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Susceptibility and molecular characterization of *mecA*- and *mecB*-positive community acquired methicillin-resistant *Staphylococcus aureus* isolates from students

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Abstract
Staphylococcus aureus is an organism of great public health importance. It is widely studied because it is virulent, causes life threatening disease and has ability to adapt to diverse environmental conditions and so develops resistance to antibiotics easily. As a result, there is a need for surveillance of its antibiotic resistance and resistance genes. The susceptibility and molecular characterization of methicillin resistant *Staphylococcus aureus* recovered from urine samples of healthy students were undertaken. Standard procedures were employed for isolation, identification, susceptibility, and polymerase chain reaction analyses. Out of 217 samples collected, 73 were confirmed *Staphylococcus aureus.* Most of the isolates were susceptible to ciprofloxacin and vancomycin followed by gentamicin and co-trimoxazole and least susceptible to penicillin, cefotaxime, ofloxacin and cefoxitin. Thirty-two (32) isolates were resistant to 5 antibiotics while 3 isolates were resistant to the 11 antibiotics used in this study. Sixteen phenotypically methicillin resistant isolates contained *mecA* gene while ten of the isolates also showed the presence of *mecB* gene. The characteristic *Sa442* and *nuc* genes of *Staphylococcus aureus* and the presence of *spa* gene confirmed MRSA. Continous surveillance for antibiotic resistance and resistance genes is paramount at local, regional and national levels. Surveillance data will assist in implementing interventions.

Keywords: Antibiotic resistance; Methicillin-resistant *Staphylococcus aureus, mecA, mecB,* CA-MRSA; Surveillance

INTRODUCTION

Infections are as diverse as the microorganisms causing them. Antimicrobial agents, natural, semi-synthetic and synthetic are widely used to treat the infections causing organisms. However, these organisms develop resistance rapidly and widely. Antibiotic resistance describes the ability of an organism to grow in the presence of an antibiotic it otherwise used to be susceptible to. Antibiotic resistance was facilitated by the increasing use of antibiotics in clinical settings (human and animals) and agriculture as bacteria acquired resistance by various means. Worldwide, infections due to antimicrobial resistant organisms kill at least 700,000 patients each year and it is predicted that by 2050, resistant infections will kill 10,000,000 per year [1].

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This postulation is due to the facts that resistance develop faster than new antibiotics are developed and there are limited antibiotic stewardship and surveillance in most countries, especially the developing countries.

Staphylococcus aureus is one of the common bacteria responsible for nosocomial- and community-acquired infections. It is an organism of great public health importance. It is widely studied because it is a pathogen of concern due to its virulence, its ability to cause various life-threatening infections and its ability to adapt to diverse environmental conditions [2]. Its remarkable survival and persistence can be attributed to a host of strategies, one of which is metabolic versatility—their ability to rapidly alter their metabolism in the presence of transient or long-term bacteriostatic and bactericidal conditions and facilitate cellular homeostasis. These attributes contribute to their widespread dissemination and challenging eradication particularly from clinical settings [3,4].

Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is currently a clinically significant and virulent organism associated with infections such as serious skin and soft tissue infections [5]. MRSA infections are referred to as community acquired if there are no healthcare associated risks such as a recent hospitalisation or surgery, dialysis, residence in a long-term care facility, and the presence of a permanent indwelling catheter or percutaneous medical device at the time of culture [6,7]. There are increasing concerns over CA-MRSA as they cause outbreaks and are displacing nosocomial acquired MRSA as pathogens in infections [8,9]. Individuals can be carriers with no symptoms and CA-MRSA can be contracted from members of households or associations through shared facilities such as sports environment and equipment, swimming pools, clothing, and towels. The risk factors associated with acquiring CA-MRSA include close contact, abrasion injuries and activities related to poor communal hygiene such as sharing towels [5]. People at risk of CA-MRSA infections are university and school students, adolescent athletes, military trainees, jail inmates, same sex sexual relationships, members of infected families, people with tattoos and storm/flood displaced people [7].

Resistance of *S. aureus* to methicillin and other β-lactam antibiotics is due the presence of *mecA* gene which encodes the production of low-affinity penicillin-binding protein 2a (PBP2a) [10]. The *mecA* is found on the staphylococcal cassette chromosome *mec* (SCC*mec)* [10], the mobile genetic element woven into the chromosome of *S. aureus.* Clusters of CA-MRSA may persist and be asymptomatic in majority of individuals for months or years without development of clinical infections. While there are studies on identification and susceptibility of MRSA isolates in Nigeria, there is the need for the understanding of the molecular features of such isolates recovered in Nigeria. Consequently, this study was undertaken to assess the susceptibility and molecular characteristics of CA-MRSA isolates recovered from students of University of Jos, Nigeria. While CA-MRSA has been predominantly isolated from skin and tissue samples from patients [11-15], in this study, CA-MRSA was isolated from urine samples obtained from healthy volunteers.

EXPERIMENTAL METHODS

Sample collection. Healthy volunteers who have not been to any hospital and were not on any antibiotics at least four weeks prior to collection of samples were employed in this study. The volunteers were male and female students of science and art courses of the University of Jos. Convenience sampling method was used to obtain 217 urine samples. Volunteers were given new sterile universal bottles and instructed to collect mid-stream urine. The volunteers consented to providing urine samples after detailed explanation of the

study and the approval of the appropriate ethical committee had been obtained.

Isolation of organism. Nutrient broth was prepared, and urine samples were inoculated into it and incubated for 18-24 h at 37ºC. Thereafter, the cultures were streaked onto mannitol salt agar and incubated for another 24 – 48 h. Re-streaking of characteristic colonies was undertaken on mannitol salt agar and incubated. The characteristic colonies were isolated and inoculated into nutrient broth and nutrient agar slants and incubated at 37ºC for 24 h. These were further characterized to establish their identities.

Characterization and identification of Staphylococcus aureus isolates. Known standard tests such as Gram-staining, catalase, coagulase, and β-lactamase tests were used to characterize and identify *S. aureus*. The BactiStaph latex test kit was employed for the coagulase test. The Microgen™ STAPH-ID Rapid Test Kits was used for the final identification of the isolates. Single colonies of 24 h cultures of the 135 coagulase positive isolates were dispersed in the suspending medium of the Microgen test kit and agitated thoroughly. Three drops of the bacterial suspension were added to each of the 12 wells of the strips and wells 10 and 11 were covered with 3 drops of mineral oil. The top of the test strips was secured with an adhesive tape and incubated at 37º C. Thereafter, the test strips were interpreted against a template, the findings were fed into the Microgen software and the isolates were identified to sub-species level.

Antibiotic susceptibility test. The disc diffusion test method of the Clinical Laboratory Standards Institute [16] was used to determine the antibiotic susceptibility patterns of 171 staphylococcal isolates. Prepared Mueller-Hinton agar was sterilized, and 20 mL aliquots were poured into sterile petri dishes and allowed to solidify. Then, 24 h cultures of the isolates were dispersed in normal saline to 0.5 mcFarland turbidity standard (ca. 10^8 cfu/mL) and were used to cover the Mueller-Hinton agar plates. The excess inoculum was discarded, and the plates were dried in the incubator for 10 min. Thereafter, antibiotic discs were placed firmly on the agar and the plates were kept for 1 h at room temperature before they were incubated in inverted positions at 37ºC for 24 h. The diameters of the zones of inhibition were measured to the nearest millimetres and classified as sensitive, intermediate, or resistant based on the CLSI interpretative chart for zones sizes [16]. This test was done in triplicates for each isolate.

Plasmid curing test. Serial dilutions of ethidium bromide were made in Mueller-Hinton broth. Standardized 24 h cultures of methicillin (cefoxitin/oxacillin) resistant isolates were inoculated into the broths and incubated for 24 h at 37ºC and thereafter the MICs were recorded. Subcultures based on the dilutions just below the MIC were made and re-tested for susceptibility to the test antibiotics by the diffusion method.

Extraction of DNA from S. aureus isolates. The diffusion test indicated that 16 of the isolates were phenotypically methicillin resistant – resistant to cefoxitin/oxacillin. These isolates were then used for all the molecular analysis. Cultures prepared in tryptic soya broth (TSB) overnight were centrifuged at 2000 rpm for 10 min and the supernatant was discarded into a disinfectant jar. Thereafter, alkaline (pH 8) 480 µL of 50 mmoL EDTA was added to the sediments and vortexed to re-suspend the cells. The cells-EDTA mixtures were transferred to 1.5 mL microcentrifuge tubes. A master mix comprising 30 µL of lysostaphin, 15 µL mutanolysin and 75 µL lysozyme per reaction were vortexed and 120 µL of the master mix was added into each of microcentrifuges labelled to correspond with the isolates for weakening of the cells for efficient lysis. The samples were incubated for 60 min at 37ºC and

then centrifuged for 3 min at 13500 rpm. The supernatant was discarded into a disinfectant jar. To the sediments, 600 µL nuclei lysis solution was added, and the tubes were agitated to re-suspend the cells. The samples were incubated for 5 min at 75ºC in heatblock for lysis of the cells. The samples were cooled to room temperature and thereafter, 2 µL of RNA solution was added to the cell lysate, agitated back and forth for about 5 times and incubated at 37ºC for 60 min. Then, 200 µL of protein precipitating solution was added to the sample and agitated at high speed using vortex for 20 s. The mixture was kept in ice for 5 min and allowed to warm up to room temperature. The protein precipitating solution/cell lysate was centrifuged at 13500 rpm for 3 min. Then, 800 µL of the supernatant containing the DNA was transferred into 600 µL isopropanol in a clean 1.5 mL microcentrifuge tube. The tube was agitated back and forth and centrifuged for 3 min. On decanting of supernatant, the DNA pellets were washed with 600 µL 70% ethanol and stored at – 20ºC.

Polymerase Chain Reaction tests. The staphylococcal protein A (*spa*) sequence typing as described by Shopsin and co-workers [17] was undertaken for the polymorphic region of protein A. The number of DNA templates determined the master mix prepared. For each reaction, the volume of the reagents was 48 µL and 2 µL DNA templates were added. The final volume of the master mix was obtained by multiplying the number of isolates for spa typing with the volume of each reaction. The master mix was vortexed and the Taq polymerase was added, the mix agitated back and forth 5 times and then 48 µL was dispensed into PCR tubes. Thereafter, 2 µL of each template was added to the corresponding PCR tube. After agitation, the PCR tubes were placed in a thermocycler which was operated according manufacturer's instructions. The primers used are shown in Table 1 [16]. Each *spa* PCR program commenced with activation of the Taq polymerase at 95ºC for 10 min, followed by 3-step cycling – denaturation for 30 s at 95ºC, annealing for 30 s at 60ºC and extension step for 1 min at 72ºC. There was a total of 30 cycles and the final extension time was 10 min at 72ºC.

Prepared agarose gel was loaded with the *spa* PCR product, electrophoresed, and then viewed using transilluminator (Bio-Rad Gel Doc XR) with pictures taken. The *spa* products were purified as described on the purification kit (Qiagen PCR purification kit - QIAquick). Five volumes of buffer PB were added to one volume of the *spa* PCR reaction and mixed. PCR DNA was applied to a QIAquick column placed in a 2 mL collection tube which was centrifuged for 1 min so that the DNA can bind to the QIAquick column. Thereafter, the flow-through was discarded with the QIAquick column left in the tube. A washing buffer, PE (750 µL) was added to the tube and centrifugation was undertaken for 1 min. The flow-through was discarded and centrifugation undertaken to ensure the washing buffer was removed as much as possible. Thereafter, 50 µL eluting buffer (EB) was added and centrifugated twice (at 1 min interval) for 1 min to elute DNA. The eluate was collected for quantification.

Spectrophotometry was used to determine the concentration of each PCR sample needed in each reaction to include 5 ng/100 bp of the amplified product. The amplified *spa* region was ~ 425 bp in length, hence, 20 ng of product was needed. Two wells were used for the sequencing protocol – one for forward primer and the other for reverse primer. The formula – template + Primer A + dH_2O = 11 µL total volume. The dH_2O for each reaction was added into the wells of 96 well plates in aliquots. Then, 1 µL of the appropriate primer was added to the wells and the determined quantity of DNA was added to the corresponding wells. Thereafter, the sequencing was undertaken, and *spa* results were analysed using the *spa*-RIDOM website and recorded.

The seven housekeeping genes: *arcC, aroE, glpF, gmk, pta, tpi, and yqiL* were detected using the protocol described by Enright and co-workers [18]. Seven master mixes were prepared for each housekeeping gene. The final volume for each master mix was obtained from the number of reactions by the DNA templates. The master mix was agitated to mix and then Tag polymerase was added followed by agitation back and forth. Thereafter, 48 µL each was added into PCR tubes, followed by the template DNA and agitation before loading into the thermocycler. The positive control employed was *S. aureus* USA300 and the primers used are shown in Table 2.

The optimization process commenced with initial heat activation of the Taq polymerase at 94ºC for 5 min, followed by 3-step cycling – denaturation for 30 s at 94ºC, annealing for 30 s at 55ºC and extension step for 1 min at 72ºC. There was a total of 30 cycles and the final extension time was 5 min at 72ºC.

A large gel comprising of 3 g of agarose heated to dissolve in 200 mL of TAE buffer and 8 µL of ethidium bromide added to stain the DNA was used to run the amplified DNA product for each of the seven housekeeping genes. The PCR products loaded on the gel was run on a Bio-Rad electrophoretic machine at 120 V for 1.5 h. Thereafter, the gel bands were viewed using the Bio-Rad Gel Doc XR transilluminator. The PCR products were purified and quantified as described earlier and then sent out for sequencing at the Bioscience Centre of the International Institute of Agriculture, Ibadan Nigeria.

mecA for methicillin resistance was detected and the type of staphylococcal cassette chromosome *mec* (SCC*mec*) determined by undertaking a multiplex PCR. The primers used are shown in Table 3, following the protocol described by Ghaznavi-Rad and co-workers [19]. The master mix was prepared to include *mecA* and the SCC*mec* types I-V primers according to the number of DNA templates. After agitation, HotStarTaq was added to the master mix followed by further agitation and then volumes of 48 µL was transferred into PCR tubes. Then, 2 µl of the template DNA was added, agitated, and then loaded in the thermocycler. MRSA USA300 was the positive control employed.

The optimization process involved initial heat activation of the Taq DNA polymerase at 95ºC for 15 min; then 3-step cycling, denaturation for 30 s at 94ºC, thereafter, annealing for 90 s at 57ºC and extension step for 90 s at 72ºC. The total cycles were 30 and the final extension time was 10 min at 72ºC. The amplified DNA product was run on a large gel of 3 grams of agarose dissolved in 200 mL of TAE at elevated temperature and then 8 µL of ethidium bromide was added to stain the DNA. A Bio-Rad electrophoretic machine was used to run the PCR products loaded on the gel at 120 V for 1.5 h. Thereafter, the bands were viewed using the Bio-Rad Gel DocXR transilluminator. The SCCmec type was determined based on the band pattern obtained.

RESULTS

Isolation and characterization. Out of 217 samples collected, 171 were positive for staphylococcus. Out of the 171 isolates, 84 were catalase positive, 135 were coagulase positive and 98 were β-lactamase producers. Of the 135 coagulase positive isolates tested with the Microgen™ STAPH-ID kit, 73 were confirmed to be *S. aureus*. The conventional methods such as the coagulase test identified staphylococcus species giving a high positive value for the prevalence of *S. aureus*, however, the Microgen™ STAPH-ID kit provided a more specific approach to identification of *S. aureus.*

Antibiotic susceptibility of staphylococcal isolates. Using the Fluka zone interpretive chart based on CLSI standards [16], the zone diameters were interpreted and the varying

degrees of susceptibility of the isolates to antibiotics are as shown in Figure 1. Most of the isolates were susceptible to ciprofloxacin and vancomycin followed by gentamicin and co-trimoxazole and least susceptible to penicillin, cefotaxime, cefoxitin and ofloxacin.

Figure 2 shows the multi-antibiotic resistant pattern of the isolates. Thirty-two (32) isolates were resistant to 5 antibiotics while 3 isolates were resistant to the 11 antibiotics used in this study. Figure 3a – d shows some of the multi-drug resistance profiling of the isolates specifying the antibiotics to which the isolates were resistant. The number and antibiotic(s) to which resistance was shown differed from one isolate to the other. The multi-drug resistance profiling chosen to be displayed started with antibiotics having more isolates resistant to them (penicillin, cefotaxime, ofloxacin and cefoxitin). The Multi-antibiotic resistant indices (MARI), obtained by dividing the number of antibiotics to which an isolate is resistant by the total number of antibiotics to which it was exposed were in the range of 0.09 – 1.00 as shown in Table 4. Only 10 (5.85%) of the isolates had MARI below 0.2 while 161 (94.15%) had values greater than 0.2 (0.27 – 1.0).

Plasmid curing. Of the resistant isolates subjected to curing, increased susceptibility was observed but more prominent with ciprofloxacin, cotrimoxazole, vancomycin and erythromycin and very less so with cefoxitin, ofloxacin and penicillin (Figure 4).

PCR Analysis. PCR analysis indicated that 16 phenotypically methicillin resistant isolates contained *mecA* gene (Figure 5) with bands at 500 base pair (bp) while six of the isolates (isolates 6, 10, 13, 14, 15 and 16) also showed the presence of *mecB* gene at 200 bp (Figure 6). Weak signals of *mecB* were observed with isolates No 5, 7, 11, 12. The characteristic Sa442 gene showed bands below 100 bp (Figure 7), nuc gene was present with bands below 100 bp (Figure 8), Figure 9 showed the presence of *spa* gene at 100 bp, and Figure 10 showed the presence of the 16SrRNA gene.

DISCUSSION

Of interest is the number of isolates (35) susceptible to ofloxacin even though it has the same mechanism of action with ciprofloxacin. This unexpected significant disparity between the antibacterial activities of ciprofloxacin and ofloxacin were also observed by another study [3]. In a study spanning over 50 medical centres in USA and Canada, more isolates were more sensitive to ofloxacin than ciprofloxacin [20]. The researchers suggest that ofloxacin has a lower mutational rate-to-resistance among *S. aureus* isolates compared to ciprofloxacin. Perhaps over years, *S. aureus* known for its ability to adapt to changes in its environment had developed changes to enable it to be resistant to ofloxacin. In another study, the degree of susceptibility for ciprofloxacin and ofloxacin was dependent on the *S. aureus* strains [21]. Both quinolones were similarly effective against *S. aureus* 3094 and 5030; ciprofloxacin was more effective against 5001 and ofloxacin was more effective against 5252 (20).

However, some studies showed same degree of susceptibility between the two quinolones [2,22]. It may appear that as the years go by, resistance to these antibiotics increase due to misuse and abuse, a common occurrence in a developing country like Nigeria where antibiotics are not strictly on prescription. It is interesting that *S. aureus* is still susceptible to ciprofloxacin considering that it is one of the most prescribed and purchased over the counter antibiotic. *S. aureus* isolates were more susceptible to ciprofloxacin than gentamicin although, the reverse was envisaged due to the complexity of gentamicin and its route of administration which limits its usage.

Table 1. Primers sequence for *spa* typing

Gene	Primer sequence	Expected amplicon size
spa 2F	GAACAACGTAACGGCTTCATCC	(250-637bp)
spa 1514R	CAGCAGTAGTGCCGTTTGCCT	~425bp

Table 2. Primers used for multilocus sequence typing

Gene	Primer sequence	Expected amplicon size
arcC-Forward	TTG ATT CAC CAG CGC GTA TTG TC	456bp
arcC-Reverse	AGG TAT CTG CTT CAA TCA GCG	
AroE-Forward	ATC GGA AAT CCT ATT TCA CAT TC	456bp
AroE-Reverse	GGT GTT GTA TTA ATA ACG ATA TC	
glpF Forward	CTA GGA ACT GCA ATC TTA ATC	465bp
glpF Reverse	TGG TAA AAT CGC ATG TCC AAT TC	
gmk, Forward	ATC GTT TTA TCG GGA CCA TC	429bp
gmk, Reverse	TCA TTA ACT ACA ACG TAA TCG TA	
pta, Forward	GTT AAA ATC GTA TTA CCT GAA GG	474bp
pta, Reverse	GAC CCT TTT GTT GAA AAG CTT AA	
tpi Forward	TCG TTC ATT CTG AAC GTC GTG AA	402bp
tpi Reverse	TTT GCA CCT TCT AAC AAT TGT AC	
yqiL Forward	CAG CAT ACA GGA CAC CTA TTG GC	516bp
yqiL Reverse	CGT TGA GGA ATC GAT ACT GGA AC	

Source: [18]

Table 3. Primers used for *SCCmec* typing

Gene	Primer sequence	Expected amplicon size
Type I Forward	GCTTTAAAGAGTGTCGTTACAGG	
Type I Reverse	GTTCTCTCATAGTATGACGTCC	613bp
Type II Forward	GATTACTTCAGAACCAGGTCAT	
Type II Reverse	TAAACTGTGTCACACGATCCAT	287bp
Type III Forward	CATTTGTGAAACACAGTACG	
Type III Reverse	GTTATTGAGACTCCTAAAGC	243bp
Type IVa Forward	GCCTTATTCGAAGAAACCG	
Type IVa Reverse	CTACTCTTCTGAAAAGCGTCG	776bp
Type IVb Forward	AGTACATTTTATCTTTGCGA	
Type IVb Reverse	AGTCATCTTCAATATGGAGAAAGTA	1000bp
Type IVc Forward	TCTATTCAATCGTTCTCGTATT	
Type IVc Reverse	TCGTTGTCATTTAATTCTGAACT	677bp
Type IVd Forward	AATTCACCCGTACCTGAGAA	
Type IVd Reverse	AGAATGTGGTTATAAGATAGCTA	1242bp
Type IVh Forward	TTCCTCGTTTTTTCTGAACG	
Type IVh Reverse	CAAACACTGATATTGTGTCG	663bp
Type V Forward	GAACATTGTTACTTAATGAGCG	
Type V Reverse	TGAAAGTTGTACCCTTGACACC	325bp
mecA Forward	TCCAGATTACAACTTCACCAGG	
mecA Reverse	CCACTTCATATCTTGTAACG	162bp
Sa442 Forward	AATCTTTGTCGGTACACGATATTCTTCACG	
Sa442 Reverse	CGTAATGAGATTTCAGTAGATAATACAACA	108bp

Source: [19].

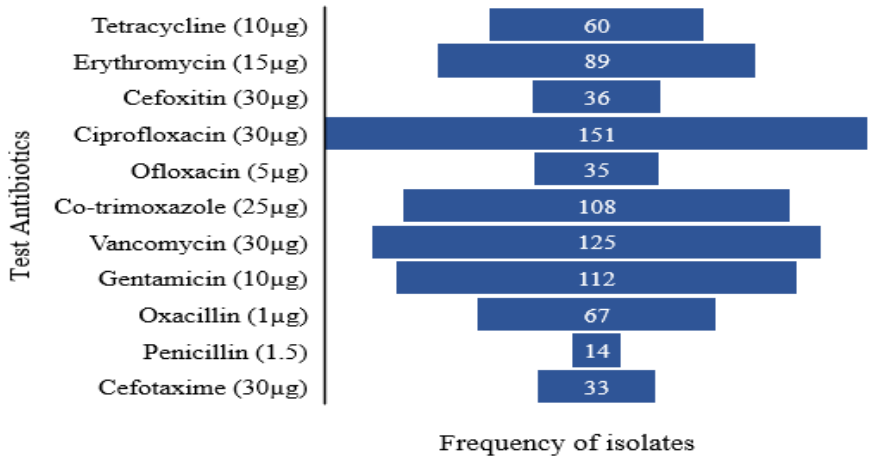


Figure 1. Susceptibility of staphylococcus isolates to given antibiotics using disc diffusion test (n = 171).

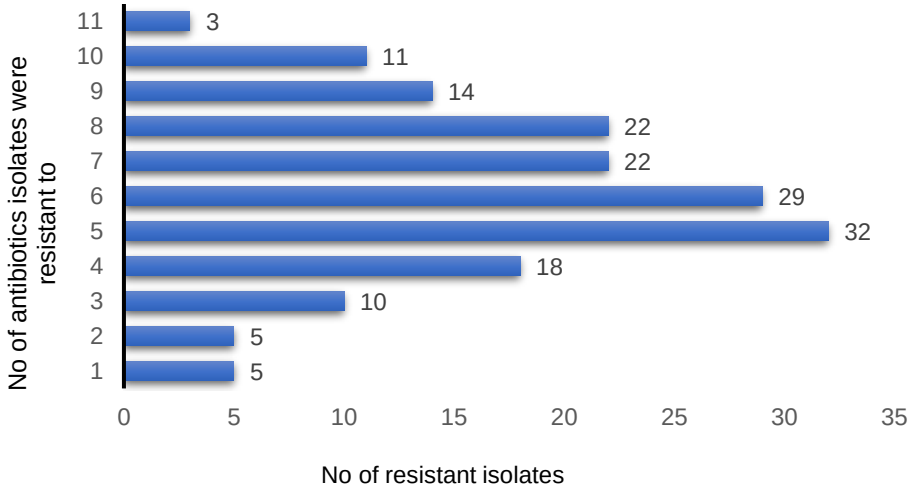


Figure 2. Multiple antibiotic resistant patterns of the staphylococcal isolates

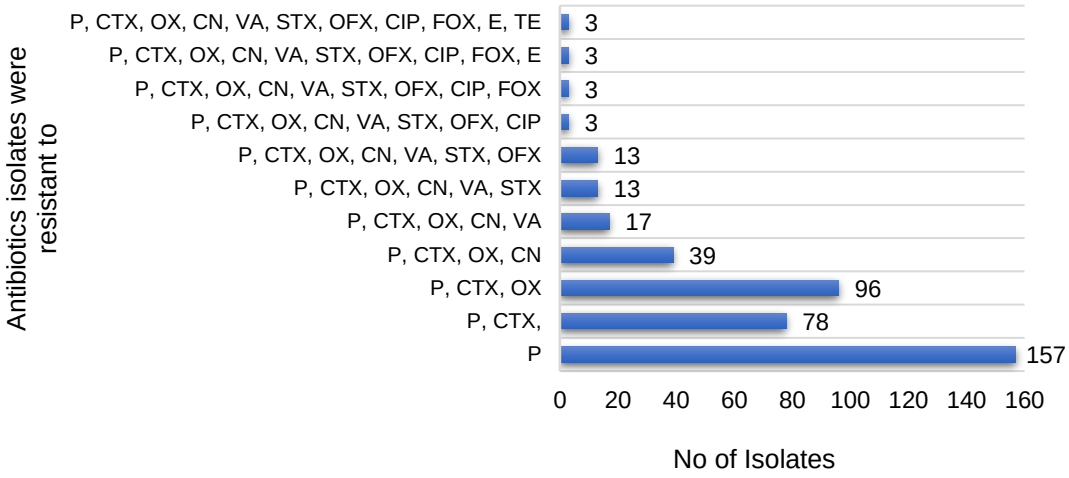


Figure 3a. Multidrug resistance profiling of staphylococcal isolates to antibiotics starting with penicillin
P – Penicillin; CTX – Cefotaxime; OX – Oxacillin; CN – Gentamicin; VA – Vancomycin; STX – Co-trimoxazole; OFX – Ofloxacin; CIP – Ciprofloxacin; FOX – Cefoxitin; E – Erythromycin; TE – Tetracycline.

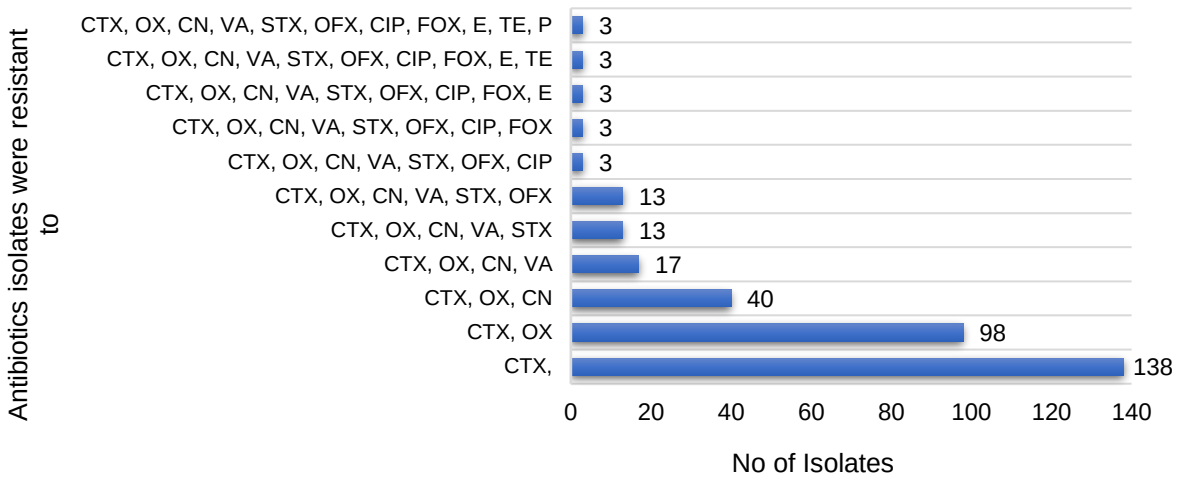


Figure 3b. Multidrug resistance profiling of staphylococcal isolates to antibiotics starting with cefotaxime

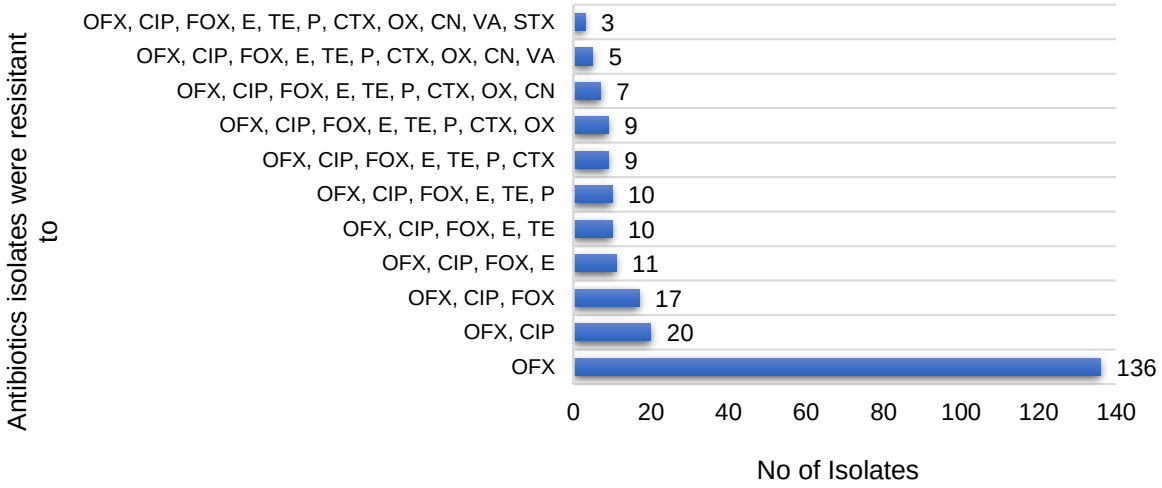


Figure 3c. Multidrug resistance profiling of staphylococcal isolates to antibiotics starting with ofloxacin

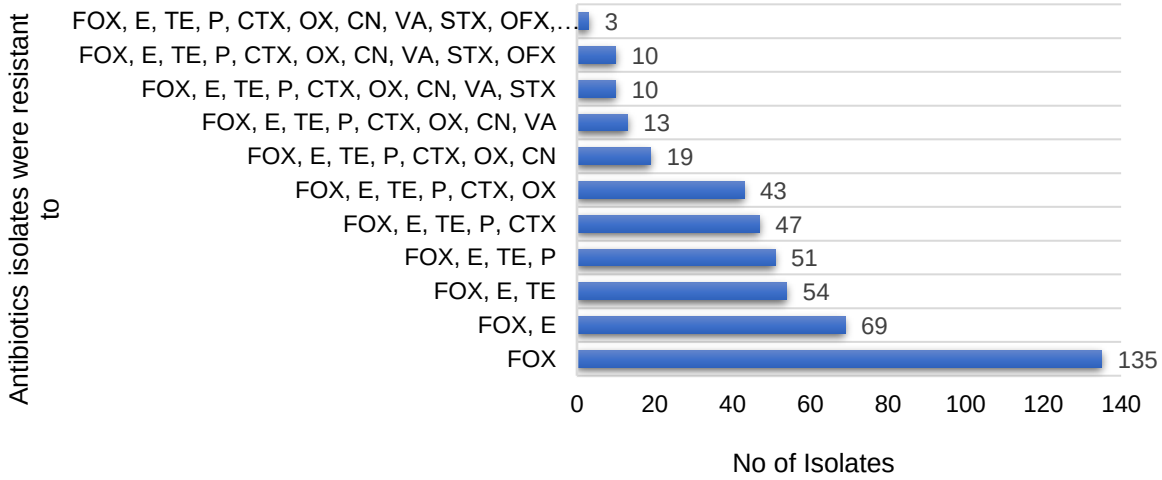


Figure 3d. Multidrug resistance profiling of staphylococcal isolates to antibiotics starting with cefoxitin

Table 4. Multi-antibiotic resistant indices (MARI) of staphylococcal isolates

No. of Antibiotics isolates are resistant to	MARI	No. of Isolates	Cumulative % at the MARI
1	0.09	5	2.92
2	0.18	5	5.85
3	0.27	10	11.70
4	0.36	18	22.22
5	0.45	32	40.94
6	0.54	29	57.84
7	0.64	22	70.76
8	0.73	22	83.63
9	0.81	14	92.81
10	0.91	11	98.24
11	1.0	3	100.0

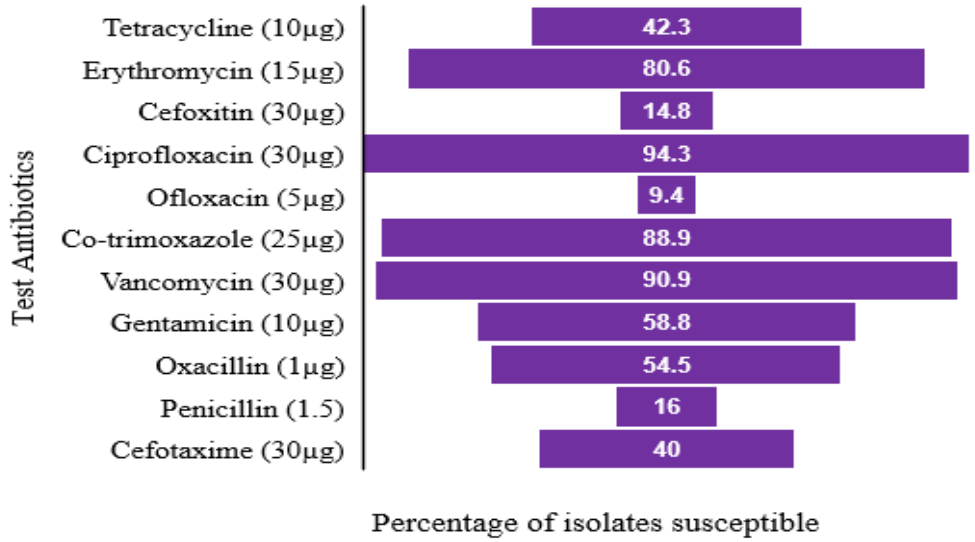
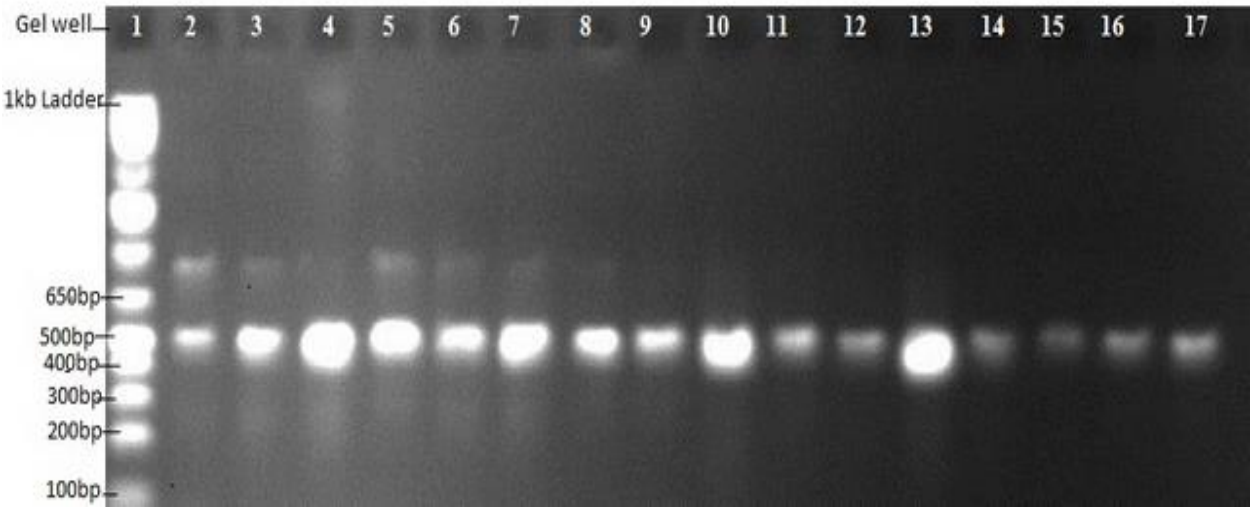


Figure 4. Percentage susceptibility of ethidium bromide treated staphylococcal isolates to antibiotics.



Key: Lane 1 is the ladder and lanes 2 – 17 are *S. aureus* isolates

Figure 5. Electrophoretogram of *mecA* gene at 500 bp amplification products *S. aureus* isolates

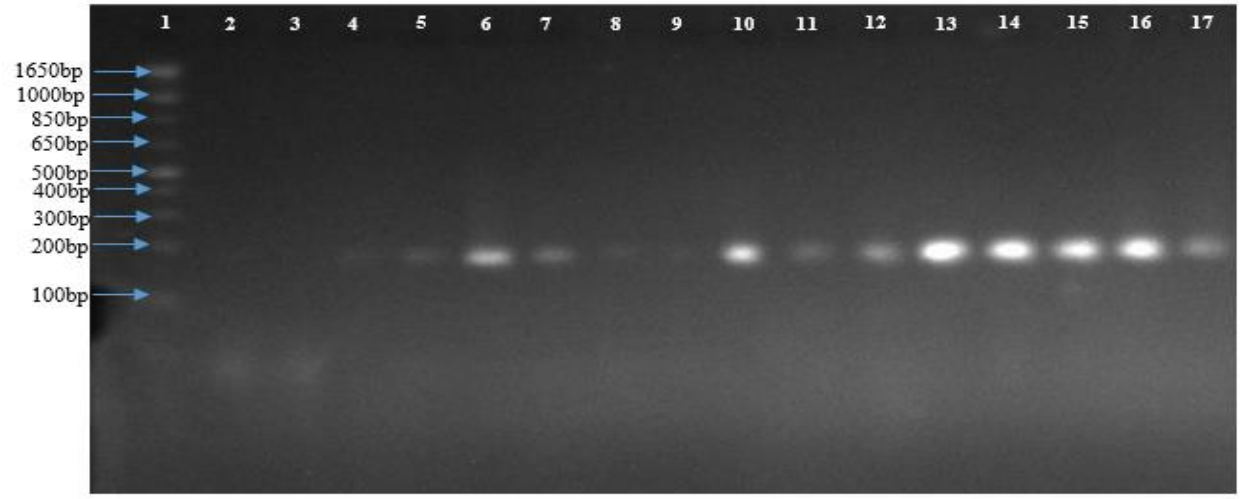


Figure 6. Electrophoretogram of *mecB* gene at 200 bp amplification products *S. aureus* isolates

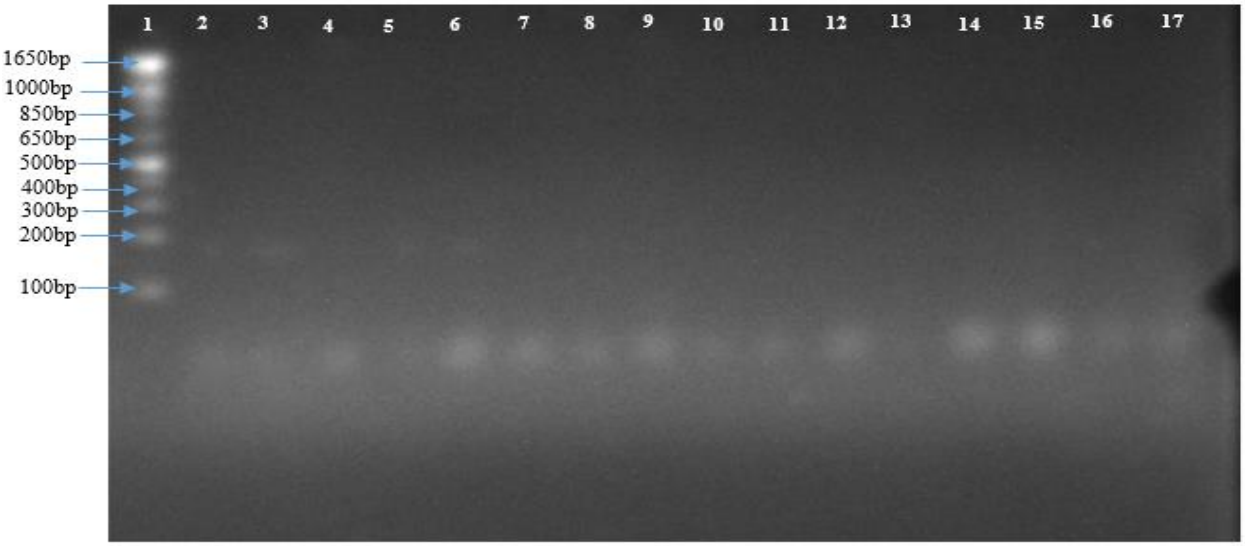


Figure 7. Electrophoretogram of *Sa442* gene at 200 bp amplification products *S. aureus* isolates

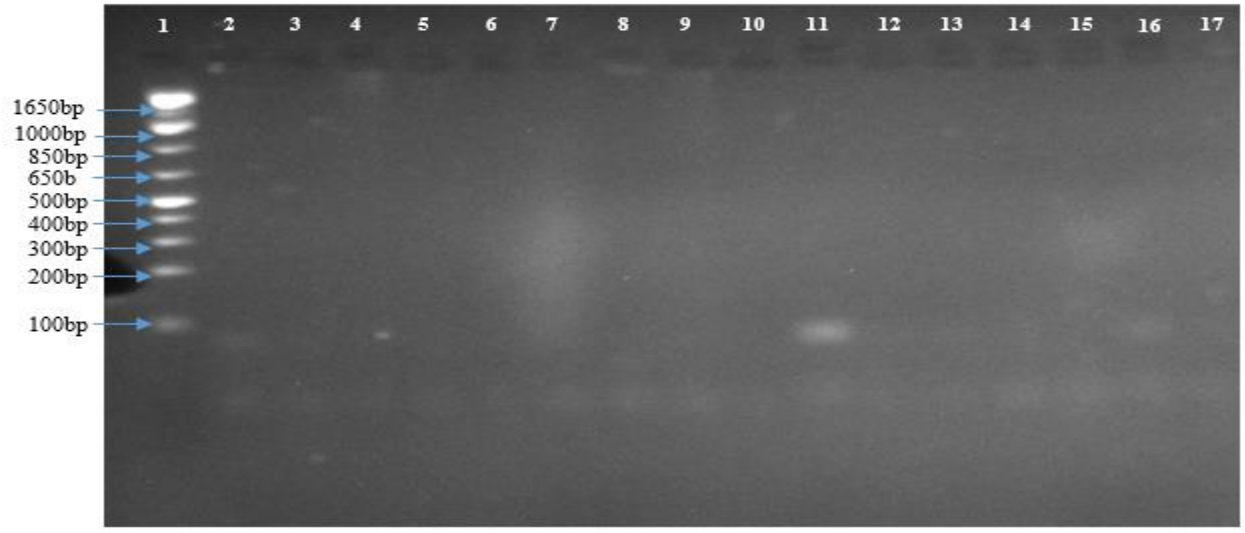


Figure 8. Electrophoretogram of *nuc* gene amplification products *S. aureus* isolates

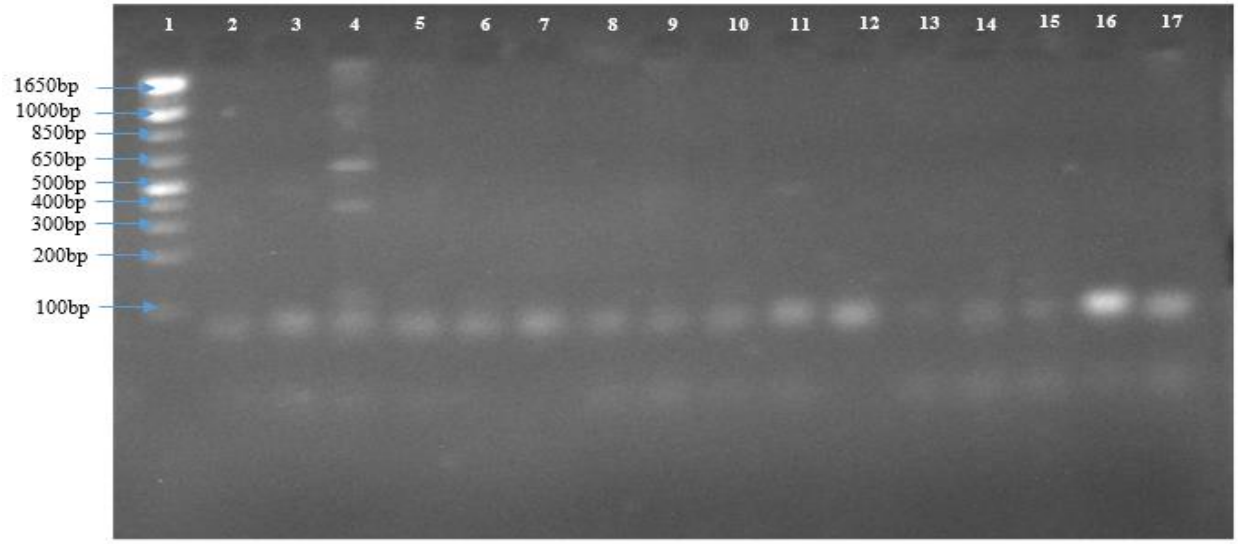


Figure 9. Electrophoretogram of *spa* gene (250-637 bp) amplification products *S. aureus* isolates

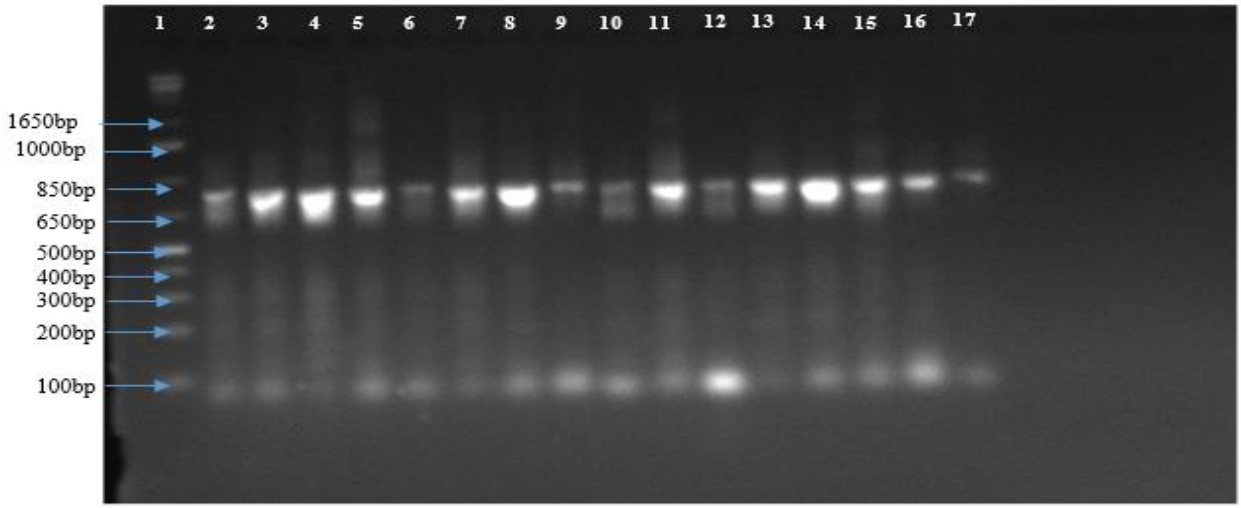


Figure 10. Electrophoretogram of 16SrRNA gene amplification products *S. aureus* isolates

Another antibiotic of interest is co-trimoxazole, which may be a better oral alternative to vancomycin for the treatment of infections due to MRSA. The performance of this old drug being better than cefotaxime may be due to limited prescription and as a result, limited exposure to the bacterium in recent times. A study of isolates from hospitalized (hospital) and outpatients (public health laboratory) in Barbados showed even higher degree of susceptibility to co-trimoxazole - 100% of hospital isolates and 94.3% of community isolates [23]. In another study in Nigeria, 78.7% of the community isolates were susceptible to co-trimoxazole [24]. However, in a study in Southern India that spanned 6 years, 2012 – 2017, the resistance of *S. aureus* (isolated from HIV patients) to co-trimoxazole ranged from 63% (2012) to 78.2% (2016) and 71% (2017) averaging 74.7% over these years [25]. A study in North India showed resistance of *S. aureus* to co-trimoxazole to be as high as 93.3% (isolated from health care workers) and 66.6% (isolated from outpatients) [26]. In a study in localities near Jos city, Nigeria, 80.6% of the MRSA isolates were resistant to cotrimoxazole [27].

Considering a study in Yenagoa, Nigeria where both ciprofloxacin and cotrimoxazole were tested, percentage resistance to both were 32.6% and 80.4% respectively [28] while this study had percentage resistance to both as 11.7% and 36.8% respectively. The findings may seem conflicting, however, the period of study, sources of *S. aureus* (urine, blood, pus, mucus or swap), the population studied, the prevalent strains of *S. aureus* in each environment, and antibiotics in frequent use in each study area among other factors determine the outcome of each study.

Other studies also observed that only three (3) isolates were susceptible to all antibiotics tested [2,27]. CA-MRSA are resistant to β-lactam antibiotics and cross resistant to other antibiotics, findings further buttressed by this study. One approach community acquired methicillin susceptible *Staph. aureus* (CA-MSSA) become CA-MRSA is by exposure of the organism to sub-inhibitory concentrations. In an environment where individuals obtain antibiotics without prescription, self-medicate, administer as they deem fit and not completing the dosage regimen, antimicrobial resistance is expected. Sub-inhibitory concentrations can precipitate multidrug resistance. A study undertaken with MSSA by exposing the isolates to sub-inhibitory concentrations of antibiotics showed that sub-inhibitory concentrations of ciprofloxacin and gentamicin led to resistance to ciprofloxacin, gentamicin, clindamycin, and azithromycin [29]. Exposure to fluoroquinolones such as ciprofloxacin can lead to *gyrA* mutations thereby activating several multidrug efflux pumps in isolates. The same study also showed that sub-inhibitory concentrations of cefotaxime led to resistance to gentamicin, ciprofloxacin, and azithromycin.

Cross resistance of staphylococcal isolates to many antibiotics includes acquisition of determinants by horizontal gene transfer of mobile genetic elements (plasmids, transposons and the staphylococcal cassette chromosome), mutations that modulate the drug binding sites on molecular targets and by increasing expression of endogenous efflux pumps [30].

The efficiency of the curing depends on the type of plasmid and the bacterial host. Plasmid-mediated resistance is reversed by spontaneous segregation and deletion and in cases of stable plasmids, curing agents are required to eliminate the plasmids. Curing is one approach used to identify the genetic location of resistance determinants. The isolates susceptible to ciprofloxacin, cotrimoxazole, erythromycin and vancomycin after curing indicate that the resistance is plasmid-coded while isolates still resistant suggest that the resistance may be chromosomal-mediated.

The 16 methicillin resistant strains subjected to PCR analysis were confirmed resistant by the detection of *mecA.* About 15 – 20% of the genome of *S. aureus* comprises mobile genetic elements which include bacteriophages, pathogenicity islands, plasmids, transposons, integrative conjugative elements, integrons and staphylococcal chromosome cassettes [31]. These genetic elements may possess antiboitic resistance genes except the bacteriophages. The methicillin resitance gene *mecA* is common in the staphylococcal chromosome cassettes and is named SCC*mec* confering resistance to β-lactams in MRSA strains. *MecA* gene is found in CA-MRSA as it is widely diseeminated by horizontal transfers. Horizontal gene transfer facilitates the acqusition of new genetic materials from outside of a specie's clonal lineage [1]. Horizontal gene transfers occurs through conjugation, transduction and natural transformation. Conjugation can be stimulated in *S. aureus,* transduction is efficient in the organism, and it is capable of natural transformation [1,32,33]. Such capabilities may explain the rapidly acquired resistance to

antibiotics by the different strains of *S. aureus.* *MecA* gene cassette codes for high level methicillin resistance and harbours genes coding for resistance to other non-β-lactams which explains the degree of resistances observed with tetracycline (64.9%), and ofloxacin (79.2%) and moderate level of resistance to erythromycin (34.1%), co-trimoxazole (36.8%) and gentamicin (34.5%). Staphylococcal cassette chromosomes (SCC) is found in staphylococcal and macrococcal species. It is a vehicle and mobile element for methicillin resistance genes and other drug resistance genes. *MecA* was first identified in MRSA, however, it has also been found in other saphylococcal species such as *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus*, and *S. fleurettii* [34]. There is > 98% sequence identity of *MecA* identified in these species with the *MecA* found in MRSA strain N 315, the first completely sequenced prototype. *MecA* has also been found in non-staphylococcal species such as *Pseudomonas, aeromonas and Escherichia (E. coli)* species [35,36], *Enterococcus, Proteus,* and *Morganella* species [37], and *Salmonella enterica* Serotype Choleraesuis [38] buttressing development of resistance by horizontal gene transfer.

The *mecB* usually found on chromosome and plasmids of *Macrococcus caseolyticus* [39] was observed in some isolates in this study. Four groups of *mecA* have been identified, *mecA1, mecA2, mecB, mecC*. The prototype strains depicting each *mec* gene are *S. sciuri* K11 for *mecA1*; *S. vitulinus* CSBO8 for *mecA2*; *M. caseolyticus* JCSC5402 for *mecB*, and *S. aureus* LGA251 for *mecC* [34]. While *mecA* and *mecC* have been reported to be present in staphylococcal species, *mecB* is usually not reported, until recently. Some isolates (Nos 2, 3, 4, 8 and 9) did not indicate the presence of *mecB* corroborating the findings of Hiramatsu and co-workers who did not find *mecB* [40,41]. Hiramastu and co-workers [40] suggested that the absence of *mecB* in staplylococcal species may be due to it being carried by trasposon while *mecA* and *mecC* are carried by SCC. However, recent findings of *mecB* in *S. aureus* [42,43] corroborates this study.

Becker and co-workers [42] recovered an *S. aureus* isolate from a nasal-throat swab from a cardiology inpatient with no infection. Plasmid encoded *mecB* was detected and neither *mecA* nor *mecC* was detected. Comparative analysis of the DNA sequence showed 100% sequence identity of *mecB* of *S. aureus* with *that of M. caseolyticus* indicating a possible gene transfer between the two genera. In a study undertaken by Delorme and co-workers [43], out of 521 MRSA isolates collected from a laboratory and obtained from inpatients, outpatients and nursing homes over a 12 month period, *mecA* was detected in 190 isolates, *mecB* in 54 isolates, *mecA* and *mecB* in 261 isolates and no *mec* amplification in 25 isolates. *MecB* confers methicillin resistance and so the isolates in which they were identified are definitely MRSA. The isolates in which *mecB* was detected also had *mecA* in this study and these isolates were obtained from healthy volunteers. Irrational use of antimicrobial agents provides the enabling environment for the acquisition of more resistance strains thereby increasing resistance profiles for other species either by selecting for mutant strains and/or by hroizontal gene transfer from related and unrelated species. The characteristic *sa442* and *nuc* gene for *S. aureus* and spa typing confirms the identity of the isolates.

SCC*mec* type comprises the type of cassette chromosome recombinase (*ccr*) gene complex and the class of *mec*-gene complex, and it is the defining feature of MRSA. MRSA appear in 5 types of SCC*mec* lineage. Types I, II and III are associated with HA-MRSA while types IV and V are associated with CA-MRSA. Types IV and V associated with CA-MRSA are short and typically do not carry any antibiotic resistance genes other than *mec*-gene

complex. Although it is reported that CA-MRSA in comparison to HA-MRSA are faster in growth, more virulent, non-multidrug resistant and lower in the degree of β-lactam resistance [43], isolates in this study were multidrug resistant. Such current display of CA-MRSA may be due to increasing exposure to antibiotics. Selective pressure of antibiotics facilitates the adaptation of the organism to its environment through mutation and horizontal gene transfer. It appears that even if antimicrobial agents are yearly produced, none will be immune to resistance and so there is need for antibiotic stewardship and strategic infection preventive measures and containment of virulent and disease causing pathogens.

Conclusion. *S. aureus* isolates were recovered from urine samples of asymptomatic carriers in a community and identified. Susceptibility test indicated multidrug resistance and the molecular charcaterization detected the presence of methicillin resistant genes *mecA* and *mecB*. Presence of MRSA in asymptomatic carriers prediposes them to CA-MRSA infections and it suggests that for certain medical procedures, MRSA eradication should be undertaken first. The study buttresses the fact that antibiotic stewardship needs to be institutionalized. Prescription by clincians and use of anitbiotics by patients must improve to regress the irrational use of antibiotics and contain antibiotic resistance.

Colonization in certain parts of the body enhances transmission between individuals and serves as potential source of contamination of the environment and infections. Education/awareness and promotion of personal hygiene may assist in containing MRSA. It is envisaged that this study will inform clinical therapy decision, policy making and implementation and antimicrobial resistance containment measures.

Horizontal gene transfer suggest continuous development of resistance across pathogens. Continous surveillance of antibiotic resistance and resistance genes is paramount. At local, regional and national level, there should be on-going organized experiments, collection of antibiotic resistance, analysis and intepretation of data to assist in planning, implementation and assessment of public health care and practice thereby facilitating profitable clinal therapy decisions. Such data will assist in implementing measures such as infection prevention and control practices, rational prescribing, capacity building, development of antimicrobial agents and diagnostic kits and public awarenes and engagement. Monitoring and evaluation of interventions should be done to assess the effectiveness and impact of the interventions and determine the need to re-strategize or implement new interventions.

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