ANTIMICROBIAL SUSCEPTIBILITY PROFILE AND OCCURRENCE OF VANCOMYCIN RESISTANCE AMONG CLINICAL ISOLATES OF*STAPHYLOCOCCUS AUREUS*IN ENUGU METROPOLIS, NIGERIA

ABSTRACT

Introduction:*Staphylococcus aureus* is a significant cause of community-acquired and healthcare-acquired infections worldwide. Methicillin-resistant Staphylococcus aureus infections have been treated with vancomycin, a last-resort antibiotic. This study aimed to investigate the occurrence of vancomycin resistance among *Staphylococcus aureus* isolates obtained from different clinical samples. **Material and Methods:**The study was conducted from February 2022 to August 2022 in the Microbiology Laboratory of the University of Nigeria Teaching Hospital Ituku-Ozalla, Enugu. A total of 150 *Staphylococcus aureus* isolates from different clinical samples were used for the study. The antimicrobial susceptibility profile was done using the Kirby-Bauer disc diffusion procedure. Methicillin resistance was detected using the cefoxitin disc diffusion technique. VRSA was confirmed using the broth dilution method. **Results:** Isolates showed high resistance to cephalosporins; 100% resistance to cefixime, to cefotaxime 98.7%, to cefuroxime 93.3%, and ceftriaxone 80%. There was also high resistance of isolates to imipenem 98.7%, Augmentin 92.2%, erythromycin, and azithromycin 81.3% and 80.0% respectively. Of the 150 isolates, 51(34.0%) were methicillin-sensitive *Staphylococcus aureus* (MSSA) and 99(66.0%) were MRSA. Of the 99 MRSA strains, 9 (9.1%) were Vancomycin-resistant (VRSA), 24 (24.2%) were Vancomycin intermediate (VISA) and 66 (66.7%) were vancomycin-sensitive (VSSA). The overall prevalence of VRSA was 6%. **Conclusion:** The isolates had a high resistance to the antibiotics used in our study. The proportion of MRSA was high. A high percentage of VRSA/VISA was also detected. To prevent the further spread of VRSA, rigorous monitoring of vancomycin treatment, response, and the development of appropriate control guidelines is strongly recommended and required.

KEYWORDS: Antimicrobial Resistance, *Staphylococus aureus,* MRSA, VRSA, Enugu Metropolis

1. INTRODUCTION

Staphylococcus aureus is a significant pathogen found across hospital-acquired and community-acquired infections and causes numerous infectious diseases including mild skin and soft tissue infections, osteomyelitis, joint infections, cardiovascular diseases, infectious endocarditis, bacteremia, life-threatening pneumonia in both apparent individuals and those with underlying diseases. [1,2]. It is an endogenous microbe that colonizes the skin, nose, anus, intestine, and vulva [3]. The rising incidence of both community and nosocomial staphylococcal infections overlap with the advent of multidrug-resistant *S. aureus*, making antibiotic therapies impotent [4].

S. aureus has lately acquired resistance to a spectrum of antibiotics, particularly the beta-lactam group [5]. The development of methicillin-resistant S. aureus (MRSA) and vancomycin-resistant *S. aureus* (VRSA) is a major international health problem. Presently, VRSA and MRSA isolates are regarded as highly powerful and harmful agents that may have the capacity to cause severe damage globally in the absence of suitable alternatives [6].

Methicillin-resistant *S. aureus* (MRSA) is a strain of *S. aureus* that has failed to respond to earlier generations of penicillin (oxacillin, methicillin, dicloxacillin, cloxacillin, and nafcillin) antimicrobial drugs. The resistance is mediated by the conjugated staphylococcal cassette chromosome mec (SCCmec) gene. All these SCCmec have the mecA gene, which encodes the low-affinity penicillin-binding protein 2a [7].

S. aureus uses a variety of resistance mechanisms, including the development of beta-lactamase enzymes to render beta-lactam antibiotics ineffective, an efflux pump for thrusting out drugs like tetracyclines[8], synthesis of aminoglycosides modifying enzymes to deactivate aminoglycoside antibiotics, modifications of DNA gyrase and topoisomerase IV expression of fluoroquinolone antibiotics, expression of Mec genes that alter penicillin-binding proteins [9] and decreased accumulation of macrolides antibiotics [7, 10].

In the late 1980s, vancomycin became the preferred antibiotic for treating patients infected with MRSA in hospitals. [11] Nevertheless, after several years on the market, vancomycin-resistant *S. aureus* strains evolved in Japan. VRSA strains emerged in the United States five years after that of Japan [12]. *S. aureus* acquires vancomycin resistance plasmid gene from vancomycin-resistant Enterococcus (VRE) via transposon Tn1546 [7, 13].Two types of glycopeptide-resistant *S. aureus* emerged as a result of increased use of vancomycin [4]. The vancomycin-intermediate *S. aureu*(VISA) which is one, is associated with a cell wall that is thicker and weakly cross-linked, leading to the formation of targets at the cell margin that sequester glycopeptides. The second type is vancomycin-resistant *S. aureus*(VRSA), which causes high-level resistance [14]. As the infections caused by VRSA increase yearly, there is growing evidence that they will become more common over time [15, 16] .*S. aureus*heteroresistance to vancomycin has been reported, which could contribute to the generation of more resistant strains in the future. The global prevalence of VRSA ranges from 9.8% to 52.4% [7, 17, 18]. Resistance was mostly recorded among patients from intensive care units, followed by those from medical wards and then surgery[5]. Patients with bacteremia and/or endocarditis are more likely to die from VRSA infections; hence a treatment plan that has high bactericidal performance must be evaluated while considering therapy options. VRSA has adverse consequences for patients due to *S. aureus*' aggressive features. In addition to the aggressiveness of *S. aureus* and the limited options for therapy in MRSA infections, the emergence of VRSA constitutes a significant threat to the general public. Moreover, the likelihood of these microbes spreading within patients is disturbing [14].

In Nigeria, multi-drug resistance infection has continued to rise due to numerous causes such as abuse of drugs, indigence, self-medication, and a shortage of trained medical personnel. As the global community faces the continuing growth in antimicrobial resistance, enough statistics regarding AMR must be available; this might serve as the essential framework for establishing successful measures for managing the issue of antimicrobial resistance [1].

However, data are scarce on susceptibility patterns for *S. aureus*, particularly MRSA and VRSA, in our research region. Even worse, there has been no appropriate regulation of medications, and self-administration of antibiotics is common, contributing to antibiotic resistance in the research area. Periodic and increased surveillance of antibiotic susceptibility of bacterial isolates from various clinical sources is required. Hence, this study was aimed to determine the susceptibility profile of *S. aureus*, and the occurrence of MRSA, VRSA, and VISA among the isolates in the Enugu metropolis [3].

2. MATERIALS AND METHODS

2.1 Study design

The study was conducted from February 2022 to August 2022 in the Microbiology Laboratory of the University of Nigeria Teaching Hospital Ituku-Ozalla,

2.2 Study Area

Enugu Metropolis is in Enugu State, Nigeria's South-East geopolitical zone. It is bordered by the states of Ebony to the east, Abia to the south, Kogi and Benue to the north, and Anambra to the west. It is located at the foot of the Udi Plateau.Enugu State has three senatorial zones: Enugu North, Enugu East, and Enugu West with seventeen local government areas. There are four referral hospitals within the metropolis and many private hospitals.

2.3 Laboratory Examinations

2. 3.1Collection of Isolates and identification

A total of 150 laboratory-confirmed *S. aureus isolates* were randomly collected from a variety of clinical specimens, including blood, urine, wounds,semen, high-vaginal swab (HVS), Endocervical smear (ECS), Urethral smear (US) and catheter tips. *S. aureus* colonies were collected aseptically using a sterile wire loop and sub-cultured onto nutrient agar slants before being incubated at 37^0C for 24 hours. They were sub-cultured again onto mannitol salt agar and blood agar (Oxoid, UK).The plates were incubated at 37^oC for 24 hrs. Following incubation, colonies with yellowish pigments from the agar were characterized using standard microbiological procedures such as Gram stain, catalase test, and coagulase test [19, 20].

2.3. 2 Antimicrobial Susceptibility Test

Antimicrobial susceptibility was determined using the Kirby-Bauer disc diffusion method by Clinical and Laboratory Standard Institute (CLSI) standards [21]. The antimicrobial susceptibility profile of each isolated S. aureus was assessed using the conventional disc diffusion method on Mueller-Hinton agar and aerobically incubated at 370C for 18-24 hours; The following antibiotics were included Cefuroxime (30µg), Cefotaxime (25µg), Ceftriaxone (45µg), Augmentin (30µg)), Cefixime (5µg), Cefoxitin (30µg), Erythromycin (15µg), Gentamicin (1µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Imipenem (10µg), Levofloxacin (5µg), Azithromycin (5µg), Vancomycin (30µg).

2.3.3 The Detection of Methicillin Resistance

Methicillin resistance was established using the disc diffusion susceptibility test. This was accomplished using Cefoxitin 30µg antibiotics disc (Oxoid, UK) on Mueller-Hinton agar plate and interpreted according to CLSI recommendations. The growth of *S. aureus* with a zone of inhibition surrounding cefoxitin disc ≤ 21mm was classified as methicillin-resistant S. aureus, while isolates with a zone of inhibition ≥ 22mm were characterized as methicillin-susceptible *S. aureus* (MSSA) [22].

2.3.4 The determination of minimum inhibitory concentration (MIC) of vancomycin

The minimal inhibitory concentration (MIC) was determined for VISA and VRSA. The MIC of vancomycin for MRSA was measured using the broth dilution method employed by Anosike et al. [23]. One gram of vancomycin powder in a bottle was dissolved in 20 ml of sterile water to yield a vancomycin concentration of 50 mg/ml according to the manufacturer's guidelines. 1 ml of 50 mg/ml vancomycin was diluted with 50 ml of water to get 1 mg/ml (1000 µg/ml) of vancomycin. This was then treated to a two-fold serial dilution in test tubes using sterile nutrient broth.Vancomycin concentrations were graded as follows: 0.98µg/ml, 1.95µg/ml, 3.9µg/ml, 7.8µg/ml, 15.63µg/ml, 31.25µg/ml, 62.5µg/ml, 125µg/ml, 250µg/ml, 500µg/ml, and 1000 µg/ml. The inoculums were prepared using 0.5 McFarland as standard. Three control culture tubes were also included: one with sterile nutrient broth, one with the test organism, and one with vancomycin (1000 µg/ml) exclusively. All were labeled appropriately. After 24 hours of incubation, all test tubes were inspected for evidence of growth. Turbidity in the test tubes after incubation indicated bacterial growth. The absence of turbidity in the test tube showed that growth was inhibited. The MIC was the lowest concentration of vancomycin that inhibited the growth of the test organism. In the controls, growth was observed in the test tube containing nutrient broth and the test organism alone. Evidence of growth was not seen in the tubes containing sterile nutrient broth alone, and nutritional broth with vancomycin antibiotic alone. Vancomycin MIC values of < 2 µg/ml were considered susceptible, ≥4 to 8 µg/ml were intermediate resistant, and ≥16 µg/ml were resistant [22].

3. RESULTS

Fig 1 shows the distribution of clinical isolates of *Staphylococcus aureus* according to the source of the isolates. The highest number of isolates was recovered from urine 78 (52.0%), followed by HVS 18 (12.0%), ECS 14 (9.3%), urethral smear 12 (8.0%), and the least was from catheter tips 2 (1.3%).

Fig 2 shows the antimicrobial susceptibility profile of the total isolates. Isolates showed high resistance to cephalosporins; 100% resistance to cefixime, 98.7% to cefotaxime, 93.3% to cefuroxime, and 80% to ceftriaxone. There was also high resistance of isolates to carbapenem; imipenem at 98.7%, Augmentin at 92.2%, and macrolides; erythromycin and azithromycin at 81.3% and 80.0% respectively.

Fig 3 shows the results of Cefoxitin susceptibility. Of the 150 clinical isolates of *S. aureus*, 99 (66.0%) were methicillin-resistant (MRSA) and 51 (34.0%) were methicillin-sensitive (MSSA).

Table 1 shows the prevalence of MRSA and MSSA according to the source of the isolate. Out of the 99 samples that were MRSA, isolates from catheter tip ranked highest at 2 (100%), followed by HVS 14 (77.8%), ECS 10 (71.4%), and the least blood culture 2 (33.3%). Of the 52 isolates that were MSSA, urine isolates ranked highest 4 (66.7%).

Table 2 shows the MIC results of MRSA isolates in the graded concentration of vancomycin. There was no growth in the tubes of 66.7% (66/99) of MRSA isolates with concentrations of 0.98-500 µg/ml, 9.1% (9/99) showed evidence of growth from 0.98-7.8 µg/ml, 24.2% (24/99) showed evidence of growth in all the tubes except positive control.

Table 3 shows the distribution of Vancomycin sensitive (VSSA), Vancomycin intermediate VISA, and Vancomycin-resistant (VRSA) isolates according to the source of the isolates. Of the 99 isolates that were MRSA, 9.1% (9/99) were VRSA, 17.2% (17/99) were VISA, and 66.7% (66/9) were VSSA. The highest occurrence of VRSA was seen in the urethral swab 16.7% (1/6) followed by wound swabs 14.7% (1/7) and the least was in the urine 12.9% (7/54). There was no VRSA in isolates from catheter tips, blood culture, and semen, HVS, and ECS.

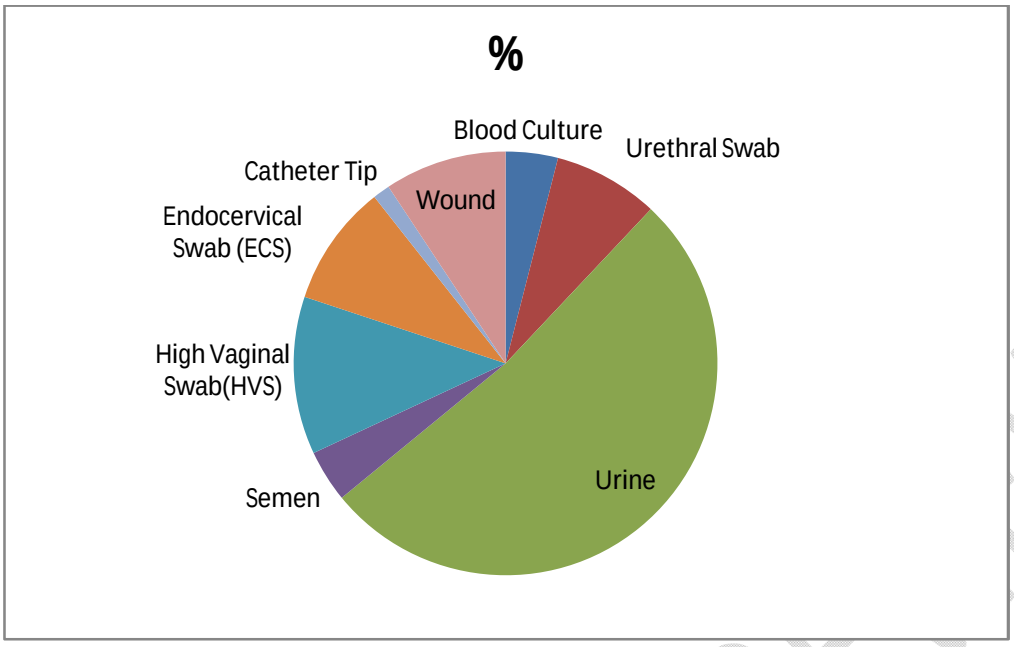

Fig1: Distribution of Clinical Isolates of *Staphylococcus aureus* according to Isolate Source

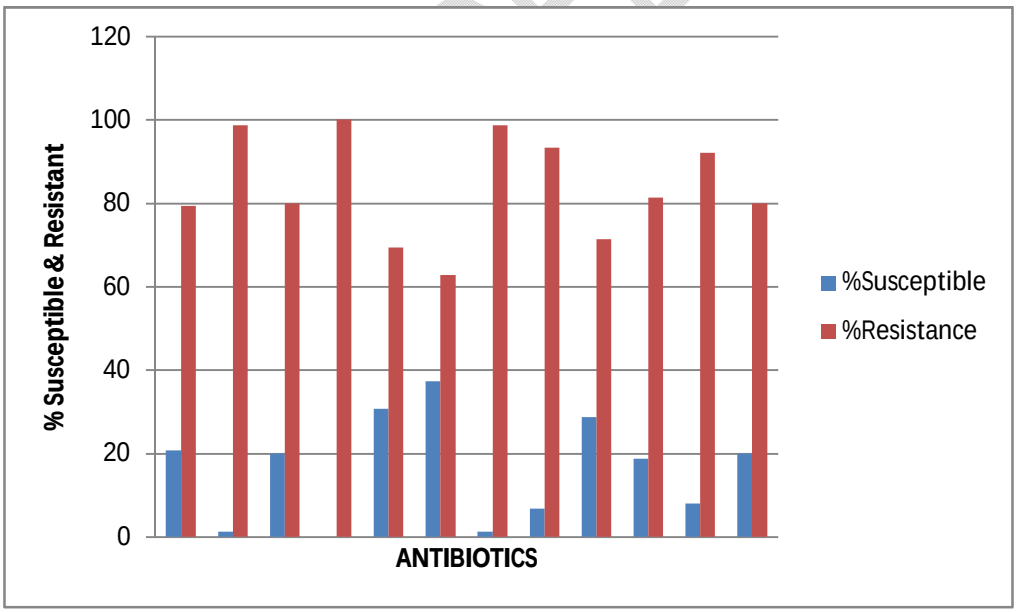

Fig 2: Resistant and Susceptibility Profiles of Clinical Isolates of *Staphylococcus aureus*

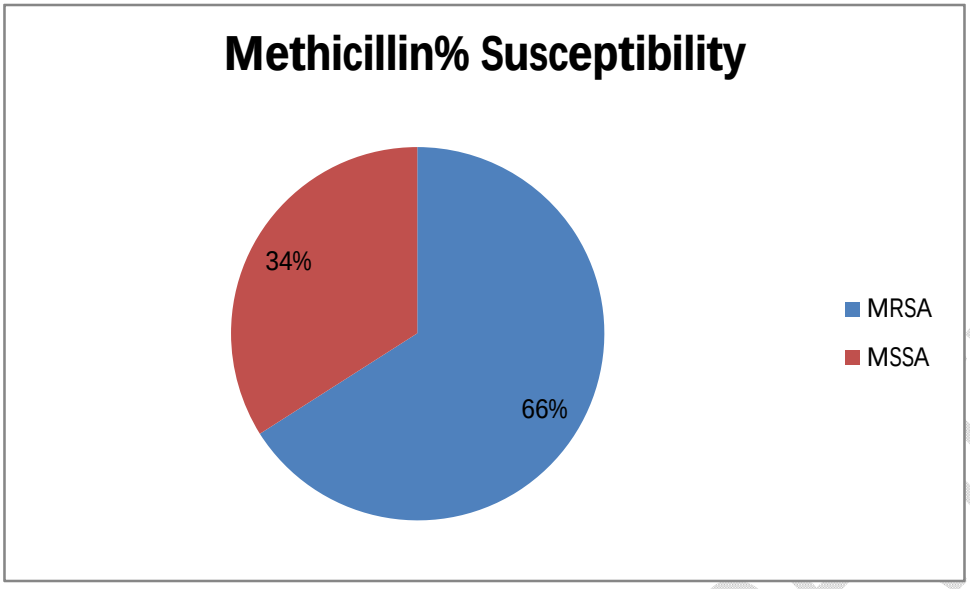

Fig 3: Distribution of Methicillin Resistance and Methicillin sensitive isolates of S. aureus

Table 1: Distribution of MRSA and MSSA according to isolate source

Sample Source	MRSA: No/%	MSSA: No/%	Total
Blood Culture	2 (33.3)	4 (66.7)	6 (4.0)
Urethral smear	6 (50.0)	6 (50.0)	12 (8.0)
Urine	54 (69.2)	24 (30.8)	78 (52.0)
Semen	4 (66.7)	2 (33.3)	6 (4.0)
High Vaginal Swab(HVS)	14 (77.8)	4 (22.2)	18 (12.0)
Endocervical Swab (ECS)	10 (71.4)	4 (28.6)	14 (9.3)
Catheter Tip	2 (100)	0 (0.0)	2 (1.3)
Wound	7 (50.0)	7 (50.0)	14 (9.3)
Total	**99 (66.0)**	**52 (34.7)**	**150 (100.0)**
P= 0.290	**X^2= 8.51**		

Table 2: MIC of Vancomycin for MRSA Isolates

Concentration (µg/ml)	No/%	Result
0.98-500	66(66.7)	No growth
0.98-7.8	24 (24.2)	Turbid
0.98-500	9 (9.1)	Turbid

Table 3: Distribution of MRSA Isolates according to Vancomycin Susceptibility

Sample Source	MRSA: No/%	VRSA*: No/%	VISA*:No/%	VSSA*: No/%
Blood Culture	2	0 (0.0)	0 (0.0)	2 (100)
Urethral Swab	6	1 (16.7)	1 (16.7)	4 (66.7)
Urine	54	7 (12.9)	11 (20.4)	36 (66.7)
Semen	4	0(0.0)	0 (0.0)	4 (100)
High Vaginal Swab(HVS)	14	0 (0.0)	3 (21.4)	11 (78.6)
Endocervical Swab (ECS)	10	0 (0.0)	8 (80.0)	2 (20.0)
Catheter Tip	2	0(0.0)	0 (0.0)	2 (100)
Wound	7	1 (14.2)	1 (14.2)	5 (71.4)
Total	**99 (100)**	**9 (9.1)**	**24 (24.2)**	**66 (66.7)**

*VRSA=Vancomycin Resistant S. aureus; *VISA= Vancomycin intermediate; *VSSA= Vancomycin Sensitive/susceptible

4. DISCUSSION

Staphylococcus aureus is a major cause of nosocomial infections and poses a substantial threat to human health all over the world. This is owing to the organism's highly developed virulence factors, pathogenicity, and the ability to repeatedly acquire resistance to overcome the obstacles that new drugs present [24]. Even though vancomycin is the antimicrobial agent of choice used to treat staphylococcal infections, especially MRSA, there has been a reported decrease in S. aureus susceptibility to the drug, as well as the isolation of intermediate- and resistant strains of the bacteria in many countries around the world. In the absence of proper management and treatment alternatives, MRSA and VRSA have become agents of extraordinary relevance, with the possibility of causing very serious global mortality. Furthermore, VRSA often exhibits multidrug resistance (MDR) against a range of antimicrobial drugs available in the market [25]. Since its first description in 1991, vancomycin resistance in *S. aureus* has been the focus of intensive research and a challenge to the healthcare sector[26].

Our study showed that the *S. aureus* isolates were highly resistant to most of the antibiotics used such as cefotaxime, cefixime, imipenem, Augmentin, azithromycin, erythromycin, and ceftriaxone. S. aureus has always been an important pathogen capable of developing resistance to novel antimicrobial drugs. Many variables may contribute to this organism's great antibiotic resistance. Self-medication, the availability and usage of antibiotics without a prescription from a competent professional, unreasonable antibiotic intake, noncompliance with prescriptions, and the sale of counterfeit or substandard medications are all contributory factors [27]. All of these are common in Nigeria.

The prevalence of MRSA in our study was 66.0% (99/150), this is consistent with the reports in some parts of the world such as 60.8% and 72% in Bangladesh [28, 29]. Tefera et al reported a lower prevalence of 45.1% in Ethiopia [7]. MRSA has become a global health concern, as indicated by the high prevalence of antibiotic resistance reported in different nations such as 76% in Lahore, India, 21.5 % in Turkey, 72% in Eritrea, and 77.9% in Iran, [17, 30, 31, 32]. The rising trend of MRSA stresses the need for severe infection control methods such as stringent compliance with hand cleanliness, prevention of antibiotic abuse, and a regular MRSA surveillance program [33].

In our study, the overall prevalence of VRSA in the whole isolates was 6.0% (9/150), This is consistent with the work of Bamigboye et al who reported 1.4% in Osogbo, Southwest Ogefere, et al who reported VRSA of 4.5% in Benin City South South while Alo et al reported 5.3% in Abakiliki, Southeast all in Nigeria [3, 24, 34]. Saderi et al also reported a lower occurrence of 3.6% in Iran 3.6% [35]. A higher prevalence of 44.5% was also reported in Nigeria by Olufunmisoet al.[26]. However, higher prevalence had been reported in some parts of the world including Pakistan 25% [36], Ethiopia 14.1% [7], and Nepal

21% [37]. The influencing factors responsible for the rate of variance could be different geographical areas, variation in sample size and length of study, source of isolates, methodology, antibiotic policy, and status of infection control. Despite these geographical variances in incidence, these different degrees of resistance to vancomycin have resulted in increasing concern about its efficacy for treatment in MRSA infections [26].

The highest occurrence of VRSA was found among urethral swab isolates at 16.7% (1/6) followed by a wound at 14.2% (1/ 7). Some researchers have reported catheter tips [24, 36] and some in Urine [26]. The variation could be due to sample size and may also be earlier exposure of this drug to isolates which may have enhanced the development of resistance. There is a significant degree of antibiotic abuse in the study area resulting from self-medication, which is frequently accompanied by poor dosage, inability to comply with treatment, and the availability of antibiotics to customers across the counters with or without prescription [38].

Conclusion

The isolates had a high resistance to most of the antibiotics used in our study. The proportion of MRSA was high. The overall occurrence of VRSA among clinical isolates of *Staphylococcus aureus* was 6.0%. The cautious use of vancomycin and other antimicrobial drugs is recommended. The importance of continual monitoring, screening, and susceptibility testing, cannot be overstated. Antibiotics and other prescription pharmaceuticals should be made available to the public under strict regulations. Efforts should also be targeted towards the development of novel drugs to tackle the problem of multidrug failure.

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