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Prevalence and Antimicrobial Susceptibility of Bacterial Isolates in Diabetic Foot Ulcers: A Comparative Study of Povidone Iodine vs. Normal Saline Dressings

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Abstract

Background and Aims: Diabetic foot ulcers (DFUs) are a major complication of diabetes mellitus, often complicated by bacterial infections leading to prolonged healing and increased amputation risk. This study aimed to assess the prevalence and antimicrobial susceptibility of bacterial isolates in DFUs and compare the efficacy of povidone iodine versus normal saline dressings on wound healing.

Materials and Methods: A prospective study was conducted at GMKMCH, Salem, from 2021 to 2023, involving 100 diabetic patients with non-healing DFUs. Pus and wound swabs were collected for aerobic culture and antibiotic susceptibility testing. Patients were randomized into two groups: Group I (povidone iodine dressing) and Group II (normal saline dressing). Wound healing was assessed using the modified Perfusion, Extent, Depth, Infection, Sensation (PEDIS) scoring system. Statistical analysis was performed using SPSS version 24.

Results: Of 100 samples, 90 yielded aerobic bacterial growth, with Staphylococcus aureus (18.4%) and Proteus species (23.2%) being the most common isolates. Polymicrobial infections were observed in 31% of cases. Povidone iodine dressings resulted in a 27.2% wound reduction score compared to 13.9% for normal saline ($p=0.001$). Gram-negative isolates showed high sensitivity to piperacillin-tazobactam (100% for Proteus), while S. aureus exhibited 55% methicillin resistance.

Conclusion: Povidone iodine dressings significantly improved wound healing compared to normal saline in DFU patients. The high prevalence of methicillin-resistant S. aureus (MRSA) and multidrug-resistant Gram-negative bacteria underscores the need for targeted antimicrobial therapy and effective wound care strategies.

KEYWORDS: Diabetic Foot Ulcer, Bacterial Isolates, Povidone Iodine, Normal Saline, Antimicrobial Susceptibility, Wound Healing.

INTRODUCTION

Diabetes mellitus, a chronic condition affecting over 500 million people globally, with prevalence continuing to rise, particularly in low- and middle-income countries [1]. Approximately 15% of diabetic individuals will develop a foot ulcer during their lifetime, a statistic that reflects the profound clinical and socioeconomic burden of this condition [1]. DFUs arise from a multifactorial pathophysiology, primarily driven by peripheral neuropathy, peripheral vascular disease, and impaired immune responses, which collectively compromise tissue integrity and healing capacity [2].

Peripheral neuropathy leads to loss of protective sensation, increasing the risk of unnoticed trauma, while vascular insufficiency hampers oxygen and nutrient delivery to tissues, delaying repair. Additionally, diabetes-related immune dysfunction, including reduced neutrophil activity and cytokine production,

predisposes patients to infections, which further exacerbate ulcer chronicity and severity [2]. These factors converge to create a vicious cycle of tissue breakdown, infection, and delayed healing, making DFUs a leading cause of morbidity in diabetic populations.

Infected DFUs are particularly concerning, as they account for a significant proportion of diabetes-related hospitalizations and are implicated in up to 60% of non-traumatic lower limb amputations [3]. The microbial profile of DFUs is typically complex, often involving polymicrobial infections comprising both aerobic and anaerobic bacteria. Common pathogens include Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Proteus species, with polymicrobial infections reported in up to 50% of cases [4].

Infections are not only difficult to treat due to their diverse etiology but are also complicated by the growing threat of antimicrobial resistance. Methicillin-resistant S. aureus (MRSA) and multidrug-resistant Gram-negative bacilli have become increasingly prevalent, particularly in hospital settings, where they contribute to treatment failures and poor clinical outcomes [7]. This rise in resistance underscores the urgent need for microbiological surveillance to identify local pathogen profiles and their susceptibility patterns, enabling clinicians to change antibiotic therapy effectively, especially in regions where empirical treatment is common due to resource constraints.

Effective wound management is a cornerstone of DFU care, aimed at controlling infection, reducing bacterial bioburden, and promoting tissue regeneration. Among the myriad wound care modalities, topical antiseptics like povidone iodine and isotonic solutions such as normal saline are widely utilized due to their accessibility, cost-effectiveness, and ease of application. Povidone iodine, a broad-spectrum antiseptic, exerts its bactericidal effects through the release of free iodine, which disrupts microbial cell walls, proteins, and metabolic pathways [5].

However, concerns about potential cytotoxicity to fibroblasts and keratinocytes have prompted debates about its safety in long-term use, particularly in wounds with delicate granulation tissue [5]. In contrast, normal saline is favored for its biocompatibility, low toxicity, and ability to maintain a moist wound environment, which is critical for epithelialization and tissue repair [6]. However, normal saline lacks inherent antimicrobial properties, potentially limiting its effectiveness in heavily infected ulcers where bacterial colonization is a primary barrier to healing.

The comparative efficacy of povidone iodine versus normal saline remains a subject of ongoing debate, as clinical outcomes depend on factors such as wound severity, infection status, and patient comorbidities. While povidone iodine is hypothesized to offer superior infection control due to its antimicrobial activity, normal saline may be adequate for less severe ulcers or as a maintenance therapy in the absence of significant infection.

This study aimed to evaluate the prevalence and antimicrobial susceptibility of bacterial isolates in DFUs and compare the efficacy of povidone iodine versus normal saline dressings in promoting wound healing. By addressing these objectives, we seek to inform clinical practice and improve outcomes for DFU patients.

MATERIALS AND METHODS

Study Setting: This prospective study was conducted at the Department of General Surgery, Government Mohan Kumaramangalam Medical College Hospital (GMKMCH), Salem, Tamil Nadu, India, in collaboration with the Department of Microbiology, from 2021 to 2023. The study was approved by the Institutional Ethics Committee, and informed consent was obtained from all participants.

Study Participants: A total of 100 diabetic patients aged >20 years with non-healing DFUs (duration >4 weeks) were enrolled. Inclusion criteria included patients with Type 1 or Type 2 diabetes and DFUs classified as Wagner's Grades I to V. Exclusion criteria comprised patients on antibiotics, those with non-diabetic ulcers, evidence of gangrene, osteomyelitis, or significant comorbidities (e.g., liver failure, renal impairment, malignancies), and those with low serum albumin (<2.5 g/dL), hemoglobin (<10.5 mg/dL), or platelet count (<100 x 10^9/L).

Sample Collection and Microbiological Analysis: Wound swabs and pus samples were collected after cleaning the ulcer with sterile normal saline and removing superficial debris. Samples were inoculated into Brain Heart Infusion broth and cultured on 5% sheep blood agar, MacConkey agar, and nutrient agar. Bacterial isolates were identified using standard microbiological techniques, including Gram staining and biochemical reactions [8]. Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method per Clinical Laboratory Standards Institute (CLSI) guidelines, with antibiotics including penicillin,

erythromycin, ampicillin, amoxyclav, gentamicin, amikacin, linezolid, cefotaxime, cephalexin, ciprofloxacin, vancomycin, co-trimoxazole, oxacillin, piperacillin-tazobactam, cefoperazone-sulbactam, ceftriaxone, ceftazidime, and meropenem.

Intervention and Wound Assessment: Patients were randomized into two groups: Group I (n=50, povidone iodine dressing) and Group II (n=50, normal saline dressing). Dressings were applied daily for six consecutive days after cleaning with hydrogen peroxide and normal saline. Wound healing was assessed using the modified PEDIS scoring system, which evaluates perfusion, extent, depth, infection, and sensation. Assessments were conducted at baseline (pretest) and after six days (posttest).

Statistical Analysis: Data were analyzed using SPSS version 24. Categorical variables were expressed as frequencies and percentages, and continuous variables as means ± standard deviations. The McNemar test assessed changes in wound grades, while the Student's t-test compared mean wound healing scores between groups. A p-value <0.05 was considered statistically significant.

RESULTS

Demographic and Clinical Characteristics

The study included 100 patients (70 males, 30 females) with a mean age of 54.5 years. Most patients were in their 5th (37%) and 6th (28%) decades of life. Type 2 diabetes was predominant (85%), and 76% of patients had HbA1c levels >8%, indicating poor glycemic control. Hypertension (43%) and coronary artery disease (30%) were common comorbidities (Table 1).

Table 1: Demographic and Clinical Characteristics of Study Participants (N=100).

Characteristic	Mean (SD) / N (%)
Age (years)	54.5 (12.3)
Male	70 (70%)
Female	30 (30%)
BMI (kg/m²)	24.8 (3.2)
HbA1c (%)	9.2 (1.8)
Duration of Diabetes (years)	6.4 (4.1)
Hypertension	43 (43%)
Coronary Artery Disease	30 (30%)
Type 2 Diabetes	85 (85%)

Distribution of Ulcers by Wagner's Classification

Most ulcers were classified as Wagner's Grade II (40%) or Grade III (38%), with fewer in Grades IV (16%) and V (6%) (Table 2).

Table 2: Distribution of Ulcers According to Wagner's Classification.

Wagner's Grade	No. of Patients (%)
Grade I	0 (0%)
Grade II	40 (40%)
Grade III	38 (38%)
Grade IV	16 (16%)
Grade V	6 (6%)

Bacterial Isolates and Growth Patterns

Of 100 samples, 90 yielded aerobic bacterial growth, with 62 (69%) showing monomicrobial and 28 (31%) polymicrobial infections. Staphylococcus aureus (18.4%) was the most common Gram-positive isolate, while Proteus species (23.2%) predominated among Gram-negative isolates, followed by Escherichia coli (16.8%) and Pseudomonas aeruginosa (16%) (Table 3).

Table 3: Distribution of Bacterial Isolates.

Bacterial Isolate	No. of Isolates (%)
Staphylococcus aureus	23 (18.4%)
Coagulase-Negative Staphylococci	6 (4.8%)
Enterococcus faecalis	1 (0.8%)
Proteus species	29 (23.2%)
Escherichia coli	21 (16.8%)
Pseudomonas aeruginosa	20 (16%)
Klebsiella species	11 (8.8%)
Acinetobacter species	3 (2.4%)
Citrobacter freundii	2 (1.6%)

Antimicrobial Susceptibility Patterns

Gram-positive isolates showed 100% sensitivity to vancomycin and linezolid. S. aureus exhibited 55% methicillin resistance (MRSA). Gram-negative isolates, particularly Proteus species, were highly sensitive to piperacillin-tazobactam (100%) and cefoperazone-sulbactam (84%). Pseudomonas aeruginosa showed 100% sensitivity to meropenem (Table 4).

Table 4: Antimicrobial Susceptibility Pattern of Key Isolates.

Antibiotic	S. aureus (n=23) (%)	Proteus sp. (n=29) (%)	P. aeruginosa (n=20) (%)
Piperacillin-Tazobactam	-	100	94
Cefoperazone-Sulbactam	-	84	68
Meropenem	-	-	100
Amikacin	69.5	46	10.5
Ciprofloxacin	47.8	53	47
Vancomycin	100	-	-
Linezolid	100	-	-

Wound Healing Outcomes

Povidone iodine dressings significantly improved wound healing compared to normal saline. Post-test results showed 63.3% of Group I patients achieving Grade I wound scores versus 33.3% in Group II (p=0.001). The mean wound reduction score was 27.2% for povidone iodine and 13.9% for normal saline (Table 5).

Table 5: Comparison of Wound Healing Outcomes.

Group	Pretest Mean (SD)	Post-test Mean (SD)	Mean Difference (95% CI)	Wound Reduction (%)
Povidone Iodine (n=50)	5.80 (0.48)	2.53 (1.38)	3.27 (2.73–3.80)	27.2 (22.8–31.7)
Normal Saline (n=50)	5.97 (0.18)	4.30 (1.42)	1.67 (1.14–2.20)	13.9 (9.5–18.3)

DISCUSSION

This study provides compelling evidence of the high burden of bacterial infections in diabetic foot ulcers (DFUs) and the superior efficacy of povidone iodine dressings over normal saline in promoting wound healing. The observation that 90% of samples yielded aerobic bacterial growth highlights the pervasive role of infection in DFU chronicity and complications. The predominance of Staphylococcus aureus (18.4%) among Gram-positive isolates and Proteus species (23.2%) among Gram-negative isolates is consistent with previous studies, which identify these pathogens as frequent culprits in DFU infections [9].

The significant prevalence of polymicrobial infections (31%) further underscores the microbiological complexity of DFUs, as multiple pathogens can act synergistically to exacerbate tissue damage, impair immune responses, and delay healing [10]. This complexity necessitates routine microbiological evaluation, including culture and susceptibility testing, to ensure that treatment strategies are tailored to the specific microbial profile of each ulcer.

The 55% prevalence of methicillin-resistant S. aureus (MRSA) is a particularly concerning finding, aligning with global trends of escalating antimicrobial resistance, especially in hospital-acquired infections [11]. MRSA is associated with increased morbidity, including prolonged wound healing, higher rates of systemic infection, and greater risk of amputation, due to its resistance to many first-line antibiotics. The 100% sensitivity of S. aureus to vancomycin and linezolid provides a critical therapeutic lifeline, but the reliance on these agents raises concerns about potential resistance emergence, particularly in settings with limited antibiotic stewardship.

For Gram-negative isolates, the high sensitivity of Proteus species to piperacillin-tazobactam (100%) and cefoperazone-sulbactam (84%), and of Pseudomonas aeruginosa to meropenem (100%), offers valuable guidance for empiric therapy [12]. However, the use of broad-spectrum antibiotics like meropenem must be judicious to prevent further resistance development, particularly in resource-limited settings where access to advanced antibiotics is restricted. These susceptibility patterns emphasize the importance of local microbiological data in informing treatment protocols, as resistance profiles can vary significantly by region and healthcare setting.

The most striking clinical finding of this study is the superior efficacy of povidone iodine dressings, which achieved a 27.2% wound reduction score compared to 13.9% for normal saline ($p=0.001$). This significant difference is likely attributable to povidone iodine's broad-spectrum bactericidal activity, which effectively reduces wound bioburden, a primary barrier to healing in infected DFUs [13].

The modified PEDIS scoring system revealed that 63.3% of patients treated with povidone iodine achieved Grade I wound scores, compared to only 33.3% in the normal saline group, highlighting the antiseptic's ability to promote rapid improvements in wound status. These findings are consistent with prior research, such as Shetty et al., which reported enhanced epithelialization and reduced infection rates with povidone iodine in non-healing ulcers [14].

In contrast, normal saline's limited antimicrobial activity likely explains its inferior performance, despite its role in maintaining a moist wound environment conducive to tissue repair. While normal saline is biocompatible and minimizes toxicity to healing tissues, its inability to actively combat bacterial colonization may allow persistent infections to hinder granulation and epithelialization. The marked difference in wound healing outcomes suggests that povidone iodine should be prioritized in the management of infected or high-risk DFUs, while normal saline may be reserved for less severe cases or as a maintenance therapy once infection is controlled.

The strong association between poor glycemic control (HbA1c >8% in 76% of patients) and higher Wagner's grades underscores the critical role of systemic factors in DFU outcomes [15]. Hyperglycemia impairs neutrophil function, delays collagen synthesis, and exacerbates neuropathy and vascular disease, all of which increase infection susceptibility and delay healing. This finding highlights the need for a multidisciplinary approach to DFU management, integrating effective wound care with aggressive glycemic control, nutritional optimization, and vascular interventions where indicated. The predominance of Wagner's Grade II and III ulcers in this study reflects a patient population with moderate-to-severe disease, emphasizing the importance of early intervention to prevent progression to higher grades, which are associated with greater morbidity and amputation risk.

This study has a few limitations. The focus on aerobic bacteria excludes the potential contribution of anaerobic pathogens, such as Bacteroides or Peptostreptococcus species, which are increasingly recognized in deep or chronic DFUs. The short intervention period of six days, while sufficient to detect early healing trends, does not capture long-term outcomes such as complete ulcer closure, recurrence rates, or amputation incidence. Additionally, patient-specific factors, such as compliance with offloading, socioeconomic barriers, or psychological factors, were not assessed, despite their potential impact on healing.

The clinical implications of these findings are significant, particularly in resource-limited settings like India, where DFUs contribute to substantial healthcare costs and disability. The cost-effectiveness and accessibility of povidone iodine make it an attractive option for widespread adoption, especially in tertiary care centers managing complex cases. However, its use should be guided by proper dilution and application protocols to minimize potential cytotoxicity. Furthermore, the integration of advanced wound care technologies, such as negative pressure wound therapy or bioactive dressings, could enhance outcomes when combined with povidone iodine, offering a synergistic approach to infection control and tissue regeneration.

CONCLUSION

Povidone iodine dressings are more effective than normal saline in promoting wound healing in DFUs, with significant reductions in wound scores. The high prevalence of MRSA and multidrug-resistant Gram-negative bacteria emphasizes the need for routine culture and susceptibility testing to guide therapy. Integrating effective wound care with glycemic control and targeted antibiotics is essential to reduce morbidity and prevent amputations in DFU patients.

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