Original Article

Prevalence of Methicillin and Vancomycin resistant *Staphylococcus aureus* on the touch screen of automated teller machines in Dhaka city

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The present study was undertaken to observe the transmission of microbial contaminants through different electronic devices. Five different bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas* spp. and *Bacillus* spp. were cultivated from the surface of 50 different automated teller machines (ATMs) of 10 different banks located in Dhaka City. Among them, the number of *Pseudomonas* spp. and *S. epidermidis* were found up to 10^4 cfu/ml while the *S. aureus* was quantified up to 10^6 cfu/ml. Fungal contamination was also observed in all cases within the range of 10^4 and 10^5 cfu/ml. Most of the isolates were found to be resistant against more than one antibiotic. Only Streptomycin and Gentamicin were found to be effective against all the bacteria. Out of 50 strains (coagulase positive) of *S. aureus*, 40 (80%) were found as Oxacillin and Methicillin resistant. Among 40 MRSA strains, 25 (62.5%) were found to be resistant against vancomycin which is referred to as VRSA. The isolated MDR bacteria from the surface of the ATM may be a health concern for the users.

Keywords: Multi drug resistance, Automated teller machine, Antibiotic, VRSA, MDR.

Introduction

In today's world, the emerging infectious diseases are the major threat to human health and cause serious fatal outcome in both local and global perspective[1,2]. The proliferation of pathogenic microorganisms including bacteria, virus, fungi and parasites into the human body through contaminated hand, food, water and dust particles may cause diseases like diarrhea and dysentery [3-5]. The use of electronic device such as taps, ATM machine, computer, telephones etc. are increasingly being used to reduce the work load and time [6,7]. In public health point of view the surface of these devices may act as one of the major and common routes of pathogenic microbes by which they can easily spread[8,9]. Several studies have already been conducted throughout the world on the presence of pathogenic contamination on the touch screen of electronic devices like ATM booth, public telephone booths, keypad of mobile phones and computers [3,9]. As described in early studies the mobile phones, computer keyboards and automated teller machines (ATM) are now becoming the source of infection because of the vast dermal contact with the key panel[10-13]. Previous study suggested that most noteworthy pathogens such as *Staphylococcus epidermidis*, *Staphylococcus aureus* (MRSA), *Staphylococcus alpha haemolyticus*, *Enterococcus faecalis*, *Bacillus subtilis*, coliforms, *Pseudomonas aeruginosa*, *Escherichia coli* and *Acinetobacter calcoaceticus* have been found from the surface of different electronic devices [14]. The probability of dissemination of such microbial contaminates is very high in densely populated country like Bangladesh where the major community doesn't maintain the personal hygiene and sanitation procedure[8]. Therefore, increased use of Automated Electronic devices by the people who are not aware of the sanitation and hygiene may pose potential threat to public health. [15-19]. Considering all the facts, this study attempted to find out the frequency of multidrug resistant strains (MDR) as well as methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Staphylococcus aureus* (VRSA) on the touch screen of automated teller machines (ATMs) of different banks.

Materials And Methods

The samples were collected from 50 ATM booths of 10 different banks (5 ATM booths of each bank e.g 5×10=50) such as Dutch Bangla Bank, City bank, Standard Chartard bank, BRAC Bank, estern Bank, AB Bank, Islamic Bank, Marchentile Bank, HSBC and First Security Bank in Dhaka city, Bangladesh, from June 2017 to September 2017. The samples were collected from the surface of the keypad and touch screen of ATMs through sterile cotton swabs[20].The sample containing swabs were then immediately inoculated into test tubes containing nutrient broth as enrichment media and transferred in to the laboratory by following thermostatic condition. Within 1 hr the samples were processed for microbiological analysis according to the standard guideline [21].

The 0.1 ml of each sample from the dilution 10^{-3} was introduced on to the nutrient agar and Sabouraud dextrose agar for the isolation of total viable bacteria and fungi, respectively. Subsequently, MacConkey agar, Manitol Salt agar, Cetrimide agar

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and Starch agar (Manufactured by Hi-media Laboratories Pvt. Ltd., Mumbai) were used as selective media for the quantification of coliforms (*E. coli*), *Staphylococcus* spp., *Pseudomonas* spp., and *Bacillus* spp., consecutively [21,22]. All the inoculated plates were incubated at 37 °C for 24 hours except SDA plates, which were incubated at 25 °C for 48 hours. For the confirmation of *E. coli* the lactose fermenting pink colonies from the MacConkey agar were further introduced on to the EMB media (Manufactured by Hi-media Laboratories Pvt. Ltd., Mumbai) to observe the green metallic sheen.

The colony characteristics of the isolates (size, shape, arrangement and color along with their gram reaction) on different selective media were presumptively examined according to the standard microbiological laboratory manual written by James G. Cappuccino[21]. Finally, the standard biochemical tests were performed (triple sugar iron test, citrate test, IMVIC test, MIU test, catalase test, nitrate, urease, gelatine, starch and oxidase) for the identification of all the pathogens (Table 2). Coagulase test was performed for *S. aureus*, 10 µl of the antiserum was taken on the slide with the suspension of the organism. Finally, agglutination was observed against light and the results were recorded [21,23,19].

According to the guidelines provided by the Clinical Laboratory Standard Institute (CLSI), the antibiotic susceptibility pattern of the isolates was examined using disc diffusion method [24,25]. The overnight inoculums of the isolates (*Escherichia coli*, *Pseudomonas* spp., *Staphylococcus aureus, Staphylococcus epidermedis* and *Bacillus* spp.) were adjusted to the turbidity of 0.5 McFarland standards (10^8 CFU/mL) and swabbed onto Mueller-Hinton agar (MHA) Manufractured by (Hi-media Laboratories Pvt. Ltd., Mumbai)[20]. The antibiotic disc used in the study included polymixin B (300 unit), streptomycin (10 µg), gentamycine (10 µg), nalidixic acid (30 µg), azythromycine (15 µg), penicillin G (10 µg), erythromycin (15 µg), amoixicilln (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), ampicillin (10 µg), chloramphenicol (30 µg) and cefixime (5 µg). All the plates were incubated at 37 °C for 12-18 hours and examined for formation of the zone of inhibitions (mm).

For the detection of MRSA and VRSA, overnight incubated suspension of *Staphylococcus aureus* (cell turbidity was adjusted to 0.5 McFarland standards) were introduced on to the Mueller Hinton Agar (MHA) and the antibiotic discs (methicillin 5 mg and vancomycine 30 mg) were placed onto the plates at spatial distance of 5 mm to observe the range of zone diameter for the detection of strain as MRSA and VRSA[19]. A blank disc was introduced as a negative control. All plates were incubated for 24 h at 37 ºC to observe the oxacillin & methicillin and vancomycin resistant *S. aureus*.

Results and Discussion

The dissemination rate of diseases causing bacteria is very high in densely populated countries where sanitation and hygiene condition are poor[12,13,20].

Existence of microbial contamination on the touches screen of ATMs

Total five isolates such as *E. coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas* spp. and *Bacillus* spp. were presumptively identified from the 50 ATM booths of 10 different banks (5 ATM booths were selected for each different bank) (Table 1). *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas* spp. were found in the surface of all Automated teller machine of 50 ATM booths while the existence of *E. coli* was found in the device of 30 ATM booths out of 50 and *Bacillus* spp. was present in 20 ATM booths. Adjacent to the bacterial load all the surface of ATMs exhibited the overall fungal contamination those were not further identified specifically (Table 1).

Among the five isolates *Pseudomonas* spp., *Staphylococcus aureus* and *Staphylococcus epidermidis* were found in high number than *E. coli and Bacillus* spp. The colonization rate of *Staphylococcus aureus* was observed up to 10^6 cfu/ml while *Staphylococcus epidermidis* was found up to 10^4 cfu/ml. For the both species of *Staphylococcus* the maximum load was observed on the ATMs pad of Dutch Bangla Bank, Standard Chartard bank and estern Bank. Among the ten Bank, *E. coli* was present in 6 ATMs up to 10^4cfu/ml except BRAC Bank, estern Bank, AB Bank, Islamic Bank, while *Bacillus* spp. was found on the device of ATMs of Dutch Bangla Bank, City bank, Islamic Bank, Marchentile Bank up to 10^3 cfu/ml. *Pseudomonas* spp. was found in all the ATMs up to 10^4 cfu/ml (Table 1). The morphological characteristics of isolates were observed through microcopy and different biochemical test (Tables 2 & 3). Previously it was reported that skin diseases and hospital acquired infection can be transmitted from infected person to the healthy host through unclean touch screen of electronic devices [26,27]. Another research group reported that mouse of computer and the key pad of mobile phone can serve as potential vector for transmission of infectious pathogens like *Acinetobacter* spp., *S. aureus* as well as extended-spectrum ²-lactamase ESBL-positive Enterobacteriaceae [28-33,7,13].

Antibiotic susceptibility pattern of the isolates

Present study tried to unveil the efficacy of the 12 commonly available drugs against the isolates found from the surface area of the ATMs of different banks. Although some antibiotics used in this study had narrow spectrum of activity against either Gram positive or Gram negative bacteria. Most of the isolates (*E. coli*, *Staphylococcus aureus*, *Staphylococcus epidermidies* and *Pseudomonas* spp.) were found to be resistant against maximum number of antibiotics. Only Streptomycin (10 µg), Nalidixic acid (30 µg), Azithromycin (15 µg), Erythromycine (15 µg), Gentamicin (10 µg), Amoxicillin (30 µg), Ceftriaxone (30 µg) and Ciprofloxacin (5 µg) were found highly effective against *Bacillus* spp. (Table 3). Streptomycin (10 µg) and Gentamicin (10 µg) were found to be effective against most of the isolated strain except *Staphylococcus aureus* (Table 4).

Drug resistant aspect of Staphylococcus aureus against Methicillin and Vancomycin

Total 50 *S. aureus* were isolated from the ATM booths, among them, 40 were found to be resistant against methicillin & oxacillin that indicated 80% MRSA. Among the 80% of MRSA, 62.5% showed resistance against vancomycin as VRSA (Table 5). As described in earlier studies, dissemination of such MRSA & VRSA strain through the dirty surface of the ATMs devices is the main reason to produce several diseases[19,34].

From the previous findings and current investigation, it can be concluded that surface area of different electronic devices harbor several bacteria including MDR strain. In most of the findings *Staphylococcus* spp. were found as foremost bacteria with resistant mechanisms against more than one antibiotic. However, the present study successfully revealed the propagation of MRSA and VRSA on the surface area of ATMs of bank which has generated a new dimension of this study. This finding has massive impact in Bangladesh perspective because there is no sufficient reports have published yet on the MRSA and VRSA identified from the surface of electronic device especially from ATMs booth. Some research groups have been reported previously on the presence of methicillin and vancomycin resistant *S. aureus* isolated from different food and clinical samples [35,36].

Table 1. *Detection of microrganisms on the surface of ATMs (cfu/ml).*

Name ofSamples	Fungi	*E. coli*	*Pseudomonas* spp.	*Staphylococcus aureus*.	*Staphylococcus epidermidis*.	*Bacillus* spp.
Duch Bangla Bank (n=5)	2.0×10^5	2.0×10^3	3.8×10^3	2.8×10^6	2.8×10^4	2.3×10^2
City bank (n=5)	4.0×10^5	2.7×10^3	1.0×10^3	4.3×10^3	1.7×10^3	1.9×10^3
Standard Chartard bank (n=5)	7.5×10^5	4.7×10^4	5.5×10^3	7.0×10^5	4.5×10^4	0
BRAC Bank (n=5)	2.5×10^4	0	1.8×10^4	2.9×10^3	1.1×10^3	0
Estern Bank (n=5)	4.5×10^5	0	4.5×10^3	4.7×10^3	4.7×10^4	0
AB Bank (n=5)	3.5×10^5	0	3.7×10^4	3.5×10^3	5.7×10^3	0
Islamic Bank (n=5)	2.0×10^4	0	2.0×10^3	2.8×10^3	2.0×10^3	3.0×10^2
Marchentile Bank (n=5)	1.5×10^5	4.0×10^3	4.5×10^3	1.5×10^3	4.4×10^3	2.7×10^2
HSBC (n=5)	4.5×10^4	7.0×10^3	6.7×10^4	4.0×10^3	6.0×10^3	0
First security Bank (n=5)	2.0×10^5	2.0×10^3	4.7×10^3	2.8×10^3	2.7×10^3	0

All the experiments were performed in triplicates and the results were reproducible.
Microbial load were enumerated from 50 ATM booth (5 booth under 10 bank) and Average microbial load from each bank has been given in the table

Table 2. *Biochemical identification of the microorganisms*

Microorganisms	TSI Slant	TSI Butt	TSI Gas	H$_2$S reaction	Indole	MR	VP	Citrate	Motility	Oxidase	Catalase	Nitrate	Unease	Starch hydrolysis	Manitol	Coagulase	Gelatin utilization
E. coli	Y	Y	+	-	-	+	-	-	+	-	+	+	-	-	+	ND	-
Staphylococcus aureus	Y	R	-	-	-	+	+	+	-	-	+	+	+	-	+	+	+
Staphylococcus epidermidis	Y	R	+	+	-	-	+	-	-	-	+	+	+	-	-	–	-
Bacillus spp.	Y	R	-	-	+	+	-	-	+/-	-	+	+	-	+	-	ND	+ (rapid)
Pseudomonas spp.	R	R	-	-	-	-	-	+	+	+	+	+	-	-	+	ND	+ (rapid)

ND: Not done; TSI: Triple Sugar Iron Test; Y: Yellow (Acid); R: Red (Alkaline); MR: Methyl red; VP: Voges-Proskauer

Table 3. *Colony morphology of the isolates*

Sample no	Microorganisms	Morphological characteristics	Gram staining result
1	*E. coli*	Flat, smooth pinkish colonies on MacConkey agar and rod shaped motile organism	Gram-negative
2	*Staphylococcus Aureus*	Large, yellow, irregular, endulate, raised, cocci, cluster on Manitol salt agar	Gram-positive
3	*Staphylococcus epidermidis*	Large, pink, irregular, endulate, raised, cocci, cluster on Manitol salt agar	Gram-positive
4	*Pseudomonas* spp.	White colorless to golden colonies on MacConkey agar and rod shaped organism	Gram-negetive
5	*Bacillus* spp.	Moderate , violet, irregular, lobate, raised, rod, chain on Starch agar	Gram-positive

All the experiments were performed in triplicates and the results were reproducible

Table 4. *Antibacterial susceptibility test of the isolates*

Antibiotic	Disc content	*E. col* n=30		*Staphylococcus aureus* n=50		*Staphylococcus epidermidis* n=50		*Bacillus* spp. n=20		*Pseudomonas* spp. n=50	
		R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Polymixin B	300 units	100	0	100	0	0	100	100	0	0	0
Streptomycin	10 µg	0	100	100	0	0	100	0	100	0	100
Gentamicin	10 µg	0	100	100	0	0	100	0	100	0	100
Nalidixic acid	30 µg	100	0	100	0	100	0	0	100	0	100
Azithromycin	15 µg	100	0	100	0	100	0	0	100	100	0
Erythromycine	15µg	100	0	100	0	100	0	0	100	100	0
Amoxicillin	30µg	100	0	100	0	100	0	0	100	100	0
Ceftriaxone	30µg	100	0	100	0	100	0	0	100	100	0
Ciprofloxacin	5µg	100	0	100	0	100	0	0	100	100	0
Ampicillin	10µg	100	0	100	0	100	0	100	0	100	0
Chloramphenicol	30µg	100	0	100	0	100	0	100	0	100	0
Cefixime	5µg	100	0	100	0	100	0	100	0	100	0

All the experiments were performed in triplicates and the results were reproducible
R   Resistant
S   Sensitive

Table 5. *Detection of methicillin and vancomycin resistant Staphylococcus aureus (MRSA &VRSA)*

Microorganism	No of isolates	Resistance against Oxacillin& Methicillin	Resistant presentence (%)
Staphylococcus aureus	n=50	40	80
	Detection of Vancomycine resistant strain among MRSA		
Isolate	No of MRSA isolates	Resistance againstVancomycin	Resistant presentence (%)
Staphylococcus aureus (n=50)	n=40	25	62.5

All the experiments were performed in triplicates and the results were reproducible

Conclusion

As a final point, this study unveiled the existence of different microorganisms on the surface of the touches screen of ATMs of different bank. Based on the antibiogram study of the isolates some bacteria were found to be resistant against more than one antibiotics. The most significant findings of this study was the incidence of methicillin and vancomycin resistant *S. aureus*, which is so alarming for the daily users. To minimize the rapid spread of such drug resistant pathogen, the surface of ATM devices should be cleaned regularly by applying proper disinfectant and people should more suspicious regarding their personal cleanliness. However, further molecular study is required to detect the resistant gene to take the necessary action against the bacteria.

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