Contamination of automated teller machines surfaces with multi-drug resistance, including esbl and quinolone-resistant gram-negative bacteria in dar es salaam, tanzania

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CONTAMINATION OF AUTOMATED TELLER MACHINES SURFACES WITH MULTI-DRUG RESISTANCE, INCLUDING ESBL AND QUINOLONE-RESISTANT GRAM-NEGATIVE BACTERIA IN DAR ES SALAAM, TANZANIA

By

Regan Shayo

A Dissertation Submitted in (Partial) Fulfillment of the Requirements for the Degree of Master of Science (Epidemiology and Laboratory Management) of

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CERTIFICATION

The undersigned certify that they have read and hereby recommended for acceptance by Muhimbili University of Health and Allied Sciences entitled: **‘‘Contamination of Automated Teller Machines surfaces with multi-drug resistance, including ESBL and quinolone-resistant gram-negative bacteria in Dar es Salaam, Tanzania”,** (partial) fulfilment of the requirements for the degree Master of Science (Epidemiology and Laboratory Management) of Muhimbili University of Health and Allied Sciences.

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Date: _____

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Date: _____

DECLARATION AND COPYRIGHT

I, **Regan Z. Shayo**, declare that this **dissertation** is my original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

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ABSTRACT

Background: Commonly touched public surfaces such as Automated Teller Machines (ATMs) are reported to be contaminated with a variety of pathogenic bacteria including multi-drug resistant (MDR) gram-negative bacteria, with potential for transmission of such dangerous microorganisms among users. In Tanzania little is known about the proportion of MDR gram-negative bacteria contaminated on ATMs, associated factors, and antimicrobial resistance patterns. This study aimed to determine the proportion of MDR gram-negative bacteria contaminated on ATM surfaces, associated factors, and antimicrobial resistance patterns in Dar es salaam, Tanzania.

Methodology: This was a cross-sectional study, conducted between January and March - 2021 at Dar es Salaam. A total of 298 ATMs from NMB, NBC, and CRDB banks were studded. Observation checklist was used to collect information. Sterile cotton swabs were used to collect samples from the mostly touched ATM Metallic keypads/screen, placed in nutrient broth. Samples were cultured on Mac-Conkey agar, and antimicrobial susceptibility was done using the Kirby Bauer disc diffusion method as per the CLSI guideline. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were standard organisms used as control. ESBL production was done using the combination disk method and MDR was identified when bacteria were resistant to three or more antimicrobial classes. Chi-square and modified poisson regression were performed to show factors associated with MDR contamination. P-value <0.05 was considered statistical significance

Results: More than half (55.4%) of ATMs in Dar es Salaam are contaminated with gram negative bacteria. *K. pneumoniae* was the most predominant bacteria, 18.5% (31/168). The highest level of resistance was observed against ampicillin (68.9%). About one-third 34.5% (58/168) of the isolates were MDR. ESBL produces were 14.1%(10/71) and more significantly resistant to meropenem (30%), while quinolone resistant isolates were 19.6%(33/168) and were more resistant to ampicillin (54.8%), trimethoprim/ sulfamethoxazole (37.1%), and meropenem (20.9%). Risk factors for contamination of ATMs included location at Ubungo (PR adj = 3.62, 95%CI = 1.58-8.30, P=0.002), Kigamboni (PR adj = 2.78, 95%CI = 1.20-6.42, P=0.017), and Temeke (PR adj = 2.75,

95%CI = 1.04-3.72, P=0.023), and less frequent cleaning (PR adj = 1.98, 95%CI = 1.04-3.73, P=0.04)

Conclusions: More tha half of ATMs in Dar es Salaam are contaminated with gram-negative bacteria including multi-drug resistant, especially those located in highly populated areas and the less frequently cleaned ones. These findings indicate the potential role of ATMs in Dar es Salaam in spreading multi-drug resistant bacteria that can cause infections that are difficult to treat, which should alert customers and owners of these machines. Clear instructions are urgently needed regarding disinfection of the machines and clients' precautionary measures, mainly hand sanitation.

Keywords: Multi-drug resistance, Gram-negative bacteria, Automated Teller Machine (ATM), Extended-Spectrum Beta-Lactamases (ESBL), Quinolone/ flouraquinolone-resistant.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AMR	Antimicrobial Resistance
AST-	Antibacterial susceptibility testing
ATM	Automated Teller Machine
CLSI	Clinical and Laboratory Standards Institute
COVID-19	Corona Virus Disease-2029
CRDB	Cooperative Rural Development Bank
E. coli	*Escherichia Coli*
ESBL	Extended Spectrum Beta Lactamases
GNB	Gram negative bacteria
GPB	Gram-positive bacteria
LMIC	Low middle-income countries
MCA	MacConkey Agar
MDR	Mult-Drug Resistant
MHA	Mueller Hinton agar
MoHCDGEC	Ministry of Health Community Development Gender Elder and Children
MRSA	Methicillin resistance staphylococcus aureus
MUHAS	Muhimbili University of Health and Allied Sciences
NBC	National bank of commerce
NMB	National Microfinance Bank
NPHL	National Public Health Laboratory
SOP	Standard operating procedure
SSA	Sub-Saharan Africa
TFELTP	Tanzania Field Epidemiology and Laboratory Management Program
VRE	Vancomycin Resistance Enterococci

DEFINITION OF TERMS

Multi-Drug Resistance: Bacteria resistant to at least one antimicrobial in three or more antibiotic classes

Antibiogram: An overall profile of antimicrobial susceptibility testing results of a specific organism to a battery of antimicrobial drugs. This profile is generated by the laboratory using aggregate data from a hospital or healthcare system, data are summarized periodically and presented showing the percentage of organisms tested that are susceptible to a particular antimicrobial drug**.**

Inanimate surfaces: Refers to anything that have no life (ATM keypads or screen).

Community-Acquired Infection: This are infections which are contracted outside the hospital

Contamination: The presence of an infectious agent on a surface such as ATM kepads, ATM screen, or any innominate surfaces.

Automated Teller Machine: Computerized telecommunication device that enables the clients of the financial institution to perform a financial transaction without a need of a cashier, human clerk, or bank teller.

Quinolone/Flouraquinolone resistance: Resistance to either Ciprofloxacin or Nalidixic acid

Extended-spectrum beta-lactamases (ESBL): Enzymes that confer resistance to most beta-lactam antibiotics, including penicillins, cephalosporins, and the monobactam aztreonam.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The emergence and spread of multidrug-resistant (MDR) bacteria in the community has a higher impact on modern medicine (1). Multi-drug resistant (MDR) resistance organisms have been associated with increased morbidity and mortality, increased length of hospitalization, costs, and loss of productivity(1,2). Often treatment of infections caused by drug-resistant organisms requires drugs that are expensive, more toxic, and rarely afforded by many, especially in Low- and Medium-Income Countries (LMICs)(2).

Gram-negative bacteria, specifically Enterobacteriaceae, are common causes of both community-acquired and hospital acquired Urinary Transimission Infections (UTIs) (3). *Klebsiella pneumoniae, Escherichia coli* as well as *Enterobacter spp*. and non-lactose fermenting bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter spp*. have been identified as major cause of multi-drug resistant bacterial infections(4,5) World Health Organisation (WHO) reported these bacteria to become resistant to a large number of antibiotics, including carbapenems, flouraquinolones and third generation cephalosporins –the best available antibiotics for treating multi-drug resistant bacteria(6). Extended-spectrum, which cause resistance to extended-spectrum cephalosporins (ESCs), are of considerable concern in veterinary and human medicine. This is because resistance to ESCs and co-resistance to other antimicrobial families (e.g., fluoroquinolones) limits the treatment options for infections with ESBL-producing bacteria(7).

Escherichia coli and *Klebsiella pneumoniae* reported to express 10%–40% and 40%-45% extended-spectrum beta-lactamases (ESBLs) and quinolones resistance respectively (8–10). This is complicating the treatment of serious infection and threatening to create resistance to all current available microbial agents(11,12) Thus, prevention of further community spread of MDR bacteria is of the utmost importance(13).

The environment has been cited to play a key role in the spread of Anti-microbial resistance (AMR) and Multi-drug resistance (MDR) micro-organisms, including resistance to extended-spectrum beta-lactamase (ESBL) producing bacteria and quinolone/ flouroquinolone resistant bacteria (14,15). Human hands reported to play role on habouring and transimiting variety of pathogenic bacteria in the community including MDR bacteria(16). Commonly touched public surfaces such as banknotes and Automated Teller Machines (ATMs) have been reported to be contaminated with a variety of pathogenic bacteria(17–19). Due to urbanization and the increase in population, ATMs are the most widely banking system used (20,21). The increased use of ATMs has been considered a potential source of bacterial contamination including MDR bacteria(22). Several studies has reported ATMs to be contaminated with MDR bacteria, including gram-negative bacteria and considered as potential source of community-acquired infections (19,23,24). In Tanzania literatures indicating the presence of ESBLs and quinolone resistance bacteria in the hospital serting and in the community. For example study done in Dar es Salaam on domestic pig and poultry showed that 51.6% of *E.coli* isolates were MDR while 65.3% and 53.7% were ESBL and quinolone resistance respectively (25).This is alarming on the presense of high prevelance MDR bacteria within the community which can also be cross-transimited among individuals. Therefore this study aimed to determine the proportion of MDR gram-negative bacteria contamination on ATMs surfaces, associated factors, and antimicrobial-resistant patterns in Dar es Salaam, Tanzania.

1.2 Problem statement

Dar es Salaam is the most populated city in Tanzania, with approximately more than seven million people (26). The use of ATMs is significantly higher as Dar es Salaam is a commercial city in Tanzania. In 2019 Dar es Salaam had 290 bank branches, which constituted 30.3% of all branches in the country. The use of ATM observed to be 6.4 ATMs per 100,000 adults(27,28). These ATMs, which are used by people of various backgrounds, are lacking constant and frequent monitoring of hygienic measures. Some of the ATMs are not provided with disinfectants and have no instructions to clients. This scenario raises the potential of these machines to be vehicles for the transmission of microorganisms, including gram-negative MDR bacteria, which causes infections that are difficult to treat.Literatures shows that the burden of MDR bacteria reported to range from 50%- 63% in the studies done in in Dar es Salaam(25,29) but little is known on the proportion of MDR bacteria contamination on environment and commonly touched public surfaces. This study aimed to determine the proportion of MDR gram-negative bacteria contaminated on ATMs surfaces, associated factors, and antimicrobial-resistant patterns in Dar es Salaam, Tanzania.

1.3 Conceptual framework

Figure 1 summarizes the conceptual framework of the study. ATMs surface contamination can be associated with factors such as lack of best hygiene practice among ATM users, and incompliance to standard methods of cleaning monitoring, improper disinfection of the surface's location of ATM, and Type of ATM. The gram-negative bacteria that were isolated from contaminated ATM surfaces have been reported to contribute to MDR hence resistance strains, which lead to treatment failure.

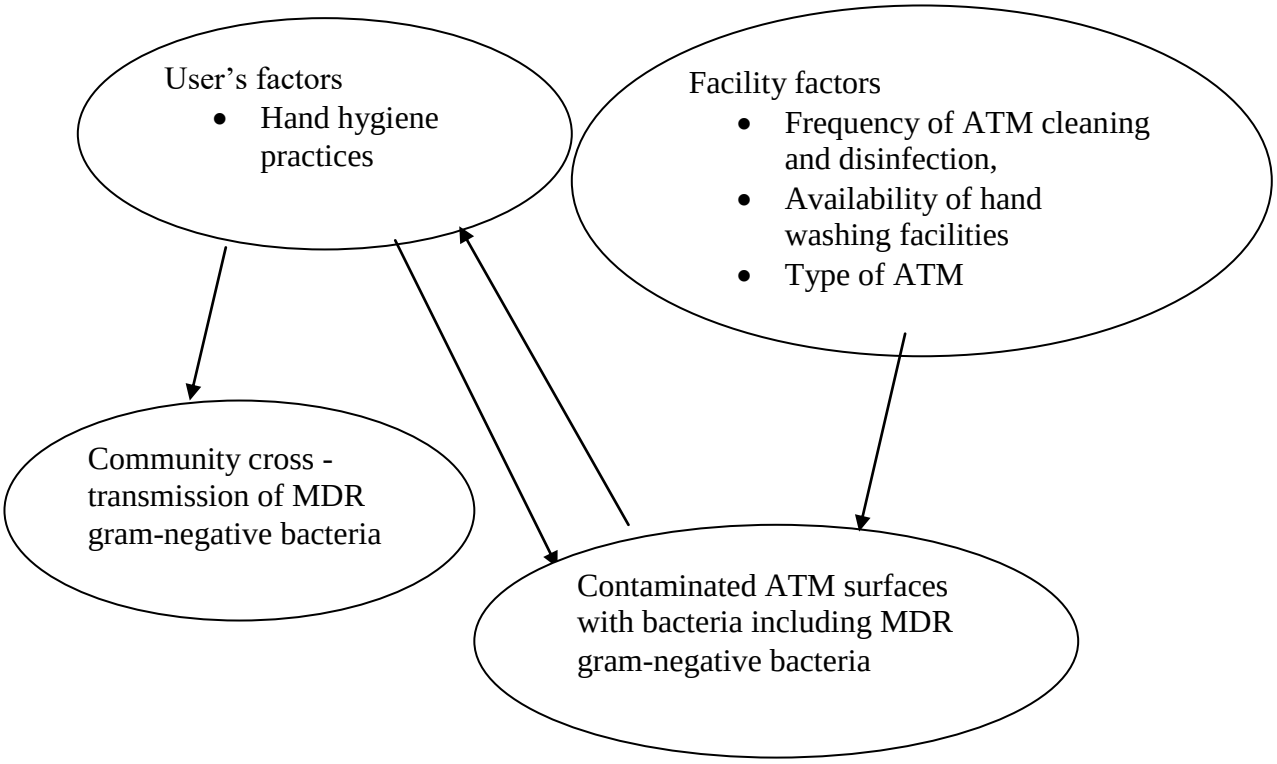

Figure 1: Conceptual Framework of the study

1.4 Justification

Surveillance of AMR and MDR pathogens is one of the strategic objectives in the National action plan on antimicrobial resistance 2017-2022(30). In its part, this study will provide data on the burden of MDR gram-negative bacteria contamination on ATMs in Dar es Salaam, where the use of these machines is highest in the country. Data emanating from this study will sensitize both owners and users of these machines, of their potential to transmit pathogens. The study will provide evidence for better management of the ATMs to curb potential transmission of infectious agents among users, including dangerous infections such as COVID-19.

1.5 Study hypothesis

1.5.1 Null

Automated Teller Machines surfaces are not contaminated with multi-drug resistance (MDR) gram-negative bacteria.

1.5.2 Alternative

Automated Teller Machines surfaces are contaminated with multi-drug resistance (MDR) gram-negative bacteria.

1.6 Objectives

1.6.1 Broad objective

To determine the proportion MDR gram-negative bacteria contaminated on ATMs surfaces, associated factors, and antimicrobial resistance pattern.

1.6.2 Specific objectives

Specific objectives of this study were;

1. To determine the proportion MDR gram-negative bacteria contaminated on ATMs surfaces in Dar es Salaam from January to March 2021.
2. To determine the factors associated with contamination of ATMs with MDR gram-negative bacteria in Dar es salaam, Tanzania from January to March 2021
3. To determine antimicrobial resistance patterns for gram-negative bacteria isolated from ATM surfaces in Dar es salaam City Tanzania from January to March 2021

1.7 Research questions

1. What is the proportion MDR gram-negative bacteria contaminated on ATMs surfaces in Dar es Salaam Tanzania?

2. Which factors are associated with MDR gram-negative bacterial contamination of ATMs in Dar es Salaam, Tanzania?

3. What is the antimicrobial resistance pattern for gram-negative bacteria isolated from ATMs in Dar es Salaam, Tanzania?

CHAPTER TWO

2.0 LITERATURE REVIEW

The emergence and spread of the resistance in bacteria are complicating the treatment of infections with currently available antimicrobial agents (12). Recent studies have reported increasing identification of MDR bacteria in cultures from non-hospitalized persons (31,32). Quinolone resistance bacteria and ESBL-producing bacteria, particularly *E. coli* and *K. pneumoniae*, have been reported to emerge in the community and linked to healthcare-associated infections, but are repeatedly isolated from community-acquired bacterial infections (33–35).

According to the European Antimicrobial Resistance Surveillance Network (EARS-Net) the resistance of *E. coli* and *K. pneumonia* to most common antibacterial classes used in clinical practice is high in some countries, where these drugs can no more be empirically used for the treatments of infections such as UTIs. For example, in Italy, rates of antibiotic resistance to aminopenicillins, aminoglycosides and fluoroquinolones in *E. coli* were 65%, 19% and 44%, and rates of antibiotic resistance to aminoglycosides and fluoroquinolones in *K. pneumonia* were 49% and 56% in 2014(36). In resourse limited countries including Tanzania studies shows, there is high antibiotic resistance among Gram-negative bacteria to commonly used antibiotics, ranges from 30% and 75%, leading to a loss of efficacy for treatment of common infections(29,37,38).

In Tanzania several studies have been conducted on MDR bacteria in community and hospital settings. A study done in Dar es salaam revealed that almost a quarter of private and shared latrines in an informal urban settlement in Tanzania are contaminated with ESBL-producing micro-organisms, suggesting a high prevalence of human ESBL fecal carriage in the community (39). A study done in Dar es Salaam on domestic pig and poultry showed that 51.6% of *E.coli* isolates were MDR while 65.3% and 53.7% were ESBL and quinolone resistance respectively(25). Another study showed that the prevalence of ESBL carriage was significantly higher among hospitalized children (50.4%), compared to community children

(11.6%),(40). A study showed that 16.5% of the community in Mwanza region was contacted with ESBL producing bacteria. *Escherichia coli* was significantly higher (15.1%) than that of *Klebsiella pneumoniae* (3.8 %). In addition, 88.1% of ESBLs isolates were carrying resistant genes (41).

Contaminated surfaces have been reported as an established route of transmission for high-risk pathogens, including those with pandemic potential (39,40,42,43). Bacteria can persist on an inanimate surface for days (44). Forexample, it has been established that *E. coli 0157:H7* can survive for up to eleven days on the inanimate surface(45). Human beings have a marked tendency to pick up microorganisms from environmental objects, and hands have been shown to play an important role in their transmission(46). Automated Teller Machine (ATM) surfaces, like any other inanimate surfaces in the community, are likely to be contaminated with micro-organisms since they are used frequently and by many people, and no restriction or hygienic guidance is provided to people when they are accessing these facilities.

Although there are studies reports on the bacteriological examination of various surfaces including paper currency (17,47), there are few reports on the examination of MDR bacteria on ATMs surfaces in Africa(19), and there is no study on the examination of MDR bacteria on ATMs surfaces in Tanzania. A study by Duraipandia et al-(2015) on contamination of ATMs surfaces with pathogenic and resistant microbial reviled that 10.6% of isolates were *E. coli* of which about 70% of isolated *E. coli* were resistant to amoxiclav and 100% resistant to co-trimoxazole(48). A study by Nachimuth et al 2025; showed that ATMs surfaces in India were contaminated with variet of gram negative pathogenic bacteria such as *E.coli* and *K.pneuminiae* which also showed high resistance level towards Cefotaxime and Meropenum(24). Automated Teller Machine (ATM) metallic keypads in Ebonyi state Nigeria revealed the presence of pathogenic microbes including MDR bacteria, which were found to be resistant to some commonly used antibiotics. *Pseudomonas aeruginosa* showed 68.75% resistance to the antibiotics and *E.coli* showed 43.75% resistance (19).

A study done in Korogwe and Mombo towns in Moshi-Tanzania revealed that paper currency notes are contaminated with gram-positive bacteria (GPB) and gram-negative bacteria (GNB), predominantly *E. coli* (49). However, the isolated bacteria were not tested for antimicrobial resistance.

In Tanzania, there is limited information regarding the proportion of MDR gram-negative bacteria contamination on ATM surfaces, its associated factors, and antimicrobial resistance pattern.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design

This was a cross-sectional study, carried out at Dar es Salaam City Tanzania for 3 months between January and March 2021.

3.2 Study area

The study area was Dar es Salaam City. Dar es salaam is a highly populated city in Tanzania, with approximately 7 million people. Dar es salaam is a business city with a high number of ATMs located in various parts.

3.3 Study units

The study units were ATMs buttons/screen surfaces in public areas in Dar es Salaam city Tanzania.

3.4 Criteria for inclusion and exclusion

3.4.1 Inclusion Criteria

All ATMs in public areas were eligible for inclusion in the study

3.4.2 Exclusion Criteria

ATMs that are located in hospital compounds

3.5 Sampling method

ATMs of the three largest banks in Dar es Salaam namely: National Microfinance Bank (NMB), Cooperative Rural Development Bank (CRDB), and National bank of commerce (NBC) in all five districts in Dar es Salaam City were the sampling frame. This banks were puporsevely selected because they contribute high number of ATMs in Dar es Salaam. A list of all ATMs was obtained from respective banks, which summed up to 432. ATMs located on

hospital compound were excluded. The proportion of ATMs of specific banks included in the sample size (298) depended on the proportion of specific bank ATMs contributed to the sample frame. Banks with a high number of ATMs in the sample frame contributed a higher number of ATMs in sample size. A simple random technique was used on specific bank ATMs to select the ATMs that were contributed in sample size (298). Samples were distributed as follows: NMB 121 out of 176, CRDB 119 out of 173, and NBC 58 out of 83. Factors associated with ATMs contamination was collected using an observation checklist (Appendix I)

3.6 Sample size estimation

The sample size will be calculated by using Kish Leslie formula;

$$n = \frac{z^2 p (1-p)}{\varepsilon^2}$$

Where;

z= level of confidence (1.96 for 95% confidence level).

p = expected proportion (prevalence of *E. coli* bacteria on ATMs surfaces was 21.4%)(19).

ε = margin of error = 5%.

$$n = \frac{1.96^2 x 0.214 (1-0.214)}{0.05^2} = 258$$

Therefore, the minimum required sample size for ATMs was 258. (Samples were raised to 298 to increase the power of the study)

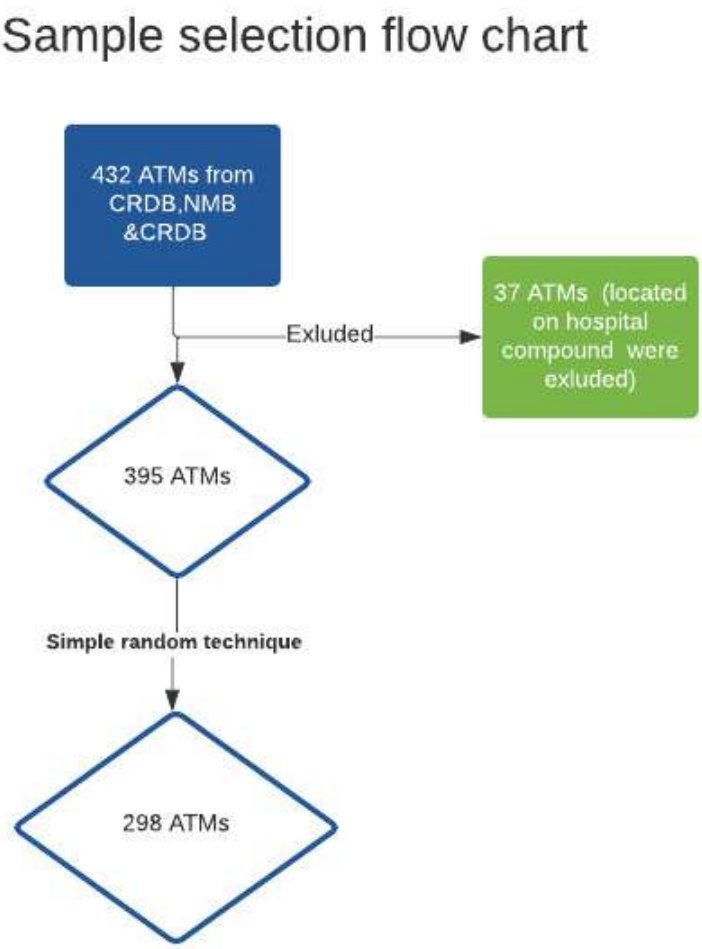

Figure 2: Sample selection flowchart

3.7 Study variables

3.7.1 Dependent variables

1. MDR gram-negative bacteria isolated from ATMs surfaces.
2. AMR pattern of isolated MDR gram-negative bacteria.

3.7.2 Independent variables

Frequency of ATM cleaning and disinfection, availability of hand-washing and cleaning facilities, Location, and Type of ATM (Stand alone ATMs or Branch ATMs).

3.8 Sample collection method, Transportation and laboratory processes

3.8.1 Sample collection and Transportation

A Sterile swab (Himedia, Mumbai, India), was moistened in sterile saline and then moved several times over the surfaces of some selected frequently-used keys on the ATM keypad/screen in aseptic procedure and placed into nutrient broth media (Oxoid, Hampshire, United Kingdom) and transported to National Public Health Laboratory (NPHL) in ice bag for processing.

3.8.2 Sample processing

Samples in nutrients broth were incubated at 37°C for 18-24 hours before culture. The culture was performed on Mac-Conkey (MCA) agar (Oxoid, Hampshire, United Kingdom) with crystal violet and bile salt. Culture plates were incubated aerobically at 37°C for 18-24 hours.

3.8.3 Bacteria isolation and Identification

Isolated bacteria were characterized by performing standard biochemical tests, which included oxidase, urease, Indole, Citrate test, and Triple Sugar Iron following Clinical Laboratory Standards Institute (CLSI 2020) guideline(50). For identification of gram-negative bacteria with ambiguity, API 20 E system (Bio-Merieux, France) was used as per manufacture instruction.

3.8.4 Antibiotic susceptibility

Identified gram-negative strains were subjected to antibiotic sensitivity test using agar diffusion method on Muller Hinton agar (Oxoid, Hampshire, United Kingdom) to determine their susceptibility patterns against selected antimicrobial agents, as described by CLSI, 2020(50).the antimicrobial agent used were gentamicin(10µg), ciprofloxacin(30 µg), doxycycline (30 µg), nalidixic acid (30 µg), cefotaxime (30 µg), meropenem (10µg), ampicillin(10µg). chloramphenicol (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), and cefotaxime/clavulanic acid(30/10µg), (Bioanalyse, Turkey) for phenotypic ESBL confirmation. The potential ESBL producing gram-negative bacteria were screened by cefotaxime (30 µg) and was confirmed for ESBL production by combination disk method of

phenotypic detection of ESBL test, where cefotaxime (30 μg) and the combination disc cefotaxime + clavulanic acid (30 μg+ 10 μg) were placed with 25 mm apart. An increase of ≥ 5 mm in the zone of inhibition for cefotaxime + clavulanic acid compared to cefotaxime alone was confirmed as an ESBL producer(51). Bacteria showing resistance against ciprofloxacin and nalidixic acid were regarded as quinolones/flouraguinolones resistance(52).

3.8.5 Quality Assurance

The culture media used for isolation and identification of organisms (Mac Conkey agar, Biochemical tests) was controlled using standard organisms (*E. coli* ATCC 25922 strains). For ESBL producing gram-negative bacteria, ESBL producing *K. pneumonia* ATCC 700603 and non-ESBL producing *E. coli* ATCC 25922 were used as a positive and negative control as per CLSI 2020(50). Two readers to minimize bias performed plate reading for isolation and identification of organisms. Senior microbiologist at the laboratory assured quality by reviewing the results.

3.9 Data analysis

Data management and analysis were done by using STATA version 15.1. Frequencies and proportions of bacteria isolated and their antibiograms were determined. A Chi-square test was used to determine the univariate association with factors that are associated with MDR contamination on ATM surface. Any variable with P<0.25 was subjected to multivariate analysis. Since the proportion of MDR bacteria was above 15% we used modified Poisson analysis to determine independent predictors of ATM surface contamination. Results from modified poisson regression analysis were presented as risk ratio and 95% Confidence Interval. A p-value of < 0.05 was considered statistically significant. Analysed data were summarized in tables and figures.

3.10 Ethical considerations

Ethical approval for this study was obtained from MUHAS Senate Research and Publications Committee (Ref. No.DA.282/298/01.C/). Authorization to conduct the study was requested and granted from the management of selected Banks.

CHAPTER FOUR

4.0 RESULTS

4.1 Overall proportional of Gram-negative bacteria recovered from ATM surfaces

Of the 298 swabs collected from ATM surfaces(screen/key-pads), 165 (55.4%) showed microbial growth. A total number of 168 microorganisms were recovered. The distribution of bacteria recovered from ATM surfaces is shown in Table 1. *Klebsiella pneumoniae* (18.5%) was the predominant isolate followed by *Acinetobacte*r spp and *E. coli*, while *Proteus* and *Providencia species* showed the least percentage (0.6%) each.

Table 1: The pattern of Gram-negative bacteria recovered from ATM surfaces in Dar es Salaam Tanzania

Organism	Number of isolates	Per cent
Klebsiella pneumoniae	31	18.5
Acinetobacter sp	21	12.5
Escherichia coli	17	10.1
Pseudomonas aeruginosa	14	8.3
Enterobacter aerogenes	13	7.7
Shigella sp	13	7.7
Enterobacter sp	12	7.1
Serratia sp	11	6.6
Klebsiella oxytoca	9	5.4
Salmonella sp	8	4.8
Citrobacter sp	7	4.2
Pseudomonas sp	4	2.4
Yersinia sp	4	2.4
Morganella sp	2	1.2
Proteus sp	1	0.6
Providencia sp	1	0.6
Total	168	100

4.2 Antimicrobial resistance pattern of isolated bacteria

The overall highest percentage of resistance was observed on ampicillin (68.9%) followed by cefotaxime (26.8%) while gentamicin showed the least resistance (1.3%). *K. pneumoniae*, *Acinetobacter sp*, *E. coli* and *P. aeruginosa*, showed high, moderate and low levels of resistance ranging from 3.2% to 87.1%. (Table 2)

Table 2: Antibiotic resistance pattern among the 168 isolates recovered from ATMs

Organism	#Isolates	AMP %R	CIP %R	MEM %R	CTX %R	SXT %R	GEN %R	CHL %R	NAL %R	DOX %R
K. pneumoniae	31	87.1	3.2	0	32.3	16.1	0	3.2	0	6.5
Acinetobacter sp.	21	28.6	4.8	4.8	14.3	38.1	4.8	9.5	47.6	0
Escherichia coli	17	70.6	5.9	0	23.5	41.2	0	11.8	17.6	11.8
P. aeruginosa	14	78.6	0	0	42.9	21.4	0	28.6	7.1	14.3
Enterobacter sp.	13	61.5	15.4	7.7	15.4	30.8	7.7	7.7	30.8	7.7
Shigella sp.	13	38.5	15.4	7.7	38.5	15.4	0	7.7	38.5	0
E. aerogenes	12	83.3	8.3	8.3	58.3	16.7	8.3	8.3	16.7	8.3
Serratia sp.	11	81.8	0	0	36.4	0	0	0	0	0
Klebsiella oxytoca	9	88.9	0	0	11.1	44.4	0	11.1	22.2	0
Salmonella sp.	8	62.5	12.5	12.5	62.5	12.5	0	0	37.5	25
Citrobacter sp.	7	71.4	14.3	0	42.9	0	0	14.3	14.3	28.6
Pseudomonas sp.	4	100	0	0	25	25	0	0	0	25
Yersinia sp.	4	50	0	0	25	0	0	0	0	0
Morganella sp.	2	0	0	0	0	0	0	0	0	0
Proteus sp.	1	100	0	0	0	0	0	0	0	0
Providencia sp.	1	100	0	0	0	0	0	0	0	0
Total	168	68.9	4.9	2.6	26.8	16.4	1.3	6.4	14.7	7.9

Key: CIP, ciprofloxacin; CHL, chloramphenicol; NAL, nalidixic acid; GEN, gentamycin; AMP, ampicillin; DOX, doxycycline; SXT, trimethoprim/sulfamethoxazole; CTX, cefotaxime; MEM, meropenem; ESBL, Extended spectrum beta-lactamase;

4.3 Proportion of MDR Gram-negative bacteria

Out of the 168 isolates, 34.5% (58/168) were MDR against three to seven classes of the tested drugs. *Enterobacter aerogenes* had the highest proportion of MDR isolates 53.8% (7/13) compare to other gram-negative bacteria, which ranged between 22.5% and 50%. From the most frequently isolated bacteria, common resistance pattern observed was CEPH3/ PEN/PHEN, CEPH3/ FOLATE/ FQ/ PEN/ QUIN, and FOLATE/ PEN/ QUIN. One isolate each from *E. coli, P. aeruginosa, and Acinetobacter* species, were resistant to six and above classes of antimicrobials as shown on Table 3.

Table 3: Multi-drug resistance pattern among most frequently isolated ram negative bacteria recovered from ATM surface

Organisms	Profile	Resistance classes	# Isolates
Acinetobacter sp	CEPH3, FQ, QUIN	3	2
	CEPH3, PEN, PHEN	3	1
	FOLATE, PEN, QUIN	3	3
	CEPH3, PEN, PHEN, QUIN	4	1
	AG, CEPH3, FOLATE, PEN, PHEN	5	1
	CEPH3, FOLATE, FQ, PHEN, QUIN	5	1
	CARB, CEPH3, FOLATE, FQ, PEN, QUIN	6	1
E coli	CEPH3, PEN, PHEN	3	1
	FOLATE, PEN, PHEN	3	2
	FOLATE, PEN, QUIN	3	1
	FOLATE, FQ, PEN, QUIN	4	1
	CEPH3, FOLATE, FQ, PEN, QUIN	5	2
	CARB, CEPH3, FOLATE, FQ, PEN, QUIN	6	1
Klebsiella oxytoca	FOLATE, FQ, PEN, QUIN	4	2
	CARB, CEPH3, FOLATE, PEN, PHEN, QUIN	6	1
Klebsiella pneumoniae	CEPH3, FOLATE, PEN	3	2
	FOLATE, FQ, PEN	3	1
	CARB, CEPH3, PEN, QUIN	4	1
	CEPH3, PEN, PHEN, QUIN	4	1
	CEPH3, FOLATE, FQ, PEN, QUIN	5	2
Pseudomonas aeruginosa	CEPH3, FQ, PEN	3	1
	CEPH3, FQ, QUIN	3	1
	CEPH3, PEN, PHEN	3	2
	FOLATE, PEN, PHEN	3	1
	CEPH3, FOLATE, PEN, PHEN	4	1
	CARB, CEPH3, FOLATE, FQ, PEN, PHEN, QUIN	7	1

Key: QUIN, quinolones; PHEN, phenicol's; AG, aminoglycosides; PEN, penicillin's; FQ, Fluoroquinolone; FOLATE, sulphonamides; CEPH3, cephalosporins; CARB, carbapenems

4.4 Isolation frequency of ESBL-Producing gram-negative from ATM surfaces

Out of 71 isolates from the most common ESBL producing gram-negative bacteria, (*K. pneumoniae, E. coli, K. oxytoca and P. aeruginosa*) screened for ESBL, 14.1%(n=10/71) were ESBL producers. The Proportion of ESBL was observed to be high among *E. coli* isolates 23.5%(n=4/17) followed by *K. pneumoniae* 16.2%(n=5/31) and *K. oxytoca* 11.1% (n=1/9).

4.5 Quinolone-Resistant gram-negative bacteria recovered from ATM surfaces

Out of 168 isolates 19.6% (n = 33/168) were found to be quinolone/fluoroquinolones - resistant. *Shigella* species were observed to be more resistant to quinolones/fluoroquinolones (53.9%) followed by *Acinetobacter sp* (52.4%). Furthermore, about 50% of ESBL producers were resistant to quinolone.

4.6 Antibiotic resistance level among ESBL, and quinolone resistance isolates

Externded Spectrum Beta Lactamase producer's bacteria were more significant resistant to meropenem(P=0.04), while quinolone/flouraguinolone resistance isolates were more significantly resistant to trimethoprim/sulfamethoxazole;(P <0.001), and meropenem (P<0.001).(Table 4)

Table 4: Comparison resistance levels between ESBL vs non-ESBL producers, and Quinolone's resistance versus non-Quinolone's resistance among gram-negative bacteria

Drug	ESBL producers (n=10)	Non-ESBL producers(n=18)		Quinolone's resistance (n=33)	Non-quinolone resistant (135)	
	%R (n)	**%R(n)**	**P-Value**	**%R(n)**	**%R(n)**	**P-Value**
STX	50(5)	22.2(4)	0.23	51.5(17)	14.8(20)	<0.001
MEM	30(3)	0(0)	0.04	27.3(9)	3.7(5)	<0.001
DOX	20(2)	5.6(1)	0.24	5.4(9)	4.4(6)	0.38
GEN	0(0)	0(0)	1.00	0.0(0)	2.2(3)	1.00
CHL	10(1)	11.1(2)	1.00	9.1(3)	8.15(11)	1.00

Key: CHL, chloramphenicol; GEN, gentamycin; AMP, ampicillin; DOX, doxycycline; SXT, trimethoprim/sulfamethoxazole; MEM, Meropenem; ESBL, Extended spectrum beta-lactamase, %R percentage resistance, (n); number of isolates

4.8 Factors associated with MDR bacteria contamination on ATM surfaces

Table 5 shows univariate and multivariate modified Poisson regression of the factors associated with MDR bacteria contamination on ATM surfaces. At the univariate level, ATMs with less frequency of cleaning were more likely to be contaminated with MDR gram-negative bacteria compare to those cleaned at least once a day (PR_{crude} = 1.82, 95%CI = 1.16-2.84, P=0.009). ATMs located at Ubungo municipal (PR_{crude} = 3.52, 95%CI = 1.59-7.84, P=0.02) Kigamboni municipal (PR_{crude} = 3.25, 95%CI = 1.38-7.67, P=0.007) and Temeke municipal (PR_{crude} = 2.5, 95%CI = 1.03-6.06, P=0.04) were more likely significantly associated with MDR bacteria contamination compared to ATMs located at Ilala municipals.

Remote ATMs were observed to be 1.49 times more likely to be contaminated with MDR bacteria compare to ATMs located at the branch (PR crude = 1.49, 95%CI = 0.98-2.28, P= 0.06).

At multivariate level, ATM surfaces contamination were more likely significantly associated with ATMs located in Ubungo ($PR_{adjusted}$ = 3.62, 95%CI = 1.58-8.30, P=0.002), Kigamboni ($PR_{adjusted}$ = 2.78, 95%CI = 1.20-6.42, P=0.017), and Temeke ($PR_{adjusted}$ = 2.75, 95%CI = 1.04-3.72, P=0.023) compared to ATMs located at Ilala municipal. On the other hand, ATMs with less frequency of cleaning were significantly associated with an increased likely hood of MDR bacteria contamination compare to those cleaned at least once a day ($PR_{adjusted}$ = 1.98, 95%CI = 1.04-3.73, P=0.04). There was a decreased risk of MDR bacteria contamination on remote ATMs but was not statistically significant ($PR_{adjusted}$ = 0.79, 95%CI = 0.43-1.46, P=0.46).

Table 5: Univariate and multivariate analysis of factors associated with contamination of the ATMs with gram-negative MDR bacteria

			Univariate analysis			Multivariate analysis		
Variable	**Categories**	**n(%)**	**cPR**	**95% CI**	**P-value**	**aPR**	**95% CI**	**P-value**
ATM type	Stand alone ATMs	80(48.5)	1.49	0.98-2.28	0.06	0.79	0.43-1.46	0.46
	Branch ATMs	85(51.5)	*Ref*			*Ref*		
Frequency of ATM cleaning	At least once a week	81(49.1)	1.82	1.16-2.84	0.009	1.98	1.04-3.73	0.04
	Once a day	84(50.9)	*Ref*			*Ref*		
Location (Districts)	Kinondoni	39 (23.6)	1.89	0.8-4.48	0.46	1.98	0.8-4.72	0.12
	Ubungo	35(21.2)	3.52	1.59-7.84	0.02	3.62	1.58-8.30	0.002
	Kigamboni	20(12.1)	3.25	1.38-7.67	0.007	2.78	1.20-6.42	0.017
	Temeke	26 (15.8)	2.5	1.03-6.06	0.04	2.75	1.04-3.72	0.023
	Ilala	45(27.3)	*Ref*			*Ref*		

Key: cPR - Crude Privelance ratio, aPR - Adjusted Privelance ratio, CI-confidence interval, Ref-reference category

CHAPTER FIVE

5.0 DISCUSSION

This study reviled that more than half of ATMs in Dar es Salam are contaminated with gram-negative bacteria and one-third of this bacteria were MDR against three to seven classes of antibiotics used. Location of ATMs and cleaning practice was observed to be a risk factor for MDR bacteria contamination. This calls for interventional measures about public awareness of the ATMs as potential vehicles in the transmission of infections including those which are difficult to treat.

Our study revealed that 55.4% of ATMs in Dar Es Salaam city Tanzania are contaminated with gram-negative bacteria, which is lower than findings reported in a study done in India, where 95.7% of ATMs were found to be contaminated with such bacteria (48). This variation is probably contributed by the fact that the current study took place during the COVID-19 pandemic, where the use of hand sanitisers was high. Nonetheless, this poses a public health risk given the fact that half of the machines were contaminated with pathogenic bacteria incluging multi-drug-resistant bacteria.

In this study *K. pneumoniae* was the most predominant isolate, accounting for 18.5% followed by *Acinetobacter* sp. and *E. coli*. These results conform with observation reported in a study in india (48) where *K. pneumoniae* (42.5%) was mostly isolated bacteria from ATM surfaces, but contrary to the finding of a study conducted by Nachimuth *et al*-(2015) and showed *E.coli* (49%) as predominant isolate followed by *Klebsiella* sp (30%) (24). However, collectively these studies show the predominance of *K. pneumoniae* and *E. coli* as the most significant gram-negative bacteria in contamination of ATM surfaces.

The current study revealed that the risk of contamination of ATM surfaces was higher in the less cleaned ATMs (ATMs cleaned atleast once a week), which conforming with a study that showed cleaning and disinfection of surfaces can reduces microbial contamination by 94.1% (53).

The risk of contamination of ATMs with MDR bacteria was also significantly associated with the location in densely populated areas namely Ubungo, Kigamboni, and Temeke. These observations are in keeping with a study conducted in Nigeria where ATMs from Abakaliki metropolis had higher isolation (78.6%) compare to a less densely populated Afikpo town (19). Collectively these findings support the need for maintaining strict hygienic measures on frequently touched public surfaces and overcrowded areas, which is effective in other studies (30,54,55).

Concerning AMR pattern, isolates recovered from this study showed high levels of resistance against the ampicillin, moderate level of resistance against, cefotaxime (CTX) trimethoprim/sulfamethoxazole(SXT) and nalidixic acid (NAL), and low level against meropenem (MEM) gentamicin (GEN) . An estimated one-third of all isolates were MDR. Some of the MDR isolates exhibited resistance to more than six different classes of antibiotics and could be classified as pan-drug resistant (PDR)(56). Notably, most MDR combinations included penicillin, tetracycline, and ciprofloxacin, which is in keeping with several studies conducted in Dar es Salaam, showing high resistance to these antibiotics (57,58). Resistance to these antibiotics can be explained by the fact that they are relatively cheap and they can be obtained over the counter without a prescription (59), which fuels the occurrence of resistance (60). Furthermore this study showed *Salmonella* spicies had high to moderate level of resistance against CTX and MEM respectively. This observation supports other study findings, where emergence of ESBL- producing *Salmonella sp* and carbapenum resistance have been reported in the community(61,62). Increase in resisance to *Salmonella* sp especially to MEM is alarming, as there are few option avilable to treat extensive drug-resistance (XDR) Typhoid. This is high time to take important step to study resistance pattern of salmonella sp to detect new stain timely.

Our study showed that among isolates screened for ESBL, 14.1% were ESBL producers. Compared with non-ESBL producers, ESBL producing bacteria had insignificant resistance to trimethoprim/sulfamethoxazole, chloramphenicol; gentamycin, doxycycline except meropenem. On the other hand, 19.6% of isolates were quinolone/ flouraquinolones-resistant

whereby quinolone/flouraquinolones resistance isolates were more significant resistant to trimethoprim/sulfamethoxazole, and meropenem except for gentamicin, doxycycline, and chloramphenicol compare to non-quinolone/flouraquinolones resistance. These findings were contrary to a study in Dar es salaam(25) showing ESBL producers and quinolone resistance isolates were more significantly resistant to all other tested antibiotics including, gentamicin, meropenem, chloramphenicol, doxycycline, and trimethoprim/sulfamethoxazole (25). This variation is presumably because the current study uses samples from inanimate surfaces while the other study uses poultry and pig, whose farming has been associated with intense use of antibiotics(63).

Nonetheless, 50% of ESBL producers were also resistant to quinolones, indicating and supporting shared mechanisms of resistance(64). These findings are important since beta-lactams and quinolones are the cornerstones for treatment of the majority of the infections occurring in humans and animals (65,66) and resistance to them has severe consequences on public health and animal production (67,68).

This study provides important preliminary information about the proportion of gram-negative MDR bacteria contamination on ATM surfaces, as well as associated factors. However, the study has several limitations. Users' hand hygiene practices were not observed, which could have provided evidence on the association of hand hygiene practices with contamination of ATMs with MDR bacteria. Finally, the preparation of the sanitisers, their composition, and expiry dates could not be verified. Hand sanitisers and water were treated the same. However this will not remove the fact that ATMs in Dar es Salaam are contaminated with pathogenic bacteria including those which are difficult to treat.

CHAPTER SIX

6.1 Conclusion

More than half of ATMs in Dar es salaam are contaminated with gram-negative bacteria, especially those which are not regularly cleaned and those located in densely populated areas, posing a danger to users and the potential spill-over to the community at large. One-third of these bacteria exhibit multi-drug resistance to commonly used antibiotics. This calls for interventional measures about public awareness of the ATMs as potential vehicles in the transmission of infections, including COVID-19. Clear instructions are urgently needed on disinfection of the machines and clients' precautionary measures, mainly hand sanitation. The owners of the ATMs need to ensure constant application of hygienic measures, including the provision of sanitisers, and constant monitoring of compliance.

6.2 Recommendations

Based on the observation made during this study the following recommendations are necessary to implement:

- Regular decontamination of the ATMs with freshly prepared disinfectants to reduce the microbial contamination of these devices.
- Availability and use of hand-washing/hand sanitiser before and after using ATM may reduce the risk of ATM contamination and possibility reduces transmission of potential pathogens.
- Increase public awareness on the potential of ATMs as vehicles in the transmission of infections, including COVID-19 and advocate for compulsory handwashing
- Research on molecular characterization of MDR isolates recovered from ATM surface to detect specific resistance genes and their potential transmission.

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APPENDICES

Appendix 1: Observation checklist

TITLE OF THE STUDY: CONTAMINATION OF AUTOMATED TELLER MACHINES SURFACES WITH MULTI-DRUG RESISTANCE GRAM-NEGATIVE BACTERIA INCLUDING ESBL AND QUINOLONE-RESISTANT IN DAR ES SALAAM, TANZANIA

Name of the bank……

Sample Number……

S\No.	**DESCRIPTION**	**KEY (YES / NO) where applicable**	**COMMENTS**
Observational Checklist			
1	Is a handwashing/cleaning facility available at the ATM		
2	Is the handwashing/cleaning facility functional?		
3	Location of ATM (District) (Ubungo, Ilala, Kinondoni, Kigamboni, and Temeke)		
4	Type of ATM (Remote ATM or Branch ATM)		

Appendix 2: Ethical clearance

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Direct Line: +255-22-2152489
Telefax: +255-22-2152489
E-mail: drp@muhas.ac.tz

Ref. No.DA.282/298/01.C/ Date: 18/11/2020

MUHAS-REC-11-2020-424
REGAN Z. SHAYO
MSc. Epidemiology and Laboratory Management, Shool of Public Health and Social Sciences
MUHAS

RE: APPROVAL FOR ETHICAL CLEARANCE FOR A STUDY TITLED: DETERMINATION OF CONTAMINATION OF AUTOMATED TELLER MACHINES SURFACES WITH MULT-DRUG RESISTANCE ESCHERICHIA COLI IN DAR ES SALAAM CITY, TANZANIA

Reference is made to the above heading.

I am pleased to inform you that the Chairman has on behalf of the University Senate, approved ethical clearance of the above-mentioned study, on recommendations of the Senate Research and Publications Committee meeting accordance with MUHAS research policy and Tanzania regulations governing human and animal subjects research.

APPROVAL DATE: 18/11/2020
EXPIRATION DATE OF APPROVAL: 17/11/2021

STUDY DESCRIPTION:
Purpose:
The purpose of this cross-sectional study is to determine the proportion of ATMs surfaces contaminated with MDR E. coli bacteria, its associated factors, and antimicrobial resistance pattern in Dar es salaam city

The approved protocol and procedures for this study is attached and stamped with this letter, and can be found in the link provided:
https://irb.muhas.ac.tz/storage/Certificates/Certificate%20-%20310.pdf and in the MUHAS archives.

Appendix 3: Permission email 1

Benedicto Haule-Physical Security Manager

NMB

NMB-Hq
Dar Es Salaam - Tanzania

Tel: 0222322058, EXT: 2058
Cell: 0756906827

From: Benedicto Haule
Sent: Tuesday, January 26, 2021 2:55 PM
To: 'Moku Security Services Ltd'copy <moksslтd98@gmail.com>; david.otullo@mokusecurityservicesltd.co.tz
Cc: Linda Msaki <Linda.Msaki@nmbbank.co.tz>; Harid Gange <Harid.Gange@nmbbank.co.tz>; Samweli Mwasabwite <Samweli.Mwasabwite@nmbbank.co.tz>; obard joseph <obard22@gmail.com>; Shadrack Mijjinga <Shadrack.Mijjinga@nmbbank.co.tz>; Joefrey Haule <Joefrey.Haule@nmbbank.co.tz>
Subject: RE: Utafiti wa vimelea vya bakteria katika mashine za kutolea huduma za fedha

Otullo

Kindly take note on the attached introduction letter and ID including OSA list, there will be a research on the listed ATM kindly allow the introduced Regan Shayo to proceed with research under guards supervision

Regard

From: Harid Gange
Sent: Tuesday, January 26, 2021 1:50 PM
To: Elizabeth Mhina <Elizabeth.Mhina@nmbbank.co.tz>
Cc: Benedicto Haule <Benedicto.Haule@nmbbank.co.tz>; Linda Msaki <Linda.Msaki@nmbbank.co.tz>
Subject: FW: Utafiti wa vimelea vya bakteria katika mashine za kutolea huduma za fedha

Hi Elizabeth,

Appendix 4: Permission email 2

regan shayo 5 Jan

Recended

J

John Nyaindi (NBC) 5 Jan

to me, Christine

Ok, the permission has been granted ; you can start your research from Thursday this week. All the Branch Managers will be informed, so you will need to report to respective Branch Manager before starting the research. For the Remote ATMs you will report to Security Guards

I would suggest that you start with Kawe ATM; located at the building where I am working

Regards

John Nyaindi | Manager: Learning & Development | Human Resources

Tel : +255 (0)768 980 211 | Ext : 1211 | Mobile : 075 4210416 | E-mail : John.Nyaindi@nbc.co.tz

NBC House, Sokoine Drive/Azikiwe Street ,Head Office