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Antibiotic Susceptibility Profile and Beta Lactamase Production of *Staphylococcus aureus* Isolated from Mobile Phones of Students from College of Natural and Pharmaceutical Sciences, Bayero University Kano, Nigeria

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Abstract

Staphylococcus aureus is a Gram-positive bacterium that is commonly found in both community and healthcare-associated infections. One of the most common mechanisms that S. aureus develops resistance against antibiotics, especially beta-lactam antibiotics, by producing beta-lactamase enzymes. Mobile phones serve as potential fomites for pathogens. The present study was conducted to ascertain the antibiotic sensitivity profile in beta-lactamase-producing S. aureus from mobile phones of some students from the College of Natural and Pharmaceutical Sciences, Bayero University, Kano, Nigeria. One hundred and twenty (120) samples were randomly collected from the surface of mobile phones of some students using sterile swab stick, 40 each from the three faculties within the college. The samples were cultured and isolates were characterized using appropriate morphological and biochemical standard methods. Antibiotic sensitivity testing was evaluated using disc agar diffusion method as described previously. Beta-lactamase production was determined using starchiodometric methods. A total of 45 (37.5%) isolates were identified as S. aureus. The highest percentage, 18 (40.0%), was from the faculty of physical sciences. The result of antibiotic sensitivity revealed the highest susceptibility to Augmentin, Ofloxacin, and Perloxacin (91%), Ciprofloxacin (87%), and Ceptriazone (82%). The highest resistance was observed against Ampicillin (95%), followed by Cephalexin (73%), Cotrimaxazole (68%), Gentamycin, and Streptomycin (62%). Out of 35 resistant isolates, 91.4 % were found to be beta-lactamase producers. Statistically there is no significant difference in antibiotic resistance pattern across the faculties (P=0.56, F = 0.592). It was concluded that there is a high prevalence of S. aureus, and the production of Beta-lactamase enzyme is the most common biochemical mechanism by which S. aureus inactivates beta-lactam antibiotics. There is a need for physical and personal hygiene guidelines while using mobile phones to prevent the spread of resistant bacteria.

Key words: Mobile phones, beta lactamase, Staphylococcus aureus, antibiotic, resistance

INTRODUCTION

Recently, mobile phones have become indispensable in our daily life activities and are found to harbour various types and numbers of bacteria, including *Staphylococcus aureus*. They are usually carried along with their owner to places that are often loaded with potential pathogenic microorganisms, such as the restroom (Muzslay *et al.*, 2013). Mobile phones have become part of our professional and social life, accessories that are usually kept in pockets and female handbags. Mobile phones are usually handled and held to the face (Ilusanya *et al.*, 2012). Users consistently handle mobile phones, which makes it possible for microorganisms to grow and multiply, and serves as a place for transmission of infections (Jana *et al.*, 2018). Evidence from previous research has shown that contaminated inanimate objects or surfaces play a vital role in the dissemination of bacterial infections (Enemuor *et al.*, 2012). How people handle their mobile phones may introduce microorganisms that may be potentially pathogenic to the surfaces of the phones (Odiete *et al.*, 2018). Considering the widespread adoption and benefits of mobile phones, people often disregard their potential health hazards. People use mobile phones while eating or waiting for food at restaurants, even after washing their hands. In the process, microorganisms are transferred from phone to hands (Ibrahim *et al.*, 2013). Mobile phone sharing also makes the spread of pathogenic organisms, nosocomial and opportunistic

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infections through it very possible (Muzslay *et al.*, 2013). Normal body flora is considered to be non-pathogenic, but recently some reports indicated that, they are being considered as opportunistic pathogens causing both community and nosocomial infections either through direct and indirect contact (Elkholy and Ewees, 2010). Among the microorganisms which had been isolated from mobile phone surfaces are: *Staphylococcus. aureus, S. epidermidis*, *Klebsiella spp*, *E. coli* and *Pseudomonas aeruginosa* (Jayachandra *et al.*, 2011). *S. aureus* is ubiquitous in nature anduses different resistance mechanisms to inactivate all antibiotic classes used for its treatment (Akindolire *et al.*, 2018). Production of beta-lactamase enzyme is a major mechanism used by *S. aureus* to confer resistance to all beta lactam antibiotics (Khan *et al.*, 2014). The Staphylococci have two primary resistance mechanisms with respect to the ß-lactam antibiotics. One is the expression of ß-lactamase enzymes, which destroy ß-lactam by hydrolysis, and are expressed by activation of the blaZ gene. Methicillin resistance in *S. aureus* is an indication of higher level ß-lactam resistance that results from the acquisition of the mecA gene, which encodes the penicillin-binding protein 2a (PBP 2a) (Khan *et al.*, 2014). The present study aims to determine the antibiotic susceptibility profile in beta-lactamase-producing *S. aureus* from mobile phones of some students from the College of Natural and Pharmaceutical Sciences, Bayero University, Kano, Nigeria.

MATERIALS AND METHODS

Study Area and Population

The study was conducted at College of Natural and Pharmaceutical Sciences, Bayero University, Kano, Nigeria, between December 2024 and February 2025 using the mobile phones of some students (120) from the three Faculties within the college. The sampling point includes all the departments within the three faculties, and the students were randomly selected.

Sample Size Determination

The sample size for the study was determined by the formula as described by Naing et al. (2022):

$N = [Z^2 (pq)]/d^2$
Where: N = the desired sample size
Z = Normal standard distribution that corresponds to confidence interval as 1.96
P = Prevalence
q = 1-p
d= degree of accuracy / precision expected at 0.05

Sample Collection

An experimental study design was adopted where a total of one hundred and twenty samples (120) were randomly collected from mobile phones of some students from the College of Natural and Pharmaceutical Sciences, Bayero University, Kano, Nigeria. Forty each from the three faculties within the college. A sterile cotton swab stick was soaked in sterile normal saline to moisten it. The selected phone was swabbed over its surface and edges, and then the swab stick was quickly placed into its container and sealed. The swab sticks were then incubated at 37 ^{0}C for 24 hours in sterile nutrient broth Cheesbrough, (2017). This served as the stock culture. The procedure was repeated for all the samples.

Cultural, Morphological and Biochemical Characterization of the Bacterial Isolates

Bacterial isolates were determined using the serial dilution technique Cheesbrough, (2017). From the stock culture in the nutrient broth medium, 1.0 ml of the sample was aseptically pipetted into a sterile test tube containing 9.0 ml of sterile normal saline and mixed thoroughly. One (1.0 ml) of the dilution from each of the test tubes (10^{-1} – 10^{-3}) was aseptically pipetted and transferred into the corresponding petri dishes. This was followed by pouring of prepared, molten and cooled nutrient agar (Oxoid, England) medium onto the plates. The contents were gently swirled and allowed to solidify at room temperature. The plates were incubated at 37 °C for 24 hours. Gram's staining technique was carried out according to the method described by Cheesbrough (2017) to determine their Gram's reaction. Loopful of discrete colonies on nutrient agar (Oxoid, England) medium was selected and aseptically sub-cultured onto selective media (Mannitol salt agar; Oxoid, England). The following biochemical tests were carried out according to the methods described by Cheesbrough (2017) to authenticate their identity:

Gram Staining

Gram staining was used for the differentiation of Gram-positive and Gram-negative bacteria by microscopy. Smears were made on microscopic slides from the colonies. The smears were air-dried and heat fixed, and flooded with crystal

violet staining reagent for 1 minute. The slides were then washed gently in running water for 2 seconds. The slides were then flooded with gram's iodine for 1 minute and thereafter washed in a gentle and indirect stream of tap water for 2 seconds. The slides were decolorized for 15 seconds with ethanol and counterstained with safranin for 1 minutes. The slides were finally washed in running water and blotted on an adsorbent filter paper. Cheesbrough (2017)

Catalase Test

The catalase test was used to differentiate Staphylococci from Streptococci bacteria. Briefly, a small amount of bacterial colony was added to a glass using a sterile loop. A drop of 3% H_2O_2 was then added onto the slide and mixed with the colony (Khatoon *et al.*, 2022).

Coagulase Test

The coagulase method was used as a confirmatory test for *S. aureus*. A drop of the staphylococcal colony was emulsified on a glass slide. A sterile wire loop was dipped into undiluted plasma and then emulsified onto the staphylococcal suspension. Coarse clumping was confirmatory for *S. aureus* Cheesbrough (2017)

Deoxyribonuclease (DNAse) test

Deoxyribonucleic acid agar media (containing 0.2 % W/V of deoxyribonucleic acid) was used for the test following the method as described by Cheesbrough (2017).

Antibiotic Susceptibility Test

Antibiotic susceptibility of the isolates was determined using the modified Kirby-Bauer agar disc diffusion method (Cheesbrough, 2017). The isolatess were standardised by making a turbid suspension of each in sterile normal saline, and were compared with 0.5 McFarland Standard. A sterile swab was dipped into the standardized suspension, pressed on the side of the bottles to allow excess to drip-off, and then used to evenly streak the entire surface of the Mueller-Hinton agar (Oxoid, England) plate. Sterile forceps were then used to place the antibiotic discs in a circular pattern on the surface of the inoculated agar plate; the plate was allowed to stand at room temperature for 15 minutes and thereafter incubated at 37°C for 24 hours. This procedure was carried out for all the isolates. After incubation, the zone of inhibition in diameter for each antibiotic was measured, and the results were interpreted using Clinical and Laboratory Standards Institute (CLSI, 2017) recommendations. The following antibiotic discs (Oxoid, England) were used: Augmentin (5µg), Ofloxacin (5µg), Perfloxacin (10µg), Ciprofloxacin (10µg), Ceftriaxone(30µg), Ampicillin (10µg), Cephalexin (10µg), Cotrimaxazole (30µg), Gentamycin (10µg) and Streptomycin (30µg).

Test for ß-lactamase Production: Starch Iodometric Method

The ability of the identified *S.aureus* to produce ß-lactamase enzymes was determined using the plate iodometric method as described by Cheesbrough (2017). Hundred (100 µl) of penicillin solution was dispensed into a well of microtitre plate. Several colonies of the organism to be tested were emulsified into the solution to get a dense suspension. Two drops of starch were added, and then the plate was kept at room temperature for 30-60 minutes. One drop of iodine was then added, which turned the solution blue. If the blue color disappeared in 10 minutes, the organism was considered as ß lactamase positive. Negative control with penicillin alone was kept without any culture suspension. A known ß-lactamase-producing strain of *S. aureus* was used as a positive control.

Determination of the Multiple Antibiotic Resistance Index (MARI)

Multiple antibiotic resistance is the resistance of isolates to three or more antibiotics. Multiple antibiotic resistance index was determined for all the isolates by using the formula as described by Sampson *et al.*, 2022: number of antibiotics to which the isolate is resistant divided by the total number of antibiotics tested.

MAR = A/B

Where A denoted the number of antibiotics to which the test isolates depicted resistance

B = Total number of antibiotics to which the test isolate has been evaluated for susceptibility

Statistical Analysis

Data were collected, summarized, and analyzed using u descriptive statistics (mean and standard deviation, percentage occurrence, and resistance) in Microsoft Excel 2019 and SPSS version 20 software. The results were presented using tables, charts, and figures, with statistical parameters such as percentages, means, standard deviations and confidence intervals were calculated. A one-way Analysis of Variance

(ANOVA) was performed to assess significant differences in antibiotic resistance across faculties. A 95% confidence level was used, and a P-value of less than 0.05 was considered statistically significant.

RESULTS

Out of 120 mobile phones examined, a total of 45 (37.5%) isolates were identified and characterized as *S. aureus* on the basis of microscopic and biochemical tests. The percentage distribution was depicted in Figure 1.0. The highest percentage was from the faculty of physical sciences followed by pharmaceutical sciences and then life sciences, 40%, 33.3%, and 26.7% respectively. The prevalence of the isolates was shown in Table 1.0. The number of isolates identified on Gram reaction, catalase, DNAse, coagulase positive and mannitol fermentation was shown in Table 2.0.

The antibiotic susceptibility results showed that augmenting, perfloxacin and ofloxacin had the highest sensitivity (91%), followed by ciprofloxacin and ceftriazone. On the other hand the highest resistance level was observed against ampicillin and cephalexin. Additionally, the result shows moderate susceptibility to streptomycin and cotrimaxazole. The number of isolates resistant/sensitive to each of the antibiotic is shown in Table 2.

The result for beta-lactamase production indicated that out of the 35 resistant isolates, thirty two (91.4%) were beta-lactamase producers and only 3 were non-beta-lactamase producers. The result is depicted in Figure 2. The beta-lactamase positive*S. aureus*isolates showed variable sensitivity and resistance to the antibiotics tested. The high percentage observed points to the role of this enzyme in mediating resistance.

The multiple antibiotic resistant index (MARI) of the isolates with reference to the ten antibiotics tested showed that the value for all the isolates was higher than 0.2. Generally, MARI revealed that 84.4% isolates were resistant to three or more antibiotics (MARI ≥ 0.3). Three isolates showed 100% resistance to the entire antibiotic tested. Resistance was consistently observed against ampicillin and cotrimaxazole. This is an indication of the misuse and overuse of these antibiotics in the environment.

Table 1.0: Prevalence of *S. aureus* from Mobile Phones Across Faculties, College of Natural and Pharmaceutical Sciences.

S/N	Faculty	Number of Sample	Number of Isolates (%)
1.	Pharmaceutical Sciences	40	15(33.3%)
2.	Life Sciences	40	12(26.7)
3.	Physical Sciences	40	18(40%)
	Total	120	45

Table 2.0: Morphological and Biochemical Characteristics of the Bacterial Isolates

Faculty	GR	Cat	DNAse	Coag	GM	NI	Isolates identified
Pharmaceutical science	+	+	+	+	+	15	*S. aures*
Life science	+	+	+	+	+	12	*S. aures*
Physical sciences	+	+	_	+	+	18	*S. aures*

GR: Gram's reaction; **Cat:** Catalase; **Coag:** Coagulase; **NI:** No. of isolates; **GM:** Growth on mannitol

Table 3: Antibiotic Susceptibility Pattern of *S.aures* Isolated from Mobile Phones of Students of College of Natural and Pharmaceutical Sciences

Antibiotics	*S. aureus* (n=45)	Sensitivity Level (%)	Resistance Level (%)
Augmentin(5µg)	41(91)		4(9)
Perfloxacin(10µg)	41(91)		4(9)
Gentamycin(5µg)	23(51.1)		22(48.9)
Ciprofloxacin(10µg)	39(87)		6(13)±4.76
Ceftriaxone(30µg)	37(82)		8(18)±9.65
Cephalexin(10µg)	12(27)		33(73)± 0.00
Ampicillin(10µg)	2(5)		43(95)± 0.00
Cotrimaxazole(30µg)	14(31.1)		31(68.9)± 0.10
Streptomycin(30µg)	17(37.8)		28(62.2)± 0.10
Ofloxacin(5µg)	41(91)		4(9)±9.00

± =Standard deviation

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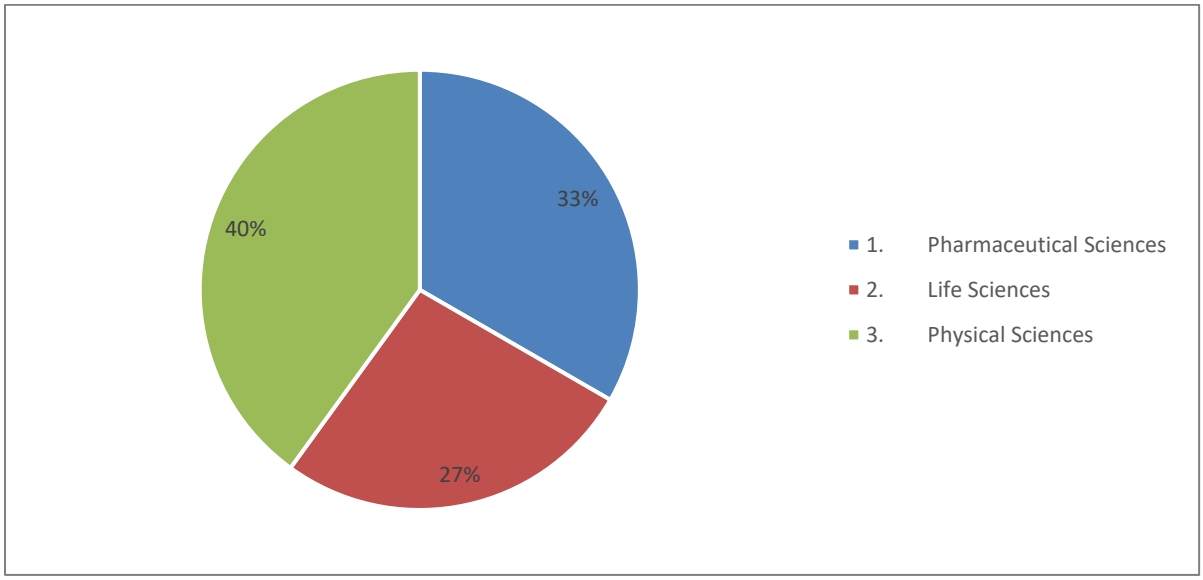

Figure 1.0: Percentage Distribution of *S. aureus* isolated from Mobile Phones Across Faculties.

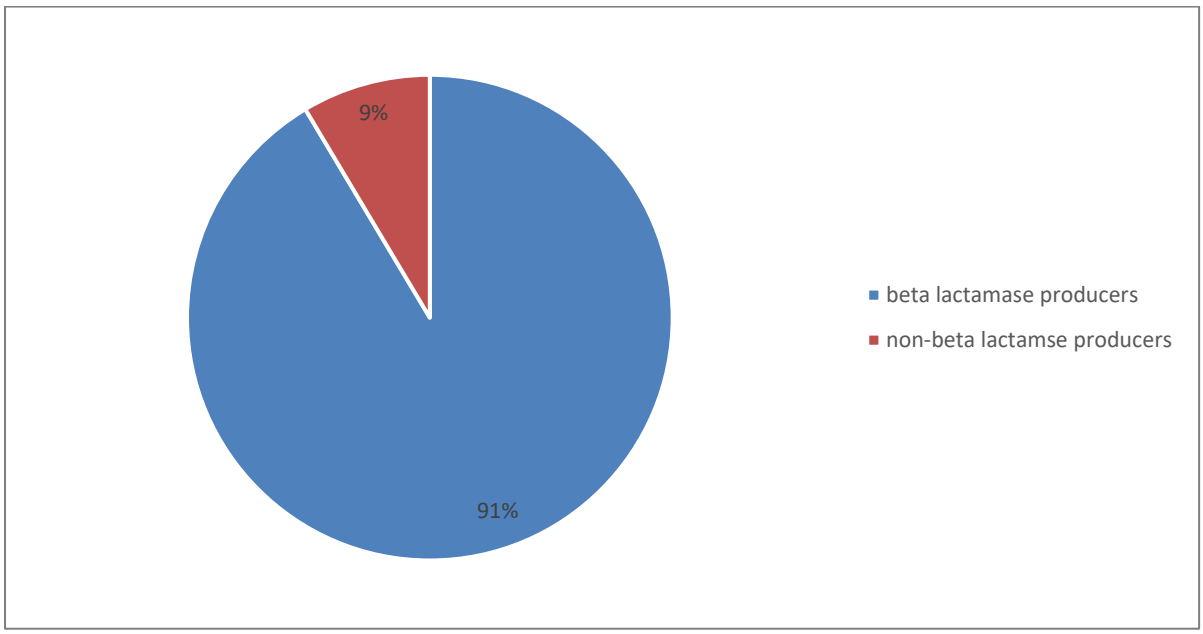

Figure 2.0: Result of Beta lactamase production test

Table 4: MARI of mobile phone bacteria isolated from students of the College of Natural and Pharmaceutical Sciences

No.antibiotics to which resistance	Mar index	No. of isolates (%)
1	0.1	3(6.7)
2	0.2	4(8.9)
3	0.3	3(6.7)
4	0.4	4(8.9)
5	0.5	5(11.1)
6	0.6	6(13.3)± 0.01
7	0.7	5(11.1)± 0.00
8	0.8	7(15.5)±13.76
9	0.9	5(11.1)± 0.01
10	1.0	3(6.7)±7.01
		45(100)

± = Standard deviation

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Statistical analysis revealed no significant difference in the resistance pattern of S. aureus isolates across the three faculties at P = 0.56, F = 0.592<F-Critical = 3.35. The standard deviation indicates variability in resistance within each faculty, with higher variation seen in ciprofloxacin in pharmacy compared to amoxicillin resistance across the three faculties.

DISCUSSION

The increasing use of mobile phones has raised significant concern regarding their potential role as vectors for bacterial contamination (Ezemba *et al.*, 2022). This is possible as mobile phones are frequently held at hand, infrequently cleaned and thus provide an environment for bacteria to thrive and possibly spread among students (Anagboso *et al.*, 2023). The high prevalence of bacteria isolated from the mobile phones of students from the physical sciences might be as a result of their location and the number of students who wander around the faculty. The magnitude of contamination of mobile phones could be determined by the level of usage and exposure of mobile phones to environmental surfaces, hands and skin of users (Adebayo *et al.*, 2023). It was documented that the combination of constant handling and heat generated by mobile phones provides a favorable condition for growth and multiplication of microbes that are normally found on our skin (Ezemba *et al.*, 2022). Generally, all the sampled faculties had a moderate number of *Staph aureus*. WHO (2020) reported that *S. aureus* was the most prevalent bacterial agent isolated, accounting for 45 (37.5%) of all the mobile phones examined in the study. This corresponds with our findings and that of Adebayo *et al.*, (2023) in which *S. aureus* was the most frequently encountered bacterial agent isolated from mobile phones of students in Southern Nigeriawith multidrug resistance, especially to beta lactams and aminoglycosides. *S. aureus*increases in optimum temperature. Mobile phones are usually kept warm in pockets, handbags, and briefcases (Anagboso *et al.*, 2023). In addition, the high occurrence rate of *S. aureus* could be attributed to the fact that it is abundant in the human body as a normal flora of the skin. The highest resistance of the *Saureus* isolated from this study against some of the tested antibiotics (ampicillin, cephalexin and cotrimaxazole) and the low susceptibility of the isolates generally to streptomycin (Table 3) could be attributed to the common use of these antibiotics as observed in previous studies presenting public health problems (Tagoe *et al.*, 2011). In addition, gross misuse of these antibiotics in chemotherapy, especially in this part of the country, could explain the reason for this observation as earlier reported by Kumurya *et al* (2010).

The bacterial isolates were generally resistant to the beta-lactam antibiotics used in this study; similar results were reported by Elmanama (2015). The isolates in this study demonstrated a high level of resistance to gentamicin, this concur to the findings reported by Zakarie (2020). The reason for this resistance may be that the bacteria might have had previous exposure to gentamicin. Quinolones were the most active antibiotics observed in this study. They act by inhibiting DNA synthesis of the bacteria by binding to two enzymes DNA gyrase and topoisomerase IV (Arumugam *et al.*, 2017). Lower percentage showed resistance to ofloxacin, pefloxacin and ciprofloxacin. Contrary to our study, Nwankwo *et al.*,(2014) reported high resistance rate of *Staph. aureus* isolates from mobile phones of healthcare workers in Kogi state, and the our result concur with the lower/moderate resistance level against gentamicin (45%) and ofloxacin (9.0%).

The detection of ß- ß-lactamase enzymes in 91.4% of the *S. aureus* isolates points to the possible role of this enzyme in mediating resistance, especially to ß-lactam antibiotics by opening (hydrolysis) the ß-lactam ring (Kok *et al.*, 2010) and are responsible for many failures of antimicrobial therapy. Most of the *S. aureus* isolated in this study (Figure 2) were positive for beta-lactamase production as observed in a previous study (Adeleke and Olarinde, 2013). The percentage is higher than in other earlier reports (Akindele *et al.*, 2010). The spread of beta-lactamase genes had been enhanced by their integration within mobile genetic elements such as plasmids and transposons which facilitate the rapid transfer of genetic materials between microbes (Drawz and Bonomo, 2010). It is surprising that in this study, all of the augmenting resistant strains were negative for beta-lactamase production, yet were resistant to beta-lactamases. This suggests that beta-lactamase production is not the only factor responsible for resistance in *S.aureus*, indicating that other mechanisms of resistance may play a major role as well. Subsequent studies on molecular characterisation of beta-lactamase production by the S. aureus isolates, as well as other mechanisms of resistance to beta-lactam, demonstrated by these isolates from inanimate objects (mobile phones) in Kano, Nigeria, should be carried out.

The bacteria isolated from mobile phones in this study exhibited multiple antibiotic resistances that is clear indication of the misused and overused of antibiotics which might have an important role in the development of antibiotic resistance. MAR index is an indication of the level of exposure of a given organism to different antibiotics, as it is an index to measure the antibiotic resistance level. The MAR indices determined in this study is a good indication that a very large proportion of the isolated bacteria had been exposed to several antibiotics. An index of ≥ 0.2 is an indication of resistance to more than one drug, and increasing values relate to the number of drugs the isolate is resistant to (Garcia-Migura *et al.*, 2014). High MAR indices have been reported to contribute to the development of superbugs (Hao *et al.*, 2014). The MARI rate observed in this study was higher than that reported by Sujan *et al.* (2018). From this study's MARI result, it can be said that these bacterial isolates originated from an environment where antibiotics are being abused. The problem of misuse and abuse of antibiotics in our society is highly disturbing. This is the probable leading cause of multidrug resistance being observed.

CONCLUSION

There is high prevalence of *S. aureus* on mobile phones, and their high level of resistance against notable antibiotics used in this study indicates that mobile phones are potential reservoir of antimicrobial-resistant pathogenic bacteria. The production of inactivating enzymes is the major mechanism that conferred resistance to most of the antibiotics used in this study. Also, the level of multidrug-resistant *S. aureus* isolates is high and should therefore be taken into consideration.

RECOMMENDATIONS

1) There is need to educate students on personal hygiene with respect to the use of mobile phones and to further expand this study to a larger community covering several pathogenic microbial species.

2) Frequent hand cleansing, especially with instant hand sanitizers is the most significant step to help prevent feco-oral and droplet transmissions.

3) Mobile phones should be handled in a manner that does not get contaminated with dirt and/or disease-causing agents.

4) Mobile phones should be regularly cleaned with relevant disinfectants.

5)There is a need for disinfection guidelines for mobile phones to stop the transmission of resistant bacteria.

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