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PHENOTYPIC AND GENOTYPIC INSIGHTS: ISOLATION OF *PSEUDOMONAS AERUGINOSA* FROM BURN WOUNDS INFECTIONS

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Abstract

Introduction: *The Pseudomonas aeruginosa is gram negative pathogen commonly associated with burn wound infections. It poses a serious clinical challenge due to its resistance to aminoglycosides.*

Objective: *Analyzing phenotypic and genotypic insights to P. aeruginosa.* ***Methodology:*** *Wound swab samples were collected from 80 burn patients following ethical approval (ERC/01/04/2023). Microbiological techniques according to the Clinical and Laboratory Standards Institute (CLSI) were used to identify bacteria. The Miniprep bacterial DNA extraction kit was used to extract the DNA, and then PCR amplification was performed to target aminoglycoside resistance genes, such as aac(6')-Ib and aph(3')-IIa. The phenotype pattern of resistance was assessed by antibiotic susceptibility testing. Patient demographics data were analyzed, using SPSS, and with a focus on age, gender, and clinical severity.*

Results: *Fifty P. aeruginosa isolates were confirmed. Phenotypic analysis was performed and 80% of isolates exhibited multidrug resistance, particularly aminoglycoside resistant. The aac(6')-Ib and aph(3')-IIa genes were identified in 70 and 65 per cent of resistant isolates, respectively, through genotypic analysis. Taken together, these results suggest a positive correlation between phenotypic resistance and the presence of resistance genes.*

Conclusion: *The findings highlight the high prevalence of aminoglycoside resistance in P. aeruginosa isolated from burn wound infections, making it prototypical of resistance in clinical isolates. This point to the pressing need for alternate therapies and strict antimicrobial stewardship programs to mitigate the development of resistance.*

Keywords: *Aminoglycoside resistance, Burn wound infections, Genotypic analysis, Multidrug resistance, Pseudomonas aeruginosa*

INTRODUCTION

Gram negative bacteria (GNB) have a characteristic envelope structure consisting of an inner membrane, peptidoglycan cell wall, and an outer membrane containing lipopolysaccharides, lipoproteins and phospholipids (1). Together, these structural components contribute to their pathogenicity and intrinsic resistance to antibiotics (2). *P. aeruginosa* is a major opportunistic pathogen for which GNB have been implicated in a significant number of severe hospitals acquired infections, particularly in burn patients (3). Resistance in *P. aeruginosa* involves several mechanisms: efflux pumps, biofilm formation, and pore protein modifications, that allow the bacterium to survive in hazardous conditions and evade antibiotic therapy (4).

P. aeruginosa has the ability to produce a hallmark biofilm — extracellular polymeric substances that enable bacterial adherence and resistance to antimicrobials (5). The Genetic determinants of algD, pelA, and pslA are important for biofilm formation and are associated with multidrug resistance (MDR) (6). Another important mechanism involves efflux pumps, which actively expel antibiotics and toxic chemical substances from the bacterial cell (7).

P. aeruginosa is killed by aminoglycosides, a common class of antibiotics that bind bacteria ribosomes to inhibit protein synthesis (8). However, the increasing prevalence of aminoglycoside resistance, caused by mechanisms such as aminoglycoside modifying enzymes and resistance genes like aac(6')-Ib,

aph(3′)-IIa and ant(2″), poses a significant clinical challenge (9). Treatment is further complicated by resistance to other antibiotic classes, including β-lactams, carbapenems and polymyxins (10). *P. aeruginosa* is particularly concerning in burn wound infections, as the pathogen is able to evade immune responses and resist numerous antibiotics (11). In addition to delaying wound healing, these infections increase the risk of sepsis and mortality. The use of phenotypic antibiotic susceptibility testing and genotypic PCR is essential to identify resistance mechanisms and recommend appropriate therapy (12).

The study aims at Analyzing phenotypic and genotypic insights to *P. Aeruginosa*. By elucidating resistance mechanisms and identifying genetic determinants, this work seeks to contribute to the development of targeted therapeutic approaches and guide antimicrobial stewardship programs

METHODOLOGIES

Distilled water was used to prepare all media, and all glassware was soaked in hot water, heat sterilized, and fumigated prior to use. Sterilization was achieved at 121°C under 15 psi pressure for 15 minutes, including the sterilization of transfer pipettes and gauze. The same conditions were applied to autoclave reagents. Lower respiratory tract and burn wound aspirate samples were cultured for routine bacterial analysis. Culture plates were prepared using conventional microbiological media, such as sheep blood agar and MacConkey's bile salt agar base, for sampling. The plates were incubated at 37°C for 18–24 hours. Colony counting and examination of morphological characteristics, including color, texture, margin, and surface, were conducted.

For further purification, some samples were subcultured onto media selective for bacterial colonies. Phenotypic and genotypic characterization of the pure isolates was performed using the obtained cultures. Routine biochemical tests, including catalase, oxidase, motility, citrate utilization, urease, TSI, and AST tests, were conducted. Genomic DNA was extracted using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, REF: K0791), following the manufacturer's protocol. Chromatographic separation was performed using 2% agarose gel. Resolution was achieved in Tris-EDTA buffer at 70 volts for one hour. The gel was stained with ethidium bromide (5 µg/ml), and PCR amplicon sizes were visualized under UV light using a 1 kbp DNA ladder as a reference. To validate the findings, selected PCR products were sequenced through genomic analysis. Statistical analyses were performed using SPSS v 22. Descriptive statistics, including frequency distributions, means, standard deviations, and bar charts, were used to present patient demographics and resistance patterns. Gender-based analysis was conducted to identify trends in aminoglycoside resistance. The small sample size presents a limitation to these preliminary insights into aminoglycoside resistance, which lay the foundation for future investigations.

RESULTS

SPSS version 22 was used for descriptive statistical analysis on the age distribution of the participants, generating frequency distribution curves. Quantitative analysis included calculating percentages, means, standard deviations, and creating bar charts to visualize trends and patterns. The sample was intentionally diverse, encompassing different demographics and clinical settings to minimize bias.

The study included 50 participants, with a near-equal gender distribution: 54% male and 46% female. Age-based analysis showed that 36% of participants were older adults, 22% were young adults, 20% were middle-aged adults, and 22% were children.

Pseudomonas aeruginosa isolates from pneumonia and non-pneumonia cases were examined microscopically as long, slender gram-negative rods. These isolates were catalase-positive, oxidase-negative, nonmotile, and typically fermentative, resulting in mucoid, pink to red colonies on MacConkey agar, characteristic of *P. aeruginosa* as described in Table I.

Antibiotic susceptibility testing revealed a resistance (high pattern), especially to aminoglycosides.

This data highlights the importance of combining phenotypic and genotypic examinations to elucidate antimicrobial resistance and to develop targeted therapeutic strategies for Pseudomonas aeruginosa infections.

Table I. Biochemical tests

Sr. No	Tests		Results	Interpretation
1	Catalase Test		Positive	Bubbling
2	Oxidase Test		Negative	Oxidized reagent
3	Citrate Test		Positive	Utilizing citrate
4	Urease Test		Positive	ammonia production
5	SIM TEST	Sulphur	Negative	-
		Indole	Negative	No indole production
		Motility	Negative	Non-motile
6	TSI TEST (Triple Sugar Iron)	Slant	Acidic	fermentation
		Butt	Acidic	fermentation
7	Gas production		Positive	-
8	H_2S production		Negative	-

The total number of participants included was 50 of which 54.0 % (n = 27) were male while 46.0 % (n = 23) were female. The respondents were categorized into four distinct age groups: children, young adults, middle aged adults and the elderly. The distribution across age groups was children (n = 11), young adult (n = 11), middle-aged adults (n= 10), and older adults (n=18). These results are disclosed in the histogram of the demographic data observed earlier in Fig. 1 Below is a breakdown of this histogram.

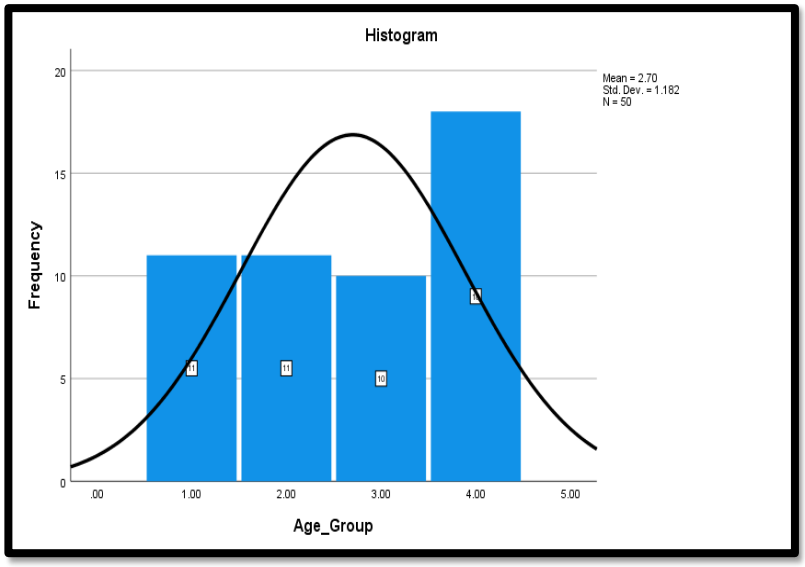

Fig. 1. Histogram showing frequency of age group using SPSS

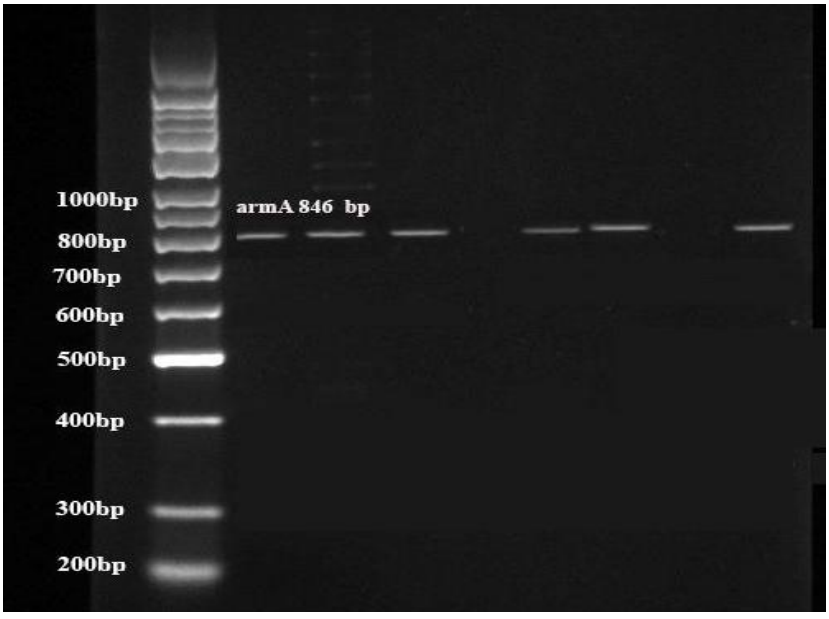

Fig. 2. Bands of armA gene on gel electrophoresis

The study examined resistance patterns for three aminoglycosides: Gentamycin, tobramycin and amikacin. Of the analysed samples, the most frequent was the ArmA antibiotic-resistant gene, identified in 86% of cases. All isolates positive for the ArmA gene also showed a very high resistance to gentamicin, amikacin and tobramycin. This result shows that there is higher association between presence of the gene ArmA and resistance to these antibiotics. The antimicrobial resistance genes observed in the study are illustrated in Figure 2 as gel electrophoresis bands of the specific band size of the ArmA gene as shown in Fig. 2.

DISCUSSION

Aminoglycoside-resistant *P. aeruginosa* isolates from burn wound infections were characterized for both phenotypic and genotypic resistance mechanisms in this study (13). Antibiotic resistance in *P. aeruginosa* is on the rise and remains a global healthcare challenge, particularly among patients in intensive care units, such as those in burn wards (14). Notably, all isolates exhibited high resistance to aminoglycosides, including gentamicin, tobramycin, and amikacin, with PCR confirming the presence of the *armA* gene in the majority of isolates (15). This finding aligns with previously documented evidence of *armA*'s role in aminoglycoside resistance (16).

Microscopic examination revealed *P. aeruginosa* as gram-negative, long, slender rods that formed typical mucoid colonies on MacConkey agar (17). Catalase and urease activity were confirmed biochemically, while oxidase negativity further validated the identification (18). The resistance patterns observed in this study reflect global trends, with multidrug resistance (MDR) posing significant therapeutic challenges (19). Similar studies from other regions have reported analogous resistance patterns, indicating the widespread nature of MDR in *P. aeruginosa (20)*. While greater precision in genetic analysis was achieved with advanced techniques such as PCR and gel electrophoresis, the study was limited by a small sample size and a single-center design. Therefore, future research should focus on increasing sample diversity and incorporating whole genome sequencing to identify new resistance mechanisms (21).

Additionally, further exploration is needed to examine environmental factors in burn wards that contribute to the emergence of resistance (22). The results underscore the urgent need for novel antimicrobial therapies and highlight the importance of considering *P. aeruginosa* resistance in infection control practices (23). Healthcare providers can integrate advanced diagnostic methods and genomic studies to develop targeted strategies aimed at mitigating the impact of multidrug-resistant (MDR) microorganisms and improving patient outcomes in burn care units (24).

However, further study is needed to understand various variables that prevail in burn wards and form the basis of resistance, such as biofilm formation and antimicrobial stewardship. These results highlight the ongoing burden of resistant infections and the urgent need for the development of new antimicrobial treatments and the implementation of surveillance of resistance into infection prevention and control programs. Overcoming *P. aeruginosa* resistance is important in enhancing patients' outcomes since it continues to be a major concern to burn care units. Further steps should be made towards raising the number of diagnostics that will help to recognize resistance profiles and create corresponding treatment approaches using molecular biology techniques. The use of such approaches will allow providing a better approach to the management of multidrug-resistant (MDR) microorganisms and improve the quality of health-care in such essential facilities.

CONCLUSION

The obtained results contribute new evidence to the current body of knowledge regarding *P. aeruginosa* resistance to antimicrobial agents and can be used to predict changing resistance trends. The findings reaffirm that this pathogen has long been considered multidrug-resistant (MDR) and highlight the continued need for in-depth research and improved antimicrobial management. Additionally, the study provides detailed insights into the action of antibiotics against *P. aeruginosa*, along with the corresponding resistance mechanisms.

References:

1. Martin JK, Sheehan JP, Bratton BP, Moore GM, Mateus A, Li SH, Kim H, Rabinowitz JD, Typas A, Savitski MM, Wilson MZ. A dual-mechanism antibiotic kills gram-negative bacteria and avoids drug resistance. Cell. 2020;181(7):1518-32.
2. López-Hernández I, López-Cerero L, Fernández-Cuenca F, Pascual Á. The role of the microbiology laboratory in the diagnosis of multidrug-resistant Gram-negative bacilli infections. The importance of the determination of resistance mechanisms. Medicina Intensiva (English Edition). 2022;46(8):455-64.
3. Laffont C, Wechsler T, Kümmerli R. Interactions between Pseudomonas aeruginosa and six opportunistic pathogens cover a broad spectrum from mutualism to antagonism. Environmental Microbiology Reports. 2024;16(5):e70015.
4. Lorusso AB, Carrara JA, Barroso CD, Tuon FF, Faoro H. Role of efflux pumps on antimicrobial resistance in Pseudomonas aeruginosa. International journal of molecular sciences. 2022;23(24):15779.
5. Vetrivel A, Ramasamy M, Vetrivel P, Natchimuthu S, Arunachalam S, Kim GS, Murugesan R. Pseudomonas aeruginosa biofilm formation and its control. Biologics. 2021;1(3):312-36.
6. Schinner S, Engelhardt F, Preusse M, Thöming JG, Tomasch J, Häussler S. Genetic determinants of Pseudomonas aeruginosa fitness during biofilm growth. Biofilm. 2020;2:100023.
7. Avakh A, Grant GD, Cheesman MJ, Kalkundri T, Hall S. The art of war with Pseudomonas aeruginosa: targeting Mex efflux pumps directly to strategically enhance antipseudomonal drug efficacy. Antibiotics. 2023;12(8):1304.
8. Webster CM, Shepherd M. A mini-review: environmental and metabolic factors affecting aminoglycoside efficacy. World Journal of Microbiology and Biotechnology. 2023;39(1):7.
9. Zhang Y, Zhang N, Wang M, Luo M, Peng Y, Li Z, Xu J, Ou M, Kan B, Li X, Lu X. The prevalence and distribution of aminoglycoside resistance genes. Biosafety and Health. 2023;5(01):14-20.
10. Ardebili A, Izanloo A, Rastegar M. Polymyxin combination therapy for multidrug-resistant, extensively-drug resistant, and difficult-to-treat drug-resistant gram-negative infections: is it superior to polymyxin monotherapy?. Expert Review of Anti-infective Therapy. 2023;21(4):387-429.
11. Hasannejad-Bibalan M, Jafari A, Sabati H, Goswami R, Jafaryparvar Z, Sedaghat F, Ebrahim-Saraie HS. Risk of type III secretion systems in burn patients with Pseudomonas aeruginosa wound infection: A systematic review and meta-analysis. Burns. 2021;47(3):538-44.
12. Abushaheen MA, Fatani AJ, Alosaimi M, Mansy W, George M, Acharya S, Rathod S, Divakar DD, Jhugroo C, Vellappally S, Khan AA. Antimicrobial resistance, mechanisms and its clinical significance. Disease-a-Month. 2020;66(6):100971.
13. Brzozowski M, Krukowska Ż, Galant K, Jursa-Kulesza J, Kosik-Bogacka D. Genotypic characterisation and antimicrobial resistance of Pseudomonas aeruginosa strains isolated from patients of different hospitals and medical centres in Poland. BMC Infectious Diseases. 2020;20:1-9.
14. Langendonk RF, Neill DR, Fothergill JL. The building blocks of antimicrobial resistance in Pseudomonas aeruginosa: implications for current resistance-breaking therapies. Frontiers in Cellular and Infection Microbiology. 2021;11:665759.
15. Saeli N, Jafari-Ramedani S, Ramazanzadeh R, Nazari M, Sahebkar A, Khademi F. Prevalence and mechanisms of aminoglycoside resistance among drug-resistant Pseudomonas aeruginosa clinical isolates in Iran. BMC Infectious Diseases. 2024;24(1):680.
16. Jouybari MA, Ahanjan M, Mirzaei B, Goli HR. Role of aminoglycoside-modifying enzymes and 16S rRNA methylase (ArmA) in resistance of Acinetobacter baumannii clinical isolates against aminoglycosides. Revista da Sociedade Brasileira de Medicina Tropical. 2021;54:e05992020.
17. Mahto KU, Das S. Microscopic techniques to evaluate the biofilm formation ability of a marine bacterium Pseudomonas aeruginosa PFL-P1 on different substrata. Microscopy Research and Technique. 2021;84(10):2451-61.
18. Shoaib M, Muzammil I, Hammad M, Bhutta ZA, Yaseen I. A mini-review on commonly used biochemical tests for identification of bacteria. International Journal of Research Publications. 2020;54(1):1-7.
19. Ou ZJ, Yu DF, Liang YH, He WQ, Li YZ, Meng YX, Xiong HS, Zhang MY, He H, Gao YH, Wu F. Trends in burden of multidrug-resistant tuberculosis in countries, regions, and worldwide from 1990 to 2017: results from the Global Burden of Disease study. Infectious Diseases of Poverty. 2021;10:1-0.
20. Giovagnorio F, De Vito A, Madeddu G, Parisi SG, Geremia N. Resistance in Pseudomonas aeruginosa: a narrative review of antibiogram interpretation and emerging treatments. Antibiotics. 2023;12(11):1621.
21. Yudianto A, Furqoni AH, Nzilibili SM, Harjanto P. The use of touch DNA analysis in forensic identification focusing on Short Tandem Repeat-Combined DNA Index System loci THO1, CSF1PO and TPOX. Infectious disease reports. 2020 ;12(Suppl 1):8716.

22. Nasser M, Ogaili M, Palwe S, Kharat AS. Molecular detection of extended spectrum β-lactamases, metallo β-lactamases, and Amp-Cβ-lactamase genes expressed by multiple drug resistant Pseudomonas aeruginosa isolates collected from patients with burn/wound infections. Burns Open. 2020;4(4):160-6.
23. Tchakal-Mesbahi A, Metref M, Singh VK, Almpani M, Rahme LG. Characterization of antibiotic resistance profiles in Pseudomonas aeruginosa isolates from burn patients. Burns. 2021;47(8):1833-43.
24. Yu T, Jiang G, Gao R, Chen G, Ren Y, Liu J, van der Mei HC, Busscher HJ. Circumventing antimicrobial-resistance and preventing its development in novel, bacterial infection-control strategies. Expert Opinion on Drug Delivery. 2020;17(8):1151-64.