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Detection of mecA and PVL-Resistant Genes in *Staphylococcus aureus* from Clinical Samples in Bayelsa State, Nigeria

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ABSTRACT

Background and Objectives: Methicillin-resistant bacteria *Staphylococcus aureus* (MRSA) is known to cause serious infections with a wide range of severity and antibiotic susceptibility. The purpose of this study was to identify mecA and PVL genes in MRSA. **Materials and Methods:** Using standard bacteriological techniques, MRSA was isolated from clinical samples. The boiling method was used to extract genomic DNA. In an AB1970 applied biosystem thermal cycler, mecA and PVL were amplified. A total of 250 chemical specimens were collected, with urine samples, wound samples and endocervical swabs. **Results:** High vaginal swabs accounting for 136 (54.4%), 49 (19.6%), 4 (16%) and 25 (10), respectively. Of the 25 MRSA isolates, 11 (44%) came from urine, 10 (40%) from the wound, 4 (16%) from the higher vaginal swab and two from the endocervical swab. The MRSA was found in the highest concentrations in urine. The susceptibility pattern of MRSA isolates revealed that the organisms were highly resistant to Ampiclox 25 (100%), Amoxicillin 21 (84%), Streptomycin 19, (76%) and Gentamycin 5 (20%). None of the 25 MRSA isolates contained mecA or PVL genes. **Conclusion:** The findings highlight the importance of continuously monitoring the methicillin resistance pattern of MRSA isolates, as well as the need for stringent infection control measures to prevent transmission and spread.

KEYWORDS

Staphylococcus aureus, mecA, PVL, susceptibility, ciprofloxacin, cephalosporins, carbapenem

INTRODUCTION

The emergence of antibiotic-resistant strains of *Staphylococcus aureus*, such as methicillin-resistant *Staphylococcus aureus* (MRSA), is a global clinical problem. Despite extensive research, no vaccine for *S. aureus* has been approved. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a gram-positive bacterium that is genetically distinct from other *Staphylococcus aureus* strains. The MRSA is to blame for several difficult infections in humans. The MRSA is any strain of *S. aureus* that has developed resistance to multiple drugs, including beta-lactam antibiotics, as a result of horizontal gene transfer and natural selection. (-lactam antibiotics are a broad-spectrum class of antibiotics that includes some penams (penicillin derivatives like methicillin and oxacillin) and cephems like cephalosporins[1].

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The MRSA infections are most commonly contracted in hospitals and other healthcare facilities. This problem is exacerbated by MRSA's proclivity for cross-infection. The intensive use of antibiotics, particularly cephalosporins and carbapenem, to which organisms are resistant, creates strong selection pressures for MRSA infection[2]. Methicillin resistance is a major cause of morbidity and mortality in *S. aureus* infections.

According to a Nigerian survey, *Staphylococcus aureus* resistance to ampicillin ranges between 0 and 95.6%, with Oyo State having the lowest resistance and Cross River State having the highest resistance[3]. Similarly, in Kano and Borno, both in Nigeria's North-East and North-West, Coagulase Negative *Staphylococci* (CONS) showed 100% resistance to ampicillin[4]. *Staphylococcus species* exhibited varying degrees of resistance to aminoglycosides. In studies where both antimicrobials were tested, there was less resistance to amikacin than to gentamicin[3]. Resistance to quinolones was also variable, with ciprofloxacin resistance ranging from 0% in Osun State to 73.4% in Edo State[5]. *Staphylococcus epidermidis* isolates from two states in the North-Eastern and North-Western Zones were all resistant to cefuroxime, whereas *S. aureus* showed 0 and 60.5% resistance at the same center[4]. All studies found that *Staphylococcus species* were resistant to chloramphenicol. The methicillin sensitivity of *Staphylococcus species* was not mentioned in the majority of the studies. Unless otherwise stated, the isolates were assumed to be methicillin-sensitive. MRSA isolates in Oyo State were highly resistant to gentamicin but completely susceptible to amikacin, ciprofloxacin and chloramphenicol[3]. Among the *enterococci* tested, *Enterococcus faecalis* demonstrated very high sensitivity to the antimicrobial gentamicin, ampicillin, cefuroxime and ceftriaxone. However, ampicillin and cefuroxime resistance was widespread in Kano State[6]. There was high resistance to ampicillin, gentamicin, amikacin, ciprofloxacin, cefuroxime and ceftazidime in a study in which the *Enterococci* were not speciated[3].

The mecA is a biomarker gene that causes resistance to methicillin and other -lactam antibiotics. Following mecA acquisition, the gene must be integrated and localized on the *S. aureus* chromosome. The mecA encodes Penicillin-Binding Protein 2a (PBP2a), which does not bind methicillin or other -lactam antibiotics like other penicillin-binding proteins[7]. As a result, PBP2a can continue to catalyze the transpeptidation reaction required for peptidoglycan cross-linking in the presence of antibiotics, allowing cell wall synthesis to occur. Because PBP2a is unable to interact with -lactam moieties, the acquisition of mecA confers resistance to all -lactam antibiotics, including methicillin antibiotics. This work, therefore, seeks to detect the presence of the mecA gene in a clinical isolate of *Staphylococcus aureus* in Bayelsa State.

MATERIALS AND METHODS

Study area: This research project was conducted from February to August, 2018. The research was conducted at the Federal Medical Center (FMC) Yenagoa, Niger Delta University Teaching Hospital (NDUTH), Okolobiri and Diette-Koki Memorial Hospital, all of which are located in Yenagoa Local Government Area, the state capital of Bayelsa. Yenagoa is located at 4°55'29" N and 6°15'51" E latitude. It covers an area of 706 km^2 and is home to a variety of ethnic groups from across the country.

Ethical clearance: The hospital's ethical committee granted permission for the procedure.

Sample size: The sample size using Taro Yemen's formula[8]:

$$n = \frac{N}{1} + Ne2$$

Where:

n	=	Sample size required
N	=	Population size
e	=	Allowable error
Assuming N	=	670
e	=	0.5

Therefore,

$$n = \frac{670}{1} + 200\ (0.05)$$

Where:
n = 250 (minimum sample size)

Sample collection: One hundred and fifty clinical samples were obtained from the Department of Medical Laboratory Science at FMC, Yenagoa, Diete-Spiff Memorial Hospital and NDUTH, Okolobiri.

Sample processing

Isolation and Identification of *Staphylococcus aureus*: The samples were cultured in blood agar and incubated at 37°C for 24 hrs. Following incubation, the colonies were subcultured into Mannitol Salt Agar (MSA) and incubated for 24 hrs at 37°C. Colonies on Mannitol Salt Agar were identified using colonial morphology, Gram staining, biochemical tests and molecular analysis.

Gram staining: A clean grease slide was coated with a drop of normal saline and a colony from the overnight culture was picked with a sterile wire loop and emulsified on the slide. The smear was allowed to dry before being heat-fixed by passing it through the flame of a Bunsen burner 2-3 times. Crystal violet was applied to the smear for 60 sec before being washed away with water. Lugol's iodine was applied to the slide for 60 sec before being washed away with water. The smeared substance was rapidly decoloured with acetone and washed with water. The slide was washed with water after being counterstained with neutral red for two minutes. The back of the slide was wiped with cotton wool and allowed to dry, immersion oil was applied and the slide was viewed under the microscopic with X100 objective lens.

Catalase test: In a test tube, two to three millilitres of H_2O_2 were dispensed. Colonies of organisms were immersed in the solution using a sterile applicator stick. A positive test resulted in immediate gas bubbling.

Coagulase test: Two separate drops of normal saline were placed on a clean slide and the organism's colonies were emulsified on each drop to form thick suspensions. Drops of plasma were added to one of the suspensions and immediate coarse clumping was observed for a positive test.

Antibiotics susceptibility testing: The isolate's antibiotic susceptibility was tested using agar diffusion and the Kirby-Buer Method on commercially available discs. Ciprofloxacin (CPX), Amoxicillin (AM), Chloramphenicol (CH), Norfloxacin (NB), Gentamycin (GN), Streptomycin (S), Rifampicin (RD), Ampiclox (APX), Erythromycin (E) and Levofloxacin are among the antibiotics used (LEV). The Muller Hinton Agar was flooded with peptone broth containing the isolate, the excess was drained and the antibiotic disc was placed on the surface and incubated at 37°C for 24 hrs. A clear zone of inhibition around the disc is indicative of particular antibiotic sensitivity or susceptibility.

DNA extraction (boiling method): The overnight broth culture of the bacterial isolate in Luria Bertani (LB) was transferred into a 1.5 mL Eppendorf tube and spun in a micro-centrifuge at 14000 rpm for 3 min. The supernatant was discarded and 1000 µL of 0.5% normal saline was added to the sediment before being vortexed on an El Tech XH-B vortexer. The cells were resuspended in 500 µL of normal saline and heated for 20 min at 950°C. The heated bacterial suspension was cooled on ice before being spun at 14000 rpm for 3 min. The supernatant containing the DNA was transferred to a 1.5 mL microcentrifuge tube and stored at -20°C for later use.

DNA quantification: The extracted genomic DNA was quantified using a spectrophotometer, the Nanodrop 1000. By double-clicking on the Nanodrop icon, the equipment's software was launched. The equipment was blanked with normal saline after being initialized with 2 µL of sterile distilled water. On the lower pedestal, two microlitres of extracted DNA were loaded. The upper pedestal was lowered to make contact with the extracted DNA on the lower pedestal. By clicking on the "measure" button, the DNA concentration was determined.

Determination of 16S rRNA: The isolates' rRNA gene's 16s rRNA region was amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on an ABI 9700 Applied Biosystems thermal cycler for 35 cycles at a final volume of 40 microlitres. The PCR mix included the Inqaba, South Africa-supplied X2 Dream Taq Master mix (Taq polymerase, dNTPs, MgCl), the primers at 0.5 µM concentration and the extracted DNA as a template. Initial denaturation at 95°C for 5 min, denaturation at 95°C for 30 sec, annealing at 52°C for 30 sec, extension at 72°C for 30 sec for 35 cycles and final extension at 72°C for 5 min were the PCR conditions. For 30 min, the product was resolved on a 1% agarose gel at 130V and visualized on a blue light transilluminator.

Amplification of mecA genes: The mecA genes were amplified from the isolates using mecA-F (5 -CAAGATATGAAGTGGTAAATGGT-3) and mecA-R (5 -TTTACGACTTGTTGCATACCATC-3). 'Primers were thermally cycled for 35 cycles on an ABI 9700 Applied Biosystems thermal cycler with a final volume of 30 microlitres. The PCR mix included the Inqaba, South Africa-supplied X2 Dream Taq Master mix (Taq polymerase, dNTPs, MgCl), the primers at 0.4 µM concentration and 50 ng of the extracted DNA as a template. The following were the PCR conditions: Initial denaturation at 95°C for 5 min, subsequent denaturation at 95°C for 30 sec, annealing at 51°C for 40 sec, extension at 72°C for 50 sec for 35 cycles and final extension at 72°C for 5 min. The product was resolved on a 1% agarose gel at 130V for 30 min before being visualized under a blue light transilluminator for a 533 bp product size.

Amplification of PVL gene: The PVL genes from the isolates were amplified for 35 cycles on an ABI 9700 Applied Biosystems thermal cycler using the staph PVL F: GCTGGACAAAACTTCTTGGAATAT-3' and staph PVL R: 5'-GATAGGACACCAATAAATTCTGGATTG-3' primers. The PCR mix included the Inqaba, South Africa-supplied X2 Dream Taq Master Mix (Taq polymerase, dNTPs, MgCl), the primers at 0.4 µM concentration and 25 ng of the extracted DNA as a template. Initial denaturation at 95°C for 5 min, denaturation at 95°C for 30 sec, annealing at 60°C for 40 sec, extension at 72°C for 50 sec for 35 cycles and final extension at 72°C for 5 min were the PCR conditions. The product was resolved on a 1% agarose gel for 25 min at 130V and visualized on a blue light transilluminator for an 1100 bp product size.

RESULTS

Gender distribution of specimens: Table 1 shows that females had more specimen loads than males, with urine being the most common specimen, accounting for 68.4% of females and 31.6% of males, respectively.

The distribution of methicillin-resistant *Staphylococcus aureus* isolates by age and gender (Table 2). According to Table 2, the most isolates retrieved were 11, representing 64.7% of the female patients between the ages of 21 and 30. This was followed by three isolates from both sexes but of various ages: 41-50 and 31-40 from men and women, respectively.

The distribution of methicillin-resistant *Staphylococcus aureus* by the specimen (Table 3). According to Table 3, the methicillin-resistant *Staphylococcus aureus* distributed across the specimens shows that urine presented with the highest distribution, representing 56% and the lowest, 16%, recorded with the HVS.

Table 1: Distribution of specimens by gender

Specimen	Male (%)	Female (%)	Total (%)
Urine	24 (31.6)	52 (68.4)	76 (52.1)
Wound swab	15 (75)	5 (25)	20 (13.7)
High vaginal swab	-	35 (100)	35 (23.9)
Endocervical swab	-	15 (100)	15 (10.3)
Total	39 (26.7)	107 (73.3)	146 (100)

Table 2: Distribution of methicillin-resistant *Staphylococcus aureus* isolates by age and gender

	Male (%)		Female (%)	
Age range (years)	Isolate	Total	Isolate	Total
01-10	-		1	1 (5.9)
11-20	1 (100)	1 (12.5)	2	2 (11.8)
21-30	2 (100)	2 (25)	11	11 (64.7)
31-40	1 (100)	1 (12.5)	3	3 (17.6)
41-50	3 (100)	3 (37.5)	-	-
51-60	-	-	-	-
61-70	-	-	-	-
71-80	1 (100)	1 (12.5)	-	-
Total	8 (100)		17 (100)	

Table 3: Distribution of methicillin-resistant *Staphylococcus aureus* isolate by specimens

Specimen	*Staphylococcus aureus* (%)
Urine	14 (56)
Wound swab	7 (28)
High vaginal swab	4 (16)
Total	25 (100)

Table 4: Antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* isolated

NE	CPX	NB	CN	AMX	S	RD	E	CH	APX	LEV
Isolate 25	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)
	10 (40)	18 (72)	5 (20)	21 (84)	19 (76)	6 (24)	17 (68)	15 (60)	25 (100)	9 (36)
	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)
	15 (60)	7 (28)	20 (80)	4 (16)	6 (24)	19 (76)	8 (32)	10 (40)	0(0)	16 (64)

CPX: Ciprofloxacin, NB: Norfloxacin, CN: Gentamycin, AMX: Amoxicillin, S: Streptomycin, RD: Rifampicin, E: Erythromycin, CH: Chloramphenicol, APX: Ampiclox, LEV: Levofloxacin, NE: Number of isolates examined, S (%): Percentage of susceptibility and R (%): Percentage of resistance

Antibiotic susceptibility pattern of methicillin-resistant *Staphylococcus aureus*: The distribution of the mecA and PVL genes in methicillin-resistant *Staphylococcus aureus* isolates (Table 4). Table 4 shows that out of the 25 isolates assayed across the different antibiotics, 40 and 60% were resistant and sensitive to CPX, respectively, 72 and 28% were resistant and sensitive to NB, respectively, 20 and 80% were resistant and sensitive to CN, respectively, 84 and 16% were resistant and sensitive to AMX, 76-24% for S, 24-76% for RD, 68-32% for E, 60 and 40% for CH, 100% for APX and 36-64% for LEV.

The distribution of the mecA and PVL genes in methicillin-resistant *Staphylococcus aureus* isolates (Table 5). None of the twenty five isolates carried the mecA or PVL genes.

DISCUSSION

One hundred and seventy (73.3%) of the 146 available clinical specimens collected were from females, while 39 (26.7%) were from males, as shown in Table 1. This demonstrates how frequently women visit the hospital and how much more health-conscious they are than men, which is consistent with the findings of Jay and Inskeep[9], who found that women are more likely than men to visit the doctor and use healthcare services. The total number of methicillin-resistant *Staphylococcus aureus* isolates was 25, with 17 (68%) coming from females and 8 from males (32%) as shown in Table 2-3. The majority of isolates

Table 5: Distribution of mecA and PVL genes in methicillin-resistant *Staphylococcus aureus* isolates

											Genes	
Specimen	CPX	NB	CN	AMX	S	RD	E	CH	APX	LEV	mecA	PVL
W8	R	R	R	R	R	S	R	S	R	R	-	-
H19	R	R	S	R	R	S	R	R	R	R	-	-
U21	R	R	S	R	R	S	R	R	R	R	-	-
U23	S	R	S	R	R	S	R	R	R	R	-	-
U24	S	R	S	R	R	S	R	R	R	S	-	-
U51	R	R	R	R	R	S	R	R	R	R	-	-
H32	S	S	S	R	R	R	R	R	R	S	-	-
H33	S	R	S	R	R	R	R	S	R	S	-	-
H34	S	R	S	R	R	S	R	R	R	S	-	-
U30	S	S	S	R	S	S	R	R	R	S	-	-
U52	R	R	R	R	R	S	R	R	R	R	-	-
U56	R	R	R	R	R	R	S	S	R	R	-	-
U79	R	R	S	R	R	R	R	R	R	S	-	-
U60	R	R	S	R	R	R	R	R	R	R	-	-
U11	S	S	S	S	S	S	S	S	R	S	-	-

CPX: Ciprofloxacin, NB: Norfloxacin, CN: Gentamycin, AMX: Amoxicillin, S: Streptomycin, RD: Rifampicin, E: Erythromycin, CH: Chloramphenicol, APX: Ampiclox and LEV: Levofloxacin

(37.5%) came from males between the ages of 41 and 50 (Table 2). This could be because people in this age group are more likely to come into contact with this organism as a result of their daily physical activities. The highest number of isolates from females (11) (64.7%) were obtained between the ages of 21-30 years (Table 2), which could be due to their sexual lifestyle, according to Bassetti *et al.*[10], which stated that high sexual activity, multiple sex partners and physical activities are risk factors for *staphylococcal* infections.

The incidence of methicillin-resistant *Staphylococcus aureus* in clinical specimens in this study revealed that urine (56%) had the highest frequency, followed by wounds (28%) and then higher vaginal swabs (16%) as presented in Table 3. This is consistent with the findings of Ekwealor *et al.*[11], who discovered that Urinary Tract Infection (UTI) is one of the leading causes of hospitalization.

The antibiotic susceptibility pattern revealed that Gentamycin (80%) was the most effective antibiotic for the study of methicillin-resistant *Staphylococcus aureus,* followed by Rifampicin (76%), Norfloxacin (64%) and Ciprofloxacin (64%) (60%) Chloramphenicol (40%) was the least effective, followed by Erythromycin (32%), Streptomycin (24%) and Amoxicillin (16%), but the organism was resistant to Ampiclox (0% susceptibility) as shown in Table 4-5. Multiple drug resistance is the ability of *Staphylococcus aureus* to be resistant to multiple drugs, which can be caused by prolonged antibiotic use as well as the presence of genes such as mecA and PVL, according to the word line of study by Havaei *et al.*[12], which stated that the gene mecA is believed to be the major cause of methicillin and methicillin-like resistance.

Though several genes associated with antibiotic resistance in *Staphylococcus aureus* are known, the genes of interest in this study were the mecA gene, which is responsible for methicillin resistance and codes for the penicillin binding protein 2a and the PVL genes, which play a role in the bacteria's skin and soft-tissue infections and severe necrotizing pneumonia. These strains produce hemolysin, a pore-forming toxin that lyses many types of eukaryotic cells, as well as -type Phenol-Soluble Modulins (PSMs), amphipathic peptides that recruit, activate and destroy leucocytes. Both -hemolysin and PSMs are overexpressed in CA-MRSA compared to HA-MRSA and are important factors in its pathogenesis and virulence[13].

The mecA and PVL genes were not found in isolates with phenotypic methicillin resistance. This could be due to the presence of other resistant genes that encode for resistance to methicillin and methicillin-like antibiotics, such as the mecC gene, according to Kim *et al.*[14], who found that Methicillin-sensitive *Staphylococcus aureus* (MSSA) confers high Minimum Inhibitory Concentration (MIC) values against a

variety of Beta lactams due to mecC-encoded PBP2a/2'. Furthermore, the blaZ gene is consistent with the findings of Al-Tamimi *et al.*[15], who discovered that it confers a high level of resistance to multi-drug antibiotics, including beta-lactams.

A previous study in Nigeria reported the complete absence of five major SCCmec types and mecA genes, as well as the gene product of PBP2a, in isolates that were phenotypically MRSA, implying that -lactamase hyperproduction was the cause of the phenomenon[16].

We, therefore, recommend that personal hygiene be taken seriously, especially during the COVID-19 pandemic, as looking for new therapeutic alternatives and policies to control antibiotic use and hospital-acquired infections should be a constant habit for researchers and healthcare practitioners.

CONCLUSION

Staphylococcus aureus isolated from clinical specimens, microbiological characterization, susceptibility testing of *Staphylococcus aureus* and mecA and PVL gene amplification by polymerase chain reaction were all performed in this study. None of the twenty five isolates carried the mecA or PVL genes but was still resistant, possibly due to a different gene. Early detection could thus reduce morbidity and mortality, as well as the development of multi-drug antibiotic resistance.

SIGNIFICANCE STATEMENT

This study attempted to discover the presence of mecA and PVL-resistant genes in *Staphylococcus aureus*. The development of drugs that target these genes will reduce mortality and morbidity associated with antimicrobial resistance across the world.

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