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RESEARCH ARTICLE

MOLECULAR CHARACTERIZATION AND ANTIBIOGRAM OF METHICILLIN AND VANCOMYCIN RESISTANT *STAPHYLOCOCCUS AUREUS* FROM CLINICAL AND COMMUNITY ISOLATES IN ABAKALIKI, NIGERIA

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ABSTRACT

This study was aimed at isolating and molecularly characterizing *Staphylococcus aureus* exhibiting methicillin and vancomycin-resistance traits from clinical and community samples in Abakaliki, South Eastern Nigeria. Exactly 303 clinical samples from wounds, pus, urine, HVS (high vaginal swab), ear swabs, sputum and semen of hospital patients; and 406 community samples (nasal and ear swabs) were obtained for this study. Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) isolates were detected using Kirby-Bauer disc diffusion method. Multiplex PCR was used to detect *mec*A, SCC*mec*, and PVL genes in the HA-MRSA and CA-MRSA isolates. 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 MRSA and VRSA were highly prevalent in wound samples and less prevalent in HVS, pus and sputum samples while none was observed in semen samples. The MRSA and VRSA isolates exhibited high resistance to nitrofurantoin, tetracycline, penicillin, clindamycin, sulphamethoxazole and ceftazidime. Gentamycin was the most effective antibiotic against the MRSA and VRSA isolates obtained from hospitals samples while ciprofloxacin was the most effective against MRSA and VRSA isolates obtained from community samples. Our study has shown that mecA and SCCmec IVa genes are present in the HA-MRSA and CA-MRSA isolates from our study area. In contrast, PVL genes were detected only in CA-MRSA. The high resistance of the clinical and community isolates to most of the antibiotics used in this study shows that such antibiotics are now ineffective in treating people with MRSA and VRSA infections. This situation is very worrisome and could result in grave public health problem if not quickly addressed. Therefore, it is pertinent to closely monitor MRSA and VRSA emerging from Abakaliki, Nigeria.

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INTRODUCTION

Globally, antibiotic resistant bacteria have increasingly become a growing concern in public health sector. It may spread and create broader infection control problems, both within healthcare institutions and in the community. Infections, especially the one caused by *Staphylococcus aureus*, has affected substantial proportions of the human population, causing significant mobility and mortality (Fred, 2006). Strains of *Staphylococcus aureus* that are resistant to multiple antimicrobial compounds and some antiseptics are major threats to patients' care owing to their stubborn intransigence to chemotherapy and disinfection (Slade *et al.,* 2009).

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Prolonged therapy with vancomycin and methicillin 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). *Staphylococcus aureus* has been inplicated in a wide range of infection ranging from acute to chronic infections such as boils, bacteriuria, osteomyelitis, pneumonia, endocarditis, meningitis, septicaemia 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). The bacterium is frequently found on the skin and anterior nares of healthy individuals. 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 disease including gastroenteritis, staphylococcal scaleded skin syndrome and toxic shock syndrome, meningitis, septicemia and arthritis (Amghalia *et al.,* 2009). Methicillin resistant *S. aureus* (MRSA) and Vancomycin resistant *S. aureus* (VRSA) has become a major public health problem all over the world. It is a major human pathogen associated with nosocomial and community infections. It is correlated with incremented morbidity and mortality, compared to other pathogenic bacteria. The elevated colonization rates lead to the incrimination of infection rates in the community and medical centers which leads to a significant increase in treatment cost. Common features of CA-MRSA strains are the presence of the Panton-Valentine leucocidin (PVL) gene and the methicillin-resistance locus (staphylococcal cassette chromosome mec (SCCmec) (Vandenesch *et al*., 2003). Panton Valentine leukocidin-producing MRSA usually cause mild skin or soft tissue infections; however, severe cases of necrotizing pneumonia and sepsis have also been reported (Maltezou *et al*., 2006). Panton Valentine leukocidin is commonly used as a marker for community acquired MRSA, responsible for soft-tissue and deep dermal infections (Havaei *et al*., 2010). Panton Valentine leukocidin is usually present in majority of community associated MRSA isolates and rarely present in hospital isolates; therefore, it is recognized as marker of community acquired strains (Vandenesch *et al*., 2003). Epidemiological data suggest that high virulence of community acquired MRSA is associated with PVL genes but direct evidence of association of PVL to pathogenesis has been limited (Li *et al*., 2010). However, due to the recent resurgence of MRSA and VRSA in hospitals, this study, is therefore designed to evaluate the prevalence of *Staphylococcus aureus* with both methicillin and vancomycin-resistance traits in healthcare institutions and community within Abakaliki metropolis, South East Nigeria.

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 37^0C for 48 hours. A loopful of the inoculated mannitol salt broth was later streaked on mannitol salt agar (MSA) and sheep blood agar, and incubated at 37^0C 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 24hours 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 Methicillin resistant *Staphylococcusaureus* (MRSA)

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 its 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 discs impregnated with oxacillin and cefoxitin were placed on Mueller-Hinton agar plate for MRSA 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.

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.

Molecular characterization of the *S. aureus* isolates

DNA extraction

Chromosomal DNA was extracted using CTAB (Cetyltrimethylammonium Bromide) method (Abashi *et al*., 2010). Nucleic acid concentration was estimated through the use of spectrophotometer. Exactly 800 µl of broth culture of the isolate was spun twice at 12,000 rpm for 10 minutes and the supernatant discarded before the addition of 1,000 µl of extraction buffer (EB) consisting of 2 % CTAB, 100 mM Tis-HCl, 20 mM EDTA, 1.4 M NaCl and 0.2 % β-mercaptoethanol. The sample was poured into new 1.5 ml sterile eppendorf tube and briefly vortexed. After that, the mixture was incubated in water bath at a temperature of 60 ^{o}C for 10 minutes. Immediately after the incubation, it was brought to room temperature and an equal volume of phenol, chloroform and isoamyl alcohol in the ratio of 25:24:1 was added, vortexed and centrifuged at 12,000 rpm for 10 minutes. Then 450 µl of the supernatant was transferred into new sterile 1.5 ml eppendorf tube and 400 µl of ice-cold isopropanol was added to precipitate the DNA. It was mixed gently by inversion for up to ten times and subsequently incubated for 1 hour at 20 ^{o}C. Each eppendorf tube that is containing the mixture was centrifuged at 2,000 rpm for 10 minutes to sediment the DNA. After centrifuging, the supernatant was carefully decanted, leaving the pellet undisturbed in the eppendorf tube. In washing the pellet, 500 µl of 70 % ethanol was added and centrifuged at 12,000 rpm for 5 minutes. The ethanol (supernatant) was decanted and the pellet containing the DNA was air-dried at room temperature. The pellet was then suspended in 100 µl of nuclease-free water for PCR.

PCR detection of *mec*A, SCC*mec*, and *PVL* Gene

The isolated genomic DNA of methicillin resistant *Staphylococcus aureus* (MRSA) was analyzed for the presence of *mec*A, SCC*mec*, and *PVL* genes in the MRSA isolates. Amplification reaction were carried out in a volume of 25 µl of a PCR mixture containing 2.0µl DNA, 2.5 µl of 10 x Buffer (Bioline), 1.25 µl of 50mM $MgCl_2$ (Bioline), 2.0 µl of mM dNTPs (Bioline), and 0.2 µl *Taq* DNA polymerase (Bioline), 1.0 µl DMSO (dimethyl sulfoxide), 1.0 µl of each primer and 17.05 µl of DEPC-treated water (Invitrogen Corporation). After mixing, the PCR tubes were briefly vortexed for thorough mixing of its contents and spun down to bring the mixture to the bottom of the tubes. After that, the tubes were then placed in thermo-cycler well (Eppendorf, Germany, Model 5332A). The PCR was performed as follows: Initial denaturation at 94 °C for 2 minutes, 40 cycles of denaturation at 94 °C for 20 seconds, annealing temperature of 72 °C for 1 min, elongation at 40 °C for 2 min, and a 5 min final extension period at 72 °C. Gel electrophoresis was used to detect amplified DNA products. The amplified PCR products were electrophoresed in 1.5 % agarose gel containing 0.5 mg/ml ethidium bromide at 80 volts for about 2 hours, and thereafter photographed in an Ultra violet-transilluminator (Fotodyene Incorporated, Heartland WI USA).

RESULTS

DISCUSSION

Infections caused by vancomycin and methicillin resistant *S. aureus* have been associated with high morbidity and mortality rates. Regardless of serious efforts to control antibiotic resistant organisms by aggressive infection control methods, antibiotic resistant Staphylococci especially MRSA and VRSA, have become the most frequent cause of hospital and community acquired infections worldwide (Anupurba *et al*., 2003; Taiwo *et al*., 2005). In this study, the highest prevalence of MRSA was recorded in FETHA I (47.4 %). This was closely followed by FETHA II (36.8 %) and Mile Four Hospital being the least (15.8 %). In contrast, the highest prevalence of VRSA was recorded in FETHA II (41.9 %). This was closely followed by FETHA I (35.5 %) and Mile Four Hospital being the least (22.6 %) (Figure1). The prevalence of MRSA in community isolates was 20.8 % while that of VRSA was 20 % (Table 1). Similar observation was also made by

The primers used are:

Specific gene for Amplification	Primer sequence	Annealing temperature	Expected Size Of Amplicon (bp)
MecA	Forward: 5′-TGGCTATCGTGTGTCACAATCG-3′ Reverse: 5′-CTGGAACTTGTTGAGCAGAG-3′	52	167
SCCmec	Forward:5′-AAAGGATCCATTAGCCGATTTGG TAATTGAA-3′ Reverse:5′-AAAGGATCCATTAGCCGATTTGG TAATTGAA-3′	50	776
PVL	Forward: 5′-GTAAAATGTCTGGACATGATCCA3′ Reverse: 5′-CAA(C/G)TGTATTGGATAGCAAAAGC3′	50	433

Reference: (Dias *et al*., 2004)

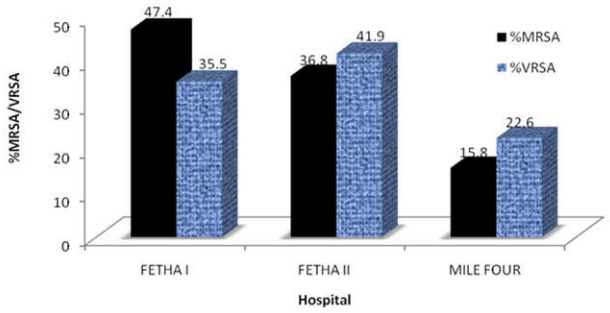

Figure 1. Distribution of MRSA/VRSA obtained from Federal Teaching Hospital (FETHA I), FETHA II and Mile Four General Hospital Abakaliki

Table 1. Prevalence of MRSA and VRSA among Community Isolates

	MRSA		VRSA	
Source	No of Isolate Tested	No Positive	No of Isolate Tested	No Positive
Community	120	25 (20.8 %)	120	24 (20 %)

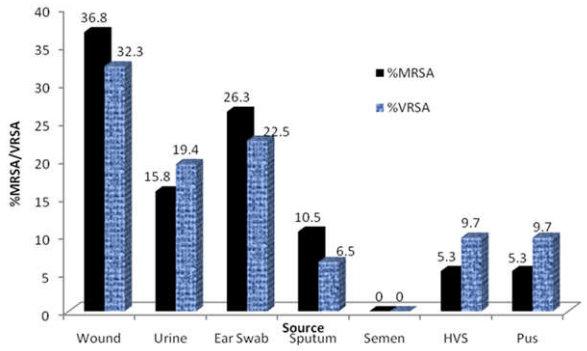

Figure 2. Distribution of MRSA/VRSA obtained from different clinical specimens collected from FETHA I, II and Mile Four General Hospital Abakaliki

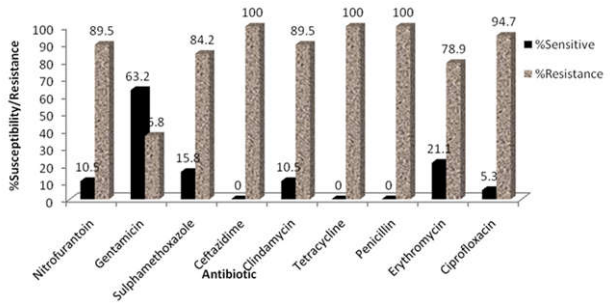

Figure 3. Percentage susceptibility and resistance pattern of Hospital Acquired Methicillin Resistant *Staphylococcus aureus* (HA-MRSA) to different antibiotics

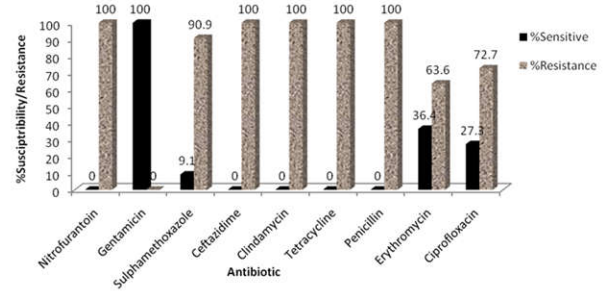

Figure 4. Percentage susceptibility and resistance pattern of Hospital Acquired Vancomycin-Resistant *Staphylococcus aureus* (HA-VRSA) to different antibiotics

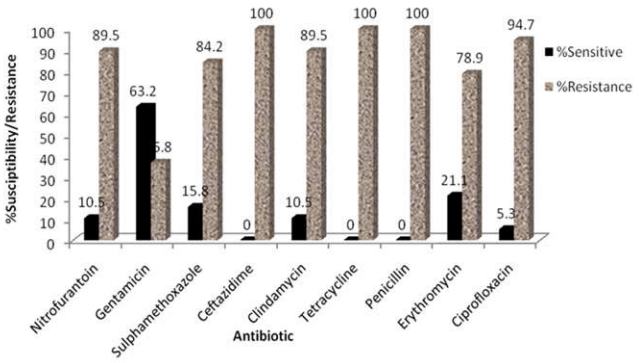

Figure 5. Percentage susceptibility and resistance pattern of isolates that are both HA-MRSA and HA-VRSA to different Antibiotics

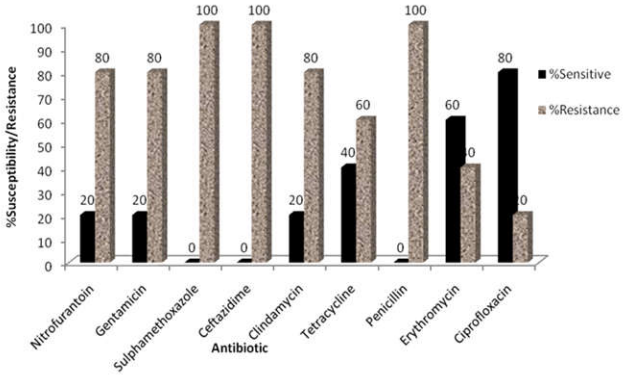

Figure 6. Percentage susceptibility and resistance pattern of Community Acquired Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) to different antibiotics

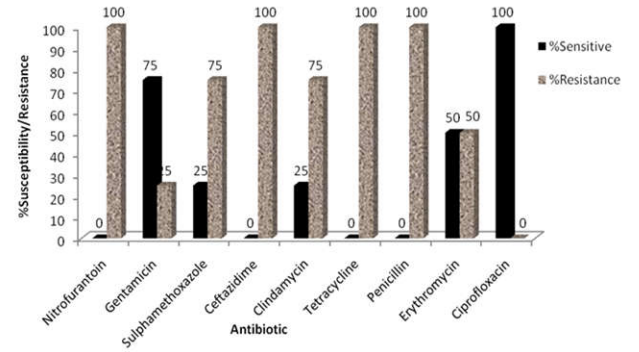

Figure 7. Percentage susceptibility and resistance pattern of Community Acquired Vancomycin Resistant *Staphylococcus aureus* (CA-VRSA) to different antibiotics

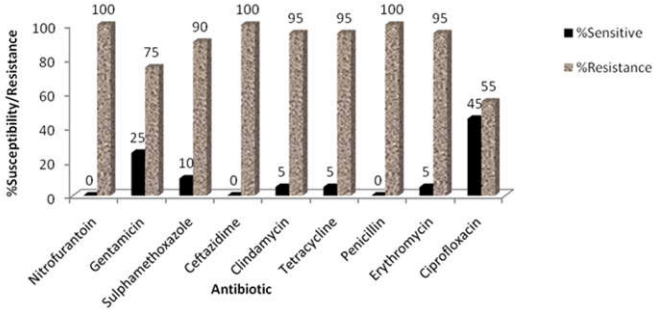

Figure 8. Percentage susceptibility and resistance pattern of isolates that are both CA-MRSA and CA-VRSA to different antibiotics

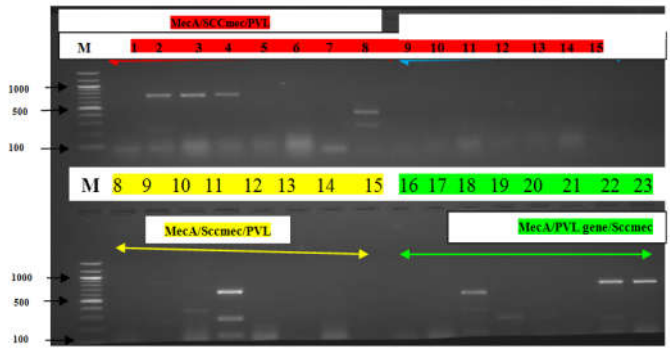

Fig. 9. Multiplex Polymerase Chain Reaction (PCR) amplification gel for *mec*A (167 bp) SCC*mec* (776 bp), and PVL (433 bp) genes. M= Marker (100 bp); Lane 1 = Positive control (*mec*A); Lane 15 = Negative control; Lane 2-14, 16-19, 22-23 = mecA (167 bp); Lane 2-4, 8, 11, 22 and 23 = SCC*mec*IVa (776 bp); Lane 8, 10, 11 and 18 = PVL gene (433 bp)

Key: Lane 2= 27C (CA-MRSA), Lane 3= 5A (HA-MRSA),Lane 4=26C (CA-MRSA), Lane 5=21B (HA-MRSA), Lane 6=1B (HA-MRSA), Lane 7=18A (HA-MRSA), Lane 8=23B (CA-MRSA), Lane 9= 3C (CA-MRSA), Lane 10= 24A (CA-MRSA), Lane 11= 12A (CA-MRSA), Lane 12= 23C (CA-MRSA), Lane 13=13A (HA-MRSA), Lane 14= 11C (CA-MRSA), Lane 16= 26B (HA-MRSA), Lane 17= 34A (HA-MRSA), Lane 18=48C (CA-MRSA), Lane 19= 16B (HA-MRSA) Lane 20= 25C (CA-MRSA), Lane 21= 11B (HA-MRSA), Lane 22=10C (HA-MRSA), 23=28A (HA-MRSA)

Borg (2007) who recorded a lower prevalence rate of 18 % for MRSA in a research they conducted using 4 hospitals in Tunisia as sampling points. The present research recorded lower prevalence rate when compared with the result of Olowe *et al*. (2007) where they recorded a higher prevalence of 47.7 % MRSA. This could be because they included samples from patients in referrals. Another report that is in agreement with this study was conducted by Aligholi *et al* (2008) who reported that VRSA showed remarkable prevalence rate of 40 %. A research conducted in Zagazig university of Egypt in 2012 recorded a lower prevalence of VRSA. In that report, VRSA strains were isolated from 4.5 % of patients infected with *S. aureus*. Another research conducted in south west Nigeria is also in agreement with the report of the present study. The research reported a prevalence rate of 20.23 % for MRSA where 70 out of 346 *S. aureus* were methicillin resistant (Ghebremedhin *et al*., 2013). The prevalence rate of 20.8 % for CA-MRSA reported in this work is similar to that reported in another research conducted by Lucianne who reported a CA-MRSA prevalence rate of 27.8 % (Lucianne *et al*., 2013).The prevalence of vancomycin resistance may be related to under usage of vancomycin in Abakaliki (study area), Ebonyi State where it is used as drug of choice for the treatment of VRSA infections. The relatively high prevalence could also be explained by administration of multiple prophylactic and post operative antibiotics with prolonged hospitalization. The highest prevalence of MRSA was observed in wound samples 7(36.8 %). This was closely followed by ear swab 5(26.3 %), urine 3 (15.8 %) and sputum 2(10.5 %) samples. HVS and pus recorded the least prevalence of MRSA with frequency value of 5.3 % each. There was no MRSA or VRSA isolated from semen samples. Similarly, the highest prevalence of VRSA was also observed in wound samples 10 (32.3 %); followed by ear swab 7(22.5 %) and urine 6(19.4%) samples. HVS and pus samples recorded the same prevalence of VRSA 3(9.7 %) while sputum samples recorded the least number of VRSA 2(6.5%) isolates (Figure 2). This is similar to the report by Olowe *et al*. (2007) who reported that the highest prevalence of MRSA was found in wound samples with a prevalence frequency of 19 %. They also reported that the lowest prevalence was observed in stool samples with a prevalence rate of 3 %. Many researchers have reported an increase in the incidence of MRSA which originated from wounds (Vidhani *et al*., 2001). It was observed that all the nineteen (19) HA-MRSA isolates were completely resistant to ceftazidime, tetracycline, and penicillin (100 %). They were also resistant to ciprofloxacin (94.7 %), clindamycin (89.5 %), nitrofurantoin (89.5 %), sulphamethoxazole (84.2 %) and erythromycin (78.9 %). Gentamicin (CN) was the most active antibiotic as 63.2 % of the HA-MRSA isolates were susceptible to this antibiotic (Figure 3).All the Eleven (11) HA-VRSA isolates were completely resistant (100 %) to nitrofurantoin, ceftazidime, clindamycin, tetracycline and penicillin. They were also resistant to sulphamethoxazole (90.9 %), ciprofloxacin (72.7 %) and erythromycin (63.6 %). Gentamycin was again effective against all the HA-VRSA isolates as all the isolates were completely susceptible (100 %) (Figure 4). These findings are in agreement with that of Ibrahim *et al*. (2013) who reported that *S. aureus* exhibited absolute resistance (100%) against ampicillin, and high resistance rate against cefotaxime (81 %), while resistance to ceftriaxone, erythromycin, ciprofloxacin, trimethoprim, gentamicin, levofloxacin and clindamycin were at a frequency of 59 %, 59 %, 41 %, 41 %, 35 %, 23 % and 18 % respectively. The adverse effect like kidney problem and congenital deafness in children (Lopez *et al*., 2011) associated with gentamicin suggests why it is not often prescribed except when the benefit is seen to outweigh the risk before it is administered to patients.

This equally suggests why it is the most active among the antibiotics used in this study. Hospital isolates that are both methicillin and vancomycin resistant showed the same percentage resistance and susceptibility patterns with HA-MRSA because all the isolate from the hospital that were methicillin resistant were also vancomycin resistant (Figure 5).All the CA-MRSA isolates were completely resistant (100 %) to sulphamethoxazole, ceftazidime and penicillin. They are also resistant (80 %) to nitrofurantoin (80 %), gentamycin (80 %), clindamycin (80 %) and tetracycline (60 %). Interestingly, the most active antibiotic was ciprofloxacin as 80 % of the CA-MRSA isolates were susceptible to this antibiotic (Figure 6). All the CA-VRSA isolates were completely resistant (100 %) to nitrofurantoin, ceftazidime, tetracycline and penicillin. They were also resistant to sulphamethoxazole (75 %), clindamycin (75 %) and erythromycin (50 %). Ciprofloxacin was the most effective antibiotic as all the isolates were completely susceptible (100 %) to this antibiotic (Figure 7). Isolates identified as both CA-MRSA and CA-VRSA were resistant (95 %) to clindamycin, tetracycline and erythromycin. They were also resistant to sulphamethoxazole (90%), gentamycin (75 %) and ciprofloxacin (55 %). None of the antibiotic used had an above average effectiveness against the tested isolates (Figure 8). 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 this is similar to the findings of our research. HA-MRSA and VRSA isolates were multi-drug resistance. The report of isolates that are both MRSA and VRSA positive is not strange as many researchers have reported the same. Aligholi *et al*. (2008) reported a high prevalence of vancomycin-resistant *S. aureus* (41.85 %) in Tehran where 2 out of the 6 strains of *S. aureus* were found to

be resistant to both MRSA and VRSA. This resistance has been attributed to inappropriate prescriptions because of the absence of standard treatment guidelines. Again this could occur due to the fact that patients do not take drugs as recommended or when they indulge in self-medication (WHO, 2015). The mechanism of methicillin-resistance in *S. aureus* is based on the production of an additional low-affinity penicillin-binding protein encoded by the mecA gene. Methicillin resistance is conferred by the acquisition of the **mec**A gene, which is carried by a mobile genetic element called staphylococcal cassette chromosome **mec** (SCC**mec**). There are five major types of SCC**mec** elements (I–V). The majority of hospital-acquired MRSA (HA-MRSA) strains carry SCC**mec** types I, II, or III, whereas community-acquired MRSA (CA-MRSA) strains carry SCC**mec** types IV or V.Some studies have reported the spread of CA-MRSA SCC**mec** type IV strains in hospital settings in Europe, United States, and Switzerland (Liu *et al*., 2008).

CA-MRSA is most likely carried in the upper respiratory tract or various cutaneous and mucosal sites and introduced into the hospital by patients or nursing staff (Skov *et al*., 2009; Miller *et al*., 2005). Most CA-MRSA infections have been associated with strains bearing the SCC**mec** type IV element and PVL genes (Bukharie *et al*., 2010). In this study, SCC**mec** IVa was present in 3 (13 %) HA-MRSA strains (Figure 9). SCC**mec** IVa was more prevalent in the CA-MRSA strains. Although SCC**mec** IV is associated with CA-MRSA (Naimi *et al*., 2003), SCC**mec** IV has also been shown to be distributed among HA-MRSA strains. Our study is in agreement with the work of Carleton *et al*. (2004) who also reported SCCmec IV in HA-MRSA isolates.Beta-lactam resistance is attributed mostly to mutations in the mecA gene. However, when amplifying a 167 bp fragment of the mecA gene by PCR in this study, 20(87 %) out of the 22 MRSA selected were positive for mecA gene while 2(8.7 %) isolates (lane 20 and 21) were negative (Figure 9). Many other researchers all over the world: in Sudan (Maimona *et al*., 2014), in Egypt (Hafez *et al*., 2009), in Saudi Arabia (Meshref *et al*., 2011), in Spain (Del-Valle *et al*., 1999), in England (Wongwanich *et al*., 2000), and in Australia (Cloney *et al*., 1999) have all reported mecA gene in MRSA isolates. Our study is in agreement with the work of Hawraa *et al*. (2014), who also reported the absence of mecA gene in some MRSA isolates in their study. Panton Valentine leukocidin (**PVL**) is considered one of the important virulence factors of **S. aureus** responsible for destruction of white blood cells, necrosis and apoptosis and as a marker of community acquired MRSA. None of the HA-MRSA isolates in this study was positive for the **PVL** genes (Figure 9). Four CA-MRSA isolates (17.4 %) were positive for PVL gene in our study. The prevalence of PVL gene among MRSA isolates in this study is lower than reports from other researchers. Reports from various countries show the increasing prevalence of **PVL** among MRSA isolates (Eckhardt *et al*., 2003). Souza *et al*. (2010) from Mumbai, India, reported prevalence of 64 % **PVL** positive isolates among MRSA which shows a higher prevalence among MRSA isolates than our findings in Abakaliki, Nigeria (17.4 %). A lower prevalence of **PVL** has also been reported in other parts of the world (5 % in France (Lina *et al*., 1999), 4.9 % in UK (Holmes *et al*., 2005), 8.1 % in Saudi Arabia (Moussa *et al*., 2008) and 14.3 % in Bangladesh (Afroz *et al*., 2008), reflecting that the prevalence of PVL gene among MRSA isolates varies greatly between geographical locations and populations.

Conclusion

This report has now established that MRSA and VRSA are present in all the major hospitals and in the community within Abakaliki metropolis, Ebonyi State, Nigeria. Our study has shown that *mec*A and SCC*mec* IVa genes are present in the HA-MRSA and CA-MRSA isolates. In contrast, *PVL* genes were detected in CA-MRSA isolates while no *PVL* gene was detected in HA-MRSA isolates. The presence of PVL gene among multidrug resistant bacteria like MRSA may be involved in virulence and increase the challenges for clinicians. This study has also demonstrated that gentamycin and ciprofloxacin are still very effective in treating MRSA and VRSA infections. The high prevalence of MRSA and VRSA observed in this study could possibly lead to a change in microbial resistance characteristics causing treatment failure and increase in the cost of infection control. Obviously, if the infection is not controlled, it will spread to a larger population of people in the community; thereby causing a very serious health care problem especially when the immune system of people is compromised.

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