OPPORTUNISTIC BACTERIA TYPES AND SENSITIVITY ON FREQUENTLY USED FOMITES IN THE UNIVERSITY OF EASTERN AFRICA BARATON IN NANDI COUNTY

A Thesis Proposal Submitted to the Department of Biological Sciences and Agriculture

School of Science and Technology

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In partial fulfillment of the Requirement of

Master of Science in Biological Sciences: Biomedical

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DECLARATION

DECLARATION BY THE CANDIDATE

This thesis is my original work and to the best of my knowledge, it has not been published and/or presented to any University for an award of a degree.

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Richard Ngaru Magondu Date

DECLARATION BY THE SUPERVISORS

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

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APPROVAL SHEET

This thesis entitled **Opportunistic Bacteria Types on Frequently Used Fomites in University of Eastern Africa Baraton in Nandi County**, is written and submitted by **Richard Ngaru Magondu**, in partial fulfillment of the requirements for the degree of Master of Science: Biology (Biomedical) is hereby accepted and approved.

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ABSTRACT

Outside and indoor settings, bacteria are found to be the ubiquitous microorganisms causing microbial contamination. Bacteria infect, transmits bacterial infections while they are in direct contact with vulnerable people. Fomites can act as environmental reservoirs to increase the ability of pathogens to be transferred from host to host. An inanimate object, which can transmit an infectious agent, is known as a fomite. The main objective of this study was to identify opportunistic bacteria types on frequently used fomites in University of Eastern Africa Baraton in Nandi County. The study area which was University of Eastern Africa Baraton was purposively selected as it is the post-secondary institution of higher learning, in the region. The research design that was employed was experimental design where the bacteria were isolated, characterized and antimicrobial activity the antibacterial sensible nature of the samples were performed through the disc diffusion method. Bacteria was isolated from various fomites, Stratified sampling technique was used to divide the population of fomites in subgroups (or strata) within the University and, due to the big number of the sampling sites. The researcher isolated, characterized and determined the antibacterial sensitivity of fomites bacteria. 365 swabs were obtained in different facilities inside the University by swabbing of the toilet cistern handles, office doors faucets and shopping baskets (sterile swabs moistened with buffered peptone water). They were then correctly labelled with reference numbers and transported in peptone water transport medium to the Biology Laboratory. This was done in the second semester of the academic year 2020/2021. The data was analyzed using SPSS Version 23. Descriptive statistics was used to analyze the prevalence of bacteria types isolated from fomites within the University buildings. All values were expressed as means and findings were presented in the form of frequency tables. The study found out that 90.2% of the samples were bacteria with 9.8% being fungi. Doors had the greatest number of gram-positive cocci (78.9%) followed by faucets and cisterns. Doors had the greatest number of gram-negative cocci (17.6%) bacteria followed by faucets and cisterns. Doors had the greatest number of gram-negative rod (3.5%) bacteria as compared to doors. Doors had gram positive rod bacteria. Faucets did not have gram negative and gram-positive rods. The gram-positive bacterial isolates were *Streptococcus pyogenes*, *Streptococcus epidermidis* and *Streptococcus aureus*. The gram-negative bacterial isolates were *Escheichia coli* and *Morexella catarrhalis*. 66.8% of the bacteria were resistant to Penicillin, Cotrimoxazole, Ampicillin, Erythromycin, Methicillin, Lincomycin, Minocycline and Chloramphenicol.

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DEDICATION

I would like to dedicate this thesis to my late father, Francis Magondu Ngaru whose wish and dedication was to see me through the highest level of education possible. I also dedicate this Thesis to my wife, Jane Wangu Ngaru and my two sons, Erick Francis Macharia Ngaru and Patrick Murimi Ngaru for their moral support,encouragementg and prayers.

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LIST OF ABBREVIATIONS

HVAC- Heating Ventilation air Conditioning

FCV – Feline calicivirus

ARGs- Antibiotic Resistant Genes

DNA- Deoxyribonucleic Acid

NoV- Norovirus

MRDO Multiple Drug Resistant Organisms

SNP Single Nucleotide Polymorphisms

VRE Vancomycin Resistant Enterococcus

MRSP Methicillin Resistant Staphylococcus Pseudo-intermedius

MRSI Methicillin Resistant staphylococcus Intermidus

CHAPTER ONE

INTRODUCTION

Background of the Study

Bacteria found to be the ubiquitous microorganisms causing microbial contamination (Knecht, McGinniss, Shankar, Clarke, Kelly, Imai & Collman, 2019). Bacteria infect, transmits bacterial infections while they are in direct contact with vulnerable people (Pessi et al., 2002). Water, food and fomites can act as environmental reservoirs to increase the ability of pathogens to be transferred from host to host (Knecht et al., 2019). An inanimate object, which can transmit an infectious agent, is known as a fomite (CDC, 2012). Fomites include surfaces such as doors, toilets, chair handles, laboratory bench, railings etc. In heavily visited places such as schools, hospitals, market places and malls and any other place where human traffic is heavy contamination of inanimate objects is usually very high (CDC, 2012). Recent epidemiology studies have documented that fomite are responsible for high exposure in bacterial transmission, in hospitals, children's health centers, long-term care centers, and educational institutions and sports facilities (Bloomfield, 2017). Different types of microorganisms, including rotaviruses, rhinoviruses, Staphylococcus aureus methicillin-resistant, and Serratia marcescens were identified to cause gastrointestinal disease, the common cold, necrotic fasciitis and the related bacteremia of catheters respectively (Bures et al., 2010).

Antimicrobial resistance is an important public health concern around the world. According to field research and health records, antimicrobial-resistant bacteria are prevalent in numerous parts of Kenya. Even so, due to a lack of dependable surveillance data, the pressure of

antimicrobial resistance has still not been ascertained. According to a latest report, the nation has over 200 antibiotic-resistant genes, with a substantial proportion these being mobile genetic components that are communicable between different bacteria. There is a lack of knowledge about the factors that influence the incidence, maintenance, and transmission of antimicrobial resistance, but it is presumed that antimicrobial misappropriation, a high prevalence of communicable diseases, and a lack of access to proper healthcare are among the key drivers (Omwenga, Aboge, Mitema, Obiero, Ngaywa, Ngwili & Bett, 2021).

Institutions of higher learning being in the category of schools have not been considered much when it comes to considering the vulnerable groups. In heavily visited places such as schools, hospitals, market places and malls and any other place where human traffic is heavy, the rate of contamination of inanimate objects is usually very high (Otter, Yezli & French, 2014). Infections can be indirectly acquired by contact between surfaces and the mouth through contaminated fingers to mouth or hand-to-mouth, hand-to-eye, or hand-to-nose contact or can be transmitted directly from contaminated devices or surface to humans or, less frequently, by aerosols, water, and/or foodstuff (Otter et al., 2014). Fluids like saliva, mucus, nasal secretions, blood, urine, and feces all can likely contain fomite pathogens (Otter et al., 2014).

Most fomite transmitted infections arise from products that are supposed to be sterile but are infected with pathogens (Barrie et al., 2014). The outbreak of population (community) acquired infections and nosocomial infections have been proven to be emanating from surface bio-contamination of fomites while in constant contact with human or natural environments of pathogenic organisms according to studies (Nwankiti et al., 2012). Hidden microorganisms in indoor and outdoor sites are unavoidable and pose harmful health hazards in our different human activities. In recent years, apprehension has increased with the implementation of new

technology in households, hospitals, industry and other settings (Eickhoff, 2014). There has been increased interest in assessing the risk of microbial types and pollution and is considered an important step towards infection prevention (Eickhoff, 2014).

In various indoor/outdoor settings, microbial contaminations are commonly documented. The bacterial contaminations of 50 public telephones in the City of Afyon, Turkey, were investigated by Tunc and Olgun (2016). Twelve different types of bacteria were present on the telephone surface, including *Escherichia (E) coli*, *Pseudomonas (P.) aeruginosa* and *Staphylococcus (S.) aureus*. Similar findings for hospital phones and personal pagers have also been recorded (Namias et al., 2010). Rutala et al. (2016) studied the scope, performance and cosmetic impacts of the disinfectant on the computer keyboards' levels of microbial contamination. Results showed that microbial on keyboard contamination were ubiquitous and disinfectant could clean up the contamination that was isolated and identified. Narmeen, Melo and Melo (2019) reported *S aureus* pathogen in multiple locations in the Azadi General Hospital with bacteriological contamination as well as molecular markers. *S. aureus* may normally cause infections in newborns, surgical, burns, diabetics, and those taking drugs to avoid immune deficiency disorders. Harrison et al. (2013) also reported that *Micrococcus luteus* and *Serratia marcescens* both have a distinctive colonial morphology on plate counts used. Results showed that bacteria zig-zag transfer between the distributors and hands can occur if either of these is contaminated. The possibility of cross-contamination of the hands, towels, and dispenser if any of these is infected has to be tackled (Harrison et al., 2013).

There have been several factors that influence the bacterial transfer rates from the surface to another surface. These involve the form of bacteria, source and target area, post-inoculation time and humidity level (Rusin, Maxwell & Gerba, 2012). The key factor influencing the

transmission rate of opportunistic bacteria is the determination of the bacterial groups. Against this context, this study aimed at researching the types of opportunistic bacteria in a selected institution of higher education in the Nandi County. These fomites included office door handles, toilet door handles, toilet water faucets, cistern handles and shopping baskets. Awareness of opportunity bacteria in various locations and particles can help to choose the necessary hygiene steps in order to remove possible cross contamination by the bacteria.

Statement of the Problem

The notion that environmental microorganisms contribute to human disease comes from our contact with the inanimate environment and the difficulty in establishing the type of organisms that cause human disease in the environment (Rhame, 2012). Every year, 1.7 million deaths from diarrhea and 33,000 deaths from antibiotic resistant bacteria infections occur worldwide (Pruss-Ustun & Covalan, 2016). Bacteria cause an estimated 60% of human infections, and enteric bacteria develop the most common diseases (McElhaney, 2013). Furthermore, population growth and increased mobility have increased bacterial transmission and the challenge to interrupting the spread of diseases (Butcher & Ulaeto, 2015). For decades, bacterial diseases have been thought to be mainly transmitted by direct contact and the environment played little or no part in the transmission of diseases (Cozad & Jones, 2013).

In institutions of higher learning as University of Eastern Africa Baraton, most people spend their time indoors. The most important fomites for contamination and transmission tend to be those found indoors and humans frequently come into direct contact with, such as doorknobs, countertops, medical equipment, handrails, clothing, and mobile phones (Cozad & Jones, 2013). As our understanding of microbes in the built environment has greatly expanded in the last

decade, so has our understanding of fomites and their role in the transmission of infectious agents and other microbial matter to and from humans (Springthorpe & Sattar, 2010). Therefore, the fundamental question is what types and sensitivity of opportunistic bacteria that are present on fomites found in the selected post-secondary institution of higher learning. As a result, this study examined the types of opportunistic bacteria by isolating and characterizing them with the aim of determining their antibacterial sensitivity to various antibacterial preparations.

Objectives of the Study

Broad Objective

To determine the opportunistic bacteria types on frequently used fomites in University of Eastern Africa Baraton in Nandi County, Kenya.

Specific Objectives

i. To isolate the bacteria, present on frequently used fomites in University of Eastern Africa Baraton in Nandi County, Kenya.

ii. To characterize the bacteria isolated on frequently used fomites in University of Eastern Africa Baraton in Nandi County, Kenya.

iii. To determine the antibacterial sensitivity of the isolated bacteria pathogens to various antibacterial preparations (drugs).

Significance of the Study

In modeling potential for transmission of bacterial pathogens into the fomite side, the efficiency of transfer is significant (Nicas & Best, 2018). This knowledge can be used to understand disease transmission indoors and the possible design of surfaces that decrease transfer efficiency and/or antimicrobial effects (Nicas & Sun, 2016). The results of this study are expected to be important to provide insight in different in ways of management of bacterial infections and how the problems of bacterial fomites can be effectively treated. Policymakers are aware of how well they can easily include the outcomes of this study in the health sector so that it is completely enforced. The results of this study also will contribute to the literature and provide a basis for citations in other studies in Microbiology and related health sciences.

Justification of the Study

In crowded indoor buildings, including schools, corporate offices and hospitals, the rapid spread of bacterial diseases constantly encourages disease mortality (Seki, et al, 2013). In Maki's analysis of the relationship between an organism and nosocomial infections. Maki virtually removed the environment as the key vector for nosocomial infections. However, the limits of the analysis were that two pathogens, aspergillus and legionella were not evaluated for which environmental interaction was paramount, nor was anaerobic environmental culture analysed eg. *Clostridium deficile*, *klebsiella* (Turner, Craddock & Klebsiella, 2013). Pseudomonas (Goldmann, 2010) and other Gram-negative organisms from hospital areas may be recovered (Bauer, Ofner, Just, Just & Daschner, 2010).

While bacterial pathogens on fomites have always been recognized as a potential risk factor in major infections, no research is carried out in this locality to confirm their existence. However, there is a lack of basic knowledge on the role of fomites in the transmission of bacterial diseases and further research is needed on the type of bacteria found on fomites. There is very little data on opportunistic bacteria types and their antibacterial sensitivity in Nandi County, so it is necessary to have classified basic line information in that area in Nandi County, particularly in University of Eastern Africa Baraton. University of Eastern Africa Baraton was chosen because it was convenient for the researcher.

Scope of the study

The area of study is Nandi County at University of Eastern Africa Baraton, which was selected purposively. The fomites, which were included in this study, were purposively selected based on their direct interaction with human beings. The study focused on isolating, characterizing and determining the antibacterial sensitivity of fomites bacteria. The design of the study was experimental.

Limitations of the Study

Despite the study being carried out successfully, it had several limitations. First, insufficient funds to carryout additional test on sensitivity and typing of the bacteria. Secondly, the time was limited due to the work load hence workload extended to late nights. Lastly, investigation of fomites is still new and therefore it was not easy to get sufficient literature. Additionally, insufficient funds limited the study to a small sample size and inability to establish the bacteria which were multidrug resistant.

Definition of Terms

Bacteria	These are single celled microorganisms. As there are no nucleus or membrane organelles, the cell structures are simpler than those of other species. Their genetic information control center is located in a single DNA.
Opportunistic Bacteria	Opportunistic bacteria are a group that create infections in hospitals, immune-compromised and patients who are presenting underlying diseases like cystic fibrosis, which favors infection and are typically not infecting healthy hosts loop.
Fomites	Fomites are inanimate objects that can become contagious and act as a transmission mechanism between various hosts.
Institutions of Higher Learning	Institution of higher learning is a college or university.
Sensitivity	Sensitivity is the inability of tolerating the adverse influence of a drug mostly at a therapeutic dose.
Resistance	This refers to when a bacteria develop the ability of defeating the designed drugs to kill them.

CHAPTER TWO

REVIEW OF RELATED LITERATURE AND STUDIES

Types of Microorganisms found in Fomites

We exist in a world of microbes. In all the habitats we live there are viruses, bacteria, protists, fungi and archaea (Kelley & Gilbert, 2013). We directly transport microbes into building areas (Adams, Bhangar, Pasut, Arens, Taylor & Lindow, 2015) from outside (Adams, Miletto & Taylor, 2013) into the indoor air and from our surroundings (Adams, Miretto & Taylor, 2013) (Lax et al., 2017). The abundance and diversity of microbial in buildings or what is known as the indoor microbiome are affected by human activities, the environment outside, architecture and management (Adams, Bateman, Bik & Meadow, 2015). Many molecular analyses display a considerable variety of microbes on constructed surfaces. Most indoor microbes tend to be sleeping, inactive or dead (Gibbons, 2016), either have no known effect on human health or are likely to support human health (Lynch et al., 2014).

Microorganisms on Surfaces

Microbial Community Ecology on Fomite Surfaces

Inanimate artifacts may be used as microbial reservoirs in the built environment. These objects contain a large array of bacterial, viral, archaeal, protists and fungal species including possible pathogens and human-hazardous microbial metabolic products. Many micro-organisms originating from other environments are usually considered impossible to live on indoor surfaces

that lack abundant moisture and nutrients. These viable microbes that survive are usually considered to be inactive or dormant until moisture and nutrients help it proliferate or are moved to different places in the host (Gibbons et al., 2015). Surveys carried out with high throughput molecular sequences of fungal populations in indoor environments have shown that they are mainly powered by transportation from the local outside environment (Adams, Miletto, Taylor & Bruns, 2013).

Similar studies of buildings and surfaces with a higher human occupancy as well as frequency of encounters have nevertheless reported elevated levels of skin related bacteria (Adams, Bateman, Bik & Meadow, 2015). The efforts made to trace the sources of the bacteria that lie on different indoor surfaces have also been provided. Urine and feces bacteria were more popular on toilet seats and lavatory handles than on other surfaces (Flores, 2011). Fresh produce bacteria have been shown to be more prevalent in kitchen counters and refrigerators (Flores, 2011). In the interior and exterior door trims of doors which open outside domestic surfaces locations are more frequently associated with bacteria associated with leafs and soil (Dunn, Fierer, Henley, Leff & Menninger, 2013). In comparison, rich microbial biofilms in baths and kitchens may form communities closely similar to those found in plumbing and water reservoirs on surfaces which frequently have high humidity levels. (Kelley, Theisen, Angenent, St. Amand & Pace, 2014).

Lax et al. (2014) showed evidently that on some surfaces, but not on others bacterial communities on different surfaces in an individual home have clear similarities (Lax et al., 2014). Moreover, as families moved into houses, the bacterial composition of the new bacterial population converged on the surfaces of the new house quickly into that of surface bacteria,

which indicates that new inhabitants rapidly deposited in the new space their own special signatures of related human bacteria.

While in recent years a great deal has been revealed on microbial communities in indoors, bacterial communities and fomites' kind of bacteria are much less known (Prussin, Garcia & Marr, 2015). However, a great deal needs to be known about the types of fomite bacteria that raise concerns about transmission of infectious diseases and other new microbial threats.

Bacterial Hazards on Fomite Surfaces

Opportunistic Bacteria

On fomite surfaces, opportunistic and antibiotic resistant bacteria that are also not mutually exclusive, bacterial dangers have been found. Chance infections occur when host defenses are affected or infection has been treated. A certain understanding of the underlying defects and of normal mechanisms defending against infection with specific microorganisms can be used to predict the pattern of infection in a compromised host.

Marks et al. (2014) found Streptococcus pyogenes and Streptococcus pneumoniae in daycare samples and then in laboratory research checked that isolates from the two species remained viable and infectious for a long period in a mouse model while present as a biofilm (Marks, et al, 2014). These results indicate that environmental fomite transmission may be a significant route when oropharyngeal secretions of the fomites contain streptococci biofilm as contaminants.

In the laminate, glass and stainless-steel surface areas Jones and Lutz (2014) estimated average survival time at 3.75, 5.75 and 6.75 h respectively. Pseudomonas aeruginosis (Marks, et al, 2014). The non-Tuberculous Mycobacterium abscess, in the presence of mineral particles, kaolin, halloysite, silicone dioxide and house dust, was evaluated by Malcolm, Caceres, Honda, Davidson, Epperson and Strong (2017). Mycobacterium abscess interacted with the particulates, with improved house dust survival rates and desiccation lasting for 2 weeks (Malcolm et al., 2017). These studies show that opportunistic bacteria can live on fomites for long periods of time in the built environment.

Antibiotic-Resistant Bacteria

More thoroughly than opportunistic bacteria, antibiotic resistant bacteria have been explored. An overview of the major risks to antibiotics in the United States was released in 2013 by the United States Center for Disease Control and Prevention (CDC) (CDC, 2013). Davis et al. (2012) have reviewed published work on the transmission of *Staphylococcus aureus* and other *staphylococci* to households and have indicated that domestic microbial populations may have a role in antimicrobial resistance genes transfer and may be human repositories. (Davis et al., 2012). In two Portugal cities, it was found that the Public Transit environment is also critical as the hand-railing (Conceição, Diamantino, Coelho, de Lencastre & Aires-de-Sousa, 2013) of public buses (Simões et al., 2013) and bus riders' hands tested positive to contamination with Methicillin Resistant Staphylococcus aureus, (MRSA).

In the health environment there can be pollution and contamination by various fomites (from mobile phones – Bhoonderowa, et al, 2014), medical devices (Kanamori, et al, 2017) through surgery tapes (Harris, et al, 2011) and physicians’ bags. The most common issues are:

transmission of antibiotic-resistant bacteria in health environments (Feldmanet al, 2012). While several of these fomites were believed to be essential sources, closer study also shows a more complex understanding. Julian et al (2011) Samples were taken for MRSA as well as Staphylococcus pseudintermedius methicillin resistant surfaces of cellular phones brought by staff in a veterinary hospital (MRSP). Only 2 of 123 telephones were insulated and only 1 of 123 was insulated from MSRP (Julian et al., 2011). Similarly, Missri, et al (2018) sampled the healthcare worker's bacterial colonization in hospitals sampled directly before and 5 minutes after sanitization with bactericidal wipes (Missri et al., 2018).

The bacterial colonization was higher on both telephones and health workers than administrative personnel. On about one-third of the phones, however, opportunistic pathogens were identified and only one phone with MRSA was colonized. Smibert et al. (2018) swabbed the medical staff of 94 previously cultured ICU patients, including 11 MRSA, 2 VRE, and 81 Gram negative bacteria, for their personal cell phones, department phones and ICU keyboards, as well as cultivated multi-drug resistance (MRDOs) (Missri et al., 2018). The isolates on cell phones had different single nucleotide polymorphism (SNP's) in the isolation of the whole genome of mobile phone isolates, compared with the isolates that show that these fomites would probably not lead to hospital acquired MRDOs. Based on the omnipresent presence of bacteria in the built environment, experiments with bacterial colonization alone appear to be less useful than those with particular pathogens and other microbial dangers to provide mechanistic or medical insights.

Several methods have been investigated for control of antibiotic-resistant bacteria and other microbial hazards on fomite surfaces, including UV light, cleaning agents, material coating, and other methods. For instance, the doses of UV light needed to inactivate Methicillin

Resistant Staphylococcus Pseudo-intermidius (MRSP), Vancomycin Resistant Enterococcus (VRE), stainless steel, laminate Formica fomite surfaces noroviruses and murine and were quantified by Mitchell et al. (2019). The ability to destroy bacterial and fungal disease pathogens on the surface of disposable medical handle gloves characterized the ability of novel chlorhexidine and gentian violet antiseptics coating to kill bacteria and fungal pathogens Reitzel, Rosenblatt, Jiang, Hachem and Raad, 2014), and found that the coating eradicated MRSA, VRE and multi-resistant Pseudomonas aeruginosa, among other things. Reitzel, Rosenblatt. Aeruginosa (Reitzel et al., 2014).

Almost all research indicates that consideration should be exercised in their use, considering the efficacy of antimicrobial cleaners. It was also found that Antibiotic Resistance Genes (ARG) abundance was positively correlating with antimicrobial chemical levels of the same dust samples and that Hartmann et al. (2016) identified ARGs (Hartmann, et al 2016). Likewise, the microbial communities and their surface resistance to clinical environments with the application of the metagenomic genome and plasmid reconstruction were contrasted and the microbioma of highly maintained buildings had an alternative resilience compared with the other buildings, and a greater diversity of their resistant genes, (Mahnert et al., 2019). As ARGs are also natural components of environments rich in bacteria (e.g., land), its function as forming bacteria in interior environment is not yet well known, the best application of these findings is still an active area of research.

An alternative to conventional cleaning methods is possible in one exciting research field that has developed over past years. Unlike antimicrobials which destroy microbes, probiotic cleaners containing Bacillus species are considered to be mainly biologically competing in order to prevent pathogenic bacteria from surviving and proliferation (Falagas & Makris, 2019). There

have been numerous studies showing that their use of lower pathogenic surfaces has decreased by an average of 90 percent above traditional chemical cleaners, varying between 70% and 99 percent Caselliet al (2016). Furthermore, in hospitals making use of probiotic cleaners, the sum of antibiotic resistance genes on treated surfaces was cut to 99 percent by Caselli et al (Caselli, et al 2019). Secure sterilization or eradication on fomite surfaces of antibiotic-resistant bacteria remains an active study area. The above studies prove that not only opportunistic bacteria deposits and fomites are available for hours or even days in the built environment, rely on fomite content, type of microorganism and indoor environmental properties.

Movement of Microorganisms between Fomites and Humans

Three main methods were used to provide insight into the significance of microbial hazards and their effects on human health and other possible modes of transmission. Experimental measurements of microbial movement from/to fomites and humans. Mathematical modeling and the resulting health risk in relation to other exposure mechanisms for a microbial exchange between fomites and people. The significance of various transmission methods to cause the disease is demonstrated by epidemiological studies. Many experiments have characterized the transmission of microbial from and to fomites and humans, including those based on surface and room dynamics (Stephens, Azimi, Thoemmes, Heidarinejad, Allen & Gilbert, 2019).

The Surface-Scale

The transmission of human norovirus (No V) between fingers and fomites as well as between fingers and food products was measured in Tuladhar, et al (2013) in one surface level

transmission dynamics study. They poisoned human finger pads and squeezed them on laminate surfaces, stainless steel surfaces, whole tomatoes, and slices of cucumber. They even contaminated the surfaces and pressed clean human finger pads against them (Tuladhar et al., 2013). The first pressing of finger pads averaged the efficiency of transfer by about 13% and was decreased over time and after drying. The efficiency of transfer from surfaces to finger pads of viable infectious viruses was on average between 2 and 4%, even after 40 minutes of drying the surfaces that were contaminated. Several other complex surface research focused on the efficacy transfer of various organism types to the various types of fomites common in health care, such as medical gloves. MRSA was tested by Moore, Dunnill and Wilson (2013) between different types of gloves worn by HCW and fomite surfaces and found that bacterial transfer ranged from ~0 to ~20 per cent depending on the material of the glove and material hydrophobicity while bacterial transfer increased as well as increased uniform transfer between different glove types (Moore et al., 2013). Greene, et al (2015). Estimated the efficiency of transfer performance of Acinetobacter baumannii from a finger pad and a fomite into a finger pad to a finger pad with and without the use of latex gloves (Greene et al., 2015).

Without handkerchiefs, transfer efficiency from fomite to finger pad was about 24 percent and transfer efficiency from finger pad to fomite was about 6 percent. These two transfer efficiencies were reduced by about half by latex gloves and the material form is not a significant determinant. Koenig, et al (2016). Koenig, Korir-Morrison and Hoffman, (2016) measured *Staphylococcus aureus'* transfer efficiency between nitrile gloves and non-porous fomites through hand shaken, touched the cell phone back and touched the stainless-steel rod with another man with his or her hands (Koenig et al., 2016). The steel pole, followed by cellular phone, had the highest transfer efficiency. Glove-to-glove transfer was performed, but of the

three scenarios examined the lowest transfer performance. The fomite-to-finger microbial transmission of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus thuringiensis* spores or poliovirus 1 is assessed in the form of fomite-to-finger microbial transfers of *Escherichia coli, staphylococcus aureus,* and poliovirus 1 seeded on ceramic tile, laminate and granite following the treatment with disinfectant wipes (Lopez et al., 2014). These studies and others provide valuable quantitative information for the physical microbial transmission into and from fomites and humans.

The Room-Scale

Room-level dynamics experiments have employed many experimental methods to demonstrate to people the importance of fomite transmission. Rhinovirus infection in the atmosphere was examined by Winther, et al (2017) in a hotel for 15 adults with normal rhinovirus colds and in which a mixture of natural and scripted activities is performed. (Winther et al., 2007). Of the 150 environmental sites in the rooms sampled 35%, rhinovirus infected. Furthermore, 60% of the samples taken 1 hour after screening to actively move the Virus from person to surface were successfully transferred from the surface to the fingertips and 33 percent were taken 18 hours after screens. Quantified viral loads recovered from the nose of people infected with influenza A(H1N1) pdm09 and compared those quantities in populations and hospital environments with those recovered from their immediate area, (Killingley et al., 2016). The average length of virus shedding was approximately 6 days by PCR and approximately 4 days by culture (viability detection) (Killingley et al., 2016).

However, room air in the vicinity of a subset of subjects were also sampled and the PCR for influenza was positive in around 40% of samples. This suggests that the importance of aerosol influenza transmission is possibly more significant than indirect transmission through fomite. Only ~ 5% of swabs have PCR for influenza and only 0.3% have been viable viruses. Suwantarat, et al 2017, combine microbial sampling with hospitalization observations and state that regular patients communicate directly or indirectly with medical equipment and other fomites shared by patients, frequently polluted with pathogens associated with healthcare (Suwantarat et al., 2017). Medication carts, wheelchairs, food bins and cleaning carts were the surfaces that patients interacted most often, resulting in interactions between ~0.2 and ~0.4 per hour (Suwantarat et al., 2017).

Microbial tracers were also used with some success for the investigation of fomite transmission in the space. In the unocupated unit operating with four separate particulate filters mounted in the recirculating central forced air heating, ventilation and air conditioning (HVAC) system, Kunkelet al, (2017) used a human respiratory activity simulator to aerosolize the two models Escherichia coli K12 and bacteriophage T4 (Kunkel et al., 2017). Multiple locations have been carried out with size-resolved aerosol sampling and plate settles swabbing and DNA extraction and qPCR samples were analyzed. In all the air specimens up to 7 m away from the biological aerosol source, DNA from both species was observed at all test conditions, and concentrations decreased more widely. A larger fraction of T4 DNA from the aerosol fractions of less than 1 μm has been recovered than E. Coli K12, which shows at all air sampling sites that smaller virus-like species can carry longer distances than large bacterial organisms. In addition, the improved efficiency of particulate filters in the HVAC system reduced the amount of recovered DNA in air samples and settled plates 3–7 m from the source (Kunkel et al., 2017).

In another microbial tracer analysis, the toilet flushing was achieved using the coliphage MS2 applied to the bowel by Sassi, et al (2018) for surface contamination (Sassi et al., 2018). In all experiments where no disinfectant was applied to bowl water prior to the flushing, the injection of the disinfectant into the toilet bowl was contaminated by the toilet bowl before the flushing decreased the fomite concentration after the flushing. Similarly, Booth and Frost (2019) used a vomit simulator to investigate the dissemination and survival, by means of norovirus, of Feline Calicivirus, which demonstrates that almost all samples taken from the ground up to 3 meters from the source have recovered viable virus from virus viable air samples (Booth & Frost, 2019).

During a new tracer experiment, Reynolds, et al (2019) measured microbial transmission in an ambulatory and the effect on a viral tracer (bacteriophage MS2) in an ethanol-based disinfectant, putting two fomites at dawn: the door handles in the patient's room and the front desk in the pen (Reynolds et al., 2019). Patients and workers sampled Fomites and the hands after 2, 3.5 and 6 hours. High-touch surfaces were washed four hours after seeding for the disinfectant interference tests and sampled 2 hours later. On all surfaces and hands, the viral tracer was found in all three-time scans with door handles and the arms in the nursing station yielding the highest amounts. MS2 levels after inoculation were higher 2 hours, and concentration on viruses declined by approximately 94 percent after spraying. If it is believed that the efficiency of MS2 microbial transfer is comparable to those of other species that are important to health (Pitol et al 2018) then the possibilities for fomite transmission in the built environment can be found in tracer studies like this. In addition, both surface and room experiments indicate very clearly that fomites play an important role in transmitting microbes to humans (Pitol et al 2018). The present study was carried out at University of Eastern Africa

Baraton to investigate the opportunistic bacteria types and their antibacterial sensitivity to bridge both the geographical and methodological this gap reviewed in this chapter.

CHAPTER THREE

RESEARCH METHODOLOGY

Research Design

The research design that was employed was experimental design in which, due to simplicity, effectiveness and expense, the antibacterial sensible nature of the samples was performed through the disc diffusion process. Disc diffusion is perhaps the most commonly used process in private clinics for evaluating antimicrobial resistance. Bacteria was also isolated and characterized. The design was also effective in isolating and characterizing the opportunistic pathogens from fomites.

Study Area

The analysis was carried out at the University of Eastern Africa Baraton in Nandi County. The county is in North Rift, Kenya. It is bordered by the County of Kakamega to the west, and the County of Uasin Gishu to the northeast. Baraton is located 47 Km from Eldoret town. Baraton is at altitude. 0.2552° or 0° 15' 19" North; Longitude. 35.0827° or 35° 4' 58" East. Baraton has a Maximum minimum temperature:22°/11° and Precipitation 9.9 mm. The population of Nandi County is 885,711 people. The chief economic activity in Baraton is tea farming and cattle rearing. Baraton also has athletics as the main sporting activity.

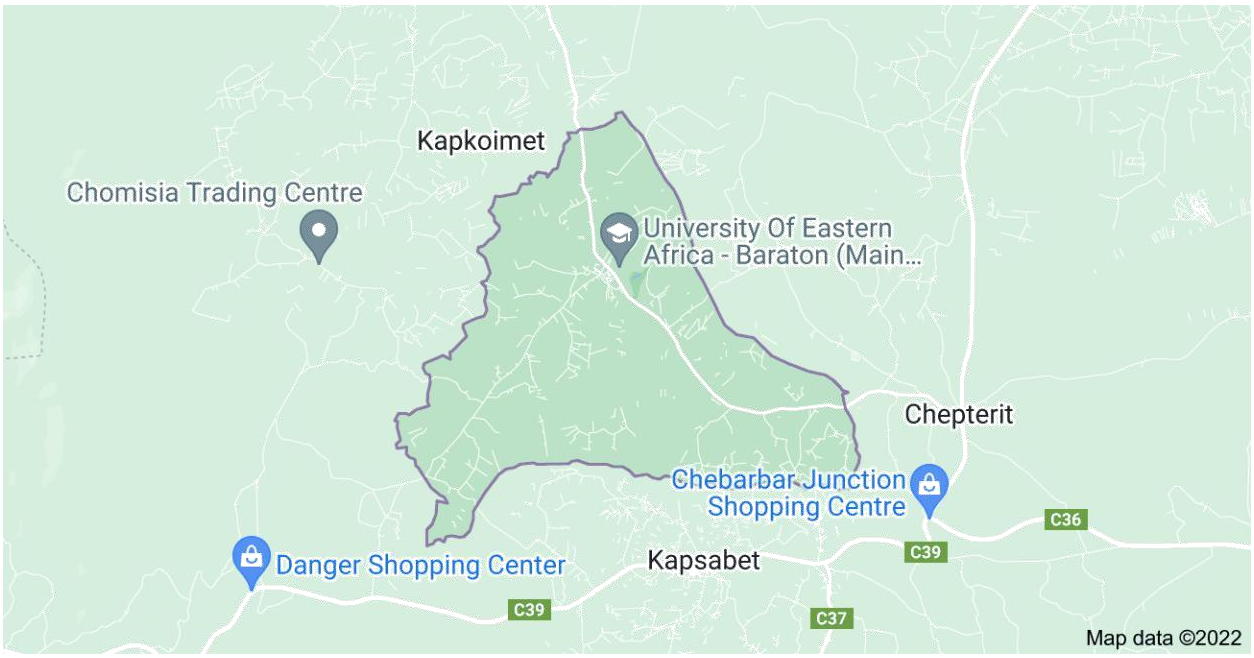

Figure 1: Map of Baraton

Study Population and Sampling Technique

The study target was the regularly contacted places like office doors, classroom doors, toilet doors, shopping baskets and toilet water faucet handles in different buildings of all the buildings within the learning and students' halls (Table 1) residence. Purposive sampling technique was used to select the study area. Stratified sampling technique was used to divide the population of fomites in subgroups (or strata) within the University and, due to the big number of the sampling sites, 20% of all the sites were selected randomly to improve the accuracy and representativeness of the results by reducing sampling bias as the burden is lessened (Krejcie & Morgan, 2010).

Table 1 Sampling Technique

Building/code	Fomite code	Doors	Faucets	Cisterns	S/Basket
ADM	OD, TD, TWF	45	10	10	-
SCI	OD, CD, TD, TWF	49	16	12	
HUM	OD, CD, TD, TWF	47	12	12	-
LIB	OD, CD, TD, TWF	53	8	7	-
TECH	OD, CD, TD, TWF	22	11	11	-
STUD/C	ED, TWF	7	4	6	-
SNKT	TD, ED, TWF	12	-	-	-
CAF	ED, OD, TD, TWF	8	2	4	-
L/A	ED, TD, TWF	61	33	28	-
L/D	ED, TD, TWF	173	112	14	-
OM/D	ED, TD, TWF	682	89	56	-
NM/D	ED, TD, TWF	86	15		
SUP	ED, SB	2	-	-	5
Fam & Cons	ED, TWF, TF	27	4	1	-
AUD	ED, TD, TWF	22	6	9	-
AMP	ED, TD, TWF	2	-	-	-
TH	ED, TD, TWF	22	2	2	-
S/B	ED	4	-	-	-
TEX	ED,	6	-	-	-
Total samples		**1330**	**320**	**172**	**5**

Key: OD= Office doors, CD= Classroom doors, TD= Toilet doors, TWF= Toilet water faucet, SB=Shopping baskets, ED=Entrance Door.

Grand total=1827 samples

The samples were collected in phases where it depended on the number of samples to be collected with each phase collecting 50 samples per day and processing them till the end before embarking on collecting the next batch till all the total number of the representative 20% of 1827.

Where: 20% of 1827=365.4. The 20% sample size was chosen by the researcher as recommended by Krejcie & Morgan, (2010).

The number of samples collected and analyzed were 365 samples. A batch of 50 samples collected and analyzed to the end took one week and therefore, 365 samples took approximately 365/50=8 weeks. Collection of samples and treatment and collection of data took 2 months.

Data Collection Procedures

Sample Collection

Of the total sites identified to be swabbed 20% were randomly picked and swabbed randomly as the representative sample,

365 swabs were obtained in different facilities inside the University by swabbing of the toilet cistern handles, office doors faucets and shopping baskets (sterile swabs moistened with buffered peptone water). They were then correctly labelled with reference numbers and transported in peptone water transport medium to the Biology Laboratory. This was done in the second semester of the academic year 2020/2021.

Culture and Isolation

The samples were suspended in buffered peptone water. After suspension and incubation for 18-24 hrs. The obtained growth marked by turbidity were inoculated in the blood agar (HIMEDIA), MacConkey Agar (HIMEDIA) and Nutrient Agar (HIMEDIA) and then incubated at 35°c. MacConkey agar and Nutrient agar were used to isolate coliforms in Swabs and bacteria of public health significance. These helped in determining the types of bacteria in each site.

Biochemical Identification

The isolates of bacteria were subjected for the purposes of differentiating gram negative and positive bacteria with standard methods of microbiology, such as morphological characteristics of the colonies. Biochemical studies were conducted on the isolates for further identification and characterization. The isolates' morphological and biochemical characteristics were compared to the Bergey's Determinative Bacteriology Manual (1994).

Antibacterial Sensitivity

Müller-Hinton agar was planted uniformly across the plates and the bacteria identified were diluted at normal concentrations (approximately 1 to 2 x 108 colony forming units per ml) (Conceicao et al., 2013). The commercially prepared disk was uniformly dispensed lightly onto the agar surface, each of which was pre-impregnated with the standardized concentrations of Chloramphenicol, Ampicillin, Lincomycin, Penicillin, Minocycline, Erythromycin, Methicillin and Co-trimoxazole. The bacterial growth around each disk was observed after a night incubation. The zone around an antibiotic disk with no growth is known as the inhibition zone. They approximate the minimum concentration of the antibiotic that was adequate to prevent test isolate growth. These zones were measured to the nearest millimeter (mm) and were compared with a standard interpretive chart which was used to identify the bacteria which were sensitive to Chloramphenicol, Ampicillin, Lincomycin, Penicillin, minocycline, Erythromycin, Methicillin and Co-trimoxazole.

Statistical Treatment of Data

The data was analyzed using SPSS Version 26. Descriptive statistics was used to analyze the bacteria types isolated from fomites within the University buildings. All values were expressed as means and analyzed descriptively; findings were presented in the form of frequency tables.

Ethical Consideration

Clearance for the study was sought from the University of Eastern Africa Baraton Review Ethics committee. Thereafter, the researcher got clearance from NACOSTI. Privacy and confidentiality were highly maintained during the research process. Unique numbers were given to each building for the purpose of anonymity.

CHAPTER FOUR

PRESENTATION OF FINDINGS, ANALYSIS AND INTERPRETATION

Introduction

This chapter presents the analysis of the findings from the study on identifying opportunistic bacteria types on selected fomites in a selected post-secondary institution of higher learning in Nandi County.

Objective 1: To Isolate and Characterize Opportunistic Bacteria Present on Frequently Used Fomites

The examination of bacterial contamination was carried out in 16 different locations. These locations were categorized as buildings: ADM, SCI, HUM, LIB, TECH, STUD/C, SNKT, CAF, L/A, L/D, OM/D, NM/D, SUP, Fam & Cons, AUD, AMP, TH, S/B and TEX.

In each building, samples were obtained from fomites which were categorized as (1) Faucets; (2) Cisterns and (3) Doors. Growth of bacteria was determined under nutrient and potato dextrose agar.

The type of contaminants was determined based on their growth on nutrient and potato dextrose agar. The samples were suspended in buffered peptone water. After suspension and incubation for 24 hrs. The obtained growth marked by turbidity were inoculated in nutrient and

potato dextrose agar and then incubated at 35°c based on site of collection as the labelling indicated. These helped in determining the growth of bacteria in each site.

Table 2: Bacteria isolates in Nutrient and Potato Dextrose Agar

Fomite of Collection	**Growth (+)/No growth (-)**	**Nutrient Agar**	**Potato Dextrose Agar**	**Total**
Faucets	+	52(96.3%)	8(14.8%)	**60(55.5%)**
	-	2(3.7%)	46(85.2%)	**48(44.5%)**
Cisterns	+	37(82.2%)	7(15.6%)	**44(48.9%)**
	-	8(17.8%)	38(84.4%)	**46(51.1%)**
Doors	+	142(90.4%)	29(18.5%)	**171(54.5%)**
	-	15(9.6%)	128(81.5%)	**143(45.5%)**
Total	**+**	**231(90.2%)**	**44(17.2%)**	
	-	**25(9.8%)**	**212(82.8%)**	

As displayed in table 2 above, a total of 231 samples obtained grew in nutrient agar with 96.3%, 82.2% and 90.4% of samples collected from faucets, cisterns and doors respectively grew in nutrient agar. However, a few (25) samples obtained did not grow in nutrient agar with 3.7%, 17.8% and 9.6% of samples obtained from faucets, cisterns and doors respectively not growing in nutrient agar. Also, a total of 44 samples that were obtained grew in potato dextrose agar with 14.8%, 15.6% and 18.5% of the samples obtained from faucets, cisterns and doors growing in potato dextrose agar.

As displayed in table two above, the results suggested that 231 (90.2%) of the samples obtained were bacteria with only 44 (17.2%) of the samples obtained were suspected to be fungi as they grew in in Potato Dextrose agar. The results therefore called for isolation and characterization of the 231 samples of bacteria which were obtained from the fomites.

Objective 1: Identification and Characterization of opportunistic Bacteria Present in the Fomites

Morphological Characterization

Pure cultures were obtained from the 231 samples of bacteria that grew on the nutrient agar by isolating individual colonies with streak plate technique using an inoculating loop to streak colonies on nutrient agar plates in one of several patterns. Successful isolation depended on spatial separation of single colonies.

The isolates of bacteria were then subjected for the purposes of morphological characterization based on gram staining. Gram staining was done, followed by microscopic examination under oil immersion. This was done to identify the general type of bacteria and classify bacteria for further identification tests.

Table 3: Morphological characterization of Bacteria by Gram Staining

Fomite of Collection	Bacterial Isolates	Frequency (n)	Percentage (%)
Faucets	Gram positive cocci	**44**	84.6
	Gram negative cocci	8	15.4
Cisterns	Gram positive cocci	**31**	83.3
	Gram negative cocci	3	8.1
	Gram negative rods	3	8.1
Doors	Gram positive cocci	**112**	78.9
	Gram positive rods	**1**	0.7
	Gram negative cocci	25	17.6
	Gram negative rods	5	3.5

From table 3 above, 84.6% of isolates from faucets were gram positive cocci with only 15.4%-gram negative cocci. 83.8% of the isolates from cisterns were gram positive cocci as compared to only 8.1%-gram negative cocci and rods. 78.9% of the isolates from doors were

gram positive cocci as compared to 0.7%-gram positive rods, 17.6%-gram negative cocci and 3.5%-gram negative rods.

Specifically, the results indicate that doors had the greatest number of gram-positive cocci followed by faucets and lastly cisterns. The results indicate that doors had the greatest number of gram-negative cocci bacteria followed by faucets and cisterns. Doors had the greatest number of gram-negative rod bacteria as compared to cisterns. Lastly, isolates from the door had 1-gram positive rod bacteria. However, faucets did not have gram negative and gram-positive rods. For further characterization of the bacterial isolates, biochemical characterization was conducted.

Biochemical Characterization of Gram-Positive Bacteria

Gram positive bacterial isolates were subjected for the purposes of characterization based on biochemical reaction as per the standard methods of microbiology. Selective biochemical tests were conducted on the isolates. These included differential growth in Blood Agar, catalase, coagulase, MSA and oxidase reactions. The isolates' biochemical characteristics were compared to the Bergey's Determinative Bacteriology Manual (1994).

Growth on Blood Agar

The gram-positive contaminants were determined based on their differential growth in blood agar. 188-gram positive isolates from nutrient agar were inoculated aseptically in blood agar and then incubated at 35°c based on fomite of collection. These helped in testing the ability of the bacteria to produce hemolysins which are enzymes that lyse the erythrocytes. The degree

of hemolysis differentiated *Staphylococcus* bacteria, *Streptococcus* bacteria and *Enterococcus* bacteria from each other.

Table 4: Biochemical Characterization of Isolated Bacteria (Blood Agar)

Source of Isolates		Frequency(n)	Percentage (%)
Faucets	Haemolysis type on Blood Agar		
	Beta-hemolysis	25	56.8
	Alpha-hemolysis	15	34.1
	Gamma-hemolysis	4	9.1
	Total	**44**	**100**
Cisterns	Haemolysis type on Blood Agar		
	Beta-hemolysis	21	65.6
	Alpha-hemolysis	8	25
	Gamma-hemolysis	3	9.4
	Total	**32**	**100**
Doors	Haemolysis type on Blood Agar		
	Beta-hemolysis	84	75
	Alpha-hemolysis	8	7.1
	Gamma-hemolysis	20	17.9
	Total	**112**	**100**
	Grand Total	**188**	

As indicated in table 4 above, 56.8%, 65.6% and 75% of the isolates from faucets, cisterns and doors respectively exhibited beta-hemolysis on blood agar. On the other hand, with 34.1%, 25% and 7.1% of the isolates from faucets, cisterns and doors respectively exhibited alpha-hemolysis on blood agar. However, 9.1%, 9.4% and 17.9% of the isolates from faucets, cisterns and doors respectively exhibited gamma-hemolysis on blood agar.

The above results gave a general suggestion that the isolates contained *Staphylococcus, Streptococcus* and *Enterococcus* bacteria. To confirm the presence of *Staphylococcus, Streptococcus* and *Enterococcus* bacteria, the researcher proceeded with further characterization and conducted biochemical characterization on the isolates.

Catalase Test

The Catalase-test was used to differentiate between *Staphylococcus* which are catalase-positive from *Streptococcus* which are catalase-negative. The isolates' biochemical characteristics were compared to the Bergey's Determinative Bacteriology Manual (1994).

Table 5: Reaction of Gram-Positive Bacteria to Catalase Test

Fomite of collection		Frequency(n)	Percentage (%)	Type of Bacteria
Faucets	Catalase Positive	32	21.8	*Staphylococcus spp.*
Cisterns	Catalase Positive	25	17	*Staphylococcus spp.*
	Catalase Negative	1	0.7	*Streptococcus pyogenes.*
Doors	Catalase Positive	89	60.5	*Staphylococcus spp.*

From table 5 above, the results indicate that 21.8%, 17% and 60.5% of the gram-positive bacteria obtained from the faucets, cisterns and doors were positive to catalase test hence this confirmed them to be *Staphylococcus spp*. However, 0.7% of gram-positive bacterial isolate obtained from cistern was catalase negative confirming it to be *Streptococcus pyogenes*. Therefore, from the gram-positive bacterial isolates, one bacterial isolate obtained from cisterns was *Streptococcus pyogenes*.

Coagulase

Coagulase test was used to identify the *Staphylococci* where *S. aureus* is a coagulase-positive and *S. epidermidis* is a coagulase-negative bacteria species. The isolates' biochemical characteristics were compared to the Bergey's Determinative Bacteriology Manual (1994).

Table 6: Reaction of Gram-Positive Bacteria on Coagulase Test

Fomite of collection		Frequency(n)	Percentage (%)	Bacterial Type
Faucets	Coagulase Negative	32	21.9	*S. epidermidis*
Cisterns	Coagulase Positive	1	0.7	*S. aureus*
	Coagulase Negative	24	16.4	*S. epidermidis*
Doors	Coagulase Positive	5	3.4	*S. aureus*
	Coagulase Negative	84	57.5	*S. epidermidis*

From table 6 above, the results indicate that 21.9%, 16.4% and 57.5% of the gram-positive bacteria were coagulase positive which was a confirmation that they were *S. aureus*. However, 0.7% and 3.4% of the gram-positive bacterial isolate obtained from cistern were coagulase negative confirming them to be *Streptococcus epidermidis*. Therefore, from the gram-positive bacterial isolates obtained from faucets, cisterns and doors were *Streptococcus epidermidis* and *Streptococcus aureus*.

Biochemical Characterization of Gram-Negative Bacteria

Gram negative bacterial isolates were subjected to biochemical tests for the purposes of characterization based on their biochemical reaction as per the standard methods of microbiology. Selective biochemical tests were conducted on the isolates. These included growth on MacConkey Agar, Chocolate agar, Blood agar and Eosin Methylene Blue agar and reactions with IMVic, Nitrate, Oxidase and Catalase media. The isolates' biochemical characteristics were compared to the Bergey's Determinative Bacteriology Manual (1994).

The isolates were confirmed to be gram negative by growth on MacConkey agar and morphological analysis. The isolates were inoculated aseptically on MacConkey agar and then incubated at 35°c based on fomite of collection. Results were observed under microscope and

morphological analysis were conducted through gram staining to confirm the morphology of the gram-negative bacteria based on the fomite of collection.

Table 7: Gram Negative Isolates Growth on MacConkey Agar

Fomite of collection		Frequency(n)	Percentage (%)
Faucets	Gram negative cocci	8	18.2
Cisterns	Gram negative cocci	3	6.8
	Gram negative rods	3	6.8
Doors	Gram negative cocci	25	56.8
	Gram negative rods	5	11.4
	Total	44	100

From table 7 above, the majority of gram-negative isolates were cocci (81.8%) as compared to rods which were 18.2%. However, faucets had only gram-negative cocci while cisterns and doors had both gram-negative cocci and rods. Faucets had 18.2%-gram negative cocci, cisterns had 3%-gram negative cocci and gram-negative rods and doors had 56.8%-gram negative cocci and 11.4%-gram negative rods.

Biochemical Reaction of Gram-Negative Rods

IMViC Tests

A traditional method called IMViC tests and was used to identify the bacteria from the 3-gram negative rods isolates from cisterns and 5-gram negative rods isolates from doors. This is a set of tests used for the differentiation of the Enterobacteriaceae family. IMViC is an abbreviation for Indole, Methyl red (MR), Voges-Proskauer (VP), and Citrate utilization tests. These tests are used to differentiate Enterobacteriaceae. When they are used alone, they are commonly used in identification of coliform bacteria species.

Table 8: Results for IMViC Tests

Fomite of collection	Morphology	Indole	MR	VP	Citrate
Cisterns	Gram negative rods	+	+	−	−
Doors	Gram negative rods	+	+	−	−

Key: MR- Methyl red, VP- Voges-Proskauer

From table 8 above, all the gram-negative rods isolates had a significant growth on the test media. Additionally, all of them were indole and MR positive, VP and Citrate negative. The results of the IMViC test were a confirmation that the gram-negative rod isolates were coliform bacteria. To isolate fecal coliforms, EMB (Eosin Methylene Blue) agar was used. On EMB, colonies appeared either coloured or colourless indicating their fermentation of lactose or sucrose and showed whether the isolates were fecal coliforms or not. All the isolates produced a green metallic sheen on EMB and hence were identified as *Eschrichia coli.*

Biochemical Reaction of Gram-Negative Cocci

The identification of gram-negative cocci was done based on the biochemical reaction. Nitrate reduction, catalase, DNase and Oxidase were the biochemical tests performed. The gram-negative cocci bacteria were differentiated using DNase and how they grew on nutrient agar at 35℃.

Table 9: Biochemical Reaction of Gram-Negative Cocci

Fomite of collection	Morphology of Isolates	Nitrate	Catalase	DNase	Oxidase
Faucets	Gram negative cocci	+	+	+	+
Cisterns	Gram negative cocci	+	+	+	+
Doors	Gram negative cocci	+	+	+	+

Out of 36 isolates, all of them had significant growth. The primary tool which was used for identification was morphology of the colonies. The isolates were grown well on chocolate agar and blood agar. On blood agar, all the colonies ranged from gray to white and 1-3 mm in diameter after they were incubated for 24 hours. The colonies were pinkish brown on chocolate agar. With their large kidney shape, the isolates were identified as *Morexella catarrhalis.* They were all positive for oxidase, DNase, and catalase tests and they also reduced nitrate to nitrite.

Objective 3: Antibacterial Sensitivity to Various Antibacterial Preparations

Table 10: Antibacterial Sensitivity

Antibiotic	Frequency	Percentage
Chloramphenicol	3	1.9%
Ampicillin	30	19.4%
Lincomycin	10	6.5%
Penicillin	36	23.2%
Minocycline	7	4.5%
Erythromycin	19	12.2%
Methicillin	18	12.2%
Cotrimoxazole	32	20.6%
Total	**155**	**100%**

From table 10 above, majority of the bacteria (23.2%) were sensitive to Penicillin, 20.6% were sensitive to Cotrimoxazole, 19.4% were sensitive to Ampicillin, 12.2% were sensitive to Erythromycin and Methicillin, 6.5% were sensitive to Lincomycin, 4.5% were sensitive to Minocycline and 1.9% were sensitive to Chloramphenicol.

The finding of this study shows that 155 (66.8%) of the bacteria were resistant to various antibiotics. This implies that these bacteria have changed and no longer respond to the antibiotics making it impossible for the infections caused by these bacteria to be treated with the various antibiotics they are resistant to.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

Introduction

This chapter is a presentation of the summary of the research, conclusions and recommendations made from the research findings. The summary, conclusion and recommendation were made in line with the research questions.

Summary

This study was focused on identifying opportunistic bacteria types on selected fomites in a selected post-secondary institution of higher learning in Nandi County which is The University of Eastern Africa Baraton. Specifically, the study was to isolate, identify, characterize and determine the antibacterial sensitivity to various antibacterial preparations. The study was conducted at the post-secondary institutions of higher learning in Nandi County namely, University of Eastern Africa Baraton, which was were selected purposively. The fomites, which were included in this study, were purposively selected based on their direct interaction with human beings. The design of the study was experimental. The study population were some of the regularly contacted places like doors, faucets and cisterns. Of the total sites identified to be swabbed 20% were randomly picked and swabbed randomly as the representative sample. The samples were suspended in buffered peptone water and incubated for 18-24 hrs. The obtained growth marked by turbidity were inoculated into various media for isolation and identification.

The isolates of bacteria were subjected to gram staining with standard methods of microbiology. Biochemical studies were conducted on the isolates which were compared to the Bergey's Determinative Bacteriology Manual. A disk diffusion test was used to find out the antibacterial sensitivity through Müller-Hinton agar. The commercially prepared disks were uniformly dispensed lightly onto the agar surfaces, each of which were pre-impregnated with Chloramphenicol, Ampicillin, Lincomycin, Penicillin, Minocycline, Erythromycin, Methicillin and Co-trimoxazole antibiotics. The data was analyzed using Excel for Windows 10. Descriptive statistics was used to analyze the frequencies of bacteria types isolated from the fomites within the selected University buildings. All values were expressed as frequencies and percentages and findings presented in form of frequency tables.

The study found out that 90.2% of the samples obtained were bacteria with only 9.8% being fungi. Thereafter, isolation and characterization of the bacterial samples was done. The study found out that doors had the greatest number of gram-positive cocci followed by faucets and lastly cisterns. Doors had the greatest number of gram-negative cocci bacteria followed by faucets and cisterns. Doors had the greatest number of gram-negative rod bacteria as compared to cisterns. Lastly, isolates from doors had one-gram positive rod bacteria. However, faucets did not have gram negative and gram-positive rods. The study found out that from the gram-positive bacterial isolates, one bacterial isolate obtained from cisterns was *Streptococcus pyogenes*, bacterial isolates obtained from faucets, cisterns and doors were *Streptococcus epidermidis* and *Streptococcus aureus*. The study found that the gram-negative rod isolates were *Eschrichia coli* and the gram-negative cocci bacterial isolates were *Morexella catarrhalis.* Lastly, the study found that 66.8% of the bacteria were resistant to the various antibiotics.

Conclusions

Based on the findings of this study, it was concluded that 90.2% of the samples obtained were bacteria and only 9.8% of the samples obtained were fungi. It can be concluded that doors had the greatest number of gram-positive cocci followed by faucets and cisterns. Doors had the greatest number of gram-negative cocci bacteria followed by faucets and cisterns. Doors had the greatest number of gram-negative rod bacteria as compared to cisterns. Doors had gram positive rod bacteria. However, faucets did not have gram negative and gram-positive rods. The gram-positive bacterial isolates were *Streptococcus pyogenes*, *Streptococcus epidermidis* and *Streptococcus aureus*. The gram-negative bacterial isolates were *Eschrichia coli* and *Morexella catarrhalis*. Based on the findings of this study, 66.8% of the bacteria obtained from the formites were resistant to various antibiotics. Specifically, the isolated bacteria were resistant to Penicillin, Cotrimoxazole, Ampicillin, Erythromycin, Methicillin, Lincomycin, Minocycline and Chloramphenicol.

Recommendations

Further identification and characterization of the isolates to be conducted to confirm the presence of any other bacterial types that might be obtained from the fomites. Based on these findings and the antibacterial resistance of the bacterial isolates, further and broader research covering wider areas should be done on these fomites in order to shed more light on their contamination. It will be of great importance to carry out antibacterial susceptibility tests of the isolates using a different method other than disk diffusion method which will help confirm the antibacterial resistance of the bacteria obtained from the fomites. A similar study is also recommended with focus on determining multidrug resistance of the isolates.

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APPENDECIES

Appendix 1: Curriculum Vitae

RICHARD NGARU MAGONDU

Address: P.O. BOX 872, KERUGOYA

Email: Ngaru.richard@gmail.com

Nationality: Kenyan

Gender: MALE

Marital status: Married

Personal Profile Statement

A meticulous serious worker, determined to improve and advance academically and also set standards for the youth. Ready and willing to work with others in a team playing spirit. Accommodative to corrections and instructions and, willing to share.

Proffesional Qualifications

- 2016-presently, on masters of science in Biomedical sciences

- 2011-2014 BSc Medical Laboratory Sciences at University of Eastern Africa Baraton

- 1984-1987 The Kenya polytechnic ordinary Diploma in Medical Laboratory Technology

  Chemical pathology 4
  Hematology and blood transfusion 5
  Microbiology 4
  Parasitology 3

Education

- 1980-1981 EAACE
  1 principle in Biology and
  1 subsidiary in chemistry Certificate
  3 points at Kirangari High School.
- 1976-1979, EACE
  Mathematics 5
  English8
  Biology3
  P.Science6

Agriculture 4
Swahili 7
Fasihi 4
Geography 5

2nd Division, 27 points at Nyahururu High School

- 1969-1975 CPE:
  Mathematics A-
  English B
  General Paper A-

  At Karaini Primary School

Work Experience

1982-1983, laboratory assistant in Endarasha secondary school

1983-194, laboratory assistant in Njabini secondary school

1984-1987, trainee technician at Kabete university campus

1987-1988, technician in Kenya Medical Research Institute

1988-1990, Diagnostics sales Representative at E.T. Monks Ltd

1990-2010, Private practice as the proprietor

UEAB

12/08/2011 - 25/02/2013, Lab Technologist at Baraton Hospital

07/04/2013 - 17/11/1014, lab asst. in Dept. of biology

18/01/2014 - --/04/2016, Lab assistant MLS department

01/09/2016 – present, clinical instructor in department of MLS

Hobbies and Interests.

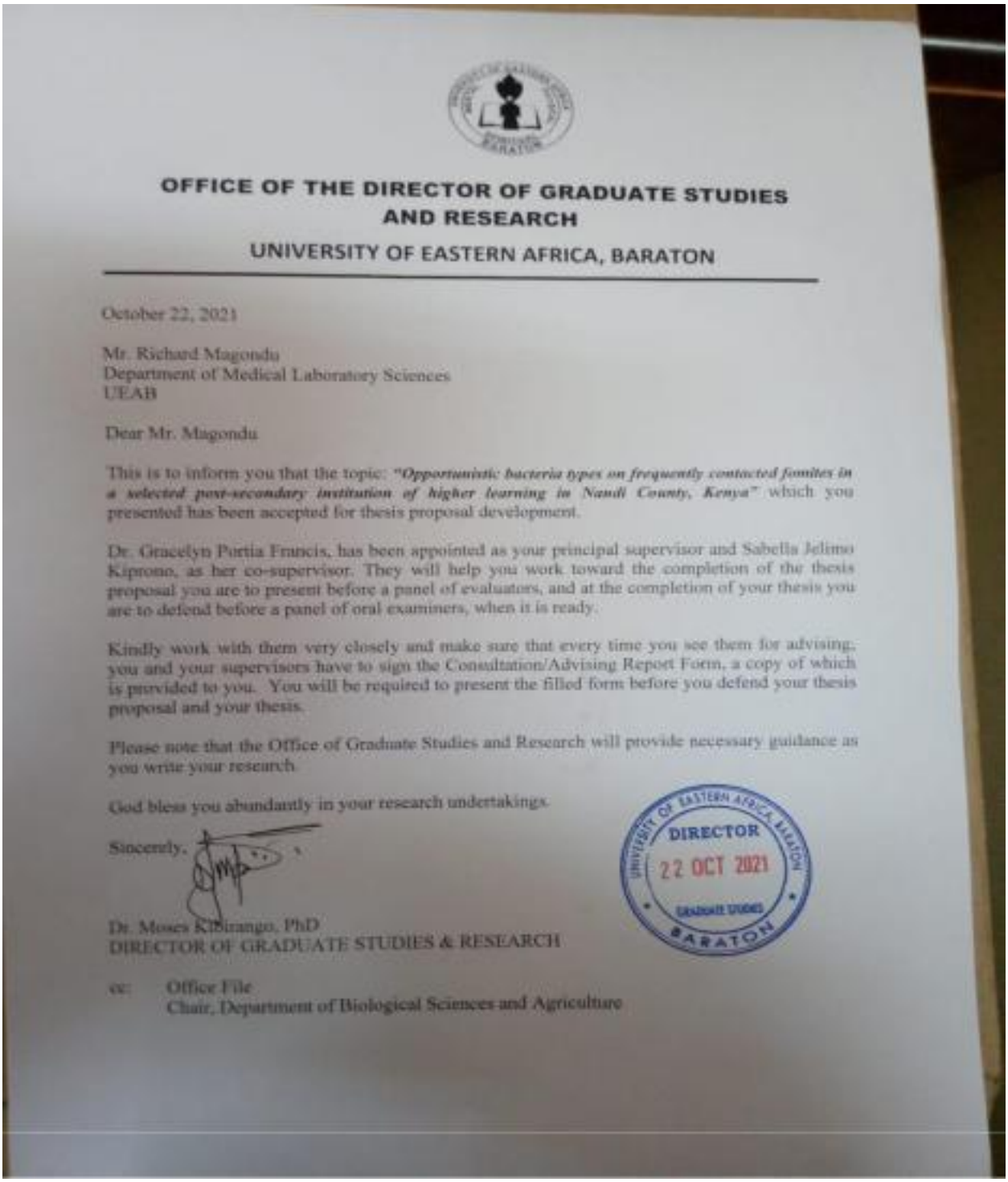
I enjoy Hiking and Picnicking a lot in company of good friends and also riding and site seeing.

Referees

(1) Prof. Jackie obey,
University of Eastern Africa, Baraton
P. O. Box 2500,
Eldoret.

(2) Peter Mwai
Gachoki, P. O. Box
47, Kerugoya.

Appendix 2: Letter from Graduate Studies



OFFICE OF THE DIRECTOR OF GRADUATE STUDIES AND RESEARCH

UNIVERSITY OF EASTERN AFRICA, BARATON

October 22, 2021

Mr. Richard Magondu
Department of Medical Laboratory Sciences
UEAB

Dear Mr. Magondu

This is to inform you that the topic: ***"Opportunistic bacteria types on frequently contacted fomites in a selected post-secondary institution of higher learning in Nandi County, Kenya"*** which you presented has been accepted for thesis proposal development.

Dr. Gracelyn Portia Francis, has been appointed as your principal supervisor and Sabella Jelimo Kiprono, as her co-supervisor. They will help you work toward the completion of the thesis proposal you are to present before a panel of evaluators, and at the completion of your thesis you are to defend before a panel of oral examiners, when it is ready.

Kindly work with them very closely and make sure that every time you see them for advising, you and your supervisors have to sign the Consultation/Advising Report Form, a copy of which is provided to you. You will be required to present the filled form before you defend your thesis proposal and your thesis.

Please note that the Office of Graduate Studies and Research will provide necessary guidance as you write your research.

God bless you abundantly in your research undertakings.

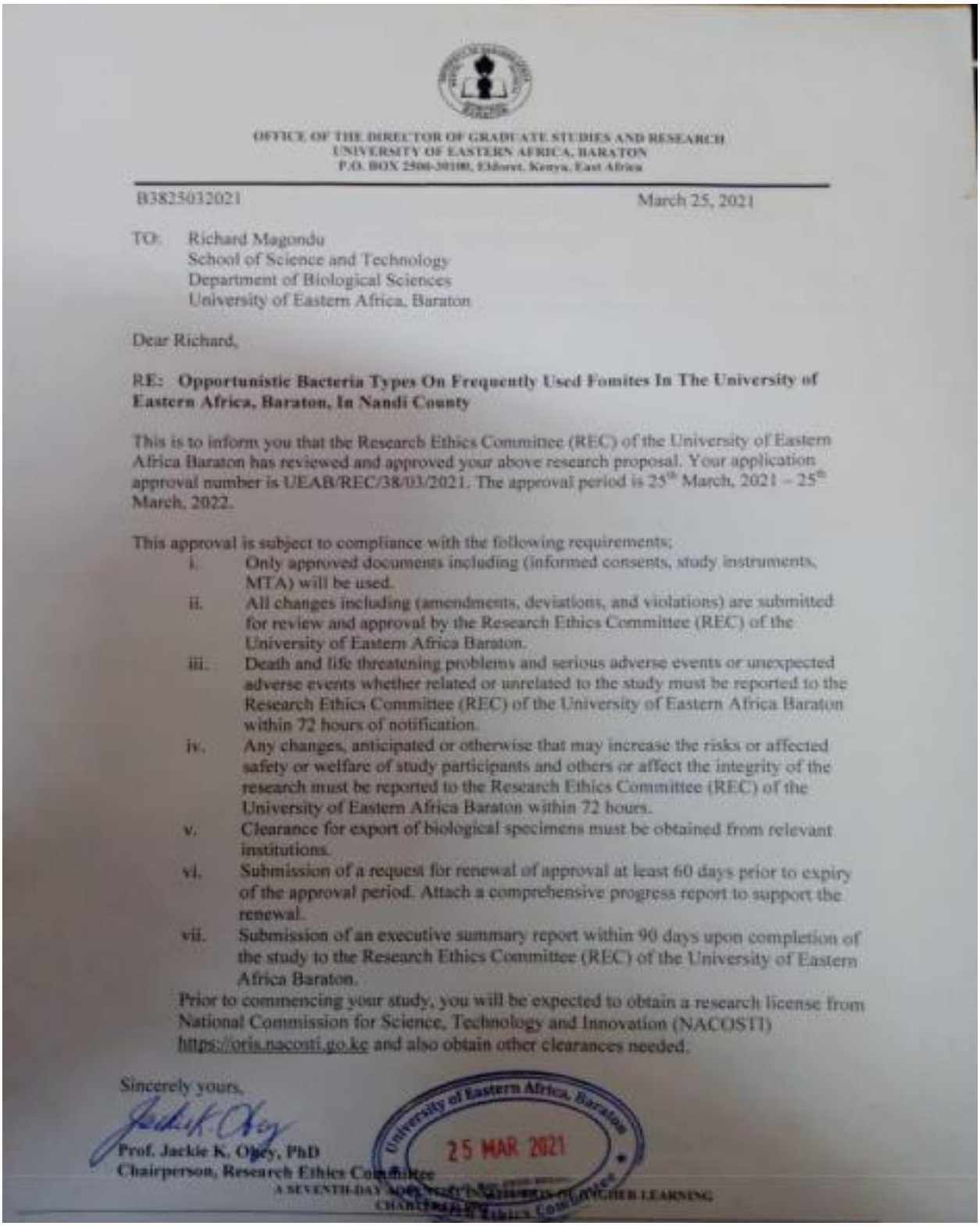
Sincerely,

Dr. Moses Kibirango, PhD
DIRECTOR OF GRADUATE STUDIES & RESEARCH

DIRECTOR
22 OCT 2021
GRADUATE STUDIES
BARATON

cc: Office File
Chair, Department of Biological Sciences and Agriculture

Appendix 3: Ethics Clearance Letter



OFFICE OF THE DIRECTOR OF GRADUATE STUDIES AND RESEARCH
UNIVERSITY OF EASTERN AFRICA, BARATON
P.O. BOX 2500-30100, Eldoret, Kenya, East Africa

B3825032021 March 25, 2021

TO: Richard Magondu
School of Science and Technology
Department of Biological Sciences
University of Eastern Africa, Baraton

Dear Richard,

RE: **Opportunistic Bacteria Types On Frequently Used Fomites In The University of Eastern Africa, Baraton, In Nandi County**

This is to inform you that the Research Ethics Committee (REC) of the University of Eastern Africa Baraton has reviewed and approved your above research proposal. Your application approval number is UEAB/REC/38/03/2021. The approval period is 25th March, 2021 – 25th March, 2022.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by the Research Ethics Committee (REC) of the University of Eastern Africa Baraton.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to the Research Ethics Committee (REC) of the University of Eastern Africa Baraton within 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to the Research Ethics Committee (REC) of the University of Eastern Africa Baraton within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. 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.
vii. Submission of an executive summary report within 90 days upon completion of the study to the Research Ethics Committee (REC) of the University of Eastern Africa Baraton.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Sincerely yours,

Prof. Jackie K. Obey, PhD
Chairperson, Research Ethics Committee

University of Eastern Africa, Baraton
25 MAR 2021

A SEVENTH-DAY ADVENTIST INSTITUTION OF HIGHER LEARNING

Appendix 4: NACOSTI Research License

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 357382

Date of Issue: 16/April/2021

RESEARCH LICENSE

This is to Certify that Mr., Richard Ngaru Magonda of University of Eastern Africa, Baraton, has been licensed to conduct research in Nandi on the topic: OPPORTUNISTIC BACTERIA TYPES ON FREQUENTLY USED FOMITES IN UNIVERSITY OF EASTERN AFRICA BARATON IN NANDI COUNTY, for the period ending : 16/April/2022.

License No: NACOSTI/P/21/9952

357382

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

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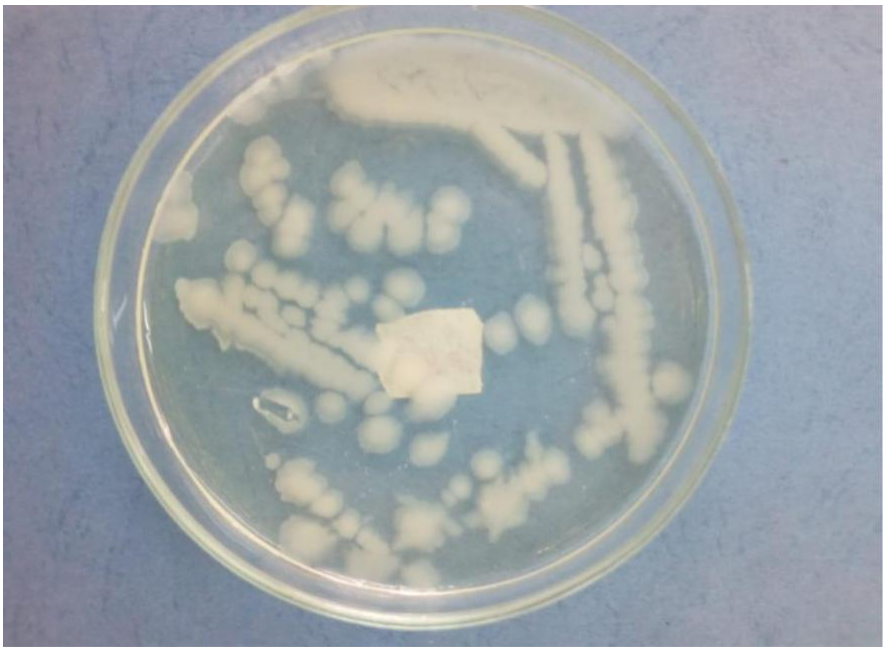
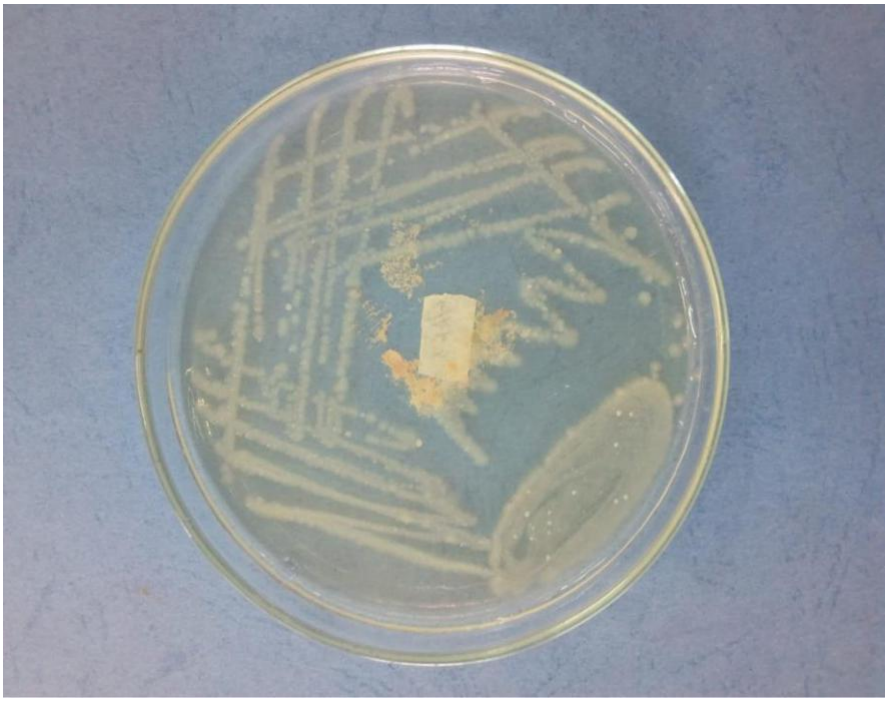
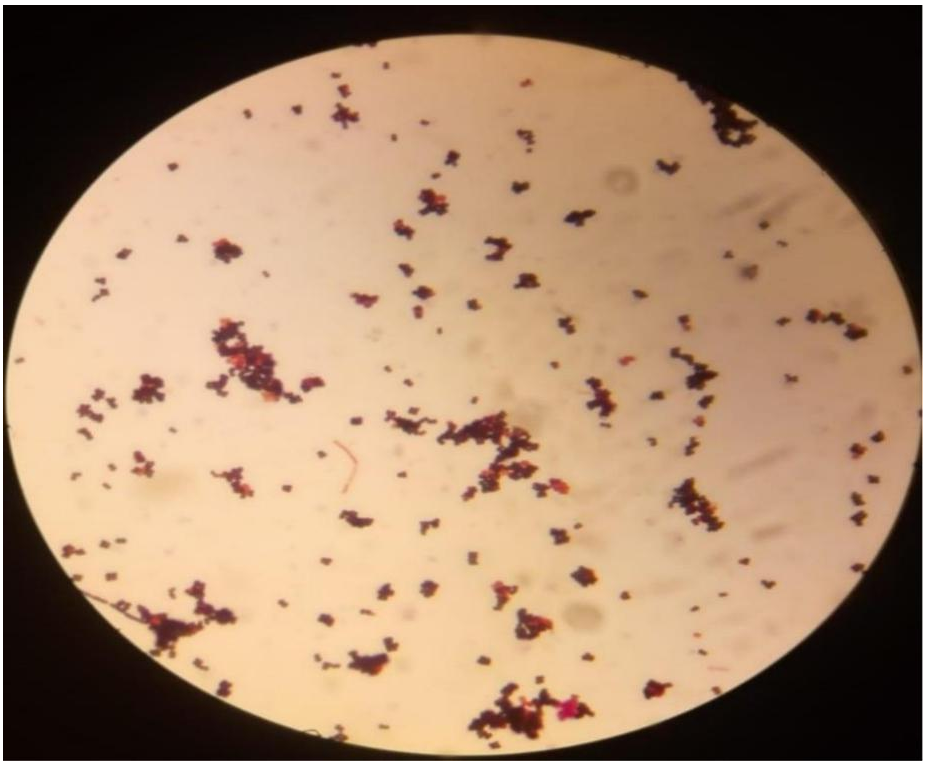
Appendix 4: Results of Bacteria Growth on Agar



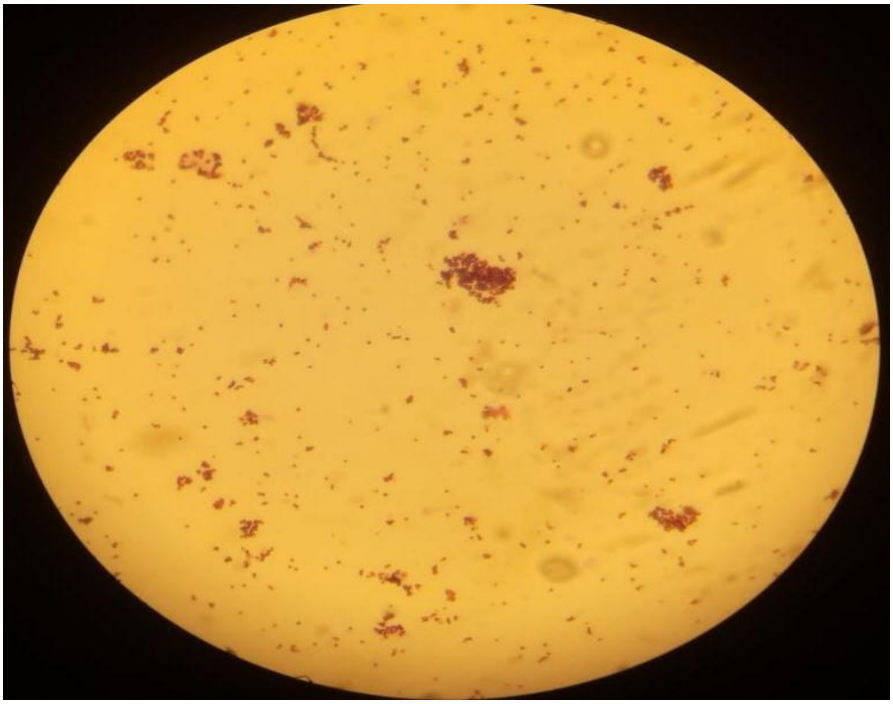
Growth on Potato Dextrose Agar from SCI



Growth on Nutrient Agar from AUD



Gram Positive Bacteria (Cocci) from OM/D



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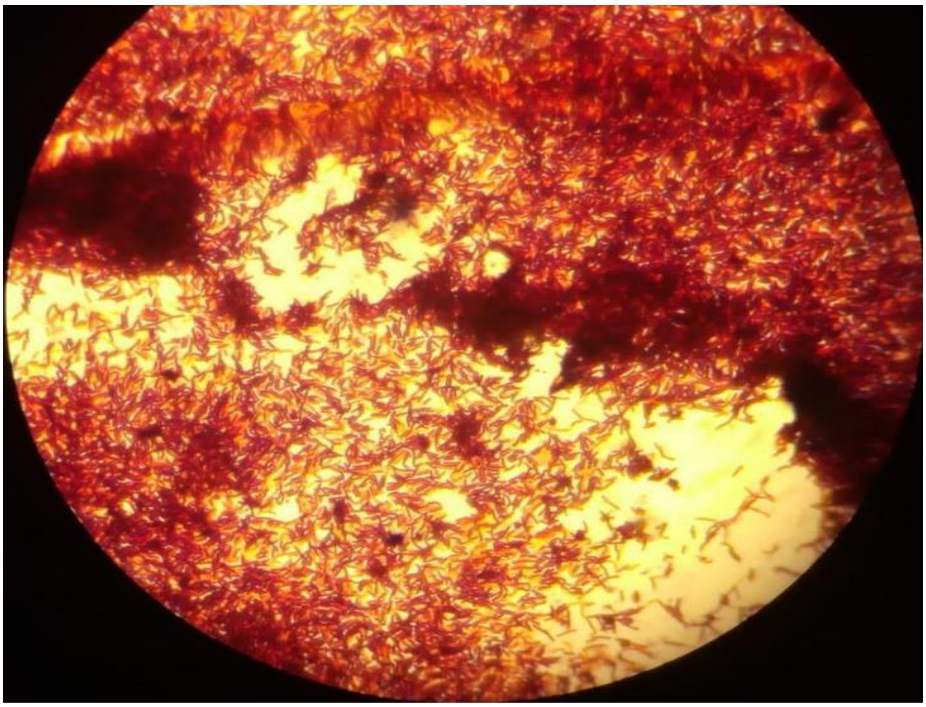
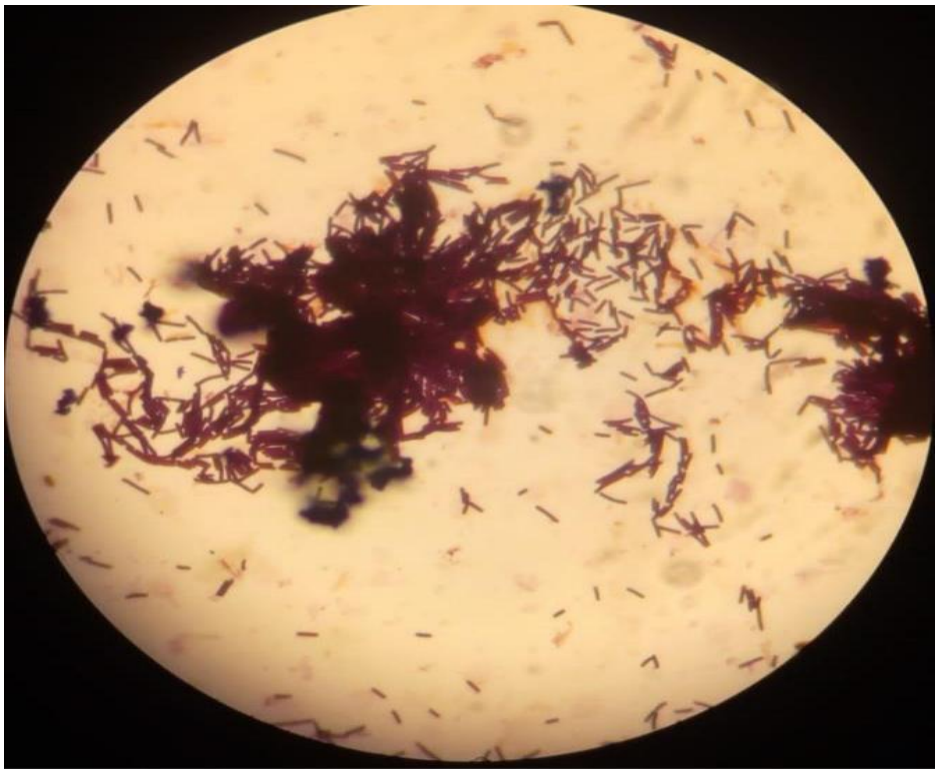


Gram Negative Rods from STUD/C



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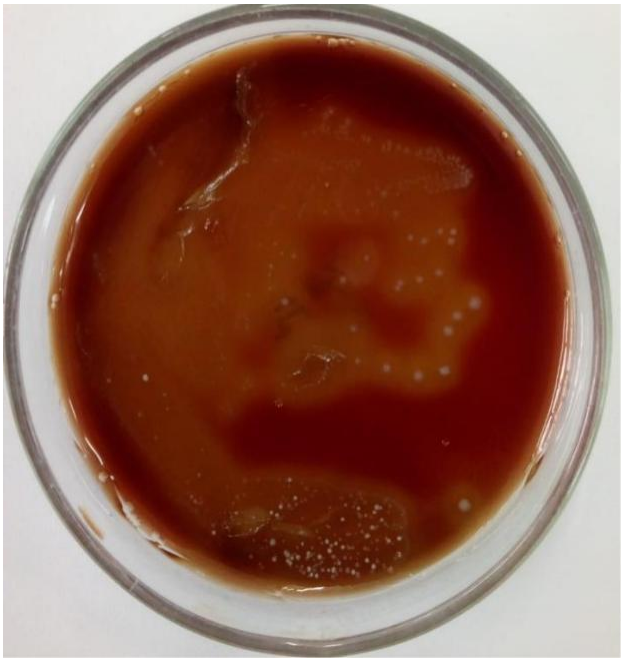
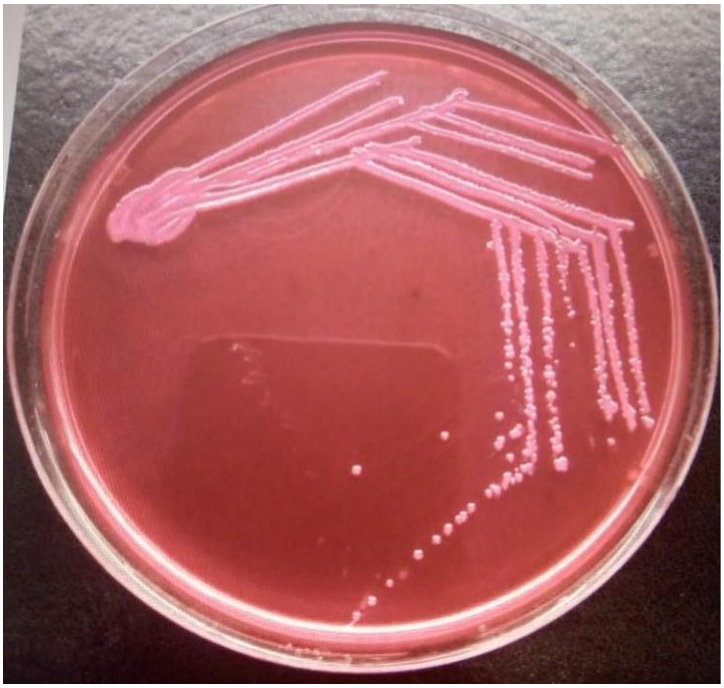
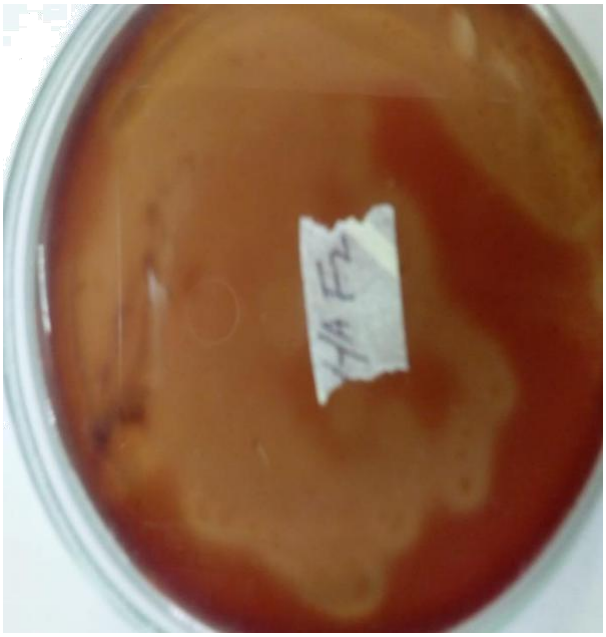


Growth on Blood Agar from SCI



Growth on MacConkey Agar from HUM



Growth on Blood Agar from STUD/C

Appendix 5: Plagiarism Certificate

Curiginal

Document Information

Analyzed document	RICHARD THESIS FINAL.docx (D142074598)
Submitted	2022-07-15 10:59:00
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Isolation and Identification of Bacteria Present on Frequently Used Fomites in University of Eastern Africa Baraton in Nandi County, Kenya

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Abstract: *Bacteria are found to be the ubiquitous microorganisms causing microbial contamination in indoor and outdoor settings. Fomites act as environmental reservoirs to increase the ability of pathogens to be transferred from host to host. The aim of this paper was to isolate and identify bacteria present on frequently used fomites in University of Eastern Africa, Baraton. Experimental research design was employed. Three hundred and sixty five (365) swabs were obtained in different facilities by swabbing of the toilet cistern handles, office doors faucets and shopping baskets. They were labelled with reference numbers and transported in peptone water transport medium to the Laboratory for analysis. Descriptive statistics was used to analyze the prevalence of bacteria types isolated from fomites. All values were expressed as means and findings were presented in the form of frequency tables. The study found out that cisterns had the greatest number of gram positive cocci followed by faucets and doors. Doors had the greatest number of gram negative cocci bacteria followed by faucets and cisterns. Cisterns had the greatest number of gram negative rod bacteria as compared to doors. Doors had gram positive rod bacteria. The gram positive bacterial isolates were Streptococcus pyogenes, Streptococcus epidermidis and Streptococcus aureus. The gram negative bacterial isolates were Eschrichia coli and Morexella catarrhalis. The study recommended that there was need for further identification and characterization of the isolates to be conducted to confirm the presence of any other bacterial types that might be obtained from the fomites.*

Keywords*: Isolation, Identification, Bacteria, Frequently, Fomites*

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1. Introduction

Bacteria are ubiquitous microorganisms causing microbial contamination (Pessi, Suonketo, Pentti, Kurkilahti, Rantio-Lehtimaki, 2002). Bacteria infect, transmits bacterial infections while they are in direct contact with vulnerable people (Pessi et al., 2002). Water, food and fomites can act as environmental reservoirs to increase the ability of pathogens to be transferred from host to host (Pessi et al., 2002). An inanimate object, which can transmit an infectious agent, is known as a fomite (CDC, 2012). Fomites include surfaces such as doors, toilets, chair handles, laboratory bench, railings etc. In heavily visited places such as schools, hospitals, market places and malls and any other place where human traffic is heavy contamination of inanimate objects is usually very high (CDC, 2012). Recent epidemiology studies have documented that fomite are responsible for high exposure in bacterial transmission, in hospitals, children's health centers, long-term care centers, and educational institutions and sports facilities (Bloomfield, 2017). Different types of microorganisms, including rotaviruses, rhinoviruses, Staphylococcus aureus methicillin-resistant,

and Serratia marcescens were identified to cause gastrointestinal disease, the common cold, necrotic fasciitis and the related bacteremia of catheters respectively (Bures et al., 2010).

Institutions of higher learning, being in the category of schools, have not been considered much when it comes to considering the vulnerable groups. In heavily visited places such as schools, hospitals, market places and malls and any other place where human traffic is heavy, the rate of contamination of inanimate objects is usually very high (Otter, Yezli & French, 2014). Infections can be indirectly acquired by contact between surfaces and the mouth, through contaminated fingers to mouth or hand-to-mouth, Hand-to-eye, or hand-to-nose contact or can be transmitted directly from contaminated devices or surface to humans or, less frequently, by aerosols, water, and/or foodstuff (Otter et al., 2014). Fluids like saliva, mucus, nasal secretions, blood, urine, and feces all can likely contain fomite pathogens (Otter et al., 2014).

Most fomite transmitted infections arise from products that are supposed to be sterile but are infected with pathogens (Barrie et al., 2014). The outbreak of population (community) acquired infections and nosocomial infections have been proven to be emanating from surface bio-contamination of fomites while in constant contact with human or natural environments of pathogenic organisms according to studies (Nwankiti et al., 2012).

Hidden microorganisms in indoor and outdoor sites are unavoidable and pose harmful health hazards in our different human activities. In recent years, apprehension has increased with the implementation of new technology in households, hospitals, industry and other settings (Eickhoff, 2014). There has been increased interest in assessing the risk of microbial types and pollution and is considered an important step towards infection prevention (Eickhoff, 2014).

In various indoor/outdoor settings, microbial contaminations are commonly documented. The bacterial contaminations of 50 public telephones in the City of Afyon, Turkey, were investigated by Tunc and Olgun (2016). Twelve different types of bacteria were present on the telephone surface, including *Escherichia (E) coli*, *Pseudomonas (P.) aeruginosa* and *Staphylococcus (S.) aureus*. Similar findings for hospital phones and personal pagers have also been recorded (Namias et al., 2010). Rutala et al. (2016) studied the scope, performance and cosmetic impacts of the disinfectant on the computer keyboards' levels of microbial contamination. Results showed that microbial on keyboard contamination were ubiquitous and disinfectant could clean up the contamination that was isolated and identified. Narmeen, Melo and Melo (2019) reported *S aureus* pathogen in multiple locations in the Azadi General Hospital with bacteriological contamination as well as molecular markers. Of the samples collected, patients, medical and hospital personnel just 52 isolates of 224 specimens were found to be *S. aureus* collected at different sites making up 23.21% of the overall isolates. *S. aureus* may normally cause infections in newborns, surgical, burns, diabetics, and those taking drugs to avoid immune deficiency disorders. Harrison et al. (2013) also reported that *Micrococcus luteus* and *Serratia marcescens* both have a distinctive colonial morphology on plate counts used. Results showed that bacteria zig-zag transfer between the distributors and hands can occur if either of these is contaminated. The possibility of cross-contamination of the hands, towels, and dispenser if any of these is infected has to be tackled (Harrison et al., 2013).

There have been several factors that influence the bacterial transfer rates from one surface to another. These involve the form of bacteria, source and target area, post-inoculation time and humidity level (Rusin, Maxwell & Gerba, 2012). The key factor influencing the transmission rate of opportunistic bacteria is the determination of the bacterial groups. It is against this context that this study aims at researching the types of opportunistic bacteria in a selected institution of higher education in Nandi County. These fomites include office door handles, toilet door handles, toilet water faucets, cistern handles and shopping baskets. Awareness of opportunity bacteria in various locations and particles can help to choose the necessary hygiene steps in order to remove possible cross contamination by the bacteria.

The notion that environmental microorganisms contribute to human disease comes from two facts: firstly, our contact with the inanimate environment is continuous and similar. Secondly, even though the prevalence of microorganisms in the ecosystem is fairly straightforward to determine, it is relatively difficult to establish the type of organisms that cause human disease in the environment (Rhame, 2012). Every year, 1.7 million deaths from diarrhea and 33,000 deaths from antibiotic resistant bacteria infections occur worldwide (Pruss-Ustun & Covalan, 2016). Bacteria cause an estimated 60% of human infections, and enteric bacteria develop the most common diseases (McElhaney, 2013). In comparison to the viral disease, the use of antibiotics will overcome bacterial diseases. Bacterial disease prevention and control relies heavily on antibiotics (McElhaney, 2013). Both antibiotics and antibacterial medicines only function 60% (McElhaney, 2013).

Cases of bacterial resistance to most common antibacterials have been documented to date. Furthermore, population growth and increased mobility have increased bacterial transmission and the challenge to interrupting the spread of diseases (Butcher & Ulaeto, 2015). Bacterial diseases control requires a good understanding of the environmental types of bacteria (Goldmann, 2010). For decades, bacterial diseases have been thought to be mainly transmitted by direct contact and the environment played

little or no part in the transmission of diseases (Cozad & Jones, 2013).

The perspectives on bacterial transmission have evolved over the years to include a more dynamic, multipurpose disease propagation model (Cozad & Jones, 2013). The spread of microbial infections includes infected fomites or surfaces (Springthorpe & Sattar, 2010). Therefore, the fundamental question is, what types and sensitivity of opportunistic bacteria that are present on fomites found in the selected post-secondary institution of higher learning. As a result, this study examined the types of opportunistic bacteria by isolating and characterizing them with the aim of determining their antibacterial sensitivity to various antibacterial preparations.

2. Literature Review

Humans exist in a world of microbes. In all the habitats we live in, there are viruses, bacteria, protists, fungi and archaea (Kelley & Gilbert, 2013). Humans, directly transport microbes into building areas (Adams, Bhangar, Pasut, Arens, Taylor & Lindow, 2015), from outside (Adams, Miletto & Taylor, 2013), into the indoor air and from our surroundings (Adams, Miretto & Taylor, 2013; Lax et al., 2017). The abundance and diversity of microbial in buildings or what is known as the indoor microbiome are affected by human activities, the environment outside, architecture and management (Adams, Bateman, Bik & Meadow, 2015). Many molecular analyses display a considerable variety of microbes on constructed surfaces. Most indoor microbes tend to be sleeping, inactive or dead (Gibbons, 2016), either have no known effect on human health or are likely to support human health (Lynch et al., 2014). Inanimate artifacts may be used as microbial reservoirs in the built environment. These objects contain a large array of bacterial, viral, archaeal, protist and fungal species including possible pathogens and human-hazardous microbial metabolic products.

Many micro-organisms originating from other environments are usually considered impossible to live on indoor surfaces that lack abundant moisture and nutrients. These viable microbes that survive are usually considered to be inactive or dormant until moisture and nutrients help it proliferate or are moved to different places in the host (Gibbons et al., 2015). Surveys carried out with high throughput molecular sequences of fungal populations in indoor environments have shown that they are mainly powered by transportation from the local outside environment (Adams, Miletto, Taylor & Bruns, 2013).

Similar studies of buildings and surfaces with a higher human occupancy as well as frequency of encounters have, nevertheless, reported elevated levels of skin related bacteria (Adams, Bateman, Bik & Meadow, 2015). The efforts made to trace the sources of the bacteria that lie on different indoor surfaces have also been provided. Urine and feces bacteria were more popular on toilet seats and lavatory handles than on other surfaces (Flores, 2011). Fresh produce bacteria have been shown to be more prevalent in kitchen counters and refrigerators (Flores, 2011). In the interior and exterior door trims of doors which open outside domestic surfaces locations are more frequently associated with bacteria associated with leafs and soil (Dunn, Fierer, Henley, Leff & Menninger, 2013). In comparison, rich microbial biofilms in baths and kitchens may form communities closely similar to those found in plumbing and water reservoirs on surfaces, which frequently have high humidity levels (Kelley, Theisen, Angenent, St. Amand & Pace, 2014).

Lax et al. (2014) showed evidently that on some surfaces, but not on others, bacterial communities on different surfaces in an individual home have clear similarities (Lax et al., 2014). Moreover, as families moved into houses, the bacterial composition of the new bacterial population converged on the surfaces of the new house quickly into that of surface bacteria, which indicates that new inhabitants rapidly deposited in the new space their own special signatures of related human bacteria. While in recent years a great deal has been revealed on microbial communities in indoors, bacterial communities and fomites' kind of bacteria are much less known (Prussin, Garcia & Marr, 2015). However, a great deal needs to be known about the types of fomite bacteria that raise concerns about transmission of infectious diseases and other new microbial threats.

3. Methodology

Experimental research design was employed in this study. The analysis was carried out in University of Eastern Africa Baraton in Nandi County, Kenya. The study was carried out in regularly contacted places like door knobs, faucet handles and cistern handles. Purposeful sampling technique was used. Stratified sampling technique was used to divide the population of fomites in subgroups (or strata) within the University and, due to the big number of the sampling sites, 20% of all the sites were selected randomly to improve the accuracy and representativeness of the results by reducing sampling bias as the burden is lessened (Krejcie & Morgan, 2010). Samples were collected from the office doors, classroom doors, toilet doors, shopping baskets and toilet water faucet handles in different buildings of all the buildings within the learning and students' halls of residence. There were 1827 sampling sites in the university and only 20% were used giving a total of 365 swabs. The materials and instruments used in the study included gloves- as a protective wear to ward off contamination while collecting samples, sterile swabs- for sample collection through swabbing, distilled water- for media preparation, bacterial culture media -for culturing the samples and sterile petri dishes and tubes - for containing the requisite media. 365 swabs were

obtained in different facilities inside the University by swabbing of the toilet cistern handles, office doors faucets and shopping baskets (sterile swabs moistened with buffered peptone water). They were then correctly labelled with reference numbers and transported in peptone water transport medium to the Biology Laboratory. The samples were suspended in buffered peptone water and incubated for a period of 18-24 hrs. The obtained growth marked by turbidity were inoculated in the blood agar, MacConkey Agar and Nutrient Agar and then incubated at 35°C. MacConkey agar and Nutrient agar were used to isolate coliforms in Swabs and bacteria of public health significance. These helped in determining the types of bacteria in each site.

The isolates of bacteria were subjected for the purposes of differentiating gram negative and positive bacteria with standard methods of microbiology, such as morphological characteristics of the colonies. Biochemical studies were conducted on the isolates for further identification and characterization. The isolates' morphological and biochemical characteristics were compared to the Bergey's Determinative Bacteriology Manual (1994).

The data was analyzed using SPSS Version 26. Descriptive statistics was used to analyze the bacteria types isolated from fomites within the University buildings. All values were expressed as means and findings were presented in the form of frequency tables. Clearance for the study was sought from the University of Eastern Africa Baraton Review Ethics committee. Thereafter, the researcher got clearance from NACOSTI. Privacy and confidentiality was highly maintained during the research process. Unique numbers were given to each building for the purpose of confidentiality.

4. Results and Discussion

4.1 Isolation and Characterization of Bacteria Present in the Fomites

Pure cultures were obtained from the 231 samples of bacteria that grew on the nutrient agar by isolating individual colonies with streak plate technique using an inoculating loop to streak colonies on nutrient agar plates in one of several patterns. Successful isolation depended on spatial separation of single colonies. The isolates of bacteria were then subjected for the purposes of morphological characterization based on gram staining. Gram staining was done, followed by microscopic examination under oil immersion. This was done to identify the general type of bacteria and classify bacteria for further identification tests. The study found out that 84.6% of isolates from faucets were gram positive cocci with only 15.4%-gram negative cocci. 83.8% of the isolates from cisterns were gram positive cocci as compared to only 8.1%-gram negative cocci and rods. 78.9% of the isolates from doors were gram positive cocci as compared to 0.7%-gram positive rods, 17.6%-gram negative cocci and 3.5%-gram negative rods. Specifically, the results indicate that cisterns had the greatest number of gram-positive cocci followed by faucets and lastly doors. The results indicate that doors had the greatest number of gram-negative cocci bacteria followed by faucets and cisterns. Human hands usually harbor microorganisms both as part of body normal flora as well as transient microbes contacted from the environment and given that people move from place to place they must handle doors leading to high presence of gram-negative bacteria (Abdulwasiu et al., 2022). Cisterns had the greatest number of gram-negative rod bacteria as compared to doors and this was attributed to high humidity levels in cisterns as pointed by Kelley, et al, (2014). Lastly, isolates from the door had 1-gram positive rod bacteria. However, faucets did not have gram negative and gram-positive rods. For further characterization of the bacterial isolates, biochemical characterization was conducted.

4.2 Biochemical Characterization of Gram-Positive Bacteria

Gram positive bacterial isolates were subjected for the purposes of characterization based on biochemical reaction as per the standard methods of microbiology. Selective biochemical tests were conducted on the isolates. These included differential growth in Blood Agar, catalase, coagulase, MSA and oxidase reactions. The isolates' biochemical characteristics were compared to the Bergey's Determinative Bacteriology Manual (1994).

The gram-positive contaminants were determined based on their differential growth in blood agar. 188-gram positive isolates from nutrient agar were inoculated aseptically in blood agar and then incubated at 35°c based on fomite of collection. These helped in testing the ability of the bacteria to produce hemolysins which are enzymes that lyse the erythrocytes. The degree of hemolysis differentiated *Staphylococcus* bacteria, *Streptococcus* bacteria and *Enterococcus* bacteria from each other.

The results showed that 56.8%, 65.6% and 75% of the isolates from faucets, cisterns and doors respectively, exhibited beta-hemolysis on blood agar. On the other hand, with 34.1%, 25% and 7.1% of the isolates from faucets, cisterns and doors respectively exhibited alpha-hemolysis on blood agar. However, 9.1%, 9.4% and 17.9% of the isolates from faucets, cisterns and doors respectively exhibited gamma-hemolysis on blood agar. The results gave a general suggestion that the isolates contained *Staphylococcus, Streptococcus* and *Enterococcus* bacteria. To confirm the presence of *Staphylococcus, Streptococcus* and *Enterococcus* bacteria, the researchers proceeded with further characterization and conducted biochemical characterization on the isolates.

4.3 Catalase Test

The Catalase-test was used to differentiate between *Staphylococcus* which are catalase-positive from *Streptococcus* which are catalase-negative. The study findings showed that 21.8%, 17% and 60.5% of the gram-positive bacteria obtained from the faucets, cisterns and doors were positive to catalase test hence this confirmed them to be *Staphylococcus spp*. However, 0.7% of gram positive bacterial isolate obtained from cistern was catalase negative confirming it to be *Streptococcus pyogenes*. Therefore, from the gram positive bacterial isolates, one bacterial isolate obtained from cisterns was *Streptococcus pyogenes*.

4.4 Coagulase test

Coagulase test was used to identify the *Staphylococci* where *S. aureus* is a coagulase-positive and *S. epidermidis* is a coagulase-negative bacteria species. The isolates' biochemical characteristics were compared to the Bergey's Determinative Bacteriology Manual (1994). The results indicated that 21.9%, 16.4% and 57.5% of the gram positive bacteria were coagulase positive which was a confirmation that they were *S. epidermidis*. However, 0.7% and 3.4% of the gram positive bacterial isolate obtained from cistern were coagulase negative confirming them to be *Streptococcus aureus*. Therefore, from the gram positive bacterial isolates obtained from faucets, cisterns and doors were *Streptococcus epidermidis* and *Streptococcus aureus*.

4.5 Biochemical Characterization of Gram Negative Bacteria

Gram negative bacterial isolates were subjected for the purposes of characterization based on biochemical reaction as per the standard methods of microbiology. Selective biochemical tests were conducted on the isolates. These included growth on MacConkey Agar, Chocolate agar, Blood agar and Eosin Methylene Blue agar and reactions with IMVic, Nitrate, Oxidase and Catalase media. The isolates' biochemical characteristics were compared to the Bergey's Determinative Bacteriology Manual (1994). The isolates were confirmed to be gram negative by growth on MacConkey agar and morphological analysis. The isolates were inoculated aseptically on MacConkey agar and then incubated at 35°c based on fomite of collection. Results were observed under microscope and morphological analysis were conducted through gram staining to confirm the morphology of the gram-negative bacteria based on the fomite of collection.

The results pointed out that the majority of gram-negative isolates were cocci (81.8%) as compared to rods which were 18.2%. However, faucets had only gram negative cocci while cisterns and doors had both gram negative cocci and rods. Faucets had 18.2%-gram negative cocci, cisterns had 3%-gram negative cocci and gram-negative rods and doors had 56.8%-gram negative cocci and 11.4%-gram negative rods.

4.6 Biochemical Reaction of Gram-Negative Rods

Indole, Methyl red (MR), Voges-Proskauer (VP), and Citrate utilization tests (IMViC) tests and was used to identify the bacteria from the 3 gram negative rods isolates from cisterns and 5 gram negative rods isolates from doors. This is a set of tests used for the differentiation of the Enterobacteriaceae family. The results showed that all the gram-negative rods isolates had a significant growth on the test media. Additionally, all of them were indole and MR positive, VP and Citrate negative. The results of the IMViC test was a confirmation that the gram negative rod isolates were coliform bacteria. To isolate fecal coliforms, EMB (Eosin Methylene Blue) agar was used. On EMB, colonies appeared either coloured or colourless indicating their fermentation of lactose or sucrose and showed whether the isolates were fecal coliforms or not. All the isolates produced a green metallic sheen on EMB and hence were identified as *Eschrichia coli.*

4.7 Biochemical Reaction of Gram-Negative Cocci

The identification of gram negative cocci was done based on the biochemical reaction. Nitrate reduction, catalase, DNase and Oxidase were the biochemical tests performed. The gram negative cocci bacteria were differentiated using DNase and how they grew on nutrient agar at 35℃. The results pointed out that out of 36 isolates, all of them had significant growth. The primary tool which was used for identification was morphology of the colonies. The isolates were grown well on chocolate agar and blood agar. On blood agar, all the colonies ranged from gray to white and 1-3 mm in diameter after they were incubated for 24 hours. The colonies were pinkish brown on chocolate agar. With their large kidney shape, the isolates were identified as *Morexella catarrhalis.* They were all positive for oxidase, DNase, and catalase tests and they also reduced nitrate to nitrite.

iv. Conclusion and Recommendation

• Conclusion

The study concluded that cisterns had the greatest number of gram positive cocci followed by faucets and doors. Doors had the greatest number of gram negative cocci bacteria followed by faucets and cisterns. Cisterns had the

greatest number of gram negative rod bacteria as compared to doors. Doors had gram positive rod bacteria. However, faucets did not have gram negative and gram positive rods. The gram positive bacterial isolates were *Streptococcus pyogenes*, *Streptococcus epidermidis* and *Streptococcus aureus*. The gram negative bacterial isolates were *Eschrichia coli* and *Morexella catarrhalis*.

5.2. Recommendations

The study recommended that there was need for further identification and characterization of the isolates to be conducted using a different method to confirm the presence of any other bacterial types that might be obtained from the fomites.

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