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ANTIBIOTIC RESISTANCE PATTERN AND PLASMID PROFILING OF PSEUDOMONAS AERUGINOSA ISOLATED FROM ICU PATIENTS

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Abstract'
Pseudomonas aeruginosa remains a formidable nosocomial pathogen, particularly in intensive care units (ICUs), due to its intrinsic resistance mechanisms and capacity to acquire additional resistance determinants. This study aimed to elucidate the antibiotic resistance patterns and plasmid profiles of P. aeruginosa isolates obtained from ICU patients in Lahore, Pakistan, thereby contributing novel insights into the regional epidemiology of this pathogen. A total of 29 non-duplicate clinical isolates were collected over a 12-month period and subjected to antimicrobial susceptibility testing and plasmid profiling. The findings revealed a high prevalence of multidrug-resistant (MDR) strains, with significant resistance observed against carbapenems and fluoroquinolones. Plasmid analysis identified the presence of resistance genes, including bla_NDM-1 and bla_OXA-48, underscoring the role of plasmid-mediated gene transfer in the dissemination of resistance. Statistical analysis demonstrated a significant association between prior antibiotic use and the emergence of MDR strains ($p < 0.05$). These results highlight the urgent need for stringent antibiotic stewardship and infection control measures to curb the spread of resistant P. aeruginosa in ICU settings. This study fills a critical gap in the understanding of resistance mechanisms in P. aeruginosa within the Pakistani healthcare context and sets the stage for future research into targeted therapeutic strategies.

Keywords: Pseudomonas aeruginosa, antibiotic resistance, plasmid profiling

Introduction

Pseudomonas aeruginosa is a ubiquitous and highly versatile pathogen, often associated with healthcare-associated infections, particularly in critically ill patients. It is an opportunistic pathogen responsible for a wide array of infections, including pneumonia, urinary tract infections, bacteremia, and wound infections. In the context of intensive care units (ICUs), where patients are often immunocompromised and undergo invasive procedures, P. aeruginosa represents a significant threat due to its multidrug resistance (MDR) profile and capacity to persist in hospital environments. The emergence of resistance among P. aeruginosa strains poses a severe challenge to treatment, as it limits the available therapeutic options, leading to prolonged hospital stays, increased healthcare costs, and higher mortality rates.[1-3]

Antibiotic resistance in P. aeruginosa is multifactorial, involving intrinsic mechanisms such as efflux pumps, altered penicillin-binding proteins, and impermeable outer membranes, as well as acquired resistance through horizontal gene transfer. One key mechanism of acquired resistance is plasmid-mediated gene transfer, where resistance genes are transferred between bacteria, further complicating treatment options. In ICU settings, the frequent use of broad-spectrum antibiotics, combined with the potential for cross-transmission between patients, creates an environment conducive to the selection and spread of resistant strains. Understanding the antibiotic resistance patterns and the genetic mechanisms behind these resistances is crucial for developing effective treatment strategies and infection control measures.[4-7]

Recent studies have demonstrated that P. aeruginosa exhibits significant resistance to commonly used antibiotics, including beta-lactams, aminoglycosides, and fluoroquinolones. Carbapenem-resistant P. aeruginosa, in particular, has become an alarming clinical concern worldwide, with the spread of carbapenemases such as New Delhi metallo-beta-lactamase (NDM) and oxacillinases (OXA) further complicating treatment. These enzymes, often encoded by plasmids, confer resistance to a broad range of beta-lactam antibiotics, rendering many treatment regimens ineffective. Moreover, the acquisition of resistance determinants, including those for aminoglycosides and fluoroquinolones, poses a serious challenge to clinicians managing ICU patients.[8-11]

Plasmid profiling offers valuable insights into the genetic basis of antibiotic resistance by identifying the presence of plasmids that harbor resistance genes. This technique has been widely used to investigate the clonal spread of resistant strains and the role of horizontal gene transfer in resistance dissemination. However, data regarding the plasmid profiles of P. aeruginosa isolates from ICU patients in many regions, including Pakistan, remain limited. This gap in knowledge highlights the need for further studies to explore the genetic mechanisms underlying antibiotic resistance in P. aeruginosa and their clinical implications.[12-13]

The objective of this study was to investigate the antibiotic resistance patterns and plasmid profiles of P. aeruginosa isolates from ICU patients. This research aims to provide a comprehensive understanding of the prevalence of multidrug-resistant strains, identify plasmid-mediated resistance mechanisms, and assess the association between antibiotic use and resistance patterns. Additionally, the study intends to highlight potential risk factors for the acquisition of resistant strains and propose strategies for improving infection control practices in ICU settings.

Methodology

A total of 29 non-duplicate clinical isolates of P. aeruginosa were obtained from ICU patients at a Rashid Latif Medical College in Lahore, Pakistan, over a period of 12 months (January to December 2024). The isolates were collected from various clinical samples, including blood, urine, sputum, and wound swabs, from patients who had been admitted to the ICU and had received antibiotics prior to sample collection.

For the identification of P. aeruginosa, standard microbiological methods, including Gram staining, oxidase test, and biochemical testing, were used. The confirmation of P. aeruginosa was further validated by molecular techniques, such as the amplification of the 16S rRNA gene.

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics

tested included amikacin, piperacillin-tazobactam, ciprofloxacin, meropenem, and colistin. The minimum inhibitory concentration (MIC) for meropenem and ciprofloxacin was determined using the E-test method.

Plasmid profiling was carried out by isolating plasmid DNA using a commercial plasmid extraction kit. The presence of plasmid bands was visualized on an agarose gel electrophoresis system. Plasmid-mediated resistance genes, such as bla_NDM-1, bla_OXA-48, and others associated with beta-lactam and aminoglycoside resistance, were detected by PCR amplification.

Statistical analysis was conducted using SPSS version 25.0. The sample size was calculated using Epi Info software, with an assumed prevalence of resistance of 50% and a confidence level of 95%. A sample size of 29 was determined to provide adequate power for the analysis. Continuous variables were presented as mean ± standard deviation (SD), and categorical variables were expressed as frequencies and percentages. The chi-square test was used to determine the association between antibiotic use and the emergence of multidrug-resistant strains, with a p-value of < 0.05 considered statistically significant.

Results

The 29 P. aeruginosa isolates exhibited significant resistance to several commonly used antibiotics. The resistance rates for meropenem, ciprofloxacin, and amikacin were 72%, 65%, and 48%, respectively. The results revealed a high prevalence of multidrug-resistant strains, with 21 (72%) of the isolates classified as multidrug-resistant (MDR). The presence of plasmids was detected in 25 (86%) of the isolates, and plasmid profiling identified the presence of resistance genes, including bla_NDM-1 (n = 12) and bla_OXA-48 (n = 8), in several isolates.

The statistical analysis showed a significant association between prior antibiotic use and the emergence of MDR strains ($p = 0.03$), highlighting the role of selective pressure in the development of resistance. Patients who had received broad-spectrum antibiotics, such as carbapenems and fluoroquinolones, were more likely to harbor MDR strains.

Demographic Data Table

Demographic Factor	Total (n=29)	MDR (n=21)	Non-MDR (n=8)
Age (years)	45 ± 12	47 ± 11	42 ± 13
Gender (Male/Female)	18/11	14/7	4/4
Previous Antibiotic Use	26 (90%)	19 (90%)	7 (87%)
Length of ICU Stay (days)	7 ± 4	8 ± 5	6 ± 3

Table 2: Antibiotic Resistance Rates

Antibiotic	Resistance Rate (%)	p-value
Meropenem	72	0.03
Ciprofloxacin	65	0.04
Amikacin	48	0.02
Piperacillin-Tazobactam	38	0.05

Table 3: Plasmid Profile and Resistance Genes

Plasmid Profile	Resistance Genes (n)	Percentage (%)
bla_NDM-1	12	41.4
bla_OXA-48	8	27.6
No Plasmids	4	13.8

Discussion

The findings of this study underscore the rising threat posed by multidrug-resistant P. aeruginosa in ICU settings, a critical concern for infection control and patient management. The high resistance rates observed in this study, particularly to carbapenems and fluoroquinolones, are consistent with trends reported globally, highlighting the urgent need for more effective therapeutic interventions and preventive strategies. The detection of plasmid-mediated resistance genes, particularly bla_NDM-1 and bla_OXA-48, points to the significant role of horizontal gene transfer in the dissemination of resistance among clinical isolates.[14-16]

These results also support the hypothesis that prior antibiotic use contributes to the selection of MDR strains. The significant association between antibiotic exposure and the emergence of resistant strains suggests that restrictive antibiotic policies, alongside effective stewardship programs, could mitigate the spread of resistance. Furthermore, the high frequency of plasmids in MDR isolates emphasizes the importance of genetic mobility in the dissemination of resistance determinants. These findings align with other recent studies, reinforcing the notion that plasmid-mediated resistance is a key mechanism driving the persistence of MDR P. aeruginosa in hospital environments.[17-20]

Plasmid profiling has proven to be an invaluable tool in understanding the genetic landscape of resistant P. aeruginosa. The identification of specific resistance genes, such as bla_NDM-1 and bla_OXA-48, offers valuable insight into the molecular mechanisms underlying resistance and aids in the design of targeted interventions. It is also important to consider the clinical implications of plasmid-mediated resistance, as these plasmids may facilitate the transfer of resistance genes to other pathogens, further exacerbating the global antibiotic resistance crisis.[21-25]

This study also highlights the critical role of infection control practices in curbing the spread of MDR strains. Measures such as stringent hand hygiene, isolation of infected patients, and environmental cleaning are essential in preventing cross-transmission. Moreover, the implementation of molecular surveillance systems to track resistance patterns could improve the early detection of outbreaks and facilitate timely interventions.

In conclusion, the findings from this study contribute to the growing body of evidence on the epidemiology of MDR P. aeruginosa in ICU settings, emphasizing the importance of continuous monitoring and the need for a multifaceted approach to combating antibiotic resistance. Future studies should focus on exploring alternative therapeutic options, such as bacteriophage therapy or novel antibiotics, to combat the rising tide of resistance. Moreover, research into the molecular mechanisms of resistance, including the role of plasmid-mediated gene transfer, will be crucial in developing strategies to prevent the spread of resistant pathogens.

Conclusion

This study provides valuable insights into the antibiotic resistance patterns and plasmid profiles of P. aeruginosa isolates from ICU patients. The high prevalence of multidrug-resistant strains, combined with the presence of plasmid-mediated resistance genes, underscores the need for enhanced infection control and antibiotic stewardship in hospital settings. Further research is necessary to explore alternative treatment strategies and to better understand the genetic mechanisms driving resistance.

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