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Antimicrobial Susceptibility Patterns of *Pseudomonas Aeruginosa* Isolates in A Tertiary Care Hospital, Peshawar, Pakistan

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ABSTRACT

Background. *Pseudomonas aeruginosa* is an opportunistic pathogen that takes advantage of the host's weakened system and causes many life-threatening, persistent infections including cystic fibrosis and other lung infections that account for high mortality rates. The presence of several resistant genes (multiple MDR efflux pumps, beta-lactamases) in the genome of *P. aeruginosa* makes it resistant to many available antibiotics, thus making the currently used treatment options ineffective.

Method. The current study was cross-sectional and focused on examining patients with reported *Pseudomonas* infections and the analysis of their antibiotic susceptibility profile. Convenient random sampling technique was used.

Results. A total of 101 male and 74 female patients were analyzed and admitted at Rehman Medical Institute. Among them, patients in the age group 41-60 years were the most affected. Antibiotic sensitivity testing reported colistin sulphate as a highly sensitive drug since all the isolates were sensitive to it, followed by imipenem and amikacin.

Conclusion. It was concluded that the most effective antibiotics reported against pseudomonal infections were colistin sulphate and imipenem, whereas aminoglycosides yielded variable outcomes.

Keywords: beta-lactamases, efflux pumps, *Pseudomonas aeruginosa*

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1. INTRODUCTION

P. aeruginosa is a gram-negative, encapsulated, motile, rod shaped, and facultative aerobe of the family Pseudomonadaceae [1]. It is found in moist environments, such as water, soil, and skin. However, studies have also reported its continued presence on dry inanimate surfaces for several days or even months [2]. It grows well at 37°C but can survive up to 42°C [3]. It forms characteristically smooth, blue-green colonies with grapes like smell which helps in its identification on the growth media [4, 5]. Its isolates may produce three types of colonies. 'Natural isolates' taken from soil and water typically produce small and rough colonies. Whereas, 'clinical samples' either yield colonies with a fried egg like appearance (large, smooth with flat edges and elevated appearance) or colonies with mucoid appearance (isolates from respiratory and urinary tract secretions) [4, 6]. The two different solubles of *P. aeruginosa*, namely blue pyocyanin and pyoverdine, are causes of blue pus which is the typical feature of *P. aeruginosa* suppurative infections which can interfere with the human respiratory epithelium and nasal cilia, thereby leading to pro-inflammatory responses [7].

P. aeruginosa capitalizes on the weakened immune system of human beings and thrives in various vulnerable conditions, such as burn wounds, long-lasting wounds, chronic obstructive pulmonary disorder (COPD), the presence of implanted biomaterials [8], ventilator-associated pneumonia (VAP), bloodstream infections leading to sepsis, infections in soft tissues of burns, open wounds, and post-surgery patients, urinary tract infections (UTIs), diabetic foot ulcers, and keratitis related to the extended use of contact lenses [9]. The primary cause of life-threatening and long-lasting infections in individuals with cystic fibrosis and other lung conditions with high rates of illness and death is primarily attributed to it [10]. When a catheter is inserted, it puts the patient at a higher risk of acquiring a UTI known as catheter-associated urinary tract infection (CAUTI). This is because pathogens utilize the catheter as a platform for colonization and the formation of biofilms [8, 9], which results in bacteriuria and increases the chances of developing secondary bloodstream infections [11]. Among all the healthcare-associated infections, *P. aeruginosa* has an estimated prevalence of 7.1–7.3% [12–14]; however, different prevalence studies have shown that over the past decade, this rate has been increasing. Moreover, in ICU (intensive care unit) patients, *P. aeruginosa* accounts for 23% of all the acquired infections [12, 15].

The genome of *P. aeruginosa* encodes a range of resistant genes, such as multiple MDR efflux pumps and antibiotic inactivating enzymes [16, 17], broad spectrum beta-lactamases that provides resistance against beta-lactam antibiotics, aminoglycosides, fluoroquinolones, carbapenems, chloramphenicol, tetracycline, macrolides, trimethoprim sulfamethoxazole, rifampin, and cephalosporins, making the available antibiotics for its treatment ineffective [17, 18]. Once it enters inside the host it can become challenging to treat because a variety of mechanisms for establishment, adaptation, and survival exist in *P. aeruginosa*, such as quorum sensing (QS), motility-sessility switch, biofilm formation, antibiotic resistance mechanisms, adaptive radiation for persistence, and the CRISPR-Cas systems [19]. Therefore, its infections can be a serious threat to the healthcare system, worldwide.

The primary methods employed by *P. aeruginosa* to defend against antibiotic assault can be categorized as intrinsic resistance, acquired resistance, and adaptive resistance [20, 21]. This bacterium possesses inherent mechanisms of resistance, such as reduced permeability of its outer membrane, the presence of efflux pumps that actively expel antibiotics from the cell, and the production of enzymes that can deactivate antibiotics. On the other hand, acquired resistance in *P. aeruginosa* can occur through the horizontal transfer of resistance genes or through mutational changes in its genetic material. Additionally, this bacterium exhibits adaptive resistance by forming biofilms in the lungs of infected individuals. These biofilms act as barriers, impeding the diffusion of antibiotics and limiting their effectiveness against bacterial cells [21]. Consequently, there is an urgent need to develop new antibiotics or to explore alternative therapeutic strategies to effectively treat *P. aeruginosa* infections that are resistant to traditional antibiotics [20, 22]. Furthermore, proper preventive measures including screening procedures, patient care and cleaning, proper antibiotic use and waste disposal, hand hygiene, cleaning, isolation, and proper examination and inspection of patients' rooms and other shared facilities such as hydrotherapy suite and physiotherapy room can help control the infections to a large extent [2].

2. METHODOLOGY

2.1. Study Design

The current study was a descriptive cross-sectional study conducted at the pathology department of Rehman Medical Institute (RMI), Peshawar. A total of 175 isolates of *P. aeruginosa* were included in this study using the convenient sampling technique.

2.2. Data Collection Procedure

Samples were collected after obtaining approval from the hospital's ethical and research committees. Individuals of all age groups infected with *Pseudomonas* who had submitted their samples to the Microbiology Department of RMI were included in the study.

2.3. Sample Collection and Handling

Samples were isolated from six (06) different sites including pus/swab samples, sputum, urine, fluid, blood, and others using proper aseptic techniques, as done by [23, 24]. All samples were properly labeled and were sent to the laboratory for further processing.

2.4. Sample Processing

All the collected samples were inoculated separately on different growth media including blood agar, chocolate agar, MacConkey agar, and CLED (Cysteine-Lactose-Electrolyte-Deficient) agar for their morphological identification. Once inoculated, the samples were incubated at 37°C for 24 hours. Inoculation was followed by the biochemical identification of clinical isolates which was done using Gram staining, catalase, oxidase, SIM (sulfur indole motility), citrate utilization test, and urease test. Later, bacterial inoculums were prepared in comparison with 0.5% of McFarland standard. Furthermore, antibiotic susceptibility testing (AST) was performed on Muller-Hinton agar using the Kirby-Bauer (KB) disc diffusion method.

3. RESULTS

The clinical isolates formed different types of colonies (specific to *P. aeruginosa*) on different media after incubation at 37°C for 24 hours, which are listed in the table below.

Table 1. Colony Morphology of *P. aeruginosa* on Different Media

Media	Colony Morphology of Clinical Isolates
Blood agar	Large beta-hemolytic colonies with undulate margins
Chocolate agar	Mucoid colonies
MacConkey agar	Colorless (non-lactose fermenter), flat and smooth colonies
CLED agar	Bluish, lactose non-fermenting colonies

Among 175 samples, the most frequent category was of 'pus/swab' including 69 samples (39.4%), followed by 'others' including 32 samples (18.3%), 'blood' including 29 samples (16.6%), 'urine' including 23 samples (13.1%), and 'sputum' including 16 samples (9.1%). The lowest number of samples were from the 'fluids' category which included only 6 samples (3.4%). This categorization of clinical isolates is indicated in Figure 1 below.

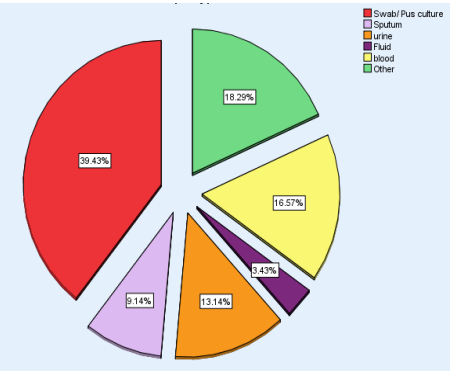

Figure 1. Percentages of Clinical Isolates

Gender-wise distribution of the study population involved a total of 101 male patients and 74 female patients. These male and female patients belonged to different age groups summarized in Table 2.

Antibiotic susceptibility testing (AST) showed that all the *P. aeruginosa* isolates were sensitive to colistin sulfate (100%). Whereas, 64.6% isolates were sensitive to imipenem, 61.14% to amikacin, 60.6% to meropenem, and 22.5% to levofloxacin. Similarly, by analyzing the resistance pattern of the isolates, it was found that 77.5% were resistant to levofloxacin, 64.1% to ciprofloxacin, 60.4% to gentamicin, 56.7% to aztreonam, 52.6% to cefepime, and 51.6% to ceftazidime. The sensitivity and resistance patterns of these antibiotics are depictd in Table 3 below.

Table 2. Age-wise Distribution of the Study Population

Age Group	Total Patients	Total Male Patients	Total Female Patients
1day-20 years	30	15	15
21-40 years	29	18	11
41-60 years	52	27	25
61-80 years	49	35	14
Above 80 years	15	06	09

Table 3. Different Antibiotics Used for AST of *P. aeruginosa*

Antibiotics	Sensitive	Resistant
Imipenem (10ug)	113 (64%)	62 (36%)
Meropenem (10ug)	105 (60%)	70 (40%)
Aztreonam (30ug)	75 (43%)	100 (57%)
Ceftazidime (30ug)	84 (48%)	91 (52%)
Cefepime (30ug)	83 (47%)	92 (53%)
Gentamicin (10ug)	70 (40%)	105 (60%)
Amikacin (30ug)	107 (61%)	64 (39%)
Ciprofloxacin (5ug)	63 (36%)	112 (64%)
Levofloxacin (5ug)	40 (23%)	135 (77%)
Piperacillin-Tazobactam (100/10ug)	104 (59%)	71 (41%)
Cefoperazone-Sulbactam (75/30ug)	100 (57%)	75 (43%)
Colistin sulphate (10ug)	75 (100%)	0

4. DISCUSSION

The inherent resistance of *P. aeruginosa* to many antibiotics is the cause of higher morbidity and mortality rates. This resistance is attributed to the presence of multi-drug efflux systems, production of β-lactamases, as well as reduced outer membrane permeability [25]. Irrespective of the advancements in the sanitation facilities and availability of several antimicrobial agents with anti-pseudomonal potential, life-threating, hospital associated, pseudomonal infections still account for high mortality rates. The antibiotics resistance issue has been greatly amplified over the past few years. Therefore, regular assessment is required to have a clear opinion of the clinical outcome of different therapeutic options [26].

The current study showed a high rate of pseudomonal infection in men as compared to women and this is in line with the studies previously conducted by Abdul Samad in Karachi, Pakistan [27]. In the current study, the age group 41-60 years had the highest frequency of patients. Similar results were also been obtained previously during the studies conducted in AFIP, Rawalpindi [28], where the highest frequency age group was 41-60 years. The AST carried out in the current study indicated 100% susceptibility to colistin sulphate followed by imipenem, while the highest resistance was observed to levofloxacin and ciprofloxacin. This is in accordance with the study conducted in India [29] (except for colistin sulphate used nowhere in the protocol but RMI).

4.1. Conclusion

The study concluded that aminoglycosides showed variable outcomes since the isolates showed resistance to gentamicin and were sensitive to amikacin. Fluoroquinolones, such as levofloxacin and ciprofloxacin, were observed as resistant antibiotics in the current investigation. Whereas, the combinations of antibiotics including cefoperazone-sulbactam and piperacillin-tazobactam showed less effectiveness, as compared to the antibiotics imipenem and meropenem.

CONFLICT OF INTEREST

The author of the manuscript has no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

DATA AVALIABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

REFERENCES

1. Klebba PE, Newton SM, Six DA, et al. Iron acquisition systems of gram-negative bacterial pathogens define TonB-dependent pathways to novel antibiotics. *Chem Rev*. 2021;121(9):5193–5239. https://doi.org/10.1021/acs.chemrev.0c01005
2. Decraene V, Ghebrehewet S, Dardamissis E, et al. An outbreak of multidrug-resistant Pseudomonas aeruginosa in a burns service in the North of England: challenges of infection prevention and control in a complex setting. *J Hosp Infec*. 2018;100(4):e239–e245. https://doi.org/10.1016/j.jhin.2018.07.012
3. Diggle SP, Whiteley M. Microbe profile: pseudomonas aeruginosa: opportunistic pathogen and lab rat. *Microbiology*. 2020;166(1):30–33. https://doi.org/10.1099/mic.0.000860

4. Ezeador CO, Ejikeugwu PC, Ushie SN, Agbakoba NR. Isolation, identification and prevalence of pseudomonas aeruginosa isolates from clinical and environmental sources in onitsha metropolis, anambra state. *Eur J Med Health Sci*. 2020;2(2):1–5. https://doi.org/10.24018/ejmed.2020.2.2.188
5. Rommes H, van Saene R, de la Cal MA. Identification and naming. In: Rommes H, van Saene R, de la Cal MA, eds. *Selective Decontamination of the Digestive Tract*. Springer; 2021:137–152.
6. Fodor A, Varga I, Hevesi M, et al. Novel anti-microbial peptides of Xenorhabdus origin against multidrug resistant plant pathogens. In: Bobbarala V, ed. *A Search for Antibacterial Agents*. Books on Demand; 2012:147–196.
7. Wu M, Li X. Chapter 87 - Klebsiella pneumoniae and pseudomonas aeruginosa. In: Tang Y-W, Sussman M, Liu D, Poxton I, Schwartzman J, eds. *Molecular Medical Microbiology*. Academic Press; 2015:1547–1564.
8. Nakkala JR, Li Z, Ahmad W, Wang K, Gao C. Immunomodulatory biomaterials and their application in therapies for chronic inflammation-related diseases. *Acta Biomat*. 2021;123:1–30.
9. Morin CD, Déziel E, Gauthier J, Levesque RC, Lau GW. An organ system-based synopsis of pseudomonas aeruginosa virulence. *Virulence*. 2021;12(1):1469–1507. https://doi.org/10.1080/21505594.2021.1926408
10. Vetrivel A, Ramasamy M, Vetrivel P, et al. Pseudomonas aeruginosa biofilm formation and its control. *Biologics*. 2021;1(3):312–336. https://doi.org/10.3390/biologics1030019
11. Roy S, Chowdhury G, Mukhopadhyay AK, Dutta S, Basu S. Convergence of biofilm formation and antibiotic resistance in acinetobacter baumannii infection. *Front Med*. 2022;9:e793615. https://doi.org/10.3389/fmed.2022.793615
12. Reynolds D, Kollef MJD. The epidemiology and pathogenesis and treatment of Pseudomonas aeruginosa infections: an update. *Drugs*. 2021;81(18):2117–2131. https://doi.org/10.1007/s40265-021-01635-6
13. Magill SS, Edwards JR, Fridkin SK. Survey of health care-associated infections. *New Eng J Med*. 2014;370(26):2542–2543. https://doi.org/10.1056/nejmc1405194
14. Weiner LM, Webb AK, Limbago B, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the national healthcare safety network at the centers for disease control and prevention, 2011–2014. *Infec Cont Hospital Epidemiol*. 2016;37(11):1288–1301. https://doi.org/10.1017/ice.2016.174
15. Vincent J-L, Sakr Y, Singer M, et al. Prevalence and outcomes of infection among patients in intensive care units in 2017. *JAMA*. 2020;323(15):1478–1487. https://doi.org/10.1001/jama.2020.2717
16. Gil-Gil T, Martínez JL, Blanco P. Mechanisms of antimicrobial resistance in Stenotrophomonas maltophilia: a review of current knowledge. *Exp Rev Anti-Infec Ther*. 2020;18(4):335–347. https://doi.org/10.1080/14787210.2020.1730178

17. Azam MW, Khan AU. Updates on the pathogenicity status of pseudomonas aeruginosa. *Drug Discovery Today*. 2019;24(1):350–359. https://doi.org/10.1016/j.drudis.2018.07.003
18. Reygaert WC. An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiol*. 2018;4(3):482–501. https://doi.org/10.3934/microbiol.2018.3.482
19. Moradali MF, Ghods S, Rehm BH. Pseudomonas aeruginosa lifestyle: a paradigm for adaptation, survival, and persistence. *Front Cell Infect Microbiol*. 2017;7:e39. https://doi.org/10.3389/fcimb.2017.00039
20. Langendonk RF, Neill DR, Fothergill JL. The building blocks of antimicrobial resistance in Pseudomonas aeruginosa: implications for current resistance-breaking therapies. *Front Cell Infect Microbiol*. 2021;11:e65759. https://doi.org/10.3389/fcimb.2021.665759
21. Coyne AJK, El Ghali A, Holger D, Rebold N, Rybak MJ. Therapeutic strategies for emerging multidrug-resistant Pseudomonas aeruginosa. *Infect Dis Ther*. 2022;11:661–682. https://doi.org/10.1007/s40121-022-00591-2
22. Pang Z, Raudonis R, Glick BR, Lin T-J, Cheng Z. Antibiotic resistance in pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. *Biotechnol Adv*. 2019;37(1):177–192. https://doi.org/10.1016/j.biotechadv.2018.11.013
23. Tara T, Ullah A, Haq IU, Ullah N, Hassan M, Umair M. Frequency and susceptibility profile of pathogen causing urinary tract infection at tertiary care hospital, Peshawar Pakistan. *Ann Allied Health Sci*. 2022;8(1):23–27.
24. Khan M, Shah SH, Abdullah M, et al. Current Epidemiological status and antibiotic resistance profile of urinary tract infection. *J Biores Manag*. 2022;9(1):123–132.
25. Livermore DM. Of *Pseudomonas*, porins, pumps and carbapenems. *J Antimicrob Chemother*. 2001;47(3):247–250. https://doi.org/10.1093/jac/47.3.247
26. Sarwar S, Sohail M, Ahmed M. Recent trends in antibiotics susceptibility pattern of Pseudomonas sp. isolated from clinical samples of Punjab, Pakistan. *Latin Am J Pharm*. 2013;32(8):1244–1248.
27. Samad A, Ahmed T, Rahim A, Khalil A, Ali I. Antimicrobial susceptibility patterns of clinical isolates of pseudomonas aeruginosa isolated from patients of respiratory tract infections in a tertiary care hospital, Peshawar. *Pak J Med Sci*. 2017;33(3):670–674. https://doi.org/10.12669%2Fpjms.333.12416
28. Ghani E, Mushtaq S, Khan SA. Multiplex polymerase chain reaction-based serotype analysis of dengue virus during 2015 dengue outbreak in Pakistan. *East Mediterr Health J*. 2017;23(9):594–597.
29. Sharma HP, Patel H, Sharma S. Enzymatic extraction and clarification of juice from various fruits—a review. *Trends In Post Harvest Technol*. 2014;2(1):1–14.