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Molecular Detection of mecA Gene in Methicillin Resistant *Staphylococcus aureus* Isolated from Surfaces of some Public Hospitals in Katsina State, Nigeria

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Abstract

The increasing rate of antibiotic resistance demonstrated by bacterial pathogens is an emerging problem that is considered a major health concern globally, especially in low-and middle-income countries. The aim of this study is to determine the prevalence of Methicillin Resistant Staphylococcus aureus from hospital environment and to detect the presence of resistance genes in the MRSA isolates. One hundred and ninety-two (192) swab samples of doorknob, bedrail, table tops and drip stand were collected from 4 hospitals within Dutsin-Ma and Kurfi local government. Antibiotic Susceptibility testing and phenotypic detection of MRSA were conducted using disc diffusion method. MecA gene was detected using Polymerase Chain Reaction. One hundred and eleven 111(57.8%) out of 192 samples were positive for S. aureus. The result showed that cefoxitin was the most effective antibiotic (66.6%) against the isolates from all the four hospitals while erythromycin was the less effective against the isolates (35.1%). A total of six (5.40%) Methicillin Resistant S. aureus and MDR (12.61%) were detected from the four hospitals. In conclusion, all the four hospitals were found to be contaminated with Methicillin resistant S. aureus, with Kurfi General Hospital having the highest number of MRSA (3). Out of the 6 phenotypically detected isolates of MRSA screened, mecA gene was detected in five (5) isolates. It is recommended that, proper hygiene practice should be improved in the healthcare settings, and proper use of antibiotics should be highly encouraged among individuals in both community and hospital.

Keywords: Methicillin resistant S. aureus, mecA gene, Hospital Environment

INTRODUCTION

Antibiotics have been a crucial part of chemotherapy throughout the 20th century, saving many lives every day (Odonkor and Addo, 2018). A major global health concern, according to Jans *et al.* (2013), is the rising rate of bacterial pathogen resistance. This is particularly true in some countries, where the use of antibiotics is on the rise due to rising incomes, accessible antimicrobials, and lax regulation of over-the-counter sales (Lim *et al.*, 2016).

A growing number of multidrug resistant organisms have been linked to the indiscriminate use of antibiotics in both human medicine and livestock management, particularly for chemotherapy, prophylaxis, and growth promoters in animal production (Aliyu *et al.*, 2022). This could be due to the lack of knowledge on antibiotics stewardship and poverty in some African countries, which leads to self-medication and misuse of antibiotics (Valentine *et al.*, 2021). According to research by Li *et al.* (2019), patients with infections caused by resistant microorganisms are found to have higher fatality rates, longer hospital stays, and higher costs to the health system.

Staphylococcus aureus is a minor member of the mucosal flora and a natural component of human skin (Fey and Olson, 2010).

Interactions with contaminated surfaces in the hospital environment led to the spread of *S. aureus* in the healthcare setting (Boyce, 2007). *S. aureus* has the potential to survive in hospital environments for weeks or months while infecting uniforms, drip stands, bed rails, doorknobs, computer keyboards, mobile phones, table tops, and drip rails (Landers *et al.*, 2014). Due to their unique pathogen-host-environment connections, *S. aureus* and other bacteria from the same genera can adhere to surfaces (Hidron *et al.*, 2018). Due to the difficulties in treating infections from colonization devices, *Staphylococcus aureus* has developed a wide range of antibiotic resistance, leading to a very major clinical issue (Mbim *et al.*, 2016). Methicillin, one of the most widely used antibiotics today, has lost some of its power against *Staphylococcus aureus*. According to Fitzpatrick *et al.* (2015), the presence of the mecA gene, which codes for a penicillin-binding protein, in the bacterial genome mediates methicillin resistance.

Despite several studies carried out in hospitals close to Dutsin-Ma and Kurfi local government, there is a dearth of information on the detection of Methicillin resistant *Staphylococcus aureus* from hospital environments there by phenotypically identifying Methicillin- and Multi-drug-resistant *Staphylococcus aureus* in the hospital setting inside the Dutsin-Ma and Kurfi local government, Katsina State, Nigeria, this study aimed to close the gap.

MATERIALS AND METHODS

Study Area

Dutsin-ma local government in Katsina state of Nigeria, Katsina is on latitude 12.513932, and longitude 7.611422. Katsina as a city is located in Nigeria with the GPS coordinates of 12° 30' 50.1552" N and 7° 36' 41.1192" E. The local government has a Federal University with State College of Education within the metropolis. Furthermore, there are three major hospitals in the area namely; General Hospital Dutsin-MA, Comprehensive Clinic and a University Clinic (unpublished data).

Sample size

The sample size was calculated using a Fischer's formula from Tuza *et al*. (2023):

$$N = \frac{Z^2PQ}{I^2}$$

Where;

N- is the sample size

Z - is 1.96, which is the score corresponding to 95% confidence interval

P- is the assumed prevalence, which is taken to be 50% for unknown.

Q = (1-P)

I - the accepted error term corresponding to 5%.

Therefore:

$$N = \frac{3.8416 \times 0.5 \times 0.5}{0.0025}$$

=384.16

N --384 samples.

However, the sample size was used for clinical and environmental study, where the 384 was

divided into two, 192 for clinical and 192 for environmental respectively. Therefore, in this study, the sample size of 192 was used.

Sample Collection and Sampling Technique

The ethical clearance was sought from Health Research Ethics Committee under Katsina State Ministry of Health, with number MOH/ADM/SUB/1152/1/756. Another ethical clearance was granted by the Research and Ethics Committee of Federal University Dutsin-ma, for the FUDMA Clinic with a given number FUDMA/CLINIC/001/NOV. 2023.

Using cluster random sampling (by including four equal surfaces mentioned below from each hospital into the study). Fourty eight (48) environmental swab samples were obtained from each of the four hospitals (Dutsin-Ma General Hospital, Comprehensive Clinic Dutsin-Ma, Federal University Dutsin-Ma Clinic, and Kurfi General Hospital). A total of 48 swab samples were obtained from environmental surfaces (12 bedrails, 12 door knobs, 12 table tops, and 12 drip stands, respectively) in various wards of each hospital.

Isolation and Identification of *Staphylococcus aureus*

The cotton swabs were immediately placed in the sterile screw cap test tubes containing 5 ml of nutrient broth at 37°C for 2 to 6 h after swabbing the environmental surfaces. Samples were inoculated on Mannitol Salt Agar and allowed to sit for 24 hours at 37°C. Gram staining, pigment production, tube coagulase test (TCT), catalase test and acid production from mannitol were used to further validate the *S. aureus* isolates (Rushdy *et al.*, 2007).

Antibiotic Susceptibility Pattern of *S. aureus* Isolated from Environmental Surfaces

Antibiotic susceptibility test was carried out using the Kirby-Bauer disc diffusion method with five antibiotics (Ciprofloxacin 10ug, Erythromycin 10ug, Gentamycin 10ug, Amoxicillin 30ug, and Cefoxitin 30ug). Following the recommendations of the Clinical and Laboratory Standard Institute (CLSI, 2021) the result were interpreted as Sensitive, Intermediate and Resistance. Phenotypic identification of MRSA was carried out by selecting the isolates resistant to Cefoxitin 30ug antibiotic following the CLSI (2021) guideline.

Molecular Detection of mecA gene in Methicillin Resistant Isolates of *Staphylococcus aureus*

DNA Extraction:

The bacterial colonies were picked and placed on a clean 1.5ml tube containing 200 µl of 1x PBS. Two hundred (200µl) genomic cell lysis buffer, (GB Buffer) 20µl proteinase K and 10µl of RNase A was added to the mixture and incubated at 60°C for 15 m. After the incubation, 400 µl of ethanol was added and mixed by vortexing. It was then transferred to a binding column attached to a collection tube and centrifuged at 8000rpm for one minute, the contents of the collection tube was discarded and the it was attached to the binding column, 500µl of wash buffer A1 was added to the binding column and centrifuged at 8000rpm for one minute, the collection tube was emptied again and the process was repeated using wash buffer 2.The collection tube was reattached to the binding column and without adding anything it was centrifuged at 13000rpm for one minute to remove any remaining ethanol. The collection tube was discarded and a new 1.5ml tube was attached to the binding column. Then 60µl of DNA elution buffer was added to the binding column and allowed to stay at room temperature for 5 minutes before being centrifuged at 8000rpm for one minute and the DNA collected (Valentine *et al.*, 2021).

Polymerase Chain Reaction

For the PCR, accupower™ hotstart PCR premix (Bioneer corporation, South Korea) was used. The premix contains all the PCR components except the template, primers and water. Three (3 µl) of DNA template, 1bµl each of the forward and reverse primers (mecA) and 15 µl of nuclease free water was added to the premix and was transferred to the thermal cycler the following conditions were used for the PCR; 95°c for 5minutes (predenaturation) then 35 cycles of (94°C for 30secs, 46°C for 40secs, 72°C for 45secs then final extension at 72°C for 7minutes, following the procedure describe by Aliyu *et al.* (2022) with some modifications.

After the PCR, the amplicons were separated on 1% agarose gel stained with ethidium bromide at 100 v for 1 h. A 50 bp molecular ladder was used to compare the band obtained. A PCR amplification of about 150 - 155 bp was obtained after running the gel electrophoresis.

RESULTS

The frequency and distribution of *S. aureus* isolates from surfaces of four public hospitals are shown in Table 1 below. From the table below, General Hospital Dutsin-Ma has the highest distribution of *S. aureus* (17.5%), while FUDMA Clinic has the lowest percentage (11.3%) respectively. However, among the surfaces sampled from, table tops were found to be more contaminated with *S. aureus* (19.0%), and door knobs were the least contaminated surfaces (8.2%).

Table 1: Distribution of *S. aureus* from surfaces of some Public Hospitals in Katsina State

Hospitals	Various hospital surfaces where swab samples were collected (n=111)				
	Table tops	Bedrails	Door knobs	Drip stand	Total
FUDMA clinic	10	05	03	04	22 (11.3%)
GH Dutsinma	10	09	08	07	34 (17.5%)
CHC Dutsinma	10	12	03	06	31 (16.0%)
GH Kurfi	07	09	02	06	24 (12.4%)
Total	37 (19.1%)	35 (18.0%)	16 (8.2%)	23 (11.9%)	111 (57.2%)

Key: FUDMA= Federal University Dutsin-Ma, GH= Genral Hospital, CHC= Comprehensive Health Care.

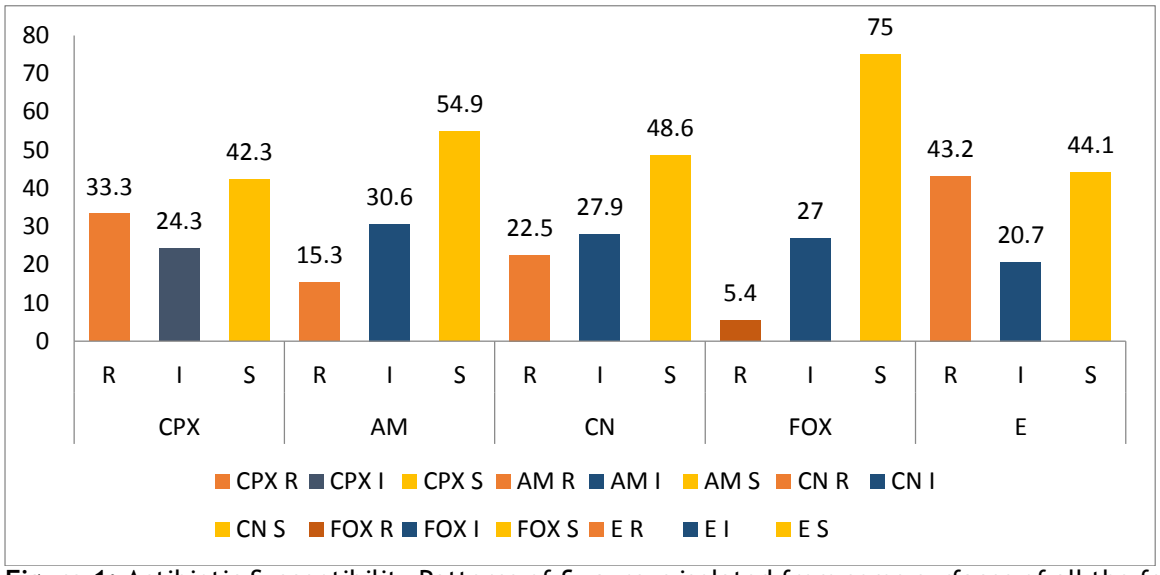


Figure 1: Antibiotic Susceptibility Patterns of *S. aureus* isolated from some surfaces of all the four hospitals combine against some common antibiotics

Key: CPX= Ciprofloxacin, AM= Amoxicillin, CN= Gentamycin, FOX= Cefoxitin, E= Erythromycin, R = Resistance, I = Intermediate, S = Susceptible

The percentages of each antibiotic tested against the total isolates of *S. aureus* from all surfaces of four hospitals were shown in the above figure. According to the graph, Cefoxitin has a higher proportion of sensitivity (75%) than Amoxicillin (54.9%). The least effective antibiotics, however, was erythromycin, which was followed by Ciprofloxacin with resistance rates of 43,2 and 33,3 percent, respectively.

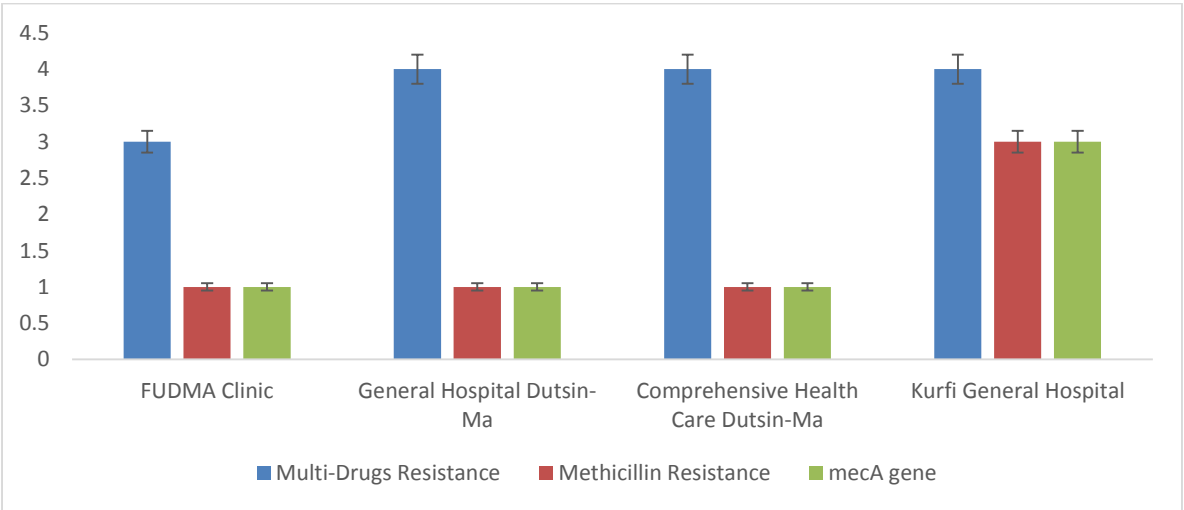
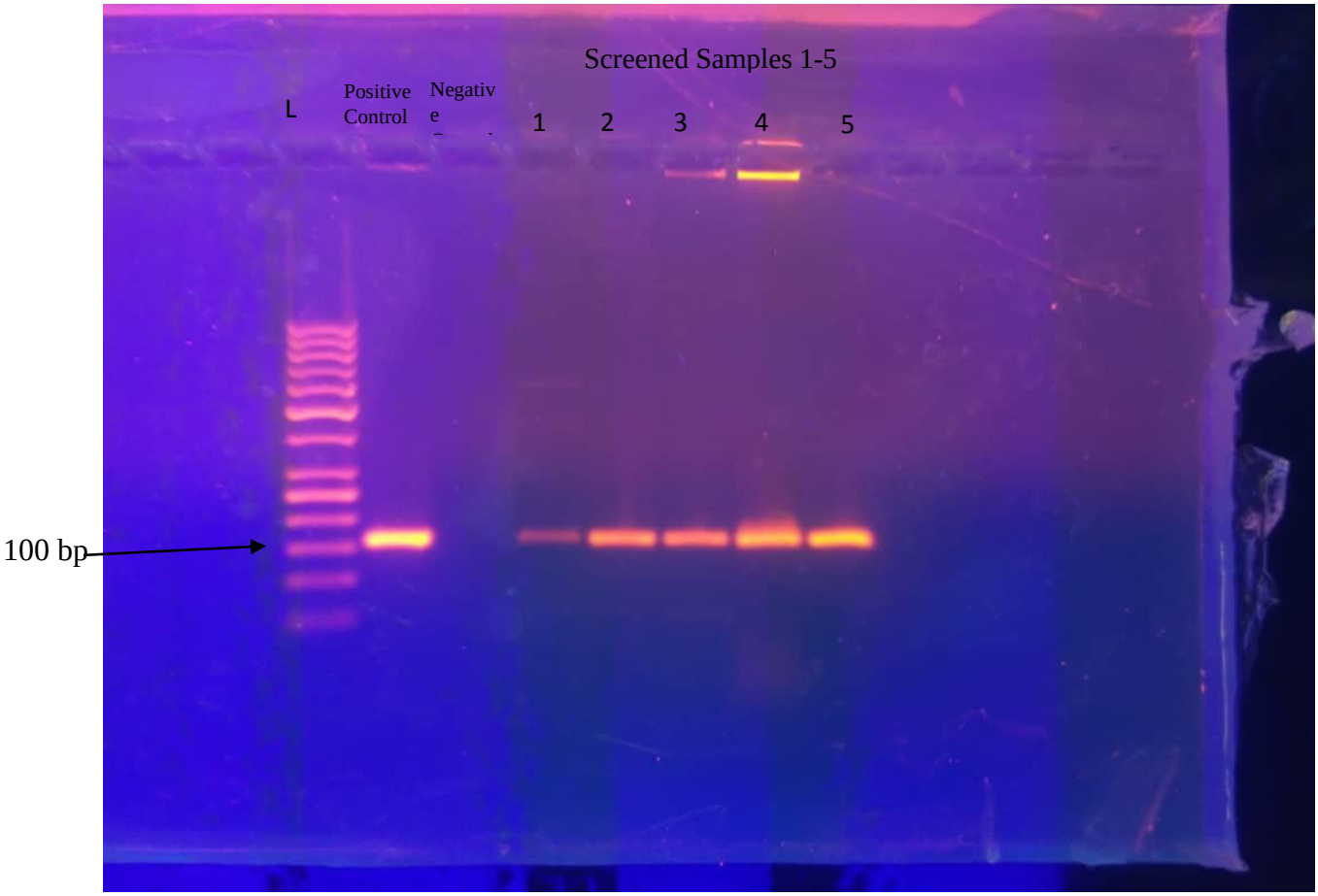


Figure 2: Histograph showing the level of Multi-drugs and Methicillin resistance as well as presence of mecA gene among *Staphylococcus aureus* isolated from some surfaces of four different hospitals within Dutsin-Ma and Kurfi Local Government, Katsina State

The figure above shows how all four hospitals were harboring both multi-drug-resistant and methicillin-resistant S. *aureus*. In contrast to the other three Hospitals, Kurfi General Hospital has the greatest prevalence of methicillin- and multi-drug-resistant S. *aureus*. Additionally, it demonstrates that five of the six mecA gene-positive S. *aureus* isolates from the four hospitals were methicillin resistant.



Key: L= Ladder, bp= base pairs

Figure 3: Detection of mecA gene in Methicillin *Staphylococcus aureus* isolated from surfaces of four different hospitals within Dutsin-Ma and Kurfi Local Government Area

The Figure above demonstrates that 5 (83.33%) of the 6 examined isolates tested positive for the mecA gene. The gene had a molecular weight of 100 bp and was found in the isolates. However, distilled water was employed as the negative control whereas mecA primers were used as the positive control.

DISCUSSION

Out of 192 samples obtained from four separate hospitals, 111 (or around 58%) were found to be positive for S. *aureus*. Although there is paucity of data with regards to the prevalence of MRSA in Nigeria, However, Reena *et al.* (2012) reported 14.4% of S. *aureus* isolated from hospital surfaces in Nepal, while Atsedewoyn *et al.* (2021) reported 13.2% prevalence of S. *aureus* isolated from hospital surfaces in Ethiopia. These findings are in contrast to the findings of this study. The results of this study are consistent with Anyadoh *et al.* (2011) finding that 59.6% of hospital surfaces were infected with S. *aureus*. Furthermore, compared to the reports of Ochie and Ohagwu (2009) in Nigeria (12.7%) and Wojtyczka *et al.* (2011) in Ghana (17.2%), our study found a greater proportion of S. *aureus*. The outcome is commendable because it addressed the issue of the bacteria's abundance in the investigated hospital's surrounding environment and revealed a higher proportion of the samples analyzed. This abundance may be explained by the bacteria's ubiquity, the volume of visitors, the scale of the hospital setting, and the facility's placement within the community.

According to this study, the occurence of S. *aureus* (35.5%) on bed rails, table tops and door knobs are consistent with the findings of Adam *et al.* (2020) in Ishaka, Uganda, who found that bedrails had the greatest percentage of S. *epidermidis* (39.2%) compared to other surfaces.

The results, however, are in direct opposition to Carvalho *et al.* (2017) 100% success rate for bedrails from Nigeria. In a similar vein, Carvalho *et al.* (2017) found a distribution rate of 53.8% and 38% of staphylococci on door knobs/handles. The variations of this study with other findings may be due to the hygienic practice of different hospitals as well as the environmental factors such as temperature, PH and Oxygen in a hospital's surrounding environment Atsedewoyn *et al.* (2021)

According to Allegranzi and Pittet (2019), the prevalence of *S. aureus* discovered on door knobs in our present investigation was seemingly greater. Since door knobs are the surfaces that both visitors and visiting medical staff contact most frequently, the percentage rate of *S. aureus* on them is probably related. The majority of healthcare facilities also did not often disinfect the knobs and handles after washing them. According to Carvalho et al. (2017), Allegranzi and Pittet (2019), environmental contamination in healthcare settings happens when staff members touch surfaces with contaminated hands or gloves, especially after treating patients, or when patients come into direct contact with the surfaces.

Erythromycin and Ciprofloxacin both had the greatest rates of resistance in this research, with 43.2% and 33.3%, respectively. This could be due to the availability, affordability and misuse of these antibiotics in the study are. This is in contrast to Adam *et al.* (2020) finding, which indicated that Trimethoprim-Sulfamethoxazole had the greatest rate of resistance in their research. Cefoxitin, Amoxicillin, and Gentamycin all had susceptibility percentages in this research that ranged from 75% to 54.9 and 48.6 respectively. The proportion of Gentamycin in this study is comparable to Andrea *et al.* (2010) reported 50.8% but lower than Adam *et al.* (2020) 86.7%. On the other hand, comparable studies by Rodrguez *et al.* (2013) and Amita *et al.* (2018) revealed high susceptible rates of Gentamycin against *S. aureus* with 91.8% and 96.2%, respectively, which were higher than the result which could be attributed to the antibiotics' status as second-line medications and their accessibility, which limits patients' options for treatment and lowers their exposure to resistant bacteria.

As a co-inducer of the mecA gene regulator, the cefoxitin antibiotic is usually utilized as a phenotypic indication for the identification of methicillin resistance in Staphylococcus species (Baguma *et al.*, 2017). Out of 111 isolates examined, this study found 6 (5.4%) methicillin-resistant *Staphylococcus aureus*. This study's proportion of methicillin-resistant *Staphylococcus aureus* was lower than that of Adam *et al.* (2020) and greater than that of Muge *et al.* (2015), who reported 2.1%. However, five of the six isolates in this investigation were found to carry mecA gene (83.33%). This higher percentage support the fact that methicillin resistance is regulated by mecA gene presence in the bacterial genome, and pose a threat of treatment complication in both healthcare and community acquired infections caused by *Staphylococcus aureus*. This is consistent with the Samah *et al.*, 2019 research, which found that all 300 isolates resistant to cefoxitin tested positive for the mecA gene. However, this study's percentage was greater than that of Peacock *et al.* (2015) and Natalia *et al.* (2011), who found 95.12% and 93.75%, respectively, of *S. epidermidis* resistant to cefoxitin. The highest susceptibility of cefoxitin antibiotic to *S. aureus* in this study could be due to the fact that the drug is a second-generation antibiotic which is not commonly prescribe in the health-care settings (Baguma *et al.*, 2017). Moreover, the occurrence of Methicillin resistant *S. aureus* in the studied hospitals, is a threat to both health-care and community settings, through cross-contamination between surfaces to the health care personnel, or the patients and to the visitors, which may finally result in the spread of the resistant bacteria in the community.

CONCLUSION

In this study, all the four hospitals were found to be contaminated with *S. aureus* with General Hospital Dutsin-Ma having the highest percentage (17.5%) and FUDMA Clinic with the lowest percentage (11.3%) respectively. Among the antibiotics tested, Cefoxitin and Amoxicillin were found to be more effective against the isolates, while the isolates demonstrated a high resistance to Erythromycin and Ciprofloxacin respectively. However, Methicillin and Multi-drug resistant *S. aureus* were isolated from the four hospitals, and five Methicillin resistant *S. aureus* were found to be harboring mecA gene.

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