Carbapenem-resistant *Acinetobacter baumannii* contamination in an intensive care unit

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**Abstract**

**Introduction:** *Acinetobacter baumannii* is a major pathogen causing infections in intensive care units (ICUs). In this study, we aimed to evaluate the presence of *A. baumannii* in an ICU environment and gloves from ICU workers and to characterize the antimicrobial resistance of the isolates in comparison with those isolated from ICU patients at the same hospital. **Methods:** ICU samples were collected from March to November 2010. Isolates biochemically characterized as *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex were evaluated by PCR targeting the 16S rDNA and *bla* _OXA-51_ genes. Antimicrobial susceptibility was determined using the disk diffusion method, and carbapenem-resistant isolates were also evaluated for the minimum inhibitory concentration of imipenem using broth microdilution. The presence of the *bla* _OXA-23_ gene was evaluated in isolates with reduced susceptibility to carbapenems. **Results:** *A. baumannii* was detected in 9.5% (84) of the 886 samples collected from the ICU environment, including from furniture, medical devices, and gloves, with bed rails being the most contaminated location (23.8%; 20/84). Multidrug-resistant (MDR) *A. baumannii* was found in 98.8% (83/84) of non-clinical and 97.8% (45/46) of clinical isolates. Reduced susceptibility to carbapenems was detected in 83.3% (70/84) of non-clinical and 80.4% (37/46) of clinical isolates. All isolates resistant to carbapenems harbored *bla* _OXA-23_. **Conclusions:** We found a strong similarity between the antimicrobial susceptibility profiles of non-clinical and clinical *A. baumannii* isolates. Such data highlight the ICU environment as a potential origin for the persistence of MDR *A. baumannii*, and hence the ICU may be a source of hospital-acquired infections caused by this microorganism.

**Keywords:** *Acinetobacter baumannii*. Antimicrobial resistance. Carbapenems. Hospital environmental contamination. Hospital infections.

**INTRODUCTION**

*Acinetobacter baumannii* is an important opportunistic pathogen commonly associated with several health care-associated infections that affect debilitated patients admitted to intensive care units (ICUs)[1,2]. Besides being able to survive in dry environments for a long time, this microorganism can spread by air, water droplets over short distances, the peeling skin of colonized patients[3], and the hands of hospital workers. Hands carrying pathogens are the main source of the spread of infectious agents, even though hand hygiene is a simple, standardized, and low-cost measure[4,6]. Furthermore, infections caused by *A. baumannii* in ICUs may be related to the lack of environmental surface cleanliness and the continuous use of medical devices, such as endotracheal tubes and intravascular and urinary catheters, which lead patients to be more susceptible to intensive handling by healthcare workers[7].

The persistence of *A. baumannii* in a hospital environment exposes it to constant selective pressure imposed by antibiotics, which, in addition to its remarkable ability to acquire resistance determinants, has led to the emergence of multidrug-resistant (MDR) *A. baumannii*[8]. Resistance to carbapenems has been reported worldwide[9-11], causing special concern because it is the chosen drug to treat infections caused by *A. baumannii*. Carbapenem resistance in *A. baumannii* is mainly mediated by the production of class D β-lactamases (oxacillinases)[12], particularly via the acquisition of genes encoding OXA-23, OXA-24/40, OXA-58, OXA-143, and OXA-51, which are intrinsically located on the chromosome[13,14]. The *bla* _OXA-23_ gene was originally identified on the chromosome of *Acinetobacter radioresistens*[15] and is currently widespread in *A. baumannii*, where it is associated with different genetic structures in the chromosome and plasmids[10,16,17]. In this context, this study evaluated the presence of *A. baumannii* in an ICU environment and gloves from ICU workers, characterized the antimicrobial resistance of the isolates obtained from different ICU locations, and compared these with clinical isolates from patients in the same ICU.
Methods

Samples

Samples were collected weekly during the period from March to November 2010 in an adult ICU of a 603-bed university hospital in Porto Alegre, Southern Brazil, resulting in 886 samples from the hospital environment (n = 858) and from the gloves (n = 28) of healthcare workers. Floor samples (n = 33) from hospital rooms were collected using a sterile drag swab (4” × 4” gauze pads) moistened with 0.1% saline peptone, which was rolled over an area of approximately 8m². The gloves were collected and transported in sterilized plastic bags, and remaining samples were collected with a sterile swab moistened with 0.1% saline peptone, which was rolled once over the entire exposed surface to be sampled. All samples were collected by the same individual. The Department of Microbiology of the Clinical Pathology Laboratory of the hospital provided 46 strains of Acinetobacter calcoaceticus-Acinetobacter baumannii complex from the following clinical specimens: biliary secretion (n = 1), bronchial lavage (n = 5), bronchoalveolar lavage (n = 2), central venous access (n = 4), nasopharyngeal aspirate (n = 2), oropharyngeal secretion (n = 3), peripheral venous access (n = 4), pleural liquid (n = 1), skin biopsy (n = 1), sputum (n = 12), tracheal aspirate (n = 8), tracheal secretion (n = 1), and urine (n = 2). The clinical specimens included in the study were isolated during the same period in which the environmental samples were collected.

Isolation and identification

Swabs collected from the hospital environment were placed in 3mL BHI broth (Himedia, India) and incubated at 37°C with constant rotation (150rpm) for 24h according to Baumann18 and were then spread on MacConkey agar (Oxoid, England). An aliquot of 5mL nutrient broth was added to the inside of each glove, homogenized, and incubated for 5 min at 22-25°C. After this, 100µL was spread on blood agar (Himedia, India) and incubated at 37°C for 24h. A volume of 100µL saline peptone obtained from each floor sample was spread on blood agar and incubated at 37°C for 24h. Colonies were preliminarily screened by morphology and characterized by oxidase (Laborclin, Brazil) and triple sugar iron (Oxoid, England) tests. All tests were standardized using the reference culture A. baumannii ATCC 19606.

Isolates presenting a biochemical profile compatible with A. calcoaceticus–baumannii complex were subjected to deoxyribonucleic acid (DNA) extraction using guanidine isothiocyanate according to Rademaker and de Brujin59. Purified DNA was used as a template for polymerase chain reaction (PCR) targeting the 16S ribosomal deoxyribonucleic acid (rDNA)20 and blaOXA-51 genes to identify Acinetobacter spp. and A. baumannii, respectively. Primers targeting blaOXA-51 were designed in this study (5’-GACGGGGCAAAAAAGGCTA-3’ and 5’-GGGTCTACATCCCATCCC-3’) (Integrated DNA Technologies, USA) to amplify a 347-bp fragment. PCR amplifications were performed in a final volume of 25µL containing 1µL target DNA, 0.2mM each deoxynucleoside triphosphate, 50mM KCl, 10mM Tris-HCl (pH 8.3), 1.5mM MgCl₂, 1U Taq DNA polymerase (Invitrogen Life Technologies, USA), and 0.8µM each primer. Amplifications were carried out in a thermocycler (MiniCycler™, MJ Research, USA) using the following conditions: initial denaturation at 95°C for 5 min; followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 51°C for 1 min, and extension at 72°C for 1 min and 30 s; with a final extension at 72°C for 10 min. Positive and negative controls were included using DNA from A. baumannii ATCC 19606 and a DNA-free reaction, respectively. Amplicons were analyzed by electrophoresis using agarose gels stained with 0.5µg/mL ethidium bromide and visualized under UV radiation. The amplification products targeting blaOXA-51 from two isolates were purified with ammonium acetate and sequenced in an automated DNA sequencer (ABI 3130 XL Genetic Analyzer, Applied Biosystems, USA). Searches and alignments of nucleotide sequences were performed with the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST).

Antimicrobial susceptibility testing

The antimicrobial susceptibility of each isolate was determined using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines21. The antimicrobials tested were the following: amikacin (AMI), ampicillin-sulbactam (AMS), aztreonam (ATM), cefepime (CPM), cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRO), ciprofloxacin (CIP), doxycycline (DX), gentamicin (GEN), imipenem (IPM), levofloxacin (LVX), meropenem (MEM), minocycline (MIN), piperacillin-tazobactam (PTZ), trimethoprim-sulfamethoxazole (COT), tetracycline (TET), ticarcillin-clavulanic acid (TCC), and tobramycin (TOB). MDR was defined as the absence of susceptibility to at least one agent in ≥ 3 antimicrobial categories23.

Carbapenem-resistant isolates characterized by the disk diffusion test were also evaluated regarding the minimum inhibitory concentration (MIC) of imipenem by broth microdilution, which was interpreted according to the CLSI guidelines21. All antimicrobial susceptibility analyses were performed using Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 as reference strains for antibiotic quality control.

Detection of blaOXA-23

Isolates that showed reduced susceptibility to carbapenems were evaluated for the presence of the blaOXA-23 gene by PCR as previously described22, with the exception of the MgCl₂ concentration, which was changed to 2.5mM. In order to ensure the specificity of PCR, one amplification product targeting blaOXA-23 was purified and sequenced as described above.

Statistical analysis

Antimicrobial susceptibility data were analyzed and compared by χ² test or Fisher’s exact test. In order to analyze differences among sources (hospital non-clinical or clinical) in resistance to all antimicrobials tested, drugs were grouped into four clusters based on resistance rates (0-25%, 25-50%, 50-75%, and 75-100%) and evaluated by Cochran-Mantel-Haenszel test. The data were analyzed using SAS software version 9.4 (SAS Institute Inc., USA), and the level of significance was set at 0.05 for all tests.
RESULTS

Acinetobacter baumannii was detected in 9.5% (84) of the 886 samples collected from the ICU environment, including furniture, medical devices, and gloves, referred to as non-clinical isolates. Among the A. baumannii-contaminated samples, 4.8% (4) were isolated from the floor and 2.4% (2) were isolated from gloves. Other strains (78) were isolated mainly from bed rails (23.8%), bedside tables (10.7%), and ambu bags (8.3%). However, this microorganism could be found in many other locations in the ICU environment, such as the monitors of mechanical ventilators, valves (of oxygen, air, and vacuum), intravenous pumps, door handles, stethoscopes, biological sewage, doors, ventilators, tables for nursing staff support, bedside chairs, equipment and medical carts, biological waste, pressure gauges, overbed tables, vital sign monitors, staff clipboards (for patient information), and serum support apparatuses.

All 84 non-clinical and 46 clinical isolates biochemically compatible with the A. calcoaceticus-baumannii complex were confirmed as Acinetobacter spp. and identified as A. baumannii by 16S rDNA and bla\textsubscript{OXA-51} PCR, respectively. Two bla\textsubscript{OXA-51} amplification products were sequenced and showed at least 98% identity with other A. baumannii OXA-51 sequences deposited in GenBank (accession numbers AJ309734.2, KJ584920.1, and KJ584925.1).

The antimicrobial susceptibility profiles of the A. baumannii isolates are summarized in Table 1. The reduced susceptibility rates (i.e., the sum of full and intermediate resistance rates) of clinical and non-clinical isolates to most drugs were not significantly different (p > 0.05), with the exceptions of ceftazidime and amikacin. Furthermore, both groups of isolates showed the highest resistance rates to the same five antimicrobials (aztreonam, ceftriaxone, cefotaxime, cefepime, and ticarcillin-clavulanic acid), as well as the lowest resistance rates to doxycycline and minocycline.

The results of the clustering analysis of reduced susceptibility rates for all antimicrobials tested within each isolate source (non-clinical or clinical) are shown in Table 2, and significant differences (p < 0.05) were found among all clusters (I, II, III, and IV). The results further indicated strong similarity between the non-clinical and clinical clusters in their susceptibility to most drugs, with the exceptions of tetracycline (in clusters I and II for clinical and non-clinical isolates, respectively) and levofloxacin and ceftazidime (in clusters III and IV for non-clinical and clinical isolates, respectively). Non-clinical isolates were resistant to more antimicrobial categories than clinical isolates (p < 0.05).

In this study, 98.8% (83) of non-clinical and 97.8% (45) of clinical isolates were classified as MDR. Only one strain isolated from the floor was susceptible to all drugs tested. Among the non-clinical isolates, 49 different resistance profiles were obtained, whereas 33 were obtained from among clinical isolates. The most common profile found among all isolates presented resistance to at least 14 antimicrobials, with the

### Table 1
Reduced susceptibility rates of hospital non-clinical and clinical Acinetobacter baumannii isolates.

<table>
<thead>
<tr>
<th>Antimicrobial Drugs</th>
<th>Non-clinical isolates (n = 84)</th>
<th>Clinical isolates (n = 46)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIN</td>
<td>7 (8.3)</td>
<td>2 (4.3)</td>
<td>0.49</td>
</tr>
<tr>
<td>TCC</td>
<td>76 (89.4)</td>
<td>43 (93.5)</td>
<td>0.53</td>
</tr>
<tr>
<td>IPM</td>
<td>68 (80)</td>
<td>37 (80.4)</td>
<td>1</td>
</tr>
<tr>
<td>LVX</td>
<td>62 (72.9)</td>
<td>39 (84.8)</td>
<td>0.19</td>
</tr>
<tr>
<td>MEM</td>
<td>70 (82.3)</td>
<td>36 (78.3)</td>
<td>0.74</td>
</tr>
<tr>
<td>CAZ</td>
<td>45 (52.9)</td>
<td>35 (76.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>TET</td>
<td>34 (40)</td>
<td>10 (21.7)</td>
<td>0.05</td>
</tr>
<tr>
<td>DX</td>
<td>4 (4.8)</td>
<td>0 (0)</td>
<td>0.16</td>
</tr>
<tr>
<td>TOB</td>
<td>55 (64.7)</td>
<td>28 (60.9)</td>
<td>0.81</td>
</tr>
<tr>
<td>CTX</td>
<td>79 (92.9)</td>
<td>45 (97.8)</td>
<td>0.42</td>
</tr>
<tr>
<td>CIP</td>
<td>76 (89.4)</td>
<td>41 (89.1)</td>
<td>1</td>
</tr>
<tr>
<td>AMI</td>
<td>61 (72.6)</td>
<td>23 (50)</td>
<td>0.01</td>
</tr>
<tr>
<td>CRO</td>
<td>82 (96.5)</td>
<td>45 (97.3)</td>
<td>1</td>
</tr>
<tr>
<td>CPM</td>
<td>77 (90.6)</td>
<td>44 (95.6)</td>
<td>0.49</td>
</tr>
<tr>
<td>PTZ</td>
<td>73 (85.9)</td>
<td>40 (86.9)</td>
<td>1</td>
</tr>
<tr>
<td>ATM</td>
<td>81 (95.3)</td>
<td>46 (100)</td>
<td>0.30</td>
</tr>
<tr>
<td>GEN</td>
<td>61 (71.8)</td>
<td>32 (69.6)</td>
<td>0.95</td>
</tr>
<tr>
<td>AMS</td>
<td>62 (72.9)</td>
<td>29 (63)</td>
<td>0.33</td>
</tr>
<tr>
<td>COT</td>
<td>74 (87.1)</td>
<td>36 (78.3)</td>
<td>0.29</td>
</tr>
</tbody>
</table>

MIN: minocycline; TCC: ticarcillin-clavulanic acid; IPM: imipenem; LVX: levofloxacin; MEM: meropenem; CAZ: ceftazidime; TET: tetracycline; DX: doxycycline; TOB: tobramycin; CTX: cefotaxime; CIP: ciprofloxacin; AMI: amikacin; CRO: ceftriaxone; CPM: ceftime; PTZ: piperacillin-tazobactam; ATM: aztreonam; GEN: gentamicin; AMS: ampicillin-sulbactam; COT: trimethoprim-sulfamethoxazole. *Reduced susceptibility rate is the sum of the full and intermediate resistance rates.
most (11.8%) prevalent profile among non-clinical isolates characterized by reduced susceptibility to the following 17 drugs: TCC, IPM, LVX, MEM, CAZ, TET, TOB, CTX, CIP, AMI, CRO, CPM, PTZ, ATM, GEN, AMS, and COT. Among the clinical isolates, there were two other profiles that were more prevalent (10.9% each). One of them presented reduced susceptibility to the same antimicrobials found in the predominant profile of non-clinical isolates, except for the absence of resistance to tetracycline, while the other presented only two differences (absence of resistance to amikacin and tetracycline).

Reduced susceptibility to carbapenems (imipenem and meropenem) was detected among 83.3% (70) of non-clinical and 80.4% (37) of clinical isolates, and no significant differences were found in the rates of reduced susceptibility to imipenem (p = 1) or meropenem (p = 0.74) between the two groups of isolates. The MIC of imipenem in carbapenem-resistant isolates ranged from 8µg/mL to 64µg/mL, and all A. baumannii isolates resistant to carbapenems harbored the blaOXA-23 gene. The specificity of the PCR amplification targeting blaOXA-23 was confirmed by sequencing one amplicon that showed at least 96% identity with other blaOXA-23 sequences deposited in GenBank (GenBank accession numbers JN207493.1, GQ861438.1, and AJ132105.1).

### DISCUSSION

The ability of A. baumannii to survive for a long time in the hospital environment and to readily acquire antimicrobial resistance has driven increased concern over this species in the medical community. In this study, we evaluated the presence of this microorganism in the ICU environment, including furniture, medical devices, and gloves, as well as characterizing the antimicrobial resistance profiles of these isolates and comparing them with those obtained from clinical sources in the same hospital and over the same period. A. baumannii was detected in a wide variety of samples collected from the ICU rooms, indicating the spread of this microorganism across the hospital locations evaluated. The bed rail was the most commonly contaminated location, suggesting it may act as a possible environmental reservoir of A. baumannii in the ICU. This reinforces the importance of disinfecting and cleaning protocols for hospital medical devices and surfaces to control and reduce the cross-transmission of A. baumannii.

In addition to the ICU environment and medical devices, the hands of healthcare workers are an important mode of dissemination for many pathogens, acting as a source of cross-infection and re-infection. Thus, we analyzed the insides of gloves used by ICU workers as an indicator of hand contamination, and we found that 7.4% were contaminated with A. baumannii. Hands may be even more contaminated than this estimate, since the analysis of gloves may underestimate the real prevalence of bacteria on the hands. It is also important to note that as only the inside of each glove was sampled, we cannot evaluate the possible transmission of A. baumannii via gloves, but this may certainly be occurring, especially considering the presence of A. baumannii in various locations in the ICU.

The surfaces of the hospital environment have been proposed to act as reservoirs of MDR bacteria for patient-to-patient transmission. In this context, we found a strong similarity in the antimicrobial susceptibility profiles of non-clinical and clinical A. baumannii isolates, reinforcing the idea that many locations in ICU rooms must be observed with considerable concern. Both clinical and nonclinical isolates showed high rates of antimicrobial multidrug resistance, especially when compared to other studies of clinical isolates that used the same criteria to define MDR.

The low susceptibility rates to carbapenems detected among both clinical and non-clinical isolates from the ICU environment indicates a significant limitation in the treatment options for Acinetobacter infections, particularly when resistance is mediated by mobilizable determinants that can be promptly transferred and spread between isolates. All carbapenem-resistant A. baumannii isolated in this study harbored the blaOXA-23 gene, as has been also reported by other authors worldwide. The high prevalence of blaOXA-23 may be due to its horizontal transfer, since it has been also identified on plasmids. Moreover, the presence of blaOXA-23 may constitute a larger problem, as in addition to its ability to hydrolyze carbapenems, OXA-23 is involved in the resistance to ticarcillin and amoxicillin, which not only affects patient care but possibly also increases resistance selection rates.

In contrast, minocycline and doxycycline are therapeutic options for the treatment of carbapenem-resistant A. baumannii infections. Minocycline and doxycycline have already been recommended for the treatment of MDR infections.
found to be effective against *Acinetobacter* spp., with isolates presenting susceptibility rates of 80-90%37,38. In this sense, minocycline has been used successfully for the treatment of wound infections and ventilator-associated pneumonia caused by MDR *A. baumannii*29.

The high resistance rates found among non-clinical isolates are of great concern since they identify the ICU environment as a possible major reservoir of resistant *A. baumannii* and, consequently, a source of healthcare-associated infections. Thus, it should be emphasized that hospital infection prevention and control measures, such as intensified hand hygiene adherence, patient cohorts or isolation, active surveillance, and especially better cleaning and disinfection of the environment may prevent outbreaks of MDR bacteria in ICUs.

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**Conflicts of interest**

The authors declare that there is no conflict of interest.

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