PREVALENCE AND ANTIBIOGRAM OF PSEUDOMONAS AERUGINOSA ISOLATED FROM CLINICAL SAMPLES AT A TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Pseudomonas aeruginosa is an important pathogen isolated from various clinical samples. It continues to cause complication in nosocomial infections. **Objective:** To determine the prevalence and antibiotics resistance pattern of P.aeruginosa isolated from a clinical samples at a tertiary care hospital. **Methodology:** This was a cross sectional study. A total of hundred clinical samples comprising urine, pus, blood and wound swabs were collected from ICU & burn unit, surgical ward, medical ward and OPD of Sheikh Zayed Medical College/Hospital, Rahim Yar Khan. These samples were cultured on MacConkey and Blood agar. Urine was cultured on CLED agar. Plates were aerobically incubated at 35–37 °C. Positive cultures were identified by culture characteristics and biochemical reactions. Antimicrobial resistance of all isolated bacteria was done by the disk diffusion testing. Pure culture was obtained then inoculated on Nutrient agar plate using disks of amoxicillin: clavulanic acid, ceftazidime, ciprofloxacin, amikacin, imipenem, piperacillin tazobactam and gentacin. After 24 hrs incubation plates were examined to read the inhibition zones. **Results:** From these samples Pseudomonas aeruginosa was isolated from urine (17%), pus (26%), blood (9%) and wound swab (48%). Pseudomonas aeruginosa showed resistance against Piperacillin Tazobactam (99%), Amoxicillin Clavulanic Acid 91%, Tigecycline 89%, Amikacin 83% and Ceftazidime 79%. **Conclusion:** Our Study showed that P. Aeruginosa, is the common microorganisms isolated resistance pattern is against common antibiotics. To cope with antimicrobial resistance against critically ill patients with pseudomonas infections it is necessary to follow firm antibiotic policies and SOPs while implementation of surveillance programmes for MDR bacteria and infection control and prevention procedures are needed.

Key words: P. aeruginosa, Antibiotics, Zone of Inhibition, Disk Diffusion Method

INTRODUCTION

Pseudomonas aeruginosa infection may cause life threatening conditions and mostly it is a hospital acquired infection.[1,2] It is ranked among top five hospitals acquired infections.[3,4] P. aeruginosa has the ability to survive on a wide variety of physical conditions, so this organism can live and develop in hospital settings. P. aeruginosa can contaminate many items; the floors, sinks in hospitals, bed rails and also isolated from the hands of staffs.[5] Additionally injured patient, soaps, respiratory equipment's and physiotherapy pools, are also important source of infections.[5] It is also common infection among immune compromised patients.[6,7,8,9] P. aeruginosa may develops resistance by different mechanisms which includes biofilm formation, production of β-lactamases, MDR efflux pumps and aminoglycoside enzymes modification.[7] P. aeruginosa also acquire resistance to antibiotics either through mutational processes which alter the expression of chromosomally encoded enzymes and plasmids.[10-13] P. aeruginosa presents severe challenge for treatment of community acquired and hospital acquired diseases. Unfortunately, selection of the appropriate antibiotic is complicated by ability of the P. aeruginosa to with stand against antibacterial agents, still during the course of disease.[14,15] Due to scarcity of information on resistance against P. aeruginosa, from various clinical samples, this study was conducted to assess the frequency and antibiotic resistance pattern against Pseudomonas aeruginosa.

METHODOLOGY

This was a cross sectional study conducted in the department of Pathology (Microbiology section) Sheikh Zayed Medical College/Hospital, Rahim Yar Khan, from 1st October 2015 to 31st January 2016. Ethical approval was sought from Institutional Review Board of Institute. A total of hundred clinical samples which included blood, wound swabs, pus and urine, were collected from, different wards and OPD patients in teaching hospitals of Rahim Yar Khan. Urin, sample was taken by Midstream urine. Sterile cotton swab, was used to collect the wound and other samples was transported to the laboratory within 30 minutes. Profusion tube was used for collection of pus and aspirates. The pus was transferred to a sterile container, labeled and sent to laboratory with request form. The blood samples were collected before medication. 20 ml blood from adult patients and 2 ml from a child with the sterile syringe and dispensed in 25ml broth medium bottle. Quarter plate of CLED agar used to inoculate urine samples, wound swabs, blood and pus samples were

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inoculated on Blood and MacConkey agar. These culture plates were incubated aerobically at 35–37 °C for 24 hrs. Positive cultures were identified by colony morphology and confirmed by biochemical reactions using the standard procedures(SOPs).

The Standard disk diffusion test was used for the susceptibility testing of all clinical isolates. Pure colonies were used to prepare bacterial suspension. A loopful bacteria was taken from an isolated colony and was transferred into 5ml of normal saline tube, mixed gently until it formed a homogenous mixture. The turbidity of the solution was then adjusted according to the McFarland standard. The cotton swab (sterile) was used to spread the bacterial suspension evenly over the entire area of Muller Hinton agar (Oxoid). The inoculated plates were left at 37 C to dry for 3-5 minutes. The Antibiotic disks of AMC (30 ug), CAZ (30 ug), CIP (5 ug), AK (30 ug), IPM (10 ug) TPZ (10ug) and CN (10ug) were applied to the inoculated plates according to following SOPs;

- 15 mm distance from the edge of the petri dish.
- Not nearer than 24 mm from center of all antibiotics.

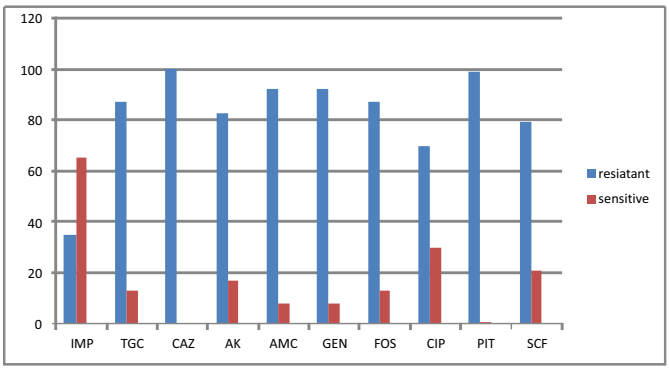
Then soft pressure applied on the antibiotic disks with sterile forceps. Plates were incubated at 37°C in an incubator aerobically for 24 hours. After 24 hrs incubation plates were examined according to CLSI 2016. The frequency of P. Aeruginosa infection was presented as percentage, similarly source and type of sample and resistance pattern was presented as percentage. The data was entered and analyzed by using SPSS version 20.

RESULTS

A total of hundred samples were collected from burn unit (22%), surgical ward (16%), ICU (16%), OPD (28%) and medical ward (18%) patients. Clinical samples included blood (10%), wound swabs (36%), pus (28%) and urine (26%).

From these samples Pseudomonas aeruginosa was isolated from urine (17%), pus (26%), blood (9%) and wound swab (48%) as shown in the table I. Pseudomonas aeruginosa showed resistance against ceftzidime (100%), Piperacillin Tazobactam (99%), Amoxicillin Clavulanic Acid 91%, Tigecycline 89%, Amikacin 83% and Ceftazidime 79%. (Figure I).

Figure I: Antibiotics Resistance Pattern of Pseudomonas aeruginosa



IMP: Imipencem, TGC: Tegecyclin, CAZ: Ceftazidime, AK: Amikacin, AMC: Amoxicillin Clavulanic Acid, GEN: Gentamycin, FOS: Fosfomycin, CIP: Ciprofloxacin, PIT: Piperacillin-Tazobactum, SCF: Cefoperazone-Sulbactam

Table I: Isolation of Pseudomonas aeruginosa from different clinical samples

Clinical Sample	No. of Sample in which Pseudomonas aeruginosa Isolated	(%) age
Wound swabs	11	48
Pus swabs	6	26
Urine	4	17
Blood	2	9
Total	23	100

DISCUSSION

The frequency and resistance to P. aeruginosa was determined in this study. P. aeruginosa is responsible for most of the nosocomial infections and is important cause of morbidity and mortality amongst patients admitted in hospital. As reported in a study, the infection was more frequent in middle and young age group. Infection was directly proportional to hospital stay.[16] We found that 23 P.aeruginosa were isolated from 100 samples and tested for antibiotic sensitivity and resistance.

In present study, the most of the clinical isolates of P. aeruginosa were isolated wound swab (48%) and pus (26%). These results are in accordance with studies carried by Jamshaid et al and other studies.[17-21]

In our study, resistance to different antibiotics against P. aeruginosa isolated from various samples was CAZ (100%), TP2 (99%), AMC (91%), AK (82%) and CIP (70%). In our study, over 65% of isolates were sensitive to IPM and 35% showed resistance to imepenem. These results were compared with results of a study, that showed CAZ resistance at 25%.[18] These results mismatched with our results in which

CAZ resistance was 100%. In another study, showed resistance of P. aeruginosa from various samples was IPM (68%) followed by CN (63%), TPZ (50%), CIP (49%) and CAZ (43%).[11] In a similar study, showed resistance for AK, CIP and CN were 48.9%, 45.2%, and 88.5% respectively.[20] Rahimi and colleagues reported, that among 100 isolates of P. aeruginosa, resistance to CAZ, CN, AK IPM and CIP was 53%, 38%, 36%, 12%, and 46%, correspondingly.[8]

Additionally in a study, 49% and 79% isolates were resistance to CN and CIP respectively.[15] According to Landman resistance pattern of P. aeruginosa isolates during 2001 to 2006 in Brooklyn was 29% for CAZ , 44% for CIP and 60 to 71% for IPM.[22] These mentioned studies showed low resistance of antibiotics against pseudomonas that are in contrast to present study.

A high level of resistant against TPZ (99%) as compared to previous studies was found in our study. Carbapenem (IMP) shows remarkable activity in our study and this could be due to its infrequent and proper use in the treatment.

AMR correlates through the high frequency of drug use and in Pakistan where anibiotics are easily available, resistance to antibiotics increases rapidly. Antibiotics be losing their worth because of the spread of resistant pathogens because of indiscriminate use of antibiotics.

CONCLUSION

Our Study showed that P. Aeruginosa, is the most common microorganisms isolated and resistance against common antibiotics is high. To cope with antimicrobial resistance against critically ill patients with pseudomonas infections it is necessary to follow firm antibiotic policies and SOPs while implementation of surveillance programmes for MDR bacteria and infection control and prevention procedures needed.

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