ISOLATION OF BACTERIA AND PREVALENCE OF RESISTANT GENES IN ISOLATES FROM HOSPITAL WASTE AND SURFACES AND ITS MANAGEMENT IN HOSPITALS IN KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with our approval as the University Supervisor.

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DEDICATION

This research project is dedicated to my family, parents and all patients and families that have ever been affected by nosocomial diseases in their hospital stay. It has been sad through the loss of precious lives and the grievous experiences of the affected families in Kenya and the whole world. The tragedies symbolize just how great a need exists for further research on this largely ignored topic, with respect to hospital environment, waste management and public health. It is therefore my best regards to all research scientists to deal with this emerging threat of nosocomial infections in relation human health and environment in Kenya and as well as the whole world.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AHL	Acyl-homo serine lactone
AIDS	Acquired Immune Deficiency Syndrome
ANOVA	Analysis of Variance
API 20E	Analytical profile Index for *Enterobactericeae* members
ATCC	American Type Culture Collection
AUG	Augmentin (Amoxicillin/clavulanic acid)
AX	Ampicillin
AZT	Azidothymidine
C	Chloramphenicol
CAUTI	Catheter associated urinary tract infections
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CLABSIs	Central line-associated bloodstream infections
CLED	Cysteine Deficient Electrolyte Deficient Agar
CLSI	Clinical and Laboratory Standards Institute
CN	Gentamicin
CONS	Coagulase negative *Staphylococcus*
COT	Cotrimoxazole

CPM	Cefepime
CRO	Ceftriaxone
CTX-M	Bla Cefotaximase gene
CXM	Cefuroxime
DNA	Deoxyribose Nucleic Acid
Dntp	Deoxynucleotide triphosphates
E	Erythromycin
EMB	Eosin Methylene Blue
ERC	Ethical Regulatory Committee
ESBL	Extended Spectrum Beta lactamase
HAI	Health Associated Infections
HCWM	Hospital Care Waste Management
HIV	Human Immuno Virus
HW	Health Waste
IBM	International Business Machine
ICU	Intensive Care Unit
IMI	Imipenem
IPR	Institute of Primate Research
JKUAT	Jomo Kenyatta University of Agriculture and Technology

KCPE	Kenya Certificate of Primary Examination
KCSE	Kenya Certificate of Secondary Education
KMH	Kikuyu Mission Hospital
KNH	Kenyatta National Hospital
LE	Levofloxacin
LNZ	Linezolid
MBC	Minimum Bacterial Concentration
MC	MacConkey agar
MDRTB	Multi Drug Resistant Organisms
$MgCl_2$	Magnesium Chloride
MIC	Minimum Inhibitory Concentration
MOH	Ministry of Health
MRSA	Methicillin –resistant *Staphylococcus aureus*
MR-VP	Methyl Red/ Voges Proskauer
MSA	Mannitol Salt Agar
MW	Medical waste
MWM	Medical waste Management
NA	Nalidixic Acid
NACOSTI	National Commission of Science, Technology and Innovation

NCCLS	National Committee for Clinical Laboratory Standards
NEMA	National Environment Management Authority
OTA	Office Technology Assessment
PCEA	Presbyterian Church of East Africa
PCR	Polymerase Chain Reaction
PHO	Public Health Officers
POPs	Persistent Organic Pollutants
SHV	Sulfhydryl variable
SPSS	Statistical Package for Social Scientist
SS	*Salmonella/Shigella* Agar
Taq	Thermus aquaticus
TBE	Tris Borate buffer
TE	Tetracycline
TEM	Bla Temoniera gene
TSI	Triple Sugar Iron Agar
UK	United Kingdom
UON	University of Nairobi
UON	University of Nairobi
USA	United States of America

USEPA United States Environmental Protection Agency

UTI Urinary Tract Infections

VRE Vancomycin-resistant Enterococci

VRSA Vancomycin Resistant *Staphylococcus aureus*

WHO World Health Organization

ABSTRACT

Control of hospital environment is key to success of healthcare quality. Increasing emergence and spread of pathogenic bacteria is of great concern and continues to challenge infection prevention and epidemiology practice. This study aimed at providing information about the management of hospital environment and wastes in selected hospitals in Kenya, determine prevalence of pathogenic bacteria and their antibiotic susceptibility while detecting presence of resistant genes. A cross sectional study was conducted at Kenyatta National Hospital (KNH)(public) and Kikuyu Mission Hospital (KMH)(private) in Kenya from May 2015 to April 2017. Simple random sampling technique was used to distribute a semi structured questionnaire among 246 health workers in each of the hospitals to capture data on knowledge and management of hospital waste. In microbiological analysis, a total of 246 samples from each of the two hospitals was obtained using sterile cotton swabs from random sampling of hospital different surfaces, drainages, hands of healthcare givers and hospital waste dump site among others. The samples were aseptically collected, transported and processed following standard procedures. Colony morphology and biochemical characterization of bacteria was also determined. The identified microbes were subjected to antibiotics susceptibility test. Detection of genes of interest in this study Bla $_{TEM}$, Bla $_{CTX-M}$ and Bla $_{SHV}$ in Gram negative bacteria was done using polymerase chain reaction. Data was registered and entered in to SPSS version 16 computer program and analyzed using ANOVA. Results from the study revealed that doctors and public health officers had the highest knowledge in hospital waste management matters. In addition, healthcare facilities whether public or private practiced inappropriate medical wastes management skills. A total of 471 bacterial isolates were recovered, and were distributed as follows; *Providentia sp* 99 (21%) followed by *Staphylococcus aureus* 87 (18 %), *Escherichia coli (E. coli*) 61 (13 %), other Gram negative bacteria were 45 (10%), *Pseudomonas sp* 44 (9 %), coagulase negative *Staphylococcus* (CONS) 43 (9%), *Serratia sp* 31 (7%), *Klebsiella sp* 30 (6 %), *Proteus sp* 19 (4%) and *Enterobacter sp* 12 (3%). Susceptibility test revealed that *Escherichia coli* isolates were the most sensitive isolate to antibiotics. Imipenem drug showed 100% sensitivity for Gram negative, while Gram-positive isolates, linezolid antibiotic was the most sensitive drug. Extended- spectrum beta lactamases Bla $_{TEM}$ (10%), Bla $_{SHV}$ (3%) and Bla $_{CTX-M}$ (5%) genes were detected in this study. In conclusion, knowledge is above average in the studied hospitals. Positive attitude of doctors and public health officers towards the operational aspects of medical waste management system can be attributed to the microscopic vision of these professionals. The current practices are inappropriate due to lack of proper facilities and information of the individuals concerned. There is high bacterial contamination of objects frequently touched by patients and healthcare workers in hospitals and medical waste harbor potential pathogens and may act as a source of infectious. Antibiotic resistance bacteria reported in tetracycline, cefuroxime and ampicillin. Potencies of gentamicin, ceftriaxone and cefepime decreasing, multidrug existence- suggesting poor hygienic practices. Among the resistant genes detected in this study included *Bla* $_{TEM}$ (10%), *Bla* $_{CTX-M}$ (5%) and *Bla* $_{SHV}$ (3%) which was low. There is need for stringent review of hospital waste management system in Kenya. The frequency of ESBL producing strains among clinical isolates has been steadily

increasing. Continued drug resistance surveillance and molecular characteristics of ESBL isolates are necessary to guide the appropriate and judicious antibiotic use.

CHAPTER ONE

INTRODUCTION

1.1 Background information of the study

Hospital acquired infections are also called nosocomial infection; is an infection acquired in hospital by a patient who was admitted for a reason other than that infection (Medubi *et al*., 2006). Nosocomial pathogens are organisms causing diseases that are acquired from the hospital and healthcare environment within few days of admission and are responsible for nosocomial infections (Medubi *et al*., 2006).

Epidemiology of nosocomial infections affects huge number of patients globally, elevating mortality rate and financial losses significantly. According to estimate reported of WHO, approximately 15% of all hospitalized patients suffer from these infections (Emily and Sydnor, 2011). These infections are responsible for 4% –56% of all death causes in neonates, with incidence rate of 75% in South-East and Sub-Saharan Africa (WHO, 2016). The incidence is high enough in high income countries i.e. between 3.5% and 12% whereas it varies between 5.7% and 19.1% in middle and low income countries. The frequency of overall infections in low income countries is three times higher than in high income countries whereas this incidence is three times higher in neonates (Nejad *et al*., 2011). With increasing infections, there is an increase in prolonged hospital stay, long-term disability, increased antimicrobial resistance, increase in socio-economic disturbance, and increased mortality rate (Allegranzi, 2011).

There are various types of nosocomial infections: the most frequent types of infections include central line- associated bloodstream infections, catheter-associated urinary tract infections, surgical site infections and ventilator-associated pneumonia. A brief detail of these is given below: Central line-associated bloodstream infections (CLABSIs) are deadly nosocomial infections with the death incidence rate of 12 %–25 % (Khan *et al*., 2015). Catheters are placed in central line to provide fluid and medicines but prolonged use can cause serious bloodstream

infections resulting in compromised health and increase in care cost (WHO, 2016). Catheter associated urinary tract infections (CAUTI) is the most usual type of nosocomial infection globally (Warren, 2001). According to acute care hospital statistics in 2011, UTIs account for more than 12% of reported infections (CDC, 2015). CAUTIs are caused by endogenous native microflora of the patients. Catheters placed inside serves as a conduit for entry of bacteria whereas the imperfect drainage from catheter retains some volume of urine in the bladder providing stability to bacterial residence (Warren, 2001).

Surgical site infections (SSIs) are nosocomial infections that fall in 2%–5% of patients subjected to surgery. These are the second most common type of nosocomial infections mainly caused by *Staphylococcus aureus* resulting in prolonged hospitalization and risk of death (CDC, 2015). Ventilator associated pneumonia (VAP) is nosocomial pneumonia found in 9–27% of patients on mechanically assisted ventilator. It usually occurs within 48 hours after tracheal incubation (Hunter, 2012). 86% of nosocomial pneumonia is associated with ventilation (Steven & Koenig, 2006).

Bacteria are the most common pathogens responsible for nosocomial infections. Some belong to natural flora of the patient and cause infection only when the immune system of the patient becomes prone to infections. *Acinetobacter* is the genre of pathogenic bacteria responsible for infections occurring in ICUs. It is embedded in soil and water and accounts for 80% of reported infections (Sridhar & Olajumoke, 2003). *Bacteroides fragilis* is a commensal bacteria found in intestinal tract and colon. It causes infections when combined with other bacteria (Jayanthi, 2014). *Clostridium difficile* cause inflammation of colon leading to antibiotic-associated diarrhea and colitis, mainly due to elimination of beneficial bacteria with that of pathogenic. *C. difficile* is transmitted from an infected patient to others through healthcare staff via improper cleansed hands (Jayanthi, 2014). *Enterobacteriaceae* (carbapenem- resistance) cause infections if it travels to other body parts from gut; where it is usually found. *Enterobacteriaceae* constitute *Klebsiella* species and *Escherichia coli (E. coli*). Their high resistance towards carbapenem causes the defense against them more difficult (Aitken, 2001). Methicillin-resistant *S. aureus*

(MRSA) transmit through direct contact, open wounds and contaminated hands. It causes sepsis and pneumonia. It is highly resistant towards antibiotics called beta-lactams (Aitken, 2001).

Besides bacteria, viruses are also an important cause of nosocomial infection. Usual monitoring revealed that 5% of all the nosocomial infections are because of viruses (Aitken, 2001). They can be transmitted through hand-mouth, respiratory route and fecal-oral route (Ducel and Nicolle, 2002). Hepatitis is the chronic disease caused by viruses. Healthcare delivery can transmit hepatitis viruses to both patients and workers. Hepatitis B and C are commonly transmitted through unsafe injection practices (CDC, 2016). Other viruses include influenza, HIV, rotavirus, and herpes-simplex virus (Ducel & Nicolle, 2002).

Fungal parasites act as opportunistic pathogens causing nosocomial infections in immune-compromised individuals. *Aspergillus spp.* can cause infections through environmental contamination. *Candida albicans, Cryptococcus neoformans* are also responsible for infection during hospital stay (Ducel & Nicolle, 2002). Candida infections arise from patient's endogenous microflora while Aspergillus infections are caused by inhalation of fungal spores from contaminated air during construction or renovation of health care facility (Emily & Sydnor, 2011).

The hospital exists as a closed community, it is therefore not surprising that certain microorganisms become predominant and cause diseases. The pathogens can be expelled from an infected or colonized patient either through direct contact, aerosols droplets or faeces to the environmental surfaces (Dwivedi & Pandey, 2008). These pathogens can be contracted by the healthcare workers and even by the patients. Hospital waste means any solid, fluid or liquid waste materials including its container and other product generated during short term healthcare consisting observational, diagnostic, therapeutic and rehabilitative services for a person suffering from diseases or injury and during research testing and immunization of human beings (Jayanthi, 2014). Improper handling and management of the hospital waste is also an important cause of nosocomial infections. Proper management means proper collection, segregation, storage, transportation, treatment and disposal of waste in

safer manner to prevent nosocomial or hospital acquired infection (Dwivedi & Pandey, 2008).

Therefore, environmental surfaces in healthcare centers act as reservoir for bacteria and can as well serve as vectors of the bacterial pathogens (Bakalli *et al*., 2015). Depending on the environmental conditions, these pathogens may remain infectious on the surfaces for weeks after the contamination event (Carvahlo *et al*., 2007). The transmission of microorganisms from the environmental surfaces to patients is largely via hand contact with the surfaces (Kampf & Kramr, 2004). Otter *et al*., (2011) reported that surfaces can play important role in the epidemic and endemic transmission of the major pathogens linked to healthcare associated infections. Nosocomial infection caused by the nosocomial pathogens pose a problem of enormous magnitude globally; hospital localities have proven favorable in transmission of infection due to existing suitable pathogens-host environment relationship (Samuel *et al*., 2010).

Micro-organisms universally attach to surfaces and produce extracellular polysaccharides, resulting in the formation of a biofilm. Biofilms pose a serious problem for public health because of the increased resistance of biofilm associated organisms to antimicrobial agents and the potential for these organisms to cause infections in patients with indwelling medical devices (Rodney, 2001). Biofilms have great significance for public health, because biofilm associated microorganisms exhibit dramatically decreased susceptibility to antimicrobial agents. Many bloodstream infection and urinary tract infections are associated with indwelling medical devices and therefore biofilm associated and the most effective strategy for treating these infections may be removal of the biofilm contaminated devices (Donlan, 2000).

1.2 Statement of the problem

Today's modern hospital is a large and complex institution offering a wide variety of diagnostic and therapeutic services to many patients. The hospital environment has become heavily contaminated with different types of pathogens and acts as a reservoir of nosocomial pathogens that may infect patients during their stay in the

hospital. The identification of the predisposing factors for acquiring an infection is therefore vital in the development of a preventive strategy for nosocomial infections (Dwivedi & Pandey, 2008). In Kenya, only a few studies have reported Hospital acquired infections (HAI) rates in relation to hospital waste management. The few reported that up to 17% of neonatal patients and 40-50% of Intensive care units (ICU) patients become infected. As in other countries, surgical site infections, infections of urinary tract (UTI) and pneumonia are the most common (Dwivedi & Pandey, 2008).

Nosocomial infections have impacted a great burden in the healthcare system where they have led to deteriorating health condition, prolonged hospitalization days, increased cost of healthcare, disabilities and high morbidity and mortality. This problem of multidrug- resistant pathogens usually carries antimicrobial resistance plasmids, which can spread within the same and to other species and are the major causes of cystitis (Kariuki *et al*., 2007) boosts the adverse impact of these infections. This in turn has created a large burden economically due to loss of productivity and increased financial input in treatment of these diseases.

Research studies shows that in developed countries a system of waste disposal that is able to ensure proper sorting at the source and disposal has been developed. In Africa such facilities lack, medical waste is mixed from collection to disposal (WHO, 2005) in addition to lack of awareness among personnel. In Kenya, mismanagement of hospital waste (HW) or medical waste (MW) is due to insufficient medical equipment's and facilities, hence need to recycle, lack of enforcement of legislation for handling, treatment and disposal. The purpose of the study was to establish the level of knowledge in management of MW and current practices, characterize bacteria present, their susceptibility to antibiotics and detect resistant genes in order to explain the risks associated with poor MW management.

1.3. Justification of the study

Hospital waste management (HWM) is still a major problem mostly in developing countries. Previous studies done have shown that first world countries have developed a system of waste disposal that is able to ensure proper sorting at the

source and disposing them. In Africa these facilities are lacking and therefore all types of wastes are mixed up together along the whole disposal chain from collection to disposal (WHO, 2005). Kenya like many developing countries experience the problem of getting sufficient medical supply and even worse is the disposal of hospital waste. This is due to lack of enforcement of legislation for handling, treatment and disposal. Healthcare surfaces and wastes acts as the store house of harmful infectious pathogens. Potential health risk includes spreading of diseases by these pathogens and wide dissemination of antimicrobial resistance genes. The incidence of infections caused by Beta lactam resistant organisms due to the production of various enzymes has increased in recent years (Nordmann *et al*., 2012). Detection of ESBL production is of paramount importance both in hospital and community isolates. Infection control practitioners and clinicians need to identify and characterize different types of resistant bacteria in order to treat them. The present study was carried out to investigate the resistance among the bacterial strains that were isolated and identified from the hospital waste environment and surfaces of Kenyatta National Hospital (KNH) & Presbyterian Church of East Africa (PCEA) Kikuyu Mission Hospital. The ongoing emergence of resistance in the community and hospital is considered a major threat for public health. Presence of bacteria especially Gram negatives is crucial in this aspect because they are the most common causes of hospital and community acquired infections and they are inherently resistant to many hydrophobic antibiotics (Bastopal *et al*., 2008). In Kenya, few studies have been undertaken to determine the prevalence of ESBL-producing bacteria (Kaftandziera *et al*., 2009). Therefore, the present study was carried out to determine the prevalence of ESBL in the Kenyan hospitals as well as detection of genes for the *bla* $_{TEM}$, *bla* $_{SHV}$ and *bla* $_{CTX-M}$ types of ESBL enzymes. The data from this study will create public awareness regarding the health risk of the MW and surfaces in hospital environments. The study will also make relevant recommendations to hospitals and medical centers on possible ways of preventing and controlling nosocomial infections.

1.4 Research questions

1. What is the level of knowledge and current practices among healthcare personnel on management of selected hospitals waste in Kenya?
2. Which are the bacterial pathogens that can be isolated in selected hospitals wastes in Kenya?
3. What are the susceptibility profiles of various bacterial isolates from selected hospitals waste to various antibiotics?
4. Which resistant genes (*Bla* $_{TEM}$, *Bla* $_{SHV}$ and *Bla* $_{CTX-M}$) can be detected from isolated Gram negative bacteria within selected hospitals waste in Kenya?

1.5 General objective

To determine knowledge and current practices of hospital waste management, prevalence of bacteria pathogens and their antimicrobial susceptibility patterns and detection of beta lactamase resistant genes (*Bla* $_{TEM}$, *Bla* $_{CTX-M}$ and *Bla* $_{SHV}$) in bacteria isolates from selected hospitals waste in Kenya.

1.5.1 Specific objectives

1. To evaluate knowledge and current practices of management of hospital waste amongst healthcare workers in selected Kenyan hospitals.
2. To determine prevalence of bacterial pathogens in selected hospital wastes in Kenya.
3. To determine antibiotic susceptibility pattern of the bacterial isolates from selected hospitals wastes in Kenya.
4. To detect the presence of *bla* $_{TEM}$, *bla* $_{SHV}$ and *bla* $_{CTX-M}$ genes in extended spectrum β lactamase (ESBL) producing Gram negative bacteria isolated from selected hospitals waste in Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1. Introduction to hospital waste

Hospital waste (HW) is any waste generated during the diagnosis, treatment or immunization of human beings or in research activity (Rao, 2008). The waste produced in the course of health care activities carries a higher potential for infection and injury than any other type of waste (Park, 2005). Hospital waste generated in the hospital falls under two major categories namely, non-hazardous and bio hazardous. Constituents of nonhazardous waste are non-infected plastic, cardboard, packaging material (WHO, 2005). Bio hazardous waste again falls into two types: the infectious waste sharps, non-sharps, plastics disposables, liquid waste, etc. and the non-infectious waste-radioactive waste, discarded glass, chemical waste, cytotoxic waste, incinerated waste (Acharya & Singh, 2000). Approximately 75- 90% of the hospital waste is non-hazardous and as harmless as any other municipal waste (Singh *et al*., 2007). The remaining 10-25% is hazardous and can be injurious to humans or animals and deleterious to environment. It is important to realize that if both these types are mixed together then the whole waste becomes harmful (Singh *et al*., 2007).

2.2 Methods of health care waste management

There are various methods of hospital waste management; incineration is the controlled method of burning the waste in the high temperature producing mainly gaseous emissions and certain toxic substances. When an incinerator is operated at optimum temperature it kills the pathogen but otherwise it causes more harm than benefit (Rao, 2008). Another method is chemical disinfection which is used to kill microorganisms on medical equipment, floors and walls. It's appropriate for treating liquid medical waste e.g. blood. Autoclaving is the process of treatment in pressurized condition. This method can be used to sterilize solids, liquids and instruments of various shapes and sizes (Al-Mutair *et al.*, 2004). Land disposal either in open dumps which sometimes are unmanaged and risk further transmission of the

infection or sanitary landfills which prevents contamination of soil and of surface water and groundwater (Al-Mutair *et al*., 2004).

Hospital wastes are extremely hazardous type of waste and if not managed properly, can lead to serious health and environment problems (Abbas *et al*., 2018). The improper management of hospital waste causes serious environmental problems in terms of air, water and land pollution (Park, 2005). The nature of pollutants can be classified as biological, chemical and radioactive (Al-Mutair *et al*., 2004). Environmental problems can arise from the mere generation of hospital waste and from the process of handling, treatment and disposal (Al-Mutair *et al*., 2004). It is ironic that the healthcare delivery system, which is established to provide treatment and safeguard the health of the people against illnesses, becomes a source of infection and means of spreading diseases in the process of healthcare delivery (Park, 2005).

Healthcare institutions and facilities generate different types of infectious and/or hazardous hospital waste that poses enormous risk to patients, healthcare providers, waste pickers, and the community at large, if their disposal is not comprehensively and scientifically managed (Rao, 2008). Major hospitals contribute substantially to the quantum of hospital waste generated. Smaller hospitals, nursing homes, clinics, pathological laboratories, blood banks, etc. also contribute a major chunk (Razdan & Cheema, 2009). Hospital waste constitutes a larger portion of infectious wastes, which is potentially dangerous, because they may be resistant to treatment and possess high pathogenicity or ability to cause disease (Fayez *et al*., 2008). It is also a source of contamination of land and water sources if not rendered harmless before its burial on land or disposal in water. Furthermore, hospital waste emits harmful gases, which leads to atmospheric pollution, when treated in open burning in dump sites or burning in incinerators. These emissions can cause respiratory and skin diseases or even cancer, if precautionary protocols are ignored (Manyele, 2004).

Over the decade, the growth of the medical sector around the world combined with an increase in the use of disposable medical products has contributed to the large amount of hospital waste generated (Coker *et al*., 2009). As such, poor hospital waste

management causes environmental pollution, unpleasant smell, growth and multiplication of insects, rodents and worms, and may lead to transmission of diseases like typhoid, cholera, and hepatitis through injuries from sharps contaminated with blood (Coker *et al.*, 2009). The problem of hospital waste disposal in the hospitals and other health establishments has become an issue of increasing concern, prompting to stake holders to seek ways of scientific, safe and cost-effective management of waste (Yitayel *et al.*, 2012).

2.3 Burdens of hospital waste

In developing countries, healthcare waste has not received the much needed attention that it deserves (Coker *et al.*, 2009). This is because of the inadequate resources, poor management of the available resources and lack of transparency in administration in these countries resulting into low priority for HCWM (Diaz *et al*, 2008). In many countries there is limited segregation of hazardous and medical waste and usually mixed with non-infectious waste (Coker *et al*., 2009). Inadequate knowledge and unsatisfactory management practices among the healthcare workers are major challenges in the management of HCWs (Patil & Parokhrel, 2005). Previous research indicate that HCW management may be affected by lack of formal training, lack of knowledge on HCW management, limited interest from hospital administration. Poor waste management has been implicated in an increase in the number of epidemics and waste related diseases in the past years (Nkonge *et al*, 2012).

In Africa continent the situation is more critical as it indicates poor hospital waste management practices as described by (Manyele, 2004) and (Leonard, 2004) and especially in Tanzania. The study posted that the general awareness on issues related to hospital waste management was generally lacking among generators and handlers (Leonard, 2004). Even though reported hospital waste management systems in Tanzania was said to be poor, more recently, moves to confront the problems posed by poor management led to the construction of 13 pilot small scale incinerators (SSI) in various parts of the country. The success achieved through this program motivated the government to extend the SSI to all referral regional and district hospitals (Manyele, 2004).

Hospital waste in South Africa is seen as a mounting problem. In recent times, there have been numerous press statements about hospital waste being disposed of in an incorrect manner (Coker *et al*., 2009). This situation has adversely affected the poor, disadvantaged members of society. The incineration of hospital waste has also caused much concern (WHO, 2005). Report from unconfirmed study indicate that (WHO, 2005), incinerators are known to pollute the air by releasing toxic metals to the atmosphere, polluting soil and water and also have been associated with a wide variety of health problems in South Africa, such as disrupting the body's hormonal, immune and reproductive systems, and even caused cancers. It is estimated that about 45% of healthcare waste generated in the Province of KwaZulu-Natal alone, for instance, cannot be accounted for, indicating that it is being illegally dumped, buried or burnt in unknown locations, thus affecting the health of the people and the environment (Leonard, 2004). The disposal of hospital waste constitutes a problem in other parts of the country (Leonard, 2004).

There has been lack of capacity in South Africa to properly dispose of the huge amounts of hospital waste generated. There have been numerous instances where hospital wastes have been dumped in residential areas (Leonard, 2004). Illegal dumping of hospital wastes in disadvantaged residential areas has resulted in situations where children have been found playing with hospital waste materials such as syringes (Odeyemi, 2012). In Tygerberg Hospital treated 48 children with AZT after some were pricked with needles and others ate potentially lethal pills they found in a field in Elsie's River (Leonard, 2004). Medical facilities waste poses a risk to healthcare workers, patients and the local communities in South Africa (Leonard, 2004).

In Kenya, the actual burden of HAIs has not been accurately quantified, but it is projected to account for about 10-25 % admissions in public health facilities according to National Health Care Waste Management Plan 2015-2021 (MOH, 2007). Some of the risks to staff from patients through such waste include HIV and AIDS, TB, Hepatitis B and C and viral hemorrhagic fever such as Ebola. Previous study showed that quantity of waste, 39 % of the waste was infectious, while 61 % was non-infectious (MOH, 2007). The varying levels of waste segregation practices

observed in the sampled hospitals brought out the difference which doesn't agree with the WHO health care waste proportions where 80 % of the waste is considered non-infectious while 20 % is infectious. The total waste produced per patient per day was estimated to be 0.525kg (WHO, 2008).

2.4 Policies and programs that govern HWM in Kenya

National plan was developed to provide viable technical options as well as a roadmap for the management of healthcare waste for 5 years in Nairobi, Kenya (MOH, 2007). This was done by individuals and concerned organizations, revised edition, National Health Care Waste Management Plan 2015-2021. The National Health care waste Management Plan of Action is a document intended for use by health managers and program officers across the health sector (including those in the private health sector (MOH, 2007). This plan was to provide a tool that gives health managers guidance in planning, implementing and monitoring the activities of health care waste management in health facilities.

Protocols developed towards management of wastes included, (National Policy on Injection Safety and Hospital Waste Management, 2007) by (MOH, 2007). The protocol was to ensure safety of health workers, patients, and the community and to maintain a safe environment through the promotion of safe injection practices and proper management of related hospital waste. This was the first document of the Ministry of Public Health and Sanitation that is explicit on the need to address health waste management problems (MOH, 2007). This set of ideas spelled out the need to advocate for support and implementation of proper management of hospital waste among others.

Strategies indicated in this protocol is the need for appropriate financial mobilization and allocation of the components of injection safety and hospital waste management for effective policy implementation. The provision of sustained supplies and equipment for waste management through strengthened logistics system addresses the purpose for commensurate investment in waste handling requirements. Some strategy that were unique recommended also is the advocacy of best waste

management practices through behavior change communication as a key element in the strategy (WHO, 2005).

This Plan therefore, underscores the need for serious involvement of health managers at all levels of health care service delivery system in order to invoke the desired high-level commitment. It was envisaged that the implementation of this plan over every five years would result in improvement of health care waste management and the general cleanliness within the health care facilities and hence reduce risks and hazards associated with poor HCWM in the community (MOH, 2007).

2.5 Nosocomial infections (NAIs)

2.5.1 1ntroduction to Nosocomial Infections

"Nosocomial" term is used for any disease acquired by patient under medical care (Krishna, 2014). It is an infection acquired by patient during hospital stay. Recently, a new term, "healthcare associated infections" is used for the type of infections caused by prolonged hospital stay and it accounts for a major risk factor for serious health issues leading to death (Krishna, 2014). Patients are primarily admitted into hospital wards or proper management of their ailments, but while on admission some patients acquire other ailments than the one they were admitted for ((WHO, 2002). Nosocomial pathogens can be transmitted through person to person, environment or contaminated water and food, infected individuals, contaminated healthcare personnel's skin or contact via shared items and surfaces (Ekhaise *et al*., 2010). Dust, which is a good vehicle of airborne contamination, may arise from human activities, such as sweeping, movement, waving of handkerchief and bed making. Sneezing has been described as the most vigorous mechanisms of generating millions of droplets into the environment. While the larger droplets fall to the ground or on nearby objects, the smaller ones are rapidly evaporated to their non-volatile residual forms and remain suspended as droplet nuclei (Odimayo *et al*., 2008).

2.5.2 Epidemiology of Nosocomial infections (NAIs)

Nosocomial infections are also known as healthcare-associated Infections (HAIs) remain a significant hazard for patients and families visiting a hospital or healthcare

facility (Adebolu & Vhriterhire, 2002). A continuously increasing prevalence, 10% of patients on general hospital units will acquire a nosocomial infection during their hospital stay. The risk for infection escalates to 15-20% for those patients on intensive care units. Presently, two million patients each year acquire a nosocomial infection, approximately 90,000 succumbing to death. The risk for these infections poses a potential patient safety threat (WHO, 2002). The impact of HAIs is more severe in resource poor settings, where the rate of infection is estimated to range from 25% to 40% (WHO, 2008). HAIs have been reported to exact a tremendous toll on patients, families and systems of care, resulting in increased morbidity and mortality and increased healthcare costs (Pitout & Nordman, 2005).

2.5.3 Agents of Nosocomial infections

Nosocomial infections are caused by many microbes and each one can cause infection in healthcare settings. Bacteria are responsible for about ninety percent infections, whereas protozoans, fungi, viruses and mycobacteria are less contributing compared to bacterial infections (Murray *et al*, 2003). Bacteria are responsible for about ninety percent infections, whereas protozoans, fungi, viruses and mycobacteria are less contributing compared to bacterial infections (Murray *et al*., 2003). Historically, *Staphylococci*, *Pseudomonads*, and *Escherichia coli* have been the nosocomial infection causal bacteria for infections such as nosocomial pneumonia, surgical wound infections and vascular access related bacteremia have caused the most illness and death in hospitalized patients; and intensive care units have been the epicenters of antibiotic resistance (WHO, 2002). In recent years, nosocomial infections have reached epidemics proportions and are one of the main concerns in the health care arena.

These infections are most commonly associated with invasive medical devices or surgical procedures. Lower respiratory tract and blood stream infections are the most lethal; however, urinary tract infections are the most common (Astal *et al.*, 2002). These bacteria predominate in cases of ventilator associated pneumonia (47%) and urinary tract infections (45%). In intensive care units (ICUs) in the United States, Gram negative bacteria account for 70% of these types of infections, and similar data

are reported from other parts of the world (WHO, 2002). A range of Gram-negative organisms are responsible for hospital acquired infections, the *Enterobacteriaceae* family being the most commonly identified group overall. Unfortunately, multidrug – resistant organisms, including *Pseudomonas species, Acinetobacter baumanii* and extended spectrum β-lactamase (ESBL) producing carbapenemase-producing *Enterobacteriaceae*, are increasingly being reported worldwide (WHO, 2002).

Selected antibiotic-resistant nosocomial pathogens include multi-drug-resistant MRSA, vancomycin-resistant enterococci, *P. aeruginosa* and *K. pneumonia*, whereas *C. difficile* which shows natural resistance. In the 1940s, the problem of drug resistance came into light and in the past few years, a rapid increase of multi-drug-resistant pathogens was seen.

Fifty to sixty percent of hospital-acquired infections are caused by resistant pathogens in the United States. Improper use of antibiotics is thought to be the major cause of this drug resistance.

2.6 Biofilm Formation

This is an organization of bacterial cell communities enclosed in an extracellular polysaccharide matrix that forms a slippery solid coat around the bacterial community. It is usually attached to an inert or living surface that normally protects the bacterial community against hostile environments, such as in the human lungs. These bacterial communities cause many persistent and chronic infections, such as *Pseudomonas* infection in immunocompromised patients (Neidig *et al.,* 2013). Biofilms can manipulate the level of inflammatory response in a host by modulating its chemical appearance and altering its cell-to-cell activity pathways through quorum sensing. Acyl-homo serine lactone (AHL) is an example of a quorum sensing molecule found in *Pseudomonas aeruginosa* (Stinson, 2010). Bacterial cells located deep inside biofilms exist in a starved or slow growing state due to nutrients limitations. This results into a state of dormancy by the bacterial cells leading to antibiotic resistance. In some instance, biofilm matrix may slow down or retard the rate of antibiotic penetration into bacterial cells

leading to expression of genes within the biofilms that mediate resistance (Taraszkiewicz *et al.,* 2013).

Though some antibiotics are more effective at penetrating and destroying cells in biofilms, biofilms have inherent resistance to antibiotics through multiple mechanisms. These include, failure of the antibiotics to fully diffuse through the biofilm, changes in the osmotic environment causing an osmotic stress response, which changes the degree of permeability into *Pseudomonas* cell envelope and porins. This effectively stops majority of antibiotics from gaining entrance in to the bacterial cells and some cells in the biofilm may adopt a distinct phenotype due to environmental factors which protect them from the action of antimicrobial agents (Sachan *et al.,* 2015).

2.7 Current approaches in combating antimicrobial resistance

New therapeutic approaches alternative to antimicrobials have been proposed for the treatment of pathogenic microbes. Some of these approaches include the use of antivirulence medicines, which do not directly kill bacteria but deprive them of their virulent factors for easy elimination by the host immune system (Nordberg *et al.,* 2013). The use of non- antibiotics like efflux pump inhibitors that will act by reducing or reversing antibiotic resistance to drugs which the target microbe has developed intrinsic resistance against it. In addition, the use of vaccines to induce immunity against pathogenic microbes and the use of genetically engineered bacteriophages to reverse a pathogen drug resistance thereby restoring bacterial sensitivity to antibiotics. Finally host pathogen interactions where the host releases cationic antimicrobial peptides at the infected sites which have multiple antimicrobial activities (Nordberg *et al.,* 2013).

Interventions which have been intended to decrease antibiotic resistance rates in hospitals, and which are now drawing considerable interest to most researchers is antibiotic cycling or rotation. Cycling is the programmed rotation of one class of antibiotics with one or more different classes displaying similar spectra of action, in a repeated order in that cycle. A class or a specific antibiotic is withdrawn from use for a defined period then reintroduced later in an effort to limit resistance to the

cycled drug. In so doing antibiotics that had greater susceptibility against pathogenic microbes turn out to be effective in treating bacterial infections (Kollef, 2006). A qualitative meta-analysis has argued that cycling could be beneficial for preserving drug susceptibility in *Pseudomonas aeruginosa*. However, there are no current published theoretical models of antibiotic cycling in hospital settings and the greater community suggests that cycling will actually facilitate the spread of resistant strains (Brown & Nathwani, 2005). In a study conducted by (Viechtbauer *et al.*, 2014), it was found that cycling could only prevent the evolution of multiple resistance but not in cases where multiple resistance is wide spread.

Antibiotic mixing is another approach in combating antibiotic resistance. Antibiotic mixing involves random allocation of appropriate antibiotics to patients in a manner with no correlations across patients or time in the used drugs (Beardmore *et al.*, 2016). Antimicrobial stewardship programs have proven in some instances to optimize antimicrobial therapy, reduce or stabilize resistance to antimicrobials. Studies have demonstrated the potentiality of these programs in restricting the occurrence and spread of resistance. They have further maintained a relationship between the usage of antimicrobials and the occurrence of resistance (Lee *et al.*, 2013).

Modification of target drugs to make them more effective against drug resistant bacteria is also being considered. A study conducted by (Barrios *et al.*, 2014) on doripenem, a beta lactam carbapenems which has four membered beta lactams in one ring connected to a second ring in structure. Based on the five membered thiazolidinic ring, they found out that this structural arrangement offers protection to beta lactamase producing bacteria as well as a wide spectrum of activity and chemical stability. Another study (Lee *et al.*, 2013) showed that the polymixin remained the most consistently effective agents against multidrug-resistant *Pseudomonas*. The study demonstrated that drugs with higher molecular weight such as colistin were able to infiltrate by active transport into the outer cell membrane of Gram-negative bacteria.

2.8 Modes of activities of the antibiotics against the NIs causing pathogens

Although uncomplicated UTIs caused by *E. coli* and other *Enterobacteriaceae* should be treated empirically with co-trimoxazole (Karlowsky *et al.,* 2006) the rates of community- acquired strains with decreasing susceptibility to first-line agents such as ampicillin, nitrofurantoin, and co-trimoxazole have been on the increase. More recently resistance to fluoroquinolones such as ciprofloxacin and levofloxacin has been on the increase (Karlowsky *et al.,* 2006). It is therefore important that antibiotic resistance profiles at the local level must be known in order to support an empiric approach to the management of these infections. Antibiotics are classified based on their chemical structure. Each class of antibiotics is characterized by a typical core structure and the various members of the class are differentiated by the addition or subtraction of secondary chemical structures from the core structure. The main classes of antibiotics currently used in clinical practice include penicillins, cephalosporins, tetracycline, aminoglycosides, fluoroquinolones, potentiated sulfonamides, macrolides and glycopeptides (Dudhagara, 2011). The major mechanisms of action are inhibition of the cell wall synthesis, damage of the cell membrane function, inhibition of protein synthesis, inhibition of the nucleic acid synthesis, and metabolic antagonism (Gaynor & Mankin, 2003). Bacteria exhibit activity to antimicrobial agents in various ways (Gilmore, 2002), (Table 2.1).

Table 2.1: Mechanisms of action of antimicrobial agents

Mechanism	Examples of Antimicrobial(s) used
Interference with cell wall synthesis	β-lactams: Penicillins, Cephalosporins, Carbapenems, Monobactams. Glycopeptides: Vancomycin, Teicoplanin
Protein synthesis inhibition	Macrolides, Chloramphenicol, Clindamycin.
Bind to 50S ribosomal subunit	
Bind to 30S ribosomal subunit	Aminoglycosides, Tetracycline.
Interference with nucleic acid sythesis	Quinolones. Rifampin
Inhibit DNA synthesis	
Inhibit RNA synthesis	
Inhibition of metabolic pathway	Sulfonamides, folic acid analogues
Disruption of bacterial membrane Structure	Polymyxins, Daptomycin.
Interference with Efflux pumps	β-lactams, fluoroquinolones, Chloramphenicol

Adapted from (McDermott *et al.*, 2003). Different methods of action of antimicrobial agents on bacteria structure and physiology.

2.9 Antimicrobial resistance

Antimicrobial resistance is the resistance of a microorganism to an antimicrobial drug that was originally effective for treatment of infections caused by it. This results to ineffective treatment and persistence in infections, increasing the risk of spread to others (Kim & Jeong, 2007). Evolution of resistant strains is a natural phenomenon that occurs when microorganisms replicate themselves erroneously or when resistant strains are exchanged between them (Gilmore, 2002). The use and misuse of antimicrobial drugs sanitary conditions and inappropriate food handling encourage further spread of antimicrobial resistance. It is important to understand the trend in

resistance pattern so as to establish adequate control program since antimicrobial resistance is not only an emerging major challenge but also a worldwide public health concern (Gilmore, 2002).

2.9.1 Impact of antimicrobial resistance

Antimicrobial resistance is among the 21st century biggest challenges. This has posed a great risk to the public (Dudhagara, 2011). Any use of antibiotics can increase selective pressure in a population of bacteria, causing vulnerable bacteria to die thereby increasing the relative numbers of resistant bacteria and allowing for further growth (Chagas *et al*., 2011). As resistance to antibiotics becomes more common there is greater need for alternative treatments (WHO, 2014). Examples of common types of drug resistant include: methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *S. aureus* (VRSA), extended spectrum beta lactamase (ESBL), vancomycin-resistant Enterococcus (VRE), multidrug resistant tuberculosis (MDRTB) (WHO, 2014).

2.9.2 Prevalence of including ESBLs resistant genes

Extended-spectrum beta-lactamases (ESBLs) are the rapidly evolving group of β-lactamase enzymes which have the ability to hydrolyze all cephalosporins and monobactams, but are inhibited by β-lactamase inhibitors, such as clavulanic acid (Rezai *et al*., 2015). ESBLs are undergoing continuous mutation causing the development of new enzymes showing expanded substrate profiles (Wang *et al*., 2012). At present, there are more than 300 different ESBL variants. *Bla* $_{TEM}$ (Temoniera) and *bla* $_{SHV}$ (Sulphy- dryl variable) were the major types. However, *bla* $_{CTX-M}$ type (predominantly hydrolyze cefotaxime) is increasingly becoming important (Livermore *et al*., 2008). Variants derived from *bla* $_{TEM}$ and *Bla* $_{SHV}$ enzymes and bla $_{CTX-M}$-ESBLs (derived from other sources) are defined as "classical ESBLs". *bla* $_{TEM}$ and *bla* $_{SHV}$ are the most common variants of ESBLs. *Bla* $_{TEM}$ encodes for β- lactamases with extended spectrum with *bla* $_{TEM-1}$ been responsible for up to 90%. The *bla* $_{TEM}$-type ESBLs are derivatives of *bla* $_{TEM-1}$ and *bla* $_{TEM-2}$. It is the most commonly encountered β-lactamase among Gram-negative bacteria. *Bla* $_{SHV}$ refers to the Sulfhydryl variable. *Bla* $_{SHV}$ types of ESBLs have

been detected in a wide range of *Enterobacteriaceae* and outbreaks of bla $_{SHV}$ – producing *Pseudomonas sp* and *Acinetobacter sp* have been reported (Rawat & Nair, 2010). The *bla* $_{SHV-1}$ β-lactamases are most commonly found in *K. pneumoniae* and are responsible for up to 20% of the plasmid-mediated Ampicillin resistance in this species. *Bla* $_{CTX-M}$ is derived from "CefoTaXimase Munich" family and constitutes a complex and non- homogeneous group of enzymes (Gutkind *et al*., 2013). The *bla* $_{CTX-M}$ is a recently described family of ESBLs; these enzymes hydrolyze cephalosporin especially Cefotaxime with high efficiency (Alobwede *et al*., 2003). More than 113 *bla* $_{CTX-M}$ varieties are currently known (Bonnet, 2004).

In Kenya, the predominant ESBL genotype is *bla* $_{CTX-M}$, most of which is isolated from isolates obtained from the urinary tract (Maina *et al*., 2012), and it's the most important ESBL gene among *E. coli* and in *Salmonella enteric serovar typhimurium* (Wang *et al*., 2012). It's believed that *bla* $_{CTX-M}$ was acquired from Genus *Kluyvera* via mobile genetic elements and strains producing such enzymes should be monitored closely in hospital and community settings (Kiiru *et al*., 2012). To date, *bla* $_{CTX-M-15}$ and *bla* $_{CTX-M-14}$ enzymes are the most predominant types of ESBLs with *bla* $_{CTX-M-15}$ showing global distribution (Lahlaoui *et al*., 2014). These organisms pose a therapeutic challenge, since they are frequently resistant to other kinds of antimicrobial drugs, including aminoglycoside- sides, quinolones, and cotrimazole (Gniadkowski, 2001).

2.10 Antimicrobial Susceptibility Tests

Antibiotic sensitivity or susceptibility is the susceptibility of bacteria to antibiotics. It varies within a species as some strains are more resistant than others. It is usually carried out to determine which antibiotic will be most successful in treating bacterial infection in vivo. Testing for antibiotic sensitivity is often done by the Kirby-Bauer method (Hudzieki, 2009) Small wafers containing antibiotics are placed onto a plate upon which bacteria are sensitive to the antibiotics are placed onto a plate upon which bacteria are growing. The various methods include;

2.10.1 Agar diffusion method

The antibiotic diffuses from a paper disc or small cylinder into an agar medium that contains test organisms. A common application of these methods is the Kirby-Bauer test; where paper discs containing known concentrations of antibiotics are applied to the surface of seeded Mueller-Hinton a g a r and the plate incubated. After overnight incubation, zone of inhibition sizes is measured in millimeters (Jorgensen *et al*., 2017). Zone of inhibition is observed as a failure of the organism to grow in the region of the antibiotic (Jorgensen *et al*., 2017).

2.10.2 Broth Dilution Method

The broth dilution method involves subjecting the isolate to a series of concentrations of antimicrobial agents in a broth environment. This method depends upon inoculation of broth containing antibiotics at varying levels; usually, doubling dilutions are used. This method is used to determine Minimum Inhibitory Concentration (MIC) or breakpoint of an antimicrobial agent required to inhibit the growth of a bacterial isolate (Mahon *et al,* 2007). It can also be used to measure the minimum bactericidal concentration (MBC) which is the lowest concentration of antimicrobial required to kill bacteria (Wiegand *et al,* 2008). A dilution test is carried out by adding dilutions of an antimicrobial to a broth or agar medium. Standardized inoculums of the test organism are then added. After overnight incubation, the MIC is reported as the lowest concentration of antimicrobial required to prevent visible growth (Wiegand *et al,* 2008). To determine the MBC, a 0.01ml aliquot of each clear tube or well from the MIC determination is sub cultured to an agar medium and incubated at 37^{o}C for 24 hours. After overnight incubation, the numbers of colonies that grow on subculture are compared with the actual number or organisms inoculated into the MIC test tubes (Mahon *et al*., 2007).

2.11 Significance of the study

Within the hospital, the surveillance of infection, observation check list and record keeping were vital tools that provided indications of inadequate hygiene practices or of contamination of the immediate environment. Surveillance would allow an

outbreak of infection to be recognized and investigated and will provide a basis for introducing control measures, assessing their efficacy and of the routine preventive measures taken by the hospitals and for reducing the level of avoidable infection. Antimicrobial resistance is driving up health care costs, increasingly the severity of disease, and increasing the death rates from certain infections. The research will also provide evidence that bacteria resistant to commonly used antibiotics and their resistance genes that are usually present in the hospital can reach the environment. This study would highlight and demonstrate the extent of hospital environment contribution to the resistance phenomena in Kenya, thus providing data for policy makers and local authorities. This may assist in future planning in an attempt to reduce antibiotic resistance and associated burdens.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

This research study was carried out among eligible healthcare workers working in the two various hospitals within the Nairobi City and surrounding county, Kiambu. The hospitals were conveniently classified based on their ownership (public or private) and the diversity of their facilities and services rendered. The research study site included Kenyatta National Hospital (KNH) situated in Nairobi County and PCEA Mission Hospital, Kiambu County. Kenyatta National Hospital was chosen due to the fact that it's the largest public teaching and referral hospital in Kenya, founded in 1901, with over 1800 beds, over 6000 staff, 50 wards and 24 theatres (www. knh.or.ke). It produces the greatest amount of hospital waste in Kenya and hence there is a need for proper hospital waste management to minimize the risks involved (www. knh.or.ke). The PCEA Kikuyu Mission Hospital (KMH) represented a private hospital serving Kiambu County and surrounding Counties. It was founded in 1908, with about 218 beds, around 346 staff, 5 wards, 4 theatres (www.pceakikuyuhospital.org). KMH was also selected to represent private referral hospital. High population density in the study sites assures that a large number of people are exposed to toxic level of hospital waste (www.pceakikuyuhospital.org). Therefore, to describe, evaluate and compare the existing practices of hospital management in a government and private hospitals these hospitals were selected and to detect any form of surface contamination in the hospitals.

3.2 Study design

A cross-sectional study design utilizing a systematic random sampling technique was adopted. All members of the study population had an equal and independent chance of being selected.

3.3 Target population

The study participants included health workers who are involved in medical waste management in their service and currently working the two health facilities. For microbiological analysis working surfaces where hospitals wastes are handled and disposed were swabbed.

3.4 Inclusion criteria

All the health care providers' who gave consent to participate, those working in the health-care establishments of KNH and KMH, those directly involved in hospital waste management process and surfaces and sites that were located in selected hospital departments of the study.

3.5 Exclusion criteria

Hospital staff handling wastes and surfaces/sites that were under isolation within the selected hospital departments and surfaces that were not in close contacts with healthcare personnel's in their line of service e.g. roof tops and ceilings.

3.6 Sample size determination

The samples collected for this study were classified into two; respondents' questionnaires and the environmental samples which were swabs from various hospital surfaces, devices and hospital sites. The sample size was determined based on a prevalence of population estimated to be at risk (0.20) which is the 20% of health workers at risk in Kenya, (WHO, 2002). The sample size was calculated using the Fischer's *et al* 1998 formulae.

$n = z^2/p\ (1-p)/d^2$ where,

n = Total sample population,

z = score of confidence interval (1.96 at 95% C.I),

p= Prevalence of population estimated to be at risk (0.20) which is the 20% of health workers at risk in Kenya, (WHO, 2002).

d = tolerable error (5%)

$n = z^2 /p (1-p)/d^2 = 1.96^2 \text{ x}0.20 \text{ x } (1-0.20)/0.05^2 = 246.$

3.7 Ethical consideration and recruitment of participants

Enrollment to the study was on voluntary basis. Scientific approval of the study was obtained from Kenyatta National Hospital Ethics and Review Committee Permit Number P730/12/2014 reference number KNH/UON ERC/A/169 (Appendix C i). Ethical clearance to carry out the study was obtained from KNH and KMH hospitals administration (Appendix C (ii) Approval letters). All procedures were carried out in accordance to the standard biosafety guidelines and waste disposal (WHO, 2014). Informed consent was obtained from the participants and the information collected was in confidence. There were no monetary gains for those who participated in the study and there were no penalties for those who declined participation.

3.8 Sampling method

The stratified sampling technique was used in the questionnaire, whereby a listing of eligible participants was conducted according to their respective departments. Proportionate allocation was done to determine the number to be sampled per stratum (department). In each stratum, random sampling was carried out to select the required sample (Table 3.1). In case the recruited participant did not give consent, the next consenting participant was included in the study. Sampling was done in repeated visiting days until the desired numbers of respondents was achieved. Simple random sampling method was used to collect samples from ten sections in each hospital (Table 3.1). At least twenty-five samples were collected per week over a period of ten weeks for each hospital guided by the number of samples. The second day of the week were the day of collection for KMH while the fourth day of the week was for KNH. The random samples were labeled as from the stratified source sections using the formula as shown below (Table 3.2).

Table 3.1: Various sample collection sites for KNH and KMH

Site	Location
Site A	Waste water drainage flowing out of the hospital
Site B	Waste water from Intensive Care Unit
Site C	Surgery (operation theatre)
Site D	Hospital Sterilization room
Site E	Pediatric ward
Site F	Gynecology and Obstetrics ward
Site G	Internal Medicine ward
Site H	General ward
Site I	Orthopedic and Surgical ward
Site J	Waste dumping site was also selected in each hospital

Various departments in each of the selected hospitals (KNH and KMH) showing the samples collected from ten different departments.

Table 3.2: Stratified job cadres and sample size for KNH and KMH

Job cadre	**KNH-Number available (N_i)**	Proportion $p = \left(\frac{n}{N}\right) N_i$	**KMH-Number available (N_i)**	**Proportion** $p = \left(\frac{n}{N}\right) N_i$
Nurses	1520	171	167	119
Laboratory technologists	125	14	17	12
Clinical officers	76	8	17	12
Doctors and Dentists	302	34	24	17
Cleaners	125	14	111	79
Public health officers	31	3	7	5
Waste pickers	9	2	3	2
Total	N=2188	n=246	N=346	n=246

www.knh.or.ke and www.pceakikuyuhospital.org, 2015) KNH (Kenyatta national hospital and KMH (Kikuyu Mission hospital) describing the formula used: P is the proportion of each job cadre to be selected using the given formula where n is the sample size (246), N is the total number of all selected hospital staffs for the purpose of this study, while N_i is the total number of a particular profession e.g. nurses in each hospital.

3.9 Data and sample collection

This section was divided into two; the first section that participants responded with the help of a questionnaire and observational check list for investigate level of knowledge and current practices of medical waste management from respondents who gave consent (Appendix I).

The second section was laboratory work that involved isolation of bacteria, their antibiotic sensitivity and detection of resistant genes from hospital waste.

3.9.1 Knowledge of management and current practices of medical waste management

A structured questionnaire (Appendix II) was used to collect the data with consent from respondents. It consisted of questions regarding the demographic variables: gender, level of education, profession and work experience, Questionnaires were divided into two major parts dived as; Five questions on knowledge regarding hospital waste management and the rest of the questions on current practices included segregation, color coding, storage and transportation, labeling, treatment and disposal, records of the wastes, final waste disposal among others. About 246 respondents were interviewed from each hospital from the calculated sample size.

Observation checklist was used based on Kenya Ministry of Health medical waste management policy and guided by literature. The doctors were interviewed regardless of their qualifications in terms of whether they are consultants, registrars, or general practitioners.

3.9.2 Environmental samples from hospital waste and surfaces

A total of 246 samples from solid and liquid wastes of the two hospitals were swabbed from the selected public and private hospital in Kenya. Solid waste samples were swabbed from surfaces such as door handles, toilet and bathroom knobs, bed rails, cabinet locks and handles, water dispensers' taps, tables including operating tables, scrubber surfaces, sink surfaces, theatre equipment surfaces, different types of hospital waste bin surfaces door handles and knobs, and floor surfaces and dump sites etc. The liquid hospital waste had sterile swabs dipped and quickly capped. The collected solid swab samples were obtained by rubbing sterile cotton wool swabs on different materials, surfaces and sites. Each department each hospital was taken samples nine to ten times to get enough samples from different surfaces. They were then put into sterile tubes, tightly capped and labeled appropriately as above. The collected samples were transported in ice cooler box to the medical microbiology laboratory (JKUAT) for processing. They were refrigerated as soon as they were transported until when they were needed for processing, isolation and identification of bacteria. Each sample was analyzed in triplicate.

3.9.3 Quality control

Quality control was performed according to USEPA (2017) guidelines waste management procedures as standard controls.

In microbiological analysis procedure for quality control was performed according to Onyango *et al.* (2018) guidelines of indicator microorganisms in hospital waste procedures as standard controls. Microorganisms present in hospital waste treatment systems a broad spectrum of pathogens present in indoor and outdoor environments. The zones of inhibition were measured and compared with National Committee for Clinical Laboratory Standards (NCCLS) guidelines. *Escherichia coli* ATCC 25922 and *Pseudomonas species* ATCC 27853 were used for quality control.

3.9.4 Coding of Sample

A combination of alphabets and numerals were randomly selected and used to indicate the name of the hospital and type of sample as indicated in Table 3.3.

Table 3.3: Interpretation of codes used to label samples

Hospital/department/Number of samples taken	Interpretation
KNH-A 1,2,3	Kenyatta National Hospital (Site A, 3 samples)
KNH-B 1,2	Kenyatta National Hospital (Site B, 2 samples)
KNH-C 1,2,3	Kenyatta National Hospital
KNH-D 1,2,3	Kenyatta National Hospital
KNH-E 1,2,3	Kenyatta National Hospital
KNH-F 1,2	Kenyatta National Hospital
KNH-G 1,2,3	Kenyatta National Hospital
KNH-H 1,2	Kenyatta National Hospital
KNH-I 1,2 ,3	Kenyatta National Hospital
KNH-J 1,2	Kenyatta National Hospital
KMH-A 1,2,3	Kikuyu Mission Hospital
KMH B 1,2	Kikuyu Mission Hospital
KMH-C 1,2,3	Kikuyu Mission Hospital
KMH-D 1,2,3	Kikuyu Mission Hospital
KMH-E 1,2,3	Kikuyu Mission Hospital
KMH-F 1,2	Kikuyu Mission Hospital
KMH-G 1,2,3	Kikuyu Mission Hospital
KMH-H 1,2	Kikuyu Mission Hospital
KMH-I 1,2,3	Kikuyu Mission Hospital
KMH- J 1,2	Kikuyu Mission Hospital
A,B,C,D,E,F,G,H,I and J,	Departments in the hospitals
Surfaces included: BDS, DOR,MACH, HADS, BIS, TROL, WST,EB, S, DS, CS	BDS-Beds, DOR-door handles surfaces, MACH-machine, HADS-hands, BIS-bins, TROL-trolley (surfaces) WST- waste water, EB-elevator buttons, S-sinks, DS-devices surfaces, CS-cabinet surfaces etc.

Interpretation of codes used, Key: KNH-Kenyatta National Hospital, KMH Kikuyu Mission Hospital, BDS-Beds, DOR-door handles surfaces, MACH-machine, HADS-hands, BIS-bins, TROL-trolley (surfaces) WST- waste water, EB-elevator buttons, S-sinks, DS-devices surfaces, CS-cabinet surfaces 1. Waste water drainage flowing out of the hospital (site A), 2. Waste water from Intensive Care Unit (site B), 3. Surgery (operation theatre) (site C) 4. Hospital Sterilization room (site D), 5. Pediatric ward (site E), 6. Gynecology and Obstetrics ward (site F), 7. Internal Medicine ward (site G), 8. General ward (site H), 9. Orthopedic and Surgical ward (site I), 10. Waste dumping site was also selected in each hospital (Site J) etc.

For example, KMH-B 3DS means- A sample taken from Kikuyu mission hospital, B is ICU department, 1,2,3 means three samples, from device surfaces.

3.10 Cultivation and isolation of bacteria

Swab samples from various surfaces were streaked on different agar containing media according to their protocols such as MacConkey (appendix V a), Mannitol salt agar (MSA) (appendix F ii) Eosin methylene blue (EMB) (appendix V c), Salmonella- Shigella agar (SS) (appendix V F) (Oxoid microbiological media, UK) using the streaking method. All the media were prepared according to the manufacturer's instructions (Biolab, Merck, South Africa). All inoculated plates were incubated for 24 hours at 37°C). All identified colonies were sub cultured onto freshly prepared nutrient agar medium to obtain pure culture of the microbes (Guardabassi and Dalsgaard, 2002).

3.10.1 Gram-staining procedure

The colonies obtained were Gram-stained (Guardabassi & Dalsgaard, 2002). This was done by picking 2-3 colonies from each plate by the help of a sterile wire loop and making a smear on a clean glass slide with a drop of distilled water. The smears were made and then air dried and heat fixed; they were then flooded with crystal violet for 1 minute and then washed off. The slides were then flooded with lugol's iodine for 1 minute and then washed off gently by decolorisation using 95% ethanol for 30 seconds and lastly, counterstaining using safranin for 1 minute after which

they were washed gently and dried. The slides were observed using ×100 magnifying lens (oil emulsion). They stained either pink (Gram negative) or purple (Gram positive). On the microscope (Nikon, standard bright field condenser) the morphology of the bacterium was also visible as rods or cocci.

3.10.2 Biochemical test analysis

The pure cultures obtained were subjected to different biochemical tests, as per the protocol by (Tolaro, 2005); the biochemical tests included citrate utilization (Appendix VI F), indole test (Appendix), triple sugar iron test (Appendix VI F), methyl red - voges proskauer test (Appendix VI q), oxidase test (Appendix VI J), catalase test (Appendix VI J) and coagulase test (Appendix F xii) and API 20E test (Appendix xiii) for confirmation of *Enterobactericeae* family as described by Cheesborough, 2006. *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27953) and *Staphylococcus* (ATCC 2593) were the control microorganisms used. The pure cultures of Gram negatives were confirmed identity using Analytical Profile Index (API) 20E test (Appendix F m). The API 20E test was performed in accordance with the manufacturers protocol (BioMerieux, 69280, Marcy I' Etoile, France) and the organisms identified to species level using API software (Appendix VI).

Preservation of pure bacterial isolates was done from the heavy growth area cells where they were pulled together by scratching with a sterile loop and suspended thoroughly in 30% glycerol supplemented nutrient broth contained in a vial. The samples were afterwards stocked up at -80^{o} C until when needed.

For short term preservation, the pure culture was streaked on MacConkey agar plates and grown overnight at 37^{o} C. From the heavy growth area, cells were accumulated scratching with a sterile needle and stabbed in a cryo-tube or vial. Then the samples were stocked up at room temperature (Tolaro, 2005).

Cultures were purified by twice sub culturing using the streaking plate method. After sub culturing, new and fresh bacterial isolates were used for Gram staining and all isolates were identified biochemically and the rest archived in 30% glycerol in

nutrient broth and stored at -80^{0}C for further processing in an Eppendorf storage rack (Appendix V).

3.11 Antimicrobial susceptibility testing

All the isolates obtained were standardized using 0.5 Mc Farland turbidity standards. This was prepared by picking about three colonies from each sample of the freshly grown bacteria in 5 ml sterile nutrient broth and the turbidity was adjusted to a 0.5 McFarland standard. Bacterial susceptibility testing was done by the disk diffusion method according to Jan Huduzieki method (Huduzieki, 2009) following the NCCLS assessment criteria (CLSI, 2012). The inoculum suspension was spread by streaking in three directions on a Mueller Hinton agar (Oxoid, UK) (Appendix VI d) plate surface with a sterile swab filter paper disks (6mm) containing designated amounts of the antimicrobial drugs obtained from commercial supply firm (All from Oxoid). Drugs used and were put in the following discs containing antibiotics/antimicrobial agents were used; (Table 3.3) Impregnated antibiotic discs were carefully and aseptically placed on the inoculated agar plates. The antibiotic susceptibility testing for each isolate was carried out in triplicate plates. All the plates were then incubated at 37^0 C and the results were observed after 24 hours as per the protocol of (CLSI, 2012). The diameter of the zone of inhibitions was measured in millimeters using a transparent meter ruler. The test organisms were classified as either sensitive or resistant according to the interpretive standard of the clinical and laboratory standards institute (CLSI, 2012).

The antimicrobial agents were chosen on the basis of treatment of Gram negative and Gram positive bacteria and were based on routine antimicrobials used for bacteria and beta lactamase detection antibiotics. The following antibiotics were used; Beta lactams, quinolone, carbapenems, aminoglycosides, cephalosporolins, tetracycline, sulfonamide trimethoprim etc. (Marwa *et al.*, 2015) were tested at the concentrations indicated in Table 3.3. These antibiotics were chosen because they are either used in both human medicine and animal veterinary practice or because previous studies have reported microbial resistance to them (Ateba *et al*., 2008).

Table 3.4: Acceptable susceptible zone of inhibition values used based on CLSI

Antimicrobial agent/code		Dosage	MIC-Interpretive Criteria(µg/ml)		
			Susceptible	Intermediate	Resistant
Imipenem	IMI	10ug	≥16	14-15	≤13
Cefepime	CPM	30ug	≥18	15-17	≤14
Cotrimoxazole	COT	30ug	≥21	14-20	≤13
Tetracycline	TE	30ug	≥19	15-18	≤14
Ampicillin	AX	10ug	≥17	14-16	≤17
Chloramphenicol	C	30ug	≥18	13-17	≤12
Nalidixic acid	NA	30ug	≥18	------	≤17
Levofloxacin	LE	5ug	≥17	14-16	≤13
Erythromycin	E	15ug	≥23	14-22	≤13
Ceftriaxone	CTR	30ug	≥21	14-20	≤13
Amoxicillin-clavulanic acid	AMC	30ug	≥18	14-17	≤13
Gentamicin	CN	30ug	≥15	13-14	≤12
Cefuroxime	CXM 0	30ug	≥18	15-17	≤14
Cefotaxime	CTX	30ug	≥21	16-20	≤15
Linezolid	LNZ	30ug	≥22	0	≤21

(Table derived from, Marwa et al., 2012) and CLSI 2014 (Appendix G). Various classes of antimicrobial agents ranges of susceptibility.

3.12 ESBL screening and confirmation by phenotypic methods

This test was done according to procedure by Helene *et al*., in 2011, where two antimicrobial disks were placed 30mm apart (center to center). One of the disks contained amoxicillin/clavulanic acid and the other contained an expanded-spectrum cephalosporin (for example, ceftriaxone, cefotaxime or ceftazidime) in this case ceftazidime was used. The test was positive if, after 24-hour incubation, the zone of inhibition in between the disks was enhanced. The enhancement was due to the inhibition of the ESBL by clavulanic acid (provided by the amoxicillin/clavulanic acid disk) and the subsequent action of the expanded-spectrum cephalosporin. A 5 millimeter increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was designated as ESBL positive. A previously identified *Klebsiella pneumonia* ESBL positive isolate

was used as a positive isolate was used as a positive control and a negative control nuclease free water was included in each run (Hudzieki, 2009).

3.13 DNA extraction and PCR amplification

The method used in this study was that by Unno *et al.*, 2010. Template in PCR reactions was prepared from five colonies heated in 100 ml distilled water (95^0C for 10 minutes) followed by centrifugation step of cell suspension at 12000 rpm for 5 minutes. The supernatant was discarded. The pellets were suspended in 2 ml of sterile distilled water and transferred to 2 ml Eppendorf tubes. The tubes were placed in a heating block and the suspension allowed boiling at 95^0 C for 5 minutes. The mixture was allowed to cool down before centrifugation at 14000 rpm for 2 minutes. The supernatant was transferred to a new tube and stored at -20^0C until further use as a source of template DNA. PCR amplification was carried out by using DNA thermal cycler (Biometra, Singapore) using specific primer for *Bla* TEM, CTX-M and SHV (Table 3.4). In a 50 µl volume containing 5µL of 10X, PCR buffer, 4µl deoxynucleoside triphosphates (dNTP) (2.5mmol/L), 1.2 µL forward primer (10umol/L), 0.5 µL Taq DNA polymerase (5 U/µL) 2µL extracted DNA (20 ng/µL) distilled water to a total volume of 50 µL (Cinagen, Iran) of PCR reaction, Amplification of *bla* TEM, *bla* SHV and *bla* CTX-M genes was performed by the DNA thermal cycler under the conditions shown in the table below (Table 3.4). Appropriate positive controls strains were used for each reaction. After PCR products were analyzed by agarose gel electrophoresis in 1.5% agarose gel. DNA was stained with ethidium bromide (1 µg/ml) for 15 minutes and visualized under UV light for 1 hour at 100 V in 0.5 × TBE buffer. PCR amplicon size was calculated by comparison to molecular weight size marker (100 bp) DNA molecular weight marker (Fermentas, USA) and the image recorded with the aid of a digital camera.

Table 3.5: Primers used in this study

Primer	Sequence (5'-3')	References	Amplicons Size (bp)
Bla $_{CTX-M-F}$	ATGTGCAGYACCAGTAARGT	Pagani *et al.*, 2003	593
Bla $_{CTX\ M-R}$	TGGGTRAARTARGTSACCAGA		
Bla $_{SHV-F}$	GGTTATGCGTTATATTCGCC	Oliver *et al,* 2002	867
Bla $_{SHV-R}$	TTAGCGTTGCCAGTGCTC		
Bla $_{TEM-F}$	ATGAGTATTCAACATTTCCG	Oliver *et al*, 2002	867
Bla $_{TEM-R}$	CTGACAGTTACCAATGCTTA		

Primers used in this study include Key-SHV-Sulphur hydroxyl Variable, CTX-M, -Cefotaximase-M, TEM- Temoniera enzyme. Various Bla genes with their primers both forward and reverse, with different molecular weight in relation to their references.

3.14 Data management and analysis

Data was presented using tables, graphs and pie charts. Data was entered into a Microsoft® Excel spread sheet, exported and analyzed using the statistical package for the social science (SPSS) package version 13.0 (IBM, Chicago). Chi square was used for testing the significance of the findings between the two study facilities.

CHAPTER FOUR

RESULTS

4.1. Level of Knowledge and current practices on management of hospital waste and environment amongst healthcare workers in Kenya

4.1.1 Social demographic variables of the respondents

The respondents comprised of healthcare workers working in various departments within the hospitals. Majority of the participants were males in Kenyatta National hospital (56%, KNH), while in Kikuyu Mission hospital majority were females (53%, KMH, p=0.072).

In gender distribution the proportion on average of males (52%) and females (48%) had a near parity in their proportions among the recruited respondents. A high proportion of respondents in terms of their profession were nurses. Respondents with Kenya certificate of primary education (KCPE) level of education were more than a half (61%), a small proportion had degree and above certificates (16%), while 23% were diploma certificates with about 40% having worked for 5-10 years in healthcare facilities (Table 4.1).

Results from this study revealed that knowledge on hospital waste management among the health workers in both hospitals was above average (over 50%) in most enquired aspects in the questionnaire. On knowledge of government rules on MW among the professionals the (public health officers) P.H.O had the highest level of knowledge in both hospitals (100%) while the cleaners were the least (35.7%) in KNH (Appendix IV). PHO had the most knowledge on Government plan rules on MW management 1998.

Table 4.1: Distribution of health care workers according to their socio-demographic variables on hospital waste knowledge and current practices issues at KNH and KMH

Character		KNH		KMH		x^2	df	p-value
		Count	Column (N %)	Count	Column (N %)			
Gender	Male	137	55.7	117	47.6	3.255	1	0.072
	Female	109	44.3	129	52.4			
Profession	Nurses	171	69.5	119	48.4	61.875	6	0.000*
	Laboratory technicians	14	5.7	12	4.9			
	Clinical officer	8	3.3	12	4.9			
	Doctors/Dentists	34	13.8	17	6.9			
	Cleaners	14	5.7	79	32.1			
	Public health officers	3	1.2	5	2			
	Waste handlers	2	0.8	2	0.8			
Level of education	Degree and above	68	27.6	40	16.3	21.828	2	0.000*
	Diploma	23	9.3	57	23.2			
	KCSE/ KCPE Certificate	155		149	60.6			
Job experience	1-5 years	60	24.4	74	30.0	2.01	2	0.36
	5-10 years	105	42.7	97	39.5			
	Over 10 years	81	32.9	75	30.5			

Key: *the asterisk means the p value is less than 0.05 there is statistical difference. Performance of level of education among the health workers according to gender, profession, level of education and job experience to show significance difference.

On source of segregation of hospital waste, the P.H.O profession had majority (100 %.) knowledgeable in both hospitals while the least were the waste handlers (50%). Knowledge of improper hospital waste management can lead to health problems was most familiar with doctors (88%) and public health officers (100%). On recognition of the international biohazard sign, the issue had the P.H.O. in KNH being most familiar with (66.7%) while the least knowledgeable being the waste handlers in each hospital with (50%). On the last issue of requirement of a waste manager in a hospital scored highly with the doctors having most knowledge on this issue with KNH (85.3%) and the waste handlers the least with (50%.). There was a high significance difference among the yes and no respondents, p=0.000* (Table 4.2).

Results revealed that generally KNH hospital had more knowledge on medical waste management than KMH however, there was no significance difference in responses among the participants in both hospitals, p=0.245) (Table 4.3).

Table 4.2: Knowledge of health care workers about some important aspects regarding hospital waste

		KNH		KMH		x^2	df	p-value
Knowledge on:	Responses	Frequency (246)	Percentage (%)	Frequency (246)	Percentage (%)			
Question 1: Government plan rules on MW management 1998	No	39	15.9	35	14.2	0.254	1	0.614
	Yes	207	84.1	211	85.8			
Question.2: Source of segregation of hospital waste	No	38	15.4	34	13.8	0.26	1	0.61
	Yes	208	84.1	212	86.2			
Question.3: Improper hospital waste management can lead to health problems	No	29	11.8	41	16.7	2.398	1	0.121
	Yes	217	88.2	205	83.3			
Question.4: Recognition of the international biohazard sign	No	60	24.4	61	24.8	0.011	1	0.917
	Yes	186	75.6	185	75.2			
Question.5: Presence of a waste manager in your hospital	No	34	13.8	65	26.4	12.152	1	0.000*
	Yes	212	86.2	18,	73.6			

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Various questions on some aspects regarding hospital waste such as government plan rules on MW management, source of segregation, improper management can lead to health problems, recognition of biohazard sign and need of a waste manager. All this realized above average percentages, meaning respondents were very much familiar in these aspects in both hospitals, there was a significance difference (*) between the two hospitals on the question of need for a hospital waste manager.

Table 4.3: Overall hospitals performance on knowledge of MW management issues

Grading	Hospitals		x^2	df	p- value
	KNH Column N %	KMH Column N %			
Poor (less than 50%)	2.4	1.6	2.815	2	0.245
Fair (51-70%)	16.7	22.4			
Good (71-100%	80.9	76			
Total	100	100			

Grading of different scores of knowledge of MW management issues among health workers in both hospitals. KNH scored the highest knowledge, however there was no significance difference among the two hospitals p=0.245.

4.2 Current practices on management of hospital waste

This included results from respondents, questionnaire and observation checklist on the current practices on hospital waste management collection, storage, segregation transport, treatment and disposal in both hospitals (Table 4.4).

Awareness on practices on medical waste management from generation to disposal scored in most cases more than 50% which was above average in the questionnaires while from observation during field trip in the hospitals, the practices were poorly implemented.

In generation of hospital waste, it was observed that, weighing records for the hospital waste in both hospitals was practiced, and that the records were well maintained however this was not done regularly. KNH recorded over 1000kg daily while KMH had between 90-300kg daily.

Table 4.4: Current practices on issues of MW management

	Description	Expected correct answer according to WHO standards	KNH (Yes/positive response)	KMH (Yes/positive response)
Segregation	Place where it takes place(point of generation)	in wards	99(40%)	98(48%)
	Biohazard symbol	in both hospitals the containers were health care waste containers, with plastic bags inside	241(98%)	148(60%)
	Correct colour coding of infectious bin	Red	172(70%)	167(68%)
Collection	how often is the collection from the ward	Daily	241 (98%)	226(92%)
	Use of protective clothing's by waste handlers gloves, masks, gumboots	Yes	244(99%)	234(95%)
Storage of MW	Knowledge of location	Yes	near main gate	inside near laundry room away from busy places
		Located inside hospital premises	234(95%)	241(98%)
	Presence of a special equipment for sharp waste and an efficient storage facility		221 (90%)	172 (70%)
Treatment	Does it occur in the hospital premises		Yes 212 (86%)	No 236(96%)
Transport	By use of			
	a) open tractors		(192, 78%)	180(73%)
	b) private licensed van		open tractors	private licensed van
Disposal	Incinerator	Yes	177(72%)	197(80%)
Problems faced by waste management staffs in the hospitals	Those who have experienced a hospital waste health related problem in their line of duty	Yes	108(44%)	32(18%)
	Those who have experienced problems like	Respiratory	60(56%)	20(64%)
		eye problems	5(5%)	3(8%)
		skin rashes	27(25%)	8(25%)
		cuts/piercing/bruises	13(12%)	1(3%)
		all of them	3(2%)	0(0%)

Performance according to various steps of hospital waste management current practices as practiced by the various health workers in the two selected hospitals: the steps include segregation, collection, storage, treatment, transport, disposal and problems faced by waste management health workers.

Segregation of hospital waste took place during the time of collection and was done by waste handler/cleaner. Generation of MW according to 99 (40%) KNH response and 98 (39.83%) KMH and was mostly in the wards (Table 4.5). In KNH, MW plastic containers and bags that were used were marked with international biohazard symbols. This hospital was compliant with OSHA standards and plastic containers were labeled but in KMH it was not according to OSHA standards with complete lack of international biohazard sign. The correct color coding (red) of the infectious MW bin was known by most health workers' professionals (KNH, 70% and KMH, 68% (Table 4.4, Appendices V a).

Collection of hospital waste took place from the wards in both hospitals. The use of protective devices such as gloves, masks and gumboots was recorded as being used and practiced in both hospitals.

Knowledge on presence of storage facilities either temporal or permanent in the two hospitals was high (95% KNH and 98% KMH) in this study. In KNH storage area was protected from unauthorized entry with a secured gate, while in KMH there was unrestricted entry. Both storage facilities were not marked with biohazard symbol at the time of study. It was observed that after the container for hospital waste fills it was removed and replaced by another one immediately such that, there were no spill over in both hospitals. In some instances, there were spill overs depending with the quantity of work per day. Storage time of hospital waste in the containers before replacing was about 6 hours in both hospitals (Appendices V, d, e).

Treatment of medical waste was in the hospital premises at least according to KMH majority (96%) of the respondents who knew treatment did not take place in the hospital premises (Table 4.5). In KNH treatment of MW took place in the storage area located in the hospital premises (86%), where there was an incinerator while at KMH there was no incinerator and hospital waste was collected by a licensed private

company. Offsite treatment of medical waste was permitted in both hospitals. In case of offsite treatment, the person responsible for disposal was supposed to obtain a disposal site receipt to ensure it was disposed to the right place however none of these receipts was available at least during the research period.

Quality control process for hospital waste management was noted to be once per year in KNH hospital while it was completely absent in KMH hospital. Tracking system for the medical waste from generation to disposal was completely lacking in both health establishments. The person responsible for medical waste management at the time of study was a housekeeper with a diploma in housekeeping at KMH, while at KNH there was an infection control officer in charge of medical waste management who was a qualified doctor. It was observed that a hand washing facility in every working station was evident in KNH but unavailable in KMH.

KNH transported the hospital waste by use of wheeled trolleys from the wards then open tractors to the disposal area. The tractors were not marked with the international biohazard symbols. At the disposal site they possessed a licensed permit with written operating plan for handling and transport of MW. At KMH MW was transported using unlabeled international biohazard symbol wheeled trolley from the ward to the temporal disposal site. In this hospital, the private company responsible for disposal is licensed and used a closed van labeled with international biohazard symbol however it lacked the written operating plan for handling and transport of MW as required in the international standards.

The budgetary allocation of the medical waste management and incinerator at KNH was about Kenya shillings 1-1.5 million each month, while the budgetary allocation at KMH was of about Kenya shillings 100,000 to Kenya shillings 600,000 each month. This cost varied with the amount of MW generated from time to time. In this study, it was observed that there were no properly constructed landfills and ash pits, there was indiscriminate release of toxic pollutants. It was observed that spent syringes, sharps, needles, medicine bottles, bloody bandages etc. were recovered in city garbage though the source of the hospital was unknown as there was no indicator (Appendices V, d). The results from this study revealed that healthcare worker faced

various problems during their day today activities in line with the process of waste management. Some of the illnesses that the respondents identified during the research study included respiratory problems eye problems, skin rashes, cuts/piercing/bruises and a combination of all the problems (Unconfirmed data from the hospital sources).

4.3 Prevalence of bacteria in hospital environment and wastes in KNH and KMH

A total of 471 bacteria isolates were identified from environmental samples of hospital surfaces, waste water dumpsites etc. KNH had the most number of positive plates that indicated presence of bacteria with a total of 197 (80.08%) while KMH had 163 (66.26%) (Table 4.5). There was no significant increase in the prevalence of contamination in private hospital compared to public hospital ($p = 0.38$). Out of the 246 samples, each site for each hospital had a total of 25 samples collected, majority of positive plates for presence of bacteria in both hospitals were from site A (waste from hospital main drainage) with 25 (100%) and site I (Orthopedic unit) for KNH with 25 (100%) level of positive growth plates, while the least was site C (operation theatre) for both hospitals with KNH only 6 plates with positive growth out of 25 (24%), while KMH had 9/25 (36%) (Table 4.5). In total Gram negative bacteria were most abundant in most departments 341 (72.3%) as compared to Gram positive bacteria 130 (27.7%). Results indicated isolation of various isolate from their positive biochemical tests (Table 4.6).

Table 4.5: Distribution of positive samples taken from hospital environments and waste in KNH and KMH.

Sample sites	**Name of Hospital**	**Samples positive with bacterial colonies.** Total no. of samples N=25 (100%)	**Samples Average N (%)**
A (waste hospital main drainage)	KNH	25(100%)	25(100%)
	KMH	25(100%)	
B (ICU)	KNH	16(64%)	15.5(62%)
	KMH	15(60%)	
C (Operation theatre)	KNH	6(24%)	7.5(30%)
	KMH	9(36%)	
D (Sterilization area)	KNH	20(80%)	15(60%)
	KMH	10(40%)	
E (Pediatrics ward)	KNH	22(92%)	22.5(90%)
	KMH	21(84%)	
F (Gynecology/obstetric)	KNH	16(67%)	17.5(70%)
	KMH	19(79%)	
G (Internal medicine)	KNH	22(92%)	16(64%)
	KMH	10(42%)	
H (General ward)	KNH	23(92 %)	18.5(75 %)
	KMH	14(58%)	
I (Orthopedic/surgical unit)	KNH	25(100%)	23(92%)
	KMH	21(84%)	
J (Hospital dump site)	KNH	22(88%)	20.5(80%)
	KMH	19(76%)	
Totals	KNH	197 (80.08%)	73.17%
	KMH	163(66.26%)	

Table 4.5 Percentage of bacterial contamination with site A had the highest, Presence of bacteria colonies indicated contamination. The least contaminated department was operation theatre.

Table 4.6: Results of biochemical tests of the bacterial isolates

Isolate Bacteria	Isolate N=471	MacConkey agar)	Gram stain	Indole test	MR/VP	citrate test	Oxidase	TSI test	catalase	Coagulase
Isolate A *Providentia sp*	102	pink-red colony-LF	-	+	+/-	+	_	A/A	+	N/A
Isolate B *E. coli*	67	Pink-red colony-LF	_	+	+/-	_	_	A/A	+	N/A
Isolate C *Pseudomonas sp*	44	Colorless NLF	_	_	-/-	+	+	A/NC	+	N/A
Isolate D *Serratia species*	34	pink red colony-LF	_	_	-/+	+	-	H_2S negative	+	N/A
Isolate E *Klebsiella species*	37	pink red colony-LF	_	_	-/+	+	-	A/A	+	N/A
Isolate F *Enterobacter sp*	18	pink red colony-LF	_	_	-/+	+	_	A/A no H_2S	+	N/A
Isolate G *Proteus sp*	22	Colourless NLF	_	_	+/-	_	_	K/A H_2S	+	N/A
Isolate H *Shigella species*	13	Colourless NLF	_	+	+/-	_	_	K/A H_2S	+	N/A
Isolate I *Salmonella species*	6	Colourless NLF	_	_	+/-	_	_	K/A H_2S	+	N/A
Isolate J *Staphylococcus aureus*	87	MSA- yellow colonies	+	N/A	N/A	N/A	N/A	N/A	+	+
Isolate K *Coagulase negative Staphylococcus*	43	MSA-small pink colonies	+	N/A	N/A	N/A	N/A	N/A	_	_

Table 4.6: Key Various biochemical results are illustrated in MR- Methyl Red, VP-Voges Proskaeur, Triple Sugar Iron Test, (Appendix V g, r). Appendix LF- lactose fermentors, NLF- non lactose fermentors, A means acid for slant (yellow colour), A means acid for butt + for production of H2S gas, K means alkaline for slant (red colour)/ K means for butt + for production of H_2S, NC means no change.

Results from API 20E test confirmed presence of the following Gram negative bacteria, *Klebsiella pneumoniae* 21, *Klebsiella oxytoca* 9, *Pseudomonas aeruginosa* 31, *Pseudomonas fluorescens* 7, *Pseudomonas oryzihabitans* 6, *Escherichia coli* 61, *Providentia rettgerri* 83, *Providentia alcalifaceans* 4, *Serratia marscens* 27, *Serratia liquafaceans* 4, *Enterobacter cloaca* 12, *Proteus vulgaris* 15, *Proteus milabilis* 3, and other Gram negatives included *Roultella ornithylitica* 17, *Ochrobactrum anthropic* 15, *Pantoea sp* 13 (Table 4.7, Appendix E xix).

Table 4.7: Total number of bacteria identified

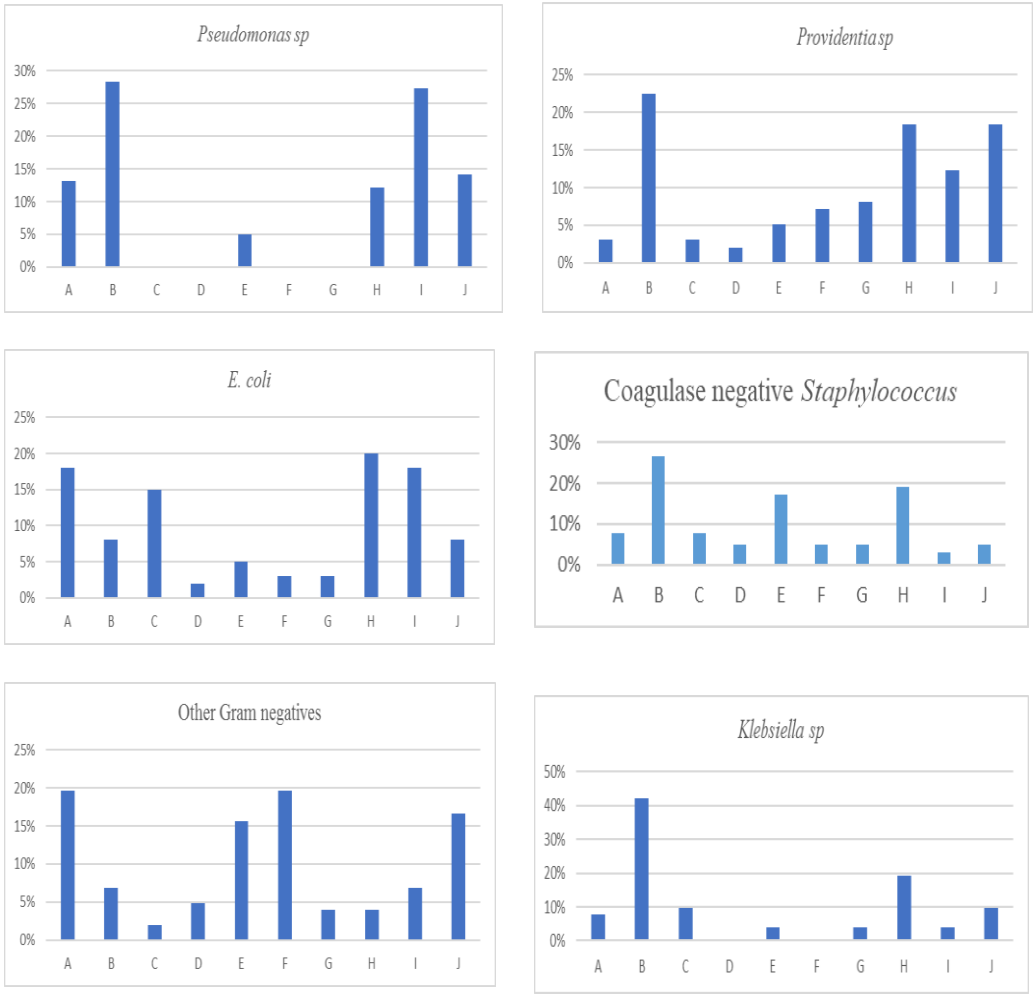
Bacteria identified	Total number	Percentage (%)
Providentia sp	99	21
S. aureus (Gram-positive)	87	18
Escherichia coli	61	13
Other Gram negatives	45	10
Pseudomonas sp	44	9
CONS (Gram positive)	43	9
Serratia sp	31	7
Klebsiella sp	30	6
Proteus sp	19	4
Enterobacter sp	12	3

Various types of bacteria isolated from hospital waste and environment. The most commonly isolated bacteria in the hospital waste environment was *Providentia sp,* while the least was *Enterobacter sp.*

On average, *Providencia sp* was the most abundant bacterial isolate (21%), followed by *S. aureus* (18.5%) while the least was *Enterobacter cloaca* at (3%) followed by *Proteus species* (4%) (Figure 4.1).

At KMH the most bacterial isolate was *S. aureus* and *Providentia species* (7.2% in both) while the least was *Enterobacter cloaca* at (1.1%).

E. coli was isolated mostly from waste water main drainages (A), *Providentia species, Pseudomonas species Klebsiella species* was the most abundant in ICU (B), *Proteus species* in operation theatre (C), *Enterobacter cloaca* in operation theatre (C). *Serratia species* in ICU (B) while *S. aureus* and other coagulase negative Gram positives were uncommon in ICU (B), general ward (H) and Orthopedic (I) (Figure 4.1).



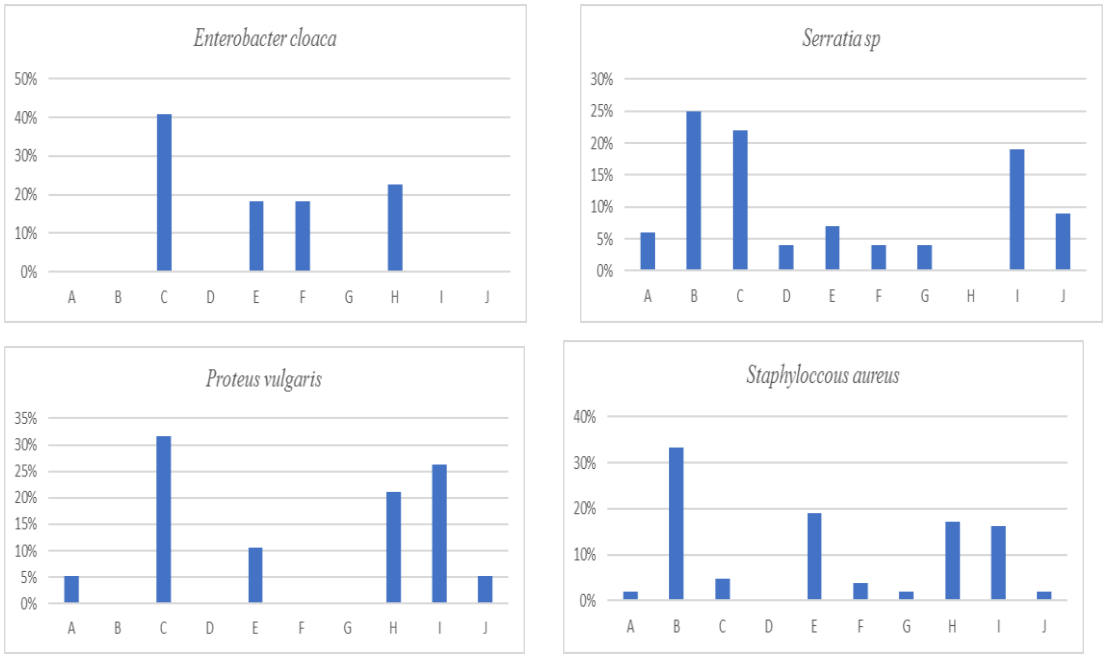


Figure 4.1: Frequency of each bacterium isolated from hospitals departments

Level of various bacterial contamination in various departments, A (waste hospital main drainage), B(ICU), C (Operation theatre), D (Sterilization area), E (Pediatrics ward), F (gynecology), G (internal medicine), H (general ward), I (orthopedic/surgical unit), J (Hospital dump site). ICU has the most number of different isolated bacteria.

The distribution of the isolates in both hospitals is listed below, with *Providentia sp* with the highest percentage (13.8%) in KNH and *Enterobacter* sp in KMH with the least percentage (Figure 4.2).

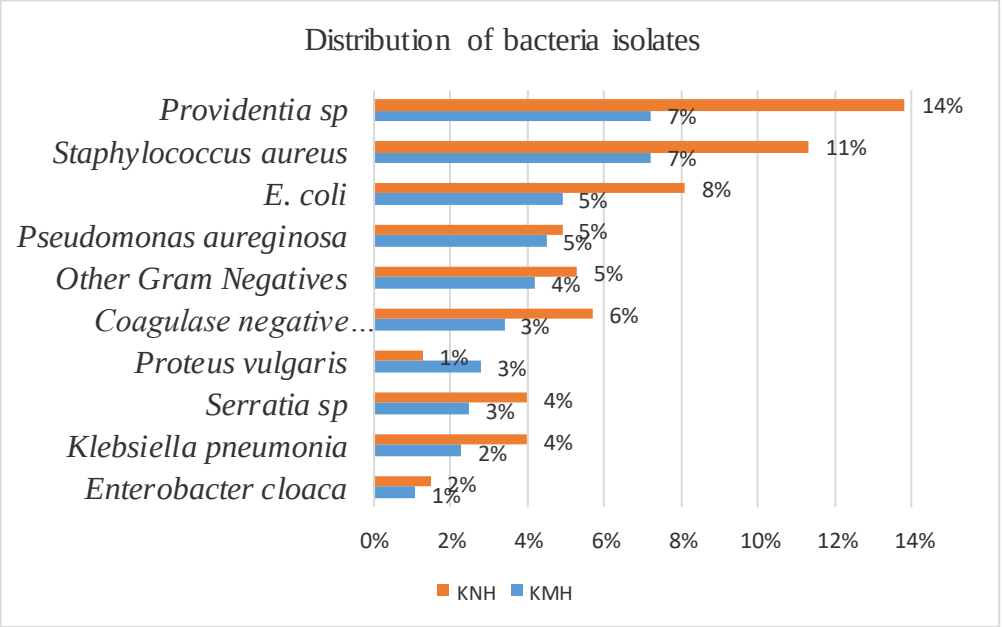


Figure 4.2: Frequency of various bacterial isolates from the sampled sites

Pseudomonas species were most abundant in sinks (20.4%) followed by operation table (18.18%) (Table 4.8). *Klebsiella species* were mostly found in operation table (26.66%) then door handles (20%). *E. coli* was isolated in nurses' hands surface (NHS) at (22.9%), followed by nurses' staff table (NST) at 19.6%. *Proteus species* were mostly found in stretchers and elevator buttons in 26.31% in both, and *Enterobacter cloaca* was abundant in nurses' hands surfaces at 50%, *Providentia species* were mostly isolated in door handles at 14.14%, *Serratia species* were most abundant in waste water samples and door surfaces at 22.58%. Other Gram negative isolates were found in waste water and door surfaces etc. *S. aureus* was abundantly found in nurses' hands surface in about 21.83%. Other coagulase negative *Staphylococcus* was most abundant in elevator buttons (39.53%) and door handles in 25.58% occurrence (Table 4.8).

Table 4.8: Occurrence of bacterial isolates in relation to their source of collection in the two hospitals environment and waste.

SS	HOSP	SZ	*PD*	*KB*	*EC*	*PT*	*ENC*	*PS*	*ST*	OGM	*SA*	CONS	Total number of bacteria	Overall	%
NHS	KNH	19	0	0	10	0	2	3	0	2	10	3	30	50	11
	KMH	19	0	0	4	0	4	2	0	0	9	1	20		
NST	KNH	19	0	4	8	1	1	4	0	3	8	0	29	46	10
	KMH	18	0	1	4	1	0	3	0	3	4	1	17		
DH	KNH	19	0	5	4	0	0	6	0	0	8	4	27	59	13
	KMH	19	0	1	6	0	2	8	0	1	7	7	32		
TS	KNH	19	2	0	0	1	1	8	2	1	7	2	24	40	8
	KMH	19	5	0	0	0	0	3	2	2	4	0	16		
OT	KNH	18	5	4	7	0	0	6	1	1	2	0	26	44	9
	KMH	19	3	4	1	2	0	2	0	1	4	1	18		
Sink	KNH	19	6	1	0	2	0	7	1	4	0	0	21	36	7
	KMH	19	3	1	0	1	0	4	4	2	0	0	15		
Stretcher	KNH	19	0	0	5	3	1	10	0	1	1	2	23	37	8
	KMH	19	0	0	4	2	0	3	1	2	2	0	14		
floor surface	KNH	19	5	0	0	1	0	7	1	1	2	1	18	28	6
	KMH	19	2	1	0	0	0	4	2	0	1	0	10		
EB	KNH	19	3	0	4	2	1	0	1	0	0	2	14	36	7
	KMH	19	1	0	1	3	4	1	1	0	0	1	12		
WW	KNH	19	2	1	0	2	0	4	6	5	0	0	20	35	7
	KMH	19	4	3	0	2	0	2	1	3	0	0	15		
DS	KNH	19	2	1	0	0	0	4	4	3	0	0	14	26	5
	KMH	19	0	3	0	0	0	1	3	5	0	2	12		
BR	KNH	19	0	3	1	1	0	1	2	1	5	0	14	18	4
	KMH	19	0	0	0	0	1	1	0	0	1	1	4		
CB	KNH	19	0	0	1	0	0	3	1	2	2	0	9	16	3
	KMH	19	0	0	1	0	0	0	0	0	5	1	7		
Total		492	44	30	61	19	12	99	31	45	87	43	471	471	100

Key- SS-sample source, SZ-sample size, PD-*Pseudomonas sp*, KB-*Klebsiella sp*, EC-*E. coli*, PT-*Proteus sp*, ENC-*Enterobacter cloaca*, PS-*Providentia sp*, ST-*Serratia sp*, OGN-other Gram negatives, SA *Staphylococcus aureus* CONS-coagulase negative *Staphylococcus*-NHS-nurse's hands surface, NST-nurses/staff table, DH-door handle, TS-table surfaces, OT- operation table, EB- elevator button, WW- waste water, DS- Device surfaces, BR- beddings/ bedrails, CB- cupboard surfaces. Most bacteria were isolated on nurse's hands surfaces, while the least was found cupboard surfaces. *Providentia sp* were the most abundant, while *Enterobacter cloaca* was the least detected.

The isolation of bacteria was highest on door handles (12.5%) followed by nurse's hands surfaces (10.6%), while the least was cupboard surfaces (3.39%) followed by bed rails (3.82%).

4.4 Antibiotic susceptibility test patterns of isolated bacteria strains

On average the most sensitive bacteria were *E. coli* species among the Gram negatives (66%) and Gram positives were *S. aureus* with 56 % isolates while, the most resistant among the Gram negatives included *Proteus species* (68%) followed by *Pseudomonas aeuruginosa* species and *Serratia* species (48% each) and the least resistant was *S. aureus* with 37. (Table 4.9). Overall results indicate that KNH had more sensitive bacteria (52.12%) as compared to KMH (47.61. Overall *E. coli* was the most susceptible bacteria that was isolated (Table 4.10).

Table 4.9: Susceptibility patterns among the various bacteria species isolated from both hospitals

Bacterial isolate	Source	Sensitive	Intermediate	Resistant	Total
E. coli	KNH	25	8	5	38
	KMH	15	5	3	23
Providentia sp	KNH	33	10	22	65
	KMH	17	5	12	34
Enterobacter cloaca	KNH	3	2	2	7
	KMH	2	2	1	5
Pseudomonas sp	KNH	8	4	11	3
	KMH	8	3	10	21
Proteus sp	KNH	1	1	4	6
	KMH	3	1	9	13
Serratia sp	KNH	11	1	7	19
	KMH	3	1	8	12
Klebsiella sp	KNH	9	4	6	19
	KMH	5	2	4	11
other Gram negatives	KNH	12	2	11	25
	KMH	9	2	9	20
S. aureus	KNH	15	2	10	27
	KMH	9	1	6	16
Other coagulase negatives *Staphylococcus* (CONS)	KNH	30	4	19	53
Totals	KMH	19	3	12	34
	KNH	147	38	97	282
	KMH	90	25	74	189
	GRAND TOTAL	237 (51%)	63(13%)	171 (36%)	

Total percentage of resistant isolates was 36% while the sensitive isolates were 51%. KNH had more resistant isolates than KMH hospital.

Table 4.10: Overall percentage level of susceptibility among the various isolated bacteria

	Percentage level of susceptibility among various antibiotics in percentages		
Bacterial isolates	Sensitive	Intermediate	Resistant
E.coli	66	21	13
Providentia species	51	15	34
Enterobacter cloaca	42	33	25
Pseudomonas species	36	16	48
Proteus species	21	11	68
Serratia species	45	7	48
Klebsiella species	47	20	33
Other Gram negatives	47	9	44
S. aureus	56	7	37
Coagulase negative *Staphylococcus* (CONS)	56	8	36

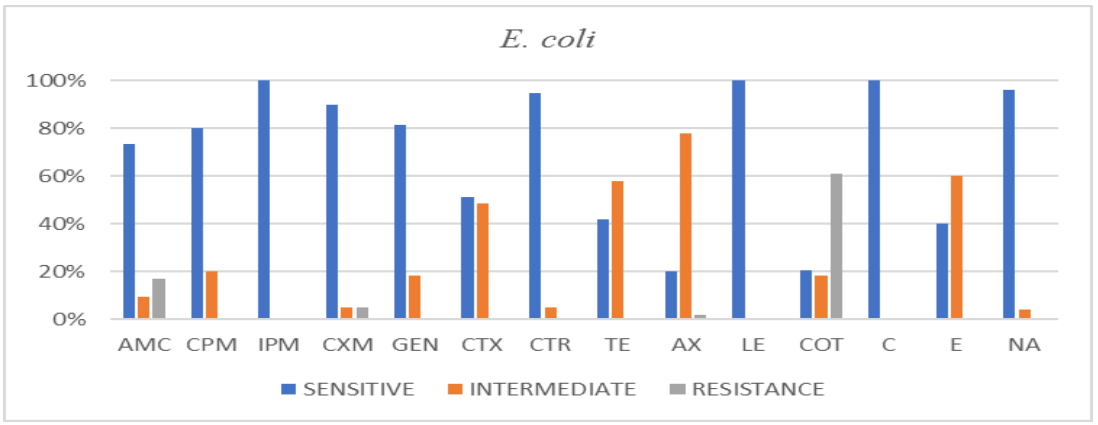
Percentages of susceptibility patterns among the various isolated isolates. *Proteus* species were amongst the most resistant isolate while the most sensitive was *E. coli.*

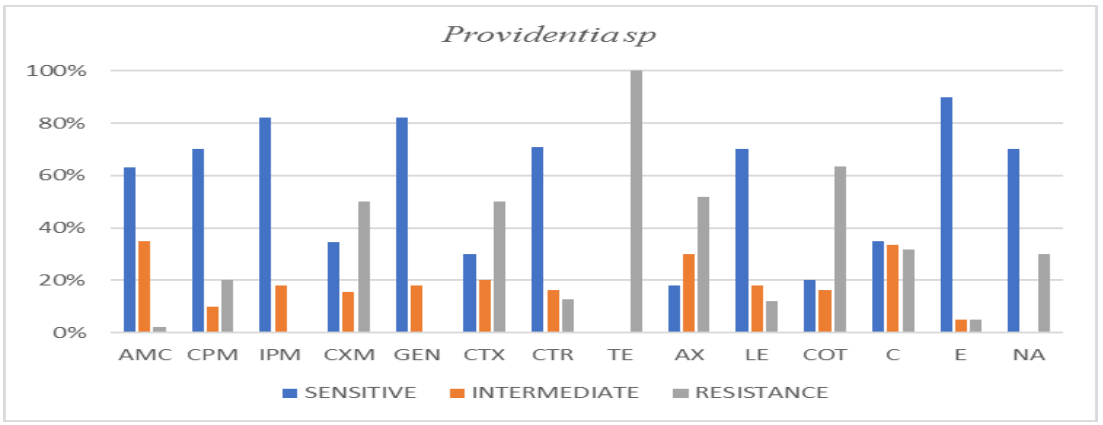
E. coli had 100% sensitivity to imipenem, cefuroxime, levofloxacin, and chloramphenicol antibiotics, and it showed high level of resistance to ampicillin (80%), cotrimaxazole (60%) and erythromycin (40%) (Figure 4.3). In *Providentia* species erythromycin was the most effective drug (94%) while tetracycline was the least effective drug (100%). In most isolates for example, *Enterobacter cloaca*, *Pseudomonas species and Klebsiella sp*, imipenem had (100%) sensitivity and resistance of 88% in cefotaxime, erythromycin and ampicillin had 100% resistance (Figure 4.3). In *Serratia* the highest sensitivity was at 100% in imipenem, cefotaxime, levofloxacin, chloramphenicol and nalidixic acid while tetracycline, ampicillin and erythromycin had 100% resistance. In other Gram negatives sensitive to antibiotics were cefepime, imipenem, levofloxacin and nalidixic acid had 100% while they were resistant amoxicillin/clavulanic acid, tetracycline, ampicillin, cotrimaxazole and erythromycin (80%) (Table 4.11) (Figure 4.3).

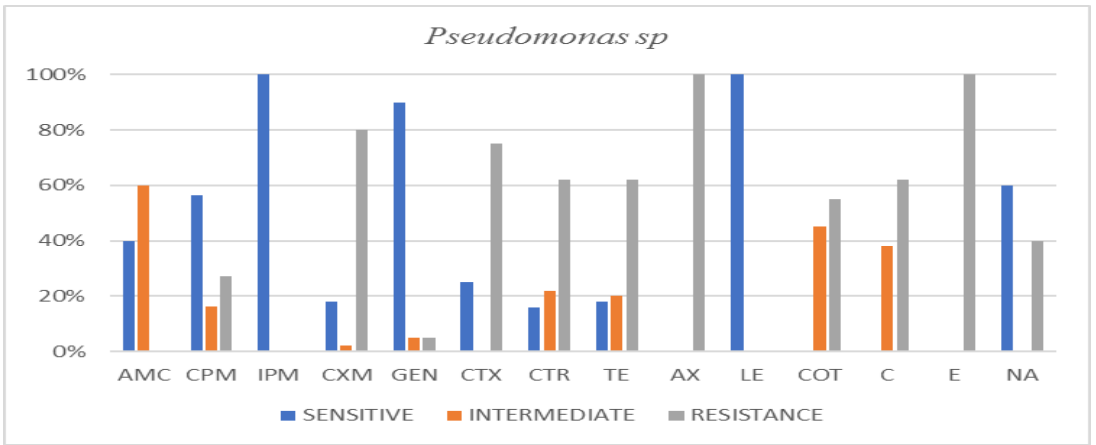
Among the Gram negatives were sensitive to imipenem with 96% followed by cefepime (68%) and levofloxacin 65%, with tetracycline 71% followed by cefotaxime 70% (Table 4.4).

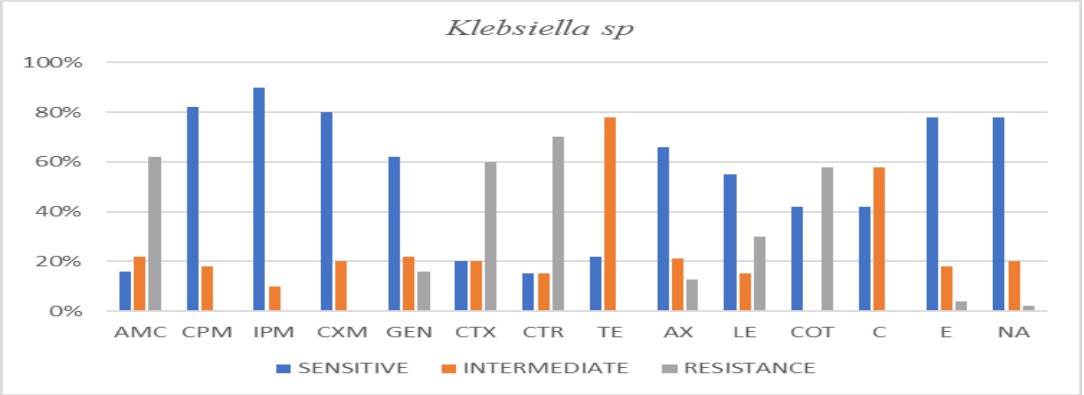
The drug of choice for most Gram negatives is the most effective imipenem antibiotic with 96% with 0% resistance with tetracycline being the least effective with 4% sensitivity.

The following are patterns of antimicrobial susceptibility in Gram negative isolates from both hospitals environment and waste in Kenya (Figure 4.3)









Other Gram negatives for example *Roultella ornithylitica, Ochrobactrum anthropi, and Pantoea species*

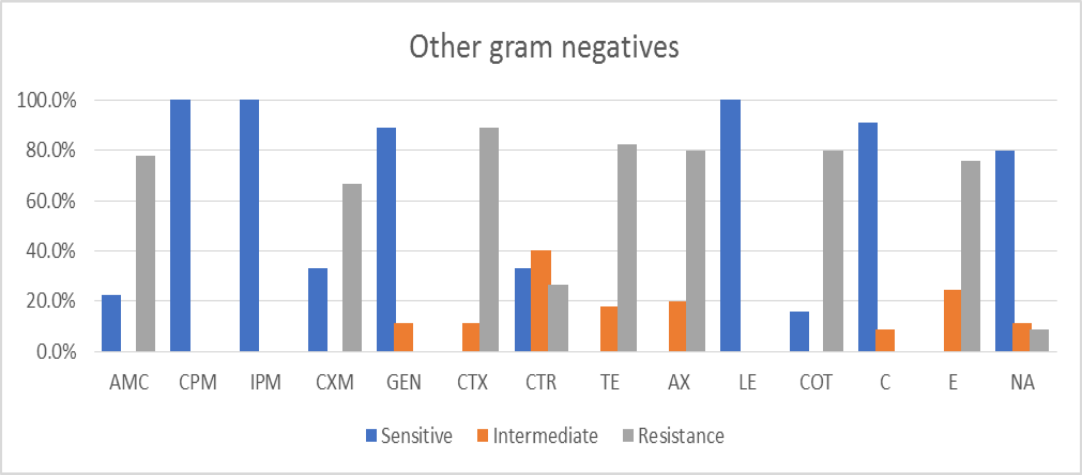


Figure 4.3: Patterns of antimicrobial susceptibility in Gram negative isolates

Key: AMC-amoxicillin/clavulanic acid, CPM-cefepime, IPM-iminepem, CXM-cefuroxime, GEN-gentamicin, CTX- cefotaxime, CTR-ceftriaxone, TE-tetracycline, AX- ampicillin, LE- levofloxacin, COT-cotrimoxazole, C- chloramphenicol, E-erythromycin, NA- nalidixic acid. Imipenem is the most active antimicrobial agents among the Gram negatives.

Results from Gram negative bacteria activity against the various classes of antibiotics reveals that there was no significance difference among the organisms in the susceptibility. x^2= 1.1674, df= 2, p=0.5578 not significant (Figure 4.3).

Among Gram positive bacteria, *S. aureus* and coagulase negative *Staphylococcus* were most sensitive to linezolid (99%) followed by gentamicin 90%, while the most resistant drug to ampicillin with 96% (Figure 4.5). In Gram positives linezolid had 99% sensitive drug while least effective drug was ampicillin at 96% for (Figure 4.4) (Appendix V x).

Among the Gram positives were most sensitive to linezolid antibiotic with 100% then gentamicin and chloramphenicol with 90% each respectively. Gram positives were most resistant to ampicillin with 100%. The drug of choice for Gram positives was linezolid with 100%, and the least effective was ampicillin with 0% sensitivity (Table 4.12).

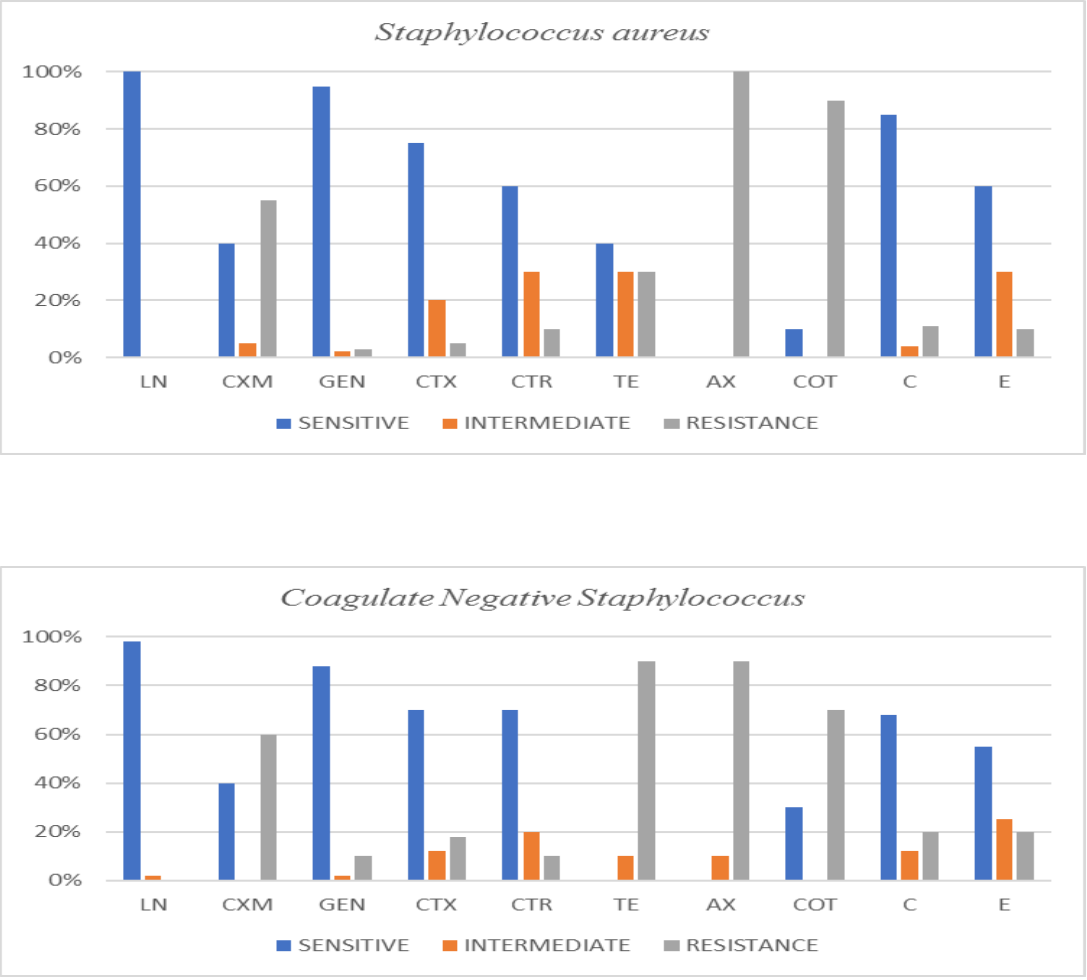


Figure 4.4: Susceptibility patterns of Gram positive bacteria

Linezolid was the most potent drug among the Gram positives, followed by gentamicin.

It was reported in this study that some bacterial isolates recorded resistant to more than three classes of antibiotics and this indicated multidrug resistance as shown in the table below (Table 4.11).

Table 4.11: Summary of resistant bacteria to different antibiotics

Bacterial isolates	**Resistant antimicrobial agent with over 30%**
Providentia sp	CXM, CTX, TE, AX, COT, NA
Enterobacter cloaca	CTX, CTR, AX
Pseudomonas sp	CXM, CTX, CTR, TE, AX, COT, C, E, NA
Proteus sp	CPM, GEN, CTX, CTR, TE, AX, LE, COT, C, E, NA
Serratia sp	AMC, CTX, TE, AX, E
Klebsiella sp	AMC, CTX, CTR, TE, LE, COT, C
Other Gram negatives	AMC, CXM, CTX, CTR, TE, AX, COT, E
Staphylococcus aureus	CXM, TE, AX, COT
coagulase negative *Staphylococcus* (CONS)	CXM, TE, AX, COT

Key: AMC-amoxicillin/clavulanic acid, CPM-cefepime, IPM-iminepem, CXM-cefuroxime, GEN-gentamicin, CTX- cefotaxime, CTR-ceftriaxone, TE-tetracycline, AX- ampicillin, LE- levofloxacin, COT-cotrimoxazole, C- chloramphenicol, E-erythromycin, NA- nalidixic acid.

Multi drug resistant isolates were considered to be resistant to more than three antimicrobial agents. In this case all the isolates isolated in this study were multidrug resistant.

4.4.1 Frequency of ESBL positive strains.

Susceptibility testing against ceftazidime and ceftazidime/clavulanate with ESBL strains showed distinct zone clearance areas with increased diameters of more than or

equal to 5mm indicating presence of an ESBL (Appendices V, w). Most of the resistant strains are found in drainages from waste water, internal medicine and the operation table areas. The areas with less resistant isolates included sterilization room and pediatrics areas. An increase in zone diameter of 5 mm for antimicrobial agent tested in combination with Clavulanate versus its zone when tested alone indicated a positive result or presence of an ESBL (Appendix V, w). The resistant ESBL and non-ESBL strains were further analyzed using PCR to detect the presence of the *Bla* resistant genes under study (Table 4.12).

Table 4.12: Distribution of ESBL and non-ESBL strains as tested from the resistant bacterial isolates

Departments	**Total number resistant strains (N= 171)**	**ESBL strains (N= 80)**	**Non ESBL strains (N= 91)**
A drainage from waste water	43	25	18
B ICU	17	5	12
C operation table	28	12	16
D sterilization room	5	2	3
E pediatrics ward	6	4	2
F Gynecology ward	7	5	2
G internal medicine	30	8	22
H General ward	7	2	5
I Orthopedic surgery	19	11	8
J Hospital dump site	9	6	3
Total	171	80	91
%	100%	46.80%	53.20%

Table 4.12. Drainage from waste water (site A) had the most ESBL positive strains, while general ward and sterilization room had the lowest number of ESBLs. Non ESBLs were mostly found in internal medicine department. 35 out of 80 (44 %) ESBL strains were from KMH, while 45 out of 80 (56%) ESBL strains were from KNH. Non- ESBL strains from KNH were 41 out of 91(45%), while in KMH 50 out of 91(55%) were isolated.

The distribution of ESBLS and non-ESBLs was 46.8% and 53.2% respectively. There was no significance difference among the isolates (Figure 4.5).

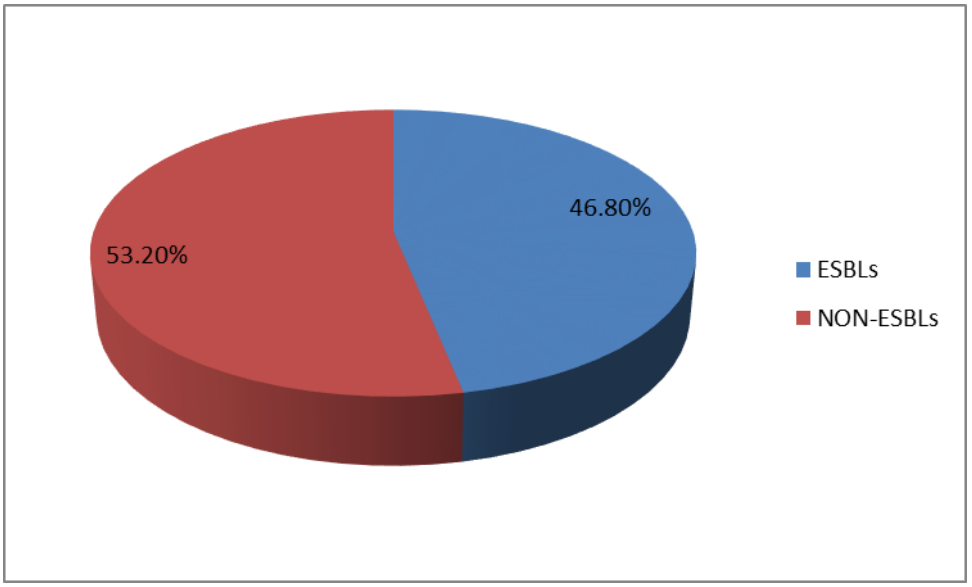


Figure 4.5: Distribution of ESBLs and non ESLBs isolates. ESBLs that were positive were more than the non ESBLs.

4.5 Detection of resistant bla genes TEM, bla CTX-M and bla SHV

PCR amplification was performed to detect the bla $_{TEM}$, bla $_{CTX-M}$ and bla $_{SHV}$ genes from 80 resistant strains due to implication of cost and manageable sample size, from the resistant 171 isolates. Out of the randomly selected resistant, 80 strains (46.7%) were ESBL positive and 91(53.2%) were non ESBL isolates producing using phenotypic tests, with the difference not significant p-value = 0.3326 (Table 4.13).

The 80 resistant strains that were selected randomly, 37 ESBLs and 43 non ESBLs. The resulting amplifications demonstrated that 6 out of 37 ESBL isolates and 2 out of 43 from non ESBL isolates carried *bla* $_{TEM}$, while 2 out of 37 from ESBL and 2 out of 43 from non ESBL isolates possessed *bla* $_{CTX-M}$ each and only 2 out of 37 were found to possess *bla* $_{SHV}$ gene while non ESBL isolates carried *bla* $_{SHV}$ (Table 4.13).

Table 4.13: Distribution of various *bla* resistant genes compared to the ESBLs

	Total isolates	bla $_{TEM}$	bla $_{CTX-M}$	bla $_{SHV}$	Total
ESBL positives (out of 80 (80/171*80	37	6	2	2	10
Non ESBL Positives (out of 91) 91/171*80)	43	2	2	0	4
Total	80	8 (10%)	4 (5%)	2 (3%)	14 (18%)

Bla $_{TEM}$ had the highest percentage among the isolates with 10%, while *Bla* $_{SHV}$ had the least (3%). The sample that proceeded to molecular work due to cost was a total of 80 resistant isolates from a total of 171. The 80 isolates were 37 ESBLs, 43 non ESBLs.

Results in this study reveal that, *bla* $_{TEM}$ was the most abundant gene with 10 %, followed by *bla* $_{CTX-M}$ 5 % and *bla* $_{SHV}$ had 3%. *Klebsiella pneumonia* had the highest number of *bla* genes 5 out of 14 (36%) followed by *Pseudomonas aeuriginosa* with 3 out of 14 *bla* genes (Figure 4.6, 4.7, 4.8 and 4.9), while the third was *Escherichia coli* and the rest with 2 out of 14, 14% and the rest as shown in the table below (Table 4.13). The distribution of the various resistant *bla* genes from their source of collection is explained in the table below (Table 4.14).

Table 4.14: Frequency of detection of genes in bacteria according to their antimicrobial resistance

Sources of bacteria N= 80	*bla* $_{TEM}$	*bla* $_{CTX-M}$	*bla* $_{SHV}$
Klebsiella. Species	3	1	1
Pseudomonas species	2	0	1
Escherichia coli	1	1	0
Providentia species	1	0	0
Serratia species	0	1	0
Enterobacter cloaca	0	1	0
Proteus vulgaris	1	0	0
Other Gram Negatives	0	0	0
Total	8	4	2
Grand total		14	

Bla $_{TEM}$ was most abundant in *Klebsiella* sp and *Pseudomonas* sp, *Bla* $_{CTX-M}$ was present in equal counts in *Klebsiella*, *E. coli*, *Serratia, Enterobacter* species.

Results from PCR experiments and gel electrophoresis showed various bands of *bla* $_{TEM,\ CTX-M}$ and $_{SHV}$ genes. The following are bands recorded from gel electrophoresis amplification for *bla* genes $_{TEM,\ SHV}$ and $_{CTX-M}$. It shows the distribution of the *bla* $_{TEM,\ SHV}$ and $_{CTX-M}$ genes among the various bacteria (Figure 4.6, 4.7, 4.8, 4.9).

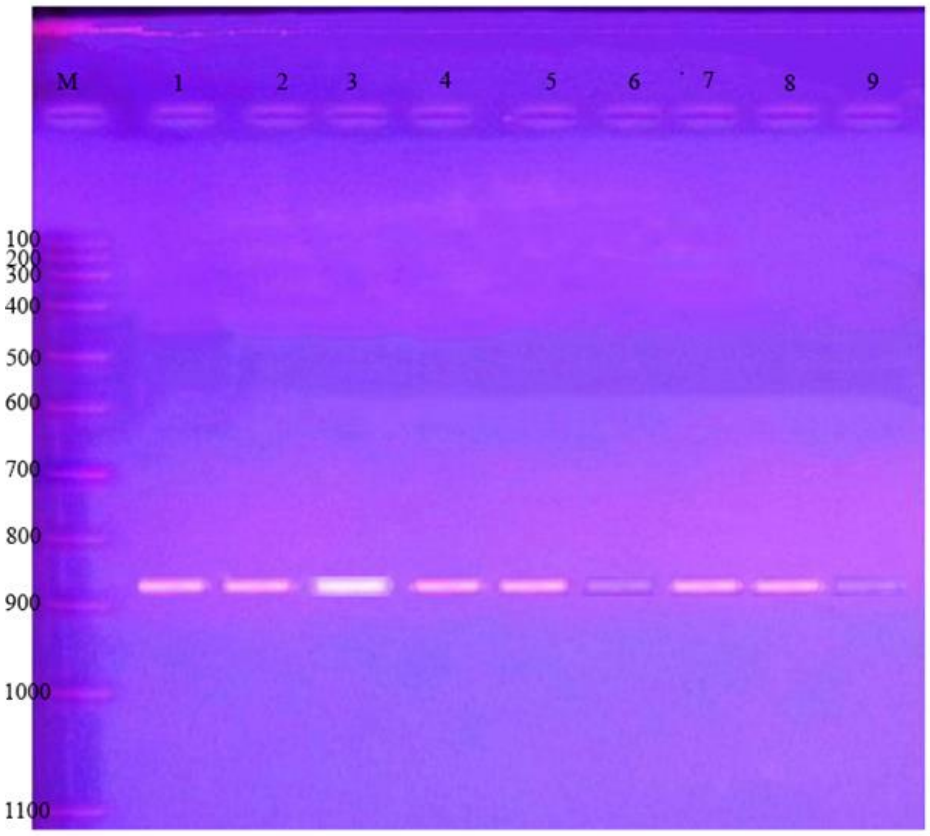


Figure 4.6: Gel electrophoresis of the PCR products of *bla* TEM gene

First lane, molecular weight marker; ladder, lane number 1 shows positive control of *E. coli*, 2,3,4, 5, 6 7, 8 and 9 shows 867 bp bands of PCR products.

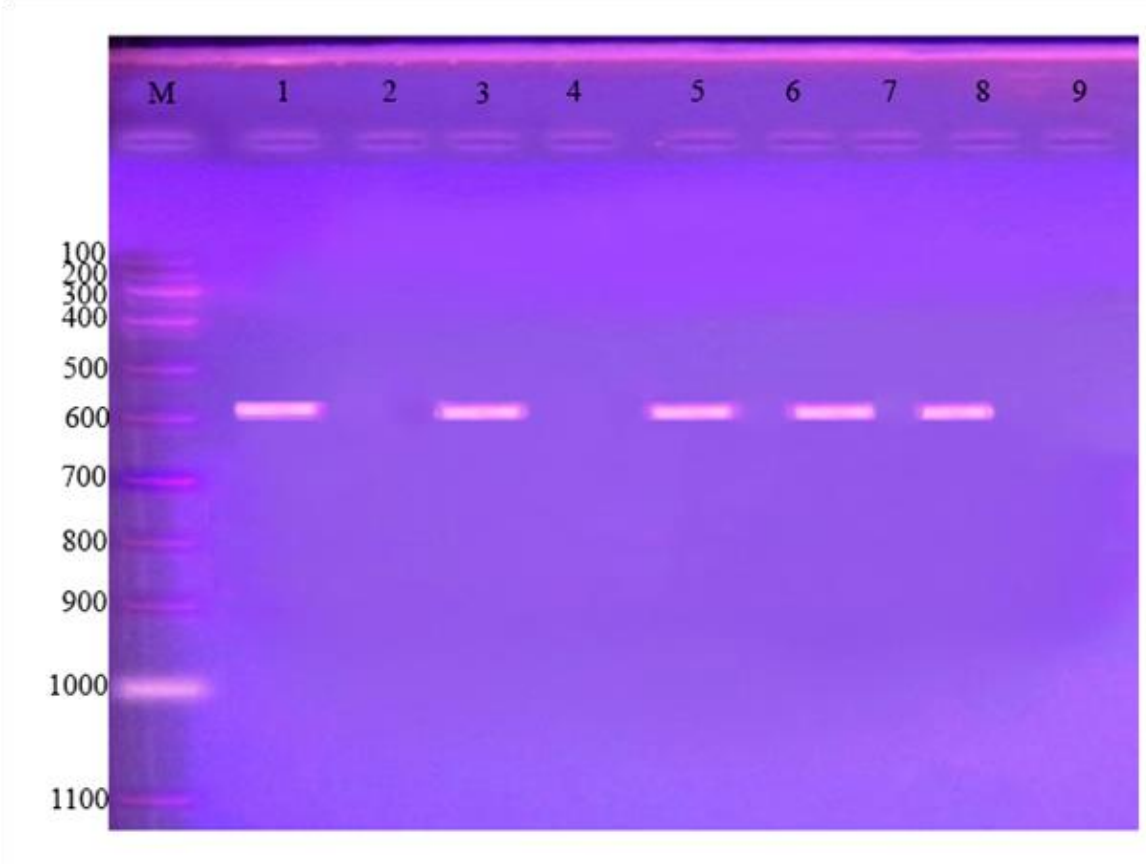


Figure 4.7: Gel electrophoresis of the PCR products of *bla* CTX-M gene

First lane, molecular weight marker; ladder, lanes numbered 1 positive control, 3, 5, 6, 8 show 593 bp bands of PCR products while lane number 2, 4, 7 and 9 shows negative result.

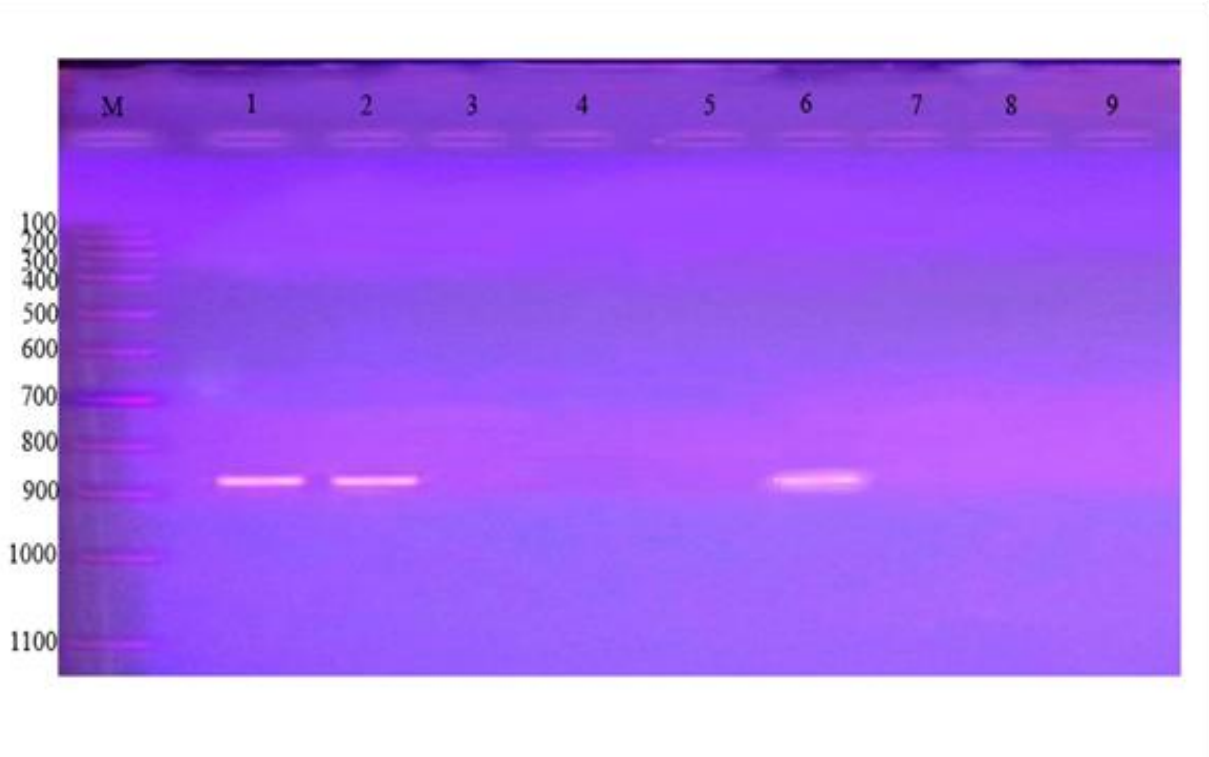


Figure 4.8: Gel electrophoresis of the PCR products of *bla* SHV gene

First lane, molecular weight marker; ladder, lanes numbered 1 positive control, 2, and 6 shows 867 bp bands of PCR products while lane number 3,4,5, 7,8 and 9 shows negative result.

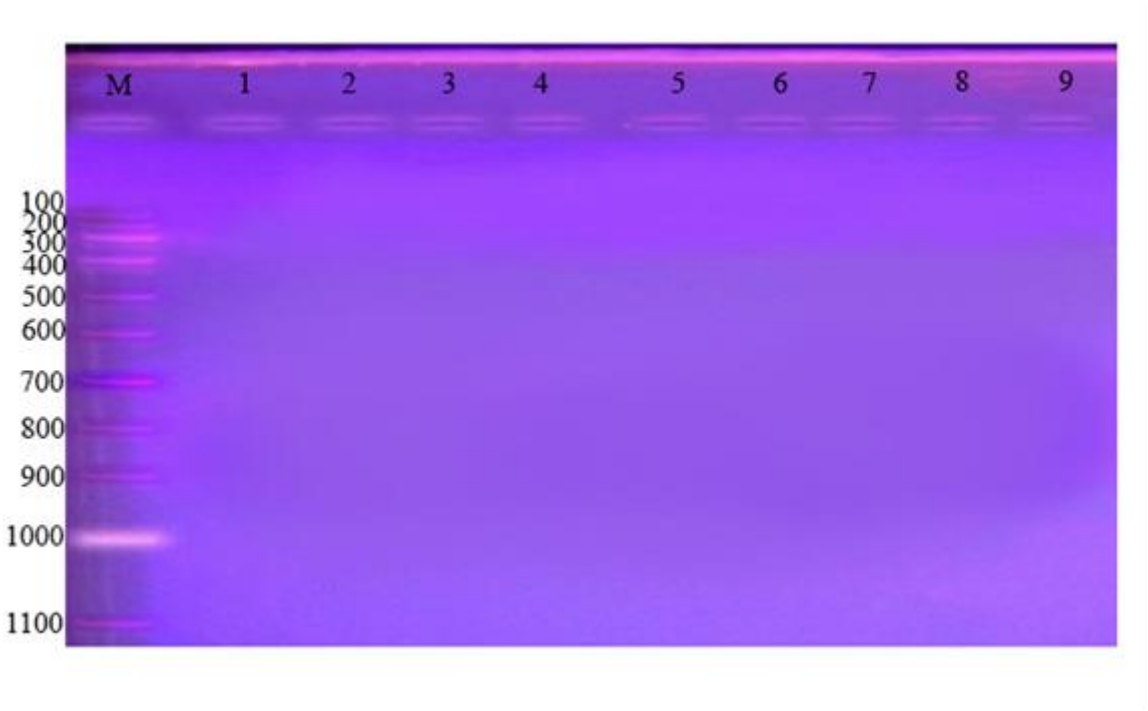


Figure 4.9: Gel electrophoresis of the PCR products showing negative

Amplification samples of bla $_{TEM, SHV}$ gene and $_{CTX-M}$.

First lane, molecular weight marker; ladder showing negative amplification for all the samples

CHAPTER FIVE

DISCUSSION

5.1 Knowledge of level of hospital waste management policies

The hospital personnel were chosen in this research of knowledge of hospital waste management considering the fact that these groups were more involved in medical waste generation, major contact with the waste and they are involved in its management than public (Oli *et al*., 2013). From this study, a high level of knowledge of hospital waste management policies was recorded. Among the respondent's level of education, 9.4% were university graduates, followed by diploma holders, at 82.5% and finally KCSE/KCPE certificates at 78.18%. The p value (p = 0.0416) indicated a significant relationship between level of education and knowledge of hospital waste management policies. This was similar to the findings by (Mathew *et al*., 2012), where awareness about the knowledge on medical waste and practices was quite high 62.5% and 100% amongst the technicians and paramedical staff respectively. The study on knowledge of health waste management gives a unique opportunity to provide information about a topic which is lacking in our country and identifies the gaps between the current knowledge among healthcare workers involved in waste management and the future desired state that should be reached.

The number of years' healthcare personnel had worked in the hospital did not indicate any significance to good knowledge of hospital waste management. On average 83.25% of personnel who have one to five years of work experience were aware of correct medical waste standards and practices, those with five-ten years of work experience had a mean score of 80.51%, while over ten years and above scored an average of 83.35%. However, the mean chi square results (p= 0.2834) indicated that there was no significant relationship between knowledge and number of years served as the p value was greater than 0.05. Training and duration of work experience were not significantly associated with knowledge scores. Mostafa *et al*, 2009, suggested that most training and orientation programs emphasize theoretical aspects with numerous lectures but minimal hands on training.

In the current study doctor, public health officers and nurses were more aware of good medical waste management policies. The p value 0.0484 indicated that there was a significant relationship between profession and knowledge on proper practices and standards of medical waste. This is consistent with a study carried out among hospital staff in medical college hospital in Bangalore showing that doctors and nurses had better knowledge than other staff regarding healthcare waste management (Madhukumar & Ramesh, 2012). This might be explained by the fact that more in-depth and detailed information is usually the concern of individuals with higher education and professional levels.

Government plan rules on medical waste management rule 1998 that the MW all health care institutions had to ensure safe disposal and environmentally sound management of waste produced by them as specified in the rules for proper disposal of hospital waste. This government plan rule on medical waste management rule was known in both studied health institutions at least according to KNH, 81.10%, KMH, 82.81%. This is slightly higher from the results reported in the study conducted in Bhopal which showed that 54.5% of health professionals were aware about the existence of medical waste management and handling rules 1998. The knowledge about MW and handling rules was above average in public health officers and doctors as compared to other staff like waste generators and handlers in this study. These findings were similar to other studies by (Pandit *et al*., 2005) and (Rao, 2008) in which technically qualified personnel like the doctors, nurses and lab staffs had high knowledge regarding these rules but was low among sanitary staff. This shows that the people with higher education had more knowledge about MW management and the rules prescribed in them (Saini *et al*., 2005). MW management knowledge was generally lacking among the waste generators and handlers who are classified as personnel with low level of education (Leonard, 2004). Training programs should take into consideration the educational level of housekeepers and waste handlers (those with lower education) since in developing countries usually a significant proportion are not well learned (Mostafa *et al*, 2009).

Segregation of hospital waste at the source was the most known issue in both hospitals with an average of 85.07% in KNH and 84.06% in the KMH. This finding

is supported by the observations made in the study done at Bangalore which stated that 87.5% of the study subjects were in favor of segregation of MW being done at source the of generation (Manyele *et al*., 2003). It was most known among the professionals for example, the (Public health officers) P.H.O.100%, clinical officers 100%, cleaners 92.9%, and nurses 83.0%. This undoubtedly reflects doctors lack of awareness of the problem in general and their role in waste management in particular. This may be attributable to their lack of training, as fewer doctors in this study reported receiving training on proper waste management at the hospital than did nurses. Another reason for deficient practices among doctors might be patient overload, due to the fact that KNH and KMH are referral hospitals to the Kenyan public. Segregation of different types of MW took place in wards which was also the point of generation. This ensures that infectious wastes do not get mixed with non-infectious wastes as this would infect the entire waste. The segregation and identification of the waste is the primary and most important step to be taken in the process of MW management.

Improper management of medical waste causes serious environmental problems in terms of air, water and land pollution and has a greater chance of causing infectious diseases (Deo *et al*., 2006) With respect to risks that workers could be exposed to due to improper management of MW, the study revealed that the issue scored 68.75% at KNH while it scored 84.06 % in KMH with public health officers (100%) and doctors (100%, KMH, 94.1% KNH) respectively scoring the highest. This result is consistent with the study done in S. Africa (Deo *et al*., 2006) that indicated that most health care workers (98.5%) were in agreement that improper management of medical waste could lead to transmission of infections in health care workers and patients.

Respondents were required to know whether infectious waste must be labeled with biohazard symbol and separately segregated from non-infectious waste (Manyele *et al*., 2003). This policy scored in terms of knowledge was the poorest with about 56.49% in KNH and 78.49% at KMH. It scored the highest with high level of education (KNH, 94.1, KMH 97.5%). Doctors scored the highest in KNH, 88.2%, and KMH, 100%). These results are in agreement with study done by (Molelekwa,

2001) who found that persons with higher level of education were more aware regarding this issue of recognizing the international biohazard sign than the less educated ones (Manyele *et al*., 2003).

There was a requirement in the questionnaire to know whether there was need to have a medical waste manager in a hospital which had KNH with 81.57% and in KMH 74.33%. At KNH the figure issue was most popular among P.H.O 100% followed by nurses 89.5% while at KMH it best known by doctors, lab technicians, clinical officers (all 100%). The high statistical difference on this issue among other health personnel's can be explained by individual interests and differences. A waste manager would encourage staff to make active contribution towards the proper medical waste management; can help prepare other health workers in handling and disposing of medical waste products from the health centers which can effectively minimize the risk of spread of hazardous diseases. Similar study done in Kings George Hospital in Visakhapatnam in India to assess the awareness about medical waste management among health care workers who were aware about the methods, guidelines of segregation and collection of wastes, they concluded that in order to improve the existing conditions a hospital control committee headed by a hospital manager, is necessary to supervise all aspects of medical waste management (Ramokate & Basu, 2009).

Significance of MW knowledge and their skill for proper waste management could be a fruitful exercise to quantify and minimize occupational associated risks. Adequate knowledge, proper techniques and safety practice measures can go a long way toward safe disposal and protection of the community from various adverse effects of hazardous waste (Padmaja, 2017).

5.2 Current practices on hospital waste management

Poor health care waste (HCW) practices from generation to disposal pose great health risks for the health workers, patients, waste handlers, scavengers and the community (Johannessen *et al.*, 2000). Results from the current study indicated that record keeping and weighing of medical waste took place in both hospitals, however, health workers need to be encouraged to update the records daily and

regularly. From the results it was found that KNH recorded over 1000kg daily while KMH had between 90-300kg daily of medical waste. The amount of waste generated in hospitals depended upon various factors; such as number of beds, types of health services provided, economic, social and cultural status of the patients and the general condition of the area where the hospital was situated (Askarian *et al.,* 2004), this could be the reason why KNH had more medical waste.

Segregation reduces the amount of waste and needs special handling and treatment (Sreegri & Babu, 2009). Data from the questionnaire and observation check list from the current study indicated that, segregation of MW took place in the ward with KNH (40%) and KMH 98(39.8%) and that it was done by cleaners. It was further revealed that the hospitals used plastic containers (Appendix E i and ii). Some containers were marked with international biohazard symbol (KNH), while others were not (KMH). Practices of high priority to segregation from source of infectious waste and sharp wastes by use of color coding system were used in KNH and KMH. It was observed that there was no uniformity in color coding of hospital waste in both hospitals. Despite the commendable level of segregation of medical waste currently achieved in the hospitals, media revealed that segregated medical waste are sometimes mixed together by collectors either at the point of collection or at the dumpsites as revealed by the researcher through the media (Appendix E, vi). The whole mixed volume therefore could be considered as being infectious which poses serious risks to the general public as recommended by WHO, 2005. Poor segregation and waste storage if not well managed allows easy access to scavengers hence high infection rates of diseases (Pescod & Saw, 1998). The hospitals basically separate hospital waste from general waste stream at the waste production points Therefore, they are stored and disposed of separately. However, the hospital did not segregate medical waste into different categories. In the wards, doctors and nurses who used sharps were required to drop them into different containers, but this was not diligently followed hence the low level of awareness on segregation point in the current study. KMH hospital did not label infectious waste with a biohazard symbol, no control measures existed for the management of these wastes. Separation of medical waste and general waste was however practiced to a fair extent. WHO rules demand that, hospitals have to provide plastic bags and strong plastic containers for infectious waste such as empty

containers of antiseptics used in the hospitals (UNEP, 2000). In relation, bags and containers for infectious waste should be marked with a biohazard symbol (Nwachukwu *et al.,* 2013). Results from this study under practices revealed that infectious wastes bins color red was the type of waste that the study population was most aware according to 172, 70% for KNH and 167, 68% KMH respectively The black color bin waste for general waste scored a low percentage right after the red color at 69, 28% (KNH) and 74, 30% (KMH) while the yellow bin for anatomical waste was the type of color code which was known by the minority i.e. 2% in both hospitals. This could be because the red color was what they would encounter often as opposed to the chemical; genotoxic wastes supposed to be in the yellow color. There was no harmony in the allocation of color codes for the different wastes categories in both hospitals and this is similar to the findings in a study done in Lagos (Longe & Williams, 2006). Waste segregation is poorly conducted in these facilities, yet rigorous segregation would minimize wastes, pollutant emissions and allow for recycling. It was noted that the only wastes that were properly segregated in both facilities were sharps. These were placed in rigid containers separate from all the other categories of wastes.

It should be noted that with proper segregation of medical waste reduces the cost of treatment and disposal as 80% of a hospital waste is general waste, which does not require special treatment, provided it is not contaminated with other infectious waste.

The collection of medical waste involved use of different types of containers from various sources like operation theatre, wards and kitchen etc. in this study. From the observations made in this study there was also a routine schedule for the collection of medical waste in KNH and it was done daily unlike in KMH where it was not regularly done. Hospital wastes generated in the hospitals was collected daily and transported to a temporary storage area by hospital's staff according to (241, 98%) for KNH and (226, 92%) for KMH. This is in line with a survey done in 2007 by the government of Kenya on hospital waste management in various hospitals, results indicated that the frequency of collection of waste in most hospitals was done once daily (MOH, 2007). In addition, the MOH 2007 survey revealed that hospitals visited were found to have refuse storage areas/rooms. In some of these hospitals,

unused rooms, some with leaking roofs were used to store waste. Use of protective clothing by waste handlers during collection scored highly, with (KNH, 244, 99%) and (KMH, 234, 95%). This was not similar to a study in India that revealed that 22.92% of the respondent was aware that the wearing personal protective equipment minimizes sharp injuries (Nwachukwu *et al.*, 2013). It is important to note that the lack of suitable and sufficient protective equipment, incorrect usage of equipment and the lack of pertinent understanding of the personnel regarding the benefits of using protective equipment exposes personnel to serious dangers (MOH, 2007).

The place where the hospital waste was stored before transporting to the final disposal site was termed as a temporary waste storage area. In this study most health personnel knew the exact location of medical waste temporal storage. From the researcher's observation KNH had a well secured but poorly sanitized temporary storage area while KMH had unsecured and not sanitized storage area. This contradicts with the study done by Nwachukwa *et al.*, 2013, who revealed that temporal storage place for medical waste must be well sanitized and secured for easy access for staff in charge of handling the waste and secured to prevent access for animals, insects and birds. A similar study done in South Africa at Tygerberg Hospital to assess the hospital waste management practices, results indicated that they had a well secured but poorly sanitized temporary storage area (Leonard, 2004).

In both hospitals the medical waste was emptied after every six to eight hours at KNH and about twenty-four hours of temporal storage. Bins and sharps containers were disposed when three quarters to full capacity in most occasions. This is similar to study conducted in India where a descriptive study was conducted to assess the knowledge on preventive practice regarding needle stick injuries among ninety-six staff nurses at Mangalore (Sristhi, 2000). KNH had its medical waste containers properly labeled with an international biohazard symbol unlike KMH hospital. WHO standards 2007 requires that segregated wastes of different categories need to be collected in identifiable containers, the duration of storage should not exceed for 8-10 hours in big hospitals (more than 250 beds) and 24 hours in nursing homes. It was paramount that container may be clearly labeled to show the ward or

room where it was kept The reason for this labeling was that it may be necessary to trace the waste back to its source (Nwachukwa *et al*., 2013).

During the study, it was observed that KNH used incineration as the main method for the treatment of hospital waste especially infectious and sharp wastes for the hospital, however in a period of one month of this study the incinerator had broken but later repaired. Treatment of medical waste was done within the hospital premises according to 212 (86%). In KMH medical waste was not treated within the hospital according to 236(96%) of the study. When incinerator had broken down and incase of lack of an incinerator in a medical facility altogether this health facility relied on offsite treatment by a private licensed company as was also revealed by a research done by (Nwachukwa *et al*., 2013). Study found that KMH subcontracted waste treatment and disposal to a private company. The company was licensed to handle hospital waste. Incinerator at KNH was located near a residential area and did not have adequate air pollution control devices. Incineration is associated with many negative environmental and health effects (Rao, *et al*., 2004). Autoclaves were better option for treating part of the wastes. The one incinerator that was in good condition during the time of study at KNH had a capacity to hold less than 500kgs of waste. Kenyatta National Hospital had a waste handler who keeps record of the waste generated while KMH had a hospital matron who keeps record of the waste generated (Hospital records). Other waste treatment facilities available in these hospitals included; compost pits for non- hazardous biodegradable waste, and shredders which were found in only KNH but not in KMH. It was recorded that most of the hospitals in Kenya did not have an alternative waste treatment option apart from incineration (MOH Kenya, 2007). Incineration is the best treatment method for HCWs in third world countries because it has the highest volume and weight reduction, requires no prior processing, renders most of the waste unrecognizable, can be used to treat different waste categories and can also be a source of energy (Manyele, 2004). Private investors should be encouraged to invest in incineration facilities for HCWs, so that hospitals are left to do their core business which is patient management and get only involved in waste segregation.

There was also availability of a hand washing facility in KNH but there were no enough hand washing facilities in KMH. At KNH the availability of a hand washing facility in every work station was a good indicator that there was emphasis on hygiene for the staff. Quality assurance standards should be enhanced in the whole medical waste management (MWM) process to ensure efficiency. Through handwashing with adequacies of water and soap removes more than 90% of the transient, superficial flora including most contaminants, since hands of healthcare workers are the most frequent vehicle of nosocomial infections, handwashing is the primary preventive measure.

It is in order for hospital waste to be transported within the hospital by means of wheeled trolleys, containers, carts or in covered wheelbarrows that are not used for any other purpose as researched by Johannessen *et al.* (2000). It was noted that a truck/lorry was the means of transport used to ferry hospital waste in the KNH hospital, while unlabeled wheeled carts were used to carry waste inside the hospital at KMH. It was recommended that manual loading should be avoided as far as possible. The bags/ containers containing medical waste should be tied before transportation and should be accompanied with a signed document by nurse / doctor mentioning date, shift, quantity and destination. Special vehicles conspicuously marked with an international biohazard symbol must be used to prevent access to and direct contact with the waste by the transportation operators, the scavengers and the public (Nwachukwu *et al*., 2013). The transport containers must be properly enclosed. The vehicles must possess a licensed permit from the government (Nwachukwu *et al*., 2013). All these measures lacked completely in this study.

According to the results, plastic containers are used for disposal as indicated, (Appendix E i, ii) and as recommended by UNEP, 2000 in its research. A storage/disposal facility that is in good condition ought to be well fenced, big enough, well ventilated and that only authorized personnel were allowed in the facilities. This was unlike in KMH which scored averagely low owing to lack of unsecured and non-sanitized facility, unlike KNH. Open damp sites were the commonly used method in the two sites of the study.

According to research done by Abas *et al*., 2018, the committee for medical waste management in a hospital should be properly constituted comprising all representatives of health workers headed by a qualified doctor who is the head of infection control (Abas *et al*., 2018). In the case of KNH, unlike KMH a housekeeper was responsible for hospital waste management. It should set guidelines and policies to be followed in medical waste borrowed from WHO standards (WHO, 2008). Situational analysis of this was contained in a study by Ministry of health, Kenya (MOH, 2005).

Poorly managed MW is reported to have contributed to hazards in healthcare establishments of bacteria resistant to antibiotics. Patients environment serves as a major reservoir of microorganisms. Plasmids from laboratory strains contained in HCW could be transferred to indigenous bacteria via the waste disposal systems. Reducing bacterial contamination in the environment reduces the risk of acquiring hospital acquired infections (Nwachukwu *et al.,* 2013).

5.3 Isolation and characterization of bacterial isolates in hospital waste

Occurrence of bacterial isolates generated from different departments, wards and effluent sites at the both hospitals were revealed in the current study. The department with the highest level of contamination was main drainage water in both hospitals (100%) with all the plates indicating positive culture. The department, with the least contamination was site C (operation room) probably because of the level of efficiency in use of disinfectants and sterilization in the operation room as confirmed by research done by Moges *et al*., 2014 in Ethiopia, who found the operation room with lesser bacterial contamination compared to other hospital units.

There was significant difference in the number bacteria isolates of bacteria in various departments in both hospitals; several factors may contribute to this. The difference in quality of the ventilation system and secondly, the difference in cleaning procedures; thirdly the difference in traffic in these areas (Moges *et al*., 2014). It was also noted that about 20% of hospital dump site waste samples, from units in public and private hospital respectively, showed no indication of bacteria presence

at all. This could probably be due to the nature of the organism or the effect of possible pre-treatment given to wastes as researched by Sridhar and Olajumoke in 2003).

Bacteria isolated from the KNH (282, 59.8 %) is more than in the KMH (189, 40.1 %). This is similar to studies done by Anitha and Jayraaj in 2012, who also found government hospitals having more bacteria as compared to private hospitals. This is a reflection of the practices in these establishments and may be attributed to improper or insufficient treatment of the wastes before disposal.

From the findings, more Gram- negative organisms (especially members of the *Enteriobacteriaceae*) 341, (72.3%) were isolated than Gram positive organisms (130, 27.7%). This agrees with research done in Addis Ababa Ethiopia by (Sintayehi, 2011), who revealed that more Gram negatives were present than Gram positive in samples collected from hospital settings. The high frequency of bacteria in this study may be due to high admission of cases with bacterial infections, which is common in developing countries like Kenya. surfaces and hands of health professionals. That could be explained by diminished survival time of Gram-negatives in the environment (Gastmeier *et al*., 2006). In fact, Gram-negative bacteria other than *Acinetobacter* sp (Wen *et al*., 2015) survive on dry surfaces for few hours only, while the survival time can be several days for *Staphylococci* (Onyango & Alreshidi, 2018).

In this study, bacteria isolated from the hospital waste and environment included Providentia sp (21%), Staphylococci aureus (18.5%), Escherichia coli (13%), Pseudomonas sp (9.3%) other coagulase negative Staphylococci (9.13%), Serratia species (6.6%), Klebsiella sp (6.4%), Proteus species (4%). Enterobacter cloaca (3%) among others. Some of these isolates have been reported by earlier researchers (Yagoub *et al.*, 2010). Similar reports by Ekhaise and Omavwoya in Benin hospital showed that the bacterial genera, Klebsiella, Pseudomonas and Serratia were the most frequent isolates in the hospital waste (Ekhaise & Omavwoya, 2008, Vichal and Shalini, 2011). In another study, Pseudomonas sp was found to be the most prevalent by 20.7 % (Ashfaq *et al.*, 2013). In a study carried out in Erbil city, Rhizgari by Aziz

et al, 2014, revealed that *E. coli* was mostly isolated (100%) from a hospital wastewater.

In hospital wastes, *E. coli* strains were obtained (13%), a similar observation was made in hospitals in Ethiopia (Yismaw *et al*, 2010) where *E. coli* was reported to be among the most frequent isolates. This study also conforms to the work of Anitha and Jayraaj, 2012 who reported *E. coli* a Gram-negative as the predominant organism in hospital wastes. The high occurrence of *Providentia species* and *E. coli* isolates in these samples could be attributed to poor hygienic conditions in the hospitals studied and the conditions in other hospitals are not different as the country lacks adequate number of healthcare facilities. These may be as a result in overcrowding in the few hospitals available and hence the unhygienic conditions (Oyeleke & Istafanus, 2009).

In device surfaces, *Providentia sp* were the most frequently isolated from elderly patients or patients with urinary catheters. In a review done by Kim *et al* 2007, This study confirmed that *Providentia sp* is uncommon and that the incidence rate was high as reserved Kim *et al*., in 2007 where incidence was 0.16%. *Providentia sp* were the most frequently isolated from elderly patients or patients with urinary catheters. The reason for the variable incidence of *Providentia* is not apparent, but the types of patients and institution might contribute to such a difference. *Serratia sp* is an opportunistic, Gram negative pathogen, was discovered in ICU device surfaces and were often involved in the epidemics of the colonization and the infection with *Serratia marcescens*. The important reservoirs in epidemics are the digestive tract, respiratory tract.

In the current study nurse's/doctors hands and nurses' staff table were colonized by *Staphylococcus* and was the second most frequently isolated bacterium (18.5%). Similar findings were also observed in a previous study by (Perwaiz *et al*., in 2007) where an isolation rate of 13%. The high prevalence of the *S. aureus* from hand swabs and door handles in this work might be as a result of inadequate hand hygiene and this could be one of the attributing factors of the distribution of the pathogen in the hospital environmental surfaces as reported earlier by (Olalekan *et al*., 2011). The

low prevalence rate of *S. aureus* on beddings and bed rails in this hospitals is not in agreement with 100% prevalence on bedrail as reported by (Boyce, 2007). Also, 26 % of *S. aureus* reported on door handle by (Carvalho *et al.* 2007) is higher to the prevalence rate of the *S. aureus* on door knob/door handle of 17% from these hospitals in the current study. *Staphylococcus sp* has been incriminated in various diseases such as post-operative infections, urinary tract infections, skin diseases, respiratory infections and food poisoning (Murray *et al*., 2003). Elevator buttons had more coagulase negative *Staphylococcus species* (9.3%) than other surfaces, this could be due to the fact that they are touched repeatedly by ungloved hands by multiple individuals who will later go on to contact patients colonized by bacteria that were not pathologic.

In door knobs/door handles, the prevalence rate of 16% of *E. coli* confirms the early report of Nworie *et al* (2012) from some parts of Abuja metropolis that the contamination of door knob/door handle can be as a result of poor hand hygiene after using the toilet. Several studies have reported the importance of frequent and adequate hand washing to reduce rates of hospital acquired infections (Rupp *et al*., 2008). Lack of washing hands regularly acquire bacterial pathogens responsible of nosocomial infections and can survive on dry surfaces for several weeks (Frost and Sullivan, 2010). It has been reported that bacterial isolates are capable of surviving on hands of health care workers for at least several minutes following contamination (Allegranzi and Pittet, 2009) hence the necessity of a hand washing facility at most points in a health care institution.

Sinks, floors and waste water drainages had the highest number of *Pseudomonas* species had a prevalence rate of 9.3 %. Similar prevalence rate of 9.3% was reported by Srinivas *et al*., 2012 in Andhra Pradesh, India. In comparison, higher prevalence rate of 32.1% and 20.3 % was reported by Rajat *et al*., 2012 in Gujarat, India respectively. This varied prevalence of *P. aeruginosa* in different places may attribute to the type of swab received for examination, type of hospitals and geographical positions (Obritsch *et al*., 2005). It's an opportunistic pathogen for humans lead to a broad spectrum of disease such as urinary, burn, respiratory infections and septicemia (Srinivas *et al*., 2012). The isolation of *Pseudomonas*

species from the sinks agrees with report of (Udeze *et al*. 2012). that sinks were the most common place in hospital environment for growth of *Pseudomonas sp*, and the most common article of contact by the people. *Pseudomonas sp* thrived on moist surfaces (Pal *et al*., 2010), it is therefore and not surprising that the report gave high *P. aeruginosa* isolates since people with wet hands (water or sweat) could easily come into contact with it. Also, the prevalence rate of *Pseudomonas* species on operation table of the hospitals in the current study hospital was still higher (18.18%) than a work reported by (Pal *et al*., 2010) that 9.6% of the pathogen was isolated from operation table in a hospital in India. The presence of this pathogen on operation table can contaminate open wounds of the patients in course of the operation (Pal *et al*., 2010). The current study *S. marcescens* were found mostly in ICU in samples taken from sinks, door surfaces and waste in ICU and this was confirmed by research done by (Mlynarczyk *et al* 2007) it accounts for only 1-2% of the nosocomial infections and caused by instrumentation.

Floor surfaces had a significant number of bacteria especially *Providentia species* especially in the operating suite It was found that *Staphylococcus aureus* and coagulase-negative *Staphylococci species* was the major species contaminating floors and other surfaces in the operating rooms. Several factors may contribute to this, first, the difference in quality of the ventilation system; secondly, the difference in cleaning procedures; thirdly, the difference in traffic in these areas. We consider the major contributing factor to be the difference in cleaning procedures. Based on researchers' observations, it is recommendable that, there should be regular use of disinfectant in cleaning the operating room floor after every operation. There are several reports on the use of disinfectant on cleaning the floors (Matinyi *et al.,* 2019) reported a significant reduction in floor bacteria with the use of a germicidal detergent. He also reported that the floors in the inner zones of the operating suite cleaned with disinfectant showed low level of bacterial contamination. In the current study, one instance *Staphylococcus aureus* was found on a pillow (beddings and bed rails category). Since operating theatres surfaces were cleaned daily with disinfectant, it was not found holding any contamination.

The surface swabs and wastes collected in KNH and KMH hospitals identified most Gram negative bacteria using API-20E. This may probably due to the nature of the organism; viable but non-cultivable or the effect of possible pre-treatment given to wastes (Anitha & Jayraaj, 2012) also reported variety of pathogenic bacteria in sewage sludge, however, *Shigella* species were not detected in their study due to low sensitivity of enrichment procedure and high temperature which decreased its survival in their study. Other Gram negative bacteria species for example, *Pantoea sp* was also isolated (12 isolates) in this study. Several studies have reported the association of this germ with nosocomial infections (Liberto, *et al*., 2009).

The recovery of members of genera *Escherichia, Enterobacter, Klebsiella, Proteus* and *Serretia* directly points to these sites as high risk (Karak *et al.,* 2012), and therefore sites investigated in this study may pose a public health risk if not put under appropriate management. The presence of these microbes has previously been shown to demonstrate the accumulation of unsorted garbage (Karak *et al.,* 2012). Comprehensive assessments on pathogenic organisms must be established to build local knowledge about public health issues and trends in medical waste management and dumpsites from other studies (Santamaria & Toranza, 2010).

5.4 Antimicrobial susceptibility test patterns of isolated bacteria strains

5.4.1 Antibiotic Resistance on Gram negative bacteria

Antibiotic resistance has become a major clinical and public health problem within the lifetime of most people living today (Namboodiri *et al.,* 2011). Determining their antibiotic resistance profiles is fundamental to understand the risks these organisms represent to public health (Olayinka *et al*., 2004). In this study, percentage of Gram negative isolates resistant to tetracycline, cefotaxime was 1%, and 52% respectively. Highest rate of sensitivity pattern was found to be in imipenem antimicrobial agent

A total of 61%, 60% and 50 % of the *E. coli* isolates exhibited resistance to cotrimoxazole erythromycin and tetracycline (Weber & Piddock, 2001) respectively; this is in contrast to (Yismaw *et al*., 2010) who also reported resistance of *E. coli* to gentamicin (47%), ciprofloxacin (43%) and ceftriaxone

(26%). According to (Namboodiri *et al.,* 2011), these antibiotics have been subjected to widespread abuse a possible reason as to why high rates of resistance are being reported. Co-trimoxazole and erythromycin are largely misused in the country and hence it is not surprising that many of the *E. coli* strains isolated in the study were resistant to it. The resistance of *E. coli* to ampicillin (10%) could be because of production of β-lactamase enzyme which has the ability to deactivate the efficacy of this β-lactam drug as reported (Hassan *et al*., 2011).

The resistance pattern for each bacterium varied according to the site which the bacteria was isolated. For example *Proteus sp* the highest resistance rate to cefotaxime, ceftriaxone, tetracycline, ampicillin, levofloxacin, and nalidixic acid from the water waste of drainages (in this study 21% is among the most prevalent collection site for *Proteus* species), the same result was reported in previous study and indicated that the highest resistance rates to tetracycline and chloramphenicol were found in strains of a domestic sewage treatment plant from El- Goela oasis in Algerian Sahara (Hacene *et al*., 2004). *E. coli* resistance to various antimicrobial agents, renders amoxicillin clavulanate could be used as an alternative to the above antibiotics for treatment of *E. coli* infections, particularly nosocomial infections (Yang *et al*., 2009). Regarding *Pseudomonas sp* the resistance rate was shown to be high for most antibiotics particularly for ampicillin, cefotaxime and chloramphenicol (Mukhtar & Saeed, 2011). High resistant rate for *Pseudomonas species* isolated from clinical sources against the same antibiotics was also demonstrated in another study conducted in Gaza Strip hospitals (Astal, 2004). The high resistance rate of *Klebsiella* species was against amoxicillin (100%). The high resistance rate found in samples collected from waste drainages is likely due to heavy metals biocides, antibiotics and various chemicals that are discharged in drainages of these hospitals and these substances have the potential to select for antibiotic resistance as researched by (Kummerer & Henninger, 2003).

The resistance rate for tetracycline was high for most of the isolated bacteria with an average of 74% among the gram negatives and 40% in gram positives tetracycline resistance rates are similar or higher than those found in other studies reported as low 23 and quinolone resistance was less than 25% among environmental isolates (Yang

et al., 2009). The low resistance rate for nalidixic acid may be due to the fact that quinolones antibiotics are excreted mostly as unchanged substances, and they are among the most persistent antibiotics in the environment thus losing its potency (Nyangacha *et al*., 2017). Low resistance rate for chloramphenicol was recorded and is rare in most studies (Dang *et al*., 2008) possibly as the result of the restricted use of this drug.

This high resistant rate (89.18%) for bacteria isolated from drainages could be due to the fact that only few compounds were partially biodegraded in under test conditions in aquatic systems (Ekhaise & Omavwoya, 2008) and most were persistent. This can be attributed to the fact that, drainages contain a high content of both organic and inorganic matter, as well as high densities of living organisms, including pathogenic, commensal and environmental bacteria. Generally, waste water and drainages are rich in nutrients, which enhance the multiplication of microorganisms facilitating gene exchange due to cell to cell contact (Serensen *et al*., 2005) making waste disposal sites important reservoirs of antibiotics resistance genes that can be exchanged by bacteria from different environmental compartments (Miranda *et al.*, 2015). Furthermore, unknown amount of antibiotics enters the sewers by waste derived from disposal of a surplus of drugs. This result is quite similar to that reported by another author Harman *et al*., 1998 who recorded that, indeed, various antibiotics have been found in municipal sewage, including fluoroquinolones, sulfonamides and erythromycin metabolites (Allen *et al*., 2010).

The carpabenem (imipenem) drug (100%) used in the study was found to be most sensitive drug against the Gram negative and Gram positive bacteria respectively. These antibiotic susceptibility results correlate with other studies. (Jolly 'Guiller *et al*., 2010) reported imipenem and meropenem with 100% and 98% respectively. Imipenem antibiotic had a low resistance rate in Kenyan hospitals probably because it is very restricted for life threatening infections therefore no resistance at all (Omulo, 2015).

The study showed that Gram negative bacteria were more resistant to the tested antibiotics than the Gram positive organisms. It is the remarkable difference in

structure and composition of the cell wall's murein layer between the Gram negative and the Gram positive bacteria that is responsible for this trend (Omulo, 2015).

This study indicated presence of multiple drug resistance for majority of the isolated strains, this result is consistent with that reported in another study done in Gaza strip but the isolated bacteria were from patient samples and indicated a high percentage of multiple drug resistance (Astal *et al*., 2004). The emergence, selection and dissemination of resistant organisms have been reported to occur in areas where antibiotics have been heavily used such as human, veterinary and agriculture (Woolhouse *et al*., 2013). Bacteria have shown the capability of attaching themselves onto surfaces in the waste water thereby forming biofilms, which enables the bacteria to withstand environmental stresses (Wen *et al*., 2015).

Biofilms are characterized by high bacterial density and diversity, which provide suitable conditions for horizontal gene transfer and genetic exchange of resistant traits (Thomas *et al*., 2007). In this study isolates recovered from waste drainages and sites records the highest numbers of antibiograms, indicating that they present the best selection sites for antibiotic resistance. (Reinthaler *et al*., 2003) has shown that biofilm formation increases the rate of genetic exchange for antibiotic resistance traits in matrixes such as drainages. Microbes have also been shown to acquire antimicrobial resistance as one of the mechanisms which help them survive in hostile environments (Anatoly *et al.,* 2016). Efflux pumps have been reported as one of the mechanisms responsible for the antimicrobial resistance in biofilm structures due to diffusion of antibiotics through the biofilm among others. Efflux pumps allow the microorganisms to regulate their internal intracellular antibiotic concentration, allowing bacteria to survive at higher antibiotic concentrations (Suzuki *et al*., 2014). They are site- specific recombination systems capable of recruiting open reading frames in the form of mobile genes cassettes (Suzuki *et al*., 2014).

5.4.2 Antibiotic Resistance on Gram positive bacteria

The high percentage of ampicillin resistant *S. aureus* in this research (96 %) confirms the earlier report of Dudhagara *et al*. (2011), that the resistance of the *S. aureus* to

this ampicillin antibiotic, may be as result of the ability of β-lactamase enzyme to break the β-lactam ring in the antibiotic and rendered it ineffective. *S. aureus* produces β-lactamase in the presence of ampicillin (Oncel *et al*., 2004). The 100% susceptibility of *S. aureus* to linezolid in this finding agreed with the findings of (Terry-Alli *et al*., 2011). Linezolid has an advantage over other antibiotics like vancomycin for treating MRSA because it has an intravenous preparation and an oral tablet that has excellent bioavailability (Seza & Fatma, 2012). The 10% resistance of *S. aureus* to gentamicin in this finding is not similar to the report of (Akindele *et al*., 2010) that reported 39% of this pathogen was resistant to gentamicin. As indicated by (Abulreesh, 2011), multidrug resistant *Staphylococci* (*S. aureus* and coagulase negative *Staphylococci* have been a common problem and recovered from diverse environmental sources (Tula *et al*., 2013), such as drinking water supplies, foodstuffs, the mucosa of humans and farm animals and hospital environments which can be important public health concern (Abulreesh, 2011).

5.5 Detection of resistant bla genes

5.5.1 Frequency of ESBL strains

Resistance to an extended spectrum beta-lactams among Gram negatives pathogens is increasingly associated with ESBLs (Bali *et al*., 2010). In the current study 37, 47% ESBL positive strains were identified while 43, 53% non ESBL strains were identified. This is slightly higher than in Asia (Samyyia *et al.*, 2018). Where the prevalence of ESBL producing *K. pneumoniae* and *E. coli* vary from 5% in Japan to 20–50% in other countries (Samyyia *et al.*, 2018). In Europe, the prevalence of these organisms varies from country to country (3% in Sweden to 34% in Portugal) (Coque *et al.*, 2008). In this study *E. coli* strains were more frequently isolated than *K. pneumoniae* strains, the production of ESBLs was more often present in *K. pneumonia (*Banno *et al.*, 2004*).*

The prevalence of ESBL positive strains in the current study indicated that there was higher number of ESBL strains in orthopedic surgery unit compared to that in internal medicine 8/80, 10% and respectively. Points for intervention could be reduction of personnel during surgery, better treatment of wounds and reduction of

the time between surgical site shaving and the intervention (Scherrer, 2003). The increase of boda boda public service vehicles as a result of legalization could also contribute to the increase in accidents as noted during the study. This difference was not statistically significant ($p > 0.05$). This observation confirms findings in other studies that ESBL producing *Enterobactericeae* are detectable in different environments and hospitalized patients with varying preference levels as researched in Ghana 43% (Feglo *et al*., 2013) and 26% in Kenya (Kariuki *et al*., 2007). Routine use of an ultra-clean air system exhaust ventilated clothing is frequently recommended. However, other less costly measures, including the reduction of the number of persons in the operating room, probably may ensure similar preventive effect (Scherer, 2003).

5.5.2 Distribution of bla TEM, CTX-M and SHV genes

The *bla* $_{\text{CTX-M, SHV}}$, and $_{\text{TEM}}$ genes are the most common genes that encode ESBL; their detection was based on phenotypic and molecular methods (Bali *et al*., 2010, Mshana & Imirzaliozgly, 2009). The $_{\text{TEM}}$ gene has been associated with 90% of ampicillin resistance (Livermore *et al*., 2008). In this study, a low association of ampicillin resistance with $_{\text{TEM}}$ gene of 10% was observed. It was also observed in this study that there was an *E. coli* that was positive for the production of B-lactamase (ESBL positive) did not have any *bla* genes. This is an indication that the isolate could be harboring other variant genes such as $_{\text{SHV, OXA, PER}}$ types implicated in ESBL production that was not included in this study.

Analysis of the resistant genes in the current study indicated that majority of the strains harbored *bla* $_{\text{TEM}}$ 8/80 (10%), followed by *bla* $_{\text{CTX-M}}$ 4/80 (5%) and *bla* $_{\text{SHV}}$ with 2 (3%) and overall prevalence 18%. In our study, the phenotypically positive ESBL strains that lacked *bla* $_{\text{TEM, SHV,}}$ and/or $_{\text{CTX-M}}$ genes can be explained by the possible presence of other ESBL-encoding genes in the studied Kenyan bacterial population (Kiiru *et al*., 2012). These results are similar to other results obtained in studies in tertiary institutions in Ghana where higher Bla $_{\text{TEM}}$ were the most abundant than the Bla $_{\text{CTX M}}$ among the *E. coli* strains (Labi *et al*., 2016).

Only one isolate of *E. coli* was positive for ESBL production and harbored the *bla* $_{\text{CTX-M}}$ gene, while one *E. coli* was ESBL negative strains harbored *bla* $_{\text{TEM}}$ gene. There were no two or three combined ESBL genes of *bla* $_{\text{SHV}}$ *bla* $_{\text{CTX-M}}$, and *bla* $_{\text{TEM}}$ that were detected in any single isolate.

In the current study no PCR amplification was observed with most of the isolates of *Providentia species* and the primers targeted to *bla* $_{\text{TEM, SHV}}$ and $_{\text{CTX-M}}$ except one. The negative amplification may be due to the presence of other resistant genes which could not be detected by primers provided for this study and were not further explored.

In the present study, the resistant $_{\text{TEM}}$ bla genes were found in *E. coli* (1/8), *Providentia sp* (1/8), *K. Pneumoniae* (3/8), *Pseudomonas sp* (2/8), $_{\text{CTX-M}}$ types were found in *Klebsiella sp, E. coli, Serratia sp* and *Enterobacter sp, with one* each respectively while the two $_{\text{SHV}}$ bla genes were found in *K. Pneumoniae* (1/2), *Pseudomonas sp* (1/2. Overall, *bla* $_{\text{TEM}}$ was present in 10 % (8/80), predominantly in *Klebsiella sp* while *bla* $_{\text{CTX-M}}$ in 5 % (4/80) and *bla* $_{\text{SHV}}$ was present in 3% (2/80) all alone and non in combinations. These results are in contrast to Saini *et al.,* 2005 who reported an 87.5 % high prevalence of *bla* $_{\text{CTX-M}}$ enzyme among ESBL producers in a tertiary care hospital in Lebanon, but in agreement with Rezai *et al*., in 2015 from Iran who observed a low prevalence of *bla* $_{\text{CTX-M}}$ of 28%.

In general antibiotic resistant microorganisms is an increasing problem due to overuse and misuse of antibiotics often spread through hands of health workers and environment. Knowing the chain of infection helps identify effective points to prevent disease transmission by observing standard precautions of hospital waste management. Detection of the novel genes demonstrates the value of phenotypic characterization of clinically significant resistance genes.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

6.1.1 Level of knowledge among healthcare personnel's on medical waste matters

Managing hospital waste requires effective knowledge among the people who produce the waste, not just those who handle it. Our study revealed that knowledge and attitude among Kenyan health workers toward medical waste management is average. The attitude of doctors, public health officers and other intellectuals in the health profession towards the operational aspects of medical waste management system can be attributed to the microscopic vision of these professionals that is mainly focused on the curative aspects of the patient care services. Nursing professionals had an upper hand over other professionals, this can be attributed to their accountability and commitment in ward management and the predominance of female workforce. The para-medical staff, including waste handlers had less understanding on the subject which may be attributed to strict instructions by authorities and fear for any punitive action. There is an urgent need for raising awareness and to use it to uplift better health practices on MWM among the hospital staff in all health-care setups.

6.1.2 Current practices on management and hospital waste

Practice of waste generation, segregation up to waste disposal is poor. No appropriate strategy exists for proper management of medical waste in the studied health establishments. Effective implementation of rules, close monitoring of guidelines with regular audit and continuous education can improve medical waste management practice. Clearly there is need for education as to the hazards associated with improper waste management. There was non-compliance of some of the healthcare facility investigated with the existing national regulatory requirements. The practices are inappropriate due to lack of proper facilities and interest of the

individual. Poor current practices increase level of surface contamination, a health risk.

6.1.3 Isolation and characterization of pathogenic bacteria found in hospital waste

This study reported bacterial contamination in the various departments of the target hospitals is a serious problem, especially significant because the contaminations were identified in some areas that should be clean like the sterilization and operation room and should contain a minimal number of microbes at all times for the safety of the patients and the health workers. The levels of contamination observed in this study carry a high risk for the development of nosocomial infections. These observations justify more attention need to be paid to infection control efforts in our hospitals. The presence of high number of microorganisms in the hospital environment and disposed wastes is a threat to the population in such environment and has a serious public health implication especially due to high resistance to commonly used antibiotics in our hospitals.

6.1.4 Susceptibility pattern of various bacteria isolates from hospital waste to various antibiotics

The prevalence of multidrug resistance obtained for strains of these organisms suggests that proper hygienic practices are not adhered to. ESBL strains are usually multi-drug resistant, so the practice of routine ESBL testing along with conventional antibiogram would be useful for all cases; it will help in the proper treatment of patients and also prevent further development of bacterial drug resistance.

6.1.5 Resistant genes present in Gram negative isolates detected in hospitals waste in Kenya

Among the resistant genes detected in this study included *bla* $_{\text{TEM}}$ type, that was the most common resistant genes (10%) found in both *Pseudomonas sp*, *E. coli* and *K.*

pneumonia among others in this study. Other resistant *bla* genes present included $_{CTX-M}$ (5%) and $_{SHV}$ (3%).

Molecular detection and identification of β- lactamases would be essential for a reliable epidemiological investigation of antimicrobial resistance. It is necessary to control hygiene and antibiotics consummation in hospital centers.

6.2 Recommendations

This study brings to point important aspect to consider when designing management strategies of hospital waste. The researcher hopes that this study will create awareness regarding the problem of hospital waste management in hospitals and will generate interest for control effort for effective hospital waste management. The researcher recommends that;

- ✓ Health care workers be made aware on recommended practices on management of medical waste from generation to disposal and of the increasing resistance of nosocomial microorganisms and the proper administration of antibiotics in treating such infections through imparting knowledge by training all healthcare workers on HWM matters.
- ✓ More hospitals in the studied counties and the country at large must also be studied in order to generate enough data which will help in the development of a holistic control programme in dealing with the threat posed by resistant nosocomial pathogens.
- ✓ Antibiotics currently administered in our hospitals should be added more as the ones in the study are the commonly used in the Kenyan hospitals are not enough to determine the level of resistance of microorganisms.
- ✓ Future studies should be done to investigate the prevalence of anaerobic and fastidious growing bacteria and other microbial contaminants such as fungi, in our hospital departments and also to expand the study to include other items such as air samples. Research should also be done on resistant genes present in Gram positive bacteria present in hospital environment and waste in Kenya, virulence traits e.g. specific enzyme production, adhesion properties of isolates obtained from hospital environments.

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APPENDICES

Appendix I: Research Consent Form

Title: Knowledge and management of hospital waste in selected hospitals in Kenya.

Introduction

In this consent form I will learn about the proposed research and my rights before, I agree to participate in it. I will read this form carefully and I agree to be interviewed.

The knowledge accrued from this study will go a long way in identifying the management of hospital waste and possible sources of infection, and provide data on appropriate modes of prevention and control of such diseases.

The participation in this study is free you need not pay any money to investigator, similarly she will no pay money for allowing us to include you in the study.

You are the one to decide for or against participation in this study. Your names and identity will not be used in analyzing results of the study, and /or in sharing results with others, including publications in journals. By signing the consent, you are authorizing granting of such access only to these specific groups mentioned above.

Declaration by the participant

The investigator has explained to me and I have fully understood the purpose, procedures, risks and benefits of participating in this study, the rights of research participants, and the confidential handling of the information. I understand that I may withdraw from the study at any time without giving any reason. I understand that I would also be able to get further information in future, and that the name and identity will not be used in the analysis of data and in sharing the results with others, based on the above, I have voluntarily agreed to enroll in this research study

Name of participant……

Signature/ left thumb print ……

Date……
. Name of the Researcher SUSAN MAINA

Address JKUAT-62000 Nairobi

Email address; muthonisusanmukiri@yahoo.com

Appendix II: Questionnaire Form

1. Questionnaire to determine knowledge and current practices on hospital waste in selected hospitals

(Tick in the box where appropriate).

A. PERSONAL INFORMATION

Name of hospital…….

Type of hospital

i) Public……

ii) Private/ church sponsored…….

iii) Others (specify)……

a) Gender male……. female…….
b) Title of job in your health profession……
c) Level of education

KCSE/certificate……

Diploma…….

Degree……

d) Job experience in years

Below 5 Years……....

Above 5 years to 10 years……...

Above 10 years……

e) How long has the healthcare service been in existence?

0-5years	6-10years	10-15years	15+years

B. Questions on level of knowledge of management of medical/hospital waste (MW) in hospitals.

1. Are you aware or familiar of existence of a government plan rules on MW management 1998?

Yes…….

No……...

2. Where does the source of segregation of hospital waste take place in the hospital in

- operating, room,
- laboratory
- wards

3. Do you believe that improper hospital waste management can lead to health problems

Yes……

No…….

I don't know…….

4. By use of drawing a diagram, are you able to recognize the international biohazard sign Yes…….

No…….

5. Do you think there is a waste manager in your hospital?

Yes……

No…….

I don't know

C. Current practices involved in MW management (segregation, collection, storage, treatment, transport, disposal, effects of MW on human health and environment).

Segregation

a) Where does segregation take place (point of generation of MW)?

Wards…….

During collection……

During disposal…….

b) Are the containers marked with a biohazard symbol?

Yes…….

No…….

c) Which is the correct colour coding of infectious bins

Black……..

Yellow……

Red…….

Collection

a) How often is the collection of MW from the ward

Daily…….

Twice a week……

Once a week…….

b) Do waste handlers often use protective clothing (gloves, masks and gumboots)?

Yes……

No……

Storage

a) Do you know the location of MW storage temporal/ permanent in your hospital?

Yes……

No…….

Briefly describe where located…….

b) Is there special equipment for sharp waste handling and an efficient storage facility?

Yes …….

No…….

Treatment

a) Is the MW treated in the hospital premises?

Yes……

No……

Transport

a) What is the means of transport of MW to the final disposal area?

Use of licensed van……

Use of open tractors……

Disposal

a) What means of disposal of MW is used in your hospital

Incinerator…….

Autoclaving……

Burning…….

What are some of the problems or risks that health workers undergo related to hospital waste management?

a) Do you think MW can cause risks and health hazards to human and environment?

Yes……

No…….

b) Have you ever encountered a health problem in your healthcare duties?

Yes…….

No……

c) For those whose answer is yes in the above question, what type of a problem have you ever experienced among the following

Respiratory problem…….

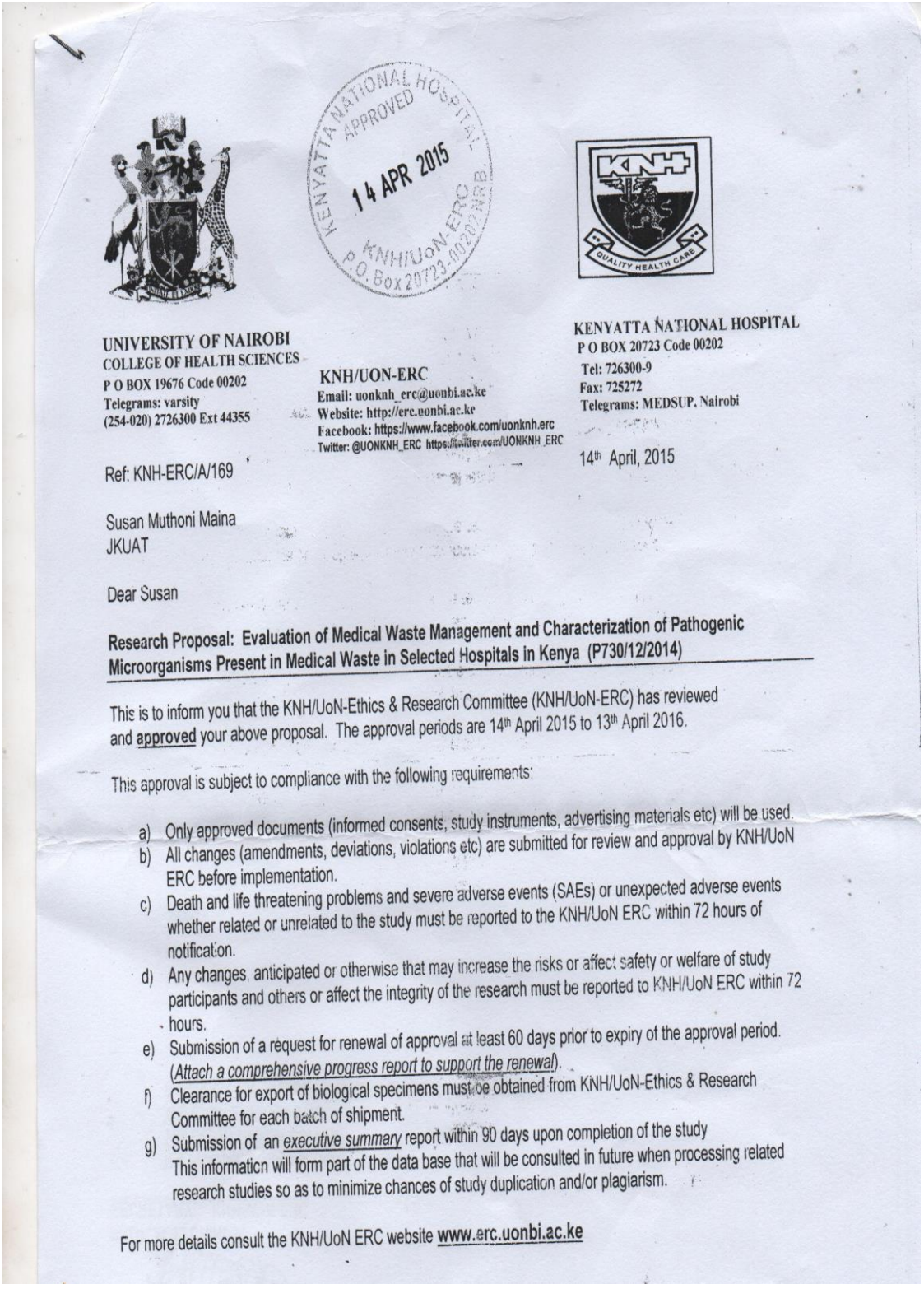
Eye problems…….

Skin rashes……

Cuts/ piercing…….

All of them…….

Appendix III A): Approval Letter KNH



KENYATTA NATIONAL HOSPITAL APPROVED 14 APR 2015 KNH/UoN-ERC P.O. Box 20723-00202 Nairobi

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14th April, 2015

Susan Muthoni Maina
JKUAT

Dear Susan

Research Proposal: Evaluation of Medical Waste Management and Characterization of Pathogenic Microorganisms Present in Medical Waste in Selected Hospitals in Kenya (P730/12/2014)

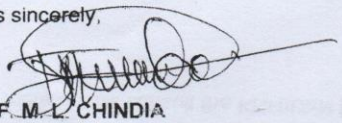
This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 14th April 2015 to 13th April 2016.

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 severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

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

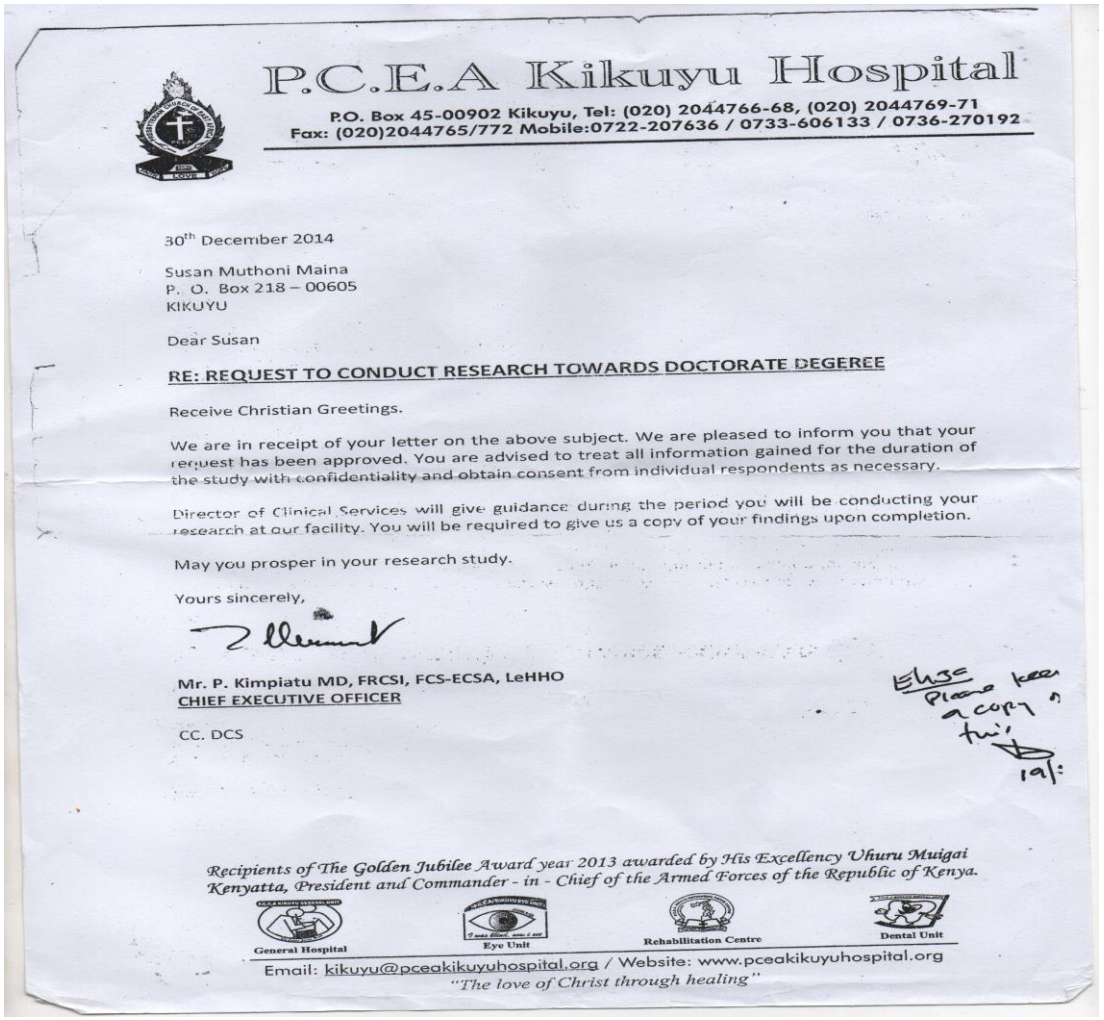
Yours sincerely,



PROF. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Chair, KNH/UoN-ERC
Supervisos: Dr. Andrew K. Nyerere, Dr. Caroline W. Ngugi

Appendix IV B): Approval Letter KMH



P.C.E.A Kikuyu Hospital

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Fax: (020)2044765/772 Mobile:0722-207636 / 0733-606133 / 0736-270192

30th December 2014

Susan Muthoni Maina
P. O. Box 218 – 00605
KIKUYU

Dear Susan

RE: REQUEST TO CONDUCT RESEARCH TOWARDS DOCTORATE DEGEREE

Receive Christian Greetings.

We are in receipt of your letter on the above subject. We are pleased to inform you that your request has been approved. You are advised to treat all information gained for the duration of the study with confidentiality and obtain consent from individual respondents as necessary.

Director of Clinical Services will give guidance during the period you will be conducting your research at our facility. You will be required to give us a copy of your findings upon completion.

May you prosper in your research study.

Yours sincerely,

Mr. P. Kimpiatu MD, FRCSI, FCS-ECSA, LeHHO
CHIEF EXECUTIVE OFFICER

CC. DCS

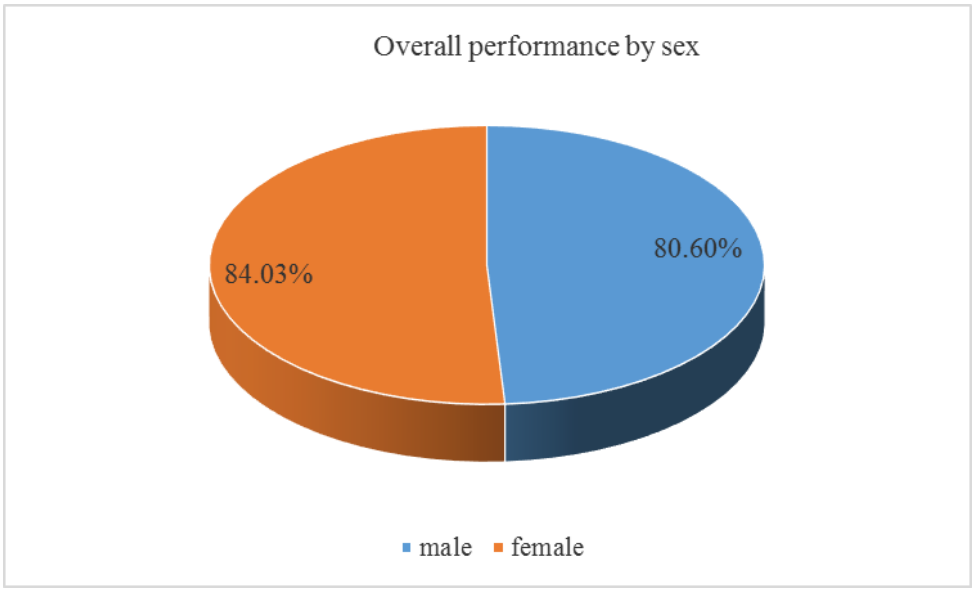
Elise Please keep a copy of this 19/:

Recipients of The Golden Jubilee Award year 2013 awarded by His Excellency Uhuru Muigai Kenyatta, President and Commander - in - Chief of the Armed Forces of the Republic of Kenya.

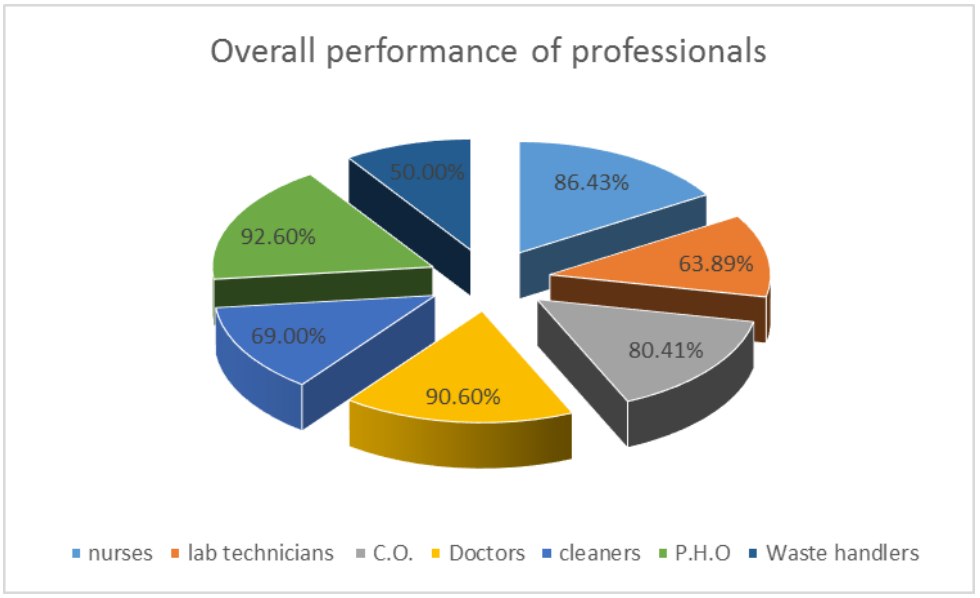
General Hospital | Eye Unit | Rehabilitation Centre | Dental Unit

Email: kikuyu@pceakikuyuhospital.org / Website: www.pceakikuyuhospital.org
"The love of Christ through healing"

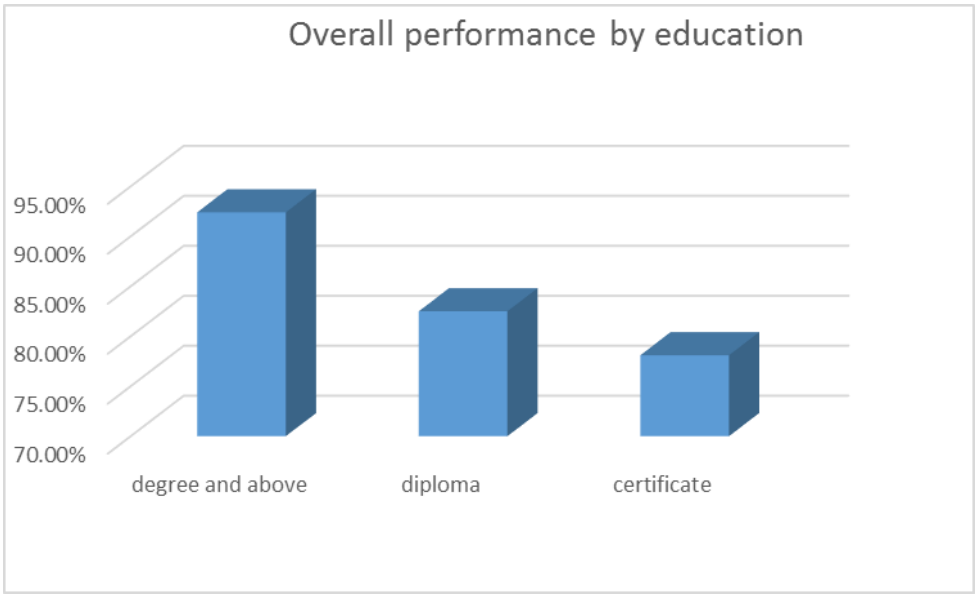
Appendix V: Performance of knowledge of on management of medical waste.



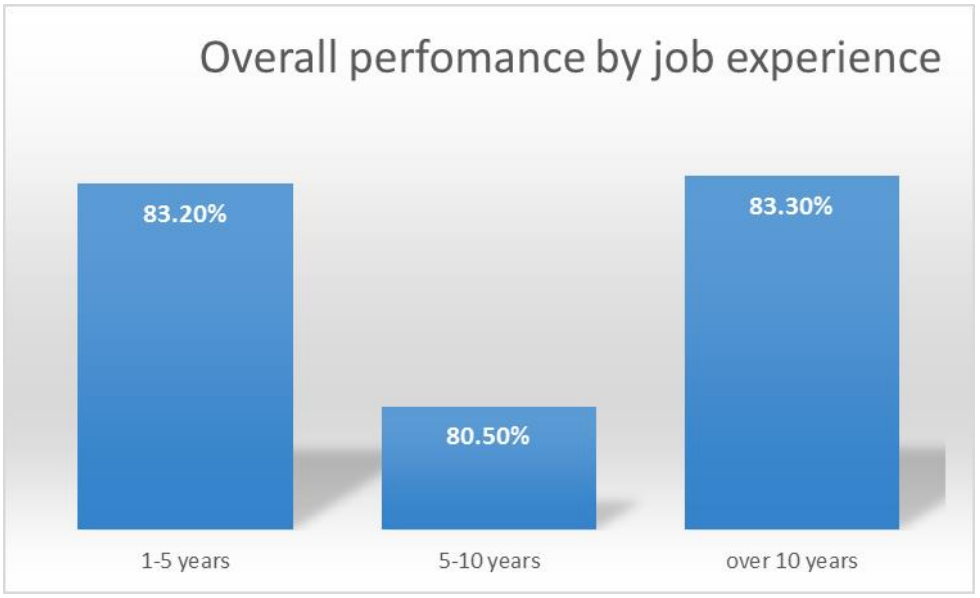
Appendix a): Distribution of study population by gender



Appendix (b): Participants population distribution by profession



Appendix (c): Participants distribution according to their level of education



Appendix (d): Study participant's performance by job experience

Appendix VI: Photographs indicating different stages of the research study.

Appendix a): Different color coded containers in KNH clearly marked with the international biohazard symbol

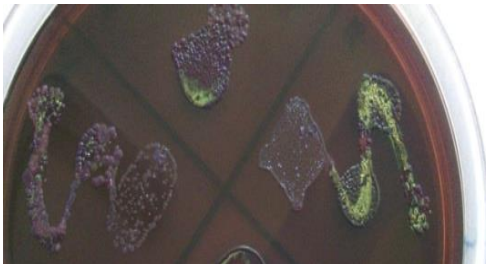
Appendix b): KMH MW containers not labeled with international biohazard symbol

Appendix c): MW in a temporally storage area in KNH

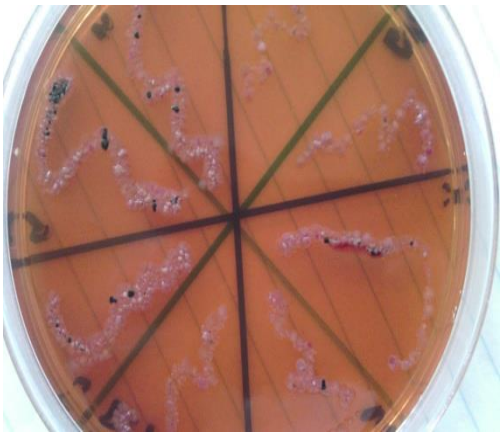
Appendix d): KNH dumpsite area

Appendix e): KMH temporal storage area

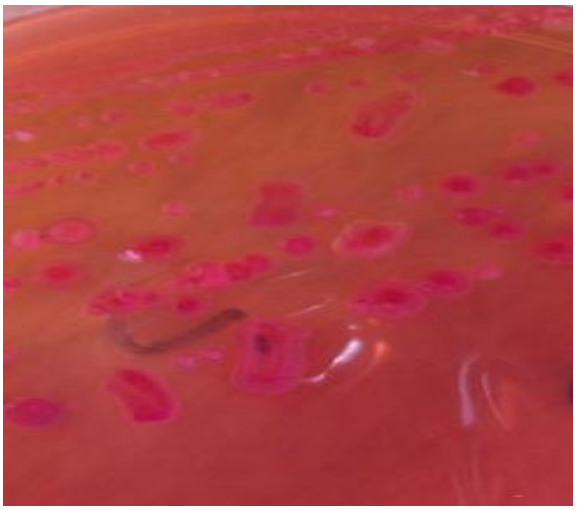
Appendix f): Dumpsite in a Nairobi area with people selecting some items from hospital waste garbage.



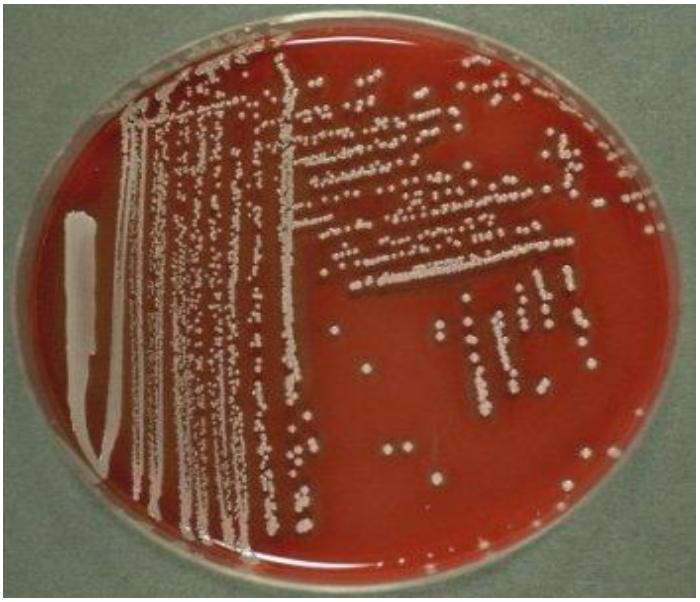
Appendix g): EMB agar, Colony morphology of *Escherichia coli* (*E. coli*) showing green metallic colour and E. coli on MacConkey agar



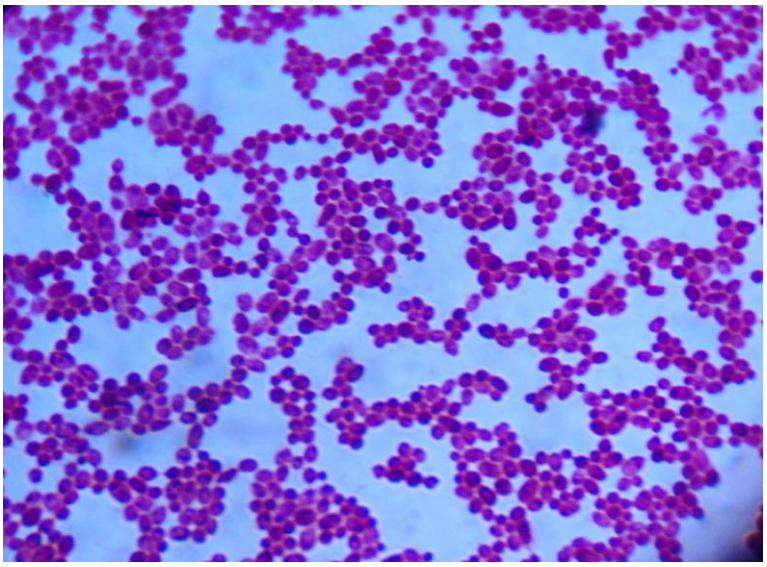
Appendix h) *Salmonella/Shigella* agar: showing pink colonies of *Shigella* and pink colonies with dark centers for *Salmonella* collected from waste drainages and dumpsite surfaces area.



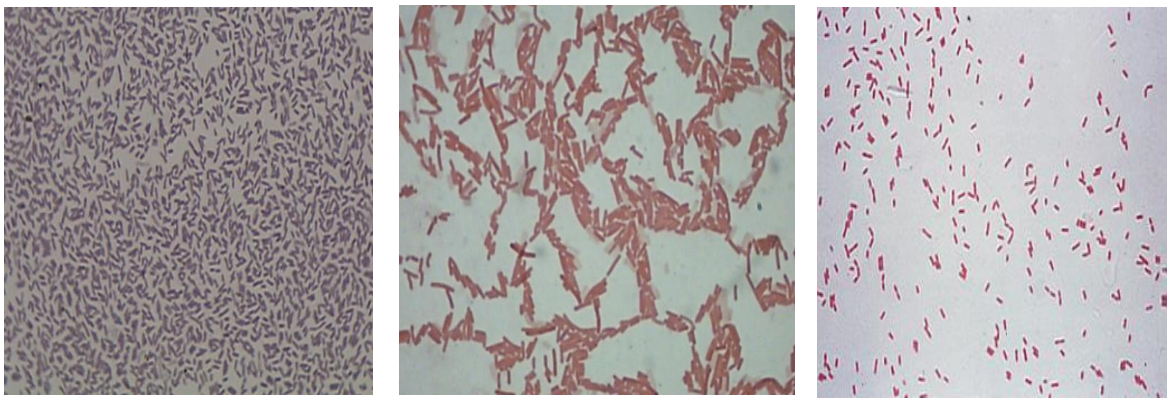
Appendix i) Colony morphology of *Klebsiella* sp. Colony with pink and mucoid colony on MacConkey agar.



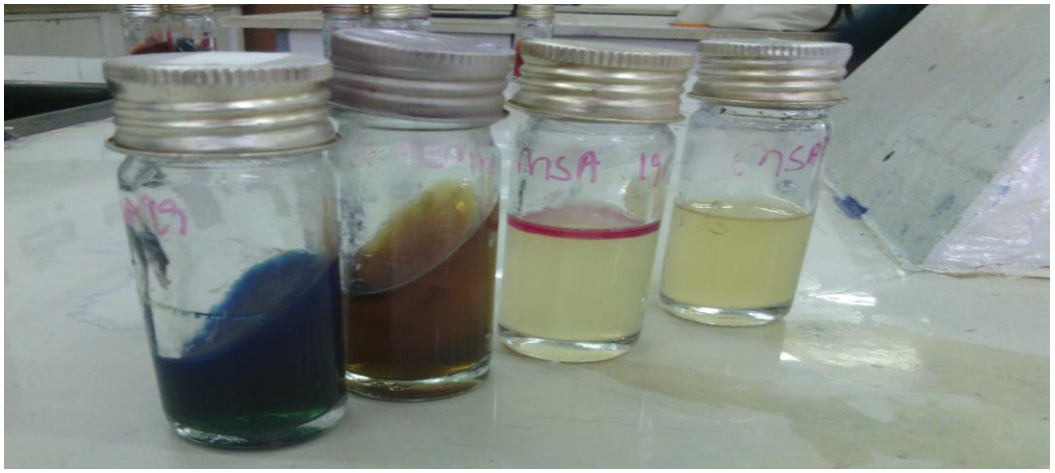
Appendix j) *Staphylococcus aureus* isolates on an MSA agar plate



Appendix k) Gram positive *Staphylococcus* species bacteria

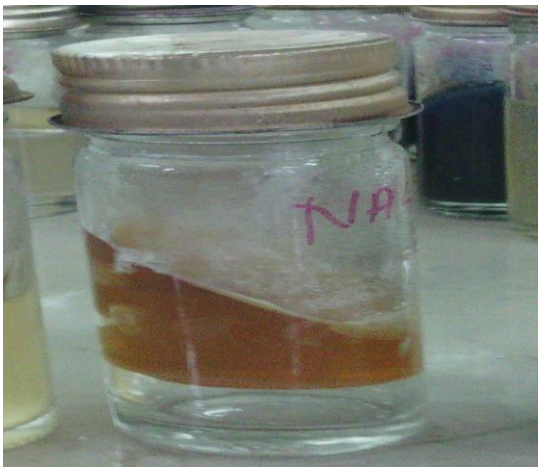


Appendix l) Gram negative rod shaped bacteria of *Providentia* species, *E. coli* and *Pseudomonads* cells respectively.



Appendix m) Results of biochemical tests regarding a bacterial isolate.

MR/VP positive indicated by the red colour

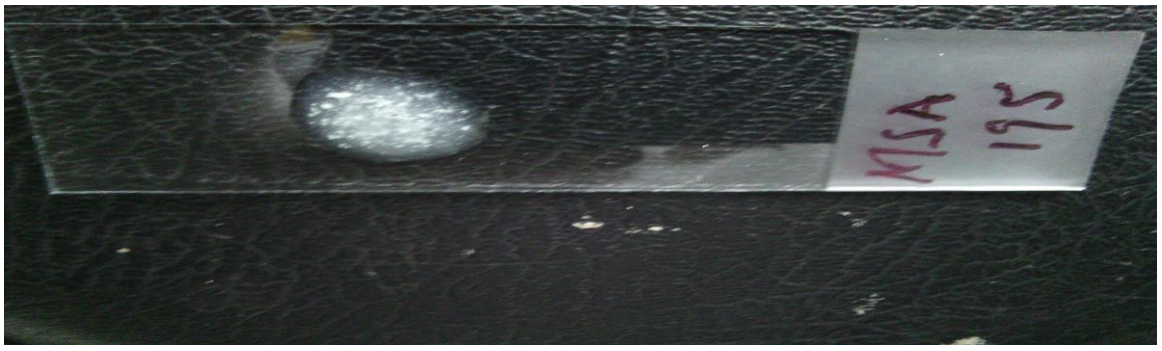


MR/VP negative indicated by yellow

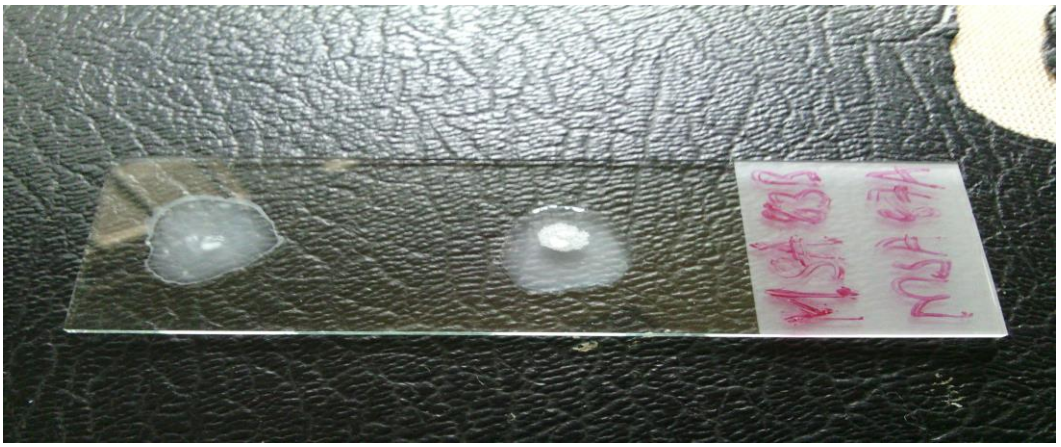
Appendix n) Biochemical results showing photos of MR/VP test in bacteria

Appendix o) Results for Triple Sugar Iron Test

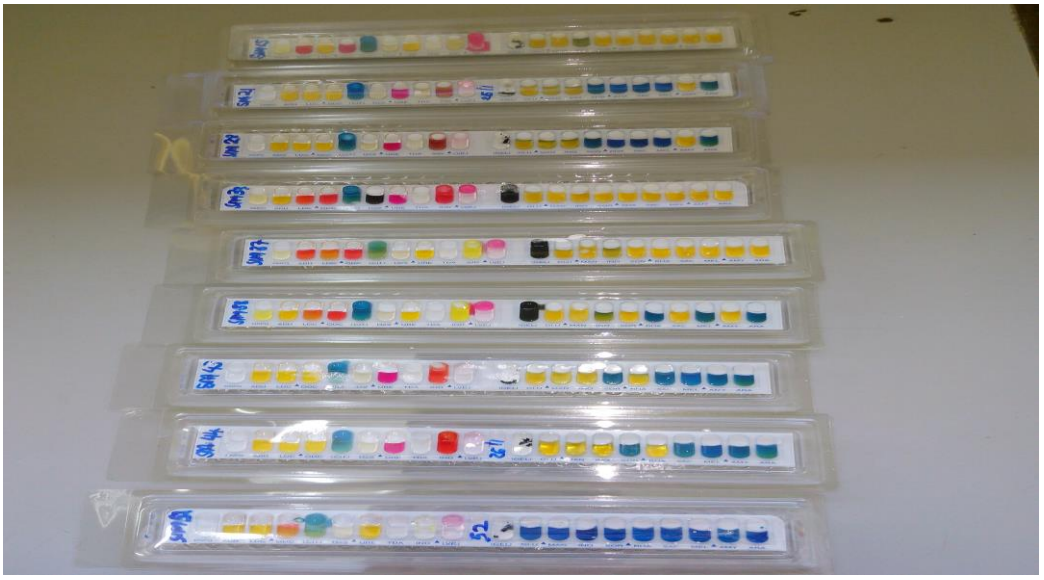
Appendix q) Oxidase positive test to identify *Pseudomonas* species



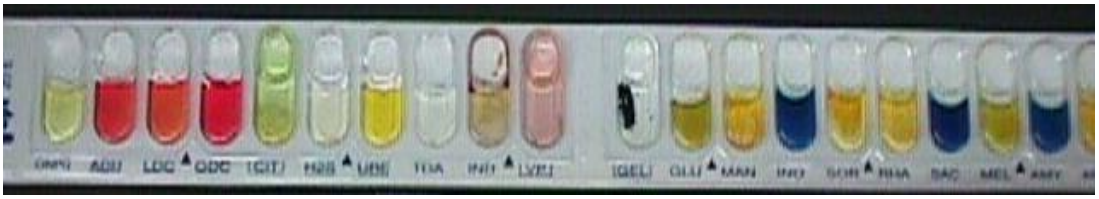
Appendix r) Catalase test indicating positive/ presence of a *Staphylococcus aureus* bacteria



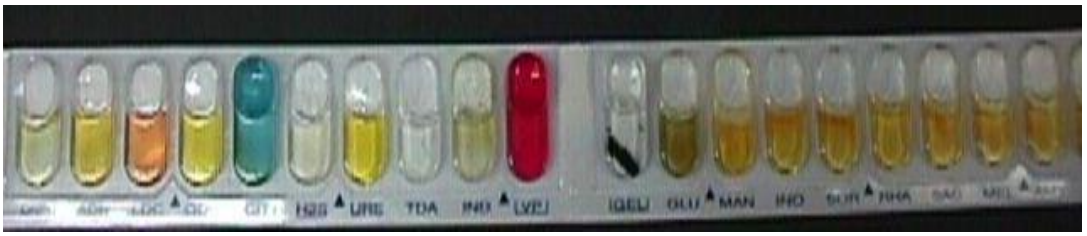
Appendix s) Coagulase test indicating positive



Appendix t) Assortment of combined confirmatory API-20E strips to confirm various bacterial species



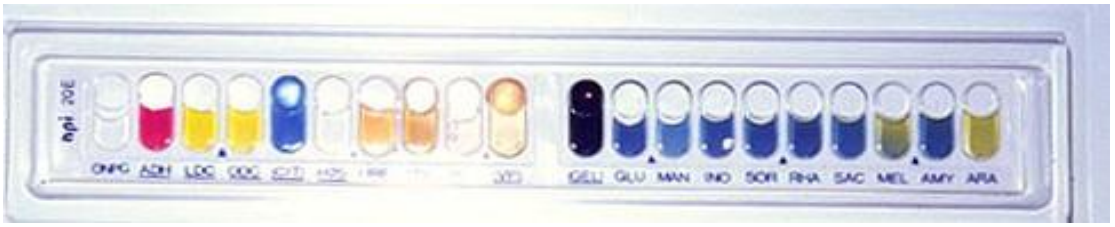
Escherichia coli



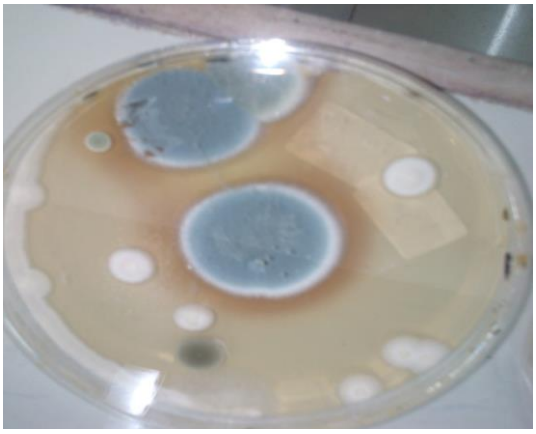
Klebsiella pneumonia

Proteus milabilis

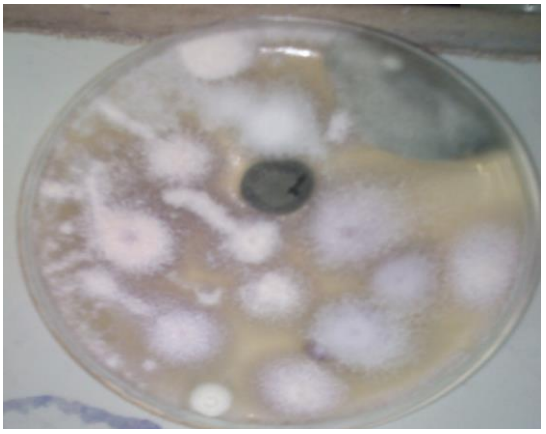
Providentia rettgerri



Pseudomonas species



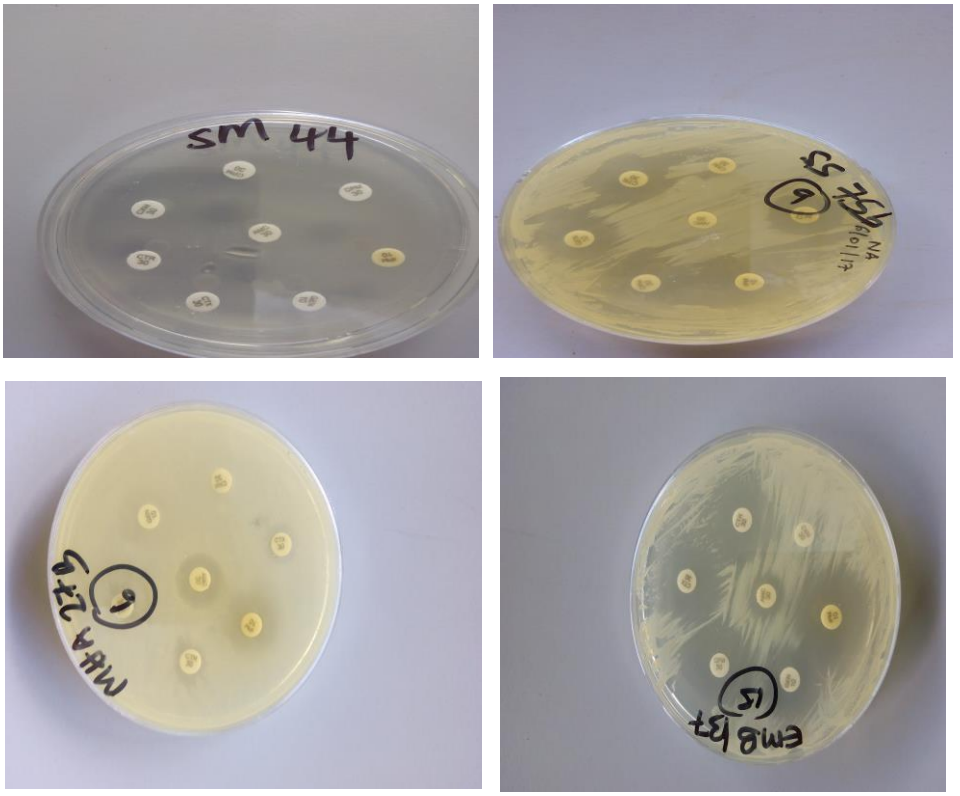
Penicilin species isolates



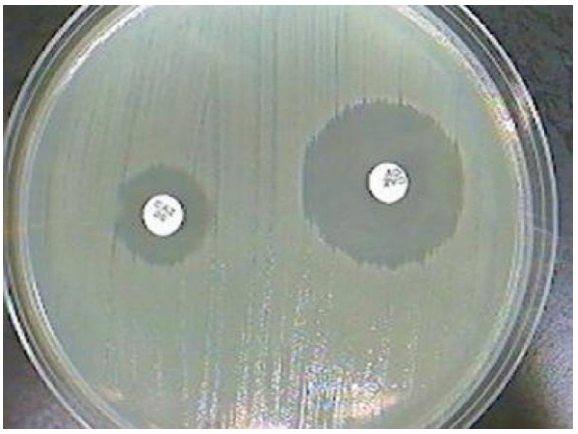
Fusarium species isolates

Appendix E (xx); Fungal growth isolates observed in a sample swab from waste water

Appendix u): Archiving of bacterial pure isolates using nutrient broth and glycerol



Appendix v): Patterns of antimicrobial susceptibility in Gram negative isolates from hospitals environments and wastes in Kenya.



Appendix w): Disc diffusion confirmation for phenotypic test characteristic using Ceftazidime disc 30 ug and Ceftazidime/ Clavulanate disc

Appendix VII: Bacteria Media Preparation

i) MacConkey agar

Formula

Gelatin peptone 17.0

Bile salts No: 3 1.5g Lactose 10.0g Neutral red 0.03g

Sodium chloride 5.0g

Peptone mixture 3.0g

Bacteriological agar 13.5g

Preparation

Suspend 50 g in 1 litre of distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 121℃ for 15 minutes. Cool to 45-50

℃ and pour in 15 – 20 ml amounts into Petri dishes.

Use:

For selection of enteric bacteria.

ii) Mannitol Salt Agar (MSA)

Ingredients

Pancreatic casein 5g

Peptic digest 5g

Beef extract 1g

Sodium chloride 75 g

D-mannitol 10 g

Phenol red 0.025g

Agar 15 g

Procedure

Suspend 111 grams of mannitol salt agar in 1000 mls of distilled water.

Boil to dissolve the medium completely.

Sterilize by autoclaving at 15 lbs. pressure(1210c) for 15 minutes.

If desired sterile egg yolk emulsion can be added to a final concentration of 5% v/v after autoclaving.

Uses

For selective and differential medium for the isolation and identification of Staphylococcus aureus from clinical and non-clinical specimens.

iii)**Eosin methylene blue agar (EMB)**

Ingredients

EMB agar g/l

Methylene blue 0.065g

Eosin Y 0.4 g

Lactose 5 g

Preparation

Suspend 36 grams of EMB agar in 100mls of distilled water.

Heat to boil to dissolve the medium completely.

Dispense and sterilize by autoclaving at 15 lbs. pressure (121^0C) for 15 minutes.

Uses

Isolation and differentiation of lactose fermenting and non-lactose fermenting enteric bacilli.

iv) Mueller Hinton Agar.

Ingredients

Beef infusion 300.0g

Acid hydrolysate of casein 17.5g

Starch 1.5g

Agar 17.0g

Preparation

Dissolve 42 g in 1 litre of distilled water. Boil to dissolve the medium

Completely. Sterilize by autoclaving at 121℃ for 15 minutes

Use- For sensitivity testing.

v) Salmonella Shigella (SS) agar (OXOID)

Ingredients

Meat extract 5g

Peptone 5g

Lactose 10g

Ox bile dehydrated 8.5g

Sodium citrate 10g

Sodium thiosulphate 8.5g

Ferric 1g

Brilliant green 0.003g

Neutral red 0.025g

Agar 15g

Final pH 7.0+/- 37^0C

Preparation

63g of SS agar is dissolved in 1000ml distilled water by placing its container for 10-15 minutes with constant agitation (stirring). It is then heated directly to boil to dissolve the medium completely. It is autoclaved at 1210C for 15 minutes, then dispensed (20mls0 on each Petri dish. Prepared appearance tan orange to tan-red, clear to moderately hazy.

Use

It is a differential selective medium recommended for the isolation of Salmonella and *Shigella*.

vi) Citrate utilization test

This test is used to determine the ability of an organism to utilize sodium citrate as its only carbon source and inorganic ammonium salts as its nitrogen source. Bacteria that can grow on this medium turn bromothymol blue indicator from green to blue.

Method

Inoculate Simmons citrate agar lightly on the slant by touching the tip of a needle to a colon that is 18-24 hours old. Broth culture is not recommended as the inoculum will be too heavy. Incubate at 35-37ºC for up to 7 days.

observe for development of blue color, denoting alkalization.

Expected Results

Positive: Growth on the medium, with or without change in color of the indicator. The color change of the indicator is due to acid or alkali production by the test organism as it grows on the medium. Growth usually results in the bromothymol blue indicator, turning from green to blue.

Quality control:

Known citrate positive; *Klebsiella pneumonia* and citrate negative; *Escherichia coli*. Both *S. enteritidis* and *S. typhimurium* do not utilize citrate as their source of carbon hence they do not grow in this medium.

vii) Indole test

Bacteria that produce the enzyme tryptophanase are able to degrade the amino acid tryptophan into pyruvic, ammonia and indole. Indole is detected by combining with an indicator, aldehyde (1 % paradimethylaminoaldehyde), that results in a blue color formation. This test is used in many identification schemes, especially to presumptively *Escherichia coli*, the many gram negative bacilli most commonly encountered in diagnostic bacteriology.

Use

This test is used to determine the ability of an organism to split tryptophan to form the compound indole.

Method

Inoculate tryptophan broth with a drop from a 24-hour brain heart infusion broth culture.

Incubate at 35ºC in ambient air for 24- 48 hours. Add 0.5 ml of Kovacs reagent

Expected results

Positive: Pink to wine colored red ring after addition of appropriate reagent Negative: No color change after addition of the appropriate reagent. S. *Tyhimurium* and *S. enteritidis* are indole negative

viii) Triple Sugar Iron (TSI) Principle

TSI is used to determine whether a gram negative bacilli utilizes glucose and lactose or sucrose fermentation and forms hydrogen sulfide (H_2S). TSI contains 10 parts lactose: 10 parts sucrose: 1-part glucose and peptone. Phenol red and ferrous sulfate serve as indicators of acidification and (H_2S) formation respectively. When glucose is utilized by a fermentative organism, the entire media becomes acidic (yellow) in

18 to 12 hours. The butt remains acidic after the recommended 18 to 24 hours'

incubation period because of the presence of organic acids resulting from the fermentation of glucose under anaerobic conditions in the butt of the tube. The slant however reverts to the alkaline (red) state because of oxidation of the fermentation products under aerobic conditions in the slant. This change is a result of the formation of carbon dioxide and water and the oxidation of peptones in the medium to alkaline amines. When in addition to glucose, lactose and/or sucrose are fermented, the large amount of fermentation products formed on the slant will more than neutralize the alkaline amines and render the slant acidic(yellow), provided the reaction is read in 18 to 24 hours. Reactions in TSI should not be read beyond 24 hours of incubation, because aerobic oxidation of the fermentation products from lactose and/or sucrose does not proceed and the slant will eventually revert to the alkaline state. The formation of carbon dioxide and water (hydrogen gas) is indicated by the presence of bubbles or cracks in the agar or by separation in the tube. The production of H2S requires an acidic environment and is manifested by blackening of the butt of the medium.

Method

1) With a straight inoculation needle, touch the top of a well isolated colony.

2) Inoculate TSI by first stabbing through the center of the medium the bottom of the tube and then streaking the surface of the agar slant.

3) Leave the cap on loosely and incubate the tube at 35º - 37ºC in ambient air for

18 -24 hours.

Expected Results

Alkaline slant/no change in the butt (K/NC = glucose, lactose, and sucrose non-utilizer: this may also be recorded as K/K (alkaline slant /alkaline butt).

Alkaline slant/acid butt (K/A) = glucose fermentation only. Acid slant/acid butt (A/A) = glucose, sucrose, and /or lactose fermenter.

Note: A black precipitate in the butt indicates production of ferrous sulfide and H2Sgas (H2S+)

Bubbles or cracks in the tube indicate the production of carbon dioxide or hydrogen. Drawing the circle around the A for acid butt, this is A/A; usually this means the organism ferments glucose and sucrose, glucose and lactose, or sucrose and lactose, with the production of gas

Quality control

A: *Escherichia coli*

K/A H_2S+: *Salmonella typhi, S. typhimurium*

K/NC: *Pseudomonas species*

S. typhimurium produces H2S while *S. enteritidis* does not

5. TE (Tris hydro methyl amino methane EDTA (ethylene diamine tetra acetic acid) For 100 ml TE 10 mM Tris-HCI pH 8.0, 1 mL 1 M Tris-HCI, pH 8.0, 1 mM EDTA pH 8.0 0.2 ml 0.5M EDTA pH 8.0, Q.S to 100 ml with H2O Filter sterilize, store at room temperature.

ix) Methyl red/Voges proskauer Test

The test is carried out on organisms to determine their ability to oxidize glucose with the production and stabilization of high concentration of acid end products. The isolated organisms were inoculated into tubes with MR-VP broth by means of loop inoculation. The tubes were incubated at 37^o C for 24 hours. Three drops of methyl red indicator are added to all the tubes cultures and color change observed and recorded. A red coloration is a signal for a positive reaction.

x) Oxidase Test

This test was used to identify microorganisms containing the enzyme cytochrome oxidase and is used to distinguish between oxidase positive *Pseudomadaceae* and

oxidase negative *Enterobacteriaceae*. The isolates were placed on filter paper disk impregnated with 1% Kovac's oxidase reagent and it was observed for colour changes. The cytochrome oxidase transfers electrons from the electron transport chain to oxygen as the final electron acceptor and reduces it to water. In this test, artificial electron donors and acceptors are provided and when the electron donor is oxidized by cytochrome oxidase by cytochrome oxidase it turns to dark purple and this is considered an oxidase positive.

xi) Catalase Test (slide test)

The catalase test was done by placing a drop of 3% hydrogen peroxide on a microscopic slide. Using a sterile wooden stick, the colony was picked and then smeared into the hydrogen peroxide drop. If the mixture produced bubbles or froth, the organism was said to be catalase positive while a negative result showed no bubbles. The used slides were disposed in the biohazard disposal container.

xii) Coagulase test

This test was done to differentiate potentially pathogenic Staphylococci such as *Staphylococcus aureus* from other Gram positive cocci (coagulase negative Staphylococcus, CONS). A *Staphylococcal* colony was emulsified in a drop of water on a clean and grease free glass slide with minimal spread, if the isolate did not form a smooth milky suspension, the test did not proceed. Similar suspensions of control positive and negative strains were made to confirm the proper reactivity of the plasma. The isolates were inoculated in plasma and incubated at 37^{o} C for 24 hours. After incubation, a coarse clumping of cocci visible to the naked eye (clot formation) was observed as positive results, while absence of clumping or any reaction taking more than 10 seconds to develop was recorded as negative results. Clot formation indicated production of coagulase enzyme which is an enzyme that clots blood plasma and is a virulence factor of *S. aureus*.

xiii) API (Analytical Profile Index) 20E TEST.

API 20E presented a biochemical panel for identification and differentiation of members of the family *Enterobacteraceae.* In API 20E for identification of members, the plastic strip holds twenty mini tests chambers containing dehydrated media having chemically defined compositions for each test. These include ONPG (Onitrophenyl b-D-galapyranoside), ADH- (amino acid arginine dihydrolase), LDC-lysidine decarboxylation, ODC-(ornithine decarboxylase), CIT(citrate utilization), H2S-(hydrogen sulphide production), URE-(urease enzyme),TDA, (Tryptophan deaminase), IND-(indole test), VP-(Voges proskauer test), GEL-(gelatinase liquefying to gelatin), GLU-(Glucose fermentation), MAN-(Mannose fermentation), INO-(Inositol fermentation), SOR- (sorbitol fermentation), RHA-(rhamnose fermentation), SAC-9sucrose fermentation), MEL-(melibiose fermentation), AMY-(amygdalin fermentation), ARA-(Arabinose fermentation).

Setting up an API 20E biochemical test strip

Pick up a single pure colony and make a suspension of it in sterile distilled water.

Take the API 20E biochemical test strip which contains dehydrated biochemical reagents in 20 separate compartments (commercially available). Bacteria will react with them and give them different colors which will help to identify bacteria to the species level.

Take a Pasteur pipette and fill up the brim with the bacterial suspension.

Add sterile oil into the ADH, LDC, ODC, H2S, and URE compartments.

Put some drops of water in the tray and put the API strip and close the tray.

Mark the tray with identification number (patent ID or organism ID) date and initials

Incubate the tray at 37^0C FOR 18 TO 24 hours.

Results and interpretation

- For some of the compartments you can just read the change in colour after 24 hours but for some you have put reagents before reading.
- Add the following reagents to these compartments TDA- put one drop of ferric chloride, IND –one drop of Kovacs reagent, VP-40%KOH-reagent 1 and one drop of VP reagent- reagent 2(alpha –Naphtha) wait for 10 minutes before telling it negative.
- Get the API reading scale – colour char, mark either positive or negative, and allocate scores as guided, add up the scores for positive wells only in each triplet and get the digit code.
- Identify the organism by using API catalogue or API web software an online database.

xiv) Gel electrophoresis

Requirements.

10mg/ ml Ethidium Bromide

Dissolve 0.5g of ethidium bromide in 50 ml deionized water in dark bottle. Store at 4^0C.

70% Ethanol

Mix 140 ml ethanol and 60 ml sterilized deionized water in the sterilized bottle.

Loading buffer

25mg bromophenol blue or xylene cyanol

4g sucrose or 4ml glycerol

Procedure

Add water to the ingredients above to make up 10mls

The loading buffer gives colour and density to the sample to make it easy to load into the wells. The dyes are negatively charged in neutral buffers and thus move in the same direction as the DNA during electrophoresis. This makes it possible to monitor

the progress of the gel. The most common dyes are bromophenol blue (Sigma B8026) and xylene cyanol (sigma X4126) Density is provided by glycerol or sucrose.

Table 2A. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter, Nearest Whole mm			Equivalent MIC Breakpoints (µg/mL)		Comments
			R	I	S	R	S	
AMINOGLYCOSIDES								(14) **WARNING:** For *Salmonella* spp. and *Shigella* spp., aminoglycosides may appear active *in vitro*, but are not effective clinically and should not be reported as susceptible.
A	Gentamicin	10 µg	≤ 12	13-14	≥ 15	≥ 8	≤ 4	
B	Amikacin	30 µg	≤ 14	15-16	≥ 17	≥ 32	≤ 16	
C	Kanamycin	30 µg	≤ 13	14-17	≥ 18	≥ 25	≤ 6	
C	Netilmicin	30 µg	≤ 12	13-14	≥ 15	≥ 32	≤ 12	
C	Tobramycin	10 µg	≤ 12	13-14	≥ 15	≥ 8	≤ 4	
O	Streptomycin	10 µg	≤ 11	12-14	≥ 15	-	-	
TETRACYCLINES								
C	Tetracycline	30 µg	≤ 14	15-18	≥ 19	≥ 16	≤ 4	(15) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline or minocycline or both.
O	Doxycycline	30 µg	≤ 12	13-15	≥ 16	≥ 16	≤ 4	
O	Minocycline	30 µg	≤ 14	15-18	≥ 19	≥ 16	≤ 4	
FLUOROQUINOLONES								(16) Fluoroquinolone-susceptible strains of *Salmonella* that test resistant to nalidixic acid may be associated with clinical failure or delayed response in fluoroquinolone-treated patients with extraintestinal *salmonellosis*. Extraintestinal isolates of *Salmonella* should also be tested for resistance to nalidixic acid. For isolates that test susceptible to fluoroquinolones and resistant to nalidixic acid, the physician should be informed that the isolate may not be eradicated by fluoroquinolone treatment. A consultation with an infectious disease practitioner is recommended.
B	Ciprofloxacin or	5 µg	≤ 15	16-20	≥ 21	≥ 4	≤ 1	
B	levofloxacin	5 µg	≤ 13	14-16	≥ 17	≥ 8	≤ 2	
U	Gatifloxacin	5 µg	≤ 14	15-17	≥ 18	≥ 8	≤ 2	
B	Gemifloxacin	5 µg	≤ 15	16-19	≥ 20	≥ 1	≤ 0.25	(17) FDA-approved for *Klebsiella pneumoniae*.
U	Lomefloxacin or	10 µg	≤ 18	19-21	≥ 22	≥ 8	≤ 2	
U	norfloxacin or	10 µg	≤ 12	13-16	≥ 17	≥ 16	≤ 4	
U	ofloxacin	5 µg	≤ 12	13-15	≥ 16	≥ 8	≤ 2	
O	Enoxacin	10 µg	≤ 14	15-17	≥ 18	≥ 8	≤ 2	
O	Grepafloxacin	5 µg	≤ 14	15-17	≥ 18	≥ 4	≤ 1	
Inv.	Fleroxacin	5 µg	≤ 15	16-18	≥ 19	≥ 8	≤ 2	

Table 2A
Enterobacteriaceae
M2-Disk Diffusion