Enferm Infecc Microbiol Clin. 2009;**27(5)**:269–274

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc

Original

Nosocomial outbreak by imipenem-resistant metallo-β-lactamase-producing *Pseudomonas aeruginosa* in an adult intensive care unit in a Brazilian teaching hospital

Renata Cristina Cezário [a,*], Lea Duarte De Morais [b], Joseane Cristina Ferreira [c], Rogério M. Costa-Pinto [d], Ana Lúcia da Costa Darini [d] and Paulo P. Gontijo-Filho [a]

[a] *Area Immunology, Parasitology and Microbiology at Universidade Federal de Uberlândia, Uberlândia-Minas Gerais, Brazil*
[b] *Microbiology Laboratory of Hospital de Clinicas da Universidade Federal de Uberlândia, Uberlândia-Minas Gerais, Brazil*
[c] *Faculty of Mathematics, Statistical and Biometric Studies at Universidade Federal de Uberlândia, Uberlândia-Minas Gerais, Brazil*
[d] *Clinical, Toxicologic and Bromatologic Analyses of Pharmaceutical Sciences at Universidade de São Paulo/USP, Ribeirão Preto, Brazil*

INFORMACIÓN DEL ARTÍCULO

Historia del artículo:
Recibido el 25 de enero de 2008
Aceptado el 1 de septiembre de 2008
Available online 21 de abril de 2009

Keywords:
Nosocomial outbreak
Imipenem-resistant *P. aeruginosa*
Metallo-β-lactamase
Intensive care unit

A B S T R A C T

Objective: To describe an outbreak of imipenem-resistant metallo-β-lactamase-producing *Pseudomonas aeruginosa*, enzyme type *bla*, by horizontal transmission in patients admitted to a mixed adult ICU.

Methods: A case-control study was carried out, including 47 patients (cases) and 122 patients (control) admitted to the mixed ICU of a university hospital in Minas Gerais, Brazil from November 2003 to July 2005. The infection site, risk factors, mortality, antibiotic susceptibility, metallo-β-lactamase (MBL) production, enzyme type, and clonal diversity were analyzed.

Results: A temporal/spatial relationship was detected in most patients (94%), overall mortality was 55.3%, and pneumonia was the predominant infection (85%). The majority of isolates (95%) were resistant to imipenem and other antibiotics, except for polymyxin, and showed MBL production (76.7%). Only *bla SPM-1* (33%) was identified in the 15 specimens analyzed. In addition, 4 clones were identified, with a predominance of clone A (61.5%) and B (23.1%). On multivariate analysis, advanced age, mechanical ventilation, tracheostomy, and previous imipenem use were significant risk factors for imipenem-resistant *P. aeruginosa* infection.

Conclusions: Clonal dissemination of MBL-producing *P. aeruginosa* strains with a spatial/temporal relationship disclosed problems in the practice of hospital infection control, low adherence to hand hygiene, and empirical antibiotic use.

Brote nosocomial causado por *Pseudomonas aeruginosa* resistente a imipenem productora de metalo-β-lactamasa en unidad de cuidados intensivos para adultos de un hospital universitario brasileño

Palabras clave:
Brote nosocomiales
Pseudomonas aeruginosa resistente la imipenem
Metallo-β-lactamase
Unidad de cuidados intensivos

R E S U M E N

Objetivo: Describir un brote causado por *Pseudomonas aeruginosa* resistente a imipenem productora de metallo-β-lactamasa (MBL), tipo de bla de la enzima, con transmisión horizontal de la muestra epidémica en pacientes ingresados en una unidad de cuidados intensivos para adultos mixta.

Métodos: Durante el período comprendido entre noviembre de 2003 y julio de 2005, se realizó un estudio de casos y controles en el que se incluyó a 47 (casos) y 122 (control) pacientes en una unidad de cuidados intensivos para adultos mixta, de un hospital universitario de Uberlândia (Minas Gerais [Brasil]). Se analizaron los sitios de la infección, los factores de riesgo, la mortalidad total, la susceptibilidad a los antibióticos, la producción del tipo MBL, de la enzima y de la diversidad clonal.

Resultados: En la mayoría de los pacientes (94%) se detectó una relación temporal/espacial. El índice de mortalidad total fue del 55,3% y la neumonía era la infección predominante (85%). La mayoría de las cepas (95%) era resistente a imipenem y a otros antibióticos, excepto al polimixin y la producción de MBL (76,7%). Únicamente se identificó bla_{SPM-1} en los 15 especímenes analizados. Además, se detectaron 4 clones, con predominio del clon A (61,5%) y B (23,1%). En análisis multivariados, la vejez, la ventilación mecánica, la traqueotomía y el uso previo del carbapenem son factores de riesgo significativos para el desarrollo de la infección de *P. aeruginosa* resistente a imipenem.

* Autor para correspondencia.
Correo electrónico: cezariorenata@yahoo.com (R.C. Cezário).

0213-005X/$ - see front matter © 2008 Elsevier España, S.L. Todos los derechos reservados.
doi:10.1016/j.eimc.2008.09.009

Conclusiones: La diseminación clonal de cepas de *P. aeruginosa* productora de MBL entre los pacientes con una relación temporal/espacial mostró problemas en el control de la infección del hospital, probablemente relacionado con una adherencia baja a la higiene de las manos y el uso empírico de los antibióticos.

Introduction

Pseudomonas aeruginosa is an opportunistic pathogen that can cause severe invasive disease in critically ill and immunocompromised patients.[1] This microorganism is an important cause of nosocomial infections, including pneumonia, wound infections, bacteremia, and urinary tract infection,[2,3] *P. aeruginosa* is frequent cause of ventilator-associated pneumonia in intensive care units (ICUs), the associated risk factors being mechanical ventilation lasting longer than 7 days, severity of the underlying condition, and previous antibiotic treatment, surgery, or immunosuppression.[3,4]

P. aeruginosa is a uniquely problematic microorganism because of a combination of inherent resistance to many drug classes and an ability to acquire resistance to all relevant treatments.[5] Thus, carbapenems remain one of the best therapy options, although their use is threatened by the emergence of carbapenem-hydrolyzing, enzyme-producing strains and dissemination of multidrug-resistant clones. One reported outbreak of *P. aeruginosa* was only susceptible to polymyxin.[6,7] In Brazilian hospitals, *P. aeruginosa* is a leading cause of nosocomial infection and the first cause of nosocomial pneumonia, notably in ICUs.[8–10]

This study describes a nosocomial outbreak of imipenem-resistant, metallo-β-lactamase-producing *P. aeruginosa* with horizontal transmission in patients hospitalized in a mixed ICU.

Methods

Hospital

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

Study design

From November 2003 to June 2005, a case-control study was carried out to investigate an imipenem-resistant *P. aeruginosa* nosocomial outbreak, affecting 47 patients.

The index case was a 77-year-old woman with a diagnosis of pulmonary thromboembolism, who had received cefepime, ceftriaxone, and ampicillin antibiotic treatment in the ICU of another Uberlandia hospital in November 2003. She was referred to the ICU of the university hospital with a clinical diagnosis of respiratory failure and acute abdomen. Forty-eight hours after starting mechanical ventilation, ventilator-associated pneumonia due to *P. aeruginosa* resistant to imipenem and other antimicrobial classes was detected (isolate 1). The patient remained in the unit for approximately 3 weeks. She received imipenem treatment for 24 hours, and was then switched to polymyxin B® for 30 days' time; despite this treatment, she died.

The risk factors associated with infection by imipenem-resistant *P. aeruginosa* (IRPa) were evaluated in a case-control study. Cases were defined as patients for whom culture had identified at least one IRPa strain (n = 47). For each case, 2 or 3 controls were selected (n = 122), defined as patients without IRPa infection, whose hospital stay coincided the closest in time with that of the infected patient. Only the first isolate per patient was considered for the analysis. During the study period, contamination of the health staff's hands (23), patients hospitalized during this period (86), and the surfaces in the ICU near the patients (mechanical ventilation unit, headboard, sinks) was investigated. The Institutional Ethics Committee gave their approval to conduct the study (CEP nº186/2005).

Data collection

Data for each patient were collected from the medical charts. The following variables were analyzed: age, sex, duration of ICU stay, comorbid conditions (diabetes mellitus, AIDS, renal failure, immunosuppression, cardiovascular disease), surgery, invasive device use (mechanical ventilation, central venous catheter, nasogastric tube) and prior use of antimicrobial drugs.

Identification and susceptibility testing procedures

Bacterial strains were identified by conventional biochemical tests, as described elsewhere.[11] Susceptibility testing was performed by agar diffusion with the following antimicrobials agents (CECON®): aztreonam (30 μg), ciprofloxacin (5 μg), cefepime (30 μg), imipenem (10 μg), meropenem (10 μg), ceftazidime (30 μg), gentamicin (10 μg), piperacillin/tazobactam (100/10 μg), and polymyxin B (300 U); the interpretative criteria used were those described in the CLSI (formerly NCCLS) guidelines.[12] Quality control was conducted using *P. aeruginosa* ATCC 27853. *P. aeruginosa* was defined as being multidrug-resistant when the organism was resistant to imipenem/meropenem and 2 or more of the other antimicrobial classes studied, except polymyxin.

Phenotypic metallo-β-lactamase production

All strains resistant to ceftazidime and/or imipenem were screened for metallo-β-lactamase (MBL) production by the disk approximation test, using filter disks with ceftazidime (30 μg) and imipenem (10 μg) at 25 mm or 10 mm equidistant, respectively, and disks containing the following inhibitors: 2 μL of undiluted 2-mercaptopropionic acid (2-MPA) solution (Aldrich Chemical Co, Milwaukee, USA) or 5 μL of EDTA 500 mM solution, as described by Arakawa.[13] The positive control strain was an IMP-1-producing *P. aeruginosa* (PSA 319).[14]

Detection of the bla gene

Because of cost constraints, 15 MBL-positive isolates were selected as representative strains for the detection of genes encoding Ambler class B β-lactamase of the VIM, IMP, or SPM type by PCR using the following specific primers (reading 5′-3′): *vim* forward, GTCTATTTGACCGCGTC, *vim* reverse; CTACTCAACGACTGAGCG; *imp* forward, ATGAGCAAGTTATCTGTATTC, *imp* reverse: GTCGCAACGACTGTGTAG and *spm* reverse: TCGCCGTGTCCAGGTATAAC, *spm* forward: CCTACAATCTAACGGCGACC. The cycling parameters were 95 °C for 5 min, followed by 30 denaturation cycles at 95 °C for 1 min, annealing at 40 °C for 1 min, and extension at 68 °C for 1 min. PCR products were visualized by electrophoresis on 0.8% agarose gels stained with 1% ethidium bromide.[14,15]

Genotyping by pulsed-field gel electrophoresis

Among 47 IRPa isolates, 15 randomly selected isolates with a positive MBL-producing phenotype were typed using pulsed-field gel electrophoresis (PFGE), as described by Denton et al,[16] with modifications. Each plug was digested with 30 U of *SpeI* restriction endonuclease (Invitrogen, Carlsbad, CA) at 37 °C for 12 h. Briefly, electrophoresis was performed by a 1% PFGE agarose gel run on the Gene Navigator (Pharmacia, Amersham Biociences) instrument at 164 V for 20.1 h, at 14 °C. The equipment was adjusted for a pulse of 5 s for 20 h and 15 s for 0.1 h. Band patterns were visualized by ethidium bromide staining and ultraviolet transillumination. Cloning was evaluated according to the Tenover criteria,[17] based on visual comparisons of the band patterns of samples run together in the same gel. Genetic similarities in samples were analyzed with a multivariate statistical package (MVSP 3.0).

Statistical analysis

Categorical variables were assessed with the chi-square test and Fisher's exact test; significance was set at a P value of $\leqslant 0.05$. Odds ratios and 95% confidence intervals were calculated with Epi-Info software, version 5.0. Variables were selected by forward logistic regression for the multivariate analysis.

Results

Following the index case, 46 additional cases of hospital-acquired infection were documented, including mechanical ventilation-associated pneumonia (41), bloodstream infection (3), surgical wound infection (1) and urinary tract infection (1) caused by *Pseudomonas aeruginosa* resistant to imipenem and other antimicrobial agents, including cephalosporins, aminoglycosides, and fluoroquinolones (data not shown).

Fig. 1 shows the mortality data and the temporal/spatial relationships (ICU stay) among the 47 patients, which provides evidence of cross-transmission of IRPa.

During the scrutiny for contamination, *P. aeruginosa* was not detected on culture of samples from the hands of 23 professionals, although initially this was suspected as the route by which the microorganism had been disseminated. However, *P. aeruginosa* was found colonizing 15/86 (17.4%) of the patients examined and on surfaces located near the patients, including the mechanical

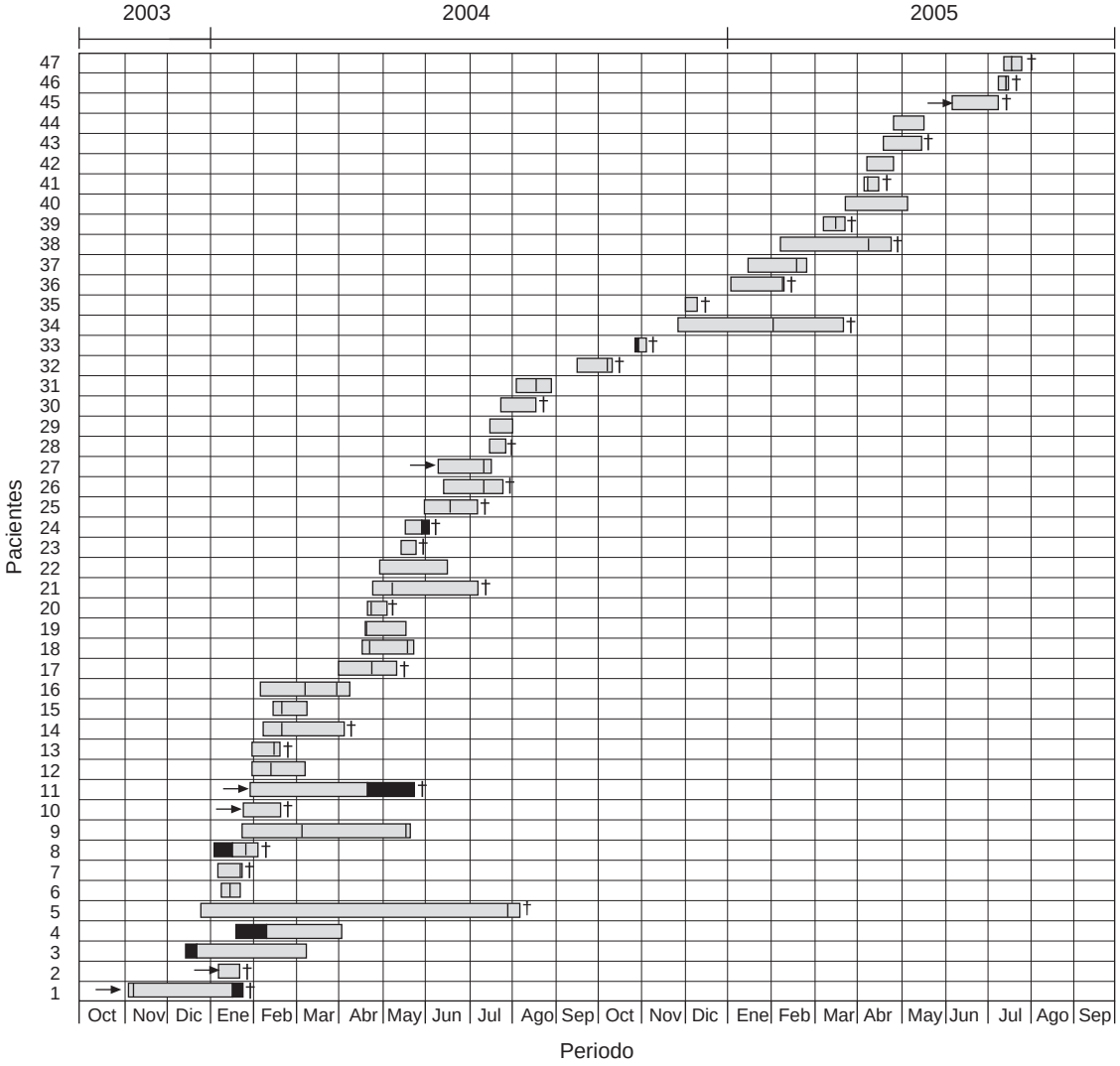

Fig. 1. Schematic representation of temporal and spatial relationships among patients infected with imipenem-resistant *Pseudomonas aeruginosa* during the study period. The arrows represent referral of patients from other hospitals to the ICU in Uberlandia Federal University Hospital, black sections indicate the time the patient was another ward, sections with hatched lines indicate time the patient was in the ICU and infected by imipenem-resistant *P. aeruginosa*, and crosses indicate death of the patient.

ventilation nebulizer in 10/86 (11.6%) and the headboard of the bed in 3/86 (3.5%), but not the sinks.

The majority of imipenem/ceftazidime-resistant *P. aeruginosa* strains in infected patients (33/43; 76.7%) had an MBL-positive phenotype, with no differences in detection between mercaptopropionic acid or EDTA (Table 1). In the contamination scrutiny, 17.8% (5/28) of *P. aeruginosa* imipenem/ceftazidime-resistant strains had an MBL-positive phenotype. Only 26.7% (4/15) of MBL-producing strains were positive for the bla_{SPM-1} gene. The bla_{VIM-2}, bla_{IMP-1}, and bla_{SPM} genes were not found.

Genotyping

Two strains collected from the environment and strains from tracheal secretions were MBL-producers. A total of 15 strains were typed; however, 2 specimens remained undefined. Four clones and their subclones were found on PFGE, with a predominance of clone A, A1, A2 (61.5%), followed by B, B1 (23.1%), C and D (1 sample each), and 2 indeterminate results (Table 2).

Table 3 shows the univariate analysis. On multivariate analysis, age $\geqslant$60 years ($P \leqslant 0.02$; OR 2.4; 95% CI 1.10–5.49), use of mechanical ventilation ($P \leqslant 0.001$; OR 7.2; 95% CI 2.74–19.10), presence of a tracheotomy ($P \leqslant 0.02$; OR 4.01; CI 1.17–13.72), and carbapenem use ($P < 0.001$, OR 5.7; 95% CI 2.31–10.08) were risk factors for acquiring imipenem-resistant *P. aeruginosa* infection

Discussion

The presence of MBL-producing *P. aeruginosa* has been described in many hospitals around the world.[18] Nonetheless, dissemination of an *SPM-1* MBL-producing *P. aeruginosa* epidemic has been demonstrated only in hospitals of different Brazilian regions.[8,14,19] As compared to other studies carried out in Brazil, the presently reported nosocomial outbreak of imipenem-resistant *P. aeruginosa* infection involved the largest number of cases to date (47 patients) and was associated with 4 clones. This study is the first to include an expressive number ($\approx$60%) of critical IRPa-infected patients who died within 30 days after the infection.

Pseudomonas aeruginosa is a leading pathogen in mechanical ventilation-associated nosocomial pneumonia, with an associated mortality rate of 20% to 80%.[8,9,20] During the outbreak in our hospital, pneumonia was the predominant infection, accounting for more than two-thirds (85%) of the cases, followed by bloodstream infection and surgical wounds.

The multivariate logistic regression analysis showed that patients with nosocomial IPRa infection were more likely to have been exposed to mechanical ventilation, and that tracheotomy and use of carbapenems were predisposing factors for the infection. These findings are consistent with those of other studies.[4,20–22]

Although the advent of carbapenems in the 1980s heralded a new treatment option for serious bacterial infections, carbapenem resistance is now observed in Enterobacteriaceae and *Acinetobacter* spp., and is becoming commonplace in *P. aeruginosa*.[18] *P. aeruginosa* possessing MBLs has been increasingly reported worldwide, mainly in ICUs and including Brazil,[14,18,19] but at rates comparatively lower than those found in our study ($\approx$90%).

These enzymes are clinically relevant because of their ability to hydrolyze most β-lactams, including carbapenems (but excluding aztreonam),[18] and their association with mobile genetic elements, increasing the possibility of rapid spread.[19,23–25] In the Americas, 6 different MBLs have been described in *P aeruginosa*, including SPM-1, IMP-1, IMP-16, VIM-2, VIM-8 and VIM-11[18] and three MBL types (SPM-1, VIM-2 and IMP-1) have been detected in Brazilian hospitals, with most strains expressing bla_{SPM-1}, which is disseminated among our hospitals.[8,14,19,22] We found that MBL-producing strains accounted for a substantial number of cases (76.7%), and that the strains were clinically relevant, because 70.0% of the patients died. In contrast to the reported results of Sader et al,[26] neither bla_{IMP-1} or bla_{VIM-1} genes were found in this study.

The acquisition of resistant bacteria in hospitals may be a consequence of selective pressure exerted by the use of antibiotics and/or horizontal dissemination.[27] Molecular typing of strains presenting the bla_{SPM-1} gene showed the presence of a single clone (A). In the present study, multidrug-resistant *P. aeruginosa* strains were clustered in two major genotypes; these showed coresistance to most of the antimicrobial agents tested. Multidrug resistance can be explained by the accumulation of several resistance mechanisms, including gene mutation, over-expression of efflux pumps, loss or modification of porins, and acquired extended-spectrum β-lactamases.[28] This suggests that the selective pressure of previous antimicrobial use in the unit contributed to the emergence of resistant clones.

Table 1
Infections by imipenem-resistant metallo-β-lactamase-producing *Pseudomonas aeruginosa* in ICU patients at Uberlandia Federal University Hospital

Infection	n/n IRPa (%)*	MBL n (%)
Ventilation-associated pneumonia	41/38 (93.0)	29 (87.0)
Bloodstream infections	3/3 (100.0)	2 (67.0)
Surgical wound	2/1 (50.0)	1 (50.0)
Urinary tract	1/1 (100.0)	1 (100.0)
Total	47/43 (94.0)	33 (76.7)

* *Pseudomonas aeruginosa*-infected patients/number of infections by IRPa.

Table 2
Data on 15 metallo-β-lactamase-producing *Pseudomonas aeruginosa* isolates at the Uberlandia Federal University Hospital ICU

Isolation period	Source (n isolates)	IRPa positive for bla_{SPM-1}	PGFE profile
December 2003	Tracheal secretion (3)	2	A
March, April 2004	Breath (1)	1	A
January, February 2005	Intestine (1)	1	A
March 2004	Headboard (1)	ND*	A_1
February 2004	Tracheal secretion (1)	ND	A_2
January 2005	Intestine (1)	ND	A_2
May 2004	Tracheal secretion (2)	ND	B
October 2004	Tracheal secretion (1)	ND	B_1
May 2005	Tracheal secretion (1)	ND	C
February 2004	Intestine (1)	ND	D
January, November 2004	Tracheal secretion (2)	ND	ND

* Not determined.

Table 3
Univariate analysis of risk factors for nosocomial infection by resistant *Pseudomonas aeruginosa* in ICU patients at Uberlandia Federal University Hospital

Variables	Patients			
	Infected n = 47 (%)	Noninfected n = 122 (%)	*P**	OR (95% CI)
Sex				
Female/Male	17/30 (36.2/63.8)	62/60 (50.8/49.2)	0.12	0.55 (0.56–1.16)
Age, y				
⩾60	11 (23.4)	50 (41.0)	0.05	0.44 (0.19–1.00)
Length of stay, days				
⩾7	44 (93.6)	20 (16.4)	<0.001	29.14 (10.59–83.71)
Comorbid conditions				
Diabetes mellitus	03 (6.0)	07 (5.7)	1.00	1.08 (0.41–2.89)
Cardiac disease	03 (6.0)	27 (22.0)	0.02	0.32 (0.11–0.95)
AIDS	02 (4.2)	00 (0.0)	0.07	3.71 (2.89–4.76)
Immunosuppression	03 (6.0)	03 (2.4)	0.35	1.85 (0.80–4.29)
Invasive Devices				
Central venous catheter	30 (63.8)	110 (90.2)	<0.001	0.37 (0.24–0.57)
Mechanical ventilation; MV	45 (95.7)	80 (65.5)	<0.001	7.92 (2.00–31.30)
Duration of MV, days				
⩾7	40 (85.1)	14 (11.5)	<0.001	0.10 (0.04–0.22)
Trauma	12 (25.5)	22 (18.0)	0.381	1.36 (0.80–2.33)
Surgery	28 (59.5)	30 (24.6)	<0.001	2.82 (1.73–4.60)
Antibiotic use				
⩾2	28 (59.6)	48 (39.3)	0.02	2.27 (1.08–4.79)
Cephalosporin (3rd/4th generation)	30 (64.0)	48 (39.3)	0.007	2.06 (1.23–3.44)
Carbapenem	22 (46.8)	04 (3.2)	<0.001	4.84 (3.27–7.16)
Quinolone	05 (10.6)	07 (5.7)	0.14	1.95 (0.95–4.02)
Vancomycin	13 (27.6)	14 (11.5)	0.019	2.01 (1.23–3.28)

OR: odds ratio; 95% CI: 95% confidence interval.
* $P \leqslant 0{,}05$.

Both the SPM and non-SPM-infected patients were clustered for 18 months in a single unit, with overlapping during their hospitalization. These data suggest that horizontal transmission between these patients may have played a role in the dissemination of the infection. Clonal dissemination was detected within and between intensive care units in São Paulo (4) and Brasilia,[10] but with unrelated strains recovered from one of them. These data differ from those of Pellegrino et al[29] who reported clonal dissemination between private and public institutions in Rio de Janeiro, in which both presented an apparently endemic background.

Detection of clonal dissemination usually indicates inadequate nosocomial infection control practice.[10] This outbreak of *P. aeruginosa* infection was probably linked to transmission of the microorganism via the hands of healthcare workers. We did not identify hand carriage among the workers examined, but a spatial/temporal relationship was seen among the cases in our unit, and low adherence (23%) to proper hand washing[30] practice was detected in a recent study.

In conclusion, to our knowledge, this is the first study to conduct an analysis using both classical and molecular techniques of an outbreak of infection associated with a highly prevalent MBL-producing *P. aeruginosa*, including mainly bla_{SPM-1} strains clustered in 2 major genotype, resulting in a significant increase in mortality of elderly patients and in previous use of antibiotics. The clonal dissemination among patients in our unit indicates problems in nosocomial infection control practice, likely associated with low adherence to hand hygiene, and selective pressure that provides resistant strains with an advantage over their susceptible ancestors.

References

1. Bukholm G, Tannaes T, Kjelsberg ABB, Smith-Erichsen N. An outbreak of multidrug-resistant *Pseudomonas aeruginosa* associated with increased risk of patient death in an intensive care unit. Infect Control Hosp Epidemiol. 2002;23:441–6.
2. Andrade SS, Jones RN, Gales AC, Sader HS. Increasing prevalence of antimicrobial resistance among *Pseudomonas aeruginosa* isolates in Latin American Medical Centers: 5 years report of the SENTRY antimicrobial Surveillance Program (1997–001). J Antimicrob Chemother. 2003;52:140–1.
3. Arruda EA, Marinho IS, Boulos M, Sinto SI, Caiffa HH, Mendes CM, et al. Nosocomial infection caused by multiresistant *Pseudomonas aeruginosa*. Infect Control Hosp Epidemiol. 1999;20:620–3.
4. Cao B, Wang H, Sun H, Zhu Y, Chen M. Risk factors and clinical outcomes of nosocomial multidrug resistant *Pseudomonas aeruginosa* infections. J Hosp Infect. 2004;57:112–8.
5. Rossolini GM, Mantengoli E. Treatment and control of severe infections caused by multiresistant *Pseudomonas aeruginosa*. Clin Microbiol Infect. 2005;11(S4): 17–32.
6. Livermore DM. Multiple mechanism of antimicrobial resistance in *Pseudomonas aeruginosa:* our worst nightmare? Clin Infect Dis. 2002;34:634–40.
7. Queenan AM, Bush K. Carbapenemases: the versatile β-lactamases. Clin Microbiol Rev. 2007;20:440–8.
8. Zavaski AP, Barth AL, Fernandes JF, Moro ALD, Gonçalves ALS, Goldani LZ. Reappraisal of *Pseudomonas aeruginosa* hospital-acquired pneumonia mortality in the era of metallo-β-lactamase-mediated multidrug resistance: a prospective observational study. Critical Care. 2006;10:100–10.
9. Teixeira PJZ, Hertz FT, Cruz DB, Caraver F, Hallal RC, Moreira JS. Pneumonia associada à ventilação mecânica: impacto da multirresistência bacteriana na morbidade e mortalidade. J Bras Pneumol. 2004;30:540–8.
10. Figueredo-Mendes CM, Sinto S, Mello-Sampaio JL, Cardoso-Leão S, Oplustil CP, Turner P, et al. *Pseudomonas aeruginosa* clonal dissemination in Brazilian intensive care units. Enferm Infecc Microbiol Clin. 2005;23:402–5.
11. Deanna L, Gilligan PH. *Pseudomonas aeruginosa*. En: Murria P, Baron EJ, Jorgensen JH, Pfaller MA, Yhocken RH, editors. Manual of Clinical Microbiology. 8th ed. Washington: American Society for Microbiology; 2003. p.719–8.
12. NCCLS Performance Standards for Antimicrobial Susceptibility Testing; fourteenth Informational Supplement M100-S14. Pennsylvania: NCCLS; 2004.
13. Arakawa Y, Shibata N, Shibayama K, Kurokawa H, Yagi T, Fujiwara H, et al. Convenient test for screening metallo-β-lactamase—producing Gram negative bacteria by using thiol compounds. J Clin Microbiol. 2000;38:40–3.
14. Gales AC, Menezes LC, Silbert S, Sader HS. Dissemination in distinct Brazilian regions of an epidemic carbapenem-resistant *Pseudomonas aeruginosa* producing SPM metallo-β-lactamases. J Antimicrob Chemother. 2003;52:699–702.
15. Toleman MA, Simm AM, Murphy TA, Gales AC, Biedenbach DJ, Jones RN, et al. Molecular characterization of SPM-1, a novel metallo-β-lactamase isolated in Latin America: report from the sentry antimicrobial surveillance programme. J Antimicrob Chemother. 2002;50:673–9.

16. Denton M, Tood NJ, Kerr KG, Hawkey PM, Littlewood JN. Molecular epidemiology of *Stenotrophomonas maltophilia* isolated from clinical specimens from patients with cystic fibrosis and associated environmental samples. J Clin Microbiol. 1998;36:1953–8.
17. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol. 1995;33:2233–9.
18. Wash TR, Toleman MA, Poriel L, Nordmann P. Metallo-β-lactamases: the quiet before the storm? Clin Microbiol Rev. 2005;18:306–25.
19. Mendes RE, Castanheira M, Pignatari ACC, Gales AC. Metallo-β-lactamases. J Bras Patol Med Lab. 2006;42:103–13.
20. Ruiz CM, Guerrero PJ, Romero PC. Etiologia de la neumonia associada a ventilación mecânica em um hospital clinico. Rev Chil Infect. 2007;24: 131–6.
21. Zavascki AP, Cruz RP, Goldani LZ. Risk factors for imipenem-resistant *Pseudomonas aeruginosa*: a comparative analysis of two case-control studies in hospitalized patients. J Hosp Infect. 2005;59:96–101.
22. Nouér SA, Nucci M, De-Oliveira MP, Pellegrino FLPC, Moreira BM. Risk factors for acquisition of multidrug-resistant *Pseudomonas aeruginosa* producing SPM Metallo-β-lactamase. Antimicrob Agents Chemother. 2005;49: 3663–7.
23. Livermore DM. Of *Pseudomonas,* porins, pumps and carbapenems. J Antimicrob Chemother. 2001;47:247–50.
24. Lariki N, Gallenni M, Thamm L, Riccio ML, Amicosante G, Frere JM, et al. Structure of In 31a bla_{IMP} containing *Pseudomonas aeruginosa* integron phyletically related to In5 which carries an unusual array of gene cassettes. Antimicrob Agents Chemother. 1999;43:890–901.
25. Poriel LM, Magalhaes M, Lopes M, Nordmann P. Molecular analysis of metallo-β-lactamase gene bla_{SPM-1} surrounding sequences from disseminated *Pseudomonas aeruginosa* isolates in Recife, Brazil. Antimicrob Agents Chemother. 2004;48:406–9.
26. Sader HS, Reis AO, Silber S, Gales AC. IMPs, VIMs, SPMs: the diversity of metallo-β-lactamases produced by carbapenem-resistant *Pseudomonas aeruginosa* in a Brazilian hospital. Clin Microbiol Infect. 2005;11:73–6.
27. Paterson DL. Looking for risk factors for the acquisition of antibiotic resistance: a 21st-century approach. Clin Infect Dis. 2002;34:1564–7.
28. Hocquet D, Bertrand X, Kohler T, Talon D, Plésiat P. Genetic and phenotypic variations of a resistant *Pseudomonas aeruginosa* epidemic clone. Antimicrob Agents Chemother. 2003;47:1887–94.
29. Pellegrino FLPC, Teixeira LM, Carvalho MGS, Noúer AS, Oliveira MP, Sampaio JLM, et al. Occurrence of a multidrug-resistant *Pseudomonas aeruginosa* clone in different hospitals in Rio de Janeiro, Brasil. J Clin Microbiol. 2002;40: 2420–4.
30. Borges LFA, Rocha LA, Gontijo Filho PP. Adesão á pratica de higienização das mãos e sua associação ás taxas de infecção hospitalar em um hospital universitário mineiro. Anais do 2.º Congresso Mineiro de Infectologia. 2006. Uberlândia, Minas Gerais, Brazil.