Outbreaks, persistence, and high mortality rates of multiresistant *Pseudomonas aeruginosa* infections in a hospital with AIDS-predominant admissions

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ABSTRACT

Introduction: Authors have reported increased incidence of multiresistant *Pseudomonas aeruginosa* (MR-PA) infections worldwide over the last decade. Researchers have proposed multifaceted approaches to control MR-PA infections, but none have been reported in the acquired immunodeficiency syndrome (AIDS) setting. **Objective and Methods:** Herein we report the impact of a multifaceted intervention for controlling MR-PA over five years in a hospital with AIDS-predominant admissions and describe the clinical characteristics of MR-PA infection in our patient population. The clinical outcomes of infected patients and molecular characteristics of the isolated strains were used as tools for controlling MR-PA infection rates. **Results:** Significant temporary decrease of new infections was achieved after intervention, although a high level of diagnostic suspicion of nosocomial infection was maintained. We obtained 35 *P. aeruginosa* isolates with multiresistant profiles from 13 infected and 3 colonized patients and 2 environmental samples. Most of the patients (94%) were immunocompromised with AIDS (n = 10) or HTLV-1 infections (n = 5). Of the followed patients, 67% had persistent and/or recurrent infections, and 92% died. We observed differences in the antibiotic-resistance pattern of MR-PA infection/colonization during two outbreaks, although the genetic profiles of the tested strains were identical. **Conclusions:** Therefore, we concluded that early multidisciplinary interventions are essential for reducing the burden caused by this microorganism in patients with AIDS. Prolonged or suppressive antibiotic-based therapy should be considered for MR-PA infections in patients with AIDS because of the persistence characteristic of MR-PA in these patients.

Keywords: *Pseudomonas aeruginosa*; disease outbreaks; infection control; molecular epidemiology; acquired immunodeficiency syndrome.

INTRODUCTION

Multiresistant *Pseudomonas aeruginosa* (MR-PA) is one of the leading pathogens that cause nosocomial infections and outbreaks worldwide.[1-8] These infections are usually associated with high mortality rates.[1-6] Also, in patients with human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS), authors have described persistent and recurrent infections caused by non-MR-PA strains.[9,10] Pulsed-field gel electrophoresis (PFGE) is the recommended technique for determining the genotypes of nosocomial pathogens.[11] Researchers have used it in several studies to confirm the similarity of *P. aeruginosa* strains, suggesting the importance of cross-colonization,[11,12] and as a tool to control nosocomial infections.[3,12] Multifaceted strategies using molecular epidemiology approach for controlling MR-PA infection outbreaks in immunocompromised patients, especially those with cancer or admitted to intensive care units (ICUs),[3,5] have been adopted but none were described in patients with AIDS. In this report, we describe the surveillance and intervention measures used for controlling MR-PA infections over five years in an infectious disease hospital in Rio de Janeiro, Brazil, in which the majority of admitted patients have AIDS. We also describe the clinical evolution of patients with MR-PA infections or colonization and the antimicrobial susceptibility profiles and molecular characteristics of MR-PA strains recovered from the patients and their environments. Increased knowledge of the epidemiology and clinical presentations

of MR-PA infection in this patient population may improve early interventions.

MATERIAL AND METHODS

Institution and surveillance program

A prospective program for controlling nosocomial infections, which used an adaptation of methodologies developed by the Centers for Disease Control and Prevention, was initiated in April 2002 in a 26-bed (including 2-4 ICU beds) hospital from Fundação Oswaldo Cruz (FIOCRUZ).[13-15] The adaptation concerned the application of surveillance in the entire hospital and used to identify nosocomial infections in patients with AIDS considered to be acquired at the hospital.[16] Infections were considered nosocomial if they first appeared 48h after admission. Infections that were likely incubating before hospital admission were not considered nosocomial. All clinical and surveillance cultures obtained from every admitted patient were observed daily for early identification and control of multiresistant pathogens from April 2002 to February 2007. Three antibiotic susceptibility patterns of MR-PA were considered: I) multidrug-resistant *P. aeruginosa* (MDR-PA) was indicated by strains resistant to carbapenems and, concomitantly, at least two other classes of antipseudomonal antibiotics: penicillins, cephalosporins, monobactams, aminoglycosides, and fluoroquinolones; II) extensive drug-resistant *P. aeruginosa* (XDR-PA) was indicated by strains resistant to all classes of antipseudomonas antibiotics with the exception of polymyxin B; III) carbapenem-resistant *P. aeruginosa* (CR-PA) was indicated by strains resistant only to carbapenems. All the patients' medical records were reviewed to confirm the prospectively collected data.

During MR-PA outbreaks, in addition to clinical cultures a differential surveillance was performed, which included rectal swab samples for MR-PA detection obtained from patients hospitalized in the same room or unit whenever an MR-PA isolate was identified. Cultures of clinical surface samples were obtained during the first outbreak in the ICU where the majority of the patients with acquired MR-PA were hospitalized. These samples were collected before cleaning from furniture, equipment, sinks, ventilation ducts, air conditioning intake grills, air conditioning filters, compressed air tubes, patients' nebulizers and respiratory circuits with sterile cotton-tipped swabs moistened with transport media. Water from air conditioner compressors and humidifiers was also sampled. During the second outbreak, in November 2006, the following samples were collected from three areas of the ICU and from two wards as part of another research protocol also approved by the institutional ethics committee: 250 L of air samples taken using an MAS-100 air sampler (Merck & Co., Inc., Whitehouse Station, NJ, USA) at a distance of 1.5-2.0 m from the patients beds and 1.5 m from the floor, two surface samples taken in each ICU and ward area using the in print technique with RODAC plates, and samples taken from the hands (washed in a 100 mL 0.1% peptone solution in sterile bags) of personnel who dealt with MR-PA-positive patients during environmental sampling.

Laboratory procedures

Clinical samples and rectal swabs were inoculated onto MacConkey agar (Merck & Co., Inc.) and cetrimide agar (Merck & Co., Inc.) plates. Environmental surface swabs were plated on sheep blood agar. Cetrimide agar, MacConkey agar, and salt mannitol agar (Merck & Co., Inc.) were used for air sampling. The plates were incubated at 37ºC for 48h. Colonies that contained Gram-negative rods were subcultured onto blood and MacConkey agar plates and incubated for 24h. All oxidase-positive bacilli and non-lactose fermenting growths were identified as *P. aeruginosa* using conventional biochemical tests.

The antibiotic susceptibility of the isolates was tested using the disk-diffusion method according to Clinical and Laboratory Standards Institute recommendations.[17] Susceptibility to polymyxin B was verified using the E-test. Resistant strains included those with intermediate susceptibility. Screening of the strains for metallo-β-lactamase (MBL) production using a ceftazidime disk in the presence of 2-mercaptopropionic acid and polymerase chain reaction for detection of the blaSPM-1, blaIMP, and blaVIM genes were performed.[18] DNA macrorestriction using SpeI followed by PFGE for molecular typing of isolates was carried out as well.[19] Restriction fragment profiles of the genomic DNA of MR-PA strains were compared using visual inspection and the GelCompar II software program (version 3.5; Applied Maths, Sint-Martens-Latem, Belgium) and analyzed using the Tenover criteria (1995).[20]

Clinical characteristics of MR-PA infections

The clinical characteristics of MR-PA infections were classified according to three categories: I) persistent infection, in which the microorganism was maintained in the same or another clinical material for at least 21 days regardless of symptoms or treatment; II) recurrent infection, in which clinical symptoms of infection reinitiated and the MR-PA was isolated in the same or another biological material after completion of treatment; and III) single infection, which was an unique infectious episode without persistence or recurrence. Treatment failure was considered in cases with any clinical deterioration or persistence of the microorganism in clinical materials resulting in a change in the antibiotic-based therapy or death.

RESULTS

Admitted patients

HIV (850 patients) and HTLV-1 (130 patients) infections were the main clinical conditions of the admitted patients (54% of admissions). Also, readmissions occurred in a high proportion of the patients (654 readmissions among 1,814 admissions) during the 5-year study period. An average of 35 clinical bacteriological cultures per 100 patient-days was performed in the hospital over this period.

Description of outbreaks

Figure 1 shows the monthly distribution and resistance patterns of *P. aeruginosa* isolates during the study period. The mean incidence density of MR-PA infection (using for calculation only the first MR-PA isolate obtained from each patient) was 0.53/1,000 patient-days from April 2002 to February 2007. However, it was 2.8/1,000 patient-days from November 2004 to March 2005 (first outbreak) and 2.1/1,000 patient-days from November 2006 to February 2007 (second outbreak).

The onset of the first MR-PA outbreak coincided with identification of the XDR-PA-infected index patient in the ICU in September 2004 (Figure 1). This patient came from another nearby hospital; two months before detection of the first outbreak with recovery of 15 MDR-PA and 2 XDR-PA strains from six patients. After an intervention program for controlling the MR-PA outbreak initiated in November 2004, the occurrence of MR-PA decreased significantly, with only one new CR-PA infection detected over a 1-year period (September 2005 to October 2006). During the second outbreak, which began in November 2006, we had seven XDR-PA isolates from five patients but detected no MDR-PA or CR-PA strains.

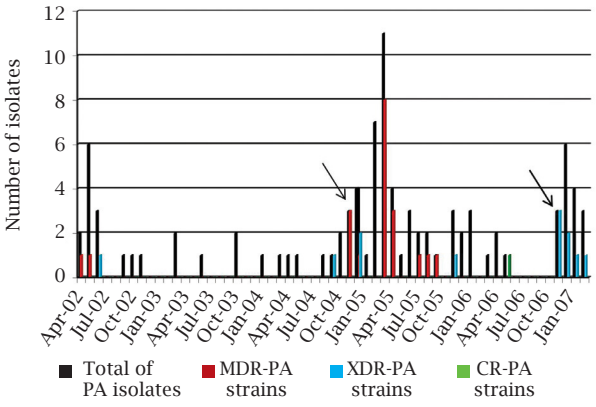

Figure 1: Epidemiologic curve showing the *P. aeruginosa* isolates obtained monthly from patients. Total *P. aeruginosa* isolates (PA, black bar) and MDR-PA (red bar), XDR-PA (blue bar), and CR-PA (green bar) isolates are shown. The arrows indicate when the infection control interventions were initiated.

Culture results, clinical presentations and outcomes

Forty-four patients had *P. aeruginosa* infections or colonization during the study. Twenty-six patients had isolates with non-CR-PA/MDR-PA/XDR-PA susceptibility patterns. Two patients had isolates with these resistance patterns detected before 24 h of hospital admission. One of these patients had been admitted previously during the first outbreak, but a rectal swab obtained from her was negative for MR-PA at that time. Of 16 patients in whom MR-PA developed during hospitalization in the study period, 15 were immunocompromised (94%) with AIDS (n = 10) or HTLV-1 (n = 5) with urinary tract infections (UTIs) (Table 1). Thirteen patients had MR-PA infections, whereas three had MR-PA colonization. Rectal swabs were positive for MR-PA in only one patient, who had MDR-PA isolates recovered from other clinical samples. Table 1 shows the age, sex, underlying and comorbid condition, length of hospital stay and admission to the ICU prior to MR-PA infection, and laboratory data for the MR-PA isolates obtained from these 16 patients.

Among six patients in whom MR-PA infection/colonization developed during the first outbreak, four were undergoing hemodialysis (patients #5 to #8 in Tables 1 and 2) and four (patients #3 to #5 and #7) were in the ICU when they acquired it. In comparison, among the five patients in whom these infections/colonizations developed during the second outbreak, none were undergoing hemodialysis and three were in the ICU when they acquired it (Table 1). Each new MR-PA-positive patient had been hospitalized in close proximity to another previously MR-PA-positive patient in the ICU or other hospital ward. Table 2 lists the types of infections, therapies for Gram-negative bacterial infections after MR-PA isolation, MR-PA infection treatment failures, infection classifications, and deaths associated with MR-PA infection and/or colonization during the study period.

Septicemia or infections related to the use of invasive devices predominated among patients (13/19, 68%). Excluding one patient who was lost to follow-up (transferred to another hospital), 58% (n = 7) of the remaining 12 infected patients had one MR-PA infection treatment failure. Also, eight patients (67%) had persistent and/or recurrent MR-PA infections, and 11 (92%) died despite receiving therapy and medical support (Table 2). The overall mortality rate associated with MR-PA infection during the study period was 80% (12/15). The average time span between obtaining the first and last clinical MR-PA-positive samples in eight patients who had persistent and/or MR-PA recurrent infections was 55.3 days (median, 29 days; range, 21-195 days). Five patients in the first outbreak (83%) and one in the second outbreak (20%) died. The patient who survived in the first outbreak died of recurrent MDR-PA septicemia six months after his first MR-PA infection (patient #8). The patient who died during the second outbreak (patient #14) had untreated XDR-PA bacteremia one month before development of XDR-PA ventilator-associated pneumonia (VAP).

*Table 1. Epidemiological data and laboratory results of patients with MR-PA infection and/or colonization**

Patient	Sex/ age (years)	Clinical condition	Other diagnoses	Length of hospital stay†/ ICU admission†	Sample - susceptibility pattern, date of sample	Time span between first and last MR-PA positive cultures (days)
1	M/43	HAM/TSP with recurrent UTI	MRSA sepsis, renal failure, respiratory failure	4/No	Urine: MDR-PA, 05/21/02; CVC tip: XDR-PA, 06/10/02	21
2	M/33	AIDS	Cryptococcal meningitis, infected dermatitis, *Pneumocystis carinii* pneumonia, *Respiratory failure*; last CD4:406 cells/µL‡	36/Yes	Blood: XDR-PA, (PFGE tested) 09/08/04; BAL: XDR-PA, 09/09/04	2
3	M/45	AIDS	Neurologic disease, DM type 2, COPD, HCV-positive, *Enterococcus faecalis* UTI, respiratory failure; last CD4: 98 cells/µL; viral load: 19,000 copies/mL‡	5/Yes	Tracheal aspirate: MDR-PA, 11/12/04; BAL: MDR-PA (PFGE tested), 11/29/04; tracheal aspirate: XDR-PA, 12/08/04; vascular catheter tip: XDR-PA, 12/08/04	27
4	M/25	AIDS	CMV esophagitis, pneumothorax, respiratory failure, MRSA colonization; last CD4: 324 cells/µL; viral load: 710,000 copies/mL‡	9/Yes	Tracheal secretion: MDR-PA (PFGE tested), 11/30/04	-
5§	M/50	AIDS	Febrile neutropenia, disseminated *Mycobacterium tuberculosis*, renal failure, hepatic failure, pancreatitis	20/Yes	Tracheal secretion: MDR-PA, 20,000 CFU, 12/19/04	-
6§	F/43	AIDS	Neurologic disease, *Salmonella* and *Enterococcus* species sepsis, MRSA CVC sepsis, bronchoaspiration, renal failure	8/No	Vascular catheter tip: MDR-PA, 03/03/05; urine MDR-PA, 03/30/05	28
7§	M/59	Leptospirosis	Renal failure, atrial fibrillation, MRSA CVC sepsis	33/Yes	Two vascular catheter tips: MDR-PA, 03/21/05; urine: MDR-PA, 03/28/05 and 03/31/05; rectal swab: MDR-PA, 04/06/05; vascular catheter tip: MDR-PA, 04/18/05; tracheal secretion: MDR-PA, 04/18/05	29
8§	M/54	AIDS	Febrile neutropenia, cryptococcosis, high blood pressure, chronic renal failure, MRSA colonization	8/No	Blood: MDR-PA, 03/10/05; urine: MDR-PA, 03/22/05; blood: MDR-PA, 9/21/05	195

(Cont.)

Table 1. Epidemiological data and laboratory results of patients with MR-PA infection and/or colonization*

Patient	Sex/ age (years)	Clinical condition	Other diagnoses	Length of hospital stay†/ ICU admission†	Sample - susceptibility pattern, date of sample	Time span between first and last MR-PA positive cultures (days)
9	F/60	HAM/TSP with recurrent UTI	Cutaneous T-cell lymphoma, hemorrhagic stroke, high blood pressure, DM-type II, MDR *Escherichia coli* UTI, MRSA osteomyelitis	53/Yes	Urine: MDR-PA, 07/06/05	-
10	F/64	HAM/TSP with recurrent UTI	*E. coli* UTI, hydronephrosis with renal calculi, decubitus ulcers and osteomyelitis, MRSA colonization	9/No	Urine: MDR-PA (PFGE tested), 08/15/05; urine: XDR-PA, 11/10/05	88
11	F/47	AIDS	Hepatitis C, decubitus ulcers, UTI, MRSA colonization	2‖/Yes	Urine: CR-PA, 05/31/06	-
12	M/56	AIDS	Hyperparathyroidism, HIV dementia, CDAD; last CD4: 9 cells/μL; viral load: > 100,000 copies/mL‡	85/Yes	Blood: XDR-PA, 11/16/06; blood: XDR-PA (PFGE tested), 12/06/06	21
13	F/75	HAM/TSP with urinary sepsis	*Streptococcus* species sepsis	28/No	Urine: XDR-PA, 50,000 CFU/mL (PFGE tested), 11/11/06	-
14	M/31	AIDS	CMV meningitis, CDAD (pseudomembranous colitis), MDR *Klebsiella pneumoniae* CVC sepsis, respiratory failure; last CD4: 6 cells/μL‡	174/Yes	Blood: XDR-PA, (PFGE tested), 11/24/06; BAL: XDR-PA, 12/26/06	33
15	M/26	AIDS	Febrile neutropenia, MRSA sepsis	32/No	Urine: XDR-PA, 80,000 CFU/mL, 01/25/07	-
16	M/69	HAM/TSP with urinary sepsis	Iatrogenic pneumothorax, decubitus ulcers	31/Yes	Urine: XDR-PA, 02/02/07	-

BAL, bronchoalveolar lavage; CDAD, *Clostridium difficile*-associated diarrhea; CFU, colony-forming unit; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; CVC, central vascular catheter; DM, *diabetes mellitus*; F, female; HAM/TSP, HLTV-I associated myelopathy/tropical spastic paraparesis; HCV, hepatitis C virus; M, male; MRSA, methicillin-resistant *Staphylococcus aureus.*

*MR-PA includes all resistance patterns(MDR-PA, XDR-PA, and CR-PA).

†Previous to MR-PA infection or colonization.

‡CD4 and viral load for HIV-positive patients when available and collected less than 6 months before MR-PA infection/ colonization.

§Performance of hemodialysis when MR-PA was acquired.

‖Discharged from the study hospital 5 days before this admission.

*Table 2. Clinical presentations, antibiotic regimen, and outcomes in patients with MR-PA infection or colonization**

Patient	Type of infection/ colonization	Antibiotic regimen†	Treatment failure‡	Infection classification§	Death during hospitalization
1	UTI	Empiric cefepime and guided by culture for 21 days, associated with empiric amikacin and guided by culture for 7 days	No	Persistent infection	Yes‖
	CVC sepsis	Not treated	N/A	-	
2	VAP and septicemia	The patient had received cefepime and levofloxacin for 14 days and was receiving treatment for *Pneumocystis carinii* pneumonia	N/A	Single episode	Yes‖
3	VAP	Empiric levofloxacin for 2 days, cefepime for 20 days, empiric polymyxin B for 2 days, amikacin for 16 days	Yes	Persistent infection	Yes‖
	CVC infection	Not treated	N/A	Recurrent infection	
4	VAP	Empiric cefepime for 3 days, empiric imipenem for 4 days	Yes	Single episode	Yes‖
5	Respiratory tract colonization	N/A	N/A	N/A	Yes
6	CVC infection	Not treated. The patient was receiving treatment for *Salmonella* and *Enterococcus* species sepsis with ampicillin and gentamicin	N/A	Persistent infection	Yes‖
	UTI	Empiric imipenem for 2 days, empiric cefepime for 1 day	Yes	-	
7	CVC sepsis	Cefepime for 15 days	Yes	Persistent infection	
	Septicemia	Piperacillin-tazobactam for 4 days	Yes	-	Yes
8	CVC sepsis	Cefepime for 8 days for febrile neutropenia, MDR-PA growth in blood, had CVC removed and then received cefepime for 14 days, piperacillin-tazobactam for 11 days, and piperacillin-tazobactam and levofloxacin for 14 days	No	Single episode	No
	Septicemia	Empiric cefepime for 1 day	N/A	Recurrent infection	Yes‖
9	Urinary sepsis	Empiric imipenem for 2 days, cefepime for 9 days	Yes	Single episode	Yes
10	UTI	Cefepime for 22 days, transferred to another hospital for J ureteral stent insertion	No	Single episode	No
	UTI	Polymyxin B for 16 days	Not determined	Recurrent infection	Yes

(Cont.)

*Table 2. Clinical presentations, antibiotic regimen, and outcomes in patients with MR-PA infection or colonization**

Patient	Type of infection/ colonization	Antibiotic regimen†	Treatment failure‡	Infection classification§	Death during hospitalization
11	Urinary sepsis	Empiric cefepime and guided by culture for 14 days	Yes	Single episode	Yes
12	Septicemia	Empiric piperacillin-tazobactam for 7 days, ciprofloxacin for 6 days	N/A	Persistent infection	No
		Cefepime and polymyxin B for 3 days, polymyxin B alone for 6 days	No		
13	Urinary tract colonization	N/A	N/A	N/A	No
14	Bacteremia	Not treated; the patient was receiving treatment for MDR *K. pneumoniae* CVC sepsis with piperacillin-tazobactam for 10 days and meropenem for 7 days	N/A	Persistent infection	Yes
	VAP	Empiric cefepime for 3 days, polymyxin B for more than 21 days	Yes	-	
15	Urinary tract colonization	N/A	N/A	N/A	No
16	UTI	Empiric cefepime for 2 days; patient transferred to another hospital	Lost to follow-up	Lost to follow-up	No

CVC, central vascular catheter; N/A, not applicable.
*MR-PA includes the entire resistance pattern (MDR-PA, XDR-PA, and CR-PA).
†Described therapy only for Gram-negative bacteria after MR-PA detection.
‡Treatment failure: clinical deterioration or persistence of the microorganism in clinical materials resulting in a request to change the antibiotic-based therapy or resulting in death.
§Infection classification: I) recurrent infection: clinical symptoms of infection reinitiated and isolation of the MR-PA in other or same biologic materials after completion of treatment; II) persistent infection: maintenance of the microorganism in the same or other clinical materials for at least 21 days independent of symptoms or treatment; III) single infection: unique episode of infection with no recurrent or persistent infection.
‖Death before last microbiological result with recovery of MR-PA.

Six patients (patient #1, #3, #8 to #11) with MR-PA infections were treated with cefepime alone or in combination therapy with other antipseudomonal antibiotics considered adequate according to laboratory test results and time of initiation and duration of therapy. However, three of these patients – two with urinary sepsis (patients #9 and #11) and one with VAP (patient #3) – had no responses to 9, 14, and 20 days into antibiotic treatment, respectively. In addition, in patient #3, MDR-PA regrew in a bronchoalveolar lavage (BAL) sample on the 15th day of treatment with cefepime, and XDR-PA grew in a tracheal aspirate and on a catheter tip on the 4th day after discontinuation of cefepime-based treatment.

Results of culture of environmental samples

MDR-PA was cultured from 2 of 12 (17%) environmental samples (water obtained from a humidifier in one patient and a respiratory circuit condensate from the other) collected during the first outbreak in the ICU, which had only two beds at that time. None of the cultures of clinical surfaces, air, or health care workers' hands in the ICU and wards obtained during the second outbreak were positive for *P. aeruginosa*.

Antibiotic susceptibility profiles and molecular analysis of MR-PA isolates

All *P. aeruginosa* isolates (n = 94) underwent antibiotic susceptibility testing. Thirty-three isolates from patients – 20 MDR-PA, 12 XDR-PA, and 1 CR-PA – had antibiotic-

resistant profiles (Figure 1). The MDR-PA strains were universally resistant to gentamicin, amikacin, cefotaxime, ticarcillin-clavulanic acid, imipenem, meropenem (tested since January 2005), ciprofloxacin, levofloxacin (tested since September 2004), norfloxacin, and trimethoprim-sulfamethoxazole. Fifteen MR-PA strains isolated from six patients in the first outbreak were susceptible to cefepime, ceftazidime, and polymyxin B; one was susceptible to aztreonam; and six were susceptible to piperacillin-tazobactam.

The index case, five of 11 patients in the outbreaks and one patient from the interepidemic period had one MDR-PA or XDR-PA isolate each analyzed by PFGE (Table 1, Figure 2). The two MR-PA-positive environmental strains also underwent PFGE. Figure 2 shows that all of the PFGE-tested strains had identical genetic profiles and belonged to the São Paulo clone (the SPM-1 strain was matched with MR-PA isolates). However, all of these strains were negative for tested MBL production.

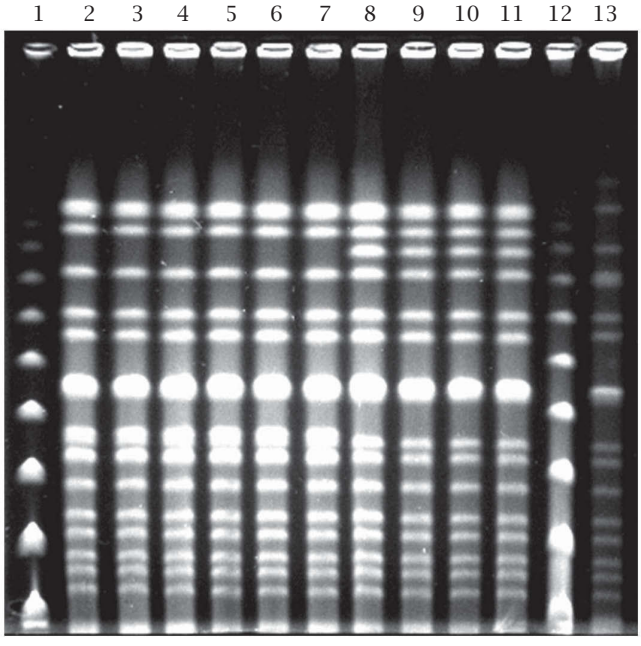

Figure 2: PFGE patterns of 10 isolates of MDR-PA and XDR-PA. *Lanes 1 and 12*, PFGE Lambda (molecular markers). *Lanes 2-11*, PFGE-detected patterns in 10 strains of MDR-PA or XDR-PA: *2,* XDR-PA, patient 1 (index case), blood; *3*, XDR-PA, patient 2 (1º outbreak), BAL; *4*, MDR-PA, patient 3, tracheal aspirate (1º outbreak); *5 and 6*, MDR-PA, swab from condensate of the ventilator circuit of patient 2 (1º outbreak); *7*, MDR-PA, water sample from a humidifier (1º outbreak); *8*, XDR-PA, patient 6, urine (non-outbreak case); *9*, XDR-PA, patient 7, urine (2º outbreak); *10*, XDR-PA, patient 8, blood (2º outbreak); *11*, XDR-PA, patient 9, blood (2º outbreak). *Lane 13,* São Paulo MLB strain.

Infection control intervention and education program

Infection-control measures involved outbreak notification, cohort of patients and professionals, temporary closing of the ICU to new admissions during the first outbreak, contact precautions for all colonized and/or infected patients, environmental disinfection, and a review of all disinfection and sterilization processes. During the first outbreak, the infection control team reviewed the health care providers' work process to minimize cross-transmission of MR-PA, including technicians involved in performing hemodialysis from a contracted service. Hospital personnel repaired a water leak in the compressed air system and changed the air conditioning filters in the ICU. Furthermore, the hospitals newly constructed ICU opened in June 2006. Members of the infection control committee systematically presented the surveillance results, including results of molecular analysis of the *P. aeruginosa* strains and the patients' outcomes, to the hospital staff in educational activities carried out over the entire study period to ensure their commitment to infection control measures. However, the hospitals' nursing staff underwent a great turnover in March 2005, whereas the physician and nursing staff did so in November 2006.

DISCUSSION

Researchers have demonstrated the difficulty of controlling MR-PA infections in hospitals where the agent is already endemic.[21] However, a multifaceted infection control program can temporarily control the transmission of this infection.[3,5] To the best of our knowledge, this is the first report to describe the epidemiological characteristics of MDR-PA/XDR-PA infection outbreaks that were temporarily controlled but not eradicated in patients with AIDS, in whom persistent MR-PA infections likely increase the risk of transmission.

The reported overall incidence of *P. aeruginosa* carriage in ICUs and oncological/hematological or medical wards has varied from 11.7% to 37.0%.[22,23] Many authors have advocated the use of surveillance cultures to improve the control of infections with multiresistant pathogens in high-risk settings, but the benefit of this strategy remains to be evaluated.[24] We agree with previous observation that surveillance of patients with MR-PA infections must be accompanied by an early implementation of an intervention program whenever required.[3] In the present study, we based the surveillance program on clinical microbiological examinations of patients' samples requested by infectious disease specialists, who usually have high diagnostic suspicion of nosocomial infections. In addition, we systematically used surveillance cultures with rectal swabs during the two outbreaks; however, we used them in a non-systematic manner between outbreaks. We performed rectal swabs only, as respiratory and urinary samples were normally used for diagnosis of infections. In addition, gastrointestinal carriage appears to

be a prerequisite for *P. aeruginosa* colonization and/or infection at other sites, and the intestinal tract is considered the most important reservoir of *P. aeruginosa*.[22,25] However, the sensitivity of rectal swabs in detecting small inocula is suboptimal,[22] and patients with colonization at sites other than the intestinal tract may have been missed.[25,26] Our intention was to perform surveillance of patients with MR-PA infections in a real-life hospital setting with limited resources so that the surveillance program would be easy to accomplish. This may have been a limitation of our study, though, as well as our lack of use of selective antimicrobial media for rectal swabs to detect antibiotic-resistant *P. aeruginosa*.[23]

Patients with MR-PA colonization and/or infections frequently have contamination of their hands, clothes, and/or immediate surroundings, which raises the possibility of transmission to other patients either directly or via contact with hospital staff or contaminated equipment.[1,2,6,27] During the first outbreak, four patients were undergoing hemodialysis performed by the same technician. We controlled this epidemic after we reviewed this technician's work and assigned different technicians for each patient. Nevertheless, we did not obtain cultures from the hands of the original technician. This horizontal transmission of MR-PA infections was supported by the findings of genotyping analysis of MR-PA isolates obtained from patients and environmental and of the temporal and spatial association among MR-PA cases.

As previously described, we obtained samples of water and wet surfaces contaminated with *P. aeruginosa* in our surveillance report only in close proximity to infected/colonized patients.[3,27] Studies showed that airborne dissemination can have a role in the transmission of *P. aeruginosa*.[27,28] However, we did not detect any *P. aeruginosa* isolates using an air sampler that has equivalent results of the sixth stage of an Andersen air sampler.[29] The small number of air samples obtained in our study may have influenced this result. The newly renovated ICU in our hospital (with isolation rooms and a modern ventilation system) may have contributed to temporary control of the infection, as suboptimal ICU designs layouts such as our previous one have been associated with MR-PA infection outbreaks.[4] The organism's panantibiotic-resistance pattern and biofilm formation most likely played important roles in extending the outbreaks.[2] Also, hospital personnel shortage[30] or, as in our case, high hospital staff turnover may be related to MR-PA infection outbreaks or initial difficulty in controlling them. In addition, the second outbreak may have been related to readmission of an MR-PA colonized patient which was likely colonized during hospitalization at the time of the first outbreak; however, we cannot rule out other explanations not fully explored in this study.

The continuous training program on prevention and control of nosocomial infections given to all health care professional categories was an essential tool for controlling MR-PA infections in our hospital, as well.[5,12] We used several educational activities and resources such as posters, folders, and signs during the study period. Continuous education is important even in hospitals specialized in treating infectious diseases, where all health care workers have some knowledge about the risk of nosocomial infections. Keeping the clinical staff informed about the surveillance results, clinical outcomes of infected patients, and, when feasible, molecular characteristics of isolated strains is essential to increase and maintain their commitment to controlling nosocomial infection. This is especially important for high-risk populations while new preventive and treatment strategies for MR-PA infections are being developed.

We observed some differences in the antibiotic susceptibility patterns between the two outbreaks, although the genetic profiles of the tested strains were identical. The antibiotic-resistance patterns of MR-PA isolates changed during the five-year study period, with MR-PA strains becoming resistant to almost all of the antibiotics tested except polymyxin B. Six patients had *P. aeruginosa* isolates susceptible or less resistant to the antibiotics tested prior to detection of more antibiotic-resistant strains. All of these patients had received cefepime alone or in combination therapy before isolation of *P. aeruginosa* strains with the patterns of greater antibiotic resistance. Therefore, although cross-colonization was an important feature of these outbreaks, antimicrobial pressure was also likely responsible for these differences in antibiotic resistance. However, in settings of infrequent antimicrobial use, frequent cross-transmission events can contribute to high antibiotic resistance rates.[31] We did not completely assess the resistance mechanism in our tested strains, but all of the isolates were negative for MBL production. Unfortunately, we did not have the necessary resources to study other antibiotic-resistance mechanisms previously described in MR-PA.[8,32] MDR-PA infections probably acquired new or derepressed resistance mechanisms during the study period. Sequential emergence of resistance is likely due to the administration of different antibiotics at different time periods following the development of resistance.[33]

The high mortality rates in this study demonstrated the burden of MR-PA infections in immunocompromised patients. This may have reflected the immunocompromised states of the patients as well as the multidrug resistance and possible enhanced virulence of the outbreak strain.[2] All patients who died had severe comorbidities or opportunistic diseases that likely contributed to the high mortality rates. Inappropriate empirical antimicrobial-based therapy and delays to initiate appropriate antimicrobial-based therapy are both detrimental to patient outcome.[34] Authors have described failure of cefepime-susceptibility breakpoints to predict clinical outcomes[35] as well as the requirement of achieving adequate drug exposure to be successful in treating patients with

P. aeruginosa infections.[36] Additionally, a multivariate logistic regression analysis demonstrated *P. aeruginosa* infection to be independently associated with treatment failure in cefepime-patients.[37] We did not evaluate any of these features in the present study; therefore, we cannot attribute the high mortality rates to the antibiotics used.

Investigators have observed relapsed or recurrent infections caused by non-multiresistant *P. aeruginosa* in patients with AIDS but without any impact on mortality.[9] In addition, authors have described persistent respiratory infections with sequentially obtained non-multiresistant *P. aeruginosa* strains in AIDS patients.[10] The incidence of treatment failure and persistent or recurrent infections was markedly elevated in the present study. Also, persistent and recurrent infections were observed in patients with and without AIDS. The criteria used for identification of persistent and recurrent infections were based on clinical and antibiotic susceptibility data but not the genetic profiles of the isolated strains, as we did not perform molecular typing of all of the MR-PA isolates (only of randomly preserved strains). In addition, we obtained rectal swabs only from patients who were negative for MR-PA in close contact with a MR-PA positive patient. Consequently, we did not investigate colonization of *P. aeruginosa* in the gastrointestinal tract of patients with MR-PA infections or colonization at other sites. The investigation of MR-PA colonization in the gastrointestinal tract may contribute to understanding the characteristics of persistent and/or recurrent infections caused by MR-PA in these patients. Therefore, more investigations of persistent infections caused by *P. aeruginosa* are necessary in this era of antimicrobial resistance in patients other than those with cystic fibrosis in whom this feature is well recognized.[38,39]

Regarding patients with symptomatic HTLV-1 infections, studies have suggested that they are at increased risk for UTIs.[40] Physicians have used prolonged urinary catheterization in these patients because of their myelopathy and/or tropical spastic paraparesis. Urinary catheterization and UTIs are both recognized risk factors for MR-PA infection.[41,42]

CONCLUSIONS

A multifaceted infection control program can control and prevent temporarily the transmission of MR-PA infection in the AIDS setting. Broader investigation of patient environments as well as risk factors for MR-PA infection and/or colonization may help increase understanding of the multifactor origin of MR-PA infection and develop local recommendations for prevention and control of the infection. Infection control programs and diagnosis and treatment approaches of MR-PA infections in AIDS patients must consider the persistent characteristic of MR-PA infections in this population.

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