ANTIBIOGRAM PATTERNS AND RESISTANCE GENES ASSOCIATED WITH AEROBIC BACTERIAL CONTAMINANTS PRESENT IN CIRCULATING KENYAN BANKNOTES IN NYERI TOWN

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DECLARATION

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

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DEDICATION

I dedicate this thesis to my late father Nelson Githinji, my mother Elizabeth Njeri, my loving in-laws late James Wamathai and Charity Mumbi loving wife Rosemary who has been my strong pillar of support and to my lovely children Githinji, Njeri, Mumbi and Njoroge who have been my inspiration.

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ABBREVIATIONS AND ACRONYMS

AMP	Ampicillin
AMR	Antimicrobial resistance
ATCC	America Type Culture Collection
BHI	Brain Heart Infusion
bla	Beta-lactamase
CBK	Central Bank of Kenya
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CNS	Coagulase Negative Staphylococci
CRO	Ceftriaxone
DNA	Deoxyribonucleic Acid
E. coli	*Escherichia coli*
ESBL	Extended-spectrum beta-lactamase
GM	Gentamicin
KEMRI	Kenya Medical Research institute
LB	Lysogeny Broth
MDR	Multidrug-resistant
mL	Milliliter
MR	Methyl Red
NCBI	National Centre for Biotechnology
NCCLS	Clinical and Laboratories Standards Institute

OTRH	Outspan Teaching and Referral Hospital
PCA	Plate Count Agar
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
rRNA	Ribosomal Ribonucleic acid
RSIC	Regional Sophisticated Instrumentation Center
Shs	Shillings
Spp	Species
SPSS	Statistical Package for Social Sciences
SXT	Co-trimoxazole
Tm	Melting Temperature
VGS	Viridans Group Streptococci
VP	Voges-Proskauer
WHO	World Health Organization
μL	Microliter

ABSTRACT

Transmission of pathogens through currency notes has become very relevant in today's world due to COVID-19 pandemic. Money users often contaminate these notes with several microflora including viruses, fungi, protozoa, and bacteria via unhygienic conditions and habits. Currency notes represent a universal medium for the transmission of bacteria in the environment and among humans. Antibiotic resistance genes (ARGs) should be considered a biological contaminant of emerging concern (CEC). The high and sometimes inappropriate use of antibiotics has accelerated the development of antibiotic resistance, creating a major challenge for the sustainable treatment of infections world-wide. The objective of the study was to determine the antibiogram patterns and resistance genes associated with bacterial contaminants present on Kenyan banknotes in circulation in the Nyeri town in Nyeri County. A cross-sectional study design was conducted during the period between March, 2019 and April, 2019 and a total of 125 currency notes consisting of different denomination notes collected randomly among shops across Nyeri town. Bacterial isolation, identification and antibiotic susceptibility was done at Outspan Teaching and Referral Hospital (OTRH) laboratory and determination of resistance genes done at Kenyatta University. A total of 125 currency notes of 5 different denominations were collected from different marketing sources such as butcheries, restaurants, health facilities, M-pesa outlets and transport Savings and Credit Cooperative Organization (SACCOS) then dropped in sterile bags. Spread plate technique, specific media and biochemical tests were used for the bacterial isolation and identification. Aerobic bacterial isolates were tested with rapid multiplex polymerase chain reaction (PCR) assays for detection of associated antibiotic resistance genes. Total of 18 different bacterial species were isolated from five Kenyan banknote currencies. Of these, 37 (52.2%) was *Staphylococcus aureus* followed by *Staphylococcus sciuri* spp. 9 (11.3%), *Staphylococcus gallinarum* 2 (2.8%), *Staphylococcus intermedius* 6 (8.5%) *Micrococcus* spp. 1 (1.4%), *Staphylococcus schleiferi* spp. 2 (2.8%), *Kluyvera ascorbata* 1 (1.4%), *Proteus penneri* 1 (1.4%), *Aeromonas media* 3 (4.2%), *Burkholderia cepacia* spp. (1.4%), *Aeromonas enteropelogenes* 1 (1.4%), *Enterobacter cloacae* 1 (1.4%), *Klebsiella oxytoca* 2 (2.8%), *Leclercia adecarboxylata* 1 (1.4%), *Raoultella ornithinolytica* 1 (1.4%), *Vibrio metschnikovii* 1 (1.4%), *Myroides odoratus* 1 (1.4%) and *Yersinia pestis* 1 (1.4%). Overall, Gram positive and Gram negative bacterial isolates exhibited resistance to vancomycin, clindamycin and amoxycilin with percentages 40 (71%), 28 (50%), and 37 (66%) and 9 (64%), 8 (57%) and 6 (43%) respectively. Thirty isolates were subjected to polymerase chain reaction for detection of resistance genes. Overall, isolates exhibited resistance to vancomycin, amoxycilin and clindamycin with percentages of 40 (71%), 28 (50%), and 37 (66%) respectively. The gene that predominated was bla TEM (20%), followed by vancomycin (6.7%) while none of the erythromycin resistance gene was found. This research found that the Kenyan banknotes in circulation within Nyeri County were contaminated with 18 distinct species of pathogenic bacteria, some of which exhibited resistance to vancomycin, amoxicillin, and clindamycin, along with their related resistant genes. The study advocates for proper handling, storage, frequent monitoring, and disinfection of the circulating banknotes.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Money is any commonly accepted medium of exchange as a recompense for goods, services, and debt settlement (Sadawarte *et al.,* 2014). Around the world, banknotes are frequently traded for products and services (Fisher-Høyrem, 2022). Paper currency also acts as a unit of measurement for determining the corresponding worth of products and services (Sarfraz, 2015; Telalbasic, 2017). Scientists hypothesized a link between handling money and the disease transmission (Limaye, 2020). Subsequently, these hypotheses were proven through current scientific techniques, revealing that pathogens could be recovered from bank notes surfaces (Cozorici, *et al*., 2022; Meister *et al*., 2023). According to Cozorici, *et al.* (2022), the adherence of bacterial strains was lower for polymer-based banknotes British pounds and Romanian Leu, in contrast to the cotton-based U.S dollars and Euro banknotes. Current scientific methods have validated these hypotheses and demonstrated that it is possible to isolate viable infectious organisms from the surfaces of coin and paper money (Sharon and Sethu, 2017; Moore and Millar, 2021).

Based on their composition, age, and the environment of the country under study, contaminated currency can act as a channel for spread of disease (Meister *et al.,* 2023). Bank notes and coinage can get contaminated with these pathogens through the atmosphere when being passed around, during storage, and while at general use (Mek *et al*., 2015; Gedam *et al*., 2018; Ofoedu *et al.,* 2021). Some Poor banknotes handling culture exposes money to contamination such as

; keeping money in socks, shoes and lubricating of fingers with saliva when counting (Vriesekoop *et al.,* 2016; Alabbasy, 2019).

Previous studies have shown that banknotes in circulation serve as an ideal breeding ground for both pathogenic and non-pathogenic microorganisms for number of rationale. The paper currency offer an extensive amount of surface area for accumulation of both microorganisms and organic debris (Feng *et al.,* 2015; Ofoedu *et al.,* 2021). Folds and/or intentional indentations or projections that are purposely designed into the paper currency as anti-counterfeiting techniques act as settling places for organic materials extending the life of the microorganisms (Mek *et al.,* 2015). Eventually, before being returned to the central bank, banknotes are used by the public for a lengthy period.

Research shows that the degree of microbial contamination on banknotes is closely linked to both their age and the materials they are made from (Cozorici *et al.,* 2022). In their study, Cozorici *et al.* (2022) examined various denominations of currency, including British pounds (£5, £10, and £20), Romanian lei (1 leu, 5 lei, and 10 lei), U.S. dollars ($1, $5, and $10), and Euros (€5, €10, and €20), to assess bacterial survival rates and adherence. The findings showed that bacterial survival rates are affected by the banknote substrate, with polymer-based notes exhibiting lower survival rates, particularly for *Salmonella enterica*, *Listeria monocytogenes*, and *Enterococcus* sp. Additionally, bacterial adherence was found to be reduced on polymer-based British pounds and Romanian lei compared to the cotton-based U.S. dollars and Euro banknotes.

Górny *et al.* (2021) conducted a study in three money sorting facilities in Poland. The study confirmed that means of payment methods serve as significant sources of microbial emissions within these environment. The findings indicated that employees in these facilities are at risk of exposure to microorganisms that could potentially lead to negative health effects. To safeguard their health, it is essential to implement stringent hygiene measures in the workplace to mitigate both unwanted contamination and the risk of secondary microbial emission from sorted currency and tabletop surfaces .

All types of people handle currency, and it can get contaminated when someone coughs, sneezes, touches it with their hands, or places it on unclean surfaces (Limaye, 2020). In the 1970s, it was proposed that currency notes could serve as environmental carriers of potentially harmful microbial organisms (Sunil *et al.,* 2020). Iyevhobu *et al.* (2020) have reported that currency is the source of certain pathogenic microorganisms linked to UTI, URTI and gastrointestinal infection .

Currency notes may become contaminated during the processing of food in the food supply chain, especially when aerosols from coughing and sneezing are involved (Ofoedu *et al.,* 2021; Yohanna, 2024). A person with unsanitary living conditions and unsanitary behaviors will contaminate the notes with harmful microorganisms (Barolia and Saini, 2018; Limaye, 2020). Because contagious diseases can spread quickly to multiple individuals, paper money poses a special risk to public health (Badvi *et al.,* 2017). Commonly isolated bacteria from currency in earlier studies include: family enterobacteriacea (Abd Alfadil *et al.,* 2018), mycobacterium (Hasan *et al.,* 2021) and in recent studies gram positive coci (Khalil and Attitalla, 2023).

Antimicrobial resistance spread across continents and international borders and has grown to be an international priority (Alemayehu *et al.,* 2019). According to projections, antimicrobial resistance causes over 10 million deaths annually globally. It also has a significant direct or indirect impact on low- and middle-income nations (Collaborators, 2022). The management of infectious diseases is severely affected by the global issue of pathogenic microbes becoming resistant to widely used antibiot\ics (Shaikh *et al.*, 2015).

Whenever extended-spectrum beta-lactamases (ESBLs) have been generated, bacterial resistance has been observed (Shaikh *et al.,* 2015). Suicide inhibitors, including clavulanic acid, sulbactam, and tazobactam, block these enzymes' ability to hydrolyze extended-spectrum beta-lactam antibiotics like penicillins, cephalosporins, and a monobactam (aztreonam) (Abbas *et al.,* 2022). Genes for the narrow-spectrum beta-lactamases (TEM-1, TEM-2, or SHV-1) can be rearranged to yield extended-spectrum beta-lactamases by changing the structure of amino acids surrounding the active region of the enzyme (Bajpai *et al.,* 2017). The earliest beta-lactamase genes discovered in Gram-negative bacteria were called *blaTEM* (Dirar *et al.,* 2020). Over two hundred subtypes, primarily encoding enzymes that hydrolyze penicillin and first-generation cephalosporins, have been found. They are specially delivered via plasmids (Clasen *et al.,* 2019).

Global surveillance has shown considerable widespread antibiotics resistance bacteria (Firoozeh *et al.,* 2017; Morehead and Scarbrough, 2018). The study aimed at establishing the antibiogram patterns and resistance genes associated with aerobic bacterial contaminants present on Kenyan banknotes circulating in Nyeri town, Nyeri County.

1.2 Statement of the problem

In most developing countries, such as Kenya, documentation on the levels of microbiological contamination of money is still inadequate. Due to this inadequate information, there is no public health policy on currency handling (Sunil *et al.,* 2020). The failure of different governments to regularly remove old, damaged, or deformed currency notes from circulation—which are a major factor in the spread of pathogenic microorganisms and pose a threat to public health—further exacerbates the issue (Mändar *et al.,* 2016; Sunil *et al.,* 2020; Yar, 2020). In addition, banknotes are handled by people with varying levels of health and personal hygiene, hence predisposing the population to the infection (Chomba and Mwamainda, 2016; Mändar *et al.,* 2016; Singh *et al.,* 2023).

Nyeri, being an agricultural administrative hub with a dense populated, experiences a significant currency notes circulation (KNBS, 2019). The local residents' poor handling and storage of banknotes could predispose the general public. Kenya faces a high levels of foodborne illiness burden attributed to poor food handling practices, lack of hygiene among other many factors (Kariuki *et al.,* 2017). The town has a substantive number of fast food and street vending businesses where individuals often handle both money and food simultaneously (Misiko and Kisiang'ani, 2024). This situation is further exacerbated by the widespread practice of purchasing antibiotics over the counter without a prescription (Murigi et al., 2021). As a result, this study was triggered with aim of investigating the potential microbial contamination of banknotes in Nyeri town and the presence of resistance genes.

Antimicrobial resistance has remainded a worldwide concern in recent years. The missuse of antibiotics has resulted in treatment failure and expensive treatment costs (Chandra *et al.,* 2021; Salam *et al.,* 2023). The resilience, spread, and transfer of infection-causing microorganisms are significantly influenced by antibiotic resistance. Infections by drug-resistant pathogenic bacteria cause long-term infections in humans (Ofori-Asenso, 2017). Currency notes have been shown to contain bacteria, including drug-resistant species, indicating that currency can be a vehicle and fomite for the spread of drug-resistant strains in the community (Hiko *et al.,* 2016). The study aims at establishing the antibiogram patterns and resistance genes associated with aerobic bacterial contaminants present on Kenyan banknotes circulating in Nyeri town, Nyeri County.

1.3 Justification

Everybody virtually uses currency to purchase goods and services all over the world. Money contamination is crucial for public health since it might facilitate an easy spread of infections among handlers. From purchasing milk at the neighborhood grocery to purchasing medications, banknotes are utilized in all business forms. Because so many people handle paper money, there is a greater chance that it will work as a vehicle for the spread of potentially pathogenic microbes in the environment.

To address the increasing concern about antibiotic resistance in human and veterinary health, it is necessary to identify and keep track of genes that encode resistance to antibiotics and possible reservoirs. Both natural and artificial environments have shown evidence of the transmission of antibiotic-resistance genes, occasionally at levels higher than those observed before antibiotics were produced in large quantities (Sanderson *et al.,* 2016; Larsson and Flach, 2022). Valuable scholarly and the urgency associated with the need to investigate the environmental dissemination of these resistance genes has been triggered by increasing knowledge and the need to stop resistance to antibiotics and make related policies. Understanding the variety of microorganisms and antibiotic-resistant genes linked to the circulating banknotes in use can serve as the foundation for increasing people's awareness of their health when handling banknotes and successfully preventing the spread of infections. The current study aims at establishing the antibiogram patterns and resistance genes associated with aerobic bacterial contaminants present on Kenyan banknotes circulating in Nyeri town, Nyeri County.

1.4 Hypotheses

i. There is no significant difference between source of money, value and microbial contamination

ii. There is no significant difference in antibiogram patterns among the aerobic bacterial isolates

iii. There is no significant difference in resistance genes associated with aerobic bacterial contaminants among the bacterial isolates.

1.5 General objective

To establish the antibiogram patterns and resistance genes associated with aerobic bacterial contaminants present on Kenyan banknotes circulating in the Nyeri town in Nyeri County.

1.5.1 Specific objectives

i. To isolate and identify aerobic bacterial contaminants present in circulating Kenyan banknotes in Nyeri town

ii. To determine the antibiotic susceptibility profiles of isolates from Kenyan banknotes circulating in Nyeri town.

iii. To determine the presence of resistance genes associated with aerobic bacterial contaminants present in circulating in Kenyan banknotes in Nyeri town

1.6 Significance of the study

The study recommends that Kenyans wash their hands after dealing with circulating banknotes to increase their awareness of their health. The study prompts a massive public health drive to educate food handlers on improving their hygiene, particularly for individuals who handle both food and money, to reduce the risk of infection to the consumers.

The study also creates awareness of the danger of using saliva while counting paper circulating banknotes. The research's conclusions make the Central Bank of Kenya more cognizant of the need to inform the public and appropriately implement regulations around handling money and personal hygiene. The study creates awareness of the need for regular testing of circulating banknotes in circulation for the existence of microorganisms. In addition, implementing methods to sterilize contaminated banknotes on a large scale and considering the introduction of washable and easily sterilizable plastic banknotes, similar to the approach used in Australia, could be effective strategies to reduce the spread of bacteria.

CHAPTER TWO

LITERATURE REVIEW

2.1 Money handling as a Potential Source of infections

Research on the contamination of paper currency and coins has been scarce (Tamele *et al.*, 2021; Marquès and Domingo, 2021). The microbiological condition and persistence of viruses on coins and currency notes have been studied over the past 20 years, and the results showed that this may be a major factor resulting in random globally observed food-borne illness cases (Nepal and Tsomo, 2022). Most underdeveloped countries lack sufficient (Akoachere *et al.*, 2014) and conclusively reliable data on studies related to microbiological contamination of currency (Angelakis *et al.*, 2014). Again, shortage and, in most cases, absence of public health regulations set to govern the use, handling, and circulation of currency, coupled with a lack of public awareness, may exacerbate the problem of antibiotic resistance (Chomba and Mwamainda, 2016; Sunil *et al.*, 2020).

In selling and trading, paper money is widely utilized (Alemu, 2014). Data that circulating banknotes have the capacity to behave as fomites and carry microorganisms is widely reported (Jalali *et al.*, 2015; Meister *et al.*, 2023). Due to its rough surface, paper money offers a favorable environment for bacteria and other particles to settle and build up over time, potentially becoming an infection source. The length of time that currency notes are in circulation, how they are handled, and their texture all affect the amount and variety of microbiological contamination (Jalali *et al.*, 2015). Additionally, the tendency of banknotes to absorb moisture promotes the development and survival of bacteria (Vriesekoop *et al.*, 2016).

Characterizing the presence of various microorganisms on currency notes in different locations worldwide has been the subject of several current attempts (Jalali *et al.*, 2015; Pandey *et al.*, 2015). Substantial amounts of microorganisms are recovered from paper money because they are in circulation for a long time (Hanash *et al.*, 2015), even though a majority of them are infused with disinfectants to avert the development of microbes (Adinortey *et al.*, 2019).

Considering that paper money may contribute to spreading and transmitting diseases, the presence of harmful microbes on them is quite concerning. Unsafe food storage facilities, diseased food handlers (Saad *et al.*, 2019), and cross-contamination were shown to be the primary entrance points for pathogens (Alimi *et al.*, 2022). Additionally, keeping the paper currency in damp, dark environments in polyethylene, cotton, or leather bags encourages the proliferation of bacteria and fungi (Girma, 2014). All kinds of transactions involve using paper notes, with lower-denomination notes being handled the most because of how frequently they are exchanged (Kitamura, 2022). While all transactions are done using money, notes of a smaller denomination are more popularly used and exchanged amongst the populace of any locality(Sands, 2016; Kitamura, 2022).

A number of variables, including the organisms' load and capacity to endure in the dry environment of the banknotes, determine the kind of microbes that reside on coins and banknotes (Cozorici *et al.*, 2022). The main exposure points could include contact with contaminated food, water, and hands. Canadian research found no bacterial contamination on recently issued banknotes (Lamichhane *et al.*, 2009). Contaminated currency notes could pose a risk when exposed to potential sources of infection such as blood, urine, faeces and food (Andrew, 2016).

Banknotes made from cotton or linen fibers like those used for euro and dollar bills, have a rougher surfeces texture and higher moisture retention capabilities compared to their polymer counterparts, which helps improve adhesion (Cozorici *et al.,* 2022). Most countries have transitioned from paper to plastic polymer banknotes (Cozorici *et al.,* 2022). Consequently, has been suggested that itmicrobial testing for banknotes takes place and that contaminated notes be replaced, in addition to ther regular withdraw of damaged currecy by federal authorities (Alemu, 2014). To maintain Hygienic, it is advised to wash hands thoroughly with soap after handling cash, coins, or using ATM machines. Morever, storing money in shoes, socks, or under carpets should be avoided (Alemu, 2014).

2.2 Types of biological contaminants isolated from banknotes

Numerous countries' currency studies revealed that bacterial contamination was far more common than contamination with other microorganisms (Alemu, 2014; Sharma and Sumbali, 2014; Agarwal *et al.,* 2015). Additionally, there are regional variations in the general trend of bacterial contamination of currency (Pal *et al.,* 2013; Agarwal *et al.,* 2015).

According to studies, methicillin-resistant bacteria are among the several multidrug-resistant microorganisms found in banknotes (Djouadi *et al.,* 2020; Al-Nuaimi *et al.,* 2022; Iyevhobu *et al.,* 2023). *Staphylococcus aureus* has frequently been identified from the currency banknote (Mändar *et al.,* 2016; Sunil *et al.,* 2020). Moreover, scientific studies on the microbial contamination of money are still limited in most developing countries (Alemu, 2014; Segura *et al.,* 2015).

Another possible cause of infection that could result in food poisoning is microbial contamination of banknotes with pathogenic microbes (Alemu, 2014; Raees *et al.,* 2014). Furthermore, the possibility of contamination of parasites like Ascaris lumbricoides and Taenia species on money notes has been investigated. Others studied include *Enterobius vermicularis* and *Trichuris trichiura* (Oo and Bello *et al.,* 2016; Orababa *et al.,* 2021 Akeredolu *et al.,* 2024). The presence of microorganisms reduces the lifespan of the banknotes (Vriesekoop *et al.,* 2016; Cozorici *et al.,* 2022). It has also been noted that these microbes can lead to infections of the skin, eyes, digestive system and respiratory infection (Sharifipour *et al.,* 2020; Sharifipour *et al.*, 2020; Orababa *et al.,* 2021; Yohanna, 2024).

Microorganisms from the Enterobacteriaceae family are commonly isolated from the banknotes that include *Vibrio cholerae* and *Micrococcus* spp. (Agarwal *et al.,* 2015; Hanash *et al.,* 2015; Yohanna, 2024). Other types of bacterial isolated from currency notes include *Salmonella* spp., *Bacillus* spp., *Klebsiella* spp, *Pseudomonas* spp. and *Mirabilis* spp (Abd Alfadil *et al.*, 2018; Satya *et al.*, 2018; Iyevhobu *et al.*, 2023). Furthermore, other studies have been able to isolate *Pseudomonas* spp. and *Corynebacterium* from circulating currency notes (Boidya *et al.,* 2015; Akond *et al.,* 2015; Qayyum & Batool, 2017; Firoozeh *et al.*, 2017; Satya Lakshmi *et al.*, 2022). Besides that, certain fungal species were found on paper money notes, like *Aspergillus* spp., *Rhizopus* spp., *Penicillium* spp (Alemu, 2014; Sharm;a and Sumbali, 2014; Ozoude and Ugbogada, 2024).

Studies conducted worldwide have documented high levels of microbiological contamination in circulating currency notes (Girma, 2014; Iteku *et al.*, 2020; Ofoedu *et al.*, 2021; Al-Nuaimi *et al.*, 2022). Environmental microorganisms, particularly *Bacillus* species and *Staphylococcus aureus*,which originates from the normal flora of individual's skin were common baseline contamination of paper money (Raees et al., 2014; Iteku *et al.*, 2020; Górny *et al.*, 2021; Iyevhobu *et al.,* 2023).

Beginning in the early 1970s, an investigation on contaminated currency revealed contamination on 13% of coins while only 58% of paper money was uncontaminated amongst all samples obtained from lab workers (Alemu, 2014; Sharon and Sethu, 2017). A study conducted in india paper currency had 121 isolates consisting six different bacteria. These bacterial are *Micrococci*, *Escherichia coli*, *Pseudomonas* spp, *Klebsiella* spp, *Bacillus* spp and *Staphylococcus aureus* (Sucilathangam *et al.*, 2016). The two most often isolated microbes from Indian money were coagulase-negative *staphylococcus* and *Bacillus* (Agarwal *et al.*, 2015). In Nagpur, it was found that *Escherichia coli* was the predominant microbe from currency notes.

Environmental microorganisms of the *Bacillus* species and *Staphylococcus aureus*, which originate from the human skin, were significant baseline contamination of paper money (Jafer *et al.,* 2015). This result aligns with the information reported from research which isolated pathogenic microorganisms from Bangladesh paper currency, which causes various diseases with antibiotic resistance (Akond *et al.*, 2015).

Many researchers have documented that currency notes can transfer bacteria to humans through food handling (Angelakis *et al.*, 2014; Girma, 2014). Specific bacterial isolates, like *Shigella dysenteriae* and *S. aureus*, are disease-causing microbes that can introduce pollutants to food, confirming assertions from studies conducted in other regions of the world that suggest paper money is typically contaminated by disease-attributing microorganisms that lead to a variety of illnesses (Raees *et al.*, 2014; Górny *et al.*, 2021).

Money contamination is linked to unsanitary behaviors, indicates considerable fecal contamination of currency, and represents inadequate local environmental sanitation, all of which point to a possible nosocomial infection hazard (Al-Nuaimi *et al.,* 2022). Almogbel *et al.* (2025) on knowledge, attitudes, and practices of the Saudi Arabian Population Regarding Contaminated Banknotes found that the currency harbored potentially harmful microbes, such as *S. aureus* and *Klebsiella pneumonia* which together with *Staphylococcus aureus* were also reported to contaminate Egyptian banknotes (Saleh *et al.*, 2018).

Sunil *et al.* (2020) assert that Sixty per cent of the microorganisms that contaminated all 100 per cent of the open marketplace notes were conceivably pathogenic with bacteria like *S. aureus* and *E. coli*. According to research by Djouadi *et al.* (2020), there were potentially pathogenic microorganisms present in ninety-six % of the currency notes that were examined where the dominant recovered microbes were coagulase-negative *Staphylococci* and *Micrococcus* spp. In addition, other potential pathogenic microbes like *Salmonella typhi* and acid-fast bacilli were reported.

Numerous pathogens, such as *Vibrio cholera* and *Pseudomonas aeruginosa,* were noted in naira currency notes. Furthermore, Orukotan and Yabaya (2011) examined all denominations of naira notes for microbiological contamination in Kaduna. The microbes documented in the study include fungi, *Fusarium*, *Penicillium*, *Aspergillus* and *Rhizopus*. The study led to the discovery that Ghanaian paper-based currency had been contaminated with dangerous microbes that might cause illnesses in people (Adinortey *et al.*, 2019). Therefore, the goal of the current investigation is to present the kind, nature, and degree of contamination of the Kenyan currency that is in use in Nyeri Town.

Some research have identified very virulent spore-forming pathogenic bacteria from currency notes belonging to the genus Bacillus (Toba *et al.*, 2007).Several studies have documented presence of bacterial, viral, fungal and parasites on currency notes (Alemu, 2014; Girma, 2014). Since food vendors in Ghana prepare food with their hands while also handling currency notes while selling, pathogenic bacteria that might live on Ghanaian currency notes could be a source of enteropathogens that cause food poisoning (Adinortey *et al.*, 2019). Some of bacteria isolated from GH¢1 notes include *E. faecalis, S. dysenteriae, C. freundii* and *Bacillus species.*

Ahmed *et al.* (2017), in research on Saudi Arabian paper currency (Riyal notes), reported *Klebsiella* sp., *Bacillus* sp., *E. coli* and *Staphylococcus* spp. pathogenic microbes. In Iran, Ndubuisi *et al.* (2016) and Orababa *et al.* (2021) took note of the existence of some pathologically important bacteria and fungi, such as *Salmonella, Shigella*, *Aspergillus, Micrococcus*, and *Penicillium*. To top it up, Pugazhendhi *et al.* (2020) indicated that

Staphylococcus spp. lead to the appearance of superficial skin lesions on individuals, resulting in conditions and infections like food poisoning, boils, deep-seated infections, styes, UTIs, toxic shock syndrome, among others. Another research was carried out by Elsharief and Haider (2018) and found the β- haemolytic *Streptococcus, Proteus* spp and *Salmonella* spp. Abirami *et al.* (2012) also documented a number of illnesses brought on by fungi that were isolated from Indian banknotes. Therefore, the likelihood of viruses on currency and their ability to endure continual handling cannot be understated.

2.3 Level of contamination of different currency

Numerous countries and sources have reported on the microbiological load of various currency notes (Alemayehu *et al.*, 2019). There have been reports of contamination of several national currency notes from South Africa with different disease causing pathogens Ethiopian (Alemayehu *et al.*, 2019), Democratic Republic of the Congo (Iteku *et al.*, 2020), Nigeria (Ofoedu *et al.*, 2021), Indian (Agarwal *et al.*, 2015), Bangladesh (Barua *et al.*, 2019), Iran (Firoozeh *et al.*, 2017), Sudan (Andrew, 2016) and Europe (Mändar *et al.*, 2016).

The geographical region's hygienic conditions, microorganism endemism, and the texture of the material used in the money all play a role in influencing the extent of contamination and the kinds of microorganisms found on the banknotes notes (Al-Kolaibe and Al-Shorgani, 2021). Sharma and Sumbali (2014) revealed that 84-100% of all the banknotes were contaminated with microorganism. This 100% banknote contamination was also reported by Djouadi *et al.* (2020) with pathogenic organisms like *Escherichia* spp. and *Staphylococcus* spp. Agarwal *et al.* (2015) reported that all the currency notes were contaminated, which is consistent with other researcher findings. Other researchers have found fungal and bacterial banknote contamination ranging from 60% to 96% (Sharma and Sumbali, 2014). A study in Tamil Nadu, India, established that 86.4 % of banknotes harboured pathogenic microbes (Sucilathangam *et al.,* 2016).

According to another study, contamination was similarly correlated with the physical condition of the currency, where a 73.8% incidence rate of bacterial contamination was witnessed on unclean and mutilated notes. In comparison, the lowest percentage of bacterial contamination was found on clean notes (Alemu, 2014). Currency notes that are folded or crumpled form pockets or voids that may contain dust particles and microbes.

Cellulose is the primary component that makes paper. The breakdown of cellulose by microorganisms that release the cellulase enzyme. Environmental factors have greatly impacted on the growth of disease causing microbes that cause cellulose to biodegrade because they create a favorable environment for their survival (Badvi *et al.,* 2017). For this reason, currency contamination is significant for public health since it can facilitate the easy spread of pathogens among handlers (Badvi *et al.,* 2017).

It has been postulated that the length of time in circulation has an impact on the proportion of microorganisms found on notes (Maritz *et al.,* 2017). These percentage differences show that sanitary practices and currency handling differ greatly between different areas and that in fact microbial contamination of banknotes is a common problem. A range of studies have also established difference in microbial contamination in different occupation groups (Umoru *et al.,* 2015; Barua *et al.,* 2019). Money can spread disease since it is handled by many people in various personal and environmental circumstances (Alemu, 2014; Meister *et al.,* 2023). The current study isolated and identified aerobic bacterial contamination in circulating Kenya banknotes in Nyeri.

Investigations have found a substantial correlation between contamination and note denomination. Compared to larger denomination notes, lower denomination notes proved increasingly contaminated (Badvi *et al.,* 2017). Earlier studies showed that when lower-value notes are traded more frequently and remain in circulation longer, they are more likely to become contaminated by microorganisms (Khalil *et al.,* 2014; Akoachere *et al.,* 2014). Agarwal *et al.*(2015) established 100 % contamination among the Rupees 10 notes and coins which are commonly used. Lower levels of 20% contamination were found in Rupees 50 and Rupee 100, which are considered a comparatively higher denomination. These studies corroborate with the findings of Ofoedu *et al.*(2021) that found the higher denominations notes of ₦500, ₦200, and ₦100 note, with the exception of ₦1,000 note, recorded increased degree of contamination over the lower denominations of ₦50, ₦20, ₦10, and ₦5 note. An investigation carried out in Australia discovered that among the currencies obtained from ten various nations, the greater the currency's microbiological content, the lower the denomination of the currency (Vriesekoop *et al.,* 2010).

The total number of pathogens increases with the age of paper notes (Agarwal *et al.,* 2015). This contamination occurs in the process of production, storage and transactions (Angelakis *et al.,* 2014). Compared to other notes, denominations that are handled and traded frequently are more likely to be contaminated (Gedam *et al.,* 2018). When money is traded through hands, the potential pathogenic microbes join the food supply chain (Mahunu *et al.*, 2024).

2.4 Antibiotics

Chemicals known as antibiotics prevent pathogens from proliferating (Bush, 2010). They are classified as either bacteriostatic (growth inhibitor) or bactericidal (killing agent) based on how they affect a target cell (Davies, 2010; Etebu Arikekpar, 2016). Antibiotics have been employed for decades to manage and control emerging and established infections (Zaman *et al.,* 2017). As a result, life expectancy has grown and worldwide disease management has improved (Klenk *et al.*, 2016; Adedeji, 2016). The actions by which antibiotics impact bacterial growth are numerous, including: 1. bacterial cell wall disintegration (e.g., Penicillin); 2. protein synthesis suppression (e.g., aminoglycosides and tetracyclines); and 3. DNA replication and transcription inhibition (e.g., quinolones and rifampin) (Table 2.1).

Table 2.1: Major antibiotic categories and mechanisms of action

Mechanisms of action	**Antibiotic categories**
Interference with cell wall synthesis	penicillin, cephalosporins, carbapenems, monobactam, vancomycin, teicoplanin
Protein synthesis inhibition	Tetracyclines, aminoglycosides, Oxazolidonones, streptogramins, ketolides, macrolides, lincosamides
Interference with nucleic acid synthesis	Fluoroquinolones
Inhibition of metabolic pathway	Sulphonamides, trimetrophim
Disruption of bacterial membrane structure	polymyxins, daptomycin

Adapted from Kohansi *et al.* (2010)

2.5 Antimicrobials resistance

According to the WHO's investigation into antimicrobial resistance (AMR), AMR is currently regarded as one of the most significant worldwide risks to the health of humankind (WHO, 2018). In recent years, multidrug resistance characteristics of microbes have emerged to be a severe risk to the general populace since various infections have become more challenging to combat (Uddin *et al.*, 2013). The issue of resistance to antimicrobial medication is of utmost importance in the area of AMR in bacteria (Dadgostar, 2019). Antimicrobial resistance hinders the capacity of the immune system to fight contagious infections and causes various problems for susceptible patients with underlying medical conditions (Dadgostar, 2019). Due to AMR's tenacity, antibiotics are becoming less effective, prompting doctors to take last-resort medication classes that are never available in developing nations. These medications have a wide range of adverse effects and are quite costly (Kashefieh *et al.*, 2021).

Antibiotics have demonstrated remarkable efficacy in controlling bacterial illnesses since they were discovered. Antibiotics targeting a wide variety of infections have been more widely available recently. New types of antibiotic resistance have arisen due to the extensive usage of these medications (Larsson and Flach, 2022; Muteeb *et al.*, 2023). One of the primary risk variables for the emergence of high levels of antibiotic resistance, prevalent in both industrialized and developing nations, is the negligent utilization of antibiotics by medical professionals or through self-prescribing and over-the-counter supply (Alenezi *et al.*, 2023). *Escherichia coli* bacteria that were resistant to many antibiotics and exhibited ampicillin resistance above 57% were identified from water (Sharma and Rai, 2012). In the study investigation, *Klebsiella* spp. demonstrated high sensitivity to gentamicin, but in another experiment in Agra, multidrug-resistant *Klebsiella* spp. were isolated from water and demonstrated maximal resistance to gentamicin (Verma *et al.*, 2011).

Antibiotic-resistant *Enterococci* is the leading causes of hospital-acquired infections of bloodstream and urinary tract since 1980s (Raza *et al.*, 2018). Three main reasons behind this emergence of multidrug-resistant enterococci includes, intrinsic resistance to antimicrobial agents like *b*eta-lactams and aminoglycosides, and acquired resistance through mobile elements like transposon and plasmids against glycopeptides, quinolones, tetracyclines, macrolides and streptogramin or through the horizontal transfer of resistance genes (Raza *et al.*, 2018)

Multidrug resistance strains of various isolates were common in the currency, according to a research study by Pavani and Srividya (2014). These bacteria included methicillin-resistant *S. aureus* (30.76%), methicillin-resistant coagulase-negative staphylococci (16%), and vancomycin-resistant enterococci (2.86%). Even though the study's MRSA incidence was significantly lower than that of Tagoe *et al.* (2011), hospital patients are always at risk from its existence. The geographical variation of widespread organisms and the various antibiotic regimens employed to treat those microbes can also account for the variance in antibiotic resistance patterns between the investigation and other findings (Olesen *et al.*, 2018; Baquero *et al.*, 2021).

The pathogenic microbes *Entrobacter*, *S. Staphylococcus, Citrobacter*, *Klebsiella*, and *Proteus* were found by Wang *et al.* (2022); Liu *et al.* (2022); Ullah *et al.* (2025) to be resistant to Tetracycline and Cotrimoxazole, but sensitive to Amoxoftine, Gentamicin, Nalidixic acid, and Ofloxacin. Antimicrobial resistance has become a significant health concern due to bacteria developing antibiotic resistance, which makes certain infections incurable today (Powers, 2004). Numerous *S. aureus* strains are thought to exhibit phenotypic resistance to numerous antibiotic classes, and these visible characteristics have been linked to various molecular genes, including the erm gene, blaZ for beta-lactam, and mecA for methicillin-resistant *Staphylococcus aureus* (MRSA) (Faden, 2019).

Kisang (2021) carried out a study to isolate, characterize and determine the antimicrobial susceptibility profiles of foodborne bacterial pathogens isolated from money and cellphones of food handlers in Nairobi, Kenya. The study concluded that the monies and phones of food handlers had antibiotic resistant strains of the *E. coli*; ampicillin (16%) and sulphamethoxazole (15%) and 7(12.5%) isolates of MRSA which were all resistant to oxacillin, the highest antimicrobial resistance was observed in cefuroxime and erythromycin (21%). Therefore money and cell phones are possible vectors of food borne antibiotic resistant pathogens and can act as sources of outbreaks if cross contamination is not addressed.

The study was conducted in Meru Teaching and Referral Hospital, Meru County during the period between April 2017 and August 2018 by Miriti *et al.* (2023). Upper respiratory infections were characterized by acute infection of nasal cavity, pharynx and larynx while lower respiratory infections were characterized by chest pains, prolonged cough, productive sputum, difficulty in breathing, fever and weight loss. Bacterial respiratory infections were prevalent in the study area and the isolates obtained showed resistance to commonly used antibiotics such as amoxicillin, ampicillin, ciprofloxacin piperacillin ciprofloxacin, ceftazidime, piperacillin-tazobactam and cephalexin (Miriti *et al.*, 2023).

2.6 Occurrence of antibiotic resistance genes

Antibiotic resistance factors have long existed before the invention of human antibiotics, regardless of the present issue of clinical antibiotic resistance. Together, the cultured Gram-positive and Gram-negative bacteria from a 4 million-year-old cave in New Mexico showed resistance to over a dozen publicly accessible antibiotics, including semisynthetic macrolides, streptomycin, clindamycin, sulfamethoxazole, and the last-resort medication daptomycin (Bhullar *et al.,* 2012). Similarly, bacterial genes that conferred resistance to beta-lactams, tetracycline, and vancomycin were found in permafrost that was 30,000 years old. Interestingly, vanA, the vancomycin resistance determinant, looked like contemporary forms (Stogios and Savchenko, 2020).

The tendency of pathogenic bacteria to withstand the effects of antibiotic agents is known as antibiotic resistance (AR), and it can result from natural, acquired, or clinical resistance (Nolte, 2014). Acquired resistance arises from genetic alterations induced by prior exposure to mutagens, antibiotics, or genetic material transfer. In contrast, natural resistance is an inherent trait that allows bacteria to withstand antibiotics without prior exposure or genetic exchange (Sandoval-Motta *et al.,* 2016).

For example, chromosome-encoded intrinsic genes for beta lactamases and multidrug efflux pumps serve a variety of protective roles for bacteria, including detoxification of metabolic intermediates, trafficking of signaling molecules, and cell wall biosynthesis (Roy *et al.*, 2021).

Furthermore, the spread of antibiotic characteristics across various bacteria is promoted by the existence of mobile elements with related AR genes, such as integrons, transposons, and plasmids, which leads to natural resistance (Partridge *et al.*, 2018). Inaccurate diagnosis, irrational antibiotic usage, and intermittent antibiotic consumption possibly as a result of a misprescription or noncompliance are some other factors that lead to antibiotic resistance (Chamoun *et al.*, 2016). Therefore, addressing these issues can stop antibiotic resistance from spreading.

2.7 Prevalence and incidences of antibiotic resistance genes in bacteria

The majority of drugs have underlying mechanisms for antimicrobial resistance (Blair et al., 2015). Antibiotic resistance has been documented in Saudi Arabia in Gram-negative bacteria obtained from hospitals as well as communities (Zowawi *et al.*, 2013), namely *Escherichia coli* that produces ESBL (Alghoribi *et al.*, 2015).

Fifty-one percent of *E. coli* that is resistant to available antibiotics recovered from this nation generated both CTX-M and TEM enzymes, according to Bindayna *et al.* (2010). Another study found that 22% of the bacterium strains that displayed resistance to anitbiotics contained both metallo-beta-lactamase (MBL) and ESBL (Marie *et al.*, 2013). The studies also revealed that United Arab Emirates (UAE) has the greatest prevalences of ESBL in the Arabian Gulf . According to an investigation conducted in 2018, *E. coli* isolates had the blaCTX-M, blaTEM, and blaSHV genes (Alfaresi *et al.*, 2018). Additionally, NDM, OXA-48, and VIM were discovered in *E. coli* from the Arabian Peninsula (Sonnevend *et al.*, 2015). According to a study conducted in Kuwait, *E. coli* isolates had a high incidence rate of CTX-M. Additionally, VIM and NDM-1 were reported to be present (Taqi *et al.*, 2017). According to a number of

Omani studies, NDM and OXA-48 carbapenemases help *E. coli* develop carbapenem resistance (Dortet *et al.*, 2015; Zowawi *et al.*, 2014). In Qatar, the prevalence of microorganisms resistant to antibiotics is rising quickly among people (Eltai *et al.*, 2018).

In Iran, strains derived from bile specimens showed frequencies of SHV and TEM of 8.4% and 50%, respectively, according to one report (Komijani *et al.*, 2017) and 92% and 70%, respectively, based to another study (Fatemi *et al.*, 2015). Furthermore, as stated by Kazemian *et al.* (2019), of the *E. coli* strains, 21.5% had CTX-M, 16.9% had TEM, and 16.9% had SHV. As reported by Borah *et al.* (2016), the overall prevalence of CTX-M among *E. coli* isolates in India was 57.7%, TEM 30.1%, SHV 10.6%, and NDM-1 18.2%. Recent research from Cuba found that among clinical strains of *E. coli*, 31.7% had TEM and 0.7% had NDM-1 (Quiñones *et al.*, 2020). In line with a study conducted in Turkey, 72.72% and 22.72% of *E. coli* isolates, respectively, have TEM and CTX-M (Bali *et al.*, 2010).

Metagenomic analysis of U.S. soil bacteria revealed resistance genes similar to those found in human pathogens. The study by Forsberg *et al.* (2012), the genes were not reactive to tetracyclines, beta-lactams, aminoglycosides, amphenicols, and sulfonamides. The chromosomal structures of the soil and water-dwelling Kluyvera are also known to have plasmid-borne beta-lactamases of the CTX-M group, suggesting that this clinically significant resistance has environmental origins (McKinnon, 2020). The oxacillinase group of beta-lactamases and the ciprofloxacin resistance gene qnrA have been found to be isolated from aquatic Shewanella (Taha *et al.*, 2019; Shahid *et al.*, 2022) (Poirel *et al.*, 2004; Poirel *et al.*, 2005).

Typically, the inheritance of van genes triggers vancomycin resistance. VanA (80%–90%) and vanB (10%–20%) are the two most common van genotypes among the nine that have been documented thus far (McCracken *et al.*, 2013; Cattoir and Leclercq, 2013). The vanA operon is often carried by a Tn3-type transposon, Tn1546. It typically comprises two regulatory genes (vanRS), a transposase (orf1)/resolvase (orf2) region, and five genes (vanHAXYZ) for glycopeptide resistance (Cattoir and Leclercq, 2013). There have been reports of genetic variants in Tn1546, including deletions and/or adding certain insertion sequences (Hsieh *et al.*, 2010).

2.8 Method of characterization of resistance genes

Antibiotic-resistant genes in bacteria are found using phenotypic (Georgios *et al.*, 2014) and molecular techniques (Anjum *et al.*, 2017). While some techniques are regularly employed in diagnostic labs, others are utilized as study instruments as described by Anjum (2015). Nowadays, test methods including disk diffusion, Etest, and Sensititer^{TM} broth microdilution are used to identify resistant bacterial phenotypes that proliferate in the face of antibiotics (Mills *et al.*, 2022).

Nucleic acid hybridization and amplification are the foundation of genetic techniques (Georgios *et al.*, 2014). The target antibiotic-resistant genes are found using certain primers and probes (Georgios *et al.*, 2014). Among these comprise metagenomics, whole genome sequencing, DNA microarrays, and polymerase chain reaction (Georgios *et al.*, 2014; Anjum *et al.*, 2017). Due to its ease of use, the polymerase chain reaction is frequently employed (Anjum *et al.*, 2017). Southern blotting and polymerase chain reaction (PCR), which can be laborious and only identify one or a small number of genes at a time, have traditionally been the only methods used to identify the genes that produce resistance (Tarik *et al.*, 2022). Therefore, screening for hundreds of potential antibiotic resistance genes might be time-consuming to find the genes causing resistance.

High-throughput sequencing techniques have made the recently popular method of metagenome sequencing more viable (Li *et al.*, 2021). It also offers the ability to detect the microbial population in a given sample without requiring time-consuming microbial culture and isolation. In this regard, this technology presents a special chance to investigate either challenging or impossible microorganisms to cultivate in a lab setting (Ercolini, 2013).

Metagenomes have been studied using a range of complementary approaches (Petriz and Franco, 2017). Recent shotgun sequencing techniques offer more information about the gene repertoire, even though the traditional 16S rRNA sequencing methods still offer essential insights into the taxonomic identities of the species in the microbiome (Jovel *et al.,* 2016; Tessler *et al.,* 2017). Furthermore, the gene repertoire may be utilized to investigate and describe biological pathways (Jovel *et al.*, 2016; Tessler *et al.,* 2017) and enable the identification of new metabolic enzymes that may catalyze processes that result in the formation of new metabolites (Kennedy *et al.*, 2008). Furthermore, the public resources for gene function annotation and compendia of particular actions, such as antibiotic resistance gene annotations, provide new ways to integrate shotgun metagenomics sequence data and investigate the annotation of environmental niche diversity and its genes (Hendriksen *et al.*, 2019).

Owing to the latest advances in technology, DNA microarrays may now detect thousands of genes at once (Bumgarner, 2013). According to a number of research, PCR product probes have been used to build microarrays, including arrays for the identification of antibiotic resistance genetic factors (Bumgarner, 2013). A functional microarray must first be created, which takes time, and involves a template for every gene of interest, the design and synthesis of primers unique to that gene, PCR synthesis, gel scoring, and purification of PCR products. Synthetic oligonucleotide probes, which have been widely characterized for gene expression and comparative genomic hybridizations, circumvent these challenges in a microarray (Negi *et al.,* 2022).

According to research, oligonucleotide microarrays have been designed to detect antibiotic resistance genes in Gram-positive or *Salmonella* enteric bacteria (Strauss *et al.,* 2015; Fink *et al.,* 2019). However, in order to investigate the spread of antibiotic resistance in all bacteria, a standard detection technology must be developed due to the possibility of horizontal gene exchange between distantly related bacteria (Jian *et al.,* 2021).

2.9. Interventions to control microbial contamination on banknotes

Part of the intervention by various governments include; the awareness programs frequently on how to handle currency notes. The program encourages individuals to practice hand hygiene before handling food at any stage and the general public should also be informed of the danger of handling food with the money (Cortese *et al.,* 2016) The Banknote Disinfection System is very useful when receiving notes, from flooding, fires, drugs or other kind of contamination to ensure the health and safety of the Banks' employees working with these notes (Thaenkaew and Ruangsiri, 2023).

CHAPTER THREE

MATERIAL AND METHODS

3.1 Study area

Nyeri County, a central Kenyan county, encompasses 3,337.2 square kilometres. It shares borders with Laikipia, Kirinyaga, Murang'a, Nyandarua, and Meru counties (Figure 3.1). It is between longitudes 36^{o}38” east and 37^{o}20” east and between the equator and latitude 0^{o} 38^{o} south. Nyeri town is the capital and the largest town in Nyeri County. Nyeri town is the county's capital and largest city. With a population of 759,164, Nyeri County is one of Kenya's most densely populated regions(KNBS, 2019). The absence of prior research on antibiotic resistance in aerobic bacterial isolates from banknotes within Nyeri County prompted the selection of this region for the study.

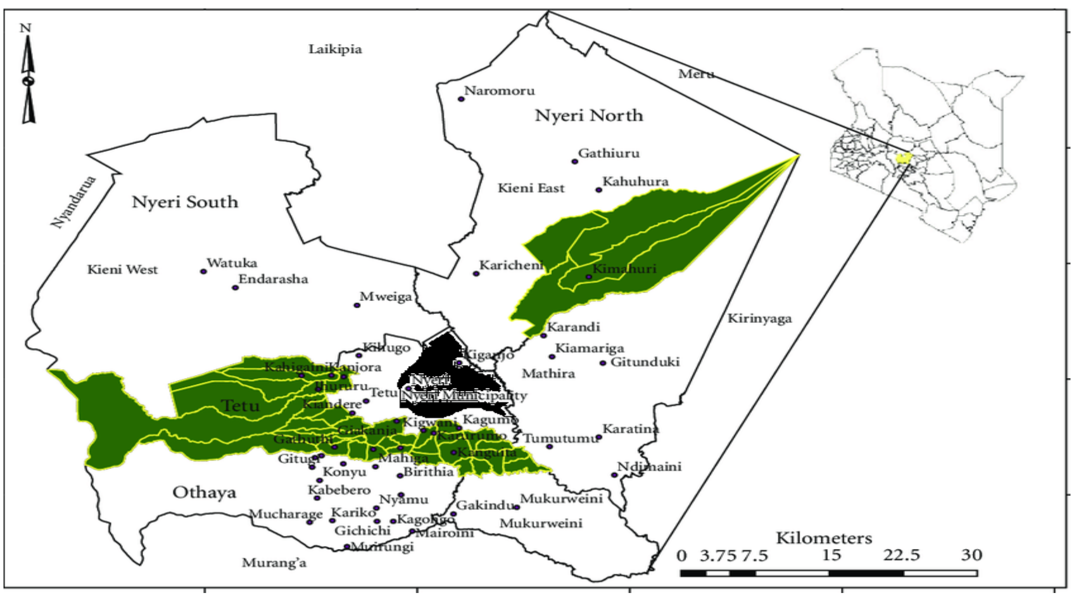

Figure 3.1 Map showing Nyeri municipality (highlighted in black) in Nyeri County, Kenya

3.2 Study design

This study employed descriptive cross-sectional design to assess aerobic bacterial contamination on Kenyan bank notes and their antibiotic susceptibility in Nyeri town.

3.3 Sampling design

The study used Stratified sampling method where the researcher divided the sources and currency denominations before collection was done. Banknotes for the experiment were sourced from a variety of market outlets, including butcheries, restaurants, healthcare facilities, M-Pesa outlets, and transport SACCOS. The banknotes were also stratified into various denomination of Ksh. 50, 100, 200, 500 and 1000.

3.4 Sample collection and processing

To collect the banknotes, the individuals were asked to drop the currency into a sterile zipped plastic bags, which were sealed and immediately transported. A total of 125 samples of Kenyan banknotes in five denominations; Ksh. 50, 100, 200, 500 and 1000 were collected and screened for bacteriological contamination at Outspan Teaching and Referral Hospital (OTRH) laboratory. The control consisted of one Kenyan banknote of Ksh. 50, 100, 200, 500 and 1000 from Central Bank of Kenya (CBK), Nyeri branch which had not be released for circulation. The samples were collected in sterile zipped plastic packets which were marked. The banknotes were dipped in sterile normal saline and vigorously shaken for 3 minutes. A sterile cotton swab was dipped and inoculated in blood agar and Mac Conkey agar for each note. The plates were incubated at 37°C for 18- 24 hours. After 18-24 hours the plates were observed for bacterial colonies (Kawo *et al.*, 2010).

3.5 Sample size

3.5.1 Sample size determination

The sample size was determined using the Fisher's formula (Lwanga *et al.*, 1991).

$N= Z^2 \times p\ (1- P)/d^2$

Prevalence of 9% was used according to a Nigerian study (Fashae *et al.*, 2010).

Where; N is the sample size

Z is 1.96 the standard normal distribution

P is 9% (expected proportion in population based on previous studies)

d is 5% the absolute degree of precision

$N= (1.96^2 \times 0.09\ (1-0.09)/0.05^2=125$

The study had a total sample size of 125 banknotes and 5 control banknotes (Central Bank of Kenya) in each denomination.

3.6 Isolation

For the primary isolation of bacteria, a loop full of the enriched broth streaked onto blood agar and McConkey's agar using sterile swap. Blood agar is a nutrient-rich medium that provides essential growth factors for many microorganisms while MacConkey agar is a selective medium used for the isolation of non-fastidious Gram-negative rods, particularly members of the family *Enterobacteriaceae* and the genus *Pseudomonas* which are very important microorganism in this study. The blood agar and McConkey's agar isolation media prepared plates were procured from Kenya Medical Research institute (KEMRI).

3.6.1 MacConkey agar

MacConkey medium (51.63 grams) was suspended in 1000 ml distilled water in a conical flask. It was then heated to boiling in a water bath and gently swirled to dissolve the medium completely. It was sterilized by autoclaving at 15 lbs pressure at 121°C for 15 minutes. It was cooled to 45 – 50°C and poured into sterile Petri plates. The agar was allowed to settle and the surface of the agar had to be dry before inoculation.

3.6.2 Blood agar

Blood agar medium (40.0 grams) was suspended in 1000 ml distilled water in a conical flask. It was then heated to boiling in a water bath and gently swirled to dissolve the medium completely. It was sterilized by autoclaving at 15 lbs pressure at 121°C for 15 minutes. It was cooled to 50°C and 5% (v/v) sterile defibrinated blood aseptically added. It was mixed well and poured into sterile Petri plates. The agar was allowed to settle and the surface of the agar had to be dry before inoculation. The cultures were incubated aerobically at 37^{o}C for 18-24 hours. Cultures on semi-solid media were examined grossly for colonial morphology and haemolysis on blood agar.

3.7 Morphological and biochemical characterization of the isolates

The bacterial isolates were characterized on the basis of their morphology, staining and biochemical tests.

3.7.1 Morphological characterization of the isolates

Examination of all cultures on solid media was performed for detection of growth, pigmentation, colonial morphology as well as changes in the media. Plates which showed visible growth were subjected to further bacteriological tests while those which did not show visible growth were incubated for further 48 hours. Gram's technique was done as described by Barrow and Feltham (1993) to differentiate Gram negative and Gram positive bacterial (Appendix I). All isolated microorganisms were subjected to microscopic examination and the shape, arrangement and Gram's reaction were detected and recorded (Dimri *et al.*, 2020).

3.7.2 Biochemical characterization of the isolates

A Bacterial Identification System was employed, utilizing the Gram positive/anaerobes (Bis-plus) and Gram negative (Bis-Neg) standardized identification panels. These panels facilitated the identification of common Gram positive organisms (including Gram positive cocci), corynebacteria (Gram positive rods), and anaerobes (both Gram positive and Gram negative) (see Appendix I). Throughout the incubation period, the metabolic activity of the inoculated bacteria was linked to specific colorimetric reactions based on the test conducted. A supplementary color code was provided to assist in determining whether the results of the tests were positive or negative, as illustrated in Appendix II. The technique consisted of 24 miniaturized biochemical tests in order to facilitates the identification of bacteria through the genus and up to species levels (Rakotovao-Ravahatra *et al.,* 2021) (Appendix III). The results profiles were then keyed on the cypress identification software (Appendix IV).

3.8 Antimicrobial testing

Antimicrobial susceptibility of the bacterial isolates was determined against various antibiotics using the Kirby-Bauer disk diffusion method according to guidelines by the Clinical and Laboratory Standards Institute (CLSI, 2013). The 0.5 McFarland standards was used to adjust the turbidity concentration of the inoculums for antibiotic test (Appendix IV).

The identified isolates were tested against commonly used and available antibiotics; ceftriaxone 30ug, tetracycline 30ug, amoxycilin 30ug, ciprofloxacin 5ug, gentamycin, clindamycin 2ug, vancomycin 30ug and erythromycin 15ug.

A sterile platinum loop was used to pick overnight bacterial colonies from the culture plate and emulsified in 4 ml of sterile peptone water to match with 0.5 McFarland turbidity standards (1.0 x 10^8cfu/ml). Using a sterile swab, the surface of Mueller Hinton agar (Oxoid, Basingstoke, UK) was evenly inoculated with the suspension and let to air dry for 10 minutes. Using multichannel disc dispenser (Oxoid, Basingstoke, UK) antibiotics discs were deposited onto the surface of the inoculated medium and plates incubated at 37 °C for 24 hours. The diameters of the zones of inhibition were measured in millimetres. The tests were replicated and the results compared with the clinical and laboratories standards institute chart. *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were used as control (CLSI, 2016). The sensitivity status of bacteria, defined as susceptible (S) (a status in which the growth of the bacteria is inhibited *in vitro* by the applied concentration of the antibiotic and the antibiotic dose is therapeutically effective), moderately susceptible (M) (bacterial response to the drug is lower, and the drug may fail to achieve a therapeutic response) and resistant (R) (bacteria are not responsive to the given antibiotic, which clearly indicates therapeutic failure) to antibiotics, was determined according to the specifications of the Clinical and Laboratory Standards Institute (CLSI, 2013 which are listed in appendix VI. Thirty isolates that showed notable resistance to commonly used antibiotics was selected and subjected to DNA extraction process for testing availability of the antibiotics resistance genes.

3.8.1 Mueller Hinton agar

Mueller Hinton agar medium (38.2 grams) was suspended in 1000 ml distilled water in a conical flask. It was then heated to boiling in a water bath and gently swirled to dissolve the medium completely. It was sterilized by autoclaving at 15 lbs pressure at 121°C for 15 minutes. It was mixed well and then poured into sterile Petri plates. The agar was allowed to settle and the surface of the agar had to be dry before inoculation.

3.9 Determinant of antimicrobial Resistance Genes

3.9.1 Genomic DNA extraction of isolates

Microbial DNA was extracted from isolates that demonstrated significant antimicrobial resistance, as determined by the Kirby-Bauer disk diffusion method, to one or more of the following antibiotics: tetracycline, erythromycin, and vancomycin. Bacterial DNA was therefore extracted using Zymo research Quick-DNA™miniprep kit according to the manufacturer's instructions. The quantity and quality of extracted bacterial DNA was determined using NanoDrop spectrophomotor method (ThermoFisher, 2000c) (Vesty *et al.*, 2017) Agarose (1%, w/v) gel electrophoresis was performed in 20 mM Tris–acetate, 2 mM EDTA, pH 8.2 (TAE). Experiments were carried out under light protection. DNA samples were incubated for ∼10 min with SG before loading the gels and UV trans-illuminator (ThermoFisher UVP, 95045201) used in viewing the bands (Vitzthum and Bernhagen, 2002).

3.9.2 Amplification of antimicrobial resistance genes

The resistance genes for various antibiotics were analysed (appendix VII). The antimicrobial resistance genes coding for tetracycline, erythromycin and vancomycin were amplified using specific primers (Table 3.1) in After optimization of the procedure, PCR (ProFlex™ PCR

System, 96-well) was carried out in a 25 µl reaction volume containing 19.875µl sterile PCR water, 0.5 µl of 10 µM reverse primer and 0.5 µl of 10 µM Forward primer, 2.5 µl 10X standard reaction buffer, 0.5 µl of 10mM dNTPS, 2.0 mM $MgCl_2$, 0.125 µltaq polymerase (ThermoFisher) and 1.0 µl of template DNA. Amplification was conducted under the following conditions; one cycle 94 °C for 5 min and 35 of 94 °C for 45 seconds, annealing at (Table 3.1) for 45 seconds and 72 °C for 2 minutes with final extension at 72 °C for 5 minutes. Amplified DNA was held at 4^{o}C in the thermocycler before storage at -20 °C (Tripathi *et al.*, 2015).

Table 3.1: Primer sequences and annealing temperature for tetracycline, erythromycin and vancomycin resistance genes for isolated aerobic bacteria

Primer	Sequence	Bases	Annealing temperature	Reference
TEM-P1-757-F	GCG GAA CCC CTA TTT G	16	55.00 °C	(Bush *et al.*, 1995)
TEM-C-NY-686-	ACC AAT GCT TAA TCA GTG AG	20		
ERMC-F	AAT CGT CAA TTC CTG CAT GT	20	53.90 °C	Sutcliffe *et al.*,1996)
ERMC-R	TAA TCG TGG AAT ACG GGT TTG	21		
VAN-A-15	GGG AAA ACG ACA ATT GC	17	51.60 °C	(Dutka-Malen *et al.* 1995)
VAN-A2-5	GTA CAA TGT GGC CGT TA	17		

3.10 Gel electrophoresis of PCR products

Using agarose gel electrophoresis on a 1.5% agarose gel in 1× Tris-EDTA solution, all PCR results were examined. 1.5 µl (1µg/ml) of ethidium bromide was added to the gel in order to stain the DNA (Lee *et al.*, 2012). Ten microliters of each PCR product were combined with gel loading dye and then added into the gel's wells. To assess the size of the DNA band, three microliters of Bioline's 100 bp DNA ladder were added. For forty minutes, the electrophoresis was operated at a steady 100 volts. Following electrophoresis, ultraviolet (UV) light was used to view the bands in the gel (Invitrogen Safe Imager 2.0 Blue Light Transilluminator). The size of the DNA fragments were measured using a radiograph and compared to the positive control and DNA ladder (Magdeldin, 2012).

3.11 Data analysis

Descriptive statistical technique was used to analyze various data from the laboratory. These included averages, percentages and frequencies. Continuous data were expressed as mean and categorical data expressed as proportion. Statistical analysis was performed using statistical package for social sciences (SPSS) software for Windows, ver. 21 (SPSS, IBM, USA). Chi-square was used to evaluate the variables correlation.

3.12 Ethical considerations

The research proposal was submitted to Kenyatta University and Outspan Teaching and Referral Hospital (OTRH) for approval to conduct the research (Appendix VII). During the current research, consent from the institution was obtained prior to the study. The protection of the privacy of research participants and institutions was ensured and adequate level of confidentiality of the research data maintained. This was achieved by ensuring anonymity of individuals and organisations participating in the research by giving code name and not actual names. Voluntary participation of respondents in the research is allowed and participants had a rights to withdraw from the study at any stage if they wish to do so. Every works of other authors used in any part of the thesis was acknowledged.

CHAPTER FOUR

RESULTS

4.1 Introduction

The study was carried out to establish the antibiogram patterns and resistance genes associated with aerobic bacterial contaminants present on Kenyan banknotes circulating in the Nyeri town in Nyeri County. Bacterial isolation, identification and antibiotic susceptibility was done at Outspan Teaching and Referral Hospital (OTRH) laboratory and resistance genes done at Kenyatta University. A total of 125 circulating Kenyan banknotes of 5 different denominations were collected from different marketing sources such as butcheries, restaurants, health facilities, M-pesa outlets and transport Savings and Credit Cooperative Organization (SACCOS) then dropped in sterile bags. In the study, 69 (55.2%) were dirty and 56 (44.8%) were clean. The five control Kenyan banknote were from Central Bank of Kenya, Nyeri branch. These 5 control banknotes were mint (not in circulation).

4.2 Aerobic bacterial contaminants present in circulating Kenyan banknotes in Nyeri town

One hundred and ten (88%) of the sampled Kenyan circulating banknotes produced bacterial growth in blood agar while 95 (76%) of the samples produced bacterial growth in Mac Conkey. The control banknotes obtained from the Central Bank of Kenya, Nyeri branch did not show any bacterial colonies on both Mac Conkey and blood agar. A total of 71 isolates were obtained from circulating banknotes with 56 (78.9%) Gram’s positive and 15 (21.1%) Gram’s negative bacteria (Table 4.1).

Table 4.1: Percentage aerobic bacterial growth on media plates and bacterial Gram type

Growth	N=125	%
Blood agar	110	88
Mc Conkey	95	76
Gram Type	**N=71**	**%**
Gram's positive	56	78.9
Gram's negative	15	21.1

The study isolated 56 (78.9%) Gram positive bacteria from Kenyan banknotes: *Staphylococcus aureus* 37 (66.1%), *Staphylococcus sciuri* spp. 8 (14.3%), *Staphylococcus intermedius* 6 (10.6%), *Staphylococcus schleiferi* spp 2 (3.6%), *Micrococcus* spp. 1 (1.8%), and *Staphylococcus gallinarum* 2 (3.6%) (Figure 4.1).

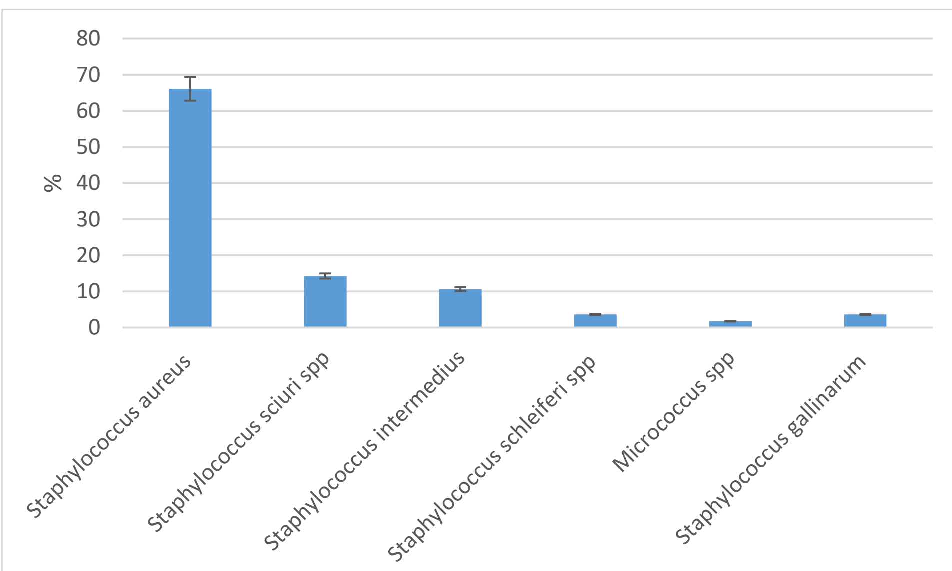

Figure 4.1: Gram positive bacteria isolated from Kenyan banknotes circulating in Nyeri town between March, 2019 and April, 2019

In addition, 15 (21.1%) Gram negative bacteria were isolated from Kenyan banknotes. They included: *Burkholderia cepacia* spp 1 (6.67%), *Aeromonas enteropelogenes* 1 (6.67%), *Kluyvera ascorbata* 1 (6.67%), *Aeromonas media* 3 (20.0%), *Raoultella ornithinolytica* 1 (6.67%), *Enterobacter cloacae* 1 (6.67%), *Klebsiella oxytoca* 2 (13.3%), *Leclercia adecarboxylata* 1 (6.67%), *Vibrio metschnikovii* 1 (6.67%), *Proteus penneri* 1 (6.67%), *Myroides odoratus* 1 (6.67%) and *Yersinia pestis* 1 (6.67%) as shown in Figure 4.2. The dominant Gram negative bacterial isolates from this study was *Aeromonas media* (20.0%) followed by *Klebsiella oxytoca* (13.3%) (Figure 4.2).

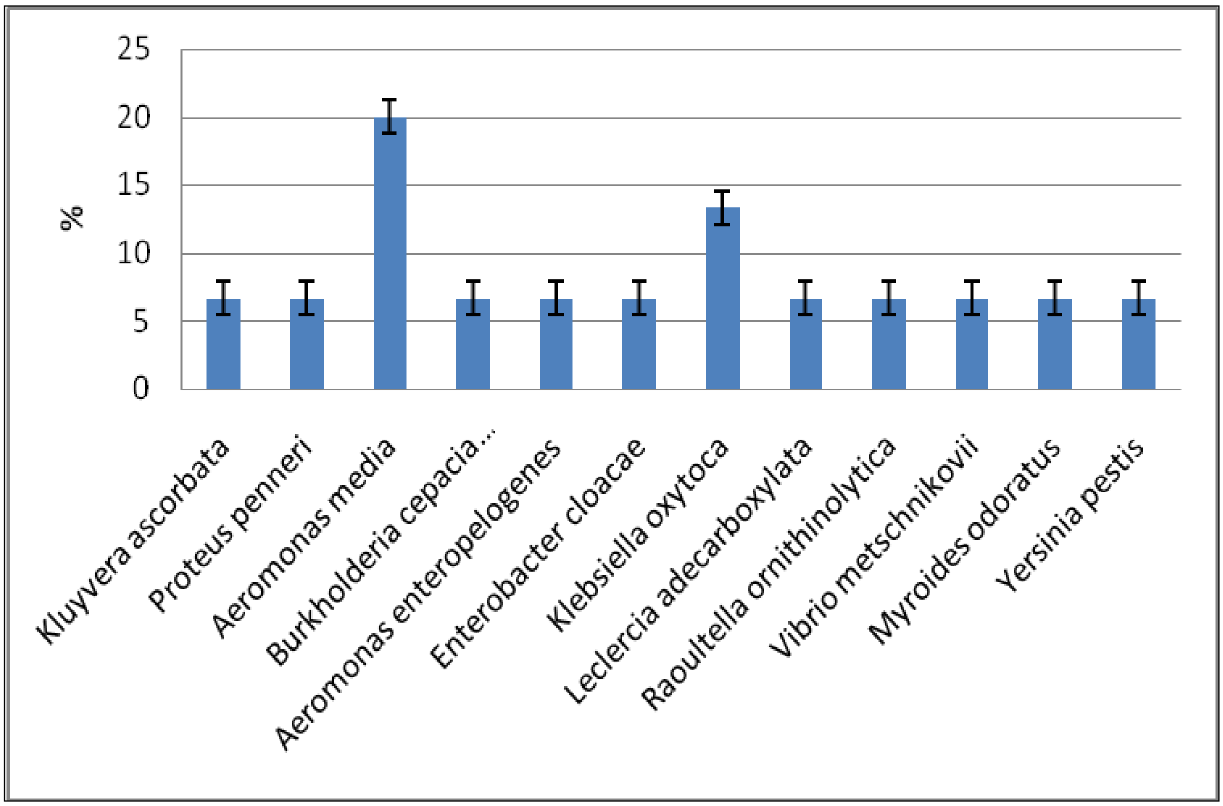

Figure 4.2: Gram negative bacteria isolated from Kenyan banknotes circulating in Nyeri town between March, 2019 and April, 2019

Staphylococcus aureas was the most common isolate, according to the relative distribution of 71 isolates taken from contaminated circulating Kenyan banknotes. The highest number of aerobic bacterial isolates 19 (26.7%) were recorded from fifty banknote and the least 12 (16.9%) were recorded from both one thousand and two hundred banknotes respectively (Table 4.3). Eighteen species of aerobic bacteria were isolated that included; *Staphylococcus aureus, Staphylococcus sciuri* spp*, Staphylococcus gallinarum, Staphylococcus intermediu, Micrococcus* spp., *Staphylococcus schleiferi* spp, *Kluyvera ascorbata, Kluyvera ascorbata, Proteus penneri, Aeromonas media, Burkholderia cepacia* spp., *Aeromonas enteropelogenes, Enterobacter cloacae, Klebsiella oxytoca, Leclercia adecarboxylata, Raoultella ornithinolytica, Vibrio metschnikovii, Myroides odoratus* and *Yersinia pestis* (Table 4.3). The majority (77.9%) of the aerobic bacteria found on banknotes from Nyeri County were *Staphylococcus* species (Table 4.2).

Table 4.2: Occurrence of bacterial species on banknotes of different denominations

Currency Denomination (Ksh)	**50**	**100**	**200**	**500**	**1000**	**Number (%**
Aerobic bacteria	**N=25**	**N=25**	**N=25**	**N=25**	**N=25**	
Staphylococcus aureus	10	6	5	8	8	37 (52.2%)
Staphylococcus sciuri spp.	2	2	2	1	0	8 (11.3%)
Staphylococcus gallinarum	1	0	1	0	0	2 (2.8%)
Staphylococcus intermedius	0	2	0	3	1	6 (8.5%)
Micrococcus spp.	0	0	1	0	0	1 (1.4%)
Staphylococcus schleiferi spp.	1	0	0	0	1	2 (2.8%)
Kluyvera ascorbate	0	1	0	0	0	1(1.4%)
Proteus penneri	1	0	0	0	0	1(1.4%)

Currency Denomination (Ksh)	50	100	200	500	1000	Number (%
Aerobic bacteria	**N=25**	**N=25**	**N=25**	**N=25**	**N=25**	
Aeromonas media	1	0	1	0	1	3 (4.2%)
Burkholderia cepacia spp.	1	0	0	0	0	1(1.4%)
Aeromonas enteropelogenes	0	1	0	0	0	1(1.4%)
Enterobacter cloacae	1	0	0	0	0	1(1.4%)
Klebsiella oxytoca	0	1	0	1	0	2 (2.8%)
Leclercia adecarboxylata	0	1	0	0	0	1(1.4%)
Raoultella ornithinolytica	0	0	1	0	0	1(1.4%)
Vibrio metschnikovii	0	0	0	1	0	1(1.4%)
Myroides odoratus	0	0	0	0	1	1(1.4%)
Yersinia pestis	1	0	0	0	0	1(1.4%)
Total	**18**	**14**	**12**	**14**	**12**	**71(100%)**

The study established a higher rate of banknotes aerobic bacterial contamination from butcheries with 38.0% and 25.4% from transport Saccos. The least aerobic bacterial contamination (2.82%) was recorded in the hotels sampled in Nyeri County (Table 4.3).

Table 4.3: Distribution of bacteria among various sources in Nyeri County

Source **Aerobic bacteria**	**Butchery** **N=25**	**Hotel** **N=25**	**Health Facilities** **N=25**	**Mpesa agents** **N=25**	**Transport SACCOs** **N=25**	**Number (%)**
Staphylococcus aureus	16	2	1	6	12	37 (52.2%)
Staphylococcus sciuri spp	3	0	2	2	1	8 (11.3%)
Staphylococcus gallinarum	1	0	0	0	1	2 (2.8%)
Staphylococcus intermedius	3	0	3	0	0	6 (8.5%)
Micrococcus spp.	1	0	0	0	0	1 (1.4%)
Staphylococcus schleiferi spp.	0	0	1	0	1	2 (2.8%)
Kluyvera ascorbate	1	0	0	0	0	1(1.4%)
Proteus penneri	0	0	1	0	0	1(1.4%)
Aeromonas media	0	0	2	0	1	3 (4.2%)
Burkholderia cepacia spp.	0	0	1	0	0	1(1.4%)
Aeromonas enteropelogenes	0	0	1	0	0	1(1.4%)
Enterobacter cloacae	0	0	0	1	0	1(1.4%)
Klebsiella oxytoca	0	0	0	2	0	2 (2.8%)
Leclercia adecarboxylata	0	0	0	0	1	1(1.4%)
Raoultella ornithinolytica	0	0	0	0	1	1(1.4%)
Vibrio metschnikovii	1	0	0	0	0	1(1.4%)
Myroides odoratus	0	0	0	1	0	1(1.4%)
Yersinia pestis	1	0	0	0	0	1(1.4%)
Total	**27**	**2**	**12**	**11**	**18**	**71(100%)**

4.3 Antibiotic susceptibility profiles of isolates from Kenyan banknotes circulating in Nyeri town using Kirby-Bauer disk diffusion method

This study revealed that antibiotic resistant strains of different aerobic bacterial isolates. Isolates of Gram positive bacteria species recorded high rates of resistance collectively as 100%, 83.3% and 66.7% against clindamycin, vancomycin and amoxycilin respectively (Table 4.4). The majority of the isolated bacteria showed sensitivity to most of the antibiotics used while highest resistance was seen against Gram positive bacteria. In addition to the above, *Klebsiela* spp., showed 100% resistance to amoxycilin, clindamycin, vancomycin and erythromycin (Table 4.5) The sensitivity test for Gram-positive bacteria (Table 4.4 and 4.5) showed that the sensitivity of *Staphylococcus* spp and *Klebsiella* spp. to the antibiotic gentamycin was 100% respectively.

Table 4.4: Antibiotic susceptibility patterns of Gram positive bacterial isolates (No. of R or S isolates/ n)

Bacteria	**CRO (n)**	**TE (n)**	**AML (n)**	**CIP (n)**	**CN (n)**	**CD (n)**	**VA (n)**	**E (n)**
Staphylococcus aureus	S (36/37)	S (35/37)	R (20/37)	S (36/37)	S (37/37)	R (24/37)	R (26/37)	S (21/37)
Staphylococcus intermedius	S (5/6)	S (6/6)	R (4/6)	S (6/6)	S (6/6)	R (4/6)	R (5/6)	R (3/6)
Staphylococcus sciur spp	S (7/7)	S (7/7)	S (6/7)	S (7/7)	S (6/7)	R (4/7)	S (5/7)	S=(5/7
Staphylococcus gallinarum	S (2/2)	S (2/2)	R (2/2)	R (2/2)	S (2/2)	R (2/2)	R (2/2)	S (2/2)
Staphylococcus sciur spp.	S (1/1)	S (1/1)	S (1/1)	S (1/1)	S (1/1)	R (1/1)	R (1/1)	S (1/1)
Micrococcus spp.	S (1/1)	S (1/1)	R (1/1)	S (1/1)	S (1/1)	R (1/1)	R (1/1)	R (1/1)
Staphylococcus schleiferi spp.	S (2/2)	S (2/2)	S (2/2)	S (2/2)	R (1/2)	R (1/2)	R (1/2)	S (2/2)

Key *:S=Sensitive, R=Resistant, CRO= Ceftriaxone, TE= Tetracycline, AML= Amoxycilin, CIP= Ciprofloxacin, CN= Gentamycin, CD= Clindamycin, VA= Vancomycin, E= Erthromycin.*

Table 4.5: Antibiotic susceptibility patterns of Gram negative bacterial isolates (No. of R or S isolates/ n)

BACTERIA	CRO	TE	AML	CIP	CN	CD	VA	E
Kluyvera ascorbate	S (1/1	S (1/1)	S (1/1)	S (1/1)	S (1/1)	R (1/1)	S (1/1)	S (1/1)
Proteus penneri	S (1/1)	S (1/1)	S (1/1)	S (1/1)	S (1/1)	S (1/1)	S (1/1)	S (1/1)
Aeromonas media	S (3/3)	S (3/3)	S (2/3)	S (3/3)	S (3/3)	S (2/3)	R (2/3)	S (3/3)
Burkholderia cepacia spp.	S (1/1)	S (1/1)	S (1/1)	S (1/1)	S (1/1)	S (1/1)	R (1/1)	S (1/1)
Aeromonas enteropelogenes	S (1/1)	S (1/1)	R (1/1)	S (1/1)	S (1/1)	R (1/1)	R (1/1)	R (1/1)
Enterobacter cloacae	S (1/1)	S (1/1)	R (1/1)	S (1/1)	S (1/1)	R (1/1)	S (1/1)	R (1/1)
Klebsiella oxytoca	S (2/2)	S (2/2)	R (2/2)	S (2/2)	S (2/2)	R (2/2)	R (2/2)	R (1/2)
Leclercia adecarboxylata	S (1/1)	S (1/1)	R (1/1)	S (1/1)	S (1/1)	R (1/1)	R (1/1)	S (1/1)
Raoultella ornithinolytica	R (1/1)	S (1/1)	R (1/1)	S (1/1)	S (1/1)	S (1/1)	S (1/1)	S (1/1)
Vibrio metschnikovii	S (1/1)	S (1/1)	S (1/1)	S (1/1)	S (1/1)	R (1/1)	R (1/1)	S (1/1)
Myroides odoratus	S (1/1)	S (1/1)	S (1/1)	S (1/1)	S (1/1)	S (1/1)	R (1/1)	S (1/1)

Key: *S=Sensitive, R=Resistant, CRO= Ceftriaxone, TE= Tetracycline, AML= Amoxycilin, CIP= Ciprofloxacin, CN= Gentamycin, CD= Clindamycin, VA= Vancomycin, E= Erthromycin.*

The study recorded 30 (24.4%) samples that show notable resistance against tetracycline, erythromycin and vancomycin. Among the 30 samples, healthy facilitates had the highest resistance of 30%, followed by 23.3% from transport SACCOs, 20% for butcheries, 16.7% from M-pesa outlets and 10% from restaurants (Figure 4.3).

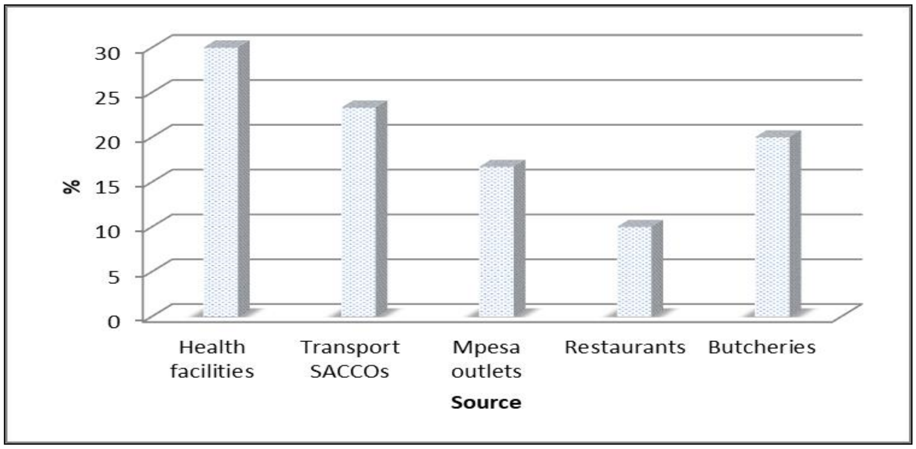

Figure 4.3: Percentage notable bacterial resistance against tetracycline, erythromycin and vancomycin from five sources using the Kirby-Bauer disk diffusion method

Among the 30 samples, fifty banknote denominations had the highest resistance of 40%, followed 20% from five hundred denomination, 16.7% from two hundred denomination, 13.3% from one hundred denomination and 10% from one thousand denominations (Figure 4.4).

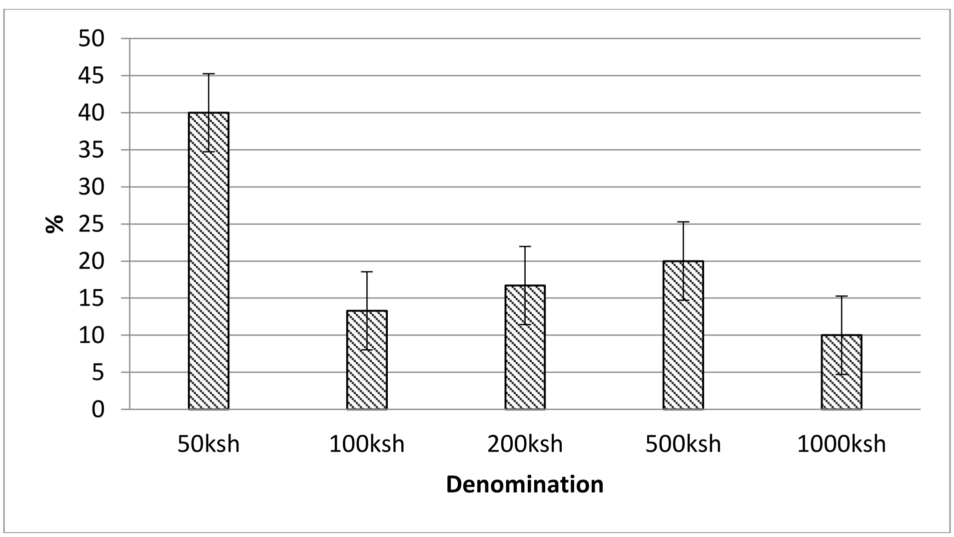

Figure 4.4: Percentage notable bacterial resistance against tetracycline, erythromycin and vancomycin from five denominations using the Kirby-Bauer disk diffusion method in Nyeri town between March, 2019 and April, 2019

4.4 Resistance genes associated with bacterial contaminants present in circulating in Kenyan banknotes in Nyeri town

Kirby-Bauer disk diffusion was used to select 30 isolates that showed remarkable resistance. From the PCR results, 8 (26.7%) had resistance genes. 2 (6.7%) isolates carried vancomycin resistance gene while, 6 (20.0%) had bla_{TEM} resistance gene (Table 4.6). Out of the 8 resistant gene, 2 (25%) were vancomycin resistant genes while 6 (75%) were resistant against tetracycline.

Table 4.6: Frequency of resistance gene isolated from circulating banknote in Nyeri Town, Nyeri County

Resistance gene tested	Sample size	Percentag
Erythromycin	30	0 (0.0%
Vancomycin	30	2 (6.7%
TEM 1	30	6 (20.0%

Two vancomycin resistance genes were isolated from two different health facilities one from fifty banknote and another from one thousand banknote (Plate 1). Six TEM 1 resistance genes were isolated from various sources. The resistance gene isolates were as follows; two from transport SACCOs; one from one thousand banknote and one from fifty banknote; one from one hundred banknote from M-pesa source, one from one hundred banknote from the restaurant; health facility yielded one TEM 1 resistance gene from fifty banknote and one TEM 1 resistance gene from fifty banknote from the butchery (Plate 1).

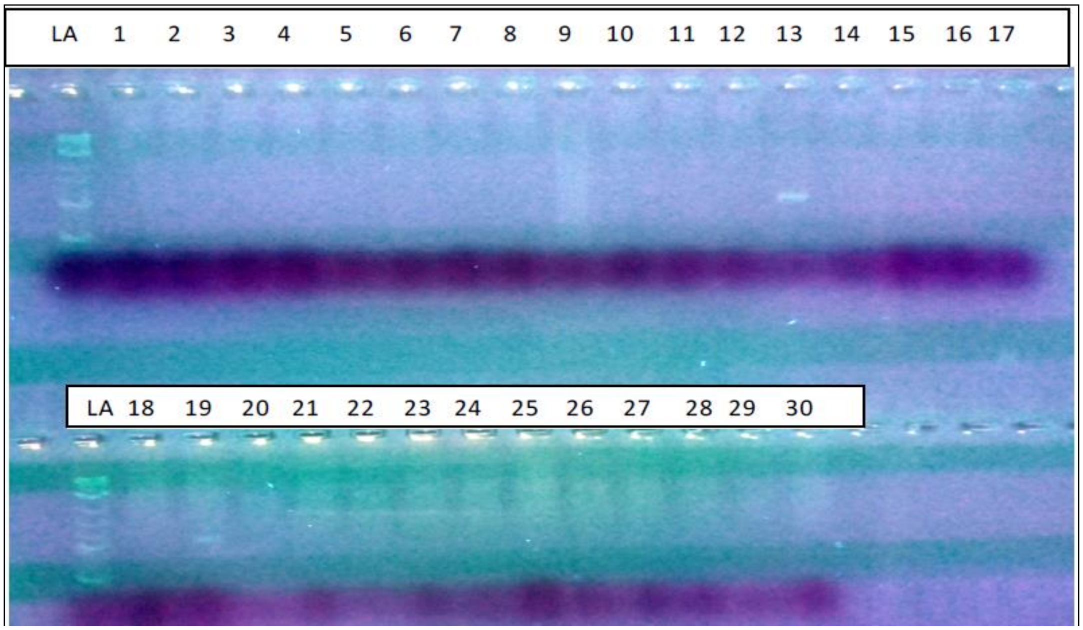

Plate 4.1: Vancomycin resistance genes from selected sources on various circulating Kenyan banknotes from Nyeri town, Nyeri County.

KEY: Source of bacterial isolate: *LA- Ladder;1-butchery one thousand banknote; 2-Health facility fifty banknote, 3-transport sacco one thousand banknote, 4-M-pesa fifty banknote 5-butchery two hundred banknote; 6- Health facility five hundred banknote; 7- M-pesa five hundred banknote; 8- transport sacco five hundred banknote; 9- transport sacco two hundred banknote; 10- transport sacco fifty banknote; 11- M-pesa fifty banknote; 12- transport sacco fifty banknote; 13- Health facility one thousand banknote; 14- Restaurant fifty banknote; 15-Health facility five hundred banknote; 16- M-pesa one hundred banknote; 17- Health facility five hundred banknote; 18- butchery one hundred banknote; 19- Health facility fifty banknote; 20- Health facility two hundred banknote; 21- Health facility one hundred banknote; 22-transport sacco fifty banknote; 23- butchery fifty banknote; 24- Restaurant one hundred banknote; 25- M-pesa two hundred banknote; 26- butchery five hundred banknote; 27-transport sacco two hundred banknote; 28-Restaurant fifty banknote; 29- Health facility fifty banknote; 30- butchery fifty banknote.*

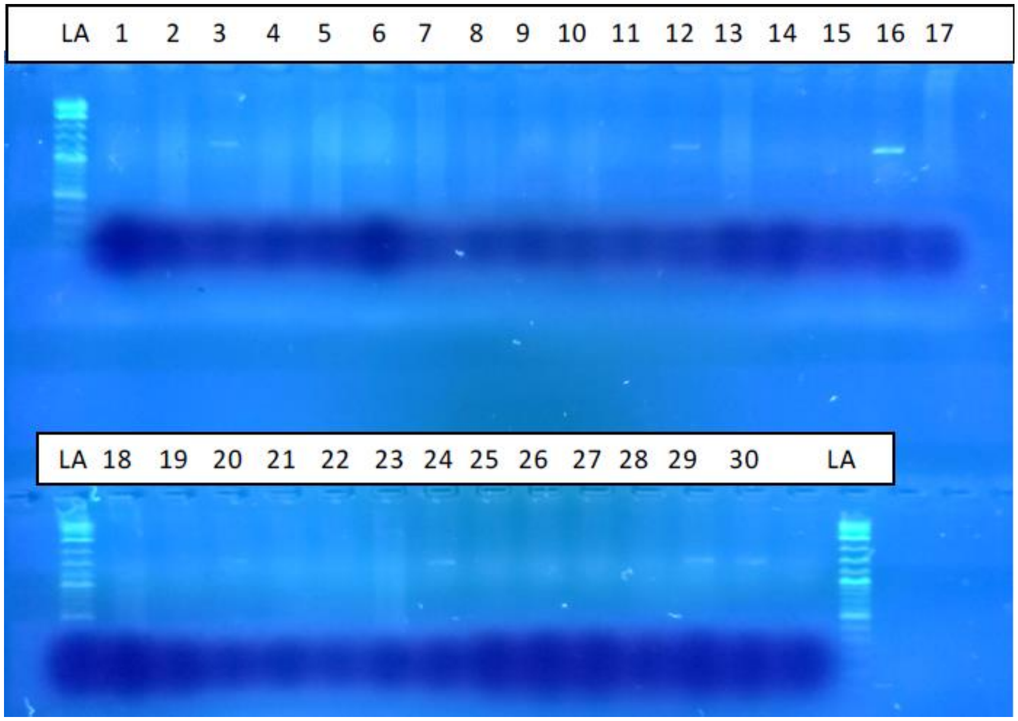

Plate 4.2: TEM 1 resistance genes from selected sources on various circulating Kenyan banknotes from Nyeri town, Nyeri County.

KEY: Source of bacterial isolate: *LA- Ladder;1-butchery one thousand banknote; 2-Health facility fifty banknote, 3-transport sacco one thousand banknote, 4-M-pesa fifty banknote 5-butchery two hundred banknote; 6- Health facility five hundred banknote; 7- M-pesa five hundred banknote; 8- transport sacco five hundred banknote; 9- transport sacco two hundred banknote; 10- transport sacco fifty banknote; 11- M-pesa fifty banknote; 12- transport sacco fifty banknote; 13- Health facility one thousand banknote; 14- Restaurant fifty banknote; 15-Health facility five hundred banknote; 16- M-pesa one hundred banknote; 17- Health facility five hundred banknote; 18- butchery one hundred banknote; 19- Health facility fifty banknote; 20- Health facility two hundred banknote; 21- Health facility one hundred banknote; 22-transport sacco fifty banknote; 23- butchery fifty banknote; 24- Restaurant one hundred banknote; 25- M-pesa two hundred banknote; 26- butchery five hundred banknote; 27-transport sacco two hundred banknote; 28-Restaurant fifty banknote; 29- Health facility fifty banknote; 30- butchery fifty banknote.*

The study established that, 22.2% of the Kenyan banknote sampled from hospital facilitates had vancomycin resistant gene while 11.1% had TEM 1 resistant genes. Transport SACCOs, M-pesa outlets, restaurants, and butcheries did not have any vancomycin resistant genes but had TEM 1 resistant genes occurrence of 28.6%, 20%, 33.3% and 16.7% respectively (Figure 4.5).

Figure 4.5: Resistance genes isolated from aerobic bacteria from circulating Kenya banknotes in Nyeri town, Nyeri County

The result showed that, 8.3% of fifty-shilling banknotes sample had vancomycin resistant genes while 25% had TEM resistant genes. One-thousand-shilling denomination recorded 33.3% occurrence of resistant genes for both vancomycin and TEM 1. One-hundred-shilling banknotes recorded 50% occurrence of vancomycin resistant genes. Both denomination of two hundred shilling and five-hundred-shilling banknote did not have any resistant gene (Figure 4.6).

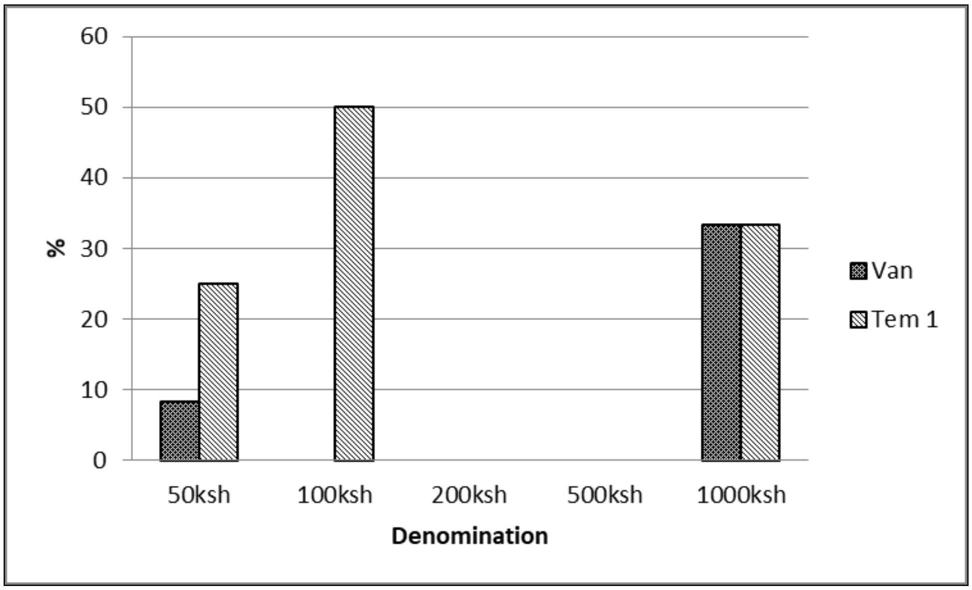

Figure 4.6: Resistance genes isolated from aerobic bacterial contaminant on Kenyan banknotes circulating in Nyeri town, Nyeri County.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

Due to their high mobility as inanimate objects in human civilisation, circulating banknotes have the ability to spread bacteria that cause disease (Meister *et al.*, 2023). In both affluent and developing nations, the unchecked use of antibiotics to treat diseases has a negative impact on public health and leads to drug resistance. Therefore, the current study's objective was to continuously assess the state of antibiotic resistance among the aerobic bacterial isolates found on circulating banknotes.

5.1.1 Aerobic bacterial contaminants present in circulating Kenyan banknotes

One hundred and ten (88%) of the sampled Kenyan circulating banknotes produced bacterial growth in blood agar while 95 (76%) of the samples produced bacterial growth in Mac Conkey. The reported levels of currency contamination in this study are higher than those found in other developing nations, such as Nigeria (52.5%), Nepal (75%), and Saudi Arabia (72.3%). However, they are similar to the 94% reported in the United States (Pope *et al.*, 2011), and lower than the 100% recently documented in india (Sunil *et al.*, 2020) and Pakistan (Badvi *et al.*, 2017). The study was limited toThe source of contamination on the used notes must be from handling and use. The high presence of aerobic bacteria on Kenyan banknotes is indicative of poor hygiene in among individuals who have recently handled the banknotes. The quality of the Kenyan banknotes and poor hanling practices of circulating banknotes could be attributable to a high level of bacterial growth in the current study.

Bangladeshi paper currency notes have a higher bacterial contamination rate of 93.7%, as per Akon *et al.* (2015). Based on earlier research, between 70 and 94 percent of coins and banknotes from countries like China, India, and the United States have diverse pathogenic organisms on their surface (Sharma and Sumbali, 2014). These disparities demonstrate that microbiological contamination of currency is a worldwide issue and mirror variations in sanitary procedures and currency handling in various regions. Practices such as placing banknotes under body surfaces, failing to wash hands thoroughly after using the restroom, sneezing or coughing onto hands while handling banknotes, storing them on dirty surfaces during transactions, and simultaneously handling money while dealing with food, meat, or related products can contribute to the contamination of banknotes at the study site (Sunil *et al.,* 2020; Ovuru *et al.,* 2024).

The central bank's control banknotes may not have developed because they were not in circulation, which would have exposed them to handling and usage. According to another investigation, there was no microbiological contamination on the new notes (Elemam *et al.,* 2016). Uncirculated notes, as to some researchers, are contaminated with fastidious organisms, and the culture conditions or substrate used were not suitable for isolating them (Akoachere *et al.,* 2014).

A total of 71 isolates were obtained from circulating banknotes with 56 (78.9%) Gram positive and 15 (21.1%) were Gram negative bacteria. Agarwal *et al.* (2015) reported Gram positive bacterial as predominant isolates from currency notes. The identification of both Gram-positive and Gram-negative bacteria in the current study confirms that circulating banknote poses a risk to public health since it may be a significant factor in the spread of microorganisms in the population. This result confirms information from around the globe that currency notes are typically contaminated with bacteria that can lead to a variety of illnesses (Iyevhobu *et al.*, 2023). Currency contamination is caused by a number of activities, including coughing, sneezing, incorrect hand washing, storing currency on clothing, body surfaces, or unclean surfaces, and using saliva to wet fingers when counting currency (Akoachere *et al.*, 2014).

The study isolated and identified 18 species of aerobic bacteria that include; *Staphylococcus aureus*, *Staphylococcus sciuri* spp., *Staphylococcus gallinarum*, *Staphylococcus intermediu*, *Micrococcus* spp., *Staphylococcus schleiferi* spp. *coagulans*, *Kluyvera ascorbata*, *Kluyvera ascorbata*, *Proteus penneri*, *Aeromonas media*, *Burkholderia cepacia* spp, *Aeromonas enteropelogenes*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Leclercia adecarboxylata*, *Raoultella ornithinolytica*, *Vibrio metschnikovii*, *Myroides odoratus* and *Yersinia pestis*. Various bacteria species identified in this study were very similar to those documented in previous investigations (de Carvalho *et al.*, 2014; Firoozeh *et al.*, 2017; Khalil and Attitalla, 2023; Elshebani *et al.*, 2024).

The study isolated 56 (78.9%) Gram positive bacteria from Kenyan banknotes: *Staphylococcus aureus* 37 (66.1%), *Staphylococcus sciuri* spp 9 (16.1%), *Staphylococcus intermedius* 6 (10.6%), *Staphylococcus schleiferi* spp. 2 (3.6%), *Micrococcus* spp. 1 (1.8%) and *Staphylococcus gallinarum* 2 (3.6%). Prior research on banknotes revealed elevated levels of pathogens in circulation, particularly Gram-positive bacteria (*Bacillus* species) and typical skin flora (*Staphylococcus aureus*) Saadabi *et al.*, 2017; Minakawa *et al.*, 2021).

This research concurs with previous investigation on bacterial contamination of paper currency notes circulating in zliten area and their antibiotic resistance (Elsharief *et al.*, 2018). Gram-positive bacteria accounted for 78.9% of all bacteria detected in the investigation, while the remaining isolates were determined to be Gram-negative bacteria. Studies carried out in Saudi Arabia (Nasser and Alwakeel, 2012), Pakistan (Ali *et al.*, 2015), Ghana (Yar, 2020), India (Sunil *et al.,* 2020), Iraq (Abdulla, 2017), and Libyan (Elemam *et al.*, 2016) found that Gram-positive bacteria were the major microbes from the contaminated banknotes.

Staphylococcus spp. was the prevalent (77.9%) aerobic bacterial contaminants isolated from banknote from Nyeri County. In line with Olusola-Makinde *et al.* (2024), the presence of *Staphylococcus* on paper money may result due to skin contant. Given this, there is a significant chance that circulating banknotes will become contaminated frequently if proper hygiene procedures are not followed (Abdallah and Sulieman, 2024). It has been documented that *S. aureus* is one of the microbes isolated from paper money in several nations (Demirci *et al.,* 2020; Yar, 2020). The most prevalent *Staphylococcus aureus* has been shown to have cross-implications in a number of infection types (Kumar *et al.,* 2009). Based on a study by Qayyum and Batool (2017), the most common microbial contaminant on banknotes was *Staphylococcus aureus* (100%) in the notes. Considering *Staphylococcus aureus* thrives in the human nose, throat, and skin, there is a significant chance that paper money will become contaminated frequently if proper hygiene procedures are not followed (Abdallah and Sulieman, 2024). It is recommended that people take measures that minimize currency contamination, particularly in areas where environmental factors encourage the growth of several infections. The adoption of easily washable plastic cash, as was done in Australia, is one such strategy.

The bacterial species isolated from this study were *Staphylococcus* spp, and *Klebsiella* spp. These findings were similar to those reported by Agarwal *et al.* (2015); Saadabi *et al.* (2017); Demirci *et al.* (2020). This study reported 13.3% *Klebsiella* spp. isolate from the banknotes. A study from Tamil Nadu reported a higher prevalence of 31.7% *Klebsiella* spp. isolates from currencies (Sucilathangam *et al.*, 2016). These bacteria' contamination of currency notes is consistent with the finding that enteropathogens can contaminate currency notes, which can serve as a reservoir for enteric infections (Mofolorunsho *et al.*, 2023). Poor hygiene practices, such as counting banknotes with saliva, placing them on unclean surfaces, not properly washing hands after using the restroom, coughing and sneezing on hands before exchanging money, etc., may be the cause of this. Due to its widespread distribution and inclusion in our typical flora, *Staphylococcus aureus* was discovered to be the most common bacterium among the isolates (Prescott *et al.,* 2008). As stated by Pal *et al.* (2021), *Staphylococcus aureus* is often a non-pathogenic strain that is found in the nose and frequently contaminates hands, fingers, faces, and nasal carriers, which can readily turn into skin carriers.

The isolated bacteria obtained from this study are in agreement with many other studies suggesting cross contamination is a common phenomenon (Thiruvengadam *et al.,* 2014; Neel *et al.,* 2018; Gedam *et al.,* 2018). As per to the study, the majority of Kenyan banknotes in circulation in Nyeri town have been contamination with a variety of microorganisms, including opportunistic infections that could infect the population and prospective pathogens that could infect healthy people. This is consistent with a research that found that 97% of Pakistani bank notes were contaminated with bacteria, raising the possibility that both healthy people and people with impaired immune systems could contract infectious diseases from currency (Ejaz *et al.*, 2018).

The study findings are in line with other research on Ghanaian and Indian currency, which found contamination rates of 98.6% and 93.9%, respectively (Feglo, 2010; Sadawarte *et al.*, 2014). Potentially harmful bacteria, including many intestinal types, were discovered to be present in the money obtained for this study. These bacteria may also cause diseases linked to oral and dental healthcare, including *Staphylococcus aureus, Klebsiella* spp.*, and Enterobacter* spp. (Tigabu and Getaneh, 2021). The most common isolated bacteria were *Staphylococcus* spp. This finding was in agreement with the findings in Saudi Arabia (Alwakeel and Nasser, 2011) and in Iran (Firoozeh *et al.*, 2017).

The study established that lower value denomination currencies; 50 ksh and 100 ksh were more contaminated with aerobic bacterial than higher value denomination ones like 200 ksh, 500 ksh and 1000 ksh. The current study supports a study by Ofoedu *et al.*, (2021) that found lower denomination currency notes to be the more contaminated. This is likely due to the fact that lower denomination notes are handled more frequently than higher denomination notes throughout the course of their existence. Based to a prior study, because lower denomination notes are exchanged more frequently and remain in circulation longer, they are more likely to become contaminated by microorganisms (Khalil *et al.*, 2014). Similar findings were also reported by Barua *et al.* (2019) in Bangladeshi and Badvi *et al.* (2017) in Pakistan, who discovered that lower value currency notes were the most contaminated since they were handled more frequently than higher denomination notes.

This was in line with research conducted in Cameroon that indicated lower denominations also had a higher propensity to be mishandled (Akoachere *et al.*, 2014). The degree of contamination was also affected by the physical state of the currency in addition to their denomination. In keeping with earlier research that found soiled notes, particularly those kept together with pieces of sticky tape, were most harmful, old, ragged, and dirty notes were more contaminated (Morka, 2021). The current study established a negative correlation between the value of banknotes and the number of microorganisms isolated, which is in agreement with a study carried out by Hammadi *et al.* (2020). The current study's result that microbial contamination was higher in lower denominations is consistent with studies from other researchers in different nations Pranaya *et al.* (2025).

The fast circulation of these notes among different social groups of the society could explain the observation. Currency notes, even before it would reach the bank and in the process of circulating and passing through hands during daily transactions, can equally transmit the pathogenic microbes (Grossman, 2019; Tooke, 2024). The lower banknotes denomination obtained from this study had the highest number of bacteria isolates and the frequency of antibiotic resistance genes which is consistent with the report of Pranaya *et al.* (2025) attributable to its higher frequency of high usage in today’s Kenyan and across her society’s daily transactions.

These lower banknote denominations are utilized for a variety of everyday minor transactions. Additionally, smaller denominations are more likely to be misused and mishandled, which makes them dirty and worn and increases the likelihood that they may become infected. Similar findings were reported in Iranian (Firoozeh *et al.*, 2017) and Bangladeshi (Barua *et al.*, 2019) where it was discovered that lower value currency notes were the most contaminated because they were handled more frequently than higher denominations.

Because of the frequent exchanges between buyers and sellers in the market and small shops, Adinortey *et al.* (2019) and Musa *et al.* (2019) hypothesize that lower currency values are more contaminated than higher denominations. They came to the conclusion that lower denominations circulating within the community are more contaminated than higher denominations. The variability in banknote contamination can vary greatly depending on the material, environment, and age of the currency may be the cause of the inconsistent findings in the literature about the level of contamination of different currency notes (Elleboudy *et al.*, 2021; Cozorici *et al.*, 2022). This investigation also showed that none of the control currency notes (Central bank) produced any bacterial growth, which is consistent with the findings of Adinortey *et al.*(2019) and Barbaruah *et al.*(2021).

The dominant Gram negative bacterial isolates from this study was *Aeromonas* spp. (20.0%) followed by *Klebsiella oxytoca* (13.3%). Bacterial agents found to contaminate Kenyan banknotes include; *Enterobacter cloacae*, *Klebsiella ozaenae*, *Cedecea davisae*, *Yersinia pseudotuberculosis*, *Acinetobacter iwoffii*, S*taphylococcus warneri* and *Enterobacter agglomerans*. In a number of surveys various bacterial contaminant like *Enterobacter* *cloacae, Acinetobacter iwoffii and Staphylococcus warneri* were isolate from currency (Bendjama *et al.*, 2020; Jiménez-Velásquez *et al.*, 2024; Chen *et al.*, 2024).

Lower respiratory tract infections, infections of the skin and soft tissues, urinary tract infections, endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis, and ocular infections can all be brought on by *Enterobacter cloacae*. *E. cloacae* infections have the highest fatality rates of any Enterobacter infection, reaching up to 87% (Ioannou *et al.*, 2022). The research of Tsuzukibashi *et al.* (2023), *Klebsiella ozaenae* is solely thought to be a nasopharyngeal colonizer or a possible cause of ozena, or atrophic rhinitis. But based on recent findings, it can produce pituitary abscess and be an invasive infection, particularly in immunocompromised hosts (Gonzales *et al.*,2016). It has been observed that *Cedecea davisae* can induce super-infection, particularly in people with weakened immune systems. According to Iredell, *et al.*(2016), Paterson and Doi (2017) and Moxley (2022), these are novel members of the Enterobacteriaceae family that are naturally resistant to antibiotics.

According to reports, *Yesinia pseudotuberculosis* was isolated from postaneurysmal prosthetic vascular infection, indicating the species' possible predilection for endovascular tissues (Hashimoto *et al.,* 2021). Additionally, it is known that human neutrophils cannot destroy *Yersinia pseudotuberculosis* (Hashimoto *et al.,* 2021). Adults with a high mortality risk can develop bacterial meningitis due to *Acinetobacter iwoffii* (Gutiérrez-Gaitán *et al.,* 2022). Due to its low virulence, *Staphylococcus warneri* often does not cause significant infections in humans; nonetheless, it was recently found that *S. warneri* can induce a rare endocarditis in humans even in the absence of risk factors (Yamamoto *et al.*, 2020; Ravaioli *et al.,* 2021). A novel cause of primary pneumonia and peritonitis is *Enterobacter agglomerans*, commonly referred to as *Pantoea agglomerans* (Sastre *et al.*, 2017). Bacterial species were found to be sensitive to the majority of the tested antibiotics in a study by Mishu *et al*. (2025) on bacterial susceptibility analysis on paper currencies. This acts as a guideline for treating individuals who will have infections brought on by these agents.

5.1.2 Antibiotic susceptibility profiles of isolates from Kenyan banknotes in Nyeri town

The person to person transmission of antimicrobial resistant bacteria via banknotes could be of great concern. Our antibiotic susceptibility test results revealed existence of antimicrobial resistance to commonly used antibiotics clindamycin, vancomycin, erythromycin, amoxycilin and tetracycline. Isolates of Gram positive bacteria species recorded high rates of resistance collectively as 100%, 83.3% and 66.7% against clindamycin, vancomycin and amoxycilin respectively. While the greatest resistance was observed against Gram-positive bacteria, the majority of the isolated bacteria demonstrated sensitivity to the majority of the antibiotics utilized.

Furthermore, *Klebsiela* species demonstrated complete resistance to erythromycin, vancomycin, clindamycin, and amoxycilin. Although the precise causes of resistance dissemination may not be entirely understood, social and behavioral factors (Schwartz and Morris, 2018) well as travel (Di Lodovico *et al.,* 2022) may contribute to the spread of microbes that are resistant to many drugs. In the past few years, antimicrobial resistance has become an important issue worldwide. There have been reports of antibiotic-resistant bacteria on banknotes worldwide (Akoachere *et al.*, 2014; Firoozeh *et al.*, 2017; Mishu *et al.*, 2025).

As per Akoachere *et al.* (2014) and Angelakis *et al.* (2014), it was proposed that "banknotes could serve as a vehicle for transmission of drug resistant pathogenic." As stated by Sharma and Dhanashree (2011), treatment failure may be caused by the careless administration of antibiotics. Owing to reports elsewhere, paper notes are typically tainted with pathogenic microbes that are in circulation, the majority of which are resistant to widely used antibiotics (Firoozeh *et al.,* 2017).

Due to its impact on increasing expenses for healthcare, patient morbidity, and infectious disease mortality, antibiotic resistance (AR) has garnered attention in clinical settings across the globe in recent years. Because data on the antibiotic susceptibility patterns of bacterial isolates are inconsistent, the impact is exacerbated in developing nations (Chamoun *et al.*, 2016). Nonetheless, it is crucial to note that irrational use of antibiotics accounts for nearly half of all patient prescriptions, and overuse or misuse of antibiotics due to factors like incorrect diagnosis are significant contributors to the spread of antibiotic resistance (Chamoun *et al.*, 2016; Hala, 2024). In underdeveloped nations, evidence-based practice is still relatively new. Without laboratory studies, such as determining the causative agent, testing for antibiotic susceptibility, or looking for specific resistance markers, antibiotics are administered. Similar to this, a number of antibiotics are readily available over-the-counter in a number of pharmacies, which raises the possibility of the current outbreak of antibiotic resistance.

The study results are consistent with findings obtained from tracheal swabs, sputum, wound swabs, pus, blood and urine samples of hospitalized patients that found *Klebsiella* spp. were 100% resistant to ampicillin, cloxacillin, penicillin, and cefuroxime (Chakraborty *et al.*, 2016). It is not surprising that bacterial resistance to antibiotics is frequently reintroduced; for instance, Gram-positive bacteria, also known as opportunistic gastroenterology, have demonstrated resistance to the antibiotic vancomycin, which was once used to treat methicillin-resistant *Staphylococcus aureus* (Holmes *et al.*, 2015; McGuinness *et al.,* 2017). Paper currency may encourage the spread of infectious diseases and antibiotic resistance, so it should be handled with caution.

The study recorded 30 (24.4%) samples that showed notable resistance against tetracycline, erythromycin and vancomycin. Healthy facilities had the highest bacterial resistance of 30% to antibiotics, followed by 23.3% from transport SACCOs, 20% from butcheries, 16.7% from M-pesa outlets and 10% from restaurants. In a studies carried out by Agarwal *et al.* (2015) and Yar (2020) on the currency notes, established that hospital billing counters and tempo drivers were maximally contaminated.

In response to reports elsewhere, paper notes are typically tainted with pathogenic microbes that are in circulation, the majority of which are resistant to widely used antibiotics (Firoozeh *et al.,* 2017). There could be major public health risks if these antibiotic-resistant microbes are spread from person to person via paper money. The main selective factor for resistance acquisition has often been assumed to be the use of antibiotics in clinical, veterinary, and agricultural settings (Kuppusamy *et al.*, 2018; Servia-Dopazo *et al.,* 2021). In addition to discouraging the misuse of antibiotics, money notes should be handled carefully to avoid spreading infections.

In regard to bacterial infections and diseases that are extremely consequential when contracted by the disabled, the emergence of multidrug-resistant strains presents a significant threat to human life and continuous existence. Comparable to a study in which the isolated Gram-positive bacteria exhibited antibiotic resistance, the greatest resistance was shown against Gram-positive bacteria (Kakoullis *et al.*, 2021; Biswas *et al*., 2021).

The study established *Staphylococcus* spp. were mostly resistance to clindamycin, vancomycin and amoxycilin. These findings are closely related to the many studies which concluded that *Staphylococcus aureus* was found resistant to many antibiotics (Ali *et al.,* 2015; Mion *et al.,* 2016). Firoozeh *et al.* (2017) found high resistance rates of *Staphylococcus* spp. and *Enterococci* spp. against tetracycline, ampicillin and erythromycin isolated from paper currency. Among the 30 samples, fifty banknote denominations had the highest resistance of 40%, followed 20% from five hundred denomination, 16.7% from two hundred denomination, 13.3% from one hundred denomination and 10% from one thousand denominations.

5.1.3 Resistance genes associated with bacterial contaminants present in circulating in Kenyan banknotes in Nyeri town

Two vancomycin resistance genes were isolated from two different health facilities one from fifty banknote and another from one thousand banknote. Six TEM 1 resistance genes were isolated from various sources. The resistance gene isolates were as follows; two from transport SACCOs; one from one thousand banknote and one from fifty banknote; one from one hundred banknote from M-pesa source, one from one hundred banknote from the restaurant;

health facility yielded one TEM 1 resistance gene from fifty banknote and one TEM 1 resistance gene from fifty banknote from the butchery.

The study established that, 22.2% of the Kenyan banknote sampled from hospital facilitates had vancomycin resistant gene while 11.1% had TEM 1 resistant genes. In addition to diseased individuals, infected objects like knives, surgical tools, and a variety of other equipment may act as sources of contamination and transmission in hospitals, where microorganisms have favorable circumstances for life. If infection control procedures are insufficient, organisms from these sources can spread from one person to another, resulting in illness (Wilson, 2019; Banik *et al.,* 2020). Medical personnel have isolated antibiotic-resistant pathogenic germs from a variety of coins and currency (Satya *et al.*, 2022; Mishu *et al.,* 2025. This study places a strong focus on washing your hands after handling cash because sharing tainted notes can spread infection. Transport SACCOs, M-pesa outlets, restaurants, and butcheries did not have any vancomycin resistant genes but had TEM 1 resistant genes occurrence of 28.6%, 20%, 33.3% and 16.7% respectively.

The result showed that, 8.3% of fifty-shilling banknotes sample had vancomycin resistant genes while 25% had TEM resistant genes. One-thousand-shilling denomination recorded 33.3% occurrence of resistant genes for both vancomycin and TEM 1. One-hundred-shilling banknotes recorded 50% occurrence of vancomycin resistant genes. The denomination of two hundred shillings and five-hundred-shilling banknotes did not have any resistant gene. Vancomycin use has been reported consistently as a risk factor for colonization and infection with VRE (Shenoy *et al.,* 2014; Flokas *et al.,* 2017: Melese *et al.,* 2020) and may increase the possibility of the emergence of vancomycin-resistant *S. aureus* or *S. epidermidis*.

TEM-1 is widespread not only amongst bacteria of the family *Enterobacteriaceae* (*K. pneumoniae*, *Enterobacter* spp.), but also in non-fermentative bacteria such as *P. aeruginosa* (Du *et al.,* 2020). This study supports the previous findings that bla_{TEM} is one of the most prevalent resistant gene among bacterial isolates (Quan *et al.*, 2017). As a guidance for switch therapy, a 10% resistance level against an empirically used antibiotic has been suggested (Patini *et al.,* 2020). In light of this, extrapolating the trends in antibiotic resistance against routinely used antibiotics should indicate that empirical therapy against these diseases will soon become challenging. Antibiotic abuse, which revealed that the vast majority of the population examined obtains antibiotics on the open market without a prescription and uses them for the wrong infections and ailments, may be the cause of the observed antibiotic resistances (Nardulli *et al.*, 2022). Currency notes would always be in circulation, particularly in the food supply system. This unavoidable circumstance may make it easier for microbial contamination to persist and increase the likelihood of currency note and food contamination (Thiruvengadam *et al.,* 2014; Agarwal *et al.,* 2015).

5.2 Conclusions

i. The circulating Kenyan banknotes in Nyeri town, Nyeri County harbour bacterial contaminants that include; included; *Staphylococcus aureus, Staphylococcus sciuri* spp, *Staphylococcus gallinarum, Staphylococcus intermediu, Micrococcus* spp., *Staphylococcus schleiferi* spp, *Kluyvera ascorbata, Kluyvera ascorbata, Proteus penneri, Aeromonas media, Burkholderia cepacia* spp., *Aeromonas enteropelogenes,* *Enterobacter cloacae, Klebsiella oxytoca, Leclercia adecarboxylata, Raoultella ornithinolytica, Vibrio metschnikovii*, *Myroides odoratus* and *Yersinia pestis.*

ii. The study revealed existence of antimicrobial resistance to commonly used antibiotics clindamycin, vancomycin, erythromycin, amoxycilin and tetracycline

iii. Kenyan circulating banknotes from Nyeri town have vancomycin and TEM resistant genes

5.3 Recommendations

i. Based on the risk of contaminated notes, proper sanitary handling should be done in possible cases to reduce community acquired infection in our society.

ii. Given the level of the antimicrobial resistance genes and their associated antibiotics regular microbial testing of paper currency notes should be established.

iii. The study recommends continuously monitoring of antibiotic resistance patterns and frequency of antibiotic resistance genes.

5.4 Recommendations for further study

i. Further studies on the public health implication of banknotes currency contamination is therefore recommended to unveil more issues surrounding paper currency contamination infections.

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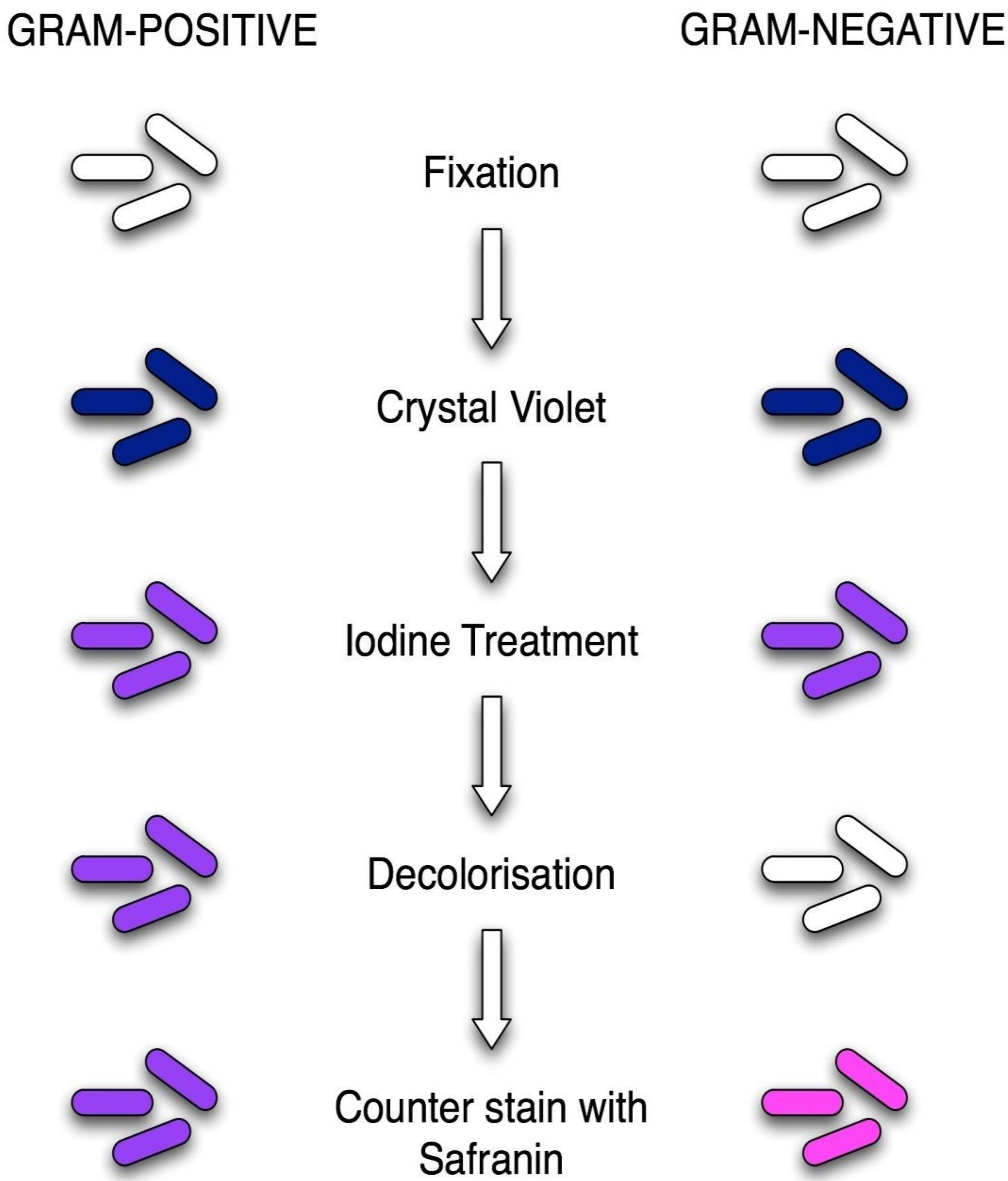
APPENDICES

Appendix I: Gram stain protocol



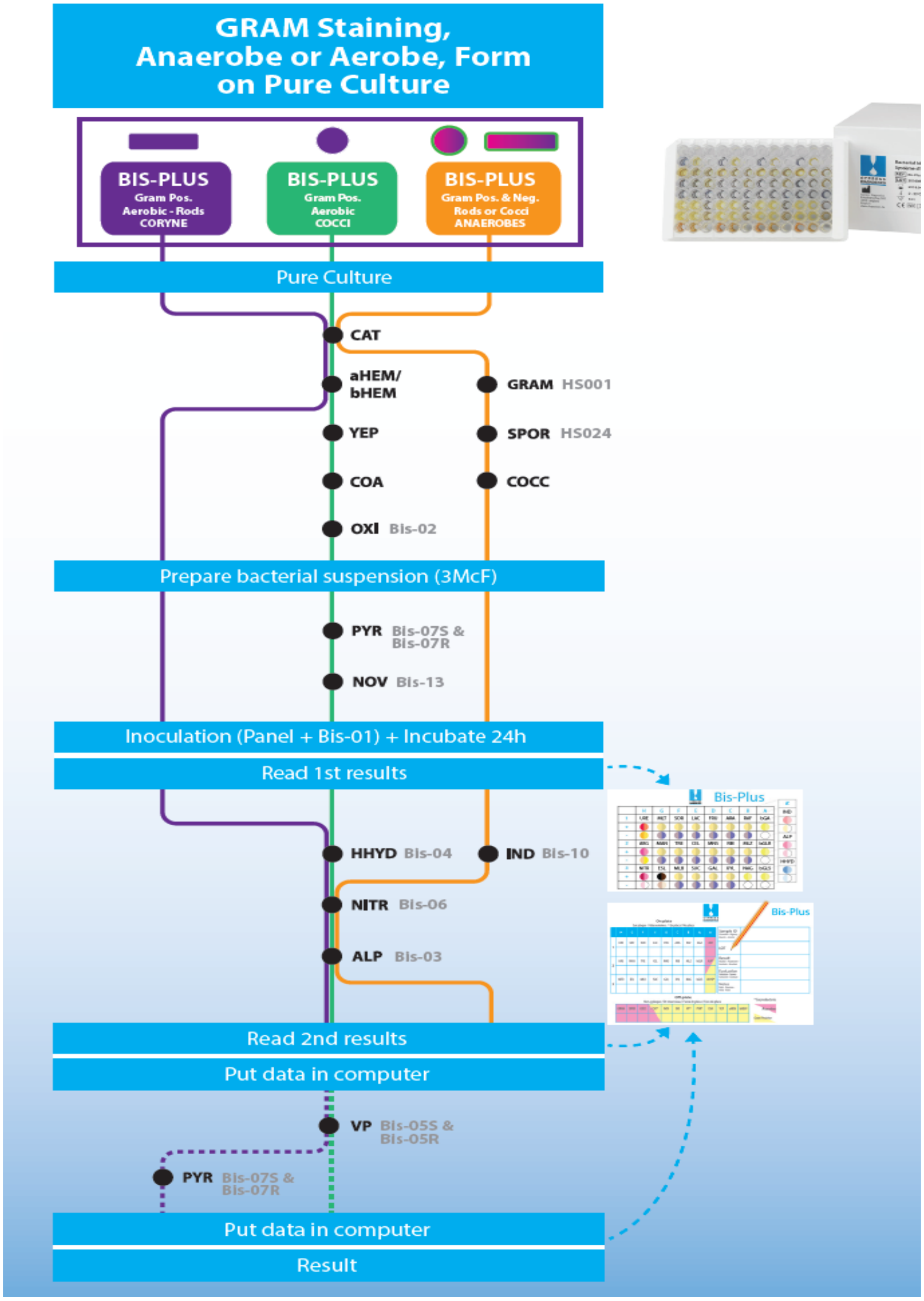
Gram Staining Procedure/Protocol according to Barrow and Feltham (1993)

1. Flood air-dried, heat-fixed smear of cells for 1 minute with **crystal violet** staining reagent. Please note that the quality of the smear (too heavy or too light cell concentration) will affect the Gram Stain results.
2. Wash slide in a gentle and indirect stream of tap water for 2 seconds.
3. Flood slide with the mordant: **Gram's iodine.** Wait 1 minute.
4. Wash slide in a gentle and indirect stream of tap water for 2 seconds.
5. Flood slide with **decolorizing agent (Acetone-alcohol decolorizer)**. Wait 10-15 seconds or add drop by drop to slide until decolorizing agent running from the slide runs clear .
6. Flood slide with counterstain, **safranin**. Wait 30 seconds to 1 minute.
7. Wash slide in a gentile and indirect stream of tap water until no color appears in the effluent and then blot dry with absorbent paper.
8. Observe the results of the staining procedure under oil immersion (100x) using a Bright field microscope.

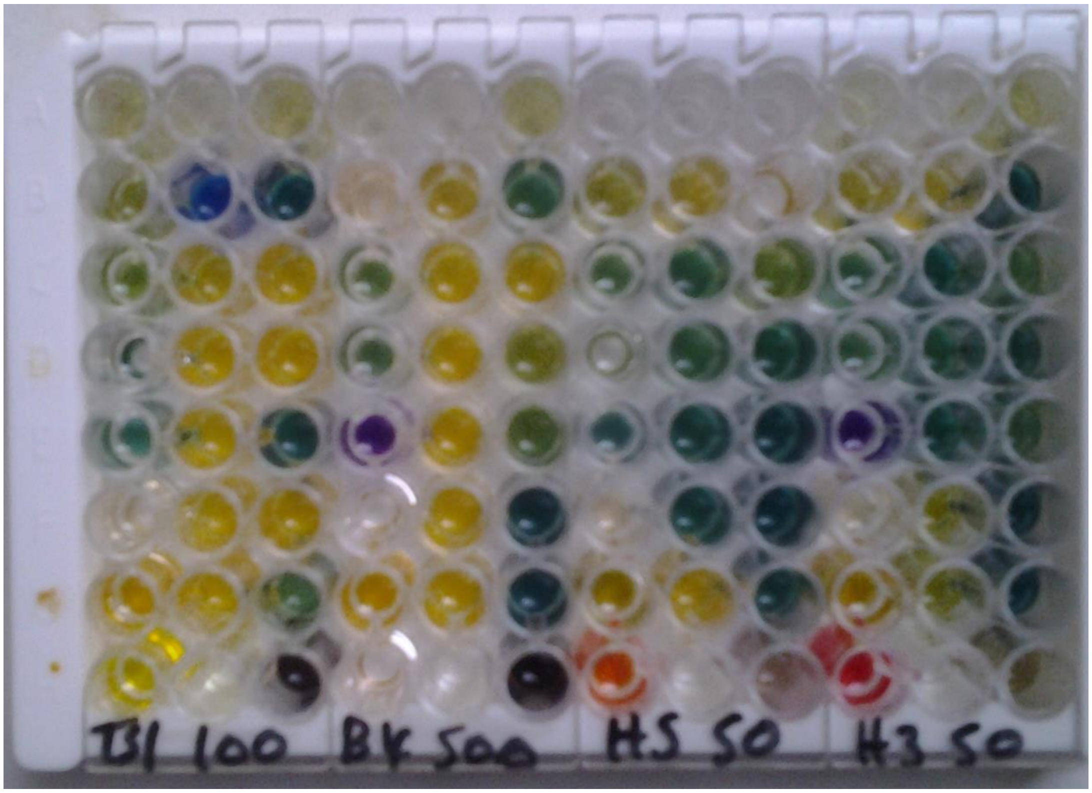
Results:

- Gram-negative bacteria will stain pink/red and
- Gram-positive bacteria will stain blue/purple.

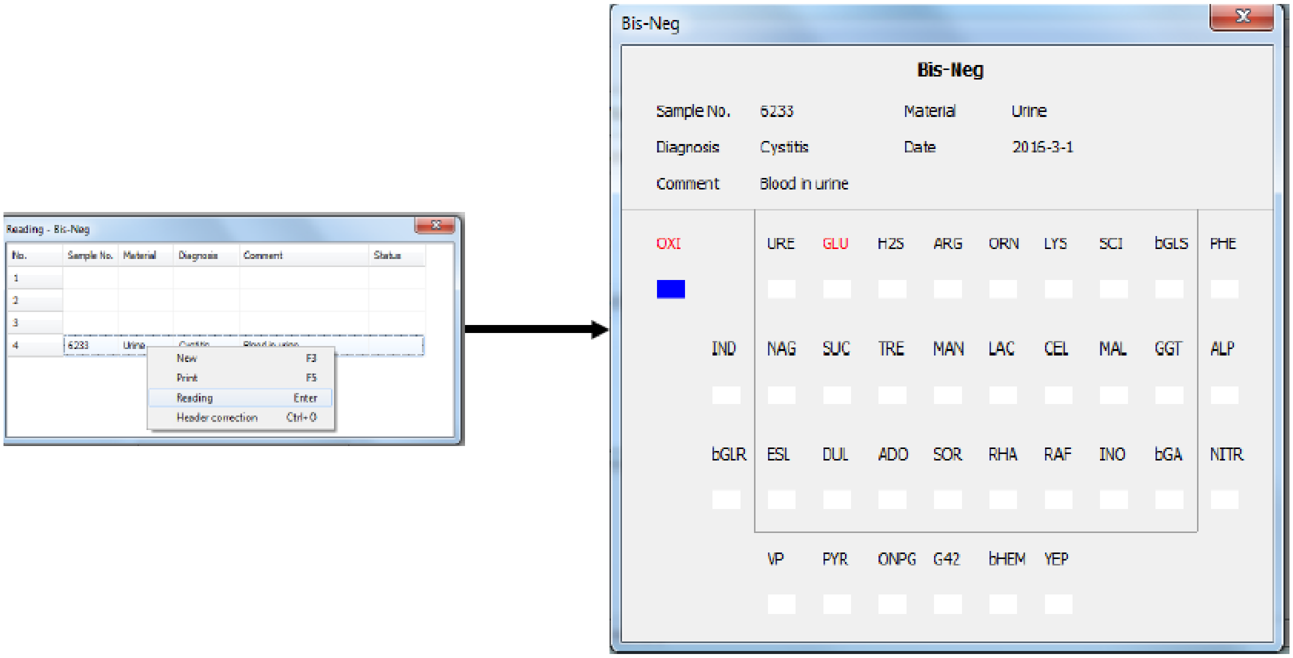
Appendix II: Cypress procedure



Appendix III: Bacterial Identification System with 24 miniaturized biochemical tests



Appendix IV: Cypress identification software



Appendix V: McFarland Turbidity Standard

INTENDED USE

The 0.5 McFarland standard is used to adjust the turbidity (concentration) of the inoculum for antimicrobial susceptibility tests.

SUMMARY AND EXPLANATION

One of the earliest uses of turbidity for the estimation of bacterial populations was in the preparation of vaccines.[1] In 1907, McFarland developed a series of barium sulfate solutions to approximate the numbers of bacteria in solutions of equal turbidity, as determined by plate counts.[2,3]

Susceptibility testing requires the use of standardized inocula. The 0.5 McFarland standard is recommended for use in the preparation of inocula for performing the antimicrobial disk diffusion susceptibility test.[4]

PRINCIPLES OF THE PROCEDURE

The 0.5 McFarland turbidity standard is prepared by adding barium chloride to sulfuric acid. The mixture of the two chemicals forms a precipitate, that when in suspension is equivalent to approximately 1.5×10^8 colony forming units/ml.

TYPICAL FORMULA AND APPEARANCE

Appearance =clear fluid, slightly turbid

(Approximate formula per liter of processed water)

Barium Chloride	1.0 g
Sulfuric Acid (concentrated)	10 ml

PRECAUTIONS

This product is for IN VITRO diagnostic use only. Culture specimens may contain microorganisms, which can be potentially infectious to the user. Strict adherence to aseptic techniques and established precautions against microbiological hazards should be followed throughout the procedure. Carefully dispose of all items which contact patient specimens or isolated bacteria.

STORAGE/SHELF LIFE

Tubes should be stored at 2-25^{0}C, protected from light. DO NOT FREEZE. Product that has exceeded the assigned expiration date noted on the label should not be used.

MATERIALS PROVIDED

0.5 McFarland Turbidity Standard tubes (10 each per box)

MATERIALS REQUIRED BUT NOT PROVIDED

Ancillary culture media, reagents and laboratory equipment as required for susceptibility testing.

PROCEDURE

Before each use, shake well, mixing the fine white precipitate of barium sulfate in the tube. The accuracy of the density of a prepared McFarland standard should be checked by using a spectrophotometer with a 1-cm light path; for the 0.5 McFarland standard, the absorbance at a wavelength of 625 nm should be 0.08 to 0.1. Alternately, the accuracy of the McFarland standard may be verified by adjusting a suspension of a control strain (e.g., *E. coli* ATCC 25922) to the same turbidity, preparing serial 10-fold dilutions, and then performing plate counts. The adjusted suspension should give a count of 10^8 colony forming units/ml.[4]

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USER QUALITY CONTROL PROCEDURES AND INFORMATION

HealthLInk recommends that the following quality assurance and quality control procedures be performed on each batch of product.

QUALITY ASSURANCE

The following quality assurance procedures must be performed to assure the product will perform according to its intended use within the assigned expiry date:

Daily, document that product storage refrigerator maintains temperature within the recommended range: 2-8^{0}C.

QUALITY CONTROL

The following incoming inspection procedure must be performed for each batch (batch = same lot, same shipment) of culture media received in the laboratory:

Examine tubes for signs of deterioration: microbial contamination, discoloration, evaporation or other signs of deterioration.

Note: Notify Technical Service immediately if media does not meet the inspection criteria.

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HealthLink provides a toll free technical service line (1-800-638-2625) to assist with product usage. To have technical questions answered; please call between the hours of 9:00 am to 5:00 pm EST.

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Appendix VI: Clinical and laboratory standards institute interpretation table for antimicrobial susceptibility test

Name of antibiotics (dose)	Inhibitory zone diameter to nearest millimeter (mm)		
	Sensitive (S)	Moderately sensitive (MS)	Resistant (R)
Amoxicillin (30 μg/disk)	≥18	14–17	≤13
Cloxacillin (5 μg/disk)	≥25	22–24	≤21
Cephalothin (30 μg/disk)	≥18	15–17	≤14
Cephradine (25 μg/disk)	≥18	13–17	≤12
Cefuroxime (30 μg/disk)	≥23	15–22	≤14
Cefixime (5 μg/disk)	≥19	16–18	≤15
Kanamycin (30 μg/disk)	≥18	14–17	≤13
Streptomycin (10 μg/disk)	≥15	12–14	≤11
Neomycin (30 μg/disk)	≥17	13–16	≤12
Vancomycin (30 μg/disk)	≥12	10–11	≤9
Erythromycin (15 μg/disk)	≥23	14–22	≤13
Azithromycin (15 μg/disk)	≥18	14–17	≤13
Ciprofloxacin (15 μg/disk)	≥21	16–20	≤15
Levofloxacin (5 μg/disk)	≥17	14–16	≤13
Tetracycline (30 μg/disk)	≥15	12–14	≤11
Doxycycline (30 μg/disk)	≥14	11–13	≤10
Cotrimoxazole (25 μg/disk)	≥16	11–15	≤10
Chloramphenicol (30 μg/disk)	≥18	13–17	≤12

Appendix VII: Antibiotic Resistance Genes

TABLE 1 Antibiotic groups considered in this study and the relevant antibiotic resistance genes for each group present on the microarray

Antibiotic	Relevant antibiotic resistance genes[a]
Amikacin	***aac(6')-Ib*, *armA*, *rmtC***
Gentamicin	***aac(6')-IIc*, *aadB*, *aac(6')-aph(2')*, *armA*, *rmtC***, *aac(3')-Ia*, *aac(3')-IVa*, ***aac(6')-Ib***
Streptomycin	*aadA1*-like, *aadA2*-like, *aadA4*-like, *strA*, *strB*
Tobramycin	***aadB*, *armA*, *rmtC***, *aac(3)-IVa*, ***aac(6')-Ib***
Ampicillin	**bla_{CMY}**, **$bla_{\text{PER-1}}$**, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$, $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-26}}$, bla_{DHA}, $bla_{\text{OXA-1}}$, $bla_{\text{OXA-2}}$, $bla_{\text{OXA-7}}$, $bla_{\text{OXA-9}}$, $bla_{\text{PSE-like}}$, bla_{SHV}, bla_{TEM}, **bla_{IMP}**, **$bla_{\text{OXA-23}}$**, **$bla_{\text{OXA-40}}$**, **$bla_{\text{OXA-48}}$**, **$bla_{\text{OXA-51}}$**, **$bla_{\text{OXA-58}}$**, **$bla_{\text{SPM-1}}$**, **$bla_{\text{GIM-1}}$**
Aztreonam	**bla_{CMY}**, **$bla_{\text{PER-1}}$**, **$bla_{\text{VEB-1}}$**, bla_{DHA}, bla_{SHV}, bla_{FOX}, $bla_{\text{ACC-1}}$, **$bla_{\text{GES-1}}$**, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$, $bla_{\text{CTX-M-26}}$, $bla_{\text{CTX-M-9}}$, $bla_{\text{OXA-7}}$, bla_{TEM}
Cefotaxime	$bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$, $bla_{\text{CTX-M-26}}$, $bla_{\text{CTX-M-9}}$, bla_{DHA}, bla_{ACC}, bla_{FOX}, bla_{SHV}, **bla_{CMY}**, **$bla_{\text{GES-1}}$**, **$bla_{\text{PER-1}}$**, **$bla_{\text{VEB-1}}$**, **bla_{IMP}**, **$bla_{\text{GIM-1}}$**
Cefoxitin	**bla_{CMY}**, **$bla_{\text{GES-1}}$**, **$bla_{\text{PER-1}}$**, bla_{DHA}, **bla_{IMP}**, bla_{FOX}, bla_{MOX}, **bla_{KHM}**, **$bla_{\text{GIM-1}}$**, **$bla_{\text{SPM-1}}$**
Cefpirome	**$bla_{\text{GES-1}}$**, **$bla_{\text{PER-1}}$**, bla_{ACC}, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$, $bla_{\text{CTX-M-26}}$, $bla_{\text{CTX-M-9}}$, bla_{FOX}, $bla_{\text{OXA-1}}$, bla_{SHV}, **bla_{IMP}**
Ceftazidime	**bla_{CMY}**, **$bla_{\text{GES-1}}$**, **$bla_{\text{PER-1}}$**, **$bla_{\text{VEB-1}}$**, bla_{ACC}, bla_{DHA}, bla_{FOX}, bla_{SHV}, **bla_{IMP}**, **$bla_{\text{GIM-1}}$**, **$bla_{\text{SPM-1}}$**
Amoxicillin-clavulanic acid	$bla_{\text{OXA-1}}$, bla_{TEM}, **bla_{IMP}**
Imipenem	**bla_{CMY}**, **$bla_{\text{GES-1}}$**, **bla_{IMI3}** , **bla_{IMP}**, **$bla_{\text{KPC-4}}$**, **$bla_{\text{OXA-23}}$**, **$bla_{\text{OXA-40}}$**, **$bla_{\text{OXA-48}}$**, **$bla_{\text{OXA-51}}$**, **$bla_{\text{OXA-58}}$**, **$bla_{\text{GIM-1}}$**
Meropenem	**$bla_{\text{GES-1}}$**, **bla_{IMI3}** , **bla_{IMP}**, **$bla_{\text{KPC-4}}$**, **$bla_{\text{OXA-23}}$**, **$bla_{\text{OXA-40}}$**, **$bla_{\text{OXA-48}}$**, **$bla_{\text{OXA-51}}$**, **$bla_{\text{OXA-58}}$**, **$bla_{\text{GIM-1}}$**
Piperacillin	**bla_{CMY}**, **$bla_{\text{VEB-1}}$**, bla_{ACC}, bla_{DHA}, $bla_{\text{OXA-1}}$, **bla_{IMP}**, **$bla_{\text{KPC-4}}$**, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$, $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-26}}$, bla_{SHV}, **$bla_{\text{PER-1}}$**, **$bla_{\text{GIM-1}}$**, **bla_{SPM}**
Chloramphenicol	*catA1*, *catB3*-like, ***catB8***, *catA3*, *cmlA1*-like, *floR1*
Ciprofloxacin	*qnrA*, *qnrB*, *qnrS*
Sulfonamide	*sul1*, *sul2*, *sul3*
Tetracycline	*tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(E), *tet*(G)
Trimethoprim	*dfrA01*, *dfrA05*, *dfrA07*, *dfrA12*, *dfrA14*, *dfrA15*, *dfrA17*, *dfrA19*

Appendix VIII: Approval letter

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School

DATE: 13th November, 2015

TO: Silas Githenji Muguongo
C/o Microbiology Department
Kenyatta University

REF: I56/CE/24951/12

SUBJECT: APPROVAL OF RESEARCH PROPOSAL
==

This is to inform you that Graduate School Board, at its meeting of 4th November 2015, approved your Research Proposal for the M.Sc. Degree Entitled, **"Antibiogram Patterns and Resistance Genes Associated with Bacterial Contaminants in Circulating Kenyan Bank Notes in Nyeri Town, Nyeri County, Kenya".**

You may now proceed with data collection, subject to clearance with the Director General, National Commission for Science, Technology and Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking forms per semester. The form has been developed to replace the progress report forms. The supervision Tracking Forms are available at the University's website under Graduate School webpage downloads.

Thank you.

ANNBELL MWANIKI
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Department of Microbiology Department

Supervisors:

1. Dr. Anthony Kebira
   C/o Department of Microbiology
   Kenyatta University

2. Dr. John Maingi
   C/o Department of Microbiology
   Kenyatta University

AM/nn

Appendix IX : Authorization

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
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P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: I56/CE/24951/12

DATE: 13th November 2015

Director General,
National Commission for Science, Technology & Innovation
P.O Box 36023-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR SILAS GITHENJI MUGUONGO– REG. NO. I56/CE/24951/12.

I write to introduce Mr. Silas Githenji Muguongo who is a Postgraduate Student of this University. He is registered for M.Sc degree programme in the **Department of Microbiology.**

Mr. Githenji intends to conduct research for a M.Sc. Proposal entitled, **"Antibiogram Patterns and Resistance Genes Associated with Bacterial Contaminants in Circulating Kenyan Bank Notes in Nyeri Town, Nyeri County, Kenya".**

Any assistance given will be highly appreciated.

Yours faithfully,

MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

AM/nn

Appendix X: Nacosti Research License

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **328568**

Date of Issue: **21/August/2025**

RESEARCH LICENSE

This is to Certify that Mr.. silas githenji Muguongo of Kenyatta University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Nyeri on the topic: ANTIBIOGRAM PATTERNS AND RESISTANCE GENES ASSOCIATED WITH AEROBIC BACTERIAL CONTAMINANTS PRESENT IN CIRCULATING KENYAN BANKNOTES IN NYERI TOWN for the period ending : 21/August/2026.

License No: **NACOSTI/P/25/29977**

328568

Applicant Identification Number

Ag. Director General
NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

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