THE EFFICACY OF ANTISEPTIC AGENTS UTILIZED AT KENYATTA NATIONAL HOSPITAL BURNS UNIT AGAINST BACTERIA ISOLATED FROM INFECTED WOUNDS

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN TROPICAL AND INFECTIOUS DISEASES, COLLEGE OF HEALTH SCIENCES, UNIVERSITY OF NAIROBI

DECLARATION

I declare that this research study is my original work and has never been published or presented for a degree in any other University. All works done by others and referred has been duly acknowledged and cited correctly.

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DEDICATION

To my precious and lovely daughter Hanna Faysal Mohamed.

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My foremost acclaim to my ALLAH, for it is by His Grace and Sprit that I have been able to accomplish this work. It is for His Glory to conceal a matter and to our honors to find it out.

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OPERATIONAL DEFINITIONS

Antiseptic: Antimicrobial substance that can be used as disinfectants on living tissue such as skin to lower possibility of infection**.**

Antimicrobial: Any element of natural, synthetic or semisynthetic origin that inhibits or kills the growth of microorganism with little or no damage to the host.

Burn: Skin or tissue injury induced by cold, electricity, heat, chemicals friction or radiation.

Blood agar: Enriched medium that is used to cultivate bacteria organisms which do not grow easily.

Disinfectant: Antimicrobial substance that can be applied on the surface of non-living objects to destroy microorganisms such as bacteria, fungi and viruses.

Minimum Inhibitory Concentration (MIC): The lowest concentration of an antimicrobial agent that can inhibit bacterial growth.

Minimum Bactericidal Concentration (MBC): The lowest concentration of an antimicrobial agent that can kill the bacteria.

Nutrient Broth: A liquid basal medium composed of beef extracts and peptone that allows many types of micro-organisms to grow.

Pour Plate Technique: A method used to count the number of colony-forming bacteria present in a liquid specimen.

LIST OF ABBREVIATIONS

BWI: Burn wound infection

ERC: Ethics and Research Committee

KNH: Kenyatta National Hospital

MIC: Minimum Inhibitory Concentration

MBC: Minimum bactericidal Concentration

SPSS: Statistical Package for Social Sciences

UoN: University of Nairobi.

WHO: World Health Organization.

CoNS: Coagulase Negative *Staphylococcus*

MRSA: Methicillin-resistant *Staphylococcus aureus*

NaDCC: Sodium dichloroisocyanurate

SOP: Standard Operating Procedure

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ABSTRACT

Background: Burn injury in the Kenyan population is associated with extended hospitalization, deformity and disabilities, which induces stigma and rejection. In addition, the highly resistant and opportunistic bacteria that infect the wounds complicates patient management. Burn-injury patients with bacterial infections, especially those associated with multidrug-resistant (MDR) strains are likely to be at higher risk for untreatable or difficult to treat infections. Therefore, use of antiseptics at an effective concentration, diluted in a clean environment and cessation of their applications when the clinical features of infection immediately fade results in reduction of burn wound infections.

Broad Objective: To identify the type, concentration, sterility and efficacy of antiseptic solutions against bacteria isolated from burn wounds in patients admitted at Kenyatta National Hospital (KNH) burns unit.

Methodology: This was a cross-sectional study carried out at KNH Burns unit. A total of 81 wound swab samples were consecutively collected from patients presenting with acute burn wounds after carefully cleaning with normal saline over a period of three months. Growth on Blood agar and MacConkey agar was evaluated for colonial morphology; gram stain and biochemical tests were used for species identification. The isolated bacteria were subjected to antiseptics to determine the efficacy. Discrete colonies were then stored at -20℃ in Skimmed Milk media in the department of Medical Microbiology UoN. Descriptive analysis was done to determine the frequencies and proportions of the variables and presented in tables and graphs where appropriate. Chi Square test was used to determine correlation between concentration of antiseptics and susceptibility of isolated bacteria. A p-value of < 0.05 was considered statistically significance.

Results: A total of 81 swabs collected from burn wound patients had bacterial growth. *Staphylococci aureus* (48.1%, 39/81), *Proteus* species (30.9%, 25/81), *Pseudomonas aeruginosa* (16.0%, *13*/81), and *Klebsiella* species (1.2%, 1/81) were the most predominant species isolated. The unidentified organisms were (3.7%, 3/81). Among the positive samples more than eighteen percent had mixed bacterial growth. The effective antiseptics tested against isolated bacteria were acetic acid 4% and chlorhexidine digluconate 5% w/v when their concentrations are

increased gradually, while 1% of silver nitrate 0.01% recorded low bacteriostatic activity. This study revealed that in comparison to 5% chlorhexidine digluconate w/v and silver nitrate 0.01% w/v, acetic acid solution is a much more effective antiseptic against bacteria isolates infecting burn wounds as it showed 100% bactericidal activity against all the infecting bacterial agents at a concentration 4%. The study also confirmed that a combination of the different concentrations of the three and/or two consecutively tested antiseptics had higher microbiocidal efficacy than when each was tested individually.

Conclusion: *Staphylococcus aureus* and *Proteus species* were the most frequently isolated bacteria infecting the burn wounds. The bactericidal and bacteriostatic activity of the antiseptics solutions utilized at KNH burns unit was different depending on the concentration and organism isolated. Four percent of acetic acid and 5% chlorhexidine digluconate w/v solutions were found to be more effective both showing bactericidal and bacteriostatic activities while 1% silver nitrate 0.01% w/v was found to be bacteriostatic. In addition, we also noted bactericidal activity when the antiseptic agents were combined against all the bacteria isolated.

1.0 INTRODUCTION

Burn is a skin injury induced by chemicals, electricity, heat, radiation light or friction. The magnitude of the skin injury due to a burn relies on the heat level and the duration of contact with the heat (Pham, Cancio, and Gibran 2008). If proper treatment is provided on time, the burn injury would not lead to deformity. In contrast, if the injury is inadequately treated it might threaten the patient‘s life, and further complications can arise such as disabilities which are burden to the family, community and to the nation (Roth and Hughes 2004). Studies have revealed that the incidence of burn injuries has been increasing in the developing countries and this has negatively impacted public health (Kuiri et al. 2015).

A disinfectant is known as a diverse chemical substance which suppresses the development of pathogenic microorganisms in the vegetative or non-sporing form. However, disinfectants do not kill all organisms but rather reduces them to a level that will not be harmful to the health or the quality of medical and surgical equipment (Nagoba et al. 2013). Disinfectants can be classified into two methods: physical methods such as ultraviolet irradiation, filtration, boiling and chemical methods e.g**.** halogens, quaternary ammonium compounds, alcohols, etc. (Tytler et al. 2006).

An antiseptic is a type of chemical agent applied to the body that suppresses or reduces the growth of micro-organisms residing on tissues without resulting damage to the skin (Sheldon 2005). For instance, chlorhexidine which has bacteriostatic and bactericidal activity is effective towards Gram-negative and Gram-positive bacteria, although, other studies have reported that this antiseptic is less efficient towards certain bacterial species such as *Pseudomonas* and *Proteus* and surprisingly inactive towards mycobacteria (Nagoba et al. 2013). Antiseptics are

generally used in dilutions, but it has been shown that certain Gram negative bacteria can still survive when some of these agents are diluted, making them ineffective against nosocomial infections (Helal and Khan 2015). The emerging microbial resistance in medical clinics or hospitals and in the community as well is instigating problems in patient management and infection control.

The mode of action of biocides shows different levels of antibacterial activity that acts on several target sites within the microbial cells with an overall of a bactericidal effect (Maillard 2002). However, most antimicrobial agents have activity on intracellular cells. The extensive use of the biocides causes theoretical issue on the development of their resistant to microbes in intrinsic in nature. For instance, antimicrobial resistance is frequently conferred by resistance genes transferred through transposons located on plasmids, which leads to rapid and wide spread to all over the world (Alkolaibe, Al-ameri, and Alkadasi 2015).

Burn injured wounds have been shown to play a role in increasing rates of morbidity and mortality in Kenya, including extended hospitalization, deformity and disability which induces stigma and rejection (Amakobe and Moronge 2016). In addition, patients who are hospitalized due to burn wounds have increased risk of nosocomial infections from opportunistic and drug resistant bacteria. Infections of bacteria, particularly multidrug-resistant strains of *Pseudomonas aeruginosa* in burn patients are more difficult to treat due to its resistance (Church et al. 2006). An immunocompromised person who has high percentage of burn wounds frequently develops life-threatening infections. Many studies have shown that, Gram-negative bacteria has major health concerns globally, particularly when they infect burn wounds causing complications with

significance increase of morbidity and mortality (Azzopardi et al. 2014; Pagani, Colinon, and Migliavacca 2005).

1.1 PROBLEM STATEMENT

Burn wound being a commonly presented clinical condition causes burdensome critical problem in hospital settings. It has been shown that burn injured wound play a role in increasing rates of morbidity and mortality in Kenya, including extended hospitalization, deformity and disability that induces stigma and rejection (Amakobe and Moronge 2016). Efficacy of antiseptic solutions against bacteria isolates might have contributed to this high mortality. Despite improvement in antiseptic solutions used against bacteria isolates, the incidence of infections in the KNH burns unit caused by specific bacteria species is increasing gradually. Management of infection remains a challenge with the increasing resistance of specific bacteria species. At KNH, very few studies have focused on burn wound infections and none of these studies have evaluated the efficacy of antiseptics used in the burns ward. Therefore, lack of this information might have negative implications of treatment outcome of burn wound infections and development of bacterial resistance to antiseptics.

2.0 LITERATURE REVIEW

2.1 Common bacterial isolates encountered in infected burn wounds

Certain species of bacteria including *Acinetobacter*, *Staphylococcus aureus*, *Proteus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella* species are predominantly isolated in infected wounds (Ngugi 2013).

The importance of evaluating the efficacy of antiseptics towards these pathogenic organisms is their ability to survive and develop resistance when lower levels of antiseptics are used and/or when they are not diluted effectively and in a clean environment.

2.2 Overview of common bacteria isolates

2.2.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an opportunistic pathogen with innate and acquired resistance to the commonly used antibiotics and some of the common disinfectants such as quaternary ammonium compounds, chloroxylenol and hexachlorophene (Kumar 2012). Studies have revealed that some isolates of this organism are resistant to Chlorhexidine and Povidone-iodine used in hospitals (Ananthnarayan and Jayaram Panikar 2009). In addition, this organism is the commonest pathogen that causes nosocomial infections in burn units and in hospitals in general (Ayres, Furr, and Russell 1993) (Article 2014). It has been shown to cause severe secondary disease in patients with critical conditions, especially patients with cancer, immunodeficiency diseases and burns (Sawa 2014) (Gellatly and Hancock 2013). Because of its high ability to invade the tissues, it produces toxins that causes complicated infection (Levinson 2016).

2.2.2 *Staphylococcus aureus*

Staphylococcus aureus is an important pathogen with increasing concern as a causative agent of nosocomial infections because of antimicrobial resistance. This species has distinctive features from CoNS (e.g. S. *epidermis*). The name 'aureus' defined gold for their elaboration of a golden pigment on solid media (i.e. Blood agar), whereas CoNS colonies appear pale, white and translucent on culture media. It has also been shown to be more virulent regardless of their phylogenic similarities (Lowy 1998). Currently, the genome databases of this species has been concluded for 7 strains, COL, Methicillin-resistance *Staphylococcus aureus* (MRSA), Methicillin-susceptible *Staphylococcus Aureus* (MSSA), 8325, N315, Mu50, and MW2 (1-6). Hence, the mean dimension of *Staphylococcus aureus* genome is 2.8Mb and the cell wall which has tough protective coat with relatively lacking definite form is about 20-40nm thick. Beneath the cell wall, there is cytoplasm which enclosed by the cytoplasmic membrane (Harris, Foster, and Richards 2002).

2.2.3 *Acinetobacter species*

Acinetobacter species are gram-negative, cocco-bacilli that are free-living and are easily found in soil, food, water and sewage. At the biotechnological site, the metabolic usefulness of this organism implies its value as a numerous commercially significant industrial manners and the degradation of a wide range of ecological toxins (K. Towner 1996). For instance, this organism is ubiquitous opportunistic pathogen that is commonly associated with infections from healthcare providers in the clinical setting, where it can easily be isolated as commensals from their skin. Therefore, some members of the genus *Acinetobacter* such as *A. baumannii* are now considered nosocomial pathogens affecting individuals with impaired host defense and/or in patients on respiratory therapy equipment because, it has ability to survive and colonize in different environmental conditions (K. J. Towner 1997).

2.2.4 *Klebsiella species*

Klebsiella is a well-known organism that causes severe pyogenic infections mainly in chronic alcoholics in which their X-ray presents characteristics abnormalities, if left untreated this might increase the mortality rate. *K. pneumoniae*; the medically most important species of the genus is associated with nosocomial infection in hospitals (Carpenter 1990). Nevertheless, this opportunistic pathogen primarily targets hospitalized immunocompromised host who has been suffering from severe underlying illnesses such as diabetes mellitus and/or chronic pulmonary obstruction. *K. oxytoca* is another species of the genus which has been isolated from human clinical specimens less than those for *K. pneumoniae*. Some studies reported that *Klebsiella* species causes eight percent of all nosocomial bacterial infections in the United States and in Europe (Podschun and Ullmann 1998).

2.3 Different types of burn wound infection

Incidence of burn wound injuries shows discrepancies across countries, populations, and time. The severity of a burn relies on the thickness of the skin involved, the level of heat and the duration of exposure (Atiyeh, Costagliola, and Hayek 2009). The management of burns requires a multidisciplinary approach and it varies depending on the psychological and physiological status of the patient. The main components are surgical intervention such as early excision and/or skin grafting, management of sepsis and multi-organ failure, nutrition, and rehabilitation (Church et al. 2006).

Burns are the most well-known and destructive forms of the skin injury. A serious of thermal injured patients require urgent care to reduce morbidity and mortality rates. There is data obtained on the National Center for Injury Prevention and Control in the United States that shows approximately two million fires occur every year and leads to 1.2 million people getting burn injuries (National Center for Injury Prevention and Control 2002). Approximately 100,000

cases with moderate to severe burn injuries requires hospitalization, around 5,000 patients of these cases die each year due to burn-associated complications (Kuiri et al. 2015). As many as 13% of burn patients present with shock at admission in Kenyatta National Hospital (KNH) (Mung'ara 2004). Out of the 46 adult patients who presented with severe burns [Percentage of Total Body Surface Area (%TBSA) range of 30% to 110%, mean 52.3%] only 6 survived, with 20 dying within one week (Ishisanya 2007).

2.3.1 Burn wound impetigo

Impetigo is a skin infection due to burn injured wound that causes loss of epithelium from a previously re-epithelialized skin surface of individuals including skin grafted burns, healed donor sites or partial-thickness burns that promotes secondary intention. This infection is not linked to mechanical stress following skin graft, hematoma and/or inadequate excision of the wound site (Church et al. 2006).

2.3.2 Burn-associated surgical wound infection

Surgical wound infections among burn patients that produce purulent exudate becomes culture positive after being cultivated from excised burns that have not yet epithelized and those from donor sites. Open areas of surgical burn wound infections shows skin erythema in the undamaged site covering the wound, loss of natural epithelial cells surrounding the wound and hyperemia (Church et al. 2006).

2.3.3 Cellulitis associated with burn wound infection

Cellulitis that is associated with burn wound infection occurs when there is delay of infection to the healthy, undamaged epidermis and soft tissues covering the wound or donor sites. This form of ailment has definitive clinical features such as erythema in the intact site covering the wound with no extensive damage to the tissue. Despite this, the clinical features can be manifest with at

least one of these signs: tenderness with localized pain, heat and swelling at the injured site and signs of lymphangitis (Church et al. 2006).

2.3.4 Un-excised burn wound invasive infections

It has been reported that an individual with areas of un-excised burn wound infections either deep-partial or full-thickness might have a greater threat of developing invasive infection (Elamenya et al. 2015). Complications of this invasive infection might cause a rapid change to the shape of the burn wound features or character like separation of the eschar or dark brown, black, or violaceous discoloration of the eschar. Clinical manifestations of un-excised burn wound invasive infections include inflammation of the intact skin such as; hotness, tenderness, redness, edema and systemic signs of sepsis i.e. hypotension, unexplained hyperglycemia, tachypnea, reduced urine-output and/or mental confusion. In addition, positive blood cultures with the isolation of a pathogen in the absence of another identifiable source of infection and evidence of microbial invasion into adjacent viable tissue on histological examination have confirmed the diagnosis of this disease. Surgical excision is the effective treatment in invasive infection of un-excised burn wounds (Church et al. 2006).

2.4 Antiseptics

An antiseptic is a type of chemical agent that suppresses the growth of micro-organisms residing on living tissues without causing any harm when applied to surfaces of the body and/or to exposed tissues. Antiseptics are applied to burns, mucous membrane or unbroken skin and to open wounds to prevent sepsis by eliminating or removing microbes from these areas. Iodine is an antiseptic that has been modified for use against infectious organisms found on skin surfaces or tissues (Sheldon 2005).

There are several classes of commonly used antiseptic agents in the hospital setting that include: hydrogen peroxide, acetic acid, chlorhexidine, iodine releasing agents (**i.e.** Povidone-iodine [PVP-1] and Iodophore), bisphenol compounds (Triclosan), silver-releasing agents (i.e. silver sulfadiazine) and chlorine-based biocides (**i.e**. sodium hypochlorite solution and Dakin's solution) (Bowler, Duerden, and Armstrong 2001; Williamson, Carter, and Howden 2017).

The iodophore is a complex containing povidone and iodine (povidone-iodine). This antiseptic solution is known to be effective against bacteria, viruses, fungi, protozoa, cysts and spores and significantly decreases surgical wound infections by releasing iodine on contact with the skin (Nagoba et al. 2013).

Some studies have identified that chlorhexidine has bacteriostatic and bactericidal activity which is effective towards both Gram-negative and Gram-positive bacteria. Despite this, other studies have reported that this antiseptic is less effective against some species of *Proteus* and *Pseudomonas* and rather inactive against mycobacteria (Alkolaibe, Al-ameri, and Alkadasi 2015). In addition, this chlorhexidine is inactive against bacterial spores, and also incompatible with soaps and other anionic substances, such as chlorides, phosphates and bicarbonates, forming salts of low solubility which may precipitate out of solution. Ethanol is an antiseptic that has bactericidal activity used to disinfect skin prior to injection, surgical procedures and venipuncture (Tytler et al. 2006).

Acetic acid is a non-toxic topical antiseptic agent that is easily available and inexpensive comparing to other topical agents. Diluted acetic acid is magnificently used by health care providers mostly for the management of wound infections caused by *Pseudomonas aeruginosa*. Lineaweaver et al. determined that a 0.25% acetic acid solution destroys 100% of exposed

fibroblasts in an in vitro model. Hence, impaired wound healing might occur at any clinically effective concentration. Some studies also reported, the acetic acid ability to slow down the wound epithelization and to limit polymorph- nuclear cells (Al-azzawi and Abdullah 2018).

Hydrogen peroxide is a potent antimicrobial agent that can rapidly cross cell membrane and is active against bacteria, bacteria spores, protozoa cyst, viruses and prions. It can be used as liquid form or gas form. The liquid form is used as an antiseptic on the skin and dental disinfectant at concentrations of 3-6% vol/vol and 0.4%-1% respectively (Williamson, Carter, and Howden 2017). Endozime AW Triple Plus is an enzymatic disinfectant that has unique feature with advanced proteolytic action by removing blood, fat, carbohydrates, starches and protein from all surgical instruments and scopes in as little as 2 minutes. This product is designed to clean the most demanding instruments (i.e. orthopedic, laparoscopic) nonetheless it is safe for use on the most delicate (i.e. ophthalmic, microsurgical) equipment. Low-sudsing, neutral pH Endozime AW Triple Plus was developed for universal applications eliminating the need for all other cleaners and detergents (Ruhof Corporation 2011).

Some of chemicals used as antiseptics have toxic effects on certain cells that delay healing and deteriorate the patient's condition when they are used at a low concentration level, because they are toxic to fibroblasts which interferes with the normal healing process by permitting more virulent microbes to dominate. Hence, the consequences of these observations have often been criticized practically with utilization of some antiseptics in burn-injured wound patients. Therefore, with the use of effective concentration of antiseptics diluted in a clean environment and cessation of their application when clinical features of infections immediately fade, a great reduction in burn wound infections could be achieved.

2.5 Past studies on resistance of bacteria isolated from burn wounds to antiseptic solutions

A study carried out in Yemen hospitals on the bacteriology of burn wound infection and their susceptibility patterns to commonly used disinfectants revealed that the most susceptible bacteria being tested against disinfectants and antiseptics were *Escherichia coli and Proteus* while *P. aeruginosa, Staphylococcus aureus* were the most resistant bacteria to disinfectant agents. In addition, the same study showed *Pseudomonas aeruginosa* (35.7%); *Escherichia coli* (23.8%); *Staphylococcus aureus* (21.4%) and *Proteus* species (19.1%) were the predominant microorganisms isolated from infected burn wound patients (Alkolaibe, Al-ameri, and Alkadasi 2015).

A study conducted in Baghdad confirmed Claradone (Povidone-iodine) and Sarttol (Dettol) affects bacterial growth. Hence, this study reveals the lowest concentration of Claradone (Povidone-iodine) which restrain the growth of *Pseudomonas aeruginosa* pathogen was minimum inhibitory concentration of 30% and 3% of the lowest effective concentration of Sarttol (Dettol) respectively. Furthermore, this study investigated the number of survival colonies of the *P. aeruginosa* after being preserved with high concentration of Claradone (Povidone-iodine) and Sarttol (Dettol) to test their susceptibility to antibiotics. They indicated, the colonies of *Pseudomonas aeruginosa* which are resistant to antibiotics were sensitive before treatment with the antiseptics mentioned above (Al-Jailawi, Ameen, and Al-Jeboori 2013).

In Ethiopia (Mitiku, Ali, and Kibru 2014) *P. aeruginosa* isolates from hospital environments (32.%) and clinical samples (47.5%) that were tested for their susceptibility to disinfectants and antibiotics showed reduced sensitivity to commonly used antibiotics; Trimethoprim-sulphametoxazole (95.1%), Gentamicin (62%) and Ceftriaxone (58%) and increased sensitivity to the antibiotics namely, Imepenem, Meropenem and Ticarcillin /Clavulanate. Hence, this study

determined *Pseudomonas aeruginosa* isolated from clinical environment were more resistant to particular antibiotics compared to those isolated from clinical sample.

A prospective longitudinal analytic study conducted in Kenya looked at association of BWI and their mortality in patients hospitalized at Kenyatta National Hospital (KNH). The study confirmed that there was a strong relationship between infected burn wound and mortality due to burn injuries. The study determined that the rate of burn wound infections was higher than that of previous retrospective study of 1995, and the overall infection rate was 23.6%. This study revealed common organisms isolated from burn wounds; *Pseudomonas aeruginosa* (36.4%), *Staphylococcus aureus* (27.3%), *Escherichia coli* (13.6%), *Proteus* (9.1%) and others mixed growth (13.6%) (Ngugi 2013).

A descriptive retrospective study carried out at Kenyatta National Hospital (KNH) in 1992 found 18.7% of the burn wound infections and the common causative organisms encountered in the infected burn wounds were *Staphylococcus aureus* (32%), *Pseudomonas aeruginosa* (21%), *Proteus* (11%), *Escherichia coli* (7%) while *Acinetobacter, Klebsiella* and *Streptococcus pyogenes* were 4%. This study also showed that the risk factors predisposing to wound infections included age of the patients and extent of burn surface area (BSA). In addition, these pathogenic micro-organisms isolated were sensitive to the commonly used antibiotics including cephalosporin, aminoglycoside and newer penicillin (Kimani 1995).

Another study carried out in Kenya identified antimicrobial sensitivity patterns of organisms encountered in wound sepsis at KNH Pediatric surgical wards. This study established that 18% was the occurrence of wound infections. *Staphylococcus aureus* (52.7%) was the most prevalent infective organism followed by *Pseudomonas aeruginosa* (17.3%). *S. aureus* was the most

resistant organism with susceptibility of less than 50% to most drugs. About 50.6% of the *Staphylococcus* isolates were methicillin resistant. *Streptococcus* was less resistant with more than 80% susceptibility to all tested drugs except cefuroxime. *E. coli* isolates were effective to ciprofloxacin. All gram negative bacteria were highly sensitive to ciprofloxacin with the following susceptibilities: *Pseudomonas aeruginosa* (92.3%), *Proteus mirabilis* (71.4%) and others 100%. Imipenem which is a new and relatively expensive Monobactam demonstrated reduced activity with the following susceptibilities: *Staphylococcus aureus* (38%), *Streptococcus* (80%) and at the gram-negative bacteria (70%) (Elamenya et al. 2015).

In a study carried out in Nigeria which evaluated the effect of disinfectants on antimicrobials of certain micro-organisms. The disinfectants were centered on concentration to antimicrobial activities and the result showed that disinfectants have broad activities to these organisms; *Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans,* and *Epidermophyton floccosum*. Dettol was more effective towards *Pseudomonas aeruginosa*, *Staphylococcus aureus, Candida albicans and Epidermophyton floccosum.* Kerosene was effective against *Candida albicans* and *Epidermophyton floccosum*. Methylated spirit and JIK had moderate effect against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Therefore, this study concluded that, a great reduction of nosocomial infections and other infective diseases will be achieved if the disinfectants are effectively diluted in a clean environment (Olufunmilayo and Precious 2017).

2.6 JUSTIFICATION

Antiseptics are broadly used in all health care facilities including hospitals and other health care centers for a variety of topical and hard-surface applications. Specifically, they are a crucial part for infection control programs that help in the prevention of nosocomial infections. Recently, multidrug-resistant strains have been reported as the reasons for nosocomial infection outbreaks

in burn units or as a colonizers of the infected wounds in burn-injured patients (Helal and Khan 2015). Past studies have established that certain routinely used antimicrobials are not effective against most common organisms infecting burn-injured wounds (Elamenya et al. 2015). Despite this, little is known about the efficacy of antiseptic solutions and resistance of bacteria to these antiseptics in the region. Therefore, the study addressed this gap by identifying the type, concentration, sterility and efficacy of antiseptics against bacterial isolates at KNH burns unit. The findings of this study will in the future guide on the utilization of effective concentrations of antiseptics that can kill bacteria associated with secondary infection of burn wounds.

2.7 RESEARCH QUESTIONS

What is the efficacy of antiseptic solutions used at KNH burns unit against bacterial isolates from infected wounds?

2.8 STUDY OBJECTIVES

2.8.1 Broad objective

To identify the type, concentration, sterility and efficacy of antiseptic solutions used at KNH burns unit against bacteria isolated from infected burn wounds.

2.8.2 Specific objectives

1. To determine the type, concentration and sterility of antiseptic solutions used at KNH burns unit.
2. To identify the bacterial pathogens isolated from infected burn wounds of inpatients seeking services at KNH burns unit.
3. To determine the efficacy of antiseptic solutions identified against the isolated bacteria organisms.

3.0 MATERIAL AND METHODS

3.1 Study design

This was a hospital based cross sectional study.

3.2 Study setting

The study was conducted at Kenyatta National Hospital (KNH) Burns Unit. KNH has a burn unit that caters for patients with burn injuries. The burn unit is well equipped and the nurses provide health care services in collaboration with plastic surgeons, psychologists, physiotherapists, occupational therapists and nutritionists. Most patients with severe burns from other hospitals in the country are referred to KNH for proper treatment. It admits both children and adults in the same unit; the unit has a bed capacity for 20 patients. The patients are admitted in the burn unit in their acute phase, where they are managed until they are stable enough to be transferred to another ward which has a large bed capacity of 100 patients to continue with management

3.3 Study population

The sample of infected burn wounds were obtained from patients admitted at KNH burns unit. The pus swabs were analyzed for bacterial growth associated with the infection and subjected to antiseptics used at the center during the study period.

3.4 Inclusion and exclusion criteria

3.3.1 Inclusion criteria

1. All infected burn-injured patients admitted at KNH during the study period.
2. Patients or patient's guardians accepting to give informed consent/assent.

3.4.2 Exclusion criteria

1. Burn-injured patients at the healing stage during the study period.
2. Refusal to give informed consent/assent.

3.5 Sample size determination

Sample size was determined by adopting Fisher's formula (Fisher et al, 1998)

$$n = \frac{NZ\alpha P(1-P)}{d2(N-1) + Z\alpha P(1-P)}$$

Description

n = Minimum required sample size

α = Level of significance (0.05)

N = total accessible population is 165 (an average of 55 burn wound patients are seen at KNH burns unit per month, hence 55×3months = 165).

Zα= Standard normal deviate at 95% Confidence level (standard normal deviation is 1.96)

P = Estimated prevalence of burn wound patients is 23.6%= 0.236 according to rate of burn wound infection at KNH by Ngunga study, 2013)

d = Absolute precision (Margin of error at 5%), standard value of 0.05

$$\mathbf{n} = \frac{165 \times 3.8416 \times 0.236 \times 0.764}{0.0025 \times 164 + 1.96 \times 0.236 \times 0.764} \qquad \mathbf{n} = 72.5 \sim 73 \text{ patients.}$$

3.6 Data collection procedure

3.6.1 Sample collection

Pus swabs were consecutively collected from 73 infected burn wound patients admitted at KNH burns ward using commercially available sterile swabs. Only one swab per patient was collected after carefully cleaning the burn wound with normal saline in order to prevent surface

contamination. The specimens were labelled and transported within 2 hours of collection to UoN microbiology laboratory for microbiological analysis.

3.6.2 Culture and identification of infected organism

The samples were inoculated directly on blood agar and MacConkey agar and subsequently incubated under aerobic and/or anaerobic condition at 37◦C for 18-24 hours. Culture plates were re-incubated for another 24 hours where no growth was depicted in the initial incubation period.

The bacterial growth on Blood agar and MacConkey agar were evaluated for their phenotypic characteristics such as colonial morphology on culture media, gram stain and biochemical tests. Discrete colonies were revived by streaking on MacConkey media and subjected to antiseptics to determine the efficacy of antiseptics to the isolated bacteria. Finally, the isolates were stored at -20◦C in Skimmed Milk media in the department of medical microbiology UoN.

Sample collection, media and identification tests were quality controlled according to the relevant SOP (Appendix II).

3.6.3 Sterilization test of antiseptic solution

Antiseptic disinfectant solutions used in KNH burns unit were tested for their sterility using the pour plate technique. The antiseptic solutions used in the burns unit were:

1. **1.** Chlorhexidine digluconate 5% w/v.
2. **2.** Acetic acid – vinegar (Zesta).
3. **3.** Silver nitrate 0.01%- Ionic silver solution (Qurion).

To evaluate sterility, 1ml of each of the antiseptic solution was placed at the center of a sterile petri dish. Approximately 15ml of molten cooled nutrient agar was then poured into the petri dish and mixed gently. The agar was then allowed to solidify and incubated under aerobic condition at 35◦C -37◦C for 18-24 hours.

3.6.4 Antiseptic efficacy testing

The susceptibility of selected bacteria isolates from burn wounds were tested against antiseptic solutions. The bactericidal concentration of the antiseptic solutions were determined using the classic method of successive dilutions. In a sequence of seven test tubes (labeled 1-7), 1 ml of sterile nutrient broth was dispensed into each of the tubes except for the tube labeled 1. Consequently, 1 ml of known concentration of the antiseptic was added into the 1st and the 2nd tubes of the series. The contents in tube labeled 2 were mixed and 1 ml of the mixture was transferred to tube 3. This successive transference was repeated until tube 5 where 1ml of content from tube 5 was discarded into sink. Finally, 0.1 ml of each selected bacteria suspension was added to all tubes except tube # 7. Tube #6 was used as positive control with its content (nutrient broth + test organism) and Tube #7 as negative control (nutrient broth + distilled water). The contents of the tubes were then incubated at 37◦C for 18-24 hours. Following the incubation period, 1ml of the contents in each of the tubes was sub-cultured on nutrient agar and observed for bacterial growth after 24 hours of incubation at 37◦C. The bactericidal concentration was considered as the concentration of the tube in which no bacteria growth was observed after sub-culturing on nutrient agar. With respect to quality control, the standard reference strain of the selected bacteria was used in order to check quality of nutrient broth. Additionally, all antiseptic disinfectants were kept in a dark area at room temperature and were freshly prepared prior to testing.

3.6.5 Data management and analysis

The data was entered into Microsoft Excel 2010 and a copy of the entry was made for backup purpose. SPSS version 23 was used for data analysis. Descriptive analysis was done to determine the frequencies and proportions of the variables and presented in tables and graphs where appropriate. The dependent variable of the study was the concentration of antiseptic solutions

and the independent variable were bacteria isolates obtained from the infected burn wound. Chi Square test was used to determine correlation between concentration of antiseptics and susceptibility of isolated bacteria. P-value of < 0.05 was used to determine the significance level.

3.7 Ethical considerations

Ethical approval was sought from KNH-UoN Ethics and Research Committee (Ref: KNH-ERC/RR/1002). Permission to conduct the study were obtained from the administrative heads of KNH and the in-charge at the burns unit. Data was stored only in a computer with a password to facilitate confidentiality.

3.8 Limitation of the study

Though burn wound infections are contaminated by different bacteria, selected bacterial isolates were used to determine the efficacy and concentrations of antiseptic solutions against those bacteria isolates from infected burn wound patients. We therefore did not consider other agents including strict anaerobic bacteria, fungi and species identification for mixed growth such as API-20/VITEK systems.

3.9 Dissemination of the study finding

The results of this study will be presented at Institute of Tropical and Infectious Diseases. The finding will also be shared with the KNH research team, KNH Burns unit and published in a peer reviewed journal

4.0 RESULTS

4.1 Distribution of isolated bacterial organisms

A total of 81 wound swabs were consecutively collected from patients presenting with acute burn wounds and analyzed for bacterial growth.

The most common bacterial organisms isolated from these wounds was *Staphylococci aureus* which was present in 39/81 (48.1%) of the samples followed by *Proteus* species in 25/81 (30.9%), *Pseudomonas aeruginosa* 13/81 (16.0%), *Klebsiella* species 1/81 (1.2%) of the samples and 3/81 (3.7%) unidentified organisms were isolated. A summary of the distribution is shown in figure 1 below

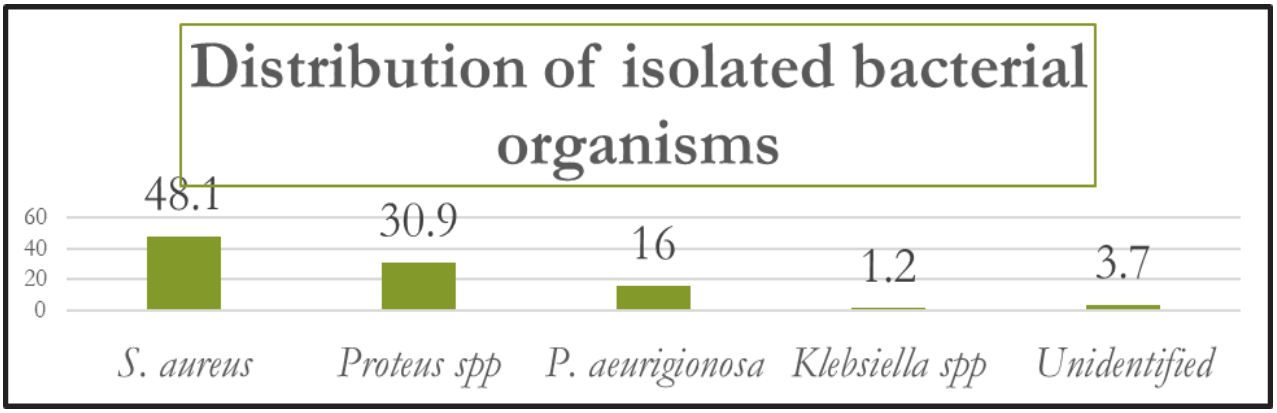

Figure 1. Distribution of isolated bacterial organisms.

4.2 Monomicrobial versus Polymicrobial infection

The pattern of mixed bacterial growth is summarized in Table 1. Samples received were analyzed for the presence of single or multiple bacteria species from the same wound using colony characteristics, gram stains and biochemical identification. More than eighteen percent of positive samples had mixed bacterial growth and approximately eighty-two percent was monobacterial growth. Among the mixed growth 14 samples had two different bacteria species

while one sample had three different species isolated from same wound. There are two different samples among the fourteenth samples of mixed growth that had two growths of *proteus* species in each, but we were not able to identify by their species level because of the study limitation.

Table 1: Burn wound infection profile

Organisms	**No. of Samples with Growth**	**Proportion of Samples with Growth**
Mono-bacteria growth	**50/81**	**61.73%**
S. aureus	27	33.3%
Ps. Aeruginosa	9	11.1%
Proteus spp	13	16.1%
Klebsiella spp	0	0%
Unidentified	1	1.23%
Mixed bacterial growth	**15/81**	**18.52%**
S. aureus + *Ps. aeruginosa*	4	4.94%
S. aureus + *Proteus spp.*	7	8.64%
S. aureus + Unidentified*	1	1.23%
Proteus + *Proteus* spp.	2	2.5%
Proteus + *Klebsiella* spp.	1	1.23%
Organisms	**No. of Samples with no growth**	**Proportion of samples with no growth**
No bacterial growth	**16/81**	**19.8%**

* A sample from same wound isolates had 2 unidentified and one *S. aureus* growth.

4.3 Sterility test of antiseptic solution

Table 2 shows that all the three antiseptics tested i.e. Chlorhexidine digluconate 5% w/v, Acetic acid 4% and Silver nitrate 0.01% w/v were not contaminated before use.

Table 2: Sterility test of the antiseptics

Type of Antiseptics	Concentration (%)	Sterility of Antiseptic Contaminant
Chlorohexidine digluconate	**5%**	**None**
Acetic Acid	**4%**	**None**
Silver nitrate 0.01%	**1%**	**None**

4.4 Different antiseptic concentration used to test isolated bacteria organisms

Results presented in table 3 shows that bacterial growth was affected by the antiseptic solutions tested. As the concentration of chlorhexidine digluconade and acetic acid increased the susceptibility of tested bacteria increased. At a concentration of 5% chlorhexidine digluconade showed a susceptibility proportion of 98.8%, while at concentration of 2.5%, 1.25% and 0.625% the susceptibility proportion was 92.6% for each. Additionally, concentrations of 0.3125%, 0.156% and 0.07% showed significant decreased susceptibility proportions of 87.7%, 76.5%, 68.0% respectively. In acetic acid solution, 100% susceptibility was observed at a concentration of 4%, while at concentrations of 2% and 1% susceptibility of 90.1% and 80.2% were observed respectively. A low susceptibility proportion of 42.0% was observed at a concentration of 0.5%.

Table 3: Concentration and susceptibility of the antiseptic solutions tested

Concentration (%)	Susceptibility n (%)*
Chlorhexidine	
5%	80 (98.8%)
2.5%	75 (92.6%)
1.25 %	75 (92.6%)
0.625 %	75 (92.6%)
0.3125 %	71 (87.7%)
0.0156 %	62(76.5%)
0.07 %	47 (68.0%)
Acetic Acid (Vinegar)	
4 %	81 (100%)
2 %	73 (90.1%)
1 %	65 (80.2%)
0.5%	34 (42%)

n* represents the number of the isolated organisms susceptible to the antiseptic solutions.

Silver nitrate solution 0.01% w/v showed a susceptibility proportion of 76.5% as shown in table 4.

Table 4: Susceptibility of the silver nitrate 0.01% w/v solution tested

Concentration (%)	Susceptibility n (%)
Silver nitrate 0.01%	
1%	62 (76.5%)

n* represents the number of the isolated organisms susceptible to the antiseptic solutions.

4.5 Effect of antiseptic solutions on selected bacteria isolates

The study revealed that the acetic acid was the most effective antiseptic agent against all the bacterial organisms (81) isolated and have susceptibility of 100% to a 4% dilution concentration. Comparatively chlorhexidine digluconate inhibited most of the organisms (80) isolated at a higher dilution of 5% and had shown susceptibility of 98.8%. However, Silver nitrate solution 0.01% w/v was the least effective antiseptic solution against some of the isolated bacteria organisms 62 (76.5%).

All *S. aureus* isolates were susceptible (100%) to chlorhexidine digluconate at concentrations of 5%, 2.5%, 1,25%, 0.625%, however the susceptibility proportion decreased from 95% to 90% and 74% at concentrations of 0.3125% to 0.156% and 0.07% respectively. Therefore, the lowest concentration of chlorhexidine digluconate that inhibited growth of *S. aureus* was 0.3125%. Silver nitrate 0.01% w/v solution was the least effective antiseptic against *S. aureus* (67%) in this study while acetic acid solution was the second most effective antiseptic against *S. aureus* (100%) specifically at a concentration of 4%. On the other hand, 2% of acetic acid solution was the lowest concentration that inhibited growth of *S. aureus* (95%).

There was no growth of *Pseudomonas aeruginosa* when treated with chlorhexidine digluconate at a concentration of 5%, but susceptibility decreased from 92%, 69% to 32% at 2.5%, 0.156% to 0.07% concentrations respectively. However, growth of *Pseudomonas aeruginosa* (85%) was observed when treated with acetic acid at a concentration of 2% and silver nitrate 0.01% w/v at a concentration of 1%. In addition, growth of *Proteus* species was observed when tested against all concentrations of chlorhexidine digluconate 5% w/v, acetic acid and silver nitrate 0.01% w/v with the exception of 4% concentration of acetic acid that showed no bacterial growth. *Klebsiella* species isolated showed high susceptibility (100%) to chlorhexidine digluconate 5% w/v, acetic

acid and silver nitrate 0.01% w/v. There was significant association between bacteria isolated from burn wound and concentrations of chlorhexidine digluconate 5% w/v (2.5%, 1.25% & 0.625%, 0.7%) and acetic acid 0.5% ($p < 0.05$) (Table 5).

Table 5: Susceptibility pattern of bacterial isolates to antiseptics

Dilutions (%)	**No* (%) of isolates susceptible to antiseptics used in burns unit**					
	S. aureus	***Proteus spp***	***P. aeruginosa***	***Klebsiella spp***	**Unidentified**	***P value***
Chlorhexidine digluconate						
5%	39 (100%)	24 (96%)	13 (100%)	1 (100%)	3 (100%)	0.666
2.5 %	39 (100%)	21 (84%)	12 (92%)	1 (100%)	2 (67%)	**0.042**
1.25 %	39 (100%)	21 (84%)	12 (92%)	1 (100%)	2 (67%)	**0.042**
0.625 %	39 (100%)	21 (84%)	12 (92%)	1 (100%)	2 (67%)	**0.042**
0.3125 %	37 (95%)	19 (76%)	12 (92%)	1 (100%)	2 (67%)	0.175
0.156 %	35 (90%)	15 (60%)	9 (69%)	1 (100%)	2 (67%)	0.063
0.07%	29 (74%)	11 (44%)	4 (31%)	1 (100%)	2 (67%)	**0.020**
Acetic acid						
4 %	39 (100%)	25(100%)	13 (100%)	1 (100%)	3 (100%)	-
2 %	37 (95%)	22 (88%)	11 (85%)	1 (100%)	2 (67%)	0.538
1%	30 (77%)	21 (84%)	11 (85%)	1 (100%)	2 (67%)	0.843
0.5 %	11 (28%)	10 (40%)	10 (77%)	1 (100%)	2 (67%)	**0.015**
Silver nitrate 0.01%						
1%	26 (67%)	21 (84%)	11(85%)	1 (100%)	3 (100%)	**0.244**

*N (%) represents the number and proportion of bacterial isolates from burn wound: *S. aureus* was 39 isolates, *Proteus* spp. 25 isolates, *P. aeruginosa* was 13 isolates. *Klebsiella* spp. was 1 isolate and three unidentified organisms.

4.6 Efficacy of antiseptics to isolated organism when they are combined together

A combination of the different concentrations of the three and/or two consecutively tested antiseptics showed high susceptibility (100%) to all organisms isolated than when each was tested separate as shown in Table 6.

Table 6: Susceptibility of combined antiseptics solutions against bacterial isolates from burn wounds

Combination of different antiseptics concentration	Bacterial growth
Chlorhexidine digluconate 5% w/v + 1% Silver nitrate 0.01% w/v + Acetic Acid 4%	No growth
Chlorhexidine digluconate 5% w/v + 1% Silver nitrate 0.01% w/v	No growth
Chlorhexidine digluconate 5% w/v + Acetic Acid 4%	No growth
Acetic Acid 4% + 1% Silver nitrate 0.01% w/v	No growth

5.0 Discussion

The aim of this study was to determine the type, concentration, sterility and efficacy of antiseptic solutions used to clean infected burn wounds for patients seeking services at KNH. We noted that in the burns unit of this facility three antiseptic agents were in use: 5% w/v Chlorhexidine digluconate, 4% Acetic acid- vinegar (Zesta) and 1% Silver nitrate 0.01% w/v - Ionic silver solution (Qurion). From the infected wounds we isolated *S. aureus* (48.1%), *Proteus* species (30.9%), *P. aeruginosa* (16%), *Klebsiella* species (1.2%) and other unidentified organisms (3.7%). Previous studies have reported the predominance of some of these bacteria in infected burn wounds where P. *aeruginosa* was the highest species isolated, while others included *E.coli*, *S. aureus* and *Proteus* species (Alkolaibe, Al-ameri, and Alkadasi 2015; Ngugi 2013). Other studies have also shown *S. aureus* and gram-negative bacteria especially *P. aeruginosa* were the commonest cultured agents from infected burn wounds (Gajadhar et al. 2003; Wang et al. 2008).

In this study, the efficacy of antiseptics acetic acid 4% and chlorhexidine digluconate 5% w/v being tested against all isolated bacteria was found when their concentarions are increased gradually. The isolates did show significant susceptibilty to the antiseptics, while 1% Silver nitrate 0.01% recorded low bacteriostatic activity .

Chlorhexidine digluconate 5% w/v was observed to have ability to kill nearly all isolated organisms (80) with susceptibilty of 98.8%. However, as the concentration of chlorhexidine digluconate decreased, the overall susceptibilty of the isolates decreased rapidly, for example 0.07% chlorhexidine was observed to have a susceptibility of 68.0%. Although, there was statistically significant association between 0.07% concentration of chlorhexidine digluconate 5% w/v and the all different organisms isolated (p value= 0.020) , the data showed the growth of organisms can not be inhibited. Furthermore, the data showed there was significant association

between Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of both chlorhexidine digluconate 5% w/v concentrations of 2.5%, 1.25% and 0.625% and the different organisms isolated ($p < 0.05$). Considering that the isolated organisms are also predominantly isolated in hospital infections makes the use of chlorhexidine an important antiseptic/disinfectant to use in hospiatls. Similarly , it has been suggested in a previous study that 4% chlorhexidine gluconate have higher effectiveness on *S. aureus* and *Enterococcus* species and can be used safely in bacteria causing nosocomial infections (Eryılmaz, Akın, and Arıkan Akan 2011). Another study reported, chlorhexidine gluconate is the potent disinfectant against bacteria while high concentration of chlorhexidine cetramide (1g/ml) displayed no efficiency agains all isolated bacteria (Saleh, Naher, and Saad 2012). This was contrary to the finding of Fakhriddeen who arranged disinfactants according to their potency as chlorhexidine cetramide, chlorhexidine gluconate (CHX), PVP-I, chloroxylenol, formaldehayde and H2O2 and he stated chlorhexidine cetramide was the potent antiseptic against bacteria. Therefore, the effectiveness of antiseptic disinfectants can be affected by their formulation (i.e. chlorhexidine cetramide versus chlorhexidine gluconate) and how these solutions are diluted and stored.

This study revealed that in comparison to chlorhexidine digluconate 5% w/v and silver nitrate 0.01% w/v, acetic acid solution is much more effective antiseptic against bacteria isolates infecting burn wounds as it showed 100% bactericidal activity against all the infecting bacterial agents at a concentration 4%. Additionally, the MBC of acetic acid was 2% to the most isolated bacteria proportion of 90.1%. Acetic acid is easily accessible to the general population from outlets like supermarkets and chemists, and it is significantly much cheaper than the other antiseptic agents. Its utilization in wound disinfection in this and other hospital in low resource settings, can ease patient mangement and reduce the economic burden to the patients and their

families. A previous study reported that at a concentartion of 0.5-5% acetic acid showed bacterostatic activity against *P. aeruginosa* with effective removal of the bacteria from the apparent infection site (Nagoba et al. 2013). There have only been a limited number of studies that have investigated reduced susceptibility to chlorhexidine and acetic acid longitudinally. We observed *Proteus* spp. and *P. aeruginosa* were the most resistant bacteria against chlorhexidine digluconate (0.07%) and acetic acid (0.5%) while *S. aureus, Klebsiella* spp. and unidentified organisms were the most susceptible bacteria to chlorhexidine digluconate. However, a previous study showed reduced susceptibility to chlorhexidine against *S. aureus* isolates (Wang et al. 2008).

Further, we established that *S. aureus* was the most resistant bacteria against acetic acid at 0.5% concentration, as opposed to the other bacterial isolates. Previous studies have shown that due to the capacity of surviving in unfavorable environment conditions and its high resistance to antiseptics, *P. aeruginosa and S. aureus* continue to be important pathogens in hospital acquired infections (Alkolaibe, Al-ameri, and Alkadasi 2015). This study has confirmed that *S. aureus*, *Proteu*s spp and *P. aeruginosa* which commonly infect burn wounds are still able to grow when subjected to 1% silver nitrate solution 0.01% w/v. This means, silver nitrate 0.01% w/v at a concentration of 1% has low bacteriostatic activity against these three bacterial organisms.

Only one organism of *Klebsiella* species isolated from infected burn-injured wound was tested against antiseptics and showed high susceptibility to chlorhexidine digluconate 5% w/v, acetic acid and silver nitrate 0.01% w/v, but this does not mean it has high significance level of bactericidal activity since the isolated organism was only one. Furthermore, there were three organisms that we were unable to identify through the process that showed low susceptibility to chlorhexidine digluconate w/v and acetic acid unless their concentration were increased to 5%

and 4% respectively. In addition, there was no minimum inhibitory concentration of chlorhexidine digluconate and acetic acid solutions that inhibit the growth of unidentified organisms because we observed a high to moderate bacterial growth when subjected to concentrations below 5% and 4% respectively. However, they were highly susceptible to 1% silver nitrate 0.01% w/v.

A study reported, the combination of different antiseptics have no clinical advantage due to the concerns relating to potential chemical interactions (Lachapelle et al. 2013). However, the current study shows a clinical efficiency in vitro when 5% chlorhexidine digluconate w/v was combined with either acetic acid/ silver nitrate 0.01% w/v, or acetic acid was combined with silver nitrate 0.01% w/v and/or when all of the three antiseptics being tested was combined together. This means combination of these antiseptics either two or more will have higher susceptibility to kill the growth of all isolated bacteria which will prevail good clinical advantage to infected burn wound if it is constituted in a controlled environment. However, further studies to prove this hypothesis need to be undertaken.

It is clear that microorganisms can adapt to a different concentrations of antiseptic agents used in hospitals consequently resulting in resistance. Therefore, there is an urgent need for well-designed studies directly comparing the clinical and economic profiles of antiseptic agents when used as stand alone or in combination for considerations as a first choice agents in the management of infected burn wounds.

6.0 Conclusion

Staphylococcus aureus and *Proteus species* were the most frequently isolated bacteria infecting the burn wounds. The bactericidal and bacteriostatic activity of the antiseptics solutions utilized at KNH was different depending on the concentration and organism isolated. Four percent of Acetic acid and 5% of chlorhexidine digluconate w/v were found to be more effective both showing bactericidal and bacteriostatic activities while 1% Silver nitrate 0.01% w/v was found to be bacteriostatic. In addition, we also noted bactericidal activity when the antiseptic agents were combined against all the bacteria isolated.

7.0 Recommendation

- With growing concerns about the development of biocidal resistance and cross-resistance with antiseptics, clinical isolates should be under continual surveillance and other possible mechanism of resistance need to be investigated.

- It is crucial to use biocides at appropriate concentrations and carry out subsequent surveillance studies to track resistance or low susceptibility patterns of *S. aureus*, *Proteus* spp. *P. aeruginosa* and *Klebsiella* spp. as well as other hospital acquired infection isolates.

- Our study finding revealed that the 4% acetic acid and 5% chlorhexidine digluconate w/v were the most potent effective antiseptic disinfectant when their concentrations were increased gradually and/or when they were used in combination. They did show bactericidal activities to the all isolated bacteria if either two or all the three antiseptics utilized in the setting were combined together. Therefore, the study recommends:

    - ✓ An increase in the concentration of acetic acid and chlorhexidine digluconate 5% w/v solutions if they are using each one separately. For example, the standard dilutions

used in the setting should be increased from 0.67% concentration of acetic acid (4%) to concentration of 2% and from 0.06% concentration of chlorhexidine digluconate 5% w/v to concentration of 0.625%; which are the lowest concentrations that inhibit bacterial growth (MIC) respectively ($p < 0.05$).

- ✓ Combine acetic acid 4% with chlorhexidine digluconate 5% w/v alone or use combination of chlorhexidine digluconate 5% w/v with acetic acid 4% and 1% Silver nitrate 0.01% w/v or acetic acid 4% and 1% Silver nitrate 0.01% w/v. i.e. each different concentration of these two/three antiseptics mentioned in Table 3 were combined simultaneously (As shown the procedure in Appendix III, figure 3). This promotes significance bactericidal activity 100% against nosocomial isolates and it will be more effective for sterilization pattern in the clinical setting. Therefore, routine disinfection protocols do not need to be alerted in the setting. However, further studies to prove this hypothesis need to be undertaken.

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APPENDICES

APEENDIX I. INFORMATION AND CONSENT FORM

Appendix 1a: Information and Consent Form – ENGLISH

TITLE OF THE STUDY:
THE EFFICACY OF ANTISEPTICS TO BACTERIAL ISOLATES FROM INFECTED BURN WOUND PATIENTS AT KENYATTA NATIONAL HOSPITAL BURNS UNIT

Principal Investigator: Dr. Faisa Salah (MSc Tropical and Infectious Diseases, UON)

Co-Investigators: Ms. Susan Odera (Department of Medical Microbiology, UON)

INTRODUCTION:

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

May I continue? YES / NO

WHAT IS THIS STUDY ABOUT?

The researchers listed above are examining burn patients undergoing treatment at KNH Burns unit. The purpose of the research is to assess the efficacy of antiseptic used in management of burn wounds to bacterial isolates from infected burn wound patients in the ward. Participants in

this research study will be asked to take a pus swab from infected burn wound. There will be approximately 73 participants in this study randomly chosen. We are asking for your consent to consider participating in this study.

WHAT WILL HAPPEN IF YOU DECIDE TO BE IN THIS RESEARCH STUDY?

If you agree to participate in this study, the following things will happen:

Sterile swabs will be used to collect pus specimen from the infected burn wound site. These samples will be cultured to isolate bacteria infected in the burn wounds. Once these bacteria are isolated, it will be used to determine the efficacy of antiseptic to that isolated bacteria infected in burn-injured wound. Any remaining pus specimen will be destroyed. There will be no health consequences of sudden withdrawal from the study. You can stop participating at any time.

ARE THERE ANY RISKS, HARMS DISCOMFORTS ASSOCIATED WITH THIS STUDY?

Medical research has the potential to introduce psychological, social, emotional and physical risks. Effort should always be put in place to minimize the risks. One potential risk of being in the study is the loss of privacy. We will keep everything you tell us as confidential as possible. We will use a code number to identify you in a password-protected computer database and will keep all of our paper records in a locked file cabinet. However, no system of protecting your confidentiality can be secure, so it is still possible that someone could find out you were in this study and could find out information about you.

There will be no risk to the participants, but there is direct involvement of the patient in this study by swabbing pus from infected burn-injured wound patients. It may be embarrassing for you to allow us to take pus from some private parts of your body. We will do everything we can to ensure that this is done in private. Furthermore, all study staff and examiners are professionals with special training in these examinations/interviews.

If any discomfort is experienced during the examination, inform the study staff immediately.

ARE THERE ANY BENEFITS BEING IN THIS STUDY?

You may benefit by receiving free health information about the antiseptic efficacy. We will refer you to a hospital for care and support where necessary. Also, the information you provide will help us better understand the management of burn wounds using the antiseptic. This information is a contribution to science and will aid in the management of burn wounds.

The finding of this study will be communicated to the head of Kenyatta National Hospital burns unit which may help in reducing the occurrence of the resistance of bacteria infected burn-injured wound patient to antiseptics used at KNH burns unit.

WILL BEING IN THIS STUDY COST YOU ANYTHING?

The study will cost you nothing but just 10 – 15 minutes of your time**.**

WILL YOU GET REFUND FOR ANY MONEY SPENT AS PART OF THIS STUDY?

There will be no refund as no expense will be involved in participating in this study.

WHAT IF YOU HAVE QUESTIONS IN FUTURE?

If you have further questions or concerns about participating in this study, please call or send a text message to the study staff Faisa Salah 0721 227002.

For more information about your rights as a research participant, you may contact the Secretary/Chairperson, Kenyatta National Hospital-University of Nairobi Ethics and Research Committee Telephone No. 2726300 Ext. 44102 email uonknh_erc@uonbi.ac.ke.

The study staff will pay you back for your charges to these numbers if the call is for study-related communication.

WHAT ARE YOUR OTHER CHOICES? Your decision to participate in research is voluntary. You are free to decline participation in the study, and you can withdraw from the study at any time without injustice or loss of any benefits such as care and treatment needed

CONSENT FORM (STATEMENT OF CONSENT)

Participant's statement

I have read this consent form or had the information read to me. I have had the chance to discuss this research study with study staff. I have had my questions answered in a language that I understand. The risks and benefits have been explained to me. I understand that my participation in this study is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study. I also give permission for my sample and achieved isolates to be used for further research,

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

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

I agree to participate in this research study: **Yes** **No**

I agree the isolates from my wounds to be stored and used for Further research **Yes** **No**

Participant printed name: _____

Participant signature / Thumb stamp _____ Date _____

Researcher's Statement

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

Researcher 's Name: _____ **Sign: _____ Date**: _____

Role in the study: _____

Witness (*If witness is necessary, a witness is a person mutually acceptable to both the researcher and participant*)

Name _____ Contact information _____

Signature /Thumb stamp: _____ Date; _____

APEENDIX 1b: INFORMATION CONSENT FORM– SWAHILI
MAELEZO KUHUSU UTAFITI/WARAKA WA IDHINI

Maarifa, Tabia na Mazoezi ya Uchangaji wa Watoto kati ya mama mchanga katika Vitogoji duni jijini Nairobi

Mtafiti mkuu: Dkt. Faisa Salah (Chuo Kikuu cha Nairobi)

Watafiti weza: Madam Susan Odera (Chuo Kikuu cha Nairobi)

UTANGULIZI

Ningependa kukueleza juu ya utafiti unaofanywa na watafiti waliotajwa hapo juu. Madhumuni ya fomu hii ya idhini ni kukupa maelezo unayohitaji ili kukusaidia uamuzi ikiwa Utahusishwa kwa utafiti huu au la. Jisikie huru kuuliza maswali yoyote kuhusu madhumuni ya utafiti, kinachotokea ikiwa unashiriki katika utafiti, hatari na faida iwezekanavyo, haki zako kama kujitolea, na kitu kingine chochote kuhusu utafiti au fomu hii ambayo haijulikani. Tunapojibu maswali yako yote kwa kuridhika kwako, unaweza kuamua kuwa katika utafiti au la. Utaratibu huu unaitwa 'kibali cha habari'. Mara unapoelewa na kukubali kuwa katika utafiti, nitakuomba kusaini jina lako kwenye fomu hii. Unapaswa kuelewa kanuni za jumla ambazo zinatumika kwa washiriki wote katika utafiti wa matibabu: i) Uamuzi wako wa kushiriki ni kikamilifu kwa hiari ii) Unaweza kujiondoa kwenye utafiti wakati wowote bila ya kutoa sababu ya uondoaji wako iii) Kukataa kushiriki katika utafiti hauathiri huduma unazostahili kwenye kituo hiki cha afya au vifaa vingine. Tutakupa nakala ya fomu hii kwa rekodi zako.

Naweza kuendelea? NDIO/LA

UTAFITI HUU UNAUSU NINI?

Mtafiti aliotajwa hapo juu atawaoji akina mama wachanga. Lengo la utafiti ni kuhusu dawa ya kusafisha vidonda vya mwili kuhusiana na moto. Karibu wagonjwa 70 walio na vidonda za miguu waliochaguliwa kwa nasibu watashiriki katika utafiti huu. Tunaomba ridhaa yako kufikiria kushiriki katika utafiti huu.

NI NINI KITAKACHO FANYIKA UKIAMUA KUHUSIKA KWA UTAFITI HUU?

Ikiwa unakubali kushiriki katika utafiti huu, mambo yafuatayo yatatokea:

Utashughulikiwa na mhojiwaji mwenye mafunzo katika eneo la kibinafsi ambako unajisikia kujibu maswali. Mahojiano itaendelea dakika takriban tano ama dakika kumi.

Baada ya mahojiano, atakupea mafunzo kuhusu chanjo zinazo idhinishwa na shirika la chanjo.

KUNA MADHARA YOYOTE YANAYOTOKANA NA UTAFITI HUU?

Utafiti wa matibabu una uwezo wa kuanzisha hatari za kisaikolojia, kijamii, kihisia na kimwili. Jitihada zinapaswa kuwekwa daima ili kupunguza hatari. Hatari moja ya kuwa katika utafiti ni kupoteza faragha. Tutaweka kila kitu unachotuambia kama siri iwezekanavyo. Tutatumia namba ya nambari ili kukutambua kwenye darasani ya kompyuta iliyohifadhiwa na nenosiri na tutahifadhi rekodi zote za karatasi kwenye baraza la mawaziri lililofungwa. Hata hivyo, hakuna mfumo wa kulinda siri yako inaweza kuwa salama kabisa, kwa hiyo bado inawezekana kwamba mtu anaweza kujua wewe ulikuwa katika utafiti huu na anaweza kupata habari kukuhusu.

Pia, kujibu maswali katika mahojiano inaweza kuwa na wasiwasi kwako. Ikiwa kuna maswali yoyote utaki kujibu, unaweza kuruka. Una haki ya kukataa mahojiano au maswali yoyote yaliyoulizwa wakati wa mahojiano.

Inaweza kuwa aibu kwa wewe kutoa maelezo ya kibinafsi. Tutafanya kila kitu tunaweza kuhakikisha kuwa hii imefanywa kwa faragha. Zaidi ya hayo, wafanyakazi wote wa utafiti ni wataalamu wenye mafunzo maalum katika mitihani/mahojiano haya.

Unaweza kujisikia wasiwasi wakati wa mahojiano, mwambie mtafiti.

KUNA MANUFAA YOYOTE KWA KUHUSIKA KWA UTAFITI HUU?

Manufaa ya utafiti huu si ya moja kwa moja kwa mtu binafsi, ila itawezesha kujua kama dawa hii ina manufaa kwa vidonda vya moto. Taarifa hii ni mchango kwa sayansi na msaada katika kuelimisha kina mama wachanga kuhusu chanjo na magonjwa yanayo zuiwa na chanjo.

KUHUSIKA KWA UTAFITI HUU KUTAGHARIMIA CHOCHOTE?

Kujihusisha na utafiti huu hautakugarimu chochote il muda wako kama dakiki kumi hadi kumi na tano.

UTAPATA MALIPO YOYOTE AU FIDIA?

Hakuna malipo au fidia yoyote kwa kujiusisha na utafiti huu

UKITAKA KUULIZA SWALI BAADAYE KUHUSU UTAFITI HUU?

Wasiliana na Mtafiti mkuu, Dkt. Faisa Sala kwa nambari ya simu: +254 721 227002. Ama mwenyekiti au katibu msimamizi, utafiti, Hospitali ya Kitaifa ya Kenyatta na Chuo kikuu cha Nairob kupitia nambari 2726300/44102; au kwa anuani uonknh_erc@uonbi.ac.ke. Watafiti watakurejeshea pesa zilizotumika kwa mawasiliano kuhusu utafiti huu

HUNA HIARI GANI?

Uamuzi wako wa kushiriki katika utafiti ni wa hiari. Una uhuru wa kushiriki katika utafiti na unaweza kujiondoa kwenye utafiti wakati wowote bila mateso yoyote mabaya. Utaendelea kupata huduma na matibabu zinahitajika hata kama hutaki kushiriki katika utafiti huu.

IDHINI

Nimesoma au kusomewa waraka huu na nimweulewa kabisa. Nimepata nafasi ya kujadiliana na mtafiti na akajibu maswali yangu kwa lugha ninayoelewa. Nimearifiwa kuhusu faida na madhara ya utafiti huu na kwamba nitapewa nakala ya waraka huu baada ya kutia sahihi. Pia naelewa kuwa nahusika kwa hiari yangu na ninaweza kujitoa kwa utafiti huu wakati wowote. Pia nimepeana kibali kutumia tarakibu kwa utafiti wa kisansi na teknolojia.

Kwa kusaini fomu hii ya kibali, sijaacha haki yoyote ya kisheria niliyoshiriki katika utafiti huu.

Nakubali kushiriki katika utafiti huu:	Ndio	La
Nakubali sampuli zangu zitumika kwa utafiti zaidi	Ndio	La

Jina la kuchapishwa la Mshiriki:_____

Sahihi ya Mshiriki: _____ Tarehe:_____

KAULI YA MTAFITI

Nimemueleza mhusika taarifa zinazofaa kuhus utafiti huu na naamini kuwa ameelewa vyema na kukubali kuhusika kwa hiari yake.

JINA:_____**TAREHE**:_____**SAHIHI**:_____

UKUMU LAKO KWA UTFITI HUU:_____

SHAHIDI (*Ikiwa atahitajika kama vile kutasfiri)*_____

Sahihi:_____ Tarehe:_____

Appendix 2: Standard Operating Procedure (SOP) for Efficacy of Antiseptics to Bacterial Isolates

1.0 Purposes:

This SOP describes procedures followed for the study protocol (Efficacy of antiseptics to bacterial isolates from the patients with infected burn wound at KNH burns unit).

2.0 Scope:

This procedure applies to Quality Assurance Protocol for Microbiological Culture and Sensitivity at University of Nairobi Department of Medical Microbiology.

3.0 Responsibility:

All individuals who are involved in research, including but not limited to:

3.1 Laboratory technologies

3.2 Medical doctor

3.3 Nurse

3.4 Medical Microbiologist

4.0 Safety

4.1For safety measures use Personal Protective Equipment

4.2 All contaminated materials must be disposed into appropriate biohazard bags/containers

4.3 No contaminated waste should be thrown on the floor

4.4 Do not eat or smoke while collecting samples

5.0 Equipment, Materials and Reagents

5.1 Equipment and Materials:

5.1.1 Leak proof standard transportation container

5.1.2 Sterile sample collection swab

5.1.3 Clinical waste dustbin

5.1. 4 Well-fitting non-sterile latex gloves

5.1.5 Sanitizer/Hand washing soap and clean running water disposable tourniquets

6.0 Procedure

The patients will be informed on the sample collection from the research purposes

6.1.1 Assemble the required equipment and materials for sample collection

6.1.2 Put on the non-sterile latex gloves

6.1.3 Identify the staff to perform the pus swab procedure

6.1.4 Inform the staff about the risk and benefits of the study

6.1.5 Inform the patients risk and benefits of the study

6.1.6 Randomly collect the sample by swabbing the burn wound infected patients using sterile swab

6.1.7 The sample will be labelled a unique code (Wound/BU/RM1/01)

6.1.8 After the wound of the patient has been cleaned with normal saline, collect the sample using a sterile cotton swab by swabbing entire surface of the wound once

6.1.9 Return the swab into the container

6.1.10 The procedure shall be continued until enough sample size obtained

6.1.11 Dispose the gloves into the yellow or red coded bin

6.1.12 Transport the samples within two hours using a labelled col box

to Medical Microbiology Laboratory, UoN for microbiologist.

7.0 Quality Control

In order to ensure and maintain good quality control the following should be observed

7.1.1 Do not use expired and no-sterile swabs

7,1.2 Follow the order of sample collection

7.1.3 Transport to the laboratory within two hours.

8.0 References:

8.1 Ohio state Medical Centre, Sample collection procedure

8.2 Layola University Medical Centre anatomic pathology/clinical laboratories sample

8.3 University of Nairobi Department of Medical Microbiology Quality Assurance Protocol for Microbiological Culture and Sensitivity

Appendix 3: Figures

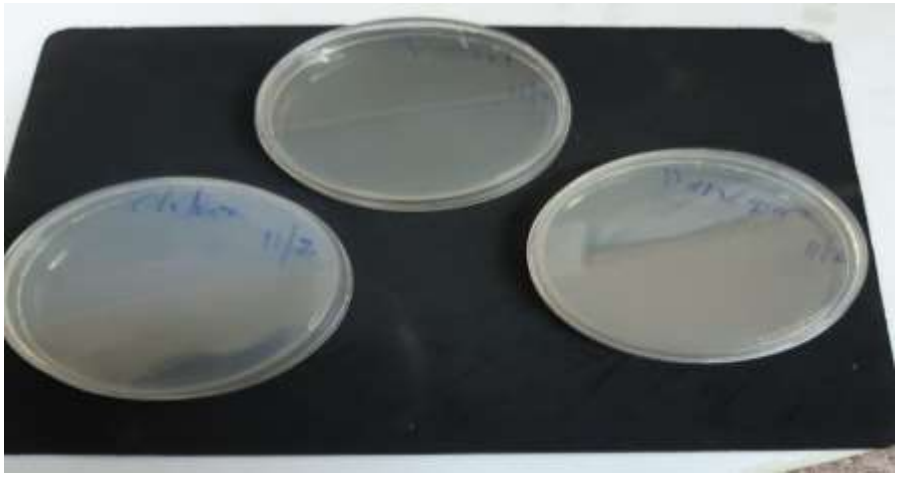

Figure 2: Shows no bacterial growth in the plates when three antiseptic solutions used in the study were tested their sterility from microorganisms; ***(a)*** Right: Chlorohexidine. ***(b)*** Top: Silver nitrate 0.01% (Quiron) and ***(c)*** Left: Acetic acid solution.

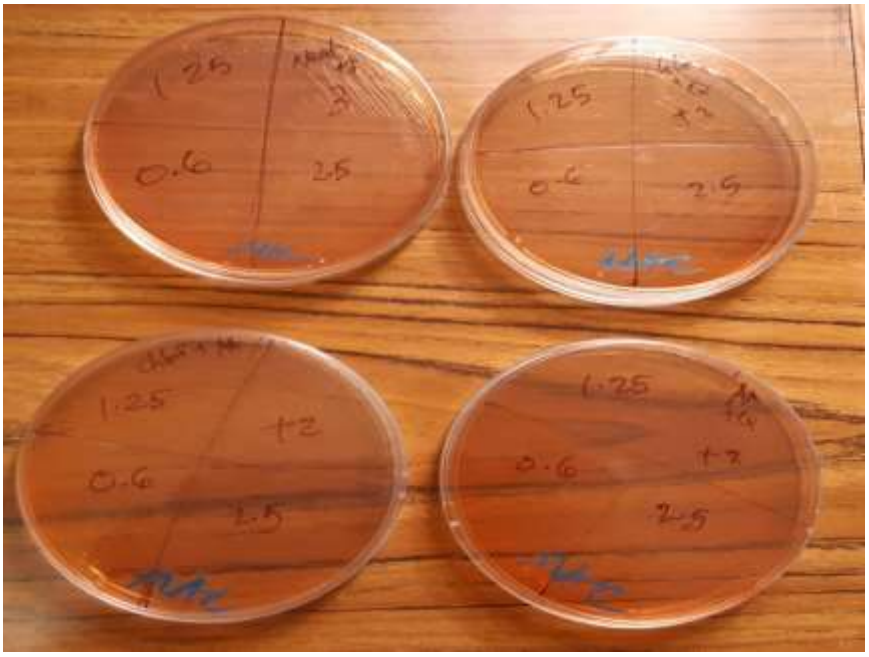

Figure 3: When all three antiseptic solutions being tested in this study were combined together have shown no bacterial growth in the plates. i.e. (a) Chlorohexidine was combined with Acetic acid and Silver nitrate 0.01% (Qurion). (b) Chlorohexidine was combined with Silver nitrate 0.01% (Qurion). (c) Chlorohexidine was combined with Acetic acid, (d) Acetic acid was combined with Silver nitrate 0.01% (Qurion).

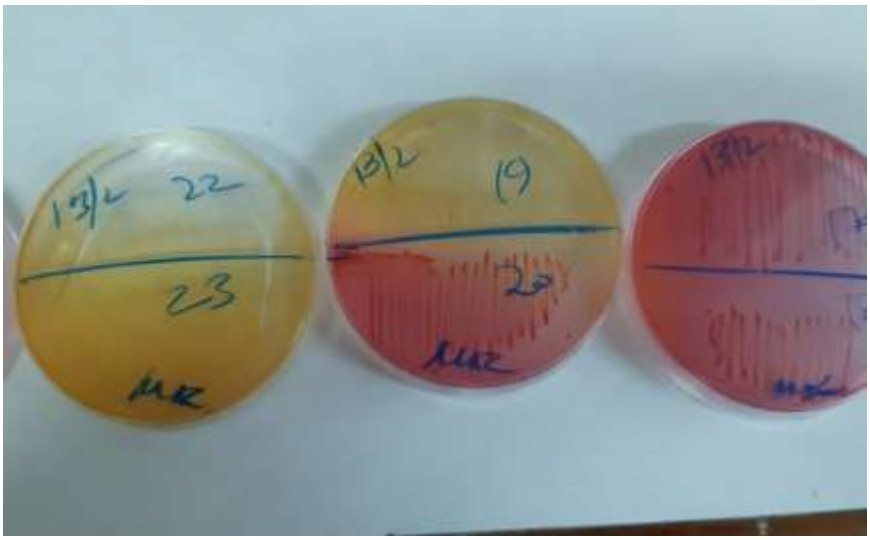

Figure 4: Shows colony of bacterial growth isolated from different patients**;**

(a) *Proteus* species and *P. aeruginosa.* **(b**) *P. aeruginosa* and *S. aureus.* **(c)** *S. aureus*

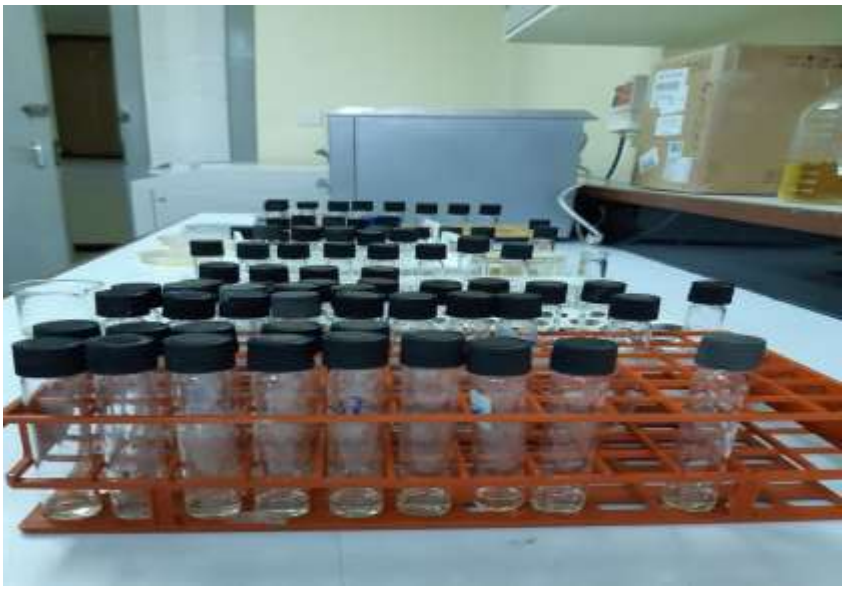

Figure 5: Quantitative Serial Dilution Technique

Appendix 4: Pictures of Burn wound Patients

Figure 6: Patients presenting with burn wound infection; Second and Third degree burns