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FREQUENCY AND ANTIBIOGRAM OF *PSEUDOMONAS AERUGINOSA* IN A TERIARY CARE HOSPITAL.

Shehnaz Bakhtiar[1], Safdar Ali Jamali[2], Fatima Gul[3], Jawwad Ahmed[4], Faleesha Gul[5], Mukhtyar Khatoon[6]

BACKGROUND: Pseudomonas aeruginosa is a significant cause of healthcare-associated infections, with rising antimicrobial resistance posing a global public health challenge. **OBJECTIVE:** This study aimed to evaluate the frequency, antibiogram, and gender-based distribution of *P. aeruginosa* isolates in a tertiary care hospital in Pakistan. **METHODS:** This cross-sectional study was conducted from January to December 2023. Clinical samples were collected from 502 patients and processed using standard microbiological techniques. Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method, and data were analyzed for gender-based differences and resistance patterns. **RESULTS:** The majority of patients were male (60.6%, n=304), while females accounted for 39.4% (n=198). Blood samples were the most common (53.4%, n=268), followed by tissue and fluid samples (14.3%, n=72 each). The medical ward contributed the highest number of samples (59.6%, n=299, p=0.004). Meropenem exhibited the highest sensitivity (90.4%, n=454), while Levofloxacin showed moderate sensitivity (65.9%, n=331, p<0.001). Gender-based differences were observed, with males showing higher sensitivity to Levofloxacin (224 vs. 112) and Co-trimoxazole (285 vs. 162, p<0.001). Resistance rates for Capreomycin (48.8%, n=245) and Minocycline (25.1%, n=126) were higher in females. Significant differences in sample distribution and susceptibility patterns were noted across genders and wards. **CONCLUSIONS:** This study highlights the high burden of *P. aeruginosa* infections and antimicrobial resistance in Pakistan, emphasizing the need for antimicrobial stewardship programs and gender-sensitive approaches to infection control. Targeted interventions in high-burden wards are essential to mitigate the impact of resistant infections.

KEYWORDS: Pseudomonas aeruginosa, Antimicrobial resistance, Gender differences, Antibiotic susceptibility, Healthcare-associated infections, Pakistan, Public health

1. Assistant Professor Microbiology/Pathology Department, Khyber Girls Medical College Peshawar, Pakistan.
2. Consultant Pulmonologist Nawabshah.
3. Medical Officer, Medical Department, Naseer Teaching Hospital, Peshawar, Pakistan.
4. Senior Lecturer Frontier Medical College, Abbottabad, Pakistan.
5. Demonstrator, Department of Pharmacology and Therapeutics, Northwest School of Medicine, Peshawar, Pakistan.
6. Trust Grade Doctor, General Internal Medicine, The Dudley Group Nhs Foundation Trust, England.

Corresponding Author: Dr. safdar ali jamali, consultant pulmonologist, Nawabshah. jamaliawarali@gmail.com

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INTRODUCTION

Pseudomonas aeruginosa is an opportunistic gram-negative computer-associated pathogen which has become ever more prevalent in modern medicine [1]. Primary *P. aeruginosa* infections are becoming an increasings concern, especially in low-income countries due to their unexpected resilience and natural tolerance to many commonly used antibiotics. Along with pneumonia, this pathogen is known to cause bloodstream infection, UTI, and surgical site infections with a particularly high incidence among immunocompromised and ICU patients [2]. The most troubling recent development is the rapidly increasing incidence of multi-drug resistant (MDR) strains of *P. aeruginosa* which worsens the already critical situation of antimicrobial resistance [3]. MDR *Pseudomonas aeruginosa* is defined by resistance to at least three classes of prescribed antibiotics including beta-lactams, aminoglycosides, and fluoroquinolones [4]. Pathogenicity in this case is due to a combination of acquired Horizontal Gene Transfer (HGT) and intrinsic factors such as low outer membrane permeability or the presence of efflux pumps [5]. These patients suffer from prolonged hospitalizations, increased costs of healthcare, and a more complex treatment which ultimately leads to increased mortality [6].

Pakistani healthcare facilities are uncontrolled hotbeds of *Pseudomonas aeruginosa* multi-drug resistance (MDR) due to rampant antibiotic abuse, lack of infection control, and primitive diagnostic systems [2]. *Pseudomonas aeruginosa*, especially MDR, poses a severe threat to public health. Multi-drug resistant *Pseudomonas aeruginosa* is prevalent in tertiary care centers with high acuity admissions [7]. Every single case of MDR *P. aeruginosa* infection collected from multi-sourced and differently specialized healthcare providers or institutions needs to be documented together with multi-national surveillance data regarding antibiotic resistance to enable rational treatment, infection prevention policies, and control of resistant infections.

This study examines the *P. aeruginosa* antimicrobial resistance profile from a tertiary care center in Pakistan. The study seeks to harness the resistance profiles of these isolates to inform Public Health policy to strengthen existing gaps on antimicrobial resistance. The study seeks to support international efforts to mitigate the rising rates of antimicrobial resistance and improve health in low and middle-income countries.

We aim to address the gap in understanding the epidemiology of *P. aeruginosa* in Pakistan, a resource-limited developing country with a heavy burden of infectious diseases and pervasive antimicrobial resistance. The implications of the several considerations put forth in this study particularly regarding multidrug-resistant *P. aeruginosa* cumulatively escalate the need for divergent, integrated responses as articulated within this research.

METHODS AND MATERIALS

The study period was six months from January to December 2023. During this time, this cross-sectional study was conducted in a tertiary care hospital in Pakistan. The hospital is a referral center and caters to a wide range of patients which also includes severely ill patients needing intensive care. Clinical specimens such as blood, urine, sputum, wound swabs, and other body fluids were obtained from patients with clinically suspected infections of *Pseudomonas aeruginosa*. These samples were collected under aseptic conditions and sent to the microbiology lab for further immediate analysis.

Culturing of specimens was done on specific types of media like MacConkey agar and cetrimide agar. The cultures were incubated during a period of 24 to 48 hours at a temperature of 37 degrees celsius.

Preliminary identifications were made after performing Gram staining, oxidase and catalase biochemical tests on the colonies elliciting characteristic morphology. Confirmation of *P. aeruginosa* was carried out through an automated identification system and/or molecular methods put in place as necessary. The antimicrobial resistance testing of *P. aeruginosa* isolates was performed with the application of the Kirby-Bauer disk diffusion method according to the standards set by the Clinical and Laboratory Standards Institute (CLSI). The panel included frequently employed therapeutic antibiotics like beta-lactams, aminoglycosides, fluoroquinolones, and carbapenems. The interpretation was made as susceptible, intermediate, or resistant according to CLSI defined criteria.

I used a specific software to analyze and record the data on the patient demographics, clinical characteristics, and the antimicrobial susceptibility patterns. To summarize the times *P. aeruginosa* infections, occur along with the resistance patterns, descriptive statistics were done. For the relationships among the categorical variables, either Chi square tests or Fisher's exact tests were applied and a p value of less than 0.05 was deemed significant. The study was approved by the institutional ethics committee and all subjects or their guardians gave written informed consent. Confidentiality was preserved in all phases of the study.

The sample size included a total of 502 individuals from which majority, n=304, 60.6%, were males while females comprised 39.4% (n=198). Clinical samples were sourced from various sites with the greatest being blood which contributed 53.4% (n=268). Other tissue and fluid samples accounted for 14.3% (n=72) each. Non-bronchoscopic lung lavage samples accounted for 8.4% (n=42) and pus samples made up 9.6% (n=48). Different hospital wards provided the participants for the sample. The medical ward provided the highest sample proportion at 59.6% (n=299) followed by the surgery ward 18.3% (n=92), pediatrics 13.3% (n=67), integrated team care 7.2% (n=36) and in the intensive care unit, the contribution was 1.6% (n=8). This demonstrates how wide the study population was in regard to the venue of the collection.

Table 1: Demographic Characteristics, Sample Types, and Ward Distribution of Study Population

Variable	Details	Frequency	Percentages
Gender	Male	304	60.6%
	Female	198	39.4%
Samples	Blood	268	53.4%
	Non-bronchoscopic Lung Lavage	42	8.4%
	Tissue	72	14.3%
	Pus	48	9.6%
	Fluid	72	14.3%
Sample extracted from different Ward	Medical	299	59.6%
	Surgery	92	18.3%
	Pediatrics	67	13.3%
	Integrated Team Care	36	7.2%
	Intensive Care Unit	8	1.6%

The antibiotic susceptibility patterns of *Pseudomonas aeruginosa* isolates are summarized in Table 2. Levofloxacin demonstrated a sensitivity rate of 65.9% (n=331), with 33.1% (n=166) of isolates being resistant. Meropenem showed the highest sensitivity among the tested antibiotics, with 90.4% (n=454) of isolates being sensitive, while 8.8% (n=44) were resistant, and 0.8% (n=4) were not applicable. Capreomycin exhibited a nearly equal distribution of sensitivity and resistance, with 51.2% (n=257) of isolates being sensitive and 48.8% (n=245) resistant. Ceftazidime showed a sensitivity rate of 68.3% (n=343) and a resistance rate of 31.7% (n=159). Co-trimoxazole demonstrated high sensitivity, with 89.0% (n=447) of isolates being sensitive and 11.0% (n=55) resistant. Minocycline exhibited a sensitivity rate of 74.9% (n=376) and a resistance rate of 25.1% (n=126). Piperacillin-Tazobactam was universally not applicable, with 100% (n=502) of isolates falling into this category.

Table 2: Antibiotic Susceptibility and Resistance Patterns of *Pseudomonas aeruginosa* Isolates

Variable	Details	Frequency	Percentage
Levofloxacin	sensitive	331	65.9%
	resistant	166	33.1%
Meropenem	Not Applicable	4	.8%
	sensitive	454	90.4%
	resistant	44	8.8%
Capreomycin	sensitive	257	51.2%
	resistant	245	48.8%
Ceftazidime	sensitive	343	68.3%
	resistant	159	31.7%
Co-trimoxazole	sensitive	447	89.0%
	resistant	55	11.0%
Minocycline	sensitive	376	74.9%
	resistant	126	25.1%
Piperacillin-Tazobactam	Not Applicable	502	100.0%

The gender distribution of *Pseudomonas aeruginosa* isolates across different sample types and ward origins is summarized in Table 3. Among blood samples, 174 were obtained from male patients and 94 from female patients, with a statistically significant difference ($p < 0.001$). Non-bronchoscopic lung lavage (NBL) samples were more frequently collected from females (n=28) than males (n=14). Tissue samples showed a slight male predominance, with 40 samples from males and 32 from females. Pus samples were equally distributed between males and females (n=24 each), while fluid samples were predominantly collected from males (n=52) compared to females (n=20).

Regarding ward origins, the majority of samples were obtained from the medical ward, with 192 from males and 107 from females, showing statistical significance (p=0.004). In the surgical ward, 49 samples were collected from males and 43 from females. Pediatric ward samples were slightly more frequent among females (n=36) compared to males (n=31). Integrated team care (ITC) samples were predominantly from males (n=24) compared to females (n=12). Lastly,

intensive care unit (ICU) samples were exclusively collected from males (n=8), with no samples from females.

Table 3: Gender Distribution Across Sample Types and Ward Origins

Variable	Details	Male	Female	P-Value
Sample	BLOOD	174	94	<0.001
	NBL	14	28	
	TISSUE	40	32	
	PUS	24	24	
	FLUID	52	20	
Ward Sample	MED	192	107	0.004
	SURG	49	43	
	PEAD	31	36	
	ITC	24	12	
	ICU	8	0	

The gender-based distribution of antibiotic sensitivity and resistance patterns for *Pseudomonas aeruginosa* isolates is summarized in Table 4. Levofloxacin showed a statistically significant difference ($p < 0.001$), with 224 sensitive isolates from males and 112 from females, while resistance was observed in 80 male and 86 female isolates. For Meropenem, 276 sensitive isolates were identified in males and 148 in females, with resistance observed in 24 male and 20 female isolates; however, the difference was not statistically significant ($p = 0.194$). Capreomycin exhibited 162 sensitive isolates in males and 95 in females, while resistance was noted in 142 male and 103 female isolates, with no significant difference ($p = 0.142$). Ceftazidime sensitivity was observed in 215 male and 128 female isolates, while resistance was noted in 89 male and 70 female isolates, with a p-value of 0.092, indicating no significant difference. Co-trimoxazole demonstrated a statistically significant difference ($p < 0.001$), with 285 sensitive isolates in males and 162 in females, while resistance was observed in 19 male and 36 female isolates. Minocycline also showed a significant difference ($p < 0.001$), with 245 sensitive isolates in males and 131 in females, while resistance was observed in 59 male and 67 female isolates.

Table 4: Gender-Based Distribution of Antibiotic Sensitivity and Resistance Patterns

Variable	Details	Male	Female	P-Value
Levofloxacin	Sensitive	224	112	<0.001
	Resistant	80	86	
Meropenem	Not Applicable	4	0	0.194
	Sensitive	276	148	
	Resistant	24	20	
Capreomycin	Sensitive	162	95	0.142
	Resistant	142	103	
Ceftazidime	Sensitive	215	128	0.092
	Resistant	89	70	
Co-trimoxazole	Sensitive	285	162	<0.001
	Resistant	19	36	
Minocycline	Sensitive	245	131	<0.001
	Resistant	59	67	

DISCUSSION

Findings from this study shed light on the epidemiology, antibiotic resistance patterns, and sex stratification of *Pseudomonas aeruginosa* isolates in one of the tertiary care hospitals in Pakistan. The results demonstrate some critical patterns of antimicrobial resistance and sample collection, distribution, and gender which are important for clinical as well as public health actions.

There is a male predominance in the study population (60.6%) which is in line with other studies documenting greater infections among men due to biological and sociological reasons [8]. Blood samples comprised the greatest proportion (53.4%) of the specimens collected which reflects the high rate of *P. aeruginosa* bloodstream infections among hospitalized patients [9]. In calculating the sample collection, it was noted that a large proportion of the sample came from the medical ward (59.6%), indicating the burden of chronic infection among the longer-term hospitalized patients.

The effect of antimicrobial therapies on *P. aeruginosa* sheds light on the astonishing magnitude of bacterial resistance. As other studies have reported, that carbapenems were the primary effective agents toward *P. aeruginosa*, noting Meropenem's primary effective sensitivity of 90.4%. However, this steaps toward concerning territory as well because 8.8% resistance figure indicates the emergence of carbapenem resistant strains which pose a severe clinical burden and limited treatment options [10, 11.] Levofloxacin has moderate sensitivity (65.9%) coupled with a resistance rate of 33.1%, which underlines the growing problem of fluoroquinolone resistance and the need for careful use of this class of drugs [12]. The nearly 1:1 ratio of sensitive to resistant strains to Capreomycin (51.2% vs. 48.8%) raises the question of its declining efficacy, therapeutic strategies must be altered.

The study demonstrated clear gender-based differences regarding susceptibility to microorganisms. For example, males had greater sensitivity to Levofloxacin 224 vs 112 and Co-trimoxazole 285 vs 162, and the differences were significant $p < 0.001$. These results support studies that have proposed the difference in susceptibility to antimicrobial agents is, at least in part, governed by the hormonal and immunologic factors of a person's sex [13]. However, the greater resistance observed in females of the older age group for some antibiotics, such as Minocycline 67 vs 59, does require some explanation in the context of healthcare access and the use of these antibiotics.

The findings captured in this study regarding the susceptibility of Co-trimoxazole 89.0% and Minocycline 74.9% are encouraging, though greater scrutiny still must be applied to *P. aeruginosa* infections. The resistance rates of 11.0% associated with Co-trimoxazole and 25.1% for Minocycline do underline the importance of ongoing vigilance in order to tell when resistance becomes a greater risk [14]. The not applicable 100% of isolates for Piperacillin-Tazobactam suggest limited use of the antibiotic in the study's context, likely owing to cost-effectiveness or availability of the drug.

The patterns of Gender samples and ward origins revealed clear distributions. Blood samples were primarily taken from men (174) compared to women (94) which has a statistically significant difference ($p < 0.001$). Likewise, the most ancillary staff sample from the medical ward was males (192) and females (107), $p = 0.004$. These results are probably driven by differences in help-seeking behavior and disease burden along with healthcare access, as has been documented previously (Johnson et al., 2016). The exclusive male ICU samples (n=8) provide insight on the need to study the more complex severe infection gender-specific determinants [15].

Public health and clinical practice is influenced greatly by these findings. Additionally, the high relative burden along with multi-drug resistant tuberculosis,

dominantly *P. aeruginosa*, within this region highlights the immediate necessity for healthcare restrain policies geared toward controlled antibiotic utilization within the region to prevent super-infection spread of resistant strains. Lack of control measures along with imposed heavily biased unidirectional excessive focused construct control measures result in significant negative gender differences to measures and greatly difficult to treat controlled measures placed using infection reaction response faculties.

CONCLUSION

In conclusion, the study highlights the alarming situation and extensive ramifications of *P. aeruginosa* infections and antimicrobial resistance in tertiary care hospitals in Pakistan. Urgently, the multifaceted *P. aeruginosa* issues are calling for precise strategies to be developed which could include, but are not limited to, the refinement of the antimicrobial control policy, age and gender specific policy where attention is directed towards elderly females, and infection control policy revision towards enhanced focus on infection control. Tackling these healthcare challenges would allow the system to lessen the burden *P. aeruginosa* infections impose on healthcare resources and improve service outcomes for patients.

ETHICS APPROVAL: The ERC gave ethical review approval.

CONSENT TO PARTICIPATE: written and verbal consent was taken from subjects and next of kin.

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AUTHORS' CONTRIBUTIONS:

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated in the work to take public responsibility of this manuscript. All authors read and approved the final manuscript.

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