

Bacterial profiles and antimicrobial susceptibility patterns of isolates recovered from surfaces of automated teller machine, bus and hospital waiting area in Addis Ababa, Ethiopia

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Abstract

Introduction: Microorganisms inhabiting in public areas, hospitals and transport facilities can be a concern in public health. Transfer of microorganisms from such reservoirs may lead to mild to severe infection in humans.

Objectives: To assess bacterial profiles and antimicrobial susceptibility patterns of isolates recovered from frequently hand touched surfaces of public transport buses, Automated Teller Machines and Hospital waiting areas.

Methods: A cross-sectional study was conducted from January to July, 2018 in selected Anbessa public transport city buses, Commercial Bank of Ethiopia -Automated Teller Machines and Hospital waiting areas in Addis Ababa. A total of 423 swabs were collected from mostly touched surface and standard microbiology procedures were used to isolate bacteria and conduct antimicrobial susceptibility testing. SPSS V.23 was used for data entry and analysis.

Result: A total of 588 bacterial isolates were isolated of which 75.7% (n= 445) were Gram-positive. Majority of the bacteria were isolated from hospital inanimate objects (40.1%; n = 236). Coagulase negative staphylococci were the most frequently recovered isolates (54.1%; n=318). *Proteus* species were the predominant isolates among the Gram-negative rods (9.9 %; n=58). Most Gram-positive bacteria showed higher proportions of resistance to tetracycline (71.3%) and penicillin (67.3%). Gram negative bacteria exhibited resistance level ranging from 6.3% (tobramycin) to 66.9% (ceftriaxone).

Conclusion: Pathogenic microorganisms on the frequently touched surfaces are of great public health concern considering the ease with which they are capable to transmit in the community and also the nature of antimicrobial resistance traits they harbor. Regular disinfection of most frequently touched object both in hospital and public areas is recommended.

Key words: Bacterial profile, automated teller machine, public transport and waiting area, automated teller machine, antimicrobial susceptibility, Ethiopia

Introduction

Inanimate objects have been shown to play a role in the transmission of bacterial pathogens either directly or indirectly to humans (Reynolds et al. 2005). 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. (Yeh et al. 2011). The concern will increase if the microbes are pathogenic and harbor antimicrobial multidrug resistance traits.

Public transportation is considered as one of the most important services in urban life that is widely used. (APTA 2012). Unhygienic and humid conditions of buses help build up microbial load on touch surfaces and interpersonal transfer of microorganisms from such reservoirs may lead to mild to severe infection in humans (Chowdhury et al. 2016). Automated teller machines (ATMs) are likely to be contaminated with various microorganisms due to the vast dermal contact by multiple users and these interfaces are, therefore,

potential vehicles for the transmission of clinically important pathogens (Onuoha et al. 2014). In Ethiopia, according to a report from the Commercial Bank of Ethiopia (CBE), active ATM cardholders reached more than 3 million (CBE, 2017). The potential role of ATMs in the transmission of pathogenic microbes in the Ethiopian setting is, therefore, worth investigating. Hospital environments and equipment are also critical for transmission of through the healthcare workers and the patients (Hammuel et al. 2014).

In Ethiopia, to the best of our knowledge, there was only one study conducted to investigate the potential role of public transports in the transmission microbes in the community (Atsebaha et al. 2019) and information on the role of ATMs and hospital waiting areas is lacking. This study is, therefore, conducted to assess bacterial profiles and antimicrobial susceptibility patterns of isolates recovered from frequently hand touched surfaces of public transport buses, Automated Teller Machines and Hospital areas in Addis Ababa city.

Materials and Methods

This cross-sectional study was conducted in Addis Ababa city from Jan, 2018 – July 2018. The sample size was calculated based on a single sample size estimation. A total of 423 swabs from 47 public transport buses, 141 commercial bank ATMs and 141 waiting area surfaces of Saint Paul Millennium Medical Collage (SPHMMC) were sampled purposively. Anbessa public buses that were not functional for more than a week; CBE ATMs installed before two weeks of sample collection day and hospital patient waiting places that are already on maintenance or on rebuilding processes were excluded from being samples. Swab sample collection method: Sterile wet cotton socked in sterile physiological normal saline tipped stick was used to collect all surface swab samples. The samples were then processed in the laboratory following the standard microbiology technique for bacterial identification and antimicrobial susceptibility testing. Questionnaires and observation checklist were used to assess the cleaning practice.

Samples were taken using sterile swab before the areas were cleaned and transported timely and then inoculated into appropriate plates with positive and negative control. Results were interpreted using standard controls and procedures following Clinical Laboratory Standard Institute guideline. All data were recorded using hard copy and Microsoft excel and later on analyzed by using SPSS version 23.0. The chi-square distribution statistical technique was employed for the analysis of count or frequency of the isolate.

Ethical clearance was obtained from the review committee of Addis Ababa University, SPHMMC and Addis Ababa Health Bureau. The Consents were asked from each sample source -bus drivers and CBE branch managers and hospital chief executive officer. Confidentiality was protected by giving a unique code and no identifier was attached to results. The responsible stakeholders were communicated for the finding in each site.

Results

Bacterial profile: A total of 588 bacterial isolates were recovered from the 423 processed swabs. 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 objects (40.1%; n = 236) followed by ATM (31.0%; n =182) and Bus (28.9%; n=170). Of all bacterial isolates, Coagulase negative Staphylococci accounted for 54.1% (n=318) followed by *Staphylococcus aureus* (18.2%; n=107). *Proteus* species (9.9%, n=58) *Klebsiella* species (4.3%, n=25) *Pseudomonas* species (3.2%, n=19) *Acinetobacter* species (2.0% n=12) and *Enterobacter* species (1.7% n=10) were the predominant isolates representing Gram negative rods.

Most of Gram positive bacteria (27.7%; n=163) were isolated from ATMs followed by Hospital environment (25.2 %; n=148) and Buses (22.8%; n= 134), however, majority of the gram negative isolates were recovered from Hospital inanimate objects (15.0%, n= 88), followed by from buses (6.1%, n=36) and ATMs (3.2%, n=19) (Table 1).

Table 1: The distribution of bacterial isolates from frequently touched surfaces of ATM, Buses and hospital waiting area inanimate objects at Addis Ababa, Ethiopia, January-July, 2018.

Bacterial isolate	Sample source			
	ATM N (%)	Bus N (%)	Hospital N (%)	Total N (%)
Gram-positive	163(27.7)	134(22.8)	148(25.2)	445 (75.7)
CoNs	135(23.0)	85(14.5)	98(16.7)	318(54.1)
<i>Staphylococcus aureus</i>	28(4.8)	33(5.6)	46(7.8)	107(18.2)
<i>Bacillus</i> 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)
<i>Acinetobacter</i> Species	0(0.0)	2(0.3)	10(1.7)	12(2.0)
<i>Citrobacter</i> Species	0(0.0)	1(0.2)	4(0.7)	5(0.9)
<i>E.coli</i>	0(0.0)	2(0.3)	6(1.0)	8(1.4)
<i>Enterobacter</i> Species	0(0.0)	4(0.7)	6(1.0)	10(1.7)
<i>Klebsiella</i> Species	1(0.2)	2(0.3)	22(3.7)	25(4.3)
<i>Proteus</i> Species	3(0.5)	25(4.3)	30(5.1)	58(9.9)
<i>Pseudomonas</i> Species	15(2.6)	0(0.0)	4(0.7)	19(3.2)
*Others	0(0.0)	0(0.0)	6(0.9)	6(0.9)
Total	182(31.0)	170(28.9)	236(40.1)	588(100.0)

Key: CoNs- Coagulase negative *Staphylococcus aureus*; *others: *Salmonella* Species, *Alcaligenes* and *Shigella* Specie

A total of 141 surface samples were collected from 47 buses. Coagulase-negative staphylococci were the predominantly isolated bacteria (n=34; 20.0%)

followed by *S. aureus* and of these 33 isolates, 9.4% (n=16) were recovered from grab rail. *Proteus* species were dominant isolates from the Gram-negative rods

(9.9%; n=58) of which 6.5% (n=11) of them were recovered from the back-door surfaces. In the hospital environment, Gram-positive bacteria were predominantly recovered from chairs and door

surfaces. Coagulase-negative staphylococci were the frequently isolated bacteria followed by *S. aureus* (Table 2).

Table 2: Distribution of bacteria in different parts of ATM (key pad and touch screen), buses (door, grape rail and vinyl seat) and hospital waiting area (chair, door and window) in Addis Ababa, Ethiopia, January-July, 2018.

Bacterial isolate	ATM			BUS			HOSPITAL				Hospital total	Total
	key pad	Touch screen	ATM total	Door	Grail	Vinyl seat	Bus total	Chair	Door	Window		
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)
<i>S.aureus</i>	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)
<i>Bacillus</i> 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)
<i>Acinetobacter</i> 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)
<i>Alcaligenes</i> 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(0.3)	2(0.3)
<i>Citrobacter</i> 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)
<i>E.coli</i>	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)
<i>Enterobacter</i> 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)
<i>Klebsiella</i> Species	10(5.0)	0(0.0)	10(5.0)	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)
<i>Proteus</i> 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)
<i>Pseudomonas</i> 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)
<i>Salmonella</i> Species	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)	2(0.8)	2(0.3)
<i>Shigella</i> 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.)	7(3.)	182(100)	54(31.8)	59(34.)	57(33.)	170(100)	180(76.)	40(16.)	16(6.)	236(100)	588(100)

Antimicrobial susceptibility test: Overall, 568 bacterial isolates were tested against 17 different selected antibiotic discs. Antimicrobial drug resistance profiles of Gram-positive bacterial isolate shows higher resistance proportions against tetracycline (71.3%), penicillin (67.3%) and cefoxitin (40.2%). *S.*

aureus showed resistance to tetracycline and Penicillin at a rate of 82.2% and 79.4%, respectively. Coagulase-negative staphylococci (CoNs) resistance to Tetracycline (67.60%) and Penicillin (63.20%) were found to be lower than *S. aureus*. On the other hand, CoNs showed higher resistance to cefoxitin (44.0%) compared to *S. aureus* (17.8%) (Table 3).

Table 3: Antimicrobial resistance patterns of Gram-positive bacteria on frequently touched surfaces of ATM, buses and hospital inanimate objects at Addis Ababa, Ethiopia, January-July, 2018.

Bacterial isolate	N	Tetracycline No.(%)	Penicillin No.(%)	Erythromycin No.(%)	Clindamycin No.(%)	Cefoxitin No.(%)	Chloranpinicol No.(%)	Gentamycin No.(%)
CoNs	ATM	318	215(67.6)	201(63.2)	71(22.3)	38(11.9)	140(44.0)	28(8.8)
	BUS	85	56(65.9)	60(70.6)	34(40.0)	14(10.4)	45(33.3)	15(11.1)
	HOSP	98	77(78.6)	71(72.4)	22(22.4)	12(12.2)	48(49.0)	4(4.1)
		107	88(82.2)	85(79.4)	40(37.4)	19(17.8)	31(29.0)	17(15.9)
<i>S.aureus</i>	ATM	28	18(64.3)	17(60.7)	5(17.9)	1(3.6)	2(7.1)	4(14.3)
	BUS	33	24(72.7)	26(78.8)	18(54.5)	8(24.2)	9(27.3)	6(18.2)
	HOSP	46	46(100.0)	42(91.3)	17(37.0)	10(21.7)	20(43.5)	7(15.2)

Key: ATM- Automatic Teller Machine, CoNs- Coagulase negative staphylococcus Species.

More than half of cefoxitin resistant CoNs were isolated from the internal surfaces of buses while relatively higher proportion (43.5%) of cefoxitin resistant *S. aureus* was recovered from hospital inanimate objects/environment. Of all Gram-negative isolates, *Proteus* species showed a relatively higher resistance rate to most frequently used antibiotics (Table 4).

General cleaning method and practice in sample source points/places: From the hospital setting, all the 30 hospital cleaners responded that they have three

cleaning program every day but none of them responded that they clean those frequently touched surfaces which explains why we got higher proportion of bacterial isolates from these surfaces. Based on our cleaning practice assessment, most ATM surfaces are not cleaned regularly but we observed that the technical staff opens and does some maintenance after that they remove dust by using blower machines. Also, these frequently touched keypads are visibly soiled especially. All of the observed buses have very dirty and soiled floor surfaces and some driver cleans with mops during the hand over time.

Table 4: Antimicrobial resistance patterns of Gram negative bacterial on frequently touched surfaces of ATM, buses and hospital inanimate objects at Addis Ababa, Ethiopia, January-July, 2018.

Isolated organism	Chl n (%)	Gen n (%)	Cipro n (%)	Ceftra n (%)	AMC n (%)	Pip n (%)	Mer n (%)	Tob n (%)	Nor n (%)	CTX n (%)	CTZ n (%)	Amp n (%)
<i>Acinetobacter</i> Species(n=12)	4(33.3)	0(0.0)	0(0.0)	12(100)	6(50.0)	6(50.0)	4(33.3)	0(0.0)	0(0.0)	8(66.7)	4(33.3)	-
<i>Alcaligenes</i> (n=2)	0(0.0)	0(0.0)	0(0.00)	2(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(100.0)	0(0.0)	-
<i>Citrobacter</i> Species (n=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)
<i>E.coli</i> (n=8)	4(50.0)	2(25.0)	0(0.0)	4(50.0)	2(25.0)	0(0.0)	0(0.0)	0(0.0)	2(25.0)	7(87.5)	2(25.0)	5(62.5)
<i>Enterobacter</i> Species (n=10)	0(0.0)	1(10.0)	1(10.0)	2(20.0)	6(60.0)	5(50.0)	2(20.0)	0(0.0)	5(50.0)	4(40.0)	6(60.0)	8(80.0)
<i>Klebsiella</i> Species (n=25)	5(20.0)	1(4.0)	2(8.0)	12(48.0)	12(48.0)	20(80.0)	1(4.0)	1(4.0)	3(12.0)	8(32.0)	6(24.0)	18(72.0)
<i>Proteus</i> Species (n=58)	27(46.6)	27(46.6)	25(43.1)	49(84.5)	29(50.0)	34(58.6)	18(31.0)	6(10.3)	46(79.3)	35(60.3)	49(84.5)	45(77.6)
<i>Pseudomonas</i> Species (n=15)	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)	0(0.0)
<i>Salmonella</i> Species (n=2)	1(50.0)	0(0.0)	0(0.0)	2(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(100.0)	0(0.0)	1(50.0)
<i>Shigella</i> spp (n=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(0.0)	2(100.0)	0(0.0)	0(0.0)
Total (n=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)	79(55.2)

Key: Amp: ampicillin, Cipro: ciprofloxacin, Gen: gentamicin, Chl: chloramphenicol, Ceftra: ceftriaxone AMC: amoxicillin-clavulanic acid, Pip: piperacillin, Tob: tobramycin, Nor: norfloxacin CXM: cefotaxime CTZ: ceftazidime Mer: meropenem

Discussion

The isolation of pathogenic bacteria from fomites indicates that they can be vehicles for disease transmission (Shiferaw et al. 2013). Different studies suggested that ATMs, public buses, and different hospital environmental surfaces 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 (Gelaw et al. 2014; Nagajothi et al. 2015; Chowdhury et al. 2016; Atsebaha et al. 2019). This study also revealed that frequently touched surfaces of public areas and objects were contaminated by various pathogenic bacteria with higher frequencies and antimicrobial resistance level. In the present study, all swab samples from the different sources yielded bacterial growth which is line with a study from Gondar that involves hospital computer keyboard and mice surfaces (Alemu et al. 2015). The rate of isolation of clinically important bacteria from the present study was 54.1% which is higher than a report by Shiferaw et al. 2013 (40.2%). This can be due to the difference in the study area.

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%). A Study from China involving metro station, hospital, and park environment reported that more Gram-positive bacteria were isolated. This could be due to their nature that most of these bacteria are skin colonizers (Zhou & Wang, 2013).

In the present study, CoNs (74.2%), *S. aureus* (15.2%), *Pseudomonas* spp. (8.2%), *Klebsiella* spp (0.5%) and *Proteus* spp(1.6%) were predominantly

isolated from ATM surfaces which is agreement with a study conducted in Nigeria (Nawas & Dakroub, 2017). Among Gram-negative bacteria, *Pseudomonas* spp were frequently isolated organisms from ATMs and this finding is similar to the reports from Nigeria (Mahmoudi et al. 2017) but quite different from the results of another study (Nagajothi et al. 2015).

From the public bus surface samples, the rate of contamination of grab rail was found slightly higher compared to the other two surfaces which is agreement with a study elsewhere (Chowdhury et al. 2016). The prevalence rate of CoNs and *S. aureus* in the present study is comparable with study in China (Peng et al. 2015). The isolation rate of *S. aureus* from all internal busses surfaces in this study (19.4%) is in line with a report from Midwestern city, USA (APTA 2012).

The majority of the bacteria (40.1%; 236/588) were isolated from hospital inanimate objects which is similar to other studies conducted in Ethiopia (Gelaw et al. 2014; Shiferaw et al. 2013; Alemu et al. 2015). Similarly, like ATMs and buses, different hospital environmental surfaces also demonstrate higher Gram-positive bacterial contamination. The isolation of CoNs and *S.aureus* from the waiting area has a similar prevalence rate with the reports of another study from Ethiopia (Gelaw et al. 2014).

Staphylococcus strains, especially CoNs, do not often cause disease, however, can be concerning due to their multidrug resistance traits (Gillespie et al. 2009). Over 83% of *Staphylococci* in the present study showed resistance to at least one antibiotic which is in agreement with another study (Zhou & Wang, 2013). In the present study, a high level of antibiotic resistance was detected against tetracycline, penicillin and cefoxitin which is consistent with earlier reports

from Ethiopia (Shiferaw et al. 2013; Alemu et al. 2015). Cefoxitin resistance rate in *S. aureus* from bus surfaces is in agreement with the reports from Portugal (Simoes et al. 2013)

The Increasing rates of multidrug resistance among Gram-positive and negative bacterial pathogens are being documented in many reports including WHO global surveillance reports (WHO 2017). In the present study also, multidrug resistance (MDR = resistance in ≥ 2 drug class) were recorded in 418 (73.6%) of all bacterial isolates with Gram-negative bacteria having 84.6% and Gram-positive bacteria 70% MDR rate. As observed from this study, ATMs, hospital waiting areas and public transport buses are less frequently sanitized which could be public health concern considering the large number of communities they serve on a daily basis.

Conclusion

Frequently hand touched surfaces of ATMs, public transports buses, and hospital waiting area inanimate object/environment were found to harbor bacteria with a high level of resistance to commonly prescribed antimicrobial. Thus, the presence of pathogenic microorganisms on these frequently touched surfaces is of great public health concern considering the ease with which they can transmit in the community and also the nature of antimicrobial resistance traits they harbor. Regular disinfection of most frequently touched objects in hospitals, ATMs and public transport buses is recommended.

Conflict of Interest: Authors declared no conflict of interest.

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