UNIVERSITY OF NAIROBI

BACTERIAL AND FUNGAL ISOLATES FROM OPERATING THEATRES AT KENYATTA NATIONAL HOSPITAL

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DECLARATION

I hereby declare that this is my original work and has not been presented for any award in any other college, institution or university.

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LIST OF ABBREVIATIONS AND ACRONYMS

BAP	Blood Agar Plate
CAP	Chocolate Agar Plate
CDC	Centres for Disease Control and Prevention
Cfu/M^3	Colony Forming Units per Cubic Meter
GBD	Global Burden of Disease Study
HAI	Health Care-Associated Infection
HCF	Health Care Facility
HCW	Health Care Worker
HEPA	High Efficiency Particulate Air
ICU	Intensive Care Unit
IPC	Infection Prevention and Control
KNH	Kenyatta National Hospital
MRSA	Methicillin Resistant *Staphylococcus Aureus*
PACU	Post Anaesthesia Care Unit
PPE	Personal Protective Equipment
QC	Quality Control
SPSS	Statistical Package for Social Sciences
SSI	Surgical Site Infection
W.H.O	World Health Organization

OPERATIONAL DEFINITIONS

An effective and safe operating theatre environment: - is a surrounding in which all sources of contamination by bacteria, fungi, viruses and any micro-environmental changes are strictly kept under control.

Bacteria: - are microscopic single-celled organisms which thrive in diverse environments like an operating theatre.

Fungus: - is a group of eukaryotic organisms such as yeasts and moulds.

Infection: - is invasion of micro-organisms into host tissues and their replication accompanied by symptoms or signs such as pain fever, swelling, or discharge.

Methicillin-resistant *Staphylococcus Aureus (MRSA):* - is a bacterium which is resistant to methicillin (a semi- synthetic penicillin). It may also be resistant to a number of antibiotics and other closely-related antibiotics or quinolones like oxacillin and flucloxacillin.

Microbe: - is a microscopic organism which is either multi-cellular or single-celled which can be found in theatre environment for example bacteria, protozoa and fungi. Microbes can either be beneficial or harmful.

Nosocomial infections: is an infection that is not present or incubating when a patient is admitted to a hospital

Operating theatre: - is also known as an operating room, or operation suite, which is a facility in a hospital where surgical operations are carried out in a sterile environment.

Staphylococcus Aureus: - is a bacterium that is a major cause of healthcare related infections with pathogenic potential ranging from mild skin infections such as boils to serious systemic illness such as osteomyelitis, sepsis and endocarditis.

Theatre environmental isolate: - is a microbial examination or testing of air, surface and equipment in the theatre in order to identify changing trends of microbial counts.

Theatre microbial isolates: - environmental pathogens found in air, inanimate objects, water and dust in theatres which survive well in the air, dust, and moisture such as *Aspergillus spp*. Microorganisms can make their way into the theatre environment from one person to another in form of droplets.

ABSTRACT

BACKGROUND: Hospital operating theatre equipment and the environment air may harbour bacteria, viruses, yeasts, and fungal spores. Susceptible patients undergoing surgery in such an environment may develop post-operative infections. Environmental surveillance to detect varying microbial levels for possible eradication is therefore necessary.

OBJECTIVES: To identify major fungal and bacterial isolates and to determine their patterns in different specialized operating theatres at the Kenyatta National Hospital (KNH).

METHODS: This was a cross-sectional study done at KNH. A total of 1372 samples from 12 operating theatres were collected between December 2017 and February 2018. Surface samples were taken with sterile wet swabs from different equipment and exposure of agar plates in the air. Settle plate method and colony forming unit per cubic metre was used to detect fungal and bacterial levels in different specialities. Student t-test was used to examine statistical significance for the varying bacterial and fungal isolate in different specialities against established normal levels in operating theatres.

RESULTS: There were 10 different bacterial genera isolated in swab samples notably: *Coagulase Negative Staphylococci* 86(44.5%) and *Staphylococci aureus* 44(22.8%), which are the major theatre equipment contaminants. Air contaminant in agar plates was dominated by *Staphylococcus epidermidis* 185(73%) and *coliforms* (21%). Additionally, 5 genera and 10 species of fungi were also isolated. *Aspergillus spp* 81(71.64%) predominated fungal isolates with *Aspergillus fumigatus* alone constituted 36 (31.9%) of the fungal isolates. There was no statistical significance in theatre isolates between different specialties. The mean settle rate was < 0.5 and <15cfu/m^3 which was within international standards.

CONCLUSION: This study showed that the KNH theatres had significantly low bacterial and fungal contamination rate on equipment swabbed and agar plate exposure as cfu/m^3 and settle rate were within international acceptable limits.

RECOMMENDATION: Effective cleaning, fumigation, dumb-dusting, regular change of disinfectants and contamination checks are necessary for operating theatres. The Presence of undesired microbial isolates (*Staphylococci spp, Aspergillus spp*) necessitates effective cleaning and disinfection of equipment and floors in operating theatres at all times.

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CHAPTER ONE

1.0 INTRODUCTION

Surgical procedures are part of medical treatment which requires uncontaminated environment and operating theatres should be clean as possible (Mishra, 2016) (Spagnolo, 2013). Theatre environment with its air may contain viruses, bacteria, yeasts, and fungal spores. The air in the operating theatre plays an essential role as it contributes to a third of all postoperative infections which can be minimized through contamination preventive measures and proper practices (Kasdekar et al., 2016).

The following are four main different methods used in counting microbes in the air and other surfaces (Pasquarella et al., 2000);

i. The count of colony forming units per meters cubic (cfu/m^3)
ii. The count of colony forming units (cfu) on settle plates
iii. Measurement of a chemical component of the microbial cell of air
iv. The count under the microscope

Bacterial bio-load for a clean operating room should not surpass 35 cfu/m3 in an empty theatre and 180 cfu/m3 during an operation (Anjali et al., 2015). According to the World Health Organization (WHO), most microbial pathogens acquired from theatre environment are Multiple Drug Resistant strains (WHO, 2002). Invasive techniques in operating theatres create routes for the pathogenic invasion which may lead to transmission of drug-resistant bacteria amongst patients. These require adequate treatment and preventive measures intended to control infection in different theatres. Methicillin-resistant *Staphylococcus aureus* (MRSA), a resistant strain not only to methicillin but also to aminoglycosides, macrolides, tetracycline, chloramphenicol, and lincosamides, may also be resistant to disinfectants and is a major source of nosocomial infections (Hiroshi et al., 2010).

1.1 PROBLEM STATEMENT

Microbial contamination of the theatre environment increases the prevalence of nosocomial infections, contributing to complications of surgery. As a result, the nosocomial infections raise the expenses and time spent at the hospital for the patient, while at the same

time devastating caregivers and their respective healthcare facilities. Moreover, an elevated rate of post-operative infection increases morbidity and mortality rates (Singh et al., 2013). In the United States of America for example, about 300,000 surgical site infections occur per year constituting 17% of all nosocomial infections (CDC, 2008). These nosocomial infections are among the top ten main causes of mortalities reported in US healthcare facilities (Humphery et al., 2014).

About 30% of patients admitted to hospitals and nursing homes in India acquire nosocomial infections contrary to an impressive 5% in resources-rich countries according to members of the Hospital Infection Control Society. This alarming scenario is attributed to the reluctance to put in place proper infection control measures during surgery (Mishra et al., 2016).

The Aga Khan University Hospital study in Kenya observed 6.8% incidence rate of surgical sites infection (S. Kariuki et al., 2009). Similarly, *E. coli*(18%) was found to be a major cause of urinary tract infections in a study carried out on incidence of nosocomial infections in the ICU at Kenyatta National Hospital (Inyama et.al 2011). Another study in Kenya observed 10.2% respiratory infections and 4.7% urinary tract infection linked to nosocomial infections (Ndegwa et al., 2015).

The cited studies link nosocomial infections to microbial contaminations, which necessitates routine studies in healthcare facilities especially operating theatres to put in checks to minimize microbial contamination.

1.2: JUSTIFICATION

It is known that the theatre environment is a potential reservoir of bacterial and fungal pathogens. This is because it harbours diverse microbial pathogens bundled together with a large number of susceptible individuals (Spagnolo et al. 2013).

Theatre environment is dynamic depending on surgical procedures underway and maintenance of infection control measures. This needs continuous monitoring of air, equipment, and surfaces to detect any change in microbial load (Kumar et al., 2015). Some bacterial and fungal isolates which can survive in the theatre environment for a long period of time and resistant to disinfectants are the main cause of nosocomial infections (Gelaw et al., 2014). In most nosocomial infections, surgical site infections account for 14%

to 17% of which 38% are attributable to the theatre environment (Spagnolo et al. 2013). Such microbial contamination in operating theatres augments the frequency of surgical site infection (Sabharwal & Sharma, 2015).

In addition, treating postoperative sepsis can be costly and time-consuming for the majority of individual patients, resulting in an increased cost to patients and lengthening hospital stay. It is estimated that additional expenses of $3000 to $29000 per person are incurred for approximately 7-10 extra days. On average, the total annual cost of about $10 billion in the United States is incurred due to postoperative sepsis (Anderson et al., 2011). This rate of nosocomial infections is usually markedly higher in resource-poor countries (Gelaw et al., 2014).

Regular monitoring of microbial levels in the theatre environment is considered a basic step towards the prevention of nosocomial infections (Sabharwal & Sharma, 2015). The meticulous effort by use of infection control measures in theatres is necessary to minimize postoperative sepsis.

Thus, this study serves as baseline research endeavoured to isolate the major theatre bacterial and fungal isolates in some of the operating theatres at Kenyatta National Hospital. Some of the isolates may be the source of theatre contaminants.

1.3: RESEARCH QUESTIONS

1. Were the major fungal and bacterial isolates in the Kenyatta National Hospital Theatres within the internationally acceptable standard levels?
2. What were the patterns of microbial isolates in different specialized theatres in Kenyatta National Hospital?

1.4: OBJECTIVES

General Objective

To isolate major fungal and bacterial pathogen levels and to determine their pattern in different specialized operating theatres in Kenyatta National Hospital.

Specific Objectives

1. To identify major bacterial and fungal isolates at Kenyatta National Hospital Operating Theatres and establish whether they are within international acceptable standard levels.
2. To establish and compare patterns of major pathogenic bacteria and fungi in different specialized theatres.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 BACKGROUND

Global Burden of Disease Study (GBD) (2010) estimated that at least 321.5 million surgical procedures were needed to address the burden of disease for a global population of 6.9 billion. Minimum rates of surgical demand vary over regions, ranging from 3383 operations per 100 000 in central Latin America to about 6495 operations per 100 000 in western sub-Saharan Africa (Rose et al., 2010).

The operating theatre environment plays an important role in the causation of post-operative infections, constituting a third of all nosocomial infections which can be minimized through preventive measures practiced by theatre personnel and other infection control units (Kasdekar et al., 2016). Postoperative sepsis is the major cause of morbidity and mortality in many operating theatres, necessitating strict aseptic techniques to minimize these infections. In developing countries, the maintenance of asepsis in many theatres is limited to mopping and fumigation. Several studies have shown that 80 to 90% of bacterial contaminants found in the wound after surgery emanate from air contaminated with microbes present in operating theatres (Bali et al., 2014).

Environmental monitoring refers to microbiological testing of air, surfaces, and equipment in operating theatres to detect varying patterns of microbial counts and micro-flora. Assessing the quality of theatre cleanliness is necessary to aid in the reduction of air contaminants in form of aerosols which may harbour bacteria, viruses, yeasts, moulds or fungal spores (Kasdekar et al., 2016).

Globally, more than 1.4 million people have developed nosocomial infection complications due to resistant strains of the so-called 'superbugs.' Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the superbugs and is a strain of bacterium which does not respond to methicillin drugs (semi-synthetic penicillin) and other antibiotics like oxacillin and flucloxacillin among others (Coia, Duckworth, Edwards, & Farrington, 2006). Healthcare facilities are the main reservoirs of MRSA, especially theatre environment. The

colonization of personnel, as well as patients and visitors, can act as carriers who can also harbour the organisms and act as the transmitters of these infections. MRSA can be harboured in the mucosal membranes of the nostrils, throat and even on the skin in these asymptomatic carriers. The portal of entry is mainly through the damaged or diseased skin, which may lead to dermatitis, eczema or chronic wounds. The unclean theatre environment, its surfaces or shared patient equipment and other items are also reservoirs for its transmission (Coia et al., 2006).

Poorly controlled infections in a crowded hospital, for instance, coupled with uncontrolled theatre environmental contamination may lead to chronic or acute nosocomial infection (Ducel et al., 2002). A survey conducted in 14 countries by WHO attributed 8.7% prevalence of nosocomial infections to the hospitalization of patients in 55 hospitals (WHO, 2002). These poorly controlled theatre infections can originate from the use of medical devices and procedures. These illnesses prolong hospital stay of affected patients, drain healthcare resources and may lead to loss of lives (WHO, 2011).

Reducing the burden of nosocomial infections is a necessity, the operating theatres are at the centre of such efforts, since surgical procedures, with both environmental impact and equipment like anaesthetic apparatus, operating devices or instruments, personnel among others occurring there may be a source of sepsis to patients(WHO, 2011). Infection Control Units and other mandated departments in hospital facilities, both in developing and developed countries ought to continue playing an essential role to avert mortalities caused by nosocomial infections. This involves isolation of pathogenic bacterial strains causing life-threatening ailments, most of which are Multi-Drug Resistant strains. Use of preventive measures and treatments designed to lower infection in healthcare facilities and theatres ought to be preceded in reducing such infections (Smith et al., 2014).

Despite efforts by public health and infection control units, infections have persisted to arise in hospitalized patients. Lowered immunity in hospitalized patients is a factor contributing to persistent nosocomial infections.

Medical procedures and invasive techniques during surgical procedures create potential routes for pathogenic invasion; hence, the transmission of drug-resistant bacteria among patients (WHO, 2002). Modern surgical procedures and therapeutic interventions are also sources of nosocomial infections (Gniadek et al., 2011).

Taken together, infection control and surveillance are supposed to be conducted periodically in order to assess the general status of theatres, necessary for infection prevention.

2.2. Nosocomial infections

Definition

The term nosocomial comes from a combination of Greek words "gnosis" meaning, (disease) and "komein" (to take care of) translated into the English word as "nosocomial" (pertaining to a hospital) (Feldman et al., 1982). They are hospital-acquired infections which may be discovered following patient hospitalization. These may be transmitted to a patient by their fellow infected patient, healthcare worker or a contaminated facility within the hospital set up and are among the leading cause of patient mortality and increased morbidity among inpatients (WHO 2002).

Nosocomial infections are common and occur worldwide, affecting both developed and developing countries in equal proportions. This is a major burden for public health authorities as well as patients economically as a result of prolonged hospital stay. Each year, 2.2 million people in Europe and about 3.0 million individuals in the United States of America (USA), develop nosocomial infections, and approximated 1 in 20 to 1 in 16 of these infection result in mortality, while the rest suffer the consequences of increased morbidity in an average of 7 - 9 days extra hospital stay (Ducel et al., 2002). This prolonged hospital stay in both public and private health systems raises hospital costs and may lead to litigation which worsens disease burden to the affected families.

In the United States alone, approximately 300,000 SSIs occur annually of which 17% are attributable to nosocomial infections, after UTI, in which 2%-5% of patients acquire it while undergoing surgery (CDC, 2008). It is also estimated that about 77% of deaths among patients with SSI in the USA are directly attributable to surgical site infections (SSI). Attributable costs of SSI vary depending on the type of operative procedure and infectious pathogen ranging from $3,000 to $29,000. SSIs are believed to account for up to $10 billion annually in health care expenditures (Anderson et al., 2011).

Mortality rates are even higher in resources poor countries, especially in multidrug-resistant strains of organisms like tuberculosis. In Africa, it has been reported that over 25% of nosocomial infections are acquired in the hospital setting leading to severe illness and deaths particularly in developing countries. In a study conducted in a specialized hospital in North-western Nigeria, pathogenic bacteria were isolated from fomites in the operating rooms. These contributed to causes of nosocomial infections which were among significant healthcare problems in the health care units (Nwankwo et al, 2012). In Kenya, a research carried out at Aga Khan University Hospital, Nairobi observed an incidence rate of surgical site infection of 6.8% predominated with the following bacterial isolates: *S. aureus* (30%), *Coagulase-negative Staphylococcus* (16%), *P. aeruginosa* (3%) and *Klebsiella spp.* 9% (Kariuki et al., 2009).

2.3. Pathogenesis of nosocomial infection

The following are different sources and factors that influence microbial pathogenesis in theatres (WHO, 2002):

i. Endogenous route from patient flora such as those from the skin, mucous membranes and GI tract

ii. Exogenous route from surgical personnel such as surgeon(s) and other team members from their soiled attires and breaks in aseptic technique and inadequate hand hygiene.

iii. Physical environment and ventilation in the operating room.

iv. Equipment tools brought to the operative area

2.4. Humidity and temperature

Temperature and humidity control are some factors which influence the growth of theatre microorganisms which need continuous monitoring and there are set standards in the developed countries. In the U.S, for example, they specify that, air temperature of 70 to 75 F (21-240C) with humidity of 50-60% which provides a balance between the patient's safety and operator comfort (Ellis, 1963). In Kenya, temperatures of 19-210C and humidity of 50-60% are ideal for theatre environment as it inhibits microbial growth. Other recent studies recommend varying humidity of 20-60% and temperature of 68-750F or18-210C (Arthur, 2015).

2.5: Approaches in theatre surveillance:

In infection control, frequent monitoring and evaluation of theatres need to be done where counting of microbes in the air and other surfaces involves various methods enlisted below (Pasquarella et al., 2000):

i. Count of colony forming units per cubic metres (cfu/m3);
ii. Count of colony forming units (cfu) on settling plates;
iii. Measurement of a chemical component of microbial cells/m3 of the air;
iv. Count under the microscope

There is no internationally accepted or agreed bioload on theatre microorganisms in isolation of bacterial and fungal levels in theatre. A well cleaned High-Efficiency Particulate Air (HEPA) need a minimum filtration efficiency of 99.97% and removal of particles greater than 0.3 microns and a standard laminar flow over the operating zone with proper air exchange. Operating room and directional flow of air should minimize colony forming units in theatres (Joseph et al., 2006). In a conventional operating room, bio-load ought not to surpass 35 cfu/m^3 in an un-occupied theatre and 180 cfu/m^3 during surgery (Anjali et al. 2015). In ultra-clean operating theatres bio-loads ought to be less than 5 cfu/m^3 in an empty theatre and should be less than 10 cfu/m^3 during operation and should not exceed 20 cfu/m3 at the periphery of theatres (Baird et al., 1996). Literature also suggests that for conventional operating theatres, bioload in an empty theatre should not go beyond 35 cfu/ m^3 of a particular time (Bali et al., 2014).

Air sampling, therefore, plays an important role in the monitoring of hygienic conditions in operating theatres. There are two ways in which air samples can be collected: Active air samplers and Passive air sampling known as settle plates (Kasdekar et al., 2016). An effective and safe operating theatre environment is where all sources of contamination by microbial pathogens are strictly kept under control. This requires careful periodic checks, planning, maintenance as well as proper ongoing training for theatre staff (Spagnolo et al., 2013).

Settle plate

In a controlled environment like theatres, air serves as a reservoir for bacteria and fungi, hence settle plate is the best method in measuring air quality and identification of critical hospital environment (Napoli, Marcotrigiano, & Montagna, 2012). Microbiological quality of air is a reflection of the hygienic state of an operating theatre and evaluation of the level of microbial air contamination is considered the basic step towards prevention (Sabharwal & Sharma, 2015).

The concentration of airborne bacteria and fungi on agar is expressed as colony forming units per cubic metres (cfu/m3). Cell counting using haemocytometer (direct microscopic) is where all cells, dead and living, are counted but cfu measures only viable cells. The results for liquids are given as (cfu/m^3) colony-forming units per gram (Rafik, 2016). Living organism's ≥50 µm in minimum dimension can be counted manually under a microscope by three experienced analysts who compare their results for accuracy and precision.

2.6 Normal microbial count:

Various international scientific societies have different guidelines regarding operating theatre environmental monitoring. These guidelines include;

i. Positive pressure
ii. ii. Exchanges of filtered air per hour
iii. Air-conditioning systems with High-Efficiency Particulate Air (HEPA) filters

Recommendations have also been given on healthcare-associated infection regarding surveillance methods, intervention to limit SSI and methods of monitoring the implementation of such guidelines (Spagnolo et al., 2013). Ensuring adequate ventilation in patient-care areas like theatres other than providing good quality air is necessary for effective prevention and control measures during construction and renovation (Joseph et al., 2006). Effective measures include:

- Using movable HEPA filters,
- Establishing barriers between the patient-care and construction areas,
- Using negative air pressure in construction or renovation areas around patient care spaces
- Sealing windows

A well cleaned High-Efficiency Particulate Air (HEPA) need a minimum filtration efficiency of 99.97% and removal of particles greater than 0.3 microns and a standard laminar flow over the operating zone with proper air exchange. Operating room and directional flow of air should minimize colony forming units in theatres (Joseph et al., 2006).

Developed countries have their standards, protocols, and guidelines available in theatre microorganisms' surveillance, with the existence of some controversies over the frequency and extent of monitoring of operation theatres (Pondoni, 2016). However, in developing countries like India, where there are no uniform guidelines. A number of operating theatres are built and maintained according to the individual's knowledge level, availability of funds, technical staff, and the equipment (Bali et al., 2014). No data, however, has been shown in Kenya as guidelines for an operating room.

2.7 Environmental cleaning

This refers to general cleaning of environmental surfaces and maintenance of cleanliness in an HCF (WHO, 2012). It is the physical or manual removal of organic materials such as dust and dirt, which removes a large proportion of microorganisms. Warm water with detergent is sufficient to remove all organic contamination. Theatres also require the use of disinfectants for environmental disinfection precisely formulated according to institutional guidelines (WHO, 2012). General infection control strategies are necessary

for preventing microbial infections. This involves risk assessment of the facility to determine if patients, especially severely immunocompromised can be affected (Bali et al., 2014).

2.8 Common microorganisms in the operating theatre

According to CDC the common SSIs microorganism's isolates in descending order are as follows (Collaboratives, 2009):

Microorganism	**Percentage (%)**
Staphylococcus aureus	*30.0*
Coagulase Negative Staphylococci	*13.7*
Enterococcus spp	*11.2*
Escherichia coli	*9.6*
Pseudomonas aeruginosa	*5.6*
Enterobacter spp	*4.2*
Klebsiella pneumoniae	*3.0*
Candida spp	*2.0*
Klebsiella oxytoca	*0.7*
Acinetobacter baumannii	*0.6*

Table 1: Microorganism in order of occurrence

A research study done in India found that the predominant bacteria in air sampling was Staphylococcus aureus out of 83 samples collected, 76 were colonized, followed by streptococcus while Aspergillus was the main fungal isolate found (Bali, et al., 2014). A study done in Ethiopia found that most of the bacterial isolates, 142 (52.9%) were from theatre environments and the remaining 77 (28.8%) from the health professionals and 49 (18.3%) were from the patient (Gelaw et al., 2014). Another Study done in Ethiopia on micro-organism isolates, shows similar findings of "*Coagulase-negative Staphylococcus* at 88.4% and *Staphylococcus aureus* 11.6%" (Gelaw et al., 2014). Eight bacterial genera and four species of the fungus were isolated during a study done in Nigeria, with higher isolates of *Coagulase negative Staph.aureus* (28.3%) and *Pseudomonas aeruginosa* (23.3%). Others included *Escherichia coli* (10.0%) and *Proteus mirabilis* (8.33%).

A study was conducted in Baghdad to distinguish and isolate bacterial in 11 theatres of two busy city hospitals over a two year period, with 1216 swabs collected from surfaces, equipment and antiseptic solutions in different operating theatres. This study found that; *Staphylococcus epidermidis* was the most common isolated (39.1%), followed by *Pseudomonas aeruginosa* (30.4%), whereas in 2002, *coliform* bacteria were found to be the most common isolates (62.5%), followed by *P. aeruginosa* (25.0%). The *coliforms* were mainly from delivery theatres which may be attributed to the normal flora in the gut which may contaminate theatres during delivery (Ensayef, et al., 2009). A study done in Turkey in two hospitals during a 3 month sampling period, found out that the average number of live microorganisms collected in the two hospitals were 224.44 and 536.66 cfu/m3. They isolated *Aerococcus sp.* and *Enterococcus sp. Pseudomonas spp.* as the common organisms and were greatly contributed by a number of people in the environment and air conditioning system (Çakir et al., 2013).

Therefore, taken together, these studies show that theatre is a potential environment where nosocomial infections, sepsis and other risk factors for patients are acquired. Operating theatre requires proper and close surveillance effective disinfection and decontamination of the theatre environment and it's inanimate to reduce this risk. This surveillance is important to theatre users for necessary control measures, planning and implementation of policies and standards such processes include sampling and doing microbial loads routinely.

CHAPTER THREE

METHODS AND MATERIALS

3.0: METHODS

3.1 Study design

This was a cross-sectional study in which 1372 study samples from swabs and agar plates were collected. There were 1200 laboratory tests conducted for study samples collected compared to 1772 controls. This was done in 12 operating theatres to identify fungal and bacterial isolates and compare their varying pattern levels with internationally acceptable standards.

3.2 Study Site

The study was carried out at Kenyatta National Hospital (KNH) which is located in the Upper Hill area of Nairobi City County, the capital city of Kenya. Nairobi is the most populous city in East Africa, with an estimated urban population of between 3 -4 million. Kenyatta National Hospital is a level 6 hospital and is the largest referral hospital in East and Central Africa with a 2000 bed capacity receiving referrals from all the 47 counties and their associated county hospitals. It also provides specialized services across the spectrum of health services and it is mandated to act as a teaching and referral hospital, participate in research and national planning and policy making (KNH strategy 2009 – 2013). The KNH has 12 operating theatres, with each theatre having a bed, scrubbing area, and anaesthetic room.

3.3 Study population

The population comprised of 12 operating rooms, one receiving area, and one recovery ward (PACU). Each theatre of the 12 has one operating bed, one scrubbing area, 6 sterile instruments setting areas as each is shared by two theatres and two sterile instruments stores serving all theatres with three sluice rooms each serving 4 theatres (figure 1).

3.4 Sample collection

There were 12 scrubbing areas, operating theatres, anaesthetic rooms and machines and 6 setting areas. There were also 3 sluice rooms, 2 sterile area stores, 1 PACU and 1 receiving area as shown in the flow chart below (figure 1).

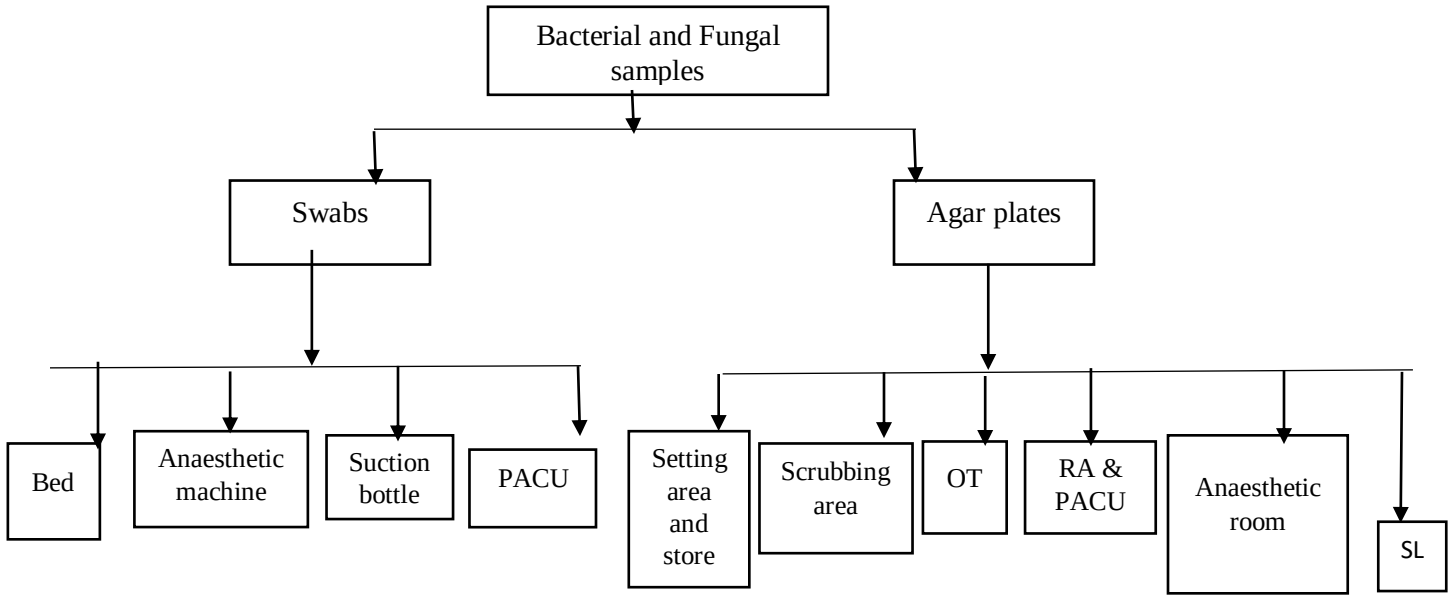

Figure 1: Flow Chart sample collection areas

KEY

Bed – Operating bed

OT – Operating theatre

PACU – post anaesthesia care unit or recovery room

RA – Receiving area

SL – Sluice rooms

3.5 Sampling process

3.5.1: Inclusion criteria

The equipment and surfaces in 12 operating theatres in the hospital main theatre:

I. Operating beds, scrubbing area, suction bottles, operating room, sluice rooms, sterile setting rooms, receiving area, Post anaesthetic care unit (PACU)

II. Monitors', anaesthetic machines and sterile supply rooms in KNH main theatres.

III. Air in the operating rooms, anaesthetic rooms, sluice rooms, scrubbing area, sterile setting area, receiving area, theatre stores and PACU

3.5.2: Exclusion criteria

1. 12 peripheral theatres outside main theatre.
2. Beds, anaesthetic machines, suction bottles outside main theatres.
3. In-animates objects or items within and outside sampling areas, walls and ceilings

3.6 Sample Size

A total of 51 samples of bacterial swabs and 50 samples of fungal swabs dipped in sterile ringers lactate solution were used to collect samples from theatre equipments. Similarly 50 samples of bacterial agar plates and 50 samples of fungal agar plates were collected from exposure of 30 minutes each time from 12 operating rooms, PACU sluice rooms, sterile instrument store and receiving area for bacterial and fungal isolation respectively. This process was repeated every 2 weeks for a period of 3 months. This was done to enable the acquisition of more than 200 swabs and 200 agar plates per month to make a total of more than 1200 samples during the 3-month study period.

3.6.1 Swabs samples per theatre

Theatre	Bed	Anaesthetic machine	Suction bottle	Receivers	PACU patients monitors
1	1	1	1	1	
2	1	1	1	1	
3	1	1	1	1	
4	1	1	1	1	
5	1	1	1	1	
6	1	1	1	1	1
7	1	1	1	1	
8	1	1	1	1	
9	1	1	1	1	
10	1	1	1	1	
11	1	1	1	1	
12	1	1	1	1	1
Total	**12**	**12**	**12**	**12**	**2**

Table 2: Swabs samples per theatre *Total Swabs = 50*

The tables above provide details of sample collection distribution per sample collection area.

The bacterial and fungal swabs were taken from the 12 theatres areas as follows each theatre (operating rooms beds, anaesthetic machines, suction bottle and used operating theatre wastes receivers) one receiving area, and one recovery ward (PACU). Each theatre

of the 12 has one operating bed, one scrubbing area, 6 sterile instruments setting areas as each is shared by two theatres and two sterile instruments stores serving all theatres with three sluice rooms each serving 4 theatres, adding up to 50 swabs.

Agar plates samples per theatre

The agar plate exposures were done in the following areas; The 12 (operating rooms, Anaesthetic room, Scrubbing room), 6 Setting area as each were shared by two theatres,3 sluice rooms each sluice is shared by four theatres, 2 sterile instrument store, one PACU and 1 receiving area.

Theatre	**Anaesthetic room**	**Operating room**	**Scrubbing room**	**Setting area**	**Sluice room**	**Sterile store**	**PACU**	**Receiving area**
1	1	1	1					
2	1	1	1	1				
3	1	1	1					
4	1	1	1	1	1			
5	1	1	1					
6	1	1	1	1		1	1	
7	1	1	1					
8	1	1	1	1	1			
9	1	1	1					
10	1	1	1	1				
11	1	1	1					
12	1	1	1	1	1	1	1	1

Total	12	12	12	6	3	2	2	1

Table 3: Agar plates samples per theatre T*otal Agar plates = 50*

3.6.2: Samples collection

A total of 50 samples of bacterial swabs from theatre equipments and 50 samples of fungal swabs were collected. Similarly 50 samples of bacterial agar plates and 50 samples of fungal agar plates were collected from exposure of 30 minutes each time from 12 operating rooms, PACU sluice rooms, sterile instrument store and receiving area for bacterial and fungal isolation respectively. This process was repeated every 2 weeks for a period of 3 months. This was done to enable the acquisition of more than 200 swabs and 200 agar plates per month to make a total of more than 1200 samples during the 3-month study period with its controls.

	Samples at each collection				**Samples per month (2 collections per month)**			
	Bacterial	**Fungal**	**Total**		**Month 1**	**Month 2**	**Month 3**	**Total**
Swabs	50	50	100		200	200	200	600
Agar plates	50	50	100		200	200	200	600
Total	**100**	**100**	**200**		**400**	**400**	**400**	**1200**

Table 4: The samples collected in the study

3.7 Data collection procedure

The principal investigator and research assistants collected samples twice per month for three consecutive months commencing December 2017 to February 2018. This was done purposely to adequately cover at least all theatre environmental pattern and microbial changes over a stipulated study period.

The 400 study samples were carried out twice a month as enumerated below:

i. Each study sample for fungal and bacterial agar plates in the 12 operating theatres, induction and scrubbing areas. The 6 samples from sluice rooms and sterile setting area rooms.

ii. Agar plate samples, each from receiving area, PACU , its monitors and the remaining 2 from the two theatre sterile supplies stores

iii. Swabs from anesthetic machines, suction bottles, kick about receivers and sterile setting rooms and beds.

iv. Four study samples from PACU monitors.

The samples in each specified areas were collected early in the morning when all areas had been cleaned adequately ready for the daily operating procedures.

3.8 Instruments and laboratory procedures

There were 25 areas identified by the researcher as the key areas of data collection in order to cover the basic areas in the theatres where microorganism can be isolated as key zones for identification of fungal organisms.

3.8.1: Collection and Transport of specimens

The evaluation of bacterial contamination in an operating theatre was performed using settle plate and swab method.

Settle plate method:

Air sampling was done with agar plate methods since in a controlled environment like theatres, air plays a central role as a reservoir for microorganisms hence settle plate was the best method for measuring air quality and identification of critical situations. Then incubation microbial isolates was done and results expressed as cfu/m^3, which allowed air particles to be collect on the settle plate.

Formula for Conversion of Colony Count (on settle plate) to Counts /m^3

Cfu/m^3 = a × 1000 /p × t × 0.2 where

a = number of colonies on Settle plate.

p = surface measurement of plate used and

t = Time of exposure of settle plate

Petri dishes containing Blood Agar, Mac-Conkey and Sabouraund Dextrose agar media which support survival and bacterial and fungal growth respectively were transported to the operating theatres in strictly aseptic sealed plastic bags. The agar plates were labelled with sample number, site of the theatre, time and date of sample collection.

Both agar plates and controls were placed in specific areas like the centre, the corners of the operating theatre floor and exposed for 30 minutes to maximize exposure time and collected sediment biological particles. After this exposure, the agar plates were taken back using sterile technique, covered and ferried to the laboratory in sealed plastic bags and incubated at 37° C for 48hours for bacteria and at 25° C for 5 days for fungi. The concentration of airborne bacteria and fungi on agar was expressed as colony forming units per cubic metre (cfu/m^3). Colony forming unit (cfu) is a measure of viable bacterial or fungal cells. This is unlike in the direct microscopic counts (cell counting using haemocytometer) where all cells, dead and living. The results were then given as cfu/30minutes (colony-forming units per m^3 of exposure).

Swab method

A swab soaked in sterile nutrient broth was used to collect samples from the theatres, anesthetic machines, recovery patient room monitors, operation tables, suction bottles and receivers used for operating room procedures. All the samples were labeled properly and immediately transported to the Department of Medical Microbiology (Bacteriology and Mycology) Laboratory for processing.

3.8.2 Quality control

The quality of exposure agar plates were controlled by including sterile agar plates that were not opened but exposed and incubated together with sampling agar plates for exposures from the various theatres. Sterile swabs were used and also soaked in sterile normal saline. Positive controls included known bacterial like *Staphylococcus aureus (ATCC29213/ATCC25922)*, *E.coli* (ATCC25922), and *Pseudomonas aeruginosa* (ATCC25923) obtained from the University of Nairobi, Department Microbiology laboratory where they were used during identification of study bacterial isolates.

3.9 Processing of Samples

Swabs taken from the different theatre environment were streaked on blood agar plates and Mac-Conkey agar for bacterial growth and Sabouraund Dextrose Agar for fungal growth. These culture plates were incubated under aerobic conditions for 48 hours at 37^0c for bacteria and at 25^{o}C for 5 days for fungi. After incubation the colonies were counted and identification of isolates done based on their macroscopic colonial morphological characteristics like size, colour and elevation margin effect on media and biochemical tests (citrate utilization test, catalase test, coagulase test, and urease test).

3.10 Variables

The explanatory/independent variables were the site of sample collection and the outcome/dependent variables were the microbial growth and the number isolated.

3.11 Ethical Consideration

This study was approved and reviewed by Kenyatta National Hospital and University of Nairobi Ethics and Research Committee (KNH-UoN ERC approval number for this study is P777/11/2016).

3.12 Data management and analysis

Data entry was done using Microsoft Excel for windows, followed by editing and analysis using statistical package for social sciences (SPSS) version 17. Descriptive statistics incorporated sample collection sites and the different samples collected. Student t-test and Analysis of Variance (ANOVA) was used in the analysis of the different microbial growth obtained from different study Samples. The level of significance of all statistical analysis was 95% and the p - Value of < 0.05 was considered statistically significant.

CHAPTER FOUR

RESULTS

RESULTS

4.0: OBJECTIVE 1: IDENTIFICATION OF THE MAJOR PATHOGENIC BACTERIA AND FUNGI AT KNH THEATRES

4.1: SECTION 1: BACTERIAL SWAB DATA

For this section 1, the results analysed are from the Bacterial swabs that were conducted during the study period.

4.1.1: Equipment Swabs Data

A total of 51 pieces of equipment were swabbed. Anaesthetic machines formed majority of the swabbed equipment at 29.4%.

Equipment examined	Number of samples	%
Anaesthetic Machines	15	29.4
Suction Machines	12	23.5
Operating Beds	12	23.5
Receivers	12	23.5
Total	51	100.0

Table 5: Equipment Swabbed during the study period

4.2 Proportion of the total bacterial growth in the operating room

61% of the equipment swabbed over three months period in the operating room had bacterial growth as shown in Figure 2.

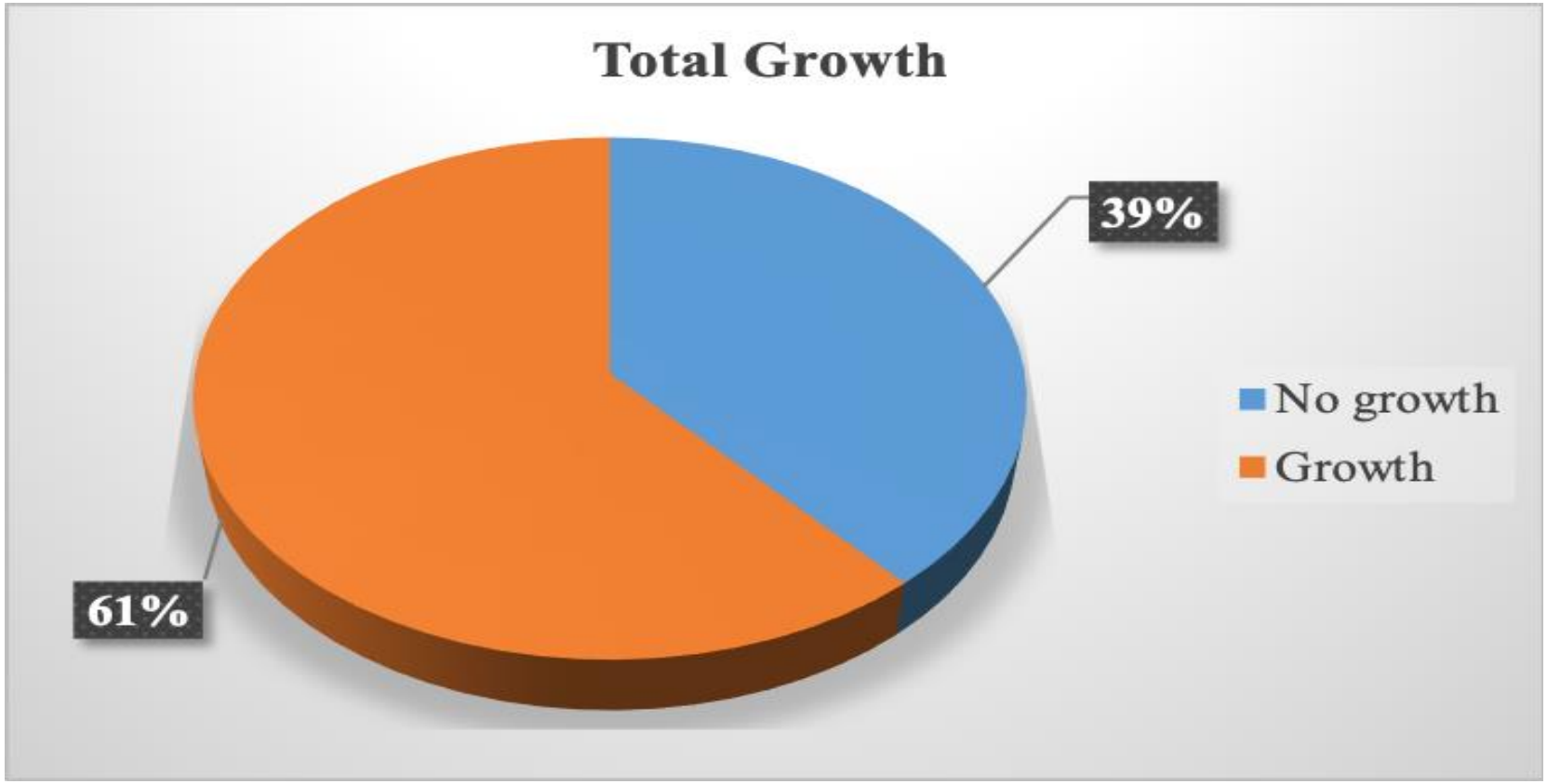

Figure 1: Proportion of the total bacterial growth in the equipment swabbed

4.3: Proportion of the total bacterial growth of microorganisms in the Post Anaesthesia Care Unit (PACU)

89% of the equipment swabbed over three months period in PACU showed growth of bacterial microorganisms.

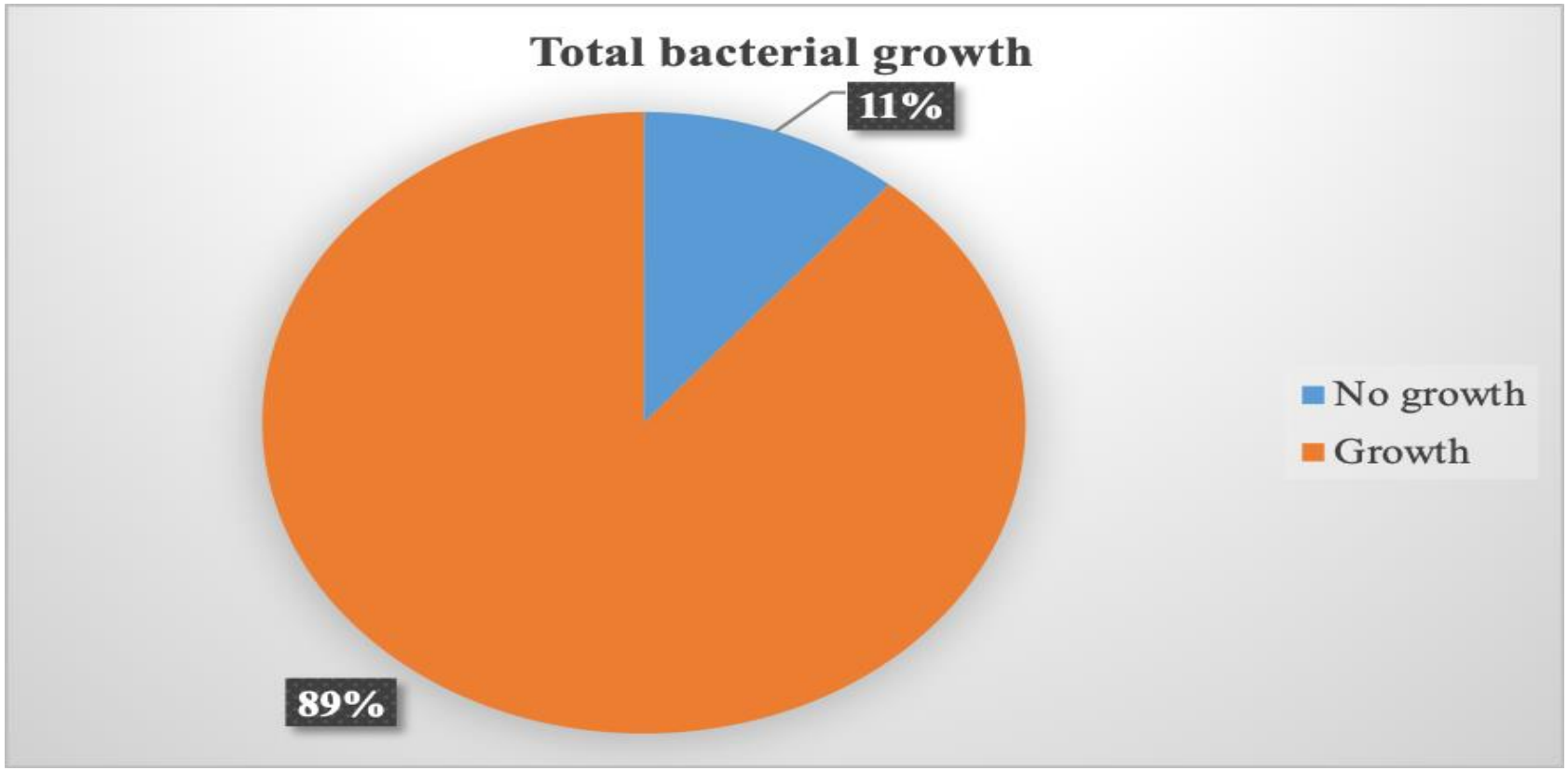

Figure 2: Total bacterial growth of microorganisms in the Post Anaesthesia Care Unit

4.4: Comparison of samples from equipment reporting growth of bacterial organisms in both PACU and operating rooms

The proportion of growth of organisms in the operating rooms and PACU was the same in the first week. There was variability in terms of growth between the 2nd and 6th sample. PACU showed a constant increase in growth than the operating room from sample 2 to sample 6.

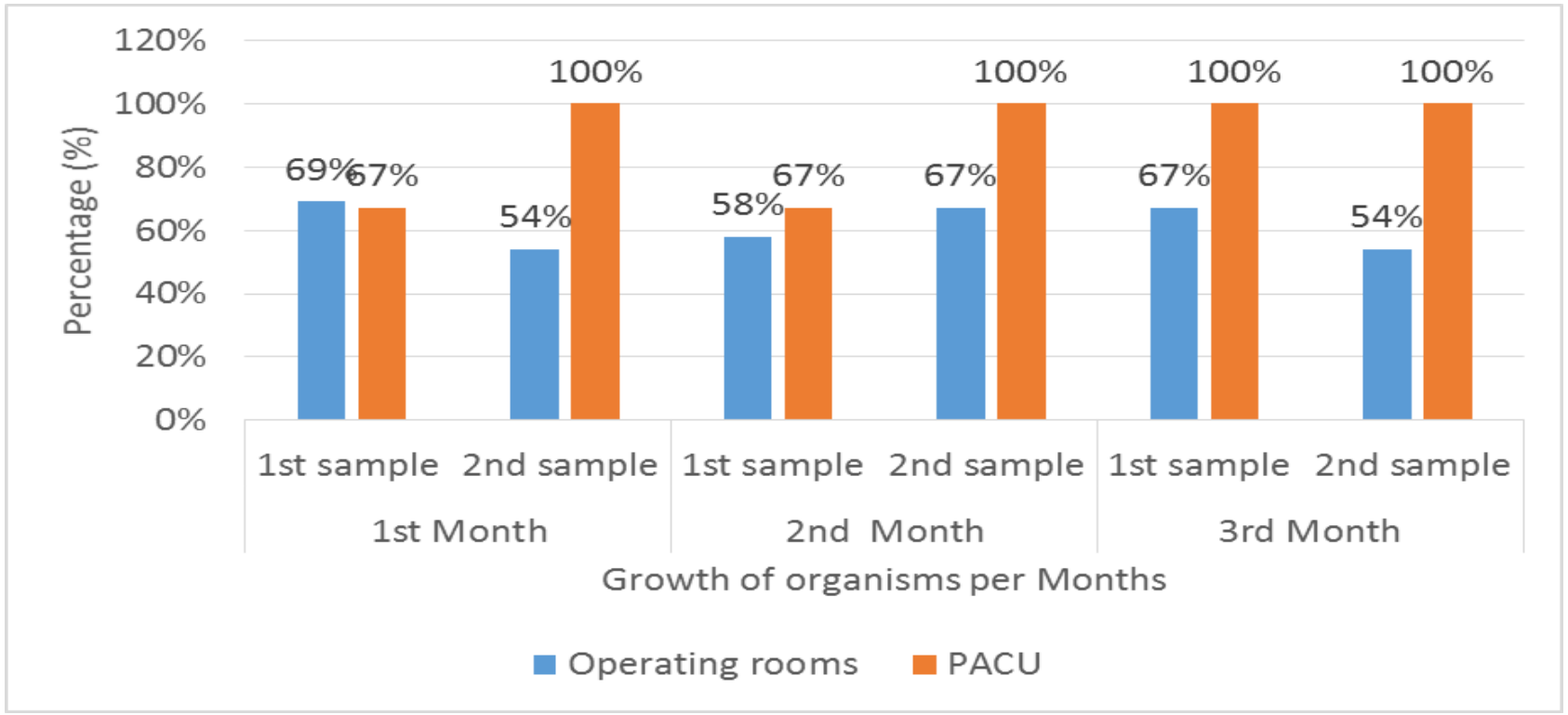

Figure 3: Samples from instruments reporting growth of organisms in both PACU and operating rooms

4. 5: Major bacterial isolates in KNH theatres

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Table 6: Swabbed bacterial isolates in the studied KNH the studied KNH theatres

Type of Microbial Isolates	**Sample1(n)**	**Sample 2 (n)**	**Sample 3(n)**	**Sample 4(n)**	**Sample 5(n)**	**Sample 6 (n)**
Number of None-Growth	16(31%)	22(43%)	21(41%)	16(31%)	16(31%)	22(43%)
Coagulase negative staphylococci	15(29%)	14(28%)	20(39%)	9(18%0	11(22%)	11(22%)
Staphylococcus aureus	5(10%)	5(10%0	3(6%)	6(12%)	11(21.6%)	7(14%)
Micrococcus spp	0	2(4%)	0	7(14%)	2(3.9%0	1(2%)
Klebsiella spp	3(6%)	2(4%)	1(2%)	0	1(2.0%)	0
Pseudomonas spp	2(4%)	1(2%)	1(2%)	1(2%)	3(5.9%)	0
Candida spp	1(2%)	0	0	0	1(2.0%)	1(2%)
Bacillus spp	2(4%)	1(2%)	0	0	1(2.0%)	0
E faecalis	0	1(2%)	0	0	2(3.9%)	0
E Coli	0	0	1(2%)	0	0	0
Granulicatella elegans	0	0	0	1(2%)	0	0
Coagulase negative staphylococci **and** ***Staphylococcus aureas***	4(8%)	2(4%)	3(6%)	4(8%)	2(4%)	5(10%)
Coagulase negative staphylococci **and** ***Micrococcus spp***	0	0	0	2(4%)	0	0
Coagulase negative staphylococci **and** ***Klebsiella***	0	1(2%)	1(2%)	0	0	0

spp						
Coagulase negative staphylococci **and** ***Candida spp***	1(2%)	0	0	0	0	0
Coagulase negative staphylococci **and** ***E. feacalis***	0	0	0	0	0	1(2%)
Staphylococcus aureas **and** ***Micrococcus spp***	0	0	0	2(4%)	1(2%)	0
Staphylococcus aureas **and** ***Klebsiella spp***	0	0	0	0	0	1(2%)
Staphylococcus aureas **and** ***Pseudomonas spp***	0	0	0	1(%)	0	0
Staphylococcus aureas **and** ***E Feacalis***	0	0	0	0	0	1(2%)
Staphylococcus aureas **and** ***E Coli***	0	0	0	1(2%)	0	0
Pseudomonas spp **and** ***Candida spp***	0	0	0	1(2%)	0	0
Candida spp **and** ***Bacillus spp***	1(2%)	0	0	0	0	0
E Feacalis **and** ***E Coli***	0	1(2%)	0	0	0	0
Staphylococcus aureus, Klebsiella spp **and** ***E Feacalis***	0	0	0	0	0	1(2%)

The three month study period indicated an average of 32.2% growths observed and from this, 10 different organisms were isolated, however there were also 15 mixed growths identified of organisms as tabled above (Table 7).

Most common bacterial organisms

The most common organism identified was *Coagulase negative staphylococci aureus* organism which formed an average 44.5% of the growths identified. It was also identified in 6 of the 15 mixed growth as shown in table 6 below.

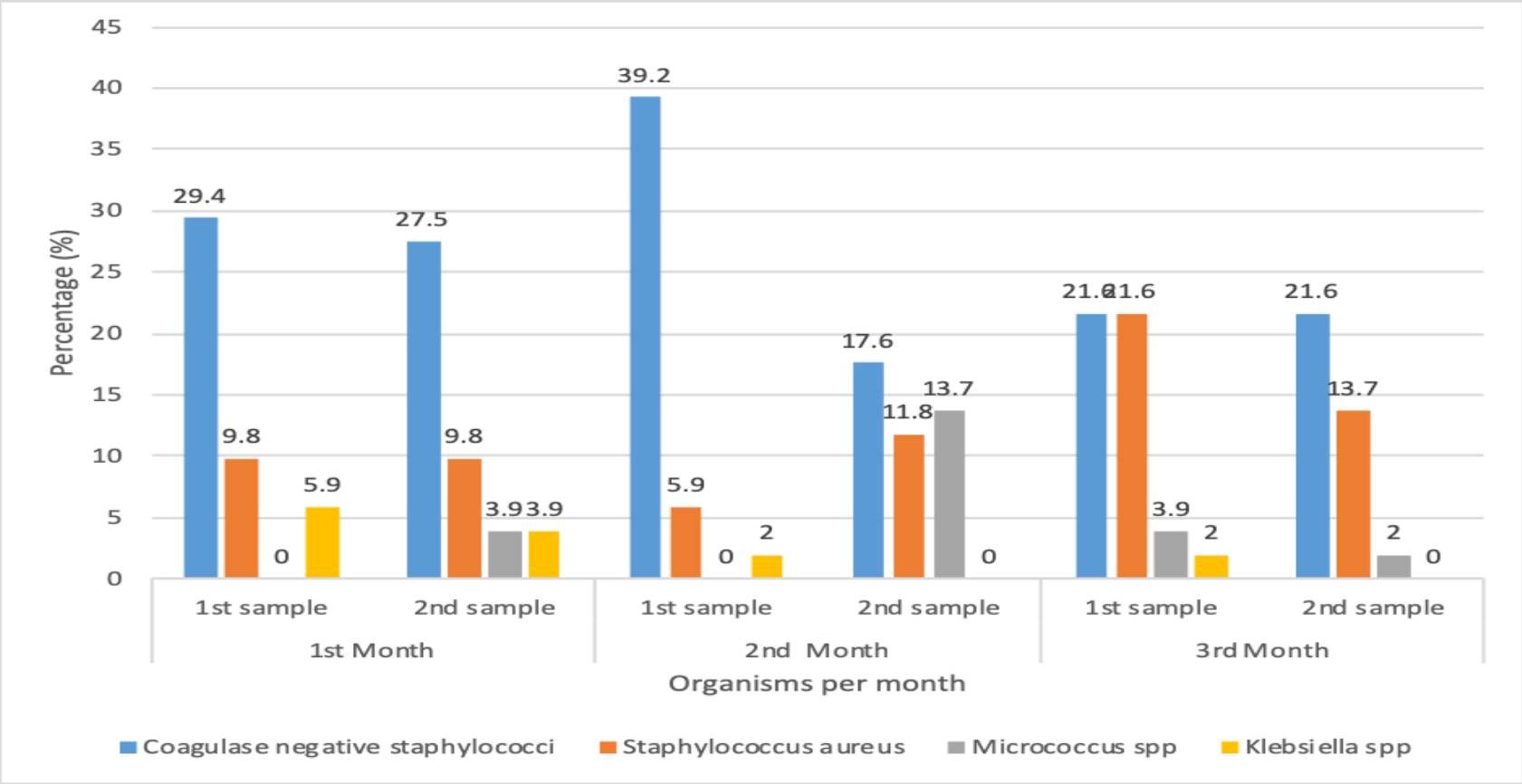

Figure 4: Major bacterial isolates

4. 6 The Proportion of equipment with microorganisms over time

High percentage of micro-orgasms growth of 68.6% were experienced in week 1, 4 and 5.week while 2 and 6 had the lowest micro-organisms of 56.9% each.

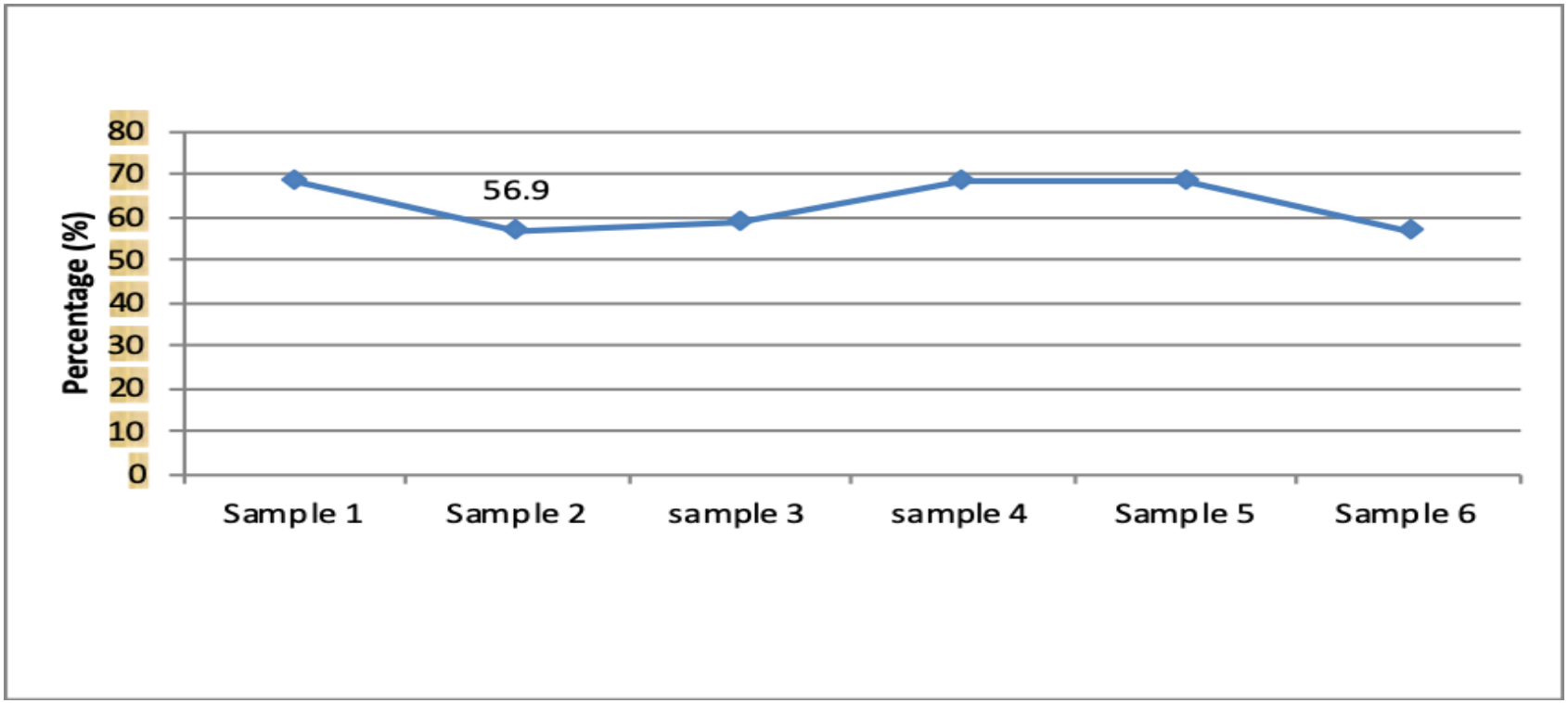

Figure 5: Proportion of equipment with organisms over time

4.7 Mean week's swabs growth observed

Anaesthetic machines and suction machines grew organisms in almost 5 samples of the 6 samples collected while receivers grew the least in only one sample.

Sites	N	Mean (weeks growth)	Std. Deviation	p-value
Anaesthetic Machines	15	4.67	.976	<0.0001
Suction Machines	12	4.67	1.073	
Operating Beds	12	3.58	1.165	
Receivers	12	0.42	1.443	

Table 7: Mean week's swabs growth

4.8 Frequency of observed organisms from equipments in the six samples collected

The most common micro-organism identified in bacterial equipments swabs were *coagulase* negative staphylococci at 80 and 86 with mixed growth respectively followed by a total of 44 (37 pure and 7 mixed growth) *staphylococci aureus* and the least was *Granulicatella elegans* identified once over the three month period.

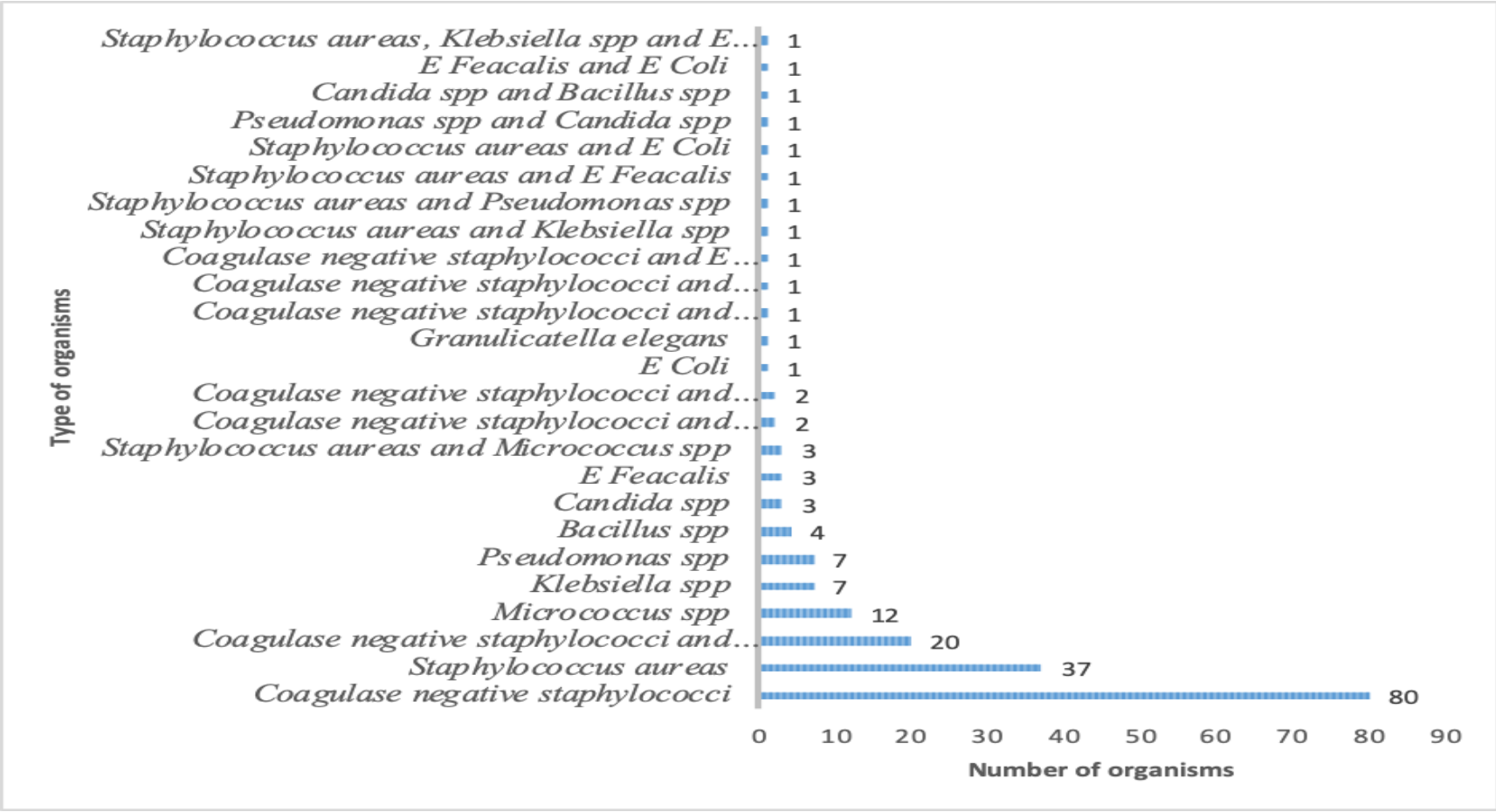

Figure 6: Frequency of observed organisms from the swabbed equipment

4.9 Pattern of micro-organisms in different specialty theatres

Theatre one (Emergency theatre) had grown organisms on average 5 times over the six samples taken while Urology theatres 3and 6 had the least organism. PACU especially the female had also grew in almost all the six samples. There was no significant difference in mean growth between different specialized theatre and any necessary intervention should be applied across all the specialties.

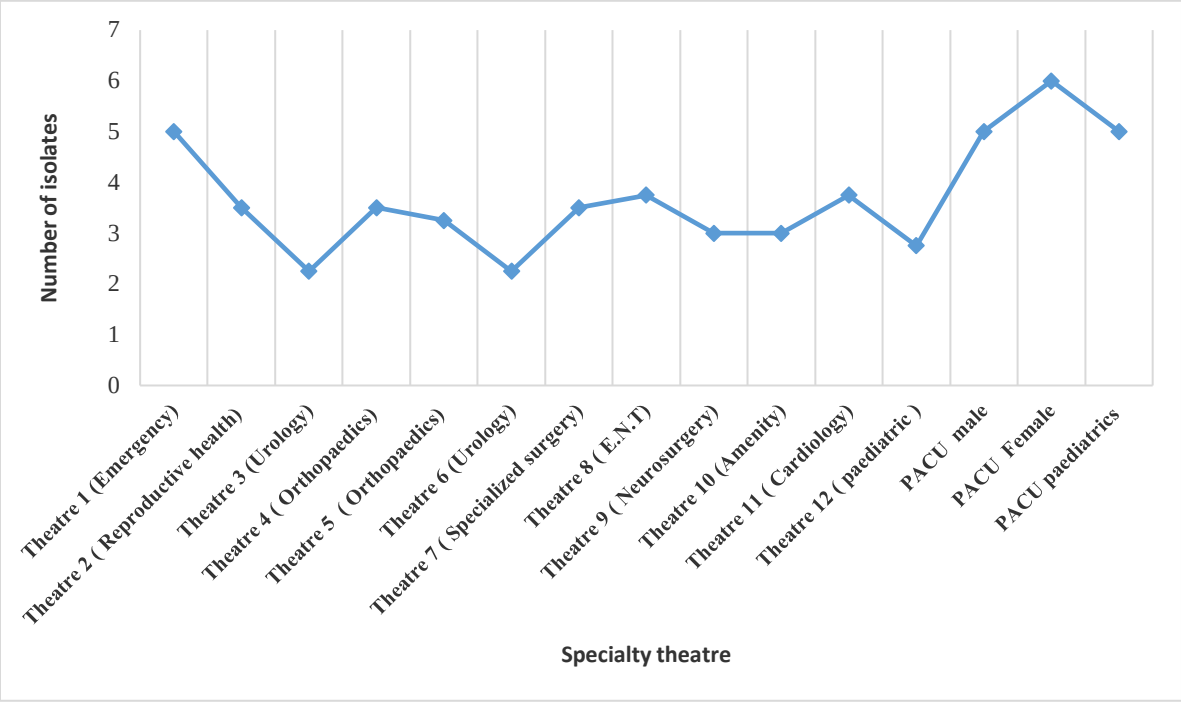

Figure 7: Pattern of micro-organisms in different specialty theatres

4.10: Bacterial agar plates samples test taken and its controls.

A total of 439 samples **(**348 tests **samples and** 91 controls **samples)** were taken in the 3 months period. Third sample being the highest number at 17.8% and the least being the first sample at (15.9%).

Sample	Group					
	Test		Control		Total	
	n	%	n	%	n	%
1	55	15.8	15	16.5	70	15.9
2	55	15.8	15	16.5	70	15.9
3	60	17.2	18	19.7	78	17.8
4	60	17.2	13	14.3	73	16.6
5	58	16.7	13	14.3	71	16.1
6	60	17.2	17	18.6	77	17.5
Total	348	100.0	91	100.0	439	100.0

Table 8: Bacterial agar plates samples

4.11 Major bacterial isolate organisms in Agar plates in KNH theatres

The most common air contaminant in KNH theatres isolated over the period was *Staphylococcus epidermidis* identified in 185 agar plate samples with growth of organisms representing 73%.

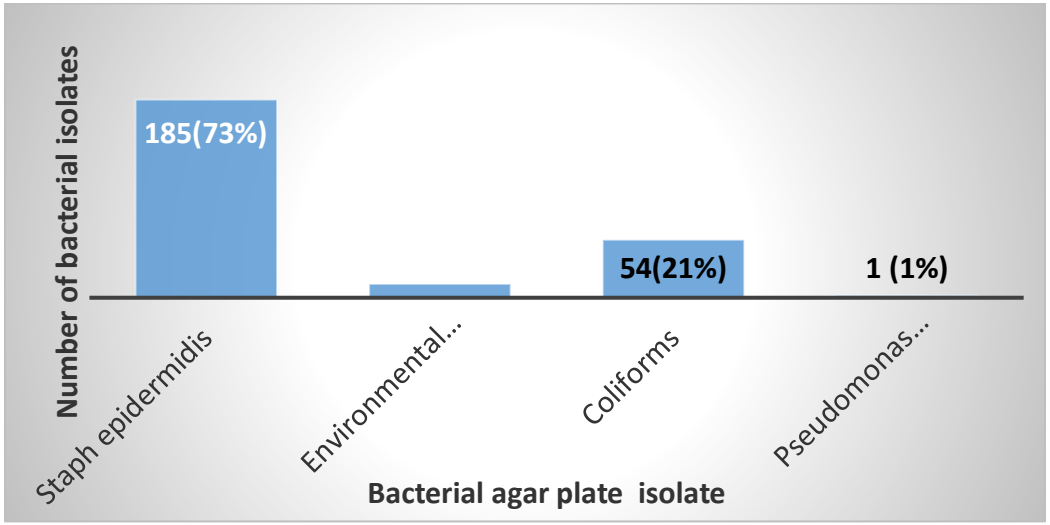

Figure 8: Pattern of Microorganisms in operating rooms

4.12: Pattern of Microorganisms in operating rooms

Theatre 4(orthopaedics) and 12(Paediatrics) had higher isolates of *coliforms* in the entire period of data collection while theatre 10(amenity) reported the highest times when there were no growths of organisms.

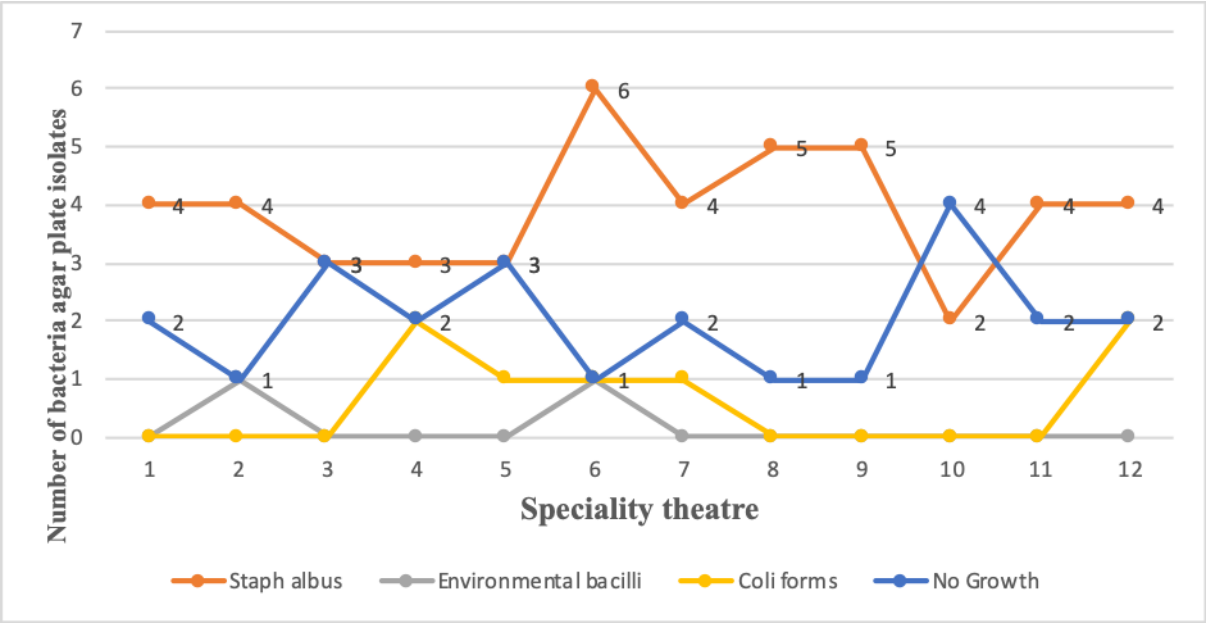

Figure 9: Pattern of Microorganisms in operating rooms

4.13 Agar Plate sample analysis in the three months period

During the data collection period the microbial contamination which exceeded 15cfu/m^3 in average were less than 2 samples hence KNH theatres air contaminants were within the international acceptable limit of less than 15cfu/m^3 in an empty theatre of about 8 hours in a busy hospital.

This was isolated during the 3rd sample in Paediatrics theatre where 20 colonies of coliforms with Settle rate: 0.67 particles/plate/min in the anaesthetic induction room and in scrubbing area of theatre 8 with mixed growths of 15 colonies of *staphylococcus epidermidis* and 6 colonies of coli-forms with settle rate: 0.7 particles/plate/min.

Generally there is no difference between different theatres in the air contaminations and same interventions can be applied uniformly across different specialties/ areas. The mean settle rate is< 0.5 which is within internationally acceptable limit of less than 15 cfu/m^3 in all the areas organisms were isolated.

1St	**Microorganisms**	**Up to 5cfu/m^3**	**6-15 cfu/m^3**	**Above15 cfu/m^3**	**Total**
	Staph epidermidis	41	8	0	49
	Environmental bacilli	1	0	0	1
	Coli-form	7	0	0	7
	No Growth	19	0	0	19
2nd	*Staph epidermidis*	30	3	0	33
	Environmental Bacilli	0	0	0	0
	Coli-form	5	0	1	5
	No Growth	31	0	0	31
3rd	*Staph epidermidis*	13	28	1	42
	Environmental bacilli	0	0	0	0
	Coli-form	7	0	0	7
	No Growth	19	0	0	19
	Pseudomonas aeruginosa	0	1	0	1
4th	*Staph epidermidis*	37	7	0	44

	Environmental bacilli	4	0	0	4
	Coli-form	15	0	0	15
	No Growth	26	0	0	26
	pseudomonas aeruginosa	0	0	0	0
5th	*Staph epidermidis*	16	1	0	17
	Environmental bacilli	3	0	0	3
	Coli-form	4	0	0	4
	No Growth	42	0	0	42
	Pseudomonas aeruginosa	0	0	0	0
	Environmental bacilli	5	0	0	5
	Coli-form	15	1	0	16
	No Growth	46	0	0	46
6th	*Pseudomonas aeruginosa*	0	0	0	0
	epidermidis(Non MRSA	1	0	0	1
	Fungi	2	0	0	2
	Total				439

Table 9: Average number of agar plate sample analysis in the three months period.

4.14 Bacterial bio-load levels in KNH theatres

Most colony forming units ranged less than 5cfu/m^3 in all the areas where data was collected and less than 30% between 6-15cfu/m^3 whereas those more than 15cfu/m^3 were less than 4 agar plates in total which is within acceptable clean operating theatres.

	Colony Forming Units				Settle rate			
	Up-to-cfu/m^3		Above 5 cfu/m^3		Up- to 0.5		Above 0.5cfu/m^3	
AREAS	n	%	N	%	n	%	n	%
PACU, Stores & Receiving area	7	53.8	6	46.2	13	100.0	0	0
Theatre 1	12	54.5	10	45.5	20	100.0	0	0
Theatre 2	19	65.5	10	34.5	28	100.0	0	0
Theatre 3	11	73.3	4	26.7	13	100.0	0	0
Theatre 4	9	90.0	1	10.0	9	100.0	0	0
Theatre 5	8	100.0	0	0	8	100.0	0	0
Theatre 6	11	73.3	4	26.7	13	100.0	0	0
Theatre 7	10	66.7	5	33.3	15	100.0	0	0
Theatre 8	9	64.3	5	35.7	12	92.3	1	7.7
Theatre 9	13	76.5	4	23.5	15	93.8	1	6.3
Theatre 10	11	100.0	0	0	10	100.0	0	0
Theatre 11	10	90.9	1	9.1	10	100.0	0	0
Theatre 12	13	81.3	3	18.8	15	93.8	1	6.3
Setting area 1 and 8	3	75.0	1	25.0	4	100.0	0	0
Sluice room 1 and 2	3	75.0	1	25.0	4	100.0	0	0
Setting area 2 and 7	2	50.0	2	50.0	4	100.0	0	0
Sluice room 3 and 4	6	85.7	1	14.3	7	100.0	0	0
Setting area 3 and 6	5	71.4	2	28.6	7	100.0	0	0

Setting area 4 and 5	1	100.0	0	0	1	100.0	0	0
Sluice 5 and 6	5	100.0	0	0	5	100.0	0	0
Sluice room 7 and 8	5	71.4	2	28.6	7	100.0	0	0
Sluice room 9 and 10	2	40.0	3	60.0	5	100.0	0	0
Setting area 9 and 12	5	83.3	1	16.7	6	100.0	0	0
Setting area 10 and 11	2	100.0	0	0	1	100.0	0	0
Sluice room 11 and 12	4	50.0	4	50.0	7	100.0	0	0

4.15 Total Colony forming unit/m3 and settle rate

Theatre 6(urology) followed by theatre 8 and 9 (ENT and Neurology) respectively had the highest number of *Staphylococcus epidermidis* but lowest number of coliforms. Theatre 10 amenity theatre had the highest number of coliforms. Theatre 3(urology/ophthalmology) remains cleaner theatre on average control of all the organisms at 3 cfu/m^3.

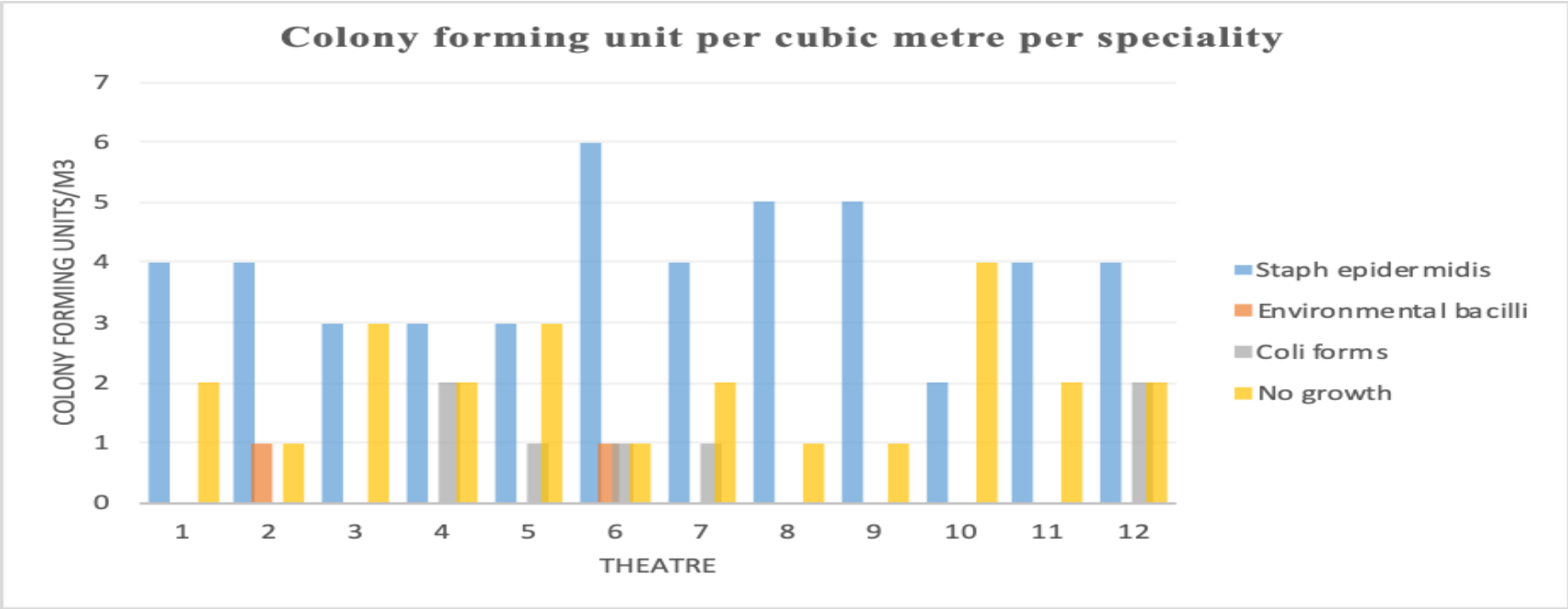

Figure 10: Colony forming unit/m3 and settle rate per theatre

4.16 Mycology Agar Plates taken twice each month for the three months duration

A total of 52 agar plates samples were taken twice each month for the three months duration, the largest samples being operating rooms, scrubbing area and induction rooms forming (212) 68% of the 312 samples taken. The distribution is as shown in the table below.

Table 8: Mycology Agar Plates

	Area and number of samples collected	**n**	**%**
Area of isolation	PACU	1	1.9
	Setting Areas	6	11.5
	Sluice Rooms	6	11.5
	Operating Rooms	12	23.1
	Scrubbing Areas	12	23.1
	Inductions Rooms	12	23.1
	Receiving Area	1	1.9
	Store Room 1	1	1.9
	Store Room 2	1	1.9
	Total	52	100.0

4.17 Number of Fungal Isolates per each sample

The second sample showed higher growth at 33 of the 115 growths isolated constituting 28.7% of the total growths.

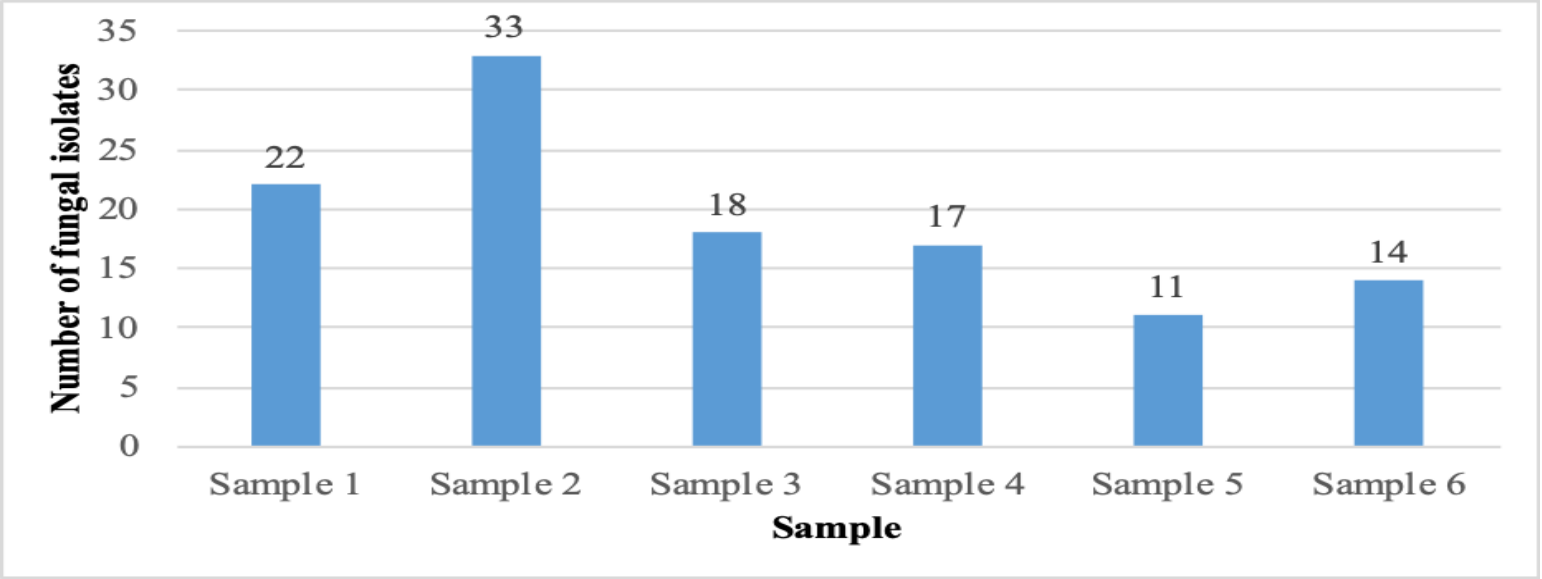

Figure 11: Fungal Isolates

4.18 Fungal bio-load levels in KNH theatres

Receiving area reported the most colonies forming unit per metres cubic of all the exposures forming 26.5% of the fungal isolates identified 4 times in the six samples. Sterile setting rooms had the least isolates though it reported growths in all the six samples taken over the three month period.

Table 11 below indicates the colony forming units in different areas in the Kenyatta National Hospital. There was only one area of agar plates sample collection in the receiving area.

Table 9: Fungal bio-load levels

Area	Sample 1 cfu/m^3	Sample 2 cfu/m^3	Sample 3 cfu/m^3	Sample 4 cfu//m^3	Sample 5 cfu/m^3	Sample 6 cfu/m^3	Total cfu/m^3
PACU	1	0	5	0	1	2	9
Setting Areas	1	2	1	1	1	1	5
Sluice Rooms	2	1	0	0	2	1	6
Operating Rooms	2	2	1	1	1	1	8
Scrubbing Areas	1	1	1	2	1	1	8
Inductions Rooms	1	2	1	2	2	1	8
Receiving Area	0	5	7	2	0	4	18
Store Room 1	0	0	0	1	0	0	1
Store Room 2	0	0	3	1	1	0	5

4.19 Pattern of fungal isolates over the three month period.

The colony forming unit in the third sample had the highest number and the least was the fifth sample as shown in figure 13 below on the next page.

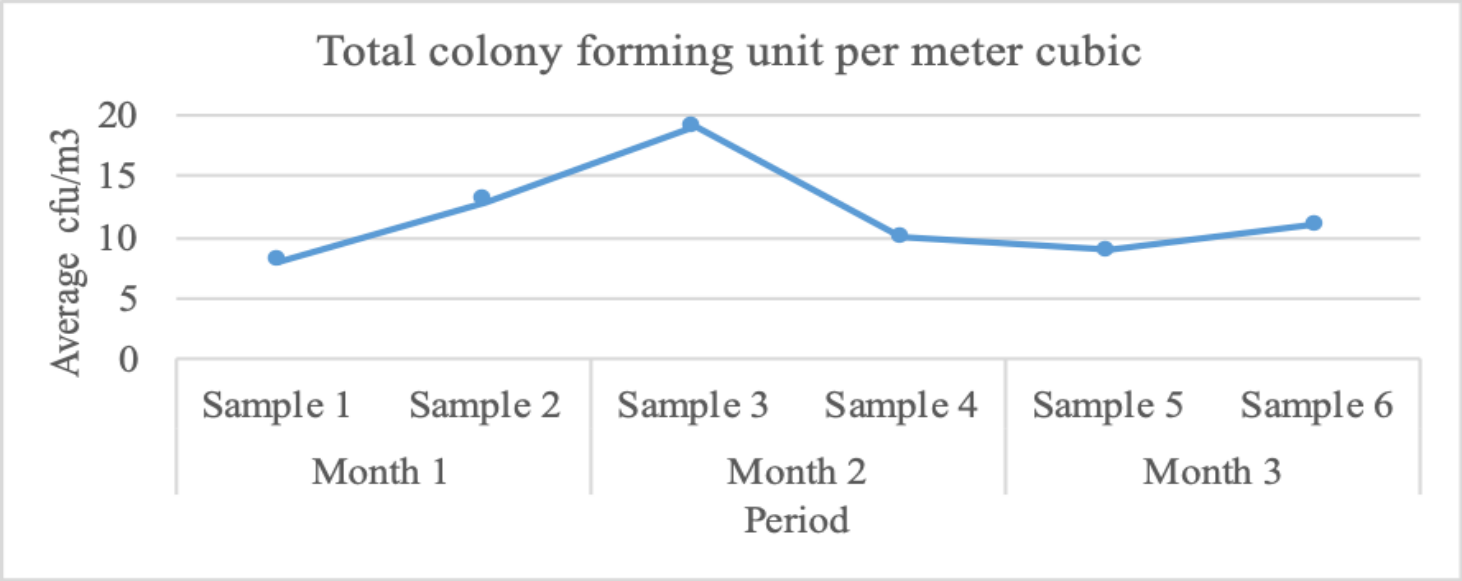

Figure 12: **Pattern of fungal isolates**

4.20 Fungal organisms isolated in different areas of theatres

Sterile supplies area stores had the least fungal organisms isolated followed by sluice rooms while receiving area had the most fungal organisms in total isolated over the three month period.

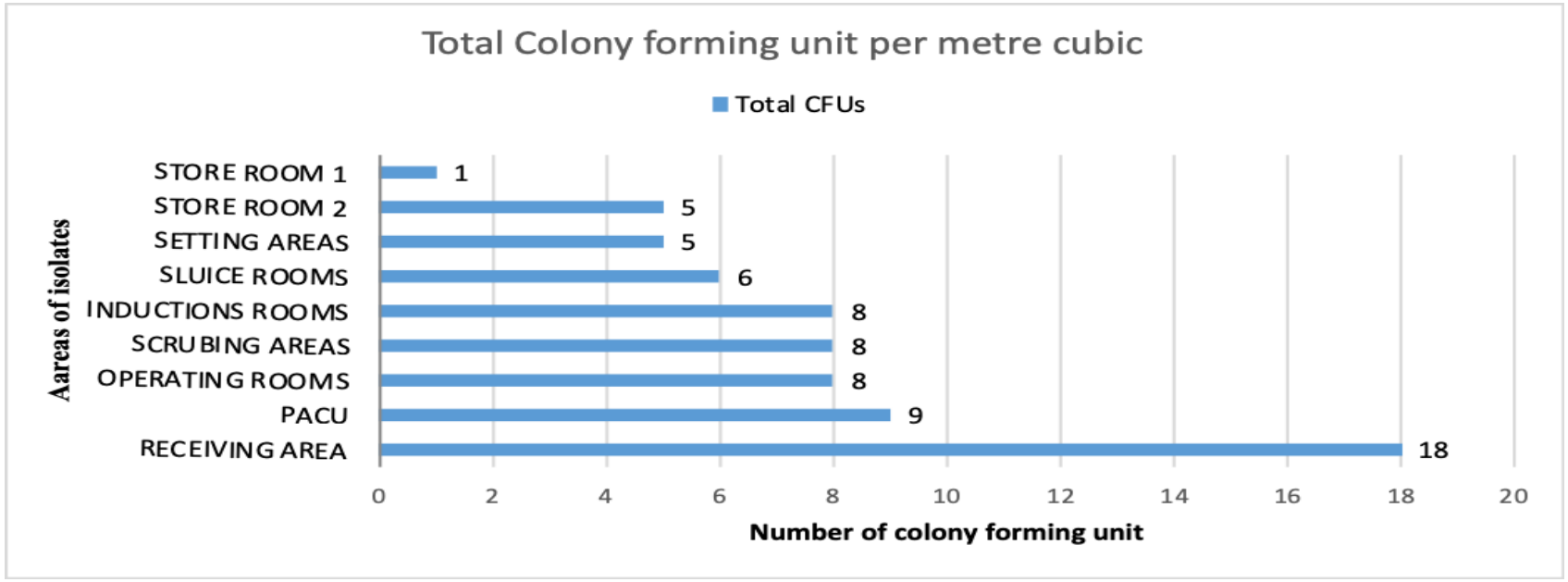

Figure 13: Fungal isolates

4.21 Types of fungi isolated during the entire three months period.

Aspergillus fumigatus (102) isolates, followed by *Aspergillus spp (47)* isolates, were found to be the most common organisms isolated over the entire period; *Penicillium* and *Acremonium* were also common.

Table 10: Types of fungi isolates

Microorganisms	**Sample 1 Growth**	**Sample 2 Growth**	**Sample 3 Growth**	**Sample 4 Growth**	**Sample 5 Growth**	**Sample 6 Growth**
Acremonium spp		3	2	2	2	1
Aspergillus fumigatus	15	19	20	23	15	10
Aspergillus Niger	1	3	0	2	0	2
Aspergillus spp	2	2	14	15	2	12
Aspergillus spp & candida spp	0	1	1	1	1	0
Aspergillus terreus	4	2	0	1	2	0
Candida spp and Aspergillus spp	0	0	1	1	1	1
Penicillin spp and Fusarium spp	0	1	1	1	6	0
Yeast	0	0	0	0	1	0

4.22: Major fungal isolate organisms in Agar Plates in KNH theatres

The most common fungi isolated in KNH theatres were Aspergillus species followed by *Aspergillus fumigatus* and the least being candida's (yeast). Mixed growths of Penicillium were also identified as common organisms.

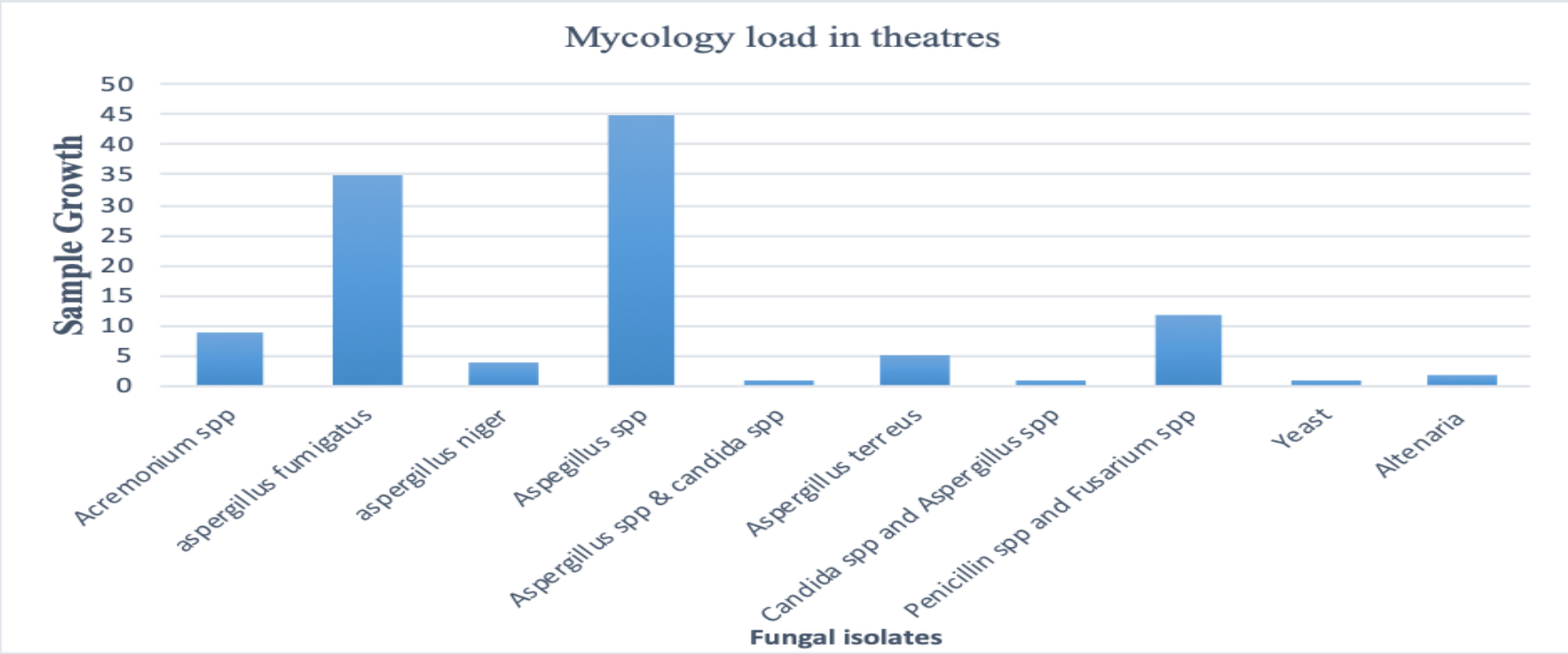

Figure 14: Major fungal isolate organisms in Agar Plates

4.23 Pattern of micro-organisms growth in different specialty theatres

Theatre 4 which is orthopaedic theatre followed by amenity which does all specialties combined had the most isolates of the fungal infection while theatre 7 grew organism in the entire period.

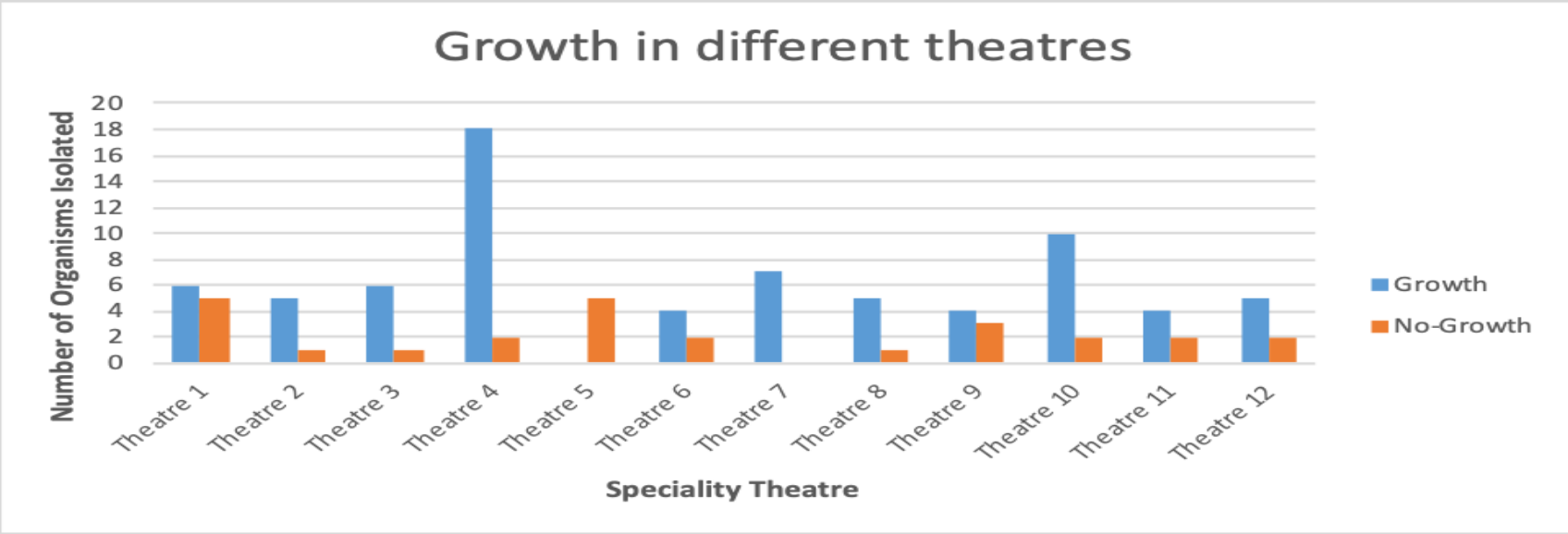

Figure 15: Pattern of micro-organisms growth

Key

Theatre 1-Emergecy Theatre	Theatre 8-ENT Theatre
Theatre 2- Reproductive Health Theatre	Theatre 9-Neurology Theatre
Theatre 3 and 6-Eurology theatres	Theatre 10-Amenity Theatre
Theatre 4 and 5-Orthopaedic Theatres	Theatre 11-Cardiothoracic Theatre
Theatre 7-Specialist Theatres	Theatre 12-Pediatric Theatre

4.24 Proportion of samples with over 5 cfu/m^3

A high proportion (7%) of samples with over 5 cfu/m^3 was reported in the third week while none (0%) was reported in the sixth week.

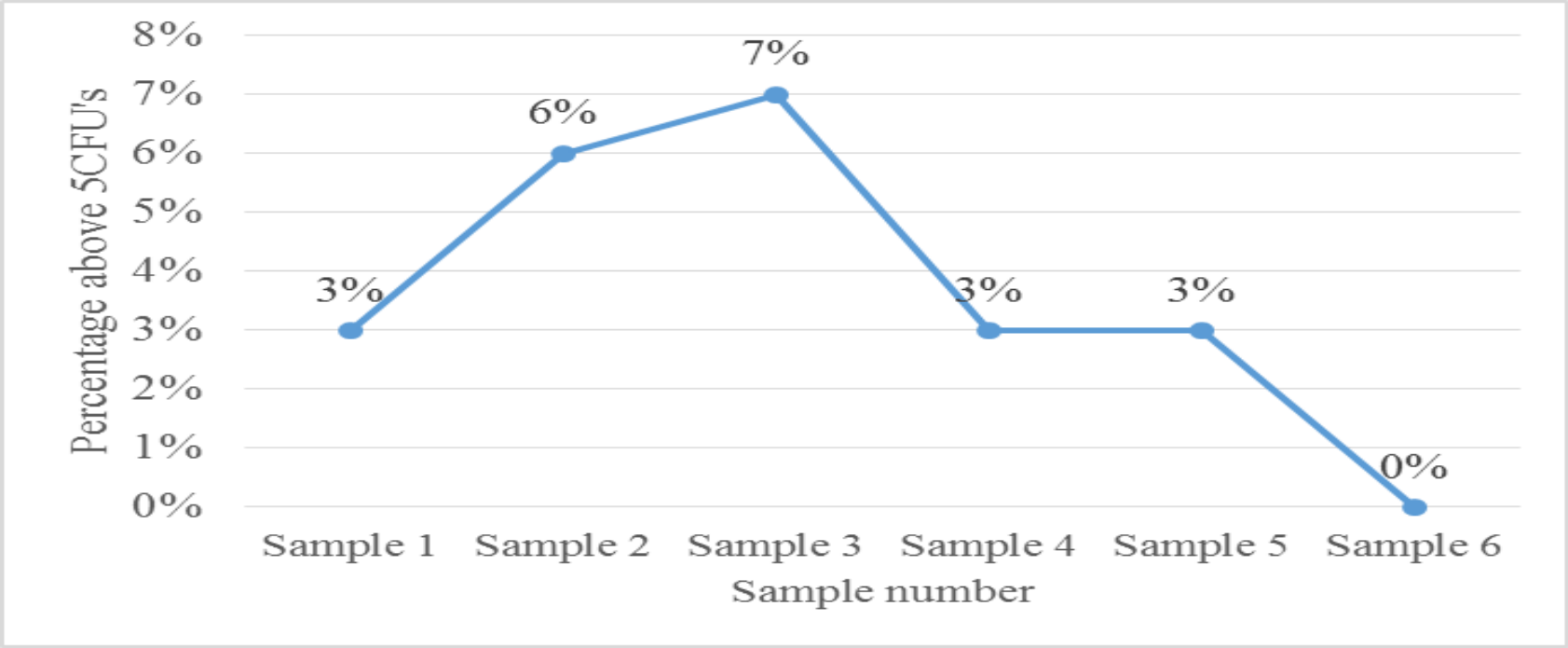

Figure 16: Proportion of samples with over 5 cfu/m^3

4.25 Average number of colony forming units in different areas.

Operating rooms had significant difference in the number of organisms identified over the period while sluice rooms had least difference in terms of organisms isolated at any particular period. P-value of 0.027 statistically significant because of outliers in PACU and receiving area which had no comparisons.

Sites of Fungal Isolates	Mean Isolates(N)	Mean cfu/m^3	Std. Deviation	p-value
PACU	1	9.00	.	
Setting Areas	6	5.33	3.266	
Sluice Rooms	6	6.17	1.472	
Operating Rooms	12	7.92	4.188	
Scrubbing Areas	12	7.67	2.309	0.027
Induction Rooms	12	7.58	3.315	
Receiving Area	1	18.00	.	
Store Room 1	1	1.00	.	
Store Room 2	1	5.00	.	

Table 11: Mean fungal cfu/m3

4.26 Areas with colony forming units and settle rate

Area	Colony Forming Units		Settle Rate	
	Up-to 5 cfu/m^3	Above 5 cfu/m^3	Up To 0.5	Above 0.5
PACU	2	6	8	0
Setting Areas	21	5	25	0
Sluice Rooms	24	13	36	0
Operating Rooms	47	7	52	0
Scrubbing Areas	37	14	43	2
Inductions Rooms	37	14	46	1
Receiving Area	10	3	13	0
Store Room 1	3	2	5	0
Store Room 2	4	2	6	0
Recovery Area	2	4	6	0
P-Value	0.009		0.740	

Table 12: Areas with colony forming units and settle rates

4.27 FUNGAL SWABS:

A total of 300 fungal swabs and 38 controls were taken over three month study period and only 4 *candida albicans* were isolated.

CHAPTER FIVE

DISCUSSION

5.0 DISCUSSION

A total of 51 pieces of equipment were swabbed in the KNH theatres over a three months study period. Sixty-one percent (61%) of the equipment showed microbial growth. The proportion of microbial growth in the operating rooms and PACU was the same in the first week, but there was variability in 2nd, 4th, 5th and 6th weeks. PACU showed high levels of microbial growth of up to 89%.

The high percentage of microbial growth of up to 68.6% were observed in the 1st, 4th, and 5th samples while sample 2 and sample 6 had the lowest microbial growth at 56.9% each. This can be explained by the fact that the theatre cleaning was supervised before the 6th sample was collected. Though fumigation had just been done before sample 3 was collected, its effect was only realized in PACU. Anaesthetic machines and suction machines grew microorganisms in almost 5 of the 6 samples collected, while receivers grew the least number of microorganisms in only one Sample. Theatre one (Emergency theatre) grew microorganisms on average 5 times over the six samples taken. Theatre 11(Cardiothoracic theatre) and theatre 8 (ENT) grew microorganisms 4 times while Urology theatre 3 and 6 had the least microbial growth. PACU especially the female section grew microorganisms in almost all the six Samples.

Study swabs isolate 10 different bacterial spp and 15 mixed growths. The most common swabbed bacterial isolates identified were *coagulase-negative staphylococci* constituting 44.5 % of isolates identified followed by *staphylococci aureus* at 22.8%. The least was *Granulicatella elegans*. These findings are closely related to the Ethiopian study by Gelaw et al. (2014) who studied hospital environment, patients and staff and isolated *coagulase-negative staphylococci* at 68.3% followed by *S. aureus* at 30.7%. Likewise, a higher number of *coagulase-negative staphylococcus* levels were also reported in a Nigerian study at 28.3% (Nwanko et al., 2009). In Nigeria where they had a lower level of *Staph. Aureus* (0.83%) and high levels of *Pseudomonas aeruginosa* at 23.3% (Nwanko et al., 2009). Our study reported the second most common microorganism being *Staphylococcus aureus* at 22.8% and lower levels of *Pseudomonas aeruginosa* at 4.1%.

A total of 439 agar plate samples were isolated, 348 tests were carried out with 91 study controls within the three months study period. Out of the study samples, the third sample had the highest number of agar plates (77, 17.8%) collected and the least was sample 1 and 2 which had only 70(15.9%) agar plates collected. From the 439 agar plate samples, 256 (58%) grew microorganisms.

The most common bacterial species in agar plates collected during the study period were *Staphylococcus epidermidis*, *coliforms*, and *Pseudomonas*. Urology, Neurology, Ear, Nose, and Throat theatres had the highest number of *Staphylococcus epidermidis*, with the lowest number of *coliforms*. Amenity, Orthopaedic, and Paediatric theatres had the highest number of *coliforms*.

The current study findings are contrary to a study done in Bagdad, where the coliforms were mainly found in the delivery theatres attributed to the normal flora in the gut that contaminated the theatres during delivery (Ensayef, et al. 2009).

The microbial contamination in agar plate samples collected by the current study exceeding 15cfu/m3 on average was found in less than 2 samples. Most colony forming units were less than 5cfu/m3 in all the areas studied and those between 6 and 15cfu/m3 were less than 30%. These ranges of colony forming units were within international acceptable limits.

Generally, there was no statistical difference (p-value=0.896) between different operating theatres in terms of air contaminants. Thus, the same interventions can be applied uniformly across different operating theatres. The mean settle rate was< 0.5 in all the areas of bacterial isolates, which was within the internationally acceptable limit of less than 15 cfu/m^3.

The current study results were way below that of the Bali's maxillofacial operating theatre results, who proposed that conventional operating theatres bio-load should not exceed 35 cfu/m^3 in an empty theatre. This explains that both the fumigation and disinfection methods in KNH theatre were effective during this period as colony forming units were below 15 cfu/m^3 of exposure (Bali et al. 2014). There were two unique occurrences of higher levels of bacterial isolates in this study: the Paediatric theatre had 20 colonies of coliforms with Settle rate of 0.67 particles/plate/min were isolated in the anaesthetic induction room; theatre 8 scrubbing area had mixed growths of 15 colonies of *Staphylococcus epidermidis* and 6 colonies of *coliforms* with settle rate of 0.7 particles/plate/min;

The most common fungi isolated in KNH theatres were *Aspergillus species* in general where 45(39.8%) isolates were identified, with *Aspergillus fumigatus* constituting 36(31.9%) with the least being Candida observed only once((<1%). Mixed growths of *Penicillium* were also identified in (9.7%). Receiving are had the highest number of fungal isolates (26.5%) identified in 4 samples collected, while sterile setting rooms had the least isolates. These study findings relate Poland's study, which found *Aspergillus species* at 35% of the isolates (Gniadek & Macura, 2011). High proportions (7%) of samples with over 5 colony forming units were reported in the third week while none (0%) was reported in the sixth Sample. The mean settle rate was< 0.5 and the colony forming units were less than 5cfu/m3 in all the study areas where data was collected and less than 30% between 6-15cfu/m3, which was within acceptable clean operating theatres.

Study limitations

1. Microorganisms within the operating theatre may differ over time depending on the contaminants. Therefore, what was captured over the 3 month period of the study may be limited in the representation of the entire period of theatre status.
2. The study only concentrated in the main theatres leaving out peripheral theatres which may have different microbial distribution.

Study delimitation

The study samples were collected early in the morning before morning operating procedures and at least twice in a month for the period of three months. Most specialty surgeries are conducted in the main theatre, though some of them could be done in the peripheral theatres.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1: CONCLUSION

Most bacterial and fungal isolates levels were below 10 cfu/m^3 and settle rate was less than 0.5, which were within internationally acceptable levels. This implies infectious control measures deployed by Kenyatta National Hospital are effective and need to be maintained. Moreover, the presence of *Staphylococci spp* and *Aspergillus spp* evidenced by this study calls for more effective cleaning and disinfection of equipment and floors to minimize postoperative sepsis.

6.2: RECOMMENDATION

The study findings aimed at establishing the functionality of the infection control measures of KNH. Following this, steps are expected to be undertaken to refine existing infection control measures by hospital policymakers and concurrently educate staff to the same effect. Moreover, the study was expected to stimulate further research on other causes of nosocomial infections in the operating theatres at KNH in Kenya, with the overall aim of advancing better healthcare services to the patients and the caregivers in the healthcare facilities.

Presence of potential pathogenic microbial isolates (*Coagulase Negative Staphylococci aureus, Staphylococcus epidermidis* and *Aspergillus spp*) in the operating theatres necessitates:

1. Effective cleaning and disinfection of equipment and floors to minimize postoperative sepsis.
2. Regular change of disinfectant i.e. Presept and Hexanios
3. Regular theatre contamination checks

4. Daily proper high-level dumb-dusting of theatre equipment
5. Close supervision of cleaning and fumigation of theatres.

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8.0 APPENDICES

8.1 Appendix 1

8.1.1 INFORMATION AND CONSENT FORM

Introduction & Purpose of Study

Hello, my name is Francis Mariach Kenimak from the University of Nairobi, Microbiology department. I am conducting operating theatre environmental bacterial and fungal isolates at Kenyatta national hospital (KNH). The aim is to determine the levels & pattern of bacterial and fungal microorganisms in KNH theatres. The study will include taking 200 swabs in specific areas in the theatres and taking 200 Agar plates per month to a total of 1200 samples in a period of 3 months to isolate the organisms which will be processed in the university of Nairobi and /KNH laboratories in the 3 month period. This will only be done if you consent for the study.

Procedures

If you accept me to carry out the study you will be asked to sign this form.

Risk

The study will not involve human subject hence minimal to no risk at all.

Benefits

The study will be a baseline research that endeavours to isolate major theatre micro-organism, some of which are the root cause of theatre contaminants. Consequently, steps will be undertaken to control theatre contaminants by educating staff, informing policy makers and possibly recommend areas of further studies.

Confidentiality

The samples taken from KNH main theatre shall be collected, transported to laboratory, processed and analysed in consideration to institutional policy & guidance on handling of specimens. No information shall be divulged to other un-authorised persons or organization. The results of the study shall be communicated to KNH and disseminated with their consent and information that we collect from this study will be kept private. The institution can ask any question regarding the study if you wish to for clarification.

Whom do I call if I have questions or problems?

If you need to contact the investigator on any matter relating to the study please call or contact. Francis Mariach Kenimak from the University of Nairobi, Microbiology department or KNH main theatre. Tel: 0725927751 or my supervisors Dr. Anne Maina Tel: 0727490540, Dr. Mureithi Marianne Tel: 0703704711.

For questions about your rights as a volunteer, contact Prof. M. I. Chindia, the secretary of the Ethics Committee at Kenyatta National Hospital, Tel: 020 – 2726300, extension 44102 or 44103.

Head of Department (HOD) Anaesthesia and Theatres KNH

Declaration

I have read the above information and had the opportunity to ask questions to my satisfaction. I voluntarily give consent the researcher to carry out the study at KNH theatres.

Name----------

Designation ----------

Signature ----------

Date----------

Investigators, Name----------

Signature ---------- **Date----------**

8.2 Appendix 2

8.2.1 Ethical Consideration

This study took place upon review and approval from Kenyatta National Hospital and University of Nairobi Ethics and Research Committee (approval number for this study from KNH-UoN ERC is P777/11/2016). Permission to perform the research was also obtained from the institution and theatre administration (KNH/theatres/05/2015), where full disclosure of the nature and the rationale for the study was explained to the concerned theatre users for their corporation.

8.3 Appendix 3

8.3.1 Questionnaire

1. Study number:
    a. Area of sample collection
    b. Theatre No:
       1 2 3 4 5 6 7 8 9 10 11 12
    c. PACU
    d. Recovery Area
2. Week:
    1 2 3 4 5 6
3. Specific site of sample collection
    a. Agar plate exposure within the room
    b. Scrubbing area
    c. Setting area
    d. Anaesthetic machine
    e. Basin
    f. Operating table
    g. Sluice Room
4. Specify the sample type:
    a. Swab b. Agar plate exposure

5. Results of culture:

   Bacteria: Yes/No

   Specify type……..

   Fungi: Yes/No

   Specify type……..

6. Bacterial load (Where applicable)……

8.4 Appendix 4

CONFERENCE PRESENTATIONS

This research has been presented in KNH/UON international conference on health in the month of November 2018 and in the Nurses National peri-operative conference in the month of November 2018 at Mombasa, the abstract is shown below;8.4.1 Abstract

1st Annual International Conference on Health and Nurses National Peri-operative Conference November 2018 held in Kenya.

AUTHORS: Mariach FK, Maina AM, Musingi J, Mburugu M, Musyoka F, Nkirigacha V, Mureithi MW.

BACKGROUND

Hospital operating theatre equipments and air may harbour bacteria, viruses, yeasts, moulds and fungal spores. Susceptible patients undergoing surgery in such environment may develop post-operative infections. Environmental surveillance to detect varying microbial levels for possible eradication is quite necessary.

OBJECTIVE

To identify major fungal and bacterial isolates as well as determine their patterns in different specialized operating theatres in Kenyatta National Hospital.

METHOD

This was a prospective cohort study done at Kenyatta National Hospital. A total of 1372 samples from 12 operating theatres were collected between December 2017 and February 2018. Surface samples were taken with sterile wet swabs from different equipments and exposure of agar plates in air. Settle plate method and colony forming unit per cubic metre was used to detect fungal and bacterial

patterns and levels in different specialities. Student t-test was conducted to examine any statistical significance for the varying bacterial and fungal isolate levels in different specialities against established normal bacterial and fungal levels in operating theatres.