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ISOLATION, ANTIBIOGRAM AND CHARACTERIZATION OF VANCOMYCIN-RESISTANT *STAPHYLOCOCCUS AUREUS* FROM CLINICAL AND COMMUNITY ISOLATES IN ABAKALIKI, EBONYI STATE, NIGERIA

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Abstract:

This study was aimed at isolating and characterizing vancomycin-resistant Staphylococcus aureus from clinical and community samples in Abakaliki, Ebonyi State, Nigeria. Seven hundred and nine (709) clinical and community samples were obtained for this study. Antibiotic susceptibility test was done using Kirby Bauer disc diffusion method. Vancomycin resistant Staphylococcus aureus (VRSA) isolates were detected using Kirby-Bauer disc diffusion method with vancomycin antibiotic disc (30 µg). Isolates were also screened for β-lactamase enzyme production. A total of 84 (27.7 %) and 120 (29.5 %) Staphylococcus aureus isolates were obtained from the clinical and community samples respectively using standard microbiological techniques. Results showed that 55 VRSA isolates were obtained from the samples with prevalence frequency of 36.9 % and 20 % for clinical and community isolates respectively. The clinical isolates were completely resistant (100 %) to nitrofurantoin, clindamycin, ceftazidime, tetracycline and penicillin. Gentamicin was the most effective antibiotic against the S. aureus isolates obtained from clinical samples as all the isolates were completely susceptible (100 %). Ciprofloxacin was the most effective antibiotic against the S. aureus isolates obtained from community samples with a susceptibility frequency of 100 %. This was closely followed by gentamicin (75 %) and erythromycin. Exactly 38.1 % and 24.2 % of the clinical and community S. aureus isolates were positive for beta-lactamase production respectively. The HA-VRSA and CA-VRSA isolates had MARI values within the range of 0.5 to 1.0. This present discovery of multi-drug resistant VRSA with high multiple antibiotic resistance indices is in Abakaliki is a serious public health issue. Therefore, there is an urgent need to keep a strict watch on VRSA emerging from Abakaliki.

Keywords: *CA-VRSA, HA-VRSA, MARI, beta-lactamase, antibiotics*

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INTRODUCTION:

Staphylococcus aureus has been implicated in a wide range of infection ranging from acute to chronic infections such as boils, bacteriuria, osteomyelitis, pneumonia, endocarditis, meningitis, septicemia and arthritis. This organism is a leading cause of human bacterial infection worldwide and is endemic in both hospital and the community (Chambers and Deleo, 2009). Multiple antibiotic resistant *Staphylococcus aureus* are major threats to patients' care, owing to their stubborn intransigence to chemotherapy and disinfection (Slade *et al*., 2009). The resistant bacteria may spread and create broader infection control problems, both within healthcare institutions and in the community. The infections especially caused by *Staphylococcus aureus* have affected substantial portions of the human population, causing significant mobility and mortality (Fred, 2006). Prolonged therapy with vancomycin may lead to development of low-level resistance that compromise therapy, but that may not be detected by routine susceptibility testing methods used in hospital laboratory (Tenover, 2007). Most Staphylococcal infections are associated with serious community-acquired and nosocomial diseases which arise often in individuals with predisposing risk factors such as haemodialysis or surgery. It causes superficial, deep-skin, soft tissue infections, endocarditis and bacteremia with metastatic abscess formation and a variety of toxin–mediated diseases including gastroenteritis, staphylococcal scalded skin syndrome, toxic shock syndrome, meningitis, septicaemia and arthritis (Amghalia *et al*., 2009). After several attempts, scientist discovered the use of vancomycin as the frontier in the fight against *Staphylococcus aureus* infection. Until recently, vancomycin was the only uniformly effective antibiotic for the treatment of *Staphylococcus* infection. The euphoria was however cut short in 1997 by the detection of VRSA in Japan from clinical isolates. Based on the recognition that management of severe *Staphylococcus aureus* disease is challenging, research is needed to further evaluate the prevalence of vancomycin resistant *Staphylococcus aureus* (VRSA) in Abakaliki, Ebonyi State.

MATERIALS AND METHODS:

Sample collection

A total of 709 samples were collected for this study. Three hundred and three (303) were clinical samples (wound (36), pus (16), urine (99), HVS (41), ear swab (37), sputum (35) and semen (39) of patients visiting Federal Teaching Hospital Abakaliki (FETHA I and II) and Mile Four General Hospital, Abakaliki while 406 (nasal (197) and ear swabs (209) were community samples. Wound, pus, HVS and ear swab samples were collected using sterile swab sticks while urine, semen and sputum samples were collected using sterile specimen bottles. In the same way, the four hundred and six (406) community samples (nasal and ear swabs) were collected from apparently healthy tutors (121), artisans (62), secondary school students of Abakaliki high school (153) and petty traders from Kpirikpiri Market (70). The Hospital patients' group includes individuals who are at greater risk of becoming infected by this opportunistic pathogen. These individuals are generally older, have chronic underlying illnesses, and require more frequent interactions with healthcare facilities; all of which predispose them to more serious infections. The Community group includes individuals who, in general, are otherwise healthy. They are usually not predisposed by age or underlying illness to these infections, but predisposed by specific activities and community interactions that place them at an increased risk for infection contraction.The collected samples were immediately transported to the department of Applied Microbiology laboratory unit of Ebonyi State University, Abakaliki for bacteriological analysis.

Culturing, isolation, characterization and identification of the isolates

The clinical and community samples were aseptically inoculated on Mannitol Salt broth and incubated at 37 ^{0}C for 48 hours. A loopful of the inoculated mannitol salt broth was later streaked on mannitol salt agar (MSA) and incubated at 37 ^{0}C for 24 hours. The plates were observed for creamy golden β-haemolytic colonies which are typical characteristic of *Staphylococcus aureus*. These suspected *S. aureus* isolates were further characterized using conventional/standard microbiology techniques such as colony morphology, Gram-staining, catalase test, motility test and other biochemical tests which include oxidase test, indole test, Simmon's citrate test, H_2S production test, voges proskauer test, methyl red test, sugar fermentation test, coagulase test and *Staphylococcus* lactase agglutination assay (Cheesbrough, 2004).

Antibiotic Susceptibility Test

The susceptibility patterns of isolated *S. aureus* isolates were determined by the Kirby and Bauer disc diffusion method as recommended by CLSI (CLSI, 2009). Each of the isolate was standardized to 0.5 Macfarland equivalent and aseptically inoculated on prepared Muller-Hinton agar plates using sterile swab stick. The inoculated plates were allowed to stand for 10-15 minutes. Antibiotic impregnated discs namely penicillin G (10 µg), tetracycline (30 µg), gentamicin (30 µg), nitrofurantoin (300 µg), erythromycin (15

µg), ciprofloxacin (5 µg), ceftazidime (30 µg), sulphamethoxazole (25 µg) and clindamycin (2 µg) (Oxoid, UK) were placed on the inoculated plates using sterile forceps. The plates were incubated at 37 ^{0}C for 24 hours after which the zones of inhibition around each disc were measured to the nearest mm with a metre rule, recorded and interpreted according to the Clinical Laboratory Standard Institute (CLSI, 2009) guidelines.

Detection of vancomycin resistant *Staphylococcus aureus* (VRSA)

This was done using Kirby Bauer disc diffusion method according to Clinical and Laboratory Standard Institute (CLSI, 2009) guidelines. A Mueller-Hinton agar plate was prepared according to manufacturer's specification. Colonies of the isolated bacteria were suspended in 5 ml of nutrient broth. The turbidity of the broth culture was adjusted to 0.5 McFarland standard, which approximately equals 1.5 10^8 CFU/ml.Standardized inoculum was swabbed onto the prepared Mueller-Hinton agar plate. After at least 3 minutes, antibiotic disc impregnated with vancomycin (30 µg) was placed on Mueller-Hinton agar plate for VRSA detection. The plate was then incubated at 37 ^{0}C for 24 hours. Inhibition zone diameter was measured to nearest millimeter and interpreted according to CLSI guidelines.

Beta-Lactamase Detection Using Nitrocefin Stick

This was done to detect the production of beta-lactamases by the *S. aureus* isolates. Before inoculation, the nitrocefin stick was allowed to cool to room temperature. A drop of distilled water was used to moderately moisten the tip of the nitrocefin stick. After this, the colour coded end of the stick was used to touch the colony by making sure that the reagent on the brown tip of the stick was rotated to pick mass of the cells. The colour coded tip was observed after 5 minutes for pink-red colour development to show the production of beta-lactamase.

Determination of multiple antibiotic resistance index (MARI)

Multiple antibiotic resistance indices (MARI) of the *S. aureus* isolates were calculated using the technique described by Christopher *et al*. (2013) and Subramani *et al*. (2012). This was calculated as the number of antibiotics to which the tested isolate was resistant to (a), divided by the total number of antibiotics that was tested on the isolates (b).

RESULTS:

Table 1: Clinical samples collected from Federal Teaching Hospital Abakaliki (FETHA I)

Sample source	No. of samples collected	No. of *S. aureus* isolated	No. of VRSA isolated
Wound swab	21	11	5
Urine	33	6	2
High vaginal swab	11	2	1
Sputum	7	2	0
Ear swab	17	9	2
Pus	7	2	1
Semen	21	4	0
TOTAL	**117**	**36 (30.8 %)**	**11 (30.6 %)**

Table 2: Clinical samples collected from Federal Teaching Hospital Abakaliki (FETHA II)

Sample source	**No. of samples collected**	**No. of *S. aureus* isolated**	**No. of VRSA isolated**
Wound swab	10	7	3
Urine	35	7	2
High vaginal swab	13	4	2
Sputum	8	2	1
Ear swab	10	5	3
Pus	9	4	2
Semen	18	2	0
TOTAL	**103**	**31 (30.1 %)**	**13 (41.9 %)**

Table 3: Clinical samples collected from Mile Four General Hospital Abakaliki

Sample source	**No. of samples collected**	**No. of *S. aureus* isolated (%)**	**No. of VRSA isolated**
Wound swab	5	3	2
Urine	31	4	2
High vaginal swab	17	3	0
Sputum	20	3	1
Ear swab	10	4	2
Pus	0	0	0
Semen	0	0	0
TOTAL	**83**	**17 (20.5 %)**	**7 (41.2 %)**

Table 4: Samples collected from apparently healthy individuals in the Community

Occupation	Ear Swab		Nasal Swab		Total	
	No of Samples	No of *Staph.* Isolated	No of Samples	No of *Staph.* Isolated	Sample	Isolate
Tutors	66	25	55	19	121	44
Artisans	32	13	30	9	62	22
Students	73	18	80	14	153	32
Petty Traders	38	12	32	10	70	22
Total	**209**	**68**	**197**	**52**	**406**	**120 (29.6 %)**

Table 5: Prevalence of VRSA in Hospital and Community isolates

Sample source	VRSA	
	No. of isolate tested	No. positive
Hospital	84 (27.7 %)	31 (36.9 %)
Community	120 (29.6 %)	25 (20 %)

Table 6: Occupational distribution of population with Community Acquired Vancomycin Resistant *Staphylococcus aureus* (CA-VRSA)

Occupation	Nasal Swab		Total		Total		
	M	F	M	F	M	F	
Tutor	1	3	2	4	3	7	10
Artisan	1	2	3	0	4	2	6
Petty Trader	0	1	1	1	1	2	3
Student	1	2	1	1	2	3	5
TOTAL	**3**	**8**	**7**	**6**	**10**	**14**	**24**

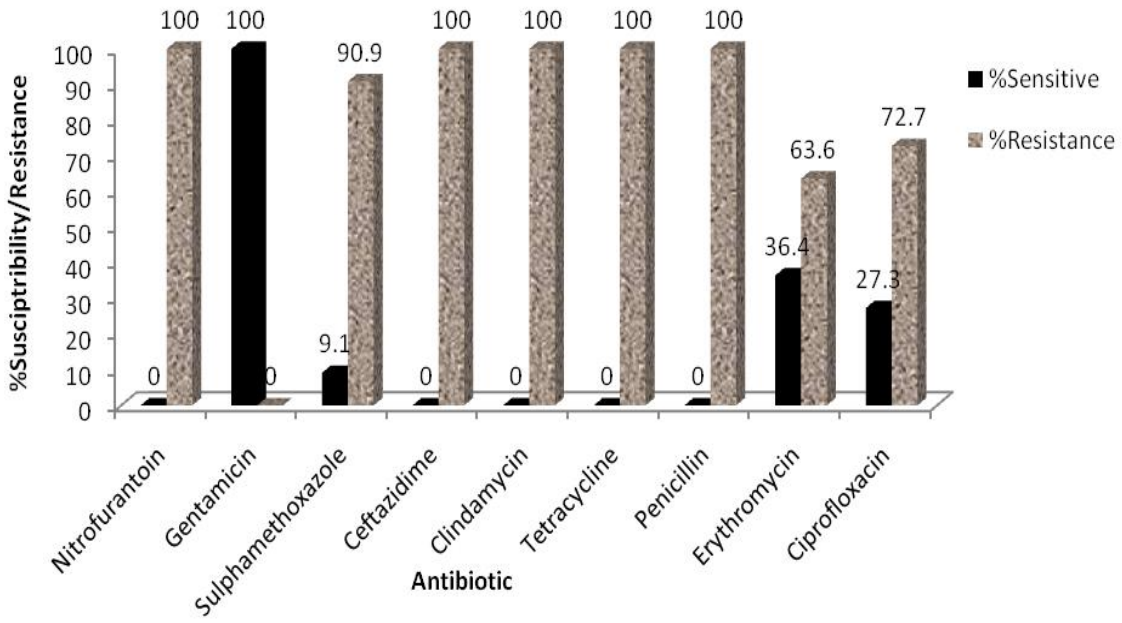

Fig 1: Percentage Susceptibility and Resistance patterns of Hospital Acquired Vancomycin Resistant *Staphylococcus aureus* (HA-VRSA) to antibiotics

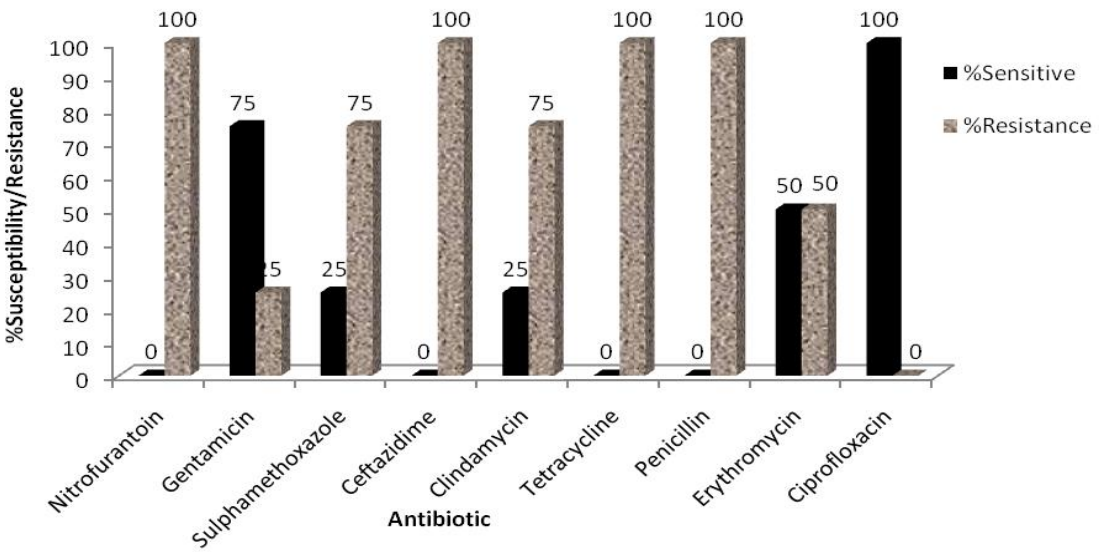

Fig 2: Percentage Susceptibility and Resistance patterns of Community Acquired Vancomycin Resistant *Staphylococcus aureus* (CA-VRSA) to antibiotics

Table 7: Beta-lactamase detection in Hospital and Community isolates

Source	No of isolates tested	Beta-lactamase positive
Hospital	84	32
Community	120	29
Total	**204**	**58**

Table 8: Multiple Antibiotic Resistance Indices (MARI) of Hospital Acquired Vancomycin Resistant *Staphylococcus aureus* (HA-VRSA) isolates

S/NO	ISOLATE CODE	F	CN	SXT	CAZ	DA	TE	P	E	CIP	MAR Index(a/b)
1	39A	14	20	18	NI	NI	17	NI	19	23	0.5
2	12A	15	22	NI	NI	NI	11	6	8	19	0.8
3	15A	14	18	10	NI	NI	13	12	16	21	0.7
4	18A	13	19	NI	NI	NI	NI	4	19	NI	0.7
5	34A	15	19	9	NI	8	12	8	19	17	0.7
6	5A	16	22	NI	NI	5	11	NI	17	19	0.6
7	6A	12	20	16	NI	14	11	9	20	14	0.7
8	11B	10	18	NI	NI	15	12	14	NI	14	0.8
9	16B	NI	18	NI	NI	NI	5	11	NI	22	0.7
10	21B	15	19	NI	NI	14	9	6	27	18	0.7
11	23B	16	23	NI	NI	15	9	11	14	18	0.8
12	13A	17	10	7	NI	12	NI	7	18	NI	0.7

Key: F= Nitrofurantoin, CN= Gentamicin, SXT= Sulphamethoxazole, CAZ= ceftazidime, DA= Clindamycin, TE= Tetracycline, P= Penicillin G, E= Erythromycin, CIP= Ciprofloxacin NI= No Inhibition

Table 9: Multiple Antibiotic Resistance Indices (MARI) of CA-VRSA isolates

S/NO	ISOLATE CODE	F	CN	SXT	CAZ	DA	TE	P	E	CIP	MAR Index(a/b)
1	3C	16	9	4	NI	15	20	4	11	24	0.7
2	30C	6	NI	NI	NI	NI	NI	NI	NI	19	1
3	47C	10	14	NI	NI	NI	NI	NI	NI	22	0.8
4	13C	16	19	6	NI	19	15	9	23	24	0.5
5	11C	17	14	NI	NI	5	30	6	24	26	0.5

Key: F= Nitrofurantoin, CN= Gentamicin, SXT= Sulphamethoxazole, CAZ= ceftazidime, DA= Clindamycin, TE= Tetracycline, P= Penicillin G, E= Erythromycin, CIP= Ciprofloxacin NI= No Inhibition

DISCUSSION:

Staphylococcus aureus has long been recognized as one of the major human pathogens responsible for hospital and community acquired infections. Infections caused by vancomycin resistant *S. aureus* (VRSA) have been associated with high morbidity and mortality rates. In view of the fact that *Staphylococcus aureus* has the capacity to change over time, VRSA will keep on being a problem in the future, as it has been long-ago. In this study, 36 (30.8 %) *S. aureus* isolates were obtained from 117 clinical samples collected from FETHA I. Eleven (30.6 %) out of these isolates were identified as vancomycin resistant *Staphylococcus aureus* (VRSA) (Table 1). In FETHA II, 31(30.1 %) *S. aureus* isolates were obtained from the 103 clinical samples collected from patients. Thirteen (41.9 %) of these isolates were identified as VRSA (Table 2). In Mile Four Hospital, 17 (20.5 %) *S. aureus* isolates were obtained from 83 clinical samples. Seven (41.2 %) of the isolates were identified as VRSA (Table 3). One hundred and twenty (29.6 %) *S. aureus* isolates were obtained from the 496 community samples (nasal and ear swabs) collected from tutors, artisans, students and petty traders (Table 4). This study is in agreement with the work of Olowe *et al*. (2007) in Osogbo, South-western Nigeria. They reported the isolation of 42.9 % *S. aureus* from Staphylococci isolates. Thirty one (36.9 %) isolates were confirmed to be vancomycin resistant *Staphylococcus aureus* (VRSA) out of the 84 *Staphylococcus aureus* isolated from the clinical samples (wound, pus, urine, HVS, ear swab, sputum and semen) (Table 5). Similarly, 24 (20 %) community-associated vancomycin resistant *S. aureus* (CA-VRSA) were detected from the 120 *Staphylococcus aureus* isolates obtained from the community samples of nasal and ear swabs (Table 5). The distribution of VRSA isolated from the three major hospitals in Abakaliki showed that the highest prevalence frequency of VRSA was found in FETHA II (13 (41.9 %)). This was closely followed by FETHA I (11 (35.5 %) and Mile Four General Hospital ((7 (22.6 %) being the least (Table 5). The highest number of VRSA was isolated from wound samples (10); followed by ear swabs (7), urine (6), HVS (3) and pus (3). The least prevalence of VRSA was observed in sputum samples (2). There was no VRSA isolate from semen samples (Tables 1, 2 and 3). This is similar to the report of Olowe *et al*. (2007) who reported that wound samples had the highest prevalence of *S. aureus*. Many researchers have reported an increase in the incidence of *S. aureus* most of which originated from wounds (Vidhani *et al*., 2001). CA-VRSA was most predominant among tutors (10). The overcrowded nature of tutors' staff rooms and irrational use of antibiotics might possibly be the reason for the high prevalence of VRSA among them. This is because overcrowded living places have been seen as one of the risk factors for community-acquired *S. aureus* (Scerri *et al*., 2013). The least number of CA-VRSA was observed among petty traders (Table 6). CA-VRSA isolates were more prevalent in nasal swabs than ear swabs (Table 6)**.**This study is in concord with the report of Aligholi *et al*. (2008) who reported a high prevalence of VRSA (41.85 %) in Tehran. The VRSA isolates (HA-VRSA) obtained from the clinical samples in the hospitals were multidrug resistant as they were resistant to at least two classes of antibiotics (Figure 1). The clinical isolates were completely resistant

(100 %) to nitrofurantoin, clindamycin, ceftazidime, tetracycline and penicillin. They also exhibited high level of resistance to sulphamethoxazole-trimethoprin (90.9 %). Gentamicin was the most effective antibiotic against the *Staphylococcus aureus* isolates obtained from clinical samples as all the isolates were completely susceptible (100 %) (Figure 1). This is in agreement with the work of Ibrahim *et al*. (2013) where antibiotic susceptibility results of *S. aureus* showed absolute resistance (100 %) against ampicillin, and high resistance against cefotaxime (81 %), while resistance frequencies to ceftriaxone, erythromycin, ciprofloxacin, trimethoprim, gentamicin, levofloxacin and clindamycin were 59 %, 59 %, 41 %, 41 %, 35 %, 23 % and 18 % respectively. The susceptibility frequency of VRSA isolates to gentamicin in this study is similar to the findings of Tiwari *et al*. (2006) who reported a high susceptibility frequency of multidrug *S. aureus* isolates to gentamicin. The CA-VRSA isolates were also multidrug resistant. All the HA-VRSA isolates were completely resistant (100 %) to ceftazidime, sulphamethoxazole and penicillin. This was closely followed by gentamicin (80 %), nitrofurantoin (80 %), clindamycin (80 %) and tetracycline (60 %). Interestingly, ciprofloxacin was the most effective antibiotic against the *Staphylococcus aureus* isolates obtained from community samples with a susceptibility frequency of 100 %. This was closely followed by gentamicin (75 %) and erythromycin (Figure 2). Most notable results of VRSA that was recorded by Chakraborty *et al*. (2011) which included eight pathogenic VRSA strains isolated from post operative pus sample in India and that is similar to the findings of our research. These resistances might result from inappropriate prescriptions due to lack of standard treatment guidelines (WHO, 2015). In developing countries, an estimated 50 % of those who need antimicrobials do not have access to them due to cost. Sometimes, these drugs are not taken by patients as prescribed and some people indulge in self-medication (WHO, 2009). Relentless exposure of bacterial strains to a large number of β-lactams has induced active and continuous production and mutation of β-lactamases in these bacteria, thus expanding their activity even against the newly developed β-lactam antibiotics. Thirty-two (32) VRSA (or HA-VRSA) isolates out of the 84 clinical samples of *S. aureus* isolates were positive for beta-lactamase production while 29 CA-VRSA isolates were positive for beta-lactamase production (Table 7). This is in agreement with the findings of Terry *et al*. (2011) in South-East Nigeria who reported that 124 (64 %) of their *S. aureus* isolates were able to produce β-lactamase enzyme by changing colour from yellow to red on the addition of nitrocefin solution. Production of β-lactamase in *S. aureus* is reported to have been consistently high in Nigeria (Torimiro *et al*., 2013). They reported that 70-80 % of *S. aureus* isolates produced β-lactamase. Other researchers reported such high β-lactamase prevalence (Kesah *et al*., 1997; Akindele *et al*., 2010). The spread of β-lactamase genes had been enhanced by their integration within mobile genetic elements such as plasmids and transposons which facilitate the rapid transfer of genetic materials between microbes (Wilke *et al*., 2005). Multidrug resistant bacteria have created therapeutic problems especially to healthcare providers and, its consistent emergence will only cause hazard to the public health (Amalia *et al*., 2016). The HA-VRSA isolates had MARI values within the range of 0.5 to 0.8. These high MARI values depict the high resistances of these isolates to antibiotics (Table 8).The MARI values for CA-VRSA ranged from 0.5 to 1.0. These MARI values indicate that the CA-VRSA isolates have higher antibiotic resistance potential than the HA-VRSA isolates (Table 9). Our study is in agreement with the report of Subramani and Vignesh (2012) who revealed that all the *S. aureus* isolates in their study had very high MARI values of above 0.2. This signified that the bacterial isolates have been exposed to several antibiotics and the selective pressure of the antibiotics used in the management of bacterial infections could be a vital reason for resistance apart from the bacteria acquiring the resistance gene through mutation or interspecies gene transmission.

CONCLUSION:

This study has been able to show that VRSA isolates are present in some major hospitals in Abakaliki and within the community in Abakaliki, Ebonyi State, Nigeria. The report of this study has established that nitrofurantoin, clindamycin, ceftazidime, tetracycline and penicillin are now ineffective in treating VRSA infections whereas gentamycin, ciprofloxacin and erythromycin are still very effective. The high resistance frequencies exhibited by these *S. aureus* isolates to most of the antibiotics used in this study is an indication that such antibiotics are now ineffective in the treatment of patients and other people with VRSA infections in Abakaliki. The high MARI values of the VRSA isolates also shows that these isolates have high pathogenic potential. Misuse of antibiotics has been linked to the evolution of multidrug resistant bacteria which usually have strong pathogenic potential. This could in turn lead to treatment failures and increase in the cost of infection control. Obviously, if the infections caused by VRSA are not controlled, it will spread to larger population of people in the community, thereby causing a very

serious public health problem. Therefore, it is pertinent to strictly monitor the prevalence of VRSA emerging from Abakaliki, Nigeria.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest

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