Journal of Health and Rehabilitation Research 2791-156X JHRR

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Original Article

Prevalence and Antibiotic Susceptibility Pattern of Pseudomonas Aeruginosa from the Foot Ulcer of Diabetic Patients in Hayatabad Medical Complex Peshawar, Pakistan

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Conflict of Interest: None.

Qasim M., et al. (2024). 4(2): DOI: https://doi.org/10.61919/jhrr.v4i2.877

ABSTRACT

Background: Diabetic foot ulcers (DFUs) are a major complication of diabetes mellitus, affecting 15% of diabetic patients and often leading to severe outcomes like amputation. These ulcers frequently become infected with bacteria such as Pseudomonas aeruginosa, which is known for its robust antibiotic resistance.

Objective: To investigate the prevalence and antibiotic susceptibility patterns of Pseudomonas aeruginosa isolated from the foot ulcers of diabetic patients in Hayatabad Medical Complex, Peshawar.

Methods: A total of 103 clinical samples from diabetic foot ulcers were collected and analyzed for the presence of Pseudomonas aeruginosa using culture techniques and biochemical tests. The antibiotic susceptibility was assessed using the Kirby-Bauer disk diffusion method to identify effective treatments.

Results: Pseudomonas aeruginosa was identified in 48 (46.60%) of the samples. High resistance was noted against amoxicillin-clavulanic acid (100%), and notable sensitivity was observed to gentamicin (79.16%) and cefoperazone-sulbactam (87.5%). The susceptibility to other tested antibiotics varied, with moderate resistance seen in agents like cefotaxime (25%) and trimethoprim-sulfamethoxazole (33.33%).

Conclusion: The study highlights a significant presence of Pseudomonas aeruginosa in diabetic foot ulcers with substantial antibiotic resistance, emphasizing the need for precise susceptibility testing to guide effective treatment strategies.

Keywords: Antibiotic resistance, Diabetic foot ulcers, Pseudomonas aeruginosa, Susceptibility testing, Peshawar

INTRODUCTION

Diabetes mellitus, affecting approximately 382 million individuals globally as of recent estimates, is projected to impact 592 million by 2035 (1). In 2013, complications associated with this metabolic disorder led to over 5.1 million deaths, highlighting the severe global health challenge it poses (1). Among the myriad complications, diabetic foot infections stand out due to their frequency and severity, affecting about 15% of those with diabetes and often resulting in amputation (2). This critical condition primarily arises from impaired cellular function due to elevated glucose levels, which detrimentally affects insulin production and exacerbates health issues such as diabetic foot ulcers (4).

Diabetes mellitus manifests in several forms, including Type 1, an autoimmune disorder leading to insulin deficiency, and Type 2, characterized by insulin resistance and constituting the majority of cases. Additionally, conditions such as impaired glucose tolerance and impaired fasting glucose serve as precursors to full-blown diabetes, marked by elevated hemoglobin A1C levels that signal long-term impaired glucose regulation (7, 8). The chronic hyperglycemia characteristic of diabetes can inflict substantial damage on

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various bodily systems, particularly the nerves. This damage is a consequence of biochemical reactions initiated by the conversion of excess glucose into sorbitol and fructose, leading to the depletion of vital molecules like NADPH, reduced blood flow, and increased oxidative stress, culminating in nerve cell damage and contributing to the development of diabetic foot ulcers (9, 10).

The pathogenesis of diabetic foot infections is complex, often involving the bacterial colonization of ulcers, with Pseudomonas aeruginosa being a predominant pathogen. Isolated first in 1882, this bacterium thrives under specific conditions, preferring temperatures between 30-37°C and a pH of 6.6 to 7.0, and is known for its resistance to phagocytosis and its capability to secrete various toxins and proteases that enhance its virulence (3, 11, 12). Compounded by the commonly impaired immune responses in diabetics, such as reduced leukocyte function, there is an increased susceptibility to infections (11, 13, 14).

The discovery and subsequent widespread use of antibiotics marked a significant advancement in treating infections. Initially derived from natural compounds produced by microbes, the term now also includes synthetic molecules that perform similar functions, such as β-lactamase inhibitors, cephalosporins, and carbapenems (15-17). However, the rise of antimicrobial resistance, particularly among pathogens like Pseudomonas aeruginosa, poses a formidable challenge. This organism often exhibits resistance to multiple antibiotics, complicating the treatment and management of infections and increasing the risk of severe outcomes such as sepsis (18-22).

This study aims to investigate the isolation and antimicrobial susceptibility of Pseudomonas aeruginosa in diabetic foot ulcers of patients in Hayatabad Medical Complex, Peshawar, using the Kirby Bauer-Disk Diffusion method. This approach addresses the critical challenge posed by this pathogen, emphasizing the need for precise microbial identification and susceptibility testing to guide effective management strategies.

MATERIAL AND METHODS

The study was conducted at Hayatabad Medical Hospital in Peshawar, Pakistan, selected for its strategic importance and the diverse demographics it serves, including diabetic patients from Khyber Pakhtunkhwa and neighboring Afghanistan. This location provided a representative environment for investigating Pseudomonas aeruginosa in diabetic foot ulcers due to the substantial influx of diabetic patients. Over the course of the study, 103 clinical samples from diabetic foot ulcers were collected, adhering to stringent inclusion criteria that encompassed all diabetic patients undergoing treatment at the hospital, except those critically ill prior to the commencement of the study.

Sample collection employed rigorous aseptic techniques to minimize contamination risks. Sterile swabs were used to collect the samples, which were immediately cooled and transported to the Microbiology Laboratory at Wazir Muhammad Institute of Paramedical Sciences, Gandhara University, for detailed analysis. Upon arrival, each sample was cultured on nutrient agar plates, incubated at 37°C for 24 hours, where the presence of Pseudomonas aeruginosa was suggested by the emergence of a green pigment and a fruity scent (23, 24). Subsequent isolation employed MacConkey agar, followed by another 24-hour incubation period.

Microscopic examination of suspected colonies involved Gram staining, which revealed Gram-negative rods. These isolates were further tested for oxidase activity; those testing positive underwent Triple Sugar Iron Agar tests to confirm the presence of Pseudomonas aeruginosa (25). For long-term studies, pure cultures were maintained on nutrient agar slants, incubated at 37°C for 24 hours, and stored at 4°C.

Phenotypic identification of bacterial isolates was meticulously performed. Fresh bacterial cultures were subjected to Gram staining using Crystal violet and Gram's iodine, followed by decolorization with ethanol and counterstaining with safranin. The motility of bacterial isolates was assessed using a motility indole urease test, where growth patterns in the MIU medium provided evidence of bacterial motility (29).

Further biochemical identification included a series of tests: the catalase test observed for gas bubble formation upon adding bacterial inoculum to a 3% hydrogen peroxide solution, indicative of catalase activity (30). Oxidase activity was assessed by introducing isolates to an oxidase reagent and noting the color change. The urease test measured the ability to hydrolyze urea, indicated by a shift in medium color from yellow to pink, while the indole production test utilized Kovacs' reagent to detect indole production, evident from a pink to red color transition. Citrate utilization was monitored on Simmons Citrate Agar, looking for a change from green to blue (32). The Triple Sugar Iron test differentiated members of the Enterobacteriaceae family based on sugar fermentation and hydrogen sulfide production, marked by medium color changes and the formation of cracks (33).

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Antimicrobial susceptibility of Pseudomonas aeruginosa was determined using a McFarland 0.5 standard to standardize inoculum density for the modified Kirby-Bauer disc diffusion method. The method assessed inhibition zones after incubation at 37°C to evaluate susceptibility to various antibiotics (20, 35). This comprehensive methodological approach was designed to provide robust data on the prevalence and resistance patterns of Pseudomonas aeruginosa in diabetic foot ulcers at Hayatabad Medical Hospital.

RESULTS

In this study, a total of 103 samples from diabetic foot ulcer patients were analyzed, with Pseudomonas aeruginosa identified as the predominant organism in 48 cases. The demographic and clinical characteristics of the patients, including age, gender, and type of diabetes, are detailed in Table 1. Notably, the majority of patients were aged between 70-80 years and had been living with diabetes for 10-20 years. Most ulcers developed within three months prior to the study.

Table1: Demographic and clinical data of diabetic foot ulcer patients (N=103)

Characteristic	Number isolates (%)
Gender	
Male	68 (66.01)
Female	35 (33.9)
Age(years)	
40-50	14 (13.59)
60-70	39 (37.86)
70-80	50(48.54)
Type of diabetes	
Type1	81 (75.7)
Type2	22 (24.3)
Duration of diabetes (years)	
<10	47 (45.63)
10-20	50 (48.54)
>20	6 (5.82)
Duration of ulcer	
<3 month	70(67.96)
>3 month	33(32.03)

The morphological characteristics of P. aeruginosa isolates were assessed using MacConkey Agar, revealing colonies with a brownish coloration and irregular edges, as depicted in Figure 1. Gram staining further identified these isolates as gram-negative rods, which do not retain the primary stain crystal violet but take on the color of the secondary stain, safranin, indicating their gram-negative nature(36).

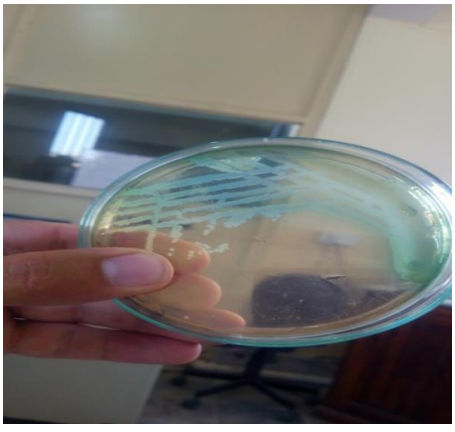

Figure 1: colony morphology of Pseudomonas aeruginosa

Biochemical testing was instrumental in characterizing the isolates. The oxidase test confirmed the presence of the enzyme oxidase, as indicated by a dark purple coloration of the smeared colony on filter paper within seconds. The catalase test showed positive results, with bubble formation confirming the enzyme's activity. In the citrate test, Pseudomonas aeruginosa utilized sodium citrate and ammonium phosphate as sole carbon and nitrogen sources respectively, causing the medium to shift from green to blue, signifying a rise in pH (Figure 2, 3.4.5)

Motility, indole, and urease tests were conducted with MIU medium, confirming that the isolates were motile and did not produce indole or urease. The Triple Sugar Iron (TSI) test demonstrated that Pseudomonas aeruginosa is a non-sugar fermenter that does not produce gas or hydrogen sulfide, maintaining an alkaline pH on both the slant and the butt of the medium.

Antibiotic susceptibility testing revealed varying resistance patterns among the isolates, with the highest resistance observed against amoxicillin-clavulanic acid and significant resistance to trimethoprim-sulfamethazole. The susceptibility pattern of Pseudomonas

aeruginosa to various antibiotics is comprehensively presented in Table1 and Figure 2. Notably, isolates showed substantial sensitivity to gentamicin, piperacillin-tazobactam, cefotaxime, ceftazidime, and cefoperazon-sulbactam, highlighting potential treatment options for managing infections caused by this pathogen in diabetic foot ulcers(28).

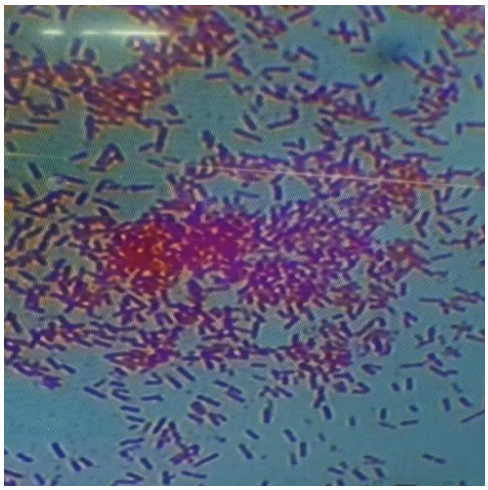

Figure 2

Figure 3

Figure 4

Figure 5

Table 2 Susceptibility pattern of Pseudomonas aeruginosa to different antibiotics

Antimicrobial agent used	Resistant No. (%)	Sensitive No. (%)
Amoxicillin-clavalunic acid	48 (100)	0 (0)
Ceftazidime	9 (18.75)	39(81.25)
Cefotaxime	12 (25)	36 (75)
Trimethoprim-sulfamethazole	16(33.33)	32(66.66)
Piperacillin-tazobactam	8 (16.66)	40 (83.33)
Gentamicin	10(20.83)	38 (79.16)
Cefoperazon-sulbactam	6(12.5)	42 (87.5)

DISCUSSION

Diabetic foot ulceration (DFU) is a common complication among diabetic patients, often stemming from poor diabetes management and inadequate healthcare services (37, 38). Such ulcers frequently become colonized by various microorganisms, which play a significant role in their pathogenesis and influence treatment strategies. This study has provided insight into the prevalence and antibiotic resistance patterns of Pseudomonas aeruginosa, a microorganism frequently encountered in hospital settings and isolated from diabetic foot ulcers.

The findings revealed a higher incidence of DFUs among males compared to females, aligning with data from Karachi which identified male gender as a risk factor for DFU (40). The mean age of the patients was 60 years, with the highest incidence observed in those aged 70-80 years. This demographic is particularly vulnerable due to factors such as increased activity levels, nutritional deficiencies, and diminished immunity, which elevate the risk of ulceration (40). Notably, Type 1 diabetic patients exhibited a higher rate of

ulceration (75.7%) compared to Type 2 (24.3%), suggesting that genetic predispositions, possibly exacerbated by consanguineous marriages common in Pakistan, might influence these patterns (41, 42).

Pseudomonas aeruginosa, a gram-negative, rod-shaped, aerobic bacterium, was identified in 46.60% of the DFUs sampled. Known for its virulence and antibiotic resistance, it produces distinctive pigments such as pyocyanin and pyoverdin which are indicative of its presence (43, 44). The bacterium's prevalence in diabetic ulcers could be attributed to prior antibiotic exposure, which may have facilitated the selection of resistant strains (45, 46).

The study underscored the challenge of multidrug resistance (MDR) in P. aeruginosa, primarily due to its low outer membrane permeability and its ability to adapt resistance mechanisms, such as enzyme production, efflux pumps, and chromosomal mutations under antibiotic pressure (47, 48). Our results corroborated other findings, showing complete resistance to amoxicillin and clavulanic acid, while resistance to piperacillin/tazobactam was relatively lower at 16.66% (49, 50). Cefotaxime and ceftazidime exhibited resistance rates of 25% and 18.75%, respectively, with ceftazidime emerging as the most effective cephalosporin. Combination antibiotics like cefaperazone/sulbactam and piperacillin/tazobactam demonstrated a high susceptibility rate of 87.5%, suggesting their potential utility in treatment protocols. Additionally, gentamicin displayed a high susceptibility rate of 79.16%, consistent with results for amikacin in similar studies (49, 51, 52).

The observed resistance to trimethoprim-sulfamethoxazole at 33.33% was lower compared to other settings, underscoring the importance of local antibiograms in guiding effective management of diabetic foot infections (53). The variability in resistance patterns across different regions highlights the necessity for ongoing surveillance and tailored antibiotic stewardship programs to combat the rise of antibiotic resistance effectively.

This study contributes valuable data to the understanding of Pseudomonas aeruginosa in diabetic foot ulcers, yet it is not without limitations. The sample size, although substantial, was limited to a single medical facility, which may affect the generalizability of the findings. Additionally, the study's focus on hospitalized patients might not fully represent the community-based prevalence and resistance patterns. Despite these limitations, the research provides critical insights into the management of DFUs, emphasizing the need for comprehensive microbial and resistance profiling in diabetic care settings.

CONCLUSION

This study has demonstrated that Pseudomonas aeruginosa isolated from diabetic foot ulcers exhibits significant antibiotic resistance, underscoring its role in nosocomial infections and highlighting the elevated risk it poses to hospitalized diabetic foot ulcer patients. The findings emphasize the critical need for tailored antibiotic susceptibility profiling to guide the selection of effective treatments for managing diabetic foot ulcers. Such targeted interventions are essential to enhance treatment outcomes, reduce complications, and mitigate the risk of severe nosocomial infections. The study advocates for the integration of precise antimicrobial susceptibility tests in routine clinical settings, thereby improving the clinical management and prognosis of diabetic patients with foot ulcers.

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