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Full Length Research Paper

The prevalence of tuberculosis and rifampicin resistance among the *Mycobacterium tuberculosis* clinical isolates at Federal Medical Centre Makurdi, Benue State, Nigeria

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Tuberculosis (TB) has become a global health challenge; it is a serious problem in sub-Saharan Africa, where rates of Human Immunodeficiency Virus (HIV) co-infection and drug resistance are high. The prevalence and rifampicin (RIF) resistance of *Mycobacterium tuberculosis* (MTB) among patients attending Federal Medical Centre Makurdi, Benue State was investigated. Sputum samples collected from 200 patients were tested for acid-fast Bacilli (AFB) using Ziehl-Neelsen stain. GeneXpert machine was used to test the resistance of the AFB to rifampicin. The prevalence of TB was 25.5% and the infection rate was higher in males (28.3%) than in females (23.1%), even though the difference was not statistically significant ($\chi^2=0.684$; $p>0.05$). The prevalence of TB among HIV patients was also investigated. Prevalence of TB in HIV sero-positive patients was 22.2 and 28.9% in HIV sero-negative patients. This difference, however, was not statistically significant ($\chi^2=4.453$; $p>0.05$). Most (70.6%) of the AFB positive specimens were susceptible to rifampicin; only a few (29.4%) were resistant to it ($\chi^2=43.377$; $p<0.05$). With the high prevalence of TB and the high rifampicin resistance (MDR-TB) in Benue State, policy makers and government should increase budgetary allocations for TB control and prevention through sourcing or appealing to international organizations for finance. In addition, the government should encourage research for the development of new and better TB vaccines by financing the project.

Key words: Prevalence, resistance, *Mycobacterium tuberculosis*, Makurdi.

INTRODUCTION

Tuberculosis (TB) is a disease caused by the bacterium *Mycobacterium tuberculosis*. It generally affects the lungs, but can also affect other parts of the body (WHO, 2005). TB which occurs in the lungs is known as

pulmonary TB while extrapulmonary TB occurs outside of the lungs, although extrapulmonary TB may coexist with pulmonary TB (Mandell et al., 2010). TB is transmitted through the air when people with active TB cough, spit,

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speak, or sneeze (WHO, 2005; CDC, 2011). Symptoms of active TB are chronic cough with blood-stained sputum, fever, night sweats, weight loss, chills, loss of appetite and fatigue (Mandell et al., 2010).

One-third of the world's population is infected with TB (WHO, 2005). New infections occur in about 1% of the population each year (WHO, 2009). There were more than 10 million cases of active TB which resulted in 1.3 million deaths in 2016, making it the number one cause of death from an infectious disease (WHO, 2017). TB is one of the leading causes of death with people living with HIV/AIDS, HIV patients may be more susceptible to TB and HIV promotes latent TB to active TB disease (WHO, 2010; Iliyasu and Babashana, 2009). In 2014, 480,000 new cases of Multi Drug Resistant Tuberculosis (MDR-TB) were estimated, while 190,000 people died of MDR-TB (WHO, 2015).

TB is a serious public health problem in Nigeria. Nigeria has the second highest TB disease burden in Africa, ranking fifth among the high TB burden countries in the world (WHO, 2008). As high as 210,000 new cases of TB occurred in the country in 2010, with Lagos, Kano, Oyo and Benue State having the highest prevalence rates (WHO, 2012). Benue State is one of the states with a high level of TB infections. People with HIV/AIDS are mostly vulnerable to the disease with some of them infected with the MDR-TB (NMA, 2013).

M. tuberculosis does not retain any common bacteriological stain due to high lipid content in the cell wall, thus it is neither Gram-positive nor Gram-negative. Ziehl-Neelsen staining or acid fast staining is used (Nester et al., 2007). GeneXpert MTB/RIF is also used (WHO, 2008, 2011). The GeneXpert MTB/RIF test is a new molecular test for TB which diagnoses TB by detecting the presence of TB bacteria, as well as testing for resistance to the drug rifampicin (WHO, 2010). Standard anti-TB drugs have been in use for years now and resistance to the drugs had been discovered. Disease strains that are resistant to a single anti-TB drug have been discovered in every country surveyed. Primary resistance occurs when a person becomes infected with a resistant strain of TB. A person with susceptible TB may also develop secondary or acquired resistance during therapy because of inadequate treatment, not taking the prescribed treatment appropriately or lack of compliance on the part of the patient, or using low-quality medication (O'Brien, 1994). MDR-TB is caused by bacteria that do not respond to, at least, isoniazid and rifampicin, the two most powerful first-line anti-TB drugs (Peter et al., 2009). Extensively drug-resistant TB (XDR-TB) is also resistant to three or more of the six classes of second-line drugs (CDC, 2009). XDR-TB is a term sometimes used to define extensively resistant TB and constitutes one in ten cases of MDR-TB. Cases of XDR TB have been identified in more than 90% of countries (Kielstra, 2014). Another term is totally drug-resistant TB which is resistant to all currently used drugs (Maryn, 2012). It was first observed

in 2003 in Italy (Milgiri et al., 2007). It was not widely reported until 2012 (Maryn, 2012; WHO, 2016).

Justification of the study

A number of factors make people more susceptible to TB infections. The most important risk factor globally is HIV; 13% of all people with TB are infected by the virus (WHO, 2011). This in particular is a serious problem in sub-Saharan Africa, where rates of HIV are high (Chaisson and Martinson, 2008). 30% of those co-infected with HIV develop the active disease (Gibson et al., 2005). Drug resistance has made treatment of TB much more difficult. Therapy requires the use of second-line drugs which have a greater risk of adverse effects and lower potency than first-line drugs (Gler et al., 2012; WHO, 2011). Drug resistant TB is a significant and growing public health threat. The additional costs of drug-sensitivity testing, second-line drugs and the medical care associated with them are expensive; it was discovered that the cost of treating a patient carrying MDR strains is hundreds of times greater than that for patients carrying drug sensitive strains. Since most cases of TB infection result in latent infection, it was estimated that there may be as many as 50 million people who are now infected with MDR-TB (Zumla et al., 2013; WHO, 2005, 2010).

TB was declared a "global health emergency" in 1993 by the WHO (2017). In 2006, the Stop TB Partnership developed a global plan to stop TB that aimed to save 14 million lives between its launch and 2015 (WHO, 2011). It was discovered that targeted objectives were not achieved by 2015, due to the increase in HIV co-infection and the emergence of MDR-TB (WHO, 2017).

The purpose of this study was to determine the prevalence of TB and rifampicin resistance among the *M. tuberculosis* clinical isolates in Makurdi metropolis.

MATERIALS AND METHODS

Study area

Makurdi, Benue State, Nigeria, was the study area. Makurdi metropolis is located in North Central Nigeria along the River Benue. It lies at Latitude: 7° 43' 32" N and Longitude: 8° 33' 51" E. Makurdi is the capital of Benue State and covers an area of 34,059 km² and has an estimated population of 500,797.

Research design

This study was a three month hospital based prospective longitudinal and cross-sectional survey which was carried out in the laboratory of Federal Medical Centre (FMC) Makurdi, Benue State, Nigeria.

Sample size

Sample size was determined using this formula (Araoye, 2004):

$$n = \frac{Z^2 P q}{d^2}$$

Where, n=desired sample size, Z=standard and normal deviation usually set at 1.96 or approximately 2.0 which correspond to 95% (0.05) confidence level, P=proportion in the target population estimated to have a particular characteristics, q=1.0-p, d=degree of accuracy usually set at 0.05, p=144/10000 people are infected with TB in Benue State (NMA, 2013).

Study population

The study population consists of 200 out patients who attended Federal Medical Centre, Makurdi for diagnosis and treatment between February and May, 2015. Ethical clearance was sought and obtained at FMC, Makurdi.

Sample collection

Three sputum specimens were collected from each patient and were examined in the laboratory for tubercle bacillus. Each patient produced 10 ml of sputum for each sputum container (20 ml). Sputum specimens were collected in an air-tight sputum container. Patients were asked to inhale deeply and cough from within and before carefully spitting into the container to avoid contamination from the outside part. After sputum collection, the containers were firmly closed and the patient's hands washed with soap and water. Sputum specimens were stored in the refrigerator until test was to be carried out. Protective precautionary laboratory procedures like using of laboratory coats, hand gloves, face mask as well as frequent hand washing with the use of disinfectants were ensured.

Laboratory test

The diagnosis was carried out using Ziehl-Neelsen staining technique on triplicate early morning sputum samples collected under standard bio-safety procedures (Morello et al., 2006; Nester et al., 2007).

M. tuberculosis GeneXpert test was used to test for rifampicin resistance among the *M. tuberculosis* clinical isolates.

The Xpert MTB/RIF has been developed by the foundation for innovative new diagnostics (FIND), who have partnered with the Cepheid Corporation and the University of Medicine and Dentistry of New Jersey (WHO, 2011).

The sample to be put in the GeneXpert machine was prepared by pouring the sputum into the already prepared MTB/RIF cartridge and mixing vigorously. After clicking "create test" in the GeneXpert DS system window, the scan barcode dialog box appeared and the barcode on the xpert MTB/RIF cartridge was scanned by the scanning machine.

The sample ID was typed in the sample ID box. This sample ID was associated with the test results and was shown in the "view results" window of all the reports. The password was typed in when the dialog box appears, and this happened after clicking "start test". The instrument module door with the blinking green light was opened and the cartridge loaded. The door was closed and the test starts when the green light stopped blinking. When the test is finished, the lights went off on their own and the system released the door lock at the end of the run. The module door was then opened to remove the cartridge.

To view results on the GeneXpert software, "view results" was clicked on the menu bar in the GeneXpert DS system window and the results appears with the sample ID and could read MTB detected low, MTB detected medium or high and RIF resistance detected or not detected. It was then instructed to print out the result (WHO, 2011).

Statistical analysis

Data collected were analyzed using descriptive and inferential statistics. Chi square test was used to determine associations between variables; correlation coefficients were used to determine relationships between variables. Significance was held at 0.05 level.

RESULTS AND DISCUSSION

Two hundred patients were screened for *M. tuberculosis* (MTB). The prevalence of TB among them was 25.5% (n= 51). As shown in Table 1, the prevalence of TB was 28.3% in the male and 23.1% in the female. The difference in the prevalence of *M. tuberculosis* was however not statistically significant.

The prevalence of TB among HIV patients is shown in Table 2. Prevalence of TB in HIV sero-positive patients was 22.2%, while it was 28.9% in HIV sero-negative patients.

The susceptibility profile of the acid fast bacilli (AFB) to rifampicin is shown in Table 3. Most of the AFB positive specimens were susceptible to rifampicin (70.6%), while 29.4% were resistant to rifampicin.

This study was designed with the aim to determine prevalence of TB and rifampicin resistance among the *M. tuberculosis* clinical isolates. Findings of this study confirms the high prevalence of TB among the patients in the state, although WHO (2009) and NMA (2013), had earlier reported that Benue State is one of the states with a high prevalence of the disease. Prevalence rate of TB was observed to be 25.5% in this study. This high prevalence in the state could be attributed to the high HIV prevalence. This study observed as high as 22.2% co-infection with HIV sero-positive patients. Previous studies had also reported high prevalence among the HIV sero-positive patients (Kumar et al., 2007; WHO, 2009, 2010; NMA, 2013). This has made screening of TB patients for HIV mandatory. Ojjezeh et al. (2015) recorded 14.0% co-infection with HIV sero-positive patients in Ondo State. Studies from other parts of Nigeria showed lower prevalence, (Nwobu et al., 2004; Onubogu et al., 2014) from Edo and Lagos states, respectively. Tsaku et al. (2011) recorded a high rate of 24.2% of patients with HIV/TB co-infection in Nasarawa State. Aweke et al. (2016) also recorded as high as 27.7% of HIV/TB co-infection in Ethiopia. Lowering of immunity due to HIV/AIDS could enhance TB infection (Iliyasu and Babashani, 2009; Aweke et al., 2016). Other studies also recorded high prevalence of TB/HIV co-infection (Ifebunandu et al., 2012; Rajasekaran et al., 2007). Awoyemi et al. (2002) recorded as high as 32.8% TB/HIV co-infection. TB is among the living causes of death for people living with HIV (WHO, 2009).

This study also recorded a higher prevalence of TB among males than females. This agrees with the study of Ojjezeh et al. (2015). They stressed that males are highly exposed to the bacilli than females.

Table 1. Prevalence of tuberculosis by sex.

Sex	Number examined (%)	Number positive (%)	Number negative (%)
Male	92 (100)	26 (28.3)	66 (71.7)
Female	108 (100)	25 (23.1)	83 (76.9)
Total	200 (100)	51 (25.5)	149 (74.5)

$\chi^2=0.684$, $df=1$; $p>0.05$ ($p=0.408$).

Table 2. Prevalence of tuberculosis according to HIV status.

HIV status	Tuberculosis infection rate		Total (%)
	Number negative (%)	Number positive (%)	
Sero-Negative	27 (71.1)	11 (28.9)	38 (100)
Sero-Positive	112 (77.8)	32 (22.2)	144 (100)
Unknown	10 (55.6)	8 (44.4)	18 (100)
Total	149 (74.5)	51 (25.5)	200 (100)

$\chi^2=4.453$, $df.=1$, $p>0.05$ ($p=0.108$).

Table 3. Susceptibility profile of rifampicin in tuberculosis patients.

Rifampicin susceptibility	Number of sputum specimens (%)
Susceptible	36 (70.6)
Resistant	15 (29.4)
Total	51 (100)

$\chi^2=43.377$, $df. =1$, $p<0.05$ ($p=0.000$).

Rifampicin resistance appeared to be higher than that reported by Hatfull and Jacobs (2000) and WHO (2013). Drug resistant TB is a significant and growing public health threat. According to Gursimrat (2011), MDR-TB and HIV are among the major challenges facing the control of TB. Crofton (1959) observed that MDR-TB and XDR-TB could threaten the success of TB control. In 2007, the United States recorded 36% of MDR-TB and 56% of XDR-TB. MDR-TB and XDR-TB have very complex pathway of diagnosis and treatment. They are resistant to a large number of medications, care was also complicated and patients were highly infectious (CDC, 2014). Treatment also takes a longer time (not less than 2 years), and also requires hospitalization. Drug resistance has made treatment of TB more difficult and expensive (CDC, 2014). Treatment uses the second-line drugs which have a greater risk of adverse effects and lower potency than first-line drugs (Gler et al., 2012; WHO, 2011).

Conclusion

Prevalence of TB was high in Makurdi. It was also observed that there was a high rate of MDR-TB and TB/HIV co-infection in the state. These factors could

generate new challenges, complicate treatment greatly, and make it difficult to eliminate TB. More attention should be given to financing the development of new TB drugs by governments and other institutions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Detection of pathogenic bacteria and fungi on biometric surface of Automated Teller Machines located in Brazilian public hospital

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The Automated Teller Machine (ATM) is used by millions of people as an alternative to gain time instead of using traditional banking systems in Brazil and ATMs are frequently localized in São Paulo city around the hospitals. However, ATMs might be potential devices for microbial accumulation and transmission in the community. The objective of the present study was to evaluate forty-two ATMs, in two hospital areas (A and B) in São Paulo city for the presence of pathogenic fungi and bacteria. Samples were collected from biometric surfaces of the devices with sterile cotton swabs soaked in the sterile physiologic saline and were cultured on selective agar for yeasts, filamentous fungi and bacteria in the period of January 2017 to March 2018. Complementary biochemical tests were applied to confirm the bacteria and the taxonomic identification of molds was performed considering the morphological characteristics by microscopic observation. Our results suggest that the biometric surfaces in ATMs is an important environmental source of microbes, once that the genera *Staphylococcus* was predominant in all agencies of both hospital areas (83.3%), following of *Streptococcus* spp. (57%) and *Enterococcus* spp. (50%). The group of Enterobacteriaceae (Gram negative bacilli) were most frequent in both areas studied (57%). Seven different fungi genera were isolated from ATMs in area A and B and yeasts were predominant in all samples collected (47%), comparing with filamentous fungi (23%). We conclude that biometric ATM surfaces play an important role in microbial transmission in hospital settings, and healthcare professionals should wash and disinfect their hands carefully before touching patients.

Key words: Automated teller machine, bacteria, fungi, contamination, hospital.

INTRODUCTION

Contamination of environmental objects and surfaces is a common phenomenon. Human beings have a marked

tendency to pick up microorganisms from environmental objects, and the hand has been shown to play a role in

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the transmission of microorganisms (Onuoha and Fatokun, 2014). Few investigators, however, have examined in detail the bacterial species found in other surfaces or environments (Reacher et al., 2000).

Since the year 2000, infection with methicillin-resistant *Staphylococcus aureus* (MRSA) was becoming increasingly common in UK hospitals, contributing significantly to morbidity and mortality (Reacher et al., 2000). The risk of environmental contamination has also been highlighted by the isolation of *S. aureus* from commonly used hospital items, included stethoscopes, pens, tourniquets, computers and blood pressure cuffs (Devine et al., 2001; Cohen et al., 1997; French et al., 1998; Berman et al., 1986; Layton et al., 1993; Al-Ansari et al., 1999).

Blomeke et al. (2007) investigated bacterial recovery and transfer from fingerprint biometric sensors and the survivability of bacteria on the devices. Two species of bacteria, *S. aureus* and *Escherichia coli* and the survivability was investigated by sterilizing the sensor surface, applying a known volume of diluted bacterial culture to the sensor and allowing it to dry. Bacteria were recovered at 5, 20, 40 and 60 min after drying by touching the contaminated device with a sterile finger cot. The result of this comparison between *S. aureus* and *E. coli* is that neither bacterial species survived for a long time on the device surface, but even at 60 min a small quantity of bacteria was still recoverable (Blomeke et al., 2007).

Due to the ongoing development and expansion of urbanization, as well as the increasing population, people do not have enough time to use traditional banking systems and have embraced new developments in electronic banking, such as Automated Teller Machine (ATM) (Mahmoudi et al., 2017). The ATM is a computerized telecommunications device that enables the clients of a financial institution to perform financial transactions without the need for cashier, human clerk or bank teller (Rasiah, 2010). Today, the extended use of electronic technologies is considered a source of bacterial contamination. In general, microbes can persist or grow on many surfaces, such as those found in restaurant kitchens and hospital environments, as well as on standard office equipment such as computer keyboards, telephones, cellphones, and ATMs (Abban and Tano-Derach, 2011; Anastasiades et al., 2009; Anderson and Palombo, 2009).

Nowadays the ATM has been an important device in the banking sector and other financial institutions. The keyboards and screens of these devices are contaminated with pathogenic or non-pathogenic microorganisms (Mbajiuka, 2015). Hundreds of people whose socio-economic levels and hygienic status are quite different with each other use ATMs daily. Customers contact with their hand the surfaces of keypad and/or screen of these devices. However, there is limited

data about their status for microbial colonization. Therefore, investigation of the bacterial load of these devices may be valuable to increase our awareness about the possible transmission ways of pathogens, some of them are pathogenic, especially in people with weakened immune system (Tekerekoğlu et al., 2013; Mahmoudi et al., 2017).

Bank ATMs are often located around hospitals. The objective of the present study was to evaluate the fungal and bacterial contamination of the biometric system of ATMs located around two public hospitals in the city of São Paulo and to investigate the risks of microbial transmission between health professionals and community that share banking branch services.

MATERIALS AND METHODS

Sample location

São Paulo is a large city in South hemisphere (latitude -23.533773 and longitude -46.625290) and is the capital of São Paulo state of Brazil. Two large general hospitals (A and B) located in São Paulo were chosen because of the high complexity of care and belong to the Brazilian Unified Health System (UHS), in addition both are school hospitals. The hospital A is a large complex clinic comparing to hospital B, in despite that the hospital B was inaugurated in 1936. The hospital A was inaugurated in April 1944 and is pioneer in medical-hospital procedures with a great number of hospitalization and intensive care units. The considered research area was 300 m around the hospitals and swab samples were collected in the period of January 2017 to March 2018, from 14 different commercial banks situated in delimited area with the aid of sterile cotton swab sticks moistened with sterile physiological saline before swabbing the biometric fingerprint buttons of the ATM machines. All cotton swab sticks were immediately transported in sterile tubes and transferred to the microbiology laboratory of Nove de Julho University for analysis.

Isolation of bacteria

A total number of forty-two (n=42) samples were collected. The swabs (21 for each area) were inoculated onto nonselective media for bacteria (Nutrient agar and Blood agar) and selective medium (MacConkey agar, *Enterococcus* agar, Baird Parker agar and Mannitol agar) in triplicate plates and incubated at 37°C for 24 h.

Bacterial identification of single colonies grown or colony-forming units (CFU) on MacConkey agar plates were tested using colonial morphology, Gram stain colonial morphology, triple sugar iron agar (TSI) test and complementary biochemical tests according to Cheesbrough (2006) for Gram negative bacteria. To detect Gram positive bacteria, colonies on Blood, *Enterococcus* agar, Baird Parker agar and Nutrient agar were tested using colonial morphology with Gram stain and after confirmed in catalase and coagulase tests (Bartholomew and Mitterer, 2008). A selective media Mannitol and Baird Parker media was used to differentiate *S. aureus* from other coagulase-negative staphylococci.

Fungal isolation

To determine the presence of *Candida* species the samples were inoculated onto *CHROMagar Candida* and incubated at 37°C for 24

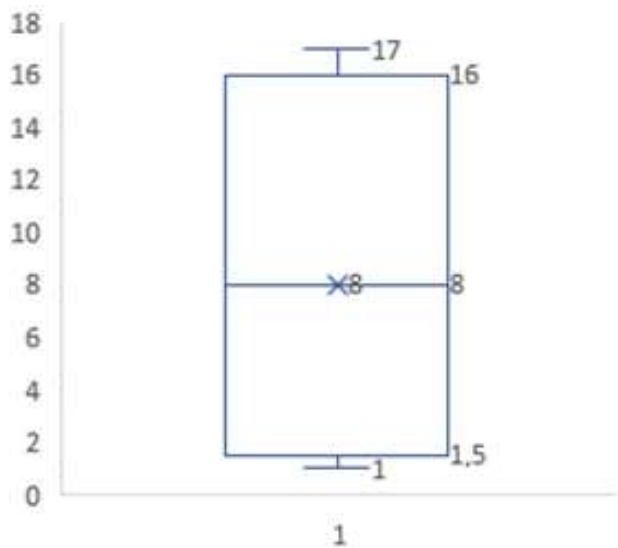


Figure 1. Distribution of bacteria recovered from ATMs in hospital A.

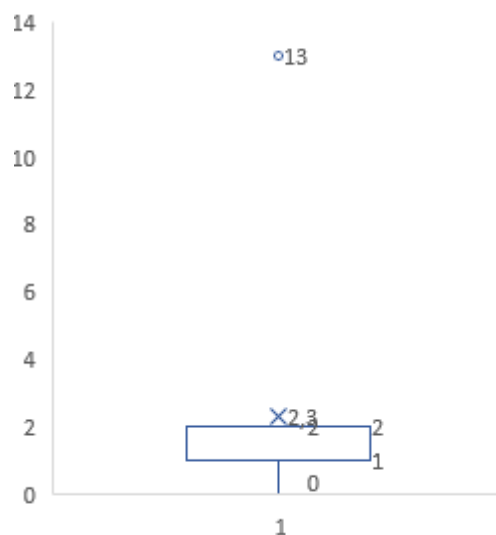


Figure 3. Distribution of fungi recovered from ATMs in hospital A area.

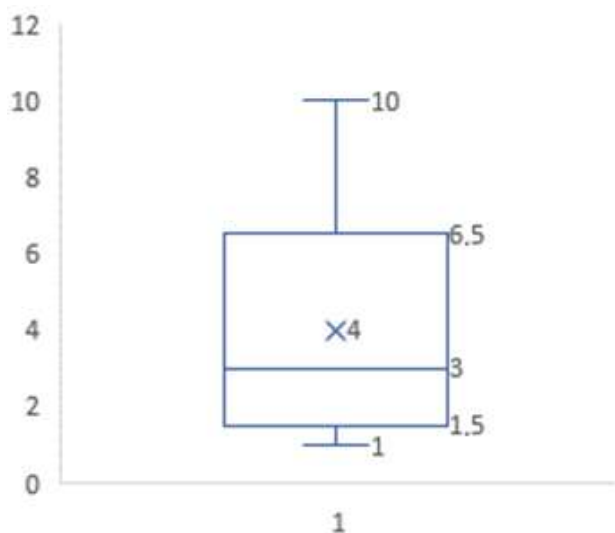


Figure 2. Distribution of bacteria recovered from ATMs in hospital B area.

assigned values (+, ++, +++) to colony counting according to Nwankwo and Offiah (2016).

Statistical analysis

Data statistical analysis obtained from the isolation of bacteria in this study were analyzed by the T test applied to the two-tailed hypothesis was the best way to compare the mean between the two samples obtained for the hospital areas A and B. The statistical analysis of the data was performed in SPSS version 16.0 (SPSS Inc., USA).

RESULTS AND DISCUSSION

From the 42 samples collected ($N = \sum N^A + N^B$), 21 samples were obtained near the hospital area A and 21 from hospital area B. Forty samples (95%) presented growth and 2 (5%) showed no bacterial growth. Different fungi (molds and yeasts) were isolated from ATMs in areas A and B. The frequency of yeasts was predominant in all samples collected (47%) compared to filamentous fungi (23%). The number of microorganisms recovered from ATMs located near hospitals A and B was compared to each other, as shown in the Figures 1, 2, 3 and 4.

The ATM is used by millions of people in a day and it is meant to be a public utility device, but dirty surfaces such as ATMs can bring them into the source of infection and may pose a risk to public health. Mbajiuka (2015) gave the definition of ATMs as “pathogen city” and the risk of transmission of pathogens from devices or computer keyboards to patients would be prevented by compliance with current hand hygiene guidelines. Unfortunately, studies have demonstrated that the mean rate of compliance with the Centers for Disease Control and

h to identification of mixed cultures by the colour of the colony and differentiate various *Candida* species. To isolation of filamentous fungi, the swabs were inoculated in Potato Dextrose Agar (PDA) and incubated at 25°C for 7 days. A portion of the colony was stained with lactophenol cotton blue. For the microscopic observation of the fungal morphology, the direct technique of mycological examination of the aerial mycelium, conidia and conidiogenic cells under a light microscope with an increase of 100 and 400-fold (100-400x). The taxonomic identification of fungi was performed considering the morphological characteristics of the vegetative mycelium and the reproductive structures (Pitt and Hocking, 2009). After incubation the CFU were counted and

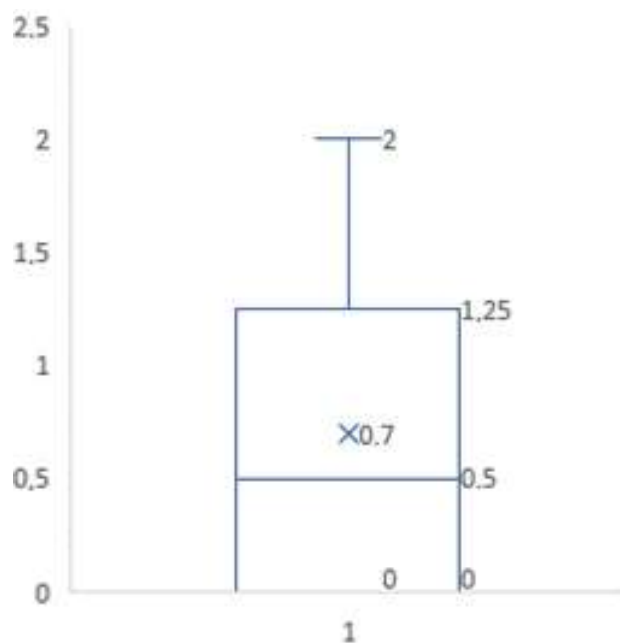


Figure 4. Distribution of fungi recovered from ATMs in hospital B area.

Table 1. Enumeration and isolated bacteria from ATMs around hospital A and B areas.

Bacteria		Hospital A area						
		Bank 1	Bank 2	Bank 3	Bank 4	Bank 5	Bank 6	Bank 7
Gram positive	<i>Staphylococcus</i> spp.	+++	+++	+++	+++	+++	+++	+++
	<i>Streptococcus</i> spp.	++	+++	++	+	+++	+	++
	<i>Enterococcus</i> spp.	++	+	++	+	0	++	+
Gram negative	Enterobacteriaceae	++	++	0	+	+	++	+
	<i>Non-fermenting Gram-negative bacilli</i>	0	+	0	0	0	+	0

Bacteria		Hospital B area						
		Bank 8	Bank 9	Bank 10	Bank 11	Bank 12	Bank 13	Bank 14
Gram positive	<i>Staphylococcus</i> spp.	+++	+++	+++	+++	+++	+++	+++
	<i>Streptococcus</i> spp.	0	++	++	+	0	++	0
	<i>Enterococcus</i> spp.	0	0	0	+	0	+	0
Gram negative	Enterobacteriaceae	+	++	+	+	++	0	+
	<i>Non-fermenting Gram-negative bacilli</i>	+	0	0	+	0	0	0

+ = Scanty growth (1-30 colonies); ++ = Moderate growth (31-70 colonies); +++ = Profuse growth (71 and above); 0= no growth.

Prevention (CDC) guidelines on hand hygiene is approximately 40% among healthcare workers, which is a likely explanation for the frequent contamination of computer keyboards (Boyce and Pittet, 2002).

In the present study, the Gram-positive *Staphylococcus*

genera showed a profuse growth (above 71 colonies) in both areas in all ATMs, following by *Streptococcus* spp. The group of Gram-negative bacteria, the family Enterobacteriaceae was present in a moderate growth (31-70 colonies). The Table 1 shows the enumeration of

Table 2. Enumeration and isolated bacteria from ATMs around hospital A and B areas.

Bacteria		Hospital A area						
		Bank 1	Bank 2	Bank 3	Bank 4	Bank 5	Bank 6	Bank 7
Gram positive	<i>Staphylococcus</i> spp.	+++	+++	+++	+++	+++	+++	+++
	<i>Streptococcus</i> spp.	++	+++	++	+	+++	+	++
	<i>Enterococcus</i> spp.	++	+	++	+	0	++	+
Gram negative	Enterobacteriaceae	++	++	0	+	+	++	+
	Non-fermenting Gram-negative bacilli	0	+	0	0	0	+	0

Bacteria		Hospital B area						
		Bank 8	Bank 9	Bank 10	Bank 11	Bank 12	Bank 13	Bank 14
Gram positive	<i>Staphylococcus</i> spp.	+++	+++	+++	+++	+++	+++	+++
	<i>Streptococcus</i> spp.	0	++	++	+	0	++	0
	<i>Enterococcus</i> spp.	0	0	0	+	0	+	0
Gram negative	Enterobacteriaceae	+	++	+	+	++	0	+
	Non-fermenting Gram-negative bacilli	+	0	0	+	0	0	0

+ = Scanty growth (1-30 colonies); ++ = Moderate growth (31-70 colonies); +++ = Profuse growth (71 and above); 0= no growth.

Table 3. Morphological and biochemical results used to identify pathogenic bacteria isolated from ATMs.

Isolated bacteria	pathogenic	Gram test	Morphology	Colony in culture media	TSI test	Catalase test	Oxidase test
<i>Staphylococcus</i>		Positive	cocci in clusters	Positive in mannitol agar (yellow)	NA	Positive	NA
<i>Streptococcus</i>		Positive	cocci in chains	Beige in Nutrient agar	NA	Negative	NA
<i>Enterococcus</i>		Positive	cocci in chains	Positive in Enterococcus media (maroon)	NA	Negative	NA
<i>Enterobacteriaceae</i> family		Negative	Bacillus	Positive in MacConkey agar	Glucose positive (yellow)	NA	Negative
<i>NFGNB</i>		Negative	Bacillus	Positive in MacConkey agar	Glucose negative (red)	NA	Positive

NA- Not applicable; GNNFB- Non-fermenting Gram-negative bacilli.

each genera or bacteria group isolated from ATMs in hospital A area and hospital B area. The Gram-positive *Staphylococcus* genera showed a profuse growth (above 71 colonies) in both areas in all ATMs, following by *Streptococcus* spp. The group of Gram-negative bacteria, the family Enterobacteriaceae was present in a moderate growth (31-70 colonies). Table 2 shows the enumeration of each genera or bacteria group isolated from ATMs in hospital A area and hospital B area in São Paulo city. Between of Gram-positive bacteria, the genera *Staphylococcus* was predominant in all agencies of both hospital areas (83%), following of *Streptococcus* spp. (57%) and *Enterococcus* spp. (50%). Among the Gram-negative bacilli, the group of Enterobacteriaceae were

most frequent in both areas studied (57%). Table 3 represents the morphological and biochemical characteristics of isolated bacteria in ATMs.

The Gram positive bacteria (*Staphylococcus*, *Streptococcus* and *Enterococcus*) and Gram negative bacteria (Enterobacteriaceae group, *Escherichia coli*, *Salmonella* spp., *Klebsiella* spp., and *Pseudomonas aeruginosa*) in ATMs revealed that there is no statistical significance between the means of these bacteria in samples collected near hospitals A and B. However, the number of isolated fungi was significant, showing that the results of hospital A area was more expressive than those collected near the B hospital region, as shown in Table 4.

Table 4. Analysis of the mean in Student's t test of bacteria and fungi recovered from ATMs in hospital area A and B.

Microorganism	Hospital A area	Hospital B area	Total	P value	Statistical significance
<i>Staphylococcus aureus</i>	16	10	26	<0.05	Not significant
<i>Coagulase Negative Staphylococci (CoNS)</i>	8	1	9	<0.05	Not significant
<i>Enterococcus</i> spp.	17	4	21	<0.05	Not significant
<i>Streptococcus</i> spp.	16	8	24	<0.05	Not significant
Enterobacteriaceae (group)	9	5	14	<0.05	Not significant
<i>Escherichia coli</i>	2	3	5	<0.05	Not significant
<i>Salmonella</i> spp.	1	2	3	<0.05	Not significant
<i>Klebsiella</i> spp.	1	1	2	<0.05	Not significant
<i>Pseudomonas aeruginosa</i>	2	2	4	<0.05	Not significant
Total bacteria isolates			108		
<i>Rhodotorula</i> spp.	13	0	13	>0.05	significant
<i>Candida krusei</i>	1	0	1	>0.05	significant
<i>Candida glabrata</i>	1	2	3	>0.05	significant
<i>Candida tropicalis</i>	1	1	2	>0.05	significant
<i>Candida albicans</i>	1	0	1	>0.05	significant
<i>Aspergillus niger</i>	2	1	3	>0.05	significant
<i>Penicillium</i> spp.	1	0	1	>0.05	significant
<i>Trichoderma</i> spp.	2	0	2	>0.05	significant
<i>Cladosporium</i> spp.	1	1	2	>0.05	significant
<i>Rhizopus</i> spp.	0	2	2	>0.05	significant
Total fungi isolates			30		

T value bacteria = 1.190; T value fungi = 3.804; gL = 40; T – critical value = 1.684 (were common to the two groups of microorganisms analyzed).

In occupational activities environments many individuals are exposed to risks of microbial origin, in a special concern is transmission of pathogens that have been demonstrated to be present on environmental surfaces in proximity to colonized or infected patients (Rutala et al., 2006). In a study performed in New York city, Bik et al. (2016) suggested that ATM keypads amalgamate microbial assemblages from different sources, including the human microbiome, eukaryotic food species, and potentially novel extremophilic taxa adapted to air or surfaces in the built environment. The authors obtained microbial DNA from ATM keypads may thus provide a record of both human behavior and environmental sources of microbes.

In this present study, it was observed a great variety of microbes in ATMs biometric surfaces around hospital areas. It is important to note that in a same surface there were various bacteria and fungi that represents a potential pathogenicity. The findings are represented in Table 5. Regarding the bacteria species, *S. aureus* were the species most frequent among the bacteria (Figure 5) and the results obtained in the present study are according of the isolates obtained from the data reported by Onuoha and Fatokun (2014) that observed that *S.*

aureus (28%) was the commonest organism isolated from ATMs of seventeen different banks in two major towns in Nigeria.

Nwankwo and Offiah (2016) reported that the ATM machines are one of the most commonly touched surfaces today. Onuoha and Fatokun (2014) described six different bacteria isolates in ATMs located in Nigeria, such as *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp., *Enterobacter* spp. and *Escherichia* spp.

Mahmoudi et al. (2017) evaluated 96 ATM keyboards at four locations in Hamadan (Iran) and they observed that all tested ATM keyboards were contaminated with at least one species of bacteria. The most frequently isolated bacteria were *Staphylococcus epidermidis* in 12 (18.5%) ATMs, *Pseudomonas aeruginosa* in 12 (18.5%), *Bacillus subtilis* in 11 (16.9%), *Escherichia coli* in 6 (9.2%), *Klebsiella* spp. in 8 (12.3%), *Enterobacter* spp. in 2 (3.1%), *Bacillus cereus* in 6 (9.2%), *Staphylococcus aureus* in 3 (4.6%), and *Micrococcaceae* spp. in 5 (7.69%) cases. The bacteria contamination of 14 commercial banks randomly scattered within the Umuahia Metropolis in Southeastern Nigeria showed a total of 102 bacterial organisms comprising nine different

Table 5. Microorganisms isolated from each ATMs in hospital area A and B.

Hospital A area	
Bank 1	
ATM 1	<i>Staphylococcus aureus</i> , <i>Enterococcus</i> spp., <i>Streptococcus</i> spp., <i>Rhodotorula</i> spp.
ATM 2	<i>Staphylococcus aureus</i> , <i>Enterococcus</i> spp., <i>Streptococcus</i> spp., <i>Klebsiella</i> spp., <i>Rhodotorula</i> spp.
ATM 3	<i>Staphylococcus aureus</i> , <i>Enterococcus</i> spp., <i>Coagulase-negative staphylococci</i> (CoNS), <i>Streptococcus</i> spp., <i>Salmonella</i> spp., <i>Candida krusei</i>
Bank 2	
ATM 4	<i>Staphylococcus aureus</i> , <i>Enterococcus</i> spp., <i>Coagulase-negative staphylococci</i> (CoNS), <i>Streptococcus</i> spp., <i>Candida glabrata</i> , <i>Candida tropicalis</i> , <i>Rhodotorula</i> spp.
ATM 5	<i>P. aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Enterococcus</i> spp., <i>Coagulase-negative staphylococci</i> (CoNS), <i>Rhodotorula</i> spp.
ATM 6	Enterobacteriaceae, <i>Staphylococcus aureus</i> , <i>Enterococcus</i> spp., <i>Streptococcus</i> spp., <i>Rhodotorula</i> spp.
Bank 3	
ATM 7	<i>Staphylococcus aureus</i> , <i>Enterococcus</i> spp., <i>Staphylococcus</i> spp., <i>Candida albicans</i> , <i>Rhodotorula</i> spp.
ATM 8	<i>Staphylococcus aureus</i> , <i>Enterococcus</i> spp., <i>Streptococcus</i> spp., <i>Coagulase-negative staphylococci</i> (CoNS), <i>Aspergillus niger</i> , <i>Penicillium</i> spp.
ATM 9	<i>Staphylococcus aureus</i> , <i>Enterococcus</i> spp., <i>Streptococcus</i> spp., <i>Rhodotorula</i> spp., <i>Aspergillus niger</i> , <i>Penicillium</i> spp.
Bank 4	
ATM 10	<i>Staphylococcus aureus</i> , Enterobacteriaceae, <i>Streptococcus</i> spp.
ATM 11	<i>Enterococcus</i> spp., <i>E. coli</i> , <i>Streptococcus</i> spp.
ATM 12	<i>Enterococcus</i> spp., <i>Coagulase-negative staphylococci</i> (CoNS), <i>Rhodotorula</i> spp.
Bank 5	
ATM 13	Enterobacteriaceae, <i>Streptococcus</i> spp., <i>Coagulase-negative staphylococci</i> (CoNS), <i>Rhodotorula</i> spp.
ATM 14	Enterobacteriaceae, <i>Streptococcus</i> spp., <i>Staphylococcus</i> spp., <i>Trichoderma</i> spp., <i>Cladosporium</i> spp.
ATM 15	Enterobacteriaceae, <i>Streptococcus</i> spp.
Bank 6	
ATM 16	<i>Staphylococcus aureus</i> , <i>Streptococcus</i> spp., <i>Enterococcus</i> spp., Enterobacteriaceae, <i>Trichoderma</i> spp.
ATM 17	<i>Staphylococcus aureus</i> , <i>Enterococcus</i> , Enterobacteriaceae, <i>Coagulase-negative staphylococci</i> (CoNS), <i>Rhodotorula</i> spp.
ATM 18	<i>Staphylococcus aureus</i> , <i>Enterococcus</i> , <i>P. aeruginosa</i> , Enterobacteriaceae, <i>Coagulase-negative staphylococci</i> (CoNS), <i>Rhodotorula</i>
Bank 7	
ATM 19	<i>Staphylococcus aureus</i> , <i>Enterococcus</i> , <i>E. coli</i> , <i>Streptococcus</i> spp., <i>Rhodotorula</i> spp.
ATM 20	<i>Staphylococcus aureus</i> , <i>Enterococcus</i> , Enterobacteriaceae, <i>Coagulase-negative staphylococci</i> (CoNS)
ATM 21	<i>Staphylococcus aureus</i> , <i>Enterococcus</i> , <i>Streptococcus</i> spp., <i>Rhodotorula</i> spp.
Hospital B area	
Bank 8	
ATM 22	<i>Escherichia coli</i>
ATM 23	<i>Coagulase-negative staphylococci</i> (CoNS), <i>P. aeruginosa</i>
ATM 24	<i>Staphylococcus aureus</i> , <i>Candida tropicalis</i>
Bank 9	
ATM 25	Enterobacteriaceae, <i>Streptococcus</i> spp., <i>Candida glabrata</i>
ATM 26	Enterobacteriaceae, <i>Streptococcus</i> spp.
ATM 27	Enterobacteriaceae, <i>Staphylococcus aureus</i>

Table 5. Contd.

Hospital A area	
Bank 10	
ATM 28	<i>Streptococcus</i> spp., <i>Rhizopus</i> spp.
ATM 29	<i>Streptococcus</i> spp., <i>Staphylococcus aureus</i>
ATM 30	<i>Salmonella</i> spp., <i>Staphylococcus aureus</i> , <i>Streptococcus</i> spp.
Bank 11	
ATM 31	Enterobacteriaceae, <i>Staphylococcus aureus</i>
ATM 32	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i>
ATM 33	<i>Enterococcus</i> spp., <i>Aspergillus niger</i>
Bank 12	
ATM 34	Enterobacteriaceae, <i>Staphylococcus aureus</i>
ATM 35	<i>Staphylococcus aureus</i> , <i>Candida glabrata</i> , <i>Salmonella</i> spp.
ATM 36	<i>Klebsiella</i> spp., <i>Staphylococcus aureus</i> , <i>Rhizopus</i> spp.
Bank 13	
ATM 37	<i>Streptococcus</i> spp., <i>Enterococcus</i> spp., <i>Cladosporium</i> spp.
ATM 38	<i>Staphylococcus aureus</i> , <i>Enterococcus</i> spp., <i>Streptococcus</i> spp.
ATM 39	<i>Enterococcus</i> spp., <i>Streptococcus</i> spp.
Bank 14	
ATM 40	<i>Staphylococcus aureus</i>
ATM 41	<i>Escherichia coli</i>
ATM 42	<i>Staphylococcus aureus</i>

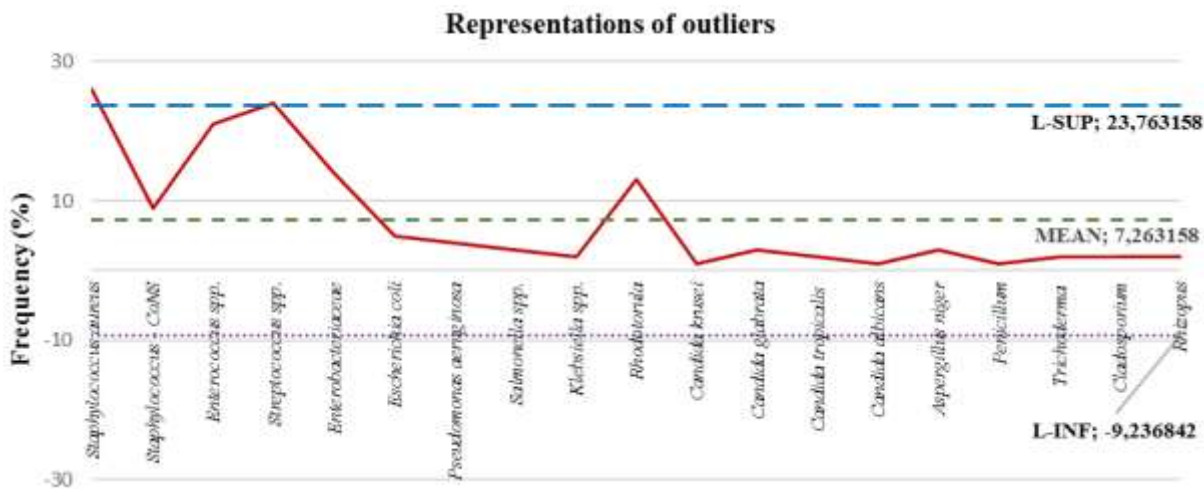


Figure 5. Frequency and outliers of bacteria and fungi isolated from 42 ATMs around hospital A and B areas (superior limit = 23,763158; mean 7,263158 and inferior limit -9,236842).

species in ATMs.

Two Gram positive bacteria, *S. aureus* and *Streptococcus* spp. showed 17.6 and 13.7% respectively while two Gram negative bacteria, *E. coli* and *Pseudomonas* spp. showed 26.5% and 9.8% respectively

(Nwankwo and Offiah, 2016). The results revealed by Mbajiuka (2015) in Abia state (Nigeria) showed that 14 ATM devices were contaminated, being a potential disease dispensing machines as bacterial isolates such as *Staphylococcus* spp. about 82.5%, *Bacillus* spp.

Table 6. Enumeration and isolated fungi from ATMs around hospital A and B areas.

Fungi		Hospital A area						
		Bank 1	Bank 2	Bank 3	Bank 4	Bank 5	Bank 6	Bank 7
Yeasts	<i>Non-albicans Candida</i>	+	+	0	0	0	0	0
	<i>Candida albicans</i>	0	0	++	0	0	0	0
	<i>Rhodotorula</i>	+++	+++	+++	+++	+++	+++	+++
	<i>Aspergillus</i> spp.	0	0	++	0	0	0	0
Filamentous fungi	<i>Penicillium</i> spp.	0	0	+	0	0	0	0
	<i>Trichoderma</i> spp.	0	0	0	0	+++	+++	0
	<i>Cladosporium</i> spp.	0	0	0	0	+++	0	0

Fungi		Hospital B area						
		Bank 8	Bank 9	Bank 10	Bank 11	Bank 12	Bank 13	Bank 14
Yeasts	<i>Non-albicans Candida</i>	+	+	0	+	+	0	0
	<i>Aspergillus</i> spp.	++	0	0	++	0	0	0
Filamentous fungi	<i>Cladosporium</i> spp.	0	0	0	0	0	+++	0
	<i>Rhizopus</i> spp.	0	0	+++	0	++	0	0

+ = Scanty growth (1-30 colonies); ++ = Moderate growth (31-70 colonies); +++ = Profuse growth (71 and above); 0= no growth.

62.5%, *Escherichia* spp. 22.5% and *Streptococcus* spp. 15%.

Okoro et al. (2018) showed that there is a strong relationship between the isolated pathogenic bacteria and the ATMs. Pathogenic bacteria such as *E. coli*, *P. aeruginosa*, *Shigella dysenteriae*, *Salmonella typhimurium*, *S. aureus* and *Klebsiella pneumoniae* were isolated from seven (7) different banks within Kaduna Metropolis. The samples (n= 200) were contaminated with *K. pneumoniae*, that had the largest percentage of isolates with 46 (23%), followed by *S. dysenteriae* with 37 (18.5%). *S. aureus*, *S. typhimurium*, and *P. aeruginosa* had 33 (16.5%), 32 (16%) and 29 (14.5%) respectively, while *E. coli* had the smallest percentage of isolates with 22 (11%).

Onuoha and Fatokun (2014) observed that *S. aureus* (28.57%) was the commonest organism isolated followed by *E. coli* (21.43%), *Coagulase negative Staphylococcus* (CoNS) (21.43%) and *Streptococcus* species (14.29%). *P. aeruginosa* and *Enterobacter* species showed the least percentage occurrence with (7.14%) respectively, in ATMs of Nigeria. Nagajothi et al. (2015) collected 92 swabs from ATM centers in Puducherry (India) and observed microbial growth in 88 (95.7%) swabs. One hundred and sixty (160) microorganisms were isolated: *Klebsiella* species (42.5%); *Coagulase-negative Staphylococcus* (CoNS) 20.62%; *P. aeruginosa* (15%); *E. coli* (10.6%) and *S. aureus* (3.75%).

The family Enterobacteriaceae consists of a number of species that are gram-negative bacilli (GNB) such as *Salmonella*, *E. coli*, *Klebsiella* and others that cause a

wide variety of intestinal and extra-intestinal infections. Clinically, many members of the family of Enterobacteriaceae are among the most potent and prevalent pathogens, indeed, many of them have acquired resistance to most antibiotics (Al-Kharousi et al., 2016).

Non-fermenting Gram-negative bacilli (NFGNB) have emerged as important healthcare-associated pathogens. NFGNB have emerged as important healthcare-associated pathogens and is important to identify these bacilli members considering the intrinsic multidrug resistance exhibited by these bacteria and NFGNB are representing by *Pseudomonas aeruginosa*, *Acinetobacter* spp. and *Burkholderia cepacian*. The importance of isolation of non-fermenters has increased after reports that are correlating them with the respiratory infection outbreaks in hospitals, or healthcare-associated infections (Chawla et al., 2013).

It was observed that yeasts were predominant in all samples collected from area A, with *Rhodotorula* spp. (50%), and *Candida albicans* (7%). *Non-albicans Candida* group was isolated in both areas (48%). Among the filamentous fungi, *Aspergillus* spp. were predominant (21%) and *Trichoderma* spp., *Cladosporium* spp. and *Rhizopus* spp. were present in 14% of all ATMs, respectively. *Penicillium* spp. were isolated in only 7% of samples. The results of CFU fungal enumeration is demonstrated in Table 6. Data about fungal contamination in ATMs were described in a few studies. Nagajothi et al. (2015) reported a small frequency in ATMs in Puducherry (India), such as *Aspergillus* spp.

(4.76%) and *Mucor* spp. (2.38%). The ATMs in Madurai city (India) fungal contamination included *Aspergillus* spp., *Mucor* spp., *Penicillium* spp. and *Fusarium* spp. (Mabel et al., 2014). Mbajiuka (2015) described that on the keyboards of ATMs in Nigeria were contaminated with fungi such as *Rhizopus* spp. (47.5%), *Aspergillus* spp. (22.5%) and *Penicillium* spp. (20%), corroborate the data of the present study in São Paulo city.

Özkan (2016) compared the fungal contamination on 30 ATMs and 30 bank cards in Marmaris (Turkey). The *Aspergillus* genera were the most dominant followed by *Alternaria*, *Cladosporium* and *Penicillium* genera. According to the author, *Aspergillus* spp. are widespread in nature because they produce many spores, have wide ecological tolerance, can easily spread through the atmosphere, and they can contaminate almost any kind of surface. It was observed *Cladophialophora*, *Cunninghamella*, *Drechslera*, *Fusarium*, *Geotrichum*, *Rhizopus*, *Scopulariopsis* and *Trichoderma* in ATMs located in Turkey.

Filamentous fungi vary quantitatively and qualitatively because of different geographical and climatological factors and the number and diversity of users. However, the author did not research yeasts in samples. In the present study, the yeasts *Candida albicans*, Non-*albicans* *Candida* and *Rhodotorula* spp. were isolated from ATMs in São Paulo city. Some authors have been documented that even low levels of bacteria such *Salmonella* spp. and some *E. coli* strains survive on dry surfaces for long periods and can easily be transferred from the fingers to food surfaces, which lead to acute ailments. *E. coli* serovars has been implicated in major food borne disease outbreaks and infections, mainly from eating contaminated hamburger. *S. aureus*, *K. pneumoniae* and *P. aeruginosa* are all well documented for their high pathogenicity, causing death in outbreaks and respiratory infections. Other microbes isolated such *Streptococcus* spp. are known opportunistic pathogens in infections and food spoilers (Rusin et al., 2002; Mead et al., 1999; Kaluski et al., 2006; Mabel et al., 2014).

About the fungi, *Cladosporium* species show a world-wide distribution and they are the most common airborne fungi and they are isolated at high frequency as contaminants. *Penicillium* species are common contaminants on various substrates and are known as potential mycotoxin producers. Opportunistic infections leading to mycotic keratitis, otomycosis and endocarditis have been reported. Its spores and components in atmosphere entering from respiratory tract affect the human health and may cause allergic reactions. *Rhizopus* species is one of the factors of opportunistic fungal infection, as skin and mucous membranes allergies. *Trichoderma* is a very common genus particularly in soil and decaying wood that attacks to hair keratin by boring hyphae (Özkan, 2016; Domsch et al., 1980; Ellis et al., 2007). Table 7 describes the

microbiological risks of pathogens isolated in the present study.

Özkan (2016) found in ATMs a fungi mycotoxin producer which can cause allergic diseases and various mycoses in humans and pointed the importance of complying with environmental and personal hygiene, because the ATMs were more contaminated than the bank cards in terms of fungal contamination. This can be explained by the situation of ATMs which are open to the atmosphere (without being in a cabin) and are cleaned rarely.

Many devices as cell phones are used in hospital indoor areas and presents risks to external population or community. Therefore, they can be vehicles for transmitting pathogens to patients and these contaminants moved by people may cause an epidemic or even pandemic situation (Zakai et al., 2016). This gains more importance in tourism and resort cities which is visited by several million people each year. Therefore, the common tools and goods used by people must be cleaned necessarily for not being a source of infection. Because visitors can bring any infection and can lead the infection spread where they contact (Özkan, 2016).

Frequent disinfection of the keyboards and screen parts, using antibacterial covers for the contact surfaces, or using alcohol wipes before and after use may be benefit for limiting the bacterial accumulation and transmission with cash machines (Tekerekoğlu et al., 2013). Based on these findings, it is recommendable to disinfect the hands after entering work area, mainly in a hospital, in order to hinder the spread of critical pathogens in the personal environment or in the hospital (Mahmoudi et al., 2017).

ATMs must be cleaned frequently with appropriate disinfectant for not being the source of infection. Such technological tools which are used by a large part of the population should be cleaned carefully to supply personal and environmental hygiene to not take the risk of causing epidemics or pandemics. The promotion of such research in order to protect the public health and to control the fungal disease is becoming a necessity. It is expected that this study will provide benefits to other studies and serve as a resource (Özkan, 2016).

According to Mbajiuka (2015) no reported outbreak of infectious or no-infectious epidemic has been noted in connection with the use of ATM, there is all indications that this device that serve as a cash dispensing machine may also serve as disease dispensing machine due of various hygienic conditions users, for daily financial transactions. It is imperative to adopt some measures aimed at curtailing the outbreak of disease transmission by the ATM surfaces and the author suggested some measures:

(i) Regular washing of hands before and after using the machines.

Table 7. Microbiological risks of pathogens isolated in ATMs in São Paulo city.

Gram positive coccus	Major pathogenic risk to human health
<i>Staphylococcus aureus</i> ; Coagulase-negative staphylococci (CoNS); <i>Streptococcus</i> spp.; <i>Enterococcus</i> spp.	Infections caused by multidrug-resistant Gram-positive bacteria represent a major public health in terms of morbidity and mortality, but also in terms of increased expenditure on patient management and implementation of infection control measures. <i>Staphylococcus aureus</i> and <i>Enterococcus</i> spp. are established pathogens in the hospital environment, and their frequent multidrug resistance complicates therapy. Vancomycin-resistant enterococci are also widespread (Woodford and Livermore, 2009).
Gram negative bacilli	Major pathogenic risk to human health
<i>Pseudomonas aeruginosa</i> Enterobacteriaceae (<i>Escherichia coli</i> ; <i>Salmonella</i> spp.; <i>Klebsiella</i> spp.)	Rising rates of drug resistance in Gram-negative bacteria and patients who develop drug-resistant hospital-acquired infections are at greater risk for longer hospital stays, complications, and mortality. Prevention of infections caused by GNB including <i>Klebsiella pneumoniae</i> and <i>Pseudomonas aeruginosa</i> , are particularly critical due to the limited treatment options. In early 2017, the World Health Organization declared that <i>P. aeruginosa</i> and Enterobacteriaceae were priority pathogens that urgently required new antibiotic development due to widespread multi-drug resistance (Agarwal and Larson, 2018).
Yeasts	Major pathogenic risk to human health
<i>Candida albicans</i> ; Non- <i>albicans</i> <i>Candida</i> (<i>Candida krusei</i> ; <i>Candida tropicalis</i> ; <i>Candida glabrata</i>)	Invasive candidiasis (IC) encompasses severe diseases such as candidemia, endocarditis, disseminated infections, central nervous system infections, endophthalmitis, and osteomyelitis. Immunosuppressive diseases, hematopoietic stem cell or solid organ transplantation, the use of wide-spectrum antibiotics or corticosteroids, invasive interventions, aggressive chemotherapy, parenteral alimentation, and internal prosthetic devices increase the risk of candidiasis and the infection still causes high mortality rates (Pappas, 2006).
<i>Rhodotorula</i> spp.	Most of the cases of infection due to <i>Rhodotorula</i> in humans were fungemia associated with central venous catheter (CVC) use. The most common underlying diseases included solid and haematologic malignancies in patients who were receiving corticosteroids and cytotoxic drugs, the presence of CVC, and the use of broad-spectrum antibiotics. Unlike fungemia, some of the other localized infections caused by <i>Rhodotorula</i> , including meningeal, skin, ocular, peritoneal, and prosthetic joint infections, are not necessarily linked to the use of CVCs or immunosuppression (Wirth and Goldani, 2012).
Filamentous fungi	Major pathogenic risk to human health
<i>Aspergillus</i> spp.; <i>Penicillium</i> spp.; <i>Trichoderma</i> spp.; <i>Cladosporium</i> spp.; <i>Rhizopus</i> spp.	Filamentous fungi occur widely in the environment, contaminating soil, air, food and other substrates. Some filamentous fungi have been reported to cause both superficial infections in the case of skin and nail infections, as well as invasive infections particularly in immunocompromised individuals. <i>Aspergillus</i> , <i>Penicillium</i> and <i>Cladosporium</i> are some of the genera of fungi which belong to the family of filamentous fungi have been reported in association with infections and disease. For example, the genus <i>Aspergillus</i> causes diseases including localized infections, fatal diseases, allergic responses, and inhaled conidia in humans and their associated health hazards in various indoor environments worldwide (Mousavi et al., 2016; Egbuta et al., 2017).

- (ii) Regular maintenance of cleaning regime of these devices by the managements.
- (iii) Avoiding using hands to eat snacks before and after using this device as it may lead to cross-contaminations of customers fingers.
- (iv) Using alcohols and other antiseptics to clean all the surfaces that people can place their hands while using the ATM.
- (v) Persons who have sign of contagious or infectious diseases should refrain from using this device until health conditions becomes stable again.

Conclusion

Such technological tools which are used by a large part of the population should be cleaned carefully to supply personal and environmental hygiene to not take the risk of causing epidemics. ATMs are used by many people must be cleaned frequently with appropriate disinfectant for not being the source of infection. Therefore, adequate personal hygiene and regular routine cleaning of these machines by the authorities is recommended. The promotion of such research in order to protect the public

health and to control the fungal disease is becoming a necessity. It is expected that this study will provide benefits to other studies and serve as a resource to prevent the spread of infection diseases in hospital areas.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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