MAKERERE UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF INTERNAL MEDICINE

ANTIMICROBIAL SUSCEPTIBILITY OF POTENTIALLY PATHOGENIC BACTERIA ISOLATED FROM DIABETIC FOOT ULCERS OF PATIENTS AT MULAGO AND KIRUDDU NATIONAL REFERRAL HOSPITALS

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(2020/HD07/19911U)

A DISSERTATION SUBMITTED TO THE COLLEGE OF HEALTH SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF A DEGREE OF MASTER OF MEDICINE IN INTERNAL MEDICINE OF MAKERERE UNIVERSITY

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July 2026

DECLARATION

I, Dr. Oluka Edwin Noah, hereby declare that the work presented in this Dissertation is my original work. The contribution of other people towards the preparation of this work is duly quoted and acknowledged. This Dissertation has not been submitted for the award of any degree in any other university.

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APPROVALS

The undersigned certify that they have read and hereby recommend acceptance of this Dissertation by Makerere University titled Antimicrobial Susceptibility of Potentially Pathogenic Bacteria isolated from Diabetic Foot Ulcers of Patients at Mulago and Kiruddu National Referral Hospitals

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ACKNOWLEDGMENT

I wish to express my profound and sincere gratitude to my supervisors**,** Dr. Freddie Bwanga and Dr. Mutebi Edrisa Ibrahim**,** for their unwavering guidance, intellectual mentorship, and scholarly support throughout the entire course of this research. Their critical insights, patience, and dedication to academic rigor greatly shaped the design, implementation, and final presentation of this work. I am deeply indebted to them for their encouragement and for consistently challenging me to uphold high scientific and ethical standards.

I am also grateful to Dr. Ssekabembe Richard, a colleague whose valuable contributions, professional support, and constructive discussions significantly enriched this study. His willingness to share knowledge and provide technical assistance was instrumental to the successful completion of this research.

I extend my sincere appreciation to the administration of Mulago National Referral Hospital and Kiruddu National Referral Hospital for granting permission to conduct this study within their institutions. I am particularly thankful to the staff of the Department of Internal Medicine for their cooperation, clinical support, and assistance during patient recruitment, sample collection, and data acquisition.

I wish to acknowledge the School of Medicine Research Ethics Committee (SOMREC) of Makerere University College of Health Sciences for granting ethical approval and providing oversight, thereby ensuring that this study was conducted in accordance with established ethical principles and regulatory requirements.

Finally, I extend my heartfelt appreciation to all the patients who voluntarily participated in this study. Their willingness to contribute, despite illness and personal challenges, made this research possible. I remain deeply grateful for their trust and cooperation.

DEDICATION

This work is dedicated, with profound love and gratitude, to my best friend, my confidante, and my forever partner**,** my beloved wife, Ruth Kiiza Oluka**.** Through seasons of exhaustion, uncertainty, and sacrifice, she stood beside me with unwavering faith, patience, and grace. Your encouragement became my strength when my resolve faltered, and your belief in me carried me through moments when the finish line felt distant. This dissertation is not merely an academic achievement; it is a testament to your love, resilience, and the quiet sacrifices you made so that I could endure and succeed.

I also dedicate this work to my parents, Dr. Emuron Francis and Professor Emuron Lydia**,** whose lives embody dedication to learning, discipline, and service to humanity. Your tireless sacrifices, guidance, and unconditional support shaped not only my academic journey but my character and values. You planted in me the conviction that knowledge is a responsibility, not a privilege, and that excellence must always be pursued with humility and purpose. Every page of this work reflects the foundation you laid and the ideals you instilled in me.

This dissertation stands as a humble tribute to your love, sacrifices, and belief in my potential. It reflects your legacy and my promise to uphold it through service, learning, and lasting impact.

To my siblings, the struggle continues. When you light a candle, put it on the table.

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LIST OF ABBREVIATIONS AND ACRONYMS:

Abbreviations	**Full form**
AMR	Antimicrobial Resistance
CDC	Center for Disease Control and Prevention
DFI	Diabetic foot infection
DFU	Diabetic foot ulcer
DM	Diabetes mellitus
ESBL	Extended Spectrum Beta lactamase-producing bacteria
HBO	Hyperbaric oxygen
HCA	Health Centre Associated
IDF	International Diabetes Federation
KNRH	Kiruddu National Referral Hospital
LPS	Lipopolysaccharide
MDR	Multiple Drug Resistance
MNRH	Mulago National Referral Hospital
MRSA	Methicillin-resistant *Staphylococcus aureus*
NPWT	Negative pressure wound therapy
PAMPs	Pathogen-Associated Molecular Patterns
PCR	Polymerase chain reaction
PDGF	Platelet-derived growth factor
spp	Species
WHO	World Health Organization
XDR	Extended Drug Resistance

DEFINITION OF TERMS:

STANDARD DEFINITIONS

Diabetic Foot Ulcer:

A full-thickness wound occurring below the ankle in a patient with diabetes mellitus, resulting from neuropathy, peripheral arterial disease, trauma, or pressure, irrespective of the presence or absence of clinical infection. (Petersmann et al., 2019).

Potentially Pathogenic Bacteria:

Microorganisms isolated from diabetic foot ulcer specimens that are capable of causing infection in compromised hosts, including both classical pathogens and opportunistic organisms, irrespective of whether overt clinical infection was present at the time of sample collection. (Pouget et al., 2020).

Antimicrobial Susceptibility:

The in-vitro response of bacterial isolates to selected antimicrobial agents, determined using standard laboratory methods and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines, with intermediate results classified as resistant for analysis. *(Giske, Turnidge, Cantón, & Kahlmeter, 2022)*

Multidrug Resistance (MDR):

Acquired non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012).

Extensively Drug-Resistant (XDR):

Non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (Magiorakos et al., 2012).

OPERATIONAL DEFINITIONS

Potentially Pathogenic Bacteria (as applied in this study):

Any bacterial organism isolated from a diabetic foot ulcer specimen on culture, irrespective of whether overt clinical infection was documented at the time of sampling. This operational threshold was adopted because the study characterized all cultivable organisms rather than only those meeting a clinical infection definition.

Resistant (for analysis):

For the purpose of analysis, isolates reported as intermediate were grouped with resistant isolates (i.e. classified as non-susceptible). This conservative operational decision was made to avoid overstating the effectiveness of agents with borderline activity (Giske et al., 2022).

Culture-positive patient:

A participant from whom one or more bacterial organisms were isolated. Polymicrobial and monomicrobial status was determined per patient, not per isolate.

ABSTRACT:

Background: Diabetic foot infections are a major cause of morbidity among patients with diabetes mellitus. Knowledge of local microbial profiles and antimicrobial susceptibility patterns is essential for guiding effective empirical therapy. This study aimed to determine the common microbial pathogens and their antimicrobial susceptibility patterns in diabetic foot ulcers at Mulago and Kiruddu hospitals.

Methods: A cross-sectional study was conducted among 80 patients with diabetic foot ulcers. Wound specimens were collected and processed using standard microbiological techniques. Data were analyzed descriptively to determine the common microbial isolates and their susceptibility patterns.

Results: Out of 80 samples, 168 bacterial isolates were obtained from 79 culture-positive patients, with 58 (73.4%; 95% CI 62.8–81.9%) patients having polymicrobial and 21 (26.6%) monomicrobial infection. Overall, Gram-negative bacteria predominated (132/168; 78.6%; 95% CI 71.8–84.1%), with *Escherichia coli* (39/168; 23.2%) being the most common isolate, followed by Gram-positive *Enterococcus spp.* (23/168; 13.7%) and *Klebsiella pneumoniae* (20/168; 11.9%). Multidrug-resistant organisms were identified in 74.4% of isolates. Linezolid and chloramphenicol were effective against Gram-positive organisms, including MRSA and VRE, while carbapenems and amikacin were effective against Gram-negative isolates. High resistance rates were observed to penicillins, erythromycin, fluoroquinolones, and cephalosporins.

Conclusion: The study found a high prevalence of polymicrobial infections, predominantly Gram-negative bacteria, with approximately three-quarters of isolates being multidrug-resistant. Common first-line antibiotics showed high resistance, leaving limited effective treatment options.

CHAPTER ONE: INTRODUCTION

1.0 Introduction

Diabetic foot ulcers are a significant health risk for individuals with diabetes, impacting their quality of life and increasing the likelihood of infections (Afonso, Oliveira, Saavedra, Borges, & Simões, 2021). This places a heavy burden on both patients and the healthcare system, affecting them socially, psychologically, and economically. According to the International Diabetes Federation (2023), in 2021, 1 in 10 adults globally was living with diabetes. One quarter of those living with diabetes lives in low- and middle-income countries, and approximately 6.7 million people die from diabetes related complications every year (Arokiasamy, Salvi, & Selvamani, 2021). A major complication of poorly controlled diabetes is diabetic foot ulcers (DFU). It is estimated that more than 16% of people living with diabetes will develop foot ulcers in their lifetime (Maldonado-Valer et al., 2023)

The pathophysiology underlying diabetic foot complications is complex, involving disruptions in the host's systems, such as neuropathy, immunopathy, and arteriopathy, alongside factors related to pathogens (Ibrahim, 2018). Foot ulcers typically result from trauma that breaches the foot's protective skin barrier, allowing bacterial colonization of the underlying tissues (Ibrahim, 2018). Subsequently, infections occur due to microbial overgrowth, leading to complications such as delayed healing, gangrene, osteomyelitis, limb amputation, and potentially death (Ibrahim, 2018; Hitam et al., 2019).

A comprehensive review of 112 studies revealed that the microbiology of diabetic foot infections (DFI) varies globally. Gram-negative bacteria are more common in low-income countries, while Gram-positive species are more prevalent in high-income countries (Macdonald et al., 2021). The review also highlighted that the most predominant bacteria across different settings were Staphylococcus aureus, Pseudomonas species, E. coli, and Enterococcus species.

Diabetic foot infections are typically treated with broad-spectrum antibiotics in cases of moderate to severe infection, and with narrow-spectrum antibiotics for mild infections (Kwon and Armstrong, 2018a). It's important to note that wounds of diabetic patients are more likely to be colonized or infected by drug-resistant strains of bacteria compared to non-diabetic patients (Lin et al., 2017). In 2019, up to 18% of isolates from diabetic foot ulcers (DFU) globally were found to be resistant to the antibiotic methicillin, which is effective against a wide range of bacteria (Macdonald et al., 2021). Antibiotic resistance is a significant concern, as it is linked to increased treatment costs, as well as morbidity and mortality rates. The excessive use of antibiotics has led to a significant increase in antibiotic resistance in recent years (Dadgostar, 2019). Proper antibiotic use is therefore crucial for improving the cure rate of diabetic foot infections, reducing the incidence of adverse reactions, and minimizing the occurrence of bacterial resistance (Akash et al., 2020).

The need to understand the bacterial profile and drug resistance patterns of bacteria isolates from DFU is important. However, there is a lack of recent data on this topic, with a few studies available, one at Mulago National Referral Hospital and another at Mbarara Regional Referral Hospital (Makeri et al., 2024). These studies reported that approximately 33% of bacteria showed resistance to methicillin. Most bacteria were susceptible to Imipenem, Vancomycin, Ciprofloxacin, and Clindamycin, but resistant to Cotrimoxazole and Ampicillin (Makeri et al., 2024). More recent data is needed for a national referral hospital, as bacterial profiles and drug resistance patterns may vary. Therefore, this study aimed to determine the bacterial profile and drug susceptibility patterns of bacteria isolates from DFU in patients living with diabetes at KNRH and MNRH in Uganda.

1.1 Statement of the problem

Whereas diabetic foot ulcers (DFUs) remain a significant global health concern, contributing to morbidity and mortality among individuals with diabetes, the scarcity of recent data on bacterial profiles and antimicrobial susceptibility patterns

in Uganda hinders effective management. This dearth of information poses significant challenges for healthcare providers, making it difficult to develop targeted treatment strategies. A recent systematic review in Sub-Saharan Africa highlighted a concerning increase in antimicrobial resistance among bacteria causing DFU infections (FW Wada et al., 2023). This trend underscores the urgent need for updated region-specific data to inform clinical decision-making. In Uganda, specifically, only two studies (Pario et al., 2011; Sikhondze et al., 2022) have addressed DFUs, leaving significant knowledge gaps (Makeri et al., 2024). Given the limited scope of these studies — Pario et al. (2011) focused exclusively on Mulago National Referral Hospital and Sikhondze et al. (2022) was conducted in western Uganda, the generalisability of their findings to current conditions at Mulago and Kiruddu National Referral Hospitals is limited. Specifically, neither study categorised resistance using current CLSI breakpoints, neither characterised the MDR/XDR burden, and resistance patterns under the 2020 National Guidelines for Antimicrobial Consumption and Use Surveillance in Human Health warrant updated, site-specific data from both centres. This study addressed the critical need for region-specific insights into pathogens and their susceptibility patterns, investigating antimicrobial resistance among bacteria isolated from diabetic foot ulcers at these two major healthcare centers. By identifying prevalent bacterial species, assessing resistance profiles, and evaluating current treatment regimens, the findings from this study informed clinical decision-making, guided local healthcare practices, and contributed to evidence-based strategies for managing diabetic foot infections.

1.2 Research questions:

1. What are the common potentially pathogenic microorganisms isolated from diabetic foot ulcers among patients attending Mulago and Kiruddu National Referral Hospitals?

2. What is the antimicrobial susceptibility pattern of common potentially pathogenic bacteria isolated from diabetic foot ulcers among patients at Mulago and Kiruddu National Referral Hospitals?

1.3 Research objectives

1.3.1 General objective

To determine the spectrum of potentially pathogenic microorganisms and their antimicrobial susceptibility patterns among diabetic foot ulcer patients attending Mulago and Kiruddu National Referral Hospitals?

Specific objectives

1. To identify the common potentially pathogenic microorganisms isolated from diabetic foot ulcers among patients at Mulago and Kiruddu National Referral Hospitals?
2. To determine the antimicrobial susceptibility patterns of common potentially pathogenic bacteria isolated from diabetic foot ulcers at Mulago and Kiruddu National Referral Hospitals?

1.4 Justification

The data generated from this study contributed to the development of policies on the management of diabetic foot infections. Despite the Ministry of Health's recommendation for laboratory surveillance on antimicrobial resistance in Uganda (Government of Uganda, 2018), it was not being implemented at the time of this study.(Ampaire, Nduhura, & Wewedru, 2017; Chaplain, Asutaku, Mona, Bulafu, & Aruhomukama, 2022).

This study also emphasised the importance of practitioners adhering to proper diagnostic procedures for identifying potentially pathogenic pathogens in foot ulcers of diabetic patients and subsequently prescribing effective treatments.

The results of this study offered valuable insights into the treatment of Diabetic Foot Ulcers (DFU) for different stakeholders, such as clinicians, hospital

administrators, microbiologists, and the public. This took into consideration the current patterns of antibiotic resistance in bacteria isolated from DFUs of patients at Kiruddu and Mulago National Referral Hospitals.

These findings also helped in the development and improvement of local guidelines and protocols for using antibiotics to treat DFIs. This could lead to clinicians customizing antibiotic treatments for DFIs, resulting in better clinical outcomes for patients with DFUs.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

2.1.1 Diabetes Mellitus

In developing countries, diabetes mellitus (DM) is a leading cause of mortality, with a growing burden in low- and middle-income countries (LMICs) (Herman, 2017). In 2021, a staggering 1 in 10 adults were living with diabetes globally (Sun et al., 2022), while in Africa, 1 in 22 adults were living with diabetes. The number is projected to rise by 12.9% by the year 2045 (International Diabetes Federation, 2023). Moreover, in Sub-Saharan Africa, it is believed that more than two-thirds of patients with DM are unaware of their diagnosis (Guariguata et al., 2024). The prevalence of DM in Uganda has been approximated to be between 2.7% and 7.4% (Bahendeka et al., 2016; Mayega et al., 2013; Ministry of Health Uganda, 2014), with a rapid rise of 166.9% expected between 2013 and 2035 (Guariguata et al., 2024).

The classification of diabetes distinguishes between two main types: type 1 and type 2. Type 2 diabetes is the most common, accounting for over 85% of all cases of diabetes (Forouhi and Wareham, 2019). Maturity-onset diabetes of the young (MODY) is a rare type of diabetes that affects young people and early adults, making up only about 2% of all diabetes cases (Petersmann et al., 2018).

The classification of diabetes mellitus has continued to evolve. Whereas type 1 and type 2 diabetes remain the predominant forms, the International Diabetes Federation now formally recognises additional categories, including type 3c (pancreatogenic) and gestational diabetes. Most recently, at the IDF World Diabetes Congress 2025 in Bangkok, the Federation endorsed malnutrition-related diabetes as a distinct entity, designated type 5 diabetes (International Diabetes Federation, 2025).

Type 5 diabetes is a severe insulin-deficient form arising from impaired pancreatic development following chronic undernutrition in early life, and is estimated to

affect 20 to 25 million people, predominantly lean adolescents and young adults in low- and middle-income countries across Asia and Africa (Hawkins et al., 2025). Its recognition is particularly relevant to the Ugandan and wider sub-Saharan context, where undernutrition is prevalent and where such patients may be misclassified as having type 1 diabetes. While the present study did not classify participants by diabetes type, this evolving taxonomy underscores the heterogeneity of the diabetic population from which diabetic foot ulcers arise. This study did not focus on identifying the specific type of diabetes in the patients or its management.

Diabetes mellitus (DM) is linked to a range of long-term complications that can impact different organ systems, resulting in significant morbidity and mortality, particularly when poorly controlled (American Diabetes Association, 2020). These complications can be broadly divided into microvascular and macrovascular complications, each with distinct pathophysiological mechanisms and clinical manifestations (Fowler et al., 2011).

Macrovascular complications involve large blood vessels and contribute to the increased risk of cardiovascular disease (CVD) in individuals with diabetes (Jyotsna et al., 2023). These complications include coronary artery disease (CAD), peripheral arterial disease (PAD), and cerebrovascular disease (Zakir et al., 2023). These macrovascular complications are driven by a combination of traditional cardiovascular risk factors, such as hypertension, dyslipidemia, and obesity, as well as diabetes-specific factors, including hyperglycemia-induced endothelial dysfunction and inflammation (Zakir et al., 2023).

Microvascular complications primarily involve small blood vessels and include conditions such as diabetic retinopathy, nephropathy, and neuropathy (Avogaro and Fadini, 2019). Diabetic retinopathy, characterized by progressive damage to the retinal microvasculature, is the leading cause of blindness in adults, potentially leading to vision impairment and blindness if left untreated (Teo et al., 2021). Similarly, diabetic nephropathy, marked by progressive decline in renal function

due to glomerular damage and proteinuria, is a leading cause of end-stage renal disease (ESRD) worldwide (Selby and Taal, 2020).

Diabetic neuropathy affects both peripheral and autonomic nerves, leading to sensory loss, pain, and impaired motor function. This makes individuals more prone to developing foot ulcers, infections, and lower limb amputations (Rosenberger et al., 2020). These complications are a result of chronic high blood sugar levels causing endothelial dysfunction, oxidative stress, and inflammation, which lead to structural and functional abnormalities in the affected tissues (American Diabetes Association, 2020). This is where diabetic foot ulcers occur.

These macrovascular and microvascular derangements converge on the diabetic foot and directly shape both the development of ulceration and the response to antimicrobial therapy. Peripheral arterial disease, a macrovascular complication, reduces lower-limb perfusion and thereby limits the delivery of systemically administered antibiotics to infected tissue, contributing to persistent infection and impaired healing (Volmer-Thole and Lobmann, 2016). Concurrently, microvascular dysfunction and diabetic peripheral neuropathy impair the local inflammatory response, blunt leucocyte recruitment, and diminish tissue oxygenation, creating a wound environment that favours bacterial proliferation and the establishment of polymicrobial, frequently resistant, infection (Pouget et al., 2020).

Chronic hyperglycaemia further impairs neutrophil chemotaxis and phagocytosis, weakening host defence against both Gram-positive and Gram-negative organisms. The combined effect of compromised perfusion, neuropathy and immunopathy is that diabetic foot infections are often more severe, slower to resolve, and less responsive to empirical antimicrobial therapy than infections in non-diabetic hosts, reinforcing the need for culture-guided treatment.

2.1.2 Diabetic foot ulcers

Diabetic foot ulcers (DFUs) are one of the serious microvascular complications experienced by individuals with diabetes mellitus (DM), posing a significant threat to their health and well-being (Schofield et al., 2021). A systematic review and meta-analysis reported that globally, 6.3% of diabetic patients had DFUs, with more males affected than females (4.5% vs 3.5% respectively) (Zhang et al., 2017). The study also indicated that in Africa and Uganda, 5.5% and 1-4% of patients with diabetes respectively had DFU.

The development of diabetic foot ulcers (DFUs) is a result of the harmful effects of high blood sugar and abnormal lipid levels on various bodily systems (Rubitschung et al., 2021). Elevated blood glucose levels create reactive oxygen species, which damage nerves and cause neuropathy (Volpe et al., 2018). Furthermore, nerve proteins undergo auto-glycosylation, hastening neurological decline in the foot, disrupting the balance between flexors and extensors, and leading to skin ulceration (Rubitschung et al., 2021).

Chronic hyperglycemia also damages the autonomic nerves, reducing sweating, decreasing skin moisture, and causing cracks that can lead to ulcers (Ziegler, 2017). Meanwhile, damage to peripheral sensory nerves reduces foot sensation, resulting in unnoticed foot wounds that may become chronic due to impaired healing mechanisms, worsened by poor blood circulation (Bandyk, 2018). These mechanisms collectively contribute to the formation of foot ulcers in patients with diabetes mellitus.

The dysregulation of immune responses also contributes to delayed wound healing in diabetic foot ulcers (DFUs). T-cells, which are crucial for wound healing, show increased apoptosis in diabetic patients, especially in Type 2 diabetes mellitus, leading to delayed wound healing (Hu et al., 2022). Additionally, endothelial cell dysfunction caused by high blood glucose levels results in decreased levels of intrinsic vasodilators and increased thromboxane A2 plasma levels in the arteries

of the feet. This promotes ulcer formation by causing vasoconstriction and increasing the risk of ulceration (Zubair and Fatima, 2021).

Diabetic foot ulcers (DFUs) were characterized using Wagner's Classification, a widely recognized system for assessing the severity of DFUs and guiding their management (Wagner et al., 1981). The system categorizes DFUs into six grades based on the depth of the ulcer and the presence of infection or ischemia. Grade 0 indicates no open lesions, but the presence of pre-ulcerative conditions such as callus, corn, or deformities that could lead to ulcer formation. Grade 1 refers to a superficial ulcer confined to the skin and subcutaneous tissue without involvement of deeper structures. Grade 2 involves an ulcer extending into deeper tissues, such as tendons or bone, but without abscess or osteomyelitis. Grade 3 is characterized by a deep ulcer with abscess, osteomyelitis, or joint involvement. Grade 4 refers to gangrene affecting part of the foot, typically involving the toes, while Grade 5 indicates gangrene of the entire foot, often necessitating amputation.

Although the Wagner classification is widely applied, its distribution and prognostic value vary by setting, and local contextualisation is important. In Ugandan and regional series, patients frequently present late and at higher Wagner grades, reflecting delayed health-seeking, limited access to specialised foot care, and a high burden of undiagnosed or poorly controlled diabetes. A tertiary study in south-western Uganda reported that a substantial proportion of diabetic foot ulcers presented at advanced grades requiring surgical intervention (Sikhondze et al., 2022), and similar late presentation has been described at Mulago (Pario et al., 2011).

This pattern is clinically significant because higher Wagner grades are associated with deeper tissue involvement, greater likelihood of polymicrobial and resistant infection, and increased risk of amputation. Interpreting the present study's findings against the Wagner grade distribution of the enrolled population therefore situates the susceptibility data within the locally observed severity profile, rather than assuming the milder grade distribution typical of high-income settings.

Wagner's classification system is valuable for assessing the progression of diabetic foot ulcers (DFUs) and determining appropriate treatment strategies (Zhu et al., 2019). Additionally, a study by Husers et al. (2020) highlighted that the system plays a key role in predicting the risk of complications, including infections and amputations.

2.1.3 Potentially Pathogenic Bacteria

Diabetic foot ulcers (DFU) offer a very conducive environment for the growth of many different bacteria (Huang et al., 2023). A recent study at a Tertiary hospital in South-Western Uganda had 46.8% of DFUs being clinically infected (Sikhondze et al., 2022). Moreover, the types of bacteria colonizing vary depending on the duration of the diabetic foot infections (DFI), for example Gram-positive cocci (Streptococcus and Staphylococcus) were isolated in cases of shorter durations of DFIs compared to the numerous aerobic (Streptococcus, Staphylococcus, Pseudomonas and Enterococcus species) and anaerobic (Bacterioides fragilis) bacteria isolated from chronic DFIs (J Jneid, Lavigne, La Scola, & Cassir, 2017).

Tascini and others isolated S. aureus as the commonest Gram-positive bacteria(Tascini et al., 2011). These findings are very similar to another more recent study (Joanne Jneid et al., 2018). Earlier studies however isolated S. epidermidis as the predominant bacterial species (Citron, Goldstein, Merriam, Lipsky, & Abramson, 2007; Yao et al., 2005). Because of the more recent studies, this study looked for S. aureus as one of the PPB. This study will also consider Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae as potentially PPB since these are the main bacterial species isolated from DFUs in Uganda (Sikhondze et al., 2022). Acinetobacter baumannii was also considered as one of the PPB as together with the previously selected bacteria, was identified as a priority pathogen by the World Health Organization (da Silva et al., 2021).

2.2 Antimicrobial resistance of the Potentially pathogenic bacteria

Antimicrobial resistance (AMR) is a significant global threat to public health and healthcare systems. Studies have linked AMR to approximately 700,000 deaths annually worldwide, highlighting the urgent need for preventive measures (O'Neill, 2017). Additionally, the prevalence of antibiotic-resistant infections is increasing in low- and middle-income countries, where limited resources hinder access to appropriate investigations and treatment (Temkin et al., 2018; Coates et al., 2020).

The results of the antibiogram test from a recent study in Southern Iran, specifically the Bandar Abbas area, showed that *E. coli* was most sensitive to meropenem and least sensitive to ciprofloxacin. *Enterococcus* was most sensitive to gentamicin and ciprofloxacin, while *Klebsiella* was most sensitive to amikacin and cotrimoxazole. *Enterobacter* was most sensitive to cotrimoxazole and equally sensitive to amikacin and ciprofloxacin. *Staphylococcus aureus* showed sensitivity to vancomycin and doxycycline, while *Acinetobacter* was found to be 100% resistant to all antibiotics except for amikacin and gentamycin (Ahmadishooli et al., 2020).

In a study conducted in Nigeria, 108 specimens of diabetic foot lesions were examined. The findings showed that all Gram-positive aerobes were sensitive to doxycycline, while the Gram-negative bacteria were sensitive to amikacin, cefoperazone/sulbactam, and meropenem (Sekhar et al., 2014). Additionally, antimicrobial susceptibility tests of swabs from diabetic foot ulcers in Tanzania revealed a polymicrobial pattern with high antimicrobial resistance to commonly used antibiotics, except for meropenem and imipenem (Chalya et al., 2011).

A cross-sectional study conducted in Eastern Uganda on post-surgical wounds found that *Staphylococcus aureus* was the predominant isolate. This bacterium was susceptible to vancomycin and clindamycin, but resistant to multiple other drugs. The Gram-negative organisms isolated showed sensitivity to imipenem, but were resistant to tetracycline, trimethoprim/sulfamethoxazole, and ceftriaxone (George et al., 2018).

A study conducted at Mulago National Referral Hospital outpatient department found that *S. aureus* is the main microorganism responsible for causing wound sepsis. All Gram-positive bacteria were found to be susceptible to Linezolid, while all Gram-negative organisms were susceptible to Ciprofloxacin and Piperacillin. Among the *Staphylococcus aureus* isolates, 100.0% were susceptible to Linezolid, 64.2% to Chloramphenicol, 54.7% to Gentamycin, 50.9% to Piperacillin, 49.1% to Ciprofloxacin, Vancomycin, and Ceftazidime each, and the lowest susceptibility of 41.5% was reported against Amoxicillin (Harunah, 2019).

The previous studies did not focus on foot ulcers in patients with diabetes. This study aims to identify the patterns of antimicrobial resistance in the potentially harmful bacteria found in diabetic foot ulcers. This information is crucial for developing effective treatment strategies and maintaining the effectiveness of antimicrobial drugs (Ibrahim et al., 2023a).

2.2.1 Risk factors for antimicrobial resistance

The World Health Organization (WHO) has stated that the inappropriate use of antibiotics is one of the most common risk factors for antimicrobial resistance (Nepal and Bhatta, 2018). Antibiotics are often prescribed to patients with viral infections or undiagnosed infections who persistently request medication (Kianmehr et al., 2019; Kohut et al., 2020). This is due to the easy access to over-the-counter antibiotics, particularly in low-income countries such as Uganda (Kohut et al., 2020).

Inadequate diagnostics are a risk factor for the development of antimicrobial resistance (Ayukekbong et al., 2017). Low- and middle-income countries have reported an increase in antimicrobial resistance due to poor availability of diagnostics (Gandra et al., 2020). The absence of adequate diagnostic tests leads to clinicians guessing the causative organisms and the appropriate drugs for treatment. In these countries, healthcare providers often rely on partial or insufficient information to diagnose infections, leading to the prescription of broad-spectrum antibiotics (Wagner et al., 2020) instead of specific antibiotics targeting the

causative organisms. This contributes significantly to selective pressure and accelerates antimicrobial resistance.

Critically ill patients are highly vulnerable to infections and often need antimicrobial treatment (Tosi et al., 2018; Stewart and Allen, 2019; Abdul-Aziz et al., 2020). These patients often require higher doses and longer duration of antibiotic treatment, which can accelerate antimicrobial resistance.

2.2.3 Consequences of antimicrobial resistance

Antibiotic resistance is a serious problem that limits the effectiveness of treatments for bacterial infections. This means that antibiotics that were once effective are no longer able to fight resistant strains of bacteria. As a result, managing infections becomes more complicated, leading to longer illness, increased sickness, and higher death rates among affected individuals. The decreasing number of effective antibiotics threatens to undo many years of medical progress, leaving doctors with few options to treat life-threatening infections (Ponte-Sucre et al., 2017; Mancuso et al., 2021).

The consequences of antimicrobial resistance in diabetic foot disease are particularly grave because infection is itself a principal driver of poor outcomes. Approximately half to two-thirds of diabetic foot ulcers become infected during their course, and once infection is established the trajectory toward tissue loss accelerates (Lipsky et al., 2020). The mortality associated with diabetic foot ulceration is striking: a meta-analysis of almost 125,000 patients reported five-year mortality of approximately 49% following an incident ulcer, with cardiovascular disease and infection as the leading causes of death (McDermott et al., 2023).

These five-year mortality rates exceed those of several common malignancies, including breast, prostate and colorectal cancer (Armstrong et al., 2020), a comparison that conveys the severity of the condition more forcefully than ulcer statistics alone. Resistance compounds this burden at every stage. When first-line agents fail, patients endure longer hospitalisation, repeated debridement, and

escalation to reserve antibiotics that are costly and not always available in resource-limited settings.

Infected, poorly controlled ulcers also sustain chronic systemic inflammation and intermittent bacteraemia, commonly from Staphylococcus aureus and Gram-negative bacilli, which can damage the vascular endothelium and worsen pre-existing cardiovascular disease, the leading overall cause of death in this population. At the limb level, suboptimal antimicrobial treatment increases the likelihood of amputation, with its attendant long-term disability, loss of livelihood, and profound psychological and financial impact on patients and families. In the Ugandan context, where out-of-pocket expenditure predominates and access to second-line agents is constrained, these consequences fall disproportionately on patients least able to bear them, underscoring the value of local susceptibility surveillance to preserve the effectiveness of available first-line therapy.

In addition, antibiotic resistance creates a significant economic burden. Healthcare costs go up because more intensive and longer treatments are needed. This leads to more hospital stays, longer hospital visits, and more medical procedures, which increases healthcare spending. This strains healthcare systems that are already overburdened and creates financial difficulties for individuals and families (Friedman et al., 2016) (Ventola, 2015).

Antibiotic resistance enables resistant bacteria to spread, making it hard to control infectious diseases and increasing the risk of widespread illness and death. It also complicates surgical procedures and medical interventions, leading to treatment failures and higher rates of illness and death among surgical patients (Majumder et al., 2020).

CHAPTER THREE: METHODOLOGY

3.1 Study design

This was a cross-sectional descriptive research study.

3.2 Study Site and setting

The study was conducted at the DM clinics, and the endocrinology wards in Mulago and Kiruddu National Referral Hospital in Uganda and the culture and sensitivity tests were done at MBN Clinical Laboratories, Kampala branch. These hospitals were selected because they are major referral centers for the management of diabetes and its complications, and they represent diverse patient populations.

Mulago National Referral Hospital, located in Mulago, Kampala, Uganda, is the largest hospital in the country. It has a total bed capacity of approximately 1,500 beds and serves as a teaching hospital for the College of Health Sciences at Makerere University. As a public hospital and tertiary referral center, Mulago offers specialized medical care, including diabetes mellitus. The Mulago Hospital DM clinic runs once a week to enroll new patients and following up with previously registered patients, with an average attendance of 90 patients on a clinic day. At each visit, blood pressure measurement is usually performed by a nurse using either a digital BP machine or a mercury sphygmomanometer after at least 30 minutes of rest. Fasting blood sugar levels are also measured using a portable glucometer. The patients were then seen by either an endocrinologist, internal medicine residents, or intern doctors rotating on the Endocrinology unit.

Kiruddu National Referral Hospital, located in Salama, Kampala, Uganda, is a subsidiary of Mulago National Referral Hospital. It was established to reduce the patient load at Mulago Hospital and provide specialized healthcare services. Like Mulago, Kiruddu Hospital primarily offers specialized care in the internal medicine department. The diabetes mellitus (DM) clinic operates once a week for enrolling new patients and following up with previously registered patients, with an average attendance of 200 patients on clinic days. During each visit, blood pressure (BP) is

usually measured using either a digital BP machine or a mercury sphygmomanometer by a nurse after at least 30 minutes of rest. Fasting blood sugar levels are also measured using a portable glucometer. After these measurements, patients were seen by either an endocrinologist, internal medicine residents, or intern doctors rotating through the Endocrinology unit.

MBN Clinical Laboratories (MBN) is an AABB (Association for the Advancement of Blood and Biotherapies)-Accredited facility that was incorporated in Uganda in February 2010. It is a complex of laboratories that serve as referral facilities for medical and biomedical Testing for individuals, clinics, other labs, hospitals, corporate entities, universities, government, NGOs and International agencies worldwide. The core function of MBN is to offer specialized medical laboratory testing services with a focus on DNA/relationship testing, PCR/molecular diagnostics, and microbiology (culture and sensitivity testing). The Lab in Kampala is the headquarters and is located on Plot 28 along Nakasero road.

3.3 Population

3.3.1 Target Population

Adult patients with a diagnosis of diabetic foot in Uganda.

3.3.2 Accessible Population

Adult patients with a diagnosis of diabetic foot attending care at the DM clinics, and the Endocrinology wards at Mulago and Kiruddu National Referral Hospital during the study period.

3.3.3 Study Population

Adult patients with diabetic foot attending care at the DM clinics, surgical clinics, surgical wards and the Endocrinology wards at Mulago and Kiruddu National Referral Hospital during the study period who met the eligibility criteria.

3.4 Sample size estimation

The sample size for this study was calculated using the Kish–Leslie (1965) formula for estimating a single population proportion, which is appropriate for cross-sectional studies assessing prevalence of microbiological outcomes.

$$n = \frac{Z^2 \times p \times (1-p)}{d^2} \text{x}$$

Where:

- n = required sample size
- Z = standard normal deviate at 95% confidence level (1.96)
- p = estimated prevalence of culture-positive diabetic foot infections
- d = margin of error (precision)

Assumptions Used

- Estimated prevalence (p): 0.55 (55%)
  This was based on findings from similar studies in sub-Saharan Africa, where the prevalence of culture-positive diabetic foot infections ranged from 50–65% (Atlaw et al., 2022a; Wada et al., 2023a)
- Confidence level: 95%
- Margin of error (d): 0.10 (10%), acceptable for hospital-based microbiological studies.

Sample Size Calculation

$$n = \frac{(1.96)^2 \times 0.55 \times (1-0.55)}{(0.10)^2} \text{x} n = \frac{3.84 \times 0.55 \times 0.45}{0.01} \text{x} n = \frac{0.9504}{0.01}$$
$$= 95.04\text{x}$$

Adjustment for Feasibility and Laboratory Yield

Given that:

Not all collected diabetic foot ulcer samples yield positive cultures,

Some samples may be contaminated or show no growth,

Laboratory capacity and study duration were limited,

$$64 \div (1 - 0.20) = 80$$

In addition, because the number of patients with diabetic foot ulcers presenting to the two clinics during the study period was finite and countable (approximately N = 190 over the enrolment window), a finite population correction was applied following Cochran (1977). Applying the correction to the unadjusted estimate of 95 gave a corrected requirement of 64, which after the 20% non-yield inflation yielded a minimum of 80 participants. The non-yield adjustment correctly increases, rather than reduces, the required number.

Final Sample Size

Eighty participants with diabetic foot ulcers were included in this study.

3.5 Sampling methods

KNRH received about twice the number of diabetic patients with DFU in a week as MNRH and therefore a 2:1 ratio was used to recruit patients from each of the facilities. A total of 25 participants were enrolled from MNRH and 51 from KNRH. Within each facility, consecutive sampling was used due to the small number of patients with DFU.

3.6 Eligibility criteria

Inclusion criteria:

- Adult patients with DM with foot ulcers receiving health care at MNRH and KNRH

Exclusion criteria

- Adult patients with DM with clinically healthy granulation tissue implying healing foot ulcers.

3.7 Study Variables

3.7.1 Outcome variables

This was a descriptive study and did not test associations between independent and dependent variables; the variables collected therefore served to characterize the study population and answer the two specific objectives, rather than to support regression analysis. The two outcome variables central to the study objectives were the potentially pathogenic bacteria isolated from diabetic foot ulcer specimens, and their corresponding antimicrobial susceptibility profile, both determined by culture and disk-diffusion susceptibility testing at MBN Clinical Laboratories.

3.7.2 Participant characteristics

In addition to the outcome variables, descriptive data were collected on sociodemographic characteristics (age, sex, smoking history, and heavy alcohol consumption), diabetes mellitus characteristics (duration of DM, presence of peripheral neuropathy), comorbidities (hypertension, HIV, cancer), and antibiotic usage (type of antibiotic used, over-the-counter prescription history, recent use). These characteristics were summarised descriptively and were not analysed as predictors of the outcome variables.

3.8 Study flow diagram

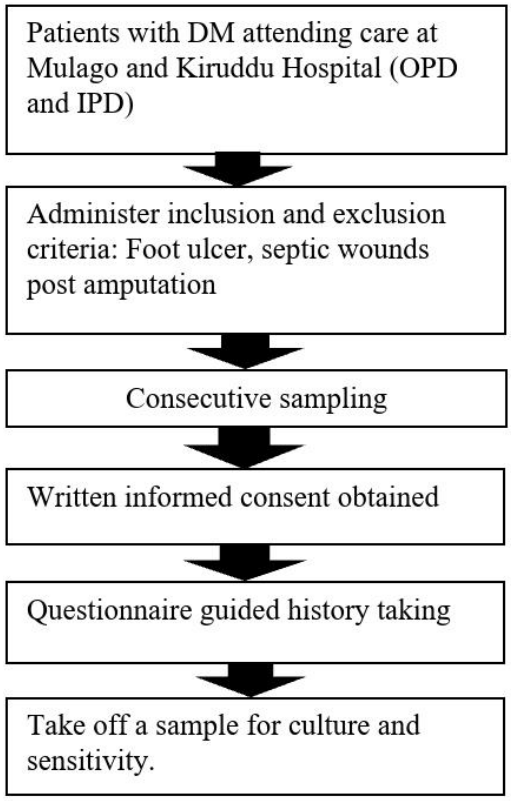

Figure 1: Study flow diagram

3.9 Data collection procedures

Trained research assistants who were intern medical doctors gathered data using semi-structured questionnaires and sterile swabs. Before collecting data, the research assistants were trained on the study objectives, questionnaire administration, sample collection and handling, and ethical considerations. They approached eligible patients with DM at Mulago and Kiruddu National Referral Hospital, explained the purpose of the study, and obtained informed consent from each participant. A study identification number was given to each participant before proceeding with data collection. The research assistants then conducted face-to-face interviews to administer the semi-structured questionnaires and obtain sociodemographic and other relevant data. They guided the participants through the questionnaire to ensure clarity and understanding of the questions, record the

responses accurately and completely, and verified the medical information provided by reviewing existing patient medical records at the facilities.

3.9.1 Data collection tool

The following information was captured in the questionnaire: the patient's sociodemographic factors (such as age, sex, smoking history, and alcohol consumption), characteristics related to diabetes mellitus (DM) (such as duration of DM), and comorbidities (such as hypertension, HIV, and cancer). Additionally, information on antimicrobial usage, including the type of antibiotic used, over-the-counter self-prescriptions, and recent antibiotic usage was recorded. The patient's temperature, blood pressure, pulse rate, respiratory rate, and the nature of discharge from the ulcer was also noted. The ulcer was graded and documented using Wagner's classification.

Once the data collection was complete, the research assistants carefully reviewed the questionnaires for completeness and accuracy. The collected data was securely stored and made accessible only to the principal investigator.

3.9.2 Specimen collection

Samples were collected from patients during the surgical wound dressing procedure, prior to cleaning the wound with an antiseptic solution. Sterile cotton swabs soaked in sterile normal saline (0.9% NaCl) were first used to remove any debris and clean the surrounding skin. Sterile swabs were used to collect discharge from clinically suspected infection sites, such as beneath the wound edges or at the center of the wound— for Gram staining and for culture. The swabs were placed in Amies Transport Media (Amies, 1967) and stored in a cool box, without ice packs, for transportation to MBN Clinical Laboratories.

3.9.3 Specimen transport

The swabs were securely transported in a cool box (without ice packs) by a reliable motorcycle courier to MBN Clinical Laboratories, located at 28 Nakasero Road, Kampala, Uganda. The samples were delivered within 4 to 24 hours of collection to ensure optimal integrity for analysis.

3.9.4 Culture procedures in the laboratory

Upon receipt at the laboratory, the swabs were inoculated onto Blood, MacConkey, and Chocolate agars and incubated at 35°C to 37°C in an ambient incubator. When no growth was observed on the agar plates within 72 hours, the sample was considered negative for pathogenic bacteria. For plates exhibiting growth of suspected bacterial pathogens, further identification was carried out to determine the genus and, where possible, the species of the bacteria.

3.9.5 Bacterial identification

Bacterial identification was performed based on colony characteristics, Gram morphology, and biochemical reactions, following the methods outlined in Cheesbrough et al. (2006). Colony characteristics assessed included morphology, hemolysis patterns on blood agar, and changes in the physical appearance of colonies on differential media (e.g., the pink coloration of lactose-fermenting colonies on MacConkey agar). Gram stain morphology was also considered.

For Gram-negative bacteria, identification was based on colony features such as the mucoid appearance of *Klebsiella pneumoniae*, lactose fermentation, Gram nature, motility, and biochemical reactions. These reactions were assessed using media such as Triple Sugar Iron (TSI) agar, citrate, sulphur-indole-motility (SIM) medium, urease tests, and oxidase tests.

Staphylococcus aureus was identified based on colony characteristics, Gram-positive cocci, a positive catalase test, and a slide/tube coagulase test. *Enterococcus* species were identified by their Gram-positive, catalase-negative cocci morphology, along with a positive bile-esculin test.

3.9.6 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method (Hudzicki, 2009). The choice of antimicrobials tested for each organism, along with the respective disc concentrations, was based on the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) to the extent possible (Wayne, 2017).

Briefly, the bacterial inoculum was prepared and standardized in sterile normal saline to match a 0.5 McFarland standard. A sterile swab was dipped into the inoculum suspension, excess fluid was removed by squeezing the swab against the side of the tube, and the swab was then evenly spread over the surface of a Mueller–Hinton agar plate. Antimicrobial discs were placed onto the inoculated agar plates, which were then incubated at 35°C–37°C for 16–18 hours (Wekesa, 2020). After incubation, the inhibition zone diameters were measured to the nearest millimeter using a ruler.

For Gram-negative bacterial pathogens, susceptibility was tested against the following antibiotics: ampicillin (10 µg), amoxicillin/clavulanate (20/10 µg), cefuroxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), gentamicin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), chloramphenicol (30 µg), imipenem/meropenem (10 µg), and amikacin (30 µg).

To screen for Extended-Spectrum Beta-Lactamase (ESBL) production in *Escherichia coli* and *Klebsiella* species, resistance mechanisms to ceftriaxone or ceftazidime were evaluated. This was done by comparing the zone diameters of ceftazidime (30 µg) alone with ceftazidime/clavulanate (30/10 µg) on Mueller–Hinton agar (Wayne, 2017). A difference of at least 5 mm in the inhibition zone diameter between ceftazidime alone and the combination disc was considered indicative of positive ESBL production in the bacterial pathogen.

When no increase in the zone diameter was observed, the organism was classified as exhibiting a non-ESBL mechanism of resistance to ceftazidime, which was due to ampC or carbapenemase production, as neither of these resistance mechanisms is inhibited by clavulanic acid. If a non-ESBL, ceftazidime-resistant organism was found to be susceptible to carbapenems, ampC was considered as the likely resistance mechanism. However, when the organism was also resistant to carbapenems, carbapenemase production was considered as the underlying mechanism of resistance to ceftazidime.

For Gram-positive organisms, susceptibility testing was conducted against the following antibiotics: penicillin (10 units), vancomycin (30 µg), linezolid (30 µg), gentamicin (10 µg), erythromycin (15 µg), tetracycline (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), and chloramphenicol (30 µg).

Induced clindamycin resistance among *Staphylococcus aureus* was detected using the double disc diffusion method on Mueller–Hinton agar. Specifically, an erythromycin disc (15 µg) and a clindamycin disc (2 µg) were placed 15–20 mm apart and incubated at 35°C ± 2°C for 16–18 hours. Flattening of the inhibition zone adjacent to the erythromycin disc (referred to as the D-zone) was interpreted as a positive D-test, indicating inducible clindamycin resistance.

Methicillin-resistant *Staphylococcus aureus* (MRSA) was identified by polymerase chain reaction (PCR) testing for the mecA gene, which was responsible for resistance to methicillin and related antibiotics.

3.10 Data Management

3.10.1 Data Entry

Completed questionnaires were reviewed daily by a trained study coordinator to ensure completeness and accuracy, with additional verification conducted by the principal investigator every two days. Data were double-entered into EpiData version 4.4 by two independent data entry personnel to minimize entry errors.

A structured data entry template with predefined variable labels, coding schemes, and validation rules was used. Built-in checks in EpiData, including range and consistency checks, were applied during entry. Discrepancies identified during double entry were resolved by cross-checking against the original questionnaires

3.10.2 Data Cleaning

Following data entry, the dataset was exported to Microsoft Excel and subsequently imported into Stata version 17 (StataCorp, College Station, TX, USA) for cleaning and analysis. Data cleaning involved checking for completeness, verifying variable

coding, identifying out-of-range values, and ensuring internal consistency across variables.

Any inconsistencies or errors were corrected by referencing the original data collection tools. Missing data were identified and documented, and duplicate entries were removed where necessary. Each participant was assigned a unique identifier to ensure accurate linkage of clinical, microbiological, and antimicrobial susceptibility data.

3.10.3 Data Security

All study data were handled in accordance with ethical standards to ensure confidentiality and data protection. Electronic data were stored in password-protected files with access limited to authorized study personnel. Backup copies were maintained on a secure external drive to prevent data loss.

Hard copy questionnaires were stored in locked cabinets accessible only to the research team. Personal identifiers were replaced with unique codes to maintain participant anonymity.

3.11 Data Analysis

Data were analysed using Stata version 17 (StataCorp, College Station, TX, USA). Prior to analysis, data cleaning procedures were performed, including consistency checks, verification of coding, and identification of missing or out-of-range values.

Demographic and clinical characteristics, including age, duration of diabetes, duration of foot ulcer, vital signs, and ulcer grade, were analysed as either continuous or categorical variables depending on their nature and distribution. Continuous variables were assessed for normality using graphical methods and summary statistics. Normally distributed variables were summarised using means and standard deviations, while non-normally distributed variables were summarised using medians and interquartile ranges. Categorical variables were summarised using frequencies and percentages. Where appropriate, continuous variables were categorised using clinically relevant cut-off points.

Bacterial isolates were classified into Gram-negative and Gram-positive bacteria. Their distribution was analysed overall and stratified by ulcer grade, mono-microbial versus polymicrobial infection, and multidrug resistance (MDR) status. Polymicrobial infection was defined as the isolation of more than one organism from a single specimen.

Antimicrobial susceptibility results were coded as sensitive (1), resistant (0), intermediate (2), or not tested (9). For analysis, intermediate results were reclassified as resistant. Results coded as "not tested" were excluded for the respective antibiotic. Binary variables were generated to represent susceptibility and resistance. MDR was defined as resistance to at least one agent in three or more antimicrobial classes, while extensively drug-resistant (XDR) isolates were defined as resistance to all but one or two classes. ESBL-producing Enterobacterales, methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant Enterococci (VRE) were identified using standard laboratory criteria.

Antimicrobial susceptibility patterns were summarised overall and stratified by organism type, Gram classification, ulcer grade, MDR status, and infection type (mono-microbial or polymicrobial). Cross-tabulations were used to explore relationships between microbial characteristics and ulcer severity. Results were presented using tables and graphical methods, including bar charts.

All analyses were descriptive, as the primary objective was to characterize microbial profiles and antimicrobial susceptibility patterns.

3.12 Quality control

3.12.1 External Quality control

The study assistants underwent a five-day training to ensure they could gather accurate information. The questionnaires for assessing PSI *(Appendix 5)* were pretested at KNRH. Data was double entered to ensure accuracy. The questionnaires were reviewed for accuracy and completeness by the principal investigator and research assistants before participants left. Once the study begun,

daily discussions were held with study assistants to assess progress and address any challenges.

3.12.2 Laboratory quality control

Laboratory procedures at MBN Clinical Laboratories were performed under a documented quality management system. Internal quality control was maintained using standard reference strains from the American Type Culture Collection (ATCC), including Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, and Pseudomonas aeruginosa ATCC 27853, which were tested in parallel with each new batch of culture media and each run of antimicrobial susceptibility testing. Acceptablezone-diameter ranges for these control strains, as specified by the Clinical and Laboratory Standards Institute (CLSI), were verified before patient isolates were reported.

Each batch of prepared culture media was subjected to sterility and growthperformance checks prior to use, and expired or contaminated media were discarded. Identification of isolates was performed using conventional biochemical methods and, where available, automated identification systems, with results cross-checked against colony morphology and Gram-stain appearance. Antimicrobial susceptibility was determined by the Kirby–Bauer disk-diffusion method and interpreted using current CLSI breakpoints, with intermediate results recorded and handled as described in the operational definitions.

Externally, MBN Clinical Laboratories participated in recognised external quality assessment schemes for clinical microbiology and is an AABB (Association for the Advancement of Blood and Biotherapies)-Accredited facility. This combination of internal reference-strain control and external proficiency testing provided assurance of the accuracy and reproducibility of the culture and susceptibility results reported in this study.

3.13 Ethical considerations

- Administrative approval was obtained from the department of Medicine SRC, Kiruddu and Mulago National Referral Hospitals *(Appendices 2 and 7).*
- Ethical approval was obtained from School of Medicine Research Ethics committee (SOMREC) of Makerere University college of Health sciences *(Appendix 1).*

- Written informed consent was obtained from study participants *(Appendices 3 and 4).*
- Confidentiality of participants ensured by using unique identifiers.

3.14 Dissemination Plan

The findings of this study were disseminated through multiple channels to ensure broad visibility and impact. First, the results were submitted for publication in a peer-reviewed journal. Additionally, the findings will be presented at both national and international conferences, as opportunities arise, to engage with the global research community.

The study's findings were also shared with key stakeholders, including the Department of Medicine at Makerere University, the participating hospitals (Mulago and Kiruddu), healthcare professionals, and at large meetings involving policymakers. Furthermore, the report was shared with the School of Medicine Research and Ethics Committee (SOMREC), the participating hospitals, the Sir Albert Cook Library, and the Ministry of Health for further review and consideration

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CHAPTER FOUR: RESULTS

4.1 Participant flow chart

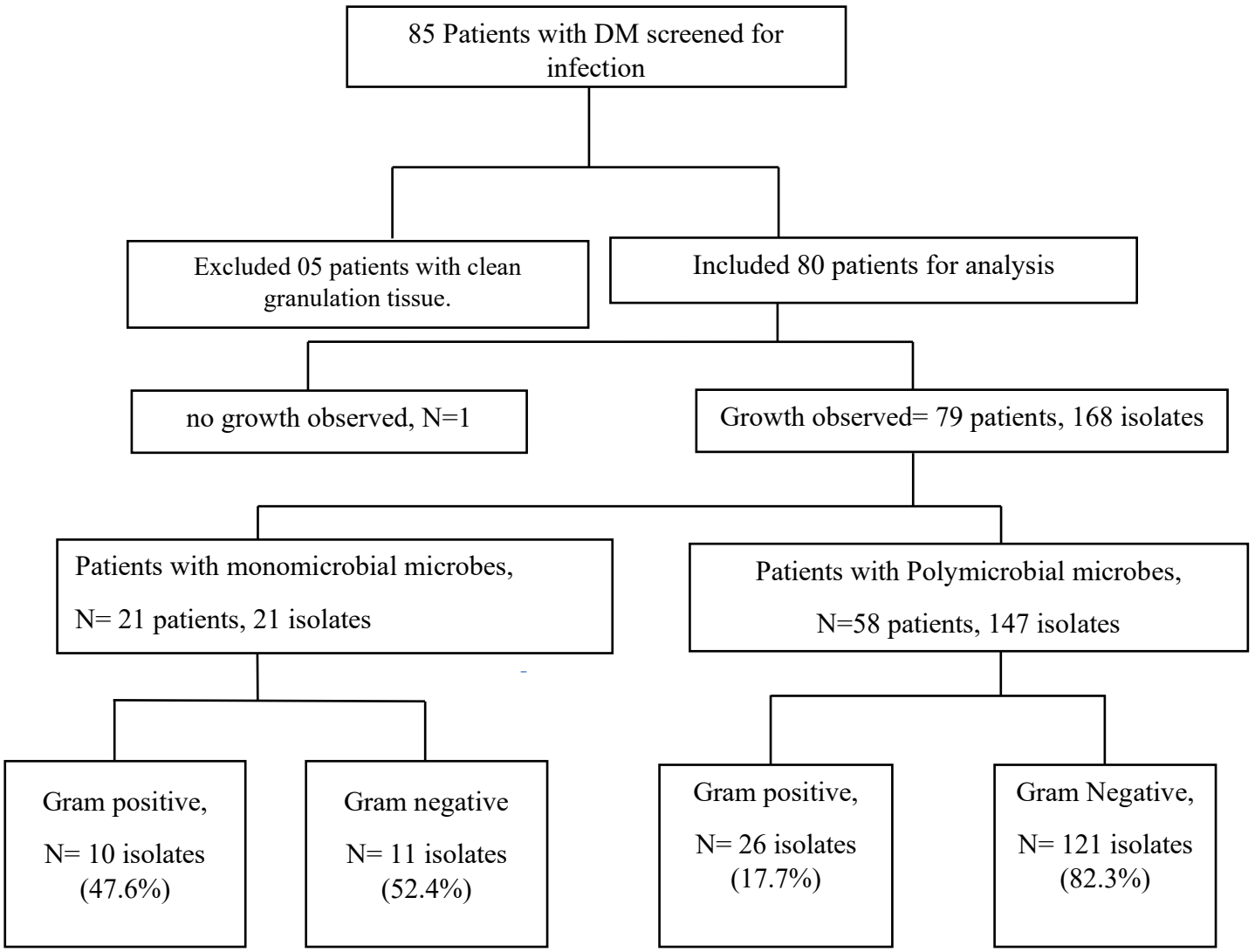


Figure 2: Participant flow chart.

During the study period, 85 adult patients with diabetic foot ulcers were screened for foot infection. Of these, five patients were excluded because their foot ulcers

had clean granulation tissue, leaving a final sample of 80 patients for analysis. Among the 79 patients with foot ulcers, 21 (26.6%) had monomicrobial infections, yielding 21 bacterial isolates (11 Gram-negative [52.4%] and 10 Gram-positive [47.6%]), while 58 (73.4%) had polymicrobial infections, yielding 147 bacterial isolates (121 Gram-negative [82.3%] and 26 Gram-positive [17.7%]). Microbiological growth was observed in 79 patients, with only one case showing no growth. The study profile is summarized in Figure 2 above.

4.2 Sociodemographic characteristics of the study participants.

Table 1: Sociodemographic characteristics of study participants.

Variable	Category		Frequency (n)	Percentage (%)
Sex	Male		37	46.25
	Female		43	53.75
Age	Mean ± SD (53.35 ± 11.9) years			
	< 50 years		29	36.25
	50–59 years		23	28.75
	≥ 60 years		28	35.00
Hospital	Mulago		29	36.25
	Kiruddu		51	63.75
Education	Not educated		04	5.00
	Educated N=76	Primary	38	50.00
		Secondary	29	38.16
		Tertiary	08	10.53
Employment	Not employed		28	35.00
	Employed N=52	Civil service	06	7.5
		Self employed	32	61.54
		Others	09	17.31
District of residence	Wakiso		39	48.75
	Kampala		15	18.75
	Others		26	32.50

The study included slightly more females 43 (53.75%) than males 37 (46.25%). The average age was 53.35 years (±11.9) with the majority 29 (36.25%) under 50

years. Most participants were from Kiruddu Hospital (63.75%), while half of the participants had primary education 38 (50%) and 29 (38.16%) had secondary education. 28 (35%) were not employed. Among the employed 52 (65%), the majority were self-employed 32 (61.54%), followed by others (17.31%) and civil servants (7.5%). Nearly half were from Wakiso (48.75%), 18.75% from Kampala.

4.2 Clinical characteristics of study participants

Table 2: Clinical characteristics of study participants

Variable	Category			Frequency (n)	Percentage (%)
DM duration	Median:7 (IQR: 4-11.5) years				
	< 5 years			23	28.75
	5–10 years			32	40.00
	> 10 years			25	31.25
Medication	Not on medication			04	5.00
	On medication N= 76	Insulin		53	69.74
		Orals N=39	Metformin	33	43.42
			Glimepiride	06	7.89
			Dapagliflozin	02	2.63
			Others	03	3.94
Foot ulcer duration	Median:2 (IQR: 1-3) months				
	Acute (≤1 month)			23	28.75
	Subacute (2–3 months)			42	52.5
	Chronic (>3 months)			15	18.75
Admission	Admitted			44	55.00
	Not admitted			36	45.00
Herbal medicine	Received			60	75.00
	Not received			20	25.00
Prior antibiotic exposure	Not exposed			06	7.50
	Exposed N=74	Amoxicillin		12	16.21
		Metronidazole		67	90.54
		Co-amoxiclav		19	25.68
		Ceftriaxone		48	64.86
		Levofloxacin/ciprofloxacin		38	51.35
		Flucamox		11	14.86
		Others		19	25.68

The median duration of diabetes mellitus among participants was 7 years (IQR: 4–11.5), with nearly 30% of the participants (23/75) having had the disease for less than 5 years, 32 (40.00%) for 5–10 years, and 25 (31.25%) for more than 10 years. Most patients 76 (95%) were on medication, predominantly insulin 53 (69.74%), while 33 (43.42%) used metformin and smaller proportions used other oral agents. The median duration of foot ulcers was 2 months (IQR: 1–3), with 23 (28.75%) presenting with acute ulcers (≤1 month), 42 (52.5%) with subacute ulcers (2–3 months), and 15 (18.75%) with chronic ulcers (>3 months). More than half of the participants 44 (55.00%) were admitted to hospital during the study period. Herbal medicine use was common, reported by 60 (75.00%) of patients. Prior antibiotic exposure was high, with 74 out of 80 participants having received antibiotics, most frequently metronidazole 67 (90.54%), ceftriaxone 48 (64.86%), and levofloxacin/ciprofloxacin 38 (51.35%).

4.3 Vital signs of study participants

Table 3: Vital signs of study participants

Variable	Category		Frequency (n)	Percentage (%)
Temperature	Mean ± SD (37.02 ± 0.87) °C			
	Normal (<37.5°C)		55	68.75
	Fever (≥37.5°C)		25	31.25
Pallor	No pallor		25	31.25
	Had Pallor N= 55	Mild	39	48.75
		Moderate	12	15.00
		Severe	04	5.00
Systolic Blood Pressure	Mean ± SD = 120.55 +/- 20.17 mmHg			
	N = 80	Hypotension (<90 mmHg)	5	6.25
		Normal (90 - 139 mmHg)	61	76.25
		Elevated (≥ 140 mmHg)	14	17.50
Diastolic Blood Pressure	Median (IQR) = 81.5 (70.75–88.0) mmHg			
	N = 80	Hypotension (<60 mmHg)	8	10.00
		Normal (60 – 89 mmHg)	58	72.50
		Elevated (≥90 mmHg)	14	17.50
Pulse Rate	Median: 96 (IQR: 86-104) bpm			
	Normal (≤100 bpm)		54	67.50
	Tachycardia (>100 bpm)		26	32.50
Respiratory Rate	Median: 19 (IQR: 17-21.5) breaths/min			
	Normal (≤ 20 bpm)		55	68.75
	Tachypnea (> 20 bpm)		25	31.25

Assessment of vital signs among the study participants revealed a mean temperature of 37.02°C (±0.87), with 55 (68.75%) exhibiting normal temperature and 25

(31.25%) presenting with fever. Systolic hypotension (<90 mmHg) was observed in 05 (6.25%) of patients, while 75 (93.75%) had normal or elevated systolic blood pressure. Diastolic hypotension (<60 mmHg) was present in 08 (10.00%) of participants, with the remainder 72 (90.00%) having normal or elevated diastolic pressure. The median pulse rate was 96 beats per minute (IQR: 86–104), with 54 (67.5%) of patients within the normal range (≤100 bpm) and 26 (32.5%) experiencing tachycardia (>100 bpm). The median respiratory rate was 19 breaths per minute (IQR: 17–21.5), with 55 (68.75%) of participants having a normal rate (≤20 bpm) and 25 (31.25%) showing tachypnoea (>20 bpm). Pallor was present in 55 (68.75%) of patients, with 39 (48.75%) classified as mild, 12 (15%) as moderate, and 04 (5%0 as severe, while 25 (31.25%) had no pallor.

4.4 Foot ulcer Characteristics:

Table 4: Foot ulcer Characteristics:

Variable	Category	Frequency (n)	Percentage (%)
Ulcer location	Heel	24	30
	Toes	47	58
	Arch	39	48.75
	Ball of foot	27	33.75
	Others	06	7.5
Depth of ulcer	Superficial	13	16.25
	Deep	58	72.50
	Bone exposed	10	12.50
Signs of infection	Redness	66	82.50
	Swelling	73	91.25
	Pus	75	93.75
	Fever	23	28.75
	Foul smell	65	81.25
Ulcer grade	1	10	12.50
	2	33	41.25
	3	25	31.25
	4	8	10.00
	5	4	5.00
Growth observed	Growth observed	79	98.75
	No growth	01	1.25

The majority of foot ulcers among the study participants were located on the toes 47 (58%), followed by the arch 39 (48.75%), heel 24 (30%), and ball of the foot 27 (33.75%), with a small proportion 06 (7.5%) occurring at other sites. In terms of depth, most ulcers were deep 58 (72.5%), while 13 (16.25%) were superficial and 10 (12.5%) had bone exposure. Clinical signs of infection were prevalent, with pus

observed in 75 (93.75%) of cases, swelling in 73 (91.25%), redness in 66 (82.5%), and a foul smell in 65 (81.25%); fever was present in 23 (28.75%) of participants. Regarding ulcer grading, 33 (41.25%) of ulcers were classified as grade 2, 25 (31.25%) as grade 3, 10 (12.5%) as grade 1, 08 (10%) as grade 4, and 04 (5%) as grade 5. Microbiological growth was detected in 79 (98.75%) of ulcers, with only one case showing no growth.

4.5 Microbiological growth characteristics

Table 5: Microbiological growth characteristics

Variable	Category		Frequency (n)	Percentage (%)
Gram positive N=36	*Enterococcus* spp.		23	63.89
	Staphylococcus aureus		09	25
	Others		04	11.11
Gram negative N=132	*Escherichia coli*		39	29.54
	klebsiella pneumoniae		20	15.15
	Acinetobacter spp.		13	9.84
	Citrobacter spp.		18	10.71
	Proteus mirabilis		11	8.33
	Pseudomonas aeruginosa		10	7.57
	Morganelli morganii		08	6.06
	Others		21	15.9
Polymicrobial	Monomicrobial		21	26.6
	Polymicrobial		58	73.4
Resistance level	Moderate		28	80.00
	High		07	20.00
Resistance subgroups	MDR N=168	Non-MDR	43	25.6
		MDR	125	74.4
	XDR N=168	Non-XDR	108	64.3
		XDR	60	35.7
	MRSA N=09	MSSA	02	22.22
		MRSA	07	77.78
	VRE N=21	Non-VRE	14	66.67
		VRE	07	33.33

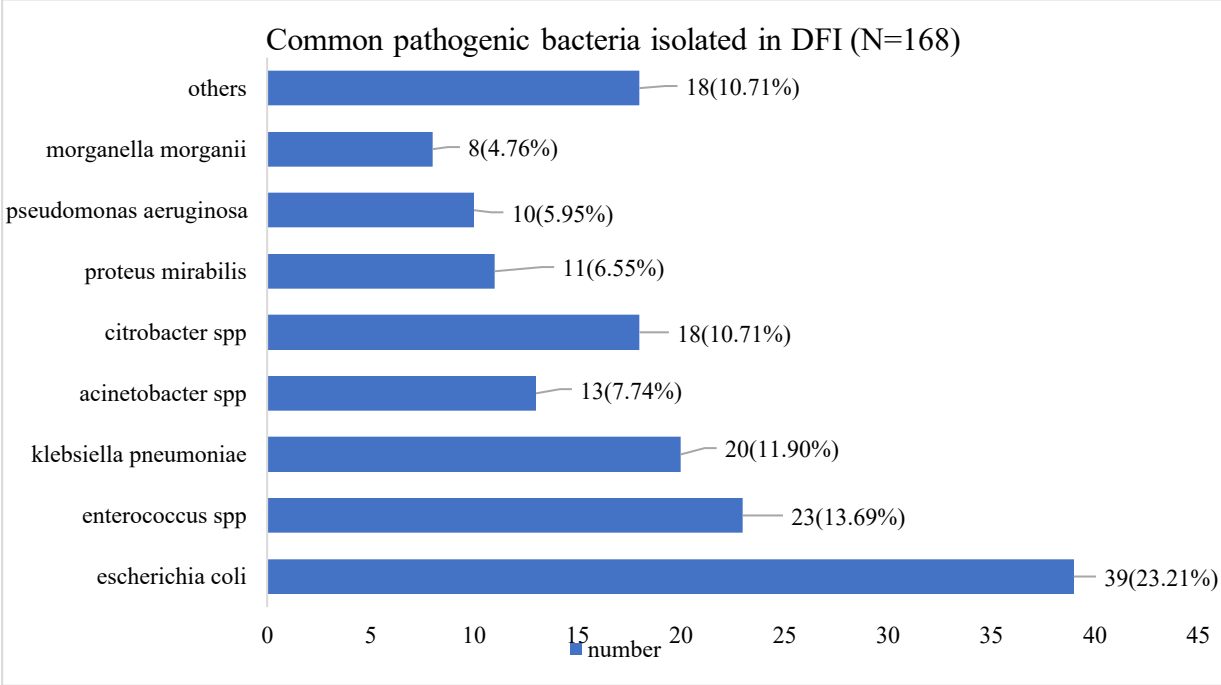
Microbiological analysis of foot ulcer samples revealed a predominance of polymicrobial infections, accounting for 58 (73.4%) of culture-positive patients, while monomicrobial infections were observed in 21 (26.6%) of patients. Gram-negative bacteria were the most frequently isolated organisms, N=132 (78.57%), with *Escherichia coli* 39 (29.54%), Klebsiella pneumoniae 20 (15.15%), and *Acinetobacter* spp. 13

(9.84%) being the most common. Other notable Gram-negative isolates included *Citrobacter* spp. 18 (10.71%), *Proteus mirabilis* 11 (8.33%), *Pseudomonas aeruginosa* 10 (7.57%), and 08 *Morganella morganii* (6.06%). Gram-positive bacteria, N=36 (21.43%) were also prevalent, with *Enterococcus* spp. 23 (63.89%) and *Staphylococcus aureus* 09 (25.00%) representing most isolates.

Antimicrobial resistance profiling revealed that 28 (80%) of isolates exhibited moderate resistance, while 07 (20%) demonstrated high resistance levels. Among resistant subgroups, multidrug-resistant (MDR) organisms constituted 125 (74.4%; 95% CI 67.3–80.4%) of isolates, and extensively drug-resistant (XDR) strains accounted for 60 (35.7%; 95% CI 28.9–43.2%). Methicillin-resistant Staphylococcus aureus (MRSA) was identified in 07 (77.78%) of Staphylococcus aureus isolates, and vancomycin-resistant Enterococcus spp (VRE) in 7 (33.33%) of *Enterococcus* spp. isolates.

4.6 Common pathogenic bacteria isolated in DM foot ulcers

Figure 3: Common pathogenic microbes isolated in DM foot ulcers



Analysis of bacterial isolates from diabetic foot ulcers revealed that Gram-negative bacteria were the most prevalent pathogens, accounting for 132 (78.57%) of the

168 total bacterial isolates. *Escherichia coli* was the most frequently isolated organism, accounting for 39 (23.21%) isolates, followed by *Enterococcus* spp. 23 (13.69%), Klebsiella pneumoniae 20 (11.90%), and *Acinetobacter* spp. 13 (7.74%). Other Gram-negative bacteria included *Citrobacter* spp. 18 (10.71%), *Proteus mirabilis* 11 (6.55%), *Pseudomonas aeruginosa* 10 (5.95%), and *Morganella morganii* 8 (4.76%). A further 18 (10.71%) isolates were classified as others which included *Proteus vulgaris* (5), *Enterobacter* spp. (4), *Klebsiella oxytoca* (4), beta hemolytic streptococcus, coagulase negative staphylococcus, *Corynebacteria* spp. and *Streptococcus pyogens* (group A strep) of which each had one isolate.

4.7 Antimicrobial susceptibilities for common pathogenic microbes.

4.7.1 Antimicrobial susceptibilities for common pathogenic gram-positive microbes.

Table 6: Antimicrobial susceptibilities for common pathogenic gram-positive microbes.

	enterococcus spp	**staphylococcus aureus**	**MRSA**	**VRE**
Total strains (Denorminator)	23	09	07	07
Vancomycin	14 (60.87%)	//	//	//
ciprofloxacin	03 (13.04)	04(44.44)	02(28.57)	02(40.00)
Chloramphenicol	17(73.91)	09(100)	07(100)	04(57.14)
Tetracycline	04(17.39)	//	03(42.86)	03(60.00)
Erythromycin	01(4.35)	02(22.22)	01(14.29)	00(00)
Linezolid	22(95.65)	09(100)	07(100)	06(85.71)
Penicillin	16 (69.57)	01(11.11)	00(00)	//
Gentamycin	//	04(44.44)	02(28.57)	//
Trimethoprim sulfamethoxazole	//	07(77.78)	06(85.71)	//

Note: * //: antibiotic not tested

Antimicrobial susceptibility testing of gram-positive isolates revealed varied patterns across different antibiotics. For *Enterococcus* spp. (n=23), high

susceptibility was observed to linezolid (22, 95.65%), chloramphenicol (17, 73.91%), and penicillin (16, 69.57%), while moderate susceptibility was noted for vancomycin (14, 66.67%). Lower susceptibility rates were seen for tetracycline (4, 30.77%), ciprofloxacin (3, 23.08%), and erythromycin (1, 4.35%). For *Staphylococcus aureus* (n=9), all isolates were susceptible to linezolid (9, 100%) and chloramphenicol (9, 100%), with substantial susceptibility to trimethoprim-sulfamethoxazole (7, 77.78%), gentamycin (4, 44.44%), and ciprofloxacin (4, 44.44%). Susceptibility to erythromycin (2, 22.22%) and penicillin (1, 11.11%) was lower. Among MRSA isolates (n=7), linezolid (7, 100%) and chloramphenicol (7, 100%) showed complete efficacy, while trimethoprim-sulfamethoxazole (6, 85.71%), tetracycline (3, 42.86%), gentamycin (2, 28.57%), and ciprofloxacin (2, 28.57%) demonstrated moderate activity. Erythromycin (1, 14.29%) and penicillin (0, 0%) were less effective. For VRE isolates (n=7), linezolid (6, 85.71%) and chloramphenicol (4, 57.14%) were the most effective agents, with moderate susceptibility to tetracycline (3, 60.00%) and ciprofloxacin (2, 40.00%). No susceptibility was observed for erythromycin (0, 0%). linezolid and chloramphenicol were the most consistently effective agents against gram-positive pathogens, while resistance to erythromycin and penicillin was common, especially among MRSA and VRE isolates.

4.7.2 Antimicrobial susceptibilities for common pathogenic gram-negative microbes.

Table 7: Antimicrobial susceptibilities for common pathogenic gram-negative microbes.

	Escherichia coli	*klebsiella pneumoniae*	*Citrobacter* spp.	*Acinetobacter* spp.	*proteus mirabilis*	*pseudomonas aeruginosa*	*Morganelli morganii*
Total strains	39	20	18	13	11	10	08
Cefuroxime	02/35 (5.71)	03/17 (17.65)	03/13 (23.08)	00/01 (0.0)	06/11 (54.55)	//	01/07 (14.29)
Ceftriaxone	05/38 (13.16)	04/18 (22.22)	08/17 (47.06)	00/12 (0.0)	07/11 (63.64)	//	02/05 (40.00)
Ceftazidime	05/36 (13.89)	06/20 (30.00)	07/17 (41.18)	00/12 (0.0)	07/11 (63.64)	08/10 (80.00)	04/08 (50.00)
Cefotaxime	05/37 (13.51)	06/19 (31.58)	08/18 (44.44)	00/10 (0.0)	07/11 (63.64)	//	04/08 (50.00)
Cefepime	05/38 (13.16)	05/18 (27.78)	08/18 (44.44)	00/10 (0.0)	07/11 (63.64)	08/09 (88.89)	06/07 (85.71)
Piperacillin tazobactam	13/33 (39.39)	08/20 (40.00)	07/15 (46.67)	01/10 (10.00)	09/11 (81.82)	07/09 (77.78)	05/05 (100)
Imipenem	31/37 (83.78)	15/19 (78.95)	04/13 (30.77)	02/12 (16.67)	07/10 (70.00)	08/09 (88.89)	02/05 (40.00)
Meropenem	01/01 (100)	02/02 (100)	03/03 (100)	00/01 (0.0)	03/03 (100)	01/01 (100)	03/03 (100)
Chloramphenicol	29/39 (74.36)	12/20 (60.00)	11/18 (61.11)	//	02/11 (18.18)	//	04/08 (50.00)
Amikacin	20/37 (54.05)	17/19 (89.47)	13/18 (72.22)	04/13 (30.77)	07/11 (63.64)	03/03 (100)	07/07 (100)
Trimethoprim sulfamethoxazole	04/39 (10.26)	06/20 (30.00)	02/18 (11.11)	01/11 (9.09)	04/11 (36.36)	//	02/08 (25.00)
Gentamycin	13/37 (35.14)	14/20 (70.00)	10/18 (55.56)	02/12 (16.67)	07/11 (63.64)	02/02 (100)	05/08 (62.50)
Tetracycline	05/38 (13.16)	07/20 (35.00)	06/17 (35.29)	00/01 (0.0)	01/11 (9.09)	//	04/08 (50.00)
Ciprofloxacin	02/35 (5.71)	08/20 (40.00)	05/18 (27.78)	01/11 (09.09)	05/11 (45.45)	07/10 (70.00)	02/06 (33.33)
Aztreonam	10/38 (26.32)	07/20 (35.00)	13/18 (72.22)	//	09/11 (81.82)	06/09 (66.67)	07/08 (87.50)

Note: * //: antibiotic not tested

Antimicrobial susceptibility testing of gram-negative isolates demonstrated considerable variability across different antibiotics and species. For *Escherichia coli* (n=39), the highest susceptibility was observed for imipenem (31, 83.78%), followed by amikacin (20, 54.05%) and chloramphenicol (29, 74.36%). Susceptibility to gentamycin (13, 35.14%), piperacillin-tazobactam (13, 39.39%), and cefepime (5, 13.16%) was moderate to low, while ciprofloxacin (2, 5.71%), tetracycline (5, 13.16%), and trimethoprim-sulfamethoxazole (4, 10.26%) showed poor activity. *Klebsiella pneumoniae* (n=20) exhibited the highest susceptibility to amikacin (17, 89.47%) and imipenem (15, 78.95%), with moderate susceptibility to gentamycin (14, 70.00%), cefepime (5, 27.78%), and ciprofloxacin (8, 40.00%). Lower susceptibility rates were seen for piperacillin-tazobactam (8, 40.00%), tetracycline (7, 35.00%), and trimethoprim-sulfamethoxazole (6, 30.00%).

For *Acinetobacter* spp. (n=13), susceptibility was generally low, with the highest rates for amikacin (4, 30.77%) and imipenem (2, 16.67%). *Citrobacter* spp. (n=18) showed the greatest susceptibility to amikacin (13, 72.22%) and aztreonam (13, 72.22%), followed by cefotaxime (8, 44.44%) and cefepime (8, 44.44%), with gentamycin (10, 55.56%) and piperacillin-tazobactam (7, 46.67%) also demonstrating moderate activity. *Proteus mirabilis* (n=11) demonstrated moderate susceptibility to amikacin (7, 63.64%), imipenem (7, 70.00%), and piperacillin-tazobactam (9, 81.82%), but lower rates for other agents. Pseudomonas aeruginosa (n=10) was most susceptible to amikacin (3, 100%), imipenem (8, 88.89%), and cefepime (8, 88.89%), with moderate susceptibility to piperacillin-tazobactam (7, 77.78%) and ceftazidime (8, 80.00%). Morganella morganii (n=8) showed high susceptibility to amikacin (7, 100%), cefepime (6, 85.71%), and piperacillin-tazobactam (5, 100%).

Overall, carbapenems (imipenem and meropenem) and amikacin were the most effective agents against gram-negative isolates, while resistance to fluoroquinolones, trimethoprim-sulfamethoxazole, and several cephalosporins was common across most species.

CHAPTER FIVE DISCUSSION

5.0 Summary of the key findings.

This study characterised the bacterial pathogens and their antimicrobial susceptibilityprofiles in diabetic foot ulcers among 80 patients at Mulago and Kiruddu National Referral Hospitals. Of these, 79 (98.8%) yielded bacterial growth, from which 168 bacterial isolates were recovered. Gram-negative bacteria predominated, accounting for 132 (78.6%) of isolates, while Gram-positive bacteria accounted for 36 (21.4%; 95% CI 15.9–28.2%). Escherichia coli was the single most common organism (39; 23.2% of all isolates), followed by Enterococcus spp. (23; 13.7%), Klebsiella pneumoniae (20; 11.9%) and Acinetobacter spp. (13; 7.7%). Polymicrobial infection was common, present in 58 of 79 culture-positive patients (73.4%). Against this organism profile, the susceptibility data identified a narrow set of agents that retained reliable activity, which forms the basis of the interpretation that follows.

5.1 Common pathogenic bacteria isolated in DM foot ulcers

5.1.1 Predominance of Gram-Negative Bacteria (Escherichia coli and Enterobacteriaceae)

In the current study, of *Escherichia coli* was the most frequently isolated organism. This finding is consistent with several other studies. For instance, Ahmadishooli and others, in a cross-sectional study involving 83 patients with diabetic foot infections at Shahid Mohammadi Hospital in Bandar Abbas, southern Iran, reported *E. coli* as the leading bacterial isolate (20.5%), followed by *Enterococcus* spp. and *Klebsiella* spp. (Ahmadishooli et al., 2020). Similarly, a retrospective analysis at King Abdulaziz University Hospital in Saudi Arabia identified *E. coli* as the most frequently isolated pathogen (16.9%), surpassing *Staphylococcus aureus* and *Klebsiella pneumoniae* (Alkhatieb et al., 2022).

Additionally, a descriptive study from Basrah, Iraq, reported *E. coli* as the most common species among Enterobacteriaceae isolated from diabetic foot infections,

accounting for approximately 36.8% of isolates (Hussein & Naser, 2023). These findings collectively support the predominance of Gram-negative organisms in certain settings.

5.1.2 Pathophysiological Basis for Gram-Negative Dominance

The predominance of Escherichia coli and other Gram-negative organisms in diabetic foot infections can be attributed to several pathophysiological mechanisms associated with diabetes and chronic wound environments (Alkhatieb et al., 2022). Persistent hyperglycaemia impairs neutrophil function, reduces chemotaxis, and compromises phagocytosis, thereby weakening host defence mechanisms, particularly against Gram-negative bacilli (Ahmadishooli et al., 2020).

Furthermore, diabetic foot ulcers are often deep, chronic, and ischaemic, creating a hypoxic and nutrient-rich environment that favours the growth of facultative anaerobic and enteric organisms such as *E. coli* and *Klebsiella* spp. (Hussein & Naser, 2023). Complications such as peripheral vascular disease and neuropathy further exacerbate tissue necrosis and delay wound healing, facilitating bacterial colonization.

These organisms possess virulence factors such as adhesins, endotoxins, and biofilm-forming capabilities, which enhance their persistence and resistance to host immune responses (Macdonald et al., 2021).

5.1.3 Epidemiological and Environmental Factors Influencing Gram-Negative Prevalence

From an epidemiological perspective, the high prevalence of *Escherichia coli* may reflect hygiene practices, healthcare exposure, and antimicrobial use patterns within the study population (Al-Mijalli, 2021). In many low- and middle-income settings, diabetic foot ulcers are prone to environmental contamination due to delayed healthcare seeking, barefoot walking, poor wound care practices, and inadequate sanitation (Sharma et al., 2025)

Additionally, prior and often inappropriate antibiotic use may suppress susceptible Gram-positive organisms, allowing resistant Gram-negative bacteria to predominate. Frequent hospitalisation, prolonged wound exposure, and repeated

clinical interventions further increase the risk of colonisation with nosocomial Gram-negative pathogens (Hussein & Naser, 2023).

5.1.4 Evidence of Gram-Positive Dominance in Other Settings (Staphylococcus aureus)

In contrast to studies highlighting Gram-negative predominance, several investigations have reported *Staphylococcus aureus* as the leading pathogen in diabetic foot infections, demonstrating regional variability in microbial patterns.

For example, a cross-sectional study conducted in Karachi, Pakistan by Nageen (2016) identified *S. aureus* as the most prevalent organism (23.2%), followed by *Escherichia coli* and *Klebsiella* spp (Nageen, 2016). Similarly, a retrospective cohort study in a tertiary hospital in Jeddah, Saudi Arabia reported *S. aureus* as the most frequently isolated pathogen (27.7%), followed by *E. coli* and *Pseudomonas aeruginosa* (Alkhatieb et al., 2022).

A systematic meta-analysis by Macdonald and others, which included over 100 studies globally, also identified *Staphylococcus aureus* as the most common isolate (~18%), with a significant proportion being methicillin-resistant (*MRSA*) (Macdonald et al., 2021). Furthermore, a systematic review focusing on Africa reported a pooled prevalence of approximately 19.9% for *S. aureus*, reinforcing its importance in the region (Makeri et al., 2023).

5.1.5 Pathophysiological Mechanisms Underlying Gram-Positive Infections.

The frequent isolation of *Staphylococcus aureus* can be attributed to its ability to exploit compromised host defences in individuals with diabetes. Chronic hyperglycaemia impairs neutrophil activity, including chemotaxis, phagocytosis, and oxidative killing, thereby reducing bacterial clearance (Makeri et al., 2023).

Additionally, diabetic neuropathy and microvascular disease contribute to tissue hypoxia and delayed wound healing, creating favourable conditions for bacterial growth (Alkhatieb et al., 2022). From a microbiological standpoint, *S. aureus* produces multiple virulence factors, including adhesins, coagulase, and biofilms, which enhance its survival and resistance to both host immunity and antimicrobial therapy (Makeri et al., 2023).

5.1.6 Epidemiological Factors Supporting Gram-Positive Prevalence

The predominance of *Staphylococcus aureus* also reflects epidemiological and healthcare-related factors. As a common skin commensal, it easily invades underlying tissues following skin breakdown due to trauma or neuropathy (Al-Mijalli, 2021).

Risk factors such as prolonged hospitalization, repeated antibiotic exposure, poor wound hygiene, and delayed medical care contribute to colonisation and infection. Additionally, variations in infection control practices, antibiotic stewardship, and community carriage rates influence the distribution of Gram-positive organisms across different regions (Hussein & Naser, 2023).

5.1.7 Summary and Interpretation of Findings

Overall, the findings of this study demonstrate a predominance of Gram-negative organisms, particularly *Escherichia coli*, in diabetic foot infections within the study population. This pattern aligns with evidence from several studies conducted in similar settings, suggesting a shift toward Gram-negative dominance in chronic and complicated wounds, especially in low- and middle-income contexts. However, the continued prominence of *Staphylococcus aureus* in other regions highlights important geographical variations in microbial aetiology.

The observed microbial profile in this study is likely the result of a complex interplay between host-related factors such as immunosuppression and poor glycaemic control, wound-related factors including chronicity and tissue hypoxia, and external influences such as healthcare exposure, hygiene practices, and antibiotic use. These findings underscore the importance of context-specific microbiological surveillance and culture-guided therapy in the management of diabetic foot infections.

Importantly, the predominance of Gram-negative organisms in this study has significant clinical implications, particularly in guiding empirical antibiotic selection and infection control strategies. It further highlights the need for early intervention, improved wound care practices, and strengthened antimicrobial

stewardship to reduce the burden of infection and prevent the emergence of resistant pathogens

5.2 Antimicrobial Susceptibility Patterns of Common Pathogens

5.2.1 Gram-negative susceptibility

Among Gram-negative isolates, the carbapenems and amikacin retained the most useful activity, although none approached universal susceptibility. Meropenem was active against 94.1% (16/17) of the Gram-negative isolates tested, and imipenem against 65.2% (75/115); the difference reflects the smaller number of isolates tested against meropenem rather than a true divergence in class activity, and both should beread together as carbapenem activity. Amikacin retained activity against 68.6% (83/121) of isolates, making it the most useful aminoglycoside, whereas gentamicin was active against only 52.9% (64/121).

By contrast, the agents most commonly used empirically in our setting performed poorly: ciprofloxacin retained activity against only 28.5% (35/123) of Gram-negative isolates, ceftriaxone 32.7% (37/113), and trimethoprim–sulfamethoxazole only 20.0% (24/120). This pattern is clinically important because it means that the fluoroquinolones and third-generation cephalosporins frequently prescribed empirically for diabetic foot infection in Uganda would be expected to fail in the majority of Gram-negative infections at these centres.

5.2.2 Gram-positive susceptibility

Among Gram-positive isolates, linezolid was the most active agent, retaining activity against 97.1% (34/35) of isolates tested, followed by chloramphenicol at 82.4% (28/34). Vancomycin retained activity against 70.8% (17/24) of isolates tested, a lowerfigure than is typically expected for Gram-positive cover and one that warrants cautious interpretation given the modest number tested and the inclusion of intermediate results as non-susceptible.

Penicillin was active against only 54.3% (19/35) of isolates and erythromycin against just 11.8% (4/34), confirming that these older agents are no longer dependable for empirical Gram-positive cover in this population. The strong performance of linezolid and chloramphenicol is notable: chloramphenicol, an inexpensive and widely available agent, may have a renewed role in resource-limited management of resistant Gram-positive diabetic foot infection, while linezolid should be reserved for confirmed resistant cases to preserve its activity.

5.2.3 Interpretation and local relevance.

Taken together, these findings depict a setting in which empirical options are substantially constrained by resistance. The predominance of Gram-negative organisms, combined with low susceptibility to fluoroquinolones, cephalosporins and cotrimoxazole, means that empirical regimens built around these agents are likely to be inadequate. The data instead support empirical Gram-negative cover with amikacinor a carbapenem for moderate-to-severe infection, pending culture, with linezolid or chloramphenicol added where resistant Gram-positive organisms are suspected.

These conclusions align with the direction of the 2020 National Guidelines for Antimicrobial Consumption and Use Surveillance in Human Health, in that they reinforce the need for culture-guided therapy and local susceptibility surveillance rather than fixed empirical prescribing. Compared with earlier Ugandan reports in which Gram-negative organisms also predominated, the present data add contemporary, breakpoint-current susceptibility figures from two major referral centres and quantify the extent to which first-line oral agents have lost reliability, which is the principal new contribution of this work.

5.2.4 General Susceptibility Trends Across Studies

Findings from this study are consistent with global evidence on antimicrobial susceptibility in diabetic foot infections. Several studies report that Gram-positive organisms, particularly *Staphylococcus aureus*, remain highly susceptible to agents

such as vancomycin, linezolid, and tigecycline, while showing high resistance to penicillin, erythromycin, and clindamycin (Sultana et al., 2023).

Similarly, Gram-negative organisms including *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are generally sensitive to carbapenems (e.g., imipenem), aminoglycosides (e.g., amikacin), and piperacillin-tazobactam, but demonstrate significant resistance to penicillins, cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole (Liu et al., 2023). These patterns highlight the increasing limitations of commonly used antibiotics and the importance of targeted therapy (Sharma et al., 2025).

5.2.5 Biological Basis of Antimicrobial Susceptibility and Resistance

Differences in susceptibility between Gram-positive and Gram-negative organisms are largely explained by microbial structure and resistance mechanisms (Alkhatieb et al., 2024). Gram-positive bacteria remain susceptible to drugs like linezolid due to their mechanism of action on ribosomal protein synthesis, which bypasses common resistance pathways such as β-lactamase production (Makeri et al., 2023).

In contrast, Gram-negative bacteria possess an outer membrane, efflux pumps, and porin channels that limit drug penetration. Carbapenems retain effectiveness due to their stability against β-lactamases, while aminoglycosides remain active through ribosomal inhibition (Kagwa et al., 2018).

Resistance mechanisms include:

- β-lactamase production (e.g., ESBL, AmpC)
- Target site mutations (e.g., fluoroquinolone resistance)
- Efflux pumps and reduced permeability (Zambelli et al., 2025).
- Enzymatic modification of antibiotics

These mechanisms collectively explain why resistance is common for older antibiotics but lower for reserved drugs.

5.2.6 Epidemiological Drivers of Resistance Patterns

Antimicrobial susceptibility patterns are strongly influenced by antibiotic use and healthcare practices (Wada et al., 2023b). Frequent and often inappropriate use of commonly available antibiotics—such as penicillins, cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole—creates selective pressure that promotes resistant strains (Mohiuddin et al., 2025).

Additional risk factors such as prior hospitalization, chronic wounds, repeated surgical interventions, and the presence of indwelling medical devices increase patient exposure to multidrug-resistant organisms, which frequently colonize wounds and subsequently precipitate infection (Liu et al., 2023).

In many low-resource settings, delayed healthcare seeking, poor wound care, and weak antimicrobial stewardship systems further accelerate resistance (Kagwa et al., 2018). In contrast, restricted use of drugs such as carbapenems and linezolid helps preserve their effectiveness

5.2.7 Variations in Susceptibility Across Regions

Despite general trends, antimicrobial susceptibility patterns vary across settings. Some studies report higher effectiveness of fluoroquinolones and gentamicin, while others show significant resistance to these agents. For example, studies from Indonesia (Yulia et al., 2025) and South Africa efficacy (Turner et al., 2024) have demonstrated good sensitivity to fluoroquinolones and aminoglycosides, contrasting with resistance patterns observed in this study.

Other reports indicate extreme resistance in certain organisms, such as *Acinetobacter* spp. (Sekhar et al., 2014), or greater reliance on alternative agents like doxycycline or tigecycline (Sharma et al., 2025). These differences reflect variations in local prescribing practices, healthcare systems, and microbial ecology.

5.2.8 Explanation for Observed Differences

Variations in antimicrobial susceptibility can be explained by both biological and epidemiological factors. Chronic diabetic wounds often involve biofilm formation,

tissue necrosis, and poor vascular supply, which limit antibiotic penetration and reduce drug efficacy (Anvarinejad et al., 2016).

Additionally, regional differences in resistance genes—such as *mecA* in MRSA or ESBL production in Enterobacterales—directly influence treatment outcomes (London, 2026). Differences in patient characteristics, ulcer severity, and prior antibiotic exposure further contribute to variability across studies.

5.2.9 Implications for Clinical Practice

The findings of this study highlight the importance of culture-guided therapy in the management of diabetic foot infections. The high resistance to commonly used antibiotics limits their effectiveness for empirical treatment.

The preserved activity of carbapenems, aminoglycosides, and selected Gram-positive agents suggests their continued relevance in severe infections. However, their use should be guided by antimicrobial stewardship principles to prevent emerging resistance.

Overall, these results emphasise the need for:

- Routine microbiological surveillance
- Development of local antibiograms
- Rational antibiotic use
- Strengthened infection prevention and control measures

CHAPTER SIX CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- Gram-negative bacteria were the predominant pathogens isolated from diabetic foot infections in this study, with *Escherichia coli* being the most frequently identified organism, followed by *Enterococcus* spp., *Klebsiella pneumoniae*, and *Acinetobacter* spp. This pattern reflects the influence of chronic wound conditions, host-related factors, and local epidemiological dynamics.
- Antimicrobial susceptibility testing revealed that linezolid and chloramphenicol demonstrated the most consistent activity against Gram-positive organisms, including MRSA and VRE. Among Gram-negative isolates, carbapenems (imipenem and meropenem) and amikacin showed the highest effectiveness.
- High levels of resistance were observed against commonly used antibiotics, including penicillins, erythromycin, fluoroquinolones, trimethoprim-sulfamethoxazole, and several cephalosporins, limiting their utility for empirical therapy.
- Overall, the findings highlight a concerning burden of antimicrobial resistance and underscore the importance of culture-guided treatment, routine surveillance, and strengthened antimicrobial stewardship in the management of diabetic foot infections.

6.2 Recommendations

- Empirical treatment of diabetic foot infections should prioritise Gram-negative coverage, given the predominance of Gram-negative organisms in this study, particularly *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter* spp.
- Linezolid should be reserved for confirmed or strongly suspected resistant Gram-positive infections, including MRSA and VRE, as it demonstrated the highest and most consistent susceptibility among Gram-positive isolates in this cohort.
- Carbapenems or amikacin should be considered for severe Gram-negative infections, especially in patients with advanced ulcers or prior antibiotic exposure, based on their superior activity against Gram-negative isolates in this study.
- Antibiotic selection for moderate to severe infections should be guided by routine culture and susceptibility testing, to avoid using antibiotics with high resistance rates such as penicillins, fluoroquinolones, trimethoprim-sulfamethoxazole, and cephalosporins.
- Hospitals should regularly update local guidelines for treating diabetic foot infections, using current culture and susceptibility data, to improve antibiotic use and reduce resistance.

6.3 Study limitations

- The cross-sectional study design captures antimicrobial susceptibility at a single point in time and does not account for temporal changes in resistance patterns or treatment outcomes.
- Clinical outcomes such as wound healing, amputation rates, and mortality were not assessed, preventing correlation between antimicrobial susceptibility patterns and patient outcomes. This was not part of this study.

Glycated haemoglobin (HbA1c), body mass index (BMI), and detailed comorbidity data (including specific cardiovascular diagnoses, renal function, and cancer history) were not systematically captured in this study. The absence of HbA1c measurements in particular limits the ability to correlate glycaemic control with organism profile and resistance patterns, which would have provided important clinical context. Future studies should incorporate these variables.

The study enrolled only patients with clinically infected diabetic foot ulcers presenting with purulent or inflamed wounds. Patients with wounds showing clean granulation tissue were excluded as a deliberate eligibility criterion, given that swab culture from non-infected wounds yields limited actionable microbiological information. While this approach enriched the sample for culture-positive results, it means the findings apply specifically to patients with infected-appearing diabetic foot ulcers at tertiary referral centres and may not be generalisable to the broader population of all patients with diabetic foot disease, including those with cleaner wounds or those managed at lower-level facilities.

The cross-sectional design did not include a follow-up period. As a result, clinical outcomes such as response to prescribed antibiotic therapy, wound healing trajectories, hospital readmission rates, and rates of amputation could not be assessed. A short prospective follow-up period of three to four weeks would have substantially strengthened the study by enabling correlation between susceptibility profiles and treatment outcomes, and is recommended for future work.

- Molecular methods for resistance detection (e.g., ESBL, carbapenemase, mecA, and van genes) were not performed, and resistance was inferred solely from phenotypic susceptibility testing.

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Appendix

Appendix 1: IRB approval letter

MAKERERE UNIVERSITY
P.O. Box 7072 Kampala, Uganda
E-mail: rresearch9@gmail.com
Phone: 256 414 533541
Fax: 256 414 541036/0414 532204
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICINE
RESEARCH ETHICS COMMITTEE

16/06/2025

To: Edwin Oluka

0701424705

Type: Initial Review

Re: Mak-SOMREC-2025-1273: Antimicrobial Susceptibility Of Potentially Pathogenic Bacteria Isolated From Diabetic Foot Ulcers Of Patients At Mulago and Kiruddu Hospitals

I am pleased to inform you that at the **202** convened meeting on **25/03/2025**, the MAK School of Medicine REC (Mak-SOMREC) meeting voted to approve the above referenced application.
Approval of the research is for the period of **16/06/2025** to **16/06/2026.**

As Principal Investigator of the research, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the protocol or the consent form must be submitted to the REC for re-review and approval **prior** to the activation of the changes.
3. Reports of unanticipated problems involving risks to participants or any new information which could change the risk benefit: ratio must be submitted to the REC.
4. Only approved consent forms are to be used in the enrollment of participants. All consent forms signed by participants and/or witnesses should be retained on file. The REC may conduct audits of all study records, and consent documentation may be part of such audits.
5. Continuing review application must be submitted to the REC **eight weeks** prior to the expiration date of **16/06/2026** in order to continue the study beyond the approved period. Failure to submit a continuing review application in a timely fashion may result in suspension or termination of the study.
6. The REC application number assigned to the research should be cited in any correspondence with the REC of record.
7. You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.

The following is the list of all documents approved in this application by MAK School of Medicine REC (Mak-SOMREC):

No.	Document Title	Language	Version Number	Version Date
1	Revised consent form	English	2	2025-05-27
2	Revised Research Budget	English	2	2025-05-27
3	Clean copy of revised proposal	English	6.0	2025-05-12
4	Revised study work plan	English	pdf	2025-05-11
5	Covid 19/Ebola risk management plan	English	pdf	--
6	Data collection tools	English	pdf	--
7	Translated consent	Luganda	pdf	--

Yours Sincerely

Prof. Ponsiano Ocama
For: MAK School of Medicine REC (Mak-SOMREC)

Appendix 2: Hospital approval letter

TELEPHONE: 0414-672315
: 0414-672308
: 0414-672309
Email : kiruddurh@gmail.com
Website : www.kiruddu.hosp.go.ug
Ref: KRD/ADM/3/101/1

THE REPUBLIC OF UGANDA

Kiruddu National Referral Hospital
Salaama Road Munyonyo.
P.O BOX 6588, Kampala, Uganda

14th August, 2025

Dr. Oluka Edwin Noah
Principal investigator

Re: Permission to conduct Research at Kiruddu National Referral Hospital.

Reference is made to your letter dated 12th August, 2025 in which you requested for permission to conduct a study at Kiruddu National Referral Hospital. This study got approval from Makerere university Research and Ethics Committee with effect from june16th,2025 through June 16th, 2026.

Please be informed that your proposal titled ***"Antimicrobial susceptibility of potentially pathogenic bacteria isolated from diabetic foot ulcers of patients at Mulago and Kiruddu Hospitals."*** was reviewed and you were granted permission to conduct your research at this facility. Please take note of the following:

i. The research team will be governed by the rules that govern the facility.
ii. You are expected to obtain final approval of your study from the National Council for Science and Technology (UNCST).
iii. You are expected to abide by the UNCST regulations for conducting research involving human participants.
iv. You should alert the hospital administration when you start your study.
v. Upon completion of your study, you are expected to provide the hospital with a summary report your findings and to acknowledge the hospital on your publication(s).
xiv. No payment required (due to MOU between Kiruddu and Makerere University)

By Copy of this letter, the Head- Endocrinology, Head- Laboratory Services and Head-Clinical Services are informed about your study and are encouraged to co-operate with your team.

Sincerely,

Dr. Akabwai George. P
Head, Research

KIRUDDU NATIONAL REFERRAL HOSPITAL
14 AUG 2025
HOSPITAL DIRECTOR

Sign.....
Dr. Kabuga Charles
Hospital Director

C.c Head- Endocrinology
c.c Head- Laboratory Services
C.c Head-Clinical Services

"Leading with innovation and serving with compassion in health care delivery"

Appendix 3: informed consent form (English)

INFORMED CONSENT

Title of proposed study: The antimicrobial drug susceptibility patterns of the bacteria isolated from foot ulcers of patients with diabetes mellitus in mulago and kiruddu national referral hostpitals.

Investigator:

Dr. Oluka Edwin Noah,

Department of Medicine, College of Health Sciences, Makerere University

Background and rationale for the study:

My name is Dr. Oluka Edwin Noah from the Department of Medicine, Makerere University. I am conducting a study to find out the clinical and social characteristics of patients with diabetes mellitus with foot ulcers and also determine which antibiotics work best against the bacteria isolated from the ulcers of these patients attending care at Kiruddu and Mulago National Referral Hospitals. The aim of this study is to identify the most common bacteria involved as well as their sensitivity to antibiotics, and this study will eventually enable health workers to better treat these infections with the appropriate antibiotics.

Sponsors:

This study is being sponsored partly by the Merck foundation which offers various scholarships to medical professionals, and partly by the lead investigator.

Purpose:

One of the most common complications of diabetes resulting in long hospital stay of diabetic patients in our setting is diabetic foot infections. This study is the only study that has focused on the commonly isolated bacteria and how they respond to antibiotics in patients with diabetic foot ulcers in central Uganda. As such, it is expected to provide the most recent information on antibiotic effectiveness in this population which is unavailable. It should therefore form useful material for reference to other researchers and other readers in general.

Duration:

This study will be done over a duration of three months, August, September and October 2025.

Study Procedure:

If you agree to participate:

You will be requested to answer questions about your current and past medical illnesses. A physical examination will be performed on you. This may take about 15mins of your time.

Your wound will be cleaned with Normal saline then a swab of pus will be taken from it; The sample will be taken for culture and sensitivity.

Who will participate in the study:

This study is being carried out on 132 patients with diabetes mellitus and foot ulcers attending health care at Mulago and Kiruddu National referral hospitals. The patient has therefore been identified based on the description above.

Risks

The patient may feel some pain as the pus swab is taken off.

However, most care and precautions shall be taken to ensure the patient feels as little pain and discomfort as possible. Also, clean techniques will be used when picking the sample to minimize the risk of infection.

Benefits

The patient will benefit from this study by having this important investigation done at no cost. The results shall be given to the primary care team to help in the management of the patient. There is no monetary benefit to the patient.

Alternatives:
Participation in this study is entirely voluntary and should you choose not to participate, you will still receive appropriate care from the doctors in this facility

Confidentiality:

The identity of the patient will be concealed in as far as the law allows. The patient's name will not appear anywhere on the coded forms with the information. The results of this study will be kept strictly confidential on a password protected computer and the paper records in a securely locked cabinet only accessible to the lead investigator. They will be used only for research purposes. These documents will also be accessed by the School of Medicine Research and Ethics Committee (SOMREC) and the Uganda National Council for Science and Technology (UNCST) for verification.

Rights to refuse or withdraw

Participation in this study is entirely voluntary, and there will be no penalty for refusal to participate or withdrawal from the study.

Cost:

The cost of laboratory investigation such as HBA1C will be met by the investigator.

Compensation

Participants in this study will receive a token of appreciation of 10,000 Uganda Shillings (UGX) to compensate for their time and effort. This compensation will be provided as a gesture of gratitude and will be given to participants upon collection of the sample.

Reimbursement:

Cost of travel will not be compensated for since participants will be recruited during their routine out-patient's visit at Mulago or Kiruddu National Referral Hospital.

Questions:

In case of any problems or any questions related to this study you may ask now or later by contacting Dr. Oluka Edwin Noah by telephone on +256 701424705 or by email oedwinnoah@gmail.com at any time during the study.

Questions about participants rights:

Questions regarding your right as a study participant should be addressed to the Chairperson of the School of Medicine Research and Ethics Committee, the committee that deals with the rights of participants and which also approved this study. His name is Prof. Ponsiano Ocama and his phone number is +256772421190.

Statement of voluntariness:

Participation in this study is entirely voluntary and should you choose not to participate, you will still receive appropriate care from the doctors in this facility. There will be no penalty for with drawing from the study.

Dissemination:

The findings of this study will be disseminated through multiple channels to ensure broad visibility and impact. First, the results will be submitted for publication in a peer-reviewed journal. Additionally, the study will be presented at both national and international conferences, as opportunities arise, to engage with the global research community.

The study's findings will also be shared with key stakeholders, including the Department of Medicine at Makerere University, the participating hospitals (Mulago and Kiruddu), healthcare professionals, and at large meetings involving policymakers. Furthermore, the report will be shared with the School of Medicine Research and Ethics Committee (SOMREC), the participating hospitals, the Sir Albert Cook Library, and the Ministry of Health for further review and consideration.

Ethical approval:

This study has been approved by the Makerere University School of Medicine Research and Ethics Committee (MAK-SOMREC) which is accredited by the Uganda National Council for Science and Technology (UNCST).

STATEMENT OF CONSENT

..... has described to me what is going to be done, the risks, the benefits involved and my rights regarding this study. I understand that my decision to participate in this study will not alter my usual medical care. In the use of this information, my identity will be concealed. I am aware that I may withdraw at any time. I understand that by signing this form, I do not waive any of my legal rights but merely indicate that I have been informed about the research study in which I am voluntarily agreeing to participate. A copy of this form will be provided to me.

Name of participant:.....

Signature..... .....Date .....

Name of witness:.....

Signature..... .....Date .....

Name of interviewer: .....

Signature .....Date .....

Appendix 4: informed consent form (translated)

OKUKKILIZA OKUTEGEEZEDDWA

Omutwe gw'okunoonyereza: Eddagala erirwanyisa obuwuka mu nkola y'okukwatibwa obuwuka buno ey'awuliddwa ku mabwa mu bigere ga abalwadde ba ssukaali mu malwaliro ga mulago ne kiruddu ewa sindikibwa abalwadde.

Omunoonyereza:
Dr. Oluka Edwin Noah
Department of Medicine, College of Health Sciences, Makerere University.

Ensibuko n'Ensonga Enkulu:

Amannya gange nze Dr.Oluka Edwin Noah okuva mu kitongole ky'obusawo mu yunivaste y'e Makerere. Nkola okunoonyereza okuzuula ebyafaayo by'abalwadde n'embeera z'abantu mu balwadde ba ssukaali abalina amabwa mu bigere era n'okumanya engeri obuwuka obuziyiza ekirwadde kino gye bukwatibwako nga bwawuddwa okuva mu mabwa g'abalwadde bano abajjanjabirwa mu malwaliro ga Kiruddu ne Mulago.

Ekigendererwa ky'okunoonyereza kuno kwe kuzuula obuwuka obuleeta endwadde nga kwotadde n'obuwulize bw'abwo eri eddagala eritta obuwuka, era okunoonyereza kuno okukkakkana nga kusobozeseza abakozi b'ebyobulamu okujjanjaba obulungi yinfekisoni/endwadde zino n'eddagala erisaanidde eritta obuwuka obwo.

Abawagira:

Okunoonyereza kuno kuwagirwa ekitundu ku kitongole kya Merck foundation egaba sikaala ez'enjawulo eri abakugu mu by'obusawo, ate ekitundu ku munoonyereza akulembera.

Omugaso:

Ekimu ku bizibu ebisinga okuva mu ssukaali ekiviraamu okumala ebbanga eddene mu ddwaliro eri abalwadde ba ssukaali mu mbeera yaffe, ye yinfekisoni z'amabwa mu bigere.

Okunoonyereza kuno kwe kwokka okutadde essira ku buwuka obutera okuzuulibwa n'okuziyiza eddagala mu balwadde abalina amabwa g'ebigere aga ssukaali mu masekkati ga Uganda. Bwe kityo, kisuubirwa okuwa obubaka obusembayo obukwata ku kuziyiga eddagala okw'obuwuka buno mu bantu bano obutaliiwo.

N'olw'ensonga eyo, kuteekwa okuba okw'omugaso eri abanoonyereza abalala n'abasomi abalala okutwaliza awamu.

Ebbanga:

Okunoonyereza kuno kugenda kukolebwa mu bbanga lya emyezi esatu, Ogwomusanvu

, Ogwomunana n'ogwekumi 2025.

MAKERERE UNIVERSITY
SCHOOL OF MEDICINE
INITIAL APPROVAL
VALID UNTIL
★ 16 JUN 2026 ★
RESEARCH & ETHICS COMMITTEE
P.O. BOX 7072,KAMPALA

Enkola y'Okunoonyereza

Bw'okkiriza Okw'etabamu, ojja kusabibwa okuddamu ebibuuzo ebikwata ku bulwadde bw'olina kati n'obwo bwe walina edda. Ojja kukeberebwa mu mubiri.

Kino kiyinza okutwala eddakiika nga kumi na ttaano ez'obudde bwo. Ekiwundu kyo kijja kuyonjebwa n'omunnyo ogwa bulijjo olwo kiggyibwemu amasira amatono. Ekyokulabirako kino kijja kutwalibwa okukeberebwa n'okumanyisibwa.

Ani agenda okwetaba mu kunoonyereza kuno:

Okunoonyereza kuno kukolebwa ku balwadde 132 abalina obulwadde bwa sukaali n'amabwa mu bigere abagenda mu by'obulamu mu malwaliro agasindikibwa e Mulago ne Kiruddu National. N'olwekyo omulwadde azuuliddwa okusinziira ku kunnyonnyola waggulu.

Obulabe:
Omulwadde ayinza okuwulira obulumi ng'aggyiddwaako ekikuta ky'amasira. Kyokka, okufaayo n'okwegendereza bijja kukolebwa okukakasa nti omulwadde awulira obulumi n'obuzibu bitono ddala nga bwe kisoboka. Ate era, enkola ya Aseptic eyo kutangira yinfekisoni okusaasana ejja kukozesebwa nga balonda sampuli okukendeeza ku bulabe bw'okukwatibwa yinfekisoni.

Emiganyulo:
Omulwadde ajja kuganyulwa mu kunoonyereza kuno ng'okunoonyereza kuno okukulu kukolebwa awatali kusasula ssente. Ebinaava mu kunoonyereza kuno birina kuweebwa ttiimu y'abajjanjabi abasookerwako okuyamba mu kulabirira omulwadde. Omulwadde talina ssente z'afuna.

Enkola endala:

Okwetaba mu kunoonyereza kuno kwa kyeyagalire ddala era singa osalawo obuteetaba mu kunoonyereza kuno, ojja kukyafuna obujjanjabi obusaanidde okuva mu basawo abali mu kifo kino

Okukuuma Ebyama:
Endagamuntu y'omulwadde ejja kukwekebwa mu kifo amateeka we gakkiriza. Erinnya ly'omulwadde terijja kulabika wonna ku foomu eziriko enkoodi n'amawulire ago. Ebinaava mu kunoonyereza kuno bijja kukuumibwa nga bya kyama nnyo ku kompyuta ekuumibwa ekigambo ky'okuyingira era ebiwandiiko by'empapula mu kabineti eriko kkufulu ennungi ebisobola okutuusibwako omunoonyereza akulembera yekka. Zijja kukozesebwa mu kunoonyereza kwokka. Ebiwandiiko bino era bigenda kufunibwa akakiiko akanoonyereza ku mpisa mu ssomero ly'obusawo (SOMREC) n'ekitongole kya Uganda National Council for Science and Technology (UNCST) okukakasibwa.

Eddembe ly'Okugaana oba Okuvaamu:
Okwetaba mu kunoonyereza kuno kwa kyeyagalire kwonna, era tewajja kubaawo kibonerezo kya kugaana kwetaba mu kunoonyereza oba okuva mu kunoonyereza.

Omuwendo:

Ebisale by'okunoonyereza mu laboratory oluvannyuma lw'okusiimuula ekiwundu kyo bijja kusasulwa omunoonyereza

Okuliyirira:

Abaneetaba mu somo lino bajja kufuna akasiimo ka 10,000 eza silingi ya Uganda okubaliyirira olw'obudde n'amaanyi gaabwe Okuliyirira kuno kujja kuweebwa nga akabonero akokusiima era kujja kuweebwa abanaaba betabye bwebanaaba bajibwaako sampo zaabwe.

Okuddizibwa ssente:

Ssente z'entambula tezigenda kuliyirirwa okuva abeetabye mu mpaka zino bwe bagenda okuwandiikibwa mu kulambula kwabwe okwa bulijjo okw'abalwadde abatali balwadde mu ddwaaliro e Mulago oba Kiruddu National Referral Hospital.

Ebibuuzo:

Singa wabaawo ekizibu kyonna oba ekibuuzo kyonna ekikwata ku kunoonyereza kuno oyinza okubuuza kati oba oluvannyuma ng'otuukirira Dr. Oluka Edwin Noah ku ssimu ku +256 701424705 oba ku email oedwinnoah@gmail.com essaawa yonna mu kiseera ky'okunoonyereza.

Ebibuuzo ebikwata ku ddembe ly'abeetabye mu kutendekebwa:

Ebibuuzo ebikwata ku ddembe lyo ng'omuntu eyeetabye mu kunoonyereza birina okuweebwa Ssentebe w'akakiiko k'okunoonyereza n'empisa mu ssomero ly'obusawo, akakiiko akakola ku ddembe ly'abeetabye mu kunoonyereza era nga nako kakkiriza okunoonyereza kuno. Amannya ge ye Prof. Ponsiano Ocama ate essimu ye +256772421190.

Ekiwandiiko ky'obwannakyewa:

Okwetaba mu kunoonyereza kuno kwa kyeyagalire ddala era singa osalawo obuteetaba mu kunoonyereza kuno, ojja kukyafuna obujjanjabi obusaanidde okuva mu basawo abali mu kifo kino. Tewajja kubaawo ekibonerezo eky'okuva mu kunoonyereza kuno.

Okubunyisa:

Ebizuuliddwa mu kunoonyereza kuno bijja kusaasaanyizibwa okuyita mu mikutu mingi okulaba nga birabika bulungi era nga bikwatibwako. Okusooka, ebyavaamu bijja kuweebwayo okufulumizibwa mu katabo akatunuulirwa bannaabwe. Okugatta ku ekyo, okunoonyereza kuno kugenda kwanjulwa mu nkiiko z'eggwanga n'ez'ensi yonna, ng'emikisa gijja, okukwatagana n'abanoonyereza mu nsi yonna.

Ebizuuliddwa mu kunoonyereza kuno era byakugabana n'abakulu abakwatibwako omuli ekitongole ky'obusawo ku yunivasite y'e Makerere, amalwaliro ageetabye mu kunoonyereza kuno (Mulago ne Kiruddu), abakugu mu by'obulamu, ne mu nkiiko ennene ezirimu abakola enkola. Ekirala, lipoota eno egenda kugabibwa n'akakiiko akakola ku kunoonyereza n'empisa mu ssomero ly'obusawo (SOMREC), amalwaliro ageetabye mu mpaka zino, etterekero

ly'ebitabo erya Sir Albert Cook, ne Minisitule y'ebyobulamu okwongera okwekenneenya n'okulowoozebwako.

Okukkirizibwa mu mpisa:

Okunoonyereza kuno kukkiriziddwa akakiiko ka Makerere University School of Medicine Research and Ethics Committee (MAK-SOMREC) akakkirizibwa ekitongole kya Uganda National Council for Science and Technology (UNCST).

EKITABO KY'OKUKKIRIZIGANYA

..... Ntegedde nti okusalawo kwange okwetaba mu kunoonyereza kuno tekijja kukyusa bujjanjabi bwange obwa bulijjo. Mu kukozesa amawulire gano, endagamuntu yange ejja kukwekebwa. Nkimanyi nti nnyinza okuvaamu essaawa yonna. Ntegedde nti bwe nssa omukono ku ffoomu eno, sivaako ddembe lyange lyonna ery'amateeka wabula ntegeeza bulaga nti ntegeezeddwa ku kunoonyereza kw'okunoonyereza kwe nzikirizza okwetabamu kyeyagalire. Kopi ya foomu eno ejja kunweebwa.

Erinnya.....

Omukono.....Olunaku lw'owezi.....

Erinnya ly'omujulizi.....

Omukono.....Olunaku lw'owezi.....

Erinnya ly'abuuza ebibuuzo.....

Omukono.....Olunaku lw'owezi.....

MAKERERE UNIVERSITY
SCHOOL OF MEDICINE
INITIAL APPROVAL
VALID UNTIL
16 JUN 2026
RESEARCH & ETHICS COMMITTEE
P.O. BOX 7072,KAMPALA

Appendix 5: Data collection form

ANTIMICROBIAL SUSCEPTIBILITY OF POTENTIALLY PATHOGENIC BACTERIA ISOLATED FROM DIABETIC FOOT ULCERS OF PATIENTS AT MULAGO AND KIRUDDU HOSPITALS

Study ID Number: ..... IP Number: .....

DATE (dd/mm/yy):.....

SECTION 1: Socio-demographic characteristics and History

1. Sex [1] M _____ [2] F_____
2. Age (completed years) _____

SOCIAL/OCCUPATIONAL HISTORY

3. Employment:

[1] Not employed _____ [2] Health worker _____ [3] Civil servant _____

[4] Peasant _____ [5] Other _____

4. Level of Education:

[1] None ____ [2] Primary ____ [3] O' level____ [4] A' level____ [5] Tertiary____

5. Current district of residence: _____
6. Tribe_____

Past medical history

7. How long have you had diabetes mellitus? (Years) .....
8. Which medication are you on for the diabetes mellitus?
   [1] None [2] Insulin [3] Oral medications (specify.....)
9. How long have you had a foot ulcer? (months).....
10. Have you been admitted to hospital within the last 3 months? (Cross-check from patient's notes)

[1] Yes ____ [2] No ____ [3] Unknown ___

11. Have you been on antibiotics within the last 3 months?

If yes, state drugs [1] Ciprofloxacin ____ [2] Amoxil____ [3] Flagyl____ [4] Amoxil-Clavulanic acid ____ [5] Septrin ____ [6] Ceftriaxone____ [7] other ____ state _____

12. Have you used herbal medicines to treat your wound?

[1] Yes ____ [2] No ____

15. Have you had a fever in the last 1 week?

[1] Yes ____ [2] No ____

SECTION 2: Physical exam (fill in answer)

16. Febrile
[1] Yes ____ [2] No ____ Temperature [in °C] ____

17. Pallor of mucus membranes:

[1] None____ [2] Mild ____ [3] Moderate ____ [4] Severe ____

18. Low blood pressure:
[1] Yes ____ [2] No ____ [mmHg] ____/____

19. Increased pulse rate
[1] Yes ____ [2] No _____ PR: _____

20. Increased respiratory rate
[1] Yes ____ [2] No _____ RR: _____

21. Where is the ulcer located?
[1] Heel [2] Toes [3] Arch [4] Ball of the foot [5] Other (specify:.....

22. How deep is the ulcer? (circle one)
[1] Superficial (only the skin is affected)
[2] Deep (involves deeper tissues such as tendons or muscle)
[3] Penetrates to the bone or joint.

23. Is there any evidence of infection? (circle all that apply)
[1] Redness around the ulcer
[2] swelling around the ulcer
[3] Pus or discharge from the ulcer
[4] Fever or chills
[5] Foul odor

24. What is the current classification of the ulcer? (Based on Wagner's system)

[1] Grade 0 (No open ulcer)
- No ulcer present, but high risk (e.g., callus, deformities, or neuropathy)

[2] Grade 1 (Superficial ulcer)
- Ulcer is confined to the skin layer; no deep tissue involvement.

[3] Grade 2 (Deep ulcer with involvement of tendons, muscles, or joints)
- Ulcer is deep, extending into tendons, muscles, or joints, but no bone involvement.

[4] Grade 3 (Deep ulcer with osteitis or abscess)
- Ulcer has infected bone (osteitis) or an abscess.

[5] Grade 4 (Gangrene of part of the foot)
- Partial foot gangrene (e.g., toes or parts of the foot affected).

[6] Grade 5 (Gangrene of the whole foot)
- Entire foot is affected by gangrene.

SECTION 3: Laboratory results

25. Pus swab culture results:

Appendix 6: Study work plan

Study workplan Oluka Edwin Noah MINT III

Study title: Antimicrobial Susceptibility Of Potentially Pathogenic Bacteria Isolated From Diabetic Foot Ulcers Of Patients At Mulago and Kiruddu Hospitals.

Activity	Months																
	A 2024	S	O	N	D	J 2025	F	M	A	M	J	J	A	S	O	N	D
Proposal writing																	
Review and approval by supervisors																	
Presentation at department of Medicine Scientific Review Committee (SRC)																	
Response to SRC comments																	
SRC approval																	
Submission to MAK School of Medicine REC																	
Response to REC comments																	
REC approval																	
Data collection and analysis																	
Interpretation and write up (Thesis)																	
Thesis defence																	
Manuscript writing and publication.																	
Mentorship																	

Appendix 7: Mulago hospital administrative clearance letter

TELEPHONE: +256-41554008/1
FAX: +256-414-5325591
E-mail: admin@mulago.or.ug
Website: www.mulago.or.ug

IN ANY CORRESPONDENCE ON THIS SUBJECT PLEASE QUOTES NO.....

MULAGO NATIONAL REFERRAL HOSPITAL
P. O. Box 7051
KAMPALA, UGANDA

29th August 2025.

Dr. Oluka Edwin Noah
Principal Investigator
Department of Medicine
Makerere University

Dear Dr. Oluka,

RE: ADMINISTRATIVE CLEARANCE TO CONDUCT A STUDY AT MULAGO NATIONAL REFERRAL HOSPITAL.

The Mulago Hospital Management is pleased to inform you that you have been offered clearance to conduct the study titled **MHREC 3010: "Antimicrobial Susceptibility of Potentially Pathogenic Bacteria Isolated from Diabetic Foot Ulcers of patients at Mulago and Kiruddu Hospitals".**

The above clearance is granted to you on the following conditions;

- That you will follow the research ethical processes
- Agreed to comply with all institutional policies and regulations of Mulago National Referral Hospital
- Agreed to provide end of study report and acknowledge Mulago hospital in all publications
- Submit a copy of filled in participant compensation log after recruiting one quarter of the approved research sample size.

Administrative clearance is valid for one (1) year effective from 28th August 2025 to 27th August 2026.

By copy of this letter, we reiterate our commitment to support this study.

DR. BYANYIMA ROSEMARY
EXECUTIVE DIRECTOR
MULAGO NATIONAL REFERRAL HOSPITAL