



Research Article

Phenotypic and Molecular Detection of Methicilin Resistant *Staphylococcus aureus* Isolated from Clinical Samples in some Selected Hospitals in Dutsin-Ma and Kurfi LGAS, Katsina State, Nigeria

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ABSTRACT

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The methicillin resistant *Staphylococcus aureus* (MRSA) is becoming increasingly important as a nosocomial pathogen; it has been associated with high mortality and present significant difficulty in the management of patients due to its cross resistance with other antibiotics. A total of 192 clinical samples were collected and cultured on mannitol salt agar (MSA). Gram positive cocci in cluster that shows mannitol fermentation on MSA and are both catalase and coagulase positive were identified as *S. aureus*. Disc diffusion method was employed to test for the sensitivity of the isolates against five antibiotics (erythromycin, ciprofloxacin, ampicillin, gentamicin and ceftiofloxacin), the isolates that showed resistance to ceftiofloxacin 30µg were noted as MRSA, and were further confirmed for the presence of *mec A* using specific PCR primer. A total of 52(27.1%) *S. aureus* isolates were recovered from the 192 samples collected. Thirty three (63.5%) of the 52 isolates were identified as MRSA, and 24 (46.2%) of these were positive for the *mec A* gene. Among the 52 isolates, 26 (50.0%) were sensitive to ciprofloxacin, 5 (9.6%) to amoxicillin, 20 (38.5%) to gentamicin, 14 (26.9%) to erythromycin and 19(36.5%) to ceftiofloxacin. The highest level of resistance was observed for ampicillin (90.4%) and thirty-three (63.5%) were identified as multidrug resistant (show resistance to 3 or more classes of antibiotics). Our finding shows the evidence of MRSA both by phenotypic and molecular method in the four selected hospitals in Dutsin-Ma and Kurifi LGAs. This necessitates the need for increased surveillance, tracking and reporting of infections and antimicrobial resistance in our hospitals. A more detail and wider scope study should be carried out to generate a comprehensive data on antimicrobial resistance; such research should be targeted to genes not covered by the present study.

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INTRODUCTION

Antibiotics refer to products secreted naturally by microorganisms that have the ability to either kill or inhibit the growth of other microbes (Foster, 2017). A few of these products are synthetic while others undergo chemical modifications (Foster, 2017) to interfere with bacterial cell wall synthesis, translation, transcription and replication of DNA (Vestergaard *et al.*, 2019) and have proven helpful in the treatment of microbial infections. Antibiotics have also been successfully used in animals and poultry production in prophylaxis, therapeutic and growth promoter purposes (El Seedy *et al.*, 2017).

The Gram-positive bacterium *Staphylococcus aureus* (*S. aureus*) has demonstrated commensal relationship (Akya *et al.*, 2020) and is also implicated in infections of the skin and various tissues as well as toxin-mediated diseases such as food poisoning and toxic shock syndrome (Kyany'a *et al.*, 2019; Akya *et al.*, 2020;). The bacterium have cause considerable morbidity and mortality in both healthcare and community settings (Valle *et al.*, 2016; Ahmadishoar *et al.*, 2020) and the emergence of antibiotic resistant strains of *S. aureus* has pose a significant challenge in therapeutic options and management of infections (Schito, 2006; Kyany'a *et al.*, 2019). The increasing resistance to antibiotics by bacterial pathogens is now a global problem (Huebner *et al.*, 2016). The Methicillin-resistant *S. aureus* (MRSA) was first reported in the 1960s (Wang *et al.*, 2012), with a significant increase in prevalence observed in the past twenty years (Girmay *et al.*, 2020) making MRSA a

worldwide problem (Amini *et al.*, 2012). It is said to be the leading cause of death due to microbial infections (Wang *et al.*, 2012). MRSA strains are classified as hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA) (Valle *et al.*, 2016; Girmay *et al.*, 2020), however, infection and colonization of horses, dogs, cats, birds, dairy cows, and chicken have also been reported (Girmay *et al.*, 2020).

Mechanism of antibiotic resistance of MRSA is a function of *mec A* gene; a 2.1kb DNA segment non-inherent to *S. aureus* inserted in a large block of exogenous DNA, called staphylococcal cassette chromosome *mec* (SCC*mec*) (Girmay *et al.*, 2020). This gene is translated into a penicillin-binding protein PBP2a with low affinity for β -lactam antibiotics (El-gendi and Amin, 2019; Girmay *et al.*, 2020), thereby enabling the bacterium to bypass the activity of β -lactam antibiotics such as methicillin, nafcillin, oxacillin, and cephalosporins (Akya *et al.*, 2020). This gene is reported (Amini *et al.*, 2012) to primarily mediates resistance towards methicillin, but recently resistance to penicillin, oxacillin, and erythromycin has also been observed.

Both phenotypic and molecular techniques are used in differentiating MRSA from other strains of *S. aureus*. Oxacillin and cefoxitin are indicators of non-susceptibility to all categories of β -lactams, and are currently used in the identification of MRSA phenotypically (Valle *et al.*, 2016), whereas techniques such as restriction fragment length polymorphism (RFLP) analysis, ribotyping, binary typing, and PCR-based methods have all been used for the

characterization of MRSA at molecular level (Valle *et al.*, 2016). Although the frequency and impact of MRSA is well documented in western countries, there is a major gap in monitoring and tracking antibiotic resistance, with data gathered in only limited number of countries across the African continent (Garoyet *et al.*, 2019). This study employed antimicrobial susceptibility testing (AST) and PCR primer for the phenotypic and molecular characterization of MRSA isolated from clinical samples in four selected hospitals in Dutsin-Ma and Kurfi LGAs, Katsina State.

MATERIAL AND METHODS

Study Design and Area

The study is a cross sectional study conducted in Dutsin-MA and Kurfi Local Government Areas (LGAs), in the northern zone of Katsina State, Nigeria. Four (4) hospitals; General Hospital Dutsin-Ma (GHD), Comprehensive Health Center Dutsin-Ma (CHCD), Federal University Dutsin-MA (FUDMA) Clinic and General Hospital Kurfi (GHK) were used for the study. Samples (ear swab, hand swab, nasal swab and wound swab) for bacterial isolation were collected from patient admitted in the selected facilities.

Inclusion and Exclusion Criteria

Patients admitted in the four selected hospitals that consented to be part of the study were included. Any individual that fail to meet the inclusion criteria was excluded.

Ethical consideration

Ethical approval for the study was sought and obtained from the Katsina State, Ministry of

Health. with assign number MOH/ADM/SUB/1152/1/756.

Sample Collection

A total of 192 swab samples were collected using sterile swab sticks. Forty eight (48) samples were purposively collected from each facility, and within each facility, a stratified sampling technique was adopted such that twelve (12) samples each of ear swab, nasal swab, hand swab and wound swab were collected. The purposive sampling technique was chosen to ensure the equal representation of the four major hospitals in Dutsin-Ma and Kurfi. All samples collected were transported under cold condition to the microbiology laboratory of Federal University Dutsin-MA within two hours of collection; the inoculation of samples in MSA was done immediately on arrival.

Isolation and Identification of *S. aureus*

All samples collected were inoculated onto mannitol salt agar (MSA) by streak plate method and the inoculated plates were incubated for 24 hours at 37°C. Colonies that demonstrated growth features of *S. aureus* (golden yellow color) on MSA were gram stained and subjected to catalase and coagulase tests as describe by Garoyet *et al.* (2019). Gram positive bacteria in cluster that ferment Mannitolin culture and were positive for catalase and coagulase were identified as *S. aureus*.

Antimicrobial Sensitivity Testing and MRSA screening

Standard antibiotics disk belonging to different classes were tested for activity against each of the isolates identified as *S aureus* using the Kirby-

Bauer disk diffusion method. These include ciprofloxacin, amoxicillin, gentamycin and erythromycin. A bacterial suspension equivalent to the 0.5 McFarland turbidity standards was prepared and evenly spread on Muller–Hinton Agar (MHA) plate using a sterile swab stick. There after the antibiotic disk were placed at approximately equal distance on the MHA to avoid overlapping of activity. The antibiotic-seeded MHA were then incubated for 24 hours at 37°C. The zones of inhibition were interpreted as resistant (R), intermediate (I), and sensitive (S) according to CLSI guideline 2022. Multidrug resistance was defined as resistance to 3 or more different classes of antibiotics (Aliyu *et al.*, 2022), while MRSA was screened using cefoxitin disc (30µg) (Ahmadishoar *et al.*, 2020).

Bacterial DNA Extraction and Polymerase Chain Reaction (PCR)

Bacterial DNA extraction was done using GeneJET Genomic DNA Purification Kit according to the manufacturers' instruction. *Mec A* forward (5'-TCCAGATTACAACCTTCACCAGG-3') and reverse (5'-CCACTTCATATCTTGTAACG-3') primers previously described (El Seedy *et al.*, 2017) were used for the amplification of *mecA* gene at 100bp. Four hundred microliter (400 µl) of PCR product and 100 µl of 6X loading dye was loaded into well comb of 1% agarose gel. Also 600 µl of 100 bp DNA ladder (5X dilution) was loaded into the first well to serve as a marker. The electrophoretic condition was set at 80 V and let to run for 45 min. The gel was stained with ethidium bromide for 10 min and shaken at 45 rpm. The gel was viewed under

ultraviolet (UV) and the photo was taken using high performance UV Transilluminator with exposure time of 6s.

RESULTS

A total of 192 patients were recruited from the four selected hospitals in Dutsin-Ma and Kurfi LGAs of Katsina State, 86(44.8%) and 106 (55.2%) of the population are male and female respectively. The age range of the participants were recorded between 0 to 91, ages 0-20 recorded 90 (46.9%) number of participants, followed by 21-40 with a record of 79(41.1%). The least number of participants is observed in the age group 81-100 with a record of 4(2.1%). (Table 1).

This study established the overall prevalence of *S. aureus* at 27.1%. Higher prevalence was recorded in male (34.9%) than female (20.8%) subjects. Isolation rate of *S. aureus* in relation to age shows higher prevalence in age group 41-60(33.3%) followed by 0-20(30.0%) (Table 1). Wound Swab(41.7%) and General Hospital Kurfi (37.5%) show more positive cultures for *S. aureus* in relation to sample type and hospital respectively (Table 1).

The present study defined methicillin resistant *S. aureus* (MRSA) as isolate that shows resistance to cefoxitin (30µg); which was interpreted as a zone of inhibition ≤ 21mm (CLSI, 2009). A total of 33(17.2%) of the positive cultures of *S. aureus* screened positive for MRSA phenotypically; 19(22.1%) of this number are male and 14(13.2%) are female. Wound swab, nasal swab, hand swab and ear swab recorded 12(25.0%), 10(20.8%), 7(14.6%) and 4(8.3%) MRSA respectively. Phenotypic distribution of MRSA

among the four selected hospitals shows the highest and least prevalence in CHCD (22.9%) and FUDMA clinic (12.5%), there was even distribution of MRSA between GHK (16.7%) and GHD (16.7%) phenotypically (Table 2).

All the 33 isolates that screened positive for MRSA phenotypically were tested for *mecA* gene using PCR method. Twenty four (24) of this number demonstrated the presence of *mecA*, where as 9 were negative. All the isolates that tested positive for *mecA* were distributed between the ages of 0 to 40. All isolates from hand swab that were resistant to cefoxitin (30µg) are carriers of *mecA*. Furthermore, 100% agreement was observed between phenotypic and molecular presence of MRSA in GHD (Table 2).

The antimicrobial activities of five representative antibiotics from five different classes were tested against the 52 isolates of *S. aureus*. These are ciprofloxacin, amoxicillin, gentamycin, erythromycin and cefoxitin. While other four antibiotics are interpreted as sensitive, intermediate or resistant, cefoxitin (30µg) is interpreted as either sensitive or resistant according to CLSI guideline 2009. The highest activity was demonstrated by ciprofloxacin (50.0%), followed by gentamycin (38.5%) and cefoxitin (36.5%). On the other hand, the bacterial isolates were most resistant to amoxicillin (90.4%).

Table 1: Prevalence of *S. aureus* in relation to gender, age, sample type and location

Parameter	Frequency	Presence of <i>S. aureus</i>	Prevalence of <i>S. aureus</i>
Gender			
Male	86(44.8%)	30	34.9%
Female	106(55.2%)	22	20.8%
Age (years)			
0-20	90(46.9%)	27	30.0%
21-40	79(41.1%)	19	24.1%
41-60	9(4.7%)	3	33.3%
61-80	10(5.2%)	2	20.0%
81-100	4(2.1%)	1	25.0%
Sample type			
Ear swab	48(25.0%)	8	16.7%
Nasal swab	48(25.0%)	14	29.2%
Hand swab	48(25.0%)	10	20.8%
Wound Swab	48(25.0%)	20	41.7%
Hospital			
GHD	48(25.0%)	9	18.8%

CHCD	48(25.5%)	15	31.3%
FUDMA Clinic	48(25.0%)	10	20.8%
GHK	48(25.0%)	18	37.5%

GHD; General Hospital Dutsin-Ma, CHCD; Comprehensive Health Center Dutsin-MA, FUDMA Clinic; Federal University Dutsin-Ma Clinic, GHK; General Hospital Kurfi.

Table 2: Phenotypic and molecular distribution of MRSA among the positive cultures

Parameter	Presence of <i>S. aureus</i>	Phenotypic distribution of MRSA	Molecular distribution of MRSA
Gender			
Male	30	19(63.3%)	16(53.3%)
Female	22	14(63.6%)	8(36.4%)
Age (years)			
0-20	27	14(51.9%)	11(40.7%)
21-40	19	17(94.5%)	13(68.4%)
41-60	3	1(33.3%)	0(0.0%)
61-80	2	0(0.0%)	0(0.0%)
81-100	1	1(100%)	0(0.0%)
Sample type			
Ear swab	8	7(87.5%)	5(62.5%)
Nasal swab	14	10(71.4%)	6(42.9%)
Hand swab	10	4(40.0%)	4(40.0%)
Wound Swab	20	12(60.0%)	9(45.0%)
Hospital			
GHD	9	8(88.9%)	8(88.9%)
CHCD	15	11(73.3%)	4(26.7%)
FUDMA	10	6(60.0%)	5(50.0%)
Clinic			
GHK	18	8(44.4%)	7(38.9%)

GHD; General Hospital Dutsin-Ma, CHCD; Comprehensive Health Center Dutsin-MA, FUDMA Clinic; Federal University Dutsin-Ma Clinic, GHK; General Hospital Kurfi.

Table 3: Antibiotic sensitivity pattern of the 52 *S. aureus* isolates

Antibiotic	Disc Concentration	Sensitive	Intermediate	Resistant	Total
CPX	10µg	26(50.0%)	19(36.5%)	7(13.5%)	52(100%)
AM	30µg	5(9.6%)	0(0.0%)	47(90.4%)	52(100%)
CN	10 µg	20(38.5%)	2(3.8%)	30(57.7%)	52(100%)
E	10 µg	14(26.9%)	12(23.1%)	26(50.0%)	52(100%)
FOX	30 µg	19(36.5%)	0(0.0%)	33(63.5%)	52(100%)

CPX; ciprofloxacin, AM; amoxicillin, CN; gentamycin, E; erythromycin

Multidrug resistant isolates are defined as isolates

that shows resistant to 3 or more antibiotics belonging to different classes (Aliyuet *et al.*, 2022). A total of 33(63.5%) out of the 52 isolates

demonstrated resistance to 3 or more antibiotics from different classes, while 19 (36.5%) were resistant to 1 or 2 antibiotics only.

Table 4: Prevalence of multidrug resistant *S. aureus* among the 52 isolates

Resistance pattern	Frequency	Prevalence
≤ two antibiotics	19	36.5%
≥ three antibiotics	33	63.5%
Total	52	100.0%

DISCUSSION

The bacterium *S. aureus* is a normal flora of the skin, however, its invasion of sterile body sites such as the blood stream and internal tissues results in various clinical manifestations (Cong *et al.*, 2020) involving the skin, cardiovascular tissue, and osteoarticular tissue infections as well as pneumonia and bacteremia (Wang *et al.*, 2012) making it a leading pathogen. The emergence and increasing prevalence of antibiotic resistant *S. aureus* is of great concern, because existing antibiotics are becoming less effective against this pathogen (Odonkor and Addo, 2018). This study isolated *S. aureus* from clinical samples and screened for methicillin resistant strains (MRSA) using cefoxitin-disk (30µg) and thereafter confirms the presence of *mecA* gene by PCR method.

Out of the 192 samples collected; 52(27.1%) were positive cultures for *S. aureus*. The findings of this study agree closely with Ariom *et al.* (2019) who reported 30.8% in Federal Teaching Hospital Abakaliki (FETHA I), 30.1% in Federal Teaching Hospital Abakaliki (FETHA II) and 20.5% in mile four general hospital Abakaliki. This finding is however much lower than the

63.1% in Eritrea (Garoy *et al.*, 2019), 42.9% reported in table eggs (El-gend and Amin, 2019) and the 50% hospital acquired bacteremia in Thailand (Lim *et al.*, 2016). This wide disparity could be attributed to differences in geographical locations and sample source. The prevalence of *S. aureus* is higher in male (34.9%) than female (20.8%) in this study, a contrast of Garoy *et al.* (2019) who reported more prevalence in female (64.8%) than male (61.8%). Wound swab recorded more positive cultures (41.7%) than other (Ear swab, Nasal swab and Hand swab) sample types.

Cefoxitin-disk (30 µg) is the recommendation of Clinical and Laboratory Standards Institute (CLSI) for the screening of MRSA (Loomba *et al.*, 2010), and in agreement with other literatures (Nicole *et al.*, 2009; Loomba *et al.*, 2010; Lim *et al.*, 2016; Garoy *et al.*, 2019) we defined resistance as a zone of inhibition ≤ 21mm on Mueller-Hinton agar. Out of the 52 positive cultures of *S. aureus*, 33(63.5%) were screened as MRSA. The findings of this study is higher than the 22.6% (Ariom *et al.*, 2019) reported in Abakaliki (Nigeria), 40.8% and 57.1% (Ahmadishoar *et al.*, 2020) reported in Tabriz and

Kerman as well as the 33.3% (Girmay *et al.*, 2020), 36.6% (Shimel *et al.*, 2014) and 35.6% (Suleiman *et al.*, 2012) reported in dairy cows in Tigray (Ethiopia), Selale/Fitche Area (Ethiopia) and Plateau (Nigeria) respectively. It is however, lower than the 72.0% reported in Eritrea (Garoy *et al.*, 2019), 84.2% recovered from milk and meat (El Seedy *et al.*, 2017), and the 92% reported in Thailand (Lim *et al.*, 2016). These disparities in prevalence of MRSA correlate with existing literature suggesting a significant geographical variation in the frequency of the pathogen within and between countries (Garoy *et al.*, 2019), which can partly be explained by the dissemination of clonal strains, temporary hospital outbreaks (Schito, 2006), differences in infection control measures and inappropriate use of antibiotics (Ahmadishoar *et al.*, 2020).

There is a varied distribution of MRSA in the four hospitals considered in this study; 8(88.9%) in GHD, 11(73.3%) in CHCD, 6(60.0%) in FUDMA clinic and 8(44.4%) in GHK. This conforms to the study of Schito (2006) that also showed considerable variation in MRSA proportions among hospitals. The prevalence of MRSA from cultures of ear swab, nasal swab, hand swab and wound swab were 7(87.5%), 10(71.4%), 4(40.0%) and 12(60.0%) respectively. Our findings is higher than the 27.8% (ear swab), 26.4% (nasal swab) and 33.3% (wound swab) reported in Abakaliki, Nigeria (Ariom *et al.*, 2019), again establishing the intra country variation of MRSA. On the other hand, this study agrees with Garo *et al.* (2019) who reported 62.5 % and 60% in abscess and burns respectively. Previous studies (Shahraz *et al.*,

2012; Jamali *et al.* 2015; ; El Seedy *et al.*, 2017; Kyany'a *et al.*, 2019) have established 100% correlation of phenotypic characterization of MRSA and the presence of *mec A* gene, however, only 24 out of the 33 isolates identified as MRSA were positive for *mec A* in the present study, our findings agree with others literatures (Umaru *et al.*, 2013; Jahan *et al.*, 2015; Ishaq *et al.*, 2016) that reported a similar trend of lower *mec A* gene detection by PCR than the phenotypic prevalence of MRSA, confirming the assertion of Garoy *et al.* (2019) that genotypic detection by PCR tends to report comparatively lower MRSA prevalence.

The sensitivity of the 52 isolates of *S. aureus* were tested against different classes of antibiotics which include fluoroquinolones (ciprofloxacin), penicillin (ampicillin), aminoglycoside (gentamycin) and macrolide (erythromycin). Out of the 52 isolates; 26(50.0%), 5(9.6%), 20(38.5%) and 14(26.9%) were sensitive to ciprofloxacin, amoxicillin, gentamycin and erythromycin respectively. The isolates were most resistant to ampicillin (90.4%), which closely agree with the 100% reported previously (Ariom *et al.*, 2019). Seven (13.5%), 30(57.7%), 26(50.0%) and 33(63.5%) out of the 52 isolates were resistant to ciprofloxacin, gentamycin, erythromycin and ceftazidime respectively. This resistance pattern showed a wide disparity with the study of Ariom *et al.* (2019) that established 94.7% and 78.9% resistance against ciprofloxacin and erythromycin respectively. Our findings also disagree with some authors (El Seedy *et al.*, 2017; Garoy *et al.*) who reported 100% and 93.9% sensitivity of *S. aureus* to gentamycin, but

aligned with Akya *et al.* (2022) who on the other hand reported 70% resistance of *S. aureus* to gentamycin. The variability observed in the 52 isolates of *S. aureus* showed resistant to 3 or more antibiotics (Table 4) and were defined as multidrug resistant bacterial pathogens in agreement with other literatures (Aliyu *et al.*,

CONCLUSION

Bacterial resistance to antibiotics has been a topical issue in the globe, it is a major public health problem worldwide as many antimicrobial agents are becoming ineffective resulting in limited treatment option, increase mortality due to infection as well as economic lost. Our study established 33(63.5%) prevalence of MRSA in the four selected hospitals in Dutsin-Ma and Kurfi LGAs, Katsina State, and 24(42.6%) were confirm to harbor *mec A* gene from our findings. Furthermore, 33 (63.5%) of the isolates of *S. aureus* demonstrate resistance to 3 or more different classes of antibiotics considered in this study. The implications of our findings suggest that our hospitals could be potential source of infection by this highly resistant pathogen, and the management of patients in our hospitals will become more complicated and difficult to handle.

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sensitivity and resistant pattern of *S. aureus* in different studies can be attributed to differences in prescription processes. Thirty three (63.5%) of 2022; Akya *et al.*, 2022), this correspond with the report of Aliyu *et al.* (2022) that the prevalence of MDR in North West Nigeria ranges between 3.21 -97.90%.

RECOMMENDATION

Generating data on the trend of antimicrobial resistance, an effective drugresistance surveillance and containment system, identifying high risk patients and reporting infections from different health care sector is a sure way that will help in mitigating antimicrobial resistance. It is our recommendation that a more detail study on the characterization and sequencing of MRSA with consideration to other genes not covered in the present study should be carried out in a broader scope. Public health education on the dangers of non-prescribed drugs, indiscriminate use of antibiotics, taking incomplete dosage of drugs, among others should be encouraged, and there should be improvement in hygiene and decontamination practices in our health care facilities.

COMPETING INTERESTS

The authors declare no conflict of interests

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