THE ANTIBIOTIC RESISTANCE PATTERN OF PSEUDOMONAS AERUGINOSA TO CIPROFLOXACIN AND MEROPENEM IN KPK

Syed Luqman Shuaib, Shahina Mumtaz, Muhammad Asghar, Haroon-ur-Rashid, Naheed Asghar, Aurangzeb

ABSTRACT

Introduction: It causes disease mainly in immune compromised persons. *Pseudomonas aeruginosa is* a common hospital acquired infection. It has many inherent mechanisms to protect itself from the action of antibiotics.

Objective: This study is designed to analyse resistance of strains of *Pseudomonas aeruginosa* in KPK.

Material Methods: Type of study was Descriptive study. Sampling procedure was nonprobable consecutive sampling. Pus samples were collected from different wards of Khyber Teaching Hospital. Collection period was 2015. Susceptibility of *Pseudomonas aeruginosa* to antimicrobial agents was determined by Disc Diffusion method of Kirby Bauer.

Results: A total of 34 specimens of organism from different wards were collected.The study shows specimens resistant to meropenem were 04 (11.76%), specimens resistant to ciprofloxacin were 18 (52.94 %) and specimens sensitive to ciprofloxacin were 16 (52.94 %).

Conclusion: We tested in our study a ciprofloxacin) and meropenem. In our study specimens resistant to meropenem were 11.76% and specimens resistant to ciprofloxacin were 52.94%. I suggest mass education of patients their attendants and health care personals for preventive measures of *Pseudomonas aeruginosa* infection.

INTRODUCTION

Pseudomonas aeruginosa is present in soil and many artificial environments. It often infects animals/ humans and plants. It causes disease mainly in immune compromised persons. When severe infection occurs in the vital organs (kidney, lungs, urinary tract) it may lead to death. It is notorious for cross infections in health care facilities. It is among the causative organism of hot tub rash. The most common species of Pseudomonas infecting human is Pseudomonas aeruginosa. The infections may be of blood, urinary tract, respiratory tract (pneumonia) & surgical infection, that may prove to be fatal.[1]

Pseudomonas aeruginosa is well adopted to grow in low oxygen pressure.[2] It is citrate, catalase & oxidase +. It mainly produces four types of pigments.[3] Blue green (pyocyanin) pigment, fluorescein production and ability to grow at 42 °C are major identifying points.[4] On MacConkey or EMB agar it gives non-lactose-fermenting (colourless) colonies.

Pseudomonas aeruginosa *is* a common hospital acquired infection e.g. in burn patients, patients with damaged skin defences. Persons with disturbed airway clearing mechanism (e.g. cystic fibrosis) in chronic respiratory disease; the immunocompromised.[6,7] Those microorganisms which cause septicaemia can invade any organ of the body and can be fatal for the patient.[5]

Pseudomonas aeruginosa is one of the most common infecting microorganisms of the burn wounds worldwide.[8] Multidrug resistant strains of Pseudomonas aeruginosa is a major problem in burn units of Pakistan also.[15] There are mechanisms by which microorganisms (like pseudomonas aeruginosa) evade body immune mechanisms. One such mechanism is biofilm formation.[9]

Increasing resistance to antibiotics in burn wound bacterial pathogens is a worldwide problem for clinician treating burn patients.

The important virulence factors are endotoxins, exotoxins, and enzymes. These all play important role in the pathogenesis. Among enzymes, elastase and proteases are produced.

Risks and spread of pseudomonas infection

In immunocompetent people Pseudomonas aeruginosa can cause ear infection. The skin lesions are associated with improperly chlorinated water in swimming pools. One of the places of spread of Pseudomonas aeruginosa infections is in hospitals. This is mainly through the hands of health care workers and improperly cleaned/manipulated equipments. In hospitals the major devices involved are catheters (venous, arterial, and urinary) and breathing machines. Burn wounds are another target of serious infections.[10]

Pseudomonas aeruginosa has largest resistance islands in its genome.[10] It has many inherent mechanisms to protect itself from the action of

Department of Dermatology Unit, HMC, Peshawar

Address for correspondence:
Dr. Naheed Asghar
Senior Registrar Dermatology Unit HMC, Peshawar
Cell: 0321 9011487
Email:drazghar@gmail.com

antibiotics. These resistance mechanism include (i) Increased expression of efflux pumps, (iii) increased expression of drug degrading enzymes, (iv) modification of drugs, (v) mutation/alteration of the target sites.

Emergence of Antibiotic resistance

A problematic property of Pseudomonas aeruginosa is its high antimicrobial resistance.[11] The gathering in integrons of many antimicrobial resistance genes favour the formation of antimicrobial resistance factors.[12]

In one study 23.1% of organisms isolated from burn wounds were Pseudomonas aeruginosa.[8] The frequency of Pseudomonas aeruginosa infection varies in wards. This may be due to the period of stay in the hospital and the pattern and duration of antibiotics taken.

There is reported resistance to all antibiotics off and on from over the world. The ratio of resistance to different antibiotics may depend upon the extensive use of an antibiotic (Amikacin, gentamicin and ciprofloxacin) and the costly and non available antibiotics of choice.

Although results in literature vary, the resistance to two commonly used antipseudomonal antibiotics is generally as follows.

Sensitivity pattern of P. aeruginosa isolates (n =44)

Antibiotic	Disk content	Sensitive (%)	Resistant (%)
Ciprofloxacin	05 µg	24 (54.5)	20 (45.5)
Carbapenem (Imipenem)	10µg	34 (77.3)	10 (32.7)[13]

Due to the ever changing resistance pattern Pseudomonas aeruginosa Today the best plan for the treatment of resistant Pseudomonas aeruginosa is first to check the sensitivity and then to use combination therapy: e.g. a β-lactamase inhibitor (sulbactam etc.) combined with other antimicrobials.[14]

In USA 5100 hospital acquired infections of Pseudomonas aeruginosa occur. The ratio of multi-drug resistant strains is above 13% (6,000), and morbidity is 400 per year.[15]

The ability of Pseudomonas aeruginosa to produce drug resistance is a global health problem.[16] More intensive check and more research into the mechanisms of drug resistance in Pseudomonas aeruginosa isolates are necessary in order to provide information for the development of good diagnostic methods and new drugs against infection.[17]

If isolated from sterile sites e.g. blood,[1] bone, deep collections; it indicate infection and require treatment. Pseudomonas aeruginosa is inherently resistant to many antimicrobials and show additional resistance in case of unsuccessful treatment.[18]

Antibiotics for- *Pseudomonas aeruginosa* (as recommended by CLSI 2014)

Ceftazidime, ofloxacin and norfloxacin are among the first line antibiotics. Among second line antibiotics are ciprofloxacin, enoxacin, levofloxacin, Imipenem and meropenem. Third line antibiotics include moxifloxacin and colistin.[22]

This study is designed to detect resistant strains in tertiary care hospital in KPK. Detection of resistant strains will help to categorise and hence deal with infection of Pseudomonas aeruginosa.

How to prevent *Pseudomonas aeruginosa* infections:

In general hot tubs and swimming pools should be properly maintained. Contact lenses, medical equipments and solutions used for different purposes should be saved from contamination. In ICUs proper antibiotic prophylaxis can delay the infections. The best way is to minimize exposure.[19]

OBJECTIVE

This study is designed to analyse resistance of strains of Pseudomonas aeruginosa in tertiary care hospital in KPK.

MATERIAL METHODS

Type of study was Descriptive study. Sampling procedure was nonprobable consecutive sampling .Place of sampling was different wards of Khyber Teaching Hospital Peshawar and burn units, (patients were from different parts of KPK).

Pus samples were collected from surgical, orthopaedics and medical wards and burn wound swabs from Burn unit of Khyber Teaching Hospital. Collection period was January 2015 to December 2015.

All patients irrespective of sex and ages between 5-65 years were included. Patients below 5 years and above 65 years of age and patients with multiple diseases were excluded.

Laboratory Diagnosis

The specimens were inoculated on, a blood agar plate and selective differential medium MacConkey medium. By Gram Staining procedure specimens Gram negative rods were identified. Other identification and confirmatory tests performed were oxidase test and TSI, which are explained as follows.

Wound swab samples from burn patients were collected according to standard laboratory procedures Institute (CLSI, 2014). The samples received were inoculated onto bacterial culture media i.e. Blood agar and MacConkey agar. After 24 hr aerobic incubation at 37°C. Isolates was identified to the species level using gram staining, oxidase test and triple sugar iron assay. Susceptibility of *Pseudomonas aeruginosa* to

antimicrobial agents was determined by Disc Diffusion method of Kirby Bauer (Kirby-Bauer disc diffusion test) on Mueller-Hinton agar (MHA) as described by the clinical and laboratory standard institute (CLSI, 2014). Discs used were

DRUG SUSCEPTIBILITY TESTING by Disc diffusion method

Drug susceptibility of the bacterial isolates was tested on Mueller-Hinton agar (CM337-Oxoid, England). MHA medium was inoculated with the test organism, and filter paper discs with the antibiotics to be tested were placed on the medium. The sensitive antibiotic produce clear zone, due to inhibition of the organism. MHA was prepared as directed by the manufacturer, (38g in 1L distilled water). The medium prepared was sterilized in autoclave at 121^{0}C for 15 minutes, pressure was 15 psi. The Petri dishes with MHA was dried in incubator at 37^{0}C. The quantity of medium in each Petri dish was 25 ml.

All the data was recorded in a specially designed proforma.

RESULTS

The study shows the sensitivity/resistance pattern for Pseudomonas aeruginosa of antibiotic meropenem and ciprofloxacin in isolates of Pseudomonas aeruginosa; obtained from pus of surgical patients. It shows the effectiveness of the two antimicrobials for Pseudomonas aerugi*nosa*.

A total of 34 specimens of organism from different wards were collected.The study shows specimens resistant to meropenem were 04 (11.76%), specimens sensitive to meropenem were 30 (88.23%), specimens resistant to ciprofloxacin were 18 (52.94 %) and specimens sensitive to ciprofloxacin were 16 (52.94 %). Sensitive to both antimicrobials (tested separately) = 15 (44.12%) and resistant to both were 03 (8.82%).

The above chart shows the sensitivity/ resistance pattern for Pseudomonas aeruginosa of antibiotic meropenem in isolates of Pseudomonas aeruginosa.

The above chart shows the sensitivity/ resistance pattern for Pseudomonas aeruginosa of antibiotic ciprofloxacin in isolates of Pseudomonas aeruginosa. It indicate the activity of the antimicrobial for Pseudomonas aeruginosa.

DISCUSSION

Pseudomonas aeruginosa is recognized as an opportunistic pathogen and one of the significant agents of nosocomial infections in recent years. It has been involved in different infections like Urinary tract infection, pulmonary diseases, and particularly in burn infections. In fact, *P. aeruginosa* can develop multidrug resistance and the capability for developing antibiotic resistance is the main problems regarding to prevention and treatment of infections.

Pseudomonas aeruginosa once make home in a hospital ward (especially burn unit), it then remain for

Species	Urea	Lact	Ox	Cit	Mot	Ind	TSI Medium			
							Slope	Butt	H2s	Gas
Escherichia Coli	-	+	-	-	+5	+2	Y6	Y	-	+2
Pseudomonas	+	-	+	+	±	-	R	R	-	-

S. No	Drug	Abre	Strength	Resistance zone
1.	Ciprofloxacin	CIP	05ug	≤15 mm
2.	Imipenem	IPM	10ug	≤13 mm
3.	Meropenem	MEM	10ug	≤13 mm

Antibiotic	Total Specimens	No. of R Sp	%age of R Sp	No. of Sen. Sp	%age of Sen. Sp
Meropenem	34	4	11.76%	30	88.23%
Ciprofloxacin	34	18	52.94%	16	47.05%
Both(Tested separately)	34	3	8.82%	15	44.12%

Statistical test applied to sensitivity of ciprofloxacin ,"P value"[21]

	Category	N	Observed Prop.	Test Prop.	P Value
Cipro Group 1	S	16	.5	.8	.000
Group 2	R	18	.5		
Total		34	1.0		

Statistical test applied to sensitivity of meropenem ,"P value"[21]

	Category	N	Observed Prop.	Test Prop.	P Value
MEM Group 1	S	30	.9	.2	.000
Group 2	R	4	.1		
Total		34	1.0		

Statistical test applied to sensitivity of meropenem ,"P value"[20]

	Category	N	Observed Prop.	Test Prop.	Asymp. Sig. (1-tailed)
MEM Group 1	S	30	.9	.6	.000
Group 2	R	4	.1		
Total		34	1.0		

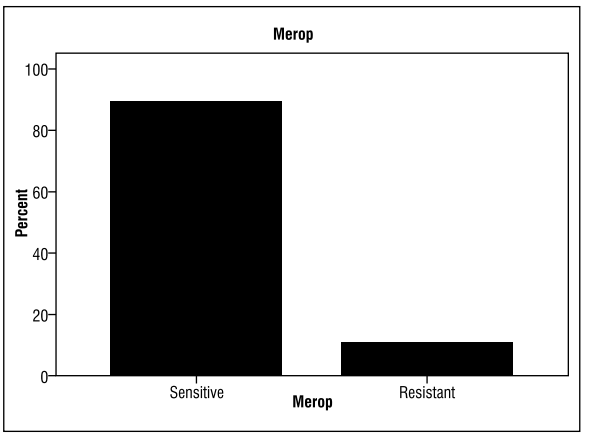

Fig. 5.5, shows the relative frequency of sensitivity and resistance of Pseudomonas aeruginosa to meropenem.

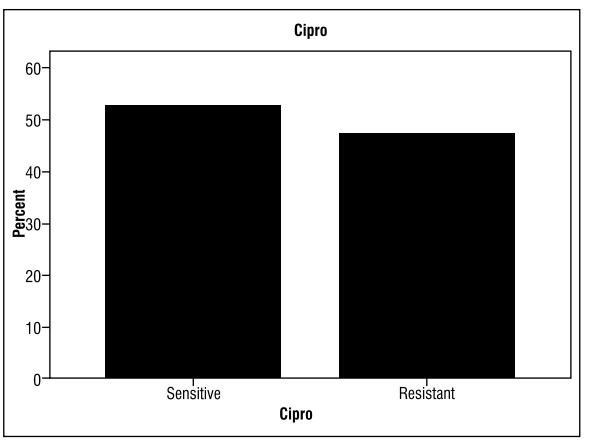

Fig.5.6, shows the relative frequency of sensitivity and resistance of Pseudomonas aeruginosa to ciprofloxacin.

months. These organisms flourished in hospitals are often multidrug resistant. They are constant threat to the patients treated in the ward.

Among the antibiotics that have the quality of being cheap, easily available, with low side effects and orally administrable and effective are fluoroquinolones. Another commonly available antibiotic class which is widely used is carbapenem. We tested in our study a fluoroquinolone (ciprofloxacin) and a carbapenem (meropenem).

In this study specimens resistant to meropenem were 04 (11.76%), specimens sensitive to meropenem were 30 (88.23%), specimens resistant to ciprofloxacin were 18 (52.94 %) and specimens sensitive to ciprofloxacin were 16 (52.94 %). Sensitive to both (tested separately) were 15 (44.12%) and resistant to both were 03 (8.82%).

There is reported resistance to all antibiotics off and on from over the world. The ratio of resistance to different antibiotics may depend upon the extensive use of an antibiotic (Amikacin, gentamicin and ciprofloxacin) and the costly and non available antibiotics of choice.

In one study the sensitivity to carbapenem (imipenem) was 67.3% and sensitivity to ciprofloxacin was 54.5% which coincide with my results.[13]

Another study shows sensitivity to carbapenem (imipenem) 60% and sensitivity to ciprofloxacin 75% which also coincide with my results.[20]

In a study 60 cases of *Pseudomonas aeruginosa* were detected in a total of 355 cases. *Pseudomonas aeruginosa* thus obtained were tested for antibiotic sensitivity. Resistance to imipenem was 80% and to ciprofloxacin was low (20%).[21] This result does not coincide with my result.

CONCLUSION

We tested in our study a fluoroquinolone (ciprofloxacin) and a carbapenem (meropenem). In our study specimens resistant to meropenem were 04 (11.76%) and specimens resistant to ciprofloxacin were 18 (52.94%).

I suggest mass education of patients their attendants and health care personals for preventive measures of *Pseudomonas aeruginosa* infection. The preventive measures include hand- washing after attending patients, use of antiseptic handwash and sterile disposal of hospital waste etc. Before treatment culture and sensitivity is must.

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