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.

**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 (1×10⁶ 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), ceftriaxone (30 µg), ceftizidime (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. Chlorof orm/isooamy l alcohol (24:1) was added and spinned. To the supernatant equal volume of phenol/chloroform/ isooamy l 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
Polynucleotide 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′-ACCCGGTCGCGAAGCTGAGC A C-3′ and R:5′ATGCCGGGCCTATG AGTGGATTCG-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, ceftriaxime, ceftazidime, 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).

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 breakpoint 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

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 48.95%, 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 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.

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 48.95%, 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

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Organism</th>
<th>Morphological characteristics</th>
<th>Gram-staining result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E. coli</td>
<td>Flat, smooth pinkish colonies on MacConkey agar and rod shaped motile organism</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>2</td>
<td>Klebsiella sp.</td>
<td>Round mucoid pinkish colonies on MacConkey agar and rod shaped non-motile organism</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>3</td>
<td>Pseudomonas sp.</td>
<td>White colorless to golden colonies on MacConkey agar and rod shaped motile organism</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>4</td>
<td>Acinetobacter sp.</td>
<td>White colorless colonies on MacConkey agar and rod shaped non-motile organism</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>5</td>
<td>Proteus sp.</td>
<td>White colorless colonies on MacConkey agar and rod shaped motile organism</td>
<td>Gram-negative</td>
</tr>
</tbody>
</table>

E. coli: Escherichia coli

Table 2: Results for antibiotic susceptibility test with standard antibiotics

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

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.

REFERENCES