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IDENTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF METHICILLIN AND ERYTHROMYCIN RESISTANT GENES IN CLINICAL AND ENVIRONMENTAL STRAINS OF *Staphylococcus aureus* IN MINNA NIGERIA

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ABSTRACT
Staphylococcus aureus that is resistant to the antibiotic methicillin (MRSA) is a growing global health threat. The disc diffusion method was used to investigate the antibiotic susceptibility profile of Staphylococcus aureus. From clinical and environmental samples, Staphylococcus aureus was detected in 21.9% (73/360) of the cases. Staphylococcus aureus predominance in environmental samples was 24%, compared to 20.5 in clinical samples. The prevalence of Staphylococcus aureus was highest among people aged 18 to 49 (74%) and lowest among those aged 0 to 17 (42%) and 50 to 70 (4%). Staphylococcus aureus was more common in females (22.4%), compared to males (20%). Staphylococcus aureus showed 88.60%, 45.60%, 34.20%, 21.50%, 18.90%, 11.40%, 8.90%, 6.30%, and 5.10%, respectively, resistance to Oxacillin, Cefoxitin, Ampicillin, Vancomycin, Erythromycin, Norfloxacin, Rifampicin, and Gentamycin. All 79 of the Staphylococcus aureus isolates were 100% responsive to septrin and levofloxacin. The isolates were used to molecularly identify the genes for methicillin (mecA) and erythromycin (ermA and ermC). The clinical and environmental samples revealed a comparatively high frequency of Staphylococcus aureus.
KEY: Methicillin, Resistant, Staphylococcus aureus, gene, antibiotics resistant, erythromycin

INTRODUCTION

Coagulase-positive, Gram-positive cocci called *Staphylococcus aureus* develop clusters that resemble grapes. *S. aureus* is a bacterium that inhabits healthy people's mucous membranes, nostrils, stomachs, and skin glands (Sahreena and Kunyan, 2018). Methicillin resistance *Staphylococcus aureus* (MRSA) is defined as any strain of *S. aureus* that has developed resistance to methicillin and other beta lactam antibiotics (Cuny *et al.,* 2015; Bitrus *et al.,* 2017). Several hard-to-treat human diseases are caused by MRSA (Bale *et al.,* 2018). The formation of penicillin-restricting protein 2a (PBP2a), which is expressed by the mecA gene on the mobile gene element (MGE) of the Staphylococcal chromosomal cassette mec (SCCmec), and has a poor affinity for beta-lactam antibiotics, is the cause of *S. aureus* resistance to methicillin (Akanbi *et al.,* 2017).

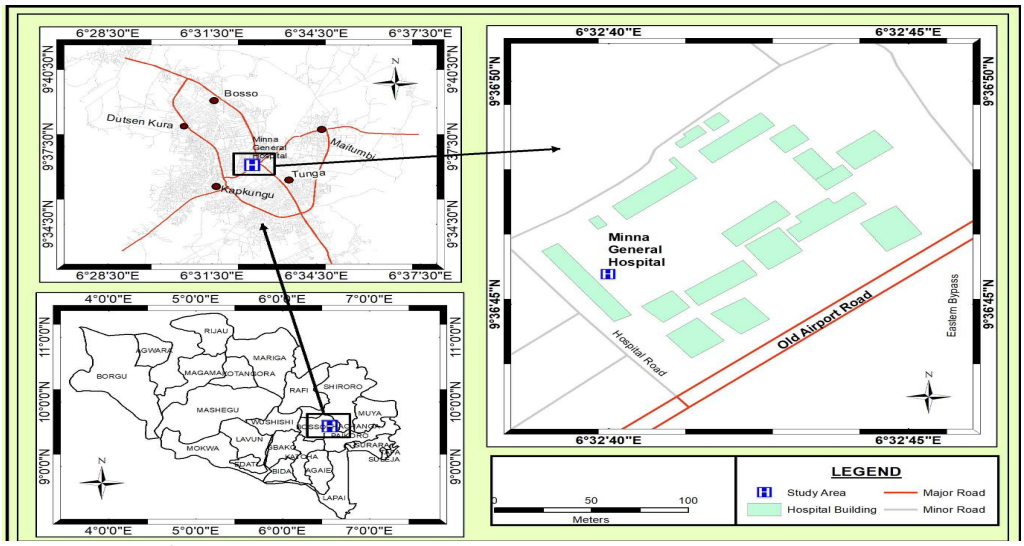
The aim of this study was to identify and determine antibiotic susceptibility profile of methicillin and erythromycin resistant *S. aureus* Four clinical strains of *S. aureus* were reported to produce spiramycin in response to erythromycin. This trait in *S. aureus* has been demonstrated to be caused by an erythromycin resistance methylating (erm gene product) that makes newly produced ribosomes resistant to macrolide-lincosamide-streptogramin B antibiotics by methylating a particular adenosine residue of the 23S rRNA (Akanbi *et al.,* 2017). The genes that produce the methylase in *S. aureus* have been given the names ermA, ermB, and ermC (Akanbi *et al.,* 2017).

It has been discovered that *S. aureus* strains that are resistant to erythromycin and methicillin are also resistant to other antibiotics such oxacillin, amoxicillin, and penicillin. Additionally, these bacteria might become resistant to medications such gentamicin, cotrimoxazole, and clindamycin (Bale *et al.,* 2018; Rasheed and Hussein, 2020; Cheung *et al.,* 2021). genes from clinical and environmental samples of Minna Niger State, Nigeria.

MATERIALS AND METHODS

Study Site

The study was carried out at Minna, Niger State, Nigeria. It is situated on Latitude 9.61 N and Longitude 6.56 E at an elevation of 299 m above sea level. It is bordered to the North by Sokoto State, west by Kebbi State, and South by Kogi and South-West by Kwara State. Niger State has a common boundary with the Republic of Benin along New Bussa, Agwara and Wushishi Local Government Area. Samples were collected from General hospitals in Minna Nigeria (GH) shown in (Figure 1).



Source: GIS Achiever, 2021

Figure 1 Map of study area

Study Design

The study was a cross sectional study using a convenience sampling technique among clinical and environmental samples. Three hundred and sixty (360) samples comprising wound, ear, nose, skin, urine and environmental samples such as air, work bench, patients bed were collected from in and outdoor patients attending General Hospital Minna, Nigeria. Ethical clearance was obtained from the research and ethics committee of Niger State Hospital Management Board for the study.

Sample collection

The clinical samples and environmental samples were obtained using sterile swab sticks. The swab was transferred to the Laboratory for processing while being stored at 4^{o}C in a Coleman box. Each sample that was gathered has the proper labels on it.

All the samples were taken to Microbiology Laboratory Federal University of Technology Minna Niger Sate and processed according to standard microbiological procedure (Cheesbrough, 2018).

Sample Processing

All the collected samples were inoculated onto Mannitol Salt Agar (MSA) and incubated for 24 hours at 37°C. Using the conventional bacteriological process, which comprises the Gram reaction, catalase reaction, coagulase test, and mannitol fermentation, the suspected different colonies of Staphylococci isolates were verified. (Cheesbrough, 2018).

Preparation of Mcfarland Turbidity Standard

Making a 0.5 Mcfarland standard (turbidity standard) as a turbidity standard, sulfuric sulfate standard suspension (1% v/v) prepared using Cheesbrough's instructions was employed (Cheesbrough, 2018)

Detection of methicillin resistant *Staphylococcus aureus* (MRSA)

Oxacillin (1 µg), Cefoxitin (30 µg), and Vancomycin (30 µg) disk phenotypic detection of MRSA (Oxoid, UK) were used for the MRSA screening (CLSI, 2019). *Staphylococcus aureus* pure isolate was suspended in sterile water and diluted 1:10 to obtain turbidity equal to the 0.5 Mcfarland (a density of 1x10^{8} cells/ml) prior to inoculation. Using sterile forceps, the antibiotic disc was uniformly positioned into the surface of the inoculated Agar plate and gently pressed down to ensure full contact with the agar surface. Incubation took place for 24 hours at 37^{0}C with the plates inverted. The findings were categorized as susceptible, intermediate, or resistant Clinical and Laboratory Standard Institutes using a ruler and the diameters of the guidelines from the CLSI (2019).

Antibiotic susceptibility testing test

The Clinical Laboratory Standard Institute's Kirby-Bauer disc diffusion techniques were used to conduct the antibiotic susceptibility test on *S.*

aureus isolates (2019). Prior to inoculation, *Staphylococcus aureus* pure isolate was suspended in sterile water and diluted at a ratio of 1:10 to achieve a turbidity of 0.5 Mcfarland (a density of $1x10^8$ cells/ml). On the Muller-Hinton agar that had been inoculated, a filter paper disk was placed that contained Ciprofloxacin (10 µg), Chloramphenicol (30 µg), Gentamacin (10 µg), Amoxil (20 µg), Streptomycin (30 µg), Rifampicin (20 µg), Erythromycin (30 µg), Nofloxacin (10 µg), Ampiclox (20 µg), and Levoflaxacin (20 µg). All the plates were incubated at 37°C for 18 hours over night. Then, with the plate held a few inches above a black, non-reflective surface illuminated by reflected light, each zone's measurement was taken with the unassisted eye while looking at the petri dish's black background while using a ruler. The outcome was noted and evaluated against the zone diameter interpretive criteria of CLSI (2019).

Detection of *mecA, ermA,* AND *ermC* Coding Genes

The MecA, ermA, and ermC coding genes in the 10 molecularly characterized *Staphylococcus aureus* are investigated using simple PCR on the extracted DNA MecA, ermA, and ermC coding areas. The final concentration of the 8000U taq DNA polymerase, $MgCl_2$, 10pM DNTP, 10pM, 5X PCR SYBR green buffer, 10pM forward and back primer, 5X PCR SYBR green buffer, and sterile distilled water was 10.5 to which 2 l template was added.

Polymerase chain reaction

The preparation cocktail for PCR sequencing contained 10 l of a 5x GoTaq colorless reaction, 3 l of 25 mM $MgCl_2$, 1 l of a 10 mM dNTPs mix, and 1 l of 10 pmol each. 27F Primer sets of 5'-AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5'- AAGGAGGTGATCCAGCC-3' and 0.3 units of Taq DNA polymerase (Promega, USA) were prepared in a total volume of 42 l using sterile distilled water and 8 l of DNA template. A GeneAmp 9700 PCR System was used to perform the PCR (Nadeen *et al.,* 2018). Applied Biosystem Inc.'s (USA) Thermalcycler has a profile that includes an initial 5-minute denaturation at 94°C for 5 min, 30 cycles of 94°C for 30 sec, 50°C for 60 sec, and 72°C for 1 min 30 sec, and a final termination at 72°C for 10 min and chill at 4°C. (Ouyang *et al.,* 2021).

RESULTS

The result obtained from this study showed, the total prevalence of *S. aureus* isolated was 44.5%. The prevalence of environmental samples 24.0% *S. aureus* was higher than clinical samples 20.5% S*. aureus* from General Hospital Minna (Table 1).

The age group 0-17 years had the highest prevalence of *S. aureus* (25.9%), followed by the age group 18-49 years (20.6%). The age range 50-70 had the lowest prevalence (8.3%). Females were found to have a higher prevalence of *S. aureus* (22.4%) than males (20.0%) (Table 2). A 88.6% were found to be Methicillin Resistant *Staphylococcus aureus* (MRSA) by oxacillin disc diffusion method, 45.6% of the isolates were found to be MRSA by cefoxitin disc diffusion method, while 21.5% were found to vancomycin resistant by disc diffusion method and 3.8% were found to be erythromycin resistant (Table 3 and 4).

Multiple Antibiotic Resistant Indices (MARI) revealed that 20 isolates (26.3%) were resistant to three or more antibiotics. MARI ≥ 0.3 indicated that the isolates came from a setting where antibiotics were widely used. Therefore, Multi drug resistant (MDR) is determined by the isolate that show resistant to three and above antibiotics as shown in (Table 4).

Table 1: Sample sources, distribution and prevalence of *S. aureus*

Sample sources	Number (%)	Number of *S. aureus* isolate	Prevalence of *S. aureus* (%)
Clinical	210 (58)	43	20.5
Environmental	150 (42)	36	24.0
Total	360 (100)	79	21.9

Table 2: Prevalence of *Staphylococcus aureus* in different age groups and gender

Parameter	Number of Sample	Number of isolated *S. aureus*	Prevalence of *S. aureus*
Age Groups			
0-17	81	21	25.9
18-49	180	37	20.6
50-70	24	2	8.3
Gender			
Male	160	32	20.0
Female	125	28	22.4

Table 3: Antibiotic susceptibility profile of *Staphylococcus aureus*

No. of *S.aureus*	Susceptibility profile (n=79)	S	I	R	$\chi 2$	P-value
N=79	Ciorofloxacin	71(89.9)	5(6.3)	3(3.8)	636.1	0.001
	Nalidixic acid	65(82.3)	5(6.3)	9(11.4)		
	Gentamycin	73(92.4)	2(2.5)	4(5.1)		
	Amoxacillin	67(84.8)	7(8.9)	5(6.3)		
	Septin	79(100)	0(0.0)	0(0.0)		
	Rifampicin	70(88.6)	2(2.5)	7(8.9)		
	Erythromycin	56(70.9)	20(25.3)	3(3.8)		
	Chlorophenicol	68(86.1)	8(10.1)	3(3.8)		
	Apiclox	41(51.9)	11(13.9)	27(34.2)		
	Levofloxacin	79(100)	0(0.0)	0(0.0)		
	Vancomycin	21(26.6)	41(51.9)	17(21.5)		
	Oxacillin	9(11.4)	0(0.0)	70(88.6)		
	Cefoxitin	25(31.6)	18(22.8)	36(45.6)		

The results show that there were significant difference in the activity of the various antibiotics against *S. aureus* (P<0.0.

Key: S= sensitive, I= intermediate, R= resistant

Table 4: Susceptibility of *Staphylococcus aureus* to Methicillins

Antibiotics	Susceptibility	Intermediate	Resistance
Vancomycin VA	26.6%	51.9%	21.5%
Oxacillin OX	11.4%	0	88.6%
Cefoxitin FOX	31.6%	22.8%	45.6%

Table 5: Multiple Antibiotic resistant indices (MARI) of *S. aureus* isolates

No. of antibiotics resistant to (n=13)	Number of isolates with same no. of antibiotic resistance	MAR index	Percentage of *S. aureus* with corresponding MARI
1	24	0.1	31.6
2,3	40	0.2	52.6
4	4	0.3	5.3
5	2	0.4	2.6
6,7	6	0.5	7.9
TOTAL	76		100

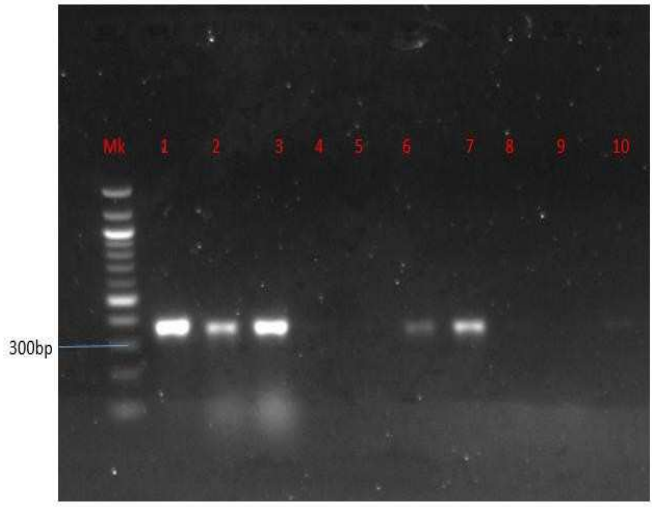


Plate 1. PCR of the mecA gene amplified from bacteria isolates on an agarose gel electrophoresis identified as *Staphylococcus aureus*

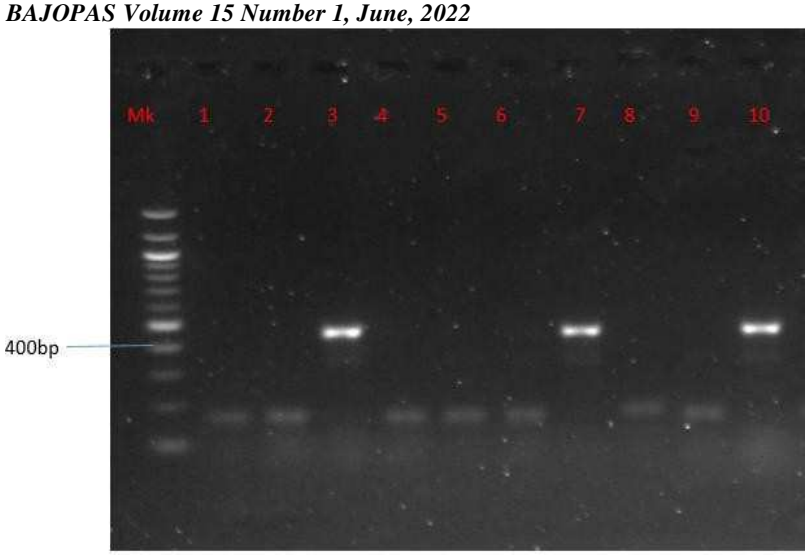


Plate 2. PCR of the ermA gene amplified from bacteria isolates as *Staphylococcus aureus* on an agarose gel electrophoresis.

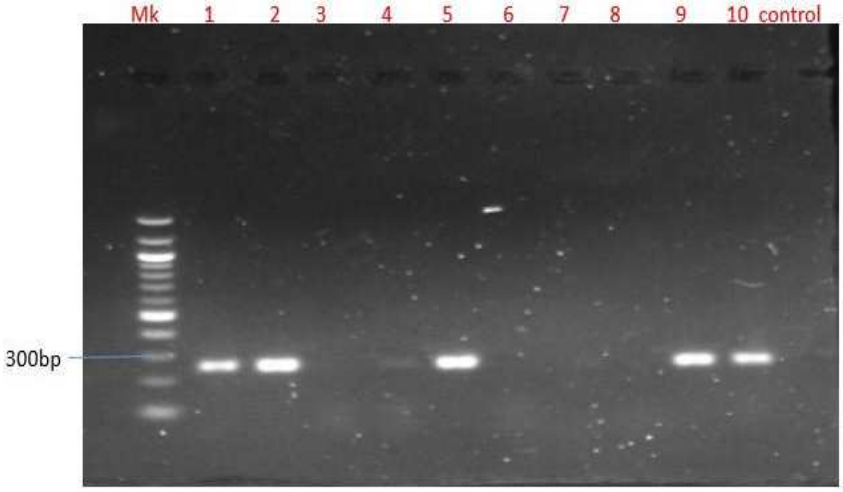


Plate 3. Agarose gel electrophoresis of the PCR of *ermC* gene amplified from bacteria isolates identified as *Staphylococcus aureus*

DISCUSSION

The total prevalence of *S. aureus* isolates in this investigation was 44.5%. Environmental samples had a higher prevalence of *S. aureus* (24.0%) than clinical samples (20.5%). Poor infection management (sanitation) in the hospital environment, which could act as a reservoir of the organism, may be to responsible for the high prevalence seen in environmental samples. Thongchai *et al.* (2018) further stated that the isolation of *S. aureus* from clinical and environmental sources, such as blood, a workbench, the air, and a laboratory floor, demonstrates the organism's ubiquity.

The age group 0-17 years had the highest prevalence of *S. aureus* (25.9%) while age group 50-70 had the lowest prevalence (8.3%). The high prevalence among age group of 0-17 this could be due to the fact that children are most vulnerable in an area where there is lack of water and poor environmental hygiene's.

S. aureus was found to be more common in females (22.4%) than in males (20.0%). The difference in prevalence between the sexes may be explained by the fact that *S. aureus* populating the vagina can easily contaminate females due to their anatomical makeup. The results of Tong *et al.* (2015), who revealed that endogenous pathogens are easily able to colonize the vaginal vault of healthy women, are in agreement with this finding.

Methicillin-resistant *Staphylococcus aureus* (MRSA) accounted for 45.6% of the isolates in this investigation, whereas 88.6% were oxacillin-resistant. Onemu and Ophori (2013) claimed a higher prevalence of 79% in Benin, whilst Muralidharan (2009) reported a prevalence of 40.6% to 59.3% in India. Kshetry *et al.* (2016) reported a prevalence of 43% in Jos.

The high levels of oxacillin resistance found in this study may be the result of excessive lactamase synthesis, which may cause phenotypic oxacillin resistance and produce oxacillin-resistant clinical and environmental isolates without the usual genetic basis for such resistance. Such strains are likely to grow into totally resistant strains when exposed to antibiotics. According to Kshetry *et al.* (2016), variations in MRSA prevalence rates between researches may result from variations in study locations, time periods, as well as hygiene standards upheld in various hospitals.

Studies by Faiqa *et al.* (2016), CLSI (2017), and Adeiza *et al.* (2020) have suggested that disc diffusion tests using cefoxitin are superior because it is a more effective inducer of mecA expression, is less affected by test conditions and penicillinase hyperproduction, provides simpler end points, is easier to read, and is more repeatable than tests with Oxacillin disk.

In this investigation, the prevalence of ermA was 3.8% and ermC was 6.3% in *Staphylococcus aureus* isolates from clinical and environmental samples. Weisblum and Demohn (1995) and Nicola et al. (1998) discovered a high prevalence of ermA (82–94%) in erythromycin-resistant *S. aureus.* During a clinical investigation, *S. aureus* was found. In *S. aureus*, the ermA gene was more common, according to Lim *et al* (2002). The majority of coagulase-negative Staphylococci isolates included the erm C gene (CoNS). In a research by Martineau *et al.* (2000) the ermC gene was also found to be more common in CoNS. This study shows that the most prevalent erythromycin resistance determinant in *S. aureus* bacteria is now ermC, surpassing ermA. It can be caused by ribosomal alteration caused by 23S rRNA methylases, principally ermA and ermC, or by active antimicrobial agent efflux caused by an ATP-dependent pump, primarily msrA (methionine sulfoxide reductase A) gene.

CONCLUSION RECOMMENDATION

Staphylococcus aureus strains from clinical and environmental samples were used in this investigation to isolate the genes for the antibiotics erythromycin (ermA and ermC) and methicillin (mecA). Consequently, it is necessary to give a better understanding of the frequency and epidemiology of MRSA. Rapid and precise identification of methicillin resistance in *S. aureus* is crucial for the use of appropriate antimicrobial therapy and for the control of hospital- and community-acquired MRSA.

Conflict of interest: None declared

Author's contribution:

Mamman Godiya Peter and Angulu Caleb Ndako and Angulu Samuel: Conducted the research.

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