Original Article

Antibiotic Resistance Patterns of *Pseudomonas aeruginosa* Bacterial Species Isolated from Various Clinical Samples

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ABSTRACT

Pseudomonas aeruginosa infections have become a real burden in healthcare settings, contributing substantially to nosocomial infections. The emergence of several drug-resistant strains was the major issue causing massive mortality. Multiple-drug resistant Pseudomonas aeruginosa isolates containing beta-lactamase were becoming more prevalent. **Objective:** To investigate and characterize the antibiotic resistance patterns of *Pseudomonas aeruginosa* isolated from different clinical samples. **Methods:** A total of 618 different clinical samples including blood, pus and urine samples were collected from the patients visiting Indus Medical College Hospital Tando Muhammad Khan, Sindh, Pakistan. All the clinical samples were processed for the isolation of bacterial species using specific culture media. The identification of *Pseudomonas aeruginosa* strains was achieved based on Gram's staining and certain biochemical tests including Cetrimide test. Moreover, antibiotic susceptibility testing was determined by Kirby-Bauer disc diffusion method. **Results:** A total of 452 bacterial strains were isolated and pure cultured from different clinical samples. Among these, 60.62% were Gram-negative and 25% of the total isolates were found to be *Pseudomonas aeruginosa* strains. Antibiotic sensitivity testing results revealed the highest resistance ratio of *Pseudomonas aeruginosa* strains against Ticarcillin (46.02%), while Colistin showed the lowest resistance (3.54%). Overall, 14.15% of the isolates of *Pseudomonas aeruginosa* strains were found to be multi-drug-resistant bacteria. **Conclusions:** The results of the present study reveal an increased ratio of antibiotic resistance particularly of Ticarcillin, piperacillin and imipenem in *Pseudomonas aeruginosa* isolated from various clinical samples.

INTRODUCTION

Pseudomonas aeruginosa are classified under the group of Gram-negative bacteria and are characterized with rod-shaped structure, aerobic in nature, motile and non-lactose fermenting bacteria [1]. The mucoid strains of *Pseudomonas aeruginosa* are encapsulated in a mucoid slime layer and produce extracellular polysaccharides that are primarily alginate polymers [2]. These bacteria are found in diverse environments including water, soil, plants and humans [3]. *Pseudomonas aeruginosa* is a versatile and opportunistic pathogen, capable of causing several types of infections, including cystic fibrosis, septicemia, and urinary tract infections especially in immunocompromised patients [4]. Mainly, Quorum Sensing (QS) system also known as communication system has been identified as a key factor in pathogenesis of *Pseudomonas aeruginosa* infections particularly in clinical isolates [5]. The characteristics of *Pseudomonas* species to grow at very low nutrient requirements and ability to withstand a vast array of physical parameters empowered these bacteria to prevail in different environments. Mainly, long-term infections caused by *Pseudomonas aeruginosa* have been associated with substantial mortality and morbidity rates. Irrespective of providing hygiene facilities and broad-spectrum antibiotics, the infections caused by

Pseudomonas species are yet uncontrolled [6, 7]. Several *Pseudomonas aeruginosa* bacterial strains are reported to show resistance against a broad group of antibiotics [7]. With the widespread use of 3rd generation cephalosporins by the health practitioners, several isolates of *Pseudomonas aeruginosa* have become resistant to this class too[8].

Antibiotic resistance in *Pseudomonas aeruginosa* is mediated by the production of specific genes, genetic mutations and biofilm formation [4, 9]. These characteristics collectively contribute to the ability of this bacterium to evade antibiotic therapy. Additionally, overuse of antibiotics enhances the development of Multidrug-Resistant (MDR) *Pseudomonas aeruginosa* strains leading to inadequate treatment and failure to combat this pathogen[10]. The elevated resistance ratio in *Pseudomonas aeruginosa* strains against a wide range of antibiotics poses a considerable threat causing community acquired and nosocomial infections [11, 12]. The studies on antibiotic sensitivity testing and occurrence ratio of *Pseudomonas* species in different regions world-wide would play a pivotal role in preventing such types of infections.

In view of these facts, the present study was carried out to investigate antibiotic resistance patterns of *Pseudomonas aeruginosa* isolated from various clinical sample at Indus medical college hospital, Tando Muhammad Khan (TMK), Sindh, Pakistan.

METHODS

This cross-sectional prospective study was carried out from January 2022 to December 2022 at the Research Laboratory, Institute of Microbiology, University of Sindh, Jamshoro, Pakistan. The study was approved by the Advanced Studies and Research Board (ASRB) of the University vide letter no ORIC/SU/872. A total of 618 patients suspected with bacterial infections visiting OPD at Indus medical college hospital, TMK, were registered for this study. This study only included patients who had not received antibiotic therapy, while those on antibiotic therapy were excluded. Notably, prior to collecting clinical samples, consent was obtained from all patients. Among the pathogenic bacteria, *Pseudomonas aeruginosa* was renowned for its extensive infectivity range and elevated resistance ratio against various antibiotics. Despite the significance of this issue, very limited studies have been conducted in TMK district, Sindh, Pakistan. In this regard, the present study underscores the necessity of investigating antibiotic resistance profiles of *Pseudomonas aeruginosa*, including the emergence of multi-drug-resistant strains, to suggest more effective treatment strategies in this region. Primarily, the clinical samples consisting of blood, pus and urine were brought and processed within three hours of the collection time at the research lab, Institute of Microbiology, University of

Sindh, Pakistan. All the chemical reagents, culture media and antibiotic discs utilized in this study were procured from Oxoid Ltd. The isolation of bacteria was achieved using multiple culture media, including nutrient agar, CLED, MacConkey's agar, and cetrimide agar. The isolated bacterial strains were further sub-cultured using four-way culturing method in order to obtain pure cultures. The identification of the pure cultured strains particularly for *Pseudomonas aeruginosa* bacterial strains was carried out primarily based colony morphology and Gram's staining reaction followed by different biochemical tests including oxidase, indole, citrate and production of pigments [13]. Further, *Pseudomonas aeruginosa* strains were confirmed by cetrimide test. Antibiotic resistance testing was conducted using Kirby-Bauer disk diffusion method according to CLSI guidelines [14]. Antibiotic discs applied in this were listed as Piperacillin (100µg), Ticarcillin (TIC) (75µg), Piperacillin/Tazobactam (100/10µg), Ticarcillin/ Clavulanic acid (85µg), Cefepime (30µg), Ceftazidime (30µg), Imipenem (10µg). Gentamicin (10µg), Amikacin (30µg), Ciprofloxacin (5µg), Levofloxacin (30µg), Ofloxacin (5µg) and Colistin (5µg).
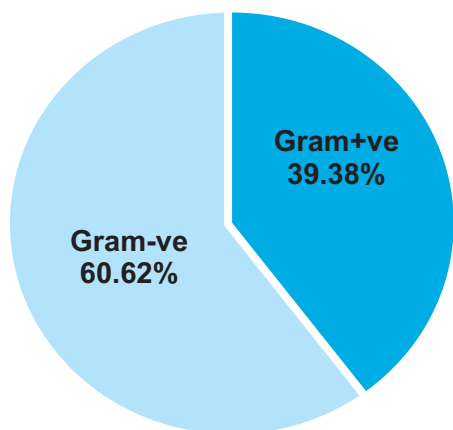
RESULTS

A total of 618 different clinical samples were collected from the patients registered under this study. Out of 618 clinical samples, 32.45% were blood, 38.33% pus samples and 29.22% were urine samples (Figure 1).

Distribution of Clinical Sample
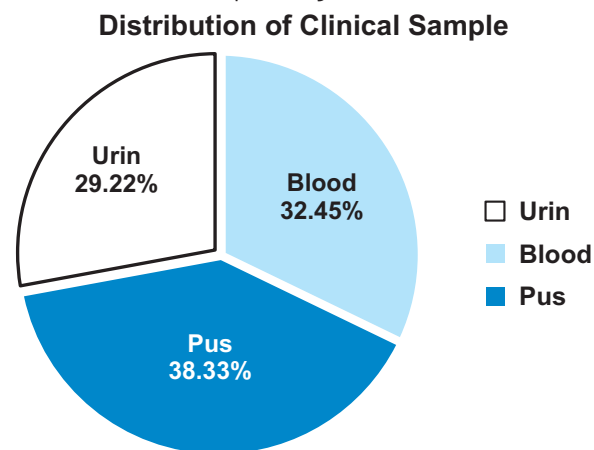
Figure 1: Distribution of Clinical Samples

Isolation frequency of the bacterial growth revealed 73.14% of the samples positive for growth with single and mixed colonies, while 26.86% of the clinical samples showed no growth. To avoid duplicates, only bacterial colonies with different colony morphologies were selected for further analysis. Gram's staining results showed 60.62% as Gram-negative and 39.38% Gram-positive bacterial species (Figure 2).

Figure 2: Results of Gram's Staining

Among the Gram-negative bacteria, 41.25 % (n=113) were identified as *Pseudomonas aeruginosa* strains, while 58.75% were other types of Gram-negative bacteria. In general, this study reveals the isolation and identification of 113 clinical isolates of *Pseudomonas aeruginosa* from various clinical samples collected from the patients suspected with bacterial infections. The isolation frequency of *Pseudomonas aeruginosa* was found highest in pus samples followed by blood and urine samples, respectively. All the *Pseudomonas aeruginosa* strains showed excellent growth on cetrimide agar medium with production of different pigments (Figure 3).
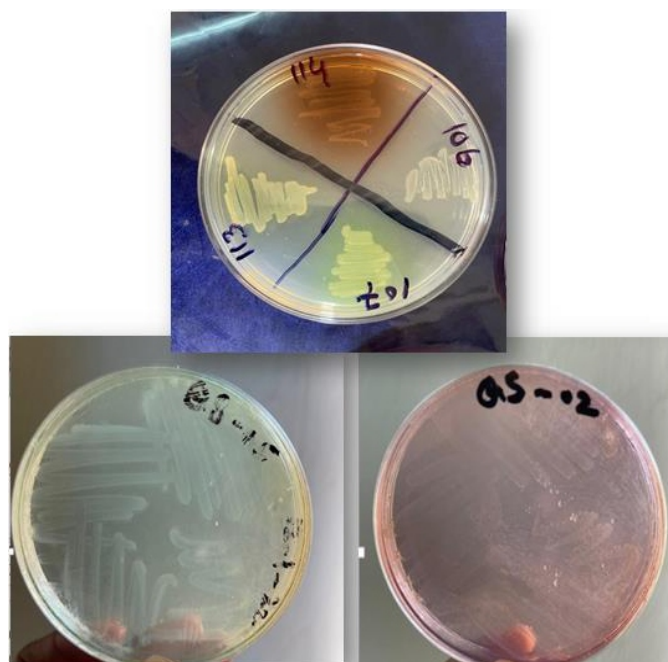
Figure 3: Pure Cultures of *Pseudomonas aeruginosa*

Biochemical identification of *Pseudomonas aeruginosa* strains revealed positive for oxidase, catalase, and citrate utilization test, while negative for indole and H2S (Figure 4). Moreover, all the *Pseudomonas aeruginosa* strains showed motility in both wet mount and SIM test.
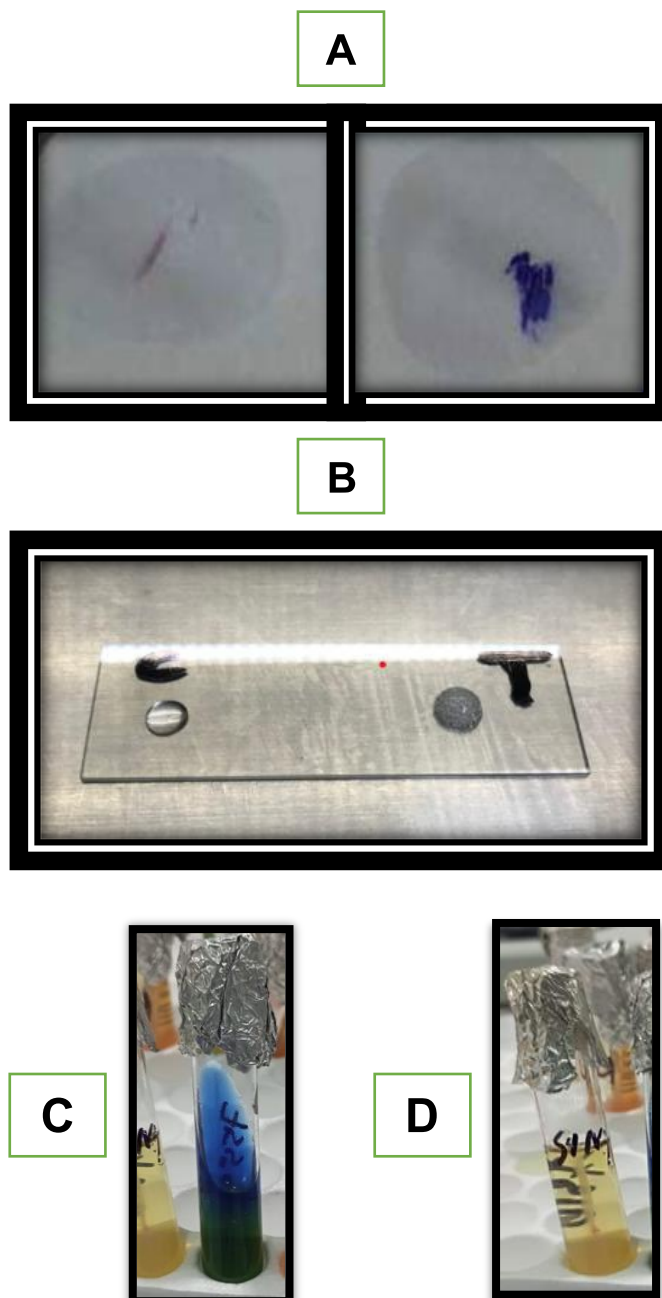
Figure 4: Biochemical Testing Results, Where "A" Shows Oxidase Test, "B" Catalase Test, "C" Citrate Utilization Test, and "D" Indicates SIM Test.

The antimicrobial resistance patterns of *Pseudomonas aeruginosa* isolates from clinical samples revealed the highest resistance ratio against Ticarcillin (46.02%), followed by Imipenem (39.83%) and Piperacillin (34.52%). The lowest resistance ratio was observed against Colistin (3.54%) followed by piperacillin/Tazobactam (13.28%), and Tic/Clavulanic acid (18.59%). *Pseudomonas aeruginosa* strains also exhibited resistance to various other antibiotics, including Ciprofloxacin (31.86%), Ofloxacin (30.09%), Gentamicin (23.99%), and Ceftazidime (23.01%), Amikacin (22.13%), Levofloxacin (20.36%) and Cefepime

(19.45%) (Table 1). Overall, 14.15% of the *Pseudomonas aeruginosa* isolates were confirmed as MDR exhibited resistance to more than three antibiotics (Table 1).

Table 1: Demographic Details of study Participants

S. No.	Antibiotics	Concen-tration (µg)	Sensitive Zone Diameter/mm	Sensitive (%)	Resist-ance (%)
1	Amikacin (AK)	30 µg	≥14	77.87%	22.13%
2	Gentamicin (CN)	10 µg	≥12	76.10%	23.9%
3	Cefepime (FEP)	30 µg	≥12	80.53%	19.47%
4	Ceftazidime (CAZ)	30 µg	≥16	76.99%	23.01%
5	Ciprofloxacin (CIP)	5 µg	≥25	68.14%	31.86%
6	Levofloxacin (LEV)	30 µg	≥18	79.64%	20.36%
7	Ofloxacin (OFX)	5 µg	≥15	69.91%	30.09%
8	Piperacillin (PRL)	100 µg	≥21	65.48%	34.52%
9	Pip/Tazobactam (TZP)	100/10 µg	≥15	86.72%	13.28%
10	Ticarcillin (TIC)	75 µg	≥17	53.98%	46.02%
11	Ticarcillin Clavulanic acid (TIM)	85 µg	≥15	81.41%	18.59%
12	Imipenem (IMP)	10 µg	≥20	60.17%	39.83%
13	Colistin (CT)	5 µg	≥11	96.46%	3.54%

Disc diffusion method CLSI-M 100

DISCUSSION

Pseudomonas aeruginosa infections have become a real burden for nosocomial infections, especially in immunocompromised and critically ill patients. The emergence of several drug-resistant strains is the major issue causing massive mortality. Multiple-drug resistant *Pseudomonas aeruginosa* isolates containing beta-lactamase were becoming more prevalent. The present study was carried out to investigate antibiotic resistant patterns of *Pseudomonas aeruginosa* strains isolated from various clinical samples including blood, pus and urine samples. Overall, 618 patients suspected with bacterial infections with different age groups were registered in this study who visited OPD at Indus medical college hospital, TMK, Sindh, Pakistan. Antibiotic resistance patterns were observed using Kirby-Bauer disk diffusion method according to CLSI guidelines [14]. A total of thirteen different antibiotics were tested against clinical isolates of *Pseudomonas aeruginosa* strains either individually or in combination of another drug (two-drug combinations). This study has revealed the isolation and identification of 113 clinical isolates of *Pseudomonas aeruginosa* strains. The isolation frequency of *Pseudomonas aeruginosa* was found highest in pus samples as compared to blood and urine samples. Previously, several studies have also recovered the highest ratio of *Pseudomonas aeruginosa* from pus samples [15, 16]. The findings of this study were consistent with results of the earlier studies conducted worldwide. However, some studies have shown the highest prevalence of *Pseudomonas aeruginosa* from sputum and urine samples [3, 17]. The antimicrobial sensitivity testing results of *Pseudomonas aeruginosa* strains showed the highest resistance ratio against Ticarcillin (46.02%),

Imipenem (39.83%) and Piperacillin (34.52%). While the lowest resistance ratio was recorded against Colistin (3.54%). Ticarcillin was found to be the least effective antibiotic against *Pseudomonas aeruginosa*. Previously, Odoi H *et al.*, also revealed same results showing Pseudomonas aeruginosa highly resistant to Ticarcillin [18]. Out of 113 *Pseudomonas aeruginosa* strains, 14.15% showed resistance against several antibiotics and thus revealed as MDR bacterial strains. However, this ratio of MDR was much lower than that reported by Ahmad S *et al.*, who reported 20% of MDR in clinical isolates of *Pseudomonas aeruginosa*. One of the studies has shown the highest resistance patterns to Gentamicin (65.5%) and another study has shown highest resistance ratio against Imipenem [15, 19, 20]. Moreover, a combination of two drugs such as piperacillin / Tazobactam indicated 13.28% resistance by *Pseudomonas aeruginosa* as to alone piperacillin (34.52%). Furthermore, *Pseudomonas aeruginosa* were also found highly sensitive against another combination of two drugs such as Ticarcillin / Clavulanic acid. These results suggest that the combination of two drugs may reduce the resistance ratio and increase the sensitivity among clinical isolates of *Pseudomonas aeruginosa* strains. Qayoom S *et al.*, has also shown the similar results with higher sensitivity of *Pseudomonas aeruginosa* strains against combination of piperacillin / Tazobactam and Ticarcillin / Clavulanic acid. Moreover, Pseudomonas aeruginosa isolates exhibited 23.01% resistance to ceftazidime (3rd generation cephalosporin) and 19.47% to cefepime (4th generation cephalosporin) [15]. Previously, two studies on *Pseudomonas aeruginosa* also recorded similar results against 3rd and 4th generation of cephalosporins [21, 22].

CONCLUSIONS

In conclusion, the present study revealed the resistance ratio exhibited by *Pseudomonas aeruginosa* strains isolated from different clinical samples. The highest resistance ratio was observed against Ticarcillin (46.02%), Imipenem (39.83%) and Piperacillin (34.52%). While the lowest resistance ratio was recorded against Colistin (3.54%). In addition, the combinations of two drugs such as Piperacillin/Tazobactam and Ticarcillin/Clavulanic acid demonstrated efficacy against *Pseudomonas aeruginosa* strains. The findings of the present study will be highly beneficial to identify the drug-resistant patterns in *Pseudomonas aeruginosa* strains in healthcare associated infections and will help the health professionals to suggest the proper antibiotic for the treatment of patients suffering from severe infections caused by *Pseudomonas aeruginosa* bacteria and to prevent the emergence of antibiotic resistance. Early recognition and implementation of infection management measures were critical for preventing the spread of drug-resistant pathogens.

Authors Contribution

Conflicts of Interest

Source of Funding

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