ADDIS ABABA UNIVERSITY
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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

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This is to certify that the thesis prepared by Mamo Akele, BSc:

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 and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

External Examiner__________________ Signature ___________Date ____________

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Abbreviations

AAU	Addis Ababa University
ATM	Automated Teller Machine
CA	Community associated
CBE	Commercial bank of Ethiopia
CLSI	Clinical and Laboratory Standards Institute
DMLS	Department of medical laboratory sciences
EPHI	Ethiopian public health institute
FMOH	Federal Ministry Of health
HA	Health care associated
HAIS	Healthcare associated infections
MRSA	Methicillin-resistant *Staphylococcus aureus*
SPHMMC	St' Paul Hospital Millennium Medical College
WHO	World Health Organization

Abstract

Background: Frequently touched surfaces on Automated Teller Machines, in public transport buses and hospital environment can constitute reservoir for healthcare and community associated infections and may represent a mechanism for the spread of these infection in the community.

Objective: To assess bacterial profiles and antibacterial susceptibility patterns of hand touched surfaces in public transport buses, Automated Teller Machines and Hospital waiting areas inanimate object in Addis Ababa city.

Methods: A cross sectional study was conducted from Jan 2018 to Sep 2018. A total of 423 samples were collected from frequent hand touch surfaces of selected Automated Teller Machine, public transport buses and hospital waiting area inanimate objects located in Addis Ababa, Ethiopia by using sterile cotton swaps. The bacterial isolates were examined and identified by colonial morphology, Gram reaction and biochemical characteristics. Antibiotic susceptibility test was done by disc agar diffusion method. Data analysis was done by using SPSS version 23

Results: Growth was seen in all samples. From the total (n=588) bacterial isolates, 75.7% and 24.3% were Gram-positive and Gram-negative, respectively. *Coagulase negative Staphylococcus species*, *Staphylococcus aureus*, *Proteus species*, *Klebsiella species*, *Pseudomonas species*, *Acinetobacter species* and *Enterobacter species* were commonly isolated bacteria. It is very dreadful to observe that and some of these bacteria 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.

Conclusion: These bacteria identified have pathogenic potential and hence their presence on those object 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: *Automated Teller Machine, Public transport bus, Hospital inanimate object, Antimicrobial resistant, Bacterial profiles, Ethiopia.*

1. Introduction

1.1 Background of the study

Inanimate objects (fomites) have been shown to play a role in the transmission of human pathogens either directly, by surface-to-mouth contact, or indirectly, by contamination of fingers and subsequent hand-to-mouth contact. Other routes of exposure include the eyes, nose, and cut or abraded skin [1]. As part of living in society, many common spaces are shared with other people. This makes it possible to spread diverse microorganisms that can lead to infections [2]. Public areas such as restaurants, public transportation systems, parks, schools, daycare centers, health care facilities and other community areas can bring a large number of people together and facilitate the transmission of microbes [3].

Public transportation is perhaps one of the most important services in urban life and this critical infrastructure is widely used [4]. And this transportation networking system is expanding continually to meet up the need of huge load of passengers and goods carried. Mass transportation systems create an environment in which large numbers of persons on a daily basis share space and interact with surfaces found within system vehicles [5]. Unhygienic and humid condition of buses help to build up microbial load on touch surfaces and interpersonal transfer of microorganisms from such reservoirs may lead to mild to severe infection in human [6].

Commercial banks are an important public institutions that are visited by large number of people daily to get financial related services and those banks provide or have developed different electronics based modern banking systems technologies such as; mobile banking, internet banking and ATMs in order to embrace and attract more customers and those devises mainly ATMs are frequently used by or shared between customers. The ATM machine is likely to be contaminated with various microorganisms due to their vast dermal contact by multiple users and these interfaces are therefore potential vehicles for the transmission of clinically important pathogens [7].

Frequently touched surfaces in hospital environment can constitute reservoir for healthcare associated infections (HAIS) [8]. The pathogens can be contracted by the contaminated environmental surfaces and equipment of the hospital through the healthcare workers and even by the patients [9]. Presence of MRSA and some other pathogenic bacteria, poor hand hygiene and heavy contamination of some important surfaces are the most important problems in hospitals [10].

Gastrointestinal infections can occur by direct hand to mouth transfer of infectious agents that are transmitted via hands or environmental surfaces. Infectious agents spread via these routes include bacterial strains such as *Salmonella*, *Shigella*, *Campylobacter*, *E. coli* (including *E. coli* O157 and *E. coli* O104) and *Helicobacter pylori*. *Staphylococcus aureus* bacteria is also the most common cause of skin and soft tissue infections, which are mostly self-limiting, but a small proportion of cases, lead to severe invasive bacteremia or pneumonia[11].

Methicillin-resistant *S.aureus* (MRSA) was first identified over 50 years ago and has become a major nosocomial and community pathogen. MRSA strains are *S.aureus* that have a mecA gene which codes for a unique penicillin-binding protein that has decreased affinity for b-lactams. This protein allows for cell growth in the presence of penicillins and other b-lactam antibiotics, which are the antibiotics of choice for *Staphylococcal* skin and soft tissue infections [12].

1.2 Statement of the problem

United States of America's intercity bus industry served an estimated 60.9 million passengers in 2015[13] and the urban mobility of Addis Ababa populations (98%) also highly dependent on public transport, 26% of the city's population is bus user [14]. And an estimated 80% of the human population are either permanently (approximately 20-33%) or transiently colonized (approximately 60%) with *S.aureus* with an approximately estimates 2% of the general population carries MRSA, and everyone is susceptible to infection [15].

Since those public transportation vehicles which have high levels of crowding and significant hand-to-fomite contact may represent an important site for MRSA transmission [16]. The transmission of *Staphylococci* including methicillin-resistant strains from carriers or infected persons primarily occurs via direct contact and it has been shown that *Staphylococci* can survive for days to months on inanimate objects [17]. Which may indicates that many populations throughout the world are at risk of exposed to a multiple verities of bacterial infection causing agent including MRSA because of using those public transports vehicles especially public buses.

Due to the ongoing development and expansion of urbanization, as well as the increasing population, people do not have enough time to use traditional banking systems and have embraced new developments in electronic banking, such as ATMs, mobile, internet banking and likes. Among these technologies ATMs are the longest standing and most widely used form of computer driven public technology with an estimated over 2.4 million units in use, since their invention and use in the late 1960's [18]. In Ethiopia, CBE's (Commercial Bank of Ethiopia) active ATM card holders reached more than 3 million (61% active users) by the end of 2015 [19].

Some studies shows that the extended use of electronic technologies is considered a source of bacterial contamination [20]. According to a study conducted by different authors, Gram positive *Staphylococcus species*, and Gram negative bacteria such as *Escherichia coli* and *Klebsiella* species [20] [28] are most frequently isolated organisms from ATM's user interface surfaces. In fact 80% of infections are spread through hand contact with hands or other objects. ATM once contaminated becomes vehicles for transmission of infection [7]. Infectious intestinal diseases are amongst the leading causes of mortality in children worldwide, latest estimates put deaths from diarrhea at about 1.9 million annually [21].

Environmental surfaces near infected or colonized patients in hospitals are frequently contaminated by potentially pathogenic micro-organisms such as vancomycin-resistant enterococci (VRE) and meticillin-resistant *Staphylococcus aureus* [22]. For instance healthcare-associated infections (HCAIs) can occur in care homes, hospitals or in a patient's own home with a prevalence level of 6.4% and 1 000 000 cases reported each year in England [23].

According world health organization(WHO) 2014s survey report there is high rates of resistance(79%) have been observed in bacteria that cause common (HCAIs) and community-acquired infections(CAI) [24]. Carbapenem-resistant and 3rd generation cephalosporin-resistant *Enterobacteriaceae* and *Staphylococcus aureus*, methicillin-resistant, vancomycin intermediate and resistant are categorized as critical and high, respectively, by WHO priority pathogens list for research and development of new antibiotics [25].

Microbes in public areas can be a critical issue in public health, because of the ease of transfer of pathogens from individual to individual and those places especially their hand touch surfaces are showing increasingly contaminating by various bacterial species including with MDR strains and becoming an important site for research/microbial investigation. In Ethiopian context there are some health care area related information but no enough evidences regarding the burden about the bus and ATMs. Investigating for bacterial contamination of hand touch surfaces has its own important factor in order to know which species type and resistant strains are circulating with in the community in order to take an effective intervention action.

1.3 Significance of the study

The major concern of this study was microbial investigation in to public places especially on frequently hand touched surfaces and the finding may provide evidence based knowledge/information about the common microbes that is circulating with in the community and becoming a public health threat.

Having basic information regarding antibacterial susceptibility status of those microbes that are commonly contaminating public places might also help to health care providers on the selection of efficient treatments. Further contribution of this study will be to design effective preventing strategies which help to control and or decrease the transmission of those microbes including the MDR ones with in the community.

2 Literature review

Microbiological investigation into public hand touch surfaces has been gaining significant attention from researchers because contaminated surfaces may act as potential reservoirs of pathogens and varies studies have already been conducted throughout the world focusing on presence and abundance of microbial contamination on different public places hand touch surfaces but for the purpose of this study the review mainly focus on public buses, ATM and hospital environments.

2.1 Bacterial isolation and susceptibility patterns of public buses

Some study conclude that public transport buses can be taken as an important factor for *S.aureus* transmission with in the community for example;

In USA a total of 237 surface samples (stanchions; seats; seat rails; back door; driver's area; heating, ventilating, and air conditioning return vent) were collected from 40 buses from July – October,2010 to determine the background prevalence and phenotypic and genotypic characteristics of MRSA strains circulating on buses. Of 40 buses; 27(68%), 25(63%) and of 237 surfaces; 41 (17%), 35 (15%) shows, *S.aureus* and MRSA contamination, respectively. Additionally only 1 unique MRSA isolate was selected from each 35 positive surfaces to characterize genotypically based on this 62.9% (22/35) and 22.9% (8/35) of the isolates were associated with CA MRSA and HA MRSA clones, respectively. Antibiotic resistance profiles indicated that 65% (13/20) were multidrug resistant (resistant to ≥3 classes). The conclusion was; buses may act as potential mixing vessels for MRSA strains in the community [26].

In Chittagong City, Bangladesh there was a study which were conducted on Bacterial contamination on hand touch surfaces of public buses with the main objectives of isolating MRSA and three enteric bacteria representatives i.e. *E.coli, Salmonella typhi and Shigella* including their antibacterial pattern. A total of 45 swab samples from three hand touch surfaces: grab rail, armrest and vinyl seat of 15 buses were collected and analyzed. The results showed that among the enteric bacterial isolates 33 were identified as *E. coli*, 17 as *Salmonella typhi* and 11 as *Shigella*. Sensitivity patterns of enteric bacterial isolates were upsetting enough as almost all isolates exhibited resistance against ampicillin, amoxicillin, ceftriaxone and chloramphenicol. 12 MRSA isolates were recovered and the isolates did not show resistance against any test antibiotics except ceftazidime. Finally they concluded, the results indicate poor hygienic condition of the buses under study and presence of enteric bacteria and MRSA potent onset of community-acquired diseases [6].

Another study which was conducted at Shanghai, China, which has mainly focus on comparing the characteristics of antibiotic resistance of airborne *Staphylococcus* isolated from Metro stations with hospital treatment rooms and public parks. Overall finding shows that 135 out of 163 (or 82.82%), 69 out of 90 (76.67%) and 60 out of 102 (58.89%) bacterial colonies from Metro station, hospital treatment rooms and parks, respectively, were Gram-positive. and The antimicrobial susceptibility data showed a higher proportion of drug-resistant strains in treatment rooms and metro stations compared with those from parks based on those findings they concluded that; closed indoor environment and crowded passenger volumes may accelerate the spread of antibiotic resistant strains[27].

Between May 2009 and February 2010, handrails of 85 public urban buses circulating in Oporto, Portugal, were screened for the occurrence of MRSA and 22 (26%) buses showed MRSA contamination [28].

A similar study performed in another urban area of Portugal– Lisbon, a year later also confirms that out of 199 buses MRSA contamination was shown in 72 (36.2%) buses. the later study includes screening of 575 passengers hands who frequently use these bus lines and the hands of 15 individuals were contaminated with MRSA and 11(2%) of these individuals were not nasal carriers of MRSA. the Further molecular technique isolates of clonal types results provide evidence that, the major hospital-associated MRSA clone in Portugal is escaping from the primary ecological niche of hospitals to the community environment. Thus public buses in major cities are a reservoir of HA- and CA-MRSA clones and may represent a mechanism for the spread of MRSA clones in the community [29].

2.2 Bacterial isolation and susceptibility patterns of ATM user interfaces

ATMs harbor more bacterial contaminants than other electronic hardware; this can be attributed to their structural design and large surface area, coupled with the fact that ATMs are usually located in open places exposed to wind and rain. These interfaces are therefore potential vehicles for the transmission of clinically important pathogens through human own hands [30].

Regarding these problems there are different studies conducted in some places for instance in India there is one study which was concerned about prevalence of multi drug resistant strains on touch screen of automated teller machine. They have collected a total of 47 samples from ATMs of 12 different banks in Vellore, Tamil Nadu state for a period of 3 months from January 2014 to March 2014. A total of 488 isolates were obtained and morphologically characterized. Among these 488 isolates, 239 *E. coli* (49%), 148 *Klebsiella sp*., (30%), 77 *Pseudomonas sp*., (16%), 17 *Acinetobacter sp*., (3%) and 7 *Proteus sp*. (1%) were the identified bacteria [31].

During summer (n = 50) and winter (n = 42) a total of 92 swab specimen were collected from different ATM centers in and around Puducherry, other city of India. Of these 92 sample 88 (95.7%) showed microbial growth and *Klebsiella* species (42.5%) was the predominant isolate followed by *CoNS* (20.62%) and *Pseudomonas aeruginosa* (15%). In the summer and winter season, *Klebsiella* species and *CoNS* were the predominant isolates, respectively. Significant number isolate showed resistance microbes colonized those ATM surfaces, for incidence all the isolates of *Klebsiella* were resistant to amoxyclav and co-trimoxazole. Regular disinfection were one of the recommendations of this study [32].

A similar study conducted from August to October, 2013 to investigate ATM as a potential source of bacterial contamination at Ebonyi State, Nigeria. Findings were; *Staphylococcus auerus* 4 (28.57%), *Coagulase-negative Staphylococcus* 3 (21.43%), *Streptococcus species* 2 (14.29%), *Pseudomonas species* 1 (7.14%), *Enterobacter species* 1 (7.14%) and *Escherichia coli* 3 (21.43%). Susceptibility of isolates ranged from 6.25% to of 25.0%, indicating that most of the antibiotics used were ineffective [7].

2.3 Bacterial isolation and susceptibility patterns of hospital environment

MRSA and other pathogens are shed by infected and colonized patients leading to contaminated hospital surfaces, which can contribute to nosocomial transmission [33]. Frequently touched surfaces and objects in the immediate vicinity of patients are more frequently and heavily contaminated [34].

Hospital based a cross sectional study conducted in Western General Hospital, UK about bacterial contamination of bed-control handsets present within the colorectal surgical unit. Within two weeks of study period they collect 70 samples by using cotton tipped moisturized swaps and inoculated on two blood agar plate one for aerobic and the other for anaerobic incubation. The result showed from these 70 bed-control handsets, 67 (95.7%) demonstrated bacterial growth with an average of 1.5 different bacterial species identified per hospital bed-control handset. In addition 21 (41.4%) bed control handsets grew bacteria known to cause nosocomial infection, including 22 (31.4%) handsets grew *Enterococcal species,* 9 (12.9%) that grew MRSA, 2 (2.9%) that grew MSSA, 2 (2.9%) grew coliforms, and 1 (1.4%) handset grew anaerobic bacteria. Based on the findings one of the conclusion was a potential inanimate object, ubiquitously present in many patient environments, could be used as a marker of environmental contamination [35].

A study conducted between the months of December 2015 to February 2016 in Bingham University teaching hospital, Nigeria also can be taken as an example. Out of the 100 surfaces sampled which were obtained from different types of materials surfaces 84(84.0%) yielded growth of bacteria. *Proteus spp*. 39 (46.43%) ranking highest, others were *Klebsiella species* 26 (30.95%), *Escherichia coli* 8 (9.50%), *Staphylococcus aureus* 4 (4.76%) *Pseudomonas species* 4 (4.76%) and Coagulase negative *Staphylococci* 3 (3.57%) were the isolated bacteria. A bacterial growth according to sample source shows that all the tables, chairs, cupboards, sphygmomanometers, waste bins, window, floor, wall, wheel chairs and kardex had 100.0% growth, soft surfaced textiles had 97.2% growth, trolley had 75.0% growth, doors had 55.5%, light switch and drip stand had 25.0% but thermometers shows no growth of bacteria[8].

Another cross-sectional study of indoor surfaces of pediatric wards at the university of Ilorin Teaching hospital in Nigeria was carried out to determine the pattern of bacterial contamination of surfaces according to material make of surfaces, last time of contact (with patient, patient relative and health care provider) and last time of cleaning and disinfection. A total of 201 samples from 8 frequently touched areas (i.e. bed rail, bedside locker, trolley, medical table, incubator, radiant warmer, wash sink and door handles) were collected. The pattern of contaminants according to the material make of sampled surfaces across the wards shows that *S.aureus, K.pneumonia and A.baumannii* were the predominant contaminants on aluminum, ceramic, wooden surfaces sampled respectively. Bacterial contamination pattern according to the time lapse of cleaning or disinfection time, surfaces that had been cleaned for greater 4 h and less than 2h prior to sample collection were found to have been contaminated with 86.3% of the isolates (n=138) aerobic bacteria were retrieved but <2h surfaces sample shows largely uncontaminated with only 1 *S aureus* [36].

E.coli, K.pneumoniea, proteus spp, P.aeruginosa and *S.aures* were recovered from cancer patient and radiation and isotope center environment of Khartoum, Sudan in 2015. The prevalence of nosocomial infection among cancer patient were high (48.1%) having these finding one of the conclusion states that hospital environment may be a source of infection to the patients and to reduce microbial contamination intervention program is needed to develop cleaning method [37].

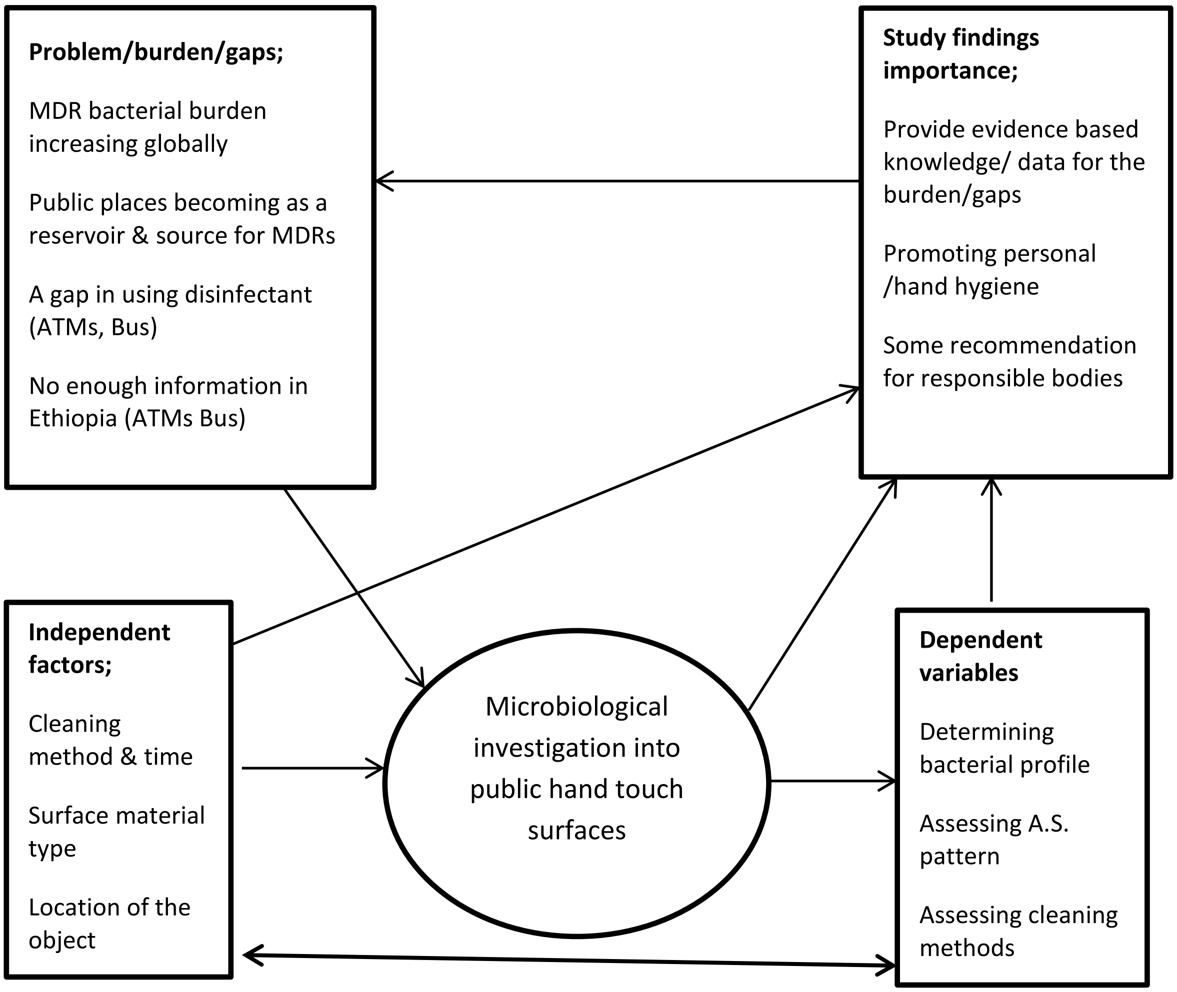
In Ethiopia a hospital based cross sectional study was conducted at the University of Gondar Teaching Hospital from November 2010 to February 2011. From a total of 220 specimens of pus, nasal, hand and surfaces swabs 142 (64.5%) were from medical devices, air and inanimate objects (such as floor areas, walls, bed-frames, door handles, light switches, sinks, stands etc.) and 118 (83.1%) of this inanimate objects had demonstrated evidences of bacterial contaminations. A total of 268 bacterial pathogens isolated and 142 (53%) were from those environmental samples. Gram-negative organisms that comprises of 41 (28.9%) of the environmental isolate were found contaminating surfaces of sink drains, door handle, bed-frames, and floor areas of the wards and operating rooms [38].

Another cross sectional study conducted at Gonder university computer surfaces finding were all collected specimen showed 100% bacterial growth. Of these 60.5% were Gram positive and the rest 39.5% were Gram negative bacteria. Some of the bacteria showed higher resistance for commonly used antibiotics, based on these and other findings they recommend that infection control guidelines must also address about disinfection and hand hygiene practices.[39].

Shiferaw et al from Jimma university health care professionals stethoscopes, isolated a total of 256 bacterial strains and Gram positive (*CoNS* and *S.aureus*) and *Klebsiella spp* from Gram negative isolate were commonly isolated species. According to the study participant response almost all of them weren't follow disinfection practice based on this they have concluded that those stethoscope can be a potential vehicle for infection transmission [40].

Over all, most finding indicates that highly hand touched surfaces of public transport buses shows with contamination of Gram-positive cocci mainly *S.aureus* and frequently isolated microorganisms from ATM surfaces and multiple inanimate objects which are fund in hospital environment were Gram-positive and negative bacteria especially among Gram-negative rods (*Enterobacteriacea*) furthermore the antibiogram data of the isolate shows significantly resistant for different antimicrobial agent that has been used in the above studies.

2.4 Conceptual framework of the study



3. Objectives of the study

3.1 General objective

To assess bacterial profiles and antibacterial susceptibility patterns of mostly used and hand touched surfaces in public transport buses, ATMs and Hospital environment areas in Addis Ababa city from January to September, 2018.

3.2 Specific Objectives

- To identify common bacterial profile contaminating the environment of public transport buses, ATM surfaces and hospital waiting area's inanimate objects.
- To assess the antimicrobial susceptibility pattern of bacterial isolates
- To assess the cleaning practices in each of facility

4. Hypothesis

The prevalence and frequency of isolating some medically important bacteria from public palaces especially from health facility in our study may not be different from the study conducted previously in Ethiopia [38].

5. Materials and Methods

5.1 Study area

The study was conducted in Anbessa public transport buses, commercial bank ATMs and St Paul's Hospital Millennium Medical College **(**SPHMMC) in Addis Ababa, Ethiopia. Addis Ababa has a population size of 2,738,248 with annual growth rate of 2.1[41].The city is divided into ten sub-cities and 99 kebeles (Lowest level administrative unit in the city.

A) Anbessa City Bus Service Enterprise is a major public transport operator in Addis Ababa. It has started as a share company founded in the year 1943 by collecting and modifying used trucks of Fascist Italians. In 1959 it was re-established as a share company and owned by Emperor Haile Selassie and members of the royal family, before it was nationalized in 1974. It came to be a public enterprise only after it was re-established in 1994 [42].

B) The history of the **Commercial Bank of Ethiopia (CBE)** dates back to the establishment of the State Bank of Ethiopia in 1942. CBE was legally established as a share company in 1963. In 1974, CBE merged with the privately owned Addis Ababa Bank. Since then, it has been playing significant roles in the development of the country. Pioneer to introduce modern banking to the country. It has more than 1160 branches stretched across the country. Currently CBE has more than 13.3 million account holders and the number of Mobile and Internet Banking users also reached more than 1,352,000 as of September 30^{th} 2016 (68% active users). Active ATM card holders reached more than 3 million (61% active users) [19].

C) St Paul's Hospital Millennium Medical College (SPHMMC), as it is known today, was established through a decree of the Council of Ministers in 2010, although the medical school opened in 2007 and the hospital was established in 1968 by the late Emperor Haile Selassie. The college has more than 2800 clinical, academic and administrative and support staffs that provide medical specialty services to patients who are referred from all over the country, teaching medicine and nursing students and doing basic and applied researches. While the inpatient capacity is more than 700 beds, The College sees an average of 1200 emergency and outpatient clients daily [43]

5.2 Study design and period

A cross-sectional study was conducted from Jan, 2018 – Sep, 2018 in Addis Ababa, Ethiopia.

5.3 Population

5.3.1 Source samples

Anbessa Addis Ababa City public transport buses, commercial bank of Ethiopia ATMs and FMOH; SPHMMC hospital inanimate object located at patient waiting areas surfaces was the source populations of this study.

5.3.2 Study samples

Randomly selected Anbessa Addis Ababa City public transport buses, commercial bank of Ethiopia ATMs and FMOH; SPHMMC Inanimate object located at patient waiting areas which is subjected to mostly hand touched surface collected swab sample was the study samples.

5.4 Inclusion and exclusion criteria

5.4.1 Inclusion criteria

All selected surface wet swab samples of study population was included

5.4.2 Exclusion criteria

New buses which start services on sampling day, ATMs on installation processes and hospital inanimate object or environmental surfaces on maintenance on sampling day and located out of waiting area were excluded.

5.5 study variables

5.5.1 Dependent variables

Bacterial isolates and drug susceptibility pattern

5.5.2 Independent variables

Source of sample places, location of sample source object and cleaning/disinfection practices

5.6 Measurement and Data collection

5.6.1 Sample size calculation and sampling method

Sample size calculation; the sample size has been calculated based on single sample size estimation. The value of P has taken as 0.831 (83.1% estimated prevalence) from the previous study conducted at the University of Gondar Hospital, Northwest Ethiopia [38]. Considering 95% confidence interval, 5% margin of error and using single sample size estimation formula the sample size is calculated as follows;

$$Sample\ size\ n = \frac{z^2 * p(1-p)}{d^2}$$

Where

n = Sample size

α = level of significance

z = at 95% confidence interval Z value (a = 0.05) =>Z α/2=1.96

p = Proportion of occurrence of the event estimated 0.831

d = Margin of error at (5%) (0.05)

$$n = \frac{1.96^2 * 0.831(1-0.831)}{0.05^2}$$

n≈ 216

And to minimize the research design effect and to increase the sensitivity of the study 10% of the sample size and additional 185 samples has been add to the normal sample size then the final required sample was 423.

Sampling method; we have divided the total sample size equally for the three major sample sources (buss, ATMs and Hospital environments 141 for each) by using multi stage simple random sampling method and a total of 423 surface swab samples were collected

5.6.2 Data collection procedure

Data collection: Related information to study surface has been collected bay using sample source information sheet from the total of a ninety different volunteer personnel's such as, bus drivers, from CBE branch manager, cleaner and guards and SPHMMC cleaners also observational check list was used to collect more specific information from each sample source during visiting and sampling day. In addition a total of 423 swab samples were collected from each randomly selected sample source surfaces for microbiological investigation.

A) Swab sample collection method; a sterile wet cotton (socked in sterile physiological normal saline) tipped stick was used to collect all surface swab samples.

i) Anbessa public transport buses; sampling was conducted on more than 10 sampling days, from three mostly populated buses stations that are located in the city such as Merkato, Piassa and Legehar areas. In each sampling day, 4 or 5 buses were randomly selected from those available buses at the time of sampling. From 3 predetermined sample locations (such as; grab rail (handrail), on the back door and vinyl seat) that are found in each buses and frequently touched by hands surfaces swab has been collected and to enhance sampling efficiency pooled sample was collected. During the whole sampling day a total of 47 buses were selected and from those buses a total of 141surface swab specimens were collected.

ii) CBE ATMs; Based on the ATMs location information that is available on CBE web page and/or obtained from E-payment head office, 141 ATMs were selected randomly like by using simple lottery methods and from each selected ATMs touch screens and/or keypads surfaces swab sample were collected for more than 10 sampling days.

ii) SPHMMC; one hundred and forty one swab (141) specimens were collected from the following three categories of frequently hand touched inanimate objects surfaces (door surface/handles, window, chair) that are found in different patient waiting area's environments i.e. outpatient departments (OPDs), pharmacy, laboratory, card room, daily casher rooms and radiology departments. From each of 3 major categories of the objects a total of 141 surface swabs specimens has been collected.

B) Specimen transportation; in all situation sampling has been conducted starting between 5:30 AM and 9:30PM local time then those collected surface swab have been transported to EPHI clinical bacteriology and mycology laboratory within 2 hours using an appropriate transport media.

5.6.3 Microbiological analysis

A) Bacterial isolation and identification; From each sample source collected surface swab specimen were inoculated on MacConkey, Blood, and Mannitol salt agar following incubation at 37^{o}C from 24h-48h then the growth was observed for isolation of bacteria. Identification of bacteria was based on colony characteristic, hemolytic appearance, differential properties of the cultures media's and Gram reaction. Gram positive cocci has been identified by their catalase and coagulase test result and for identification of Gram negative rod the following tests were done; catalase, oxidase, urease, indole, citrate utilization, lysine decarboxylation, glucose & lactose fermentation, gas & H_2S production and motility tests

B) Antibacterial susceptibility test; has been performed from primary isolation which shows growth and identified as important pathogenic bacterial species based on the standard Kirby-Bauer disk agar diffusion (DAD) method on Mueller Hinton agar according to the clinical and laboratory standards institute guidelines (CLSI)[45]. A standardized inoculum of the bacteria were swabbed onto the surface of a Mueller Hinton agar (MHA) plate. Filter paper disks impregnated with antimicrobial agents (ampicillin (10µg), ciprofloxacin (5µg), gentamicin (10µg), cefoxitin 30(µg), tetracycline 30(µg), chloramphenicol (2µg), ceftriaxone (30µg), piperacilline/tazobactam(100/10µg), meropenem(10µg), norfloxacin(10µg) tobramycin(10 µg), cefuroxime(30 µg), Ceftazidime(30 µg), penicillin(10µg), erythromycin(15µg), clindamycin (2µg), and amoxicillin-clavulanic acid(10µg)) are placed on the agar. After overnight incubation, the diameter of the zone of inhibition around each disk is measured. By referring to the standardized tables compiled by CLSI 2018, a qualitative report of susceptible or resistant has been obtained.

5.7 Data quality control

A) Pre analytical phase; The required sample source surface's information were collected by using standardized sample source information sheet from related persons and additional information included using standardized observational check lists. For all microbiological investigation surface swab collection has follow aseptic technique including using of sterile tubes to transport, sterile cotton tipped stick and clean powder free glove. Each sample also controlled and recorded by using sample or specimen information format that contains all information starting from the collection up to results of each swab specimen.

B) Analytical phase; All laboratory processes, material and equipment quality has been guaranteed by performing a quality control (QC) activity according to the standard bacteriology laboratories SOPs. All swabs and isolation procedures were performed aseptically, media preparation was based on the manufacturer's instruction and sterility has been tested. International control bacteria strains**,** *E. coli* (ATCC 259), *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853) are used in controlling the tests carried out.

C) Post analytical phase; All laboratory microbiological investigation result documented and the findings were cross checked by experienced microbiologist supervisor(advisor) of this study every day also the progress has been presented to primary advisor as much as possible. Purified bacterial cultures were stored in nutrient broth with 20% glycerol at –70°C until use. Bacterial cultures may be stocked in this condition for 2 years.

5.8 Data analysis and interpretation.

All data were recorded by hard copy, Microsoft excel and analyzed by using SPSS (Statistical Package for social sciences statistical software) version 23 for the analysis of count or frequency of the isolated bacterial species including with its specimen source types. Comparisons were made using Chi-square test. P-value of <0.05 was considered indicative of a statistically significant difference.

5.9 Ethical consideration.

Ethical clearance had obtained from the review committee of College of Health Science DMLS AAU, SPHMMC and Addis Ababa health bureau. The permissions were asked from each sample source bus drivers and CBE branch managers. Those sample source privacy protected by giving a unique code.

5.11 Definition of terms

An Automated Teller Machine or Automatic Teller Machine (ATM) is a computerized telecommunications device that enables the clients of a financial institution to perform financial transactions without the need for a cashier, human clerk or bank teller. ATMs are known by various other names including ATM machine, automated banking machine cash dispenser and various regional variants derived from trademarks on ATM systems held by particular banks [18].

Hand grab(Rails) is found with in public transport buses that is used for passengers for those who do not get a seat and grab by hands in order not to fall on the surface when the bus make a move.

Multi Drug Resistance (MDR) is defined as non-susceptibility to at least two agent in three or more antimicrobial categories [39].

6. Results

6.1 Bacterial isolates of frequent hand touches surfaces

A total of 423 (141 from each three sample source places) swab specimens were collected from ATMs, Buses and hospital environments. A total of 588 bacterial species were recovered from all specimens processed. Of these, 75.7% (n = 445) were Gram-positive and 24.3% (n = 143) were Gram-negative bacteria. Majority of bacteria were isolated from hospital inanimate object 40.1% (n = 236). The rest (n =182) 31.0% and (n=170) 28.9% were recovered from ATMs and Buses, respectively. Among Gram positive isolates *Coagulase negative Staphylococcus species* (*CoNS*) (n=318) 54.1% were frequently isolated bacteria followed by *Staphylococcus aureus* (n=107) 18.2%. *Proteus species* (n=58) 9.9%, *Klebsiella species* (n=25)4.3%, *Pseudomonas species* (n=19)3.2%, *Acinetobacter species* (n=12)2.0% and *Enterobacter species* (n=10)1.7% were the predominant isolates of gram negative rod (table 1).

Table 1. Bacterial profile isolated from frequently touched surfaces of ATM, buses and hospital inanimate objects at Addis Ababa, Ethiopia from January to Sep, 2018

Bacterial isolate	Total	Percentage
Gram-positive	**445**	**75.7%**
Coagulase negative staphylococcus	318	54.1%
Staphylococcus aureus	107	18.2%
Bacillus species	20	3.4%
Gram-negative	**143**	**24.3%**
Acinetobacter	12	2.0%
Alcaligens	2	0.3%
Citrobacter	5	0.9%
E.coli	8	1.4%
Enterobacter spp	10	1.7%
Klebsiella spp	25	4.3%
Proteus SPP	58	9.9%
Pseudomonas spp	19	3.2%
Salmonella spp	2	0.3%
Shigella spp	2	0.3%
Total	**588**	**100.0%**

Most of Gram positive bacteria (n=163) 27.7% were isolated from ATMs followed by hospital environment (n=148) 25.2 % and buses (n= 134) 22.8% however majority of Gram negative (n= 88) 15.0% were isolated from hospital environment, (n=36) 6.1% and (n=19) 3.2% were recovered from buses and ATMs respectively. The prevalence and distribution of Gram positive and Gram negative bacteria significantly ($\chi2$ test, $p < 0.05$) differed between the three locations.

Table2.The distribution pattern of bacterial isolate on frequently touched surfaces of ATM, buses and hospital inanimate objects at Addis Ababa, Ethiopia from Jan to Sep, 2018

Bacterial isolate	**Sample source**			**Total (Percentage)**
	ATM	BUS	Hospital	
Gram-positive	**163 27.7**	**134 22.8**	**148 25.2**	**445 (75.7)**
Coagulase negative staphylococcus	135 23.0	85 14.5	98 16.7	318 54.1
Staphylococcus aureus	28 4.8	33 5.6	46 7.8	107 18.2
Bacillus species	0 0.0	16 2.7	4 0.7	20 3.4
Gram-negative	**19 3.2**	**36 6.1**	**88 15.0**	**143 24.3**
Acinetobacter	0 0.0	2 0.3	10 1.7	12 2.0
Alcaligens	0 0.0	0 0.0	2 0.3	2 0.3
Citrobacter	0 0.0	1 0.2	4 0.7	5 0.9
E.coli	0 0.0	2 0.3	6 1.0	8 1.4
Enterobacter spp	0 0.0	4 0.7	6 1.0	10 1.7
Klebsiella spp	1 0.2	2 0.3	22 3.7	25 4.3
Proteus SPP	3 0.5	25 4.3	30 5.1	58 9.9
Pseudomonas spp	15 2.6	0 0.0	4 0.7	19 3.2
Salmonella spp	0 0.0	0 0.0	2 0.3	2 0.3
Shigella spp	0 0.0	0 0.0	2 0.2	2 0.2
Total	**182 31.0**	**170 28.9**	**236 40.1**	**588 100.0**

From Gram positive bacterial isolate *CoNS* were most frequently recovered from ATMs and higher number *Staphylococcus aureus* isolate were from hospital environments. Of the total 148 Gram positive hospital isolate 46 (31.1%) were identified as *S.aureus* also 33 out of 134(24.6%) and 28 out of 164(17.2%) Gram positive isolate from buses and ATMs, respectively, were *S.aureus*. The prevalence difference between hospital environment and ATM surfaces was statistically significant ($p < 0.05$) but but no significant difference was observed between hospital and buses as well as bus and ATM surfaces.

From both palaces, hospital and buses environments, *Proteus species* were predominantly isolated bacteria, similarly *Klebsiella spp*, *Acinetobacter* and *E.coli* from hospital environments and *Pseudomonas spp* from ATMs surface were more commonly recovered Gram negative rods were (table 2).

From 141 (138 key pad and 3 touch screen) ATMs a total of 141 surface sample were collected. Of these 588 total isolate 182(31%) were from those ATM surfaces; *CoNS*(135/182;74.2%), *S.aureus*(28/182;15.2%), *Pseudomonas spp.*(13/182;8.2%) were common isolates of ATM surfaces.

A total of 141 surface sample were collected from 47 buses. For each of these buses, samples were collected from three distinct locations: grab rail, back door and vinyl seat. *CoNS* (85/170; 50%) were more frequently recovered bacteria followed by *S.aureus* (33/170; 19.4%). Of Gram negative rods most of the isolate were *Proteus Spp* (25/170; 14.7%) and slightly more (11/170; 6.5%) number recovered from the back door surfaces followed by vinyl seat that were (8/170; 4.7%).

One hundred forty one (141) hospital environment surface swab specimen also collected from different patient waiting area's inanimate object (chair) and environment surfaces(Door and window). Gram positive bacteria were predominantly recovered from chairs and door surfaces and in all objects *CoNS* were frequently isolated bacteria followed by *S.aureus*. Most of Gram negative rod were isolated from chairs and windows surface. Chair surfaces shows all gram negative bacterial growth which are isolated in this study and *Proteus species*, *Klebsiella species* and *Acinetobacter species* were frequently isolated from those chair surfaces but from Door and windows surface only *Proteus species, Klebsiella species* and *E.coli* were recovered(table 3).

Table 3. Bacterial profile from ATM key pad and touch screen, buses door, grape rail and vinyl seat and hospital waiting area chair, door and window in Addis Ababa; January to Sep 2018 N/%

Source of sample place	**ATM**			**BUS**				**HOSPITAL**				**Total**
Source of sample object **Bacterial isolate**	**key pad**	**Touch screen**	**Total**	**Door**	**Grape rail**	**Vinyl seat**	**Total**	**Chair**	**Door**	**Wind ow**	**Total**	
Gram-positive	**158 86.8**	**5 2.7**	**163 89.6**	**41 24.1**	**49 28.8**	**44 25.9**	**134 78.8**	**110 46.6**	**32 13.6**	**6 2.5**	**148 62.7**	**445 75.7**
CoNS	131 72.0	4 2.2	135 74.2	21 12.4	30 17.6	34 20.0	85 50.0	72 30.5	22 9.3	4 1.7	98 41.5	318 54.1
S.aureus	27 14.8	1 0.5	28 15.2	9 5.3	16 9.4	8 4.7	33 19.4	34 14.4	10 4.2	2 0.8	46 19.5	107 18.2
Bacillus species	0 0.0	0 0.0	0 0.0	11 6.5	3 1.8	2 1.2	16 9.4	4 1.7	0 0.0	0 0.0	4 1.7	20 3.4
Gram-negative	**17 9.3**	**2 1.1**	**19 10.4**	**13 7.6**	**10 5.9**	**13 7.6**	**36 21.2**	**70 29.7**	**8 3.4**	**10 4.2**	**88 37.3**	**143 24.3**
Acinetobacter species	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	2 1.2	2 1.2	10 4.2	0 0.0	0 0.0	10 1.2	12 2.0
Alcaligens species	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	2 0.8	0 0.0	0 0.0	2 0.8	2 0.3
Citrobacter species	0 0.0	0 0.0	0 0.0	0 0.0	1 0.6	0 0.0	1 0.6	4 1.7	0 0.0	0 0.0	4 1.7	5 0.9
E.coli	0 0.0	0 0.0	0 0.0	1 0.6	1 0.6	0 0.0	2 1.2	2 0.8	2 0.8	2 0.8	6 2.4	8 1.4
Enterobacter species	0 0.0	0 0.0	0 0.0	1 0.6	0 0.0	3 1.8	4 2.4	6 2.5	0 0.0	0 0.0	6 2.5	10 1.7
Klebsiella species	1 0.5	0 0.0	1 0.5	0 0.0	2 1.2	0 0.0	2 1.2	16 6.8	2 0.8	4 1.7	22 9.3	25 4.3
Proteus species	3 1.6	0 0.0	3 1.6	11 6.5	6 3.5	8 4.7	25 14.7	22 9.3	4 1.7	4 1.7	30 12.7	58 9.9
Pseudomonas species	13 7.1	2 1.1	15 8.2	0 0.0	0 0.0	0 0.0	0 0.0	4 1.7	0 0.0	0 0.0	4 1.7	19 3.2
Salmonella species	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	2 0.8	0 0.0	0 0.0	2 0.8	2 0.3
Shigella species	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	2 0.8	0 0.0	0 0.0	2 0.8	2 0.2
Total	**175 96.2**	**7 3.8**	**182 100**	**54 31.8**	**59 34.7**	**57 33.5**	**170 100**	**180 76.3**	**40 16.9**	**16 6.8**	**236 100**	**588 100**

6.2 Antimicrobial susceptibility test

Over all 568 bacterial isolate were tested against 17 different selected antibiotic discs. Antimicrobial drug resistance profiles of Gram positive bacterial isolate shows higher proportions resistance rate against tetracycline (71.3%), penicillin (67.3%) and oxacillin (40.2%). *S.aureus* resistance rate to tetracycline (82.20%) and penicillin (79.40%) higher than *CoNS* resistance to tetracycline (67.60%) and penicillin (63.20%) on the other hand *CoNS* shows more resistance rate to oxacillin(44.0%) than *S.aureus* (17.8%). Relatively *S.aureus* (15.90%) and *CoNS* (8.80%) showed least resistance to chloramphenicol.

The distribution of the antibiotic resistant Gram positive bacteria is shown in table 4. There were higher proportion of *CoNS* and *S.aureus* species were resistance to tetracycline, penicillin and gentamycin in hospital environments and all of the patient waiting area *S.aureus* isolate was resistant to tetracycline on the other hand higher frequency of *CoNS* and *S.aureus* that were recovered from buses surfaces shows resistance to erythromycin and clindamycin and both isolate resistance to chloramphenicol with higher frequency was isolated from ATM surfaces. 53.3% of oxacillin resistance *CoNS* isolated from buses internal surfaces but relatively higher (43.5%) of oxacillin resistant *S.aureus* isolates were recovered from hospital inanimate objects/environment followed by buses (27.3%) and ATMs (7.1%).

Gram negative rods recovered from ATM, bus and hospital frequently hand touched surfaces varied in their patterns of resistance to the antibiotics used (Table 5). Resistance to these antibiotics ranged from 6.3% (tobramycin) to 66.9% (ceftriaxone) and relatively high resistance rate ware observed against ceftriaxone, cefuroxime, ceftazidime and ampicillin

Table 4. Antimicrobial resistance patterns of Gram positive bacterial isolate on frequently touched surfaces of ATM, buses and hospital inanimate objects at Addis Ababa, Ethiopia from January to Sep, 2018. N(%)

Bacterial isolate	**Place**	**No**	**Tetracycline /30**	**penicilin /10**	**Erythromycin /15**	**Clndamicn /2**	**Oxacillin /30**	**Chloranfinicol /30**	**Gentamycin /10**
Coagulase negative Staphaylococcuse species		**318**	**215(67.6)**	**201(63.2)**	**71(22.3)**	**38(11.9)**	**140(44.0)**	**28(8.8)**	**41(12.9)**
	ATM	135	82(60.7)	70(51.9)	15 (11.1)	14 (10.4)	45(33.3)	15(11.1)	8(5.9)
	BUS	85	56(65.9)	60(70.6)	34(40.0)	12(14.1)	47 (55.3)	9(10.6)	9(10.6)
	HOSP	98	77(78.6)	71(72.4)	22(22.4)	12(12.2)	48(49.0)	4(4.1)	24(24.5)
Staphaylococcus aureus		**107**	**88(82.2)**	**85(79.4)**	**40(37.4)**	**19(17.8)**	**31(29.0)**	**17(15.9)**	**18(16.8)**
	ATM	28	18(64.3)	17(60.7)	5(17.9)	1(3.6)	2(7.1)	4(14.3)	2(7.1)
	BUS	33	24 (72.7)	26(78.8)	18(54.5)	8(24.2)	9(27.3)	6(18.2)	5(15.2)
	HOSP	46	46(100.0)	42(91.3)	17(37.0)	10(21.7)	20(43.5)	7(15.2)	11(23.9)
Bacillus species		20	ND	ND	ND	ND	ND	ND	ND
TOTAL		**425**	**303(71.3)**	**286(67.3)**	**111(26.1)**	**57(13.4)**	**171(40.2)**	**45(10.6)**	**59(13.9)**

ND Not Done

Table 5. Antimicrobial resistance patterns of Gram negative bacterial isolate on frequently touched surfaces of ATM, buses and hospital inanimate objects at Addis Ababa, Ethiopia from January to Sep, 2018. N(%)

Bacterial isolate	place	N# isolate	Chloramphenicol/30	Gentamicen/10	Ciprofloxacin/5	Ceftriaxone/30	Amoxicillin-cavulanic	Piperacilline /10	Meropenem/10	Tobramycin/10	Norfloxacin/10	Cefuroxime/30	Ceftazidime/30	Ampicillin/10
Acinetobacter		**12**	**4(33.3)**	**0(0.0)**	**0(0)**	**12(100)**	**6(50)**	**6(50)**	**4(33.3)**	**0(0)**	**0(0)**	**8(66.7)**	**4(33.3)**	**ND**
	B	2	2(100)	0(0)	0(0)	2(100)	0(0)	2(100)	0(0)	0(0)	0(0)	2(100)	0(0)	ND
	H	10	2(20)	0(0)	0(0)	10(100)	6(60)	4(40)	4(40)	0(0)	0(0)	6(60)	4(40)	ND
Alcaligens		**2**	**0(0)**	**0(0)**	**0(00)**	**2(100)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**2(100)**	**0(0)**	ND
	H	2	0(0.0)	0(0.0)	0(0.0)	2(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(100)	0(0.0)	ND
Citrobacter		**5**	**2(40.0)**	**2(40.0)**	**1(20.0)**	**4(80.0)**	**4(80.0)**	**0(0.0)**	**0(0.0)**	**0(0.0)**	**0(0.0)**	**4(80.0)**	**2(40.0)**	**2(40.0)**
	B	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	H	4	2(50)	2(50)	1(25.0)	4(100)	4(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	4(100)	2(50)	2(50)
E.coli		**8**	**4(50.0)**	**2(25.0)**	**0(0)**	**4(50)**	**2(25)**	**0(0)**	**0(0)**	**0(0)**	**2(25)**	**7(87.5)**	**2(25)**	**5(62.5)**

	B	2	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)	0(0)	0(0)
	H	6	4(66.7)	2(33.3)	0(0)	4(66.7)	2(33.3)	0(0)	0(0)	0(0)	2(33.3)	6(100)	2(33.3)	5(83.3)
Enterobacter spp		**10**	**0(0)**	**1(10)**	**1(10)**	**2(20)**	**6(60)**	**5(50)**	**2(20)**	**0(0)**	**5(50)**	**4(40)**	**6(60)**	**8(80)**
	B	4	0(0)	1(25)	0(0)	2(50)	2(50	2(50	2(50	0(0)	0(0)	0(0)	2(50	3(75)
	H	6	0(0)	0(0)	1(16.7)	0(0)	4(66.7)	3(50)	0(0)	0(0)	5(83.3)	4(66.7)	4(66.7)	5(83.3)
Klebsiella spp		**25**	**5(20)**	**1(4)**	**2(8)**	**12(48)**	**12(48)**	**20(80)**	**1(4)**	**1(4)**	**3(12)**	**8(32)**	**6(24)**	**24(96)**
	A	1	0(0)	0(0)	0(0)	0(000)	1(100)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	B	2	1(50)	1(50)	0(0)	2(100)	1(50)	1(50)	1(50)	1(50)	1(50)	2(100)	0(0)	2(100)
	H	22	4(18.2)	0(0)	2(9.1)	10(45.5)	10(45.5)	18(81.8)	0(0)	0(0)	2(9.1)	6(27.3)	6(27.3)	22(100)
Proteus SPP		**58**	**27(46.6)**	**27(46.6)**	**25(43.1)**	**49(84.5)**	**29(50)**	**34(58.6)**	**18(31)**	**6(10.3)**	**46(79.3)**	**35(60.3)**	**49(84.5)**	**45(77.6)**
	A	3	2(66.7)	1(33.3)	0(0)	2(66.7)	0(0)	0(0)	0(0)	0(0)	3(100)	3(100)	3(100)	3(100)
	B	25	11(44)	6(24)	10(40)	23(92)	1(4)	12(48)	16(64)	2(8)	24(96)	18(72)	18(72)	17(68)
	H	30	14(46.7)	20(66.7)	15(50)	24(80)	28(93.3)	22(73.3)	2(6.7)	4(13.3)	19(63.3)	14(46.7)	28(93.3)	25(83.3)
Pseudomonas		**19**	**2(10.5)**	**2(10.5)**	**2(10.5)**	**11(57.9)**	**3(15.8)**	**2(10.5)**	**2(10.5)**	**2(10.5)**	**2(10.5)**	**3(15.8)**	**10(52.6)**	ND
	A	15	0(0)	0(0)	0(0)	7(46.7)	1(6.7)	0(0)	0(0)	0(0)	0(0)	1(6.7)	6(40)	ND

spp	H	4	2(50)	2(50)	2(50)	4(100)	2(50)	2(50)	2(50)	2(50)	2(50)	2(50)	4(100)	ND
Salmonella spp		**2**	**1(50)**	**0(0)**	**0(0)**	**2(100)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**2(100)**	**0(0)**	**1(50)**
	H	2	1(50)	0(0)	0(0)	2(100)	0(0)	0(0)	0(0)	0(0)	0(0)	2(100)	0(0)	1(50)
Shigella spp		**2**	**0(0)**	**0(0)**	**0(0)**	**2(100)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**2(100)**	**0(0)**	**0(0)**
	H	2	0(0)	0(0)	0(0)	2(100)	0(0)	0(0)	0(0)	0(0)	0(0)	2(100)	0(0)	0(0)
Total		**143**	**45(31.5)**	**35(24.5)**	**31(21.7)**	**100(69.9)**	**62(43.4)**	**67(46.9)**	**27(18.9)**	**9(6.3)**	**58(40.6)**	**75(52.4)**	**79(55.2)**	**85(59.4)**

A- ATM, B- Bus, H- Hospital, ND- not done, 0- 100% susceptible

Table 6. Multi drug resistance patterns of bacterial isolate on frequently touched surfaces of ATM, buses and hospital inanimate objects at Addis Ababa, Ethiopia from January to Sep, 2018. N(%)

Bacterial isolate	No	Anti- biogram patterns (%)					
		R0	R1	R2	R3	R4	≥R5
Gram positive	**425**	**75(17.6)**	**53(12.5)**	**97(22.8)**	**83(19.5)**	**62(14.6)**	**55(12.9)**
CoNS	318	64(20.1)	43(13.5)	68(21.4)	64(20.1)	40(12.6)	39(12.3)
S.aureus	107	11(10.3)	10(9.3)	29(27.1)	19(17.8)	22(20.6)	16(15)
Gram negative	**143**	**7(4.9)**	**24(16.8)**	**23(16.1)**	**21(14.7)**	**23(16.1)**	**45(31.5)**
Acinetobacter	12	0(0.0)	4(33.3)	2(16.7)	1(8.3)	3(25.0)	2(16.7)
Alcaligens	2	0(0)	0(0)	2(100)	0(0)	0(0)	0(0)
Citrobacter	5	1(20)	0(0)	1(20)	1(20)	1(20)	1(20)
E.coli	8	1(12.5)	1(12.5)	1(12.5)	2(25)	1(12.5)	2(25)
Enterobacter spp	10	0(0)	2(20)	0(0)	3(30)	3(30)	2(20)
Klebsiella spp	25	0(0)	4(16)	11(44)	6(24)	2(8)	2(8)
Proteus SPP	58	0(0)	0(0)	4(6.9)	8(13.8)	12(20.7)	34(58.6)
Pseudomonas spp	19	5(26.3)	10(52.6)	2(10.5)	0(0)	0(0)	2(10.5)
Salmonella spp	2	0(0)	0(0)	1(50)	0(0)	1(50)	0(0)
Shigella spp	2	0(0)	1(50)	1(50)	0(0)	0(0)	0(0)
Total	**568**	**82(14.4)**	**77(13.6)**	**120(21.1)**	**104(18.3)**	**85(15)**	**100(17.6)**

R0- No antibiotic resistance, R1- Resistance to one antimicrobial, R2-Resistance to two antimicrobials, R3 Resistance to three antimicrobials, R4-Resistance to four antimicrobials, R5-resistance to five and more antimicrobials, *CoNS*-coagulase negative staphylococcus.

The predominant (n=58), *Proteus SPP* isolate relatively shows higher resistant rate to mostly used antibiotics. Of this isolate, 29(50%), 34(58.6%), 35(60.3%), 45(77.6%), 45(79.3%), 49(84.5%) and 49(84.5%), demonstrated resistance to amoxicillin-calavulanic acid, piperacillin, cefuroxime, ampicillin, ceftriaxone and ceftazidime, respectively. Majority of this isolate recovered from hospital and bus specimens however hospital isolate shows more resistance percentage. Even though majority of *Pseudomonas spp* recovered from ATM surface, most resistance isolate to antibiotics used were recovered from hospital surfaces. All isolates of *Alecaligens*, *Salmonella spp* and *Shigella spp* were only from hospital environment and all these isolate were resistance to ceftriaxone and cefuroxime.

The antibiogram of Gram positive (17.6%) bacterial isolates weren't developed resistance against to any of the seven (tetracyclines, penicillines, macrolide, lincosamide, cephems, phenicols and aminoglycoside) antibiotic class used and the rest of isolate showed higher multi drug resistance rate with a minimum 22.8% and maximum 12.9%, to two class and five and more class of drugs used respectively but none of the isolate showed resistance to all of used antibiotics. Relatively higher, 21.4% and 20.1% of *CoNS* demonstrates resistance to two and three class also 27.1% and 20.6% of *S.aureus* isolate were resistant to two and four drugs. More susceptible *CoNS*(30.4%) and *S.aureus*(28.6%) bacteria were from ATM surfaces when compared to bus and hospital environment and all *S.aureus* recovered from these hospital objects didn't show susceptible to any of drugs used.

Similarly gram negative bacteria shows higher frequency of resistance rate such as, more than 95% of the isolate were resistance at list to one antibiotics class (of the seven antibiotic class; Phenicols, Aminoglycosides, Fluoroquinolones, Cephems, Beta-lactam combination, Penicillin and penems) used. More frequent *Protues Spp* (58.6%) at least to five and/or more and *Klebsiella Spp* (44%) and (8%) were resistance to two and five and more antibiotic class, respectively. Of these 5% of susceptible bacteria *Pseudomonas Spp* (n=5) from ATM surfaces also *Citrobacter* (n=1) and *E,coli* (n=1) were from busses. Over all, of the total isolate (n=568) multi drug resistance (resistance to ≥2 antibiotic class) were recorded in 409 (72.0%) isolates. In (297/425: 70%) of Gram positive and (112/143:78.3%) of Gram negative bacterial isolates were also observed MDR.

6.3 General cleaning method and practice in each sample source points/places

A total of 90 different volunteer personnel which have different profession and duties in these sample source places has interviewed about cleaning practices. From 30 bank staff majority were bank branch manager, three were responded that, they have observed every morning these ATM surfaces are cleaned with dry wiping method, one respondent also confirmed that they use glass cleaner solution and one bank personnel response were, these surfaces are cleaned according to service/maintenance program by technical stuff the rest has responded that, they didn't observe or have no any information about this cleaning method used. Of those 30 bus driver all of them responded that. These buses has lavaijo day but the program is not regular and chair and doors are flushed by clean water and none of hand grab are cleaned. And 30 hospital cleaners responded, they have two cleaning program every day (this information is only about the patient waiting areas) but none of them responded they clean those frequently touched sample source surfaces unless if there is visible dirt or blood splash.

Based on our cleaning assessment most of ATM surface did not cleaned regularly but we observed that these technical stuff opens and do some maintenance after that they remove dusts by using blower machines. Also these frequently touched key pads are visibly soiled specially those metallic or aluminum surfaces. Almost all of observed buses has very dirty and soiled flour surfaces and some driver cleans with mopes during the hand over time. But during lavaiajo day those sample source surfaces (only vinyl seat and back door) are cleaned (flushed) only with high pressure tap water. None of hospital inanimate object (specimen source) observed wen cleaned by assigned person. Over all we didn't observe any disinfection practice to those sample sources and some of cleaners uses there heavy duty gloves to move chairs during cleaning time.

7. Discussion

The isolation of pathogenic bacteria from fomites indicates that they can be vehicles for disease transmission [46]. Some studies suggest that, ATMs [32] public buses [6] and different hospital environmental surfaces [38] contaminated with various types of bacterial pathogens, might be potential areas for pathogen accumulation and they might have a role in microbial transmission in the community. This study also confirmed that, frequently touched surfaces of these public palace have shown contamination by varies pathogenic bacteria with higher frequencies and different distribution rates.

A total of 423 swab specimens were collected from all three major sample sources places and all these swab specimen yielded bacterial growth (588; 100%) has similar findings with the reports of Alemu et al from Gonder university hospital computer keyboard and mice surfaces [39]. However, higher than the previous reports in Ethiopia and Nigeria hospital environment which has a prevalence of 83.1% and 84%, respectively [38], [8].

The majority of bacteria cultured from materials taken from all touch surfaces of sample source were Gram positive bacterial species (75.7%) as compared to Gram negative rods (24.3%). Zhou F. et al, from China metro station, hospital and parks environment isolated more Gram positive bacteria with the prevalence of 74.4% which has quite similar result with our finding [27]. Similarly, ATM surface, busses and hospital environments also demonstrates higher rates of Gram positive bacteria contamination over Gram negative rods. Similar findings have been documented by Chukwudozie et al from Nigeria ATMs and Shiferaw et al from stethoscope[7] [40]. Isolation of more Gram positive organisms is probably because they are members of the body flora of both asymptomatic carriers and sick persons [46]. Furthermore, *Staphylococci species* which is also the predominant isolate of this study, can survive for days to months on inanimate objects [17]

Of 588 overall isolate or thirteen different bacterial species pattern which are; *Coagulase negative Staphylococcus spp, Staphylococcus aureus, Bacillus spp, Acinetobacter spp, Alcaligens spp, Citrobacter spp, E.coli, Enterobacter spp, Klebsiella spp, Proteus spp, Pseudomonas spp, Salmonella spp and Shigella spp, CoNS* was the most predominant pathogen with over all isolation rates of, 54.1%. Higher than Shiferaw et al report from stethoscope 40.2%

[40] however, 64.4 % from metro station of china and 68.3% in different surgical units of Gondar University Hospital, which is relatively higher to the present findings[17][38].

Higher number of Gram positive bacteria were recovered from ATM surfaces and majority (135 out of 318) of the predominant isolate *CoNS* also recovered from those ATM surfaces. Of these 588 total isolate 182 (only five different species) were from ATM surfaces which are; *CoNS*(74.2%), *S.aureus*(15.2%), *Pseudomonas spp.*(8.2%), *Klebsiella spp*(0.5%) and *Proteus spp*(1.6%). has similar species patterns with the reports of Nwankwo et al a study conducted in Nigeria ATMs [30]. Among Gram negative bacteria *Pseudomonas spp* were frequently isolated organism this finding also similar with the reports of Oluduro et al in Nigeria and Mahmoudi et al in Iran ATM surfaces[20] but quite different from the results of Nagajothi et al from India[32], which is *Klebsiella spp* were predominant isolate from ATM surfaces followed by *Pseudomonas spp*.

From forty seven public buses (grab rail, back door and vinyl seat) surfaces a total of 170(28.9%) bacterial isolate were recovered when compared to the other two places, which is lower frequency but demonstrates more bacterial species pattern relatively to ATM surfaces. of these 170 isolate 59(34.7%) were from grab rail which is slightly higher compared to the other two surfaces. Agrees with reports of Chowdhury et al that is, more bacterial load was observed on grab rails [6]. Of these 170 bacteria isolated from those bus surfaces 50% (n=85) and 19.4% (n= 33) were, *CoNS* and *S.aureus* respectively. This finding is in agreement with a study in China has a prevalence of *CoNS* and *S.aureus* 64.4% and 11.3%, respectively [47]. Isolation of *S.aureus* from all internal busses surfaces prevalence also in line with report from Midwestern city, USA buses has a prevalence 17% [26]. *Proteus spp* (25/170; 14.7%) most frequent isolate among Gram negative bacteria in this study also most common isolates of these buses environment and recovered more commonly from the door and venial seat surfaces, that is more soiled and humid area relative to hand grab rail. This might be because of unhygienic and humid condition of buses help to build up those *Proteus spp* load on touch surfaces. Moreover, *Proteus spp.* bacteria have been isolated from different human and non-human environments and their presence in higher organisms, soil, and water is well documented in different studies [48]

Health care facility environment contaminated with infectious bacteria are known to be common cause of HAI. Of the overall isolate majority (40.1%:236/588) of bacteria (all the 13 species) many of which are documented as a common cause of HAI, were isolated from hospital inanimate objects. Similarly like ATM and buses, different hospital environmental surfaces and inanimate object also demonstrates higher Gram positive bacterial contamination. Of these total (n=236) waiting area isolate, *CoNS* 98 (41.5%) were predominant bacteria followed by *S.aureus* 46 (19.5%). Which has similar prevalence pattern with the reports of Gelaw et al which are 68.3% and 30.7%, respectively [38]. However Nurain et al from Sudan and Segujja et al from Uganda records, *S.aureus* were predominantly recovered over *CoNS* bacteria, different from our report [37] [49].

Although, majority of Gram positive organisms were isolated from ATM surfaces, more frequent (n=88) and different species of Gram negative bacterium were from hospital environment. Of these *Proteus species* 30/236 (12.7%) was the most prevalent isolates and followed by *Klebsiella spp* 22/236 (9.3%). Again this finding agree with the percentage pattern reports of Essien et al which has a prevalence of 46.4% and 30.9% respectively[8]. Different findings were reported from Iran [10]. Furthermore these two predominant isolate are documented as a cause of different nosocomial infection. For example, the predominant causes of postoperative SSIs [38], predominant cause nosocomial infection among cancer patient [37] overall hospital environments demonstrates higher bacterial frequency and species diversity relatively ATM and buses. This difference may because of infected patients, colonized with bacteria and health care workers are the major source of environmental contamination [50]. Over crowdedness of these waiting areas and occupied mostly by infected patients with varies types of pathogenic bacteria every day.

With the appearance of antibiotic-resistant bacteria, increasing numbers of infections are causing huge losses to both economic concerns and social resources over recent decades, and this has become a global problem [51]. Although *Staphylococcus strains*, especially *CoNS*, do not often cause disease, they can have strong levels of resistance to many antibiotics [52]. 82.3% of the present study's predominant isolate *Staphylococcus species* (*CoNS and S.aureus*) showed resistance to at least one antibiotics used. Agrees with the above statement and with the reports of Zhou et al. that is over 90% of *Staphylococcus strains* were resistance at least to one drug [27]. The highest frequencies of antibiotic resistance were detected against tetracycline, penicillin and oxacilline. Has consistency with earlier report from Ethiopia [39][40].

The predominant isolate, *CoNS* species also showed higher resistance prevalence to oxacilline over *S.aureus*, has consistency with previous reports in Ethiopia[40] and quite different with the finding of Mbim et al and Alemu et al which is higher prevalence oxacillin resistant *S.aureus* were recorded over *CoNS* [53][39]. Although majority of *CoNS* were from ATM surfaces, higher prevalence (55.3%) of oxacilline resistance were observed from busses surfaces followed by hospital environments that is 49%. again has similar pattern with reports of Zhou et al, which is more resistant bacteria were recovered from metro station over hospital treatment room environments[27].

More number of *S.aureus* species were from hospital area, similarly highest percentage (43.5%) of oxacilline resistant *S.aureus* isolated from hospital objects followed in busses surfaces which is 27.3%. Prevalence of oxacillin resistance *CoNS* and *S.aureus* from hospital environment in this study higher than the previous finding from stethoscope which has resistance level 30.1% and 26.6%, respectively [40]. However a study from Ethiopia hospital computer surface, *CoNS* (83%) and *S.aureus* (87%) [39]. and from Iran different hospital environments *S.aureus* (60%) [10]. Yielded higher report than the present study. However isolating oxacilline resistance *S.aureus* from buses surfaces has consistency with the reports of Simoes et al and Conceicao et al that has 26% and 36.2% prevalence [28] [29].

The predominant Gram negative *Proteus species*, also frequent isolates of buses and hospital environment showed higher resistance to most of drugs tested and all of these ATM isolate were resistant to meropenem, tobramycin, norfloxacin, cefuroxime, ceftazidime and ampicillin. On the other hand gentamycin, meropenem and tobramicen were effective to *Klebsiella species*, recovered from hospital surfaces. All *Salmonella* and *Shigella* species were resistance to ceftriaxone and cefuroxime. The predominant isolates of all *Pseudomonas species* also demonstrated resistance to tobramicen and meropenem. Has consistency with report of other works that is mention else were [32], [37], [40], [50].

The increasing rates of multi drug resistance among Gram positive and negative bacterial pathogens are being documented in many reports, including WHO global surveillances reports [24]. In the present study also, among the total isolates (n = 568) multi drug resistance (MDR = resistance in ≥ 2 drug class) were recorded in 409 (72.0%) of all bacterial isolates. Gram negative bacteria, 78.3% and Gram positive bacteria, 70% showed resistance for two or more antimicrobials tested. Which is relatively higher MDR rate were observed in Gram negative isolate [40].

In our study, all ninety personnel (bank branch manager, bank guards, bus drivers, cleaners) responded that they didn't observe and/or practice any disinfection practice specifically on those highly hand touched surfaces. Based on our cleaning practice assessment observation period we confirmed that, cleaning habit of frequently hand touched surfaces was poor. Also the predominant isolate *Staphylococcus* and *Proteus species* can be taken as an indicative for poor hygienic practices. This was also indicated by several other investigators. For instance in the reviews of Drzewiecka D, *Proteus spp.* bacteria present in soil or water habitats are often regarded as indicators of fecal pollution[48]. Even though *Staphylococci* represent a regular part of the microbiota of the skin and mucous membranes of humans and animals, had prefer areas of higher humidity [17] [46]. Also isolated well from hospital and other environments by different investigators. To reduce these and other microbial load in the environment as much as possible, the primary recommendation of several investigators were promoting hygiene specially hand hygiene and followed by regular disinfection practices.

8. Strength and limitation of the study

8.1 Strength

Hospital different patient waiting areas that has high and unrestricted human traffic flow environmental bacterial contamination investigation, assessing of additional public places like ATM and transport buses and processing of higher samples size can be taken as the strength of this study.

8.2 Limitation

The study was limited to government institution, observational bias may be occurred during cleaning observational practice and antibiotic susceptibility test were not done for Gram positive rod isolates.

9. Conclusion

Frequently hand touched surfaces of ATMs, public transports buses, and hospital waiting area inanimate object/environment harbored microorganisms such as *Coagulase negative Staphylococcus, Staphylococcus aureus, Bacillus species, Acinetobacter, Alcaligens, Citrobacter, E.coli, Enterobacter spp, Klebsiella spp, Proteus SPP, Pseudomonas spp, Salmonella spp and Shigella spp*. In this study most of the isolated bacteria were highly resistant to the commonly used antibiotics. High rate of multiple antimicrobial resistance to majority of the common antimicrobial agents were also found. The attitude towards disinfection practice were poor. Thus presence of pathogenic microorganisms on these frequently touched surfaces capable of transmitting pathogens through the community.

10. Recommendations

Based on the study findings, the following recommendations are forwarded to city bus, commercial bank, hospital administrator, and other interested governmental and nongovernmental organization.

It is imperative that administrative bodies and all professionals should take an active role in infection control within their organization

Those frequently hand touched surfaces of ATMs, buses and hospital waiting area inanimate objects should be cleaned regularly with solutions that has disinfectant properties.

Each facility should promote personal hygiene especially hand hygiene and provide enough resources such as hand sanitizers to their customers/users/visitors.

Antibiotic resistant bacteria on ATM, public buses and hospital inanimate object and environmental surface are being reported in the study. Therefore, continuous microbiological surveillance is needed to monitor these organisms and put them under control.

In order to confirm the role of contaminated inanimate surfaces as real source of bacterial cross infection in these public places, further study with the aid of molecular technique has recommended.

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12. Annex

12.1 sample source information sheet

Hello! My name is Mamo Akele. I'm studying my second degree in Addis Ababa University. Now, I'm conducting research on Bacterial profiles and antibacterial susceptibility patterns of frequently hand touched surfaces on public transport buses, Automated Teller Machines and Hospital patient waiting areas, I need you to give me some information concerning the study.

You are directly responsible for the cleaning procedures of this surface or by any means you have a chance of observing the cleaning practices near to the place this is why I am interested to ask you some information about cleaning activities which is performed on this surface on daily bases. No names are required.

Do you agree? Yes ☐ No☐

Places; Bus ☐ Bank ☐ Hospital ☐ surface/specimen ID ___________________

Profession/duty; bus driver ☐ cleaner ☐ guard ☐

Have you ever cleaned/observed cleaning of this specific surface? Yes ☐ No ☐

If yes, how often? Once a day ☐ once a week ☐ not at all ☐ other ________________

What cleaning method do you use/observe?

Only dry wiping ☐ With clean water ☐ With soap & clean water ☐ With bleach ☐ or other methods ______________________________________

12.2 Sample/data collection information form

1. Source of sample; Bus ☐ ATM ☐ Hospital ☐

1.1 Bus; grab rail ☐ armrest ☐ vinyl seat ☐

1.2 ATM; Key Pad ☐Touch screen ☐

1.3 Hospital patient waiting area; OPDs ☐ P.card room areas ☐pharmacy dispensing areas ☐ radiology area☐laboratory area ☐

1.3.1 Chair ☐ door surface/handles ☐ window ☐

1.4 types of surface material made from; metal ☐aluminum ☐plastic ☐ wood ☐ glass ☐ other ____________________

2. Sample unique ID number __________________ 2.1 additional code/place_________________

3. Colony characteristics of cultured organisms on;

3.1Blood Agar ____
3.2 MacConkeyAgar ____
3.3 Manitole Sult Agar ____

4. Gram reaction result ____

5. A. Catalase test: Positive ☐Negative ☐Coagulase test; Positive ☐Negative ☐

B. Catalase test: Positive ☐Negative ☐Coagulase test; Positive ☐Negative ☐

6. Biochemical characteristics of Gram negative rod;

tests	TSIA	Gas Glucose	Indole	SCA	LIA	Urea	Manitol	Motility	Oxidase
Results									
Positive									
Negative								`	

7. Bacteria isolated A,______________________B,______________________C,_______________

8. Drug susceptibility pattern;

Disc used / Result	Bacteria isolated	tetracycline	penicillin	erythromycin	clindamycin	Cefoxitin	Oxacillin	Cefoxitin/	chloramphenicol	gentamicin	ciprofloxacin	ceftriaxone	amoxicillin-clavulanic acid	Piperacillin	Meropenem	Tobramycin	Norfloxacin	Cefuroxime Na	Ceftazidime	ampicillin
Sus																				
Res																				

12.3 Dummy tables

Table 3; Distribution of bacterial isolates from specimen sources hand touched inanimate objects

Bacterial isolate	N# of buses specimen culture positive N#(%)	N# of ATMs specimen culture positive N#(%)	N# of hospital patient waiting areas specimen culture positive N#(%)	Total n# of bacterial isolate

Table 4; Drug susceptibility pattern of bacterial isolate

Bacterial isolate	Disc used / Result	ampicillin	ciprofloxacin	gentamicin	Ceftazidime	tetracycline	chloramphenicol	ceftriaxone	trimethoprim-sulfamethoxazole	methicillin	penicillin	erythromycin	clindamycin	vancomycin	amoxicillin-clavulanic acid	Cefoxitin/MRSA
	Sus															
	Res															
	Sus															
	Res															

12.4 Observation checklists

Observation/visiting date _______________ Bus Side Number _________________

ATM place of location ____

Hospital inanimate object name _______________ Place of location ____________

Buses internal environment cleaning	**Practices performed/ event observed**		**If the Answer is Yes will use for recommendation**
Frequently touché surfaces are not cleaned and disinfected	**Yes**	**No**	
ATM surfaces			
Frequently touched surfaces are not cleaned with care			
Key pads and touch screens are visibly soiled			
Hospital environment			
Frequently touch surfaces are not cleaned and disinfected			
Cleaners use their heavy duty gloves to clean this surfaces			

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Mamo Akele (B.Sc.)

Signature:___________________________

Date of submission:__________________

This proposal has been submitted with approval as advisor.

Advisor:

Kassu Desta (MSc, PhD candidate, Associate Professor)

Signature: _____________________

Date: _________________________

Place: Addis Ababa, Ethiopia.

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