

Original Research Article

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## Plasmid Profile and Antimicrobial Susceptibility Pattern of *Staphylococcus* sp. Isolated from High Touch Areas within the University of Port Harcourt Nigeria

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### ABSTRACT

The present study was conducted to determine and to compare the microbial population of ATM and computer keyboard within the University of Port Harcourt and also to determine the role of plasmid and resistance in *Staphylococcus* sp isolated from the high touch areas. A total of 70 swabbed samples was collected and cultured on different media and the organisms were identified using its colonial, morphological biochemical characteristics. The identified bacteria were subjected to antibiotic sensitivity test using the disc diffusion method. The plasmid of isolated *S. aureus* was cured by treatment with acridine orange. Results obtained from the study indicated that some of the ATM and the keyboards were positive for the presence of microorganisms which include *Staphylococcus aureus*, *Streptococcus* sp, *Klebsiella* sp, *Escherichia coli*, *Pseudomonas* sp and *Micrococcus* sp. The antibiogram study was carried out on the identified organisms and the result showed that majority of the bacteria were resistant to most of the standard antibiotics including cloxacillin, followed by augmentin, erythromycin, tetracycline, and streptomycin. *Staphylococcus* sp were subjected to plasmid profiling with the use of acridine orange to detect the role of plasmid in their resistance and it was observed that they were susceptible to chloramphenicol (98%), Gentamycin (94%), cotrimoxazole (78%), streptomycin (60%), tetracycline (58%), augmentin (52%) and cloxacillin (40%) because some antibiotic markers in *Staphylococcus* isolates are plasmid mediated. Hand washing after ATM and computer usage should be adopted to avoid contamination.

#### Keywords

Antibiotics, Disc diffusion, High touch area, Plasmid profile, *S. aureus*.

#### Article Info

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### Introduction

Today, ATMs are widely used by many people, and they tend to harbor the microorganisms on their surfaces. The hand borne transmission through ATM is one of the most important routes for the spread of infectious agents in the community (Forbes *et al.*, 2012). Contamination occurs everywhere including environment and all its objects. Computers keyboards and mouse, ATM keyboards, escalator handrails and other high

touch areas are the most open surface parts of computer which show 100% contamination. Computers continue to have an increased presence in almost every aspect of our occupational, recreational and residential environments. They are used in almost every facility, in various university environments, students have indicated 100% access to computers, 92.1% regularly use internet and 73.3% regularly use e-mail especially in

developed countries (Palmer and Bray, 2001). The ability of these high touch areas may act as a reservoir for the transmission of potential hazardous or pathogenic microorganisms (Hartman *et al.*, 2004). Inanimate surfaces can act as fomites, studies on this has been carried out previously in hospital and community environment (Huber and Pelon, 2005). About 80% of infections are spread through hand contact with hands or other objects.

Most high-touch areas are touched by the bare hands of people who are in various hygienic conditions. People most times believe that microbes are only present in research labs or in hospitals and clinics and often have a misleading feeling of security in other places. Lack of knowledge of the source of germs could be the main cause of health problems (Reynolds *et al.*, 2000). Bacteria can be transmitted from tapping on the keys and regular usage of the device and ATM machines which may incur contaminants such blood, secretions, or other various sticky substances in the environment (Fukata *et al.*, 2008).

*Staphylococcus aureus* are usually found on skin or in the nasal environment and only survives on dry skin on the outside of the body. They can appear on keyboards quite a bit as a result of usage. The computers keyboard and a mouse have a very dynamic environment. Food and eventually settle on the keyboard through the air or from physical contact. Cleaning our keyboard and mouse regularly is very important, although most people ignore it. Only about half of computer users' clean their computer keyboards at least once a month. Often time's keyboards are shared with multiple people; this makes it much more dangerous surface when it comes to passing diseases. If we use our own keyboard and mouse and nobody else uses it, the chances of that keyboard and mouse serving as a method of transmission is fairly

small, but where sharing keyboards is concerned there is a higher probability of transmission occurring. Some of the microbes that can be found in the finger and in the hand are *Enterobacter*, *Klebsiella* sp, *Aeromonas* sp, *Serratia* sp, *Pseudomonas* sp (Wargo *et al.*, 2006). An Automated Teller Machine (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). Contamination of environmental objects and surfaces is a common phenomenon. Humans have a marked tendency to pick up microorganism from environmental object, and the hand has been shown to play a role in the transmission of organisms. The ATM machine is likely to be contaminated with various microorganisms due to their vast contact by multiple users. There is no restriction as to who has access to the facility and no guideline to ensure hygiene usage. But like all surfaces microbial colonization of these metallic keypads are eminent, particularly when there are no proper cleaning regimes in place for most of these facilities (Abban and Tano-Derah, 2011).

## **Materials and Methods**

### **Sample collection**

A total of 70 swabs were used for the study, 10 swabs from FDB ATM gallery in Ofirima the Faculty of Science, 10 from UBA ATM gallery in Abuja campus and 10 from FBN ATM on Choba. Twenty swabs were obtained from computer keyboards in Choba Park Business center, 20 from computer keyboards in Abuja campus.

### **Isolation *S. aureus***

Swabs collected were placed in a test-tube containing sterilized normal saline and a ten-fold serial dilution was carried out. 0.1ml

each of the samples were cultured on solidified nutrient agar plate (Oxoid) and mannitol salt agar. Plates were incubated at 37°C for 24 hours. Colonies were observed and counted in the plates. The isolates were subcultured onto nutrient agar and incubated at 37°C for 24 hours. Biochemical tests were carried out on the isolates for identification.

### **Antibiotic susceptibility testing**

Antibiotic susceptibility testing was carried out using the disc diffusion method, in brief; 3mls of normal saline was dispensed into test tubes and pure culture of *S. aureus* was inoculated into each of the test tubes containing the normal saline and was compared with the prepared McFarland standard to check if they were of equivalence turbidity. After this, swab sticks were placed into each of the test tubes and then were drained a little to discard excess liquid before swabbing on the surface of the nutrient agar plate. The antibiotic Gram positive disc was gently picked from the container using sterile forceps and was placed at the center of the Muller Hinton (Oxoid) agar plates and incubated at 37°C for 24 hours. After incubation the diameter zone of inhibition was measured.

### **Plasmid Curing of *S. aureus* isolates**

The plasmid was cured by treatment with acridine orange according to the method of Brown (2000). Fifty (50) mg of acridine orange was dissolved in 10ml of distilled water to prepare a stock solution which was stored in the refrigerator. From the stock solution 1ml of acridine orange solution and 0.5ml of glacial acetic acid was added to 50ml of distilled water for the preparation of the working solution.

Nutrient broth was prepared and supplemented with 0.1mg/ml acridine orange. 20microliter of overnight culture of the

bacteria was subcultured into 5mls of the nutrient broth containing acridine orange. The samples were incubated at 37°C for 72 hours. The isolates were sub-cultured onto Muller Hinton agar and an antibiotic sensitivity disc was placed on the media. The plates were inverted and incubated at 37°C for 24 hours. Zones of inhibition on the plate were measured using a transparent meter rule.

### **Results and Discussion**

The findings of this study showed that ATM keyboards can be considered a source of bacterial infections, similar to other contaminated surfaces in public places, such as telephones and door handles. Because most people with different levels of hygiene and health standards use these machines, they can be widely involved in absorbing, harboring, and transferring infectious microorganisms. Contaminated hands touching an ATM keyboard can transfer pathogens to the keyboard and bills, ultimately facilitating the spread of infectious diseases (Chirman *et al.*, 2011; Rusin *et al.*, 2002). A total of 70 swabs were obtained from ATM button and computer keyboard.

The bacterial load on the FDB ATM in Ofirima was from  $1.75 \times 10^5$ - $2.06 \times 10^5$  CFU/ml and the bacterial load of UBA ATM button in Abuja campus range from  $2.53 \times 10^5$ - $2.75 \times 10^5$  CFU/ml. Further From the FBNATM machine in Choba area, the bacterial load was  $2.25 \times 10^5$ -  $2.85 \times 10^5$  CFU/ml. The computers used by the computers (more than 12-18 persons were using that particular computer per day) in Choba showed a bacterial load of  $2.25 \times 10^5$ - $2.73 \times 10^5$  CFU/ml and the computer used in Abuja campus by more than 5-10 persons per day showed a bacterial load of  $1.95 \times 10^5$ - $2.47 \times 10^5$  CFU/ml. From the study it was observed that the computers operated by multiple clients in the computer center at

Choba and the FBN ATM in Choba are at high risk of transferring the pathogenic bacteria to the users and as ATM machines are operated by all sections of people frequently. Microorganisms can be transmitted into computer keyboards by the air the computer user breathes out; sneezing, digging nose during work by hands or other reactions leaves the keyboard contaminated. Detailed result of microbial area count of computer keyboard and ATM button in Ofirima, Abuja campus and in Choba are shown in Table 1.

The microorganism were identified using their morphology characteristics and the biochemical test and the highest number of organism that occurred was *Staphylococcus aureus* followed by *Klebsiella* sp, *Bacillus* sp, *Micrococcus* sp, *E.coli*, *Proteus vulgaris* and *Pseudomonas* sp (Table 2).

All the *Staphylococcus aureus* were tested for their sensitivity to eight (8) types of antibiotics and it was observed that some of these *Staphylococcus* are highly resistant to standard antibiotics such as cloxacillin followed by Augmentin, erythromycin, and tetracycline. Gentamycin and chloramphenicol was highly susceptible as shown in Figure 1. The *Staphylococcus* isolates were also subjected to plasmid curing and sensitivity

testing was to detect the role of plasmid in their resistance. Result obtained showed that *Staphylococcus aureus* was more susceptible to the antibiotics Chloramphenicol (98%), Gentamycin (94%), Cotrimoxazole (78%), Streptomycin (60%), Tetracycline (58%) and Augmentin (52%) as indicated in Figure 2.

ATM machines and computer keyboard have been used all over the world. Today the ATM machines are not only used for cash supply to the customer but also they give service on different kind of monetary procedures such as money transfer, stock market transaction and bill payment. In a systematic review, Kramer *et al.*, (2006), reported that many bacterial and viral pathogens could survive on the inanimate objects and such pathogens could cause epidemic infections as a result of direct or indirect transmission in hand-object susceptible patient. Dogon *et al.*, (2008), found many types of pathogens on the computer keyboard which were used in education institutes and Tekerekoglu *et al.*, (2011), reported that ATM buttons and computer keyboard carried multi-drug resistant hospital pathogens including, *Acinetobacter* sp, *S. aureus* and extended-spectrum beta lactamase ESBL-positive enterobacteriaceae and hence suggested that frequent disinfection on computer keyboard to reduce bacteria reservoir.

**Table.1** Microbial Area Count of computer keyboard and ATM button in Ofirima, Abuja campus and Choba

S/No	Area	Number of Swab Samples	Quantity(CFU/ml) (minimum-maximum)	Isolate Code
1.	Fidelity ATM gallery, Ofinima	10	1.75×10 <sup>5</sup> -2.06×10 <sup>5</sup>	FIO
2.	UBA, ATM gallery, Abuja	10	2.53×10 <sup>5</sup> -2.75×10 <sup>5</sup>	UIA
3.	First Bank ATM gallery, Choba	10	2.25×10 <sup>5</sup> -2.85×10 <sup>5</sup>	FIC
4.	Computer keyboard in Abuja park	20	1.95×10 <sup>5</sup> -2.47×10 <sup>5</sup>	CKA
5.	Computer keyboard in Choba	20	2.25×10 <sup>5</sup> -2.73×10 <sup>5</sup>	CKC

KEY: FIO: Fidelity Isolate OfirimaUIA: UBA Isolate AbujaFIC: First Bank Isolate ChobaCKA: Computer Keyboard AbujaCKC: Computer keyboard Choba

**Table.2** Biochemical reaction test

S/N	Isolate Code	Catalase	Coagulase	Oxidase	Citrate	Indole	MR	VP	Urease	Motility	TSIA			Sugar Fermentation			Probable Genera	
											Slant	Gas	H <sub>2</sub> S	Sucrose	Lactose	Glucose		
1	FIO1	+	+	-	+	-	-	+	-	-	A	A	-	-	+	+	+	<i>S.aureus</i>
2	FIO2	+	-	-	+	-	-	+	-	-	A	A	+	-	+	+	+	<i>Klebsiella sp</i>
3	FIO3	+	-	-	+	-	-	+	-	+	A	B	-	-	-	-	+	<i>Bacillus sp</i>
4	FIO4	+	+	-	+	-	-	+	-	-	A	A	-	-	+	+	+	<i>S.aureus</i>
5	UIA1	+	-	-	-	+	+	-	-	+	A	A	-	-	+	-	+	<i>E.coli</i>
6	UIA3	+	-	-	+	-	-	+	-	-	A	A	+	-	+	+	+	<i>Klebsiella sp</i>
7	FIO5	+	+	-	+	-	-	+	-	-	A	A	-	-	+	+	+	<i>S.aureus</i>
8	UIA4	+	-	-	-	+	+	-	+	+	A	A	+	+	-	-	+	<i>Proteus sp</i>
9	FIC1	+	-	-	-	+	-	+	-	-	A	A	+	-	+	-	+	<i>Streptococcus sp</i>
10	FIC3	+	-	-	+	-	-	+	-	+	A	B	-	-	+	-	+	<i>Serratia marsecens</i>
11	FIC5	+	-	-	+	-	-	+	-	+	A	A	-	-	+	-	+	<i>Micrococcus sp</i>
12	CKC2	+	-	-	+	-	-	+	-	-	A	A	+	-	+	+	+	<i>Klebsiella sp</i>
13	UIA2	+	-	-	-	+	-	-	-	+	A	A	-	-	+	-	+	<i>S.aureus</i>
14	UIA5	+	+	-	+	-	-	+	-	-	A	A	-	-	+	+	+	<i>S.aureus</i>
15	UIA6	+	+	-	+	-	-	+	-	-	A	A	-	-	+	+	+	<i>S.aureus</i>
16	FIC2	+	+	-	+	-	-	+	-	-	A	A	-	-	+	+	+	<i>S.aureus</i>
17	CKC3	+	-	+	-	+	-	+	-	-	A	B	-	-	-	-	+	<i>Pseudomonas sp</i>
18	FIC3	+	+	-	+	-	-	+	-	-	A	A	-	-	+	+	+	<i>S.aureus</i>
19	FIC6	+	+	-	+	-	-	+	-	-	A	A	-	-	+	+	+	<i>S.aureus</i>
20	CKA1-10	+	+	-	+	-	-	+	-	-	A	A	-	-	+	+	+	<i>S.aureus</i>

Key: + = positive VP = VogesProskauer - = negative MR = Methyl Red

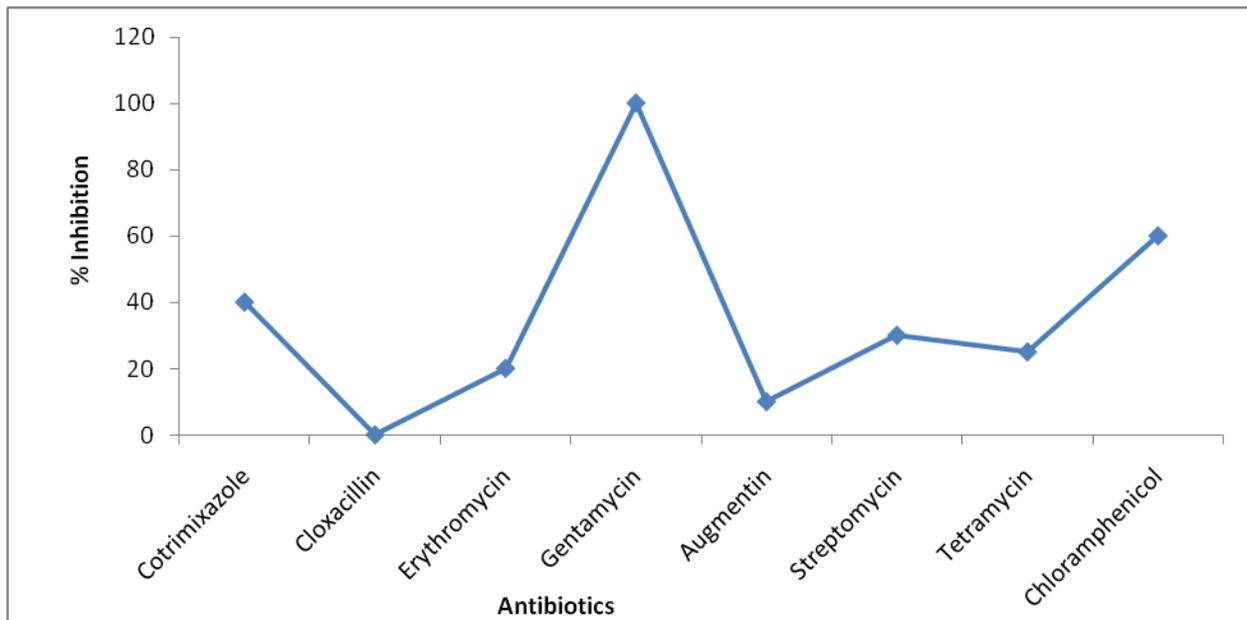
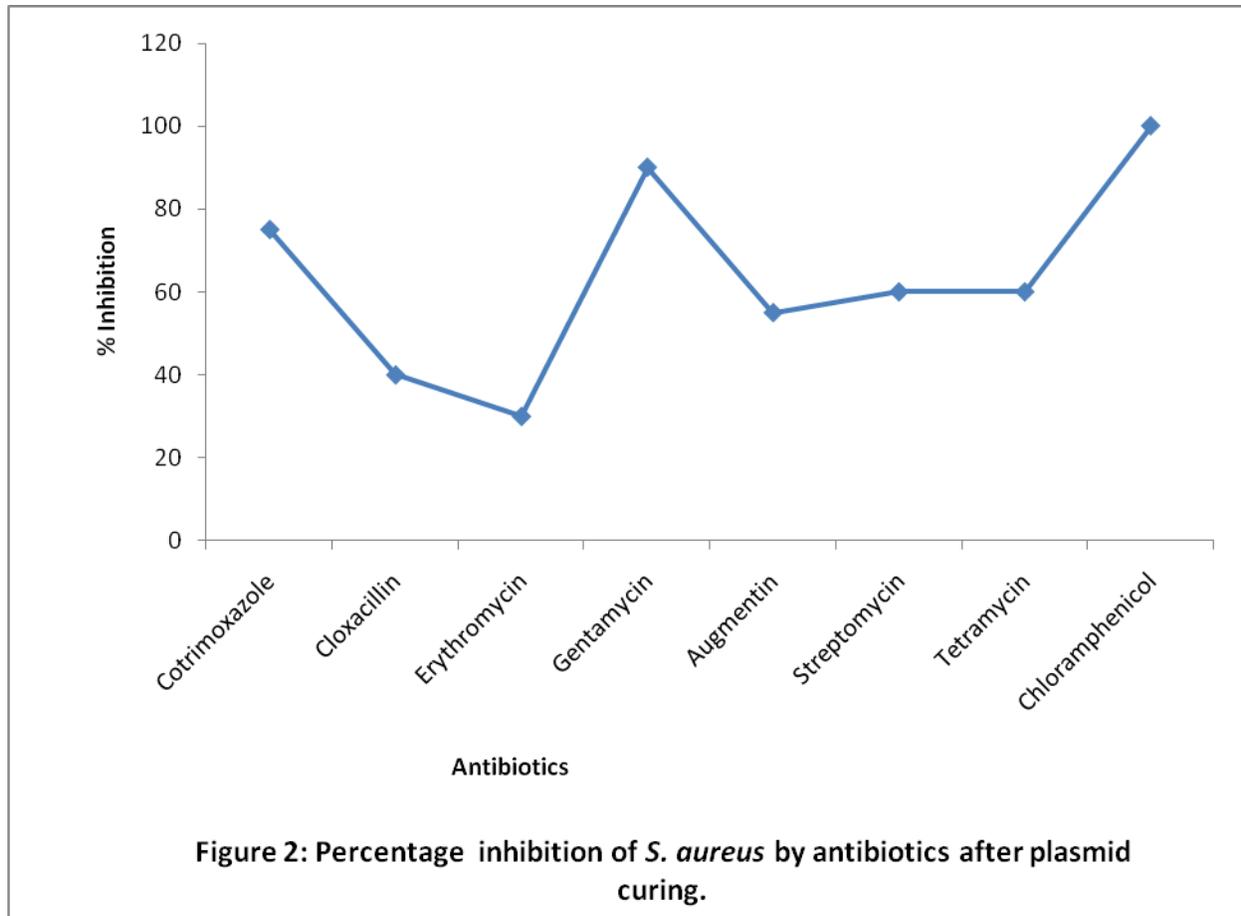


Figure 1: Percentage inhibition of *S. aureus* by antibiotics before curing.



In this study, we found out that all the studied ATMs and computer keyboards were positive for a bacterial strain and is also in agreement with the study carried out by Abban and Tano- Debrah (2011) who reported the presence of *Staphylococcus* species, *Escherichia coli* and *Klebsiella* species on the keypads of ATM machines, together with Anastasiades *et al.*, (2009) who reported that *Staphylococcus aureus* are prevalent on computer keyboards. Although the majority of the organisms detected were low virulent strains such as *Bacillus* sp, and some other pathogens including *S. aureus*, *Klebsiella* sp, *E. coli*, *Proteus vulgaris* and *Micrococcus* sp.

The present study revealed that *S. aureus* was the prevalent, this contradict the study carried out on ATM in Melli and Saderat banks in Iran by Mahmoudi *et al.*, (2017) showed that

the most frequently isolated bacteria were *Staphylococcus epidermidis* (18.5%), *Pseudomonas aeruginosa* (18.5%), *Bacillus subtilis* (16.9%), *Escherichia coli* (9.2%), *Klebsiella* spp. (12.3%), *Enterobacter* spp. (3.1%), *Bacillus cereus* (9.2%), *Staphylococcus aureus* in 3 (4.6%), and *Micrococcaceae* spp (7.69%). All isolated bacteria were susceptible to gentamicin, cephalotin, tobramycin, amikacin, norfloxacin, and vancomycin while *S. aureus* resistance rate to trimethoprim/sulfamethoxazole was 50%. Their study also showed the presence of higher percentage of Gram positive organisms to Gram negative which is also in confirmatory to the present study. A study carried out by Mbajiuka (2015) on ATM keyboards in Nigeria revealed the presence *Staphylococcus* spp been the highest in occurrence (82.5% (33),

*Bacillus* spp 62.5% (25), *Escherichia* spp, 22.5% (9) *Streptococcus* spp 15% (6). Fungal species were also isolated in their study such as *Rhizopus* spp with about 47.5% (19), *Aspergillus* spp, 22.5% (9) and Penicillin spp, 20% (8) were found on the keypads.

In the study carried out by Nwankwo and Offiah (2016) *E. coli* was the highest in occurrence (26.5%) followed by *S. aureus* (17.6%) and other organisms. This is contrary to our findings and some other researchers (Oluduro *et al.*, 2011). Their findings of *E. coli* been the highest in occurrence may be due to the fact *E. coli* is a normal flora of the gastrointestinal tract and thus makes it easily to be picked up from toilet door handles etc. due to low hygiene measures. This may explain its preponderance as a bacterial contaminant of shared user hardware interfaces. It has also been associated with numerous infectious disease conditions and nosocomial infections. It follows that since users constantly touch interfaces there is every chance of introducing *E. coli* onto the interface in use. Methicillin resistant *S. aureus* is known to be notorious in their acquisition of resistance to drugs. Many strains of *S. aureus* carry a wide variety of multi drug resistant genes on plasmid and so the resistances of *S. aureus* isolated from high touch areas such as the ATM, and computer keyboard have been widely reported. In this study, twenty *Staphylococcus aureus* was isolated and most of them were derived from First bank ATM gallery in Choba and computer keyboard in Abuja campus. The antibiogram result of this study showed that *Staphylococcus aureus* was 60% resistant to Cotrimoxazole, Erythromycin (80%), Gentamycin (10%), Augmentin (90%), Streptomycin (70%), Tetramycin (70%), Chloramphenicol (50%). After the plasmid curing, the *Staphylococcus aureus* was 70% susceptible to Cotrimoxazole, 40% in Cloxacillin, 30% in Erythromycin, 100% in

Gentamycin, 50% in Augmentin, 65% in Streptomycin, 60% in Tetramycin and 100% in Chloramphenicol because the antibiotic resistance markers in *S. aureus* isolates are plasmid mediated. A study carried out by Nagajothi *et al.*, (2015) Among *S. aureus*, 40% were resistant to Methicillin, 55% to penicillin followed by resistance to co-trimoxazole (27%), gentamycin (25%), erythromycin (20%), amikacin (15%), ciprofloxacin (5%), and clindamycin (5%). Twenty percent of CoNS were resistant to gentamicin followed by co-trimoxazole (15%), amikacin (10%), and ciprofloxacin (2%). Methicillin resistance was not observed in CoNS. There is a need to minimize the antibiotics pressure that favors the selection of methicillin resistant *S. aureus* (MRSA) strains in ATM gallery and computer keyboard. Although *S. aureus* are resistant to other antibiotics most especially Erythromycin followed by Cloxacillin, Augmentin, Streptomycin and Tetramycin.

Most high-touch areas are touched by the bare hands of people who are in various hygienic conditions. The public should be conscious that ATM, computer keyboards and other public high touch areas are not only useful but they are source of microbes and thus preventive and good hygiene measures should be put into consideration when using these high touch areas.

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