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Antimicrobial Resistance Pattern of *Pseudomonas Aeruginosa* Species in Patients of Shaikh Zayed Hospital Lahore, Pakistan

Muhammad Roman[1], Atif Ahmad Khan[2], Husnul Hayat[3], Abdul Waheed[1], Muhammad Nadeem[4], Naveed Abbas Akhtar[5], Shah Jahan[6] , Hafiz Ullah Khan[2]

[1]*Department of Microbiology, University of Health Sciences, Lahore Pakistan*
[2]*Department of Pathology, Bannu Medical College, Bannu KP, Pakistan*
[3]*Pediatric Department, Lahore General Hospital Lahore, Pakistan*
[4]*Faculty of life sciences university of Central Punjab Lahore*
[5]*Department of Histopathology, Shaikha Fatima Institute of Nursing &Health Sciences, Sheikh Zayed Hospital Lahore, Pakistan*
[6]*Department of Immunology, University of Health Sciences, Lahore Pakistan*

Correspondence: *Muhammad Roman*
Contact: m_roman63@yahoo.com

Abstract

Objective: To determine the exact resistance pattern of the rod shaped gram negative bacilli, pseudomonas aeruginosa for different anti-microbial used to treat against the pseudomonal infections among the patients.
Material and Methods: Present retrospective hospital record based cross sectional analytical study included a total of 2680 clinical samples was compile from patients of Shaikh Zayed Hospital, Lahore Pakistan from January 2013 to December 2014. Samples were processed and identified by standard protocol. The P. aeruginosa was tested for antibiotic resistance by Kirby Bauer disc method according to CLSI guidelines.
Results Three groups which are described in detail, as a result of studying among the 200 isolated Pseudomonas aeruginosa the following antibiotics was used i.e. IPM, MEM, AK, CN, CIP, CAZ, SCF and TZP. We finally came to a conclusion that IPM, MEM, CAZ, SCF, TZP are having less resistivity and more sensitivity, and the AK, CN, CIP is more resistant while the sensitivity is less. The percentage detail for resistivity in IPM was 33%, MEM was 29%, CAZ was 45%, SCF was 40.5%, the TZP was 40.5%, AK was 51.5%, CN was 64%, and the CIP was 58%.
Conclusion: This hospital based epidemiological data will help to follow better infection control strategies and improve the knowledge of antibiotic resistance patterns among clinicians. Thus there is a need for antimicrobial surveillance to monitor the resistance patterns in local hospitals.
Keywords: Pseudomonas aeruginosa, Multi Drug-Resistant, Nosocomial Infections

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Introduction

Pseudomonas aeruginosa is gram negative rod, motile, and an aerobic bacterium. It is the leading cause of opportunistic Nosocomial infections. It is a typical pathogen correlated with respiratory tract infections, cystic fibrotic patients and is also the causative of pseudomonal pneumonia.

Pseudomonas aeruginosa (P. aeruginosa) is an aerobic, motile, gram-negative rod shaped bacilli known as the leading cause of opportunistic Nosocomial infections. [1] It is important bacterial pathogen especially in hospital environment and cause 10% to 20% infections in hospitals especially prevalent among immunocompromised patients like cystic fibrosis, patients with burn wounds, organ transplants, intravenous drug addiction and acute leukemia. In immunocompromised subjects it has been involved in different infections i.e. Urinary tract infection, pneumonia, skin infections in rigorous burns. [2]

The negligent professional behavior results in low-cost antimicrobial to vanished its effectiveness and more complex antibacterial were introduced and marketed to fight against simple infection[3] for that reason, area-specific monitoring studies of P. aeruginosa is required for its resistance patterns and consequently the data generated would help clinicians and to provide correct pragmatic treatment for patients.

Authorship Contribution: [1,3]Conceived and planned the idea of the study, data collection and writing of the manuscript, [2]Supervised the study all along and reviewed the manuscript, [4,6]Data analysis & interpretation of the statistics.

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Keeping these facts in view, undertaken to find out the prevalence of drug resistance and antibiotic susceptibility patterns of pathogenic *P.* aeruginosa isolated from various clinical specimens in a tertiary care hospital revealed that 84.7% of P. aeruginosa were MDR and most of these isolates were susceptible to imipenem, meropenem and piperacillin/tazobactam.[4] Elderly, in-patients and associated with invasive procedure were found to be risk factors in the setup investigated.[5] The patients that are receiving incorrect empirical therapy for MDR strains have high mortality rate and had more severe underlying diseases than the patients infected by some susceptible Pseudomonas aeruginosa strains. Patients with MDR strains stay longer in the hospital than the patients infected with non-MDR strains. [6]

The treatment with different antibiotic may vary the risk of emergence of antibiotic resistance. To compare the risks of emergence of resistance we associated four antibiotics to compare the risks of antibiotic resistance emergence that are anti-pseudomonal agents, ciprofloxacin, ceftazidime, imipenem, and piperacillin, for assessing the relative risks for emergence of resistant *P. aeruginosa* in patients treated with any of these drugs. It is concluded that there were noticeable differences among antibiotics that their use would allow the emergence of resistance in *P. aeruginosa*. The antibiotic ceftazidime was associated with the lowest risk than the antibiotic imipenem which had the highest risk. [5]

Multidrug Efflux Pumps, Expression Patterns and the contribution to Antibiotic Resistance can be seen in the Pseudomonas aeruginosa Biofilms. *P. aeruginosa* biofilms are resistant to antibacterial therapies. The resistance of *P. aeruginosa* biofilms to various antibacterial agents proposes that efflux pumps may add to the inherent resistance of biofilms. These biofilms substrates the subject to efflux from the plank tonic cells.[7] The present study was aimed to determine the exact resistance pattern of the rod shaped gram negative bacilli, pseudomonas aeruginosa for different anti-microbial used to treat against the pseudomonal infections among the patients.

Materials and Methods

The current study was conducted at the Department of Microbiology, Shaikh Zayed Hospital Lahore, Pakistan from Jan 2013 to Dec 2014. The sample size selected for the present study was 200 Pseudomonas positive samples carried out for microbiological examination. The samples were collected from the patients admitted to different wards of Shaikh Zayed Hospital and come for routine checkup to the outdoor patient department of Shaikh Zayed Hospital using standard procedure according to CLSI. History of the patients was also recorded from their corresponding wards.

Processing of Samples: In the present study, 2680 samples were brought to the department of microbiology for general bacterial investigation, these sample includes pus/wound, sputum, urine, tracheal aspirates, central venous (CV) catheter tip, broncho-alveolar lavage (BAL) fluid, catheters and high vaginal swabs. After culturing on routine media, e.g. Blood, MacConkey, CLED and Chocolate (Oxoid) 1480 (55 %) of the total specimens shown bacterial growth, of which 200 (13%) specimens were Pseudomonas aeruginosa isolates. On MacConkey agar isolates shown lactose Non-fermented colorless Oxidase positive colonies and biochemical identification tests were performed on these strains for further identification of Pseudomonas aeruginosa. The specimen taken from the growth on primary media were inoculated on biochemical media i.e. Citrate media, Urease media, (Oxoid) Triple Sugar Iron media (TSI), and Peptone water then incubated for 18 hrs at 37°C. After incubation the results were recorded. 'Hanging drop' method used for motility and Indole test was performed using Kovac's reagent on growth on Peptone water. Alkaline slant and no reaction in butt of TSI had shown a positive strain of Pseudomonas. It exhibits negative reaction for unease test, Indole and positive reaction for citrate. Acid produced due to utilization of glucose oxidatively. On Muleur Hinton agar, the colonies were pigmented/non pigmented and Oxidase positive.

Antimicrobial susceptibility: The antibiotics used for susceptibility testing of *P. aeruginosa* through disc-diffusion (Kirby Baur disc diffusion method) are Imipenem, Meropenem, Gentamicin, Cefoperazone, Amikacin, Ceftazidime, Piperacillin/tazobactam, and Ciprofloxacin according to CLSIs guidelines. 0.5 mc Farland standard was applied for putting the anoculam on sensitivity media.

Results

In the current study, a total of 200 isolates of pseudomonas aeruginosa (PA) were isolated from different sites as shown in table I upon which antibiotics IPM, MEM, AK, CN, CIP, CAZ, SCF and TZP were putted up to find out their resistance pattern on pseudomonas aeruginosa. Among the isolates of PA, 129 were males and 71 females. Out of 129 male patients there were 4 infants, 18 newborns and 107 others. Out of 71 female patients there were 4 infants, 20 newborns and 47 others as shown in table 3. the ratio of male was higher than female as shown in table I.

Mean, standard deviation and median, for age of the infants in month, newborn in days and others in year were

determined. In which the mean for infants is 4.1 month, for newborn is 1.7 days and for others is 45.7 years. Standard deviation for these three group is 2 months in infants, 0.6 days in newborn and 20 years in others. The median is 4 months in infants, 2 days in newborn, and 45.5 years in others respectively shown in table II.

Table I: Classification of data into groups.

Group	Male	Female	Total	%
Infants	4	4	8	4.0
N.B	18	20	38	19.0
Others	107	47	154	77.0
Total	129	71	200	100.0

Table II: descriptive measures of age for three groups.

AGE	Group		
	Infants in Month	N. B in Days	Others in Years
Mean	4.1	1.7	45.7
Standard Deviation	2.0	0.6	20.0
Median	4.0	2.0	45.5

Table III: Antibiotic resistance pattern of pseudomonas aeruginosa specie.

		Groups				
		Infants in month	N.B in days	Others in years	Total	
		N	N	N	N	%
IPM	Sensitive	7	22	94	123	61.5
	Intermediate	1	6	4	11	5.5
	Resistant	0	10	56	66	33.0
	Total	8	38	154	200	100.0
MEM	Sensitive	5	20	77	102	51.0
	Intermediate	3	9	28	40	20.0
	Resistant	0	9	49	58	29.0
	Total	8	38	154	200	100.0
AK	Sensitive	2	7	80	89	44.5
	Intermediate	0	1	7	8	4
	Resistant	7	29	67	103	51.5
	Total	9	37	154	200	100.0
CN	Sensitive	2	9	49	60	30.0
	Intermediate	0	1	11	12	6.0
	Resistant	6	28	94	128	64.0
	Total	8	38	154	200	100.0
CIP	Sensitive	1	10	63	74	37.0
	Intermediate	0	2	7	9	4.5
	Resistant	7	26	84	117	58.5
	Total	8	38	154	200	100.0
CAZ	Sensitive	7	35	61	103	51.5
	Intermediate	0	1	6	7	3.5
	Resistant	1	2	87	90	45.0
	Total	8	38	154	200	100.0
SCF	Sensitive	4	27	69	100	50.0
	Intermediate	3	2	14	19	9.5
	Resistant	1	9	71	81	40.5
	Total	8	38	154	200	100.0
TZP	Sensitive	7	29	64	100	50.0
	Intermediate	1	2	16	19	9.5
	Resistant	0	7	74	81	40.5
	Total	8	38	154	200	100.0

To find out the antimicrobial resistance pattern among the 200 isolated PA, patients in Shaikh Zayed Hospital Lahore, Pakistan, the following antibiotics were used i.e. IPM, MEM, AK, CN, CIP, CAZ, SCF and TZP. The sensitivity and resistance shown by the above antimicrobial discs was recorded and found resistivity for IPM (33%), MEM (29%), AK (51.5%), CN (64%), CIP (58%), CAZ (45%), SCF (40.5%) and TZP (40.5%) as shown in the table III.

Discussion

Pseudomonas aeruginosa is the most common pathogen of Nosocomial infections. It is associated with high rate of morbidity and mortality among hospitalized patients because it's commonly resistant to routine antibiotics and antiseptics. It's the capability of pseudomonas to grow more and multiply easily in moisture environment in hospital therefore, multiple studies were conducted to determine the susceptibility pattern of antibiotics for pseudomonal infections that would definitely help clinicians for better treatment strategies.

The current study was conducted in Shaikh Zayed Hospital Lahore, Pakistan to detect the resistivity pattern of antibiotics to Pseudomonas aeruginosa and was comparable with other studies. In this study 200 strains of Pseudomonas aeruginosa were found. Males were (64.5%) and females (35.5%). The study was also conducted by [8] and reported that the percentage was 61 % in male and 31% in female. In present study the age wise prevalence of clinical isolates shows that most of patients 77% were adults, new born and infants are 19%, and 4% respectively This is comparable with the study conducted by [2] and mentioned that 92 % were adults and 8% were infants and new born.

The gender wise and age wise distribution of patients diagnosed with infections followed the natural epidemiological pattern. In our study, Pseudomonas aeruginosa were mostly identified from blood, pus, and urine. Similar results have also been reported in another study.[9]

We found the maximum isolation of Pseudomonas aeruginosa infections from surgical unit, followed by pediatric unit and then medical unit. The isolation of pseudomonas aeruginosa in surgical unit was mostly from pus, blood and urine. This was also observed in a study in USA which shows that most sample for bacterial growth was from surgical units.[10] In present study it is evident from a comparison with other it is observed that pseudomonas aeruginosa are becoming resistant to Amikacin, Gentamicin, Ciprofloxacin and Ceftazidime which is

commonly used.[11] These antimicrobial drugs are losing their efficacy due to randomly use of antibiotics, inappropriate dose, lack of knowledge and patient poor compliance. So therefore it's the challenge of the time to make authentic policies for antibiotics used and its implementation to control this current problem and resistance lying behind already observed by which shows that resistant was increased to Amikacin, Gentamycin, and Ciprofloxacin.[12] So it is compulsory to have reliable antibacterial policies during observation programmers for multi-drug resistant microorganisms and infection control actions need to be implemented.

Table I: Classification of data into groups.

Group	Male	Female	Total	%
Infants	4	4	8	4.0
N.B	18	20	38	19.0
Others	107	47	154	77.0
Total	129	71	200	100.0

Besides this useful information it's important to monitored susceptibility pattern of antibiotic for common organisms specially pseudomonas aeruginosa in hospitals. This effort would help in control of spreading the resistance and help clinicians for managing the infectious diseases properly. It's the responsibility of Microbiologist, Physician, Pharmacist and community to solve this issue and provide best surgical and medical care to patients during hospitalization.

Conclusion

We finally came to a conclusion that IPM, MEM, CAZ, SCF, TZP are having less resistivity and more sensitivity, and the AK, CN, CIP is more resistant while the sensitivity is less.

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