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Risk Factors for Acquisition of Multidrug-Resistant *Pseudomonas aeruginosa* Producing SPM Metallo-β-Lactamase

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To evaluate risk factors for colonization or infection due to multidrug-resistant *Pseudomonas aeruginosa* (MDRPa) carrying the bla_{SPM} gene (SPM-MRDPa) among hospitalized patients, we undertook a case control study at a 480-bed, tertiary-care university hospital. Two different case definitions were used. In the first definition, a case patient (SPM case patient) was defined as a patient who had at least one isolate of SPM-MDRPa (14 patients). In the second, a case patient (non-SPM case patient) was defined as a patient who had at least one isolate of non-SPM-MDRPa (18 patients). For each case patient, we selected two controls, defined as a patient colonized and/or infected by a non-MDRPa isolate during the same study period and with the closest duration of hospitalization until the isolation of *P. aeruginosa* as cases. The use of quinolones was the single independent predictor of colonization and/or infection by bla_{SPM} MDRPa (odds ratrio [OR] = 14.70, 95% confidence interval [95% CI] = 1.70 to 127.34, *P* = 0.01), whereas the use of cefepime was the single predictor of colonization and/or infection by non-bla_{SPM} MDRPa (OR = 8.50, 95% CI = 1.51 to 47.96, *P* = 0.01). The main risk factor for MDRPa was a history of antibiotics usage. Stratification of risk factor analysis by a precise mechanism of resistance led us to identify a specific antibiotic, a quinolone, as a predictor for SPM-MDRPa.

Over the past few years, a notable increase in antibiotic resistance among gram-negative bacteria recovered from hospitalized patients has been reported, especially for critically ill patients (12). Infections caused by multidrug resistant (MDR) gram-negative bacteria, especially MDR *Pseudomonas aeruginosa* (MDRPa) have been associated with increased morbidity, mortality, and costs (28, 30). In Latin America, *P. aeruginosa* is a major cause of nosocomial infections, ranking first in nosocomial pneumonia, second in wound infections, third in urinary tract infections, and fifth in bacteremia (1, 29). Infections caused by *P. aeruginosa* are particularly challenging because this organism has a natural susceptibility to a very limited number of antimicrobial agents.

β-Lactam resistance in *P. aeruginosa* may result from production of various β-lactamases, including metallo-β-lactamases (Mbla) (26). These enzymes are clinically relevant because of their systematic hydrolysis of carbapenems and the association of Mbla genes with mobile genetic elements, increasing the possibility of rapid spread (23, 35). Indeed, Mbla genes have spread among *P. aeruginosa* and *Enterobacteriaceae* isolates throughout southeast Asia (21, 41), Europe (11, 40), Australia (33), the United States (37), and Canada (16). Recently, a new Mbla gene, bla_{SPM} (for São Paulo metallo-β-lactamase), was identified in an epidemic MDRPa strain (SPM-MDRPa) disseminated among 12 Brazilian hospitals (13, 35). We detected this same strain among *P. aeruginosa* isolates from Hospital Universitário Clementino Fraga Filho, a public hospital, and three other private hospitals in Rio de Janeiro, Brazil, between 1999 and 2000 (34). These isolates had a unique DNA pattern by pulsed-field gel electrophoresis (PFGE) and were highly resistant to antimicrobial agents, being consistently susceptible only to polymyxin. While little is known about the biology and epidemiology of bla_{SPM} acquisition, the development of measures to prevent the dissemination of MDRPa strains in Brazilian hospitals represents a major public health challenge. Recognition of these resistant clones in our population provides a unique opportunity to identify risk factors for their acquisition and dissemination.

Previously reported risk factors for colonization and invasive disease caused by *P. aeruginosa*-resistant strains include antimicrobial use, previous hospitalization, severity of illness, surgery, and immunosuppression (2, 5, 20, 39).

The acquisition of resistant bacteria in the hospital may be a consequence of selective pressure exerted by the use of antibiotics and/or horizontal dissemination. The distinction between these two mechanisms may be made by the use of molecular typing: a unique genotype is expected to be present in horizontal transmission, and multiple clones are detected when selective pressure is the major mechanism (32). Most of the studies published so far assessed risk factors for MDRPa without distinction of the genotype of the isolate. The objective of the present study was to evaluate if risk factors for nosocomial

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colonization or infection by MDRPa differed among patients carrying SPM-MDRPa or non-SPM-MDRPa isolates. We were especially interested in investigating if previous use of a specific antibiotic (or class of antibiotics) would be a predictor for the isolation of an SPM-MDRPa or a non-SPM-MDRPa isolate from a patient with a *P. aeruginosa* infection.

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MATERIALS AND METHODS

The study was performed at the Hospital Universitário Clementino Fraga Filho, a 480-bed public tertiary-care teaching hospital in Rio de Janeiro, Brazil, with ~120,000 patient-days per year. The study was approved by the Ethical Committee of the Hospital. A surveillance study was conducted with the microbiology laboratory database of the hospital to identify all positive cultures for *P. aeruginosa* between March 1999 and April 2000. *P. aeruginosa* was recovered from various biological materials, collected according to the discretion of the attending physicians. No routine surveillance culture was performed during this period. MDRPa was defined as an isolate resistant to at least eight of the following antibiotics: amikacin, aztreonam, ceftazidime, cefepime, ciprofloxacin, gentamicin, imipenem, meropenem, piperacillin-tazobactam, and ticarcillin-clavulanate. MDRPa isolates were further characterized as carrying the bla_{SPM} gene or not, as described under microbiological procedures.

An infectious disease physician evaluated all the patients and classified the *P. aeruginosa* isolate according to established diagnostic criteria for nosocomial infections (Centers for Disease Control and Prevention) (15). Colonization was defined when these criteria were not fulfilled. When the isolate was determined as causing colonization but the attending physician prescribed an antimicrobial agent, the patient was classified as having a possible infection. The overall use of the different classes of antibiotics did not change during the study period and was equally distributed through all hospital wards.

To identify risk factors associated with the acquisition of MDRPa and particularly MDRPa expressing the bla_{SPM} gene, we performed a case control study using two different case definitions. In the first analysis, a case was defined as a patient who had at least one isolate of MDRPa carrying the bla_{SPM} gene (SPM case patient). In the second analysis, a case was defined as a patient who had at least one isolate of MDRPa that did not carry the bla_{SPM} gene (non-SPM case patient). For each case patient, we selected two controls, defined as patients colonized and/or infected by a non-MDRPa isolate during the same study period and with the closest duration of hospitalization until the isolation of *P. aeruginosa*, as cases. Only one *P. aeruginosa* isolate per patient was considered for this analysis. If more than one *P. aeruginosa* isolate was recovered from the same patient, we selected only one isolate, in the following preferential order: first, an SPM-MDRPa isolate; second, a non-SPM-MDRPa isolate; and third, a non-MDRPa isolate. If a patient had only non-MDRPa isolates, the first *P. aeruginosa* isolate was considered.

Microbiological procedures. Bacterial isolates were identified with the GNI VITEK system card (BioMérieux Vitek, Inc., Hazelwood, Mo.) and by conventional biochemical tests (22). Antimicrobial susceptibility was determined by disk diffusion in accordance with CLSI (formerly NCCLS) guidelines (27). Interpretation of zone diameters obtained for polymyxin B followed a protocol suggested by Gales et al. (14). Evaluation of chromosomal polymorphisms was performed by PFGE, as described previously, with SpeI used to digest the DNA (34). Banding patterns were interpreted by visual inspection and with the GelCompar II program, version 3.5 (Applied Maths, Kortrijk, Belgium) using the Dice index and the unweighted-pair group method with arithmetic averages. The bla_{SPM} gene was detected by PCR (13) and colony blotting (F. L. P. C. Pellegrino et al., submitted for publication). Carbapenemase activity was evaluated by a biological method (6).

Collection of data. Data were collected by reviews of the patients' medical charts and the laboratory database. The following variables were collected and analyzed: age, gender, underlying disease, clinical specimen from which *P. aeruginosa* was isolated, length of hospital stay, transfer from another hospital, invasive procedures in the preceding week prior to *P. aeruginosa* isolation, admission to an intensive care unit, and antibiotic exposure. Previous hospitalization or home care assistance for the preceding year were examined.

Statistical analysis. We compared the characteristics of cases and controls by univariate analysis, and variables with P values of <0.05 were entered in a logistic regression analysis. The chi-square or Fisher's exact test was used for the comparison of dichotomous variables, and continuous variables were compared by the Wilcoxon test. The odds ratio (OR) and the 95% confidence intervals (95% CI) were calculated. All tests were two tailed, and P values of <0.05 were considered significant. The statistical analyses were performed using Epi-Info, version 6.04b (Centers for Disease Control and Prevention, Atlanta, Ga.) and SPSS 10.0 for Windows (SPSS, Inc., Chicago, Ill.).

RESULTS

During the study period, we identified 32 MDRPa isolates from 32 patients. In 18 patients (56%), the isolate was classified as causing infection, whereas in 10 patients (31%), the isolates were classified as colonization, and in 4 patients (12%), the isolates were classified as possible infections. A surgical wound was the most frequent source (11 isolates), followed by blood (5 isolates) and urine (3 isolates). All MDRPa isolates were resistant to carbapenems. Fourteen (44%) of the 32 patients with MDRPa were colonized or infected by isolates harboring the bla_{SPM} gene, all presenting carbapenemase activity (SPM case patients), and 18 patients were colonized or infected by isolates that did not express the bla_{SPM} gene and did not reveal carbapenemase activity (non-SPM case patients). By PFGE, all SPM isolates had a unique pattern that we called genotype A. The isolates from the 18 non-SPM patients belonged to nine different genotypes, including three isolates with genotype A.

SPM case patients were found across seven hospital wards, but 7 of the 14 patients were hospitalized in the same ward during a 2-month period (January and February 2000). Twelve patients had been hospitalized previously: 7 at the Hospital Universitário Clementino Fraga Filho and 5 in other hospitals. Ten SPM case patients were immunosuppressed: 5 were solid organ transplant recipients (4 renal and 1 liver transplant recipients), 4 had leukemia and had received chemotherapy (1 underwent hematopoietic stem cell transplantation), and 1 had AIDS. In 8 of the 14 SPM case patients, the *P. aeruginosa* isolate was considered infection; in isolates from 3 patients, the isolate represented colonization; and in isolates from 3 patients, the isolates were considered possible infections.

Table 1 shows the univariate analysis of cases and controls. In the first analysis, the 14 SPM case patients were compared to 28 control (non-MDRPa) patients. Compared to the controls, SPM case patients were more likely to have chronic renal failure, to have received solid organ transplant or dialysis, to be immunosuppressed, to have drains, and to have been hospitalized in the preceding year. In addition, SPM case patients were more frequently exposed to the following antibiotics: narrow-spectrum cephalosporins, cefepime, quinolones, and vancomycin. The median duration of antibiotic use until the isolation of *P. aeruginosa* was significantly longer in SPM case patients. By multivariate analysis, previous exposure to quinolones (OR = 14.70, 95% CI = 1.70 to 127.34, $P = 0.01$) was the single variable associated with colonization or infection by MDRPa isolates harboring the bla_{SPM} gene.

In the second analysis, 18 patients with MDRPa isolates that did not harbor the bla_{SPM} gene (non-SPM case patients) were compared to 36 control patients with non-MDRPa isolates. There were no differences between cases and controls with regard to demographics, underlying conditions, or coexisting exposures. Regarding the exposure to antibiotics, case patients were more likely to have received cefepime, carbapenems, and

TABLE 1. Univariate analysis of risk factors for colonization and/or infection by bla_{SPM} multidrug-resistant *Pseudomonas aeruginosa* (MDRPa) and non-bla_{SPM} MDRPa

Variable	First analysis[c]				Second analysis[c]			
	SPM patients (n = 14)	Controls (n = 28)	P value	OR (95% CI)	Non-SPM patients (n = 18)	Controls (n = 36)	P value	OR (95% CI)
Categorical[a]								
Gender (male:female)	7:7	17:11	0.50	0.65 (0.18–2.36)	12:6	17:19	0.18	2.24 (0.69–7.26)
Underlying condition								
Cancer	4 (28)	7 (25)	1.00	1.20 (0.28–5.07)	5 (28)	9 (25)	1.00	1.15 (0.32–4.14)
Chronic renal failure	5 (36)	0	0.002	NA	1 (5.5)	3 (8)	1.00	0.65 (0.06–6.70)
Diabetes	1 (7)	3 (11)	1.00	0.64 (0.06–6.79)	1 (5.5)	5 (14)	0.65	0.36 (0.04–3.38)
Cardiac disease	2 (14)	4 (14)	1.00	1.00 (0.16–6.26)	4 (22)	9 (25)	1.00	0.86 (0.22–3.28)
Pulmonary disease	2 (14)	3 (11)	1.00	1.39 (0.20–9.45)	2 (11)	5 (14)	1.00	0.77 (0.13–4.45)
Neurologic disease	2 (14)	8 (28)	0.45	0.42 (0.07–2.30)	3 (17)	10 (28)	0.51	0.52 (0.12–2.19)
Organ transplant	6 (45)	0	0.0006	NA	2 (11)	2 (5)	0.59	2.12 (0.27–16.50)
Coexisting exposure								
Immunosuppression	10 (71)	4 (14)	0.0004	15.00 (3.12–72.10)	5 (28)	8 (22)	0.74	1.35 (0.37–4.92)
Surgery	6 (45)	5 (36)	0.13	3.45 (0.82–14.50)	2 (11)	9 (25)	0.30	0.37 (0.07–1.96)
Central venous catheter	9 (64)	11 (39)	0.13	2.78 (0.73–10.50)	7 (39)	15 (42)	0.84	0.89 (0.28–2.83)
Mechanical ventilation	3 (21)	8 (28)	0.72	0.68 (0.15–3.11)	6 (33)	9 (25)	0.52	1.50 (0.43–5.17)
Dialysis	4 (28)	1 (3.5)	0.03	10.80 (1.07–109)	1 (5.5)	3 (8)	1.00	0.65 (0.06–6.70)
Foley catheter	7 (50)	10 (38)	0.37	1.80 (0.49–6.62)	9 (50)	17 (47)	0.88	1.12 (0.36–3.47)
Drains	8 (57)	6 (21)	0.04	4.89 (1.22–19.70)	3 (17)	10 (28)	0.51	0.52 (0.12–2.19)
Any invasive procedure	13 (93)	20 (71)	0.23	5.20 (0.58–46.60)	13 (72)	28 (78)	0.74	0.74 (0.20–2.72)
Continuous nurse assistance	7 (50)	9 (32)	0.26	2.11 (0.57–7.86)	7 (39)	14 (39)	1.00	1.00 (0.31–3.19)
Hospitalization within the preceding yr	11 (78)	7 (25)	0.0009	11.00 (2.37–51.10)	7 (39)	10 (28)	0.41	1.65 (0.50–5.47)
Use of antibiotics								
Any	14 (100)	21 (75)	0.07	NA	17 (94)	28 (78)	0.24	4.86 (0.56–42.30)
Narrow-spectrum cephalosporin	5 (36)	1 (3.5)	0.01	15.00 (1.54–146)	2 (11)	2 (5)	0.59	2.12 (0.27–16.50)
Broad spectrum or cephalosporin	6 (45)	9 (32)	0.49	1.58 (0.42–5.94)	8 (44)	14 (39)	0.69	1.26 (0.40–3.95)
Cefepime	5 (36)	2 (7)	0.03	7.22 (1.19–44.00)	6 (33)	2 (5)	0.01	8.50 (1.51–48.00)
Any cephalosporin	10 (71)	14 (50)	0.18	2.50 (0.63–9.90)	12 (67)	19 (53)	0.33	1.79 (0.55–5.81)
Broad spectrum or cefepime	9 (64)	11 (39)	0.13	2.78 (0.73–10.50)	12 (67)	16 (44)	0.12	2.50 (0.77–8.14)
Carbapenem	4 (28)	2 (7)	0.15	5.20 (0.82–33.00)	7 (39)	4 (11)	0.03	5.09 (1.25–20.80)
Quinolone[b]	8 (57)	2 (7)	0.0008	17.30 (2.91–103)	5 (28)	6 (17)	0.47	1.92 (0.50–7.44)
Aminoglycoside	2 (14)	3 (11)	1.00	1.39 (0.20–9.45)	7 (39)	6 (17)	0.10	3.08 (0.84–11.20)
Vancomycin	7 (50)	4 (14)	0.02	6.00 (1.35–26.60)	8 (44)	5 (14)	0.02	4.96 (1.32–18.70)
Continuous[a]								
Age (years), median (range)	48.5 (11–88)	50 (12–97)	0.62	NA	58 (22–85)	52 (12–97)	0.51	NA
Median no. of days (range) to *P. aeruginosa* isolation	21.5 (1–69)	17 (0–89)	0.67	NA	29 (0–127)	22 (0–115)	0.35	NA
Median no. of days (range) of antibiotic use until *P. aeruginosa* isolation	28 (1–62)	7.5 (0–170)	0.02	NA	22.5 (0–55)	9 (0–170)	0.03	NA

[a] Categorical variables are shown as number (percentage) unless otherwise stated. Continuous variables are median days (range). NA: not applicable.
[b] Quinolones used were ciprofloxacin, norfloxacin, ofloxacin, and pefloxacin.
[c] Risk factors by multivariate analysis are as follows: for the first analysis, use of quinolones (OR = 14.70, 95% CI = 1.70 to 127.34, P = 0.01); for second analysis, use of cefepime (OR = 8.50, 95% CI = 1.51 to 47.96, P = 0.01).

vancomycin. Multivariate analysis showed that the use of cefepime was associated with non-SPM-MDRPa isolates (OR = 8.50, 95% CI = 1.51 to 47.96, P = 0.01).

DISCUSSION

This is the first attempt to characterize the clinical and epidemiological context of colonization and invasive disease by *P. aeruginosa* isolates expressing the bla_{SPM} gene, which is widely disseminated among Brazilian hospitals. We observed that bla_{SPM} isolates accounted for a substantial number of MDRPa cases (44%), and most of these isolates were clinically relevant, since 57% of the patients were considered to have invasive disease.

Half of the SPM case patients were clustered in a 2-month period in a single ward. In addition, most of the patients had been previously admitted to our hospital (seven patients) or to other institutions (five patients). In a previous paper, we identified patients with colonization or invasive disease by bla_{SPM} from three other hospitals in Rio de Janeiro during the same period as our cases (34). Taken together, these data suggest that cross-transmission between patients and the transfer of patients between institutions may have played a major role in the dissemination of bla_{SPM}. Indeed, after the identification of the problem, infection control measures were implemented (basically, educational aspects); in a subsequent analysis of 107 isolates between October 2002 and August 2003, no SPM-MDRPa isolate was identified (Pellegrino et al., submitted).

In the present study, we used as controls patients colonized and/or infected with susceptible isolates of *P. aeruginosa* (non-MDRPa isolates) because we sought to investigate the probability that a patient with an isolate of *P. aeruginosa* had an MDRPa isolate. This information would be a useful guide for selection of empirical antibiotic treatment and early institution of contact precautions. By univariate analysis, we observed that bla_{SPM} colonization and/or infection was associated with immunosuppression, receipt of hemodialysis, and hospitalization in the preceding year. On the other hand, for non-SPM-MDRPa isolates, there were no differences among cases and controls regarding underlying conditions or invasive procedures. By multivariate analysis, risk factors for SPM and non-SPM-MDRPa differed but in both situations involved the use of antibiotics: quinolones for the acquisition of SPM-MDRPa (bla_{SPM}) and cefepime for non-SPM-MDRPa. It is possible that quinolones could select for other resistance mechanisms present in Mbla-producing isolates and thus indirectly select for SPM-MDRPa. On the other hand, to our knowledge, the use of cefepime has not been identified as a risk factor for resistant *P. aeruginosa*. Exposure to various antibiotics has been associated with MDRPa, including carbapenems (4, 5, 18, 20, 38), piperacillin-tazobactam (18, 20), vancomycin (18), aminoglycosides (18, 20), cephalosporins (18), ceftazidime (36), and quinolones (3, 8, 31, 39). The mechanisms of resistance of MDRPa in these studies were not defined.

The mechanism by which the use of a specific antibiotic predisposes to the development of infection by resistant organisms is not well known. One explanation for the association between antibiotic exposure and MDRPa is that the antibiotic itself has a potential to induce or select for resistance. It is well known that previous exposure to a particular antibiotic is associated with the acquisition of resistance to itself (10) or to antibiotics belonging to a different class (7, 25). This is particularly true for *P. aeruginosa*, which is a pathogen harboring multiple mechanisms of resistance (24). On the other hand, it would be expected that in the case of colonization or infection by bla_{SPM}, antibiotic pressure could play a lesser role, since horizontal transmission would be the main mechanism of acquisition (32). However, it is possible that exposure to a particular antibiotic increases the burden of colonization by a clonal strain, increasing the possibility of horizontal transmission. This phenomenon has been reported with vancomycin-resistant enterococci and the use of antianaerobic antibiotics (9).

Our study has some limitations. First, since we did not perform surveillance cultures, we cannot rule out the possibility that some of the control subjects might have been colonized by MDRPa and should have been classified as case patients instead of controls. Second, since our controls were those with susceptible strains of *P. aeruginosa*, they were much less likely to have been exposed to antibiotics that are active against this organism. Therefore, it is possible that the OR is overmagnified in this analysis, as discussed by Harris et al. (17, 19). However, since our main interest was to evaluate the probability that a patient with *P. aeruginosa* had an MDRPa isolate, the most appropriate control group seemed to be non-MDRPa patients. Another limitation of our study is that since most cases were clustered in place, it is possible that other variables associated with breakdowns in infection control measures played a role in the acquisition of bla_{SPM}. Also, because of the small number of patients with bla_{SPM}, we cannot rule out the possibility of a beta error in some analyses. On the other hand, our study seems to be the first to analyze by multivariate analysis the clinical scenario associated with a highly prevalent metallo-β-lactamase-producing *P. aeruginosa* strain.

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