PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF *Staphylococcus aureus* ISOLATED FROM DIABETES MELLITUS PATIENTS WITH FOOT ULCERS AT VIHIGA COUNTY REFERRAL HOSPITAL, KENYA

BY

TUVEI SUSAN MAMUSAVU

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MASENO UNIVERSITY

DECLARATION

Declaration by the Candidate

I hereby declare that this thesis is my original work and has not been presented for the award of a degree in any other university or institution of learning. I have done all the work carried herein and all sources of information have been acknowledged by means of references.

Susan Mamusavu Tuvei **Signature** **Date**

(MSC/PH/00155/2014)

Declaration by the Supervisors
This thesis has been submitted for examination with our approval as University Supervisors.

Prof. Bernard Omondi Abong'o **Signature:**  **Date: 24th July 2017**
National University of Lesotho
Faculty of Science and Technology

Dr. Bernard Guyah **Signature** **Date**
School of Public Health and Community Development
Maseno University

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DEDICATION

To my beloved family, husband Evans Ovamba and my two children Keith and Patience for their support and patience hoping that they will gain from my acquired skills.

ABSTRACT

Staphylococcus aureus is a predominant pathogen isolated from diabetic foot ulcers. In recent years, complications-related to diabetic foot infections has increased due to increased incidence of antibiotic-resistant bacteria. Management of these infections requires appropriate antibiotic selection on the basis of culture and susceptibility test results. Although diabetes is among the top ten causes of mortality in Vihiga, most public hospitals in Kenya do not isolate and conduct antimicrobial susceptibility tests for diabetic foot ulcers. This makes the prevalence and antimicrobial susceptibility of *S. aureus* infection uncertain. This study investigated prevalence and antimicrobial susceptibility of *S. aureus* isolated from diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya. Specifically the study determined prevalence of *S. aureus* infection and susceptibility of *S. aureus* isolated from Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya to penicillin G, ceftriaxone, gentamicin, vancomycin and linezolid. The study adopted a hospital based cross- sectional study design with a population of 225 participants. A sample size of 156 participants was recruited using simple random sampling technique. A questionnaire was administered for bio-data collection while a laboratory form was used to gather antimicrobial susceptibility information. Pus swabs were collected for *S. aureus* screening. Laboratory tests involved Gram staining, inoculation on Blood agar plates at 37˚C for 24 - 48 hours, coagulase test and Kirby- Bauer disk diffusion on Mueller Hinton Agar. Percentages were used to present prevalence and antimicrobial susceptibility. Results indicated 60.3% prevalence of *S. aureus*. There was 100% resistance to penicillin G, a susceptibility rate of 35.1 % to ceftriaxone, 36.2% to vancomycin, 38.3% to gentamicin and 60% to Linezolid. The study concluded that diabetes mellitus patients with foot ulcers are prone to *S. aureus* infection. *S.aureus* isolated from diabetic patients with foot ulcers at Vihiga County referral hospital was resistant to penicillin G and linezolid was the most effective first line antibiotic. In view of this, the study informs health practitioners the importance of *S.aureus* screening for appropriate antibiotic prescription to reduce infection rate among diabetes mellitus patients and recommends adoption of diabetic foot ulcers screening to ensure that bacterial infections are detected and appropriate medication prescribed to patients in good time.

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ABBREVIATIONS AND ACRONYMS

BA	Blood Agar
CNS	Central Nervous System
CONs	Coagulase Negative Staphylococcus
COPs	Coagulase Positive Staphylococcus
MHA	Mueller Hinton Agar
OPD	Out Patients Department
S. aureus	*Staphylococcus aureus*
SGS	School of Graduate Studies

OPERATIONAL DEFINITIONS

Education: Classroom based formal training attained by diabetic patients with foot ulcers at any level from primary to college

Diabetes: A disease caused by metabolic disorder by immunological deficiency in pancreas leading to insufficient insulin production or when the body becomes insulin resistant or it does not respond to the insulin produced

Foot ulcer: A wound below the ankle in a person with diabetes, irrespective of duration.

Isolation: Infection by *S.aureus*

Patient: An individual suffering from diabetes and has foot ulcer

Prevalence: The frequency of *S. aureus* isolation or infection among Diabetes mellitus patients with foot ulcers for the two and half months study period.

Staphylococcus aureus: A Gram-positive bacterium that can live as a commensal organism on the skin and in the nose and throat

Susceptible: *S. aureus* is inhibited by the usually achievable concentrations of the antimicrobial agent when the dosage recommended to treat the site of infection is used.

Resistance: *S. aureus* not inhibited by the usually achievable concentration of the agent with normal dosage.

Antibiotics: Penicillin G (Penicillin), gentamicin (aminoglycoside) and ceftriaxone (cephalosporin) are the antibiotics used in treating Diabetes foot ulcers at Vihiga County Referral Hospital while Vancomycin and linezolid are the first line antibiotics for treating *S.aureus*

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CHAPTER 1:INTRODUCTION

1.1 Background Information

It has been widely thought that diabetic patients are prone to infections due to hyperglycemia induced immunodeficiency (Moazezi *et al*., 2014). Although there are different types of diabetes, diabetes mellitus has become among the biggest health problem in many countries, especially in the low- and middle-income countries which have a major impact on the quality of life for hundreds of millions of people and their families, overwhelms the capacity of many national healthcare systems, and adversely impacts the economy of countries that are in most need of development (Ahmed *et al*., 2014). Diabetes mellitus is a chronic medical condition comprising of three main types; type I, type II and gestational diabetes (Ebere, Kimani, & Imungi, 2017). Type II is the most common form of diabetes mellitus accounting for 85% of diabetes mellitus cases worldwide, 90% of cases in Sub-Saharan Africa and 95% of diabetes mellitus cases in adults (Mathangi & Prabhakaran, 2013; Ebere *et al*., 2017).

The study focused on type II diabetes which according to Steyn *et al*. (2014) is a non-insulin-dependent diabetes that accounts for most cases of diabetes mellitus worldwide. It is estimated that in 2000 there were approximately 150 million individuals with the disease and that the numbers are estimated to reach 552 million by 2030 (Yerat & Rangasamy, 2015). Type II diabetes is the fourth leading cause of death after Human Immunodeficiency Virus, Tuberculosis and malaria in most developing countries and it has reached epidemic proportions (Steyn *et al*., 2014). Changes in the prevalence of type II Diabetes Mellitus being observed in communities is attributed to genetic and non genetic factors associated with family history of diabetes and

behavioural factors including unhealthy diet, obesity and lack of physical activity (Ebere et al., 2017 ; Steyn *et al*., 2014). These factors are strongly influenced by the socio-demographic status of the individuals which include age, gender, marital status and education level (Rao *et al*., 2010; Ebere *et al*., 2017; Veghari, et al., 2010).

The risk of Type II Diabetes Mellitus increases with age as a result of weight gain, reduced physical activity, ageing pancreas and insulin resistance by cells (Donato *et al*., 2003; Tanaka & Seals, 2008; Booth *et al*., 2011). Marital status has also been linked with increased risk of Type II Diabetes Mellitus especially among the widowed possibly due to poor lifestyle (Ebere *et al*.,2017). Type II Diabetes Mellitus has been found to be inversely associated with level of education such that the higher the education level, the lower the prevalence rate possibly due to observation of healthy diet and increased physical exercise (Lessmann *et al*., 2012; Veghari, et al., 2010). Overweight especially abdominal obesity in females has been associated with increased risk of Type II Diabetes Mellitus mainly because of associated insulin resistance (Hussain *et al*., 2010).

Lots of complications are associated with Type II Diabetes Mellitus, these complications arise from the disruption of the vascular system which can result in inadequate circulation to the peripheral body placing the foot at higher risk of ulceration and infection (Deribe, Woldemichael, & Nemera, 2014). One of the major Type II Diabetes Mellitus complications is the development of diabetic foot ulcer mainly related to trauma, neuropathy and deformity (Yusuf, et al., 2016). According to Iversen (2009), people with diabetes are more likely to develop foot ulcers than people without diabetes. These factors together can lead to abnormal pressure on the skin, bones, and joints of the foot during walking and can lead to breakdown of

the skin of the foot. Sores may develop. Damage to blood vessels and impairment of the immune system from diabetes makes it difficult for wounds to heal. Bacterial infection of the skin, connective tissues, muscles, and bones can then occur. About 15% of Type II Diabetes Mellitus develop foot ulcers during their lifetime, and this constitutes the most common cause of disability and hospitalization given the greater risk of being infected with *Staphylococcus aureus* (Yerat & Rangasamy, 2015). Diabetes mellitus has been associated with a susbstantially increased risk of *S. aureus* bacteremia. *S.aureus* infection worsens the wound condition, delays the healing mechanism and, if appropriate measures are not taken in time, could lead to systemic infection, septicaemia, amputation or even death (Tiwari *et al*., 2012). *S. aureus* is both a commensal bacterium and a major human pathogen with an ability to cause wide range of clinical diseases across all ages (Tong *et al*., 2015). It is always necessary to evaluate different microorganisms infecting the wound on a routine basis in addition to administering regular glycemic control, wound care, surgical debridement, pressure offloading, and maintaining adequate blood supply (Apelqvist *et al*., 2008).

Diabetic foot infections are often polymicrobial where *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas* species, *Staphylococcus aureus, Streptococcus pyogens, Citrobacter koseri, Klebsiella Pneumoniae, Providencia* species, *Citrobacter freundii*, *Serratia* species and *Enterococcus* species are the most frequent pathogens that contribute to progressive and widespread tissue destruction (Dwedar *et al*., 2015; Hena and Growther, 2010; Mahgoub and Omer, 2015). In addition to these isolates, Wang *et al*. (2010) also isolated, *Acinetobacter* species*, Klebsiella* species*, S. haemolyticus, S.* epidermidis, *Enterococcus* species and *Streptococci.* Other limb-threatening diabetic ulcer infections according to Yerat and

Rangasamy (2015) included *Bacteroides fragilis*, *Peptococcus* and *Peptostreptococcus*. Although various bacterial species have been implicated in wound infections, *S. aureus* remains the most predominant pathogen in diabetic foot ulcers (Oh and Tan, 2013). Hena and Growther (2010) established *S. aureus* prevalence rate of 42.3%, Wang *et al*. (2010) a prevalence rate of 63% and Mahgoub and Omer (2015) a prevalence rate of 46%.

It has been estimated that approximately 20–30% of the general population are *S. aureus* carriers (Rutare, 2013; plata *et al*., 2009). *S. aureus* infection increases the risk of mortality, morbidity, medical care costs and loss of productivity (Rutare, 2013). The increased medical care costs accrue directly as expenses caused by extension of hospital stay, additional diagnostic or therapeutic procedures, and additional antibiotic use while loss of productivity is due to absence from work during hospitalization (Rutare, 2013). *Staphylococcus aureus* (*S. aureus)* is a Gram-positive bacterium that can live as a commensal organism on the skin and in the nose and throat (Ryu *et al*., 2014). *S. aureus* is a 1 μm, Gram-positive cell in the laboratory may be observed as single cells, in pairs or as grape-like irregular clusters and it is characterized as coagulase- and catalase positive, non-motile, non-spore-forming and as facultative anaerobe (Stark, 2013).

An estimated 1.2 million Kenyans live with diabetes (3.3 % prevalence) and by 2025 the number is expected to rise to 1.5 million (4.5% prevalence) (El-busaidy *et al.,* 2014). In urban and rural Kenya the prevalence of diabetes mellitus is rising and has been estimated at 12% and 16% respectively (Dalal *et al*., 2011; El-busaidy *et al*., 2014). According to County Government of Vihiga (2013) diabetes is among the top ten causes of morbidity and mortality due to increased alcohol intake, smoking, changes in dietary factors with a shift from traditional foods to

westernized diet of fast foods, hypertension, and change in life style factors where patients have reduced physical activity. About three Diabetes mellitus patients with foot ulcers visit Vihiga County referral hospital per day for treatment and dressing of their wounds. However, the hospital does not conduct isolation of *S. aureus* in diabetic patients with foot ulcers regularly therefore its presence could be increasing thereby seriously deteriorating patients' quality of life. This makes the prevalence of *S. aureus* infection in Vihiga County uncertain. This study therefore sought to bridge *S. aureus* prevalence knowledge gap by determining prevalence of *S. aureus* infection in diabetic patients with foot ulcers at Vihiga County Referral Hospital, Vihiga County, Kenya.

S. aureus infection requires careful management because of its ability to acquire antibiotic resistance (Almeida *et al*., 2014). The discovery of antibiotics in the early 20th century fundamentally transformed human medicine; however, the rise of antibiotic-resistant bacterial strains represents a serious threat to public health (CDC, 2013). In recent years, there has been an increasing need for antimicrobial agents active against resistant Gram-positive bacteria (Moellering, 2003). *S. aureus* being the most important Gram-positive bacteria has become resistant to most of the therapeutic agents that have been developed in the recent years (Murugan *et al*., 2008).

Scientists continue to disagree on the most effective antibiotic for the treatment of *S.aureus,* whereas Ako-Nai *et al*. (2005) advocates for pencillin, Rajaduraipandi *et al*. (2006) advocates for aminoglycoside. Vihiga County referral hospital uses penicillin G (penicillin), ceftriaxone (cephalosporin) and gentamicin (aminoglycoside) to treat diabetic wound infections. However, the hospital does not conduct antimicrobial susceptibility testing for Diabetes mellitus patients with foot ulcers which makes antimicrobial susceptibility of *S. aureus* in Vihiga County

uncertain. This study therefore examined susceptibility of *S. aureus* to antibiotics used in the treatment of diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya for the purposes of generating knowledge on the susceptibility of *S.aureus* isolated from diabetic foot ulcers.

Due to the increasing complexity of treating *S.aureus* infections, knowledge of the real-world clinical impact of newer agents is needed for informed decision making (Caffrey *et al*., 2010). Vancomycin, a glycopeptide and linezolid an oxazolidinone being the first line antibiotic agents have become the standard therapy to which other antibiotics are compared (Watkins *et al*., 2012). Vancomycin though established as a treatment of choice for *S. aureus* infection, emergence of *S. aureus* strains with reduced vancomycin susceptibilities led to the discovery of linezolid (Kishore *et al*., 2014). Linezolid discovered in 1990s was approved in 2000 as the first line antibiotic for treatment of Gram positive infections including *S.aureus* (Ehsan *et al*., 2014). The clinical benefit of vancomycin and linezolid is threatened by the emergence of resistant strains of *S.aureus* (Swoboda *et al*., 2005).

Scientific studies on the susceptibility of *Staphylococcus aureus* to vancomycin and linezolid have divergent views given that some scientists established *S.aureus* to be more susceptible to vancomycin while others established *S.aureus* to be more susceptible to linezolid. Whereas Kishore *et al*. (2014) argued they have similar *in vitro* susceptibility, Wunderink *et al*. (2012) concluded that the treatment of *S.aureus* was significantly higher with linezolid than with vancomycin. This makes it impossible to single out the most effective antibiotic between vancomycin and linezolid in treatment of *S.aureus* isolated from diabetic foot ulcers raising the

need to establish susceptibility of *S.aureus* isolated from diabetic patients with foot ulcer at Vihiga County Referral Hospital, Kenya to vancomycin and linezolid.

1.2 Statement of the Problem

Over 50% of all hospital admissions and 55% of hospital deaths in Kenya are attributed to non-communicable diseases with diabetes being among the leading condition and this is because people with diabetes are prone to infections due to hyperglycemia induced immunodeficiency. This puts diabetic patients with foot ulcers at a greater risk of being infected with *S. aureus* which needs adequate management through proper diagnosis and conducting of antimicrobial susceptibility tests given that *S. aureus* has the ability to acquire antibiotic resistance. Although diabetes is among the top ten causes of morbidity and mortality and about three diabetic patients with foot ulcers visit Vihiga County referral hospital per day for treatment and dressing of their wounds, the hospital does not regularly isolate *S. aureus* and conduct its antimicrobial susceptibility test for these diabetic patients with foot ulcers, therefore *S. aureus* presence and its resistance to antibiotics could be increasing thereby seriously deteriorating patients' quality of life. Further, most of the available studies on bacterial infections and antimicrobial susceptibility of *S. aureus* lack consensus on the antibiotic to which *S. aureus* is more susceptible and most studies used specimens which were not specific to diabetic foot ulcers since the focus was on hospital personnel and other non diabetic patients. This makes the prevalence and antimicrobial susceptibility of *S. aureus* infection in Vihiga County uncertain. This study therefore investigated the prevalence and antimicrobial susceptibility of *S. aureus* isolated from diabetic patients with foot ulcers at Vihiga County Referral hospital, Kenya.

1.3 Justification of the study

In spite of the introduction of antimicrobial agents and improvements in the frequency and morbidity of staphylococcal diseases in the twentieth century, staphylococci have persisted as an important hospital and community pathogen (Farzana and Hameed, 2006). Infections by Staphylococcus are common in developing countries with variations in antimicrobial susceptibility making it difficult to determine the effective antibiotic for treatment (Almeida *et al*., 2014). The genus Staphylococcus includes pathogenic organisms in which *S. aureus* is the most important that has become resistant to most therapeutic agents that have been developed in the recent years threatening their clinical benefit (Murugan *et al*., 2008). Given that the prevalence and antimicrobial susceptibility of *S. aureus* is uncertain, this study investigated the prevalence and antimicrobial susceptibility of *S. aureus* isolated from diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya. The study findings inform health practitioners and other stakeholders on the prevalence of *S. aureus* in Vihiga County and most effective antibiotics for its treatment. This knowledge creates awareness on the importance of *S.aureus* screening for appropriate antibiotic prescription to reduce infection rate among diabetic patients. This will have some life-saving potential and enable the county to come up with appropriate measures in relation to treatment of diabetic patients with foot ulcers.

1.4 Objectives of the Study

1.4.1 General Objective

To investigate the prevalence and antimicrobial susceptibility of *S. aureus* isolated from Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya.

1.4.2 Specific Objectives

The specific objectives were to;

1. Determine prevalence of *S. aureus* infection in Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya.

2. Determine the susceptibility of *S. aureus* isolated from Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya to penicillin G, gentamicin, ceftriaxone, vancomycin and linezolid.

1.5 Research Hypothesis

1. H_0 : There is no prevalence of *S. aureus* infection among Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya

   H_1 : There is prevalence of *S. aureus* infection among Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya

2. H_0 : *S.aureus* isolated from Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya is not susceptible to penicillin G, gentamicin, ceftriaxone, vancomycin and linezolid.

   H_1 : *S.aureus* isolated from Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya is susceptible to penicillin G, gentamicin, ceftriaxone, vancomycin and linezolid.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The chapter captures an overview on diabetes, foot ulcers, information on the bacterial infections among patients with diabetes and antimicrobial susceptibility of *S. aureus* to antibiotics used in treatment of diabetic patients with foot ulcers.

2.2 Diabetes Prevalence

Diabetes is a chronic illness requiring continual health care and patient self-management education to prevent the development of acute complications and reduce the risk of long-term complications (Iversen, 2009). Diabetes and its complications impose a large economic burden on diabetic individuals, their families and social health system (Ning, 2013). Diabetes is a disease caused by metabolic disorder and classified in two basic forms Type I and Type II diabetes (Mathangi *et al*., 2013). Type I is caused by immunological deficiency in pancreas leading to insufficient insulin production and Type II occurs when the body becomes insulin resistant or it does not respond to the insulin produced and it accounts for 95% of diabetes in adults (Mathangi *et al*., 2013).

Over the past 30 years, the status of diabetes has changed from being considered as a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting the youth and middle aged people with the rise in its prevalence in the world (Mohan, 2007). In 2011, an estimated 366 million adults aged 20-79 years had diabetes; this number is predicted to rise to 552 million by 2030 (Ning, 2013). Sub-Saharan Africa, like the rest of the world, is experiencing an increasing prevalence of diabetes alongside other non-communicable diseases. In 2010, over

12 million people were estimated to be living with diabetes in Africa, and this is projected to increase to 23.9 million by 2030 (Hall *et al*., 2011).

According to the Ministry of Public Health of Kenya as reported by El-busaidy *et al*. (2014), over 50% of all hospital admissions and 55% of hospital deaths in Kenya are attributed to non – communicable diseases with diabetes being among the leading condition. The prevalence of diabetes in Kenya was estimated to be about 3.3% in 2007 (Maina *et al*., 2010). Data from the Ministry of Health as reported by McFerran (2008) in Kenya indicated that, of all humans living in Africa, an estimated 1.2 million Kenyans live with diabetes, and if the trend continues, by 2025 that number is expected to rise to 1.5 million (4.5% of the population). The incidence of diabetes is increasing in Kenya, however, its awareness is still demanding in many rural parts of the country. Furthermore, majority of the rural population in Kenya is highly ignorant of the common etiologies/risk factors for diabetes which include foot ulcers and infections (El-busaidy *et al*., 2014). Vihiga County being one of the rural parts of Kenya, diabetes is among the top ten causes of morbidity and mortality for the population above five years old (County Government of Vihiga, 2013).

2.3 Diabetic Foot Ulcers and Diabetes

As explained by Iversen (2009), a diabetic foot ulcer as a wound below the ankle in a person with diabetes, irrespective of duration as depicted in Figure 2.1. Foot lesions and foot ulcers are the most commonly observed impediments with diabetic patients (Mathangi *et al*., 2013). The diabetic foot has been considered the major complication of diabetes care, and the International Diabetes Federation (IDF) dedicated the year 2005 to foot care of people with diabetes in order

to raise awareness of foot disease among people with diabetes (Iversen, 2009). Pervasiveness of these ulcers in diabetic patients is as high as 25% and requires constant monitoring and patients have to visit a hospital frequently which is exorbitant (Mathangi *et al*., 2013). Iversen (2009) asserted that infection can complicate any type of diabetic foot ulcer and is one of the most common causes of hospital admission among people with diabetes.

Figure 2.1. Foot ulcer as a result of diabetes complication

2.4 Prevalence of Bacterial Infections

Almeida *et al*. (2014) investigated the prevalence of wound colonization by *Staphylococcus* especially *S. aureus,* in hospitalized diabetic patients, and to identify the factors associated with such colonization. They used a cross-sectional study that enrolled 125 patients with wounds who were hospitalized in a remote and underdeveloped inland region of northeastern Brazil with extreme poverty. The results indicated that twenty-five wounds (20%) were colonized by *S. aureus.* Although the researchers established colonization of wounds by *S. aureus* their study

focused on hospitalized patients which necessitates a study on overall prevalence based on both hospitalized and outpatient.

Thomsen (2004) established that a total of 225 (17.1 %) diabetics had bacterium. The bacterium among diabetes was greatest in adults under 65 years and the risk appeared to be higher in diabetic females than in males. Although the researchers established bacterium infection in diabetics, their study failed to establish *S. aureus* infection which necessitates the need of a study to establish *S. aureus* prevalence in a rural village of Vihiga County.

Simkhada (2013) designed a descriptive, cross sectional study to know the prevalence of culture positive infection in diabetic patients at Tribhuvan University Teaching Hospital in Nepal. Among 100 patients included, 53 were female and 47 were male. In total, 21% of them had culture positive infection. Infection was more in female (P=0.047) and asymptomatic bacterium was found more common in female as compared to male. Although the researchers established bacterium infection in diabetics, their study generalized bacterium infection and was not specific to *S. aureus* infection.

Peter and Chin- Hong (2006) reviewed the spectrum of infectious complications in patients with diabetes in the United States. They established that the most common infections in patients with diabetes are foot infections. These patients are particularly prone to foot infections because peripheral neuropathy is present in about 50% of patients with long-standing diabetes because of foot ulcers. Although the researchers established most common infections in diabetics, their

study was generalized and fell short of being specific to *S. aureus* infection to establish the prevalence rate.

Rashid *et al.* (2012) determined the prevalence of nasal *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* in hospital personnel and associated factors in Pakistan. A total of 129 nasal swabs and epidemiological information concerning risk factors for nasal carriage were obtained from physicians, nurses, sanitary workers and administrative staff. Antibiotic susceptibility testing was performed using disk diffusion method. The prevalence of *S. aureus* was higher in physicians (51.8%), nurses (66.6%) and sanitary workers (59%) as compared to administrative staff (27.6%). The prevalence was higher in females (53%) as compared to males (43%). Based on the age, it was higher in those aged over 50 years (60%), 30 – 40 years (52%) and less than 30 years (44%). Similarly, Gebremedhn *et al.* (2016) conducted a cross sectional study from September 2014 to February 2015 in three selected health centers and one general hospital in Ethiopia using 249 HIV patients. Out of 249 study participants, *S. aureus* was isolated from 81 (32.5 %) patients. Individuals in the age group of 70–79 years were more colonized by *S. aureus, S. aureus* colonization rate was higher among females 62 (35.6 %) as compared to males 19 (25.3 %) attributed to rare visits of patients to health facilities. The studies though attempting to analyze prevalence based on socio demographics, the focus was on hospital personnel and HIV patients necessitating a study on diabetic patients with foot ulcers.

Aedh (2016) assessed the prevalence of *S. aureus* colonization besides determining antimicrobial susceptibility. A total of 158 hospitalized intensive care unit patients were recruited to participate

in this study. *S. aureus* colonization was more common among males than females (7.08% vs. 5.19%). Based on age the prevalence was noted for ages of 0-15 (5.6%), 16-30 (6.5%), 31-45 (11.5%), > 45 (8.2%). It was also noted that business people had a prevalence rate of 11.1%, farmer (21.9%), retirees (7.8%), others (10.4%). Married participants had the highest prevalence rate as compared to single and widowed participants. Those with no formal education had the highest prevalence at 10.7%, primary level (12.2%), secondary (9.8%) and university (3.1%). Focus on hospitalized patients who were not diabetic patients with foot ulcers justifies a study on the group to establish the prevalence of *S. aureus* infection.

Oguzkaya-Artan *et al.* (2015) determined prevalence of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) nasal carriage among emergency department workers, and bacterial contamination on hand-touch surfaces at Erciyes University Hospital, Kayseri, Turkey. They enrolled 105 workers and 190 hand-touch surfaces. The *S. aureus* carriage rate was 18.1% (n=19). A higher prevalence rate of 23.3% was noted for those aged over 30 years compared to 16% for those less than 30 years; males had a higher prevalence at 27.3% as compared to females at 11.5%. Based on profession, physicians had a highest rate at 23.8% as compared to others at 18.4%. Focus on workers rather than patients motivate a study based of diabetic foot ulcer patients to establish the prevalence rate among the group.

Rutare (2013) determined the prevalence of *Staphylococcus aureus* among pediatric patients admitted in neonatal intensive care unit of Kenyatta National Hospital using a cross sectional descriptive study design. Results indicated that children aged more than five years had the highest level of *S. aureus* isolation rate 13(34.2%), while the age group 2-5 years had the lowest rates 2(12.5%). The rate of isolation in males was 22.2% compared to 21.6% in females. The

focus on pediatric patients necessitates a study on diabetic patients with foot ulcers for the generation of knowledge on the prevalence rate of *S. aureus* among the group.

Based on the reviews done, it is evident that various studies on bacterial infections among diabetic patients with foot ulcers and *S. aureus* infection have been conducted. However, most of the studies did not establish prevalence of *S. aureus* infection among diabetic patients with foot ulcers since the focus was on hospital personnel and other non diabetic patients making the prevalence among the group uncertain. This study sought to bridge the knowledge gap of uncertainty by determining overall and socio-demographic based prevalence of *S. aureus* infection in Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Vihiga County, Kenya.

2.5 Susceptibility of S. aureus to Pencillins, Cephalosporins, Aminoglycosides, Vancomycin and Linezolid

In Vihiga County Referral Hospital penicillin (penicillin G), cephalosporin (ceftriaxone) and aminoglycoside (gentamicin) are the antibiotics used to treat diabetic patients with foot ulcers. Ako-Nai *et al*. (2005) in determining the treatment and antibiotic resistant profile of *S. aureus* in Nigeria used penicillin and tetracycline. The study established penicillin as more effective than tetracycline where out of the 178 staphylococcal isolates evaluated, 68% of *S. aureus* isolates were resistant to penicillin and 71% to tetracycline. Although their analysis established that *S. aureus* was more susceptible to penicillin than tetracycline, they did not compare with ceftriaxone and gentamicin which are also used to treat Diabetes mellitus patients with foot

ulcers at Vihiga County Referral Hospital. Further, the susceptibility rate was not correlated with socio – demographic factors such as age, gender, occupation and education level creating uncertainty regarding the relationship.

Rajaduraipandi *et al*. (2006) reported the prevalence and treatment of *S. aureus* for a total of 7172 clinical specimens using penicillin, aminoglycoside, cephalosporins and erythromycin. Out of 906 strains of *S. aureus* isolated from clinical specimens, the results indicated that aminoglycoside, cephalosporins, erythromycin was more effective in treatment followed with penicillin recording 99.6% resistance. This study focused on clinical specimens which were not specific to diabetic patients with foot ulcers and did not consider comparing antimicrobial susceptibility with socio demographic factors.

Mir and Srikanth (2013) conducted a retrospective study from June 2011 to November 2012 in a tertiary care hospital in south India to find out the prevalence rate of *S. aureus* and antibiotic susceptibility pattern. A total of 210 Staphylococcus strains were isolated from various clinical samples, 180 were coagulase positive Staphylococcus (CoPS) and 30 were coagulase negative Staphylococcus (CoNS). Among 180 CoPS, 58 (32.22%) were penicillin resistant. Although they concluded that there is need for continuous monitoring of the antimicrobial susceptibility pattern of *S. aureus* for the selection of appropriate therapy, they did not compare with ceftriaxone and gentamicin which are also used to treat Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital hence this study conducted antimicrobial susceptibility of *S. aureus* on ceftriaxone and gentamicin in comparison to the socio demographic factors.

Farzana and Hameed (2006) conducted a study at Pakistan Institute of Medical Sciences, Islamabad, Pakistan during a period of two years to establish the sensitivity pattern of Gram-positive cocci isolated from the samples brought to Pathology Laboratory against commonly used antibiotics using disc diffusion method, at the hospital's laboratory. Samples comprised of blood, pus and urine, from Out Patient Department (OPD) as well as indoor patients from different wards of the hospital. Out of 5069, 1688 were *S. aureus.* Out of these Gram-positive cocci 56% were resistant to penicillin group, 73% were susceptible to cephalosporin group, 78% were susceptible to aminoglycoside group, 15% were resistant to quinolone group and 31% were resistant to other antibiotics. However, their analysis of the susceptibility rate was not specific to specimens from Diabetes mellitus patients with foot ulcers and failed to consider susceptibility based on socio demographic factors making the susceptibility pattern uncertain justifying a study on diabetic foot ulcers.

Vancomycin and linezolid are the first line drugs used in management of resistant strains of *S. aureus.* Wunderink *et al*. (2012) assessed efficacy and safety of linezolid, compared with a dose-optimized vancomycin regimen, for treatment of *S. aureus* involving 1184 patients. The results revealed 57.6% susceptibility to linezolid as compared to 46.6% susceptibility to vancomycin. Although they concluded that the susceptibility of *S. aureus* in the population was significantly higher with linezolid than with vancomycin, their study participants were not specific to diabetic patients with foot ulcers which makes the susceptibility of *S. aureus* isolated from Diabetes mellitus patients with foot ulcers to vancomycin and linezolid uncertain.

Olivares *et al*. (2011) studied the activity of vancomycin, ciprofloxacin, daptomycin and linezolid in methicillin-resistant strains, isolated from blood cultures using 87 specimens. They

established that fifty-three (53%) strains showed loss of susceptibility to vancomycin and ten strains (11.5%) resistant to linezolid were observed. Although they concluded low susceptibility to vancomycin was frequent, their study specimens were not specific to diabetic patients with foot ulcers which make the susceptibility of *S.aureus* isolated from diabetic patients with foot ulcers to vancomycin and linezolid uncertain.

Kishore *et al*. (2014) compared in vitro activities of linezolid and vancomycin against *S. aureus* in order to help in formulating a better treatment. They isolated 200 strains of *S. aureus* from different clinical specimens. Antibiotic sensitivity testing was performed against linezolid and vancomycin for all 200 strains. Similarly, Rutare (2013) determined the prevalence and antimicrobial susceptibility of *Staphylococcus aureus* among paediatric patients admitted in neonatal intensive care unit of Kenyatta National Hospital using a cross sectional descriptive study design. Results indicated that the susceptibility of *S.aureus* to both vancomycin and linezolid was the highest at 98.6% followed by amikacin at 94.4%. ampicillin showed the highest resistance (94.4%), followed by erythromycin (47.9%), gentamycin at 38%, amoxicillin-clavulanic acid (38%), cefotaxime (38%) and none of the tested antibiotics had 100% susceptibility. Although they concluded that linezolid and vancomycin had similar in-vitro susceptibility for *S. aureus* treatment, their study specimens were not specific to pus swab from diabetic patients with foot ulcers and antibiotic sensitivity was not compared with socio demographics of patients which make the susceptibility of *S. aureus* isolated from Diabetes mellitus patients with foot ulcers to vancomycin and linezolid uncertain.

Ehsan *et al*. (2014) determined the efficacy of vancomycin and linezolid to Staphylococci using a total of 2989 specimens of blood, pus and wound swab. Results showed antimicrobial

resistance of these isolates were amoxicillin (74.8%), amoxicillin+clavulanate (32.8%), ciprofloxacin (35.2%), ofloxacin (33.6%), ceftriaxone (30.4%), erythromycin (58.3%), clindamycin (16.3%), kanamycin (52.2%) fusidic acid (41.7%), doxycycline (24.7%), vancomycin (2.6%) and linezolid (0.8%) respectively. They concluded that linezolid was more superior to vancomycin. This study's specimens were not specific to pus swab from diabetic patients with foot ulcers which makes the susceptibility of *S.aureus* isolated from Diabetes mellitus patients with foot ulcers to vancomycin and linezolid uncertain.

Al-Zoubi *et al*. (2015) assessed the antibiotic susceptibility pattern of *Staphylococcus aureus* isolated from clinical specimens in local hospitals of Northern Province in Jordan from various clinical specimens of different body sites from 358 patients. Results indicated that 31% of isolates were multidrug resistance with most of these strains having been isolated from wound specimens. All isolates were susceptible to vancomycin (100%). They were also susceptible to chloramphenicol, linezolid, nitrofurantoin, rifampicin and teicoplanin (>80%), but showed resistance to erythromycin and penicillin. Similarly, Okon *et al*. (2013) investigating the epidemiology and antibiotic susceptibility pattern of *Staphylococcus aureus* recovered from tertiary hospitals in Northeastern, Nigeria established that, out of the 96 *S.aureus* isolates the overall antibiotic resistance pattern was noted as; penicillin (91.5%), gentamicin (13.6%), erythromycin (15.3%), cotrimoxazole (18.6%), ciprofloxacin (22.0 %), and vancomycin (0%). Although the studies established vancomycin as the most effective antibiotic, comparison of socio demographics and antimicrobial susceptibility was not considered with study specimens being not specific to diabetic foot ulcers hence a justification for a study on antimicrobial susceptibility of *S. aureus* to bridge the uncertainty gap.

Heffernan *et al*. (2015) in undertaking a survey on basic demographic data on patients with *S. aureus* infections to provide information on antimicrobial resistance among *S. aureus* in New Zealand using 1185 *S. aureus* isolates established that resistance to fusidic acid, erythromycin and ciprofloxacin was common at 57.6%, 25.3% and 16.1% respectively. It was also noted that there was no resistance to vancomycin. *S. aureus* resistance to antibiotics was more prevalent among isolates from patients in the youngest age group (<5 year) and more prevalent among isolates from healthcare facility patients than isolates from community patients. Although age was determined as the key socio demographic factor influencing antimicrobial resistance of *S. aureus*, the study was not specific to isolates from diabetic foot ulcers which create uncertainty on the antimicrobial susceptibility rate among the group a justification for the study.

Shahina, Chowdhury and Arifuzzaman (2014) ascertained the current situation of growing antibiotic resistance against bacteria, ex-gram positive cluster forming cocci where 271 isolates were tested for antimicrobial susceptibility testing. Results indicated that antimicrobial resistance was higher in male than female and higher in the age group 21-40 years (38.37%), 0 -20 years (28.98%), 41-60 (27.30%) and above 60 years (10.33%). Observation of susceptibility test indicated that ampicillin, amoxycillin and co-trimoxazole showed increased rate of resistance which were 34, 26 and 19% respectively. On the other hand, positive cluster cocci were susceptible to cephalosporins (cephalexin, cefradine, cefixime and doxycycline at 7, 6, 3 and 2% respectively). Failure to involve vancomycin and linezolid indicated lack of knowledge on *S. aureus* susceptibility rate in relation to the socio demographics justifying a study that includes the first line antibiotics of vancomycin and linezolid.

Aboud *et al*. (2015) investigated the prevalence and antibiotic sensitivity of *S. aureus* infection in children using 212 clinical samples of pus and skin swabs collected from patients enrolled from the out-patient department of Khartoum dermatology and venereal teaching hospital over a period of 6 months. Antibiotics sensitivity test was carried out using Kirby-Bauer Disk Diffusion Technique in accordance with Clinical and Laboratory Standard Institute. Sex differences in the prevalence and antimicrobial sensitivity of *S. aureus* were not significant whilst age differences were significant. The most effective antimicrobial agent was gentamicin (97.3%) efficacy, then fusidic acid (78%) efficacy, erythromycin (50%), amoxy-clav (21.3%) and bacitracin (12.7%). Use of specimens not isolated from diabetic foot ulcers creates uncertainity of antimicrobial susceptibility among the group a justification for a study using isolates from diabetic foot ulcers.

Abdalla *et al*. (2012) assessed the factors affecting antimicrobial sensitivity in *Staphylococcus aureus* clinical isolates from Asser region, Saudi Arabia involving 81 patients. Erythromycin in *Staphylococcus aureus* positive cases (50) showed 10% resistant for age (0-15 years), 32% in age group (16-50 years) and 24% in age group with over 50 years. Similarly, Naghavi-Behzad *et al*. (2015) by investigating *Staphylococcus aureus* resistance pattern and risk factors involving 475 patients who were confined to bed in the surgery ward noted that vancomycin with a 0.4% resistance rate was the least resistant with linezolid showing a resistance rate of 2.9%. Age of patients and hospitalization duration among the demographics showed statistically significant relation with antimicrobial resistance. Although the studies established the key socio demographic factors influencing antimicrobial resistance of *S. aureus*, the studies were not

specific to isolates from diabetic foot ulcers which create uncertainty on the antimicrobial susceptibility rate among the group a justification for the study.

Scientists have tested the susceptibility of *S. aureus* to pencillins, cephalosporins, aminoglycosides, vancomycin and linezolid used in the treatment of Gram-positive bacteria. However, it is evident that they lack consensus on the antibiotic to which *S. aureus* is more susceptible to. It was also noted that most studies used specimens which were not specific to diabetic foot ulcers and failed to correlate the relationship between socio demographics and antimicrobial susceptibility rate. This study therefore examined susceptibility of *S. aureus* to pencillins (penicillin G), cephalosporins (ceftriaxone) and aminoglycosides (gentamicin) to generate knowledge on the uncertain susceptibility rate of *S. aureus* isolated from diabetic foot ulcers at Vihiga County Referral Hospital, Kenya.

2.6 Summary of the Knowledge Gaps

Studies on bacterial infections among diabetic patients with foot ulcers, antimicrobial susceptibility of *S. aureus* have been conducted world over. However, most of the studies did not establish prevalence of *S. aureus* infection in diabetic patients with foot ulcers, lacked consensus on the antibiotic to which *S. aureus* is more susceptible to and most studies used specimens which were not specific to diabetic foot ulcers since the focus was on hospital personnel and other non diabetic patients. This makes the prevalence and antimicrobial susceptibility of *S.aureus* uncertain. This study therefore investigated the prevalence and antimicrobial susceptibility of *S. aureus* isolated from Diabetes mellitus patients with foot ulcers at Vihiga County Referral hospital, Kenya.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study Area

The study was conducted at the Vihiga County Referral Hospital, Vihiga County, Kenya. According to County Government of Vihiga (2013), diabetes is among the top ten causes of morbidity and mortality for the population above five years old. About 3 diabetic patients with foot ulcers visit the hospital on a daily basis. Vihiga County whose headquarters are in Mbale is located in the Western Region of Kenya. Vihiga County lies between longitudes 34º41' and 35º0' East and between latitudes 0º and 0º02' North (County Government of Vihiga, 2013). The county covers a total area of 531.0 KM^2 and it boarders Nandi to the East, Kisumu County to the South, Siaya County to the West and Kakamega County to the North and has five Constituencies; Luanda, Emuhaya, Hamisi, Sabatia and Vihiga. The County's population stands at 612,000 (County Government of Vihiga, 2013). See Figure 3.1.

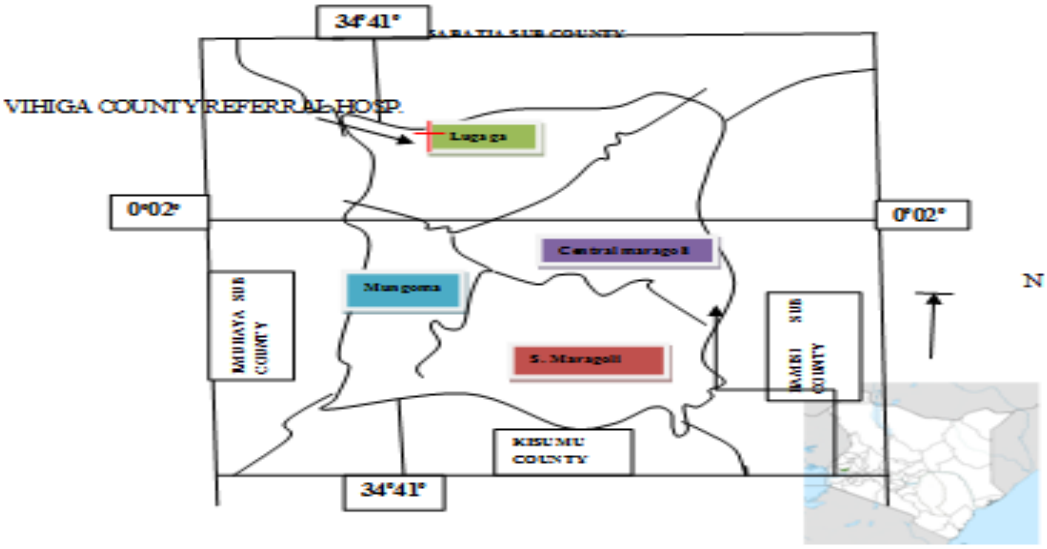

Figure 3.1: Map of Vihiga County (County Government of Vihiga, 2013)

3.2 Study Design

This study employed a hospital based cross- sectional study design of Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Vihiga County, Kenya. The study determined the prevalence rate of *S. aureus* and antimicrobial susceptibility using frequency distribution tables.

3.3 Study Population

The target population comprised of 225 adult (aged 18 years and above) diabetic patients with foot ulcers who attended Vihiga County Referral Hospital for treatment for the two and half month study period from 6th February 2016 to 20th April 2016.

3.4 Inclusion and Exclusion Criteria

3.4.1 Inclusion Criteria

Consenting Diabetes mellitus patients with foot ulcers above 18 years.

3.4.2 Exclusion criteria

Diabetes mellitus patients with foot ulcers who had dressed their wounds using antiseptics.

3.5 Sample Size and Sampling Technique

The sample size for the study was determined following Fisher *et al*. (1998) formula as utilized by Mugenda and Mugenda (2003) as shown below. According to Mugenda and Mugenda (2003), Fisher *et al.* (1998) provides a simplified formula for computing sample size.

$$n = \frac{z^2 p(1-p)}{d^2}$$

Where

n = sample size of the target population which is greater than 10,000;

$z = 1.96$ Value of the standard normal distribution curve corresponding to 95% level of significance

$p = 0.5$, Prevalence of *S. aureus* infection among diabetics with foot ulcers at Vihiga County Referral, Vihiga County, Kenya. Fisher *et al*. (1998) as captured by Mugenda and Mugenda (2003) stated that where the data on the proportion of respondents with characteristic being investigated is not available, $p = 0.5(50\%)$ is regarded as appropriate.

d = Margin of error which is 5% (0.05)

Therefore, at 95% confidence interval assuming a target population of more than 10,000, the desired sample size is

$$n = \frac{(1.96)^2(0.5)(0.5)}{(0.05)^2}$$
$$= 384$$

Mugenda and Mugenda (2003) further showed that, if the target population is less than 10,000, the appropriate formula is

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

Where

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

n = desired sample size for target population of more than 10,000; and

N = estimate of population size in the current study,

Hence the sample size in this study was,

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

To ensure equitable representation of the population in the sample, this study employed simple random sampling. Participants were assigned unique numbers which were place in a bowl and thoroughly mixed then blind folded assistant researcher selected the numbers. The participants corresponding to the numbers picked were included in the sample. The sample size was adjusted by adding 10% of the calculated sample size to take care of sampling error. Thus the corrected sample size was 156.

3.6 Data Collection Methods

Diabetic patients with foot ulcers who consented to participate in the study were subjected to an interview using a structured questionnaire (Appendix 3). The questionnaire had questions on socio-demographic characteristics (age, gender, education level and marital status), diabetes diagnosis and antibiotics medication history. Laboratory request form was used to collect data on prevalence and antimicrobial susceptibility (Appendix 4). Secondary data on antibiotics used in relation to socio-demographic factors of Diabetes mellitus patients was obtained from patients' files in the diabetic clinic at Vihiga County Referral Hospital, Kenya.

3.7 Validity and Reliability Tests

For the purpose of determining the reliability and validity of the questionnaire, test-retesting was carried out with 5 identical respondents, sampled within the study area. The test – retest method of assessing reliability of data involves administering the same instrument twice to the same group of subjects with a time lapse between the first and second test (Mugenda and Mugenda, 2003). Respondents selected for pre-testing had been admitted at the hospital for one week and did not participate in the actual study. Score obtained by each respondent on the first and the second test were correlated by use of Cronbach's alpha to assess reliability of the questionnaire. After applying the Cronbach's Coefficient Alpha test, the study obtained a 0.654 for the first test and 0.671 Cronbach's alpha coefficient for the second test after small improvement on the tool which is approximately equal to 0.7 as shown in Table 3.1. According to Maina (2015) , scales in the questionnaire of 0.7 and above indicate satisfactory reliability.

Table 3.1: Instrument Reliability Test Results

Cronbach's Alpha	N of Items
0.671	18

The study employed construct validity technique where the questionnaire was reviewed by two experts in the field who made amendments with regard to the content of the questionnaire.

3.8 Laboratory Techniques

Pus specimens from foot ulcers of diabetic patients were swabbed aseptically for *S. aureus* screening. The wound was cleaned with sterile saline and the swab moistened with sterile saline water before swabbing the wound. Using a 'zig-zag' motion the whole wound surface was

swabbed. The specimens were aseptically handled and processed. Gram staining was done to determine the organism present. Subsequently, the specimens were inoculated on to Blood Agar (BA) plates incubated at 37˚C for 24 - 48 hours. Isolated colonies were further tested for the production of free coagulase enzyme using the tube coagulase test, based on conventional microbiological methods. *Staphylococcus aureus* ATCC 25923, a known coagulase producer was included as a control strain. All the confirmed *S. aureus* strains were subsequently tested for antimicrobial susceptibility based on the Kirby-Bauer disk diffusion method using antimicrobial discs (penicillin G, ceftriaxone, gentamicin, vancomycin and linezolid) on Mueller Hinton Agar (MHA).

3.8.1 Culture

Blood agar media was prepared by weighing appropriate 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^{o}C for 15 minutes in an autoclave. The blood agar was cooled at 55^{o}C after autoclaving, and then 5% sheep blood was added and mixed. Petri dishes were laid on a flat surface and approximately 20 mls of blood agar was dispensed into the Petri dishes, left to solidify, labeled and stored at 2-8^{o}C in a refrigerator.

A smear of pus and positive control were made at the periphery of blood agar plate, and then streak of parallel lines made along the edge of the plates using disposable plastic loops, after inoculation the plates were placed upside down in aerobic incubator for 24-48 hours at 37◦C. Plates were checked after 24 hours then after 48 hours for growth of round golden yellow colonies with beta haemolysis.

3.8.2 Gram staining

A clean glass slide was labeled and a smear was made by putting a drop of normal saline on the slide and mixed with a small amount of the colony and left to air dry for 5 minutes at room temperature, heat fixed the smear by rapidly passing through tip of the blue portion of the bunsen flame 3-4 times and left to cool. The heat fixed smear was stained with crystal violet for 1 minute, rinsed with running tap water, stained with Grams iodine for 1 minute, rinsed with running tap water, decolorised with acetone for 30 seconds rinsed with running tap water then counterstained with safranin for 2 minutes, rinsed with running tap water, left to dry then examined under $100\times mg$ oil immersion (Gram stains were procured already prepared from Sure check supplies).

3.8.3 Coagulase Tube Method

Citrated rabbit plasma (0.3 milliliters) was put into two test tubes and labeled as control and sample. Tube labeled as sample was inoculated with a colony of Gram positive cocci and mixed by shaking for 3 minutes to make a cloudy suspension, control tube was inoculated with a positive control of *S. aureus* and both tubes were incubated at 37°C for 1-4 hours in an incubator. A positive coagulase test was represented by clotting.

3.8.4 Antimicrobial Susceptibility Testing

3.8.4.1 Inoculum preparation

The sample was again cultured on nutrient agar plate and a sterile straight wire was used to transfer 3-5 isolated colonies to 5ml of sterile saline and mixed, its turbidity was adjusted using 0.5 McFarland turbidity standards.

3.8.4.2 Inoculation on agar plate

Within 15 minutes of inoculum preparation, a sterile cotton swab was dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess volume of the inoculum. Entire surface of Mueller Hinton agar was swabbed to ensure even distribution, without re-immersing the swab in the suspension. Inoculated Muller Hinton (MH) plate was allowed to stand for 3 minutes to dry. Antibiotic discs (penicillin G (10 μg); gentamicin (10 μg) and ceftriaxone (30 μg) were applied using sterile forceps with gentle pressure to ensure complete contact of disc with MH agar. Plates were incubated within 15 minutes of application of discs as diffusion of antibiotics and bacterial growth commences at the same time, plates were incubated at 37°C for 18 - 24 hours. Diameter of zones of inhibition was measured using a transparent plastic ruler. Any growth around the disc was considered resistant, measured zones of inhibition were compared with that of Clinical and Laboratory Standards Institute (2015) values as shown in appendix 6.

3.9 Data Analysis

Descriptive statistics involving frequency distribution tables and percentages were used to present prevalence and antimicrobial susceptibility of *S. aureus* infection. The study further conducted correlation analysis to ascertain significant relationship between susceptibility of *S. aureus* socio-demographic factors.

3.10 Ethical Considerations

Approval for the study was obtained from Maseno University Board of School of Graduate Studies (SGS). Ethical approval for this study was sought from Maseno University Ethical Review Committee and further permission sought from Vihiga County Referral Hospital management through the Chief Officer Ministry of Health-Vihiga County (Appendix 1). Written informed consent (Appendix 3) was obtained from each participant before enrollment. Participation in the study was voluntary and participants were not penalized for withdrawing from the study. Confidentiality was maintained during the study by assigning code numbers to the participants and information obtained from the participants was not disclosed deliberately or accidentally in ways that might identify the participant. Biological samples and data obtained from the participants were kept in secure cabinets with access to the research data being limited to the investigator and information obtained only used for academic purposes. Free education on diabetes was also provided to the participants with assurance on the confidentiality of their responses given and study results.

CHAPTER 4: RESULTS

4.1 Socio-Demographic Characteristics of the Study Participants

The study involved examining diabetic patients based on their socio-demographic characteristics which included; age, gender, marital status and education level. Table 4.1 results indicated the age bracket of diabetic patients with foot ulcers as; 17(10.9%) within 18-30 years; 34(21.8%) within 31-44; 37(23.7%) within 45-60 and 68 (43.6%) over 60 years. Gender based analysis indicated that; 80(51.3%) were males and 76(48.7%) were females. The study also established that 8(5.1%) had no education at all, 94(60.3%) had up to primary education, 41(26.3%) had up to secondary education and 13(8.3%) up to college education. Marital status analysis showed that 133(85.3%) were married, 15(9.6%) were widowed and 8(5.1%) were divorced.

Table 4.1: Socio-demographic characteristics of the study participants

Socio-demographics	Frequency (n)	Percentage (%)
Age group in years		
18-30	17	10.9 %
31-44	34	21.8 %
45-60	37	23.7 %
Over 60	68	43.6 %
Total (N=156)	**156**	
Gender		
Male	80	51.3 %
Female	76	48.7 %
Total (N=156)	**156**	
Marital status		
Single	0	0.0 %
Married	133	85.3 %
Widowed	15	9.6 %
Divorced/Separated	08	5.1 %
Total (N=156)	**156**	
Education		
None	08	5.1 %
Primary	94	60.3 %
Secondary	41	26.3 %
College	13	8.3 %
Total (N=156)	**156**	

Note*. N represents total sampled Diabetes mellitus patients with foot ulcers, n Diabetes mellitus patients with foot ulcers per socio- demographic category. % obtained by* $\frac{n}{N} \times 100\%$

4.2 Prevalence of *S. aureus* infection among Diabetes Mellitus Patients with Foot Ulcers at Vihiga County Referral Hospital, Kenya.

Following cultures of pus swab of 156 specimens by inoculation onto Blood Agar (BA) plates and incubated at 37^0C for 24 – 48 hours a total of 118(75.6%) of the pus swabs had growth as shown in Figure 4.1 while 38 (24.4%) pus swab specimens did not have any growth as shown in Figure 4.2. Gram staining done on selected colonies of the 118 plates with growths and

examined microscopically revealed that 102 pus swabs specimens had Gram positive cocci in grape like clusters as shown in Figure 4.3 while 16 were not Gram positive as shown in Figure 4.4. Coagulase test was conducted on Gram positive cocci isolated from the 102 pus swab plates to confirm for *S. aureus.* It emerged that 94 of the pus swab Gram positive cocci were coagulase positive while 9 were coagulase negative. This implied that the prevalence of *S. aureus* was 94 (60.3%) among Diabetes mellitus patients with foot ulcers seeking treatment at Vihiga County Referral Hospital, Kenya. Based on the socio demographic factors as shown in Table 4.2, the study established that *S.aureus* infection was most prevalent among; those aged over 60 years (63.8 %), females (57.4%), those married (84.0%) and those with primary level education (51.1%).

Table 4.2: Prevalence of *S. aureus* in Diabetes mellitus patients with foot ulcers attending Vihiga County Referral Hospital, Kenya.

Characteristic	**Positive for *S.aureus* n = 94**	**Negative for *S.aureus* n = 9**	**No Bacteria n = 53**	**Total N = 156**
Age group in years				
18-30	0 (0.0%)	1 (11.1%)	16(30.2%)	17(10.9%)
31-44	13 (13.8%)	0 (0.0%)	21(39.6%)	34(21.8%)
45-60	21 (22.3%)	0 (0.0%)	16(30.2%)	37(23.7%)
Over 60	60 (63.8%)	8 (88.9%)	0(0.0%)	68(43.6%)
Gender				
Male	40 (42.6%)	9 (100%)	31 (58.5%)	80 (51.3%)
Female	54 (57.4%)	0 (0.0%)	22 (41.5%)	76 (48.7%)
Marital Status				
Single	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Married	79 (84.0%)	9 (100%)	45 (84.9%)	133 (85.3%)
Widowed	15 (16.0%)	0 (0.0%)	0 (0.0%)	15 (9.6%)
Divorced/ Separated	0 (0.0%)	0 (0.0%)	8 (15.1%)	8 (5.1%)
Education Level				
None	8 (8.5%)	0 (0.0%)	0 (0.0%)	8 (5.1%)
Primary	48 (51.1%)	9 (100%)	37 (69.8%)	94 (60.3%)
Secondary	32 (34.0%)	0 (0.0%)	9 (17.0%)	41 (26.3%)
College/ University	6 (6.4%)	0 (0.0%)	7 (13.2%)	13 (8.3%)
Total (N = 156)	**94 (60.3%)**	**9 (5.8%)**	**53 (34.0%)**	

***Note**. N and n refers to total sampled Diabetes mellitus patients with foot ulcers and Diabetes mellitus patients with foot ulcers per bacterial infection respectively, value outside bracket is number of Diabetes mellitus patients with foot ulcers while value in bracket is the prevalence rate per socio-demographic category. Overall prevalence (%) given by* $\frac{n}{N} \times 100\%$ *and prevalence by socio-demographic given by* $\frac{patients}{n} \times 100\%$

Figure 4.1. *S. aureus* bacterial growth in Blood Agar

Figure 4.2. Blood Agar without any *S. aureus* growth

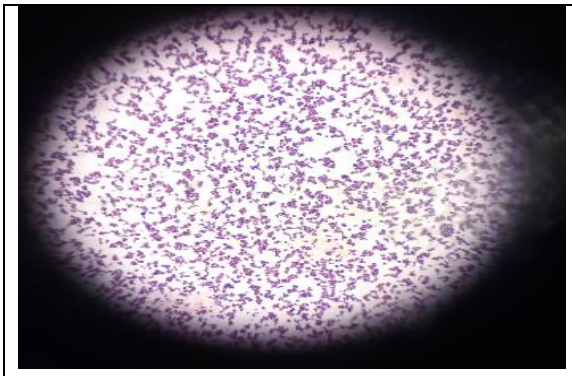

Figure 4.3. *S. aureus* Gram positive cocci in cluster

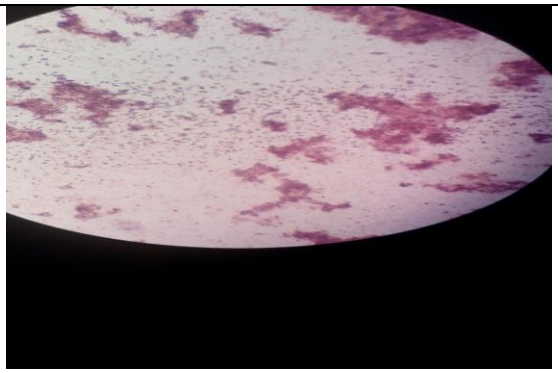

Figure 4.4. Gram negative rods

4.3 Antibiotics used in treatment of Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya.

The study by review of patient's files established that penicillin (penicillin G), cephalosporin (ceftriaxone) and aminoglycoside (gentamicin) were used in the treatment of diabetic foot ulcers. Table 4.3 results indicated that a total of 132 diabetic patients with foot ulcers were exposed to antibiotic treatment where; 112(71.8%) were on penicillin G, 8(5.1%) were on ceftriaxone and 12(7.7%) were on gentamicin. Based on the socio demographic factors, the study established that diabetic patients with foot ulcers in the age bracket of over 60 years (42.9%), 18 - 30 years (87.5%) and 45-60 years (41.7%) were highly exposed to penicillin (penicillin G), cephalosporin (ceftriazone) and aminoglycoside (gentamicin) respectively. Males were highly exposed to penicillin G (59.8%) and ceftriaxone (75%) with similar exposure being reported in both males and females for gentamicin (50%). Married patients were highly exposed to penicillin G (98.2%) and gentamicin (75%) while divorced/separated patients were highly exposed to ceftriaxone (87.5%). It was also noted that those who had attained primary level education were highly exposed to pencillin G (66.1%) and ceftriazone (62.5%) while those with college or university education were highly exposed to gentamicin (50%).

Table 4.3: Type of antibiotic used in the treatment of Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya

Characteristic	Penicillin G n=112	Ceftriaxone n=8	Gentamicin n=12	None n=24	Total N=156
Age group in years					
18-30	17 (15.2%)	7 (87.5%)	0(0.0%)	0(0.0%)	24 (15.4%)
31-44	26 (23.2%)	0 (0.0%)	1(8.3%)	0(0.0%)	27 (17.3%)
45-60	21 (18.8%)	0 (0.0%)	6(50.0%)	9(37.5%)	36 (23.1%)
Over 60	48 (42.9%)	1 (12.5%)	5(41.7%)	15(62.5%)	69 (44.2%)
Gender					
Male	67 (59.8%)	6 (75.0%)	6 (50.0%)	24 (100%)	103 (66.0%)
Female	45 (40.2%)	2 (25.0%)	6 (50.0%)	0 (0.0%)	53 (34.0%)
Marital Status					
Single	-	-	-	-	-
Married	110 (98.2%)	1 (12.5%)	9 (75.0%)	8 (33.3%)	128 (82.1%)
Widowed	2 (1.8%)	0 (0.0%)	2 (16.7%)	16 (66.7%)	20 (12.8%)
Divorced/ Separated	0 (0.0%)	7 (87.5%)	1(8.3%)	0 (0.0%)	8 (5.1%)
Education Level					
None	7 (6.3%)	1 (12.5%)	0 (0.0%)	2 (8.3%)	10 (6.4%)
Primary	74 (66.1%)	5 (62.5%)	1 (8.3%)	14 (58.3%)	94 (60.3%)
Secondary	30 (26.8%)	0 (0.0%)	5 (41.7%)	1 (4.2%)	36 (23.1%)
College/ University	1 (0.8%)	2 (25.0%)	6 (50.0%)	7 (29.2%)	16 (10.2%)
Total (N = 156)	**112 (71.8%)**	**8 (5.1%)**	**12 (7.7%)**	**24 (15.4%)**	

***Note**. Antibiotics Diabetic Patients were exposed to. Sourced, From "Vihiga County Referral Hospital Diabetic Clinic Patient's files". N and n refers to total sampled Diabetes mellitus patients with foot ulcers and Diabetes mellitus patients with foot ulcers exposed to specific antibiotic respectively, value outside bracket is number of patients per socio-demographic category while value in bracket is the frequency (%) of antibiotic usage. Overall frequency (%) given by* $\frac{n}{N}\times 100\%$ *and frequency by socio-demographic given by* $\frac{patients}{n}\times 100\%$

4.4 Susceptibility of *S. aureus* isolated from Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya to penicillin G, gentamicin, ceftriaxone, vancomycin and linezolid

To dertermine susceptibility of *S. aureus* isolated from Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya to pencillin G (pencillin), gentamicin (cephalosporins), ceftiaxoze (aminoglycosides), vancomycin and linezolid, all confirmed *S. aureus* strains were tested for antimicrobial susceptibility based on the Kirby-Bauer disk diffusion method using antimicrobial discs of Penicillin G (10 µg), Gentamycin (10 µg), Ceftriaxone (30 µg), vancomycin (30 µg) and linezolid (30µg) on Mueller Hinton Agar (MHA) as shown in Figure 4.5 and zone of inhibition diameter measured in mm in comparison to Clinical and Laboratory Standards Institute (2015) for Interpretation of Zones of inhibition (diameter in mm) in Appendix 6.

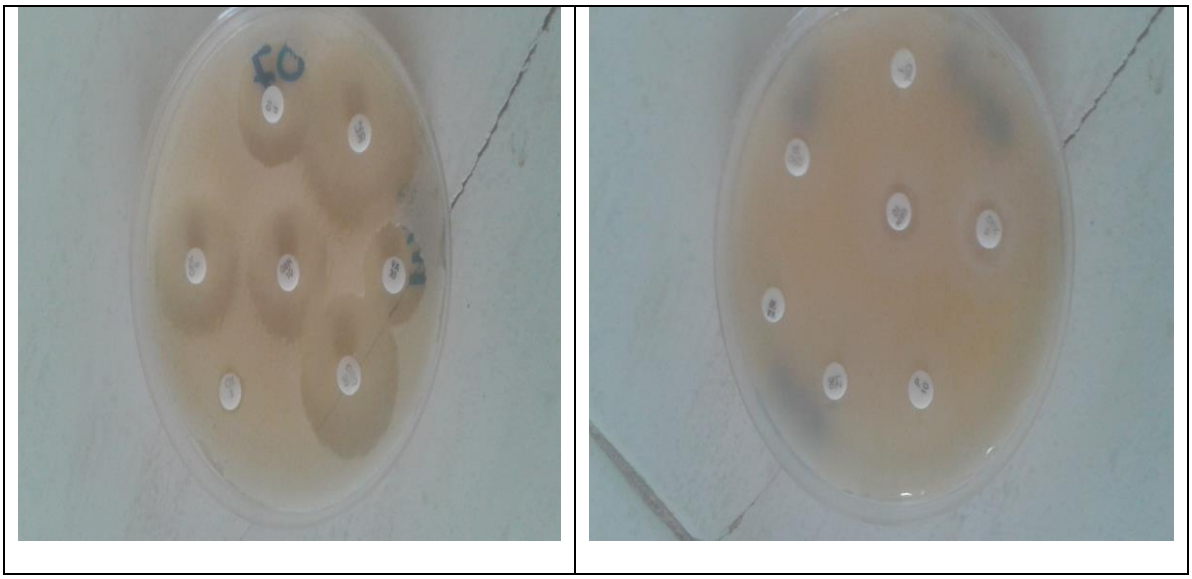

Figure 4.5: Antibiotic Discs on Muller Hinton Agar after Growth Showing Susceptibility test Result.

Table 4.4 test results on the susceptibility of *S. aureus* isolated from Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya to penicillin G, gentamicin, ceftriaxone, vancomycin and linezolid showed that, *S. aureus* isolated from the 94 (100%) Diabetes mellitus patients were resistant to penicillin G, 33 (35.1%) were susceptible to Ceftriaxone, 36 (38.3%) were susceptible to Gentamicin , 34 (36.2 %) were susceptible to vancomycin while 56 (59.6%) were susceptible to linezolid in comparison to Clinical and Laboratory Standards Institute (2015) for Interpretation of Zones of inhibition (diameter in mm) in Appendix 6.

Based on the socio demographic factors, *S.aureus* isolates from Diabetes mellitus patients with foot ulcers in the age bracket of over 60 years were most resistant to pencillin G (63.8%) and highly susceptible to ceftriazone (16.0%), gentamicin (18.1%), vancomycin (22.3%) and linezolid (39.4%). Isolates from Males were highly susceptible to gentamicin (20.2%), vancomycin (19.1%) and linezolid (35.1%) while isolates from females were highly resistant to penicillin G (57.4%) and more susceptible to ceftriaxone (22.3%). Isolates from married patients were highly resistant to penicillin G (85.1%) and highly susceptible to ceftriazone (27.7%), gentamicin (38.3%), vancomycin (27.7%) and linezolid (43.6%). It was also noted that those who had attained primary level education were highly resistant to pencillin G (56.4%) and highly susceptible to ceftriazone (19.1%) and linezolid (30.9%) while those who had attained secondary education were highly susceptible to gentamicin (20.0%) and vancomycin (19.1%).

Table 4.4: Susceptibility of *S. aureus* to pencillin G, ceftriaxone, gentamicin, vancomycin and linezolid

Characteristic	**Penicillin G n=94**		**Ceftriaxone n=94**		**Gentamicin n=94**		**Vancomycin n=94**		**Linezolid n=94**	
Disc Concentration	**10µg**		**30µg**		**10µg**		**30µg**		**30µg**	
	S	R	S	R	S	R	S	R	S	R
Age										
18-30	-	-	-	-	-	-	-	-	-	-
31-44	0 (0.0%)	12 (12.8%)	5(5.3%)	7 (7.4%)	12(12.8%)	0(0.0%)	12(12.8%)	0(0.0%)	12(12.8%)	0 (0.0%)
45-60	0(0.0%)	22 (23.4%)	13(13.8%)	9 (9.6%)	7(7.4%)	15(16.0%)	1(1.1%)	21(22.3%)	7(7.4%)	15 (16.0%)
Over 60	0(0.0%)	60 (63.8%)	15(16.0%)	45 (47.9%)	17(18.1%)	43(45.7%)	21(22.3%)	39(41.5%)	37(39.4%)	23 (24.5%)
Gender										
Male	0(0.0%)	40 (42.6%)	12(12.8%)	27 (28.7%)	19(20.2%)	21 (22.3%)	18(19.1%)	22 (23.4%)	33(35.1%)	7 (7.4%)
Female	0(0.0%)	54 (57.4%)	21(22.3%)	34 (36.2%)	17(18.1%)	37 (39.4%)	16(17.0%)	38 (40.4%)	23(24.5%)	31 (33.0%)
Marital Status										
Single	-	-	-	-	-	-	-	-	-	-
Married	0(0.0%)	80 (85.1%)	26(27.7%)	53 (56.4%)	36(38.3%)	43 (45.7%)	26(27.7%)	53 (56.4%)	41(43.6%)	37 (39.4%)
Widowed	0(0.0%)	14 (14.9%)	7(7.4%)	8 (8.5%)	0(0.0%)	15 (16.0%)	8(8.5%)	7 (7.4%)	15(16.0%)	1 (1.1%)
Divorced/ Separated	-	-	-	-	-	-	-	-	-	-
Education										
None	0(0.0%)	7 (7.4%)	0(0.0%)	7 (7.4%)	0(0.0%)	7 (7.4%)	0(0.0%)	7 (7.4%)	0(0.0%)	7 (7.4%)
Primary	0(0.0%)	53 (56.4%)	18(19.1%)	35 (37.2%)	17(18.1%)	36 (38.3%)	16(17.0%)	37 (39.4%)	29(30.9%)	24 (25.5%)
Secondary	0(0.0%)	31 (33.0%)	12(12.8%)	19 (20.2%)	19(20.0%)	13 (13.8%)	18(19.1%)	13 (13.8%)	23(24.5%)	7 (7.4%)
College	0(0.0%)	3 (3.2%)	3(3.2%)	0(0.0%)	0(0.0%)	2 (2.1%)	0(0.0%)	3 (3.2%)	4(4.3%)	0(0.0%)
Total (n = 94)	**0(0.0%)**	**94 (100%)**	**33 (35.1%)**	**61 (64.9%)**	**36 (38.3)**	**58 (61.7%)**	**34 (36.2)**	**60 (63.8%)**	**56 (59.6%)**	**38 (40.4%)**

Note. *n refers to total diabetes mellitus patients with foot ulcers infected with S.aureus, R is resistance, S is susceptibility . Value outside bracket is number of Diabetes mellitus patients with foot ulcers and infected with S.aureus based on socio demographics while value in bracket is the antibiotic susceptibility or resistance rate. Susceptibility or resistance rate (%) given by* $\frac{patients}{n} \times 100\%$

The study further conducted correlation analysis to ascertain significant association between susceptibility of *S. aureus* isolated from Diabetes mellitus patients with foot ulcers at Vihiga County referral hospital, Kenya to pencillin G, ceftriaxone, gentamcin, vancomycin and linezolid with socio-demographic factors (age, gender, marital status and education level). Table 4.5 results indicated that there was a significant negative association between age and susceptibility of *S. aureus* to antibiotics while the other socio-demographic factors of gender, marital status and education level had no significant association with susceptibility of *S. aureus* to antibiotics. This implied susceptibility of *S. aureus* to antibiotics decreased with increase in the age of the patient as indicated by correlation coefficients of ($r = -0.59$) with a p-value of 0.026 for penicillin G, ($r = -0.59$) with a p-value of 0.000 for ceftriaxone, ($r = -0.63$) with a p-value of 0.034 for gentamicin, ($r = -0.60$) with a p-value of 0.000 for vancomycin and ($r = -0.55$) with a p-value of 0.000 for linezolid.

Table 4.5: Correlation r (p-values) test results for socio-demographics with Penicillin G, Ceftriaxone, Gentamicin, Vancomycin and Linezolid

Socio-demographic	Penicillin G	Ceftriaxone	Gentamicin	Vancomycin	Linezolid
Age	-0.59(0.026)*	-0.59(0.000)*	-0.63(0.034)*	-0.60(0.000)*	-0.55(0.000)*
Gender	-0.083(0.808)	0.111(0.744)	0.043(0.900)	0.043(0.900)	-0.07(0.837)
Marital status	0.289(0.389)	0.293(0.382)	0.179(0.599)	0.343(0.302)	0.422(0.196)
Education	0.13(0.704)	0.36(0.276)	0.08(0.814)	0.08(0.814)	0.35(0.292)

Note. *Values in parenthesis ( ) indicate p-values and p-value <0.05 and * implies significant at 5% level of significance. Values outside parenthesis indicate correlation coefficients (r)*

CHAPTER 5: DISCUSSION

5.1 Prevalence of *S. aureus* infection among diabetes mellitus with foot ulcers at Vihiga County referral hospital, Kenya.

The study established an overall 60.3 % prevalence of *S. aureus* infection among diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital. This indicates that diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya are prone to *S. aureus* infection. However, this study finding was much higher than the studies conducted in Ethiopia, and Nigeria which found diabetic foot ulcer prevalence to be 31.1% and 41.1%, respectively (Amogne *et al*., 2011, Ogbera *et al*., 2005, Mariam *et al*., 2017). This variation might be due to difference in sample size or could be attributed to differences in geographical location of the studies as well as socio-cultural variation of the study participants. This relatively higher prevalence of *S. auerus* infection among diabetes patients in Vihiga County Referral Hospital might be due to poor dressing of their wounds and inconsistency in clinic visits as depicted from the review of diabetes mellitus patient's files at the Vihiga County Referral Hospital Diabetic Clinic. Based on age, a higher prevalence rate was noted among those in the age group of over 60 years at 63.8%. This was similar to Rashid *et al.* (2012) who established a higher prevalence for those aged over 50 years. This observation could be attributed to these group of patients having other preexisting medical conditions like hypertension and rare visits to the diabetic clinic.

The influence of gender on foot ulcers has been controversial, with some studies demonstrating male gender as a risk factor, while other studies have shown no difference. Prevalence analysis

of *S. aureus* infection based on gender indicated that females had a higher prevalence of 57.4% as compared to their male counterparts at 42.6%. This conformed to the findings of Gebremedhn *et al.* (2016); Rashid et al. (2012); Simkhada (2013) and Thomsen (2004). The results contradicted Aedh (2016), Oguzkaya-Artan *et al*. (2015) and Rutare (2013) who found out a high prevalence in males than females. However, these studies focused on hospital workers and children unlike the other studies that focused on adult patients. Highest prevalence in females could be attributed to rare visits of female patients to health facilities.

Based on marital status, it was noted that those married had a higher prevalence at 84.0%. This finding was similar to Aedh (2016). Education level analysis indicated that those with primary level education had the highest prevalence rate at 51.1% which was similar to Aedh (2016). Low level of education may attribute to poor dressing of their wounds.

5.2 Susceptibility of *S. aureus* isolated from Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya to penicillin G, gentamicin, ceftriaxone, vancomycin and linezolid.

S. aureus isolated from diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya had a susceptibility rate of 0% (100% resistant) to penicillin G. Although these results are not similar to Ako-Nai *et al*. (2005) and Mir and Srikanth (2013) who established that *S. aureus* was susceptible to penicillin to some degree i.e. 32% and 67.78% respectively this study's findings were consistent with the findings of Rajaduraipandi *et al*. (2006) who established that *S. aureus* was 99.6% (100%) resistant to penicillin. This may be attributed to the fact that *S. aureus* is a penicillinase producing bacteria causing resistance to penicillin G.

S. aureus had a susceptibility rate of 35.1% and 38.3% to ceftriaxone and gentamicin respectively which was similar to Rajaduraipandi *et al*. (2006); Farzana and Hameed (2006) who showed that *S. aureus* was susceptible to cephalosporins and aminoglycosides to some degree. This may be attributed to non- adherence to intake of antibiotics by patients and inconsistency in clinic visits for treatment as per the diabetic patients' records which could have exacerbated the infection and antibiotic resistance.

As first line antibiotics, *S. aureus* had a susceptibility rate of 36.2% and 60% to vancomycin and linezolid respectively. This was similar to the findings of Wunderink *et al*. (2012); Al-Zoubi *et al*. (2015) who established susceptibility of *S. aureus* to linezolid and vancomycin to some degree. Further, the finding on the association between age and antimicrobial susceptibility was similar to Abdalla *et al*. (2012); Aboud *et al*. (2015); Naghavi-Behzad *et al*. (2015); Shahina *et al*. (2014) findings attributed to high level of antibiotic abuse arising from self medication often associated with inadequate dosage and failure to comply to treatment.

CHAPTER 6: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary of the Findings

The first objective of this study was to determine prevalence of *S. aureus* infection in Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya. This was based on the null hypothesis of no prevalence of *S.aureus* infection among Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya. A prevalence rate of 60.3% *S. aureus* infection among Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital was noted implying the rejection of the null hypothesis. This was due to poor dressing wounds and inconsistency in clinic visits that increased the infection rate.

The second objective of this study was to determine the susceptibility of *S. aureus* isolated from Diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya to penicillin G, gentamicin, ceftriaxone, vancomycin and linezolid. It was established that *S. aureus* isolates were resistant to penicillin G with varying susceptibility rates to ceftriaxone at 33 (35.1%), gentamicin at 36 (38.3%), vancomycin at 34 (36.2%) and linezolid at 56 (59.6%). Age was also noted as having significant negative association with susceptibility of *S. aureus* to antibiotics that may be attributed to high level of antibiotic abuse arising from self medication and failure to comply to treatment due to rare hospital visits.

6.2 Conclusion

In conclusion these results indicated that;

i. Diabetes mellitus type II patients with foot ulcers at Vihiga County Referral Hospital, Kenya are prone to *S. aureus* infection. The prevalence varied in terms of age, gender and marital status.

ii. Linezolid was the most effective antibiotic to treat *S. aureus* given that it is the first line antibiotic. Among the antibiotics used to treat diabetes mellitus patients with foot ulcers at Vihiga County Referral Hospital, Kenya gentamicin was the most effective. It was also noted that susceptibility rate of *S. aureus* to antibiotics was dependant on age.

6.3 Recommendations from the Study

i. Vihiga County Referral Hospital, Kenya needs to develop a policy for regular surveillance and early screening of microbial infections among diabetes mellitus patients with foot ulcers to detect infections early so that healthcare providers can initiate preventive measures.

ii. Vihiga County Referral Hospital, Kenya needs to adopt use of linezolid to manage foot ulcers in diabetic patients since it was the most effective antibiotic *S.aureus* was susceptible to. The hospital needs also to develop policies geared towards ensuring effective surveillance and monitoring of the sensitivity pattern and usage of antibiotics among different age groups. This will ensure early detection of resistance incidences so that the healthcare providers can prescribe appropriate antibiotics to be used within and outside the hospital.

6.4 Study Limitations

This study focused on *S. aureus* infection and not other bacterial infections among diabetic patients with foot ulcers which need to be considered by other researchers when carrying out further research. Further, *S. aureus* has its genetic structure but the study failed to look at which gene is susceptible to the various antibiotics.

6.5 Areas for Further Research

From the results, it is evident that the study was limited to *S. aureus* infection. It should be noted that diabetic patients with foot ulcers are exposed to numerous bacterial infections. The study therefore recommends that future studies should be conducted to establish other bacterial infections and genotype analysis of *S. aureus* to know which gene is susceptible or resistant to which antibiotic.

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APPENDICES

Appendix 1: Letter of Ethical Approval

MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

Tel: +254 057 351 622 Ext: 3050
Fax: +254 057 351 221

Private Bag – 40105, Maseno, Kenya
Email: muerc-secretariate@maseno.ac.ke

FROM: Secretary - MUERC

DATE: 1st February, 2016

TO: Tuvei Susan Mamusavu
PG/MSc/PH/00155/2014
Department of Biomedical Science and Technology
School of Public Health and Community Development
Maseno University

REF: MSU/DRPI/MUERC/00268/15

RE: Antimicrobial Susceptibility of *staphylococcus aureus* Isolated from Diabetic Patients with Foot Ulcers at Vihiga County Referral Hospital, Kenya. Proposal Reference Number MSU/DRPI/MUERC/00268/15

This is to inform you that the Maseno University Ethics Review Committee (MUERC) determined that the ethics issues raised at the initial review were adequately addressed in the revised proposal. Consequently, the study is granted approval for implementation effective this 1st day of February, 2016 for a period of one (1) year.

Please note that authorization to conduct this study will automatically expire on 31st January, 2017. If you plan to continue with the study beyond this date, please submit an application for continuation approval to the MUERC Secretariat by 4th January, 2017.

Approval for continuation of the study will be subject to successful submission of an annual progress report that is to reach the MUERC Secretariat by 4th January, 2017.

Please note that any unanticipated problems resulting from the conduct of this study must be reported to MUERC. You are required to submit any proposed changes to this study to MUERC for review and approval prior to initiation. Please advice MUERC when the study is completed or discontinued.

Thank you.

Yours faithfully,

DIRECTORATE OF RESEARCH PUBLICATION & CONSULTANCIES 01 FEB 2016 MASENO UNIVERSITY

Dr. Boruke Anyona,
Secretary,
Maseno University Ethics Review Committee

Cc: Chairman,
Maseno University Ethics Review Committee.

Appendix 2: Letter of Introduction

SUSAN MAMUSAVU TUVEI (MSC/PH/00155/2014),

C/O SCHOOL OF MEDICINE MASENO UNIVERSITY,

P.O. PRIVATE BAG,

MASENO.

5TH FEBRUARY 2016.

THE MEDICAL SUPRETENDANT,

VIHIGA COUNTY REFERRAL HOSPITAL,

P.O. BOX 1069- 50300,

MARAGOLI.

THRO'

CHIEF OFFICER MINISTRY OF HEALTH- VIHIGA COUNTY.

Received. Approved on 6 mth
& MUERC approval
5/2/16

CHIEF OFFICER
MINISTRY OF HEALTH
04 FEB 2016
FORWARDED

Dear Sir/Madam,

RE: APPLICATION FOR RESEARCH PERMISSION

Being a Master of Science- Medical Microbiology student at Maseno University intending to conduct a research entitled **"Antimicrobial Susceptibility of *S.aureus* Isolated from Diabetic Patients with Foot Ulcers at Vihiga County Referral Hospital, Kenya"**; I hereby tender my application for your consideration.

This application is made taking into account that relevant approvals (see attachments) for conducting the research have been sought which include;

i. Maseno University School of Graduate Studies
ii. Maseno University Ethical Review Committee (MUERC)

More importantly, this study will provide information to the County and the country at large on the effectiveness of antibiotics used in the treatment of diabetic patients with foot ulcers.

I look forward to your positive response to see me through my master's studies. Thanking you in advance.

Yours faithfully,

Susan Mamusavu Tuvei

Appendix 3: Study Questionnaire

1. Age in Years

   18- 30 ☐ 31 – 44 ☐ 45- 60 ☐ Over 60 ☐

2. Gender: Male ☐ Female ☐

3. Marital Status

   Single ☐ Married ☐ Widowed ☐ Divorced/separated ☐

4. Level of education

   None ☐ Primary ☐ Secondary ☐ College/University ☐

5. When were you diagnosed with diabetes?

   1-6 Months ☐ 7- 12 Months ☐ Over 1 Year ☐

6. How long have you been on medication?

   Less than 3months ☐ 3 – 6 months ☐ over 6 months ☐

7. What type of medication?

   Antibiotics ☐ Others ☐

8. Which type of antibiotic

   Pencillins ☐ Cephalosporin ☐ Aminoglycosides ☐ None ☐

Appendix 4: Laboratory Request Form

Patient Number ……..

GRAM STAIN				
Gram +ve Cocci in Clusters	Present		Absent	
CULTURE				
Blood Agar (BA)	Day1		Day2	
	Growth	No Growth	Growth	No Growth
COAGULASE TEST				
	Positive		Negative	
ORGANISM ISOLATED				
S. aureus	Yes		No	
SUSCEPTIBILITY TESTING				
Antibiotic	Resistant		Susceptible	
Penicillins				
Cephalosporines				
Aminogycosides				
Vancomycin				
Linezolid				

Technologist Sign: …….. Date: ……..

Appendix 5: Consent Form

Participation Consent

Study Title: Antimicrobial Susceptibility of *Staphylococcus aureus* isolated from diabetic patients with foot ulcers at Vihiga county referral hospital, Kenya.

Dear participant,

You are hereby invited to participate in this study because you are a diabetic patient with foot ulcers. This form is meant to make you aware why this research is being carried out. Please read through and make decision if you want to take part or not. The principal investigator in this study is a master's student at Maseno University.

The purpose of this study will be to investigate antimicrobial susceptibility of *S. aureus* isolated from diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya. Pus specimens from diabetic patients with foot ulcers will be collected for *S. aureus* screening.

You are free to decide whether to take part or not. It is of importance to note that there is no financial benefit by participating and there will be no cost implications for participating. Participating in this study is important as the findings of the study may be helpful in decision making in improving public health and to prevent bacterial infection among diabetic patients with foot ulcers in Kenya.

Questions posed to the participants with regard to their socio-demographic characteristics may cause discomfort. The researcher wishes to assure the participants that their information will be treated with high level of confidentiality but not guaranteed.

……

Read and Signed

NOTE: below are the key contacts

Principle investigator: Ms. Susan Mamusavu Tuvei (0715579330)

Kiswahili Version

Mada Ya Utafiti: Kiwango cha Maambukizi na Kuhisi kwa vimelea *Staphylococcus aureus* kwa Antimikrobiell kutoka kwa wagonjwa wa kisukari na wenye vidonda miguuni hapa Hospitali ya Kata ya Rufa ya Vihiga, Kenya.

Kwako mhusika:

Unaalikwa kushiriki kwenye utafiti kwa sababu uko na ugonjwa wa kisukari na kidonda cha mguu. Fomu hii inakuelezea kwa sababu gani utafiti huu unafanywa. Tafadhali soma fomu hii halafu uamue kama utashiriki kwenye utafiti huu. Mtafiti ni mwanafunzi anaye fanya shahada ya uzamili kwenye Chuo kikuu cha Maseno.

Nia hasa ya utafiti huu nikutathimini kiwango cha maambukizi na Kuhisi kwa vimelea *Staphylococcus aureus* kwa Antimikrobiell kutoka kwa wagonjwa wa kisukari na wenye vidonda miguuni hapa Hospitali ya Kata ya Rufa ya Vihiga, Kenya. Sampuli ya usaha kutoka kwa wagonjwa wa kisukari na wenye vidonda miguuni itachukuliwa kwa ajili ya uchunguzi wa vimelea *S. aureus* .

Unaweza kutoa uamuzi wa kushiriki kwenye utafiti huu au la, Ni muhimu kufahamu kwamba hakuna faida za kifedha kwa kushiriki kwenye utafiti huu. Kushiriki katika utafiti huu ni muhimu kwa sababu, uvumbuzi ama majibu ya utafiti huu yatasaidia kwa uamuzi wa kuboresha afya ya umma na kuzuia maambukizi ya vimelea kwa wagonjwa wa kisukari na wenye vidonda miguuni hapa Kenya.

Hatari zinazoambatana na kushiriki katika utafiti huu ni kama usumbufu kutokana na maswali ya kijamii. Mtafiti anataka kukuhakikishia wewe mshirika ya kwamba maelezo utakayopeana yatahifadhiwa kwa usiri wa hali ya juu. Lakini hatuezi kuhakikisha hili.

……

Kusoma na kuweka saini

Zaidi; wasiliana na

Mtafiti Mkuu: Ms. Susan Mamusavu Tuvei (0715579330)

Appendix 6: Interpretation of Zones of inhibition (diameter in mm)

Antibiotic	Concentration (µg)	Susceptible (S)	Intermediate (I)	Resistant (R)
Penicillin G	10	≥ 29	-	≤ 28
Flucloxacillin	10	≥ 29	-	≤ 28
Gentamicin	10	≥ 15	13-14	≤ 12
Ceftriaxone	30	≥ 21	14-20	≤ 13
Vancomycin	30	≥ 17	15-16	≤ 14
Linezolid	30	≥ 21	-	≤ 20

Source: Clinical and Laboratory Standards Institute (2015)