PREVALENCE OF *Staphylococcus aureus* AND *qacA/B* GENES ISOLATED FROM BURN WOUNDS AT KENYATTA NATIONAL HOSPITAL AND THEIR ASSOCIATION WITH MINIMUM INHIBITORY CONCENTRATIONS OF CHLORHEXIDINE

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U52/81012/2015

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN MOLECULAR PHARMACOLOGY

DEPARTMENT OF PHARMACOLOGY AND PHARMACOGNOSY

UNIVERSITY OF NAIROBI

JULY, 2022

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DEDICATION

To my dad, the late Patrick Paul Muiruri and mum, Jane Muiruri. I also dedicate this work to my husband James and daughters Naomi, Jane and baby Zoey. To my son, baby Jonathan; you are in our hearts forever.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to God for his goodness and favour throughout this journey. This would not have been possible without his grace.

Special gratitude goes to my supervisors; Dr. James H. Kimotho, Dr. Kipruto A. Sinei and Dr. Margaret Oluka for their mentorship and guidance that has enabled me to complete this work. I would also like to specially thank Prof. Faith Okalebo for her mentorship and kindness that enabled me to complete this thesis.

My sincere appreciation goes to Lucy Muita, (KEMRI-Culture Media Production) for her invaluable facilitation and support as I carried out my work at KEMRI. I also appreciate other members of staff of KEMRI for their technical support including Mrs. Anne Mwangi (Culture Media), Alex Maiyo (Centre for Viral Research (CVR)) and Mr. Misiani Ochwoto (CVR).

Special thanks go to Dr. Kimani Wanjeri, (Chairman, Department of Plastic Surgery) for his support and facilitation to access the patients at KNH burns unit and ward 4D. I would also like to sincerely thank Matron Obiero of ward 4D, as well as all the nurses and staff at ward 4D for enabling me to collect swabs from the patients.

I am also grateful to my husband and children for their prayers and support.

Lastly, I must sincerely thank the burns patients who graciously permitted me to collect their wound specimens as they braved the wound-dressing process. May God bless you.

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LIST OF ABBREVIATIONS AND ACRONYMS

Ala	Alanine
Asp	Aspartic acid
ATP	Adenosine Triphosphate
blaZ	Beta lactamase gene
DNA	Deoxyribonucleic Acid
HIV	Human Immunodeficiency Virus
ICU	Intensive Care Unit
KEMRI	Kenya Medical Research Institute
KNH	Kenyatta National Hospital
MBC	Minimum Bactericidal Concentration
MFS	Major Facilitator Superfamily
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MSSA	Methicillin-Susceptible *Staphylococcus aureus*
norA	Quinolone resistance gene
PCR	Polymerase Chain Reaction
PMF	Protein-Motive Force
S. aureus	*Staphylococcus aureus*
smr	small multi-drug resistance family
TSA	Trypticase Soy Agar

OPERATIONAL DEFINITIONS

Antiseptic: a substance which prevents or arrests growth of microorganisms on living tissue.

Biocide: a chemical agent capable of killing a living organism.

Disinfectant: a chemical agent used chiefly on inanimate objects to destroy or inhibit the growth of disease-carrying microorganisms.

Fomites: Objects or materials likely to carry infection such as clothes, utensils, or furniture.

Genotypic resistance: The presence of efflux-mediated resistance genes in an organism.

Minimum Bactericidal Concentration (MBC): The lowest concentration of an antimicrobial agent needed to kill 99.9% of the initial organism inoculum.

Minimum Inhibitory Concentration (MIC): The lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation.

Methicillin Resistant *Staphylococcus aureus* (MRSA): a strain of *Staphylococcus aureus* resistant to commonly used antibiotics such as penicillins and is responsible for difficult to treat infections.

Methicillin Susceptible *Staphylococcus aureus* (MSSA): *Staphylococcus aureus* bacteria sensitive to penicillins.

Phenotypic Resistance: Reduced susceptibility of an organism to an antimicrobial agent that is not due to genetic alteration. It presents as growth in biofilms, stationary growth phase or persistence.

Virulence factors: Molecules produced by bacteria or other microorganisms that enable them to invade the host, cause disease, and evade host defenses.

ABSTRACT

Background

Staphylococcus aureus (S. aureus) is one of the leading pathogens that cause severe infection of wounds of burns patients. Chlorhexidine has a broad-spectrum of activity and is therefore frequently used as a topical antiseptic agent in patients with wounds. Over the years, there have been reports of micro-organisms with lowered susceptibility to chlorhexidine. In Kenya, the prevalence of *S. aureus* with reduced susceptibility to chlorhexidine is unknown. In addition, the association between *qacA/B* genes and chlorhexidine resistance in *S. aureus* has not been determined in Kenyan isolates.

Objective

The main objective of this study was to determine the prevalence of *S. aureus* and chlorhexidine resistance genes *qacA/B* in *S. aureus* isolated from wounds of burns patients admitted at Kenyatta National Hospital (KNH) and their association with Minimum Inhibitory Concentrations (MICs) of chlorhexidine.

Method

The study design was a cross-sectional, hospital and laboratory-based study. Patients admitted at the burns ward who gave informed consent had swab specimens of their wounds cultured on Tryptic soy agar (TSA) with 5% sheep blood (KEMRI) for 24 hours at 37°C. *S. aureus* was identified by colony arrangement, gram stain, coagulase and catalase tests. Phenotypic resistance of *S. aureus* to chlorhexidine was determined by obtaining the MIC by broth dilution as described by the Clinical and Laboratory Standards Institute (CLSI 2010). DNA extraction was done by rapid lysis method using DNeasy® blood and tissue kit (Qiagen® USA). Amplification of *qacA* genes was performed by PCR using known primer sequences in the Applied Biosystems Veriti 96 well thermal cycler (ThermoFisher Scientific). PCR products were confirmed using 2% agarose gel electrophoresis and visualization with SYBR Green dye (ThermoFisher Scientific).

Results

One hundred (100) swab samples were obtained from participants with burn wounds showing signs of infection. There were 34 (34%) patients with *S. aureus* colonization in their burn wounds. No association was found between age, gender, site of the burn wound and frequency of infection with *S. aureus* (p = 0.215, 0.161, 0.311 respectively). The median MIC for chlorhexidine was 128µg/ml and growth of less than half of the *S. aureus* isolates [41.2% (14/34)] were inhibited at this MIC. The prevalence of *qacA/B* was 52.9% (18/34). MIC was not significantly different between the samples with *qacA/B* gene and those without the gene (p = 0.878).

Conclusion

There is a high prevalence of *S. aureus* amongst admitted burns patients and emergence of chlorhexidine-resistant pathogens due to the presence of *qacA/B* genes in over half of the isolates.

MICs were not statistically significant between isolates with *qacA/B* gene and those without.

Reduced susceptibility to chlorhexidine indicated by MICs > 4µg/ml in the hospital environment requires further investigation to confirm the consequences of using lower chlorhexidine concentrations.

CHAPTER ONE: INTRODUCTION

1.1 Background

The use of antiseptics and disinfectants is essential in hospitals for infection prevention (Sekiguchi *et al.*, 2004). These infections may be serious and result in increased morbidity, mortality, length of hospital stay and use of resources. Burn wounds serve as a suitable site for multiplication of bacteria and source of infection due to the long duration of hospital stay involved. *Staphylococcus aureus* frequently causes hospital-acquired infections in burns patients (Alebachew, 2012). In developing countries, it is estimated that 75% of mortality associated with burns is due to infection (Al-aali, 2016). In KNH, an average of 60 patients are admitted to the burns unit every month with varying degrees of burn wounds. The incidence of burn-related infections, similar to trauma has been found to be 50% due to primary endogenous infection (Kibor *et al*., 2014). In 2003, a study conducted by Nthumba and Oliech in KNH found the incidence of mortality arising from burn wound infections to be 13.3%.

Staphylococcus aureus is both a commensal bacterium and a human pathogen that is frequently isolated in hospitals (Alebachew, 2012). *S. aureus* causes a range of skin and soft tissue infections such as at surgical sites, abscesses, wounds, carbuncles and boils (Tong *et al*., 2015). It is also implicated in causing systemic infections. The success of *S. aureus* in causing disease can be attributed to host factors such as age, impaired immunity and the use of invasive devices. Properties of a specific *S. aureus* strain such as antibiotic resistance, virulence factors, ease of colonization and transmission and breaches in infection control and preventive measures also contribute to high rates of infection (Tong *et al*., 2015).

Chlorhexidine is commonly used as an antiseptic owing to its wide spectrum of activity, effectiveness and safety (Horner *et al*., 2012). Studies have reported reduced levels of sensitivity to chlorhexidine, particularly in Methicillin-Resistant *Staphylococcus aureus* (MRSA) (Horner *et al*., 2012). In KNH, chlorhexidine is one of the topical antiseptics used for burn wound cleansing. Other antiseptics in use include povidone iodine 10% solution, silver sulphadiazine 1% and normal saline 0.9% solution. *S. aureus* resistance to chlorhexidine is mediated by energy-dependent efflux pumps encoded by the *qac* gene family derived from plasmids (Vali *et al.*, 2008). *QacA* is commonly responsible for reduced susceptibility to chlorhexidine in *S. aureus.* The distribution of *qac* genes in *S. aureus* differs from one geographical location to

another (Conceição *et al.*, 2016). The differences in geographical distribution are influenced by factors such as the community being sampled, the prevailing infection-control policies, clonal expansion and pressure of frequent chlorhexidine use (Horner *et al.*, 2012). The prevalence of *qacA/B* genes has not been determined in Kenya. This study aims to obtain this knowledge as a basis for guiding chlorhexidine use for burn wounds.

1.2 Problem statement

In developing countries, it is estimated that 75% of mortality associated with burns is due to infection. A study conducted in KNH by Kinyua A.M (2013) found the prevalence of burn wound infection to be 86%. The use of antiseptics and disinfectants is important for infection prevention and control in hospitals. Several studies have reported reduced levels of sensitivity to chlorhexidine, particularly in Methicillin-Resistant *Staphylococcus aureus* (MRSA). In KNH, chlorhexidine is one of the topical antiseptics used on burn wounds. The use of chlorhexidine as a topical antiseptic for burn wound care without data on its effectiveness means that there is a possibility of evolution of new clones with increasing resistance. It is important to find out the prevalence of *qacA/B* genes and the risk factors for resistance to chlorhexidine.

1.3 Research Questions

1. What is the prevalence of *S. aureus* infection in burn wounds of patients admitted in Kenyatta National Hospital?
2. What is the prevalence of *qacA/B* genes in *S. aureus* isolates?
3. Is there a relationship between chlorhexidine MICs and the presence of chlorhexidine resistance genes?

1.4 Objectives

1.4.1 Main Objective

The main objective of this study was to determine the prevalence of *qacA/B* genes in *S. aureus* isolated from wounds of burns patients admitted in Kenyatta National Hospital and their influence on the effectiveness of chlorhexidine as an antiseptic.

1.4.2 Specific Objectives

1. To determine the prevalence of *S. aureus* infection in patients admitted with burn wounds at Kenyatta National Hospital.
2. To determine the prevalence of *qacA/B* genes in *S. aureus* isolates.
3. To establish the association between the MICs of clinical isolates and isolated genes.

1.5 Study justification

Chlorhexidine is regularly used in KNH to control infection. It is therefore important to determine its effectiveness as an antiseptic especially for burn wounds which are prone to infection. Genotypic resistance to chlorhexidine has not been determined in Kenya and this study aims to fill that gap since nosocomial infections are on the rise, resulting in increased patient morbidity and mortality.

1.6 Significance of the study

This study will present new insights into the effectiveness of chlorhexidine for topical cleansing of burn wounds.

Through the findings of this study, recommendations may be made to revise the disinfection policies of Kenyatta National Hospital.

Moreover, the analysis provided in this study will convey valuable information for future research that will explore the effectiveness of other topical antiseptics used in Kenyan hospitals.

CHAPTER TWO: LITERATURE REVIEW

2.1 *Staphylococcus aureus* infection

Staphylococcus aureus is a commensal and a pathogenic bacterium that accounts for a range of human diseases (Tong *et al.*, 2015). *S. aureus* belongs to the family Staphylococcaceae. They are Gram-positive cocci characterized as coagulase and catalase-positive. Their diameters range from 0.5 – 1.5 μm and form circular clusters with hemolysis when cultured on blood agar plates. They are facultative anaerobes, non-motile, and do not form spores (Harris *et al*., 2002).

S. aureus is a versatile organism that is able to adapt to different environments and colonize the anterior nares, skin, throat, perineum and gastrointestinal tract. Approximately 30% of healthy adults are colonized with *S. aureus* and are able to spread the bacteria among populations by physical contact and aerosol spread (Tong *et al.*, 2013). Persons colonized with *S. aureus* are more susceptible to develop infections, particularly patients with Type I diabetes, intravascular medical devices, intravenous drug users, Human Immunodeficiency Virus (HIV), and hemodialysis (Lowy, 1998).

Infections arising from *S. aureus* include: skin, wound and soft tissues, pneumonia, endocarditis, septic arthritis, toxic shock syndrome, osteomyelitis and septicemia (Stark, 2013). Nosocomial infections are acquired through fomites, hands of health care workers colonized by *S. aureus*, or transfer of bacteria from an infected patient through compromised skin or mucous membrane barriers (Lowy, 1998). Strains of *S. aureus* express many virulence factors that promote adherence to damaged tissue, enable evasion of antibody-mediated immune responses by binding to proteins in blood, and promote iron uptake (Foster, 2004). Many of these nosocomial infections are caused by strains of *S. aureus* that are resistant to multiple β-lactam antibiotics and are referred to as Methicillin Resistant *Staphylococcus aureus* (MRSA).

The Centers for Disease Control and Prevention (CDC) estimated that in 2011, more than 80,000 cases of invasive MRSA infections occurred in the Unites States resulting in 11,000 deaths (Petlin *et al.*, 2014).

2.2 Pathogenesis of burn wound infections

Burn wound infections are responsible for increased complications and death. About 75% of mortalities resulting from burn injuries are attributable to infections rather than osmotic shock

and hypovolemia (Alebachew, 2012). *S. aureus* is frequently isolated from burn wounds. Other bacteria found include *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and other coliform bacilli (Alebachew, 2012).

The skin's main function is protection from external insults. Following a burn injury, this barrier is destroyed. This interrupts the innate immunity and increases susceptibility to infection (Rowan *et al.*, 2015). Burn wound surfaces provide a favourable environment for the growth of micro-organisms since they are protein-rich and consist of avascular and necrotic tissue. In addition, the lack of vascular tissue on the burn surface impairs movement of immune cells to the wound and also hinders penetration of antibiotics to the wound site (Church *et al.*, 2006).

Immediately following a burn injury, wound surfaces are sterile. Colonization of the wound by different micro-organisms follows 2-7 days after the burn injury (Macedo-viñas, 2017). The source of these micro-organisms may be the host's normal flora from the respiratory or gastrointestinal tract or the hospital environment (Church *et al.*, 2006). Once the burn wound is colonized by micro-organisms, invasion into sub-adjacent tissue occurs, resulting in destruction of granulation tissue (Rowan *et al.*, 2015). Invasion of *S. aureus* into host tissues is enhanced by virulence factors such as coagulase, leucocidins, protein A and super antigens that enable evasion of the immune system, adherence and destruction of host tissues (Church *et al.*, 2006). Invasive infection can lead to septic shock and hypotension, resulting in multi-organ failure and ultimately death (Rowan *et al.*, 2015).

The main priority for burn wound patients should be to prevent and treat infection promptly. Many methods have been employed to prevent infection of burn wounds such as early wound debridement and the use of topical antimicrobial agents such as silver sulfadiazine, silver nitrate and chlorhexidine (Rowan *et al.*, 2015).

2.3 Chlorhexidine

Chlorhexidine is an antiseptic agent possessing topical antimicrobial activity (Horner *et al.*,2012). It is divalent, positively charged, and exists as salts of gluconate, acetate and hydrochloride. Chlorhexidine is widely available at 0.5-4% concentration (Milstone *et al.*, 2008).

Chlorhexidine produces activity depending on the concentration on the skin surface (Edmiston *et al.*, 2013). At low concentrations, its activity arises from its positively charged biguanide groups

reacting with the negatively charged microbial surface, thus destroying the integrity of the cell membrane. The phospholipid bridges cause displacement of the positive ions that normally maintain the cell membrane. This causes the membrane to become leaky to protons and potassium ions. When used at higher concentrations, chlorhexidine causes morphological destruction, resulting in cell death (Horner *et al.*,2012).

Chlorhexidine has a wide spectrum of antibacterial activity. This includes gram positive and negative bacteria. It is also active against viruses and fungi but it is not lethal to acid-fast organisms. Table 1 gives a summary of some microorganism species and chlorhexidine activity against them.

Table 2.1: Bacteriostatic activity of chlorhexidine against different microbial species (Karpinski et al., 2015)

Microorganism	**MIC (µg/ml)**
Staphylococcus aureus MSSA	0.25-8
Staphylococcus aureus MRSA	2-8
Enterococcus faecalis	4-16
Streptococcus mutans	0.9-4
Lactobacillus reuteri	0.125-4
Lactobacillus fermentum	0.25-1
Lactobacillus acidophilus	0.5-2
Porphyromonas gingivalis	0.9
Fusobacterium nucleatum	1.8
Escherichia coli	2-16
Klebsiella spp	8-16
Pseudomonas aeruginosa	16-32
Candida albicans	1-16
Candida tropicalis	75
Candida krusei	150
Aspergillus spp	8-64

Chlorhexidine is useful in infection-prevention procedures such as hand hygiene to reduce bacterial flora where it is combined with 70% isopropyl alcohol. It is also used in pre-surgical disinfection and before the introduction of peripheral and central vascular catheters (Donskey *et al.*, 2016). MRSA infection control in hospitals is another important use of chlorhexidine since it is used as a body wash for decolonization (Horner *et al.*,2012). Other uses of chlorhexidine in the hospital setting are summarized in Table 2.

Table 2. 2: Uses of chlorhexidine in the hospital setting (Horner et al., 2012)

Application	Commonly used dilution of chlorhexidine
Hand disinfection General use Pre-operative	0.5% (hand rub), 4% (liquid)
Pre-procedure skin disinfection Pre-surgical Insertion of vascular catheters	2% in 70% isopropyl alcohol (liquid)
Care of vascular catheters while *in situ*	2% in 70% isopropyl alcohol (gel)
Bathing patients in ICU	4% (liquid)
MRSA decolonization	1% (dusting powder), 4% (liquid)
Prevention of vascular catheter infections Impregnation of catheter site dressing Impregnation of catheter Oropharyngeal decolonization to prevent ventilator-acquired pneumonia	 2% in 70% isopropyl alcohol (gel) 425µg/cm 0.12% (rinse), 0.2% (rinse), 2% gel

The benefits of chlorhexidine include: its safety and tolerability, rapid onset of action and prolonged residual activity (Wang *et al.*, 2017).

2.3.1 *Staphylococcus aureus* reduced susceptibility to chlorhexidine

Resistance to biocides, just as with antibiotics may be intrinsic or acquired (Horner *et al.*, 2012). Intrinsic resistance to chlorhexidine occurs in bacterial spores and Mycobacteria whose outer cell capsule forms a barrier that prevents entry of the biocide molecules. Acquired resistance to chlorhexidine is mediated through efflux pumps which arise from genes found on plasmids (Hayden *et al.*, 2016). The efflux pumps are energy-dependent and require Adenosine Triphosphate (ATP) or the proton-motive force to drive them. These efflux pumps have a broad substrate specificity that includes antibiotics and antiseptics (Horner *et al.*, 2012).

Minimum inhibitory concentrations (MICs) are used to determine the sensitivity to chlorhexidine. For Staphylococci, an MIC $\geq$ 4µg/ml indicates chlorhexidine resistance (Horner *et al.*,2012). Table 3 gives a summary of the prevailing techniques used to determine reduced sensitivity to chlorhexidine.

Table 2. 3: Methods used to determine reduced susceptibility to chlorhexidine (Horner et al., 2012)

Method used to measure MIC	**Details and comments**	**References**
Agar dilution	Different concentrations of Chlorhexidine are incorporated into nutrient agar followed by application of a controlled number of bacterial cells to the surface of the agar. 5 or more doubling dilution concentrations are used and an MIC can be determined. Chlorhexidine may be tested at specific concentrations necessary for differentiation of susceptible, intermediate or resistant isolates (breakpoint agar method). A multipoint or spiral plate method can be used. Microbial contamination and heterogeneity are easily detected.	Wiegand, *et al*., 2008
Broth dilution	Serial dilution of Chlorhexidine into broth and addition of a controlled number of bacterial cells. Macrodilution or microdilution methods can be employed.	Noguchi *et al.*, 2005
MBC broth dilution	Similar to MIC by broth dilution, including neutralization, followed by inoculation of solid biocide-free agar with an aliquot from the broth.	Longtin *et al*., 2011
Time-kill study	Involves measurement of growth of bacteria exposed to a biocide in broth for different lengths of time (hours). Studying inactivation kinetics can provide useful information about clonal populations and aggregation depending on the shape of the resultant inactivation kinetic.	Cookson *et al*., 2002

2.4 Antiseptic resistance genes in *Staphylococcus aureus*

Genotypic resistance can be determined by the presence of bacterial plasmid-borne genes encoding multi-drug efflux pumps (Shi *et al*., 2015). The multi-drug efflux pumps are membrane proteins which play a role in protecting the bacteria from toxic substances including antiseptics, disinfectants and some classes of antibiotics such as β-lactams and fluoroquinolones (Wassenaar *et al.*, 2015).

To date, there are over 12 antiseptic resistance genes described in Staphylococci: *qacA – qac J, smr* and *norA*. These genes are classified into 2; Major Facilitator Superfamily (MFS) and small multi-drug resistance family (*smr*) depending on their DNA sequence homology, substrate-specificity, protein structure and plasmid-associated proteins coded for (Horner *et al.*,2012). The *qacA* gene was the first efflux-mediated pump to be sequenced on plasmid pSK1 from *S. aureus* isolated from hospitals (Costa *et al.*, 2013). The QacA pump confers resistance to a variety of structurally diverse compounds including monovalent and divalent cations, dyes, quaternary ammonium compounds, diamidines and biguanides (Wei *et al.*, 2012). The QacA protein belongs to the MFS (Liu *et al.*, 2019). QacA consists of 14 regions that traverse the membrane. The conserved regions located on the N-terminal are thought to be involved in generating the energy required to drive the efflux pumps, while the C-terminal consists of differing sequences that confer substrate selectivity (Horner *et al.*,2012). The *qacA* gene is most commonly associated with reduced susceptibility of chlorhexidine in Staphylococci. However, the presence of the *qacA* gene does not always translate into phenotypic resistance to chlorhexidine. Conversely, the *qacA* gene may be absent and Staphylococci may express reduced sensitivity to chlorhexidine (Horner *et al.*,2012). The *qacB* gene was first isolated on plasmid pSK23 and closely resembles *qacA* in sequence homology with the difference being in only 7 nucleotides (Liu *et al.*, 2019). As a result, Aspartic acid (Asp) is replaced with Alanine (Ala) at codon 323 (Horner *et al.* 2012). Thus, *qacB* confers resistance to monovalent antiseptics and dyes, but not divalent cations (Wei *et al.*, 2012). Figure 1 illustrates the transmembrane segment of QacA, and the amino acid differences between QacA and QacB (Paulsen *et al.*, 2006).

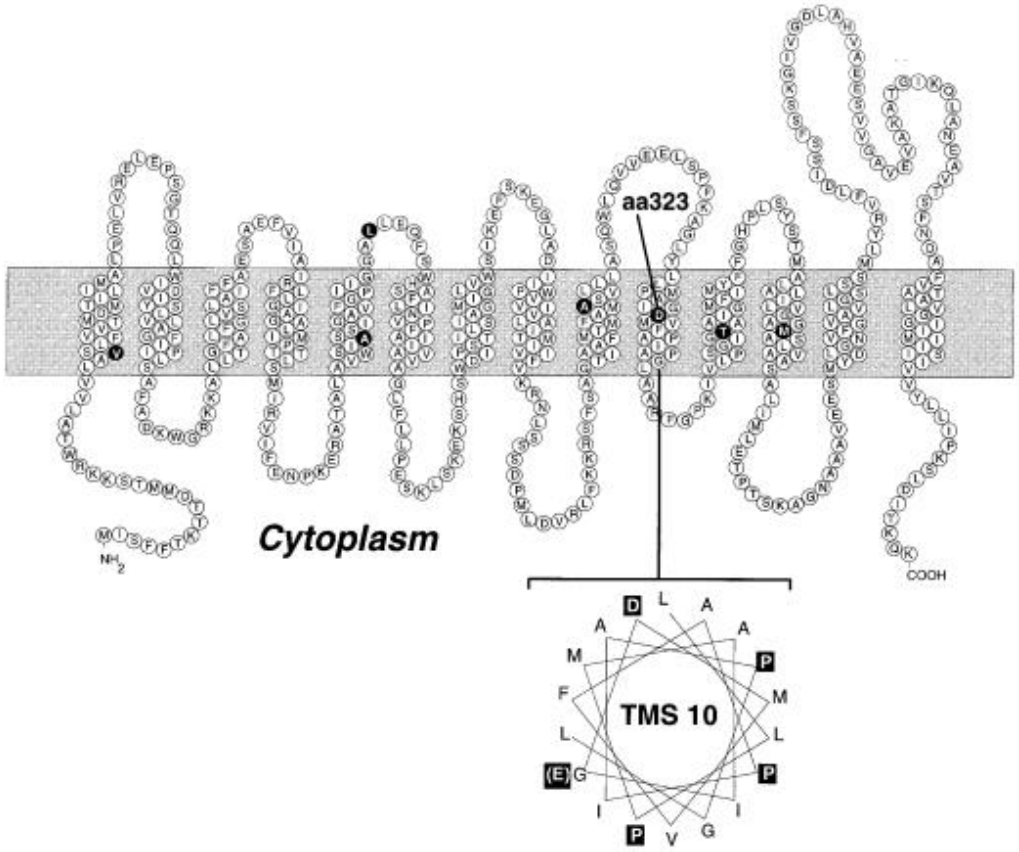

Figure 1: Two dimensional model of *S. aureus* QacA protein (Paulsen *et al.*, 2006).

The *qacC/D* gene (now known as *smr*) codes for proteins that promote efflux of quaternary ammonium compounds and intercalating dye ethidium bromide. *Smr* consists of 107 amino acids and 4 transmembrane segments and utilizes the proton-motive force (Costa *et al.*, 2013). Mutations in *smr* have been demonstrated to induce reduced susceptibility to chlorhexidine resulting in MICs > 4mg/L. The mutations are thought to arise from base-pair substitutions that result in a change in substrate specificity, but the precise mechanism of resistance remains unknown (Horner *et al*., 2012).

The *norA* gene codes for NorA protein. NorA belongs to the MFS and consists of 388 amino acids, with 12 transmembrane segments (Costa *et al.*, 2013). NorA is able to eject several compounds with differing structures and chemical compositions such as ethidium bromide, quaternary ammonium compounds, and fluoroquinolones (Costa *et al.*, 2013).

Other plasmid-borne *qac* genes, *qacG, qacH and qacJ* have been isolated from animals and food but their frequency and effect on antiseptics used in clinical practice remains unknown (Ignak *et al*., 2017).

2.4.1 Prevalence of *qacA/B* genes and importance of reduced susceptibility to chlorhexidine

The occurrence of *qacA/B* and *smr* genes in Staphylococci vary according to a country's location (Conceição *et al.*, 2016). These variations are as a result of factors such as differences in infection-prevention strategies, the nature of the population under study, clonal spread and the pressure of chlorhexidine use (Horner *et al*., 2012). Reported prevalence of *qacA/B* gene includes; 1% in the Eastern United States, 10% – 20% in the United Kingdom and 80% in Brazil (Longtin *et al.*, 2011). A summary of the prevalence of *qacA/B* genes is given in Table 4.

The widespread use of chlorhexidine in hospitals has resulted in bacteria with reduced susceptibility (Hardy *et al.*, 2018). The mechanism by which this occurs is by up-regulation of MDR efflux pumps. This may provide a survival advantage to microorganisms and low level resistance to other antimicrobial agents which are substrates of these pumps (Huet *et al.*, 2019). An increase in the numbers of MRSA isolates giving chlorhexidine MICs ≥ 4mg/L increased from 1.7% in 1990 to 46.7% in 2005 following use of 4% chlorhexidine for hand hygiene (Wang *et al.*, 2008). In addition, a U.K hospital that executed an ICU Chlorhexidine-based policy recorded an increase in transmission of MRSA in strains expressing chlorhexidine resistance, but a reduction in strains without *qacA/B* genes (Fritz *et al.*, 2013). This suggests that there is a need to monitor the possibility of bacterial strains with reduced susceptibility to chlorhexidine (Hayden *et al.*, 2016).

In Kenya, the susceptibility of *S. aureus* to chlorhexidine has not yet been determined. Chlorhexidine is regularly used in KNH to control infection. It is therefore important to determine its effectiveness as an antiseptic especially for burn wounds which are prone to infection. Genotypic resistance to chlorhexidine is most commonly conferred by *qacA/B* gene and this has not been determined in Kenya. This study aims to fill that gap since nosocomial infections are on the rise, resulting in increased patient morbidity and mortality.

Table 2. 4: Global prevalence of qacA/B (Horner et al., 2012)

Study location	**Single/Multicentre study**	**Year of isolate collection**	**Total *S. aureus***	***qacA/B* (%)**	**Clinical/Non clinical**	**Hospital (H)/Community(C)**	**Reference**
Scotland	Multi	Not specified	88	11.4	Clinical	HC	(Smith *et al*, 2008)
Taiwan	Single	2008-2009	156	28	Clinical	HC	(Ho *et al.*, 2012)
Japan	Single	2003	65	52	Unspecified	H	(Sekiguchi *et al.*, 2004)
Malaysia	Single	2011	259	27	Clinical	H	
Spain	Single	1997-2006	111	2.7	Carriage	C	(Ghasemzadeh *et al*., 2014)
China	Multi	2008-2012	499	5.2	Clinical/environmental	H	(Sekiguchi *et al.*, 2004)
Brazil	Multi	2002-2003	74	80	Unspecified	H	(Miyazaki *et al.*, 2007)
Korea	Multi	2006-2009	446	9	Clinical	H	Lee *et a*l., 2012
California	Multi	2008-2011	829	0.6	Carriage	Hospice	(Mcdanel *et al.*, 2013)
USA/Germany	Multi	2009	689	0.7	Clinical/environmental	H	(Kosmidis *et al.*, 2012)
Australia	Single	200-2009	151	65-94.7	Clinical	H	(Mcdanel *et al.*, 2013)
Canada	Multi	2005-2009	334	2	Clinical/carriage	H	(Longtin *et al.*, 2011)
Belgrade	Single	2007-2009	100	17	Clinical	H	(Opacic *et al.*, 2010)

CHAPTER THREE: METHODS

3.1 Study design

The design was a cross-sectional, hospital and laboratory-based study. It involved review of patient files to obtain their biodata, collection of wound swabs, overnight culture to isolate *S. aureus*, determination of phenotypic resistance using MICs, DNA extraction of *S. aureus* isolates, PCR to amplify *qacA/B* gene and lastly sequencing of *qacA/B* gene to establish similarities with others from clinical isolates.

3.2 Study site

The study was carried out at the burns unit and burns ward of KNH and at KEMRI, Nairobi. KNH was founded in 1901 as the native civil hospital. It is currently the largest referral and teaching hospital in Kenya with a bed capacity of 1800 and over 6000 staff members. The burns unit offers specialized treatment and care for patients with burn wounds and admits an average of 60 burn patients monthly. When these patients are satisfactorily stabilized, they are transferred to Ward 4D for continued monitoring and cleansing of their burn wounds.

KEMRI is a state corporation established through the Science and Technology (Amendment) Act of 1979. The Act established KEMRI as a national body mandated to carry out health research in Kenya. KEMRI, Nairobi is located off Mbagathi Road.

3.3 Study population

The study population was male and female patients of all ages with burn wounds admitted in KNH from January to June, 2017.

3.4 Inclusion and Exclusion criteria

Patients who were included in the study were males and females of all ages with burn wounds showing signs of infection admitted at KNH. In addition, the patients who met the inclusion criteria gave informed consent to participate, or proxy consent was given by relatives. Parents and guardians of children under 8 years of age gave informed consent for their children to participate in the study. Patients who were excluded from the study were those who declined to give consent or proxy consent was denied by their relatives. Patients who had fresh burn wounds that were less than 48 hours old were also excluded from the study.

3.5 Sample size calculation

The main outcome of interest was the prevalence of Chlorhexidine resistance genes. Therefore, the formula for estimation of sample size for prevalence studies is as shown in equation (1). (Pourhoseingholi *et al*., 2013):

$$n = \frac{Z^2 P(1-P)}{d^2}$$ ---------- Equation 1 (Fisher's formula)

Where,

n = Sample size

P = Expected prevalence or proportion (0.9%)

d = Absolute precision (5%)

$Z = Z$ Statistic for 95% confidence level (1.96)

$$n = \frac{1.96^2 \times 0.9(1-0.9)}{0.05^2}$$

$$n = 138$$

Because the study population was expected to be small, the Cochran formula for a finite population correction factor was applied: Equation (2).

$$n = \frac{n_o}{1 + \frac{(n_o - 1)}{N}}$$ ---------- Equation 2 (Cochran formula)

n = adjusted sample size

n_o = calculated sample size for infinite population

N = assumed population of patients admitted at the burns unit.

An assumption that the hospital admitted about 300 burns patients in a period of 5 months was made (average of 60 patients admitted per month). The adjusted sample size was a minimum of 95 patients. This was adjusted by about 5% to cater for biases arising from incomplete data.

$$n = \frac{138}{1 + \frac{(138 - 1)}{300}}$$

n = 94.5

As a result, 100 patients were sampled.

3.7 Sampling and participant recruitment

Patients were sampled by convenience sampling technique. This involved selecting patients who were on the daily wound-dressing list prepared by the nurses at the surgical ward 4D. The wound dressing process would begin at 9:00am thus by 8:00am, the patients would be taken through the consenting process with the help of the screening and eligibility form on Appendix A. Patients who gave their consent and met the inclusion criteria were enrolled to participate in the study. Proxy consent was obtained from relatives for patients who were unable to consent for themselves.

3.8 Data Collection

Data collection procedures were divided into five sections:

3.8.1 Collection of swabs and review of patient files

Wound samples were obtained from patients who satisfied the inclusion criteria. Specimens were collected after cleansing the wound, from the sites showing signs of infection such as malodor, redness and presence of pus. Sterile cotton swabs were used to collect specimens and these were then immersed in Stuart transport media (HiMedia Laboratories Pvt. Ltd., India) and capped tightly, labelled and protected from direct light and heat. Data pertaining to age, sex, cause and site of burn wound and duration of burn wound at the time of the study was abstracted from patient files using a case report form as outlined in Appendix B.

Samples were then transported to KEMRI-Nairobi microbiology laboratory for culture.

3.8.2 Culture of swabs to isolate *Staphylococcus aureus*

The swabs were inoculated using aseptic technique on Trypticase soy agar (TSA) with 5% sheep blood (KEMRI) and incubated at 37°C for 24 hours. The selected colonies were sub-cultured overnight on TSA at 37°C. Gram staining was performed to identify gram positive cocci. Next, catalase test was performed. Briefly, few drops of 3% hydrogen peroxide solution (Rosak industries Ltd, Nairobi, Kenya) were placed on a slide and a few colonies of

the test organism immersed onto the solution using a clean wooden stick. Active bubbling on the glass slide was considered as catalase positive.

Coagulase test was performed to identify *Staphylococcus aureus* using the tube test method. Three small test tubes were labelled T-test organism, Pos-positive control (*S. aureus* broth culture) and Neg- negative control (sterile broth). Plasma (0.2ml) was pipetted into each tube and 0.8ml test broth culture pipetted into tube 'T', 0.8ml of *S. aureus* culture into tube 'Pos' and 0.8ml of sterile broth into tube 'Neg'. The contents of each tube were mixed gently and incubated at 35°C for 1 hour up to overnight (to allow for clotting to occur). After incubation, the tubes were gently tilted to check for clotting. Clotting observed in tube 'T' positively identified *S. aureus* (Cheesbrough, 2006).

Identification of *S. aureus* was also done by incubating selected colonies on Mannitol Salt Agar (KEMRI) overnight at 37°C and checking for fermentation of mannitol on the plates.

The selected colonies of *S. aureus* were suspended in Trypticase Soy broth (TSB) in 20% glycerol (KEMRI) and stored at -80°C.

3.8.3 Determination of phenotypic resistance to Chlorhexidine

The MIC was determined by broth macrodilution method. Solutions were prepared by making 1ml two-fold dilutions of chlorhexidine 5% solution (Hexon® Biodeal Laboratories Ltd) at concentrations ranging from 0.125µg/ml to 512µg/ml using sterile distilled water in sterile tubes and caps. Next, inocula of *S. aureus* were prepared by the direct colony suspension method from a 24 hour 37°C TSA (KEMRI) plate by suspending colonies in sterile distilled water. The suspension was adjusted to achieve a turbidity equivalent to 0.5 McFarland. The 0.5 McFarland standard was prepared by combining 0.05ml of 1% barium chloride with 9.95ml of 1% sulphuric acid to form a turbid suspension. The density of the suspension of bacterial cells was compared to the 0.5 McFarland standard by holding the suspension and McFarland turbidity standard in front of a light against a white background with contrasting black lines. The density was adjusted by adding sterile distilled water or bacteria if the density was too heavy, or not sufficient respectively.

This suspension was added into each tube containing different chlorhexidine concentrations in equal volumes of 20µl. The tubes were shaken thoroughly to mix the solutions. Next, 1µl sterile loops were used to streak the tube contents onto Mueller Hinton agar plates (KEMRI) and thereafter incubated at 37°C overnight in an ambient air incubator. *S. aureus* strains

ATCC 25923 was included as a negative control with each of the samples. The MIC was determined by checking the lowest concentration of chlorhexidine in which there was no visible growth of *S. aureus* on the Mueller Hinton agar plates (Franklin *et al.*, 2012).

3.8.4 Genomic DNA extraction

DNA extraction was carried out using DNeasy® Blood & Tissue Kit (QIAGEN®) according to the manufacturer's instructions. *S. aureus* isolates previously stored at -80°C were inoculated onto Mueller-Hinton agar plates overnight at 37°C. Next, selected colonies were used to prepare suspensions using 1ml sterile distilled water in eppendorf tubes. These suspensions were centrifuged at 1500 rpm to form a pellet which was then re-suspended in 200µl Phosphate Buffered Saline (QIAGEN®), followed by 20µl proteinase K. Lysis buffer was then added to each tube in volumes of 200µl and mixed by vortexing. The samples were then incubated at 56°C for 10 minutes and thereafter 200µl absolute ethanol was added to each tube and mixed by vortexing. This mixture was pipetted into a DNeasy mini spin column placed in a 2ml collection tube and centrifuged at 8000 rpm for 1 minute. The flow-through and collection tubes were discarded and placed in new 2ml collection tubes. Wash buffer of 500µl was added to the spin columns and centrifuged for 1 minute at 8000 rpm. The flow-through and collection tubes were discarded and placed in new 2ml collection tubes as before. This process was repeated and the collection tubes were centrifuged for 3 minutes at 14000 rpm and the flow-through and collection tubes discarded thereafter. The spin columns were then transferred to new 2ml eppendorf tubes and the DNA was eluted by adding 200µl elution buffer to the centre of the spin column membrane. After incubation for 1 minute at room temperature, the columns were centrifuged at 8000 rpm for 1 minute and the eppendorf tubes capped and stored at -20°C in preparation for PCR.

3.8.5 Amplification of chlorhexidine resistance gene *(qacA/B)*

Amplification of *qacA* gene was performed by PCR using primer sequences (qacA/B-Forward primer CTATGGCAATAGGAGATATGGTGT and qacA/B-Reverse primer CCACTACAGATTCTTCAGCTACATG) (Fritz *et al.*, 2013). Primer solutions (Inqaba Biotech®) were prepared according to the manufacturer's instructions to obtain a 100µM stock solution. A working solution of each primer was prepared to 20µM. Next, a PCR master mix was prepared for each sample as shown in Table 5.

Table 3. 1: Volumes of contents of PCR master mix for amplification of qacA/B

Solution	**Volume (µl)**
10X buffer	2.5
Magnesium Chloride 10µM	2
dNTPS	0.5
Primer 1 (F)	0.5
Primer 2 (R)	0.5
Taq	0.2
Bovine Serum Albumin (BSA)	0.2
DNA	2
Nuclease-free water	16.6
Total volume	**25**

The solutions were then placed into a Veriti (Applied Biosystems) 96 well thermal cycler and denaturation was done for 7 minutes at 94°C. Thereafter, 35 cycles of PCR were performed as follows: 30 seconds of denaturation at 94°C, annealing for 30 seconds at 56°C, and extension for 30 seconds at 72°C. A final extension step was performed at 72°C for 7 minutes to give an expected amplicon of 321bp. *S. aureus* strains ATCC 25923 and NB01264 were used as negative and positive controls respectively with each PCR run. Amplification products were visualized using UV by a 2% agarose gel stained with Sybr green® (ThermoFisher Scientific).

3.9 Data Management

The quantitative data was entered into an Excel 2016 database. To ensure that data obtained remained confidential, serial numbers were used instead of names to identify patients. All information in soft copy was stored in password controlled files which were only accessible to the researcher. Data collection tools were piloted and the feedback obtained used to make the necessary adjustments.

3.10 Data analysis

Descriptive data analysis was first done using SPSS version 20. All continuous variables were summarized as the median and the Interquartile range (IQR). Categorical variables were summarized as the frequencies and percentages.

Chi - square test was used to examine the associations between categorical variables.

Odds ratios were used to determine associations between presence of *S. aureus* and categorical data of the patients.

A p value of < 0.05 was considered statistically significant.

3.11 Ethical considerations

Authority to conduct the study was obtained from Kenyatta National Hospital – University of Nairobi Ethics and Research Committee on 23rd January 2017 (Appendix I) and KEMRI Nairobi on 18th April 2017 (Appendix J). The nature of the study was fully disclosed to the participants. Patients and parents or guardians of children under the age of 18 years were required to give consent to participate in the study.

3.12 Quality Assurance

To ensure confidentiality, unique patient identifiers were used and all documents kept under lock and key.

CHAPTER FOUR: RESULTS

4.1 Overall prevalence of *Staphylococcus aureus* infection

The first objective of the study was to determine the overall prevalence of *S. aureus* infection among the patients with burn wounds in KNH. A total of 100 swabs were collected from participants with burn wounds during the study period. *Staphylococcus aureus* was isolated from 34 (34%) of the swabs while 66 (66%) were negative for *Staphylococcus aureus*.

4.1.1 Medical history and socio-demographical characteristics of patients with burn wounds

Majority (40%) of the patients admitted with burns were less than 10 years of age. The second most populous category was aged between 21-25 years at 16%, followed by ages 26-30 and 11-15 years at 10% each. The minority (6%) of patients were above 40 years, followed by the age groups of 16 -20 years (8%) and 31 – 40 years (9%).

There were more males, n = 52 admitted in the burns ward of KNH than females, n = 48 as summarized in Table 6. Majority of the patients had burn wounds on their arms and legs. The face and head were the least common sites with burn wounds (5%) and (4%) respectively (Table 4.1).

4.1.2 Prevalence of *Staphylococcus aureus* and factors associated with infection

Majority (50%) of burns patients who tested positive for *S. aureus* were less than 10 years old (17/34) followed by those between 20-25 years at 17.6% (6/34). There was only 1 patient (2.9%) between 26 and 30 years with *S. aureus* colonization.

Patients between 31-40 years had the highest *S. aureus* prevalence at 50% (5/10) This was followed closely by those who were <10 years at 42.5% (17/40). *S. aureus* was not isolated in any patient > 40 years of age.

There was no statistically significant relationship between the age of the patient and infection with *S. aureus* ($p = 0.215$).

Of the patients admitted at KNH burns ward found to have *S. aureus* infection, 21/34 (62%) were male, while 13/34 (38%) were female. Thus, out of the males admitted in the burns unit of KNH, (n = 52), 21/52 (40.4%) were found to be positive for *S. aureus* compared to female patients (n = 48) 13/48 (27.1%) as shown in *Table 4.1*. This implied that the prevalence of *S. aureus* infection was higher in males than in females. No association was found between the gender of the patients and infection with *S. aureus* ($p = 0.161$).

There were 8/34 patients (23.5%) with *S. aureus* infection on burn wounds on their arms and legs each. This was followed by the chest and head with 5/34 (14.7%). The lowest site with *S. aureus* infection was found to be the face with only 1 patient (2.9%). There was no association between the site of burn wounds and infection with *S. aureus* as shown in table 4.1.

Table 4 1:Demographic characteristics of patients with burn wounds and associations with Staphylococcus aureus

Variable	***S. aureus* present**	***S. aureus* absent**	**OR (95% CI)**	**P value**
Age group in years				
≤10	17 (42.5)	23 (57.5)	1.0	
11-15	3 (30.0)	7 (70.0)	0.6 (0.1-2.6)	0.474
16-20	2 (25.0)	6 (75.0)	0.5 (0.1-2.5)	0.364
21-25	6 (37.5)	10 (62.5)	0.8 (0.2-2.7)	0.731
26-30	1 (10.0)	9 (90.0)	0.2 (0.0-1.3)	0.085
31-40	5 (50.0)	5 (50.0)	1.4 (0.3-5.4)	0.670
>40	0	6 (100.0)	-	0.999
Gender				
Female	13 (27.1)	35 (72.9)	1.0	
Male	21 (40.4)	31 (59.6)	1.8 (0.8-4.2)	0.161
Site of burn wound				
Abdomen	3 (50.0)	3 (50.0)	2.0 (0.4-10.7)	0.406
Arm	8 (26.7)	22 (73.3)	0.6 (0.2-1.6)	0.363
Back	4 (44.4)	5 (55.6)	1.6 (0.4-6.5)	0.485
Chest	5 (27.8)	13 (12.2)	0.7 (0.2-2.2)	0.596
Face	1 (25.0)	3 (75.0)	0.6 (0.1-6.4)	1.000
Head	5 (100.0)	0	-	0.004
Leg	8 (28.6)	20 (71.4)	0.7 (0.3-1.8)	0.639

4.2 Minimum Inhibitory Concentrations of chlorhexidine

MICs were determined by broth macrodilution followed by overnight culture on Mueller-Hinton agar plates as shown in Figure 2.

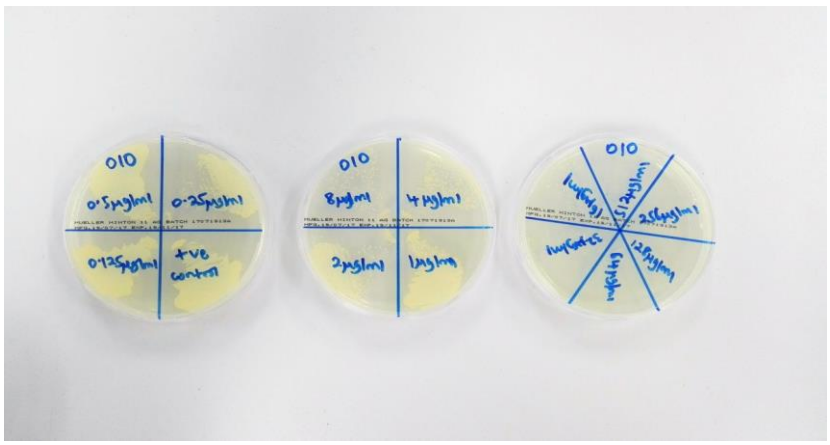

Figure 2: Mueller-Hinton agar plates showing MICs of chlorhexidine required to inhibit *S. aureus*

4.2.1 Distribution of MICs of chlorhexidine

The median MIC was 128 µg/ml. MICs were ranging from 8 - 512 µg/ml. Most (41.2%) of the isolated bacteria was inhibited at MIC of 128 µg/ml, 17.6% at 64 µg/ml and 14.7% at 32 µg/ml.

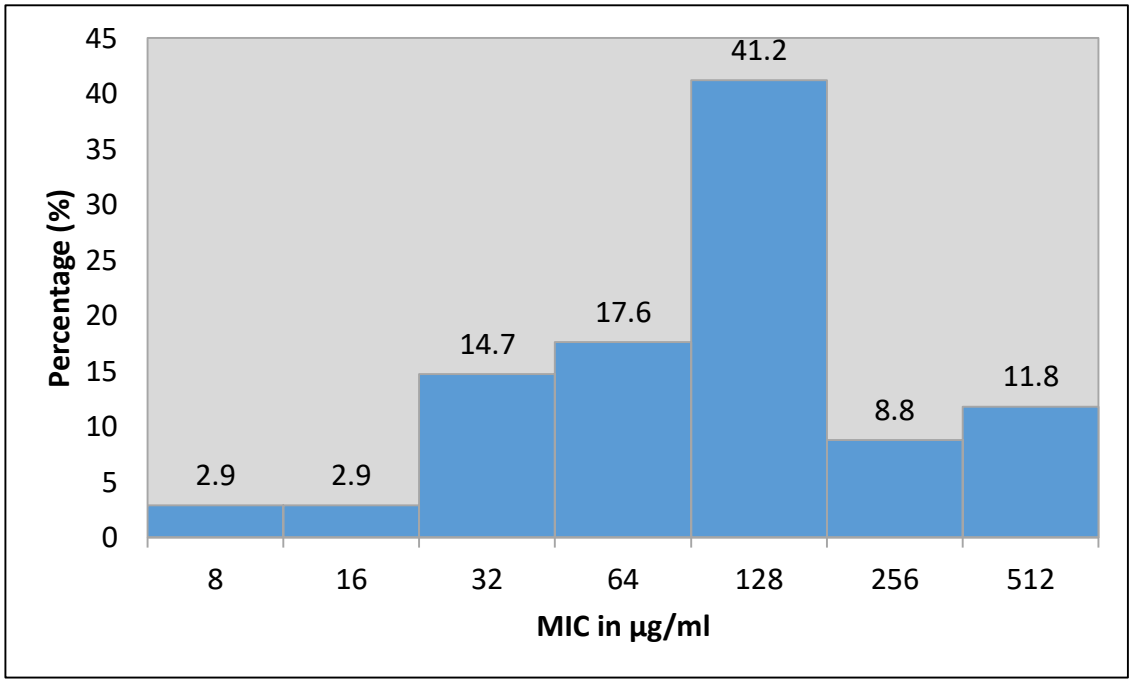

Figure 3: MICs of chlorhexidine that inhibited S. aureus obtained from burn wounds of patients admitted at KNH

4.3 Genotypic resistance of *S. aureus* to Chlorhexidine

4.3.1 Presence of *qacA/B* gene in *Staphylococcus aureus* isolated from wounds of burns patients admitted at KNH

Out of 34 isolates of *S. aureus*, 18 (52.9%) were positive for *qacA/B* gene while 16 (47.1%) were negative. A sample of the bands obtained is shown in Figure 4.

Figure 4: qacA/B DNA fragment visualization under UV light

M - 100bp molecular marker
P – Positive control (*S. aureus* NB01264)
N – Negative control (*S. aureus* ATCC 25923)
Samples 14, 26, 62, 73, 58, 79 and 93 had distinct bands. The size of amplification product was 321bp.

4.4 Association between presence of *qacA/B* and Minimum Inhibitory Concentrations of chlorhexidine

MIC was not significantly different between the samples with *qacA/B* gene and those without the gene ($p = 0.878$).

A higher proportion (66.7%) of the isolates with *qacA/B* gene were inhibited at a MIC ≥ 128 µg/ml compared to 56.3% of those without *qacA/B* gene though the difference was not statistically significant ($p = 0.549$) as shown in table 4.2 and figure 5.

Table 4.1:Association between presence of qacA/B and MICs of chlorhexidine

Variable	***qacA/B gene***		**OR (95% CI)**	**p value**
	Present	**Absent**		
MIC(µg/ml)				
Median (IQR)	128 (64-192)	128 (64-128)	-	0.878
MIC, n (%)				
8	0	1 (6.3)	-	1.000
16	0	1 (6.3)	-	1.000
32	4 (22.2)	1 (6.3)	4.0 (0.2-75.7)	0.355
64	2 (11.1)	4 (25.0)	0.5 (0.0-6.7)	0.600
128	9 (50.0)	5 (31.3)	1.8 (0.2-17.0)	0.608
256	1 (5.6)	2 (12.5)	0.5 (0.0-11.1)	0.661
512	2 (11.1)	2 (12.5)	1.0	

Chi-square X^2= 0.360 (p = 0.549)

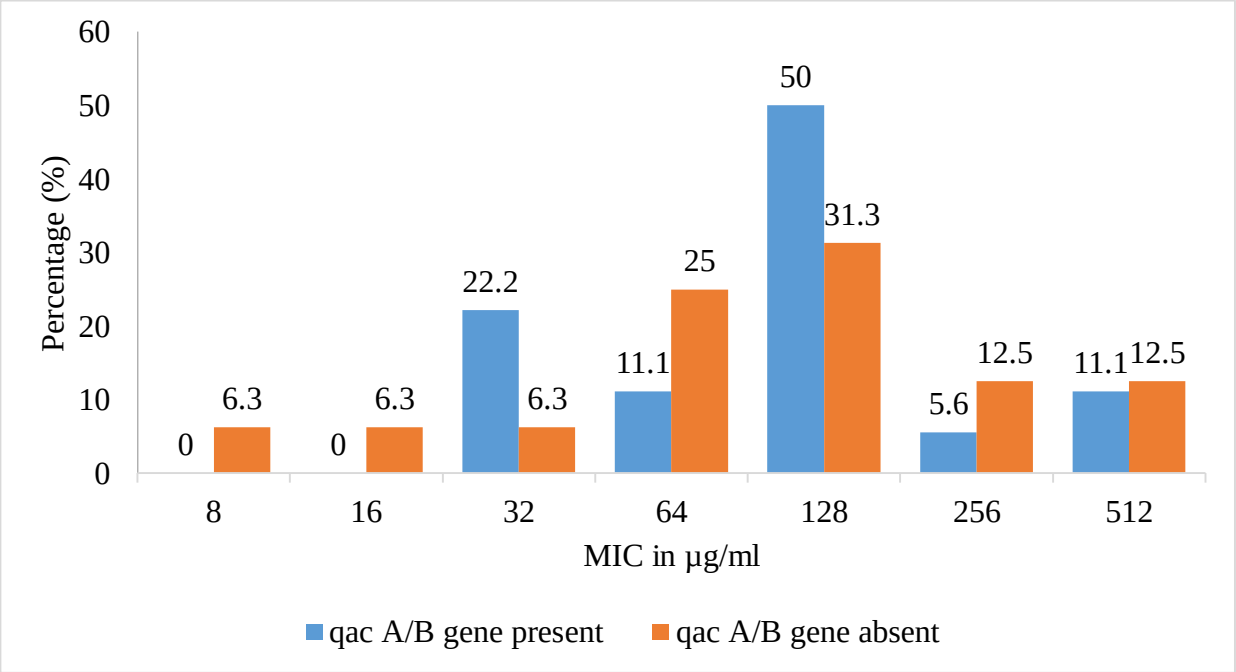

Figure 5: Relationship between MICs of chlorhexidine, and presence of qacA/B gene

CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Burn wound infection is the most common cause of morbidity and mortality in patients with burn wounds. *S. aureus* is frequently isolated from patients in the hospital environment and is identified as a significant cause of burn wound infections (Alebachew, 2012). Likewise, in this study, the findings show the necessity of urgent measures for the restriction of further spread of *S. aureus* infections in KNH burns ward.

5.1.1 Burn wound Characteristics

Children <10 years formed the majority of patients with burn wounds at KNH. This is consistent with a previous study carried out in Kijabe, Kenya that indicated that very young children have a higher risk of being burned compared to patients in other age groups (Dale *et al.*, 2013). This may be attributed to the high percentage of the population in Kenya that falls below the age of 15 years (42.2%) (Kenya demographics 2017). In addition, poverty may be a contributing factor to many families being unable to use safer sources of energy like LPG or hire help to supervise children while the parents are away at work.

The 20-25 year olds were the next largest group with a prevalence of 16%. This is slightly lower than a similar prevalence study carried out by Wong *et al.*, 2014 in Kibera; Kenya who found a prevalence of 22.9% for this age group. In addition, Wong *et al.*, 2014, found a prevalence of burn injury in the 11-15 year age group of 10.6%. This agrees with the findings of this study of 10% for this age group.

The gender distribution of burn wounds found that males had a higher prevalence of burn wounds at 52%. This is contrary to findings of a study conducted by Liwimbi *et al*., 2007 in a Malawian hospital burns unit that found a gender prevalence of 55% females, and 45% males. However, it is important to take into consideration that gender prevalence in burns varies widely according to geographical location, socio-economic status, cultural beliefs and environmental contributions (Wong *et al.*, 2014). However, the higher prevalence of burns in the male population may be attributed to the inquisitive and exploring nature of boys as compared to girls especially for those in the age group < 10 years (Agbenorku *et al*, 2011).

This study found that the upper and lower limbs were the most common areas that suffered burns at 23.5% each. These results are similar to those reported at a Malawian burns unit (Liwimbi *et al*.,2007) that found that upper and lower limbs had the highest burn prevalence at 32% (upper limbs) and 26% (lower limbs). The chest and head regions had a prevalence of

14.7% each. Majority of the burns were caused by scalding by hot liquids particularly during food preparation and this could explain why the upper limbs were most affected by burns.

5.1.2 *S. aureus* wound colonization in hospital burn units

The overall prevalence of *S. aureus* colonization in the wounds of burns patients over the study period was found to be 34%. This carriage rate is significantly higher than that found in Thika level 5 hospital in Kenya of 8.9% (85/950) in a study by Aiken *et al.*, 2014. However, this might be attributable to the sampling technique employed in Thika level 5 which might have introduced a time-dependent bias. There is a shortage of studies on the prevalence of *S. aureus* colonization in burn units across Kenya. This is an area that requires more research in order to prevent burn wound infection and increased morbidity and mortality of burns patients in the country.

A study in Mulago Hospital; Uganda reported the prevalence of MRSA in the burns unit to be 46% (41/89) (Kateete *et al.*, 2011). A similar study was carried out in Ethiopia in the Burns unit of Yekatit 12 hospital, and the prevalence of *S. aureus* carriage was found to be 57.8% (66/114) (Alebachew, 2012). In a study by Saaiq *et al.*, 2015, the prevalence of *S. aureus* in a burns centre in Pakistan was found to be 18.62% (19/95). It is therefore evident that the prevalence of *S. aureus* in burns units varies greatly according to the geographical location and the infection control policies in practice in those hospitals (Alebachew, 2012).

5.1.3 *S. aureus* susceptibility to chlorhexidine

In this study, *S. aureus* exhibited reduced susceptibility to chlorhexidine as shown by the MICs (8-512 µg/ml). These *S. aureus* isolates demonstrated resistance to chlorhexidine since chlorhexidine resistance in Staphylococci is defined as an MIC ≥ 4µg/ml (Horner *et al*., (2012)). However, Horner *et al*., (2012) further assert that this should be considered as tolerance to chlorhexidine because the *in vitro* concentrations are lower than in-use concentrations. This reduced susceptibility is further demonstrated by the low MIC (0.5µg/ml) obtained when susceptible *S. aureus* ATCC 25923 was tested under similar conditions as the test strains. MICs can be used to demonstrate decreasing susceptibility to chlorhexidine, though this method poses some challenges in that there are no standardized CLSI breakpoints, and the assay methods may vary. In addition, the correlation between MICs > 4µg/ml and the presence of *qacA/B* positive isolates may sometimes be poor (Schlett *et al.*, 2014). In this study, all 34 *S. aureus* isolates had MICs >4µg/ml, but only 52.9% (18/34) were positive for *qacA/B* gene. The remaining 47.1% (16/34) *S. aureus* isolates

despite demonstrating reduced susceptibility to chlorhexidine, were negative for *qacA/B* resistance genes. These results are consistent with studies by Horner *et al*., (2012), Schlett *et al.*, (2014) and Gann *et al.*, (2013). It is possible that the strains that did not carry *qacA/B* gene carried a different efflux mediated gene that conferred resistance to chlorhexidine (Horner *et al*., 2012). This being the first study on chlorhexidine resistance in Kenya, there is no data on the MICs of chlorhexidine from *S. aureus* isolated from hospitals or the community setting in the country.

Lastly, the clinical significance of reduced susceptibility to chlorhexidine is not fully understood, though studies have shown that repeated exposure to sub-inhibitory concentrations of chlorhexidine results in increased MICs (Horner *et al*.,2012). Further studies are warranted to investigate this conclusively.

5.1.4 *S. aureus* genetic variants and resistance

In this study, 52.9% (18/34) *S. aureus* isolates were positive for *qacA/B* gene. Studies indicate that the prevalence of *qacA/B* gene in clinical isolates varies according to geographical location. In a Taiwanese and Chinese hospital, the prevalence of *qacA/B* in MRSA isolates was found to be 43.8% (Ho *et al.*, 2012) and 7.8% (Lu *et al*., 2015) respectively. The United Kingdom, Brazil and USA have recorded a prevalence of 10-20%, 80% and 1% respectively (McGann *et al.*, 2011).

This is the first study in Kenya to investigate the prevalence of *qacA/B* genes in a clinical setting. It therefore can possibly provide a foundation from which other studies can make comparisons for future research.

5.1.5 Study Limitations

There may be some possible limitations in this study. First is that the study was carried out in a single centre. However, results from single centre trials may have an important impact on patient care and clinicians must carefully evaluate the results from single centres within the context of their clinical experience. Secondly, there are limited previous studies carried out on *qacA/B* in Kenya and this presents a need for further development in this area of study in Kenya.

5.2 Conclusion and Recommendations

5.2.1 Conclusion

This study revealed a high prevalence of *S. aureus* amongst admitted burns patients and emergence of chlorhexidine-resistant pathogens due to the presence of *qacA/B* genes in over half of the isolates.

MICs were not statistically significant between isolates with *qacA/B* gene and those without the gene.

Reduced susceptibility to chlorhexidine indicated by MICs > 4µg/ml in the hospital environment requires further investigation to confirm the consequences of using lower chlorhexidine concentrations.

5.2.2 Recommendations

In line with the findings of this study, the following is recommended:

5.2.2.1 Recommendations for policy and practice

- Given the high prevalence of *qacA/B* gene, the continued use of chlorhexidine as a key antiseptic should be monitored. The use of other more effective antiseptics should be considered.
- Routine cultures of burn wounds should be carried out in order to isolate microorganisms that infect the wounds. In addition, susceptibility tests should be carried out on these microorganisms so that only those antibiotics with proven efficacy can be prescribed to the patients.
- There needs to be continuous monitoring to ensure correct use of antiseptics. Exposure of microorganisms to sub-inhibitory concentrations of antiseptic leads to increasing levels of resistance.
- Continuous surveillance of antiseptic resistance genes is important since it will discourage indiscriminate use of antiseptics, without the corresponding efficacy data.
- Healthcare workers who come into contact with burns patients should be educated on the importance of observing strict infection-control protocols.

5.2.2.2 Recommendations for further research

- It is recommended that similar studies be carried out in other health facilities in the country to get a broad view of the situation.

- The presence of other resistance genes and their influence on antiseptics in use should be investigated.
- Finally, it is recommended that a large prospective study be carried out on the prevalence of chlorhexidine resistance genes, as well as susceptibility tests for in-use concentrations of chlorhexidine recorded in literature.

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APPENDICES

APPENDIX A: SCREENING AND ELIGIBILITY FORM

All subjects enrolled must meet eligibility criteria based on the inclusion and exclusion criteria detailed in the application approved by the KNH-UoN Ethics and Research Committee.

I. Study Information

Study Title: Prevalence of *Staphylococcus aureus* and *qacA/B* genes isolated from burn wounds at Kenyatta National Hospital and their association with Minimum Inhibitory Concentrations of chlorhexidine.

Principal Investigator: Emily Ngonyo Muiruri

Signature…….

Date of screening…….

II. Patient Information

Participant Code Number…….

Gender: Male ☐ Female ☐

III. Inclusion/Exclusion Criteria (Tick where appropriate)

Inclusion Criteria. (Items 1 to 3 must be answered **YES** for eligibility	**YES**	**NO**
1. Patients with burn wounds; either male or female		
2. Patients with burn wounds showing signs of infection such as pus, malodour and redness		
3. Patients and parents or guardians of patients who give informed consent to participate in the study		
Exclusion Criteria. (Item 1 needs to be answered **YES** for exclusion)	**YES**	**NO**
1. Patients or parents or guardians who decline consent to participate in the study		
2. Patients with fresh burn wounds less than 48 hours old		

APPENDIX B: DATA COLLECTION FORM

Participant Code Number: …….

I. Participant Demographics.

1. Date of Birth: Day……. Month…… Year……

2. Gender: Male ☐ Female ☐

II. Participant's Antibiotic History.

1. Date of admission: Day……. Month……. Year…….....

2. Cause and site of burn wound

…….

……

……

……

3. Was an antibiotic prescribed to the participant? **YES** ☐ **NO** ☐

4. Was Chlorhexidine used to clean the participant's burn wounds? **YES** ☐ **NO** ☐

APPENDIX C: PARTICIPANT INFORMATION AND CONSENT FORM

Title of study: Prevalence of *Staphylococcus aureus* and *qacA/B* genes isolated from burn wounds at Kenyatta National Hospital and their association with Minimum Inhibitory Concentrations of chlorhexidine.

Principal Investigator: Emily Ngonyo Muiruri, Postgraduate student (MSc Molecular Pharmacology, Department of Pharmacology and Pharmacognosy, University of Nairobi)

Supervisors: Dr. Kipruto A. Sinei, PhD; Dr. James H. Kimotho, PhD; Dr. Margaret N. Oluka, PhD

Consenting process

This document is a consent form that contains information about the study indicated above. The purpose of this consent form is to give you information that will help you to decide whether or not to participate in the study. Feel free to ask any questions about the purpose of the study, the possible risks and benefits that may arise, or any other clarification that you may need to help you make a decision on whether to participate in the study or not. Please understand the following principles:

i) Your decision to participate in this study is voluntary
ii) You are free to withdraw from the study at any time without feeling obliged to give a reason for your withdrawal
iii) Refusal to participate in the research will not cause you any disadvantage or affect the quality of services you are entitled to receive

When we have answered your questions to your satisfaction, you may decide to participate in the study or not. Should you agree to take part in the study, we will request you to sign your name on this form.

What is this study about?

Chlorhexidine is an antiseptic agent that is used on the skin to prevent infection of wounds with bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus pyogenes*. Of these, *Staphylococcus aureus* is the most frequently isolated micro-organism in burn wounds. Chlorhexidine is used widely at the KNH burns unit to clean the wounds of patients with burns. However, some patients' wounds get infected in spite of the use of Chlorhexidine, leading to further complications in management of the burn wounds sometimes with fatal outcomes. Infection of burn wounds with *Staphylococcus aureus* may arise from resistance to systemic antibiotics or resistance to topical antiseptic agents. In this study, I want to find out the prevalence of *Staphylococcus aureus* infection in burn wounds. Permission is sought from you to enroll in this medical study.

Purpose of the study

The main objective of this study is to determine the prevalence of Chlorhexidine resistance genes in *Staphylococcus aureus* isolated from wounds of burn patients at Kenyatta National Hospital.

Procedures to be followed

With your permission, we will go through your medical records to obtain information such as your age, date of admission, antibiotics issued to you and cause and site of your burn wounds. In addition, we will take a sample from your wound using a sterile cotton swab and this will be taken to the laboratory for analysis to establish the presence of *Staphylococcus aureus* bacteria.

Risks or/and discomforts

You may feel some discomfort when your wound is swabbed during sample collection. In case any bleeding occurs during this process, your wound will be cleaned and dressed by a qualified nurse.

Rights and safety

The Kenyatta National Hospital-University of Nairobi Research and Ethics committee (KNH/UoN - ERC) will review the study protocol and the informed consent process to ensure that your rights and safety as a participant in this study are safeguarded prior to starting the research process.

Benefits

The study will not provide any monetary benefit to you. However, you and other patients with burn wounds will contribute to the advancement of science since the information you provide will help to detect the prevailing Chlorhexidine resistance genes and in this way improve patient care by making recommendations to policy makers on the need to carry our routine cultures of burn wound surface swabs and determination of susceptibility to Chlorhexidine.

Assurance on Confidentiality

All the information you provide will be kept strictly confidential. We will use code numbers to identify you, and your name will not be used anywhere during data handling or writing reports. Your medical records will be kept under lock and key and only authorized personnel will be able to access it.

CONSENT FORM

Participant statement

I have read this consent form and discussed this research study with a study counselor. I have had my questions answered in a language that I understand. The risks and benefits have been explained to me. I understand that my participation in this study is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that all efforts will be made to keep information regarding my personal identity confidential.

By signing this consent form, I have not given up any of the legal rights that I have as a participant in a research study.

I agree to participate in this research study	**Yes**	**No**
I agree to have my wound swab taken for culture	**Yes**	**No**

Participant Signature/Thumb stamp: _____

Date: _____

Participant printed name: _____

Contacts

For further information about this study, you may contact me, my academic department, the lead supervisor, or The Kenyatta National Hospital/University of Nairobi Ethics and Research committee using the contacts provided below:

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The Chairperson,

The Kenyatta National Hospital/University of Nairobi Research and Ethics Committee

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Researcher's Statement

I, the undersigned have fully explained the relevant details of this research study to the participant named above and believe that the participant has fully understood and has freely given his/her consent.

Researcher's name: _____ **Date:** _____

Signature: _____

Role in the study: _____

For more information, contact the principal investigator: Emily Ngonyo Muiruri

Telephone: 0788-833222

APPENDIX D: FOMU YA MAELEZO NA RUHUSA YA KUSHIRIKI KATIKA UTAFITI

Kichwa cha Utafiti: Kiwango cha kusambaa cha jeni dhidi ya Chlorhexidine katika vimelea vya *Staphylococcus aureu*s kutoka majeraha ya moto ya wagonjwa waliolazwa katika hospitali kuu ya Kenyatta.

Mtafiti Mkuu: Emily Ngonyo Muiruri

Wasimamizi wa utafiti: Dkt. Kipruto A. Sinei, PhD; Dkt. James H. Kimotho, PhD; Dkt. Margaret N. Oluka, PhD

Maelezo ya makubaliano ya kushiriki katika utafiti

Fomu hii itatumika na watafiti ili kukujulisha kuhusu utafiti uliotajwa utakaoanza hivi karibuni. Nia ya fomu hii ni kutoa maelezo kuhusu utafiti huu ili uweze kuelewa na kuamua kama utashiriki au la. Uko na uhuru wa kuuliza maswali yoyote yanayohusika na huu utafiti kama vile faida zitakazopatikana, hatari zilizopo mtu anaposhiriki katika utafiti, na mengineo. Tafadhali kumbuka kanuni zifuatazo:

i) Uamuzi wa kushiriki katika utafiti huu ni kwa hiari.
ii) Uko na uhuru wa kujitoa katika utafiti huu kwa wakati wowote bila ya kupeana sababu za kufanya hivyo.
iii) Kutokubali kushiriki kwenye huu utafiti hautakuletea hasara zozote wala kuathiri ubora wa huduma utakayopokea katika hospitali ya Kenyatta.

Utakaporidhika na jinsi tutakavyo jibu maswali yako, utaamua iwapo utashiriki katika utafiti huu au la. Ikiwa utakubali kushiriki katika huu utafiti, tutakuomba uweke sahihi au kidole chako kwenye fomu kuonyesha ya kwamba umekubali kushiriki kwa hiari yako bila ya kulazimishwa na mtu yeyote.

Utafiti huu unahusu nini?

Chlorhexidine ni dawa inayotumika kuosha ngozi kwa kusudi ya kuepuka maambukizi na vimelea kama *Staphylococcus aureus, Pseudomonas aeruginosa* na *Streptococcus pyogenes.*

Kati ya vimelea hivi, *Staphylococcus aureus* ndio inayopatikana mara kwa mara kwenye majeraha ya moto. Chlorhexidine hutumika katika hospitali ya Kenyatta kuosha majeraha ya moto ili kuzuia maambukizi na vimelea. Licha ya kutumia Chlorhexidine, wagonjwa wengine hupata maambukizi ya *Staphylococcus aureus* kwenye majeraha yao inayozuia majeraha kupona na hata kusababisha mauti kwa walioathirika zaidi. Maambukizi ya majeraha ya moto na vimelea vya *Staphylococcus aureus* husababishwa na kupingana na dawa za kuangamiza vimelea au jeni zinazopingana na dawa za kuosha vidonda. Watafiti wana kusudi ya kupima kiwango cha maambukizi na vimelea vya *Staphylococcus aureus* katika majeraha ya moto ya wagonjwa waliolazwa katika hospitali ya Kenyatta na wanaomba ruhusa yako ili ushiriki katika utafiti huu.

Madhumuni ya utafiti

Lengo kuu la utafiti huu ni kuchunguza kiwango cha kusambaa cha jeni dhidi ya Chlorhexidine katika vimelea vya *Staphylococcus aureus* kutoka majeraha ya moto ya wagonjwa waliolazwa katika hospitali kuu ya Kenyatta.

Utaratibu utakaofuatwa

Utakapopeana ruhusa ya kushiriki katika huu utafiti, watafiti wataangalia rekodi yako ya matibabu ili kupata maelezo kama vile umri wako, tarehe ya kulazwa hospitalini, dawa ulizopewa na madaktari na wauguzi na sababisho na eneo la jeraha la moto.

Pia, watafiti watachukua sampuli kutoka jeraha lako la moto kwa kutumia pamba safi na kulipeleka kufanyiwa uchunguzi katika maabara ili kubaini kiwango cha kuenea kwa *Staphylococcus aureus.*

Hatari au usumbufu unaoweza kutokea

Inawezekana kuwa utahisi uchungu mdogo sampuli la jeraha lako linapochukuliwa na mtafiti wetu. Iwapo damu itatoka kwenye jeraha lako, mwuguzi atapaosha vizuri na kuweka pamba safi.

Haki zako na usalama wako

Jopo la kusimamia masilahi ya washiriki wa utafiti la hospitali kuu ya Kenyatta ikishirikiana na chuo kikuu cha Nairobi litaangalia maelezo ya makubaliano ya kushiriki katika utafiti ili kuhakikisha ya kwamba haki na usalama wako utalindwa kwa wakati wote ambapo utashiriki katika utafiti huu.

Faida ya kushiriki katika utafiti

Ijapokuwa utafiti huu hautakupatia faida ya kifedha, wewe na wagonjwa wengine walio na majeraha ya moto mtasaidia kuendelezwa kwa utafiti wa kisayansi kwani kushiriki kwenu kutasaidia watafiti kujua kuenea kwa jeni dhidi ya Chlorhexidine. Pia, utakuwa umesaidia kuboresha huduma kwa wagonjwa kwani matokeo ya utafiti huu yatasaidia kutoa mapendekezo kwa wanaounda sera za kudumisha afya ya kwamba uchunguzi wa mara kwa mara ufanyiwe wagonjwa wenye majeraha ya moto na pia kuchunguza nguvu ya Chlorhexidine dhidi ya vimelea mbalimbali.

Hakikisho la usiri

Maelezo yote utakayotoa katika utafiti huu yatawekwa katika usiri mkuu. Tutatumia nambari maalum kukutambulisha na jina lako halitatumika popote katika kuripoti matokeo ya utafiti. Pia, rekodi zako za matibabu zitafungiwa kwenye kabati ambayo itatumika na watafiti peke yao.

FOMU YA KUIDHINISHA KUSHIRIKI KATIKA UTAFITI

Kauli ya mshiriki

Nimesoma fomu hii ya kuomba ruhusa ya kushiriki katika utafiti pamoja na mshauri wa utafiti na maswali yangu yote yamejibiwa katika lugha niliyoelewa. Nimeelezwa kwa kikamilifu madhara na faida zinazoweza kutokea ninaposhiriki katika utafiti huu. Nimeelewa ya kwamba kushiriki kwangu katika utafiti huu ni kwa hiari na ninaweza kuchagua kujitoa katika utafiti huu kwa wakati wowote. Nimekubali kwa hiari yangu kushiriki katika utafiti huu.

Ninaelewa ya kwamba kila juhudi litafanywa ili kuweka maelezo kuhusu utambulisho wangu kwa usiri mkuu.

Kwa kuweka sahihi kwenye fomu hii, sijasalimisha haki zangu za kisheria nilizonazo kama mshiriki katika utafiti.

Nimekubali kushiriki katika utafiti huu	Ndio	La
Nimekubali mtafiti kuchukua sampuli kutoka jeraha langu la moto	Ndio	La

Sahihi ya mshiriki /alama ya kidole:_____

Tarehe:_____

Jina la mshiriki:_____-

Mawasiliano

Kwa maelezo zaidi kuhusu utafiti huu, unaweza kuwasiliana nami, Idara ya Pharmacology na Pharmacognosy, msimamizi mkuu, au jopo la kusimamia masilahi ya washiriki wa utafiti la hospitali ya Kenyatta ikishirikiana na chuo kikuu cha Nairobi ukitumia njia zifuatazo:

Emily Ngonyo Muiruri
Idara ya Pharmacology na Pharmacognosy
Shule ya mafunzo ya dawa
Chuo kikuu cha Nairobi
S.L.P 19676 – 00200
Nairobi
Nambari ya simu: 0788-833222

Msimamizi mkuu
Dkt. Kipruto A. Sinei
Idara ya Pharmacology na Pharmacognosy
Chuo Kikuu Cha Nairobi
S.L.P 19676-00202
Nairobi.
Nambari ys simu: 0722381639

Jopo la kusimamia masilahi ya washiriki wa utafiti la hospitali ya Kenyatta ikishirikiana na chuo kikuu cha Nairobi,
S.L.P 19676 – 00200
Nairobi
Nambari ya simu: 020-2726300 Ext: 44102

Kauli ya mtafiti

Mimi, niliyeweka sahihi hapo chini natoa kauli ya kwamba nimetoa maelezo yote kwa kikamilifu kuhusu utafiti huu kwa mshiriki wa utafiti, na ninaamini ya kwamba alielewa maelezo yote na amekubali kushiriki katika huu utafiti kwa hiari yake.

Jina la mtafiti:_____ **Tarehe:_____**

Sahihi:_____

Majukumu katika utafiti:_____

Kwa maelezo zaidi, wasiliana na mtafiti mkuu: Emily Ngonyo Muiruri
Nambari ya simu: 0788-833222

APPENDIX E: CHILD INFORMATION DOCUMENT

Title of study: Prevalence of *Staphylococcus aureus* and *qacA/B* genes isolated from burn wounds at Kenyatta National Hospital and their association with Minimum Inhibitory Concentrations of chlorhexidine.

Principal Investigator: Emily Ngonyo Muiruri, Postgraduate student (MSc Molecular Pharmacology, Department of Pharmacology and Pharmacognosy, University of Nairobi)

Supervisors: Dr. Kipruto A. Sinei, PhD; Dr. James H. Kimotho, PhD; Dr. Margaret N. Oluka, PhD;

Consenting process

This document is a consent form that contains information about the study indicated above. The purpose of this consent form is to give you information that will help you to decide whether or not to participate in the study. Feel free to ask any questions about the purpose of the study, the possible risks and benefits that may arise, or any other clarification that you may need to help you make a decision on whether to participate in the study or not. Please understand the following principles:

1. Your decision to participate in this study is voluntary
2. You are free to withdraw from the study at any time without giving a reason for your withdrawal
3. Refusal to participate in the research will not cause you any disadvantage or affect the quality of services you are entitled to receive

When we have answered your questions to your satisfaction, you may decide to participate in the study or not. Should you agree to take part in the study, we will request you to sign your name on this form.

What is this study about?

Chlorhexidine is an antiseptic agent that is used on the skin to prevent infection of wounds with bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus pyogenes*. Of these, *Staphylococcus aureus* is the most frequently isolated micro-organism in burn wounds. Chlorhexidine is used widely at the KNH burns unit to clean the wounds of patients with burns. However, some patients' wounds get infected even though Chlorhexidine is used, leading to further complications in treating the burn wounds. Infection of burn wounds with *Staphylococcus aureus* may arise from resistance to medicines called antibiotics or resistance to topical antiseptic agents. In this study, I want to find out the prevalence of *Staphylococcus aureus* infection in burn wounds. Permission is sought from you to enroll in this medical study.

Purpose of the study

The main objective of this study is to determine the prevalence of Chlorhexidine resistance genes in *Staphylococcus aureus* isolated from wounds of burn patients at Kenyatta National Hospital.

Procedures to be followed

With your permission, we will go through your medical records to obtain information such as your age, date of admission, antibiotics issued to you and cause and site of your burn wounds. In addition, we will take a sample from your wound using a sterile cotton swab and this will be taken to the laboratory for analysis to establish the presence of *Staphylococcus aureus* bacteria.

Risks or/and discomforts

You may feel some discomfort when your wound is swabbed during sample collection. In case any bleeding occurs during this process, your wound will be cleaned and dressed by a qualified nurse.

Rights and safety

The Kenyatta National Hospital-University of Nairobi Research and Ethics committee (KNH-UoN ERC) will review the study protocol and the informed consent process to ensure that your rights and safety as a participant in this study are safeguarded prior to starting the research process.

Benefits

The study will not provide any monetary benefit to you. However, you and other patients with burn wounds will contribute to the advancement of science since the information you provide will help to detect the prevailing Chlorhexidine resistance genes and in this way improve patient care by making recommendations to policy makers on the need to carry our routine cultures of burn wound surface swabs and determination of susceptibility to Chlorhexidine.

Assurance on Confidentiality

All the information you provide will be kept strictly confidential. We will use code numbers to identify you, and your name will not be used anywhere during data handling or writing reports. Your medical records will be kept under lock and key and only authorized personnel will be able to access it.

CHILD ASSENT FORM

Project Title: Prevalence of *Staphylococcus aureus* and *qacA/B* genes isolated from burn wounds at Kenyatta National Hospital and their association with Minimum Inhibitory Concentrations of chlorhexidine.

Investigator: Emily Ngonyo Muiruri

Supervisors: Dr. Kipruto A. Sinei, PhD; Dr. James H. Kimotho, PhD; Dr. Margaret Oluka, PhD

We are doing a research study about how many patients' burn wounds have been infected with a type of bacteria known as *Staphylococcus aureus.* In addition, we want to find out why the wounds are still getting infected with this bacterium, even though the wound has been cleaned with an antiseptic called Chlorhexidine. We would like to know if there is something inside the bacteria that is causing the antiseptic Chlorhexidine not to work as it should.

Permission has been granted to undertake this study by the Kenyatta National Hospital-University of Nairobi Ethics and Research Committee (KNH-UoN Protocol No._____

This research study is a way to learn more about people. At least _____ children will be participating in this research study with you.

If you decide that you want to be part of this study, you will be asked to allow a nurse to take a sample of your wound. This may cause you some discomfort, but no harm. There will be qualified doctors and nurses to take care of you and clean your wound. The sample collected will be taken to the laboratory.

Not everyone who takes part in this study will benefit. A benefit means something good happens to you. These benefits might be that the results we obtain from your sample will tell us if your wound is infected with *Staphylococcus aureus.* This information will help doctors in the future to choose the best antiseptic to clean burn wounds so that they heal faster.

If you do not want to be in this research study, you will still continue to receive the best care for your wound.

When we are finished with this study we will write a report about what was learned. This report will not include your name or that you were in the study.

You do not have to be in this study if you do not want to be. If you decide to stop after we begin, that is okay too. Your parents know about the study too.

If you decide you want to be in this study, please sign your name.

I, _____ want to be in this research study.

(Signature/Thumb stamp) _____ (Date)_____

Contacts

For further information about this study, you may contact me, my academic department, the lead supervisor, or The Kenyatta National Hospital/University of Nairobi Ethics and Research committee using the contacts provided below:

Emily Ngonyo Muiruri

Department of Pharmacology and Pharmacognosy

School of Pharmacy

University of Nairobi

P.O. Box 19676 –00200

Nairobi.

Tel: 0788-833222

Lead Supervisor:

Dr. Kipruto A. Sinei

Dept. of Pharmacology and Pharmacognosy

University of Nairobi

P.O. Box 19676-00202

Nairobi.

Tel: 0722381639

The Chairperson,

The Kenyatta National Hospital/University of Nairobi Research and Ethics Committee

P.O. Box 19676 – Nairobi. Tel: 020-2726300 Ext: 44102

APPENDIX F: FOMU YA WATOTO YA MAELEZO KUHUSU UTAFITI

Kichwa cha Utafiti: Kiwango cha kusambaa cha jeni dhidi ya Chlorhexidine katika vimelea vya *Staphylococcus aureu*s kutoka majeraha ya moto ya wagonjwa waliolazwa katika hospitali kuu ya Kenyatta.

Mtafiti Mkuu: Emily Ngonyo Muiruri

Wasimamizi wa utafiti: Dkt. Kipruto A. Sinei, PhD; Dkt. James H. Kimotho, PhD; Dkt. Margaret N. Oluka, PhD

Maelezo ya makubaliano ya kushiriki katika utafiti

Fomu hii itatumika na watafiti ili kukujulisha kuhusu utafiti uliotajwa utakaoanza hivi karibuni. Nia ya fomu hii ni kutoa maelezo kuhusu utafiti huu ili uweze kuelewa na kuamua kama utashiriki au la. Uko na uhuru wa kuuliza maswali yoyote yanayohusika na huu utafiti kama vile faida zitakazopatikana, hatari zilizopo mtu anaposhiriki katika utafiti, na mengineo. Tafadhali kumbuka kanuni zifuatazo:

i) Uamuzi wa kushiriki katika utafiti huu ni kwa hiari.
ii) Uko na uhuru wa kujitoa katika utafiti huu kwa wakati wowote bila ya kupeana sababu za kufanya hivyo.
iii) Kutokubali kushiriki kwenye huu utafiti hautakuletea hasara zozote wala kuathiri ubora wa huduma utakayopokea katika hospitali ya Kenyatta.

Utakaporidhika na jinsi tutakavyo jibu maswali yako, utaamua iwapo utashiriki katika utafiti huu au la. Ikiwa utakubali kushiriki katika huu utafiti, tutakuomba uweke sahihi au kidole chako kwenye fomu kuonyesha ya kwamba umekubali kushiriki kwa hiari yako bila ya kulazimishwa na mtu yeyote.

Utafiti huu unahusu nini?

Chlorhexidine ni dawa inayotumika kuosha ngozi kwa kusudi ya kuepuka maambukizi na vimelea kama *Staphylococcus aureus, Pseudomonas aeruginosa* na *Streptococcus pyogenes.* Kati ya vimelea hivi, *Staphylococcus aureus* ndio inayopatikana mara kwa mara kwenye majeraha ya moto. Chlorhexidine hutumika katika hospitali ya Kenyatta kuosha majeraha ya

moto ili kuzuia maambukizi na vimelea. Licha ya kutumia Chlorhexidine, wagonjwa wengine hupata maambukizi ya *Staphylococcus aureus* kwenye majeraha yao inayozuia majeraha kupona na hata kusababisha mauti kwa walioathirika zaidi. Maambukizi ya majeraha ya moto na vimelea vya *Staphylococcus aureus* husababishwa na kupingana na dawa za kuangamiza vimelea au jeni zinazopingana na dawa za kuosha vidonda. Watafiti wana kusudi ya kupima kiwango cha maambukizi na vimelea vya *Staphylococcus aureus* katika majeraha ya moto ya wagonjwa waliolazwa katika hospitali ya Kenyatta na wanaomba ruhusa yako ili ushiriki katika utafiti huu.

Madhumuni ya utafiti

Lengo kuu la utafiti huu ni kuchunguza kiwango cha kusambaa cha jeni dhidi ya Chlorhexidine katika vimelea vya *Staphylococcus aureus* kutoka majeraha ya moto ya wagonjwa waliolazwa katika hospitali kuu ya Kenyatta.

Utaratibu utakaofuatwa

Utakapopeana ruhusa ya kushiriki katika huu utafiti, watafiti wataangalia rekodi yako ya matibabu ili kupata maelezo kama vile umri wako, tarehe ya kulazwa hospitalini, dawa ulizopewa na madaktari na wauguzi na sababisho na eneo la jeraha la moto.

Pia, watafiti watachukua sampuli kutoka jeraha lako la moto kwa kutumia pamba safi na kulipeleka kufanyiwa uchunguzi katika maabara ili kubaini kiwango cha kuenea kwa *Staphylococcus aureus.*

Hatari au usumbufu unaoweza kutokea

Inawezekana kuwa utahisi uchungu mdogo sampuli la jeraha lako linapochukuliwa na mtafiti wetu. Iwapo damu itatoka kwenye jeraha lako, mwuguzi atapaosha vizuri na kuweka pamba safi.

Haki zako na usalama wako

Jopo la kusimamia masilahi ya washiriki wa utafiti la hospitali kuu ya Kenyatta ikishirikiana na chuo kikuu cha Nairobi litaangalia maelezo ya makubaliano ya kushiriki katika utafiti ili kuhakikisha ya kwamba haki na usalama wako utalindwa kwa wakati wote ambapo utashiriki katika utafiti huu.

Faida ya kushiriki katika utafiti

Ijapokuwa utafiti huu hautakupatia faida ya kifedha, wewe na wagonjwa wengine walio na majeraha ya moto mtasaidia kuendelezwa kwa utafiti wa kisayansi kwani kushiriki kwenu kutasaidia watafiti kujua kuenea kwa jeni dhidi ya Chlorhexidine. Pia, utakuwa umesaidia kuboresha huduma kwa wagonjwa kwani matokeo ya utafiti huu yatasaidia kutoa mapendekezo kwa wanaounda sera za kudumisha afya ya kwamba uchunguzi wa mara kwa mara ufanyiwe wagonjwa wenye majeraha ya moto na pia kuchunguza nguvu ya Chlorhexidine dhidi ya vimelea mbalimbali.

Hakikisho la usiri

Maelezo yote utakayotoa katika utafiti huu yatawekwa katika usiri mkuu. Tutatumia nambari maalum kukutambulisha na jina lako halitatumika popote katika kuripoti matokeo ya utafiti. Pia, rekodi zako za matibabu zitafungiwa kwenye kabati ambayo itatumika na watafiti peke yao.

FOMU YA WATOTO YA RUHUSA YA KUSHIRIKI KATIKA UTAFITI

Kichwa cha utafiti: Kiwango cha kusambaa cha jeni dhidi ya Chlorhexidine katika vimelea vya *Staphylococcus aureu*s kutoka majeraha ya moto ya wagonjwa waliolazwa katika hospitali kuu ya Kenyatta.

Mtafiti Mkuu: Emily Ngonyo Muiruri

Wasimamizi wa utafiti: Dkt. Kipruto A. Sinei, PhD; Dkt. James H. Kimotho, PhD; Dkt. Margaret N. Oluka, PhD

Tunafanya utafiti kuhusu kiwango cha kusambaa cha jeni dhidi ya Chlorhexidine katika vimelea vya *Staphylococcus aureus* kutoka majeraha ya moto ya wagonjwa waliolazwa katika hospitali kuu ya Kenyatta.

Ruhusa ya kutekeleza huu utafiti umetolewa na jopo la kusimamia masilahi ya washiriki wa utafiti la hospitali ya Kenyatta ikishirikiana na chuo kikuu cha Nairobi (Nambari ya usajili) _____.

Utafiti huu unatusaidia kupata maelezo zaidi kuhusu magonjwa ya watu. Kuna watoto _____ watakaoshiriki katika utafiti huu pamoja nawe.

Iwapo utaamua kushiriki katika utafiti huu, utaulizwa kumruhusu mwuguzi kuchukua sampuli kutoka jeraha lako la moto kwa kutumia pamba safi na kulipeleka kufanyiwa uchunguzi katika maabara ili kubaini kiwango cha kuenea kwa *Staphylococcus aureus.* Iwapo utahisi uchungu wowote wakati huu, wauguzi watakuwa karibu kuhakikisha ya kwamba wameosha jeraha lako na hutopata madhara yoyote.

Sio kila mtu atakayeshiriki kwenye utafiti huu atapata faida. Faida inamaanisha ya kwamba jambo zuri litakutendekea. Kwa maoni yangu, faida itakayopatikana kwa kushiriki kwenye utafiti huu ni kwamba watafiti watapata maelezo zaidi kuhusu dawa inayofaa kuosha majeraha ya wagonjwa ili kuepuka maambukizi.

Iwapo utaamua kutoshiriki katika utafiti huu, utaendelea kupata matibabu ya hali ya juu ya majeraha yako.

Tutakapomaliza utafiti huu, tutaandika ripoti kuhusu yale tuliyojifunza. Ripoti hii haitataja jina lako, wala kushiriki kwako katika utafiti huu.

Kumbuka ya kwamba, sio lazima ushiriki katika utafiti huu kama hutaki. Ukiamua kujitoa hata utafiti utakapokua umeanza, ni sawa pia. Tumeeleza wazazi wako kuhusu utafiti huu.

Iwapo utaamua kushiriki katika utafiti huu, tafadhali andika jina lako hapa chini.

Mimi, _____ nimeamua kushiriki katika utafiti huu kwa hiari yangu.

(Sahihi/Alama ya kidole)_____

(Tarehe)_____

Mawasiliano

Kwa maelezo zaidi kuhusu utafiti huu, unaweza kuwasiliana nami, idara ya Pharmacology na Pharmacognosy, msimamizi mkuu, au jopo la kusimamia masilahi ya washiriki wa utafiti la hospitali ya Kenyatta ikishirikiana na chuo kikuu cha Nairobi ukitumia njia zifuatazo:

Emily Ngonyo Muiruri
Idara ya Pharmacology na Pharmacognosy
Shule ya mafunzo ya dawa
Chuo kikuu cha Nairobi
S.L.P 19676 – 00200
Nairobi
Nambari ya simu: 0788-833222
Msimamizi mkuu:
Dkt. Kipruto A. Sinei
Idara ya Pharmacology na Pharmacognosy
Chuo Kikuu Cha Nairobi
S.L.P 19676-00202
Nairobi.
Nambari ys simu: 0722381639
Jopo la kusimamia masilahi ya washiriki wa utafiti la hospitali ya Kenyatta ikishirikiana na chuo kikuu cha Nairobi,
S.L.P 19676 – 00200
Nairobi
Nambari ya simu: 020-2726300 Ext: 44102

APPENDIX G: PARENTAL INFORMATION AND CONSENT FORM FOR CHILDREN PARTICIPATING IN A STUDY

Project Title: Prevalence of *Staphylococcus aureus* and *qacA/B* genes isolated from burn wounds at Kenyatta National Hospital and their association with Minimum Inhibitory Concentrations of chlorhexidine.

Investigator: Emily Ngonyo Muiruri

Supervisors: Dr. Kipruto A. Sinei, PhD; Dr. James H. Kimotho, PhD; Dr. Margaret Oluka, PhD

Introduction: I would like to tell you about a study being conducted by the researchers listed above. The purpose of this consent form is to give you the information you will need to help you decide whether or not your child should participate in the study. Feel free to ask any questions about the purpose of the research, what happens if your child participates in the study, the possible risks and benefits, the rights of your child as a volunteer, and anything else about the research or this form that is not clear. When we have answered all your questions to your satisfaction, you may decide if you want your child to be in the study or not. Once you understand and agree for your child to be in the study, I will request you to sign your name on this form. You should understand the general principles which apply to all participants in a medical research: i) Your decision to participate is entirely voluntary ii) Your child may withdraw from the study at any time without necessarily giving a reason for his/her withdrawal iii) Refusal to participate in the research will not affect the services your child is entitled to in this health facility or other facilities.

May I continue? YES/ NO

For children below 18 years of age, we give information about the study to parents or guardians. We will go over this information with you and you need to give permission in order for your child to participate in this study. We will give you a copy of this form for your records.

If your child is older than 8 years, he/she will be required to agree to participate in the study after being fully informed.

What is the purpose of the study?

The researchers listed above are interviewing individuals who have children below 8 years who have burn wounds and are admitted at Kenyatta National Hospital's burns unit. The

purpose of the study is to find out the prevalence of Chlorhexidine resistance genes in *Staphylococcus aureus* isolated from wounds of burn patients at Kenyatta National Hospital. Participants in this research study will be asked questions about their burn wounds such as the cause and site of the burn wound and the date of admission. Participants will also have the choice to undergo a swab collection from the burn wound. There will be approximately 138 participants in this study randomly chosen. We are asking for your consent to allow your child to participate in this study.

What will happen if you decide you want your child to be in this research study?

If you agree for your child to participate in this study, the following will happen:

You will be interviewed by a trained interviewer in a private area where you will feel comfortable answering questions. The interview will last approximately 10 minutes. The interview will cover topics such as what is *Staphylococcus aureus* and why is it a problem? What is Chlorhexidine and what is it used for?

A specimen will be collected from your child's burn wound using a sterile cotton swab. This sample will be transported in a special medium to Kenya Medical Research Institute (KEMRI) Nairobi microbiology laboratory for culture to isolate *Staphylococcus aureus*. You will be informed about the results. We will ask for a telephone number where we can contact you if necessary. If you agree to provide your contact information, it will be used only by people working for this study and will never be shared with others. The reason we may need to contact you is to clarify some information that may be missing from your child's medical records.

Are there any risks, harms or discomforts associated with this study?

Medical research has the potential to introduce psychological, social, emotional and physical risks. Efforts should always be put in place to minimize the risks. One potential risk of being the study is loss of privacy. We will keep everything you tell us as confidential as possible. We will use a code number to identify your child in a password-protected database and will keep all of our paper records in a locked file cabinet. However, no system of protecting

confidentiality can be absolutely secure so it is still possible that someone could find out your child was in this study and could find out information about your child.

Also, answering questions in the interview may be uncomfortable for you. If there are any questions you do not want to answer, you can skip them. You have the right to refuse the interview or any questions asked during the interview.

It may be embarrassing for you to have your child's sample taken if the burn wound is located on a private part of the body. We will do everything we can to ensure that this is done in private. Furthermore, all study staff and interviewers are professionals with special training in these examinations/interviews.

Your child may feel some discomfort when his/her burn wound is getting swabbed and may bleed a little. In case of an injury, illness or complications related to this study, contact the study staff right away at the numbers provided at the end of this document. The study staff will treat your child for minor conditions or refer the child for treatment for conditions that require more extensive care.

Are there any benefits of being in this study?

Your child may benefit by receiving a free laboratory test that isolates *Staphylococcus aureus*. This will contribute in informing the doctors on suitable antibiotic choices for your child. Also, the information you provide will help us better understand resistance prevalence of *Staphylococcus aureus* to the frequently used antiseptic in hospitals, Chlorhexidine. This information is a major contribution to science and will inform policy makers on the continued use of Chlorhexidine for burn wounds in hospitals.

Will being in this study cost you anything?

This study will not cost you anything financially. This is because your child is already admitted at the burns unit and therefore you will not incur any transport costs. In addition, all materials required to collect and transport the swab sample will be met by the investigators.

Is there any reimbursement for participating in this study?

No, there will not be any financial reimbursement for participating in this study.

What if you have questions in future?

If you have further questions or concerns about your child participating in this study, please call or send a text message to the study staff at the number provided at the bottom of this page.

For more information about your child's rights as a research participant, you may contact the secretary/Chairperson, Kenyatta National Hospital-University of Nairobi Ethics and Research Committee, Telephone No. 2726300 Ext. 44102. Email:uonknh_uonbi.ac.ke

What are your other choices?

Your decision to have your child participate in this research study is voluntary. You are free to decline or withdraw participation of your child in the study at any time without injustice or loss of benefits. Just inform the study staff and the participation of your child in the study will be stopped. You do not have to give reasons for withdrawing your child if you do not wish to do so. Withdrawal of your child from the study will not affect the services your child is otherwise entitled to in this health facility or other health facilities.

For more information, contact the head nurse at the burns unit from 8:00am to 5:00pm.

CONSENT FORM (STATEMENT OF CONSENT)

The person being considered for this study is unable to consent for him/herself because he or she is a minor (a person less than 18 years of age). You are being asked to give your permission to include your child in this study.

Parent/guardian statement

I have read this consent form or had the information read to me. I have had the chance to discuss the research study with a study counselor. I have had my questions answered by him or her in a language that I understand. The risks and benefits have been explained to me. I understand that I will be given a copy of this consent form after signing it. I understand that my participation and that of my child in this study is voluntary and that I may choose to withdraw at any time.

I understand that all efforts will be made to keep information regarding me and my child's personal identity confidential.

By signing this consent form, I have not given up my child's legal right as a participant in this research study.

I voluntarily agree to my child's participation in this research study:

	Yes	**No**
I agree to have my child undergo a swab test	Yes	No
I agree to have his/her wound exudate preserved for later study	Yes	No
I agree to provide contact information for follow-up	Yes	No

Parent/Guardian signature/Thumb stamp:_____Date:_____

Parent/Guardian printed name:_____

Researcher's statement

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given his/her consent.

Printed Name:_____ Date: _____

Signature:_____

Role in the study:_____

Witness Printed Name (*if witness is necessary*)_____

Signature:_____Date_____

Contacts

For further information about this study, you may contact me, my academic department, the lead supervisor, or The Kenyatta National Hospital/University of Nairobi Ethics and Research committee using the contacts provided below:

Emily Ngonyo Muiruri

Department of Pharmacology and Pharmacognosy

School of Pharmacy

University of Nairobi

P.O. Box 19676 –00200

Nairobi.

Tel: 0788-833222

Lead Supervisor:

Dr. Kipruto A. Sinei

Dept. of Pharmacology and Pharmacognosy

University of Nairobi

P.O. Box 19676-00202

Nairobi.

Tel: 0722381639

APPENDIX H: FOMU YA WAZAZI YA MAELEZO NA RUHUSA YA WATOTO KUSHIRIKI KATIKA UTAFITI

Kichwa cha utafiti: Kiwango cha kusambaa cha jeni dhidi ya chlorhexidine katika vimelea vya *Staphylococcus aureu*s kutoka majeraha ya moto ya wagonjwa waliolazwa katika hospitali kuu ya Kenyatta.

Mtafiti Mkuu: Emily Ngonyo Muiruri

Wasimamizi wa utafiti: Dkt. Kipruto A. Sinei, PhD; Dkt. James H. Kimotho, PhD; Dkt. Margaret N. Oluka, PhD

Utangulizi

Ningependa kukueleza kuhusu utafiti utakaotekelezwa na watafiti waliotajwa. Lengo la fomu hii ni kukupa maelezo utakayohitaji ili uweze kuamua iwapo utamruhusu mtoto wako kushiriki katika utafiti huu. Jisikie huru kuuliza maswali yoyote yanayohusiana na huu utafiti kama vile: yale utakayotarajia iwapo motto wako atashiriki katika utafiti huu, faida na madhara yanayoweza kutokea, haki za mtoto wako kama mshiriki wa utafiti na mengineo. Tutakapojibu maswali yako hadi uridhishwe na majibu yote, unaweza kuamua iwapo utamruhusu mtoto wako kushiriki katika huu utafiti au la. Ukikubali mtoto wako kushiriki karika huu utafiti, nitakuomba uweke sahihi yako kwenye fomu hii. Unafaa kuelewa kanuni zinazoongoza washiriki wote wa utafiti kama vile: Uamuzi iwapo mtoto wako atashiriki ni kwa hiari tu, unaweza kumtoa mtoto wako kutoka utafiti huu kwa wakati wowote bila ya kueleza sababu ya kumtoa, kumkataza mtoto wako kushiriki katika utafiti hauta athiri huduma ambazo mtoto wako anastahili kupewa katika hospitali hii au hospitali yoyote ingine. Ninaweza kuendelea? Ndio/La

Kwa wale watoto walio chini ya miaka 18, tunapatia maelezo kuhusu utafiti kwa wazazi au walezi wa watoto. Tutapitia fomu hii pamoja nawe na unastahili kupeana ruhusa ili mtoto wako ashiriki katika utafiti huu. Tutakupatia nakala ya fomu hii iwe rekodi yako.

Iwapo mtoto wako amefikisha miaka 8, yeye ataelezwa kuhusu utafiti huu na atajiamulia iwapo atashiriki au la.

Lengo la utafiti huu ni nini?

Watafiti waliotajwa hapo juu wanahoji watu ambao watoto wao wana majeraha ya moto. Lengo la utafiti huu ni kuchunguza kiwango cha kusambaa cha jeni dhidi ya Chlorhexidine katika vimelea vya *Staphylococcus aureus* kutoka majeraha ya moto ya wagonjwa waliolazwa katika hospitali kuu ya Kenyatta. Washiriki katika huu utafiti wataulizwa maswali kama vile sababisho la jeraha la moto, sehemu ya mwili iliyo na jeraha la moto na tarehe ya kulazwa hospitalini.

Watafiti watachukua sampuli kutoka jeraha la moto la mtoto kwa kutumia pamba safi na kulipeleka kufanyiwa uchunguzi katika maabara ili kubaini kiwango cha kuenea kwa *Staphylococcus aureus.*

Kutakuwa washiriki takriban watu 138 watakaoshiriki katika utafiti huu. Tunakuomba ruhusa ili mtoto wako ashiriki katika utafiti huu.

Utatarajia nini iwapo utamruhusu mtoto wako kushiriki katika huu utafiti?

Utakapomruhusu mtoto wako kushiriki katika huu utafiti, utahojiwa na mtu aliye na ujuzi wa mahojiano katika chumba kilichotengwa mbali na watu wengine ili uweze kujibu maswali vizuri. Mahojiano yatachukua muda wa takriban dakika 10. Mahojiano yatazungumzia kuhusu jeni za *Staphylococcus aureus* na madhara yake, matumizi na manufaa ya dawa ya kuoshea vidonda ya Chlorhexidine na mengineo.

Baada ya kumaliza mahojiano, watafiti watachukua sampuli kutoka jeraha la moto la mtoto kwa kutumia pamba safi na kulipeleka kufanyiwa uchunguzi katika maabara ili kubaini kiwango cha kuenea kwa *Staphylococcus aureus.* Utajulishwa kuhusu matokeo yatakayopatikana.

Tutakuomba nambari yako ya simu ili tuweze kuwasiliana nawe iwapo itahitajika. Ukikubali kutupatia nambari yako ya simu, tunakuhakikishia ya kwamba itatumika na watafiti wetu peke yake na haitasambazwa kwa watu wengine. Tunaweza kukupigia simu iwapo kuna maelezo tutakayokosa katika rekodi za mtoto wako.

Hatari au usumbufu unaoweza kutokea na utafiti huu

Utafiti wa kisayansi una uwezo wa kuleta madhara ya kisaikolojia, kijamii, hisia au ya kimwili. Ni lazima mikakati iwekwe ili kupunguza madhara haya. Kuna hatari ya kupoteza faragha ya rekodi za mtoto. Hatutarudia tutakayoyazungumzia na mtu yeyote mwingine na tutatumia nambari maalum kwa kumtambulisha mtoto wako itakayowekwa kwnye tarakilishi iliyo na neno la siri. Nakala zote zitafungiwa kwenye kabati maalum. Hata baada ya kuweka mikakati hii, bado kunapouwezo wa mtu mwingine kupata maelezo kuhusu mtoto wako.

Pia, unaweza kuwa na wasiwasi unapojibu maswali yetu kwa hivyo kumbuka ya kwamba unao uhuru wa kukataa kujibu maswali yale ambayo yatakuletea wasiwasi au kukataa mahojiano yote.

Unaweza kusikia wasiwasi iwapo jeraha la moto la mtoto wako lipo katika sehemu ya siri ya mwili kwa hivyo tutahakikisha ya kuwa sampuli itachukuliwa katika chumba kilicho na pazia au chumba maalum. Zaidi ya haya ni kwamba watafiti wote wana utaalam wa kufanya mahojiano.

Mtoto wako anaweza kupata usumbufu wakati mwuguzi atachukua sampuli kutoka jeraha lake la moto. Iwapo damu itatoka kwenye jeraha la moto, mwuguzi atahakisisha ya kuwa ameiosha na kuifunga na pamba safi kama itahitajika.

Faida ya kushiriki katika utafiti

Ijapokuwa utafiti huu hautakupatia faida ya kifedha, mtoto wako na wagonjwa wengine walio na majeraha ya moto watasaidia kuendelezwa kwa utafiti wa kisayansi kwani kushiriki kwenu kutasaidia watafiti kujua kuenea kwa jeni dhidi ya Chlorhexidine. Pia, utakuwa umesaidia kuboresha huduma kwa wagonjwa kwani matokeo ya utafiti huu yatasaidia kutoa mapendekezo kwa wanaounda sera za kudumisha afya ya kwamba uchunguzi wa mara kwa mara ufanyiwe wagonjwa wenye majeraha ya moto na pia kuchunguza nguvu ya Chlorhexidine dhidi ya vimelea mbalimbali.

Utahitajika kutumia fedha zozote ukishiriki katika utafiti huu?

La, hautatumia fedha zozote kwani mtoto amelazwa hospitalini tayari kwa hivyo hakuna nauli yoyote utakayotumia ili kumleta hospitalini. Pia, watafiti watatoa vifaa vyote vitakavyohitajika kwa kutekeleza utafiti huu.

Utalipwa fedha zozote kwa kushiriki katika utafiti huu?

La, hautalipwa fedha zozote utakaposhiriki katika utafiti huu. Kushiriki kutakuwa kwa hiari tu.

Iwapo utakuwa na maswali katika siku za usoni?

Iwapo utakuwa na maswali yoyote kuhusu mtoto wako kushiriki katika utafiti huu, wasiliana nasi ukitumia nambari za simu za runununu zilizowekwa mwisho wa fomu hii.

Kwa maelezo zaidi kuhusu haki za mtoto kama mshiriki wa utafiti, unaweza kuwasiliana na katibu au mwenyekiti wa jopo la kusimamia masilahi ya washiriki wa utafiti la hospitali ya Kenyatta ikishirikiana na chuo kikuu cha Nairobi ukitumia njia zifuatazo: nambari ya simu: 2726300 Ext. 44102, barua pepe:uonknh_erc@uonbi.ac.ke

Una chaguo gani lingine?

Uamuzi iwapo mtoto wako atashiriki katika utafiti huu ni kwa hiari yako. Unao uhuru wa kumkataza kushiriki katika wakati wowote bila kudhulumiwa au kubaguliwa na yeyote. Utawajulisha watafiti kuhusu uamuzi wako na mtoto wako atatolewa kutoka utafiti huu. Sio lazima upeane sababu ya kumtoa kutoka utafiti huu kama hutaki kufanya hivyo. Kumtoa mtoto wako kutoka utafiti huu hautaathiri huduma ambazo mtoto wako ana haki ya kupata katika hospitali hii au hospitali yoyote ingine. Kwa maelezo zaidi, wasiliana na mwuguzi mkuu katika idara ya majeruhi wa moto katika hospitali kuu ya Kenyatta kuanzia saa mbili asubuhi hadi saa kumi na moja jioni.

FOMU YA RUHUSA YA MTOTO KUSHIRIKI KATIKA UTAFITI (KAULI YA RUHUSA)

Mtu ambaye anatarajiwa kushiriki katika utafiti huu hana uwezo wa kujiamulia iwapo atashiriki kwa sababu ni mtoto aliye chini ya miaka kumi na minane. Tunakuomba ruhusa umkubali mtoto wako ashiriki katika utafiti huu.

Kauli ya mzazi/mlezi

Nimesoma fomu hii na nimepewa maelezo kuhusu utafiti huu. Nimeongea na mshauri wa utafiti na maswali yangu yote yamejibiwa kwa lugha niliyoelewa. Nimeelezwa faida zote na madhara yanayoweza kutokea kwa kushiriki katika utafiti huu. Nimeelewa ya kwamba kushiriki kwangu na kwa mtoto wangu katika utafiti huu ni kwa hiari na ninaweza kujitoa kwa wakati wowote.

Ninaelewa ya kwamba juhudi zote zitafanywa ili kuweka maelezo kunihusu mimi na mtoto wangu siri.

Kwa kukubali kuweka sahihi katika fomu hii, sijasalimisha haki za mtoto wangu za kisheria kama mshiriki wa utafiti huu.

Nimekubali kwa hiari yangu kuwa mtoto anaweza kushiriki katika utafiti huu:

Ndio **La**

Nimekubali mtoto wangu kuchukuliwa sampuli kutoka jeraha la moto **Ndio** **La**

Nimekubali unyevu wa jeraha kuwekwa kwa madhumuni ya kufanyiwa uchunguzi **Ndio** **La**

Nimekubali kupeana nambari ya simu ili watafiti wawasiliane nami **Ndio** **La**

Sahihi ya mzazi/mlezi au alama ya kidole _____

Tarehe:_____

Jina la mzazi/mlezi:_____

Kauli ya mtafiti

Mimi niliyeweka sahihi yangu hapa chini, nimetoa maelezo kwa kikamilifu kwa mshiriki wa utafiti aliyetajwa hapo juu na ninaamini ya kwamba ameelewa na amepeana ruhusa ya kushiriki kwa hiari yake.

Jina:_____ **Tarehe:_____**

Sahihi:_____

Jukumu katika utafiti:_____

Jina la shahidi (*iwapo shahidi anahitajika*):_____

Sahihi:_____ Tarehe:_____

Mawasiliano

Kwa maelezo zaidi kuhusu utafiti huu, unaweza kuwasiliana nami, Idara ya Pharmacology na Pharmacognosy, msimamizi mkuu, au jopo la kusimamia masilahi ya washiriki wa utafiti la hospitali ya Kenyatta ikishirikiana na chuo kikuu cha Nairobi ukitumia njia zifuatazo:

Emily Ngonyo Muiruri
Idara ya Pharmacology na Pharmacognosy
Shule ya mafunzo ya dawa
Chuo kikuu cha Nairobi
S.L.P 19676 – 00200
Nairobi
Nambari ya simu: 0788-833222

Msimamizi mkuu
Dkt. Kipruto A. Sinei
Idara ya Pharmacology na Pharmacognosy
Chuo Kikuu Cha Nairobi
S.L.P 19676-00202
Nairobi.
Nambari ys simu: 0722381639

Jopo la kusimamia masilahi ya washiriki wa utafiti la hospitali ya Kenyatta ikishirikiana na chuo kikuu cha Nairobi,
S.L.P 19676 – 00200
Nairobi
Nambari ya simu: 020-2726300 Ext: 44102

APPENDIX I: KNH - UoN ETHICAL APPROVAL FORM

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KENYATTA NATIONAL HOSPITAL APPROVED 23 JAN 2017 KNH/UoN-ERC P.O. Box 20723-00202 NRB.

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
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/17

23rd January 2017

Emily Muiruri
Reg. No.U52/81012/2015
Dept.of Pharmacology and Pharmacognosy
School of Pharmacy
College of Health Sciences
University of Nairobi

Dear Emily

Revised Research Proposal: "Prevalence of Chlorhexidine Resistance genes *in Staphylococcus aureus* isolated from wounds of burns patients at Kenyatta National Hospital (P829/011/2016)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above revised proposal. The approval period is from 23rd January 2017 – 22nd January 2018.

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) 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*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
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.

Kindly arrange to submit a copy of registration by Pharmacy and Poisons Board and approval when ready..

Protect to discover

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

Yours sincerely,

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

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director, CS, KNH
The Assistant Director, Health Information, KNH
The Chair, KNH- UoN ERC
The Dean, School of Pharmacy, UoN
The Chair, Dept. of Pharmacology and Pharmacognosy, UoN
Supervisors: Dr. James H. Kimotho, Dr. Margaret N. Oluka, Dr.Kipruto A. Sinei

APPENDIX J: KEMRI RESEARCH PROJECT ATTACHMENT

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel: (254) (020) 2722541, 2713349, 0722-205901, 0733-400003, Fax: (254) (020) 2720030
E-mail: director@kemri.org, info@kemri.org, Website. www.kemri.org

KEMRI/TR/11/15 **18th April, 2017**

Emily Ngonyo Muiruri
NAIROBI

Re: **MSc RESEARCH PROJECT ATTACHMENT**

Reference is made to your letter on the above mentioned subject.

We are pleased to inform you that the Institute has offered you an MSc Research attachment at the Production Department at Kenya Medical Research Institute, (KEMRI) with effect from **1st May to 31st July, 2017.**

We note that you will work within the Microbiology Laboratory as you execute your MSc thesis entitled *"Prevalence of chlorhexidine resistance genes in staphylococcus aureus"* isolated from wounds of burns patients at Kenyatta National Hospital, under the supervision of Dr. James Kimotho. You are expected to maintain diligence and Confidentiality in all your undertakings.

However, you are required to pay an attachment fee of **Kshs. 6,000 (Bankers Cheque or Cash)** to the **Director, KEMRI** before starting the attachment and present the payment receipt to the Training Office to facilitate your deployment. You are also expected to take up a Personal Accident cover in case your college/University has not insured you during the period of attachment.

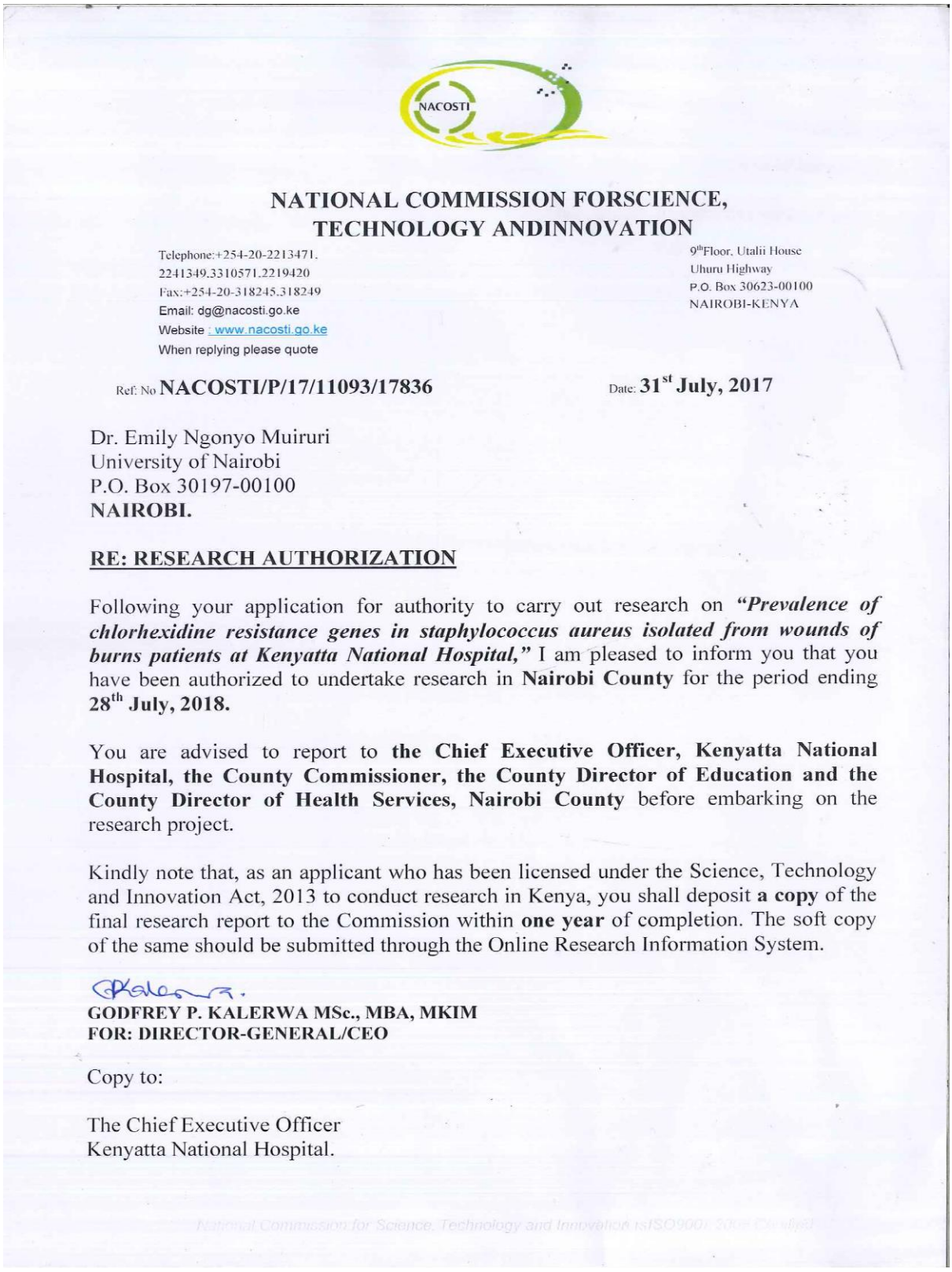
We wish you all the best even as you gain practical skills to complement your studies.

Ruth Nyambura
For: Ag. DIRECTOR
KENYA MEDICAL RESEARCH INSTITUTE

cc: Production Manager – KEMRI Production Department

In Search of Better Health

APPENDIX K: NACOSTI RESEARCH AUTHORIZATION



NACOSTI

NATIONAL COMMISSION FORSCIENCE, TECHNOLOGY ANDINNOVATION

Telephone:+254-20-2213471, 2241349,3310571,2219420
Fax:+254-20-318245,318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No **NACOSTI/P/17/11093/17836**

Date: **31st July, 2017**

Dr. Emily Ngonyo Muiruri
University of Nairobi
P.O. Box 30197-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Prevalence of chlorhexidine resistance genes in staphylococcus aureus isolated from wounds of burns patients at Kenyatta National Hospital,"*** I am pleased to inform you that you have been authorized to undertake research in **Nairobi County** for the period ending **28th July, 2018.**

You are advised to report to **the Chief Executive Officer, Kenyatta National Hospital, the County Commissioner, the County Director of Education and the County Director of Health Services, Nairobi County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The Chief Executive Officer
Kenyatta National Hospital.

National Commission for Science, Technology and Innovation is ISO9001: 2008 Certified