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Research Article

Molecular Identification of Vancomycin-Resistant *Staphylococcus aureus* Isolated from Wound Infections of Patients Attending Government Hospitals in Kaduna Metropolis

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ABSTRACT

Vancomycin-resistant S. aureus (VRSA) isolates are widely spread in both communities and hospitals, causing high morbidity and mortality worldwide. The present study aimed to identify vancomycin-resistant Staphylococcus aureus (VRSA) isolated from wound infections of patients attending Government hospitals in Kaduna Metropolis. A total of 168 wound samples were collected from patients across three selected hospitals. Staphyloccus aureus was isolated from wounds swaps and subjected to antibiotic susceptible testing using agar disk diffusion method. The VRSA isolates were detected by a combination of phenotypic and genotypic methods. Seven coagulase-positive S. aureus strains were isolated, which occurred higher among patients from YDMH (50%) compared to 31.25% among patients from RGH and 18.75% in GAGH. Wounds of male patients had higher infection with S. aureus (68.75%) than those of females (31.25); whereas those between 20-50 years old had the highest wound infection (50.00%). The isolates exhibited high resistance against vancomycin (71.42%). Seven isolates (43.75%) were categorized as VRSA. Moreover, the MIC of the seven VRSA isolates were 8 µg/mL), with MBC ranging between 8-32 µg/mL with CFU ranging from 2.9-7.2×106, 1.7-3.7 ×106 and 1.7×106 CFU/mL respectively. The isolates housed vanA and vanB gene (474bp and 800bp respectively). Given the alarming rate of resistance among VRSA isolates, monitoring of antibiotic resistance should be performed. Although vancomycin remains a drug of choice for VRSA, our study suggests that its efficacy may be limited due to development of resistance.

Keywords: Infection; Patient; Resistant; *Staphylococcus aureus;* Vancomycin; Wound

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INTRODUCTION

Staphylococcus aureus is a Gram-positive, non-motile spherical bacteria that occur in grape-like clusters. Some species are saprophytes, while others are pathogenic. *Staphylococcus aureus* is one of the most common causes of nosocomial infections like surgical wound infection, blood stream infection, pneumonia among others (Liu *et al.,* 2022). The treatment for this bacterium is a problem due to the emergence and spread of methicillin-resistance gene. Vancomycin is commonly used for treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) (Prakash *et al.,* 2016**)**. But the incidence of vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) has been increasing in various parts of the world (Tiwari *et al*., 2018; Liu *et al.,* 2022). The common species of *Staphylococcus* include

Staphylococcus aureus and *Staphylococcus epidermis* (which does not normally cause infection).

This bacterium had been isolated on the human skin, in the air, wounds and pus (Cimolai, 2021). Wound can be an injury or damage to the underlying tissue that offer access for microorganisms to cause infections. Through this, *S. aureus* had gain access into human body. Disease-associated strains often promote infections by producing potent toxins and expressing cell surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant forms of pathogenic *S. aureus* is a worldwide problem in clinical settings. The strain, popularly dubbed a "superbug", is resistant to methicillin, as well as to penicillin and other commonly used antibiotics (Hiramatsu *et al*., 2019).

Vancomycin is a glycopeptide antibiotic effective against majority of Gram-positive bacteria, particularly against multiple drug-resistant *enterococc*i and *staphylococci* which are resistant to β-lactum antibiotics (Reynolds**,** 2018**)**. These pathogens have acquired resistance to this compound by virtue of their intrinsic property especially among clinical isolates. This leads to severe complications in immuno compromised as well as surgical patients. Vancomycin resistant enterococci (VRE) were first reported in 1988 in Europe, USA (Uttley *et al***.,** 2018**)** and African countries (Unkal and Kaliwal**,** 2023**)**.

Vancomycin binds with the C-terminal D-alanyl-D-alanine (D-Ala-D-Ala) residue of pentapeptide and blocks the addition of precursors by transglycosylation to peptidoglycan chain and inhibits cross linking of cell wall by transpeptidation (Courvalin**,** 2016**)**. Resistance to vancomycin is caused by synthesis of precursors with low-affinity for these antibiotics conferred by operons present on *van* gene clusters that encode enzymes which synthesize low affinity precursors wherein C-terminal D-Ala residue is replaced by D-lactate (D-Lac) that modify vancomycin binding site (Unkal and Kaliwal**,** 2023**)**. These genes confer high- or low-level resistance to vancomycin which may be inducible or constitutive (Unkal and Kaliwal**,** 2023**)**. New rapid techniques for identification and characterization of VRSA have been developed. Consequently, the research identified vancomycin-resistant *Staphylococcus aureus* isolated from wound infections among patients attending selected government hospitals in Kaduna metropolis.

MATERIALS AND METHODS

Study Design: This study adopted a descriptive cross-sectional design to identify vancomycin-resistant *Staphylococcus aureus* isolated from wound infections of patients attending selected Government Hospitals in Kaduna metropolis.

The Study Area: The research was conducted in three selected Government Hospitals within Kaduna metropolis, namely Rigasa General Hospital (RGH), Gwamna Awan General Hospital (GAGH) and Yusuf Dantsoho Memorial Hospital (YDMH). Kaduna State is located in the Northern region of Nigeria, known for its significant healthcare institutions (Aminu *et al.,* 2017). The figure 1 below illustrates the map of Kaduna Metropolis showing Sampling locations.

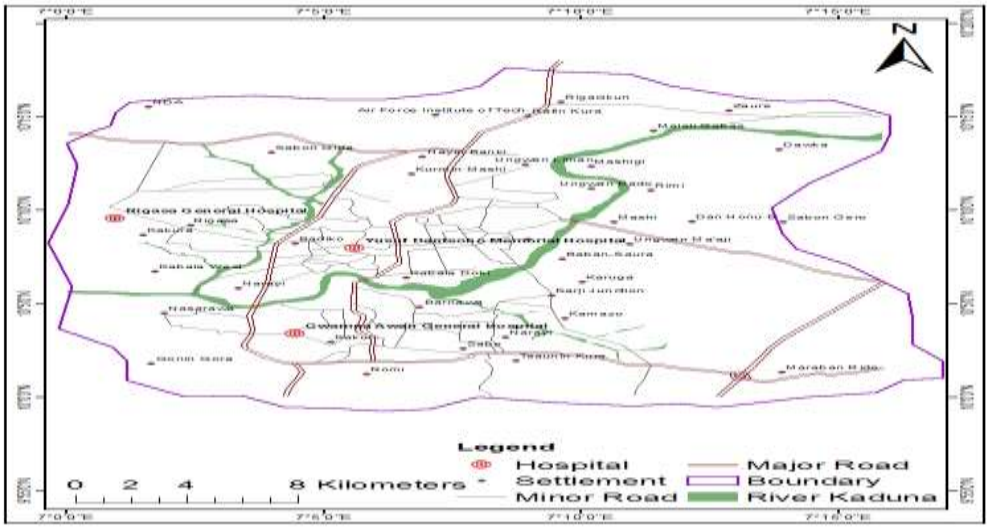

Figure 1: Map of Kaduna Metropolis showing Sampling Location

Ethical Considerations

Ethical approval was obtained from the Kaduna State Ministry of Health. Each participant was informed about the purpose of the study and provided with a consent form to ensure voluntary participation. The study followed the declaration of Helsinki guidelines to protect patient privacy and confidentiality (Aminu *et al.,* 2017).

The Inclusion Criteria: Patients diagnosed with wound infections by attending physician. Both male and female patients aged 10 years and above, as well as those who consented to the study and allowed the use of their clinical samples were included.

The Exclusion Criteria: Patients who have already received antibiotic treatment prior to sample collection, as well as patients with wounds unrelated to infections, such as surgical incisions with no signs of infection were excluded.

Target Population: The population used for this study consisted of patients with wounds attending the selected Government Hospitals.

Determination of Sample Size

The sample size was determined based on the prevalence of *Staphylococcus aureus* in wound infections reported in previous studies from similar hospital settings as reported by Aminu *et al.* (2017) with prevalence of 12.5%. A confidence level of 95% and a margin of error of 5% were applied to calculate the required number of samples for statistical relevance. The sample size was calculated using the Cochran sample size formula which was used to calculate an ideal sample size given a level of precision, desired confidence level and estimated proportion of the attribute present in the population. This was done using the formula below as adopted by (Cochran, 1977).

$$n = \frac{t^2 \times p(1-p)}{m^2}$$

Where; n = number of samples, m = margin of error= 0.05, p = percentage of existing prevalence= 12.5% (Aminu *et al.,* 2017), t = t-value at 95% Confidence Interval (CI) =1.96, Inputting the figures into the sample size formula, n= $(1.96)^2$ x 0.125 x (1- 0.125)/ $(0.05)^2$= 3.84 x 0.125 x 0.875/0.0025 =168.

Sampling technique

The study employed multistage sampling technique, where all the Government general Hospitals within Kaduna Metropolis were first clustered from which three (3) were selected through balloting system.

Administration

Structured questionnaire was administered to the patients attending the three selected general hospitals to obtain data on their socio-demography and exposure to some risk factors.

Collection of Samples and Storage

Wound swab samples were collected from patients following aseptic techniques. The procedure is as follows: a sterile cotton swab was moistened with saline solution and gently rubbed over the wound area. Care was taken to avoid contamination from surrounding skin flora. Each sample was labeled with the patient's identification code, date and hospital. Immediately after collection, the swab samples were placed in sterile transport media (e.g., Stuart's medium) to maintain bacterial viability. The samples were transported to the Microbiology Laboratory at Kaduna State University within two hours of collection. Where immediate processing was not possible, the samples were stored at 4°C to prevent bacterial overgrowth or death.

Isolation and Identification of *Staphylococcus aureus*

The collected samples were processed according to standard microbiological procedures (Isenberg, 2014). The specimens were cultured on Mannitol Salt Agar (Sigma-Aldrich, Gillingham, UK), followed by overnight incubation at 37°C for 48 hours. Presumptive *S. aureus* yellow colonies were subjected to Gram staining and standard biochemical tests (catalase, slide, and tube coagulase tests, DNase test, mannitol fermentation test and blood haemolysis test). A loopful of each biochemically confirmed *S. aureus* isolate was then inoculated into the nutrient broth (Thermo Fisher Scientific, Oxoid Ltd., Basingstoke, UK) containing 15% glycerol and frozen at 4˚C**.** The strains of *Staphylococcus aureus* were identified on the basis of colony morphology, Gram's stain and different biochemical tests. Specific identification of the isolates was performed by growth on Mannitol salt agar which is a selective media for *Staphylococcus aureus* which grows as yellow white colonies surrounded by yellow zone.

Microscopic examination of isolates

The bacteria isolates were identified based on the Gram staining techniques, which apart from differentiating an isolate as Gram positive or negative, also helped to identify whether it is rod or cocci (Pandian *et al*., 2012).

Biochemical characterization of bacterial isolates

The bacteria were subjected to biochemical characterization by conducting specific tests, including Gram staining catalase, oxidase, coangulase, and citrate tests (Boominadhan *et al*., 2009; (Pandian *et al*., 2012).

Antibiotic Susceptibility Testing for Vancomycin Resistance

A 0.5 MacFarland standard was prepared in accordance with the method of Cheesbrough (2002). About 1ml of concentrated sulphuric acid with 99ml of sterile water (1%V/V). Another 1% (W/V) Solution of barium chloride were also be prepared by dissolving 0.5 g of dehydrated barium chloride in 50ml distilled water. About 0.6mL of the barium chloride solution was mixed with 99.4ml H_2SO_4 solution to yield 1% W/V barium sulphate suspension. The turbid solution (0.5) was used as a reference to adjust the turbidity of the bacterial suspension. Some quantity of each test bacteria from overnight growth culture was added to 2mL of sterile physiological saline as suspension medium. The bacterial suspension was compared to 0.5 Mac Farland standard (1.5 x 10^8 CFU mL) under a white background with contrasting black lines.

Antibiotic susceptibility Test

All biochemically identified coagulase-positive *S. aureus* (CPS) isolates (n=65) were tested for their susceptibility to antibiotics that are commonly prescribed to treat staphylococcal infection. The antibiotic sensitivity was determined according to Clinical and Laboratory Standard Institute (CLSI) guidelines for disk agar diffusion (CLSI, 2024). Disk agar diffusion (Kirby-Bauer) method according to CLSI procedure was applied for the assessment of antibacterial effects of different antibiotics against *S. aureus* isolates (Bauer *et al.* 1996). The clinical *S. aureus* isolates (0.5 McFarland) were spread onto the surface of the Mŭller-Hinton Agar (MHA) with a sterile swab. Gentamicin (10 µg), kanamycin (20 µg), Ceftroxane (30 µg), Levofloxacin (30 µg), Imipenem (30 µg), Ampicilin (30 µg), ciprofloxacin (5 µg), Trimethoprim-Sulf (30 µg), tetracycline (30 µg) and vancomycin (30 µg) disks were used as antibiotics. Vancomycin was purchased from the Sigma Aldrich Company (USA), while the other antibiotics were purchased from the PADTAN TEB Company (Tehran, Iran). The agar plates were incubated at 37 °C for 24 h and the diameter of the zone of inhibition for each microorganism was measured. All tests were performed as triplicate.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Assays Using Microtiter Plate

Pre-preparation of Stock Colony

A single colony from each pure isolates were inoculated into Petri dish were each transferred to a tube containing approximately 3 mL of fresh Mŭller-Hinton Broth (MHB). The tubes were incubated at 37 °C for about 12 hours, until the culture reaches an optical density at 600 nm (OD_{600}) of around 0.1 (Wiegand *et al*., 2018).

Preparation of diluted standardized inoculum

The OD600 of stock culture was measured before inoculated into the 96-well microplate; If OD600 > 0.1, the culture was diluted with MHB to achieved an OD600 of 0.1. The culture was incubated for an additional 15–30 minutes if OD600 < 0.09. Ten (10) mL of MHB was prepared in a trough and add 100 µL of the standardized inoculum. Mixed thoroughly by pipetting (Balouiri *et al*., 2016).

Preparation of 96-well Microplate

One (1) g of Vancomycin was dissolved in 10 mL of distilled water to create a 1000 µg/mL stock solution. Fifty (50) µL of MHB was dispensed into wells in columns 1–10 for growth control and serial dilution, aiming for concentrations ranging from 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16, 32 µg/mL. Column 11 wells were left empty and 100 µL of MHB was added to column 12 wells for sterility control. Serial dilutions were performed by transferring 50 µL from column 11 to column 12. Using a multichannel pipette, 50 µL of the diluted standardized inoculum was added to wells in columns 1–11. The plate was sealed, labelled appropriately, and incubated at 37 °C overnight (Balouiri *et al*., 2016; Wiegand *et al.,* 2018).

Minimal inhibitory concentration (mic) assessment

The microplate was allowed to cool to room temperature for 15 minutes. The OD600 of each well was measured using a plate reader. The data was plotted with optical density on the Y-axis and antibiotic concentration on the X-axis, fitting it to a modified Gompertz model to determine the MIC (Wiegand *et al.,* 2018).

Minimal bactericidal concentration (mbc) assessment

Hundred (100) µL of wells without turbidity (8 µg/mL, 16 µg/mL and 32 µg/mL) were transferred to a new 96-well plate, tenfold serial dilutions was performed with saline solution. Twenty (20) µL were plated from each well onto Mueller-Hinton Agar (MHA) plates and incubated overnight. The MBC is identified as the lowest concentration of the antibiotic that results in bacterial death, indicated by the inability to re-culture bacteria.

Molecular Detection of *van*A and *van* B genes of VRSA by PCR

The *S. aureus* vancomycin resistance gene (*vanA*) was investigated in vancomycin- resistant and intermediate resistance isolates by PCR method using specific primers; *Van*A(474bp): Frd: 5'ATGAATAGAATAAAAGTTGC'3, Rv: 5'TCACCCCTTT

AACGCTAATA'3 Saha *et al.* (2018) and *Van*B (800bp); Frd: 5'GTGACAAACCGGAGGCGAGGA'3, Rvs: CCGCCATCCTCCTGCAAAAAA'3 (Tiwari et al., 2016). PCR amplification was carried out in a 25 µL reaction mixture with each primer. The amplification conditions were initial denaturation at 98°C for 2 min, followed by 35 cycles of denaturation at 98°C for 10 s; annealing at 50°C for 1 min; polymerization at 72°C for 1 min 30 s for *vanA* gene and initial denaturation at 94°C for 10 min; 30 cycles with a 30 s denaturation step at 94°C, a 45 s annealing step at 50°C and a 30 s extension step at 72°C and 10 min extension step at 72°C and a holding step at 4°C for *vanB* gene (Bamigboye *et al.,* 2018). The PCR products were mixed with 2µl bromophenol blue, electrophoresed in 1.2% agarose gel with 0.1% ethidium bromide and visualized by using UV transillumination.

Data Analysis

Descriptive Statistics (mean, median, mode and standard deviation) were calculated for continuous variables with the Statistical Package for Social Sciences (SPSS, 2007). Analysis of Variance (ANOVA) was applied to determine if there is significant difference between categorical variables, such as the demographic factors in vancomycin resistance and the Ducan multiple range were tested for significant difference at P≤0.05.

RESULTS

The biochemical identification of *Staphylococcus aureus* associated with wound infection was analyzed. On microscopy, the presence of spherical cocci with purple colour (Gram-positive), arranged in clusters was indicative of *Staphylococci* with coagulase positive differentiating *Staphylococcus aureus* from another *Staph.* sp **(**Table 1**).**

The percentage resistance profile of the vancomycin-resistant *staphylococcus aureus* (VRSA) to common antimicrobial agents with 30% resistance to YDMH9, 20% GAGH4, 60% to RGH6, 50% to YDMH21, 10% to RGH13, 20% to GAGH15 and 30% resistance to RGH16 isolate as shown in Table 2.

Percentage resistance of vancomycin-resistant *Staphylococcus aureus* (VRSA) to different antibiotics with GEN, KAN and CRO (28.57%), LEV, IMP and SXT (14.29%), AMP (57.14%), while VAN (71.42%) respectively (Fig. 1).

The antimicrobial resistance profile of the isolates to common antimicrobial agents is shown in Table 3. Percentage resistance of vancomycin-resistant *Staphylococcus aureus* (VRSA) to different antibiotics with GEN, KAN and CRO (28.57%), LEV, IMP and SXT (14.29%), AMP (57.14%), while VAN (71.42%)

The fitting intersection of S*taphylococcus aureus* growth against the Vancomycin concentration was determined for MIC. All the bacterial isolated showed MIC value lies on the intersection of the lower part of the jump with the jump slope (8µg/mL) (Fig. 2).

The results of the MBC are as presented in Table 4. All the 7 isolates were first sensitive at 0.125-4 µg/mL respectively but showed growth across all the concentrations used in the study in addition to the remaining isolate. A total of 7 isolates were susceptible at MBC range of 8, 16 µg/mL to 32 µg/mL with CFU ranging from 2.9-7.2×10^6 CFU/mL at 8 µg/mL and 1.7-3.7 ×10^6 CFU at 16 µg/mL as well as 8-1.7×10^6 CFU/mL 32µg/mL respectively.

A higher frequency of VRSA in males (68.75%) than females (31.25%) was observed (Table 3). However, there was no significant association between gender and the frequency of VRSA (*p*-value= 0.510). Regarding the age of the patients, the results revealed that there was a significant association between age and the frequency of VRSA strains (*p*-value= 0.021). However, a high percentage of VRSA (50%) was observed in the age group 20-50 years compared to other groups. Concerning the Hospital setting of clinical samples, RGH had a high frequency of VRSA (50.00%) compared to others. Significant correlation was observed between the clinical sample Hospital setting and frequency of VRSA strains (*p*-value= 0.012) (Table 5).

The possible risk factors associated with VRSA among wound patients. Total of 168 respondent responses to the consent form, 51.14% agreed the studied risk factors (general health challenge, wound incurred, wound been exposed to the environment, improper management of the wound) as responsible for the VRSA, while 48.86% disagreed with the risk factors. Other factors that were agreed to be responsible are wound been exposed to the environment and lack of visitation of health facilities (Table 6).

Frequency distribution of VRSA for each hospital, with RGH 2(28.57%) was observed between the frequency of VRSA and the type of hospital. However, the samples collected from YDMH showed a high frequency of MRSA 4 (57.14%) compared to GAGH 1 (14.29%) (Fig. 4).

Agarose gel electrophoresis of PCR amplified of the *vanA* and *vanB* gene using the gene-specific primers of clinical isolates that was suspected to be VRSA yielded 474 bp (van A) and 800 bp (VAN b) amplicon respectively (Plate. 1 and 2).

Table 1: Biochemical Identification *of S. aureus* Associated with Wound Infection within Kaduna Metropolis

S/N	Isolate Code	Gram R×n	Shape	Catalase	Citrate	Coagulase	Oxidase	Motility	Probable Organisms
1	RGH7	+	Cocci	+	_	_	_	_	*S. epidermidis*
2	YDMH9	+	Cocci	+	+	+	_	_	*S. aureus*
3	GAGH4	+	Cocci	+	+	+	_	_	*S. aureus*
4	RGH19	+	Cocci	+	_	_	_	_	*S. hominis*
5	RGH12	+	Cocci	+	_	_	_	_	*S. epidermidis*
6	RGH6	+	Cocci	+	+	+	_	_	*S. aureus*
7	YDMH8	+	Cocci	+	_	_	_	_	*S. epidermidis*
8	YDMH21	+	Cocci	+	+	+	_	_	*S. aureus*
9	RGH9	+	Cocci	+	_	_	_	_	*S. epidermidis*
10	YDMH3	+	Cocci	+	_	_	_	_	*S. hominis*
11	GAGH6	+	Cocci	+	_	_	_	_	*S. hominis*
12	RGH13	+	Cocci	+	+	+	_	_	*S. aureus*
13	GAGH15	+	Cocci	+	+	+	_	_	*S. aureus*
14	YDMH42	+	Cocci	+	_	_	_	_	*S. epidermidis*
15	RGH16	+	Cocci	+	+	+	_	_	*S. aureus*
16	RGH23	+	Cocci	+	_	_	_	_	*S. epidermidis*

Keys: IC: Isolate Code, RGH: Rigasa General Hospital, YDMH: Yusuf Dansoho Mermorial Hospital, GAGH: Gwanma Awan General-Hospital. + ve = tests are positive, -ve = tests are negative**,** Rxn = Reaction

Table 2: Antibiotic Percentage Resistance Pattern of the Vancomycin-Resistant *Staphylococcus aureus* (VRSA) Isolates

IC	GEN	KAN	CRO	LEV	IMP	AMP	CIP	SXT	TET	VAN	% Resistance
YDMH9	S	I	R	S	I	R	S	I	S	R	30.0
GAGH4	I	S	I	I	R	S	S	S	I	R	20.0
RGH6	R	R	R	I	I	R	R	S	I	I	50.0
YDMH21	I	I	S	R	S	R	R	R	S	R	50.0
RGH13	S	R	S	S	I	S	S	S	S	I	10.0
GAGH15	R	I	I	I	I	I	S	I	I	R	20.0
RGH16	S	I	I	I	I	R	I	S	R	R	30.0
% Antibiotic	**28.57**	**28.57**	**28.57**	**14.29**	**14.29**	**57.14**	**28.57**	**14.29**	**14.29**	**71.42**	

Keys: IC**=** Isolate Code**, %=** Percentage

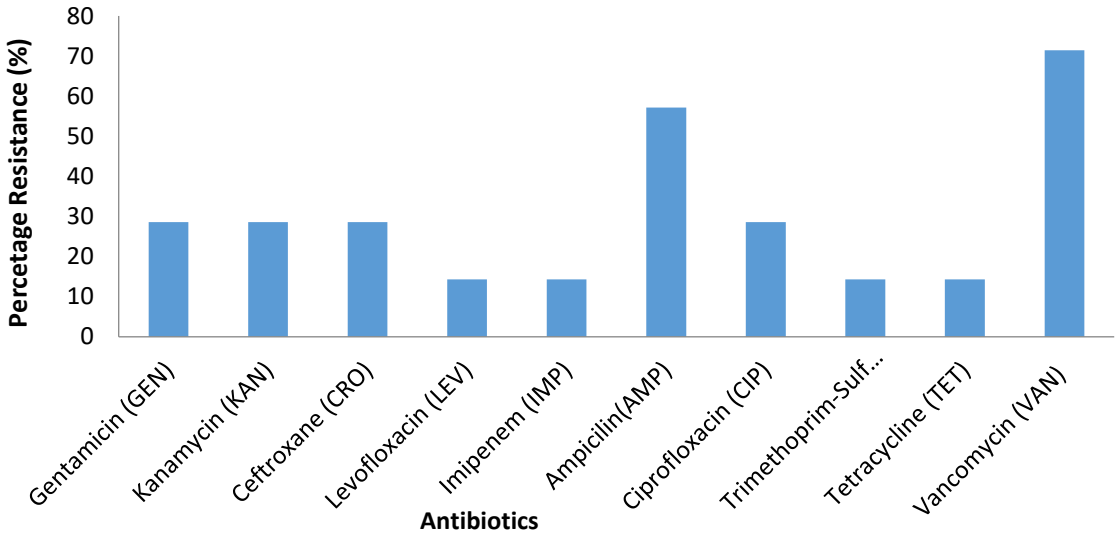

Figure 1: **Percentage Resistance of vancomycin-resistant *Staphylococcus aureus* (VRSA) to different Antibiotics**

Table 3: Antimicrobial Sensitivity Profile of VRSA from Wound Samples

S/N	Phenotypic	Disc Content (µg)	Number (%) Resistance
1	Gentamicin (GEN)	10	2 (28.57)
2	Kanamycin (KAN)	20	2 (28.57)
3	Ceftroxane (CRO)	30	2 (28.57)
4	Levofloxacin (LEV)	30	1 (14.29)
5	Imipenem (IMP)	30	1 (14.29)
6	Ampicilin (AMP)	30	4 (57.14)
7	Ciprofloxacin (CIP)	5	2 (28.57)
8	Trimethoprim-Sulf (SXT)	30	1 (14.29)
9	Tetracycline (TET)	30	1 (14.29)
10	Vancomycin (VAN)	30	5 (71.42)

Keys: S/N= Serial Number, µg = Microgram, **%=** Percentage

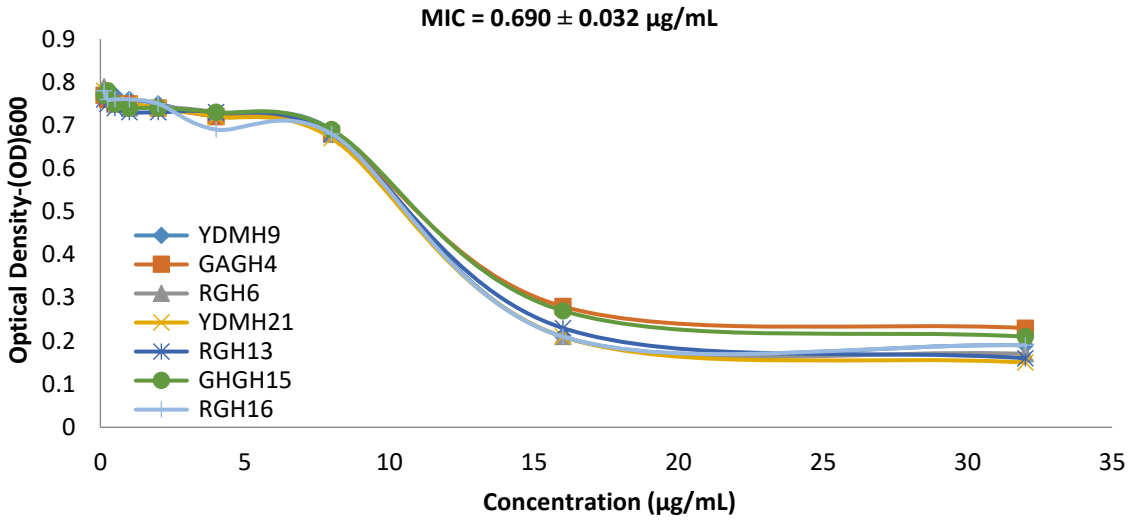

Figure 2: The Fitting Intersection of MIC Determination S*taphylococcus aureus* against Vancomycin of MIC Determination

Table 4: Vancomycin MBC of Resistant *Staphylococcus aureus*

	×10^6 CFU/Ml		
Isolate Code	**8 µg/mL**	**16 µg/mL**	**32 µg/mL**
YDMH9	4.3	3.1	1.4
GAGH4	3.1	2.3	1.2
RGH6	5.7	3.6	0. 8
YDMH21	2.8	1.9	1.1
RGH13	7.2	3.7	1.7
GAGH15	5.3	3.6	1.2
RGH16	2.9	1.7	0.9

Key: CFU: Colony Forming Unit

Table 5: Prevalence of VRSA in Relation to Demographic Factors of Patients

Variable	**Status**	**VRSA (n=7) No (%)**	**P-value**
Gender	Male	4 (57.14)	0.010
	Female	3 (42.86)	
Age	10-20	1 (14.29)	
	20-50	4 (57.14)	0.021
	>50	2 (28.57)	
Occupation	Students	4 (57.14)	0.031
	Civil Servant	3 (42.86)	
Hospital Setting	RGH	1 (14.29)	
	YDMH	5 (71.43)	0.012
	GAGH	1 (18.75)	

Key: VRSA= Vancomycin -resistant *S. aureus*;

Table 5: Risk Factors Associated with Vancomycin Resistance *S. aureus* of Patients

Risk Factors	No: Examined	VRSA (%)	P=value
Was the wound incurred			
Yes	160	5 (71.43)	0.041
No	8	2 (28.57)	
Do you stayed long before visiting the Hospital			
Yes	101	4 (57.14)	0.030
No	67	3 (42.86)	
Was their prior wound been exposed to the environment			
Yes	85	3 (42.86)	0.019
No	83	4 (57.14)	
Do you have general Health challenge			
Yes	108	4 (57.14)	0.021
No	60	3 (42.86)	
Have the wound been exposed to the environment			
Yes	90	3 (42.86)	0.045
No	78	4(57.14)	
Have you been managing the wound			
Yes	150	6 (85.71)	0.018
No	18	1 (14.29)	

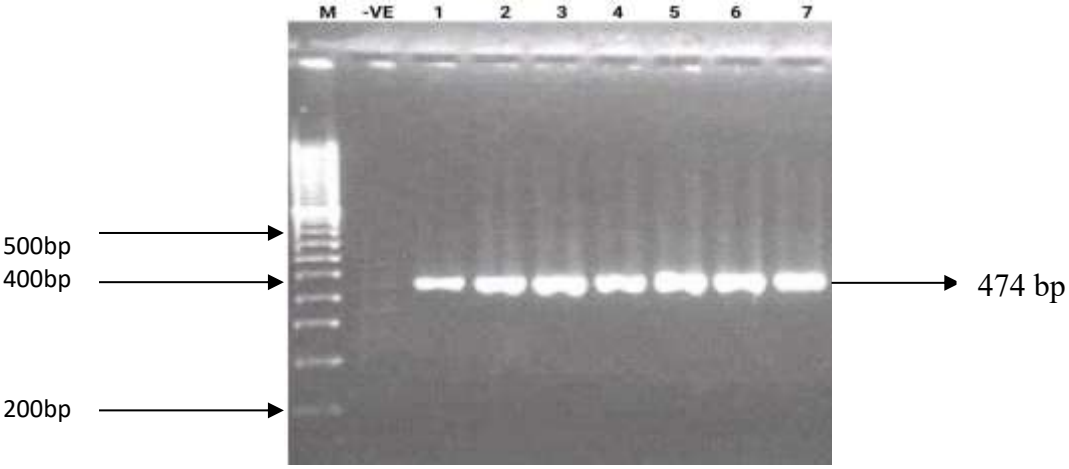

Plate 1: Agarose Gel Electrophoresis of PCR-Amplified *vanA* genes of clinically VRSA strain
Keys: M: Molecular Ladder, -VE: negative control, 1-7: VRSA Isolates.

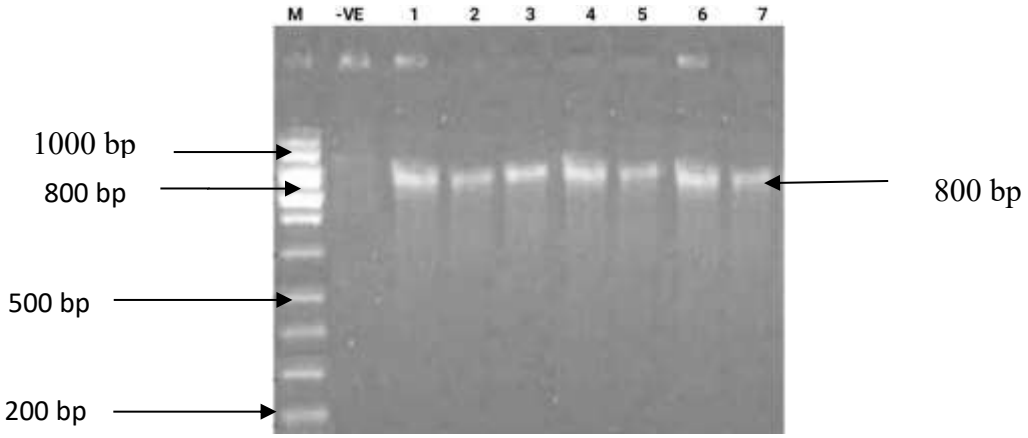

Plate 2: Agarose gel electrophoresis of PCR-amplified *vanB* gene of VRSA strain
Keys: M: Molecular Ladder, -VE: negative control, 1-7: VRSA Isolates

DISCUSSION

The isolation of coagulase-positive *S. aureus* strains from human patients in the current study indicates their role in wound infection since *S. aureus* is a normal flora of the skin and can enter the body through abrasions, cuts, surgical incisions, burn and intravenous catheter causing pyogenic infections. These findings coincided with a high frequency of *S. aureus* reported in wound and pus samples from human patients (Adhikari *et al*., 2024) in Europe and African countries (Dilnessa and Bitew, 2022). However, the presence of *S. aureus* in wound samples was nearly similar as previously reported (Abdelwahab *et al*., 2023). On the contrary to our results, Mwailunga *et al*. (2023) reported a high isolation rate of *S. aureus* from blood samples. The present research showed that 100% of Gram-positive isolates were oxidase positive, catalase positive and coagulase positive. Isolates were oxidase positive that may be due to the presence of N'-tetramethyl-phenylenediamine dihydrochloride (Kandel *et al*., 2022).

In general, studies revealed that VRSA strains are increasing in some areas around the world. The increasing resistance of *S. aureus* to the various commonly used antibiotics is a problematic issue, making infections by this bacterium difficult to treat. The current study showed a high resistance rate of the isolated *S. aureus* strains to tetracyclines (tetracycline), macrolides (erythromycin), beta-lactams (cefoxitin), fluoroquinolones (ciprofloxacin) which indicates the misuse of these antibiotics in the study area for treatment of Gram-positive organisms in hospital and community settings (Rahimi *et al*., 2018). These results correspond to other studies Kandel *et al*. (2020); Maharjan *et al*. (2022). A remarkable finding in this study is that *S. aureus* strains exhibited low resistance to levoflaxacin (1.5%) and ceftroxane (4.6%), and this is probably due to lower consumption of these antibiotics. The current research coincided with a low resistance rate (2.7%) of clinical *S. aureus* strains to some antibiotics (Abdelwahab *et al*., 2023).

The frequency of VRSA phenotypic resistant to this study (52.3%) coincided with other studies in Kerman City, Iran (Fasihi *et al*., 2018). The variation in the frequency of VRSA isolates in different studies may be attributed to factors such as the number of patients studied, the geographical location of the hospitals involved, the antibiotics used, the type of samples studied, and the methodology employed (Rahimi *et al*., 2018). The findings report that 7 VRSA strains in this study was phenotypically resistant to vancomycin and harbored the vancomycin resistance gene (*van*A and *van*B). In comparison to this study, the frequency of VRSA strains in other countries is variable and ranged from 0-6.6% (Elkhyat *et al*., 2022; Abdelwahab *et al*., 2023). Such differences in the frequency of VRSA may be due to differences in antibiotic policies (Asadpour and Ghazanfari, 2019).

The result of this study showed that 7 isolate of *S. aureus* were susceptible to vancomycin at MIC of approximately 8 µg/mL (Wiegand *et al*., 2018). A total of 7 isolates gave MIC value that range from 4 µg/mL to 8 µg/mL. The vancomycin MIC of ≤ 2 µg/mL and ≥ 16 µg/mL for vancomycin sensitive and resistance *S. aureus* (Wiegand *et al*., 2018). It therefore means that all the organisms isolated and tested with vancomycin were vancomycin-resistant *Staphylococcus aureus.* Other researchers' findings of MIC values for isolated *S. aureus* ranged from 0.5 to 2 µg/mL previously isolated by Yadav *et al* (2018) from

isolates obtained from clinical samples (wound). The present study shows that the MBC across the isolate 2.1-7.8 × 10^7 CFU/mL at 8 µg/mL, which is progressively declined as the concentration raised in µg/mL as reported by Dilnessa and Bitew, (2022).

The current study showed that the frequency of VRSA was significantly associated with patient demographic characteristics such as gender, age and Hospital setting. However, the frequency of VRSA was higher in males than in females in the age group of over 50 years compared to other age groups. This result is consistent with previous studies (Dilnessa and Bitew, 2022; Adhikari *et al*., 2023), indicating that gender and age are not risk factors for the acquisition of VRSA in human patients. On the basis of risk factors associated with VRSA among wound patients attending general hospitals. Health challenge from the patients, exposure to the environment after wound is incurred, improper management of the wound as responsible for the VRSA with a significant different on the basis of risk factors. Similar finding was recorded by Kandel *et al*. (2022).

In this study, 7 VRSA isolates were carrying the *van*A and *van*B gene as genotypically confirmed by PCR. *vanA* and *vanB* genes are highly specific for vancomycin-resistant *S. aureus.* PCR amplification of *vanA* and *vanB* gene of suspected clinically isolated VRSA strains using gene specific primer and plasmid DNA preparation yielded 474 bp and 800 bp amplicon respectively.

The high percentages of VRSA isolate possessing the *van* gene and exhibiting high resistance to different classes of antibiotics such as tetracyclines, macrolides, fluoroquinolones, aminoglycosides and vancomycin coincide with other studies (Elkhyat *et al*., 2020; Kandel *et al*., 2020; Idrees *et al*., 2023). This indicates that these antibiotics are no longer effective in the treatment of VRSA infections.

CONCLUSION

The isolation of VRSA from the clinical specimens as demonstrated in this study is an indication that the organism is widespread. In this study, the strains have shown high resistance to vancomycin. The MIC and MBC values obtained show that VRSA is increasing with alarming rate, and this accounts for the gradual decline in the effectiveness of use of vancomycin. The current study showed that the frequency of VRSA was significantly associated with patients demographic characteristics such as gender, age and hospital setting. The detection of the vancomycin resistance gene (*van*A and *van*B) in the VRSA strain in this study is alarming and indicates the risk of *van*A and *van*B spreading among *S. aureus*. Therefore, it is crucial to conduct antibiotic sensitivity tests before prescribing antibiotics and prudently use antibiotics in both hospital and community settings to combat the spread of VRSA strains.

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