A COMPARATIVE STUDY OF LEVIN VS Z STROKE WOUND SWAB TECHNIQUES IN DIAGNOSING INFECTION IN PATIENTS WITH CHRONIC WOUNDS USING WOUND BIOPSY AS GOLD STANDARD AT THE KENYATTA NATIONAL HOSPITAL.

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A dissertation submitted in partial fulfillment for the award for master of medicine degree in general surgery in the department of Surgery

University of Nairobi School of medicine

STUDENT'S DECLARATION

I, **Dr. Ogoye Madaraka,** do declare that this dissertation is my original work and has not been presented for a degree in any other university

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This dissertation has been submitted for examination with my approval as the Chairman, Department of Surgery.

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LIST OF ABBREVIATIONS

BC-	Before Christ
CFU-	Colony forming units
CLED-	Cysteine, lactose and electrolyte deficient agar
CM –	Centimeters
ERC –	Ethics and Research Committee
KNH-	Kenyatta National Hospital
MCS –	Microscopy culture and sensitivity
MRSA-	Methicillin resistant Staphylococcus aureus
NHS-	National Health Services
SBA-	Sheep blood agar
SPSS-	Statistical package for the social sciences
UK-	United Kingdom
UON-	University of Nairobi
USA-	United States of America
US$-	United states dollar
YRS -	Years
Z STROKE-	Zigzag Stroke Technique

ABSTRACT

Background: Chronic wounds reduce quality of life and increase societal socioeconomic burden by increasing cost of healthcare, reducing productivity with loss of man hours. Wound infection being a factor that leads to chronicity should be diagnosed early and managed accordingly to facilitate timely wound healing. There are a number of ways that have been devised to diagnose wound infection, with standard being wound biopsy, which is costly and painful. This study seeks to determine the wound swab technique that gives better micro-organism yield in diagnosing chronic wound infection.

Study Design: This was a comparative cross-sectional study.

Objective: The aim of the study was to compare effectiveness of Levin and Z stroke wound swab techniques in diagnosis of infection among patient with chronic wounds at KNH.

Methods:145 patients above 18 years with wounds of duration more than 4 weeks without healing and gave informed written consent were consecutively enrolled into the study at the KNH medical and surgical wards, surgical outpatient clinics and theatres. Data collected including demographics, etiology, signs of wound infection and duration of wounds were documented. Subsequently 2 swabs Levin and z-stroke plus punch biopsy of wounds were taken from each patient with specimen submitted to lab within 1hr of collection. Culture of the specimen was done and data from the laboratory was analyzed using SPSS version 21.

Results: Ninety males and fifty five females were recruited into the study. The infection rate was 50%, mean age was 37years, the youngest patient was 18 years while the oldest was 79 years. Surgical site infection was the commonest cause of chronic wounds at 31.7% while chronic osteomyelitis was least at 1.4%.The commonest isolated organism was staphylococcus aureus at 18.6% with beta hemolytic streptococcus the least at 1.4%.Most of the wounds were located in the lower limbs at 38%. The sensitivity and specificity of Levin technique were 95.8% & 61.6% respectively and diagnostic accuracy of 78.6%. Z-stroke had sensitivity of 97.2% & specificity of61.6% with diagnostic accuracy of 79.3%. Both Levin and Z-stroke are equally effective at picking infection in chronic wounds.

Conclusion: Both Levin and Z-stroke are equally effective at picking infection in patients with chronic at Kenyatta National Hospital. This is in contrast to studies in other settings which depict that Levin wound swab technique to have a better pick rate than z-stroke.

CHAPTER ONE: INTRODUCTION

Infection or bacterial colonization of wounds causes delays in healing and leads to a chronic wound state. Non-healing wounds are associated with prolonged hospitalization. They also cause patients severe chronic pain, significant emotional and physical distress, reduced mobility and social isolation. Wound scarring may have long lasting functional, cosmetic and psychological consequences for the patients. All these culminate in reduced quality of life, increased treatment cost and demands for advanced wound management practices[(1)].In the US an estimated 6.5 million people afflicted by chronic wounds consume US$ 25 billion annually from the healthcare budget while the UK spent US$ 6.12 billion in 2012 for wound management[(2)]

Need for diagnosis of wound infection arises in scenarios where there are signs and symptoms of local wound and or systemic infection, when no other explanation to delay in wound healing is feasible, and also in preparation for surgical procedures like skin grafting that maybe compromised due to presence of bacteria like Methicillin resistant staphylococcus aureus. The need for wound diagnosis for infection also arises in surveillance of multi-drug resistant bacteria. Qualitative, quantitative and semi-quantitative wound culture techniques are utilized in this assessing wound for the presence of infection.

Various diagnostic techniques identify microbes by performing culture on specimen harvested from the wounds this include needle aspiration of wound fluids, wound swab techniques of Levin and z stroke with wound tissue biopsy considered gold standard.

Gardener et al (2006) in Caucasian population that consisted of 83 wounds found Levin technique (sensitivity 90% and specificity 57%) to be superior to z stroke and needle aspiration with 78% concordance with tissue biopsy. Angel et al(2011) also in Caucasian population in prospective randomized control trial with 50 patients also noted Levin technique to have higher pick rate for both acute and chronic infection [(2, 3)]. There is paucity of comparative data on the different wound swab techniques for the predominantly African population in our setup.

CHAPTER TWO: LITERATURE REVIEW

The intact skin is the first line of defense and barrier protecting human beings from microbial invasion to a susceptible host, because of its properties like sebaceous secretions, fatty acids and low surface pH, that inhibits colonization and growth of pathogenic microbes[(3)]. Wound is tissue damage that disrupts skin compromising its protective function, this loss of protective function grants microbial species opportunity to access exposed human tissues initiating complex reaction between potential pathogens and host. Wounds come as result of damage to skin from abrasions, cuts, lacerations, bites, puncture wounds and burns [Chemical, cold, heat, friction and electricity] [(4-5).]

Wounds are categorized into acute and chronic wounds; it is theorized that acute wounds heal in a predictable manner and time frame with adequate anatomical and functional results. The healing occurs with fewer if any complications. Wounds that have failed to heal in 4 weeks to three months are considered chronic[(6)].Wound healing requires orderly integrated complex molecular and biological events of cell migration, proliferation and extracellular matrix deposition. This takes place in four overlapping phase of coagulation, inflammation, migration-proliferation and remodeling[(7)]. An optimal cellular response to inflammatory mediators, cytokines, growth factors and mechanical forces is key for timely and smooth wound healing process.

Chronic wounds heal not in an orderly process hence fail to produce adequate/satisfactory anatomical and functional results. Chronic wounds are theorized to be stagnated in the inflammatory phase that could possibly be induced by local biofilm infection that up regulates cytokines and reduce growth factors. The healing process in chronic wounds is not complete and is disorganized by various factors which delays or lengthen one or more phases of wound healing. The factors that interfere with wound healing include: infection, tissue hypoxia, wound necrosis, exudates and excess of inflammatory cytokines[(8)].

Chronic wounds include; pressure ulcers, diabetic foot ulcers, venous and arterial insufficiency ulcers. It's estimated that in developed countries approximately 1-2% of the population suffer from chronic wounds in their lifetime. Jabrink et al (2017) estimated that in the USA the annual cost of wound care is approximately US$ 20 billion while in the UK its estimated to cost approximately 5.5% of NHS expenditure[(9)]

Bedridden patients are vulnerable to pressure ulcers which are a major source of infection. Bacteremia associated with pressure ulcers leads to infectious complications like abscesses, cellulitis, osteomyelitis, septicemia and even death [(8)]. Khor et al (2014) found that

neutrophilia and stage 4 pressure ulcers were strongly correlated with occurrence of deep wound infection and therefore strong independent predictors of mortality in Malaysian population, while Mathews 2005 in the US population reported 39.7% incidence of septicemia as cause of death in pressure ulcers patients[10].

Diabetic foot ulcers are of are three types; ischemic ulcers, neuropathic ulcers and combination of the two. All are prone to infection with trauma being a risk factor.

In the US the estimated annual diabetic foot incidence is 5% with an amputation rate of 1% among diabetics. Foot infections vary widely from superficial paronychia to cellulitis, myositis, abscesses, necrotizing fasciitis, tendinitis, septic arthritis and osteomyelitis. Early detection and management of the focus can prevent progression and reduce morbidity associated with extensive debridement and amputations.

Infected diabetic foot contributes to over 90% of non-traumatic lower limb amputations and prolonged hospitalization.[8] Beverly 2016 found cellulitis requiring antibiotics, osteomyelitis and severity of diabetic foot Infection as significant contributors towards lower extremity amputations In 2004, 71000 lower extremity amputation at a cost of US$ 38,077 per procedure were performed, these occurred at mean of 5 years earlier than non-diabetic foot lower extremity amputations (66 yrs. vs. 71 yrs.)[11].

Venous ulcers arise from ambulatory venous hypertension when calf muscle pump fails, valvular dysfunction and deep venous outflow obstruction occur with estimated annual incidence of 1.7% above 65 yrs. They are the commonest etiology of chronic leg ulcers (70%) Venous ulcers are colonized by bacteria and some proceed to wound infection. Wound healing is impaired due to chronic inflammatory condition propagated by the biofilm induced tissue destruction. Reduced quality of life as well as societal and individual burdens like loss of productivity, caretaker, physician consultations, wound supplies, transportation costs and pre mature disability are immense. The financial burden to the healthcare system in the US is estimated to be 2 billion dollars annually [12-13].

The earliest documented wound care record is Clay tablet manuscript dating back to 2200 BC where 3 healing gestures are described: washing the wound, making the plaster and bandaging the wound, where plaster here means a bandaging material. Sumerians used beer as antiseptic while the Egyptians used honey, grease from animal fat and lint from vegetable oil for the same. The Greeks insisted on hygienic practices such as wounds cleansing with warm water followed by dressing with acetic acid and wine. In the 19th to 20th century introduction of antiseptics, antibiotics, aseptic techniques in surgery and modern wound care techniques have prevailed[14].

A Wound is infected when invasion by microorganisms with subsequent proliferation to a level that it invokes a local and or systemic immune response in the host. In most cases of wound infection interventions are key to assist body defenses in combating invading wound microorganisms, whose presence impairs wound healing and cause local tissue damage ([2, 16]. Wound infection continuum is a relationship between the host, wound and bacteria whose status change continuously in the wound depending on local environmental and systemic factors. Apart from bacteria other microbes are also associated with microbial virulence and wound infection. Wound continuum stages describe gradual increase in number and virulence of micro-organisms. Wound infection continuum progresses in 5 different stages;

- contamination,
- colonization,
- local infection/critical colonization,
- spreading invasive infection and
- Septicemia [4,15]

Contamination[26]	Colonisation[26]	Local infection		Spreading infection[22, 23]	Systemic infection[22, 23]
All wounds may acquire micro-organisms. If suitable nutritive and physical conditions are not available for each microbial species, or they are not able to successfully evade host defences, they will not multiply or persist; their presence is therefore only transient and wound healing is not delayed	Microbial species successfully grow and divide, but do not cause damage to the host or initiate wound infection	Covert (subtle) signs of local infection:[2, 27-36] ■ Hypergranulation (excessive 'vascular' tissue) ■ Bleeding, friable granulation ■ Epithelial bridging and pocketing in granulation tissue ■ Wound breakdown and enlargement ■ Delayed wound healing beyond expectations ■ New or increasing pain ■ Increasing malodour	Overt (classic) signs of local infection:[2, 27, 28, 35, 36] ■ Erythema ■ Local warmth ■ Swelling ■ Purulent discharge ■ Delayed wound healing beyond expectations ■ New or increasing pain ■ Increasing malodour	■ Extending in duration +/- erythema ■ Lymphangitis ■ Crepitus ■ Wound breakdown/ dehiscence with or without satellite lesions ■ Malaise/ lethargy or non-specific general deterioration ■ Loss of appetite ■ Inflammation,	■ Severe sepsis ■ Septic shock ■ Organ failure ■ Death

Figure 1: wound infection continuum table

Factors that determine progression through the continuum include; microbial types, microbial load, microbial virulence and synergistic actions as well as capability of a host to mount an immune response. Other factors like aging, obesity, poor nutritional status, chronic steroid use and diabetes mellitus encourage progression by altering efficiency and lowering activity of the immune system[14].

wound contamination is characterized by non-proliferating microbes that don't evoke host responses, all open wounds are contaminated from time of injury, while chronic wounds have exogenous exposure through nosocomial or environmental plus poor hand and wound care hygiene practices as well as endogenous sources (normal flora), of note is that in a non-compromised wound microbes are cleared by phagocytes.

Colonization refers to limited microbial proliferation without evoking host response. It may impede wound healing though, Browne et al noted in a study of diabetic foot ulcers that where there was no bacterial growth within a wound a healing rate of 0.2cm/ week was observed, while the presence 10^5–10^6 colony forming units of bacterial load was associated with 0.15 cm /week healing rate and load above 10^6 with 0.05 cm/ week healing rates [16].

Wound infection occurs when microbes proliferate and invade into deeper tissues evoking a host response. A deep tissue quantitative microbial count> 10^5 CFU/ml has higher incidence of wound infection, this however depends on dose of infecting microbes being e.g. Being lower for beta hemolytic streptococcus and pseudomonas, and higher for enterococcus. Counts > 10^6 CFU/ml markedly affects wound healing processes [17].

- Biofilms: is defined as a structured community of microbes with genetic diversity and variable gene expression (phenotypes) that creates behaviors and defenses used to produce unique infections (chronic infections). And are characterized by significant tolerance to antibiotics and biocides, while remaining protected from host immunity [17]. Biofilm forms when bacteria attach to wound and form micro colonies over time. Biofilm cycle has five stage
- planktonic phase- Pilli and flagella mediated reversible weak attachment to wound surfaces
- Irreversible attachments-extracellular polymeric substance protects growing colony
- Cell proliferation- colony numbers increase via microbial proliferation by quorum sensing
- Growth and maturation- mature colonies surrounded by ineffective immune cells
- Dispersal- mature biofilm reseed wound with planktons[18].

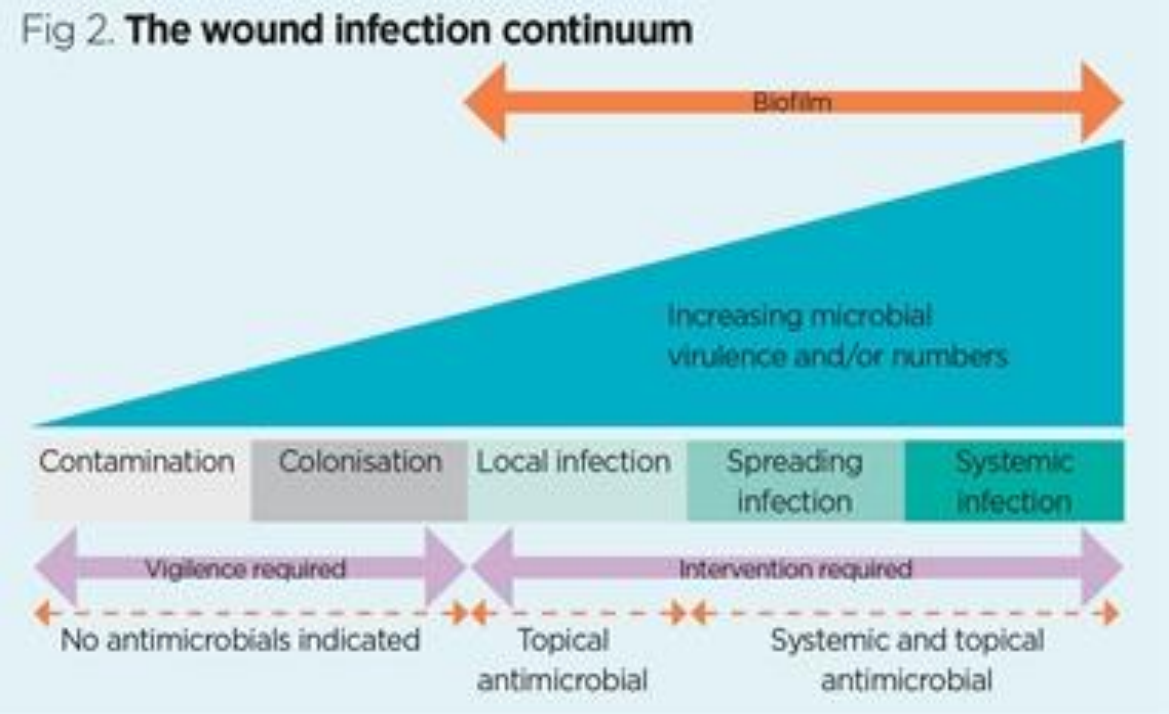

Figure 2: Stepwise antimicrobial therapy table

Biofilm is an impediment to healing of chronic wounds as bacteria within it are 50-1000 times resistance to antimicrobial therapy than unattached bacteria [(19)].Normal wound management processes become ineffective once biofilm is in mature state at the same time biofilm seeds wound surface with planktonic microbes in an active or passive dispersal process maintaining and enhancing wound infection. Visually healthy wounds with delayed healing have been shown via biopsy and microbial cultures to be harboring biofilms, biofilm deep in wound beds can't be visualized hence need for biopsies. Even with biopsies biofilm detection is still difficult requiring sophisticated techniques using scanning electron microscope and confocal laser scanning microscopy [(20, 21, 22,25)].

Localized wound infection can be suspected with worsening pain, edema, purulent/ serous exudation, friable bright red granulation tissue and wound odor. Antimicrobial dressings, topical antimicrobials plus/minus debridement are sufficed at this stage. In addition to classical signs of infection ;rubor,calor, dolor and tumor. other signs of spreading infection like cellulitis and/lymphangitis, wound breakdown with satellite lesions with or without epithelial bridging, induration and redness more than 2cm from wound margins with malaise and systemic signs indicate progression in wound infection continuum [(22, 23)].

Scenarios that necessitates wound specimen collection[24].

- Normal surveillance- resistance microbes checking protocol
- Post antimicrobial therapy- to assess response to treatment
- Local/systemic symptoms and signs of wound infection- for pathogen isolation and antimicrobial sensitivity.
- Non wound healing progress post 2 weeks of adequate therapy- rule out pathogens as a cause for stagnation[26].
- When the presence of certain bacteria would be detrimental to performance of certain surgical procedure e.g. MRSA.

Ways of assessing for wound bio burden are pertinent when dealing with a suspected wound infection as visual observation is not reliable and bio burden isn't visible to the naked eyes. This can be done by use of wound cultures. Wound cultures can be

a) qualitative,

b) semi- quantitative and

c) quantitative

Qualitative cultures only indicate the presence or absence of growth, precisely to mean species identification

Semi-quantitative cultures identify the species while grading micro-organisms presence as either/or±1(scanty), + (few), ++(moderate), +++ (numerous) or as categories such as $< 10^5$ colony forming units per gram of tissue. Qualitative and semi-quantitative methods are majorly swab based

Quantitative cultures give both species identification and an absolute quantity of micro-organisms cultured per unit volume of tissue majorly tissue biopsy based but wound swab is also employed. The technique was described by Loeb et al in 1974 by taking tissue biopsy (2 parallel incisions 1-2 cm in length and 0.5 cm apart with tissue in between weighing approximately 0.02- 0.05 grams) using scalpel on burn wound surface followed by macerating the tissue and suspending it in 2mls normal saline and subsequent serial dilution ratio of 1:10. 0.1ml of resultant solution was then inoculated in blood agar at 37 degrees for 24 hours' quantification was then done using formula below

$$\text{Organisms/Gm. of tissue} = \frac{N \times D \times 2 \times 10}{W}$$

Where N = the number of colonies on the plate chosen for colony counts

D = the dilution inoculated on the plate {i.e., 1:10, $1:10^2$, $1:10^4$, etc.}

W = Weight of the biopsy specimen in Gm.

The constant factors (2 and 10) in the numerator adjust for the preparation of the original suspension in 2 ml. of saline solution and for the inoculation of only 0.1 ml. of its dilution on the plate evaluated. [27-28].

Alternatively, the colony count per gram of tissue can be obtained by the use of Miles and Misra formula as follows

(CFU/gm of tissue = C x D x V/W x 0.01)

Where, C = the total number of Colony forming units, D

= the dilution factor, W = the weight of the tissue, V =

The volume of normal saline, 0.01 = the volume of the

Inoculum.

Wound specimen for culture can be collected in any of the following three ways

- Needle aspirated wound fluids
- Wound swabs
- Wound tissue biopsy

2.1 Needle Aspirated Wound Fluids

In this technique wound fluid is obtained by using 10ml syringe on gauge 22 needle making repeated pricks on tissues surrounding wound when taking collections around wounds like abscess/ or when copious volumes of wound fluid is present([5,29].it can also be used to sample deeper pocket of fluid beneath superficial debris. When strict aseptic technique is employed exogenous contamination is avoided. To increase yield in open cavity wounds like pressure ulcers sterile 0.85% saline solution irrigation followed by fluid aspiration can increase the yield.

2.2 Wound Swabs

The technique involves the use of cotton tipped, Dacron-rayon and calcium-alginate tipped swab to sample superficial wound fluid and tissue debris. This method has been used commonly due to its simplicity, non-invasiveness, affordability and convenience for most wounds, although questions have been raised concerning representation of wound microbiology both deep and superficial, anaerobic bacteria yield and wound contaminants from non- adequate wound cleansing and debris removal prior to swabbing

Swabs have also been postulated to collect small fraction of a millimeter of specimen(<0.1mls) as well as tendency to retain the collected specimen which greatly reduces the amount of bacteria that can be recovered from the swab for cultures, especially when fungal, mycobacterial, anaerobic and aerobic request are made from a single specimen[30].

The methods commonly used include swabs of wound exudate, Levine and Z stroke techniques. Other methods like dry and pre- soaked velvet pad, filter paper disk and cylinder scrubbing are not used routinely. The Levin technique involves rotating swab between fingertips with enough pressure to squeeze/ express wound fluid in an area $1cm^2$away from wound edges and skin margin after cleansing of wound, while in Z technique the swab is rotated between fingers and manipulated in 10-point position in zigzag manner across the wound without touching edges and skin margins [5].

The samples are then stored in appropriate container and transported to the laboratory in shortest time possible for semi-quantitative and qualitative analysis. Alginate tipped swab can facilitate performance of quantitative analysis given the right diluent as the alginate will dissolve and release all the organisms present on the swab tip Charcoal transport medium improves bacterial isolation by neutralizing toxic substances like fatty acids and is good for fastidious organisms while thioglycollate is good for anaerobes and fastidious organisms

Gardner et al (2006) in a study of 83 wounds with 36% infection rate found Levine technique to be more accurate compared to the other 2 with sensitivity of 90% and specificity of 57% at critical threshold of 37000 per swab. The concordance between Levin technique and tissue specimen was 78% in the same study with positive predictive value of 0.77 and negative predictive value of 0.91.Ronda's et al (2013) found that Levine technique identifies infection better than z stoke technique in both acute and chronic wound with biopsy as criterion standard. At a threshold of $3.7x\ 10^4$microorganisms per swab Levin had sensitivity of 0.90, specificity of 57%,positive predictive value of 0.77 and negative predictive value of 0.91. Angel et al (2011) In study on chronic wounds at 58% infection rate found Levine technique identified more organisms than z technique in both acute and chronic wounds ($p \leq 0.001$)[31].

2.3 Wound Tissue Biopsy

Biopsy is considered the gold standard for wound sampling for microbial culture both qualitative and quantitative, but at the same time is viewed as invasive, painful, labor intensive with increased financial burden to the patient, requiring killed operator to perform while exacerbating tissue damage and disrupts the wound bed from healing[27].

Wound biopsy can be done by use of scalpel, sterile curettes and punch biopsy techniques; biopsy provides both species identification(qualitative) as well as both number and density of organisms in the sample (quantitative). Nelson et al (2018)using sterile dermal curette/ scalpel reportedly identified more pathogens with tissue biopsy compared to swab technique in diabetic foot ulcers, at least one pathogen was identified in 86.1% for biopsies. 70.1% for wound swabs[32].Haalboom et al (2018) while comparing Levine technique to punch biopsy at the same site on open chronic wounds found no difference in the yield rate with swab identifying all organisms (100%) cultured from biopsy on 131wounds (72.8%)[33].

Copeland et al (2016) in a meta-analysis involving 23 studies of 2746 clinically infected and non-infected wounds of various etiologies found Levin technique (78%) to have better concordance with tissue biopsy compared to zigzag and needle aspiration[34]. At quantitative threshold of 3.7×10^4 organism Levine had an acceptable accuracy to biopsy. In the same meta-analysis tissue biopsy exhibited 100% sensitivity, 93% specificities and 95.1% accuracy in predicting wound closure after debridement and treatment of infected wound [36].

Pellizzer et al (2001) while comparing deep tissue punch biopsy vs. superficial swab culture on an ulcer base of diabetic foot wounds found biopsy had higher pick-rate of infection at 30 day follow up compared to superficial swab. Monitoring of antibiotic resistance strains was also done better with biopsy compared to swab techniques[35].Therefore, this study seeks to determine which swab technique between Levin and z strokes higher infection pick rate in chronic wounds with wound biopsy as a gold standard.

CHAPTER THREE: STUDY JUSTIFICATION

Infection is a major impediment to wound healing leading to wound chronicity. It contributes significantly to the cost of treatment and length of hospital stay [36-37]. There is paucity of comparative data on which swab techniques between z stoke and Levin gives better yield of micro- organisms in the diagnosis of chronic wound infection in our predominantly African setup. There has been no study done at Kenyatta national hospital comparing the two wound swab techniques in terms of infection pick rates on both acute and chronic wounds, this study narrowed down on assessing infection pick rates in chronic wounds.

3.1 Study Objectives

3.1.1 Main Objective

The main objective was to the compare effectiveness of Levin and z stroke wound swab techniques in diagnosis of infection with wound biopsy as gold standard among patient with chronic wounds at KNH.

3.1.2 Specific Objectives

a) To determine the sensitivity and specificity of Levine technique
b) To determine the sensitivity and specificity of z stroke swab technique

3.2 Methodology

3.2.1 Setting

The study was conducted at Kenyatta National Hospital surgical wards, medical wards, theatres and wound clinics from the 20th February to 20th of June 2020

3.2.2 Study Population

The target population were adults aged 18 years and above with wounds, that have lasted more than 4weeks' duration without healing at Kenyatta national hospital.

3.2.3 Study Design

The study was a comparative cross sectional study

3.2.4 Inclusion Criteria

Consenting patients 18 yrs. and above with wound lasting more than 4weeks' duration without healing

3.2.5 Exclusion Criteria

- Patients under 18 yrs.
- Allergy to local anesthesia
- Acute wounds
- Necrotic wounds
- Bleeding diathesis
- Pregnant women

3.2.6 Ethical Considerations

The study proposal was submitted to and approved by KNH Ethics and Research Committee. Patients were only recruited into the study after giving informed consent. All data collected was treated with confidentiality.

3.3 Sample Size

Sample size of a diagnostic study was calculated based upon literature review with local prevalence of 88% wound infection at KNH [37] and sensitivity of Levin wound swab technique at 90%(31)

$$TP + FN = z^2 \times \frac{(SN\,(1 - SN))}{W^2}$$

$$N(sN) = \frac{TP + FN}{P}$$

TP- true positive

FN- false negative

SN –sensitivity

Z – Confidence interval normal distribution value i.e. for 95% z = 1.96

P – The prevalence of disease in the test population

W- Accuracy= 0.05

N (Sn) = 3.842{0.09/0.0025}

=3.842x36/0.88

= 157

The sample size calculated was 157 patients.

3.4 Data Collection

Eligible candidates who met the inclusion criteria were consecutively enrolled in the study until sample size was achieved. Data collection was conducted by the principal researcher. It entailed consent taking then assigning a unique serial number to eligible candidates and filling in of data collection sheet. The duration of wound presence, etiology of the wound and the location of the wound was documented in the data collection sheet, before taking 2 swabs and a biopsy of the wound from each of the eligible candidates in an aseptic manner. The swabs were taken using a sterile cotton tipped swab while the biopsy was taken using sterile 3mm punches after infiltration of topical local anesthesia, the tissue obtained from biopsy was collected into a sterile bottle with 2mls of sterile saline 0.9% Personal protective equipment was used throughout data collection period with regards to COVID-19 personal

protection protocol. The specimens were delivered to the laboratory within1-2 hours of collection.

In the laboratory swabs were inoculated directly and then streaked on blood agar first then on Macconkey agar. The tissue from the biopsy bottle was weighed and homogenized and then serially diluted then with saline. 10 microliter was then retrieved from the solution. The 10 microliter solution was inoculated and streaked on both blood agar and Macconkey agar. Plates were then incubated overnight (18-24 hours) at 35-37degrees Celsius. Cultures were then examined for any microbial growth. Plates with no growth were again re-incubated a further 24hours period of time after which plates were discarded after showing no growth.

In plates that had growth isolated bacteria were first identified by carrying out identification tests given below

- **a)** Gram staining
- **b)** Coagulase test
- **c)** Indole test
- **d)** Citrate test
- **e)** Methyl red test
- **f)** Voges proskaner test

Once identified, antibiotic susceptibility tests were done, reading of which were qualified to be susceptible or resistant as per guidelines given by the European committee on antimicrobial susceptibility testing (EUCAST) Quantification of bacterial growth. CFU: shown by individual growth of colonies Levin/z-stroke- grown colonies counted. Biopsies: growth from 10 microliters picks counted and then count calculated by (miles and mistral formula Colony forming units per gram of tissue = count x dilution x volume/weight x0.01

N/B-10 microliters inoculated on agar plates was picked from a 1 in 10 diluted sample

Dilution made by adding 0.5ml from the mixed saline bottle of biopsy to 4.5mls sterile saline in test-tube.

3.5 Data Analysis

Data collected was analyzed with the aid of computer program me statistical package for social sciences (SPSS) software version 21. 2x2 contingency tables were constructed. Using data obtained from wound biopsy as gold standard, sensitivity and specificity of both Levin and z-stroke wound swab techniques were calculated, negative and positive predictive values as well as diagnostic accuracy of both Levin and z-stroke wound swab techniques were calculated.

3.6 Quality Control

The principal researcher collected the entire specimen pool using standard procedure as prescribed for each method. Patient's unique serial number was matched with both laboratory request form and specimen bottle labeling both at the bedside and upon specimen reception at the laboratory. All specimens were delivered to the laboratory within one to two hours of collection. At the laboratory specimen handling and processing was done by one laboratory technologist with knowledge and vast experience in specimen handling and processing.

CHAPTER FOUR:RESULTS

We recruited 145 eligible patients. The male to female ratio was 1.6:1 with age range of 18 -79 years, and a mean of 37.2years.Majority of the wounds were in the age group of 20-29years (see table 1) with surgical site infection predominating at31.7 %(see figure 3). Majority of the chronic wounds were in the lower limbs at 38% followed by trunk at 23.6% with abdomen distant 3rd at 18.6% head and neck was the lowest at 6.2 %(see figure 5).

Table 1: Patient Characteristics

Age	Frequency (N)	Percentage (%)
<20	7	4.8
20-29	46	31.7
30-39	38	26.2
40-49	25	17.2
50-59	14	9.7
≥60	15	10.3
Gender		
Male	90	62.1
Female	55	37.9

The mean age of the patients was 37.2 (SD=14.3) years.

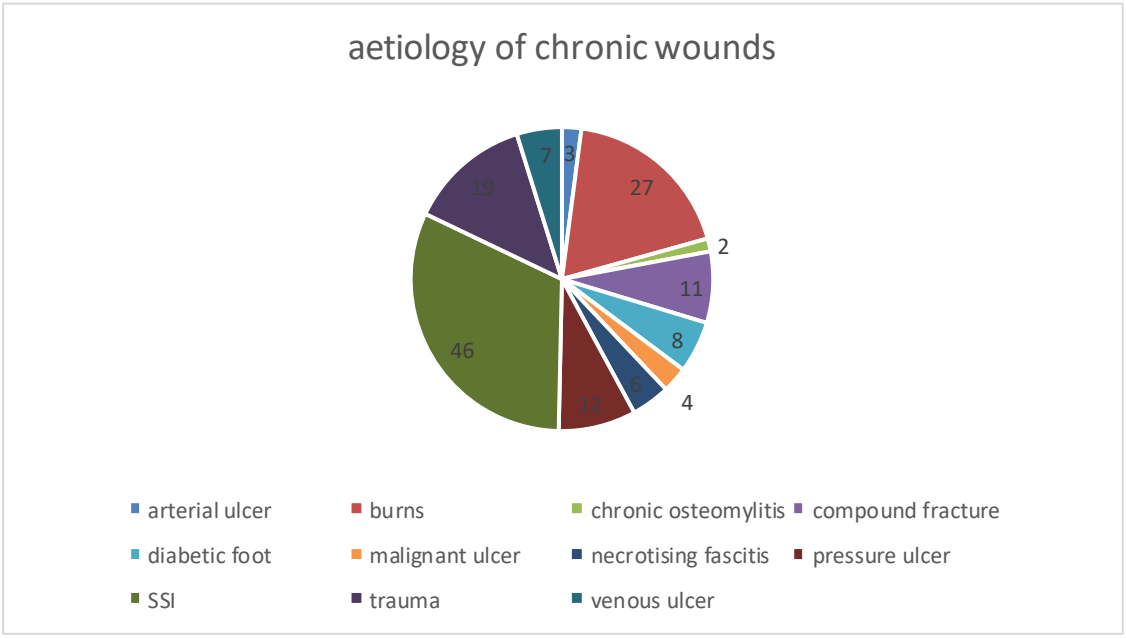

Figure 3: Etiology of chronic wounds

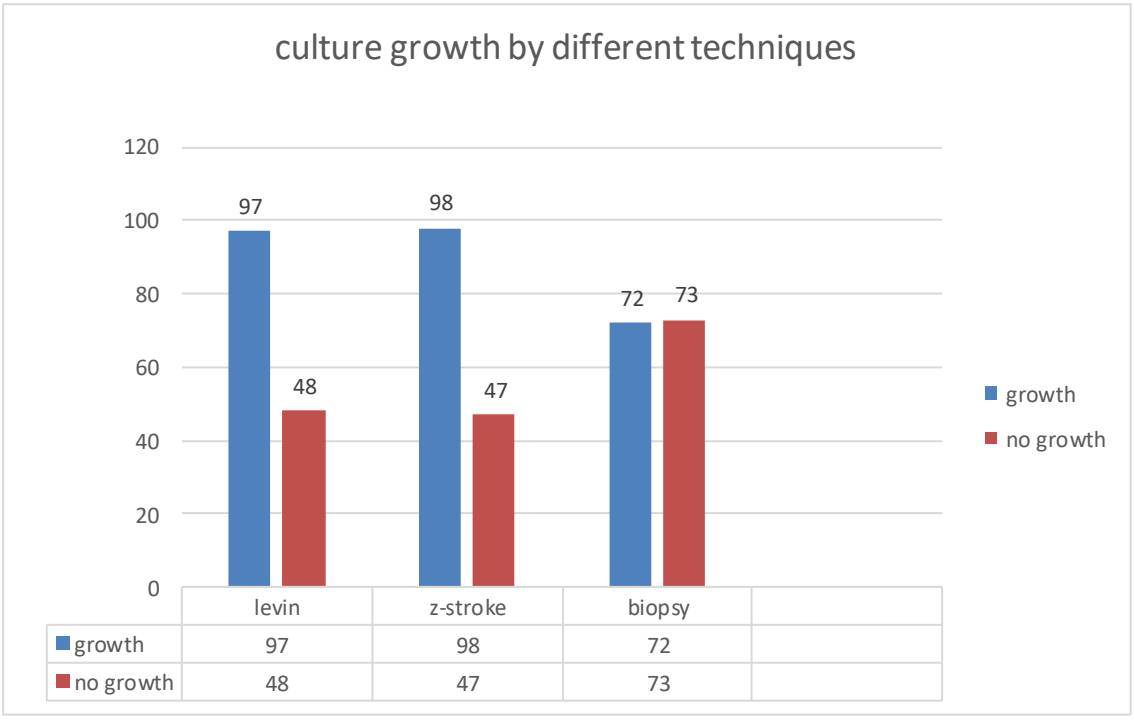

Figure 4:Culture growth by technique

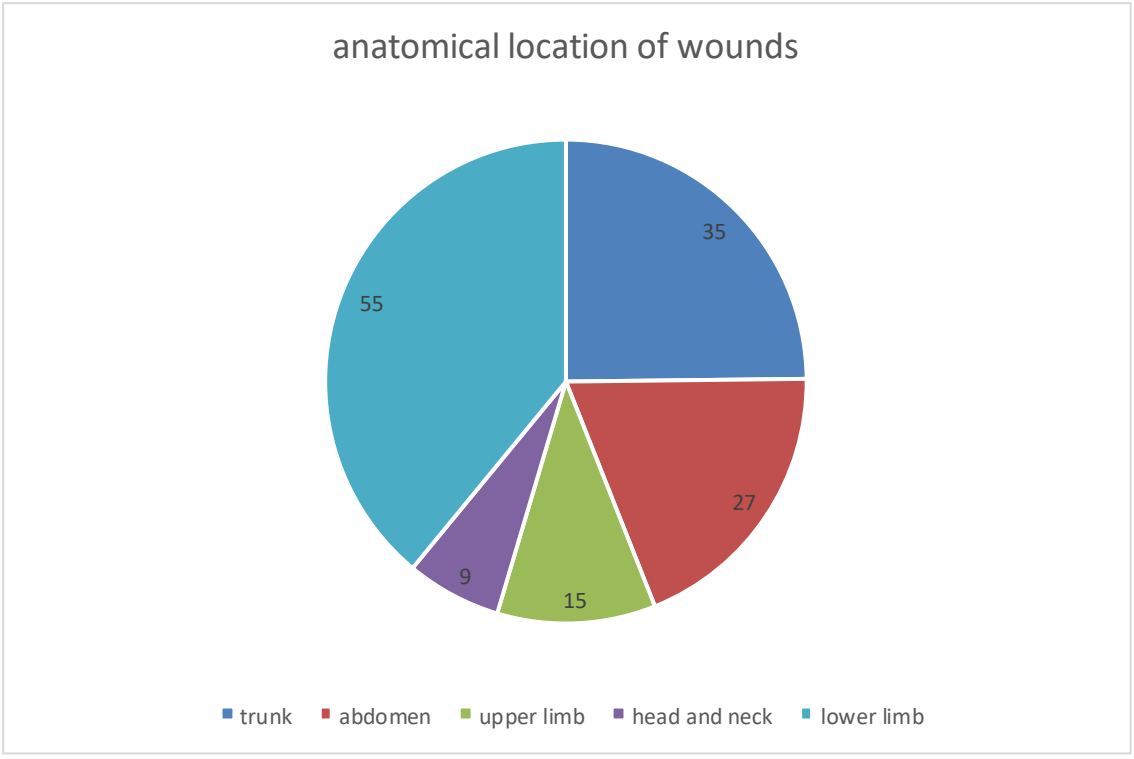

Figure 5: Anatomical location of wounds

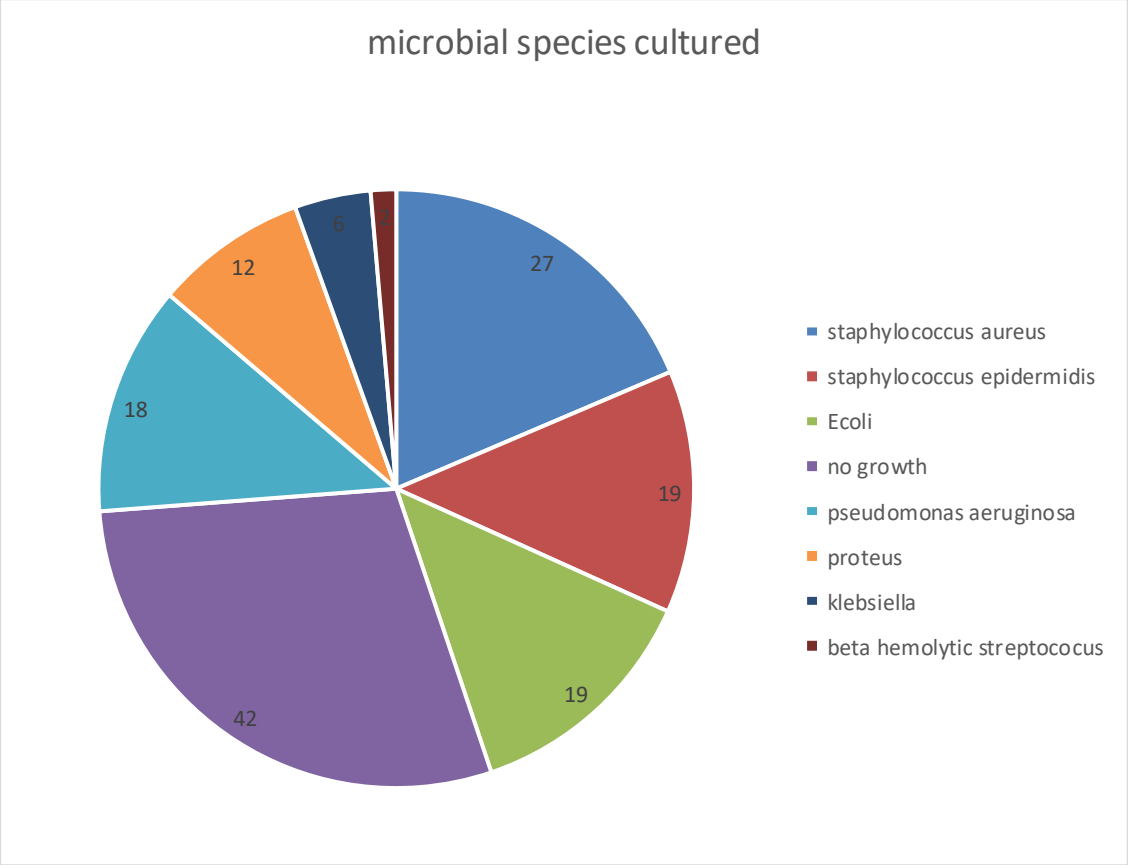

Figure 6: Microbial Species Cultured

Table 2: Sensitivity and Specificity of Levine Technique

		Biopsy				
		Positive	Negative	Total		
Levin	Positive	69	28	97	**PPV**	71.1%
	Negative	3	45	48	**NPV**	93.8%
Total		**72**	**73**	**145**		
		Sensitivity	**Specificity**			
		95.8%	**61.6%**			
Diagnostic accuracy		**78.6%**				

Table 3: Sensitivity and specificity of Z stroke swab technique

		Biopsy				
		Positive	Negative	Total		
Z Stroke	Positive	70	28	98	**PPV**	71.4%
	Negative	2	45	47	**NPV**	95.7%
Total		**72**	**73**	**145**		
		Sensitivity	**Specificity**			
		97.2%	**61.6%**			
Diagnostic accuracy		**79.3%**				

CHAPTER FIVE: DISCUSSION, CONCLUSION & RECOMMENDATIONS

5.1 Discussion

Chronic wounds reduce quality of life while increasing societal socioeconomic burden in terms of loss of productivity and actual cost of healthcare. Infections being a major contributory factor in chronic wound states among other conditions require timely diagnosis and management in both cost effective and less invasive manner. A technique that is both cost effective and less invasive is vital for both the physician satisfaction and good patient outcomes. This study was conducted to compare the effectiveness of the 2 swab techniques namely Levin and z stroke in picking wound infection in patients with chronic wounds at Kenyatta National Hospital.

The wound infection rate was 50% and majority of chronic wounds in this study were due to surgical site infection at 31.7% with burns coming 2nd at 18.6% necrotizing fasciitis was the last 0.7%. Kiplagat in his dissertation in 2018 involving 80 patients using Levin swab technique noted infection rate of 88% more than 4 x the rate in this study the difference could be due to the predominantly burns patient in his study with mixture of both acute and chronic wounds as well as the use of biopsy as gold standard in this study [37].in a previous study at KNH Bhatt in population of 292 post-operative patients found surgical site infection rate of 17.4% , the study unlike our study looked at acute postoperative wounds[36]. Regionally Oladeinde et al in rural tertiary hospital in Nigeria in a 5 year surveillance (2006-2010) in patients with wounds had an overall prevalence of wound infection of 70.1 % [3].

In terms of bacterial species staphylococcus aureus was the most common isolated organism at 18.6% followed by Escherichia coli and staphylococcus epidermidis at 13.1%, beta hemolytic streptococcus was the least isolated at 1.4%. This is in contrast to Karimi who found in population of orthopedic patients at KNH that the predominant bacteria causing wound infection was pseudomonas species at 42.6% with proteus mirabillis and staphylococcus aureus coming second both at 33% with serattia and acinetobacter species being the least causative agent at 0.9%([38]).

Kiplagat In predominantly burns patients at KNH had proteus mirabillis at 34.2% being the organism cultured most from burn wound infection with staphylococcus aureus coming second at 18.8% and pseudomonas aeruginosa coming third at10.5%.Oladeinde et al in rural tertiary hospital in Nigeria in a 5 year surveillance (2006-2010) in patients with wounds noted

staphylococcus aureus as the predominant organism in tandem with observation in our study[3,37].

Wound biopsy which was the gold standard yielded 49.7% infection rate out of the 145 specimens cultured with value of 1 x 10^5/gram of tissue organisms considered as infection. Levin had 66.9% of the sample showing positive growth for micro-organisms while z stroke had 67.6% of the samples showing positive growth for micro-organism

Levin had sensitivity of 95.8% with specificity of 61.6% with diagnostic accuracy of 78.6%. Z-stroke had sensitivity of 97.2% with specificity of 61.6% and diagnostic accuracy of 79.3%. The results from our study show both Levin and z stroke wound swab techniques to be comparable to each other in their infection pick rate in chronic wounds with biopsy as gold standard for diagnosis. As for the concordance between Levin and tissue biopsy was 95.8% while that for z-stroke and biopsy was 97.2%

The findings in these study is in contrast to a study by Gardner et al in Caucasian population who compared wound exudate, z-stroke technique and Levin technique with biopsy as gold standard, where Levin had a sensitivity of 90% with the concordance rate of Levin at 78% (in comparison with our study with sensitivity of 95.8% and diagnostic accuracy of 78.6%) which was higher than z-stroke and wound exudate while in our study rates are similar for both z –stroke and Levin wound swab techniques[25].Donna in 2009 also in a Caucasian population in comparative study involving 2 swab techniques in both acute and chronic wounds in 50 patients where Levin detected more organisms in both chronic acute wounds($t=12.04, p<0.01$) as compared to z-stroke[39].

Copeland in a systematic review of comparative studies of Levin vs. z-stroke noted that Levin was superior to z stroke swab techniques with wound biopsy was superior to Levin in antibiotic resistant wound infections and monitoring response to treatment [34].Nelson et al in a multicenter study involving 400 patients with infected diabetic foot ulcers in Caucasian population comparing concordance between Levin swab technique and wound biopsy. They noted staphylococcus aureus as the commonest micro-organisms cultured from the wounds which is in agreement with our study they also noted that wound biopsy picked more micro-organisms than Levin which is not in agreement with our study where the concordance rate for Levin was 95.8%[32].

5.2 Conclusion

The results of this study show an equal infection pick rate in chronic wounds between Levin and z-stroke swab techniques. This is in contrasts to previous studies that depict Levin wound swab technique to be superior to Z- stroke wound swab technique in diagnosis of infection in chronic wounds.

5.3 Study Limitations

a) This study focused only on chronic wounds and the results from the study are not reflective of wound infections as a spectrum because patients with acute wounds have been omitted from the study.

b) There was no growth of anaerobic organisms and hence not represented in the study results.

c) The study was conducted during COVID-19 pandemic presenting logistic challenges hence the original study population of 157 subjects was difficult to achieve due reduced patients turnover and hence only 145 patients were recruited into the study.

5.4 Recommendations

a) Following the results of the study either Levin or Z- stroke swab techniques can be used for diagnosis of infection in chronic wounds at Kenyatta national hospital as they both have high concordance rate with wound biopsy

b) A comparative study of Levin and z- stroke wound swab techniques with wound biopsy as a gold standard in diagnosis of infection in acute wounds and population under 18 years.

c) Similar study to be carried out with more emphasis towards capturing anaerobic micro- organisms in our culture medium.

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APPENDICES

Appendix: I (a) Levin Wound Swab Technique

Equipment:

Gloves

Sterile normal saline

Culture swab(s)

Sterile swab container

Laboratory requisition form(s)

10ml syringe (prefilled with 0.9% sodium chloride) – for irrigation

5 ml syringe (prefilled with 0.9% sodium chloride) – for moistening culture swab

Sterile gauze pad

Appropriate wound dressing

Biohazard bag

Procedure

1. Wash hands, apply gloves, remove soiled dressing and place in biohazard bag.
2. Cleanse wound by removing excess debris from the wound base by irrigating with normal saline. Thoroughly flush wound.
3. Gently wipe excess saline with a sterile gauze pad
4. Remove soiled gloves and cleanse with hand sanitizer
5. Apply sterile gloves
6. Moisten the culture swab with the 0.9% sodium chloride (a moist swab provides more accurate results than a dry swab).
7. Identify a small area (1 cm^2) of clean viable tissue and rotate the swab on it for 5 seconds while applying enough pressure to produce exudate. Avoid necrotic tissue and wound edges. A wound culture must be taken from clean tissue because pus or necrotic tissue will not provide an accurate profile of the micro flora contained within the tissue.
8. Insert swab into the sterile container.
9. Redress the wound and perform hand hygiene.
10. Assess the patient and ensure that any wound pain has been managed. (This is done initially and again during the process.)

11. Complete the lab slip and/or electronic document, including wound site, time the specimen was collected, and any antimicrobials the patient is receiving.
12. Send the specimen to the lab immediately (within 1 hour) to keep the specimen stable. If specimen must be stored, refrigerate immediately after specimen collection.
13. Sources: Cross, HH, Obtaining a wound swab culture specimen, Nursing. 2014 Jul;44(7):68-9

Appendix: I (b).Z Stroke Technique

EQUIPMENT:

Gloves

Sterile normal saline

Culture swab(s)

Container to transport specimen to lab

Laboratory requisition form(s)

10ml syringe (prefilled with 0.9% sodium chloride) – for irrigation

5 ml syringe (prefilled with 0.9% sodium chloride) – for moistening culture swab

Sterile gauze pad

Sterile swab container

Appropriate wound dressing

Biohazard bag

PROCEDURE

a. Wash hands, apply gloves, remove soiled dressing and place in biohazard bag.
b. Cleanse wound by removing excess debris from the wound base by irrigating with normal saline. Thoroughly flush wound.
c. Gently wipe excess saline with a sterile gauze pad
d. Remove soiled gloves and cleanse with hand sanitizer
e. Apply sterile gloves
f. Moisten the culture swab with the 0.9% sodium chloride (a moist swab provides more accurate results than dry swab).
g. Identify a small area (1 cm^2) of clean viable tissue and rotate the swab on it for 5 seconds in a zigzag motion and at the same time rotating between the fingers while applying enough pressure to produce exudate. Avoid necrotic tissue and wound edges. A wound culture must be taken from clean tissue because pus or necrotic tissue will not provide an accurate profile of the micro flora contained within the tissue.
h. Insert swab into the sterile container.
i. Redress the wound and perform hand hygiene.
j. Assess the patient and ensure that any wound pain has been managed. (This is done initially and again during the process.)

k. Complete the lab slip and/or electronic document, including wound site, time the specimen was collected, and any antimicrobials the patient is receiving.
l. Send the specimen to the lab immediately (within 1 hour) to keep the specimen stable. If specimen must be stored, refrigerate immediately after specimen collection.

Sources: Cross, HH, Obtaining a wound swab culture specimen, Nursing. 2014 Jul;44(7):68-9

Appendix I (c).How to perform a punch biopsy:

Equipment:

- Punch biopsy (2–8mm - 4mm most used)
- Basic dressing pack
- Antiseptic solution (Chlorhexidine 0.1% aqueous) or povidone iodine
- 2ml syringe and 25G hypodermic needle
- Lignocaine 1% +/- adrenaline 1:100,000
- Scalpel #15 blade
- microbiology specimen culture bottle
- Alginate dressing sheet
- Gloves, apron and goggles/face protection

Procedure:

- Use ‘aseptic, no touch technique’ with sterile gloves if
- Indicated in at risk patients
- Administer anesthetic to selected site:
- Usually a single bleb of 0.2ml into dermis immediately under chosen biopsy site/ drops of 1ml of 2% lignocaine hydrochloride at biopsy site effective within 1-3 minutes
- Wash hands and don gloves
- Prepare biopsy site (with 5cm margin if wound extensive) using aqueous chlorhexidine solution or normal saline
- Position punch vertically over the site
- Apply gentle downward pressure while rotating the barrel to cut into the tissue to the fatty dermallayer
- Withdraw punch
- Lift out the specimen by piercing with local anesthetic needle and slice off through fatty layer with scalpel
- Transfer to specimen to culture bottle. Specimen to be transported to the laboratory immediately or within one hour

Appendix II: Informed Consent (English Version)

Chronic wound infection pick rate comparing Levin and z stroke wound swab techniques with wound biopsy as gold standard at Kenyatta national hospital.

Introduction

This informed consent is for patients with chronic wounds admitted at KNH in the wards, in operation theatres and seeking consultation at various outpatient specialties clinics

We shall be requesting patients to participate in this research project at KNH wards, theatres and specialties clinics

The principal investigator is: Dr. Ogoye Makoyo Madaraka currently undertaking master of medicine degree in general surgery at the University of Nairobi.

Supervisors

Dr Ferdinard Nang'ole
MBCHB, MMED SURGERY (UON)
Lecturer department of surgery, university of Nairobi
Consultant plastic and reconstructive surgeon KNH

Dr Daniel Ojuka
MBCHB, MMED SURGERY (UON)
Lecturer department of surgery, university of Nairobi
Consultant general and breast surgeon KNH

Dr Anne Njeri Maina
MBCHB, MSC MICROBIOLOGY
Lecturer department of microbiology, university of Nairobi

This informed consent has 3 parts

- Information sheet; sharing information with those participating in the research project
- Certificate of consent; signature appended by those who agree to participating in the study
- Statement by researcher

Part 1

Information sheet

My name is Dr. Ogoye Madaraka, a postgraduate student at university of Nairobi pursuing master of medicine degree in general surgery. My work place is at Kenyatta national hospital

Purpose of the research

With the aim of improving wound care to patients presenting with chronic wounds at our facility, am conducting a study to see if these wounds are harboring bacteria and other microbes to a level that cause infection hindering the process of wound healing we will be comparing 2 swab techniques with wound biopsy as our main control for infection detection at Kenyatta national hospital.

Broad objective

The main objective of this study is to compare infection pick rate of Levin wound swab technique versus z stroke wound swab technique with wound biopsy as a control method to detect infection in patients with chronic wounds at Kenyatta national hospital

Study procedure

To conduct the study, the participants will be requested by the principal researcher or research assistant to provide information, that will consist of the patient's age and gender, what caused the wound and how long he/she has had the wound, antimicrobial use current/ recent and topical or systemic, cigarette smoking and a diagnosis of diabetes mellitus, hypertension and other comorbidities. This information will be recorded in a form. We will assign you a unique number to hide your identity and it's only the researcher who is privy to the information you give. Consent to participate in the study shall be obtained at that juncture if the participant is satisfied with the explanation and agree to participate in the study

Thereafter 2 different swabs will be taken from the wound. Local anesthesia will then be administered at the biopsy site and the participant will be given 3-5 minutes for anesthesia to take effect. During the process you may feel minimal pain which wears off immediately; wound biopsy will then be taken using a 3mm punch. The samples will then be taken for analysis at the laboratory. Where appropriate lab results will be shared with your doctor to facilitate your treatment. The rest of the information is confidential. Data analysis results arrived at from the research is used by doctors, health planners and policy makers to improve care of patients with chronic wounds locally, regionally, nationally and internationally.

Voluntary Participation

You are hereby invited to participate in the study. Your participation is voluntary and there is no penalty whatsoever for refusal to participate in the study. I and my research assistants will be glad to respond to your queries comprehensively

Confidentiality

The information the participants give are treated with confidentiality only the principal researcher and the research assistant are privy to the information given the other documents are assigned unique serial numbers for identification purposes

Benefits

There are no rewards financial and non- financial for participating in the study

Risks

There are no anticipated risks associated with participation in the study

Withdrawal of Consent

You are also free to withdraw from the study at any point that you may wish to, you are entitled to ask any question and seek clarifications before you make a decision to participate in the study

Consent

I----------confirm that the aim of this study and my role as a participant have been properly explained to me by Dr. Ogoye Madaraka/research assistant. I also acknowledge that I have read and understood the contents of this consent form/ the contents of this form have been read to me and I understood well the information contained there off, as a result I agree to the conditions explained and give consent for my participation or for---------- who is under my care by virtue of being unable to give consent or is a minor

Sign----------Ip/op no----------

Date---------- Witness----------

Sign---------- Date----------

If the patient/ participant is illiterate

I've been a witness to the accurate reading and explanation of the contents of consent form and that our concerns and questions have been addressed comprehensively by the researcher and/ his assistant, I confirm that the participant has given consent to participate in the study willingly

Witness name----------

Sign----------Date----------

If you have any queries, please feel free to contact the following:

Principal Researcher

Dr. Ogoye Madaraka

Department of surgery school of medicine university of Nairobi

P.O Box 19676 KNH Nairobi 00202

Mobile phone no 0721817238

Supervisors

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Department of surgery university of Nairobi

Mobile no 0733864249

Kenyatta National Hospital /University of Nairobi Ethics and Research Committee College of health sciences P.O Box 19676-00202 Nairobi telephone no (254-020) 2726300-9 extension

Statement by Researcher

I have gone through the information sheet/ consent form with the participant and/ translator explaining all the details in a manner that he/she understands best, the participant has also been made aware of the following

- The information given by him/her will be treated with confidentiality
- The result of data analysis from this study will be published in medical literature to be shared for academic purposes so as to help in decision making, planning and possibly change practice in management of patients with chronic wounds
- That participation or refusal to participate in the study does not compromise quality of care he/she will receive

I have given the participant an opportunity to ask questions concerning the study and that I have answered the questions correctly and in manner that the participant fully understood

I confirm that there was no coercion of the participant towards giving consent as this was voluntarily done

A copy of this informed consent has been provided to the participant

Name of researcher----------

Signature of researcher----------

Date----------

Appendix III: Informed Consent (Swahili Version)

Fomu hili la makubaliano ni la wagonjwa walio na vidonda vilivyochukua muda mrefu zaidi ya kawaida bila kupona katika wadi na kiliniki ya hospitali kuu ya Kenyatta

Tungependa kuwaomba wagonjwa wenye vidonda kama haya kushiriki katika utafiti huu ukiwa kwenye wadi, theatre au kliniki ya hospitali kuu ya Kenyatta

Mtafiti mkuu ni daktari ogoye madaraka ambaye anahitimu shahada ya upasuaji katika chuo kikuu cha Nairobi

Wahadhiri wahusika

- Daktari Daniel ojuka
- Daktari Ferdinard Nang`ole
- Daktari Anne Njeri

Fomu hili la makubaliano liko na sehemu tatu

- Maelezo kuhusu utafiti
- Sehemu ya makubaliano
- Ujumbe kutoka kwa mta

Maelezo

Jina langu ni Dk. Madaraka makoyo ogoye Nafasi yangu ya kufanya kazi iko katika hospitali ya kitaifa ya Kenyatta.nahitimu shahada ya upasuaji katika chuo kikuu cha nairobi.

Kusudi la utafiti

Kwa madhumuni ya kuboresha utunzaji wa jeraha kwa wagonjwa wanaowasilisha na jeraha sugu katika kituo chetu, ninafanya utafiti ili kuona ikiwa vidonda hivi vina bandia bakteria na vijidudu vingine kwa kiwango kinachosababisha maambukizi kuzuia mchakato wa uponyaji wa jeraha tutakuwa tukilinganisha 2 swab Mbinu zilizo na biopsy ya jeraha kama udhibiti wetu kuu wa kugundua maambukizo katika hospitali ya kitaifa ya Kenyatta.

Lengo kuu

Kusudi kuu la utafiti huu ni kulinganisha kiwango cha kuchukua maambukizi ya mbinu ya Levin jeraha la kuogelea dhidi y

a z mbinu ya jeraha la jeraha na biopsy ya jeraha kama njia ya kudhibiti kugundua maambukizo kwa wagonjwa walio na jeraha sugu katika hospitali ya kitaifa ya Kenyatta

Utaratibu wa kusoma

Kufanya utafiti, washiriki wataombewa na mtafiti mkuu au msaidizi wa utafiti kutoa habari, ambayo itajumuisha umri wa jinsia na jinsia, ni nini kilisababisha jeraha na ni muda gani alikuwa na jeraha, utumiaji wa antimicrobial / ya hivi karibuni na ya kimantiki au ya kimfumo, ya sigara ya sigara na utambuzi wa ugonjwa wa kisukari, shinikizo la damu na matibabu mengine. Habari hii itarekodiwa katika fomu. Tutakupa nambari ya kipekee ya kuficha kitambulisho chako na ni mtafiti tu anayeshughulikia habari unazotoa. Idhini ya kushiriki katika utafiti huo itapatikana katika mkutano huo ikiwa mshiriki ameridhika na maelezo na akikubali kushiriki katika utafiti

Baada ya hapo swabs 2 tofauti zitachukuliwa kutoka kwa jeraha. Anesthesia ya ndani basi itasimamiwa kwenye wavuti ya biopsy na mshiriki atapewa dakika 3-5 kwa anesthesia kuanza. Wakati wa mchakato unaweza kuhisi maumivu madogo ambayo huondoka mara moja; jeraha

biopsy kisha itachukuliwa kwa kutumia Punch 3mm. Sampuli zitachukuliwa kwa uchambuzi katika maabara. Ambapo matokeo sahihi ya maabara yatashirikiwa na daktari wako kuwezesha matibabu yako. Habari iliyobaki ni ya siri. Matokeo ya uchambuzi wa data yaliyofika kutoka kwa utafiti huo hutumiwa na madaktari, waandaaji wa afya na watunga sera kuboresha huduma ya wagonjwa walio na jeraha sugu ndani, kikanda, kitaifa na kimataifa.

Haki ya kushiriki utafiti

Kushirika katika utafiti huu ni kwa hiari ya mgonjwa hakuna anayelazimishwa.

Taadhima ya siri

Ujumbe utakaopeana ni wa siri , watu wengine hawataupokea isipokuwa mtafiti mkuu na msaidizi wake, ujumbe huo pia utatambuliwa kwa namba mahalumu na sio kwa majina yako

Gharama au Fidia

Haitakugharimu chochote wala hakuna fidia utakaolipwa kuhusika katika utafiti huu

Hatari Unayoweza Kupata

Kuhusika katika utafiti huu hautakudhuru kwa vyovyote vile

Haki ya Kujiondoa

Wale watakaokubali kushiriki pia wako huru kujiondoa kwa utafiti wakati wowote

Fomu ya Makubaliano

Nimeridhika na maelezo kuhusu utafiti huu, nahakikisha kuhusika kwa utafiti huu ni kwa hiari yangu. Nimepata muda wa kuuliza maswali nikapatiwa majibu kikamilifu.

Jina la mshriki----------

Sahihi ya mshiriki----------

Tarehe----------

Washiriki wasioweza kuandika na kusoma

Nahakikisha nimesomewa na kuelezwa kuhusu utafiti huu kwa lugha na namna ambaye ninaufahamu vyema.nimepata nafasi ya kuuliza maswali kuhusu utafiti, nimepata majibu kikamilifu ya maswali haya.nimekata kauli kuhusika kwa utafiti huu kwa hiari yangu

Jina la mshiriki----------

Sahihi au alama ya kidole ya mshiriki----------

Tarehe----------

Mshiriki wa utafiti huu akihitaji maelezo zaidi tafadhali wasiliana na:

Anwani za Wahusika

Mtafiti mkuu

Dkt. Ogoye Madaraka
Idara ya upusuaji chuo kikuu cha Nairobi
SLP 19676 KNH , Nairobi 00202
Nambari ya simu 0721817238

Dkt. Ferdinard Nang'ole
Mhadhiri idara ya upasuaji chuo kikuu cha Nairobi
SLP 19676 KNH, Nairobi 00202
Nambari ya simu 0733864249
KNH/UON- ERC
Shule ya utabibu,
SLP 19676 -00202 Nairobi
Nambari ya simu: (254-020) 2726300-9 EXT: 44355

Ujumbe Kutoka Mtafiti Mkuu

Nimemweleza mshirikikwa kusoma ujumbe ya utafiti huu ilivyochapishwa kwa maandishi, mshiriki pia amepata nafasi ya kuuliza maswali ambayo yamejibiwa kikamilifu.kujumuishwa kwa utafiti huu ni kwa hiari ya mshiriki na wala sio kwa lazimaNimehakikisha mshriki amefahamu ya kwamba kushiriki katika utafiti huu ni kwa hiari yake na anaweza kujiondoa wakati wowote ule atakavyo. Ujumbe atakaotoa mshiriki ni ya sirina Kushiriki au kutoshiriki kwa utafiti huu hakutadhuru matibabu anayopata.mwisho ni kwamba matokeo ya utafiti huu huends ukachapishwa kwa maandishi ya kisayansia ili kuboresha elimu ya mbinu za kutunza vidonda

Jina la mtafiti----------

Sahihi ya mtafiti----------

Tarehe----------

Appendix IV: Data Collection Sheet

1. code----------
2. age----------
3. Gender: male--------- female----------
4. Date of admission----------
5. Location of wound----------
6. Duration of wound presence----------
7. Date of wound biopsy and swab----------
8. Signs of wound infection noted during biopsy

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9. Antimicrobial use at time of biopsy

Topicalsystemic ☐ ☐

9.Wound Etiology

Diabetic Foot ☐ Arterial Ulcers ☐ Burns ☐

Venous Ulcer ☐ Diabetic Foot ☐ Others ☐

10. Wound biopsy microscopy culture and sensitivity

Growth Present: Yes ☐ No ☐

Microorganism grown----------

Antimicrobial sensitivity----------

Antimicrobial resistance----------

11. Levin swab technique microscopy, culture and sensitivity

Growth Present: Yes ☐ No ☐

Microorganism grown----------

Antimicrobial sensitivity----------

Antimicrobial resistance----------

12. Z stroke swab technique microscopy, culture and sensitivity

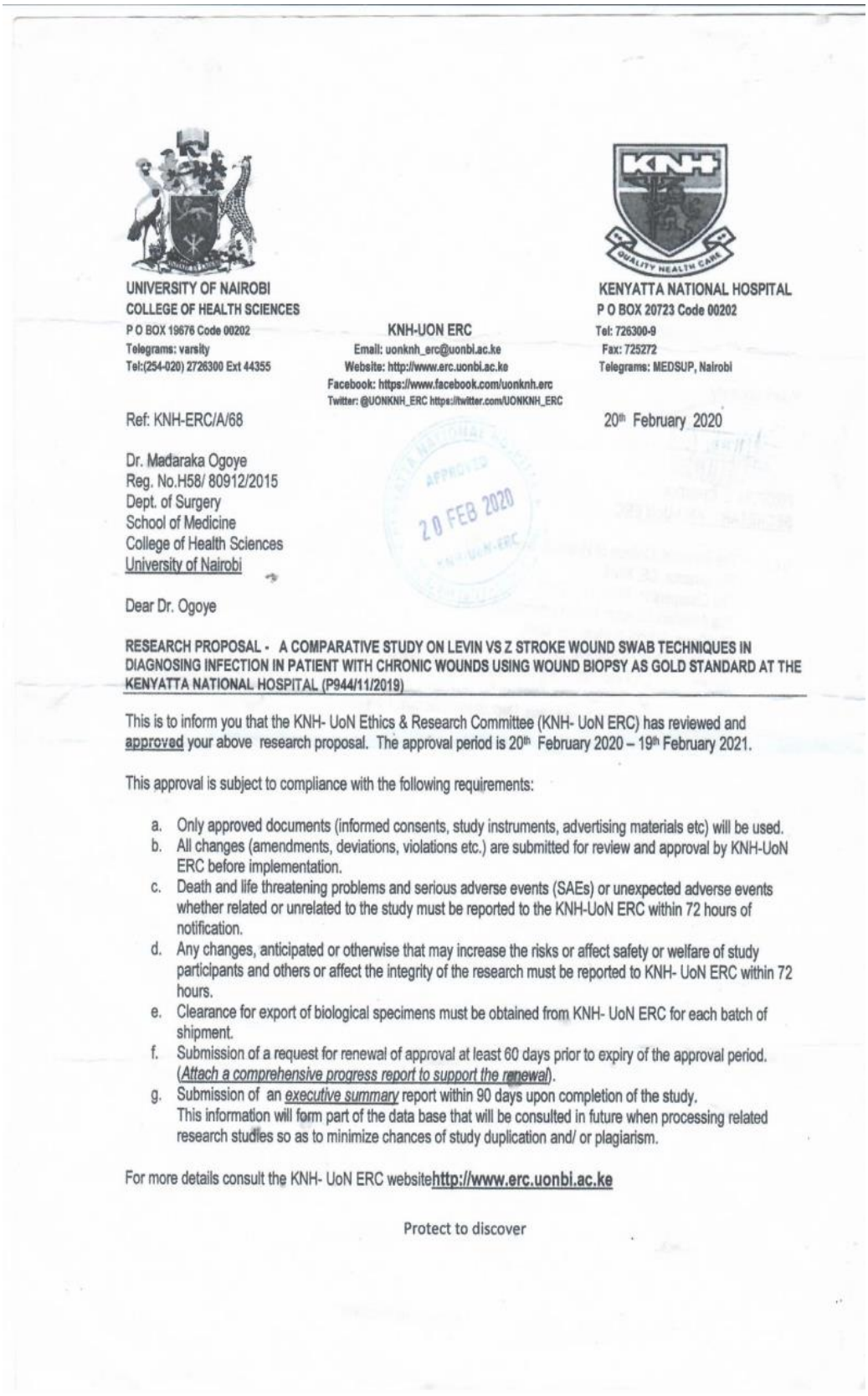
Growth Present: Yes ☐ No ☐

Microorganism grown----------

Antimicrobial sensitivity----------

Antimicrobial resistance----------

Appendix V: KNH/UON-ERC Letter of Approval



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KNH-UON ERC
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Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/68

20th February 2020

Dr. Madaraka Ogoye
Reg. No.H58/ 80912/2015
Dept. of Surgery
School of Medicine
College of Health Sciences
University of Nairobi

APPROVED
20 FEB 2020
KNH-UON-ERC

Dear Dr. Ogoye

RESEARCH PROPOSAL - A COMPARATIVE STUDY ON LEVIN VS Z STROKE WOUND SWAB TECHNIQUES IN DIAGNOSING INFECTION IN PATIENT WITH CHRONIC WOUNDS USING WOUND BIOPSY AS GOLD STANDARD AT THE KENYATTA NATIONAL HOSPITAL (P944/11/2019)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and approved your above research proposal. The approval period is 20th February 2020 – 19th February 2021.

This approval is subject to compliance with the following requirements:

a. Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b. All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
c. Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d. Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e. Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
g. Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Protect to discover

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Director, CS, KNH
The Chairperson, KNH- UoN ERC
The Assistant Director, Health Information, KNH
The Dean, School of Medicine, UoN
The Chair, Dept. of Surgery, UoN
Supervisors: Dr. Ferdinard Nang'ole, Dept.of Surgery, UoN
Dr. Daniel Ojuka, Dept. of Surgery, UoN
Dr. Anne Njeri Maina, Dept.of Microbiology, UoN

Appendix VI: Plagiarism Report

A Comparative Study On Levin Vs Z Stroke Wound Swab Techniques In Diagnosing Infection In Patient With Chronic Wounds Using Wound Biopsy As Gold Standard At The Kenyatta National Hospital.

ORIGINALITY REPORT

15%	7%	9%	11%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Loebl, E.C.. "The use of quantitative biopsy cultures in bacteriologic monitoring of burn patients", Journal of Surgical Research, 197401 Publication	2%
2	pdfs.semanticscholar.org Internet Source	2%
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