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Research Article

ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF *PSEUDOMONAS* SPECIES ISOLATED FROM VARIOUS CLINICAL SAMPLES AT A TERTIARY CARE HOSPITAL

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ABSTRACT

Considerable increase in the prevalence and multidrug-resistant (MDR) *Pseudomonas* has been observed with towering morbidity and mortality. As a consequence of the haphazard use of antimicrobials, the spread of antimicrobial resistance is now a global issue. This study aimed to access the distribution rate and antibiotic susceptibility patterns of *Pseudomonas* species isolated from various clinical specimens in Kathmandu Model Hospital, Nepal. During the study period, 1252 samples were collected, cultured and the organism was isolated and identified. The antimicrobial susceptibility testing was done using the modified Kirby-Bauer disc diffusion method as per CLSI guidelines. Out of 1252 samples, 28 clinical isolates of *Pseudomonas* species were isolated. The highest number of *Pseudomonas* spp. was isolated from swab samples that included pus, ear, and wound (46.4 %). *Pseudomonas* spp. demonstrated marked resistance against cefixime (96.4 %) and showed higher sensitivity to piperacillin/tazobactam (92.9 %). The result showed pus, wound exudates, ear discharges samples exhibit *Pseudomonas* as common etiology of infection. *Pseudomonas* spp. demonstrated highest sensitivity against piperacillin/tazobactam, amikacin, meropenem, gentamycin. The steady resistance of *Pseudomonas* spp. to most of the antibiotics, necessitates these drugs to be confined to extreme infections and hospital intensive care units to circumvent the speedy emergence of resistant strains.

Keywords: Antibiotic resistance, Cefixime, Kirby-Bauer disc diffusion, Multi drug resistance, *Pseudomonas*.

INTRODUCTION

Pseudomonas species are Gram-negative, aerobic, non-spore-forming straight or slightly rod-shaped bacteria that belong to the family *Pseudomonadacae*. It is extensively prevalent in nature including soil, aqua, and diverse flora all around the globe (Kireçci & Kareem, 2014; Pathmanathan *et al*., 2009). The most important species from a medical point of view is *Pseudomonas aeruginosa* (*P. aeruginosa*) while *Pseudomonas fluorescens* (*P. fluorescens*) and *Pseudomonas putida* (*P. putida*) occasionally cause infection in immunosuppressed hosts (Sivanmaliappan & Sevanan, 2011; Kayser *et al*., 2005). *Pseudomonas* infections can flourish in multitudinous anatomical sites including skin, subcutaneous tissue, bones ears, eyes, urinary tract, respiratory tract, cardiac valves. The site may differ with the portal of entry and the patients' susceptibility (Kireçci & Kareem, 2014).

Pseudomonas infections are often life-threatening and tough to manage due to intrinsic resistance to numerous antimicrobials. Systems responsible for antimicrobial drug resistance in *Pseudomonas* spp. are the resistance genes they harbor via. horizontal gene transfer, mutation in target sites and efflux proteins (Anil & Shahid, 2013). *Pseudomonas* spp. exhibits resistance to numerous antibiotics, thereby jeopardizing the selection of convenient therapy (Javiya *et al*., 2008). Since novel antimicrobials agents may not be promptly available in near future, it is necessary to perform region-specific survey studies to generate data that would aid clinicians to determine the accurate empirical regimen (Javiya *et al*., 2008; Ramana & Chaudhury, 2012).

In a country like Nepal, the haphazard use of antimicrobials plays an important role in the development of resistant nosocomial pathogens like *Pseudomonas*. Data on the antimicrobial susceptibility profile of *Pseudomonas* spp. is not ample in Nepal. Continued studies on antimicrobial resistance profile of *Pseudomonas* are crucial to determine susceptible pattern against regularly prescribed antimicrobials in Nepal and guide the clinicians to select the treatment options (Anil & Shahid, 2013). Therefore, this cross-sectional study determines the distribution rate and antimicrobial susceptibility patterns of *Pseudomonas* spp. isolated from various clinical samples of patients in Kathmandu Model Hospital.

MATERIALS AND METHODS

Study design, specimen collection, and bacterial identification

A descriptive cross-sectional study was undertaken at the Department of Microbiology in Kathmandu Model Hospital to isolate the bacteria and verify their antibiotic

susceptibility pattern. Twelve hundred and fifty-two samples which include urine, sputum, swab (pus, wound and ear), fluid, tips (catheter, VP shunt) were collected from patients of all ages and both genders excluding the patients already on antibiotic therapy. Properly labeled samples were obtained in a clean, sterile, and leak proof container. The samples were inoculated on MacConkey Agar and Blood Agar, and the bacteria were identified by colony morphology, Gram staining, biochemical tests; which included indole test, methyl red test, Voges-Proskauer test, citrate test, triple sugar iron test, oxidase test, and green-blue pigment production test (Golshani *et al*., 2012).

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed by modified Kirby Bauer's disc diffusion method on Mueller Hinton Agar medium according to the Clinical Laboratory Standard Institute guidelines (CLSI, 2013). The antibiotics used were ciprofloxacin ((5 µg), cotrimoxazole (25 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefixime (10 µg), amikacin (30 µg), gentamycin (10 µg), ceftazidime (30 µg), cefoperazone/sulbactum (75/10 µg), meropenem (10 µg), piperacillin/tazobactam (100/10 µg), chloramphenicol (30 µg) (HiMedia, India). Suspension of bacteria maintained to 0.5 McFarland standards was inoculated on Mueller Hinton Agar (HiMedia, India) plates using sterile swabs, and then antibiotic discs were placed on it. The plates were incubated at 37 °C for 24 hours. The diameter of the zone of inhibition was measured and compared with standard strain. The results were interpreted as sensitive, intermediate, resistant according to CLSI (2013) guidelines. *Pseudomonas* (ATCC 27853) was used as standard control strains.

Data analysis

All the results were entered in the worksheet of Statistical Package for Social Science Software (SPSS 14.0) and compared by the chi-square test as percentages with 95 % confidence intervals. A *p* value of <0.05 was considered to be statistically significant.

RESULTS

Among 1252 clinical samples, a total of 28 (2.2 %) *Pseudomonas* spp. was isolated. With regards to gender, 16 (57.1 %) isolates were from male patient while 12 (42.9 %) from female patient (Fig. 1). Isolation of *Pseudomonas* spp. with gender of patient was not statistically significant. The highest number of isolates (n=13, 46.4 %) were from the age group of 16-30 years and the least (n=2, 7.1 %) were from 46-60 years of age. Five (17.9 %) isolates were from age group of 31-45 years, 4 (14.3 %) from less than 15 years and above 60 years of age (Fig. 2).The percentage of isolation of *Pseudomonas* spp. with age of patient was not statistically significant.

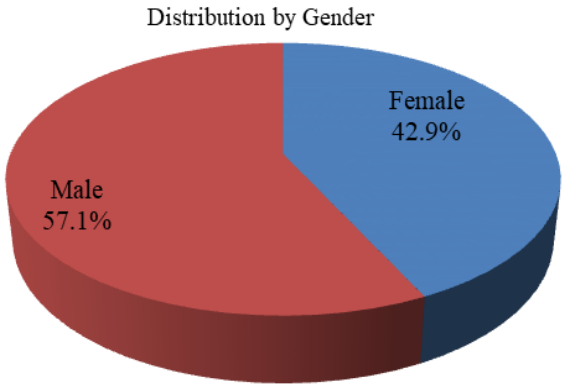

Fig. 1. Demographic characteristics of patients

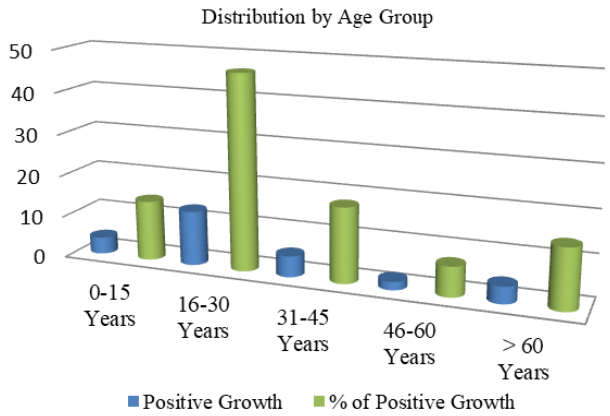

Fig. 2. Age-wise distribution of patients

Out of 1083 samples collected from outpatients, a total number of isolates were 18 (1.66 %), and out of 169 samples collected from inpatients, the total number of isolates was 10 (5.91 %) and the distribution of *Pseudomonas* spp. with patient settings was statistically significant (Table 1). Thirteen (46.4 %) isolates of *Pseudomonas* spp. were contributed from swab sample, 8 (28.6 %) from urine sample, 4 (14.3 %) from sputum sample, 2 (7.1 %) from tips that include catheter tip and VP shunt tip, and 1 (3.6 %) from the fluid sample. The isolated of *Pseudomonas* spp. with different samples was statistically significant (Table 2).

Pseudomonas spp. were susceptible to piperacillin/tazobactam 26 (92.9 %) followed by amikacin 24 (85.7 %), meropenem and gentamycin 19 (67.9 %), ciprofloxacin 18 (64.3 %), ceftazidime 16 (57.1 %), cefotaxime 14 (50.0 %), ceftriaxone 7 (25 %) and cotrimoxazole 4 (14.3 %). *Pseudomonas* isolates were resistant to cefixime 27 (96.4 %) followed by cotrimoxazole 21 (75 %), chloramphenicol and cefuperazone/sulbactam 20 (71.46 %), ceftriaxone 16 (57.1 %), cefotaxime 9 (32.1 %), gentamycin 8 (28.6 %), ciprofloxacin and ceftazidime 7 (25 %) and amikacin 3 (10.7 %), as depicted in Table 3.

Table 1. Distribution of *Pseudomonas* spp. in outpatient and inpatient

Patient	No. of Cases	Positive Growth		*p*-value
		Negative (n, %)	Positive (n, %)	
Outpatient	1083	1065, 98.33	18, 1.66	0.001
Inpatient	169	159, 94.08	10, 5.91	
Total	1252	1224	28 (2.23%)	

Table 2. Distribution of *Pseudomonas* spp. in different samples

Samples	No. of Cases	Positive Growth			*p*-value
		No.	%	% of total	
Urine	870	8	28.6	0.63	0.002
Sputum	92	4	14.3	0.31	
Fluid	92	1	3.6	0.07	
Swab	156	13	46.4	1.03	
Tips	42	2	7.1	0.15	
Total	1252	28	100	2.23	

Swab= Pus, Wound and Ear; Tip= Catheter & VP shunt

Table 3. Antimicrobial susceptibility pattern of *Pseudomonas* spp. isolates against first-line and second-line drugs

Class of Antibiotics	Potency (µg)	Susceptibility Patterns of *Pseudomonas* spp. (N=28)					
		Sensitive		Intermediate		Resistant	
		Total No.	%	Total No.	%	Total No.	%
Fluoroquinolones Ciprofloxacin	5	18	64.3	3	10.7	7	25
Sulfonamides Cotrimoxazole	25	4	14.3	3	10.7	21	75
Aminoglycosides Gentamycin	10	19	67.9	1	3.6	8	28.6
Amikacin	30	24	85.7	1	3.6	3	10.7
Cephalosporins Ceftazidime	30	16	57.1	5	17.9	7	25
Ceftriaxone	30	7	25	5	17.9	16	57.1
Cefixime	10	0	0	1	3.6	27	96.4
Cefotaxime	30	14	50.0	5	17.9	9	32.1
Carbapenem Meropenem	10	19	67.9	0	0	9	32.1
Combination Drugs Cephoperazone-Sulbactam	75/10	6	21.4	2	7.1	20	71.4
Piperacillin-Tazobactam	100/10	26	92.9	0	0	2	7.1
Miscellaneous Chloramphenicol	30	8	28.6	0	0	20	71.4

DISCUSSION

During the study period, the highest number of *Pseudomonas* spp. was isolated from swab samples (46.4 %) followed by a urine sample (28.6 %) and sputum sample (14.3 %). The isolation of *Pseudomonas* spp. with different samples was statistically significant ($p < 0.05$). The results are in line with other findings as Khan *et al.* (2008) showed maximum clinical isolates from pus swab (57.64 %) followed by urine sample (24.2 %) and Mohanasoundaram (2011) showed higher prevalence in pus swab followed by urine sample. The result was not consistent with the study carried out by Aggarwal *et al*. (2008) that showed the major source of *Pseudomonas* spp. (28.57 %) to be sputum and tracheostomy sample followed by pus (24.13 %), urine (19.04 %), and fluids (15.38 %). Wound infection, ear infection occurs on external body surfaces that make them vulnerable to contamination with dust, dirt, polluted water making the patient susceptible to community-acquired as well as hospital-acquired *Pseudomonas* spp. infection. Other parameters like poor health status, illiteracy, malnutrition, underlying diseases, and lack of proper techniques in using medical procedures may promote to *Pseudomonas* spp. infection in Nepal (Sharma *et al*., 2004).

The highest number of *Pseudomonas* isolates (46.4 %) was from the patients of the age group 16-30 years followed by 31- 45 years (17.9 %). There was no significant association of *Pseudomonas* spp. infection with age of patient ($P > 0.05$). Findings of previous studies (Mohanasoundaram, 2011; Anil & Shahid, 2013) reported the highest numbers of *Pseudomonas* isolates in different age groups. It could be due to the involvement of this age group in different practices, prolonged hospitalization, or decreased immunity (Anil & Shahid, 2013). In this study, distribution of *Pseudomonas* isolates was common in males compared to females with a male to female ratio being 1.5:1. However, there was no significant association of *Pseudomonas* spp. infection with gender ($P > 0.05$). This result is in agreement with a previous study (Khan *et al*., 2008), which showed 61.78 % male and 38.22 % female infected by *Pseudomonas*. Many studies suggested that the *Pseudomonas* species infection is more common in male than female (Ullah *et al*., 2019).

Nowadays, the prevalence of *Pseudomonas* and the new resistant strains continue in both community and hospital originated infections (Savas *et al*., 2005). In this study, the highest number of cases was in inpatients as compared to outpatients. There was a significant association between outpatient/ inpatient and *Pseudomonas* spp. infection ($p < 0.05$). Findings of previous studies conducted by Mohanasoundaram (2011) agree with the present study as they reported the highest number of cases in inpatients with 35.86 %, 57 %, and 40.7 % in the year 2008, 2009, and 2010 respectively. *Pseudomonas* spp. finds the hospital environment accommodating and is responsible for causing hospital-acquired infections. Also, inpatients are at risk due to underlying diseases, decreased immunity, and prolonged hospital stay. The use of equipment that requires a wet, body temperature environment, such as dialysis tubing and respiratory therapy equipment is particularly susceptible to colonization by the organism (Savas *et al*., 2005).

The unique feature of *Pseudomonas* spp. is its resistance to a variety of antibiotics that result from low permeability of the cell wall, the production of inducible cephalosporinases, an active efflux, and a poor affinity for the target DNA gyrase (Mohanasoundaram, 2011). In this study, *Pseudomonas* spp. isolates were more sensitive to aminoglycosides amikacin and gentamycin. These findings are in agreement with the findings of Savas *et al*. (2005); they reported that for *Pseudomonas* spp. the resistance rate against amikacin was very low, i.e., 26 %. These findings are similar to the findings of Kumar *et al.* (2020) and Tripathi *et al*. (2011) that showed less (26.66 % and 10.79 %) resistant rate to amikacin, respectively. Other studies reported that the antipseudomonal effects of amikacin were greater than gentamycin (Maes & Vanhoof, 1992).

These findings are also similar to the findings of Chand *et al.* (2020) indicating higher sensitivity of gentamycin with a resistant rate of only 20.68 %. The result is not consistent with the findings of Agbo *et al.* (2020) and Motbainor *et al.* (2020) since they reported that for *Pseudomonas* the resistance rate against gentamycin was high i.e. 73.43 % and 54.5 %, respectively. It might be due to the poor substrate property of these drugs for those enzymes that are responsible for adenylation or acetylation and phosphorylation. In this study, only 25 % *of Pseudomonas* spp. showed resistance to ciprofloxacin that is comparable to studies of Anil and Shahid (2013), and Ramana and Chaudhury (2012) that showed 27.59 % and 39% resistant to ciprofloxacin, respectively. The result is also comparable to recent studies of Agbo *et al.* (2020), Chand *et al.* (2020) and Kumar *et al.* (2020) which showed 21.68 %, 31.03 %, and 35.55 % resistant to ciprofloxacin, respectively, suggesting it could be the regular regimen for *Pseudomonas* spp infection.

Recently, increased resistance has been observed against third-generation cephalosporins for Gram-negative bacilli, especially *P. aeruginosa.* The present study also showed a high rate of resistance of isolates to cefixime (96.4 %) and ceftriaxone (57.1 %). These high values of resistance are comparable to the reports of Chand *et al.* (2020) and Motbainor *et al.* (2020) that showed 100 % resistance to both cefixime and ceftriaxone. These findings are also similar to the studies of Anil and Shahid (2013) that revealed a higher resistance rate (68.96 %) to ceftriaxone. The increased prevalence of cephalosporin resistance is

related to the increased use of beta-lactam antibiotics such as amoxicillin, enzymatic inactivation, and biofilm formation.

The present study also showed a higher resistance rate to co-trimoxazole (75 %). Less resistance to co-trimoxazole (51.72 %) had been reported in a study from Nepal (Anil & Shahid, 2013). High resistance to co-trimoxazole (100 % and 64.44 %) was reported in the studies of Motbainor *et al.* (2020) and Kumar *et al.* (2020), respectively. It might be due to alteration of the metabolic pathway by pathogen in which they might have bypassed the reactions of folic acid synthesis where the drug acts. Most *Pseudomonas* isolates were sensitive to piperacillin/tazobactam (92.9 %) followed by meropenem (67.9 %). Few *Pseudomonas* isolates were resistant to piperacillin/tazobactam (7.1 %) followed by meropenem (32.1 %). These results are similar to the study done by Tripathi *et al*. (2011) that showed 89.22 % sensitive and 4.9 % resistant isolates against piperacillin/tazobactam and 65.68 % sensitive and 20.59 % resistant against meropenem. Low rate of resistance to piperacillin/tazobactam (11.1 % and 19.4 %) was reported by Kumar *et al.* (2020) and Chand *et al.* (2020), respectively. Similarly, low rate of resistance to meropenem was reported by Agbo *et al.* (2020). These drugs are needed to be preserved as antipseudomonal agents for future aspects.

In this study, *Pseudomonas* isolates were highly resistant to chloramphenicol and cefoperazone/ sulbactam (71.4 %) which was in corroboration with a report from India (Javiya et al., 2008) that showed a 75 % resistance rate of *Pseudomonas* spp. to chloramphenicol. These results are not consistent with the study of Kumar *et al.* (2020) that showed only 20 % resistance to cefoperazone/ sulbactam. Some data showed the antimicrobial resistances of *P. aeruginosa* to cefoperazone/ sulbactam ranged between 10.4 % - 56.9 % in Asia-pacific areas (Chiang *et al*., 2016). This shows the resistance property of *Pseudomonas* against this drug is increasing day by day which may be due to plasmid-mediated transferable enzymes and extended-spectrum enzymes. Massive exploitation of antimicrobials is one of the greatest factors in the selection and persistence of antimicrobial resistance among bacterial pathogens. The limitation of this study was that *Pseudomonas* was not identified to species level and molecular typing and plasmid profiling were not carried out.

CONCLUSION

The result shows pus, wound exudates, ear discharges, urine samples exhibit *Pseudomonas* as etiology of infection. None of the isolates of *Pseudomonas* spp. were sensitive to cefixime. *Pseudomonas* isolates reveal the highest sensitivity against piperacillin/tazobactam, amikacin, meropenem, gentamycin. As *Pseudomonas* spp are gradually becoming resistant to carbapenem and other important antibiotics in many parts of the world, these drugs should be confined to extreme infections and hospital intensive care units to circumvent the speedy emergence of resistant strains. Strict policy on antimicrobial drug overuse, prescription of drugs without susceptibility testing, self-medication practice should be made by the government of Nepal. An effective strategy on the limited and provident use of antipseudomonal agents should be established by hospitals, clinicians, clinical microbiologists, public health officials to convoy empirical therapy.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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