EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
MICROBIOLOGY

G

ISSN
2090-0872

WWW.EAJBS.EG.NET

Vol. 16 No. 2 (2024)

Citation: *Egypt. Acad. J. Biolog. Sci. (G. Microbiolog) Vol.16 (2) pp.139- 149 (2024)*
DOI: 10.21608/EAJBSG.2024.393270

Egypt. Acad. J. Biolog. Sci., 16(2):139-149 (2024)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
https://eajbsg.journals.ekb.eg/

Bacteria Associated with Automated Teller Machines: Isolation and Identification

Abdulbaset E. Mostafa[1], Mervat G. Hassan[2], Mohammad M. El-Metwally[3], Sahar E. Abo-Neima[4] and Mariam Zewail[5]

[1]Institute of Graduate Studies and Environmental Research Damanhour University.
[2]Botany and Microbiology Department, Faculty of Science, Benha University.
[3]Botany and microbiology department, Faculty of Science, Damanhour University.
[4]Physics Department, Faculty of Science, Damanhour University.
[5]Department of Pharmaceutics, Faculty of Pharmacy, Damanhour University.
*E. mail: abdomostafa532@gmail.com ; walaabdu48@gmail.com

ARTICLE INFO
Article History
Received:21/10/2024
Accepted:24/11//2024
Available:28/11/2024

***Keywords*:**
Automated teller machines, pathogenic bacteria.

ABSTRACT

Numerous individuals frequent automated teller machines (ATMs) daily. This machine is accessible to the public irrespective of class, age, or race. This work sought to isolate and identify bacterial species from ATM surfaces, which may function as potential reservoirs for bacterial contamination. This investigation was conducted on the streets of Alexandria, with samples collected at various times throughout the early morning and peak afternoon periods, from diverse locations adjacent to hospitals, urban neighborhoods, and less developed regions. By using blood and MacConkey agar plates, 169 bacterial isolates have been isolated from 51 swabs, the isolates have varied colony morphologies, including creamy, grey, green, white, yellow, pink, and beige colors, exhibiting circular, irregular, and flat shapes. Gram staining identified 132 gram-positive isolates (81 cocci and 51 bacilli) and 37 gram-negative. Subsequent biochemical testing revealed a diversity of bacterial species which include seven isolates of *Klebsiella*, five isolates of *Proteus*, and nine isolates of *Pseudomonas*.

INTRODUCTION

Microorganisms are little organisms that can only be seen with the use of a microscope. They possess both beneficial and detrimental functions. Their capacity to adapt and proliferate on diverse surfaces and in varying settings is crucial to their ubiquitous presence in the biosphere. The automated teller machine (ATM) is considered a mini-bank, since it facilitates almost all types of banking transactions (Nworie *et al.*, 2012). The keypads, which may harbor harmful bacteria, provide an often-neglected reservoir for gastrointestinal disorders (Micheals, 2002). A significant number of bacteria have the capability to survive on dry fomites such as ATM keypads. They have developed several physiological resting phases that provide an advantage for survival or hibernation in conditions of low water activity. Certain Gram-negative bacteria may persist on surfaces for up to eleven days (El-Dars & Hassan, 2005). Key determinants for the persistence of infections on surfaces include the availability of organic matter, solar radiation, temperature, and humidity (Taylor *et al.*, 2013).

A review indicated that numerous Gram-positive bacteria, including *Enterococcus* spp., *Staphylococcus aureus*, and *Streptococcus pyogenes*, as well as Gram-negative bacteria such as *Acinetobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Shigella* spp., can persist on surfaces for extended periods, potentially lasting months (Neely & Maley, 2000; Kramer *et al.,* 2006).

The survival rates of various pathogens differ, with mycobacteria and Clostridium difficile enduring for months, whereas *Bordetella pertussis*, *Haemophilus influenzae,* and *Vibrio cholerae* survive for just days (Wagenvoort & Penders, 1997; Webster *et al.,* 2000). Particular bacteria, including *Salmonella* and *Escherichia coli*, have been identified as being transmitted from hands to raw, processed, and cooked meals, even in minimal quantities on the fingers (Famurewa & David, 2009; Nworie *et al.,* 2012). Research indicates that microorganisms, upon contact with hands and hard surfaces, establish favorable homes on these surfaces, making them challenging to eradicate (Kissiedu, 2002; Umeh *et al.,* 2007).

In Egypt, as in the rest of the world, and in light of epidemics that may spread at any time, it is necessary to study the presence of pathogenic microbes in one of the most widely used tools for everyone in the streets. Previous research on this topic in Egypt is very little, so the importance of this study appears.

MATERIALS AND METHODS

1. Materials:

The blood agar, MacConkey agar, and LB broth were acquired from Muller-Hinton Agar, while methylated spirit, glycerol, ethanol, and stains including safranin, crystal violet, and Gram iodine were procured. All remaining compounds were of analytical grade.

2. Methodology:

3. Comprehensive Sample Collection:

3.1. Research Area:

This investigation was conducted on the streets of Alexandria, with samples collected at various times throughout the early morning and peak afternoon periods, from diverse locations adjacent to hospitals, urban neighborhoods, and less developed regions.

3.2. Collection and Processing of Samples:

Table (1), illustrates the distribution of the complete sample. Fifty-one swab samples were taken from the Bank's automated teller machines situated on Alexandria streets using sterile cotton swab sticks saturated with sterile distilled water prior to swabbing the ATM buttons. The swab sticks were sent to the laboratory for bacteriological examination within two hours after collection. The bank's ATM machines are located near the laboratory where the investigation was conducted. Bacterial introduction. The inoculation of bacteria included the direct streaking of swab sticks onto blood agar and MacConkey agar on Petri plates, each labeled with the date and sample source code. The streaked samples were incubated for 24 to 48 hours at 37 °C, following which the colonies were examined.

Table 1: Total Samples distribution.

Location of the samples obtained from the ATM	No. of the swabs	Percentage of different swabs included in the study
Next to hospitals	10	40%
Civilized areas	23	27%
Less civilized areas	18	33%
Total	51	100%

3.4. Preparation of Media:

All glassware was cleaned with detergent and allowed to air dry. The glassware was encased in aluminum foil, put in canisters, and subjected to sterilization in a hot air oven at 200°C for one hour. The products were removed from the oven and let to cool after the attainment of sterility. They were retained for storage as required. Work surfaces were decontaminated and disinfected using 70% ethanol swabs. A sterile working environment was attained by the use of a spirit light. The media used (Blood and MacConkey agars) were measured and produced in accordance with the manufacturer's instructions. The prepared medium was meticulously placed in the autoclave and sterilized at 121°C for 15 minutes. Prior to use, the media were chilled to around 45 °C.

3.5. Isolation and Identification of Bacterial Isolates:

Plate growths were detected after 24 to 48 hours of incubation; the isolates were then sub-cultured on new medium plates until pure isolates were attained. The pure culture of isolates was stored in LB glycerol cryovial tubes at -20°C. The isolates were classified according to their morphological characteristics.

3.6. Gram Reaction and Biochemical Characteristics:

3.6.1. Gram Staining Techniques:

A thin smear was prepared by emulsifying a small sample of the organism obtained from an 18–24 hour-old culture of purity into a drop of sterilized water that had been distilled on a grease-free slide. The smear is then air-dried and fixed by heat by briefly passing it over a flame. The slide was meticulously positioned on the staining rack and immersed in primary stain (crystal violet) for 30–60 seconds, after which the smear was gently washed with tap water. Subsequently, Gram (sharp) iodine was applied for 30 seconds, followed by another gentle rinsing with tap water. Seventy percent ethanol was employed as a decolorizer for 10 to 30 seconds, followed by staining with the secondary stain (safranin) for 30 seconds, after which it was washed with tap water and let to dry. The smear was then analyzed under a microscope with an oil immersion lens (100x), revealing Gram-positive bacteria as purple and Gram-negative organisms as red.

3.6.2. Biochemical Characterization of the Isolates:

3.6.2.1. Catalase Test: This assay is used to detect organisms that synthesize the enzyme catalase. This enzyme neutralizes hydrogen peroxide (H_2O_2) by decomposing it into water and oxygen gas. This assay indicates the existence of hydrogen peroxide. A drop of 3% peroxide hydrogen solution was applied to the sterile slide holding a loopful of the organism, which is defined by the enzyme catalase that releases oxygen. Foaming or bubbling signifies a pleasant outcome. (Taylor and Achanzar, 1972).

3.6.2.2. Coagulase Test: Coagulase is a protein that induces the coagulation of blood plasma. This examination is conducted on Gram-positive Staphylococcus species. A drop of distilled water that was sterile was applied to each end of a sterile slide, and a colony of the test organism was emulsified at each location to create thick suspensions. A loop of plasma was incorporated into one of the suspensions and stirred gently. The slide was assessed for aggregation or coagulation of the organism within 10 seconds. This indicates that the bacterium is Staphylococcus aureus, and plasma was not included in the second suspension, which functions as the negative control.

3.6.2.3. Oxidase Test: This assay is used to detect bacteria that possess the enzyme cytochrome oxidase, which is crucial in the electron transport chain. It is often used to differentiate between oxidase-negative Oxidase-positive Enterobacteriaceae Pseudomonadaceae A segment of filter paper was saturated with several drops of oxidase reagent (Tetra methyl-p-phenylenediamine dihydrochloride). A colony of the test organism was then

applied to the saturated filter paper. If the organism produces oxidase, the phenylenediamine in the reagent will oxidize to a deep purple hue. The color change within five seconds signifies a happy outcome. Forbes et al. (1998)

3.6.2.4. Citrate Utilization Test: This test is often used to distinguish organisms that can utilize citrate as a carbon source. Simmons citrate agar media was produced in 10 ml tubes and let to solidify in a slanted orientation. A sterile wire loop was used to inoculate the test organism onto the slant medium, which was then incubated at 37℃ for 24 to 48 hours, following which it was assessed for color change. A vivid blue hue in the medium indicated a positive citrate test. Baron and Finegold (1990).

3.6.2.5. Indole Test: This test identifies microorganisms capable of metabolizing tryptophan into indole. It is used for the identification of bacteria belonging to the family Enterobacteriaceae. Inoculate sterile tubes with 4 ml of tryptophan broth and incubate for 24 to 28 hours. Subsequently, 0.5 ml of Kovac's reagent is introduced. The presence or absence of a red ring signifies a positive or negative test result. (Eyre, 2009).

3.6.2.6. Urease Examination: This identifies organisms capable of hydrolyzing urea (urease-producing bacteria) to generate ammonia and carbon dioxide. It is mostly used to differentiate urease-positive protease from other Enterobacteriaceae. Organisms that rapidly hydrolyze urea, such as Proteus spp., Morganella morganii, and certain strains of Providencia stuartii, will yield strong positive reactions within 1 to 6 hours of incubation. In contrast, delayed positive organisms, including Klebsiella spp. and Enterobacter species, will exhibit weak positive reactions in the slant after 6 hours of incubation, which will intensify with extended incubation. examination (Koneman *et al.,* 1983).

3.6.2.7. Sugar Fermentation Test: The carbohydrate fermentation test assesses the ability of microorganisms to ferment a particular carbohydrate. Carbohydrate fermentation patterns are useful in distinguishing between bacterial groups or species by assessing the presence of acid and/or gas generated by carbohydrate fermentation. A basal medium including a singular carbohydrate source, such as glucose, lactose, sucrose, or another carbohydrate, is used for this purpose. The pH indicator bromothymol blue (BTB) is included in the medium to detect the decrease in pH resulting from acid generation. A little inverted tube known as Durham's tube is also submerged in the liquid to assess gas generation (hydrogen or carbon dioxide). All members of Enterobacteriaceae have yielded a positive test result. (Hugh and Leifson, 1953)

RESULTS

1. Isolation Identification of Bacterial Isolates:

Upon culturing 51 swabs obtained from the ATM on blood agar and MacConkey agar plates, it was noted that all swabs exhibited growth, with the exception of the control and six swabs (1, 3, 18, 22, 25, and 36). The plates mostly exhibited creamy, grey, green, white, yellow, salmon, and beige hues, while their forms were primarily round, flat, round, and irregular. It was observed that many bacterial types were present on the majority of these plates. This was discerned from the morphology and pigmentation of the bacteria that proliferated on those plates. The gram stain test revealed that out of 169 isolates, there was an almost equal distribution between gram-positive and gram-negative classifications, with 37 isolates classified as gram-negative and 132 as gram-positive, including 51 gram-positive bacilli and 81 gram-positive cocci.

Tables 1 and 2, provide the discovered pure cultures, excluding identical bacteria based on morphology, pigmentation, and dimensions. A total of 169 cultures were cultivated on blood and MacConkey agar plates, yielding the results shown in Table 1. The hues of the colonies mostly included milky, gray, green, white, yellow, pink, and beige, with a nearly equal

distribution of irregular and circular shapes, as well as flat forms. The current investigation revealed an intriguing variety of microbial communities isolated from ATM surfaces, which were evident from the diverse growth patterns on blood agar and MacConkey agar plates. Among the 51 swabs, 45 yielded viable microbial colonies, indicating the presence of microorganisms on these high-contact surfaces. The morphological analysis indicated a broad spectrum of colony characteristics, with colors ranging from creamy, grey, green, white, yellow, salmon, and beige, alongside various colony shapes such as circular, irregular, and flat.

The distribution of isolates revealed a nearly balanced presence of gram-positive and gram-negative bacteria. Specifically, out of 169 isolates, 132 were gram-positive, with a predominance of cocci (81 isolates), followed by bacilli (51 isolates), while gram-negative bacilli accounted for 37 isolates. This observation is notable as gram-positive organisms, particularly cocci in clusters or chains, are commonly associated with the human microbiota, potentially representing opportunistic pathogens transferred from users' hands to ATM surfaces. The diversity in colony morphology and pigmentation highlights the richness of the microbial population present. For instance, colonies exhibiting metallic sheen, beta-hemolysis on blood agar, and those with distinctive colors such as green or pink are indicative of potentially pathogenic species. Notably, the observation of irregularly shaped bacilli colonies exhibiting swarming motility could suggest the presence of Proteus spp., a group well-known for their environmental adaptability and opportunistic infections.

The gram-positive cocci were further characterized using the catalase test, which differentiated staphylococci from streptococci. This distinction is clinically significant as staphylococci, particularly *Staphylococcus aureus*, are frequent culprits in skin and soft tissue infections, while streptococci are associated with respiratory tract infections and systemic conditions like bacteremia. The presence of a high proportion of gram-positive organisms suggests that these microorganisms might have originated from human skin, emphasizing the role of ATMs as a potential reservoir for microbial transmission. Meanwhile, the identification of gram-negative bacilli, such as *Escherichia coli* and *Pseudomonas aeruginosa*, both of which are commonly associated with nosocomial infections, further underscores the risk posed by contaminated surfaces in public spaces.

Previous studies (Famurewa & David, 2009; Umeh *et al.*, 2007; Nworie *et al.*, 2012) have shown that surfaces in public places are often contaminated with a variety of bacteria, including pathogenic species capable of causing infections. Factors such as inadequate cleaning protocols, high human traffic, and the diverse range of activities conducted in these areas contribute to the persistence of bacterial contaminants. These bacteria can survive on surfaces for extended periods, increasing the likelihood of transmission to individuals who come into contact with them._High-touch surfaces, such as door handles, elevator buttons, handrails, and electronic interfaces, are particularly prone to bacterial contamination. For instance, ATMs, which are used by numerous individuals daily, can harbor a wide array of bacteria, including those from human skin, respiratory secretions, and environmental sources. The presence of bacteria on these surfaces can be indicative of poor hygiene practices and insufficient cleaning measures.

Table 1. Identified pure cultures with the exclusion of similar bacteria in terms of shape, color and size.

Isolate code	Growth on blood agar	Growth on MacConkey Agar	Color (Respectively)	form	Isolate Number	Gram	Shape	Isolate code	Growth on blood agar	Growth on Mac Conkey Agar	Color (Respectively)	form	Isolate Number	Gram	Shape
2A	+	-	White	Circular	2A	+ve	***Cocci*** , Grape Like Clusters	11A	+	-	Yellow	Circular	11A	+ve	**Cocci**,Grape Like Clusters
2B	+ß hemolysis	-	Dark yellow	Circular	2B	+ve	***Cocci*** , Clusters	11B		+	Gray green metallic coloreless	+	11B	-ve	Bacilli,Rods
2C	+	-	Brown	irregular	2C	+ve	***Bacilli***, Rods	11C		-	Gray	+	11C	+ve	**Bacilli**,Rods
4A	+ ß hemolysis	-	Yellow	Circular	4A	+ve	***Cocci***, Clusters	11D	+	+	Small gray, Dark pink	irregular	11D	-ve	Bacilli, Rods
4B	+	+	Creamy Dark pink	irregular	4B	-ve	*Bacilli*, Rods	12A	+	-	Green	irregular	12A	+ve	**Bacilli**, Rods
4C	+	-	Simon	irregular	4C	+ve	***Bacilli***,Rods	12B	+	-	Simon	irregular	12B	+ve	**Bacilli**, Rods
4D	+	-	Green	irregular	4D	+ve	**Bacilli**, Rods	12C	+	-	White	Circular	12C	+ve	**Cocci**, Grape Like Clusters
5A	+	-	Gray green	irregular	5A	+ve	**Bacilli**, Rods	12D	+	-	Creamy	Circular	12D	+ve	**Cocci**, Grape Like Clusters
5B	+	-	Green	irregular	5B	+ve	**Bacilli**, Rods	13A	+	-	Gray	irregular	13A	+ve	**Bacilli**, Rods
5C	+	-	Dark gray	Circular	5C	+ve	**Cocci**, Short Chains	13B	+	-	creamy	Circular	13B	+ve	**Cocci**, Grape Like Clusters
5D	+	-	Gray	irregular	5D	+ve	**Bacilli**, Rods	13C	+	+	Gray ,pale	irregular swarming in blood	13C	-ve	Bacilli, Rods
5E	+	-	White, Pink	Circular	5E	-ve	Bacilli, Short rods	13D	+	+	Light gray	irregular	13D	-ve	Bacilli, Rods
6A	+	-	Green	Circular	6A	+ve	**Cocci**, Chains	14A	+	-	Simon	irregular	14A	+ve	**Bacilli**, Rods
6B	+	-	Green	irregular	6B	+ve	**Bacilli**, Rods	14B	+	-	Green	irregular	14B	+ve	**Bacilli**, Rods
6C	+	-	Gray	irregular	6C	+ve	**Bacilli**, Rods	14C	+	-	Dark green	irregular	14C	+ve	**Bacilli**, Rods
6D	+	-	White	Circular	6D	+ve	**Cocci**, Grape Like Clusters	14D	+	-	White	Circular	14D	+ve	**Cocci**, Grape Like Clusters
6E	+	-	Simon	irregular	6E	+ve	**Bacilli**, Rods	15A	ß hemolysis+	-	Dark yellow	Circular	15A	+ve	**Cocci**, Clusters
6F	+	-	Dark White	Circular	6F	+ve	**Cocci**, Grape Like Clusters	15B	+	+	Green colorless	irregular	15B	-ve	Bacilli, Rods
6G	+ß hemolysis	-	Yellow	Circular	6H	+ve	**Cocci**, Clusters	15C	+	-	Green	irregular	15C	+ve	**Bacilli**, Rods
6H	+	-	Creamy	Circular	6G	+ve	**Cocci**, Grape Like Clusters	15D	+	+	Dark gray Dark pink	Circular	15D	-ve	Bacilli, Rods
6I	+	-	Light yellow	Circular	6I	+ve	**Cocci**, Grape Like Clusters	15E	+	-	Creamy	Circular	15E	+ve	**Cocci**, Grape Like Clusters
7A	+	-	Green	irregular	7A	+ve	**Bacilli**, Rods	15F	+	-	Large gray	Circular	15F	+ve	**Cocci**, Grape Like Clusters
7B	+	-	Light gray	Circular	7B	+ve	**Cocci**,Grape Like Clusters	16A	+	+	Large gray Pale	Irregular swarmin in blood	16A	-ve	Bacilli, Rods
7C	+	+	White, Pink	Circular	7C	-ve	Bacilli, Short rods	16B	+	-	Small gray	Circular	16B	+ve	**Cocci**,Short Chains
8A	+ß hemolysis	-	golden yellow	Circular	8A	+ve	**Cocci**,Clusters	16C	ß hemolysis +	-	Golden yellow	Circular	16C	+ve	**Cocci**,Clusters
8B	+	-	Beige	irregular	8B	+ve	**Bacilli**,Rods	16D	+	-	Green	irregular	16D	+ve	**Bacilli**,Rods
8C	+	+	Gray,Dark pink	Circular	8C	-ve	Bacilli, Rods	16E	+	-	White	Circular	16E	+ve	**Cocci**, Grape Like Clusters
8D	+	-	Green	irregular	8D	+ve	**Bacilli**,Rods	17A	+	-	Green		17A	+ve	**Bacilli**, Rods
9A	+ß hemolysis	-	Yellow	Circular	9A	+ve	**Cocci**,Clusters	17B	+	+	Gray Dark pink	Circular	17B	-ve	Bacilli, Rods
9B	+	-	Dark green	irregular	9B	+ve	**Bacilli**,Rods	17C	ß hemolysis+	-	Beige	Circular	17C	+ve	**Cocci**, Clusters
9C	+	-	creamy	Circular	9C	+ve	**Cocci**,Grape Like Clusters	19A	+	-	Creamy	Circular	19A	+ve	**Cocci**, Grape Like Clusters
9D	+	-	Light yellow	Circular	9D	+ve	**Cocci**,Grape Like Clusters	19B	+	-	Gray	Circular	19B	+ve	**Cocci**, Short Chains
20A	+	+	Gray Dark pink	Circular	20A	-ve	Bacilli, Rods		+	+	Gray, Dark Pink	Circular	37B	-ve	Bacilli, Rods
20B	+	+	White, pink	Circular	20B	-ve	Bacilli, Short rods	37C	+	-	green	irregular	37C	+ve	Bacilli, Rods
20C	+	-	White	Circular	20C	+ve	Cocci, Grape Like Clusters	38A	+	+	Metallic colorless	irregular	38A	+ve	Cocci, Grape Like Clusters
20D	+	-	green	irregular	20D	+ve	Bacilli, Rods	39A	+	-	Large creamy	irregular	39A	-ve	Bacilli, Rods
21A	+	-	Gray	irregular	21A	+ve	Bacilli, Rods	39B	+	-	green	irregular	39B	+ve	Bacilli, Rods
21B	+	-	Simon	Circular	21B	+ve	Cocci, Grape Like Clusters	39C	+	-	yellow	irregular	39C	+ve	Bacilli, Rods
21C	+	-	Green	irregular	21C	+ve	Bacilli, Rods	40A	+	-	green	irregular	40A	-ve	Bacilli, Short rods
21D	ß hemolysis+	-	Yellow	Circular	21D	+ve	Cocci, Clusters	40B	+	-	green	irregular	40B	+ve	Cocci, Grape Like Clusters
23A	+	+	Gray, Dark pink	Circular	23A	-ve	Bacilli, Rods	40C	+	+	Brown colorless	irregular	40C	+ve	Bacilli, Rods
23B	ß hemolysis+	-	Light Yellow	Circular	23B	+ve	Cocci, Clusters	41A	+	-	white	Circular	41A	+ve	Bacilli, Rods
24A	+	-	Creamy	Circular	24A	+ve	Cocci, Grape Like Clusters	41B	+	-	green	irregular	41B	-ve	Bacilli, Rods
24B	+	+	Silver Gray colorless	irregular	24B	-ve	Bacilli, Rods	42A	+	-	Brown	irregular	42A	-ve	Bacilli, Rods
24C	+	-	White	Circular	24C	+ve	Cocci, Grape Like Clusters	42B	+	-	Creamy	Circular	42B	+ve	Cocci, Grape Like Clusters
26A	+	-	Green	irregular	26A	+ve	Bacilli, Rods	42C	ß hemolysis+	-	yellow	Circular	42C	+ve	Bacilli, Rods
26B	+	-	Beige	irregular	26B	+ve	Bacilli, Rods	42D	+	-	white	Circular	42D	+ve	Bacilli, Rods
26C	+	-	white	Circular	26C	+ve	Cocci, Grape Like Clusters	43A	+	-	white	Circular	43A	+ve	Cocci, Grape Like Clusters
27A	+	-	Simon	irregular	27A	+ve	Bacilli, Rods	43B	+	+	Gray, dark pink	Circular	43B	+ve	Cocci, Clusters
28A	+	-	Green	irregular	28A	+ve	Bacilli, Rods	43C	ß hemolysis+	-	Light yellow	Circular	43C	+ve	Cocci, Grape Like Clusters
28B	ß hemolysis +	-	Dark creamy	Circular	28B	+ve	Cocci, Clusters	44A	+	-	Dark brown	irregular	44A	+ve	Cocci, Grape Like Clusters
29A	+	+	Gray, Dark pink	Circular	29A	-ve	Bacilli, Rods	44B	+	-	Small white	Circular	44B	-ve	Bacilli, Rods
29B	+	-	White	Circular	29B	+ve	Cocci, Grape Like Clusters	44C	+	+	Large white	Circular	44C	+ve	CocciClusters
29C	+	-	green	Circular	29C	+ve	Cocci, Chains	44D	+	-	creamy	Circular	44D	+ve	Bacilli, Rods
30A	+	-	green	irregular	30A	+ve	Bacilli, Rods	45A	+	-	white	Circular	45A	+ve	Cocci, Grape Like Clusters
30B	ß hemolysis +	-	yellow	Circular	30B	+ve	Cocci, Clusters	45B	+	-	creamy	Circular	45B	-ve	Bacilli, Short rods
30C	+	+	Brown Colorless	irregular	30C	-ve	Bacilli, Rods	45C	+	-	Dark creamy	Circular	45C	+ve	Cocci, Grape Like Clusters
31A	+	-	White	Circular	31A	+ve	Cocci, Grape Like Clusters	46C	+	-	beige	Circular	46C	+ve	**Cocci**, Grape Like Clusters
31B	+	-	Creamy	Circular	31B	+ve	Cocci, Grape Like Clusters	47A	+	+	Gray, Dark pink	Circular	47A	-ve	Bacilli, Rods
31C	+	+	White, Pink	Circular	31C	-ve	Bacili, Short rods	47B	+	+	Dark green colorless	irregular	47B	+ve	**Cocci**, Grape Like Clusters
31D	+	-	Light gray	Circular	31D	+ve	Cocci, Grape Like Clusters	47C	+	+	white pink	Circular	47C	+ve	**Cocci**, Grape Like Clusters
32A	+	+	Gray, Dark Pink	Circular	32A	-ve	Bacilli, Rods	48A	+	+	Gray, Dark pink	Circular	48A	-ve	Bacilli, Rods
32B	+	-	White	Circular	32B	+ve	Cocci, Grape Like Clusters	48B	+	-	Green	irregular	48B	-ve	Bacilli, Rods
32C	+	-	Brown	irregular	32C	+ve	Bacilli, Rods	49A	+	+	Green metallic	irregular	49A	-ve	Bacilli, Short rods
33A	+	-	Creamy	Circular	33A	+ve	Cocci, Grape Like Clusters	49B	+	-	Small white	Circular	49B	-ve	Bacilli, Rods
33B	+	+	Gray Pale	Irregular swarming in blood	33B	-ve	Bacilli, Rods	49C	+	-	Small gray	Circular	49C	+ve	**Bacilli**, Rods
33C	+	-	Dark green	irregular	33C	+ve	Bacilli, Rods	49D	+	-	Small green	Circular	49D	-ve	Bacilli, Rods
34A	+	-	white	Circular	34A	+ve	Cocci, Grape Like Clusters	49E	+	+	creamy	Circular	49E	+ve	**Cocci**, Grape Like Clusters
34B	ß hemolysis+	-	Light yellow	Circular	34B	+ve	Cocci, Clusters	49F	+	+	Gray, Dark pink	Circular	49F	+ve	**Cocci**, Grape Like Clusters
34C	+	+	Green colorless	irregular	34C	-ve	Bacilli, Rods	49G	+	-	white	Circular	49G	+ve	**Cocci**, Chains
34D	+	+	Small creamy	Circular	34D	+ve	Cocci, Grape Like Clusters	50A	+	-	Light yellow	Circular	50A	+ve	***Cocci***, Grape Like Clusters
34E	+	-	gray	irregular	34E	+ve	Bacilli, Rods	50B	hemolysis+	-	Dark yellow	Circular	50B	-ve	*Bacilli*, Rods
35A	+	-	green	irregular	35A	+ve	Bacilli, Rods	50C	+	+	Gray pale	Irregular swarming in blood	50C	+ve	***Cocci***, Grape Like Clusters
35B	+	-	Golden yellow	Circular	35B	+ve	Cocci, Clusters	50D	+	-	Green	irregular	50D	+ve	***Cocci***, Grape Like Clusters
35C	+	-	Simon	irregular	35C	+ve	Bacilli,Rods	51A	+	-	beige	Circular	51A	+ve	***Cocci***, Clusters
35D	+	-	White	Circular	35D	+ve	Cocci,Grape Like Clusters	51B	+	-	white	Circular	51B	-ve	*Bacilli*, Rods
37A	+	-	white	Circular	37A	+ve	Cocci,Grape Like Clusters								

Additionally, biochemical assays were performed on 37 gram-negative isolates to differentiate between the microorganisms (Table 2). Sixteen isolates were identified as *E. coli*. Seven isolates are *Klebsiella*, five isolates are *Proteus*, and nine isolates are *Pseudomonas*. This study provides a foundation for further research on the potential health implications and the development of strategies to mitigate microbial contamination in high-contact public areas. Due to their adaptability to various climatic circumstances, they may proliferate on several surfaces (Kramer *et al.,* 2006; Agu, 2018; Aquino, 2021). The majority of bacteria constitute normal flora and do not induce significant sickness in humans; nonetheless, some strains may possess medicinal significance (Saroja *et al.,* 2013). Bacteria may survive or proliferate on many surfaces, such as computer keyboards, door handles, mobile phones, and elevator buttons (Tekerekoğlu *et al.,* 2011). Fomites represent a worldwide issue in the dissemination of environmental microorganisms. Microorganisms recovered from the hands are classified as either temporary or resident. The hands are regarded as a possible reservoir for bacteria that contribute to the transmission of infections and illnesses. Manual transmission facilitates the spread of nosocomial diseases. They are also significant in the context of foodborne infections (Tekerekoğlu *et al.,* 2013; Aquino *et al.,* 2019). Numerous studies have shown that polluted surfaces significantly contribute to the transmission of infectious illnesses (Sepehri *et al.,* 2009; Dawodu & Akanbi, 2021). Recent study indicates that the transmission of bacteria is influenced by several parameters, including surface properties, bacterial species, moisture levels, and the inoculum size (Nagajothi *et al.,* 2015). Numerous financial services are already provided via ATMs, and individuals like using ATMs to save time. Consequently, the use of ATMs is vital. Notwithstanding the prevalence of ATMs, they are mostly located in retail malls, healthcare facilities, and urban areas (Nwankwo & Offiah 2016; Monteiro *et al.,* 2021). The point of contact is the customer's hands on the surfaces of the keypad or screen of these gadgets (Tekerekoğlu *et al.,* 2011; Adedoyin, 2019). Daily, many individuals with varying health, social, and economic circumstances use ATMs. Bacteria including Bacillus spp., negative *Staphylococci*, *Staphylococcus aureus*, and *Escherichia coli* have been recovered from ATMs, indicating their potential for colonization by human diseases (Tekerekoğlu *et al.,* 2013; Abdulaziz, 2019).

Bacterial contamination in public areas presents a considerable public health issue due to the prevalent and enduring presence of many microbial species on commonly touched surfaces. Public spaces, including transit hubs, retail malls, gyms, restaurants, and ATMs, are prone to microbial buildup owing to significant human circulation. The facilitation of bacterial transmission in certain circumstances may result in the proliferation of infectious illnesses, adversely affecting population health on a significant scale.

Table 2. Biochemical tests of the 37 gram-negative isolates.

Isolate code	Catalase	Sugar fermention	citrate	Indole	Coagulase	Urease	Oxidase	Organism
4B	+	+	-	+	-	-	-	*E. coli*
8C	+	+	-	+	-	-	-	*E. coli*
11D	+	+	-	+	-	-	-	*E. coli*
13D	+	+	-	+	-	-	-	*E. coli*
15D	+	+	-	+	-	-	-	*E. coli*
17B	+	+	-	+	-	-	-	*E. coli*
20A	+	+	-	+	-	-	-	*E. coli*
23A	+	+	-	+	-	-	-	*E. coli*
29A	+	+	-	+	-	-	-	*E. coli*
32A	+	+	-	+	-	-	-	*E. coli*
37B	+	+	-	+	-	-	-	*E. coli*
42A	+	+	-	+	-	-	-	*E. coli*
44B	+	+	-	+	-	-	-	*E. coli*
48A	+	+	-	+	-	-	-	*E. coli*
49B	+	+	-	+	-	-	-	*E. coli*
50B	+	+	-	+	-	-	-	*E. coli*
5E	+	+	+	-	-	+	-	*Klebsiella*
7C	+	+	+	-	-	+	-	*Klebsiella*
20B	+	+	+	-	-	+	-	*Klebsiella*
31C	+	+	+	-	-	+	-	*Klebsiella*
40A	+	+	+	-	-	+	-	*Klebsiella*
45B	+	+	+	-	-	+	-	*Klebsiella*
49A	+	+	+	-	-	+	-	*Klebsiella*
13C	+	+	+	-	-	+	-	*Proteus*
16A	+	+	+	-	-	+	-	*Proteus*
33B	+	+	+	-	-	+	-	*Proteus*
47A	+	+	+	-	-	+	-	*Proteus*
51B	+	+	+	-	-	+	-	*Proteus*
11B	+	-	+	-	-	-	+	*Pseudomonas*
15B	+	-	+	-	-	-	+	*Pseudomonas*
24B	+	-	+	-	-	-	+	*Pseudomonas*
30C	+	-	+	-	-	-	+	*Pseudomonas*
34C	+	-	+	-	-	-	+	*Pseudomonas*
38A	+	-	+	-	-	-	+	*Pseudomonas*
41B	+	-	+	-	-	-	+	*Pseudomonas*
48B	+	-	+	-	-	-	+	*Pseudomonas*
49D	+	-	+	-	-	-	+	*Pseudomonas*

Conclusion:

Automated teller machines harbor a significant quantity of pathogenic and infectious germs. This investigation highlights the diverse bacterial communities present on ATM surfaces, with 169 isolates displaying a range of morphologies and Gram characteristics. The dominance of gram-positive cocci, identified through catalase testing, points toward the presence of *Staphylococcus* species among others. The findings underscore the significant potential for bacterial spread in public environments, reinforcing the need for hygiene awareness in shared spaces.

Recommendations:

The wide range of bacterial species identified from ATMs, coupled with the significant presence of both gram-positive and gram-negative isolates, highlights the need for regular sanitation of these devices. Furthermore, the findings

emphasize the importance of public hygiene measures to minimize the risk of disease transmission from these frequently used surfaces.

Declarations:

Ethical Approval: Not applicable.

Conflicts of Interest: The author declares no conflicts of interest.

Authors Contributions: All authors contributed towards the study design, experiment execution, data analysis, and manuscript drafting.

Funding: No funding was received.

Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

REFERENCES

Abdulaziz JM.(2019): Determination of bacterial contamination, occurrence and antimicrobial susceptibility of Staphylococcus aureus on commercial banks Automated Teller Machines (ATMs) in Kaduna Metropolis, Kaduna State, Northwestern Nigeria .*EC Veterinary Science;* 4:161–74.

Adedoyin A.(2019): A Study investigating bacterial colonization on Automated Teller Machines in Ibadan Metropolis, South-West Nigeria.*ACTA Pharmaceutica Sciencia ;*3:119–32

Aquino S, de Lima JE, da Silva MO, de Sousa GF.(2019): Detection of pathogenic bacteria and fungi on biometric surface of Automated Teller Machines located in Brazilian public hospital. *African Journal of Microbiology Research;*13:219–31

Baron, E. J. and Finegold, S. M. (1990): General microbiology; Baily and scotts Diagnostic Microbiology.8th. Ed. Mosby Co. St. Louis. Balitimore.

Chessbrough, M. (2000): Biochemical test to identify bacteria. In Chessbrough, M. (ed). Laboratory Practice in Tropical Countries, Vol. 2:63-70. Cambridge University Press, UK.

Dawodu OG, Akanbi RB.(2021): Isolation and identification of microorganisms associated with automated teller machines on Federal Polytechnic Ede campus PLoS One. ;16:e0254658.hones in teaching hospitals, Kerman, Iran. Ameriecan Journal of Applied Sciences. 2009;6:806.

El-Dars FMS Hassan WMH. (2005): A preliminary bacterial study of Egyptian paper money. *International Journal of Environmental Health Research,* 15:235–239. https://doi.org/10. 1080/ 09603120500105976 PMID : 16134486

Eyre, J. W. H. (EDIN.) (2009): The Project Gutenberg eBook of The Elements of Bacteriological Technique, A Laboratory Guide for Medical, Dental, and Technical Students. Second Edition Rewritten and Enlarged. eBook #27713 ISO-8859-1.

Famurewa O, David OM. (2009) Cell Phone: A medium of transmission of bacterial pathogens. *World Rural Observation*; 1:69–72.

Forbes, B. A.; Sahm, D. F. and Weissfeld, A. S. (1998): Bailey and Scott's Diagnostic Microbiology, 10th edition. Mosby Inc., St. Missouri, USA.

Hugh, R., and E. Leifson. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative rods. *Journal of Bacteriology,* 66:24-26.

Kissiedu KO.(2002): Evaluation of currency note as agents of microbial cross contamination. BSc. Thesis, Dissertation, University of Ghana, Legon, Ghana.

Koneman, E. W.; Allen, S. D.; Dowell, J. r.; V. R. and Somers, H. M. (1983): Color Atlas and Textbook of Diagnostic Microbiology. Second Ed. J. B. Lippincott Co., N.Y., PP. 72-73.

Kramer A, Schwebke I, Kampf G.(2006): How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. (BMC) *Biomed Center Infect Diseases*, 6:130. https://doi.org/ 10.1186/1471-2334-6-130PMID: 16914034

Micheals B.(2002): Handling Money and serving ready to eat Food. *Food Serving Technology*, 2:1–3.

Monteiro A, Almeida B, Paciência I, Cavaleiro Rufo J, Ribeiro E, Carolino E, et al.(2021): Bacterial contamination in health care centers: Differences between urban and rural settings. *Atmosphere*,12:450.

Nagajothi J, Jeyakumari D, Vigneshwaran S, Kumar RP, Bharatwaj R, Bagyalakshmi R.(2015): Study of prevalence of microbial contamination with its antibiotic resistance pattern in automated teller machine in and around Puducherry,*Journal* of *Earth, Environment* and *Health Sciences*, 1:27.

Neely AN, Maley MP.(2000): Survival of Enterococci and Staphylococci on hospital fabrics and plastic. *Journal of Clinical Microbiology*, 38:724–726. https://doi.org/10. 1128/JCM.38.2.724-726. 2000 PMID:10655374

Nwankwo E, Offiah J.(2016): Bacterial contamination of user interface of Automated Teller Machines (ATM) of various banks in Umuahia Metropolis, AbiaState, *Nigeria International Journal of Tropical Disease Health*, 13:1–9

Nworie O, Mercy M, Chukwudi A, Oko I, Chukwudum SO, Agah VM, et al. Antibiogram of bacteria isolated from automated teller machines within Abakaliki metropolis. *American Journal of Infectious Diseases,* 2012 Oct 1; 8(4):168

Ozkan VK.(2016): Determination of Microfungal Contamination on Automated Teller Machines and Bank Cards in Marmaris, Turkey. *Journal of Pharmaceutical, Chemical and Biological Sciences,* 3(4):528–53

Saroja V, Kamatchiammal S, Brinda K, Anbazhagi S.(2013) Enumeration and characterisation of coliforms from Automated Teller Machine (ATM) centers in urban areas. *Journal* of Modern Agriculture and *Biotechnology,* 2:14–22

Sepehri G, Talebizadeh N, Mirzazadeh A, Mir-shekari TR, Sepehri E.(2009); Bacterial contamination and resistance to commonly used antimicrobials of healthcare workers' mobile in teaching hospitals, Kerman, *Iran Journal of Applied Animal Science*, 6:806.

Taylor, W.; I. and Achanzar, D. (1972). Catalase Test as an Aidn to the Identification of Enterobacteriaceae. *Applied Microbiology*, 24(1), 58–61.

Taylor MW, Tsai P, Simister RL, Deines P, Botte E, Ericson G, et al.(2013 Feb.) :'Sponge-specific'bacteria are widespread (but rare) in diverse marine environments. *The (ISME)International Society For Microbbial Ecology Journal,* 7(2): 438–43. https:// doi.org/10.1038/ ismej.2012.111 PMID: 23038173

Tekerekoğlu MS, Duman Y, Serindağ A, Cuğlan SS, Kaysadu H, Tunc E, et al.(2011): Do mobile phones of patients, companions and visitors carry multidrug-resistant hospital pathogens? *Ameriecan Journal Infection Control*, 39:379–81

Tekerekoğlu MS, Yakupogullari Y, Otlu B, Duman Y, Gucluer N.(2013):

Bacteria found on banks' Automated Teller Machines (ATMs) *African Journal of Microbiology Research*, 7:1619–21

Umeh EU, Juluku JU, Ichor T.(2007): Microbial Contamination of 'Naira' Nigerian Currency Notes in Circulation. *Research Journal of Environmental Sciences*, 1(6):336–339.

Wagenvoort JHT, Penders RJR(1997): Long-term in-vitro survival of an epidemic MRSA phage-group III-29 strain. *Journal of Hospital Infection*, 35:322–325.

Webster C, Towner KJ, Humphreys H(2000): Survival of Acinetobacter on three clinically related inanimate surfaces. *Infection Control and Hospital Epidemiology*, 21:246–30 https://doi.org/10.1086/503214 PMID: 10782579