Macedonian Journal of Ecology and Environment
Vol. 22, Issue 2
pp. 139-151
Skopje (2020)
ISSN 1857 - 8330 (online)
Original scientific paper
Available online at www.mjee.org.mk

Seasonal variation in microbial profile of Automated Teller Machines (ATMs) in and around University of Port Harcourt, Choba, Nigeria

Сезонски варирања во микробниот профил кај банкомати (АТМ) во и околу Универзитетот Порт Харкорт, Чоба, Нигерија

Helen Amarachi Odoemelam*, Ogugbue Jason Chimezie & Chiaka Aniebo-Mbakwem

Department of Microbiology, Faculty of Science, University of Port Harcourt, Choba, Port Harcourt, Rivers State, Nigeria

Abstract

The Automated Teller Machine (ATMs) has been widely and publicly used for transactions by bank customers. The individuals that use this machine come from different homes and background, and there is every likelihood that some of them are carriers of diverse microorganisms. The objective of the present study was to investigate the seasonal variation in the microbial profile of the contact surface of ATMs in and around University of Port Harcourt, Choba, Nigeria. The samples were collected during the dry and rainy seasons. The sampling was done three times in a day; Morning 8–9 am, afternoon 1–2 pm, evening 5–6 pm, from different ATMs using moistened sterile swab sticks. The study recorded that during rainy and dry seasons, the total heterotrophic bacterial count ranged from $\log_{10}$ 4.08 to 9.56 CFU/m^2 and $\log_{10}$ 3.08 to 7.80 CFU/m^2 respectively. The total fungal counts in rainy and dry seasons were $\log_{10}$ 3.38 to 7.52 CFU/m^2, $\log_{10}$ 3.08 to 6.48 CFU/m^2 respectively. The frequency of occurrence of fungi during rainy and dry seasons were 30% and 28% respectively. The 16s RNA sequence analysis was carried out on the bacterial and fungal isolates. The different samples (A, B, C, D, E, F, G, H, I, J) showed significant differences ($p<0.05$) in the number of microorganisms between different collection periods, except for total fungal count for sample C during the dry season and sample D during the rainy season. Further findings showed that *Bacillus cereus* had the highest occurrence of 10 (15.9%) and 9(17%) respectively in the rainy and dry seasons, while *Proteus mirabilis* and *Comamonas aquatica* had the lowest occurrence 2(2.63%), 2(2.63%) respectively in the rainy season. *Escherichia coli* and *Comamonas aquatica* were not isolated during the dry season. *Aspergillus niger* had the highest frequency of occurrence 8(27%) in both seasons, *Kodamaea ohmeri* had the lowest occurrence in both seasons. This study highlights the need for banks to take preventive measures against spread of infectious diseases through ATMs by regularly cleaning the ATMs surfaces with disinfectant and for users of ATM to always wash their hands after using the machine.

Key Words: Automated Teller Machine; Microbial Profile; Bacteria; Fungi

Submitted: 02.10.2020
Accepted: 07.12.2020

* Author for correspondence: amarachi.odoemelam@uniport.edu.ng

Апстракт

Банкоматите се уреди кои нашироко се користат при банкарските трансакции. Лицата кои ги користат нивните услуги доаѓаат од различни средини, па се очекува да тие се носители на различни микроорганизми. Целта на оваа студија беше да се истражи сезонското варирање во микробниот профил кој се јавува на контактниот дел од банкоматите лоцирани во и околу Универзитетот Порт Харкорт, Чоба, во Нигерија. Беа колекционирани примероци за време на сушните и дождливите периоди од годината. Примероците беа земани од различни банкомати со стерилен брис три пати на ден; наутро помеѓу 8-9 часот, на пладне, помеѓу 1-2 часот и навечер, околу 5-6 часот. Истражувањата покажаа дека за време на дождливите и сушните периоди вкупниот број на хетеротрофни бактерии варира од $\log_{10}$ 4.08 до 9.56 CFU/m^2 и од $\log_{10}$ 3.08 до 7.80 CFU/m^2, соодветно. Бројот на вкупни фунги за време на дождливи и сушни периоди се движеше од $\log_{10}$ 3.38 до 7.52 CFU/m^2, односно од $\log_{10}$ 3.08 до 6.48 CFU/m^2, соодветно, додека фреквенцијата на појавување на фунги за време на дождливи и сушни периоди беше 30% и 28%. Исто така, беше направено и 16s RNA секвенционирање на изолираните бактерии и фунги. Различните примероци (A, B, C, D, E, F, G, H, I, J) покажуваат сигнификантни разлики ($p<0.05$) во бројот на микроорганизми помеѓу различните периоди на колекционирање, освен кај вкупниот број на фунги за примерок C за време на сушниот период, и за примерок D за време на дождливиот период. Понатамошните истражувања покажаа дека *Bacillus cereus* покажува најголем степен на јавување, 10 (15.9%) и 9 (17%) соодветно во дождлива и сушна сезона, додека *Proteus mirabilis* и *Comamonas aquatica* покажуваат најмал степен на јавување, 2 (2.63%) и 2 (2.63%), соодветно, во дождлива сезона. *Escherichia coli* и *Comamonas aquatica* не беа изолирани за време на сушната сезона. *Aspergillus niger* покажа најголема фреквенција на јавување, 8 (27%) и во двете сезони, додека *Kodamaea ohmeri* покажа најмала фрекфенција и во двете сезони. Оваа студија укажува на тоа дека е потребно банките да преземат превентивни мерки и редовна дезинфекција на површините на банкоматите со цел да се спречи ширењето на инфективни заболувања преку користење на истите, како и на потребата од редовно миење на раце по користење на машината.

Клучни зборови: банкомат, микробен профил, бактерии, габи

Introduction

The world over the years has witnessed an increase in electronic gadgets and resources produced to aid people in every aspect of human facets. These inventions have virtually impacted and revolutionized every aspect of endeavor on the planet Earth. In science, agriculture, medicine and banking sectors, many sophisticated machines have been developed and deployed such as computers, mobile phones, automated teller machines, etc. The automatic teller machine (ATM) is known as "a computerized telecommunications device that enables clients of a financial institution to carry out financial transactions without the help of a cashier, human clerk or bank teller" (Veerappan et al. 2013). For many years, ATM has been widely and publicly used for transactions by bank customers (Hone et al. 1998; Okoro et al. 2018; Aquino et al. 2019), and there are millions of units of ATMs in use all over the world. The individuals that use this machine come from different homes and background, and there is every likelihood that some of them are carriers of diverse microorganisms.

Operating the ATM involves slotting in a card into a recipient hole and subsequently following instructions on the screen by punching the keys of the metallic keypads to input own secret codes and command (Stanley & Kayode 2014). The inherent danger in punching of these metallic keypads becomes imminent when microbes have colonized the surface area of the machine, especially where there is no adequate cleaning maintenance to safeguard the facilities. Though this computerized telecommunication device makes it easy and handy for clients of the bank to transact business without being in contact with bank staff, it may portend a lot of hidden dangers. Neely & Sittig (2002) had stated that individuals who are immunosuppressed or immunocompromised, arising from any number of circumstances will be more susceptible

to infections when they contact pathogens. Since there are no limitations and restrictions to the usage of this machine, the possibility of horizontal transmission of dangerous microbes from person to person is high. However, many factors such as destination surface features, bacterial species involved, moisture levels, pressure and friction between the contact surfaces and the inoculum size on surfaces may determine the level of bacterial transmissions between surfaces (Stanley & Kayode 2014). Also, users of ATMs normally punch the keyboards using their hands, and the hand has been shown to play a role in the transmission of microorganisms (Onuoha & Fatokun 2014).

Healthcare-associated infections are a major concern to clinicians and the general public. These organisms that cause infections usually live and grow in a cold and damp environment, which ATM centres are such. These centres are being used by everybody whether healthy or sick which exposes the ATM as a potential medium of spreading pathogens that cause sicknesses.

Stephen & Kwaku (2011) showed indication of possible "cross-contamination of the fingers during usage of the machines with foodborne pathogens such as species of *Aeromonas*, *Bacillus, Enterobacter, Klebsiella* and *Salmonella*". Also, on the horizon of Nigeria, Okoro et al. (2018) revealed that there is a significant relationship between the isolated pathogenic bacteria and the ATMs. The study documented *Escherichia coli, Pseudomonas aeruginosa, Shigella dysenteriae, Salmonella typhimurium, S. aureus* and *Klebsiella pneumoniae* pathogens were isolated from seven (7) different banks. A recent study by Aquino et al. (2019) on the biometric surface of ATMs located in Brazilian public hospital, evaluated forty-two ATMs, in two hospital areas (A and B) in São Paulo city for the presence of pathogenic fungi and bacteria. The study found that the biometric surfaces in ATMs are an important environmental source of microbes, that the genera *Staphylococcus* was predominant in all agencies of both hospital areas (83.3%), following of *Streptococcus* spp. (57%) and *Enterococcus* spp. (50%). Also, the group of Enterobacteriaceae (Gram-negative bacilli) were most frequent in both areas (57%). Furthermore, they reported seven different fungal genera that were isolated from ATMs in areas A and B and that yeasts were predominant in all samples used (47%), when compared to filamentous fungi (23%).

There is a yearly influx of large human population in and around the University of Port Harcourt using ATMs in various locations. Therefore, investigation of the seasonal variation in the microbial profile of these devices may be valuable to increase our awareness about the possible transmission routes of microbes, some of which may be pathogenic, especially in individuals who are immunosuppressed or immunocompromised (Neely & Sittig 2002; Tekerekoğlu et al. 2013; Mahmoudi et al. 2017).

As a result of the teeming population of students, staff, host communities and visitors of the university, bank ATMs are positioned in and around the university to aid easy transactions. In this study, the objective was to evaluate the seasonal variation in the microbial profile of ATMs in and around the University of Port Harcourt, Nigeria using microbial methods and molecular identification schemes.

Materials and methods

Sources and collection of samples

Samples were collected from contact surfaces of different ATM centres (e.g. Figure 1 & 2) in and around the University Park, University of Port Harcourt, Nigeria, during the rainy season and dry season. The

Figure 1. Automated Teller Machine gallery, University of Port Harcourt, Choba, Nigeria

Figure 2. Automated Teller Machine gallery, University of Port Harcourt, Choba, Nigeria

samples were carefully collected using sterile cotton swabs sticks moistened with sterile physiological saline by moving it over the surfaces of the contact metallic keypads of ATM (Okoro et al. 2012).

The samples were obtained at different time regimes each day (8 am to 10 am, 1 pm to 2 pm and 5 pm to 6 pm) and subsequently transported to the laboratory for microbiological analysis. The reason why the samples were obtained at different times of the day, is to ascertain the time of the day in which the number of microorganisms is high.

Enumeration and isolation of bacteria

Ten-fold serial dilutions were carried out using normal saline. 0.1ml of 10^{-1}, 10^{-3} and 10^{-5} dilution were plated on different media and incubated for 24hrs at 37°c. The total viable cell count (TVC) was determined.

Enumeration and isolation of fungi

Aliquots (0.1 ml) of 10^{-1}, 10^{-2} and 10^{-3} dilutions of the serially diluted samples were spread plated on Sabouraud Dextrose Agar and incubated at room temperature for 3–5 days. The total viable cell count (TVC) was determined.

Processing and identification of isolates

All swab samples were labelled with a laboratory number and code from the place of sampling. All samples were cultured using the spread plate method on different agar media and inoculated NA plates were incubated at 37°C for 24 hours. Inoculated SDA plates were incubated at room temperature for 3-5 days. After incubation, pure cultures of colonies that developed were obtained by sub-culturing on sterile nutrient agar plates and sabouraud dextrose agar for fungi. Isolated bacteria were identified using Gram staining, conventional biochemical identification procedures (Chessbrough 2006) and molecular identification schemes.

Molecular identification of isolates

The DNA extraction was done using a ZR fungi/bacteria DNA miniprep extraction kit supplied by Inqaba South Africa. The substantial growth of the pure culture of isolates was suspended in 200 microlitres of isotonic buffer in a ZR bashing bead lysis tubes with 750 microlitres of lysis solution added to each tube. The tubes were secured in a bead beaker fitted with a 2 ml tube holder assembly and processed at maximum speed for 5 minutes. After that, ZR bashing bead lysis tubes were centrifuged at 10.000x for 1 minute. Four hundred (4000) microlitres of the supernatant obtained were transferred to a zymo spin iv spin fitter (orange top) in a collection tube and centrifuged at 7.000x for 1 minute. Include unit of speed. 7.000 or 7,000 (correct all under molecular identification). One thousand two hundred (1200) microlitres of fungal/bacterial DNA binding buffer was added to the filtrate in the collection tubes bringing the final volume to 1600 microlitre. Eight hundred (800) microlitre was then transferred to a zymo-spin JIC column in a collection tube and centrifuged at 10.000x for 1 minute with the flow-through discarded from the collection tube. The remaining

volume was transferred to the same zymo-spin and spun. Two hundred (200) microlitre of the DNA pre-wash buffer was added to the zymo- spin ITC in a new collection tube and spun at 10.000x for 1minute followed by the addition of 500 microlitres of fungal/bacterial DNA wash buffer and centrifugation at 10.000x for 1 minute. The zymo-spin TTC column was transferred to a clean 1.5 microlitre centrifuge tube with 100 microlitres of DNA elution buffer added to the column matrix and centrifuged at 10.000x microlitre for 30 seconds to elute the DNA. The ultra-pure DNA was then stored at -20 °C for other downstream reactions. The 16s rRNA region of the rRNA genes of the bacterial isolates was amplified, whereas, the ITS (Internal Transcribed space) region of the rRNA genes of the fungal isolates were amplified. DNA sequencing (Inquaba Biotechnical Industries, South Africa) was done using the Sanger method of sequencing with 3500ABI genetic analyser.

Results and Discussion

Total Heterotrophic Bacterial Counts of the ATMs during rainy and dry seasons

The total heterotrophic bacterial counts of the ATMs during rainy and dry seasons are

Table 1. Total Heterotrophic Bacterial Count CFU/m^2 of the ATMs during rainy and dry seasons

Sample name	Time	Rainy season	Dry season
	M	$2.5 \times 10^{0}7$	3×10^{4}
A	A	2.8×10^{4}	8.3×10^{1}
	E	1.66×10^{2}	8.3×10^{1}
	M	2.22×10^{7}	1.208×10^{3}
B	A	5.08×10^{4}	8.3×10^{2}
	E	8.33×10^{2}	2.5×10^{2}
	M	2.58×10^{6}	4.58×10^{3}
C	A	8.3×10^{6}	7.375×10^{3}
	E	8.3×10^{3}	1.67×10^{3}
	M	2.5×10^{6}	4.17×10^{3}
D	A	6.0×10^{4}	2×10^{3}
	E	5.0×10^{3}	6.66×10^{2}
	M	2.275×10^{5}	1.75×10^{6}
E	A	1.66×10^{3}	1×10^{6}
	E	8.3×10^{2}	4×10^{4}
	M	2.517×10^{6}	1.87×10^{6}
F	A	$2.08\times10^{0}6$	9.3×10^{4}
	E	6.25×10^{4}	2×10^{3}
	M	7.8×10^{4}	4.3×10^{6}
G	A	2.66×10^{4}	4.3×10^{6}
	E	1.67×10^{3}	4×10^{4}
	M	7.5×10^{3}	2.8×10^{4}
H	A	5.8×10^{3}	1.875×10^{4}
	E	8.3×10^{2}	2.67×10^{3}
	M	2.0×10^{6}	2.08×10^{4}
I	A	7.0×10^{4}	2.5×10^{2}
	E	1.67×10^{3}	$1{,}66\times10^{2}$
	M	2.08×10^{6}	1×10^{4}
J	A	1.67×10^{5}	4×10^{4}
	E	5.83×10^{4}	2.5×10^{2}

Key to abbreviations: Key to abbreviations: M – Morning 8 – 9 AM; A – Afternoon 1–2 PM; E- Evening 5–6 PM; Sample A – ATM Fide Bank Ofrima; Sample B – ATM Fide Bank Abuja park; Sample C – ATM ACC Bank Abuja park; Sample D – ATM Fide Bank Accident and Emergency Unit UPTH; Sample E- ATM UB Bank UPTH; Sample F – ATM Ster Bank UPTH; Sample G – ATM UB Bank Choba; Sample H – ATM FB Bank Choba; Sample I – ATM FMB Bank Choba; Sample J – ATM EC Bank Choba

presented in Tab. 1. The table revealed that sample A had the highest bacterial count of 2.5 $\times 10^7$ CFU/m^2 in the rainy season during the morning period. Samples B, E and H had the lowest bacterial count of 8.3×10^2, 8.3×10^2 and 8.3×10^2 CFU/m^2 respectively during the evening period. In the dry season, sample G had the highest bacterial count of 4.3×10^6 CFU/m^2 in morning and afternoon while sample A had the lowest count of 8.3×10^1 in the afternoon and evening.

Total Fungal Counts of the ATMs during rainy and dry seasons

Tab. 2 shows that in the rainy season sample E had the highest fungal count of 2.22×10^6 CFU/m^2 in the morning period while sample C and sample D had the lowest count of 1.66×10^2 CFU/m^2 in the evening and morning period respectively. In the dry season, sample H had the highest fungal count of 2.08×10^5 CFU/m^2 and samples A, B and E had the lowest fungal count of 8.3×10^1 CFU/m^2, 8.3×10^1 CFU/m^2 and 8.3×10^1 CFU/m^2 respectively.

Table 2. Total Fungal Count (CFU/m^2) of the ATMs during rainy and dry seasons

Sample name	Time	Rainy season	Dry season
A	M	1.25×10^3	1.67×10^2
	A	5.83×10^2	8.3×10^1
	E	4.16×10^2	3.33×10^2
B	M	5.83×10^2	8.3×10^1
	A	6.66×10^2	1.67×10^2
	E	3.33×10^2	1.67×10^2
C	M	1.67×10^3	1.67×10^2
	A	1.3×10^4	1.67×10^2
	E	1.66×10^2	8.3×10^1
D	M	1.66×10^2	2.5×10^4
	A	3.3×10^3	1.67×10^3
	E	8.3×10^3	8.33×10^2
E	M	2.25×10^6	8.3×10^1
	A	2.58×10^5	1×10^2
	E	4.25×10^5	2.5×10^2
F	M	2.92×10^4	3.6×10^4
	A	1.75×10^4	2.4×10^4
	E	3.75×10^2	1.2×10^4
G	M	1.17×10^5	2.67×10^3
	A	5.83×10^3	5.8×10^4
	E	8.33×10^2	3.3×10^3
H	M	1.417×10^4	2.08×10^5
	A	1×10^4	4.16×10^2
	E	8.33×10^2	1.66×10^2
I	M	1.25×10^4	9.16×10^2
	A	2.5×10^3	5.83×10^2
	E	1.67×10^3	1.67×10^3
J	M	1.75×10^4	1.75×10^4
	A	1.67×10^3	1.67×10^3
	E	8.33×10^4	8.33×10^2

Key to abbreviations: M – Morning 8 – 9 AM; A – Afternoon 1–2 PM; E- Evening 5–6 PM; Sample A – ATM Fide Bank Ofrima; Sample B – ATM Fide Bank Abuja park; Sample C – ATM ACC Bank Abuja park; Sample D – ATM Fide Bank Accident and Emergency Unit UPTH; Sample E- ATM UB Bank UPTH; Sample F – ATM Ster Bank UPTH; Sample G – ATM UB Bank Choba; Sample H – ATM FB Bank Choba; Sample I – ATM FMB Bank Choba; Sample J – ATM EC Bank Choba

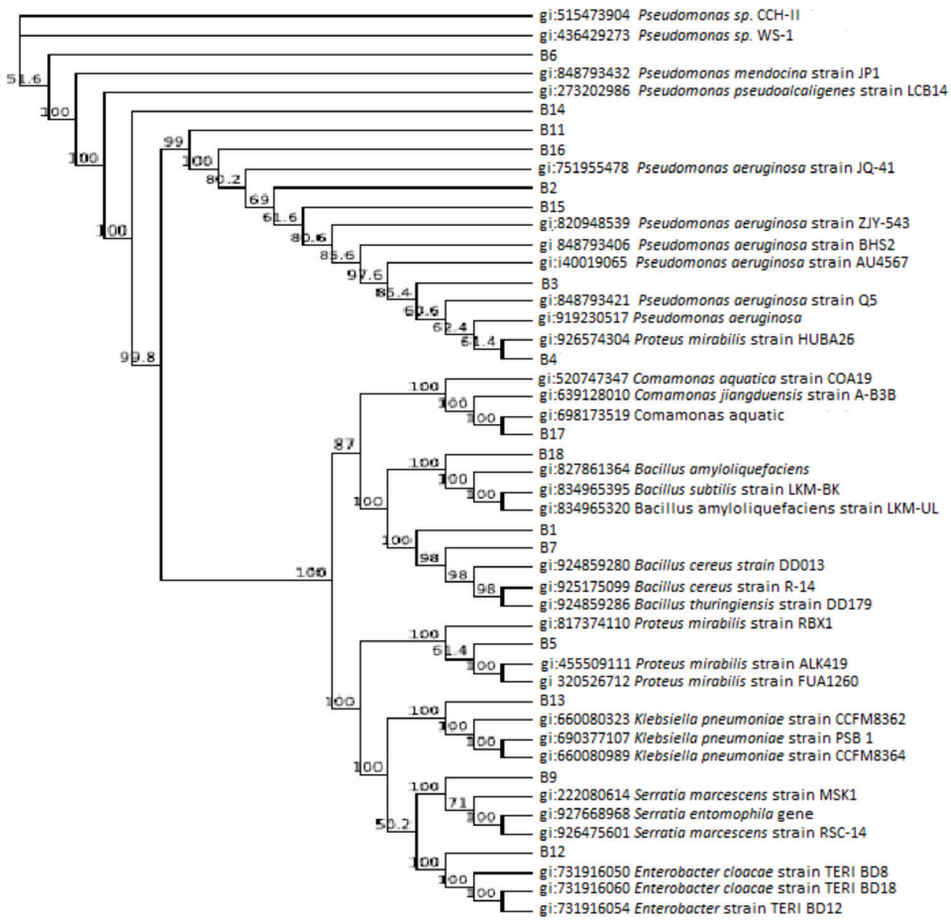

Figure 3. Neighbor – joining phylogenetic tree of the bacterial isolate. Bootstrap values of ≥ 50 % (based on 1000 replicates) are given in the nodes of the tree. NCBI accession numbers are given.

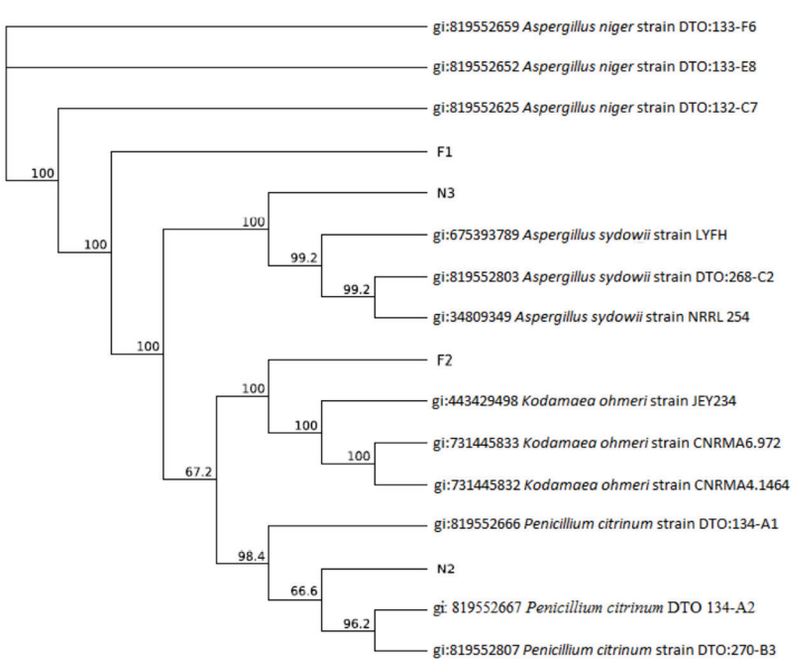

Figure 4. Neighbor – joining phylogenetic tree of the fungal isolate. Bootstrap values of ≥50% (based on 1000 replicates) are given in the nodes of the tree. NCBI accession numbers are given.

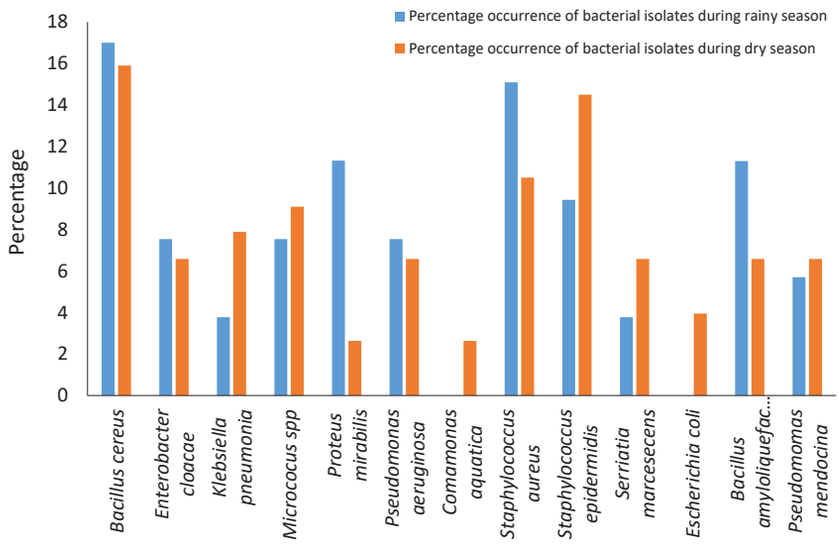

Figure 5. Percentage frequency of occurrence of Bacterial isolates during the rainy and dry seasons

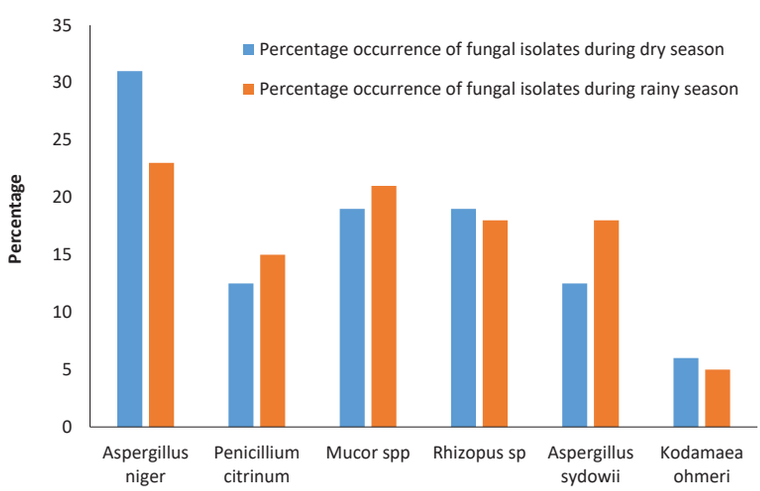

Figure 6. Percentage frequency of occurrence of Fungal isolates during the rainy and dry seasons

Analysis of variance for the Total Heterotrophic Bacterial count and Total Fungal count during the rainy season and dry season.

Tab. 3 revealed that samples (A, B, C, D, E, F, G, H, I, J) showed significant difference at P< 0.05 between the different collection periods (Morning, Afternoon, Evening), except for total fungal count for sample C during the dry season and also sample D during the rainy season.

Data obtained on the microbial load of contact surfaces of cash dispensing machines at various locations within Port Harcourt suggest the public health risks associated with the use of ATMs especially for immunocompromised or immunosuppressed individuals who patronize these machines. The colonization of these contact surfaces by pathogenic microbes may predispose clients to infection by these microbes. Oluduro et al. (2011) and Okoro et al. (2012) showed that the microbial load present on a surface is among the microbe-associated factors that determine whether an infection will occur or not when individuals come in contact with such surfaces. A similar study has shown a high level of bacterial and fungal contamination of metallic keypads of ATMs (Neely & Sittig 2002).

Table 3 Analysis of variance for the Total Heterotrophic Bacterial count and Total Fungal count during the rainy season and dry season

		Sum of Squares	df	Mean Square	F	Sig.
A_THBC_RS	Between Groups	1.199E+15	2	S.993E+14	99.986	.002
	Within Groups	1.800E+13	3	6.000E+12		
	Total	1.217E+15	5			
A_THBG_DS	BetweenGroups	1718413333	2	859206666.7	143.193	.001
	WithinGroups	18000970.00	3	6000323.333		
	Total	1736414303	5			
A_TFC_RS	Between Groups	1120000.000	2	560000.000	60.327	.004
	Within Groups	27648.000	3			
	Total	1147848.000	5			
A_TFC_DS	Between Groups	93333.333	2	46666.667	245.614	.000
	WithinGroups	570.000	3	190.000		
	Total	93903.333	5			
B_THBC_RS	Between Groups	9.412E+14	2	4.706E+14	31.956	.009
	WithinGroups	4.418E+13	3	1.473E+13		
	Total	854E+14	5			
B_THBG_DS	BetweenGroups	1343333.333	2	671666.667	82.582	.002
	WithinGroups	24400.000	3	8133.333		
	Total	1367733.333	5			
B_TFC_RS	BetweenGroups	304033.333	2	152016.667	52.723	.005
	WithinGroups	8650.000	3	28983.333		
	Total	312683.333	5			
B_TFC_DS	BetweenGroups	120000.000	2	60000.000	87.805	.002
	WithinGroups	2050.000	3	683.333		
	Total	122050.000	5			
C_THBC_RS	BetweenGroups	1.039E+14	2	5.194E+13	292.513	.000
	WithinGroups	5.327E+11	3	1.776E+11		
	Total	1.044E+14	5			
C_THBC_DS	BetweenGroups	46930000.00	2	23465000.00	117.442	.001
	WithinGroups	599400.000	3	199800.000		
	Total	47529400.00	5			
C_TFC_RS	BetweenGroups	299253333.35	2	1496236666.7	55.607	.004
	WithinGroups	8072400.000	3	2690800.000		
	Total	307325733.3	5			
C_TFG_DS	BetweenGroups	.000	2	.000	.000	1.000
	WithinGroups	6252.000	3	2084.000		
	Total	6252.000	5			
D_THBC_RS	BetweenGroups	1.169E+13	2	5.845E+12	24.351	.014
	WithinGroups	7.201E+11	3	2.400E+11		
	Total	1.241E+13	5			
D_THBC_DS	Between Groups	17973333.33	2	8956666.667	267.460	.000
	Within Groups	100800.000	3			
	Total	18074133.33	5			
D_TFC_RS	Between Groups	12333333.33	2	6166666.667	.452	.674
	Within Groups	40923400.00	3	13641133.33		
	Total	53256733.33	5			
D_TFC_DS	Between Groups		2	420166666.7	708.067	.000
	Within Groups	840333333.3	3	593400.000		
	Total		5			
E_THBC_RS	Between Groups	99737333333	2	49565666667	54.639	.004
	Within Groups	2738100000	3	912700000.0		
	Total		5			
E_THBC_DS	Between Groups	4.900E+12	2	2.450E+12	26824.409	.000
	Within Groups	274000000.0	3	91333333.33		
	Total	4.900E+12	5			
E_TFC_RS	Between Groups	8.531E+12	2	4.265E+12	778.748	.000
	Within Groups	16432000000	3	5477333333		
	Total	8.547E+12	5			
E_TFC_BS	Between Groups	48533.333	2	24266.667	56.000	.004
	Within Groups	1300.000	3			
	Total	49933.333	5			

		Sum of Squares	df	Mean Square	F	Sig.
F_THBC_RS	Between Groups	9.883E+12	2	4.941E+12	183.303	.001
	Within Groups	80872000000	3	26957333333		
	Total	9.964E+12	5			
F_THBC_DS	Between Groups	6.365E+12	2	3.182E+12	10630.536	.000
	Within Groups	898096800.0	3	299365600.0		
	Total	6.366E+12	5			
F_TFC_RS	Between Groups	1208003333	2	604001666.7	80.527	.002
	Within Groups	22501800.00	3	7500600.000		
	Total	1230505133	5			
F_TFC_DS	Between Groups	4000000.000	2	2000000.000	67.265	.003
	Within Groups	89200.000	3			
	Total	4089200.000	5			
G_THBC_RS	Between Groups	8805333333	2	4402666667	29.262	.011
	Within Groups	451368200.0	3	150456066.7		
	Total	9256701533	5			
G_THBC_DS	Between Groups	3.5359E+13	2	1.770E+13	265.399	.000
	Within Groups	2.000E+11	3	66674833333		
	Total	3.559E+13	5			
G_TFC_RS	Between Groups	24697333333	2	12345666667	739.663	.000
	Within Groups	50085000.00	3	16695000.00		
	Total	24747418333	5			
G_TFC_DS	Between Groups	5879253333	2	2939626667	1336.032	.000
	Within Groups	6600800.000	3	2200266.667		
	Total	5885854133	5			
H_THBC_RS	Between Groups	69333333.33	2	34666666.67	191.106	.001
	Within Groups	544200.000	3			
	Total	69877533.33	5			
H_THBC_DS	Between Groups	966920000.0	2	454460000.0	155.858	.001
	Within Groups	9325000.000	3	3108333.333		
	Total	978245000.0	5			
H_TFC_RS	Between Groups	268000000.0	2	134000000.0	100.375	.002
	Within Groups	4005000.000	3	1335000.000		
	Total	272005000.0	5			
H_TFC_DS	Between Groups	83100253333	2	41550126667	2492.988	.000
	Within Groups	500003688.00	3	16666796.00		
	Total	83150253721	5			
I_THBC_RS	Between Groups	7.414E+12	2	3.707E+12	2206.247	.000
	Within Groups	5040680000	3	1680226667		
	Total	7.415E+12	5			
I_THBC_DS	Between Groups	752093333.3	2	3760466667	564.000	.000
	Within Groups	2000250.000	3			
	Total	754093583.3	5			
I_TFC_RS	Between Groups	209333333.3	2	1046666667	603.846	.000
	Within Groups	520000.000	3			
	Total	209853333.3	5			
I_TFC_DS	Between Groups	813333.333	2	406666.667	120.055	.001
	Within Groups	10162.000	3			
	Total	823495.333	5			
J_THBC_RS	Between Groups	7.715E+12	2	3.857E+12	255.730	.000
	Within Groups	45250000000	3	15083333333		
	Total	8.760E+12	5			
J_THBC_DS	Between Groups	139320000.0	2	69660000.00	100.446	.002
	Within Groups	2080512.000	3	693504.000		
	Total	141400512.0	5			
J_TFC_RS	Between Groups	508000000.0	2	254000000.0	167.952	.001
	Within Groups	4537000.000	3	1512333.333		
	Total	512537000.0	5			
J_TFC_DS	Between Groups	3706720000	2	1853360000	2223.054	.000
	Within Groups	2500200.000	3	833400.000		
	Total	3708220200	5			

Note: Key to abbreviations: M – Morning 8 – 9 AM; A – Afternoon 1–2 PM; E- Evening 5–6 PM; Sample A – ATM Fide Bank Ofrima; Sample B – ATM Fide Bank Abuja park; Sample C – ATM ACC Bank Abuja park; Sample D – ATM Fide Bank Accident and Emergency Unit UPTH; Sample E- ATM UB Bank UPTH; Sample F – ATM Ster Bank UPTH; Sample G – ATM UB Bank Choba; Sample H – ATM FB Bank Choba; Sample I – ATM FMB Bank Choba; Sample J – ATM EC Bank Choba

THBC, Total Heterotrophic Bacterial Counts; TFC, Total Fungal Counts; RS, Rainy Season; DS, Dry Season

The total heterotrophic bacterial count (CFU/m^2) during the rainy and dry seasons ranged from $\log_{10}$ 7.398 to 2.919 and from $\log_{10}$ 6.633 to 1.919 respectively (Tab. 1) whereas, the total fungal count in the rainy and dry seasons ranged from $\log_{10}$ 6.352 to 2.220, and from $\log_{10}$ 5.318 to 1.919 respectively (Tab. 2). However, there were no significant differences ($p>0.05$) in total heterotrophic bacterial counts and total fungal counts of sample A and H during both rainy season and dry season, the number of occurrence of bacterial isolates was 72 and 53 respectively (Tab. 2). This trend may be ascribed to the fact that these microorganisms survive and proliferate faster in the rainy season due to availability of moisture whereas, only endospore bearing cells survive in a dry environment. Generally, the THBC of ATM keypad surfaces in the rainy season were higher than the THBC in the dry season (Fig. 5). This trend may be ascribed to the fact that these microorganisms survive and proliferate faster in the rainy season due to availability of moisture whereas, only endospore bearing cells survive in a dry environment for a long time. Fomites have been shown to have the ability to harbour microbes and sustain their survival for several months (French et al. 2004) with incidences of cross-contamination of these organisms among the public facilities and host documented (Hardy et al. 2006). Neely & Maley (2000) had demonstrated that microorganisms could survive for a more extended period on plastics. The total numbers of occurrence of fungi in rainy and dry seasons were 39 and 16 respectively.

In Fig. 5 it is shown that *Bacillus cereus* was isolated from all the ATM keypad surfaces and had the highest occurrence of 10 (15.9%) in the rainy season. Also, *Bacillus cereus* had the highest occurrence of 9(17%) among the bacteria isolated in the dry season. *Bacillus cereus* is a gram-positive aerobic or facultatively anaerobic, motile spore-forming, rod-shaped bacterium that is widely distributed in the environment. The bacterium associated mainly with food poisoning has also been reported to be a cause of severe and potentially fatal non–gastrointestinal tract infection (Edward 2010). *Bacillus cereus* is responsible for some of the foodborne diseases (2-5%), causing acute, nausea, vomiting, and diarrhoea (Edward 2010). Fig. 6 further shows that *Proteus mirabilis* and *Comamonas aquatica* had the lowest occurrence during the rainy season. *Comamonas aquatica* is a novel microorganism that was isolated from ATM UB bank (UPTH) and ATM FMB Bank, Choba, and which was first isolated from respiratory tract samples, urogenital tract samples and digestive tract samples where it causes intrabdominal infection (Wauters et al. 2003). Other bacteria isolated from the ATM keypads include *Staphylococcus* sp., *Escherichia* sp., and *Klebsiella* sp. Abban and Tano – Debrah (2011), in their study, also documented the presence of these bacteria on ATM keypads. *Bacillus cereus, Staphylococcus aureus, Klebsiella pneumonia, Enterobacter* sp. and *Pseudomonas aeruginosa* are all well documented for their high pathogenicity, causing even death in some major outbreaks and infections (Edward 2010; Mead et al. 1999). The higher frequency of occurrence of 8 (10.5%) obtained for *Staphylococcus aureus* may be attributed to the fact that it is a significant component of the normal flora of the skin and nostrils and hence, can quickly be discharged by several human activities (Itah and Ben 2004). *Staphylococcus aureus* can be transmitted easily through processes such as sneezing, talking, and contact with moist skin and has also been associated with numerous infections. Therefore, as users always touch interfaces and often sneeze, there is every chance of introducing *Staphylococcus aureus* on to the interface in use (Oluduro et al. 2011).

Considering the percentage frequency of occurrence of fungi isolated during the rainy season, (Figure 6) *Aspergillus niger* had the highest percentage frequency of occurrence 9(23%) whereas, *Kodamaea ohmeri* had the lowest percentage of occurrence 2(5%) during the rainy season. Also, in the dry season, *Aspergillus niger* had the highest occurrence of 5 (31%), and *Kodamaea ohmeri* had the lowest occurrence of 1 (6%) (Fig. 6). Generally, the frequency of occurrence of fungal isolates reduced during the dry season as follows: *Aspergillus niger* 5 (31%), *Penicillium citrinum* 2 (13%), *Mucor* sp. 3 (19%), *Rhizopus* sp. 3(19%), *Aspergillus sydowii* 2(12.5%) and *Kodamaea ohmeri* 1(6%) and this could be attributed to the low moisture content of the atmosphere during the dry season which limits the growth of these fungi. Some of the fungal species obtained in this study had been isolated from ATM keypads around Aluu Community Rivers State and Jos Plateau State, Nigeria (Okoh 2013; Maori et al. 2013).

Kodamaea ohmeri has been implicated in causing fungaemia, and wound infection (Han et al. 2004) and this study herald the first report of *K. ohmeri* being isolated from ATMs surfaces during the rainy and dry seasons.

It was isolated from an ATM in a Teaching Hospital which suggests cross-contamination of keypads as a result of ATM use by an infected individual in the hospital. The high bacterial load on ATM keypads obtained in this study agrees with the findings of other similar surveys (Oluduro et al. 2011; Okoro et al. 2012) where they reported that keypads of ATMs harboured more bacteria than computer keyboards due to the strategic location of ATM in public places and along the roadside.

Generally, there were significant differences in microbial counts of ATM keypads obtained during the rainy season and dry season and during the different diurnal time regimes (morning, afternoon, and evening) monitored. During the rainy season, sample A (ATM Ofrima Hall) had the highest total heterotrophic bacterial count in the morning hour, which was ascribed to the massive population of students in that hall. Increased use of ATMs at particular times of the day or during the rush hour resulted in increased microbial loads of keypads. In the dry season, sample G (ATM UB Bank Choba) had the highest total heterotrophic bacteria owning to the installed air conditioners in the ATM rooms. Sample E (ATM UB Bank UPTH) had the highest total fungal count in the rainy season whereas, Sample G (ATM UB Bank Choba) had the highest total fungal count during the dry season.

Conclusion

There is a shortage of reports on seasonal variation of the microbial profile of automated teller machines. In this study, first, we documented that there is a significant variation in loads of microbial contaminants of ATMs between rainy and dry seasons. Second, the study is the first to describe the isolation and characterization of a strain of *Comamonas aquatica* and *Kodamaea ohmeri* microorganisms from ATMs. These microorganisms were identified and confirmed using morphological, physiological and biochemical tests. This study agreed that ATM surface harbours a community of bacteria with different virulence and pathogenicity, thereby increasing the risk of infection and also the severity of the infection. The organisms isolated from this work are all pathogenic. Therefore there is a need for banks to initiate preventive measures against the spread of infectious diseases through ATMs surface by disinfecting ATM surface with disinfectant. Creating customers awareness is of great importance since it will avail the general public of information on the hygienic usage of ATM.

Funding

We did not receive any grant.

References

Abban, S. & Tano-Debrah, K. (2011). Automatic Teller Machines (ATMs) as potential sources of food-borne pathogens - a case from Ghana. *Nature and Science* **9**: 63–67.

Aquino, S., Alves de Lima, J., Oliveria da Siva, M., & Fabricio de Sousa, G. (2019). Detection of pathogenic bacteria and fungi on biometric surface of automated teller machines located in Braxilian public hospital. *African Journal of Microbiology Research* **13**(11): 219–231.

Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries*. Cambridge University Press. 434 pp.

Edward J. B. (2010). *Bacillus cereus*, a volatile Human Pathogen. *Clinical Microbiology Review* 23(2) 382–398.

French, G. L., Otterb, J.A., Shannona, K. P., Adamsb, N.M.T., Watlingb, D. & Parksb, M. J. (2004). Tackling Contamination of the hospital environment by methicillin-resistant *Staphylococcus aureus* (MRSA): A comparison between conventional terminal cleaning and hydrogen peroxide vapour decontamination. *Journal Hospital Infection* **57**: 31–37.

Han, X. Y., Tarrand, J. J. & Eseudero, E. (2004). Infections by the yeast *Kodamaea (Pichia) ohameri*. Two cases and literature review*. European Journal Clinical Microbiol Infection* **23**:121– 130.

Hardy, K. J. Oppenheim, B.A., Gossain, S., Gao, F. & Hawkey, P. M. (2006). A study of the relationship between environmental contamination with methicillin Resistant *Staphylococcus Aureus* (MRSA) and patients' acquisition of MRSA. *Infection Control and Hospital Epidemiology* **27**: 127–132. .

Hone, K. S., Graham, R. Maguire, M. C., Baber, C. & Johnson, G.I. (2008). Speech technology for automatic teller Machines: *an investigation of user attitude and performance Ergonomics* **41**(7): 962–981.

Itah, A. Y. & Ben, A. E. (2004). Incidence of enteric bacteria and *Staphylococcus aureus* in day care centres in Akwa- Ibom State, Nigeria. *Southeast Asian Journal Tropical Medical Public Health* **35**(1): 202–9.

Mahmoudi H., Arabestani, M.R., Alikhan, Y.M., Sedighi, I., Kohan, H.F. & Molavi, M. (2017). Antibiogram of bacteria isolated from automated teller machines in Hamadan, West Iran. *GMS Hygiene and Infection Control* **12**:1–6.

Maori, L., Agbor, V. O. & Ahmed, W.A. (2013). The prevalence of Bacteria organism on toilet door handles in Secondary School in Bokkos Local Government Area, Jos Plateau State, Nigeria. *Journal of Pharmacy and Biological Sciences* **8**(4): 85–91.

Mead, P. S., Slutesker, L., Dietz, V., McCaig, L. F., Bresee, J.S., Shapiro, C, Griffin, P. M. & Tauxe, R, V. (1999). Food-Related Illness and Death in the United States. *Emerging Infectious Diseases* **5**(5): 607–625.

Neely, A. N. & Sittig, D. F. (2002). Basic microbiologic and infection control information to reduce the potential transmission of pathogens to patients via computer hardware. *Journal of American Medical Information Association* **9**(5): 500–508.

Neely, A.N. & Maley, M. P. (2000). Survival of *Enterococci* and *Staphylococci* on Hospital fabrics and plastic. *Journal* of *Clinical Microbiology* **38**(2): 724–726.

Onuoha, S.C. & Fatokun, K. (2014). Bacterial contamination and public health risk associated with the use of banks' automated teller machines (ATMs) in Ebonyi State, Nigeria. *American Journal of Public Health Research* **2**(2):46–50.

Okoh, N. V. (2013). *Microbiological profile on doorknobs in some hostels in Aluu Community Rivers State*. B.sc thesis University of Port Harcourt Choba.

Okoro, J., Oloninefa, S.D., Ojonigu, A.F. & Sani, M. (2018). Assessment of some selected automated teller machines in Kaduna metropolis for pathogenic bacteria contamination. *British Journal of Environmental Sciences* **6**(1):19–35.

Okoro, N. , Mbaba, M., Anyim .C., Inya .O., Okoli. S, Victor. M. A. and Uchecukwu ,O. E. (2012). Antibiogram of Bacteria Isolated from Automated Teller Machines within AAKALIKI Metropolis. *American Journal of Infectious Diseases* **8**(4:) 168–174.

Oluduro, A. O., Ubani, E.K. & Ofoezie, I.E. (2011). Bacterial Assessment of Electronic Hardware user Interfaces in Ile-Ife, Nigeria. *Journal of Basic and Applied Pharmaceutical Sciences* **32**(3): 323–334.

Stanley, C. O & Kayode, F. (2014). Bacterial Contamination and Public Health Risk Associated with the use of (ATMs) In Ebony State, Nigeria. *American Journal of Public Health Research* **2**(2):46–50.

Tekerekoğlu, M.S., Yakupogullari, Y., Otlu, B., Duman, Y. & Gucluer, N. (2013). Bacteria found on banks' automated teller machines (ATMs). *African Journal of Microbiology Research* **7**(16): 1619–1621.