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Increasing Trend of Antimicrobial Drug-Resistance in *Pseudomonas aeruginosa* Causing Septicemia

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Abstract

The emergence of multi-drug resistant strains of *Pseudomonas aeruginosa* has complicated treatment decision and may lead to treatment failures. In this study, we describe the trends of drug-resistant *P. aeruginosa* isolated in blood cultures from patients detected in a tertiary teaching hospital and evaluated the prevalence of resistance to amikacin, ampicillin, carbenicillin, cefixime, cefotaxime, ceftazidime, ceftizoxime, ceftriaxone, gentamicin, imipenem, and trimethoprim/ sulfamethoxazole in sixty-nine strains of *P. aeruginosa* isolated from neonates with septicemia in Kashan, Iran, from April, 2000 to June 2004. In assessment of the current breadth of multi-drug resistance in *P. aeruginosa* isolated from neonates with septicemia, 4.3% were susceptible to all studied agents, 10.1% were resistant to a single agent. Multi-drug resistance (MDR) isolates accounted for 73.9% of isolates. The majority of MDR isolates (41.2%) were resistant to three antimicrobial agents, which accounted for 30.4% of all isolates. Nineteen MDR isolates from fifty-one (37.3%) were resistant to four agents (19 out of 69; 27.5% of all isolates) and 21.6% to five agents (15.9% of all isolates). Statistical analysis confirmed that there were no significant differences between multi-drug resistance phenotypes of isolates with age**,** gender, gestational age, outcome of septicemia, and application of respirator in neonates. Continued local surveillance studies are urged to monitor emerging antimicrobial resistance and to guide interventions to minimize its occurrence.

Keywords: *Neonatal septicemia,* Pseudomonas aeruginosa*, Multi-drug resistance, Iran*

Introduction

Pseudomonas aeruginosa is the leading cause of nosocomial septicemia. Despite improvement in recent years, prognosis of bacteremia due to *P. aeruginosa* remains poor, with case-fatality rates about 20% or more (1). Due to ubiquitous nature of *P. aeruginosa*, its high affinity to moist environments, and ability to survive at various conditions, it remains as a common pathogenic agent in neonatal intensive care units. Immunodeficiency status of newborn, invasive characteristics of diagnostic and therapeutic procedures and indiscriminating use of antimicrobials are predisposing factors with significant morbidity and mortality rates in *P. aeruginosa* septicemia (2, 3). *P. aeruginosa* exhibits intrinsic resistance to several antimicrobial agents. It produces some broadly specific multi-drug efflux systems, including MexAB-OprM and MexXY-OprM (4). The anti- *Pseudomonas* β-lactams represents an effective solution against *P.* spp. infections. Therefore, acquired resistance to these agents constitutes a major challenge for anti-*Pseudomonas* chemotherapy, especially when it is associated with resistance to other classes of drugs, such as aminoglycosides (5). Antimicrobial resistance to clinical isolates of *P. aeroginosa* may complicate the treatment of infections and can adversely affect clinical outcomes and treatment costs for patients. New antimicrobial agents with activity against *P. aeroginosa* will not be available in the near future, making ongoing surveillance of the activities of currently avail-

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able agents of critical importance (6). In recent years, an increased rate of colonization and infection with *P.aeruginosa* was noted in the neonatal intensive care unit in Beheshti Hospital. For these reasons, Multi-drug resistance (MDR) has not been commonly addressed in our previously surveillance program. We investigated activities of antimicrobial susceptibility status and determined the frequency rates of MDR phenotypes. In recent years, an increased rate of colonization and infection with *P. aeruginosa* was noted in the NICU in Beheshti Hospital (7).
The aim of the present study was to determine the current susceptibility patterns of *P. aeroginosa* to antibiotics used for septicemia, as well the prevalence of the MDR phenotypes of antibiotic resistant *P. aeruginosa* in Kashan, Iran.

Materials and Methods

In this study, we analyzed demographic,clinical and laboratory characteristics of hospitalized septic newborns in Beheshti hospital, Kashan, Iran, which is a 400-bed, tertiary-care teaching hospital with a 20-bed neonatal unit, capable of ventilating up to three neonates at once.
Two hundred and nine neonates with clinical diagnosis of septicemia, from April 2000 to June 2004 were included in this study.Blood cultures were performed routinely on all neonates with clinical signs indicating sepsis such as poor feeding, respiratory distress, fever and hypothermia. Blood was cultured using brain heart infusion (BHI) broth. Subcultures were performed on days 1, 2, 3, 5, 7 and 10. The isolates were identified by standard biochemical tests. Anti- bacterial resistance pattern of the isolates was studied by Kirby-Bauer disk diffusion technique. Susceptibility of the isolates were done and interpreted according to NCCLS recommendations (8).The antibiotic concentration per disk was as follows: amikacin (30µg), ampicillin (10µg), carbenicillin (100µg), cefixime (5µg), ceftazidime (30µg), ceftizoxime (30µg), ceftriaxone (30µg), gentamicin (10µg), imipenem (10µg), and trimethoprim/ sulfamethoxazole (1.25/23.75µg) (HiMedia India).

Results

P. aeruginosa was the common etiologic agent, which was isolated from 69 cases (33%), of them, 44(63.8%) were boys, and 38(55.1%) preterm. Early onset disease (0-7 d) was seen in 64 cases (92.8%), 12 cases (17.4%) of them died.
The overall resistance rate to all ten agents in this study was high, and the resistance rates for sixty-nine *P. aeruginosa* isolates analyzed are provided in Table1. Of the agents tested, amikacin (23.2%) and gentamicin (27.9%) demonstrated the lowest, while ampicillin, cefixime and ceftizoxime (100%) demonstrated the highest rates of resistance. Among the total isolates, 4.3% were susceptible to all studied agents, and 10.1% were resistant to a single agent, predominantly to ceftriaxone (Table 2). MDR isolates accounted for 73.9% of the 69 isolates. The majority of MDR isolates (41.2%) were resistant to three antimicrobial agents, and this group accounted for 30.4% of all isolates. Isolates were also identified as resistance to four agents (37.3% of MDR isolates; 27.5% of all isolates) and to five agents (21.6% of MDR isolates; 15.9% of all isolates). Statistical analysis confirmed that there were no significant differences between isolates to one-drug, two-drugs, and multi-drug resistance phenotypes with age**,** gender, gestational age, outcome of septicemia, and application of respirator in neonates (Table 3).

Table 1: Antimicrobial susceptibility tests on *P. aeruginosaisolated* from neonatal sepsis

Antimicrobial agents	Reistant isolates No. (%)	Susceptible isolates No. (%)
amikacin	16(23.2)	53(76.8)
ampicillin	12(100)	0(0)
carbenicillin	30(81.1)	7(18.9)
cefixime	10(100)	0(0)
ceftazidime	37(86)	6(14)
ceftizoxime	8(100)	0(0)
ceftriaxone	48(92.3)	4(7.7)
gentamicin	19(27.9)	49(72.1)
imipenem	10(83.3)	2(16.7)
trimethoprim/ sulfamethoxazole	23(63.9)	13(36.1)

Table 2: Resistance to one or more antimicrobials among *P. aeruginosa* isolated from neonatal sepsis

No. of agents to which isolates were resistant	Number and Total percent of isolates	amikacin No. (%)	ampicillin No. (%)	carbenicillin No. (%)	ceftazidime No. (%)	ceftriaxone No. (%)	gentamicin No. (%)	imipenem No. (%)
0	3(4.3)							
1	7(10.1)		1(14.3)		1(14.3)	5(71.4)		
2	8(11.6)	1(12.5)	3(37.5)		2(25)	6(75)	1(12.5)	
3*	21(30.4)	5(23.8)	5(23.8)	7(33.3)	11(52.4)	13(61.9)	9(42.8)	4(19)
4*	19(27.5)	4(21)	1(5.3)	17(89.4)	16(84.2)	16(84.2)	3(15.8)	2(10.5)
5*	11(15.9)	6(54.5)	2(18.2)	6(54.5)	7(63.6)	8(72.7)	6(54.5)	4(36.4)

* 51 out of 69 (73.9%) isolates were resistant to three or more antimicrobials and defined as MDR.

Table 3: Clinical characteristic and resistance rates of *P. aeruginosa* isolated from neonatal sepsis

characteristics	Sensitive No. (%)	Resistant to one drug No. (%)	Resistant to two drugs No. (%)	MDR phenotype isolates No. (%)	Total No. (%)
Patient's age					
Early onset	3(100)	6(85.7)	8(100)	47(92.1)	64(92.8)
Late onset	0(0)	1(14.3)	0(0)	4(7.9)	5(7.2)
Gender					
Male	2(66.7)	5(71.4)	5(62.5)	32(62.7)	44(63.8)
Female	1(33.3)	2(28.6)	3(37.5)	19(37.3)	25(36.2)
Gestational age					
Term	1(33.3)	2(28.6)	5(62.5)	23(45)	31(44.9)
Preterm	2(66.7)	5(71.4)	3(37.5)	28(55)	38(55.1)
Outcome					
Survival	0(0)	0(0)	3(37.5)	42(82.3)	57(82.6)
Mortality	3(100)	7(100)	5(62.5)	9(17.7)	12(17.4)
Respiratory therapy					
Yes	0(0)	2(28.6)	5(62.5)	13(25.5)	20(29)
No	3(100)	5(71.4)	3(37.5)	38(74.5)	49(71)

Discussion

The overall resistance rate to all antimicrobial agents in this research was significant, as has been reported by others (5, 6). The antimicrobial resistance of *P. aeruginosa* has been reported to be increasing in several studies (5, 6). In Europe, significant decline in susceptibility rates to ß-lactams, aminoglycosides, and quinolones was recently observed in this pathogen, and nosocomial outbreaks of MDR *P. aeruginosa* have been described in various European hospitals (9, 10). The mechanisms of resistance to beta-lactams, quinolones and aminoglycosides are different so the emergence of resistance to two or three drugs should theoretically be low. Combination therapy appears to prevent the emergence of resistance. Resistance in *P. aeruginosa* has been shown to lead to an increased morbidity and mortality (11). Multi-drug-resistance caused by a variety of resistance mechanisms implies that there are few alternatives for some patients (12, 13). The production of carbapenemases and AmpC enzymes reported as the main cause for multi-drug-resistance in *P. aeroginosa* in China (14). In addition, imipenem resistance in *P. aeruginosa* is

considered associated with loss of the porin OprD combined with activity of chromosomal beta-lactamase (AmpC), while overexpression of multidrug efflux pumps is considered to confer meropenem resistance. Carbapenem resistance can also result from production of metallo beta- lactamases (15).

The results of a comparative analysis of two case-control studies in hospitalized patients in brazil on 93 patients with imipenem-resistant *P. aeruginosa* (IRPA) and 93 control patients with a related study on 93 IRPA patients and 65 imipenem-sensetive *P. aeruginosa* (ISPA) patients showed that carbapenem exposure was the main risk factor for IRPA, and found that the use of both carbapenem and vancomycin can increase this effect (16).

There is also conflicting evidence about the ability of combination therapy to prevent the emergence of antimicrobial resistance in patients with *P. aeruginosa* bacteremia. Emergence of resistance during therapy for *P. aeruginosa* infections can occur, resulting in increased rates of morbidity and mortality and higher costs. Combination therapy with two anti- pseudomonal antimicrobial agents limited the risk of emergence of resistant pseudomonal strains compared to monotherapy in an animal model of pseudomonal peritonitis (17, 18).

However, there are little or no clinical confirmatory data. Most studies of human *P. aeruginosa* infections have been underpowered to address this question. An observational study reported that resistant *P. aeruginosa* emerged in 10.2 percent of 271 cases during treatment with four individual antipseudomonal agents (19). Ceftazidime was associated with the lowest risk and imipenem with the highest risk. Addition of an aminoglycoside did not alter this risk.

The choice of empiric therapy varies with the likelihood of *Pseudomonas* resistance. This is an important issue since the rate of resistance is increasing. This was illustrated in the prospective analysis cited above of nosocomial bloodstream infections occurring in 49 hospitals in the United States between 1995 to 2002; the proportion of *P. aeruginosa* isolates resistant to ceftazidime increased from 12 percent in 1995 to 29 percent in 2001 (20).

There is an urgent need to document all these factors, control the use of antimicrobials in the area and select the best strategies for prevention to hinder the development of drug resistance.

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