MICROBIAL CONTAMINANTS ISOLATED FROM ITEMS AND WORK SURFACES IN THE POST- OPERATIVE WARD ATKAWOLO GENERAL HOSPITAL, UGANDA

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DECLARATION

I declare that the contents of this report were compiled originally by myself and to the best of my knowledge have never been submitted to any other institution or university for any award or qualification.

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This dissertation has been compiled under the careful supervision and guidance of the following supervisor

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DEDICATION

To my mother Norah Sserwadda for the encouragement offered during the course, to my brother Mawanda Allan for constantly standing by my side through all the pain and struggles, and lastly to my fiancé Brenda Nakamwadda for constantly keeping me on the right path I am destined to follow. My deepest appreciation and love to all of them and the rest of my friends.

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LIST OF ABBREVIATIONS AND ACRONYMS

BA…………………………………………………...Blood Agar

CDC…………………………………………………Centre for Disease control

CLSI………………………………………………...Clinical Laboratory Standards institute

HAI…………………………………………………. Hospital Acquired Infections

IDC…………………………………………………. Infectious Disease Control

MDR..Multi drug resistant

MOH………………………………………………...Ministry of Health

MRSA…………………………………………….Methicillin Resistant Staphylococcus aureus

PDR...Pandrug resistant

SPPSS………………………………………………. Statistical Package for Social Sciences

SOP…………………………………………….......Standard Operating Procedures

Spp...Species

TSI…………………………………………………...Triple Sugar Iron

VRE………………………………………….............Vancomycin Resistant Enterococci

XDR... ..Extensively drug resistant

ABSTRACT

Introduction: Medical care equipment and hospital work surfaces play a major role in the accommodation of pathogenic bacteria. As such, they act as modes of transmission of these organisms among patients giving rise to nosocomial infections. Consequences of such infections include morbidity and mortality of the patients and increased antibiotic resistances amongst others.

Aim of the study: This research was aimed at determining the microbial contaminants present on medical items and work surfaces in the post-operative surgical ward at Kawolo general hospital and their susceptibility patterns to antibiotics.

Methodology: A cross sectional and laboratory based study was conducted for the research. Sterile swabs were used to collect samples from medical equipment including scissors, infusion stands, beds and work surfaces which were composed of tables, sinks and taps. The samples were processed and the antimicrobial susceptibility patterns of the identified isolates were assessed and further classified as MDR, XDR and PDR.

Results: Out of the total 138 swabs that were collected and processed, the overall prevalence of bacterial contaminants was 44.2% (61/138). The Gram positive organisms constituted of 75.4% (46/61) while the Gram negative organisms accounted for 24.6% (15/61). All the Gram positive organisms isolated were *Staphylococcus aureus*, while the Gram negative bacteria isolated had *Klebsiella pneumoniae* with 7(11.5%), *Proteus vulgaris, Enterobacter species* and *Serratia merscescans* representing 5(8.2%), 2(3.3%) and 1(1.6%) respectively. The items that had the highest level of bacterial contaminants that were isolated were infusion stands and patient beds from which 12 swabs (19.67%) had isolates followed by tables and sinks represented by 10 (16.39%), scissors with 9 (14.75%) and light switches with 8 (13.11%). Statistically, contaminated patient beds were the most likely medical care equipment that were capable of transmitting organisms to the patients with OR: 20.1 and *p value* 0.0008. Antibiotic susceptibility patterns for Gram positive isolated revealed that vancomycin was the most effective antibiotic with 100% while ciprofloxacin and ceftriaxone were the most effective antibiotics for Gram negative isolates both with 80% sensitivity. MDR for *Staphylococcus aureus* accounted for 52% (24/46) with 4% (1/24) classified as a possible XDR. Gram negative isolates had 27% (4/15) MDR strains out of which 50 %(2/4) were classified as possible PDR.

Conclusion: The high prevalence of bacteria present on the medical equipment at Kawolo hospital is an indicator of ineffective decontamination. A microbiology unit should be put in place for routine isolation and susceptibility testing of bacteria before antibiotic treatment to combat increasing drug resistance trends together with an infection control surveillance system.

CHAPTER ONE
INTRODUCTION

1.1 Background to the study

The contribution of the hospital or health facility environment as a reservoir to the transmission of bacterial pathogens is very significant as it accommodates patients with both diverse microorganisms and a large number of immune- compromised individuals. As such, equipment used in hospital post-operative surgical wards such as forceps, scissors, catheters, scalpels and speculums among others are often contaminated with a variety of bacterial pathogens and act as fomites, or transmission vehicles to the humans.

The ultimate consequence is acquisition of nosocomial infections, which are infections that patients were not infected with initially, but rather acquired them as a result of staying within the hospital facilities (Girard *et al,* 2002).

These nosocomial infections, also called hospital acquired infections (HAI) have a lot of devastating implications affecting both developed and developing countries which range from increased patient morbidity to elevated mortality rates of the patients. These infections also lead to prolonged hospital visits, increased antimicrobial resistance, and disabilities in the affected patients which greatly reduce the quality of life.

It is estimated that 90% of the nosocomial infections identified in humans are caused by bacteria while the remaining 10% is caused by fungi and protozoans (Bereket *et al,* 2012). The global burden of these infections has been difficult to determine however in 2010, CDC reported that the mortality rates due to nosocomial infections alone are higher than the deaths due to motor accidents, HIV and breast cancer combined annually. It further emphasized that closely 271 deaths per day occur as a result of such infections with a morbidity of over 1.8 million people per year. A WHO (2011) factsheet indicates that the incidence of these nosocomial infections at any one time varies from 3.5% - 12% and 5.7% - 19% in developed and developing countries respectively.

A variety of bacterial pathogens have been isolated and identified as nosocomial over the years among which *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, Klebsiella species, Salmonella species, Shigella species, Proteus vulgaris* and *Proteus* *mirabilis* were the most commonly isolated contaminants found on hospital equipment (Ummu *et al,* 2013).

Another study by William *et al* (1996) carried out in North Carolina showed that Coagulase negative *Staphylococcus* (56%), *Bacillus* (22%) *and Diptheroids* (14%) were the prevalent bacteria that were isolated from surgical equipment causing nosocomial infections whereas in another research done in Tokyo Japan, *Staphylococcus species, Micrococcus species, Enterococcus species, Stenotrophomonas maltophilia* and *Candida utilis* were among the bacteria isolated from the forceps used in surgery (Saito *et al*, 2014).

In a study performed in Ghana to assess the environmental impact on post-operative wound infections in a private hospital found that, 86% of the post-operative samples that were collected had bacterial isolates with a very strong correlation between the wound isolates and environmental isolates for (Acheampong *et al*, 2011). In Uganda relatedly, studies performed in Jinja Hospital and Mulago Hospital revealed than 58% and 68.8% respectively of the post-operative wounds had bacterial infections with *S. aureus* and *E. coli* respectively being most prevalent (Anguzu *et al*, 2007; Kajumbula *et al*, 2011).

Whereas these infections pose life-threatening consequences to the public, these HAIs can be prevented or minimised through education of the public about the infection chain and the importance of cleanliness. Adhering to strict aseptic surgical procedures throughout the entire patient care period can help minimize or prevent the occurrence of such infections. Emphasis on effective hand washing before contact with patients or inanimate objects as the most effective nosocomial infection control measure is paramount since nosocomial infections transmission are exacerbated by hand touches of health workers (Ekrami *et al,* 2011; Acheampong *et al,* 2011).

1.2 Scope of the study

Kawolo Hospital is a General Hospital run by the Buikwe District council under the decentralization system. The hospital opened in 1968 and currently has an in-patient capacity of 112 beds. It serves a population of about 1.2 million people from the districts of Buikwe, Buvuma and Mukono. It has a wide range of services which include: outpatient, ICU, radiology, surgery, gynecology and laboratory among others however, emphasis was put on the post-operative surgical ward.

Commonly used equipment in the ward like infusion stands as well as the work surfaces for instance tables and sink taps among others were swabbed to establish the level of contamination in relation to nosocomial infections. The study was carried out from April to June 2015 and the data obtained was analyzed and compiled by the end of June 2015.

1.3 Statement of the problem

During surgical interventions, patients often undergo traumatic and invasive procedures involving minor and deep incisions thus rendering them extremely vulnerable to attacks by nosocomial microorganisms. In case the equipment and or surfaces used during patient recovery in the post-operative ward are contaminated with such organisms, the patients are highly susceptible to nosocomial infections. Closely, 99,000 lives are claimed by HAIs per annum with a heavy economic impact of closely 40 million dollars globally (Brannigan *et al*, 2012). Research has further emphasized a strong correlation of organisms in the post-operative environment to those isolated in the patient wounds (Acheampong *et al*, 2011).

Currently, antibiotic treatment of bacterial nosocomial infections has been challenged by the development of resistance to the various antimicrobial agents used of which methicillin-resistant *S. aureus*, penicillin resistant pneumococci and vancomycin resistant *S. aureus* among others have been identified to pose the greatest threat (Aschalew, 2011; Weber *et al*, 1999). These findings therefore, suggest that poor disinfection of the post-operative surgical ward articles and environment can predispose the patients in recovery to infections consequently leading to nosocomial infections.

Kawolo hospital has a well-established post-operative surgical unit yet there has not been any study performed to assess the significant role the environment plays in the harboring nosocomial pathogens as well as their antibiotic susceptibility profile. Therefore, this study was aimed at contributing towards identification of the most prevalent pathogens and their antimicrobial susceptibility patterns.

1.4 Main objective

To identify the microbial contaminants isolated from medical items and work surfaces in the post-operative surgical ward at Kawolo general hospital.

1.4.1 Specific objectives

1. To establish the predominant bacterial contaminants present on medical care articles.
2. To determine the medical care instruments and work surfaces with the highest bacterial contamination.
3. To determine the antimicrobial susceptibility pattern of the bacterial isolates to commonly used antibiotics in the surgical ward at Kawolo general hospital.

1.5 Significance of the study

The research was performed to provide necessary information about the predisposure of nosocomial infections in the post-operative surgical ward and as such, provide evidence based information about the most prevalent bacterial contaminants as well as common hospital equipment contaminants. Such information is necessary for formulation, designing and implementation of effective prevention, control and monitoring programs to minimize spread of hospital acquired infections at Kawolo general hospital.

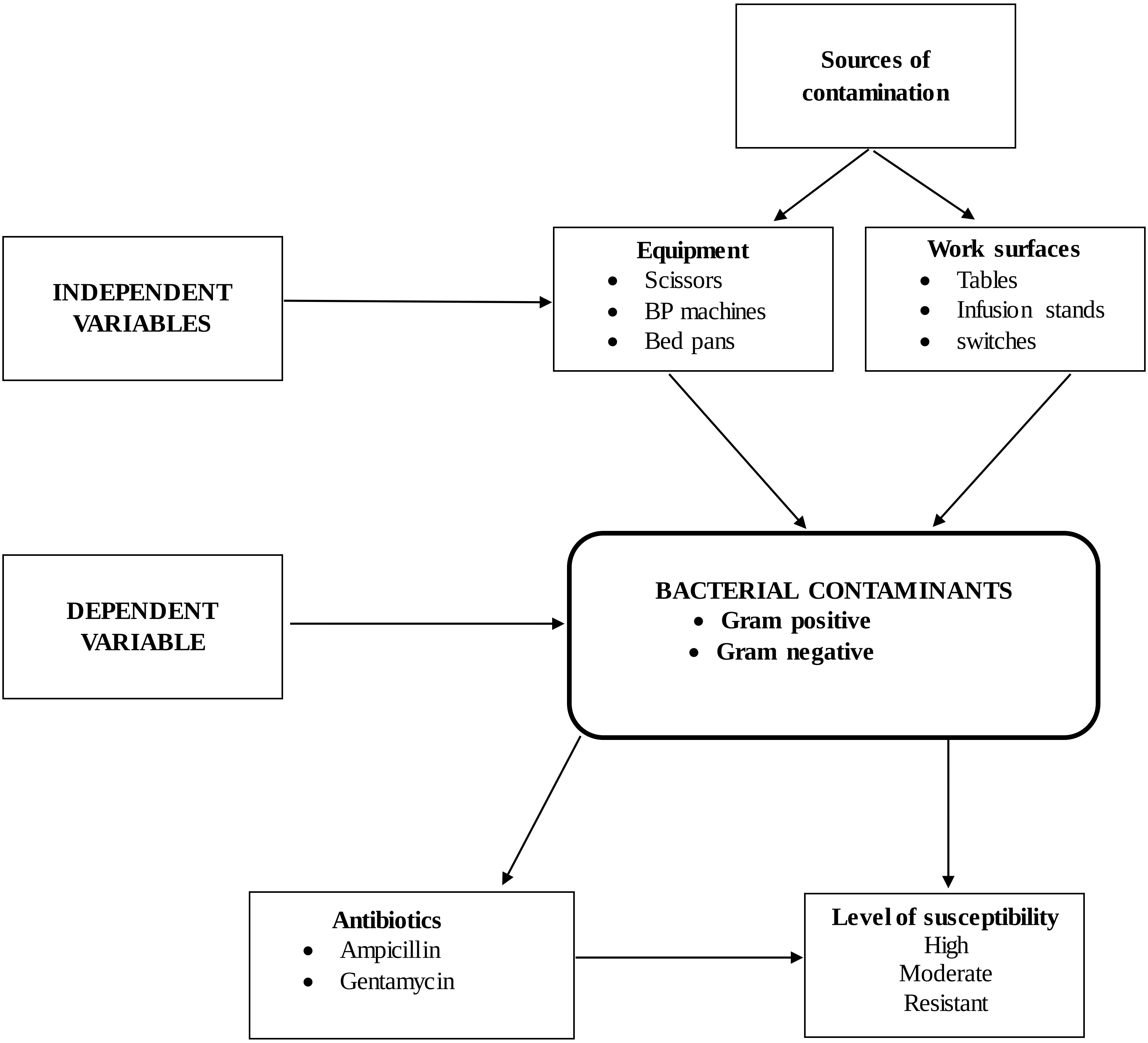
The antibiotic susceptibility profile in this study will provide knowledge of the antibacterial agents that can be used to effectively treat the patients and minimize wastage of medical resources during the management of nosocomial infections brought about by empirical use of antibiotics at Kawolo general hospital.

Since no such research had ever been done before by any one at Kawolo general hospital, the information from this study will be used as a source of knowledge to fill the information gap at the health facility and a reference for other researchers who wish to undertake similar studies.

1.6 Research questions

1. What is the predominant bacteria type isolated from medical ward?
2. Which is the most commonly contaminated post- operative surgical ward article and work surface?
3. What is the antimicrobial susceptibility pattern for the isolates identified to the commonly used antibiotics at Kawolo general hospital?

1.7 Conceptual framework



Medical care equipment like scissors and bed pans together with work surfaces for instance working tables and infusion stands acted as sources of infection in the transfer of microbial agents to the patients in the ward. The type of bacteria (dependent variable) isolated (Gram negative or positive) influenced which antibiotic was utilized. The type of bacteria and the antibiotic agent utilized thereafter influenced the susceptibility patterns.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of equipment microbial contaminants

The surgical ward is a very critical and by far the most important section in any medical facility. As such, absolute sterility of all items ranging from the medical equipment used, to the medical personnel involved is a must if quality health care is to be achieved. However, in African setting, there has been failure to achieve this goal mainly due to the widespread of microorganisms within the favorable environmental conditions that are prevalent within the continent. According to a study done in Nigeria, nosocomial infections which are the ultimate consequence of such bacterial agents have gained public health attention worldwide due to the increase crowding of people, immunocompromization of patients as a result of illness and senescence (Ochie, 2009).

2.2 Global burden of nosocomial infections

Over the years it has been difficult to establish the worldwide prevalence of nosocomial infections at an incident point in time. However, many studies have been done in different regions at different times to establish the rates and types of infections that are prevalent. The nosocomial rates of infection have the largest impact on developing countries especially in Africa than the already developed countries in Europe and America. Actually, recent systematic reviews have estimated that the prevalence of HAIs is 7.6% and 10.1% in high-income countries and low/middle-income countries respectively (Nejad *et al*, 2011). Only 23 developing countries out of 147 accounting for 15.6 % were identified to have a functional national surveillance for HAIs from a survey conducted by WHO First Global Patient Safety Challenge.

In European countries, the European Centre for Disease control reported a HAIs overall prevalence of 7.1% in which 4131000 patients are affected by about 4544100 episodes on average per year.

A study in Pakistan revealed a nosocomial infection frequency of 29.13% with urinary tract infections contributing 39.1%, respiratory tract infections 30.1% and blood stream infections 27.3% within the intensive care unit (Shaikh *et al*. 2008).

Another study done in selected hospitals in the Gaza strip in Palestine revealed that 24.7% from the total 243 of the swabs collected from equipment, environment and personnel were contaminated with microorganisms with each having an overall prevalence of 45%, 48.3% and 6.7% respectively (Nahed, 2011).

2.3 Burden of nosocomial infections in Africa

A systematic review to establish the incidence of nosocomial infections in Africa from 2004 - 2009, the prevalence of HAIs was reported to range between 2.5 - 14.8% among the hospitals that were studied. Furthermore, the surgical wards had the largest number of studies and had a cumulative incidence of HAIs ranged from 5.7 – 45.8% with the highest focus placed on SSI that reported a prevalence of 2.5 – 30.9% (WHO, 2011).

Another research to identify the pathogenic bacteria in wounds from patients in Nigeria had *Staphylococcus aureus* isolated from 33(42.30%), *Pseudomonas aeruginosa, Proteus mirab ilis, Escherichia colI* from 25(32.90%), 10(12.80%), and 10(12.80%) respectively from a total of 45 surgical wound specimens that were analyzed (Nwachukwu *et al*, 2009). In Addis Ababa, a prospective study to identify the prevalence of microbial agents responsible for causing infections in a tertiary hospital, a total of 250 patients from ICU and surgical ward demonstrated that 35.8% developed nosocomial infections with the SSI accounting for 29.8%. *E. coli* 11.4%, P. *aeruginosa* 22.7%, *K. pneumoniae* 15.9%, *P. vulgaris* 13.6%, *E. cloacae* 2.3%, *K. oxytoca* 4.5%, C. *braakii* 4.5%, and S. *aureus* 15.9% were some of the isolated bacterial pathogens in SSI (Endalafer *et al*, 2011).

2.4 Burden of nosocomial infections in Uganda

Information about the incidence and prevalence of such infections in Uganda is very limited. In particular, little or no recent information has been found for studies done in the surgical wards of hospitals.

A cross sectional study was carried out in Jinja Hospital, Uganda on drug sensitivity patterns of bacterial isolates from septic postoperative wounds. 58.5% of the specimens contained pathogenic bacteria among which included: *S. aureus* 45.1%, *P. aeruginosa* 9.9%, *Klebsiella pneumoniae 7.0*%, Coliforms 16.9%, *Proteus mirabilis* 11.3% and *Enterobacter spp* 2.82% (Anguzu *et al*, 2007).

At Mulago national referral hospital, a descriptive cross sectional study was done from September 2011 to April 2012 to establish the microbial resistance in hospitalized patients for 314 patients where 68.8% of the swabs collected yielded bacterial isolates of which *E. coli and S. aureus* accounted for 23.7% and 21.1% respectively (Kajumbula *et al*, 2011).

No information is available for the prevalence of such nosocomial infections as no studies have been published yet at Kawolo general hospital.

2.5 Methods used in recent studies

Previous similar studies have been done to assess the microbial contaminants found on medical equipment and have involved the use of various methods for sampling and processing of the samples collected. The basis for choosing their respective sample sizes was a decision left unto the researchers. The table below summarizes their methods and respective findings:

Author	Country	Study design	Sample size	Results
Tesfaye *et al* 2011	Ethiopia	Cross-sectional	176	93% prevalence
Nahed Al Laham 2011	Palestine	Cross-sectional	243	24.7 % prevalence
Anywar *et al* 2011	Uganda	Cross-sectional	122	11% prevalence
Ochie *et al* 2009	Nigeria	Cross-sectional	142	27.2% prevalence

2.6 Causative agents of nosocomial infections

It is estimated that 90% of the nosocomial infections identified in humans are caused by bacteria among which include: *Staphylococcus aureus, Streptococcus spp, Bacillus cereus, Klebsiella pneumonia, Escherichia coli, coagulase-negative Staphylococcus, Pseudomonas aeruginosa* and *Enterococci* (Bereket *et al*, 2012), while the remaining 10% is accounted for by protozoan and fungal infections like Candidiasis.

Available data from recent studies has indicated that *Escherichia coli* and *Staphylococcus aureus* are the most frequently isolated organisms responsible for causing those nosocomial infections in surgical wards. However, over the past decades, coagulase-negative Staphylococcus have greatly emerged at tremendous rates among the affected patients. A study conducted in China showed that nosocomial infections occurred in 26 out of the 923 investigated patients giving an overall prevalence of 2.8%, with 50% originating from the ICU and *Escherichia coli* accounting for 23.1% of the isolates obtained (Xiang *et al,* 2011). Another study done in Jacksonville Florida (Magill *et al*, 2011) reported a nosocomial infection prevalence of 6% with surgical site infections (SSI) accounting for 18% being most frequent.

2.7 Routes of Nosocomial infections

There are various mechanisms through which nosocomial pathogens gain entry into the body of human beings and cause infections the commonest being direct contact mode of transmission. It involves a direct body surface-to-body surface contact and physical transfer of microorganisms between a patient with the infection and another person who acts as a susceptible host.

Indirect contact transmission is one where inanimate instruments or fomites such as needles, stethoscopes, medical dressings and gloves become contaminated with nosocomial pathogens which come into contact with susceptible hosts and later manifest as infections in patients. This is the most common infection pathway accounting for the highest infection rates in the surgical ward.

Droplet transmission occurs when organisms contaminating droplets from an infected person are propelled to a susceptible host via sneezing, coughing and talking during procedures like bronchoscopy. On addition, transmission of organisms like *Mycobacterium tuberculosis, Legionella* and *Varicella* viruses can occur by airborne transmission where air currents containing infective microbes are dispersed from one host to another.

Other mechanisms of transmission include vehicular intercession where food, medical invasive devices, and water act as the sources of the nosocomial infections and vector borne mode which involves the spread of nosocomial microbes to susceptible hosts through mosquitoes, flies and rats among others.

2.8 Prevention and control of nosocomial infections

HAIs have a deleterious impact on the health of the patients and the medical workers which exerts a negative impact on productivity and the economy of the nation. Because of that, protocols to minimize the spread of such infections were formulated by Rosenthal *et al* (2014) some of which can be summarized as below:

- Effective screening of patients who are known carriers of epidemic bacterial strains requiring special isolation in intensive care units to prevent spread of infections to other patients.
- Hand hygiene by thorough washing hands with soap and water and also used of alcohol based hand-rubs before and after touching the patients.
- Following standard precaution measures like the use of protective wear for example gloves, closed shoes, clinical gowns and masks before coming into contact with any patient regardless of their health status.
- Thorough cleaning and decontamination of the health facility floors, walls, bedrails, bedside tables, doorknobs, medical equipment and all patient-care areas using EPA registered disinfectants and regular cleaning 2-3 times a day.
- Ventilation of rooms with HEPA filters that are regularly monitored and frequently maintain to reduce on entry of pathogens into the ICU and surgical wards.
- Continuous monitoring and surveillance of pathogens to minimize infections and prevent any outbreaks within the hospital and public settings through observations, interviews, surveys, inspections and quality assurance activities.
- Provision of prescheduled trainings to the health workers and the general public about the dangers of nosocomial infections and their control measures.
- Continuous monitoring of the antibiotic prescriptions at all levels and their usage to combat drug resistances among the patients.

CHAPTER THREE
METHODS AND MATERIALS

3.1 Study design

The study was a cross sectional where samples were collected from medical articles and work surfaces, and analyzed between May and June 2015. The antimicrobial susceptibility of the isolated organisms was assessed by the study.

3.2 Study location

The study was done at Kawolo Hospital which is run by the Buikwe District council under the decentralization system. The hospital was started in 1968 and currently has an in-patient capacity of 112 beds serving a population of about 1.2 million people from the districts of Buikwe, Buvuma and Mukono. It has a wide range of services which include: outpatient, ICU, radiology, surgery, gynecology and laboratory among others. A number of surgery patients are handled by the hospital daily with majority admitted in the post-operative ward.

3.3 Study population

Areas with high probability of constant hand contact for medical devices including scissors, infusion stands, and beds; work surfaces including tables, sink taps and light switches were swabbed. Air was not sampled for any contaminants.

3.4 Sample size determination

The study sample size was determined using a formula adopted from (Keish *et al*, 1965) stated as follows:

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

Where: N is the sample size

Z is the score corresponding to 95% confidence interval

P is the assumed prevalence = 11%

Q = (1-P)

I is the accepted error term corresponding to 5%

Therefore;

$$N = \frac{1.96^2 \times 0.1 \times 0.9}{0.05^2}$$

$$=138.29$$

$$N \approx 138 \text{ samples}$$

3.5 Study variables

The dependent variable or outcome of the research was the bacterial contaminants that were isolated from the post-operative surgical ward.

The independent variables that exerted an effect on the outcome of the study were the work surfaces, ward equipment and antibiotics used.

3.6 Sample unit

A swab picked from medical equipment and work surfaces was considered as the sample unit of the research.

3.7 Sampling technique

Simple random sampling technique was used for the selection criteria. Emphasis was put on areas that encountered constant contact with hands. Sterile swabs moistened in normal saline were used on an area of approximately 10cm from either the medical care devices including scissors, infusion stands and beds, or work surfaces including light switches, sink taps and tables following standard operating procedures. For each equipment or surface mentioned above, a total of 23 swabs picked randomly was collected to constitute the total sample size calculated.

The swab samples were recapped immediately to prevent contamination and labeled using a marker with the identification number and the site from which they had been collected.

The samples were temporarily placed in a cool box maintained at 4^oC and transported immediately to the Microbiology laboratory and processed within 30 minutes.

3.8 Sample processing

On arrival at the laboratory, the swabs were inoculated onto the prepared culture media which consisted of blood agar plates and MacConkey plates. These plates were then incubated at 37^0C for 24 – 48 hours and inspected for bacterial growth shown in picture 1 in appendix IV.

3.9 Preliminary identification methods

Presumptive characterization of bacteria was done following the Gram stain reactions, colonial characteristic features like hemolysis on blood agar, variations in differential media shown by color changes and enzymatic activities of the organisms (Cheesbrough, 2000; Elmer *et al*, 1997).

3.10 Biochemical tests

Colonies from primary cultures were extracted and used for carrying out biochemical tests for the final isolation and identification of the organisms.

Gram positive cocci were isolated and identified based on their reactions in Gram stain, catalase and coagulase tests (Cheesbrough, 2000).

Gram negative rods were identified by performing a range of tests that included; motility tests, TSI, urease, indole, oxidase, and Simon's citrate agar illustrated by picture 2 in appendix IV

3.11 Susceptibility testing

The drug diffusion disc technique developed by Bauer *et al* (1966) was used to assess the susceptibility of the bacterial isolates to the common antibiotics used for treatment at Kawolo hospital. The test organism was picked using a sterile wire loop, emulsified in sterile broth and incubated for two hours to let the organisms attain their log growth phase. The density of the suspension was matched with the standard opacity of 0.5 McFarland barium sulphate solution, after which a sterile swab containing the isolate was uniformly seeded onto Muller Hinton agar plate (appendix III). Antibiotic discs impregnated with different drugs were placed at prescribed regions on the plate and incubated for 18 – 24 hours at 37^0C. Zones of inhibition which were indicated by the levels of clearing around the antibiotic discs as shown in the pictures 3 and 4 in appendix IV, and interpreted using CLSI 2014 guidelines where sensitivity was shown by clearance around the antibiotic disc and resistance indicated by no clearance measured in millimeters.

The Gram positive isolates obtained were tested against trimethoprim sulfamethoxazole (1.25/23.5µg), penicillin (10IU), erythromycin (15µ g), vancomycin (30µ g), cefoxtin (30µ g) and clindamycin (2µ g).

Gram negative bacterial isolates were tested with ciprofloxacin (5µ g), gentamicin (10µ g), trimethoprim sulfamethoxazole (1.25/23.5µg), chloramphenicol (30µ g), nalidixic acid (15µ g) and ceftriaxone (30µ g).

3.12 Quality control

The authenticity and quality of the results to be obtained was dependent on the integrity of the procedures and protocols that were involved from the time of collection to analysis of the samples collected. As such, SOPs as per CLSI 2014 were strongly adhered to during the pre-analytical, analytical and post-analytical processing of the samples.

Also all the materials and reagents used in the study were adequately controlled to ensure sterility throughout the entire process. On addition to that, all prepared culture media batches were tested for sterility and performance.

As stated by Bauer *et al.* (1966), standard inoculums of bacterial suspension for susceptibility testing were prepared using reference from 0.5 McFarland barium sulphate solution.

Internationally recognized control strains including *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were incorporated into the study.

3.13 Inclusion and exclusion criteria

Only equipment and surfaces used within the post-operative surgical ward environment were sampled and included in the study. Swabs from medical items and work surfaces that grew only fungi after culture were excluded.

3.14 Data analysis

The bacteria isolated were classified according to the colonial characteristics, Gram reactions and biochemical identification tests. The origins of the bacteria identified for example medical equipment and the different working surfaces were identified and noted. The most prevalent isolate was related to the particular surface or equipment from which it was obtained. For the bacteria exposed to antibiotics, the length of clearance was determined to establish the resistant and susceptible isolates to each antibiotic. All the data obtained was analyzed using Stata version 13 statistical package alongside Microsoft Excel. Chi-square tests were used to compute which equipment were most likely to transmit bacteria where *p values* < 0.05 were declared significant. The study findings were represented in graphical illustrations like pie charts, bar graphs, tables and word explanations. Data was also presented in proportions, percentages, absolute numbers and fractions.

3.15 Ethical consideration

Permission was sought from the IHSU ethical committee before engaging in the study. The medical superintendent of Kawolo general hospital was requested and permitted the study to take place at the medical facility. All information collected was treated with extreme confidentiality at each level.

CHAPTER FOUR
RESULTS

4.1 Introduction

A total of 138 swabs were collected from medical care instruments consisting of scissors, beds, infusion stands and work surfaces consisting of tables, sink taps and light switches in the post-operative surgical ward at Kawolo hospital between May and June 2015. The samples were cultured, identification biochemical tests were performed to obtain the respective bacterial isolates, and drug susceptibility testing was done. The results obtained were analyzed using Stata version 13 and Microsoft excel software and presented in form of tables, graphs, pie charts and descriptive statistics as elaborated.

4.2 Growth patterns of the swabs collected

All the collected swab samples were cultured and examined for growth after 24 hours. Out of the total 138 swab samples collected, 61 (44.2%) of them presented with bacterial growth whereas the remaining 77 (55.8%) of the swabs had no growth after 48 hours of incubation.

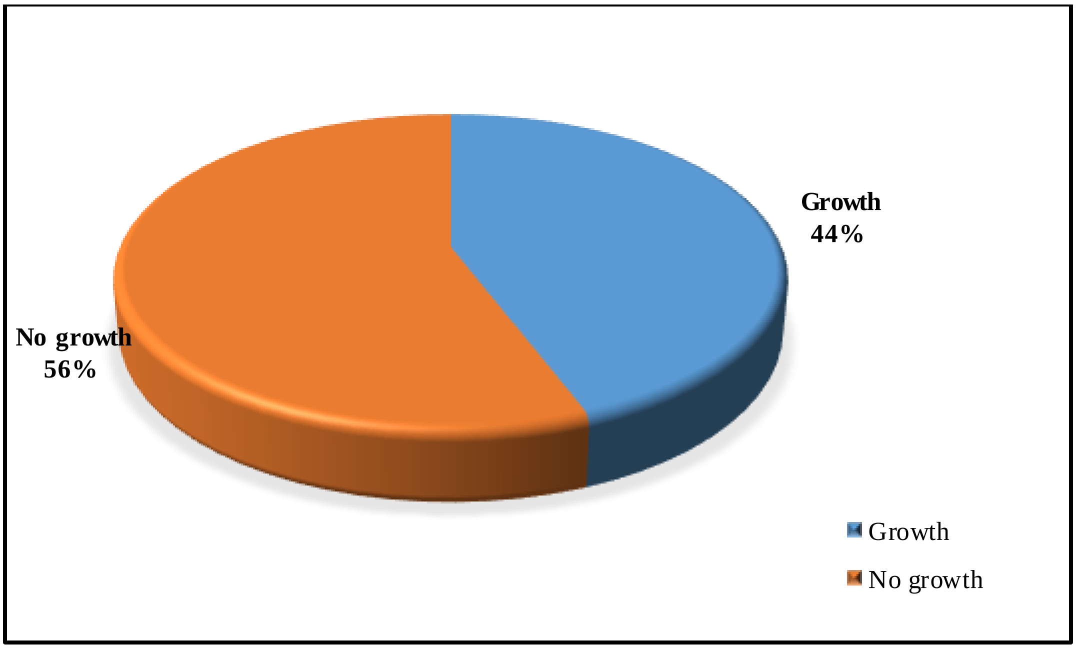


Figure 1: Incubated sample swabs with and without growth after 48 hours

4.3 Gram stain reaction of the organisms cultured

From a total of 61 samples that showed bacterial growth, only 15 (24.6%) were Gram negative while the rest 46 (75.4%) were Gram positive (Table 1).

Table 1: Gram reactions of the isolates obtained

		Frequency	Percent
Valid	Positive	46	75.4
	Negative	15	24.6
	Total	**61**	**100.0**

4.4 Frequency of bacterial isolates

Staphylococcus aureus was the most predominant isolate with 46(75.4%) followed by *Klebsiella pneumoniae* with 7(11.5%), *Proteus vulgaris, Enterobacter species* and *Serratia merscescans* representing 5(8.2%), 2(3.3%) and 1(1.6%) respectively (Figure 2) isolated using the chart in appendix II.

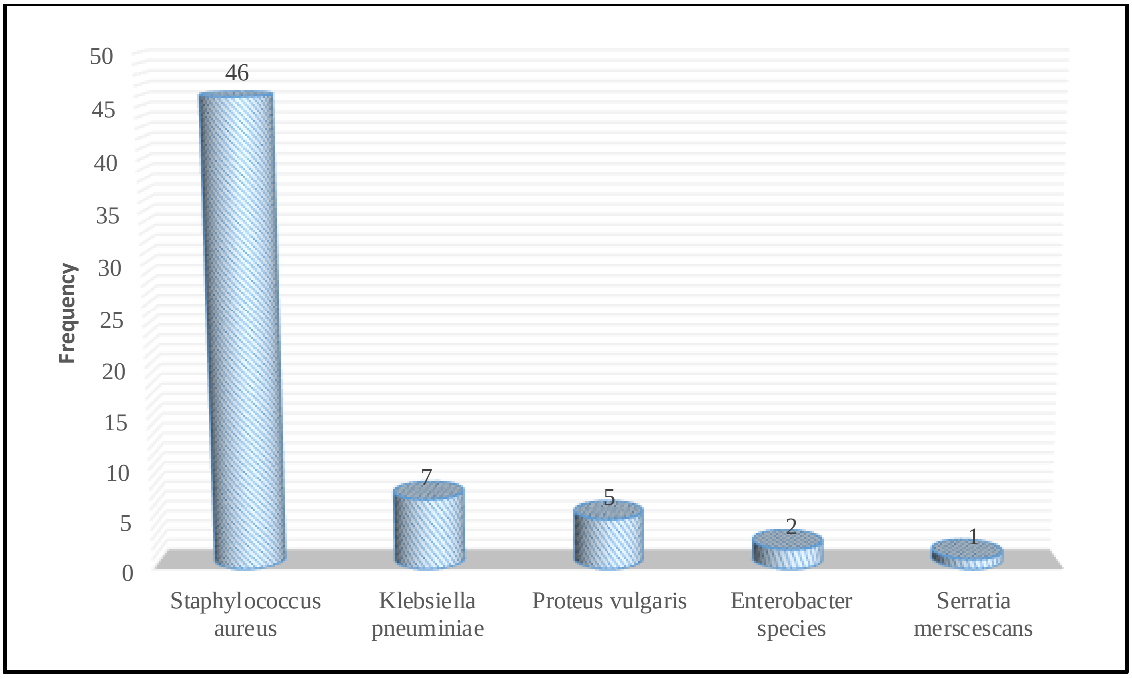


Figure 2: Frequency of the bacteria isolated from the swabs with bacterial growth

4.5 Collection sites of the isolates

The study population was divided into two categories namely: work surfaces which consisted of tables, light switches and sink taps and medical care equipment which consisted of scissors, infusion stands and patient beds. 23 swabs were picked at random from each of the following items with focus placed on areas with constant hand contact in order to increase chances of collecting organisms: tables, patient beds, infusion stands, scissors, sink taps and light switches. Between the two categories, medical equipment generally had the highest levels of bacterial isolates which represented 33(54%) while work surfaces accounted for 28(46%).

The items that had the highest level of bacterial contaminants that were isolated were infusion stands and patient beds from which 12 swabs (19.67%) had isolates followed by tables and sinks represented by 10 (16.39%), scissors with 9 (14.75%) and light switches with 8 (13.11%) accounting for the least contaminated item that was sampled by the study (Figure 3).

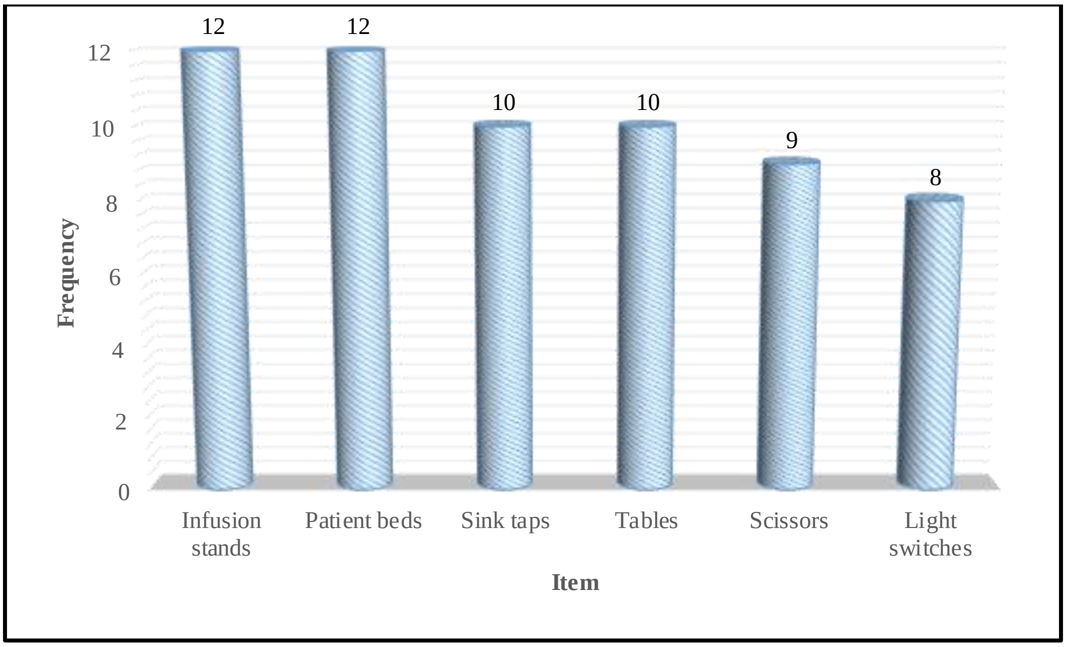


Figure 3: Frequency of isolates recovered from each item sampled

Staphylococcus aureus was the most prevalent bacteria isolated from all the sampled surfaces and equipment. Its distribution and that of the rest of the isolates for each item sampled in the study was tabulated and illustrated (Table 2).

Table 2: Distribution of bacterial isolates on each respective medical item and work surface

Isolate	Infusion stands	Patient beds	Sink taps	Tables	Scissors	Light switches
Enterobacter spp	1	0	0	0	0	1
K. pneumoniae	1	0	1	3	1	1
Proteus vulgaris	0	1	1	1	0	2
S. merscescans	1	0	0	0	0	0
S. aureus	9	11	8	6	8	4

In an attempt to determine which of the items had the highest likelihood to transmit bacterial contaminants to the patients, *Staphylococcus aureus* which was the most predominant isolate that was present and recovered from all the items from both categories was used. Statistical computations revealed that contaminated patient beds were the most likely medical care equipment that were capable of transmitting organisms to the patients with OR: 20.1 and *p value* 0.0008.

Scissors followed with 11 times possibility of introducing the organisms into the patients (OR: 11.7, *p value* 0.0093). Tables, sinks and infusion stands presented with (OR: 7.7, *p value* 0.04), (OR: 5.6, *p value* 0.0320), and (OR: 4, *p value* 0.0439) respectively. However, light switches had OR: 1 and *p value* 1.00 which was not statistically significant.

4.6 Susceptibility patterns of the bacterial isolates

4.6.1 Gram positive antimicrobial susceptibility pattern

Staphylococcus aureus was the only Gram positive bacteria isolated. Their susceptibility patterns were determined against a profile set of 6 antibiotics. Vancomycin showed sensitivity in all 46 (100%) of the Gram positive isolates followed by clindamycin with 38(83%) sensitivity and 8(17%) resistance, trimethoprim sulfamethoxazole with 28(61%) sensitivity and 18(39%) resistance. Both cefoxtin and erythromycin presented each with 22(48%) sensitivity and 24(52%) resistance while the least effective antibiotic was penicillin G with 3 (7%) sensitivity and 43(93%) resistance. Vancomycin together with clindamycin, and trimethoprim sulfamethoxazole were the most effective antibiotics from the results.

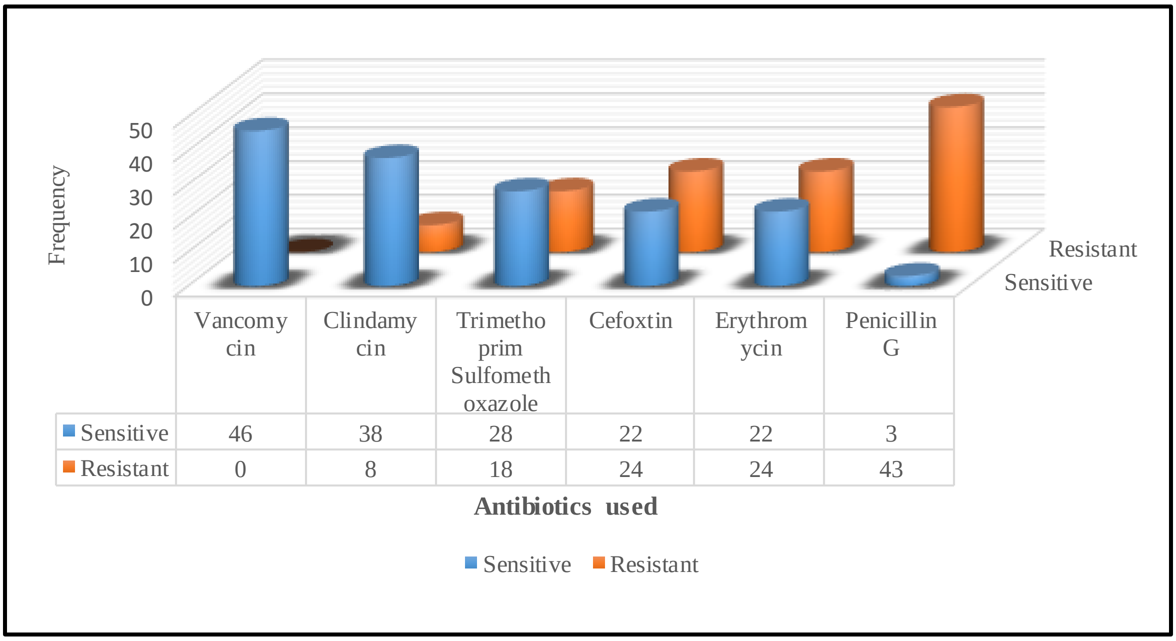


Figure 4: Gram positive antimicrobial susceptibility pattern

MRSA isolates accounted for 24(52%) of *Staphylococcus aureus* isolates which was indicated by their resistance patterns to cefoxtin (Figure 5). Out of the 24 MRSA isolates, 1 isolate (4%) was defined as a possible XDR as per the guidelines provided by the European center for disease control.

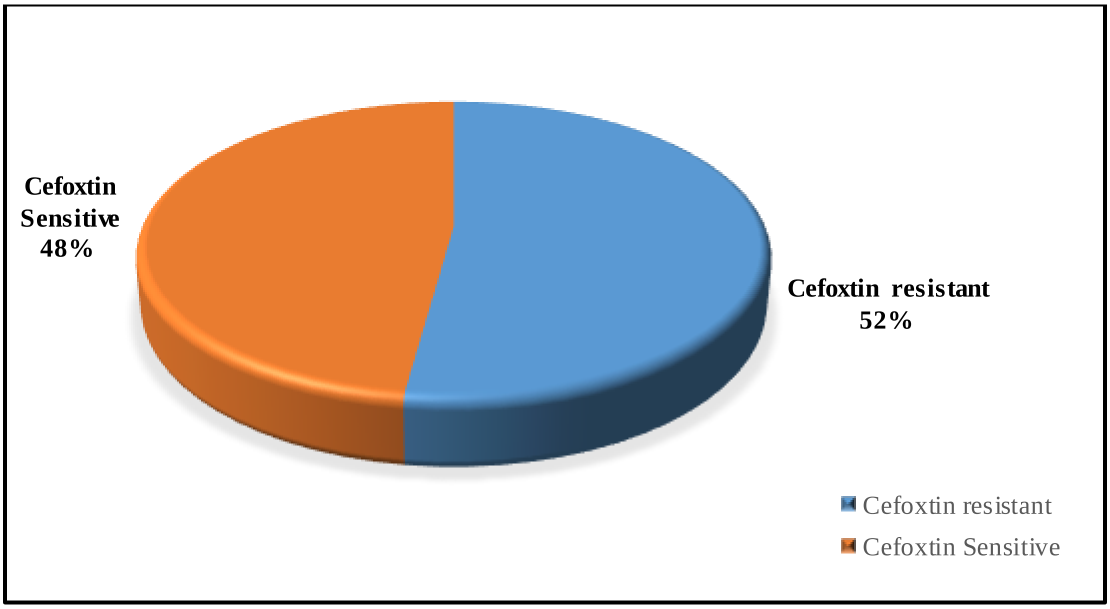


Figure 5: MRSA isolates obtained from susceptibility patterns to cefoxtin

4.6.2 Gram negative antimicrobial susceptibility pattern

Klebsiella pneumoniae 7(47%), *Proteus vulgaris* 5(33%), *Enterobacter species* 2(13%) and *Serratia merscescans* 1(7%) were the isolates obtained in the study representing making a total of 15 isolates. The drug susceptibility patterns for the isolates were tested against a panel of 6 antibiotics and the results revealed that ciprofloxacin and ceftriaxone had the highest sensitivity and lowest resistances of 12(80%) and 3(20%) respectively. Gentamycin had sensitivity of 11(73%) and resistance of 4(27%), trimethoprim sulfamethoxazole with 10(67%) and 5(33%), nalidixic with 9(60%) and 6(40%) while chloramphenicol had the least sensitivity of 5(33%) and highest resistance of 10(67%). This information showed that ceftriaxone, ciprofloxacin and gentamycin are the best drugs of choice for Gram negative isolate infections.

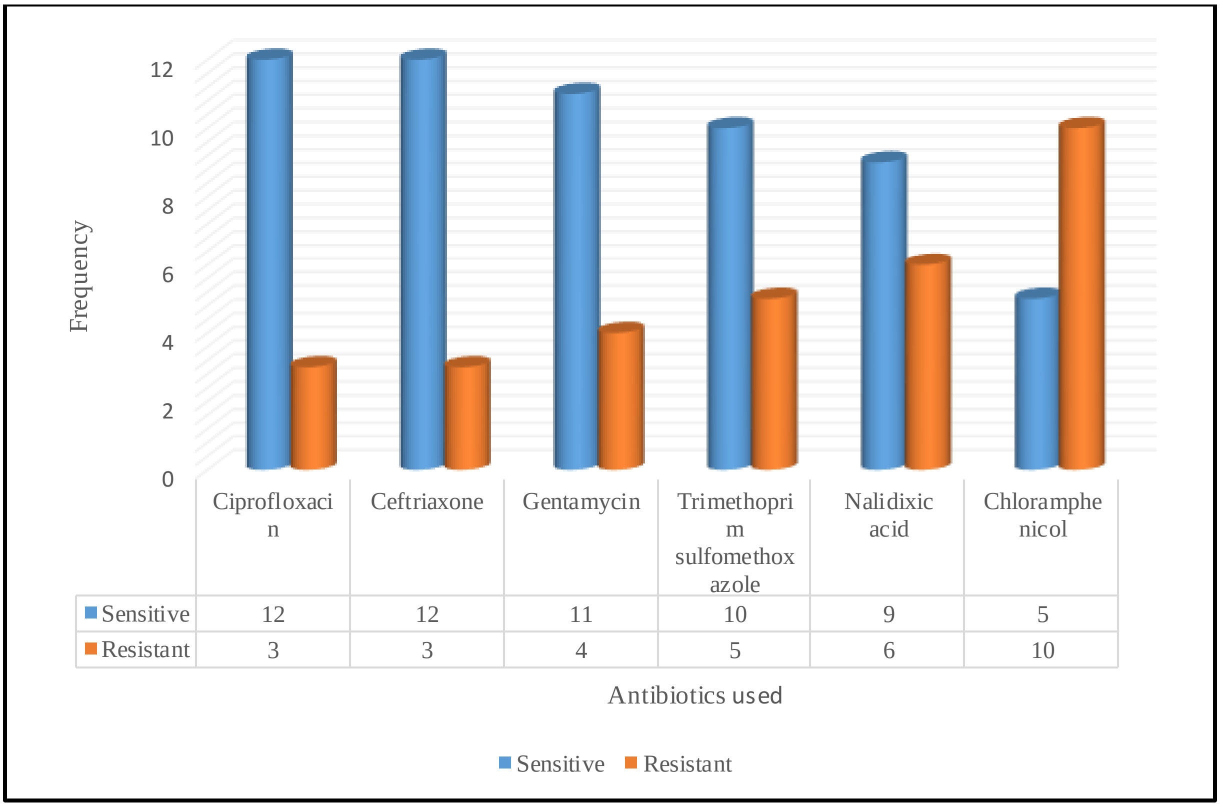


Figure 6: Gram negative isolate antimicrobial susceptibility pattern

The CLSI 2014 guidelines were used to further group the Gram negative isolates according to their resistance degrees; out of the 15 isolates, 4(27%) were classified as MDR as they showed resistance to 3 or more antibiotics classes used. Of the 4 MDR isolates, *Klebsiella pneumoniae* had 1(25%) MDR and 2(50%) being further classified as PDR and while *Proteus vulgaris* accounted for the remaining 1(25%) of the MDR strains.

Table 3: Classification of Gram negative isolates according to their degree of resistance

Isolate	Frequency	Resistance degree
Klebsiella pneumoniae	2	PDR
Klebsiella pneumoniae	1	MDR
Proteus vulgaris	1	MDR

CHAPTER FIVE

DISCUSSION

The results obtained from the cross sectional study carried out at Kawolo hospital from May to June 2015 revealed that the overall prevalence of the bacterial isolates in the post-operative surgical ward was 44% (61/138) which was a considerably high level of bacterial contamination capable of causing nosocomial infections. This high level of contamination is suggestive of poor decontamination, poor sanitation practices and ineffective sterilization of medical care articles. On a related note, the absence of a microbial pathogen surveillance system at the hospital to monitor and control the transmission of potential nosocomial pathogens is another possible situation exacerbating the prevalence as no mitigation strategies are laid ahead of time.

Recent related studies performed within Uganda hospitals have revealed indifferent figures. Jinja and Mulago hospitals reported 58.5% and 68.8% respectively (Anguzu *et al*, 2007; Kajumbula *et al*, 2011) probably because the conditions in all these health facilities are somewhat similar. The prevalence from this study has shown a close relationship with findings in some other African countries. In a study carried out in Nigeria, a prevalence of 47% was reported (Oochie *et al*, 2009) whereas 85.8% was reported from a research done at Jimma university teaching hospital in Ethiopia (Shiferaw *et al*, 2013). Elsewhere in the world, a research performed in Taiwan reflected a prevalence of 63.5% (Chen *et al*, 2014) and 57% from a study carried out in 7 hospitals in Iran (Ekrami *et al*, 2010).

The Gram positive bacteria isolated from the medical equipment and work surfaces was *Staphylococcus aureus* with 75.4% (46/61) constituting the highest prevalence in all the isolates obtained. These results coincide with those from other studies where *Staphylococcus aureus* had the highest prevalence of 30.2% in a study done in Nigeria (Ummu *et al*, 2013), 26.2% in Ethiopia (Wondemagegn *et al*, 2012) and 28.4% in Cameroon (Kihla *et al*, 2014). The high survival and diversity of these organisms is attributed to the presence of surface proteins present in their cell walls which give the bacteria their biofilm formation properties, evasion of host immune responses and tissue adhering abilities enabling them to proliferate both in commensal and invasion modes (Foster *et al,* 2014).

Amongst the Gram negatives*, Klebsiella pneumoniae* 11.5% (7/15), was the most prevalent isolate. Others including *Proteus vulgaris* 8.2% (5/15), *Enterobacter spp* 3.3% (2/15), and *Serratia merscescans* 1.6% (1/15) *were* amongst the isolates obtained. The findings are in harmony with a similar study done by Ekrami *et al* (2014) in which *Klebsiella pneumoniae* was the most predominant Gram negative isolate identified. A study in Algeria demonstrated that the presence of pilli encoded by *mrkD* gene in *Klebsiella pneumoniae* aid in adherence of the organism to host tissues during invasion and biofilm bacterial forms are 10 times more resistant to antibiotics than the uncapsulated forms (Bellifa *et al*, 2013). Furthermore, their ability to resist destruction from many chemical decontaminants due to mutations could have contributed to their survival in the environment.

Both patient beds and infusion stands had the highest bacterial contamination levels of 19% (12/61) compared to the other surfaces and equipment swabbed. This can be explained by the fact that these are some of the articles most frequently in contact with hands during patient care in any hospital setting as most authors have reported that medical workers' hands are the most probable means of transfer (Webber *et al,* 2013). Statistical analysis using Chi-squares to determine the chances for transmission of *Staphylococcus aureus* in medical equipment and work surfaces, it was found that patients' beds were 20 times more likely to transmit the bacteria (OR: 20.1, *p value* 0.0008) as compared to the infusion stands which had just 4 times likeliness (OR: 4, *p value* 0.0439) despite the fact that they both had the same level of bacterial contamination as per the findings of the study. The most probable explanation for these results could be the fact that patient beds have a higher frequency to patient contact than the infusion stands. The beds are responsible for accommodating a vast number of the patients that are admitted within the post-operative ward and yet rarely or no sterilization is done to ensure their decontamination. This renders them highly exposed and most likely responsible for harboring such organisms that can later result into nosocomial infections. Scissors, tables, sinks and light switches also contribute in harboring such organisms that can later gain access into patients and cause infections with devastating effects both health wise and economically.

According to the study, the most effective antibiotic for Gram positive isolates was vancomycin with 100% sensitivity, followed by clindamycin and trimethoprim sulfamethoxazole with 83% and 61% sensitivities respectively. However, an increased resistance was observed for penicillin, cefoxtin and erythromycin with 93%, 48% and 48% resistances respectively. Similar studies revealed 100% resistance rates for penicillin G, and clindamycin (Abdallah *et al*, 2015) and 100% resistance to penicillin G (Kajela, 2013). These trends could be as a result of the increasing poor use and abuse of antibiotics by the general population resulting in a rise in the number of resistant bacterial strains and their relatively high availability as they are cheap to obtain.

The study revealed that 52% (24/46) of *Staphylococcus aureus* isolates were MRSA which accounted for more than half of the organisms isolated. Similarly, another study at Mbarara hospital in Uganda found out that 31.3% of the *Staphylococcus aureus* isolated were resistant to methicillin

(Bazira *et al*, 2014). These two results are indicative of increasing trends of microorganism resistance towards the antibiotics used since this kind of medication is readily available over the counter with limited regulatory use. MDR *Staphylococcus aureus* accounting for 52% (24/46) were isolated in the study of which 4% (1/24) was described as a possible XDR as it was only sensitive to one antibiotic, vancomycin. This shows that there is a very big challenge that is existing in regards to treatment as there is no Microbiology laboratory for isolation and determination of the most effective drug of choice for patients before prescriptions are made thereby giving rise to very high resistance patterns.

The study deduced the most effective antibiotic for Gram negative isolates was ciprofloxacin, ceftriaxone and gentamycin with 80%, 80% and 73% sensitivities respectively. However, the least effective antibiotic was chloramphenicol and nalidixic acid with 67% and 40% resistances respectively. Other similar studies reported low susceptibilities of erythromycin 7.1% and chloramphenicol 6.5% (Kihla *et al*, 2014). The high resistances are reflective of the ready availability of these drugs in the population and increasing abuse of prescriptions.

Some Gram negative organisms were described as MDR due to their ability to resist 3 or more of the antibiotics used and represented 27%(4/15). Further classification was done and it was found out that *Klebsiella pneumoniae* had 1(25%) possible PDR resistant to all the antibiotics in the drug profile. Isolation of enterrobacteria for instance Gram negative rods like *Klebsiella pneumoniae, Proteus vulgaris* and *Enterobacter spp* is a good indicator of fecal contamination of medical articles and work surfaces for this case and therefore suggests very poor sanitation and hygiene amongst the patients and medical workers in the hospital. It was also observed by the researcher during the study that there was a poor attitude of the medical workers towards hand washing and use of gloves whenever handling patients. As a result, such a practice could highly facilitate the transmission of such organisms from person to person.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

The overall general prevalence of the isolated microbes was high with 44%. The results from the study also showed that *Staphylococcus aureus* was the most predominant bacteria found contaminating the medical articles and work surfaces. Other bacterial contaminants included *Klebsiella pneumoniae, Proteus vulgaris, Serratia merscescans* and *Enterobacter spp.* Patient beds and sink taps had the highest bacterial contamination rates amongst the medical equipment and work surfaces respectively that were sampled in the study, with patient beds having the greatest probability of harboring and transmitting the potential nosocomial causing organisms.

Vancomycin was the most recommended antibiotic of choice for treatment of Staphylococcal infections followed by clindamycin. Ciprofloxacin was the recommended drug of choice for treatment of gram negative infections. From the study, increasing resistance trends of bacterial against single or multiple antibiotics were observed. This is evidently due to the absence of a functional microbiological isolation unit in the hospital, abuse of antibiotics described and poor decontamination and sterilization protocols.

6.2 Recommendations

The following recommendations were put forward to the hospital administration, government and non-governmental organizations charged with the improvement of the health welfare based on the findings of the study:

1. A Microbiology laboratory unit with competent staff should be put in the hospital to make routine isolation of bacteria and sensitivity before patients are initiated on any treatments for routine monitoring of the post-operative ward.
2. A high prevalence of potential nosocomial infection causing pathogens observed implies insufficient sterilization and decontamination protocols and therefore, we recommend reconsiderations about the type, strength, usage and quantity of disinfectant used by the hospital administration.
3. Several organisms were resistant to multiple antibiotics from the drug susceptibility patterns from the study. We therefore recommend the use of vancomycin for Gram positives, ciprofloxacin and ceftriaxone for Gram negatives which can come in handy most especially in the empiric treatment as bacterial culture results tend to take a while before returning to the ward.

4. Additionally, routine infection control surveillance and monitoring systems should be put in the hospital and the whole country at large to identify emerging pathogens and their respective susceptibility patterns to antibiotics.
5. To confirm the role of medical equipment and work surfaces in harboring potential nosocomial pathogens, genotypic molecular methods should be introduced to accurately isolate specific strains and other organisms like fungi that were excluded from the study. Further research in this field within Kawolo hospital and other hospitals across the country is highly encouraged to give a clear picture and to deduce other ways such infections can be controlled.

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APPENDIX I

HOSPITAL APPROVAL LETTER

IHSU INTERNATIONAL HEALTH SCIENCES UNIVERSITY
sanitas per scientiam
PART OF INTERNATIONAL MEDICAL GROUP

MEDICAL SUPERINTENDENT
KAWOLO HOSPITAL
BUIKWE DISTRICT

making a difference to health care

Dean's Office-Institute of Allied Health Sciences

Kampala, 20th May 2015

The Medical Director
Kawolo Hospital

Allowed to Conduct his study from the Hospital

Dear Sir,

RE: ASSISTANCE FOR RESEARCH

Greetings from International Health Sciences University.

This is to introduce to you **Sserwadda Ivan**, Reg. No. **2012-BMLS-PT-004** who is a student of our University. As part of the requirements for the award of a Bachelors of Medical Laboratory Science of our University, the student is required to carry out a research in partial fulfillment of his award.

His research topic is: **Microbial contaminants isolated from items and work surfaces in the postoperative ward at Kawolo general Hospital, Uganda.**

This therefore is to kindly request you to render the student assistance as may be necessary for his research.

I, and indeed the entire University are grateful in advance for all assistance that will be accorded to our student.

Sincerely Yours,

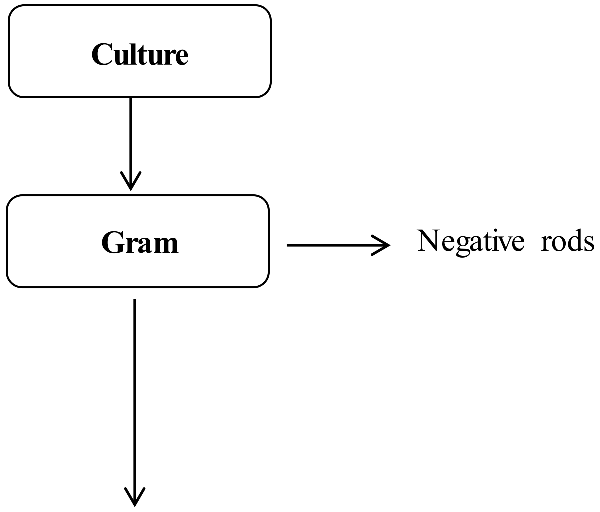
20 MAY 2015

Okiria John Charles
Senior Lecturer / Dean, Institute of Allied Health Sciences

The International Health Sciences University
P.O. Box 7782 Kampala – Uganda
(+256) 0312 307400 email: jokiria@ihsu.ac.ug
web: www.ihsu.ac.ug

APPENDIX II

GRAM NEGATIVE BACTERIA ISOLATION CHART



TSI	Citrate	Urea	Motility	VP	Indole	Oxidase	Isolate
Slope-Y Butt-Y	+	+	-	+	-	-	*K. pneumoniae*
Slope-Y Butt-Y Gas +	+	-	+	+	-	-	*Enterobacter*
Slope-R Butt-Y Gas +	+	+	+	+	-	-	*S. merscescans*
Slope-R Butt-Y Gas+/-	+	+	+	-	+	-	*P. vulgaris*

KEY

VP- Voges Proskauer

TSI- Tripple sugar iron agar

Y- Yellow

R- Red

APPENDIX III

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Procedure

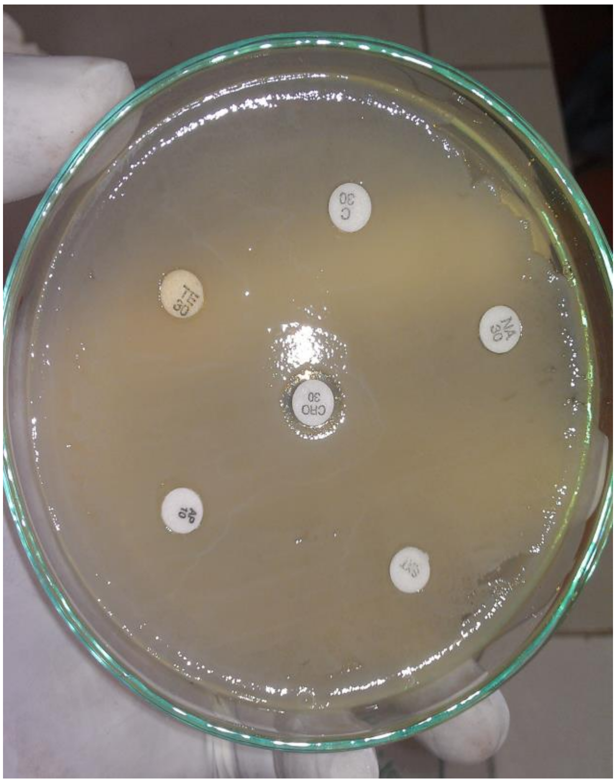
1. Prepare a suspension of the test organism by emulsifying 3-5 colonies of the organism in a small volume of sterile peptone water.
2. Compare the tube suspension with that of standard 0.5 McFarland and make sure their turbidities are similar.
3. Inoculate the plates by dipping a sterile swab into the inoculum, remove excess inoculum by pressing and rotating the swabs firmly against the side of the tube.
4. Spread the inoculum evenly over the Muller-Hinton agar plate with the swab three times, and finally round the edge of the agar plate.
5. Place the antimicrobial disc at even distances on the inoculated plate using sterile forceps or needle.
6. Incubate the plates at 37^{o}C for 18-24 hours.
7. Read the plates by measuring the diameters of each zone around the antibiotics and recorded in mm.
8. Results should be interpreted using CLSI standard susceptibility tables with measurements of the diameters reported as sensitive, intermediate or resistant.

APPENDIX IV

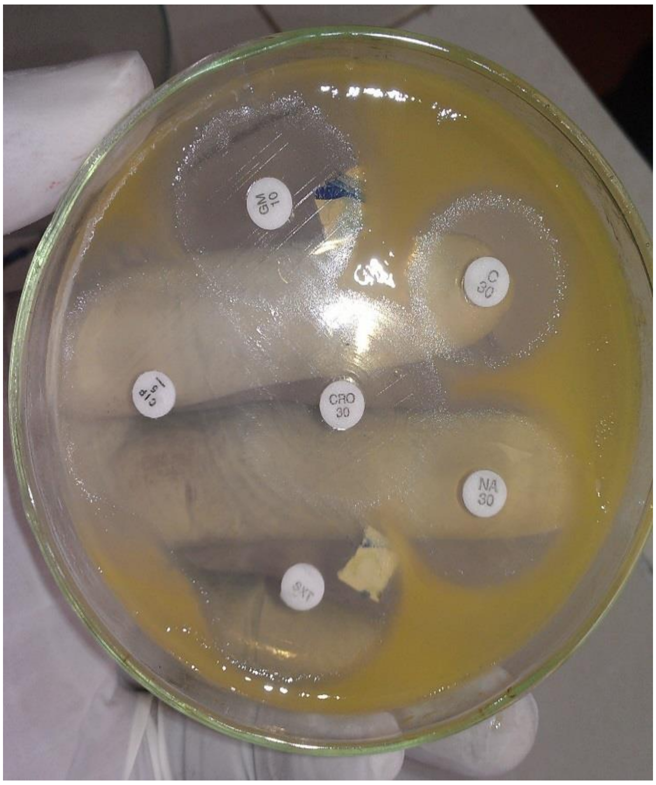
CULTURE, ISOLATION AND SUSCEPTIBILITY PICTURES

Picture 1: Colonies of *K. pneumoniae* on BA.

Picture 2: TSI, Simon's Citrate and Urease



Picture 3: Resistant bacterial pattern.



Picture 4: Sensitive susceptibility pattern.