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Vancomycin Resistant *Staphylococcus aureus* from Clinical Isolates in Zaria Metropolis, Kaduna State

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Abstract

Vancomycin antibiotic is a well-known drug of last resort in the treatment of methicillin-resistant *S. aureus* (MRSA), but recent studies have shown the emergence of resistance to vancomycin and other antibiotics. This study determines the phenotypic and genotypic prevalence of vancomycin resistant *S. aureus* (VRSA) among clinical isolates in Zaria Metropolis. A total of 350 suspected *Staphylococcal* isolates from clinical specimens (blood, urine, high vaginal swab, wound swab, ear swab, urethral swab) submitted to the Medical Microbiology Unit of the selected hospitals in Zaria were collected for the period of 6 months. The antibiotic susceptibility profile of MRSA isolates were determined using disc diffusion method while the minimum inhibitory concentration (MIC) for vancomycin was determined using Etest® gradient method. PCR and sequencing were conducted on the isolates to molecularly detect the presence of *mecA* and *van* genes (*vanA*, *vanB*, *vanC*, *vanD*, *vanR* and *vanXY*). Phenotypic VRSA evaluation showed that 3.92% of *S. aureus* isolates were VRSA, 19.6% of isolates were VISA (vancomycin intermediate *S. aureus*) and 76.47% of isolates were VSSA (vancomycin susceptible *S. aureus*). Twelve (12) isolates that had vancomycin MIC range of 4-16 µg/ml were selected for genotypic evaluation of virulent genes. All isolates amplified with *16SrRNA* signifying all are *S. aureus*. The result shows that 58% of the isolates harbors *mecA* gene, 25% of isolates harbors *vanA* gene, 67% *vanB* gene, 41.7% *vanC*, 58.3% *vanD*, 83.3% *vanR* and 83.3% *vanXY*. The presence of *van* gene was implicated in generation of Vancomycin resistance isolates.

Keywords: *Staphylococcus aureus;* Vancomycin resistance; Clinical isolates

Introduction

The emergence of methicillin resistant *Staphylococcus aureus* and its multidrug resistant profile has put pressure on agents to treat the organism [1]. The treatment of the MRSA infections has become problematic because of the emergence of resistance to vancomycin and other antibiotics, which were effective against MRSA. In clinics, determination of the anti-microbial susceptibility of an isolate is crucial for an optimal therapy [2]. Vancomycin has been considered the mainstay of therapy for infections associated with methicillin-resistant *S. aureus* (MRSA) as well as infections due to methicillin-susceptible *S. aureus* (MSSA) in patients with intolerance to beta-lactam antibiotics [3]. However the increase use of vancomycin has led to the emergence of various vancomycin resistance phenotypes and to the consequent reduction in its efficacy against MRSA in the clinical environment [4]. Various vancomycin resistance phenotypes includes VISA(vancomycin-intermediate *S. aureus*), hVISA (heterogenous vancomycin-intermediate *S. aureus*), VRSA(Vancomycin-resistant *S. aureus*) *S. aureus* strains are defined to be vancomycin resistant(VRSA) at minimum inhibitory concentration (MIC) ≥ 16 µg/ml and vancomycin intermediate *S. aureus* (VISA) at MIC between 4-8 µg/ml by the Clinical and Laboratory Standards Institute (CLSI 2014). Also, the MIC for heterogenous VISA (hVISA) strains was defined by the presence of subpopulations of VISA at a rate of 1 organism per 10^5 to 10^6 organisms [5]. Some case reports had found VRSA in absence of Vancomycin exposure (Whitener, 2003). The increasing prevalence of VRSA is a threat to clinical medicine. Sepsis and other infection caused by Vancomycin resistant strains are very difficult to manage and as there are few options left available to deal with VRSA [6]. Therefore, present study will be conducted to determine the extent of Vancomycin resistance in this locality.

Material and Methods

Study area

The study was carried out using three selected hospitals within Zaria metropolis. The following hospitals were selected for this study base on patients' population, distance apart, good representation of Zaria metropolis: Gambo Sawaba General Hospital Kofangayan, (MIBA) Major Ibrahim B. Abdullahi memorial hospital Sabon Gari Zaria, St. Luke Anglican Hospital Wusasa Zaria, Kaduna State.

Sample size

Sample size was calculated using sampling technique [7] and approximately 350 specimens was used for the research.

Collection of isolates

A total of three hundred and fifty (350) suspected *Staphylococcal* spp. isolated from clinical specimens (blood, urine, high vaginal swab, wound swab, ear swab, urethral swab) submitted to the laboratory of the selected hospitals were collected over a period of 3 months and transported in a sterile ice park to Pharmaceutical Microbiology laboratory, A.B.U, Zaria.

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Identification of *S. aureus* isolates

Microgen *Staphylococcus* identification kit (bioMerieux, Inc, Durham, USA) was used to identify the *S. aureus* isolates. The procedures were carried out according to the manufacturer's instructions.

Antibiotic susceptibility test

Kirby-Bauer Disk diffusion method was employed to perform antibiotics susceptibility test for each of the isolates previously identified as *Staph. aureus* and result interpreted by the Clinical Laboratory Standard Institute. List of antibiotics used are: Cefoxitin (30 µg), Vancomycin (30 µg), Chloramphenicol (30 µg), Amoxicillin (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Teicoplanin (30 µg), Erythromycin (15 µg), oxacillin (30 µg), Clindamycin (30 µg) and Cefixime (30 µg) (Oxoid Ltd.).

Determination of Multiple Antibiotics Resistance (MAR) index

The Multiple Antibiotic Resistance (MAR) index was determined for each isolate by dividing the number of antibiotics to which the organisms are resistant to by the total number of antibiotics tested (Figure 1) [8,9].

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

Determination of minimum inhibitory concentration (MIC) of vancomycin using vancomycin MIC strip

All isolates were tested with Vancomycin MIC Evaluator strip (Oxoid) on Mueller-Hinton agar plates (Figures 2-9). For each isolates a bacterial suspension adjusted to 0.5 McFarland standards were used. The zones of inhibition were read after 24 hours incubation at 37°C.

Bacteria cell preparation

The preparation of the bacteria cell was carried out using the method described by [10]. An overnight culture of the bacteria isolates were cultured in LB broth for 48 h. This is to ensure increased cell mass and yield.

DNA extraction

Genomic DNA extraction was carried out using the method described by Zymogen^{R} manufacturer protocols.

Detection of resistant gene using primers by PCR

mecA and *van* genes (*vanA*, *vanB*, *vanC*, *vanD*, *vanX*, *vanXY*, *vanR*) [11,12] including *16SrRNA* [13] primers were used for PCR. PCR was performed with the following thermal settings conditions:

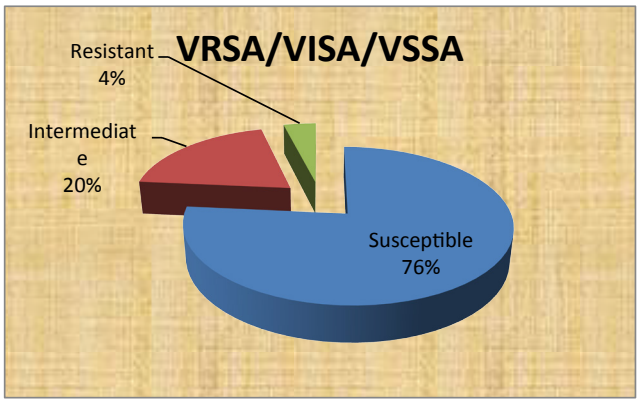

Figure 1: Occurrence of VRSA among *S. aureus* isolated from hospitals in Zaria.

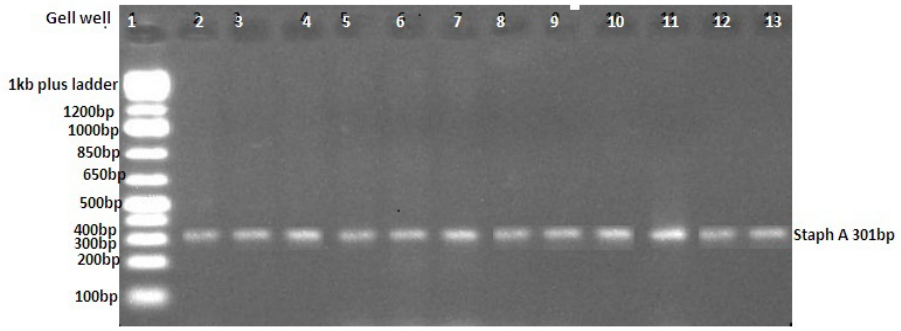

Figure 2: Electrophoretic gel of *Staph A* (301bp) amplified from *S. aureus* isolates.

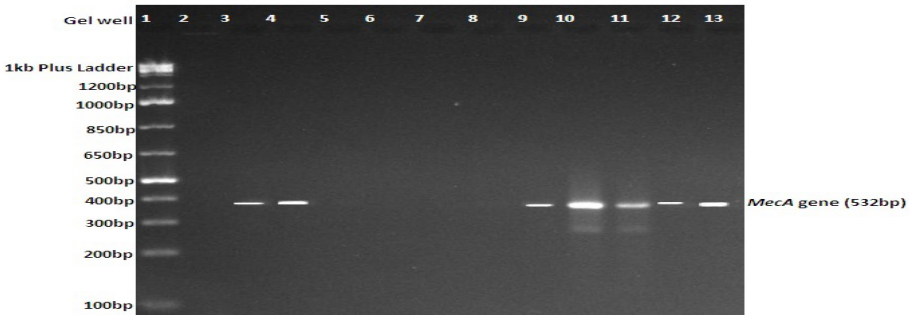

Figure 3: Electrophoretic gel of *mecA* (532bp) amplified from *S. aureus* isolates.

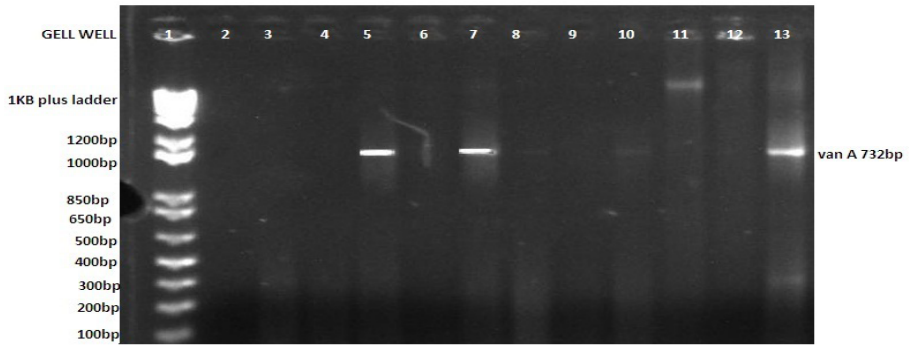

Figure 4: Electrophoretic gel of *vanA*(732bp) amplified from *Staphylococcus aureus* isolates.

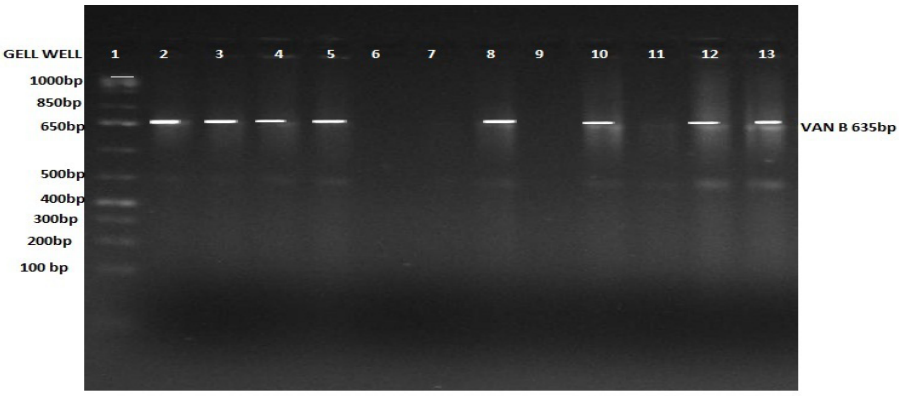

Figure 5: Electrophoretic gel of *vanB* (635bp) amplified from *Staphylococcus aureus* isolates.

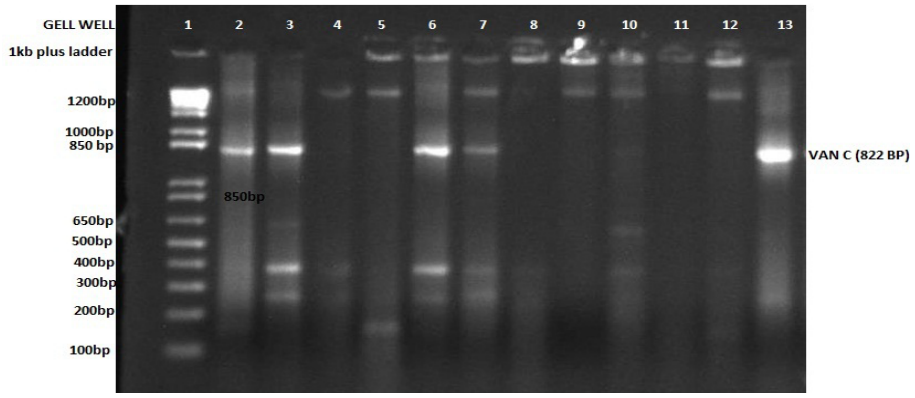

Figure 6: Electrophoretic Gel of *vanC* (822bp) amplified from *Staphylococcus aureus* isolates.

5 min at 94°C for initial enzyme activation, followed by 40 cycles of amplification consisting of denaturation at 94°C for 30s for *mecA* and 1 min for *van genes*, annealing at 57°C for 45s for *mecA* and at 55°C for 1 min for *van* genes, and extension at 72°C for 30 s for *mecA* and 2 min for *van* genes, with a final extension at 72°C for 5 min (Table 1) [14].

Results

Occurrence of *S. aureus* among the collected isolates from the Hospitals

A total of 350 isolates were collected from three Hospitals in Zaria and were analyzed for *S. aureus*. The prevalence of *S. aureus* in this study was observed to be 14.6% of the suspected *Staphylococcal* isolates collected. HGSGH had the highest occurrence of *S. aureus* (20.67% (31)), followed by MIBA [11% (11)] while SLAH had the least occurrence (Table 2).

Keys: SLAH=St. Luke Anglican Hospital. Wusasa, MIBA=Major Ibrahim B. Abdullahi memorial hospital Sabon Gari Zaria, HGSGH= Hajiya Gambo Sawaba General Hospital, Kofan-Gayan.

Distribution of *S. aureus* isolates by source

Base on sample source, HVS (39%) and urine (31%) samples had the highest occurrence of *S. aureus* followed by wound (17%) and Ear

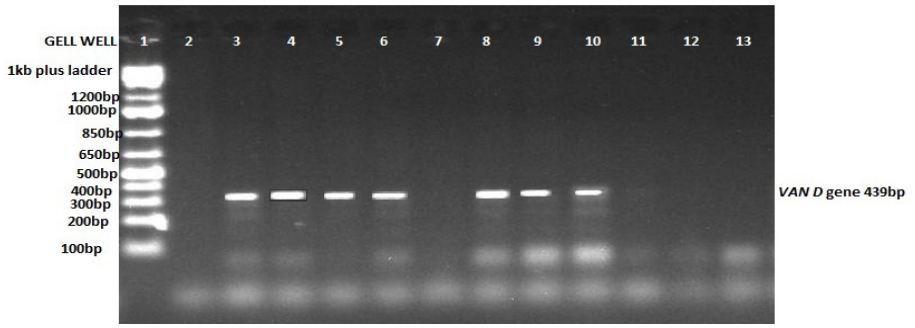

Figure 7: Electrophoretic gel of *vanD* (439bp) amplified from *Staphylococcus aureus* isolates.

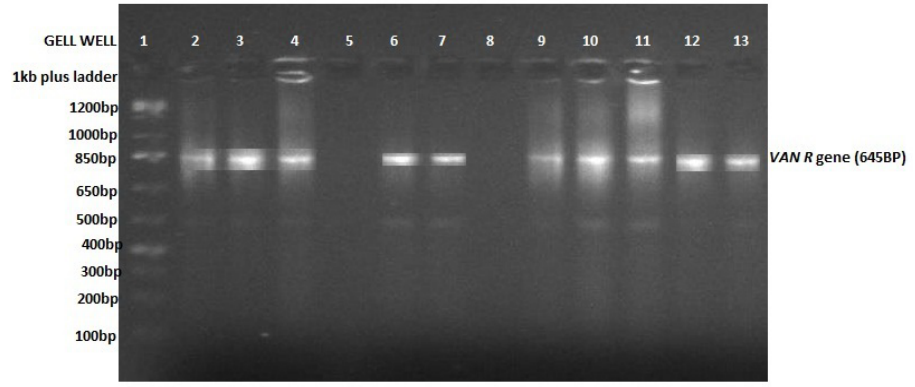

Figure 8: Electrophoretic gel of *vanR* (645bp) Amplified from *S. aureus* isolates.

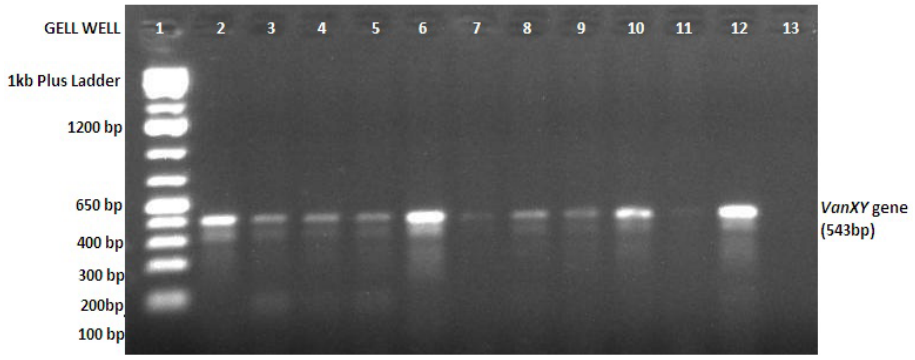

Figure 9: Electrophoretic gel of *vanXY* (543bp) amplified from *S. aureus* isolates.

swabs (9.8%) samples while Urethral swab had the least occurrence of *S. aureus* (2%) (Table 3).

Antibiotics susceptibility profile of *S. aureus* isolates

The isolates were susceptible to Gentamycin (96.1%), Chloramphenicol (82.4%), Ciprofloxacin (78.4%), Vancomycin (76.5%), Erythromycin (58.8%) and Teicoplanin (52.9%), while resistant to Cefoxitin (98%), Cefixime (100%), Amoxicillin (100%) and Oxacillin (100%) as shown in (Table 4).

Multiple antibiotic resistance (MAR) index of isolates

The multiple antibiotic resistant indexes (MARI) for each of the 51 isolates were determined as:

$$\text{MARI} = \frac{\text{Number of antibiotics to which isolate is resistant}}{\text{Total number of antibiotics used}}$$

MARI showed that all 51 (100%) isolates were resistant to three or more antibiotics. MARI ≥ 0.3 indicated that the isolates originated from an environment where antibiotics were frequently used. Two isolates showed 90% resistance to the eleven antibiotics tested. All the isolates were consistently resistant to amoxicillin, cefixime and oxacillin (Table 5).

Phenotypic prevalence of VRSA among *S. aureus* isolates

The result shows that the MIC (minimum inhibitory concentration) obtained for 51 isolates of *S. aureus,* 39 (76.5%) were susceptible to vancomycin, 10 (19.6%) were intermediate resistant

Primer	Forward	Reverse	Bp	Reference
16SrRNA	AATCTT TGTCGG TAC ACG ATATTCTTC ACG	CGTAATGAG ATT TCA GTA GAT AATACA ACA	107	Martineau *et al*. [13]
mecA	AAA ATCGAT GGTAAA GGT TGGC	AGT TCT GCA GTACCG GAT TTG C	532	Strommenger *et al*. [11]
vanA	GGGAAAACGACAATTGC	GTACAATGCGGCCGTTA	732	Nicole *et al*. [12]
vanB	ATGGGAAGCCGATAGTC	GATTTCGTTCCTCGACC	635	
vanC	GGTATCAAGGAAACCTC	CTTCCGCCATCATAGCT	822	
vanD	CTCCTACGATTCTCTTG	CGAGCAAGACCTTTAAG	439	
vanXY	AATAGCTATTTTGATTTCCCCGTTA	TCCTGAGAAAACAGTGCTTCATTAA	543	
vanR	AGCGATAAAATACTTATTGTGGA	CGGATTATCAATGGTGTCGTT	645	Anahita *et al*. [19]

Table 1: Primers sequence and base pair.

Hospital	No. of Isolates screened	No of *S. aureus* isolated	% Prevalence of *S. aureus*
SLAH	100	11	11.00
MIBA	100	9	9.00
HGSGH	150	31	20.67
Total	350	51	14.6

Table 2: Occurrence of *S. aureus* among presumed *Staphylococci* isolates from the hospitals.

S/No	SOURCE	NUMBER (%)
1	High Vagina Swab	20 (39%)
2	Urine	16 (31%)
3	Wound	9 (17%)
4	Ear Swab	5 (9.8%)
5	Urethral Swab	1 (2%)
	TOTAL	**51**

Table 3: Distribution of *S. aureus* isolates by source.

S/No	Antibiotic (µg)	Sensitive (%)	Intermediate (%)	Resistant (%)
1	Vancomycin (30)	39(76.5)	0(0)	12(23.5%)
2	Tecoiplanin (30)	27(52.9)	18(35.3)	6(11.8%)
3	Erythromycin (15)	30(58.8)	8(17.6)	13(23.5%)
4	Gentamycin (30)	49(96.1)	0(0)	2(3.92%)
5	Amoxicillin(10)	0(0)	0(0)	51(100%)
6	Chloramphenicol(30)	42(82.4)	3(5.9)	6(11.8%)
7	Cefoxitin(30)	1(1.96)	0(0)	50(98%)
8	Clindamycin(2)	18(35.3)	21(43.1)	12(21.63%)
9	Ciprofloxacin(5)	40(78.4)	2(3.9)	9(17.65%)
10	Oxacillin(30)	0(0)	0(0)	51(100%)
11	Cefixime(30)	0(0)	0(0)	51(100%)

Table 4: Antibiotics susceptibility profile of *S. aureus* isolates.

to vancomycin and 2 (3.9%) were totally resistant to vancomycin, as shown in Figure 1.

Molecular characterization of antibiotic resistant genes in isolated *S. aureus*

Molecular characterization of *Staph A 30* (16S rRNA)

Molecular detection of *Staph A* 30 (16S rRNA) was carried out using the specific primers for chromosomal fragment of *Staphylococcus aureus.* This study revealed that all isolates on gel electrophoresis in the DNA extracted were *Staphylococcus aureus* (Figure 2).

Keys: Lane 1=1 kb DNA ladder, Lane 2=U8, Lane 3=U12, Lane 4=W18, Lane 5=U53, Lane 6=W19, Lane 7=U5, Lane 8=U4, Lane 9=U9, Lane 10=HVS2, Lane 11=SBU10, Lane 12=U45, Lane 13=W17.

No. of antibiotic to which resistant	Resistant isolates	MAR index	% of *S. aureus* to MARI
1	0	0	0
2	0	0.1	0
3	1	0.2	1.96
4	14	0.3	27.45
5	21	0.4	41.17
6	10	0.5	19.6
7	1	0.6	1.96
8	1	0.7	1.96
9	1	0.8	1.96
10	2	0.9	3.92
11	0	1.0	0

Table 5: Multiple Antibiotic Resistance (MAR) index of isolates.

Primers	Number (%)
MecA	7 (58)
vanA	3 (25)
VanB	8 (67)
VanC	5 (41.7)
VanD	7 (58)
VanR	10 (83.3)
VanXY	10 (83.3)

Table 6: Percentage occurrence of virulent genes.

Molecular characterization of *mecA* gene

Molecular detection of *mecA* gene revealed that only 7 (58%) of the isolates amplified with *mecA* gene on agarose gel electrophoresis. Hence amplification on Lane 3, 4, 9, 10, 11, 12 and 13 (Figure 3)

Keys: Lane 1=1 kb DNA ladder, Lane 2= U8, Lane 3=U12, Lane 4=W18, Lane 5=U53, Lane 6=W19, Lane 7=U5, Lane 8=U4, Lane 9=U9, Lane 10=HVS2, Lane 11=SBU10, Lane 12=U45, Lane 13=W17.

Molecular characterization of *vanA* gene

Molecular detection of *vanA* gene revealed that only 3 (25%) of the isolates amplified with *vanA* gene on agarose gel electrophoresis. Hence amplification on Lane 5, 7 and 13 (Figure 4).

Keys: Lane 1=1 kb DNA ladder, Lane 2= U8, Lane 3=U12, Lane 4=W18, Lane 5=U53, Lane 6=W19, Lane 7=U5, Lane 8=U4, Lane 9=U9, Lane 10=HVS2, Lane 11=SBU10, Lane 12=U45, Lane 13=W17.

Molecular characterization of *vanB* gene

Molecular detection of *vanB* generevealed that only 8(67%) of the isolates amplified with *vanB* gene on agarose gel electrophoresis. Hence amplification on Lane 2, 3, 4, 5, 8, 10, 12 and 13 (Figure 5).

Keys: Lane 1=1 kb DNA ladder, Lane 2= U8, Lane 3=U12, Lane 4=W18, Lane 5=U53, Lane 6=W19, Lane 7=U5, Lane 8=U4, Lane 9=U9, Lane 10=HVS2, Lane 11=SBU10, Lane 12=U45, Lane 13=W17.

Molecular characterization of *vanC* gene

Molecular detection of *vanC* gene revealed that only 5(41.7%) of the isolates amplified with *vanC* gene on agarose gel electrophoresis. Hence amplification on Lane 2, 3, 6, 7 and 13 (Figure 6).

Keys: Lane 1=1 kb DNA ladder, Lane 2= U8, Lane 3=U12, Lane 4=W18, Lane 5=U53, Lane 6=W19, Lane 7=U5, Lane 8=U4, Lane 9=U9, Lane 10=HVS2, Lane 11=SBU10, Lane 12=U45, Lane 13=W17.

Molecular characterization of *vanD* gene

Molecular detection of *vanD* gene revealed that only 7(58.3%) of the isolates amplified with *vanD* gene on agarose gel electrophoresis. Hence, amplification on Lane 3, 4, 5, 6, 8 (Figure 7).

Keys: Lane 1=1 kb DNA ladder, Lane 2= U8, Lane 3=U12, Lane 4=W18, Lane 5=U53, Lane 6=W19, Lane 7=U5, Lane 8=U4, Lane 9=U9, Lane 10=HVS2**,** Lane 11=SBU10, Lane 12=U45, Lane 13=W17.

Molecular characterization of *vanR* gene

Molecular detection of *vanR* gene revealed that only 10 (88.3%) of the isolates were positive for *vanR* gene on agarose gel electrophoresis. Hence amplification on Lane 2, 3, 4, 6, 7, 9, 10, 11, 12 and 13 (Figure 8).

Keys: Lane 1=1 kb DNA ladder, Lane 2= U8, Lane 3=U12, Lane 4=W18, Lane 5=U53, Lane 6=W19, Lane 7=U5, Lane 8=U4, Lane 9=U9, Lane 10=HVS2, Lane 11=SBU10, Lane 12=U45, Lane 13=W17.

Molecular characterization of *vanXY* gene

Molecular detection of *vanXY* gene revealed that only 10 (88.3%) of the isolates were positive for *vanXY* gene on agarose gel electrophoresis. Hence amplification on Lane 2, 3, 4, 5, 6, 7, 8, 9, 10 and 12 (Figure 9).

Keys: Lane 1=1 kb DNA ladder, Lane 2= U8, Lane 3=U12, Lane 4=W18, Lane 5=U53, Lane 6=W19, Lane 7=U5, Lane 8=U4, Lane 9=U9, Lane 10=HVS2, Lane 11=SBU10, Lane 12=U45, Lane 13=W17.

Percentage occurrence of virulent genes

The percentage of occurrence for vancomycin resistant genes detected is presented below in (Table 6).

Discussion

The rate of resistant strains occurrence is associated to lack of strict measures in the use of antibiotics in these hospitals by both health practitioners and the community. It is a well-known fact that development of antibiotics resistance in most organisms is associated with unjustified, irrational and irregular use of antibiotics by the human population, over the counter accessibility without recommendations and unrestricted use of antimicrobials in poultry, farm animals, and fisheries [15]. This study shows that phenotypic occurrence of VISA isolates is more than VRSA and this agrees with the study of [16], who reported that higher rate of VISA occurred than VRSA. VRSA reports in this study agrees with the study of [17] who reported phenotypic occurrence of VRSA in southern parts of Nigeria ranging from 0% to 6% among clinical isolates. But contrary to the reports of [18] who reported phenotypic prevalence rate of VRSA to be 57.7% in Zaria using disc methods. Though disc method is sometime not reliable, further finding are recommended to validate the superiority of MIC strip over disc method. Further evaluation of the findings of this study showed that the twelve (12) isolates that were multidrug resistant and exhibited either VISA or VRSA phenotypically, expressed three or more of the vancomycin resistant determinant genes (*mecA, vanA, vanB, vanC, vanD, vanR and vanXY*). This is in line with the reports of [19-21] who reported the expression of more than one of these genes in the isolates tested. However, there are limited reports on expression of *vanXY* in *S. aureus.* The carriage of *van* genes by intermediate resistance isolates [21] and none expression of the *van* genes [22] have been reported. Other reports supporting the findings of this study suggest that isolates with v*anA* gene expresses high level of resistance while isolates with *VanB* gene exhibits variable resistance to most antibiotics tested. More so, carriage of *vanC* and *vanXY* signifies low resistance while *vanD* shows moderate resistance [23-26]. Nevertheless some of the isolates that were vancomycin resistant are still susceptible to other antibiotics like gentamicin, chloramphenicol, ciprofloxacin and erythromycin. This could be as a result of adaptation to a tailored resistant mode, which give other antibiotic with different mechanism of action advantage over then. Some Case reports have also found VRSA in the absence of Vancomycin exposure [19,21] which is similar to this study. Vancomycin antibiotic are rarely used in the hospitals where the isolates were obtained, however the current study has exposed the presence of phenotypic and genotypic vancomycin resistant *S. aureus* strains in these hospitals. This might be an indication that a large proportion of the bacteria isolates have been pre-exposed to several antibiotics, and also, may be due to a combination of microbial characteristics such as selective pressure on antimicrobial usage, societal and technological changes that enhance the transmission of drug resistant organisms [26]. Other reasons could be due to increase in irrational consumption of antibiotics and transmission of resistant isolates between people [9]. It has therefore become necessary for further surveillance studies to be performed especially in this locality where there is limited documentations of procedures and preservation of clinical data. Due to the presence of VRSA in this locality, it is however necessary to impose restrictions on the use of the available antibiotics and other prescription drugs, to patients in both hospitals and community practice and to develop new drugs to combat the menace of multidrug failure.

Conclusion/Recommendation

This study has exposed the presence of Vancomycin resistant *S. aureus* encoding genes other than *vanA* gene in hospitals where vancomycin is rarely used, and also show cases limited reports on the prevalence of *van* resistant genes in our environment. The presence of these other cluster genes (*vanB, vanC, vanD, vanR* and *vanXY*) exhibited high level of resistance to the antibiotics tested. It has therefore become necessary for further periodic surveillance studies to be performed in this locality and to impose restrictions on prescriptions and abuse of available antibiotics by patients and professionals in both hospitals and community practice.

Ethical Approval

Ethical clearance was obtained from Ethical committee of the Ministry of health, Kaduna State.

Conflict of Interest

The authors declare that they have no conflict of interest on the findings reported in this study.

Reference

1. Thati V, Shivannavar CT, Gaddad SM (2011) Vancomycin resistance among methicillin resistant Staphylococcus aureus isolates from intensive care units of tertiary care hospitals in Hyderabad. Indian J Med Res 134: 704-708.
2. Mathews AA, Thomas M, Appalaraju B, Jayalakshmi J (2010) Evaluation and comparison of tests to detect methicillin resistant Staphylococcus aureus. Indian J Pathol Microbiol 530: 79-82.
3. Uttley AH, George RC, Naidoo J, Woodford N, Johnson AP, et al. (1989) vancomycin-resistant enterococci causing hospital infections. Epidemiol Infect 103: 173-181.
4. Howden BP, Peleg AY, Stinear TP (2014) The evolution of vancomycin intermediate Staphylococcus aureus (VISA) and heterogeneous VISA. Infect Genet Evol 21: 575-582.
5. Hiramatsu K (2001) Vancomycin-resistant Staphylococcus aureus: a new model of antibiotic resistance. Lancet Infect Dis 1: 147-155.
6. Appelbaum PC (2007) Reduced glycopeptide susceptibility in methicillin-resistant Staphylococcus aureus (MRSA). Int J Antimicrob Agents 30: 398-408.
7. Kadam P, Bhalerao S (2010). Sample Size Calculation. Int J Ayurveda Res 1: 55-57.
8. Paul S, Bezbaruah RL, Roy MK, Ghosh AC (1997) Multiple antibiotic resistance (MAR) index and its reversion in Pseudomonas aeruginosa. Lett Appl Microbiol 24: 169-171.
9. Christopher AF, Hora S, Ali Z (2013) Investigation of plasmid profile, antibiotic susceptibility pattern multiple antibiotic resistance index calculation of Escherichia coli isolates obtained from different human clinical specimens at tertiary care hospital in Bareilly-India. Annals of Tropical medicine and public health 6: 285-289.
10. Dubey RC (2009) Multicolour Textbook of Biotechnology.4th Edition S. Chand and Co. India Pp: 71-80.
11. Strommenger B, Kettlitz C, Werner G, Witte W (2003) Multiplex PCR Assay for simultaneous Detection of Nine clinical Relevant Antibiotic Resistance genes in Staphylococcus aureus. J Clin Microbiol 41: 4089-4094.
12. van den Braak N, van Belkum A, van Keulen M, Vliegenthart J, Verbrugh HA, et al. (1998) Molecular characterization ofvancomycin-resistant enterococci fromhospitalized patients and poultry products in the Netherlands. J Clin Microbiol 36: 1927-1932.
13. Martineau F, Picard FJ, Lansac N (2001) Correlation between the mecA gene determined by multiplex PCR assays and the disc diffusion susceptibility patterns of Staphylococcus aureus. Chemother 44: 231-238.
14. D Neasy (2006) Animal Tissues (spin-column protocol): purification of Total DNA from animal tissue. Blood and Tissue Handbook Pp: 28-31
15. Elimam MA, Rehan S, Elmekki MA, Elhassan MM (2014) Emergence of Vancomycin Resistant and Methcillin Resistant *Staphylococus aureus* in Patients with Different Clinical Manifestations. J Am Sci 10: 106-110.
16. Liaqat F, Sheikh AA, Nazir J, Hussain T, Rabbani M, et al. (2015) Isolation identification and control of vancomycin resistant Staphylococcus aureus. Pak J Pharm Sci 28: 997-1004
17. Terry-Alli OA, Ogbolu DO, Akorede E, Onemu OM, Okanlawon BM (2011) Distribution of mec A gene amongst Staphylococcus aureus isolates from Southwestern Nigeria. African J Biomed Res 14: 9-16.
18. Olayinka BO, Olayinka AT, Onaolapo JA, Olurinola PF (2005) Pattern of resistance to vancomycin and other antimicrobial agents in staphylococcal isolates in a university teaching hospital. African Clinical Experiment Microbiol 6: 46-52.
19. Dezfulian A, Aslani MM, Oskoui M, Farrokh P, Azimirad M, et al. (2012) Identification and characterization of a high vancomycin-resistant Staphylococcus aureus harboring VanA gene cluster isolated from diabetic foot ulcer. Iranian J Basic Medical Sci 15: 803-806.
20. Saadat S, Solhjoo K, Norooz-Nejad MJ, Kazemi A (2014) VanA and vanB positive vancomycin-resistant Staphylococcus aureus among clinical isolates in Shiraz, South of Iran. Oman Med J 29: 335-339.
21. Zhu X, Liu C, Gao S, Lu Y1, Chen Z, et al. (2015) Vancomycin intermediate-Resistant Staphylococcus aureus (VISA) Isolated from a patient who never received Vancomycin treatment. Int J Infect Dis 33: 185-190.
22. Liaqat F, Sheikh AA, Nazir J, Hussain T, Rabbani M, et al. (2015) Isolation identification and control of vancomycin resistant Staphylococcus aureus. Pak J Pharm Sci 28: 997-1004.
23. Abu Shady HM, El-Essawy AK, Salama MS, El-Ayesh AM (2012) Detection and molecular characterization of vancomycin resistant Staphylococcus aureus from clinical isolates. Afr J Biotechnol 11: 16494-16503.
24. Howden BP, Davies JK, Johnson PD, Stinear TP, Grayson ML (2010) Reduced vancomycin susceptibility in Staphylococcus aureus, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. Clin Microbiol Rev 23: 99-139.
25. Peleg AY, Monga D, Pillai S, Mylonakis E, Moellering RC, et al. (2009) Reduced susceptibility to vancomycin influences pathogenicity in Staphylococcus aureus infection. J Infect Dis 199: 532-536.
26. Orozova P, Chikova V, Kolarova V, Nenova R, Konovsk, M, et al. (2008) Antibiotic resistance of potentially pathogenic Aeromonas strains. Trakia J Sciences 6: 71-78.