IDENTIFICATION, CHARACTERIZATION AND ANTIMICROBIAL PROFILING OF *Staphylococci aureus* ISOLATED FROM HIGH-CONTACT SURFACES IN KISII UNIVERSITY

BY:

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A RESEARCH PROPOSAL SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE IN BIOMEDICAL SCIENCE AND TECHNOLOGY OF THE DEPT OF APPLIED HEALTH SCIENCES, SCHOOL OF HEALTH SCIENCES, KISII UNIVERSITY

DECLARATION

DECLARATION BY THE CANDIDATE

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

EMMANUEL OCHIENG' OWINO Sign: _____Date: _____

Reg. No**: HE12/00026/21**

DECLARATION BY THE SUPERVISORS

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

Dr. ERIC O. OMWENGA, PhD Sign: _____Date: _____

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DEDICATION

This work is dedicated to my dear guardian, Chrispine Owino and Cynthia Opiyo for their tireless effort to see me up to this stage. God bless you.

ACKNOWLEDGEMENTS

First and foremost, I give all glory and honour to God Almighty for granting me wisdom, strength, and good health throughout this research journey. His guidance and grace have been my source of inspiration and perseverance.

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ABSTRACT

High-contact surface-associated infections remain one of the leading causes of increased morbidity and mortality globally amongst individuals in public settings. Although many pathogenic microbial species are common in the environments, their distribution, frequency, and antimicrobial susceptibility patterns from high-touch surfaces, remain largely unknown. All surfaces in public settings despite being visibly clean always are contaminated with invisible residues for example, body fluids such as blood, urine, saliva and mucus which can remain on the surfaces in the environment in very small microscopic amounts, posing a serious risk on the transmission and spread of infections causing pathogens. Therefore, apparent purity does not always correlate with microbiological purity. Surfaces that are considered clean may be microbiologically contaminated and constitute a reservoir of infectious agents. The risk of infections is related to microbial contamination of the surface by Gram-negative bacteria, such as *Acinetobacter*, Gram-positive such as *Staphylococcus aureus*, viruses, such as *Coronavirus*, *Norovirus* and *rotaviruses*, and fungi such as *Candida albicans*. By integrating standard bacteriological procedures; 200 samples will be collected from the high-touch surface using swabs and will be inoculated on nutrient agar, and incubated at 37 °C for 24 hours. Integrating the various bacterial species identification methods using the morphological characteristics, Gram stain, biochemical tests (catalase, oxidase and coagulase) and antimicrobial susceptibility tests using modified Kirby-Bauer disk diffusion technique following the Clinical Laboratory Standards Institute 2024 guidelines will be applied as used before. Therefore, this study aims at assessing the prevalence of microorganisms on high-touch surfaces, identify them and profile their antimicrobial susceptibility patterns of bacterial isolates from high contact surfaces. Data obtained from this study will be analyzed using Graph-Pad Prism software and will be presented as Mean ±SD as the experimental work will be done in triplicates that will be independent of each other.

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LIST OF ABBREVIATION AND ACRONYMS

ABB – Antibacterial Biofilm

AMR – Antimicrobial Resistance

ATCC – American Type Culture Collection

BHI – Brain Heart Infusion

CAI – Community-Acquired Infection

CLSI – Clinical Laboratory Standards Institute

CFU – Colony-Forming Units

DNA – Deoxyribonucleic Acid

HAI – Healthcare-Associated Infection

IRB – Institutional Research Board

KSH – Kenyan Shillings

KSUERC – Kisii University Ethics Review Committee

MDR – Multidrug-Resistant

MRSA – Methicillin-Resistant *Staphylococcus aureus*

MSSA – Methicillin-Sensitive *Staphylococcus aureus*

PBS – Phosphate-Buffered Saline

SARS – Severe Acute Respiratory Syndrome

SD – Standard Deviation

SPSS – Statistical Package for the Social Sciences

TSB – Tryptic Soy Broth

UV – Ultraviolet

VHP – Vaporized Hydrogen Peroxide

VRE – Vancomycin-Resistant *Enterococcus*

CHAPTER ONE
INTRODUCTION

1.0 Background of the Study

High-touch surfaces are frequently contacted by students, lecturers, workers, patients, and visitors, which may be a reservoir for pathogens and a source for transmission of surface-associated pathogens, leading to multiple outbreaks of surface-acquired infections (Weber & Rutala, 2013). Environmental contamination has been documented to be the main contributor towards bacterial transmission more so when individuals contaminate their hands or gloves by touching contaminated objects, or when patients come into direct contact with contaminated surfaces (Weber & Rutala, 2013).

The transmission of pathogenic microorganisms via high-contact surfaces is a growing concern in modern infection control, particularly in light of recent global pandemics and the increasing prevalence of surface-contracted infections. High-contact surfaces, often referred to as "fomites," are common in both public and private spaces, ranging from hospital beds and waiting room chairs to frequently touched items in households like light switches and TV remotes (Boyce, 2016).

These surfaces can become contaminated with microorganisms through direct contact with infected individuals or indirectly through airborne droplets and hands carrying pathogens. Once contaminated, these surfaces act as vectors for the transmission of infections, especially in high-risk environments such as hospitals, schools, and public transport systems (Rusin et al., 2020).One of the critical aspects of surface contamination is the resilience of various microorganisms, including viruses like *SARS-CoV-2*, which can remain viable on surfaces for extended periods under certain conditions (van Doremalen et al., 2018).

Bacteria such as *Staphylococcus aureus, Escherichia coli, Enterococcus, Streptococcus, Acinetobacter, Salmonella, Shigella Klebsiella pneumonia, Proteus, and Pseudomonas species*, which are known to cause opportunistic infections, have also been Isolated from high-contact surfaces, especially in hospital environments where antibiotic-resistant strains are prevalent (Kramer et al., 2021).

All surfaces in the public settings despite being visibly clean i.e., free of visible residues, e.g., body fluids, they are not microbiologically clean for several

microorganisms thrive on these surfaces. However, apparent purity does not always correlate with microbiological purity. Surfaces that are considered clean may be microbiologically contaminated and constitute a reservoir of infectious agents. The risk of infections is related to microbial contamination of the surface by Gram negative bacteria, e.g., *Acinetobacter*, Gram positive, e.g., *Staphylococcus aureus*, viruses, such as *corona-*, *noro-* and *rotaviruses*, and fungi, e.g., *Candida*.

Even a single contact of human skin with a contaminated surface can contribute to the transmission of these pathogens. The most easily transmitted diseases from inanimate surfaces to the skin are: *Escherichia coli*, *Salmonella spp*., *Staphylococcus aureus* (100% of cases), *Candida albicans* (90%), *rhinoviruses* (61%), HAV (33%) and *rotaviruses* (16%) (Weber & Rutala, 2013). Therefore, Microorganisms on the hands can be transferred to various surfaces, from which they can re-infect other people. The risks associated with contaminated surfaces cannot be overlooked given the very low hand-washing compliance rate among individuals in the public space (Weber & Rutala, 2013). Therefore, the identification of these potentially pathogenic microorganisms on surfaces is essential for understanding their role in the spread of infections and for developing targeted cleaning protocols to reduce contamination.

This is compounded by the fact that we are currently experiencing, increasing concern surrounding antimicrobial resistance (AMR) compounds the risks associated with high-contact surfaces. Pathogens such as multidrug-resistant *Acinetobacter baumannii* and Vancomycin-resistant *Enterococcus* (VRE) are particularly concerning due to their ability to survive on surfaces and resist conventional disinfection methods (Neely & Maley, 2017).

Identifying these organisms and understanding their survival dynamics on different surfaces are crucial in mitigating the spread of resistant strains, which pose a serious threat to public health. This study seeks to systematically investigate high-contact surfaces in various settings, identify the microorganisms present, and assess their pathogenic potential. The results will provide insights into the contamination patterns and persistence of pathogenic microorganisms, with a focus on improving sanitation measures and preventing the spread of surface-borne infections. As high-contact surfaces continue to be a major route of microbial transmission, the outcomes of this

research will have implications for public health policies, particularly in the design of cleaning protocols, disinfection strategies, and infection control practices.

1.1 Statement of the Problem

Staphylococcus aureus is a common, adaptable pathogen capable of surviving on various surfaces, posing significant health risks, especially within shared environment like Kisii University. Kisii University has a high density of foot traffic and human interaction on its surfaces, particularly in areas such as restrooms, desks, door handles, elevator buttons, computer keyboards, and laboratory equipment are frequently used by students, staff, and visitors, creating potential hubs for microbial contamination and pathogen transmission to students, visitors, and staff.

Studies have consistently shown that high-contact surfaces are often contaminated with *Staphylococcus aureus*, with variations in prevalence depending on maintenance and hygiene practices. However, specific data on the prevalence of this pathogen on surfaces within Kisii University remains limited. Identifying the presence of *S. aureus* will help assess the risk level of pathogen exposure and inform the university's public health response.

Staphylococcus aureus, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), poses a growing public health challenge due to its role in antibiotic resistance and its ability to cause various infections (Gunnarsdóttir & Briem, 2014). The persistence of this pathogen on frequently touched surfaces can contribute to nosocomial infections and accelerate the spread of antimicrobial-resistant strains within the university environment. Investigating the epidemiology of *S. aureus* will provide insight into its contribution to antimicrobial resistance, disease occurrence, and the potential for outbreaks.

The spread of antimicrobial-resistant strains like MRSA leads to increased healthcare costs, as treatments become more complex, and hospital stays are prolonged hence straining resources (Boyce, 2007).This economic burden impacts both healthcare facilities and patients. Identifying the presence of *S. aureus* within Kisii University could emphasize the need for preventive measures, which may help reduce healthcare costs and alleviate the strain on hospitals and affected individuals.

Previous studies done elsewhere (India, USA, Ethiopia and greater parts of Kenya respectively), targeting *Staphylococcus aureus* isolated from high-contact, have documented the prevalence of *Staphylococcus aureus* (50 %) on high-contact surfaces particularly in areas such as hospitals, schools, and public transportation where frequent human contact increase the risk of contamination and transmission (Otter & French 2009; Dancer, 2014). However, to the best of my knowledge, there are no published reports examining the presence of *Staphylococcus aureus* on high-contact surfaces specifically at Kisii University. Therefore, this study aims to fill the gap by investigating the characterization, antimicrobial & antibiofilm profiles and distribution of *Staphylococcus aureus* isolates obtained from high-contact surfaces within Kisii University.

1.2 Justification

High-contact surfaces, such as door handles, handrails, and shared equipment, can serve as reservoirs for the transmission of Staphylococcal infectious. Identifying *Staphylococcus aureus* on these surfaces is crucial to ensure the health and safety of students, faculty, and staff.

By identifying and addressing the presence of *Staphylococcus aureus*, Kisii University can implement appropriate infection control measures. This may include increased cleaning and disinfection protocols, isolation of affected areas, or even temporary closure of facilities if necessary. Early identification helps prevent the spread of infections and protects vulnerable individuals.

Universities have a responsibility to maintain a safe and healthy environment for their community. Identifying potentially pathogenic microorganisms on high-contact surfaces aligns with regulatory requirements and standards set by health authorities. Failure to address such issues may result in legal consequences or loss of accreditation.

The identification of *Staphylococcus aureus* on high-contact surfaces allows Kisii University to take prompt action to mitigate the risk of infection. By addressing these issues proactively, the university can minimize disruptions to academic operations and ensure continuity of education.

The identification of *Staphylococcus aureus* provides an opportunity for research and innovation within the field of microbiology and public health. Kisii University can collaborate with external partners to study the prevalence, characteristics, and transmission dynamics of these microorganisms. This research can contribute to the development of new detection methods, treatment options, or preventive strategies.

1.3 Study Objectives

1.3.1 General Objective:

To identify, characterize and elucidate the antimicrobial resistance patterns of potentially pathogenic *Staphylococcus aureus* bacterial isolates on high-contact surfaces.

1.3.2 Specific Objectives:

a. To deduce the prevalence and distribution of *Staphylococcus aureus* bacterial isolates from high-contact surfaces of Kisii University.
b. To determine the resistance profiles of the isolated *Staphylococcus aureus* bacterial isolates from high-contact surfaces of Kisii University.
c. To establish the biofilm formation ability of the resistant *Staphylococcus aureus* bacterial isolates obtained from high contact surfaces of Kisii University.

1.4 Research Questions

a. What is the prevalence and distribution of *Staphylococcus aureus* on high contact surfaces of Kisii University?
b. What are the antimicrobial susceptibility patterns of *Staphylococcus aureus* isolates from these surfaces of Kisii University and there signs of antibiotic resistance?
c. How capable are *Staphylococcus aureus* isolates from high-contact surfaces of Kisii University of forming biofilms?

1.5 Limitations of the Study

The study might not cover all high-contact surfaces, as some locations may be overlooked or difficult to access, leading to an incomplete representation of *S. aureus* contamination across the university. Contamination levels per site of sample collection may vary depending on the time of day or day of the week due to fluctuating human traffic and cleaning schedules. A single sampling period may not capture this variability.

Testing for resistance may be limited to a select number of antibiotics, potentially overlooking resistance to newer or less commonly used drugs. Results of *in vitro* susceptibility testing may not perfectly predict in vivo efficacy. Assay sensitivity and accuracy can vary based on the methods and materials used, potentially affecting the consistency of biofilm quantification. Findings from Kisii University may not be generalizable to other settings with different environmental factors, sanitation practices, or microbial communities. This limits the ability to apply the results broadly to other institutions.

CHAPTER TWO

LITERATURE REVIEW

High-contact surfaces, commonly referred to as frequently touched objects, are critical in the transmission of pathogens, particularly in environments with high human interaction. These surfaces include restrooms, door handles, light switches, elevator buttons, countertops, and shared equipment, which facilitate the transfer of microorganisms from person to person or between surfaces (Weber & Rutala, 2013).

Globally, *S. aureus* has been extensively studied on high-contact surfaces in healthcare, transportation hubs, schools, and other communal spaces. In North America and Europe, MRSA has become particularly notable on hospital surfaces due to its association with healthcare-associated infections (HAIs) and community-acquired infections (CAIs) (Otter et al., 2015; Kourtis et al., 2019). Studies by Boyce (2013) and Dancer (2014) found *S. aureus* contamination levels as high as 40-60% on surfaces such as bed rails, patient charts, and medical devices, linking this presence to hand hygiene lapses among healthcare workers. The World Health Organization (WHO) has since emphasized regular surface disinfection, citing *S. aureus* contamination as a major infection control challenge worldwide (WHO, 2016).

In Africa, studies show that contamination rates on high-contact surfaces are comparable to those in high-income countries but are complicated by limited resources and often insufficient infection control measures (Aiken et al., 2014). Moyo et al. (2019) found that 75% of tested surfaces in Nigerian hospitals were contaminated with pathogens, with *S. aureus* as one of the predominant bacteria. Limited studies in South Africa, Nigeria, and Ghana reveal significant challenges related to cleaning infrastructure, which further exacerbates surface contamination risks. This has been linked to increased rates of HAIs in these regions, highlighting the need for standardized disinfection protocols (Amissah et al., 2020).In Uganda and Tanzania, studies in public health centres have reported high rates of *S. aureus* persistence on surfaces such as IV stands, shared beds, and public bathrooms, often due to challenges in maintaining routine cleaning with appropriate disinfectants (Mongodin et al., 2017).

In Kenya and neighbouring countries, research on high-contact surfaces shows *S. aureus* prevalence rates of 40-70% in hospitals and public institutions, with both methicillin-sensitive *S. aureus (*MSSA) and MRSA strains identified. Kariuki et al. (2018) highlighted a lack of consistent disinfection routines, particularly in rural settings, as a primary driver of surface contamination. Research specific to Kisii is limited, though a study by Ogola et al. (2021) in similar rural Kenyan regions demonstrated that high-contact surfaces are frequently contaminated with *S. aureus*. Given healthcare limitations and lower economic resources, Kisii presents an area of interest for future studies to improve understanding of regional surface contamination dynamics and infection control needs.

High-contact surfaces are ideal reservoirs for pathogens such as *S. aureus, Escherichia coli*, and *Klebsiella pneumoniae*. Studies consistently indicate that surfaces like door handles, bed rails, and shared equipment often test positive for these pathogens (Humphreys, 2016). The material composition of high-contact surfaces plays a significant role in determining the survival and growth of microorganisms. Studies have shown that smooth, non-porous surfaces, such as plastic and stainless steel, provide a more conducive environment for microbial survival compared to porous surfaces like wood or fabric (Kramer et al., 2006). However, advancements in material science have led to the development of antimicrobial surfaces that can actively reduce microbial contamination. Copper, for example, has been found to possess natural antimicrobial properties, significantly reducing the survival of bacteria and viruses on its surface (Warnes et al., 2023). These antimicrobial surfaces have shown promise in healthcare settings, where reducing surface contamination is a priority. Pathogens' ability to persist on surfaces for extended periods significantly contributes to their role in disease transmission. The survival time of microorganisms depends on factors such as the type of surface, environmental conditions (humidity, temperature), and the characteristics of the pathogen itself. For example, Kramer et al. (2006) found that bacteria like *Staphylococcus aureus* can persist for days to weeks on non-porous surfaces such as plastic and metal, while *Escherichia coli* and *Enterococcu*s spp. Can survive for similar durations.

A review by Otter and French (2010) found that 55-85% of high-touch surfaces in healthcare facilities across Europe and the U.S. harboured various pathogens, with *S. aureus* identified on 70% of these surfaces. In school settings, *S. aureus* contamination was documented on desks, chairs, and other shared surfaces, often with strains capable of causing skin infections and respiratory issues (Becker et al., 2018).

In healthcare facilities, studies report *S. aureus* as a prominent contaminant, especially in emergency rooms, surgery wards, and waiting areas, with prevalence rates ranging between 60-80% on surfaces in high-patient-volume areas (Ateba et al., 2019). This underscores the importance of surface hygiene in preventing the spread of *S. aureus* and other infectious agents in these settings. The spread of antimicrobial-resistant *S. aureus* strains, particularly MRSA, remains a global challenge. Chambers and DeLeo (2022) documented a substantial increase in multidrug-resistant *S. aureus* isolates on high-contact surfaces, with resistance extending to fluoroquinolones, cephalosporins, and even linezolid in some cases. Worldwide studies have observed reduced efficacy of disinfectants due to resistance, especially among biofilm-forming *S. aureus* strains, which complicates surface decontamination efforts (Weber et al., 2016).

In African institutions, studies have highlighted high resistance levels among *S. aureus* isolates to commonly used antibiotics like penicillin, erythromycin, and clindamycin (Aiken et al., 2014). In Kenyan hospitals, MRSA prevalence is reportedly high, with strains showing resistance to tetracycline, sulfamethoxazole, and macrolides, raising significant concerns for patient safety and infection control (Ogola et al., 2021).

The high prevalence of resistant *S. aureus* strains in East Africa underscores the need for better AMR surveillance and more stringent antibiotic stewardship practices, especially in environments where high-contact surface contamination is prevalent (Kariuki & Dougan, 2018).Biofilm formation by *S. aureus* is a well-documented phenomenon, especially on medical devices, high-contact surfaces, and in healthcare settings. Biofilms allow bacteria to persist longer on surfaces and resist removal through standard cleaning and disinfection, complicating infection control (Donlan & Costerton, 2017).Studies in healthcare settings have shown that biofilm-producing *S. aureus* isolates are more resistant to antimicrobials and disinfectants, increasing the

risk of HAIs. This is particularly concerning in regions where disinfection resources are limited and cleaning practices are suboptimal (Rupp & Archer, 2021).

In African settings, the prevalence of biofilm-producing *S. aureus* strains has been linked to increased infection rates, particularly in resource-constrained healthcare facilities where disinfectant efficacy may be compromised (Ateba et al., 2020). These findings suggest a need for disinfection methods capable of disrupting biofilms, such as enzymatic cleaners or physical removal through rigorous scrubbing.

Effective cleaning and disinfection are essential in managing surface contamination, particularly in environments with high foot traffic. Dancer (2009) emphasized that routine cleaning may be insufficient to eliminate pathogens from surfaces, particularly in healthcare settings where vulnerable patients are at risk of infection. The choice of disinfectant, contact time, and concentration play crucial roles in the efficacy of cleaning protocols. For example, Rutala and Weber (2016) found that bleach-based disinfectants are highly effective against pathogens such as *Clostridium difficile,* while quaternary ammonium compounds are more effective against other bacteria and viruses. The implementation of these protocols in high-contact areas is vital for curbing the growth and survival of these pathogenic microorganisms on the high contact surfaces (Kramer et al., 2006).

Alcohol-based solutions, quaternary ammonium compounds, and chlorine-based disinfectants are commonly used on high-contact surfaces in healthcare settings. However, biofilm formation and increasing microbial resistance have raised concerns about the efficacy of these disinfectants, especially in settings with frequent use (Dancer, 2014; Weber et al., 2016). Advanced disinfection methods, including UV light and vaporized hydrogen peroxide (VHP), have shown promising results against resistant *S. aureus* and other pathogens, though their use is often limited to high-resource settings due to cost and availability constraints (Rutala & Weber, 2013).

In African institutions, disinfectants such as bleach and alcohol-based cleaners are commonly used. Studies report variable efficacy, often affected by inconsistent application and lack of access to higher-efficacy disinfectants like VHP (Moyo et al., 2019). Kenyan hospitals have inadequate staff training and frequent lapses in cleaning

protocols hence contribute to persistent *S. aureus* contamination (Ogola et al., 2021). There is growing interest in alternative and sustainable disinfection strategies for settings with limited resources. For example, a study on electrostatic sprayers and UV light in South African hospitals demonstrated significant reductions in surface contamination, suggesting potential applications for similar interventions in Kenya and other East African regions (Msimanga et al., 2022).

Despite advancements in cleaning technologies and molecular identification methods, challenges remain in fully controlling surface contamination. The persistence of certain pathogens, the rise of antimicrobial resistance, and the variability in cleaning efficacy all contribute to ongoing infection risks. Future research should focus on optimizing disinfection protocols, developing more effective antimicrobial surface materials, and enhancing our understanding of how environmental factors influence microbial survival on surfaces (Weber & Rutala, 2013).

CHAPTER THREE

METHODOLOGY

3.1 Study Area

The study will be conducted in Kisii University, Kisii county, Kenya (0º40´49.7352"S and 34º46´37.4196”E), encompassing areas such as the Academic blocks; Science complex, Tuition complex, Sakagwa, Amphitheatre, Library, Lecture halls, Laboratory and the Medical annex. The study area is located in Kisii town, in the South Western Kenya region. This region was selected due to its high probability of visitations by students, visitors and university’s staff on daily basis, offering a diverse range of socioeconomic and demographic characteristics that provide a comprehensive context for understanding the interactions between the individuals and the high contact surfaces.

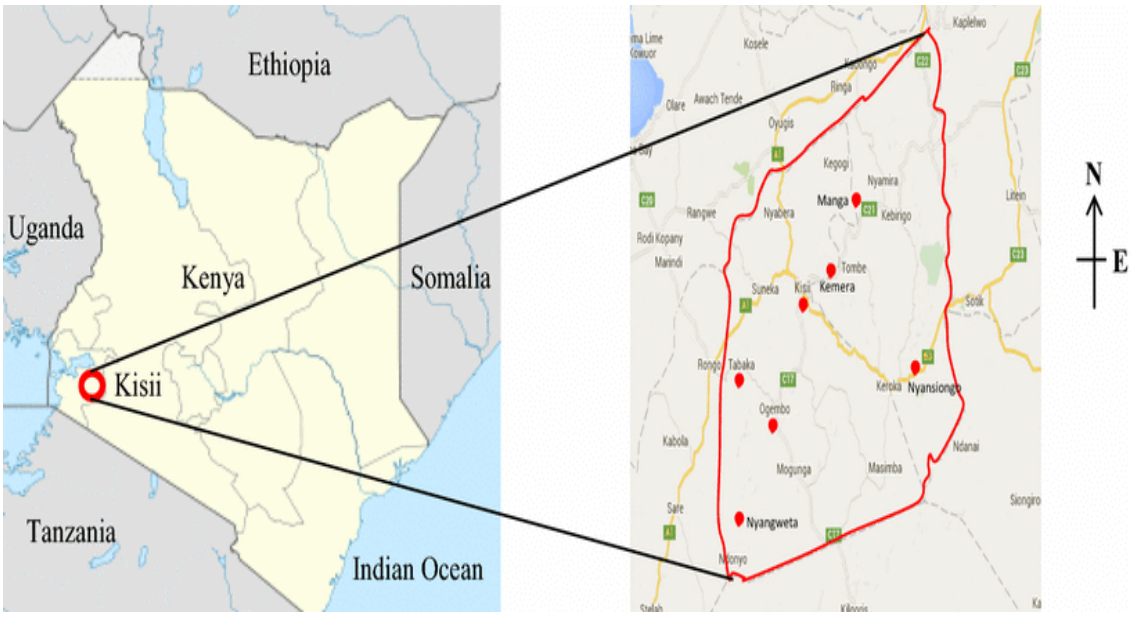

FIGURE 1: KENYAN MAP: [SOURCE: HTTPS://MAPS-KENYA-KE.COM

3.2 Study Design

The study will employ a cross-sectional study design as used before (Cohen et al 2007). It will integrate laboratory experimental works that will involve culturing and identification of *Staphylococcus aureus* microorganisms swabbed from the high contact surfaces within the targeted sites within Kisii University. This design is ideal

because enables the collection of data at a single point in time, to estimate the level of contamination on high contact surfaces, allowing for the examination of associations between variables, such as prevalence, geographic location, nature of the surfaces and type of microorganisms, without requiring follow-up. The approach is well-suited for identifying trends and correlations, providing a snapshot of the current state of the microbial prevalence in the study sites.

3.3 Sample Population

The study will focus on surfaces that presumably exhibits a high frequency in its contact with human individuals at selected sites within Kisii University. The samples will include those attained from all across the contact surfaces regardless of the frequency of contact in the day and period of collection. This diverse samples is crucial for analysing distribution patterns of microorganisms in the aforementioned sites and identifying the most prevalent species. These insights can guide targeted control or management strategies and public health interventions.

3.3.1: Inclusion Criteria:

Sampling should focus on frequently touched surfaces (door handles, light switches, handrails, elevator buttons, desks, computer keyboards) in high-traffic areas like lecture halls, libraries, laboratories, cafeterias, restrooms, and administrative offices. Shared spaces with diverse users, such as communal areas, should be prioritized to capture surfaces with higher contamination risks. Samples should include a range of materials (plastic, metal, wood, glass) as these may impact bacterial attachment and biofilm formation. Consistency in environmental conditions (temperature, humidity) and timing (aligned with cleaning schedules and usage patterns) is essential. Analysis will be done in a lab equipped to detect and measure pathogenic microorganisms, with results clearly reporting microbial presence and concentration levels across sampled areas.

3.3.2: Exclusion Criteria:

The rarely touched surfaces (like walls and ceilings), restricted-access areas (such as laboratories and clinics), and recently cleaned or sanitized surfaces (within the past 1-2 hours) will be excluded from sampling as there could be high chances of minimal contamination. Also, surfaces in specialized hygiene areas with controlled conditions (e.g., UV sterilization) and those that are difficult to access or pose safety risks will also be omitted from sampling.

3.4 Sample Size Determination

Fishers' formula will be used to calculate the sample size (Fishers *et al.,* 1991) based on prevalence of 50% (MOPHS, 2018) and a standard error of 0.05.

A sample size of 384 will be calculated as follows:

$$N = \frac{Z^2pq}{d^2}$$

$$= \frac{1.96^2 \times 0.5 \times 0.5}{0.05^2}$$

$$= 384$$

Where:

N = Sample size required

Z = Confidence level at 95% (standard value of 1.96)

p = Estimated average prevalence of *Staphylococcus aureus* (50 %)

q = 1-p, proportion of those high-contact surfaces (1-0.5=0.5)

d = Required error (0.05)

n = Calculated sample size

N_C = Sample size required 10% of 384 will be added on the minimum sample size to account for non-response, refusals or bias bringing the sample population to a total of 422

Target population (N) = $(N_c \times 422) \div (N_c + 422 - 1) = 200$

3.5: Sampling Procedure

The study will utilize a multistage sampling approach, beginning with a random selection of the study site within Kisii University, followed by systematic sampling of specific surfaces presenting the high touch factor. The procedure is as follows;

Stage 1: Random Selection of Study Sites: A list of high-access buildings (e.g., library, administration blocks, fruit centre, tuition complex, science complex, etc.) will be compiled based on their potential for high-touch surfaces. Random sampling will then be used to select a diverse representative sample from this list, considering facility size, access frequency, and geographic location. *Stage 2: Systematic Sampling of Eligible Surfaces*: Each selected site will have its surfaces reviewed to identify high-touch areas, with data collected through manual records. Inclusion criteria will ensure that only sites accessed within the past fortnight are considered. Eligible surfaces will be selected systematically (e.g., every 5^{th} site) to maintain consistency and representativeness across all locations.

3.6 Sample Collection

Sterile collection procedures that will entail usage of sterile containers, swabs, gloves, and labelling materials will be used in sample collection and sample collection procedures will be done according to established protocols as used before (Grady et al.2021).

Swabbing will be performed on surfaces like door handles and railings using sterile swabs moistened with saline, and samples will be placed in sterile containers promptly. After collection, each container will be securely sealed and labelled with a unique identification number, date, time, and site details, and stored at 4°C to maintain bacterial viability, avoiding freezing. Samples will be transported to the laboratory

within 6 hours using insulated containers to maintain temperature and adhering to regulations for biological specimens. Upon arrival, the integrity of each sample will be checked for leaks or contamination, and proper labelling will be verified. Samples will then be logged, homogenized with sterile equipment to ensure even bacterial distribution, and accurate records will be maintained throughout all stages of collection and handling. Quality control measures will ensure compliance with protocols, with regular reviews based on any feedback or issues encountered.

3.7 Isolation and Characterization.

Samples collected using sterile swabs will be transferred and cultured by use of streaking method unto the Nutrient culture media (HIMEDIA, Ref MM012-500G) to encourage the growth of *Staphylococcus aureus* bacteria by use of streaking method (Benson., 2001). The plates will then be incubated at the right conditions i.e. temperature of 37°C for 24-48 hours, aerobically. Upon growth, the colonies will be isolated and sub-cultured using streak plate techniques to obtain pure colonies. After growth, characterization of the isolated colonies will be done using morphological examination to observe the colony morphology, colour, size and texture will be done that will be followed by Gram staining following standard operating procedures as used before (Holt et al, 1994). The suspected *S. aureus* isolates will be subjected to biochemical testing using such tests like catalase, oxidase and coagulase to identify the bacterial species as previously used (Karmakar.2016). *S. aureus* ATCC 25923 will be used as positive control for identification purposes and antimicrobial susceptibility tests.

3.8 Bioassays.

3.8.1 Disc Diffusion (Kirby-Bauer) Test.

Disc diffusion will be done on the isolates obtained by use of various antibiotics discs (Methicillin, Ciproflaxin, Erythromycin, Vancomycin, Trimethoprim-Sulfamethoxazole, Gentamicin, Rifampicin, Tetracycline) as done earlier (Clinical and Laboratory Standards Institute; CLSI, 2024). Briefly, the bacterial suspension

equivalent to a 0.5 McFarland standard (approximate bacterial concentration of 1.5 x 10^8 CFU/mL) will be prepared and used for disc diffusion tests. From this suspension, 100µls of the *S. aureus* bacteria isolates will be dispensed unto the sterile Mueller Hinton agar Medium and then spread on the surface of the medium by use of the spread plate technique (Marlon.2024). Then the inoculum will be left for about 15min before the antibiotic discs impregnated with the various antibiotics will be placed onto the surface of the inoculated agar plate using sterile forceps or a disk dispenser. The plate will then be incubated at 37°C for 16-18 hours aerobically.

After incubation, the diameter of the zones of inhibition (clear areas around the disks) will be measured in millimetres. The zone diameters will be compared to a standard chart provided by organizations like the Clinical and Laboratory Standards Institute (CLSI, 2024). *S. aureus* ATCC 25923 will be used as positive control for antimicrobial susceptibility tests and all bioassays will be done in triplicates that are independent of each other.

3.8.2 Antibiofilm Determination

A fresh culture of *Staphylococcus aureus* will be grown in a suitable medium, such as Tryptic Soy Broth (TSB) or Brain Heart Infusion (BHI), often supplemented with glucose to encourage biofilm formation. The culture will then be standardized to a specific optical density (e.g., OD 600 ~0.5) to ensure consistent bacterial concentration across wells. The standardized bacterial suspension will then be added to each well of a sterile 96-well microtiter plate, with controls included (positive control for biofilm formation and negative control with only media. The agent will be added at various concentrations to separate wells containing *S. aureus*. Plates will then be incubated under appropriate conditions (usually 24–48 hours at 37°C) to allow for biofilm formation.

After incubation, the wells will be gently washed (three times) with phosphate-buffered saline (PBS) to remove non-adherent cells, leaving only the biofilm attached to the plate surface. Crystal violet stain (0.1–1% solution) will be added to each well and left for around 15–30 minutes to bind to the biofilm matrix. Excess crystal violet will be removed by washing the wells with water or PBS. The bound dye will be then

solubilized, often with ethanol, acetic acid, or a combination, to release the stain into solution.

The absorbance (optical density) of the solubilized crystal violet solution will be measured at 570 nm (or sometimes 595 nm) using a microplate reader. Higher absorbance values correspond to greater biofilm biomass whereas a reduction in absorbance relative to the control indicates antibiofilm activity.

3.9 Data Analysis and Presentation

The data analysis will involve systematic documentation of the location, type, and frequency of high-contact surface samples to assess *Staphylococcus aureus* distribution at Kisii University. Positive samples will be identified through various morphological, biochemical, and molecular tests, with a quantitative analysis determining contamination rates and statistical methods highlighting high-risk surfaces. Antimicrobial susceptibility testing will evaluate resistance patterns among isolates, while biofilm formation will be categorized based on absorbance data from microtiter plate assays. The findings will be presented through detailed tables outlining prevalence rates, susceptibility profiles, and biofilm strength, alongside graphs and charts illustrating contamination rates and resistance levels. Descriptive statistics will provide further context to the data, aiding in the interpretation of potential transmission risks and informing sanitation protocols.

3.9.1 Data Management and Quality Control:

Records of the sampling process will be maintained, documenting the number of buildings contacted, eligible contact surfaces identified, and surfaces sampled. Any issues or deviations from the planned procedures will be documented, and adjustments will be made as necessary. Quality control measures will be implemented to monitor the sampling process. Regular reviews of sampling procedures and data will ensure consistency and adherence to the study protocol. Any discrepancies or challenges

encountered will be addressed promptly to minimize their impact on the study outcomes

Additional Considerations**:** Decide on a strategy for managing missing data (e.g., imputation, complete case analysis). Verify assumptions for statistical tests (e.g., normality for t-tests, independence for chi-square tests). Set significance thresholds (e.g., $\alpha = 0.05$) and adjust for multiple comparisons if necessary.

3.10 Ethical Clearances.

The initial ethical clearance for this research will be sought from the Ethical Review Committee at Institutional Research Board (IRB) and from the Kisii University Ethics Review Committee (KSUERC). This will ensure adherence to ethical principles such as informed consent, and confidentiality.

To maintain the confidentiality of the study sites, a unique identification code will be assigned to each building targeted with the study. Informed consent will be obtained from the university staff's offices in a case where the study is yet to capture samples from their offices or restrooms when necessary. It will be made explicit to them that the collected data will be used exclusively for research purpose.

APPENDICES

APPENDIX 1.0 WORK PLAN

ACTIVITY	Period 2024 -25							
	Sept.	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Apr.**
Proposal development								
Approval of Proposal by Kisii University Ethics Review Committee								
Pilot study and Field work								
Data analysis								
Write up of project result and discussions								
Research presentation								

TABLE 1: WORK PLAN

APPENDIX 1.0 BUDGET

Item	Cost Estimation (KSH)
Sampling Materials (swabs, tubes)	**5,000**
Culture Media and Reagents	**25,000**
Culture plates(petri dishes)	**2,000**
Gloves	**1,800**
Antibiotics discs	**4,000**
Microtitre plates	**3,000**
Ethical approval costs	**1,100**
Literature search	**200**
Thesis production	**2,000**
Bench space costs	**4,000**
TOTAL	**KSH. 48,100**

TABLE 2: BUDGET

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