surrounding a resistance gene are crucial for the understanding of the flow of such genes among strains belonging to different lineages and species.

In March 2014, a carbapenem-resistant *Acinetobacter* isolate (IEC338SC) was recovered from the quantitative endotracheal aspirate culture (>10^5 CFU/mL) of a 71-year-old female patient admitted to the ICU of a tertiary teaching hospital in the city of Belém, Pará state, North Region, Brazil. The patient was diagnosed with ventilator-associated pneumonia and died from causes unrelated to the infection.

The IEC338SC strain was identified as *A. pittii* by *gyrB* multiplex PCR and partial *rpoB* sequencing, as previously described (Gundi et al., 2009; Higgins et al., 2010). The minimum inhibitory concentrations (MICs) were determined using the VITEK 2 System or ETEST (bioMérieux),

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and the results were interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2018). The strain showed resistance against imipenem (MIC ≥32 μg/mL), meropenem (MIC ≥32 μg/mL), and piperacillin-tazobactam (MIC ≥128 μg/mL). In contrast, it showed susceptibility to ceftazidime, cefepime, amikacin, gentamycin, ciprofloxacin, tetracycline, sulfamethoxazole/ trimethoprim, ampicillin/sulbactam, and polymyxin B. PCR assays for CHDL genes ($bla_{OXA-23-like}$, $bla_{OXA-24-like}$, $bla_{OXA-58-like}$, and $bla_{OXA-143-like}$) and metallo-β-lactamase genes (bla_{NDM}, bla_{VIM}, bla_{IMP}, and bla_{SPM}) were exclusively positive for the $bla_{OXA-24-like}$ gene (Mendes et al., 2007; Woodford et al., 2006). In addition, DNA sequencing showed the presence of bla_{OXA-72}.

In order to examine the totality of genetic information present in IEC338SC strain, total DNA was extracted from the wild-type strain and used as input for library preparation using the Nextera Mate Pair kit (Illumina). The sequencing was carried out on an Illumina MySeq system. Contig assembly was carried out with SPAdes v3.7.1 and were annotated with Prokka. The contigs were ordered and scaffolded using SSPACE, and the gaps were closed using Gapfiller. The assembly and the gaps were curated manually using Geneious.

Strain IEC338SC was found to bear 3 small plasmids pIEC338SCox, pIEC338SC2, and pIEC338SC3, in addition to its 3,935,326-bp long chromosome (accession number CP015145). The strain was found to be most closely matched to the *A. pittii* strain PHEA-2 (accession number CP002177.1), an environmental strain isolated from China. For screening of other antibiotic resistance genes, the complete sequence of the genome was submitted to the ResFinder database (http://cge.cbs.dtu.dk/services/ResFinder/). In addition to the bla_{OXA-72} gene, the only other resistance-related gene observed was the $bla_{OXA-213}$, present on the chromosome. The OXA-213 enzyme is able to hydrolyze penicillins and carbapenems (Figueiredo et al., 2011).

We performed multilocus sequence typing (MLST) on IEC338SC strain by MLST 1.7 server of Center for Genetic Epidemiology (https://cge.cbs.dtu.dk/services/MLST/). We found that the strain belongs to sequence types (STs) 1582 and 1076, which were first described in this study and were deposited in the MLST databases developed by the University of Oxford and the Pasteur Institute, respectively.

The bla_{OXA-72} gene is located on the 10,498-bp pIEC338SCox (CP015146) plasmid and is flanked by XerC/XerD-binding sites (Fig. 1). This recombination system is chromosomally encoded and forms a part of the core genome of many Gram-negative species, and involves the use of tyrosine recombinases to resolve chromosome dimers during cell division. However, it has been exploited by mobilizable elements such as plasmids and integrative mobile elements (Midonet and Barre, 2014).

Interestingly, the pIEC338SCox plasmid showed 100% sequence similarity to pAP10253-1 (accession number KY499579) isolated from a strain of *A. pittii* found in Brazil in 2012 in Espírito Santo state, located approximately 3000 km away from Belém city. Although sharing high-similarity plasmids, these 2 OXA-72–producing strains of *A. pittii* isolated in Brazil belong to different STs according to the schemes of the Pasteur Institute and Oxford University of molecular typing.

The pIEC338SCox plasmid also showed 99% similarity to a 4.5-kb region of the pMMD plasmid (accession number GQ904226; 10 kb) and 100% similarity to a 4.3-kb region of the pAB-ML plasmid (accession number KT022421; 12 kb). These DNA modules consist of *mobA, OriV*, iterons, *rep*A, hypothetical proteins, and bla_{OXA-72} gene and are flanked by XerC/XerD-like binding sites, suggesting that they may have been acquired through recombination events. According to Grosso et al. (2012), XerC/XerD-like binding sites might be responsible for *bla*OXA-24/40 genes and larger contiguous modules of DNA mobilization, and may clarify the identification of *bla*OXA-24/40 and/or common modules in different plasmid or in chromosomes.

The other 2 plasmids found in the IEC338SC strain were both smaller than pIEC338SCox. The pIEC338SC2 plasmid (5562 pb; CP015147) bears *traK*, a gene coding for a type IV secretion system coupling protein (IPR019476). It also bears a gene encoding an integrase, which is perhaps involved in transposition of the acquired regions of the other plasmids. The third plasmid, pIEC338SC3 (5813 pb; CP015148), carries a resolvase, which may be involved in resolving catenation or dimerization of replicons resulting from the exploitation of the chromosomal XerC/XerD recombination system by the plasmid.

Given that *Acinetobacter* has been recognized as an important organism for the dissemination of resistance genes (Chagas et al., 2015) and the emergence of OXA-72 producers in Brazil and others countries, the occurrence of an OXA-72–producing *A. pittii* should be seen with concern by the health authorities. In conclusion, in this article, we reported on the sequencing of the complete genome of a carbapenem-resistant *A. pittii* clinical isolate carrying bla_{OXA-72} found in Belém, North Region, Brazil. These data point towards the relevance of non-*baumannii Acinetobacter* species in healthcare-associated infections and their role as a reservoir of resistance genes.

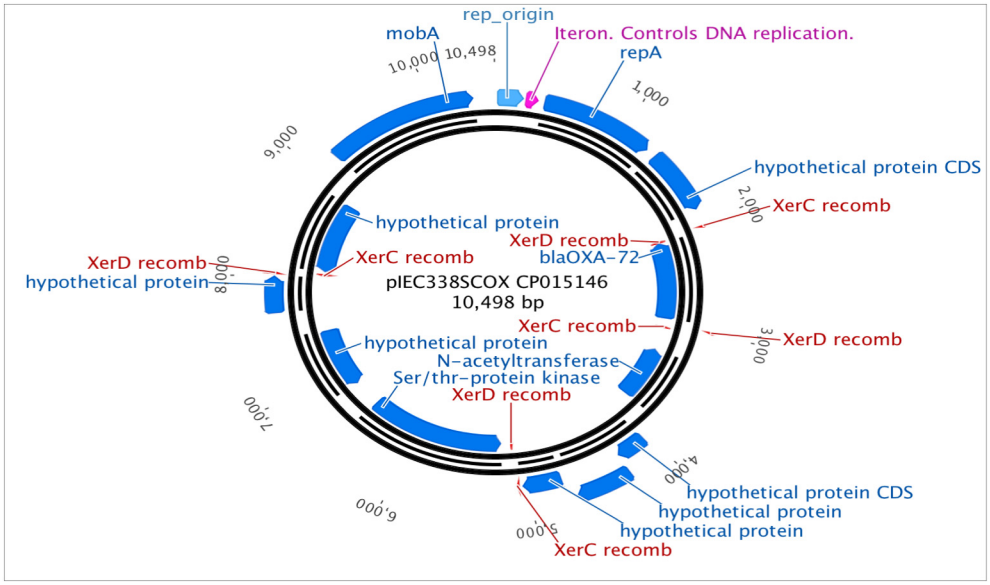

Fig. 1. Plasmid map showing the CDS encoded by pIEC338SCox. The XerC/XerD binding sites are shown in red.

Nucleotide sequence accession numbers

All the replicons found in the IEC338SC strain were deposited in GenBank under the respective accession numbers CP015146, CP015147, and CP015148, linked to BioProject PRJNA316135.

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Conflict of interest

All authors declare that they have no conflict of interest.

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