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Prevalence of *qnr* Genes among Multidrug Resistance *Staphylococcus aureus* from Clinical Isolates

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Authors' contributions

This work was carried out in collaboration between both authors. Author TYM designed the study, while author ABA wrote the protocol, the first draft of the manuscript and together with author TYM performed the statistical analysis. Both authors ABA and TYM managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Objectives: *Staphylococcus aureus* (*S. aureus*) is regarded as an important aetiological agent of various human infections. Fluoroquinolones are routinely used in the chemotherapeutic management of these infections; nonetheless, in recent years, a growing rate of resistance to these drugs has been reported worldwide. The aims of this study were to isolate and discover the prevalence of plasmid-mediated (*qnrA, qnrB*, and *qnrS*) genes among the quinolone-resistant clinical *S. aureus* isolates in Bayelsa State, Nigeria.
Methods: A total of 25 (31.25%) clinical isolates of *S. aureus* were collected from hospitalized patients. The bacterial isolates were identified through standard laboratory protocols and further confirmed using the API Staph system (bioMérieux, France) test strips. The antimicrobial susceptibility and minimum inhibitory concentration (MIC) were determined by the standard disk

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diffusion and serial dilutions methods respectively. Polymerase chain reaction (PCR) was used for detecting *qnrA, qnrB*, and *qnrS* genes.

Results: Of the 25 *S. aureus* isolates, 19(76.00%) were resistant to ampicillin-cloxacillin, while 14 (56.00%) each were resistant to norfloxacin and Amoxicillin, 13 (52.00%) each to gentamicin and erythromycin, 11 (44.00%) were resistant to streptomycin, rifampicin and ciprofloxacin, respectively. The resistance pattern among the isolates to chloramphenicol and levofloxacin were 10 (40.00%) and 7 (28.00%) respectively. All the eleven ciprofloxacin resistant were high-level (1000 µg/mL) resistance isolates and only one (9.00%) of these isolates was positive for the *qnrB* gene.

Conclusion: The study results were indicative of the presence of low frequency of *qnr* genes among the clinical isolates of *S. aureus* in Yenagoa, indicating that other mechanisms are employed in resisting to these fluoroquinolones. This, however, emphasizes the need for establishing discreet policies associated with infection-control measures in hospital settings.

Keywords: Staphylococcus aureus; clinical isolates; polymerase chain reaction; multidrug resistance; qnr genes.

ABBREVIATIONS

AMP : Ampicillin-cloxacillin,
AMO : Amoxicillin,
NOR : Norfloxacin,
CHL : Chloramphenicol,
CIP : Ciprofloxacin,
ERY : Erythromycin,
GEN : Gentamicin,
LEV : Levofloxacin,
RIF : Rifampicin,
STR : Streptomycin

1. INTRODUCTION

Historically, *Staphylococcus aureus* (*S. aureus*) (including drug-resistant strains, such as methicillin-resistant *S. aureus*; MRSA) are habitat of the skin and mucous membranes, and humans are considered the major reservoir for these organisms [1,2,3,4]. It is estimated that up to half of all adults are colonized, and approximately 15% of the population persistently carries *S. aureus* in the anterior nares [5,6,7]. Some populations such as healthcare personnel, persons who use needles on a regular basis (i.e., diabetics and intravenous (IV) drug users), hospitalized patients, and immunocompromised individuals tend to have higher rates of *S. aureus* colonization (up to 80%). *S. aureus* can be transmitted from person-to-person by direct contact or by fomites [8,9,10]. In addition to humans and domestic animals, livestock and fomites may also serve as supplement reservoirs, giving this bacterial pathogen dramatic relevance in veterinary medicine [9,11,12].

Despite the close association between *S. aureus* and humans, *S. aureus* has been regarded as a major human pathogen and continues to be one of the most commonly implicated Gram-positive bacteria causing human disease throughout the world [10,13]. *S. aureus* are the causative agents of multiple human infections, including bacteremia, infective endocarditis, skin and soft tissue infections (e.g., impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome, and others), osteomyelitis, septic arthritis, prosthetic device infections, pulmonary infections (e.g., pneumonia and empyema), gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections [2,8] Depending on the strains involved and the site of infection, these bacteria can cause invasive infections and/or toxin-mediated diseases [8,14].

Management of *S. aureus* infections therapeutically largely depends on the type of infection as well as the presence or absence of drug resistant strains and when antimicrobial therapy is desired, the duration and mode of therapy are largely dependent on the infection type as well as other factors [8].

The emergence of various isolates of the drug-resistant *S. aureus* occurs due to the inappropriate and unnecessary administration of these antibiotics leading to limiting the treatment options. A situation leading to the introduction of Fluoroquinolones in the therapeutic regimes. The Fluoroquinolones as documented are synthetic chemotherapeutic agents with wide and effective antibacterial activity against gram-negative and -positive organisms and have been used against various bacterial infections. The current members of this compounds have the greatest potency against aerobic gram-negative pathogens and less activity against gram-positive

pathogens, such as *Staphylococcus aureus*, has become a problem [15,16]. Despite the potential activity of these groups, it is bothersome that within a relatively brief period after their introduction into clinical practice, rising levels of resistance to these antimicrobial agents were noted in some organisms [17,18]. Serious infections caused by these resistant organisms have been associated with considerable morbidity and mortality [19].

Resistance to fluoroquinolones typically arises as a result of alterations in the target enzymes (DNA gyrase and topoisomerase IV) and of changes in drug entry and efflux. Mutations are selected first in the more susceptible target: DNA gyrase, in Gram-negative bacteria, or topoisomerase IV, in Gram-positive bacteria [20]. Additional mutations in the next most susceptible target, as well as in genes controlling drug accumulation, augment resistance further, so that the most-resistant isolates have mutations in several genes. Resistance to quinolones can also be mediated by plasmids that produce the Qnr protein, which protects the quinolone targets from inhibition. As reported by Kim et al. [21] and Minarini et al. [22], the three major groups of qnr determinants are *qnrA*, *qnrB*, and *qnrS*.

Evidently, the broad emergence of the growing trend associated with the prevalence of plasmid resistance in *S. aureus* isolates is irrefutable, however, only a limited number of studies, if any of Nigerian origin is about the prevalence of *qnr* gene among clinical isolates *S. aureus* has been reported. Consequently, warranting this study in investigating the prevalence of MDR and the presence of *qnr* genes among *S. aureus* isolated from clinical specimens of patients attending tertiary healthcare facilities in Yenagoa, South-southern region, Nigeria.

2. MATERIALS AND METHODS

2.1 Population Study

After obtaining Ethical clearance, we explained the reason for the study to the participants (patients), and asked them for their consent. After providing informed consent, specimens were collected from one hundred and forty-six (146) patients. The specimens collected were: Endocervical swab (21), High vaginal swab (19), Sputum (22), Throat swab (4), Urethral swab (5), Urine (61), and Wound swab (14). The mean age of patients was 30 ± 18.71 years (range, 1-60 years). Forty-eight percent were males, and 52% were females.

2.2 Specimen Collection, Isolation, and Identification

Specimens for Endocervical, high vaginal, throat, urethra, and swab were obtained with sterile cotton swabs moistened with sterile saline; while the mid-stream clean catch urine and sputum samples were collected into a sterile wide mouth universal (Sterilin, England) container. The samples were transported to the Laboratory under ice.

In the Laboratory, Soy Trypticase broth (Oxoid, England) was inoculated with the swab samples; after overnight incubation at 37°C, the broth was subcultured onto 5% blood agar plates and phenol red mannitol salt agar (Oxoid, England) and incubated aerobically at 37°C for 18 to 24 h. Mannitol fermentation-positive isolates were further analysed. Haemolysis was scored as positive if a clear zone of beta-hemolysis was observed on blood agar. The isolates were stored at −84°C in Trypticase soy broth containing 20% glycerol and subcultured prior to testing.

Standard microbial protocols as described by Hamdan-Partida et al. [5] was employed in biochemical identification of isolates, Single colony from pure cultures were subjected to Gram staining reaction, catalase test, and tube coagulase test. The biochemical properties were further determined using the API *Staph* system (Biomérieux, France). *S. aureus* NCTC6571 and *Staphylococcus epidermidis* ATCC 14990 were used as positive and negative controls, respectively.

2.3 Antimicrobial Susceptibility Testing

The Kirby–Bauer disk diffusion method was performed according to the Clinical Laboratory Standards Institute guidelines [23]. All isolates were screened for resistance to ten (10) different antibiotics: ampicillin-cloxacillin (30 µg), amoxycillin (25 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (30 µg), levofloxacin (5 µg), norfloxacin (5 µg), rifampicin (5 µg), and streptomycin (10 µg) (Oxoid, England). MIC for fluoroquinolones (ciprofloxacin, levofloxacin and norfloxacin) were determined according to the CLSI publication M100 [23].

2.4 DNA Extraction and Detection of *qnr*-Encoding Genes

The detection of *qnrA*, *qnrB*, and *qnrS* plasmid-mediated quinolone-resistance genes was performed using polymerase chain reaction (PCR) and specific primers (Table 1) [24]. Plasmid DNA was extracted by boiling [25]. PCR amplifications were performed in a thermocycler (Gene Amp PCR System 9700, Applied Biosystems, USA) as follows: 94°C for 5 minutes and 35 cycles of 5 minutes at 72°C, 1 minute at specific annealing temperature for each primer, and 1 minute at 72°C. A final extension step of 10 minutes at 72°C was performed. Amplification reactions were prepared in a total volume of 25 µL (24 µL of PCR master mix plus 1µL of template DNA) including 5 ng of genomic DNA, 2.0 U of Taq DNA polymerase (Fermentas, Vilnius, Lithuania), 10 mM deoxyribose nucleoside triphosphate mix at a final concentration of 0.2 mM, 50 mM $MgCl_2$ at a final concentration of 1.5 mM, 1 µM of each primer, and 1×PCR buffer (final concentration). PCR products were electrophoresed on 1.5% agarose gel and stained with ethidium bromide solution and finally visualised in gel documentation system (UVItec Limited, Cambridge, UK).

2.5 Statistical Analysis

Statistical data analysis was performed for descriptive statistics including frequencies, demographic characteristics and chi-square independent test for $P < 0.05$ using the computer software program SPSS version 20 (SPSS Inc., Chicago, IL, USA).

3. RESULTS

In total, 25(31.25%) *S. aureus* strains were isolated and identified in this study. Fig. 1, shows the rate of recovery of *S. aureus* from the specimens. As shown, *S. aureus* was more frequently isolated among the urine specimens with 8 (32.00%). This is followed by isolates from the wound, Endocervical, and sputum with 6(24.00%), 5(20.00%), and 4 (16.00%) respectively. In addition, the recovery rate of *S aureus* from the throat and urethral was 1 (4.00%) each, while no isolate was recovered from the higher vaginal swabs.

Table 1. Primers for detection of *qnr A, qnrB* and *qnrS* genes in *S. aureus*

Target Gene	Primer	Sequence (5′-3′)	Length of product (bp)
qnrA	*qnrA*	F: 5ˈGATAAAGTTTTTCAGCAAGAGG R: 5ˈATCCAGATCCGCAAAGGTTA	628
qnrB	*qnrB*	F:5ˈATGACGCCATTACTGTATAA R: 5ˈGATCGCAATGTGTGAAGTTT	408
qnrS	*qnrS*	F: 5ˈATGGAAACCTACAATCATAC R: 5ˈAAAAACACCTCGACTTAAGT	428

Whichard et al. [23]

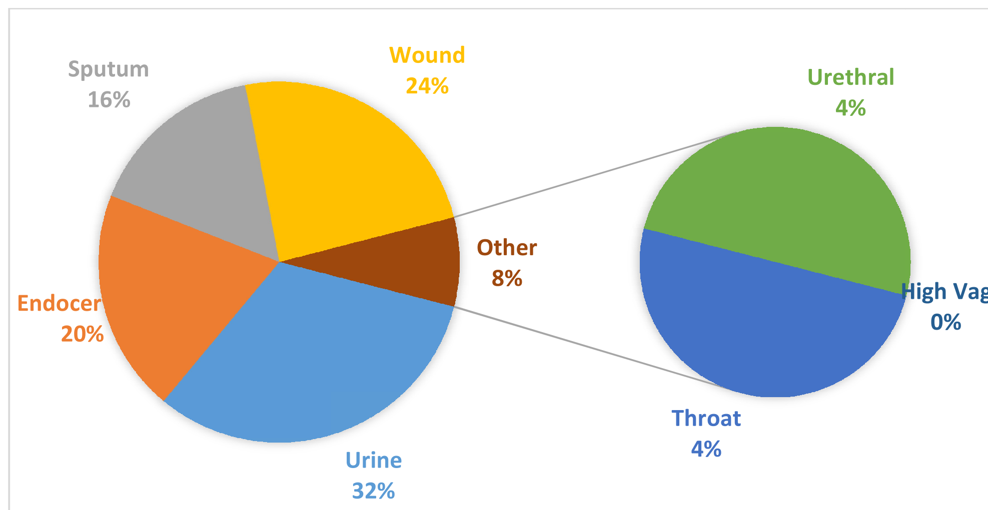
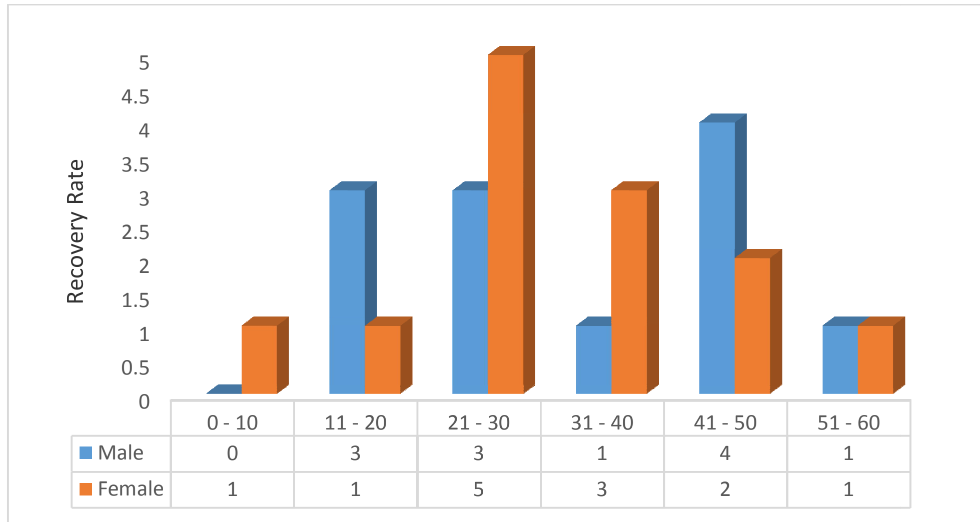


Fig. 1. Recovery rate of *Staphylococcus aureus* from specimens

Fig. 2 shows the age and gender-wise distribution of the isolated *S. aureus*. As revealed, *S. aureus* was more common amongst the age group 21-30 with 32% (20% females, 12% males), closely, this is followed by the age group 41-50 with 24% (8% females, 16% males), while age grouped 11-20, 31-40 were with 16% (4%:12% females, 12%:4% males) each, while the distribution rate amongst the age group 51-60 and 0-10 years had 8% (4% females, 4% males) and 4% (4% females, 0% males), respectively.

As highlighted in Fig. 3, the antimicrobial susceptibility rates of *S. aureus* isolated strains were ampicillin-cloxacillin 19(76.00%), amoxicillin 14(56.00%), norfloxacin 14(56.00%), erythromycin 13(52.00%), ciprofloxacin 11(44.00%), rifampicin 11(44.00%), streptomycin 11(44.00%) and chloramphenicol 10(40.00%) resistant. Resistance was ≤28% for levofloxacin 7(28.00%). Among the isolates, 14(56.00%), 11(44.00%) and 7(28.00%) strains were resistant to fluoroquinolones: norfloxacin, ciprofloxacin and levofloxacin, respectively. Fifty-six percent of the *S. aureus* isolates were multidrug resistant strains. All the eleven (100%) ciprofloxacin resistant *S. aureus* isolates had high MIC ≥1000 μg/mL. PCR showed that only 1(9.09%) *S. aureus* isolate carried *qnrB* gene. The *qnrA* and *qnrS* genes were not found among the clinical isolates of this study (Fig. 4).



	0 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 60
Male	0	3	3	1	4	1
Female	1	1	5	3	2	1

Fig. 2. Distribution of *Staphylococcus aureus* by age and gender-wise

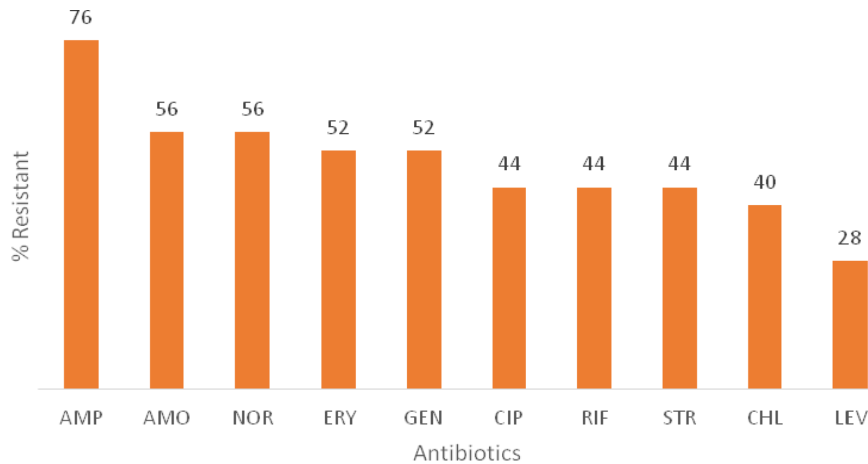


Fig. 3. Antimicrobial susceptibility of *S. aureus* from clinical isolates

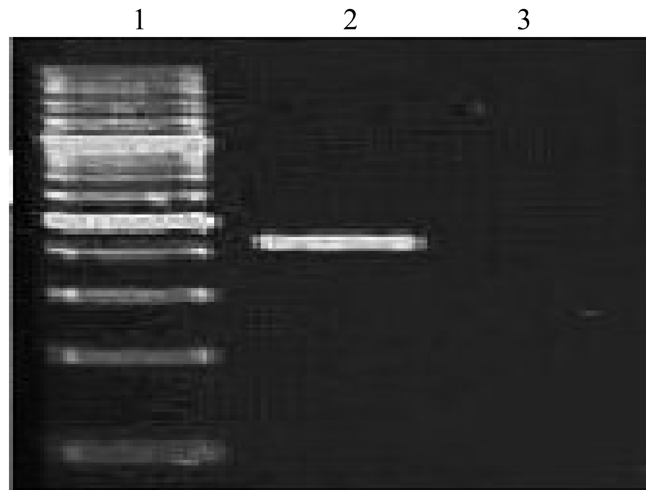


Fig. 4. Gel documentation of polymerase chain reaction assay for qnr gene
Lane 1: Molecular mass marker (1000 bp deoxyribonucleic acid ladder), Lane 2: Positive (amplicon size – 408 bp), Lane 3: Negative control

4. DISCUSSION

The pathological relevance of MDR *S. aureus* has increased the importance of screening for this pathogen among clinical isolates. Screening to search for demonstrating the importance of *S. aureus* as the common isolated human bacteria pathogens in both community and hospital base infections has been well documented [26,27,28,29,30,31,32].

This study showed a high level of resistance against Ampicillin-cloxacillin (76%), amoxicillin and norfloxacin (56%) each, erythromycin and gentamicin (52%) each among *S. aureus* isolates recovered from the patients.

Among the 25 isolates, resistances to fluoroquinolones were as demonstrated: 14(56%) strains were resistant to norfloxacin, 11(44%) to ciprofloxacin and the least of 7(28%) strains were resistant to levofloxacin.

Diekema et al. [32] showed that 89.5%, 88.6%, and 60.5% of clinical *S. aureus* isolates were resistant to ciprofloxacin in Europe, USA and Canada respectively. On the other hand, the resistance of *S. aureus* isolates from infections was 92.5% to ciprofloxacin in an India study [33], while in Pakistan, the prevalence of ciprofloxacin resistance among *S. aureus* isolates was over 90% [34]. Beforehand, Mehta et al. [35] had reported a steady increase in resistance to ciprofloxacin from 39% in 1992 to 68% in 1996. The implication for such increase in resistance in healthcare units as observed in this study and others is that ciprofloxacin may not be useful as a first-line antibiotic. As such, there will be need for a full review of hospital management and further evaluation of monitoring systems. It has been reported that ciprofloxacin resistant *S. aureus* isolates tend to show increased resistance to other antibiotics, including aminoglycosides [36,37].

With the recovery rate of 9.09%, this study showed a low prevalence rate of plasmid-mediated quinolone resistance (*qnrS*) when compared with other studies [38,39]. We did not find the *qnrA* and *qnrB* genes in our clinical isolates. This outcome indicates that the frequency of *qnrS* genes in our study was higher than the frequency of *qnrA* and *qnrB* genes, hence, corroborating earlier studies reporting higher frequency of *qnrS* among *S. aureus* isolates. This rate of *qnrS* is lower than those found in earlier study previously conducted among clinical isolates in same Bayelsa by Alade et al. [38], however the none recovery of *qnrA* and *qnrB* is same with the present findings, the implication of this indicates that *QnrS* is considered as one mechanism of quinolones resistance.

In another study, a low frequency for *qnr* gene isolation was also reported among *E. coli* by Pereira et al. [40] in Brazil. Furthermore, currently, resistance against quinolones and *qn*r genes has increased in many parts of the world including places like in Iran among *E. coli* [41],

China among *Shigella* [42], Togo among *Klebsiella* spp., [43]; *Salmonella* isolates [44], and in Hungary among *E.* coli, Klebsiella, Citrobacter, Enterobacter, and Serratia species [45,46].

In this study, *qnrS*-positive isolates showed high-level resistance, however other mechanisms such as secondary changes in DNA gyrase or topoisomerase IV, and porin or efflux systems, which was not evaluated in this present study have also been documented as an alternative mechanism deployed by *S. aureus* and other gram-positive bacteria in establishing resistance against quinolones [47,48,49,50,51,52,53,54, 55,56,57]. We are of the opinion that our isolated clinical *S. aureus* could be deploying those mechanisms other than *qnrS* to acquire resistance to the quinolones.

5. CONCLUSION

In conclusion, our finding showed high frequencies of fluoroquinolone resistances but low qnr genes among *S. aureus* isolated from patients in Federal Medical Centre hospital in Yenagoa, Bayelsa state, Nigeria. The circulation of strains of *S. aureus* with a resistance plasmid gene can be considered as a risk for the spread of these types of genes among other bacteria, which requires special considerations. Correspondingly, the appropriate use of antibiotics may be useful to limit the potential spread of these resistant genes.

6. LIMITATION OF STUDY

The study could not determine if the isolated *S. aureus* is Methicillin Resistance (MRSA) due to the unavailability of cefoxitin disks.

CONSENT AND ETHICAL APPROVAL

The Research and Ethical Committee of The Federal Medical Centre Yenagoa approved the study, (Ref. No. FMC/REC/19/018). Informed consent was obtained from all individual that partook in the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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