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Prevalence of *Pseudomonas Aeruginosa* and its Antibiotic Sensitivity Pattern

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ABSTRACT

Background: *Pseudomonas aeruginosa* is an opportunistic pathogen which causes most of the chronic infection in humans. This study was undertaken to determine the prevalence rate of *Pseudomonas aeruginosa* that is isolated from various clinical specimens along with its antibiotic susceptibility pattern.

Methods: This descriptive cross sectional study was conducted in Kathmandu Medical College and Teaching Hospital (KMCTH) from February to May 2018. *Pseudomonas aeruginosa* isolated from various clinical specimens were processed in clinical laboratory, Department of Microbiology, KMCTH. Isolation, identification and sensitivity of *Pseudomonas aeruginosa* to antibiotics were measured.

Results: A total of 7527 samples were been processed of which 46 isolates of *Pseudomonas aeruginosa* were obtained. *Pseudomonas aeruginosa* was isolated mainly from Pus, Wound swab, Sputum and Tracheal aspirate. Here 63.04% *Pseudomonas aeruginosa* isolates were resistant to Ceftazidime, 65.21% to Cefixime, 56.52% to Ceftriaxone and Cefotaxime followed by 56.52% to Piperacillin. Furthermore, the current study reveals antibiotics like Imipenem, Meropenem, Piperacillin/Tazobactam, Ciprofloxacin, Gentamicin, Amikacin and Tobramycin were found to be good choice for the treatment of infection caused by this organism.

Conclusions: Continuous monitoring of antibiotic susceptibility pattern of *Pseudomonas aeruginosa* is essential and rational treatment regimens prescription by the clinicians is required to limit the spread of antimicrobial resistance.

Keywords: Antibiotic resistance; clinical isolates; *Pseudomonas aeruginosa.*

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic gram negative rod that can cause acute and chronic infection in human.[1]

Pseudomonas aeruginosa is one of the nosocomial pathogen with increased prevalence rate as well as increased mortality rate in the hospital setting and commonly among patients with wounds and burns.[2] *Pseudomonas aeruginosa* is considered as a common source for hospitalized and non hospitalized patients throughout the world.[3] Life threatening infections are caused by *Pseudomonas aeruginosa* it possess resistance to many antibiotics which occurs as a result of overuse and misuse of antibiotics.[4] Due to increase drug resistance cure of infection is getting difficult.[5] Availability of the therapeutic options has limit due to increase in rate of multiple drug resistance strains of *Pseudomonas aeruginosa*.[6]

We aimed to determine prevalence rate of *Pseudomonas aeruginosa* isolated from various clinical specimens along with its antibiotic susceptibility pattern.

METHODS

A descriptive cross sectional study was carried out in Clinical Microbiology Laboratory of Kathmandu Medical College and Teaching Hospital from the month of February to May 2018. Ethical approval was taken from Institutional Review Committee (IRC), KMCTH, Ref no: 10/1/2018. The clinical specimens like Urine, Blood, Sputum, Pus/Wound swab, Cerebrospinal fluid, High Vaginal swab, Ascitic fluid, Pleural fluid, Tracheal aspirate, Catheter tip, Drain tip and Endotracheal tip were collected following the Clinical and Laboratory Standards Institute (CLSI) guideline. Immediately after collection of specimens, specimens were transferred to the Clinical Microbiology laboratory of KMCTH without delay for processing. Specimens were inoculated in

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Nutrient agar (NA), MacConkey agar (MA) and Blood agar (BA) culture plate incubated at 37ºC for 24 hours.

Identification of isolates were done by following standard microbiological techniques which involves morphological appearance of the colonies, Gram's staining reaction, with other standard biochemical test such as oxidase test, catalase test, motility test, haemolysin production,[7-9] triple sugar iron agar (TSI) media, sulphide indole motility (SIM) media, Simmon's citrate media, Chirstensen's urea. Nutrient Agar (NA) was observed for pyocyanin pigment and MacConkey Agar (MA) for lactose non-fermenter and blood agar (BA) to record haemolysis.

An antibiotic sensitivity tests of the pathogen isolated from clinical specimen against different antibiotic was performed using Muller Hinton Agar (MHA) by the standard disk diffusion technique of Kirby-Bauer method.[10] *Pseudomonas aeruginosa* ATCC 27853 was used for quality control.

The antibiotic sensitivity test was done as per CLSI guideline, 2007. Few colonies from culture plate were inoculated into 2ml of peptone water. It was then incubated at 37ºC for 2 hours. Turbidity was then compared with 0.5 Mc Farland Standard. A sterile cotton swab was dipped into broth and the swab rotated several time and pressed firmly on the inner side of the tube above the fluid level to remove excess inoculums from the swab. Then the dried surface of a MHA plate was inoculated by streaking the swab over the entire agar surface three times, turning the plate 60 degree between streaking. Commercially prepared antibiotic disc 6mm in diameter was used. Disc was not placed closer than 24mm (center to center) on the Muller Hinton Agar plate. 5 disc on 100mm plate was placed. Disc was applied to plates making close contact with the medium. Antibiotics such as Ceftazidime (30mcg), Ceftriaxone (30mcg), Cefotaxime (5mcg), Cefixime (5mcg), Imipenem (10mcg), Meropenem (10mcg), Piperacillin (30mcg), Piperacillin/Tazobactam (100mcg), Ciprofloxacin (5mcg), Gentamicin (30mcg), Amikacin (30mcg), Tobramycin (10mcg), Polymyxin B (300units), Colistin (10mcg), Ampicillin/Sulbactam (10mcg) and Co-trimoxazole (25mcg) were tested.

After overnight incubation of test organism *Pseudomonas aeruginosa* along with *Pseudomonas aeruginosa* ATCC 27853 for quality control, the diameter of Zone of Inhibition (ZOI) of disk was measured and was recorded in millimeter.

The descriptive statistical analysis was done by entering data in Microsoft office excel and was analyzed using SPSS (Statistical Package for Social Services) 17.0.

RESULTS

Total 7527 samples were processed of which 1009 were Gram negative bacilli.

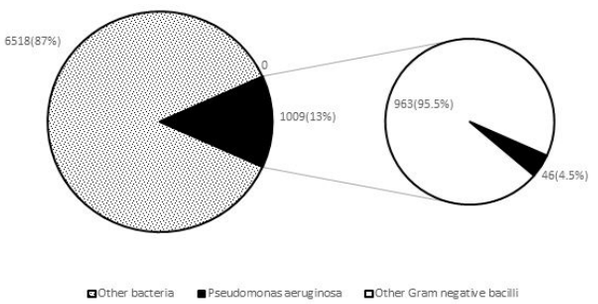

Figure 1. Prevalence of Pseudomonas aeruginosa.

Out of 1009 Gram negative bacilli strain, 4.5% isolates were found to be *Pseudomonas aeruginosa*.

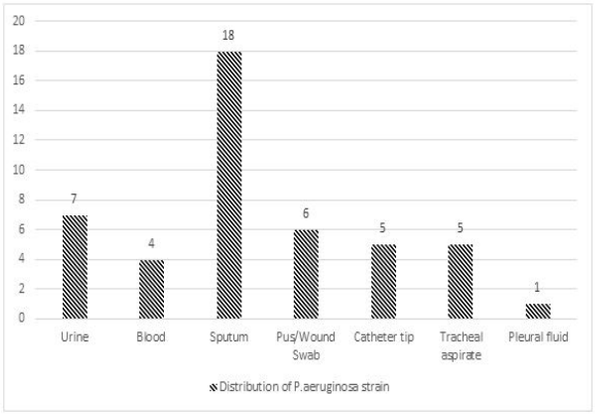

Figure 2. *Pseudomonas aeruginosa* strain isolated from different samples.

Maximum number of *Pseudomonas aeruginosa* strain were isolated from Sputum followed by Urine, Pus/ Wound swab, Catheter tip, Tracheal aspirate, Blood and Pleural fluid.

Pseudomonas aeruginosa showed resistance to antibiotics such as Ceftazidime (63.04%), Ceftriaxone (56.5%), Cefotaxime (56.5%), Cefixime (65.2%) and Piperacillin (56.5%). Polymyxin-B (100%) and Colistin (100%) were found to be more effective as compared to other antibiotics. Similarly, *Pseudomonas aeruginosa* are more sensitive toward antibiotics like Imipenem (65.2%), Meropenem (65.2%), Piperacillin/Tazobactam (76.0%), Ciprofloxacin (60.8%), Gentamicin (58.6%) , Amikacin (71.7%), Tobramycin (71.7%).

Table 1. Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* clinical isolates (n= 46).

Name of antibiotics	Total sample	Sensitivity n(%)	Resistant no(%)
Ceftazidime (Caz) 30mcg	46	17 (36.9)	29 (63.0)
Ceftriaxone (Ctr) 30mcg	46	20 (43.4)	26 (56.5)
Cefotaxime (Ctx) 5mcg	46	20 (43.4)	26 (56.5)
Cefixime (Cfm) 5mcg	46	16 (34.7)	30 (65.2)
Imipenem (Imp) 10mcg	46	30 (65.2)	16 (34.7)
Meropenem (Mrp) 10mcg	46	30 (65.2)	16 (34.7)
Piperacillin(Pi) 30mcg	46	20 (43.4)	26 (56.5)
Piperacillin/ Tazobactam (Pit) 100mcg	46	35 (76.0)	11 (23.9)
Ciprofloxacin (Cip) 5mcg	46	28 (60.8)	18 (39.1)
Gentamicin (Gen) 30mcg	46	27 (58.6)	19 (42.2)
Amikacin (Ak) 30mcg	46	34 (71.7)	12 (26.0)
Tobramycin (Tob) 10mcg	46	33 (71.7)	13(28.2)
Polymyxin-B (Pb) 300units	46	46 (100)	0 (0)
Colistin (Cl) 10mcg	46	46 (100)	0 (0)
Ampicillin/ Sulbactam (A/S) 10mcg	46	13 (28.2)	33 (71.7)
Co-Trimoxazole (Cot) 25mcg	46	9 (19.5)	37 (80.4)

Regarding the multidrug resistance pattern of different specimens, *Pseudomonas aeruginosa* isolated from specimen like Tracheal aspirate, Catheter tip and Blood were multidrug resistance.

Table 2. Distribution of MDR, *Pseudomonas aeruginosa* isolates among clinical specimens.

Clinical samples	Total isolation	MDR strain	MDR isolation %
Sputum	18	5	26.3
Pus/Wound swab	6	2	40
Tracheal aspirate	5	5	100
Catheter tip	5	3	60
Urine	7	1	14.2
Blood	4	3	75
Pleural fluid	1	0	0

DISCUSSION

In this study, a total of 7527 samples were being processed, of which 46 isolates were of *Pseudomonas aeruginosa*. Similar type of study was conducted by Saroj et al,[11] in which 120 (24%) *Pseudomonas aeruginosa* was isolated over the period of 9 months, which is around six times more than our result that could be due to hospital acquired *Pseudomonas aeruginosa* infection.

This study was performed in tertiary care hospital, *Pseudomonas aeruginosa* were examined with respect to the specimen, maximum number of specimen from which *Pseudomonas aeruginosa* was isolated were Sputum, Pus/Wound swab, Catheter tip and Tracheal aspirate. In some studies it is been reported that patient having resistant *Pseudomonas aeruginosa* have a poor prognosis so it is important that close attention should be given to *Pseudomonas aeruginosa* strains having multidrug resistance.[12] Similar type of study was conducted by Yadav et al,[13] which show 70% of *Pseudomonas aeruginosa* isolates have obtained from Pus, Wound swab, Urine, Sputum and Tracheal aspirate.

The isolated pathogens showed resistance to Co-trimoxazole 37 (80.43%), Ampicillin/Sulbactam 33 (71.73%), Piperacillin 26 (56.52%), Cefotaxime 26 (56.52%), Ceftriaxone 26 (56.52%) and Ceftazidime 29 (63.04%). Similar type of result was found in the study of Anil et al,[6] which reveals multidrug resistance rate of more than 50% on *Pseudomonas aeruginosa* isolates. Furthermore, in this study antibiotics like Imipenem, Meropenem, Piperacillin/Tazobactam, Ciprofloxacin, Gentamicin, Amikacin, Tobramycin were found to be good choice for the treatment of infection caused by *Pseudomonas aeruginosa*. Similar type of hospital based study was conducted by Vasundharaet al,[14] from April 2013 to April 2014 in which of 38 isolates of *Pseudomonas aeruginosa* 16 (42%) were resistant to Imipenem.

In this study, predominance of *Pseudomonas aeruginosa* which is one of non- lactose fermenter is seen in respiratory sample, which is similar to the study conducted by Sharma et al,[15] from March 2013 to March 2015, in which they reported highest number of *Pseudomonas aeruginosa* isolated from respiratory sample followed by urine.

Many studies have figured varying degree of resistance to Imipenem.[16] But Yadav et al,[4] in their study figured out Meropenem 83.5% sensitive followed by Ciprofloxacin only 51% sensitive, Amikacin 78% sensitive were detected to be the most effective drug for routine prescription against *Pseudomonas aeruginosa* strains, which is similar to our study.

On the basis of resistance pattern, *Pseudomonas aeruginosa* strains isolated from specimen like Tracheal aspirate, Catheter tip and Blood shows multidrug resistance. The study conducted by Bhandari et al,[17] reported an increased rate of multidrug resistant (83.1%) *Pseudomonas aeruginosa* isolated from tracheal aspirate samples.

Continuous monitoring for multidrug resistance among *Pseudomonas aeruginosa* isolates is important because outbreak caused by *Pseudomonas aeruginosa* strains including Carbapenem resistance have been reported elsewhere.[18] The limitation of this study was patients were not categorized as outpatient or inpatient.

CONCLUSIONS

The result confirms Imipenem, Meropenem, Piperacillin/Tazobactam, Ciprofloxacin, Gentamycin, Tobramycin, Colistin and Polymyxin B as most effective antibiotics. *Pseudomonas aeruginosa* showed increased resistance to antibiotics like Ampicillin/Sulbactam, Co-Trimoxazole and Ceftazidime. Imipenem and Meropenem can be used as first line drug. In case of high drug resistant strain of *Pseudomonas aeruginosa* antibiotics such as Colistin and Polymyxin B will be beneficial. So, rational treatment prescription by physicians is required to limit the spread of antimicrobial resistance of *Pseudomonas aeruginosa* strain.

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