ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 8, Issue 2, 2015

ISSN - 0974-2441

Research Article

PREVALENCE OF MULTI DRUG RESISTANT STRAINS ON TOUCH SCREEN OF AUTOMATED TELLER MACHINE

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Received: 25 December 2014, Revised and Accepted: 30 January 2015

ABSTRACT

Multiple drug resistant (MDR) bacteria have spread globally even on non-porous surfaces such as mobile phones, computers, public telephone booths, keypads and touch screens of automatic teller machine (ATM). In this study, occurrence of different groups of MDR bacteria from metallic keypad and touch screens of ATM machine was examined. The samples were collected from different ATMs located in Vellore, Tamil Nadu. Swabs were taken from 47 ATMs and 488 isolates were identified as *Escherichia coli* (49%), *Klebsiella* sp., (30%), *Pseudomonas* sp., (16%), *Acinetobacter* sp., (3%) and *Proteus* sp., (1%) and they were subjected to antibiotic susceptibility testing with ampicillin, cefotaxime, ceftriaxone, ceftizidime and meropenem of which 46 isolates showed high level of resistance toward cefotaxime, and meropenem by plate assay. Further polymerase chain reaction amplification of NDM-1 and CTX-M genes for all 46 isolates showed no amplified product, which showed the possibility for the presence of other types of extended spectrum β-lactamases or metallo beta-lactamase. Our results showed the prevalence of MDR bacteria in ATM centers and most importantly awareness toward the public regarding the spread of pathogenic bacteria in the environment.

Keywords: Multiple drug resistance, Automatic teller machine centers, Public health, Antibiotic susceptibility pattern, Resistance gene.

INTRODUCTION

The increasing threat to human health is by an emergence of infectious diseases, and they are one of the sources that cause a fatality globally [1]. Pathogenic microorganisms including bacteria, virus, fungi and parasites plays a vital role in causing infectious disease by entering into the body and starts to proliferate [2]. Non-porous surfaces such as taps, telephones, doorknobs etc., facilitate a surface for the transmission of pathogenic microorganisms. Public restroom plays a vital role in spreading disease to a person by contact through hand on mouth or hand to food contact [3,4]. The residence of pathogens like *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus alpha haemolyticus*, *Enterococcus faecalis*, *Bacillus subtilis*, coryneforms, *Pseudomonas aeruginosa*, *Escherichia coli* and *Acinetobacter calcoaceticus* were found on the surface of public telephone booths since they are constantly utilized by the users and also observed in the door knobs, the mouthpiece and keypad of mobile phones and computer [5]. Microbes develop resistance to available antibiotics due to its irrational use and they started to spread via horizontal gene transfer. The bacteria shows reluctant toward antibiotic are increasing with each passing year [6]. Beta-lactamases produced by these pathogenic microbes to overcome the activity of beta-lactam group of antibiotics pose the greatest resistance ability even to last resort of antibiotics including carbapenems. Some microbes have progressed resistance to multiple drugs and is known as multiple drug resistance (MDR). MDR permits disease-causing microbes to resist antimicrobials, which are targeted at eliminating the microbes [7]. The antibiotic resistant pathogens widened worldwide and strains, which pose resistant to four or more frontline antibiotics called extremely drug resistant have found recently [8]. Totally drug-resistant strains have also been reported in later years [9]. It is found that the mobile phones are now becoming the source of infection since the users pay less attention for their personal hygiene as well as the use of mobile phones by the users in all the places provides an open array for breeding potential pathogenic microbes, and thus it becomes a great public health hazard [10]. The computer keyboards and mouse acts as fomites and the contaminated surface cause illness which has been recorded in health care and hospital environments [11]. Recently, it is noticed that the automatic teller machine (ATM) also serve as a mediator for the transmission of disease by vast dermal contact with the key panel [12,13]. Several works have been reported to analyze the microbial contamination on the screens and metallic keypads on ATMs. There is a possibility that the ATMs may be contaminated with MDR strains of bacteria, however very few works have been reported on the same. Hence, the aim of this study was to find out the prevalence of MDR strains on the touch screen of ATMs.

METHODS

Sample collection and processing

The samples were collected from 47 ATMs of 12 different banks from Vellore, Tamil Nadu for a period of 3 months from January 2014 to March 2014. The samples were collected from the keypad and touch screen of ATMs using sterile swabs [12]. The swabs were then immediately transferred to test tubes within a period of 1 hr, and the bacterial isolates were examined for colony morphology and Gram-staining.

Antibiotic susceptibility testing

The antibiotic susceptibility for the bacterial isolates was examined using disk diffusion method as per the Clinical Laboratory Standard Institute (CLSI, 2009) guidelines. The inoculums were adjusted to the turbidity of 0.5 McFarland (1×10^8 CFU/mL) and swabbed onto Mueller-Hinton agar (MHA) (Hi-media Laboratories Pvt. Ltd., Mumbai). The antibiotic disk's used were ampicillin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), and meropenem (10 µg) respectively.

Plate assay

Plate assay was done as per [14], accordingly 2 µg/mL concentration of cefotaxime and 4 µg/mL concentration of meropenem (concentration of antibiotics selected as per the break points) were amended into 25 mL of MHA plate and isolates grown after overnight incubation was considered to be resistant.

Genomic DNA isolation

DNA isolation was done by Ausubel *et al*. method [15], 1995. Briefly, overnight grown culture was centrifuged and to the pellet, TE buffer, 10% sodium dodecyl sulfate and proteinase K were added and incubated. After 1 hr, 5 M NaCl was added, followed by the addition

of CTAB/NaCl solution, it was placed in the water bath at 65°C for 10 minutes. Chloroform/isoamyl alcohol (24:1) was added and spinned. To the supernatant equal volume of phenol/chloroform/ isoamyl alcohol was added and centrifuged. Ice cold isopropanol was added to the collected supernatant, and the precipitated DNA was then washed with 70% ethanol.

Amplification of resistance gene

Polymerase chain reaction (PCR) amplification for NDM-1 gene [16] and CTX-M group gene was carried out using PCR conditions and primers as previously described [17]. The primers used to amplify NDM-1 gene are F:5'-ACCCGGTCGCGAAGCTGAGCAC-3' and R:5'ATGCGGGCCGTATG AGTGAGTGATTGC-3'and the CTX-M group of gene includes; CTX-M 1, CTX-M 2, CTX-M 9, CTX-M 26 and CTX-M 8 respectively.

RESULTS

Sample collection and processing

There were totally 47 samples collected from different ATM machines and a total of 488 isolates were obtained and morphologically characterized (Table 1). 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 identified (Fig. 1).

Antibiotic susceptibility pattern

Ampicillin, cefotaxime, ceftazidime, ceftriaxone, and meropenem were the antibiotic disk's used to access the susceptibility pattern of all the isolates, which showed that organisms were resistant to more than one antibiotics tested (Table 2).

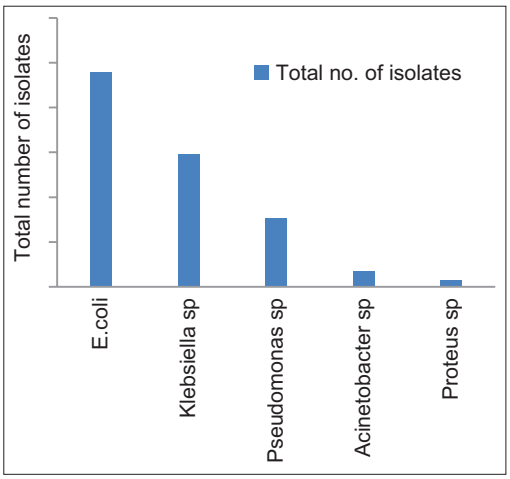

Fig. 1: Total bacterial strains identified from collected samples

Plate assay

Among 120 isolates which showed high level of resistance by disk diffusion method was selected for plate assay and out of 120 isolates 46 showed resistance to cefotaxime (2 µg/mL) and meropenem (4 µg/mL) at its break point concentration.

Amplification of resistant genes

The strains which showed resistance phenotypically to cefotaxime and meropenem at its breakpoint concentrations were selected. Then the selected 46 isolates were amplified for resistance gene using NDM-1 and CTX-M gene primers. It was observed that none of 46 isolates were amplified for NDM-1 and CTX-M genes.

DISCUSSION

Only few studies have been carried out in India on isolation and identification of microbes from ATM. Hence, the present study focused mainly on to determine the existence of MDR strains on the surface of metallic keypad and touch screen of the ATMs located within the city. The scope of this study was to prompt awareness to the general public and ATM users on the possible spread of diseases due to the existence of pathogens, particularly MDR pathogens on ATM touch screen and keypads.

The consequence of this study revealed high level of contamination on the surface of the ATMs with *E. coli*, *Klebsiella* sp., *Pseudomonas* sp., *Acinetobacter* sp., and *Proteus* sp., The percentage distribution of the bacterial isolates showed that occurrence of *E. coli is* about 48.97%, followed by *Klebsiella* sp., 30.32%, *Pseudomonas* sp.,15.77%, *Acinetobacter* sp., 3.48% and *Proteus* sp., 1.43%. Our results coincide with Abban *et al.,* reported that on the surface of ATM was contaminated by food borne pathogen such as *Aeromonas, Bacillus*, *Enterobacter*, *Escherichia*, *Klebsiella* and *Salmonella* and the possibilities of cross contamination when contact with such environment [18] and another study by Onuoha and Fatokun examined the possibility of ATM as a source of bacterial contamination including *Staphylococcus* sp., *Streptococcus* sp., *E. coli*, *Enterobacter* sp., *Pseudomonas* sp., thus to create an awareness among the public regarding the pathogen occurrence even on the keypad and touch screen of ATMs [19]. Identified drug-resistant *E. coli* and other pathogens at highest percentage reveal the possibility for spread of most common diseases though there was no detection of extended spectrum β-lactamases (CTX-M) and metallo beta-lactamase (NDM) resistant genes. There is also a possibility for the presence of other types of beta-lactamase genes because the identified organisms showed resistance phenotypically.

Table 1: Colony morphology of isolates

S. no.	Organism	Morphological characteristics	Gram-staining result
1	*E. coli*	Flat, smooth pinkish colonies on MacConkey agar and rod shaped motile organism	Gram-negative
2	*Klebsiella* species	Round mucoid pinkish colonies on MacConkey agar and rod shaped non-motile organism	Gram-negative
3	*Pseudomonas* species	White colorless to golden colonies on MacConkey agar and rod shaped organism	Gram-negative
4	*Acinetobacter* species	White colorless colonies on MacConkey agar and rod shaped non-motile organism	Gram-negative
5	*Proteus* species	White colorless colonies on MacConkey agar and rod shaped motile organism	Gram-negative

E. coli: Escherichia coli

Table 2: Results for antibiotic susceptibility test with standard antibiotics

Organism	Total number of isolates	Ampicillin resistant (%)	Cefotaxime resistant (%)	Ceftriaxone resistant (%)	Ceftizidime resistant (%)	Meropenem resistant (%)
E. coli	239	178 (75)	145 (61)	172 (72)	181 (75)	24 (10)
Klebsiella sp	148	108 (73)	103 (69)	112 (75)	110 (74)	14 (9)
Pseudomonas sp	77	57 (74)	59 (76)	62 (80)	64 (83)	9 (11)
Acinetobacter sp	17	12 (70)	14 (82)	12 (70)	11 (65)	3 (17)
Proteus sp	7	5 (72)	5 (74)	5 (71)	6 (86)	1 (14)

E. coli: Escherichia coli

Hence, there is a great risk of spreading antibiotic reluctant bacteria through contact with such highly contaminated public device like ATM machines. This can be overcome only when we maintain personal hygiene like washing hands regularly using soap or alcohol. The regular wiping of the screens and keypads of the ATMs using disinfectant may help in reducing the spread of -MDR pathogens.

CONCLUSION

This study established an existence of microbial contamination on the surface of the keypad and touch screen of ATMs. The phenotypic resistance to some antibiotics was observed by these pathogenic organisms. Hence, it is needful to take instant steps towards the widespread of pathogenic microbes, especially against the prevalence of MDR pathogens. The precautions to be taken in order to avoid the spread of pathogenic microbes and are made feasible by proper cleaning regimen using appropriate sanitizers and the surface of ATM devices should regularly be cleaned.

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