Scientific African 15 (2022) e01111

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

Occurrence, antibiotic susceptibility and resistance genes among *Staphylococcus aureus* isolated from keypads of automated teller machines (ATM) in a private university, Nigeria ☆

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ARTICLE INFO

Article history:
Received 14 June 2021
Revised 18 January 2022
Accepted 28 January 2022

Editor DR B Gyampoh

Keywords:
Antibiotic pattern
Automated teller machines
Cross-contamination
Staphylococcus aureus
Resistant genes

ABSTRACT

Environmental surfaces and objects associated with dermal contact by multiple users are suspects of being reservoirs of microorganisms; the ATM is likely to be one of such surfaces. *Staphylococcus aureus* is an opportunistic organism and causes infection. *S. aureus* is a major public health interest because of its antibiotic resistance attributes. Considering this fact, a study on the occurrence, susceptibility pattern, and resistant genes among *S. aureus* isolates from ATM keypads of three different banks located in a private university in Nigeria, was carried out. A total of 56 swabbed samples were collected from seven ATM keypads belonging to the three different banks, and cultured on Mannitol Salt Agar for the isolation of *Staphylococcus aureus*. Using disk diffusion technique, 0.5 McFarland of each isolate was screened for antibiotic susceptibility, on Mueller-Hinton agar. Also, six of the isolates were screened for genotypic characterization and the detection of β-lactam (*blaZ*) and macrolide (*ermB*) resistance genes. Out of 56 isolates obtained, 30 (54%) were phenotypically identified as *Staphylococcus aureus* using the standard biochemical test. The isolates were 100% resistant to augmentin, ceftazidime, ceftriaxone, cloxacillin and cefuroxime but were susceptible to gentamycin and ofloxacin. On the molecular level, four out of the six randomly selected isolates showed amplified base pair product for *S. aureus, blaZ* gene as well as the absence of the gene encoding *ermB*. The present result has shown the potential contamination of ATM keyboards with *Staphylococcus aureus* and the ATM keypads can also serve as prospective platforms for cross-contamination with antibiotic-resistant strains especially when frequently used by clients of affiliated financial institutions. Therefore, this implies that the hygiene practice of handwashing with soap and water after ATM usage should be further reinforced to reduce the level of contamination and spread of this pathogen.

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https://doi.org/10.1016/j.sciaf.2022.e01111

Introduction

An Automated Teller Machine or Automatic Teller Machine (ATM) is an electronic banking outlet that allows the customer to complete transaction without the aid of cashier or teller. ATMs can be found in city centers, business areas, and also, in and outside bank premises. People of different social levels and hygienic status visit ATMs daily for a quick-service transaction and the hands is a point of contact on the surfaces of the keypad devices.

The human surface tissue (skin) is constantly in contact with environmental microorganisms and becomes readily colonized by certain microbial species [1]. Human hands can spread microorganisms among persons and places in the environment. In this vein, human hands can spread normal microflora organisms and transit organisms that have been picked from the environment [2]. Pathogens spread among people through direct or indirect contact on hands or inanimate objects [3] and the longer a pathogen persists on a surface, the more it may be a source of transmission and thus endanger susceptible persons. Hence, cross-infection of microorganisms between environmental surfaces and a host can be established [4].

Staphylococcus aureus is a Gram-positive, rod-shaped, coagulase-positive, commensal bacterium that is usually found on the skin and nasal membranes of humans as well as on most mammals [5,6]. *S. aureus* is an opportunistic pathogen that can cause a variety of self-limiting to life-threatening diseases in humans [7]. It can cause superficial skin lesions such as boils and styles, deep-seated infections, urinary tract infections, toxic shock syndrome, and food poisoning [8]. *S. aureus* can survive for up to 12 days on abiotic surfaces [9] which may serve as a reservoir for contamination in people who come into contact with these surfaces. Several *S. aureus* strains are reputed to be phenotypically resistant to many groups of antibiotics [10], and different molecular genes such as *erm* gene, *blaZ* for beta-lactam and *mecA* for methicillin-resistant Staphylococcus aureus (MRSA) have been associated with these observable traits.

This research work targets the occurrence of *Staphylococcus aureus* on ATM keypads, the antibiogram pattern, and the resistant genes present on the implicated strains. This research is novel in that it is the first study to report the detection of chromosomal encoded *blaZ* genes in *S. aureus* isolates from community-acquired environment in the Nigerian environment especially from the ATM in a tertiary institution and this has several public health implications beyond Nigeria. Hitherto, other researchers have reported isolations that have been limited to clinical and animal sources.

Materials and methods

Sample collection

Swab sticks moistened in sterile Nutrient broth were used to collect samples from the keypads of seven different ATM booths belonging to three different banks; namely Bank A, Bank B, and Bank C, all situated within the premises of a private University in Nigeria. Bank C has five separate booths at different position on campus while banks A and B have only one booth each. The entire surface of the keypad at each ATM booth, was double swabbed, from left to right, during each sampling process. Samples were collected in the morning and evening sessions of a day, for four weeks in September 2017. After each sample collection, the swab sticks were taken for analysis, in the laboratory.

Isolation and identification of bacteria

Isolation of the micro-organism was carried out using the spread plate technique, as described by [11]. Each swab stick was streaked on Petri plates on Mannitol salt agar (Lab M, UK), and incubated at 37 °C for 24 h to get single colonies. Growth of pure yellow-pigmented colonies, which were also Gram-positive, coagulase, and catalase-positive, were phenotypically identified as *S. aureus* [12].

Susceptibility testing

The standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility profiles of the phenotypically identified isolates [13]. The inoculum of each test *S. aureus* isolate was prepared by suspending the freshly grown bacteria in 5 ml sterile peptone water and the turbidity adjusted to 0.5 McFarland standard. The antimicrobial susceptibility test was performed by inoculating the test organism on labeled plates containing sterile Mueller-Hinton medium and standard discs of gentamicin (10 µg), augmentin (30 µg), ofloxacin (5 µg), ceftazidime (30 µg), and cefuroxime (30 µg) added. Incubation of the inoculated plates was at 37 °C, for 24 h. Measurement of inhibition zones produced was with a transparent meter rule and recorded in millimeters and the values interpreted following the Clinical Laboratory Standard Institute [14] standards.

Molecular characterization of S. aureus

To validate the identification of *S. aureus*, six out of the phenotypically identified isolates were randomly picked and subjected to genotypic characterization, using PCR amplification of the 16S rRNA, for the identification of *S. aureus* using methods described by [15]. The boiling method as described by [16] was used for genomic DNA extraction. The list of primers

used in the study were Staph756F (5-AACTCTGTTATTAGGGAAGAACA-3) 756 bp, Staph750R (5-CCACCTTCCTCCGGTTTGTCACC-3)756 bp, MecA1(5-GTAGAAATGACTGAACGTCCGATAA-3) and MecA2 (5-CCAATTCCACATTGTTTCGGTCTAA-3) 532 bp [17].

PCR amplification of resistant genes

Furthermore, these same six, randomly selected phenotypically identified *S. aureus*, as described above, were subjected to a polymerase chain reaction (PCR) for detection of resistance gene that encoded for β lactams and macrolides via *bla*Z and *ermB* using previously published primers and methods by [18,19]. The list of primers used in the study was *Bla*Z 1F (5-TGAGGCTTCAATGACATATAGTGATAA-3), BlaZ 1R (5-GTTCAGATTGGCCCTTAGGA-3) 404 bp, BlaZ 2R (5-ACTTCAACACCTGCTGCTTTC-3) 173 bp, Erm B1 (5-CATTTACGACGAAACTGGC-3) and Erm B2 (5-GGAACATCTGTGGTATGCCG-3) 836–1260 bp.

Data analysis

One-way between-groups analysis of variance (ANOVA) was conducted to determine if there was a statistically significant difference in the occurrence of *Staphylococcus aureus* on the ATM keypads of the different banks at each of the stipulated time sessions and regardless of the time sessions, while an independent-samples *t*-test was carried out to compare the occurrence of the pathogen on the ATM keypads of the different banks, between the different time sessions.

The total number of isolates utilized for the molecular study was six, in the entire research work. This limited number of isolates did not make performing data analysis on the molecular aspect of the work possible.

Results and discussion

Identification of target isolates

Out of 56 surface swab samples from ATM keypads collected, 30 (54%) bacterial isolates produced golden-yellow pigmentation on MSA.These were phenotypically affirmed *S. aureus* after showing positive for Gram-positive, catalase-positive, and coagulase-positive [20].

Occurrence of Staphylococcus aureus

S. aureus was widespread on all the ATM keypads examined. The occurrence (54%) of *S. aureus* in this research correlates with the report of Oluduro et al. [21] who, not only reported on the occurrence of 35.8% for *S. aureus* isolated among other bacteria from electronic hardware such as computer keypads and ATM at Ile-Ife, Nigeria but that *S. aureus* was also the most frequent bacterial contaminant. Similarly, Anele et al. [22] have reported 17.5% occurrence of *S. aureus* among other bacterial organisms isolated from ATM keypads in Port Harcourt in Nigeria.

The occurrence of *S. aureus* on the sampled ATM has supported the reports that these keypads could be platforms for bacterial cross-contamination and infection, as have been ascribed to surfaces of mobile phones, computers and doorknobs. Furthermore, [23,24] have revealed that the use of an ATM can serve as a possible health risk associated with dermal contact, particularly when contaminated hands touch ATM keypads. The same authors have suggested that contaminated hands could be vehicles that spread infectious agents to keypads, and subsequently, susceptible human hosts.

The 54% occurrence obtained in this study, may be attributed to the fact that *S. aureus* is a normal skin microflora and easily transferable to surfaces through simple dermal contact, via abrasives or touch. Also, the human palm is usually moist due to varying degrees of perspiration, containing sodium chloride that sustains the growth of this halophilic organism [25]. sneezing, talking and touch are other activities that can spread *S. aureus* [26]. Furthermore, the growth, survival and infectious rate of *S. aureus,* via the air/ atmosphere, is also dependent on a number of environmental factors such as temperature, relative humidity, and the presence of oxygen; though these physical growth parameters may vary for different *S. aureus* strains.

Fig. 1, showed the occurrence of *S. aureus* on the keypads of the different bank's ATMs, in morning and afternoon samplings. In the morning samplings, there was a 100% occurrence of *S. aureus* on the keypads belonging to Bank C located at positions 4 and 5 on the university campus. For afternoon sampling, only the keypads belonging to Bank C, located at position 1 had a 100% incidence of *S. aureus*.

The observation that the percentage occurrence of *S. aureus* varied with the time of sampling and the location of a bank's ATM may be attributed to its level of patronage by the bank's clients or the functional condition of the ATM or the combination of both. When a bank's ATM is unable to dispense cash or perhaps the ATM is "out of service", such ATM booths experience less patronage by users. Furthermore, many users rush to the ATM, in early morning or afternoon to fulfill pressing financial obligations. Nevertheless, there was no significant difference in the occurrence of *S. aureus* on the ATM keypads among the different banks either in the mornings, afternoons, or between these same specified sessions.

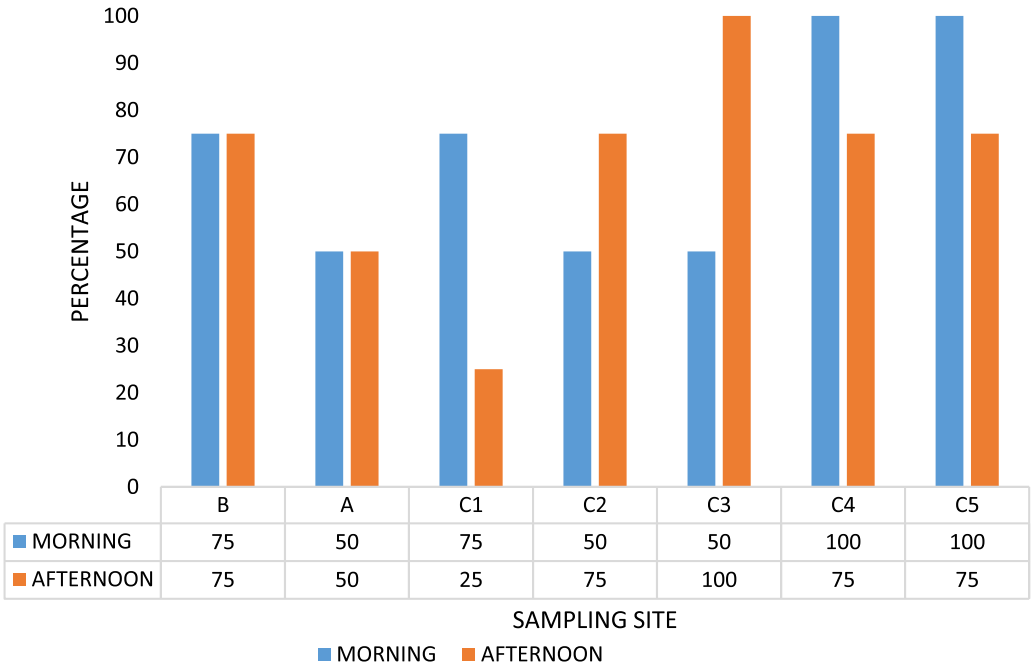

Fig. 1. Percentage occurrence of *Staphylococcus aureus* on the ATM keypads of different banks in the morning and afternoon periods.

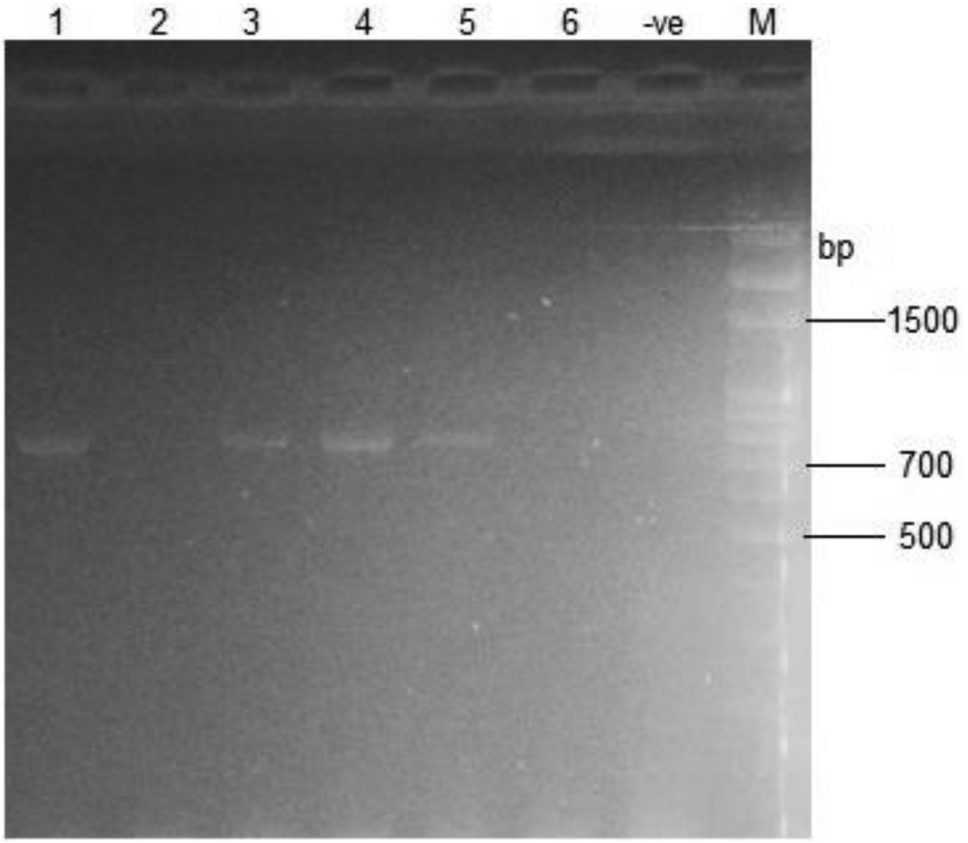

Fig. 2. 16S rRNA Fingerprint of *Staphylococcus aureus* amplified on 1.5% agarose gel
Lane M: Marker. (100 bp DNA ladder); Lane 1: Bank C4 ATM keypad, Lane 2: Bank A ATM keypad, Lane 3: Bank C3ATM keypad, Lane 4: Bank C5 ATM keypad, Lane 5: Bank B ATM keypad, Lane 6: Bank C1ATM keypad.

Table 1
Weekly antibiotic susceptibility (%) profile of *Staphylococcus aureus*.

	Week 1			Week 2			Week 3			Week 4		
Antibiotics	R	I	S	R	I	S	R	I	S	R	I	S
CAZ	100	0	0	100	0	0	100	0	0	100	0	0
CRX	100	0	0	100	0	0	100	0	0	100	0	0
GEN	0	0	100	0	0	100	11	11	78	0	9	91
CTR	100	0	0	100	0	0	100	0	0	100	0	0
ERY	70	30	0	90	10	0	100	0	0	64	36	0
CXC	100	0	0	100	0	0	100	0	0	100	0	0
OFL	0	0	100	0	0	100	0	0	100	0	0	100
AUG	100	0	0	100	0	0	100	0	0	100	0	0

Key: Ceftazidime(CAZ), Cefuroxime (CRX), Gentamicin (GEN), Ceftriaxone (CTR), Erythromycin (ERY), Clooxacillin (CXC), Ofloxacin (OFL), Augmentin (AUG). n: Number of *Staphylococcus aureus* screened each week. R-Resistance, Intermediate, S- Susceptibility.

PCR amplification of S. aureus

Fig. 2 showed the PCR amplification of six *S aureus* isolates using 16S rRNA primer.

In the present study, four out of six *S. aureus* isolates were amplified, and classified by PCR using specific primers 16S rRNA region for housekeeping genes to molecularly confirm *S. aureus*. Results indicated that four *S aureus* in Lanes 1, 3, 4, and 5 had coagulase genes while isolates on Lane 2 and 6 do not. Coagulase testing has been known to be the most reliable method for identifying *Staphylococcus aureus* [27]. However, the observation that two out of the selected six phenotypically affirmed *S. aureus* isolates were negative for PCR amplification of *S. aureus* is not surprising. This negative result, at molecularly level, is indicative that these strains do not contain *S. aureus* identification marker. Nevertheless, other physiologically, coagulase-positive staphylococci (CoPS) strains besides *S. aureus*, have been known to include *S. intermedius, S. schleiferi* subsp *coagulans, S. delphini,* and *S. hyicus*; (of which these two isolates on lane 2 and 6 might be) and are commensals of animals [28]. It is likely that one or several users of the ATM booths had previous dermal contact with animals and hence, the subsequent transmission to the keypads [28–30]. The university campus has animal breeding farms and attendant workers. 16S rRNA sequencing has been suggested to be the gold standard for the identification of bacteria [31].

Antibiotic resistance pattern of S. aureus *isolates*

Antibiotic resistance pattern for *S. aureus* isolates obtained in the weeks of study is shown in Table 1 and interpreted as resistance, intermediate, and susceptibility. For each of the weeks sampled, *S. aureus* showed consistent 100% resistance to augmentin, ceftazidime, ceftriaxone, cefuroxime, and cloxacillin. Some *S. aureus* showed varied susceptibility to different antibiotics as the weeks progressed.

The antibiotic resistance pattern of *S. aureus* obtained in this study corroborates with the report of Agbagwa and Ibeachu [32], who also recounted that *S. aureus* showed resistance to similar antibiotics. It was observed in this work that *S. aureus* showed 100% resistance to commonly used antibiotics such as ceftazidime, cefuroxime, ceftriaxone, erythromycin, cloxacillin, and augmentin. Khan and Malik [33] have also reported similar trend with the use ofsimilar antibiotics.

Furthermore, Onuoha and Fatokun [34] have reported 100% susceptibility to gentamicin and ofloxacin, an observation similarly made in this study. This may suggest the use of these antibiotics as therapeutic drugs for the treatment of *S. aureus* infection within this locality.

The antibiotic resistance pattern of *S. aureus* obtained at the different bank's ATM booths was the same, despite the sampling time. Possibly because, perhaps the implicated antibiotics may be frequently used by the populace patronizing the ATMs or as a result of misuse of antibiotics and indiscriminate purchase.

The result of amplification for the resistant genes *bla*Z and ermB responsible for resistance to β lactam and macrolides is shown in Fig. 3 and Fig. 4, respectively. The result revealed *bla*Z gene in four out of the six isolates; isolate on Lanes 1 and 6 showed specific DNA bands of *bla*Z gene (173 bp) while Lanes 4 and 5 showed specific DNA bands of *bla*Z gene (404 bp), while no ermB gene was detected in all the six isolates amplified.

*Bla*Z gene is a plasmid or chromosome inducible enzyme that encodes for the production of β-lactamase capable of hydrolyzing β-lactam antibiotics [35] which include ceftazidime, cefuroxime, ceftriaxone, cloxacillin, and augmentin. In this study, *S. aureus* had developed 100% resistance to these specified antibiotics. β-lactam antibiotics are the cheapest, easiest to produce, and most effective classes [6]. When staphylococci habouring b*laZ* gene produce beta-lactamase enzyme, the cleavage of the β-lactam ring contained in the β-lactam antibiotics becomes inactivated, and antibiotic resistance developed [6]. In other words, presence of blaZ genes in strains implies development of resistance to all beta-lactam antibiotics which includes the penicillins and cephalosporins (with the exceptions of ceftobiprole and ceftaroline), and hence, pose serious threat to the control of infections by *S. aureus* in the community.

It is worth noting that research on the molecular detection of *bla*Z genes in *S. aureus* in Nigeria, hitherto, has been focused on clinical and animal body sources. This is to the best of our knowledge the first report of such detection from

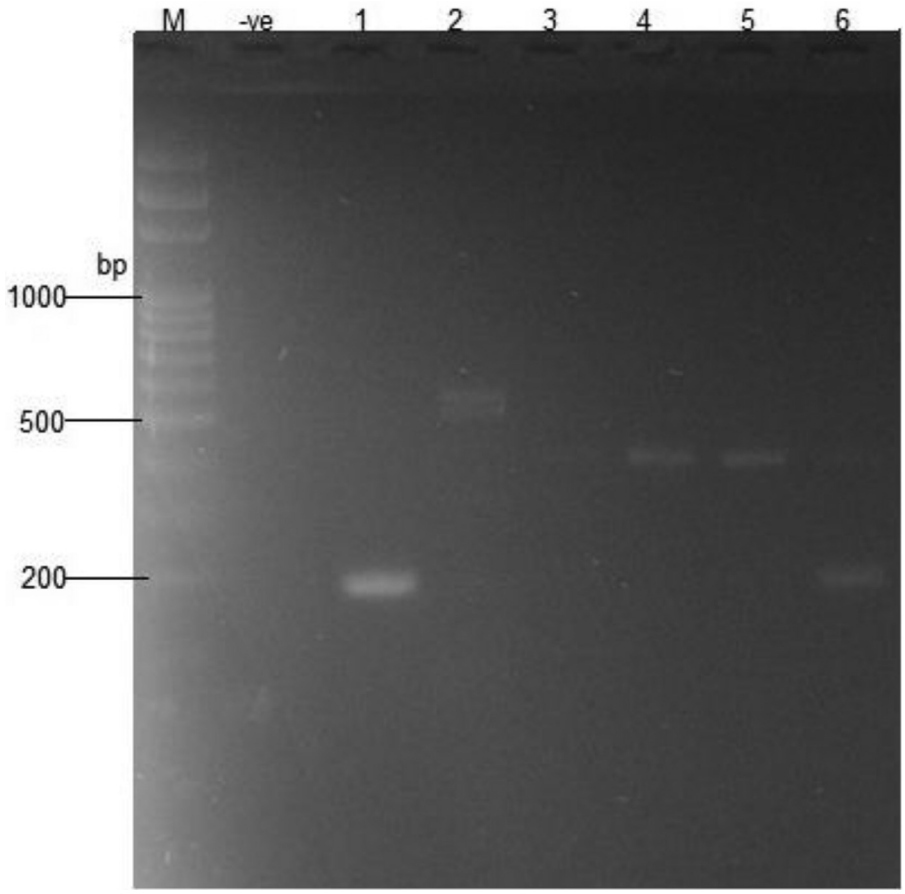

Fig. 3. 16S rRNA Fingerprint of *blaZ* Gene amplified on 1.5% agarose gel
Lane M: Marker. (100 bp DNA ladder); Lane 1: Bank C4 ATM keypad, Lane 2: Bank A ATM keypad, Lane 3: Bank C3 ATM keypad, Lane 4: Bank C5 ATM keypad, Lane 5: Bank B ATM keypad, Lane 6: Bank C1 ATM keypad.

environmental surfaces. Probably, the fact that the ATM keypads are public utility facilities always open for the use of all and sundry (regardless of the history of occupation, antibiotic use pattern or animal contact), a contributory factor which makes these keypads, via the hands of the users, a reservoir for the collection and spread of this organism with diverse molecular and antibiotic resistance characteristics.

Detection of *blaZ* gene by a molecular method such as PCR is a gold standard for confirmation of β-lactams when comparing phenotypic tests [36].

S. aureus from this study were most susceptible to gentamicin and ofloxacin. Gentamicin is an aminoglycoside and a ribosome-targeting antibiotic; mainly bactericidal. Ofloxacin is a fluoroquinolone and its molecular targets in *S. aureus* have been reported to be (i) DNA gyrase that introduces negative supercoils into chromosomal DNA and (ii) topoisomerase IV which promotes chromosome decatenation following replication [37]. Fluoroquinolones produce bactericidal effect. The susceptibility of *S. aureus* to gentamicin and ofloxacin antibiotics indicate that the mechanisms of action by the antibiotics are still effective. Gentamicin and ofloxacin could be the recommended drug for use in situations of staphylococcal infection in the locality.

It is worthy of note that no *erm* (erythromycin ribosomal methylation) B genes were detected among the strains which were molecularly typed. This implies that the genes coding for the enzymes causing macrolide resistance, via enzymatic modification of the target binding site, are not present. Nevertheless, this result may change if a statistically significant number of molecularly characterized *S. aureus* isolates were screened for this same resistant genes.

The observation that four of the six molecularly screened *S. aureus* were not only negative for ermB genes but also phenotypically showed resistance to erythromycin, may be explained. *S. aureus*, besides having core chromosomes, also possess mobile genetic elements (MGEs) or accessory genomes such as plasmids, insertion sequences, transposons, pathogenicity islands, bacteriophages and staphylococcal cassette chromosomes [38]. These MGEs may also harbor genes for antibiotic resistance. It must be remembered that the molecular screening, undertaken in this research work, was limited only to the core genomes.

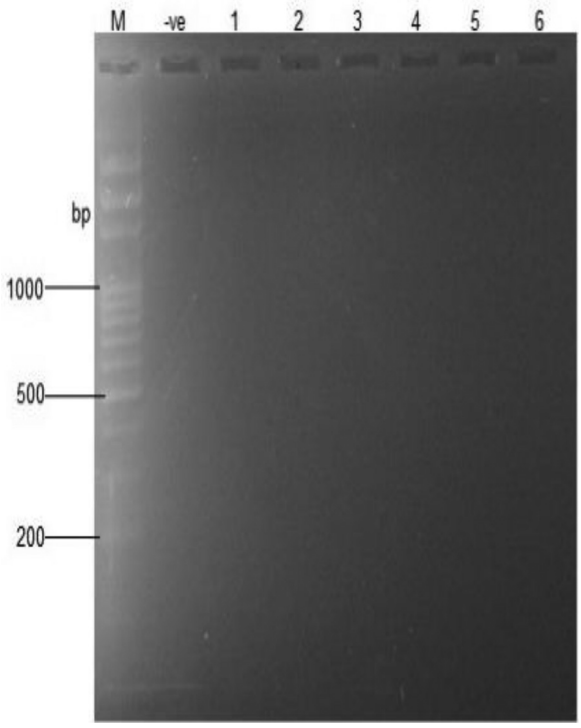

Fig. 4. 16S rRNA Fingerprint of ermB Gene amplified on 1.5% agarose gel
Lane M: Marker. (100 bp DNA ladder); Lane 1: Bank C4 ATM keypad, Lane 2: Bank A ATM keypad, Lane 3: Bank C3 ATM keypad, Lane 4: Bank C5 ATM keypad, Lane 5: Bank B ATM keypad, Lane 6: Bank C1 ATM keypad.

Conclusion

This study has shown a high prevalence of *S. aureus* on ATM keypads and that the same organism is resistant to commonly used antibiotics. Some of these organisms have been shown to carry the *blaZ*gene and are important in producing penicillin-resistant strains. These keypads can be sources of cross-contamination for users of bank ATMs. Consequently, ATM keypads are a platform for the acquisition and spread of antibiotic-resistant bacterial strains through dermal contact. It is suggested that cross-contamination from this platform may be minimized through the maintenance of personal hygiene practices like washing of hands regularly using soap or sanitizing with alcohol. The study therefore conclude that it is important to create, execute and target the populace who use the ATM facilities with educational awareness programmes that address the contamination/cross-contamination and persistence of *S. aureus* on inanimate surfaces. Such efforts will not only be of tremendous benefit to the public, but will also help make realizable one of the African Union's (AU) agenda 2063 goals which is impacting the citizens with proper education and revolutionizing skills founded on Science, Technology and innovation.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

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