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Original Article **Open Access**

Emergence of clinical *vanA*-type vancomycin-resistant *Staphylococcus aureus* isolates in National Orthopaedic Hospital Dala, Kano, Nigeria

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Abstract:

Background: The increasing prevalence of multi drug resistance (MDR) in strains of *Staphylococcus aureus* is a major challenge in the selection of an appropriate therapeutic agents, especially in persistent orthopaedic infections. This study investigated the patterns of antimicrobial resistance and identified the genetic determinants of resistance in *S. aureus* isolates from orthopaedic patients.
Methodology: This was a descriptive cross-sectional study of hospitalized patients at National Orthopaedic Hospital Dala (NOHD), Kano, Nigeria from whom urine samples, and nasal and wound swabs were collected for isolation of *S. aureus*. Samples were cultured on standard media and *S. aureus* isolated and identified using both conventional biochemical tests and a standard rapid diagnostic kit. The antibiotic susceptibility was determined to a panel of 15 antibiotics using the modified Kirby-Bauer disc diffusion method. Vancomycin minimum inhibitory concentration (MIC) of each isolate was determined using vancomycin Epsilon-test strip. *mecA* and *vanA* were detected by multiplex polymerase chain reaction (PCR) assay.
Results: From the total of 134 samples, *S. aureus* was isolated from 36 (26.8%); 10 (7.4%) from urine, 13 (9.7%) from nasal swab, and 13 (9.7%) from wound swab. Thirty-four (94.4%) isolates were phenotypically methicillin (cefoxitin) resistant (MRSA), while 2 (5.6%) isolates were methicillin sensitive (MSSA). Phenotypic resistance rate of the *S. aureus* isolates was highest to gentamicin (94.4%), followed by penicillin (88.8%), cephalosporins and fluoroquinolones (87.4%), while rate was lowest to vancomycin (11.1%, 4/36). Seventeen (47.2%) were MDR, 16 (44.4%) were extensively drug resistant (XDR), and 2 (5.6%) were pan-drug resistant (PDR) *S. aureus* isolates. The *mecA* gene was detected in 4 (11.8%) of the 34 phenotypic MRSA isolates and *vanA* genes in 2 (50.0%) of the 4 phenotypic VRSA isolates.
Conclusion: The detection of *vanA* and *mecA* in clinical *S. aureus* isolates in this study is an indication that clinical VRSA has emerged in MRSA population in Nigeria. This emergence can pose a major threat to primary care-givers and a public health challenge among the daily inhabitants of National Orthopaedic Hospital Dala (NOHD), Kano and the community at large.

Keywords: *Staphylococcus aureus*, *vanA*, *mecA*, MDR, XDR, PDR, orthopaedic

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Émergence d'isolats cliniques de *Staphylococcus aureus* résistants à la vancomycine de type-*vanA* à l'Hôpital National d'Orthopédie de Dala, Kano, Nigeria

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Résumé:

Contexte: La prévalence croissante de la multirésistance aux médicaments (MDR) dans les souches de *Staphylococcus aureus* est un défi majeur dans la sélection d'agents thérapeutiques appropriés, en particulier dans les infections orthopédiques persistantes. Cette étude a examiné les modèles de résistance aux antimicrobiens et identifié les déterminants génétiques de la résistance dans les isolats de S. aureus provenant de patients orthopédiques.
Méthodologie: Il s'agissait d'une étude transversale descriptive de patients hospitalisés à l'Hôpital National Orthopédique de Dala (NOHD), à Kano, au Nigeria, auprès desquels des échantillons d'urine et des écouvillons nasaux et de plaies ont été prélevés pour l'isolement de *S. aureus*. Les échantillons ont été cultivés sur des milieux standard et S. aureus a été isolé et identifié à l'aide d'un test biochimique conventionnel et d'un kit de diagnostic rapide standard. La sensibilité aux antibiotiques a été déterminée sur un panel de 15 antibiotiques en utilisant la méthode de diffusion sur disque de Kirby-Bauer modifiée. La concentration minimale inhibitrice (CMI) de vancomycine de chaque isolat a été déterminée à l'aide d'une bandelette de test de vancomycine Epsilon. *mecA* et *vanA* ont été détectés par un test de réaction en chaîne par polymérase (PCR) multiplex.
Résultats: Sur un total de 134 échantillons, *S. aureus* a été isolé à partir de 36 (26,8%); 10 (7,4%) d'urine, 13 (9,7%) d'écouvillonnage nasal et 13 (9,7%) d'écouvillonnage de plaie. Trente-quatre (94,4%) isolats étaient phénotypiquement résistants à la méthicilline (céfoxitine) (SARM), tandis que 2 (5,6%) isolats étaient sensibles à la méthicilline (MSSA). Le taux de résistance phénotypique des isolats de *S. aureus* était le plus élevé à la gentamicine (94,4%), suivie de la pénicilline (88,8%), des céphalosporines et des fluoroquinolones (87,4%), tandis que le taux était le plus faible à la vancomycine (11,1%, 4/36). Dix-sept (47,2%) étaient multirésistants, 16 (44,4%) étaient extrêmement résistants aux médicaments (XDR) et 2 (5,6%) étaient des isolats de *S. aureus* pan résistants aux médicaments (PDR). Le gène *mecA* a été détecté dans 4 (11,8%) des 34 isolats phénotypiques de SARM et les gènes *vanA* dans 2 (50,0%) des 4 isolats phénotypiques de VRSA.
Conclusion: La détection de *vanA* et *mecA* dans les isolats cliniques de *S. aureus* dans cette étude est une indication que le VRSA clinique est apparu dans la population de SARM au Nigeria. Cette émergence peut constituer une menace majeure pour les dispensateurs de soins primaires et un défi de santé publique parmi les habitants quotidiens de l'hôpital national d'orthopédie de Dala (NOHD), de Kano et de la communauté dans son ensemble.

Mots clés: *Staphylococcus aureus*, *vanA*, *mecA*, MDR, XDR, PDR, orthopédique

Introduction:

Clinically significant bacterial isolates, particularly *S. aureus*, are progressively becoming resistant to almost all antibiotics currently on the market, making them practically ticking time bombs in the near future unless an alternative therapeutic method is offered. This situation poses a threat to antibiotics of last resort. *Staphylococcus aureus* is a member of the human microbiota that has been linked to a variety of illnesses, including mild skin and soft tissue infections (SSTIs) and severe, life-threatening conditions such as infective endocarditis, surgical site infection (SSI), periprosthetic joint infections (PJI), osteomyelitis, and toxic shock syndrome (TSS), in both healthy people and in those with underlying illnesses (1,2).

Despite the difference in the hospital mortality rates for methicillin-susceptible *S. aureus* (MSSA) and the methicillin-resistant *S. aureus* (MRSA) (3), infections caused by MRSA has steadily been on the rise worldwide, resulting in increase consumption of vancomycin (4). The antibiotic 'selective pressure' among overcrowded inhabitants creates an environment that is suitable for the rapid development and efficient spread of numerous multidrug-resistant (MDR) pathogens in both the community and hospital settings (5). This has remained a huge challenge for healthcare professionals.

In the past ten years, there has been an exponential rise in the prevalence of vancomycin-resistant *S. aureus* (VRSA), vancomycin intermediate *S. aureus* (VISA), and heterogeneous vancomycin intermediate *S. aureus* (hVISA) infections. This is supported by *invitro* research that points to multiple vancomycin resistance mechanisms in MRSA, the main ones being decreased permeability and increased cell wall thickness, which reduce vancomycin availability for intracellular target molecules (1). Another type of resistance was brought on by plasmid-mediated vancomycin resistance genes (*vanA*, *vanB*, *vanD*, *vanE*, *vanG* and *vanL*), which may have been acquired from enterococci species (1).

The risk of surgical infections, morbidity, mortality, and financial burden for orthopaedic patients are all known to be greatly increased by MSSA/MRSA colonization and admission into healthcare institutions (6,7). Vancomycin is still regarded as the most effective therapeutic agent against infections caused by MRSA, but its prolonged and extensive usage, dependence on haemodialysis, intensive care units (ICU), and use in patients with indwelling devices have led to the rise of VRSA infections (8).

High frequency of clinical VRSA infec-

tions have been reported in the American continent (1), and the molecular mechanism of resistance in these VRSA strains has been well characterized. In order to establish the hypothesis that "VRSA may emerge more frequently than expected in Nigeria", many researchers have investigated and determines its prevalence using phenotypic methods, with many reporting culture isolations of clinical VISA and VRSA among patients with different infective conditions (9-12). However, only few studies have used molecular methods to investigate the occurrence of VISA and VRSA in Nigeria and none has detected *van* genes in any *S. aureus* isolate (13). The objective of this study is to investigate the patterns of antimicrobial resistance in clinical *S. aureus* isolates from National Orthopaedic Hospital Dala, Nigeria, and identify the genetic determinants of MRSA and VRSA using multiplex PCR approach.

Materials and method:

Study setting and design:

The study setting is National Orthopaedic Hospital Dala (NOHD), Kano located in the north-western region of Nigeria but also a referral hospital for orthopaedic patients from the north-west, north-east, and north-central regions of the country. The hospital has 9 wards and an emergency unit, which can accommodate nearly 2500 inpatient admissions and more than 9000 outpatients per year.

The study design is descriptive cross-sectional conducted between September 2018 and March 2019 on all hospitalized patients in the hospital while excluding patients who declined to participate in the study.

Ethical approval:

The hospital research ethics committee granted permission to conduct the study (NOHD/RET/ETHIC/60). In addition, informed consent of each patient who participated in the study was obtained.

Sample collection, isolation and identification:

Urine sample, nasal and wound swabs, were collected from a total of 134 hospitalized patients according to standard procedures. The specimens were inoculated onto nutrient broth and mannitol salt agar, and incubated aerobically at 37^{o}C for 24 hours.

Suspected staphylococcal colonies on the culture plates were preliminary identified by conventional method of Gram stain, catalase and coagulase tests (Staphytect Plus Test Kit). Standard rapid diagnostic kit (Microgen™ Staph-ID System) and the software probability test results were used to conclusively identify each isolate as *S. aureus*.

Determination of antibiotic susceptibility:

Antibiotic susceptibility test (AST) was performed on pure cultures of the *S. aureus* isolates using the modified Kirby-Bauer disc diffusion method (14). Recommended classes of antibiotics including benzylpenicillin (1µg), amoxicillin-clavulanic acid (30µg), oxacillin (1 µg), cefoxitin (30µg), ceftriaxone (30µg), norfloxacin (10µg) ciprofloxacin (5µg), gentamicin (10µg), erythromycin (15µg), clindamycin (15µg), quinupristin-dalfopristin (15µg), tetracycline (30µg), linezolid (10µg) and trimethoprim-sulfamethoxazole (25µg) discs were used. Vancomycin E-test strip was used to determine vancomycin minimum inhibitory concentration (MIC).

The discs and strips were aseptically placed using sterile forceps on Muller Hinton agar plates that have been inoculated with standardized inoculum of pure colonies of *S. aureus* isolates, and incubated at 37°C for 24 hours. The plates were examined for the presence or absence of zones of inhibition of bacterial growth, and the inhibition zone diameters were interpreted as sensitive or resistance in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guideline (15).

DNA extraction and PCR amplification:

DNA extraction was performed on *S. aureus* isolates that phenotypically resistant to antibiotic discs. A typical isolate was cultivated in 5ml Luria-Bertani (LB) for 24 hours at 37°C. The genomic DNA (gDNA) of the isolates was extracted with a ZR Fungal/Bacterial DNA Mini Prep™ (USA) using the protocol described by the manufacturer (16).

Amplification of the antibiotic-resistant *vanA* and *mecA* genes was performed in a PCR thermal cycler (Bio-Rad DNA Machine) after an external optimization of the reaction to ensure amplification with specific *vanA* and *mecA* primers (17,18) (Table 1). The PCR master mix contained 1.0 µl each of forward and reverse primers, 1 x PCR buffer, 1.5 mM $MgCl_2$, 0.15 mmol/L dNTP, 1.25 IU Taq DNA polymerase, and 1 L of prepared DNA (0.5g) template, which was added to the final volume. The cycling conditions (denaturation, annealing, and extension) were as previously described by Pournajaf et al., (19).

Results:

Of the total of 134 clinical samples cultured, 78 (58.2%) yielded growth of Staphylococcus isolates. *Staphylococcus aureus* was isolated from 36 (27.6%), *Staphylococcus hyicus* from 1 (0.7%), and coagulase negative staphylococci (CoNS) from 28 (20.8%) (Fig 1).

The percentage resistance of isolated *S. aureus* to the classes of antibiotics tested is shown in Fig 2, with resistance rates exceeding 80% to penicillins, cephalosporins, fluoroquinolones and aminoglycosides, and over

70% resistance rates to macrolide-lincosamide-streptogramin (MLS), tetracycline and sulfamethoxazole. Resistance rates to oxazolidinones (linezolid) exceeded 50% while resistance to glycopeptide (vancomycin) was 11.1% (4/36).

Table 1: Primers used to amplify and detect *mecA* and *vanA* genes

Target gene	Primer name	Oligonucleotide sequence	Amplicon size (bp)	Reference
mecA	MecA-F	GGGATCATAGCGTCATTATTC	527	17
	MecA-R	AACGATTGTGACACGATAGCC		
vanA	VanA-F	CATGAATAGAATAAAAGTTGCAATA	1030	18
	VanA-R	CCCCTTTAACGCTAATACGATCAA		

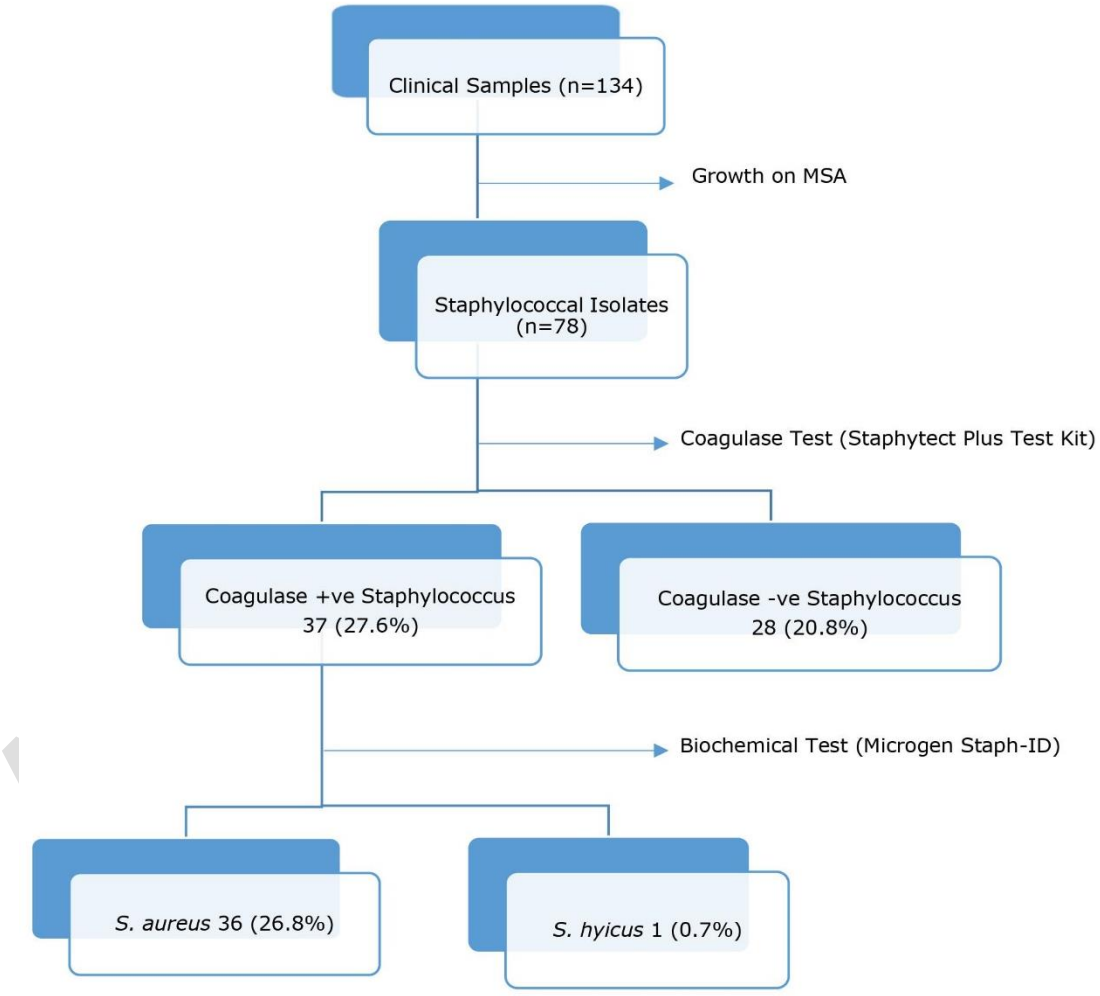


Fig 1: Workflow for isolation and identification of *Staphylococcus aureus*

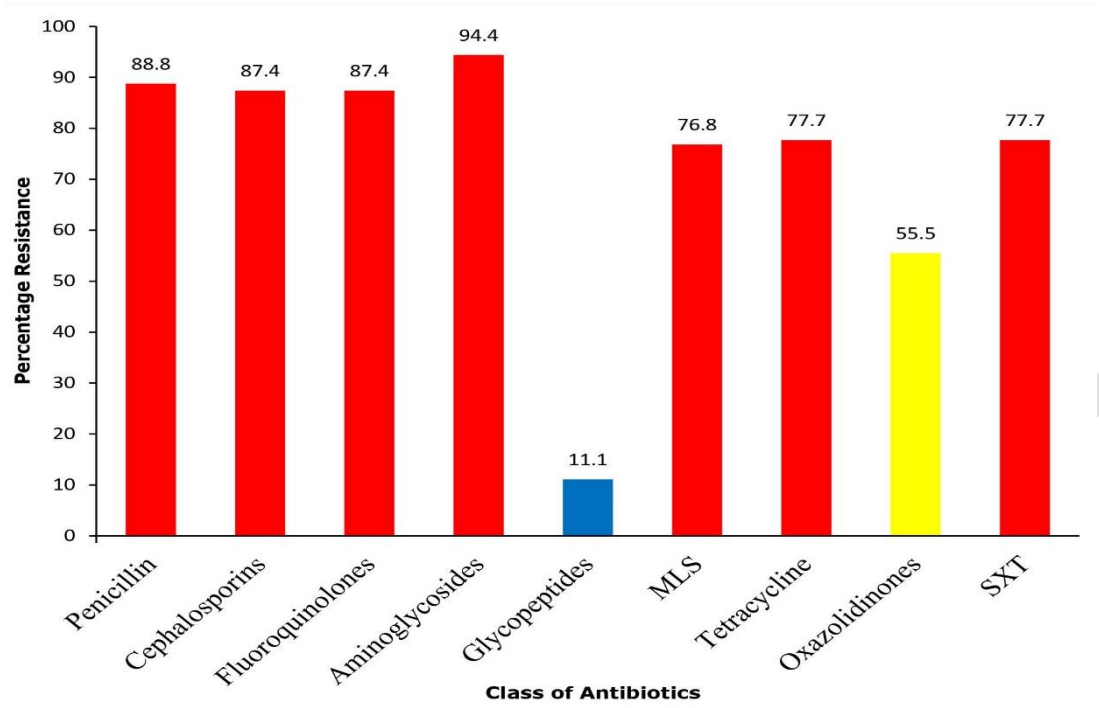


Fig 2: Percentage resistance of isolated *Staphylococcus aureus* to selected antibiotic classes

Table 2: Percentage distribution of isolates by methicillin susceptibility and antibiotic resistance classification

Specimen type	MRSA (%)	MSSA (%)	MDR (%)	XDR (%)	PDR (%)
Urine sample (n=10)	9 (25.0)	1 (2.8)	6 (16.7)	6 (16.7)	1 (2.8)
Wound swab (n=13)	13 (36.1)	0	8 (22.2)	7 (19.4)	1 (2.8)
Nasal swab (n=13)	12 (33.3)	1 (2.8)	3 (8.3)	3 (8.3)	0
Total (n=36)	**34 (94.4)**	**2 (5.6)**	**17 (47.2)**	**16 (44.4)**	**2 (5.6)**

MRSA: methicillin-resistant *S. aureus*; MSSA: methicillin sensitive *S. aureus*; MDR: multi-drug resistant; XDR: extensively drug-resistant; PDR: pan-drug resistant

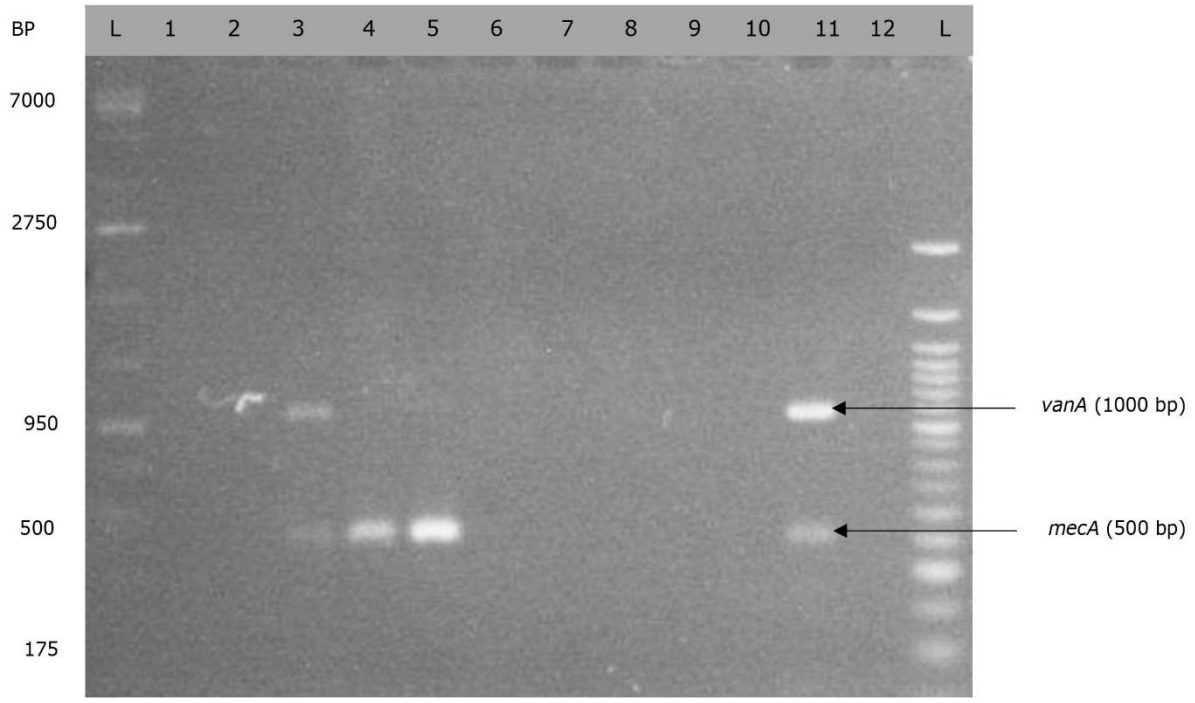
As shown in Table 2, resistance to cefoxitin was used to classify the isolates into MRSA and MSSA according the 2021 EUCAST guideline (15). Among the tested *S. aureus* isolates, 34 (94.4%) were MRSA, and only 2 (5.6%) were MSSA. The isolates were also classified based on their antibiotic resistance profile as described by Magiorakos (20), with 17 (47.2%) being MDR, 16 (44.4%) extensively drug resistant (XDR), and 2 (5.6%) pan-drug-resistant (PDR).

Fig 3 represents the gel electrophoresis pattern of the multiplex PCR amplicons which showed that 4 *S. aureus* isolates tested harbored *mecA* gene while 2 harbored *vanA* gene.

Discussion:

Staphylococcus aureus is one among the bacteria that is most frequently found in orthopaedic patients, especially those with implants, and it typically calls for a challenging and expensive course of therapy. It is the second leading global cause of death for people and can set off a number of infectious diseases (21,22). This has frequently presented its victims with significant economic and health challenges. In this study, we investigated the antibiotic resistance profile of *S. aureus* recovered from hospitalized orthopaedic patients at the NOHD, and reported a prevalence of 26.8%. In a systematic review, a lower prevalence of 18.2% was reported for nasal carriage and urinary tract infection among patients with HIV/AIDS in Nigeria (23). Majority of orthopaedic patients require implants, and since wounds such as burns are frequently left exposed and heal slowly, these circumstances may have contributed to the high prevalence observed.

According to a previous research, *S. aureus* is capable of developing resistance to



Lane 3: N18, Lane 4: U15, Lane 5: U21, Lane 11: W56, Lane L: 100bp Molecular DNA ladder
Keys: N= Nasal, U= Urine, W= Wound, BP= Base pair

Figure 3: Gel electrophoresis of the PCR amplicons of the *vanA* and *mecA* genes from representative resistant *Staphylococcus aureus* isolates

every class of antibiotic now in use and resistance can arise through *de novo* chromosomal gene changes or from the acquisition of horizontally transferred resistance determinants (24). Except for oxazolidinones and glycopeptides, resistance of *S. aureus* isolates was over 70% to almost all of the classes of antibiotics evaluated in this study. The high resistance observed is caused by the ability of these *S. aureus* isolates to horizontally acquire genetic elements, that results in emergence of MRSA strains, which is a serious issue and threat to public health. According to this study, linezolid is becoming a less effective alternative for treating orthopaedic MRSA infections. However, vancomycin still remains a reserve antibiotic under hospital treatment policy and guidelines (25).

MRSA strains are known to cause a variety of infections, including surgical site infection (SSIs), bones and joints infection, bacteraemia, meningitis, pneumonia, endocarditis, and toxic shock syndrome (TSS), some of which have a major impact on patient morbidity, mortality, and financial burden (26). Although, the high level of resistance by some of the isolates is attributed to over expression of penicillin binding protein 2a (PBP2a) due to duplication or enhanced transcription of the *mecA* gene, the high percentage resistance to penicillins and cephalosporins, indicates that MRSA may have become resistant to beta-lactams through acquisition of specific genetic determinant (*mecA*) (24) and other multi-drug resistant genetic determinants.

Despite the efficacy of clavulanic acid in combination with other antibiotics to treat hospital-acquired MRSA infections, resistant strains and those with diminished susceptibility to ceftaroline (a fifth-generation cephalosporin) have been identified (27). Vancomycin and daptomycin, two supposedly 'last-resort' antibiotics, have been made available to clinicians as therapeutic choices as a result of this 'selection pressure' (28). In spite of the fact that daptomycin has broad-spectrum effectiveness against Gram-positive organisms such as MRSA, there are no published randomized control studies or data to support its usage in infections following orthopaedic surgery (25).

Vancomycin is an alternate treatment option for MRSA infections; however, some strains of *S. aureus* (VRSA) have acquired the *vanA* operon, which encodes enzymes that helps them hydrolyze the normal D-ala-D-ala precursors for vancomycin, and to synthesize new D-ala-D-lactate precursors that have reduced binding affinity to vancomycin and other glycopeptides (29). Although *vanA* was detected in only 2 (5.6%) of the 36 *S. aureus* isolated among the participants in this study, these VRSA isolates were resistant to all the prescribed and accessible antibiotics, implying those orthopaedic patients harboring such strains will have fewer or no treatment options available to them, and there is also a high risk

that they can horizontally spread the resistant genes to other bacteria (including *S. aureus*) in the microbiome of the hospital. As such, the hospital MRSA and VRSA strains are particularly a threat to patients, guardians and primary caregivers (nurses and doctors). Detection of these strains in Nigerian hospitals may not be unrelated to increase use of glycopeptides (vancomycin) in recent times, unsanitary conditions, unclean ward beddings, and non-compliance with prescribed antibiotic medications (30). However, vancomycin is not available for prescription in NOHD, and nosocomial preventive measures are usually enforced within the hospital environment.

About 50% of the *S. aureus* isolates recovered from all the samples (urine, nasal and wound swabs) were both MDR and XDR, indicating that the isolates in this study may have arisen from previous antibiotics exposure or may have picked up resistant genes from other organisms such as vancomycin resistant enterococci (VRE) in the environment, which are known to be reservoirs of antimicrobial resistance (AMR) genes that are carried on plasmids, transposons and integrons. In contrast to our study, 90.2% and 71.6% of the *S. aureus* isolated from urine and wound samples of sick patients respectively in several Nigerian teaching hospitals were MDR (9). The type of infectious diseases, the locales, or community pressure on the antibiotics tested could all be contributing factors to variations in MDR rates. Although, 37.1% MDR, 13.8% XDR and 0% PDR were reported among bacterial strains in the study by Basak et al., (31), these rates were still slightly lower than the rates reported in our current study.

The emergence of VRSA and occurrence of XDR and PDR *S. aureus* strains in our study constitute significant financial burden to orthopaedic patients (and/or their guardians) who require implants and gradual recovery process. There are limited number of alternatives for potent antibiotic medications required to combat the threats of these strains in Nigerian hospitals. Unfortunately, this high prevalence of pathogenic MDR *S. aureus* strains may cause NOHD, a referral center for orthopaedic patients, to eventually develop into a center for nosocomial infections.

Conclusion:

This study reports the emergence of clinical *vanA*-type VRSA in NOHD, Kano, Nigeria and found that the majority of the *S. aureus* isolated from orthopaedic patients were MDR and XDR strains from their *in vitro* resistance to EUCAST-recommended antibiotics.

The detection of *vanA* and *mecA* in a few *S. aureus* isolates raises the probability of horizontal transmission to other isolates and increased risk of nosocomial infection in the NOHD. Although there are ongoing research studies on developing novel therapeutic approaches that may be effective against VRSA strains, for now, clinicians are limited to very few treatment options.

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Contributions of authors:

AU, AGO and OBO were involved in study conceptualization; AU and ODA were involved in data acquisition; AU, ODA and KM were involved in analysis of data; AU, KM, AGO and OBO were involved in drafting and critical review of the manuscript. All the authors approved the final manuscript submitted for review.

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Conflicts of interest:

Authors declared no conflict of interest

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