

**BACTERIOLOGICAL EVALUATION OF NIGERIA CURRENCY FROM PARK
BANK AND PETROL STATION**

BY

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DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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**A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY,
FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN
PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF
BACHELOR OF SCIENCE DEGREEE OF B.Sc (Hons), MICROBIOLOGY**

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CERTIFICATION

I hereby certify that this project was carried out by **Blessing Labe KAJIANG (MISS)** with Matriculation number **LSC2103963** of the Department of Microbiology, Faculty of Life Sciences under the supervision of **Prof C.E. Oshoma**, University of Benin, Benin City.

Prof C.E. Oshoma

(Project Supervisor)

Date

PROF E.O. IGBINOSA

(Head of Department)

Date

DEDICATION

This project is dedicated to God almighty for his love and his kindness in my life and for his supervision throughout my course of study in school. I also dedicate this work to my parents for their love, care and support may the almighty continue to bless them.

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ABSTRACT

The handling of currency notes is an everyday activity that exposes them to various sources of microbial contamination. This study was carried out to evaluate the bacteriological quality of Nigerian currency notes collected from different sources banks, park, and petrol stations. A total of samples of different denominations (₦1000, ₦500, ₦200, and ₦100) were randomly collected from banks, park and petrol station and analyzed using standard microbiological procedures. The currency notes were swabbed, and the isolates were cultured on nutrient agar, MacConkey agar, and mannitol salt agar plates. The bacterial isolates were identified based on their phenotypical characteristics. the isolates were evaluated for their virulence factors and antibiotic susceptibility profile. The results revealed a high level of bacterial contamination on most of the notes examined, with isolates including loads compared to those from banks, possibly due to frequent handling and poor hygiene practices. The presence *Staphylococcus aureus*, *Klebsiella pneumoniae*. Notes obtained from petrol stations and park showed higher microbial of potential pathogens on currency notes indicates their role as fomites in the transmission of infectious diseases. This study emphasizes the need for improved public hygiene awareness, regular replacement of old and damaged notes, and the promotion of cashless transactions to reduce the risk of disease spread.

CHAPTER ONE

1.0. INTRODUCTION

1.1 Background of study

The most popular way to exchange goods and services is through bank currency notes, especially in developing nations (Vidiati *et al.*, 2022). People from different backgrounds and occupations always conduct physical transactions using legal tender in a range of denominations to exchange goods and services, and it gives a nation some degree of autonomy over its exchange policies (Schueffel and Patrick). During transactions, cash and payment cards are the most commonly passed goods that are routinely passed from one hand to the other (Aboubaker and Muhamed, 2022). The handling habits associated with currency notes have significant implications for their longevity and hygiene. Currency notes often exhibit a limited lifespan due to various factors, including the improper treatment they receive during daily transactions. The way currency is handled such as squeezing notes, storing them in unhygienic locations (Iyevhobu *et al.*, 2023) (e.g., brassieres, dirty apron pockets, or socks), and engaging in activities that compromise their cleanliness (such as handling raw meat or greasy materials) contributes to their degradation and microbial contamination. These items facilitate the transfer of value between individuals or entities in a wide range of commercial and personal exchanges. Improper handling practices such as concurrent handling of banknotes and food items, use of saliva to wet fingers during counting, placing or storing paper notes in or on dirty surfaces among others has led to widespread contamination of banknotes with various microbial species. Globally, contamination of banknotes with microbial species is due to one of the most concerning groups of bacteria associated with such contamination is the *Enterobacteriaceae* family, which includes various opportunistic and pathogenic organisms such as *Escherichia coli*, *Klebsiella spp.*,

Enterobacter spp., and *Salmonella spp.* These organisms are commonly found in the intestinal tract of humans and animals and can cause serious infections when introduced to other body sites, particularly in immunocompromised individuals.(Alemu *et al.*, 2014) A large multi-national study involving 1280 banknotes obtained from 10 different countries showed that bacterial contamination of banknotes is greatly influenced by age of the notes and the nature of material used to produce the notes (polymer-based versus cotton-based) The contaminated banknotes have been demonstrated to be a viable source of cross contamination and a vehicle for transmission of infectious agents in the community .Bacteria causing foodborne diseases such as typhoid fever, gastroenteritis, shigellosis, etc. have been isolated from banknotes (Ejaz and Zubair, 2018). Studies across Nigeria have documented varying rate of microbial contamination of banknotes. Enteric infections and diarrheal diseases constitute pervasive health burdens globally. Much of the disease burden is attributed to enteric infections, including Salmonella, rotavirus, and many other bacterial, viral, and protozoan organisms These fecal pathogens are transmitted through different routes, which may be direct (contaminated fingers and fomites) or indirect (food, flies, water currency note. These bacteria are known to cause various diseases ranging from food poisoning, wound and skin infections, and respiratory and gastrointestinal problems to life-threatening diseases such as meningitis and septicemia Infections caused by enteric pathogens are common and usually treated with antibiotics. However, as the rate of antibiotic resistance in this group of organisms increases, healthcare costs increase as well as the length of hospital stay, morbidity, and mortality An improved understanding of the bacteriological quality of currencies would help us better understand the role of money as a medium of transmitting infections also Paper currency which is routinely passed among individuals during exchange of goods and services may result in the spread of microbes from contaminated paper currency to other surfaces. Once these currencies are contaminated with microorganisms, there is the

possibility of spreading such organisms across different population. Items that are circulated from dirty hands are likely to be contaminated. Particularly, contaminated bank notes can serve as a vehicle for transmission of infections depending on the material and age of the banknotes as well as the climate and country of study. Fomites are inanimate objects that are capable of absorbing, harboring and transmitting infectious microorganisms. Dust and dirt that commonly accumulate on such Currencies might contain spores of infectious agents. Contamination of objects by pathogens is of public health concern due to disease transmission. Currency notes might present a particular risk to public health because communicable disease can spread through contact with contaminated banknotes. Everyone handles money in the form of coins or notes, and "dirty" money, money tainted with harmful microbes is constantly in use. Production, post production storage, and consumption can all result in contamination. Cashiers, salespeople, and members of the general public can spread skin microbes to the currency notes they handle. Potential sources of germ transfer to currency notes during handling include anal contamination, wounds, nasal secretions, and aerosols produced by coughing and sneezing *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Klebsiella aerogenes* have been reported to survive well on the skin, and are known to be transferred from fabrics to hand as well as from hands to fabrics (Badvi *et al.*, 2017). Contamination of objects by pathogenic microorganisms is of much public health concern as contaminated materials can be sources of transmitting pathogens. Items that are passed from hand to hand are likely to be contaminated with disease causing microorganisms especially if handled with unclean hands, or kept in dirty surroundings. Paper money, therefore presents a particular risk to public health, since communicable diseases can spread through contact with fomites. The survival of various microorganisms of concern on money is such that it could serve as a vehicle for transmission of disease and represents an often overlooked enteric disease reservoir. With low infectious doses capable of causing illness noted

for a number of different infectious intestinal diseases, failure to adequately sanitize hands, or use food handling tools (spoons, utensils or bakery/serving papers) between handling money and serving food, could put persons at risk (Prasad, 2021). Cross contamination by simultaneous handling of money and animal yields and poor sanitation observed in markets, slaughter houses and bistros too rise the risk of infection. Add ally, simultaneous handling of food and change via servers or vendors can have solemn costs as the food they serve is equipped to eat and its does not entail any additional heating. Research has shown that contaminated fomites in general and paper currency in particular, plays a key role in the spread of bacterial infections with antimicrobial resistance Most factors influencing the persistence of microorganisms on currency notes population of microorganisms and their survival on surfaces, counting of currency notes with saliva, improper hand washing after using the toilets, sneezing and coughing on hands leading to contamination of currencies and transmission of microorganism users (Ekeleme *et al.*, 2021). Other quality of materials used to produce the notes and the duration of storage conditions together with other environmental conditions may influence the load of microbial contamination. Other are quality of materials used to produce the notes and the duration of storage conditions together with other environmental conditions may influence the load of microbial contamination Recently currency notes denominations are more in circulation harbor more of the infectious agents.

1.2 Aim and Objectives of the Study

The aim of this study was the bacteriological assessment of currency notes.

The specific objectives of the study were to:

1. isolate and identify bacteria present on selected Nigerian currency denominations.
2. determine the prevalence of isolated bacteria from different currency denominations and sources (bus park, petrol station and bank).

3. assess the antibacterial susceptibility patterns of the isolated bacteria.
4. determine phenotypic virulence characteristics of the isolated bacteria.

CHAPTER TWO

2.0. LITERATURE REVIEW

Money is one of the most frequently passed items in the world, money is simply any commodity esteemed by all persons, any articles of food, clothing, or ornament which any person will readily receive, and which every person desires to have by him in greater or less quantity, in order that he may have the means of procuring necessaries of life at any time (Ogbonna,2015). Bacteriological studies to ascertain the survival has largely experimented on bacteria including *Staphylococcus aureus*, *Escherichia coli*, and Vancomycin- Resistant Enterococci (VRE) on banknotes from different sources and the transmission of bacteria to people who come in contact with the banknotes (Janda and lopez, 2021). Globally, money is one of the items most frequently passed from hand to hand. During its passing, money can get contaminated and may thus plays a role in the transmission of microorganisms to other people. For example, money may get contaminated with microorganisms from the respiratory- and gastro-intestinal tract during counting (Germa *et al.*, 2014). Money is not usually suitable for the survival of microorganisms, except for some that are resistant to external conditions and non-resistant forms of spores (Sharma and Sumbali, 2013). In addition, the general hygiene levels of a community or society may contribute to the amount of microbes found on coins and notes, and thus the chance of transmission during handling of money. While antimicrobial resistance has steadily been increasing, e.g. with Extended-Spectrum Beta Lactamases (ESBL) producing *Escherichia coli* and *Klebsiella spp* (Gedik *et al.*, 2010) contaminated banknotes and coins contribute to the transmission of these multi-drug-resistant microorganisms in the community. While the kind of isolated bacteria between

studies can vary, due to the methods used, season, environmental conditions, sort of money (coin or banknote) or local community flora, in general, Gram negative bacteria were the most dominants.

2.1. MONEY IN THE HOSPITAL OUTLET

Healthcare-associated infections are one of the most extreme affected problems in healthcare these days, pathogens are capable of living on surfaces that can act as agents of pathogen transmission if no disinfection is completed (Sikora and Zahra, 2021). Fomites is one of the most sources of nosocomial infections. The pathogens persist on surfaces or months and can thereby be a non-faecal supply of transmission (Elshebani *et al.*, 2024). Therefore, the possibility that money can also act as environmental fomite for the transmission of disease is very high during exchange of coins are treated through individuals could be handled under non hygienic requirements and possibly contaminated with different microbes (Abia and Ubomba, 2019). Microorganisms on the skin can be transferred from cashiers, salespeople and most people to currency that manipulates material switch of cloth from arms, surfaces, and the surroundings can contaminate the currency, therefore contamination of pathogenic microorganisms is of public health importance as infected materials may be feasible resources of transmission of such pathogens according to (Hassan, 2013) Human pathogens can be transferred immediately through physical contact, or in a roundabout way through many other environmental substances which consist of: air, water, food or other inanimate objects, that may bring about significant infection and illness in human being the tainted money get in flow and therefore unfold contaminated microbes to others hand and therefore, transmitting the pathogens by means of this procedure. Consequently, this look at targets to decide the extent of the presence of bacterial contamination associated with Nigeria foreign exchange coins that could play a massive position in an effort to discover the opportunities of transmission of infectious marketers to circulate round medical services in Nigeria.

2.2. THE ENTEROBACTERIACEAE FAMILY

Enterobacteriaceae is a large family of Gram negative facultative anaerobic, rod-shaped bacteria. Members are typically oxidase negative, catalase-positive, and ferment glucose (Janda and Lopez, 2021). They are commonly found in gastrointestinal tract of humans and animals but can also survive in soil and water and on various surfaces of currency. These pathogenic species include; *Escherichia coli*, *Salmonella*, *Shigella*, *klebsiella* *Enterobacter proteus*. They are capable of causing diseases such as diarrhea, urinary tract infections, pneumonia and septicemia (Janda and Abbott, 2021). In Nigeria, the prevalence of these bacteria along with their antibiotic resistance patterns poses significant public health challenges. This aims to evaluate the bacteriological profile of Enterobacteriaceae in various environments including clinical settings food sources, water supplies to inform public health strategy (Hasman *et al.*,2013). Enterobacteria are not only associated with a wide range of clinical syndrome but also a major causative agents of foodborne enteritis and zoonotic infection. The family Enterobacteriaceae was subsequently created with a single type genus (Enterobacter) to house a collection of 112 species previously referred to as the “colony-typhoid group” (Janda and Abbot, 2021). This group was originally defined by set of unifying phenotypic traits, including Gram stain reaction (negative), good growth on artificial media, acid formation from D-glucose (often with gas), and production of nitrites from nitrate.

2.3. MICROBIAL CONTAMINATION OF CURRENCY NOTES

A Paper currency note is widely exchanged for goods and services in countries worldwide and it was first developed in China (Almed *et al.*, 2010). An individual living in unhygienic conditions having unhygienic habits will contaminate the notes with bacteria and these notes will act as a vehicle delivering bacteria to contaminate the hands of the next user. improper hand washing after using the toilet, counting paper notes using saliva, coughing and sneezing on hands then exchanging money, and placement or storage of paper notes on dirty surfaces

leads to the contamination and these notes will act as a vehicle delivering bacteria to contaminate the hands of the next user. The money makes for easy transfer of microorganisms and thus cross contamination. Paper notes of currency which is handled by a large number of people, under a variety of personal and environmental conditions thus increase the possibility of acting as environmental vehicle for the transmission of potential pathogenic microorganism. Important factors for the survival of pathogenic agents on surfaces are the presence of organic matter, solar irradiation, temperature and humidity (Taylor *et al.*, 2016). Recently its was discover that many Gram-positive bacteria, such as *Enterococcus spp.*, *S. aureus* and *Streptococcus pyogenes*, and Gram-negative bacteria, such as *Acinetobacter spp.*, *Escherichia coli*, *Klebsiella spp.*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Shigella spp.*, can survive for months on surfaces In addition, mycobacteria and *Clostridium difficile* can survive for months, while other pathogens, such as *Bordetella pertussis*, *Haemophilus influenzae*, *Proteus vulgarisor*, *Vibrio cholera*, persist only for days(Yar, 2020) *Candida albicans* can survive for some times on surfaces, whereas respiratory tract viruses, such as Coronavirus, Coxsackievirus, Influenza virus, severe acute respiratory syndrome-associated virus or Rhinovirus, can persist on surfaces for a few days. Noroviruses are environmentally stable, able to survive both freezing and heating (although not thorough cooking), and resistant to many common chemical disinfectants, and can persist on surfaces for up to 2 weeks (Lopman *et al.*, 2012). Herpes viruses persist for only a few hours leading to one week, and viruses of the GI tract, such as Astrovirus, HAV, Poliovirus and Rotavirus, persist for appropriately two months.

2.4. FORMITES OF CURRENCY

Infectious diseases, the leading cause of death worldwide are caused by pathogenic microorganisms, such as bacteria, viruses, parasites or fungi. They can be spread, directly from one person to another or from animals to humans or indirectly through food, water or

many other inanimate objects scientifically referred to as fomites (Darwood, 2014). These routes of transmission are of great importance in the health of many populations where the frequency of infection is a general indication of local hygiene and environmental sanitation levels (Baronia and Saini, 2018). Banknotes and coins represent fomites which can become contaminated with infectious agents emanating from the atmosphere, dust, dirt or droplets resulting from coughing or sneezing, as they are passed on from hand to hand. Everyday, cash money in the form of notes and coins is passed on from one person to another for the payment of goods or services, for settlement of debts, or for deferred payment in economic activities.

2.5. STUDIES OF NIGERIAN CURRENCYS FOR BACTERIAL CONTAMINATION

Currency notes when not used and in circulation, remain clean and fresh but when in use and have changed hands, may be soiled and liable to tear and microbial contamination. As unrelated to human health as it seems, money could serve as a vehicle in the transmission of pathogenic microbial organisms as well as disease if handled unhygienically during its transit (Chukwu, 2010)

Currency notes are widely exchanged daily, for goods and services, by many people in communities all over the world (Allan *et al.*, 2018) which expose these banknotes to microbial contamination as a result handling and storage, thereby making them potential sources of microbial infection to other people, more especially the immunocompromised (Moses *et al.*, 2018). Banknotes, the most frequently used and circulated substance in the world, are usually contaminated with various microbes as they are handled by people of diverse health and hygienic standards and are stored under varying environmental and personal hygienic conditions (Tosin *et al.*, 2019). Contamination of banknotes may occur generally from the public in communities, hospitals.

During handling and transactions, contamination from skin, wounds, nasal secretions and aerosols, and the anal region, are some of the main sources of contamination of banknotes (Agholor *et al.*, 2020). Improper handling may be as a result of handlers coughing, sneezing onto hands and touching the notes: improper storage may come in the way of placement in or on dirty surfaces (Moses *et al.*, 2018). Practices which are unhygienic such as wetting fingers with saliva prior to counting notes (Moses *et al.*,8) and handling currency notes with filthy and stained fingers by market traders, automobile mechanics, meat-sellers and so on, may also introduce an array of bacteria to the notes, these could have a great impact on public health in a developing country like Nigeria, where microbial infection is ubiquitous and access to adequate healthcare is beyond the majority of citizens (Abd Alfadil *et al.*, 2018). microbial contamination takes place during the various stages of food preparation.

2.6. PUBLIC HEALTH IMPLICATIONS

Currency notes could play a role in the transmission of faeca-oral pathogens (Ukong *et al.*, 2019). Infectious agents get to vulnerable subjects and hosts through various routes such as direct contact, food, water, insects and a variety of inanimate objects called fomite (Nwoke, 2018). The role of fomites in disease transmission is significant, being, on the one hand, easily contaminable from various sources in the environment, and also readily available for direct contact with human subjects, on the other; especially in the pathogenic microbial infection chain. (Boone and Garba, 2007). While many parasites take the cyclical or biological pattern involving active transmission by vectors, others that take the faecal-oral route also have fomites as vehicle for transmission

Transmission of pathogens through currency notes has become very relevant in today's world due to COVID-19 pandemic. Globally, paper currency notes are widely used to exchange for goods and services (Alemu, 2014). Users often contaminate these notes with several microflora including viruses, fungi, protozoan, and bacteria via unhygienic conditions and

habits Some of these practices and habits include nose-picking, coughing and sneezing on palms, applying saliva on fingers to aid notes counting, improper hand washing after using toilets, and storage of paper notes on contaminated surfaces. Microorganisms are known to spread through air, water, food, etc., an important mechanism of the spread of pathogens by fomites. (Pradeep, 2012).

2.7. PREVENTIVE MEASURES

To reduce the risk of contamination and infection while using the naira notes, it's important to always practice good personal hygiene, being mindful of how u handle and store money, frequently and thorough hand washing with soap and water or alcohol base sanitizer especially after handling money and before touching face, nose and mouth. Also avoid squeezing money into one's pocket, using saliva in counting money this act can upset the gastrointestinal tract which can lead to harboring of pathogenic bacteria from money such as Escherichia coli salmonella shigella and proteus. Minimize direct contact inculcate the used of gloves or tissue when handling large amount of money, especially in food or health related environments, use of electronics such as mobile banking, point of service, to reduce the risk of physical handling of money. Avoid handling money while handling food this is directed to mostly food vendors who attend to customer at the same time collecting money knowing fully well that bacteria can be transfer through inanimate objects like money which can cause serious problems to one's health. Money should be properly store in a clean bag wallet and not in pockets which may be expose to sweat or dirty and contamination. Ensure public awareness by educating the public on the risk of unhygienic handling of money and the importance of clean currency practices, promotes sanitary habits in markets shops and public places.

2.8 GAPS IN EXISTING LITERATURE

There is a need for more comprehensive studies that not only identify bacterial species but also analyze their resistance to key antibiotics using standardized susceptibility testing (e.g., disc diffusion, minimum inhibitory concentration (MIC) determination). These findings could guide appropriate antibiotic stewardship efforts and raise awareness about environmental reservoirs of antimicrobial resistance (AMR) organisms. Most bacteriological studies in Nigeria rely on traditional culture-based methods and biochemical tests for the identification of isolates. While useful, these methods may lack the specificity and sensitivity of molecular techniques such as PCR, 16S rRNA gene sequencing, or whole genome sequencing (WGS). Molecular tools can confirm species identity, detect virulence genes, and identify specific resistance genes (e.g., bla_{CTX-M}, bla_{NDM}, mcr-1, etc.). Understanding the genetic makeup of Enterobacteriaceae isolates from currency is essential for tracking the spread of high-risk clones and resistance mechanisms. It also allows for the identification of possible genetic linkages between environmental and clinical strains, which can inform epidemiological studies and infection control strategies (Wilkie *et al.*, 2024). Currently, very few Nigerian studies have employed molecular approaches for bacterial level data are crucial for high-resolution surveillance and global comparisons.

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. MATERIALS USED

Petri dish

Slant bottle

Conical flask

Beaker

Measuring cylinder

Ethanol

Freezer

Autoclave

Immersion oil

microscope

Ruler

Naira notes

Bunsen burner

Inoculum loop

Spatula

forceps

Gram staining reagent

Biochemical reagents

Antibiotic susceptibility disk

Nutrient agar Muller-Hinton agar,

MacConkey agar

Peptone water

3.2. Study Area

This study was conducted around the University of Benin campus, Ugbowo, with samples collected from park, bank and petrol station. These areas are commercial and densely populated environments, where cash-based transactions are predominant.

3.3 Sample Collection

A total of 12 samples of the Nigerian notes consisting of four (4) pieces of Naira denomination “of one hundred” (“~~₦~~100“)”two hundred” (“~~₦~~200 “)” five hundred (“~~₦~~500 “)” and “one thousand (“~~₦~~1000)” notes were collected from each of the three locations. The nature of the notes collected were sorted and analyzed into different denomination “(₦100, ₦200, ₦500, and ₦1000)”. Each sample was collected using sterile gloves and stored in sterile polythene bags to avoid cross-contamination.

3.4. Preparation of Culture media

The culture media used in this study include; Nutrient agar, Mueller-Hinton agar, MacConkey agar and peptone water. These media were autoclaved at 121°C for 15mins. All media used were prepared according to the manufacturer’s instruction.

3.5. Sample Processing

Currency samples were processed using the swabbing method with sterile moistened swabs. Each currency notes was swabbed at a particular area of the money and soaked in a 5ml of sterile peptone water, the swabbed stick was then cut into the peptone water following a period of 10minutes at room temperature. The container bearing the swabbed portion was then cover and rotated to dislodge the cells into suspension. 0.2ml of water from each soak naira notes were collected and dispense in 108(one hundred and eight) sterile petril dishes before pouring molten agar. Molten MacConkey agar was poured into the one hundred and eight petri dishes that contain 0.2ml of the stock from each soaked naira notes while molten Nutrient agar was poured into 12 petri dishes also Molten Mannitol salt agar was poured into the petri dish a serial dilution of 10^{-1} was done and its was then pour plated all were incubated at 37°C for 24hr. After 24hr incubation the number of colony forming unit was recorded and bacterial load of each naira notes was determined.

3.6 Characterization and identification of Bacteria

3.6.1 Gram Staining Test

This test differentiates the bacteria into Gram Positive and Gram-Negative Bacteria, which helps in the classification and differentiations of microorganisms. Gram positive bacteria are known to retain the purple crystal violet color due to the presence of a thick peptidoglycan layer (Sagar and Aryal,2022) unlike Gram negative bacteria that lacks or have a thin peptidoglycan layer. To perform gram staining procedure:

Firstly, a smear of bacteria isolate was prepared on a grease-free slide. This was allowed to air-dry and then flushed with crystal violet stain and left for 1 minute, which was thereafter rinsed with distilled water. The slide was flushed with iodine solution for 30 seconds, then washed off with distilled water. This was then decolorized with 95% of alcohol for 10

seconds and immediately rinsed with distilled water. Finally, the smear was counterstained with safranin for 1 minute then washed off. The slide was allowed to air dry before examining it under the microscope using an immersion oil with an objects lens at 100x magnification.

3.6.2 Biochemical Tests

3.6.2.1 Oxidase Test

The oxidase test helps identify microorganisms that produce the enzyme cytochrome oxidase. It differentiates between oxidase-positive *Pseudomonadaceae* and oxidase-negative *Enterobacteriaceae*. Cytochrome oxidase facilitates the transfer of electrons from a donor (in the electron transport chain) to the final acceptor (oxygen), producing water as a byproduct. The presence of cytochrome oxidase causes the electron donor to be oxidized, resulting in a color change to dark purple. To perform the test, filter paper was impregnated into 1% tetramethyl-pphenylenediamine dihydrochloride, smear pure colony into the paper strip and observe for a color change after 10 seconds (Barton and Fauque, 2009).

3.6.2.2 Catalase Test

The catalase test determines if bacteria produce the catalase enzyme, which neutralizes hydrogen peroxide. When catalase is present, it breaks down hydrogen peroxide into water and oxygen, producing bubbles as an indicator of a positive result. This enzyme is typically produced by obligate aerobes and facultative anaerobes. The test was performed by placing a pure colony on a slide in which a few drops of 3% hydrogen peroxide (H₂O₂) was dropped and observed for bubble formation within 10 seconds.

3.6.2.3 Indole Test

The indole test identifies bacteria that produce the enzyme tryptophanase, which converts the amino acid tryptophan into indole. To determine the presence of indole Kovac's reagent was

used which contains para-dimethylaminobenzaldehyde thereafter some of the bacterial colony of pure culture was smear on the paper strip and allow to air dry the Kovac's reagent was added to its to allow for some times for color change, when indole reacts with these reagents, it forms a red indole dye, indicating a positive result.

3.6.2.4. Urease Test

The urease test detects bacteria that produce the enzyme urease, which is part of the amidohydrolase and phosphoesterase superfamily. Urease breaks down urea into ammonia (NH_3) and carbon dioxide (CO_2). The increase in ammonia raises the pH of the medium to an alkaline level, turning the color pink at pH 8.1, which indicates a positive result. This test is particularly useful for identifying *Helicobacter pylori* strains that produce urease. To perform the test, immerse infected stomach mucosa or bacterial colonies in urea broth and observe for a color change within 30 minutes to confirm a positive result.

3.6.2.5 Triple Sugar Iron Test

The Triple Sugar Iron (TSI) test helps identify bacteria in the *Enterobacteriaceae* family. The test medium contains three sugars: 0.1% glucose, 1% lactose, and 1% sucrose, along with red phenol as a pH indicator and ferrous sulfate for detecting hydrogen sulfide production. The medium was prepared with a slant and butt configuration. Bacteria use glucose first, causing the medium to turn yellow within 6-8 hours if glucose is utilized both aerobically (on the slant) and anaerobically (in the butt). If the bacteria also ferment lactose and sucrose, the entire medium will remain yellow. If the bacteria cannot ferment these sugars, they will deaminate amino acids, turning the medium alkaline and red due to phenol red. Strict aerobes will only change the color of the slant, while facultative anaerobes will change both the slant and the butt. Additionally, if the bacteria produce hydrogen sulfide by reducing thiosulfate, this will be indicated by blackening or cracking of the agar.

3.6.2.6 Methyl Red

The Methyl Red or MR test is a diagnostic tool to identify specific enteric gram-negative rods species. This test helps determine whether an organism can effectively maintain a stable level of acidity, as certain organisms undergo a process known as mixed acid fermentation. Methyl red serves as a pH indicator to determine acid levels, displaying red at low pH, indicating a positive outcome, and turning yellow at high pH, indicating a negative result. On the other hand, Voges Proskauer test is used to identify specific types of organisms, such as enteric gram-negative rods, *Aeromonas*, and *Vibrio*, as well as the viridans group of streptococci and staphylococci, at the species level. While a pink-red hue at the surface is a positive result, a lack of a pink-red color is a negative result.

3.6.2.7 Citrate Utilization Test

The citrate utilization test is a biochemical method used to evaluate whether microorganisms can utilize citrate as the sole carbon source and ammonium salts as the sole nitrogen source. The procedure is carried out on Simmons' citrate agar, all isolates were streaked onto the surface of Simmons citrate agar in test tubes and incubated at 37°C for 48 hours. The change in the medium's colour from green to blue within 24 to 48 hours indicated a positive reaction.

3.2.6.8 Sugar Fermentation tests

The sugar fermentation test is a biochemical method used to assess the capacity of microorganisms to metabolize specific carbohydrates with the consequent production of acid and/or gas. The sugars analyzed in this study were glucose, lactose, maltose, sucrose, mannitol, and xylose. A 100 ml fermentation medium was prepared by combining 1.5 g of peptone water, 1 g of the respective sugar, 0.5 g of disodium phosphate, and 0.72 ml of phenol red indicator. The medium was sterilized at 121 °C for 5 minutes, as longer sterilization causes the sugars to caramelize. Phenol red appears red in an alkaline

environment but turns yellow (gold) in an acidic one, while disodium phosphate helps maintain an alkaline pH. The medium was then inoculated with 50 μL of bacterial suspension standardized to McFarland 1×10^8 and incubated for 24 hours. Sugar fermentation resulted in acid production, which lowered the pH and caused the phenol red indicator to change from red to yellow.

3.2.6.9 CO₂ Gas Production

Durham tubes were placed inverted in the glucose fermentation tubes prior to sterilization at 121°C for 5 minutes. During fermentation, if the bacterium produces gas, the released gas displaces the liquid inside the Durham tube, creating a visible air bubble or clear space that indicates gas production.

3.2.6.10 Voges Proskeurs test

this test determines if an organism produces acetyl methyl carbinol from the fermentation of glucose. To a test tube containing 48h glucose broth culture, 6 drops of 5% α -naphthol solution was added. The tube was shaken well to aerates its content, after which 2 drops of potassium hydroxide was added, and tube mixed well. The formation of a pink or red colour at upper part of medium indicates a positive result (AL-Joda and Jasim,2021)

3.7 Virulence factors

virulence factors are molecules and traits produced by pathogens (like bacteria and viruses) that enable them to cause disease by colonizing a host, invading host cells, obtaining nutrients, and evading or subverting the host's immune system. These factors can include toxins, adhesins (for attachment), capsules (to evade immune detection), enzymes that break down host tissues, and structures that facilitate entry into host cells.

After biochemical test was done, virulent factor was performed to determine the enzyme utilization of the following: gelatinase, protease, lipase, and hemolysin.

3.7.1 Gelatinase: Gelatinase is a type of proteolytic enzyme that is capable of breaking down gelatin and type IV collagen, which is a crucial component of the basement membrane

Procedures: using the following; conical flask, distilled water, dissolved 0.5g to 100ml of distilled water, allowed the mixture to boil in order to completely dissolved the gelatinase/gelatin as its granular in nature. After boiling, add two gram of Nutrients agar to the boil gelatin and autoclave for 15minutes at 121°C

3.7.2 Protease:

Any enzyme that breaks down protein into its building blocks, amino acids, is called protease, which is a general term. Your digestive tract produces a number of these enzymes, but the three main proteases are pepsin, trypsin

Procedures: preparing protease in the laboratory using the following media Casein, with tryptic soy broth, with Agar Agar the standard which is to every 100ml of water add 15g of Agar Agar, into a conical flask add 3g of tryptic soya broth 2g of Caesin and 15g of Agar Agar, after dissolving the agar and autoclave at 121°C for 15minutes

3.7.3 Hemolysins:

are substances, primarily proteins, that cause the lysis (destruction) of red blood cells, leading to the release of hemoglobin, the protein responsible for carrying oxygen in the blood. While often associated with bacteria, fungi can also produce hemolysins. These substances contribute to a pathogen's ability to cause disease

Procedures: while preparing hemolysis made used of tryptic soy broth with blood with Agar Agar into a conical flask add 3g of tryptic soy broth, 2g of Agar Agar to 100ml of water and

autoclave at 121°C for 15minutes. After the agar has cooled did a cheek test to see if the agar is ready to used then add blood to it

3.7.4 Lipase:

Lipase is an enzyme that breaks down triglycerides into free fatty acids and glycerol by catalyzing the hydrolysis of the ester bonds In triglycerides. Lipases are present in pancreatic secretions and participate in fat digestion and metabolism. They play an essential role in lipid transport and serve individual functions in several tissues, including hepatic lipase in the liver, hormone-sensitive lipases in the adipocytes, lipoprotein lipase in the endothelial cells, and pancreatic lipase in the small intestine.

3.8: Antibiotic Susceptibility

Antimicrobial susceptibility testing is a laboratory procedure performed by medical technologists (clinical laboratory scientists) to identify which antimicrobial regimen is effective for individual patients. On a larger scale, the testing aids in evaluating treatment services provided by hospitals, clinics, and national programs to control and prevent infectious diseases (Lagier *et al.*,2015), Identified isolates were subjected to antibiotic susceptibility testing using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar.

To test for antibiotic susceptibility test, the following media were used according to manufacturer's instruction: nutrient agar, tryptic suya broth, Muller Hinton agar.

To perform this test, firstly, tryptic suya agar was prepared and the organism was inoculated which was observed for 24hr. The were subculture into nutrient agar and incubated for 24hr. Sterile preparation of 1×10^8 MacFarland Standard concentration of each bacterial isolates was made and 0.5ml of inoculum was spread on Mueller Hinton agar plates with a sterile glass rod. The inoculum was allowed to dry for 10minutes before sterile disk impregnated with various antibiotics concentrations (20mcg Rifampicin, 30mcg Cefotaxime, 10mcg

Streptomycin, 10mcg, Azithromycin, 20mcg Amoxil, 10mcg Ciprofloxacin, 30mcg Erythromycin, 20mcg Cefuroxime, 10mcg Gentamycin, 20ug Levofloxacin, 10ug Ceflotaxin, 10ug Spaeefloxacin, 30ug Ciprofloxacin, 30ug Amoxicillin, 10ug Augmentin, 30ug Gentamycin, 30ug Pefloxacin, 10ug Tarivid, 12ug Azithromycin.) were placed on the surface with the help of sterile forceps. The plates were incubated for 24hr after which reading of zone of inhibition was taken to be twice the measured value. (CLSI, 2025).

CHAPTER FOUR

RESULTS

Table 4.1: shows the means and standard deviation of bacterial counts from the different denomination of naira notes. The highest count recorded was 71.48 ± 69.27 cfu/cm², while the lowest was 1.65 ± 2.33 cfu/cm².

Table 4.2: Shows the cultural, morphological and biochemical features of the bacteria isolated from currency notes. The isolated bacteria include *Staphylococcus* spp. 1, *Staphylococcus* spp 2, *Acinetobacter* spp, *klebsiella aerogenes*, *staphylococcus spp3*, *staphylococcus epidermis1*, *klebsiella pneumonia*, *staphylococcus epidermis2*, *Serratia* spp

Table 4.3: Shows the antibiotic susceptibility of the isolated bacteria from currency notes. The bacteria showed varying levels of resistance with the lowest resistance index being 0 which was recorded by *staphylococcus aureus*, while the highest was 0.4 which was recorded by *Serratia* spp.

Table 4.4: Shows the Percentage of occurrence of the bacterial isolates obtained from currency notes. The highest occurrence was 11.76% recorded by *Staphylococcus spp1*, *staphylococcus spp2* *Acinetobacter*, *Staphylococcus epidermis* while the lowest was 5.88% recorded by *klebsiella aerogenes*, *klebsiella pneumonia*

Table 4.1: Total Heterotrophic Counts (cfu/cm²) of Isolated from Currency Notes from petrol Station, park and Bank

Currency (₦)	LOCATION (cfu/cm ²)		
	Park	Petrol	Bank
1000	1.00±0.22	2.22±0.39	NG
500	78.71±26.62	3.59±0.04	NG
200	36.00±12.88	71.48±69.27	NG
100	8.33±0.33	27.77±0.93	NG

NG

Table4.2: Total Coliforms Counts of Bacteria Isolated from Currency Notes from Bus Park petrol station and banks

Currency (₦)	LOCATIONS (cfu/cm ²)		
	Park	Petrol	Bank
1000	NG	NG	NG
500	NG	NG	NG
200	NG	3.84±1.68	NG
100	NG	NG	NG
NG			

Table4.3: Total Staphylococci Counts of Bacteria Isolated from Currency Notes from Bank, Bus park, and Petrol

Mannitol salt Agar	LOCATIONS (cfu/cm ²)		
	Park	Petrol	Bank
1000	NG	NG	NG
500	NG	NG	NG
200	17.68±12.61	NG	NG
100	NG	12.12±9.31	NG

Table4.4: Cultural, Morphological and Biochemical Characteristics of Bacteria Isolated from Currency Notes

Characteristics	1	3	7	8	10	14	17	21
Elevation	Convex	Convex	Raised	Raised	Convex	Convex	Raised	Convex
Margin	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Color	Golden yellow	Golden yellow	Creamy	Mucoid	Golden yellow	Whites	Creamy	Whites
Shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Size	2-4mm	2-4mm	1-3mm	2-4mm	2-4mm	1-2mm	2-4mm	1-2mm
Transparency	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Gram stain	+	+	-	-	+	+	-	+
Cell type	Cocci	Cocci	Cocco bacillus	Bacilli	Cocci	Cocci	Bacilli	Cocci

Cell arrangement	Cluster	Cluster	Diplococci	Rods	Cluster	Cluster	Rods	Cluster
TSI (Triple sugar iron)	-	-	-	-	-	-	-	-
H ₂ S	-	-	-	-	-	-	-	-
SCI (Simon Citrates)	-	+	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-
Glucose	+	+	+	+ ₋	+	-	+	-
Maltose	-	+	+	-	+	-	+	-
Lactose	-	+	-	-	+	-	+	-
CO ₂	-	+	+	-	+	-	+	-
Indole	-	-	-	-	-	-	-	-
Xylose	-	-	-	-	+	-	+	-
D-mannitol	-	+	-	-	+	-	+	-
KOH (Potassium Hydroxides)	-	-	+	+	-	-	+	-
Oxidase	-	-	+ ₋	-	-	-	-	+
Catalase	+	+	+	+	+	+	+	+

MR(Methyl Red)	+	+	+	-	-	-	-	-
VP(Voges Prokourse)	+	+	+	+	+	+	+	+
Probable organism	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	<i>Acinetobacter</i>	<i>Klebsiella aerogenes</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermis</i>	<i>Klebsiella pneumonia</i>	<i>Staphylococcus epidermis</i>

Key: _ Negative

+ Positive

Table4.5: Antibiotic Susceptibility of Bacteria Isolated from Currency Notes

Gram positive isolates	RD (20µg)	CTZ (30µg)	S (30µg)	AZM (10µg)	AMX (20µg)	CPX (10µg)	E (10)	LEV (20µg)	CN (10µg)	CEF (30µg)	Resistance index%
<i>Staphylococcus aureus</i>	S	I	S	S	S	S	S	S	S	R	0.2
<i>Staphylococcus aureus</i>	S	R	S	S	S	S	S	S	S	S	0.1
<i>Staphylococcus aureus</i>	S	S	S	S	S	S	S	S	S	S	0
<i>Staphylococcus epidermis</i>	S	R	S	I	S	S	S	S	S	S	0.2
<i>Staphylococcus epidermis</i>	S	I	S	R	S	S	S	S	S	S	0.1
<i>Serratia</i>	S	S	I	R	I	R	S	S	S	S	0.4

Table 4.6: Antibiotic Susceptibility of Bacteria Isolated from Currency Notes

Gram positive isolates	RD(10µg)	CT(30µg)	S(30µg)	AZ(10µg)	AM(20µg)	CP(10µg)	E(30µg)	LE(20µg)	C(10µg)	CE(30µg)	Resistance index%
<i>Acinetobacter</i>		R	R	R	R	R	R	R	R	R	1.0
<i>Klebsiella aerogenes</i>	S	S	S	S	S	S	I	I	I	S	0.3
<i>Klebsiella pneumonia</i>	R	R	R	R	R	R	R	R	R	R	1.0

Key:

- 0-12 Resistant
 - 13-16 Intermediate
 - 17-36 Susceptible
- (CLSI, 2025)

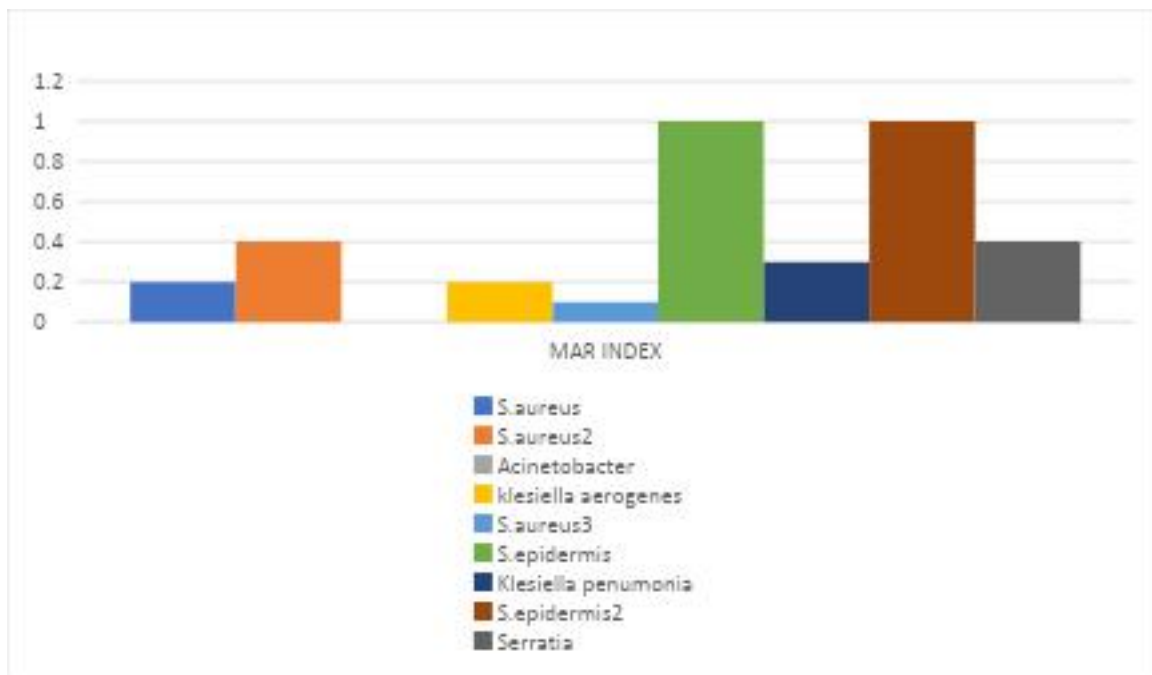


Figure 4.6: Shows the Resistance index of Antibiotic susceptibility test isolated from currency

Table4.7: Occurrence of the Bacteria Isolated from Currency Notes

Bacterial identified	Occurrence (%)
<i>Staphylococcus aureus</i>	11.76
<i>Staphylococcus aureus</i>	11.76
<i>Acinetobacter</i>	11.76
<i>Staphylococcus marcescens</i>	5.88
<i>Staphylococcus aureus</i>	29.41
<i>Klebsiella aerogenes</i>	17.65
<i>Klebsiella pneumonia</i>	5.88
<i>Staphylococcus epidermis</i>	11.76

Table 4.8: Virulent factors isolated from currency notes

Isolates	Gelatinase	Hemolysin	Protease	Lipase
<i>Staphylococcus aureus</i>	+	Hollow	-	Growth
<i>Staphylococcus aureus</i>	-	-	-	Growth
<i>Acinetobacter</i>	-	Hollow	-	Growth
<i>Klebsiella aerogenes</i>	Growth	-	+	Growth
<i>Staphylococcus aureus</i>	+	Hollow	+	-
<i>Staphylococcus epidermis</i>	Growth	Hollow	+	Growth
<i>Klebsiella pneumonia</i>	Growth	Hollow	+	Growth
<i>Staphylococcus epidermis</i>	-	Hollow	+	Growth
Serratia	+	Hollow	+	-

Key: - = No Zone of Inhibition

+ = Zone of Inhibition

Hollow = Clear Zone of inhibition

Growth = No clear Zone

CHAPTER FIVE

DISCUSSION

This study aimed to assess the prevalence and potential public health risks associated with bacterial contamination on different denominations and sources of Nigerian currency (Chigozie *et al.*, 2021). Since currency notes are widely circulated and frequently handled, they can act as vehicles for the transmission of pathogenic microorganisms, particularly in areas with poor hygiene standards (Alemu, 2014). Findings revealed significant contamination of Nigerian banknotes with both pathogenic and opportunistic bacteria (Mofolorunsho *et al.*, 2023), consistent with previous research showing that currency notes serve as potential vectors for microbial transmission due to frequent handling and environmental exposure.

The antibiotic susceptibility patterns of the bacterial isolates indicated a generally high level of sensitivity, with most isolates responding well to the antibiotics tested. The resistance index ranged from 0 to 1, suggesting varying degrees of susceptibility among the isolates. However, the detection of bacteria such as *Klebsiella* spp. and *Staphylococcus aureus* points to fecal contamination and poor hygiene practices among currency handlers (Paterson and Yohei, 2017). The presence of multidrug-resistant strains further underscores the potential public health threat posed by contaminated currency notes. The observed resistance patterns likely reflect the widespread misuse and overuse of antibiotics in both healthcare and community settings, highlighting the importance of implementing antibiotic stewardship programs.

Lower denomination notes showed higher microbial loads compared to higher denominations, likely due to their more frequent circulation and handling. This observation supports previous studies (Mbajiuka *et al.*, 2014; Agholor *et al.*, 2020) which reported that lower-value notes

harbor more microbes because they are used more often in everyday transactions. Samples collected from petrol stations and bus parks exhibited greater contamination than those from banks, reflecting poor hygiene, as well as increased exposure to dirt, grease, and dust in these environments.

The study identified several bacterial species on Nigerian currency notes, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Acinetobacter* spp., *Klebsiella pneumoniae*, *Klebsiella aerogenes*, and *Serratia* spp. These organisms suggest contamination from diverse sources such as human skin, the respiratory tract, and the gastrointestinal tract. The detection of *S. aureus* and *S. epidermidis* points to contamination through direct hand contact and poor personal hygiene. Although these species are part of the normal skin flora, they can cause opportunistic infections such as abscesses, wound infections, and septicemia when introduced into vulnerable sites on the body.

The isolation of *Klebsiella* spp. and *Acinetobacter* spp. also indicates possible fecal contamination and inadequate sanitation practices. These bacteria are common in the environment and often linked to hospital acquired infections like pneumonia, urinary tract infections, and bloodstream infections. Their presence on banknotes from public locations such as bus parks and petrol stations raises concerns about cross-contamination between individuals and different environments.

Antibiotic susceptibility testing showed that most isolates were sensitive to the antibiotics used, though some demonstrated resistance. Resistance indices varied between 0.0 and 1.0, with multidrug resistance observed in *Acinetobacter* spp. and *Klebsiella pneumoniae*. This aligns with previous findings (Imarenezor *et al.*, 2018; Paterson and Yohei, 2017) that multidrug-resistant bacteria can persist on inanimate objects like currency notes. The

detection of these resistant strains highlights the risk of spreading antimicrobial resistance within communities, especially in areas where antibiotics are frequently misused.

Additionally, several isolates produced virulence factors such as gelatinase, protease, hemolysin, and lipase, which enhance their ability to survive and cause disease. Hemolysin facilitates red blood cell lysis, while protease and lipase aid tissue invasion and nutrient utilization. These virulence traits not only improve bacterial survival on surfaces like currency notes but also increase their potential to cause infections upon contact with humans.

5.2 CONCLUSION

This study has shown that Nigerian currency notes are heavily contaminated with a variety of bacterial species, including both pathogenic and opportunistic organisms such as *Staphylococcus aureus*, *Klebsiella* spp., *Acinetobacter* spp., and *Staphylococcus epidermidis*. The presence of these microorganisms some of which displayed antibiotic resistance and virulent characteristic indicates that currency notes can act as potential carriers for the transmission of infectious diseases within the community.

The level of contamination varied depending on the denomination and the source of the currency. Lower denominations (₦100 and ₦200) and notes collected from highly interactive environments like motor parks and petrol stations showed the highest microbial loads, likely due to frequent handling and exposure to unhygienic conditions. Conversely, notes obtained from banks exhibited lower levels of contamination, reflecting relatively better sanitary conditions.

The identification of multidrug-resistant and enzyme producing bacteria on the currency notes poses a significant public health risk, especially in areas where hygiene is poor and antibiotic misuse is widespread. These findings highlight the role of paper currency as a fomite capable of transmitting infectious agents and contributing to the spread of

antimicrobial resistance in the environment. The study concludes that improper handling, poor personal hygiene, and unhygienic storage practices are major contributors to the bacterial contamination of currency notes. To minimize the risk of disease transmission through money handling in Nigeria, it is important to promote good hygiene practices, encourage regular handwashing, increase the use of electronic payment methods, and replace damaged or worn out notes promptly.

5.3 RECOMMENDATIONS

Based on the outcomes of this study, the following recommendations are proposed:

1. **Public Awareness:** Efforts should be made to educate the public on the health risks associated with handling currency notes and to emphasize the importance of regular handwashing after touching money.
2. **Improved Currency Management:** The Central Bank of Nigeria should consider introducing polymer-based notes, which are less porous and easier to clean, or establish more frequent currency replacement programs.
3. **Enhanced Hygiene Practices:** Individuals, especially those in high-contact areas such as bus parks and petrol stations, should be encouraged to maintain proper hygiene by using hand sanitizers and avoiding direct hand-to-mouth contact.
4. **Further Research:** Ongoing surveillance and more comprehensive studies across different regions of Nigeria should be carried out to better assess the extent of microbial contamination on currency notes and its implications for public health.

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