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Isolation and Identification of Microorganisms Associated with Automated Teller Machines in Calabar Metropolis

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Authors' contributions

This work was carried out in collaboration between all authors. Author RCA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors COA and AAN managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The metallic keyboards of automated teller machines situated within Calabar metropolis were investigated for microbial contamination due to their vast dermal contact by multiple users. Swab sticks soaked with normal sterile saline were used to collect specimens from the keypads. Isolation was done on nutrient agar and eosine methylene blue (EMB) for bacteria and Sabouraud dextrose agar for fungi using standard microbiological procedures. Identification of microorganisms was done using colonial, microscopic and biochemical characteristics. The results reveal that these machines that serve as cash dispensing device can be a potential disease dispensing machines as samples analyzed revealed bacterial isolates such as *Staphylococcus spp* (32%)), *Bacillus spp* (24%), *Escherichia spp* (20%), *Pseudomonas spp* (18%) and *Salmonella spp* (6%) while fungal isolates of the samples analyzed include microorganisms such as *Rhizopus spp* (56%), *Aspergillus*

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spp, (25%) and *Penicillium spp* (19%). These findings necessitate the need for adequate personal hygiene by users and frequent routine cleaning of these machines by the bank's authorities is highly recommended.

Keywords: Microorganisms; Automated teller machines (ATM); Calabar; isolates; routine cleaning.

1. INTRODUCTION

Automated teller machines (ATMs) are the longest standing and most widely used form of computer driven public technology which makes banking and other financial transactions easier [1]. 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. A typical usage of the ATM involves slotting a card into a recipient hole and following on-screen instructions by punching the keys of the metallic keypads to enter secret codes and commands; thus instructing the machine as to the kind of service one requires [2].

Microorganisms exist everywhere in the environment and are able to persist or even grow on any surface [3]. Although most of them are harmless, some are pathogenic, especially in people with weaken immune system. The ATM is likely to be contaminated with many different kinds of microorganisms both pathogenic and non-pathogenic due to their vast usage and dermal contact by many people in a day especially in an overcrowded environment [4]. ATM once contaminated becomes vehicles for the transmission of infection, such that the user may succeed in picking these pathogens after making use of the Automated Teller Machine, since there is no restriction as to who has access to the facility, and no guidelines to ensure hygienic usage [5].

Contamination of environmental objects and surfaces is a common phenomenon. Many factors have been shown to influence the bacteria transfers between surfaces, including the source and destination surface features, bacterial species involved, moisture levels, pressure and friction between the contact surfaces and inoculums size on surfaces [6]. Human beings have a marked tendency to pick up microorganisms from environmental objects, and the hand has been shown to play a role in the transmission of organisms [7].

The reservoir of any organism, which may be animate or inanimate objects, in the epidemiology of any infection is very important [8]. Many epidemiological studies have shown that many contaminated surfaces played a major role in the spread of infectious diseases [9].

The presence of viable pathogenic bacteria on inanimate objects has been reported by earlier investigators [10]. *Salmonella* species and *Escherichia coli* have also been shown to be transferred from hands to raw processed and cooked foods, even at low levels on the fingers [11]. Furthermore, microorganisms found to contaminate fomites has also been shown to persist on environmental surfaces in varying periods of time ranging from hours to months [12]. In addition, bacteria that can cause severe gastroenteritis have been found on ATM keypad and cross infection of microorganisms between environmental surfaces and a host has equally been established [13]. Furthermore, microbes once attached to hands and to some surfaces may survive for a while and may be difficult to remove [14].

It has been shown that snacks eaten with the fingers can easily be cross contaminated by bacteria from the hands after handling dirty currency notes [15]. It has also been shown that hard, nonporous surfaces such as ATM have the highest bacteria transfer rates to hands [12].

However, personal hygiene and good hand washing technique have been found to be an effective method of preventing the transmission of pathogens through fomites such as the user interface of automated teller machines.

2. MATERIALS AND METHODS

2.1 Study Site

Automated teller machines in four banks at different locations in Calabar metropolis were used in this study and permission was given by the banks' management.

2.2 Sample Collection and Processing

Sterile swab sticks were used to collect samples from four different ATM points within calabar metropolis namely:

- Bank A ATM located at Calabar road
- Bank B ATM located at Mary Slessor road
- Bank C ATM located within UNICAL main Campus
- Bank D ATM located within the University of Calabar Teaching Hospital

Sterile swab sticks moistened with sterile saline were used to clean the surfaces of the Automated teller Machines. The Swab Sticks were transferred immediately to the laboratory within one hour of samples collected to prevent drying of the samples for microbiological analysis. All the samples were processed in the laboratory according to the standard microbiological methods under very strict complete aseptic conditions. The swabs were inoculated on appropriate media.

2.3 Isolation of Microorganisms

The method described by Okoro et al. [16] was used. The swab stick bearing the sample was dipped in a test tube containing of sterile normal saline and agitated for 5 min before inoculations were collected from there and cultured by the streak and spread plate techniques. An inoculum was taken out aseptically in duplicates from each sample used in normal saline. One inoculum was placed on the surface of a solid, sterile nutrient agar plate and streaked totally using a flamed wire loop. The second inoculum was placed on the surface of Eosine Methylene Blue Agar (EMB) and spread evenly with the aid of a glass holder.

The inoculation was repeated on solid Sabouraud Dextrose Agar (for Fungi). The inoculated plates were incubated accordingly. Bacteria culture plates were incubated at 37^{o}C for 24 to 48 hours while fungal culture plates were incubated at room temperature for 2 to 5 days. The plates were examined daily for growth. On the establishment of growth, each culture plate was examined for distinct colonies from which sub-cultures were made on fresh solid agar media and incubated as described earlier. When there was new growth, they were examined for uniformity as a mark of purity. The resulting pure cultures were used for characterization and subsequent identification.

The culture media used were nutrient agar which was used for the cultivation of non-fastidious organisms, Sabouraud Dextrose Agar which was used for the cultivation of Fungi and Eosine Methylene Blue Agar (EMB) which was used as a differential medium in the culture of lactose fermenting organisms such as *E. coli*. The various media were prepared according to the manufacturer's instruction as described above.

2.4 Characterization and Identification of Bacterial Isolates

Bacterial isolates were characterized as described by Mehmet et al. [17] using colonial, microscopic and biochemical characteristics. All tests were done using standard basic media and reagents [18]. The isolates were identified following a check on their characteristics which matched those of existing taxa in standard manuals. Characteristics of bacteria in Bergy's Manual of Determinative Bacteriology were used as a standard in this case. Isolates were thus identified in line with those contained in the manual and were named according to their matching species.

2.5 Identification of Fungal Isolates

This test was used in the characterization and identification of fungi isolates from Sabouraud Dextrose Agar media-plates based mainly on the colony features and microscopic examinations. These colonies were for the presence of fungal mycelia, whether it is septate or non-septate, or it conidia, also if the fungal has black spores, green pigmentation and the arrangement of its conidiophores. Mounts of the fungal isolates were made each with lactophenol cotton blue and examined individually microscopically using 10X and 40X objective lens and compared with mycological atlas.

2.6 Determination of Occurrence

The occurrence of each bacterium and each fungus species isolated from the test samples was determined as a percentage ratio of their prevalence relative to the total number of samples examined. The formula below was used:

$$\% \text{ Occurrence} = \frac{\text{No. of positive test}}{\text{Total No. tested}} \times \frac{100}{1}$$

The total occurrence of bacteria and fungi in each bank was determined as a percentage ratio of their prevalence in each bank relative to the total number of samples examined in all banks. The formula below was used:

$$\% \text{ Occurrence} = \frac{\text{No. of positive test in a bank}}{\text{Total No. tested in all banks}} \times \frac{100}{1}$$

3. RESULTS

The results revealed that both pathogenic and non-pathogenic microbes colonized the metallic surfaces of the Automated teller machines used in the study. Microorganisms such as bacteria which included *Escherichia coli, Salmonella sp., Bacillus subtilis, Pseudomonas sp.* and *Staphylococcus aureus* were identified (Table 1) and fungi which included *Penicillium sp., Rhizopus sp.* and *Aspergillus niger* were also identified (Table 2) from the various automated teller machines within Calabar metropolis.

Table 3 shows the total percentage occurrence of bacterial Isolates from ATM keyboards. Across the 4 banks, *Staphylococcus spp* has the highest occurrence with a total of 32%, *Bacillus subtilis* has a total occurrence of 24%, *Escherichia sp.* has a total occurrence of 20%, *Pseudomonas sp.* has a total occurrence of 18% and *Salmonella sp.* with the least occurrence of 6%.

The total number of bacterial isolates in Bank A ATM is 38%, Bank ATM has 27% of the total isolates, Bank C ATM has 16% of the total isolates and Bank D ATM has 19% of the total number of isolates.

Table 4 shows the total percentage occurrence of fungal isolates from ATM keyboards. Across the 4 banks, *Rhizopus spp* has the highest occurrence with a total of 56%, *Aspergillus spp* has a total occurrence of 25%, and *Penicillium spp* with the least occurrence of 19%.

Table 1. Morphological and biochemical characteristics of bacterial isolates

Characteristics	Isolates				
	1	**2**	**3**	**4**	**5**
Pigment characters	-	-	Blue-green; Fluorescent Yellow-greenish	-	Golden yellow to orange
Motility	Motile	Motile	Motile	Motile	Non-Motile
Shape	Rod	Straight rod	Rod	Rod	Cocci
Gram Reaction	Gram negative	Gram negative	Gram positive	Gram positive	Gram positive
Spore formation	Negative	Negative	Negative	Positive	Negative
Catalase	Positive	Positive	Positive	Positive	Positive
Citrate	Negative	Positive	Positive	Negative	Positive
Glucose utilization	Positive	Positive	Positive	Positive	Positive
Voges Proskauer	Negative	Negative	Negative	Negative	Positive
Coagulase	Negative	Negative	Positive	Negative	Positive
Methyl red	Positive	Positive	Positive	Positive	Positive
Indole production	Negative	Negative	Negative	Negative	Negative
Probable organism	*Escherichia coli*	*Salmonella sp.*	*Pseudomonas sp.*	*Bacillus subtilis*	*Staphylococcus aureus*

Table 2. Morphological characteristics of fungi isolates

Morphological characters	Isolate 1	Isolate 2	Isolate 3
Colony	Blue-green colonies	Brownish colonies	Black colonies
Morphological characters	Septate branched, mycelium, with conidiophores	Non-septate, forms chlamydospores and cottony surface.	Septate branched, mycelium, Blackish conidia, Ascopores are produced
Probable fungus	*Penicillium sp.*	*Rhizopus sp.*	*Aspergillus niger*

Table 3. Total percentage occurrence of bacterial Isolates from ATM keyboards

Isolates	Bank A ATM at Calabar Road Number (%)	Bank B ATM at Mary Slessor Number (%)	Bank C ATM at UNICAL Number (%)	Bank D ATM at UCTH Number (%)	Total	% Occurrence
Esherichia coli	7 (23%)	4 (19%)	2 (14%)	3 (20%)	16	20%
Staphylococcus aureus	9 (30%)	7 (33%)	4 (29%)	5 (33%)	25	32%
Bacillus subtilis	8 (27%)	5 (24%)	3 (21%)	3 (20%)	19	24%
Pseudomonas spp.	4 (13%)	3 (14%)	4 (29%)	3 (20%)	14	18%
Salmonella spp.	2 (7%)	2 (10%)	-	1 (7%)	5	6%
Total Number of isolates	30 (38%)	21 (27%)	13 (16%)	15 (19%)	79	100

Table 4. Total Percentage occurrence of fungal Isolates from ATM keyboards

Isolates	Bank A ATM at Calabar Road Number (%)	Bank B ATM at Mary Slessor Number (%)	Bank C ATM at UNICAL Number (%)	Bank D ATM at UCTH Number (%)	Total	% Occurrence
Aspergillus spp	3 (23%)	2 (22%)	1 (33%)	2 (29%)	8	25%
Rhizopus spp	7 (54%)	5 (54%)	2 (67%)	4 (57%)	18	56%
Penicillium spp	3 (23%)	2 (22%)	-	1 (14%)	6	19%
Total Number of isolates	13 (41%)	9 (28%)	3 (9%)	7 (22%)	32	100

The total number of fungal isolates in Bank A ATM is 41%, Bank B ATM has 28% of the total isolates, Bank C ATM has 9% of the total isolates and Bank D ATM has 22% of the total number of isolates.

4. DISCUSSION

Bank A ATM has the most bacterial and fungal abundance because of the high number of individuals that use the ATM as the area is thickly populated and close to the calabar main market with poor sanitation level while bank C ATM has the least number of bacterial and fungal abundance as the ATM is only used by fewer individuals who spend few hours within the environment and with probable good sanitation level.

The high level of microbial load seen in this study which includes*: Staphylococcus aureus, E. coli, Bacillus subtilis, Pseudomonas sp., Rhizopus sp.* and *Aspergillus niger* is in line with the study of Fraser and Girling [19], who reported that keyboards of ATM harbored more bacteria than computer keyboards and this may be due to the fact that they are exposed to many users, environmental factors such as rain and climatic factors such as wind.

This study is also in conformity with Anastasiades et al. [20] who reported that *Staphylococcus aureus* is prevalent. High levels of microorganisms such as *E. coli*, *Staphylococcus* and *Rhizopus* have also been reported by Mbajiuka [21]. Humphrey et al. [22] disclosed that even low levels of *Salmonella spp* and some *Escherichia coli* strains can easily be transferred from the fingers to surfaces. *Pseudomonas aeruginosa* is well documented for their high pathogenicity, causing even death in some major outbreaks and infections [23]. People must keep in mind that the ATM devices might be potential areas for pathogen accumulation.

The number of microorganisms present on a surface is amongst the microbe-associated factors that determine whether an infection will occur or not. Apart from the number of microorganisms, the type and quality of microorganisms present on a surface is also an important factor to know if an infectious outbreak will occur or not [19].

These findings necessitate the need for frequent disinfection of automated teller machines and its accessories along with periodical microbiological surveillance.

5. CONCLUSION

Automated teller machines were invented to ease the stress associated with banking, therefore, the general public should maintain good hygienic conditions to avert any possible outbreak of diseases, as it has been proved that microorganisms are associated with the use of ATM. Cleaning regimen aimed at reducing the population and presence of microorganisms on the surfaces of automated teller machines should be developed using appropriate sanitizers and it should strictly be adhered to by all automated teller machines operators.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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