Genotypic characteristics of *Pseudomonas aeruginosa* strains circulating in the tertiary referral Children's Medical Hospital in Tehran, Iran

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ABSTRACT

Pseudomonas aeruginosa is an important pathogen with the ability to cause infection in all departments of the hospital, especially in intensive care units (ICUs). The aim of this study is to analyse the epidemiological relationships among clinical *P. aeruginosa* strains isolated from different wards of the Children's Medical Center Hospital (Tehran, Iran). These isolates were identified by standard laboratory procedures and tested for antimicrobial resistance to several antibiotic agents. The genetic similarity of the strains was investigated by amplification of the enterobacterial repetitive intergenic consensus sequence (ERIC-PCR). During the study period, 87 non-duplicate patients were colonised or infected with *P. aeruginosa*. Among the isolates, resistance to piperacillin/tazobactam was low (27%), followed by amikacin (31%), gentamicin (33%), imipenem (33%), ciprofloxacin (36%) and meropenem (39%). Thirty-five patients (40.2%) were either colonised or infected with a multidrug-resistant *P. aeruginosa* strain (MDRP) over a one-year period, and 17 isolates were non-susceptible to all the tested antibiotics. One predominant profile (D) was identified in 59 strains. This profile first appeared in the paediatric intensive care unit (PICU) and infection ward in June 2010, and circulated around all wards up to the end of the study period. Of the 35 MDRP, 22 (62.8%) were found to be profile D. Molecular typing of the isolates suggests considerable cross-transmission of *P. aeruginosa* not only between patients in one ward but also between patients from different wards. This can be explained partly by the high number of patients transferred between different wards of the hospital.

KEY WORDS: Genotyping techniques.
Pseudomonas aeruginosa.
Transmission.

Introduction

Pseudomonas aeruginosa is an important pathogen with the ability to cause infection across all departments of the hospital, especially in the intensive care unit (ICU).[1] The overall rate of nosocomial *P. aeruginosa* infection has been found to be increasing[2] and the high frequency of multidrug resistance among these strains is a major problem in hospitals and makes eradication difficult.[3] Several nosocomial infections and outbreaks caused by multidrug-resistant *P. aeruginosa* (MDRP) strains have been reported,[4–6] and cross-transmission plays a major role in the spread of MDRP in different units of the hospital over a long period of time.[7] Molecular typing is essential to track the dissemination of specific strains and may facilitate the analysis of transmission.[8] The aim of this study is to analyse the epidemiological relationships among clinical *P. aeruginosa* strains isolated from different wards of the Children's Medical Center Hospital in Tehran, Iran.

Materials and methods

The case definition for inclusion in this study was admission to the hospital between March 2010 and February 2011, and a culture positive for *P. aeruginosa* with clinical and laboratory evidence of infection. *P. aeruginosa* strains isolated from various clinical samples were collected from the paediatric intensive care unit (PICU), neonatal intensive care unit (NICU), infection ward, urology ward, gastroenterology ward and surgical ward. All *P. aeruginosa* isolates, excluding those from patients with cystic fibrosis, were collected.

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No attempt was made to differentiate carriage, colonisation or clinical infection. These isolates were identified by standard laboratory procedures[9] and tested for antimicrobial resistance to cephalothin (30 µg), cefepime (30 µg), ciprofloxacin (5 µg), meropenem (10 µg), ceftazidime (30 µg), piperacillin-tazobactam (110 µg), imipenem (10 µg), gentamicin (10 µg) and amikacin (30 µg). The susceptibility of these antibiotics against clinical isolates of *P. aeruginosa* was determined using appropriate antibiotic discs and the disc-diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI).[10] Multiresistant *P. aeruginosa* (MRPA) was defined as strains resistant to three or more of the following classes of antibiotic: antipseudomonal penicillins,

Table 1. Frequency of different sources of *P. aeruginosa* strains isolated from different wards of the Children's Medical Center Hospital.

	Respiratory		Urine		Wound		Eye		Blood		Other		Total	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
PICU	16	18.4	7	8	5	5.7	2	2.3	1	1.1	2	2.3	33	38
Infection	2	2.3	4	4.6	5	5.7	1	1.1	2	2.3	1	1.1	15	17.2
NICU	0	0	0	0	2	2.3	6	6.9	0	0	0	0	8	9.2
Urology	1	1.1	7	8	1	1.1	0	0	0	0	0	0	9	10.3
Surgical	2	2.3	7	8	1	1.1	0	0	1	1.1	0	0	11	12.6
Gastroenterology	10	11.5	1	1.1	0	0	0	0	0	0	0	0	11	12.6
Total	31	35.6	26	30	14	16	9	10.3	4	4.6	3	3.4	87	100

NICU: Neonatal intensive care unit; PICU: Paediatric intensive care unit.

antipseudomonal oxyimino-β-lactams, fluoroquinolones, aminoglycosides and carbapenems.[11]

The genetic similarity of the strains was investigated by amplification of the enterobacterial repetitive intergenic consensus sequence (ERIC-PCR).[12] Comparison of banding patterns was performed using Gelcompar II, version 6.5 (Applied Maths, Sint-Matens-Latem, Belgium). The similarity matrix was calculated using the Dice coefficient and the clustering of the similarity matrix, analysed via the UPGMA (unweighted pair-group method with average linkages). Clustering and relatedness among genetic clones was defined as 70% similarity.

Results

During the study period, 87 patients were colonised or infected with *P. aeruginosa*. Thirty-three (38%) of these were hospitalised in the PICU and the remainder were distributed across the infection ward (n=15; 17.2%), gastroenterology ward (n=11; 12.6%), surgical ward (n=11; 12.6%), urology ward (n=9; 10.3%) and NICU (n=8; 9.2%). The respiratory tract was the most common source of *P. aeruginosa* isolates (35.6%), followed by urine (30%), wounds (16%), the eye (10.3%), blood (4.6%), and others areas (<4%) (Table1).

The monthly incidence of patients with *P. aeruginosa* strains isolated from clinical samples varied from two to 17. Incidence remained low until November, with the exception of June. The highest number of patients infected or colonised by *P. aeruginosa* was seen in November (n=17) and December (n=17), following by June (n=10).

Among all *P. aeruginosa* isolates, resistance to piperacillin/tazobactam was the lowest (27%), followed by amikacin (31%), gentamicin (33%), imipenem (33%), ciprofloxacin (36%) and meropenem (39%). Carbapenem-resistant *P. aeruginosa* isolates from hospitalised patients ranged from 9% to 46%. Imipenem resistance among all isolates was 33% whereas meropenem resistance was 39% (Table 2).

Thirty-five patients (40.2%) were either colonised or infected with MRPA over a one-year period and 17 isolates were resistant to all the antibiotics tested (multidrug-resistant). Of the 35 patients, 16 (45.7%) were in the PICU, six (17.1%) were on the infection ward, four (14.2%) were in NICU, three (8.5%) were on the urology ward, three (8.5%) were on the gastroenterology ward, and three (8.5%) were on the surgical ward (Table 2).

Molecular typing of 87 isolates identified 16 ERIC-PCR profiles (A–O; Fig. 1). One predominant profile (D) was identified in 59 strains. Patients who were colonised/infected with these strains could be found on all wards. This profile first appeared in PICU and the infection ward in June 2010 and was present on all wards up to the end of the study. During November and December, all isolates (except one in December) were of the same *P. aeruginosa* genotype (profile D).

Table 2. Antimicrobial resistance of *P. aeruginosa* strains isolated from different wards.

	Total	CF		FEP		CP		MEM		CAZ		PTZ		IMP		GM		AM		MDR		PDR	
	(*n*)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)
PICU	33	29	87	21	63	14	42	14	42	25	75	10	30	13	39	9	27	9	27	16	48.5	6	18
Infection	15	15	100	11	73	6	40	7	46	11	73	5	33	6	40	7	46	6	40	6	40	3	20
NICU	8	8	100	2	25	2	25	1	12.5	1	13	1	13	2	25	1	12.5	1	13	4	25	1	13
Urology	9	9	100	3	33	3	33	3	33	3	33	3	33	2	22	3	33	4	44	3	33.3	3	33
Surgical	11	11	100	6	54	5	45	4	36	5	45	3	27	5	45	6	54	5	45	3	45	3	27
Gastroenterology	11	9	81	10	90	2	18	5	45	9	81	2	18	1	9	3	27	2	18	3	27	1	9
Total	87	81	93	53	61	32	36	34	39	54	62	24	27	29	33	29	33	27	31	35	40.2	17	20

CF: Cephalothin; FEP: Cefepime; CP: Ciprofloxacin; MEM: Meropenem; CAZ: Ceftazidime; PTZ: Piperacillin-tazobactam;
IMP: Imipenem; GM: Gentamicin; AM: Amikacin; MDR: Multidrug resistance; PDR: Pan-drug resistance.
NICU; Neonatal intensive care unit; PICU: Paediatric intensive care unit.

Twenty-two out of the 35 MDRP isolates (62.8%) were found to be profile D. Sixteen were isolated from patients in PICU, four patients were positive in NICU, three on the surgical ward, and just one across the other wards.

Among the 17 patients suffering from multidrug-resistant infection, the attributable mortality rate was 23.5%. All *P. aeruginosa* strains isolated from NICU and the urology, surgical and infection wards were resistant to ceftriaxone.

Discussion

The findings of this study suggest that cross-transmission may be an important route of colonisation or infection for *P. aeruginosa*. In Iran, frequent patient movement may be an important risk factor in the dissemination of bacteria. In the authors' hospital, normally patients will be assigned to the appropriate ward according to medical specialty, but may then move to other wards such as PICU when the level of care required changes. In addition, patients may be admitted briefly to a ward that does not match the medical care needed because no bed is available on the appropriate ward and immediate admission is necessary. This patient movement may result in the dissemination of bacteria around the hospital, especially if proper infection control procedures are not instituted.[5] In this situation, genotyping can help to clarify the epidemiology of the *P. aeruginosa* infection.

Molecular typing of the isolates studied here suggested considerable cross-transmission of *P. aeruginosa* isolates, not only between patients in one ward but also between patients from different wards. It can partially be explained by the high number of transfers of patients between different wards.[13,14] In the study by Czekajlo-Kolodziej *et al*., clonally related strains were isolated from patients on the same ward, different wards, and even different hospitals.[14] In another study, *P. aeruginosa* circulated in the NICU ward in an endemic pattern.[15] In the study by Avetisian *et al*., 72.6% of *P. aeruginosa* isolates in ICU belonged to the same genotype and indicated intrahospital transmission.[16]

In the present study, strains with a D profile were detected across the study period except in March, April and May. In November and December, the increased incidence of patients with P. *aeruginosa* was noticed on different wards, especially PICU. All these isolates (except one in December) were of the same *P. aeruginosa* genotype (profile D).

Rapid emergence of resistance in *P. aeruginosa* isolates creates a significant problem for treatment.[17] Several studies have found that MDRP strains appear after prolonged exposure to antipseudomonal agents.[18,19] In the present study, 40.2% of the isolates were MDRP. According to the European Antimicrobial Resistance Surveillance System, the incidence of MDRP isolates in Europe is 4.7%, while in the ICU setting it ranges from 50% in Turkey to ≤3% in the UK, Spain, Germany, Bulgaria and Malta.[20]

Of most concern in recent years is the emergence of carbapenemases in MDRP.[21] In many European countries, mostly around the Mediterranean, carbapenem-resistant *Pseudomonas* spp. have become endemic over the past decade.[6] In the present study, the rate of infection due to carbapenem-resistant *P. aeruginosa* across different wards varied from 9% to 46%. Elsewhere, resistance to carbapenems above 25% among *P. aeruginosa* isolates have been reported in Italy, Turkey, Germany and Greece.[6] In addition, resistance to cephalosporins among *P. aeruginosa* isolates in this study was higher than that reported in the UK, Sweden, Finland, Denmark, Switzerland and The Netherlands.[6]

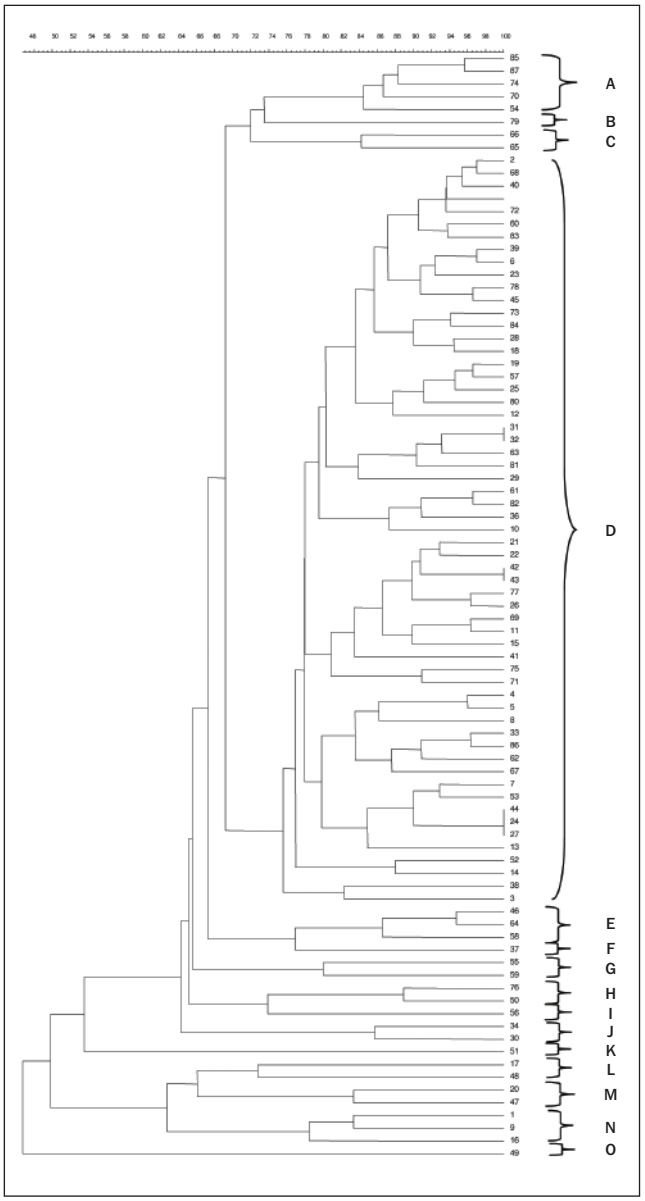

Fig. 1. Dendrogram of genotype analysis derived from 87 *P. aeruginosa* isolates. The scale at the top represents the genetic distance between isolates.

Finally, molecular typing of isolates obtained from the authors' hospital suggests that cross-transmission plays a major role in the spread of *P. aeruginosa* between wards, and therefore priority should be given to improvements in infection control practices. □

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