CONTAMINATION LEVELS, BACTERIA DIVERSITY AND SUSCEPTIBILITY OF BACTERIA ISOLATED FROM POS MACHINE OPERATED AROUND MEDICAL JUNCTION TO NEW BENIN MARKET, BENIN CITY, EDO STATE.

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BENIN CITY

OCTOBER, 2025

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCE IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) DEGREE (BSC) IN SCIENCE LABORATORY TECHNOLOGY

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CERTIFICATION

This is to certify that this project work carried out by Gabriel Adewale FOLORUNSHO with the matriculation number, LSC2009845 of the department of Science Laboratory Technology (Microbiology Techniques), Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

This book is dedicated to God Almighty whose unwavering love, guidance, direction and strength have fueled my journey of discovery.

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ABSTRACT

Point of Sale (POS) terminals have become a common feature of commercial transactions in urban environments such as Benin city, Edo State, Nigeria. This study was aimed at investigating the microbial contamination, bacterial diversity and antibiotic susceptibility patterns of microorganisms isolated from Point of Sale (POS) machines keypads within Medical Junction to New Benin Market axis of Benin City, Edo State, Nigeria. Twenty POS machines were randomly sampled using sterile swab sticks and the samples were analyzed using standard microbiological procedures and biochemical tests for bacterial Identification. The total heterotrophic counts ranged from 3.3 x 10^3 to 9.9 x 10^3. Antibiotics susceptibility pattern of the bacteria isolated was performed using Kirby-Bauer disk diffusion method Four main bacterial genera were isolated and identified: *Escherichia coli* (32.5 %), *Neisseria* spp. (27.5 %), *Staphylococcus* spp. (20 %), and *Streptococcus* spp. (20 %). *E. coli* showed the highest prevalence with (32.5 %) Antibiotic susceptibility testing, performed using the Kirby–Bauer disk diffusion method, revealed high resistance to β-lactam antibiotics such as ampicillin, augmentin, and ampiclox, while the isolates exhibited greater sensitivity to fluoroquinolones including ciprofloxacin, ofloxacin, and levofloxacin. The findings confirm that POS terminals in Benin City serve as potential reservoirs for pathogenic and drug-resistant bacteria, posing significant public health risks, particularly in densely populated commercial settings. Regular disinfection of POS devices, improved hand hygiene among operators and customers, and public awareness campaigns on microbial contamination are recommended to mitigate the spread of infectious agents and antimicrobial resistance.

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CHAPTER ONE

1.0 BACKGROUND OF STUDY

Point of Sale (POS) terminals have become a common feature of commercial transactions in urban environments such as Benin City, Edo State, Nigeria. These devices enable cashless payments in various public spaces, markets and retail settings. However, their frequent handling by both sellers and customers makes them likely hotspots for microbial contamination, as human hands are well-established vectors for the transmission of bacteria (Ufuoma *et al*., 2023). In densely populated and economically active zones like the stretch from Medical junction to New Benin Market, POS terminals are often used by multiple individuals throughout the day, increasing the possibility of bacterial colonization and subsequent cross-contamination.

Numerous studies in Nigeria have demonstrated the microbial contamination of frequently touched surfaces such as door handles, mobile phones and currency. These studies commonly report the presence of pathogens like *Staphylococcus aureus*, *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, which are known to cause a range of infections, including those affecting the skin, gastrointestinal tract and respiratory system (Olodu *et al.,* 2023). Like mobile phones, POS machines are rarely disinfected despite being heavily used in crowded, open-air environments. This increases their potential to harbour antibiotic-resistant bacteria (ARB). For instance, research in Nasarawa State found that 70 % of mobile phones tested carried *S. aureus* and *E. coli,* with 85% of isolates displaying resistance to multiple antibiotics (Ufuoma *et al.,* 2022). Given the similar usage patterns and cleaning neglect of POS devices, similar microbial risks are expected.

Antibiotic resistance in Nigeria is a growing public health concern, driven by the misuse of antibiotics, poor hygiene and limited infection control in public and community settings. Local studies in Benin City have identified high bacterial loads on communal surfaces such as toilet seats and market water sources, where isolates of *E.coli* and *S. aureus* showed resistance to widely used antibiotics like ampicillin and cefuroxime. According to the World Health Organization (2020), antimicrobial resistance could be responsible for as many as 10 million deaths annually by 2050 if not urgently addressed. This threat is particularly critical in low-resource regions like Nigeria, where access to healthcare and sanitation infrastructure is often inadequate. Environmental studies in the Niger Delta, including parts of Edo State, have also detected resistant strains of *Salmonella*, *Shigella* and *Vibrio* species, with up to 100 % resistance reported against antibiotics such as amoxicillin and augmentin *(*Frieri *et al.,* 2017*)*. Given these findings, POS machines, which are frequently touched and rarely sanitized, may act as vectors for the transmission of drug-resistant pathogens within communities.

1.1 AIM OF STUDY

The aim of this study was to determine the contamination levels, bacterial diversity and susceptibility of bacteria isolated from POS machine around medical junction to New Benin Market in Benin City, Edo State, Nigeria.

The specific objectives of this study were to :

1. determine total heterotrophic bacterial count from the POS machines
2. isolate, enumerate and identity the bacterial isolates from the POS machines
3. determine the frequency distribution of the bacterial isolates from the different POS machines
4. determine the susceptibility pattern of the bacterial isolates against some conventional antibiotics

CHAPTER 2

2.0 LITERATURE REVIEW

The widespread use of Point of Sale (POS) machines for financial transactions in Nigeria, especially in urban areas such as Benin City, Edo State, has sparked concerns about their role as possible carriers of microbes (Ajayi and Ezeanya, 2018). These devices, handled frequently by both vendors and customers, often in unhygienic conditions, may harbor harmful microorganisms similar to other high-touch surfaces like mobile phones and keyboards (Adams and Dancer, 2019). Previous studies have shown that such devices can support bacterial growth, including antibiotic-resistant strains, posing significant public health risks (Alemu, 2014).This literature review investigates the extent of microbial contamination, the variety of bacteria present and the antibiotic resistance patterns of microbes found on such surfaces, with a focus on POS machines. It draws on both global and local studies of microbial contamination on shared objects, examines bacterial diversity in public spaces and addresses the rising issue of antimicrobial resistance (AMR). Additionally, it underscores the health risks linked to contaminated POS machines and emphasizes the urgent need for proper hygiene measures, particularly in the context of areas like the Medical to New Benin Market in Benin City.

2.1 Global Perspectives on Fomite Contamination

Fomites have been widely recognized around the world as key contributors to both hospital-acquired and community-spread infections. A thorough review by Kramer *et*

al. (2006) revealed that bacteria like *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* can survive on non-living surfaces for extended periods ranging from several days to months depending on factors such as temperature and humidity. The research highlighted that frequently touched surfaces in public settings, including ATMs and point-of-sale (POS) machines, are especially vulnerable to microbial contamination due to constant contact.

In Saudi Arabia, Dahiya *et al.* (2024) examined bacterial presence on mobile phones used by healthcare staff in intensive care units. The study detected *S. aureus, E. coli and Klebsiella* species, with *S. aureus* being the most prevalent. Researchers attributed the contamination largely to inadequate hygiene habits, such as not regularly cleaning mobile devices and recommended routine disinfection of electronics. Likewise, research by Reynolds *et al.* (2007) in the United States showed that public keyboards and touchscreens were heavily contaminated, including with drug-resistant strains like MRSA, reinforcing the idea that electronic devices can act as fomites.

In many African urban areas, where overcrowding and poor sanitation are common, public surfaces serve as potential breeding grounds for pathogens. For instance, a Kenyan study reported widespread contamination of public health facility surfaces with organisms such as *E. coli, Pseudomonas* species and *Klebsiella* species (Mutonga *et al*., 2019).

2.2 Microbial Contamination in Nigeria

The growing concern around POS (Point of Sale) machines as potential carriers of microbes stems from their rising usage and frequent handling. According to the Central Bank of Nigeria (CBN), POS transactions surged by over 200 % between 2018 and 2023, particularly in informal market areas. These devices are touched by

numerous individuals each day, making them high-contact surfaces with significant contamination risk (CBN, 2023). Unlike personal items such as mobile phones, POS terminals are passed from person to person, increasing the chances of microbial transmission.

While direct studies on microbial contamination of POS machines are scarce, research on similar devices suggests they could act as reservoirs for bacteria. For instance, (Carretto *et al.,*2013) reported high levels of bacteria such as *Bacillus* spp., *Proteus* spp. and coagulase-positive *Staphylococci* on ATM keypads in Lagos. Given the comparable usage patterns of ATMs and POS devices, it is reasonable to assume POS machines may host similar microbial communities.

In Nigeria, most research on fomite contamination has concentrated on surfaces like currency, phones and hospital tools. Ufuoma *et al.* (2022), for example, studied bacterial presence on mobile phones among university students in Nasarawa State, finding a high occurrence of *S. aureus* (27.5 %)*, E. coli* (12.5 %) and *Salmonella* sp. (7.5 %). The study linked the contamination to poor hand hygiene and lack of device cleaning, with 70 % of participants rarely washing their hands and half never cleaning their phones—conditions that also apply to POS machines.

Similarly, a study by Olodu *et al.* (2023) in Benin City found that suya meat sold along New Benin Market was contaminated with bacteria like *Bacillus cereus, S. aureus*, *Klebsiella* spp. and *Proteus* spp., largely due to poor handling and environmental exposure. This implies that other public surfaces in the area, such as POS terminals, may also be at risk due to similar environmental and hygiene issues.

Another investigation by Eke *et al.* (2013) in Ekpoma, Edo State, revealed bacterial counts ranging from 0.3–0.85 × 10^5 CFU/ml in suya meat, with *S. aureus* and coliforms being the most prevalent. The study pointed to human handling as a key source of contamination an issue likely relevant to POS machines in commercial

environments. Supporting this, Okwelle *et al.,* 2024 found high coliform levels (4–20 MPN/100 ml) in borehole water from Benin City's automobile spare-parts markets, suggesting widespread environmental contamination that could easily extend to surfaces like POS devices in similar contexts.

2.3 Bacteria Variety on Frequently Touched Surfaces

The types of bacteria found on commonly handled objects are influenced by surrounding environmental factors, patterns of human behavior and cleanliness standards. Research has detected various Gram-positive and Gram-negative bacteria on public surfaces, highlighting their potential role in spreading infectious diseases.

Frequently Isolated Bacteria

Research conducted throughout Nigeria has consistently identified the following bacterial species as prevalent in various environments:

Staphylococcus aureus commonly linked to skin infections, respiratory illnesses like pneumonia and bloodstream infections.

Escherichia coli a key indicator of fecal contamination, often implicated in gastrointestinal illnesses.

Klebsiella pneumoniae a healthcare-associated pathogen known for causing pneumonia and urinary tract infections.

Pseudomonas aeruginosa an opportunistic organism frequently found in damp settings, posing risks especially in immunocompromised individuals.

Salmonella and *Shigella* species causative agents of typhoid fever and bacillary dysentery, respectively.

A study by Mutonga *et al.* (2019) utilized biochemical assays and molecular tools such as API-20E and PCR to analyze Enterobacteriaceae present on surfaces within healthcare facilities. The investigation revealed a variety of potentially harmful species, including members of the genus Providencia.

Consistently, pathogens like *S. aureus*, *E. coli*, *Klebsiella* spp. *P. aeruginosa and Bacillus* spp. Are reported as frequent contaminants on frequently touched surfaces. In the same study by Mutonga *et al.* (2019), samples collected from hospital surfaces and the hands of healthcare workers revealed notable presence of Providencia rettgeri (21.01 %), *S. aureus* (18.47 %) and *E. coli* (13 %). Molecular identification techniques confirmed the presence of diverse opportunistic pathogens, highlighting concerns regarding hygiene in clinical environments.

In Nigeria, Okwelle *et al.* (2022) investigated microbial contamination in well and stream water in Rivers State and isolated *E. coli, Salmonella* spp. *Staphylococcus* spp. and *Klebsiella* spp. with *E. coli* being the most frequently detected. The researchers attributed these contaminations largely to human activities and environmental exposure conditions similar to those found around high-traffic areas such as point-of-sale (POS) terminals in commercial zones like the New Benin Market.

2.4 Factors Influencing Bacterial Diversity

The diversity of bacteria present on surfaces is shaped by several factors, including the type of surface material, how frequently the surface is touched and surrounding environmental conditions. Materials such as plastic and stainless steel, which are commonly used in point-of-sale (POS) devices, are non-porous and can harbor bacteria for prolonged periods. Research has demonstrated that pathogenic bacteria like *Staphylococcus aureus* can remain viable on plastic surfaces for several days,

while *Escherichia coli* may persist for over a month under favorable conditions (Van Elsas *et al.,* 2011).

Environmental factors such as high humidity and the presence of organic matter, often observed in open market settings like New Benin Market, further contribute to bacterial survival and proliferation on surfaces (Zhou *et al.,* 2019).

Human activity is another critical contributor. Improper hygiene practices, such as infrequent handwashing or handling food and money simultaneously, can lead to microbial contamination. Studies have highlighted that frequent human contact with surfaces, especially in commercial environments, significantly increases bacterial load, including the presence of fecal indicators like *E. coli* (Ahmed *et al.,* 2022).

Additionally, surfaces like plastic and rubber, which are prevalent in POS device design, have been shown to support longer bacterial survival compared to porous materials (Choi *et al.,* 2020).

2.5 Antimicrobial Susceptibility and Resistance Patterns

Antimicrobial resistance (AMR) has become a major global health concern and surfaces such as point-of-sale (POS) machines may act as potential sources for the spread of resistant bacterial strains. Research indicates that bacteria collected from frequently touched public surfaces display different levels of antibiotic susceptibility, with resistance trends often attributed to the excessive and inappropriate use of antimicrobial agents (World Health Organization, 2020).

2.6 Global Trends in Antibiotic Resistance

Antibiotic resistance continues to be a serious global health issue, particularly among Gram-negative bacteria such as *Escherichia coli, Klebsiella pneumoniae and*

Pseudomonas aeruginosa. According to the World Health Organization, antimicrobial resistance (AMR) poses a critical challenge to public health, especially with the emergence of multidrug-resistant (MDR) organisms that hinder effective treatment of infections (WHO, 2024). Research conducted in Mozambique by (Faife *et al.* 2024) investigated diarrheagenic *E. coli* (DEC) strains from the Buffalo River and revealed a high prevalence of resistance to commonly used antibiotics like ampicillin and amoxicillin-clavulanate. Using the disk diffusion method, the study found that approximately 86 % of isolates exhibited multidrug resistance, underlining the difficulties in managing infections originating from environmental sources.

In clinical settings, resistance among Gram-positive bacteria is also alarming. For example, investigations in Kenyan hospitals by (Odhiambo *et al.* 2023) identified the presence of vancomycin-resistant Staphylococcus aureus (VRSA) and vancomycin-resistant Enterococcus (VRE) on various surfaces. These findings stress the importance of infection control practices, as contaminated environments may facilitate the spread of these resistant strains.

2.7 Mechanisms of Antibiotic Resistance

Environmental microorganisms develop resistance through several pathways, including:

1: Horizontal gene transfer via plasmids

2: Synthesis of extended-spectrum beta-lactamases (ESBLs)

3: Formation of biofilms that promote persistence and antimicrobial resistance on inanimate surfaces

Key mechanisms that drive resistance include the production of beta-lactamases, activation of efflux pumps and the horizontal transfer of resistance genes. A recent

investigation conducted in Nigeria by (Ahmed *et al.,* 2022) identified sulfonamide resistance genes sul1 and sul2 in 31.7 % and 21.7 % of bacterial samples collected from pharmaceutical wastewater, respectively. Additionally, 43.3 % of these isolates carried the class 1 integron gene (intI1), which facilitates the spread of resistance determinants. These results imply that frequently touched surfaces such as point-of-sale (POS) machines could act as reservoirs for resistance genes, potentially contributing to their transmission within community environments.

2.8 Public Health Risks Linked to Contaminated POS Machines

The contamination of Point-of-Sale (POS) machines presents notable public health concerns, particularly in crowded commercial hubs such as the area spanning from Medical Junction to New Benin Market. These devices are frequently touched by numerous individuals throughout the day, making them potential vectors for microbial cross-contamination. Harmful microorganisms like Staphylococcus aureus can lead to skin infections and foodborne illnesses, while *Escherichia coli* and *Salmonella* are well-known for causing gastrointestinal infections. Moreover, the presence of multidrug-resistant (MDR) bacteria on these surfaces increases the risk of treatment-resistant infections, potentially raising both illness rates and mortality.

In settings like Nigeria, where sanitation and healthcare access are often inadequate, contaminated surfaces can significantly intensify the spread of infectious diseases. According to (Nwachukwu *et al.* 2025) the environmental hygiene challenges in urban Nigeria contribute to a higher prevalence of pathogen transmission via shared objects. The World Health Organization (2022) emphasizes the importance of routine cleaning and disinfection of high-contact surfaces to minimize the spread of microbes, yet such measures are frequently overlooked in busy marketplaces.

Additionally, the potential for outbreaks of skin and gastrointestinal infections, along with the transfer of MDR organisms from informal public spaces to healthcare facilities, highlights the public health threat posed by inadequately sanitized POS machines. As (Rossi *et al.,* 2017) explain, indirect transmission routes such as via shared electronic devices can act as significant contributors to microbial exposure, especially in areas where basic hygiene infrastructure is lacking.

2.9 Hygiene Practices and Control Strategies

Implementing proper hygiene measures is essential to minimizing the presence of microbes on commonly touched surfaces. Research indicates that routine disinfection using agents such as alcohol-based cleaners can markedly decrease microbial contamination (Van Arkel *et al.,* 2021). Despite this, a significant proportion of individuals do not practice regular device cleaning. For instance, Adebayo *et al.,* (2024) found that nearly half of mobile phone users in urban Nigeria rarely or never sanitize their phones a habit that likely extends to the handling of point-of-sale (POS) terminals by vendors.

Encouraging hand hygiene and promoting surface sanitation through public awareness initiatives may help reduce transmission risks. In medical environments, the International Federation of Infection Control (IFIC) advises the use of personal protective equipment and strict cleaning regimens to curb hospital-acquired infections *(IFIC, 2023).* These best practices can be tailored to informal sectors such as open markets where shared devices like POS machines are frequently used. Additionally, while solar disinfection (SODIS) has been investigated As a low-cost sanitation method, recent findings by Tong *et al.* (2021) suggest that it has limited effectiveness against resistant bacterial strains in environmental samples, highlighting the need for integrated disinfection approaches.

Vulnerable Groups

Populations such as young children, older adults, individuals with weakened immune systems and those suffering from chronic illnesses are more susceptible to health risks. Additionally, informal sector workers handling point-of-sale (POS) devices without adequate protection may unknowingly act as carriers of resistant microbial strains (WHO 2022).

Preventive and Control Strategies

Effective prevention requires both hygiene education and behavior change, supported by public awareness campaigns that promote:

1: Regular handwashing

2: Use of alcohol-based hand sanitizers

3: Routine disinfection of electronic devices using 70 % isopropyl alcohol.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample location

The study was carried out within the commercial areas stretching from Medical Junction to New Benin Market, Benin City, Edo State, Nigeria.

3.2 Sample Collection

POS keypads were randomly selected within the study area 20 Sterile cotton swab sticks moistened with sterile physiological peptone water were used to swab the surface of the keypads. Each swab sample was introduced into sterile swab tubes, properly labelled and immediately transported to the microbiology laboratory of for further microbiological analysis within two hours of collection.

3.3 Serial Dilution of Swab Samples

The swab stick samples collected from the POS keypads were subjected to serial dilution to obtain countable microbial colonies. Each swab stick was first immersed in

a sterile test tube containing 9 ml of normal saline and allowed to soak for a few minutes to ensure proper release of microorganisms into the solution. This stepwise dilution helped to reduce microbial load systematically, thereby facilitating accurate isolation, enumeration and characterization of bacteria during subsequent plating on appropriate culture media.

3.4 Preparation of media

3.4.1 Nutrient Agar

Twenty-eight grams (28 g) of dehydrated nutrient agar powder was weighed and dissolved in 1 Liter of distilled water. The mixture was gently heated with continuous stirring until completely dissolved. The prepared medium was dispensed into clean conical flasks and sterilized by autoclaving at 121 °C for 15 minutes. After sterilization, the medium was allowed to cool to 45–50 °C before being aseptically poured into sterile Petri dishes at a uniform depth of about 20 ml per plate. The plates were allowed to solidify inverted to prevent condensation and stored at 4 °C until required for inoculation.

3.4.2 MacConkey Agar

Fifty grams (50 g) of dehydrated MacConkey agar powder was weighed and dissolved in 1 liter of distilled water. The solution was heated gently with constant agitation until fully dissolved. The medium was then dispensed into sterile conical flasks and sterilized by autoclaving at 121 °C for 15 minutes. After sterilization, the agar was allowed to cool to 45–50 °C and aseptically poured into sterile Petri dishes at a depth of about 20 ml per plate. The plates were left to solidify on a level surface inverted to avoid condensation.

3.5 Isolation of Samples Using the Pour Plate Method

A total of twenty (20) swab samples collected from POS keypads were processed for microbial isolation using the pour plate technique. From each serially diluted sample, 1.0 ml aliquots were aseptically transferred into sterile Petri dishes in duplicates. About 15–20 ml of sterile molten nutrient agar cooled to 45–50 °C, was poured into each plate, gently mixed with the inoculum by swirling to ensure uniform distribution, and allowed to solidify. The plates were then inverted and incubated at 35–37 °C for 24–48 hours under aerobic conditions.

3.6 Enumeration of Microorganisms

Microbial enumeration was carried out on plates containing colonies to ensure accuracy and reliability of counts. The number of colonies on each plate was recorded and the average count from duplicates was calculated. The colony-forming units per milliliter (CFU/ml) of the original sample were determined using the formula:

$$cfu/ml = \frac{\text{Mean number of colonies x Dilution factor}}{\text{Volume plated (ml)}}$$

3.7 Morphological Characteristics of Isolates

Preliminary characterization of the bacterial isolates was based on their observable macroscopic morphology. Features noted included colony shape (circular, irregular, round), margin/edge (entire, undulate, lobate, filamentous), elevation (flat, raised,

convex), colour (milky, pale green), colony consistency (dry, wet) and opacity (transparent, translucent, or opaque). Representative colonies with distinct morphological differences were subsequently sub-cultured onto fresh nutrient agar plates to obtain pure cultures for further microscopic and biochemical identification.

3.8 Gram staining test

The Gram staining technique was used for differentiation between gram positive and gram negative bacterial strains. A drop of sterile distilled water was placed on a neat and clean glass slide and a single isolated colony of 24 hours old culture was mixed in it. The smear was made by spreading the culture. This smear was air dried and fixed by rapidly passing the slide three times over the flame. It was then flooded with crystal violet for 1 minute and then washed off with distilled water. Then gram's iodine solution was added to the smear and the glass slide was left for one minute and rinsed with distilled water. This step was followed by the application of decolorizing agent (ethanol) for 30 seconds. Decolorizing agent was immediately washed with distilled water and the smear was counter stained with safaranine for one minute. The slide was washed with distilled water; air dried and was observed under the microscope.

3.9 Biochemical Tests

3.9.1 Catalase Test

A small amount of a pure bacterial colony was transferred onto a clean glass slide using a sterile loop. A drop of 3 % hydrogen peroxide (H_2O_2) was added to the colony. The appearance of immediate effervescence (bubbling) indicated a positive catalase reaction while the absence of bubbles indicated a negative result.

3.9.2 Indole Test

Sterile test tubes containing 5 ml of peptone water were inoculated with pure bacterial isolates and incubated at 37 °C for 24–48 hours. After incubation, 0.5 ml of Kovac's reagent was gently added to each culture tube. The formation of a red-coloured layer at the surface indicated a positive indole reaction, while a yellow layer indicated a negative result.

3.9.3 Citrate Utilization Test

Simmons citrate agar slants were inoculated by streaking the slope with a sterile loop containing the test organism. The tubes were incubated at 37 °C for 24–48 hours. A colour change from green to blue indicated a positive citrate utilization test while no colour change (medium remaining green) indicated a negative result.

3.9.4 Oxidase Test

A piece of filter paper was soaked in freshly prepared oxidase reagent (1 % tetramethyl-p-phenylenediamine dihydrochloride). Using a sterile loop a portion of the bacterial colony was smeared on the filter paper. The appearance of a deep purple colour within 30 seconds indicated a positive oxidase reaction while no colour change indicated a negative result.

3.9.5 Glucose Fermentation Test

Test tubes containing glucose broth supplemented with phenol red as a pH indicator and inverted Durham tubes were inoculated with the test organism and incubated at 37 °C for 24–48 hours. A colour change from red to yellow indicated acid production

(positive glucose fermentation). The presence of gas bubbles in the Durham tube indicated gas production.

3.9.6 Mannitol Fermentation Test

Test tubes containing mannitol broth with phenol red indicator and inverted Durham tubes were inoculated with the isolates and incubated at 37 °C for 24–48 hours. A colour change from red to yellow indicated acid production due to mannitol fermentation, while the presence of gas in the Durham tube confirmed gas production. A negative result was indicated by no colour change (medium remaining red) and absence of gas.

3.10 Antimicrobial sensitivity bioassay

The antibiotic susceptibility of the bacterial isolates was determined using the Kirby–Bauer disk diffusion technique on Mueller Hinton agar, prepared following Clinical and Laboratory Standards Institute (CLSI, 2021) guidelines. Each pure bacterial isolate was standardized by suspending colonies in sterile normal saline to match 0.5 McFarland turbidity standard. Using the pour plate method 1 ml of the standardized inoculum was introduced into sterile Petri dishes and approximately 20 ml of molten Mueller Hinton agar (cooled to 45–50 °C) was poured and mixed to ensure even distribution of the inoculum. After the agar solidified Gram-positive and Gram negative antibiotics sensitivity disc was bought from the pharmaceutical shopping store. Antibiotic disc used and their concentrations were as follows: Gram positive discs contained; Ciprofloxacin (10 µg), Norfloxacin (10 µg), Gentamycin (10 µg), Lincocin (20 µg), Streptomycin (30µg), Riflampicin (20 µg), Erythromycin (30 µg), chloramphenicol (30µg), Ampiclox (20 µg) and Floxapen (20 µg). The Gram negative discs contain, Tarivid (10 µg), Peflacine (10

µg), ciproflox (10 µg), Augumentin (30 µg), Gentamycin (10 µg), Stretomycin (30 µg), ceporex (10 µg), Nalidixic acid (30 µg), Septrin (30 µg) and Ampicilin (30 µg). Commercially prepared antibiotic-impregnated discs were aseptically placed on the surface using sterile forceps. Plates were incubated in an inverted position at 35–37°C for 18–24 hours. Following incubation, the diameter of the inhibition zones around each antibiotic disc was measured in millimeters using a transparent ruler. The results were interpreted as Resistant (R), Intermediate (I), or Susceptible (S) based on CLSI interpretive criteria. A clear inhibition zone of sufficient diameter indicated susceptibility, reduced zone size indicated intermediate response and absence or very small inhibition zones indicated resistance. Resistance implied the organism could grow and multiply despite the presence of the antibiotic intermediate suggested a borderline effect where higher doses or specific body sites may still achieve efficacy while susceptibility indicated the antibiotic would likely be effective in treatment.

CHAPTER FOUR

4.0 RESULTS

This chapter presents the findings obtained from the microbiological analysis of POS keypads collected from various locations from Santana Market to Ring Road axis of Benin City. The results are organized into tables showing the cultural and biochemical characterization of the isolates. Table 4.1 represents the bacterial count of each sample, expressed in colony-forming units per milliliter (cfu/ml). This quantifies the microbial load present in the samples, which is essential for evaluating the level of contamination or microbial presence.

Table 4.2 presents the represents the morphological, cultural, and biochemical characteristics of the bacterial isolates obtained from the various samples. This includes colony appearance, Gram reaction, cell arrangement, and results from biochemical tests such as catalase, indole, and sugar fermentation. These parameters were used for the presumptive identification of the bacterial species.

Table 4.3 illustrates the distribution of bacterial isolates across the different sample types. This highlights which microorganisms were recovered from specific samples and helps determine contamination sources.isplays the cultural and biochemical characterisation of isolates.

Table 4.4 displays the frequency of occurrence of each bacterial isolate, giving insight into the most prevalent organisms across all the samples analyzed in this study.

Table 4.5 shows the antibiotics susceptibility pattern (antibiogram) of the isolates , this includes the response of each bacterial species to various antimicrobial agents, indicating their resistance or sensitivity profiles based on standard zone of inhibition.

TABLE 4.1 Heterotrophic bacterial count of POS machines

S/N	cfu/ml
1	3.3×10^3
2	3.1×10^4
3	2.5×10^4
4	5.2×10^4
5	1.5×10^4
6	1.5×10^3
7	2.5×10^3
8	2.1×10^4
9	1.8×10^4
10	4.5×10^4
11	5×10^2
12	5×10^2
13	1.5×10^3
14	1.6×10^3
15	2.2×10^4
16	2.9×10^4
17	2.0×10^4
18	1.5×10^4
19	9.9×10^3
20	8.8×10^3

TABLE 4.2 Morphological, Cultural, and Biochemical Characteristics of Isolates

	A	B	C	D
Shape	Circular	Circular	Round	Round
Colour	Creamy	Whitish	Creamy	Creamy
Margin	Entire	Entire	Entire	Entire
Opacity	Opaque	Opaque	Opaque	Opaque
Elevation	Raised	Flat	Flat	Flat
Wet/dry	Wet	Wet	Wet	Wet
Gram stain	+	-	+	-
Cell shape	Cocci	Cocci	Cocci	Rod
Arrangement	Clusters	Single	Clusters	Single
Catalase	+	-	-	+
Indole	+	-	+	-
Citrate	-	-	-	-
Oxidase	-	+	+	-
Spore	-	-	-	-
Glucose	+	-	+	-
Mannitol	-	+	-	+
Lactose	+	-	+	-
Suspected isolates	*Staphylococcus* spp.	*Neisseria* spp.	*Streptococcus* spp.	*Escherichia coli*

TABLE 4.3 Distribution of bacterial isolates from POS machines

S/N	A	B	C	D
1	X	✓	X	X
2	✓	✓	✓	✓
3	✓	✓	✓	✓
4	✓	✓	✓	✓
5	X	✓	X	X
6	✓	X	X	X
7	X	X	X	X
8	✓	X	X	✓
9	X	X	✓	✓
10	✓	✓	✓	✓
11	X	X	X	✓
12	X	X	X	✓
13	X	✓	X	X
14	X	✓	X	X
15	✓	✓	✓	✓
16	✓	✓	✓	✓
17	X	X	✓	✓
18	X	✓	X	X
19	X	X	X	X
20	X	X	X	✓

TABLE 4.4 Percentage occurrence of bacterial isolate from POS machines

	Frequency	Percentage Occurrence %

Staphylococcus spp.	8	20.00
Neisseria spp.	11	27.50
Streptococcus spp.	8	20.00
E . Coli	13	32.50

TABLE 4.5 ANTIBIOGRAM TEST FOR BACTERIA ISOLATE FROM POS MACHINES

	A	B	C	D
Pefloxacin	Susceptible	Susceptible	Susceptible	Susceptible

Ofloxacin	Not Determined	Susceptible	Susceptible	Susceptible
Azithromycin	Susceptible	Susceptible	Susceptible	Susceptible
Levoflaxcin	Susceptible	Susceptible	Susceptible	Susceptible
Cefaloxcin	Not Determined	Susceptible	Not Determined	Susceptible
Spiramycin	Not Determined	Susceptible	Not Determined	Susceptible
Ciprofloxacin	Susceptible	Susceptible	Susceptible	Susceptible
Amoxicillin	Resistant	Susceptible	Resistant	Susceptible
Augmentin	Not Determined	Susceptible	Not Determined	Susceptible
Gentamicin	Susceptible	Susceptible	Susceptible	Susceptible
Ampiclox	Resistant	Not Determined	Resistant	Not Determined
Zinnacef	Resistant	Not Determined	Resistant	Not Determined
Rocephin	Resistant	Not Determined	Resistant	Not Determined
Erythromycin	Susceptible	Not Determined	Susceptible	Not Determined

CHAPTER 5

5.0 DISCUSSION

The findings of this research provide valuable insight into the microbial contamination of Point of Sale (POS) keypads along Medical junction to New Benin

Market corridor in Benin City, highlighting their potential public health implications. The results showed the presence of both Gram-positive and Gram-negative bacteria, with variations in microbial load, frequency of occurrence, and antibiotic resistance patterns. These results are consistent with earlier studies on other frequently touched devices such as ATMs, mobile phones, and computer keyboards, which have been implicated as fomites in the spread of infectious agents (Ebuara *et al*., 2020)

The study demonstrated that POS keypads within the study area were contaminated with a variety of bacterial species, including *Neisseria* spp., *Staphylococcus* spp., *E . coli* and *Streptococcus* spp. Such contamination is expected, given that POS terminals are high-contact surfaces handled repeatedly by both customers and operators. Many of these microorganisms are normal residents of the skin, nose, and mouth but can become opportunistic pathogens. The presence of *Staphylococcus* and *Streptococcus* species is particularly noteworthy because they are well-documented causes of skin, respiratory, and foodborne infections.

As presented in Table 1, microbial counts ranged from 3.3×10^3 to 8.8×10^3 cfu/ml, indicating a high level of bacterial contamination. This observation aligns with findings from previous studies on high-touch surfaces such as ATM machines, mobile phones, and computer keyboards, which consistently report that these devices act as reservoirs for both commensal and potentially pathogenic microorganisms (Anderson and Palombo, 2009). These findings suggest poor hygiene practices associated with the handling of POS machines in the study area. The elevated bacterial loads recorded here further suggest that POS devices could act as reservoirs for pathogens and aid in their transmission, particularly in busy market areas with diverse hygiene practices.

Cultural and biochemical tests (Table 4.2) confirmed the presence of *E. Coli*, *Staphylococcus*, *Streptococcus* and *Neisseria* species. The coexistence of Gram-positive and Gram-negative organisms indicates contamination from multiple sources, including human skin, respiratory droplets, and the surrounding environment. These organisms are commonly associated with human skin, mucous membranes, and fecal matter, suggesting that contamination of POS keypads likely results from frequent hand contact by multiple individuals. Poor hand hygiene, particularly after handling money or using restrooms, can easily transfer these bacteria to shared surfaces (Reynolds *et al.,* 2012).

The frequent detection of *Staphylococcus aureus* was expected, as this bacterium commonly colonizes human skin(Coates *et a.l* 2014). While about 20–40% of healthy individuals carry *S. aureus*, its pathogenic potential should not be underestimated—it is a major cause of boils, abscesses, wound infections, and pneumonia. The growing concern of Methicillin-Resistant *Staphylococcus aureus* (MRSA) further highlights its clinical significance. In immunocompromised individuals, *S. aureus* can cause severe diseases such as endocarditis (Valenzuela *et al.,* 2018).

The isolation of *Staphylococcus epidermidis* is also noteworthy. Although it is part of the skin's normal flora, it is a recognized opportunistic pathogen capable of forming biofilms on medical devices, leading to infections such as catheter- and prosthetic valve-associated infections (Otto, 2009). Its presence on POS terminals suggests contamination through frequent skin contact and highlights their potential as fomites. This agrees with reports identifying *S. epidermidis* as one of the most frequent contaminants on high-touch surfaces (Fritz *et al.,* 2014).

The detection of *Neisseria* spp. carries public health importance, as this genus contains both harmless and pathogenic members. Commensal species such as *N. sicca* and *N. lactamica* can occasionally cause opportunistic infections (Vaneechoutte *et al*., 2011), while *N. meningitidis* is responsible for serious conditions like meningitis and septicemia (Stephens *et al.,* 2007). Their presence on POS keypads suggests contamination from respiratory secretions and reflects poor hygiene practices. Even though not all *Neisseria* strains are pathogenic, their detection points to possible health risks, especially in light of growing antimicrobial resistance (WHO, 2024).

The occurrence of *Streptococcus* spp. is also significant, given that some species—such as *S. pyogenes* and *S. pneumoniae*—cause pharyngitis, pneumonia, and other severe infections (Cunningham, 2000). Their presence on POS keypads indicates contamination via skin contact or respiratory droplets, emphasizing the potential for community spread in crowded areas.

As shown in Table 4.3, bacterial contamination was widespread across all sampled POS locations. While the types of organisms identified were similar, variations in contamination levels likely resulted from differences in hygiene practices, environmental exposure, and customer flow. These results mirror findings from *Ebuara et al.*, 2020) who observed consistent contamination across various public devices.

According to Table 4.4, *Escherichia coli* was the most frequently isolated organism, reflecting its strong adaptability and ability to survive in diverse environments (*Jozic et al.*, 2017). *Neisseria* spp. appeared less frequently, likely due to its fastidious growth requirements (Stephens *et al.,* 2007). The presence of *Staphylococcus* and

Streptococcus spp. confirms that human-derived microbes contribute significantly to surface contamination (Otter *et al.,* 2013).

Antibiotic susceptibility tests (Table 4.5) revealed high resistance to commonly used antibiotics such as ampicillin, augmentin, and erythromycin, while greater sensitivity was observed to fluoroquinolones (ciprofloxacin, ofloxacin, and levofloxacin). These findings are consistent with reports by Motayo *et al.,* (2012) who documented widespread beta-lactam resistance and emerging multidrug resistance among community isolates. The detection of resistant bacteria on POS terminals underscores their potential role as reservoirs for antibiotic resistance genes and transmission pathways in public spaces.

Overall, contamination was found across all POS locations,

5.2 CONCLUSION

This study has demonstrated that POS keypads within Medical Junction to New Benin Market axis of Benin City are contaminated with diverse bacterial species, including *E. coli*, Staphylococcus, Streptococcus, and Neisseria. The microbial load recorded was considerably high, indicating that these devices are potential reservoirs for pathogenic organisms. The predominance of *E. coli .* highlights its strong environmental persistence, while the recovery of antibiotic-resistant isolates raises concern about the potential spread of antimicrobial resistance in the community. The contamination observed across all sampled POS locations underscores that the problem is widespread and not restricted to specific sites. Collectively, these findings highlight the public health risks posed by contaminated

POS keypads and the urgent need for improved hygiene practices to prevent disease transmission.

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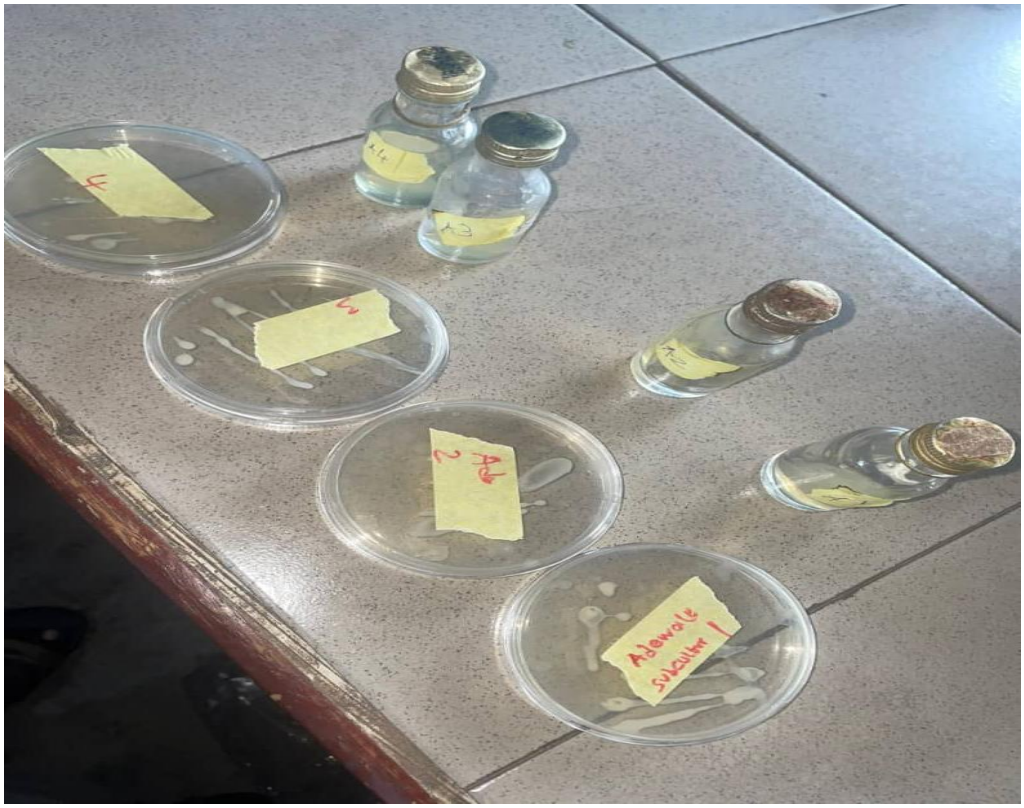
APPENDIX I

LABELING OF PETRI-DISHES (Field Work, 2025)

INOCULATION OF SAMPLES (Field Work, 2025)



ISOLATE SAMPLE (Field Work, 2025)