Iranian Journal of Clinical Infectious Diseases
2011;6(1):18-23

ORIGINAL ARTICLE

Detection of ESBLs and MDR in *Pseudomonas aeruginosa* in a tertiary-care teaching hospital

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ABSTRACT

Background: *Pseudomonas aeruginosa* is characterized as an important nosocomial pathogen with increasing antimicrobial resistance. The multidrug-resistant (MDR) phenotype in *P. aeruginosa* is increasing worldwide. The purpose of this study was to evaluate the prevalence of antibiotic susceptibility, ESBLs (Extended spectrum beta lactamases) producing and multidrug resistant (MDR) *P. aeruginosa*, isolated from clinical specimens of patients and environment of hospital.

Patients and methods: This descriptive study was carried out on 76 isolates of *P. aeruginosa* from a 500-bed tertiary-care general teaching hospital in Kashan, Iran in 2010. Susceptibility testing according to the CLSI (Clinical Laboratory Standards Institute) recommended to eight antipseudomonal agents was performed. ESBLs producing strains were confirmed by double disk diffusion method. Multidrug-resistant isolates were defined as those resistant to three or more classes of antipseudomonal agents.

Results: The highest resistance rates from the isolated *P. aeruginosa* were shown against piperacillin, imipenem, cefotaxime, ceftriaxone, gentamicin, ceftazidime, aztreonam, and ciprofloxacin, respectively. Seven isolates (9.2%) were ESBL producers. More than 30% of the isolates were resistant to at least three classes of antibiotics, and 13% of MDR strains were resistant to all eight tested classes of antimicrobials. Among the total isolates, 6.6% were susceptible to all studied agents, and 9.2% were resistant to a single agent. The isolated bacteria from the tracheal samples showed the highest MDR rate.

Conclusion: Prevalence of *P. aeruginosa* producing ESBL and MDR strains from our clinical samples and environment is still low.

Keywords: *Pseudomonas aeruginosa; Antibiotic resistance, ESBL, Multiple drug resistance (MDR).*
(Iranian Journal of Clinical Infectious Diseases 2011;6(1):18-23).

INTRODUCTION

Pseudomonas aeruginosa is an important pathogen commonly implicated in serious nosocomial infections such as pneumonia and sepsis (1). In addition, it is a major cause of chronic lung infections and death in children and adults with cystic fibrosis (2). The occurrence of multidrug-resistant *P. aeruginosa* strains is increasing worldwide and limiting our therapeutic options (3). The spread of this organism is often difficult to control, as *P. aeruginosa* exhibit intrinsic resistance to several antimicrobial agents (4). Drug resistance in *P. aeruginosa* may be mediated via several distinct mechanisms (1,5). An

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increasing prevalence of infections caused by multidrug-resistant (MDR) isolates has been reported in many countries (6). The resistance mechanisms of *P. aeruginosa* including producing of β-lactamases, efflux pumps, and target-site or outer membrane modifications (7). β-lactamases are bacterial enzymes that are encoded by chromosomal or by plasmid-born genes, and protecting the microorganisms against the lethal effects of β-lactam antibiotics by hydrolyzing the β-lactam rings, thus rendering the drug effect(8). Extended-spectrum β-lactamases (ESBLs) are enzymes that mediate resistance to extended-spectrum cephalosporins (ESCs), such as cefotaxime, ceftriaxone, and ceftazidime, and the monobactam aztreonam (9). ESBLs are inactivated by the β-lactamases inhibitor, clavulanate(8). Resistance to multiple drugs is usually the result of the combination of different mechanisms in a single isolate or the action of a single potent resistance mechanism (7). Antimicrobial resistance to clinical isolates of *P. aeruginosa* may complicate the treatment of infections and can adversely affect clinical outcomes and treatment costs for patients. New antimicrobial agents with activity against *P. aeruginosa* will not be available in the near future, making ongoing surveillance of the activities of currently available agents of critical importance (10). The occurrence and detection of ESBLs in *P. aeruginosa* are undefined in Kashsn. The aim of this study was to evaluate the prevalence of antibiotic susceptibility, ESBL producing and multidrug resistant (MDR) *P. aeruginosa*, isolated from clinical specimens of patients and environment of hospital.

PATIENTS and METHODS

This descriptive study was carried out in Beheshti hospital, a 500-bed tertiary-care general teaching hospital in Kashan, Isfahan province, Iran. A total of 76 *P. aeruginosa* isolates were collected from 60 patients and 16 from environmental specimens enrolled in this study.

Identification was based on colonial morphology, oxidase positivity, the presence of characteristic pigments, and growth at 42°C. The strains isolated from urine 17(22.4%), the trachea 14(18.4%), wound 9(11.8%), stool 7(9.2%), bronchoscopy fluid 3(4%), blood 3(4%), sputum 3(4%), pleural fluid 1(1.3%), vaginal discharge 1(1.3%), gastrointestinal fluid 1(1.3%), pus 1(1.3%) and environment 16(21%).

Antimicrobial susceptibility testing was performed on all 76 isolates according to the standard method established by the CLSI. Isolates were tested using Mueller-Hinton agar. Imipenem (10μg), ciprofloxacin (5μg), ceftazidime (30μg), ceftriaxone(30μg), cefotaxime(30μg), aztreonam(30μg), piperacillin(100μg), and gentamicin(10μg) disks(Mast Group Ltd., Merseyside, U.K.) were used. *P. aeruginosa* ATCC 27853 was used as quality control strain in susceptibility determination. Multidrug-resistant (MDR) isolates were defined as those showed resistant to three or more classes of antipseudomonal agents (carbapenems, fluoroquinolones, penicillins/cephalosporins, and aminoglycosides). ESBL production in all of the isolates was detected by double disk synergy test as described by Jarlier (11). Synergy was determined between a disk of amoxiclav(20μg amoxicillin and 10μg clavulanic acid) and a 30μg disk of each third generation cephalosporins. Test antibiotic placed 20 mm apart on a lawn culture of the isolate under test on Mueller-Hinton agar. The test organism was considered to produce ESBL if the zone size around the antibiotic disk increased towards the amoxiclav disk. This criterion also fulfills the CLSI guidelines (12). This increase occurs because the clavulanic acid present in the amoxiclav disk inactivates the ESBL produced by the test organism (13).

RESULTS

P. aeruginosa was isolated from 60 patients and 16 specimens from the hospital environment and instruments. *P. aeruginosa* demonstrated the highest resistance rate to piperacillin (36.8%), imipenem (29%), cefotaxime (27.6%), ceftriaxone (25%), gentamicin (23.7%), ceftazidime (21%), aztreonam (19.7%) and ciprofloxacin (11.9%), respectively (table 1).

Table 1. Antimicrobial susceptibility tests on *P. aeruginosa* isolated from patients and environment of Shahid Beheshti hospital in Kashan

Antimicrobial agents	Resistant isolates(%)	Intermediate isolates (%)	Susceptible isolates (%)
Piperacillin(100µg)	28(36.8)	0(0)	48(63.2)
Imipenem (10µg)	22(29)	2(2.6)	52(68.4)
Cefotaxime (30µg)	21(27.6)	45(59.2)	10(13.2)
Ceftriaxone (30µg)	19(25)	8(10.5)	49(64.5)
Gentamicin(10µg)	18(23.7)	1(1.3)	57(75)
Ceftazidime (30µg)	16(21)	3(4)	57(75)
Aztreonam (30µg)	15(19.7)	5(6.6)	56(73.7)
Ciprofloxacin (5µg)	9(11.9)	11(14.5)	56(73.6)

Of 76 *P. aeruginosa* isolates, 7 (9.2%) were found to be positive for ESBL production. Maximum ESBL production was found in strains isolated from trachea and urine samples (table 2).

Table 2. Distribution of *P. aeruginosa* ESBLs producing and MDR strains according to the type of specimens

Specimens	ESBL (n=7)	MDR(n=21)	Total(n=76)
Urine	2	1	17
Trachea	2	8	14
Wound	0	4	9
Stool	0	1	7
Bronchoscopy fluid	1	1	3
Blood	0	2	3
Sputum	0	0	3
Gastrointestinal fluid	1	1	1
Pus	0	1	1
Vaginal discharge	0	0	1
Pleural fluid	0	0	1
Environment	1	2	16

ESBL: Extended spectrum beta lactamases, **MDR**: Multidrug resistant

Of 7 ESBL-positive isolates, 4 (57.1%) were multi-drug resistant. Maximum resistance (85.7%) was seen with piperacillin. Of 76 isolates, 21 (27.6%) were multi-drug resistant. The majority of MDR isolates (38.1%) were resistant to seven antimicrobial agents, and this group accounted for 10.5% of all isolates. Table 3 shows antibiotic sensitivity pattern of 21 strains of MDR *P. aeruginosa.*

Table 3. Antibiotic sensitivity pattern of 21 strains of MDR *P. aeruginosa*

Antibiotics	Sensitive(%)	Intermediate (%)	Resistant (%)
Piperacillin(100µg)	1(4.8)	0(0)	20(95.2)
Imipenem (10µg)	1(4.8)	0(0)	20(95.2)
Gentamicin(10µg)	2(9.5)	1(4.8)	18(85.7)
Cefotaxime (30µg)	3(14.3)	1(4.8)	17(80.9)
Ceftriaxone (30µg)	4(19)	1(4.8)	16(76.2)
Ceftazidime (30µg)	6(28.6)	2(9.5)	13(61.9)
Aztreonam (30µg)	6(28.6)	3(14.3)	12(57.1)
Ciprofloxacin (5µg)	2(9.5)	10(47.6)	9(42.8)

DISCUSSION

Pseudomonas aeruginosa is one of the most important nosocomial pathogens in health care settings. The presence of multidrug resistance *P. aeruginosa* is trend to increase, which rendering many antimicrobial agents ineffective (1). Our study reported the highest resistance rate to piperacillin, imipenem, cefotaxime, ceftriaxone, gentamicin, ceftazidime, aztreonam, and ciprofloxacin, respectively. The prevalence of resistance to piperacillin in *P. aeruginosa* as reported by Javiya *et al*. (73.21%) is much higher than that reported in our study (14). In this study, notable resistance to *P. aeruginosa* was observed against imipenem. Carbapenems are considered to be the treatment of choice against serious ESBL-associated infections. The resistance to carbapenems, especially in *P. aeruginosa*, results from reduced levels of drug accumulation or increased expression of pump efflux or production of metallo-β-lactamases (15-17). In our study, gentamicin resistance value was lower than values reported in study with other studies (18,19). In this study, ciprofloxacin exhibited nearly high

susceptibility pattern (73.6%), while Gul *et al*., reported that more than 90% of isolates were sensitive to ciprofloxacin (20). Cephalosporins, especially the third-generation ceftadizime, are known as anti-pseudomonal drugs has demonstrated high susceptibility pattern about 75% with *P. aeruginosa* isolates; however, cefotaxime and ceftriaxone, two members of the cephalosporin drug tested in this study, showed susceptibility rate of 13.2% and 64.5%, respectively. These low resistance rates were comparable with the report from Malaysia of 40% and 31% (21). In contrast, cephalosporins tested in a study conducted in Ibadan, southwestern Nigeria, showed that 90% of the isolates were sensitive (22).

In present study more than 50% of isolates were found to be susceptible to aztreonam. Some local studies showed that *P. aeruginosa* was sensitive to aztreonam (20). In our study, the prevalence of MDR *P. aeruginosa* isolates, was 27.6% which is lower than values reported in other studies in Iran, Malaysia and Pakistan (4,23,24) and this prevalence rate is lower than the previous study which was performed in this hospital in 2005(25).

Our results presented 9.2% ESBL production among *P. aeruginosa* isolates. The studies conducted by others depicted very lower rates of ESBL production in *P. aeruginosa*, 3.7%, 4.2%, and 7.7%, respectively (4,26,27). In addition, the results of others, showed more higher rates (20.3% to 46%) of ESBLs in *P. aeruginosa* isolated in their investigations (13,23,24,28).

About 17 (22.4%) *P. aeruginosa* isolates out of 76 isolates were obtained from urine samples, indicating that urinary tract infection (UTI) is the most common hospital acquired infection (29). The distribution of the source of the isolates differs with studies and clinical specimens (30,31). In general, there is a considerable geographic difference in the prevalence of ESBLs in different countries. The emergence of resistance in *P. aeruginosa* strains that had been consistently susceptible to standard antimicrobial therapy is of growing clinical concern, as is the alarming trend to multidrug resistance. With this in mind, the early detection of ESBLs, the judicious use of appropriate antibiotics, and the implementation of infection control strategies are major concerns to avoid the spread of this threat in the hospital.

ESBLs producing isolate are not prevalent in our hospital. Our results showed that decreasing resistance to some antibiotics and the decrease of MDR strains to previous report in this hospital clearly indicate the need for continuous monitoring of antibiotic susceptibility in *P. aeruginosa* strains.

ACKNOWLEDGEMENT

The study is supported by Kashan University of Medical Sciences Research fund.

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