TITLE: CHARACTERIZATION OF PAC1-BRL: A PLASMID RESPONSIBLE FOR *bla*$_{OXA-72}$ DISSEMINATION IN DIFFERENT ENVIRONMENTS OF SÃO PAULO STATE

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CHDL-production *Acinetobacter baumannii* is the main mechanism of carbapenem resistance in Brazil. An increasing number of reports have documented the emergence of *bla*$_{OXA-72}$ in Southeast of Brazil. Additionally, we recently detected OXA-72-producing isolates in the microbiota of migratory birds that visit the São Paulo zoo lakes, demonstrating the dissemination of this clone in distinct ecological niches. The aim of this study was to understand the genetic mechanisms involved in the dissemination of *bla*$_{OXA-72}$ by comparing clinical and environmental isolates from São Paulo. We selected thirteen OXA-72-producing *A. baumannii* isolated, respectively, from microbiota of birds (n=9) and human bloodstream infections (n=3). The clinical isolates were recovered in 2000, 2012 and 2013. PFGE and MLST were performed to verify the clonal relationship among these isolates. Plasmid analysis followed by Southern Blot Hybridization were used to determine the location of *bla*$_{OXA-72}$. Based on the clonal relationship, two isolates were fully sequenced by Illumina HiSeq Platform. The plasmids were assembled by Newbler and Ray tools. SABIA pipeline was used to plasmid automatic annotation. The isolates were strongly related according Tenover criteria A1 (n=6), A2 (n=5), A3 (n=1). According to MLST analysis the representative isolates of each clone detected showed that the isolates belonged to ST 79 and ST 957, all grouped under the CC79. Plasmid and hybridization analysis showed that *bla*$_{OXA-72}$ was harbored by a small plasmid (≅16 Kb) in all isolates. The plasmids harboring *bla*$_{OXA-72}$ of both environmental and clinical isolates were named pAC1-BRL and pA61817-BRL, respectively. By whole genome sequence, both plasmids were identical (16.6 Kb and 36% GC) and showed 99% of identity with a 7 Kb region contained in different plasmids deposited in NCBI. This region was composed by *bla*$_{OXA-72}$ and accessory genes, which were probably responsible for the mobilization of this region. Plasmids incompatibility group analysis showed that the plasmids belonged to GR2 group. No insertion sequences were detected in these plasmids. However, the recombination site XerC/XerD was detected flanking the *bla*$_{OXA-72}$. Besides *bla*$_{OXA-72}$, a gene encoding for a macrolide phosphotransferase, *mph*(E), was also detected. The toxin-antitoxin system AbkAB was responsible for plasmid maintenance. We conclude that single small plasmids have been responsible for *bla*$_{OXA-72}$ in different environments in São Paulo.

Keywords: *Acinetobacter baumannii*, OXA-72, migratory birds, pAC1-BRL, pA61817-BRL