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First detection of metallo-β-lactamases in nosocomial isolates of *Pseudomonas aeruginosa* in Alagoas, Brazil

Primeira detecção de metalobetalactamases em isolados nosocomiais de Pseudomonas aeruginosa *em Alagoas, Brasil*

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ABSTRACT

Introduction: *Pseudomonas aeruginosa* is a leading cause of opportunistic infections in humans, and the choice of effective antimicrobial agents to control this bacterium has been limited, mainly due to its ability to produce metallo-β-lactamases (MβL), enzymes capable of inactivating many antimicrobials through hydrolysis. **Objective:** This study aimed to detect the presence of multidrug-resistant (MDR) *P. aeruginosa* strains and the MβL-encoding genes (*bla*SPM, *bla*IMP and *bla*VIM) in nosocomial isolates in Maceió (AL). **Methods:** The isolates were collected from four public institutions/hospitals in Maceió, and cultures were identified by conventional methods. Antibiotic susceptibility was determined by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI), and polymerase chain reaction (PCR) was used to identify the presence of the MβL-encoding genes *bla*SPM, *bla*IMP and *bla*VIM. **Results:** Forty-three strains of *P. aeruginosa* were MDR among 85 identified nosocomial isolates (50.6%), 79.1% and 20% of which were resistant to carbapenem (imipenem and meropenem) and aztreonam, respectively. PCR was performed in susceptible or resistant isolates and we identified nine (20.9%) MDR strains with *bla*SPM gene, whereas only one strain had *bla*IMP and none *bla*VIM positive was found. **Conclusion:** Production of MβL is an important mechanism of resistance to carbapenems and other β-lactams among *P. aeruginosa* strains in the evaluated samples. We reported the first identification of MβL-encoding genes in *P. aeruginosa* from nosocomial environments in Maceió, a new insight for the epidemiology of MβL in the Northeastern region of Brazil.

Key words: *Pseudomonas aeruginosa*; carbapenems; β-lactamases.

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative bacterium and one of the most common causes of human infections. In Brazilian hospitals, it is the first cause of pneumonia[1] and it often presents an antibiotic resistance profile that makes its eradication difficult. The indiscriminate use of antibiotics has contributed to the selection and subsequent spread of strains resistant to carbapenems (mainly imipenem and meropenem), considered an important therapeutic option for multiresistant *P. aeruginosa*, limiting therapy options and compromising the patient treatment[2].

Multidrug-resistant (MDR) isolates of *P. aeruginosa* can also be metallo-β-lactamase (MβL) producers[3]. Those metalloenzymes are able to inactivate β-lactam antibiotics such as cephalosporins, penicillins and carbapenems through hydrolysis using one active site with zinc ion[4]. Additionally, mechanisms of intrinsic and acquired resistance have been described, such as efflux pumps (mainly MexXY OprM and Mex ABprM), porin down-regulation and ampicillin class C β-lactamase (AmpC) overproduction[5]. Several types of MβL have been identified among *P. aeruginosa* strains, such as imipenemase (IMP), Verona imipenemase (VIM), São Paulo metallo-β-lactamase (SPM) and German imipenemase (GIM), encoded by specific genes into plasmids and transferable genetic elements that contribute to the

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dissemination of these MDR strains[6,7]. MβL such SPM-1 and IMP have already been isolated in many regions of Brazil[8-10], but in the Northeast, the blaSPM-1 gene has been detected only in samples from Pernambuco and Sergipe[11-15].

OBJECTIVE

The aim of this study was to detect the presence of MDR *P. aeruginosa* strains and the MβL-encoding *bla*SPM, *bla*IMP and *bla*VIM genes in nosocomial isolates from four public institutions/hospitals in Maceió (Alagoas), using disk-diffusion and polymerase chain reaction (PCR) approaches, respectively.

METHODS

Collection and identification of bacterial isolates

Isolates of *Pseudomonas aeruginosa* were recovered from patients admitted into four hospitals in the state of Alagoas, Brazil, from August to July 2009. The isolates were previously identified by conventional procedures at Santa Casa de Misericórdia and Centro de Patologia Médica e Laboratorial (CPML), responsible for bacterial identification of samples from the three major public hospitals in Maceió: Hospital Geral do Estado Professor Osvaldo Brandão Vilela, Hospital Escola Dr. Hélvio Alto and Maternidade Escola Santa Mônica. After bacterial identification (morphological characteristics, pyoverdin and pyocyanin production, oxidase and Gram stain), *P. aeruginosa* strains were re-isolated on Mueller-Hinton agar (Difco) for the next laboratorial steps.

Antimicrobial susceptibility testing

The resistance profile of each isolate was evaluated by Kirby Bauer's disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI)[16], with five classes of antimicrobial agents such as carbapenems (imipenem [IMP], meropenem [MEM]), monobactam (aztreonam [ATM]), flourquinolones (ciprofloxacin [CIP], levofloxacin [LVX]), aminoglycosides (gentamicin [GEN], amikacin [AMC]) and cephalosporins (ceftazidime [CAZ]), in duplicates. For the interpretation of susceptibility test results, all isolates that were resistant to two or more classes of antibiotics were considered MDR, according to CLSI[15]. *P. aeruginosa* ATCC 27853 was used as the quality control strain.

DNA extraction and screening for metallo-β-lactamase genes by PCR

Total deoxyribonucleic acid (DNA) of *P. aeruginosa* isolates was extracted through the boiling of bacterial cells[16] and checked by 1.2% agarose gel electrophoresis. The isolates were analyzed by PCR for *bla*SPM (sense 5'-CCTACAATCTAACGGCGACC-3' and antisense 5'-TCGCCGTGTCCAGGTATAAC-3'), *bla*IMP (sense 5'-CTACCGCAGCAGAGTCTTTGC-3' and antisense 5'-GAACAACCAGTTTTGCCTTACC-3'), and *bla*VIM (5'-ATGTTCAAACTTTTGAGTAGTAAG-3' and 5'-CTACTCAACGACTGAGCG-3'), as previously described by Sader *et al*.[1]. PCR conditions were performed as indicated by Toleman *et al*.[8] for *bla*SPM and *bla*VIM, and by Scheffer *et al*.[17] for *bla*IMP. MβL-positive strains for IMP (16 IMP-1 and 319 IMP-1), VIM (285 VIM-2) and SPM (43 SPM-1) were used as positive controls, whereas ATCC 27853 of *P. aeruginosa* was the negative control. The obtained PCR products were electrophoresed on a 2% agarose gel and evaluated.

RESULTS AND DISCUSSION

Isolates of MDR *P. aeruginosa* and the prevalence of MβL genes were evaluated in clinical specimens recovered from patients admitted into institutions /hospitals of Maceió. Eighty-five *P. aeruginosa* isolates were obtained from different clinical specimens, mainly urine (29.4%), tracheal aspirates (28.2%), and wounds (25.9%), as well as bloodstream, catheter tips, sputum and other sources (16.5%). The highest detected rate of *P. aeruginosa* resistance was to meropenem (34.1%), followed by gentamicin (31.8%); and the lowest was to aztreonam (20%). Among the total screened isolates, 43 (50.6%) were MDR and 79.1% of them were resistant to at least one tested carbapenem (60.5% to imipenem and 69.8% to meropenem). Resistant and susceptible isolates of *P. aeruginosa* were evaluated, and only one of them was positive for *bla*IMP gene (**Figure 1A**); nine isolates with the MDR phenotype were positive for *bla*SPM gene (20.9%) (**Figure 1B**), but *bla*VIM was absent in all. The Pa130, a *bla*IMP-positive strain, was MDR and resistant to all tested antibiotics, including carbapenems. The highest prevalence of SPM-producing strains was observed among clinical isolates from urine tract infections (five), whereas eight showed resistance to imipenem and meropenem, except for one sample that was only resistant to meropenem. The resistance profile of the 10 MβL-positive strains (SPM and IMP), with additional information of clinical specimens, is depicted in **Table**.

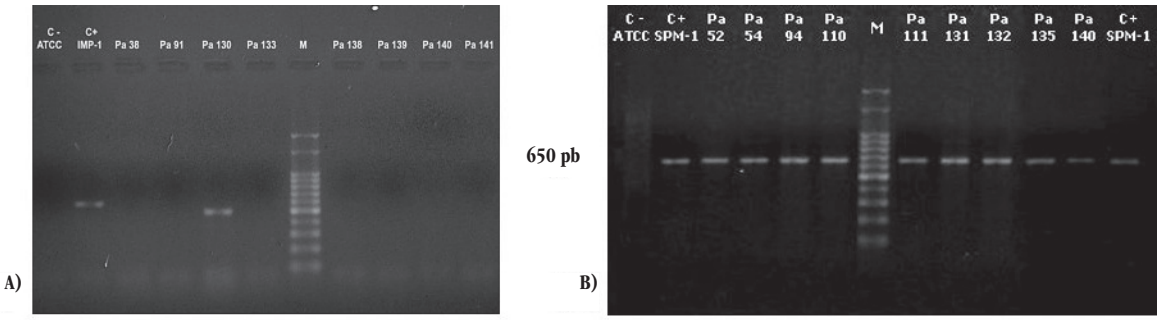

FIGURE – *PCR products (*bla *genes) in* Pseudomonas aeruginosa *strains. The negative control (C-) is the* P. aeruginosa *ATCC 27853;* P. aeruginosa *positive for the presence of* bla*IMP or* bla*SPM is C+ A) amplification of* bla*IMP gene (600 pb) in one strain; B)* bla*SPM gene (650 bp) in nine strains. Ladder 100 bp (M).*

PCR: polymerase chain reaction; IMP: imipenemase; SPM: São Paulo metallo-β-lactamase.

TABLE – Resistance profile of MβL-positive *Pseudomonas aeruginosa* strains from Alagoas

Strain	Origin	*bla* gene	Sample	IMP	MER	AZT	CAZ	CIP	LVN	GEN	AMC	Type*
Pa52	StªC**	*bla*SPM	Urine	R	R	S	-	R	R	R	R	MDR
Pa54	StªC/ICU	*bla*SPM	Urine	R	R	S	-	-	R	R	R	MDR
Pa94	HGE	*bla*SPM	Urine	R	R	S	R	R	R	R	R	MDR
Pa110	HGE	*bla*SPM	Wound secretion	R	R	S	R	R	R	R	R	MDR
Pa111	HGE	*bla*SPM	Urine	R	R	S	R	R	R	R	R	MDR
Pa130	HEHA	*bla*IMP	Tracheal aspirate	R	R	R	R	R	R	I	R	MDR
Pa131	HGE/ICU	*bla*SPM	Urine	R	R	S	R	R	R	R	R	MDR
Pa132	HGE/ICU	*bla*SPM	Wound secretion	R	R	S	R	R	R	R	R	MDR
Pa135	StªC/ICU	*bla*SPM	Tracheal aspirate	-	R	S	-	R	R	R	R	MDR
Pa140	StªC/ICU***	*bla*SPM	Urine	R	R	S	-	R	R	R	R	MDR

MβL: metallo-β-lactamase; IMP: imipenem; MER: meropenem; AZT: aztreonam; CAZ: ceftazidime; CIP: ciprofloxacin; LVN: levofloxacin; GEN: gentamicin; AMC: amikacin; Pa: Pseudomonas aeruginosa*; StªC: Santa Casa de Misericórdia; SPM: São Paulo metallo-β-lactamase; R: resistant; S: susceptible; ICU: intensive care unit; HGE: Hospital Geral do Estado; HEHA: Hospital Escola Dr. Hélvio Alto; I: intermediate; -: not available; *MDR (multidrug-resistant); **oncology unit; ***neurological intensive care unit.*

P. aeruginosa is highly adapted to the hospital environment and the emergence of MDR isolates, mainly due to the production of MβLs, has been associated with high mortality rates among infected patients. Endorsing this fact, Zavascki *et al*.[18] confirmed the mortality rate of 51% among the patients infected with metalloenzyme-producing isolates, against 32% in the patients infected with metalloenzyme-nonproducing isolates in Porto Alegre (RS). Likewise, Marra *et al*.[19] reported a high mortality rate (86%) in patients with bloodstream infection by *P. aeruginosa* MβL-positive strains (São Paulo, Brazil). Such data suggest that the spread control of the MβL-positive isolates can substantially reduce the number of post-infection deaths. In this context, associated with the increase of bacterial resistance and immunocompromised individuals, it is important to implement a regular monitoring of antimicrobial susceptibility of nosocomial *P. aeruginosa* isolates to establish protocols for prophylaxis and empirical treatment. Higher resistance to many antimicrobials in *P. aeruginosa* MβL-producing isolates was observed more frequently than in those MβL-nonproducing.

All over Brazil, the *bla*SPM gene is highly prevalent among isolates of MβL-producing *P. aeruginosa*. In São Paulo, Sader *et al*.[9] identified the *bla*VIM-2 gene in 31% of the screened strains, whereas Picão *et al*.[10] identified MβL-encoding genes *bla*SPM-1 and *bla*IMP-1 in 27 isolates (62.8%) of *P. aeruginosa*. Researchers of Rio Grande do Sul found imipenem/ceftazidime resistant strains with SPM-1[20] and attest the high prevalence of the SPM-1 type (50%) and 8% of IMP strains in 60 isolates[21].

After the evaluation of different cases from many hospitals in Rio de Janeiro, Nouér *et al*.[22] identified antibiotics usage as the major risk factor associated with acquisition of multidrug resistance when *P. aeruginosa* expresses the *bla*SPM-1 gene, mainly the administration of quinolones. Perez *et al*.[23] recovered seventy-nine MβL-producing *P. aeruginosa* (86.8%) from patients of Porto Alegre that showed the ability to produce biofilm *in vitro*, what can represent a new problem. Hence, we can observe different epidemiological data in Brazil, mainly about *bla*VIM prevalence.

In the northeastern states, 2% (4/198) of the *P. aeruginosa* strains recovered from patients in João Pessoa and 7.71% (24/311)

from those in Fortaleza were classified as metallo-β-lactamase producers by phenotypic methods[11, 14], whereas PCR was carried out for the detection of MβL-encoding genes in Recife[13]. Poirel *et al*.[12] identified eleven carbapenem-resistant *P. aeruginosa* isolates from hospitals of Recife, and the VIM, IMP and SPM screening through PCR only detected the *bla*SPM-1 gene. The genetic elements surrounding the *bla*SPM-1 gene in these strains were sequenced and described as possibly involved in the MβL dissemination.

The antibiotic susceptibility profile associated to MβLs is generally characterized by resistance to imipenem, meropenem and most β-lactams, with the exception of aztreonam[9, 12, 13, 24]. Among the isolates of *P. aeruginosa* obtained in this survey, Pa130 was positive for the *bla*IMP gene and also resistant to imipenem, meropenem and aztreonam, unlike most of the previously described MβL samples. In the study by Yan *et al*.[25], 90% of *Klebsiella pneumonia* isolates were resistant to aztreonam, with a resistant profile similar to that of the Pa130 sample, what might be due to other mechanisms, such as the production of β-lactamases or AmpC oxacillinases[25, 26]. These researchers draw attention to metalloenzymes in carbapenem-susceptible strains or with low-level resistance phenotypes.

In our research, the *bla* gene-positive samples were obtained from the neurological intensive care unit (ICU), the general ICU and the oncology unit at three major hospitals in Maceió (Table), and the application of specific techniques will be further evaluated in order to discuss the clonality of the strains.

The emergence of different mechanisms of resistance in *P. aeruginosa* is an important public health problem, particularly due to the expression of MβLs by nosocomial isolates, encoded by genes often connected to mobile elements, which facilitate their spread[27]. Here we report the identification of genes encoding metalloenzymes SPM and IMP in strains resistant to carbapenems and other β-lactams, recovered from hospitals of Alagoas. Since meropenem and imipenem are the most active and potent β-lactams against Gram-negative bacteria, besides being currently the only carbapenems commercially available in Brazil, this resistance profile represents a challenge to any treatment.

The adequate identification of strains with MβL-encoding genes is important to guide therapeutic choices in public and private hospitals and can give support to better treatments against these infections. Thus, control measures should be implemented to prevent further spread of metallo-β-lactamase-producing strains.

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RESUMO

Introdução: Pseudomonas aeruginosa *é a principal causa de infecções oportunistas em seres humanos, e a escolha de agentes antimicrobianos eficazes para controlar essa bactéria tem sido limitada, principalmente devido à sua capacidade de produzir metalobetalactamases (MβL), enzimas capazes de inativar muitos antimicrobianos por meio de hidrólise.* ***Objetivo:*** *Este estudo objetivou detectar a presença de cepas de* P. aeruginosa *multirresistentes e os genes codificadores de MβL (*bla*SPM,* bla*IMP e* bla*VIM) em isolados nosocomiais em Maceió (AL).* ***Métodos:*** *Os isolados foram coletados de quatro instituições públicas/hospitais em Maceió, e as culturas foram identificadas por métodos convencionais. A sensibilidade aos antibióticos foi determinada pelo método de disco-difusão de acordo com o Clinical and Laboratory Standards Institute (CLSI), e a reação em cadeia da polimerase (PCR) utilizada para identificar a presença de genes que codificam MβL* – bla*SPM,* bla*IMP e* bla*VIM.* ***Resultados:*** *Quarenta e três cepas de* P. aeruginosa *foram multirresistentes entre os 85 isolados nosocomiais identificados (50,6%); destes, 79,1% e 20% foram resistentes aos carbapenêmicos (imipenem e meropenen) e ao aztreonam, respectivamente. A PCR foi realizada em isolados suscetíveis ou resistentes, e nós identificamos nove (20,9%) cepas multirresistentes com gene* bla*SPM, enquanto apenas uma possuía* bla*IMP e nenhuma* bla*VIM positiva foi encontrada.* ***Conclusão:*** *A produção de MβL é um importante mecanismo de resistência aos carbapenêmicos e a outros betalactâmicos entre cepas de* P. aeruginosa *nas amostras avaliadas. Relatamos a primeira identificação de genes codificadores de MβL em* P. aeruginosa *de ambiente hospitalar de Maceió, uma nova visão para a epidemiologia de MβL na região Nordeste do Brasil.*

Unitermos: Pseudomonas aeruginosa*; carbapenêmicos; betalactamases.*

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