*Nigerian Journal of Pharmaceutical and Applied Science Research, 12(4):41-49; December 2023 p-ISSN: 2971-737X; e-ISSN: 2971-7388. Available at www.nijophasr.net*

# Prevalence of methicillin and vancomycin-resistant *Staphylococcus aureus* in goat meat and environment of sales in Uyo metropolis, Akwa Ibom State, Nigeria.

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**ABSTRACT**

***Background***: There has been an increasing incidence of drug resistance associated with Methicillin-Resistant *Staphylococcus aureus* (MRSA) and the emergence of vancomycin resistance trait in humans leading to concerns to detect other sources which this resistant could emanate. This study was carried out to determine the prevalence, antibiotic susceptibility profile of MRSA and vancomycin-resistant *Staphylococcus aureus* (VRSA) in goat meat, its handlers and environment of sales within Uyo Metropolis, Akwa Ibom State.

***Methods*:** *Staphylococcus aureus* were isolated from the samples using mannitol salt agar (MSA) and standard laboratory methods. Antibiotic susceptibility pattern was determined using modified Kirby Bauer disc agar diffusion method.

***Results:*** Ninety-five (63.3%) isolates out of the 150 samples were confirmed and characterized as *Staphylococcus aureus*. One hundred percent (100 %) of the isolates were resistant to norfloxacin (10 µg), One hundred percent (100 %) were susceptible to rifampicin (20 µg) and levofloxacin (20 µg) respectively. Fifty-two (54.7 %) of the isolates were MRSA. The multiple antibiotics resistance (MAR) index indicated that 39 (75 %) were resistant to 3 or more antibiotics (MARI $\geq$ 3). Further PCR analysis of *Staphylococcus aureus* isolates that were MRSA indicated the presence of mecA gene in all the samples screened. There was a hundred percent (100 %) susceptibility of confirmed MRSA to vancomycin.

***Conclusion*:** Goat meat, its environment of slaughter and sales in Uyo metropolis were confirmed a reservoir of Methicillin-Resistant *Staphylococcus aureus* (MRSA) but not of Vancomycin-Resistant *Staphylococcus aureus* (VRSA).

***Keywords:*** Antibiotics, Goat Meat, Resistance**,** *Staphylococcus aureus*, Vancomycin.

## 1.0 INTRODUCTION

The decreasing effectiveness of antibiotics in treating common infections has become a worldwide problem. These increase in resistance is catalyzed by interspecies gene transmission, lack of good sanitation and hygiene in hospitals, animal habitats and our communities, thereby causing an increase in the frequency of disease transmission (1). *Staphylococcus aureus* is a Gram-positive bacterium and the most common cause of *Staphyloccocal* infection. It is responsible for different diseases such as skin infection, wound infections and toxin mediated diseases (2). *Staphylococcus aureus* is one of the major cause of infections in hospitals and in communities across the world, this has made *Staphylococcus aureus* develop resistance to commonly prescribed antimicrobial agents. It has the ability to acquire resistance to new antimicrobial agents. The first serious emergence of antibiotic resistant *Staphylococcus aureus* occurred as Methicillin-Resistant *Staphylococcus aureus* (MRSA). The strain expressed a modified penicillin-binding protein encoded by mecA gene. Due to widespread prevalence of MRSA, the empirical therapy for MRSA has been changed to vancomycin. The first incidence of reduced susceptibility of *Staphylococcus aureus* to vancomycin was reported in Japan in 1997 (3). Thereafter, various cases were reported in every continent except Oceania (4). Due to the continued use of vancomycin, the first case of vancomycin-resistant *Staphylococcus*

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*aureus* (VRSA) was reported in the United States in Michigan in 2002, followed by cases in New York, New Jersey, and Delaware (5). Further studies indicated that the origin of VISA was preceded by heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA) (6). There were also cases of hVISA, vancomycin-intermediate *Staphylococcus aureus* (VISA), and VRSA infections reported from every continent. This indicates that resistance to vancomycin, which is currently a highly reliable antibiotic for the treatment of MRSA infections, is a call for global concern. Antibiotics are being introduced into animal feed as growth promoters and in the treatments of different forms of ailments, most times in sub-therapeutic doses, thereby leading to the development of resistance (7). These goats feeds on water and plants which might have been contaminated with urine and other excretes from patients and other animals which have been placed on antibiotic thereby harboring these resistant strains of the organism. There is an urgent need to look into what people eat around us. Goat meat is a known delicacy among the people of Akwa Ibom State. Hence the choice of goat meat and the environment in which they are prepared in order to detect the possible source of *Staphylococcus aureus* resistance to antibiotics. This study investigated the Methicillin and Vancomycin-Resistant profile of S*taphylococcus aureus* from goat meat, its handlers and the environment of sales.

## 2.0 MATERIALS AND METHODS

### 2.1 Materials

*2.1.1 Sample Collection Sites*

A total of one hundred and fifty (150) samples were collected for this study. Sampling places include; abattoir at Itam, Itam market, Akpan Andem market and the market at Urua Ekpa junction. The sites include aprons of the seller, tables were meat are displayed for sale, chopping stick surface, hands of the sellers, meat and bodies of the animal already slaughtered and washed.

*2.1.2 Sampling and Cultivation Technique*

Samples were collected using moistened swab sticks from various sites ranging from slaughter house to market places where the goats are dissected to customers. The swabs were properly labeled and immediately transported to the Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, University of Uyo. The swabs were streaked unto sterile Mannitol Salt Agar (MSA) plates and incubated at 37℃ for 24 hours after which the colonial characteristics were observed.

### 2.2 Methods

*2.2.1 Identification of Staphylococcus aureus*

Isolates that grew on the mannitol salt agar were purified by sub-culturing on nutrient agar for 24 hours. The isolates were identified by Gram staining and biochemical reaction to identify *Staphylococcus aureus* (8). The shape, arrangement, and Gram reactions of the isolates were observed under a light microscope (at a magnification of 100x). Confirmatory biochemical tests including catalase and coagulase were performed to identify suspected *Staphylococcus aureus* following standard microbiology protocols (9).

*2.2.2 Antibiotic sensitivity test by Kirby-Bauer Disc Diffusion*

Antibiotic sensitivity testing was carried out using Kirby-Bauer disc diffusion method. *Staphylococcus aureus* isolates were sub-cultured in nutrient broth and incubated at 37 ℃ for 24 hours. The turbidity of the overnight broth culture was adjusted to the turbidity 0.5 MacFarland standards by two-fold serial dilution. A sterile pipette was used to aseptically transfer 0.1 mL of each isolate into already labelled petri dishes before introducing about 20 mL sterile nutrient agar. The plates were swirled to mix and allowed to set. Gram positive and Gram negative antibiotic disc were aseptically placed on the surface of the plate using sterile forceps. The plates were incubated inverted for 24 hours at 37 ℃. Antibiotic resistance and susceptibility were determined after 24 hours by measuring the zone of microbial inhibition in millimeters.

*2.2.3 Determination of Multiple Antibiotics 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 antibiotics tested (10).

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

*2.2.4 Detection of Methicillin-Resistant Staphylococcus aureus (MRSA) and Vancomycin-Resistant Staphylococcus aureus (VRSA***)**
Disc diffusion method was used to identify MRSA and VRSA. All the confirmed *Staphylococcus aureus* was tested for MRSA and VRSA using antimicrobial susceptibility cefoxitin (30 µg) and vancomycin disc (30 µg) by Kirby Bauer disc diffusion method respectively. Muller Hinton agar plates were inoculated with the bacterial suspension which was adjusted to the turbidity of 0.5 McFarland standards. Sterile forceps were used to place the cefoxitin and vancomycin disc on the agar plates. The plates were incubated at 37 ℃ for 24 hours. The diameter of the zones of microbial growth inhibition was measured in millimetres and compared with a standard which state that cefoxitin is sensitive when the diameter of the zone of inhibition nearest to whole millimetre is ≥ 22 while that of vancomycin is ≥ 15. (11, 12, 13).

*2.2.5 Detection of mecA Gene by PCR Technique*
Selected isolates found to be MRSA by specific phenotypic features was further tested by PCR confirmation using specific primer pairs. The mecA-specific primer pairs used are Forward, 5′-GTTGTAGTTGTCGGGTTTGG-3′, and Reverse, 5′-CTTCCACATACCATCTTCTTTAAC-3′. The extracted DNA cells was amplified beginning with an initial denaturation step at 94 ºC for 30 seconds, followed by 33 cycles of amplification at 94 ºC for 30 seconds, annealing at 47 ºC for 30 seconds and extension at 72 ºC for 30 seconds, followed by final extension step at 72 ºC for 5 minutes. The amplfied products was visualised by electrophoresis in 1.5 % agarose gel stained with ethidium bromide.

**2.3 Statistical Analysis**
Statistical analysis was done using electronic calculator to determine the percentage resistance and susceptibility.

**3.0 RESULTS**

**3.1 Confirmation of Staphylococcus aureus**
All the one hundred and fifty (150) swabs cultured yielded growth (100%) after 24 hours of incubation. The characteristic colonies ranged from 1 mm to 3.0 mm in size with varying shades of colour ranging from pale yellow to golden yellow. Ninety-five (63.3%) of the isolates were Gram positive and appeared as cocci in clusters when viewed under the microscope. They were catalase and coagulase positive, thus confirming them to be *Staphylococcus aureus*.

**3.2 Antibiogram**
Antibiogram of the 95 confirmed isolates of *Staphylococcus aureus* indicated that 95 of the isolates were resistant to norfloxacin (10 µg) (100 %), 76 were resistant to Amoxicillin (20 µg) (80.0 %), 63 were resistant to ampicillin/cloxacillin (20 µg) (66.3 %), 13 were resistant to chloramphenicol (30 µg) (13.7 %), 8 were resistant to streptomycin (30 µg) (8.4 %), 7 were resistant to erythromycin (30 µg) (7.4 %), 6 were resistant to gentamycin (10 µg) (6.3 %) while one hundred (100.0 %) of the samples were susceptible to rifampicin (20 µg) and levofloxacin (20 µg) as shown in Table 1. The pattern of resistant is expressed in figure 1.

Table 1: Number of resistant *Staphylococcus aureus* and their percentages

| ANTIBIOTIC | NUMBER OF RESISTANT ISOLATES | PERCENTAGE (%) |
|---|---|---|
| Ciprofloxacin (10 µg) | 4 | 4.2 |
| Amoxicillin (20 µg) | 76 | 80.0 |
| Chloramphenicol (30 µg) | 13 | 13.7 |
| Ampicillin/cloxacillin (20 µg) | 63 | 66.3 |
| Erythromycin (30 µg) | 7 | 7.4 |
| Norfoxacin (10 µg) | 95 | 100.0 |
| Gentamycin (10 µg) | 6 | 6.3 |
| Levofloxacin (20 µg) | 0 | 0.0 |
| Streptomycin (30 µg) | 8 | 8.4 |
| Rifampicin (20 µg) | 0 | 0.0 |
| Cefoxitin (30 µg) | 52 | 54.7 |
| Vancomycin (30 µg) | 0 | 0.0 |

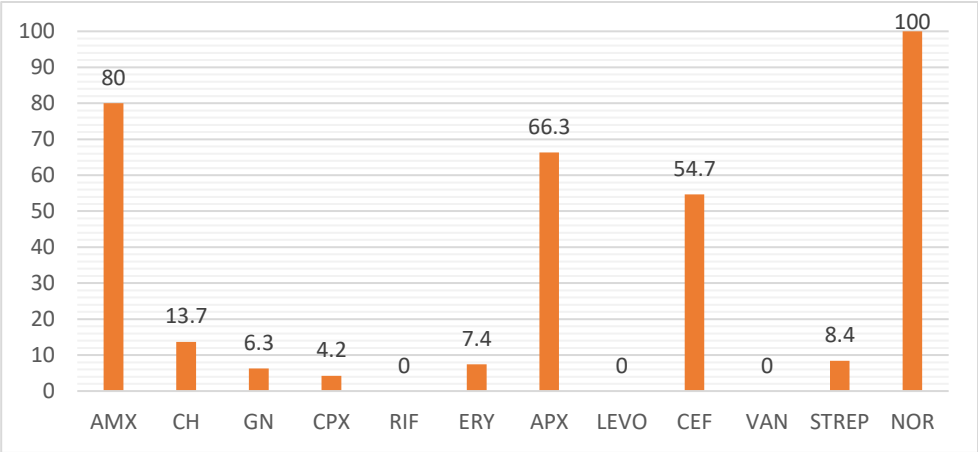



Figure 1: Percentage resistant of *Staphylococcus aureus* to antibiotics

Table 2: Antibiotics resistance pattern and multiple antibiotics resistance index of MRSA isolates

| Sample | Antibiotics Resistance Pattern | NART | ARC | MAR |
|---|---|---|---|---|
| I1 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| I2 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| I5 | NOR, AMX, STREP, AMP | 4 | MDR | 0.4 |
| I6 | NOR, AMX, STREP, AMP | 4 | MDR | 0.4 |
| I8 | NOR, AMX | 2 | MDR | 0.2 |
| I11 | NOR, AMX, ERY, GN | 4 | MDR | 0.4 |
| I15 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| I16 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| I18 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| I19 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| I20 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| I24 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| H8 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| H9 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| H10 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| H13 | NOR, AMX, AMP, CH | 3 | MDR | 0.3 |
| H14 | NOR, AMX, AMP | 4 | MDR | 0.4 |
| H15 | NOR, AMX, CH, AMP | 3 | MDR | 0.3 |
| H16 | NOR, AMX, CH | 4 | MDR | 0.4 |
| H17 | NOR, AMX | 3 | MDR | 0.3 |
| H18 | NOR, AMX, AMP | 2 | MDR | 0.2 |
| H20 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| H21 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| H22 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| H25 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| K2 | NOR | 1 | NIL | 0.1 |
| K8 | NOR, AMP | 2 | MDR | 0.2 |
| K14 | NOR | 1 | NIL | 0.1 |
| B20 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| S7 | NOR, AMX, AMP, CPX, CH | 5 | MDR | 0.5 |
| T1 | NOR | 1 | NIL | 0.1 |
| T4 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| T5 | NOR, AMX | 2 | MDR | 0.2 |
| T6 | NOR | 1 | NIL | 0.1 |
| T9 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| T11 | NOR, AMP, STREP | 3 | MDR | 0.3 |

| | | | | |
|---|---|---|---|---|
| T12 | NOR | 1 | NIL | 0.1 |
| T13 | NOR, AMX | 2 | MDR | 0.2 |
| A1 | NOR, AMX, AMP, STREP | 4 | MDR | 0.4 |
| A2 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| A4 | NOR, AMP, ERY | 3 | MDR | 0.3 |
| A5 | NOR, AMX | 2 | MDR | 0.2 |
| A7 | NOR | 1 | NILL | 0.1 |
| A8 | NOR, AMX, AMP, STREP | 4 | MDR | 0.4 |
| A15 | NOR, AMX, AMP, STREP | 4 | MDR | 0.4 |
| A16 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| H1 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| H2 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| H3 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| H4 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| H6 | NOR, AMX, AMP | 3 | MDR | 0.3 |
| H7 | NOR, AMX | 2 | MDR | 0.2 |

NART: Number of antibiotics resistant; ARC: Antibiotics resistance classification; MAR: Multiple antibiotic index; MDR: Multiple drug resistance.

KEYS: CEF-cefoxitin, AMP-ampicillin, AMX-amoxicillin, CPX-ciprofloxacin, CH-chloramphenicol, RIF-rifampicin, STREP-streptomycin, ERY-erythromycin, NOR-norfloxacin, LEV-levofloxacin, GN-gentamycin, VAN-vancomycin

### 3.3 Identification of MRSA

From the 95 isolates of confirmed *Staphylococcus aureus*, 52 isolates were found to be resistant to cefoxitin (30 µg), confirming them as phenotypic MRSA (54.7 %), (Table 1).

### 3.4 Multiple Antibiotic Resistance (MAR) Index

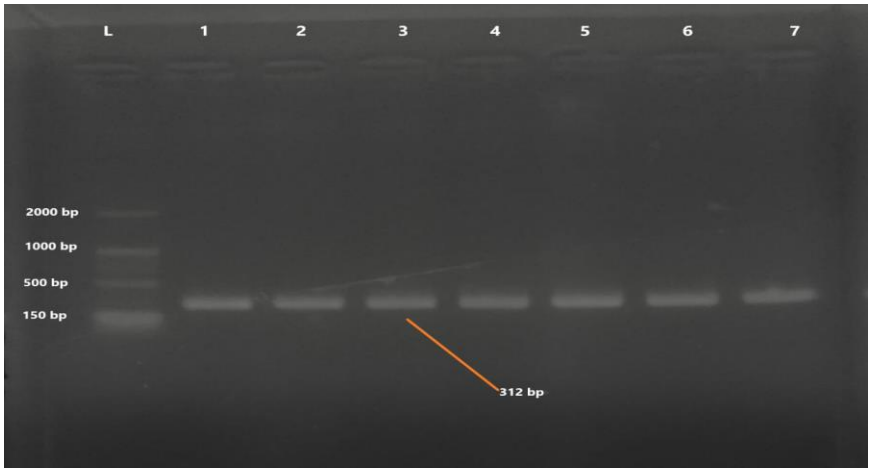
Out of the 52 samples that exhibited MRSA, 39 (75 %) showed MAR index as they were resistant to 3 or more antibiotics as presented on Table 2.

### 3.5 Antibiogram of confirmed *Staphylococcus aureus* Resistant to Cefoxitin (30 µg)

Comparison was done for MRSA with that of multiple drugs, results revealed that only 1 (1.9 %) out of the 52 MRSA isolates was resistant to ciprofloxacin (10 µg) showing lowest resistance, while 52 (100 %) out of 52 MRSA isolates were resistant to norfloxacin (10 µg) showing the highest resistance.

### 3.6 Detection of mecA Gene by PCR Technique

The PCR result obtained revealed that all the isolates carried mecA gene, confirming them as MRSA as shown in figure 2.




Lane L: Molecular weight ladder. Lane 1, 2, 3, 4, 5, 6 and 7 are tested isolates with positively amplified mecA (indicated by 312 bp PCR amplicon).

Figure 2. Amplicon of mecA gene:

### 3.7 Identification of VRSA

All the isolates of *Staphylococcus aureus* were susceptible to vancomycin (30 µg) (Figure 3) as the zones of inhibition were greater than CLSI specification (≥ 15mm).

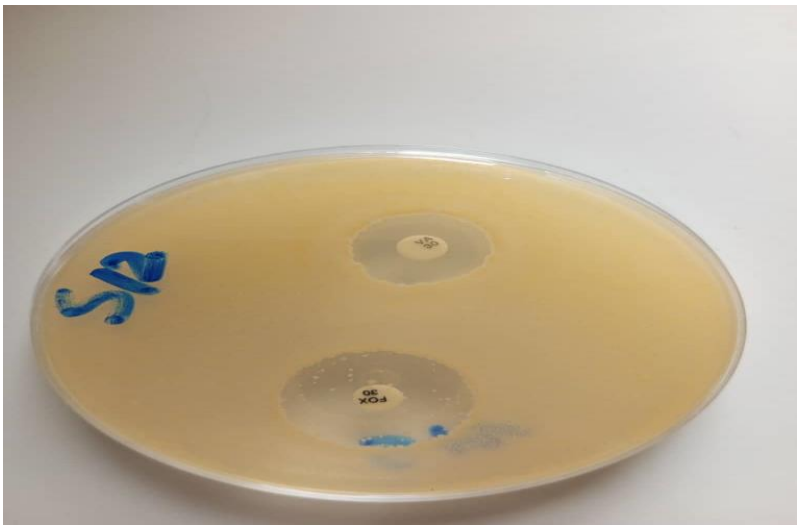



Figure 3: Cefoxitin and vancomycin disc showing zones of inhibition.

## 4.0 DISCUSSION

*Staphylococcus aureus* has been one of the most problematic pathogens and a major threat to human health worldwide due to its anti-microbial resistance, infectivity and possession of virulence factors (14, 15) as well as its ability to repeatedly acquire resistance to overcome the challenges presented by the new anti-staphylococcal antibiotics (16). Vancomycin is the main antimicrobial agent available to treat serious staphylococcal infections, especially those of MRSA, but a decrease in vancomycin susceptibility of *Staphylococcus aureus* and isolation of vancomycin intermediate and resistant *Staphylococcus aureus* from many countries have been reported (17). Since its first report in 1997, the threat of vancomycin resistance in the organism has been the topic of intensive research and discussion, attracting attention in the health care community (18). In his survey (19), interviewed animal keepers in Indonesia and reported a high incidence of antibiotics administration to animal. Sadly, most of the antibiotics are bought over-the-counter and are given to the animal without a proper dose regimen, leading to abuse and resistance. These antibiotics are given to treat infection or to prevent an outbreak and as growth promoter by the animal keepers (7). This investigation was aimed at exploring the prevalence of methicillin and vancomycin resistant *Staphylococcus aureus* in goat meat abattoir, its environment of sale and its handlers. *Staphylococcus aureus* was isolated from the samples as expected, susceptibility testing indicated the presence of resistant species. This is in tandem with the findings of (20) who isolated *Staphylococcus aureus* from horse and its handlers. Other researchers have reported the detection, screening and identification of *Staphylococcus aureus* following growth on Mannitol salt agar, catalase and coagulase test (21, 22). The multi-drugs antibiotics disc has found usefulness in the routine detection of antibiotic resistance in clinical laboratories. Similar procedure was employed in this study for the detection of methicillin and vancomycin resistance. Results obtained from the multi-drugs test showed 100 % resistance to norfloxacin and 80 % resistance to amoxicillin (table 1). Several reports (19) states that tetracyclines and amoxicillin are the most commonly used antibiotics in animal husbandry. Amoxicillin, a penicillin antibiotic is employed in the treatment of bacterial infections in humans. Unfortunately, animal breeders also use this drug in the management of these conditions in animals without using the proper dose determination. This is in consonant with the study by (23) who reported that *Salmonella paratyphi*-infected broiler barn was resistant to tetracycline, fluoroquinolones, trimethoprim, and cefoxitin. Nalidixic acid, the prototype quinolone and the second-generation quinolones (e.g. ciprofloxacin and norfloxacin) are predominately active towards Gram negative bacteria while third generation (e.g. levofloxacin) and fourth generation (e.g. moxifloxacin, gemifloxacin) quinolones exhibited improved and greater activity against Gram-positive bacteria (24). This has been seen in levofloxacin having 100 % susceptibility. Rifampicin, an ansamycin antibiotic also exhibited a strong potency (100 %) against *Staphylococcus aureus*. This may be attributed to it rare use in medical practice, making it still very effective. The multiple drugs resistance may be due to selective pressure from inappropriate use. (25). The overall MRSA prevalence of 54.7 % observed in this study may be considered high although it falls within the range determined in a previous report (26, 27) which put the prevalence in Nigeria at the range of 34.7 %, 43 %, and 79 % from Ilorin, Jos, and Benin, respectively. Reports by (28, 29) puts the prevalence in Nigeria to be at the range of 21 %–50 %. Similar proportions of 28.6 % and 28 % has been reported from studies in Kano and Bauchi respectively (30, 31). High resistance to methicillin might be connected to cross resistance which

is common among beta lactam antibiotics. The presence of mecA gene was used to confirm MRSA as all the representative samples screened using PCR method were positive for mecA. In this study, the susceptibility of *Staphylococcus aureus* isolates to vancomycin was 100 %, none was resistant indicating that the prevalence rate of VRSA is 0 %. In Nigeria, screening for methicillin and vancomycin resistant *Staphylococcus aureus* among clinical isolates was done in Enugu state, results obtained showed 100 % susceptibility to vancomycin, with 27.6 % susceptibility to multi drugs (25). Also, research carried out by (32) on clinical and community isolates within University of Port Harcourt showed 100 % susceptibility to vancomycin and resistant to other antibiotics. Vancomycin is less common and expensive, meaning it is less abused and could be the reason it has not yet developed resistance in goat and its environment of sale in this part of Nigeria, compared to the report of resistance to vancomycin by (20) in Kano and Zaria in *Staphylococcus aureus* isolated from Horses and it handlers.

## 5.0 CONCLUSION

Once upon a time, methicillin was effective against *Staphylococcus aureus* infections, not so again. Vancomycin is celebrated today due to *Staphylococcus aureus* being susceptible to it, but this may not last long because of abuse and misuse. The current findings contribute to the understanding of the resistance of *Staphylococcus aureus* from goat meat, its handlers and environment of sales in Uyo metropolis to commonly prescribed antibiotics, thus, confirming it as a reservoir of MRSA and not of VRSA. Therefore, vancomycin is still very effective in the treatment of infections due to *Staphylococcus aureus* in this part of Nigeria.

**Acknowledgment**

The authors acknowledge the assistance given by Prof. Emmanuel Etim of the Department of Pharmaceutical Chemistry, University of Uyo, Akwa Ibom State and Mrs Ekaette Nkanang of the University of Uyo medical center.

**Conflict of Interest**

The authors declare that there is no conflict of interest.

**Contribution of the Authors**

Edet E. Akpanenang – Idea generation, laboratory analysis and manuscript development; Ememobong G. Asuquo - Idea generation, supervision and manuscript editing; Chinweizu E. Udobi - Idea generation, supervision and manuscript editing; Chibuzor N. Nwosu – supervision; Ugochukwu D. Onele - supervision and manuscript editing.

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