JRIIE Journal of Research Innovation and Implications in Education
Center for Research Implications & Practice

Website:www.jriiejournal.com ISSN 2520-7504 (Online) Vol.6, Iss.2, 2022 (pp. 382 - 388)

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*

How to cite this work (APA):

Magondu, R. N., Ramesh, G. P. & Kiprono, S. J. (2022). Isolation and Identification of Bacteria Present on Frequently Used Fomites in University of Eastern Africa Baraton in Nandi County, Kenya. *Journal of Research Innovation and Implications in Education, 6*(2), 382 – 388.

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.

5. Conclusion and Recommendation

5.1. 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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