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ABSTRACT

Background: **Diabetic foot infections are a common complication of diabetes mellitus. The microbiology of diabetic foot infections and sensitivity to antimicrobials varies geographically.**

Objective: **To identify causative organisms and sensitivity to commonly available antimicrobials**

Design: **This was a cross-sectional descriptive study conducted over a one-year period.**

Study Setting: **The Nakuru Level 5 Hospital wound clinic.**

Subjects/Participants: **The study recruited individuals with diabetic foot ulcers from the Nakuru Level 5 Hospital wound clinic. Deep tissues obtained during the debridement of infected diabetic foot ulcers were sent for culture and sensitivity. Micro-organisms grown in culture were identified and sensitivity to antibiotics was tested using the disk infusion method.**

Results: **More than half (54%) of the diabetic foot infection cases were caused by multiple microbes, while 34% were caused by a single organism. Only aerobic bacteria were isolated and gram-negative bacteria were predominant. Specifically, *Staphylococcus aureus* and *E. coli* were the most common organisms isolated. Most organisms (68.8%) were susceptible to ciprofloxacin. The bacterial isolates were resistant to some of the commonly use antibiotics including cefuroxime (23.5%), ampicillin (19.6%), gentamicin (17.6%), and ceftriaxone (13.7%).**

Conclusion: **Ciprofloxacin is an ideal choice for empiric antimicrobial therapy.**

The etiology of DFU infections is predominantly polymicrobial. *Staphylococcus aureus* **and** *E. coli* **are the main causes of diabetic foot infections.**

Keywords: **Diabetic Foot Ulcers (DFU), Microbiology, Antimicrobial Sensitivity**

INTRODUCTION

Diabetic foot ulcers (DFU) are a major health problem for persons living with diabetes. It is estimated that at least a quarter of all diabetics develop diabetic foot ulcers in their life. In addition to increasing the risk of mortality among diabetics, DFUs cause significant morbidity with at least one in every five cases resulting in lower limb amputation (1, 2). The risk of lower limb amputation increases further when the foot ulcer is infected and the foot is ischemic (3).

The pathophysiology of DFU involves an interplay of vascular, neurological and biomechanical factors. The major risk factors for developing DFU include older age, male gender, longer diabetes duration, poor glucose control, poor foot care, foot deformities, impaired circulation and peripheral neuropathy (4). DFUs are also more common among individuals of low social-economic status (1). A recent systematic review of local studies found that 13.4% of diabetic individuals in sub-Saharan Africa develop foot ulcers. Peripheral neuropathy, longer duration of illness, living in rural areas, and poor self-care practice were the main risk factors to developing DFUs (5).

The management of DFU is guided by the following principles; debridement, pressure relief, addressing lower limb ischemia and controlling wound infection (1). Addressing the wound infection problem in

the management of DFUs calls for clinicians to understand the microbiology of DFUs, and the antimicrobial sensitivity patterns.

The microbiology of DFUs is diverse and may vary with geographical location. Globally, Staphylococcus aureus is the most commonly isolated organism in cases of DFU infections. Other common causes of DFU infections are Pseudomonas spp., E. coli and Enterococcus spp (6). Similarly, cases of DFU infections in sub-Saharan Africa are attributed to S. aureus (34.34%), *E. coli* (21.16%), and *P. aeruginosa* (20.98%) (7).

Infection control is an important aspect of DFU care. Infection control is however complicated by concerns of antimicrobial resistance (8). A systematic review of DFUs by Li *et al* identified Staphylococcus aureus as the most common cause of infections with 288 out of 1336 isolates exhibiting multidrug-resistance (9). In an analysis of studies conducted in 12 Asian countries, Sultana *et al* also highlighted a worrying trend of antimicrobial resistance displayed by both gram negative and gram positive organisms (10). Similarly, a review of studies in sub-Saharan Africa highlighted a high prevalence of antimicrobial resistance. The authors noted that more than 50% of isolated organisms were resistant to commonly used antibiotics such as gentamicin, and ciprofloxacin (7). The high prevalence of antimicrobial resistance in cases of DFU infections highlights the need for regular culture & sensitivity testing.

Therefore, a comprehensive understanding of the local microbiology of DFU and antimicrobial sensitivity patterns is essential to guide the management of DFU. This study was conducted to map out the most common microbes in DFU among diabetic patients in Nakuru, Kenya. Further, the study investigated the sensitivity patterns to commonly available antibiotics, to guide empiric therapy.

MATERIALS AND METHODS

Study design: This was a descriptive cross-sectional study conducted over a one-year period.

Study Setting: The study was conducted at the Nakuru Level 5 hospital, Nakuru, Kenya.

Subjects/Participants: Convenient sampling was used to recruit 41 individuals with diabetic foot ulcers from the local wound care clinic. The study included individuals with diabetic foot ulcers, and excluded individuals with non-diabetic wounds. The study analyzed forty-one wounds; all participants had only one area affected by ulceration.

Sampling Procedure

The surgical procedures were performed in an operating room while observing aseptic techniques. Wound debridement was performed removing all infected and non-viable tissues. The deeper tissues were obtained and sent for culture and sensitivity in sterile containers within less than an hour of the collection.

The samples were cultured in a variety of agars including Sabouraud agar, blood agar, EMB agar and anaerobic agar. Inoculums were incubated for 24-48 hours at optimum temperature 35^0C-37^0C. Specific species of gram-positive bacteria were identified using catalase test, optochin test, hemolysis reaction and bacitracin test. Oxidase test was used to identify specific species of gram-negative bacteria. The disk infusion method was used to test susceptibility to antibiotics. Disks impregnated with commonly available antibiotics were placed on the surface of agar plates with micro-organisms isolated from swabs. The size of the inhibition zone was interpreted using the Clinical and Laboratory Standards Institute (CLSI) guidelines (11).

Data Analysis: Microsoft Excel®, Microsoft Office 21 version, was used to analyze the data. The data was represented as frequencies, percentages, in tables and charts.

Ethical Consideration

The study obtained approval from the Nakuru Level 5 hospital research ethics committee.

Informed consent was obtained from the study participants. The financial cost of performing the culture & sensitivity tests was covered by the researchers.

RESULTS

Demographics

This study analyzed samples from forty-one patients with diabetic foot ulcers. The study analyzed forty-one wounds, all participants had only one area affected by ulceration.

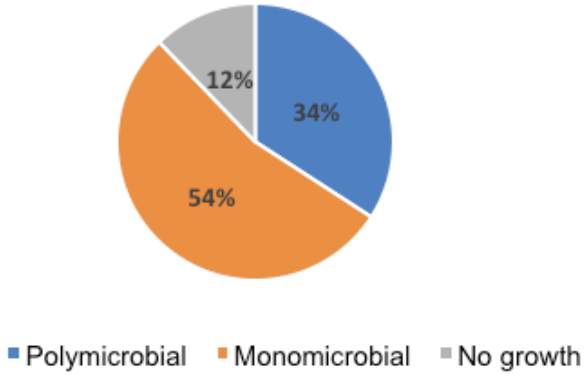
Male patients accounted for 49% of the study participants, while 51% were female. The average age of the participants was 61.7 years (SDV=12.30).

Microbiology

The cultures results revealed that DFU infections were caused by multiple organisms. More than half of (54%) of the cultures yields were poly-microbial, with 34% resulting

in mono-microbial growth. No growth was reported in 12% of the cultures.

Figure 1: Culture growth status



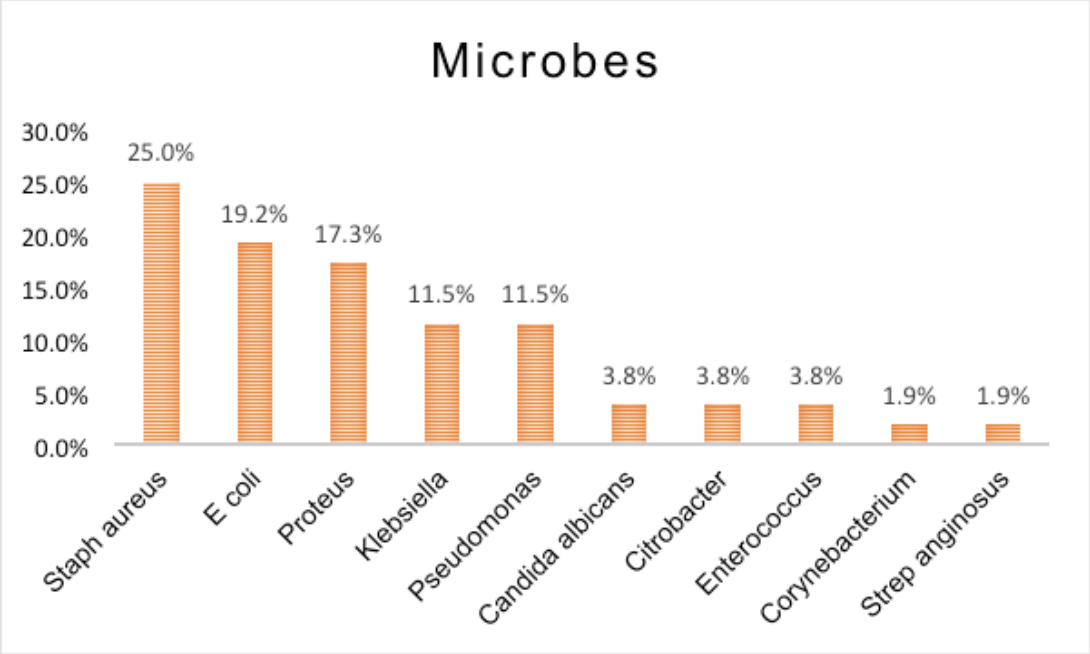
Gram negative rods accounted for most of the pathogens grown at 59.5%, followed by gram positive *cocci* at 33.3%.

Table 1: Microbes classification by gram staining

Organisms	Count	%
Positive cocci	14	33.3%
Negative rods	25	59.5%
Positive rods	1	2.4%
Positive yeast	2	4.8%

Breaking down individual microbes isolated, *Staphylococcus aureus* and *E.coli* dominated at 25% and 19.2% respectively. Figure 3 below highlights the frequency of individual microbes isolated.

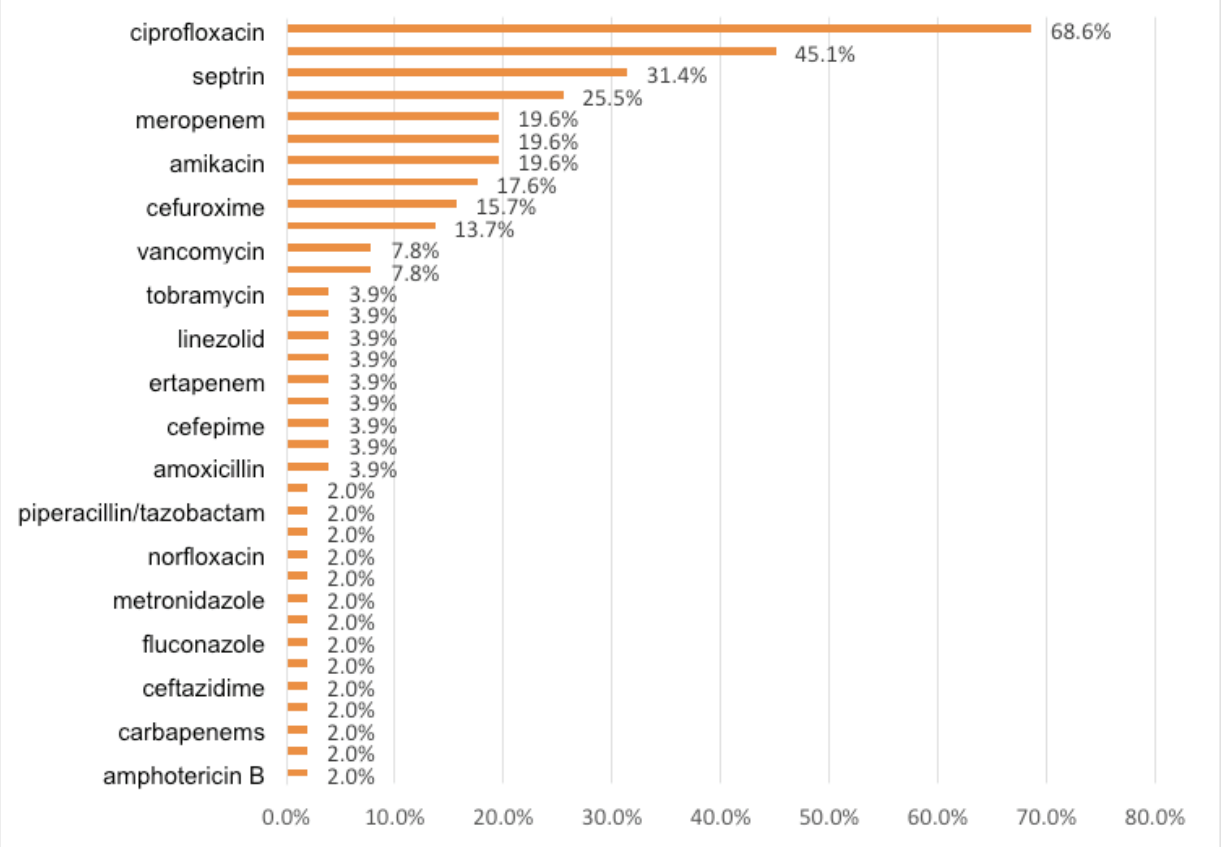
Figure 2: Individual microbes isolated



Antimicrobial Sensitivity Patterns

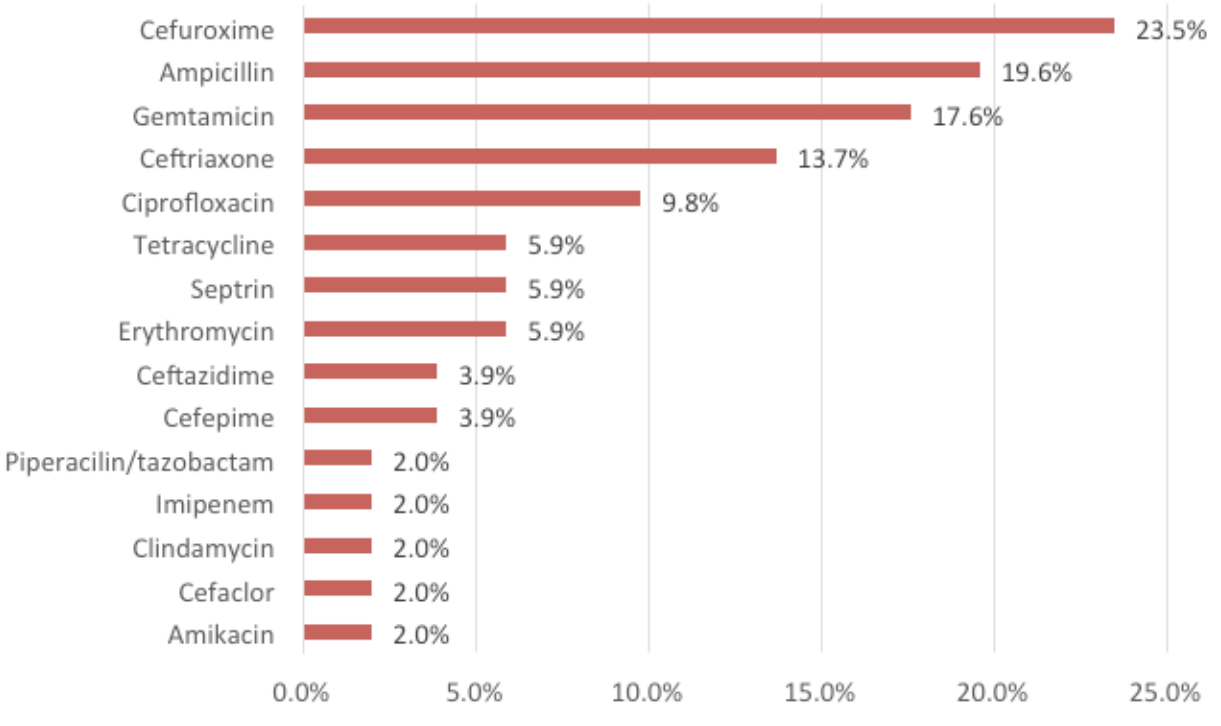
Ciprofloxacin demonstrated the highest level of sensitivity, with 68.8% of the microbes being susceptible, followed by Septrin (trimethoprim/sulfamethoxazole) (45.1%). Figure 4 below summarizes the sensitivity of different antibiotics.

Figure 3: Sensitive Antimicrobials



Microbes showed greatest resistance to cefuroxime (23.5%), ampicillin (19.6%), gentamicin (17.6%), and ceftriaxone (13.7%). Figure 4 below summarizes the pattern of antimicrobial resistance.

Figure 4: Resistant antimicrobials



DISCUSSION

This study was about mapping out the microbiology of DFU infections as well as the sensitivity profiles of commonly used antibiotics in controlling DFU infections. The study demonstrated that most diabetic foot infections in our setting are polymicrobial. Specifically, *Staphylococcus aureus* and *E. coli* are the predominant pathogens. The microbes were highly sensitive to ciprofloxacin, and resistant to common beta lactams such as cefuroxime, ampicillin and ceftriaxone.

On the microbiology of DFU, the study noted most infections were polymicrobial. The observation mirrors the findings of a US-based multicenter clinical trial where 83.8% of diabetic foot infections were polymicrobial (12). Broadly, the study found that gram negative bacteria were more prevalent than gram positive bacteria. The most common gram-negative bacteria were *E. coli,* Proteus and Pseudomonas. A study by Mutonga *et al* on the bacteriology of DFU in Kenya also found gram negative bacteria comprised 65% of isolated microorganisms (13). Likewise, a meta-analysis found gram negative bacteria were the most common cause of diabetic foot infections in low and middle income countries such as Kenya (14).

In analyzing individual pathogens, the study identified *Staphylococcus aureus* and *Escherichia coli* as the most common causes of DFU infections. The findings corresponds to the results of an earlier study in Kenya that also identified *S. aureus* and *E. coli* as the main cases of diabetic foot infections (15). A multicenter study in Korea similarly identified *S. aureus* as the predominant microorganism (16). While the microbiology of DFU varies geographically, *S. aureus* has been credited as the main cause of diabetic foot infections globally (14). The findings of this study confirm this assertion.

The study found a higher prevalence of gram negative than gram positive bacteria. The most common gram-negative bacteria were *E. coli,* Proteus and Pseudomonas. A study of DFU in India similarly reported a predominance of gram negative pathogens (17). A study by Mutonga *et al* on the bacteriology of DFU in Kenya also found gram negative bacteria comprised 65% of isolated microorganisms. The findings are also consistent with the findings of a meta-analysis that found gram negative bacteria were the most common cause of diabetic foot infections in low and middle income countries such as Kenya (14).

Infection control is one of the main goals of DFU management. In recent years, this has been made difficult by high rates of antimicrobial resistance. The trend has emphasized the need for routine culture and sensitivity, as well as center-specific antibiograms (Spichler *et al*., 2015). This study was a first step in establishing a baseline sensitivity trend in our local context. We noted that most bacterial isolates were highly susceptible to ciprofloxacin. This observation is supported by Kengne *et al's* report, wherein bacteria were sensitive to second generation quinolones such as ciprofloxacin and aminoglycosides (18). A similar trend of sensitivity to fluoroquinolones was reported in a Nigerian study (19).

There was a varying degree of sensitivity and resistance to other commonly used antibiotics. While some microbes were sensitive to gentamicin, ceftriaxone, and trimethoprim–sulphamethoxazole (Septrin), an equally good number of pathogens were resistant to the same antibiotics. Equally, a good number of isolates were resistant to cefuroxime, ampicillin, ceftriaxone, ceftazidime. The results mirror findings of an earlier study in Kenya where isolates were resistant to common antibiotics such as ampicillin, amoxicillin, ceftazidime, cefuroxime and trimethoprim–sulphamethoxazole (13). Similar to a study by Yefou *et al*, this study noted that gram negative bacteria were highly susceptible to amikacin, imipenem and meropenem (20). The variation in sensitivity to these antibiotics highlights the need for an evidence-based approach to management of diabetic foot infections through culture and antibiotic sensitivity testing.

CONCLUSION

The etiology of DFU infections is predominantly polymicrobial. Gram negative bacteria were the predominant microorganisms in diabetic foot infections. *Staphylococcus aureus* and *E. coli* are the main causes of diabetic foot infections. Microorganisms were resistant to commonly available antibiotics. Routine wound culture and sensitivity testing should be used to guide antimicrobial therapy.

Ciprofloxacin demonstrated a high degree of sensitivity to DFU microbes. From this study it would be recommended as the antibiotic choice *for* empiric therapy of diabetic foot infections.

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