CARBAPENEM-RESISTANT ACINETOBACTER BAUMANNII OUTBREAK AT UNIVERSITY HOSPITAL

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SHORT COMMUNICATION

ABSTRACT

Nineteen clonally related imipenem-resistant Acinetobacter baumannii isolates were recovered from eight intensive care unit patients. All isolates harboured blaOXA-51-like ß-lactamase genes and showed the absence of 22 kDa fraction in outer membrane porin profile analysis. It suggests a combination of two mechanisms as responsible for carbapenem–resistant phenotypes.

Key words: Acinetobacter, blaOXA-type genes, carbapenemases, nosocomial infection, Brazil

INTRODUCTION

In Brazilian hospitals, multidrug-resistant (MDR) Acinetobacter baumannii constitute a serious cause of nosocomial infection, comprising 8.8% of the total nosocomial bacterial isolates that cause infections in ICU patients, according to the MYSTIC Program Brazil (11). In this respect, carbapenems remain as the widest spectrum therapeutic option for treatment of such infections. However, resistance to these antimicrobial agents has increased, resulting in the use of potentially more toxic agents such as the polymyxins (7). Although high carbapenem resistance rates have been reported among Acinetobacter spp. isolated in Brazil, very little is known about their mechanisms of resistance. Recently, it has been reported that IMP-1 metallo-beta lactamase-producing Acinetobacter strains emerged in 1998 in some Brazilian hospitals (15). Regarding OXA-type carbapenemases, only the blaOXA-23-like gene has been associated with imipenem resistance in Brazil (6). We hereby report the combination of the naturally intrinsic harboured blaOXA-51-like gene and impermeability as mechanism responsible for imipenem–resistant phenotype in clonally related A. baumannii recovered from an outbreak, in a Brazilian teaching hospital.

From September 2005 to February 2006 eleven MDR, Acinetobacter baumannii isolates were recovered from six ICU patients hospitalized at the Hospital Universitário da Universidade de São Paulo (HU-USP). Species identification and antimicrobial susceptibility (Table 1) were evaluated using the Vitek system (BioMérieux, Hazlewood, Mo.) and the disk diffusion method, respectively. Molecular typing was performed by Pulsed Field Gel Electrophoresis (PFGE) of ApaI-digested genomic DNA of A. baumannii isolates (17). PFGE band profiles were identical for all carbapenem-resistant strains. Minimum inhibitory concentrations (MICs) for all isolates were determined by the agar dilution method (4). Additionally, some combinations antibiotic/ß-lactamase inhibitors were tested as follows: ceftazidime/clavulanic acid (4.0 mg/L) (4), imipenem/EDTA (320 mg/L) (22), imipenem/NaCl (200 mM) (16). All strains were found to be resistant to more than 3 antimicrobial groups (Table 1), presenting MICs ≥ 32 and ≥ 64 mg/L for imipenem and ceftazidime, respectively. The inhibitors tested did not affect MIC’s values when associated with imipenem or ceftazidime.

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Research on carbapenemase production and outer membrane porin profile was performed using imipenem-susceptible and non-susceptible isolates recovered from HU-USP.

Carbapenemase activity was evaluated using a bioassay (9). This test involved satellite growth of *Staphylococcus aureus* ATCC 25923 around the putative carbapenemase-producing *A. baumannii* strains growing on Muller-Hinton agar plates containing 10⁸ CFU of ATCC strain/mL and imipenem at a concentration of 0.06 or 0.12 mg/L. Imipenemase activity was confirmed in all imipenem resistant isolates.

Metallo-β-lactamase (MBL) production was then screened by a double disk synergy test using ceftazidime and imipenem as substrates and EDTA and thiol compounds as β-lactamase inhibitors (1,12). Imipenemase activity was not inhibited by EDTA or thiol compounds, suggesting that a serine-type β-lactamase was responsible for the hydrolysis of imipenem.

Imipenem-susceptible and resistant *A. baumannii* were screened by PCR for the *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub>, *bla*<sub>SPM-1</sub>, *bla*<sub>OXA-23</sub>-like, *bla*<sub>OXA-21</sub>-like, *bla*<sub>OXA-51</sub>-like and *bla*<sub>OXA-58</sub>-like genes, as previously described (21,23). The *bla*<sub>OXA-51</sub>-like gene was the only one detected, even in imipenem-susceptible strain, confirming global reports of the intrinsic presence of class D carbapenemase in *A. baumannii* (10,20). IS<sub>Aba</sub>1 was also found by PCR, but the insertion sequence was not upstream of the *bla*<sub>OXA-51</sub>-like gene in any isolate (21).

Alterations in permeability were evaluated by outer membrane porin (OMP) analysis in imipenem susceptible (MIC 0.5 mg/L) and resistant *A. baumannii* isolates. The OMP fractions were prepared by the N-Lauryl-sarcosinate method (5). Total OMP concentration was measured according to Bradford (3). OMP profiles were analyzed by SDS-PAGE and showed absence of an expected 22 kDa fraction in the imipenem-resistant isolates.

*A. baumannii* is recognized as playing a significant role in the colonization and infection of hospitalized patients, especially those in critical care environments. The carbapenemases, such as imipenem, have been widely used to treat infections caused by MDR *A. baumannii* clinical isolates, nevertheless, regrettably, carbapenem-resistant *A. baumannii* clinical isolates have become more prevalent. In this respect, impermeability or drug inactivation by carbapenemases belonging to metallo-beta-lactamase class B or some class D OXA-type enzyme subgroups have been described as major causes of resistance.

Additionally, a decrease in outer membrane permeability has been associated with resistance to carbapenems in *A. baumannii* clinical strains (15). It was associated to with the loss of 29 kDa OMP (13), 31-36kDa (5), 25/29 kDa corresponding to the so-called CarO (2,5,18,14). Thus, isolates with weak OXA carbapenemases could be required to bear additional co-determinants of resistance, in particular, the absence of outer-membrane proteins as demonstrated by Costa et al. (5), whose resistant isolates had acquired two β-lactamases and had also lost a protein of 31-36 kDa. Bou et al. (2) report a multiresistant isolate that produce OXA-24 with reduced expression of two proteins 22kDa, the same lacked protein in our isolate, and 33kDa.

At the ICU from HU-USP, the outbreak involved eight cases of infection by a single RAPD-PCR clone. Carbapenem resistant phenotype was related to the lack of a 22 kDa OMP and the presence of *bla*<sub>OXA-51</sub>-like β-lactamase genes. Although *bla*<sub>OXA-51</sub>-like β-lactamase genes were the only ones identified, further studies are necessary to understand the role of these genes like resistance mechanism.

Table 1. Antibiotic susceptibilities of the *Acinetobacter baumannii* strains in this study.

| Case   | Origin (Specimen) | Isolation date | Hospital unit | Antimicrobial susceptibility |
|--------|------------------|---------------|--------------|-----------------------------|
| Case 1 | Urine            | 05/09/2005    | Adult ICU    | none                        |
| Case 2 | Tracheal secretion | 11/01/2006   | Adult ICU    | ART                         |
| Case 3 | Blood            | 12/01/2006    | Adult ICU    | SAM, ART                    |
| Case 3 | Blood            | 19/01/2006    | Adult ICU    | SAM                         |
| Case 4 | Abdominal secretion | 20/01/2006   | Adult ICU    | SAM                         |
| Case 5 | Tracheal secretion | 24/01/2006   | Adult ICU    | SAM                         |
| Case 5 | Blood            | 31/01/2006    | Adult ICU    | SAM                         |
| Case 6 | Vaginal secretion | 02/02/2006   | Adult ICU    | SAM, FEP                    |

Table captions: All strains were tested by Kirby Bauer method for: PIP, piperacillin; TZP, piperacillin/tazobactam; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; ATM, aztreonam; IMP, imipenem; MEM, meropenem; CIP, ciprofloxacin; AMK, Amikacin; GEN, Gentamicin; SXT, Trimethoprim/Sulfamethoxazole; ART, aztreonam; SAM, ampicillin/sulbactam; ICU, intensive care unit.
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RESUMO

Caracterização de cepas de Acinetobacter baumannii durante um surto de infecção hospitalar

Foram isoladas 19 cepas monoclonais de 8 pacientes da unidade de terapia intensiva, resistentes aos carbapenêmicos. Todas as cepas apresentaram o gene blaOXA-51-like e por análise do perfil de proteínas de membrana notou-se ausência da fração de 22 kDa, sugerindo a combinação de dois mecanismos de resistência aos carbapenêmicos.

Palavras-chaves: Acinetobacter, blaOXA-type genes, carbapenemases, infecção hospitalar

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