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Recent Trends in Antibiotics Susceptibility Pattern of *Pseudomonas* sp. Isolated from Clinical Samples of Punjab, Pakistan

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***SUMMARY*. The refractory nature of *Pseudomonas* infections to chemotherapy reflects its ability to rapidly acquire resistance and adapt to hostile environment. In order to keep eye on changing trends in susceptibility patterns of *Pseudomonas sp.* and to modify therapeutic choices, a retrospective study was carried out. Samples for culture and sensitivity testing were screened for presence of *Pseudomonas sp.* Resistance/susceptibility of isolates was tested against 12 drugs. A total of 256 isolates of *Pseudomonas sp.* were studied which were 3.4% of total clinical samples. In this study, milk (16.6%), ear (14%) and respiratory samples (10.9%) were the most important sources of *Pseudomonas* isolates. The resistance to cefepime, ceftazidime, aminoglycosides and fluoroquinolones has been significantly increased over the past years while resistance rates were consistent for some drugs like tazocin (13%). The recent susceptibility trends needs to be addressed periodically to help choice of most appropriate antibiotics for *Pseudomonas* infections.**

INTRODUCTION

Pseudomonas infections are a significant global concern owing to its ability to infect all body tissues. A wide variety of virulence factors have been reported that contribute to its pathogenicity. Increasing antimicrobial resistance of *Pseudomonas* sp. is threatening therapeutic improvements in these infections. This resistance is contributed by multiple mechanisms including impermeability, β-lactamases and multi-drug efflux [1]. The potential of *Pseudomonas* to rapidly acquire resistance is leading cause of gradually increasing case-fatality rates.

Unnecessary prescription of inappropriate antibiotics has raised several health and economic issues. The rapidly increasing resistance to carbapenems is also leaping forward to serious therapeutic challenges and has greatly reduced therapeutic choices. In addition, the β-lactam and aminoglycosides resistant sub-populations of *Pseudomonas* are rapidly becoming more prevalent [2]. This prevalence of resistant strains of *Pseudomonas* is more commonly seen in hospital acquired infections. Thus the changing trends need to be addressed in order to provide guidelines to clinicians, to add suitable choices in antibiotic policy. The study was aimed to identify recent trends in antimicrobial susceptibility patterns of *Pseudomonas* spp. from clinical isolates.

MATERIAL AND METHODS

Sample collection

The Chughtais Lahore Laboratory has more than 100 collection centers with a wide geographical distribution in Pakistan. The lab is receiving approximately 19,000 clinical samples for culture and sensitivity testing, annually. In this study, data of 7521 samples was collected retrospectively with a case control study design from April to October, 2012. For each case, details were collected about patient's age, gender, site of infection and city.

KEY WORDS: Antibiotic resistance, Clinical samples, Multiple drug resistance, *Pseudomonas*.

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Identification of Isolates

All clinical samples except urine were cultured on 5% sheep blood, chocolate and MacConkey agar. Urine samples were cultured on CLED Agar. *Pseudomonas* isolates were identified by routine clinical microbiology methods. *Pseudomonas aeruginosa* were identified by the production of pyocyanin on Mueller Hinton agar.

Antimicrobial Susceptibility Assay

Antimicrobial susceptibility testing was carried out using the modified Kirby-Bauer disc diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) [3]. The antibiotic discs were obtained from Oxoid (Oxoid limited, UK). The zone diameters were interpreted as sensitive and resistant according to NCCLS standards. This study included 10 different antibiotics for susceptibility testing of *Pseudomonas* isolates. Two additional antibiotics were used for *Pseudomonas* spp. isolated from urinary tract infections (UTI). A total of 12 antimicrobials from different groups were used. Cephalosporins used were cefepime (FEP) (30 µg) and ceftazidime (CAZ) (30 µg). Antimicrobials from aminoglycosides included amikacin (AK) (30 µg), tobramycin (TOB) (10 µg) and gentamicin (CN) (10 µg). One β-lactam/β-lactamase inhibitor combination piperacillin/tazobactam (TZP) (110 µg) and three generations of fluoroquinolones (*i.e.* 3rd generation levofloxacin (LEV) (5 µg), 2nd generation ciprofloxacin (CIP) (5 µg) and ofloxacin (OFX) (5 µg), and 1st generation norfloxacin (NOR) (10 µg) were used. Out of all fluoroquinolones used, norfloxacin and ofloxacin were tested in urinary tract infections (UIT) only. Two carbapenems, imipenem (IMP) (10 µg) and meropenem (MEM) (10 µg) were tested. Intermediate susceptibilities were considered as resistant in this study. Non-duplicate clinical isolates were reported in this study.

Statistical Analysis

The data were compared by using a Chi-square test with IBM SPSS software, version 20. Analysis was performed on the cross-tabulated values of the presence of the resistant/susceptible isolates. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Data of 7521 samples were analyzed over a period of 6 months and among them *Pseudomonas* sp. was identified in 256 of the samples. These isolates were 3.4% of total clinical samples submitted for culture and sensitivity testing, showing the prevalence of *Pseudomonas* infections. The total numbers of samples from different sites with samples positive for *Pseudomonas* isolates are shown in Table 1.

The majority of *Pseudomonas* isolates were obtained from respiratory, urogenital and pus samples. The respiratory samples included sputum, bronchial washing, tracheal secretion, ETT tip, pleural fluid and bronchial lavage. Urogenital samples included urine, urine catheter tip and HVS (high vaginal swab). Brain samples included CSF and brain abscess. The relative incidence rate was significantly higher in milk (16.6%), ear (14.2%) and respiratory samples (10.9%). Demographic distribution of clinical samples collected for culture and sensitivity test-

Culture Site	Total number of samples	Positive for *Pseudomonas* spp.	%
Urogenital specimens	4764	79	1.66
Respiratory source	377	41	10.88
Pus	1139	75	6.58
Ear swab	70	10	14.29
Brain source	286	4	1.40
Wound swab/secretion	278	19	6.83
Ascitic fluid	183	2	1.09
Throat swab	122	8	6.56
Cvp tip	153	6	3.92
Milk	6	1	16.67
Synovial fluid	52	0	0.00
Peritoneal fluid	20	0	0.00
Others *	71	11	15.49

Table 1. Distribution of samples from different sites with samples positive for *Pseudomonas* isolates. *Different body fluids, shunt tips, pancreatic pseudo-cyst fluid, suction catheter tip, tissue.

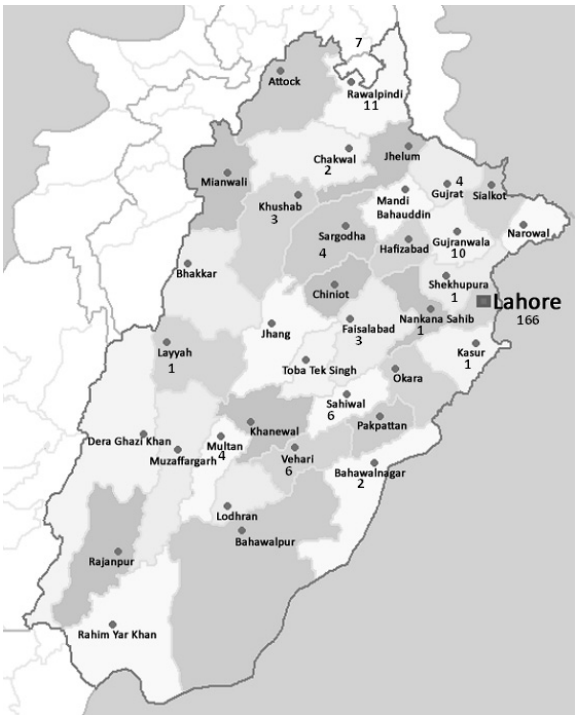

Figure 1. Demographic Distribution of clinical samples.

ing is shown in Fig. 1, that showed that isolates are from all over the Punjab province but majority are from the Lahore.

The incidence rate of *Pseudomonas* was significantly higher in males as compared to females (149 and 107, respectively). The distribution of positive cases significantly varies in different age groups in both genders shown in Fig. 2. The incidence rate was higher in males between the age of 50 and 60 years (26.1%), while same in males and females between the age of 20 and 30 years.

Resistance of isolated *Pseudomonas* sp. to various individual drugs was evaluated. The percent resistance/sensitivity to tested antimicrobial drugs is shown in Fig. 3. A total 0.4% of all samples were resistant to all tested drugs. None of isolates was sensitive to all drugs. In third generation of cephalosporin, resistance rate was 71% for ceftazidime. Significant numbers of *Pseudomonas* isolates (35%) were found to be resistant to fourth generation of tested cephalosporin (cefepime). Among aminoglycosides, resistance rate was higher for tobramycin (87%) followed by gentamicin (50.8%) and amikacin (46.8%). A significant proportion (19%) of *Pseudomonas* isolates showed resistance to carbapenems. 13% and 57% of the isolates showed resistance to tazocin and fluoroquinolones, respectively. About 95 % of isolates showed resistance to drugs used for urinary tract infections.

Resistance of isolates to different antimicrobials showed variation with respect to source of isolation Table 2. In general isolates from brain and ear infections, pus and respiratory track sources were more resistant than the isolates from other sources.

DISCUSSION

Appropriate antibiotic administration is prerequisite for the treatment of serious *Pseudomonas* infections. The resistance of *Pseudomonas* sp. to antibiotics has been increased significantly over the past years and thus need to be evaluated to have a clear perception of clinical outcomes of different therapeutic options [4]. A large number of samples were collected from Lahore city, this prevalence is not true indicator of high rate of *Pseudomonas* infections. This reflects lack of awareness and facilities in other areas of Punjab to adequately address infections and inappropriate antibiotic

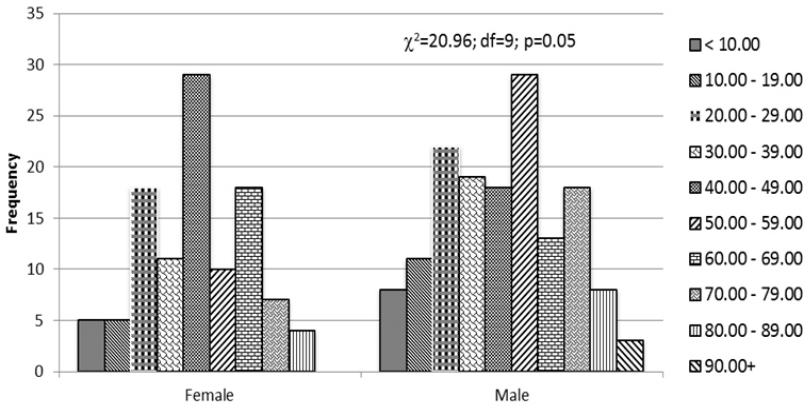

Figure 2. Age wise distribution of *Pseudomonas* infections in both genders.

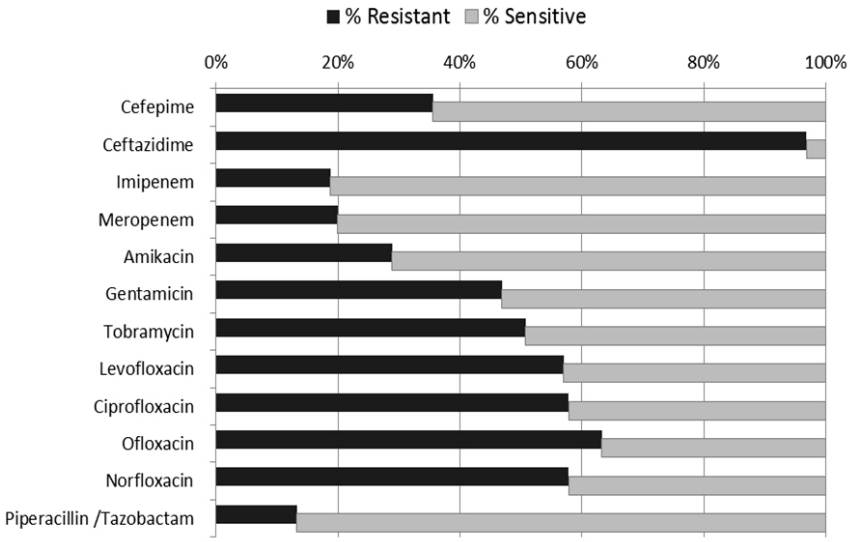

Figure 3. Incidence of resistance in *Pseudomonas* to antimicrobial drugs.

Sample source	**Brain Infections**		**Ear Infection**		**PUS**		**Respiratory Source**		**Urogenital pecimens**		**Others**	
Number of samples	**4**		**10**		**76**		**41**		**79**		**46**	
Drug	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**
Cefepime (FEP)	0	100	30	70	35.5	64.5	34.1	65.9	35.4	64.6	41.3	58.7
Ceftazidime (CAZ)	75	25	70	30	71.1	28.9	78	22	67.1	32.9	71.7	28.3
Imipenem (IMI)	0	100	0	100	13.2	86.8	19.5	80.5	16.5	83.5	37	63
Meropenem (MEM)	0	100	0	100	13.2	86.8	19.5	80.5	19	81	39.1	60.9
Amikacin (AK)	0	100	30	70	23.7	76.3	34.1	65.9	25.3	74.7	41.3	58.7
Gentamicin (CN)	25	75	50	50	48.7	51.3	41.5	58.5	40.5	59.5	60.9	39.1
Tobramycin (TOB)	25	75	50	50	51.3	48.7	43.9	56.1	46.8	53.2	65.2	34.8
Levofloxacin (LEV)	25	75	50	50	60.5	39.5	58.5	41.5	54.4	45.6	58.7	41.3
Ciprofloxacin (CIP)	25	75	50	50	60.5	39.5	58.5	41.5	55.7	44.3	58.7	41.3
Ofloxacin (OFX) (For UTI only)	25	75	50	50	60.5	39.5	61	39	55.7	44.3	58.7	41.3
Norfloxacin (NOR) (For UTI only)	0	0	0	0	0	1.3	0	0	39.2	21.5	0	0
Piperacillin /Tazobactam (TZP)	25	75	0	100	10.5	89.5	19.5	80.5	7.6	92.4	23.9	76.1

Table 2. Antibiotic resistance patterns of *P. aeruginosa* against different antibiotics isolated from different sites.Values show percentages in respective samples.

treatment without antibiotic susceptibility testing. This is significantly contributing to the development of resistant in life threatening pathogens.

In this study, resistance rate to different antimicrobials was evaluated. The high resistance reflects poor outcomes associated with these drugs. The alarming situation is the rapidly increasing resistance to cefepime and ceftazidime which is may be due to synthesis of extended spectrum β-lactamases by these pathogens [5]. The resistance to aminoglycosides has also increased significantly in past decade [6]. The resistance rate varied between different aminoglycosides and was highest for tobramycin. Resistance to gentamicin was found to be associated with resistant to tobramycin ($p < 0.001$). Although majority of the isolates (71 %) were sensitive to amikacin, but still the resistance rate is higher than result of a similar studies (21%) conducted by [7], that depicts the challenges of rapidly increasing resistance to therapeutic choice of aminoglycosides in future.

The resistance rates against tazocin and carbapenems seems to be consistent with published literature [2,8]. However for carbapenems, the resistance rate (19%) was found to be significantly higher as compared to similar studies conducted in 2010 by Anjum & Mir [7]. This showed considerable differences in susceptibility patterns according to geographical distribution. Whereas, the isolates did not show any significant fluctuation in their susceptibility trend against fluoroquinolones.

Natural ability of *Pseudomonas* species to rapidly acquire resistance, and increasing prevalence of multi-drug resistant *Pseudomonas aeruginosa* need to be debated at international level to potentiate response against fatal infections. These infections have greatly increased mortality and hospital length of stay [9]. In addition, antibiotic selection pressure on hospitalized patients and nosocomial nature of *Pseudomonas* infections is a big source of multi-drug resistant *Pseudomonas* infections [10]. Hands of nurses, contaminated equipments and unhygienic conditions pose serious threats and are rapidly increasing the prevalence of nosocomial *Pseudomonas* infections [11].

In order to enhance drug development for the treatment of multi-drug resistant *Pseudomonas* species and to develop strategies to prevent nosocomial infections, a major source of selection of drug-resistant strains of *Pseudomonas* species, on-going studies are necessary for estimation of rate of development of drug resistance. In addition, limiting access to antibiotics without doctor's prescription should be strictly implemented. Keeping in view high rate of development of resistance in past year, drug resistance should be immediately focused for adequate antibiotic treatment in future. However administration of less commonly used antibacterials (*e.g.* colistin) for the treatment of multidrug-resistant *P. aeruginosa* may be also helpful [12].

CONCLUSION

The present study showed that the *Pseudomaonas* is continuously updating itself against commonly used antibiotics. There is need to use appropriate antibiotic to cure the patient. Imipenem and meropenem were proved to be most effective against *Pseudomonas*. Furthermore, use of antibiotics in combinations is also an efficient option. Besides treatment of *Pseudomonas,* there is need of careful administration of antibiotics.

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