ORIGINAL ARTICLE

Emerging Patterns in Antimicrobial Susceptibility Profiles of *Pseudomonas SPP*- Hospital Based Study

SADIA IKRAM[1], SAIMA INAM[2], SABA SHAMIM[3], SYED ZEESHAN HAIDER[4], MUHAMMAD ATIF QURESHI[5], ASMA INAM[6]

[1]Associate Professor of Pathology, Azra Naheed Medical College, Lahore, Pakistan
[2]Associate Professor of Pathology, Sharif Medical and Dental College, Lahore, Pakistan
[3] Head of Microbiology Dept. IMBB, University of Lahore, Pakistan
[4]Associate Professor of Microbiology & Biomedical Sciences, University of Lahore, Pakistan
[5]Professor & Head Department of Medicine and Allied, Azra Naheed Medical College, Lahore, Pakistan
[6] Associate Professor of Pharmacology, Azra Naheed Medical College, Lahore, Pakistan
Correspondence to: Dr. Sadia Ikram, Email dr_sadia.sajid@hotmail.com

ABSTRACT

Background: There is an emerging trend of *pseudomonas* infections in immune-compromised patients, specifically in hospital settings. The flagship member of this family is *Pseudomonas aeruginosa*, which is a major infectious agent. This study assessed the distribution and susceptibility patterns of *Pseudomonas species* isolated from various specimens as a part of surveillance program, in order to devise antibiograms.
Aim: To determine frequency & antimicrobial sensitivities of *Pseudomonas* species in a tertiary care hospital from Lahore.
Study design: Retrospective, descriptive, cross sectional study
Place & duration of study: Conducted in a tertiary care hospital in Lahore, from January 2021 to January 2022.
Methods: Thirty two isolates of *Pseudomonas species* from different clinical specimens were isolated in microbiology section of a tertiary care hospital during a period of 13 months. MacConkey and blood agar were utilized for culturing of organisms. Gram staining, oxidase, and catalase testwere utilized for phenotypic characterization. Antibiotic susceptibility testing against anti-*pseudomonal* drugs was performed according to the Clinical and Laboratory Standards Institute guidelines (CLSI) 2021.
Results: The inference drawn at the end of study was that, out of thirty two isolates of *Pseudomonas* species received and pus was the most common specimen (46.8%), followed by 18.75% from urine specimens. 62.5% of the *pseudomonas* species were obtained from male patients. The most affected age group was 40-60 years, followed by 1-20 years. The most sensitive options turned out to be Imipenem (65.6%) and Pipericillintazobactam (62.5%), followed by 59.3% sensitivity in Amikacin. The least sensitive options in the study isolates were Aztreonam (15.6%) and Ticarcillin clavulanic acid (25%). In 18% of *pseudomonas* species isolated from urine cultures, fosfomycin was 83.3%, whereas nitrofurantoin turned out to be 50% sensitive.
Conclusions: The steadily rising resistance in *Pseudomonas* species againstavailable antibiotics optionsnecessitates their use inlife-threatening and hospital acquiredinfections.
Keywords: *Peudomonas*, Flouroquinolones, Carbapenem

INTRODUCTION

Pseudomonas species belong to the family *Pseudomonadacae* and are Gram-negative, non-fermenting rods. They are extensively prevalent around the globe commonly found in soil, water, and vegetation[1]. From clinical perspective, *Pseudomonas aeruginosa* is the most significant specie, while *Pseudomonas fluorescens* and *Pseudomonas putida* are amongst the infrequent causes of infection in immune-compromised hosts[2]. *Pseudomonas* infections can affect innumerable sites including skin, bones, ears, eyes, urinary tract, respiratory tract and they vary according to the susceptibility of patients[1] with reports of rising resistance against different classes of antibiotics[3,4].

Pseudomonas spp. have been assigned to a serious level of peril due to an ever-growing multidrug-resistance that cuts across fluoroquinolones, aminoglycosides, third and fourth generation cephalosporin and advanced beta-lactams[4]. Significant morbidity and mortality has been observed worldwide with the emergence of multidrug resistant (MDR) *Pseudomonas aeruginosa* posing serious healthcare challenges[5]. Patient survival in these infections caused by MDR pathogens can only be improved by timely and apposite therapy[6].

In patients having cancer, burns or cystic fibrosis, *Pseudomonas aeruginosa* infection is accountable for about 10% of all nosocomial infections having increasing antibiotic resistance worldwideleading to high mortality rates[7]. In this context, in developing countries, there is a dearth of data on the magnitude of this problem; hence, this study was done to assess the distribution of *Pseudomonas spp.* associated with infections in patients and their antibiotic antimicrobial susceptibility patterns in order to control the spread of MDR *pseudomonads* in our hospital setting.

Rationale & objectives: Injudicious use of antibiotics has led to emergence of these resistant strains of bacteria. The selection of appropriate treatment for these patients depends on the knowledge of prevalence of *Pseudomonas* species and their susceptibility patterns in any setting. Keeping this perspective in mind, this study was aimed to determine frequency & antimicrobial sensitivities of *Pseudomonas* species.

MATERIALS & METHODS

After approval from Ethical Review Board a retrospective, descriptive, cross sectional study was conducted in a tertiary care hospital in Lahore, from January 2021 to January 2022. All the samples received in microbiology section of pathology laboratory during this time period were processed as per microbiology standards. By using streaking method, specimens were inoculated on blood and MacConkey agar (Oxoid) and aerobically incubated for 18 to 24 h at 35–37°C. Non lactose fermenting colonies on MacConkey agar were gram stained and subjected for oxidase test. Oxidase positive Gram negative rods were further inoculated on triple sugar iron agar. Inoculated TSI were incubated at 37°C overnight and those showing red/red slant and butt on TSI were further confirmed *pseudomonas* species by inoculation on API NE.
Inclusion Criteria: All Gram negative rods confirmed as pseudomonas species on API were included in the study. *Pseudomonas* species were processedfor antimicrobial susceptibility testingby Kirby Bauer disc diffusion method as per Clinical Laboratory Standards Institute (CLSI, 2021) guidelines for following antimicrobials: Pipericillintazoactam, Cefipime, Impipenem, Meropenem, Aztreonam, Tobramycin, Gentamicin, Amikacin, Ciprofloxacin, Levofloxacin, Ticarcillinclavulanic acid. The diameter of the zone of inhibition was compared according to

Clinical and Laboratory Standards Institute guidelines (CLSI 2021)[8]. Data was compiled on Microsoft Excel and SPSS 23. Frequency and percentages were calculated for qualitative variables like age, gender, sample distribution and antimicrobial susceptibility patterns against various antibiotics.

RESULTS

The findings of this study revealed that thirty two isolates of *Pseudomonas* species were received in microbiology laboratory from January 2021- January 2022. The distribution of *pseudomonas* species in various specimens is shown in Table: 1. Pus was the most common specimen (46.8%), from which *pseudomonas* species were obtained, followed by 18.75% from urine specimens.

Table 1: Percentage of samples

| Specimens	Percentage (%)
Pus	46.8%
Urine	18.75%
Blood	12.5%
Wound swab	6.25%
Bronchial washings	6.25%
Throat swab	3.1%
CVP tip	3.1%
HVS	3.1%

As shown in figure 1, study finding showed that 62.5% of the *pseudomonas* species were obtained from male patients as compared to 37.5% of females.

Figure 1: Male to Female Ratio
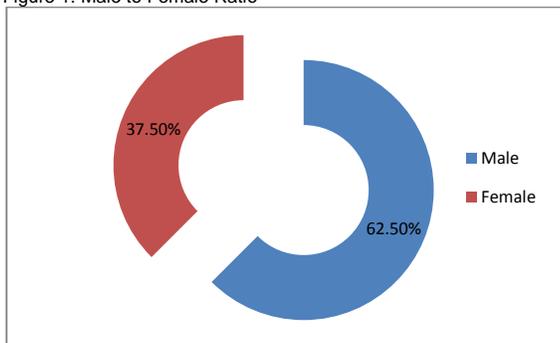
Looking at the age distribution, the most affected age range turned out to be 40-60 years, followed by the age group of 1-20 years (Fig. 2).

Figure 2: Age Distribution of *Pseudomonas* species
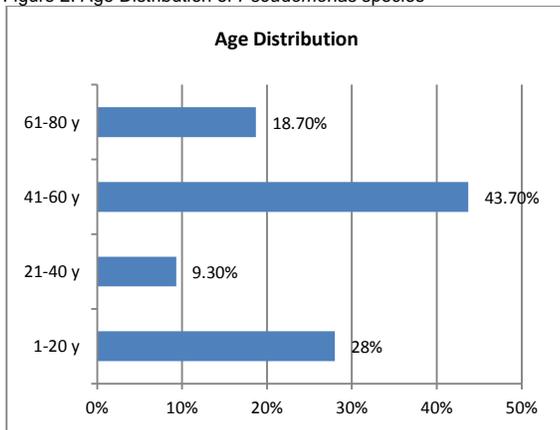
When the susceptibility profiles of study isolates were analyzed against anti-*pseudomonal* drugs, no single drug was found to be 100% sensitive. Most sensitive options turned out to be Imipenem (65.6%) and Pipericillintazobactam (62.5%), followed by 59.3% sensitivity in Amikacin. Drugs from aminogylcoside and flouroquinolone group were less than 50% susceptible. The least sensitive options were Aztreonam (15.6%) and Ticarcillin clavulanic acid (25%) (Fig. 3).

Figure 3: Percentage Sensitivity
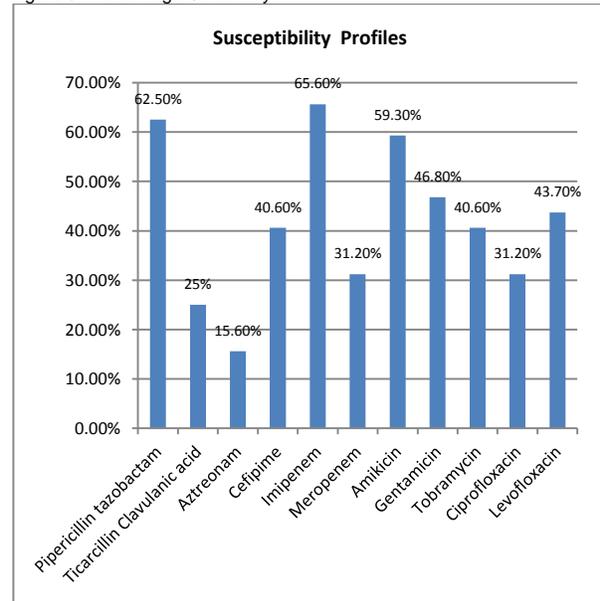
It was also analyzed the sensitivity patterns of 18% of *pseudomonas* species isolated from urine cultures against the two commonly used antibiotics for urine isolates. Amongst those, fosfomycin was 83.3%, whereas nitrofurantoin turned out to be 50 % sensitive.

DISCUSSION

During this study, thirty two *pseudomonas* species were isolated and pus was the most predominant specimen from which these were isolated, followed by urine, blood and wound swab. Studies from Nigeria and Nepal revealed wound swab and urine as predominant specimens, which is slightly different from our study findings[9,10]. *Pseudomonas* species infections are promoted by health status, illiteracy, malnutrition and underlying diseases. As an opportunistic pathogen, a breach in the first-line defense of the skin is required to initiate *Pseudomonas* infection, significantly influencing the high frequency of pus from which the isolates were recovered.

In the current study, percentage of males affected by *Pseudomonas* infection was more as compared to females. A previous study from Pakistan has also shown similar results[11], with 61.78% male and 38.22 % female infected by *Pseudomonas*. More or less same pattern was observed in study from Cyprus[12].

Maximum number of *Pseudomonas* was isolated from 41-60 years group(43.7%), followed by 1-20 years (28%). There is no agreement in this regard and different age groups can be affected based on variable factors[10,13], like prolonged hospitalization or decreased immunity[14].

Due to limited treatment options and increasing resistance to antibiotics, there is a major threat to patient care due to *Pseudomonas* species[15]. Cell wall permeability, inducible cephalosporinases, efflux of drug and a poor affinity for DNA gyrase are unique features of *Pseudomonas* species leading to the upsurge in the ongoing resistance[10]. Resistant strains of

pseudomonas species against the different classes of antibiotics were observed in the current study. According to our analysis, Imipenem and Piperacillin/tazobactam were amongst the most active antibiotic with sensitivities of 65.5% and 62.5% respectively and various other studies have shown results similar to our findings[9,15].

Amikacin, an aminoglycoside has shown better susceptibility pattern against the study isolates i.e. 59.3%. Another observation in this study was that, the isolates were more susceptible to amikacin then gentamicin or tobramycin. These outcomes are similar to the results from other centers[16,17,18]. Fluoroquinolones (Ciprofloxacin and levofloxacin) also revealed less than 50% sensitivity. There are high rates of multidrug resistance in *Pseudomonas* species especially against fluoroquinolones, aminoglycosides and third-generation cephalosporins in previous studies from Nigeria. Igbalajobi et al. reported 18-31% resistance against penicillin, aminoglycosides and fluoroquinolone, which are comparable with these study findings[19]. The observed resistance rate in the current study reflects the current antibiotic prescription patterns and the selective pressure that follows. As gentamicin and ciprofloxacin have been amongst the commonly prescribed antibiotics ending up in relatively higher rates of resistance, as compared to piperacillin/tazobactam and imipenem which are newer treatment options in hospital practice in our country.

Anti-*pseudomonas* cephalosporin was up to 40% sensitive in our study isolates, which is comparable with other studies[20] showing up to 100% percent resistance against 3rd generation cephalosporin. The most probable cause of rising cephalosporin resistance is increased use of beta-lactam antibiotics, enzymatic inactivation and biofilm formation.

CONCLUSIONS & RECOMMENDATIONS

Pseudomonas species have emerged as highly multidrug-resistant (MDR) pathogens. The most important factor for the rapidly developing antimicrobial resistance among bacterial pathogens is the massive misuse of antimicrobials.

As *Pseudomonas* species are progressively becoming resistant to most of the class of antimicrobials across the globe, hence Carbapenem group should be confined to life threatening infections and intensive care units. Stringent policies should be made by the government, overuse of drugs, and prescription of drugs without sensitivity testing and self-medication. An effective strategy should be developed by hospitals, clinicians, clinical microbiologists and public health on limiting the use of *antipseudomonal* agents in order to control this emerging drug resistance.

Authors' contribution: SI: Conception, planning of research, Data Collection, and writing of manuscript, Discussion. **SI:** Help in references, Discussion, SS & **SZH:** Proof Reading and critically revised the paper in keeping with important intellectual content. **MAQ**: Proof Reading, **AI**: Help in references, Proof Reading
Conflict of interest: Authors state no conflict of interest.

REFERENCES

1. Kireçci E, Kareem RD. Antibiotic susceptibility patterns of Pseudomonas aeruginosa strains isolated from various clinical specimens. Sky J Microbiol Research. 2014;2:13-7.
2. Sivanmaliappan TS, Sevanan M. Antimicrobial susceptibility patterns of Pseudomonas aeruginosa from diabetes patients with foot ulcers. International journal of microbiology. 2011;2011.
3. Agarwal G, Kapil A, Kabra SK, Das BK, Dwivedi SN. Characterization of Pseudomonas aeruginosa isolated from chronically infected children with cystic fibrosis in India. BMC microbiology. 2005;5(1):1-11.
4. Magiorakos A-P, Srinivasan A, Carey RB, Carmeli Y, Falagas M, Giske C, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection. 2012;18(3):268-81.
5. Ahmed MS, Hassan A, Jarir SA, Hadi HA, Bansal D, Wahab AA, et al. Emergence of multidrug-and pandrug-resistant Pseudomonas aeruginosa from five hospitals in Qatar. Infection Prevention in Practice. 2019;1(3-4):100027.
6. Peter S, Oberhettinger P, Schuele L, Dinkelacker A, Vogel W, Dörfel D, et al. Genomic characterisation of clinical and environmental Pseudomonas putida group strains and determination of their role in the transfer of antimicrobial resistance genes to Pseudomonas aeruginosa. BMC genomics. 2017;18(1):1-11.
7. Kabir SMM, Koh J. Sustainable textile processing by enzyme applications: IntechOpen London, UK; 2021.
8. Clinical, Institute LS. Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute Wayne, PA; 2017.
9. Adejobi A, Ojo O, Alaka O, Odetoyin B, Onipede A. Antibiotic resistance pattern of Pseudomonas spp. from patients in a tertiary hospital in South-West Nigeria. Germs. 2021;11(2):238.
10. Gyawali R, Khadka RB, Shrestha B, Manandhar S. Antimicrobial susceptibility patterns of Pseudomonas species isolated from various clinical samples at a tertiary care hospital. Journal of Institute of Science and Technology. 2020;25(2):49-54.
11. Khan JA, Iqbal Z, Rahman SU, Farzana K, Khan A. PREVALENCE AND RESISTANCE PATTERN OF PSEUDOMONAS AERUGINOSA AGAINST VARIOUS ANTIBIOTICS. Pakistan journal of pharmaceutical sciences. 2008;21(3).
12. Ullah N, Güler E, Güvenir M, Arıkan A, Süer K. Isolation, identification, and antibiotic susceptibility patterns of Pseudomonas aeruginosa strains from various clinical samples in a university hospital in Northern Cyprus. Cyprus J Med Sci. 2019;4(3):225-8.
13. Anil C, Shahid RM. Antimicrobial susceptibility patterns of Pseudomonas aeruginosa clinical isolates at a tertiary care hospital in Kathmandu, Nepal. Asian J Pharm Clin Res. 2013;6(3):235-8.
14. Shrestha S, Amatya R, Adhikari R. Prevalence and antibiogram of pseudomonas aeruginosa isolated from clinical specimens in a Teaching Hospital, Kathmandu. International Journal of Infectious Diseases. 2016;45:115-6.
15. Peshattiwar PD, Peerapur BV. ESBL and MBL mediated resistance in Pseudomonas aeruginosa: An emerging threat to clinical therapeutics. J Clin Diagn Res. 2011;5(8):1552-4.
16. Kumar A, Das S, Anjum N, Oraon V, Das S. Antimicrobial Susceptibility Pattern of Extended Spectrum Beta-lactamase (ESBL) and Non ESBL Producing Pseudomonas aeruginosa, Isolated from Pus Samples from a Tertiary Care Hospital in Bihar. Int J Curr Microbiol App Sci. 2020;9(6):3646-55.
17. Maes P, Vanhoof R. A 56-month prospective surveillance study on the epidemiology of aminoglycoside resistance in a Belgian general hospital. Scandinavian journal of infectious diseases. 1992;24(4):495-501.
18. Gad GF, El-Domany RA, Zaki S, Ashour HM. Characterization of Pseudomonas aeruginosa isolated from clinical and environmental samples in Minia, Egypt: prevalence, antibiogram and resistance mechanisms. Journal of antimicrobial chemotherapy. 2007;60(5):1010-7.
19. Oluborode O, Smith S, Seriki T, Fowora M, Ajayi A, Coker A. Antibiotic susceptibility pattern and molecular typing by PCR-RAPD analysis of clinical and environmental isolates of Pseudomonas aeruginosa. Microbiology and Biotechnology Letters. 2018;46(4):434-7.
20. Chand Y, Khanal S, Panta OP, Shrestha D, Khadka DK, Poudel P. Prevalence of some virulence genes and antibiotic susceptibility pattern of pseudomonas aeruginosa isolated from different clinical specimens. 2020.