Temporal evolution of carbapenem-resistant *Acinetobacter baumannii* in Curitiba, southern Brazil

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Background: In the last few years, carbapenem-resistant *Acinetobacter baumannii* isolates (CR-AB) have been identified worldwide. The first description of OXA-23–producing *A baumannii* in Brazil was from the city of Curitiba in 2003. The aim of the present study was to evaluate the persistence and dissemination of the first OXA-23–producing *A baumannii* clone isolated from patients in Hospital de Clinicas, Curitiba, Brazil.
Methods: An antimicrobial susceptibility profile of the isolates was determined by the standard agar dilution method. Molecular detection of β-lactamase genes was done by polymerase chain reaction. The clonal relationship of the isolates was analyzed by pulsed-field gel electrophoresis (PFGE). Epidemiologic and clinical features were evaluated as well.
Results: Genotypic analysis of 172 CR-AB isolates by PFGE identified 3 distinct major PFGE clusters (A, B, and C, accounting for 36, 69, and 65 isolates, respectively). All isolates carried the $bla_{OXA\text{-}23}$–like gene and were multidrug-resistant, but were susceptible to tigecycline and polymixin B. The mortality rate related to CR-AB infection was 45.4%, and ventilator-associated pneumonia and bloodstream infections were the most frequent clinical manifestations.
Conclusions: The presence of 3 clones among the CR-AB isolates suggests that cross-transmission was the main mechanism responsible for dissemination of OXA-23 producers. PFGE pattern A was genotypically similar to that of the first OXA-23–producing *A baumannii* clone identified in Curitiba in 1999. This clone persisted in the same hospital until April 2004. The presence of the bla_{OXA}-23–like gene was the main mechanism associated with carbapenem resistance among the isolates studied.
Key Words: *Acinetobacter baumannii*; carbapenem resistance; OXA-23.

Acinetobacter baumannii is an opportunistic pathogen responsible for an increasing number of health care–associated infections (HAIs), particularly ventilator-associated pneumonia (VAP) and bloodstream infections, in critically ill patients hospitalized in intensive care units (ICUs).[1] The most common risk factor for the acquisition of multidrug-resistant (MDR) *A baumannii* is prevous antibiotic use, following by mechanical ventilation, length of ICU/hospital stay, severity of illness, and use of medical devices.[2]

A baumannii has emerged as a cause of worldwide nosocomial outbreaks displaying ever-increasing resistance. MDR *A baumannii* isolates have been reported from hospitals in Europe, North America, South America and Asia.[1,3] *A baumannii* infections are often difficult to treat, because this pathogen has demonstrated high resistance to many antibiotics as a result of both intrinsic and acquired mechanisms. Carbapenems (eg, imipenem, meropenem) are usually the antibiotics of choice against *Acinetobacter* infections; however, their use has been compromised by the emergence of carbapenem-hydrolyzing β-lactamases (eg, carbapenemase) belonging to molecular class B or D.[4] Metalloenzymes (carbapenemases of molecular class B) are prevalent in some regions, especially in east Asia.[5] Carbapenemases belonging to molecular class D (OXA enzymes) have emerged globally as the main mechanism responsible for carbapenem resistance in this species.

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Three main acquired OXA carbapenemase clusters have been described in *A baumannii*, based on the variant sequence homology: OXA-23, including OXA-27 and OXA-49; OXA-24/OXA-40, including OXA-25 and OXA-26; and OXA-58.[6] OXA-51/69–like occurs naturally and is chromosomally located in *A baumannii*.[3,6] OXA-23 (ARI-1) was first identified in Scotland in 1985; since then, outbreaks of OXA-23–producing *Acinetobacter* spp strains have been reported worldwide.[3,7] Recently, OXA-23–producing *Acinetobacter* spp isolates have been reported in Germany[8] and the Asia-Pacific region.[9] In Latin America, OXA-23–producing *A baumannii* isolates have been reported in Brazil,[10-12] Argentina,[13] and Colombia.[14]

The spread of an epidemic clone of OXA-23–producing *A baumannii* was reported in 2 tertiary-care university hospitals in Curitiba, Brazil in 2003.[10] An increase in carbapenem-resistant *A baumannii* (CR-AB) has been occurred over the past few years at the Hospital de Clinicas, Universidade Federal do Paraná (HC-UFPR), a 635-bed tertiary-care teaching hospital. The aim of the present study was to evaluate whether this increased number of CR-AB isolates at HC-UFPR was due to the spread of the original OXA-23–producing clone or to the presence of new epidemic clones. Epidemiologic characteristics of *A baumannii* HAIs were evaluated as well.

MATERIALS AND METHODS

The Hospital de Clinicas Ethical Committee approved the study design (1120.159/2005-10).

Bacterial isolates

A total of 172 CR-AB isolates were obtained from patients at HC-UFPR between October 2002 and May 2005. Only one bacterial isolate per patient was included in the study. Isolates were identified using conventional biochemical tests.[15] The clinical data analyzed included patient age, sex, infection site, medical comorbidities, major risk factors, and antimicrobials used. HAI was defined according to Centers for Disease Control and Prevention (CDC) criteria, and colonization was defined as the presence of positive culture specimens in the absence of clinical signs and symptoms justifying infection.[16]

Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) were determined by the agar dilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI)[17] and interpreted according to the CLSI standards.[18] The following antimicrobial agents were tested: amikacin, ampicillin-sulbactam, ciprofloxacin, aztreonam, cefepime, ceftazidime, gentamicin, imipenem, meropenem, piperacillin-tazobactam, trimethoprim-sulfamethoxazole, and polymyxin B. MICs of tigecycline were determined with the E-test (AB BIODISK, Solna, Sweden) and the agar dilution method,[17] performed on fresh Mueller-Hinton agar plates (Difco-BD, Franklin Lakes, NJ). Tigecycline breakpoints for *Enterobacteriaceae* approved by the Food and Drug Administration (susceptible, ≤2 mg/L; resistant, ≥8 mg/L) were applied to all isolates. *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were tested for quality control purposes. Screening for metallo-β-lactamase (MBL) was done by the imipenem-EDTA disk diffusion test as described previously.[19]

Molecular detection of β-lactamase genes

All isolates were tested for the presence of the $bla_{\text{OXA-23}}$ gene by polymerase chain reaction (PCR) using the primers OXA23-F (5-GATCGGATTGGAGAACCAGA-3) and OXA23-R (5-ATTTCTGACCGCATTTCCAT-3) as described previously.[5] The presence of $bla_{\text{IMP-1}}$, $bla_{\text{SPM-1}}$, $bla_{\text{SIM-1}}$, $bla_{\text{VIM-1}}$, and $bla_{\text{VIM-2}}$ alleles was investigated in 2 isolates screened as possible MBL producers. The primers IMP1-F (5-CTACCGCAGCAGAGTCTTTGC-3), IMP1-R (5-GAACAACCAGTTTTGCCTTACC-3), SPM-F (5-CCTACAATCTAACGGCGACC-3), SPM-R (5-TCGCCGTGCCAGGTATAAC-3), VIM1-F (5-TCTACATGACCGCGTCTGTC-3), VIM1-R (5-TGTGCTTTGACAACGTTCGC-3), VIM2-F (5-ATGTTCAAACTTTTGAGTAGTAAG-3), VIM2-R (5-CTACTCAACGACTGAGCG-3), SIM1-F (5-GTACAAGGGATTCGGCATCG-3), and SIM1-R (5-TGGCCTGTTCCCATGTGAG-3) were used under conditions described previously.[20-24] Positive controls for all genes were run simultaneously.

DNA sequencing

Amplicons obtained by PCR targeting the $bla_{\text{OXA-23}}$ gene were submitted to restriction mapping with the enzyme HindIII, which produced fragments of 390 and 110 bp. Two representatives of each PFGE cluster of OXA-23–like producing *A baumannii* were randomly selected for DNA sequencing using the primers described previously. PCR products were sequenced on both strands. The nucleotide sequences and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, WI) and compared with sequences available using Blast 2.0 (http://www.ncbi.nlm.nih.gov/blast).

Molecular typing by pulsed-field gel electrophoresis and dendrogram analysis

All isolates were genotyped by pulsed-field gel electrophoresis (PFGE). DNA was prepared as described previously.[25] DNA was cleaved using 30 U of ApaI (Invitrogen, Carlsbad, CA) for 18 hours at 37 °C.