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Evaluation of Antibiotic Susceptibility and Multidrug Resistance in Clinical Isolates of Pseudomonas Aeruginosa

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Abstract

Pseudomonas aeruginosa has been designated as a species of "greatest public health concern" by the World Health Organization (WHO). While P. aeruginosa seldom causes serious infections in healthy people, it is linked to a number of invasive illnesses in patients who have been hospitalized for an extended period of time. patients with ventilator-associated pneumonia, those receiving cancer chemotherapy or wide spectrum antibiotic therapy, and critically ill intubated patients (World Health Organization, 2017). The objective of the

study was to identify and molecularly describe resistance genes in pseudomonas aeruginosa from body blood, pus patients. Twenty (20) samples were gathered for this investigation between December 2024 and march 2025 from tertiary care facilities in Bannu, KPK, Pakistan. Twelve (12) (60%) of the samples tested positive for pseudomonas aeruginosa. Isolated strains of P. aeruginosa showed complete resistance to Colistin (100%) whereas, the zone of inhibition for Amikacin was measured as 5.8mm, Vancomycin 5.5mm, Fluoroquinolones 5.6mm, imipenem (3.3mm) tobramycin 6.5mm, Gentamicin 6.0mm, tazobactam(6.9mm), linezolid (7.5 mm), Cafdnir (6.8mm) Although the majority of MDR p. aeruginosa resistance occurs to all classes of antibiotics, the study's goal is to determine which class of antibiotic is best for treating MDR p. aeruginosa.

Keywords: Pseudomonas aeruginosa, Multidrug-resistant (MDR), Gram-negative bacteria, Antibiotic resistance

Introduction

Pseudomonas aeruginosa has been designated as a species of "greatest public health concern" by the World Health Organization (WHO). While P. aeruginosa seldom causes serious infections in healthy people, it is linked to a number of invasive illnesses in patients who have been hospitalized for an extended period of time. patients with ventilator-associated pneumonia, those receiving cancer chemotherapy or wide spectrum antibiotic therapy, and critically ill intubated patients (World Health Organization, 2017). In many parts of the world, P. aeruginosa resistance to antipseudomonal medications has been rising over time. The incidence of P. aeruginosa infections within a few days of starting antimicrobial therapy is greatly influenced by antibiotic resistance in the intensive care unit (ICU). Additionally, known risk factors for P. aeruginosa infection include co-infection with other microorganisms prior to isolation, total parenteral nutrition usage, comorbid cerebrovascular disease, history of cerebrovascular disease, ICU admission, cancer, compromised immune system, and obstruction coronary disease (Alhussain et al, 2019). Additionally linked include mechanical ventilation, severe respiratory failure, infection sites in the central venous catheter and respiratory tract, intubation, and the use of several invasive devices. P. aeruginosa, however, possesses a variety of resistance mechanisms. Antimicrobial therapy is therefore restricted (Kollef et al, 2014).

Material and Method

Sample Collection

Patients with wound, burn, and pus infections were the subjects of sample collection. samples were taken from patients admitted to the District Hospital in Bannu, Khyber

Pakhtunkhwa, Pakistan's Intensive Care Unit (ICU), medical ward, Outpatient Department (OPD), and Operation Theatre (OT) under aseptic settings. A thorough foundation for ensuing microbiological analysis and antibiotic susceptibility testing was provided by this varied sampling method, which guaranteed representation across various hospital units and infection types.

Incubation

Samples were inoculated on nutrient brat. Citramide agar plates, incubated at 37c for 24 hours, and examined for morphology and microscopically.

Gram Staining

The microbial isolate ware colonies were spread on a glass slide, smeared, fixed with heat and flame, and then coated with crystal violet ware for 60 seconds before washing with tap water.

Gram's Iodine was applied to a smear for 60 seconds, followed by decolonization with ethanol or acetone for another 60 seconds, and then washed with tap water. The process involves adding safranin for 45 seconds and then washing it with tap water.

The bacteria were observed under a microscope at 400x and 1,000x oil immersion, with purple colonies indicating gram positive bacteria, and red or pink colonies indicating gram negative bacteria.

Phenotypic Identification

Pseudomonas aeruginosa was phenotypically identified using its distinctive colony morphology and specific growth patterns. Colonies looked paler and less defined after the initial inoculation, which was consistent with the organism's usual early growth stage. After additional confirmation they grow on Citramide agar, producing yellow color.

Antibiotic Susceptibility

Pseudomonas aeruginosa isolates were tested for antibiotic susceptibility using the Kirby-Bauer disk diffusion method in accordance with CLSI standards. Commercial antibiotic discs containing Amikacin, Tobramycin, Vancomycin, Imipenem, Fluoroquinolones, Colistin, Tazobactam, Linezolid, and Cefdinir were aseptically applied to the agar surface after pure cultures were inoculated onto Mueller–Hinton agar plates to produce a homogenous bacterial lawn. The plates ware incubated at 37°C for 24 hours. Then put antibiotic to diffuse into the medium and exert their inhibitory effects. After the incubation the diameter of the zone of inhibition of each disc ware measure in millimeters and interpreted as susceptible or resistance according to CLSI method.

Results

The study investigates the prevalence of pseudomonas aeruginosa in tertiary care hospital in District Bannu, Khyber Pakhtunkhwa during the period of December 2024 to

march 2025. Total 20 samples were collected from the patient across the different hospital wards including ICU, OPD and OT from different infections site such as burns, wound and pus. Out of these 20 samples 12 samples ware showing positive results for pseudomonas aeruginosa indicate a notable presence of this opportunistic pathogen within the hospital environment. Our finding highlight the clinical importance of pseudomonas aeruginosa in hospitalized patients

(shown in Table.1)

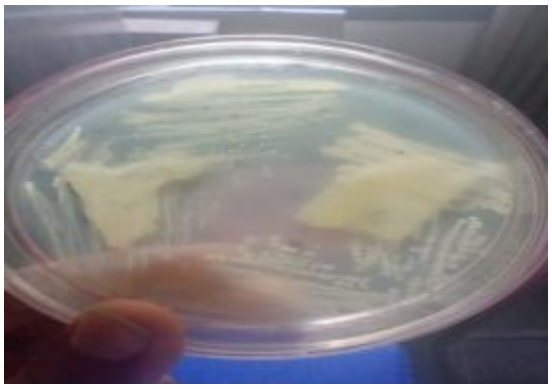
Table 1:

	Origin of sample	Nature of a samples	Number of samples	Total No of *P. aeruginosa* isolates
1	OPD	Pus	8	7
2	OT	Pus	9	4
3	ICU	Blood	3	1

Identification of Isolation

Pseudomonas aeruginosa was phenotypically identified using its distinctive colony morphology and specific growth patterns. Colonies looked paler and less defined after the initial inoculation, which was consistent with the organism's usual early growth stage. After additional confirmation they grow on Citramide agar, producing yellow color

(show in the Fig 1)



Gram's Staining

After carrying out standard Gram's staining technique the bacteria were observed under a microscope at 40x and 100x oil immersion, where p. aeruginosa colonies were observed as pink as shown in (Table 2).

Table 2: *Gram staining of P. aeruginosa*

S#	Total No of isolates	Grams Negative
1	12	12

Antibiotic Susceptibility

Alarming resistance patterns were found in the antimicrobial susceptibility tests of isolated cultures of Pseudomonas aeruginosa, with total resistance against Colistin

(100%) highlighting the isolates' multidrug resistance. Amikacin (5.8 mm), Vancomycin (5.5 mm), Fluoroquinolones (5.6 mm), Imipenem (3.3 mm), Tobramycin (6.5 mm), Gentamicin (6.0 mm), Tazobactam (6.9 mm), Linezolid (7.5 mm), and Cafdnir (6.8 mm) as (shown in table 3 & Fig 2&3). All had significantly smaller measured zones of inhibition, suggesting poor efficacy. The restricted therapeutic options against these strains are highlighted by the fact that these values are much below the standard interpretative criteria for susceptibility. The results highlight the critical need for ongoing monitoring of resistance patterns, prudent antibiotic usage, and investigation of alternate treatment approaches to counter the escalating threat of multidrug-resistant P. aeruginosa.

Table 3: *Antimicrobial resistance of isolated strains against selected antibiotics*

Antibiotic tested	Zone inhibition measured (mm)	Interpretation (Tentative) as per CSLI guidelines
Amikacin (AK) (30 µg)	5.8mm	**Resistant**
Tobramycin (TOB)(10 µg)	6.5mm	**Resistant**
Vancomycin (VA)(30 µg)	5.5mm	**Resistant**
Imipenem (IMP)(10 µg)	3.3mm	**Resistant**
Fluoroquinolones (F)(300 µg)	5.6 mm	**Resistant**
Colistin (CT)(10 µg)	0 mm	**Completely Resistant**
Tazobactam(TPZ)(110 µg)	6.9mm	**Resistant**
Linezolid(LNZ)(30 µg)	7.5 mm	**Resistant**
Cafdnir(CES) (105 µg)	6.8mm	**Resistant**

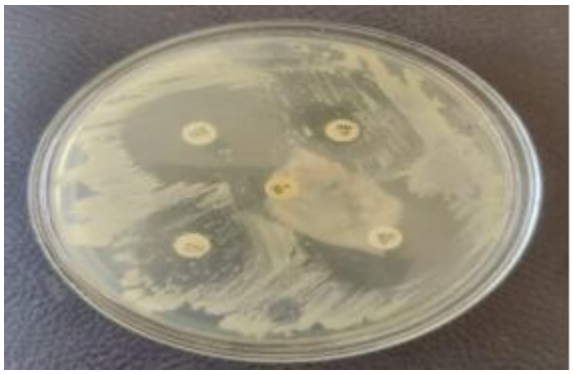


Figure.2

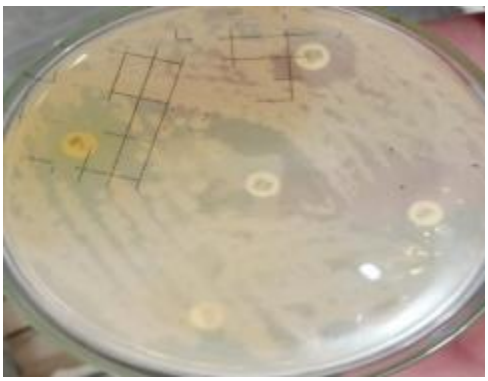
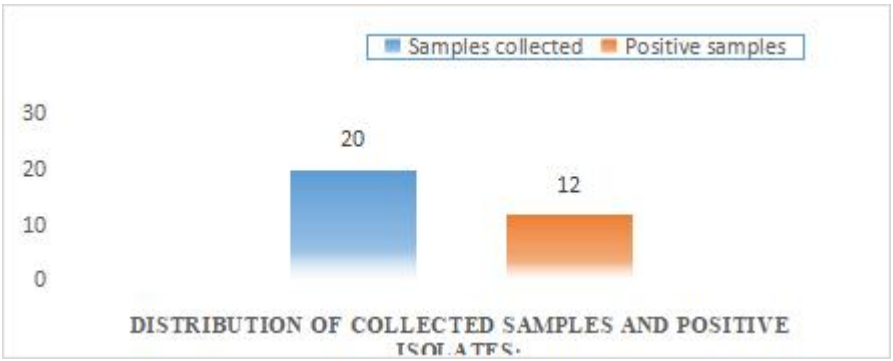


Figure.3

Distribution of Collected and Positive Isolates



Ward Wise Distribution

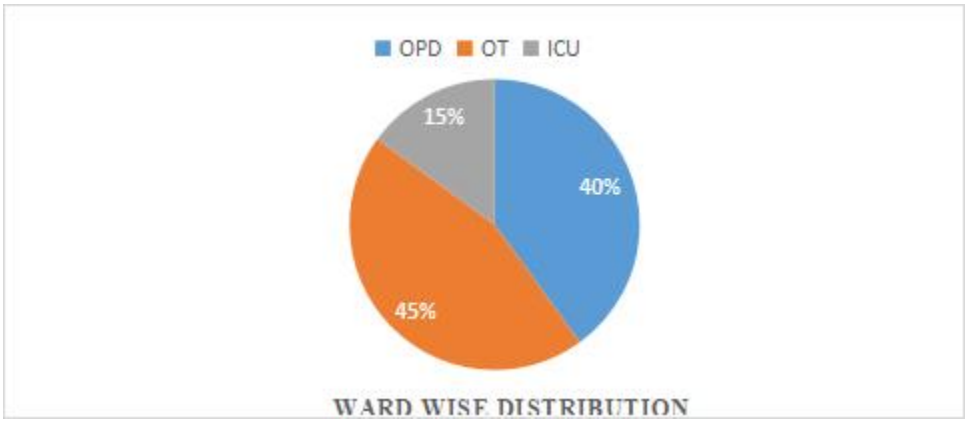


Figure. 1: Antimicrobial resistance of the isolated strains

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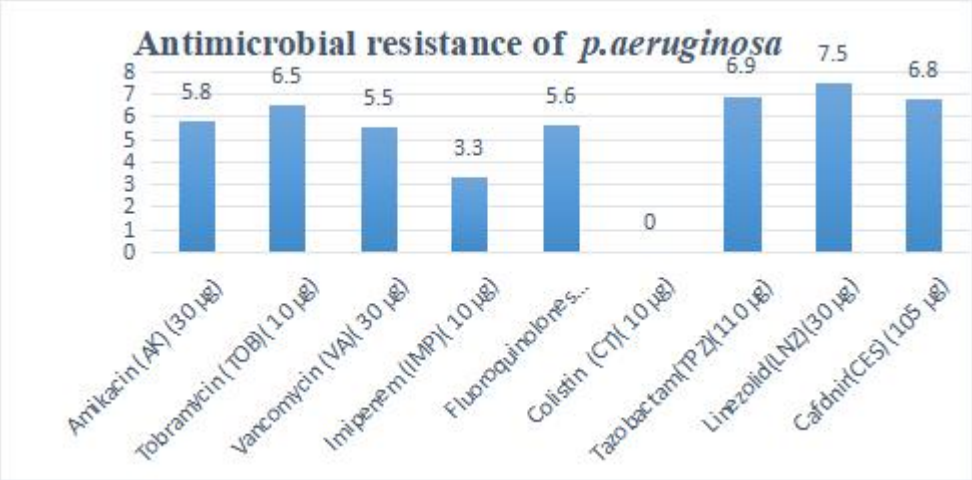

Discussion

Pseudomonas aeruginosa is an opportunistic pathogen. Its infections in hospitals mainly affect the patients in intensive care units and those having catheterization, burn, and/or chronic illnesses (Yetkin et al., 2006). The ability of P. aeruginosa to survive on minimal nutritional requirements and to tolerate a variety of physical conditions has allowed this organism to persist in both community and hospital settings (Lister et al., 2009). Though, its infects healthy tissues rarely but when defenses are compromised. This explains why most infections are nosocomial (Mesaros et al., 2007). P. aeruginosa characterized by inherent resistances to a wide variety of antimicrobials. Its intrinsic resistance to many antimicrobial agents and its ability to develop multidrug resistance and mutational acquired resistance to antibiotics through chromosomal mutations imposes a serious therapeutic problem (Gales et al., 2001; Gorgani, 2009; Al-Grawi, 2011). A number of antimicrobial agents, including several Beta–lactams are active against P. aeruginosa. Extended-spectrum Penicillin, often used to treat infections caused by this bacterium.

The study investigated the prevalence of P. aeruginosa strain in tertiary care hospitals in Bannu, KPK, Pakistan. The important of p. aeruginosa, especially in infected patient, is related to their insufficient immune response as well. Acquisition of mobile genetic element has increase the existence of both intrinsic resistance and acquired resistance among these p.aeruginosa make them more challenging. Based on different study, it is evident that the emergence of resistance p. aeruginosa strain is increasing worldwide.

Although most Cephalosporins are not active against P. aeruginosa, Ceftazidime, a third generation agent, and Cefepime, a fourth-generation agent, have excellent and about equivalent activity. Of Carbapenems, a class of broad-spectrum β-lactam antibiotics, Meropenem has slightly greater activity against P. aeruginosa (Ayalew et al.,

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2003; Shah and Narang, 2005; Baldwin et al., 2008). Of the fluoroquinolones agents, Ciprofloxacin is the most active against P. aeruginosa. Finally, the Aminoglycosides have been mainstays in the treatment of these infections (Hauser and Sriram, 2005; Katzung et al., 2009). However, P. aeruginosa adaptive ability causes difficulties for the sensitivity of microbial identification methods and it has become necessary to develop genotype-based characterization systems capable of accurately identifying these bacteria despite any phenoltypic modifications. So, molecular identification eliminates the problem of variable phenotype and allows for more accurate identification of bacteria (Drancourt et al., 2000).

16S rDNA genes are highly conserved among all organisms and they possess various unique species specific regions that allow for bacterial identification. Polymerase chain reaction (PCR) is highly sensitive, specific and rapid method which vastly improved the detection of P. aeruginosa especially when using species-specific primer for 16SrDNA (Spilker et al., 2004).

A study conducted in Iraq in which 25 isolates were identified as P. aeruginosa using Vitek 2 system. The correct identification rates of P. aeruginosa using this automated technique were 90.7%. Results from other investigators indicate that the Vitek ID-GNB cards correctly identified 85.3 to 100% of P. aeruginosa strains (Funke et al., 1998; Jossart and Courcol, 1999). Joyanes et al., tested 146 routinely isolated strains with the Vitek 2 system and ID-GNB cards and found correct identification rates of 91.6% (Joyanes et al., 2001). Using the same vitek identification card, Ines et al. (2009) found that correct identification rates of P. aeruginosa were 90.1%.

A total of 20 clinical samples were obtained from individuals of varying age groups and both genders. Out of these, 12 samples (60%) tested positive for P. aeruginosa, indicating a significant prevalence of this opportunistic pathogen in wound and burn infections within the studied population. Which are much similar to from an earlier report F.ullah, S. A. Malik, et al.(44 in thesis). That the p. aeruginosa rate was 69.5% for p. aeruginosa isolate the clinical specimens of p.aeruginosa obtain from a hospital in Karachi Pakistan. Our finding are similar to a study conducted in in Iraq by (Hassan et al., 2012; Manhal, 2006; Rauf, 2003; Miteb, 2006; Al-Derzi, 2012) [20] that showed that presence of P. aeruginosa among burn wound patient (44.4%) whereas in our study the it was recorded as 0f P. aeruginosa is 60%

During the present study all of the isolated strains showed resistance to Amikacin, vancomycin, Ampicillin, tobramycin, Gentamicin, Fluoroquinolones, Tazobactam, Linezolid, Cafdnir, 58%, 55%, 33%,65%, 60%, 56%, 69%, 75%, 68% respectively. But in a study by Jazani et al. (2010) in Iran demonstrated that gentamycin was slightly higher

than ours study results. Similarly, Gentamicin 73%, Ceftazidime 83%, Tobramycin 84%, Amikacin 40%, Imipenem 27%, Ciprofloxacin 65% and 75.4% of isolates were resistant to Cefepime respectively. In a retrospective study was carried out of Gram-negative isolate in Saudi Arabia, they found that P. aeruginosa susceptibility significantly declined after 2007, especially for carbapenem (66% in 2004 to 26% in 2009), ceftazidime (69% in 2004 to 44% in 2009) and ciprofloxacin (67% to 49%) (Al-Johani et al., 2010).

Conclusion

In conclusion, this study not only confirms the prevalence of P. aeruginosa but also underscores its high level of resistance to multiple antibiotics. These results contribute to the growing evidence that multidrug-resistant P. aeruginosa poses a major public health concern.

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