

Molecular detection on the bla_{KPC} gene in the extensively prevalent nosocomial isolates of drug resistant *Acinetobacter spp* at hospital in southeastern region of Brazil

Detecção molecular do gene bla_{KPC} dentre isolados nosocomiais de *Acinetobacter spp* extensivamente resistente a drogas isolados em um hospital na região sudeste do Brasil

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ABSTRACT:

Acinetobacter baumannii is a multiple drug resistance opportunistic pathogen who has been responsible for severe outbreaks in nosocomial environment. *A. baumannii* possesses several mechanisms of antibiotic resistance that make it resistant to β-lactamics, mainly due to OXA-type carbapenemases expression, and others antibiotics. The *bla*_{KPC} gene is an antibiotic resistance gene typical of *K. pneumoniae* and *P. Aeruginosa*. In 2009, bla_{KPC} was found in *A. baumannii* isolates in Puerto Rico and later in São Luis, Brazil. The aim of this study is to screening the presence of *bla*_{KPC} in nosocomial *A. baumannii* isolates from hospital environment monitoring. The study was approved by the Research Ethics Committee of the Universidade Estadual de Montes Claros under number 852.002/2014. Thirty-one DNA samples of *Acinetobacter* spp extensively drug resistant isolated from hospitalized patients in the southeast of Minas Gerais state – Brazil and kept at -20°C were used for PCR amplification of *bla*_{KPC} gene detection. The expected amplicon size of 876 bp was visualized on 1.5% agarose gel stained with ethidium bromide and photographed. Twenty-eight DNA samples of *Acinetobacter* spp. tested negative to *bla*_{KPC} gene and three isolates showed PCR expected amplicon of 876 bp. The presence of *K. pneumoniae* and *Acinetobacter* spp. in the same hospital environment could results in gene transfer between them. Also, the widespread and increasing uses of carbapenems could accelerate the spread of carbapenems-resistant strains by transferring resistance genes among the enterobacteriaceae and related microorganisms, impacting the public health system.

Keywords: Acinetobacter baumannii, multi-resistance, carbapenemase, blaker

RESUMO:

Acinetobacter baumannii é um patógeno oportunista de resistência a múltiplas drogas que tem sido responsável por surtos graves em ambiente nosocomial. A. baumannii possui vários mecanismos de resistência aos antibióticos que o tornam resistente aos β -lactâmicos, principalmente devido à expressão de carbapenemases do tipo OXA, e a outros antibióticos. O gene $bla_{\rm KPC}$ é um gene de resistência a antibióticos típico de *K. pneumoniae* e *P. aeruginosa*. Em 2009, o $bla_{\rm KPC}$ foi encontrado em isolados de *A. baumannii* em Porto Rico e depois em São Luís, Brasil. O objetivo deste estudo foi rastrear a presença de $bla_{\rm KPC}$ em *A. baumannii* nosocomiais isolados de ambiente hospitalar. O estudo foi aprovado pelo Comitê de Ética em Pesquisa da Universidade Estadual de Montes Claros sob o número 852.002/2014. Trinta e uma amostras de DNA de *Acinetobacter* spp. extensivamente resistentes a drogas isoladas de pacientes hospitalizados no sudeste de Minas Gerais e mantidas a -20°C foram utilizadas para detecção do gene *bla_{KPC}* por PCR. O tamanho esperado do amplicon era de 876 pb, o qual foi visualizado em gel de agarose a 1,5%, corado com brometo de etídio e fotografado. Vinte e oito amostras de DNA de *Acinetobacter* spp. foram negativos e três isolados mostraram o fragmento de 876 pb, correspondente ao gene *bla_{KPC}*. A presença de *K. pneumoniae* e *Acinetobacter* spp. no mesmo ambiente hospitalar e o uso crescente e generalizado de carbapenêmicos pode acelerar a disseminação de cepas resistentes aos mesmos pela transferindo genes de resistência entre as enterobactérias e microrganismos relacionados, com impacto no sistema público de saúde.

Palavras – chave: Acinetobacter baumannii, multirresistente, carbapenemase, bla_{KPC}

INTRODUCTION

Acinetobacter baumannii is a multiple drug resistance (MDR) opportunistic pathogen who in recent decades has been responsible for severe outbreaks in nosocomial environment (FONSECA et al. 2013; PEREZ et al. 2010; PELEG, SEIFERT and PATERSON 2008). Intensive care units, burn therapy units and long-term care units are the most associated with infections caused by A. baumannii and the most affected patients are the hospitalized one who are often elderly, debilitate, severe ill or immunocompromised (PEREZ et al. 2010; HAMMOUDI et al. 2015). A. baumannii can cause nosocomial pneumonia, urinary tract infections, endocarditis, meningitis and sepsis (MARTINEZ et al. 2016). Since its high antibiotic resistance and the precarious health condition of the patients, these infections are difficult to treat and have a mortality rate of 50% (CARVALHO et al. 2016). For this reason, the study of MDR pathogens and the control of their diffusion in a hospital environment is worldwide considered a major issue (PELEG, SEIFERT, and PATERSON 2008; CARVALHO et al. 2016).

A. baumannii possesses several mechanisms of antibiotic resistance that make it resistant to β -lactamics, aminoglycosides, fluoroquinolones and tetracyclines. These mechanisms are due to chromosomal and plasmid-acquired β -lactamases, overexpression of efflux pumps, porins down regulation, aminoglycosides modifying enzymes, gyrase and topoisomerase mutations (HANDAL et al. 2017; BRATU et al. 2008). Among them, the major antibiotic resistance mechanism is due to β -lactamases expression. They are classified by Ambler in four families: classA, Extended-Spectrum β -lactamases; classB, metal- β -lactamases (MBLs); classC, Acinetobacter-derived cephalosporinases; classD, carbapenem-hydrolyzing β -lactamases known as OXA-type carbapenemases (CARVALHO et al. 2016).

In *A. baumannii* the major part of antibiotic resistance is due to OXA-type carbapenemases expressions. These have a spectrum breadth unrivalled by other β -lactamases (QUEENAN and BUSH 2007; AZIMI et al. 2015). The genes belonging to the OXA-type family were grouped into six subgroups: bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-51}, bla_{OXA-58}, bla_{OXA-143} and bla_{OXA-235}. In particular, bla_{OXA-23} is the most diffused carbapenemases coding gene in Brazil (FONSECA et al. 2013; CARVALHO et al. 2016).

Recently two other genes coding for carbapenemases have been found in isolates of *A. baumannii*, although they are not typical of this pathogen: $bla_{NDM'}$ which encodes for a classB β -lactamase and bla_{KPC} which encodes for a classA β -lactamase (BERRAZEG et al. 2014; ROBLEDO et al. 2010; RIBEIRO et al. 2016). Their association with mobile genetic elements, transposons, suggests that both can be horizontally transferred between different pathogens (MARTINEZ et al. 2016; QUEENAN and BUSH 2007; ROBLEDO et al. 2010; RIBEIRO et al. 2016).

The bla_{NDM-1} gene is a chromosomal gene original from plant pathogens, such as *Pseudoxanthomonas*, and pathogens from the same environment. In 2007 there was the first recorded and published case of an infection caused by *K. pneumoniae* with bla_{NDM-1} gene. Since then, a worldwide spread of this gene has been recorded in other community or nosocomial pathogens, including in *A. baumannii* (BERRAZEG et al. 2014).

The $bla_{\rm KPC}$ gene is an antibiotic resistance gene typical of *K*. *pneumoniae* and *P*. *aeruginosa* and is localized on



plasmid-borne transposon Tn4401. For the first time in 2009 $bla_{\rm KPC}$ was found in *A. baumannii* isolates in Puerto Rico and later in São Luis, Brazil (ROBLEDO et al. 2010; RIBEIRO et al. 2016). *A. baumannii bla*_{KPC}-positive specimens were collected in intensive care units and general medicine units of the same hospital where, at the same time, other infections caused by $bla_{\rm KPC}$ -positive *K. pneumoniae* were found. The coincidence about common hospital environment and its association with transposon Tn4401 makes to thinking that it could be possible for this gene to be horizontally transferred between different pathogens present simultaneously in the same environment.

It is believed that the acquisition of further antibiotic resistance mechanisms in *A. baumannii* is a worldwide issue and probably not only limited to this pathogen (ROBLEDO, AQUINO, and VÁZQUEZ 2011). The aim of this study is to screening the presence of $bla_{\rm KPC}$ in nosocomial *A. baumannii* isolates to pursue a hospital environment monitoring.

MATERIALS AND METHODS

CARVALHO et al. (2014) isolated extensively drug resistant (XDR) *Acinetobacter* spp from hospitalized patients at the hospital located in the southeast of Minas Gerais state - Brazil. The total DNA samples were kept in the freezer at -20°C in the laboratory. Thirty-one samples containing enough material for detection of *bla*_{KPC} gene by PCR were selected for this study.

Primers described by Yigit et al. (2001) were used to screen for the $bla_{\kappa PC}$ gene; they generated an amplicon size of 876 bp. All primers were synthesized by Integrated DNA Technology (USA). The amplifications were performed in a single PCR containing 2× GoTaq Green Master Mix[®] (Promega, USA), 2.5 mM MgCl₂, 10 µM of each primer, and 50 ng of bacterial DNA in a final volume of 50 µL. The amplification conditions used were the same as those reported by the authors listed for each primer. The amplicons were visualized on a 1.5% agarose gel stained with ethidium bromide and photographed. As a positive control for the PCR, two nosocomial strain of K. pneumoniae previously identified by a Brazilian reference laboratory (Ezequiel Dias Foundation), encoding $bla_{\rm KPC}$, was used. Distilled and sterilized water was used as a negative control.

The research was performed in accordance with the Brazilian National Council of Research Ethics (CONEP) Resolution 466/12 and the Declaration of Helsinki. The study was approved by the Research Ethics Committee of the Universidade Estadual de Montes Claros, affiliated with the CONEP, under number 852.002/2014

RESULTS

Twenty-eight DNA samples of *Acinetobacter* spp. in this study (n = 31) tested negative to bla_{KPC} gene (Figure 1A and 1B) and three isolates (ISO 15, ISO17 and ISO21) showed PCR expected amplicon of 876 bp, corresponding to the bla_{KPC} gene (Figure 1B).

DISCUSSION

Acinetobacter spp. has been reported as extensively drug-resistant, including Meropenem and Imipenem antibiotics (CARVALHO et al. 2016) and it has acquired a huge genetic repertoire via horizontal gene transfer, resulting in virulent and resistant microorganism to any environment (HANDAL et al. 2017), especially in the hospital due to the drug pressure and patient profile (CARVALHO et al. 2016).

Since, the Bla_{KPC} belonging first of all to Klebsiella pneumoniae and it is located in the mobile plasmid containing the transposon Tn4401 (MARTINEZ et al. 2016), the presence of K. pneumoniae and Acinetobacter spp. in the same hospital environment could results in gene transfer between them. The presence of bla_{kpc} in A. baumannii described in Puerto Rico (MARTINEZ et al. 2016; ROBLEDO et al. 2010), São Luis city in Brazil (RIBEIRO et al. 2016) and Iran (AZ-IMI et al. 2015) corroborates with this study. However, in this work was not possible to determine if the Acinetobacter spp. ISO15, ISO17 and ISO21 resistant to Imipenem and Meropenem was due to $bla_{_{\rm KPC}}$ gene (this study) or bla_{OXA23} genes (CARVALHO et al. 2016). Also, it was unknown if the plasmid harboring $bla_{\mu\nu}$ gene was integrated into the Acinetobacter baumannii chromosome since we used total DNA extraction to detect the $bla_{\rm KPC}$ amplicon by PCR.

The widespread and increasing uses of carbapenems may accelerate the spread of carbapenems-resistant strains by transferring resistance genes among the



Figure 1. PCR for detection of the *bla*_{se} gene among carbapenem-resistant *Acinetobacter* spp DNA on 1.5% agarose gel. Panel A: *Lane M*: Molecular mass marker 100 base par (Ludwig biotechnology); *Lane 1 to 14: Acinetobacter* spp DNA. *Lane* 15 and 16: Positive control (*Klebsiella pneumoniae* reference strain carrying *bla*_{se} gene). Panel B: *Lane M*: Molecular mass marker 100 base pair (Ludwig biotechnology); *Lane 1 to 17: Acinetobacter* spp DNA. *Lane* 15 and 16: Positive control (*Stepsiella pneumoniae* reference strain carrying *bla*_{se} gene). Panel B: *Lane M*: Molecular mass marker 100 base pair (Ludwig biotechnology); *Lane 1 to 17: Acinetobacter* spp DNA. Lane 18: Negative control (*Distilled water*). The size of the 100bp Ludwig biotechnology marker, in base pairs, is indicated to the left. The expected 876-base pair amplified fragment corresponding to the *bla*_{se} gene is designated to the right of the gel.

enterobacteriaceae and related microorganisms with impact in the public health system. Then, we reported here by the first time in the southeast of Brazil the presence of $bla_{\rm KPC}$ gene in the *A. baumannii* isolates which are extensively drug resistant including carbapenems.

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Conflict of Interest

The authors declare that is no conflict of interest

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