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Carbapenem-resistant *Acinetobacter baumannii* in Brazil: susceptibility profile and diversity of oxacillinases

Acinetobacter baumannii *resistente aos carbapenêmicos no Brasil: perfil de suscetibilidade e diversidade de oxacilinases*

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ABSTRACT

Introduction: The *Acinetobacter calcoaceticus-baumannii* (ABC) complex includes five species, and the *A. baumannii* is the most important of them because it carries mechanisms of carbapenems resistance, especially the oxacillinases. **Objectives:** The objectives of this study were to identify the species of the ABC complex, to evaluate the susceptibility profile and to investigate the presence of oxacillinases in carbapenems-resistant isolates from four Brazilian States. **Methods:** In the study period, 92 isolates from Rio Grande do Sul (RS), Rio de Janeiro (RJ), Paraná (PR) and São Paulo (SP) were collected. The isolates were identified by matrix-assisted laser desorption ionization-time of fight mass spectrometry (MALDI-TOF MS) and sequencing of *gyr*B gene. Evaluation of susceptibility was performed by disk diffusion and broth microdilution. The presence of oxacillinases was performed by in-house multiplex polymerase chain reaction (PCR). **Results:** Ninety-one (99%) isolates were identified as *A. baumannii* by MALDI-TOF and sequencing. The majority of isolates (56; 61%) showed resistance to the six antimicrobial agents tested. Three isolates were resistant to polymyxin B [minimum inhibitory concentration (MIC) ≥ 4 µg/ml). Eighty (87%) isolates were positive to OXA-23-like, and twelve (13%) isolates to OXA-24-like. **Conclusion:** Our findings confirm the knowledge about the dissemination of the $bla_{\text{OXA-23}}$ gene in Brazil and suggest the recent emergence and spread of $bla_{\text{OXA-24}}$ gene, since it was identified in three of the four sampled states.

Key words: *Acinetobacter baumannii*; carbapenems; beta-lactamases.

INTRODUCTION

The *Acinetobacter baumannii-calcoaceticus* (ABC) complex includes the species *A. baumannii*, *A. calcoaceticus*, *A. pittii*, *A. dijkshoorniae* and *A. nosocomialis*[1, 2]. The conventional methods used in the routine laboratory are unable to distinguish between ABC species. Recently, the mass spectrometry matrix-assisted laser desorption ionization-time of fight mass spectrometry (MALDI-TOF MS) was implemented in clinical microbiology laboratory for identification of specie level[3]. The ABC complex has become increasingly important due to the carbapenems resistance mainly associated with production of carbapenemases. This isolates have been reported in many regions of the world associated with high morbidity and mortality[4].

The most prevalent carbapenemase in *A. baumannii* are OXA-carbapenemases, and the less frequently metallo-beta-lactamases. The OXA-carbapenemases already identified in the ABC complex are divided into 6 subfamilies: OXA-51-like, OXA-23-like, OXA-24-like, OXA-58-like, OXA-143 and OXA-235. In Brazil, strains producing enzymes of all these families have already been reported, except the OXA-235[5, 6]. The aim of this study was to evaluate the susceptibility profile and to determine the prevalence of oxacillinases in carbapenems-resistant ABC isolates from four Brazilian states: Rio Grande do Sul (RS), Rio de Janeiro (RJ), Paraná (PR), and São Paulo (SP).

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METHODS

From July 2013 to October 2013 a total of 92 ABC isolates resistant to imipenem and meropenem were recovered as follows: 31 (34%) isolates from Porto Alegre (RS), 28 (30%) from Rio de Janeiro (RJ), 21 (23%) from Curitiba (PR), and 12 (13%) from São Paulo (SP). Only one isolate from each patient was included in this study.

Identification of species of the ABC complex

The isolates were first identified by the Vitek® II system (BioMérieux, France). MALDI-TOF MS system (Bruker Biotyper® system – version 3.1) and *gyr*B gene sequencing were performed to confirm the identification at the specie level. To perform MALDI-TOF, a bacterial colony was placed in the polymeric matrix and inserted into the machine. The results were interpreted according to the manufacturers' recommendations. PCR amplification of *gyr*B was performed with a Applied Biosystems® Veriti® 96-Well Fast Thermal Cycler by using PCR reaction 5X Phusion HF buffer each one containing deoxynucleoside triphosphates 1.0 µl, primers at a concentration of 10 pmol/µl, deoxyribonucleic acid (DNA) 2.6 µl and 0.5 µl Phusion DNA polymerase (Perkin-Elmer) in a total volume of 50 µl[7]. A total of 35 amplification cycles were performed with denaturation of DNA template at 98ºC for 10 s, annealing at 57ºC for 30 s and extension at 72ºC for 10 min. Amplified products were purified by using QIAquick® (Qiagen) for sequencing. The sequences produced were compared with those available on the GenBank and subsequently aligned using the BioEdit software version 7.1.3.

Susceptibility profile

The antimicrobial susceptibility profile was performed by disk diffusion for amikacin, Ampicillin/sulbactam, cefepime, ceftazidime, piperacillin/tazobactam, gentamicin (Oxoid®) and by broth microdilution to polymyxin B and tigecycline. The results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) 2016, except for tigecycline wich we used the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The chi-square test or Fisher's exact test was used to statistical analyses with significance at 5% ($p \leq 0.05$).

Oxacillinases genes

The presence of oxacillinases genes ($bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$ and $bla_{\text{OXA-143-like}}$) were evaluated by multiplex polymerase chain reaction (PCR) using specific primers as previously described[8].

RESULTS

Identification of species of the ABC complex

According to *gyr*B gene sequencing, all isolates were identified as *A. baumannii*. MALDI-TOF Bruker® correctly identified 91 (99%) isolates (score > 2.0) as *A. baumannii*. One isolate (1%) had inconclusive result.

Susceptibility profile

The majority of isolates (56; 61%) showed resistance to the six antimicrobial agents tested. However, some differences among the states were identified: the isolates from São Paulo (SP) were less resistant to gentamicin (16.7%; $p = 0.001$); isolates from Rio Grande do Sul (RS) were more resistant to amikacin (51.6%; $p = 0.005$) and isolates from Rio de Janeiro (RJ) were more resistant to ceftazidime (75%; $p = 0.023$) (**Table**). Three isolates were resistant to polymyxin B [minimum inhibitory concentration (MIC) ≥ 4 µg/ml] and the MIC_{50}/MIC_{90} were 1.0 and 2.0 µg/ml, respectively. All isolates were susceptible to tigecycline (MIC_{50}/MIC_{90} were 0.5 and 1.0 µg/ml).

Oxacillinases genes

$Bla_{\text{OXA-51-like}}$ was identified in all isolates; 80 (87%) of the isolates presented $bla_{\text{OXA-23-like}}$. It was also possible to identify 12 (13%) isolates producing $bla_{\text{OXA-24-like}}$ from Rio Grande do Sul (RS), Paraná (PR) and São Paulo (SP). The $bla_{\text{OXA-58-like}}$ and $bla_{\text{OXA-143-like}}$ genes were not detected (Table).

TABLE – Resistance profile and distribution of oxacillinase genes among *A. baumannii* isolates from four Brazilian States

Antibiotic	RS (*n* = 31)	RJ (*n* = 28)	SP (*n* = 12)	PR (*n* = 21)	Total (*n* = 92)
Amikacin	16 (51.6)[a]	17 (60.7)	11 (91.7)	19 (90.5)	63 (68.5)
Ampicillin/ sulbactam	31 (100)	25 (84.3)	11 (91.7)	21 (100)	88 (95.6)
Cefepime	30 (96.8)	24 (85.7)	12 (100)	19 (90.5)	84 (91.3)
Ceftazidime	26 (83.9)	21 (75)	12 (100)	21 (100)	80 (86.9)
Piperacillin/ tazobactam	31 (100)	28 (100)	12 (100)	21 (100)	92 (100)
Gentamicin	20 (64.5)	14 (50)	2 (16.7)	18 (85.7)	54 (58.7)
Polymyxin[b]	1/2	1/2	2/2	0.75/1	
Oxacillinases[c]					
$bla_{\text{oxa-23-like}}$	30 (99)	28 (100)	9 (75)	13 (62)	80 (87)
$bla_{\text{oxa-24-like}}$	1 (3.2)	0	3 (25)	8 (38)	12 (13)

[a]: number (percentage) of isolates presenting resistance; [b]: MIC50/MIC_{90} to polymyxin (µg/ml); [c]: all isolates were positive for $bla_{\text{oxa-51-like}}$ *gene; none of the isolate was positive for* $bla_{\text{oxa-58-like}}$ *or* $bla_{\text{oxa-143-like}}$ *genes.*

RS: Rio Grande do Sul; RJ: Rio de Janeiro; SP: São Paulo; PR: Paraná; MIC: minimum inhibitory concentration.

DISCUSSION

The species prevalence and the resistance profile of the ABC complex may vary according to the geographic region. However, *A. baumannii* is the most common specie associated with nosocomial infections and high resistance to carbapenems worldwide[9]. In this study, all isolates were confirmed as *A. baumannii* by the methods used (MALDI-TOF and *gyr*B sequencing) and MALDI-TOF proved to be a quick and easy method for the identification of *A. baumannii* compared to the molecular method, obtaining high agreement score, as already reported[10].

In our study, two isolates of *A. baumannii* presented polymyxin MIC of 8 mg/ml and one isolate showed MIC of 4 mg/ml which are considered resistant to polymyxin. Despite the low prevalence of polymyxin resistance in our study, phenotypes related to polymyxin resistance are worrisome as they may impair antimicrobial therapy[11]. On the other hand, high rates of antimicrobial resistance were observed in the four states and this situation highlights the few therapeutic options to treat severe infections cause of *A. baumannii*[2, 4].

The main enzyme associated with carbapenem resistance in *A. baumannii* isolates is OXA-23[5]. In this study, the four Brazilian states confirmed the high prevalence of OXA-23 associate with carbapenems resistance. This finding corroborate data already reported in a study that evaluated the spread of OXA-23 in five geographic regions in Brazil, in which the prevalence of carbapenems-resistant OXA-23-positive *A. baumannii* was 94.2%[12].

In addition, 12 (13%) isolates were identified as OXA-24-like-producing strains from three states [Rio Grande do Sul (RS), Paraná (PR), and São Paulo (SP)] suggesting the beginning of spread of this gene in Brazil. To date, there are few reports of OXA-24/40-like in Brazil, but they are all associated with the multidrug resistance phenotype[13, 14].

In conclusion, we observed high rates of antimicrobial resistance and dissemination of OXA-23 in all evaluated states. Furthermore it should be noted that this report evidences the dissemination of the enzyme OXA-24-like in different Brazilian states, suggesting the emergence of this novel type of enzyme in *A. baumannii* strain in our country.

RESUMO

Introdução: *O complexo* Acinetobacter calcoaceticus-baumannii *(ABC) inclui cinco espécies, sendo* A. baumannii *a mais importante clinicamente por carrear muitos mecanismos de resistência aos carbapenêmicos, sobretudo as oxacilinases.* ***Objetivos:*** *Os objetivos deste estudo foram identificar as espécies do complexo ABC, avaliar o perfil de suscetibilidade e investigar a presença de oxacilinases em isolados resistentes aos carbapenêmicos provenientes de quatro estados brasileiros.* ***Métodos:*** *No período do estudo, foram coletados 92 isolados do Rio Grande do Sul (RS), do Rio de Janeiro (RJ), do Paraná (PR) e de São Paulo (SP). Os isolados foram identificados por* matrix-assisted laser desorption ionization-time of fight mass spectrometry *(MALDI-TOF MS) e sequenciamento do gene* gyr*B. A avaliação da suscetibilidade foi realizada por disco-difusão e microdiluição de caldo. A presença de oxacilinases foi realizada por reação em cadeia da polimerase (PCR)* multiplex in house*.* ***Resultados:*** *Noventa e um (99%) isolados foram identificados como* A. baumannii *por MALDI-TOF e pelo sequenciamento. A maioria dos isolados (56; 61%) apresentou resistência aos seis agentes antimicrobianos testados. Três isolados foram resistentes à polimixina B [concentração inibitória mínima (CIM) ≥ 4 µg/ml). Oitenta (87%) isolados foram positivos para OXA-23 e 12, (13%) para OXA-24.* ***Conclusão:*** *Nossos resultados confirmam a disseminação do gene* bla_{OXA-23} *no Brasil e sugerem a recente emergência e disseminação do gene* bla_{OXA-24}*, uma vez que ele foi identificado em três dos quatro estados amostrados.*

Unitermos: Acinetobacter baumannii*; carbapenêmicos; betalactamases.*

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