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Article/Artigo

Prevalence and clinical outcomes of episodes of ventilator-associated pneumonia caused by SPM-1-producing and non-producing imipenem-resistant *Pseudomonas aeruginosa*

Prevalência e evolução clínica de episódios de pneumonia associada à ventilação mecânica causada por *Pseudomonas aeruginosa* resistente a imipenem produtoras e não-produtoras de SPM-1

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ABSTRACT

Introduction: *Pseudomonas aeruginosa* is a leading cause of ventilator-associated pneumonia (VAP) and exhibits high rates of resistance to several antimicrobial drugs. The carbapenens are usually the drugs of choice against this microorganism. However, the carbapenem resistance has increased among these strains worldwide. The presence of metallo-β-lactamases (MBL) has been pointed out as a major mechanism of resistance among these strains. No previous study addressed outcomes of respiratory infections caused by these strains. **Methods:** Our group sought to analyze the epidemiology and clinical outcomes of patients with VAP caused by imipenem-resistant *P. aeruginosa*. A total of 29 clinical isolates of carbapenem-resistant *Pseudomonas aeruginosa* were screened for metallo-β-lactamase (MBL) genes. **Results:** Demographic and clinical variables were similar between the SPM-1-producing and non-SPM-1-producing group. Five (17.2%) isolates were positive for bla_{SPM-1}. No other MBL gene was found. All patients were treated with polymyxin B. The infection-related mortality was 40% and 54.2% for SPM-1-producing and -non-producing isolates, respectively. **Conclusions:** There were no differences in epidemiological and clinical outcomes between the two groups.

Keywords: *Pseudomonas aeruginosa*. Antimicrobial resistance. Outcome.

RESUMO

Introdução: *Pseudomonas aeruginosa* é uma importante causa de pneumonia associada à ventilação mecânica (PAV) e exibe altas taxas de resistência a vários antimicrobianos. Os carbapenens são usualmente as drogas de escolha para esse microorganismo. Contudo, a resistência a carbapenens tem crescido entre essas amostras em todo o mundo. A presença de metalo- β-lactamase (MBL) tem sido apontado como um importante mecanismo de resistência nessas cepas. Nenhum estudo prévio avaliou desfechos clínicos de infecções respiratórias causadas por essas amostras **Métodos:** Nosso grupo analisou a epidemiologia e evolução clínica de episódios de PAV causada por *P. aeruginosa* resistente a imipenem. Um total de vinte e nove isolados clínicos de *Pseudomonas aeruginosa* resistente a carbapenem foram avaliados quanto à presença de genes para metalo-β-lactamase (MBL). **Resultados:** Variáveis clínicas e demográficas foram similares entre o grupo produtor de SPM-1 e o não-produtor. Cinco (17,2%) isolados foram positivos para bla_{SPM-1}. Nenhum outro gene para MBL foi encontrado. Todos os pacientes foram tratados com polimixina B. A mortalidade relacionada à infecção foi de 40% e 50% respectivamente para os isolados produtores de SPM-1 e não-produtores de SPM-1. **Conclusões:** Nao houve diferença entre os dados epidemiológicos e a evolução clínica entre os dois grupos.

Palavras-chaves: *Pseudomonas aeruginosa*. Resistência antimicrobiana. Desfecho.

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INTRODUCTION

Pseudomonas aeruginosa exhibits high rates of resistance to several antimicrobial drugs. The carbapenems are usually the drugs that provide the best coverage against this microorganism. However, the carbapenem resistance has increased among these strains[1]. The presence of metallo-β-lactamases (MBL) has been pointed out as a major mechanism of this resistance[2, 3].

The São Paulo Metallo-β-lactamase (SPM-1) was described in São Paulo, in 2001. Since then, a SPM-1-producing endemic clone has been predominantly reported in several regions in Brazil[4, 5]. Its prevalence among carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) isolates has reached rates of 34.8%[5]. However, the clinical outcome of these infections compared with infections caused by non-SPM-1-harboring isolates has not been reported[6, 7]. Indeed, to our knowledge, no previous study has specifically addressed outcomes of respiratory tract infections caused by these strains.

The present study aimed to investigate the prevalence of bla_{SPM-1} gene among imipenem-resistant *Pseudomonas aeruginosa* responsible for episodes of ventilator-associated pneumonia (VAP), as well as the clinical outcomes of these infections compared with episodes of VAP caused by non-SPM-1-producing isolates.

METHODS

This study was carried out at Hospital São Paulo, a 750-bed teaching hospital in São Paulo, Brazil. It was designed to assess the clinical outcome of patients with ventilator-associated pneumonia (VAP) caused by imipenem-resistant SPM-1-producing *Pseudomonas aeruginosa*. All adult patients for whom respiratory tract cultures had grown CRPA from January 1, 2004 to December 31, 2006 and had

received a diagnosis of VAP were eligible for inclusion in the study. Only the first episode of pneumonia was included. Polymicrobial VAPs were excluded. All episodes fulfilled the criteria for nosocomial infection as defined by the Centers for Disease Control and Prevention (CDC).

Patients with VAP caused by SPM-1-producing strains were compared to patients with VAP caused by non-SPM-1-producing strains selected on the basis of hospital unit and date of admission (±30 days). From the in-patient medical record, we collected demographic, clinical, and microbiological data. Infection-related mortality was considered present when a patient died within 14 days of the diagnosis of pneumonia and the death could not be directly attributed to any other cause. Several demographic and clinical variables were evaluated.

The microbiological analysis of respiratory samples was conducted using conventional techniques. The cutoff for the bronchoalveolar lavage (BAL) and for the tracheal aspirate was 10^4 colony-forming units (CFU/)mL and 10^5 CFU/mL, respectively. The susceptibility testing was performed by the disk diffusion technique[8]. Minimum inhibitory concentration (MIC) determinations for carbapenems and polymyxin B were conducted using the agar dilution technique[9]. Isolates were considered susceptible to polymyxin B if the MIC was ≤2μg/ml. *P. aeruginosa* isolates were screened for the presence of MBLs by using Multiplex PCR. The following genes were searched: $bla_{IMP,}$ $bla_{VIM,}$ $bla_{GIM-1,}$ bla_{SIM-1}, and bla_{SPM-1}[10].

Ethical considerations

The study was approved by the local Ethics Committee.

RESULTS

A total of 29 patients with VAP caused by CRPA were identified. The clinical and demographic characteristics of these patients are shown in **Table 1**. Demographic and clinical variables were similar between the SPM-1-producing and non-SPM-1-producing isolates. The mean ages were 63 years and 51 years for the SPM-1-producing and non-SPM-1-producing groups, respectively. The mean durations of ICU stay were 25 and 29 days for the SPM-1-producing and non-SPM-1-producing groups, respectively. Three (60%) patients and 18 (75%) patients in the SPM-1-producing and non-SPM-1-producing groups, respectively, utilized carbapenems before the diagnosis of VAP.

Patient outcomes are shown in **Table 2**. There were no differences in the outcome-associated variables between the two groups. All patients in both groups were treated with polymyxin B. No combined therapy was utilized. In all cases, empirical antimicrobial therapy was determined to be adequate. The infection-related mortality rates were 40% and 54.2% in the SPM-1-producing and non-SPM-1-producing groups, respectively. No differences in the infection-related mortality rates and in the in-hospital mortality rates were observed between the two groups.

All SPM-1-producing isolates were resistant to all beta-lactams, fluoroquinolones, and aminoglycosides. Only polymyxin B remained susceptible. The MIC_{50}/MIC_{90} for imipenem, meropenem, and polymyxin B were 16/≥32μg/mL, 16/≥32μg/mL and 1/2μg/mL, respectively. Only five samples (17.2%) showed PCR amplification product for the bla_{SPM-1} gene. No other metallo-β-lactamase was detected.

TABLE 1 - Clinical and demographic characteristics of episodes of ventilator-associated pneumonia caused by imipenem-resistant *Pseudomonas aeruginosa*.

	SPM-1 (n=5)		Without SPM-1 (n=24)		
Variable	n	%	n	%	p
Age (years)	63		51		0.24
Duration of hospital stay, days	25		29		0.88
Duration of ICU stay, days	24		22		0.63
Number of antibiotics	5		4		0.32
Duration of mechanical ventilation, days	24		16		0.44
APACHE II score, mean	15		15		0.67
Time under antibiotics	31		27		0.41
Female gender	4	80.0	7	29.1	0.05
Surgery	2	40.0	17	70.8	0.30
Previous hospitalization	0	0.0	6	25.0	0.55
Corticosteroid use	2	40.0	12	50.0	>0.99
Immunossupressive drug use	1	20.0	2	8.3	0.44
Comorbidity					
diabetes *mellitus*	1	20.0	2	8.3	0.44
cirrhosis	0	0.0	3	12.5	>0.99
COPD	0	0.0	5	20.8	0.55
chronic renal failure	1	20.0	3	12.5	0.55
cancer	1	20.0	5	20.8	>0.99
transplant	1	20.0	2	8.3	0.44
Antimicrobial drug use					
third-gen chephalosporin	4	80.0	14	58.3	0.62
fourth-gen cephalosporin	3	60.0	9	37.5	0.62
carbapenem	3	60.0	18	75.0	0.59
vancomycin	5	100.0	21	87.5	>0.99
aminoglycoside	1	20.0	6	25.0	>0.99
fluoroquinolone	1	20.0	7	29.2	>0.99
piperacillin-tazobactam	0	0.0	3	12.5	>0.99

SPM: São Paulo metallo-β-lactamase, **ICU:** intensive care unit; **APACHE II:** acute physiology and chronic health evaluation II; **COPD:** chronic obstructive pulmonary disease.

TABLE 2 - Comparative analysis of factors related to clinical outcome.

	SPM-1 (n=5)		Without SPM-1 (n=24)		
Variable	n	%	n	%	p
Presence of septic shock	2	40.0	15	62.5	0.62
Presence of ARDS	2	40.0	7	29.2	0.63
Bacteremia	1	20.0	2	8.3	0.44
Acute renal failure	0	0.0	2	8.3	>0.99
Infection-related mortality	2	40.0	13	54.2	0.65
In-hospital mortality	3	60.0	18	75.0	0.59

SPM: São Paulo Metallo-β-lactamase, **ARDS:** acute respiratory distress syndrome.

DISCUSSION

The present study aimed to identify outcome differences between episodes of VAP caused by carbapenem-resistant SPM-1-producing and -non-producing *Pseudomonas aeruginosa*. As far as we know, this is the first work specifically addressing this subject. Furthermore, few studies analyzed risk factors for the presence of SPM-1 among *Pseudomonas aeruginosa* (PA) isolates in Brazil.

Noue et al., found the use of quinolones as the sole independent predictor of colonization and/or infection by bla_{SPM} multi-drug-resistant *Pseudomonas aeruginosa*[11]. Other recent study described an outbreak of imipenem-resistant MBL-producing *Pseudomonas aeruginosa* in an adult intensive care unit (ICU). Pneumonia was the predominant infection (85%), and only SPM-1 was identified in 15 specimens analyzed. Advanced age, mechanical ventilation, tracheostomy, and previous imipenem use were significant risk factors for infection[12].

Our findings demonstrate that there are no differences between SPM-1-producing and -non-producing strains causing VAP. The low incidence of SPM-1-producing strains demonstrates that other resistance mechanisms are involved, such as permeability mutations or the up-regulation of efflux systems[13, 14]. Recently, we demonstrated the importance of efflux pumps and AmpC hiperexpression among *Pseudomonas aeruginosa* strains in our setting[15].

At our hospital, the isolation of CRPA has been kept at an average rate of 60% over the past few years, and the lower respiratory tract has been its major site[16]. In addition, a previous study undertaken at our hospital demonstrated that 43.9% of these strains harbored MBLs, mostly SPM-1 (55.6%)[14]. On the other hand, our study demonstrated a lower percentage of MBL-producing strains in respiratory tract when compared with previous studies focused on blood isolates[5, 7, 14]. Interestingly, this finding was previously observed in a descriptive study at our setting[6].

Marra et al., described seven patients with bloodstream infections caused by MBL-producing CRPA (4 out 7 were SPM-1)[6]. Infection-related mortality was very high (71.4%), but the respiratory tract was the infection site in only two patients in this study. Only one of these respiratory strains was positive for the presence of bla_{SPM-1}. Other recent study investigated the outcomes of 86 MBL-producing *Pseudomonas aeruginosa* infections[7]. The presence of MBL-producing strains was associated to higher in-hospital mortality rates when compared with non-MBL-producing strains (51.2% versus 32.1%). Severe sepsis or septic shock and age were associated with a poor outcome in this study. However, only 14 isolates were tested for the presence of MBL genes, and all were positive for the presence of bla_{SPM-1}. All other strains were submitted only to phenotypic test by disc diffusion with 2-mercaptopropionic acid. In our study, the in-hospital mortality rates were higher mainly in the non-SPM-1 group. All patients in our study were critically ill with higher APACHE II scores. A lot of them presented septic shock and acute respiratory distress syndrome (ARDS). Thus, we think that these factors may justify the high mortality rates. Otherwise, there were no differences between these outcome variables between the two groups.

Our study has limitations. First, the small sample size limited our capacity to detect differences between the two groups. Finally, it is plausible that our findings may not be generalized to other settings. On the other hand, it is important to better understand the outcome of these infections, as recently, SPM-1 was reported in Switzerland. This is the first reported case of a *Pseudomonas aeruginosa* isolate possessing bla_{SPM-1} outside Brazil[17].

In conclusion, our study found no differences in clinical outcomes between SPM-1-producing and non-SPM-1-producing episodes of VAP. A reduced number of respiratory isolates harbored MBL genes (17.2%). The bla_{SPM-1} was the only gene identified among the MBL-positive strains. A relatively high infection-related mortality rate was also observed. Further studies are needed to confirm these results.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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