

**MICROBIAL PROFILE OF POINT-OF-SALE(POS) MACHINES AMONG TRADERS  
IN BENIN CITY, EDO STATE.**

**BY**

**OSAIGBOVO, OGHOSAMASE**

**BMS1903142**



**DEPARTMENT OF MEDICAL LABORATORY SCIENCE**

**SCHOOL OF BASIC MEDICAL SCIENCES**

**COLLEGE OF MEDICAL SCIENCES**

**UNIVERSITY OF BENIN, NIGERIA**

**OCTOBER, 2025**

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**A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY  
SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL  
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REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE IN  
MEDICAL LABORATORY SCIENCE**

**OCTOBER, 2025**

## **CERTIFICATION**

We the undersigned certify that this research work was carried out by **OSAIGBOVO, OGHOSAMASE** in the Department of Medical Laboratory Science, School of Basic Medical Science, University of Benin, Benin City in partial fulfillment of the requirements for the award of Bachelor of Science in Medical Laboratory Science.

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## **DEDICATION**

This project is dedicated to Almighty God.

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My deepest and unreserved reverence to Almighty God for his unconditional love, protection and guidance upon my life. My sincere appreciation goes to my amiable supervisor DR. (Mrs) N. A. Olise for her availability, encouragement and immense contributions in making this work a success.

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## ABSTRACT

The increasing prevalence of microbial contamination in objects such as Point-of-Sale (POS) machines poses significant public health risks, particularly in high traffic areas such as markets, schools, where hygiene practices may be inadequate. This study aimed to evaluate the bacterial and fungal loads on POS machines used by traders across specific markets in Benin City, Edo State to identify the types of microorganisms present, assess their prevalence, and propose measures for reducing contamination. A total of 150 POS machines were sampled within the study area using swab sticks moistened with sterile saline. These swabs of POS surfaces were collected from five different markets: Oba market, New Benin market, Uselu market, Oliha market and Ikpoba hill market. The markets covered three Local Government Area. Microbial analyses were performed using standard techniques consisting of macroscopy, microscopy, identification and susceptibility testing of isolates present, and type of microbial contamination. The results revealed that a large percentage of the samples (75.3%) showed bacterial contamination where *Klebsiella spp.* (17.3%) and *Staphylococcus aureus* (15.3%) were found to be predominant, while fungal contamination was 57.3% of the samples collected, with *Candida spp.* being the most prevalent with the percentage (38.7%). The study highlighted significant variations in contamination rates across different markets and, indicating that certain markets, such as Ikpoba-hill market, had higher levels of bacterial contamination. The findings underscore the urgent need for improved hygiene practices among traders and regulatory oversight to ensure reduced contamination on these devices thereby reducing the spread of diseases. In conclusion, this research provides critical insights into the microbial quality of POS machines in Benin City and emphasizes the importance of implementing effective and good hygienic practices to protect the health of users.

## CHAPTER ONE

### INTRODUCTION

#### 1. BACKGROUND OF STUDY

Microorganisms are present almost everywhere in our surroundings. They may propagate via food, water, air and most importantly by fomites (Ejaz *et al.*, 2018). The Point-of-sale (POS) machine is a machine that performs some human teller functions like balance enquiry, bills payment, mini statements and withdrawal and transfer of cash which is its major function. POS transactions are carried out through the use of debit/credit cards which enable the card holders to access and carry out banking transactions without a teller. Different people from different socio-economic levels and hygienic status use the POS daily and this increases the chances of hand-borne transmission of microorganisms to the machine surfaces (Yuana *et al.*, 2022).

Electronic banking, an offshoot of Information and Communication Technology (ICT) has provided the classic and associated transformation to the banking industry with benefits through ease of monetary transactions. It is rather becoming 'an essential to have' than 'a pleasure to have' service. E-banking systems evolved technologies include, Automated Teller Machines (ATMs), Point of Sales (POS) device, Electronic Funds Transfer and Telebanking with the ATMs and POS machine as the most frequently used technologies (John *et al.*, 2021).

The rise of high-touch devices, particularly Point of Sale (POS) terminals, has transformed financial transactions across Nigeria and other parts of the world. Traditionally, financial operations relied heavily on cash and banking institutions, but the introduction of POS devices, driven by technological advances and several other factors has made transactions faster and more accessible (Adekanmi *et al.*, 2021). These services are provided around various locations such as shopping centres /malls, airports, grocery stores, petrol/ gas stations, restaurants, shops, schools,

hotels, hospitals or any place that large number of people gather (John *et al.*, 2021). Therefore, the development of e-banking services has not only affected economic status of countries but has had several deep social and cultural effects on the quality of individual lives.

The advent of electronic payment systems has revolutionized financial transactions globally. In Nigeria, the adoption of Point-of-Sale (POS) machines has significantly increased, facilitating cashless transactions and enhancing financial inclusion. However, the frequent handling of these devices by multiple users daily raises concerns about their potential as reservoirs for microbial contaminants. Studies have indicated that frequently touched surfaces, such as Point-of-sale machine harbor various microorganisms, some of which are pathogenic and exhibit antibiotic resistance (Akinseye *et al.*, 2018). Studies have highlighted concerns about the contamination of various surfaces, including currency notes, with microbial species (Ejaz *et al.*, 2018). This contamination can contribute to the transmission of bacterial pathogens and antibiotic-resistant bacteria, posing risks to human health (Usman *et al.*, 2021). Given the similarities in usage patterns, POS machines may similarly serve as vectors for microbial transmission, posing public health risks. Despite this, there is a paucity of data on the microbial contamination of POS machines, particularly in regions like Benin city, Edo state. This study aims to bridge this knowledge gap by assessing the microbial profile of POS machines in the specified area.

## **1.2. STATEMENT OF PROBLEM**

Contamination of environmental objects and surfaces by microorganisms is a common phenomenon. The presence of viable pathogenic bacteria on inanimate objects has been reported by earlier researchers. Several studies of the human environment have demonstrated contamination and colonization of inanimate objects such as door handles, plastics, faucets,

phones, money, fabrics, plastics and other fomites by bacteria and, which is also responsible for the spread of various bacterial infections (Anibijuwon *et al.*, 2014).

Microbes are found on surfaces that require contact with human hands like computer keyboards, door handles, mobile phones and elevator buttons which serve as microbial reservoirs (Nwankwo and Offiah, 2016). Many bacterial, fungal and viral pathogens can survive on inanimate objects for several months and such pathogens could cause epidemic infections (Adedeji, 2019).

Microorganisms are ubiquitous and their ability to contaminate environmental objects and their surfaces is not an unusual phenomenon. There are reports indicating the presence of viable organisms on inanimate objects causing contamination, colonization and the spread of microbial infections (John and Adegoke, 2018). Human beings have a marked tendency to pick up microorganisms from environmental objects and the hands play a vital role in contact and transmission of organisms. Human hands have been shown to play a significant role in the transmission and cross-contamination of microorganisms between environmental surfaces. Furthermore, microorganisms found to contaminate fomites have been shown to persist on the surfaces for periods ranging from a few hours to several months, and have been detected and recovered from surfaces after routine conventional cleaning. The ability of inanimate objects to support viable microorganisms for a prolonged period of time is well documented and such environmental surfaces and objects, especially those in close proximity with persons and frequently touched, poses a lot of threat to human health and is a cause for public concern. Examples of such inanimate objects in the environment that are currently in frequent contact with the hands are the keypads of an automated teller machine (ATM) (Anibijuwon *et al.*, 2014). The ATM is likely to be contaminated with many different kinds of microorganisms both

pathogenic and non-pathogenic due to their vast usage by many people in a day especially in an overcrowded environment (Marbel *et al.*, 2014).

Colonization of e-banking facilities by pathogenic organisms has been reported as a potential vehicle for their transmission. Microorganisms found to contaminate fomites are known to persist on environmental surfaces for varying periods of time ranging from hours to months. Hence, cross infection of microorganisms between environmental surfaces and a host has equally been established (John *et al.*, 2021).

The public health risks posed by these contaminated surfaces are significant, especially in populated areas such as Benin city, Edo state. Edo, as one of Nigeria's commercial hubs, sees high volumes of POS transactions in its markets, businesses, and academic institutions. Given the sheer number of transactions conducted daily, the potential for POS devices to serve as vectors for disease transmission is a growing concern. The lack of regular cleaning protocols for these devices, combined with their frequent use, creates an environment conducive to the accumulation and transfer of microbial contaminants (Adekanmi *et al.*, 2021). This highlights important concerns regarding the safety of POS devices as they become an essential part of everyday financial transactions. The frequent handling of these machines emphasizes the need for research into their potential role as fomites in spreading infectious microorganisms, posing public health risks that go beyond the context of the pandemic. While POS devices are essential for both retailers and consumers, they are frequently used without adequate cleaning between transactions. This increases the risk of spreading pathogenic bacteria that may cause infections, particularly antibiotic-resistant strains such as MRSA and VRE (Yusha'u *et al.*, 2024).

### **1.3. SIGNIFICANCE OF STUDY**

The significance of this study stems from its ability to fill a critical knowledge gap, exposing the hidden threat of microbes (bacteria and fungi) that may be present in POS machines used across specific locations in Benin city, Edo state, and thereby contributing to the development of more effective strategies for limiting the spread and transmission of these pathogens through contact with them thereby protecting public health.

With regard to the bacterial and fungal contamination of POS, there are practically no studies precisely quantifying this type of contamination. With reference to the qualitative and quantitative identification of health threats caused by viruses, such data in relation to ATM surfaces having contact with means of payment are not available at all in the global literature on the subject. Hence, the aim of this study is to fill this gap by assessing bacterial, and fungal contaminations of external surfaces of POS machines in Benin city, Edo state.

Evaluating the susceptibility of bacterial agents isolated from POS devices will provide useful data to healthcare practitioners and policy makers in developing effective strategies to combat the spread of resistant strains. The aim of this study is to evaluate the bacterial and fungal contaminants on POS devices used by traders in local markets in Benin city.

### **1.4. AIM AND OBJECTIVES**

#### **1.4.1. AIM**

This study is aimed at determining the microbial profile of Point-of-sale(POS) machines among traders across markets in Benin city, Edo state.

#### **1.4.2. SPECIFIC OBJECTIVES OF STUDY**

The specific objectives of the study were,

1. to isolate and identify bacteria and fungi present on the surface of POS machines.
2. to determine the frequency and distribution of microbial contaminants across selected POS terminals in different locations.
3. to assess the antibiotic susceptibility patterns of bacterial isolates.

### **1.5. RESEARCH QUESTIONS**

1. What types of bacteria and fungi are present on the surface of POS machines in Benin city, Edo state?
2. What is the prevalence of microbial contamination across POS machines in different locations within the study area?
3. What are the antibiotic susceptibility patterns of the bacterial isolates obtained from POS machines?
4. What preventive measures can be recommended to reduce microbial contamination of POS machines?

### **1.6. RESEARCH HYPOTHESES**

#### **Alternative Hypothesis (HA):**

There is a significant difference in the microbial contamination levels of POS machines based on their location and the hygiene practices of the operators.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 OVERVIEW OF POINT-OF-SALE (POS) MACHINES

##### 2.1.1 POINT-OF-SALE (POS) MACHINES

Point-of-sale (POS) technology refers to the hardware and software systems used by merchants to process electronic payments (Frank, 2023). The term "Point of Sale (POS)" or "Point of Purchase (POP)" refers to the location and time where a retail transaction is completed (Okeke *et al.*, 2017). The POS terminal is also known as a POP terminal and is used for instant payment of goods and services, as it is user-friendly, easy to operate, and has multi-functional capabilities (Mohammed *et al.*, 2022). A point of Sales (POS) terminal is a machine used to accept cards for payment of goods and services. POS terminal allows a cardholder to have a real-time online access to funds and information in his/her bank account through debit or credit cards (Iwedi, 2017). They are considered by Awoniyi (2022) as a virtual replacement for cash transactions. The terminal keeps a record of customer purchases and deposit transactions, allowing customers to check their balance, pay for items, and perform funds transfer transactions without the need for physical cash (Ikpefan *et al.*, 2018). A POS terminal is a device deployed in a merchant location that allows users to swipe their electronic cards to make payments instead of using physical cash (Williams *et al.*, 2018). The adoption of POS terminals has significantly reduced the volume of cash-based transactions, as such adoption of POS technology allows cardholders to make payments at sales or purchase outlets without the need for physical cash (Osang, 2017). In Nigeria, these POS terminals are now also commonly used for transfer of funds to withdraw money, instead of the conventional ATM. The device is mostly used by retail shop owners where payment is being made with the use of an automated teller machine cards or via electronic bank

transfers. Other payment and transactions carried out with the device include electricity bills settlement, payment for airtime, TV or cable subscription and so on (Dabo *et al.*, 2025).

## **2.2. MICROBIAL CONTAMINATION OF INANIMATE OBJECTS**

Inanimate objects in the built environment can serve as reservoirs of microbial matter. Each of these objects is host to an entire community composed of a wide variety of bacterial, viral, archaeal, protistan, and fungal organisms, including potential pathogens and microbial metabolic products harmful to humans (Stephens *et al.*, 2019). These inanimate objects that are able to serve as reservoir for microbes and are able to transmit these pathogens to humans are known as fomites. Microorganisms may be transmitted from animated sources to inanimate environmental sources, which may become secondary reservoirs if they meet the needs of transmitted pathogens to survive and to multiply (Kramer and Assadian, 2014). In healthcare settings, bacteria, bacterial spores, viruses and yeasts are mainly transmitted from infected and/or colonized patients, but also from staff, and in some situations from visitors to the inanimate hospital environment, particularly to areas adjacent to patients and frequently touched surfaces by hands “high-touch surfaces” (Kramer and Assadian, 2014). Fomites consist of both porous and nonporous surfaces or objects that can become contaminated with pathogenic microorganisms and serve as vehicles in transmission (Zhuang *et al.*, 2023). During and after illness, viruses like the SARS-COV 2 are shed in large numbers in body secretions, including respiratory droplets, blood, feces, urine, saliva, and nasal fluid (Calvet *et al.*, 2024). Fomites become contaminated with virus by direct contact with body secretions or fluids, contact with soiled hands, contact with aerosolized virus (large droplet spread) generated via talking, sneezing, coughing, or vomiting, or contact with airborne virus that settles after disturbance of a contaminated fomite

(i.e., shaking a contaminated blanket). Humans can come into contact with viruses and other pathogens when they consume or recreate in virus-contaminated water, eat contaminated food, breathe contaminated air, or touch contaminated fomites. Infected individuals can also deposit viruses onto fomites via touch if there is virus present on their body, thereby contaminating fomites (Anderson and Boehm, 2021). Microbes such as Vancomycin-resistant *enterococcus* (VRE), Methicillin-resistant *Staphylococcus aureus* (MRSA), and gram- negative bacteria can persist on non-living objects such as hospital equipment for many months. Thus, by touching contaminated surfaces and noncritical equipment, hands may acquire and transfer microorganisms to other inanimate objects or patients (Akpepuun and Nyinoh, 2021). Hospital Acquired Infections (HAIs) arising from contamination of frequently handled hospital surfaces or equipment regarded as high-touch surfaces, such as sinks, telephones, patients' beds, and linens which serve as fomites have been reported to spread bacterial pathogens, including; *Acinetobacter baumannii*, *Staphylococcus aureus*, carbapenem- resistant *Enterobacterales*, and vancomycin-resistant *Enterococcus* species which have also been seen to show some degree of resistance (Odoyo *et al.*, 2023). It has been reported that both Gram-positive and Gram-negative bacteria are able to survive up to months on dry inanimate surfaces, with longer persistence under humid and lower-temperature conditions. Factors that may affect the transfer of microorganisms from one surface to another and cross-contamination rates are type of organisms, source and destination surfaces, humidity level, and size of inoculum (Russotto *et al.*, 2015). Bacteria are ubiquitous and are among the most common microorganisms found on fomites. Pathogens can be transferred to fomites through respiratory droplets, skin contact or bodily fluids, fecal contamination (Guleria, 2024). Microbiological contamination refers to an accidental or non-intended introduction of infectious material like bacteria, virus, fungi, yeast, mould,

protozoa or their toxins and by-products (Ejaz *et al.*, 2018). Inanimate objects like currency play a significant role in the indirect transmission of infections like trachoma, diphtheria, gastroenteritis, whooping cough and diarrhoea (Sharma and Dhanashree, 2011). Microorganisms are ubiquitous and their ability to contaminate environmental objects and their surfaces is not an unusual phenomenon (Mbajuka, 2015). There are reports indicating the presence of viable organisms on inanimate objects causing contamination, colonization and the spread of microbial infections (John and Adegoke, 2018). Human beings have a marked tendency to pick up microorganisms from environmental objects and the hands play a vital role in contact and transmission of organisms. Studies have implicated environmental surfaces in the transmission of microorganisms with the hands acting as chief organ for physical manipulation of the environment. Human hands usually harbour microbes as part of the body normal flora as well as transient microorganisms contracted from the environment (Ayalew *et al.*, 2015). Several organisms possess the potentials to survive on dry surfaces. These organisms have developed complicated separate physiologic resting stages which accord them the surviving advantage or potentials to hibernate effectively due to low water activity (John and Adegoke, 2018). Adegoke and Okoh (2011) showed that inanimate materials like currency notes can serve as vehicles for transmission of plasmid bearing vancomycin-resistant *Staphylococcus aureus*. Microorganisms found to contaminate fomites are known to persist on environmental surfaces for varying periods of time ranging from hours to months. Hence, cross infection of microorganisms between environmental surfaces and a host has equally been established (Onuoha and Fatokun, 2014). While POS devices are essential for both retailers and consumers, they are frequently used without adequate cleaning between transactions. This increases the risk of spreading pathogenic bacteria that may cause infections, particularly antibiotic-resistant strains such as MRSA and

VRE (Yusha'u *et al.*, 2024). Microbes are found on surfaces that require contact with human hands like computer keyboards, door handles, mobile phones and elevator buttons which serve as microbial reservoirs (Nwankwo and Offiah, 2016). Many bacterial, fungal and viral pathogens can survive on inanimate objects for several months and such pathogens could cause epidemic infections (Adedeji, 2019).

## **2.3. COMMON PATHOGENIC MICROORGANISMS ISOLATED FROM POS SURFACES**

### **2.3.1. *Klebsiella spp***

*Klebsiella* is a rod-shaped, lactose-fermenting, gram-negative, non-motile bacterium. *K. pneumoniae*, a facultative anaerobe that is typically found in the natural flora of the intestines, can thrive with or without free oxygen. They can be found in the skin when infection is hospital acquired or transient. Additionally, this bacterium is encased in a capsule that serves as a physical barrier to elude the host's immune response, increasing its pathogenicity (Liu *et al.*, 2025). *Klebsiella* bacteria are becoming more and more resistant to antibiotics, most notably to the carbapenem class. Patients who are ill and undergoing treatment for other ailments are frequently infected with *Klebsiella* in healthcare settings. Individuals requiring devices such as intravenous (vein) catheters or ventilators (breathing machines) and those on prolonged antibiotic courses are especially susceptible to infections caused by *Klebsiella*. *Klebsiella* infections typically do not affect healthy persons. Pneumonia, bloodstream infections, wound or surgical site infections, and meningitis are among the various infections linked to healthcare that can be brought on by the bacteria *Klebsiella* (CDC, 2016).

## **Pathogenesis**

The symptoms of a *K. pneumoniae* infection differ depending on where the infection is located, and are similar to symptoms of the same diseases caused by other microbes.

For instance, meningitis from *K. pneumoniae* produces the hallmark symptoms of bacterial meningitis, including: Confusion, Neck stiffness, and Sensitivity to bright light.

Bloodstream infections (bacteremia and sepsis) from *Klebsiella* cause:

Fever, Chills, Rash, Light-headedness, and Altered mental states.

Pneumonia from *K. pneumoniae* can result in: Fevers and chills, Flu-like symptoms, Cough, which may produce mucus that's yellow, green, or bloody, Breathing issues (Bennington-Castro, 2016).

## **Source of Contamination:**

Source of contamination: An individual needs to be exposed to *Klebsiella* in order to get the infection. *Klebsiella*, for instance, needs to enter the bloodstream to induce a bloodstream infection or the respiratory (breathing) tract to cause pneumonia. *Klebsiella* bacteria can transmit from person to person in healthcare settings (for instance, from patient to patient through infected hands of healthcare professionals or other people) or, less frequently, by environmental pollution. The air does not carry the microorganisms. Additionally, patients in medical facilities may be exposed to *Klebsiella* if they have intravenous (vein) catheters, wounds after surgery or trauma, or are on ventilators. Unfortunately, *Klebsiella* may be able to enter the body and cause infection due to several medical devices and circumstances (CDC, 2016).

### **2.3.2. *Salmonella Spp.***

*Salmonella* is a gram-negative bacterium that causes diarrhea in humans. It is a member of the Enterobacteriaceae family. Both sick humans and animals harbor the germs in their guts. The rod-shaped bacterium *Salmonella* has a peptidoglycan-based cell wall. This facultative, motile anaerobe is vulnerable to a number of medications. Many of the 107 strains of this organism that have been discovered so far have different metabolic traits, virulence levels, and multi-drug resistance genes that make treatment more difficult in regions where resistance is common. Over 2,500 different bacterial serotypes are members of the *Salmonella* family; these are minute, single-celled organisms (WHO, 2016).

#### **Pathogenesis**

Salmonellosis, often known as *Salmonella* infection, is an intestinal tract bacterial illness. Among other ailments, *Salmonella* is a bacterial group that causes typhoid fever, food poisoning, gastroenteritis, and enteric fever. Common signs of an infection with *Salmonella*: appear six to seventy-two hours after consuming tainted food, and if left untreated, persist for three to seven days. Constipation, enlarged spleen, headache, malaise, anorexia, and generalized systemic enteric fever are followed by increasingly severe stomach symptoms. In 25% of Caucasian patients, the trunk has rose patches. Ileal ulceration of Peyer's patches, which can result in bleeding or perforation, is one of the complications. Without enteric fever, non-typhoidal *Salmonella* can cause common enterocolitis can result in symptoms like headache, nausea, vomiting, diarrhea, and dehydration (Jain *et al.*, 2020).

The symptoms of typhoid fever can persist anywhere from three to sixty days and start to show up eight to fourteen days after consuming tainted food. These include a 104°F temperature,

weakness, lethargy, coughing, nosebleeds, delirium, stomach pain, and enlarged organs. According to Akullian *et al.*, (2015), typhoid fever is a dangerous disease that can be fatal especially in young children as they lack natural immunity against it.

### **Source of Contamination**

*Salmonella* lives in the intestines of birds, animals and humans. Most human infections are caused by eating food or drinking water that has been contaminated by feces (excrement). Foods that are most commonly infected are:

Uncooked meat, egg and poultry - *Salmonella* foodborne outbreaks occur in a variety of foods, according to CDC data, but they are most frequently found in poultry, including chicken, duck, and eggs and these organisms can survive when these products are undercooked (Oh *et al.*, 2023).

Poor hygiene: In general, humans are not as significant as animals as carriers of salmonellosis. Human transmission can happen if an infected food handler's fecal-contaminated hands come into contact with food that is subsequently eaten without being properly cooked, frequently after a period of time during which microbiological growth takes place. By touching them or by touching surfaces that other individuals subsequently contact, an individual with contaminated hands can spread the sickness to others (Ehuwa *et al.*, 2021).

### **2.3.3. *Staphylococcus aureus***

*Staphylococcus aureus* is a Gram Positive cocci (GPC), usually arranged in form of grape like clusters. They are usually found as part of the normal flora of the skin, nasopharynx, oropharynx (mouth and throat) of healthy individuals. *Staphylococcus aureus* is a versatile Gram-positive coccus, a facultative aero-anaerobic bacterium that is both a commensal organism and an

opportunistic pathogen, capable of causing a broad spectrum of human diseases (Touaitia *et al.*, 2025). *Staphylococcus aureus* is an important and versatile pathogen with the ability to colonize individuals and cause superficial and invasive infection. *S. aureus* can survive on environmental surfaces like glass, PVC, stainless steel, aluminum, plastics for prolonged periods of time (at least 1 week) and can be transferred to skin by fomites (Katzenberger *et al.*, 2021). Thus, environmental surfaces are potential reservoirs for *S. aureus* transmission (Eells *et al.*, 2014).

### ***Pathogenesis***

*S. aureus* is a highly adaptable and opportunistic pathogen that can colonize various anatomical sites, evade the host immune system, and cause a broad spectrum of infections. Its pathogenic potential is driven by a complex interplay of virulence factors that facilitate adherence, invasion, immune evasion, and tissue destruction (Touaitia *et al.*, 2025). *S. aureus* are one the most common bacterial infections in humans and are the causative agents of multiple human infections, including bacteremia, infective endocarditis, skin and soft tissue infections (e.g., impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome, and others), osteomyelitis, septic arthritis, prosthetic device infections, pulmonary infections (e.g., pneumonia and empyema), gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections (Taylor and Unakal, 2025).. Despite colonizing the anterior nares, skin, and mucosal surfaces in approximately 30% of healthy individuals, *S. aureus* exploits breaches in host barriers or immune defenses to establish invasive infections, underscoring its adaptability and resilience (Laux *et al.*, 2019). *S. aureus* is a well-known bacterium that develops antibiotic resistance. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of *S. aureus*, which has been noted to acquire resistance to different groups of antibiotics and become multi-drug resistant. The MRSA strains can be divided into two different groups, the ones which cause

hospital infections (healthcare-associated (HA-MRSA)) and those which cause infections in the community (community-associated (CA-MRSA)) (Pal *et al.*, 2020).

### ***Source of Contamination***

*S. aureus* can be transmitted from animal to animal, person to person, as well as from animals to humans and vice-versa. Transmission usually occurs by direct contact, often via the hands, with colonized or infected animals or people and contaminated equipment and surfaces (Pal *et al.*, 2020). *S. aureus* can contaminate food if people who carry the bacteria touch food without first washing their hands. *Staphylococcus* can multiply in contaminated food and make a toxin that causes food poisoning (CDC, 2024). When these individuals with contaminated hands come in contact with these POS machines, they may deposit this bacteria on it, which can be picked up by an uninfected person after direct contact.

### **2.3.4. *Streptococcus spp.***

The genus *Streptococcus* consists of a gram positive spherical or ovoid cells that exists in chains or pairs, that may be found in the environment and may colonize the skin and mucous membranes of humans and animals. They are usually part of the normal flora of the mouth, throat, gut, genitourinary tract depending on their groups. They are also opportunistic and they tend to cause infections in individuals with weakened immune system. Although transmission occurs usually by airborne droplets (produced by coughing or sneezing) through human-to-human interaction, reacquisition from environmental surfaces cannot be excluded as viable group. A *Streptococcus* could be isolated (Menschner *et al.*, 2020). These *Streptococcus* possess biofilm which contribute to its spread by increasing its survival during dessication and contribute to its transmission from fomites (Marks *et al.*, 2013).

## ***Pathogenesis***

The Gram-positive encapsulated bacterium *Streptococcus pneumoniae* is a common inhabitant of the human nasopharynx but can shift from commensal to pathogen when they move to surrounding tissues and even blood causing invasive diseases, including otitis media, sinusitis, pneumonia, bacteremia, and meningitis (Ayed *et al.*, 2024). *Streptococcus pyogenes* is a human pathogen responsible for a wide range of invasive and non-invasive diseases as acute pharyngitis, impetigo, erysipelas, cellulitis, meningitis, pneumonia, puerperal fever, sepsis, streptococcal toxic shock syndrome (STSS) and necrotizing fasciitis, popularly known as the flesh-eating disease (Imöhl *et al.*, 2017), and to immune-mediated diseases (e.g., acute rheumatic fever and rheumatic heart disease) (Chen *et al.*, 2017). *Streptococcus agalactiae* is a major cause of invasive infections in neonates and pregnant women associated with younger maternal age, race, ethnicity and comorbidities (Edwards *et al.*, 2019).

## ***Source of Contamination***

*Streptococcus* species are usually found as part of the normal flora in various parts of the human body. Depending on the specie, it can be transmitted via respiratory droplets, contact with infected persons or contaminated surfaces (Avire *et al.*, 2021), through contaminated food (Guntala *et al.*, 2024). These objects may get contaminated if an infected person releases their respiratory droplets on them or touches them using contaminated hands and the object may serve as a reservoir for the organism. And when an uninfected vulnerable individual comes in contact with these surfaces, they tend to get infected.

### **2.3.5. *Escherichia coli***

*Escherichia coli*, rod-shaped and gram-negative bacteria belonging to the Enterobacteriaceae family, were first isolated from infant stool and characterized by Theodor Escherich in 1885 (Pakbin *et al.*, 2021). *E. coli* is able to grow both aerobically and anaerobically, preferably at 37°C, and can either be nonmotile or motile, with peritrichous flagella. They are usually found as commensals of the gastrointestinal tract of humans without causing disease or infection in the individual. *Escherichia coli* is one of the predominant facultative commensal anaerobes that is generally considered safe under normal health conditions but has also been associated with development of some chronic intestinal conditions when the host gut milieu is altered by some genetic or environmental factors or in immunocompromised hosts (Bhat *et al.*, 2019). In the digestive tract, commensal *E. coli* strains are located in the large intestine, especially in the caecum and the colon. They reside in the mucus layer that covers the epithelial cells throughout the tract until they are sloughed into the lumen of the intestine from whence some cells are eliminated and passed out through host faeces (Conway and Cohen, 2015). Enterobacteriaceae such as the *E. coli* can contaminate surfaces and can survive for prolonged periods of time on dry surfaces (Shimoda *et al.*, 2019).

#### ***Pathogenesis***

*E. coli* is known as a harmless commensal of the gastrointestinal tract in warm-blooded animals. However, there is an alternate side to *E. coli* afforded through gene gain and loss that enable it to become a highly diverse and adapted pathogen. Pathogenic *E. coli* can cause a broad range of human diseases that span from the gastrointestinal tract to extraintestinal sites such as the urinary tract, bloodstream, and central nervous system (Croxen and Finlay, 2010). Certain *E. coli*

mediate various diseases, including intestinal and extraintestinal disorders in humans and animals worldwide. Nine pathovars have been described for *E. coli* strains isolated from humans causing diarrheagenic and extraintestinal diseases and of these, seven pathotypes have been described as enteric pathogenic *E. coli*, including Enteropathogenic *E. coli* (EPEC), Enterohaemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Diffusely adherent *E. coli* (DAEC), and, a new pathotype, Adherent-Invasive *E. coli* (AIEC), causing mostly diarrhea and intestinal disorders. However, the EHEC pathotype has been implicated in extraintestinal diseases such as Hemolytic Uremic Syndrome (HUS) (Pabkin *et al.*, 2021).

### ***Source of Contamination***

*E. coli* is an enteric pathogen and its contamination is usually through the fecal oral route and it is associated with poor hygiene. When an individual with contaminated hands touches this surface, the bacterium is deposited there. A number of studies have shown that enteric and respiratory pathogens are capable of surviving from hours to months on fomites, depending on the numbers deposited, the type of microorganism, and the variable environmental conditions (Katzenberger *et al.*, 2021). Due to their ability to survive for a period of time on these surfaces, when a susceptible individual comes in contact with these surfaces, they tend to pick up these pathogens and when they are introduced into the body, they become infected.

### **2.3.6. *Candida Spp***

*Candida* is a genus of yeasts that belongs to the phylum Ascomycota. These organisms are typically unicellular and reproduce by budding. *Candida albicans*, are members of normal human microbiota, commonly residing in the oral cavity, gastrointestinal tract, and urinary and

vaginal tracts of healthy people. *C. albicans* has extensively coevolved with humans and cohabiting microbes. Despite this, it is a major human pathogen, causing life-threatening infections, especially in immunocompromised individuals under certain conditions (Chow *et al.*, 2024). *Candida albicans* is a diploid polymorphic fungus commonly present in several human surfaces such as skin, throat, or vagina mucosa (Parambath *et al.*, 2024). Members of these species include the most frequent cause of opportunistic infections, *Candida albicans*, the drug-resistant *Candida glabrata*, the new global public health threat *Candida auris*, and other emerging species such as *Candida tropicalis*, *Candida parapsilosis*, and *Candida krusei* (Lamoth *et al.*, 2018). They exist as commensals, *C. albicans* colonizes the oral, gastrointestinal, and genital tracts asymptotically. *C. albicans* is carried without symptoms by a large fraction of the population. Indeed, *C. albicans* colonizes mucosal surfaces of healthy subjects and is considered to be a component of the normal digestive and genital floras. *C. albicans* is the leading cause of nosocomial fungal infections (Macias-Paz *et al.*, 2022).

### **Pathogenesis:**

*Candida albicans* is currently the most important opportunistic fungal pathogen of humans, responsible for both superficial and systemic infections. Clinical manifestations of *C. albicans* infections include superficial candidiasis infections (cutaneous candidiasis, oropharyngeal candidiasis, and vulvovaginitis) that are frequent but usually benign in immunocompetent hosts. The manifestation of infection is frequently mild, and this is especially the case when topical infection occurs. However, in individuals who are severely immunocompromised, systemic infections can develop, and whilst these are relatively uncommon, they are associated with high patient mortality (Henriques and Williams, 2020). Invasive candidiasis is an important contributor to neonatal morbidity and mortality. Although this fungal organism colonizes human

skin and mucosa and may cause only limited mucocutaneous disease in immunocompetent hosts, immunocompromised hosts, such as premature infants, transplant patients, chemotherapy patients with invasive candidiasis can suffer from severe and life-threatening illness (Daniel *et al.*, 2023).

1. Candida can cause various types of infections, collectively known as candidiasis. The most common pathogenic species is *Candida albicans*, although others like *C. glabrata* and *C. tropicalis* are also clinically relevant (Kabir *et al.*, 2012).
2. Typical Symptoms of Candida infection:

**Oral thrush:** white patches on the tongue and oral mucosa

**Vaginal yeast infection:** itching, burning, abnormal discharge, Invasive candidiasis: fever, chills, organ-specific symptoms.

Complications can include systemic infection in immunocompromised individuals, leading to multi-organ failure (Pappas *et al.*, 2016).

### ***Source of Contamination***

*Candida* is often part of the normal human microbiota and would cause infections under certain factors. Factors that can lead to overgrowth include: Antibiotic use, Immunosuppression, Diabetes, Indwelling medical devices (e.g., catheters).

## 2.4 ANTIMICROBIAL RESISTANCE AND ANTIBIOGRAM PATTERNS OF ORGANISMS ISOLATED FROM HIGH TOUCH SURFACES

As early as 1972, Spaulding proposed a classification of inanimate surfaces into three general categories based on the risk of infection if the surfaces were contaminated at the time of use. These categories can be applied to devices or instruments as follows: critical (exposed to normally sterile areas of the body; require sterilization), semi-critical (touch mucous membranes; may be sterilized or disinfected), and non-critical (touch skin or come into contact with people only indirectly; can be either cleaned and then disinfected with an intermediate-level disinfectant, sanitized with a low-level disinfectant or, simply, cleaned with water and soap) (Cobrado *et al.*, 2017). Over the past decade, substantial scientific evidence has accumulated that contamination of environmental surfaces in hospital rooms plays an important role in the transmission of several key healthcare-associated pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), *vancomycin-resistant Enterococcus spp.* (VRE), *Clostridium difficile*, *Acinetobacter spp.*, and *norovirus* (Otter *et al.*, 2013). Some of these organisms may show resistance to certain drugs and they are referred to as Multidrug Resistant bacteria (MDR). Multidrug-resistant bacteria are bacterial species resistant to at least one antibiotic from a different spectrum of antibiotics (Nwankwo *et al.*, 2022).

In a study by Innocent-Adiele *et al.*, (2023), *Staphylococcus* species was observed to be resistant to all discs but APX. *Micrococcus* species was susceptible only to pefloxacin, intermediate to sceptrin and Erythromycin but resistant to all other antibiotic discs. *Bacillus species* were susceptible to ciprofloxacin, pefloxacin, Rocephin, streptomycin, Sceptrin, and intermediate

Erythromycin and resistant to other antibiotics, and *Bacillus species* were susceptible to only pefloxacin and Rocephin but intermediate for ciprofloxacin, sceptrin, streptomycin, and Erythromycin. For the gram-negative isolates, *Enterobacter species* were resistant only to amoxicillin, susceptible to ciprofloxacin, augmenting, gentamicin, pefloxacin, and ciprofloxacin; and intermediate for Tanvid, sceptrin, chloramphenicol, and streptomycin. *Aerobacter spp.* was resistant to the following; SXT = Septrin, CH = Chloramphenicol, CPX = Ciprofloxacin, SP = Sporfloxacin intermidiate to AM = Amoxacillin and susceptible to AU = Augmentin, CN = Gentamicin, PEF = Pefloxacin, OFX = Tarivid, S = Streptomycin. The last isolate which turned out to be *Enterobacteria* well was observed to be susceptible only to Tanvid, intermediate only to chloramphenicol, and resistant to the other discs.

In another study by Francisca Upekiema Adie *et al.*, (2021), the *Escherichia coli* isolates were resistant to Nalidixic acid but susceptible to other antibiotics they were tested against. The *Klebsiella spp.* isolates were only resistant to Streptomycin but susceptible to other antibiotics. The *Pseudomonas aeruginosa* were resistant to Rifampicin, Gentamycin, Streptomycin and Nalidixic acid but were susceptible to Ciprofloxacin, Augmentin, Ampicillin, etc., meanwhile the *Salmonella spp.* and *Shigella spp.* did not show resistance to any of the test antibiotics. The antibiotics susceptibility profile of the Gram-positive bacteria; the *Staphylococcus spp.* isolates were resistant to chloramphenicol but susceptible to the remaining antibiotics. Meanwhile, the *Bacillus spp.* isolates were susceptible to Ciprofloxacin, Streptomycin, Erythromycin, Chloramphenicol and Levofloxacin.

In another study by Augustine Brian Odigie *et al.*, (2018), *Escherichia coli* and *P. aeruginosa* were found to demonstrate remarkable resistance to Sulfamethoxazole- Trimethoprim, Cephalexin, Ampicilin, and Nalidixic acid. Most isolates of *S. pneumoniae* demonstrated greater than 50% resistance to Gentamicin, Ampicillin and Nalidixic acid. Only Sulfamethoxazole-Trimethoprim and Nalidixic acid were found not to significantly inhibit the growth of *S. aureus*. *Bacillus species* were generally the most susceptible isolates to all the antibiotics. Findings from the pre-plasmid curing antibiotic analysis suggest that both *E. coli* and *P. aeruginosa* were resistant to majority of the antibiotics, followed by *S. pneumoniae*, *S. aureus* and *B. subtilis*. The multi-drug resistance observed in most isolates of *E. coli* and *P. aeruginosa* is in line with the earlier studies that reported them as among the major Gram-negative multi-drug resistant isolates from hospital and environmental samples (Wang *et al.*, 2017).

## **2.5 PUBLIC HEALTH IMPLICATIONS OF CONTAMINATED POS MACHINES**

Point-of-Sale (POS) machines have been earlier established to be an object that can harbor certain microbes which may include bacteria, fungi. Due to frequent contact with this machine by various individuals (both healthy and infected), microbes tend to be deposited on this machine, and when a susceptible or immunocompromised individual comes in contact with this contaminated machine, they get infected by these pathogens or microbes.

Contamination of environmental objects and surfaces by microorganisms is a common phenomenon. The presence of viable pathogenic bacteria on inanimate objects has been reported by earlier researchers. Several studies of the human environment have demonstrated contamination and colonization of inanimate objects such as door handles, plastics, faucets,

phones, money, fabrics, plastics and other fomites by bacteria and, which is also responsible for the spread of various bacterial infections (Anibijuwon *et al.*, 2014). The Point-of-sale (POS) machine is a machine that performs some human teller functions like balance enquiry, bills payment, mini statements and withdrawal and transfer of cash which is its major function. POS transactions are carried out through the use of debit/credit cards which enable the card holders to access and carry out banking transactions without a teller. Different people from different socio-economic levels and hygienic status use the POS daily and this increases the chances of hand-borne transmission of microorganisms to the machine surfaces (Yuana *et al.*, 2022). This POS is not usually cleaned after every use or between use and so they can serve as fomites and aid in transmission of diseases.

### **2.5.1 Common Pathogens Isolated from POS Machines**

Several studies have shown that various pathogens have been isolated from POS machines over the years. These microbes includes bacteria and fungi.

#### **Bacteria:**

A study by John *et al.*, (2021) shows that the bacteria associated with the assessed fomites (POS machines) included *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and species of *Bacillus*, *Streptococcus*, *Salmonella*, *Shigella*, *Micrococcus*, *Proteus*, *Pseudomonas*, *Vibrio* and *Klebsiella*.

In another study by Yusha'u *et al.*, (2024), the Bacterial species isolated include *Staphylococcus aureus* (26.56%), *Escherichia coli* (18.75%), *Bacillus subtilis* (17.19%), *Streptococcus spp.* (15.63%), *Klebsiella spp.* (14.07%), *Salmonella spp.* (4.69%), and *Pseudomonas aeruginosa*. (3.13%).

In another study by Shayehgi *et al.*, (2020), Also a total of 12 different bacterial isolates were obtained from ATMs and POS devices, consist of: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus spp.*, *Enterobacter spp.*, *Escherichia coli*, *Klebsiella pneumonia*, *Shigella dysenteriae*, *Salmonella spp.*, *Pseudomonas spp.*, *Bacillus spp.*, *Lactobacillus spp.* and *Enterococcus spp.*.

### **Fungi:**

A study by Ejeagba and Iheke, (2015) shows that fungal isolates of the samples analyzed includes microbes such as *Rhizopus spp.* with about 47.5% (19), *Aspergillus spp.*, 22.5% (9) and *Penicillin spp.*, 20% (8) were found on the keypads.

Another study by Agu *et al.*, (2018), showed that certain fungi were isolated from keypads and these fungi includes *Rhizopus spp.*, *Aspergillus spp.*, *Penicillium spp.*

Another study by Okolosi-Patani *et al.*, (2023) showed that fungi like *Candida spp.*, *Penicillin notatum.*, *Aspergillus fumigatus.*, *Aspergillus niger.*, *Mucor spp.* were isolated from keypads.

## **2.5.2 Transmission Mechanisms**

These POS machines can aid in the transmission of microbes that may cause diseases through various means. The mechanisms of transmission includes:

- **Direct Transmission :**

This mechanism of disease transmission occurs when individuals pick up microbes from the POS machines as a result of direct contact with different surfaces of the machine (keypads and screens). Human beings have a marked tendency to pick up microorganisms from environmental objects and the hands play a vital role in contact and transmission of organisms. Studies have

implicated environmental surfaces in the transmission of microorganisms with the hands acting as chief organ for physical manipulation of the environment. Human hands usually harbour microbes as part of the body normal flora as well as transient microorganisms contracted from the environment (Ayalew *et al.*, 2015).

- **Indirect Transmission**

This mechanism of transmission occurs when an individual comes in contact with a contaminated POS machine, and then pick up this microbes and through subconscious hand to face contact, these microbes can be introduced into the eyes, nose, mouth which can then result in infections or diseases. This form of transmission can be referred to as self-innoculation. Self-inoculation is a type of contact transmission where a person's contaminated hands makes subsequent contact with other body sites on oneself and introduces contaminated material to those sites (Kwok *et al.*, 2015).

- **Cross Contamination**

This mechanism of transmission occurs when an individual touches or comes in contact with a contaminated POS machine, the individual picks up these microbes and these microbes adhere to the hands of the individual. When these contaminated hands (unwashed or not properly washed hands) handles other objects like fruits, money, vegetables and others, the microbes are now deposited on those materials and this is referred to as cross-contamination. Cross-contamination occurs between raw products and food contact surfaces, food handlers, and ready-to-eat (RTE) foods (Kirchner *et al.*, 2021).

### 2.5.3 Diseases Associated with Contamination:

These devices are usually contaminated and microbes tend to inhabit them. When these microbes are then introduced into the body, they can result in certain diseases which may include:

- Diarrheal diseases:

These Point of Sale (POS) machines especially those used in market places can become contaminated with enteric pathogens that may cause these kind of diseases. This is usually as a result of poor hygiene through contamination with fecal pathogens following the use of toilets, handling of raw food. When hands after being contaminated with these pathogens are now used to touch the mouth, the pathogens can then be ingested into the body and it can then cause diarrheal diseases. Studies have shown that enteric pathogens like *E.coli*, *salmonella spp.*, *shigella spp.* that causes diarrheal diseases have been isolated from ATM and POS machines (Adie *et al.*, 2021). Egberongbe *et al.*, (2021), isolated enteric pathogens like *E.coli* (a leading cause of diarrheal diseases), *salmonella spp.*, *shigella spp.* and *enterobacter spp.* (occasional cause of diarrheal diseases) from keypads and screens of ATM and POS machines.

- Skin and soft tissue infections:

These skin infections are usually caused by the Gram positive cocci. *Staphylococcus aureus* which may be isolated from these machines due to hand contamination. *Staphylococcus aureus* is a worldwide recognized pathogenic organism which has a vital role it plays in causing diseases and its transfer within and out of the community. Transmission of *Staphylococcus aureus* is rather easy, it can be transmitted via various sources majorly fomites, human hands. One of the major sources and spread of community *Staphylococcus aureus* infections are fomites (Omololu-Aso *et al.*, 2022). *S. aureus* is part of the normal microbial flora of the skin and mucosa and when someone with a minor injury comes in contact with these contaminated machines, the

bacteria colonizes it and can cause infections like boils, cellulitis. *S. aureus* is a common cause of a wide range of human infections including, endocarditis, osteomyelitis, meningitis, bacteraemia, pneumonia, skin and soft tissue infections (Amaeze *et al.*, 2024).

- Respiratory Tract Infections

Studies have shown over time that these machines can be contaminated with certain bacteria like the *Klebsiella spp*, *Streptococcus pneumoniae* that are capable of causing respiratory infections.

- Opportunistic Infections

Opportunistic Infections (OIs) are defined as infections occurring due to bacteria, fungi, viruses, or parasites that normally do not cause a disease, but become pathogenic when the body's defense system is impaired (Riccardi *et al.*, 2019). Opportunistic pathogens belong to bacterial genera or species that cause mild or no illness in immunocompetent persons but can cause life-threatening infections in vulnerable subjects (Rossi *et al.*, 2023). Opportunistic pathogens have been isolated from these surfaces over time and so when individuals with weakened immunity comes in contact with these contaminated surfaces, they become infected.

#### **2.5.4 Populations at Risk**

Individuals in certain populations have varying susceptibility to certain infections. Some are more susceptible than others. The populations that are most likely to develop diseases from coming in contact with contaminated POS machines may include:

- Immunocompromised Individuals:

Immunocompromised (IC) individuals represent a heterogeneous group of pediatric and adult patients at increased risk of morbidity and mortality for infectious diseases compared to the general population, due to altered susceptibility to infectious agents and to impaired capacity to

fight them (Agrati *et al.*, 2023). These individuals are usually more prone to certain diseases or infections when compared to the general population and this is because their immune system is already weakened probably due to underlying disease conditions like diabetes, cancer, HIV/AIDS and so it is not strong enough to withstand pathogens that would not normally affect them. Susceptible populations, such as those with compromised immune systems including infants, elderly, transplant patients, ICU patients, and HIV-positive individuals are at increased risk of developing infections from microorganisms that persist in built environments (Stephens *et al.*, 2019).

- Children

Children have been found to be more at risk to develop these diseases from contaminated surfaces because of their careless and poor hygienic practices and their weak immune system. During exploratory play behaviour, a normal part of child development, young children often put their toys and other objects and substances including surfaces into their mouths (Parvin *et al.*, 2020). We define ‘child mouthing’ behaviours as children putting surfaces, objects and substances in their mouth during exploratory play behaviour. These hand-to-mouth and object-to-mouth behaviours, while indicative of normal childhood development, may result in the ingestion of faecal pathogens for susceptible paediatric populations. In a rural Tanzania study that modeled the hand mouthing and drinking water pathways, 99.7% of children’s *E. coli* exposure was due to contacts between children’s hands and mouths while only 0.3% of *E. coli* exposure was from drinking water (Kwong *et al.*, 2020). Due to this behaviour, they can touch these contaminated machines and then put their hands into their mouths. Some may even go as far as mouthing these machines directly. Infants and young children are more susceptible to many infections and rely primarily on innate immunity, as their adaptive immune system remains

naive due to limited pathogen exposure (Sakleshpur and Steed, 2022). Their immune system is still building up and may not be strong enough to fight and overcome some of these pathogens, hence putting them at a higher risk.

- Pregnant Women

Pregnancy have been previously associated with increased susceptibility to infection. Immunologic alterations during pregnancy may help explain the altered severity of and susceptibility to infectious diseases during pregnancy. As pregnancy progresses, hormone levels change dramatically and are considerably higher than at any other time (Robinson and Klein, 2012). The interplay between sex hormones and the immune system is complex and multi-factorial, and it affects many organ systems. Even though pregnant women do not seem to be more susceptible than non-pregnant women to initial infection in general, immunologic alterations with advancing pregnancy may impair pathogen clearance, resulting in an increased severity of disease caused by some pathogens. Increased disease severity may also be due to other physiological changes of pregnancy (e.g., decreased lung capacity, urinary stasis, and changes in blood flow) (Kourtis *et al.*, 2014). This immunologic alterations then tend to place them at a higher risk of acquiring these diseases.

## **2.6 HYGIENE PRACTICES AND MITIGATION STRATEGIES**

The spread of these infections and diseases via various contact surfaces like that of the POS machines can be mitigated and prevented through proper hygiene practices and sufficient awareness to the public about the negative impact and effect of acquiring these infections. One of the primary mitigation strategies against the spread of infections via surface contact is through proper hand hygiene.

- **Hand hygiene:** The hands have been implicated in the transmission of diseases because human beings have a marked tendency to pick up microorganisms from environmental objects and the hands play a vital role in contact and transmission of organisms (Ayalew *et al.*, 2015). The role of the hands in transmitting diseases can be mitigated via regular hand washing, as this tends to eliminate some of the microbes present there. Previous laboratory studies found that the six-step hand hygiene procedure should take 20–30 s if using alcohol-based hand rub (ABHR), or 40–60s if washing hands with soap and water, in order to ensure effective removal of transient microorganisms acquired from direct contact with patients, contaminated surfaces, or the environment (Shi *et al.*, 2023). According to the Centers for Disease Control and Prevention (CDC), hand hygiene is the single most important practice in the reduction of the transmission of infection in the healthcare setting (Toney-Butler *et al.*, 2023). Due to the risk of disease transmission owing to poor hand hygiene and using those contaminated hands to touch food meant to be ingested, touch our faces or mucous membranes, it is essential and necessary that these POS users (both owners and customers) wash their hand regularly after each use. Personal hygiene and good hand washing technique have been found to be an effective method of preventing the transmission of pathogens through fomites such as the user interface of Point-of-Sale machines (Agu *et al.*, 2018).
- **Regular Disinfection of POS Surfaces:** This is another essential mitigation strategy against the spread and transmission of diseases via POS machines. Disinfection eliminates most microbes, excluding bacterial spores, and typically involves the use of chemical agents. The degree of destruction of organisms depends on their sensitivity to

chemical disinfection. High-level disinfection involves the elimination of all but large quantities of spores, intermediate-level disinfection leads to destruction of all life except spores, and low-level disinfection will not reliably kill mycobacteria or spores. “Cleaning” is the process of removal of foreign material from a surface or object and may involve both mechanical processes and the use of detergents with water. Cleaning alone removes most harmful viruses or bacteria from surfaces. Surfaces should be cleaned before they are sanitized or disinfected because impurities like dirt may make it harder for chemicals to get to and kill germs (CDC, 2025). Three types of available solutions can be used during cleaning: detergents, which remove organic material and suspend grease or oil; disinfectants, which rapidly kill or inactivate infectious particles; and detergent-disinfectants, which achieve both aims. To achieve good hygiene, it was necessary to wipe the surface clean using a cloth soaked in detergent before applying the combined hypochlorite/detergent. When detergent cleaning alone or combined hypochlorite/detergent treatment failed to eliminate contamination from the surface and the cleaning cloth was then used to wipe another surface, the pathogen was transferred to that surface and to the hands of the person handling the cloth (Gibson *et al.*, 2012). These surfaces can also be wiped using alcohol based disinfectants to eliminate these pathogens. Disinfection of these POS machines should be done multiple times a day so as to prevent microbial accumulation on the machines.

## **CHAPTER 3**

### **MATERIALS AND METHODS**

#### **3.0 MATERIALS AND METHODS**

##### **3.1 STUDY AREA**

The study focused on Five (5) main markets in Three (3) local government areas of Benin city, Edo state. The markets included: Oba market, New Benin market, Uselu market, Oliha market, Ikpoba-hill market all located within Oredo, Ikpoba-okha, and Egor local government area, Benin city, Edo state.

##### **3.2 ETHICS**

Ethical approval was sort from The Research Ethics Committee of the College of Medical Sciences, University of Benin and an official approval was provided with Reference No. CMS/REC/2024/795. All of the experiment's procedures was conducted in accordance with the The National Health Research Ethics Committee (NHREC).

##### **3.3 SAMPLE COLLECTION**

Thirty (30) samples of the Point-of-sale (POS) machines was collected from each market using a sterile swab stick pre-moistened with sterile normal saline to ensure adequate collection of microbial flora to rub the keypads and screens of the machines. The swab stick was placed into its container and labeled with; sample number, sample location and sampling dates and transported to the Medical microbiology laboratory in the University of Benin teaching Hospital, Benin city for examination within 2 hours of collection.

### **3.4 ANALYSIS OF SAMPLES**

#### **3.4.1 Bacterial and Mycological Analysis**

##### **1. Processing of Swab Samples for Bacteria and Fungi;**

Swab sticks were placed in test tubes containing ringers solution to prepare serial dilutions.

##### **2. Determination of Total Viable Count (Miles and Misra Method);**

Serial dilutions were prepared. The colonies were counted in colony-forming units per milliliter (cfu/ml) using a colony counter after overnight incubation at 37°C.

##### **3. Culturing Technique;**

The media used in this study were MacConkey agar (selective and differential), Mueller Hilton Agar (MHA), nutrient agar (a non-selective medium) and Saboraud dextrose agar (SDA) for fungi. These were dispensed into sterile petri dishes, allowed to cool, and solidify. The melted, sterile agars were then poured into the appropriate petri dishes and allowed to solidify further. The petri dishes were inverted and placed in the incubator. The cultured media were incubated at 37°C for 24-48 hours and 72 hours for bacteria and fungi respectively. After incubation, the petri dishes were examined for initial identification based on colony characteristics. To identify the bacteria isolated from the Point-of-Sale machines, various morphological and biochemical tests were performed on the pure cultures of the bacteria while fungi isolates were identified using microscopic and macroscopic characteristics (colonial heads, phialides, conidiophores and presence or absence of rhizoides and hyphae).

### **3.5 BACTERIAL AND FUNGI ISOLATES MORPHOLOGY**

Emergent colonies were identified using standard techniques. All bacteria isolate in this study were identified by their colonial morphology in media (size, colour, opacity, shape and edge) while fungi isolates were identified using microscopic and macroscopic characteristics such as colonial heads, phialides, conidiophores and presence or absence of rhizoides and hyphae.

#### **3.5.1 Identification by Colonial Morphology**

The isolated microorganisms displayed various colony types on the agar surface, identified based on morphological features such as:

Texture: Colony surface might appear smooth, glistening, mucoid, slimy, dry, powdery, or flaky.

Transparency: Colonies may be transparent, translucent, or opaque.

Shape: Colony shapes may vary, including circular, dot-like, or irregular forms.

Elevation: Elevation might include thin film, raised, or convex.

Color or Pigmentation: Some bacteria produce intracellular pigments, resulting in distinct colony colors like yellow, pink, purple, or red. Others lack pigmentation and may appear white or gray.

#### **3.5.2 Motility Test**

Motility refers to an organism's ability to move autonomously, either using propeller-like motions or through gliding. Motile bacteria use thread-like structures called flagella, which extend from the cell's plasma membrane and wall, either as a single structure or multiple flagella.

Hanging drop method;

A drop of normal saline was placed at the center of a cover slip. A small sample of the organism from the culture medium was mixed with the saline. A glass slide with a bit of plasticine was placed over the cover slip, which was then inverted, suspending the bacterial culture. The motility of the organisms was observed under a microscope, initially at 10x magnification and then at 40x.

### **3.5.3 Microscopy of Fungal Isolates**

Microscopy played very important role in diagnosis of fungal infections. Using NEEDLE MOUNT FROM CULTURE PLATE, A drop of lactophenol cotton blue was placed on a clean, grease-free glass slides marked with a code for each isolate was used. With a needle or wire bent at a 90 degree angle, cut out a small portion of an isolated colony, removing along with it a small amount of the supporting agar. Place the portion of isolate onto a glass slides containing at drop of lactophenol cotton blue. Place a coverslip into position and apply gentle pressure by tilting with a pencil eraser to disperse the growth and the agar. Observe microscopically as above for the characteristic shape and arrangement of the spores. Use of mycology atlas to aide in identification is advocated. Note down colonial characteristics and microscopy of colony.

### **3.5.4 Gram Staining**

The Gram staining technique is a widely used method in microbiology to identify and classify bacteria based on their cell wall composition. The technique was developed by Hans Christian Gram in 1884.

Gram Staining Technique:

The Gram staining technique involves the following steps:

1. Preparation of the sample: A bacterial sample is prepared by growing the bacteria on a suitable medium, such as agar or broth.
2. Fixation: The bacterial sample is fixed onto a slide by gently passing through a flame.
3. Staining: The slide is then stained with a series of dyes, including:
  - Crystal violet: A purple dye that stains all bacteria.
  - Iodine: A brown dye that helps to fix the crystal violet stain. This component acts as a mordant.
  - Acetone: A decolorizer that removes the stain from Gram-negative bacteria. And this is used briefly.
  - Neutral red: A dye that is used as a counterstain. Neutral red was used.
4. Rinsing: The slide is rinsed with water to remove excess stain.
5. Observation: The slide is then observed under a microscope to determine the Gram reaction of the bacteria using x100 oil immersion objective.

### **3.6 BIOCHEMICAL CHARACTERIZATION**

#### **3.6.1 Biochemical Tests**

##### **1. Urease Test**

Some bacteria produce the enzyme urease, which hydrolyzes urea into ammonia (NH<sub>3</sub>) and carbon dioxide (CO<sub>2</sub>), forming ammonium carbonate in the presence of water.

## Method

The urease test detects the enzyme urease, which hydrolyzes urea into ammonia and carbon dioxide. The medium contains urea and the pH indicator phenol red. If urease is produced, the released ammonia raises the pH, making the medium alkaline. This causes the color of the medium to change from yellow/orange to pink.

## 2. Citrate Test

This test determines if the bacterium can utilize citrate as a carbon source.

Method: A tube with citrate medium is inoculated with a small bacterial sample, either by streaking or deep inoculation in a Simmons citrate tube. It's incubated at 30–37°C for 24–48 hours.

Results: A positive result is indicated by growth in the citrate medium or a color change to blue in the Simmons citrate tube. A negative result shows no growth or no color change, remaining green in the tube.

## 3. Indole Test

Indole is produced via reductive deamination of tryptophan by the bacterial enzyme tryptophanase forming indole, pyruvic acid and ammonia as an intermediate. The bacteria are cultured in sterile tryptophan or peptone broth for 24–48 hours before the test. After incubation, two drops of Kovac's reagent (isoamyl alcohol, paradimethylaminobenzaldehyde, and concentrated hydrochloric acid) are added.

Method: A bacterial colony from a pure culture is suspended in tryptophan medium and incubated at 37°C for 20–28 hours. After incubation, a few drops of Kovac's reagent are added.

Results: A positive test shows a reddish-pink ring at the top of the medium while a negative test leaves the reagent pale yellow.

#### 4. Oxidase Test

A filter paper disc impregnated with oxidase reagent was placed on a clean slide. A small amount of the bacterial colony was applied to the disc. A positive oxidase test was indicated by the development of a purple color within 10 seconds.

#### 5. Catalase Test

A colony of the bacteria was placed on a clean slide. A drop of hydrogen peroxide was then added. A positive catalase test was indicated by the immediate formation of active bubbles.

#### 5. Coagulase Test

This test is used to distinguish *Staphylococcus aureus* from other coagulase negative *staphylococcus*. *S.aureus* produces the enzyme coagulase which converts fibrinogen in plasma into fibrin thereby resulting in a clumping reaction.

Method: A drop of normal saline is placed on a clean grease free glass slide, and a small amount of test colony is emulsified on the saline. A drop of plasma is then placed on the suspension and the slide is rocked. A positive test is indicated by an immediate clumping reaction and when test is negative, no clumping is seen.

### **3.7 DATA ANALYSIS**

The data obtained were analyzed with Chi square using the statistical software INSTAT (Graph and software inc, LA Jolla, CA, USA).

## CHAPTER FOUR

### RESULTS

#### 4.1 PREVALENCE OF BACTERIAL AND FUNGAL CONTAMINATION ON POS MACHINES IN SELECTED MARKETS IN BENIN CITY

A total of 150 samples collected from selected markets in Benin City were examined for bacterial and fungal contamination. The analysis revealed the presence of a wide range of microorganisms, with both bacterial and fungal isolates varying in prevalence across markets. While some samples showed no growth, the majority yielded one or more isolates, including *Klebsiella spp.*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterobacter spp.*, *Pseudomonas spp.*, *Escherichia coli*, and several fungal species such as *Candida spp.*, *Aspergillus spp.*, *Rhizopus spp.*, and *Penicillium spp.*.

Out of 150 samples examined, six groups of bacteria were isolated. Table 1 showed that a total of 24.7% of the samples showed no bacterial growth, while the remaining samples yielded one or more bacterial contaminants. Among the isolates, *Klebsiella spp.* emerged as the most frequently encountered species, making up 17.3% of the total. *Staphylococcus aureus* was the next most common at 15.3%, followed closely by *Staphylococcus epidermidis* at 12.7%. *Enterobacter spp.* accounted for 11.3% of the isolates, while *Pseudomonas spp.* made up 10.0%. *Escherichia coli* was the least common, occurring in 8.7% of samples.

Analysis of fungal contamination presented in Table 2. revealed that 42.7% of the 150 samples showed no fungal growth. Among the positive cultures, *Candida spp.* dominated, accounting for 38.7% of isolates. Filamentous fungi were less frequent: *Aspergillus spp.* occurred in 10.0% of samples, *Rhizopus spp.* in 4.7%, and *Penicillium spp.* in 4.0%.

Table 4.1: Bacteria Isolated in Point-of-Sale (POS) Machines from Markets in Benin City

| Bacterial                         | Frequency | Percentage (%) |
|-----------------------------------|-----------|----------------|
| No Growth (NG)                    | 37        | 24.7           |
| <i>Klebsiella</i>                 | 26        | 17.3           |
| <i>Enterobacter</i>               | 17        | 11.3           |
| <i>Staphylococcus aureus</i>      | 23        | 15.3           |
| <i>Staphylococcus epidermidis</i> | 19        | 12.7           |
| <i>Pseudomonas spp.</i>           | 15        | 10             |
| <i>Escherichia coli</i>           | 13        | 8.7            |
| Total                             | 150       | 100            |

Table 4.2: Fungi Isolated in POS Machines from Markets in Benin City

| Fungal                  | Frequency | Percentage (%) |
|-------------------------|-----------|----------------|
| No Growth (NG)          | 64        | 42.7           |
| <i>Candida spp.</i>     | 58        | 38.7           |
| <i>Aspergillus spp.</i> | 15        | 10             |
| <i>Rhizopus spp.</i>    | 7         | 4.7            |
| <i>Penicillium spp.</i> | 6         | 4              |
| Total                   | 150       | 100            |

#### 4.2. Distribution of Bacterial and Fungal by Market Location

The distribution of bacterial isolates across markets showed notable differences. Table 3. showed that at Oba market, *Enterobacter spp.* accounted for the highest proportion (23.3%), followed by *Klebsiella spp.* (20.0%) and *Pseudomonas spp.* (16.7%). At Uselu market, *Klebsiella spp.*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* were evenly represented at 16.7% each, while *Enterobacter spp.* and *Escherichia coli* were the least frequent. In New Benin market, *Klebsiella spp.* and *Staphylococcus aureus* shared the highest proportion (20.0% each), while *E. coli* and *Pseudomonas spp.* were less represented. Ikpoba hill market recorded the highest proportion of *Staphylococcus epidermidis* (20.0%) and a relatively higher presence of *Escherichia coli* (16.7%) compared to other markets. Oliha market showed *Staphylococcus aureus* as the most prevalent organism (20.0%), with other isolates ranging between 10.0% and 13.3%.

Table 4. revealed that fungal distribution across markets also varied. At Oba market, both no growth and *Candida spp.* were recorded in equal proportions (46.7%), while *Aspergillus spp.* and *Rhizopus spp.* were present at 3.3% each, and *Penicillium spp.* was absent. At Uselu market, *Candida spp.* was prevalent (46.7%), with smaller proportions of *Aspergillus spp.* (10.0%), *Rhizopus spp.* (3.3%), and *Penicillium spp.* (6.7%). In New Benin market, *Candida spp.* made up 36.7%, followed by *Aspergillus spp.* (13.3%) and *Rhizopus spp.* (6.7%). Ikpoba-hill market recorded the highest no-growth proportion (50.0%), with *Candida spp.* (33.3%) and others occurring at low frequencies. In Oliha market, 43.3% of samples showed no growth, while *Candida spp.* was 30.0% and *Aspergillus spp.* 13.3%.

Table 4.3: Distribution of Bacterial Isolates by Market Location

| Market Location | No Growth (NG) | <i>Klebsiella spp.</i> | <i>Enterobacter spp.</i> | <i>Staphylococcus aureus</i> | <i>Staphylococcus epidermidis</i> | <i>Pseudomonas spp.</i> | <i>Escherichia coli</i> |
|-----------------|----------------|------------------------|--------------------------|------------------------------|-----------------------------------|-------------------------|-------------------------|
| Oba Market      | 6 (20.0%)      | 6 (20.0%)              | 7 (23.3%)                | 2 (6.7%)                     | 3 (10.0%)                         | 5 (16.7%)               | 1 (3.3%)                |
| Uselu           | 9 (30.0%)      | 5 (16.7%)              | 2 (6.7%)                 | 5 (16.7%)                    | 5 (16.7%)                         | 3 (10.0%)               | 1 (3.3%)                |
| New Benin       | 9 (30.0%)      | 6 (20.0%)              | 3 (10.0%)                | 6 (20.0%)                    | 2 (6.7%)                          | 1 (3.3%)                | 3 (10.0%)               |
| Ikpoba-hill     | 5 (16.7%)      | 5 (16.7%)              | 2 (6.7%)                 | 4 (13.3%)                    | 6 (20.0%)                         | 3 (10.0%)               | 5 (16.7%)               |
| Oliha           | 8 (26.7%)      | 4 (13.3%)              | 3 (10.0%)                | 6 (20.0%)                    | 3 (10.0%)                         | 3 (10.0%)               | 3 (10.0%)               |

Table 4.4: Distribution of Fungal Isolates by Market Location

| Market Location | No Growth (NG) | <i>Candida spp.</i> | <i>Aspergillus spp.</i> | <i>Rhizopus spp.</i> | <i>Penicillium spp.</i> |
|-----------------|----------------|---------------------|-------------------------|----------------------|-------------------------|
| Oba Market      | 14 (46.7%)     | 14 (46.7%)          | 1 (3.3%)                | 1 (3.3%)             | 0 (0.0%)                |
| Urelu           | 10 (33.3%)     | 14 (46.7%)          | 3 (10.0%)               | 1 (3.3%)             | 2 (6.7%)                |
| New Benin       | 12 (40.0%)     | 11 (36.7%)          | 4 (13.3%)               | 2 (6.7%)             | 1 (3.3%)                |
| Ikpobahill      | 15 (50.0%)     | 10 (33.3%)          | 3 (10.0%)               | 1 (3.3%)             | 1 (3.3%)                |
| Oliha           | 13 (43.3%)     | 9 (30.0%)           | 4 (13.3%)               | 2 (6.7%)             | 2 (6.7%)                |

### **4.3. Mean Bacterial Moad by market Location**

The mean bacterial load differed considerably between markets. Table 4.5. showed that Oba market recorded the lowest load ( $4.45 \times 10^5$  CFU/ml), followed by Uselu market ( $8.42 \times 10^5$  CFU/ml). Oliha market showed a moderately higher bacterial load ( $1.29 \times 10^6$  CFU/ml), while New Benin recorded  $1.73 \times 10^6$  CFU/ml. Ikpoba hill market had the highest bacterial load, reaching  $5.26 \times 10^6$  CFU/ml. These findings show that bacterial load was not evenly distributed, with Ikpoba hill demonstrating markedly heavier contamination.

Table 4.5: Mean Bacterial Load (CFU/ml) by Market Location

| Market Location | No tested | Bacterial Count (cfu/ml)<br>(Mean $\pm$ SEM) |
|-----------------|-----------|--|
| Oba Market      | 30        | $4.45 \times 10^5 \pm 2.90 \times 10^5$      |
| Urelu           | 30        | $8.42 \times 10^5 \pm 4.74 \times 10^5$      |
| New Benin       | 30        | $1.73 \times 10^6 \pm 1.12 \times 10^6$      |
| Ikpoba-hill     | 30        | $5.26 \times 10^6 \pm 2.18 \times 10^6$      |
| Oliha           | 30        | $1.29 \times 10^6 \pm 6.59 \times 10^5$      |

#### **4.4. Antibiotic susceptibility of bacterial isolates**

Antibiotic susceptibility testing presented in Table 4.6. revealed distinct sensitivity and resistance patterns between bacterial isolates. High sensitivity was observed with Erythromycin (92.9%), Pefloxacin (91.3%), and Ceftriaxone (90.5%). Other antibiotics with strong activity included Ceftazidime (88.4%), Cefuroxime (88.1%), Azithromycin (88.1%), Ofloxacin (90.5%), Augmentin (85.7%), and Ciprofloxacin (81.0%).

Resistance was most marked against Cefotaxime (42.0%), followed by Lincomycin (33.3%) and Imipenem (28.6%). Moderate resistance levels were observed against Cephalosporin, Trimethoprim, Streptomycin, and Augmentin (26.1–27.5%). Lower resistance rates occurred with Ciprofloxacin (21.7%) and Ofloxacin (21.7%), while Pefloxacin and Erythromycin had the least resistance at 8.7% and 7.1% respectively.

Table 4.6: Susceptibility of Bacterial Isolates to Antibiotics

| Antibiotic    | N Tested | Resistant n (%) | Sensitive n (%) |
|---------------|----------|-----------------|-----------------|
| Gentamicin    | 69       | 10 (14.5%)      | 59 (85.5%)      |
| Ciprofloxacin | 69       | 15 (21.7%)      | 54 (78.3%)      |
| Cephalosporin | 69       | 19 (27.5%)      | 50 (72.5%)      |
| Trimethoprim  | 69       | 19 (27.5%)      | 50 (72.5%)      |
| Streptomycin  | 69       | 18 (26.1%)      | 51 (73.9%)      |
| Cefuroxime    | 69       | 29 (42.0%)      | 40 (58.0%)      |
| Ofloxacin     | 69       | 15 (21.7%)      | 54 (78.3%)      |
| Augmentin     | 69       | 19 (27.5%)      | 50 (72.5%)      |
| Pefloxacin    | 69       | 6 (8.7%)        | 63 (91.3%)      |
| Ceftazidime   | 69       | 8 (11.6%)       | 61 (88.4%)      |
| Gentamicin    | 42       | 7 (16.7%)       | 35 (83.3%)      |
| Cefotaxime    | 42       | 6 (14.3%)       | 36 (85.7%)      |
| Ceftriaxone   | 42       | 4 (9.5%)        | 38 (90.5%)      |
| Cefixime      | 42       | 11 (26.2%)      | 31 (73.8%)      |
| Lincomycin    | 42       | 14 (33.3%)      | 28 (66.7%)      |
| Ciprofloxacin | 42       | 8 (19.0%)       | 34 (81.0%)      |
| Imipenem      | 42       | 12 (28.6%)      | 30 (71.4%)      |
| Cefuroxime    | 42       | 5 (11.9%)       | 37 (88.1%)      |
| Ofloxacin     | 42       | 4 (9.5%)        | 38 (90.5%)      |
| Erythromycin  | 42       | 3 (7.1%)        | 39 (92.9%)      |
| Augmentin     | 42       | 6 (14.3%)       | 36 (85.7%)      |
| Azithromycin  | 42       | 5 (11.9%)       | 37 (88.1%)      |

#### **4.5. The antibiotic resistance profiles of bacterial isolates recovered from Point-of-Sale (POS) machines in Benin City reveal distinct patterns between Gram-negative and Gram-positive antibiotics.**

Among the Gram-negative isolates (Table 4.7), *E. coli* displayed generally low resistance across most antibiotics, with the highest resistance observed for ciprofloxacin (30.8%), ofloxacin (38.5%), and cefuroxime (38.5%). Resistance to gentamicin (7.7%), trimethoprim (7.7%), augmentin (7.7%), ceftazidime (7.7%), and pefloxacin (15.4%) remained relatively low. *Klebsiella* species exhibited moderate to high resistance to several agents: trimethoprim showed the highest resistance at 50.0%, followed by cefuroxime and streptomycin (41.7% each). Resistance to cephalosporin (29.2%), augmentin (29.2%), ciprofloxacin (16.7%), and ofloxacin (12.5%) was also evident, while all *Klebsiella* isolates were sensitive to pefloxacin. *Pseudomonas* demonstrated considerable resistance to cefuroxime (40.0%) but lower resistance to other agents, including trimethoprim (26.7%), augmentin (26.7%), ciprofloxacin (20.0%), cephalosporin (20.0%), and gentamicin (20.0%). Resistance was lowest for streptomycin (6.7%), ofloxacin (6.7%), pefloxacin (6.7%), and ceftazidime (6.7%). *Enterobacter* isolates showed the highest overall resistance levels among the Gram-negative group, with cefuroxime (47.1%), cephalosporin (41.2%), and augmentin (41.2%) demonstrating particularly high resistance. Moderate resistance was observed for ofloxacin (35.3%), trimethoprim (11.8%), ciprofloxacin (23.5%), streptomycin (29.4%), and ceftazidime (23.5%).

For gram positive bacterial (table 4.8) *S. aureus*, resistance was highest to Lincomycin (34.8%), imipenem (30.4%), and cefixime (30.4%). Moderate resistance levels were found for ciprofloxacin (17.4%), augmentin (17.4%), and ofloxacin (13.0%), while resistance to cefuroxime (8.7%), erythromycin (8.7%), and azithromycin (13.0%) was comparatively low.

Notably, *S. aureus* isolates were fully sensitive to ceftriaxone (0% resistance). Non-pathogenic *Staphylococcus* species showed a slightly different profile, with Lincomycin resistance (31.6%) and imipenem resistance (26.3%) being the most pronounced. Resistance to ceftriaxone (21.1%), cefixime (21.1%), ciprofloxacin (21.1%), and cefuroxime (15.8%) was moderate, while resistance to gentamicin (10.5%), augmentin (10.5%), azithromycin (10.5%), and erythromycin (5.3%) was relatively low.

Overall, the data indicate that Gram-negative isolates, particularly *Enterobacter* and *Klebsiella*, demonstrate higher resistance to key  $\beta$ -lactam antibiotics (cefotaxime, cephalosporins, and augmentin) and certain fluoroquinolones. Gram-positive isolates remain largely sensitive to ceftriaxone but show notable resistance to aminoglycosides (lincomycin and gentamicin) and imipenem.

Table 4.7. Antibiotic Resistance Patterns of Gram-Negative Bacteria Isolated from Point-of-Sale (POS) Machines Used by Traders in Benin City

| Antibiotic    | <i>E. coli</i><br>(n=13) | <i>Klebsiella</i><br>(n=24) | <i>Pseudomonas</i><br>(n=15) | <i>Enterobacter</i><br>(n=17) |
|---------------|--------------------------|-----------------------------|------------------------------|-------------------------------|
| (Gentamicin)  | 7.7                      | 12.5                        | 20                           | 17.6                          |
| Ciprofloxacin | 30.8                     | 16.7                        | 20                           | 23.5                          |
| Cephalosporin | 15.4                     | 29.2                        | 20                           | 41.2                          |
| Trimethoprim  | 7.7                      | 50                          | 26.7                         | 11.8                          |
| Streptomycin  | 15.4                     | 41.7                        | 6.7                          | 29.4                          |
| Cefuroxime    | 38.5                     | 41.7                        | 40                           | 47.1                          |
| Ofloxacin     | 38.5                     | 12.5                        | 6.7                          | 35.3                          |
| Augmentin     | 7.7                      | 29.2                        | 26.7                         | 41.2                          |
| Pefloxacin    | 15.4                     | 0                           | 6.7                          | 17.6                          |
| Ceftazidime   | 7.7                      | 8.3                         | 6.7                          | 23.5                          |

*Percentages represent resistant isolates relative to total tested for each species.*

Table 4.8. Antibiotic Resistance Patterns of Gram-Positive Bacteria Isolated from Point-of-Sale (POS) Machines Used by Traders in Benin City

| Antibiotic    | <i>S. aureus</i> (n=23) | Non-path. <i>Staph</i> (n=19) |
|---------------|-------------------------|-------------------------------|
| Gentamicin    | 21.7                    | 10.5                          |
| Cefotaxime    | 21.7                    | 5.3                           |
| Ceftriaxone   | 0                       | 21.1                          |
| Cefixime      | 30.4                    | 21.1                          |
| Lincomycin    | 34.8                    | 31.6                          |
| Ciprofloxacin | 17.4                    | 21.1                          |
| Imipenem      | 30.4                    | 26.3                          |
| Cefuroxime    | 8.7                     | 15.8                          |
| Ofloxacin     | 13                      | 5.3                           |
| Erythromycin  | 8.7                     | 5.3                           |
| Augmentin     | 17.4                    | 10.5                          |
| Azithromycin  | 13                      | 10.5                          |

#### **4.6. Association between Market Location and Prevalence of bacteria and Fungal Contamination**

The association between market location and prevalence of bacteria (table 4.9) showed that Ikpoba-hill market had the highest prevalence of bacterial contamination (83.3%), while New Benin and Uselu had the lowest (70.0%). Oba and Oliha market recorded intermediate values (80.0% and 73.3% respectively). Despite these variations, chi-square analysis (chi square = 19.632,  $p = 0.717$ ) indicated that the differences were not statistically significant.

The association between market location and prevalence of fungi (table 4.10) revealed the highest prevalence in Uselu market (66.7%), followed by New Benin (60.0%) and Oliha (56.7%). Oba market recorded 53.3%, while Ikpoba-hill had the lowest prevalence (50.0%). The chi-square test (chi square = 8.174,  $p = 0.944$ ) showed no significant differences across markets,

Table 4.9. Association Between Market Location and Prevalence of Bacteria

| Market Location | Prevalence n (%) | Chi square value | p-value |
|-----------------|------------------|------------------|---------|
| Oba Market      | 24 (80.0%)       | 19.632           | 0.717   |
| Urelu           | 21 (70.0%)       |                  |         |
| New Benin       | 21 (70.0%)       |                  |         |
| Ikpoba-hill     | 25 (83.3%)       |                  |         |
| Oliha           | 22 (73.3%)       |                  |         |

Table 4.10. Association Between Market Location and Prevalence of Fungi

| Market Location | Prevalence (%) | Chi square value | p-value |
|-----------------|----------------|------------------|---------|
| Oba Market      | 16 (53.3%)     | 8.174            | 0.944   |
| Urelu           | 20 (66.7%)     |                  |         |
| New Benin       | 18 (60.0%)     |                  |         |
| Ikpoba-hill     | 15 (50.0%)     |                  |         |
| Oliha           | 17 (56.7%)     |                  |         |

## CHAPTER FIVE

### DISCUSSION

The predominance of *Klebsiella spp.* and *Staphylococcus aureus* contamination in POS machines as shown by this study is in line with a study by Obiebi *et al.* (2025 ) where *Klebsiella spp.*, *Staphylococcus spp.*, and other coliforms were isolated from surface of POS machines. Organisms like *E.coli*, *Klebsiella spp.*, *Enterobacter spp.* are enteric pathogens which are not meant to be found on the surface of these machines or devices are now found due to poor hand hygiene as their presence indicates fecal contamination. When they enter into the body, they can cause serious diarrheal diseases and Urinary Tract Infection (UTI) as *E.coli* is known to be the most common cause of UTI.

Isolation of *Pseudomonas spp.* (10%) in POS machines is in agreement with a study by John *et al.*, (2021) where *Pseudomonas spp.* was shown to be one of the highest bacterial contaminants in keypads and screens of POS machines in both dry and wet seasons. *Pseudomonas spp.* are known for their resistance against certain antibiotics and they also produce biofilm which enhance their survival rate on surfaces irrespective of environmental conditions and this can aid in their community spread. They are known to be opportunistic as they mainly cause infections in immunocompromised individuals or those using hospital equipments like catheters and patients with wounds. They may not cause infection in healthy individuals. They are able to cause infections like Respiratory tract infections, UTIs, sepsis and bacteremia.

In this study, *Enterobacter spp.* (11.3%) Was also isolated from the surface of POS machines and this is in agreement with a study by Innocent-Adiele *et al.*, (2023) which showed that

*Enterobacter spp.* Was isolated in the keypads of Automated Teller Machines (ATM). They are also opportunistic pathogens that usually cause infections in hospital settings and in immunocompromised individuals. It has been shown to cause infections like UTIs, sepsis, wound infections. It's presence on the surface of POS machines is as a result of fecal contamination and poor hand hygiene majorly.

*Staphylococcus aureus* was shown in this study to account for 15.3% of contamination on the surface of POS machines. In Uyo, a study by John *et al.*, (2021) showed *Staphylococcus aureus* to be one of the highest contaminants seen in the surface of POS machines, and this is also seen in this study. These bacteria has the ability to survive on surfaces of objects even in extreme dry conditions and this tends to enhance the risk of community spread. They are able to cause several infections like skin and soft tissue infections, RTI like pneumonia, sepsis and bacteremia. Some of these *S.aureus* tends to show resistance to certain antibiotics and they are referred to as Methicillin Resistant Staphylococcus Aureus (MRSA) thereby making treatment of infections difficult. They can survive on these surfaces for long periods of time and as such, their transmission or spread is relatively easy.

Fungal isolates that was observed in this study includes *Candida spp.* which accounts for 38.7% of fungal contamination, *Aspergillus spp.* accounting for 10.0% of contamination, *Rhizopus spp.* accounts for 4.7% of contamination and *Penicillium spp.* accounts for 4.0% of contamination and this is in agreement with a study by Mbajiuka, (2015) in Abia which showed that *Rhizopus spp.*, *Aspergillus spp.*, *Penicillium spp.*, was isolated in the keypads of ATMs. *Candida* are a genus of yeasts that are usually found as commensals in human mucosal surfaces like the oral cavity,

gastrointestinal tract, skin and the vagina. They are opportunistic pathogens and they usually cause infections under certain conditions like immunosuppression, use of antibiotics, a suitable environment that supports the survival of these organisms. Their presence on the surface of POS machines is usually as a result of poor hand hygiene and irregular disinfection. Studies have shown that *Candida albicans* are able to survive on plastic surfaces like that of the POS machines for upto 72 hours and non-albican species can survive for longer periods of time. They are able to cause infections like oral candidiasis, cutaneous candidiasis, vaginal candidiasis, diaper rash (candida dermatitis) in infants. *Aspergillus* is a genus of spore forming, filamentous fungi. They are usually seen in air, dust, soil and in surfaces of inanimate objects. Due to their spores, they can easily become aerosolized in air and dust particles and when they settle on the keypads and screens of these POS machines, they can remain there for some time, and due to lack of regular disinfection, they can be spread among individuals. They can cause infections like allergic reactions, aspergilloma, keratitis, otomycosis. *Rhizopus spp.* and *Penicillium spp.* were also isolated in small numbers , 4.7% and 4.0% respectively. Their presence on the surface of POS machines is usually as a result of poor hygiene practices as they are usually found in air, dust, soil. They pose a high risk of causing infections in immunocompromised individuals. *Rhizopus spp.* are known to cause mucormycosis in diabetic patients and *Penicillium spp.* causes allergic reactions.

Antibiotic susceptibility patterns revealed that high sensitivity was observed with Erythromycin (92.9%), Pefloxacin (91.3%), and Ceftriaxone (90.5%) making them suitable for treatment in cases of infection as they would work well in combating these pathogens. There was high resistance against Beta-lactams and Carbapenems even as high as 42% resistance which implies

that these drugs cannot be effectively used to treat these infections if it occurs. Some of these organisms showed multi drug resistance and this has proven that Anti Microbial Resistance (AMR) can be seen in community settings and not just hospital settings alone. When individuals especially those with weakened immunity get infected by these resistant strains, the outcome can be very fatal and finding a treatment becomes very difficult.

## **CONCLUSION**

This study has shown that the surfaces, keypads and screens of POS machines harbor certain microorganisms like *Klebsiella spp.*, *Staphylococcus spp.*, *Enterobacter spp.*, *E.coli*, *Candida spp.*. The level of these bacterial and fungal contamination varies across the various locations as we noticed the highest contamination in Ikpoba-hill market and the lowest contamination in Uselu market and this may be as a result of poor hygienic practices of the residents there and also due to environmental factors. This study also highlights the presence of antibiotic resistant strains on these POS machines and they can play a role in the spread of AMR in the community.

## **RECOMMENDATIONS**

1. Public enlightenment: Members of the public should be enlightened and educated on how these POS devices can serve in spreading infectious pathogens and also resistant ones so as to prevent or control its spread.
2. Regular disinfection: Users of these POS machines should ensure regular cleaning and disinfection of their devices so as to eliminate some of these pathogens that would be present there.

3. Hand hygiene: The importance of a good hand hygiene cannot be over emphasized. The public should be educated on how hand hygiene is important in curbing the spread of these infections.
4. Inclusion of POS machines in public health sanitation policy: The Ministry of Health can update their sanitation policies to include routine checks of these payment devices and health officers can inspect payment devices during routine sanitation visits to public areas.
5. Surveillance programs: Continuous monitoring should be done on these POS machines so as to detect emerging pathogens and resistant strains that may be present on these devices.

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## **APPENDIX I**

### **Media**

The media used includes commercially dehydrated products and laboratory prepared media.

#### **Macconkey Agar (CM7, Oxiod, England)**

##### **Preparation**

- 52 grams of MacConkey agar powder was weighed and suspended aseptically in 1 liter of sterile distilled water and was allowed to dissolve for 10 minutes.
- It was sterilized by autoclaving at 121°C for 15 minutes.
- The agar was cooled at 50°C, mixed and then poured aseptically into the petri dish.
- It was allowed to set and stored at 4°C for 2 weeks.

#### **Mueller Hinton Agar (LabMal, Academy)**

##### **Preparation**

- 38 grams of Mueller Hinton agar powder was weighed and suspended aseptically in 1 liter of sterile distilled water and was allowed to dissolve for 10 minutes.
- It was sterilized by autoclaving at 121°C for 15 minutes.
- The agar was cooled at 50°C, mixed and then poured aseptically into the petri dish.
- It was allowed to set and stored at 4°C for 2 weeks.

#### **Citrate Koser Medium (Himedia (M069) Laboratories, India)**

##### **Preparation**

- 5.7 grams of sodium citrate powder was weighed and suspended aseptically to 1 liter of sterile distilled water.
- This was allowed to dissolve for 10 minutes and then mixed.
- Equal volume of the broth was dispensed into sterile bijou bottles.
- It was then sterilized by autoclaving at 121°C for 15 minutes.
- It was allowed to cool and then stored at room temperature.

### **Urea Agar Base (CM53, Oxiod, England)**

#### **Preparation**

- 2.4 grams of urea agar base powder was suspended in 95ml of sterile distilled water.
- It was heated to boil to dissolve completely.
- It was sterilized by autoclaving at 121°C for 15 minutes.
- It was cooled to 50°C, 5ml of sterile 40% urea solution was added aseptically and mixed.
- It was then dispensed into sterile bijou bottles and was allowed to set in a slanted position.
- It was then stored at 4°C for 2 weeks.

### **Peptone Water (CM9, Oxiod, England)**

#### **Preparation**

- 10 grams of peptone and 5 grams of sodium chloride were weighed and added aseptically to 1 liter of sterile distilled water.
- It was mixed and dispensed into sterile bijou bottles.
- It was sterilized by autoclaving at 121°C for 15 minutes.
- It was allowed to cool and stored at room temperature.

## Media Constituent

- **Macconkey Agar (CM7, Oxiod, England)**

### Constituents

|                  |            |
|------------------|------------|
| Peptone          | 20.0grams  |
| Lactose          | 10.0grams  |
| Neutral red      | 0.075grams |
| Bile salt        | 5.0grams   |
| Sodium chloride  | 5.0grams   |
| Agar             | 12.0grams  |
| Distilled water  | 1000ml     |
| pH $7.4 \pm 0.2$ | at 25°C    |

- **Mueller Hinton Agar (LabMal, Academy)**

### Constituents

|                    |           |
|--------------------|-----------|
| Casein hydrolysate | 17.5grams |
| Beef infusion      | 2.0grams  |
| Starch             | 1.5grams  |

Agar 17.0grams

Distilled water 1000ml

pH  $7.3 \pm 0.1$  at  $25^{\circ}\text{C}$

● **Citrate Koser Medium (Himedia (M069) Laboratories, India)**

**Constituents**

Sodium ammonium phosphate 1.5grams

Potassium dihydrogen phosphate 1.0grams

Magnesium sulphate 0.2grams

Sodium citrate 3.0grams

Bromothymol blue 0.016grams

Distilled water 1000ml

pH  $6.7 \pm 0.2$  at  $25^{\circ}\text{C}$

● **Peptone Water (CM9, Oxiod, England)**

**Constituents**

Peptone 10grams

Sodium chloride 5.0grams

Distilled water 1000ml

pH  $7.2 \pm 0.2$  at 25°C

● **Urea Agar Base (CM53, Oxiod, England)**

**Constituents**

Peptone 1.0grams

Glucose 1.0grams

Sodium chloride 5.0grams

Disodium phosphate 1.2grams

Potassium dihydrogen phosphate 0.8grams

Phenol red 0.012grams

Agar 15.0grams

Distilled water 95ml

pH  $6.8 \pm 0.2$  at 25°C

## APPENDIX II

### Chemical Reagent

All chemicals used in this study were of analytical grade and they include;

- **Gram Stain Reagent**

#### Constituent

|                 |          |
|-----------------|----------|
| Crystal violet  | 0.2grams |
| Distilled water | 80ml     |
| Lugol' s iodine | 2.0grams |
| Distilled water | 100ml    |
| Acetone         | 95%      |
| Neutral red     | 1.0gram  |
| Distilled water | 100ml    |

1gram of neutral red was dissolved in a small amount of water and was made up to 100ml

- **Kovac's reagent**

#### Constituent

|                 |           |
|-----------------|-----------|
| Sodium chloride | 0.85grams |
|-----------------|-----------|

Distilled water

100ml

## APPENDIX III

### ● **Materials**

Slides

Cover slips

Grease pencil

Wire loop

Straight wire

Test tubes

Petri dish

Maccartney bottles

Forceps

### ● **Equipment**

Microscope




Hot air oven

Incubator

Autoclave

## APPENDIX IV

### ETHICAL APPROVAL

|  |   |  |
|--|---|--|
|   | <b>RESEARCH ETHICS COMMITTEE</b><br>COLLEGE OF MEDICAL SCIENCES<br>UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.  |  |
| <b>Chairman:</b> Prof. F. A Imarhiagbe<br>MBChb, FMCP<br>Cert Clin Res and ethics (NIH), MD.<br>0803449092   | <b>Email:</b> researchethics.cms@gmail.com  | P.M.B 1154, BENIN CITY   |
| <b>Our Ref:</b> CMS/REC/01/VOL.2/795   | <b>Date:</b> 13 <sup>th</sup> July, 2025  |  |
| <b>Re: MICROBIAL PROFILE OF POINT-OF-SALE (POS) MACHINES AMONG TRADERS IN BENIN CITY, EDO STATE.</b>   |   |  |
| <b>Name of Principal Investigator:</b>   | <b>OSAIGBOVO OGHOSAMASE</b><br>Department Of Medical Laboratory Science,<br>School of Basic Medical Science,<br>College of Medical Sciences,<br>University of Benin |  |
| <b>REC Approval No: CMS/REC/2024/795</b>   |   |  |
| This is to inform you that the research described in the submitted proposal, the Informed Consent Forms and other participant information materials have been reviewed and approved by the College Research Ethics Committee, University of Benin.   |   |  |
| This approval dates from <b>13<sup>th</sup> July, 2025 to 12<sup>th</sup> July, 2026</b> . In multi-year research, Endeavour to submit your annual report to the REC early in order to obtain renewal of your approval and avoid disruption of your research.  |   |  |
| The National Code of Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the code including ensuring that all adverse events are reported promptly to the REC. No, changes are permitted in the research without prior approval by REC except in circumstances outlined in the code. REC reserves the right to conduct compliance visit to your research site without prior notice. Thank you. |   |  |
|   |   |  |
| <b>PROF. F.A IMARHIAGBE</b><br>Chairman, REC   |   |  |

## APPENDIX V



