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Antibiotic Resistance and Sensitivity Pattern of *Pseudomonas aeruginosa* Obtained from Clinical Samples

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Abstract: This study aimed to determine the antibiotic susceptibility profile of *Pseudomonas aeruginosa* from fifty different isolates of varied clinical origins. A total of 700 samples of pus, urine, swab, and other samples from various patients were examined. Based on bacterial growth over routine nutrient agar and MacConkey medium, isolates with positive results on both media were chosen. Using the modified disc-diffusion method (Modified-Kirby Baur method), antimicrobial sensitivity of total isolates was operated by following CLSIs guidelines. In the current study, a large number of isolates of *P. aeruginosa* obtained from different specimens are resistant to Cefixime (82%), followed by Ampicillin (79%) and Augmentin (61%). However, the antibiogram of *P. aeruginosa* also showed that most of the isolates (86%) were highly sensitive to Amikacin. The second maximum sensitivity of *P. aeruginosa* was seen towards Tazocin (80%), followed by Tecarcilline (79%). *P. aeruginosa* offers a high risk of antibiotic resistance to a wide range of antibiotics; hence it is necessary to avoid the use of antibiotics to reduce antibiotic resistance. Also, researchers should search for the discovery of certain novel antibiotics that may provide impressive inhibition toward *Pseudomonas aeruginosa*.

Keywords: *Pseudomonas aeruginosa*; antibiotic resistance; antimicrobial agents; antibiotic sensitivity.

1. Introduction

P. aeruginosa is a versatile gram-negative bacillus belonging to the family *Pseudomonaceae* that exists in moist habitat, water, and disinfectant solutions; water immortalizes life when the condition is nutrient deficient and can utilize various organic compounds [1–3]. Different water sources like bottled mineral water, seawater, and river water may have these bacteria [4–6]. The genus *Pseudomonas* comprises above 140 species, most of which are Saprophytic. Above 250 *Pseudomonas* species have a pathogenic affinity for humans [7,8]. *P. aeruginosa* is known for hospital-acquired opportunistic infections [9,10]. In the last century, it gained importance for its pathogenicity in hospitals [11,12]. It is a common human microflora and needs minimal nutrients for its growth and reproduction. This property helps to stay for a long time in hospitals [13–15]. *P. aeruginosa* is a strategic microorganism

responsible for recurrent infections in hospitalized patients, especially burn patients [16,17]. *P. aeruginosa* causes many diseases like a wound, burn and ocular infections, bronchopneumonia, meningitis, and endocarditic [18,19]. It should be isolated from various body fluids like urine, pus, ear swab, eye, blood, and sputum due to its high infection rate in exposed body parts [20,21].

Opportunistic diseases are mostly caused by pathogenic species of *Pseudomonas* [22,23]. The most commonly affected patients by *P. aeruginosa* are those with weaker immune systems like bone marrow graft and neutropenia [24,25]. Overall 16% nosocomial infections are due to *P. aeruginosa* [26], rate of hospital-acquired UTI is 12% [27], surgical wound infections are 8% [28], and rate of bloodstream infections is 10 % [29,30]. The anti-pseudomonal products used for the treatment practices are present in a very narrow range which is an alarming point; this is due to variations in mechanisms of *P. aeruginosa* species used for resistance against antibiotics [31,32]. *P. aeruginosa* is a non-fermentative and dominant bacterial species mostly seen in hospitalized patients' specimens. It is affected by antimicrobial agents, but the resistance rate to these agents causes a demand for more such agents. Microbial drug-resistant is an ominous threat to human health [33,34].

The alarming situation of drug resistance by *P. aeruginosa* corresponds to the high fatality and morbidity rate. It is difficult to fix this problem in medical practice due to its high physiological flexibility [9,35,36]. According to the infectious disease society of America, *P. aeruginosa* is one of the 'ESKAPE' list pathogens which present a terrible threat to public health; this is due to the high prevalence and ineffectual effects of the current antimicrobial agents [37,38]. Unfortunately, the resistance due to microorganisms increases in parallel with the advancement of antibiotics; the same is the case with *P. aeruginosa*, which has different resistant mechanisms [39,40]. Due to antibiotic resistance, pathological importance, and distant habitat of *P. aeruginosa* this study was accomplished to isolate and identify *P. aeruginosa* from various clinical samples, and its susceptibility to antibiotics and resistance profile have been identified.

2. Materials and Methods

Standard and systematic review methods were followed during this work [41]. The research was conducted in the clinical laboratory of Microbiology in Khyber Teaching Hospital (KTH) Peshawar Khyber Pakhtunkhwa (KPK), Pakistan.

2.1. Samples collection.

In this study total of 700 samples were collected from patients hospitalized for more than a week. The samples were collected from different wards of the hospital through the convenience sampling method and further analyzed in the hospital's microbiology laboratory. These bacterial isolates were obtained from swabs, pus, wounds, blood, sputum, urine, and burns samples. The samples were also distributed in different groups according to the patient's age. In group 1st,166 samples were from patients up to 20 years of age; the total isolates of group second were 249 for the age range of 21 to 40 years, 222 samples were in the third group for the age range of 41 and 60 years, and the 63 samples were from patients above 60 years of age.

2.2. Processing.

Each sample was taken carefully from infection sites and transported to the hospital's clinical laboratory in sterilized tubes. Further, nutrient agar plates were streaked with these samples and incubated for 24 hours at 37 °C. Then Gram staining was done for the single suspected colonies. After staining, this was sub-cultured on MacConkey agar [42]. *P. aeruginosa* was isolated in pure form and stored in 1% nutrient agar slant at 4 °C in the refrigerator for further use.

2.3. Confirmation of Pseudomonas aeruginosa.

The differential and selective media's sub-cultured isolates were further subjected to morphological and biochemical identification through motility, Gram staining, catalase, oxidase, urease, Citrate utilization, Triple Sugar Iron (TSI), and Tryptophan hydrolysis (by the breakdown of amino acid tryptophan with the release of indole) [43,44].

2.4. Antibiotic sensitivity test.

We used Antibiotic sensitivity testing (AST) to determine the range of isolates' susceptibility to different therapeutic agents. We used the disk diffusion method for this purpose [45]. The commercially available antibiotic discs were useful for investigating the antibiotic sensitivity of *P. aeruginosa*. The susceptibility of each isolate was determined by using it with different antimicrobial agents. This study used Tazocin, Amikacin, Ticarcillin, Meronym, Tienam, Sulzine, Azactam, Cefotaxime, Cefobide, Ciproxin, Avelox, Tegacil, and Augmentin as antimicrobial agents. Each of these agents was used in the range of 10µg. *P. aeruginosa* ATCC 27853 was taken as a control [46]. For this purpose, the selected bacterial colonies were inoculated into the nutrient broth and kept in an incubator at 37 °C for about 24 hours. After incubation 0.5 McFarland turbidity standard was obtained to get a total count of $1x10^8$ CFU/ml. After being inoculated into sterilized Muller Hinton agar (MHA). The antibiotic discs were placed carefully on agar plates and incubated for 24 hours at 37°C. After the incubation period, clear inhibition zones were observed. A measuring ruler was used for inhibition zone measurement.

2.5. Identification of multidrug-resistant strains.

The *Enterobacteriaceae* CLSI standard chart was used to identify *P. aeruginosa's* resistance to multiple drugs. Different inhibition zones were measured according to the *Enterobacteriaceae* CLSI standard chart [47]. Some of the isolates were resistant to three or more antibiotics; all these isolates are in the range of MDR *Pseudomonas aeruginosa.*

3. Results and Discussion

3.1. Results.

Among the 700 processed clinical samples (322 male and 378 female), 137 (19.57%) isolates were found positive for *P. aeruginosa*, 82 (59.85%) of which were males and 55 (40.14%) of them were females, as shown in Figure 1. The percentages of *P. aeruginosa* isolates collected from various clinical samples are also given in Figure 2, while Table 1 shows the multiple drug resistance among different age groups.

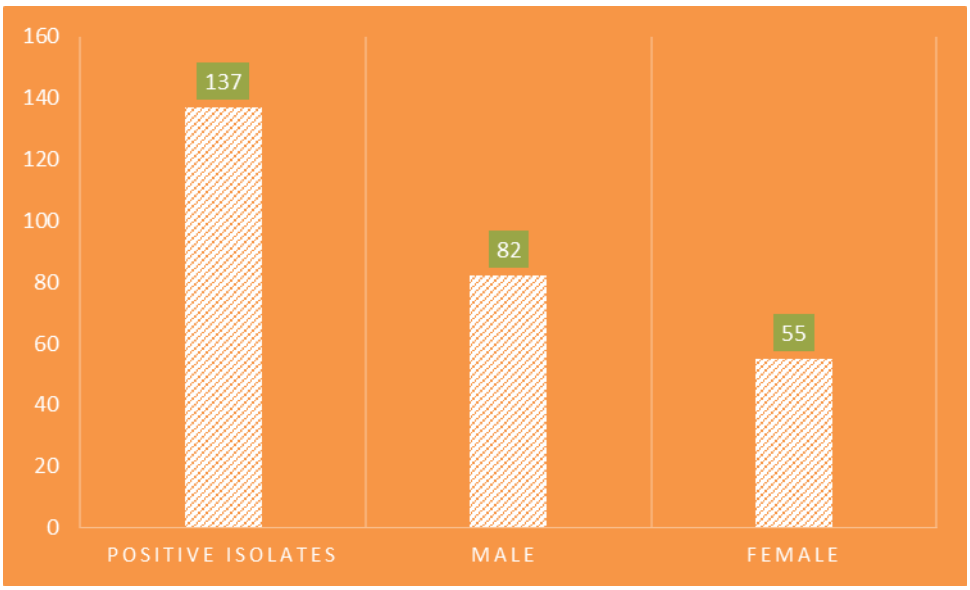

Figure 1. Frequency of the total number of clinical samples and its distribution of positive isolates on a gender basis.

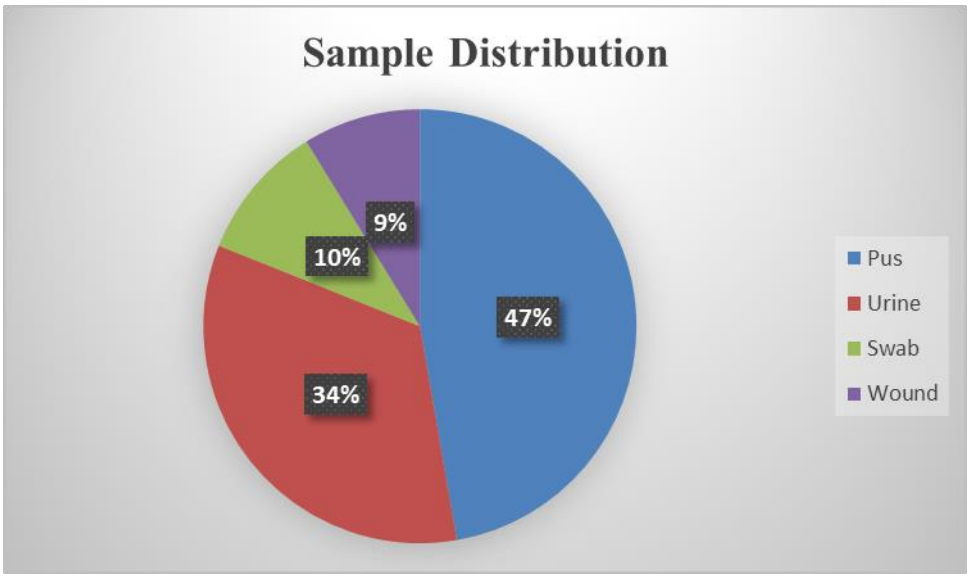

Figure 2. Frequency Percentages of *P. aeruginosa* isolates collected from clinical samples: Among the positive isolates, 64 (47 %) were from pus (the maximum positive isolates), 47 (34%) urine, 14 (10%) swab, and 9 (12%) isolates were from wounds.

Table 1. Multiple drug resistance among different age groups: Out of 137 positive isolates, 31 positive isolates were from group 1st (166 samples), group 2nd (249 samples), 3rd (222 samples), and 4th (63 samples), having 56, 21 and 29 positive isolates respectively. The most positive isolates were found in group second (21-40 years of age), and the third group (41-60 years of age) was found with the least number of positive isolates.

Age group	MDR	Non-MDR	Total
< 20	31	135	166
21-40	56	193	249
41-60	21	201	222
> 61	29	34	63
Total	137	563	700

According to the CLSI standards, the sensitivity status of *P. aeruginosa* for antibiotics showed that Tazocin is the most effective antibiotic for *P. aeruginosa*, with the highest sensitivity of about 88%. Amikacin was the second most sensitive antibiotic for *P. aeruginosa*, with 84% sensitivity, followed by Ticarcillin (79%). Isolates were found to be 78% susceptible

to Meronym, while the observed sensitivity for Tienam, Sulzone, and Azactam was about 76%, 75%, and 70%, respectively. Figure 3 represents the antibiogram of *P. aeruginosa* against some selected antibiotics; from that, the sensitivity of Cefotaxime and Cefobid was found to be 66%, which was somewhat like Ciproxin (62%). Tegacil and Augmentin were in the same range of up 58%. The sensitivity for Cefspan, Cefixime, and Ampicillin was moderate in action, i.e., their sensitivity was found to be 38%, 22%, and 20%, respectively. Similarly, the isolates of *P. aeruginosa* showed the highest resistance to Ampicillin (80%), followed by Cefixime (77%) and Cefspan (61%). Augmentin and Tygacil showed resistance up to 52%, whereas Tazocin had the lowest resistance of about 12%.

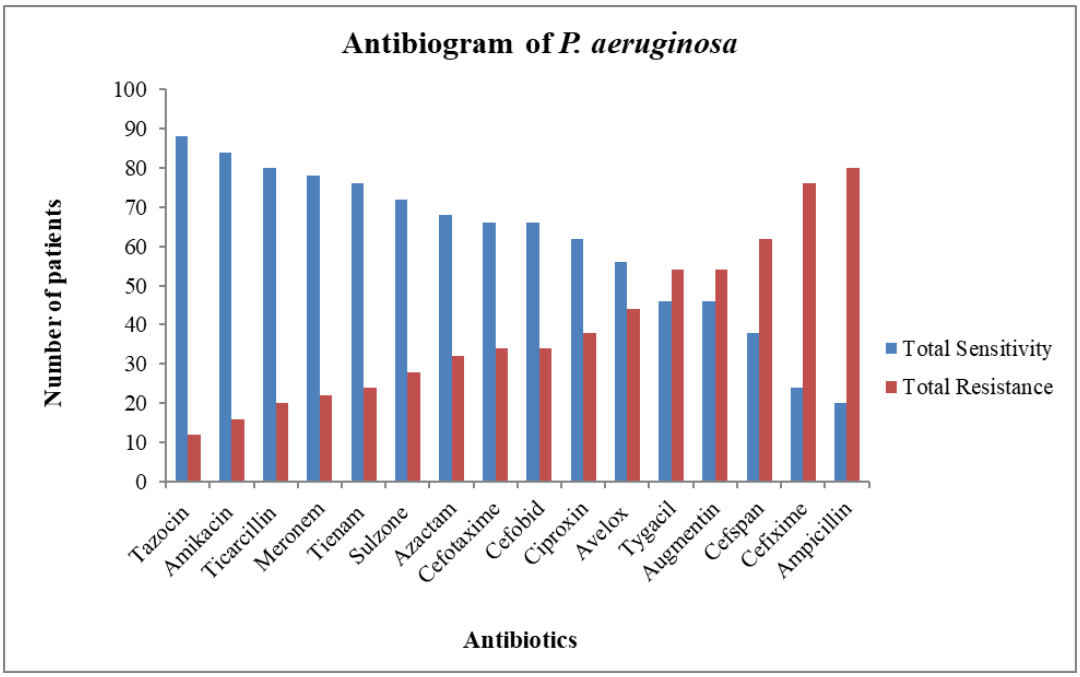

Figure 3. Antibiogram of *P. aeruginosa* against selected antibiotics in 100 positive patients from various samples.

3.2. Discussion.

Due to its pathogenic status and contribution to nosocomial infections, *P. aeruginosa* is the leading cause of mortality and morbidity in hospitalized patients. Study and research about *P. aeruginosa* are essential due to its resistance and low susceptibility to antiseptics and some antibiotics. Another factor is that it has a diverse adaptation to the hospitalized environment and its nutritional requirements are shallow in the presence of moisture. *P. aeruginosa* is the main responsible organism for nosocomial infections. It mainly contributes to an external ear inflammation, urinary tract infection, and sepsis infections. Besides these, it also presents in patients with acute leukemia, grafting, and mucoviscidosis. It causes most infections with a high fatality rate [9,11,48–50]. This study aimed to determine the antibiotic susceptibility profile of *P. aeruginosa* from fifty different isolates of different clinical origins. It was determined that *P. aeruginosa* has different susceptibility patterns for different medicines, which can help correct and better prescription of antibiotics by physicians to different patients with better results [31,43]. Therefore, antibiotic resistance and pattern of sensitivity were performed for *P. aeruginosa* of different clinical isolates, which corresponded with other studies.

This study was performed separately for males and females, which showed that the infection rate in males is more (59.8%) than in females (40.1%). Our present study is following

[51], which shows that in most of the cases, 56 (40.8%) are patients with age ranges from 21 to 40 years [45]. Most of the isolates from *P. aeruginosa* were from pus, 47%, then urine, 34%, while 10% were isolated from the swab and 9% from wounds. Similarly, blood samples displayed no results, i.e., no growth was observed, which shows the absence of *P. aeruginosa*. These outcomes are in parallel with the studies of [52] and [53]. Most cases of *P. aeruginosa* were found in surgical wards, 48%, then pediatric and medical wards, 23% and 17 %, respectively. The pus and swab samples contribute to *P. aeruginosa* infections in medical wards [54].

When an organism is frequently exposed to an antibiotic, it becomes resistant to that particular antibiotic; this might be the case with *P. aeruginosa.* The antimicrobial profile in a earlier study found that isolates of *P. aeruginosa* were not sensitive to about three classes of antibiotics [55]. At the same time, another study identified comparative findings in about 2906 clinical isolates of *P. aeruginosa* [56]. The current antibiogram study showed that *P. aeruginosa* was highly susceptible to Amikacin (86%) and was highly resistant to Cefixime (82%). A relatable antibiogram was reported by [57]. In this study, we observed that *P. aeruginosa* shows maximum resistance to Cefixime (82%), followed by Ampicillin (79%) and Augmentin (61). On the other side study performed by [58] showed that the majority of the isolates were resistant to Cefotaxime (93.5%) and Trimoxazole (93.3%). At the same time, resistance towards Ceftazidime was about to 86%, Gentamycin was 73.3%, and Ciprofloxacin was 75.5%. According to this study, the maximum resistance of *P. aeruginosa* is against ordinarily used antibiotics, and resistance against recent antibiotics is boosted continuously. The frequent uses of antibiotics, self-prescription, absence of attention, patient refusal, aimless use, and contaminated environments lead to transmission of the recalcitrant organism, which causes the weak effectiveness of antimicrobial agents [56].

4. Conclusions

The rate at which pathogens get resistant to antibiotics is increasing daily; therefore, the current results elucidate the intimidation of P. aeruginosa as it has become a highly resistant specie in hospital patients. Hospitalized patients with resistant P. aeruginosa infections have increased all-cause mortality. It is essential to develop and discover new antibiotics, the resistant pattern that will reduce the treatment cost and improve patient care quality. To overcome antibiotic resistance and prohibit the transmission of multidrug-resistant (MDR) strains of *P. aeruginosa* and other opposing bacteria, it is essential to prescribe correct medications, implement strict antibiotics policies, and launch national programs and infection control procedures. It is also necessary to regularly monitor the antibiotic-resistant and sensitivity patterns of different microorganisms like P. aeruginosa in clinical units, which will help clinicians and microbiologists reduce antibiotic resistance.

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Conflicts of Interest

The authors declare no conflict of interest.

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