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A 3-year study of Infection Profile and Anti-Microbial Resistance of *Pseudomonas aeruginosa* Isolated from various clinical samples at a tertiary care hospital in Bangladesh

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Abstract

Background: *Pseudomonas aeruginosa* is widely recognized as a significant contributor to hospital and community-acquired infections. These bacteria exhibit resistance to numerous antibiotics through both intrinsic and acquired mechanisms, complicating treatment efforts. Therefore, ongoing monitoring of infection rates and antibiotic resistance patterns is essential to effectively select appropriate therapeutic options.

Methods: This retrospective cross-sectional study was carried out at a tertiary care hospital in Bangladesh, focusing on *Pseudomonas aeruginosa* isolates identified between 2022 to 2024 at Bangladesh Medical University, Dhaka. All clinical isolates were incubated at 37 °C for 24 hours in various media, including 5% sheep blood agar, MacConkey agar, and chromogenic agar media based on types of clinical samples. The identification of bacterial species and their antibiotic susceptibility profiles were assessed using the Vitek-2 automated system from bioMérieux. Data were analyzed systematically using SPSS version 27, developed by IBM in Chicago. Descriptive statistics were employed to determine frequencies and percentages, while trends were assessed through a one-sample Chi-square test.

Results: Throughout the three years, a total of 102096 specimens, including sputum, blood, pus, urine, throat swab, wound swab, tracheal aspirate, catheter tip and endotracheal tubes, were analyzed. The yearly isolation rates of *Pseudomonas aeruginosa* showed a significant increase (p-value <0.05), with 510 (21.95%) isolates in 2022, 718 (30.90%) in 2023, and 1095(47.13%) in 2024. Notably, resistance rates to cefoprazole-sulbactam, piperacillin-tazobactam exhibited a significant decline (p-value <0.05), although the overall resistance to ceftazidime remained concerning at 59.62%. The reduction in multidrug-resistant (MDR) isolates was statistically significant over the three years, with counts of 27.65%, 16.63%, and 12.89% in 2022, 2023, and 2024, respectively (p-value <0.05).

Conclusion: The incidence of *Pseudomonas aeruginosa* infections is on the rise, yet there has been a gradual decline in both resistant and MDR isolates from 2022 to 2024, indicating a positive trend. Further investigation is necessary to identify and promote the factors contributing to this improvement.

Keywords: *Pseudomonas aeruginosa,* Bangladesh, AMR, Surveillance, VITEK-2 system

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Introduction

Pseudomonas aeruginosa is a Gram-negative, aerobic, oxidase-positive bacterium that cannot ferment carbohydrates, classifying it as a nonfermentative Gram-negative bacillus. This organism exists in a wide range of environments such as air conditioners, sucker machines, incubators, stethoscopes, phototherapy, mothers' beds, respiratory support door handles, weighing machines, doctors' mobile phones, bedside lockers, BP machines, hands of healthcare workers, switchboards, sterilizer swabs from gallipots, water supply, CPAP machines, kidney trays, medicine trolleys, pulse oximeters, sinks, and baby cots [1-3]. Its capacity to endure in both arid and moist environments, coupled with its resistance to disinfectants and antibiotics, has greatly enhanced its persistence in healthcare environments [4]. At present, this organism is a major contributor to various hospital-acquired infections leading to considerable morbidity and mortality. The swift rise and dissemination of multidrug-resistant (MDR) strains in hospitals significantly contribute to elevated mortality rates, particularly in immunocompromised individuals [5-7].

Infections can be spread by infected individuals, healthcare personnel, or visitors, as well as through different medical and surgical procedures [8]. The growing resistance of P. aeruginosa to frequently prescribed antibiotics, coupled with the increasing prevalence of multidrug-resistant strains, represents a major global health issue [9]. Resistance may arise from chromosomal mutations or the transfer of resistance genes from other bacterial species. Infections caused by these drug-resistant strains can result in treatment failures, higher healthcare expenses, and increased mortality rates. Misuse of antibiotics and insufficient infection control measures in healthcare settings exert selective pressure on bacteria, promoting the development of multidrug-resistant strains [9].

Pseudomonas aeruginosa exhibits numerous virulence factors and utilizes a range of strategies to withstand various antibiotic classes [9-10]. These strategies encompass biofilm formation, the synthesis of drug-inactivating enzymes like extended-spectrum beta-lactamases, metallo-beta-lactamases, and carbapenemases; alterations in outer membrane protein channels; and the function of efflux pumps, all of which enable it to effectively circumvent host defenses and resist antibiotic therapies [11-14]. Over time, there has been a global report of rising resistance in *Pseudomonas aeruginosa* to beta-lactams, fluoroquinolones, and aminoglycosides [5]. Nonetheless, the resistance patterns of P. aeruginosa can differ across various regions, frequently influenced by variations in antibiotic prescribing practices and infection control strategies [5]. Consequently, it is essential to possess a comprehensive understanding of local antibiotic resistance trends through consistent surveillance, regular detection, and documentation of resistance patterns among bacterial populations. This information will aid local medical practitioners in choosing the suitable antibiotics for empirical treatment and will support infection control teams in the ongoing monitoring and revision of antibiotic policies and infection control strategies. The present study investigates a three-year trend of antibiotic resistance identified in *Pseudomonas aeruginosa* isolates at a tertiary care facility in Bangladesh.

Materials and Methods

Study Design

This retrospective study was conducted a tertiary hospital located of Dhaka in Bangladesh to examine the vulnerability pattern of all *P. aeruginosa* isolates. The study was carried out over an extended 03-years period from January 2022 to December 2024. A total of 102096 clinical specimens were collected from various departments in this hospital. The samples collected included sputum, blood, pus, urine, throat swab, wound swab, tracheal aspirate, catheter tip and endotracheal aspirate.

Bacterial identification method and antibiotic susceptibility testing

All the clinical samples obtained in microbiological laboratory were processed by standard microbiological techniques. The specimens had been cultured on Chocolate agar, 5% Sheep Blood agar, MacConkey agar plates and chromogenic media (Hi-Media, India) based on type of clinical samples. Isolates were identified as *Pseudomonas aeruginosa* by standard, conventional phenotypic methods using Kligler's Iron Agar (KIA), MIU stands for Motility Indole Urea, Christensen's urease agar, Simmons citrate agar, oxidase strip and pigment production[7].After the bacteria had grown on the solid medium, a Vitek-2 (bioMérieux) automated system was employed for identification purposes and to determine the antibiotic susceptibility testing of all *Pseudomonas aeruginosa* isolates. Subsequently, 0.5% sterile NaCl was added to the bacterial suspensions and the concentration was modified at 0.5– 0.63 McFarland using the VITEK-2 system (BioMérieux). For purposes of identification, the GN-21341 cards were employed, after which the antibiotic susceptibility of the *Pseudomonas aeruginosa* isolates could be examined using AST-N291 cards. Moreover, quality control was important, and thus the reference strain *Pseudomonas aeruginosa* ATCC 27853 was employed. A number of antibiotics were tested in this examination, including Gentamicin, Amikacin, Ciprofloxacin, Ceftazidime, Piperacilin-Tazobactam, Cefoperazone sulbactam, Colistin**,** Polymixin B, and Imipenem. The interpretation of the MIC results based on Clinical Laboratory Standard Institute (CLSI) guidelines (2023) [15].

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Statistical Analysis

Data were analyzed by SPSS version 27. Categorical variables were calculated as percentages. Chi-square test was used to compare the two groups. All p-values <0.05 were considered as statistically significant.

Results

A total of 102096 specimens comprising of sputum, blood, pus, urine, throat swab, wound swab, tracheal aspirate, catheter tip and endotracheal aspirate were processed during the entire three year period. Of these, 23519 specimens were processed in the year 2022, 33584 specimens in 2023, and 44993 in 2024, which yielded 4558, 5032 and 6825 Gram-negative bacilli respectively. Out of these, *Pseudomonas aeruginosa* accounted for 510(11.18%), isolates in the year 2022, 718(14.22%) isolates in 2023, and 1095(16.04%) in 2024. The isolation rate of *Pseudomonas aeruginosa* was found to have significantly increased (p-value <0.05) over the years. Distribution of *Pseudomonas aeruginosa* according to gender, age and season is shown in table no 1. It was found that, in all 3 years, numbers of males suffering from *Pseudomonas* infection were significantly higher compared to females. *Pseudomonas aeruginosa* was isolated from subjects belonging to all age groups starting from neonates to individuals as old as or even older than 80 years. It was noticed that majority of *Pseudomonas aeruginosa* 48.21% (1120/2323) were isolated from adults (15-60 years of age), while 721(31.03%) and 482(20.74%) were from individuals of old age group (>60 years) and those of younger age group (0-14 years) respectively.

Pseudomonas aeruginosa isolation rate was more during the rainy season i.e. 1344(57.85%) when compared with other months 979 (42.14%) (p-value >0.05). Maximum number of isolates were from urine, followed by wound swab, respiratory samples, pus, endotracheal aspirate and blood samples (figure 1) though these differences were not statistically significant. The antibiotic susceptibility profile revealed gradually decreasing number of resistant isolates over the period starting from 2022 to 2024 except for imipenem to which the organisms exhibited an increasing resistance trend. The percentage resistance of isolates against imipenem showed slow but gradual increase (7.25%, 9.14% and 10.05% respectively in the years 2022, 2023 and 2024) over the years. The number of MDR isolates was 27.65%, 16.63%, and 12.89% in the years 2022, 2023and 2024, respectively (p-value <0.05).

Discussion

Pseudomonas aeruginosa is the most frequently isolated non-fermenting bacterium from clinical specimens, posing a significant challenge in the treatment of both community-

Table 1: Sociodemographic characteristics and season-wise distribution of *Pseudomonas aeruginosa* for three-year study period.

	Year of Isolation			
Gender	**2022**	**2023**	**2024**	**Total**
Male	326	430	635	1391(59.87%)
Female	184	288	460	932(40.13%)
Total	510	718	1095	2323
Age				
0-14 years	95	127	260	482(20.76%)
15-60 years	257	329	534	1120(48.21%)
>60 years	158	262	301	721(31.03%)
Total	510	718	1095	2323
Season				
Rainy season	354	379	611	1344(57.85%)
Other season	156	339	484	979(42.15%)
Total	510	718	1095	2323

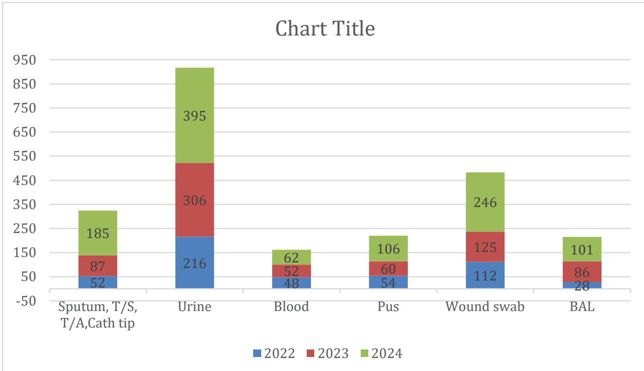

Figure 1: Distribution of *Pseudomonas aeruginosa* from different samples.

Table 2: Antibiotic resistance pattern of *Pseudomonas aeruginosa* in 2022, 2023 and 2024.

Antibiotics	Year			
	2022	2023	2024	p-value
Ciprofloxacin	36.32(%)	22.34(%)	23.83(%)	>0.05
Cefoperazone-sulbactam	47.26(%)	41.19(%)	37.21(%)	<0.05
Ceftazidime	59.22(%)	67.03(%)	52.63(%)	>0.05
Piperacillin-tazobactam	52.33(%)	43.18(%)	37.25(%)	<0.05
Gentamicin	27.65(%)	19.63(%)	17.83(%)	>0.05
Amikacin	18.47(%)	13.67(%)	10.45(%)	>0.05
Imipenem	7.25(%)	9.14(%)	10.05(%)	>0.05
Colistin	4.20(%)	2.65(%)	2.05(%)	>0.05
Polymixin B	0	0	0	-
Multidrug resistant isolates (MDR)	27.65%	16.63%	12.89%	<0.05

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acquired and hospital-acquired infections [16]. To ensure optimal clinical outcomes, it is essential to analyze and select the appropriate first-line antibiotic treatment. The issue of antibiotic resistance is critical, and the World Health Organization (WHO) report from 2015 emphasizes the need for ongoing surveillance of antimicrobial resistance (AMR) to identify infections caused by resistant organisms and to track the emergence of resistant strains [17]. This study observed an increase in the incidence of *Pseudomonas aeruginosa* infections over three consecutive years. Over the three years, a total of 45,508 males and 56,588 females were affected. Among these, 1,391 males had positive cultures for *Pseudomonas aeruginosa*, which was higher than the 932 females with similar results. Although this difference was not statistically significant (p-value >0.05), it highlighted a noteworthy trend of male predominance in disease occurrence. Yadav et al. have reported similar findings, indicating that male patients are more likely to contract *Pseudomonas aeruginosa* infections compared to females [18]. The reasons for this disparity may be multifactorial, with one significant factor being that males tend to exhibit lower compliance with hand hygiene practices, including timing, soap usage, and handwashing behavior, compared to females [19,20]. Additionally, other factors such as cultural influences, hormonal differences, and immune status may also contribute to the male predominance in both the incidence of the disease and the likelihood of poor clinical outcomes [21].

In the current research, *Pseudomonas aeruginosa* was isolated from neonates to individuals aged 80 years and older. It was found that the majority of *Pseudomonas aeruginosa* (1120, 48.21%) were sourced from adult patients, while a smaller number (721, 31.03%) were identified in the elderly population. Despite the lower numbers, it is crucial to manage the older age group with utmost care, as these individuals are often susceptible to various co-morbid conditions and are at a higher risk of infections due to seasonal climatic changes [22]. In Bangladesh, the rainy season lasts from April to October. Given that *Pseudomonas aeruginosa* is a saprophytic organism, its spread is anticipated to be more pronounced during the rainy season, leading to an increased incidence of infections [23]. In our study, 53500 cultures were conducted during the rainy season and 48596 during the non-rainy season. Notably, the isolation rate of *Pseudomonas aeruginosa* was higher in the rainy months, with 1344 (57.85%) compared to the dry months (979; 42.14%). Although this difference was not statistically significant (p-value >0.05), it remains a noteworthy observation that could have implications for the community, particularly for the elderly population.

In our study, samples from urine, wound swabs, sputum, and endotracheal secretions represented 39%, 20.79%, 14.03%, and 9.25% of the identified *Pseudomonas aeruginosa* respectively. Javiya et al. identified urine, pus, and sputum as the main sources of isolates in their research conducted in Gujarat, India, with each contributing 27%, while endotracheal secretions accounted for 14% of the *Pseudomonas aeruginosa* isolates, aligning with our findings [24]. Additionally, studies by Khan and Faiz in Pakistan and Hoque et al in Bangladesh reported a higher prevalence of isolates from respiratory samples and wound swabs, respectively [5,25]. Several factors may have contributed to these discrepancies, including the duration of the study, the demographic characteristics and size of the patient population, and the geographical context. Furthermore, antibiotic resistance remains a significant concern in bacterial infections, as highlighted by the WHO, which has classified it as a critical global issue. Notably, our study observed a declining trend in the prevalence of antibiotic-resistant *Pseudomonas aeruginosa* (Table 2), including multidrug-resistant strains, which also showed a significant decrease (p-value <0.05). This reduction in antibiotic resistance was statistically significant (p-value <0.05), particularly concerning antibiotics such as cefoperazone-sulbactam, piperacillin-tazobactam, gentamicin, amikacin, and colistin (Table 2). Our findings are consistent with similar observations reported in other studies [26].

The resistance rates observed in our study against ceftazidime, an antipseudomonal cephalosporin, were notably high, ranging from 59.22% in 2022 to 67.03% in 2023, which is concerning. Recent research from Bangladesh and other nations indicates that cephalosporins are among the most frequently prescribed antibiotics for various deep-seated infections, both by clinicians and for self-medication [27,28]. Therefore, our findings may suggest the need for a more judicious use of cephalosporins to prevent the swift emergence of resistance to this antibiotic by problematic pathogens such as *Pseudomonas aeruginosa*. The use of quinolones has been previously linked to the development of multidrug resistance [29]. It is also crucial to highlight that ciprofloxacin is increasingly utilized both as a prescribed and self-medicated antibiotic [28,30]. Additionally, resistance to β-lactam antibiotics is primarily driven by mechanisms such as β-lactamase production, which can be transferred among the same or different bacterial species, potentially leading to increased resistance to β-lactam antibiotics. A progressive rise in resistance to imipenem, one of the last-resort antibiotics, was observed, although it was present in only 8.81% of the isolates, indicating that it may still be a viable option for treating multidrug-resistant *Pseudomonas aeruginosa* infections. In contrast to our findings, studies conducted in Nepal have reported mixed results; for instance, Gyawali et al. in 2020 noted a 32.1% resistance rate to Meropenem, while Chander et al. in 2018 found all Pseudomonas isolates to be sensitive to Imipenem [31,32]. Nevertheless, our isolates showed a high sensitivity to colistin and polymyxin B, both of which are reserved by the WHO, with an overall resistance rate to colistin of only 2.96%, consistent with studies from India and Pakistan (2024), and this resistance rate has been declining over the years [33,34]. Colistin (polymyxin E) and polymyxin B are part of the same polymyxin group, sharing similar structures, mechanisms of action, and instances of cross-resistance. Various researchers from different countries have reported colistin resistance rates ranging from a minimum of 2% to a maximum of 21.3%. [35,36]. All isolated *Pseudomonas aeruginosa* were sensitive to polymyxin B, which is a positive finding. Colistin and polymyxin B, despite their inherent systemic toxicity, are considered the last resort treatment for patients in critical condition suffering from gram-negative bacterial infections [37].

According to the current data, less than 27.49% of *Pseudomonas aeruginosa* exhibited resistance to ciprofloxacin. Amikacin and gentamicin emerged as the most effective options, with resistance rates of 14.19% and 21.70%, respectively. Notably, the resistance rates of these antibiotics have decreased in 2024 compared to 2022, indicating that these antibiotics monitored by the WHO remain preferable for managing infections caused by *Pseudomonas aeruginosa.* Regarding multidrug-resistant (MDR) strains, the present study found that 19.05% of *Pseudomonas aeruginosa* were classified as MDR, which aligns with the 14% reported in the United States [38]. The rise of infections caused by MDR strains is a growing global concern, as therapeutic options are becoming increasingly limited. Research conducted by Samad et al. and Farhan et al. reported high isolation rates of MDR Pseudomonas strains at 39% and 66%, respectively [39,40]. The ongoing emergence and spread of MDR *Pseudomonas aeruginosa* is alarming. It is imperative to enforce stringent antibiotic policies to curtail the unnecessary use of antibiotics, thereby preserving the efficacy of existing medications and controlling the rise and dissemination of drug-resistant strains.

Limitation of the Study

The study is limited in scope as it was conducted at a single center, utilizing data from only one hospital. Consequently, the findings cannot be generalized to all tertiary hospitals within the region.

Conclusion

In conclusion, the incidence of *Pseudomonas aeruginosa* infections is on the rise. The excessive use of antibiotics is a primary factor contributing to the development and proliferation of drug-resistant strains. The level of resistance to cephalosporins is particularly concerning. However, there has been a notable decline in resistance rates among isolates to cefoparazone sulbactam, piperacillin-tazobactam, and imipenem. Given the scarcity of new effective medications being developed, it is essential to maintain the effectiveness of current drugs. This can be accomplished by promoting a more judicious and limited use of antibiotics. Additionally, rigorous monitoring and management of hospital-acquired infections, along with a robust antibiotic policy, regular assessments of antibiotic susceptibility patterns, and prompt communication of findings to assist physicians in making appropriate antibiotic prescriptions, are vital to mitigate the rise and spread of drug-resistant strains.

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Conflicts of Interest:

There are no conflicts of interest.

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