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Determination of Phenotypic and Molecular detection of mecA and mecC genes in *Staphylococcus aureus* isolated from patients admitted in an emergency unit of a tertiary healthcare facility in Lagos, Nigeria

Chinedu Nweke Idakari [1,*], Godwin Macueley Emelobe [2], Patrick Chinazam Nwosu [3], Ifeoma Cecilia Uche-Omovoh [4], Ikechukwu Francis Agwu [5], Chidiebere Brown Ene [6], Winifred Chinwendu Akpa [7], Christian Obasi Akpa [8], Ogochukwu Chioma [9], Ngozi Maryjane Ezekwesili [10], Chinedum Christabel Amagwu [11], Uchenna Samuel Okparaoka [12] and Ogechukwu Rejoice Idakari [13]

[1] *Department of Medical Microbiology and Parasitology, Alex-Ekwueme Federal University Teaching Hospital Abakaliki (AEFUTHA) Ebonyi State, Nigeria.*
[2] *Department of Medical Microbiology and Parasitology, Alex-Ekwueme Federal University Teaching Hospital Abakaliki (AEFUTHA), Ebonyi State, Nigeria.*
[3] *Clinical Lead Rehabilitation and Quality Department/ San Remo Nursing and Rehabilitation Center 3550 Shiloh Road, Garland Texas, USA.*
[4] *Department of Obstetrics and Gynaecology, Alex-Ekwueme Federal University Teaching Hospital Abakaliki, Ebonyi State, Nigeria.*
[5] *Department of Medical Microbiology and Parasitology, University of Uyo Teaching Hospital, Uyo Akwa Ibom State, Nigeria.*
[6] *Department of Medical Microbiology and Parasitology, Alex-Ekwueme Federal University Teaching Hospital Abakaliki Ebonyi State, Nigeria.*
[7] *Department of Medical Microbiology and Parasitology, Alex-Ekwueme Federal University Teaching Hospital Abakaliki (AEFUTHA.*
[8] *Department of Community Medicine Alex-Ekwueme Federal University Teaching Hospital Abakaliki (Abakaliki), Ebonyi State, Nigeria.*
[9] *Department of Surgery, Federal Medical Center Keffi Nasarawa.*
[10] *Department of Medical Microbiology and Parasitology, Alex-Ekwueme Federal University Teaching Hospital Abakaliki (AEFUTHA.*
[11] *Department of Medical Microbiology and Parasitology, Alex-Ekwueme Federal University Teaching Hospital Abakaliki.*
[12] *Department of Anatomic Pathology, Alex-Ekwueme Federal University Teaching Hospital Abakaliki (AEFUTHA), Ebonyi State, Nigeria.*
[13] *Department of Civil Law, Faculty of Law, Ebonyi State University, Abakaliki Ebonyi State Nigeria.*

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Abstract

Background: *Staphylococcus aureus* is a Gram-positive bacteria pathogen responsible for both community acquired and healthcare associated infections. Methicillin-resistant *S. aureus* (MRSA) remains a significant global health concern due to its high morbidity and mortality. This study investigated the phenotypic and molecular detection of mecA and mecC genes in *S. aureus* isolates from patients admitted to the emergency unit of a tertiary healthcare facility in Lagos, Nigeria.

* Corresponding author: Chinedu Nweke Idakari

Methods: This is a cross-sectional study conducted in an emergency unit of Lagos University Teaching Hospital Idi-Araba Lagos. Consenting adult patients admitted in emergency unit were enrolled into the study. It was conducted between Ist June, 2019 to 30th May, 2020.

Result: A total of 300 patients clinically diagnosed with infections were recruited for the study. *Staphylococcus aureus* was identified as the causative agent in 43 of these cases. Female patients constituted 51.2% of those infected, while males accounted for 48.8%. The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) among the isolates was 41.8%. All MRSA isolates carried the mecA gene, and one isolate was found to possess both mecA and mecC genes.

Conclusion: The observed high prevalence of MRSA and the presence of both mecA and mecC genes highlight the epidemiological significance of this study. Continuous antibiotic resistance monitoring and routine screening for methicillin-resistant *Staphylococcus aureus* are therefore strongly recommended.

Keywords: *Staphylococcus aureus*; mecA; MRSA; Patients; Antibiotics

1. Introduction

Staphylococcus aureus are gram positive bacteria that majorly causes community and health care associated infections. It is a common human pathogen that is able to elicit a wide range of infections, including skin and soft tissue infections, toxin-mediated diseases, and pneumonia [1]. *S. aureus* exhibits increasing virulence and resistance to various antibiotics, complicating prevention and treatment of infections [1,2]. However, for the past few decades, *S. aureus* has been more and more difficult to treat because of its constant evolution and resistance to treatment with drugs such as methicillin and vancomycin [3].

Methicillin resistant *Staphylococcus aureus* (MRSA) is a substantial public health problem worldwide causing significant morbidity and mortality and inflated health care costs [4]. It is considered a major health problem for many years, MRSA has been considered the prototype for multi-resistant hospital acquired pathogens, which causes infections in high-risk, hospitalized patients [5]. MRSA is one of the major causes of both hospital and community acquired infections in humans, [6], resistance of Staph aureus to methicillin occurs due to the acquisition of mecA gene that is carried on a large mobile genetic element of the staphylococcal cassette chromosome and which encodes a low affinity penicillin binding protein 2a (PBP2a) to β-lactam antibiotics except the fifth-generation cephalosporins, the mecA gene complex also contains insertion sites for plasmids and transposons that facilitate acquisition of resistance to other antibiotics such as erythromycin, clindamycin, gentamicin, cotrimoxazole, and ciprofloxacin [7], the presence of mecC genes may also mediate methicillin resistance. Methicillin resistant Staph. aureus causes skin and soft tissue infections, blood stream infection, infective endocarditis, osteoarthritis, pleuropulmonary infections, prosthetic device infections and respiratory tract infections, MRSA is equally responsible for toxin mediated diseases. MRSA infections have been reported to be responsible for increased length of hospitalization, economic issues, and high mortality rate. A number of investigations have reported that *S. aureus* is among the most frequently encountered bacterial species in microbiology laboratories in Nigeria [8,9]. In the past antibiotic susceptibility testing of *S. aureus* in Nigeria was based on phenotypic testing especially the disk diffusion technique which is now accompanied with the use of PCR for detection of the specific genes (mecA and mecC genes) for the identification and confirmation of MRSA [10]. The increase of MRSA has caused researchers to tend more towards studies of the nature of genes encoding resistance to methicillin and other antibiotics and the mechanism by which these genes spread and evolve. However, due to changes in the healthcare system along with the evolution of the microorganisms, MRSA can now also be considered a major cause of community-acquired infections (CA-MRSA) [5].

Significant increase in the prevalence and emergence of MRSA is a serious public health concern and has a serious negative impact on medical practices [11].

There was an estimate of 94,360 invasive MRSA infections in the United States in 2005, causing more than 18,000 deaths per year [12]. Methicillin-resistant *S. aureus* prevalence has increased over the last 10 years; MRSA-related hospital discharges have doubled over 10 years, with hospital discharges for MRSA skin and soft tissue infection tripling since 2004 [13].

2. Problem statement

Methicillin Resistant *Staphylococcus aureus* (MRSA) is a major public health concern and is responsible for both hospital and community associated infections worldwide, MRSA increases the use of medical resources, as well as cost of

treatment and duration of healthcare associated and community associated staphylococcal infections, it is also one of the major causes of high mortality and hospitalization rates. These are major public health problems worldwide, especially in developing countries where infection rates are higher due to overcrowding, poor infection prevention and control practices, antibiotics misuse and others leading to evolution and spread of methicillin resistant strain.

2.1. Justification of the study

Based on the increased incidence of methicillin resistant staphylococcal infections Worldwide it is important to study the spread of methicillin resistant *Staphylococcus aureus*, also to detect and understand the nature of genes encoding resistance. To help improve on our prevention and control practices and help in proper treatment of the patients positive for MRSA in the adult ward of Lagos University Teaching Hospital.

This study aimed at determining both phenotypic and molecular characteristic of methicillin resistant *Staphylococcus aureus* isolated from adult patients admitted in an emergency unit of a tertiary healthcare facility in Lagos, Nigeria.

2.2. Study area

This study was undertaken at the adult medical emergency units of the Lagos University Teaching Hospital (LUTH) Idi-Araba, Lagos state, Nigeria. This is one of the largest hospitals in the country and it is cosmopolitan city in Nigeria. The study participants were recruited from patients on admission the adult medical ward of LUTH.

2.3. Study design

This study was a cross-sectional study of all the patients at both male and female adults in the medical emergency unit of Lagos University Teaching Hospital Idi-Araba, Lagos. Samples were collected from patients based on the nature and site of infection before administration of antibiotics. Macroscopy, microscopy, culture and sensitivity testing was carried out on the specimens, Multiplex PCR for mecA and mecC gene detection was then carried out on *Staphylococcus aureus* isolates that were positive for resistance testing using cefoxitin disc diffusion test.

2.4. Type of samples collected

Clinically relevant samples including blood, urine, wound swab, wound biopsy, sputum was collected based on the nature and sites of infection in order to increase the chances of isolating the pathogen of interest.

2.5. Recruitment of patients

Adults on admission in emergency unit were recruited for this study after informed consent. Demographic data and clinical information of patients was obtained from the patient' medical record file.

2.6. Inclusion criteria

All adult patients with clinical diagnoses of infections were enrolled into the study

2.7. Exclusion criteria

Patients without clinical diagnoses of infections were exempted from the study

3. Tools and techniques for sample and data collection

3.1. Questionnaire

A structured questionnaire was administered to patients to obtain demographic and medical record review which captured data on age, sex and clinical symptoms and length of hospitalization of the patients.

3.2. Sample Collection and Transport

Blood culture bottle was given to the attending physicians to collect blood sample from adults on admission following examination by the attending physician. Sterile bottle was given to patients with urinary tract infections for urine sample collection. Wound swabs were collected from patients with any form of skin and soft tissues infections, pus, cerebrospinal fluid and all samples collected was transported to the Microbiology laboratory of the Lagos University Teaching Hospital for immediate processing.

3.3. Quality Control

Quality control strains were obtained from Department of Medical Microbiology, College of Medicine University of Lagos. Quality control of Antimicrobial Susceptibility Testing was carried out according to the clinical Laboratory Standards Institute (CLSI) guidelines using ATCC 43300 *S. aureus* as control strain [14].

3.4. Laboratory Specimen Processing

SPUTUM, WOUND BIOPSY, URINE, WOUND SWAB SPECIMEN PROCESSING

Specimens were inoculated on chocolate (incubated under 5% carbon dioxide for 24hours) and MacConkey agar (incubated under 37ºC for 24hours).

3.5. Blood Culture Processing

Incubated the blood culture bottles in the BACT ALERT machine at 37ºc, subculture broth from a positive flagged on chocolate (incubated under 5% carbon dioxide for 24hours) and MacConkey agar (incubated under 37ºC for 24hours).

Bottles with no microbial activities flagged negative on the 5th day of being loaded in the machine

3.6. Identification

The identification of the *Staphylococcus aureus* was confirmed by standard methods including, growth on mannitol salt agar, colony morphology, Gram stain and biochemical characteristics. Gram positive cocci isolates were subjected to catalase test and all gram-positive catalase positive isolates were subjected to coagulase test. Catalase and Coagulase positive organisms were identified as *S. aureus* while coagulase- negative organisms will be identified as coagulase-negative *staphylococcus*.

3.7. Mannitol salt Agar

Mannitol salt agar is a selective and differential medium it contains high concentration of (about 7.5-10%) of salt (NaCl) which is inhibitory to most bacteria, making it selective for gram positive bacteria (Staph aureus, Enterococcus) that can tolerate high salt concentration.

Staphylococcus aureus produces yellow colonies with yellow zones while coagulase negative Staph produces pink or red colonies with no change to the medium

3.8. Catalase Test

- **Procedure**: A drop of 3% hydrogen peroxide solution was placed on a clean, glass slide; the edge of another clean slide was used to pick the test organism. The organism was dipped into hydrogen peroxide. Bubble formation was regarded as positive.
- **Principle**: the enzyme catalase produced by the isolate mediates the breakdown of the hydrogen peroxide into oxygen and water leading to production of bubbles.

3.9. Coagulase Test

Coagulase test was performed to differentiate coagulase positive cocci (*Staphylococcus aureus*) from coagulase negative Staphylococci.

- **Procedure**: colony was placed on a clean, glass slide 10ul of human plasma was added. This was rocked for some few minutes and the presence of agglutination indicated a positive reaction.
- **Principle**; coagulase is an enzyme-like protein, it causes the plasma to clot by converting fibrinogen to fibrin.

3.10. Antibiotic susceptibility testing

Discrete colonies of identified isolates were emulsified in 3-5ml of normal saline and the turbidity of the resulting suspension was matched against 0.5 McFarland standard. The suspension was streaked on Mueller Hinton agar using a sterile swab stick. Ciprofloxacin (CIP), Penicillin(P), Gentamicin (CN), Clindamycin (DA), Erythromycin(E), Linezolid (LZD), Levofloxacin (LEV), Tetracycline (TE) and Trimethroprim-Sulphamethozole (SXT) were tested on all the *S. aureus* isolates. The plates were incubated for 24hours. Then check and measure the clear zone of inhibition around the antibiotic disc with a meter rule [14].

Antibiotic sensitivity and resistance were determined by the Clinical and Laboratory Standards Institute (CLSI) disk diffusion test on Mueller-Hinton Agar, using calibrated inoculum of the isolates based on McFarland standard [14].

3.11. Phenotypic detection of methicillin resistant *Staphylococcus aureus* (MRSA)

Methicillin resistance was tested in all Staphylococcus species using cefoxitin disk

- **Principle**: Cefoxitin inhibits the growth of *S.aureus* that are not methicillin resistant while resistant strains show reduced zone of inhibition to methicillins, oxacillins, and cefoxitin. Methicillin resistance is mediated by PBP-2a, alternative penicillin – binding protein encoded by mecA gene, which permits the organism to grow and multiply in the presence of methicillin and other beta- lactam antibiotics. Cefoxitin is recommended as a surrogate for disc diffusion testing of MRSA because it is a better inducer of mecA gene. The principle of this test is that the methicillin resistant *Staphylococcus aureus* (MRSA) shows reduced zone of inhibition to cefoxitin disc because of the presence of alternative binding protein.
- **Procedure**: the suspension of 0.5 McFarland standards of the isolate was inoculated on Mueller Hinton agar using sterile swab and cefoxitin disc placed on the agar. The agar was incubated at 35^0C to 37^0C for 24 hours in an ambient air.
- **Result:** the result was considered as resistant if the zone of inhibition is less than 22mm.
- **Quality control**: this was done using *S. aureus* ATCC 43300 and *S. aureus* ATCC 25923 as positive and negative controls respectively [14].

3.12. Molecular testing

Multiplex PCR, which utilizes multiple primer sets within a single PCR, was used to detect mecA and mecC gene.

- **Principle of PCR:** It is a primer mediated enzymatic amplification of DNA which involves three stages denaturation at 94˚C for 15 to 30 seconds, annealing at 54˚C-60˚C for 20 to 40seconds and elongation at 72˚C-80˚C, the DNA polymerase will synthesize new strands of DNA complementary to the offered template.

3.12.1. Extraction (kit from Nigeria institute of Medical Research)

Extraction kits content; (lysis buffer, two bottles of wash buffer, elusion buffer)

A day-old pure culture was grown on chocolate agar then introduce into tryptycase soy broth and the procedure was carried out according to the manufacturer's instruction.

3.13. DETECTION OF Meca AND Mecc GENES BY Multiplex PCR

Multiplex PCR was used to detect mecA and mecC genes in all methicillin resistant *S. aureus* strains. The mecA- specific primer set mecA1 (AAAATCGATGGTAAAGGTTGGC) and mecA2

(5'AGTTCTGCAGTACCGGATTTTGC3') with 155pb weight and mecC- specific primer set mecC1 (5'GAA AAA AAGGCT TAG AAC GCC TC3') and mecC2 (5'GAA GAT CTT TTC CGT TTTCAG C3') with 188bp weight was used for amplification. PCR was performed in a 20µl of a reaction mixture containing 1x PCR Buffer (Solis Biodyne), 1.5 mM MgCl2, 200 µM of each dNTP (Solis Biodyne), 20 pMol of each primer, 2.5 units of TaqDNA polymerase (Solis Biodyne), 20ng of extracted DNA, and sterile distilled water was used to make up the reaction mixture. After amplification agarosegels electrophoresis was used to separate the DNA strands. The PCR conditions were as follows: Initial denaturation at 95°C for 5 minutes followed by denaturation at 94°C for 30 seconds (30 cycles), annealing at 54°C for 40 seconds (cycles 30), elongation at 72°C for 4 minutes (30 cycles) and final elongation at 72°C for 10 minutes. *S. aureus* NCTC 13552 was used as the positive control strain [15,16].

3.14. Data Analysis

The statistical analysis software version 21 (SPSS) and excel was used for data analysis.

The data were entered into Excel Microsoft and statistical analysis was done using Social Sciences (SPSS) software, version 25. The variables were expressed in percentages and frequency. The p-values less than 0.05 was interpreted as statistically significant.

3.15. Ethical Approval

The ethical approval for the study conduction was obtained from Lagos University Teaching Hospital Research Ethical Committee (IREC). The study was conducted according to declaration of Helsinki. Both verbal and written informed consents were obtained from all the participants after the detail of the study was explained to them.

4. Result

4.1. Socio-demography of patients infected with *Staphylococcus aureus* in the adult medical emergency unit of LUTH

Out of the 300 patients enrolled in the study who had a clinical diagnosis of infection, *Staphylococcus aureus* was isolated from 43 cases. The age distribution of patients infected with *S. aureus* showed that the highest proportion (46.5%) were within the 41–50 years age group, followed by 23.3% in the 31–40 years group. Patients aged 51–60 years accounted for 14%, while those aged 18–30 years and above 61 years represented 11.6% and 4.6%, respectively. Among the infected patients, 22 (51.2%) were females and 21 (48.8%) were males, with a mean age of 47.7 years (SD ± 14.39). Furthermore, 18 (41.86%) of the *S. aureus* isolates were identified as methicillin-resistant *Staphylococcus aureus* (MRSA) (Table 1).

4.2. Distribution of *Staphylococcus aureus* infections among patients the adult medical emergency unit of LUTH by sample site

Staphylococcus aureus was isolated from various clinically relevant samples, majority 17() was isolated from the blood stream, followed by skin and soft tissue 15(), UTI was 7 and RTI was 4. MRSA was majorly isolated SSTI (10), followed by BSI (5), UTI was 2, RTI was 2. (Figure 1).

4.3. Antibiotic Susceptibility Pattern of MRSA isolated from patients in the adult medical emergency unit of LUTH

All the MRSA isolates were resistant to erythromycin and penicillin, followed by tetracycline and clindamycin which had 17 (94.4%) and 16 (88.9%) resistance respectively. All isolates were susceptible to vancomycin while 16 (88.9%) of them were susceptible to linezolid. See table 2.

4.4. Molecular detection of gene from MRSA isolated from patients in the adult medical emergency units of LUTH

All the isolates identified as MRSA using phenotypic method were all positive for MRSA gene detection using multiplex PCR. 17 (94.4%) of the MRSA isolates had MecA gene only while 1 (5.6%) had both MecA and MecC gene. See figure 3 and 4.

Table 1 Sociodemographic characteristics of the patients

Variable	Frequency (n=43)	Percentage (%)
Age group (Years)		
18-30	5	11.6
31-40	10	23.3
41-50	20	46.5
51-60	6	14.0
≥61	2	4.6
Means Age	47.7±14.4	
Gender		
Female	22	51.2
Male	21	48.8
MRSA Positive Po	18	41.9%

4.5. Patients (PX)

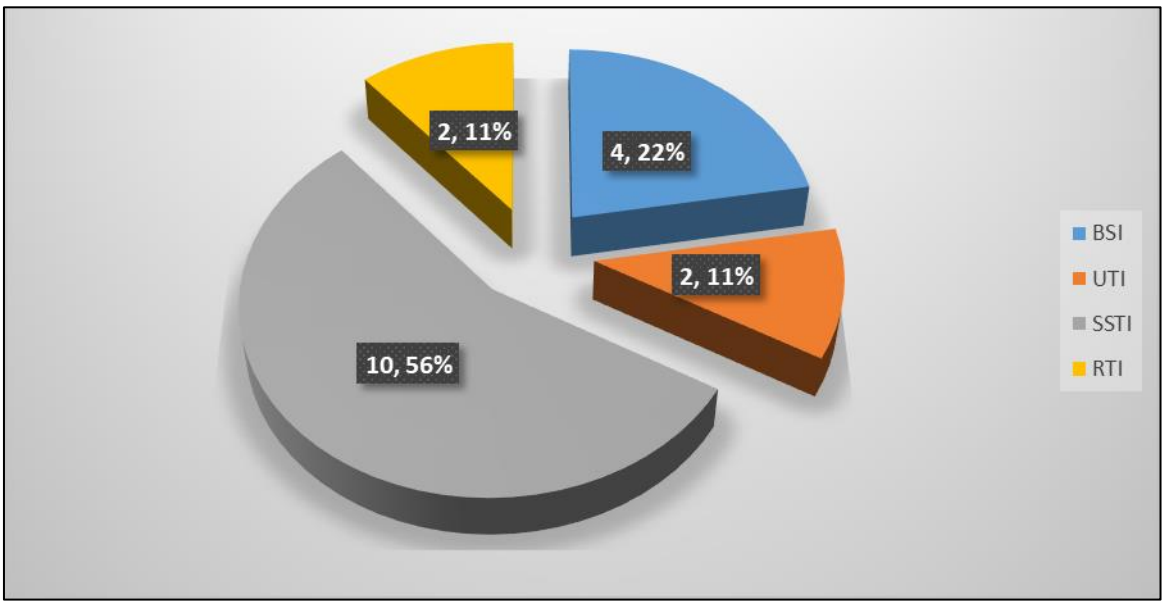


Figure 1 Distribution of 18 MRSA infections in the adult medical emergency unit of LUTH by sample type. (BSI (22%) = blood sample, RTI (11%) = sputum sample, UTI (11%) = Urine sample, SSTI (56%) = Wound swab and wound biopsy)

Table 2 Antibiotic susceptibility and resistance profile of *Staphylococcus aureus* isolated from patients the adult medical emergency of LUTH

	Antibiotics	**Sensitive Number (%)**	**Resistant Number (%)**
1	Erythromycin	10 (23.3%)	33 (76.7%)
2	Gentamicin	17 (39.5%)	26 (60.5%)
3	Tetracycline	10 (23.3%)	33 (76.7%)
4	Penicillin	13 (30.2%)	30 (69.8%)
5	Clindamycin	20 (46.5%)	23 (53.5%)
6	Levofloxacin	31 (72.1%)	12 (27.9%)
7	Vancomycin	43 (100%)	0 (0%)
8	Linezolid	41 (95.3%)	2 (4.7%)
9	Trimethroprim-Sulphamethozole	21 (48.8%)	22 (51.2%)
10	Ciprofloxacin	17 (39.5%)	26 (60.5%)

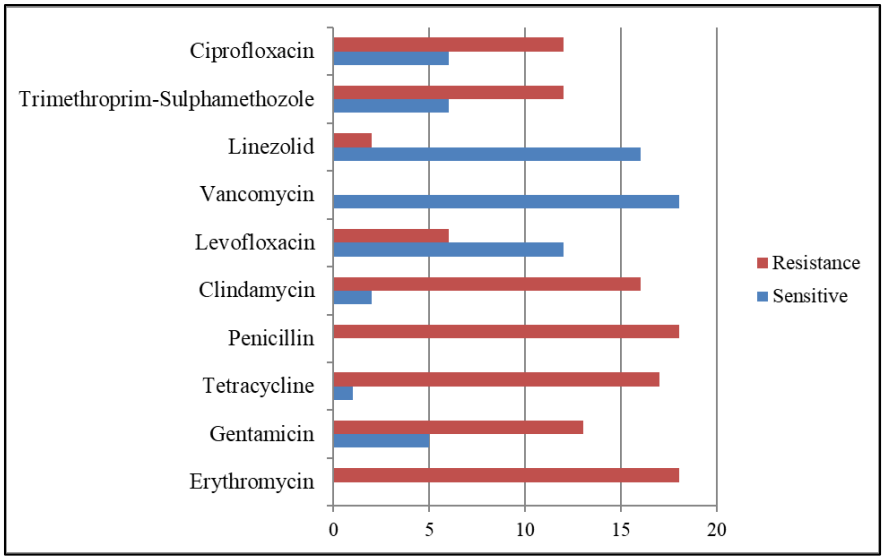


Figure 2 Antibiotic Susceptibility Profile of Methicillin Resistant *Staphylococcus aureus* isolated from patients in the adult emergency unit of LUTH

(Erythromycin and Penicillin= 100% resistance, Gentamicin =73.2%R, Tetracycline = 94.4%R, Clindamycin =11.1%S and 88.9%R, Trimethoprim-sulphametazole and Ciprofloxacin had 66.7%R, Levofloxacin had 33.3%R fortunately, Linezolid had 88.9% sensitive while all the isolates were sensitive to Vancomycin).

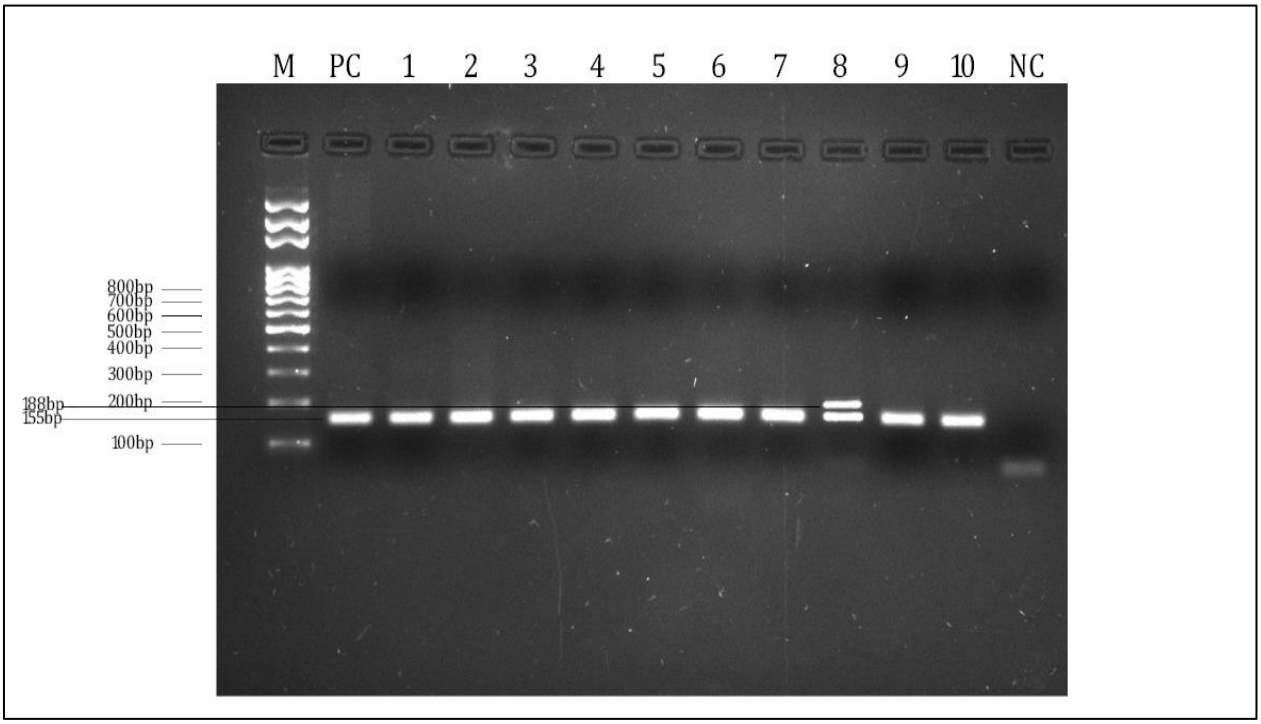
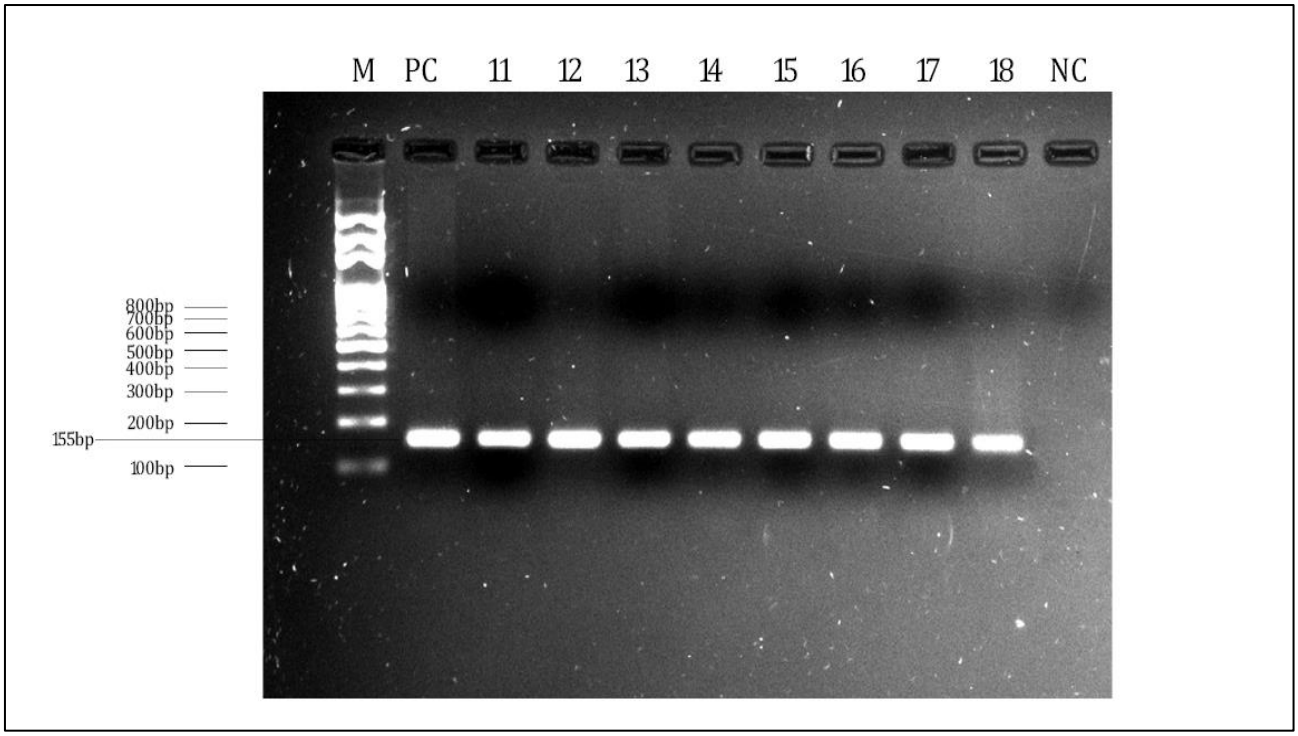


Figure 3 Agarose gel electrophoresis of PCR products of MRSA isolate 1-10

(Meca genes. M = DNA marker fragments, PC = MRSA positive control, NC= Negative control, Lane 1, 2, 3, 4, 5, 6, 7, 9 and 10 indicate the Meca positive MRSA while 8 represent both Meca and Mecca positive MRSA. The DNA fragments of 155bp were amplified from Meca gene (Meca primer weight =155bp) while the DNA fragment of 188bp were amplified from Mecca gene (Mecca primer weight =188bp).



(mecA genes. M = DNA marker fragments, PC = MRSA positive control, NC= Negative control, Lane 11, 12, 13, 14, 15, 16, 17 and 18 indicate the mecA positive MRSA. The DNA fragments of 155bp were amplified from mecA gene (mecA primer weight =155bp).

Figure 4 Agarose gel electrophoresis of PCR products of MRSA isolate 11-18

5. Discussion

Resistance of *Staphylococcus aureus* to methicillin is linked with the acquisition of mec genes known to code for resistance to beta-lactam antibiotics, *S. aureus* has been an impediment for antimicrobial chemotherapy, and the introduction of new classes of antibiotic is usually followed by the emergence of resistant forms of this pathogen [17,18]. All the Methicillin resistant *S. aureus* identified using phenotypic method in this study were all positive for resistance gene detection, this shows that resistance of *Staphylococcus aureus* isolated from the patient in the adult medical emergency unit of LUTH was mediated by mecA and mecC genes, 17 (94.4%) had mecA only while one which was isolated from the wound biopsy collected from a 52years female patient had both mecA and mecC, although the prevalence of MRSA among the *Staphylococcus aureus* in this study was high it is a great relieve that all the MRSA isolates were sensitive to vancomycin which is the drug of choice.

In this study, the prevalence of methicillin resistance among the *Staphylococcus aureus* isolated from the patients admitted in the adult medical emergency unit of LUTH was 18 which is 41.9% of the total *Staphylococcus aureus* isolates this prevalence is high and in accordance with 42.3% and falls within the range of 25% to 40% prevalence in Nigeria [19], but less than the prevalence 46.5% MRSA reported in a research carried out in Kenya [20] and 59% reported in a similar multicenter study in Lagos [21].

Staphylococcus aureus was isolated from various clinical specimens (Sputum, Blood, Urine, wound swab/ biopsy) obtained from different infection site, with the highest sample being from blood (39.5%) and the lowest being sputum(9.3%) this is in contrast with a similar study carried out in Ekiti state [22] where urine has the highest occurrence (25.5%) of *S. aureus* and also a similar study carried out by Shittu and Lin, more than 80% of the total number of isolates recovered were from infected wounds. However, MRSAs were mostly (55%) isolated from skin and soft tissue infections in this study this is in accordance to various studies in Nigeria, Africa and other part of the world [22,23]. *S. aureus* is ubiquitous in its distribution, and although it is a normal flora of the anterior nares, skin, and genital tract of humans, it can cause infections in virtually any organ or part of the body, infections of the wounds, skin and soft tissue, blood, and the lower urinary tract are particularly common.

The antimicrobial susceptibility pattern of *S. aureus* isolates varies with location and time. The highest degree of antibiotic resistance in this study was to tetracycline (76.7%), Erythromycin (76.7%) and Penicillin (69.8%) this is partially in agreement with a similar research by Olowe et al., where penicillin G (82.7%) and tetracycline (65.4%) has

the highest degree of antibiotic resistance but slightly different in the resistance profile of erythromycin which has one of the lowest degree of antibiotic resistance in this same study [18], this difference might be because of the antibiotic abuse, difference in time (2013 and 2020) or partially because this research did not classify any isolate as intermediate while 30.7% was classified as intermediate. MRSA isolates show 100% resistance to penicillin and erythromycin and 94.4% resistance to tetracycline. These drugs are available over the counter and are widely used in Nigeria without medical prescription [24]. Resistance to other antibiotics such as Levofloxacin and Linezolid appear to be relatively low, while all the isolates were susceptible to vancomycin, which agrees with previous reports. The antibiotics with low degree of resistance are not readily available for over counter purchase. Vancomycin is not available for routine clinical use in Nigeria, hence, most *S. aureus* isolates in this study including MRSA, are still susceptible to the vancomycin, although there are few reports of the emergence of vancomycin-resistant *S. aureus* clinical isolates in some centers in Nigeria.

Most of the *S. aureus* isolates in this study were multidrug resistant (MDR). The MDR among MRSA strains was higher than the methicillin sensitive strains. These results are in agreement with global findings of MRSA strains being multi-drug resistant [25,26]. This might be as a result of the fact that that a large number of the bacteria isolates have been pre-exposed to several antibiotics [26]. Resistance may also be due to a combination of microbial characteristics such as selective pressure on antimicrobial usage. Also the transmission of resistance from one organism to another might also contribute to the high resistance. Other factors may include an increase in irrational consumption of antibiotics and transmission of resistant strains between people [27].

This study showed that all the MRSA isolates were significantly less sensitive to antibiotics as compared with MSSA isolates. Although methicillin resistant *S. aureus* is not necessarily more virulent than methicillin-susceptible *S. aureus*, treatment options are often highly limited by multidrug resistance [26]. Methicillin resistant *S. aureus* isolates in this study showed higher resistance to the antibiotics used than methicillin-sensitive *Staphylococcus aureus* (MSSA) this finding is similar to an observation by Ephraim et al [28] who reported 60% multidrug resistant rate for *S. aureus* isolates.

MRSA isolates were detected by the disk diffusion method using cefoxitin disk, cefoxitin is a better inducer of the mecA gene, and tests using cefoxitin provide more reproducible and accurate than oxacillin. In addition, CLSI, recommends the cefoxitin disk for phenotypic detection of MRSA. However, detecting mec genes by PCR is the gold standard method [14].

In this study 94.4% (17 out of 18) of the MRSA isolates where positive for mecA gene only while 5.6% had both mecA and mecC. This implies that cefoxitin disk diffusion test has 100% sensitivity and specificity for mecA gene, this is in contrast with Olowe et al in drawing comparisons between methicillin, oxacillin, cefoxitin and the gold standard PCR, observed that all the antibiotics mentioned slightly overestimated methicillin resistance in *S. aureus* and a study carried out in University of Benin teaching hospital [28] where phenotypic method using cefoxitin disc had 44.0% and 30.0% was positive for mecA gene. This discrepancy between phenotypic and genotypic resistance testing may be due to the fact that most of the researchers only tested for mecA gene, it is possible that mecC gene was responsible for the resistant of those isolate, it may also be as a result of other mechanism of resistance to methicillin such as the presence or over expression of β-lactamase enzymes and chromosomal mutations like the acquisition of modified Penicillin Binding Proteins. This may also be due to the fact that screening is not usually done to detect other genes that code for resistance (mecC) compared to mecA gene (mecA specific primers can only detect mecA gene).

MRSA strains carrying the mecC gene may become a problem for public health since genotypic tests are not usually carried out to detect this gene.

In this study 5.6% (1 out of 18) of the MRSA isolate was positive for mecC gene this is similar to the findings in a study carried out in Germany where the presence of mecC was investigated in 1604 MRSA strains isolated in 2004 and 2005, and 1603 strains isolated in 2010 and 2011, and only one isolate (0.06%) was found to be mecC-positive for each period [29], also a study conducted in Denmark between 2003 and 2011, the mecC positivity was reported to be 1.5%, and the authors noted an increase in mecC positive samples from 1.9% in 2010 to 2.8% in 2011 [30]. In studies conducted in various countries, mecC was not detected in 102 MRSA strains isolated from wounded military personnel in the United States of America (USA) between 2009 and 2011 [31], 34 MRSA strains isolated from a dental clinic in Egypt [32], and any of the 500 *S. aureus* isolates collected in the UK in 2012 and 2013 [33]. In a study by Kılıc¸et al [34] to investigate mecC gene in MRSA strains isolated from humans, 1177 MSSA (Methicillin-Susceptible *S. aureus*) and 523 MRSA strains isolated from various clinical samples in a hospital between 2007 and 2014, and reported that mecC was not present in any of the samples [34].

The detection of the mecC gene in the present study is noteworthy, as most standard MRSA diagnostic assays are designed to detect only mecA. Consequently, strains carrying mecC may go undetected, leading to false-negative results and inappropriate antibiotic therapy. Hence, the inclusion of both mecA and mecC in MRSA screening protocols is essential for accurate diagnosis, effective surveillance, and the prevention of further spread within healthcare facilities and the wider community.

6. Conclusion

Staphylococcus aureus in this study, was associated with numerous infections and were multidrug resistant. All the phenotypically identified MRSA isolates harbored resistance gene. All the MRSA isolates had mecA gene while there was co-existence of mecA and mecC gene in one of the isolates. This is epidemiologically important because it the genes are carried in staphylococcal cassette chromosome mec (SCC mec) which has high tendency to cause a serious outbreak in the communities and hospital words.

Antibiotics resistance surveillance and routine screening for methicillin resistant *Staphylococcus aureus* is recommended. Vancomycin and linezolid are recommended for the treatment of methicillin resistant *Staphylococcus aureus*.

Limitation

The high prevalence of MRSA and the presence of both Meca and Mecca genes are significant findings. However, while these results can help guide local policy decisions, their generalizability is limited due to the small sample size and the fact that the study was conducted in a single facility.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed

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