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Bloodstream Infections with Metallo-β-Lactamase-Producing *Pseudomonas aeruginosa*: Epidemiology, Microbiology, and Clinical Outcomes

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***Pseudomonas aeruginosa* strains that produce metallo-β-lactamases (MBLs) are becoming increasingly prevalent. We evaluated the epidemiological and microbiological characteristics of monomicrobial bloodstream infections caused by MBL-producing *P. aeruginosa* isolates, as well as the clinical outcomes in patients with these infections.**

Since their first description in the early 1990s (14), metallo-β-lactamase (MBL)-producing organisms have been detected in many parts of the world (1, 8, 15, 18). MBL-producing *Pseudomonas aeruginosa* strains have been reported to be important causes of nosocomial infections (4, 8, 13). While the prevalence of these strains in hospitals is still unknown, they have been reported to be associated with clonal spread and nosocomial outbreaks (4, 8, 18). The appearance of MBL genes and their spread among bacterial pathogens are matters of major concern with regard to the future of antimicrobial chemotherapy (2). It is well known that a poor outcome occurs when patients with serious infections due to MBL-producing organisms are treated with antibiotics to which the organism is completely resistant (8, 15). Data extrapolated from in vitro studies suggest that polymyxin B or colistin represents the best treatment options (10).

Carbapenems are considered indicator drugs for the detection of resistance mechanisms in *Pseudomonas aeruginosa* infections (11). Due to the high prevalence of imipenem resistance noted among *P. aeruginosa* isolates causing bloodstream infections (BSIs) at our hospital, we decided to investigate whether MBL production was directly associated with this resistance mechanism and to analyze the epidemiology of these infections.

This study was carried out at the Universidade Federal de São Paulo, a 624-bed university hospital located in the state of São Paulo, Brazil. All patients for whom blood culture results were positive for *P. aeruginosa* from January 2000 to May 2002 were eligible for inclusion in the study. Each patient was included only once at the time of the first BSI. Polymicrobial BSIs and BSI episodes that represented relapses were excluded. Mortality related to bacteremia was considered present when a patient died within 14 days of the start of treatment and the death could not be directly attributed to any other cause.

Data for patients with BSIs due to a MBL genotype-producing *P. aeruginosa* isolate were collected by reviews of the patient's medical charts and the laboratory database. The data abstracted included age, sex, the number of hospital days prior to infection, the length of the hospital stay, the underlying disease, and antibiotics prescribed for at least 48 h in the 15 days prior to the onset of the BSI (17). The severity of illness was classified by using the Simplified Acute Physiology Score (SAPS) (9). The sources of infection were defined according to Centers for Disease Control and Prevention criteria (7). For the treatment of MBL-producing *Pseudomonas* BSIs, only polymyxin B or colistin was classified as adequate antimicrobial therapy.

Blood cultures were processed by using the BACTEC 9240 blood culture system (Becton Dickinson, Sparks, MD). Organisms were identified according to routine bacteriological procedures. Susceptibility testing was performed by the disk diffusion method, following the recommendations of the CLSI (formerly the National Committee for Clinical Laboratory Standards) (13). MIC determinations were performed for all blood isolates by broth microdilution techniques (13). Isolates were considered susceptible to colistin if the MIC was ≤ 2 μg/ml (6). *P. aeruginosa* isolates were also screened for the presence of the MBL phenotype by using EDTA and 2-mercaptopropionic acid (2-MPA) for MBL inhibition (1). In addition, the carbapenemase activities of cell sonicates from overnight broth cultures were determined by spectrophotometric assays (3). All strains of *P. aeruginosa* were tested by PCR analysis to confirm the presence of the bla_{IMP}, bla_{VIM}, and bla_{SPM} genes (5, 15, 18). The primers yielded PCR products, and both strands were sequenced on a Perkin-Elmer system 377 DNA sequencer (12).

During the study, 96 patients with *P. aeruginosa* BSIs were identified, of whom 76 had monomicrobial infections. The MBL phenotype was detected in *P. aeruginosa* isolates from 23

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TABLE 1. Correlation between the MBL genotypes and antimicrobial susceptibility profiles

Genotype	Antimicrobial MIC (μg/ml) (susceptibility[a])						
	Gentamicin	Amikacin	Ciprofloxacin	Aztreonam	Cefepime	Imipenem	Colistin
bla_{IMP-16}	>16 (R)	16 (R)	>4 (R)	32 (R)	>32 (R)	16 (R)	<0.5 (S)
bla_{IMP-1}	<2 (S)	<8 (S)	>4 (R)	16 (R)	8 (R)	16 (R)	<0.5 (S)
bla_{IMP-1}	<2 (S)	<8 (S)	>4 (R)	16 (R)	16 (R)	16 (R)	<0.5 (S)
bla_{SPM-1}	>16 (R)	>64 (R)	>4 (R)	8 (S)	>32 (R)	>32 (R)	1 (S)
bla_{SPM-1}	>16 (R)	>64 (R)	>4 (R)	8 (S)	>32 (R)	>32 (R)	1 (S)
bla_{SPM-1}	>16 (R)	>64 (R)	>4 (R)	>32 (R)	>32 (R)	>32 (R)	0.5 (S)
bla_{SPM-1}	>16 (R)	>64 (R)	>4 (R)	16 (R)	>32 (R)	>32 (R)	1 (S)

[a] R, resistant; S, susceptible.

of the 76 patients (30.3%) by using EDTA. The production of MBL was detected in only 6 of these 23 strains by using 2-MPA. Carbapenem hydrolysis was detected in 30.4% (7 of 23) of the EDTA-positive isolates and in all 4 isolates positive by the 2-MPA test.

Of the 76 *P. aeruginosa* isolates included in this study, MBL gene bla_{SPM-1} was the most prevalent, being recovered from four (5.3%) of the *P. aeruginosa* BSI strains, followed by the bla_{IMP-1} gene (two strains [2.6%]) and the bla_{IMP-16} gene (one strain [1.3%]). No bla_{VIM} MBL gene was detected. Details of antimicrobial susceptibility testing are shown in Table 1. Among the *P. aeruginosa* isolates causing polymicrobial BSIs that were excluded from the analysis (20.8%), only one MBL gene (bla_{SPM-1}) was identified. The strain producing that MBL was detected by both phenotypic methods (EDTA and 2-MPA) and carbapenem hydrolysis.

The clinical characteristics and outcomes of the seven MBL genotype-producing *P. aeruginosa* patients are presented in Table 2. The mean age was 63 ± 13 years (age range, 46 to 80 years). Fifty-seven percent of these patients were male. The most frequent diagnoses responsible for hospitalization were solid and hematologic malignancies (42.9%) and gastrointestinal diseases (28.6%). The most frequent sources of BSIs were gastrointestinal (42.9%). Most BSIs (57.1%) occurred before 21 days of hospitalization. The mean duration of hospitalization was 43.8 ± 37.4 days (range, 13 to 117 days). Among these patients, 85.7% were receiving antimicrobial therapy before the BSI. Before the BSI, cephalosporins (42.9%) were the most prescribed antibiotic. Seventy-one percent of the patients infected with an MBL-producing isolate (71.4%) received inadequate empirical therapy. The majority (71.4%) of the patients infected with an MBL-producing isolate had a SAPS score <40. Mortality within 14 days after the BSI occurred in 71.4% of the patients infected with an MBL-producing isolate. The crude (overall, in-hospital) mortality of MBL patients was 85.7%.

In conclusion, the overall rates of morbidity and mortality among patients infected with MBL-producing *P. aeruginosa* BSI is high. Notably, in our study, 81.1% of the strains were resistant to imipenem without MBL production. This demonstrates that other resistance mechanisms are involved, such as permeability mutations via the loss of porins or the up-regulation of efflux systems (10, 16). In addition, it is necessary to identify MBL-producing *P. aeruginosa* isolates in BSIs so that appropriate treatment will be provided and to better understand the microbiological characteristics and epidemiology of MBL-producing strains in other institutions with a high prevalence of imipenem-resistant *P. aeruginosa* strains.

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TABLE 2. Characteristics of the seven patients with MBL genotype-producing *P. aeruginosa* isolates

Patient no.	Age (yr)	Sex[a]	Diagnosis	Site of infection	Hospital stay prior to BSI (days)	Hospital stay (days)	SAPS score	Previous antibiotic treatment	Response to antibiotic therapy	DNA sequencing result	Outcome
1	46	M	Gastrointestinal disease	Gastrointestinal	8	28	30	Quinolone	Inadequate	IMP-16	Death (related to BSI)
2	63	F	Hematologic malignancy	Catheter	20	32	31	None	Inadequate	IMP-1	Death (related to BSI)
3	61	F	Neurologic disease	Respiratory	41	117	32	Carbapenem	Adequate	IMP-1	Survival
4	71	M	Hematologic malignancy	Respiratory	22	22	56	Cephalosporin	Inadequate	SPM-1	Death (related to BSI)
5	74	M	Renal failure	Catheter	33	72	44	Carbapenem	Adequate	SPM-1	Death (not related to BSI)
6	80	M	Gastrointestinal disease	Gastrointestinal	0	13	37	Cephalosporin	Inadequate	SPM-1	Death (related to BSI)
7	49	F	Hematologic malignancy	Gastrointestinal	21	23	24	Cephalosporin	Inadequate	SPM-1	Death (related to BSI)

[a] M, male; F, female.

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