ANTIBACTERIAL ACTIVITY OF *Carica papaya* AND COMMON ANTIBIOTICS AGAINST METHICILLIN RESISTANT *Staphylococcus epidermidis* ISOLATED FROM KAMPALA INTERNATIONAL UNIVERSITY TEACHING HOSPITAL WARDS SURFACES, BUSHENYI, UGANDA

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DECLARATION

I, Abubakar Sunusi Adam (M.Sc.MB/0001/151/DF) hereby declare that the work presented in this dissertation is my original work and has never been presented for any academic award in any other University.

Signature..... Date 22/10/2018

CERTIFICATION

This dissertation titled "**Antibacterial activity of *Carica papaya* and common antibiotics on Methicillin resistant *Staphylococcus epidermidis* isolated from ward surfaces of Kampala International University Teaching Hospital, Uganda**" has been submitted for examination with approval of my supervisors.

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DEDICATION

This dissertation is dedicated to the Almighty Allah who has granted me the grace to start and finish this work and also to my family whose support has been a driving force.

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ABBREVIATIONS

ACME	Arginine Catabolic Mobile Element
AMPs	Antimicrobial Peptides
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
CSF	Cerebro-Spinal Fluid
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standard Institute
CoNS	Coagulase Negative Staphylococci
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
dNTPs	Deoxy-ribonucleotide triphosphates
DR-SE	Drug Resistant *Staphylococcus epidermidis*
ICU	Intensive Care Unit
KIU-TH	Kampala International University -Teaching Hospital
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
MSA	Mannitol Salt Agar
MUST	Mbarara University of Science and Technology

PBPs	Penicillin Binding Proteins
PCR	Polymerase Chain Reaction
PIA	Polysaccharide Intercellular Adhesin
SCCmec	Staphylococcal Chromosomal Cassette mec
UGX	Ugandan Shillings
UV	Ultra-violet
WHO	World Health Organization

ABSTRACT

Staphylococcus epidermidis is coagulase-negative staphylococci that frequently cause device or surgery-associated nosocomial infections worldwide. Methicillin resistant *S. epidermidis* (MRSE) have been reported with very serious clinical implications. The antibiotics in clinical use are associated with high resistance levels and non-affordability due to high prices. *Carica papaya* that has been documented to have antimicrobial properties might be able to offer a solution. This study was therefore aimed at determining the antibacterial activity of *C. papaya* and common antibiotics against MRSE isolated from wards surfaces of Kampala International University Teaching Hospital, Uganda.

Swab samples collected from selected ward surfaces were inoculated on Mannitol salt agar for isolation of *S. epidermidis*. The isolates were tested against common antibiotics (Amikacin 30µg, Cefazolin 30µg, Cefoxitin 30µg, Trimethoprim-sulfamethoxazole 25µg, Ciprofloxacin 30µg and Gentamicin 30µg) using the disc diffusion method. Isolates resistant to Cefoxitin were subjected to *C. papaya* leaf and seed crude extracts using agar well diffusion method. Minimum Inhibitory (MIC) and Bactericidal concentration (MBC) of the *C. papaya* leaf and seed crude extracts were determined. mecA gene was detected from MRSE using conventional Polymerase chain reaction.

Out of the 363 swab samples analyzed, 112 (30.85%) prevalence of *S. epidermidis* was obtained. Both *C. papaya* leaf and seed crude extracts (methanol and acetone) exhibited antibacterial activity against MRSE with MICs and MBCs ranges of 250 to 31.2mg/ml and 125 to 31.3mg/ml for leaf and seed extracts respectively. Out of 112 *S. epidermidis* isolates, 11 (9.8%) were found resistant to Cefoxitin and all were positive for mecA gene.

This study concludes that *S. epidermidis* is present in KIU-TH wards surfaces. It was resistant to Trimethoprim-sulfamethoxazole (80.4%) and sensitive to Cefazolin (93.8%) and all the 11(9.8%) isolates resistant to Cefoxitin were positive for mecA gene. *Carica papaya* leaf and seed crude extracts (methanol and acetone) were effective against MRSE.. It is therefore recommended that KIU-TH should use stronger disinfectants such as those containing phenol, biguanides and halogens to decontaminate wards surfaces. In addition, Trimethoprim-sulfamethoxazole should not be prescribed in cases *S. epidermidis* is implicated. *Carica papaya* leaf and seed crude extracts could be a source of novel antibiotics for treatment of MRSE.

CHAPTER ONE: INTRODUCTION

1.0 Introduction

This study investigated the antibacterial activity of *Carica papaya* and common antibiotics against *S. epidermidis* isolated from KIU-TH wards surfaces. The chapter consists of the background of the study, statement of the problem, research objectives, research questions, justification/significance, diagrammatic and description of conceptual frame work, and scope of the study.

1.1 Background of the study

Staphylococcus epidermidis is one of the gram positive, coagulase-negative staphylococci that frequently cause device- or surgery-associated nosocomial infections worldwide (Hidron *et al.*, 2008). These infections include: respiratory and surgical site, urinary tract infections, meningitis, blood stream infections, gastroenteritis and endocarditis, all of which are considered life-threatening. Among these, prosthetic valve endocarditis is the highest risk with 25% mortality worldwide (Samuel *et al.*, 2010; WHO, 2011). Two million people are affected with these nosocomial infections annually, and 5% to 15% of them result in hospitalization globally (Apanga *et al.*, 2014).

The United State nationwide Surveillance and Control of Pathogens of Epidemiological Importance (SCOPE) database reported that the most common pathogens recovered from nosocomial bloodstream infections within a seven years period were *S. epidermidis* (31%), followed by *S. aureus* (20%) (Wisplinghoff *et al.*, 2004). According to Azeez (2012), the rates of nosocomial Staphylococcal infection range from 2 to 49% in Sub-Saharan Africa and vary with the environment, intensive care units (ICUs) having the highest occurrence rates of 21.2-35.6%. In Uganda, the prevalence of *S. epidermidis* is approximately 15%, according to a study conducted in Mulago Teaching and National Referral Hospital, Kampala (Okee *et al.*, 2012). Another study conducted in Makerere between August 2012 and July 2013 revealed that out of 87 isolates, 27% were coagulase-negative staphylococci of which 60% were resistant to the common antibiotics tested (Kajumbula, 2014).

The occurrence of drug-resistance among nosocomial pathogens has resulted in the emergence and re-emergence of difficult-to-treat infections in patients depicting the pre-antibiotic era (Mbim *et al.* 2016). Drug-resistant strains of *S. epidermidis* (DR-SE) have become a very serious clinical problem, due to the difficulties in eradicating their infections from colonized devices (Fitzpatrick *et al.*, 2005). *S. epidermidis* is resistant against many of today's commonly used antibiotics, including methicillin, which is mediated by the *mecA* gene encoding a penicillin binding protein with reduced affinity to beta-lactam antibiotics similar to that in *S. aureus* (Hiramatsu *et al.*, 2002). It is also resistant to Amikacin and Gentamicin by acquiring resistant genes called aac and ant plasmids which are Aminoglycoside modifying enzymes that change the target of the antibiotic. It became resistant to Ciprofloxacin through gyrA resistant gene which interferes with the activity of an enzyme gyrase. Resistance to Trimethoprim/Sulfamethoxazole is mediated by SXZ-dhps resistant gene which target enzyme modification system (Daniela *et al.*, 2015). A previous study recommends the use of Vancomycin, Gentamicin, Cefazolin, Linezolid and Telavancin for the treatment of Methicillin resistant *S. epidermidis* (Fang *et al.*, 2011). However, most of the antibiotics recommended are the second line drugs and are also expensive; hence a need for an alternative measure against infections, especially from natural sources such as Ethno-medicinal plants.

Medicinal plants such as *C. papaya, Ficussycomorus* Linn. (Moraceae), *Balanites aegyptica* L., Drel (Balantiaceae), *Sesbania sesban* Linn.(Papilionaceae), *Tamarindus indica* L. (Fabaceae), *Albiziacoriaria* Welw. ex Oliv (Fabaceae) are rich sources of medicine and can provide possible inexpensive alternatives in the treatment of resistant microbial strains, due to the presence of a multitude of phytochemical compounds which are linked to antimicrobial activities (Arujothi *et al.*, 2014; Caluwe *et al.*, 2010). *C. papaya* L. (pawpaw), which belongs to the family Caricaceae, is a medicinal plant recognized as an effective natural medicine in controlling both oedema and inflammation associated with surgical operations (Otsukia *et al.*, 2010). Phytochemicals, such as tannins, alkaloids and phenolic compounds present in different parts of *C. papaya*, have been shown to treat different ailments (Doughari *et al.*, 2007). The leaf extracts of *C. papaya* have been reported to inhibit growth of several pathogens, including Coagulase positive *S. aureus* and Coagulase negative *S. epidermidis* and also used as soap substitute for the treatment of skin infections (Anibijuwon and Udeze, 2009; Nagesh and Samreen, 2016). Furthermore, *C. papaya* seeds are known to suppress worms, hence are used in the treatment of internal parasites, and

dysentery in humans; and are also effective against *S. aureus* and *Bacillus subtilis* (Udoh *et al.*, 2005; Faisal *et al.*, 2016).

The effect of *C. papaya* seeds extract on *S. epidermidis*, however, has not been widely explored. Hence a need to evaluate the antibacterial activity of *C. papaya* on *S. epidermidis* isolated from Hospital surfaces.

1.2 Problem statement

Hospitalized patients undergoing invasive procedures are enormously susceptible to secondary infections by nosocomial pathogens from contaminated surfaces, and/or devices such as surgical equipment (Brannigam *et al.*, 2012). *Staphylococcus epidermidis* is one of the major causes of nosocomial infections that follow catheterization and other surgical procedures. Related ailments include infections of: surgical wounds/sites, the urinary and respiratory tracts, and the brain (Samuel *et al.*, 2010). *Staphylococcus epidermidis* and similar organisms have the ability to adhere to the surfaces, as a result of their unique pathogen-host-environment relationships (Hidron *et al.*, 2008).

Nosocomial infections affect two million people annually, with 25% mortality rate, and prolong hospital stay among 5 to15% of patients worldwide (WHO, 2011; Apanga *et al.*, 2014). The incidence rate of nosocomial infection caused by *S. epidermidis* in Sub-Saharan Africa range from 2-49% with Uganda having 15% (Azeez, 2012;Okee *et al.*, 2012). A study conducted by Nalwoga and others reported 13% prevalence of Coagulase negative Staphylococci including *S. epidermidis* from wound samples among the surgical ward patients of Kampala International University Teaching Hospital (Nalwoga *et al.*, 2016). However, the source of contamination by this bacteria has not been studied. Despite little attention being given to *S. epidermidis*, compared to *S. aureus* in health care settings, *S. epidermidis* is reported to have developed resistance to multiple antibiotics, including methicillin (Kozitskaya *et al.*, 2004). This complicates and increases the cost of treatments, hence the need for relatively inexpensive, alternative antibacterial agents from natural sources, such as medicinal plants.

Some research studies have shown that *C. papaya* leaves and seeds are effective against clinical isolates of *S. aureus* and *S. epidermidis* (Anibijuwon and Udeze, 2009; Nagesh and Samreen,

2016). Research on *S. aureus* isolated from hospital environments in Nigeria, Ghana, Ethiopia and Uganda have revealed widely variable susceptibility patterns against antibiotics commonly used in hospitals (Hammuel *et al.*, 2014; Saba *et al.*, 2017; Amenu *et al.*, 2014; Ivan, 2012). Similar investigations on *S. epidermidis*, however, are quite limited and none have been done in Kampala International University-Teaching Hospital.

Furthermore, there is a paucity of data on the antibacterial activity of *C. papaya* leaves and seeds extract against Methicillin resistant *S. epidermidis* isolated from hospital environments. Hence this study evaluated the antibacterial activity of *C. papaya* and common antibiotics on *S. epidermidis* isolated from KIU-TH wards surfaces.

1.3 Objectives

1.3.1 Main objective

The main objective of this study was to determine the antibacterial activity of *C. papaya* and common antibiotics against Methicillin resistant *S. epidermidis* isolated from KIU-TH wards surfaces.

1.3.2 Specific objectives

. To determine the distribution of *Staphylococcus epidermidis* from ward surfaces of Kampala International University Teaching Hospital

i. To determine the susceptibility pattern of *Staphylococcus epidermidis* isolated from different wards surfaces of Kampala International University-Teaching Hospital on the commonly used antibiotics.

ii. To determine the antibacterial activity of *Carica papaya* leaf and seed extracts against Methicillin resistant *Staphylococcus epidermidis* isolated from different wards surfaces of Kampala International University-Teaching Hospital.

v. To determine the presence of mecA gene among Methicillin resistant *Staphylococcus epidermidis* isolated from different wards surfaces of Kampala International University-Teaching Hospital.

1.4 Research questions

i. What is the distribution of *S. epidermidis* isolated from different wards surfaces of Kampala International University-Teaching Hospital?

ii. What is the susceptibility pattern of *S. epidermidis* isolated from different wards surfaces of Kampala International University-Teaching Hospital to the commonly used antibiotics?

iii. What are the antibacterial activities of *C. papaya* leaves and seeds extracts against Methicillin resistant *S. epidermidis* isolated from different wards surfaces of Kampala International University-Teaching Hospital?

iv. Is the mecA gene present in the strains of Methicillin resistant *S. epidermidis* isolated from different wards surfaces of Kampala International University-Teaching Hospital?

1.5 Justification and significance of the study

Despite *S. epidermidis* being non-pathogenic, it is a common cause of nosocomial infections (Murdoch *et al.*, 2009). Reports on the susceptibility patterns of the organism to common antibiotics have yielded inconsistent results (Koksal *et al.*, 2009; Mohammad *et al.*, 2015). Drug-resistant strains of *S. epidermidis* have been isolated from Mulago hospital, Kampala (Okee *et al.*, 2012). A study on the susceptibility pattern of *S. epidermidis* to antibiotics commonly used in KIU-TH had not been carried out; and there was no information on the mecA gene among resistant strains of *S. epidermidis* from the KIU-TH surfaces.

The development of resistant strains to common antibiotics has necessitated the use of newer expensive drugs, hence the need to explore the use of inexpensive and locally available medicinal plants, with antimicrobial activity. *Carica papaya* is one of such plants with these medicinal properties, according to studies conducted in Nigeria and India (Anibijuwon and Udeze, 2009; Nagesh and Samreen, 2016). The active ingredients of this plant, however, vary with soil types and climates/seasons; hence it's imperative to verify the antibacterial activity of leaf and seed extract of *C. papaya* grown in the Ugandan environment on *S. epidermidis*. Furthermore, as opposed to the study by Nagesh and Samreen (2016), which used clinical

pathogens, the antibacterial activity of *C. papaya* extracts on *S. epidermidis* isolated from hospital surfaces had not yet been established.

The Information generated will enrich the knowledge base of the general public on the antibacterial activity of *C. papaya* leaf and seed extracts against antibiotic resistant *S. epidermidis*. The outcome from the distribution and risk factors associated with *S. epidermidis* will benefit the management of KIU-TH to enhance the hygiene practice in the hospital. Furthermore, the susceptibility pattern of *S. epidermidis* isolated from KIU-TH will give the health workers a clue in prescribing the effective antibiotics for the treatment of infections caused by *S. epidermidis*. *Carica papaya* leaf and seed crude extracts will help both the hospital management and the community as an alternative antimicrobial agent against resistant *S. epidermidis*. Presence of mecA gene indicated the presence of Methicillin resistant *Staphylococcus epidermidis* (MRSE), this information will help the hospital management and the policy makers to come up with necessary measures to prevent the occurrence of an outbreak by MRSE. The overall information of this study will serve as a guide and source of literature to the students and other researchers.

1.6 Conceptual frame work

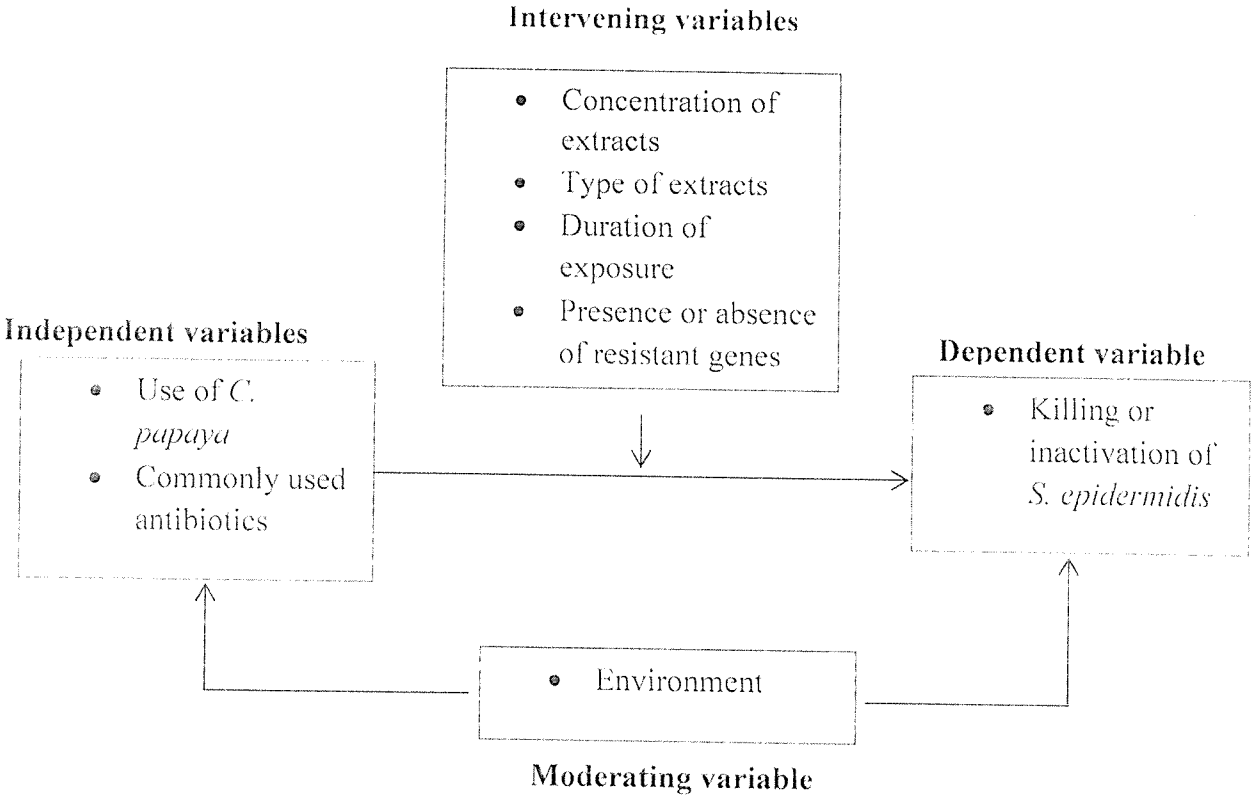


Figure 1: Conceptual frame work

Source: Adapted from Ivan. (2015) and modified by the researcher)

Description of conceptual framework

From the above illustration, it can be observed that killing, or inactivation of *S. epidermidis*, depends on the use of *C. papaya* and common antibiotics, which can both be moderated by the environment. The two variables (independent and dependent), could be affected by some set of intervening variables, including: concentration and type of extract (leaf or seed). That may affect the activity of *C. papaya*, by increasing, or decreasing the chances of killing, or inactivating the *S. epidermidis*, based on the concentration of the active component in the extracts. Duration of exposure to the common antibiotics, and presence or absence of antimicrobial resistant genes, may affect the activity of the common antibiotics.

1.7 Scope of the study

This includes the time, geography/area and content/methods scope of the study

1.7.1 Time scope

The study was carried out from March, 2017 to August, 2018 as per the research time table.

1.7.2 Geographical/area scope

The study was carried out in KIU-TH located in Ishaka Municipality. This is a well-established referral hospital with several wards including Medicine, Surgery, Paediatrics and Obstetrics/Gynecology. The plant samples were collected from Kigondo town in Bushenyi district.

1.7.3 Content/methods scope

This study concentrated on the use of *S. epidermidis* isolates and epidemiological data collected from wards surfaces of KIU-TH, antibacterial activity of commonly used antibiotics and crude extracts of *C. papaya* leaf and seed against *S. epidermidis* and mecA gene analysis. Collections of epidemiological data, swab samples, isolation of *S. epidermidis* were carried out to address objective one while antibiotic susceptibility testing addressed objective two. Collection, preparation, extraction, phytochemical screening, antimicrobial activity testing, minimum inhibitory concentration and minimum bactericidal concentration of *C. papaya* leaf and seed crude extracts were carried out to address objective three. DNA extraction and polymerase chain reaction were used to address objective four.

CHAPTER TWO: LITERATURE REVIEW

2.0 Introduction

This chapter evaluates relevant studies that had been carried out in different parts of the world with reference to *S. epidermidis* and its sensitivity patterns to *Carica papaya*, and the commonly used antibiotics in Hospitals.

2.1 Epidemiology of *Staphylococcus epidermidis*

The *S. epidermidis* as organism, the susceptible host to its infections and some environmental factors affecting its survival are described below;

2.1.1 *Staphylococcus epidermidis*

Staphylococcus epidermidis is a Gram-positive bacterium and one of over 40 species belonging to the: Kingdom: Bacteria, Phylum: Firmicutes, Class: Bacilli, Order: Bacillales, Family: Staphylococcaceae, Genus: Staphylococcus and Species: *epidermidis*. Thus, the Binomial name *S. epidermidis* (Frebourg *et al.*, 2000). Rosenbach distinguished *S. epidermidis* from *S. aureus*, initially naming *S. epidermidis* as *S. albus*. He chose aureus and albus since the bacteria formed yellow and white colonies respectively (Rosenbach, 1884; Shiroma *et al.*, 2015). The major virulence factors of *S. epidermidis* include its ability to adhere to medical devices such as catheters, prosthetic joints, fracture fixation devices, cardiac pacemakers, heart valves, artificial lenses, vascular grafts, mammary implants, Cerebro Spinal Fluid (CSF) shunts and formation of a biofilm (Chu *et al.*, 2009).

Biofilm is one of the virulence factors that enhanced mechanical, metabolic, immune, and antibiotic resistance in *S. epidermidis* and its production is associated with the production of a polysaccharide intercellular adhesin (PIA) encoded by the accessory intercellular adhesion (*ica*) operon (Kozitskaya *et al.*, 2004). The biofilm of *S. epidermidis* consists of clusters of cells that are embedded in extracellular slime substance that is up to 160 micrometers (μm) thick, exceeding 50 cells. Biofilms as such act as a diffusion barrier to antibiotics and host defense Nilsson *et al.*, 1998).

Staphylococcus epidermidis is the most frequent cause of device- or surgery-associated nosocomial infections worldwide (Hidron *et al.*, 2008). According to the World Health Organization (WHO, 2011), nosocomial infections are one of the major infections with a huge economic impact worldwide. These infections affect about 2 million people annually resulting in 5% to 15% of them requiring hospitalization (Apanga *et al.*, 2014). Prevalence rates of nosocomial infections of 7.7 and 9.0% were reported in the European and Western Pacific Regions respectively (Wood *et al.*, 2009).

In Sub-Saharan Africa, nosocomial infections rates range from 2-49%, and show considerably high figures of 21.2-35.6% which occurs in intensive care units (ICU) (Azeez, 2012). The prevalence rates of the infections reported varied between 2.5% - 14.8% in Burkina Faso, United Republic of Tanzania, Senegal and 28% in Uganda (Nejad *et al.*, 2011; WHO, 2011; Greco and Magombe, 2011).

2.1.2 Susceptible hosts to *Staphylococcus epidermidis* and possible infections

Staphylococcus epidermidis causes biofilms to grow on plastic devices placed within the body (Hedin, 1993). This occurs most commonly on intravenous catheters and on medical prostheses (Otto, 2009). Infection can also occur in dialysis patients or anyone with an implanted plastic device like central venous catheters, fracture fixation devices, cardiac pacemakers and heart valves, artificial lenses, vascular grafts, mammary implants, and CSF shunts that may have been contaminated (Rupp and Archer, 1994). It also causes endocarditis, most often in patients with defective heart valves and parts of the inside lining of the heart muscle (Darouiche, 2001).

Patient-related risk factors for infection with *S. epidermidis* are malignancy, chemotherapy, leukopenia, premature birth, bone marrow transplantation, and immunosuppression for reasons such as polytrauma, HIV infection, and transplantation (Murdoch *et al.*, 2009). Catheter infections along with catheter-induced urinary tract infections (UTIs) lead to serious inflammation and pus secretion which may lead to an extremely painful urination (Queck and Otto, 2008). Septicemia and endocarditis are also diseases caused by *S. epidermidis* with the symptoms of fever, headache, fatigue, anorexia and dyspnea (Otto, 2009). Septicemia is particularly prevalent consequential from neonatal infections, predominantly with very low birth

weights (Chu *et al.*, 2009). *Staphylococcus epidermidis* is very likely to contaminate patient-care equipment and environmental surfaces, possibly explaining the high incidence of *S. epidermidis* in the hospital setting.

2.1.3 Environmental factors for survival of *Staphylococcus epidermidis*

Staphylococcus epidermidis is a part of the normal human flora, typically the skin flora, and less commonly the mucosal flora (Fey and Olson, 2010). On a healthy adult there are between 10^3 and 10^5 colony forming units of Coagulase negative Staphylococci (CoNS) per cm^2 of skin (Kloos, 1980) and approximately 40 species of CoNS share the skin environment with a plethora of other microorganisms (Roth and James, 1988). The skin provides a harsh environment for bacteria through: constantly changing temperature, humidity and salinity, exposure to detergents and host antimicrobial peptides (AMPs) which present challenges for bacterial survival. *Staphylococcus epidermidis* possesses a wide variety of surface expressed molecules, some of which are likely to have important roles in survival and adhesion on the skin. Transmission of *S. epidermidis* in the health care setting arises through contact with contaminated surfaces in the environment (Boyce, 2007).

Some clones of *S. epidermidis* are possibly endemic in the hospital environment as numerous studies intensely propose that they are frequently caused by strains transmitted among hospitalized patients (Huebner and Goldmann, 1999). *Staphylococcus epidermidis* have the capability to survive in the intensive care unit surroundings on medical devices and medical equipment such as patient care equipment, uniforms, computer key boards, cellular phones, bedrails, door knobs, table tops and identification badges for weeks to months (Kramer *et al.*, 2006; Landers *et al.*, 2010).

2.1.4 Infections caused by *Staphylococcus epidermidis*

Staphylococcus epidermidis has been documented as one of the primary causative pathogen of bloodstream infections, surface infections, and meningitis as a result of intravascular devices (prosthetic heart valves, shunts, etc.) or more commonly occur in prosthetic joints, catheters, and large wounds (Apanga *et al.*, 2014). Catheter infections along with catheter-induced UTIs lead to serious inflammation, pus secretion and painful urination. Septicemia and endocarditis are also diseases associated with *S. epidermidis* (Shiroma *et al.*, 2015). Their symptoms run the gamut from fever, headache, and fatigue to anorexia and dyspnea. Septicemia is especially prevalent resulting from neonatal infections, particularly in very low birth weights. Endocarditis is an infection of the heart valves and parts of the inside lining of the heart muscle. *S. epidermidis* is very likely to contaminate patient-care equipment and environmental surfaces, possibly explaining the high incidence of *S. epidermidis* in the hospital setting (Okee *et al.*, 2012).

2.2 Susceptibility patterns of *Staphylococcus epidermidis* to common antibiotics

Staphylococcal infections are a common and significant clinical problem in medical practice. Most strains of *S. epidermidis* are now resistant to penicillin, and methicillin-resistant strains of *S. epidermidis* are common in hospitals and are emerging in the community (Fang *et al.*, 2011). Penicillinase-resistant penicillins (flucloxacillin, dicloxacillin) remain the antibiotics of choice for the management of serious Methicillin-susceptible *S. epidermidis* (MSSE) infections, but first generation cephalosporin (cefazolin, cephalothin and cephalexin), clindamycin, amikacin, gentamycin, ciprofloxacin, lincomycin and erythromycin have important therapeutic roles in less serious MSSE infections such as skin and soft tissue infections or in patients with penicillin hypersensitivity (Muhammad *et al.*, 2015).

All serious Methicillin-resistant *S. epidermidis* (MRSE) infections are recommended to be treated with parenteral vancomycin or teicoplanin if the patient is vancomycin allergic (Rayner, 2015). Also antibiotics such as linezolid and quinupristin/dalfopristin have good anti-staphylococcal activity but are very expensive therefore they are recommended to be used for patients who are intolerant of conventional therapy or highly resistant strains such as heterogenous vancomycin-intermediate *S. epidermidis* (hVISE) (Mack *et al.*, 2005).

A number of drug susceptibility studies for *S. epidermidis* have been done and have yielded inconsistent results for some drugs. Beta-lactam drugs including penicillins, ampicillins and oxacillin are considered to be a major group of antimicrobial drugs used in the treatment of bacterial infections but *S. epidermidis* has developed resistance to these antibiotics (Cherifi, 2014). Resistance to penicillins is mostly caused by the presence of ß-lactamases, which reduces the affinity for ß-lactams by mutations in penicillin binding proteins (PBPs) (Brinas *et al.*, 2005).

A study carried out in Turkey on blood culture of septicemic patients by Koksal *et al.* (2009), showed that *S. epidermidis* was resistant to ciprofloxacin, erythromycin, gentamycin, tetracycline, clindamycin and trimethoprim sulfamethoxazole and susceptible to vancomycin and teicoplanin. Another study carried out in Nigeria on clinical isolates by El-Mahmood, (2015), was in agreement with Koksal *et al.* (2009) on resistance of *S. epidermidis* to gentamycin and erythromycin, but not for tetracycline. The study by El-Mahmood (2015) also showed that *S. epidermidis* was resistant to augmentin, nitrofurantoin, chloramphenicol, and Ofloxacin but susceptible to ampicillin, streptomycin, pefloxacin, and co-trimoxazole.

Unlike the above studies which showed *S. epidermidis* to be resistant to gentamycin, a study carried out in India among patients suspicious of bacteremia by Mohammad *et al.* (2015) showed gentamycin to be very effective against these bacteria and so was vancomycin. However, penicillin, tetracycline, erythromycin and clindamycin were less effective on *S. epidermidis* (Mohammad *et al.*, 2015). *Staphylococcus epidermidis* isolates from hospital environment are more resistant to the antibiotics such as methicillin (oxacillin), cephradine, cefaclor, cefazolin, amikacin and streptomycin (Mack *et al.*, 2005).

It was reported that, the selective pressure exerted by the broad-spectrum cephalosporin, create rapid overgrowth of *S. epidermidis* resistant to antibiotics used, and as such cephalosporins become ineffective against *S. epidermidis* (Dancer, 2001). The fact that most of the studies give inconsistent results raised a need for susceptibility studies for this bacterium to the common used antibiotics in KIU-TH. Furthermore, the evidence of drug resistance among S. epidermidis strains called for a need to come up with cheaper effective alternatives which includes the use of ethno-medicinal plant extracts such as those from *C. papaya*.

2.3 Antibacterial activity of *Carica papaya*

Carica papaya was named by a Swedish botanist called Carl linneaus in 1753. It is a native to South America and is naturalized in Florida, Mexico, Central America, in the West Indies, in Tropical Africa and Asia (Namuddu *et al.*, 2011). *Carica papaya* called Papali in Uganda is known to be used as an ointment by the local people for the treatment of skin infection (Namuddu *et al.*, 2011). *Carica papaya* flowers have been therapeutic on jaundice (Anibijuwon and Udeze, 2009). Effectiveness of these treatments however, is reliant on the quantity of the different compounds in the preparations.

The seeds of papaya have antimicrobial activity against *Trichomonas vaginalis* trophozoites (Calzada *et al.*, 2007). The seed and pulp of papaya were shown to be bacteriostatic against *Bacillus subtilis*, *Enterobacter cloacae*, *Escherichia coli*, *Salmonella typhi*, *S. aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* by the agar cup plate method (Osato *et al.*, 2003). Purified extracts from ripe and unripe fruits also showed significant antibacterial activity on *S. aureus*, *Bacillus cereus*, *E. coli*, *P. aeruginosa* and *Shigella flexneri* (Emeruwa, 2005). The aqueous extract of fruit promoted significant wound healing in diabetic rats and the seeds have bacteriostatic activity on Gram positive and Gram negative organisms which could be useful in treating chronic skin ulcers (Dawkins *et al.*, 2003).

According to the study by Nkuo-Akeni *et al.* (2001), herbal formulations containing papaya leaves and root or leaves alone as one of the constituent were shown to have antibacterial activity against *S. typhi*, *S. paratyphi* and *S. typhimurium* but the water, acetone and ethanol extract of papaya leaves showed no microbicidal activity. Papaya fruits are used as topical ulcer dressings, which promote desloughing, granulation and healing. It also reduces the odour in chronic skin ulcers. It is cost effective and is considered to be more effective than other topical applications in the treatment of chronic ulcers (Hewitt *et al.*, 2002).

A study carried out in India by Aliya *et al.* (2016), proved that unripe *C. papaya* fruit methanolic extracts showed good antibacterial activity against nosocomial infection causing organisms including *S. epidermidis*. Study from South America revealed that, different extracts of *C. papaya* leaf showed antibacterial activities against *S. aureus*, *E. coli* and *C. albicans* with methanol extract showing more activity (Subramanian *et al.*, 2014). A study by Khan *et al.*

(2012) from India, reported that all the extracts of *C. papaya* (dry leaf, green leaf, root, stem, ripe pulp, unripe pulp, ripe peel, unripe peel and seed) were effective against *P. aeruginosa, S. aureus* and *E. coli* but hot aqueous extract of ripe peel showed best antibacterial activity against *E. coli*.

It was reported in a study carried out in India, that *S. aureus* and *S. epidermidis* were found to be highly susceptible to the ethanol and methanol extracts of leaves of *C. papaya* amongst the test organisms used in the study, with *Pseudomonas* sp. *Neisseria* sp., *Proteus mirabilis, Acinetbacter baumanni* and *Enerococcus faecium* found to be inhibited to some extent (Nagesh and Samreen, 2016). It was reported in Nigeria that, ethanolic and aqueous extract of seeds and leaves of *C. papaya* were found to be effective against *E. coli, P. aeruginosa, K. pneumoniae, S. aureus, S. pneumoniae* and *B. subtilis* with ethanolic extract giving better activity than the aqueous (Ayanfemi and Bukola, 2015).

2.3.1 Minimum inhibitory and bactericidal concentrations of *Carica papaya* extracts

Minimum Inhibitory Concentration (MIC) is define as the minimum concentration of a given antimicrobial agent required to inhibit the growth of microorganisms. Minimum Bactericidal Concentration is the minimum concentration of antimicrobial agent required to completely kill the bacterial cell (Adejuwon *et al.*, 2011). Minimal inhibitory concentrations (MIC) of *C. papaya* were demonstrated by Nirosha and Mangalanayaki (2013). The result obtained shows that the MIC of ethyl acetate of leaves extract against *S. aureus, S. pneumoniae, E. coli* and *P. aeruginosa* was 70 mg/ml, while that of root extract against *S. aureus, P. aeruginosa, S. pneumonia* was 150 mg/ml. Okunola and others. (2012) determined the MIC of dried and fresh leaves extracts of *C. papaya* against *E. coli, Salmonella, S. aureus* and *S. pyogens*. The result gave lowest MIC of 50 mg/ml against *S. aureus* while the MIC values ranging between 75-100 mg/ml was given against *E. coli, Salmonella and S. pyogens*.

A study by Doughari *et al.* (2007) revealed that the lowest MIC and MBC of 50 mg/ml were demonstrated by the root extract of *C. papaya* against *S. typhi*, while the MIC and MBC values ranging between 100-200 mg/ml were observed against *S. pyogens, S. aureus, S. pneumoniae* and *S. flexneri*. In another study carried out by Adejuwon *et al.* (2011), MIC of *C. papaya* aqueous and methanol extract were determined against *P. aeruginosa, S. typhi, E. coli, S. aureus,*

B. cereus and *K. aerogenes*. Among these bacterial species, *B. cereus* and *K. aerogenes* were found to be sensitive with MIC of 0.5 mg/ml for both extracts. Mwesigwa and co-workers. (2012) reported MIC of 100 and 3.12 mg/ml of *C. papaya* extracts and amoxacillin against *E. coli*.

Awatif (2015), reported that water extracts of *C. papaya* were less effective than the alcohol extracts, with MIC of 16 mg/ml for the treated *E. coli* and *C. jejuni*. Chima *et al.* (2016), revealed that the relatively high MIC for cold ethanol extracts of *C. papaya* of 0.92, 0.65 and 0.61 mg/ml on *K. pneumoniae*, *E. coli* and *S. aureus* respectively attests to the claim that gram negative bacteria have higher resistance to plant extracts. From the above we can deduce that, MIC and MBC vary between extract and the test organism. Therefore, in this study minimum concentration of the active extract required to inhibit or completely kill the growth of *S. epidermidis* was determined using broth dilution method.

2.3.2 Factors affecting the effectiveness of *Carica papaya* extracts

The efficacy of *C. papaya* extract is dependent on the variation in active substance content and antioxidant activity as reported by Udoh *et al.* (2005). Also temperature affects the production of secondary metabolites as reported by Bilger *et al.* (2007), that the biosynthesis of phenol was demonstrated in a plant growing in a low temperature regime. Illumination also affects the synthesis and accumulation of secondary metabolites in medicinal plants. The increase of illumination time can increase the contents of secondary metabolites. For example, the amount of flavonoids in Arabidopsis increased after long time illumination (Fuglevand *et al.*, 1996). Precipitation also affects the active components of the plants as reported by Wei *et al.* (2016), in his study which showed that the contents of tannin, rutin and total phenolics were negatively correlated to the annual average precipitation.

Some researchers have stated that the variations in the active substance contents of the plants are associated with the soil fertility (Khan *et al.*, 2012). It was reported that, the dried leaf extract of *C. papaya* was more potent than the other part of the plant against some of the bacteria to which standard antibiotics were not able to inhibit (Okunola *et al.*, 2012). Antibacterial activity in *C. papaya* is highly affected by the polarity of the solvent, nature of the extracted compounds and

extraction process (Metrouh-Amir, Duarte and Maiza, 2015). It was reported by Nirosha and Mangalanayaki (2013), that the ethanolic extract of leaves and roots of *C. papaya* were more effective against *S. aureus, S. pneumonia, B. cereus, S. typhi, E. coli* and *P. aeruginosa* than that of aqueous extract of leaves and root. Orhue and Momoh (2013), also reported that *C. papaya* leaves extract in 1% Hydrochloric acid (HCl) and ethanol, showed antimicrobial activity against *Bacillus sp. Enterobacter coacae, E. coli, S. typhi, S. aureus* and *Proteus vulgaris* while extracts in water was only active against *E. coli* and *S. aureus.*

2.3.3 Phytochemical composition of *Carica papaya*

Fruits contain the following compounds: benzylisothiocyanate, *cis* and *trans* 2, 6-dimethyl-3, 6 epoxy-7 octen-2-ol, carpaine, benzyl-D glucoside, 2-phenylethyl-D-glucoside, 4-hydroxy-phenyl-2 ethyl-D-glucoside and four isomeric malonated benzyl-D-glucosides (Nguyen *et al.*, 2013). A study by Okeniyi *et al.* (2007) showed that the seed of *C. papaya* contains; carpaine, benzylisothiocyanate, benzylglucosinolate, glucotropacolin, benzylthiourea, hentriacontane, sitosterol, caricin and Myrosin enzyme). Its root has carposide and myrosin. The bark contains sitosterol, glucose, fructose, sucrose, galactose and xylitol. The latex has proteolytic enzymes, glutamine cyclotransferase, papain and chymopapains A, B and C, peptidase A and B, and lysozymes (Antonella *et al.*, 2007). Phytochemical analysis of *C. papaya* leaf extract revealed the presence of alkaloids, glycosides, flavanoids, saponins, tannins, phenols and steroids (Natarjan *et al.*, 2014).

2.4 Antimicrobial resistance of *Staphylococcus epidermidis*

Antimicrobial resistance is a phenomenon which occurs when microorganisms, such as bacteria, viruses, fungi and parasites transform into ways that render ineffective the medications against the caused infections. This is a major problem because resistant strains of a microorganism may kill, or can be spread to others; and lead to huge costs to individuals and society (WHO, 2014). In *S. epidermidis*, the role of exo-polysaccharide matrix or ability of biofilm formation is used to cause resistance by reducing the permeability and penetration of antibiotics into the organism (Hall-Stoodley *et al.*, 2004). There are many and varied resistance mechanisms in bacteria, hence, some of them may be intrinsically resistant to certain, specific antibiotics or to more than one class of antimicrobial agents (Koll and Brown, 1993).

The most significant ways of achieving resistance, however, are innate gene mutation and acquired antimicrobial resistance genes from another microorganism (Garza *et al.*, 2010). Beta-lactam drugs, including penicillins, ampicillins and Oxacillin are considered to be a major group of antimicrobial drugs used in the treatment of bacterial infections; but *S. epidermidis* has developed resistance to these antibiotics (Cherifi, 2014). Resistance to penicillins is mostly caused by the presence of ß-lactamases, which reduces the affinity for ß-lactams by mutations in penicillin-binding proteins (PBPs) (Brinas *et al.*, 2005).

2.4.1 mecA gene in Methicillin resistant *Staphylococcus epidermidis*

The mecA gene in bacterial cells is responsible for resistance to antibiotics such as methicillin, penicillin and other penicillin-like antibiotics (Ubukata *et al.*, 2007). The best known carrier of the mecA gene is the bacterium Methicillin-resistant *S. aureus* (MRSA). Apart from *S. aureus* and other Staphylococcus species, especially *S. epidermidis*, it can also be found in *S. pneumoniae* strains resistant to penicillin-like antibiotics (Miragaia *et al.*, 2005). In Staphylococcus species, mecA is spread on the Staphylococcal chromosomal cassette mec (SCCmec) genetic element (Deurenberg *et al.*, 2009). The mecA gene does not allow the beta-lactam ring structure of penicillin-like antibiotics to bind to the enzymes that help form the cell wall of the bacterium (transpeptidases), and hence the bacterium is able to replicate as normal. The gene encodes the protein penicillin binding protein 2A (PBP2A) (Turlej *et al.*, 2011). Penicillin binding protein 2A PBP2A has a low affinity for beta-lactam antibiotics, such as methicillin and penicillin (Ubukata *et al.*, 2007). This facilitates transpeptidases activity in the presence of beta-lactams, preventing them from inhibiting cell wall synthesis (Deurenberg *et al.*, 2009).

Staphylococcus epidermidis strains circulating in hospitals have been established to be methicillin-resistant (Diekema *et al.*, 2001). The resistance of *S. epidermidis* to methicillin is usually due to the mecA gene, which is carried by staphylococcal cassette chromosome mec (SCCmec), and produces a PBP2A with low affinity for ß-lactams. Staphylococcal chromosomal cassette mec (SCCmec) can also carry genetic elements for other antibiotic resistance; most methicillin-resistant strains (MRSE) are, therefore, highly resistant to other antibiotics (Wang *et al.*, 2004). In diverse collections of *S. epidermidis* isolates, molecular characterization of this

element revealed five SCCmec types previously found in *S. aureus* (Turlej *et al.*, 2011). Additionally, there were high numbers of new and unclassified SCCmec types (Miragaia *et al.*, 2005). These results indicate a high degree of genetic diversity within the SCCmec elements carried by *S. epidermidis* (Hanssen *et al.*, 2007).

CHAPTER THREE: METHODOLOGY

3.0 Introduction

This section presents the research methodology used for this study. It consists of the research design, study area, sample size and sampling strategies, data collection methods to address the different specific objectives, quality control, data analysis, ethical considerations and limitations of the study.

3.1 Research design

The study was a cross sectional research design using quantitative method. According to Creswell and Plano Clark (2007), Quantitative method is used to generate numerical data (Creswell, 2007). In this study, quantitative approach was used in collecting data for Objective one, two and three (susceptibility pattern of *Staphylococcus epidermidis* strains collected from selected surfaces of KIU-TH and effects of C. *papaya* leaves and seeds extracts on the isolated bacterium).

3.2 Study area

The research was carried out in KIU-TH, located in Ishaka Municipality, Bushenyi District, Western Uganda, GPS location 00° 32'19"S, 30° 08'40"E. This is approximately 330 kilometres (210miles) by road, Southwest of Kampala (Arusho and Paul, 2010). It is a well-established referral hospital with several wards which includes: Medical, Surgical, Paediatric, Maternity, Psychiatric, Accident and Emergency, Private and Semi-Private Wards. In addition, there are a wide range of "Specialist" departments and clinics, including: General Surgery, Orthopaedics, Obstetrics & Gynecology, Medicine, Ophthalmology, Dentistry, Paediatrics, and Physiotherapy. This hospital has 700 beds occupancy (Unpublished data).

3.3 Sample size

The sample size was calculated using a formula from Keish *et al.* (1965):

$$N = \frac{Z^2PQ.}{I^2}$$

Where;

N- is the sample size

Z - is 1.96, which is the score corresponding to 95% confidence interval

P- is the assumed prevalence, which is taken to be 50% for unknown.

Q = (1-P)

I - the accepted error term corresponding to 5%.

Therefore:

$$N = \frac{1.96^2 X\ 0.5\ X\ 0.5}{0.05^2}$$

$=384.16$

$N \approx 384$ samples.

Fifty (52) swab samples were collected from different surfaces (bedrails, doorknobs, floors and walls) of all the seven different wards (surgical, medical, pediatrics, maternity, accident and emergency and semi-private). Fifty one (51) samples were collected from private ward due to the limited number of rooms in the ward hence less doorknobs as compared to other wards. For the epidemiological data, fourteen (14) out of thirty five (35) cleaners were recruited for the study using simple random sampling technique and Seven (7) Ward In-Charges using purposive sampling technique .

3.4 Sampling strategy

Swab samples were taken from surfaces of seven wards (Medical, Surgical, Paediatric, Maternity, Psychiatric, Accident and Emergency, Private, and Semi-Private), selected using the purposive sampling strategy based on the fact that these wards are associated with medical devices in KIU-TH.

3.5 Sample collection and storage

Swab samples were collected from floors, door knobs, walls, and bedrails from different wards in the hospital using sterile swab soaked in normal saline. However, due to the limited number of door knobs and bedrails in the hospital, three sixty three (363) swab samples were collected out of the proposed sample size (384). The samples were collected from the surfaces by moving a

sterile, pre-moistened swab, over the entire surfaces: 10 passes in a horizontal direction and 10 passes in a vertical direction. Care was taken not to overlap the previous pass, so as to ensure thorough coverage of the surfaces. The swabs were rotated slowly while making each pass; and held at a 45° angle, so that the surfaces were contacted by the full length of the swab head.

The swabs were then collected and placed into a falcon tube (15ml) containing 2 ml of normal saline. The normal saline was to prevent the bacterial cells from lysis and moisten the swabs before collection. In order to extract as much liquid as possible from the swabs, each swab was rolled on the inner edge of the cryogenic vials/tube, before removal from the container.

The samples were kept in an ice box during collection and transported in Stuart media to the laboratory promptly thereafter. Most samples were processed immediately, at the Microbiology Laboratory, Department of Microbiology and Immunology of KIU-Western Campus. The remainder was stored under refrigeration, at 2–8°C; and was processed within seven days (Valle *et al.*, 2007).

3.6 Data collection

Quantitative data was collected in the study and the data collection methods are described as per objective.

3.6.1 Distribution of *Staphylococcus epidermidis* from ward surfaces of Kampala International University-Teaching Hospital

Swab samples were collected from the seven wards of KIU-TH and transported to Microbiology laboratory for analysis.

3.6.1.1 Microbial isolation and identification

The swabs were inoculated using the streaking method to obtain colonies and sub-cultured on Mannitol salt agar (MSA) to obtain discreet colonies under a biosafety cabinet. The plates were then incubated at 37°C overnight (Anam *et al.*, 2015) after which Coagulase test was performed. This was carried out by dropping rabbit plasma onto a sterile glass slide and emulsifying a loopful of the bacterial colony on the slide using a sterile wire loop. Presence of agglutination

differentiated the coagulase negative from coagulase positive Staphylococci (Skinner *et al.*, 2009).

Desferroxiomine and Fosfomycin antibiotics, 1mg/ml each, were prepared and impregnated with 6mm discs made by using Whatman filter paper number 1 and tested against the coagulase-negative isolates to differentiate *S. epidermidis* from other species of coagulase-negative staphylococci (Thiago *et al.*, 2013).

3.6.2 Susceptibility pattern of *Staphylococcus epidermidis* isolated from different wards surfaces of Kampala International University-Teaching Hospital on the commonly used antibiotics.

The antibiotic susceptibility test was performed using the Kirby-Bauer disc diffusion method (Anjana *et al.*, 2009). Pure colonies of *S. epidermidis* collected from the selected surfaces were picked from an agar plate, transferred into a tube containing tryptic soy broth, and turbidity adjusted to 0.5 McFarland standards.

The standardized suspension of isolates was inoculated on Mueller Hinton agar plates using the spread plate method. Antibiotic discs of commonly used antibiotics (Amikacin 30µg, Cefazolin 30µg, Cefoxitin 30µg, Trimethoprim-sulfamethoxazole 25µg, Ciprofloxacin 30µg and Gentamicin 30µg) were firmly placed on the agar plates using sterile forcep. The plates were incubated at 37°C overnight after which the diameters of the zone of inhibition were measured. The results were then interpreted as per the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2012).

3.6.3 Antibacterial activity of *Carica papaya* leaf and seed extracts against antibiotic resistant *Staphylococcus epidermidis* isolated from different wards surfaces of Kampala International University-Teaching Hospital.

3.6.3.1 Identification and collection of *Carica papaya* leaves and seeds

The plant samples were collected from Kigondo village in Ishaka municipality, and taken to the Botanist at the Department of Biology and science laboratory technology at Mbarara University of Sciences and Technology (MUST), Uganda, for identification. A voucher number was

obtained as Abubakar S. Adam # 0003 and specimen was stored in herbarium of the department for future reference. Thereafter, fresh leaves and seeds (from ripe pawpaw) were collected from the village early in the morning and transported in a sterile nylon bag to the Pharmacology laboratory, Department of Pharmacology, KIU-WC.

In the KIU-WC Pharmacology laboratory, the leaves and seeds were thoroughly washed with tap water, and rinsed with sterile distilled water. The clean material was then air-dried under room temperature (Ayoola and Adeyeye, 2010). The dried leaves and seeds were pulverized using pestle and mortar to obtain a powder which was stored in air-tight glass containers and covered with aluminium foil to protect it from sunlight until required for extraction.

3.6.3.2 Preparation of *Carica papaya* leaves and seeds extract

Extraction was carried out using the maceration method as described by Gideon *et al.* (2012). One hundred grams (100g) of the leaves and seeds powder each was put in three different beakers and dissolved in 500 mls of absolute methanol, acetone and distilled water, with polarity index of 5.1, 4.1 and 10.2, respectively. The mixture was allowed to mix for 48 hours, with frequent shaking using vibratory sieve shaker to avoid pouring or evaporation of the solvents before extracting the active components. The crude extracts were filtered using a clean cotton cloth, followed by use of Whatman filter paper number 1. The filtrate was distilled and then evaporated to remove the solvent.

The percentage yield extract was obtained using the formula: W_2-$W_1/W_0 \times 100$. (Where: W_2 is the weight of the extract and the container, W_1 the weight of the container alone, and W_0 the weight of the initial dried sample (Anokwuru *et al.*, 2011).

3.6.3.3 Preparation of extract concentration

Five hundred (500) mg of each extract was dissolved in 1ml of 20% Dimethyl sulfoxide (DMSO) to obtain the concentration of 500 mg/ml as described by (Gideon *et al.*, 2012).

3.6.3.4 Phytochemical screening

The crude extracts of *C. papaya* were screened to check the presence of phytochemicals, such as: flavonoid, tannins, terpenoids, cardiac glycosides, saponins, and steroids using the standard procedures described by Gideon *et al.* (2012) and as described in Appendix I.

3.6.3.5 Antimicrobial screening of crude extract

The antimicrobial activity of the extracts was demonstrated using agar well diffusion method as described by Ogutu *et al.* (2012). Sterile Mueller Hinton agar plates were inoculated with the standardized suspension of the isolates resistant to the antibiotics used above using the same procedure as that of antibiotic susceptibility testing. Five wells of 5 mm diameter were punched into the agar plates using a sterilized cork borer (5 mm). Using a micropipette, 100µl of both extracts were added to the first, second and third well accordingly. A concentration of 7µg/ml of vancomycin was prepared according to Johnson (2012) and100µl of the prepared vancomycin was added to the fourth well as positive control while DMSO was added to the fifth wells as negative controls. The diameter of the zone of inhibition was measured and results interpreted according to the Clinical and Laboratory Standard Institute guidelines (CLSI, 2012).

3.6.3.6 Minimum inhibitory and bactericidal concentrations

The minimum inhibitory concentration (MIC) of the crude extracts was determined according to Ogutu *et al.* (2012). Two-fold serial dilution of the extract was carried out in a series of sterile tubes containing 1ml of nutrient broth to obtain different concentrations (500, 250,125, 625, 31.25 and 15.63 mg/ml). One ml suspension of the test organism compared with 0.5 McFarland standard was added to each tube. This method was modified by preparing two sterile tubes: one containing only nutrient broth and test organism without the extract, to serve as negative control; and the other containing only the broth and extract without the test organism, to serve as positive control. Each of the tests was done in triplicate in order to minimize errors.

The viability of the test organism was verified by plating out a loopful of broth suspension from positive control onto the sterile Mueller Hinton agar. The diluted tubes and the plates were incubated overnight at 37°C. After incubation, the turbidity from each diluted tube was compared with the control tubes and the highest dilution without turbidity was considered as MIC and

interpreted in mg/ml. The turbidity was determined using spectrophotometer at 460nm wavelength due to the colour of the extracts.

The result of MIC was used to determine Minimum Bactericidal Concentration by sampling clear tubes. A loopful of broth from each clear tube was inoculated onto the nutrient agar in triplicate. Nutrient agar plate was streaked with the test organism to serve as controls. All the plates were incubated at 37°C for 24 hrs. After incubation the concentration at which no visible growth was seen was taken as the MBC (Ugoh *et al.*, 2013).

3.6.4 Presence of mecA gene among Methicillin resistant *Staphylococcus epidermidis* isolated from different wards surfaces of Kampala International University-Teaching Hospital.

Presence of mecA gene responsible for methicillin resistance in *S. epidermidis* isolates was determined by Polymerase Chain Reaction (PCR) (Prasad *et al.*, 2012).

3.6.4.1 DNA extraction

Bacterial DNA was extracted by the standard protocol described by (Prasad *et al.*, 2012). Briefly, 5 ml overnight culture of *S. epidermidis* was centrifuged for 10 minutes to harvest the cells. The supernatant was discarded and 875 µl of TE buffer was added to the pellet. The cells were suspended in the buffer by gentle mixing. 100 µl of Sodium dodecyl phosphate and 5 µl of proteinase K were added to the cells. One milliliter (1ml) of phenol-chloroform mixture was added to the content and mixed well by inverting the tubes and incubated at room temperature for 5 minutes. The tubes were centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant was collected using cut tips and is transferred to a fresh tube. The process was repeated once again by using phenol-chloroform mixture and the supernatant was collected in a fresh tube. Hundred microliter (100 µl) of sodium acetate 5M was added to the tubes and mixed gently. Two milliter (2 ml) of isopropanol was added and mixed gently by inversion till a white precipitate of DNA was formed from the mixture. Ninety microliters (90µl) of the supernatant containing the Deoxyribonucleic acid (DNA) was transferred into a new clean tube and put in an ice box for Polymerase chain reaction (PCR) (Prasad *et al.*, 2012).

3.6.4.2 Polymerase chain reaction (PCR)

The presence of mecA gene in resistant *S. epidermidis isolates* was detected using conventional PCR conducted from Molecular laboratory, College of Veterinary animal resources and biosecurity, Makerere University. A known Methicillin resistant *S. epidermidis* and distilled water were used as positive and negative controls respectively as described by Arefi. (2013). The DNA of the Methicillin resistant *S. epidermidis* strains were amplified with the primers mecA-Forward(5′-AAA ATC GAT GGT AAA GGT TGG C-3′) and mecA-Reverse (5′-AGT TCT GCA GTA CCG GAT TTG C-3′). PCR was performed with a 25µl volume reaction mix containing: 3.5µl of reaction buffer containing (50mM KCl, 10mM Tris-HCl (pH 8.0), 2.5 mM $MgCl_2$), 0.5µl of Deoxyribonucleotide triphosphates (dNTP's) (10mM), 1.5µl each of forward and reverse primers, 2.5µl of Taq DNA polymerase, 2.5µl (0.4mM) of DNA template and 13µl of RNase free water. Amplification was performed by denaturation of the double stranded DNA into single strands by subjecting it at 94°C for 5 mins. This was followed by lowering temperature by thermo cycler to 55°C for 30 sec to allow the annealing of primers to the beginning (3') of each single stranded DNA (template). The extension was carried out at 72°C for 2 mins with a total of 35 cycles and an additional extension at 72°C for 10 min. The experiment was carried out within 30 mins.

The amplified DNA fragments were visualized under Ultra-violet (UV) trans-illumination following electrophoresis at 85 volts on 1.5% agarose gel stained with ethidium bromide showing bands for positive isolates and a molecular ladder (500bp) indicating the weight of base pairs (Najar *et al.*, 2013).

3.7 Quality control

All the equipment in the laboratory, such as autoclave, microscope, incubator, evaporator, etc, were used following the manufacturer's operating guidelines. Each test was done in triplicate, in order to minimize errors. Positive and negative controls were used so as to get precise and reliable results. To avoid contamination during PCR, the working surfaces were decontaminated by washing with 10% chlorine to hydrolyze possible DNA contaminants. During PCR, gloves and laboratory coats were changed often, to prevent spread of amplified DNA or contamination

with nucleases naturally occurring on skin that might degrade the sample DNA. The primers were tested on known positive/negative controls prior to use, so as to avoid false-positive result.

3.8 Data analysis

The raw data was entered in an excel sheet and cleaned off any errors. The data from objective 1 and 3 were then analyzed using statistical package for social sciences (SPSS) version 21 software. From objective one, the distribution rate of *S. epidermidis* between the wards and surfaces were compared using one way ANOVA while from objective three the inhibition zone diameter provided by leaves extract (Methanol, Acetone and Aqueous) and seeds (Methanol, Acetone and Aqueous) were compared using one way ANOVA and t-test, where $p \leq 0.05$ was used to indicate level of significance between the distribution of *S. epidermidis* and the activity of the extracts. Results of objective two were presented in percentages while that of objective four was interpreted using gel electrophoresis.

3.9 Ethical considerations

In order to make sure that the study is conducted ethically, several specific issues were addressed.

3.9.1 Institutional consent

Ethical clearance was sought from the Research Ethics Committee of KIU-WC.

3.9.2 Hospital approval

Permission to collect samples from the selected wards and qualitative data from cleaners and ward In-Charges was sought from the hospital management of KIU-TH and approval was obtained.

3.9.3 Informed consent

All wards In-Charges for the seven wards and the randomly selected cleaners were informed of the study, using the best locally understood language. The purpose of the study, methods, possible risk(s) and benefits of participation were clearly spelt out.

Involvement in the research was voluntary and participants were free to opt out at any time without penalty or loss of potential advantages. Individuals willing to be part of the study were requested to fill out and sign a pertinent Consent form, administered by the researcher, and in the presence of a witness. A copy of the signed form was given to the participants.

3.9.4 Privacy and confidentiality

Privacy of participants was ensured by protecting individual identity and information. For example, all data collected was used without names of the participants and kept safely and confidentially.

3.9.5 Justice in selection

Every respondent was given equal opportunity to participate in the study. No particular priority was given to any group.

3.9.6 Respect of rights of individuals

Each respondent had an entitlement to his/her opinion, response and comments. The researcher ensured that each and every response provided during the course of the study was respected.

3.9.7 Welfare of research participants

No conceivable risk was anticipated in this study. Every effort, however, was made to ameliorate any unforeseen harm to participants in the study. By not exposing their identity to the public.

3.9.8 Protection of research personnel and environment

Protective wear, including gloves and laboratory coats were used to protect research personnel against the test organism. Inoculation of samples was carried out in a safety cabinet to prevent environmental contamination and infection to research personnel.

All plates and any disposable materials used were properly disposed of or burnt after being autoclaved. Reusable glass wares were autoclaved so as to prevent the risk of infection; any washing was done in a sink; and the runoff disposed of in a septic tank. The surfaces of the

working benches (Inner part of safety cabinet and pouring space) were decontaminated with 70% ethanol.

3.9.9 Scientific validity

Well knowing that research cannot be ethically sound unless it is scientifically valid, reliable results were generated for each objective, using authentic and feasible methods described herein. Study operations were refrained from manipulation of findings and all the procedures and results generated were securely and accurately documented.

3.10 Limitations to the study

- Concentration of the active compounds (phytochemicals) in the leaves and seeds varies depending on the season (dry or wet season) and time (morning and afternoon). This limitation was minimized by ensuring that the leaves were collected very early in the morning) and during the wet season.
- The number of the samples collected was less than the proposed sample size due to the limited number of doorknobs in the hospital. This was minimized by collecting equal number of samples from all the seven wards.
- The specific of the PCR fragments can mutate to the template DNA, due to non-specific binding of primers. This was minimized by keeping the stipulated time given to each step of the experiment as well as the level of temperature.
- Irregularity of electric power availability affected the incubation period of the bacteria. This was minimized by working during both day and night to compensate for time lost.

CHAPTER FOUR: RESULTS

4.0 Introduction

This chapter consists of the results on; the distribution and risk factors associated with *S. epidermidis* in different wards surfaces of KIU-TH, susceptibility pattern of *S. epidermidis* against commonly used antibiotics at KIU-TH, antibacterial activity, of *C. papaya* leaf and seed crude extracts against antibiotic resistant *S. epidermidis* isolated from wards surfaces of KIU-TH and mecA gene analysis of methicillin resistant strains of *S. epidermidis*.

4.1 Distribution of *Staphylococcus epidermidis* in different wards surfaces of Kampala International University Teaching Hospital

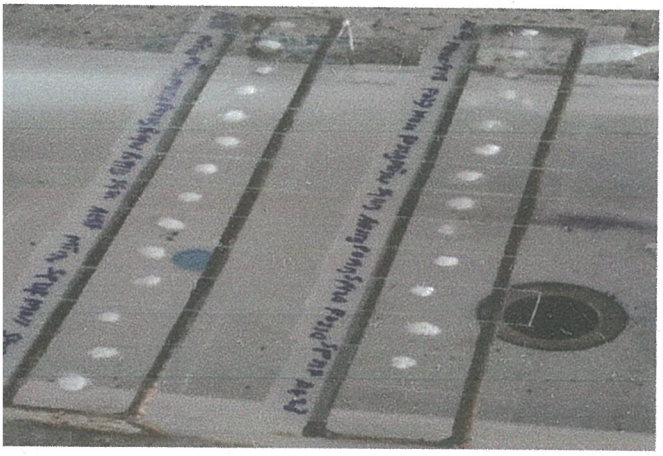
4.1.1 Identification of *Staphylococcus epidermidis* isolated from wards surfaces of Kampala International University Teaching Hospital based on the different tests

A total of three hundred and sixty three (363) swab samples were collected from four different surfaces (Wall, Bedrail, Floor and Doorknob) in seven wards. Out of these 173(47.66%) yielded pink colonies on Mannitol salt agar indicating they were coagulase negative. Following catalase and coagulase tests, all the 173 pink colonies were catalase positive and coagulase negative as indicated in table 1 and figure 2 (a,b and c) below. Out of 173 coagulase negative isolates, 143 were susceptible to Desferroxiomine antibiotic. Out of 143 isolates susceptible to Desferroxiomine, 112 (30.85%) were susceptible to Fosfomycin antibiotic which was indicative of *S. epidermidis* as shown in Table 1 and figure 2 (d and e) below.

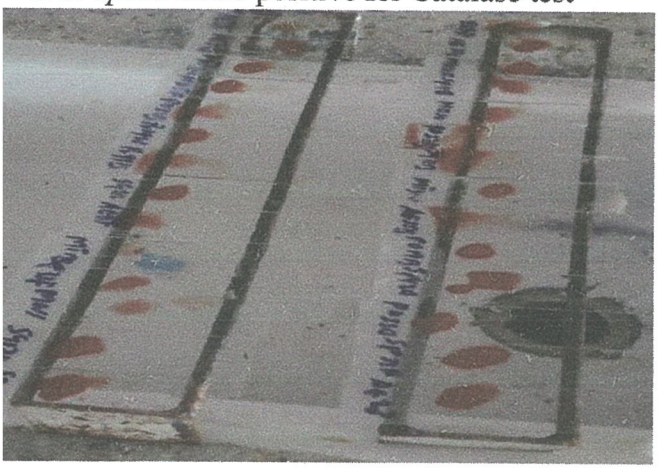
Table 1: Identification of *Staphylococcus epidermidis* isolated from wards surfaces of Kampala International University Teaching Hospital based on the different tests

Tests	Number of isolates tested	Positive isolates (%)
Growth on Mannitol salt agar	363	173 (47.66)
Catalase	173	173 (100)
Coagulase	173	0 (0.00)
Desferroxiomine	173	143 (82.66)
Fosfomycin	143	112 (78.32)

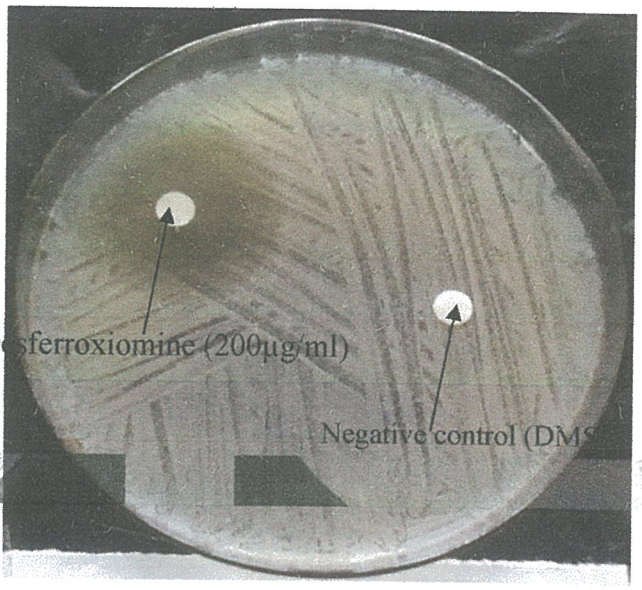
a. *S. epidermidis* on Mannitol salt agar



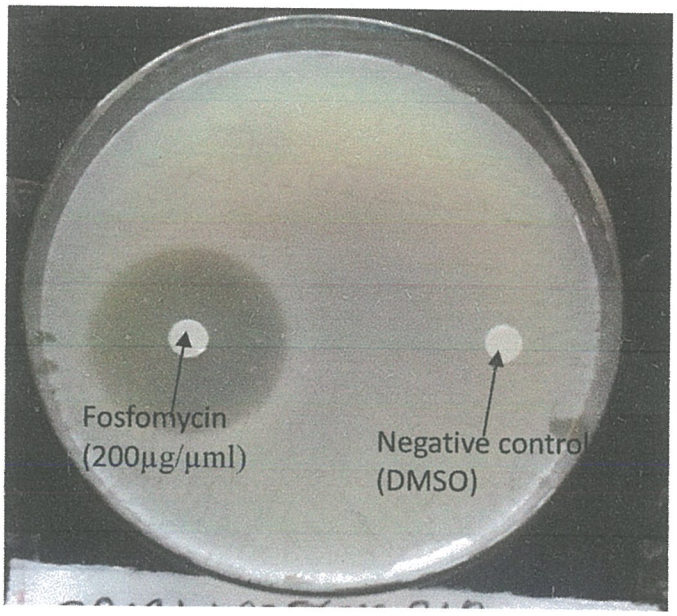
b. *S. epidermidis* positive for Catalase test



c. *S. epidermidis* negative for Coagulase test



d. *S. epidermidis* susceptible to Desferroxiomine



e. *S. epidermidis* susceptible to Fosfomycin

Figure 2: Identification of *Staphylococcus epidermidis* using different tests.

4.1.2 The distribution of *Staphylococcus epidermidis* in ward surfaces of Kampala International University-Teaching Hospital

Table 2 shows the distribution of *S. epidermidis* from all the seven wards and four surfaces selected in this study. Among the selected wards, Surgical had the highest distribution rate (25(22.32%) while Private had the lowest distribution rate 10(8.93%). However, the difference in the distribution of S. epidermidis between the wards is not statistically significance at p-value > 0.05 (refer to appendix II and III). Moreover, among the surfaces bedrail had the highest distribution rate 44(39.28%) while wall had the lowest distribution rate 11(9.82%). However, statistically there was no significant difference in the distribution of *S. epidermidis* between the surfaces at p-value > 0.05 except wall vs bedrail which are statistically significant at p-value = 0.0001 (refer to appendix II and III).

Table 2: The distribution of *Staphylococcus epidermidis* in ward surfaces of Kampala International University-Teaching Hospital

Sample source (wards)	Sample size	Surfaces	Sample collected	No. of *S. epidermidis* isolated (%)
Medical ward	52	Wall	12	1 (0.89)
		Bedrail	14	8 (7.14)
		Floor	12	5 (4.46)
		Doorknob	14	3 (2.67)
Subtotal	-	-	-	17 (15.18)
Surgical ward	52	Wall	12	2 (1.78)
		Bedrail	14	9 (8.03)
		Floor	12	6 (5.36)
		Doorknob	14	8 (7.14)
Subtotal	-	-	-	25 (22.32)
Maternity ward	52	Wall	12	1 (0.89)
		Bedrail	14	5 (4.46)
		Floor	12	5 (4.46)
		Doorknob	14	5 (4.46)
Subtotal	-	-	-	16 (14.28)
Accident and Emergency ward	52	Wall	12	3 (2.67)
		Bedrail	14	5 (4.46)
		Floor	12	5 (4.46)
		Doorknob	14	2 (1.78)
Subtotal	-	-	-	15 (13.39)
Pediatrics ward	52	Wall	12	2 (1.78)
		Bedrail	14	7 (6.36)
		Floor	12	3 (2.67)
		Doorknob	14	3 (2.67)
Subtotal	-	-	-	15 (13.39)
Private ward	51	Wall	11	0 (0.00)
		Bedrail	14	4 (3.57)
		Floor	12	1 (0.89)
		Doorknob	14	5 (4.46)
Subtotal		-	-	10 (8.93)
Semi-private ward	52	Wall	12	2 (1.78)
		Bedrail	14	6 (5.36)
		Floor	12	2 (1.78)
		Doorknob	14	4 (3.57)
Sub total	-	-	-	14 (12.5)
Total	363	-	363	112 (30.85)

4.2 The antibiotic susceptibility pattern of *Staphylococcus epidermidis* isolates

The antibiotic susceptibility pattern of isolates was determined following measurement of zone inhibition each antibiotic disc as indicated in the figure 3 below.

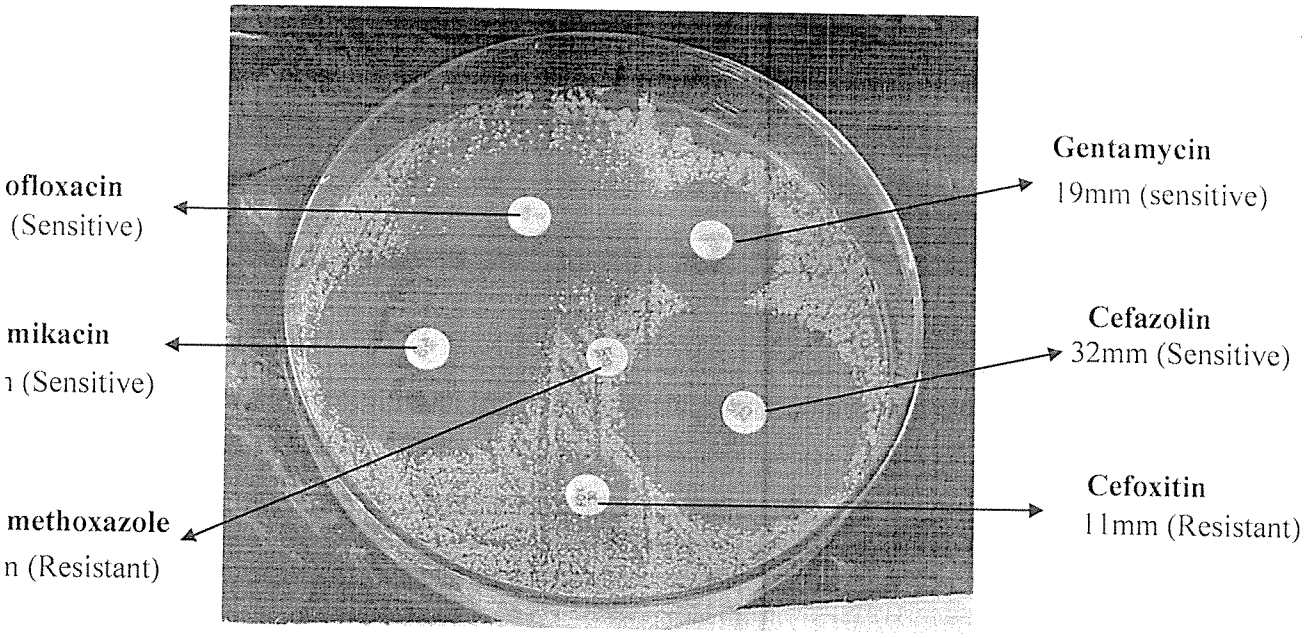


Figure 3: Zones of inhibition of some common antibiotics against *Staphylococcus epidermidis*

The percentages of resistance (R), intermediate (I) and susceptibility (Su) of *S. epidermidis* against the different antibiotics was determined as shown in Table 3 below.

Out of 112 (30.85%) of the isolates, 105(93.8%) were sensitive to Cefazolin while 90(80.4%) were more resistant to Trimethoprim-sulfamethoxazole and Cefoxitin had the highest percentage of intermediate 32(28.6%) as indicated in the table 3 below.

Table 3: The antibiotic susceptibility pattern of *Staphylococcus epidermidis* isolates from the selected wards

Isolates per ward	Antibiotics (%)																	
	Amikacin			Cefazolin			Trimethoprim-sulfamethoxazole			Ciprofloxacin			Gentamycin			Cefoxitin		
	R	I	Su	R	I	Su	R	I	Su	R	I	Su	R	I	Su	R	I	Su
SGW (N=25)	1 (4.0)	3 (12.0)	21 (84.0)	1 (4.0)	1 (4.0)	23 (92.0)	15 (60.0)	3 (12.0)	7 (28.0)	6 (24.0)	7 (28.0)	12 (48.0)	1 (4.0)	3 (12.0)	21 (84.0)	3 (12.0)	7 (28.0)	15 (60.0)
MDW (N=17)	3 (17.6)	1 (5.9)	13 (76.5)	1 (5.9)	2 (11.8)	14 (82.3)	11 (64.7)	4 (23.5)	2 (11.8)	1 (5.9)	6 (35.3)	10 (58.8)	2 (11.8)	4 (23.5)	11 (64.7)	2 (11.8)	4 (23.5)	11 (64.7)
AEW (N=15)	1 (6.7)	0 (0.0)	14 (93.3)	0 (0.0)	1 (6.7)	14 (93.3)	11 (73.3)	3 (20.0)	1 (6.7)	2 (13.3)	2 (13.3)	10 (66.7)	1 (6.7)	0 (0.0)	14 (93.3)	2 (13.3)	5 (33.3)	8 (53.3)
PDW (N= 15)	1 (6.7)	0 (0.0)	15 (93.3)	0 (0.0)	0 (0.0)	15 (100.0)	14 (99.3)	1 (6.7)	1 (6.7)	2 (13.3)	2 (6.7)	12 (80.0)	1 (6.7)	0 (0.0)	15 (93.3)	2 (13.3)	3 (20.0)	11 (73.3)
MTW (N=16)	0 (0.0)	1 (6.2)	15 (93.7)	0 (0.0)	0 (0.0)	16 (100.0)	15 (93.7)	0 (0.0)	1 (6.2)	3 (18.7)	3 (18.7)	10 (62.5)	0 (0.0)	1 (6.2)	15 (93.7)	2 (12.5)	5 (31.2)	9 (56.2)
PRW (N=10)	0 (0.0)	0 (0.0)	10 (100.0)	0 (0.0)	0 (0.0)	10 (100.0)	10 (80.0)	0 (20.0)	0 (0.0)	4 (40.0)	1 (10.0)	5 (50.0)	0 (0.0)	1 (10.0)	9 (90.0)	0 (0.0)	3 (30.0)	7 (70.0)
SPW (N=14)	0 (0.0)	1 (7.1)	13 (92.9)	1 (7.1)	0 (0.0)	13 (92.9)	14 (100.0)	0 (0.0)	0 (0.0)	3 (21.4)	1 (7.1)	10 (71.4)	0 (0.0)	1 (7.1)	13 (92.9)	0 (0.0)	5 (35.7)	9 (64.3)
Total	5(4.5)	6(5.4)	86(76.8)	3(2.7)	4(3.6)	105(93.8)	90(80.4)	11(9.8)	12(10.7)	21(18.8)	22(19.6)	69(61.6)	5(4.5)	10(8.9)	98(87.5)	11(9.8)	32(28.6)	70(62.5)

Key: R= Resistance, I= Intermediate, Su= Susceptible, SGW= Surgical ward, MDW= Medical ward, AEW= Accident and Emergency ward, PDW= Pediatrics ward, MTW= Maternity ward, PRW= Private ward, SPW= Semi-private ward.

4.3 Antibacterial activity of *Carica papaya* leaf and seed crude extracts against methicillin resistant *Staphylococcus epidermidis* isolated from wards surfaces of Kampala International University-Teaching Hospital

The results presented here includes percentage yield, phytochemical analysis, antibacterial activity and minimum inhibitory and minimum bactericidal concentrations of *C. papaya* leaves and seeds crude extract against antibiotic resistant *S. epidermidis* isolated from wards surfaces of KIU-TH.

4.3.1 Percentage yield of *Carica papaya* leaf and seed crude extracts

Table 4 below shows the percentage yield of the crude extract of *Carica papaya* leaf and seed using methanol, acetone and water as solvents. In the leaf and seed of *C. papaya*, methanolic crude extract gave the highest yield of 9% and 6.4% respectively while the aqueous gave the least yield of 5% and 4.2% respectively. However, this process was repeated 3 times in order to get enough crude extracts for the study following the same procedure.

Table 4: Percentage yield of leaf and seed crude extract of *Carica papaya* in different solvents

Crude extract	Leaf (%)	Seed (%)
Methanol	9	6.4
Acetone	6.4	5.2
Aqueous	5	4.2

4.3.2 Phytochemical analysis of *Carica papaya* leaf and seed extracts

The phytochemical analysis of the plant extract carried out in this study revealed presence of tannins, terpenoids, cardiac glycosides, alkaloids, phenols and triterpenoids in both leaf and seed extract. However, seed additionally had flavonoids, saponins and steroids as shown in Table 5 below.

Table 5: Phytochemical analysis of *Carica papaya* leaf and seed crude extracts

Phytochemicals	Test performed	Leaf crude extracts			Seed crude extracts		
		ME	AE	AqE	ME	AE	AqE
Flavonoids	Lead acetate test	-	-	-	+	-	-
Tannins	Ferric chloride test	+	+	+	+	+	-
Terpenoids	Sulphuric acid test	+	+	-	+	+	-
Cardiac Glycosides	Borntragor's test	+	-	-	+	-	-
Saponins	Water test	-	-	-	-	-	+
Steroids	Chloroform test	-	-	-	+	+	-
Alkaloids	Wagner's test	+	+	+	+	+	-
Phenols	Ferric Chloride test	+	+	+	+	+	+
Triterpenes	Salkovaski's test	+	+	-	+	+	-

Key: ME= Methanol extract, **AE**= Acetone extract, **AqE**= Aqueous extract, + represents positive, - represents negative

4.3.3 Antibacterial activity of methanol, acetone and aqueous crude extracts of *Carica papaya* leaf and seed against antibiotic resistant *S. epidermidis* isolated from wards surfaces of Kampala International University -Teaching Hospital

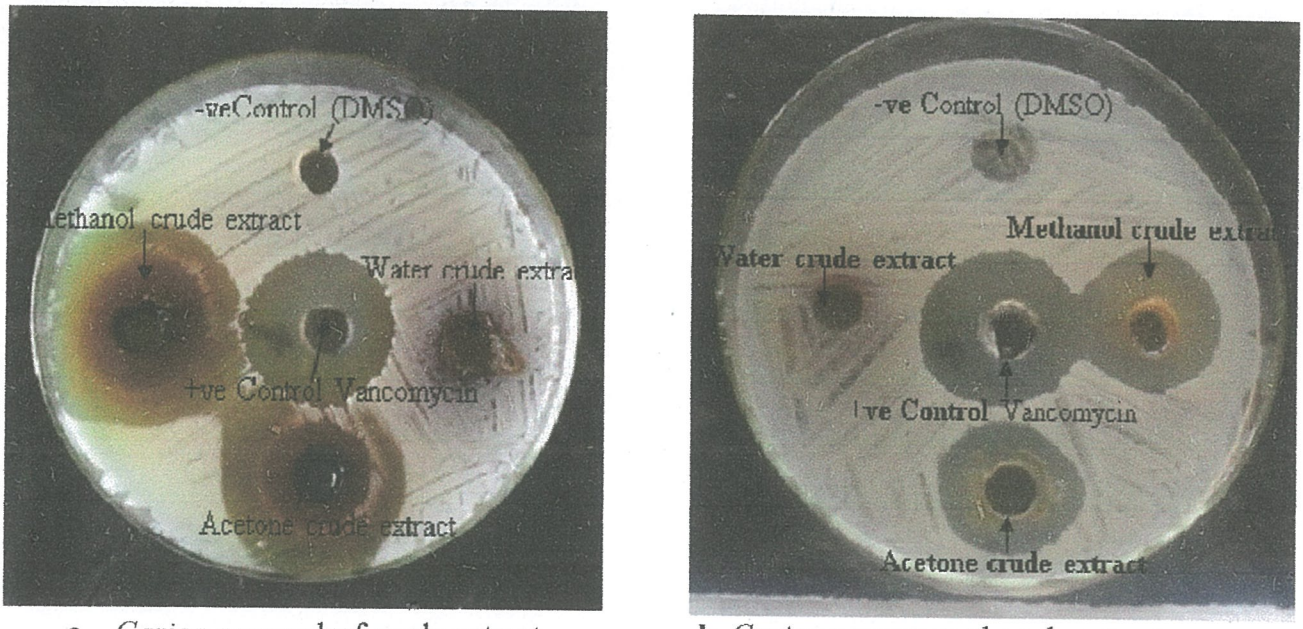
Table 6 shows the antibacterial activity of methanol, acetone and aqueous crude extracts of *C. papaya* leaf and seed against antibiotic resistant *S. epidermidis* isolated from wards surfaces of KIU-TH. The seed crude extracts showed more activity than the leaf. Both seed and leaf aqueous crude extract had no activity while methanolic crude extract had the highest activity with inhibition zones diameter of 23mm and 16.5mm respectively. Vancomycin (positive control) had inhibition zone diameter ranging from 23 to 13mm. The difference between the activity of the extracts from both leaf and seed is statistically significant at p-value < 0.05. However, there was no statistical difference between the activity of acetone and methanol leaf extracts at p-value = 0.6650. Furthermore, there was no significant difference between the activity of acetone seed extract and acetone leaf extract at p-value =0.0559 but the difference between the activity of

methanol seed extract and methanol leaf is statistically significant at p-value= 0.0040 (refer to appendix II and III).

Table 6: Antibacterial activity of leaf and seeds crude extracts of *Carica papaya* on Methicillin resistant isolates of *Staphylococcus epidermidis*

illin ant of *S. nidis*	Ward Surfaces	Mean inhibition zone of leaf crude extracts diameter (mm)			Mean inhibition zone of seed crude extracts diameter (mm)			Mean inhibition zone of positive control diameter (mm)
		AE	ME	AqE	AE	ME	AqE	Vancomycin
	Wall	12	11.5	-	15	16	-	16
	Doorknob	11	13	-	12	14	-	14
	Bedrail	13	14	-	16	18.5	-	23
	Bedrail	10.5	11	-	13	15	-	17
	Wall	13	14	-	14	21	-	14
	Floor	16	12.5	-	18	19	-	16
	Floor	14	16.5	-	21	23	-	14
	Bedrail	15	17	-	18	20.5	-	13
	Bedrail	10.5	11	-	11.5	13	-	15
	Doorknob	11	13	-	13.5	16	-	18
	Bedrail	0	11	-	10	12.5	-	20

Key: ME=Methanolic extract, **AE**= Acetone extract, **AqE**= Aqueous extract, - represents absence.



a. *Carica papaya* leaf crude extract **b.** *Carica papaya* seed crude extract

Figure 4: Antibacterial activity of *Carica papaya* leaf and seed crude extracts against Methicillin resistant *S. epidermidis*

4.3.4 Minimum inhibitory and minimum bactericidal concentrations (MIC and MBC) of leaf crude extracts of *Carica papaya* on Methicillin resistant *Staphylococcus epidermidis*

Table 7 below shows the minimum inhibitions concentration of methanol, acetone and aqueous crude extracts of *Carica papaya* leaf. The minimum inhibitory as well as the minimum bactericidal concentrations ranges from 250 to 31.3 mg/ml respectively. Methanolic extract has the lowest MIC values (31.3 mg/ml) against PD23B, MT5W, SG11B and highest MIC values (125mg/ml) against MD11B, AE48F, SG17B and SG3D. Acetone extract gave highest MIC value (125mg/ml) against AE48F and lowest MIC values (31.3mg/ml) against MD11B, PD19B, MD53F and AE28B.

The highest MBC value (250 mg/ml) of methanolic extract was shown against SG17B while the lowest (62.5mg/ml) was against MT13W, PD23B, PD19B, MT5W, MD53F and SG11B. Acetone extract gave highest MBC values (125mg/ml) against MT13W, AE48F, SG17B and SG11B, and the lowest values (31.3mg/ml) against MD11B, PD19B and MD53F.

Table 7: Minimum inhibitory and minimum bactericidal concentration (MIC and MBC) of leaf extracts of *Carica papaya* on Methicillin resistant *Staphylococcus epidermidis* isolated from wards surfaces of Kampala International University-Teaching Hospital

Methicillin resistant *S. epidermidis isolates*	Wards Surfaces	MIC values (mg/ml)		MBC values (mg/ml)	
		Methanolic extract	Acetone extract	Methanolic extract	Acetone extract
MT13W	Wall	62.5	62.5	62.5	125
PD23B	Bedrail	31.3	62.5	62.5	62.5
MD11B	Bedrail	125	31.3	125	31.3
PD19B	Bedrail	62.5	31.3	62.5	31.3
MT5W	Wall	31.3	62.5	62.5	62.5
AE48F	Floor	125	125	125	125
MD53F	Floor	62.5	31.3	62.5	31.3
SG17B	Bedrail	125	62.5	250	125
AE28B	Bedrail	62.5	31.3	125	62.5
SG3D	Door	125	62.5	125	62.5
SG11B	Bedrail	31.3	62.5	62.5	125

Key: MIC= Minimum Inhibitory Concentration, **MBC**= Minimum Bactericidal Concentration.

4.3.5 Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC and MBC) of seed extracts of *Carica papaya* on Methicillin resistant *Staphylococcus epidermidis*

The results on the minimum inhibition concentration of methanol, acetone and aqueous extracts of *Carica papaya* seed are shown in Table 8. The minimum inhibitory and the minimum bactericidal concentrations ranged from 125 to 31.3 mg/ml respectively. Methanolic extract had the lowest MIC values (31.3mg/ml) against PD23B, MD11B, MT5W, MD53F and SG3D and highest MIC values (125mg/ml) against PD19B. Acetone extract gave highest MIC value (125mg/ml) against MT13W, AE48F and SG11B and lowest MIC values (31.3mg/ml) against AE28B.

The highest MBC values (125mg/ml) of methanolic extract were shown against MT13W, PD19B, SG17B and AE28B while the lowest (31.3mg/ml) against MT5W. Acetone extract gave highest MBC values (125mg/ml) against MT13W, MD11B, AE48F, MD53F, SG3D and SG11B and the lowest values (62.5mg/ml) against PD23B, PD19B, MT5W, SG17B and AE28B.

Table 8: Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC and MBC) of seed extracts of *Carica papaya* on Methicillin resistant *Staphylococcus epidermidis*

Strains of *S. epidermidis*	Wards Surfaces	MIC values (mg/ml)		MBC values (mg/ml)	
		Methanolic extract	Acetone extract	Methanolic extract	Acetone extract
MT13W	Wall	62.5	125	125	125
PD23B	Bedrail	31.3	62.5	62.5	62.5
MD11B	Bedrail	31.3	62.5	62.5	125
PD19B	Bedrail	125	62.5	125	62.5
MT5W	Wall	31.3	62.5	31.3	62.5
AE48F	Floor	62.5	125	62.5	125
MD53F	Floor	31.3	62.5	62.5	125
SG17B	Bedrail	62.5	62.5	125	62.5
AE28B	Bedrail	62.5	31.3	125	62.5
SG3D	Doorknob	31.3	62.5	62.5	125
SG11B	Bedrail	62.5	125	62.5	125

Key: MIC= Minimum Inhibitory Concentration, **MBC**= Minimum Bactericidal Concentration.

4.4 Detection of mecA gene from Methicillin resistant *Staphylococcus epidermidis* isolated from wards surfaces of Kampala International University-Teaching Hospital

The mecA gene responsible for Methicillin resistance was detected from the isolates resistant to Cefoxitin. The bands (1-9) in the figure below were representative amplicons of the isolates at the size of 500bp molecular ladder, while NC and PC were negative and positive controls respectively.

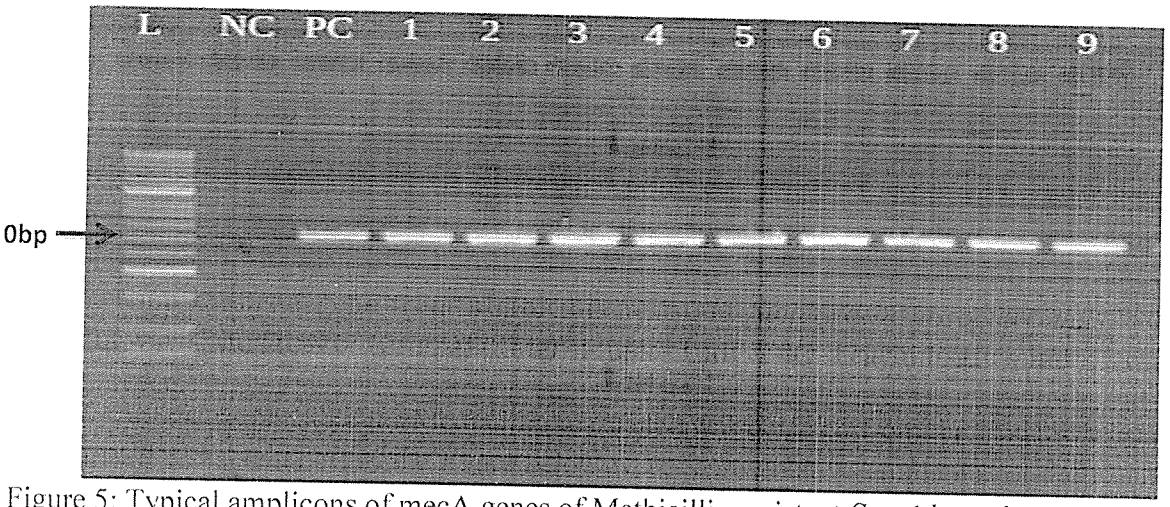


Figure 5: Typical amplicons of mecA genes of Methicillin resistant *S. epidermidis*

All the 11 isolates of *S. epidermidis* resistant to Cefoxitin which were believed to be Methicillin resistant strains were found to be carrying mecA gene as shown in Table 9 below.

Table 9: Methicillin resistant *Staphylococcus epidermidis* isolated from wards surfaces of Kampala International University-Teaching Hospital

Serial number	Isolates	Wards Surfaces	mecA gene
1	SG11B	Bedrail	+
2	SG03D	Doorknob	+
3	SG17D	Doorknob	+
4	MD11B	Bedrail	+
5	MD53F	Floor	+
6	AE28B	Bedrail	+
7	AE48F	Floor	+
8	PD23B	Bedrail	+
9	PD19B	Bedrail	+
10	MT05W	Wall	+
11	MT13B	Bedrail	+

Key: **SXT**= Sulfamethoxazole-Trimethoprim, **CIP**= Ciprofloxacin, **CZ**= Cefazolin, **AK**= Amikacin, **GEN**= Gentamicin, **SGB**= Surgical (Bedrail), **SGD**= Surgical (Doorknob), **MDB**= Medical (Bedrail), **MDF**= Medical Floor), **AEB**= Accident and Emergency (Bedrail), **AEF**= Accident and Emergency (Floor), **PDB**= Pediatrics Bedrails), **MTW**= Maternity (Wall), **MTB**= Maternity (Bedrail), + represents positive.

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.0 Introduction

The aim of the study was to determine the antibacterial activity of *Carica papaya* and common antibiotics against *Staphylococcus epidermidis* isolated from wards surfaces of Kampala International University Teaching Hospital with specific objectives of determining the: distribution and risk factors associated with *Staphylococcus epidermidis* in wards surfaces of KIU-TH, Antibiotic susceptibility pattern of *S. epidermidis* isolated from wards surfaces of KIU-TH, Antibacterial activity of *C. papaya* leaf and seed crude extracts against antibiotic resistant *S. epidermidis* isolated from KIU-TH wards surfaces and presence of mecA gene in Methicillin resistant *S. epidermidis* isolated from KIU-TH. The chapter includes discussion of the results, conclusions and recommendations in accordance to the objectives stated above.

5.1 Discussion

Staphylococcus epidermidis is one of the major causes of nosocomial infections that follow catheterization and other surgical procedures through contaminated surfaces and/or medical equipment resulting in infections of wounds or surgical sites, urinary or respiratory tracts and the brain (Brannigam *et al.*, 2012). The distribution of *S. epidermidis* in wards surfaces of KIU-TH from this study was 112 (30.85%). *S. epidermidis* was more sensitive to Cefazolin (93.8%) while more resistant to Trimethoprim-sulfamethoxazole (80.4%) among the antibiotics tested. The phytochemical analysis of the plant extract carried out in this study revealed presence of tannins, terpenoids, cardiac glycosides, alkaloids, phenols and triterpenoids in both leaf and seed extract. However, seed additionally had flavonoids, saponins and steroids. The leaf and seed crude extracts (methanol and acetone) of *Carica papaya* had activity against Methicillin resistant *S. epidermidis* while water crude extract had no activity at all. The minimum inhibitory as well as the minimum bactericidal concentrations of leaf crude extracts ranges from 250 to 31.3 mg/ml while of seed ranged from 125 to 31.3 mg/ml respectively. All the 11 isolates of *S. epidermidis* resistant to Cefoxitin which were believed to be Methicillin resistant strains were found to be carrying mecA gene

The prevalence of *S. epidermidis* in wards surfaces of KIU-TH from this study is lower compared to the 43.7% which was reported in the study by Aloma *et al.* (2016) in tertiary health

care hospital, Brazil and the 39.2% reported by Amenu *et al.* (2014) in the hospital environment of Ethiopia. However, this study showed a higher prevalence of *S. epidermidis* than a study by Ochie *et al.* (2009) in Nigeria and Wojtyczka *et al.* (2011) in Ghana which showed prevalence of 12.7% and 17.2% respectively. The prevalence reported by this study was in line with a study by Robert *et al.* (2014) in Poland, where the prevalence of *S. epidermidis* in hospital environment was reported to be 26.2%. The prevalence of *S. epidermis* attained from this study could probably be attributed to inadequate cleaning of the floor (two times in a day) or not mopping the surfaces (doorknobs, walls and bedrails) or use of detergents (Omo, Jik, Teem and Aerial) as disinfectant which are so weak to kill the bacteria (Garza *et al.*, 2010).

The higher distribution rate of *S. epidermidis* on bedrail (39.3%) in this study was in agreement with the 100% prevalence on bedrail as reported by Boyce (2007) from Nigeria. Similarly, the distribution rate of *S. epidermidis* on doorknob/handle in this study (26.8%) is lower than 53.8% and 38% of Staphylococci on door knob/handle reported by Hammuel *et al.* (2014) and Carvalho *et al.* (2007) respectively. The distribution rate noted in this study of *S. epidermidis* on door knobs/door handles may probably be because they are the most frequently touched surfaces among others or the fact that the knobs and handles were not being mopped with disinfectant after cleaning in all the seven selected wards as interviewed. Bhalla *et al.* (2004) and Boyce (2007) reported that environmental contamination in health care settings arise when healthcare workers touch the surfaces with their hands or gloves particularly after attending to the patients or when the patients come in direct contact with the surfaces. The distribution rate of *S. epidermidis* on the floor (24.12%) is higher than 8.6 and 16.7% reported by Hammuel *et al.* (2014) in two different hospitals' environment in Zaria. However, 30.8% was reported by Boyce *et al.* (1997) and 50.0% by Carvhalo *et al.* (2007) in Brazil which are higher compared to this study. Perhaps the variations of the contamination between these hospitals could be due to the difference in hygiene practices within the hospitals. In this study, walls had the least distribution rate of *S. epidermidis* (9.82%) among other surfaces, this may be due to the fact that patients and health workers rarely get in contact with the walls as compared to other surfaces.

The highest percentage of resistance by *S. epidermidis* against Trimethoprim-sulfamethoxazole (72.32%) was in agreement with the findings of Hulya *et al.* (2006), Hellmark *et al.* (2009), Xiao *et al.* (2011) and Muhammad *et al.* (2015) who reported 62.5%, 82%, 58.5% and 87% of *S.*

epidermidis resistant to Trimethoprim-sulfamethoxazole respectively. However, it is higher than 33.33% of *S. epidermidis* resistance to Trimethoprim-sulfamethoxazole reported by Mariem *et al.* (2015). Resistance to Ciprofloxacin (18.75%) in this study is lower than the one reported by Hellmark *et al.* (2008) and Mariem *et al.* (2015) with 79% and 66.67% respectively. Moreover, a set of contrary results higher than the findings of this study were reported from Iran and Argentina which ranges between 56% to 77% and 80% respectively (Hadidi *et al.*, 2008; Rodinguez *et al.*, 2003). However, it is closely related to results reported from Turkey and Brazil ranging between 20 to 59.2% and 25.5% respectively (Bayram and Balci, 2006; Mendez *et al.*, 2005). The highest percentage of resistance by *S. epidermidis* to Trimethoprim-sulfamethoxazole in this study could be as a result of frequent use of the antibiotic in the hospital due to it being cheaper and first line drug.

The percentage of *S. epidermidis* resistant to Cefoxitin (9.82%) in this study was very low compared to the previous studies carried out by Begum *et al.* (2011), Hellmark *et al.* (2009) and Akinjogunla *et al.* (2014) who reported 50.4%, 58% and 33.3% of Cefoxitin resistance by *S. epidermidis* in their studies respectively. Resistance to cefoxitin by disc diffusion can be used for the detection of Methicillin resistant *S. epidermidis* (MRSE) strains in routine testing because cefoxitin is a potential inducer of the system that regulates mecA gene (Madhusudhan *et al.*, 2011). Therefore, low percentage of cefoxitin resistance exhibited by *S. epidermidis* in this study could be as a result of insignificant number of MRSE in the hospital through which the resistance is transferred from one strain to the other.

The low percentage of S. epidermidis resistant to Gentamycin (4.46%) in this study, is similar to the study of Hammuel *et al.*, (2014) that reported 0.00% of the pathogens resistant to Gentamycin but lower than that of Akindele *et al.* (2010) with 39% resistance. Moreover, the percentage of *S. epidermidis* resistant to Amikacin (5.35%) according to this study is in support of the findings of Mariem *et al.* (2015) where 2.78% of *S. epidermidis* was resistant to Amikacin. Resistance to Cefazolin (2.67%) in this study support the report of Muhammad *et al.* (2015) where 7.2% of *S. epidermidis* was resistant to Cefazolin. However, Ibrahim *et al.* (2015) reported that 82.8% of *S. epidermidis* was resistant to Cefazolin in his study which is higher than the findings of this investigation. However, Cefazolin is the most effective antibiotic against *S. epidermidis* isolated from KIU-TH and this could be as a result of it been a rare drug, as such it

may be difficult for this bacteria to understand the drug's mechanism of action and develop resistant to it.

The susceptibility percentage of *S. epidermidis* to Cefazolin, Gentamycin, Amikacin and Ciprofloxacin (93.8%, 87.5%, 76.8%, 61.6%) in this study, is slightly higher than that of Ibrahim *et al.* (2015) who reported 50.8%, 44.3% and 17.2% for Gentamycin, Ciprofloxacin and Cefazolin respectively. Moreover, in another studies of Bilal and Srikanth (2013) and Muhammad *et al.* (2015), Amikacin, Cefazolin and Gentamycin were effective against *S. epidermidis* with 93.1%, 91.8%, and 96.2% respectively which are higher than the results of the present study. The high susceptibility of Cefazolin, Gentamycin, Amikacin and Ciprofloxacin in this study could be as a result of them being second line antibiotics and a bit more expensive, which decreases their usage by the patients and as such they are not more exposed to the bacteria through which resistance may occur. These variations in antibiotic susceptibility pattern indicates that regional differences perhaps played a role in the resistance profiles of bacteria and further justifies the necessity to embark on antibiotic susceptibility studies on bacterial isolates from different hospitals on a regular basis (Hammuel *et al.*, 2014).

The more effectiveness of the methanolic and acetone crude extracts against the test organisms as compared to the aqueous extracts may probably be due to the better solubility of the active components in organic solvent and differences in the phytochemical compounds present in the extracts (Nirosha and Mangalanayaki 2013). The more effectiveness (higher inhibition zones) of methanolic extracts than acetone and aqueous extracts for both the leaves and seeds was in agreement with Subramanian *et al.* (2014) in his study on antimicrobial properties *C. papaya* different leaf extracts against *E. coli and S. aureus*. However, it is contrary to the findings of Aruljothi *et al.* (2014) who reported that acetone extract of *C. papaya* leaf and seed had more activity than methanol and aqueous extracts. Lack of activity by aqueous extracts of the leaf and seed against all the resistant isolates of *S. epidermidis*, correlate with the report of Nirosha and Mangalanayaki (2013) that showed that aqueous extract of *C. papaya* leaves and roots were ineffective against all the organisms tested in their study. However, another study from Okunola and Alabi (2012) reported that aqueous extract of *C. papaya* leaves exhibited higher activity against *S. aureus* compare to acetone and methanol extracts. However, the difference in the finding of this study from that of Okunola and Alabi (2012) could probably be due to the

differences in the method and the plant sample used. In this study differs from that of the study mentioned above where they used disc diffusion instead of agar well diffusion method, and a fresh leaf extract instead of dried leaf extract.

The antibacterial activity of methanolic seed extracts of *C. papaya* against Methicillin resistant *S. epidermidis* is in support of a study carried out by Ayanfemi and Bukola (2015) and that of Egbuonu *et al.* (2016) that showed seed extracts to be more effective than the leaf against all the organisms tested in their study. The variation of antibacterial activities of the different extracts depends on the polarity of the solvents used, concentrations of the compounds being extracted from each solvent and in addition to their extrinsic bioactivity and by their ability to dissolve or diffuse in the media used in the assay (Anjana *et al.*,2009). Methanolic extracts were more effective in this study, this could be because it contains more phytochemicals (Flavonoids, Tannins, Terpenoids, Cardiac glycosides, Steroids, Alkaloids, Phenols and Triterpenes). However water extracts exhibited on activity against Methicillin resistant *S. epidermidis*, this could be attributed to the low quantity of phytochemicals (Phenols, Saponins, Tanins and Alkaloids) in the extracts. Plant extracts have the ability to either inhibit or completely kill the bacterial cell under study; this can be examined through the determination of minimum inhibitory and minimum bactericidal concentration of the active extracts. It was reported that the phytochemicals responsible for antibacterial activity in *C. papaya* are the Papain, Alkaloids, Flavonoids, Tannins and Steroids as reported by Natarjan *et al.* (2014)

The results of MIC and MBC from this study (250 to 31.3 mg/ml and 125 to 31.3 mg/ml) correlate with the discoveries of Ayandele and Oluwaseun (2015) which reported the MIC's and MBC's values of *Carica papaya* leaf and seed extracts against many bacterial isolates including *Staphylococcus aureus* ranging between 200 to 150mg/ml and 200 to 175mg/ml respectively. However, the MIC's and MBC's values are higher compare to other studies reported by Mwesigwa *et al.* (2012) with MIC values ranging between 100 to 3.12 mg/ml against E. coli and Okunola *et al.* (2012) reported the MIC of *Carica papaya* leaf extract against *E. coli, Salmonella, S. aureus* and *Streptococcus pyogens* ranged between 100 to 75mg/ml. The high MIC's and MBC's values observed with extracts against test organisms might be an indication of low effectiveness or that the organisms have the potential for developing resistance to the bioactive compounds (Jigna *et al.*, 2006). Therefore, the high MIC's and MBC's values

observed in this study with both the *Carica papaya* leaf and seed extracts may be due to the fact that the organism used in this study were resistant isolates.The bioactivity of plant extracts is dependent upon its phytochemical constituents.

Presence of mecA gene in all the 11 isolates resistant to Cefoxitin in this study was in line with the study by Baguma *et al.* (2017) where mecA gene was found in all the 300 isolates resistant to Cefoxitin (100%). However, the percentage obtained from the study was higher compared to studies by Natalia *et al.* (2011) and Andrea *et al.* (2010), also reported very high percentages of 95.12 and 93.75 respectively of mecA positive *Staphylococcus epidermidis* resistant to Cefoxitin. However, the result is in contrast to some studies reported 80% and 74.02% of *S. epidermidis* harbouring mecA gene among the Cefoxitin resistant isolates (Amita *et al.*, 2008; Samah *et al.*, 2009). The presence of mecA gene in all the 11 isolates in this study indicated that, the isolates were resistant to methicillin which represents all the β-lactam group of antibiotics (Peacock and Paterson, 2015).

5.2 Conclusions

This study showed presence of *S. epidermidis* in the different ward surfaces in KIU-TH with door knobs and bedrails being more contaminated. Trimethoprim-sulfamethoxazole antibiotic was less effective against *S. epidermidis* isolated from KIU-TH wards surfaces while Cefazolin was most effective. *Carica papaya* leaves and seeds (methanol and acetone crude extracts) had antibacterial activity against the antibiotic resistant *S. epidermidis* isolated from KIU-TH wards surfaces. All the eleven (11) Cefoxitin resistant *S. epidermidis* isolates had mecA gene.

5.3 Recommendations

5.3.1. All the wards surfaces investigated in KIU-TH should be mopped and decontaminated using a strong disinfectant that contains phenol.

5.3.2. Cefazolin, Gentamycin and Amikacin could be better prescriptions in the management of infections caused by *S. epidermidis*.

5.3.3. *Carica papaya* leaf and seed crude extract could be used as a source of novel antibiotics to be used in the management of infections caused by *S. epidermidis*.

5.3.4. Finally, a further study should be carried out to detect mecA gene from both Methicillin resistant and sensitive *S. epidermidis* to find out whether there are other factors responsible for Methicillin resistance in Staphylococci.

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Appendix I: Phytochemical screening

Test for flavonoids: 1.0ml of 10% lead acetate will be added to 1.0ml of the extract contained in a test-tube. A formation of a yellow precipitate will be considered as positive for flavonoids.

Test for tannins: 5.0g of dried extract will be stirred with 10.0ml of distilled water. The mixture will be filtered and ferric chloride reagent will be added to the filtrate. A blue-black precipitate will be taken as positive for the presence of tannins.

Test for terpenoids: 0.5ml 0f the dried extracts will be evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 10minutes on a water bath. Formation of grey colour will indicate the presence of terpenoids.

Test for cardiac glycosides: 0.5g of dried extract will be dissolved in 2.0ml of glacial acetic acid containing one drop of ferric chloride solution. The solution will then under lay with 1.0ml of concentrated H2SO4. A brown ring formed at the interface shows the presence of a cardenolides.

Test for saponins: This will be screened by shaking 0.5g of dried extract with water in a test tube, frothing which persist on warming will be used as evidence for the presence of saponins.

Test for steroids: 0.5g of the dried extract will be extracted with 2.5ml of chloroform in a test tube and 1ml of concentrated sulphuric acid added to form a lower layer. A reddish-brown interface will be taken as the presence of steroids.

Appendix II: Statistical analysis

Statistical difference in the distribution of *S. epidermidis* between the wards

Turkey's multiple comparisons test	Summary	Adjusted P-Value
Surgical vs. Medical	Ns	0.8871
Surgical vs. Maternity	Ns	0.8218
Surgical vs. Pediatrics	Ns	0.7430
Surgical vs. Accident and Emergency	Ns	0.7430
Surgical vs. Semi-private	Ns	0.6552
Surgical vs. Private	Ns	0.3138
Medical vs. Maternity	Ns	> 0.9999
Medical vs. Pediatrics	Ns	> 0.9999
Medical vs. Accident and Emergency	Ns	> 0.9999
Medical vs. Semi-private	Ns	0.9992
Medical vs. Private	Ns	0.9361
Maternity vs. Pediatrics	Ns	> 0.9999
Maternity vs. Accident and Emergency	Ns	> 0.9999
Maternity vs. Semi-private	Ns	> 0.9999
Maternity vs. Private	Ns	0.9687
Pediatrics vs. Accident and Emergency	Ns	> 0.9999
Pediatrics vs. Semi-private	Ns	> 0.9999
Pediatrics vs. Private	Ns	0.9874
Accident and Emergency vs. Semi-private	Ns	> 0.9999
Accident and Emergency vs. Private	Ns	0.9874
Semi-private vs. Private	Ns	0.9961

Statistical analysis of the distribution of *S. epidermidis* between the surfaces

Turkey's multiple comparisons test	Summary	Adjusted P-Value
Wall vs. Bedrail	***	0.0001
Wall vs. Doorknob	*	0.0305
Wall vs. Floor	ns	0.0830
Bedrail vs. Doorknob	ns	0.1520
Bedrail vs. Floor	ns	0.0601
Doorknob vs. Floor	ns	0.9646

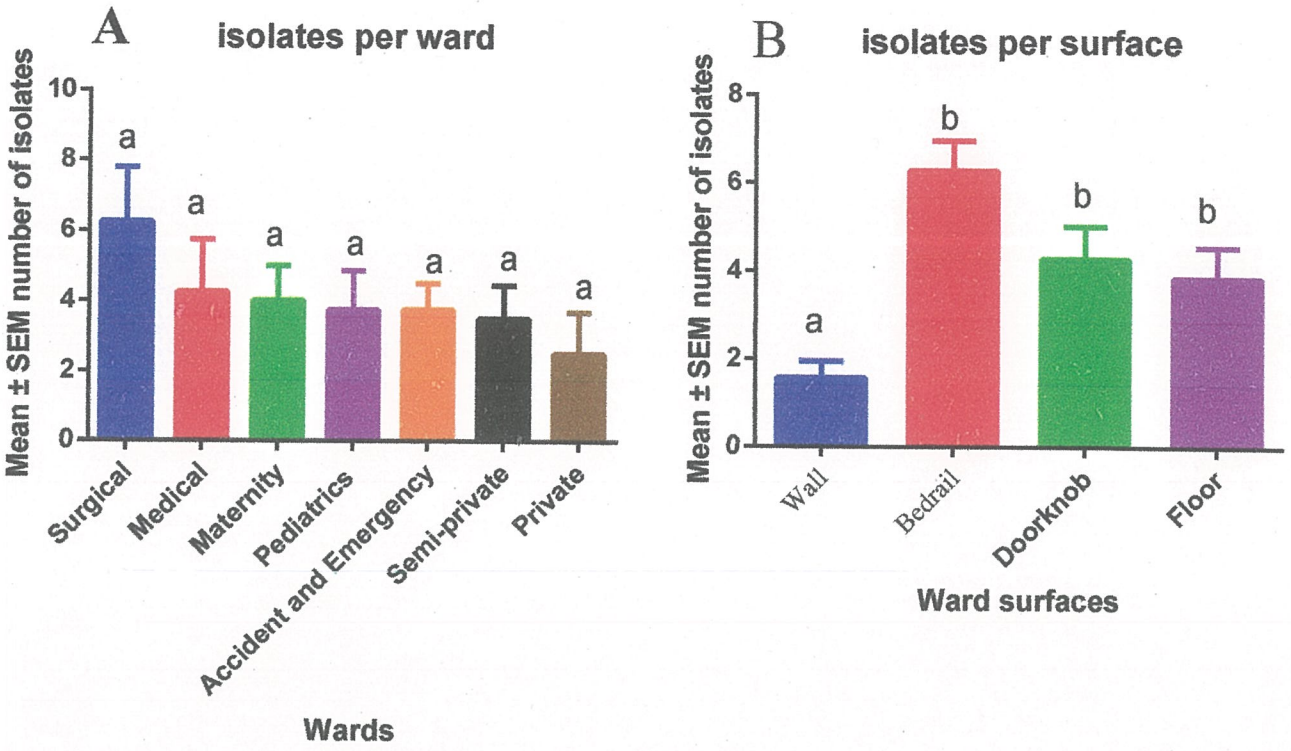
*= degree of significance

Statistical analysis between the activities of *Carica papaya* leaf and seed extracts

	P-values			
'key's multiple comparisons test	Leaf	Seed	Acetone leaf and seed	Methanol leaf and seed
tone vs. Methanol	0.6650	0.3753	0.0559a	0.0040a
tone vs. Water	< 0.0001	< 0.0001		
hanol vs. Water	< 0.0001	< 0.0001		
tone vs. Positive control	< 0.0001	< 0.0001		
hanol vs. Positive control	< 0.0001	0.0002		
er vs. Positive control	< 0.0001	< 0.0001		

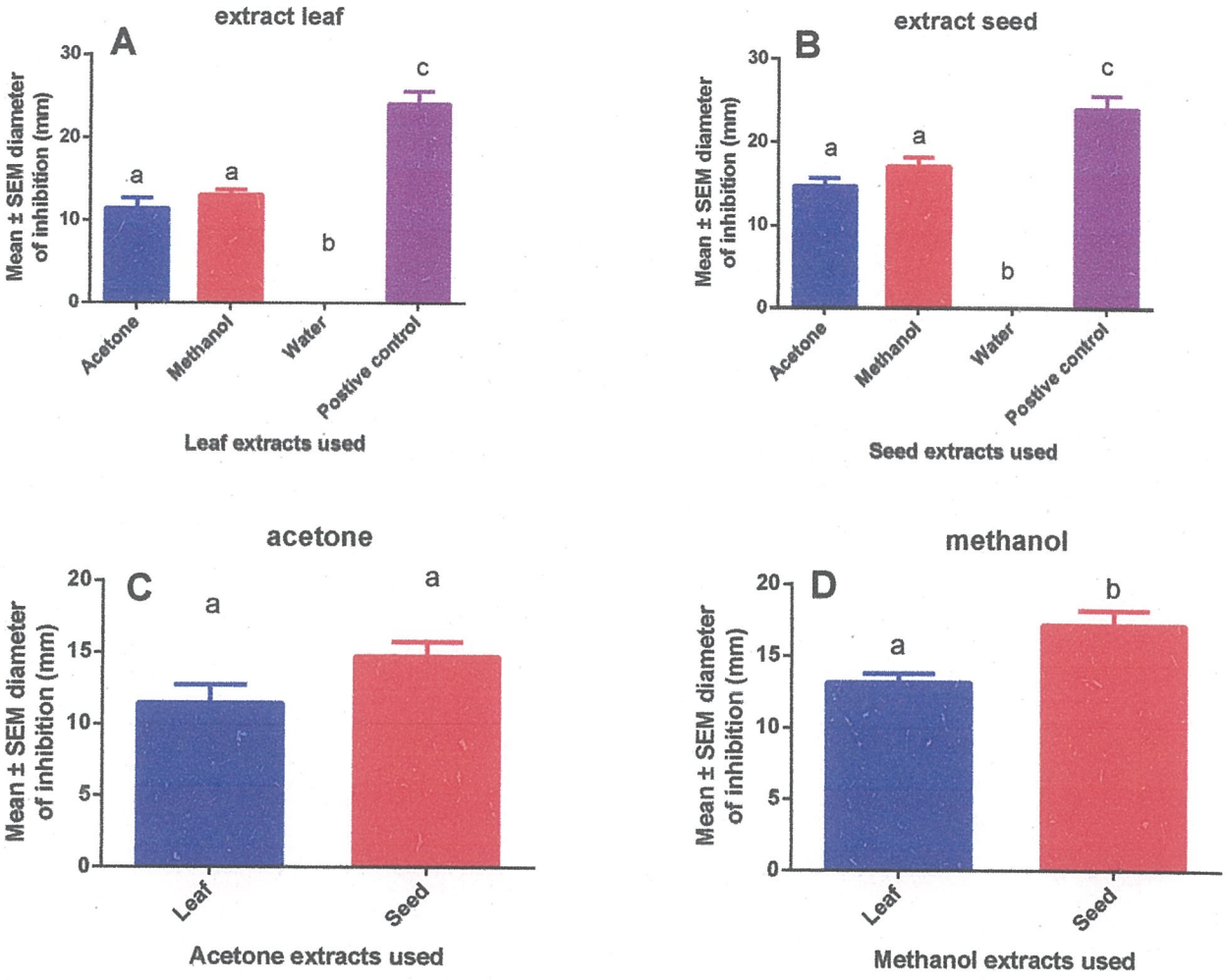
KEY: superscripts a = one sample t test.

Appendix III: Graphical presentation of results



Key: a superscript from graph (A) means no significance difference between the group while (a to b) from graph B means there is significance between the group.

Figure 6: Graphical presentation of the distribution of *S. epidermidis* between the wards and surfaces



Key: superscript (a) means no significant difference between the groups while (b) means there is significant difference between the groups

Figure 7: Diagrammatical presentation of antibacterial activity of *C. papaya* leaf and seed crude extracts

Appendix IV: Ethical clearance form

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RESEARCH ETHICS COMMITTEE (REC)

7th March 2018

Ref: 2017/06

Abubakar Sunusi Adam
Postgraduate Student
KIU WC

APPROVAL OF YOUR PROPOSAL

Submitted Proposal: "ANTIBACTERIAL ACTIVITY OF CARICA PAPAYA (LINN) AND COMMONLY USED ANTIBIOTICS AGAINST STAPHYLOCOCCUS EPIDERMIDIS ISOLATED FROM WARDS SURFACES OF KAMPALA INTERNATIONAL UNIVERSITY TEACHING HOSPITAL" Nr UG-REC- 023/2017/06

Reference is made to the above Protocol, which you submitted to the Research Ethics Committee (REC) for ethical review and approval. It has been noted that all the concerns raised earlier by the Committee, in its meeting of 17 January 2018, have been properly responded to.

This is, therefore, to inform you that your study has been approved, following an Expedited Review. You may now proceed with preparations to implement the research. Please note that this approval is for a period of one year.

As Principal Investigator, you are expected to fulfill the following conditions, which are part of the approval process regarding your study.

1. You are required to register the Protocol with the Uganda National Council for Science and Technology, according to the guidelines of the Council, for final clearance to undertake the research.
2. Any changes/amendments and/or additions to the Protocol, Consent Form and/or Data Collection Tools must be submitted to the REC for review and approval prior to activation of the changes.
3. Reports of unanticipated problems involving risks to participants should be submitted to REC.
4. Only the approved Consent Forms should be used in enrolling participants. For that purpose, therefore, you should retain all signed Consent Forms on file;

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RESEARCH ETHICS COMMITTEE (REC)

5. In order to continue with the study beyond the approved period, a Continuing Review Application must be submitted to the REC ten weeks prior to the indicated expiration date of the approval.

The documents approved in this Application Process are listed below:

Document	Language	Version
Protocol	English	Version 2
Protocol Application Form	English	Version 1
Data Collection Tools	English/Runyankole	Version 2
Informed Consent Document	English/Runyankole	Version 2

KIU RESEARCH ETHICS COMMITTEE(REC)
APPROVED
VALID UNTIL
13 MAR 2019
P.O. Box 71, Bushenyi - Ishaka

Professor John Rwomushana
KIU REC CHAIRPERSON