ANTIBIOTIC SUSCEPTIBILITY AND PREVALENCE OF *Staphylococcus aureus* IN DIABETIC WOUNDS AMONG PATIENTS AT MOI TEACHING AND REFFERAL HOSPITAL, KENYA

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DECLARATION

Declaration by the Student

This thesis is my original work and has not been presented for a degree in any other University. No part of this thesis may be reproduced without the prior written permission of the author and/or University of Eldoret

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This thesis has been submitted with our approval as the university supervisors.

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DEDICATION

Dedication to my devoted mom for her constant encouragement and prayers, which enabled me to reach this far, to my brothers John Oguda, Felix Oyoo, and George Okoth, and my sister Moline Atieno, for their persistent encouragement and support till this far.

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ABSTRACT

The frequent source of deferred healing processes in wounds is *Staphylococcus aureus* worldwide. Diabetic wounds in diabetic patients contribute to delayed healing due to antibacterial resistance, which has caused a lot of suffering and a low quality of life for patients. This study investigated the prevalence, possible risk factors, and antimicrobial susceptibility profile of *S. aureus* isolated from diabetic wounds of patients attending Moi Teaching and Referral Hospital (MTRH). It involved purposive sampling; patients with diabetic wound infections who were willing to participate in the study were selected. The study included Type 2 Diabetes patients aged 13 years and above, who presented at the MTRH diabetic clinic during the study, and had not used antibiotics for any reason. The study targeted 156 diabetic patients attending MTRH. A questionnaire was administered to collect socio-demographic data. Wound swabs were aseptically collected and cultured on blood agar, then sub-cultured on Mannitol Salt Agar to isolate *S. aureus*. It was further identified by catalase and coagulase tests. Determination of antimicrobial susceptibility was by the Agar disk diffusion method. The occurrence of *S. aureus* in diabetic wounds was significantly (p=0.025) influenced by the sex of patients attending the diabetic clinic at MTRH. However, age (p=0.6503), underlying conditions (p=0.8437), previous hospitalization (p=0.808), previous antibiotic use (p=0.6874), marital status (p=0.118), and the level of education (p=0.192) do not significantly predispose the wounds of diabetic patients attending an outpatient diabetic clinic at MTRH to *S. aureus*. 31 samples were positive for *S. aureus*. An instance of intermediate sensitivity 26 (10.48%) was shown by *S. aureus* isolated in this study, with 72 (29.03%) resistance to antibiotics. However, most of the isolates were vulnerable to the test antibiotics. A higher number of *S. aureus* isolates were susceptible to Cefoxitin (96.77%) and Clindamycin (80.65%), with lesser susceptibility to Ampicillin (25.81%). A prevalence of 19.87%, Cefoxitin being effective against most of the S. aureus isolates, and a significant relation between sex and occurrence of *S. aureus* in diabetic wounds were established. Early surveillance and screening to detect any presence of MRSA in diabetic wounds and the susceptibility profile of *S. aureus* in diabetic wounds of patients attending the diabetic clinic at MTRH is recommended. Re-evaluation of the use of Ampicillin should also be taken into consideration to prevent widespread antibiotic resistance. Female diabetic patients should take more precautions to prevent any traumatic injury, which could lead to diabetic wound infections.

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LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

ANOVA	Analysis of Variance
BAI	Body adiposity index
BMI	Body mass index
BPA	Bacterial protease activity
CA-MRSA	Community-associated- Methicillin-resistant *S. aureus*
COPD	Chronic Obstructive Pulmonary Disease
CRP	C-reactive protein
CVD	Cardiovascular disease
DFI	Diabetic foot infections
DM	Diabetic Mellitus
DNA	Deoxyribonucleic acid
GDM	Gestational diabetes mellitus
HA-MRSA	Hospital-associated- Methicillin-resistant *Staphylococcus aureus*
HCL	Hydrochloric acid
HGT	Horizontal gene transfer
HIV	Human Immunodeficiency Virus
MIC	Minimum inhibitory concentration
MHA	Mueller-Hinton agar
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSA	Mannitol Salt Agar
MSSA	Methicillin-sensitive *S. aureus*

MTRH	Moi Teaching and Referral Hospital
NACOSTI	National Commission for Science, Technology and Innovation
NIAID	National Institute of Allergy and Infectious Diseases
PCT	Procalcitonin
RTIs	Road Traffic Injuries
S. aureus	*Staphylococcus aureus*
T2D	Type 2 Diabetes
TAM	Traditional African Medicine
WC	Waist circumference
WHO	World Health Organisation
WHR	Waist-to-hip ratio
WHtR	Waist-to-height ratio

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Diabetes mellitus is a chronic metabolic disorder that usually shows signs such as persistent hyperglycemia, which results from the impairment of secretion and action of insulin, or both (Aloke et al., 2022). The condition has raised concern globally, with the number of individuals suffering increasing due to lifestyle changes, if not the food people consume, and an aging population, which constitutes the majority of our population (Standl et al., 2019). In many developing countries, including Kenya, the burden of diabetes is on the rise. Consequently, numerous complications have resulted, hence affecting patients' quality of life, if not increasing the cost of healthcare (Karugu et al., 2024).

Diabetic wounds occur when diabetic patients have experienced impaired blood circulation, neuropathy, as well as a weakened immune system, which in turn collectively delay the healing process(Rodríguez-Rodríguez et al., 2022), hence a conducive environment for microbial colonization as well as infection. Generally, once infection starts, complications in the healing process can progress to severe outcomes such as tissue necrosis, osteomyelitis, and limb amputation if not properly managed are inevitable(Burgess et al., 2021). Diabetic foot infections (DFI) are mainly polymicrobial, and *Staphylococcus aureus* remains one of the most predominant and clinically significant pathogens (Shettigar & Murali, 2020a), always recording higher chances as a pathogen of being isolated from the wounds (Dunyach-Remy *et al.,* 2016)

S. aureus is a Gram-positive, coagulase-positive bacterium that normally forms part of the normal flora on the skin and mucous membranes of healthy individuals (Fayisa & Tuli, 2023), which causes no harmful effect on the host before entering the internal body. In case of destruction of the skin, a wound materializes, which allows entry of microorganisms into the internal body and becomes pathogenic (Reid et al., 2011). Infections caused by *S. aureus* are particularly in diabetic wounds, are of concern due to the compromised immune response and delayed tissue repair associated with diabetes (Shettigar & Murali, 2020).

The development and spread of antimicrobial resistance (AMR) among *S. aureus* isolates have made the management of diabetic wound infections increasingly challenging and a great problem in the healthcare system (Akash et al., 2020). Antibiotic resistance by different bacterial species recovered from diabetic foot patients is an emerging global problem. Studies conducted before suggest that DFI is caused by multiple antibiotic-resistant methicillin-resistant *S. aureus* (Abalkhail & Elbehiry, 2022). MRSA are pathogens that have a major negative impact on all aspects of patient care and are progressively being acknowledged as difficult pathogens in the community (Lee *et al.*, 2018). *S. aureus* has developed the ability to acquire resistance to antimicrobials (Mlynarczyk-Bonikowska *et al.*, 2022). Through the acquisition of Staphylococcal cassette chromosome mec (SCCmec) that carries the mecA gene, which is responsible for methicillin resistance, MRSA is considered to have emerged from *S. aureus* (Lakhundi & Zhang, 2018). This growing resistance underscores the urgent need for local surveillance to monitor resistance trends and inform empirical therapy.

A better understanding of the prevalence and antibiotic susceptibility patterns of *S. aureus* in diabetic wound infections is therefore of importance if guiding the appropriate antibiotic use and strengthening infection control practices is to be realized. At Moi Teaching and Referral Hospital (MTRH), which serves as a major referral and teaching institution in western Kenya, there is limited published data on the antibiotic resistance profiles of *S. aureus* isolated from diabetic wounds.

In Kenya, studies by Tuvei (2017), Mutonga (2018), and Kisoi (2021) have shown that *S. aureus* is a predominant pathogen in diabetic wound infections, contributing to delayed healing and increased complications. However, the scope of existing studies remains limited, with insufficient focus on comprehensive antibiotic susceptibility testing and larger patient populations**.** The current study sought to investigate the prevalence, risk factors, and patterns shown by *S. aureus* in samples from diabetic wounds from patients attending the MTRH in response to test antibiotics. This would then help in the best administration of antibiotics, thereby reducing the rate of antibiotic resistance emergence.

1.2 Statement of the Problem

Diabetes is an expensive health issue for both the patient with diabetes and the healthcare system. (American Diabetes Association, 2018). Regrettably, both the occurrence of diabetes and the related costs are on the rise. The projected number of individuals with diabetes has almost tripled in the previous 40 years, growing from 108 million in 1980 to 352 million in 2019 (Reddy & Tan, 2020). The worldwide incidence of diabetes among adults over 18 years of age grew from 4.7% in 1980 to 8.5% in 2014 (Al-Khaledi et al., 2018). Approximately up to 25% of diabetics will advance into DFI in their lifetime. Additionally, a meta-analysis has shown a greater all-cause mortality rate in diabetics with a DFI compared to those without a DFI, showing a mortality rate of 99.9 per 1000 person-years in the DFI population, compared with 41.6 per 1000 in the diabetes-only population. (Hurlow et al., 2018a) One of the most serious complications of this disease is diabetic wound infections (Inzucchi *et al.,* 2015).

According to Hurlow *et al.* (2018), Diabetic wound infections contribute substantially to morbidity, prolonged hospital stays, and costs of healthcare globally for patients with diabetes mellitus. Approximately up to 25% of diabetics develop diabetic wound infections in their lifetime. A recent meta-analysis has shown a greater mortality rate in diabetics with diabetic wound infections (99.9 per 1000 person-year) compared to those without diabetic wound infections (41.6 per 1000 person-year) (Hurlow et al., 2018). DFIs increase the chances of hospitalization of diabetic patients for any other diabetes-related complication, making these infections a serious contributor to healthcare costs. A number of these wounds progress to chronic non-healing wounds, often characteristically linked with comorbidities such as vascular deficits, hypertension, and other chronic diseases (Gatheca et al., 2018). The increasing prevalence of DFIs, combined with the emergence of antibiotic-resistant pathogens such as *S. aureus*, poses a growing clinical and public health challenge.

At MTRH, empirical antibiotic use in managing diabetic wounds without prior bacteriological identification or susceptibility testing could risk treatment failure, hence contributing to the development of resistance by bacteria as well as delayed healing of the wounds. Despite the clinical importance of *S. aureus* in diabetic wound infections, there is limited data on its prevalence and antibiotic susceptibility patterns in this setting. Hence, this study seeks to determine the prevalence and antibiotic susceptibility profiles of *S.*

aureus isolated from diabetic wound infections among patients attending MTRH. The findings will provide essential evidence to guide effective antibiotic selection, promote rational drug use, and enhance the management of diabetic wound infections.

1.3 Justification of the Study

It has become essential to assess the antibiotic susceptibility of *S. aureus* bacterial populations in our hospitals if the increasing problem of the emergence of methicillin-resistant *S. aureus* in hospitalized patients is to be addressed. Numerous researchers have found that *S. aureus* expresses resistance to many drugs apart from methicillin. In Kenya, the presence of *S. aureus* in diabetic wounds has previously been reported by Tuvei (2017), Mutonga (2018), and Kisoi (2021). These studies have shown *S. aureus* as a predominant pathogen in diabetic wound infections, contributing to delayed healing and increased complications. Although some reports have indicated emerging antibiotic resistance among these isolates, the scope of existing studies remains limited, with insufficient focus on comprehensive antibiotic susceptibility testing and larger patient populations**.** Thus, understanding the prevalence and antibiotic profile of *S. aureus* is important in coming up with treatment strategies and guiding global antibiotic stewardship programs.

The present study sought to determine the prevalence, risk factors, and antibiotic profile of *S. aureus* in samples from diabetic wounds from patients attending the MTRH. Carrying out the study at the MTRH was therefore necessary, due to its referral status, which ensures it serves 23 counties as well as the middle-class surrounding population. The findings of this study can be instrumental in guiding clinical treatment protocols concerning the most appropriate antibiotics for dealing with diabetic wound infections, as well as refining antibiotic stewardship strategies. Ultimately, the outcomes of this study can contribute to improving DFI treatment outcomes by reducing the burden of antibiotic-resistant infections and shaping the overall management of diabetic wounds in Kenya and beyond.

1.4 Study Objectives

1.4.1 General Objective

To examine the prevalence, antibiotic susceptibility, and associated risk factors for the occurrence of *S. aureus* in diabetic wounds of patients attending MTRH.

1.4.2 Specific Objectives

i) To determine the prevalence of *S. aureus* in samples collected from diabetic wounds of patients attending outpatient diabetic clinics at MTRH.

ii) To determine the antibiotic profile of *S. aureus* isolates from diabetic wounds of patients attending outpatient diabetic clinics at MTRH.

iii) To establish the possible risk factors for the occurrence of *S. aureus* in diabetic wounds of patients attending the MTRH.

1.5 Research Questions

i) What is the prevalence of *S. aureus* in samples of wounds from diabetic patients attending the MTRH?

ii) What is the antibiotic profile of *S. aureus* isolates from diabetic wounds of patients attending outpatient diabetic clinics at MTRH?

iii) What are the possible risk factors for the occurrence of *S. aureus* in diabetic wounds of patients attending the MTRH?

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Diabetes

Diabetes is a chronic condition brought on by either insufficient insulin production by the pancreas or inefficient insulin use by the body, leading to hyperglycemia and systemic complications. (Gathecha et al., 2018). Type 1 diabetes (T1D) and Type 2 diabetes (T2D) are the two primary forms of the disease. Gestational diabetes, a condition of hyperglycemia that arises during pregnancy, pre-diabetes, and diabetes mellitus (DM), from less common causes like genetic syndromes, acquired processes like pancreatitis, exposure to specific medications, viruses, and illnesses like cystic fibrosis are the other categories. (Balaji *et al.*, 2019). The different types of diabetes vary from one to the other in their pathophysiology, clinical presentation, and management strategies, despite the common feature, which is defects in insulin secretion and insulin action causing chronic hyperglycemia.

The setting of T1D is mainly caused by destroying the pancreatic β-cells that produce insulin, thus leading to complete insulin insufficiency and requiring ongoing insulin treatment (American Diabetes Association, 2018). Insulin is one hormone that usually has a primary role in controlling blood sugar; on the other hand, Type 2 diabetes is characterized by Insulin resistance and a relative decrease in insulin production, which is closely linked to Oxidative damage, endoplasmic reticulum stress, chronic inflammation, dyslipidemia, and ectopic lipid deposition (Lu *et al.,* 2024). Elements of the metabolic syndrome and Obesity are frequently linked to T2D. Universally, there were 352 million individuals between the ages of 20 and 64 who were suffering from diabetes mellitus in 2019 (American Diabetes Association, 2018)

. This number is expected to rise to 417 million by 2030 and 486 million by 2045(Builders & Builders, 2016). Additionally, there were 111 million individuals between the ages of 65 and 99 in 2019 suffering from T2DM, which is expected to rise to 195 million by 2030 and 276 million by 2045 (Builders & Builders, 2016).

The other type of diabetes includes gestational diabetes mellitus, which is the most common medical complication of pregnancy, and the prevalence of undiagnosed hyperglycemia and even overt diabetes in young women is increasing (Casagrande et al., 2018). Around the world, up to 14% of pregnancies end in gestational diabetes. Occurs in approximately 5–15% of pregnant women, varying in ethnicity and region. It can raise the chance of delivery troubles and Associated complications, even though there are often no symptoms (Casagrande et al., 2018). After birth, gestational diabetes normally goes away, although T2D (Casagrande et al., 2018) might appear years later or shortly after the pregnancy ends. The development of gestational diabetes while pregnant increases the risk of later developing T2D. Factors such as maternal overweight and obesity, later age at childbearing, previous history of GDM, family history of T2DM, and ethnicity form major GDM risk (Casagrande et al., 2018). Multifarious aspects, which consist of genetic defects, pancreatic obstruction, surgery, and organ transplantation, contribute to the commencement of this type of diabetes. For women who have GDM, 40–60% have a chance of developing DM after 5–10 years of pregnancy. (Alam *et al.*, 2021).

Pre-diabetes is a condition in which an individual's blood glucose level some extent above the normal, though not high enough to be classified as diabetes. Left untreated, pre-diabetes may put one at risk of getting type 2 diabetes (Khan *et al.*, 2019). This might be influenced by lifestyle behaviors, as those who appear to be active than others and have diets richer in carbohydrates and saturated fats, increase the risk of T2D in all ethnic groups (Gardner *et al.,* 2023). About 10.1 million people in the US have been diagnosed with diabetes, and 3.9 million have not reached a level that can be considered diabetic; this can be linked to family history. People with a family history are 2–4 times more likely to develop prediabetes (Najafipour et al., 2018); therefore, screening them might allow for early treatment.

2.2 Prevalence of Diabetic Wound Infections

Injuries are progressively becoming a chief public health challenge globally and are responsible for 9% of deaths (Gathecha *et al.,* 2018). Diabetes is an expensive health issue for both the patient with diabetes and the healthcare system (American Diabetes Association, 2018). Regrettably, both the occurrence of diabetes and the related costs are on the rise. The projected number of individuals with diabetes has almost tripled in the previous 40 years, growing from 108 million in 1980 to 352 million in 2019 (Reddy & Tan, 2020). The worldwide incidence of diabetes among adults over 18 years of age grew from 4.7% in 1980 to 8.5% in 2014 (Al-Khaledi et al., 2018). Approximately up to 25% of diabetics will advance into DFI in their lifetime. Additionally, a meta-analysis has shown a greater all-cause mortality rate in diabetics with a DFI compared to those without a DFI, showing a mortality rate of 99.9 per 1000 person-years in the DFI population, compared with 41.6 per 1000 in the diabetes-only population (Hurlow et al., 2018a).

People with diabetes have higher chances of being hospitalized for difficulties related to DFI than for any other difficulty of diabetes, and hospitalization is an expensive aspect of DFI management**.** Some diabetic wounds have become Chronic Non-Healing Wounds

(CNHW), which are characteristically linked with comorbidities such as diabetes, vascular deficits, hypertension, and chronic disease. Given the factors of the elderly population, the sustained danger of diabetes and obesity universally, and the continued danger of infection, it is anticipated that prolonged wounds will remain a substantial clinical, social, and economic challenge (Gatheca *et al.*, 2018)**.** Hence, the need for early measures, including the use of appropriate antibiotics as well as regulations to contain the spread of bacterial resistance of bacterium.

2.3 Etiology of Diabetic Wound Infections

Wound infection is a consequence of the invasion of wounds by pathogenic organisms. The majority of these organisms can originate from the normal flora on the skin (Zegadło *et al.*, 2023), or the other parts of the body, or the outside environment (Maheswary *et al.*, 2021). Recent studies reveal that biofilm infections may directly hinder wound closure or cause defective wound closure where the wound site gives the impression of closed but repaired skin, and lacks obstruction function (Sen *et al.*, 2021). Diabetic wound infections are the most common complications of diabetic patients due to their weakened immune system (Martin *et al.*, 2024). These microorganisms are discussed in sub-sections 2.3.1 to 2.3.3.

2.3.1 Bacteria

Diabetic wounds are polymicrobial with both aerobic and anaerobic bacteria and vary by geographic region, wound severity, and prior antibiotic exposure. *Staphylococcus aureus* is a chief colonizer of DFI, produces profuse biofilm, and, in so doing, inhibits wound healing and intensifies wound infection. (Shettigar & Murali, 2020b). *S. aureus* colonization of wounds can cause infection, depending on the number per gram of wound tissue (Ladhani *et al.,* 2021), the host immune response, the number of different species present, the virulence of the organisms, and synergistic interactions between different species (Van Dyck *et al.,* 2021). In particular, in the initial phase of infections, within the first week, Gram-positive bacteria, especially *S. aureus*, appear to be the most frequent colonizers. Methicillin-resistant Staphylococci are linked to delayed healing and higher amputation risks. From the beginning of the second week, Gram-negative bacteria, such as *P. aeruginosa* and *A. baumannii*, start to colonize the wound, provoking sepsis if they enter the lymphatic system and blood vessels. (Puca *et al.,* 2021)Reports have indicated the presence of *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *Proteus* species, Clostridiales, and Bacteroides in diabetic wounds (Baral *et al.,* 2024).This could also be contributing to the delay in healing of the diabetic wounds

2.3.2 Fungi

Fungal pathogens have likewise been found to be associated with wound infection. Taking an example of the epidemiology of wound infections caused by fungi, the shift has been realized such that fungal pathogens are now more common (Ozturk *et al.,* 2019)**, with** fungal infections being reported to constitute 9–40% cause of DFIs (Allkja *et al.,* 2025). Nearly 56% of diabetic foot ulcers develop infections, and 20% of these individuals suffer from amputation as a result and still the development of various foot problems attributed to fungal infection is on the rise. Nevertheless, little data are available on the pervasiveness of fungal foot infections in patients with diabetes. The *Candida* spp is fungi that normally contribute to infections of diabetic foot ulcers are largely (Raiesi *et al.,* 2018); hence, they may contribute the hindering healing, especially in patients having underlying conditions, including diabetes mellitus. *Candida parapsilosis* can be found in ischemic or bonepenetrating wounds, while *Candida tropicalis* and *Candida glabrata* are not associated with diabetic wounds in most cases but can always be present (Puca et al., 2021)

. In addition, specific fungal pathogens cause chronic skin infections, including eumycetoma and chromoblastomycosis, often manifesting as slow-healing wounds (Krumkamp *et al.*, 2020).

2.3.3 Viruses

Traditionally, though bacterial pathogens such as *S. aureus* dominated diabetic wound infections, Viruses are increasingly being spotted in chronic wounds and may contribute directly by infecting wound tissues or indirectly by modulating local immunity and predisposing to secondary bacterial infections (Krumkamp et al., 2020). Keratinocyte function and delaying epithelialization have been impaired by HSV infection, notwithstanding that Simplex Virus (HSV-1) and HSV-2 have been detected in chronic wounds. Due to immune dysregulation, diabetic patients have become more susceptible to HSV reactivation, which results in exacerbating wound chronicity. Human Papillomavirus (HPV), Cytomegalovirus (CMV), and Other herpesvirus infections have contributed to poor healing outcomes due to their linkage to impaired angiogenesis and prolonged inflammation in diabetic wounds (Krumkamp et al., 2020).

Viruses can also help bacteria evade the immune system (Finlay & McFadden, 2006). Pseudomonas aeruginosa causes chronic wound infections and is at times usually infected by a bacteriophage —a type of virus that targets bacteria. Some phages kill the bacteria they infect, while others —Pf phage, the one that infects P. aeruginosa —use the bacteria to multiply themselves, without killing their host in the process. It has been found that P. aeruginosa can harness Pf phages to help produce biofilms (Vashisth et al., 2023), which are complex, multi-layered microbial communities that provide a protective haven for germs in the body. The infectious phages are thus able to protect the bacteria, hence surviving.

To further understand the role that these phages play in P. aeruginosa, they can harness Pf phages to help produce biofilms and wound infections; hence, tests were carried out on wounds in patients at their hospital for phages by the research team first on P. aeruginosa, and they found that Pf phages could be harnessed to help produce biofilms(Vashisth et al., 2023). Out of 37 patients in their hospital with chronic *P*. aeruginosa infections, 25 (68%) also had phage in their wounds. The longer a wound had gone without healing, the more likely it was to harbour phage-infected *P. aeruginosa* (Vashisth *et al.*, 2023). In mice, phage-infected *P. aeruginosa* was more likely to infect wounds and prove fatal than *P. aeruginosa* that didn't harbor phage. The researchers used cultured immune cells to look closely at immune cell interactions with *P. aeruginosa*. They found that the phages served as decoys for the immune system. Immune cells used virus-fighting mechanisms to engage the phages instead of mounting an attack on the bacteria.

2.4 Management of Diabetic Wound Infections

Wound healing comprises three natural stages: swelling, proliferation, and remodeling (Rodrigues *et al.,* 2019). The provocative phase starts when there is a vascular comeback that warrants homeostasis by allowing the formation of blood clots, which is slower in diabetic patients. Secondarily, grief particles produced by wounded cells play a role in attracting leukocytes, which in turn helps in the detection and elimination of infective agents, along with cytokine release, that arouses cells that take part in the multiplication phase. Once granulation tissue starts the process of covering the wound surface, it indicates the shift to the multiplication phase (Tomic-Canic et al., 2018). Important aspects in this subsequent stage are indicated by the initiation of fibroblasts, which yield collagen and other extracellular matrices, as well as by neo-angiogenesis. The third phase, which is Remodelling which is the third phase helps in re-establishment of the wound structure and plays the role of the tissue (Rodrigues *et al.,* 2019).

The commonly encountered hindrance in the recovery of wounds is the establishment of Pathogens within the wound, and also conditions like diabetes. Conversely, the majority of the bacteria that reside on the skin as microbiota have an advantageous part in averting colonization by other infectious agents. Once they attain a critical starting point at the site of a wound, they can obstruct the recuperative process. Among the commonly known colonizers, *S. aureus* and MRSA are commonly detected, which affect the early stages of wound healing, while *P. aeruginosa* and *Escherichia coli* are distinctive for chronic wounds as they infect deeper skin layers (Mihai *et al.,* 2018). Diabetic wound management approaches are further discussed in sections 2.4.1 to 2.4.2.

2.4.1 Wound Dressing

Wound dressing has been customarily applied to offer protection and a shielding barrier at the site of the wound, thus protecting it from external attack (Prete *et al.,* 2023). Consequently, typical wound covering materials such as cotton and wool, which only provide a non-resisting obstruction, are being substituted by more innovative dressings that are proficient and can allow passage of effective compounds only. Thus, different blends of both artificial and ordinary constituents have been incorporated: sponges, hydrogels, films, hydrocolloids, and hydrofiber mats. Perfect dressing must guarantee a moist and conducive environment, alongside evading the buildup of wound exudates. Therefore, a good wound bandage should be biocompatible, semi-permeable to liquid and oxygen, and should also be hypoallergenic.

2.4.2 Antimicrobial Agents

Quinolones, tetracyclines, aminoglycosides, and cephalosporins are frequently applied antibiotics in antimicrobial wound dressings. They use different mechanisms to thwart bacterial multiplication, these include alteration of protein and nucleic acid productions, which results in metabolic imbalances, and the disturbance of bacterial outer covering reliability, thus also meddling with cellular division (Buyana *et al.,* 2020). Conversely, continued application and inappropriate usage of antibiotics have resulted in the development of resistant bacterial colonies. Generally, chronic wounds are colonized by bacteria that at least show resistance to the frequently prescribed agents. (Simões *et al.,* 2018).

Considering the interrupted blood circulation nature in most chronic wounds, oral antibiotics administration may be unproductive due to low chances of reaching the damaged tissues. As a consequence, oral or topical antibiotics are not an advisable method of administering antibiotics in cases of chronic wound treatment owing to the resistance and tolerance developed by bacteria (Eriksson *et al.,* 2022). Topical non-antibiotic antimicrobials are chosen because of ease of usage as well as their mode of destruction, which includes interfering with the bacterial phospholipid cell membrane, thus interfering with cellular compartments through pH imbalance and cytoplasm outburst. Since essential oils do not promote the emergence of antimicrobial resistance, treatment for multidrug resistance can rely on their application (Visan & Negut, 2024).

Antifungal creams are used both as a palliative treatment for existing fungal infections and as a prophylactic measure in cases where there is a risk of fungal infection. (Mellinghoff *et al.,* 2018). Although bacteria cause most infections in chronic wounds, research indicates that the contribution of fungal infections is not insignificant. (Kalan & Grice, 2018).

Natural plant extracts that demonstrate antifungal activities can always be used to cure wounds. (Parham *et al.,* 2020)**.** In addition, therapeutic principles of traditional medicinal plants, which are efficient in yielding potent antiviral drug molecules and offer themselves for scientific evaluation owing to the high cost and undesirable side effects of conventional antiviral drugs, have encouraged the use of natural plant extract, as well as the absence of effective vaccines (in some cases) and the emergence of resistant microbial strains. (Biswas *et al.,* 2020).

2.5 Antibiotic Resistance in Diabetic Wounds

The ability of germs like bacteria to develop the ability to resist the drugs designed to destroy them is termed antibiotic resistance. (Dhasarathan *et al.,* 2021). Resistant infections can be problematic and sometimes unbearable for any treatment option. (Dolecek *et al.,* 2022). Antibiotic resistance has become an urgent global community health risk, documented to have claimed at least 1.27 million people worldwide and resulted in nearly 5 million deaths in 2019 (Dolecek *et al.,* 2022).

Antibiotic resistance can be developed by any bacteria to affect people at any stage of growth, as well as the healthcare, veterinary, and agriculture industries, hence the world's most urgent public health problems (Dadgostar, 2019). The ability of bacteria to fail to respond to drugs poses a danger to life, since they play a critical role in sensitive medication processes, such as organ transplant and surgical procedures (Chinemerem Nwobodo et al., 2022). Antibiotic-resistant infections that necessitate the use of second and third-line treatments can endanger patients by producing grave side effects, for example, organ failure, and prolong care and recovery, sometimes for months. In some cases, these infections do not have other treatment options. Hence, the a need for appropriate and proper stewardship in antibiotic administration

The cause of resistance in bacteria is generally due to self-medication, genetic variations, or mutations in bacteria, and also by phenotypic variation, such as in β-lactams. As antibiotics are precise to their targets, bacteria secrete certain enzymes to cleave chemical bonds within the antibiotic structure. Additionally, alterations in membrane permeability, antibiotic target modifications in bacteria, and resistant gene transfer to the next generations also cause resistance to antibiotics. (Naveed *et al.*, 2020). Biofilms are considered synonymous with antibiotic resistance as a result of proficiency in transferring genes for resistance, as well as their innate phenotypic tolerance to antibiotics. (Bowler *et al.*, 2020).

The absence of new antibiotics being discovered is also because no new class of antibiotics has received regulatory approval since the late 1980s (Kållberg *et al.*, 2018)**.** Despite continuing efforts over the last decade from global health organizations such as the WHO to introduce guidelines for the appropriate and responsible consumption of drugs, inappropriate antibiotic prescribing of up to 23% has been realized (Nepal *et al.*, 2021)**.** Among the other causes are patients not finishing the entire antibiotic course and poor infection control in the healthcare setting.

Mechanisms, such as mainly horizontal gene transfers of virulence and antibiotic resistance genes, which are also often aided by biofilm establishment, are the source of

Antibiotic resistance, which ensues as a consequence of overuse of antimicrobial agents. (Kavya *et al.*, 2023). Notwithstanding whether the biofilm is one microbe or polymicrobial, bacteria covered within a biofilm from the external environment interconnect using signal transduction pathways, which include quorum sensing or two-component systems, resulting in worldwide variations in gene expression, promoting virulence, and accelerating the acquisition of antibiotic resistance. (Singh *et al.,* 2021). While humans are considered the main reservoir of *S. aureus,* it can freely cross species barriers and infect new hosts, since the array of eukaryotic species that it can inhabit as a commensal or opportunistic pathogen remains unknown. (Nash *et al.,* 2015). The remarkable capacity of *S. aureus* to adapt to new or multiple hosts makes it a formidable bacterium that threatens animal health. (Park & Ronholm, 2021).

2.6 Overview of *Staphylococcus aureus*

The Gram-positive bacterium *S. aureus* is a major human pathogen in addition to being a commensal organism. It is the cause of many illnesses, extending from slight skin infections to serious systemic disorders. In clinical settings, its increasing antibiotic resistance presents a significant concern. (Dhasarathan et al., 2021).

S. aureus has a spherical form with a diameter of around 0.5 to 1.5 µm. It can grow in both aerobic and anaerobic environments since it is a facultative anaerobe, non-motile, and non-spore-forming (Dhasarathan et al., 2021). *S. aureus* is part of the normal human flora, commonly colonizing the skin and nasal passages of approximately 30% of the population. While asymptomatic colonization is common, breaches in the skin or mucosa can lead to infections ranging from localized skin infections to systemic illnesses like bacteremia, endocarditis, and pneumonia (Dhasarathan et al., 2021). Surface adhesins, exoenzymes, capsular polysaccharides, toxins such as toxic shock syndrome toxin (TSST-1), and exfoliative toxins are among the virulence factors of *S. aureus*. They can avoid human defenses and spread illness to these features (Dhasarathan et al., 2021).

The level of antibiotic resistance in *S. aureus* has become a serious worldwide health concern, with bacteria employing various strategies to avoid the effects of several types of antibiotics. MRSA strains are resistant to all beta-lactam antibiotics due to the acquisition of the mecA gene, which codes for a modified penicillin-binding protein (PBP2a), hence decreasing methicillin binding (Jiang *et al.*, 2023; Lade & Kim, 2023). *S. aureus* continues to play a role in being a model of adaptive resistance, even though MRSA and MDR strains present immediate risks.

The ability of S. aureus to rapidly acquire antibiotic resistance has made it a global public health crisis, as it employs diverse strategies to evade antimicrobial agents, including the mecA gene, which encodes penicillin-binding protein (PBP2a) and thereby decreases β-lactam antibiotic binding, which is further achieved through the Staphylococcal Cassette Chromosome mec (SCCmec) mobile genetic element, particularly types IV and V in community-associated MRSA. (Nash et al., 2015). Mutations in gyrA (DNA gyrase) and parC (topoisomerase IV) genes also result in drug affinity reduction, causing resistance to fluoroquinolones (Elshobary *et al.*, 2025). β-lactamase synthesis results in Penicillin derivatives hydrolysis, whereas macrolide resistance is a result of the erm genes, which consequently methylate ribosomal RNA. Some *S. aureus* strains also have proteins like NorA, which expel fluoroquinolones, tetracyclines, and macrolides from bacterial cells (Nash et al., 2015).

Prolonged wound duration and limited diagnostics correlate with 54% MRSA rates in diabetic ulcers. (Nash et al., 2015). Antibiotic resistance in *S. aureus* complicates diabetic wound management globally, with MRSA posing a critical threat in resource-limited settings. Multidrug-resistant (MDR) *S. aureus* increases hospitalization costs by 40% and amputation risks by 3.5-fold (Moya *et al.,* 2023). However, vancomycin and linezolid remain effective against MDR *S. aureus.*

Host-jumping events are repeatedly linked with the acquisition of genetic elements from host-specific gene pools that confer traits required for survival and adaptation in the new host niche. (Bruce *et al.,* 2022). These traits include a diversity of virulence aspects, such as super-antigens that are used to manipulate innate and adaptive immune responses. Resistance of Vancomycin-resistant *S. aureus* strains arises from the acquisition of the vanA gene cluster, which modifies cell wall precursors to reduce vancomycin binding. (Dhasarathan et al., 2021). Horizontal gene transfer is made easier by the prevalence of resistance genes on mobile genetic elements such as plasmids, transposons, and Staphylococcal cassette chromosomes mec (SCCmec) (Lakhundi & Zhang, 2018). By protecting bacterial colonies from drugs and immunological reactions, biofilm development further increases resistance. (Dhasarathan et al., 2021).

2.6.1 Risk Factors for the Occurrence of S. *aureus* in Diabetic Wounds

Type 2 diabetes has no specific drug that can be used to cure it; however, ways to lower blood sugar as well as manage the condition are by eating well, exercising, maintaining a healthy weight, and monitoring blood glucose levels. If nutrition and workout don't control your blood glucose, you may need medications or insulin therapy (Amanat et al., 2020a). *S. aureus* is a leading pathogen in diabetic wound infections and leads to delayed healing, sepsis, and sometimes limb amputations. Diabetic patients face a 3–7-fold higher risk of *S. aureus* infections compared to non-diabetics, driven by hyperglycemia, impaired immunity, and bacterial virulence (Amanat et al., 2020a). Some of these risk factors are discussed in subsections 2.7.1 to 2.7.6.

2.6.2 Age

Although there may not be a certain age at which T2D manifests, an individual's age significantly raises their risk of getting the disease. The danger of T2D is at its peak with an increase in age, especially after 45 years, due to less exercise, and thus gaining weight (Amanat et al., 2020a). T2D is still uncommon in young people under the age of 18, but its prevalence has sharply increased among children, adolescents, and younger adults as a result of concurrent rises in obesity and unhealthy eating and exercise habits (Lascar et al., 2018). Therefore, aging may augment T2D risk through pathophysiological mechanisms independent of obesity. (Fazeli *et al.,* 2020).

According to various studies, age-related physiological changes may predispose older diabetic patients to *S. aureus* infections as well. (Lavigne *et al.*, 2021; Lee *et al.*, 2023). Prolonged diabetes in elderly persons worsens immunological dysfunction, which makes bacteria more persistent. (Lee et al., 2018). Aging also impairs the removal of *S. aureus* biofilms by decreasing neutrophil chemotaxis and phagocytic effectiveness. (Lee et al., 2018). Peripheral artery disease and neuropathy are more common in older individuals, which results in hypoxic wound conditions that are favourable for *S. aureus* colonization. (Lee et al., 2018).

2.6.3 Sex

The majority of data from populations of American, Western European, or Asian descent indicate a slightly higher prevalence of T2D among men than women when examining only the international rates of the disease as standardized across all age groups; according to the (American Diabetes Association, 2018) Atlas, an estimated 221 million men and 204 million women worldwide were estimated to have had T2D in 2017. A higher prevalence of T2D among men than women differs from region to region; hence, region-specific (Blüher & Stumvoll, 2020). There exist some variations in sex differences in T2D incidence that vary throughout the life span. Females have significantly higher rates of T2D in youth, while males have a significantly higher prevalence of T2D in midlife. However, the rates have been documented to be fairly similar between the sexes in later life. (Blüher & Stumvoll, 2020). There is limited information linking sex to *S. aureus* infections. However, diabetic patients face a 3–7-fold higher risk of *S. aureus* infections compared to non-diabetics, particularly due to their impaired immunity. (Amanat et al., 2020a). In diabetic wounds, sex has a considerable impact on the likelihood of *S. aureus* infection due to hormonal, immunological, and socioeconomic variables. (Smit *et al.,* 2017).

2.6.4 Weight

While T2D may be caused by numerous factors, Various aspects play a vital role in the progression of both obesity and T2D (Blüher & Stumvoll, 2020). Insulin resistance, pro-inflammatory cytokines, endothelial dysfunction, deranged fatty acid metabolism, and cellular processes, such as mitochondrial dysfunction and endoplasmic reticulum stress, have been shown to suggest an array of potential relationships linking obesity and T2D. Hyperinsulinemia and T2D show a correlation with the presence of excess adiposity and fat distribution, as well as increased upper body fat, which is also associated with metabolic syndrome, T2D, and cardiovascular disease (Apovian *et al.*, 2019). Being bulky is a prime risk contributor for T2D since higher levels of fatty tissue one contains increase the level of resistance of one's cells to insulin (Bjerregaard *et al.*, 2018).

Chronic low-grade inflammation, compromised immunological responses, and altered skin barrier function are all characteristics of obesity that can make people more susceptible to infections. (Apovian et al., 2019). Hyperglycemia, poor vascularization, and neuropathy exacerbate these effects in diabetic patients, fostering an environment that is favourable for *S. aureus* colonization as well as *S. aureus* infection. Additionally, obesity increases fibrin deposition and causes a hypercoagulable condition, which has been demonstrated in animal models to increase *S. aureus* virulence and persistence in infected tissues. (Apovian et al., 2019).

2.6.5 Fat Distribution

If the body stores fat primarily in the abdomen, the chances of developing T2D are greater than if your body stores fat elsewhere. (Vasan *et al.*, 2018). Several health conditions, including T2D, hypertension, hyperlipidaemia, cardiovascular disease (CVD), arthritis, gallbladder disease, certain cancers, and non-alcoholic fatty liver disease, are clinically linked to Obesity; hence, it plays an important role as an indicator of the upsurge of risk to the aforementioned conditions. (Burhans *et al.,* 2018).

Especially, the link between adiposity and T2D is strong. Generally, obese individuals have a sevenfold while overweight individuals have roughly threefold higher risk of T2D when compared to normal-weight individuals (Piché *et al.*, 2020). Remarkably, obese women have a higher risk for T2D with a relative risk of approximately eight compared to obese men, who have a relative risk of T2D of approximately six when compared to normal-weight peers (Harreiter & Kautzky-Willer, 2018). Although the contributing factors to these notable variations are not explained, they may be related to fat distribution and mass. The longer a person has had obesity, the harder it may be for them to lose weight. Several studies, particularly on animals, have also shown a strong correlation between T2D and obesity brought on by excessive fructose consumption (Apovian et al., 2019).

Determination of obesity can be achieved through ways such as the index mass of the body (BMI), circumference of the waist (WC), waist-to-hip ratio (WHR), in addition to waist-to-height ratio (WHtR) or body adiposity index (BAI). BMI and BAI are pointers of overall mass, whereas WC and WHR are conventionally believed to capture abdominal obesity, where there are amplified levels of visceral adipose tissue (Piché et al., 2020).

According to Mieczkowski *et al.* (2022), visceral fat is recognized to release pro-inflammatory cytokines, including TNF-α and IL-6, which hinder wound healing by encouraging oxidative stress and chronic inflammation. In diabetic wounds, this inflammatory environment may promote *S. aureus* colonization and growth. Pro-inflammatory cytokines, which are higher in visceral fat, promote the production of adhesion molecules on endothelial cells. This makes it easier for immune cells to be drawn to the wound site, which promotes the growth of bacteria. (Grumann *et al.*, 2014).

Immune dysregulation can result from excess fat, especially in the visceral compartment. This includes decreased macrophage activity and compromised neutrophil function, which hinders the body's ability to eradicate *S. aureus* infections (Kalan *et al.*, 2023). The distribution of fat affects the skin microbiome. Diabetes patients frequently have dysbiosis, defined by a lower level of beneficial bacteria like *Corynebacterium* and a higher prevalence of *S. aureus* (Najafipour et al., 2018). By upsetting the body's natural defenses against infections, this imbalance can make wound infections worse.

2.6.7 Family History

The known independent risk factor for both type 1 and type 2 diabetes is a family history of diabetes. There is a two to six times higher risk of T2D in individuals with a family history of the disease compared to individuals without (Najafipour et al., 2018). Its effect extends beyond common environmental, genetic, and epigenetic mechanisms, making it a crucial indicator of disease vulnerability. The risk of T2D increases if one parent or sibling has it. (Najafipour *et al.,* 2018). Women who have suffered from gestational diabetes mellitus (GDM) are at an increased risk of developing T2D after pregnancy. Studies found that the risk of developing T2D is sevenfold higher for those with GDM compared to those who had a pregnancy without GDM. (Vounzoulaki *et al.*, 2020). Maternal diabetes is associated with higher offspring transmission, while paternal transmission shows weaker associations, though some high-risk populations report equal or excess paternal risk. (Vounzoulaki et al., 2020).

Despite the paucity of direct research connecting a history of the family to *S. aureus* infection in diabetic wounds, Family history remains a risk factor for diabetes. Because of shared genetic and environmental variables, those who have a first-degree relative with diabetes are far more likely to have the condition themselves (Vounzoulaki et al., 2020). A family history raises the risk of *S. aureus* infections indirectly by raising the chance of developing diabetes, which is the main underlying illness that predisposes people to chronic wounds and subsequent infections (Vounzoulaki et al., 2020). Additionally, genetic variables that affect skin barrier function and immune response may also run in families, which might impact a person's vulnerability to infection and colonization by *S. aureus* (Vounzoulaki et al., 2020).

2.6.7 Race and Ethnicity

The differences in the likelihood of developing T2D and the age of diagnosis may also depend on race and/or ethnic background. Worldwide, both Hispanic and Asian populations have a higher prevalence of diabetes than European and African populations in both native settings and among their diaspora. Multiple factors, such as genetic, epigenetic, lifestyle, and environmental, have led to the emergence of these differences among race/ethnicity groups could emanate from. (Cheng *et al.*, 2019). T2D is over 1.5 times more common in black Americans than in white American adults. This discrepancy is due in part to higher obesity prevalence among African American adults. (Moonesinghe *et al.*, 2018). However, limited availability of fresh produce, financial obstacles to healthcare, and a lack of health knowledge may further increase the incidence of diabetes among black American adults.

Socioeconomic status and healthcare access are critical mediators of racial disparities in diabetic wound infections. Minority patients often experience delayed diagnosis, limited access to podiatric care, and suboptimal wound management, all of which increase susceptibility to *S. aureus* colonization and infection (Clayton *et al.*, 2023). Worldwide, racial and ethnic minority groups are disproportionately affected by diabetes mellitus, which has higher incidence rates and more serious consequences, such as infections and DFIs (Clayton *et al.*, 2023). *Staphylococcus aureus*, especially methicillin-resistant strains, or MRSA, is the most common bacterium that colonizes diabetic wounds, making treatment more difficult and raising morbidity. According to this research, race and ethnicity affect the likelihood and severity of diabetic wound infections, particularly those brought on by *S. aureus*, in addition to the incidence of diabetes (Najafipour et al., 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Area of Study

The study took place at the MTRH, the second largest referral hospital in the country, just after Kenyatta National Hospital, serving the Rift Valley and Western regions of Kenya. It is located 310 km northwest of Nairobi in Uasin Gishu County (Eldoret) along Nandi Road in Eldoret Town, Uasin Gishu County, at 0.5° N and 35° E. Several clinics are run at the hospital, and the diabetic outpatient clinic (Chandaria Cancer and Chronic Diseases Centre) is one of these clinics. The average number of patients seen per day in diabetic outpatient clinics is 15; that is, on Mondays and Thursdays. This makes a total of 120 patients per month and 1440 per year. The clinics are run by endocrinologists, physicians, nurses, and clinical officers.

Uasin Gishu, which is located on a plateau and has a cool and temperate climate, Eldoret City is the County's most populated center, as well as its administrative and commercial center found in. The county borders Trans-Nzoia County to the north, Elgeyo-Marakwet and Baringo counties to the east, Kericho County to the south, Nandi County to the southwest, and Kakamega County to the west. This study area was considered since the majority of patients in this region seek medical care in the facility, owing to the available facilities and the advantage of serving the neighboring counties. The study was a one-day commitment for participants and 6 months for the entire research.

3.2 Research Design

The study employed a hospital-based cross-sectional descriptive study design, whereby data of interest such as age, sex, marital status, education, underlying conditions, and history of antibiotic therapy, were obtained from the diabetic wound clinic located at the MTRH (Chandaria Cancer and Chronic Diseases Centre), which was a common point, where the patients were reporting for routine check-ups, as well as administering questionnaires to the participants upon inclusion into the study, from which a correlation was identified and further investigation done on data of interest gathered on by the questionnaire. These patients were identified by making sure they met the inclusion criteria for the study, which were being 13 years and above, as well as having a diabetic wound. After this, recruitment was done every week. In addition, they were briefed about the research, and informed consent was obtained before their inclusion as study participants. For the patients who could not read, the consent content was read to them and explained in both English and Kiswahili. Upon enrollment, each participant was given a questionnaire, and a unique study number was assigned to identify them. This same number was used to label the corresponding laboratory sample (including identification details and date). Before commencing the study, a preliminary interview was carried out using the pretested structured questionnaire to verify the validity of the instrument.

3.3 Sampling Design

Purposive sampling was used to identify key elements for data collection and analysis relevant to the specific research questions. As a result, patients with diabetic wound infections were deliberately selected. This design was appropriate for the study as it enabled the collection of baseline data on antibiotic susceptibility patterns in bacterial diabetic wound infections among patients at MTRH.

3.4 Target Population

The target population included T2D patients aged 13 years and above who had developed diabetic wound infections and visited the MTRH diabetic clinic (Chandaria Cancer and Chronic Diseases Centre) for wound dressing during the study period from August 2024 to January 2025. Diabetic wounds with the following characteristics were considered infected: New or increased pain, swelling, erythema, purulent exudate, malodor, and localized warmth around the site of the wound.

3.5 Inclusion and Exclusion Criteria

Consenting diabetes mellitus patients with diabetic wound infections older than 13 years were all included in this study. However, patients with diabetes mellitus infected wounds who had dressed their wounds using antiseptics were excluded from participating in this study as the antiseptics could have interfered with the possibility of isolating the target bacteria if not their sensitivity to target antibiotics.

3.6 Sample Size Determination

The sample size for the study was determined following the formula by Fisher *et al.* (1991) as modified by Jung (2014) as shown in the Equation below:

$$n_f = \frac{n}{1+\frac{n}{N}}$$

Where;

n_f = desired sample for a population less than 10,000;

n = desired sample size for the target population exceeding 10,000;

and the N estimate of population size in the present study,

Hence, the sample size for this research was

$$n_f = \frac{384}{1+\frac{384}{225}} = 142$$

To ensure equitable representation of the population in the sample. The sample size was adjusted by adding 10% of the calculated sample size to take care of sampling error. (Peterson & Kaur, 2018). Thus, the corrected sample size was 156.

3.7 Collection of Patient Information

Diabetic type II patients with wound infections who consented to take part in the research were subjected to an interview using a structured questionnaire (Appendix I). The questionnaire had questions on socio-demographic characteristics (age, gender, education level, and marital status), diabetes diagnosis, and antibiotic medication history. Data on prevalence and antimicrobial susceptibility were obtained with the aid of a laboratory request form (Appendix II).

3.8 Sample Collection

Samples were collected from 156 patients at the MTRH, with the assistance of a clinical officer in charge or a nurse in charge who had the requisite training from medical school and was authorized to collect samples from patients. It included patients with T2D who were attending their check-ups at a diabetic wound care clinic with diabetic wound infections during the study. Pus specimens from infected wounds of these diabetic patients were swabbed aseptically for *S. aureus* screening. The wounds were cleaned with sterile saline, and the swab was moistened with sterile saline water before swabbing in a ‘zig-zag’ motion to cover the entire wound surface.

3.9 Isolation of *Staphylococcus aureus*

Staphylococcus aureus was isolated as described by Naureen *et al.* (2022). Swabs were inoculated into Tryptone Soy Broth for 18-24 hours and thereafter sub-cultured on blood agar. Blood agar media was prepared by weighing 40 grams and suspending the blood agar powder in distilled water as indicated by the manufacturer. The mixture was heated to boil and sterilized at 121 °C for 15 minutes in an autoclave. Blood agar was cooled to 55 °C after autoclaving, and then 5% sheep blood was added and mixed. Petri dishes were laid on a flat surface, approximately 20 millilitres of blood agar were dispensed into the Petri dishes, left to solidify, labelled, and then stored at 2-8 °C in a refrigerator. A smear of pus was made at the periphery of the blood agar plate, and then a streak of parallel lines was made along the edge of the plate using disposable plastic loops. After inoculation, the plates were placed upside down in an aerobic incubator for 24-48 hours at 37˚C. The plates were checked after 24-48 hours for the growth of round golden-yellow colonies with beta hemolysis in the laboratory.

3.10 Identification of *Staphylococcus aureus*

Colonies that developed clear, beta-hemolytic, and round characteristics of *S. aureus* were sub-cultured on Mannitol Salt Agar (MSA) for identification. These MSA plates were incubated at 37˚C for 24 hours. The yellowish appearance of colonies on MSA was an indication of positive fermentation of mannitol, which was a characteristic of *S. aureu*s (Jiwantoro & Putri, 2023).

3.10.1 Gram Staining and Microscopic Examination

Gram staining was performed as described in detail in Appendix III. A clean glass slide was labelled and a smear was made by putting a drop of normal saline on the slide and mixing it with a small amount of the colony and left to air dry for 5 minutes at room temperature (25°C), the smear was heat-fixed by rapidly passing through the tip of the blue portion of the bunsen flame 3-4 times and left to cool. The heat-fixed smear was stained using crystal violet for 1 minute, rinsed with running tap water, stained with Gram's iodine for 1 minute, rinsed with running tap water, decolorization was done by the addition of acetone for approximately 10-15 seconds, and immediately washed with distilled water. Then counterstained with safranin for 2 minutes, rinsed with running tap water, left to dry, then examined under 100x oil immersion. Observation of purple cocci-shaped bacteria arranged in clusters was characteristic of *S. aureus* (Evans et al., 2022).

3.10.2 Biochemical Tests

i. Catalase Test

The catalase test was performed as described in Appendix IV with 3% hydrogen peroxide. Using a sterilized wire loop, a single colony was picked and emulsified into a drop of hydrogen peroxide on a glass slide. The formation of bubbles indicated a catalase-positive test result since *S. aureus* is catalase-positive. This test differentiates *Staphylococci* from *Streptococci* (Pervin et al., 2019)

ii. Coagulase test

The coagulase test was performed using the procedure described in Appendix IV. Citrated rabbit plasma (0.3 milliliters) was put into two test tubes and one labelled as a control and the other as a sample. The tube labelled as the sample was inoculated with colonies of gram-positive, cocci-shaped, and catalase-positive test isolates and mixed by shaking for 3 minutes to make a cloudy suspension. The Control tube was inoculated with positive control of *S. aureus*, and both tubes were incubated at 37◦C for 1-4 hours in an incubator. If there had been no coagulation after 4 hours in the tube containing the sample, the tube incubation was extended up to 24 hours. *S. aureus* is coagulase-positive, and this differentiates it from other *Staphylococcus* species (Javid et al., 2018)

3.11 Antimicrobial Susceptibility Tests

Antimicrobial susceptibility tests on *S. aureus* isolates were performed (Cheng et al. (2019). The inoculum was prepared by transferring 5 isolated colonies to 5 mL of sterile saline from the MSA plate and mixing. The suspension was adjusted to match the 0.5 McFarland turbidity standards. Within 15 minutes of inoculum preparation, a sterile cotton swab was dipped into the adjusted suspension, and excess inoculum was removed by pressing the swab firmly on the inside wall of the tube. The dried surface of a Mueller-Hinton agar (MHA) plate was then inoculated by streaking the swab over the entire surface while ensuring even distribution. The inoculated MHA plate was allowed to stand for 3 minutes to dry. The antimicrobial discs used in the study consisted of eight (8) antibiotics of different classes/families and with different modes of action as prioritized under the MTRH protocol: Amoxicillin (30 μg), Ampicillin (10 μg), Cefoxitin (30 μg), Ciprofloxacin (5 μg), Clindamycin (2 μg), Erythromycin (15 μg), Tetracycline (30 μg), and Trimethoprim (25 μg). The discs were applied using sterile forceps with gentle pressure to ensure complete contact with the disc in MHA. The plates were then incubated at 37℃ for 15 minutes after application of the discs for 24 hours. The plates were examined, and the diameters of the zones of inhibition were measured using a transparent plastic ruler. The results were defined as susceptible, intermediate, or resistant, according to the approved guidelines of the Clinical and Laboratory Standards Institute. (CLSI, 2021).

3.12 Data Analysis

Most of the data obtained in the study were descriptive and thus were presented in frequency tables and figures. The prevalence was calculated based on the number of positive results (having *S. aureus*) from the samples divided by the total number of samples. Only *S. aureus* was isolated and recorded. Data from the questionnaire and laboratory results were coded to convert all of them into numerical data, which was entered into SPSS version 20. Data cleaning was then done to confirm accuracy and consistency. Analysis was done with the aid of SPSS Version 20. Frequencies were determined for socio-demographic characteristics. *S. aureus* carriage was the dependent variable. Factors that predispose to the susceptibility pattern of *S. aureus* were also the independent variable. Analysis of Variance (ANOVA) was used to determine the susceptibility pattern of *S. aureus*, while the Chi-square test was used to determine the association between *S. aureus* infections and the possible predisposing factors. Pearson's correlation was also used to determine the relationship between resistance, sensitivity, and intermediate sensitivity of *S. aureus* isolates. All the statistical tests were performed at a 5% level of significance. The data collection tools were stored under lock and key and were only made accessible to the candidates and supervisors (Guo et al., 2021)

3.13 Ethical Considerations

The authority to conduct research in Kenya was obtained from the National Commission for Science, Technology and Innovation (NACOSTI) (license no: NACOSTI/P/24/34462) (Appendix V). Ethical approval for research on human subjects was sought from the

MTRH/Moi University Institutional Ethics Review Committee (Approval No: 0004852) (Appendix VI). A research information sheet (Appendix VII) was shared with the participants and explained to them. Informed consent was obtained from patients who met the desired criteria and who agreed to participate in the study (Appendix VIII). A statement by the researcher (Appendix IX) was also signed once the participant had understood the entire process, and a copy was shared with the participant(s). Subjects were assured of the confidentiality of the research findings. This was adhered to by ensuring that all data stored in the database was rights-protected by the principal investigator.

CHAPTER FOUR

RESULTS

4.1 Demographic Characteristics of Participants

This study utilized a total of 156 patient specimens obtained from diabetic wounds of patients attending an outpatient diabetic clinic (Chandaria Cancer and Chronic Diseases Center) at the MTRH during the period from August 2024 to January 2025. Their demographic characteristics are described in Table 1.

Table 4.1: Demographic characteristics of selected patients at MTRH

Factor	Categories	Total (%)
Age group (Years)	13 – 30	15 (9.62)
	31 – 44	26 (16.67)
	45 – 60	60 (38.46)
	>60	55 (35.25)
Sex	Female	63 (40.38)
	Male	93 (59.62)
Underlying Conditions	Yes	88 (56.41)
	No	68 (43.59)
Hospitalized	Yes	133 (85.26)
	No	23 (14.74)
Antibiotics Use	Yes	50 (32.05)
	No	106 (67.95)
Marital Status	Single	19 (12.18)
	Married	122 (78.21)
	Other	15 (9.62)
Level of Education	Primary	69 (44.23)
	Secondary	36 (23.08)
	Tertiary	33 (21.15)
	No School	18 (11.54)

A total of 156 samples were collected throughout the study period, out of which 93 (59.62%) were males and 63 (40.38%) were females. Most of the study participants were between the ages of 45–60 years. A majority of the respondents (122) of the diabetic patients in the current study period were married. 19 (12.18%) were single, while 9.62% (15) of the positive cases were from widows/widowers. 69 (44.23%) of the patients had a primary school education, while 36 (23.08%) had a secondary school education. A total of 33 (21.15%) of the respondents had a tertiary school education, with only 18 (11.54%) having no school experience. More than half (56.41%) of the diabetic patients attending outpatient diabetic clinics at the MTRH during the duration of this study had underlying conditions. A majority of the diabetic patients, 133 (85.26%), in this study also had previously been hospitalized. A total of 50 (32.05%) had used antibiotics before they enrolled in this study.

4.2 Prevalence of *S. aureus* from Diabetic Wounds of Patients Attending MTRH

This study used 156 (100%) samples obtained from wounds of diabetic patients attending an outpatient diabetic clinic (Chandaria Cancer and Chronic Diseases Center) at the MTRH. The plates of swabs were inoculated into Tryptone Soy Broth and thereafter sub-cultured on blood agar (Figure 4.1a) and (Figure 4.1b), respectively. The yellowish appearance of colonies on MSA indicated the presence of *S. aureu*s.

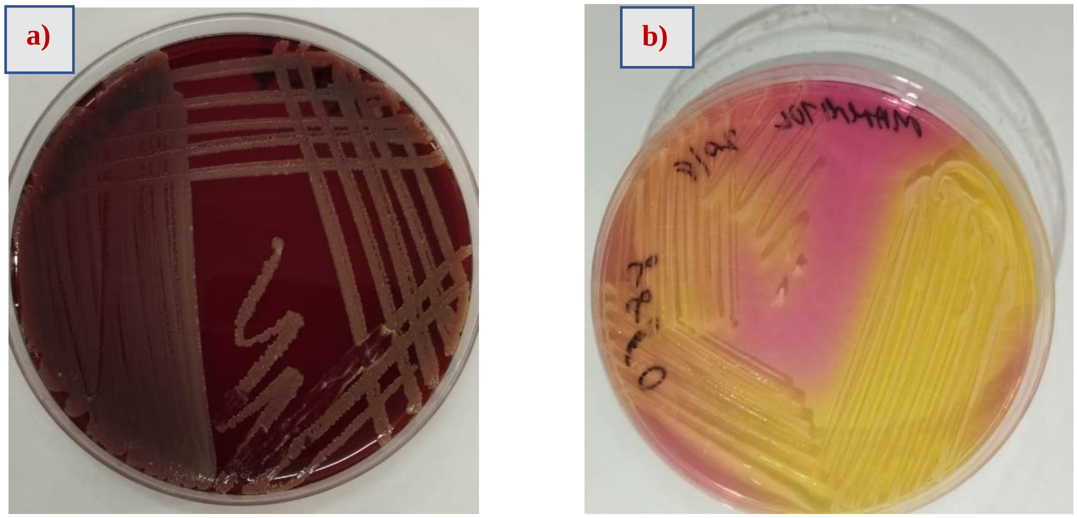

Figure 4.1: Plates showing *Staphylococcus* species on Blood agar (a) and Mannitol Salt agar (b) with golden yellow and yellowish appearance, respectively.

A loopful of each distinct colony was subjected to Gram staining, and morphological identification was revealed by microscopy. Purple, cocci-shaped cells arranged in pairs and clusters were identified as *S. aureus* (Figure 4.2).

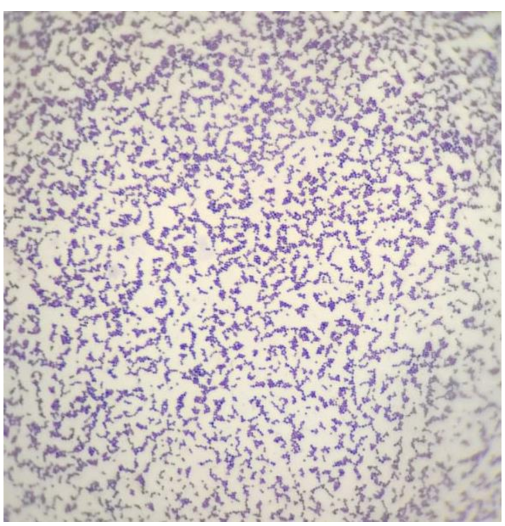

Figure 4.2: Showing a glass slide under 100×mg oil immersion, Gram-positive Cocci (purple)

Further identification was obtained through biochemical testing using the catalase test

(Figure 4.3a) and the coagulase test (Figure 4.3b).

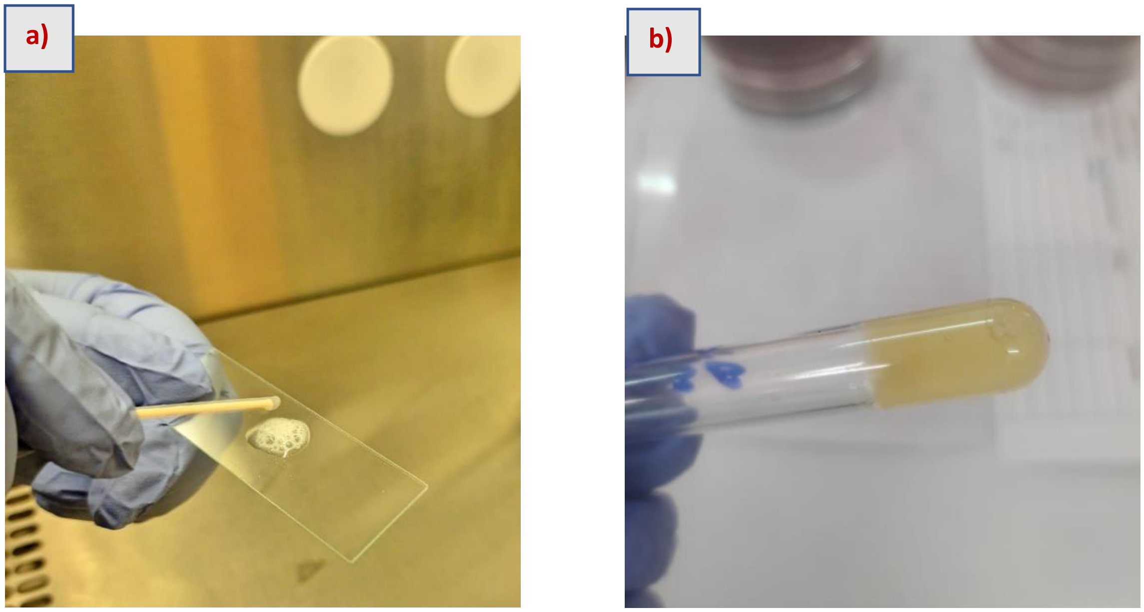

Figure 4.3: Catalase test (a) and Coagulase test (b)

Upon confirmation from the samples, this study noted that 31 (19.87%) samples were positive for *S. aureus*, while 125 (80.13%) samples tested negative for *S. aureus*. This translates to a prevalence of 19.87% of *S. aureus* as shown in Table 4.2.

Table 4.2: Prevalence of *S. aureus* in Diabetic Wound Infections

Samples examined	No.	Prevalence (%)
Positive	31	19.87
Negative	125	80.13
Total	156	100

4.3 Antibiotic Susceptibility Patterns of *S. aureus* Isolated from Wounds of selected diabetic patients attending MTRH

This study assessed the antibiotic susceptibility patterns of the isolated *S. aureus* against eight (8) antibiotics of different classes/families and with different modes of action as prioritized under the MTRH protocol. The tested antibiotics were Amoxicillin (30 μg), Ampicillin (10 μg), Cefoxitin (30 μg), Ciprofloxacin (5μg), Clindamycin (2μg), Erythromycin (15 μg), Tetracycline (30 μg), and Trimethoprim (25 μg). The clear zones formed around the discs were recorded as zones of inhibition (Figure 4.4), which were measured in millimeters using a transparent plastic ruler. *S. aureus* had varying degrees of susceptibility profiles to the antibiotics they were subjected by the disc diffusion method (Figure 4.5 & Table 4.3). *S. aureus* isolated in this study had at least one instance of intermediate sensitivity 26 (10.48%) and/or antibiotic resistance 72 (29.03%) to the other antibiotics. However, more than half of the isolates were susceptible to the test antibiotics, i.e, except Ampicillin, half of the isolates were susceptible to the other 7 test antibiotics, as shown in Table 4.3.

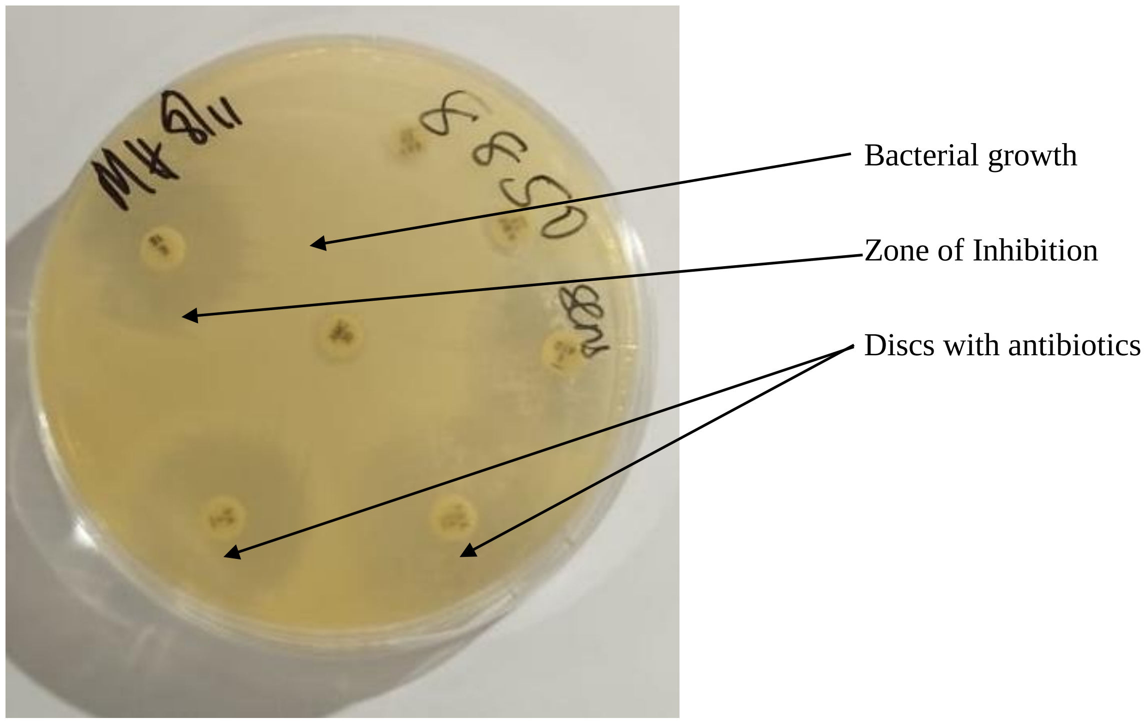

Figure 4.4: Antibacterial susceptibility test of *S. aureus* showing clear zones of inhibition, bacterial growth, as well as antibiotic discs used.

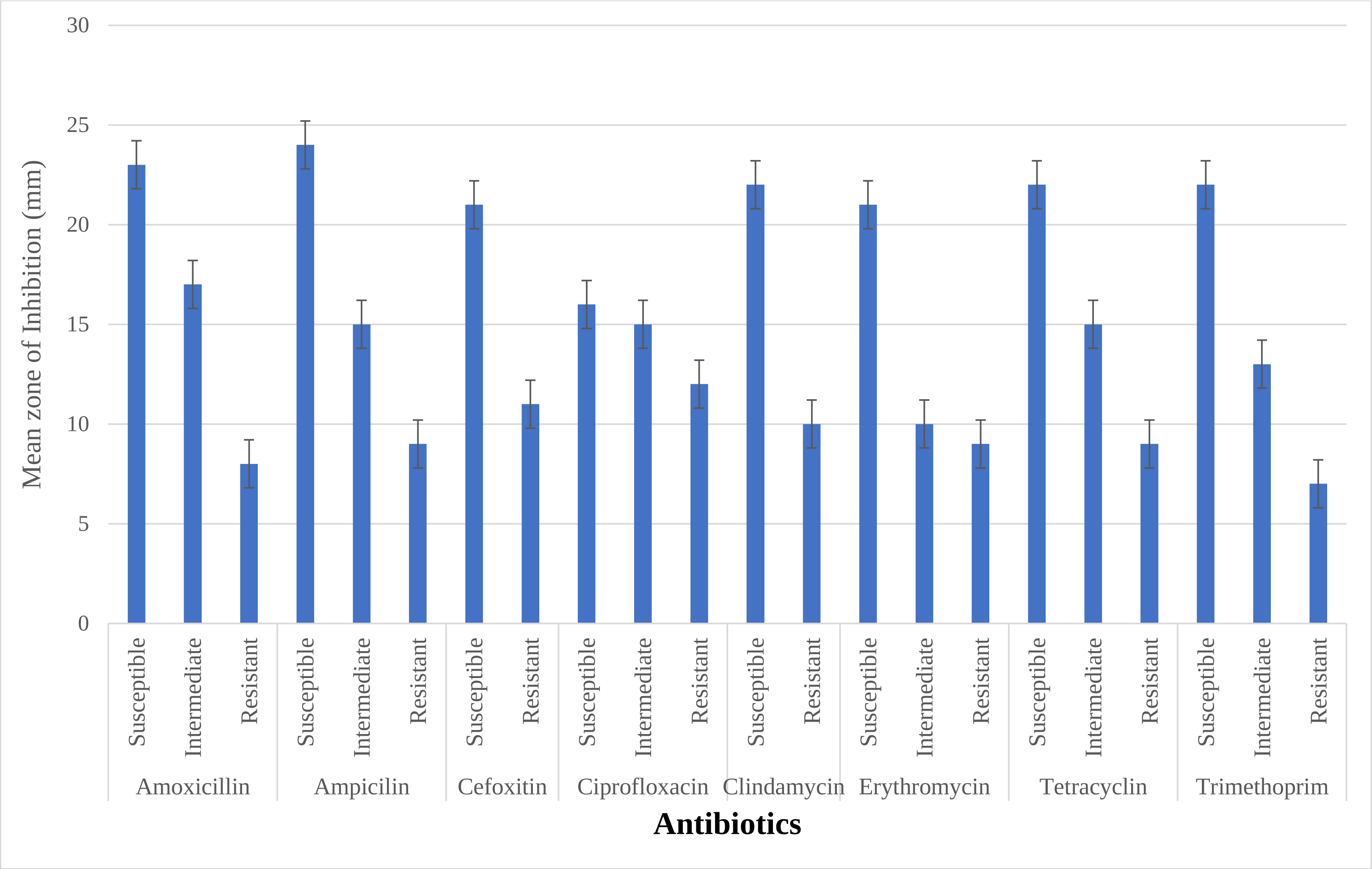

Figure 4.5: A bar graph showing the diameter of the zone of inhibition(mm) against the test antibiotics.

A higher number of *S. aureus* isolates were susceptible to Cefoxitin and Clindamycin, with lesser susceptibility to Ampicillin (Table 4.3)

Table 4.3: Susceptibility profile of *S. aureus* isolates to tested antibiotics

Antibiotic	S. (%)	I . (%)	R. (%)	Total
Amoxicillin	18 (58.06)	5 (16.13)	8 (25.81)	31
Ampicillin	8 (25.81)	11 (35.48)	12 (38.71)	31
Cefoxitin	30 (96.77)	-	1 (3.23)	31
Ciprofloxacin	16 (51.61)	2 (6.45)	13 (41.94)	31
Clindamycin	25 (80.65)	-	6 (19.35)	31
Erythromycin	20 (64.51)	1 (3.23)	10 (32.26)	31
Tetracycline	15 (48.39)	6 (19.35)	10 (32.26)	31
Trimethoprim	18 (58.06)	1 (3.23)	12 (38.71)	31

Pearson's correlation coefficients were calculated to determine the relationship between resistance, sensitivity, and intermediate sensitivity, which is presented in Table 4.4.

Table 4.4: Pearson's correlation coefficients of *S. aureus* isolates against test antibiotics

	Resistant	Intermediate	Sensitive
Resistant	1		
Intermediate	-0.09	1	
Sensitive	0.27	0.02	1

That means that an increase in antibiotic-resistant *S. aureus* isolates results in an increase in antibiotic-sensitive *S. aureus* isolates as well. On the other side, an increase in antibiotic-resistant and/or sensitive *S. aureus* isolates results in a decrease in *S. aureus* isolates with intermediate sensitivity. None of the correlations are statistically significant ($p > 0.05$), hence weak correlation as the coefficients are closer to 0 than they are to -1 or +1, as shown in Table 4.4.

ANOVA was used to determine the susceptibility of *S. aureus* isolated from diabetic wounds of patients attending outpatient diabetic clinics at MTRH. The statistically significant p-value of 0.0000 at a 95% confidence level ($P \leq 0.05$) (Table 4.5) means that the *S. aureus* isolates are statistically susceptible to the test antibiotics.

Table 4.5: ANOVA on antibiotic susceptibility of *S. aureus* from diabetic wound infections

Source of Variation	SS	Df	MS	F	P-value
Between Groups	8195.508	2	4097.754	333.7214	0.0000
Within Groups	3008.347	245	12.2789		
Total	11203.85	247			

4.4 Risk Factors Associated with *S. aureus* in Diabetic Wound Infections

4.4.1 Age

Out of the 31 (19.87%) positive cases of the presence of *S. aureus* in wounds of diabetic patients, a majority, 13 (41.9%) were patients more than 60 years of age. Age groups of 13 – 30 and 31 – 44 had 4 (2.56%) positive cases each, while those aged between 45 - 60 years old were 10 (6.41%) (Table 4.6). Despite those aged more than 60 years recording a higher positivity rate, the age of diabetic patients did not significantly influence the presence of *S. aureus* in their wounds (Chi-square test p-value = 0.6503) (Table 4.6). This means that *S.*

aureus is likely to be present in wounds of diabetic patients attending the outpatient diabetic clinic at MTRH, irrespective of their age.

Table 4.6: Relationship between Age and presence of *S. aureus* in diabetic wound infections

Age group (Years)	Total No. (%)	Positive No. (%)	p-value
13 – 30	15 (9.62)	4 (2.56)	0.6503*
31 – 44	26 (16.67)	4 (2.56)	
45 – 60	60 (38.46)	10 (6.41)	
>60	55 (35.25)	13 (8.33)	
Total	156 (100)	31 (19.87)	

* Chi-Square test.

4.4.2 Sex

A total of 93 (59.62%) male and 63 (40.38%) female diabetic patients attending the outpatient diabetic clinic at MTRH were enrolled in this study. Of the 31 positive cases, 13 (41.94) were male, while 18 (58.06) were female. The results showed that the sex of a diabetic patient significantly influenced the infection of wounds with *S. aureus* (Chi-square test p-value = 0.025) (Table 4.7). The sex of a diabetic patient, particularly those attending the outpatient diabetic clinic at MTRH, can be linked to the existence and subsequent isolation of *S. aureus* in their wounds. This is evident from the results of this study, whereby female diabetic patients recorded a significantly higher positivity rate when compared to male diabetic patients (Table 4.7).

Table 4.7: Relationship between Sex and the presence of *S. aureus* in diabetic wound infections

SEX	Total (%)	Positive (%)	Negative (%)	p-value
Female	63 (40.38)	18 (58.06)	45 (36.00)	0.025*
Male	93 (59.62)	13 (41.94)	80 (64.00)	
Total	156 (100)	31 (19.87)	125 (80.13)	

* Chi-Square test.

4.4.3 Underlying Conditions

More than half (56.41%) of the diabetic patients attending the outpatient diabetic clinic at MTRH during the duration of this study had underlying conditions. Out of the total (31) positive cases of *S. aureus* infection, 17 (54.84%) were from diabetic patients who had underlying conditions, while those without underlying conditions accounted for only 14 (45.16%) of the total patients enrolled (Table 4.8). The analysis showed no significant relation between the existence of underlying conditions and the presence of *S. aureus* in wounds (p-value = 0.8437) (Table 4.8). That means that diabetic patients are likely to get *S. aureus* infection, whether they have underlying conditions or not.

Table 4.8: Relationship between underlying conditions and the presence of *S. aureus* in diabetic wound infections

Present	Total (%)	Positive (%)	Negative (%)	p-value
YES	88 (56.41)	17 (54.84)	71 (56.8)	0.8437*
NO	68 (43.59)	14 (45.16)	54 (43.2)	
Total	156 (100)	31 (19.87)	125 (80.13)	

* Chi-Square test. Underlying conditions refer to pre-existing medical issues that can increase the risk of complications from a new illness or condition.

4.4.4 Previous Hospitalization

A majority of the diabetic patients, 133(85.26%), in this study had previously been hospitalized for various reasons. 87 (65.41%) of hospitalizations (s) were due to T2D-related illness, with 29 (21.81%) due to other illnesses, while 17 (12.78%) were due to diabetic wound-related illness. Out of the total positive cases, 26 (83.87%) were from those patients with a history of hospitalization (Table 4.9). Despite that, there was no significant relation between the existence of previous hospitalization and the presence of *S. aureus* in wounds (p-value = 0.808) (Table 4.9). This implies that those diabetic patients attending the outpatient diabetic clinic at MTRH are likely to have *S. aureus* in their wounds, irrespective of their hospitalization status before the current study.

Table 4.9: Relationship between previous hospitalization and the presence of *S. aureus* in diabetic wound infections

Hospitalized	**Total (%)**	**Positive (%)**	**Negative (%)**	**p-value**
YES	133 (85.26)	26 (83.87)	107 (85.6)	0.808*
NO	23 (14.74)	5 (16.13)	18 (14.4)	
Total	156 (100)	31 (19.87)	125 (80.13)	

* Chi-Square test. Source:

4.4.5 Prior Antibiotic Use

A total of 50 (32.05%) diabetic patients attending outpatient diabetic clinics at MTRH in this study had used antibiotics before enrolment. Of the 31 (19.87%) positive cases, 9 (29.03%) diabetic patients had used antibiotics, while 22 (70.96%) had no history of antibiotic use preceding their enrolment (Table 4.10).

Table 4.10: Relationship between antibiotic use and presence of *S. aureus* in diabetic wound infections

Antibiotics	**Total (%)**	**Positive (%)**	**Negative (%)**	**p-value**
YES	50 (32.05)	9 (29.0)	41 (32.8)	0.6874*
NO	106 (67.95)	22 (70.96)	84 (67.2)	
Total	156 (100)	31 (19.87)	125 (80.13)	

* Chi-Square test.

The results indicated that there was no significant relation between prior antibiotic use and the presence of *S. aureus* in the wounds of diabetic patients sampled in this study (p-value = 0.6874) (Table 4.10). This means that the isolates from diabetic patients at MTRH could not be directly labeled as resistant to the antibiotics used by the patients before their enrolment in this study.

4.4.6 Marital Status

During the study period, 122(78.21%) of the diabetic patients visiting the outpatient diabetic clinic at MTRH were in a marital relationship. 22 (70.96%) of them returned positive results for *S. aureus* infections in their wounds. Three (0.97%) of the positive cases were from those who were single, while 6 (19.35%) of the positive cases were from others (widows/widowers) (Table 4.11). Despite more married patients recording a higher positivity rate, the results showed that the marital status of diabetic patients was not significantly related to the presence of *S. aureus* in their wounds (p-value = 0.118) (Table 4.11). This indicates that diabetes patients who visit the MTRH outpatient diabetic clinic are likely to have S. aureus in their wounds, regardless of their marital status.

Table 4.11: Relationship between marital status and presence of S. aureus in diabetic wound infections

Marital Status	Total No. (%)	Positive No.	%	p-value
Single	19 (12.18)	3	0.97	0.118*
Married	122 (78.21)	22	70.97	
Other	15 (9.62)	6	19.35	
Total	156 (100)	31	19.87	

* Chi-Square test.

4.4.7 Level of Education

Out of the 31 (19.87%) positive cases of the presence of *S. aureus* in wounds of diabetic patients, the majority, 16 (51.61%) out of 69, had a primary school education (Table 4.12). Those with a tertiary school education were 33, with 9 (29.03%) returning positive results of *S. aureus* infections. Diabetic patients who attended the outpatient diabetic clinic at MTRH during the duration of this study and had no school experience or had a secondary school education returned 3 (0.97%) positive cases each, from a total of 18 and 36, respectively (Table 4.12). Despite the participants who were only primary school graduates registering a higher positivity rate, the level of education of diabetic patient (s) was not significantly related to the presence of *S. aureus* in their wounds (p-value = 0.192) (Table 4.12). This means that *S. aureus* is likely to be present in diabetic wounds of patients attending the outpatient diabetic clinic at MTRH, irrespective of their level of education.

Table 4.12: Relationship between the level of education and presence of *S. aureus* in diabetic wound infections

Education level	Total No. (%)	Positive No.	%	p-value
Primary	69 (44.23)	16	51.61	0.192
Secondary	36 (23.08)	3	0.97	
Tertiary	33 (21.15)	9	29.03	
No School	18 (11.54)	3	0.97	
Total	156 (100)	31	19.87	

* Chi-Square test.

CHAPTER FIVE

DISCUSSIONS

5.1 Prevalence of *S. aureus* from Diabetic Wounds of Patients Attending MTRH

The research collected a total of 156 samples from diabetic wounds of patients attending the outpatient diabetic clinic at MTRH during the study period. Thirty-one samples were positive while 125 samples tested negative, a prevalence of 19.87%. This prevalence is lower when compared to similar studies at Vihiga County Referral Hospital (Tuvei, 2017), which reported an overall 60.3 % prevalence of *S. aureus* infection among diabetes mellitus patients. Similar studies in Ethiopia also recorded a higher prevalence of 31.1% (Mariam *et al.*, 2017) and 25.19% (Atlaw *et al.*, 2022). Mutonga (2018) recorded a 98% prevalence, while Amini *et al.* (2013) reported an 87% prevalence. The fact that these studies focused on foot ulcers explains the difference in prevalence results, if not the differences in periods and settings in which the studies were conducted.

According to Mutonga (2018), about 10–15% of diabetic patients will develop DFIs at some point in their lives. Tuvei (2017), due to interconnected physiological and immunological factors such as impaired blood circulation, high blood sugar, and peripheral neuropathy, also documents that foot ulcers are more prone to infections than other wounds. These high prevalence(s) can be linked to the fact that most often, *S. aureus* colonizes on skin or mucosal surfaces. However, it has been recognized that children, HIV or diabetic patients with wounds are more prone to *S. aureus* colonization, which could be due to compromised or immature immune defenses or altered skin integrity. (Shettigar & Murali, 2020). They also have the potential to cause serious infections if not treated early. Even when the virulence and invasive capability of *S. aureus* strains recovered from diabetes patients' wounds are lower than those of strains typically seen in infections, they nevertheless retain the ability to cause and sustain invasive and deep-tissue infections. (Tuchscherr *et al.*, 2018).

5.2 Antibiotic Profile of *S. aureus* Isolated from Diabetic Wound Infections

There were instances of intermediate sensitivity 26 (10.48%) and/or antibiotic resistance 72 (29.03%) to the other antibiotics for the *Staphylococcus aureus* isolated in this research. However, most of the isolates were vulnerable to the test antibiotics. This indicates that antibiotic resistance is not widespread among diabetic patients, which has also been reported by other authors before (Amini *et al.*, 2013; Atlaw *et al.*, 2022; Mariam *et al.*, 2017; Mutonga, 2018; Tuvei, 2017). This study found that a higher number of *S. aureus* isolates were susceptible to Cefoxitin (96.77%) and Clindamycin (80.65%), with lesser susceptibility to Ampicillin (25.81%). These findings differ from those of Amini *et al.* (2013), who reported 63.9% resistance by *S. aureus* isolates to Clindamycin. Atlaw *et al.* (2022) also documented a high level of resistance of *S. aureus* to erythromycin and trimethoprim, unlike in the current study. The authors, however, reported that the *S. aureus* isolates were sensitive to clindamycin, just like in the current study, and could easily be attributed to differences in antibiotic use practices, as well as local bacterial ecology, and patient characteristics among the participants of this study

The present results concur with the findings of Fawad (2022), who also documented that the *S. aureus* they isolated showed high sensitivity to cefoxitin and clindamycin. Bhat *et al.* (2011), also documented that *S. aureus* presented better susceptibility to commonly used antibiotics like erythromycin, ceftriaxone, and clindamycin. *S. aureus* isolates showed high rates of resistance to oxacillin (95.2%), clindamycin (68.7%), and erythromycin

(65.6%) according to Owais *et al.* (2024). These differences could also be linked to the possibility in the reduction antibiotic pressure, if not the differences in infection type and source, MTRH stewardship practices, in addition to genetic variability among *S. aureus* isolates.

S. aureus isolates in our study exhibited notable resistance rates (25.81%) for amoxicillin and 38.71% for ampicillin, as well as poor sensitivity to both amoxicillin (58.06%) and ampicillin (25.81%). This is consistent with regional statistics and earlier studies conducted in Kenya, where β-lactamase generation is ubiquitous and leads to significant resistance to antibiotics of the penicillin family. For example, research conducted at a major hospital in Kenya found that 70–80% of the isolates of DFIs were resistant to ampicillin and amoxicillin. Pooled resistance rates for these antibiotics in DFI isolates are equally high, frequently above 60%, in sub-Saharan Africa (Mutonga, 2018; Wada *et al.*, 2023).

The remarkably high cefoxitin susceptibility in our study (96.77%) suggests that MRSA is not very common among diabetic wound isolates perups due to better antibiotic stewardship and restricted use of β-lactam antibiotics for T2DM patients at MTRH. This contrasts with research from Kenyatta National Hospital and other East African institutions, which show that MRSA rates in public hospitals can vary from 31% to over 50% for skin and soft tissue infections, including DFIs. Some private hospitals in Kenya, however, report MRSA rates that are far lower (3–6%) (Wangai *et al.*, 2019). The low MRSA prevalence at MTRH could be the result of good antimicrobial stewardship and infection control procedures, such as restricting unnecessary prescriptions and regular hand hygiene

MTRH isolates showed a resistance rate of 41.94% and a moderate susceptibility to ciprofloxacin (51.61%). This aligns with a previous meta-analysis conducted in sub-Saharan Africa that discovered 52.45% pooled ciprofloxacin resistance in *S. aureus* from DFIs (Makeri et al., 2023). Other investigations conducted in Kenya and the surrounding area have shown a similar pattern, which is indicative of the effects of widespread fluoroquinolone usage and the emergence of resistance (Mutonga, 2018; Wada *et al.*, 2023). In the current study, susceptibility to erythromycin was 64.51% and clindamycin susceptibility was comparatively high at 80.65%. The corresponding resistance rates were 19.35% and 32.26%. In contrast to certain regional studies, where erythromycin resistance is frequently above 30% and clindamycin resistance might surpass 40%, these results are encouraging (Mutonga, 2018; Wada *et al.*, 2023). Effective local management or less frequent application of these chemicals might be the cause of the comparatively high susceptibility at MTRH. Tetracycline and trimethoprim demonstrated significant resistance (32.26% and 38.71%, respectively) and moderate susceptibility (48.39% and 58.06%, respectively) in this study. These rates are consistent with pooled findings from sub-Saharan Africa, where *S. aureus* from DFIs frequently exhibits resistance to trimethoprim-sulfamethoxazole and tetracycline at levels exceeding 40% (Wada et al., 2023). Over-the-counter availability and usage in veterinary and human care are contributing reasons. The spread of antibiotic-resistant *S. aureus* complicates therapy, highlighting the necessity of implementing strong infection control methods like improved hygiene and creating novel therapeutic approaches. Given the variability in antibiotic resistance patterns, personalized treatment plans based on susceptibility testing are crucial for effective management.

5.3 Risk Factors Associated with *S. aureus* in Diabetic Wound Infections

5.3.1 Age

Out of the 31 (19.87%) positive cases, a majority (8.33%) were of patients aged 60 years and above. Age groups of 13 – 30 and 31 – 44 had 4 (12.90%) positive cases each, while those aged between 45 -60 years old were 10 (32.26%). This concurs with earlier studies as well, for example, Tuvei (2017) reported an elevated occurrence percentage among the age group of over 60 years at 63.8%. Amini *et al.* (2013) also realized that the majority of the participants (51/90) were more than 60 years old. This was similar to Rashid *et al.* (2012), who established a higher prevalence for those aged over 50 years. This observation could be explained by this group of patients having age-related physiological, metabolic, and immune factors that could increase the vulnerability to *S. aureus* infection. However, despite those aged more than 60 years recording a higher positivity rate, the age of iabetic patients did not significantly influence the presence of S. *aureus* in their wounds, and this could imply that other factors, such as glycemic control, duration of diabetes, immune status, hygiene practices, and presence of peripheral neuropathy or vascular complications, likely play a greater role in predisposing wounds to infection than chronological age alone.

The danger of T2D is at its peak with an increase in age, especially after 45 years, due to less exercise, thus gaining weight (Amanat et al., 2020). Therefore, aging may augment T2D risk through pathophysiological mechanisms independent of obesity. As has been reported by other authors before, as people age, their immune systems become less effective at fighting off infections. The elderly often have condensed neutrophil function and other immune impairments, making them more susceptible to infections like *S. aureus* (Thorlacius *et al.*, 2019).

5.3.2 Sex

Of the 31 positive cases in this study, 13 (41.94%) were male, while 18 (58.06%) were female. The sex of a diabetic patient significantly influenced wound infection with *S. aureus (*p-value of 0.025). The sex of a diabetic patient, particularly those attending the outpatient diabetic clinic at MTRH, can be linked to the existence and subsequent isolation of *S. aureus* in their wounds. This is evident from the outcomes of this research, whereby female diabetic patients recorded a significantly higher positivity rate (58.06%) than their male counterparts. The highest prevalence of *S. aureus* infections in diabetic wounds in females could be attributed to the kind of chores traditionally female-dominated. These chores are likely to expose them to *S. aureus* infections, particularly if they have wounds. Given that *S. aureus* is a common pathogen, it can easily be introduced into an open wound when conducting routine chores. Most studies have not explored the influence of sex in the occurrence of diabetes. That is despite an estimated 17.7 million more men than women worldwide suffering from diabetes mellitus (Ciarambino et al., 2022). However, when T2D is diagnosed, women seem to have a higher load of risk factors. Most studies have also not explored the influence of sex in the occurrence of diabetes.

Women experience greater hormonal fluctuations throughout their lives, particularly during pregnancy and menopause, which can affect glucose metabolism and increase the risk of developing diabetes (Kautzky-Willer *et al.*, 2023). The influence of sex is therefore inconclusive, as some studies demonstrated male gender as a risk factor, some female gender as a risk factor, while other studies have shown no difference. Amini *et al.* (2013) reported equal proportions of infections in both sexes. Tuvei (2017) reported that females had a higher prevalence of 57.4% as compared to their male counterparts at 42.6% just as was in this study. Rashid *et al.* (2012) and Gebremedhin *et al.* (2016) also shared the same view. However, this study's results contradicted those by Atlaw *et al.* (2022), Aedh (2016), Reveles *et al.* (2016), Oguzkaya-Artan *et al.* (2016), and Sekhar *et al.* (2014), with the authors reporting a higher occurrence in males than females.

5.3.3 Underlying Conditions

Hypertension, heart disease, and kidney disease were the most frequent underlying conditions noticed during the study. Diabetes and hypertension are closely related chronic illnesses that increase each other's risk and severity. Individuals with hypertension are 2.5 times more likely to develop diabetes than normotensive individuals. (Jia & Sowers, 2021). Just like other studies before, this study found that most of the participants had underlying conditions that perups could have raised their diabetes risk by altering metabolic health, causing insulin resistance, and complicating the care of modifiable risk factors. The higher positivity rate can be attributed to the already low immune systems among most diabetic patients. Underlying conditions weaken the patient’s immune system, rendering them highly susceptible to other infections. However, from the current study, the presence of *S.aureus* could not be directly linked to the existence of underlying conditions (Reveles *et al.,* 2016), differ with their findings, comparable to those from this study. According to the authors, hypertension (76%), dyslipidemia (52%), obesity (49%), peripheral vascular disease (37%), and kidney disease (12%) significantly predispose diabetic patients to *S. aureus* infection.

5.4.4 Previous Hospitalization

A majority of the diabetic patients, 133 (85.26%), who participated in this research were hospitalised before for various reasons, out of which 26 (83.87%) of them returned a positive result. This is slightly lower than that from the findings of Reveles *et al.* (2016), who documented a prevalence of 19% among patients with a history of recent hospitalization. This suggests that it is possible that *S. aureus* infection among diabetic patients in this and other studies could be linked to nosocomial risk. Application of improperly sterilized equipment, in addition to contaminated fomites in hospitals, could be the main reason. The present study identified previous hospitalization as an independent risk factor for *S. aureus* infection. Hospital-acquired infection is one of the notorious causes of microbial infections. (Neubeiser *et al.,* 2020). According to Liu et al., (2022), the occurrence of hospital-acquired infections is mainly due to the poor ward environment and the inadequate implementation of isolation measures for patients. The risk of hospital-acquired *S. aureus* is impacted by variations in infection control procedures in different hospitals/health facilities.

5.3.5 Antibiotic Use

The study showed that 50 (32.05%) diabetic patients attending the outpatient diabetic clinic at MTRH were on drugs before enrolment. Of the (19.87%) positive cases, 9 (29.03%) diabetic patients had used antibiotics. The relationship between prior antibiotic use and the occurrence of *S. aureus* in the wounds of diabetic patients was not statistically significant (p-value = 0.6874). This means that the *S. aureus* isolates from the current study could not be directly labelled as resistant to the antibiotics used by the patients before their enrolment in this study. That means the patients may have contracted the pathogen after completion of the prescribed dosage or may have resisted the prior medication, which may not have been among the eight tested in the current study. However, that contradicts the findings from Amini *et al.* (2013), who reported that 55.4% of the positive cases had a history of recent antibiotic therapy in the last few days. Reveles *et al.* (2016) also hold the same view, with a 43% positivity rate documented by the authors. This could be due to diabetic medications and/or the extra antibiotics utilised by diabetic patients to counter common ailments like the flu due to their compromised immune systems. According to Yuan *et al.* (2020), antibiotics can raise the risk of diabetes by altering the gut microbiota and impacting metabolic health. Additionally, the risk may be confounded by underlying diseases that require the use of antibiotics.

5.3.6 Marital Status

A majority, 122, of the participants attending the outpatient diabetic clinic at MTRH during the research were married. 22 (70.97%) of them returned positive results for *S. aureus* infections in their wounds. Three (0.97%) of the positive cases originated from the single proportion of the subjects, while 19.35% (6) of the positive cases were from others (widows/widowers). There have also been similar results by earlier studies. Tuvei (2017) noted that those married had a higher prevalence of 84.0%. Aedh (2016) also documented similar findings. According to Karimi *et al.* (2025), divorced persons have a low chance of having T2D, as well as widowed people, while, on the other hand, single people are more likely to have it. Additionally, it has been documented that single men are at risk of diabetes compared to married men, while the impact on women can be different depending on the specific marital status. Social support, which is frequently provided by marriage, has a favorable impact on health-related behaviors, including diet, exercise, and treatment compliance. Regarding the effect of marital status on *S. aureus* infection rates, the majority of studies have produced conflicting findings, with the majority suggesting that married participants had higher rates (Adeiza *et al.*, 2020). This can be linked to increased exposure and transmission opportunities within households.

5.3.7 Level of Education

Out of positive cases (19.87%) for the presence of *S. aureus* in diabetic wounds, the majority, 16 (51.61%) out of 69, had a primary school education. Those with tertiary school education were 33, with 9 (29.03%) returning positive results of *S. aureus* infections. Diabetic patients who attended the clinic during the duration of this study and had no school experience or had a secondary school education returned 3 (0.97%) positive cases each, from a total of 18 and 36, respectively. Tuvei (2017) conducted an education level analysis from their data and found that those who possessed primary level education recorded the highest prevalence rate at 51.1% just like in the current study. Aedh (2016) also reported similar findings. Reduced socioeconomic status is frequently associated with Lower education levels, which can result in more exposure to cramped living arrangements, unsanitary environments, and restricted access to medical treatment. A low level of schooling may also be linked to poor dressing of their wounds, as most do not acquire sanitary techniques. Highly schooled individuals are exposed, hence making it easier to early detection and treat early, which lowers the risk of severe infections. (Early & Seifried, 2012). These factors can increase the risk of diabetes and/or *S. aureus* infections.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The study established a 19.87% prevalence of *S. aureus* from diabetic wounds of patients attending the diabetic clinic (Chandaria Cancer and Chronic Diseases Center) at the MTRH.

2. The results of the study showed that Cefoxitin was effective against most of the S. aureus isolates, followed by Erythromycin and Clindamycin, with lesser susceptibility to Ampicillin. The other antibiotics had at least one instance of intermediate sensitivity and/or antibiotic resistance by the isolates.

3. This study also shows a significant relation between sex and the occurrence of *S. aureus* in diabetic wounds of patients attending the diabetic clinic at MTRH. However, age, underlying conditions, previous hospitalization, previous antibiotic use, marital status, and level of education do not significantly predispose the wounds of diabetic patients attending the outpatient diabetic clinic at MTRH to *S. aureus*.

6.2 Recommendations

1. This study's relatively high prevalence of 19.87% recommends early surveillance and screening to detect any presence of MRSA in diabetic wounds to get first-hand knowledge about the identification to detect infections early so that healthcare providers can initiate preventive measures. Sensitization on proper wound dressing among these patients is also recommended to help reduce wound infections.

2. It is recommended that diabetic patients at MTRH take cefoxitin, erythromycin, and clindamycin because some isolates were resistant to certain antibiotics. Re-evaluation of treatment options, particularly the use of Ampicillin, should also be taken into consideration to prevent widespread antibiotic resistance.

3. The sex of a diabetic patient was identified as a possible risk factor in this study; hence, female diabetic patients should take more precautions to prevent any traumatic injury, which could lead to diabetic wound infections

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APPENDICES

Appendix I: Questionnaire

The reason behind these questions is to obtain feedback for study purposes only. Data collected will be kept from third parties. This is to aid in the analysis and evaluation of the prevalence and antibiotic profile pattern of *S. aureus* carriage. The information will go a long way in the Implementation of infection control practices. The questionnaire consists of two sections, A and B. Carefully and thoroughly go through them while completing them, and then review them to be certain you have not left anything out.

In case of any concern, please seek clarification from us. We take note of the unclear sections for you. Feel free to ask about any unclear part before, during, or after filling in the form.

As a participant, you are free to avoid queries that sound personal or otherwise, and you

You are free to stop your participation without any penalty.

Place a tick where appropriate and fill in any blank spaces

Section A: Demographic Data

1. Gender:	**2. Marital status**	**3. Age in years**	**4. Education**
[ ] Male	[ ] Married	[ ] 13-30	[ ] Tertiary Education
[ ] Female	[ ] Single	[ ] 31-44	[ ] Secondary School Education
	[ ] Other	[ ] 45-60	[ ] Primary School Only
		[ ] More than 60	[ ] No school

5. A family member has suffered from diabetes type i

[ ] Yes

[ ] No

Section B: Possible Risk Factors

1. Have you been hospitalized in the past 10 days?

[ ] Yes

[ ] NO

3. History of Antibiotic Use

[ ] Present

[ ] Absent

4. Family history of diabetes T2D

[ ] Present

[ ] Absent

5. Underlying co-morbidities

[ ] Present

[ ] Absent

Appendix II: Laboratory Request Form

Patient number….

GRAM STAIN				
Gram +ve Cocci in Clusters	Present		Absent	
CULTURE				
Blood Agar (BA)	Blood Agar (BA)		Mannitol Salt Agar (MSA)	
	Day 1		Day 2	
	Growth	No Growth	Yellow colonies	No Yellow colonies
CATALASE TEST				
	Positive		Negative	
COAGULASE TEST				
	Positive		Negative	
ORGANISM ISOLATED				
S. *aureus*	Yes		No	

SUSCEPTIBILITY TESTING			
Antibiotic	Susceptible	Intermediate	Resistant
Amoxicillin			
Ampicillin			
Cefoxitin			
Ciprofloxacin			
Clindamycin			
Erythromycin			
Tetracycline			
Trimethoprim			

Technologist: …………………….. Sign: ……………………..

Date: ………………..

Appendix III: Gram Stain Procedure

(Source: MTRH Microbiology Laboratory Standard Operating Procedures)

Part A: Slide Preparation:

i. Wash the slide with soap and water to remove any grease or oil, wipe it with spirit or alcohol, and dry the slide.

ii. Label the slide

iii. To prepare a smear for bacterial suspensions in broth, place a loop full of the broth culture on the slide using a sterile, cooled loop. Spread using a circular motion of the inoculating loop to about one centimeter in diameter. To prepare a smear from bacterial plate cultures, use a sterile, cooled loop to place a drop of sterile water or saline solution on the slide. Sterilize and cool the loop again, pick up a very small sample of a bacterial colony, and gently stir into the drop of water/saline on the slide to create an emulsion. For primary gram staining of Swab Samples, roll the swab over the cleaned surface of a glass slide.

iv. Heat fix the smear by allowing the smear to air dry. After the smear has air-dried, hold the slide at one end, and pass the entire slide through the flame of a Bunsen burner two to three times with the smear side up.

Part B: Gram Stain Procedure

i. Place the slide with a heat-fixed smear on the staining tray.

ii. Flood the smear gently using crystal violet and let stand for 1 minute.

iii. Hold the slide in a tilted position and rinse with tap water gently or distilled water using a wash bottle.

iv. Flood the smear gently using Gram's iodine, after which let it stand for 1 minute

v. Hold the slide slightly tilted position and rinse it with tap water or distilled water gently using a wash bottle. The purple circle of the smear will appear on the slide.

vi. Apply 95% ethyl alcohol or acetone to decolorize it.

vii. Rinse with water immediately

viii. Counter-stain by gently flooding it with safranin and let stand for 45 seconds.

ix. Using tap water or distilled water, gently rinse the slide slightly while in a Tilted position.

x. Use a bibulous paper to Blot dry the slide with.

xi. Observe the smear under a light microscope in an oil immersion.

Appendix IV: Biochemical test for identification of *S. aureus*

(Source: MTRH Microbiology Laboratory Standard Operating Procedures)

a. Catalase test

This is used to differentiate the bacteria that produce the enzyme catalase, such as *Staphylococci,* from non-catalase-producing bacteria, such as *Streptococci*.

Procedure:

i. 2-3ml of hydrogen peroxide solution is poured into a test tube

ii. Using a wooden stick or a glass rod, several colonies of the test organism are removed and immersed in the hydrogen peroxide solution

iii. Active bubbling indicates a positive catalase test.

b. Coagulase test

This test is used to identify *S. aureus,* which produces coagulase.

Tube test procedure (detects free coagulase):

i. Plasma is diluted in the ratio of 1:10.

ii. The labels "test organism," "positive control," and "negative control" are placed on three test tubes.

iii. Each tube is pipetted with 0–5 ml of the diluted plasma..

iv. Five drops (about 0.1ml) of the test organism are added into the labeled positive, 5 drops of the *S. aureus* culture to the tube labeled positive, and 5 drops of sterile broth to the tube labeled negative.

v. The tubes are incubated at 35-37 degrees Celsius after mixing gently. Clotting should occur within 1 hour; if not, the examination is repeated every 30 minutes for up to 6 hours.

vi. Clotting is indicative of *S. aureus*.

Appendix V: NACOSTI Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **168885**

Date of Issue: **13/April/2024**

RESEARCH LICENSE

This is to Certify that Mr.. DOMNIC Owuor OGUDA of University of Eldoret, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Uasin-Gishu on the topic: PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF Staphylococcus aureus FROM DIABETIC WOUND INFECTIONS FROM PATIENTS ATTENDING MOI TEACHING AND REFERAL HOSPITAL for the period ending : 13/April/2025.

License No: **NACOSTI/P/24/34462**

168885

Applicant Identification Number

Director General

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

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See overleaf for conditions

Appendix VI: IREC Formal Approval

MTRH/MU-INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
COLLEGE OF HEALTH SCIENCES
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

Reference: IREC/895/2024
Approval Number: 0004852

25th July, 2024

Owuor Domnic Oguda,
University of Eldoret,
School of Sciences,
P.O. Box 1125-30100,
ELDORET-KENYA.

Dear Mr. Oguda,

PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF STAPHYLOCOCCUS AUREUS FROM DIABETIC WOUND INFECTIONS AMONG PATIENTS ATTENDING MTRH

This is to inform you that ***MTRH/MU-IREC*** has reviewed and approved the above referenced research proposal. Your application approval number is ***FAN: 0004852.*** The approval period is **25th July, 2024 – 24th July, 2025.**
This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, Material Transfer Agreements (MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***MTRH/MU-IREC***.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***MTRH/MU-IREC*** within 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***MTRH/MU-IREC*** within 72 hours.
v. Clearance for export of biological specimens must be obtained from ***MOH at the recommendation of NACOSTI*** for each batch of shipment.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to ***MTRH/ MU-IREC.***

Prior to commencing your study; you will be required to obtain a research license from the National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and other relevant clearances from study sites including a written approval from the CEO-MTRH which is mandatory for studies to be undertaken within the jurisdiction of Moi Teaching & Referral Hospital (MTRH) and its satellites sites.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

INSTITUTIONAL RESEARCH ETHICS COMMITTEE
25 JUL 2024
APPROVED
P. O. Box 4606 - 30100 ELDORET

cc	CEO	-	MTRH	Dean	-	SOP	Dean	-	SOM
	Principal	-	CHS	Dean	-	SON	Dean	-	SOD

Appendix VII: Consent Form

I am Mr. Owuor. The title of my Research Study**: Prevalence and antibiotic susceptibility pattern of *Staphylococcus aureus* from diabetic wounds in patients attending MTRH**

PART II: Certificate of Consent

Study population:

The category has been chosen since most diabetic wounds tend to take a long time to heal and often may result in diabetic foot ulcers if not long sufferings. Hence, the need to isolate the *S. aureus* from their wounds for the study

Usage of the collected sample swabs

The collected swab samples will be used to isolate *S. aureus*, from which, after identification, the isolates will be subjected to an antibiotic susceptibility test, where they are classified as sensitive, intermediate, and resistant. These results will then be correlated to demographic factors, diabetic risk factors, as well as individual underlying conditions, from which a conclusion will be made.

The study methodology will involve administering a questionnaire upon recruitment into the study, which will then be followed by the collection of swabs from the diabetic wounds. The swab aspirates will then be taken to the lab for culturing and Isolation of pure cultures of *S. aureus*. The pure cultures of *S. aureus* will then be confirmed by doing biochemical tests such as the Coagulase and Catalase tests.

The study is expected to run for six months, but for participants, it will take one day, inclusive of administering a questionnaire and collecting samples from the wounds.

If you agree, you will do the following:

You will respond to questions in the questionnaire

Avail yourself for a sample collection from the diabetic wound

Benefits:

From the result, we will be able to diagnose SA infection and recommend medication.

Risks/Discomforts:

During swabbing, there may be some little discomfort.

Payments and Reimbursements:

It is logical to anticipate receiving compensation from this research; however, there is no guarantee that your child will benefit directly from taking part in this study. Information gathered from this study may prove useful to others in the future.

I have read/ received a clear explanation about the ongoing research. I am aware that the information I will provide will be confidential. I hereby willingly decide to take part in the research.

Name of Participant:

Signature of Participant: Thumbprint

Date:

Day/month/year

Appendix VIII: Similarity Report

University of Eldoret

University of Eldoret

Certificate of Plagiarism Check for Thesis

Author Name	Owuor Domnic Oguda SSCI/BIO/M/002/22
Course of Study	Type here...
Name of Guide	Type here...
Department	Type here...
Acceptable Maximum Limit	Type here
Submitted By	titustoo@uoeld.ac.ke
Paper Title	ANTIBIOTIC SUSCEPTIBILITY AND PREVALENCE OF Staphylococcus aureus IN DIABETIC WOUNDS AMONG PATIENTS AT MOI TEACHING AND REFFERAL HOSPITAL, KENYA
Similarity	11%
Paper ID	4595159
Total Pages	103
Submission Date	2025-10-30 10:15:30

Signature of Student

Signature of Guide

Head of the Department

University Librarian

Director of Post Graduate Studies

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