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Full Length Research paper

Risk factors for ventilation associated-pneumonia by multidrug-resistant *Pseudomonas aeruginosa* producing metallo- -lactamase

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To evaluate if risk factors for ventilation associated-pneumonia (VAP) by *Pseudomonas aeruginosa* differed among patients affected by *P. aeruginosa* producing metallo- -lactamase (MBL-PA) or non-MBL-PA isolates. A 1-year retrospective case-control study was conducted in a surgical-clinical intensive care unit at a tertiary-care university hospital. Cases were patients with MBL-PA VAP and controls were those with non-MBL-PA VAP. Univariate and multivariate analysis were performed to identify risk factors. A total of 40 cases and 53 controls were included in the study. Univariate analysis showed that a high-risk ASIS score, clinical category in hospital admission, prior hospitalization, length of ICU stay, length of mechanical ventilation, exposure to three or more antibiotics, exposure to carbapenems and fluoroquinolones were risk factors for VAP by MBL-PA. After logistic regression, the prior hospitalization and length of mechanical ventilation continued significant. The mortality was also independently associated with MBL -PA VAP. Hospitalization within the preceding year and length of mechanical ventilation were risk factors for MBL-PA VAP, deserving attention and further investigations. MBL-PA VAP resulted in higher mortality rates most likely related to the commitment effective treatments of nosocomial infections by this organism. Early identification of patients with risk factors which could guide this initial appropriate empirical therapy and the development of strategies to prevent the dissemination of MBL-PA strains.

Key words: Metallo- -lactamases, risk factors, *P. aeruginosa*, bacterial drug resistance, case-control studies.

INTRODUCTION

Pseudomonas aeruginosa is one of the major causes of ventilation associated-pneumonia (VAP), and has been associated with the worst morbidity and mortality rates (Combes et al., 2007; Kollef et al., 2006). In addition, treatment options for *P. aeruginosa* infections have significantly decreased in recent years due to its ability to acquire resistance to antipseudomonal agents, such as piperacillin, ceftazidime and cefepime, fluoroquinolones, carbapenems and aminoglycosides (NNISS, 2004; Andrade et al., 2008).

The resistance to the -lactam in *P. aeruginosa* may result from production of various -lactamases, including metallo- - lactamases (MBLs) (Pellegrino et al., 2008; Livermore and Woodford, 2000). These enzymes are clinically relevant because of their systematic hydrolysis of carbapenems and association of the MBL genes with mobile genetic elements, increasing the possibility of rapid spread (Patzer et al., 2009) . Consequently, during the last decade, the emergence and dissemination of these MBL genes have been extensively documented around the world (Patzer et al., 2009), with the SPM-1 MBL-producing *P. aeruginosa* demonstrated only in hospitals in different Brazilian regions (Zavascki et al., 2006; Nouér et al., 2005; Cezário et al., 2009; Gales et al., 2003), including the Uberlândia Federal University Hospital Cezário et al., 2009).

Previously reported risk factors for VAP caused by multidrug resistant (MDR) *P. aeruginosa* include prior use of antibiotics, previous hospitalization, severity of illness,

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prolonged hospitalization, mechanical ventilation lasting more than 7 days, surgery and immunosuppresion (Zavascki et al., 2006; Nouér et al., 2005; Gales et al., 2003). The use of several antibiotics as fluoroquinolones (Zavascki et al., 2006; Nouér et al., 2005), cefepime (Nouér et al., 2005), anaerobicidal agents (Souza et al., 2008) and carbapenem (Zavascki et al., 2005) were independent predictors of infection caused by MDR *P. aeruginosa.*

The objective of the present study was to evaluate if risk factors for VAP caused by *P. aeruginosa* differed among patients carrying *P. aeruginosa* producing MBL (MBL-PA) or non-MBL-PA isolates.

MATERIALS AND METHODS Hospital

setting and study population

The Uberlândia Federal University Hospital is a 500-bed, tertiary-care teaching hospital in the city of Uberlândia, Minas Gerais State, Brazil, with a 15-bed, surgical-clinical adult intensive care unit (ICU).

Study design

A retrospective case-control study was performed in the adult ICU at the Uberlândia Federal University Hospital. The study included all patients with VAP by *P. aeruginosa* from December, 2005 to December, 2006. Cases were those patients with VAP by MBL-PA and controls were those infected by non-MBL- PA. Only one episode per patient was considered for this analysis.

The study was approved by the University Ethical Committee (016/06). Written informed consent was obtained from each participant or family member.

Definitions

Pneumonia was considered ventilator associated when its onset occurred after 48 h of mechanical ventilation, and it was diagnosed as new, when persistent pulmonary infiltrates appeared on chest radiographs plus two of the following three clinical criteria were present: temperature >38 or <36°C, leukocytosis >12,000 cells/mm^3 or leucopenia <4,000 cells/mm^3 and purulent secretion (Delclaux et al., 1997; Parker et al., 2008; Rello et al., 2006; Zhuo et al., 2008). The endotracheal aspirate was used for quantitative culture in the diagnosing pneumonia, with threshold of the $l0^6$ cfu/ml. (Zhuo et al., 2008; Nseir et al., 2008).

Lower respiratory tract colonization was defined as endotracheal aspirate culture < $l0^6$ cfu/ml with or without clinical criteria of VAP (Delclaux et al., 1997). All colonized ventilated patients were excluded from the analysis and only patients who were intubated in the ICU and were diagnosed with VAP were included.

Data collection

In order to analyze variables potentially associated with MBL-PA VAP, age, sex, average severity of illness score (ASIS) score (Rosenthal et al., 2005), underlying, immunocompromised status, trauma, surgery, length of intensive care unit stay, mechanical ventilation duration and presence of other invasive devices were evaluated. Exposure to -lactams, fluoroquinolones, aminoglycosides, vancomycin and anaerobicidal agents, over the last 14 days, prior to pneumonia onset was also analyzed.

Bacterial identification and antimicrobial susceptibility

Bacterial isolates were identified with conventional biochemical tests including oxidase production, oxidation of glucose on OF-medium, arginine utilization, nitrate reduction, growth on cetrimide agar, and production of characteristical pigments (Koneman et al., 1999). Susceptibility testing was determined by the agar diffusion method and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2008). Quality control was conducted using *P. aeruginosa* ATCC 27853.

Phenotypic metallo- -lactamase production

All isolates resistant to ceftazidime and/or imipenem were screened for metallo-beta-lactamase (MBL) production by the disk approximation test, using filter disks with ceftazidime (30 µg) and imipenem (10 µg) at 20 and 15 mm equidistant to the disks containing the following inhibitors: 2-mercaptopropionic acid (2-MPA) solution (Aldrich Chemical Co, Milwaukee, USA) and ethylenediaminetetraacetate (EDTA), respectively, as described previously (Arakawa et al., 2000; Picão et al., 2008). The positive control strain was an IMP-1-producing *P. aeruginosa* (PSA 319) (Picão et al., 2008).

Statistical analysis

Univariate analysis was performed in order to compare variables for group outcomes by the X^2 test or Fisher's exact test for categorical variables and Student's t-test for continuous variables. Variables for which the p- value was 0.2 in univariate analysis were included one by one in the logistic regression model for the multivariate analysis. Significance was set at a p-value of 0.05. Odds ratios and 95% confidence intervals were calculated with SPSS for Windows, Version 17.0 and Bioestat, Version 5.0 (Ayres et al., 2000).

RESULTS

During the study period we identified 40 patients with VAP caused by *P. aeruginosa* MBL producers according to the disk approximation tests. These cases were compared with 53 controls, patients infected by a non-MBL-PA isolate during the same study period.

Resistances to antimicrobial agents of MBL-PA and non-MBL-PA isolates are summarized in Table 1. Resistance to -lactam agents was significantly higher among MBL- PA isolates than those of non-MBL-PA. Resistance to non- - lactam agents was also significantly higher among these isolates (ciprofloxacin, 18.7% and gentamicin, 47.5%).

The results of univariate analysis of risk factors for MBL-PA VAP were shown in Table 2. Eight characteristics including ASIS score, clinical category in admission, prior hospitalization, prolonged stay in the ICU, prolonged time of mechanical ventilation, exposure of carbapenems, fluoroquinolones, use of three or more antibiotics, as well as worst clinical outcome

Table 1. Antimicrobial resistance rates of MBL and non-MBL *P. aeruginosa* isolates to antimicrobial agents.

Antibiotic	MBL-PA (n = 40)	Controls (n = 53)	p
Aztreonam	13 (32.5)	1 (1.9)	<0.001
Cefepime	24 (60.0)	3 (5.6)	<0.001
Ceftazidime	28 (70.0)	3 (5.6)	<0.001
Ciprofloxacin	20 (18.7)	03 (5.6)	<0.001
Imipenem	40 (100.0)	0	<0.001
Piperacillin/tazobactam	13 (32.5)	1 (1.9)	<0.001
Polymyxin B	0	0	-
Gentamicin	19 (47.5)	0	<0.001

Values are number (%).

Table 2. Univariate analysis of risk factors for VAP by metallo - -lactamase- (MBL**)** producing *Pseudomonas aeruginosa.*

Variables	MBL+ (n = 40)	MBL - (n = 53)	OR (95% CI)	p
Age, years[a]	52.87	48.92	0.7 (0.2 - 2.7)	0.99
Sex, male	26(65.0)	37 (69.8)	0.80 (0.33 – 1.93)	0.62
ASIS score[a]	4.60	3.69	2.43 (1.44 – 4.09)	0.001
Admission category clinical	25 (62.5)	17(32.07)	3.53 (1.49 – 8.35)	0.004
Surgical	08 (20.0)	17(32.07)	0.53 (0.20 – 1.39)	0.19
Trauma	07(17.5)	19 (35.85)	0.38 (0.14 – 1.02)	0.08
Immunocompromise	25 (62.5)	28 (52.83)	1.49 (0.64 -3.44)	0.35
Previous surgery	29 (72.5)	35 (66.04)	1.36 (0.55 -3.32)	0.51
Hospitalization within the preceding year	36 (90.0)	35 (66.04)	4.63 (1.42- 15.05)	0.011
Length of ICU stay (days)[a]	47.45	17.20	1.07(1.03-1.10)	<0.001
Duration of mechanical ventilation(days)[a]	44.25	13.07	1.09(1.04-1.13)	<0.001
Presence of ≥ 3 devices	37 (92.5)	44 (83.0)	2.52 (0.63-10.0)	0.19
Antibiotic exposure				
-lactams	40 (100.0)	45 (84.90)	6.93 (0.83-57.91)	0.07
Cephalosporins 3rd/4th generation	34(85.0)	39 (73.6)	2.03 (0.70 – 5.87)	0.19
Carbapenems	26 (65.0)	15 (28.30)	4.70 (1.95 -11.37)	0.001
Fluoroquinolones	17(42.5)	09 (16.9)	3.6 (1.39 – 9.37)	0.008
Vancomycin	22 (55.0)	21 (39.6)	1.86 (0.81 -4.28)	0.14
Aminoglycosídes	14 (35.0)	09 (16.98)	2.63 (1.00 – 6.93)	0.08
Anaerobicidal	12 (30.0)	19 (35.8)	0.77 (0.32 – 1.85)	0.55
≥ 3 antibiotics	29(72.5)	24 (45.28)	3.19 (1.32 – 7.68)	0.010

MBL, metallo- -lactamase; +, producers; - , non-producers, Values are number (%); [a] Median; OR, odds ratio; CI, confidence interval; immunocompromise, dialysis, advanced age - ≥ 60 years, AIDS; Hospitalization within the preceding year, hospitalization in any hospital; anaerobicidal antibiotics, metronidazole, clindamycin.

(crude mortality - 55.0 vs. 24.5%) (OR 3.76; 95% CI 1.55 - 9.09; p-0.03) were significantly associated with MBL-PA VAP. In contrast, remaining variables were not significant. These data were further analyzed by means of logistic regression models. After adjustment for confounding factors the following risk factors continued significant: prior hospitalization and prolonged time of mechanical ventilation (Table 3). Additionally, the mortality was also independently associated with case group (OR 4.95; 95% CI 1.50 -16.35; p-0.008).

DISCUSSION

We conducted a case-control epidemiological study in which 40 inpatients harboring metallo- -lactamase-producing *P. aeruginosa* (MBL-PA) and 53 controls with MBL-negative *P. aeruginosa* (non-MBL-PA) ventilator-associated pneumonia were investigated. The aim of this study was to evaluate the risk factors for VAP by *P. aeruginosa* producing MBL.

In spite of the incidence of imipenem-resistant

Table 3. Multivariate analysis of risk factors for VAP by metallo - -lactamase- (MBL) producing *Pseudomonas aeruginosa*.

Variables	OR (95%CI)	p
Hospitalization within the preceding year	6.91 (1.25 - 38.24)	0.026
Duration of mechanical ventilation (days)	1.09 (1.05 - 1.15)	< 0.001

OR, odds ratio; CI, confidence interval.

P. aeruginosa pneumonia and bacteremia having increased in recent years (Suárez et al., 2009; Rocha et al., 2008), whereas about half of Brazilian *P. aeruginosa* isolates were found to be imipenem-resistant (Sader et al., 2005a, 2005b; Mendes et al., 2004), being 43.9% of these metallo- - lactamases producers (Toleman et al., 2005), there are few data about risk factors for acquiring MBL-PA (Zavascki et al., 2006; Nouér et al., 2005). Recognition of these resistant clones in our institution provides an opportunity to identify risk factors for development of VAP by MBL-PA.

The antimicrobial susceptibility studies clearly had demonstrated MBL-PA not only resistant to -lactams but also frequently resistant to other classes of antibiotics suggesting the involvement of the other antibiotic-resistant mechanisms (Cezário et al., 2009; Hirakata et al., 2003). Our results related to the antimicrobial susceptibility are in agreement with the literature. Besides that all MBL-PA isolates were MDR.

The identification of risk factors for antimicrobial resistance in *P. aeruginosa* could guide clinicians to choose empirical therapeutic options for individual patients (Nouér et al., 2005; Zavascki et al., 2005) . By univariate analysis, we observed that MBL-PA VAP was associated with high-risk ASIS score, clinical category in admission, prior hospitalization, length of ICU stay and of mechanical ventilation, use of three or more antibiotics, use of carbapenems and fluoroquinolones. These findings are consistent with those of other studies (Zavascki et al., 2006; Nouér et al., 2005; Cezário et al., 2009). Neverthless, the multivariate logistic regression analysis showed that patients with MBL- PA VAP were more likely to have been exposed to prior hospitalization and prolonged time of mechanical ventilation.

Prior hospitalization in our hospital or in other institutions was an independent risk factor for VAP by MBL-PA , probably owing the detection of the MBL-PA in our ICU previously (Cezário et al., 2009), suggesting that cross-transmission could have occurred at that unit, despite the adoption of contact isolation.

Our findings pointed out that the patients in our ICU who have been exposed to prolonged mechanical ventilation were associated with factors that lead to the acquisition of MBL-PA, as described by other authors with bla_{VIM} positive bacteria (Horianopoulou et al., 2006). One possible explanation for these findings is that patients who are in ICU or in mechanical ventilation for longer periods of time have increased exposure to nosocomial pathogens MDR, as MBL-PA, and, subsequently, are at increased risk for colonization and development of infections with these organisms.

The selective pressure imposed by antibiotic use, especially to -lactams, remains an important risk factor for infections by antibiotic-resistant *P. aeruginosa*, creating a microbiologically favorable environment for bacterial strains and selecting resistant clones (Zavascki et al., 2006; Nouér et al., 2005). Our findings fail to show specific antibiotics as fluoroquinolones and -lactams as risk factor for the acquisition of MBL-PA, as reported by other studies (Zavascki et al., 2006; Nouér et al., 2005), this antibiotics were associated with MBL-PA VAP just by univariable analysis.

Infections caused by resistant organisms result in higher rates of mortality due to the negative effect of delayed administration of appropriate antibiotic therapies have been highlighted in patients with serious infections in ICUs (Ramphal, 2005). In the present study we found higher rates of crude mortality (55.0% vs. 24.5%) for patients infected with MBL-PA by univariate and multivariate analysis. It was suggested that MBL-PA isolates presented higher virulence than non-MBL-PA ones, based on higher mortality rates among MBL-PA patients as showed the literature (Kirakata et al., 2003; Laupland et al., 2005), additionally the more frequent inappropriateness of antimicrobial therapy for MBL-PA infections.

The potential limitations of this study are its retrospective nature and limited sample size, as other studies (Nouér et al., 2005), when considering mortality as an outcome measure. Secondly we used patients with non-MBL-PA as controls, a design that tends to overestimate the magnitude of the effect of antibiotic exposure, as showed the literature (Harris et al., 2001; Zavascki, 2004) . Additionally, the study was performed in a single center, raising the possibility of institutional bias either in patient selection or in other institutional practices.

In conclusion, we observed that prior hospitalization and length of mechanical ventilation were risk factors significantly associated to MBL-PA VAP after forward logistic regression in the multivariate analysis. Nevertheless, the mortality was significantly higher in this group, due to the poor prognostic, deserving attention and further investigations. Early identification of patients

with risk factors favoring antibiotic-resistant infections such as: more severe illness, prior hospitalization, long duration of mechanical ventilation as well as the knowledge of the bacterial ecology of the unit are important when initiating an empiric regimen covering these highly resistant organisms and could guide the development of strategies to prevent the dissemination of MBL-PA strains.

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