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Antimicrobial Resistance Profile and Quinolone Resistance Genes in *Staphylococcus aureus* from Patients Attending Federal Medical Centre Keffi, Nigeria

E. A. Sunday[1], Y. B. Ngwai[1*], R. H. Abimiku[2], I. H. Nkene[1], Y. Ibrahim[1], E. Y. Envuladu[3] and S. K. Kigbu[4]

[1]Department of Microbiology, Nasarawa State University, P.M.B.1022, Keffi, Nasarawa State, Nigeria.
[2]Plateau State Human Virology Research Centre (PLASVIREC), Jos, Nigeria.
[3]Department of Pharmacy, Federal Medical Centre, P.M.B.004, Keffi, Nasarawa State, Nigeria.
[4]Department of Bacteriology, National Veterinary Research Institute, P.M.B.01, Vom, Plateau State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors EAS and YBN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IHN, and YI managed the analyses of the study. Authors RHA, EYE and SKK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study investigated the antimicrobial resistance profile and quinolone resistance genes in *Staphylococcus aureus* from patients attending Federal Medical Centre, Keffi, Nigeria.
Methodology: A total of 240 clinical samples which comprised of high vaginal swabs, endocervical swabs, sputum, ear swabs, wound swabs, semen and eye swabs, were collected from the patients. *Staphylococcus aureus* was isolated and identified from these samples using standard microbiological method. Antimicrobial susceptibility testing of the isolates was performed and interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) method.

**Corresponding author: E-mail: ngwaiyb@yahoo.com;*

Ciprofloxacin-resistant *S. aureus* were screened for quinolone resistance genes using Polymerase Chain Reaction (PCR) method.

Results: Out of 240 clinical samples, the prevalence of *S. aureus* was 21.3%. The prevalence in relation to clinical samples was higher in eye swab (45.5%) and ear swab (44.4%), but lower in sputum (14.5%). The isolates were more resistant to oxacillin (88.2%), sulphamethoxazole/ trimethoprim (82.4%) and erythromycin (76.5%), but less resistant to ciprofloxacin (19.6%) and levofloxacin (5.9%). The most common resistance phenotypes in the isolates were sulfamethoxazole/trimethoprim (SXT) - clindamycin (DA) – ofloxacin (OX) - erythromycin (E) - rifampicin (RD) and SXT-DA-OX-E- streptomycin (S) -RD with an occurrence of (13.7%) each. The percentage occurrences of multidrug resistant and extensive-drug resistant isolates were 92.2% and 7.8% respectively. The occurrences of quinolone resistance genes in the ciprofloxacin-resistant isolates were: *aac(6')-Ib-cr* (60.0%), *gyr*A and *gyr*B (50.0%), *par*C (40.0%), *qnr*B (20.0%) and *qnr*S (10.0%).

Conclusion: The isolates were less resistant to levofloxacin, cefoxitin, ciprofloxacin and gentamicin in the study location. Most of the ciprofloxacin-resistant isolates harbored quinolone resistance genes with *aac(6')-Ib-cr* as the most common.

Keywords: Staphylococcus aureus; clinical; antibiotic; resistance; quinolone; genes.

1. INTRODUCTION

Staphylococcus aureus is a gram-positive bacterium recognized as a common agent of bacterial infections in both human and veterinary medicine [1]. In humans, the bacterium is associated with community and hospital-acquired infections in both immune-competent and immune-compromised individuals worldwide [2, 3,4]. This bacterium commonly cause urinary tract infections, boil, mastitis, wound infections, pustule, abscesses, osteomyelitis, septicaemia, food poisoning, vomiting, diarrhea, and meningitis [5,6,7]. Treatment of *S. aureus* infections is usually accomplished by antimicrobial agents including fluoroquinolones (FQs). However, the acquisition of resistance by the bacterium limits the continued usefulness of antimicrobial agents which affect attempts at medical control [8]. Antimicrobial resistance is amongst the most significant health care problems of this era [9,10].

Fluoroquinolones, of which ciprofloxacin is the most widely used, are broad-spectrum antibiotics with good activity against gram-positive organisms, including both methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) [11,12,13,14]. This wide usage of FQs provides a selective advantage for the emergence of FQ-resistant *S. aureus* (FQRSA), especially among methicillin resistant *S. aureus* (MRSA) strains [3]. Mutations in the gyrase or topoisomerase IV enzymes produce changes that cause resistance to FQs by two basic mechanisms: alteration in the interaction of the FQs with their target sites and alterations that affect access of the drug [15]. The mutations responsible for resistance occur in certain regions of each enzyme subunit called the Quinolone Resistance Determining Region (QRDR), particularly in the *gyr*A and *gyr*B genes of the topoisomerase IV, making the enzyme less sensitive to inhibition by fluoroquinolones. For some of the more common QRDR mutations, it appears that the amino acid changes reduce the affinity of enzyme-DNA complex to fluoroquinolones. There are several studies which suggest that a combination of mutations in both genes may be responsible for high-level resistance even to newer fluoroquinolones [16, 17]. Many strains of *S. aureus* also carry a wide variety of multi-drug resistant genes on plasmids which aid the spread of resistance even among different species [18].

Resistance of *S. aureus* isolates to commonly used antibiotics in different parts of the world has been widely reported [19,20,21,22]. A more critical *S. aureus* resistance is that of MRSA strains which demonstrate joint resistance to many commonly used antibiotic classes namely: fluoroquinolones, macrolides, chloramphenicol, tetracycline, and aminoglycosides [23]. Antibiotic resistance in bacteria have been classified into: multidrug resistance (MDR: non-susceptible to ≥1 agent in ≥3 antimicrobial categories); extensive drug resistance (XDR: non-susceptible to ≥1 agent in all but ≤2 antimicrobial categories); pan drug resistance (PDR: non-susceptible to all antimicrobial listed) [24]. The occurrence rate of infections due to multidrug resistant *S. aureus*,

vary from one country to another, between hospitals or different units of the same hospital [21]. Fluoroquinolone resistant *S. aureus* have been widely reported globally [21,25]. These studies have documented the carriage of both chromosomal and/or plasmid mediated quinolone resistance (QR) genes in the *S. aureus* isolated.

In Nigeria, antibiotic resistance in *S. aureus* have been documented in several studies [26,27,28], [29]. Furthermore, several studies have reported on FQRSA [27,30,31]. We are not aware of any previous report on the prevalence and antimicrobial resistance of *S. aureus*; and the occurrence of QR genes in *S. aureus* in the location under study. This study thus investigated the prevalence and antimicrobial resistance profile of *S. aureus* as well as the occurrence of QR genes in *S. aureus* from clinical samples from patients which accessed Federal Medical Centre, Keffi, Nigeria. Since FQs are used to treat MSSA and MRSA in Nigeria, monitoring antimicrobial resistance and multidrug resistance in *S. aureus* in our area will help in optimizing the therapeutic management of patients with infection. Furthermore, assessing QR genes in bacteria can provide alternative means of rapid detection of quinolone resistance in clinical bacterial infections.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 240 clinical samples were collected from patients in Federal Medical Centre, Keffi, Nigeria (FMCK), using appropriate sterile sample containers and transported to Microbiology Laboratory in FMCK for analyses and culture. The study included male and female Nigerian patients of all ages which attended FMCK for three (3) months from January to March 2018. The samples were: sputum (83), high vaginal swabs (49), endocervical swabs (21), semen (30), ear swabs (9), eye swabs (11) and wound swabs (37).

2.1.1 Isolation of *Staphylococcus aureus*

The specimens collected were streaked on mannitol salt agar (MSA: HiMedia Laboratories Pvt. Ltd., India) prepared based on manufacturer's instructions and incubated at 37°C for 24 h [32]. Golden yellow colonies on MSA were selected as presumptive *S. aureus*.

2.1.2 Identification of *Staphylococcus aureus*

Staphylococcus aureus isolates were identified through conventional methods [32] of Gram staining, slide catalase production, slide coagulase test; and confirmed using KB004 HiSTAPHTm commercial identification kit (HiMedia Laboratories Pvt. Ltd., India) following manufacturer's instructions. The isolates were stored in the refrigerator at 4°C on nutrient agar (Merck KGaA, Darmstadt, Germany) slants and reactivated by sub-culturing on mannitol salt agar and used in experiments.

2.2 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of the isolates was performed using disc diffusion method on Mueller-Hinton agar (HiMedia Laboratories Pvt. Ltd., India) as described [33]. A discrete colony of each isolate was picked using a wire loop and inoculated into 5 ml Mueller-Hinton broth (HiMedia Laboratories Pvt. Ltd., India) and incubated at 37°C for 24 h. The culture was standardized using sterile Mueller-Hinton broth (HiMedia Laboratories Pvt. Ltd., India) to match the turbidity of 0.5 McFarland Standard prepared by mixing 0.5 ml of 1.172% (w/v) $BaCl_2.2H_2O$ (HWATSI Chemicals PVT-Ltd., India) added into 99.5 ml of 1% (w/v) H_2SO_4 (HWATSI Chemicals PVT-Ltd., India). The standardized culture was aseptically streaked on the surface of Mueller-Hinton agar (HiMedia Laboratories Pvt. Ltd., India) plate using sterile swab stick. The culture was allowed to be absorbed within the agar with the Petri dish lid in place for 10 min; and antibiotics discs (Oxoid Ltd., Basingstoke, UK) used in the test were then aseptically placed 15 mm apart on the inoculated agar surface. The discs include: Oxacillin (OX) (1 µg), Sulphamethoxazole/ Trimethoprim (SXT) (25 µg), Erythromycin (E) (30 µg), Rifampicin (RD) (5 µg), Clindamycin (DA) (2 µg), Streptomycin (S) (30 µg), Gentamicin (CN) (30 µg), Ciprofloxacin (CIP) (5 µg), Cefoxitin (FOX) (30 µg) and Levofloxacin (LEV) (5 µg). Within 1 h of applying the disc, the plate was inverted and incubated at 37°C for 24 h. After the 24 h incubation, the diameters of the zone of inhibition for each of the isolates were measured to the nearest millimeter (mm). Inhibition zone diameters obtained were interpreted as Susceptible or Resistant based on the [33] criteria.

2.2.1 Determination of Multiple Antibiotic Resistance (MAR) index

The MAR index of the isolates was determined as described previously [34] using the formula:

$$\text{MAR Index} = \frac{\text{Number of antibiotics isolate is resistant to}}{\text{Number of antibiotics tested}}$$

2.2.2 Classification of antibiotic resistance

Antibiotic resistance in the isolates were classified into: multidrug resistance (MDR: non-susceptible to ≥1 agent in ≥3 antimicrobial categories); extensive drug resistance (XDR: non-susceptible to ≥1 agent in all but ≤2 antimicrobial categories); pan drug resistance (PDR: non-susceptible to all antimicrobial listed) [24].

2.2.3 Molecular detection of quinolone resistance genes

2.2.3.1 DNA extraction

The DNA of ciprofloxacin resistant *S. aureus* isolates were extracted using the boiling method as described [35]. Briefly, following purification, 1 pure colony of isolate was inoculated into 2 ml Luria-Bertani broth (Inqaba Biotec, South Africa) in a Bijou bottle and incubated at 37°C for 8 hours and 200 µl of LB culture was transferred into Eppendorf tube and centrifuged at 3200 rpm for 2 minutes in a microcentrifuge (Model 5417R: Touch plate Super Mixer, CAT. No. 1291, Lab-line Instrument Inc., USA) at room temperature. The supernatant was discarded living the cells and the cells were washed twice with washing buffer. Exactly 0.5 ml of sterile phosphate buffer was added to the pellet and vortex for 5 sec, after which it was heated at 90ºC for 10 min. Rapid cooling was done by transferring the tubes into freezer for 10 min. It was then centrifuge at 3200 rpm for 1 minute to separate the DNA and the cell debris and 300 µl of the supernatant was transferred into 2 ml Eppendorf tube and stored at −10°C prior to usage.

2.2.3.2 DNA amplification of target genes by polymerase chain reaction

The DNA amplification of target genes (*qnr*B, *qnr*S, *aac(6')-Ib-cr*, *gyr*A, *gyr*B and *par*C) in the quinolone resistant *S. aureus* isolates was carried out using the single-plex PCR method as described [36]. Briefly, the PCR processes were carried in 25 µl reaction volume in a reaction tube made up of 5 µl of Master Mix (Qiagen), 2.4 µl of primers (0.4 µl each of forward and backward primers), 0.5 µl of $MgCl_2$, 1.5 µl of DNA template and 15.6 µl of nuclease free water. The primers, gene sequences and amplicon sizes for the quinolone resistance genes used in this study are as shown in Table 1. The reaction tubes were placed in the holes of the Thermo-cycler and closed. Amplification conditions for *qnr*B and *qnr*S genes were: initial denaturation at 94°C for 5 min, followed by 32 cycles of amplification at 94°C for 45 seconds, 53°C for 45 sec, with final extension at 72°C for 5 minutes [37]; amplification conditions for *aac(6')-Ib-cr* were: initial denaturation at 95°C for 5 min, followed by 32 cycles of amplification at 95°C for 20 sec, annealing 59°C for 40 sec and initial extension at 70°C for 30 sec and final extension at 72°C for 5 min [37]; *par*C, *gyr*A and *gyr*B genes were amplified at: initial denaturation at 94°C for 5 min, followed by 35 cycles of amplification at 95°C for 45 sec, annealing at 51°C for 30 sec, initial extension at 72°C for 30 sec and final extension at 72°C for 10 min [38], [39].

2.2.3.3 Agarose gel electrophoresis

The PCR products (8 µl) were evaluated on a 1.5% (w/v) agarose gel (Gibco Life Technologies, Paisley, United Kingdom) at 100 mV for 60 min using BIO-RAD Power Pac 3000; and a molecular weight marker (1-kb DNA Ladder) was used as a standard. The DNA bands were then visualized and photographed under UV light using UVitec and Video copy processor after staining the gel with ethidium bromide (Inqaba Biotec, South Africa) as described [37].

3. RESULTS AND DISCUSSION

3.1 Prevalence of *Staphylococcus aureus*

The cultural, morphological and biochemical characteristics of *S. aureus* isolated from clinical samples of the patients are as shown in Table 2. Golden yellow colonies on mannitol salt agar and Gram positive cocci in clusters which were catalase and coagulase positive amongst other biochemical tests indicated *S. aureus*. The prevalence of *S. aureus* in the clinical samples is as shown in Table 3. From the 240 samples, 51 (21.3%) *S. aureus* were isolated, with the highest isolation rate (45.5%) in eye swab and the lowest (14.5%) in sputum.

3.2 Antibiotic Resistance Profile of the *Staphylococcus aureus* Isolates

The antibiotic resistance profile of the isolates is as shown in Table 4. The highest resistance was to oxacillin (88.2%; 45/51) and the lowest to Levofloxacin (5.9%; 3/51). Resistance was also low to gentamicin (21.6%; 11/51), ciprofloxacin (19.6%; 10/51) and cefoxitin (17.7%; 9/51).

3.3 Antibiotic Resistance Phenotypes of the *Staphylococcus aureus* Isolates

The antibiotic resistant *S. aureus* isolates were distributed into resistance phenotypes as shown in Table 5. The most common phenotypes were SXT-DA-OX-E-RD (13.7%) and SXT-DA-OX-E-S-RD (13.7%).

3.4 Multiple Antibiotic Resistance (MAR) Index

The MAR indices of the isolates are shown in Table 6. All the *S. aureus* isolates were MAR isolates, being resistant to at least two antibiotics tested. The most common MAR index was 0.5 (25.5%); and many (64.7%) of the *S. aureus* isolates had MAR index of 0.5 and above. None of the isolates was found to be resistant to above 8 of the tested agents.

3.5 Classification of Antibiotic Resistance of the Isolates

Antibiotic resistance in the isolates was classified into multiple drug resistance (MDR) (non-susceptible to ≥ 1 agent in ≥ 3 antimicrobial categories), non-multiple drug resistance (NMDR) (non-susceptible to a class of antimicrobial category), extensive-drug resistance (XDR) (non-susceptible to ≥ 1 agent in all ≤ 2 antimicrobial categories) and pan-drug resistance (PDR) (non-susceptible to all antimicrobial categories tested) based on the criteria described [24] is as shown in Table 7. Most (92.2%; 47/51) of the isolates were MDR and none of the isolates were PDR.

3.6 Molecular Detection of Quinolone Resistance Genes

The distribution of the quinolone resistance genes screened for in the ciprofloxacin resistant isolates is as shown in Fig. 1. Of the 10 isolates screened, many harbored *aac (6')-Ib-cr* (60.0%) (Plate 2), *gyr*A (50.0%) (Plate 5), *gyr*B (50.0%) (Plate 6) and *par*C (40.0%) (Plate 4); *qnr*B was detected in 2 isolates (Plate 1); and *qnr*S was detected in only 1 isolate (Plate 3).

Antimicrobial resistance is amongst the most significant health care problems of this era, as it is global and can spread between countries or continents [40]. This study investigated the antimicrobial resistance profile and quinolone resistance genes in *S. aureus* from patients attending Federal Medical Centre, Keffi, Nigeria.

Table 1. Target genes, primers, nucleotide sequences and amplicon sizes for the quinolone resistance genes screened

Target gene	Primer name	Primer sequences	Amplicon size (bp)	References
*gyr*A	gyrA(F)	5'-ATGGCTGAATTACCTCAATC-3'	399	[39]
	gyrA(R)	5'-CATCATAGTTATCGATGAAATC-3'		
*gyr*B	gyrB(F)	5'-TCGGCGACACGGATGACGGC-3'	583	[38]
	gyrB(R)	5'-ATCAGGCCTTCACGCGCATC-3'		
*par*C	parC(F)	5'-ACTTGAAGATGTTTTAGGTGAT-3'	459	[36]
	parC(R)	5'-TTAGGAAATCTTGATGGCAA-3'		
*qnr*B	qnrB(F)	5'-GATCGTGAAAGCCAGAAAGG-3'	469	[37
	qnrB(R)	5'-CGATGCCTGGTAGTTGTCC-3'		
*qnr*S	qnrS(F)	5'-ACGACATTCGTCAACTGCAA-3'	210	[37]
	qnrS(R)	5'-TAAATTGGCACCCTGTAGGC-3'		
aac(6')-Ib-cr	aac(6')(F)	5'-TTGCGATGCTCTATGAGTGGCTA-3'	482	[37]
	aac(6')(R)	5'-CTCGAATGCCTGGCGTGTTT-3'		

Table 2. Cultural, morphological and biochemical characteristics of *staphylococcus aureus* from patients in federal medical centre, Keffi, Nigeria

Cultural characteristics	Morphological Characteristics		Biochemical Characteristics														Inference
	Gram stain	Morphology	Cat	Coa	Vp	Akp	ONPG	Ur	Arg	Man	Su	Lac	Ar	Rf	Tr	Mal	
Golden yellow colonies on MSA	+	Cocci in cluster	+	+	+	+	-	+w	+w	+	+	+	-	-	+	+	*S. aureus*

MSA= Mannitol Salt Agar; Cat= Catalase; Coa= Caogulase; Vp= Voges-Proskauer; Akp= Alkaline phosphatase; ONPG= Ortho-nitrophenyl-β-galactoside; Ur= Urease; Arg= Arginine Utilization; Man= Mannitol; Su= Sucrose; Lac= Lactose; Ar= Arabinose; Rf= Raffinose; Tr= Trehalose; Mal= Maltose; + =Positive; +w= Positive to weak reaction; − =Negative

Table 3. Isolation rates of *Staphylococcus aureus* in relation to clinical samples from patients in Federal Medical Centre, Keffi, Nigeria

Clinical Samples	No. of Samples	No. (%) of *S. aureus*
High vaginal swab (HVS)	49	9 (18.4)
Endocervical swab (ECS)	21	6 (28.6)
Wound swab (W/S)	37	8 (21.6)
Ear swab (E/S)	9	4 (44.4)
Sputum (SP)	83	12 (14.5)
Semen (Sem)	30	7 (23.3)
Eye swab (Eye/S)	11	5 (45.5)
Total	**240**	**51 (21.3)**

Table 4. Antibiotic resistance of *Staphylococcus aureus* isolated from patients in Federal Medical Centre, Keffi, Nigeria

Antibiotics	Disc Content (µg)	No. (%) of Resistance (n= 51)
Rifampicin (RD)	5	36 (70.6)
Clindamycin (DA)	2	32 (62.8)
Erythromycin (E)	15	39 (76.5)
Levofloxacin (LEV)	5	3 (5.9)
Oxacillin (OX)	1	45 (88.2)
Cefoxitin (FOX)	30	9 (17.7)
Sulphamethoxazole/Trimethoprim (SXT)	25	42 (82.4)
Streptomycin (S)	25	23 (45.1)
Gentamicin (CN)	30	11 (21.6)
Ciprofloxacin (CIP)	5	10 (19.6)

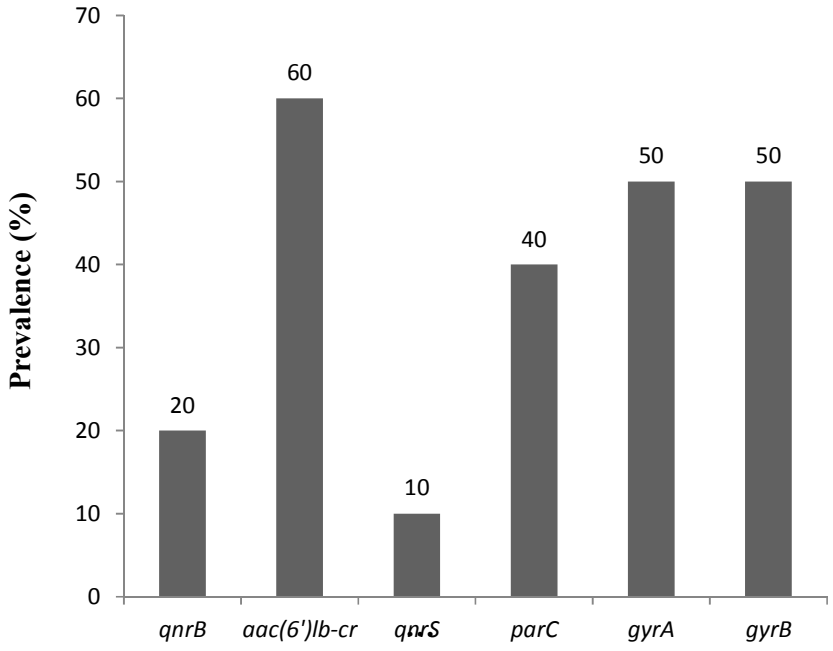

Fig. 1. Occurrence of quinolone resistance genes in ciprofloxacin resistant *Staphylococcus aureus* isolated from patients in Federal Medical Center, Keffi, Nigeria

Table 5. Antibiotic resistance phenotypes of *Staphylococcus aureus* isolated from patients in Federal Medical Centre, Keffi, Nigeria

Antibiotic Resistance Phenotypes	No. (%) Resistance of *S. aureus*
SXT, CIP	1 (2.0)
DA, OX	1 (2.0)
SXT, E	2 (3.9)
SXT, DA, OX	1 (2.0)
CN, SXT, DA	1 (2.0)
SXT, E, RD	1 (2.0)
SXT, OX, RD	2 (3.9)
OX, E, FOX	1 (2.0)
SXT, OX, E	1 (2.0)
SXT, LEV, CIP	1 (2.0)
SXT, DA, OX, RD	2 (3.9)
SXT, OX, S, RD	1 (2.0)
DA, OX, E, RD	1 (2.0)
SXT, OX, E, RD	2 (3.9)
SXT, DA, OX, E, RD	7 (13.7)
DA, OX, E, S, RD	1 (2.0)
SXT, DA, OX, E, S	2 (3.9)
SXT, OX, E, S, RD	2 (3.9)
CN, SXT, OX, E, FOX	1 (2.0)
CN, DA, OX, E, FOX, RD	1 (2.0)
SXT, DA, OX, E, S, RD	7 (13.7)
CN, SXT, OX, E, S, RD	2 (3.9)
CN, SXT, OX, E, LEV, CIP	1 (2.0)
CN, DA, OX, FOX, S, RD	1 (2.0)
CN, DA, OX, E, FOX, CIP, S	1 (2.0)
CN, DA, OX, E, FOX, S, RD	1 (2.0)
CN, SXT, OX, E, LEV, CIP, S	1 (2.0)
CN, DA, OX, FOX, CIP, S, RD	1 (2.0)
SXT, DA, OX, E, CIP, S, RD	2 (3.9)
SXT, DA, OX, E, FOX, CIP, RD	1 (2.0)
SXT, DA, OX, E, FOX, CIP, S, RD	1 (2.0)

RD= Rifampicin; DA= Clindamycin; E= Erythromycin; LEV= Levofloxacin; OX= Oxacillin; FOX= Cefoxitin; SXT= Sulphamethoxazole/trimethoprim; S= Streptomycin; CN= Gentamicin; CIP= Ciprofloxacin

Table 6. Multiple Antibiotic Resistance (MAR) Index of the *Staphylococcus aureus* isolated from patients in Federal Medical Centre, Keffi, Nigeria

No. of Antibiotics Resistant to (a)	No. of Antibiotics Tested (b)	MAR Index (a/b)	No. (%) of isolates
10	10	1.0	0 (0.0)
9	10	0.9	0 (0.0)
8	10	0.8	1 (2.0)
7	10	0.7	7 (13.7)
6	10	0.6	12 (23.5)
5	10	0.5	13 (25.5)
4	10	0.4	6 (11.8)
3	10	0.3	8 (15.7)
2	10	0.2	4 (7.8)

Table 7. Classes of antimicrobial resistance in the *Staphylococcus aureus* isolated from patients in Federal Medical Centre, Keffi, Nigeria

No. of Samples	No. (%) of *S. aureus*	No. (%) Resistance Type			
		NMDR	MDR	XDR	PDR
240	51 (21.3)	0 (0.0)	47 (92.2)	4 (7.8)	0 (0.0)

NMDR = Non-multi-drug resistance (non-susceptible to a class of antimicrobial categories); MDR = Multi-drug resistance (non-susceptible to ≥ 1 agent in ≥ 3 antimicrobial categories); XDR = Extensive drug resistance (non-susceptible to ≥ 1 agent in all but ≤ 2 antimicrobial categories); PDR = Pan drug resistance (non-susceptible to all antimicrobial listed) [24]

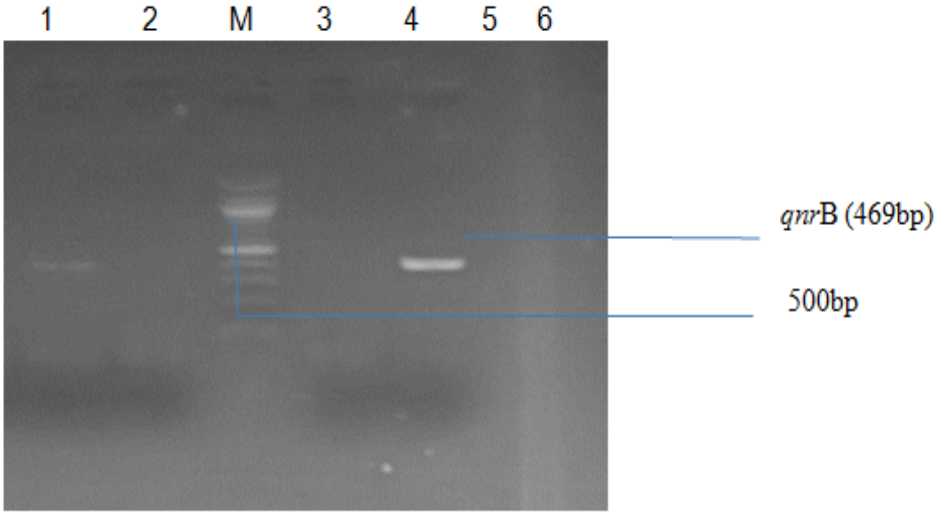

Plate 1. Agarose gel electrophoresis of the amplified *qnr*B gene from the *Staphylococcus aureus* isolates. Lanes 1 and 4 represent the *qnr*B band, Lane M represents the 1500bp molecular ladder, while other lanes show no bands

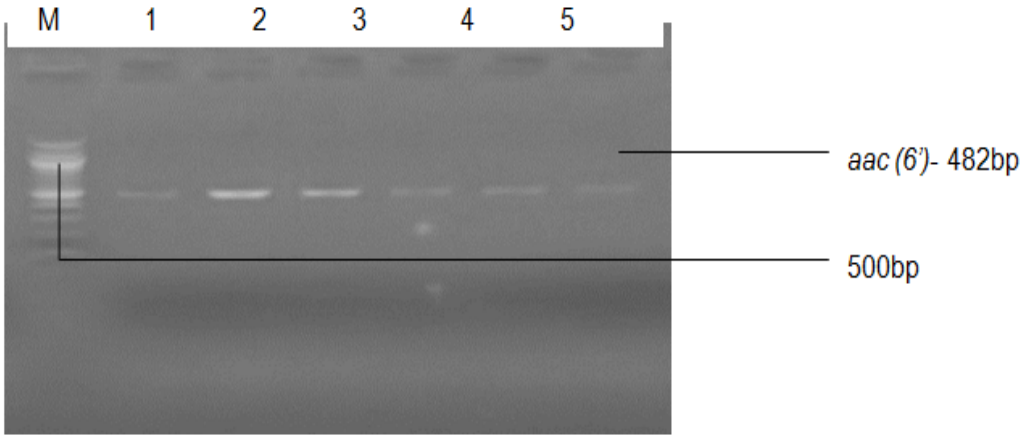

Plate 2. Agarose gel electrophoresis of the amplified *aac (6')-Ib-cr* genes from the *Staphylococcus aureus* isolates. Lanes 1, 2, 3, 4, 5 and 6 represent the *aac (6')-Ib-cr* band, Lane M represents the 1500bp molecular ladder

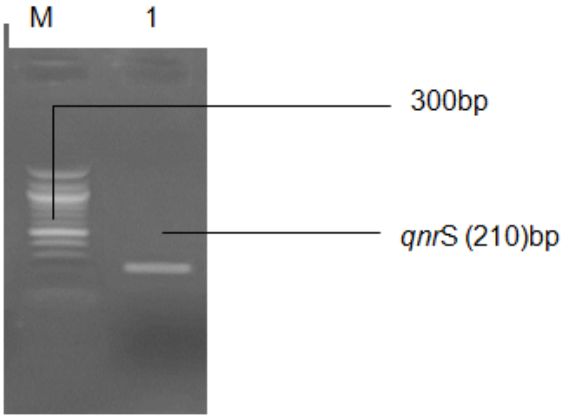

Plate 3. Agarose gel electrophoresis of the amplified *qnr*S gene from the *Staphylococcus aureus* isolates. Lane 1 represent the *qnr*S band, Lane M represents the 1500bp molecular ladder

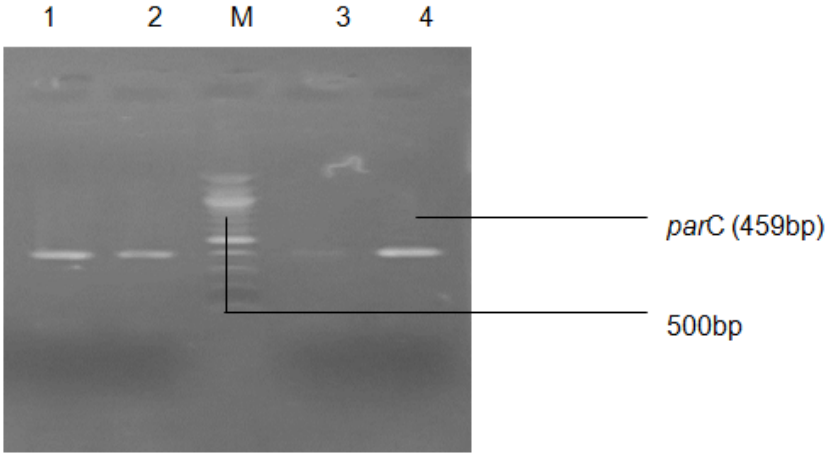

Plate 4. Agarose gel electrophoresis of the amplified *par*C genes from the *Staphylococcus aureus* isolates. Lane 1, 2, 3 and 4 represent the *par*C gene band, Lane M represents the 1500bp molecular ladder

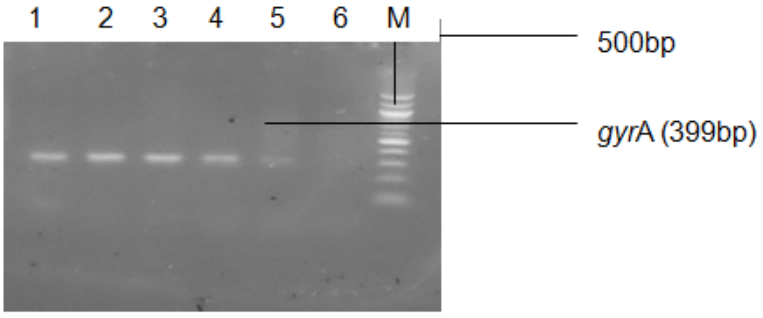

Plate 5. Agarose gel electrophoresis of the amplified *gyr*A genes from the *Staphylococcus aureus* isolates. Lane 1, 2, 3, 4 and 5 represent the *gyr*A gene band, while lane 6 shows no band. Lane M represents the 1500bp molecular ladder

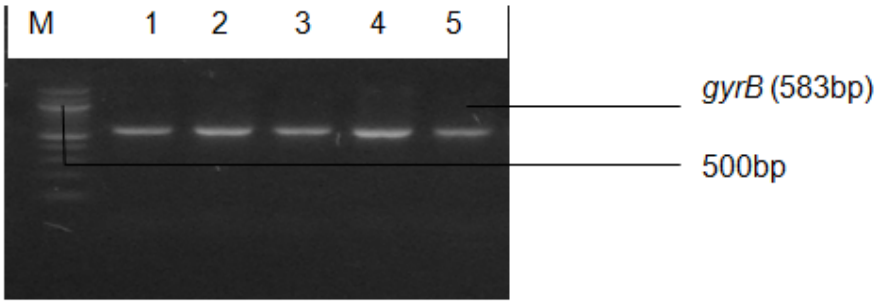

Plate 6. Agarose gel electrophoresis of the amplified *gyr*B genes from the *Staphylococcus aureus* isolates. Lane 1, 2, 3, 4 and 5 represent the *gyr*B gene band, Lane M represents the 1500bp molecular ladder

The observed (21.3%) prevalence of *S. aureus* from all the clinical samples of patients is less than the 33.6% reported [26] from patients in Yenagoa. The observed specimen-related prevalence from eye swabs (45.5%), ear swabs (44.4%) and high vaginal swabs (18.4%) are higher than the 1.8%, 21.1%, 0.9% obtained for corresponding specimens [27] among patients in Southwest Nigeria. The isolation of *S. aureus* from the clinical samples of eye swabs, ear swabs and high vaginal swabs of patients with suspected cases suggested that the organism may likely be responsible for conjunctivitis, otitis media and vaginitis respectively, since *S. aureus* has been reported as one of the bacteria associated with those infections. However, the occurrence of *S. aureus* 21.6% in wound swabs was in close agreement with 22.1% from studies reported [27] and also in agreement that *S. aureus* is also a pathogen associated with wound infections [41].

The observed resistance of the *S. aureus* isolates from our study to commonly used antibiotics is not surprising, as studies in different parts of the world have been widely reported it [8, 19], 20, 21, 22]. Also, the observation that all the isolates have MAR index above 0.2 suggest that the isolates originate from an environment where antibiotics are freely available and misused [42]. The observation from this study that 92.2% of the *S. aureus* isolates were MDR strains is in close agreement with 97.8% MDR *S. aureus* isolates reported [26] from patients in Yenagoa. The isolates were commonly resistant to the antimicrobial agent classes: penicillins, sulphonamides, macrolides, lincosamides, and rifamycins due to the 88.2% MRSA strains which have emerged with concomitant resistance among these common antibiotics as reported [24]. And only a few resistances were observed in the fluoroquinolones (ciprofloxacin and levofloxacin) 19.6% and 5.9% respectively in the test. This observation is similar to that of 10% ciprofloxacin *S. aureus* resistance reported [14] from clinical isolate from nasal infection in Kurdistan-Iran, 9% ciprofloxacin as reported [22] in public hospitals in Ghana and 6.0% levofloxacin resistance as reported [10], generally indicating low resistance to the fluoroquinolone antibiotics. This suggest that these fluoroquinolone antibiotics may not have been misused indiscriminately or abused in those study locations, and thus remains a good drug of choice for the treatment of infections caused by *S. aureus*.

Fluoroquinolone resistant *S. aureus* have also been widely reported globally [3, 8, 14, 21, 36, 43], [44]. The 19.6% (10/51) ciprofloxacin resistance observed in this study is low and less than the 32.6% and 34.8% resistance of *S. aureus* isolates to ciprofloxacin and ofloxacin respectively as reported [26], 58.0% resistance to ciprofloxacin reported [43] in methicillin-resistant *S. aureus* clinical isolates in Cairo, 92.5% and 80.4% resistance of *S. aureus* isolates to ciprofloxacin and ofloxacin respectively reported [3]. All these indicates high resistance of *S. aureus* isolates to the fluoroquinolone antibiotics in those study locations and suggests that they are commonly prescribed for the treatment of infections caused by *S. aureus* and thus implies that those antibiotics may have been misused indiscriminately and abused in those study locations [45].

The detection of both plasmid mediated quinolone resistance (PMQR) genes (*qnr*B, *qnr*S and *aac(6')-Ib-cr*) and chromosomal mediated quinolone resistance (CMQR) genes (gyrA, gyrB,

and parC) in the ciprofloxacin-resistant *S. aureus* isolates is an indication that these genes may be responsible for the observed resistance to ciprofloxacin. The detection of *gyr*A, *gyr*B and *par*C agrees with reports [14, 27, 43]. The occurrence rates of *gyr*A, and *gyr*B were similar and higher than that of *par*C [43] but not so as reported by [44]. The plasmid mediated quinolone resistance genes (*qnr*B, *qnr*S and *aac(6')Ib-cr*) detected in the ciprofloxacin-resistant *S. aureus* isolates in the study location have been reported to be commonly associated with quinolone resistance in members of Enterobacteriaceae such as *E. coli*, *Klebsiella* spp, *Citrobacter* spp, *Salmonella* spp, amongst other bacteria [16,37,46]. The resistance observed may be as a result of the spread of resistance gene elements among the bacteria via inheritance and horizontal transfer of antimicrobial resistance genes (ARGs) [16,47].

Although, the worldwide prevalence of antimicrobial resistance cannot be ascertained, constant surveillance is most pertinent in order to aid in optimizing the therapeutic management of patients with infections [48,49].

4. CONCLUSION

The prevalence of *S. aureus* from clinical samples of patients was relatively low and the isolates were less resistant to ciprofloxacin and levofloxacin. In view of this low resistance observed, such antibiotics may be useful for the treatment of infections caused by *S. aureus* in the study location. In addition, plasmid mediated quinolone resistance (PMQR) genes (*qnr*B, *qnr*S and *aac(6')-Ib-cr*) and chromosomal mediated quinolone resistance (CMQR) genes (*gyr*A, *gyr*B, and *par*C) were detected in the ciprofloxacin-resistant *S. aureus* isolates, with a higher detection rate for *aac(6')-Ib-cr*.

CONSENT

All authors declare that 'written informed consent was obtained from the patients

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The ethical approval for this study was obtained from the ethical committee of the Federal Medical Centre, Keffi, Nigeria.

Ethical clearance (nhrec/21/12/2012) was obtained after due submission and defense of the research protocol before the health research ethics committee of the federal medical centre, Keffi, Nigeria. The study was carried out in a manner that assured of confidentiality of the participants.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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