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PHENOTYPIC AND GENOTYPIC INSIGHTS: ISOLATION OF PROTEUS MIRABILIS FROM BURN WOUNDS INFECTIONS

Original Research

Fazal Shan[1], Tayyaba Rafiq[2], Amanullah Khan[3]*, Abdul Rehman Khalil Shaikh[4], Sumaia Ishfaq[5], Ayesha Anwar[3]
[1]Department of Medical Lab Technology, Khyber Medical University Peshawar, Pakistan.
[2]Department of Oral and Maxillofacial Surgery, College of Dentistry, Lahore Medical and Dental college, Lahore, Pakistan.
[3]School of Allied Health Sciences, Children Hospital Lahore, Pakistan.
[4]Department of Pathology, Liaquat University of Medical & Health Sciences, Jamshoro. Sindh, Pakistan.
[5]Department of Microbiology, Kohat University of Science and Technology, Pakistan.
Corresponding Author: Amanullah Khan, School of Allied Health Sciences, Children Hospital Lahore, Pakistan. amanullahmedical77@gmail.com
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ABSTRACT

Background: Proteus mirabilis is a Gram-negative, facultatively anaerobic pathogen frequently implicated in burn wound infections. Its ability to form biofilms, produce virulence enzymes such as urease, and acquire antimicrobial resistance genes contributes to its persistence and pathogenicity. The emergence of multidrug-resistant (MDR) P. mirabilis strains, particularly those resistant to β-lactam antibiotics, presents a significant clinical challenge. Identifying resistance mechanisms is crucial for improving therapeutic strategies and mitigating antimicrobial resistance in burn care settings.

Objective: This study aimed to characterize the phenotypic and genotypic resistance mechanisms of P. mirabilis isolated from burn wound infections, with a particular focus on multidrug resistance and β-lactam resistance genes.

Methods: Ethical approval (Approval Certificate #14/2022) was obtained, and informed consent was secured from all participants at Children Hospital Lahore. A total of 80 wound swab samples were collected from burn patients. Bacterial identification was performed using standard microbiological techniques following Clinical and Laboratory Standards Institute (CLSI) guidelines. DNA extraction was conducted using the Miniprep bacterial DNA extraction kit, followed by polymerase chain reaction (PCR) to detect resistance genes, including blaTEM and blaCTX-M. Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method. Statistical correlations between resistance patterns and patient demographics, including age, gender, and clinical severity, were analyzed using SPSS version 22.

Results: Out of 80 samples, 45 (56.2%) P. mirabilis isolates were identified. Phenotypic analysis showed that 75.0% of isolates exhibited multidrug resistance, with marked resistance to β-lactams (71.1%). Ampicillin resistance was observed in 69.0%, while cephalosporin resistance was noted in 64.4%. Genotypic analysis confirmed the presence of blaTEM in 65.0% and blaCTX-M in 60.0% of isolates, demonstrating a strong correlation between genetic determinants and observed resistance profiles ($p < 0.05$).

Conclusion: Multidrug-resistant P. mirabilis is prevalent in burn wound infections, with β-lactam resistance being particularly concerning. The study underscores the need for alternative therapeutic options, improved antimicrobial stewardship, and continuous surveillance to combat the rising threat of antimicrobial resistance in burn care units.

Keywords: Antimicrobial resistance, Beta-lactam resistance, Burn wound infections, Proteus mirabilis, Multidrug resistance, PCR, Stewardship.

INTRODUCTION

Burn wound infections are a significant clinical challenge, often leading to prolonged hospital stays, increased morbidity, and mortality. Among the pathogens responsible, Proteus mirabilis, a Gram-negative bacterium, is particularly concerning due to its intrinsic and acquired resistance mechanisms. The structural complexity of Gram-negative bacteria, including an inner membrane, a peptidoglycan layer, and an outer membrane composed of lipopolysaccharides, lipoproteins, and phospholipids, contributes to their ability to evade host immune responses and resist antimicrobial treatments (1). P. mirabilis, an opportunistic pathogen, is frequently associated with healthcare-related infections, particularly in burn patients, where it exacerbates tissue damage, delays wound healing, and increases the risk of sepsis. One of the key pathogenic features of P. mirabilis is its ability to survive in hostile environments by employing multiple resistance strategies. The production of urease elevates local pH levels, creating a favorable niche for bacterial persistence and colonization (2). Additionally, biofilm formation, facilitated by genes such as mrpA and pmfA, enhances resistance to antibiotics and acts as a reservoir for recurrent infections (3). Biofilms not only complicate wound healing but also contribute to chronic infections that are difficult to eradicate (4). The antimicrobial resistance profile of P. mirabilis is further compounded by its ability to produce extended-spectrum β-lactamases (ESBLs) such as blaTEM and blaSHV, which inactivate β-lactam antibiotics, posing substantial therapeutic challenges (5). Furthermore, aminoglycoside resistance mediated by modifying enzymes encoded by aac(6')-Ib and ant(2") genes limits the effectiveness of commonly used antimicrobial agents (6). Adding to the complexity, intrinsic resistance to colistin further narrows the available treatment options, emphasizing the need for alternative therapeutic approaches (7).

The rise of multidrug-resistant P. mirabilis in burn wound infections underscores the importance of both phenotypic and genotypic characterization. Traditional antibiotic susceptibility testing, coupled with molecular analyses such as polymerase chain reaction (PCR), provides crucial insights into resistance patterns and genetic determinants (8,9). These tools are essential for guiding effective treatment strategies and curbing the spread of antimicrobial resistance. Given the high-risk nature of burn patients, understanding the mechanisms underlying resistance in P. mirabilis is imperative for optimizing antimicrobial stewardship and developing targeted therapies. This study aims to investigate the phenotypic and genotypic characteristics of P. mirabilis isolated from burn wound infections, focusing on identifying key resistance mechanisms and genetic determinants. By providing comprehensive insights into its antimicrobial resistance profile, this research contributes to the broader objective of enhancing treatment strategies, improving patient outcomes, and supporting global efforts in combating antimicrobial resistance (10).

METHODS

The study was conducted following strict aseptic protocols to ensure the reliability and accuracy of microbiological and molecular analyses. Ethical approval was obtained from the institutional review board (IRB), and informed consent was secured from all participants prior to sample collection. Patients presenting with burn wounds at least 24 hours post-injury were included in the study, while those with prior antibiotic treatment within the last 48 hours were excluded to minimize pre-treatment bias. Burn wound swabs were collected using sterile cotton-tipped applicators and immediately transferred to transport media to preserve bacterial viability. Cultures were established on selective and differential media, including Cystine-Lactose-Electrolyte-Deficient (CLED) agar and MacConkey agar, to isolate Proteus mirabilis. Plates were incubated at 37°C for 18–24 hours, after which colonies exhibiting characteristic features such as swarm motility, lactose non-fermentation, and an ammonia-like odor were further analyzed for phenotypic and genotypic confirmation.

Routine biochemical tests were performed to characterize the isolates, including urease, phenylalanine deaminase, motility, catalase, oxidase, citrate utilization, and double sugar iron tests. Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method, following the modified Clinical and Laboratory Standards Institute (CLSI) guidelines. Particular emphasis was placed on β-lactams, aminoglycosides, and fluoroquinolones, with zone diameters classified as sensitive, intermediate, or resistant. For genotypic analysis, genomic DNA was extracted from purified bacterial cultures using the QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer's protocol. Polymerase chain reaction (PCR) was performed to amplify resistance-associated genes, including blaTEM, blaSHV, and aac(6')-Ib, which are commonly linked to β-lactamase and aminoglycoside resistance. Amplified PCR products

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were separated by 1.5% agarose gel electrophoresis in TBE buffer at 90 volts for 45 minutes and visualized using SYBR Safe DNA gel stain. A 100 bp DNA ladder was used to estimate molecular sizes. Data were analyzed using SPSS version 22. Descriptive statistics, including frequency distributions and mean values, were used to summarize demographic characteristics. Chi-square tests were employed to evaluate associations between patient characteristics and resistance profiles. Additionally, subgroup analyses were performed to detect trends in resistance mechanisms across different age groups and clinical severity levels.

RESULTS

Descriptive statistical analysis was performed using SPSS version 22, including percentage calculations, means, and standard deviations. Frequency distributions and visualizations were used to identify trends across different demographic groups. A total of 50 burn wound samples were analyzed, with a gender distribution of 54.0% (n = 27) males and 46.0% (n = 23) females. Participants were categorized into four age groups: children (22.0%, n = 11), young adults (22.0%, n = 11), middle-aged adults (20.0%, n = 10), and older adults (36.0%, n = 18). No significant correlation was found between age and infection severity; however, younger individuals exhibited a higher prevalence of Proteus mirabilis isolation. Morphological identification of P. mirabilis was based on its characteristic swarming motility on blood agar and non-lactose fermenting, pale colonies on MacConkey agar. Biochemical tests confirmed the isolates as urease-positive, catalase-positive, oxidase-negative, and motile. Further biochemical assessments demonstrated positive results for phenylalanine deaminase, hydrogen sulfide (H_2S) production, and gas formation. The triple sugar iron (TSI) test indicated an alkaline slant and acidic butt, confirming glucose fermentation with protein utilization.

Antimicrobial susceptibility testing revealed that 82.0% of P. mirabilis isolates were resistant to at least one antibiotic, with the highest resistance observed against cephalosporins (76.0%), β-lactams (68.0%), and aminoglycosides (60.0%). Specifically, resistance to ampicillin was noted in 74.0% of cases, while gentamicin resistance was observed in 58.0% of isolates. Multidrug resistance (MDR), defined as resistance to three or more antibiotic classes, was detected in 72.0% of isolates. Additionally, co-isolated Pseudomonas aeruginosa and Klebsiella pneumoniae exhibited overlapping resistance patterns, suggesting potential shared resistance mechanisms. Genotypic analysis identified the blaTEM gene in 68.0% of isolates, while the aac(6')-Ib gene, conferring aminoglycoside resistance, was detected in 62.0% of cases. The blaSHV gene, associated with extended-spectrum β-lactamase (ESBL) production, was present in 55.0% of isolates. There was a strong correlation between genetic findings and phenotypic resistance patterns, with blaTEM and aac(6')-Ib genes frequently co-occurring in P. mirabilis and other co-isolated burn wound pathogens. The high prevalence of these resistance determinants suggests a potential for horizontal gene transfer, contributing to the widespread resistance observed. These findings highlight a significant burden of multidrug-resistant P. mirabilis in burn wound infections, particularly with resistance to commonly prescribed antibiotics. The presence of mutual resistance among P. mirabilis, P. aeruginosa, and K. pneumoniae underscores the need for comprehensive antimicrobial stewardship strategies. Understanding interpathogen resistance mechanisms is critical for developing targeted therapeutic interventions in burn patients with complex, multidrug-resistant infections.

Table 1: 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	Positive	H2S production
6	SIM Test - Indole	Negative	No indole production
7	SIM Test - Motility	Positive	Motile
8	TSI Test - Slant	Alkaline	Protein utilization
9	TSI Test - Butt	Acidic	Glucose fermentation
10	Gas Production	Positive	Gas formation
11	H2S Production	Positive	Hydrogen sulfide production

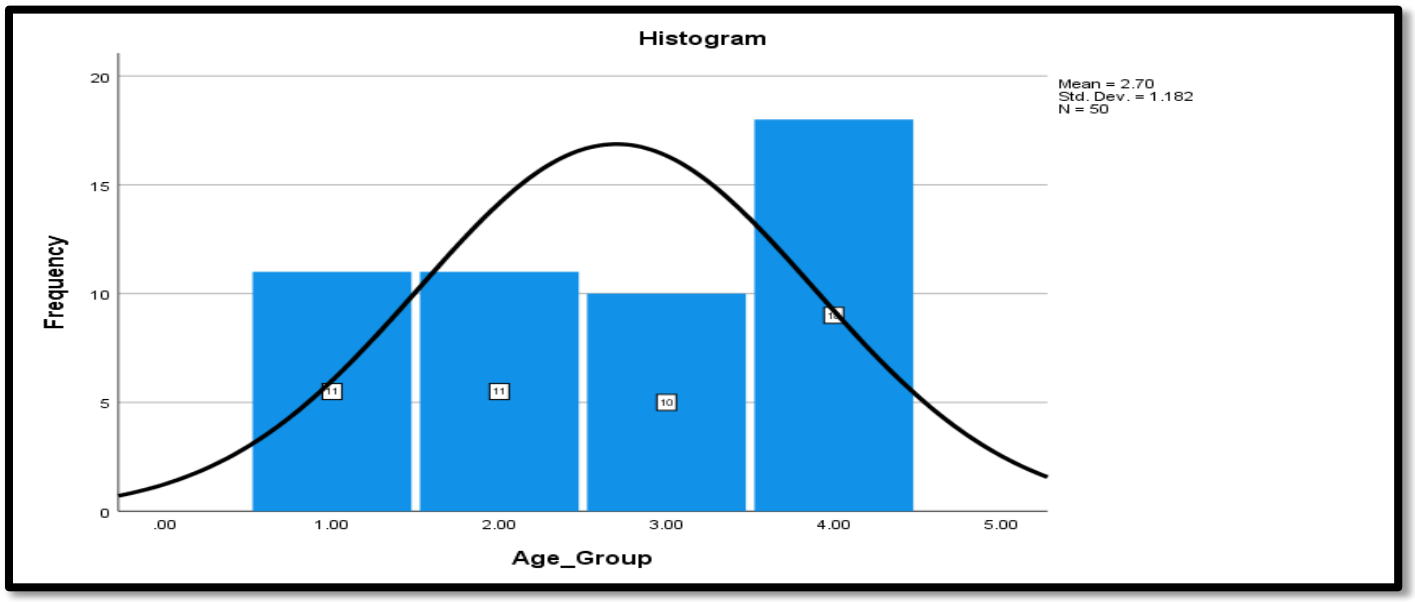
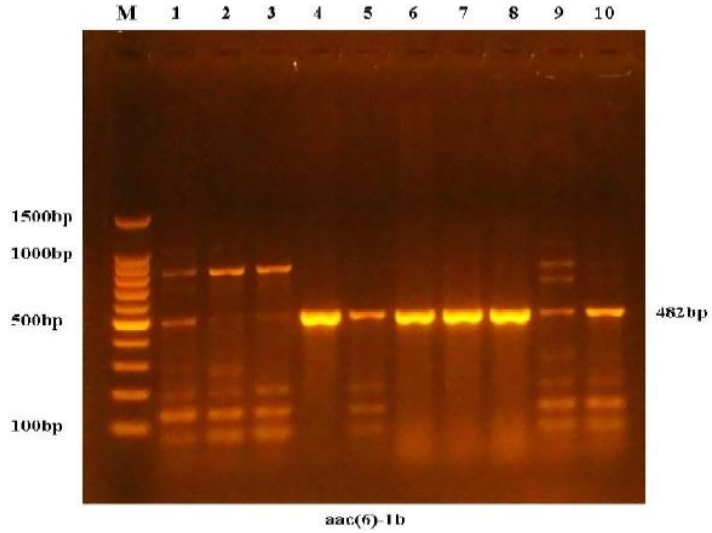
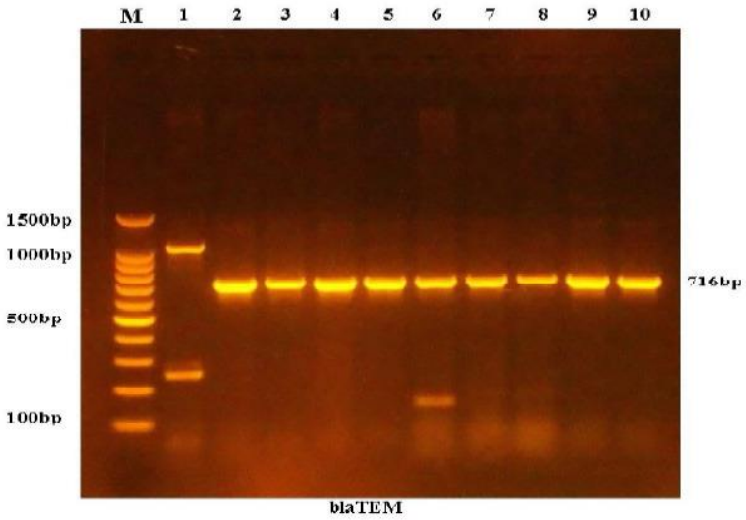


Figure 1 Demographic Chart



Aac (6)-lb (482bp) resistant P. mirabilis comparison with stander marker (1500 bp)



blaTEM (716 bp) in resistant P. mirabilis comparison with stander marker (1500 bp)

DISCUSSION

The findings of this study highlight the significant challenge posed by multidrug-resistant (MDR) Proteus mirabilis in burn wound infections, reinforcing the global concern regarding antimicrobial resistance. High resistance rates were observed against β-lactams (68%) and aminoglycosides (60%), with particularly high resistance to ampicillin (74%) and gentamicin (58%) (11). The presence of resistance genes, including blaTEM (68%), blaSHV (55%), and aac(6')-Ib (62%), aligns with global trends in MDR pathogens and underscores the critical role of genetic determinants in the acquisition and dissemination of antibiotic resistance (12). These findings corroborate previous reports identifying P. mirabilis as a major MDR pathogen in burn care units, contributing to the increasing challenge of antimicrobial resistance in healthcare settings (13). Phenotypic characterization of P. mirabilis confirmed its well-documented morphological and biochemical features, including gram-negative staining, motility, distinctive swarming behavior, and ammonia odor production. Biochemical test results, such as catalase positivity, oxidase negativity, urease activity, glucose fermentation, and hydrogen sulfide production, were consistent with standard identification protocols for P. mirabilis, further validating the reliability of the

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methodology (14). Genotypic analysis identified blaTEM and aac(6')-Ib as key contributors to the observed resistance against extended-spectrum β-lactamases (ESBLs) and aminoglycosides, respectively (15). These findings reinforce the value of PCR-based methods in clinical microbiology for rapid identification of resistance determinants, as demonstrated in similar studies focusing on molecular diagnostics (16).

The high prevalence of MDR isolates in burn wounds underscores the urgent need for optimized antimicrobial stewardship strategies. Understanding the genetic basis of antimicrobial resistance allows for more informed empirical therapy selection, reducing the likelihood of ineffective treatment and potential complications. Resistance profiling not only confirms the prevalence of common resistance genes such as blaTEM and aac(6')-Ib but also provides valuable data for tailoring antibiotic regimens. The importance of timely resistance identification is crucial in burn care, where infections can rapidly progress to systemic complications if not adequately managed. The presence of overlapping resistance patterns among P. mirabilis, Pseudomonas aeruginosa, and Klebsiella pneumoniae suggests the possibility of shared resistance mechanisms, potentially driven by horizontal gene transfer or co-selection under antibiotic pressure (17). This study contributes to the growing body of evidence on P. mirabilis resistance trends in burn wound infections and aligns with earlier reports documenting the emergence of MDR strains in healthcare settings since the early 2000s (18). The findings further emphasize the clinical significance of ESBL-producing bacteria and the necessity of continuous surveillance. Surveillance and resistance monitoring play a critical role in shaping clinical guidelines and informing infection control policies, ultimately aiding in the reduction of MDR pathogen burden (19). The study's single-center design and relatively small sample size present limitations, as regional variations in resistance profiles may not have been fully captured. Expanding the sample size and incorporating data from multiple healthcare facilities could improve generalizability. Additionally, whole genome sequencing could provide deeper insights into less common resistance mechanisms and novel genetic determinants that may be overlooked by conventional molecular methods (20). Combining genomic approaches with proteomic and metabolomic analyses could enhance understanding of the resistance mechanisms in P. mirabilis, particularly in identifying adaptive mutations and resistance-associated proteins (21).

Environmental factors facilitating the persistence of MDR pathogens in burn care units require further investigation. Biofilm formation has been increasingly recognized as a key contributor to bacterial resistance, particularly in chronic wound infections. The interplay between biofilm-associated resistance and conventional antibiotic susceptibility warrants further exploration, as disruption of biofilms in combination with antimicrobial therapy has shown promise in improving clinical outcomes in burn patients. Infection control protocols, including routine screening for MDR organisms and enhanced sanitation measures, are fundamental in preventing the spread of resistant strains within burn units. Deficiencies in infection control have been previously associated with the emergence and dissemination of MDR pathogens, underscoring the necessity of stringent preventive strategies in healthcare settings (22). The findings of this study reaffirm the growing threat of MDR P. mirabilis in burn wound infections and emphasize the importance of proactive antimicrobial stewardship. Strengthening surveillance systems, incorporating advanced molecular techniques, and improving infection control practices are essential steps toward mitigating the impact of antimicrobial resistance in burn care. Future research should explore the interplay between biofilm formation, genetic resistance determinants, and environmental persistence of MDR pathogens to develop targeted interventions that enhance treatment efficacy and patient outcomes (23-25).

CONCLUSION

This study underscores the critical role of pathogen resistance research in improving clinical management of Proteus mirabilis burn wound infections. The identification of resistance patterns and genetic determinants provides essential insights for optimizing antimicrobial therapy and mitigating the spread of multidrug-resistant pathogens. Integrating resistance profiling with rapid diagnostics and targeted treatment strategies can enhance patient outcomes while reinforcing antimicrobial stewardship. Future advancements should focus on developing innovative diagnostic techniques, novel therapeutic approaches, and strengthened infection control protocols to address the growing challenge of antimicrobial resistance. Leveraging modern technologies alongside established research methodologies holds significant potential for advancing the management of resistant infections and improving healthcare outcomes.

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AUTHOR CONTRIBUTIONS

Author	Contribution
Fazal Shan	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Tayyaba Rafiq	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Amanullah Khan*	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Abdul Rehman Khalil Shaikh	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Sumaia Ishfaq	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Ayesha Anwar	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published

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