Antimicrobial Activity of Plain and Medicated Soaps on Sale in Nairobi on Selected Microorganisms

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ABBREVIATIONS

ATCC	American Type Culture Collection
CLSI	Clinical Laboratory Standard Institute
ERC	Ethics and Research Committee
FDA	Food and Drug Administration
IZD	Inhibition Zone Diameters
KNH	Kenyatta National Hospital
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
N	Negative
^{o}C	Degrees centigrade
P	Positive
SPSS	Statistical Package for the Social Sciences
SOPs	Standard Operating Procedures
SDA	Sabouraud Dextrose Agar
SDB	Sabouraud Dextrose Broth
TCS	Triclosan
TCC	Triclocarban
UoN	University of Nairobi
US	United States
W H O	World Health Organization
Wt./vol	Weight per volume

DECLARATION

I declare that this dissertation is my original work and has, to the best of my knowledge, not been presented for a degree in any other university.

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DEDICATION

I dedicate this work to my husband Justus who has encouraged me all the way and whose encouragement has made sure that I give it all it takes to finish which I have started. To my children Lynn, Laura, Lonnex and Lenny who have been affected in every way possible by this quest. Thank you, my love for you all can never be quantified. God bless you all.

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ABSTRACT

Background: Soaps are made of fats and oils as the main ingredients and other components such as soda ash, salts and detergents. The main function of soap is cleaning, killing and removing microorganisms. Some soap additionally has antibiotics and are thus referred to as medicated soaps. Such soaps tend to be more expensive than the plain soaps. However, there is insufficient information on the enhanced efficacy of medicated soaps compared to plain soaps in Kenya.

Objectives: This study aimed at comparing the antimicrobial effects between medicated and plain soaps against selected microorganisms.

Materials and methods: This was an experimental laboratory-based study. Purposive sampling method was used to collect seven brands of medicated and seven brands of plain soaps present in the market. The samples were delivered to the Department of Medical Microbiology - University of Nairobi and were coded as either medicated (MS001 to MS007) or non-medicated (NMS001 to NMS007) before processing. Approximately, 2g of each soap sample was weighed into universal bottles, dissolved in sterile distilled water and tested for antimicrobial activities against selected pathogenic microorganisms of the species; *Pseudomonas aeruginosa, E. coli, Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus capitis, Streptococcus pneumonia* and *Candida albicans*. Diameter zones of inhibition, minimum inhibition concentration and bactericidal concentrations were compared. Analysis of variance and Fisher's exact test were utilized to analyze the antimicrobial activities of the soap samples against the test microbes and the significance considered at 95% confidence interval ($p<0.05$).

Results: All 14 (100%) of the soap brands were effective against *P. aeruginosa* with average inhibitory zone diameters of 9.1mm for non-medicated soaps and 9.0 mm for medicated soaps. Three medicated soaps (MS005, MS006 & MS007) and one non-medicated soap (NMS007)

were effective against *S. pneumonia*, *S. aureus*, *S. epidermidis* and *P. aeruginosa*. In terms of minimum bactericidal concentrations and minimum inhibitory concentrations, medicated soap 005 and 007 had low concentrations hence highly active against *S. aureus* with minimum bactericidal concentration of 62.5mg/ml and minimum inhibitory concentration of 31.25mg/ml, respectively. Non-medicated soap NMS007 was low hence active against *S. aureus, E. coli,* and *C. albicans* with MBC of 62.5mg/ml and MIC of 31.25mg/ml respectively. One non-medicated soap's antimicrobial activity was comparable to that of medicated/antiseptic soaps. There was significant association between the MIC and MBC of medicated and non-medicated soaps tested in the present study ($P \leq 005$).

Conclusions and recommendations: The findings of the present study suggest that medicated soaps are better antimicrobial agents compared to non-medicated soaps. However, some non-medicated soaps such as brand NMS007 have more antimicrobial activities as indicated by inhibition zone diameters, MBC and MIC compared to some medicated soaps. The choice of soap should be that which is effective against disease causing bacteria at low concentrations. The public should be encouraged to use effective non-medicated soaps such as NMS007 since it was widely effective in low concentrations against pathogenic microorganisms.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Soaps are cleansing products made of either animal or plant fats and oils. Some soaps can dissolve in water while others are insoluble. Soaps are formed through a process called saponification where fats and oils interact with an alkaline material preferably sodium or potassium crystals (Boyce and Pittet, 2002). Soaps are formulated differently depending on their intended purpose. They can as such be categorized as plain soaps, also called toilet soaps, or can have additional ingredient which are intended to kill bacteria and thus referred to as antibacterial (medicated/antiseptic) soaps (Obi, 2014). Medicated soaps should be able to inhibit or kill disease causing pathogens and other microorganisms whereas plain soaps are normally utilized for cleaning work (Maany *et al.*, 2015).

The main purpose of soap is to clean and, secondly, to kill and remove germs including bacteria. It is reported that soaps can remove sixty five to eighty five percent of bacterial organisms on a person's skin (Bhat *et al.*, 2011). Bacteria are found almost everywhere in the environment including animal body, plants, water, sewer and soil. Those found in the human body as normal flora are important in terms of health (Nostro *et al.*, 2007). Some bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* from the environment are deposited on the skin of human beings and can cause illness (Byarugaba, 2009). Health care workers can also spread bacteria, especially the opportunistic ones, from sick patients to healthy individuals in the hospital set up. They can also contaminate themselves, their family members and the environment (Ozdemir and Dizbay, 2015). Hand hygiene has been shown to reduce transfer of disease-causing agents and pathogens from one person to another (Riaz *et al.*, 2009). Washing hands using clean water and soap is therefore an important procedure for health care workers in order to prevent transmission

of bacteria. Food handlers in restaurants and homes as well as those who deliver the food should practice hand washing and proper hygiene (Fox and Harvey, 2008).

Due to the added ingredients to the medicated soaps, they are able to have more bactericidal activities compared to the plain/toilet soaps (Maany *et al*., 2015). Many cleaning materials and agents such as p-chloro-m-xylenol (PCMX/ chloroxylenol), trichloro carbamide (TCC) and triclosan (TCS) are found in the markets in different forms and formulations. They are the usual ingredients in medicated soaps but are usually added to soaps for preservation purposes (Poole, 2002). However, infections are still widespread despite the use of the antiseptic or medicated soaps (Yueh *et al.*, 2012). Poor hygienic conditions and widespread food related disease has not declined with the advent of medicated soaps. The reason could be that these medicated soaps have inadequate ingredients to kill the microbial agents or they could be devoid of antiseptic agents. The present study was carried out in order to shed light on this aspect. When bacteria are exposed to low levels of antimicrobials, inadequate to kill them, the resultant selection pressure favors resistant strains that end up occupying the niche. Hence the bacteria may develop drug resistant strains due to prolonged exposure. A similar phenomenon can occur due to widespread use of medicated soaps with inadequate levels of antimicrobials. The present study aimed at determining the antibacterial effects of antiseptic/medicated and plain soaps in Nairobi, Kenya.

1.2 Statement of the problem

Generally, people use antiseptic soap in order to protect themselves from harmful microorganisms on the skin and other surfaces. By so doing, they expose the normal flora to antimicrobial agents thus there is the risk of development of drug resistant strains of pathogenic microorganism within

the human skin and the environment. For decades, human beings have used plain soaps and water for cleaning purposes and currently there is no documented evidence that they do not work or have lost their potency. Washing our bodies with plain soap and water primarily protect us from bacteria and other pathogenic microbes including viruses and fungi. However, many individuals believe that antiseptic soaps are superior in controlling infectious agents (Poole *et al.*, 2008). Triclosan (TCS) is a bactericidal agent that is used in medicated soaps, plastic materials, first aid products and tooth paste among other products (Allmyr *et al.*, 2009). It is thought that its frequent widespread use may lead to development of drug resistance strains among the pathogens thus exacerbating the problem of microbial infections. Emergence of drug resistance may also be due to exposure of the microbes to low concentrations of the bactericidal agents. Currently, there is an increase in infectious diseases related to food and unhygienic environment despite the widespread use of medicated soaps (National Disease Surveillance Centre, 2004). The medicated soaps could be exposing microbial pathogens to inadequate antimicrobial concentrations hence may contribute in spreading antimicrobial resistant disease-causing micro-organisms to human and the environment. In Kenya, medicated soaps are expensive compared to plain soaps but owing to vigorous advertisement strategies as well as their perceived antimicrobial effects they are being widely used yet there is lack of information on their relative efficacy and potential of emergence of resistance (Chepsergon, 2012).

1.3 Justification

Soaps and detergents help in cleaning, removing and killing microbes attached to cloths, skin and other materials. When the body is scrubbed during washing, bacteria are removed leading to general reduction of the prevalence and incidences of skin infections. It is considered that medicated soap reduces the incidence of diseases but some studies have shown that medicated

soap may assist in the spread of drug resistant microbes (Poole *et al.*, 2008). Currently, there is an increase in communicable diseases related to food and unhygienic environment despite the widespread use of medicated soaps (National Disease Surveillance Centre, 2004). In this regard, there is a need for studies to find a solution to the problem. Understanding the problem will go a long way in preventing the spread of infectious disease. Testing different soaps solutions on different pathogenic strains of bacteria will provide information on their effectiveness in inhibiting or killing pathogenic microbes. It is anticipated that the study findings will fill the gap on the role played by medicated and plain soaps in terms of antimicrobial activities against pathogenic microorganisms. The study findings can inform policy and also may aid in the establishment of new policies to guide the use of antiseptic and plain soaps in Kenya. The present study will contribute to this by generating information on comparative antimicrobial activities of plain and medicated soaps available in the Kenyan market. In addition, it will contribute to the existing pool of knowledge regarding the efficacy of soaps in Kenya.

1.4 Research hypothesis

There is no difference in antimicrobial activities of plain and medicated soaps on sale in Nairobi on selected microorganisms.

1.5 Research Questions

1. How are antimicrobial activity of medicated soaps on selected microorganisms?
2. How are antimicrobial activity of plain soaps on selected microorganisms?
3. What is the comparison of antimicrobial activity of plain soaps with that of medicated soaps on selected microorganism?

1.6 Objectives

1.6.1 Broad objective

To compare antimicrobial activity of plain and medicated soaps on sale in Nairobi on selected microorganisms.

1.6.2 Specific objectives

1. To determine antimicrobial activity of medicated soaps on selected microorganisms
2. To determine antimicrobial activity of plain soaps on selected microorganisms
3. To compare the antimicrobial activity of plain soaps with that of medicated soaps on selected microorganism

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

Soaps and other hand washing agents have been in use for many years. These agents are used to clean the hands in order to remove dirt. Some of the hand washing agents contain antiseptic properties which kill microorganisms. Soaps are salts made through saponification of free fatty acids. During saponification, alkaline/bases reacts with fatty acids in the presence of oils or fats to form a soap base. Afterwards other substances and products are incorporated to produce different types of soaps and detergents that are present in the market. The different types of soaps available in the market are used for cleaning purpose such as bathing and cleaning surfaces (Padsalgi *et al.*, 2008).

2.2 General hand sanitation

Cleaning can be defined as the process of removing dirt, microbial flora or any other matter without necessarily killing. Cleaning of hands is a very important measure in curbing the spread of communicable disease in both the health care environment (hospitals) and the community (Boyce and Pittet, 2002). Though many research studies have been conducted on the effectiveness these products used as antiseptic agents for hand hygiene in hospital set up, a few have been studied at community levels in the society (Boyce and Pittet, 2002). Studies comparing the effectiveness of both medicated and toilet/plain soaps have reported that there were no significant association between the microbial agents studied and diseases (Luby *et al.*, 2002). Such studies have not been carried out in Kenya especially in Nairobi County.

2.3 Types of hand washing agents

2.3.1 Toilet/Plain soap

Washing hands with common soap also referred to as plain or toilet soap assists in suspending germs which are removed when rinsed with water. The usual toilet/plain bar soap, liquid soap or powdered soap are composed of detergents with strong surface tensions which hold on to and suspend microbial flora and dirt (Painter and Hoekstra, 2017).

2.3.2 Antimicrobial soap

Medicated soaps also called antimicrobial/antiseptic soaps do both the cleaning of dirt and killing microorganisms. Antiseptic ingredients in the soap such as Triclosan, Triclocarban, Chlorhexidine, Hexachlorophene and iodine generally continue to reduce microbial flora on the skin after washing hands (Larson, 2001).

2.3.3 Hand rubs

These are agents used without water application and are able to kill microbial flora present in the hands because they contain disinfectants. One is required to apply a thin layer (about 3.5mL) on the hand followed by rubbing till it dries up. Antiseptic hand rubs contain alcohol in the form of ethanol, isopropanol or n-propanolol either singly or in combination as the main ingredients (Padsalgi *et al.,* 2008). Hand rubs are present in different concentrations and blends with traces of antimicrobials/antiseptics which kill bacteria and other micro-organisms by denaturing their proteins. However, as they are not able to remove organic materials they cannot be used when the hands are dirty.

2.4 Triclosan

Triclosan has been utilized world over in preparation of medicated soaps. Hence it is basically the main active ingredient in most medicated soaps especially the liquid preparation and has been utilized in most hygienic products in the U.S.A since the 1960s (Yueh *et al.*, 2012). It is an antimicrobial phenoxyphenol agent sold as an "antibacterial" component in hygiene products but it also has antifungal as well as antiviral properties (Yu *et al*., 2010). It is bacteriostatic at low concentrations and bactericidal at higher concentrations (Ernst-moritz-arndt, 2004). Studies have shown that triclosan can inhibit both gram positive and negative bacterial flora and has varying efficacies depending on the type and species of bacteria (Yu *et al*., 2010). For instance, triclosan is generally successful in preventing the growth of gram-negative bacteria such as *Serratia marcescens* and *Pseudomonas aeruginosa* (Yu *et al*., 2010). Triclosan functions by inhibition of definite targets of bacteria called enoyl-acyl carrier protein reductase (Heath *et al.*, 1998; Murry and Dermott, 1999). The chemical isoniazid also utilizes these bacterial biosynthetic fatty acids pathway targets (Murry and Dermott, 1999).

The first study to compare the pros and cons of utilizing soap containing triclosan in a community set up was conducted by (Aiello *et al*., 2007) and reported that products which contain this chemical, in its original formulation in the market, neither killed pathogenic organisms nor reduced the incidence and signs of infections that can be passed from one person to another through contaminated hands. Its efficacy was comparable to that of plain/toilet soap in most of the studies reviewed (Boyce and Pittet, 2002; Luby *et al.*, 2002). The only difference in efficacy was when used at high concentration and long hand washing (i.e., $\geqslant$1.0% wt./vol). Concerning risks related to triclosan, many studies have proved that there is an association between the contacts of

microorganisms in the lab with increasing minimum inhibition concentration to drugs utilized in the hospital. On the contrary, there was no evidence of resistance to antimicrobials used in the community setting with home utilization of cleaning agents containing Triclosan (Aiello *et al*., 2007). These results have elicited interest in determining whether triclosan may lead to development of drug resistant microorganisms (Aiello *et al.*, 2005).

2.5 Risks and benefits of antiseptic product

The US Food and Drugs Administration have deliberated on the disadvantages and advantages of antimicrobial products, including medicated soap, marketed to the public. This has resulted in the call for studies on some antimicrobial products consumed at the community set up. Globally, more emphasis has been directed to the use of antiseptic soaps containing triclosan as the active component. Most liquid soaps marketed to the public as medicated or antibacterial contain Triclosan at 0.1% and 0.45% weight/volume (wt./vol) concentrations while bar soaps contain triclocarban as an active ingredient (Chuanchuen *et al.*, 2001; Yueh *et al.*, 2012) conducted experiments to determine the benefits and risks of using medicated soaps versus the plain soaps. They found no differences between the use of toilet/plain soap and medicated soap/antimicrobial. These two soaps yielded similar results when used under normal circumstances. It was noted that consumer products containing triclosan when used for a long time may lead to the development of resistance to triclosan and other antibiotics among the bacteria and thus emerging of pathogens/microbes resistant to antibiotics in the environment. In support of this, (Aiello *et al.*, 2005) showed a link between the usage of strains resistant medicated soap and to antibiotics among the microorganisms. Hypothetically, incorporating antibiotics into products used by the public could lead to development of antibiotic resistant strains among the bacterial strains. These strains

of bacteria could potentially become resistant to chemotherapeutic drugs as well. This could aggravate the plight of clinical drug resistance thus exacerbating the problem of drug resistance.

2.6 Test organisms in the study

The isolates are both clinical and reference strains from American type culture collection (ATCC) belonging to the genera *Escherichia, Candida, Pseudomonas, Streptococcus* and *Staphylococcus*. Among the bacterial pathogens, *S. aureus* and *E. coli* are the most important source of a range of human and animal infections (Tanih *et al.*, 2015). *Staphylococcus aureus* causes several infections including soft tissues, skin, bone and joint infections as well as surgical site infections (Bachir and Abouni, 2015). *Escherichia coli* cause cellulitis, a spreading severe illness of the skin which extends more intensely than erysipelas into the subcutaneous tissues (Tanih *et al.*, 2015). A study done on the virulence factor profile of *E. coli* recovered from soft tissue and skin infections showed that these strains of pathogens exhibited a notable virulence. The virulence factors were similar to that of *E. coli* strains recovered from infections of the urinary tract. It was also similar to E. coli isolated from cases of blood stream infection (bacteraemia) and in all cases, the entry was through the skin (cutaneous) (Sunder *et al.*, 2017). *Pseudomonas aeruginosa* is normally a bacteria that rarely causes disease in healthy individuals/persons and for an infection to take place, some disturbance of the physical barriers such as the skin, mucus linings and membranes has to occur hence opportunistic pathogen (Molecolare and Microbiologia, 2005). Candidiasis is a disease caused by *Candida albicans*. This yeast usually inhabits the skin, intestines and the vaginal canal, although it does not cause disease. Conversely, it can build up into an infection generally of the vaginal, mouth, skin leading to white or red patches, irritation and itching (Hani *et al.*, 2015).

2.7 Disc diffusion method

This is a method of inoculating agar plates with inoculum of the microorganisms to be tested which has been standardized. Discs made of filter paper of diameter 6mm having various concentrations of soap are placed on the surface of the agar. The petri dishes are then incubated at appropriate conditions and the antimicrobial agent which is the soap at various concentrations will diffuse and inhibit the development and growth of the microorganisms under the test. The inhibition diameter of the growth zones is then measured (Hudzicki, 2012).

The inhibition of growing pattern of the isolates shows the varying ability of the bacteria to resist the antimicrobial result of the soaps. These differences could be owing to changes in the nature and structures of the cell wall of the bacteria because it is the definitive target of the antimicrobial agent or antiseptic. The active component in the soap is what separates the antimicrobial agents. The dynamic antimicrobial components in most of the considered soaps are triclosan and triclocarbanide whose function is to denature cell activity and interfere with microbial absorption. This mainly depends on some properties of test organism, the time of exposure and the composition of the soap concentration (Obi, 2014).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

Nairobi is the biggest city in Kenya and headquarters of the National Government. Most soap manufacturing companies are found in Nairobi hence retailers in the city stock variety of the soap brands due to their proximity to the manufacturing firms. Nairobi County is one of the 47 counties in the country. Appendix 1 shows the map of the study area. Geographically, the city is the smallest of all the counties in Kenya but it is the most populated (Awino, 2014).

3.2 Study design

This research used a cross sectional laboratory-based experimental design. The soap samples were bought from various randomly selected vendors in Nairobi city. Samples of seven plain and seven medicated soaps were collected in May and June 2017 a period of two months.

3.3 Sampling method

The soaps were divided into two strata namely; plain and medicated soaps and collected purposively. Since medicated soaps brands were relatively few in the market, convenient sampling method was used to collect all seven (7) brands of medicated soaps available in the market and 21 plain soap brands according to a reconnaissance survey done prior to the study which was used as a sampling frame (Appendix 9). Plain soap brands were more than medicated soaps brands. Since the study was comparative, equal number of plain soaps were randomly sampled totaling to 14 different types of soaps (7 medicated and 7 plain soap brands). This was arrived at because this was the most suitable sampling method for the study (Meissner *et al.*, 2011). Only bar soaps were sampled because they were the most available and widely distributed in the study area. Liquids

and powder soaps were excluded because they were not widely distributed in all the outlets. The soaps were coded and given numbers as shown in Table 3.1

Table 3.1: List of plain and medicated soaps sampled

	Plain soaps		**Medicated soaps**
1.	NMS 01	1.	MS 01
2.	NMS 02	2.	MS 02
3.	NMS 03	3.	MS 03
4.	NMS 04	4.	MS 04
5.	NMS 05	5.	MS 05
6.	NMS 06	6.	MS 06
7.	NMS 07	7.	MS 07

Key: NMS-Non-Medicated Soaps, MS- Medicated Soaps

3.4 Collection of soaps

The different brands of medicated and plain soaps were purchased from supermarkets/ shops in their original packaging and transported to the department of Medical Microbiology Laboratory-University of Nairobi for processing. The samples were coded appropriately for analysis. In total 7 samples of different medicated soap brands and 7 plain soap brands were collected. The type, ingredients and the source of soap collected were recorded accordingly.

3.4.1 Soaps ingredients

There following were some of the common ingredients which were incorporated to both medicated and plain soap. They included: Sodium palmate, sodium palm kernelate, glycerin, perfume, sodium

sulfonate, tetra sodium EDTA, Aqua, Etidronic acid and sodium chloride. Triclocarban, triclosan and terpineol thymol was found to be the most commonly used antibacterial agent in medicated soaps.

3.5 Test organisms

Gram positive and negative bacterial isolates and yeast associated with skin infections were tested against the different brands of soaps. Clinical isolates and standard strains of *Pseudomonas aeruginosa, E. coli, Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus capitis, Streptococcus pneumonia* and *Candida albicans* were used. Isolates stocked at the University of Nairobi microbiology teaching laboratory and clinical isolates were used. The isolates are both clinical and ATCC (Horton, 2017) .

3.6 Preparation of soap sample discs

Two grams of each sample of soap was dissolved in 20mls of sterile/double distilled water in universal bottles after weighing. This made a stock solution of 1000mg/10ml (1g/10ml) which is considered 100%. The stock solution was then used for preparing different concentrations of soap samples using the formula (Selvamohan and Sandhya, 2012).

(RV*RC/OC) *Where RV- Required Volume × Required Concentration divide by the Original Concentration

This gave various concentrations such as, 800mg/10ml, 600mg/10ml, 400mg/10ml 200mg/10ml, 100mg/10ml. Filter paper disc of 6mm were prepared and sterilized in the autoclave. About 50 µl of soap solution in different concentrations were impregnated on the filter paper disc. The discs were left to dry at ambient temperature before applying on Petri dishes inoculated with

microorganism (Wootton, 2013). The current study aims at comparing the efficacy of both medicated and plain soap.

3.7 Soap assays

3.7.1 Disc diffusion method

The study used disc diffusion method according to standard operating procedure (SOP) described by (Hudzicki, 2012). A standard McFarland of 0.5 were prepared using freshly growing bacterial cultures. The bacteria were picked aseptically using a sterile straight wire and emulsified in sterile distilled water in test tubes. The suspended bacteria were inoculated by spreading evenly on the agar media surface using sterile swabs made of cotton wool. Bacteria were cultured in Mueller Hinton agar (Oxoid, U.K) while that for fungi were cultured for 2-3 days in Sabouraud Dextrose Agar (SDA) (Oxoid, U.K). The soap impregnated discs were placed in triplicates same distance to each other on the plates. Incubation of bacteria was at 37^{o}C for 12-16 hr (overnight) while candida albicans were cultured in SDA and incubated for 2-3 days at 30^{o}C. Diameters of inhibitions zones were measured using a ruler in millimeters after the end of incubation period. Standard deviations of the triplicate experiment were also computed and recorded. Antibiotic discs of Ciprofloxacin for bacteria and Ketoconazole for candida were incorporated as positive controls. This is because ciprofloxacin acts on both gram positive and negative bacteria while ketoconazole works for candida albicans, these controls were arrived at after some literature showed to have used in similar studies, discs impregnated with sterile double distilled water were used as negative controls. The experiments were done in triplicates in order to minimize bias and for consistent results.

Different bacteria appear differently in different types of media. *Pseudomonas aeruginosa* produce green pigments on nutrient agar hence appearing green as shown in Figure 3.1.

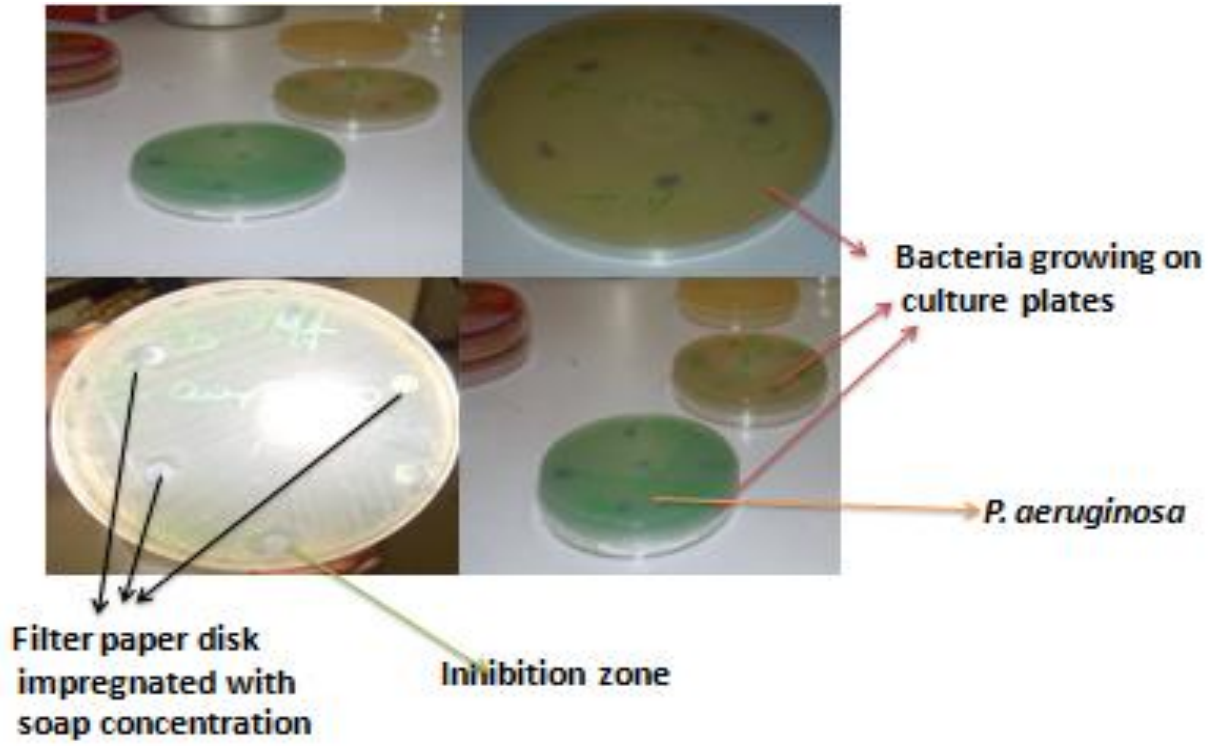

Figure 3.1: Agar plates with bacterial cultures growing

3.7.2 Minimum Inhibition Concentration (MIC)

Antimicrobial activity of the different soap brands at different concentrations were investigated using broth dilution (Appendix 6) for the purposes of MIC determination and were further sub-cultured into agar media to determine the bactericidal concentrations. Mueller Hinton broth was used for the determination of MICs for bacteria and Sabouraud Dextrose Broth (SDB) for candida albicans, using the tube method. Serial dilution for the soap from initial concentration of 1g/10ml which was the stock solution were done to obtain 500mg/10ml, 250, 125, 62.5, 31.25mg/ml concentrations. For MIC determination, 1ml of the inoculums were put in each tube using a sterile pipette and incubated accordingly. Bacteria were incubated at 37^{o}C overnight while candida

albicans were incubated at 30^{o}C for 2-3 days. Both negative and positive controls were set in separate tubes. All the entire tests were set in triplicates. The minimum inhibition concentration (MIC), lowest concentrations inhibiting the growth of the microorganisms was recorded (Farkas *et al.*, 2018).

3.8 Data Management

The data entry was done into IBM SPSS statistics version 23, cleaned and coded for analysis. Each soap was coded based on the brands and the numbering done accordingly to avoid mix up. Statistical analysis was done using IBM SPSS Statistics version 23 and results were presented as summary statistics such as averages and standard deviations. Descriptive statistics were used to describe data in frequencies and percentages.

3.9 Data analysis

One-way ANOVA test was utilized to determine if there was any significant association between variables such as inhibition zones diameters of the different bacterial strains produced by different soap brands at different concentrations. The MIC and MBC for the medicated and plain soaps were compared and standard deviations computed using Excel computer package. Analyses were done using Fisher's exact test at 95% confidence interval and a p-value of (0.05).

3.9 Dissemination

The study findings have been disseminated to the department of Microbiology University of Nairobi and KAVI- Institute of clinical research UoN in a Journal club on 15th November 2019. Also, the abstract was accepted for a poster presentation at the 4th African International Biotechnology and Biomedical Conference in Mombasa on 27th and 28th August 2019 (Appendix

7). A manuscript is being set and will soon be submitted for publication in a peer reviewed open accessed journal.

3.10 Ethical consideration

Ethical review and permission to conduct the study was obtained from Kenyatta National Hospital-University of Nairobi Ethics and Research Committee (KNH- UoN ERC) and was approved (P179/03/2017) Appendix 3. The soap brands were coded during data collection, laboratory test, data analysis and presentation for confidentiality purposes. The microorganisms were handled according to University of Nairobi biosafety guidelines and they were autoclaved before disposal after the study in order to prevent environmental contamination.

CHAPTER FOUR: RESULTS

4.1 Antimicrobial activity of medicated soaps

The 14 different types of soaps were tested in different concentrations ranging from 1000mg/10ml to 100mg/10ml (Appendix 5). Susceptibility profiles of different soap brands at different concentrations against test microbes were variable with the lowest inhibition zone diameter being 7 mm and the highest inhibition zone diameter being 10.0mm. At 1000mg/10ml concentration, all the soap brands were active against *P. aeruginosa* with zones of inhibition diameters that ranged from 7.0mm to 10.0mm. Soap brands MS005, MS006 and MS007 were active against *P. aeruginosa, S. pneumonia, S. aureus* and *S. epidermidis* with 10.0mm inhibition zone diameters. At 800mg/10ml concentration all the soaps brands were active against *P. aeruginosa* but with reduced inhibition diameters compared to 1000mg/10mlconcentration. Brands MS002, 005, 006 and 007 were active with inhibitory zone diameters that ranged from 8.0mm to 10.0mm. Brands MS005 and MS007 were also active against *S. pneumonia, S. aureus* and *S. epidermidis* with inhibitory zone diameters of 8.0mm while MS005 had 10.0mm for the same organisms. At 600mg/10ml concentration, brand MS001 had the smallest zone of inhibition against *P. aeruginosa* while MS 004 had no activity against *P. aeruginosa.* Brands MS002, 005, 006 and 007 were active against *P. aeruginosa* with 10.0mm (brand MS002) and 8.00mm (brands MS005, 006 and 007) inhibition zone diameters. Brands MS005, MS006 and MS007 were also active against *S. pneumonia, S. aureus* and *S. epidermidis* with inhibition zone diameters of 8.0mm. The susceptibility profiles of the medicated soap were directly proportional to the increase in concentration. Brands MS002, MS005 and MS007 had varied activities at lower concentrations of 100mg/10ml, 200mg/10ml and 400mg/10ml. Brands MS001, MS003 and MS004 were not active at lower concentrations as shown in Table 1.

Table 4.1: Antimicrobial susceptibility profiles of medicated soaps on test microbes

Conc. (mg/ml)	Soap Brand	Average Inhibition Zone Diameters in millimeters (mm)						
		P. aeruginosa	*S. pneumoniae*	*S. aureus*	*S. epidermidis*	*E. coli*	*C. albicans*	*S. capitis*
1000	MS001	10	-	-	-	-	-	-
	MS002	10	-	-	-	-	-	-
	MS003	7	-	-	-	-	-	-
	MS004	7	-	-	-	-	-	-
	MS005	10	10	10	10	-	-	-
	MS006	10	10	10	10	-	-	-
	MS007	10	10	10	10	-	-	-
800	MS001	8	-	-	-	-	-	-
	MS002	10	-	-	-	-	-	-
	MS003	7	-	-	-	-	-	-
	MS004	7	-	-	-	-	-	-
	MS005	8	8	8	8	-	-	-
	MS006	10	10	10	10	-	-	-
	MS007	8	8	8	8	-	-	-
600	MS001	7	-	-	-	-	-	-
	MS002	10	-	-	-	-	-	-
	MS003	7	-	-	-	-	-	-
	MS004	-	-	-	-	-	-	-
	MS005	8	8	8	8	-	-	-
	MS006	8	8	8	8	-	-	-
	MS007	8	8	8	8	-	-	-
400	MS001		-	-	-	-	-	-
	MS002	10	-	-	-	-	-	-
	MS003	-	-	-	-	-	-	-
	MS004	-	-	-	-	-	-	-
	MS005	7	-	7	7	-	-	-
	MS006	-	8	8	8	-	-	-
	MS007	7	7	7	7	-	-	-

Key: MS- Medicated soaps, +ve-positive control, -ve control, (-)-No inhibition, Conc – concentration

Table 4.1: Continued

Conc. (mg/ml)	Soap Brand	Average Inhibition Zone Diameters in millimeters (mm)						
		P. aeruginosa	*S. pneumoniae*	*S. aureus*	*S. epidermidis*	*E. coli*	*C. albicans*	*S. capitis*
200	MS001	-	-	-	-	-	-	-
	MS002	7	-	-	-	-	-	-
	MS003	-	-	-	-	-	-	-
	MS004	-	-	-	-	-	-	-
	MS006	-		7		-	-	-
	MS005	7		7	7	-	-	-
	MS007	-	-	7	7	-	-	-
100	MS001	-	-	-	-	-	-	-
	MS002	7	-	-	-	-	-	-
	MS003	-	-	-	-	-	-	-
	MS004	-	-	-	-	-	-	-
	MS005	7		7	7	-	-	-
	MS006	-	-	-	-	-	-	-
	MS007	-	-	-	7	-	-	-
-ve		-	-	-	-	-	-	-
+ve		12	12	12	12	12	12	12

Key: MS- Medicated soaps, +ve-positive control, -ve control, (-)-No inhibition, Conc – concentration

Figure 4.2 shows a soap solution with antimicrobial activities against *S. aureus* presented in triplicates. The soap solution was more active compared to the positive control.

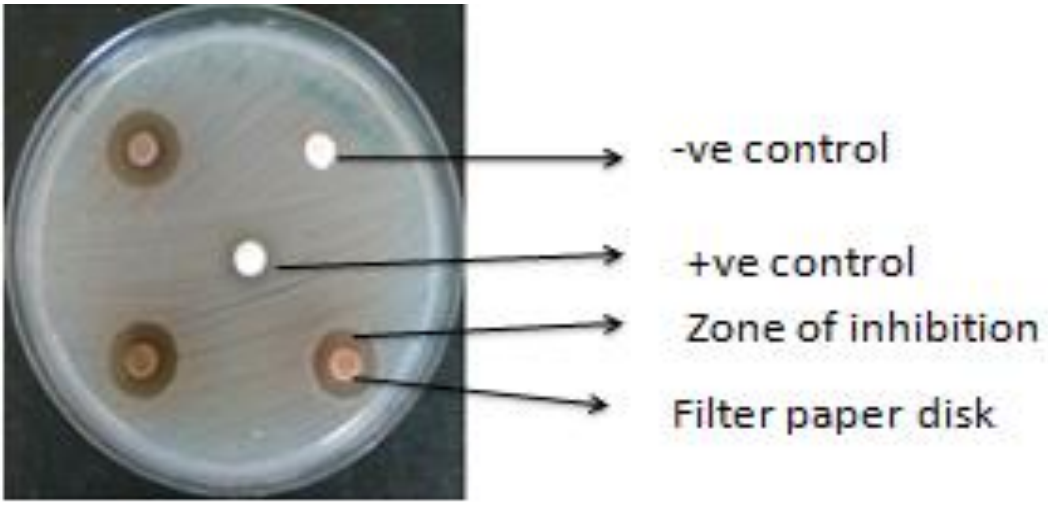

Figure 4.2: Disc diffusion method showing –ve, +ve control and active soap solution

4.1.2 MIC and MBC of medicated soaps against test microbes

The MIC and MBC for different medicated soap concentrations (1000mg/10ml, 500mg/10ml, 250mg/10ml, 125mg/10ml, 62.5mg/10ml and 31.25mg/10ml) of soaps were investigated. All the medicated soaps brands at the tested concentration were not active against *S. pneumoniae* and *S. capitis*. Figure 4.3 shows the results of MIC and MBC after incubation.

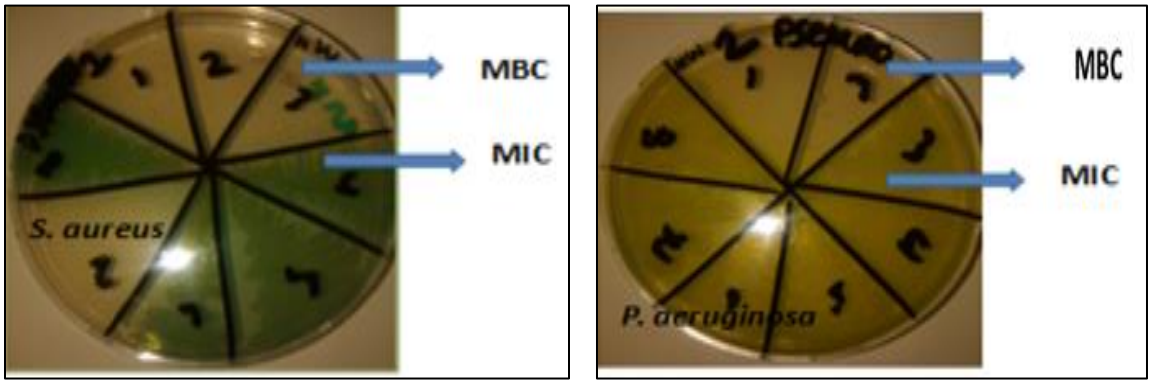

Figure 4.3: MIC and MBC concentrations after incubation and culturing on agar media

The soaps brand MS001 had an MBC of 1000mg/10ml and MIC of 500mg/10ml against *P. aeruginosa*, *E. coli*, *S. epidermidis*, *S. aureus* and *C. albicans* whereas brand MS002 and MS003

had MBC's of 1000mg/10ml and MIC of 500mg/10ml against *P. aeruginosa*, *E. coli*, *S. epidermidis* and MBC of 500mg/10ml and MIC of 250mg/10ml against *S. aureus* and *C. albicans*, respectively. Further, soap brand MS004 had an MBC of 1000mg/10ml and MIC of 500mg/10ml against *P. aeruginosa*, *E. coli*, *S. epidermidis*, *S. aureus* and MBC of 500 and MIC of 250 against *C. albicans*. The brand MS005 had MBC of 1000mg/10ml and MIC of 500mg/10ml against *S. epidermidis* and *C. albicans*, MBC of 500mg/10ml and MIC of 250 mg/10ml against *P. aeruginosa*, *E. coli* and MBC of 62.5 and MIC of 31.25 against *S. aureus*, respectively.

MS006 had MBC of 1000mg/10mland MIC of 500mg/10ml against *P. aeruginosa*, MBC of 500mg/10ml and MIC of 250 mg/10ml against *C. albicans*, MBC of 250mg/10ml and MIC of 125mg/10ml against *E. coli* and *S. aureus*, respectively. The soap brand MS007 had an MBC of 1000mg/10ml and MIC of 500mg/10ml against *S. epidermidis*, MBC of 500mg/10ml and MIC of 250mg/10ml against *C. albicans*, MBC of 250mg/10ml and MIC of 125mg/10ml against *P. aeruginosa* and *E. coli*, respectively, and an MBC of 62.5mg/ml and MIC of 31.25mg/ml against *S. aureus* (Table 4.2).

Table 4.2: MIC and MBC of medicated soaps against test microbes

Soap	Organisms	MIC (mg/ml)	MBC (mg/ml)
MS001	*P. aeruginosa*	1000	500
	E. coli	1000	500
	S. epidermidis	1000	500
	S. aureus	1000	500
	C. albicans	1000	500
	S. pneumonia, S. capitis	-	-
MS002	*P. aeruginosa*	1000	500
	E. coli	1000	500
	S. epidermidis	1000	500
	S. aureus	500	250
	C. albicans	500	250
	S. pneumonia S. capitis	-	-
MS003	*P. aeruginosa*	1000	500
	E. coli	1000	500
	S. epidermidis	1000	500
	S. aureus	500	250
	C. albicans	500	250
	S. pneumonia S. capitis	-	-
MS004	*P. aeruginosa*	1000	500
	E. coli	1000	500
	S. epidermidis	1000	500
	S. aureus	1000	500
	C. albicans	500	250
	S. pneumonia S. capitis	-	-
MS005	*P. aeruginosa*	500	250
	E. coli	500	250
	S. epidermidis	1000	500
	S. aureus	62.5	31.25
	C. albicans	1000	500
	S. pneumonia S. capitis	-	-

Key: MS- Medicated soaps, MBC-Minimum bactericidal concentration, MIC- Minimum inhibitory concentration

Table 4.2: Continued

Soap	Organisms	MIC (mg/ml)	MBC (mg/ml)
MS006	*P. aeruginosa*	1000	500
	E. coli	250	125
	S. epidermidis	250	125
	S. aureus	250	125
	C. albicans	500	250
	S. pneumonia S. capitis	-	-
MS007	*P. aeruginosa*	250	125
	E. coli	250	125
	S. epidermidis	1000	500
	S. aureus	62.5	32.25
	C. albicans	500	250
	S. pneumonia S. capitis	-	-

Key: MS- Medicated soaps, MBC-Minimum bactericidal concentration, MIC- Minimum inhibitory concentration

4.2 Antimicrobial activity of plain/non-medicated soaps

Susceptibility profiles of different concentrations of non-medicated soaps on the different test microbes were variable. The highest inhibition zone diameter was 14mm while the lowest was 7.0mm. At a concentration of 1000mg/10ml, soap brands NMS001 and NMS004 were active against *P. aeruginosa* with 14.00mm inhibition zone diameter, for both, brand NMS007 inhibition zone diameters for *S. pneumonia*, *S. aureus* and *S. epidermidis* was 12.00mm while NMS002, NMS005 and NMS006 zone diameters for *P. aeruginosa* was 6.00mm, respectively. The results showed that at 800mg/10ml concentration, brands NMS004 had 12.0mm zone of inhibition on *P. aeruginosa*, brands NMS003 and NMS007 zones of inhibition were 10.0mm for *S. pneumonia*, *S. aureus* and *S. epidermidis*, respectively. Brand NMS001 had 8.00 mm zones for *P. aeruginosa*, while NMS002, NMS005 and NMS006 exhibited 7.0mm for *P. aeruginosa*, respectively.

At concentration 600mg/10ml brands NMS003 had 10.0mm zone of inhibition for *P. aeruginosa*, NMS004 and NMS007 had inhibition zone diameters of 8.0mm on *P. aeruginosa, S. pneumonia,*

S. aureus, and *S. epidermidis*, respectively while NMS001, NMS002, NMS005 and NMS006 had 7.0mm zones of inhibition for *P. aeruginosa*. Brand NM007 was able to inhibit all the test microbes from concentration 600mg/10ml and above while there was no inhibition at lower concentrations. At concentration 400mg/ml brand NMS003 had 10.0mm zone of inhibition for *P. aeruginosa*, brands NMS007 inhibited *P. aeruginosa, S. pneumonia, S. aureus* and *S. epidermidis* at 8.00mm zones of inhibition while NMS001, NMS002, NMS004, NMS005 and NMS006 were inhibited *P. aeruginosa* with 6.00mm inhibition zones diameters. Brands NM003, 004, 006 and 007 were active against *P. aeruginosa* at 200mg/10ml and 100mg/ml concentrations with zones of inhibitions of 7.0mm as shown in Table 4.3.

Table 4.3: Antimicrobial profiles of non-medicated soaps against test microbes

Conc. (mg)	Brand	Inhibition Zone Diameters in millimeters (mm)						
		P. aeruginosa	*S. pneumonia*	*S. aureus*	*S. epidermidis*	*E. coli*	*C. albicans*	*S. capitis*
1000g	NMS001	14	-	-	-	-	-	-
	NMS002	7	-	-	-	-	-	-
	NMS003	10	-	-	-	-	-	-
	NMS004	14	-	-	-	-	-	-
	NMS005	7	-	-	-	-	-	-
	NMS006	7	-	-	-	-	-	-
	NMS007	12	12	12	12	-	-	-
800mg	NMS001	8	-	-	-	-	-	-
	NMS002	7	-	-	-	-	-	-
	NMS003	10	-	-	-	-	-	-
	NMS004	12	-	-	-	-	-	-
	NMS005	7	-	-	-	-	-	-
	NMS006	7	-	-	-	-	-	-
	NMS007	10	10	10	10	-	-	-
600mg	NMS001	7	-	-	-	-	-	-
	NMS002	7	-	-	-	-	-	-
	NMS003	10	-	-	-	-	-	-
	NMS004	8	-	-	-	-	-	-
	NMS005	7	-	-	-	-	-	
	NMS006	7	-	-	-	-	-	-
	NMS007	8	8	8	8	-	-	-
400mg	NMS001	7	-	-	-	-	-	-
	NMS002	7	-	-	-	-	-	-
	NMS003	10	-	-	-	-	-	-
	NMS004	7	-	-	-	-	-	-
	NMS005	7	-	-	-	-	-	-
	NMS006	7	-	-	-	-	-	-
	NMS007	8	8	8	8	8	-	-

Key: NMS – None medicated soap, NI – No inhibition, Conc – concentration

Table 4.3: Continued

Conc. (mg)	Brand	Inhibition Zone Diameters in millimeters (mm)						
		P. aeruginosa	*S. pneumonia*	*S. aureus*	*S. epidermidis*	*E. coli*	*C. albicans*	*S. capitis*
200gm	NMS001	-	-	-	-	-	-	-
	NMS002	-	-	-	-	-	-	-
	NMS003	7	-	-	-	-	-	-
	NMS004	7	-	-	-	-	-	-
	NMS006	7	-	-	-	-	-	-
	NMS007	7	-	-	-	-	-	-
100gm	NMS001	-	-	-	-	-	-	-
	NMS002	-	-	-	-	-	-	-
	NMS003	7	-	-	-	-	-	-
	NMS004	7	-	-	-	-	-	-
	NMS006	7	-	-	-	-	-	-
	NMS007	7	-	-	-	-	-	-
-ve		-	-	-	-	-	-	-
+ve		12	12	12	12	12	12	12

Key: NMS– None medicated soap, NI – No inhibition, Conc – concentration

4.2.2 MIC and MBC of non-medicated soaps against test microbes

Non medicated soaps also referred to as plain soap exhibited different inhibition zone diameters against different microorganisms. Some soaps were active with large inhibition zones diameters while others did not inhibit the bacteria at all. The lower the soap concentration in terms of MBC and MIC the more active the soap was and vice versa. All the seven tested non medicated soaps did not inhibit *S. pneumonia* and *S. capitis*. Brand NMS001 against *C. albicans* had an MIC of 125mg/10ml and MBC of 250mg/10ml. The following *P. aeruginosa, E. coli, S. epidermidis* and *S. aureus* had MIC's of 500mg/10ml and MBC's of 1000mg/10ml.

Brand NMS002 against *E. coli, S. aureus* and *C. albicans* had MICs of 500mg/10ml and MBCs of 1000mg/10ml. It was also active against *S. epidermidis* with 250mg/10ml MIC and MBC of 500mg/10ml however it was not able to inhibit *P. aeruginosa*. The brand NMS003 was more

active against *E. coli* with MBC and MIC of 500mg/10ml and 250mg/10ml, respectively, and less active against *P. aeruginosa, S. epidermidis, S. aureus* and *C. albicans* with MBC and MIC of 1000mg/10ml and 500mg/10ml, respectively. The soap brand NMS004 exhibited an MIC of 500mg/10ml and MBC of 1000mg/10ml against all the test microorganisms except *S. pneumonia* and *S. capitis*. The soap brand NMS005 was more active against *S. aureus* and *C. albicans* with MBC and MIC of 500mg/10ml and 125mg/10ml, respectively. It was less active against *P. aeruginosa, E. coli* and *S. epidermidis* with MBC and MIC of 1000mg/10ml and 500mg/10ml, respectively.

The soap brand NMS006 exhibited varied activities against different tested microorganisms. It was more active against *E. coli, S. aureus* and *C. albicans* with MBC and MIC of 500mg/10ml and 250mg/10ml, respectively and less active against *P. aeruginosa* and *S. epidermidis* with MBC and MICs of 1000mg/10ml and 500mg/10ml, respectively. Non medicated soap brand NMS007 was highly active compared to the other non-medicated soaps tested in this study as indicated by the minimum and bactericidal concentration although it was not active against *S. pneumonia* and *S. capitis* similar to the other tested soaps. It had an MBC and MIC of 125mg/10ml and 62.5mg/10ml, respectively, against *P. aeruginosa* and *S. epidermidis*. It was very active against *E. coli, S. aureus* and *C. albicans* with MBC and MIC of 62.5mg/10ml and 32.25mg/10ml, respectively (Table 4.4).

Table 4.4: MIC and MBC of different concentrations of plain soaps against test microbes

Soaps	Test organisms	MBC (mg/ml)	MIC (mg/ml)
NMS001	*P. aeruginosa*	1000	500
	E. coli	1000	500
	S. epidermidis	1000	500
	S. aureus	1000	500
	C. albicans	250	125
	S. pneumonia	-	-
	S. capitis	-	-
NMS002	*P. aeruginosa*	1000	500
	E. coli	1000	500
	S. epidermidis	500	250
	S. aureus	1000	500
	C. albicans	1000	500
	S. pneumoniae	-	-
	S. capitis	-	-
NMS003	*P. aeruginosa*	1000	500
	E. coli	500	250
	S. epidermidis	1000	500
	S. aureus	1000	500
	C. albicans	1000	500
	S. pneumoniae	-	-
	S. capitis	-	-
NMS004	*P. aeruginosa*	1000	500
	E. coli	1000	500
	S. epidermidis	1000	500
	S. aureus	1000	500
	C. albicans	1000	500
	S. pneumoniae	-	-
	S. capitis	-	-
NMS 005	*P. aeruginosa*	1000	500
	E. coli	1000	500
	S. epidermidis	1000	500
	S. aureus	500	250
	C. albicans	500	250
	S. pneumoniae	-	-
	S. capitis	-	-

Key: NMS– None medicated soap, MBC-minimum bactericidal concentration, MIC-minimum inhibition concentration

Table 4.4: Continued

Soaps	Test organisms	MBC (mg/ml)	MIC (mg/ml)
NMS006	*P. aeruginosa*	1000	500
	E. coli	500	250
	S. epidermidis	1000	500
	S. aureus	500	250
	C. albicans	500	250
	S. pneumoniae	-	-
	S. capitis	-	-
NMS007	*P. aeruginosa*	125	62.5
	E. coli	62.5	31.25
	S. epidermidis	125	62.5
	S. aureus	62.5	31.25
	C. albicans	62.5	31.25
	S. pneumoniae	-	-
	S. capitis	-	-

Key: NMS– None medicated soap, MBC-minimum bactericidal concentration, MIC-minimum inhibition concentration

4.3 Comparison of the antimicrobial activity of the plain and medicated soaps

4.3.2 Inhibition zone diameters

All the non-medicated soaps at concentration 1000mg/10ml were active against *P. aeruginosa* with the highest inhibition zone diameter being 14mm and an average of 9.1mm whereas the medicated soaps were active against *P. aeruginosa* with the highest inhibition zone diameter being 10mm and an average of 9.0mm at the same concentration. Again, at 1000mg/10ml concentration MS005, 006 and 007 were also active against *S. pneumonia, S. aureus* and *S. epidermidis* with average zone diameters of 10.0mm hence medicated soaps were active against a wide range of the test microbes compared to non-medicated soaps at 1000mg/10ml concentration. Generally, the two classes of soaps were active against *P. aeruginosa* with reducing activities as the concentration reduced.

Non-medicated soap MNS007 at concentration 800mg/10ml was active against, *P. aeruginosa*, *S. pneumonia*, *S. aureus* and *S. epidermidis* with average inhibition zone diameter of 12mm, 10mm at concentration 600mg/ml, and 8mm at concentration 400mg/10ml, respectively. Medicated soaps MS005, 006, and 007 at concentration 800mg/10ml were also active against *P. aeruginosa*, *S. pneumonia*, *S. aureus* and *S. epidermidis* with average inhibition of 8mm (MS005 and 007) and 10mm (MS006), respectively. Therefore, medicated soaps investigated were more active at concentration 800mg/10ml compared to non-medicated soap at the same concentration. Similarly, medicated soaps (MS005, 006 and 007) at concentration 600mg/10ml recorded average inhibition zone diameter of 8mm against *P. aeruginosa*, *S. pneumonia*, *S. aureus* and *S. epidermidis*, respectively. At concentration 400mg/ml, medicated soap MS005 and 007 had an average inhibition zones diameter of 7.0mm while MS006 had an average of 8mm inhibition zone diameter while there were no activities on non-medicated soaps against *S. pneumonia*, *S. aureus* and *S. epidermidis* at the same concentration. Generally, more medicated soaps were more active compared to non-medicated soaps in terms of inhibition zone diameters activities of medicated and non-medicated soaps. There was significant difference ($p=0.091$) in terms of antimicrobial activities between different concentrations of medicated and non-medicated soaps in this study. Generally, there was no significant difference ($p=0.043$) between activities of medicated soaps and non-medicated soaps in general (Table 4.5).

Table 4.5: Comparative analysis of the inhibition of MS and NMS soap samples

Organism	Soap	N	Mean	Std.	Std. Error	95% Confidence Interval for Mean		Min	Max	ANOVA	Sig.
						Lower Bound	Upper Bound			4.226	0.043
P. aeruginosa	NMS	42	9.1	2.443	0.407	6.73	8.38	6	14	4.327	0.091
	MS	42	9.2	1.681	0.330	6.55	7.91	6	10		
S. aureus	NMS	42	8.00	2.828	1.414	3.50	12.50	6	12		
	MS	42	7.24	1.300	0.315	6.57	7.90	6	10		
S. epidermidis	NMS	42	7.60	2.608	1.166	4.36	10.84	6	12		
	MS	42	7.63	1.821	0.455	6.65	8.60	6	12		
S. pneumonia	NMS	42	7.00	1.155	0.577	5.16	8.84	6	8		
	MS	42	6.00	0.000	0.000	6.00	6.00	6	6		

Key: Non medicated soap- NMS, MS- Medicated Soap, Std-standard deviation

All seven medicated and non-medicated soaps brands at different concentration were not active against *S. pneumonia* and *S. capitis*. For both the classes of soaps, the MBC and MIC were at concentration 1000mg/10ml and 500mg/10ml, respectively. Non-medicated soap brand NMS007 was active compared to the other non-medicated soaps tested in this study according to the minimum and bactericidal concentration although it was not active against *S. pneumonia* and *S. capitis* like the other tested soaps (Figure 4.1 and 4.2).

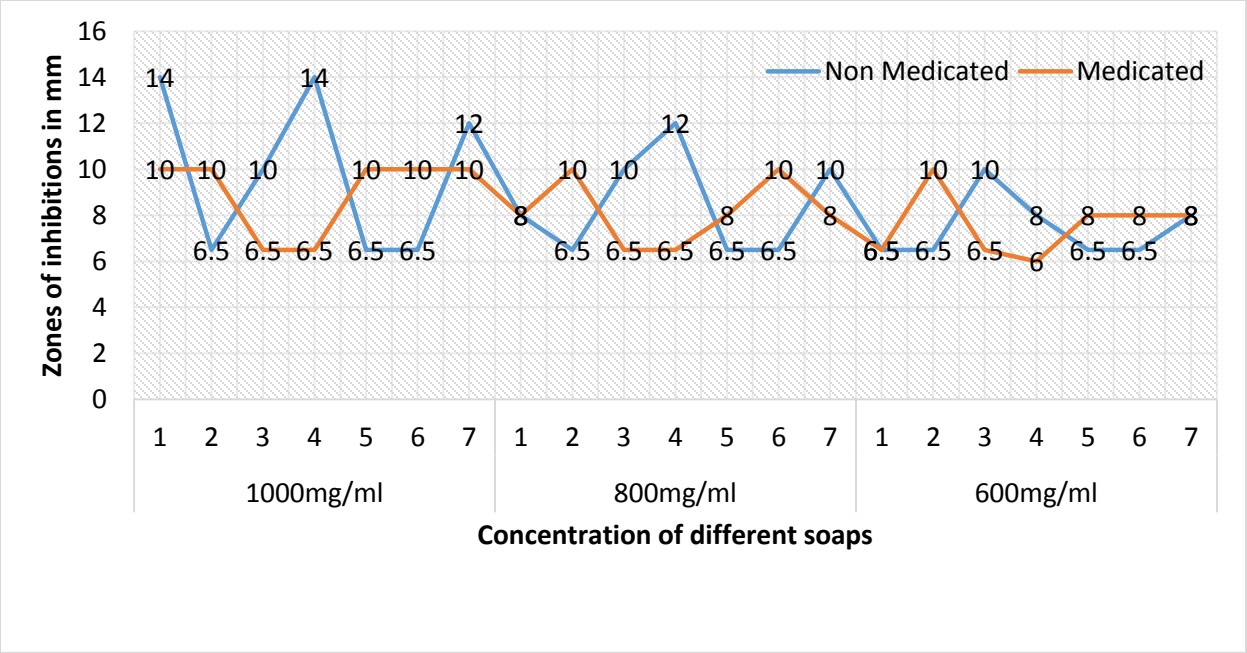

Figure 4.4: Comparative analysis of medicated and non-medicated soaps (concentration 1000mg/ml to 600mg/ml)

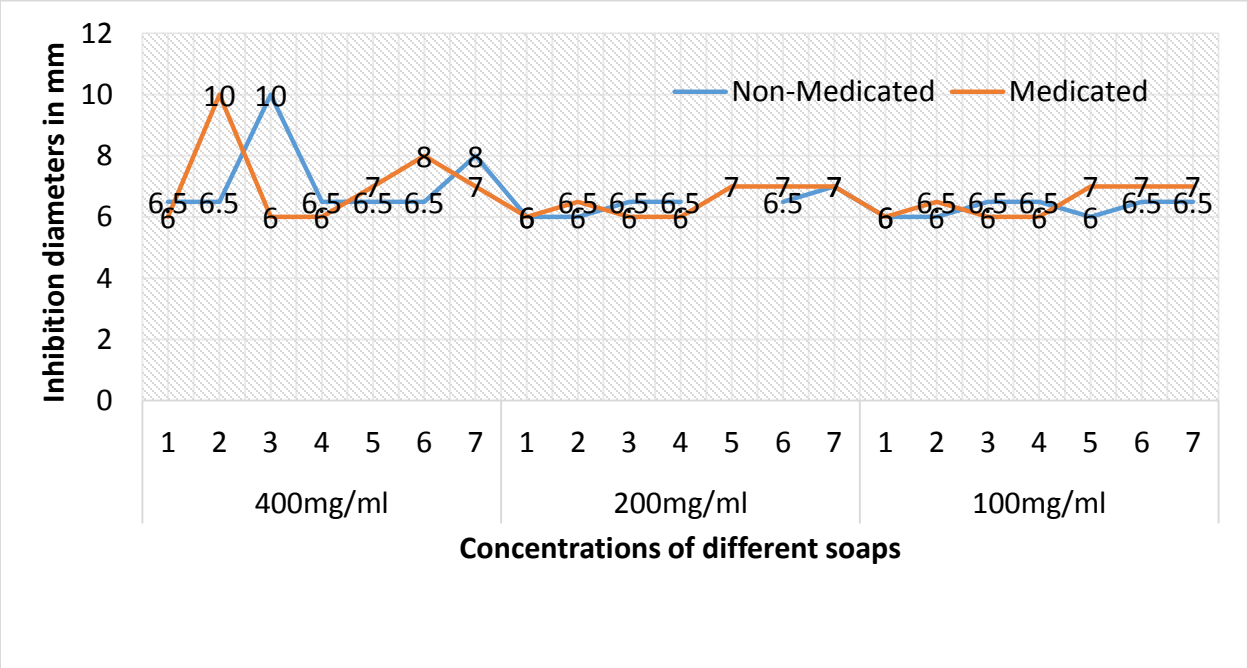

Figure 4.5: Comparative analysis of medicated and non-medicated soaps (concentration 400mg/ml to 100mg/ml)

4.3.2 Comparison of MIC and MBC of medicated and non-medicated soaps

The results showed that, there was no significant association between activities of different non-medicated soaps and also between different concentrations ($p>0.05$). The soap had an MBC and MIC of 125mg/10ml and 62.5mg/10ml, respectively, against *P. aeruginosa* and *S. epidermidis*. They were active against *E. coli, S. aureus* and *C. albicans* with MBC and MIC of 62.5mg/10ml and 32.25mg/10ml, respectively.

The brand MS007 had an MBC of 250mg/10ml and an MIC of 125mg/10ml against both *P. aeruginosa* and *E. coli*. This brand of soap was active against *S. aureus* with MIC of 32.25mg/10ml and an MBC of 62.5mg/10ml. This soap had no activity against S. *Pneumonia* and *S. capitis*. Among the medicated soaps, brand MS005 and MS007 were more active against *S. aureus* compared to the other medicated soaps. There was no significant association between antimicrobial activities of different brands of medicated soaps except MS005, 006 and 007 where their p-values were less than 0.05. When both medicated/antiseptic and non-medicated/plain soaps were compared in terms of their ability to kill and inhibit the tested microbes, non-medicated soap brand NMS007 was superior compared to the other soaps tested. Generally, more medicated soaps were more active against a wide range of the test organisms compared to non-medicated soaps ($P\leq005$) as shown in Table 4.6.

Table 4.6: Comparative analysis of the MIC and MBC of the different soap samples

Test (mg/ml)	Soap	N	Mean	Std.	Std. Error	95% Confidence Interval for Mean		F	Sig.
						Lower Bound	Upper Bound		
MIC	MS001	7	625.00	306.19	125.00	303.68	946.32	6.16	0.09
	MS002	7	541.67	245.80	100.35	283.72	799.62		
	MS003	7	625.00	410.79	167.71	193.90	1056.10		
	MS004	7	791.67	332.29	135.66	442.95	1140.38		
	MS005	7	422.04	333.86	136.30	71.67	772.41		
	MS006	7	192.88	165.66	67.63	19.03	366.72		
	MS007	7	213.71	163.26	66.65	42.38	385.03		
	NMS001	7	200.00	167.71	75.00	-8.23	408.23		
	NMS002	7	916.67	204.12	83.33	702.45	1130.88		
	NMS003	7	916.67	204.12	83.33	702.45	1130.88		
	NMS004	7	1000.00	0.00	0.00	1000.00	1000.00		
	NMS005	7	875.00	306.19	125.00	553.68	1196.32		
	NMS006	7	541.67	367.99	150.23	155.48	927.85		
	NMS007	7	73.25	42.31	17.27	28.85	117.65		

Key: Non medicated soap- NMS, MS- Medicated Soap, Std-standard deviation

Table 4.6: Continued

Test (mg/ml)	Soap	N	Mean	Std.	Std. Error	95% Confidence Interval for Mean		F	Sig.
						Lower Bound	Upper Bound		
MBC	MS001	7	875.00	250.00	125.00	477.19	1272.81	3.019	0.005
	MS002	7	900.00	223.61	100.00	622.36	1177.64		
	MS003	7	500.00	0.00	0.00	500.00	500.00		
	MS004	7	750.00	353.55	250.00	2426.55	3926.55		
	MS005	7	612.50	396.27	177.22	120.46	1104.54		
	MS006	7	385.42	331.70	135.42	37.32	733.52		
	MS007	7	427.08	326.96	133.48	83.96	770.20		
	NMS001	7	400.00	335.41	150.00	-16.47	816.47		
	NMS002	7	1000.00	331.70	135.42	37.32	733.52		
	NMS003	7	1000.00	326.96	133.48	83.96	770.20		
	NMS004	7	400.00	335.41	150.00	-16.47	816.47		
	NMS005	7	500.00	331.70	135.42	37.32	733.52		
	NMS006	7	625.00	250.00	125.00	227.19	1022.81		
	NMS007	7	145.83	85.39	34.86	56.22	235.45		

Key: Non medicated soap- NMS, MS- Medicated Soap, Std-standard deviation

Table 4.7 shows comparison of MIC and MBC of all medicated soaps. The ANOVA shows that there is significant between the medicated soaps MIC since the p-value was 0.008 which is less than 0.05 hence significant. While the MBC values according to ANOVA shows that there was no significant association between MBC of medicated soaps (p=0.068). A significant main effect was obtained for MIC level, F (1.47) = 4.226, p = 0.043 <0.05. Non-medicated soaps had significantly higher MIC scores (M = 657.06) than did Medicated soaps (M = 487.42).

Table 4.7: Comparison of MIC and MBC of medicated soaps by ANOVA

Dependent Variable:	Soap Type	Mean	Std. Deviation	N	F	Sig.	Partial Eta Squared
MIC Concentration	Medicated Soap	487.42	341.82	42	4.226	0.043	0.050
	Non medicated Soap	657.06	407.82	41			
	Average	571.22	383.19	83			
MBC Concentration	Medicated Soap	610.89	341.84	31	2.939	0.093	0.059
	Non medicated Soap	437.50	340.31	18			
	Average	547.19	348.11	49			

A significant main effect was obtained for MIC level, F (1.47) = 4.226, p = 0.043 <0.05. Non-medicated soaps had significantly higher MIC scores (M = 657.06) than did Medicated soaps (M = 487.42). This was still a minimal difference (Partial Eta Squared = 0.050).

CHAPTER FIVE: DISCUSSION

Soaps are used for cleaning, removal of microbes and dust from surfaces, skin, clothing and utensils among other surfaces. The soaps should be effective against microbes and should not be harmful to human skin. Results obtained in this study showed that majority of tested medicated soaps have antimicrobial activities. The different brands of medicated soaps were active in varying degrees as shown by the inhibitions of the growth patterns of the tested microorganisms thus concurring with a study in India and in Nigeria (Chaudhari, 2016; Olajuyigbe et al., 2016). In the present study, the medicated soaps exhibited different antimicrobial patterns against the test microorganism with the highest inhibition zone diameter being 10 mm while the lowest was 6.5 mm in diameter at the highest concentration (1000mg/ml). The activity of medicated soap was directly proportional to soap concentration.

All the medicated soaps were active against *P. aeruginosa,* which is a pathogen associated with wound infections in hospital environment. Hence, they can be used to clean hands and the skin in order to prevent *P. aeruginosa* pathogens from contaminating wounds and food and infecting patients. The medicated soaps were also active against wide range of different bacteria associated with skin infection such as *S. aureus, S. epidermidis* and *Streptococcus species*. More so, the selected clinical pathogens used for the determination of antiseptic/antimicrobial properties of medicated and plain soaps in the present study were pathogens previously reported to be predominant in skin infections (Chaudhari, 2016; Olajuyigbe et al., 2016; Olufunmiso, Tolulope and Roger, 2017). Similarly, the active components of the medicated soaps are triclosan, trichloro carbamide, terpineol thymol and chloroxylenol which have the ability to inhibit bacteria to a larger

extent by interfering with cell wall activity through disruption or denaturing the cell wall thus interfering with entire metabolism of the microorganisms (Ikpoh *et al.*, 2012).

The medicated soaps tested in this study did not inhibit the growth of *Candida albicans* which is a fungus since the active ingredients are meant to inhibit bacteria as described earlier. *Candida albicans* is a yeast like fungus and can be spread from one person to another through contaminated hands. Other means of hand sanitation should be utilized in case of *C. albicans* contamination especially on the hands. Some bacteria such as *E. coli* were not inhibited by the medicated soaps tested in the present study but were inhibited by both herbal soap and medicated soap in a similar study in India (Chaudhari, 2016). This might be due to the active ingredients which are incorporated to the various soaps, the geographical distribution of the organisms and may be the resistance of the organisms. Some brands of medicated soaps such as in this study were active at lower concentrations of 100mg/ml. An antimicrobial agent is best when it is active at a lower concentration and poor when active at high concentration.

The results of the present study regarding variation of MIC and MBC against different concentrations concurs with a study which reported that the MBC and MIC results obtained against certain strains of bacteria were varied (Nashaat AL-Saadi`, 2016). The lowest MBC (62.5mg/10ml) and MIC (31.25mg/10ml) were exhibited by soap brand MS005 and MS007 on *S. aureus*. These two brands of medicated soaps were the most effective compared to all the medicated soaps tested in this study followed by brand MS006.The antimicrobial activities of these three brands of soaps against *S. aureus* could be effective or have therapeutic potential in treating or healing of skin and wound infections which normally cause or may be involved in secondary infections as was observed in a similar study, although this factor was not tested in the current

study (Riaz *et al.*, 2015). The results of MIC and MBC of the current study concurs with other study reports in that different medicated soaps exhibited different antimicrobial activities against the test strains of bacteria (Riaz *et al.*, 2015; Chaudhari, 2016).

Antimicrobial activity of plain soaps against the test microorganisms at different concentrations was variable. Non-medicated soap brand NMS007 was more active compared to other non-medicated soaps as shown by the minimum inhibitory and bactericidal concentration. This type of soap is the best because it exhibited inhibition zone diameters, MIC and MBC that were comparable to medicated soaps. This study has proved that some non-medicated soaps such as brand NMS007 are indeed capable of inhibiting a wide range of bacteria. A similar study showed that non-medicated soaps are capable of killing microorganisms hence they also contain some antibacterial activities like medicated soaps although to a lesser extent (Riaz *et al.*, 2015)

In this study, both the non-medicated and medicated soaps at higher concentration (1000mg/10ml) inhibited the growth of *P. aeruginosa* with inhibition zone diameters of 14.0mm (average 9.1mm) and 10.0mm (average 9.0mm), respectively. This shows that plain soaps at high concentrations are capable of either killing or inhibiting the growth of microorganisms such as bacteria and yeast in a similar way to medicated soaps. A study by (Sz *et al.*, 2016) showed that both medicated and plain soaps were equally effective against a wide range of microorganisms including bacteria thus corroborating the findings of the present study.

A similar study showed that, an antibacterial soap is capable of eliminating 65% to 85% of human skin bacteria (Srinivasan, 2016). The findings of the present study also suggest that both plain and

medicated soaps are equally effective against bacteria that are commonly associated with skin/wound infections. Generally, the two classes of soaps were active against *P. aeruginosa* with reducing activity as the concentration reduced.

When compared in terms of effectiveness against different bacteria, only NMS007 was effective against a wide range of bacteria at concentration 1000mg/10ml while three medicated soaps (MS005, 006 and 007) at the same concentration were effective against; *S. pneumonia, S. aureus* and *S. epidermidis*. During medicated/antiseptic soaps formulation, the common soap base is integrated with specific quantities of antimicrobial or germicidal ingredients in order to be able to kill bacteria and other microorganisms. The ingredients have antiseptic agents which give the soap the ability to kill pathogenic microorganisms also commonly referred to as germs even when the residue remains after it has been washed from the skin (Mwambete and Lyombe, 2011).

When different concentrations were compared, there was significant positive association ($p \leq 0.05$) between inhibition zones of the same concentrations among the test organisms. There were no significant associations between inhibition zones of different microorganisms at different concentrations ($p=0.091$). Activity of different soap concentrations against the test microorganisms was directly proportional to soaps' concentration. A similar study share the same sentiments with the current study in that, antimicrobial activities of the tested soaps were directly proportional to soaps' concentration (Sz *et al.*, 2016). When the two types of soaps were compared by ANOVA, the results showed that there was no significant difference ($P>0.05$) among the medicated and non-medicated soaps. The average inhibition zones for the two categories of soaps were comparable. This implies that both medicated and plain soaps tested in this study would give

comparable results when used for cleaning and removing dirt. The soaps are able to kill pathogenic microorganisms to some degree as discussed earlier. The average inhibition zones for the two categories of soaps were comparable. When the antimicrobial activities of medicated and plain soaps were compared at concentration 1000mg/ml, average inhibition zones against *P. aeruginosa* was 9.1mm for plain soaps and 9.0mm for medicated soaps.

An important observation of the study was that, both the medicated and non-medicated soaps were inactive against some organisms. However, since they were active in terms of inhibition zones, then the MIC and MBC could be high. Generally, soaps are incorporated with ingredients that are capable of killing bacteria; reduce skin infections caused by several species of *Staphylococcus* and other gram-negative bacteria as well as other micro-organisms associated with skin infections.

There was no statistically significant difference between MIC and MBC of both medicated and non-medicated soaps tested in the present study ($P \leq 005$). This means that the two categories of soaps were comparable in terms of MIC/MBC. The two types of soaps can be used interchangeably since their ability to kill the organisms in this study was similar.

The findings of the present study suggest that medicated soaps were more effective compared to non-medicated soaps. A study done by Selvamohan and Sandhya, (2012), reported that antibacterial soap is better in removing bacteria than a plain non-medicated soap which is less effective hence concurs with the results of the current study. Similarly, Schaffner *et al.*, (2014) who compared antimicrobial and non-antimicrobial hand wash activities reported that antimicrobial hand wash agents were more effective in providing greater bacterial reductions than

non-antimicrobial agents which is in agreement with the results of the present study. In this study, some medicated soaps were not active against all microorganisms tested. This shows that we should not rely on medicated soaps for sanitization of hands and surface. It also informs us that we should employ other antiseptic agents whose efficacies have been proved. Aiello *et al*., (2007) also reported no difference in microbial decrease and therefore no difference in overall health gain between non-medicated and medicated soaps. It also supports the fact that not all the medicated soaps were effective against all the microorganisms tested. In a nutshell, we should treat all the soaps equally unless their efficacy against pathogenic microorganisms has been proved. Soaps should strictly be used for cleaning purposes and removing dirt's but not for disinfection. The present study has provided information on role played by both plain soap and medicated soap in killing or inhibiting bacteria in Kenya.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

All the medicated soaps tested in this study were able to inhibit the growth of *P. aeruginosa,* bacteria associated with skin and wound infections. Additionally, three medicated soaps (MS005, 006 & 007) had microbial activities against *S. pneumonia, S. aureus* and *S. epidermidis* plus *P. aeruginosa*. In terms of MBC and MIC, MS 005 and MS 007 were highly active against *S. aureus* with MBC of 62.5mg/10ml and MIC of 31.25mg/10ml, respectively. Hence suggesting that soap brand MS005, 006 and 007 were better in terms of antimicrobial activities.

All the non-medicated soaps were also effective against *P. aeruginosa*. For non-medicated soaps only brand NMS007 inhibited a wider range of the test microbes (*S. pneumonia, S. aureus, S. epidermidis* and *P. aeruginosa*). In terms of MBC and MIC, soap NMS007 was highly active against *E. coli, S. aureus* and *C. albicans*. Since some non-medicated soaps showed similar antimicrobial activities to those of antiseptic soaps, they can be considered equally effective and therefore useful for day to day use.

In general, more medicated soaps than non-medicated soaps were active against wide range of the test organisms. However, the present study also suggests that some non-medicated soaps such as brand NMS007 can be more effective as compared to medicated soaps.

6.2 Recommendations

- The public should be advised that not all medicated soaps have antimicrobial activities against all the microorganisms.
- The public should be encouraged to use effective non-medicated soaps such as NMS007 since it is effective against a wide range of pathogenic microorganisms and is cheaper.
- Some brands of medicated soaps such as in this study were active at lower concentrations of 100mg/ml. Hence, they can be recommended for surface decontamination and hand washing where bacteria of similar nature are suspected to be the source of contamination.
- That all the soaps (non-medicated and medicated) tested in this study did not inhibit the growth of *Candida albicans* which is a fungus since the active ingredients are meant to inhibit bacteria as described earlier. Therefore, it is not recommended for hands and cleaning surfaces where fungus is suspected as the main contaminants.
- Studies to be done to test whether the soaps really contain the antimicrobial agents and their concentration
- Further studies be done on antimicrobial resistance both phenotypic and genotypic in relation to prolonged use of medicated soaps

6.3 Limitations

The main limitation of this study was resources hence the study sampled a limited but representative sample size. This also contributed to the testing of the soaps against a limited number of micro-organisms.

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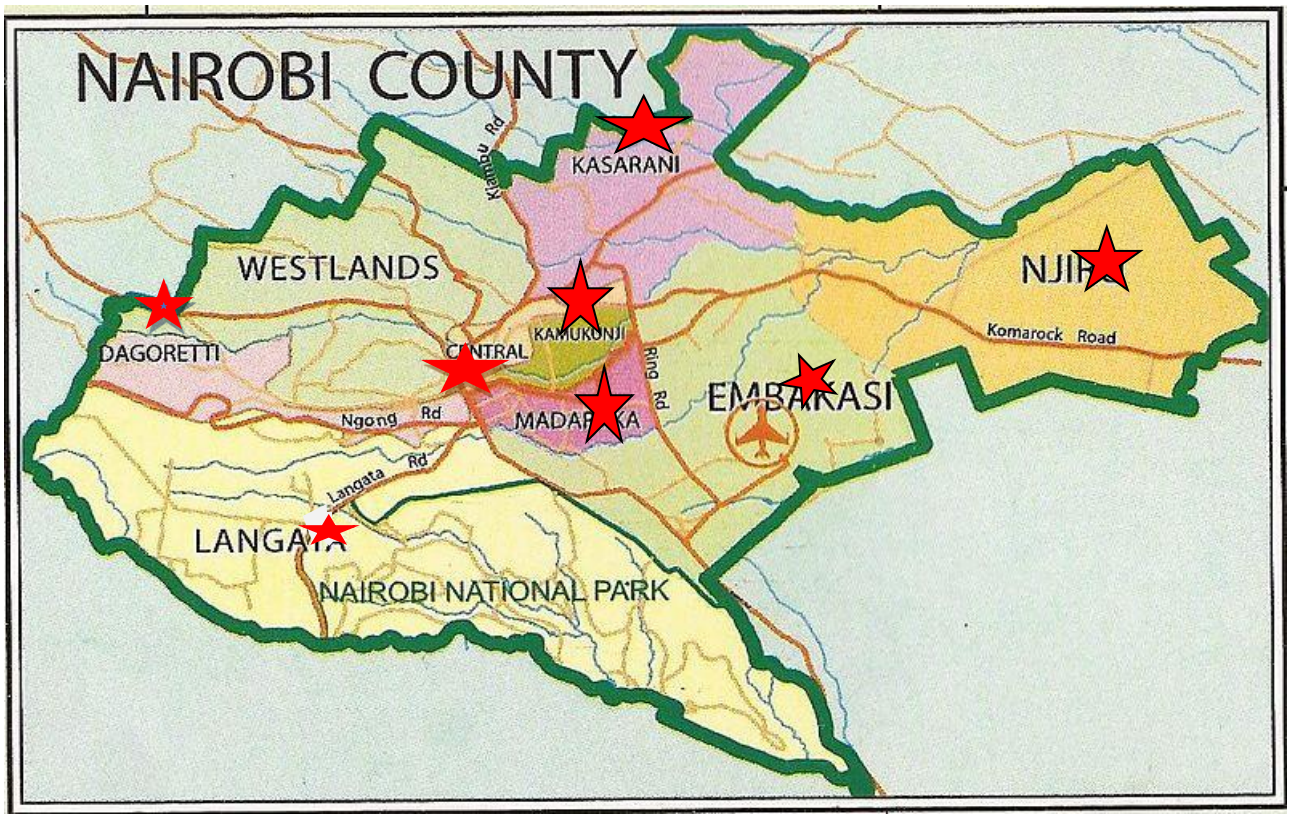
APPENDICES

Appendix 1: Map of Nairobi County

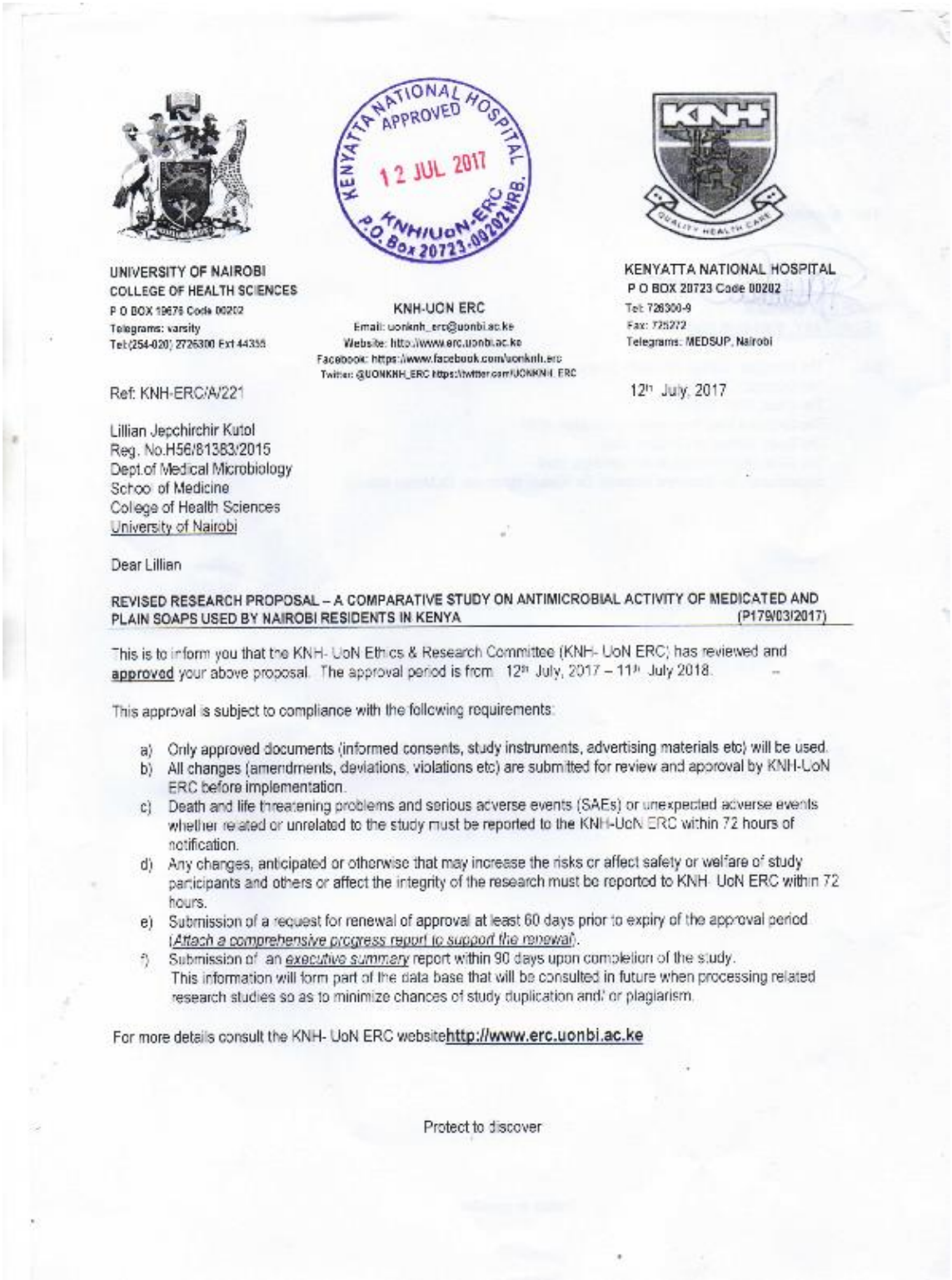


Key: ★ **sites where the soaps were purchased**

Appendix 2: Data extraction tool

	Brand names of soaps	**Codes**	**Soap type**
1.	Geisha	NMS 001	Non-medicated
2.	Flamingo	NMS 002	Non-medicated
3.	Imperial	NMS 003	Non-medicated
4.	Lux	NMS 004	Non-medicated
5.	Fa	NMS 005	Non-medicated
6.	Palmolive	NMS 006	Non-medicated
7.	Menengai	NMS 007	Non-medicated
8.	Geisha	MS 001	Medicated
9.	Diva	MS 002	Medicated
10.	Lifebuoy	MS 003	Medicated
11.	Protex	MS 004	Medicated
12.	Dettol	MS 005	Medicated
13.	Safeguard	MS 006	Medicated
14.	Kinga antibacterial soap	MS 007	Medicated

Appendix 3: Ethical clearance letter



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12th July, 2017

Lillian Jepchirchir Kutol
Reg. No.H56/81383/2015
Dept.of Medical Microbiology
Schoo of Medicine
College of Health Sciences
University of Nairobi

Dear Lillian

REVISED RESEARCH PROPOSAL – A COMPARATIVE STUDY ON ANTIMICROBIAL ACTIVITY OF MEDICATED AND PLAIN SOAPS USED BY NAIROBI RESIDENTS IN KENYA (P179/03/2017)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and approved your above proposal. The approval period is from 12th July, 2017 – 11th July 2018.

This approval is subject to compliance with the following requirements:

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

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

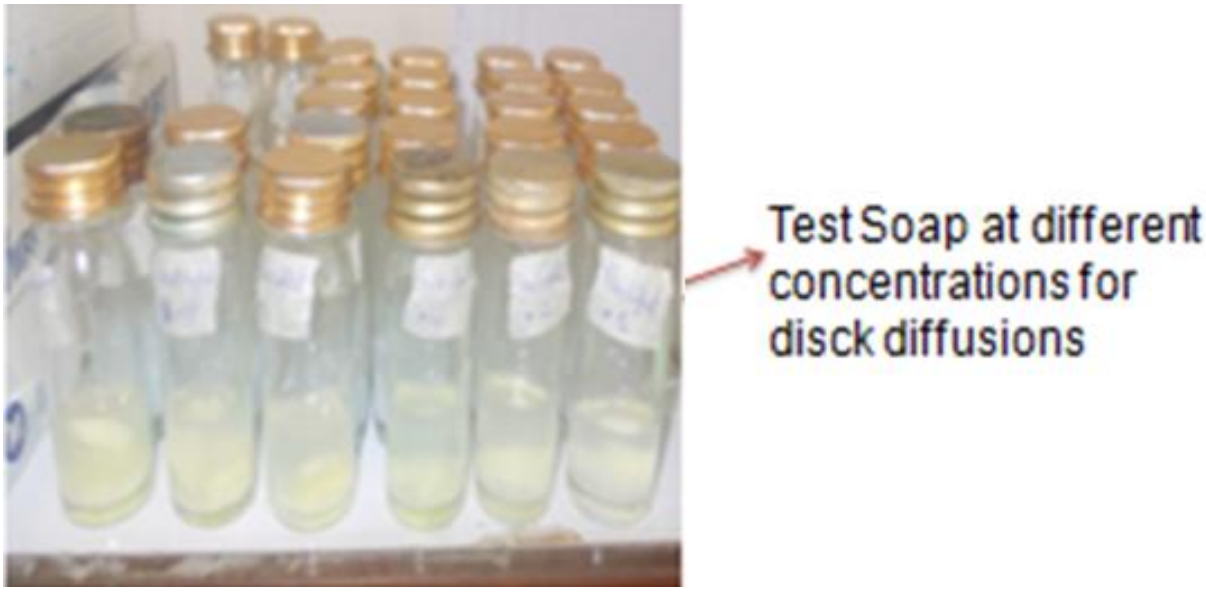
Protect to discover

Appendix 4: Codes of the different soaps tested

Soap code	Soap
MS001	Geisha Germiguard
MS002	Diva
MS003	Lifebuoy
MS004	Protex
MS005	Dettol
MS006	Safeguard
MS007	Kinga
NMS001	Geisha
NMS002	Flamingo
NMS003	Imperial Leather
NMS004	Lux
NMS005	Fa
NMS006	Sunlight
NMS007	Menengai

Appendix 5: Solutions at different concentrations

The soaps were formulated into different concentrations beginning from 1000mg/10ml, 800mg/10ml, 600mg/10ml, 400mg/10ml, 200mg/10ml and finally 100mg/10ml (Plate 4.1).



Appendix 6: Soap diluted at different concentration in broth for MIC determinations

Broth dilutions for MIC

Appendix 7: Poster presented in the 4th African International Biotechnology and Biomedical Conference

Comparison of Antimicrobial Activity of Medicated and Plain Soaps used by Nairobi Residents in Kenya

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AFFILIATIONS: 1. University of Nairobi, 2. County government of Nakuru

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Introduction

The main function of soap is cleaning, killing and removing microorganisms. Some soap's additionally have antibiotics and are thus referred to as medicated soaps. However, there is insufficient information on the enhanced efficacy of medicated soaps compared to plain soaps in Kenya.

Objective

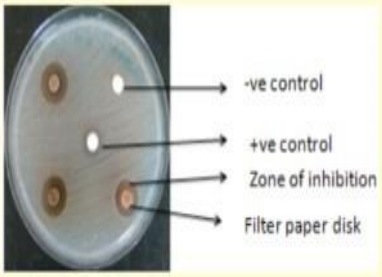
The study aimed at comparing the antimicrobial properties of medicated and plain soaps used in Nairobi, Kenya

Methodology

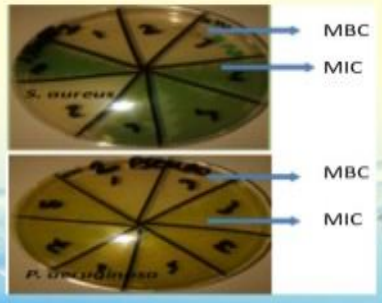
This was an experimental laboratory based design. Purposive sampling method was used to collect different brands of medicated and plain soap in the market and delivered to University of Nairobi for processing. The soaps samples were prepared using standard techniques for antimicrobial activities against selected pathogenic microorganisms as recommended by Clinical Laboratory Standard Institute

Results

All the soap brands were effective against *P. aeruginosa* with average inhibitory zone diameters of 9.1 for non-medicated soaps and 9.0mm for medicated soaps.



In terms of MBC and MIC, medicated soap 005, 007 and non medicated soap (NMS007) were very active against *S. aureus* with MBC of 62.5mg/ml and MIC of 32.25mg/ml, respectively.



Statistical analysis revealed that there was significant association between the MIC and MBC of both medicated and non-medicated soaps tested in this study (P≤005).

Conclusion and Recommendations

- The study showed that some non-medicated soap can be more effective compared to medicated soaps
- Some medicated soaps are not effective at all
- The choice of soap should be that which is effective against disease causing bacteria at low concentrations
- The public should be advised that not all medicated soaps have antimicrobial activities against all the microorganisms encountered in nature.

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Appendix 8: Plagiarism Report

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Appendix 9: Sampling frame of non-medicated soaps

	Brand names of soaps		**Brand names of soaps**
1.	Geisha	15	Gaea
2.	Flamingo	16	Nivea
3.	Rexona	17	White wash
4.	Jamaa	18	Dove
5.	Lux	19	Lido
6.	Palmolive	20	Sawa
7.	Msafi	21	Bidco cream
8.	Imperial Leather		
9.	Pears		
10.	Menengai		
11.	White wash		
12.	Sunlight		
13.	Olive		
14.	Fa		