



The Prevalence of Coagulase-Positive *Staphylococci* Colonization among Diabetic Patients with Foot Ulcers at Vihiga County Referral Hospital, Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the prevalence of *Coagulase-positive Staphylococci* colonization among diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya.

Study Design: This study employed a hospital based cross-sectional study design of diabetic patients with foot ulcers at Vihiga County Referral Hospital, Vihiga County, Kenya.

Place and Duration of Study: Vihiga County Referral Hospital, Kenya between February 2016 and April 2016.

Methodology: The study population comprised of 225 adult diabetic patients and a sample size of 156 patients with foot ulcers who attend Vihiga County Referral Hospital for treatment. Pus

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specimens from foot ulcers of diabetic patients were swabbed aseptically for *Coagulase-positive Staphylococci* screening. 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 standard methods. *Coagulase-positive Staphylococci* ATCC 25923, a known coagulase producer was included as a control strain.

Results: 94 out of 156 pus swabs were coagulase positive. This implied that the prevalence of *Coagulase-positive staphylococci* was 94(60.3%) among Diabetic patients with foot ulcers seeking treatment at Vihiga County Referral Hospital, Kenya. Socio demographics of participants indicated that majority of the participants were; male at (51.3%), over 60 years at (43.6%), married at (85.3%) and had up to primary education at (60.3%).

Conclusion: Diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya are prone to *Coagulase-positive staphylococci* colonization.

Keywords: *Coagulase-positive Staphylococci*; prevalence; socio-demographics.

1. INTRODUCTION

Over the past 30 years, the status of diabetes mellitus 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 [1]. In 2011, an estimated 366 million adults aged 20-79 years had diabetes; this number is predicted to rise to 552 million by 2030 [2]. 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 [3].

According to the Ministry of Public Health of Kenya as reported by [4], over 50% of all hospital admissions and 55% of hospital deaths in Kenya are attributed to non – communicable diseases with diabetes among the leading condition. The International Diabetes Federation estimated the prevalence of diabetes in Kenya to be about 3.3% in 2007 [5]. Data from the Ministry of Health as reported by [6] 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 this country. Further, majority of the rural population in Kenya is highly ignorant of the common complications associated with diabetes which includes foot ulcers that are often colonized by bacteria [4]. Vihiga County being one of the rural parts of Kenya continues to record increasing cases of diabetes with high

rates of morbidity and mortality reported due to rising foot ulcers complications [7].

The diabetic foot ulcer has been considered the major complication of diabetes care that requires constant monitoring and care to reduce infections resulting from bacterial colonization [8-10]. Due to hyperglycemia induced immunodeficiency, diabetic foot ulcers are often colonization by *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas* species, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Citrobacter koseri* among others which cause tissue destruction [11]. However, *Coagulase-positive Staphylococci* remains the predominant pathogen in diabetic foot ulcers which has been associated with increased mortality and hospital costs [12]. *Staphylococcus aureus* (*Coagulase-positive Staphylococci*) is a Gram-positive bacterium that can live as a commensal organism on the skin and in the nose and throat [13]. Further, *Coagulase-positive Staphylococci* is a 1 µm, Gram-positive cell that may be observed in the laboratory 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 anaerobic [14]. It has been estimated that approximately 20–30% of the general population are *Coagulase-positive Staphylococci* carriers [15].

About three diabetic patients with foot ulcers visit Vihiga County referral hospital per day for treatment and cleaning up of their wounds. However, the hospital does not conduct isolation of *Coagulase-positive Staphylococci* in diabetic patients with foot ulcers therefore its presence could be growing thereby seriously deteriorating patients' quality of life. This makes the prevalence of *Coagulase-positive Staphylococci*

colonization among diabetic patients with foot ulcers in Vihiga County uncertain hence a justification for the study.

1.1 Objectives of the Study

1.1.1 General objective

To investigate the prevalence of *Coagulase-positive Staphylococci* among diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya.

1.1.2 Specific objectives

The specific objectives were to;

1. Examine the socio-demographic distribution of diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya.
2. Determine prevalence of *Coagulase-positive Staphylococci* colonization among diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya.

1.2 Research Questions

1. What is the socio-demographic distribution of diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya?
2. What is the prevalence of *Coagulase-positive Staphylococci* colonization among diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya?

1.3 Significance of Study

Given that the prevalence of *Coagulase-positive Staphylococci* in Vihiga County, Kenya remains uncertain. This study investigated the prevalence of *Coagulase-positive Staphylococci* isolated from diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya for purposes of informing health practitioners and other stakeholders. This knowledge will create awareness on the importance of *Coagulase-positive staphylococci* screening that will have some life-saving potential by enabling the County to come up with appropriate microbial colonization preventive measures.

2. LITERATURE REVIEW

2.1 Bacterial Colonization among Diabetic Patients with Foot Ulcers

Almeida [16] Investigated the prevalence of wound colonization by *Staphylococcus* especially

Coagulase-positive Staphylococci, 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 *Coagulase-positive staphylococci*. Although the researchers established colonization of wounds by *Coagulase-positive staphylococci* their study focused on hospitalized patients which necessitated a study on overall prevalence based on both hospitalized and outpatient.

Thomsen [17], in his study on Diabetes and Community-acquired Bacteremia: Risk and Prognosis in North Jutland County, Denmark established that a total of 225 (17.1%) diabetics had bacterium. The bacterium among diabetics was greatest in adults under 65 years and the risk appeared to be higher in diabetic female than in male. Although the researchers established bacterium infection in diabetics, their study failed to establish prevalence colonization based on a specific bacterium.

Simkhada [18], in his study using a descriptive, cross sectional study investigated the prevalence of culture positive colonization in diabetic patients at Tribhuvan University Teaching Hospital in Nepal. Among 100 patients included, 53 were female and 47 were male. Results indicated that 21% of patients had been colonized by bacterium. Colonization was more in female ($P=0.047$) as compared to male. Although the researchers established bacterium colonization in diabetics, their study generalized bacterium colonization rather than focusing on specific bacterium such as *Coagulase-positive staphylococci*. Further, the study's prevalence analysis was based on gender failing to capture overall prevalence.

Based on the reviews done, it is evident that various studies on bacterial colonization among diabetic patients with foot ulcers have been conducted. However, most of the studies were not specific to *S. aureus* colonization with none having been conducted in Kenya. This creates uncertainty with regard to the prevalence of *Coagulase-positive Staphylococci* among diabetic patients with foot ulcers in Kenya.

3. MATERIALS AND METHODS

3.1 Study Design

This study employed a hospital based cross-sectional study design of diabetic patients with foot ulcers at Vihiga County Referral Hospital, Vihiga County, Kenya. The study determined the prevalence rate of *Coagulase-positive staphylococci* using descriptive statistics involving frequency distribution tables and percentages. The study population comprised of 225 adult (aged 18 years and above) diabetic patients with foot ulcers who attend Vihiga County Referral Hospital for treatment.

3.2 Inclusion and Exclusion Criteria

Consenting diabetic patients with foot ulcers above 18 years were included in the study while diabetic patients with foot ulcers who had cleaned their wounds using antiseptics were excluded.

3.3 Sample Size and Sampling Technique

The sample size for the study was determined following Fisher *et al.* (1998) formula as modified by [19], as shown below.

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

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 (384),

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. 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.

3.4 Laboratory Techniques

Pus specimens from foot ulcers of diabetic patients were swabbed aseptically for *Coagulase-positive staphylococci* 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 standard methods. *Staphylococcus aureus* ATCC 25923, a known coagulase producer was included as a control strain.

3.4.1 Culture

A smear of pus was made at the periphery of blood agar plate, and then streak of parallel lines was made along the edge of the plate using disposable plastic loops, after inoculation the plates were incubated at 24-48 hours at 37°C.

3.4.2 Gram staining

A slide was labelled and a smear was made on a clean glass slide, heat fixed the stained with crystal violet for 1 minute, rinsed, stained with grams iodine for 1 minute, rinsed decolorised with acetone for 30 seconds rinsed with water then counterstained with safranin for 2 minutes, rinsed with water, left to dry then examined under $\times 100$ oil immersion.

3.4.3 Coagulase tube method

0.3 milliliters of citrated rabbit plasma was added to two test tubes and labeled as control and sample. Tube labeled as sample was inoculated with a colony of gram positive cocci to make a cloudy suspension, control tube was inoculated with a positive control of *Coagulase-positive staphylococci* and both tubes were incubated at 37°C for 1-4 hours. A positive coagulase test was represented by clotting.

3.5 Ethical Considerations

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. Written informed consent was obtained from each participant before enrollment. 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.

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 male and 76(48.7%) were female. 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.

4.2 Prevalence of Coagulase-positive *Staphylococci* Infection among Diabetics 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°C for 24 – 48 hours a total of 118(75.6%) of the pus swabs had growth as shown in Fig. 4.1 while 38 (24.4%) pus swab specimens did not have any growth as shown in Fig. 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 Fig. 4.3 while 16 were gram negative as shown in Fig. 4.4. Coagulase test was conducted on gram positive cocci isolated from the 102 pus swab plates to confirm for *Coagulase-positive staphylococci*. It emerged that 94 of the pus swab gram positive cocci were coagulase positive while 8 were coagulase negative. This implied that the prevalence of *Coagulase-positive staphylococci* was 94(60.3%) among Diabetic patients with foot ulcers seeking treatment at Vihiga County Referral Hospital, Kenya. Details are contained in Table 4.2.

Table 4.1. Socio-demographic characteristics of the study participants

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

*Socio - demographics frequency and percentage distribution for diabetic patients with foot ulcers

Table 4.2. Prevalence of coagulase-positive *Staphylococci* in diabetic patients with foot ulcers attending vihiga County referral hospital, Kenya

	Frequency	Percent (%)
Positive for <i>Coagulase-positive staphylococci</i>	94	60.3
Negative for <i>Coagulase-positive Staphylococci</i>	62	39.7
Total	156	100.0

*Frequency and percentage distribution of diabetic patients with foot ulcers



Fig. 4.1. BA bacterial growth



Fig. 4.2. BA - no bacterial growth

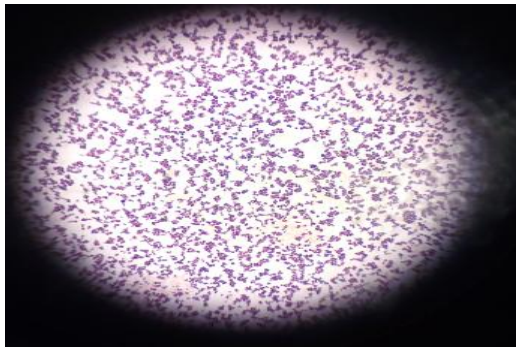


Fig. 4.3. Gram positive cocci in cluster

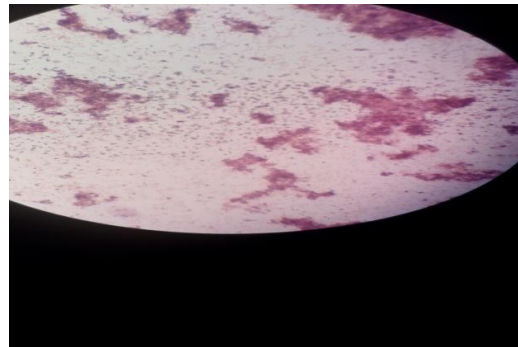


Fig. 4.4. Gram negative rods

5. DISCUSSION

The finding indicated that diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya are prone to *Coagulase-positive staphylococci* colonization given a relatively higher prevalence rate of 60.3%. This study finding was much higher than the studies conducted in Brazil, Denmark and Nepal which found *S. aureus* colonization prevalence to be 20%, 17.1% and 21% respectively [16-17]. This variation might be due to differences in geographical location of the studies as well as socio-cultural variation of the study participants.

6. CONCLUSION AND POLICY RECOMMENDATIONS

6.1 Conclusion

In conclusion, diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya are prone to *Coagulase-positive staphylococci* colonization. This relatively higher prevalence of *Coagulase-positive Staphylococci* colonization

among diabetic patients at Vihiga County Referral Hospital might be due to poor dressing of their wounds and inconsistency in clinic visits as depicted from the review of diabetic patient's files at the Vihiga County Referral Hospital Diabetic Clinic. Therefore, Vihiga County Referral Hospital, Kenya needs to develop a policy for regular surveillance and early screening of microbial colonization among diabetic patients with foot ulcers to detect bacterial colonization early so that healthcare providers can initiate preventive measures.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this paper and accompanying images. Written informed consent was obtained from each participant before enrollment. Free education on diabetes was also provided to the participants with assurance on the confidentiality of their responses given and study results.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki. 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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