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PREVALENCE OF PSEUDOMONAS AERUGINOSA IN WOUND INFECTIONS IN KHYBER PAKHTUNKHWA, PAKISTAN

Original Research

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ABSTRACT

Background: *Pseudomonas aeruginosa* is a Gram-negative, rod-shaped bacterium and a major cause of hospital-acquired infections globally. Its metabolic adaptability and multidrug resistance make it especially dangerous in immunocompromised individuals. Commonly found in water, soil, and hospital environments, it can cause severe infections including pneumonia, bloodstream infections, and chronic wound infections. Its persistence and resistance have led the World Health Organization to classify it among critical priority pathogens for the development of new antibiotics.

Objective: To determine the prevalence of *P. aeruginosa* in wound infections across three major teaching hospitals in Khyber Pakhtunkhwa (KPK), Pakistan, and evaluate key epidemiological factors contributing to its occurrence.

Methods: A cross-sectional study was conducted using 57 wound swab samples collected from Mardan Medical Complex (MMC), Khyber Teaching Hospital (KTH), and Saidu Teaching Hospital (STH). Samples were processed using standard microbiological protocols, including culturing on Blood Agar and MacConkey Agar, followed by Gram staining and biochemical testing (catalase, oxidase, citrate, and indole tests). The data were analyzed with SPSS version 26.0 and Microsoft Excel, with findings expressed in frequencies and percentages.

Results: Out of 57 samples, 22 (38.6%) tested positive for *P. aeruginosa*. Hospital-wise distribution showed 9 cases (40.9%) at MMC, 7 (31.8%) at KTH, and 6 (27.3%) at STH. All isolates displayed beta-hemolysis on Blood Agar, non-lactose fermentation on MacConkey Agar, and were confirmed as Gram-negative rods. Biochemical testing revealed 100% positivity for catalase and citrate utilization, 95% for oxidase, and 0% for indole. Infections were more frequent in males (63.6%) and in the 41–60 age group (36.4%).

Conclusion: The significant prevalence of *P. aeruginosa* in wound infections across hospitals in KPK calls for stronger infection control protocols and localized antibiotic stewardship. The higher incidence among males and middle-aged adults highlights potential occupational or comorbid risk factors. Broader regional studies are necessary to assess resistance patterns and support effective clinical decision-making.

Keywords: *Biochemical Tests, Infection Control, Khyber Pakhtunkhwa, Nosocomial Infections, Pseudomonas aeruginosa, Risk Factors, Wound Infection.*

INTRODUCTION

Pseudomonas aeruginosa is a gram-negative, rod-shaped bacillus belonging to the Pseudomonadaceae family, known for its widespread presence in nature and its remarkable capacity for antibiotic resistance. This opportunistic pathogen is capable of surviving in diverse environmental niches, including water, soil, and air, due to its metabolic adaptability, and it poses a serious threat to both human and animal health on a global scale (1,2). P. aeruginosa is frequently implicated in a range of hospital-acquired infections such as pneumonia, septicemia, meningitis, endocarditis, and endophthalmitis, particularly among immunocompromised patients, including those with cancer, cystic fibrosis, burns, organ transplants, or neutropenia (3,4). The bacterium has gained notoriety for its resistance to multiple antibiotic classes, making it increasingly difficult to treat with standard therapies. Recognizing the public health implications, the World Health Organization has classified carbapenem-resistant P. aeruginosa among the priority pathogens for which new therapeutic options are urgently needed (5). According to the Centers for Disease Control and Prevention (CDC), multi-drug resistant P. aeruginosa is responsible for approximately 32,600 infections and 2,700 deaths annually in the United States alone, contributing to a financial burden of $767 million in healthcare costs per year (6).

The bacterium's ability to form biofilms further compounds the problem, as biofilms are responsible for up to 80% of chronic infections and are highly resistant to both antimicrobial agents and host immune responses (7,8). Hospital-acquired infections caused by biofilm-producing strains of P. aeruginosa represent a major challenge in clinical settings, affecting around 65% of patients with nosocomial infections (9). Clinical studies assessing the prevalence of P. aeruginosa have reported varying rates of infection across sample types and patient demographics. For instance, one study analyzing 1,230 clinical samples found an 8.53% prevalence of P. aeruginosa, with a higher incidence in female patients (61.9%) than males (48.3%). The pathogen was most frequently isolated from pus (46.6%), followed by urine (31.4%), wound swabs (11.4%), ear and throat swabs (8.5%), and vaginal swabs (1.9%) (10-12). Moreover, it has been estimated that P. aeruginosa accounts for 10–20% of all hospital-acquired infections (13). Despite global awareness and ongoing research efforts, there is a paucity of localized data on the prevalence of P. aeruginosa in specific regions of Pakistan, such as Khyber Pakhtunkhwa. Understanding the epidemiology of this pathogen in regional healthcare settings is critical for informing treatment protocols and enhancing infection control strategies. Therefore, the objective of the present study is to determine the prevalence of Pseudomonas aeruginosa in wound infections in Khyber Pakhtunkhwa, Pakistan, in order to guide effective treatment strategies and improve local infection management practices.

METHODS

This cross-sectional study was conducted to determine the prevalence of *Pseudomonas aeruginosa* in wound infections among patients in selected hospitals of Khyber Pakhtunkhwa, Pakistan. A total of 57 wound swab samples were collected from patients admitted to Mardan Medical Complex (MMC), Khyber Teaching Hospital (KTH), and Saidu Teaching Hospital (STH). The samples were processed using standard microbiological procedures in the microbiology laboratory at Abdul Wali Khan University Mardan (AWKUM), Garden Campus. The study design was based on standard clinical research methodology, and the sample size was calculated using the WHO sample size estimation formula for infinite populations, using an assumed population proportion of 50% (P = 0.5), Z-score for 95% confidence level, and acceptable margin of error (M) (14). Informed consent was obtained from all participants prior to sample collection, and ethical approval was obtained from the institutional review board of AWKUM. Inclusion criteria comprised patients with clinically diagnosed wound infections requiring microbiological assessment, while patients already on antibiotics or with non-infective wounds were excluded to prevent false-negative outcomes. After sample collection, swabs were immediately transported under sterile conditions and cultured for bacterial growth using enriched and selective media.

Blood Agar: Blood agar was used as an enriched medium to support the growth of fastidious organisms and to observe hemolytic activity, particularly due to hemolysin production by *Pseudomonas aeruginosa*. The media was prepared using blood agar base powder and supplemented with 5–10% defibrinated sheep blood. The mixture was sterilized using an autoclave, poured into sterile petri dishes under aseptic conditions, and incubated after inoculation (15).

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Table 1: Material used for the preparation of blood agar

Sr. No	Items
1	Blood agar powder
2	5 to 10 % of sheep or horse blood
3	Distilled water
4	Autoclave use for sterilization
5	Spirit lamp
6	Sterile petri dishes

Mecconkay Agar: MacConkey agar was utilized as a selective and differential medium to isolate gram-negative bacteria and to differentiate lactose fermenters from non-fermenters. *P. aeruginosa* being a non-lactose fermenter appeared as pale or colorless colonies. The medium was prepared using MacConkey agar powder dissolved in distilled water, sterilized in an autoclave, and poured into sterile plates. Color differentiation was monitored after incubation (16).

Table 2: Material used for the preparation of Macconkey agar

Sr. No	Items
1	Macconkey agar powder
2	Distilled water
3	Glass flask
4	Autoclave use for sterilization
5	Spirit lamp
6	Sterile petri dishes

Gram Staining: Gram staining was performed to distinguish between gram-positive and gram-negative organisms. A colony from cultured plates was emulsified in a drop of normal saline on a slide, heat-fixed, and stained sequentially with crystal violet, iodine, decolorizer (ethanol), and safranin. *P. aeruginosa*, being gram-negative, retained the counterstain and appeared pink under light microscopy (17).

Biochemical Tests: The isolates were further confirmed using a series of biochemical tests to assess their metabolic characteristics. These tests helped differentiate *P. aeruginosa* from other gram-negative bacteria based on enzymatic activity and utilization of carbon sources (18).

Catalase Test: To detect catalase activity, a drop of hydrogen peroxide was added to a bacterial colony placed on a clean slide. The presence of bubble formation indicated catalase positivity, a characteristic feature of *P. aeruginosa*.

Oxidase Test: This test identified the presence of cytochrome c oxidase enzyme. A colony was transferred to filter paper, and oxidase reagent (tetramethyl-p-phenylenediamine) was applied. The development of a dark blue or purple color within 30 seconds confirmed oxidase positivity, consistent with *P. aeruginosa*.

Citrate Test: Simmons citrate agar was used to determine the ability of the isolate to utilize citrate as the sole carbon source. A color change from green to blue indicated a positive result. *P. aeruginosa* demonstrated a clear positive reaction due to its metabolic ability to utilize citrate efficiently.

Indole Test: Indole production was assessed by inoculating bacteria into tryptophan broth, followed by the addition of Kovac's reagent after incubation. A red layer at the surface indicated a positive result. *P. aeruginosa* did not produce a red color, confirming a negative indole reaction.

Data Analysis: The collected data were entered and analyzed using SPSS version 26.0 and Microsoft Excel. Descriptive statistics, including frequencies and percentages, were used to present the distribution of *P. aeruginosa* in clinical samples.

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RESULTS

Out of the 57 wound swab samples collected from three major hospitals in Khyber Pakhtunkhwa, Pakistan, 22 samples tested positive for *Pseudomonas aeruginosa*, indicating an overall prevalence rate of 38.6%. The distribution of positive cases across hospitals revealed that 9 cases (40.9%) were from Mardan Medical Complex, 7 cases (31.8%) from Khyber Teaching Hospital, and 6 cases (27.3%) from Saidu Teaching Hospital. All 22 isolates showed distinct growth characteristics on culture media, with beta-hemolytic colonies on blood agar and pale, non-lactose fermenting colonies on MacConkey agar. Microscopic examination through gram staining revealed that all isolates were gram-negative rods. Biochemical testing further confirmed the identification of *P. aeruginosa*. The catalase test was positive in all 22 isolates (100%), while the oxidase test showed positivity in 21 of the 22 isolates (95%). All isolates demonstrated citrate utilization, turning the medium blue (100% positivity). None of the isolates tested positive for indole production, confirming a 0% positivity rate.

Gender-wise analysis revealed a higher prevalence among male patients, with 14 cases (63.6%) compared to 8 cases (36.4%) in female patients. Age-wise distribution indicated the highest number of infections in the 41–60 year age group, accounting for 8 cases (36.4%). The 21–40 year group followed with 7 cases (31.8%), while 4 cases (18.2%) occurred in patients older than 60 years. The lowest prevalence was observed in the 0–20 year group, contributing 3 cases (13.6%). When combining gender and age data, it was observed that male patients consistently had higher case numbers across all age groups except the elderly category (>60 years), where cases were equally distributed between males and females (2 each). In the 0–20 year group, males accounted for 2 cases and females 1 case; in the 21–40 group, males had 5 cases while females had 2; and in the 41–60 group, 5 cases were male and 3 were female.

Table 3: Biochemical test results for *Pseudomonas aeruginosa*

S.NO	Test Name	Total Positive samples	Test Positive n= (%)
1.	Catalase	22	22 (100%)
2.	Oxidase	22	21 (95%)
3.	Citrate	22	22 (100%)
4.	Indole	22	00 (00%)

Table 4: Age wise prevalence for *Pseudomonas aeruginosa.*

Age Group (Years)	Number of Cases (n=22)	Percentage (%)
0–20	3	13.6%
21–40	7	31.8%
41–60	8	36.4%
>60	4	18.2%

Table 5: Gender and Age wise combined prevalence for *Pseudomonas aeruginosa.*

Age Group (Years)	Male Cases (n=14)	Female Cases (n=8)
0–20	2	1
21–40	5	2
41–60	5	3
>60	2	2

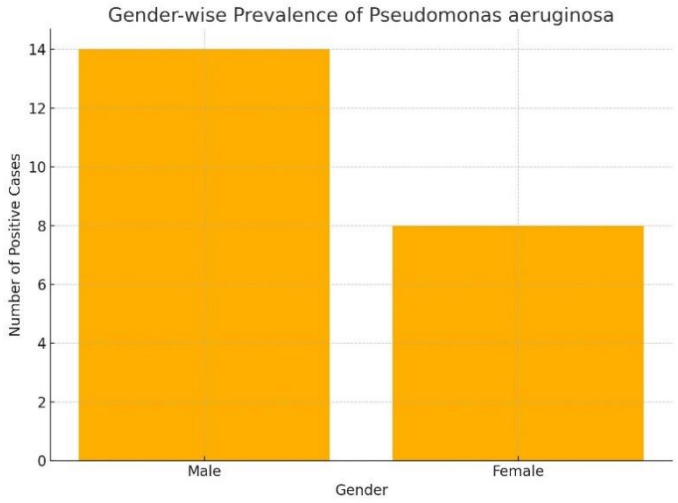

Figure 1 Gender-wise Prevalence of pseudomonas aeruginosa

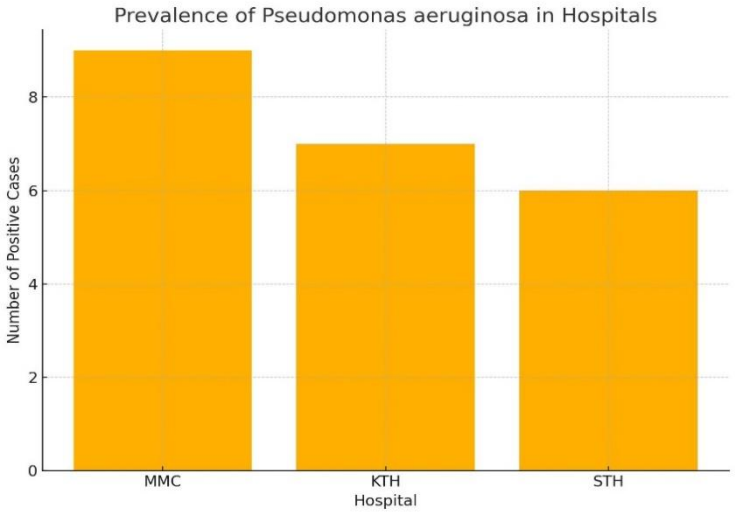

Figure 2 Prevalence of Pseudomonas aeruginosa in Hospitals

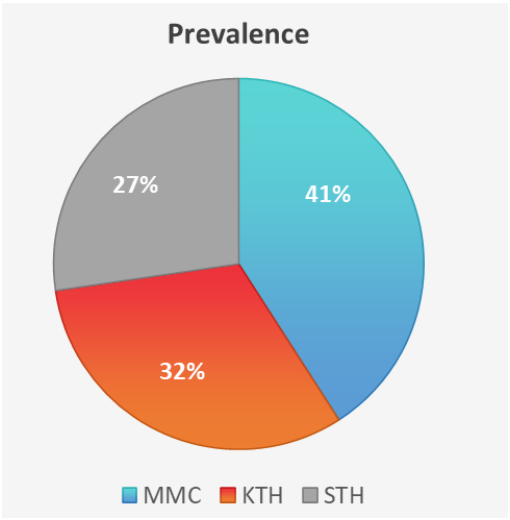

Figure 3 Prevalence

DISCUSSION

The present study aimed to investigate the prevalence of *Pseudomonas aeruginosa* in wound infections across three major teaching hospitals in Khyber Pakhtunkhwa, Pakistan. The detection of *P. aeruginosa* in 38.6% of the wound swab samples underscores its substantial role in the region's nosocomial infection burden. These findings are consistent with global surveillance data that identify *P. aeruginosa* as a leading opportunistic pathogen associated with wound infections, burn wounds, surgical site infections, and other healthcare-associated infections due to its high environmental adaptability and intrinsic resistance mechanisms (19,20). The organism's ability to survive in moist environments and resist multiple classes of antibiotics has made it a persistent threat in clinical settings. The higher prevalence among male patients (63.6%) compared to females (36.4%) may reflect gender-based occupational exposure, increased trauma-related injuries, or sociocultural variations in health-seeking behaviors. This pattern aligns with earlier epidemiological studies that report male predominance in wound-related infections, possibly due to increased outdoor exposure and delayed healthcare access. Age distribution analysis revealed that the majority of infections occurred in the 41–60 years age group (36.4%), followed by

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those aged 21–40 years (31.8%). Aged individuals (>60 years) constituted 18.2% of the positive cases, suggesting age-related immune compromise and comorbidities such as diabetes mellitus may play a contributory role in infection susceptibility and delayed wound healing. In contrast, the pediatric and adolescent group (0–20 years) represented a lower prevalence (13.6%), which may be due to relatively lower exposure to hospital environments and fewer chronic wound conditions.

Among the three healthcare institutions, *P. aeruginosa* was most frequently isolated at Mardan Medical Complex (40.9%), followed by Khyber Teaching Hospital (31.8%) and Saidu Teaching Hospital (27.3%). These inter-hospital differences may stem from disparities in infection control practices, patient demographics, antibiotic stewardship, and the presence of high-risk units such as intensive care and surgical wards. Previous studies have emphasized the association of *P. aeruginosa* outbreaks with contaminated medical equipment, poor hand hygiene, and improper sterilization practices (21,22). The predominance of the pathogen in certain facilities raises concerns regarding environmental reservoirs and the adequacy of infection prevention protocols. From a microbiological standpoint, all isolates demonstrated beta-hemolytic activity on blood agar and non-lactose fermenting colonies on MacConkey agar, with biochemical profiles consistent with *P. aeruginosa*. The uniform positivity for catalase and citrate tests, along with high oxidase positivity (95%) and universal indole negativity, reflects a typical biochemical pattern seen in clinical isolates. These findings corroborate with established identification protocols and validate the reliability of the diagnostic methods employed (23).

The study provides valuable insights into the local epidemiology of *P. aeruginosa* infections and highlights the necessity for robust infection control strategies. Its strengths include direct clinical sampling, application of standard culture and biochemical testing, and comprehensive demographic stratification. However, several limitations must be acknowledged. The study was limited to three hospitals and a relatively small sample size, which may restrict the generalizability of findings across the broader region. Additionally, the study did not assess antimicrobial susceptibility patterns, which are essential for guiding effective treatment regimens and addressing the growing concern of multi-drug resistance. Future studies should expand to include multi-center surveillance with larger populations, incorporate molecular diagnostics to detect virulence genes, and explore resistance patterns to support the development of targeted antimicrobial policies. Investigations into environmental sampling and hospital hygiene audits could further illuminate the sources of transmission. Integration of genotypic analysis would enhance understanding of strain-specific pathogenicity and resistance mechanisms, enabling a more precise approach to infection control and therapeutic intervention. In conclusion, the study emphasizes the clinical significance of *Pseudomonas aeruginosa* as a key pathogen in wound infections within healthcare settings in Khyber Pakhtunkhwa. Targeted strategies including routine surveillance, adherence to infection control protocols, and antimicrobial stewardship programs are critical to mitigating the impact of this pathogen in hospital environments.

CONCLUSION

This study concluded that *Pseudomonas aeruginosa* is a significant contributor to wound infections in teaching hospitals across Khyber Pakhtunkhwa, Pakistan. Its frequent detection in clinical samples underscores the need for strengthened infection control practices and vigilant monitoring within hospital settings. The consistent biochemical identification of the pathogen highlights the reliability of conventional diagnostic methods. Differences in infection rates between hospitals and among patient demographics suggest the influence of institutional practices and patient-related factors. These findings emphasize the importance of targeted prevention strategies, improved hygiene protocols, and informed clinical management to curb the impact of this opportunistic and adaptable pathogen in healthcare environments.

AUTHOR CONTRIBUTION

Author	Contribution
Hamad Ali*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Jahangir Khan	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
Hammad Khan	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published

Author	Contribution
Awais Javed	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Muhammad Usama	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Qaisar Ali	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published

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