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Research Article

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Drug resistance pattern of *Pseudomonas aeruginosa* isolates at PIMS Hospital, Islamabad, Pakistan

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ABSTRACT

Pseudomonas aeruginosa is an opportunistic pathogen causing serious nosocomial infections in patients. Emergence of multi-drug resistant P. aeruginosa is an increasing infection control problem leading to high morbidity and mortality. Extended spectrum beta-lactamase enzymes are the increasing cause of resistance to penicillin's, cephalosporins, and aztreonam antibiotics in P. aeruginosa. The objective of the study was to determine the prevalence of Pseudomonas aeruginosa from infected patients, antibiotic resistance and occurrence of ESBL producing P. aeruginosa among these isolates. A total of 200 specimens were received by the pathology laboratory of Pakistan Institute of Medical Sciences, Islamabad, Pakistan, which comprised of 50 tracheal 50 pus, 25 bloods, and 25 urine and 50 miscellaneous samples including sputum, swab, wounds, tissue and different body fluids. P.aeruginosa was tested against a panel of 14 antibiotics. The highest percentage of resistance to antibiotics amoxicillin+clavulanicacid, cefoperazone+sulbactum, ceftriaxone,ceftazidime, Piperacillin and tobramycin was measured. The most effective drug established were polymixine B, Nalidixic acid, meropenem, amikacin, imipenem, azetrainum were found as more effective in the order respectively. Among all 200 isolates, 150 were found to be ESBL positive and 50 were ESBL negative. Different factors like gender, age, were also related along with the patient stay in hospital. More males than females were infected having high percentage of Pseudomonas aeruginosa and highest frequency was observed in age group less than 15, gradually declined with increase in age. Since treatment proved to be difficult, prevention is considered as an appropriate means of overcoming infection. Routine detection of ESBLs and careful in vitro testing before antibiotic use may help in the prevention and treatment of patients infected with ESBL producing P. aeruginosa.

Key words: *P. aeruginosa*, Nalidixic Acid, Amikacin, Imipenem, Azetrainum

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INTRODUCTION

P. aeruginosa is found almost everywhere that is in water, in soil and also on plants. It can also be present in tap water found in patient rooms [1]. It can be isolated from various body fluids such as sputum, urine, wounds, and eye or ear swabs and from blood because it can infect almost any external part or organ of the body [2]. Strains of *P. aeruginosa* which are Multidrug-resistant (MDR) are often isolated from the patients suffering from nosocomial infections, especially from those which are present in the intensive care unit [3].That is why infections caused by *P. aeruginosa* are serious because it is inherently resistant to many antibiotics and also capable of acquiring resistance to all effective drugs classes [4]. *P. aeruginosa* is an opportunistic infectious pathogen, so often leads to chronic diseases [5]. A narrow class of antibiotics is effective against *P. aeruginosa*, including the carboxypenicillins, quinolones (ciprofloxacin, levofloxacin), the antipseudomonal cephalosporin, and aminoglycosides. Beta-lactamase

production by this organism present the major mechanism of resistance to β-lactam antibiotics is and it is reported that more than 340 β-lactamase enzymes produced by *P. aeruginosa* have been detected [6]. Some enzymes like AmpC beta-lactamases, extended-spectrum beta-lactamases (ESBLs), and metallo-beta-lactamases, make *P. aeruginosa* as serious pathogens in hospitalized patients [7]. It is essential to determine the accurate bacterial susceptibility to antibiotics for the better management of bacterial infections [8].That is why this study was conducted to find the current level of susceptibility and cross-resistance for anti-pseudomonal antibiotics which are widely used against *P. aeruginosa.* It can also help in selecting the most appropriate empirical antimicrobial therapy for infections, in terms of safety with the evaluation of the data regarding the testing for ESBLs production hence providing information about the best therapeutic options for treating such infections.

EXPERIMENTAL SECTION

The study was conducted at Pakistan Institute of Medical Sciences (PIMS), Islamabad, Pakistan. The sensitivity pattern of Gram-negative bacilli was determined against commonly used antibiotics using disc diffusion method. Samples comprised of blood, pus and miscellaneous specimens including different body fluids, high vaginal swabs, urine, tracheal secretions, wound, tissue and different types of swabs, both from outdoor patients (OPD) as well as indoor patients (IPD) from different wards of surgical and medical of the hospital were investigated for *Pseudomonas aeruginosa.* The study population consisted of hospitalized patients from different wards. The demographic information (age, sex) were obtained from the patient's medical record. Pus and tracheal samples were directly inoculated on Blood agar and MacConkey agar.

Blood samples were collected from patients and were transferred to 50 mL of Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 hours. Growth was sub cultured on Blood agar and MacConkey agar plates, and incubated for 24 hours at 37°C. Urine samples were transferred to sterile centrifuged tubes, centrifuged and streaked on Cystine-Lactose-Electrolyte Deficient (CLED) medium. Body fluids, sputum, swab, wound and tissue samples were cultured on Blood agar and MacConkey agar and incubated for 24 to 48 hours at 37°C.By using Bergey's Manual of Determinative Bacteriology, the isolates were biochemically characterized and identified.

Determination of Antibiotic Resistance Patterns of P. aeruginosa

Antibiotic resistance patterns of the bacterial isolates confirmed as *P. aeruginosa* were studied. The pattern among different groups of antibiotics was determined by employing disc diffusion method of Bauer *et al.* [9]. Bacteria were classified as susceptible, intermediate or resistant to antibiotics in accordance with current Clinical Laboratory Standard Institute (CLSI) recommendations (2010).

Disc diffusion (Kirby-Bauer) susceptibility test

Antimicrobial susceptibility testing was carried out by the standard Kirby-Bauer disk diffusion method following guidelines provided by CLSI (2010). Muller-Hinton agar (MHA) was used after sterilization by autoclaving at 121˚C for 15 minutes. Also the Double disc diffusion method was used to detect the extended spectrum β-lactamases (ESBL).

RESULTS AND DISCUSSION

Antibiotic susceptibility pattern of isolates

Antibiotic resistance patterns of the bacterial isolates confirmed to be *P. aeruginosa* were analyzed (**Table No.2, 3 and 4**). Our results are likely similar to the results of the [10] which shows that resistance of *P. aeruginosa* isolates to tested antibiotics in antibiogram test were 100% to cefpodoxime, 82.98% to ceftriaxone, 78.73% to imipenem, 75% to meropenem, 72.72% to gentamicin, 69.23% to ciprofloxacin and aztreonam, 67.57% to cefepime, 65.95% to ceftazidime, and 61.53% to piperacillin. Our results are also in accordance with the study report of [11] which shows that the resistance of *P.aeruginosa* isolates against broad-spectrum cephalosporins and monobactames were cefepime (97%), cefotaxime (92.5%) ceftazidime (51%), and aztreonam (27%). Ciprofloxacin (91.5%), imipenem (84.9%) and meropenem (82.1%) were the most effective anti-pseudomonas agents in this study. Among most commonly used antibiotics, polymixine B proved to be most effective against *P. aeruginosa* with resistance rate of only 7.9%.The study under discussion also revealed that *P. aeruginosa* showed greater resistance against drugs, which is in agreement with the findings of [12], who reported that *P. aeruginosa* showed resistance against amoxicillin+clavulanic acid and showed sensitive pattern for meropenem. Another report also showed high levels of resistance to ceftazidime (73.7% resistant) and meropenem (76.0% resistant) by *P. aeruginosa* [13].

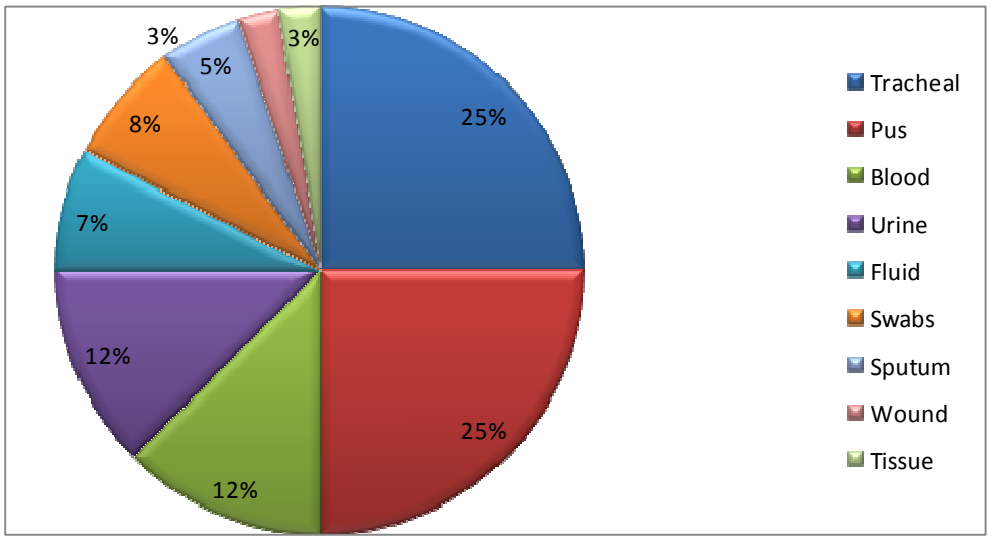

Figure No.1: Pie diagram showing the samples wise distribution of understudy specimen

Table No.1: Gender and age wise distribution of patients with *P. aeruginosa* infection

Group	Age	Number	Females	Percentage	Males	Percentage
A	1-15	87	27	31.03%	60	69.96%
B	15-30	54	24	44.44%	30	55.55%
C	30-45	34	15	44.12%	19	55.58%
D	45-60	25	5	20%	20	80%
Total	1-60	200	71	35.05%	129	64.05%

Table No.2: Antibiotics Sensitivity pattern of *Pseudomnas spp.* from the isolates of Surgical Ward

DRUG(S)	TOTAL	RESISTANT COUNT (%)	SENSATIVE COUNT (%)	INTERMEDIATE COUNT (%)
Ceftazidime	47	31 (65)	16(34)	0(0)
Ceftriaxone	44	35(79)	9(20)	0(0)
Amoxicillin/ Calvulanic acid	20	16(80)	4(20)	0(0)
Piperacillin	26	16(61)	9(34)	1(3.8)
Cefoperazone+ Sulbactum	20	20(100)	0(100)	0(0)
Piperacillin/ Tazobactam	47	21(44)	22(46)	4(8.5)
Tobramycin	15	10(66.7)	4(26)	1(6.67)
Levofloxacine	31	10(32.3)	20(64)	1(3.22)
Imipenem	31	10(32.3)	20(64)	1(3.22)
Polymixin B	31	2(6.5)	29(93.5)	0(0)
Amikacin	25	12(48)	13(52)	0(0)
Meropenem	23	7(30.4)	16(69.9)	0(0)
Ciprofloxacin	12	9(75)	2(16.7)	1(8.33)
Nalidixic acid	37	13(35.1)	23(62.3)	1(2.7)

Prevalence of ESBL producing P. aeruginosa

Among all 200 isolates, (n=150) 75% were found to be ESBL positive (n=50) 25 were found to be ESBL negative detected by double disk diffusion method. Our results are in accordance to the findings in another setting [12, 14].

Table No.3: Antibiotic Sensitivity pattern of *Pseudomnas spp.*from the isolates of Medical Ward

DRUG(S)	TOTAL	RESISTANT COUNT (%)	SENSATIVE COUNT (%)	INTERMEDIATE COUNT (%)
Ceftazidime	27	22(81.5)	5(18.5)	0(0)
Ceftriaxone	31	27(87.1)	4(12.9)	0(0)
Amoxicillin/ Calvulanic acid	9	8(88.9)	1(11.1)	0(0)
Piperacillin	16	12(75)	4(25)	0(0)
Cefoperazone+ Sulbactum	9	9(100)	0(0)	0(0)
Piperacillin/ Tazobactam	28	12(42.8)	14(50)	2(7.14)
Tobramycin	15	9(60)	5(33.33)	1(6.66)
Levofloxacine	36	12(33.3)	22(61.1)	2(5.55)
Imipenem	23	11(47.8)	12(52.2)	0(0)
Polymixin B	37	1(2.7)	36(97.3)	0(0)
Amikacin	6	2(33.3)	4(66.7)	0(0)
Meropenem	3	0(0)	3(100)	0(0)
Ciprofloxacin	3	2(66.7)	1(33.3)	0(0)
Nalidixic acid	22	1(4.5)	20(90.9)	1(4.5)

Table No.4: Antibiotic Sensitivity pattern of *Pseudomnas spp.*From the isolates of Out Door Patients

DRUG(S)	TOTAL	RESISTANT COUNT (%)	SENSATIVE COUNT (%)	INTERMEDIATE COUNT (%)
Ceftazidime	36	26(72.2)	10(27.0)	0(0)
Ceftriaxone	36	29(80.5)	7(19.4)	0(0)
Amoxicillin/ Calvulanic acid	9	9(100)	0(0)	0(0)
Piperacillin	22	15(68.2)	6(27.3)	1(4.54)
Cefoperazone+ Sulbactum	19	17(89.5)	2(10.5)	0(0)
Piperacillin/ Tazobactam	34	12(35.5)	21(61.7)	1(2.94)
Tobramycin	16	8(50)	7(43.7)	1(6.25)
Levofloxacine	28	13(46.4)	15(53.6)	0(0)
Imipenem	31	10(32.2)	21(67.7)	0(0)
Polymixin B	16	1(6.25)	15(93.7)	0(0)
Amikacin	19	9(47.4)	10(52.6)	0(0)
Meropenem	2	0(0)	2(100)	0(0)
Ciprofloxacin	4	2(50)	2(50)	0(0)
Nalidixic acid	18	1(5.6)	17(94.4)	0(0)

CONCLUSION

We conclude that antibiogrm results for the drug sensitivity patterns of the *P. aeruginosa* with these outcomes will lead to antibiotics stewardship to overcome the resistance by bacteria. The result of present study could be significant for strategic practices to prevent and address the emergence and spread of drug resistant *P. aeruginosa* in clinical environment.

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