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Research Article

Microbial Profile of Diabetic Foot Ulcers of Patients Attending the Regional Hospital Bamenda (Cameroon)

Eban-Odi Luisa Nang and Asanghanwa Milca*

Department of Medical Laboratory Sciences, Faculty of Health Sciences, University of Bamenda, Bambili, Cameroon

***Corresponding Author:** Asanghanwa Milca, Department of Medical Laboratory Sciences, Faculty of Health Sciences, University of Bamenda, Bambili, Cameroon.

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Abstract

Introduction: Diabetic foot ulcers are a common complication in patients with diabetes mellitus and are associated with a high risk of amputation.

Aim: To determine the microbial profile of diabetic foot ulcers and the antimicrobial susceptibility of isolates from infected ulcers in patients attending the Regional Hospital Bamenda.

Methods: A cross-sectional hospital-based study conducted from May 2023 to July 2023; involving 25 samples from consenting patients with diabetic foot ulcers. Pus samples were obtained from the base of the ulcers using sterile swabs and inoculated into Mannitol Salt, McConkey, Blood and Sabouraud Dextrose agar. Antibiogram was performed using the Kirby-Bauer disc diffusion technique.

Results: The study included 25 samples, and our results showed that *Staphylococcus aureus* was the most commonly isolated organism (32%); followed by *Pseudomonas* spp (24%), *Escherichia coli* (12%), *Klebsiella* spp (12%), and *Proteus* spp (8%). Fungal isolates, specifically from the Candida group were found in 4% of cases. Antimicrobial susceptibility testing revealed multidrug resistance to broad-spectrum antibiotics in most of the isolated microorganisms.

Conclusion: Our findings highlight the diverse microbial nature of diabetic foot ulcers, with *Staphylococcus aureus* being the predominant pathogen. The high prevalence of multidrug resistance emphasizes the need for alternative treatment approaches in the management of diabetic foot ulcers in this setting.

Keywords: Diabetic Foot Ulcer; Bacterial Profile; Antimicrobial Susceptibility

Introduction

Diabetic foot ulcers are among the most common complications of patients with diabetes mellitus [1]. It is usually the result of poor glycemic control, underlying neuropathy, peripheral vascular disease or poor foot care. Many foot ulcers fail to heal and lead to severe complications such as osteomyelitis. Infection is the most common cause of lower limb amputation in diabetic foot ulcers [2]. People with diabetes mellitus are at higher risk of developing diabetic foot ulcers due to impaired metabolic mechanisms that can lead to decreased cell and growth factor response, diminished peripheral blood flow, and decreased local angiogenesis (the formation of new blood vessels); and these can result in damage to the peripheral nerves, peripheral vascular disease, ulcerations, deformities, and even gangrene (tissue death) in the feet [3]. Gram-

negative bacteria and other organisms such as *Staphylococcus aureus* and *Streptococcus pyogenes* have been identified in diabetic foot ulcers [4]. Mixed flora, including strains of aerobic bacteria such as *Staphylococcus aureus*, *Streptococcus pyogens*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*, as well as anaerobic bacteria like *Bacteriodes fragilis*, *Clostridium perfringes, Peptostreptoccocus spp, and Provetella oralis*, have been implicated in diabetic foot ulcers [5]. The most common fungi found in diabetic foot ulcers are *Candida* and *Aspergillus* species. Other common fungi include *Fusarium, Mucor*, and *Rhizopus* species. These infections can occur in patients with diabetes due to the decreased blood flow and poor circulation that often accompanies the disease, which can make it difficult for wounds to heal. Additionally, people with diabetes are more susceptible to infections due to the high sugar levels in their blood, which can create a favorable environment for fungal growth [6,7]. Treatment of infected diabetic foot ulcers typically involves the use of antibiotics, debridement, and wound care. However, the emergence of antibiotic-resistant strains of bacteria has made the treatment of diabetic foot ulcers more challenging. Therefore, it is essential to monitor the bacterial flora of diabetic foot ulcers and to use appropriate antibacterial agents to achieve adequate wound healing [6]. Understanding the types of bacteria that are commonly associated with diabetic foot ulcers and their antimicrobial susceptibility patterns is essential for effective diagnosis and treatment [8]. In this context, recent studies have begun to shed more light on the microbial profile and antimicrobial susceptibility patterns of diabetic foot ulcers in Cameroon, revealing a complex and diverse range of bacteria, including both gram positive and gram-negative bacteria as well as fungi [9]. These studies have also highlighted the emergence of antibiotic-resistant strains of bacteria, emphasizing the need for effective infection control measures and targeted antimicrobial therapies to improve outcomes for patients with diabetic foot ulcers in Cameroon. This hospital based cross-sectional study aimed at providing information on the microbial picture of diabetic foot ulcers and the antimicrobial susceptibility pattern of isolates in diabetes patients from Bamenda, the North West Region of Cameroon. The results may provide new information for improving empiric therapy in patients with diabetic foot ulcers in this setting.

Methods

This study was a cross sectional, hospital-based study. The participants in the study were diabetic patients who had foot ulcers and were receiving care at the Bamenda Diabetes center admitted in wards D and E, as well as outpatients who were being treated at the center. 25 diabetic patients with foot ulcers were included in this study, which was conducted from May 2023 to July 2023 at the Bamenda Regional Hospital, Cameroon. Demographic data and clinical data of the patients were gotten using a well-structured questionnaire. Samples were collected from patients after obtaining their informed consents.

Sample collection and inoculation

To obtain samples for analysis, two sterile swabs were used to collect pus or exudate from the deeper part of the foot ulcers. The swabs were gently rotated over the base of the ulcer and one was used for gram staining, while the other was used for culture. A direct gram-stained smear of the specimen was examined. The specimens were inoculated into McConkey agar, Mannitol salt agar, Blood agar and Sabouraud Dextrose agar. The inoculated plates were incubated at 37^0C for 24 hours and the plates were examined for growth. The organisms were identified on the basis of their gram staining properties and their biochemical reactions to biochemical tests.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done using Mueller Hinton agar on isolated colonies according to 'Kirby Bauer' disc diffusion method. Antimicrobial agents used for antimicrobial susceptibility testing of isolates were: Gentamycin, Ciprofloxacin, Ceftriaxone, Imipenem, Augmentin, Doxycycline, Nitrofurantoin, Amikacin for the gram-negative bacilli and Ciprofloxacin, Imipenem and Erythromycin for the gram-positive cocci. Ketoconazole, Fluconazole, Flucytosine and Nystatin were used for fungi. Results were interpreted as resistant (R), intermediate (I), or sensitive (S) for each antimicrobial according to standard ranges of Clinical and Laboratory Standards Institute (CLSI).

Statistical analysis

The data collected was entered into a Microsoft Excel spreadsheet, then imported into the Statistical Package for Social

Sciences (SPSS) version 20.1 for analysis. The Chi-square test was used to determine differences between categorical variables, and *p*-values used to interpret the results.

Ethical consideration

The study was approved by the Ethical Review Committee of the Bamenda Regional Hospital.

Results

Description of study participants

The study involved hospitalized Diabetes mellitus patients between May 2023 and July 2023 at the Bamenda Regional Hospital – Cameroon, who had foot ulcers. A total of 25 patients were included in this study; 17 (68%) males and 8 (32%) females. Participants were aged 38 to 90 years, with majority (88%) above age 50. Most (64%) participants had a duration of Diabetes mellitus of 5-10 years; 4(16%) had a disease duration of less than 5 years and 5 (20%) a disease duration of above 10 years. According to the level of education, 7 (28%) participants had completed primary education; 15 (60%) at least secondary education and 3 (12%) had not gone to school. Twenty-three (92%) participants were clinically classified as having Type 2 diabetes, while the diabetes classification of 2 (8%) participants were unknown.

Prevalence and type of micro-organisms isolated from the diabetic foot ulcers

Overall, both gram-positive and gram-negative bacteria, and fungi contributed to infections recorded in the diabetic foot ulcers. From the 25 samples analyzed, 23 (92%) had growth. *Staphylococcus aureus* was the commonest bacterium isolated (32%), followed by *Pseudomonas* species (24%). *Klebsiella* species and *Escherichia coli* were isolated from 12% of the samples respectively. *Proteus* species was isolated from 8% of the samples. Only one sample (4%) was positive for fungi, and the isolate was from the *Candida* group (Figure 1).

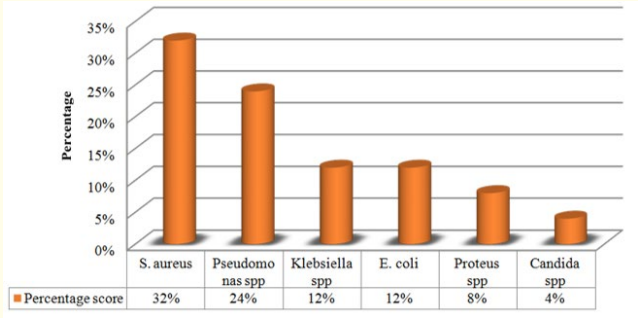

Figure 1: Different microorganisms isolated from the diabetic foot ulcersn.

Factors influencing microbial growth

Foot ulcers of patients who have had diabetes for more than 5 years had more bacterial growth compared to patients with a diabetes duration of less than 5 years (81% vs 25%, p = 0.022). Ulcers of patients who rarely had foot inspection had more growth compared to those of patients who regularly did (74% vs 0%, p = 0.03). Ulcers of patients not practicing good foot hygiene had more bacterial growth compared to those of patients practicing good foot hygiene (71% vs 23%, p = 0.028). Patients with persistent and worsening foot ulcers also had more growth compared to newly developed ulcers and ulcers undergoing healing (p = 0.006) (Table 1).

Variable		Frequency (n = 25) positive n (%)	Bacterial Growth		X^2 (p-value)
			Negative n (%)		
Duration of diabetes	0 – 5 years ≥ 6 years	4 21	1 (25) 17 (81)	3 (75) 4 (19)	5.218 (0.022)
Duration of foot ulcers	0 – 2 months ≥ 3 months	13 12	10 (77) 8 (67)	3 (23) 4 (33)	0.326 (0.568)
Regular foot inspection	Yes No	2 23	0 (0) 17 (74)	2 (100) 6 (26)	4.620 (0.031)

Antibiotic treatment	Yes No	20 5	16 (80) 2 (40)	4 (20) 3 (60)	3.175 (0.074)
Practice of good foot hygiene	Yes No	11 14	3 (23) 10 (71)	8 (77) 4 (29)	4.812 (0.028)
Current status of foot ulcer	Healing	1	0 (0)	1 (100)	12.53 (0.006)
	Persistent	17	15 (88)	2 (12)	
	Worsening	4	3 (75)	1 (25)	
	New	3	0 (0)	3 (100)	

Table 1: Factors influencing microbial growth from the diabetic foot ulcers.

Significant at $p < 0.05$.

Antimicrobial susceptibility testing

For gram-positive cocci

Staphylococcus aureus isolates were resistant (100%) to Augmentin, resistant (100%) to Erythromycin, resistant (87.5%) to Gentamycin, resistant (75.0%) to Ciprofloxacin, resistant (62.5%) to Ceftriaxone, and resistant (50.0%) to Imipenem (Figure 2).

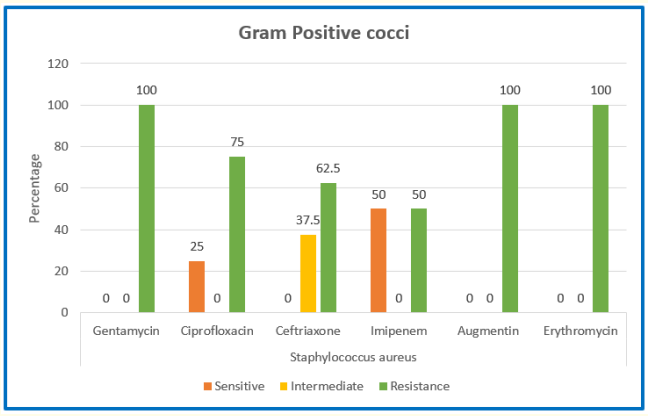

Figure 2: Gram Positive Cocci susceptibility.

For gram negative bacilli

Pseudomonas spp isolates were resistant (83.3%) to Ceftriaxone, resistant (83.3%) to Gentamycin, resistant (50.0%) to Ciprofloxacin but sensitive (100%) to imipenem. *Klebsiella spp* isolates were to a similar extent resistant to Ceftriaxone, Ciprofloxacin, Gentamycin and Imipenem (66.7%) broad-spectrum antibiotics. *Escherichia coli* isolates were resistant (100%) to Gentamycin, resistant (100%) to Doxycycline, resistant (100%) to Nitofurantoin, resistant (66.7 %) to Ceftriaxone but sensitive (100%) to Ciprofloxacin and Imipenem (100%). *Proteus spp* isolates were resistant (100%) to Imipenem, resistant (100%) to Ceftriaxone, resistant (50%) to Gentamycin and resistant (50%) to Amikacin (Figure 3).

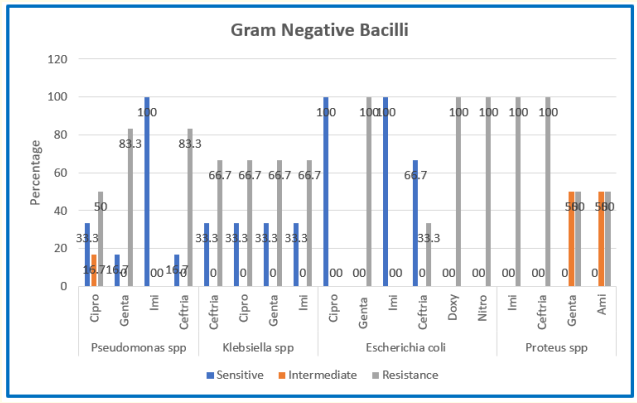

Figure 3: Gram negative bacilli susceptibility.

For fungi

The *Candida spp* isolate was resistant (100%) to Ketoconazole, Fluconazole, Flucytosine and Nystatin (Figure 4).

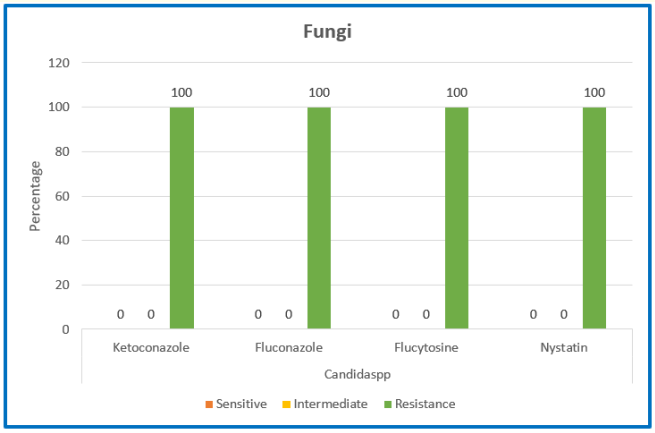

Figure 4: Fungi susceptibility.

Discussion

The aim of this study was to investigate the types of microorganisms present in diabetic foot ulcers of patients attending the Regional Hospital Bamenda, Cameroon. *Staphylococcus aureus* was the most common bacterium isolated, accounting for 32% of the cases, followed by *Pseudomonas* species (24%) and *Klebsiella* and *Escherichia coli* (12% each). These results are similar to those conducted in three selected hospitals in Addis Ababa, Ethiopia by Atlaw A., *et al.* who also identified *Staphylococcus aureus* as the most prevalent organism (25.19%), followed by *Pseudomonas* species (18. 89%) and *Escherichia coli* (16.53%) [10]. In another study by Kengue AP., *et al. Proteus mirabilis* was the most common microorganism isolated in association with *Staphylococcus aureus* [11]. This difference may be associated with the difference in geographical locations and predisposing factors.

Our study showed a male preponderance in the occurrence of diabetic foot ulcers; consistent with data from studies conducted in Indonesia and India by Murshed M., *et al.* and Shah P., *et al.* respectively [12,13]. This may be due to the fact that men are more involved in outdoor activities that increase the likelihood of injuries and the development of ulcers. Most of the participants in this study were aged 50 and above, similar to records of the afore-mentioned studies conducted in India and Indonesia. This could be due to the fact that as age increases, there is reduced sensation in the feet due to age-related peripheral neuropathy. This phenomenon can make it difficult to detect foot injuries and infections, hence the development and progression to foot ulcers.

In this study, most participants had a current status of foot ulcer that was persistent which is consistent with previous study carried out in Indonesia by Mushed M., *et al.* where the rate of bacterial isolation in the ulcer increased as the severity of the ulcer increased. This may be due to the fact that microorganisms play a significant role in the healing process of diabetic foot ulcers and the presence of bacterial infections in diabetic foot ulcers is associated with delayed healing and an increased risk of amputation [12].

Most participants in this study had a duration of diabetes mellitus of above 5years, consistent with data from a study conducted in Cameroon by Kengne AP., *et al.* where participants with diabetic foot ulcers had a duration of diabetes of over 8 years [14]. The longer a person has diabetes, the greater the risk of developing complications such as nerve damage which can contribute to the development of foot ulcers.

In this study, antimicrobial susceptibility of the various microorganisms isolated was tested using broad spectrum antibiotics. Isolates from our study showed multi-drug resistance with Augmentin Erythromycin, Doxycycline and Nitrofurantoin. This outcome coincides with those of a previous study carried out in Brazil by Alexandre F., *et al.* of multidrug resistance of bacterial isolates from diabetic foot ulcers. The reason for this may be due to the overuse and misuse of antibiotics, self-medication, insufficient hygienic practices in healthcare settings and also chronic nature of diabetic foot ulcers. The non-adherence of patients to a particular hospital for the management of their ulcers may also lead to the administration of multiple courses of antibiotics which may have an antagonistic effect on the healing process. This study confirms that antimicrobial resistance is a growing health problem and a major concern among hospitalized persons [15].

Conclusion

Staphylococcus aureus is the main bacterium isolated from a cohort of diabetic foot ulcers of diabetes patients attending the Regional Hospital Bamenda – Cameroon. Other isolates included *Pseudomonas* species, *Escherichia coli*, *Klebsiella* species, *Proteus* species and *Candida species.* Our findings confirm multidrug resistance to the different isolates, with Imipenem antibiotic being more applicable to both gram-negative and gram-positive isolates compared to the others tested.

Limitation: We could only identify 25 diabetes patients with foot ulcers within the study period.

Acknowledgement

We extend our heartfelt gratitude to the patients with diabetic foot ulcers who generously gave their consent and actively participated in this study. Their willingness to contribute their time, samples, and personal information was instrumental in advancing our understanding of diabetic foot ulcers and the microbial factors associated with them.

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