Detection and Prevalence of Methicillin and Vancomycin Resistant *Staphylococcus aureus* among Clinical Isolates in ESUTH, Enugu State, Nigeria

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Abstract

The increasing rate of drug resistance associated with methicillin resistant *Staphylococcus aureus* and the emergence of vancomycin resistant trait is a great problem in human disease treatment and management. The present study was carried out to determine the prevalence, antibiotic susceptibility profile of MRSA, and vancomycin resistant *S. aureus* among patients at ESUT Teaching Hospital, Enugu. Four hundred and fifty (450) Clinical samples were collected between April and November 2018. All *S. aureus* were recovered using standard laboratory method. Antibiotic susceptibility pattern was determined using modified Kirby Bauer disc agar diffusion method. Methicillin and vancomycin resistance were determined using cefoxitin (30µg) and vancomycin (30µg) disc diffusion, respectively. Penicillin binding protein 2a was detected through rapid latex agglutination assay while nitrocef stick was used for screening of beta lactamase production. Eighty three (18.4%) isolates out of 450 clinical samples were confirmed and characterized as *Staphylococcus aureus*. Twenty three (27.7%) of the isolates were MRSA. All (100%) the MRSA isolates were susceptible to vancomycin. The result of antibiotic susceptibility test showed much resistance to β-lactam antibiotics. The multiple antibiotics resistance index (MARI) showed that 66 (79.5 %) were resistant to 3 or more antibiotics (MARI ≥ 0.4). Seventy two percent of the MRSA were multidrug resistant. Seventy four (89.1%) isolates produced beta-lactamase while Penicillin binding protein 2a was detected in 13.2% of MRSA isolates. The results suggest the need for methicillin resistance *S. aureus* regular surveillance studies as well as institution of infection control measures, antibiotic stewardship programme and amendment of antibiotic regimen guidelines for MRSA infections.

Keywords: Antibiotic susceptibility, Methicillin-resistant *Staphylococcus aureus* prevalence, Penicillin Binding Protein, disc agar diffusion, rapid latex agglutination, Nigeria

Introduction

Staphylococcus aureus, a Gram positive cocci, is an opportunistic pathogen and frequent cause of wide range of clinical infections (Kong *et al*., 2016; Ryu *et al*., 2014). The bacteria form part of the normal flora of the skin, intestine, upper respiratory tract and vagina (Lowy, 1998). It is also a common cause of skin, wound and urinary tract infections (Sina *et al*., 2011).

Staphylococcus aureus can establish infection in the host through the expression of an inclusive set of virulence factors such as toxins, enzymes, adhesins, and other surface proteins that allow the pathogen to

survive under extreme conditions and are essential for the bacteria's ability to spread through tissues (Ryu *et al*., 2014). Toxins such as the 33-kd protein-alpha toxin, exfoliatin A, exfoliatin B and Panton-Valentine leukocidin (PVL) produced by *S. aureus* have determined its pathogenicity (Lowy, 1998). These toxins can be harmful to the host and cause skin diseases (carbuncles, boils, folliculitis and impetigo) and other complications, such as endocarditis, meningitis as well as toxic shock syndrome (TSS) (Kong *et al*., 2016; Mims *et al*., 2004).

Since 1959, treatment of *S. aureus* infections included semi-synthetic penicillin drugs, such as methicillin (Livermore, 2000). However, in the 1960's the rise of methicillin-resistant *S. aureus* (MRSA) strains was apparent (Jevons *et al*., 1963; Jevons, 1961). In 2016, over 60% of the *S. aureus* strains isolated in hospitals were resistant to this antibiotic (Kshetry *et al*., 2016). Methicillin resistance in this bacterial species represents a threat to human health as about a third of healthy individuals carry *S. aureus* on their skin and nose (Grundmann *et al*., 2002).

S. aureus is perhaps the most notorious of all the bacterial pathogens associated with human infection (Kunin, 1993). It is the first bug to battle penicillin in 1967 due to its ability to produce β-lactamase. Resistant semi synthetic penicillins in the early 1960s provided temporary respite which ended with the emergence of methicillin resistant *S. aureus*, discovered shortly after methicillin became available for clinical use (Miall *et al*., 2001; Fluit *et al*., 2001).

MRSA is of concern not only because of its resistance to methicillin but also because it is generally resistant to many other chemotherapeutic agents (Ugwuoji *et al*., 2022; Vidhani *et al*., 2016). Multidrug resistant *S. aureus* evolved following acquisition of antimicrobial resistance genes by horizontal gene transfer and resistance determinants generated by chromosomal mutation which poses great challenges in treatment of staphylococcal infections (Jesen and Lyon, 2009).

Methicillin resistant *S. aureus* (MRSA) strain carries a large heterologous mobile genetic element, staphylococcal cassette chromosome (SCC) which includes the central element of methicillin resistance, the *mecA* gene (Kumar, 2016; Ito *et al*., 1999). Six SCCmec type (I-VI) have been identified and reported in *S. aureus* which are defined by combination of the *mec* gene complex class with the *ccr*allotype (Oliveira *et al*. 2006; Ito *et al*. 2004). Several strategies have been developed for SCC mec type (Okuma *et al*. 2002) and their broad application has led to the detection of several variants or subtype of the major SCC type (Olivera and de Lancastre 2002; Ito *et al*., 2001). Community acquired methicillin resistant *S. aureus* (CA-MRSA) has a characteristic staphylococcal cassette chromosome type IV (SCCmec IV) gene, lacking in non β–lactam determinant and possessing distinct necrotizing toxin, Panton valentine leukocidin (PVL). (Hackbarth and Chambers, 2010).

The glycopeptides antibiotic,vancomycin, was introduced into the clinical setting in 1958 for the treatment of Gram positive bacterial infections (Rubinstein and Keynan, 2014; Perl, 1999). The increase in prevalence of methicillin resistance *S.aureus* has led to the dramatic increase in vancomycin usage within the last twenty years (Wijesekara *et al*., 2017; Ena *et al*., 1993).Vancomycin resistance among staphylococci was developed in laboratories even before the drug was in use clinically (Tenover *et al*., 1994).

However, this resistance was so difficult to induce that many felt it would be unlikely to occur in the clinical setting. The fact that no vancomyin resistant staphylococci were reported in the first 20 years the drug was used only strengthened this assumption. Unfortunately, this confidence was shattered by the first reports of vancomycin resistance

in coagulase negative staphylococci in 1979 and 1983 (Tuazon and miller, 1983).

Given the well-known virulence nature of *S. aureus*, the isolation of this organism generated enormous concern in the medical community and has prompted a flurry of activity aimed at limiting their emergence. This study, therefore, was aimed to determine the prevalence, antibiotic susceptibility profile of MRSA, and detection of vancomycin resistant *S. aureus* from clinical samples with a view to contributing towards controlling the emergence of new cases.

Materials and Methods

Sample Collection

Bacteriological specimens comprising wound swab (from different anatomic locations), High Vagina Swab(HVS), Urethral Swab(US) and Urine were collected from patients attending Enugu State University of Science and Technology(ESUT) Teaching Hospital situated in Enugu metropolis (longitude 6˚ 27' 10' N and latitude 7˚ 30 '40' E), between October 2017 and August 2018. Swab and urine samples were collected with sterile swab-stick and universal bottle respectively. Informed written consent was obtained from each patient prior to collecting specimen. Approval was also obtained from the hospital management board before the commencement of the research. Specimens collected were analyzed within 30 minutes of collection. The age and sex of patients were recorded.

Isolation of Bacteria

Each sample was streak-inoculated into sterile blood agar plate prepared following standard method. Using a sterile wire-loop, the sample was first smeared on the blood agar plate before making several streaks to spread the load. The loop was flamed before the next streak. The streaked plate was then incubated at 37^0C for 24 h for growth. To obtain pure isolates, the 24h culture was then sub-cultured on the surface of sterile nutrient agar (NA) prepared following standard method by streaking. The streaked NA was incubated at 37^0Cfor 24h. The stock isolates were made in NA slants and kept at 4^0C in a refrigerator.

Identification of Isolates

Identifications of isolates were by colony morphology, Gram staining, catalase test and coagulase test (Cheesbrough, 2016). Thereafter, isolates that were positive to Gram staining, catalase and coagulase tests were considered as *S. aureus* (Cheesbrough, 2000).

A loopful of 24h Nutrient broth culture of these isolates were streaked on Mannitol salt Agar (MSA) plates prepared following standard method (Cheesbrough, 2016).Plates were incubated at 37^0C for 24h. Isolates that produced colonies that exhibited characteristics deep golden yellow coloration were selected and sub-cultured onto Nutrient broth. The overnight broth culture were sub-cultured onto Nutrient agar slants and allowed to germinate for 24h at 37^0C in an incubator. The slants with visible growth were stored in refrigerator until required for further work.

Phenotypic screening of *S.aureus* for Methicillin and Vancomycin resistance

Detection of methicillin and vancomycin resistant *S. aureus*

Susceptibility of the *S. aureus* isolates was done by means of the agar diffusion method on Muller Hinton agar using Oxacillin (1µg), Cefoxitin(30µg) and Vancomycin (30µg) sensitivity discs. The isolates were standardized to 0.5 McFarland standard and suspension of which was aseptically inoculated on the Muller Hinton agar plates. The plates were incubated for 24h at 37°C.Therefater, the zones of inhibition were measured and interpreted using

Clinical Laboratory Standard Institutes (CLSI) breakpoint (CLSI, 2016).

Antibiotic susceptibility testing (AST)

Antibiotic susceptibility testing was carried out to obtain the susceptibility pattern of *S. aureus* isolated from clinical samples. The isolates were tested against a panel of seven antibiotics. The antibiotics used include ampicillin, erythromycin, oxacillin, ciprofloxacin, gentamicin, levofloxacin and ceftriazone using Kirby-Buar method as described by Chessbrough, (2002).
The diameter of the zones of growth inhibition were measured to the nearest millimeter and isolates classified as either sensitive or resistant based on clinical and laboratory standard institute(CLSI) interpretative chart zone size (CLSI, 2012).

Screening for beta-lactamase

Nitrocefin test

Oxoid identification stick was used to detect beta lactamase produced. Nitrocefin is a cephalosporin developed by Glaxo Research Ltd. This compound exhibits a rapid distinctive colour change from yellow to red as the amide bond in the beta lactam ring is hydrolyzed by a beta lactamase.

For the test, the pure culture of *S. aureus* stored in bijou bottles were removed from the refrigerator and allowed to reach room temperature. Well separated representative colonies from the primary isolation medium were selected. One stick (colour-coded black) was removed from the container, and holding the coloured end, the colonies were touched with the impregnated end of the stick and the stick was rotated picking a small mass of cells. Because the reaction required moisture, the inoculated tip of the stick was placed in the moisture condensate on the lid; one drop of sterile distilled water was added to moisten the tip of the stick. The reagent-impregnated top of the stick, were examined for up to 5mins, and if negative, the stick was re-examined after 15 minutes. This is because some *Staphylococcus* may take up to 1h before the reaction establishes a colour change (CLSI, 2012). In the presence of β-lactamase, impregnated tip of stick changes to a pink-red colour (positive result) while no colour change inability of *S. aureus* to produce β-lactamase. The reading was confirmed by comparing the colour of the used stick to that an unused one.

Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR index was determined for each isolate by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotic tested (Paul *et al*., 1997).

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

Antibiotic Susceptibility Testing of Beta Lactamase Isolates

Antibiotic susceptibility testing was carried out to obtain the susceptibility pattern of beta lactamase positive *S. aureus* isolated from clinical specimen. The isolates were tested against a panel of nine antibiotics namely Oxacillin, Cefoxitin, Ciprofloxacin, Erythromycin, Levofloxacin, Ampicillin, Augmentin, Ceftriazone and Vancomycin.

The antibiotics susceptibility pattern was determined using the Kirby-Bauer modified disc agar diffusion (DAD) technique, (Cheesbrough, 2002). Discrete colonies on Nutrient Agar plate were emulsified in 3 ml of normal saline and the turbidity was adjusted to 0.5 McFarland. Using sterile swab sticks, the surface of MHA in 90 mm-diameter plate was inoculated with the bacterial suspension by streaking the surface of the agar in three directions to ensure even distribution. The inoculated plates were allowed to dry for 10 minutes after which

the antibiotic discs were placed on the surface of the agar. The plates were left at room temperature for the pre-diffusion, inverted and incubated aerobically at 37^0C for 24h.

The diameter of the zone(s) of growth inhibition were measured to the nearest millimeter and isolates classified as; sensitive or resistant based on clinical and laboratory standard institute(CLSI) interpretative chart zone size (CLSI, 2012).

MRSA Molecular Characterization Studies

Rapid Latex Agglutination

The MRSA screen test is a latex agglutination test based on the reaction of latex particles sensitized with monoclonal antibodies against PBP 2a of *S. aureus* and PBP2a extracted from tested colonies. The test was performed according to the manufacturer's instructions (Oxoid Ltd, England).

The test tube containing the organism was suspended in micro-centrifuge with the extraction reagent and placed in boiling water for 3 min at 95ºC to obtain the supernatant. For each supernatant to be tested, 50 µL was placed separately on the circle labelled "Test" and "Control". One drop of test latex was added, swirled for three minutes and observed for agglutination under normal lighting conditions (CLSI, 2012).

Data analysis

Data was entered in Microsoft excel 2010 and then transferred to SPSS version 23 for analysis. Comparisons between proportions were made using one way ANOVA for three means. Differences showing a critical value less than F value confidence level 0.05 were considered not significant.

Results

Sample population, and isolation

A total of four hundred and fifty (450) samples collected randomly were screened for the presence of *S. aureus*. Out of the 450 samples screened, 269 were urine samples and 150 were from females while 119 were from males (Figure1). A total of 105 wound swab samples were screened out of which 20 samples were from females and 85 samples from males. High vaginal swab and urethral swab were 52 and 24 samples respectively.

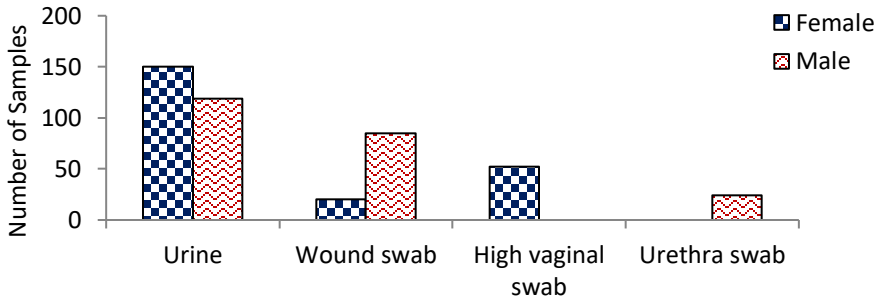


Figure 1. Collected sample distribution based on gender and sample type

The prevalence rate of *Staphylococcus aureus* from this study is 18.4%. Urine samples has a prevalence of 7.7%, wound swab 7.1%, high vaginal swab, 2.7% and urethral swab, 0.8% (Figure2).

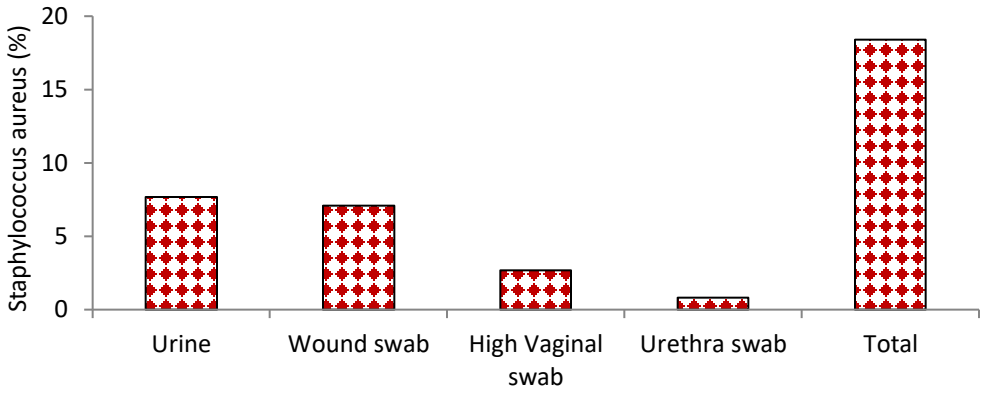


Figure 2. Prevalence of *Staphylococcus aureus* in the examined clinical samples

Phenotypic MethicillinResistant *S. aureus*

A total of 23 (27.6%) of the 83 *S. aureus* isolates were resistant to cefoxitin 30µg (zone ≤ 21mm).This shows that 27.6 % were methicillin resistant *S. aureus* (MRSA) phenotipycally. All the 23 isolates were sensitive to vancomycin. The prevalence of MRSA among the isolates screened was presented in figure 3. Urine samples had a prevalence rate of 8.4%, wound swab 14.4%, high vagina swab 3.6% and urethral swab 1.2% summing it up to a total of 27.6%.

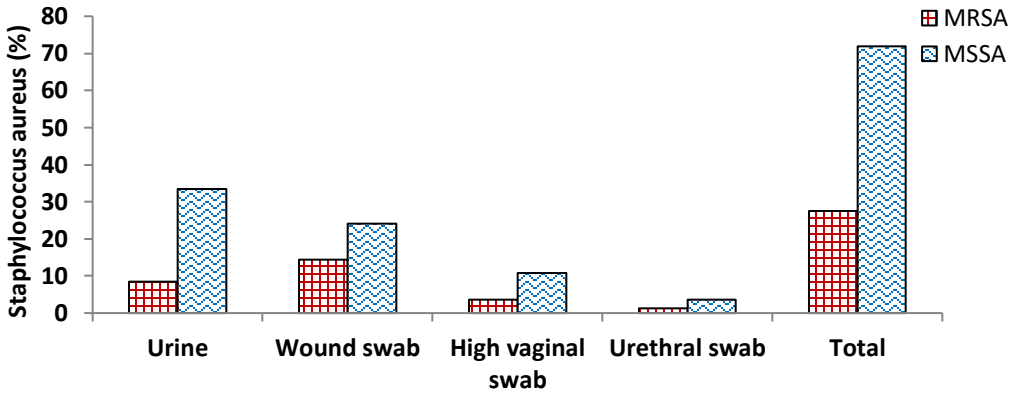


Figure 3: Prevalence of MRSA among clinical isolates screened. MRSA = Methicillin resistant *S. aureus*; MSSA = Methicillin sensitive *S. aureus*

Results of Antimicrobial Susceptibility Testing

The detailed antimicrobial susceptibility results of the 83 *Staphylococcus aureus* are shown in Table 1. Generally, out of the 83 pathogenic *S. aureus*, high percentage of resistance was observed against oxacillin (73.5%) and ampicillin (83.1%). Also, erythromycin had 54(65.1%) resistant isolates followed by ciprofloxacin, 40(48.2%) isolates; gentamycin, 39 (46.9%) isolates and augmentin with resistant rate of 36.1%. Vancomycin showed high activity

against 100% of the isolates, augmentin 63.9 %, ciprofloxacin 51.8 %, levofloxacin 66.3% and gentamicin 53.1% (Table 1).

Table 1. Antibiotic resistance/sensitivity profile of the isolated *S.aureus*

Antibiotics	**Potency(µg)**	**No. of *S.aureus*(%)**	
		Resistant	**Sensitive**
Ampicillin (AMP)	30	69 (83.1)	14 (16.9)
Augmentin (AMC)	30	30 (36.1)	53(63.9)
Levofloxacin (LV)	5	28 (33.7)	55 (66.3)
Ciprofloxacin (CIP)	5	40 (48.2)	43 (51.8)
Ceftriazone (CF)	30	21 (25.3)	62 (74.7)
Erythromycin (ET)	10	54 (65.1)	29 (34.9)
Gentamicin (GN)	10	39 (46.9)	44 (53.1)
Vancomycin (VAN)	30	Nil	83 (100)
Oxacillin(OX)	1	61 (73.5)	22 (26.5)

Beta (β)-Lactamase production

The result of β-lactamase production test (Figure 4) showed that seventy four (89.1%) isolates produced β - lactamase. Nine (9) were found to be non-β-lactamase producers.

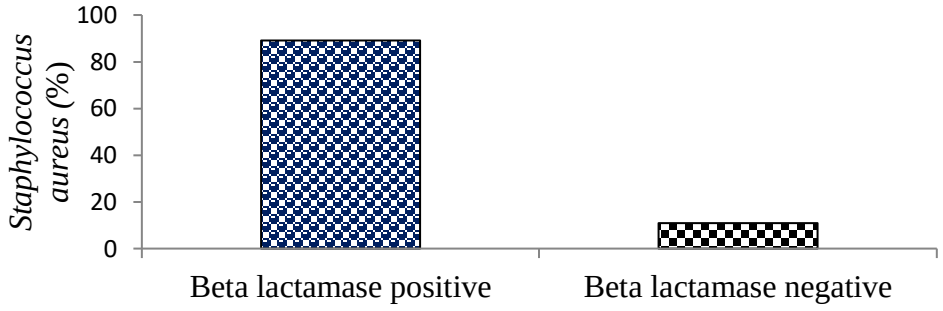


Figure 4.β-lactamase production by all the pathogenic *Staphylococcus aureus*

The Multiple Antibiotic Resistant Index (MARI) of all the isolated *S. aureus*

As calculated with the formula and indicated in Table 2, three(3) isolates were resistant to only one antibiotic, thirteen (13) isolates were resistant to two antibiotics, eighteen (18) isolates were resistant to three antibiotics. Fourteen (14) isolates were resistant to four antibiotics; fifteen (15) isolates were resistant to five antibiotics. MARI showed that sixty six (79.5 %) isolates were resistant to three or more antibiotics. MARI ≥0.4 indicated that the

isolates originated from an environment where antibiotics were frequently used. Ten isolate showed 100% resistance to the eight antibiotics tested. Percentage of *Staphylococcus aureus* to MARI was also calculated (Table 2).

Table 2. Antibiotic resistant indices of all the pathogenic *S.aureus* isolated

No. of antibiotics to which all *S. aureus* were resistant to	All resistant *S. aureus*	MAR index	% of all *S. aureus* to MARI
1	3	0.1	3.6
2	13	0.25	15.7
3	18	0.4	21.7
4	14	0.5	18.1
5	15	0.6	16.9
6	6	0.75	7.2
7	3	0.8	3.6
8	10	1.0	12.0

Detection of Penicillin Binding Protein (PBP2a)

The presence of *mecA* gene product PBP2a which is responsible for methicillin resistance was determined in isolates that showed phenotypic resistance to cefoxitin. Twenty three isolates were tested, eleven (47.8 %) methicillin resistant *S. aureus* were PBP2a positive and twelve (52.2 %) were negative (Figure 5).

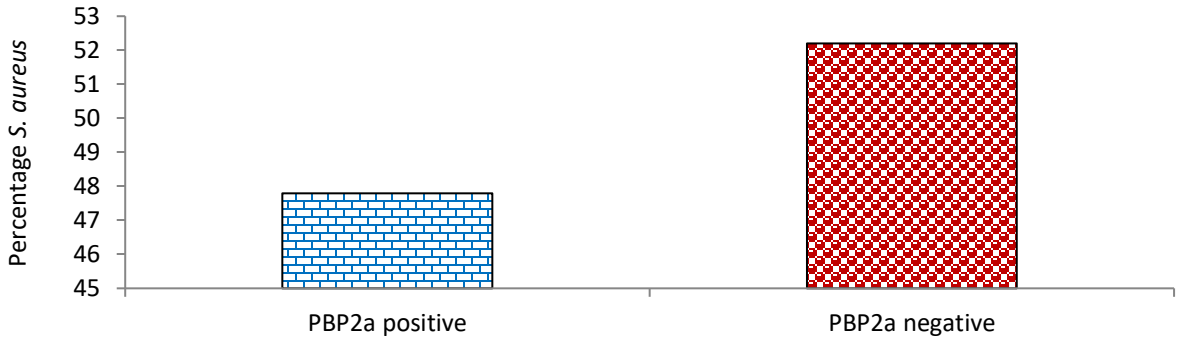


Figure 5. Result of the Latex Agglutination Test for Penicillin Binding Protein (PBP2a) in clinical isolates of methicillin resistant *Staphylococcus aureus*.

Discussion

In this study, a total of 450 samples were screened out of which 243 and 179 isolates were catalase and coagulase positive respectively. Eighty three isolates were finally confirmed ($p < 0.05$) to be *Staphylococcus aureus* among all the clinical isolates. Several works have reported the detection, screening and identification of *S. aureus* following catalase test, coagulase test and growth on Mannitol salt agar (Sahebnasagh *et al*., 2014; Jain *et al*., 2008; Athanasopoulos *et al*., 2007).The finding that the prevalence of *Staphylococcus aureus* was highest in urine

(7.7%) and wound (7.1%) has been corroborated by previous reports (Mitiku *et al*., 2021; Ali *et al*., 2019).

Wound swabs yielded the highest proportion of MRSA, and this had been established in previous studies (Ali *et al*., 2019; Fayomi *et al*., 2011). This was followed by urine and HVS in descending order. Because there is a breach in the skin epithelium in all wounds, it is therefore more prone to infection than the intact skin. Also, it has been suggested that the expanding use of invasive procedures in tertiary hospital environment, including prosthetic devices, intravascular, and urinary catheterization, might have accounted for high yields from urine (Obianuju *et al*., 2015).It is possible that the site of isolation of MRSA and specimen type could be associated with prevalence of MRSA.

The overall MRSA prevalence of 27.6% observed in this study may be considered high although it falls within the range determined in a previous report (Adeiza *et al*., 2020; Abubakar and Sulaiman, 2018) which put the prevalence in Nigeria at the range of 21%–50%. Similar proportions of 28.6% and 28% have been reported from studies in Kano and Bauchi, respectively (Nwankwo *et al*., 2014; Nwankwo *et al*., 2010). Some studies, however, reported even higher rates of 34.7%, 43%, and 79% from Ilorin, Jos, and Benin, respectively (Ibadin *et al*., 2017; Onemu and Ophori, 2013). The observed MRSA prevalence of 27.6% is close to 20.1% recorded in a study carried out in National Orthopedic Hospital Enugu also in South Eastern Nigeria but higher than 12.5% prevalence recorded in Maiduguri, North Eastern Nigeria (Onemu and Ophori, 2013).In contrast to our findings, the prevalence of MRSA was found to be very low in Switzerland (0.09%), the United Kingdom (0.005%), Spain and Portugal (Ike *et al*., 2016). However, a very high prevalence in the range of 42-51% frequency of MRSA was reported in Pakistan by (Ullah *et al*., 2016). This confirms the high regional variations in the findings from different countries and cities.

The antibiotics used in the hospital included in this study were ampicillin, erythromycin, oxacillin, ciprofloxacin, gentamicin, levofloxacin and ceftriazone. Antibiotics such as oxacillin and ampicillin were observed to have low activity against the 83 test isolates, with 85 % of the isolates being resistant. Similar patterns of antimicrobial susceptibility (92.1%) have been reported in Benin Nigeria (Igbinosa *et al*., 2016) and Zaria, Nigeria (Udobi *et al*., 2013; Okon *et al*., 2011).Medugu *et al.* (2021) and Motayo *et al*. (2012) have also recorded similar trends in previous studies. Although, the high resistance of the isolated MRSA to ciprofloxacin, erythromycin, and gentamicin have been confirmed by Motayo *et al.*(2012),it is not in line with later studies in Kano, Ekiti and Abeokuta which reported otherwise to ciprofloxacin and gentamycin (Ogundipe *et al*., 2020; Omoshaba *et al*., 2020; Nwankwo and Nasiru, 2011). These drugs are commonly prescribed, available as over-the-counter antibiotics, and may have developed resistance due to selective pressure from inappropriate use.

The finding that all the isolated MRSA were susceptible to vancomycin has been reported by previous studies in Nigeria (Ghamba *et al*., 2012, Fayomi *et al*., 2011). However, there are few reports of the emergence of vancomycin-resistant *S. aureus* in some centres in Nigeria (Olufunmiso *et al.*, 2017;Taiwo *et al*.,2011).Vancomycin has been the most reliable therapeutic agent against methicillin resistant *S. aureus* and resistance to this glycopeptides is seen as serious problem in antimicrobial chemotheraphy of MRSA infection (Mahros *et al*., 2021; Appelbaum, 2007). Various studies carried out in diverse population and different settings have reported vancomycin resistance in MRSA, however in 2011, the first MRSA to acquire resistance to vancomycin was isolated from Japanese

patient. Subsequent isolations of several vancomycin resistance *S. aureus* (VRSA) strain from USA, France, South Korea, Africa and Brazil ranging from 0-8 % has confirmed the emergence of VRSA a global issue (Hiramatsu *et al*.,2001). From 2002-2010, ten additional VRSA isolates were reported, eight from the United States, one from Iran and one from India (Gould, 2010).By the end of 2013 VRSA isolates have been reported for the first time in Europe (ECDE) and Latin America. As observed from this work, vancomycin which is the only antibiotic with 100% susceptibility, even with multidrug resistant strains of MRSA, remained the best therapeutic option for the isolates.

Bacteria often develop resistance to β-lactam antibiotics by synthesizing β-lactamase, an enzyme that attacks the β-lactam ring (Shrestha *et al*., 2021).To overcome this resistance, β-lactam antibiotics are often administered alongside β-lactamase inhibitors e.g amoxicillin (β lactam antibiotic) and clavulanic acid (β-lactamase inhibitor). The clavulanic acid is designed to overwhelm all β- lactamase enzymes, bind irreversibly to them, and effectively serve as an antagonist so that the amoxicillin is not affected by the β-lactamase enzymes (Luthra *et al*., 2018). This might have accounted for the high percentage (63.8 %) of sensitivity to Amoxicliin-clavulanic (Augmentin) in this study.

Susceptibility to cefoxitin (74.7%), followed by gentamicin (53.1%) and then ciprofloxacin (51.8 %) was high. The high resistance level by the isolates to β-lactam drugs was not unexpected in this study as 89.8 % of the *S. aureus* wereβ-lactamase producers. It was observed that *S. aureus* isolates are resistant to a large number of commonly prescribed antibiotics with the β-lactam agent taking the highest proportion. This may be due to the ability of more than 80 % of staphylococcal isolates to now produce penicillinase regardless of the clinical setting (Lakhundi and Zhang, 2018).

Ten of the isolates were observed to be resistant to all the 8 antibiotics tested (MARI ≥0.4) while one isolate was susceptible to all the antibiotics tested. These suggest that such resistant isolates originated from a high risk source of contamination where antibiotics are often used or that a large proportion of the bacterial isolates have been exposed to several antibiotics (Ayandele *et al*., 2020).

Cefoxitin(30μg) was used in this study for detection of phenotypic MRSA. It is an accepted method for detecting MRSA with high efficiency and has been used as an alternative to PCR in resource constrained areas (Ghamba *et al*., 2012; Anand *et al*., 2009). Also, recent studies have indicated that disc diffusion test using cefoxitin is far superior to most of the currently recommended phenotypic methods like oxacillin disc diffusion (Sultana *et al*, 2019; Anand *et al*., 2009). It has been reported as surrogate marker of *mecA* gene, gives clearer end points, easier to read and is more reproducible than tests with Oxacillin disc diffusion (Jain *et al*. 2008). It is the best for determining *mecA* mediated resistance in *S. aureus* (CLSI 2007). Cefoxitin will only detect MRSA with *mecA* mediated resistance mechanism (Swenson *et al*., 2007). CLSI guidelines regard the isolates as MRSA if they are found resistant to either cefoxitin or oxacillin or both regardless of the presence of *mecA* gene. CLSI, (2012) has also recommended that oxacillin be replaced by cefoxitin, a more potent inducer of mecA gene expression, which is less affected by test condition and hyper production of penicillinase (Brown *et al*., 2005), although, the gold standard for the detection of MRSA is the polymerase chain reaction (PCR) that detects *mecA* gene or alternatively, by detecting the *mecA* gene product, PBP2a, by latex agglutination test (Berger- Bachi and Rocher, 2002). Detection with cefoxitin, when compared to studies that used polymerase chain reaction

(PCR) for mecA detection in southwestern Nigeria, Ekiti in particular, which recorded prevalence of 22.2% and 19.2%, respectively (Olowe 2013; Terry, 2011), the prevalence in this study(27.6%) is higher, noteworthy as this is the first available information on this in the hospital.

Methicillin resistance in *S. aureus* is primarily mediated by the *mec*A gene which codes for the modified penicillin-binding protein 2a (PBP 2a or PBP 2') (Fishovitz *et al*., 2014; Shittu *et al*., 2011). PBP2a is encoded by the *mecA* gene located in the bacterial cell wall and has a low binding affinity for β-lactams (Fishovitz *et al*., 2014). The observed low percentage rate of PBP2a (13.3%) is in agreement with previous studies about the suitability of latex screen test for MRSA identification (Gorwitz *et al*., 2015). According to Motayo *et al*. (2012), resistance in *S. aureus* involves two mechanisms, the expression of beta lactamase and *mecA* gene. As in other kinds of resistance, this may be connected with inappropriate use of antibiotics in the hospital, lack of antibiotics policy and guidelines and poor infection control practices.

Conclusions

The prevalence of methicillin resistant *Staphylococcus aureus* in Enugu state university teaching hospital within the study period is 27.6%. All the isolated MRSA showed one hundred percent sensitivity to only vancomycin while ampicillin and oxacillin gave the highest percentage resistance. A higher percentage of the pathogenic *Staphylococcus aureus* are β - lactamase producers and almost half of these possessed PBP2a. Both β - lactamase production and presence of PBP2a are pointers to the multidrug resistance (MDR) profile observed. This was also supported by the MARI (≥0.4) calculated that indicated that the isolates originated from an environment where antibiotics were frequently used.

While it is notable that this research showed zero vancomycin resistant *Staphylococcus aureus* isolated within the period of study from ESUTH teaching hospital, Enugu, it could also serve as a valuable update for programs and policy decisions in reducing the emergence and spread of antimicrobial resistance of *S. aureus* in south eastern Nigeria. Continuous update in surveillance on antibiotic susceptibility of *S. aureus* could help public health workers in combating outbreak. Surveillance programs on the prevalence and characterization of MRSA would be of great importance in understanding its epidemiology in Nigeria.

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