Antibiotic Resistance and Molecular Typing of *Pseudomonas aeruginosa*: Focus on Imipenem

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Susceptibility tests by disk diffusion and by E-test and molecular typing by macrorestriction analysis were performed to determine the relatedness of *Pseudomonas aeruginosa* isolates from three distinct hospitals. The resistance profile of 124 isolates to 8 antimicrobial agents was determined in three different hospitals, in Porto Alegre, Brazil. Frequencies of susceptibility ranged from 43.9% for carbenicillin to 87.7% for ceftazidime. Cross-resistance data of imipenem-resistant isolates indicated that most (70%) were also resistant to carbenicillin, although 30% remained susceptible to ceftazidime and cefepime. In general, susceptibility profiles were not able to determine relatedness among isolates of *P. aeruginosa*. On the other hand, molecular typing by macrorestriction analysis demonstrated high discriminatory power and identified 66 strains among 72 isolates of *P. aeruginosa*. Imipenem-susceptible isolates were all different. However, identical clones of imipenem-resistant isolates were found in two of the hospitals, despite variable response to other antibiotics. No clustering of infection among the different medical centers was observed. In conclusion, clones of *P. aeruginosa* did not spread among the different hospitals in our city even though related isolates of imipenem-resistant *P. aeruginosa* were found.

Key Words: Pseudomonas aeruginosa, antibiotic resistance, imipenem.

Despite improvements in antibiotic therapy, *Pseudomonas aeruginosa* remains as one of the most prominent Gram-negative bacteria causing hospital-associated infections. *P. aeruginosa* is intrinsically resistant to a number of antimicrobial agents, frequently including multiple classes of antimicrobial agents [1]. Carbapenens are potent agents for the treatment of infections due to multiresistant pseudomonads. However, nosocomial isolates may easily develop resistance to carbapenens due to reduced uptake of the drug, which leads to outbreaks of multiresistant/carbapenen-resistant strains [2-4]. It is therefore important to develop surveillance programs to determine the epidemiological situation of *P. aeruginosa* in different hospitals [5].

Outbreaks caused by multiresistant *P. aeruginosa* have been reported in various nosocomial settings, such as in individual intensive care units (ICU) or other units within a hospital. These outbreaks can be brief or can persist for an extended period of time, with the same strains being identified over a one year period [3,4,6-8].

Typing techniques that establish clonal relationships between individual isolates in hospital settings are warranted in order to recognize nosocomial transmission and hence to guide infection control practice. DNA-based techniques, such as

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macrorestriction analysis by pulsed field gel electrophoresis, have been successfully applied to the epidemiological study of *P. aeruginosa* [9].

We examined *P. aeruginosa* strains isolated from hospitalized patients in Porto Alegre, Brazil, to determine their susceptibility to antibiotics and their epidemiological relatedness.

Materials and Methods

A total of 124 nonreplicate *Pseudomonas aeruginosa* clinical isolates were recovered from patients from 3 hospitals (HCPA, ISCM, and HSL), in Porto Alegre, Brazil, between September 1998 and June 1999.

Overall, 54% of the isolates were obtained from the respiratory tract, 28% from urine, 6.5% from blood and 11.5% from a variety of other sources (catheters, abdominal secretions, skin).

Identification was based upon production of characteristic pigments (blue and green). Additional biochemical tests used to identify *P. aeruginosa* included*:* oxidase, oxidation of glucose on OF-medium, arginine and nitrate, and growth in cetrimide agar [1]. Isolates were checked for purity by plating on MacConkey agar before the susceptibility tests and typing.

The susceptibility tests were performed with the agar disk diffusion method, according to NCCLS guidelines [10]. The following antimicrobial agents were tested: amikacin, aztreonam, carbenicillin, ceftazidime, cefepime, ciprofloxacin, imipenem and ticarcillin (BBL-Becton Dickinson Microbiology, Cockeysville). The resistance rates determined in this study include both intermediate and fully resistant lines. The isolates were classified into antibiotypes according to the susceptibility patterns. Isolates that had an identical response to all antimicrobial agents were considered to belong to the same antibiotype, while a single difference in resistance to any of the antimicrobial agents was considered a distinct profile.

In order to avoid false designation of resistance to imipenem, due to drug degradation during storage [11], isolates that tested resistant by the disk diffusion method were confirmed by the E-test (AB Biodisk, Solna, Sweden).

Macrorestricton analysis, followed by PFGE, was performed as described previously [12]. Briefly, the bacteria was embedded in agarose blocks, digested with restriction endonuclease *Spe*I (Gibco BRL, USA) and electrophoresed in a CHEF DR II apparatus (Bio-Rad, Richmond, USA). The gels were run at 14^{o}C, 5.9 V cm^{-1}, for 22 hours, with a switch time of 5 to 50 s. A lambda ladder (48.5 Kb, Sigma, USA) was used as a molecular weight marker. The gels were stained with ethidium bromide and the image was acquired on a Chemilmager transiluminator 4000 (Alpha Inntech Corporation). Comparison of macrorestriction profiles was performed by visual analysis.

Isolates were considered to be part of a major clone whenever they had identical macrorestriction profiles (PFGE). They were considered related (subtypes) when there were 1 to 6 differences in fragments (bands) between profiles. Isolates with more than 6 different fragments were considered distinct or unrelated strains [13]. Each major clone was coded with a capital letter and a number was added to each subtype.

Results and Discussion

Among the 124 *P. aeruginosa* isolates the degree of susceptibility varied from 43.9% for carbenicillin to 87.7% for ceftazidime. Cefepime, ceftazidime and imipenem proved to be effective against most *P. aeruginosa* clinical isolates (> 80% susceptibility).

The rates of resistance of *P. aeruginosa* to amikacin, aztreonam, ciprofloxacin, ceftazidime and cefepime were similar to those described in other studies for isolates from Brazil [14-16] and other countries [4, 5, 17-20]. However, in our study ticarcillin and carbenicillin were less effective when compared with most other surveillance studies. Although many reports suggest increasing multiresistance in *P. aeruginosa* [2-4, 8] only four isolates (3.2%) were resistant to all of the antimicrobial agents, which contrasts with 37% susceptibility to all agents.

We found 20 strains to be resistant to imipenem, most of them with MIC ≥ 32 μg/mL. In fact, only one of these isolates had a MIC = 16 μg/mL, which would also be considered resistant according to NCCLS [10]. We therefore found 100% correlation between disk diffusion and E-testing for all imipenem resistant isolates, as also described by Burns et al. [21].

The rate of susceptibility to imipenem (83.7%) was similar to rates found in the SENTRY antimicrobial surveillance program in Brazil and in Latin America [14, 15]. However, a study of 26 Brazilian isolates of *P. aeruginosa* found less than 70% susceptibility to imipenem [20]. Data from other countries indicates susceptibility to imipenem ranging from 79% [4] to 95% [20].

Cross-resistance data are useful to indicate alternative drugs for treatment. In our study, ceftazidime was the most active antimicrobial agent, and less than 50% of the ceftazidime-resistant isolates were also resistant to imipenem or cefepime (Table 1). Additionally, only 30% of the isolates resistant to imipenem were also resistant to cephalosporins, which indicates that ceftazidime and cefepime may be good options for the treatment of infections due to imipenem-resistant *P. aeruginosa*.

Bouza and cols. [5] provided cross-resistance data of *P. aeruginosa* isolated in Spain. They found cross-resistance rates to most of the agents similar to those of our study; imipenem-resistant *P. aeruginosa* were more resistant to ticarcillin and amikacin in our study. However, the percentage of individual resistance to ticarcillin and amikacin agents was also higher in our population than in the Spanish isolates. Among ceftazidime-resistant isolates Bouza et al. [5] found 80% to be resistant to cefepime, while we found only 47% (Table 1). This may be important since individual resistance to each agent was very similar in the two studies.

The high incidence of cross-resistance to antibiotics of different classes observed in our study is probably due to a combination of multiple, unrelated resistance mechanisms.

We typed 72 isolates by macrorestriction analysis (including those resistant to imipenem, ceftazidime or cefepime) and found 66 distinct strains. There were no common clones for any susceptibility pattern, unless they were also resistant to imipenem.

Several imipenem-resistant isolates were indistinguishable by macrorestriction analysis, despite variations in their susceptibility to other antimicrobial agents. The 20 imipenem-resistant isolates (9 from ISCM, 7 from HSL and 4 from HCPA) had 12 major types of DNA (Table 2).

There were 5 major types of imipenem-resistant *P. aeruginosa* in ISCM. Two isolates were of type A and were recovered within a period of 4 days, from patients hospitalized in different units. Their susceptibility profiles were very similar, differing only by resistance to aztreonam. Type B included 3 urine isolates, with the same susceptibility profile, but recovered during a longer period of time (18 days). The type C isolates were obtained during a two day period and had a similar susceptibility profile, but were isolated from patients hospitalized in different units. The remaining 2 isolates from ISCM had distinct molecular types. Although they were recovered on the same day, there was no epidemiological correlation between the patients (Table 2). Although laboratory data indicated similar clones of *P. aeruginosa* in ISCM, thus suggesting patient-to-patient transmission, the epidemiological data do not corroborate this conclusion, since the patients were admitted in distinct units and the isolates were obtained at different periods of time. These isolates may have some characteristic which improves their permanence and dissemination in a hospital environment.

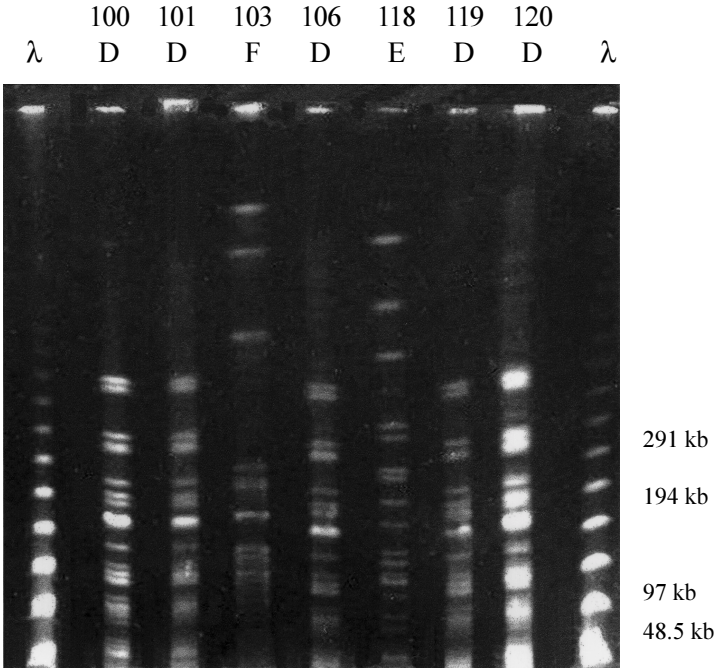
Most of the isolates from HSL (5 out of 7) belonged to the same genotype (figure 1). These isolates were obtained from the respiratory tract of patients in the same unit during a relatively long period (28 days). Unrelated strains were also obtained during this period of time, including one isolated from a patient in the same unit. Almost all isolates of HSL had distinct susceptibility profiles, regardless of their genotype. Since identification of type D *P. aeruginosa* from HSL was restricted to this period it seems that this clone came from a short duration outbreak.

Table 1. Cross-resistance among *P. aeruginosa* isolates

Drug to which isolates were resistant	No. of strains	Resistant	% Resistance [a] to					
		CAZ	CEF	IMI	AZT	CIP	TIC	AMI	CAR
Ceftazidime	15		47	40	73	47	73	60	87
Cefepime	18	39		33	83	83	100	94	100
Imipenem	20	30	30		45	50	55	45	70
Aztreonam	37	30	41	24		65	81	68	97
Ciprofloxacin	39	18	38	26	62		85	92	97
Ticarcillin	43	26	42	26	70	77		79	100
Amikacin	41	22	41	22	61	88	83		98
Carbenicillin	70	19	26	20	51	54	61	57	

[a] nonsusceptible isolates.
CAZ= ceftazidime; CEF= cefepime; IMI= imipenem; AZT= aztreonam; CIP= ciprofloxacin; TIC= ticarcillin; AMI= amikacin; CAR= carbenicillin.

Figure 1. Macrorestriction profiles of *P. aeruginosa* isolates from HSL



All lanes are from the same gel. λ = molecular weight marker (48.5 Kb).

Table 2. Distribution of imipenem resistant *P. aeruginosa* isolates according to macrorestriction (genotype), antimicrobial susceptibility profile (antibiotype) and location of the patient

Hospital	N° isolate	Genotype (*Spe*I)	Antibiotype	Date of isolation (mo/day/yr)	Location (unit)
ISCM	123	A	f	06/09/99	USC
	26	A	g	06/12/99	UTI
	24	B	j	05/29/99	USF
	27	B1	j	06/10/99	UPF
	21	B2	j	06/15/99	USC
	20	C	i	04/06/99	USC
	39	C1	j	04/07/99	UPF
	23	K	a	10/25/98	USF
	15	L	i	10/25/98	USC
HSL	100	D	e	11/10/98	UTI
	101	D	a	11/11/98	UTI
	106	D	h	11/17/98	UTI
	119	D	b	12/02/98	UTI
	120	D	c	12/06/98	UTI
	118	E	a	12/02/98	UTI
	103	F	d	11/13/98	UCL
HCPA	42	H	a	10/24/98	UCL
	62	I	i	01/11/99	UCL
	69	J	f	10/10/98	UTI
	76	G	c	10/08/98	UTI

Antibiotype of imipenem resistant isolates: a= susceptible to all other agents; b= resistant only to ceftazidime; c= resistant only to carbenicillin; d= resistant a carbenicillin and ciprofloxacin; e= resistant a carbenicillin e ticarcillin; f= resistant a ciprofloxacin and amikacin; g= resistant to aztreonam, ciprofloxacin, amikacin; h= resistant a ceftazidime, cefepime and carbenicillin; i= resistant to aztreonam, ciprofloxacin, ticarcillin, amikacin and carbenicillin; j= resistant to all agents.

Isolates from HCPA were all unrelated, according to either DNA or susceptibility profiles even though two isolates were recovered within a two day period from sputum of patients admitted to the same unit.

It is well known that macrorestriction analysis is the most powerful tool for epidemiological studies of *P. aeruginosa*, while phenotypic markers can lead to misinterpretation of strain relatedness [9, 22]. We found that in some cases both DNA and susceptibility profiles lead to the same conclusion while in others the results were conflicting. For instance, we observed agreement in the case of isolates 21 and 27, which belonged to the same major type (B) and displayed the same antibiotype (Table 2).

However, discrepancy was more frequent than agreement between the different methods. In most cases, susceptibility patterns were not able to indicate strain identity, as seen for HSL isolates belonging to clone D and ISCM isolates belonging to clone A. Furthermore, the susceptibility pattern has low

discriminatory power for establishing strain identity. In our study the antibiotype "a" was found in all hospitals, despite the absence of epidemiological relationships among isolates.

We analyzed isolates from distinct hospitals in the same city, during a relatively long period of time and found a lower frequency of resistance to antibiotics used against *P. aeruginosa* than the rates described in other studies [2-4, 8, 20].

Contrary to other studies that describe major clones of resistant *P. aeruginosa* spread among several hospitals within a country [3, 4, 7], molecular typing of our isolates revealed mainly unique strains. However, we noted a clustering of infections due to the same genotype among imipenem resistant *P. aeruginosa* isolates, suggesting possible transmission inside a hospital but not between different medical centers.

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