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Antibiotic resistance pattern and gender wise prevalence of *Pseudomonas aeruginosa* strain isolated from the tertiary health care units

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Key words: *P. aeruginosa*, Multidrug resistance, Antimicrobial sensitivity profiling, Clinical samples, Public and private health care units.

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Abstract

Antibiotic resistance is a matter of great concern and it has finally attracted the attention of scientists and mass-media worldwide. The present study aims to determine the gender wise prevalence and multidrug resistance in *P. aeruginosa* isolated from clinical samples of public and private health care units. The 280 clinical samples (118 female and 162 male) of pus, urine, pleural fluid, blood, high vaginal swabs, throat, ear, stool, cerebrospinal fluid and ascitic fluid were collected from public and private health care hospitals of Khairpur and Sukkur cities of Pakistan. The isolates were isolated and identified using conventional microbiological methods and molecular characterization using 16S rRNA gene sequence homology. The antimicrobial sensitivity was determined using Kirby-Bauer's disc-diffusion method according to the clinical laboratory standard institute. The Overall 28% samples were found positive for the isolation of *P. aeruginosa* and highest 100% prevalence was found in ascitic fluid, followed by ear swab 50% while the lowest percent prevalence was in urine (3.8%) samples. The overall prevalence was found more in male patient than female patients. The results of antibiotic sensitivity profiling revealed complete resistance against all the antibiotics tested. The phylogenetic correlation of amplified 16S rRNA gene sequence of isolate shared 99% similarity with *P. aeruginosa* strain (Gen Bank accession no. JF513146.1). Increasing multidrug resistance among Pseudomonas is an alarming situation in a hospital setting and requires prompt steps to cope up with resistance.

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Introduction

*P. aeruginosa*is an opportunistic bacterial pathogen commonly found in soil and water, and on animal's surfaces including humans (Sedighi *et al.*, 2015). This bacterium has ability to colonize healthy subjects and act opportunistically but it is particularly harmful in hospital and poses quite a significant threat. It has been known to be isolated from patients, hospital personnel as well as from medical equipment (Abreu *et al.*, 2014). It can also lead to several Nosocomial infections for example; wound infections, burns, meningitis, urinary tract infections, necrotizing pneumonia, outer ear and eye infections. The bacterium can also cause fatal septicemia and invasive infections of the blood in patients suffering from disabilities as well as in infants (Sedighi *et al.*, 2015). *P. aeruginosa,* also the main cause of infection in immune impaired patients such as patient suffering from cancer, cystic fibrosis, burns, neutropenia and acquired immune deficiency syndrome (AIDS). Subsequently lead them to the considerable morbidity, prolonged hospitalization, increased costs, and mortality (Brewer *et al.*, 1996; Vincent *et al.*, 2006; Parker *et al.*, 2008; Coggan *et al.*, 2012; Micek *et al.*, 2015; Zafer *et al.*, 2015).

Worldwide, there are growing threats to modern medicine from the emergence of multidrug resistant (MDR) bacteria such as *P. aeruginosa* causing several infections. Recently, MDR *P. aeruginosa* has been identified as a major cause of nosocomial infections. This coupled with marked decline in the discovery and development of novel antibiotics especially against gram-negative bacteria in the last two decades leads to critical challenge to clinicians. MDR bacteria are usually defined as when it is resistant to three or more group of antibiotics. In clinical practice, antibiotics commonly used in treating gram-negative infection are penicillin, cephalosporin, carbapenem, monobactem, quinolone, and aminoglycoside. Increasing incidence of resistance of gram-negative bacteria against even newer antibiotic including carbapenem has been reported in many countries (Mohan Gurjar, 2015).

Antibiotic resistance of *P. aeruginosa* is a topic of great concern and it has finally attracted the attention of mass-media and global research scientists (Schillaci1, and Cascioferro, 2015).Over the years, this particular bacterium has been known to be highly resistant to current antibiotics due to both intrinsic and acquired resistance mechanism within it (Sedighi *et al.*, 2015). *P. aeruginosa* is the most important example of a species that has extensively evolved drug resistant i.e., resistant to all standard antipseudomonal antibiotics (Carbapenemsand aminoglycosides) and is sensitive only to colistin (Cabot *et al.*, 2012; Zafer, *et al.*, 2015). Nowadays, intensive clinical use of carbapenems has caused the presence of carbapenem resistant *P. aeruginosa* populations (Oikonomou *et al.*, 2011; Zafe *et al.*, 2015) and an increase in carbapenem resistance by acquisition of different mechanisms, such as hyperproduction of chromosomal Amp C beta-lactamase, overexpression of efflux systems, alteration or lack of outer membrane proteins (such as porin Opr D), and production of carbapenemases (Lister *et al.*, 2009; Zafer *et al.*, 2015).

There are discrepancies regarding the magnitude of the risk of resistance acquisition associated with the different antipseudomonal agents. In patients previously colonized or infected with *P. aeruginosa*, carbapenems and fluoroquinolones may have a greater tendency to select resistant mutants than other agents (Carmeli *et al.*, 1999; Chastre *et al.*, 2008; Riou*et al.*, 2010 and Ong*et al.*, 2011). Previous exposure to antibiotics is considered an imperative risk factor for the acquisition of *P. aeruginosa* and the subsequent development of infection (Kollef *et al.*, 2014). According to the classical paradigm, non-antipseudomonal agents would promote acquisition of any *P. aeruginosa* strain (Bonten *et al.*, 1999; Venier *et al.*, 2014), whereas drugs with antipseudomonal activity would select those resistant to the particular class of antimicrobial drug used (El Amari *et al.*, 2001).

Resistance acquisition driven by exposure to antipseudomonal agents can be reached by either selecting mutants in patients previously colonized or infected by susceptible phenotypes (Carmeli *et al.*, 1999; Riou *et al.*, 2010) or promoting selection of an already resistant strain (Cobos-Trigueros *et al.*, 2015). Thus the present study aimed to determine the Prevalence and Antimicrobial-Resistance of *P. aeruginosa* in clinical samples of public and private health care hospitals of Khairpur Mir's and Sukkur, Pakistan.

Materials and methods

Bacterial isolates and their identification

The present study was carried out in a post graduate research laboratory (PGRL) of microbiology at Shah Abdul Latif University, Khairpur Sindh, Pakistan in 2014. The study included various clinical samples such as urine, pus, blood, high vaginal swab (HVS), stool, ear, throat, cerebrospinal fluid, ascitic fluid and pleural fluid from different patients of age and gender (male and female).

All the samples received during this period were analyzed for the isolation and antimicrobial sensitivity profiling of *P. aureoginosa*. The streaking plate technique was used for the isolation of *P. aureoginosa* on the surface of nutrient agar. The growth media and biochemical test reagents were purchased from Oxoid (Oxoid, UK) and Sigma-Aldrich (Sigma-Aldrich, USA).

Characterization of Gram Negative Bacteria

According to the society of American Bacteriologists and Nasreen *et al.*, (2015), colorless colony on Mac Conkey agar were observed for morphological characteristics and were further selected for identification. Several Physiological (Oxidase, Catalase tests) and biochemical tests such as Citrate Utilization, Hanging drop technique (HDT), Methyl Red (MR), Voges-Proskauer (VP) Test, Gram staining and Microscopic examination, were carried out for characterization of *P. aureoginosa*.

Determination of Antimicrobial Susceptibility Test by Disk Diffusion Method

Antimicrobial susceptibility tests were performed to measure the ability of an antibiotic to inhibit bacterial growth in vitro by disc diffusion method. This test was performed by modified Kirby-Bauer method. The result of the test was interpreted according to the Clinical Laboratory Standard Institute guidelines (CLSI) in 2015. Bacterial culture from nutrient agar was inoculated into 3 ml Mueller-Hinton broth with a sterile loop. The broth was incubated at 37˚C for 3 to 4 hours. Turbidity of the broth culture was adjusted to a Mac Farland 0.5 standard (1.5×10^8 CFU/ml). Mac Farland 0.5 was prepared by adding 0.5 ml of 0.048 M $BaCl_2$ (1.75% $BaCl_2$, $2H_2O$) to 99.5 ml of 0.36 NH_2SO_4 (1%) with Mueller Hinton broth. When the proper density was achieved, a sterile cotton swab was submerged in the suspension, lift out of the broth, and excess fluid was removed by pressing and rotating the swab against the wall of the tube.

The swab was then used to inoculate the entire surface of the supplemented Mueller-Hinton agar plate three times, rotating the plate 90º between each inoculation. The inoculated plate were allowed to dry (usually taking only a few minutes) before the discs was placed on the plate.

Commercial antibiotic discs (company) were placed on the Mueller-Hinton agar plates. The plates containing the discs were incubated at 37ºC for 16 to 18 hours. Only five distinct antibiotics, Gentamycin (GM), Amoxicillin clavunic acid (AMC), Sparfloxacin (SPX), Fosfomycin (FOS), Moxifloxacin (MXF), Fusidic acid (FD), Enoxacin (EN), Azomax (AZM), Piperacillin-Tazobactam (TZP) and Sulbactam (SCP), were used for testing the resistant pattern of *P. aeruginosa*. After overnight incubation, the zone diameter of inhibition was measured with a scale (in mm) and results were recorded.

Molecular characterization using 16S rRNA

The molecular identification of the selected bacterial isolate was commercially carried out by partial sequencing of the 16S rRNA gene at the Genomic Division, Macrogen Inc., Seoul, Korea.

The molecular characterization of the isolate was performed using universal primers, 27F (5'-AGAGTTTGATCMTGGC TCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGA CTT-3'). The amplified gene products were then purified and sequences were obtained through ABI PRISM Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystem, USA) by using universal sequencing primers, i.e. 518F (5' CCAGCA GCCGCGGTAATACG-3') and 800R (5'-TACCAGGG TATCTAATCC-3'). Electrophoresis of sequencing reaction was 114 s completed using the automated ABI PRISM 3730115 × l DNA Sequencer (Applied Biosystems, USA).

Phylogenetic correlation analysis

The partial sequence of the 16S rRNA gene obtained in this study was analyzed and compared with nucleotide sequence databases in the National Center for Biotechnology Information (NCBI) website using Basic Local Alignment Search Tool (BLAST) program, in order to confer the percentage sequence similarities. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (17). Evolutionary analyses were conducted in MEGA6 according to the (18).

Results

Study site and samples collection

In order to fight bacterial infections successfully, the rapid recognition of proper treatment modalities are critical. The determination of antibiotic susceptibility and resistance are keys to this process.

In present study 280 clinical samples were successfully collected in order to investigate the prevalence and antimicrobial resistance profile of *P. aureoginosa*. The 280 clinical samples (162 from male and 118 from female) were randomly collected at different health care facilities of Khairpur and Sukkur cities of Pakistan. The uniqueness of present study was the very high samples number in order to accomplish the promising results in the area of Khairpur and Sukkur.

Isolation and Identification of Pseudomonas

The 58 isolates (28%) of pseudomonas were isolated with the maximum number of isolates from the pus (52) followed by urine (2). The one isolate of pseudomonas from each sample e.g. ascetic fluid, ear swab, throat swab and HVS was isolated where as it was not isolated from the blood, stool, pleural fluid and CSF as shown in Fig. 1. The isolates were identified by the cultural characteristics as nonfluorescent, greenish, surface concave colonies on the nutrient agar surface.

Morphologically the isolates were Gram negative bacilli, non-spore former and capsulated. Based on the biochemical characteristics the isolates were Catalase, Oxidase and citrate positive whereas the indole, methyl red and vogues proskauer negative as shown in the Table 1. The isolate ferment glucose, lactose, mannose, and maltose where as it did not ferment sucrose. The based on cultural, morphological and biochemical basis, the isolates were presumed as pseudomonas.

Table 1. The preliminary identification of the bacterial isolates using microbiological conventional method.

Sample	Isolate	Morphology					Biochemical characteristics								Sugar fermentation				
		M	GR	SS	CS	FS	I	MR	VP	C	OX	C	NR	UR	G	L	M	S	M
Urine	PU-1	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
	PU-2	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
HVS	PHVS-1	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
E. S	PES-1	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
T.S	PTS-1	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
A. F	PAF-1	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
Pus	PP-1	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
	PP-2	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
	PP-3	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
Sample	Isolate	Morphology					Biochemical characteristics								Sugar fermentation				
		M	GR	SS	CS	FS	I	MR	VP	C	OX	C	NR	UR	G	L	M	S	M
	PP-4	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
	PP-5	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+

PP-5	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-7	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-8	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-9	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-10	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-11	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-12	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-13	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-14	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-15	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-16	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-17	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-18	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-19	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-20	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-21	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-22	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-23	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-24	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-25	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-26	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-27	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-28	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-29	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-30	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-31	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-32	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-33	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-34	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-35	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-36	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-37	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-38	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-39	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-40	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-41	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-42	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-43	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-44	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-45	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-46	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-47	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-48	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-49	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-50	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-51	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+
PP-52	Rod	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+

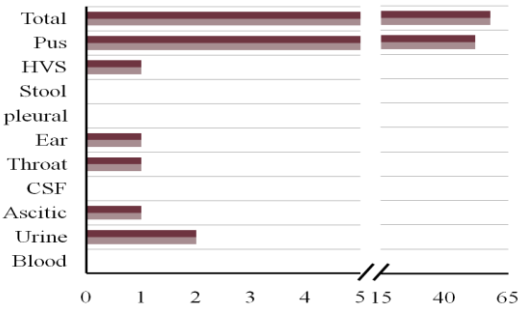

Fig. 1. Isolation of *P. aeruginosa* from the clinical samples of private and public health care hospitals.

Gender wise prevalence of Pseudomonas

The maximum (100%) prevalence of pseudomonas was found in ascitic fluid followed by the ear swab (50%) and in pus samples (39%) whereas the lowest percent prevalence was found in urine (3.8%), HVS (14%) and in throat swab (20%). The pseudomonas was not isolated from the blood, stool, pleural fluid and CSF in spite of several attempts to isolate it from these samples. The overall percent prevalence of pseudomonas was higher in the male patient then female patient.

The pseudomonas was found 100% prevalent in male samples of urine, ear swab and ascetic fluid where as in female patient it was 100% prevalent in HVS and throat swab. In pus samples the pseudomonas was 63.5% in male patient where as 36.5% in female patient in Fig. 2.

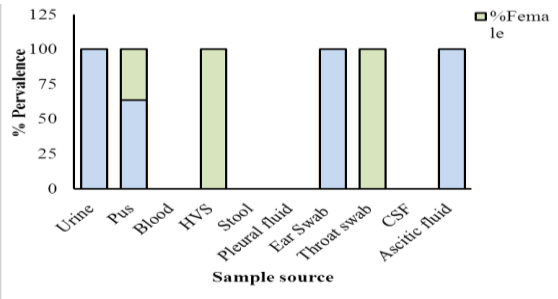

Fig. 2. Gender wise prevalence of *P. aeruginosa* in clinical samples of private and public health care hospitals.

Antimicrobial sensitivity profile (ASP) of pseudomonas

According to the CLSI (2012), the results were interpreted and it was found that antimicrobial panel used was completely ineffective and not displayed any desired results i.e. zone of inhibition against pseudomonas.

The six out of ten (AMC, GM, FOS, MXF, FD and AZM) were completely ineffective without zone of inhibition while four antibiotics (EN 15mm, SPX 13mm, TZP 10mm and SCP 8mm) has displayed zone of inhibition but it was in range of resistance as displayed Table 2 and Fig. 3.

Table 2. Antimicrobial sensitivity profile of the *P. aeruginosa* isolated from the clinical samples of private and public health care hospitals.

Drug	Potency	S	I	R	Result
Amoxicillin-clavulanic acid	AMC (30µg)	≥18	14-17	≤13	8mm
Piperacillin-Tazobactam	TZP (110µg)	≥ 21	15-20	≤14	10mm
Sulbactam	SCP (105µg)	NR	NR	NR	8mm
Sparfloxacin	SPX (5µg)	≥21	16-20	≤15	13mm
Moxifloxacin	MXF (5µg)	≥17	14-16	≤13	0
Gentamicin	GM (10µg)	≥15	13–14	≤12	0
Fosfomycin	FOS (50µg)	≥16	13–15	≤12	0
Fusidic acid	FD (10µg)	NR	NR	NR	0
Enoxacin	EN (10µg)	NR	NR	NR	15mm
Azomax	AZM (15µg)	≥22	16–21	≤15	0

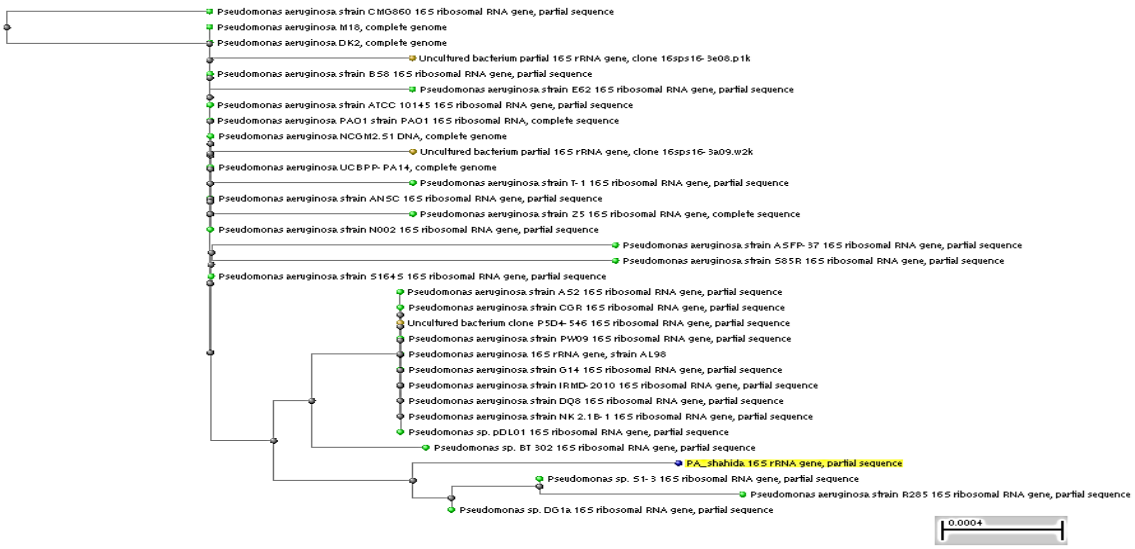

Fig. 3. Phylogenetic tree showing evolutionary relationship of PP4 isolate based on 16S rRNA sequence homology. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Neil model (1993). The percentage of trees in which the associated taxa clustered together is shown next to the branches. The analysis involved 14 nucleotide sequences.

Phylogenetic correlation analysis

Bacterial isolates sharing same cultural, morphological, biochemical and multidrug resistance pattern. The isolate (PP4) from pus has displayed complete resistance against all the antibiotics tested antibiotics was subjected to molecular characterization using 16S rRNA sequence homology. The phylogenetic correlation studies revealed that the amplified 16S rRNA gene sequence of PP4 isolate shared 99% similarity with *P. aeruginosa* strain (Gen Bank accession no. JF513146.1) available in NCBI Gen Bank database (Fig. 4).

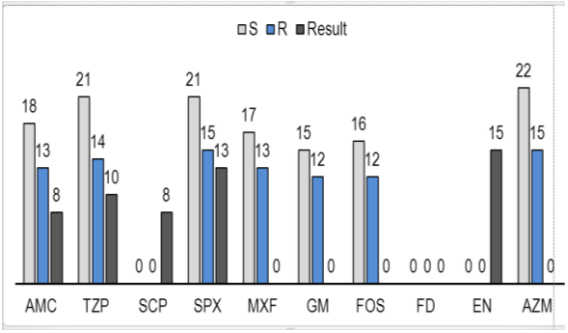

Fig. 4. Antimicrobial sensitivity profile of the *P. aeruginosa* isolated from the clinical samples of private and public health care hospitals.

Discussion

P. aeruginosa, gram-negative bacilli, highly difficult to control with antimicrobial agents. The factors make this microbe more infectious are their resistance to drugs, ability to acquire mutation, frequent involvement in serious infections, and medical devices for example, urinary catheters, intravascular medical devices, endotracheal tubes etc (Ali, *et al.*, 2105). Being a ubiquitous organism in natural environments, *P. aeruginosa* is also a familiar oppor-tunistic and nosocomial pathogen. In hospitalized patients, Pseudomonas has been considered the most common pathogen accounting for 30-33% of the total cases (Ali, *et al.*, 2105). Therefore, the present study was aimed to determine the gender wise prevalence and antimicrobial resistance profile of *P. aeruginosa* isolated from public and private health care units. The current study also highlighted the resistance of *P. aeruginosa* to most commonly used antibiotics.

The clinical specimen were collected from the patients pus, urine, pleural fluid, blood, high vaginal swabs, throat, ear, stool, cerebrospinal fluid and ascitic fluid. In order to get optimistic results the ample (Total 280, 118 female samples and 162 male) samples were collected from the private and public health care hospitals and their associated laboratories. The isolates were isolated using repetitive streak plate method, identified and selected for their antibiotic susceptibility profile (ASP). Antibiotic resistance among bacterial isolates has been known as a growing clinical problem and a serious threat to public health. Similar threat was evident from antibiotic resistance profile of *P. aeruginosa* strains isolated from different patients in the present study. The present study displayed the 28% positive samples for the isolation of *P. aeruginosa* and the study from India showed 9.3% prevalence (Manjunath *et al.*, 2011) while 9% of the bacterial dominance was found by Zakaria (2005) in Palestine.

MDR is an emerging problem in clinical settings and has been reported worldwide (Barbier and Wolff, 2010; Nseir *et al.*, 2011). These strains may be a result of the emergence of multiple mechanisms of resistance after exposure to a number of different antipseudomonal drugs and cross-resistance between these drugs.

In the present study, 58 Pseudomonas were isolated and screened, all the isolates (100%) were found resistant to the routinely used antibiotics (panel used in present study) and in the similar study conducted in Karachi, Pakistan displayed that 63% of the total strains were found multidrug resistant to commonly-used antipseudomonal drugs (Ali *et al.*, 2105). In the present study, gender wise prevalence of clinical isolates (Fig. 2) shows that infections caused by *P. aeruginosa* are more common in males compared to females. Prakash, *et al.*, (2014) and Ansari, *et al.*, (2015) have also reported in their study that male patients are at higher risk than female.

In the present study, the strains were mainly obtained from Pus samples (36.9%), ear swab (25%), throat (20%), HVS (10%), urine (3.8%) and ascetic fluid (100%) while the attempt from samples of blood,

pleural fluid, stool and CSF were remained unsuccessful for the isolation of pseudomonas. In similar study Ali *et al*., (2105) found 23% prevalence in pus samples while Khan *et al*., (2008) observed 57.64% in pus and urine 24.2% samples. This finding correlates to some extent with a previous study where higher prevalence was obtained from pus and urine samples.

Conclusion

The present study indicate that Pseudomonas aeruginosa can be regularly isolated from clinical samples with substantial prevalence and emergence of MDR *P. aeruginosa* as well as its frequent transmission is out of debate. In addition, isolated *P. aeruginosa* was resistant to a whole panel of antibiotics used against it including front-line antipseudomonal drugs. Antimicrobial research is not adequate to maintain pace with the clinical challenges of MDR microbial crises. Lack of new drug discovery and other issues are leaving disastrous consequences on the health of community. To overcome these issues, new therapeutic agents with lesser toxicity, maximum efficacy, and cost effective in nature are need of hour. Epidemiological studies and strict laws regarding antibiotic policies should be constructed to limit the unnecessary use of antibiotics so that spread of multidrug resistance can be avoided.

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