Assessment of Bacterial Profiles and Antimicrobial Susceptibility Pattern of Isolates Contaminating Metallic Keyboards of Automated Teller Machines in Adama City, Oromia, Ethiopia

Rediet Negash

A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

Presented in partial Fulfillment of the Requirements for the Degree of Master's in Biology (Applied Microbiology)

Office of Graduate Studies

Adama Science and Technology University

December, 2024
Adama, Ethiopia

Assessment of Bacterial Profiles and Antimicrobial Susceptibility Pattern of Isolates Contaminating Metallic Keyboards of Automated Teller Machines in Adama City, Oromia, Ethiopia

Rediet Negash

Advisor: Zerihun Belay (PhD)

Co-Advisor: Mesfin Bekele

A Thesis Submitted to the Department of Applied Biology School of Applied Natural Science

Presented in partial Fulfillment of the Requirements for the Degree of Master's in Biology (Applied Microbiology)

Office of Graduate Studies

Adama Science and Technology University

December, 2024

Adama, Ethiopia

DECLARATION

I hereby declare that this master thesis entitled "**Assessment of Bacterial Profiles and Antimicrobial Susceptibility Pattern of Isolates Contaminating Metallic Keyboards of Automated Teller Machines in Adama City, Oromia, Ethiopia"** is my original work. That is, it has not been submitted for the award of any academic degree, diploma, or certificate in any other university. All Sources of materials that are used for this thesis have been duly acknowledged through citation.

Rediet Negash

Name of student Signature Date

RECOMMENDATION OF ADVISORS

We, the Advisor(s) of this thesis, here by certify that I/We have read he revised version of the thesis entitled "**Assessment of Bacterial Profiles and Antimicrobial Susceptibility Pattern of Isolates Contaminating Metallic Keyboards of Automated Teller Machines in Adama City, Oromia, Ethiopia"** Prepared under our guidance by **Rediet Negash** submitted in partial fulfillment of the requirements for the degree of Master of Science in Applied Microbiology. Therefore, I/We recommend the submission of a revised version of the thesis to the department following the applicable procedures.

Zerihun Belay (PhD)	_____	_____
Major Advisor	Signature	Date
Mesfin Bekele	_____	_____
Co-Advisor	Signature	Date

APPROVAL OF ADVISORS

We, the Advisors of the thesis entitled "**Assessment of Bacterial Profiles and Antimicrobial Susceptibility Pattern of Isolates Contaminating Metallic Keyboards of Automated Teller Machines in Adama City, Oromia, Ethiopia"** and developed by **Rediet Negash**, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Zerihun Belay (PhD)	_____	_____
Major Advisor	Signature	Date
Mesfin Bekele	_____	_____
Co-Advisor	Signature	Date

APPROVAL OF BROAD REVIWERS

We, the undersigned, members of the board of Examiners of the thesis by **Rediet Negash** have read and evaluated the thesis entitled "**Assessment of Bacterial Profiles and Antimicrobial Susceptibility Pattern of Isolates Contaminating Metallic Keyboards of Automated Teller Machines in Adama City, Oromia, Ethiopia"** and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Applied Microbiology.

Chairperson	Signature	Date
_____	_____	_____
Reviewer	Signature	Date
_____	_____	_____

Finding approval and acceptance of the thesis is contingent upon submission of its final copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and School Graduate Committee (SGC)

_____	_____	_____
Department Signature	Date	
_____	_____	_____
School Dean	Signature	Date
_____	_____	_____
Office of Postgraduate Studies, Dean	Signature	Date

ACKNOELEDGMENTS

First, I would like to thank God for his mercy, and grace and for giving me good health throughout my life journey. I am also most grateful to my main research advisor Dr. Zerihun Belay for suggesting this research title. Also, I express my deepest appreciation to my advisor for his relentless and continuous guidance and mentorship right from the start of the proposal writing, through my laboratory work till the writing of my thesis. My deepest gratitude to my co advisor Mesfin Bekele for his support. I would like to extend my gratitude to Adama Science and Technology University Department of Applied Biology for providing me with a scholarship for my MSc in Applied Microbiology. My special gratitude also goes to the management and staff members of Adama Public Health Research and Referral Laboratory for their high-level laboratory for the analysis of samples collected, for providing me with all the required materials, and for their sharing of knowledge. My special gratitude also goes to the management, staff members and laboratory technicians of the National Animal Health Diagnostic and Investigation Center found in Sebeta for their enormous contribution while doing the MALDI TOF MS analysis. Finally, my special thanks go to my family, basically my mom and all my friends for their unfailing support, encouragement, and advice they provided me with throughout my studies.

TABLE OF CONTENTS

CONTENTS **PAGE**

LIST OF TABLES

TABLE **PAGE**

LIST OF FIGURES

FIGURE **PAGE**

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AbB: Abay Bank

AdIB: Addis International Bank

AIB: Awash International Bank

AmB: Amhara Bank

AMC: Amoxicillin-clavulanate

AMP: Ampicillin

ATM: Automated Teller Machine

BOA: Bank of Abissinia

BrB: Brehan Bank

BuB: Buna Bank

CAZ: Ceftazidime

CBE: Commercial Bank of Ethiopia

CBO: Cooperative Bank of Oromia

CLSI: Clinical Laboratory Standards Institute

CoNS: Coagulase negative staphylococcus

CDC: Center for Disease Control

CTX: Cefotaxime

CIP: Ciprofloxacin

DA: Clindamycin

DB: Dashen Bank

DGB: Debub Global Bank

E: Erythromycin

eDT: Extended direct transfer

FEP: Cefepime

GM: Gentamicin

HAIs: Healthcare-associated infections

HCCA: Hydroxycinnamic acid

IMP: Imipenem

KZ: Cephazolin

LSV: Logarithmic score value

MALDI-TOFMS: Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry

MRSA: Methicillin Resistant Staphylococcus aureus

NAHDIC: National Animal Health Diagnostic and Investigation Center

NIB: Nib Bank

OIB: Oromia International Bank

P: Penicillin G

PMNs: polymorph nuclear leukocyte

PTZ: Piperacilin/Tazobactam

SXT: Sulfa/Trimethazole

T: Tetracycline

TSI: Triple sugar iron test

USCDC: US Centers for Disease Control

WB: Wegagen Bank

ZB: Zemen bank

ABSTRACT

Automated Teller Machine (ATM) is a computerized telecommunication device which makes banking easier today. However, it is contaminated by potential pathogens transmitted with hand contact. In studying microbial profile of ATM machines was an interesting project. Contaminated surfaces play a significant role in the spread of infectious diseases. Given that 80% of infections are transmitted by hand contact with contaminated hands, studying microbial profile of ATM machines is an interesting project. Apart from that, the contaminating pathogenic microorganisms are becoming more resistant to antibiotics. Thus, this study was aimed to investigate the presence and types of bacteria that colonize ATMs surfaces and determine the antimicrobial resistance in Adama City and provide valuable insights into public health and hygiene practices using standard methods. Samples were collected using sterile swabs rubbed on the keypads, screens, and other surfaces of the ATMs. The swabs were streaked onto Blood agar MacConkey and Chocolate agar and incubated at 37^{o}C for 24 hours. Identification of microorganisms was done using standard biochemical characterization, Gram-staining, and Endospore-staining and MALDI-TOF MS methods. Antimicrobial susceptibility testing of the bacterial isolates was conducted using the Kirby-Bauer agar disc diffusion method. All the data were analyzed using MS excel sheet and analyzed by SPSS, version 27. Thus, a total of 77 bacterial isolates were collected from 80 samples of which 58% were gram-negative and 42% were gram-postive. Based on both cultural and biochemical tests 13 different bacterial isolates were categorized into; Escherchia coli, Psudomonas aeruginosa, Shigella dysenteriea, Salmonella tyhimurium, Staphylococcus aureus, Klebsiella pneumoniae, Bacillus spp. Shigellaspps. Listeria spp. Serattia spp. Citrobacter spp., Protous mirballis, and Providencia stuarti. The Antibibiogram study showed that the majority of the bacteria were highly resistant to standard antibiotics, of which more gram-negative bacteria were resistant than gram positive bacteria to many antibiotics. All gram-negative bacteria were resistant to penicillin G 15/15(100%) antibiotics. Gram-postive was also resistant to antibiotics clindamycin 3/3(100%) Bacillus spp, S.aureus and Listeria spp. From the result of MALDI-TOF 16151(ICBE), 16153(ICBE5), 16157(ICBO2), 16160(ICBE25) were identified as K. pneumoniae with scores 2.32, 2.36, 2.21, 2.05, respectively these 4 isolates score values indicate high confidence identification. Generally, it can be concluded that bacteria identified have

pathogenic potential and hence their presence on those objects surfaces may have a capability of transmitting pathogens through the community is an indicative of the need for awareness on cleaning of such surfaces or disinfection and adequate hand hygiene.

Keywords: Antibiotics, Automated teller machine, Bacterial isolates, MALDI-TOF MS, microbial profile, pathogens

CHAPTER ONE

1. INTRODUCTION

1.1 Background of study

The automated teller machine (ATM)s are automated banking machines, cash dispensers, and various regional variants derived from trade marks on ATM systems held by specific banks. It is a means by which a customer of a financial institution (bank) performs financial transactions without the need of a cashier, human clerk or bank teller (Bodena *et al*., 2019).Due to their widespread use by many people throughout the day and their vast usage and dermal contact by many people in a day , ATMs are likely to be contaminated with a wide range of microorganisms both pathogenic and non-pathogenic (Id and Akanbi, 2021; Stanley and Okpara,2015).

Microorganisms can survive and even grow on any surface and can be found everywhere in the environment. Although most of them are harmless, some are pathogenic, particularly to people with compromised immune systems. ATMs that are contaminated become vehicles for the transmission of infection, and the user is vulnerable to these pathogens after using the Automated Teller Machine (Agu *et al.*,2018). Some common bacteria that may be present on contaminated surfaces like ATMs include: *S.aureus, E. coli, Bacillus spp., P.aeruginosa* and others from hands that have been artificially contaminated with a single species of microorganism (Yuana *et al*., 2022) also indicated that *S.aureus* with a prevalence of (41.6%), *E.coli* (34.4%) and *K. pneumonia* (24.0%) were some of the bacterial isolates found on the keypads of the ATMs.

The isolates obtained from the data reported by (Onuoha and Fatokun 2014) observed that *S.aureus* (28%) was the commonest organism isolated from ATMs of Seventeen different banks in two major town of Nigeria (Nwankwo and offiah 2016) reported that the ATM machines are one of the most touched surfaces today (Onuoha and Fatokun 2014) described six different bacteria isolates in ATMs located in Nigeria, such as *Staphylococcus spp., Streptococcus spp., Pseudomonas spp., Enterobacter spp and Escherchia spp.*

Mohaoumdi *et al* 2017 evaluated 96 ATM keyboards at four locations in Hamadan (Iran) and they observed that all tested ATM keyboards were contaminated with at least one species of bacteria. The most frequently isolated bacteria were *S.epidermidis* in 12(18.5%) ATMs *P.aeruginosa* in 12 (18.5%) *B.subtilis* in 11(16.9%), *E.coli* in 6 (9.2%), *S.aureus* in 3 (4.6%), and *Micrococcaceaespp spp* in 5(7.69%) cases. The bacteria contamination of 14 commercial banks randomly scattered within the Umuahia Metropolis in South eastern Nigeria showed a total of 102 bacterial organisms comprising nine different species in ATMs. Two Gram-positive bacteria *S.aureus* and *streptococcus spp* showed 17.6 and 13.7% respectively while two Gram negative bacteria *E.coli* and *pseudomonas spp.* showed 26.5% and 9.8% respectively (Nwankwo and Offiah, 2016).

The results revealed by Mbajiuka (2015) in Abia state (Nigeria) showed that ATM devices were contaminated, being a potential disease dispensing machines as bacterial isolates such as *Staphylococcus spp* about 82.5%, *Bacillus spp* 62.5%, *Escherichia spp.* 22.5% and *Streptococcus spp* 15%. Okoro *et al.* (2018) showed that there is a strong relationship between the isolated pathogenic bacteria and the ATMs. Pathogenic bacteria such as *E. coli, P.aeruginosa, and S. dysenteriea, S.thyphimurium, S.aureus* and *K.pneumoniae were* isolated from seven (7) different banks within Kaduna Metropolis. The samples (n= 200) were contaminated with *K. pneumoniae*, that had the largest percentage of isolates with 46 (23%), followed by *S. dysenteriea* with 37(18.5%). *S. aureus, S. tyhimurium*, and *P. aeruginosa* had 33 (16.5%), 32 (16%) and 29 (14.5%) respectively, while *E. coli* had the smallest percentage of isolates with 22 (11%).

Onuoha and Fatokun (2014) observed that *S. aureus* (28.57%) was the commonest organism isolated followed by *E. coli* (21.43%), C*oagulase negative Staphylococcus* (CoNS) (21.43%) and *Streptococcus* species (14.29%). *P. aeruginosa* and *Enterobacter* species showed the least percentage occurrence with (7.14%) respectively, in ATMs of Nigeria. Nagajothi *et al*. (2015) collected 92 swabs from ATM centers in Puducherry (India) and observed microbial growth in 88 (95.7%) swabs. One hundred and sixty (160) microorganisms were isolated: *Klebsiella* species (42.5%); *Coagulase-negative Staphylococcus* (CoNS) 20.62%; *P. aeruginosa* (15%); *E. coli* (10.6%) and *S.aureus* (3.75%) (Reiner, 2013).

In Ethiopia there are several studies under taken on the microbial profile and antimicrobial susceptibility of these bacteria isolated from paper currencies (Gosa Girma et al, 2014; Adem Hiko et al, 2016; Haile Alemayehu and Mogesse Ashenafi 2019) automated teller maschines (Mamo Akele et al, 2019). The latter assessed the bacterial profiles and antibacterial susceptibility patterns of frequently hand touched surfaces on public transport buses, Automated Teller Machines and St' Paul Hospital patient waiting areas in Addis Ababa, Ethiopia. According to (Mamo Akele *et al*, 2019) the bacterial contaminants isolated from ATM were bacteria that were highly resistant to the commonly used antibiotics such as, cefuroxime 77.6%, ampicillin 79.3%, ceftriaxone 84.5% and ceftazidime 84.5%. Moreover, 72.0% of the isolates were multidrug resistant was observed.

They concluded that these bacteria have pathogenic potential and hence their presence on those objects surfaces. According to (Mamo, 2019) some of the bacteria observed from hand touch surfaces of selected Automated Teller Machine, public transport buses and hospital waiting area inanimate objects located in Addis Ababa, Ethiopia, are highly resistant to the commonly used antibiotics such as, 71.3%, 67.3% and 40.2% of *Staphylococcus species* showed resistant against to tetracycline, penicillin and oxacillin, respectively.

Proteus Spp was resistant to cefuroxime 77.6%, ampicillin 79.3%, ceftriaxone 84.5% and ceftazidime 84.5%. Moreover, in 72.0% of the total isolate multidrug resistance was observed. However, compared to the currently utilized number of ATM machines across the country, this report is quite insignificant. Therefore, the objective of this study is to investigate the presence of bacterial diversity and their pattern of antimicrobial resistance collected from Bank ATMs from Adama city, Oromia, Ethiopia.

1.2 Statement of the problem

Microorganisms are ubiquitous their presence on ATM keypads/buttons is unavoidable, as various researchers have reported, (Agu *et al.,* 2018) and the inclination of these microorganisms to be picked up by humans and infected through oral, nasal, or eye contacts with contaminated fingers is very high. Microorganisms found in fomites have

also been shown to persist on environmental surfaces for varying amounts of time ranging from hours to months (Agu *et al*., 2018). As a result of the expansion of urbanization and the increasing population around the cities, people are avoiding the use of traditional banking systems and are more embracing adopting electronic banking system, such as ATMs, mobile banking, internet banking, and the like.

CBE's (Commercial Bank of Ethiopia) currently CBE has more than 40 million account holders in it. Its more than 1940 branches and the number of mobile and internet banking users also reached more than 6.6 million and 37,000 active ATM holders reached more than 8.3 million and 17 million CBE birr users (Chandrasekar & Taye, 2017) BOA (bank of Abyssinia) annual report shows the bank has more than 5.16 million account holders from the total number of customers 18.79% (969,984) are ATM card holders, 17.65% (910,969) mobile banking subscribers and the rest are customers who have using the banking service branches (Chandrasekar & Taye, 2017).

The number of active ATM machines found in Adama city are 262 that give service to all bank's customers (Source; MOTI Engineering plc). People of various socioeconomic and sanitary statuses use ATMs daily, increasing the likely hood of hand borne transmission of microorganisms to the machine's surfaces (Paulo,2001).Hand-borne transmission is one of the most important routes for the spread of many infectious agents in the community. Hand transmission is a critical factor in the spread of disease-causing bacteria, pathogens, and viruses (Adedoyin, 2019). Other factors have been attributed or demonstrated to influence bacterial transfer between surfaces, including the source and destination surface features, bacterial species involved, moisture levels, pressure and friction between the contact surfaces, and the size of inoculums on surfaces (Yuana *et al*., 2022).

Bacteria that can cause severe gastroenteritis to have been discovered on ATM keypads and buttons. The presence of pathogenic bacteria on ATM user interfaces poses a risk to vulnerable, immune compromised individuals. The reservoir of any organism, whether animate or inanimate, is critical in the epidemiology of any infection. Pathogens live and multiply in the reservoir on which they rely for survival pathogens live on formite. Many epidemiological studies have revealed that contaminated surfaces played a significant

role in the spread of infectious diseases (Stanley, 2015).

Many epidemiological studies have found that contaminated surfaces play a significant role in the spread of infectious diseases (Bakterien & Von, 2017). Most people are unaware that microbes can be found on many common outdoor objects, including ATM keyboards. ATMs are typically air conditioned, and the cold and damp environment encourages the growth of a wide variety of microorganisms, ranging from pathogens to harmless microbes. The city of Adama is well-known for having an environment that is conducive to the proliferation of pathogenic microorganisms. Bacteria that can cause severe gastroenteritis to have been discovered on ATM keypads and buttons (Nwankwo & Offiah, 2016).

Given that 80% of infections are transmitted by hand contact with contaminated hands or food particles, and that many pathogenic microorganisms are becoming more resistant to antibiotics, public health management is now more concerned with preventing disease than with treating it (Division & Lumpur, 2014). According to (Mamo, 2019) some of the bacteria observed from hand touch surfaces of selected Automated Teller Machine, public transport buses and hospital waiting area inanimate objects located in Addis Ababa, Ethiopia, are highly resistant to the commonly used antibiotics such as, 71.3%, 67.3% and 40.2% of *Staphylococcus species* showed resistant against to tetracycline, penicillin and oxacillin, respectively. *Proteus Spp* was resistant to cefuroxime 77.6%, ampicillin 79.3%, ceftriaxone 84.5% and ceftazidime 84.5%. Moreover, in 72.0% of the total isolate multidrug resistance was observed.

1.3 Significance of the study

The significance of the study on the Assessment of Bacterial Profiles and Antimicrobial Susceptibility Pattern of Isolates Associated with ATMs lies in several aspects, for example in public health concern ATMs are frequently accessed by many individuals making them potential sources of bacterial contamination. Understanding the types of bacteria present and their antimicrobial susceptibility pattern is crucial for assessing the potential health risk associated with ATM usage. In identification of potential pathogens this study helps in identifying the specific bacteria present on ATMs including potential pathogenic species. This information is valuable for public health officials and healthcare

providers to understand the potential transmission of diseases in the community. In Antimicrobial resistance monitoring it helps in assessing the antimicrobial susceptibility of bacteria isolated from ATMs provides insights into the prevalence of antimicrobial resistance in the community.

This data also supports antimicrobial Stewardship programs and informs treatment guidelines, aiding in the management of bacterial infections. In hygiene and sanitation practices these study findings can raise awareness about the importance of maintaining proper hygiene and sanitation practices while using ATMs. It can contribute to educating both public and ATMs facility management about the need for regular cleaning and disinfection to minimize the risk of bacterial contamination. In Environmental monitoring also studying bacterial contamination in ATMs adds to the understanding of microbial ecology in urban environments. It provides data on the distribution of bacteria in public spaces and contributes to the broader knowledge of microbial communities associated with human interactions. Overall, this study is significant as it addresses public health concerns, antimicrobial resistance patterns, and hygienic practices and contributes to the understanding of bacteria in urban environments. It can support the development of appropriate measures to prevent and control infections associated with ATMs, ultimately benefiting the health and well-being of the community.

1.4. Objectives

1.4.1. General objective

- To assess bacterial profiles and antimicrobial susceptibility pattern of isolates associated with automated Teller Machines in Adama City, Oromia, Ethiopia.

1.4.2. Specific objectives

- To isolate and characterize bacteria found on Automated teller Machines (ATMs) in Adama City.
- To determine the antimicrobial susceptibility profile of the isolates.
- To compare the prevalence of the bacterial isolates between the Automated teller Machines (ATMs) of different banks located in Adama.
- To identify the isolates using MALDI-TOF MS.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. History of ATM and Its Introduction to Banking Operation

Automated Teller Machines (ATMs) have revolutionized the banking industry since their introduction. The concept of ATMs began in the 1960s (Konheim, 2016). The first ATM was installed by Barclays Bank in London in 1967. This machine allowed customers to withdraw cash outside of banking hours. ATMs gained popularity throughout the 1970s and 1980s as banks across the world began adopting technology Galbraith *et al*., (1999); Nworie *et al*. (2012) and Noble, (2002). Initially, they were mainly used for cash withdrawals, but soon expanded to include other functionalities like balance inquiries and deposits. Over time, ATMs evolved with technological advancements. They became more sophisticated, offering features like bill payments, fund transfers between accounts, and printing mini statements (Paul & Paul, 2013).

Different countries adopted the technology at varying rates, with some nations integrating them deeply into their banking infrastructure. ATMs significantly changed the way people interacted with banks. They reduced the need for physical bank visits, provided greater convenience, and extended banking hours. Security concerns emerged with the proliferation of ATMs, leading to the development of technologies like PIN codes and enhanced encryption to protect customer transactions. Innovations continued, such as ATMs with biometric authentication and contactless transactions. Today, ATMs continue to evolve with advancements in digital banking (Tushar & Chawla, 2022). They are part of broader networks that allow customers to access their accounts globally, often supporting multiple languages and currencies. Overall, ATMs have been a pivotal innovation in the banking sector, offering convenience, accessibility, and security to customers while reshaping traditional banking practices.

2.2. The Spread of ATMs in Ethiopia

In Ethiopia, the adoption and spread of ATMs have been relatively recent compared to more developed regions. ATMs were first introduced in Ethiopia around the early 2000s. Commercial banks began deploying ATMs in major cities like Addis Ababa, targeting urban areas with

higher population densities and economic activity. Initially, ATMs were concentrated in Addis Ababa, the capital city, and other major urban centers (Chandrasekar & Taye, 2017).

This expansion catered primarily to the growing middle class and business communities needing convenient access to banking services. Ethiopia faced challenges in ATM deployment due to infrastructural limitations, including reliable electricity and internet connectivity. These factors initially slowed the widespread adoption of ATMs outside major cities (Onuoha & Fatokun, 2014). The Ethiopian government has been supportive of banking sector modernization, including the expansion of electronic banking services like ATMs. Regulations have been developed to facilitate the deployment and operation of ATMs by banks. Efforts have been made to extend ATM services beyond urban centers to rural areas, aiming to improve financial inclusion. Mobile banking and agent banking networks have also been utilized to complement ATM services in remote regions. As of recent years, the number of ATMs in Ethiopia has been increasing steadily (Brink, 2010).

Banks continue to invest in expanding their ATM networks, improving service reliability, and integrating new technologies to enhance security and user experience (Paul & Paul, 2013). The spread of ATMs in Ethiopia has contributed to greater convenience for customers, reduced reliance on traditional bank branches, and improved access to financial services, particularly for urban residents and businesses. ATMs in Ethiopia have seen significant growth in recent years, there is ongoing effort to further expand their reach, particularly into rural areas, and to enhance their capabilities to meet the evolving needs of the population.

a b c d

Figure 1: ATMs of Some Selected Banks in Adama city (a). ATM of Commercial Bank of Ethiopia (b). ATM of Abyssinia Bank (c). ATM of Dashan Bank (d) ATM of Oromia International Bank

2.3. Bacterial colonization on ATM surfaces

Microbes are present on surfaces that come into touch with human hands, such as door handles, computer keyboards, mobile phone buttons, and elevator buttons (Bakterien & Von, 2017). Microbial reservoirs are found in different objects. According to numerous epidemiological studies, many contaminated surfaces contributed significantly to the spread of infectious diseases. Numerous fungal, bacterial, and viral pathogens can persist on inanimate objects for several months. These pathogens have the potential to spread epidemically through direct or indirect contact with "hand-object-susceptible patient rings" (Gupta *et al.,* 2005).Surface bacterial colonization can have significant effects on human health. Health care associated infections (HAIs) are infections that can cause serious illness or even death. In healthcare facilities, bacterial colonization on surfaces can aid in the spread of these infections.

According to Hu (2012) surfaces in patient rooms, such as bed rails, call buttons, and door handles, and were frequently contaminated with bacteria, including drug-resistant strains. Similarly, bacterial colonization on surfaces in public places can contribute to the

spread of infectious diseases such as the common cold and influenza. It is critical to implement effective cleaning and disinfection practices to prevent bacterial colonization on surfaces. This may entail using appropriate cleaning agents and techniques, such as disinfectants and steam cleaning. Furthermore, some surfaces may benefit from the application of antimicrobial coatings or other technologies designed to inhibit bacterial growth (Spengler & Urb, 2017).

2.4. Bacteria commonly associated with ATMs and frequently touched Surfaces and objects

According to Bakterien and Von (2017), a number of research groups have studied the microbiological surveillance of automated teller machines (ATMs) and reported that the existence of various bacterial species including *Bacillus spp,* devoid of coagulase in two separate studies conducted in Turkey and India respectively, the following bacteria were found on ATMs: *Klebsiella, P.aeruginosa, E.coli ,S.aureus, Enterobacter, Acinetobacterspp, Coagulase-negative Staphylococcus,* and Non fomenters. *Coagulase negative Staphylococcus* (CoNS) was found to be both Methicillin resistant and susceptible to the drug (Winfield & Groisman, 2003).

2.4.1. *Bacillus spp.*

A genus of rod-shaped, Gram-positive bacteria belonging to the phylum Firmicutes is called *Bacillus*. When oxygen is used or present *Bacillus spp.* which can be facultative or obligate aerobes were test positive for the Catalase enzyme. There are many species of *Bacillus* in nature, including both free-living and parasitic pathogenic ones (Adedoyin,2019).The bacteria have the ability to produce endospore in response to harsh environmental conditions, which allow them to hibernate for extended periods of time.

According to (Aquino *et al*., 2019), *B.subtilis* a significant model organism that is also well-known for being food spoiler that causes ropiness in bread and other related foods. *B.coagulans* strains found in the environment and in commercial products may contribute to food spoilage of highly acidic tomato based products. *B.anthracis*, which causes *anthrax,* and *B.cereus*, which causes food poisoning, are two *Bacillus spp.* that are considered medically significant (Aquino *et al*., 2019).

2.4.2. *Staphylococcus aureus and Coagulase- negative Staphylococcus (CONs)*

Coagulase-negative Staphylococcus that do not produce Coagulase enzyme are classified as *Staphylococci.* They are Gram-positive and can be found singly or in irregular grape-like clusters. *Coagulase negative staphylococci (CoNS)* are naturally present in human skin. Despite their low virulence, they are increasingly being recognized as agents of clinically significant infection of the bloodstream and other sites. When bacteria are present, *Coagulase-negative Staphylococci* are frequently associated with nosocomial infections *Coagulase-negative staphylococci* may be susceptible or resistant to Methicillin (Alwis & Xiaofen, 2012). *S. aureus* is a Firmicutes Gram-positive coccal bacterium that is commonly found in the nose, respiratory tract, and on the skin. It frequently tests positive for Catalase and nitrate reduction (State,2020).

Although *S.aureus* is not always pathogenic, it is a leading cause of skin infections, respiratory infections, and food poisoning also one of the major components of skin and nose micro biota, and survives long periods on inanimate objects. Pathogenic strains frequently spread infections by producing powerful protein toxins and expressing cell surface proteins that bind to and inactivate antibodies (Adedoyin, 2019).A worldwide problem in clinical medicine is the emergence of antibiotic resistant strains of *S.aureus* such as Methicillin-resistant *S.aureus* (MRSA) (Eguia and Chambers, 2003). *Coagulase-negative staphylococci aureus* and *S. aureus* are two different types of bacteria belonging to the *staphylococcus* genus.

The difference between the two like Coagulase production, *S. aureus is* Coagulase positive it produces enzyme Coagulase that cause blood plasma to clot but *CONs doesn't produce* (Yunoki.,2017). *S.aureus* is known for being a pathogenic bacterium that can cause a variety of infection, ranging from minor skin infections to severe conditions like sepsis and pneumonia but *CONs* is less pathogenic compared to *S.aureus* However *CONs* can still cause infections in certain situations particularly in immune-compromised individuals or in medical device-related infections *S. aureus* including Methicillin-resistant *s. aureus*(MRSA), is known for its ability to develop antibiotic resistance making treatment challenging *CONs* can also exhibit antibiotic resistance but have lower resistance rates as compared with *S. aureus* (Id & Akanbi, 2021).

2.4.3. *Escherichia coli*

E. coli is a Gram negative, facultative anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in lower intestines of warm-blooded organisms and mostly recovered in clinical laboratories and has been incriminated in infectious diseases involving virtually every human tissue and organ system (Lehman,2005). Many *E. coli* strains are harmless, but some serotypes can cause severe poisoning in their hosts. Virulent *E. coli* strains can cause Urinary tract and wound infections, pneumonia in immune suppressed hospitalized patients and meningitis in neonates' gastroenteritis are other common infections caused by *E. coli*. *E. coli* is one of the common organisms involved in gram-negative sepsis and endotoxin induced shock (Adedoyin., 2019).*E. coli* is a normal flora of the gastrointestinal tract which can easily be picked up from toilet door handles. In a society of low hygiene, this probably explains its preponderance as a bacterial contaminant of shared user hardware interfaces. It has also been associated with numerous infectious disease conditions and nosocomial infections. It follows that since users constantly touch interfaces there is every chance of introducing *E. coli* onto the interface in use also airborne organisms can be transported from users (Winfield & Groisman, 2003).

2.4.4. *Klebsiella spp.*

Klebsiella is a genus of Gram-negative bacteria commonly found in the environment and in the human digestive system. Some species of *Klebsiella*, such as *K.pneumoniae* can be opportunistic pathogens and cause infections in humans, particularly in healthcare settings. *Klebsiella spp*. is known for causing a range of infections including pneumonia, Urinary tract infections, blood stream infections and wound infections. *Klebsiella spp*. are typically transmitted through contact with contaminated surfaces, healthcare equipment, or person to person contact (Id & Akanbi, 2021). *K.pneumoniae* is found in the normal flora of the mouth, skin, and intestines, it can cause destructive changes to human lungs if aspirated (inhaled), specifically to the alveoli (in the lungs) resulting in bloody sputum (Rusin *et al*.,2002).In the clinical setting it is the most significant member of the *Klebsiella* genus of *Enterobacteriaceae*. In recent years, *Klebsiella* have become important pathogens in nosocomial infections(Paulo, 2001).

2.4.5. *Pseudomonas aeruginosa*

P.aeruginosa is a Gram-negative, rod-shaped bacterium commonly found in soil, water and other moist environments. It is an opportunistic pathogen that can cause infections in humans, particularly in individuals with weakened immune systems or underlying health conditions. *P.aeruginosa* is known for its ability to cause a wide range of infections, including pneumonia, urinary tract infections, skin infections, and sepsis. It is a significant cause of healthcare-associated infections, particularly in hospital settings (Jones, 2011). *P.aeruginosa* has a remarkable ability to develop resistance to multiple antibiotics, making treatment challenging. This bacterium is often resistant to many commonly used antibiotics, including carbapenems and fluoroquinolones. *P.aeruginosa* is known for its ability to form biofilms, which are communities of bacteria encased in a matrix of extracellular polymeric substances (Diagnostic, 2005). Biofilms help protect the bacteria from antibiotics and the host immune system, making infections difficult to eradicate. Due to its high level of antibiotic resistance, infections caused by *P.aeruginosa* can be challenging to treat. Treatment typically involves the use of combination therapies and antibiotics that are effective against multidrug-resistant strains (State, 2021).

2.4.6. *Salmonella spp.*

Salmonella spp. refers to a group of bacteria belonging to the genus *Salmonella*. These bacteria are known for causing food borne illnesses in humans and animals. Human infections with salmonella are most caused by ingestion of food, water or milk contaminated by human or animal excreta. Salmonella are primary pathogens of lower animals (eg. poultry, cows, pigs, pets, birds, sheep, donkeys, lilizards and snakes) which are the principal source of nontyphoidal *salmonellosis* in humans (Hu, 2012).The food borne ingestion of *S.thyphimurium* from contaminated hands also causes *Salmonellosis*. *Salmonellosis* is a food borne illness caused by infection with salmonella bacteria. *Salmonella* infection affects the intestines and causes vomiting, fever, and cramping which usually clear up without medical treatment. *Salmonella* infection can be prevented by not serving any raw meat or eggs and not keeping reptiles as pets (particularly for young children). Hand washing is a powerful way to guard against salmonella infections (Duraipandian, 2016).

2.4.7. *Shigella spp.*

Shigella is a genus of Gram-negative, non-motile, rod-shaped bacteria that can cause severe gastrointestinal infections in humans. *Shigella species* are non-lactose fermenters and tend to be biochemically inert. There are four major subgroups. The CDC classification combines *S. dysenteriea* (group A), *S.flexneri* (group B) and *S.boydii* (group C) as *shigella* sero groups A, B, C because of their biochemical similarities. *Shigella* infection (also known as *shigellosis*) is an infection of the digestive tract (or gut) caused by *shigella* bacteria (Winfield & Groisman, 2003).Symptoms started between 1 to 7 days (usually 1 to 3 days) after the ingestion of the bacteria and this typically last for between 4 to 7 days. The symptoms include dysentery (diarrhea containing mucus/blood,) nausea and vomiting, fever and stomach cramps. All species of *shigella* cause acute bloody diarrhea by invading and causing patchy destruction of colonic Epithelium.

This leads to the formation of micro ulcers and inflammatory exudates and causes inflammatory cells (polymorph nuclear leukocyte, PMNs) and blood to appear in the stool. Poor hygienic practices coupled with other unwholesome behavioral and cultural activities might be responsible for this infection (Nwankwo & Offiah, 2016). *Shigella* infections involve promoting good hygiene practices, such as hand washing with soap and water, especially after using the bathroom or changing diapers. Ensuring safe food handling and clean water sources also play a significant role in preventing the spread of *Shigella.* Health concerns related to drug-resistant bacteria, also known as antibiotic-resistant bacteria Drug-resistant bacteria, are more difficult to treat because they do not respond to standard antibiotic treatments.

This can result in prolonged illness, increased severity of infections, and higher mortality rates. Treating infections caused by drug-resistant bacteria often requires more expensive and prolonged therapies, leading to higher healthcare costs for both individuals and healthcare systems Drug-resistant bacteria can spread easily within healthcare settings, communities, and even globally (Spengler & Urb, 2017). This can lead to outbreaks of infections that are difficult to control. Infections caused by drug-resistant bacteria can result in more severe complications and longer recovery times for patients. This can impact their quality of life and overall health outcomes (Cimolai, 2008). Vulnerable

populations, such as the elderly, immune compromised individuals, and those with underlying health conditions, are at higher risk of infections caused by drug-resistant bacteria. This can lead to poorer health outcomes in these groups. The rise of multidrug-resistant bacteria, also known as superbugs, poses a significant threat as these bacteria are resistant to multiple classes of antibiotics (Saroja *et al*.,2013).

Infections caused by superbugs can be extremely difficult to treat and, in some cases, may be untreatable. The rise of multidrug-resistant bacteria, also known as superbugs, poses a significant threat as these bacteria are resistant to multiple classes of antibiotics. Infections caused by superbugs can be extremely difficult to treat and, in some cases, may be untreatable (Konheim, 2016).

2.5. Health risk associated with bacterial contamination on ATMs

Bacterial contamination on Automated Teller Machines (ATMs) has the potential to endanger the health of those who use them. ATMs are commonly used in public places by many people, and the presence of bacteria on ATM surfaces can increase the risk of bacterial transmission and the spread of infectious diseases (Onuoha & Fatokun, 2014). Numerous investigations have revealed a variety of bacteria, such as *B.cereus*, *E.coli*, and *S.aureus*, on ATM surfaces. Numerous health issues, such as diarrhea, food poisoning, and skin infections, can be brought on by these bacteria. The presence of *S.aureus* on ATM surfaces, for example, has been linked to an increased risk of skin infections (Rufa,2019).The obvious health risks associated with bacterial contamination on ATM surfaces, the presence of bacteria can also reduce the effectiveness of cleaning and disinfection procedures. Despite routine cleaning and disinfection, the level of bacterial contamination on ATM surfaces increased over time (Saroja *et al*., 2013). This suggests that current cleaning and disinfection practices may not be adequate to control bacterial growth and spread on ATM surfaces. To reduce the health risks associated with bacterial contamination on ATM surfaces, effective cleaning and disinfection practices must be implemented (Gupta *et al*., 2005).

This could include using appropriate cleaning agents and techniques, such as alcohol-based disinfectants (Hussey, 2013). Furthermore, ATM users can reduce the risk of

bacterial transmission by using hand sanitizer or washing their hands after using an ATM. Generally, bacterial contamination on ATM surfaces can pose significant health risks, and is critical to take precautions to limit the spread of potentially harmful pathogens (Id & Akanbi, 2021).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Description of Study area

The study was conducted at the Adama Public Health Research and Referral Laboratory Center, East Shoa, Oromia Region. Adama City is located at longitude and latitude of 8.54 ºN&39.27ºE, respectively at an elevation of 1712 meter, 99 km far southeast of Addis Ababa the capital City of Ethiopia. Adama City is found between the base of an escarpment to the west and Great Rift Valley to the east. The average annual temperature of the city is 20.7 ºC or 69.3 ºF and about 371 mm or 14.6 inches of precipitation falls annually. Population in Adama City: 121,254 males and 119,525 females, for a total of 240,779(CSA, 2023).

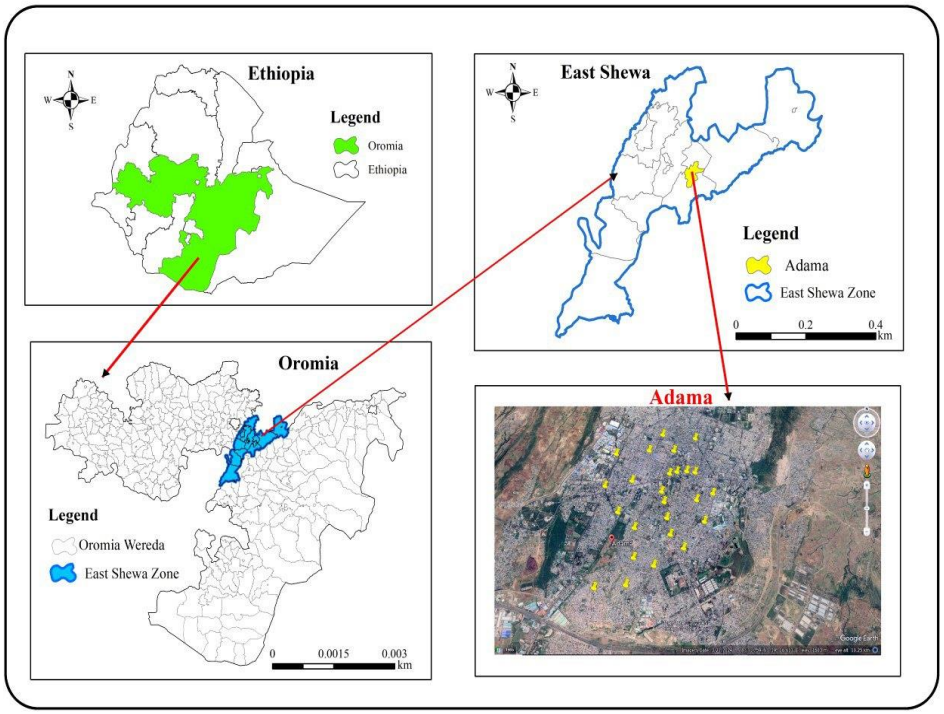


Figure 2: Map of Oromia, Adama and the Site of Sample Collection: (Miller, 2006) Google map, n.d.

3.2. Samples and sampling techniques

3.2.1. Sample size determination

A total of two hundred sixty-two ATMs found in Adama city. Out of these, Eighty (80) ATMs were randomly selected for this study. Commercial Bank of Ethiopia (109), Dashen Bank (24), Bank of Abissinia (47), Awash Bank(26), Buna Bank(14), Abay Bank(4), Brehan Bank(4), Cooperative Bank of Oromia(8), Nib Bank(7), Zemen Bank(6), Wegagen Bank(5), Debub global Bank(1), Amhara Bank(2), Oromia International Bank(4), Addis International Bank(1).(Source; MOTI Engineering PLC).

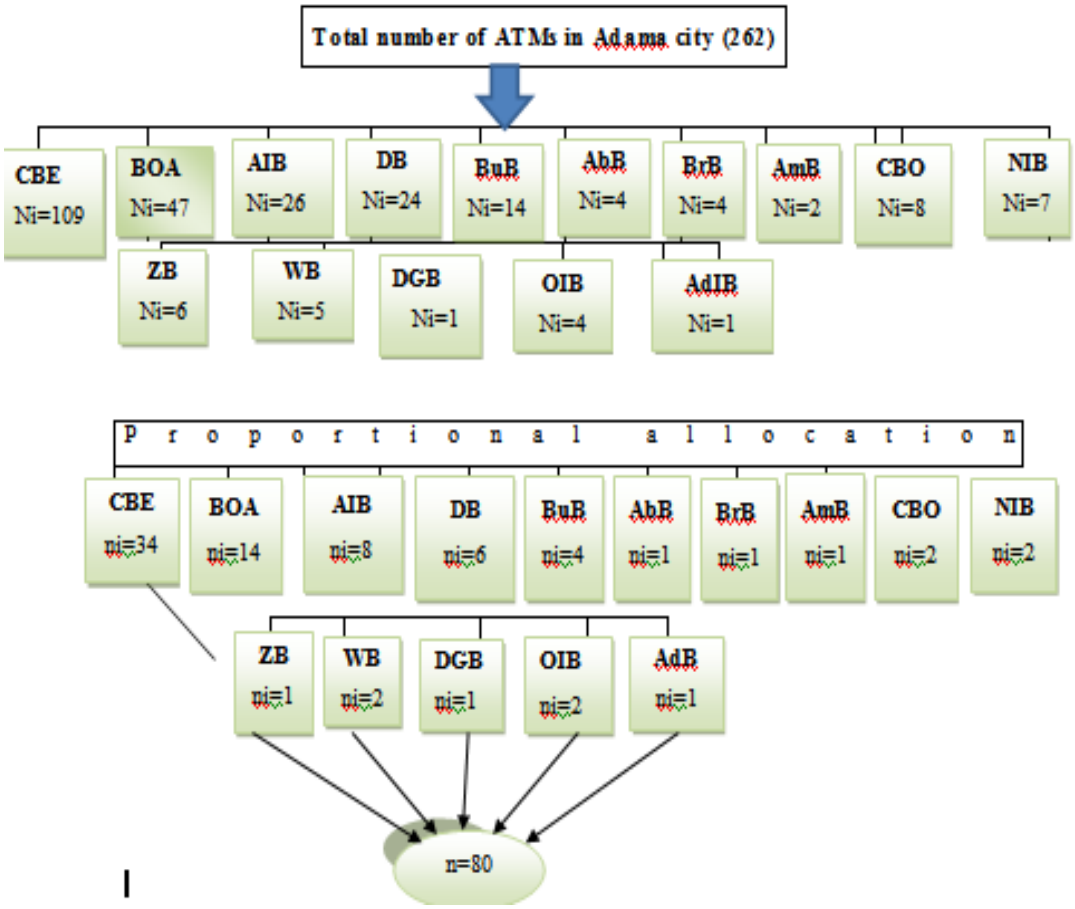


Figure 3: Schematic presentation of the sampling technique

3.2.2. Sample Collection and Analysis

Swab samples were taken from representative ATMs during the period of March to May 2024. The samples were collected using sterile swabs rubbed on the keypads and other touch surfaces of the ATMs. The sterile tube was labeled with unique sample code and immediately transported in ice box with in 2hours to the bacteriology department of Adama Public Health Research and Referral Laboratory Center for analysis within 24hours.The collected swab samples were streaked onto blood, MacConkey and Chocolate agar plates following incubation at 37°C for 24-48 hours (Hussey,2013). Colonies from the plates were sub-cultured on fresh agar plates to obtain pure cultures. The pure culture was identified using Gram staining techniques and biochemical characterization including, Catalase test, Oxidase test, Indole test, Triple sugar iron test, Citrate test, Urease test, Motility test also H_2S production test (Stanley,2015).

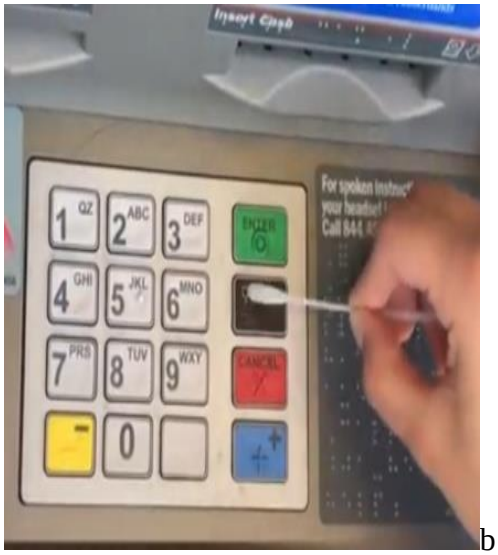
a  b 

Figure 4: Sample collection and transportation (a).Sample taking by swabbing the keypads (b). Sterilized fulken tube that had saline solution

Table 1: Bacterial isolate Sample source and code

Sample Code	Name of the bank	Branchor location of ATMs
ICBE1	CBE	Around 04
ICBE2	CBE	Around jemalmegazen
ICBE3	CBE	Around bole
ICBE4	CBE	Aroundderartutulu street
ICBE5	CBE	ASTU compound
ICBE6	CBE	Adama buta road
ICBE7	CBE	Adama buta road
ICBE8	CBE	Aba geda street
ICBE9	CBE	Aba jifar road
ICBE10	CBE	Daka-adi branch
ICBE11	CBE	Around selase church
ICBE12	CBE	Around selase church
ICBE13	CBE	Elemo branch
ICBE14	CBE	Elemo branch
ICBE15	CBE	Sar-tera branch
ICBE16	CBE	Around posta1
ICBE17	CBE	Around posta2
ICBE18	CBE	Around posta3
ICBE19	CBE	Around posta4
ICBE20	CBE	Around tikur-abay1
ICBE21	CBE	Around tikur-abay2
ICBE22	CBE	Berecha branch
ICBE23	CBE	Turban obo branch
ICBE24	CBE	Adama hospital
ICBE25	CBE	Gendegara branch
ICBE26	CBE	Around gendegara
ICBE27	CBE	Around amede1
ICBE28	CBE	Around amede 2
ICBE29	CBE	Around franko1
ICBE30	CBE	Around franko2
ICBE31	CBE	Aba-geda branch
ICBE32	CBE	Around robi hotel
ICBE33	CBE	Adama branch
ICBE34	CBE	Irrecha branch
IBOA1	BOA	Around 09 1
IBOA2	BOA	Around 09 2
IBOA3	BOA	Around 09 3
IBOA4	BOA	Around 09 4
IBOA5	BOA	Adama menaheriya branch
IBOA6	BOA	Around elemokiltu road
IBOA7	BOA	Around posta 1
IBOA8	BOA	Around posta 2
IBOA9	BOA	Around posta 3
IBOA10	BOA	Around posta 4
IBOA11	BOA	Around warka1
IBOA12	BOA	Around warka2
IBOA13	BOA	Around warka3
IBOA14	BOA	Arada branch
IAIB1	AIB	Around posta1
IAIB2	AIB	Around posta2
IAIB3	AIB	Bole branch
IAIB4	AIB	Adama General hospital
IAIB5	AIB	Around sartera
IAIB6	AIB	Selase branch
IAIB7	AIB	Gendegara branch
IAIB8	AIB	Around ASTU
IDB1	DB	Around pota road1
IDB2	DB	Adama-Ras branch
IDB3	DB	Selase branch
IDB4	DB	Kechema branch
IDB5	DB	Dashen bank building
IBuB1	BuB	Selasebanch
IBuB2	BuB	Sartera branch
IBuB3	BuB	Tikur-abay branch
IBuB4	BuB	Gendegara branch
IAbB1	AbB	Around adama hospital
IBrB1	BrB	Around mebrathaile road
IAmB1	AmB	Around posta bet road
ICBO1	CBO	Chafe branch
ICBO2	CBO	Derartutulu branch
INIB1	NIB	Sartera branch
INIB2	NIB	Adama menahariya branch
IZB1	ZB	Dada-mall building
IWB1	WB	Around mebrathaile
IWB2	WB	Around franko road
IDGB1	DGB	Dembela branch
IOIB1	OIB	Arada branch
IOIB2	OIB	Boste branch
IOIB3	OIB	Kerayu branch
AdIB1	AdIB	Adama branch

N.B: I(isolates) CBE(Commercial Bank of Ethiopia),BOA(Bank of Abissinia),AIB(Awash International Bank), DB(Dashen Bank), BuB(Buna Bank), NIB(Nib Bank), AbB(Abay Bank), DGB(Debub Global Bank), BrB(Brehan Bank), WB(Wegagen Bank), AmB(Amhara Bank), CBO(Cooperative Bank of Oromia), ZB(Zemen bank), AdIB(Addis International Bank, OIB(Oromia International Bank).

3.2.3. Characterization and Identification of Bacterial Isolates

Bacterial isolates were characterized in a three–step approaches (State ,2020).The three step approach in the characterization of bacterial isolate includes the following: Colony features like hemolytic activity, pigment, consistency, and colour.

Morphological analysis: Essentially, the shape and arrangement of each isolate's cells was examined in stained slide mounts of the isolates, during which their reaction to general dye, the Gram-stain, as well as reactions to specific dyes that showed the presence or absence of characteristic features such as capsule, flagella, spore so on was determined (State, 2020).Wet mounts was used to examine the motility of the isolate in grooved slides under a microscope.

Gram-staining: Gram's staining was performed on the bacteria to differentiate between gram-positive and gram-negative species. Bacterial colonies were smeared on a drop of water on a clean glass slide then left to dry and heat fixed. Then, the slides were flooded with crystal violet dye for 1 min followed by rinsing by tap water. Then the slides were flooded with gram's iodine for 1min as a mordant and again rinsed by tap water. Thirdly, the slides were rinsed with 70% (v/v) ethanol for 15 sec and rinsed with tap water. Lastly the slides were flooded with safranin as a counter stain for 1 min and rinsed with tap water. Next the slides were allowed to dry and were viewed under microscope examined using an oil immersion objective (x100). The results were photographed and recorded (State, 2020).

Endospore-staining: Endospore-staining procedures were used to assess their endospore-producing ability. The isolate that is characterized by endospore staining is Bacillus species. Bacterial colonies from slightly older cultures were applied to a clean slide using a drop of water. The smears was then heated and fixed. Blotting papers were cut to the size of the slides and placed on them.

The slides were flooded with the malachite green solution and were heated over aflame for 5minutes, with ample malachite green solution added as needed. Following that, the blotting papers were removed, and the slides were rinsed with tap water. The slides was then flooded with safranin for 30 seconds before being rinsed with tap water (State, 2020). Finally, the slides were allowed to dry before being viewed under an x100 microscope with oil immersion. The results were photographed and documented.

3.3. Biochemical Tests

Different biochemical tests were performed to identify the isolated bacteria strains described as follows Catalase test, Oxidase test, Citrate utilization test, H_2S production test, Indole production test, Motility test, Urease test, Triple sugar iron (TSI) test. Depending on the test results the bacteria could be categorized to the different genera they belong according to Bergey's Manual of Determinative Bacteriology (Holt, 2003).

Catalase test: For Catalase tests fresh 18–24-hour old pure bacterial colonies were used. A 3-4 ml of 3% (v/v) H_2O_2 were poured into sterile test tubes and then pure bacterial colonies taken with the stick side of cotton swab were immersed fully into the test tubes. Then, formation of bubbles due to conversion of H_2O_2 into H_2O and O_2 was checked for in the test tubes (Holt, 2003)**.**Here Slide method was also used for Catalase test. Briefly a loop full of the isolated bacteria was smeared on a clean microscope slide and 2-3 drops of 3% (v/v) H_2O_2 was added directly on the smear, the formation of visible bubbles indicates positive result and result were recorded and photographed.

Oxidase test: A piece of filter paper was soaked with a few drops of Oxidase reagent (*Tetramethyl-p-phenylene diamine dihydrochloride*). A colony of the test organism was then smeared on the soaked filter paper. If the organism could produce Oxidase, the *phenylene diamine* in the reagent was oxidized to deep purple color. The change of color within 10 seconds indicates positive result (Holt, 2003)**.**

Citrate utilization test: Simmons Citrate agar, (Bromothymolbule 0.08 g/L; $MgSO_4$ 0.2 g/L; (NH_4) $2HPO_4$ 1 g/L and Agar 15 g/L) a medium containing citrate as the sole carbon source and ammonium salts as the sole nitrogen source was used to test for the ability of the bacteria to use organic compounds other than sugars as their sole source of carbon. Fresh 18-24 hours old pure

bacterial colonies were streaked on the slants and the test tubes were incubated at 37^0C for 2-4 days. If the bacteria metabolize citrate the color of the Simmons citrate agar slants will change from green to deep blue (Holt, 2003).

Urease test: For Urease test urea agar slants were prepared using urea agar base (Dextrose 1g/L; Disodium phosphate 1.2 g/L; Mono potassium phosphate 0.8 g/L; NaCl 5 g/L; peptone 1g/L; phenol red 0.012 g/L and Agar 15 g/L) with addition of 40% (w/v) urea which was added to the agar base after sterilization and cooling down. Fresh 18-24 hours old pure bacterial colonies were streaked on the slants and incubated at 35^0C for up to 5 days. The bacterial colonies that can produce Urease and convert the urea into CO_2, H_2O and NH_4 was covert the slant colors from yellow to bright pink (Holt, 2003)**.**

Triple sugar iron (TSI) test: Slant of triple sugar iron medium was prepared in the test tube by auto calving at 121^0C. Using a sterile technique; a 20 ml of the experimental bacterium from 24h old pure culture was inoculated into the test tubes using a stab and streak inoculation method with an inoculating needle. The screw caps were not fully tightened, and the tubes were incubated for 24 h at 37 ^{0}C. Fermentation is indicated by the yellowing of the butt and the slant of the Triple Sugar Iron (TSI) agar medium. If gas was formed during the fermentation, it is shown in the butt either by the formation of bubbles or cracking of the agar (Holt, 2003)**.**

H_2S Production, motility and Indole production (SIM) test: To find out the isolated organism's ability to reduce sulfur, produce indole and for motility, SIM media was used. SIM media is a differential medium that tests for the three characteristics simultaneously. For this experiment SIM medium (Beef extract 3 g/L; $Na_2S_2O_3$ 0.025 g/L; peptic digest of animal tissue 30 g/L; Peptonized iron 0.2g/L and agar 3 g/L) was prepared and was poured into test tubes. After solidifying the test tubes were inoculated by stabbing in the center to half inch then test tubes were incubated for 24 hours at 37^0C. After 24 hours the test tubes were checked for the formation of a black precipitate (H_2S production and fuzzy area surrounding the stab line (motility) and then 2-3 drops of kovac's indole reagent was added to the tubes and the formation of red band was checked (Holt, 2003)**.**

3.3.1. Identification of *S.aureus*

For identification of *staphylococcus spp* Manitol salt agar was used. Streak a plate of Manitol salt agar with appropriate culture using the quadrant streak plate method placed a pure colony on nutrient agar and incubated at 37^0C for 24hr. The result was interpreted: yellow colour colonies on Manitol salt agar were *S.aureus* (State, 2020).

3.4. Identification of isolates using MALDI-TOF

The isolated bacteria were investigated using the Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) which analyzes the protein or peptide constituents of a sample microorganism and enables identification of the microorganisms up to genus and species level. MALDI TOF-MS identification of the bacterial isolates was carried out at the National Animal Health Diagnostic and Investigation Center (NAHDIC) found in Sebeta. Pure culture preparation was carried out in Adama public Health Research and Referral Laboratory center. Each selected bacteria colony after the biochemical test carefully picked and purified in nutrient agar and incubated at 37^0 for 24 hours (Division & Lumpur, 2014).The overnight grown bacteria were carefully covered by Aluminum foil and transported to Sebeta using ice free glass box.

The extended direct transfer (eDT) procedure as per manufacturer's instruction was used to analyze the bacteria. The extended direct transfer method uses 70% formic acid to lyses the cell membrane of the bacteria for simple extraction of the microbial proteins or peptides and makes for easier characterization of the samples. The target plate which contains 94 wells was cleaned thoroughly and was dried before starting the procedure. Then, a distinct colony from the overnight grown bacteria was smeared directly on the wells as a thin film and left to dry. Then the spot was overlaid with 1µl of 70% (v/v) formic acid and allowed to dry at room temperature. Then, immediately the spot was overlaid with 1µl of α-cyano-4hydroxycinnamic acid (HCCA) solution and allowed to dry at room temperature. Then after the samples were dried the target plate was placed inside the MALDI Biotyper and analyzed. The samples were then said adequately identified to the species level when the logarithmic score value (LSV) is greater than or equal to 1.9 (Nworie *et al.*, 2012).

3.5. Antimicrobial Susceptibility Testing

The antibiotic sensitivity of the isolates was tested using the Kirby Bauer antibiotics disc method against the following antibiotics in accordance with the Clinical Laboratory Standards Institute guidelines (Bakterien & Von, 2017).The antibiotic discs were obtained from Adama Public Health Research and Referral Laboratory Center. For gram-negative used antibiotics disk are Ampicilin(10µg); Amoxicillinclavulanate(20/10µg); Cephazolin(30µg); Cefepime (30µg); Cefotaxime (30µg); Ceftazidime (30µg); Ciprofloxacin (5µg); Gentamicin (10µg); Imipenem (10µg); Piperacilin/Tazobactam (100/10µg); Clindamycin (2µg); Erythromycin (15µg); PenicilinG(10µg); Tetracycline(30µg); Sulfa/Trimethazole(1.25/23.75µg). For gram-postive used antibiotics disk are Ampicilin(10µg); Cefepime (30µg); Ciprofloxacin (5µg); Clindamycin (2µg); Gentamicin (10µg); Erythromycin (15µg); Piperacilin/Tazobactam (100/10µg);Tetracycline(30µg) (Brook et al.,2013). In brief, the pure isolate (four to five colonies) was added to a sterile tube containing 5ml of normal saline and gently mixed until homogeneous suspension was formed. Droplet and McFarland Densitometer (Made in Germany for measurement of cell suspension's turbidity) was used to standardize the turbidity of bacterial suspension.

The turbidity of the suspension was compared against a reference 0.5 Mcfarland tube. A sterile cotton swab was dipped into the suspension and used to inoculate the bacterial suspension over the entire surface of Muller Hinton agar (Brook et al.,2013). The bacterial suspensions were left to dry at room temperature for 3to 5 minutes. The antimicrobial drug discs were then placed on Muller Hinton agar using a disc dispenser and incubated at 37^0 C for 18-24 hours. The diameter of Zone of inhibition was measured with digital caliper (Made in France by Pierre vernier also known as Vernier caliper used for making accurate linear measurements) at the end of the incubation period. Based on this measurement, the isolates were categorized as sensitive, intermediate and resistant following CLSI guidelines. A positive control was performed against specific bacterial type strains obtained from Adama public health research and referral laboratory center. These strains were; B.*subtilis*ATCC 6010^T, *E.coli*ATTC25922^T, *P.aeruginosa ATCC27853T, S.thyphimurium ATCC13311T, S.aureusATCC25923T,* and *S.pyogen ATCC12204 T.* In this study, DMSO was used as a negative control.

3.6. Determination of Occurrence

The occurrence of each bacterial species isolated from the test samples was determined as a percentage ratio of their prevalence relative to the total number of samples examined using the formula of (Stanley and Okpara 2015).

$$\% \text{ Occurrence} = \frac{\text{No. of positive test} \times 100}{\text{Total No. tested}}$$

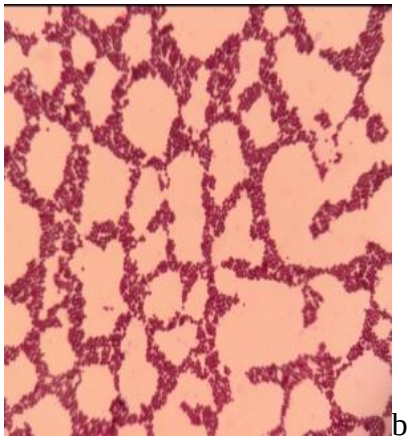
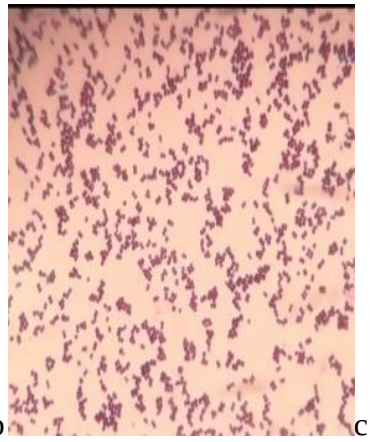
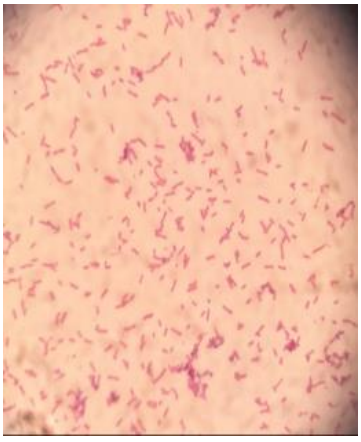
3.7. Data analysis

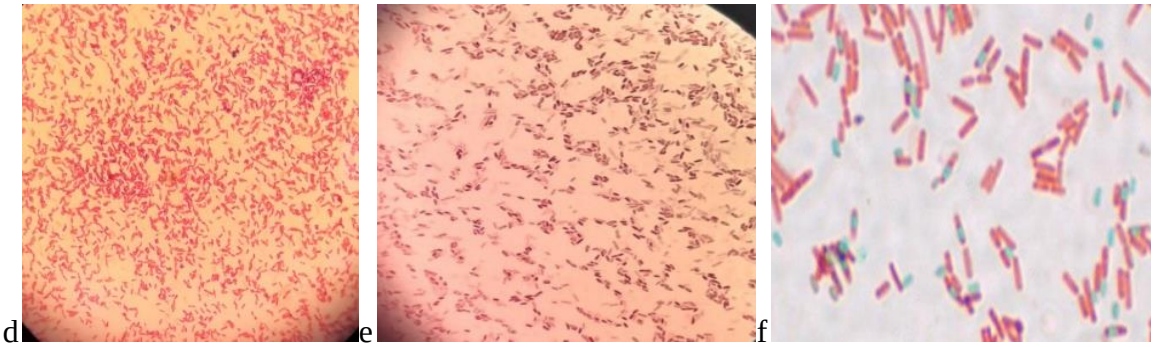
The data obtained from this study was analyzed by using Statistical Package for Social Science (SPSS, version 27) and MS excel sheet. The results were presented through texts, tables, and graphs.

CHAPTER FOUR

4. RESULTS

A total of 77 bacteria were isolated and categorized into two groups of which more than half (58.4%) were gram-negative rods followed by Gram-postive cocci and Gram-positive rods (41.5%) (Figure 8). Based on morphological and biochemical tests, they were identified: *Escherchia coli*, *Psudomonas aeruginosa*, *Shigella dysenteriea*, *Salmonella tyhimurium*, *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus spp. Shigellaspps. Listeria spp. Serattia spp. Citrobacter spp., Protous mirballis, Providencia stuarti* and from the result of MALDI-TOF MS 16151 (ICBE), 16153 (ICBE5), 16157 (ICBO2), 16160 (ICBE25) were identified *as K. pneumoniae* with scores 2.32, 2.36, 2.21, 2.05, respectively. These 4 isolates scores indicate high confidence identification, whereas 16154(IBOA2) 1.89 was identified as *K. variicola* indicating low confidence identification. From table 5 it was observed that *S.aureus* and *Listeria spp.* was the commonest organism isolated (20%) and *Listeria spp.* (16.25%), *Serattia spp.* (12.50%), *Salmonella spp.* (11.25%), *Protous mirballis* (7.50%), *Escherchia coli* (6.25%), *Klebsiella pneumonia* (3.75%), *Bacillus spp*(3.9%), *Shigella spp.(*3.75%), *Citrobacter spp.*(5.2%), *Psudomonas aeruginosa* (2.50%), *Providencia stuarti*(2.50%) , *Salmonella tyhimurium* showed the least percentage occurrence with (1.25%) respectively.

a  b  c 



Gram's staining of isolated pathogenic bacteria viewed fewer than 100 x magnifications

Figure 5: Gram's staining of isolated pathogenic bacteria viewed under 100 x magnifications (a) A Gram-positive cocci shape in cluster form (b). Gram-positive cocci shape in single form (c). Gram-postive rod shape form in cluster (d) Gram-negative rod shape in cluster form (e). Gram positive rod with spore (f).Endospore staining result of *Bacillis spp*.

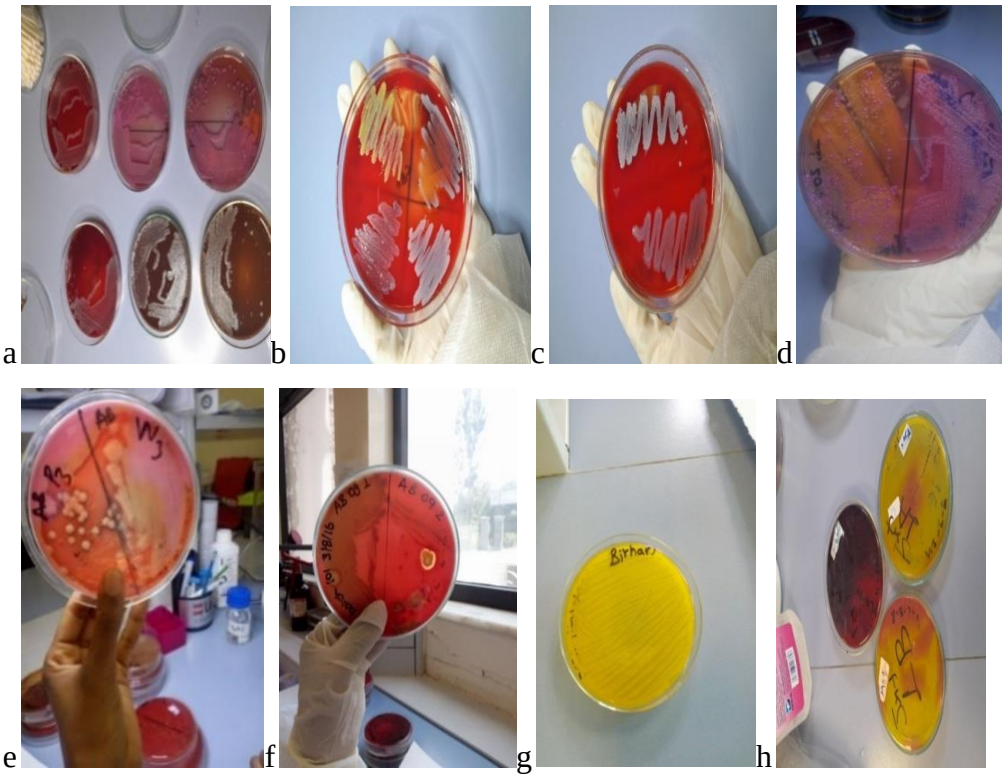


Figure 6: Cultural and Colony Appearance of pathogenic bacteria isolated from ATMs (a). Growth of bacteria on different culture media Chocolate and MacConkey and blood agar (b)&(c)Colonies that were sub-cultured on fresh media until pure microscopically (d)Growth of colonies on MacConkey agar (e)&(f).growth of colonies with hemolysis (g)&(h) growth of colonies on Manitol salt agar turn the colour to yellow *(s.aureus).*

4.1. Biochemical Tests conducted on the isolated Pathogenic Bacteria

The result of the biochemical tests conducted on the isolated pathogenic bacteria (that is, *E. coli, P. aeruginosa, S. dysenteriea, S. tyhimurium, S. aureus, K. pneumoniae, Bacillus spp., shigella spp., Listeriaspp., serattia spp., Citrobacter spp., P. mirballis, P. stuarti* presented in Table 2. Eight (8) tests which include triple sugar iron (TSI); Catalase; Oxidase; Indole; H_2S; Motility; Citrate utilization test; Urease test; were carried out on thirteen (13) isolates for each of the pathogenic bacteria isolated from the samples for confirmation and identification.

In Table 2, out of the thirteen (13) isolates tested for TSI, *p. aeruginosa, bacillus spp., shigella spp., and Listeriaspp.* It was negative. *Listeriaspp.* and *shigella spp.* are typically non fermenters of lactose and sucrose and with no gas or H_2S production also *p. aeuroginosa* don't ferment sugar with no gas or H_2S production. *Bacillusspp.* Also negative for lactose and sucrose fermentation and shows alkaline /alkaline with no gas and H_2S production. While others were positive *E. coli, S. dysenteriea, S. tyhimurium, K. pneumoniae, serattia spp., Citrobacter spp., P.mirballis, P.stuarti. E. coli ferments* glucose and lactose with a gas production (Figure 7). *S. dysenteriea* ferments glucose only with alkaline slants. *S. typhimurim* also ferments glucose and lactose without gas production.

K. pneumoniae ferments glucose and lactose with gas production. *C.freundi* is also glucose fermenter and produce both acid and gas also H_2S. *Serattia spp.* Usually, glucose fermenters produce H_2S (Figure 7). *P. stuarti* also glucose fermentor and produce acid and gas H_2S production. *P. mirballis* also a glucose fermenter and can produce acids and gas also H_2S production. Table 2 also revealed that *E. coli* and *S. dysenteriea, P.stuarti* were indole positive means they produce indole from tryptophan (Figure 7c). While S. *tyhimurium, S.dysenteriea and K.pneumoniae, Bacillusspp. C.freundi, S. aureus, shigella spp. serattia spp., Listeriaspp. P. mirballis* were indole negative means they can't produce indole from tryptophan (Table 2).

However, all the isolates were Catalase positive as revealed in (Table 2 & Figure 7e) that all exhibited Catalase activity, leading to postive Catalase result. The citrate utilization test was positive in *P.aeruginosa, S.aureus* and *K.pneumoniae, Bacillusspp., C.freundi, S. aureus, serattia spp., P. stuarti, P.mirballis* were positive they can utilize citrate as carbon sources this lead to colour change from green to blue (Figure 7h) but *E.coli, S.dysenteriea, S.thyphimurium,*

shigella spp., Listeriaspp., were negative means they can't utilize citrate as carbon sources and the media remains green without colour change (Table 2). The Urease test was positive in *E.coli, P.aeruginosa, S.aureus and K.pneumoniae, C.freundi, P.stuarti, P.mirballis* means they hydrolyzes urea to form ammonia which raises the PH of the medium and cause it turns to pink (Figure 7g) but negative in *S.dysenteriae, S.typhimurium, Bacillusspp., shigellaspp., serattiaspp., listeriaspp.*

This means they don't hydrolyze urea in the medium that's why no colour changes in the medium. Oxidase was postive in *P. aeruginosa, Bacillusspp. Serattiaspp.* that produce enzyme oxidase (Figure 7f) but negative in *E. coli, S. dysenteriea, S. tyhimurium, K. pneumoniae, C.freundi, S.aureus, Shigellaspp., P.stuarti, listeriaspp., P. mirballis* means they don't produce oxidase enzyme. The motility test was postive in *E. coli, P. aeruginosa, S. tyhimurium, Bacillusspp., C. freundi, Serattiaspp., P. mirballis* all were motile but negative in *S. dysenteri, K. pneumoniae, S. aureus, shigellaspp., P. stuarti* (Table 2).

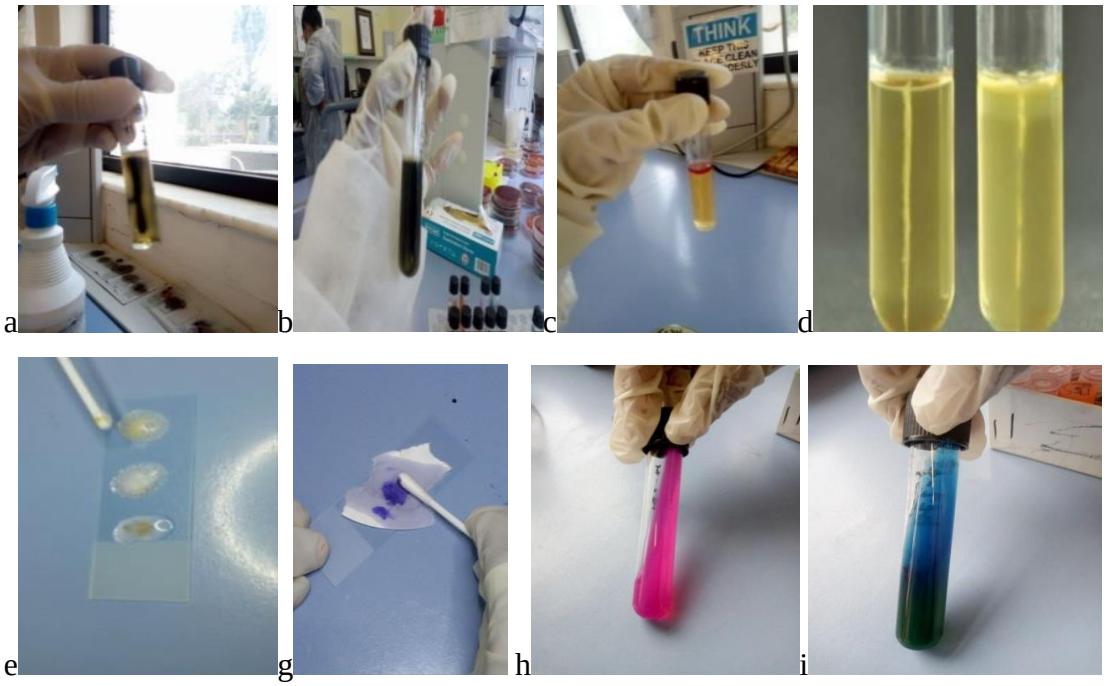


Figure 7: The results of biochemical tests performed to identify bacterial isolates recovered from ATM centers (a) & (b) SIM test with H_2S positive that black precipitate formed (*p.mirballis*) (c).Indole test that formed bright red colour ring formed that indicates presence of indole (d).Motility in SIM medium with a non-motile organism on left and a motile organism on the right (e). Catalase test the first two formed bubble that indicated production of O_2 gas the last there was no bubble negative (f). Oxidase test positive result (g). Urease test positive result (h). Citrate test results positive.

Table 2: Results of biochemical tests performed to identify bacterial isolates recovered from ATM centers

TSI/ KIA	Indole	Catalase	Citrate	Urease	Oxidase	Motility	H_2S	gas	Identified bacteria	Bacteria code
A/A	+	+	-	+	-	+	-	+	*E. coli*	**I1**
K/K	-	+	+	+	+	+	-	-	*P.aeurognosa*	**I2**
K/A	+	+	-	-	-	-		-	*S.dysentriae*	**I3**
K/A	-	+	-	-	-	+	+	+	*S.typhimurim*	**I4**
A/A	-	+	+	+	-	+	-	+2	*K.pneumonia*	**I5**
K/K	-	+	+	-	+	+	-	-	*Bacillusspp*	**I6**
A/A	-	+	+	+	-	+	+	+	*C.freundi*	**I7**
	-	+	+	+	-	-	-		*S.aureus*	**I8**
K/K	-	+	-	-	-	-	-	-	*Shigellaspp*	**I9**
K/A	-	+	+	-	+	+	-	+	*Serratia spp*	**I10**
K/A	+	+	+	+	-	-	-	-	*P.stuarti*	**I11**
K/K	-	+	-	-	-			-	*Listeria*	**I12**
K/A	-	+	+	+	-	+	+	+	*P.mirballis*	**I13**

KEY: +=positive -=negative A/A=sucrose or lactose fermented but not glucose K/A=glucose fermented but not sucrose or lactose K/K=no fermentation has occurred with glucose, lactose, or sucrose TSI=triple iron sugar ATM=automated teller machine.

4.2. Occurrence of Pathogenic Microorganisms in ATMs in the Study Area

Table 3 showed the number (percentage) of isolates obtained from ATMs from various locations within Adama city. A total of eighty (80) samples were obtained from fourteen (15) different banks found in Adama city. These banks were: Commercial bank of Ethiopia(34), Dashen bank(6), Abissinia bank(14), Awash international bank(8), Buna bank(4), Abay bank(1), Birhan bank(1), Cooperative bank of Oromia(2), Nib bank(2), Zemen bank(1), Wegagen bank(2), Debub global bank(1), Amhara bank(1), Oromia international bank(2), Addis international bank (1)as shown in Table 3 below. Out of the thirty four (34) samples obtained from CBE, the isolates recorded were 4/5(80%) *Escherichia coli*, 2/2(100%) *P.aeruginosa*, 3/3(100%) *S.dysenteriae,* 6/16(37.5%) *S.aureus*and 2/3(66.6%) *K.pneumonia*3/3(100%) *Bacillussp.*, 9/13(69.2%) *listeriaspp.,* 2/2(100%) *p.stuarti,* 2/4(50%)*Citrobacterspp.*, 3/6(50%) *P.mirballis.*

Out of the forty (14) samples obtained from Abissinia Bank the isolate recorded were (Table 3). 5/13(38.4%) *listeria*, 1/16(6.25%) *S. aureus*, 1/9(11.1%) *salmonella spp.*, 1/4(25%) *citrobacter spp.* 2/6(33.3%) *P.mirballis*, 1/5(20%)*E.coli.* Also, eight (8) samples obtained from Awash bank the isolate recorded were 1/9(11.1%) *salmonella spp.*, 3/13(23%) *listeriaspp.*, 4/10(40%) *Seratiaspp.* When seven (7) samples obtained from Dashen bank ATMs were examined (Table 3). 1/9(11.1%) *salmonella spp.*,1/13(7.6%) *listeriaspp.*,1/6(16.6%) *P. mirballis*, 1/10(10%) *S.marcessens.* Table 3 revealed that out of four (4) samples obtained from Buna bank ATMs all is 4/10 (40%) *S. marcescens.*

Out of one (1) sample obtained from Abay bank there is no bacteria isolate found. Table 3 showed that out of the One (1) sample obtained from Berhan bank the isolate recorded were 1/9(11.1%) *Salmonella spp.* Out of the One (1) sample obtained from Amhara bank 1/10(10%). Out of one (2) sample obtained from Cooperative Bank of Oromia the recorded isolate was 1/9(11.1%) *salmonella spp.* 1/3(33.3%) *K. pneumoniae.* Out of one (2) sample obtained from Nib international bank and out of one (1) sample Zemen bank there is no bacteria isolate recorded.

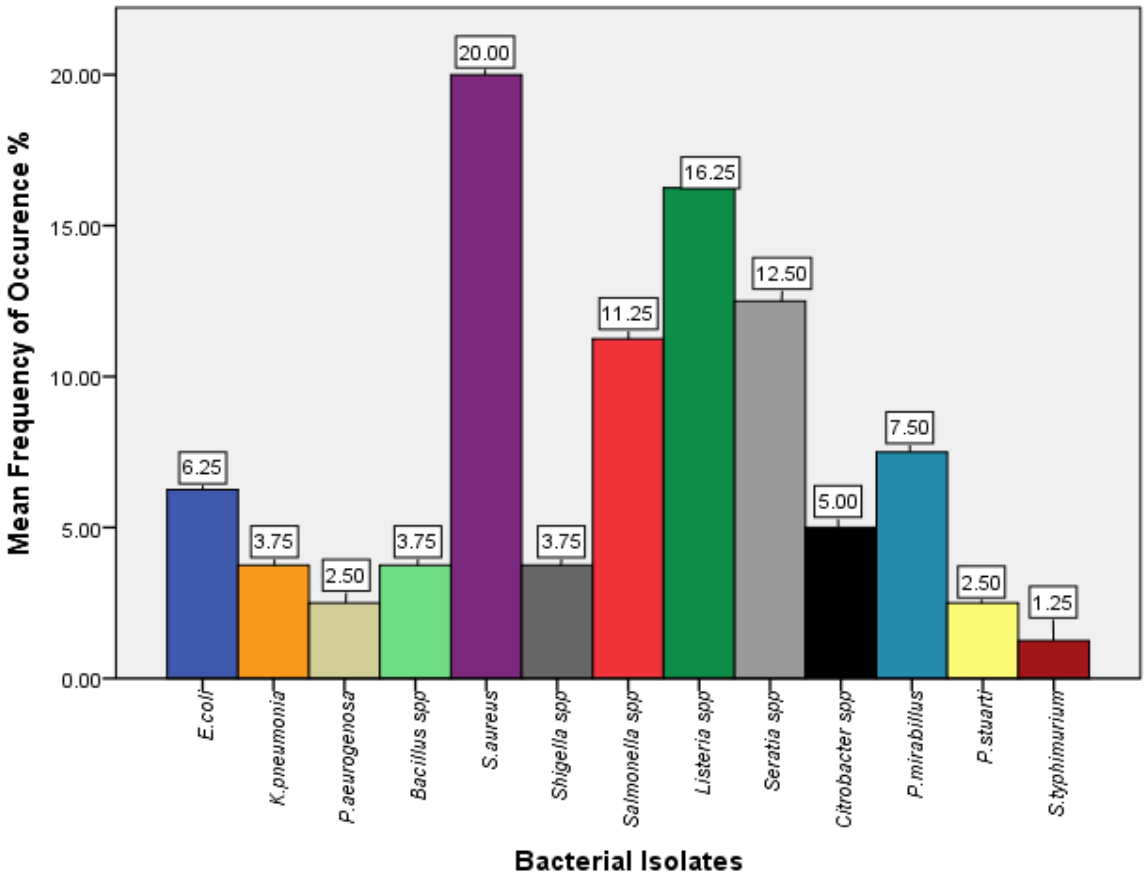
Out of one (1) sample Wegagen bank the isolate recorded were 1/16(6.25%) *S.aures.* Table 3 showed that out of the One (1) sample obtained from Debub global bank ATMs the recorded isolate were 1/4(25%) *C.freundi* (Table 3). On the other hand, the isolate recorded

were1/9(11.1%) *salmonella*, when one (1) sample is obtained from Addis international bank ATMs were examined. Out of one (2) sample obtained from Oromia international bank However, out of the 80 samples, 5(6.25%) were *E. coli*, 2 (2.50%) were *P. aeruginosa*, while *S. dysenteriae* had 3 (3.75%), *S.tyhimurium* had 1(1.25%), *S.aureus* had 16(20%) and 3(3.75%) were *K.pneumoniae* ,*Bacillusspp.*3(3.9%), *shigellaspp.* 3 (3.75%), *listeriaspp.* 13(16.25%), *Seratiaspp.* 10(12.50%), *Citrobacterspp.* 4(5.2%), *p. mirballis*6(7.50%), *P. stuarti*2(2.5%) (Table 3).

Table 3: Frequency Occurrence of bacterial isolates

Frequency	Percent	
Bacillus	3	3.75
Citrobacterspp	4	5.2
E. coli	5	6.25
K.pneumoniea	3	3.75
Listeriaspp	13	16.25
P.aerogenosa	2	2.50
P.mirabillus	6	7.50
P.stuarti	2	2.50
S.aures	16	20
S.typhimurimum	1	1.25
Salmonellaspp	9	11.25
Serratiaspp	10	12.50
Shigellaspp	3	3.75
Total	77	100.0

Figure 8: Mean Frequency Occurrence of bacterial isolates



4.3. Antimicrobial Susceptibility Test

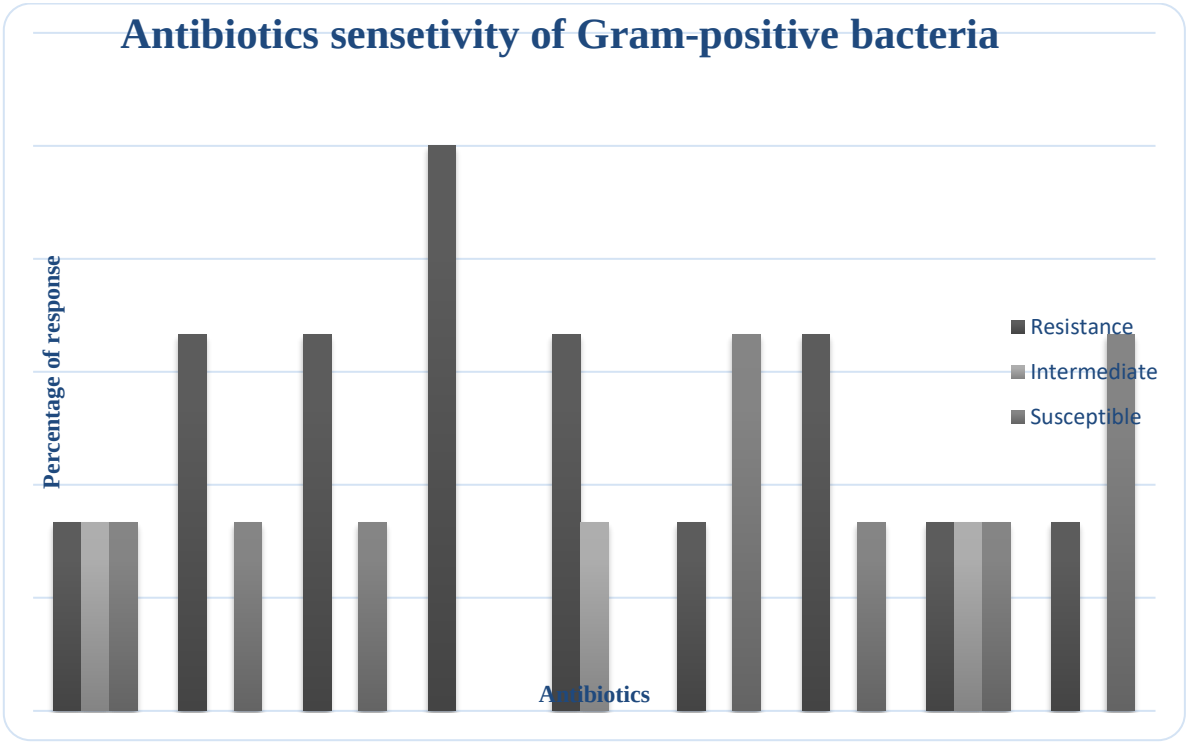
Antimicrobial susceptibility profile of Gram-positive bacteria isolates: A total of 32 Gram-positive bacteria were subjected to antimicrobial susceptibility test and many of the *listeria spp.* isolates were susceptible to gentamicin, ciprofloxacin and penicillin G 3/9(33.3%) resistant to erythromycin, clindamycin, cefepime piperacillin/tazobactam also for tetracycline 5/9(55.5%) intermediate only for ampicillin 1/9(11.1%).Many *S. aureus* isolates were susceptible to piperacillin/tazobactam, tetracycline, ampicillin and cefepime 4/9(44.4%) (Figure9). Resistant to ciprofloxacin, clindamycin, erythromycin, penicillin G and gentamicin 5/9(55.5%). Additionally, many *Bacillus spp* isolate were susceptible to tetracycline and gentamicin 2/9(22.2%) also and intermediate for pepracili/tazobactum and erythromycin 2/9(22.2%) resistant to cefepime, ampicillin, ciprofloxacin, clindamycin, and penicillin G 5/9(55.5%). Gram-positive bacteria had significantly high resistance levels to clindamycin (Table 4).

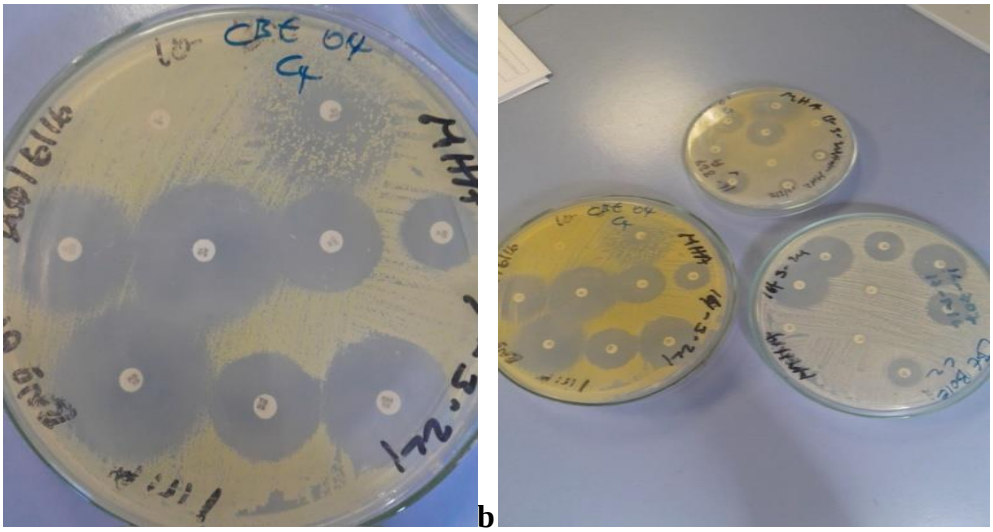
Table 4: Antibiotic sensitivity pattern of Gram-postive bacterial isolates zones of inhibition measure in millimeter

		Based on their Diameter of zone of inhibition (mm)								
Bacterial isolates	**Bacteria code**	**AMP**	**FEP**	**CIP**	**DA**	**E**	**GM**	**P**	**PTZ**	**T**
Potency	**(µg)**	**10**	**30**	**5**	**2**	**15**	**10**	**10**	**100/10**	**30**
Bacillusspp.	**I6**	R	R	R	R	I	S	R	I	S
Listeriaspp.	**I12**	I	R	S	R	R	S	S	R	R
S.aureus	**I8**	S	S	R	R	R	R	R	S	S

KEY: R resistance **I** intermediate **S** sucsseptible **AMP** Ampicilin (10 µg), **FEP** Cefepime (30µg), **CIP** Ciprofloxacin (5µg), **DA** Clindamycin (2µg) **E** Erythromycin (15µg) **GM** Gentamicin (10µg) **P** Penicilin G (10µg) **PTZ** Piperacilin/Tazobactam (100/10µg) **T** Tetracycline(30µg)

Figure 9: Antibiotics sensitivity of gram-positive bacteria in percentage





a **b**

Figure 10: Antimicrobial susceptibility test result of gram-postive(a).Antimicrobial susceptibility test for *s.aureus (*b).Antimicrobial susceptibility test for *Listeriaspp.*

Antimicrobial susceptibility pattern of the Gram-negative bacteria isolates: In this study, antimicrobial susceptibility tests were done for 42 Gram-negative bacteria isolates. All isolates of *E. coli* isolates were resistant to Amoxa-clavunate, cefazolin, ceftazidime, clindamycin, imipenem, penicillin G, cefotaxime, ciprofloxacin, Sulfa/Trimethazole 9/15(60%). while one of them were susceptible for gentamicin 1/15(6.6%) also they were intermediate for tetracycline, ampicillin and pepracilin-tazobactum, erythromycin and cefepime 5/15(33.3%) (Table 5).All isolates of *P. aeruginosa* were resistant to ampicillin, ciprofloxacin, ceftazidime, erythromycin, penicillin G, cefotaxime and Sulfa/Trimethazole 7/15(46.6%) also were susceptible to Amoxa-clavunate, gentamicin, cefepime 3/15(20%) and intermediate for imepinem, Piperacillin/Tazobactum, tetracycline, and clindamycin 4/15(26.6%).

Data also demonstrated that many *S.dysenteriea* isolates were susceptible to ampicillin, gentamicin, imipenem, cefepime, pipraclin/tazobactum, Sulfa/Trimethazole 6/15 (40%) and resistant to Amoxa-clavunate, cefazolin, ceftazidime, ciprofloxacin, clindamycin, erythromycin tetracycline and penicillin G 8/15(53.3%) intermediate only to cefotaxime 1/15 (6.6%)(Table 5). Many *S.typhimurium* and *salmonella spp.* were resistant to ampicillin, cefepime, cefotaxime, ceftazidime ciporofloxacin, clindamycin, penicillin G, pipracilin/tazobactum 9/15 (60%) also for ciprofloxacin and intermediate only for Amoxa-clavunate 1/15 (6.6%) and susceptible to Sulfa/Trimethazole, gentamicin, and imipenem, erythromycin 4/15 (26.6%)(Table 5).

All isolates of *K.pneumoniea*were resistant to Amoxa-clavunate, ceftazidime, gentamicin, imipenem piperacillin-tazobactum, tetracycline, penicillin G, clindamycin and erythromycin 9/15 (60%) intermediate only for cefazolin 1/15 (6.6%) Susceptible to ampicillin, cefepime, cefotaxime, Sulfa/Trimethazole 4/15 (26.6%)(Table 5). Many of *C.freundi* and *Citrobacter spp.* were resistant to ampicillin, cefepime, erythromycin, cefazolin, ceftazidime, penicillin G, piperacillin-tazobactumSulfatrimetazole and tetracycline 9/15 (60%) Susceptible only for gentamicin 1/15(6.6) and intermediate for Amoxa-clavunate,imipenem, clindamycin, ciprofloxacin, cefotaxime 5/15 (33.3%).

All *p. mirabilis* isolates were resistant to ampicillin, Amoxa-clavunate, cefazolin, cefepime, cefotaxime, gentamicin, imipenem, erythromycin, penicillin G, tetracycline, Sulfatrimetazole 11/15 (73.3%) and susceptible only to piperacillin/tazobactum1/15 (6.6%) only intermediate to

clindamycin1/15 (6.6%). Many of *p.stuarti* resistanttoAmoxa-clavunate, ampicillin, cefazolin, cefepime, cefotaxime, ceftazidime, imipenem, penicillin G 8/15 (53.3%) and intermediate to clindamycin, erythromycin, ciprofloxacin, piperacillin/tazobactum 5/15 (33.3%) and tetracycline only susceptible to ampicillin and ceftazidime 2/15 (13.3%) (Table 5). Many *Shigella spp.* were resistant to Amoxa-clavunate, cefepime, cefotaxime, ciprofloxacin, piperacillin/tazobactum, tetracycline, imipenem, penicillin G and tetracycline 9/15 (60%) susceptible to ampicillin, cefazolin, gentamicin, ceftazidime 4/15 (26.6%). Many *seratita spp.* isolates were resistant to Amoxa-clavunate, cefazolin, cefotaxime, gentamicin, imipenem, penicillin G, Sulfatrimetazole 7/15 (46.6%) and intermediate for clindamycin, erythromycin, ciprofloxacin, piperacillin/tazobactum, tetracycline 5/15 (33.3%) also susceptible only to two antibiotics ampicillin and ceftazidime2/15 (13.3%) (Table5)

Table 5: Antibiotic sensitivity pattern of Gram-negative bacteria isolates zones of inhibition measure in millimeter

Bacteria isolate	Bacteria code	Based on their Diameter of zone of inhibition (mm)														
		AMC	AMP	KZ	FEP	CTX	CAZ	CIP	DA	E	GM	IMP	P	PTZ	SXT	T
potency	(µg)	20/10	10	30	30	30	30	5	2	15	10	10	10	100/10	1.25/23.75	30
E.coli	I1	R	I	R	I	R	R	R	R	I	S	R	R	I	R	I
P.aurogenosa	I2	S	R	I	S	R	R	R	I	R	S	I	R	I	R	I
S.dysenteriae	I3	R	S	R	S	I	R	R	R	R	S	S	R	S	S	R
S.thyphimurium	I4	I	R	R	R	R	R	R	R	S	S	S	R	R	S	R
K.pneumonia	I5	R	S	I	S	S	R	R	R	R	R	R	R	R	S	R
C.freundi	I7	I	R	R	R	I	R	I	I	R	S	I	R	R	R	R
Shigelaspp	I9	R	S	S	R	R	S	R	R	R	S	R	R	R	R	R
Seratiaspp	I10	R	S	R	R	R	S	I	S	I	R	R	R	S	R	S
P.stuarti	I11	R	R	R	R	R	R	S	I	S	S	R	R	I	I	S
P.mirballis	I13	R	R	R	R	R	R	R	I	R	R	R	R	S	R	R

KEY:AMP-Ampicilin(10µg);**AMC**-Amoxicillin-clavulanate(20/10µg);**KZ**-Cephazolin(30µg);**FEP**-Cefepime(30µg)**CTX**-Cefotaxime(30µg);**CAZ**-Ceftazidime(30µg)**;CIP**-Ciprofloxacin(5µg);**GM**-Gentamicin(10µg);**IMP**-Imipenem(10µg);**PTZ**-Piperacilin/Tazobactam(100/10µg)**;DA**-Clindamycin(2µg);**E**-Erythromycin(15µg);**P**-PenicilinG(10µg);**T**-Tetracycline(30µg);**SXT**-Sulfa/Trimethazole(1.25/23.75µg): **S**=sensitive I=intermediate **R**=resistance

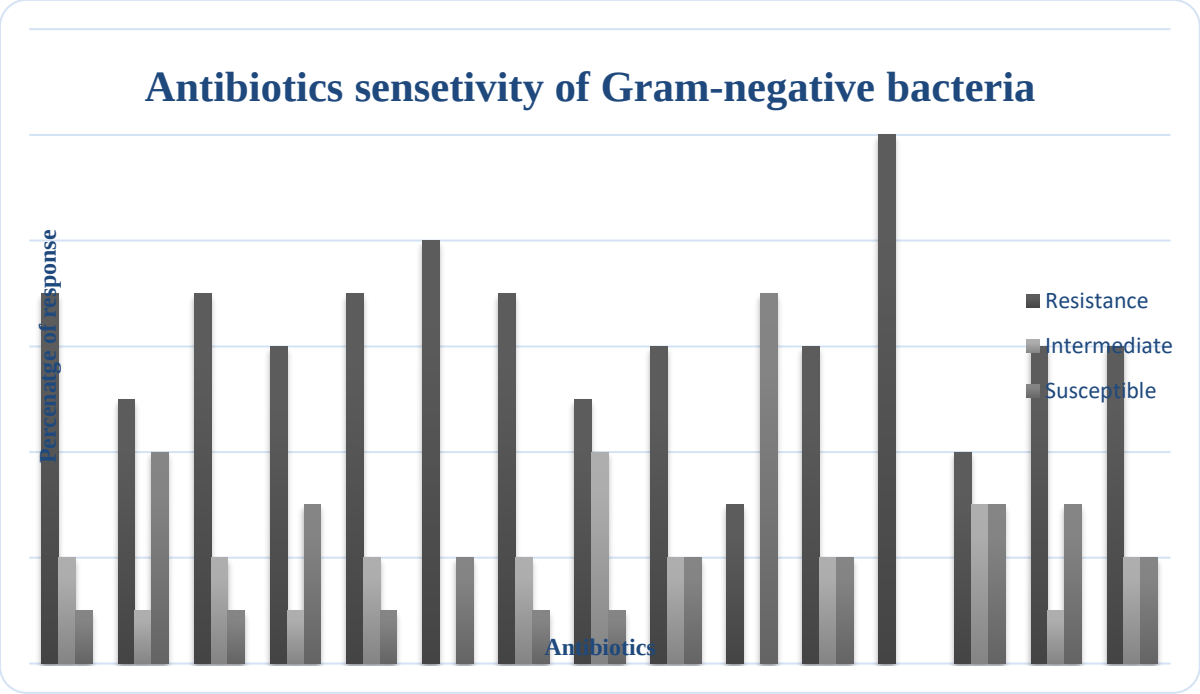


Figure 11: Antibiotics sensitivity of gram-negative bacteria in percentage

a  b 

Figure 12: Antimicrobial susceptibility test result gram-negative (a) Antimicrobial susceptibility test for *P. stuarti* (b) Antimicrobial susceptibility test for *P. aeuroginosa*

4.5 Multidrug-resistance pattern of bacterial isolates

Most gram-negative bacteria rather than gram positive bacteria resistant to many antibiotics when we compare each other. From gram-negative bacteria, *p.mirballis* is highly resistant from fifteen antibiotics it was resistant to 13 antibiotics 13/15(86.6%) all other bacterial species followed by *S.thyphimurium, shigella spp.* and others resistant around eleven antibiotics from fifteen antibiotics. All gram-negative bacteria were resistant more than 8 antibiotics. Gram-postive were also resistant to antibiotics *bacillusspp, s. aureus* and *listeria spp* were resistant to five antibiotics from nine 5/9(55.5%).

4.6 Identification of isolates using MALDITOF

From twelve isolates were subjected to MALDI-TOF MS analysis with sample Id 16150(ICBE8), 16151(ICBE7), 16152(ICBE3), 16153(ICBE5), 16154(IBOA2), 16155(ICBE24), 16156(IDB4) and 16157(ICBO2), 16158 (IAmB1) 16159(ICBE32) 16160(ICBE25), 16161 (ICBE20). According to the identification list suggested by the MALDI biotyper 3.0 software (Bruker), these isolates were identified as 16151(ICBE), 16153(ICBE5), 16157(ICBO2), 16160(ICBE25) were identified as *K. pneumoniae* with score value 2.32, 2.36, 2.21, 2.05, expectively these 4 isolates score values indicate high confidence identification. one that coded with 16154(IBOA2) 1.89 was identified as *k. variicola* indicating low confidence identification. However, 3 isolates 16150(ICBE8), 16156(IDB4), 16158(IAmB1), 16159(ICBE32) have 0.00, 0.00, 0.00 and 1.60 scores respectively which means no organism identification is possible. 16159 (ICBE32) *Exigubacteriumspp.* with a score value 2.29 indicates high confidence identification.16161 (ICBE20) was identified as *P.rettgeri*. 16152(ICBE3) and 16153(ICBE5) identified as *B.licheniformis* and *B.cereus*with score values 2.07 and 2.00 values indicate high confidence identification (Table 6).

Table 6: MALDITOF results

Sample name	Sample ID	Organism (best match)	Score value	Organism (second best match)
E6(-) (C)	16150(standard)	No peak found	0.00	No peaks found
E7(+++) (A)	16151(standard)	*Klebsiella pneumoniae*	2.32	*Klebsiella pneumoniae*
E8(+++) (A)	16152(standard)	*Bacillus licheiniforms*	2.07	*No organism identification possible*
E9(+++) (A)	16153(standard)	*Klebsiella pneumoniae*	2.36	*Klebsiella pneumoniae*
E10(+) (B)	16154(standard)	*Klebsiella pneumoniae*	1.89	*Klebsiella varicols*
E11(+++) (A)	16155(standard)	*Bacillus cereus*	2.00	*Bacillus antracis*
E12(-) (C)	16156(standard)	No peaks found	0.00	No peaks found
F1(+++) (A)	16157(standard)	*Klebsiella pneumoniae*	2.21	*Klebsiella pneumoniae*
F2(-) (C)	16158(Standard)	No peaks found	0.00	No peaks found
F3 (+++) (A)	16159(standard) **yellow**	*Exiguobacteriunspp*	2.29	*Exiguobacteriunspp*
F4(-) (C)	16159(Standard) White	No organism identification possible	1.60	No organism identification possible
F5(+++) (A)	16160(Standard)	*Klebsiella pneumoniae*	2.05	*Klebsiella pneumoniae*
F6 (+++) (A)	16161	*Providencia rettegri*	2.53	*Providencia rettegri* 2.07

CHAPTER FIVE

5. DISCUSSION

ATMs are widely used by many people, and they tend to harbor micro-organisms on their surface. The study was conducted to create awareness to the public and users of ATM machines on the possible diseases that are likely to be contacted due to the presence of pathogens at ATM centers. The hand borne transmission through ATM is one of the most important routes for the spread of infectious agents in the community (Gorný, 2024). To investigate whether ATMs can serve as potential vector for transmission of infection, the study was carried out by collecting swabs from ATM centers in Adama city.

According to the Bergey's Manual of Determinative Bacteriology (Holt, 2003) the result revealed that the growth of Microorganisms in ATM machines particularly they are pathogenic and antibiotic-resistant organisms such as *E. coli, P. aeruginosa, S. dysenteriea, S. tyhimurium, S. aureus, K. pneumoniae, Bacillus spp., shigellaspp., listeriaspp. Serattiaspp. Citrobacterspp. ,P.mirballis, P. stuarti*(Girma, 2015).The high level of bacterial contamination seen in this study is in line with the study of Oloninefa & Ati, (2020) who reported that keypads of ATMs harbored more bacteria than computer keyboards and this may be due to the fact that ATMs are usually located in the open, exposed to winds and rain.

These study also in agreement with the study of (Barbosa et al., 2020) who reported the presence of *Staphylococcus species, Escherchia spp.* and *klesiella spp.* on the keypads of ATM machines. In the same way ,Anastasiades et al., (2010) reported that *S. aureus* are prevalent on Computer keyboards and mouses. The result from this study is in agreement with study carried out in England comparing the bacteria isolated from metallic ATM keypads with those isolated from toilets seats and found that ATM machine that had comparable levels of bacteria described as heavily contaminated with both *pseudomonas species* and *Bacillus species* (Report, 2014). This could be a result of their visitation to the toilets without observing proper hygine's (Barbosa et al., 2020).

Interestingly, this study revealed where *S. aures* was observed as one of highest prevalence among other isolates. *S. aureus* is the major component of normal flora of the skin and nostril, which probably explains its high prevalence's as contaminant, can easily be discharged by several human activities, like sneezing, talking and contact with moist skin (Barbosa et al., 2020)The results obtained in Table 5 showed that pathogenic bacteria such as *E. coli, P. aeruginosa, serattia spp., S. tyhimurium, S. aureus, K. pneumoniae, Bacillus spp., shigellaspp., listeriaspp., salmonella spp., Citrobacterspp., P. mirballis, P. stuarti*were isolated from the ATMs located in Adama city. This result is in agreement with the results obtained by (Barbosa et al., 2020).

The isolated pathogenic bacteria which most of them are members of the family *Enterobacteriaceae* can cause hand-to-mouth infections in man if hands are not sanitized after using ATM. There is also a possibility of them causing nosocomial infections through medical personnel that used an ATM without thorough sanitation of hands used on ATM in the hospital and its environments. The pathogenic bacteria isolated from the ATMs located within Adama city as shown in Table 5 have the possibility of being harbored under the fingertips of the users and as a result of this, they can cause fingertips to mouth infections which can impaired the lives of the infected (Rusin*et al*.,2002).Other study reported by Yuana et al. (2022) also indicated that *S.aures*with a prevalence of 41.6%, *E.coli* 34.4% and *K. pneumonia* 24.0% were some of the bacterial isolates found on the keypads of the ATMs.

The isolates obtained from the data reported by Onuoha and Fatokun (2014) observed that *S.aureus* 28% was the commonest organism isolated from ATMs of Seventeen different banks in two major town of Nigeria (Hiko, Abdata, et al., 2016). Nwankwo and offiah (2016) reported that the ATM machines are one of the most touched surfaces today Onuoha &Fatokun (2014) described six different bacteria isolates in ATMs located in Nigeria, such as *Sthaphylococcus spp., Streptococcus spp., Pseudomonas spp., Enterobacter spp and Escherchiaspp.*Similar to these findings out of the 80 samples, *S.aureus*had the highest number (percentage) of 20% followed by *Listeriaspp.* With 16.25%, *Serratiaspp.*, 12.50% in *Salmonella spp.*, 11.25% in *P.mirballis* 7.50% in *Citrobacterspp.* 5.2%, in *K. pneumonia, Bacillus spp.,* and *shigellaspp.* 3.75%, also P. *aeruginosa* and *P. stuarti*2(2.50%) in *S. tyhimurium* the lowest number and

(percentage) of 1.25%was recorded as revealed in Table 5 and Figure 8. The highest number (percentage) recorded in *S. aureus* obtained from the ATMs located around Posta bet might probably be due to the location of the many users who have found there and high flows of people which must have led to the use of ATMs (Barbosa et al., 2020).

Although *S. aures* is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory infections such as sinusitis and food poisoning. *S. aureus* is responsible for many infections, but it may also occur as a commensal. The presence of *S. aureus* does not always indicate infection. It can survive from hours to weeks or even months on dry environmental surfaces, depending on strain (Hiko, Irsigler, et al., 2016). *S. aureus* can infect tissues when the skin or mucosal barriers have been breached. This can lead to many different types of infections, including boils and carbuncles (a collection of boils). *S. aureus* infections can spread through contact with pus from an infected wound, skin-to-skin contact with an infected person by producing hyaluronidase that destroys tissues, and contact with objects such as towels, sheets, clothing, or athletic equipment used by an infected person.

E. coli is one of the most frequent causes of many common bacterial infections including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), and traveler's diarrhoea and other clinical infections such as neonatal meningitis and pneumonia (Winfield & Groisman, 2003). *E. coli*, there is still a high possibility of infections caused by *E. coli* that could affect the users if there is poor hand washing practice (Aquino et al., 2021).Some kinds of *E. coli* can cause diarrhoea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses. In addition, *E. coli* recorded the highest number (percentage) of 4/5 (80%) at CBE and the lowest number (percentage) of 1/5(20%) (Appendix S).The highest number (percentage) obtained from CBE might be due to high population along posta bet and franko road and the lowest number (percentage) recorded in Abissinia bank might be due to less population in those areas. Poor hygienic practices coupled with unsanitized hands after leaving the toilets. More so, *P. aeruginosa* had found only in CBE number (percentage) of 2/2 (100%) from the ATMs around Adama General Hospital (Appendix S).

High number of users of ATMs around the hospital might be responsible for the numbers of *P. aeruginosa* obtained. As explained by (Guimarães & Barbosa, 2022). *P. aeruginosa* has emerged as an important pathogen during the past two decades. It causes between 10% and 20% of infections in most hospitals (State, 2021).*Pseudomonas* infection is especially prevalent among patients with burn wounds, cystic fibrosis, acute leukemia, organ transplants, and intravenous-drug addiction.*P. aeruginosa* is a common nosocomial contaminant and epidemics have been traced to many items in the hospital environment. Patients who are hospitalized for extended periods are frequently colonized by this organism and are at increased risk of developing infection (Gorný, 2024). The most serious infections include malignant external otitis, endophthalmitis, endocarditis, meningitis, pneumonia, and septicemia. The likelihood of recovery from pseudomonas infection is related to the severity of the patient's underlying disease. The introduction of the anti-pseudomonal amino glycosides and penicillin's has improved substantially the prognosis of these infections (Girma et al., 2014).

Ticarcillin and carbenicillin have been especially beneficial in neutropenic patients; however, prompt institution of therapy is mandatory for optimal benefit. Many new drugs with anti-*pseudomonal* activity, including penicillins, cephalosporins, and other beta-lactams, have been introduced in recent years and offer the potential for new approaches to therapy for these infections (Aquino et al., 2019).However, only 3/3(100%) were recorded for *S. dysenteriae*from two sites ATMs of CBE located in ASTU compound while the lowest number (percentage) 1(1.25%) was recorded from the ATMs of Commercial bank of Ethiopia along Tikurabayberecha branch.

High numbers of ATMs users, poor hygienic practices coupled with behavioral and cultural activities might be responsible for these results particularly for the Comercial bank of Ethiopia ATMs found in ASTU. The few users of ATMs along Tikurabayberecha branch and coupled with the indifferent attitude of people in using the ATMs must have accounted for the numbers of *S. dysenteriae*obtained. *Shigella* infection (also known as shigellosis) is an infection of the digestive tract (or gut) caused by *Shigella* bacteria (Duraipandian, 2016). Even though the lowest number (percentage) of 1/1 (100%) was recorded in *S. tyhimurium*from DB ATMs along

Gendehara branch found around KidistSelase Church this due to Less population patronizing the ATMs along this area compared as Posta bet and Franko.

The highest number (percentage) of 9(11.25%) obtained all *Salmonella spp.* from the ATMs of Commercial Bank of Ethiopia around 04, Awash bank around Mebrathaile, BOA around Franko, BrB around Posta bet, CBO around 04, AdIB around Sartera, DB along Posta bet road. This is due to the high number of users of ATMs around the Adama Referral Hospital and 04 also many users have found there and around Posta bet and Franko Road there are high flows of people which must have led to contributed might be responsible for this result (Appendixx S).

Salmonellosis is a food borne illness caused by infection with *Salmonella* bacteria. Most infections are spread to people through consumption of contaminated food (usually meat, poultry, eggs, or milk). *Salmonella* infections affect the intestines and cause vomiting, fever, and cramping, which usually clear up without medical treatment (Girma, 2015). Appendix 18 also revealed that the number and (percentage) of *K. pneumonia* recorded in the study showed that CBE 2/3 (66.6%) and CBO 1/3(33.3%) The highest number (percentage) was recorded from CBE around SelaseMazoriya road and the other from CBO on Shewa Dabo Building. This might be the high flow of people around Adama Referral Hospital which must have contributed to the use of ATMs (Winfield and Groisman 2003).

This could indicates that the possibility of exposing the users ATMs in the study area to pneumonia. Even though *K.pneumoniae*is found in the normal flora of the mouth, skin, and intestines, it can cause destructive changes to human lungs if aspirated (inhaled), specifically to the alveoli (in the lungs) resulting in bloody sputum (MacWilliams, 2009). In the clinical setting, it is the most significant member of the *Klebsiella* genus of *Enterobacteriaceae*. *K. oxytoca*and *K.rhinoscleromatis*have also been demonstrated in human clinical specimens. In recent years, *klebsiellae* have become important pathogens in nosocomial infections. *K. Pneumoniae can* also cause a variety of extra pulmonary infections, including enteritis and meningitis (in infants), urinary tract infections in children and adults (Ogbole et al., 2018).

According to (Mamo, 2019) some of the bacteria observed from hand touch surfaces of selected Automated Teller Machine, public transport buses and hospital waiting area inanimate objects located in Addis Ababa, Ethiopia, are highly resistant to the commonly used antibiotics such as, 71.3%, 67.3% and 40.2% of *Staphylococcus species* showed resistant against to tetracycline, penicillin and oxacillin, respectively. *Proteus Spp*was resistant to cefuroxime 77.6%, ampicillin 79.3%, ceftriaxone 84.5% and ceftazidime 84.5%. Moreover, 72.0% of the total isolates multidrug resistance was observed. Like this study All isolates obtained from touch surfaces of selected Automated Teller Machine E. *coli* were resistant to Amoxa-clavunate, cefazolin, ceftazidime, clindamycin, imipenem, penicillin G, cefotaxime, ciprofloxacin, Sulfa/Trimethazole 60%.Many*S. aureus* isolates were susceptible to pepracilin/tazobactum, tetracycline, ampicillin and cefepime 44.4%. Resistant to ciprofloxacin, clindamycin, erythromycin, penicillin G and gentamicin 55.5%.*p.mirballis* is highly resistant from fifteen antibiotics it was resistant to around thirteen antibiotics 86.6%.

CHAPTER SIX

6. CONCLUSION

In conclusion, a total of 13 spp. was identified isolated from all bank ATMs that were used for the study. The results obtained from both morphological and biochemical tests carried out that obtained from ATM machines showed different bacterial isolates. *E. coli*, P.*aeruginosa, S.dysenteriea, S. tyhimurium, S. aureus, K. pneumoniae, Bacillus spp. Shigella spps. Listeria spp. Serattia spp. Citrobacter spp., P. mirballis, P. stuarti.* There is a highly significant relationship between bacterial isolates and their respective locations which may account for several factors such as, poor hygiene practices by ATM users, rain fall, dust, wind and high concentration of users at ATM centers. They were confirmed culturally, Gram-stained and biochemically tested. This study revealed the presence of pathogenic microbes that listed above was found to be resistant to much commonly used variety of antibiotics. From ICBE13 and ICBE9, ICBE 6, ICBE26 has higher pathogenic microbes than others. Mainly CBE have most infected and found with high isolates. CBE found around postabet also around bole, elemo-branch,around 04(gendegara-branch), Adama science and technology compound high bacterial load recorded. Also, IDB1, IBrB1, IadIB (Adama branch) were most infected.

CHAPTER SEVEN

7. RECOMMENDATIONS

As a result of the relationship that exists between the Automated Teller Machines (ATMs) & the isolated pathogenic bacteria, the following recommendations will be made:

- ✓ Good hand washing, and other hygienic practices should be observed by the users of ATMs
- ✓ A sanitizer should be provided by the bank management at every ATM Location so that users can disinfect their hands after using the ATMs.
- ✓ ATM Cleaners should be employed by the bank management so that they can disinfect the Metallic buttons at intervals using compatible disinfectants.
- ✓ Administrative bodies and all professionals should take an active role in infection control within their organization
- ✓ Antibiotic resistant bacteria on ATM are being reported in this study. Therefore, continuous microbiological surveillance is needed to monitor these organisms and put them under control

References

Adedoyin, A. B. (2019). *A Study Investigating Bacterial Colonization on Automated Teller Machines in Ibadan Metropolis , South-West Nigeria*. *3*(6), 119–132. https://doi.org/10.31080/ASPS.2019.03.0286

Agu, R. C., Osondu-anyanwu, C., & Nwachukwu, A. A. (2018a). *Isolation and Identification of Microorganisms Associated with Automated Teller Machines in Calabar Metropolis Isolation and Identification of Microorganisms Associated with Automated Teller Machines in Calabar Metropolis*. *September*. https://doi.org/10.9734/JABB/2018/42855

Agu, R. C., Osondu-anyanwu, C., & Nwachukwu, A. A. (2018b). *Isolation and Identification of Microorganisms Associated with Automated Teller Machines in Calabar Metropolis Isolation and Identification of Microorganisms Associated with Automated Teller Machines in Calabar Metropolis*. *August*. https://doi.org/10.9734/JABB/2018/42855

Alwis, W. R. De, Pakirisamy, P., San, L. W., & Xiaofen, E. C. (2012). *A Study on Hand Contamination and Hand Washing Practices among Medical Students*. *2012*(Cdc). https://doi.org/10.5402/2012/251483

Anastasiades, P. S., Filippousis, G., Karvunis, L., Siakas, S., Tomazinakis, A., Giza, P., & Mastoraki, H. (2010). Interactive Videoconferencing for collaborative learning at a distance in the school of 21st century: A case study in elementary schools in Greece. *Computers and Education*, *54*(2), 321–339. https://doi.org/10.1016/j.compedu.2009.08.016

Aquino, S., Alves de Lima, J. E., Alves dos Reis, T., Correa, B., & Borrely, S. I. (2021). Mycoflora and DNA sequencing analysis of radioresistant fungi isolated from irradiated vehicular air conditioning filters collected in São Paulo, Brazil. *Microbiology Research International*, *9*(1), 1–15. https://doi.org/10.30918/mri.91.21.011

Aquino, S., Eduardo, J., Lima, A. De, & Oliveira, M. (2019). *Detection of pathogenic bacteria and fungi on biometric surface of Automated Teller Machines located in Brazilian public hospital*. *13*(11), 219–231. https://doi.org/10.5897/AJMR2019.9055

Bakterien, A. Von, & Von, V. D. T. (2017). *Antibiogram of bacteria isolated from automated teller machines in Hamadan , West Iran*. *12*, 10–15.

Barbosa, F., Voss, G., & Delerue Matos, A. (2020). Health impact of providing informal care

in Portugal. *BMC Geriatrics*, *20*(1), 1–9. https://doi.org/10.1186/s12877-020-01841-z

Bodena, D., Teklemariam, Z., Balakrishnan, S., & Tesfa, T. (2019). *Bacterial contamination of mobile phones of health professionals in Eastern Ethiopia : antimicrobial susceptibility and associated factors*. 1–10.

Brink, B. (2010). Urease Test Protocol. *American Society for Microbiology*, *November 2010*, 1–7.

Brook, I., Wexler, H. M., & Goldstein, J. C. (2013). *Antianaerobic Antimicrobials : Spectrum and Susceptibility Testing*. *26*(3), 526–546. https://doi.org/10.1128/CMR.00086-12

Chandrasekar, K. S., & Taye, E. (2017). *The Adoption of Automatic Teller Machines in Commercial Bank of Ethiopia*. *December*.

Chinedu, *, & Mbajiuka, S. (2015). Antimicrobial Effects of the Leaf Extracts of Chromolaena Ordorata (Siam Weeds) on Some Human Pathogens. *C.S. World Journal of Pharmaceutical Research World Journal of Pharmaceutical Research SJIF Impact Factor 5*, *4*(8), 209–220.

Cimolai, N. (2008). The role of healthcare personnel in the maintenance and spread of methicillin-resistant Staphylococcus aureus. *Journal of Infection and Public Health*, *1*(2), 78–100. https://doi.org/10.1016/j.jiph.2008.10.001

Diagnostic, A. (2005). *Product Specification Sheet*. *1*(800), 110906.

Division, M., & Lumpur, K. (2014). *BACTERIOLOGICAL ASSESSMENT OF SOME AUTOMATED TELLER MACHINE (ATM) KEYPADS IN ILORIN METROPOLIS*. *273*(2), 264–273.

Duraipandian, J. (2016). *Study of Prevalence of Microbial Contamination with its Antibiotic Resistance Pattern in Automated Teller Machine in and around Puducherry , India*. *June*. https://doi.org/10.4103/2423-7752.159924

Eguia, J. M., & Chambers, H. F. (2003). *Community-acquired Methicillin-resis- tant Staphylococcus aureus : Epidemiology and Potential Virulence Factors*.

Galbraith, G. M. P., Hendley, T. M., Sanders, J. J., Palesch, Y., & Pandey, J. P. (1999). Polymorphic cytokine genotypes as markers of disease severity in adult periodontitis. *Journal of Clinical Periodontology*, *26*(11), 705–709. https://doi.org/10.1034/j.1600-051x.1999.t01-1-261101.x

Girma, G. (2015). Health Risk Associated with Handling of Paper Currencies. *International*

Journal of Food and Nutritional Science, *2*(2), 1–6. https://doi.org/10.15436/2377-0619.15.014

Girma, G., Ketema, T., & Bacha, K. (2014). Microbial load and safety of paper currencies from some food vendors in Jimma Town, Southwest Ethiopia. *BMC Research Notes*, *7*(1), 1–8. https://doi.org/10.1186/1756-0500-7-843

Gorný, D. (2024). *Active commuting as a form of sustainable urban mobility : The case of the Brno Metropolitan Area*. *32*(3), 152–163.

Guimarães, P., & Barbosa, M. (2022). The state of Portuguese research in economics: 20 years after. *Portuguese Economic Journal*, *21*(3), 283–309. https://doi.org/10.1007/s10258-022-00211-4

Gupta, A., Sharma, G. G., Young, C. S. H., Agarwal, M., Smith, E. R., Paull, T. T., Lucchesi, J. C., Khanna, K. K., Ludwig, T., & Pandita, T. K. (2005). *Involvement of Human MOF in ATM Function*. *25*(12), 5292–5305. https://doi.org/10.1128/MCB.25.12.5292

Hiko, A., Abdata, K., Muktar, Y., Woyesa, M., & Mohammed, A. (2016). Contamination of Ethiopian paper currency notes from various food handlers with E. coli. *SpringerPlus*, *5*(1). https://doi.org/10.1186/s40064-016-2742-z

Hiko, A., Irsigler, H., Ameni, G., Zessin, K. H., & Fries, R. (2016). Salmonella serovars along two beef chains in Ethiopia. *Journal of Infection in Developing Countries*, *10*(11), 1168–1176. https://doi.org/10.3855/jidc.6354

Holt, P. S. (2003). Molting and Salmonella enterica serovar enteritidis infection: The problem and some solutions. *Poultry Science*, *82*(6), 1008–1010. https://doi.org/10.1093/ps/82.6.1008

Hu, N. O. N. G. (2012). *SHORT REPORT Characterization of staphylococci contaminating automated teller machines in Hong Kong*. 1366–1371. https://doi.org/10.1017/S095026881100207X

Hussey, M. A. (2013). Endospore Stain Protocol. *American Society for Microbiology*, *September 2007*, 1–11.

Id, O. G. D., & Akanbi, R. B. (2021). *Isolation and identification of microorganisms associated with automated teller machines on Federal Polytechnic Ede campus*. 1–15. https://doi.org/10.1371/journal.pone.0254658

Itah, A. Y., & Ben, A. E. (2004). Incidence of enteric bacteria and Staphylococcus aureus in

Day Care Centers in Akwa Ibom State, Nigeria. *Southeast Asian Journal of Tropical Medicine and Public Health*, *35*(1), 202–209.

Jones, S. (2011). *aeruginosa on hands in a hospital environment*. *12*(4), 146–148.

Konheim, A. G. (2016). Automated teller machines : their history and authentication protocols. *Journal of Cryptographic Engineering*, *6*(1), 1–29. https://doi.org/10.1007/s13389-015-0104-3

Lehman, D. (2005). Triple Sugar Iron Test Protocols. *American Society for Microbiology*, *September 2005*, 2–3.

MacWilliams, M. P. (2009). Citrate Test. *American Society for Microbiology*, *December 2009*, 1–7.

Metropolis, U., & State, A. (2021). *Bacterial Contamination of User Interface of Automated Teller Machines (ATM) Bacterial Contamination of User Interface of Automated Teller Machines (ATM) of Various Banks in Umuahia Metropolis , Abia State , Nigeria*. *January 2016*. https://doi.org/10.9734/IJTDH/2016/22812

Muriithi, C., Mwongera, C., Abera, W., Chege, C. G. K., & Ouedraogo, I. (2023). A scalable approach to improve CSA targeting practices among smallholder farmers. *Heliyon*, *9*(10), e20526. https://doi.org/10.1016/j.heliyon.2023.e20526

Noble, D. (2002). Modeling the heart - From genes to cells to the whole organ. *Science*, *295*(5560), 1678–1682. https://doi.org/10.1126/science.1069881

Nwankwo, E. O., & Offiah, J. C. (2016). *Bacterial Contamination of User Interface of Automated Teller Machines (ATM) of Various Banks in Umuahia Metropolis , Abia State , Nigeria*. *13*(3), 1–9. https://doi.org/10.9734/IJTDH/2016/22812

Nworie, O., Mercy, M., Chukwudi, A., Oko, I., & Chukwudum, S. O. (2012). *Antibiogram of Bacteria Isolated from Automated Teller Machines Within Abakaliki Metropolis*. *8*(4), 168–174. https://doi.org/10.3844/ajidsp.2012.168.174

Ogbole, M. E., A. Yaza, J., A. Ameh, J., Mailafia, S., H. Olabode, O., J. Adah, B., R. Okoh, G., & Maduike, S. (2018). Coagulase - Positive Staphylococci Isolated from Cattle in Maiduguri, Nigeria. *International Journal of Current Microbiology and Applied Sciences*, *7*(05), 1301–1306. https://doi.org/10.20546/ijcmas.2018.705.158

Oloninefa, S. D., & Ati, O. F. (2020). *ASSESSMENT OF SOME SELECTED AUTOMATED TELLER MACHINES IN*. *March 2018*.

Onuoha, S. C., & Fatokun, K. (2014). *Bacterial Contamination and Public Health Risk Associated with the Use of Banks ' Automated Teller Machines (Atms) in Ebonyi State , Nigeria*. *2*(2), 46–50. https://doi.org/10.12691/ajphr-2-2-2

Paul, S., & Paul, S. (2013). *ATM : The New Horizon of E-Banking*. *7522*(2000), 148–152.

Paulo, S. (2001). *IMPORTANCE OF HAND GERM CONTAMINATION IN HEALTH-CARE WORKERS AS POSSIBLE CARRIERS OF NOSOCOMIAL INFECTIONS*. *43*(3), 149–152.

Reiner, K. (2013). American Society for Microbiology, Catalase Test Protocol. *American Society for Microbiology*, *November 2010*, 1–9.

Report, F. (2014). *ITS Professional Capacity Building : Setting Strategic Direction 2010-2014*. *March 2011*.

Rufa, I. (2019). *BACTERIAL LOAD OF THE SURFACE OF SOME SELECTED AUTOMATED TELLER MACHINES (ATMs) IN KANO METROPOLIS ,*. *12*(1), 76–79.

Rusin, P., Maxwell, S., & Gerba, C. (2002). Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage. *Journal of Applied Microbiology*, *93*(4), 585–592. https://doi.org/10.1046/j.1365-2672.2002.01734.x

Saroja, V., Kamatchiammal, S., Brinda, K., & Anbazhagi, S. (2013). *Enumeration and chracterisation of coliforms from Automated Teller Machines (ATM) centres in urban areas Enumeration and Characterisation of Coliforms from Automated Teller Machine (ATM) Centers in Urban Areas*. *May 2014*.

Science, H. (2019). *COLLEGE OF HEALTH SCIENCES Bacterial profiles and antibacterial susceptibility patterns of frequently hand touched surfaces on public transport buses , Automated Teller Machines and St ' Paul Hospital patient waiting areas in Addis Ababa , Ethiopia By ; M*.

Spengler, G., & Urb, E. (2017). *Identification and Antimicrobial Susceptibility Testing of Anaerobic Bacteria : Rubik ' s Cube of Clinical Microbiology ?* 1–29. https://doi.org/10.3390/antibiotics6040025

Stanley, C. (2015). *(ATM) IN MICHAEL OKPARA UNIVERSITY OF AGRICULTURE , UMUDIKE (MOUAU) AND ITS ENVIRONS*. *4*(08), 85–99.

State, N. (2020). *BAJOPAS Volume 13 Number 1, June, 2020*. *13*(1), 46–53.

Tushar, A., & Chawla, R. (2022). *Automated Teller Machine and Impact on Banking System*.

1–7. https://doi.org/10.55041/IJSREM14288

Winfield, M. D., & Groisman, E. A. (2003). Role of nonhost environments in the lifestyles of Salmonella and Escherichia coli. *Applied and Environmental Microbiology*, *69*(7), 3687–3694. https://doi.org/10.1128/AEM.69.7.3687-3694.2003

Yuana, A., Terkimbi, T. E., Ortwer, A. B., Peter, A., Mwuese, G. G., & Inya, O. J. (2022). *Isolation and Identification of Bacteria on Automated Teller Machines (ATMs) in Makurdi Metropolis*. *8*(1), 1–5. https://doi.org/10.11648/j.fem.20220801.11

Yunoki, T., Matsumura, Y., Yamamoto, M., & Tanaka, M. (2017). *Anaerobe Genetic identi fi cation and antimicrobial susceptibility of clinically isolated anaerobic bacteria : A prospective multicenter surveillance study in Japan*. *48*. https://doi.org/10.1016/j.anaerobe.2017.09.003

APENDICES

Appendix A Representative picture of sterile swab and fulken tube used for sample transportation

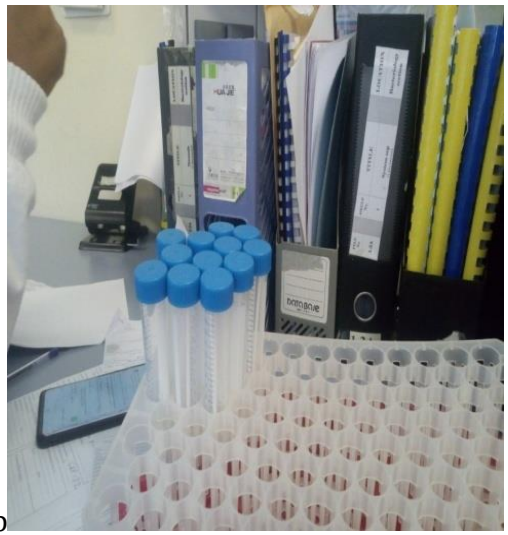
a  b 

Figure (A): sterile swab that used for saple taking and inoculation **(B):** Fulken tube that has saline solution with swab for sample holding and transport

Appendix B Representative picture of media preparation procedure

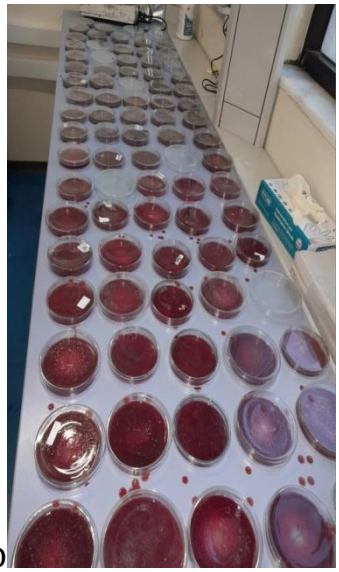
A  **B**  **C**  D 

Figure (A): sterilized petridishes waiting for media dispensing **(B):** blood agar preparation **(C):** Dispensing blood agar on sterilized petridishes**(D):** prepared media that left for dry

Appendix C Representative picture of growth of bacteria on blood and MacConkey agar

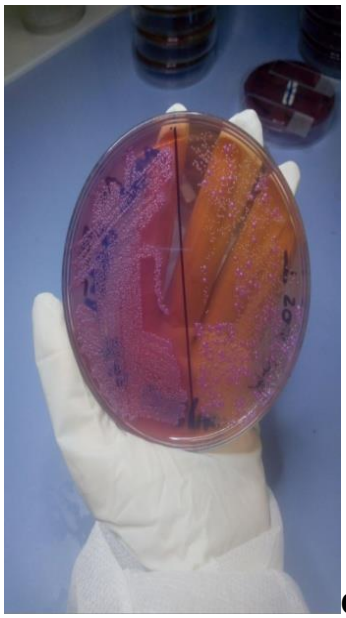
A  b  **C** c

Figure (A): non lactose fermenting colonies on MacConkey agar *S.marcessens***(B):** lactose fermenting colonies on MacConkey agar like *E.coli* **(C):** growth of colonies on blood agar

Appendix D Representative picture of growth of colonies on different selective media

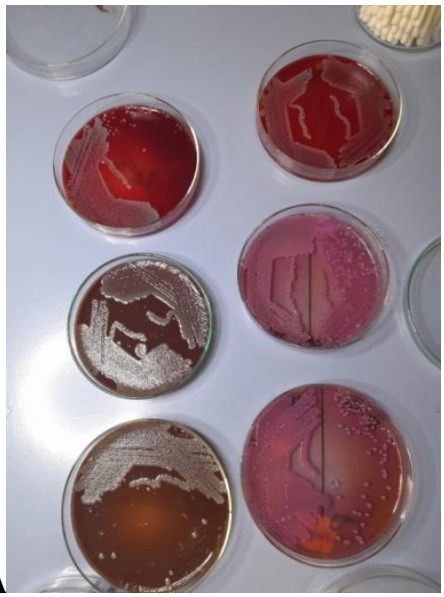

A  **B**

Figure (A): growth of colonies on Blood, Chocolate and MacConkey agar

(B):growthof colonies on Blood agar

Appendix E Representative picture of Antimicrobial Susceptibility test

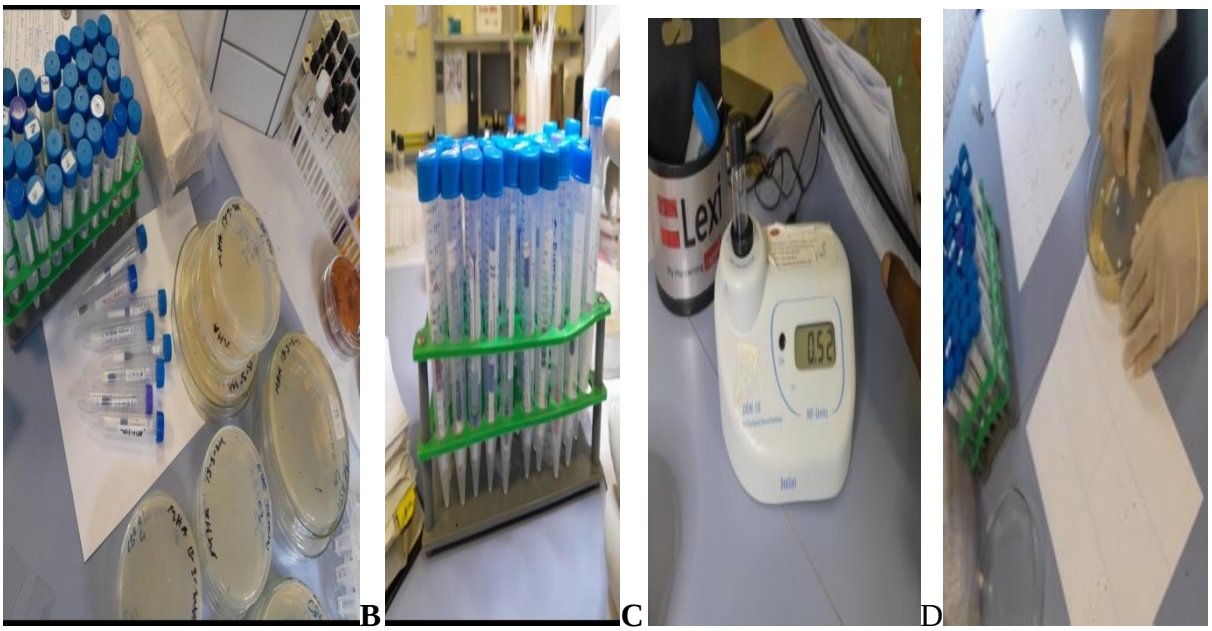


Figure (A): Antibiotics and Mulerhinton agar **(B):** Antibiotics that used for antimicrobial susceptibility test(c). McFarland Densitometer that used to standardize the density of bacterial suspension.(d).Putting the antibiotics on the Muller hinton agar plate

Appendix F: Representative picture Antimicrobial Susceptibility test result

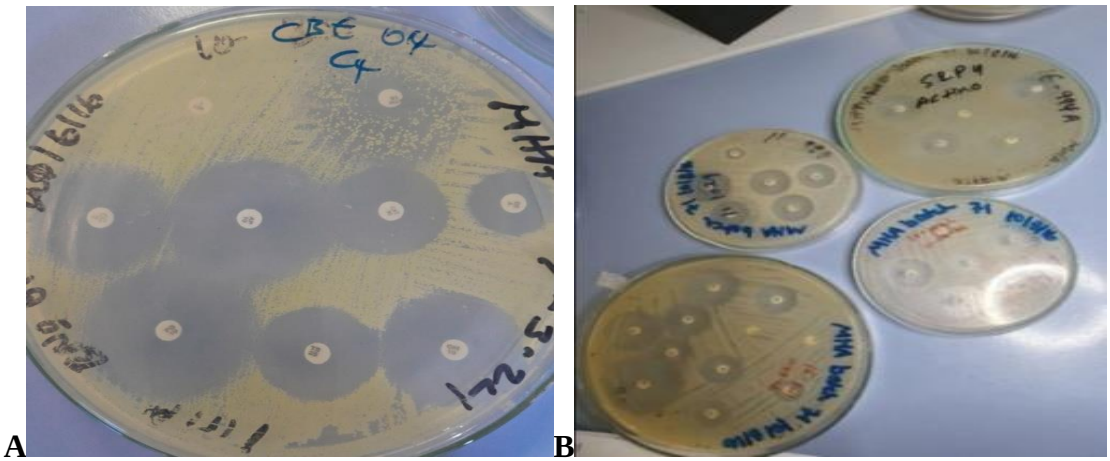


Figure (A)(B): Antimicrobial Susceptibility test result of some selected bacterial isolates

Appendix G: Representative picture of *S.aureus* on Manitol Salt agar

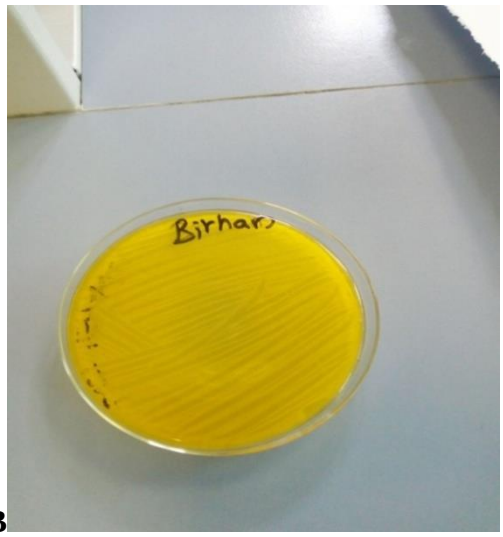
A  B 

Figure (A)(B):*S.aureus* on Manitol Salt agar that change the colour of the media in to yellow that indicate the presence of *S.aureus*

Appendix H: Representative picture of Biochemical test procedure and result

A  B  C  D 

Figure (A): Biochemical test reagents oxidase,catalase, indole **(B):**inoculating the colonies on slant and butt **(C):**results of biochemical tests **(D):**positive result of urea and and citrate test

Appendix I: Representative picture of Gram staining procedure

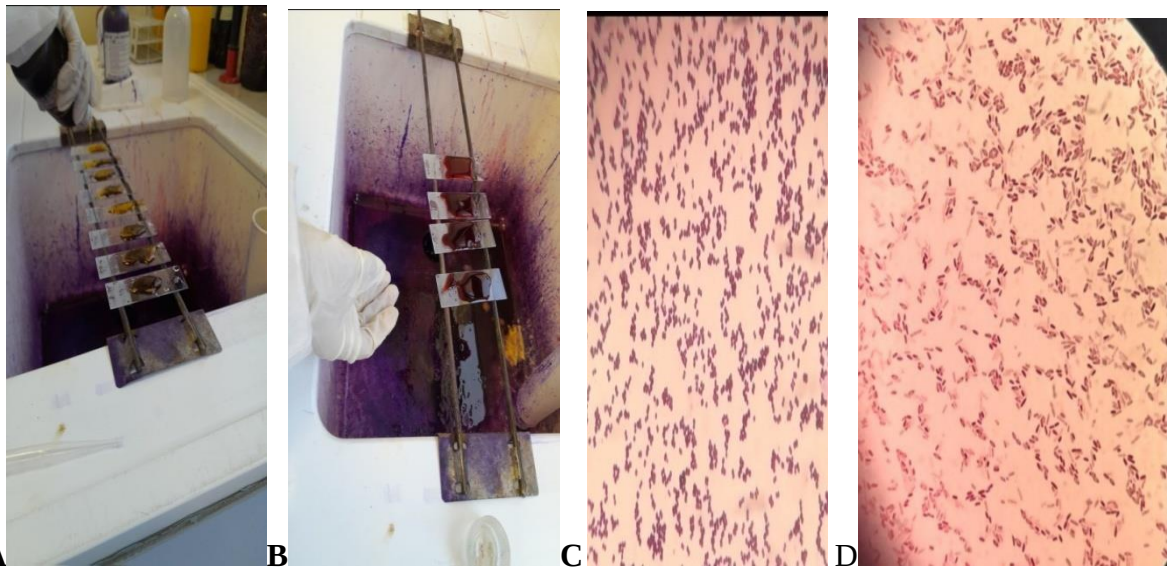


Figure (A): Gram's iodine added to the slides **(B):**Safranin added to the slides **(C):**Gram-postive cocci that were magnified under microscope with oil immersion 100x **(D):**Gram-negative rod that were magnified under microscope with oil immersion 100x

Appendix J: Representative picture of An Aerobic jar

Figure (A): An Aerobic jar used in an aerobic environment. This method of anaerobiosis, as others is used to culture bacteria that die or fail to grow in the presence of oxygen(anaerobes)

Appendix K: Representative picture of isolates that were put in container

A  B 

Figure (A) (B): Isolates that were put in container then placed in refrigerator under 20^0C

Appendix L: Representative picture of while I was working in the bacteriology labratory

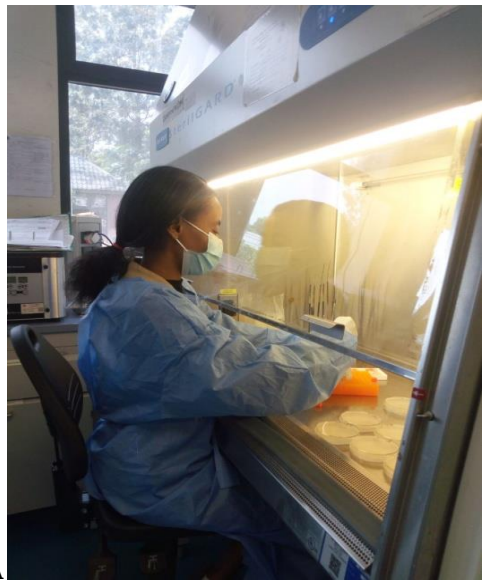
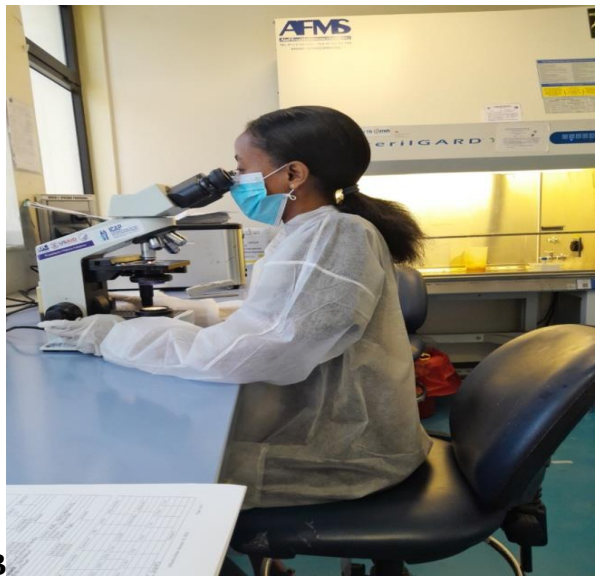
A  **B** 

Figure (A): while I was working in flame wood to prevent any contamination

(B): while I was observing microscopes to study morphology

Appendix M Representative table of CLSI guidelines

Antibiotics	R	I	S
AMP	≤16	-	≥17
AMC	≤13	14-17	≥18
FEP	≤14	15-17	≥18
CIP	≤15	16-20	≥21
CTX	≤12	23-25	≥26
DA	≤14	15-20	≥21
CAZ	≤17	18-20	≥21
E	≤13	14-22	≥23
GM	≤12	13-14	≥15
P	≤28	-	≥29
PTZ	≤17	18-20	≥21
IMP	≤19	20-22	≥23
SXT	≤10	11-15	≥16
T	≤11	12-14	≥15
KZ	≤19	20-22	≥23

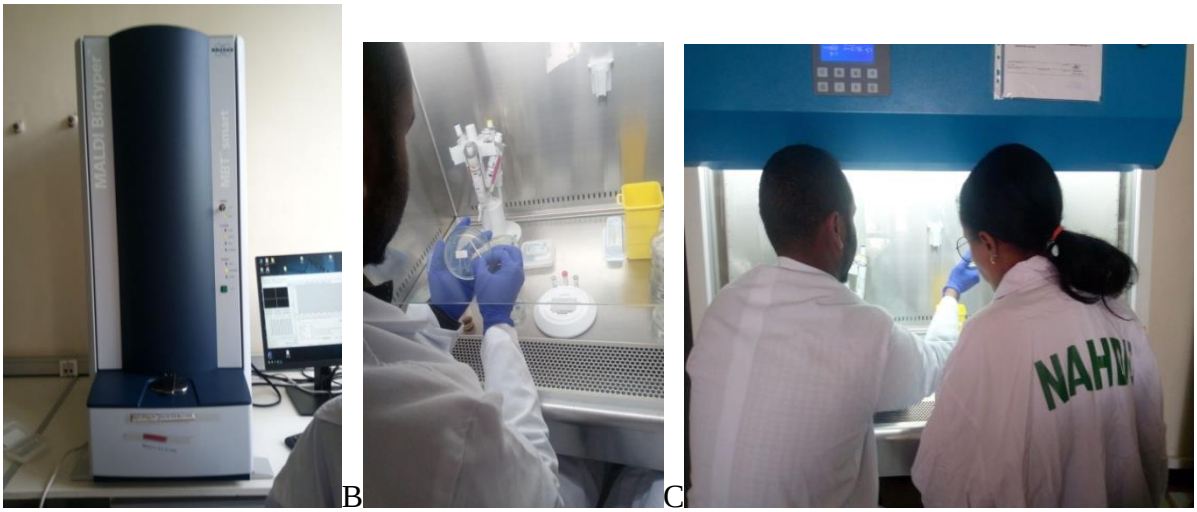
Appendix N Representative table of percentage of antibiotics used for gram-postive

	R	I	S
AMP	33.30%	33.30%	33.30%
FEP	66.60%	0%	33.30%
CIP	66.60%	0%	33.30%
DA	100%	0%	0%
E	66.60%	33.30%	0%
GM	33.30%	0%	66.60%
P	66.60%	0%	33.30%
PTZ	33.30%	33.30%	33.30%
T	33.30%	0%	66.60%

Appendix O Representative table of percentage of occurrence of antibiotics used for gram-negative

	R	I	S
AMC	70%	20%	10%
AMP	50%	10%	40%
KZ	70%	20%	10%
FEP	60%	10%	30%
CTX	70%	20%	10%
CAZ	80%	0%	20%
CIP	70%	20%	10%
DA	50%	40%	10%
E	60%	20%	20%
GM	30%	0%	70%
IMP	60%	20%	20%
P	100%	0%	0%
PTZ	40%	30%	30%
SXT	60%	10%	30%
T	60%	20%	20%

Appendix P Representative picture of MALDI-TOF MS



B C

Figure (A): MALDI biotyper **(B)** and **(C):** MALDI-TOF processing in bacteriology labratory

Appendix Q Representative table of bacterial isolation and sample code

Code	Name of the bank	Branch or location	Positive/Negative	Isolated bacteria
ICBE1	CBE	Around 04	+	***S.aureus***
ICBE2	CBE	Around jemalmegazen	+	***E.coli***
ICBE3	CBE	Around bole	+	***Bacillusspp, S.aureus***
ICBE4	CBE	Around derartutulu street	+	***S.aureus***
ICBE5	CBE	ASTU compound	+	***S.aureus, K.pneumoniea***
ICBE6	CBE	Adamabuta road	+	***E.coli***
ICBE7	CBE	Adamabuta road	+	***K.pneumoniea***
ICBE8	CBE	Aba geda street	+	***E.coli***
ICBE9	CBE	Aba jifar road	+	***P.aeuroginosa***
ICBE10	CBE	Daka-adi branch	+	***S.aureus***
ICBE11	CBE	Around selase church	+	***P.aeuroginosa***
ICBE12	CBE	Around selase church	+	***S.aureus***
ICBE13	CBE	Elemo branch	+	***S.dysenteriae***
ICBE14	CBE	Elemo branch	+	***S.dysenteriae***
ICBE15	CBE	Sar-tera branch	+	***E.coli***
ICBE16	CBE	Around posta1	+	***S.aureus,Listeria***
ICBE17	CBE	Around posta2	+	***E.coli***

ICBE18	CBE	Around posta3	+	***P.stuarti***
ICBE19	CBE	Around posta4	+	***Listeria***
ICBE20	CBE	Around tikur-abay1	+	***P.stuarti***
ICBE21	CBE	Around tikur-abay2	+	***C.freundi***
ICBE22	CBE	Berecha branch	+	***S.aureus***
ICBE23	CBE	Turban obo branch	+	***Listeria***
ICBE24	CBE	Adama hospital	+	***Bacillus spp***
ICBE25	CBE	Gendegara branch	+	***P.mirabills***
ICBE26	CBE	Around gendegara	+	***Shigella spp***
ICBE27	CBE	Around amede1	+	***Listeria***
ICBE28	CBE	Around amede 2	+	***Listeria, C.freundi***
ICBE29	CBE	Around franko1	+	***Listeria***
ICBE30	CBE	Around franko2	+	***Listeria***
ICBE31	CBE	Aba-geda branch	+	***Listeria***
ICBE32	CBE	Around robi hotel	+	***P.mirabills***
ICBE33	CBE	Adama branch	+	***Listeria***
ICBE34	CBE	Irrecha branch	+	***P.mirabills***
IBOA1	BOA	Around 09 1	+	***S.aureus***
IBOA2	BOA	Around 09 2	+	***Salmonella spp***
IBOA3	BOA	Around 09 3	+	***Listeria***
IBOA4	BOA	Around 09 4	_	_
IBOA5	BOA	Adamamenaheriya branch	_	_
IBOA6	BOA	Around elemokiltu road	_	_
IBOA7	BOA	Around posta 1	+	***C.freundi***
IBOA8	BOA	Around posta 2	+	***P.mirballis***
IBOA9	BOA	Around posta 3	+	***Listeria***

IBOA10	BOA	Around posta 4	+	***P.mirballis***
IBOA11	BOA	Around warka1	+	***E.coli***
IBOA12	BOA	Around warka2	+	***Listeria***
IBOA13	BOA	Around warka3	+	***Listeria***
IBOA14	BOA	Arada branch	+	***Listeria***
IAIB1	AIB	Around posta1	+	***S.marcessens***
IAIB2	AIB	Around posta2	+	***S.marcessens***
IAIB3	AIB	Bole branch	+	***Salmonella spp***
IAIB4	AIB	Adama General hospital	+	***S.marcessens***
IAIB5	AIB	Around sartera	+	***seratia***
IAIB6	AIB	Selase branch	+	***Listeria***
IAIB7	AIB	Gendegara branch	+	***Listeria***
IAIB8	AIB	Around ASTU	+	***Listeria***
IDB1	DB	Around pota road1	+	***S.typhi***
IDB2	DB	Adama-Ras branch	+	***Listeria***
IDB3	DB	Selase branch	_	_
IDB4	DB	Kechema branch	+	***P.mirballis***
IDB5	DB	Dashen bank building	+	***S.marcessens***
IBuB1	BuB	Selasebanch	+	***S.marcessens***
IBuB2	BuB	Sartera branch	+	***S.marcessens***
IBuB3	BuB	Tikur-abay branch	+	***S.marcessens***
IBuB4	BuB	Gendegara branch	+	***S.marcessens***
IAbB1	AbB	Around adama hospital	+	_
IBrB1	BrB	Around mebrathaileroad	+	***Salmonella spp***

IAmB1	AmB	Around posta bet road	+	***S.marcessens***
ICBO1	CBO	Chafe branch		***Salmonella spp***
ICBO2	CBO	Derartutulu branch		***K.pneumoniea***
INIB1	NIB	Sartera branch	_	_
INIB2	NIB	Adamamenahariya branch	_	_
IZB1	ZB	Dada-mall building	+	_
IWB1	WB	Around mebrathaile	+	***S.aureus***
IWB2	WB	Around franko road	_	_
IDGB1	DGB	Dembela branch	+	***C.freundi***
IOIB1	OIB	Arada branch	-	-
IOIB2	OIB	Boste branch	-	-
IOIB3	OIB	Kerayu branch	-	-
AdIB1	AdIB	Adama branch	+	***Salmonella spp***

Appendix R Representative Table of types of bacterial isolates recorded from different bank ATM centers

S/N	Name of Banks	Total bacterial isolates
1	**CBE**	*E. coli, P.mirballis, K.pneumoniea, Bacillus, S.aureus, S.dysenteriea, S.thyphimurium, Listeria, P.stuarti, Citrobacter spp, P.aeuroginosa*
2	**BOA**	*Listeria, S.aureus, Salmonella, Citrobacter, P.mirballis , E.coli*
3	**AIB**	*S.marcessens,Salmonellaspp,Listeria*
4	**DB**	*Listeria,S.Typhi, P.mirballis,S.marcessens*
5	**BuB**	*S.marcessens*
6	**CBO**	*Salmonella spp, K.pneumoniea*
7	**AbB**	_
8	**ZB**	_
9	**WB**	*S.aureus*
10	**DGB**	*C.freundi*
11	**AmB**	*S.marcessens*
12	**BrB**	*Salmonella spp*
13	**NIB**	_
14	**OIB**	
15	**AdIB**	*Salmonella spp*

Appendix S Representative table of Number and percentage of occurrence of Gram-positive bacterial isolates obtained from ATMs in Adama city

Isolated bacteria	Name of banks	Name of branch	code	occurrence
S.aureus	CBE	Ganda-gara branch,aroundderartutulu,ASTUcompound, daka-adibranch,kelechabranch,aroundposta 1,berecha branch and around bole	ICBE1, ICBE4, ICBE5, ICBE5, ICBE10, ICBE12, ICBE16, ICBE22 and ICBE3	13(16.25%)
	BOA	Around 09 1	IBOA1	1(1.25%)
	WB	Adama branch	IWB1	1(1.25%)
Listeria	CBE	Around posta 1 and 4,turbanobobranch,aroundamede 1 and 2,around franko 1and 2,around franko 1 and 2,aba-geda branch and adama branch	ICBE16,ICBE19,ICBE23,ICBE27,ICBE28,ICBE29,ICBE30,ICBE31 ,ICBE33	9(11.25%)
	BOA	Around 09 3,aroundposta 3,around warka 2 and 3,arada branch	IBOA3,IBOA9,IBOA12,IBOA13,IBOA14	5(6.25%)
	AIB	Selasebranch,gendegaabranch,around ASTU	IAIB6,IAIB7,IAIB8	3(3.75%)
	DB	Adamaras branch	IDB2	1(1.25%)
Bacillus	CBE	Around bole,aroundadamahospital,daha-di branch	ICBE3,ICBE24,ICBE10	3(3.75%)
Total				

Appendix T Representative Table of Number and percentage of Gram-negative bacterial isolates obtained from ATMs in Adama city

Isolated bacteria	Name of banks	Name of the branch	code	occurrence
E.coli	CBE	Around jemalmegazen,adamabutaroad,abagedastreet, sar tera branch around posta 2	ICBE2,ICBE6,ICBE8,ICBE13,ICBE17	4(5%)
	BOA	Around warka 1	IBOA11	1(1.25%)
K.pnemonia	CBE	Astu compound, and adamabuta road	ICBE5,ICBE7	2(2.5%)
	CBO	Derartutulu branch	ICBO2	1(1.25%)
P.aerogenosa	CBE	Around aba jifar road and kelecha branch	ICBE9,ICBE11	2(2.5%)
S.dytsenteriea	CBE	Elemobranch,elemobuilding,aroundgendegara	ICBE13,ICBE14,ICBE26	3(3.75%)
S.thypimurium	BOA	Around 09 kebele 2	IBOA2	2(2.5%)
	AIB	Bole branch	IAIB3	1(1.25%)
	DB	Around pota road 1	IDB1	1(1.25%)
	BrB	Around mebrathaile road	IBrB1	1(1.25%)
	CBO	Chafe branch	ICBO1	1(1.25%)
	AdIB	Adama branch	IadIB1	2(2.5%)

P.stuarti	CBE	Around posta3,aroundtikurabay 1	ICBE1,ICBE20	2(2.5%)
Citrobacter spp	CBE	Around tikurabay2,aroundamede 2	ICBE21,ICBE28	2(2.5%)
	BOA	Around posta 1	IBOA7	1(1.25%)
	DGB	Dembela branch	IDGB1	1(1.25%)
P.mirabilus	CBE	Gendegarabranch,aroundrobihotel,irrechabranch	ICBE25, ICBE32, ICBE34	3(3.75%)
	BOA	Around posta 2 and 4	IBOA8, IBOA10	2(2.5%)
	DB	Kechema branch	IDB4	1(1.25%)