Full Length Research Paper

Susceptibility pattern of *pseudomonas aeruginosa* against various antibiotics

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To determine current trends of antibiotic resistance among clinically significant *pseudomonas aeruginosa* strains causing various nosocomial and community acquired infections. A total of 100 clinical isolates of *P. aeruginosa* from inpatient and outpatient were studied from June 2008 to May 2009 for its prevalence and susceptibility profiles. Most of the isolates were from pus followed by urine, sputum, blood, ear swab and catheter tip. Uropathogenic *P. aeruginosa* infections were higher in females than males, ratio was found more among young and elderly debilitated patients. Ciprofloxacin, Piperacillin, Imipenem were found more effective for treatment of infections in outpatients but for inpatients, parental therapy with newer aminoglycosides and third and forth generation cephalosporins need to be advocated as the *P. aeruginosa* causing nosocomial infections exhibits a high degree of drug resistance. Ninety nine percent of the clinical isolates were resistant to six commonly used antibiotics with highest resistance to ampicillin (100%) and cefuroxime (100%) followed by amoxycillin (99%), co-trimoxazole (99%), Tetracycline (99%), Cefazoline (99%). The in vitro sensitivity pattern of 100 isolates of *P. aeruginosa* showed highest sensitivity to imipenem (97%) followed by amikacin (79%), tobramycin (70%), ceftazidime (62%), ciprofloxacin (73%), cefoperazone (60%), piperacillin (65%), Gentamycin (34%) and Cefotaxime (14%). ESBLs producing strains (33%) were also less in number but were much more resistant to -lactam and other antibiotics. The results indicate that *P. aeruginosa* is the most common gram -negative bacterium responsible for the nosocomial as well as community acquired infections. The excessive use of antibiotics has not only led to treat the *P. aeruginosa* infections but also the emergence of antibiotic resistance. The development of multidrug resistant *P. aeruginosa* is currently one of the greatest challenges to the effective management of infections. This suggests that in addition to curative measures promptly preventive measures such as hygienic as well as better hospital and postoperative care in administration should be adopted.

Key words: *P. aeruginosa,* antibiotic resistance, nosocomial infections.

INTRODUCTION

Pseudomonas aeruginosa is a versatile bacterium ubiquitous in nature and is quite innocuous in most environments. It can be frequently isolated from soil, water, (National Institute of Health, 1994) and occasionally from normal human skin (Percy and Barthold, 1993). It can inhabit the nasopharynx and lower digestive tract up to 6% (Weisbroth,1979). Normally human faecal car-

riage of *P. aeruginosa* is low, around 3% (Botzenhart and Doring, 1993). However, carriage increases with the length of stay in hospital, reaching 30 - 50% after 3 weeks and thus can present a distinct risk of endogenous infection (Neu, 1983). *P. aeruginosa*, an increasingly prevalent opportunistic human pathogen, is the most common gram- negative bacterium found in nosocomial and community acquired infections. It can infect almost any external site or organ, and therefore, can be isolated from various body fluids such as sputum, urine, wounds, eye or ear swabs and from blood (Hugbo and Olurinola,

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1992).

Unfortunately, as fast as new antibiotics have appeared and they greatly reduced the effects of infectious diseases, microorganisms have very cleverly figured out ways to escape their effects. *P. aeruginosa* may develop several mechanisms of resistance against a variety of antibiotics. The major mechanism of resistance to -lactam antibiotics is beta-lactamase production. More than 340 β- lactamase enzymes have been detected to date. These drug inhibitory agents include the emerging class A SHV and TEM - derived extended-spectrum β lactamases (ESBLs), inhibitor resistant enzymes, non - TEM, non - SHV class A ESBLs, and carbenpenemases, class B metallo-β-lactamases and some of their novel inhibitor, plasmid and chromosomally encoded class C enzyme, and finally, the OXA - type oxacillinases ESBLs, and carbapenemases of class D (Helfand and Bonomo, 2003). ESBL producing organism pose unique challenges to clinical microbiologists, clinicians, infection control professionals and scientist engaged in finding new antimicrobial agents. ESBLs are enzymes capable of hydrolyzing third and fourth generation cephalosporins such as ceftazidime, ceftoxime and cefepime as well as aztreonam. Currently, carbapenems are regarded as the drug of choice for treatment of infections caused by ESBL-producing organisms (Rupp and Fey, 2003).

AIMS AND OBJECTIVES

Despite the use of potent antibiotics still high mortality exists in case of *P. aeruginosa* infections. Nosocomial multidrug resistant *P. aeruginosa* is an important health care problem worldwide that prolongs the duration of hospitalization, thereby increasing the cost of patient care. Considering the problem of *P. aeruginosa* infection and multidrug antibiotic resistance in it, the present study has been carried out to determine current trends of antibiotic resistance among *P. aeruginosa* strains causing various nosocomial and community acquired infections.

MATERIALS AND METHOD

A total of 100 *P. aeruginosa* isolates from clinical samples of pus, urine, blood, different body fluids, and throat, sputum and ear swabs, both from outdoor patients as well as indoor patients from different wards of the hospital were aseptically collected during June 2008 to May 2009 submitted to Pathology Laboratory, Rawalpindi and identified on the basis of colony morphology according to Bergey's Manual of Determinative Bacteriology, 8th edition.

Antimicrobial susceptibility testing was carried out by disk diffusion method of Bauer et al. (1966) was used for antibiotic susceptibility testing for each bacterial isolate on Muller Hinton agar (CM337-OXOID). Medium was prepared and sterilized by autoclaving at 121° for 15 min. 25 ml of media was poured in 90 mm sterile Petri dishes and incubated at 37⁰C overnight to check sterility. Using ampicillin (10 g), amikacin (30 g), amoxycillin (25 g), cefazoline (30 g), ceftazidime (30 g), co-trimoxazole (25 g), cefotaxime (30 g), cefuroxime (30 g), cefoperazone (75 g), ciprofloxacin (5 g), gentamycin (10 g), imipenem (10 g), piperacillin (100 g) and Tetracycline(30 g). *P. aeruginosa* (ATCC27853) was used as a control strain. The plates were incubated at 37ºC for 18 h and after incubation, plates were examined and zones of inhibition and reported the organism sensitive, intermediate, resistant according to national committee for control laboratory standards (NCCLS, 1993). After 18 h of incubation, plates were examined and zones of inhibition were measured. Results were interpreted on the basis of zone sizes, as sensitive, intermediate, resistant according to National Committee for Control Laboratory standards (NCCLS, 1993).

DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES (ESBLs)

Double disc diffusion method was used to detect the extended spectrum beta lactamases (ESBL). A single, separated colony of the test organism was picked and emulsified in 0.9% normal saline in a test tube, the turbidity of the test organism was matched with 0.5% McFarland's Standard. The suspension of test organism was spread on the Mueller - Hinton agar surface in a petri plate with the help of cotton swab soaked in suspension tube. A disk of co - amoxicillin (20 μg amoxicillin/10 μg clavulanic acid) was placed in the center of the agar surface. The discs of cephotaxime, ceftriaxone, ceftazidime and aztreonam (30 μg) were arranged in such a way that the distance between the central disc and surrounding discs was approximately 30 mm. The plates were incubated at 37°C for 24 h. After an overnight incubation, the zones around 3rd generation cephalosporins discs and aztreonam were observed. If the inhibition zone around one or more cephalosporins discs was extended on the side nearest to the co- amoxiclave disc, the organism showing this synergism is an ESBL - producer. When there was no extension of zones, the test was repeated by reducing the distance between the cephalosporins and aztreonam, amoxiclave discs to 20 mm or even less. Zones of inhibition were again observed next day. If no extension of 3rd generation of cephalosporins and aztreonam towards co- amoxiclav discs was observed, the organisms were considered as non-producer of ESBLs.

RESULTS

A total of 100 isolates of *P. aeruginosa* isolated from various specimens (43 were isolated from males, 46 from females and 8 from environmental sources) are included in the present study. A series of biochemical tests were conducted for identification and Characterization of *P. aeruginosa* as outlined in the Bergey's Manual of Determinative Bacteriology (9th Edition). Culture sensitivity testing of these samples was conducted against 15 most commonly used antibiotics for *Pseudomonal* infections by means of Disc diffusion method (Bauer - Kirby, 1966) (Table 4 and Figures 1 to 5). Later on ESBL production and glycocalyx (alignate) formation was also studied in order to understand the virulence of these strains. The samples comprised of pus, urine, blood, sputum, ear swabs, catheter tips, from the hands of paramedics and also from environmental sources (Table 3). *P. aeruginosa* was found to be the most prevalent organism throughout the sampling period.

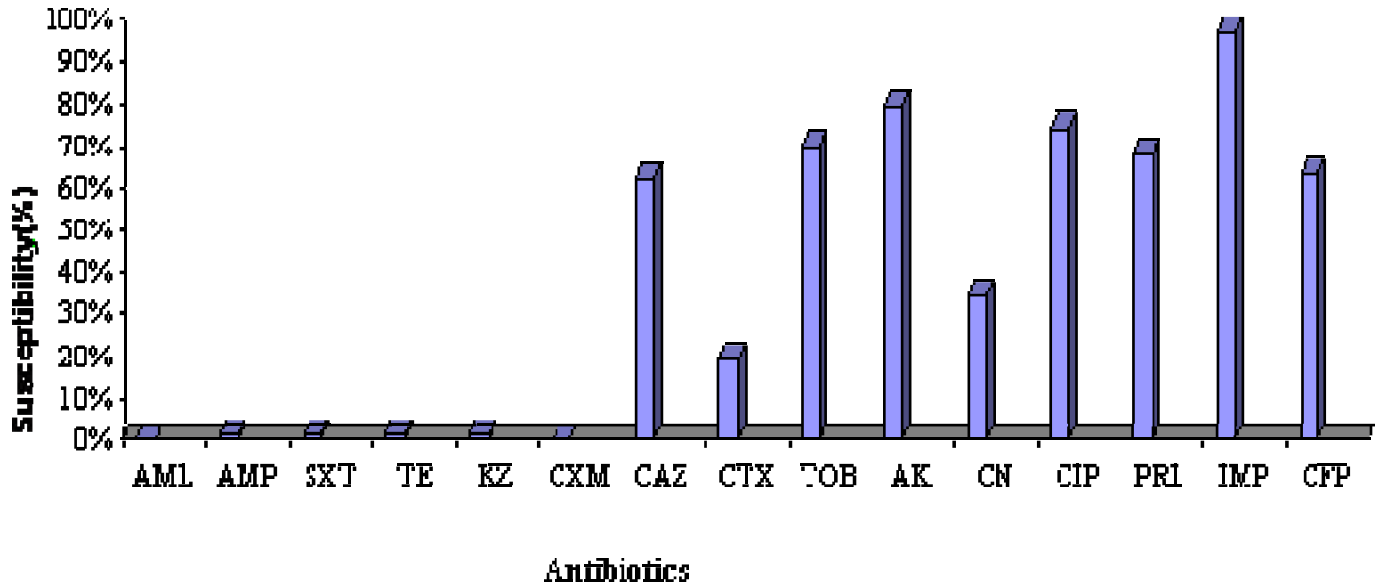Although, it is an opportunistic pathogen, responsible for,

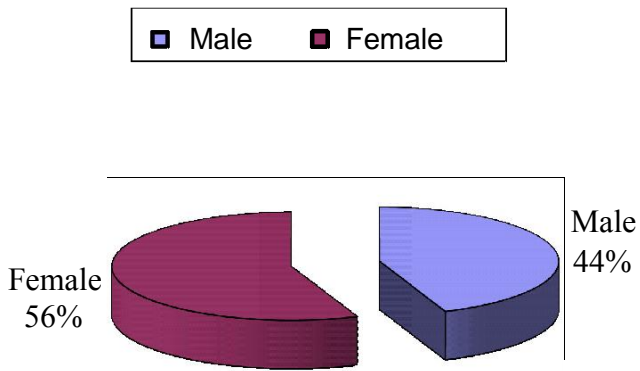**Figure 1.** Antimicrobial susceptibility pattern in *P. aeruginosa*

Figure 2. Gender wise prevalence of ESBL producers among *P. aeruginosa*
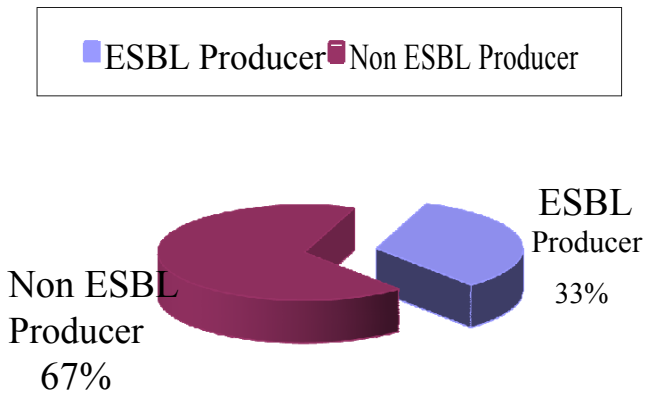

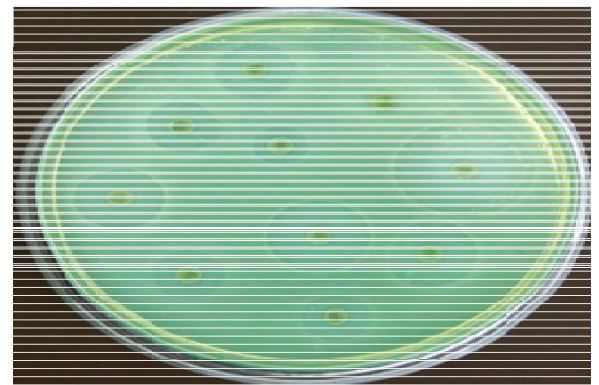**Figure 3.** Prevalence of ESBL producers among *P. aeroginosa.*

Figure 4. Susceptibility Pattern of P. aeruginosa against various antibiotics isolated from environmental source.
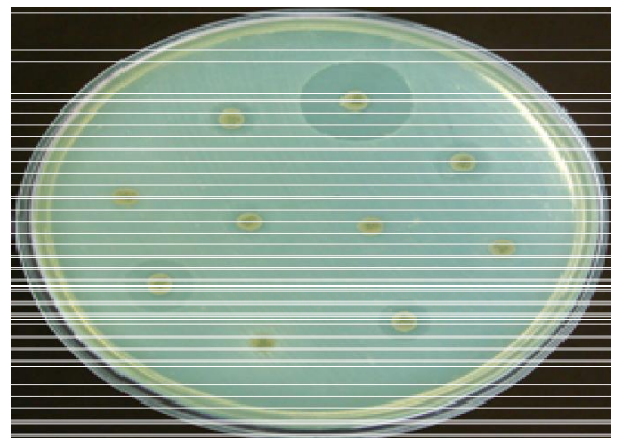

Figure 5. Susceptibility pattern of *P. aeruginosa* against various antibiotics isolated from Clinical source.

Table 1. Used culture medias and their composition

Media	Ingredients	Amount (gms/L)
Nutrient broth *CM-1*	Peptone	10.0
	Sodium chloride	5.0
	Meat extract	10.0
Nutrient agar *CM-3*	Peptone	10.0
	Sodium chloride	5.0
	Meat extract	10.0
	Agar	15.0
Muller hinton agar *CM-337*	Meat infusion	6.0
	Casein hydrolysate	17.5
	Starch	1.5
	Agar	10.0
Blood agar *CM-55*	Infusion from beef heart	500.0
	Tryptose	10.0
	Sodium chloride	5.0
	Agar	15.0
	Defibrinated blood	50.0
Tryptic soy agar *CM-131*	Phytone	500.0
	Tryptose	10.0
	Sodium chloride	5.0
	Agar	15.0
MacConkey agar *CM-7*	Peptone	20.0
	Lactose	10.0
	Bile salt no 3	5.0
	Neutral red	0.075
	Sodium chloride	5.0
	Agar	12.0
Brain heart infusion *CM-225*	Infusion from calf brain	200.0
	Infusion from beaf heart	250.0
	Peptone	10.0
	Dextrose	2.0
	Sodium chloride	5.0
	Disodium Phosphate	2.5
Pseudomonas cetrimide agar *CM-559*	Peptone	20.0
	Magnesium chloride	1.4
	Potassium sulphate	10.0
	Cetrimide	0.3
	Agar	13.6

nosocomial infection throughout the year but the present data showed that most of its occurrence was observed from July - September, covering the months of summer. It was observed that indoor patients (84%) were more infected with *P. aeruginosa* infection as compared to outdoor patients (15%). Out of these 100 samples of *P. aerugionsa,* 41 were isolated from pus, 32 from urine, 3 from ear swabs, 4 from blood, 3 from catheter tip, 5 from sputum and 8 from environmental sources (Table 3). Among clinical isolates *P. aeruginosa* was more pre-valent in pus (44%) followed by urine (34%), sputum (5%), blood (4%), catheter tips (3%) and ear swabs (3%) (Table 1).

Among 41 isolates of pus, 23 (57%) were from males

Table 2. Antimicrobial discs along with codes and potencies used in study.

Antimicrobial agent	Antibiotic group	Code	Disc potency (g)
Amoxycillin	*Penicillin*	AML	25
Cefazoline	*Cephalosporin*	KZ	30
Co-trimoxazole (Septran)	*Sulphonamide*	SXT	25
Cefuroxime	*Cephalosporin*	CXM	30
Ampicillin	*Penicillin*	AMP	10
Tobramycin	*Aminoglycoside*	TOB	10
Amikacin	*Aminoglycoside*	AK	30
Ceftazidime	*Cephalosporin*	CAZ	30
Cefotaxime	*Cephalosporin*	CTX	30
Piperacillin	*-lactamase inhibitor*	PRL	100
Gentamycin	*Aminoglycoside*	CN	10
Tetracycline	*Tetracycline*	TE	30
Ciprofloxacin	*Quinolones*	CIP	5
Cefoperazone	*Cephalosporin*	CFP	75
Imipenem	*Carbapenem*	IMP	10

Table 3. Isolation sources of *p. Aeruginosa.*

Specimen	*P. aeruginosa*
Pus	41
Sputum	5
Urine	32
Tracheal asp.	-
Blood	4
Throat	-
Catheter tip	3
Stool	-
Periton	-
Ear Swab	3
Environmental	8
Hands of paramedics	4
Total	100

and 18 (43%) were from females. Among 32 isolates of urine 18 (56%) were from females and 14 (44%) from males. Out of 5 isolates from sputum, 2 (40%) were from males and 3 (40%) were from females. In case of pus prevalence of *P. aeruginosa* was more in males (57%) as compared to females (43%). In case of urine the prevalence was higher in females (56%) as compare to males (44%). Table 2 shows the susceptibilities of the 100 isolates. Among penicillin group (–lactam antibiotics), none of the isolates were found sensitive to ampicillin and no isolate showed intermediate behavior while rest of the isolates showed highly resistant behavior (100%). For amoxycillin only 1% isolates showed sensitivity. Piperacillin (ß-lactamases inhibitor) was found effective against 65% isolates. Three aminoglycosides, that is, tobramycin, Amikacin, and gentamycin were

tested against *P. aeruginosa.* Out of these three aminoglycosides, amikacin showed 79% susceptibility, gentamycin showed 34% sensitivity and tobramycin showed 70% sensitivity. Among the quinolones and fluoroquinolones, ciprofloxacin was found effective against 73% isolates. co-trimoxazole was found effective showed 100% resistance. Among 3rd generation cephalosporins, ceftazidime showed 62% sensitivity and Cefotaxime showed 14% sensitivity.

Tetracycline was found effective against 1% of the isolates. Cefoperazone (extended- spectrum cephalosporin) inhibited 60% of the isolates so 60% isolates were sensitive to it. A total of 3% isolates showed intermediate behavior, while, 37% isolates were resistant to it. Imipenem (carbapenems) was found to be the most effective antibiotic among all the antibiotics used in this study. The percentage of sensitive organisms was 97%, whereas, only 3% were resistant to imipenem. Overall isolates exhibited 100% resistance to cefuroxime and ampicillin, 99% resistance to tetracycline, cefazoline, against only 1% of the isolates. Among first generation cephalosporins, cefazoline showed 99% resistance and Cefuroxine, the 2nd generation cephalosporin also Co - trimoxazole and amoxycillin 30% resistance to tobramycin, 21% to amikacin, 66% to gentamycin, 38% to Ceftazidime, 81% to cefotaxime, 32% to piperacillin, 26% to ciprofloxacin, 37% to cefoperazone and 3% to Imipenem (Table 2). No isolate was found sensitive to Cefuroxime. Whereas, imipenem showed highest activity (97%) followed by amikacin (79%) and ciprofloxacin (73%) . ESBL producing *P. aeruginosa* isolates are a major cause of nosocomial infections. The present study was conducted to determine the prevalence, resistance and phenotypic transfer of ESBLs among *P. aeruginosa* isolates. Out of 100 isolates, 33 (33%) were found to be ESBL producers and non-ESBL were 67% (Figure 1).

Table 4. Sensitivity pattern of *p. Aeruginosa*

Antibiotics	Sensitive (%)
Ampicillin	0
Amoxycillin	1
Septran	1
Tetracycline	1
Cefazoline	1
Cefuroxime	0
Ceftazidime	62
Cefotaxime	14
Tobramycin	70
Amikacin	79
Gentamicin	34
Ciprofloxacin	73
Piperacillin	65
Imipenem	97
Cefoperazone	60

The detection of ESBL was performed by Double disc diffusion method. Figure 2 indicates the gender wise prevalence of ESBL producers among *P. aeruginosa*. Out of total 41 pus isolates, 14 (34%) were found to be ESBLs producer, 11 (34%) from urine samples out of 32, 2 (66%) from catheter tip out of 3, 3 (60%) from sputum out of 5, 2 (50%) from blood out of 4 and 4 (50%) from Environmental sources out of 8. No ESBL producing strain was found in ear swab and fluid sample.

DISCUSSION

In our study a significant increase was found in the number of *P. aeruginosa* strains isolated from pus followed by urine. Similar pattern of isolation of *P. aeruginosa* from pus, urine, ear, nose, wound and other infection sites was reported by Ergin and Mutlu, (1999) but in a study conducted by Olayinka, (2004) *P. aeruginosa* was mostly isolated from urine samples. This could be attributed due to differences in geographical location and hygienic measures or due to the fact that most patients going for major surgery tend to get catheterised. In the present study, uropathogenic *P. aeruginosa* was found higher in females than males. Ratio was also found more among young and elderly debilitated patients. The one reason may be that *P. aeruginosa* may be a common inhabitant of lower intestinal tract and in female the distance between anal and vaginal opening is small, thus *P. aeruginosa* through fecal contamination invade and colonized urinary tracts causing infection. Thornton et al., (1970) have suggested that the ascending infection from the lumen of the drainage tube may be the major pathway by which bacteria gain access to the urinary bladder.

Garibaldi et al. (1974) have also documented a higher risk for developing bacteriuria in adult female patients, the elderly and critically ill patients with a urinary catheter. Longer stay in the hospital increases the colonization of skin and environment of the patient and may be responsible for higher incidence of Urinary catheter related infections (Tullu et al., 1998). In the present study, blood, ear swabs, and sputum samples were so less to comment on, this may be due to the low number of blood, ear swabs and sputum samples sent from wards and OPDs during the study period. However *P. aeruginosa* is said to be responsible for pneumonia and septicaemia with attributable deaths reaching 30% in immunocompromised patients (Fergie et al., 1994; Dunn and Wunderink, 1995; Brewer 1996) . The possibility of aspira-tion *Pseudomonal* pneumonia cannot be ruled out in post-surgical patients especially if immunocompromised (Olayinka et al., 2004).

In the present study, *P. aeruginosa* was also isolated from the hands of the nursing staff. This is in agreement with Cruse (1973) who reported that the hands of nurses working in wards with infected patients often carry *P. aeruginosa*. Similar kind of results was reported by (Oguntibeju and Nwobu, 2004). *P. aeruginosa* is currently one of the most frequent nosocomial pathogen and the infections due to this organism are often difficult to treat due to antibiotic resistance (Emori and Gaynes, 1993) The mechanisms of resistance to antibiotics include reduced cell wall permeability, production of chromosomal and plasmid mediated ß-lactamases (Livermore, 1989), aminoglycoside-modifying enzymes (Livermore, 1987) and an active multidrug efflux mechanism (Li, 1994; Shahid and Malik, 2004). In the present study, the susceptibility of 100 clinical isolates of *P. aeruginosa* showed highest resistance (100%) was found against cefuroxime and amoxicillin. The next most resistant antibiotics were ampicillin (99%), septran (99%), tetracycline (99%) and cefazoline (99%), cefotaxime (81%), gentamycin, (66%) Ceftazidime (38%), cefoperazone (37%), piperacillin (32%), tobramycin (30%), amikacin (21%) and imipenem (3%). Among most commonly used cephalosporins, ceftazidime and cefoperazone proved to be most effective against *P. aerugionsa*, with resistant rate of (38%) and (37%), respectively.

Among first generation cephalosporins, cephradine and cephalexin were tested against *P. aeruginosa* isolates. Most of the isolates (88%) in this study were cephradine resistant and 80% were resistant to cephalexin. Earlier studies (Wise et al., 1979; Karmali et al., 1980) also showed similar findings. Among second generation cephalosporins, *P. aerugionsa* showed 100% resistance against cefuroxime. It was previously reported that none of the first or second generation cephalosporins are active against *P. aeruginosa* (Moore et al., 1993; Jones et al., 1977). Third generation cephalosporin group included commonly used antibiotics cefotaxime, ceftazi-

dime, cefoperazone and ceftriaxone, which were tested against *P. aerugionsa* isolates. Among third-generation cephalosporins, ceftriaxone was found to be less active, as 67% isolates were resistant. Other workers (Neu et al., 1984; Moore et al., 1993) have obtained va-riable results with this antibiotic. Among cephalosporins, however, ceftazidime and cefoperazone were found to be most effective third-generation cephalosporins as only 38% and 37% isolates were resistant. This finding is consistence with other findings (Al-Lawati et al., 2000; Gencer et al., 2002). Among penicillins maximum resistance was noted for ampicillin (100%) and amoxicillin (99%). Similar kind of results had also been reported by Oguntibeju and Nwobu, (2004). An important striking feature found in this study was increased resistance to gentamycin (66%) whereas the strains were sensitive to amikacin and tobramycin. Various workers have also reported the increased sensitivity of *P. aeruginosa* strains to amikacin and resistance to gentamicin (Nagoba et al., 1997; Veenu et al., 1998). *P. aeruginosa* isolates showed resistance towards various antibiotics such as cephalosporins, tetracycline and gentamicin. Majority of the *P. aeruginosa* strains from the present study were multiple antibiotic resistant, especially the isolates recovered from pus. Such multiple resistance patterns have also been documented earlier (Gales et al., 2000).

During the study, we observed that the alginate capsules of mucoid strains of *P. aeruginosa* could not act as a barrier against imipenem. This finding is comparable to the results of Slack and Nichol's studies, in which alginate impeded the penetration of all antibiotics except the ß- lactams (Slack and Nichols, 1981; Rezaee et al., 2002). However, the alginate glycocalyx provides a barrier against penetration of cefotaxime, and this antibiotic was clearly inferior to imipenem against our *P. aeruginosa* strains. In addition, this reduced susceptibility may be related to the more extensive use of cefotaxime in hospital. On the other hand, additional resistance mechanisms especially production of extended-spectrum -lactamases (ESBLs) and other enzymes may contribute to ceftazidime resistance (Bonfiglio et al., 1998). Alginate, an extracellular glycocalyx, probably acts as a barrier against aminoglycosides (Govan and Deretic, 1996) . The mucoid strains of *P. aeruginosa* were found more resistant to amikacin, gentamicin and tobramycin than the non-mucoid strains by Rezaee et al., (2002). Similar types of results were found in the present study. Overall, there was moreresistance to gentamycin, followed by tobramycin and amikacin. The results of this study suggest that the capsule may act as a barrier against aminoglycosides (Demko and Thomassen, 1980). Nevertheless, there is evidence that alginate provides an ionic barrier against penetration of aminoglycoside antibiotics (Govan and Deretic, 1996). Slack and Nichols, (1981) used antibiotic diffusion through agar as a criterion for direct measurement of the permeability of the alginate layer to antibiotics. They found that, with the exception of

-lactams, alginate did in fact impede the penetration of antibiotics such as aminoglycosides. However, Gordon et al. (1988) observed that the alginate-to-anti-biotic ratio could greatly influence the perceived permeability barrier. When this ratio is high, aminoglycosides (but not -lactams) are retained in the alginate layer. However, low alginate- to-antibiotic ratios quickly result in disruption of the gel structure and faster penetration of amino-glycosides. Razaee et al. (2002) suggested that high levels of antibiotic saturate the negative charge of algi-nate and result in a breakdown in the permeability layer.

The development of antimicrobial resistance is a natural process, which cannot be stopped. Resistance means that people cannot be effectively treated and they remain ill for longer period of time. It also means that epidemics are prolonged and thus that there is a greater risk of infection to others. The development of resistance is accelerated when antimicrobials are misused (http://www.emro.who.net). Despite the use of potent antibiotics still high mortality exists in case of *P. aeruginosa* infections. Nosocomial multidrug resistant *P. aeruginosa* is an important health care problem worldwide. Antimicrobial resistance prolongs the duration of hospitalization, thereby, increasing the cost of patient care. There are multiple factors, which contribute to the global spread of resistance. Decreasing unnecessary antibiotic use, treating with narrow spectrum agents, improving compliance with therapy, decreasing use of antibiotic in animal and agriculture, and improving infec-tion control all have a role in confronting this problem. In addition, immunization may diminish the impact of resistance by preventing infection and also the carriage of transmission.

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