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The changing epidemiology of *Acinetobacter* spp. producing OXA carbapenemases causing bloodstream infections in Brazil: a BrasNet report

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ABSTRACT

We evaluated the epidemiology of *Acinetobacter* spp. recovered from patients diagnosed with bloodstream infections in 9 tertiary hospitals located in all Brazilian geographic regions between April and August 2014. Although OXA-23–producing *Acinetobacter baumannii* clones were disseminated in most hospitals, it was observed for the first time the spread of OXA-72 among clonally related *A. baumannii* isolated from distinct hospitals. Interestingly, *Acinetobacter pittii* was the most frequent species found in a Northern region hospital. Contrasting with the multisusceptible profile displayed by *A. pittii* isolates, the tetracyclines and polymyxins were the only antimicrobials active against all *A. baumannii* isolates.

Acinetobacter spp. have been increasingly reported as important nosocomial pathogens worldwide (Towner, 2009). According to the last report of the Brazilian Health Surveillance Agency (ANVISA), *Acinetobacter* spp. ranked as the fourth most prevalent pathogen, causing 2159 (11.8%) catheter-related bloodstream infections (BSIs) among intensive care unit (ICU) patients in 2013 (ANVISA, 2013). Unfortunately, the carbapenem resistance rates observed among these pathogens was extremely high (80.7%) (ANVISA, 2013). These findings corroborate the results of previous studies that reported a significant increase in the imipenem resistance rates in *Acinetobacter baumannii* isolated from Brazilian medical centers, from 12.6% in 1997–1998 period to 71.4% in 2008–2010 (Gales et al., 2012). This increase in resistance has been mainly associated with the spread of OXA-23–producing clones and, to a lesser extent, to OXA-143 clones (Antonio et al., 2011; Chagas et al., 2014; Werneck et al., 2011b). Considering the high prevalence of carbapenem resistance among *Acinetobacter* spp. isolates in Brazil, the diversity of carbapenemases and clones, and the continental dimensions of the country, we performed a study to characterize the microbiology and epidemiology of *Acinetobacter* spp. causing BSIs in hospitalized patients from distinct Brazilian regions.

Nine hospitals from 5 different states representative of all of the Brazilian regions were involved in this study. The distributions of the participating medical centers and the *Acinetobacter* spp. isolates are displayed in Fig. 1. The hospitals were guided by a standardized protocol to collect clinical and microbiological data from BSI episodes caused by *Acinetobacter* spp. between April and August 2014 (1 isolate per patient). The isolates were referred to research laboratories for microbiological characterization. Bacterial identification was initially performed by a Microflex LT mass spectrometer using the MALDI BioTyper 3.0 (Bruker Daltonik, Bremen, Germany) (Espinal et al., 2012) and confirmed by *rpoB* sequencing (La Scola et al., 2006). Matrix-assisted laser

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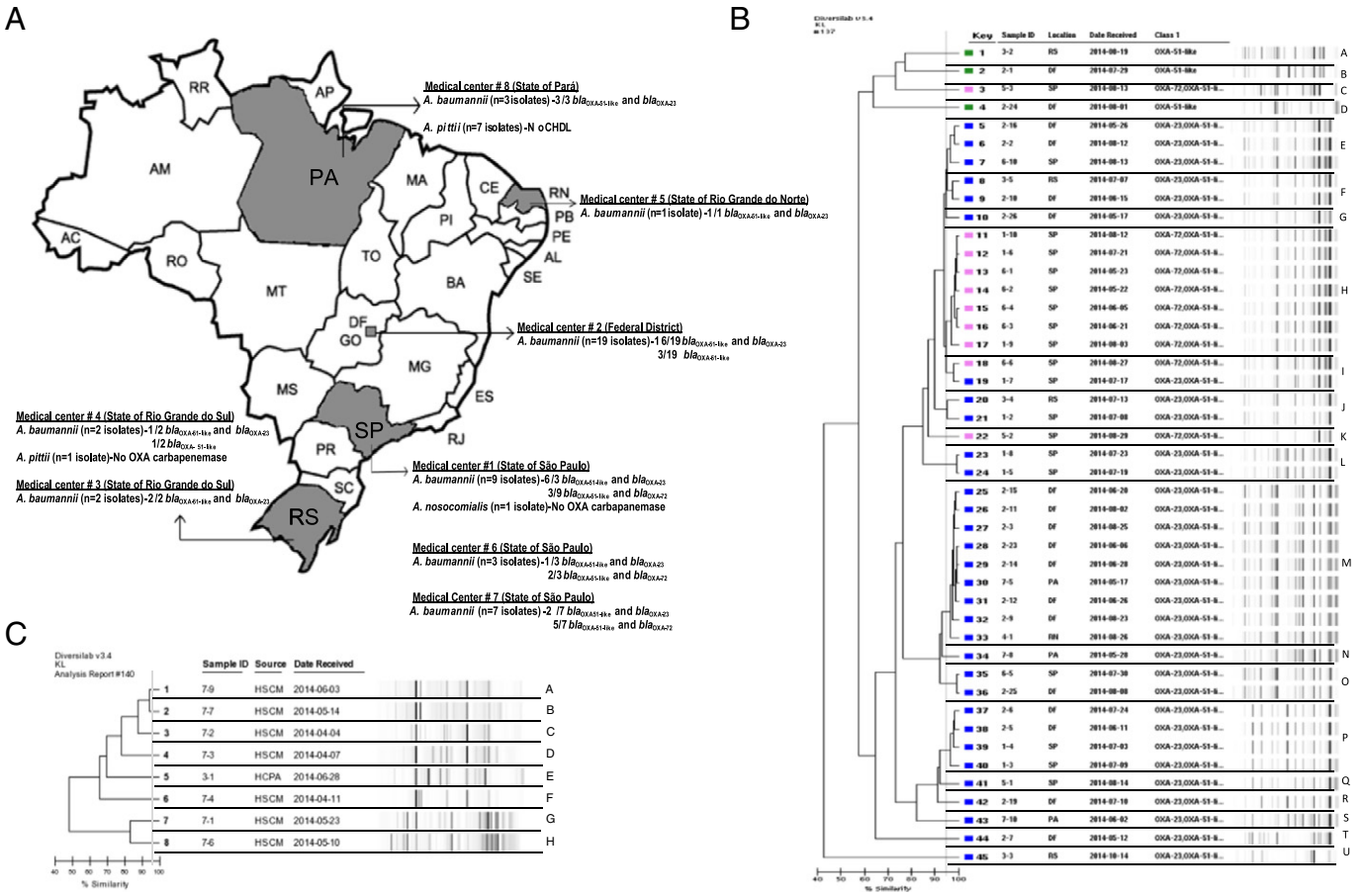

Fig. 1. Geographic distribution of *Acinetobacter* spp. isolates and CHDL-encoding genes according to the medical centers participating in the study (**A**). Dendrogram and computer-generated image of rep-PCR banding patterns of the 49 *A. baumannii* (**B**) isolates and of the 8 *A. pittii* isolates (**C**).

desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) correctly identified 54 of 55 (98.2%) isolates at the species level. *A. baumannii* (46 isolates) was the most frequently identified species, followed by *Acinetobacter pittii* (n = 8) and *Acinetobacter nosocomialis* (n = 1). Seven out of 8 *A. pittii* isolates occurred in a single neonatal ICU in the state of Pará (in the Northern Region). The antimicrobial susceptibility was evaluated using broth microdilution Trek Diagnostics Systems/Sensititre® panels (Cleveland, OH, USA). Quality control was performed by testing *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 strains. The results were interpreted according to both the CLSI and EUCAST criteria (CLSI, 2014; EUCAST, 2015) as shown in Table 1. Polymyxins (MIC_{50}, 1 μg/mL; 93.5% susceptible) and tigecycline (MIC_{50}, 0.5 μg/mL) exhibited the highest in vitro activity against *A. baumannii*, followed by minocycline (MIC_{50}, ≤2 μg/mL; 80.4% susceptible) and doxycycline (MIC_{50}, ≤2 μg/mL; 76.0% susceptible). Tobramycin (MIC_{50}, 4 μg/mL; 50% susceptible) was the most active aminoglycoside tested. Two OXA-23–producing *A. baumannii* isolates, detected in a single medical center, were also resistant to polymyxins. However, the tigecycline MICs were low in both isolates (0.5 and 1 μg/mL). Although the *A. baumannii* isolates were generally highly resistant to β-lactams, fluoroquinolones, and aminoglycosides, all *A. pittii* and *A. nosocomialis* isolates were multisusceptible.

The carbapenem-hydrolyzing class D β-lactamase (CHDL)–encoding genes were investigated by multiplex PCR followed by DNA sequencing (Higgins et al., 2010a), and none were detected in non-*baumannii* isolates. The bla_{OXA-23} gene was the most frequently acquired CHDL-encoding gene found in *A. baumannii* (69.6%; Fig. 1), in agreement with previous studies (Chagas et al., 2014; Higgins et al., 2010b). Interestingly, bla_{OXA-72} was detected in 10 *A. baumannii* (21.7%) isolates recovered from 3 different hospitals located in the state of São Paulo. No significant differences regarding the antimicrobial susceptibility were observed between *A. baumannii* isolates carrying the bla_{OXA-23} and bla_{OXA-72} genes. However, as expected, the isolates of *A. baumannii* carrying solely $bla_{OXA-51-like}$ were more susceptible to β-lactams than those carrying bla_{OXA-23} or bla_{OXA-72}. Although $bla_{OXA-143}$ is the second most frequent CHDL encoding gene described among Brazilian *Acinetobacter* isolates (Antonio et al., 2011; Werneck et al., 2011b), no isolates carrying this gene were found in the present study. OXA-143 is frequently found in the Southeast region, especially in the state of São Paulo (Antonio et al., 2011; Mostachio et al., 2012; Werneck et al., 2011a, 2011b); this enzyme has never been isolated from other Brazilian region (Chagas et al., 2014), except for a single case of an OXA-231–producing *A. baumannii* strain isolated from the city of Londrina, located in the Brazilian South region (Gionco et al., 2012). The introduction of clones carrying bla_{OXA-72} in the hospitals from the state of São Paulo might be responsible for the absence of isolates carrying $bla_{OXA-143}$ in the present study.

The genetic relatedness among the *Acinetobacter* spp. was investigated by the DiversiLab typing system (bioMérieux, Durham, NC, USA) according to the manufacturer's recommendations. A cluster was defined as at least 2 isolates with a similarity index ≥95%, and isolates having a similarity index of ≥99% were considered identical, as previously published (Higgins et al., 2010b; Schleicher et al., 2013). The occurrence of 15 clones among the 32 *A. baumannii* carrying bla_{OXA-23} was observed. Both the interhospital and intrahospital spread of epidemic clones were detected in hospitals located in distinct Brazilian regions. In addition, the spread of a single clone of *A. baumannii* carrying bla_{OXA-72} (n = 8 isolates) was detected in 2 hospitals in the city of São Paulo. In fact, in 1 of these hospitals, 5 of the 7 *A. baumannii* studied carried bla_{OXA-72}. Two isolates carrying bla_{OXA-72} recovered from a third