Carbapenem-Resistant *Pseudomonas aeruginosa* Outbreak in an Intensive Care Unit of a Teaching Hospital

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The genetic similarity of carbapenem-resistant *Pseudomonas aeruginosa* strains isolated in the Hospital Universitário São Francisco, Bragança Paulista, São Paulo, Brasil, was evaluated by pulsed field gel electrophoresis (PFGE). A unique clone was detected among 5 of 7 isolates, suggesting that cross-contamination might have played a role in the spread of carbapenem-resistant *P. aeruginosa* strains. Interestingly, a similar PFGE pattern was encountered in a *P. aeruginosa* strain isolated from Hospital São Paulo that was used as a PFGE control.
Key Words: *Pseudomonas aeruginosa*, PFGE, carbapenem.

Pseudomonas aeruginosa is a Gram-negative aerobic bacillus isolated from soil, water, plants, and animals, including humans [1]. The minimal nutritional requirements of *P. aeruginosa*, its tolerance of a wide variety of physical conditions, and its relative resistance to antimicrobial agents contribute to its ecological success and to its role as an effective opportunistic pathogen [1,2].

Pseudomonas aeruginosa is primarily a nosocomial pathogen, and it rarely affects healthy persons. It is a leading cause of nosocomial infections, ranking first as a cause of nosocomial pneumonia in Brazilian hospitals [3]. In the United States, *P. aeruginosa* ranked first among all nosocomial pathogens related to pneumonia in intensive care units reported to the National Nosocomial Infection Surveillance System [4].

Carbapenems generally remain one of the last therapeutic resorts against serious *P. aeruginosa* infections [5]. However, carbapenem use has been limited by the emergence and the dissemination of multidrug resistant clones [2]. In the Hospital Universitário São Francisco, HUSF, a 160-bed Brazilian teaching hospital, an increase in the isolation of carbapenem-resistant *P. aeruginosa* was noticed between March and April 2001. The main aim of this study was to investigate if the increase of carbapenem-resistant *P. aeruginosa* isolation was related to the emergence and spread of an epidemic strain within our institution.

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Materials and Methods

Bacterial strains

Seven *P. aeruginosa* strains were included in the study. They were collected from diverse body sites from five patients hospitalized at HUSF between March and April 2001.

Susceptibility testing

Following the species identification, antimicrobial susceptibility testing was performed using the disk-diffusion technique, as described by the National Committee for Clinical Laboratory Standards (NCCLS) [6]. The antimicrobial agents tested were amikacin, gentamicin, piperacillin/tazobactam,

ceftazidime, cefepime, imipenem, meropenem, ciprofloxacin, chloramphenicol, and polymyxin B. The antimicrobial susceptibility testing results were interpreted using the NCCLS criteria established for non-Enterobacteriaceae [7]. Quality control was performed by testing *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *P. aeruginosa* ATCC 27853.

Genotyping

The *P. aeruginosa* isolates were typed by pulsed-field gel electrophoresis (PFGE) to determine genetic similarity. The PFGE was performed using SpeI restriction endonucleases and the switch time varied from 5 to 90 seconds, as previously described [8]. Analysis of PFGE patterns was performed by visual inspection of photographs of ethidium bromide-stained gels. The isolates were classified as identical if they shared the same bands, closely related if they differed by one to three bands, and as distinct if they varied by more than three bands [8].

Results

The results of this study were summarized in Table 1. Five carbapenem-resistant *P. aeruginosa* were collected from four patients hospitalized in the intensive care unit of the HUSF, while the two remaining isolates were isolated from a patient hospitalized in the internal medicine ward. All of the carbapenem-resistant *P. aeruginosa* strains were isolated between March and April 2001, and they were responsible for causing nosocomial-acquired infections.

Regarding the antimicrobial susceptibility results, two strains collected from the same patient were susceptible to all agents tested except the carbapenems. In contrast, the five remaining carbapenem-resistant *P. aeruginosa* strains were resistant to all antimicrobial agents tested, except polymyxin B.

Among the seven carbapenem-resistant *P. aeruginosa* strains, two distinct PFGE patterns were detected. The PFGE pattern C_1 was found in five multidrug resistant *P. aeruginosa*. Curiously, the pattern C_1 was similar to the pattern C_2 displayed by a carbapenem-resistant *P. aeruginosa* isolated from the Hospital São Paulo, HSP, Universidade Federal de São Paulo/EPM, which was included in the PFGE gel as a control (P813).

Discussion

Intensive care units (ICUs) have been clearly noted as endemic settings for *P. aeruginosa*. Several factors have contributed to this, such as the presence of critically ill patients who need invasive devices, extensive use of antibiotics, and limited number of health care providers [9-12]. In our study, all carbapenem-resistant *P. aeruginosa* isolates, except two, were collected from patients hospitalized in the ICU. Two isolates were collected from a patient who was hospitalized at the internal medicine ward but had been hospitalized in ICU in November 2000. At that time, the patient was admitted to the ICU due to serious trauma injuries and developed a multidrug-resistant *P. aeruginosa* acute osteomyelitis in the left femur after a surgical correction of an open fracture.

The epidemiology of *P. aeruginosa* infections has been studied by the analysis of phenotypic markers, including antimicrobial susceptibility profiles. However, genotypic methods, such as PFGE, are the most important tools to characterize the identity of bacterial strains like *P. aeruginosa* [8,9]. We observed a good correlation between antimicrobial susceptibility results and PFGE patterns, as all multidrug-resistant *P. aeruginosa* exhibited an identical PFGE type.

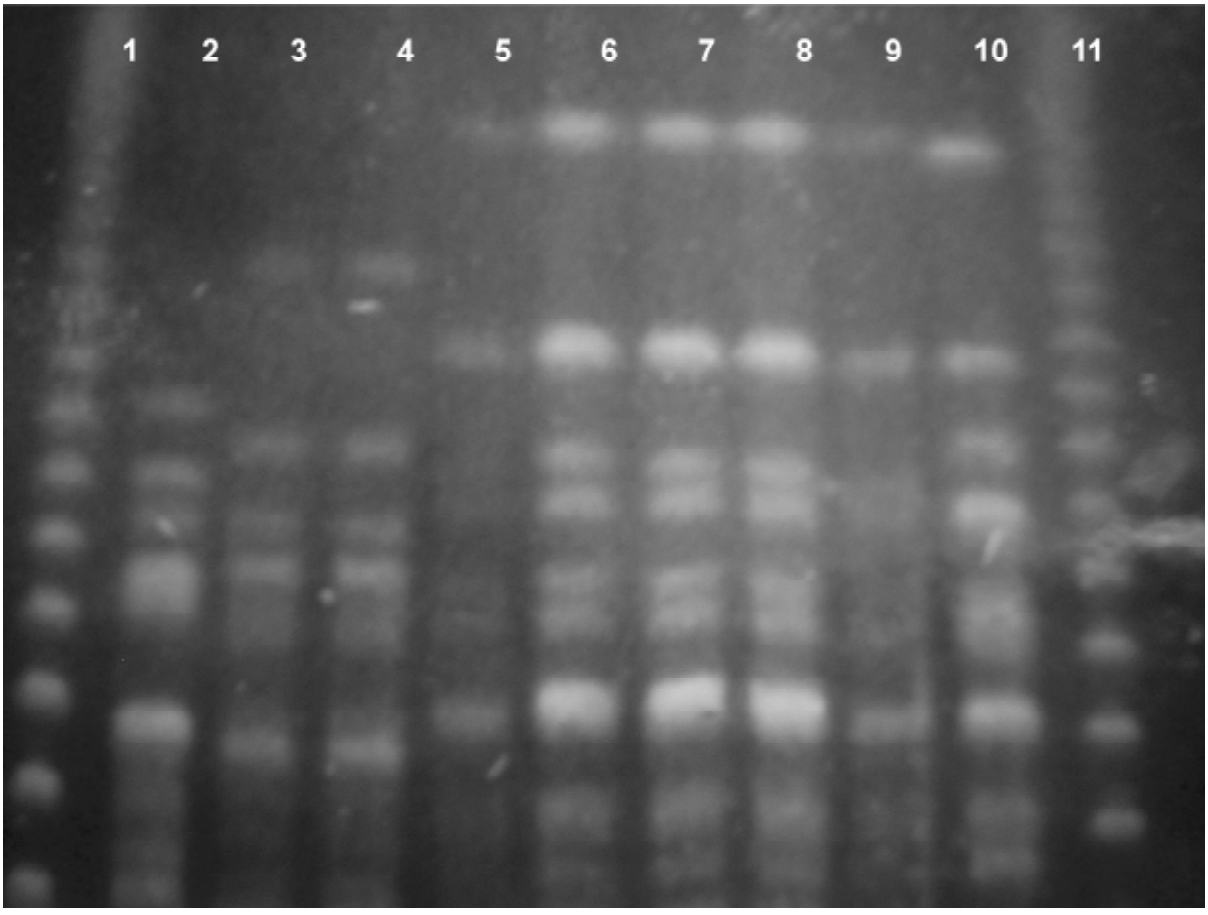
The presence of a clonal strain among the carbapenem-resistant *P. aeruginosa* suggested that the ICU experienced an outbreak due to horizontal transmission. Hands of medical personnel have been considered the major vector of propagation of *P. aeruginosa* [9-11]. Bertrand et al. showed that 50% of all cases of *P. aeruginosa* colonization in an ICU were due to cross-colonization [9]. The patient with chronic osteomyelitis could have been the index case since no multi-drug resistant *P. aeruginosa* isolates belonging to clone C_1 were detected previously to his

Table 1. Charcteristics of carbapenem-resistant *Pseudomonas aeruginosa* – Patients' demographic data, antimicrobial susceptibility profiles and PFGE patterns

									Susceptibility to Antimicrobial Agents[d]										
Patients' initials	**Sex**	**Age**	**Unit[a]**	**Diagnosis**	**Length of stay in days[b]**	**Body site of isolation[c]**	**Bank number**	**Date (d/m)**	**Am**	**Gn**	**P/T**	**Cz**	**Cp**	**Im**	**Mp**	**Cip**	**Chl**	**Pol**	**PFGE pattern**
PDL	M	35	ICU	Trauma	7	TA	P1270	4/4	S[e]	S	S	S	S	R[f]	R	S	S	S	B
					23	wound	P1271	10/4	S	S	S	S	S	R	R	S	S	S	B
HGS	F	64	ICU	Pneumonia	5	BAL	P1275	10/4	R	R	R	R	R	R	R	R	R	S	C_1
BCM	M	68	ICU	Acute Vascular Abdomen	8	urine	P1273	10/4	R	R	R	R	R	R	R	R	R	S	C_1
RCA	M	25	ICU	Trauma	27	abscess	P1282	4/5	R	R	R	R	R	R	R	R	R	S	C_1
NOP	M	52	Int. med.	Osteo-myelitis	3	skin secretion	P1274	9/4	R	R	R	R	R	R	R	R	R	S	C_1
NOP	M	52	Int. med.	Osteo-myelitis	13	bone	P1272	19/4	R	R	R	R	R	R	R	R	R	S	C_1

a. ICU, intensive care unit; Int. Med., internal medicine.
b. Hospital stay in days before the isolation of carbapenem-resistant *P. aeruginosa*.
c. TA, tracheal aspirate; BAL, bronchoalveolar lavage.
d. Am, amikacin; Gn, gentamicin; P/T, piperacillin/tazobactam; Cz, ceftazidime; Cp, cefepime; Im, imipenem; Mp, meropenem; Cip, ciprofloxacin; Chl, chloramphenicol, and Pol, polymyxin B.
e. S, susceptible.
f. R, resistant.

Figure 1. Pulsed field gel electrophoresis patterns of carbapenem-resistant *Pseudomonas aeruginosa*. Lanes 1 and 11, DNA molecular marker; lane 2, *P. aeruginosa* quality control strain 120030; lane 3, P1270; lane 4, P1271; lane 5, P1272; lane 6, P1273; lane 7, P1274; lane 8, P1275; lane 9, P1282; lane 10, carbapenem-resistant *P. aeruginosa* control strain P813.



hospitalization. However, multidrug-resistant *P. aeruginosa* isolates were detected in the HUSF previously, in November 2000, and they are still sporadically detected. Thus, clone C_1 might have emerged earlier and persisted in the hospital environment, and occasionally caused infections and outbreaks. Generally, the genotypic results will guide the actions that must be taken by the infection control team, such as eradication of a common environmental source or other control measures to avoid cross-infection [8,9]. After emphasizing compliance to infection control measures, such as handwashing and contact precaution, a reduction of the multidrug-resistant *P. aeruginosa* isolation was observed in the HUSF.

The genetic similarity among carbapenem-resistant *P. aeruginosa* isolated at HUSF and HSP was a totally unexpected finding. The occurrence of multidrug-resistant *P. aeruginosa* strains belonging to a unique genotype was also reported in various hospitals in the state of Rio de Janeiro [13]. Recently, the dissemination of an epidemic of carbapenem-resistant *Pseudomonas aeruginosa* producing SPM metallo-beta-lactamase in distinct Brazilian regions was reported [14]. A single clone of *P. aeruginosa* was reported from various Brazilian hospitals located in different parts of Brazil. The spread of *P. aeruginosa* among hospitals could be explained by the transfer of infected patients and/or sharing of common health care staff. However, to

have a better understanding about the dissemination of carbapenem-resistant *P. aeruginosa* isolates among Brazilian hospitals, it would be important to evaluate a larger number of *P. aeruginosa* isolates collected from distinct hospitals located in other Brazilian regions as well.

We confirmed the importance of *P. aeruginosa* as an important nosocomial pathogen in Brazilian hospitals and we emphasize that handwashing is a valuable measure to control an outbreak.

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