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ORIGINAL ARTICLE

Carbapenem-resistant Pseudomonas aeruginosa producing NDM-1

Pseudomonas aeruginosa resistente aos carbapenêmicos produtor de NDM-1

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ABSTRACT

Introduction: NDM-1-producing is increasingly common in new generation of multidrug-resistant bacteria. **Objective and Methods:** This study reports the occurrence of metallo-β-lactamase, especially NDM in *Pseudomonas aeruginosa* in the state of Minas Gerais, Brazil. A disc combination test used to screen isolates and Polymerase Chain Reaction was used to search for metallo-β-lactamases genes. **Results:** Among the 95 strains analyzed, all were resistant to carbapenems, 20% were phenotypically positive for metallo-β-lactamases, and 1% were positive for *bla*SPM gene and 2.1% for *bla*NDM. **Conclusions:** In Brazil, SPM carbapenemases always was predominant among P. aeruginosa clinical isolates, but not NDM. This study highlights the need to review clinical protocols, in order to contain the dissemination of this profile.

Keywords: Carbapenemase; Resistance mechanism; Metallo-β-lactamase.

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RESUMO

Introdução: A produção de NDM-1 é cada vez mais comum nesta nova geração de bactérias multirresistentes. **Objetivo e Métodos:** Este estudo avalia a ocorrência de metalo-β-lactamase, especialmente NDM em *Pseudomonas aeruginosa* no estado de Minas Gerais, Brasil, por meio de um teste do disco combinado para triagem de isolados e Reação em Cadeia de Polimerase para pesquisar os genes para metalo-β-lactamases. **Resultados:** Das 95 cepas analisadas, todas resistentes aos carbapenêmicos, 20% eram fenotipicamente positivas para metalo-β-lactamases, e 1% positivas para o gene *bla*SPM e 2,1% para *bla*NDM. **Conclusões:** No Brasil, as carbapenemases SPM sempre foram predominantes entre os isolados clínicos de *P. aeruginosa*, mas não a NDM. Este estudo destaca a necessidade de revisão de protocolos clínicos, a fim de conter a disseminação desse novo perfil.

Palavras-chave: Carbapenemase; Mecanismo de resistência; Metalo-β-lactamase.

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Introduction

Pseudomonas aeruginosa is an important and opportunistic pathogen responsible for several infections in the hospital environment and carbapenems are commonly used as antimicrobials of the last choice in infections caused by Multidrug-resistant (MDR) *P. aeruginosa*; however, carbapenem-resistant strains have been reported around the world over the last decades with the production of Metallo-β-lactamase (MBL)[1].

Acquired carbapenem resistance in Gram-negative rods is often associated with carbapenemase production (MBL, Carbapenemases other, such as *Klebsiella pneumoniae* carbapenemase – KPC or oxacillinase and AmpC β lactamase), these represent a group of enzymes that hydrolyze and inactivate the β-lactam antibiotics, detected in beginning of the 40's[1,2].

New Delhi MBL (NDM) was reported in 2008 in New Delhi, India, in *Klebsiella pneumoniae* strain, since then, its variants have been reported worldwide, being the most prevalent *bla*NDM-1, which are present largely in Enterobacterales, but also in nonfermenters (*Acinetobacter* and *Pseudomonas* spp.)[1,3]. Since then, NDM-producing bacteria have been detected in several countries[4].

In Brazil, NDM-1 was first identified in *Providencia rettgeri*, in 2013, then reported sporadically in different states of Brazil such as Rio de Janeiro, Parana, Rio Grande do Sul, Santa Catarina, Distrito Federal, São Paulo and Bahia[4].

The presence of NDM producers in health care facilities, which is a growingly important medical issue that compromises the efficacy of treatment[4]. made necessary the use of simple and reliable tests. Carbapenem resistance detection by Polymerase Chain Reaction (PCR) is gold standard, but several phenotypic techniques are still used[2]. This study reports the occurrence of metallo-β-lactamase, especially NDM in *Pseudomonas aeruginosa* in the state of Minas Gerais, Brazil.

Methods

Between May 2013 and August 2017, a total of 95 Carbapenem-resistant *Pseudomonas aeruginosa* clinical isolates exhibiting carbapenem resistance, reported at the Municipal Hospital and Maternity of Uberlandia in the state of Minas Gerais, Brazil, were recovered and included in this study. Isolates from different clinical specimens, identifiedby Maldi-Tof at the hospital laboratory, were frozen until the development of this study.

Acquired lactamases encoding genes were investigated by PCR using primers for *bla*VIM, *bla*IMP, *bla*SPM, *bla*GIM, *bla*SIM and *bla*NDM genes (Table 1).

The reaction mixture (25µL) contained 12.5µl GoTaq® Green Master Mix, 0.75µL of each pair of primers, 10µL of ultrapure water and 1µL of DNA[5]. Amplifications were performed in Mastercycler Personal (Eppendorf) using the following program: initial denaturation at 95ºC for 5 minutes, followed by 30-second cycles at 95ºC, annealing according to table 1, extension at 72ºC for 1 minute and final extension at 72ºC for 5 minutes. The amplification products were analyzed by electrophoresis on 1.5% agarose gel, 90V, stained with Diamond™ Nucleid Acid Dye for 30 minutes, revealed in a transilluminator. All assays used control samples producing the genes of interest.

The susceptibility of the isolates to imipenem, meropenem, piperacillin-tazobactam, cefepime, ceftazidime, ciprofloxacin, aztreonam, gentamicin, amikacin and polymyxin were evaluated with agar diffusion method

Table 1. Specific primers for genes encoding Metallo-β-lactamase.

Primer	Sequence (5'- 3')	Annealing temperature
*bla*IMP-F	GGAATAGAGTGGCTTAATTCTC	42ºC
*bla*IMP-R	CCAAACCACTACGTTATCT	
*bla*SIM-F	TACAAGGGATTCGGCATCG	47ºC
*bla*SIM-R	TAATGGCCTGTTCCCATGTG	
*bla*GIM-F	TCGACACACCTTGGTCTGAA	47,5ºC
*bla*GIM-R	AACTTCCAACTTTGCCATGC	
*bla*SPM-1 F	AAAATCTGGGTACGCAAACG	46,5ºC
*bla*SPM-1 R	ACATTATCCGCTGGAACAGG	
*bla*NDM-1 F	GCCAAAGTTGGGCGCGGTTG	55ºC
*bla*NDM-1 R	ACCGCCTGGACCGATGACCA	
*bla*VIM-F	GATGGTGTTTGGTCGCATA	47ºC
*bla*VIM-R	CGAATGCGCAGCACCAG	

Source: Woodford (2010)[5].

following Clinical and Laboratory Standards Institute (CLSI) guidelines according to the year of isolation (2015 to 2017), and *Pseudomonas aeruginosa* ATCC 27853 was used for control.

Carbapenemase production was detected using a combination meropenem disc test with 10μL of the EDTA (0.1 M). The interpretation of the combination meropenem disc test was based on the comparison between the inhibition zones of the meropenem discs, with and without inhibitor; MBL production becomes evident by an increase of ≥5mm in the discs that is supplemented with EDTA[6].

Results

From 95 isolates, 9.5% (n=9) was collected in 2013, 13.7% (n=13) were collected in 2014, followed by 14.7% (n=14) in 2015, 15.7% (n=15) in 2016 and 46.3% (n=44) in 2017. The isolates were collected from blood culture and catheter tip (n=28), tracheal secretion (57) and urine (n=10) (Table 2).

Only 19 isolates were identified as carbapenemase producers by the combination disc test. PCR analysis of MBL genes was performed for all carbapenem-resistant *P. aeruginosa* isolates and one positive isolate for *bla*SPM-1 and two for *bla*NDM-1 (Table 2), coming from 2013, 2015 and 2017, respectively and all of BSI (Figure 1). No amplicons were detected for the *bla*SIM, *bla*VIM, *bla*IMP, and *bla*GIM genes.

Of the isolates, 52.6% were nonsusceptible to any of the β-lactams tested, in addition to carbapenems. Of the isolates, 71.6% were nonsusceptible to aminoglycosides tested. Of the isolates 62% were nonsusceptible to piperacillin-tazobactam, 3.2% isolates were resistant to polymyxin.

All *bla*NDM-1 and *bla*SPM-1 positive isolates were defined as pan drug resistance, susceptible only to polymyxin B.

Discussion

In this study, we assessed the most significant resistance mechanisms in carbapenem resistant *P. aeruginosa* isolates. Carbapenem-resistant *Pseudomonas aeruginosa* is a major healthcare-associated pathogen worldwide. This mechanism of carbapenem resistance is important because it significantly changes the efficacy of commonly used antipseudomonal agents, including cephalosporins, piperacillin-tazobactam, ceftolozane-tazobactam, imipenem-relebactam and ceftazidime-avibactam[7].

Resistance to almost all classes of antibiotics has been reported, including aminoglycosides, cephalosporins, fuoroquinolones, β-lactams, and more recently colistin. The therapeutic options available for patients with infections caused by *Pseudomonas aeruginosa* carrying the *bla*NDM gene are scarce[1]. In the present study, Polymyxin was the most effective antimicrobial agents, however some sample are already showing resistance to it.

Shahin & Ahmadi (2021)[8], reporting *bla*NDM-1 in Iran, attribute increase of this phenomenon among Gram-negative isolates to inappropriate and excessive prescription and use of carbapenems in hospitals, which leads to selective pressure. The overall data show that the frequency of *bla*NDM-1 producing *P. aeruginosa* isolates was 2.1% (2/95). Silva et al. (2019)[4] suggest the predominance of the *bla*NDM-1 variant in Brazil; according to them, between 2012 and 2015 there was a significant increase of NDM-producing Gram-negative bacteria, and reports of *bla*NDM-1 from different countries in Europe and Asian.

Accordingly, we report the first isolate of *P. aeruginosa* producing carbapenemases *bla*NDM-1, from Minas Gerais. And Perez et al. (2021)[3] emphasize the potential for dissemination of an NDM-1–producing *P. aeruginosa* among critically ill patients in Southern Brazil, which possibly could displace other MBL enzyme (mostly SPM-1)

Table 2. Characteristics of the samples, including patients infected by Pseudomonas aeruginosa in a Brazilian public hospital.

	P. aeruginosa N=95 (%)	*P. aeruginosa* *bla*SPM-1 N=1	*P. aeruginosa* *bla*NDM-1 N=2
Age (mean) ± SD 0-20 years	63.6 ± 18.6		
0-20 years	2 (2.1)		
21-40 years	8 (8.4)		
41-60 years	24 (25.3)		46
61-80 years	48 (50.5)		70
≥81 years	13 (13.7)	82	
Female	36 (37.9)	F	F (both)
UCI	55 (57.9)	yes	no (both)
BSI	28 (29.5)	yes	yes (both)
PNM	57 (60.0)		
2013	9 (9.5)	yes	
2014	13 (13.7)		
2015	14 (14.7)		yes
2016	15 (15.7)		
2017	44 (46.3)		yes
MBL	19 (20.0)	no	only one

Legend: CI = Confidence interval; SD: Standard deviation; UCI = Intensive care unit; BSI = Bloodstream infection; PNM = Pneumonia; MBL = Metalo β-lactamase test. Source: Research database.

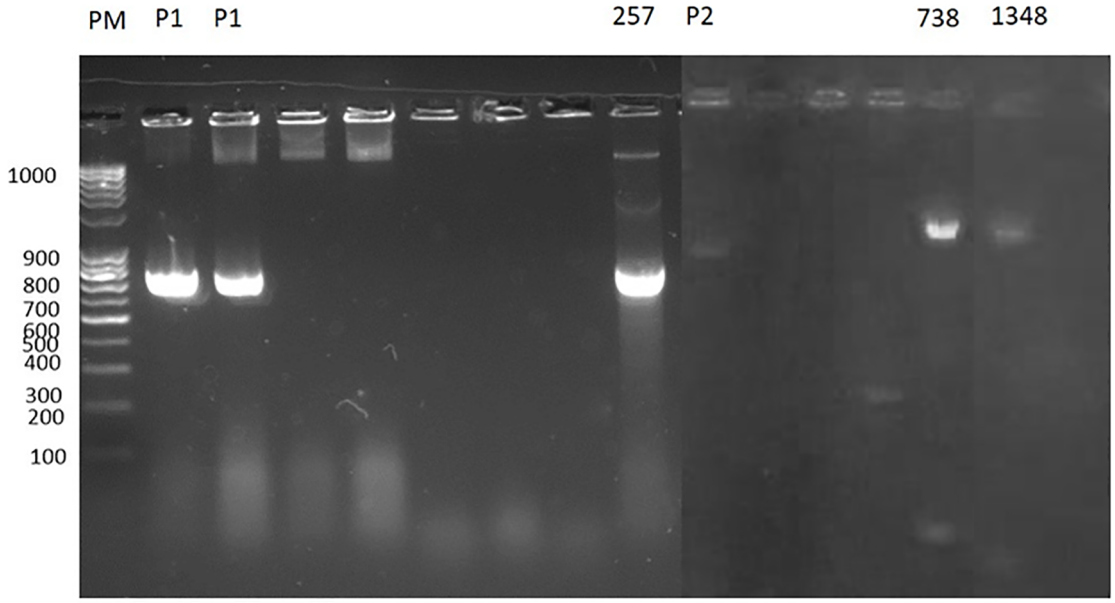

Figure 1. Agarose gel electrophoresis for *bla*SPM-1 and *bla*NDM-1 gene. Legend: PM = Molecular weight marker 100 bp; P1 = *P. aeruginosa* *bla*SPM-1 positive control; P2 = *P. aeruginosa* *bla*NDM-1 positive control; 257 = Sample of *P. aeruginosa* positive for *bla*SPM-1; 738 and 1348 = Sample of *P. aeruginosa* positive for *bla*NDM-1. Source: Research database.

or another resistance mechanism as driver for carbapenem resistance.

In this study, a few resistance mechanisms have been investigated and in these isolates, other mechanisms may be present that were not evaluated, as inactivation of the outer membrane protein OprD, overexpression of chromosome-encoded AmpC and overproduction of multidrug efflux pumps[9]. Resistance to carbapenems is influenced by several factors, not all of which were evaluated in our study; however, the results showed that mechanisms involving the production of MBLs were observed more frequently and also play an important role in the emergence of the high-level carbapenem-resistant phenotype among *P. aeruginosa* isolates.

The lack of testing specifically for carbapenemase production suggest that the prevalence of carbapenem-resistant *P. aeruginosa* may be much higher than is perceived[7]. There is a long history of β-lactamase research, and questions such as the continuing unresolved issues surrounding the mechanism; opportunities offered by new technologies; the need for new inhibitors, especially for MBLs; and the continuing clinical importance of β-lactams, mean that this remains a rewarding area of research[10].

Conclusion

It is difficult to predict the future situation of antimicrobial resistance, particularly in developing countries, and these data only reinforce the importance of continuous epidemiological surveillance. Combining both phenotypic and genotypic methods may result in better results. Yet, these tests often are not performed in many clinical laboratories. Which resulted in late findings of *P. aeruginosa* producer of *bla*NDM-1, as in this study, and sequencing of gene may reveal new disclosure.

Authors' Contributions

We describe contributions to the papers using the taxanomy (CRediT) provide above: Conceptualization, Investigation, Methodology and Editing: Paulo Ricardo Freitag Jorge; João Paulo Pimenta. Supervision and Writing: Cristiane Silveira de Brito. Validation, Funding acquisition, Formal Analysis and writing: Rosineide Marques Ribas; Paulo Pinto Gontijo Filho; Lizandra Ferreira de Almeida e Borges.

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