Original Article

PREVALENCE AND ANTIMICROBIAL RESISTANCE PATTERN OF PSEUDOMONAS AERUGINOSA ISOLATED FROM DIFFERENT CLINICAL SAMPLES AT TERTIARY CARE HOSPITAL IN NEPAL

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ABSTRACT

Background
Pseudomonas aeruginosa is one of the commonest organism causing different infections like wound infections, Lower Respiratory Tract Infection, Urinary tract infection, infections in burn patient in hospital setting. The increasing trend of antibiotic resistance in *Pseudomonas aeruginosa* poses a challenge to their empiric treatment with conventional agents. So, the objective of this study was to determine the prevalence and antimicrobial resistance pattern of *Pseudomonas aeruginosa* isolated from different clinical samples.

Methods
This was descriptive cross-sectional study carried out in the Microbiology laboratory, BPKIHS
from March –August 2022. Identification of *Pseudomonas aeruginosa* was done by standard protocol and antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method following Clinical Laboratory Standard Institute guidelines

Results
A total of 16,950 clinical samples were processed of which 198 isolates of *Pseudomonas aeruginosa* were isolated mainly from urine, pus, blood, sputum, wound swab, BAL (broncho-alveolar lavage). Of the total *Pseudomonas aeruginosa* isolated 78.3% were resistant to ceftazidime, 71.2% were resistant to cefepime, 62.1% were resistant to ceftriaxone followed by Piperacillin 59%, ciprofloxacin 43.4%, levofloxacin 39.3%, Gentamicin 36.3%, Imipenem 31.3%. None of the isolates were resistant to colistin. This study shows that the organism was highly sensitive to Amikacin (76.7%), Tobramycin (74.7%) and Piperacillin+tazobactam (PIT-71.7%) which could be the good choice for the treatment of this organism.

Conclusion
Periodic antimicrobial surveillance is essential to update the data for the prevalence and changing susceptibility pattern of the antibiotics over the period of time as this will help in choosing appropriate antibiotics for the treatment.

Keywords: Antimicrobial resistance, CLSI (Clinical Laboratory Standard Institute), *Pseudomonas aeruginosa*

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INTRODUCTION

Pseudomonas aeruginosa are gram negative non-fermentative bacteria, ubiquitous in nature. It is one of the leading causes of nosocomial infections and responsible for the 10 % of all hospital acquired infections[1]. It has been implicated in the wide variety of infections like pneumonia, urinary tract infections, blood stream infections, skin and soft tissue infections, in severe burns and in infections among immune-compromised individuals[2]. It is also associated with increased mortality and longer hospital stay mainly because of high antibiotic resistance[1].

Bacteria develop resistance because of the irrational use of antimicrobial agents and the various strategies adopted by the bacteria to overcome the actions of the antimicrobial agents. Because of the development of the resistance the choices of the antibiotics for treatment of infections has been narrowed[3]. Respiratory equipment, cleaning solutions, disinfectants, sinks, vegetables, endoscopes, and physiotherapy pools are the major reservoir of *Pseudomonas aeruginosa* in the hospital environment[4]. There are various mechanisms of antibiotic resistance like production of enzymes (extended-spectrum β-lactamases, carbapenemases), aminoglycoside-modifying enzymes, mutation in efflux pumps, derepression of ampC, modification of target site of antimicrobial agents and outer membrane permeability barrier.[2] The ability of *P. aeruginosa* to survive in vivo and in the hospital environment by producing extracellular matrix also adds to its resistance mechanism.[2] Multidrug-resistant (MDR) *P. aeruginosa,* responsible for the increasing prevalence of health care associated infection (HAI) limits the antimicrobial treatment option leading to high morbidity and mortality[5]. Combination therapy is often required to cure the infection caused by the *Pseudomonas aeruginosa*[6]. So the aim of this study is to determine the Prevalence of *Pseudomonas aeruginosa* and its antimicrobial resistance pattern from different clinical samples

METHODS

This was a descriptive cross-sectional study carried out in the Microbiology laboratory of BPKIHS, Dharan, Nepal from March-August 2022. Ethical clearance was taken from Institutional review committee (IRC/2147/021), BPKIHS. A total of 16, 950 different samples like Pus, Urine, Blood, sputum, BAL, wound swab, fluids (pleural, ascetic fluid) were received for culture and sensitivity. The samples received were inoculated on media like Blood agar, MacConkey agar, urine sample was inoculated on CLED media, Blood was inoculated in BHI media and sub-culture was done on Blood agar and MacConkey agar. Identification of *Pseudomonas aeruginosa* was done from the colony morphology, gram stain, motil-

ity, pigment production and biochemical tests like catalase, oxidase, citrate, SIM, TSI.[7] Antimicrobial sensitivity testing was done as per the CLSI guidelines using Kirby-Bauer disc diffusion method on Muller Hinton agar (MHA). The antibiotic discs used were commercially available disc of 6mm diameter from Himedia India. The antipseudomonal drugs used ere Beta-lactam[Piperacillin (100mcg)], Piperacillin+ Tazobactam (100/10mcg), Cephalosporins [ceftazidime (30mcg), ceftriaxone (30mcg), cefepime (30mcg), Aminoglycosides [Amikacin (30mcg), Gentamicin (10mcg),Tobramycin (10mcg)], Quinolones [Ciprofloxacin (5mcg), Levofloxacin (5mcg)], Carbapenems [imipenem (10mcg), Meropenem (10mcg)], Colistin (10mcg). The test organism's colony were suspended in peptone water and incubated at 37°C for 2 hours. The turbidity was adjusted to 0.5 McFarland's standard. Lawn culture from the peptone was then done on MHA plates using cotton swab and antibiotic discs were placed on it and was incubated at 37°C overnight. The zone of inhibition was measured and interpretation was done according to CLSI guidelines.[8]

All the data were entered in Microsoft office excel and were analyzed using SPSS (Statistical Package for Social Services) version 17.0.

RESULTS

A total of 16,950 samples were cultured aerobically of which 2836 (16.73%) showed significant bacterial growth. Out of total bacterial growth 198 (6.98%) isolates were *Pseudomonas aeruginosa* isolated from the different clinical samples. Majority of isolates 137 were isolated from in-patient [wards (78%) and Intesive care unit {ICU (22%)}] and 61 were isolated from out-patients (figure-1)
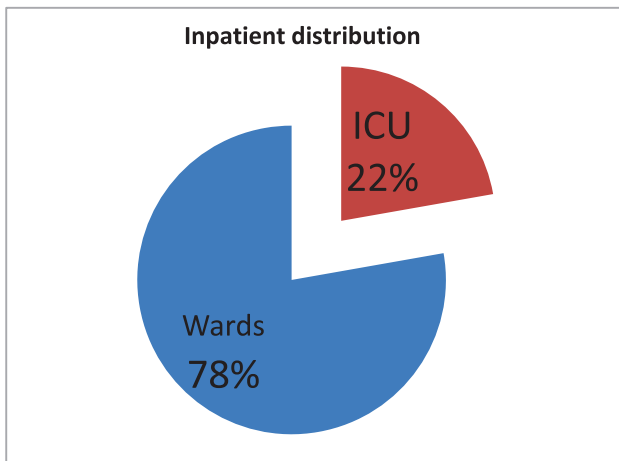

Distribution of Pseudomonas aeruginosa

OPD 31%

Inpatient 69%
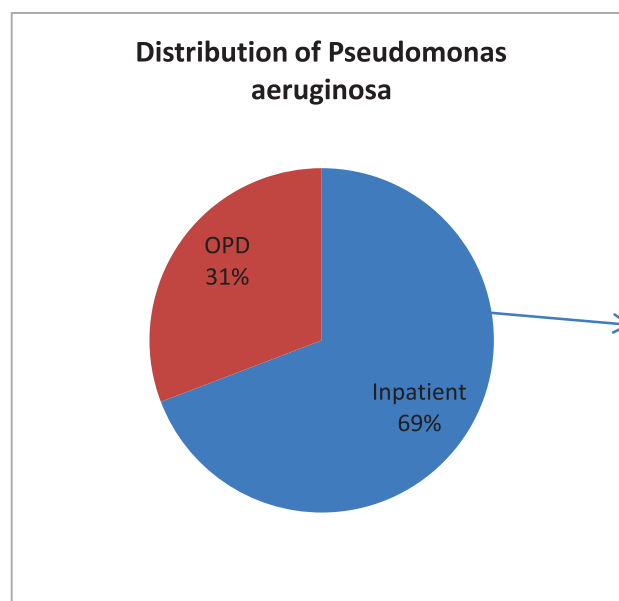
Figure 1: Distribution of *Pseudomonas aeruginosa*

Of the total *Pseudomoans aeruginosa* isolated, highest number was isolated from urine sample followed by pus, blood, sputum and BAL as shown in figure-2.
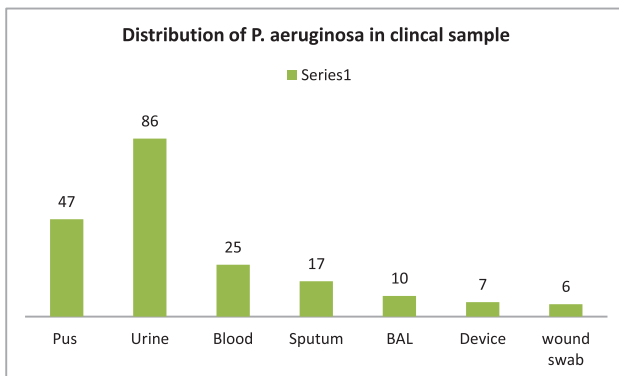

Figure 2: Distribution of *Pseudomonas aeruginosa* in clinical sample

Isolated *Pseudomonas aeruginosa* showed resistance to antipseudomonal antibiotics like ceftazidime (78.3%), cefepime (71.21%), Ceftriaxone (62.1%), Piperacillin (59%),Likewise, *Pseudomonas aeruginosa* were highly sensitive to Amikacin (76.7%), Tobramycin (74.7%), Piperacillin/Tazobactam (71.7%), Imipenem (68.6%), Meropenem (63.6%), Ciprofloxacin (56.5%), levofloxacin (60.6%). Also, it showed 100% sensitivity towards colistin as in figure-3.
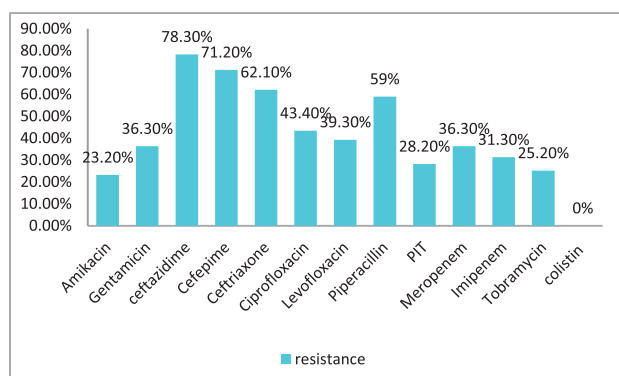

Figure 3: Antibiotic resistance pattern of *Pseudomonas aeruginosa*

DISCUSSION

Pseudomonas aeruginosa is one of the important agent causing both community acquired and nosocomial infections and presents a serious therapeutic challenge for the treatment.[2] Selection of appropriate antibiotic to initiate therapy is essential to optimize the clinical outcome.[9] So, this study will help in selecting appropriate antibiotic for the treatment of infections caused by *Pseudomonas aerugionsa*.

In our study, a total of 198 *Pseudomonas aeruginosa* were isolated with a prevalence rate of 6.98% which is similar to study by Saeed W. M et. al. where the prevalence of *Pseudomonas aeruginosa* was 6.5%.[10] Higher prevalence was reported by Dash M et. al. (9.7%), Yadav VC et. al. (13%),JS gill et.al. (14. 7%), Rajat et. al. (32.1%) respectively.[2,11,12,13] In comparision, lower prevalence was observed in study by Pokharel K et. al. which was 4.5% and Okon et. al. in Nigeria (2.1%).[14,15] The variation in the prevalence of *Pseudomonas aeruginosa* may be because variation in study population, geographical location, type of clinical specimens received.[2]

In the present study higher number of *Pseudomonas aeruginosa* were isolated from urine sample 86 (46.43%) followed by pus 47 (23.73%), blood 25(12.6%), sputum 17 (8.5%) while in a study by Bashir D *et. al.* higher number of *Pseudomonas aeruginosa* was isolated from wound swab 131 (46.3%) followed by blood 59 (20.8%), urine 35 (12.4%), CSF 18 (6.4%).[16] Another study by Dash M et. al. reported higher number of *Pseudomas aeruginosa* from pus/swab (67.6%) followed by urine (15%), sputum (9.5%).[2]

Our study revealed maximum number of isolates were resistant to ceftazidime (78.3%), cefepime (71.21%), ceftriaxone (62.1%), and piperacillin (59%) similar results have been reported by many Dash M *et. al.* and Pokharel K *et. al.* and many other studies.[2,14,17,18] While a lower resistance has been reported towards ceftazidime (3.7%) bySaeed W. M et. al.[10] and 10.9% by Raja NS et. al.[6]

The isolated *Pseudomonas aeruginosa* in our study showed lower resistance towards Amikacin ,Piperacillin/tazobactam, Imipenem, Meropenem which is in agreement with other studies.[2,6,11] respectively So these drugs could be the good treatment choice for the *Pseudomonas aeruginosa* infections.

Although colistin remains 100% sensitive in this study and other studies,[12] its use should be limited and should preserved for the future uses wthen no treatment options are available.

CONCLUSION

This study shows that the antibiotic resistance pattern of *Pseudomonas aeruginosa* is in increasing trend and requires continous monitoring and surveillance to

check for the changing susceptibility pattern. Each hospital should make their own antibiotic plans and policies based on their own data for the empirical therapy as resistance pattern tend to vary from one area to another. Rational and judicious use of antibiotic has to be done, if assess group of drugs are sensitive it has to

used and reserve group should be preserved.

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