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Research Article

Investigating the Burden of *Pseudomonas aeruginosa* Infections in COVID Patients and Resistance Profiles in Abbottabad, Pakistan

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Abstract: Nosocomial infections are a great menace for hospitalized patients and *Pseudomonas aeruginosa* has emerged as one of the most potent nosocomial pathogens due to its continuous emanation of multi-drug resistance. Conjointly, *P. aeruginosa* coinfection is a significant problem in multitudinous infections. The present study aimed to investigate the frequency and antibiotic susceptibility profile of *P. aeruginosa*, from the specimens isolated from patients from Ayub Teaching Hospital in Abbottabad, Pakistan. In this cross-sectional study, eighty strains of *P. aeruginosa* were obtained from 200 patients (urine, wounds, and pus samples), using routine microbiological methods, and antibiotic susceptibility testing was performed using the Kirby Bauer disc diffusion method. The majority of isolates (51.25%) were taken from wounds, followed by pus (38%) and urine (27.27%). Of the 80 isolates, 12 originated from individuals who were also infected with the *Coronavirus* (a 34% coinfection rate). These isolates were sensitive to levofloxacin (80%) and vancomycin (75%) but were resistant to moxifloxacin (80%) and amikacin (69%). *P. aeruginosa* is found in high frequency in clinical specimens from patients in Abbottabad, and these microorganisms are transiently resistant to routinely given antibiotics, making it critical to utilize anti-Pseudomonas medications correctly. It is concluded that the *P. aeruginosa* infections and resistance continued to increase owing to various intrinsic and extrinsic factors.

Keywords: Antimicrobial Resistance, Co-infection, Moxifloxacin, *Pseudomonas aeruginosa*, Vancomycin.

1. INTRODUCTION

Pseudomonas aeruginosa is a gram-negative, rod-shaped bacterium that has become a global health issue and widespread use of antibacterial drugs has increased bacterial resistance levels and severity [1, 2]. *P. aeruginosa* causes an opportunistic human disease that infects only a small percentage of healthy persons. It primarily affects immunocompromised individuals, such as those with HIV, cancer, or cystic fibrosis, where fatality rates can reach 90%. It is highly invasive and associated with both acute and chronic vision problems.

Bacterial coinfection is a significant problem in COVID-19 patients and *P. aeruginosa* is one of the predominant coinfecting bacteria identified [3]. *P. aeruginosa* is a common nosocomial organism that causes fatal chronic infections in immunocompromised people with diseases such as cystic fibrosis, catheter-related infections, and burn wounds [4]. A study was conducted that revealed that 43 (12.46 %) of 340 COVID-19 patients got secondary bacterial infections. The most commonly isolated bacteria included *P. aeruginosa* i.e., 9.30% [5]. Other studies also showed that *P. aeruginosa* is the second most often detected infection in COVID-19 patients. It is a frequent co-infection that causes illness aggravation in people with COVID-19 [6]. Additionally, *H. influenza, S. aureus, K. pneumoniae, Mycoplasma pneumoniae,* and *Streptococcus pneumoniae* are frequent co-infecting microorganisms [3].

Treatment options include broad-spectrum carbapenem, aminoglycosides, fluoroquinolones, and aztreonam, which are the most widely recommended antibiotics for bacterial infections caused by the bacteria *P. aeruginosa* [7]. Eradication of this organism is difficult and complicated due to its inherent resistance to many different families of chemotherapeutic agents and antibiotics [8].

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Antimicrobial resistance develops in *P. aeruginosa* by various mechanisms, including innate resistance, characterized by overexpressed efflux systems and decreased membrane permeability [9]. Resistance to beta-lactam antibiotics, carbapenem antibiotics, aminoglycoside antibiotics, and fluoroquinolone antibiotics can be acquired through acquiring resistance genes or mutations in genes producing porin efflux pumps, penicillin-binding proteins, or chromosomal beta-lactamase [10]. Multidrug-resistant *P. aeruginosa* isolates limit options for treatment and greatly increase morbidity rates [11]. Many antibiotics are ineffective against *P. aeruginosa,* due to the presence of resistance genes such as the chloramphenicol resistance gene catB and the ampicillin resistance gene ampC, which encodes a beta-lactamase enzyme that hydrolyzes ampicillin and confers beta-lactam resistance. *P. aeruginosa* has been identified as a significant concern in bacterial infections [12], especially those isolated from hospitalized patients, particularly those in critical care units [13].

Antibiotic misuse and abuse are a rising public health problem, since they may result in serious adverse effects and the rise of resistant bacteria [14]. As a consequence, developing novel treatment options for P. aeruginosa infections is a high priority that has received significant attention over the last decade. Moreover, as a result of misuse or overuse of antibiotics, the sensitivity profile of P. aeruginosa to different antibiotics is constantly changing regionally, selection antibiotics remains a challenge for the resistant strains. Additionally, no comparable data was previously accessible under this framework. This study was conducted to develop a framework to investigate the prevalence of *P. aeruginosa* in the local population of Abbottabad District, KPK, Pakistan, during the Covid-19 outbreak and its susceptibility pattern. The objectives of the present investigation were to determine the antibiotic susceptibility of *P. aeruginosa* and its prevalence in Covid-19 patients.

2. MATERIALS AND METHODS

2.1. Study Area

This cross-sectional study was conducted at Ayub Medical Complex, Abbottabad, Pakistan. Ayub Teaching Hospital is a public sector, non-profit tertiary level center of academic health sciences located in Abbottabad, Khyber Pakhtunkhwa, Pakistan.

2.2. Sample Collection

The samples were obtained from Ayub Medical Complex (Microbiology Laboratory) in Abbottabad, Pakistan. In this study, 200 urines, wound, and pus samples were isolated from several wards of the hospital, including the OPD ward, the ENT ward, and the emergency ward. 35 out of 200 samples came from coronavirus-infected patients. Samples were collected from urine, wound, and pus from different wards and OPD from the patients by using sterile swab sticks according to the standards of CLSI [15].

2.3. Samples Culturing

All samples were cultured on MacConkey, CLED, and Blood agar and incubated at 37 °C for 24 h. Isolated colonies were picked by using a sterile wire loop and suspended in 2 ml of distilled water in a sterilized tube to make a standard suspension. A sterile swab was dipped into the suspension and streaked on Muller Hinton Agar. *P. aeruginosa* isolates were confirmed by performing standard microbiological procedures such as colony characteristics, gram staining and biochemical assays including catalase, oxidase and citrate tests.

2.4. Antimicrobial Susceptibility

Antimicrobial susceptibility of isolated *P. aeruginosa* strains was performed against thirteen different commonly used antibiotics. To assess susceptibility to antibiotics, *P. aeruginosa* was sub-cultured on Muller Hinton at 37 °C for 24 h and the Kirby Bauer disc diffusion method was used as per CLSI guidelines.

2.5. Antibiotics Used

Levofloxacin 30 µg, Ciprofloxacin 30 µg, Amikacin 30 µg, Piperacillin 30 µg, Aztreonam 30 µg, Cefoperazone 30 µg, Ceftazidime 30 µg, Cefepime 30 µg, Moxifloxacin 30 µg, Tazobactam 30 µg, Imipenem 30 µg, Meropenem 30 µg, and Vancomycin 30 µg were used for screening. A zone size exceeding 22 mm was considered dignified

susceptible, whereas a zone size of more than 21 mm was considered dignified resistant. As per CLSI criteria, isolates were classified as susceptible or resistant [15]. As a control *P. aeruginosa* ATCC 27853 was taken.

2.6. Data Analysis

The data analysis was done using a variety of tools in Microsoft Excel 2016.

3. RESULTS

3.1. Growth Profile and Antibiotic Susceptibi lity of *P. aeruginosa*

The growth rate of *P. aeruginosa* was evaluated using urine samples, 41% wound swab samples, and 38% pus samples (Figure 1). The results showed that the bacteria grew in 80 of the 200 samples, as well as 35 coronavirus patients, 12 of which (34%) were also infected with *P. aeruginosa*. Susceptibility to antibiotics was determined using isolates from patient samples. Antibiotics from multiple categories were used. Total of 80 % of *P. aeruginosa* isolates were sensitive to levofloxacin, 75% to vancomycin, 70% to aztreonam, 68% to ciprofloxacin, 63% to cefepime, and cefoperazone 60%. The majority of the strains are resistant to moxifloxacin 80%, followed by amikacin 69%, ceftazidime 61%, and piperacillin 59%.

3.2. Sensitivity in COVID vs Non-COVID Patients

The sensitivity of *P. aeruginosa* in the COVID and non-COVID patient samples were showed in Figure 2. The levofloxacin showed 82% sensitivity, ciprofloxacin showed 69% sensitivity, piperacillin showed 43% sensitivity, aztreonam showed 72% cefoperazone 62%, ceftazidime 31%, cefepime 62%, moxifloxacin 19%, amikacin 31%, tazobactam 46%, imipenem 46%, meropenem 43%, and vancomycin showed 82% sensitivity in non-covid patients while in covid patients the sensitivity pattern is as follows levofloxacin 67% sensitivity, ciprofloxacin showed 58% sensitivity, piperacillin showed 33% sensitivity, aztreonam showed 58%, cefoperazone 50%, ceftazidime 83%. The sensitivity of *P. aeruginosa* in different age groups is indicated by the Levofloxacin and vancomycin show varying sensitivity profiles in each age group. In the COVID and non-COVID patients, the sensitivity pattern is 68% sensitivity, ciprofloxacin (68%), piperacillin (70%) and aztreonam (62%) with age-specific sensitivity, and 73% sensitivity in the cosmopolitan population.

3.3. Sensitivity According to Gender

The sensitivity of *P. aeruginosa* in male and female were different. The levofloxacin showed 84% and 74% sensitivity, ciprofloxacin showed 69% and

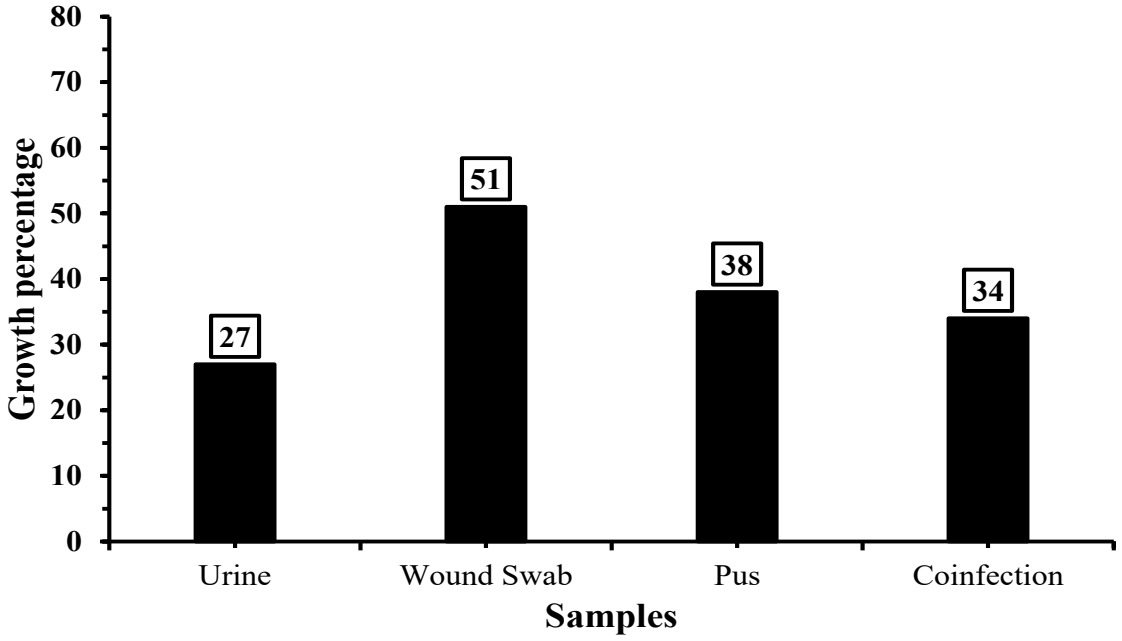

Fig. 1. Growth profile of *Pseudomonas aeruginosa* (n=80).

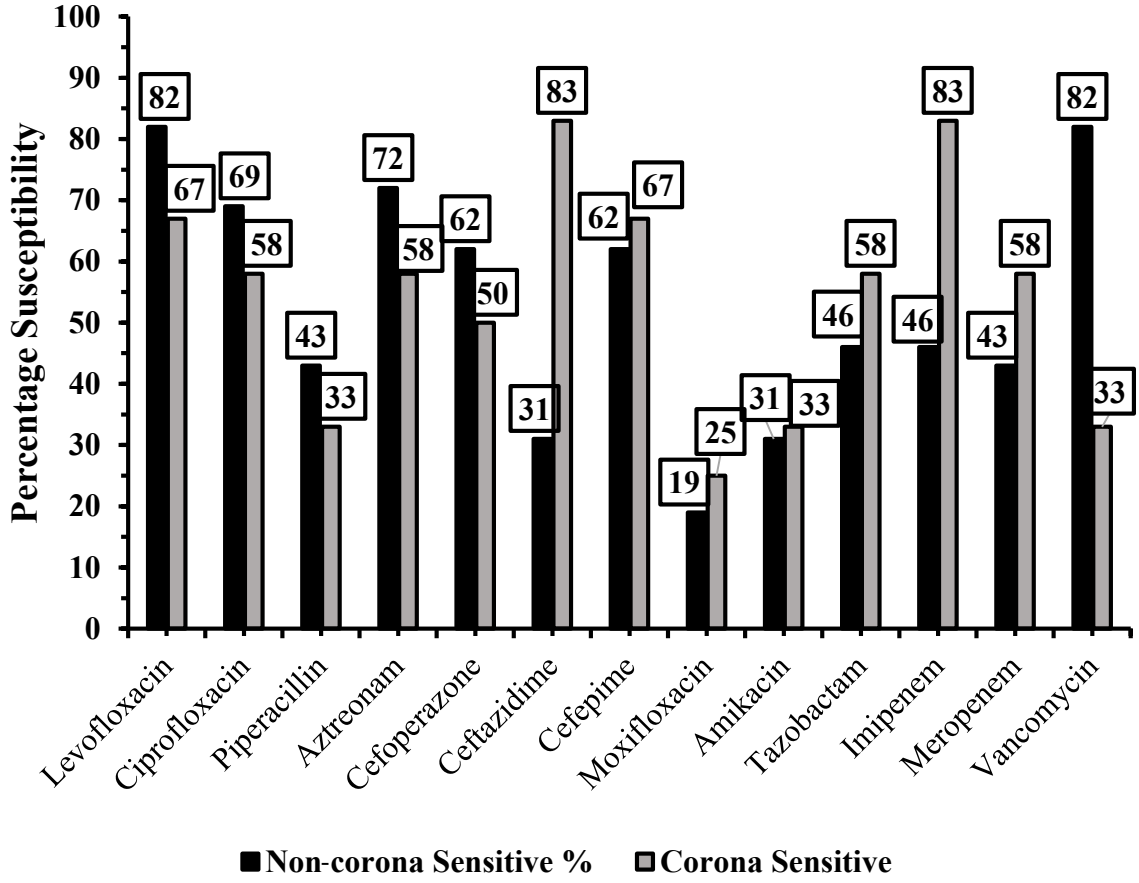

Fig. 2. Comparison of antibiotic susceptibility of *P. aeruginosa* in COVID and non-COVID patients.

66% piperacillin showed 33% and 51% sensitivity, aztreonam showed 67% and 74%, cefoperazone 60%, ceftazidime 38% and 40%, cefepime 64% and 60%, moxifloxacin 22% and 17%, amikacin 33% and 29%, tazobactam 51% and 43%, imipenem 53% and 49%, meropenem 47% and 43% and vancomycin showed 82% and 66% sensitivity pattern. The most important details from the text are the sensitivity patterns of the subjects, as well as the physical and emotional factors that affect the sensitivity.

3.4. Sensitivity in Different Areas

The sensitivity of *P. aeruginosa* in different areas is as follow; the levofloxacin showed 67% sensitivity, ciprofloxacin showed 80% sensitivity, piperacillin showed 33% sensitivity, aztreonam showed 73%, cefoperazone 67%, ceftazidime 33%, cefepime 73%, moxifloxacin 13%, amikacin 20%, tazobactam 40%, imipenem 53%, meropenem 33%, and vancomycin showed 67% sensitivity in patients living in Nawanshahr. Those patients who were living in Cantonment areas their sensitivity pattern was as follows, levofloxacin 92% sensitivity, ciprofloxacin showed 68% sensitivity, piperacillin showed 36% sensitivity, aztreonam showed 72%, cefoperazone 68%, ceftazidime 44%, cefepime 52%, moxifloxacin 20%, amikacin 28 %, tazobactam 52%, imipenem 56%, meropenem 36%, and vancomycin showed 76% sensitivity. Levofloxacin showed 72% sensitivity, ciprofloxacin showed 67% sensitivity, piperacillin showed 44% sensitivity, aztreonam showed 72%, cefoperazone 61%, ceftazidime 30%, cefepime 67%, moxifloxacin 17%, amikacin 44%, tazobactam 50%, imipenem 33%, meropenem 56%, and vancomycin showed 78% sensitivity in patients living in Jhugian. Those patients who are living in Havelian their sensitivity pattern is levofloxacin 82% sensitivity, ciprofloxacin showed 69% sensitivity, piperacillin showed 50% sensitivity, aztreonam showed 64%, cefoperazone 45%, ceftazidime 36%, cefepime For 64%, moxifloxacin 27%, amikacin 32%, tazobactam 45%, imipenem 59%, meropenem 55%, and vancomycin showed 77% sensitivity.

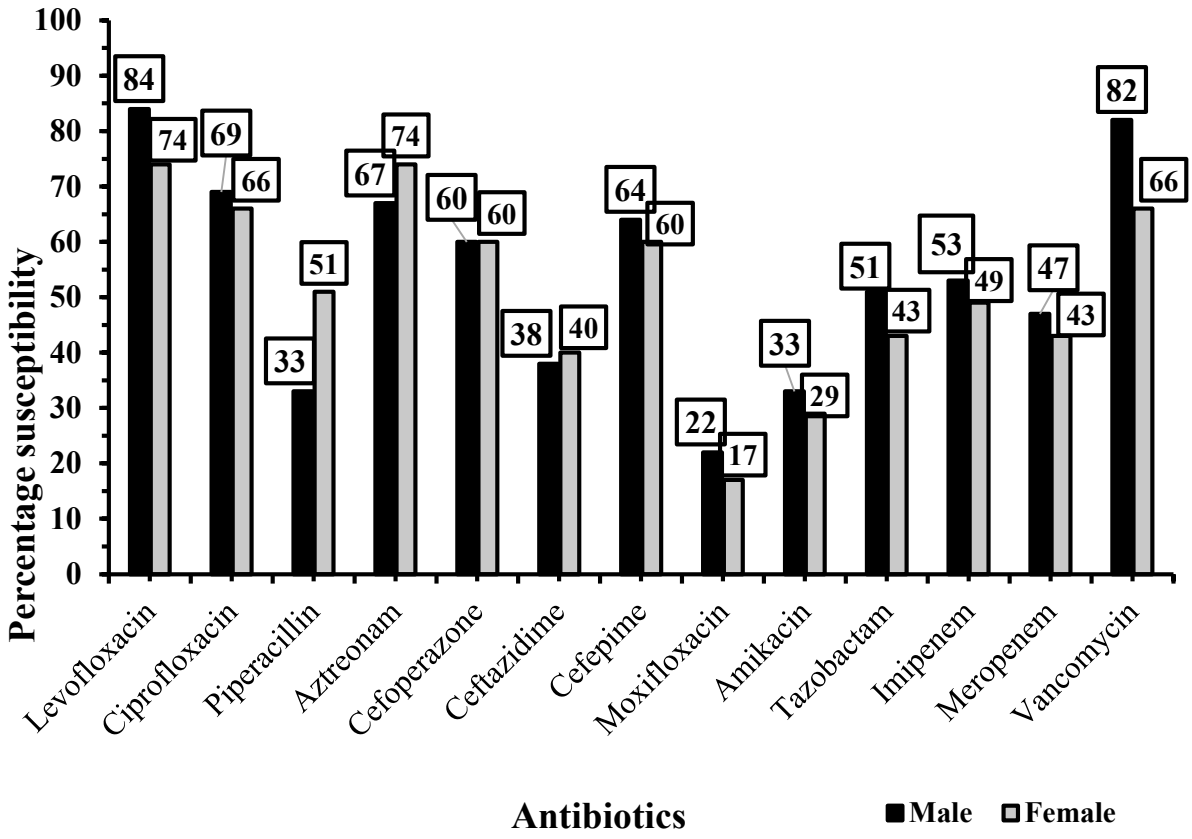

Fig. 3. Susceptibility profile of *P. aeruginosa* in different age group.

4. DISCUSSION

Pseudomonas aeruginosa is the most common infection in hospitals due to its ease of adaptation. The samples were obtained from Ayub Medical Complex (Microbiology Laboratory) in Abbottabad, Pakistan. In this study, 200 urines, wound, and pus samples were isolated from several wards of the hospital, including the OPD ward, the ENT ward, and the emergency ward. In 80 samples, *P. aeruginosa* was isolated. It was most frequently detected in wound samples (51.25%), pus samples (37.9%), and urine samples (27.27%). Previously reported results indicated that more than 70% of *P. aeruginosa* isolates originated from pus, wounds, and tracheal aspirates [16]. Our findings corroborate with [17] and [18], who found that the prevalence of *P. aeruginosa* in urine samples was lowest at 18.7% and 10%, respectively. 35 of the 200 samples came from coronavirus-infected patients. Twelve patients (34%) were co-infected with *P. aeruginosa* from these 35 samples.

Many *P. aeruginosa* infections are challenging to treat because of their drug resistance. The antibiotics levofloxacin, ciprofloxacin, aztreonam, cefoperazone, cefepime, and vancomycin were sensitive to *P. aeruginosa* isolates. Resistance to moxifloxacin, amikacin, ceftazidime, piperacillin, meropenem, tazobactam, and imipenem was identified in decreasing order. Levofloxacin was efficient against *P. aeruginosa* which was multidrug-resistant. *P. aeruginosa* isolates exhibited a 61% resistance to ceftazidime in this study. This result is higher than those reported by Mohamed and Abdelhamid (2020) and Khan and Faiz (2016), who reported resistance rates of 46% and 14%, respectively, while Mahmoud *et al*. (2013) and Pokharel *et al*. (2019) reported resistance rates of 91.2% and 63% respectively [19-22].

The results of 57.9% imipenem resistance opposed the findings of Al-Zaidi (2016), Zahoor *et al*. (2020), and Hasan et al. (2020), who reported 5.5%, 5.5%, and 5% resistance to imipenem, respectively [18, 23]. The findings contradict Feretzakis et al. study, which found resistance to cefepime and levofloxacin to be 56% and 55%, respectively [24].

Ceftazidime and imipenem were the most effective antibiotics in patients with coronavirus illness, with each having an 83% sensitivity, whereas moxifloxacin had a 25% sensitivity. Levofloxacin and vancomycin were the most effective antibiotics in non-Corona patients, with a sensitivity of 82%, while moxifloxacin has a sensitivity of just 19%. Antibiotics are more effective in non-corona patients than in corona patients. This is because of the treatment of infections in corona patients, which has resulted in increased resistance.

In contrast to a study in northern Nigeria [23, 25] and Kirkuk city of Iraq [18] this study indicated a higher prevalence of *P. infections* in male patients than in female patients (56.25 and 43.75%). Levofloxacin was the most effective antibiotic in men, with an 84% sensitivity, followed by vancomycin, with an 82% sensitivity, while ciprofloxacin and aztreonam were the most effective antibiotics in women, with a 74% sensitivity. Moxifloxacin was the most resistant antibiotic, with a sensitivity of 22% and 17%.

According to the findings, the highest frequency of *P. aeruginosa* (53.75 %) was observed in young patients (aged 15 to 35 years), while the lowest incidence was reported in patients aged 35 and older (46.25%). These findings contradict previous research conducted in Ethiopia and Al-Sulaimfania, Iraq by Shewatatek et al., 2014. Their findings indicated a higher prevalence of these bacterial isolates in elderly ill people [26]. On the other hand, our findings were consistent with those of Okon et al. in Nigeria, who reported the highest prevalence (20.7%) in patients aged 29 years [23, 27] and under and Hasan et al., who reported a 45.6% prevalence in young patients (ages 15 to 30 years), while the lowest rate (20.1%) was found in elderly patients aged 45 years and above [18]. This could be explained by the fact that the young-old group is more active and involved in a variety of clinical hygiene activities. Levofloxacin was the most effective antibiotic for adults under the age of 35, whereas vancomycin was the most effective antibiotic for people above the age of 35. Antibiotic resistance, according to our research, increases with age. This could be because older adults use an excessive number of antibiotics.

Ciprofloxacin was the most effective antibiotic in rural areas, with a sensitivity of 76%, followed by aztreonam and vancomycin, both of which have a sensitivity of 73%, whereas levofloxacin was the most effective antibiotic in urban areas, with a sensitivity of 86% and vancomycin having a sensitivity of 77%. European study, nosocomial isolates of *P. aeruginosa* showed 40% resistivity to Amikacin [28]. Amikacin has been used sparingly only in severe forms of the disease owing to high treatment costs and administrated intravenously. Therefore, drug resistance has been slow to emerge in such scenarios [29].

5. CONCLUSIONS

The findings of this research, concluded that antibiotic-resistant bacteria represent a severe public health issue around the globe. The rapid development of resistance by pathogenic bacteria in the environment has led to the rapid development of MDR, XDR, and PDR microorganisms. As a result of the rising prevalence of antibiotic resistance, alternative antimicrobial medications must be investigated as well. During the research, it was noticed that the bacteria *P. aeruginosa* was most sensitive to the antibiotic levofloxacin and the most resistant to the antibiotic moxifloxacin. *P. aeruginosa* is becoming a more dangerous disease due to its increased resistance and capacity to survive in a variety of environments, notably hospitals. Constant monitoring of the development of antimicrobial resistance, the use of suitable antibiotics, the use of combination treatments, and basic measures such as hand washing have all become critical in the management of the organism's growth and spread. *P. aeruginosa* MDR strains would be prevented from developing if the appropriate combination of chemical therapies were used.

6. FUTURE PROSPECTIVE

Antibiotic resistance is a serious problem caused by bacteria adapting to and acquiring resistance to antibiotics, which is a major source of infection. Antibiotic-resistant bacteria are capable of surviving and reproducing in the presence of antibiotics. There is a potential that microorganisms will develop antibiotic resistance with each serving that is consumed. There are many measures to avoid drug-resistant diseases, including immunizations,

proper food preparation, hand washing, and the use of antibiotics only when required. In addition, infection prevention aids in the prevention of the spread of antibiotic-resistant bacteria in the environment. Other measures for lowering antibiotic resistance exist, such as expanding antibiotic-resistant sickness surveillance. Policies and efforts for infection prevention and control should be developed and implemented more effectively. High-quality medications should be used and disposed of in a safe manner, which should be regulated and encouraged. There was evidence of multidrug resistance in several clinical specimens, including pus, wounds, and urine. These findings indicate the necessity for periodic antimicrobial susceptibility testing to monitor the resistance pattern throughout a broad geographic area. This will aid in the preservation of therapeutic efficacy as well as the health of patients. Before administering antibiotics, it is necessary to do a thorough investigation of their effectiveness. Antibiotics should be strictly regulated, and their effectiveness should be closely scrutinized as well. It is not recommended to do an antibiotic susceptibility test until after the antibiotic susceptibility test has been completed.

7. CONFLICT OF INTEREST

The authors declared no conflict of interest.

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