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MOLECULAR CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF VANCOMYCIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM FROZEN CHICKEN MEATS SOLD IN NIGER STATE

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ABSTRACT

Introduction: Community-acquired *Staphylococcus aureus* (CA-SA) has emerged as an important human pathogen in various parts of the world, particularly in developing nations including Nigeria.
Aim: This study investigated the incidence, antibiotic resistance patterns, biofilm formation ability, and molecular characteristics of *Staphylococcus aureus* isolated from chicken meat in four Local government area (L.G.A) of Niger states, Nigeria.
Methods: *Staphylococcus aureus* isolation and identification were done via inoculation on a Brain Heart Infusion media plate containing 4% NaCl and 16SrRNA PCR, while the antimicrobial susceptibility profile was determined by the disc diffusion method on Mueller-Hinton agar. The phenotypic detection of Vancomycin-resistant *S. aureus* was carried out using the Vancomycin Agar Screen (VAS) method. Vancomycin resistance and biofilm-forming genes were characterized using PCR methods.
Results: A total of 188 frozen chicken meats were screened for *S. aureus*; 50% (94 samples) were positive for *Staphylococcus aureus*. Suleja had the highest incidence of 63.8%, while Bosso had the lowest (42.5%). The isolates were highly resistant to tetracycline {87 (92.6%)}, augmentin 61 (64.9%) and 60 (63.8%) ampicillin. The resistance rates varied among locations in the State. High percentage of the *Staphylococcus aureus* isolated 80(85.1%) were susceptible to Vancomycin (VSSA), 9(9.5%) were VISA while 1(1.1%) was VRSA. The VRSA isolates harbored the vanA gene, while it does not habour the vanB and vanXY genes. Additionally, this isolate form biofilms phenotypically but does not possess the adherence determinant (icaA and icaD genes respectively).
Conclusion: This study reported incidences of 50% *Staphylococcus aureus* with high resistant profile to commonly prescribed antibiotics, 9.5% VISA and 1.1% VRSA in frozen chicken sold in Niger state. The *S. aureus* were also observed to exhibit biofilm formation ability phenotypically the genes encoding for adherence determinant (icaA and icaD genes respectively) were absent when they were molecularly characteristics. This study therefore highlight the importance of monitoring antibiotic resistance in foodborne pathogens and its implications for public health.
Keyword: Frozen chicken meat, vancomycin, Niger state, antimicrobial resistance, biofilm production

INTRODUCTION

Frozen chicken is one of the most consumed meat as source of protein in Nigeria including Niger state and 70% are smuggled into the country (1). Due to the route of importation of this frozen chicken, preservation and processing, high percentage of the meats are influenced by environmental and human factors, encouraging contamination and increases frequency of infection (2, 3). Treating these infections involves the use of chemotherapy; and resistance to these commonly prescribed drugs elicits risk from antimicrobial resistance (AMR) bacteria especially normal floral of humans or animals such as *Staphylococcus aureus, Staphylococcus epidemidis, E. coli* etc (4). Due to the rising resistant profile in all

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sphere, some drugs such as vancomycin, colistin and linezolid's are strongly regulated, and are not commonly prescribed, as they are seen as drug of last resort. However, due to poor implementation or nonexistence of antibiotic stewardship programs and regulation of the administration of antibiotics to animals been raised for food, development of resistance to these drugs of last resort with broad spectrum activities have been reported (5). Poultry has become a significant source of protein (chicken meat and eggs) in the past 20 years (6). As dietary supplements, a number of antimicrobial drugs are utilized to stimulate chicken growth. According to Davis *et al*. (7), the regular use of antibiotics on poultry promotes the growth and maintenance of a reservoir of resistant bacteria that can infect humans or transfer mobile resistance determinants to other pathogenic organisms in humans. Because these bacteria have the ability to cause difficult-to-treat foodborne diseases, consumers are directly at risk when they are found in poultry products. Compared to other animal species, microbial pollutants are more frequently documented from poultry and poultry products (8). The demand for this poultry product some of which are carriers of food-borne pathogens has increased due to changes in eating patterns, intricate and drawn-out food supply systems with increased international movement, and inadequate hygiene methods. In both humans and animals, *Staphylococcus aureus* is a commensal of the skin and mucous membranes, especially the nostrils (9). After causing damage to the skin and mucous membranes, it can result in skin infections and potentially fatal invasive illnesses (10). Worldwide, *Staphylococcus aureus* is a major cause of food poisoning (11, 12). It is possible for *S. aureus* to create a biofilm of established microorganisms. The capacity of those bacteria to adhere to a substrate or surfaces, as well as to any accessible moist solid surface, including host tissue, is what defines them. They then use extracellular polymer molecules to construct a matrix. Depending on the development phase and gene expression, these microorganisms have a unique phenotype. They are important sources of resistance to drugs and the host immune system. *S. aureus* may occasionally exhibit methicillin resistance due to the acquisition of the mecA gene, which could make treating infections in humans and animals more difficult (13). Methicillin-resistant *S. aureus* (MRSA) strains have been detected in raw and cooked meat, milk, cheese, and other food products (14, 15, 16). Because food handlers in food service facilities come into close contact with food products, there is a risk that the serving utensils will become contaminated. The recommended drug for MRSA infections is vancomycin. Over 20 years ago, vancomycin-resistant MRSA was first isolated (17). Since then, other nations in North America, Europe, Asia, Africa, and South America have reported having identified vancomycin-resistant *S. aureus* (VRSA) (18, 19, 20). This study therefore assessed the possible isolation of vancomycin resistant *S. aureus* from frozen chicken sold in Niger state to evaluate the incidence of the variant and public health importance in our environment.

MATERIALS AND METHODS

Study Area

This study was conducted in Niger state, Nigeria. It is located on $10^0$00N' $6^0$00E' with an area of 76,363km^2 and 6,783,300 million population (21). The sampling was carried out in 4 Local government areas (LGA) of the States (Bida, Bosso, Chanchaga and Suleja). They were identified and selected on the basis of divergent concomitant factors such as population density.

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Figure 1: Pictorial Representation of Different Local Government Areas in Niger State

Sample Collection and Transportation

The method of Persoons *et al*. (22) were employed for sample collection and transportation. A total of 188 frozen chicken samples were collected from markets in the 4 local government area of the state. Briefly, 50g of frozen chicken were placed in a disposable sterile bag containing 400mL of brain-heart infusion (BHI) broth supplemented with nalidixic acid and colistin each at a concentration of 10 μg/mL. All collected samples were transported aseptically in ice.

Isolation and Identification of *Staphylococcus aureus*

Following overnight incubation of the broth at room temperature (30°C), a loopful were streak-inoculated unto a BHI plate containing 4% NaCl and incubated overnight at 35°C. Presumptive *S. aureus* isolates (Yellow pigmented colonies) were purified on nutrient agar at 37˚C for 24 hours. Further identification of pure isolates of *S. aureus* was based on their growth pattern, colonial morphology on selective media, gram staining, microscopic appearance and biochemical tests. A Pure isolates of *S. aureus* were inoculated onto agar slant and stored in -4^{0}C for further use.

Antimicrobial Susceptibility Testing (AST)

Antibacterial susceptibility test was carried out by using disc diffusion (Kirby-Bauer) method on Mueller-Hinton agar plates. Bacterial suspension was prepared in 0.5 McFarland turbidity standard for each isolate and was swabbed on the already prepared Mueller-Hinton agar plates containing the antibiotic discs (Oxiod, UK), which were incubated at 37˚C for 24h. The experiment used 11 antibiotics namely: Ampicillin, Augumentin, Azithromycin, Cefoxitin,

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Clindamycin, Erythromycin, Gentamycin, Linozolids, Oxacillin, Trimethoprim/Sulphamethoxazole and Tetracycline. The results were interpreted according to the CLSI (23) guidelines. Intermediately-susceptible isolates were classified as resistant while isolate resistant to three or more classes of antimicrobial agents were considered MDR (24)

Phenotypic Detection of Vancomycin Resistant *Staphylococcus aureus* by Vancomycin Agar Screen (VAS) and Minimum Inhibitory Concentration Strip Methods

The Clinical and Laboratory Standards Institute (CLSI) recommended vancomycin agar screen (VAS) for identification of VRSA were adopted for the detection of vancomycin resistant S. *aureus*. Briefly, vancomycin supplement (Oxoid, Basingstoke, UK) were incorporated into BHI agar to a concentration of 6.0 mg/L. Four VAS plates were inoculated per test sample to provide duplicate plates for incubating temperature. 10µL drop of the standardized (0.5 McFarland) inoculum were inoculated using a micropipette onto the agar surface, spotting an area of 10mm to 15 mm at the centre of the VAS plate. One set of duplicate plates were incubated at 30°C and second set at 35°C. After 24 hours incubation the plates were examine for evidence of small colonies (>1 colony) or a film of growth, suggesting reduced susceptibility to vancomycin. Standard reference *Enterococcus faecalis and Staphylococcus aureus* were used as vancomycin susceptible and resistant quality control strains. Isolates phenotypically identified as vancomycin resistant *S. aureus* were subjected to further species confirmation and identification of vancomycin resistance genes using molecular methods. According to CLSI, *S. aureus* isolates for which vancomycin MIC ≤ 2 µg/mL are VSSA, 4-8 µg/mL are classified as VISA, and ≥16 µg/mL are classified as VRSA.

Detection of Biofilm Formation

Biofilm formation was determined using Congo red agar and Tube method;

a. Congo Red Agar method (CRA)

Production of slime from all vancomycin resistant *S. aureus* were investigated by culturing them on Congo Red Agar (CRA). The inoculated CRA plates were incubated at 37°C for 24 h. After incubation, appearance of bright black colonies were observed and the result recorded as described by Gundogan, *et al.*, (25).

b. Tube Adherence Method

All vancomycin resistant *S. aureus* organisms were inoculated in 5 mL trypticase soy broth in test tubes and incubated overnight at 37°C. After incubation, the tubes were decanted, dried and stained with 0.1% crystal violet, washed gently and placed upside down for drying. Observation were made and the result recorded as described by Christensen, *et al.,* (26).

Polymerase Chain Reaction (PCR) for the Detection of Vancomycin Resistance Genes

PCR technique using Vancomycin resistance genes specific primers for vancomycin were used to screen isolates that were phenotypically identified as vancomycin resistant for the presence of 3 vancomycin resistance genes (vanA, vanB, and vanXY) in their extracted DNA. The primers and their sequences are shown in Table 1a while that of biofilm adherence are shown in Table 1b below. Reaction cocktail that were used for all PCR per primer set included (reagent volume µl) - 10X PCR SYBR green buffer (2.5), 25mM $Mgcl_2$ (1.0), 5pMol forward primer (1.0), 5pMol reverse primer (1.0), DMSO (1.0), 2.5Mm DNTPs (2.0), Taq 5u/ul (0.1), DNA template (3.0). The admixture

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was made up to 22 µl using sterile distilled water. The amplicon from the PCR was observed on 1.5% agarose gel. The bands that appeared were compared with that of the ladder and the observation were recorded.

Table 1a: Primers Used to identify Vancomycin Resistance Genes

S/N	Gene	Forward Primer	Reverse Primer	Base Pair
1	vanA	GGGAAAACGACAATTG	GTACAATGCGGCCGTTA	550
2	vanB	ATGGGAAGCCGATAGTC	GATTTCGTTCCTCGACC	500
3	vanXY	GGTATCAAGGAAACCTC	CTTCCGCCATCATAGCT	800

Table 1b: Primer for Adherence of Biofilm Formation

S/N	Gene	Forward Primer	Reverse Primer	Base Pair
1	icaA	ACACTTGCTGGCGCAGTCAA	TCTGGAACCAACATCCAACA	250
2	icaD	ATGGTCAAGCCCAGACAGAG	GCTGTTGAAGTTAATACTGTACCTGC	400

RESULTS

Staphylococcus aureus Incidence from Chicken Meat

From the 188 samples screened, 94 (50%) were positive for *Staphylococcus aureus* (Table 2). Frozen chicken meat from Suleja LGA recorded the highest *S. aureus* incidence (63.8%) followed by Chanchaga and Bida with 46.8% each respectively and the least was Bosso with 42.5% incidence (Table 2).

Table 2: Distribution of *Staphylococcus aureus* in Chicken Meat sold in Niger State

S/N	Location	NSS	NPS	%
1	Chanchaga	47	22	46.8
2	Suleja	47	30	63.8
3	Bosso	47	20	42.5
4	Bida	47	22	46.8
Total		**188**	**94**	**50**

Keys: % = Percentage NSS = Non Staphylococcus Species NPS = number of positive *Staphylococcus aureus*

Antibiotics Resistance Profiles of *Staphylococcus aureus* Isolated from Chicken Meats from Niger State

The antimicrobial resistance profiles (ARPs) of the 94 *S. aureus* isolated from frozen chicken meats sampled in Niger State to 11 antibiotics revealed that 87 (92.6%) of the isolates were resistant to tetracycline while 61 (64.9%) and 60 (63.8%) were resistant to augmentin and ampicillin, respectively. Resistance to azithromycin, gentamycin and linozolids was demonstrated by 47.9%, 47.9% and 46.8% of the isolates, respectively.

Sulphamethoxazole/trimethoprim and erythromycin resistance were demonstrated by 36.2% and 29.8% respectively while 22.3% of the isolates were concurrently resistant to cefoxitin and oxacillin (Table 3).

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Table 3: Antibiotics Resistance Profiles of *Staphylococcus aureus* Isolates from Niger State

Antibiotic	*S. aureus* from various Location (% Resistant to Antibiotic)				Total (n=94)
	Chanchaga (N=22)	Suleja (N=30)	Bosso (N=20)	Bida (N=22)	
AMP	13(59.1)	15(50.0)	13(59.1)	19(63.3)	60(63.8)
AUG	21(95.5)	13(43.3)	21(95.5)	6(20.0)	61(64.9)
AZN	13(40.9)	9(30.0)	13(40.9)	10(33.3)	45(47.9)
CEP	5(22.7)	6(20.0)	5(22.7)	5(16.7)	21(22.3)
CLN	7(31.8)	7(23.3)	7(31.8)	6(20.0)	27(28.7)
ERY	8(36.4)	6(20.0)	8(36.4)	6(20.0)	28(29.8)
GEN	8(36.4)	15(50.0)	8(36.4)	14(46.7)	45(47.9)
LIN	10(45.5)	12(40.0)	10(45.5)	12(40.0)	44(46.8)
OX	5(22.7)	6(20.0)	5(22.7)	5(16.7)	21(22.3)
SXT	9(40.9)	10(33.3)	9(40.9)	6(20.0)	34(36.2)
TEM	20(90.9)	23(76.7)	20(90.9)	24(80.0)	87(92.6)

Key: n = number of resistant isolates, N = number of *S. areus* recovered per location, AMP = Ampicillin, AUG = Augumentin, AZN = Azithromycin, CEP = Cefoxitin, CLN = Clindamycin, ERY = Erythromycin, GEN = Gentamycin, LIN = Linozolids, OX = Oxacillin, SXT = Sulphamethoxazole/trimethoprim, TEM = Tetracycline.

Distribution of Vancomycin Resistance in Chicken Meat from Niger State

The minimum inhibitory concentration result revealed that out of the 94 *Staphylococcus aureus* isolated, 80(85.1%) were susceptible to Vancomycin, 9(9.5%) were Vancomycin intermediate *Staphylococcus aureus* while 1(1.1%) was Vancomycin Resistant *Staphylococcus aureus*. Bida had the highest susceptible strains 22(100%). Suleja had the highest percentage of VISA 16.6%. Suleja had 1(3.3%) of VRSA while Chanchaga, Bosso and Bida had none (Table 4).

Table 4: Distribution of the Susceptibility of *S. aureus* Isolated from Chicken Meat to Vancomycin

L.G.A	VSSA (%)	VISA (%)	VRSA (%)
Chanchaga	18(80.8)	2(9.1)	0(0)
Suleja	24(80)	5(16.6)	1(3.3)
Bosso	18(90)	2(10)	0(0)
Bida	22 (100)	0(0)	0(0)
Total	80(85.1)	9((9.5)	1(1.1)

Keys: n = Number of isolates, VSSA = Vancomycin susceptible *Staphylococcus aureus*, VISA = Vancomycin intermediate *Staphylococcus aureus*, VRSA = Vancomycin resistant *Staphylococcus aureus*

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Distribution of Biofilm Producing *S. aureus* isolated from Frozen chicken in Niger State

The results revealed that out of the 94 *Staphylococcus aureus* isolated, 28(29.7%) were biofilm producers, 20(21.2%) of the isolates were intermediate biofilm producers and 46(48.9%) were non biofilm producers. Overall, Suleja had the highest biofilm producers 10(33.3%), followed by Chanchaga 8(36%), then Bosso 5(25%), while Bida 5(22.7%) had the least biofilm producing isolates. There was correlation between the two methods adopted in this study (Congo red agar method and Tube method).

Table 5: Biofilm Formation Ability Using Congo Red Agar and Tube Method

Location	NPS	Congo Red Agar Method			Tube Method		
		Positive	Intermediate	Negative	+++	++	+
Chanchaga	22	8(36%)	4(18.1%)	10(45%)	7(31.8%)	4(18.1%)	11(50%)
Suleja	30	10(33.3%)	5(16.6%)	15(50%)	10(33.3%)	5(16.6%)	15(50%)
Bosso	20	5(25%)	6(30%)	10(50%)	5(25%)	6(30%)	10(50%)
Bida	22	5(22.7%)	6(27.2%)	11(50%)	6(27.2%)	6(27.2%)	10(45.4%)
Total	94	28(29.7%)	21 (22.3%)	46(48.9%)	28(29.7%)	21(22.3%)	46(48.9%)

Key: +++ = Strong biofilm producers, ++ = moderate biofilm producers, + = weak biofilm producer

Molecular Confirmation of *S. aureus*

The morphologically and biochemically identified Vancomycin Resistant *S. aureus* (VRSA) isolates revealed the presence of the 16SrRNA gene of *Staphylococcus aureus* by the amplification of the 1.5 kb product of the bacteria-specific (Plate 1). Further analysis showed that the *S. aureus* had 92.9 identity compatibility with S. *aureus* strain SIRST56 with association number MK780044 identified and submitted in the gene bank (Table 6)

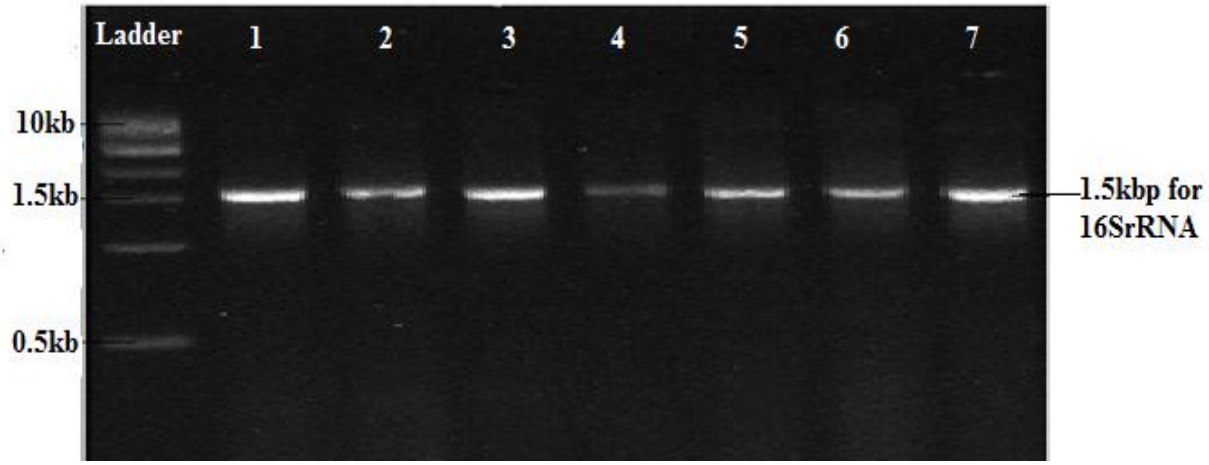

Plate 1: Polymerase chain reaction amplification of bacteria-specific 16SrRNA gene of presumptive *Staphylococcus aureus* isolates.

Lane M – 10 kp DNA ladder.

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Table 6: BLAST result of 16S rRNA gene: Sequencing ID in a gene bank, and compatibility of DNA sequences obtained from National Center Biotechnology Information (NCBI)

Isolate	Sequence lengths	Most closely related type strain[a]	Compatibility (%)	Accession numbers[d]	Country
1	1417	*S. aureus* strain SIRST56	92.91	MK780044	India

[a]GenBank references accession numbers are shown in parentheses; taxonomic connection was established by comparing the sequence with those published by the National Center for Biotechnology Information (NCBI).
[b]The similarity to the closest type strain sequence is exhibited as a percentage.
[c]GenBank accession numbers for this study.

Characterization of Vancomycin Resistance (*vanA*, *vanB* and *vanXY*) and Biofilm Production of *S. aureus* isolated from chicken meat in Niger State

The result of the molecular analysis of *vanA* gene is shown in plate 2a to 2c below. Agarose gel electrophoresis revealed a major band with 550 bp in positive isolates with *vanA* gene. Molecular analysis of *vanA* gene in VRSA showed that the VRSA 1(100%) was positive for *vanA* gene (Figure 2a) while it does not harbor *vanB* and *vanXY genes* (Figure 2b and 2c). Figure 3 showed that VRSA observed in this study, although could form biofilm phenotypically but does not possess the adherence determinant genes (icaA and icaD genes respectively).

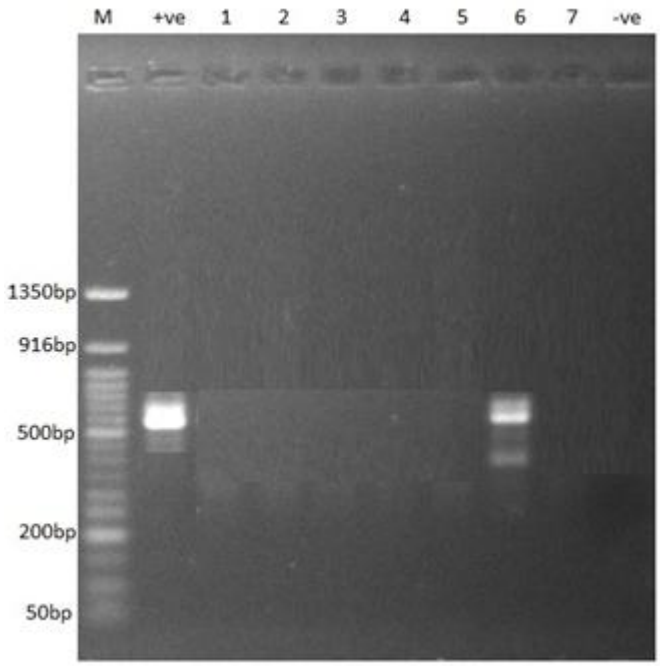

Plate 2a: Agarose gel electrophoresis of PCR-amplified vancomycin resistance gene (*vanA*)

Lanes: M= 100-bp ladder; Lane 1= *S. aureus* isolates showing 550bp *vanA* amplicon +ve control

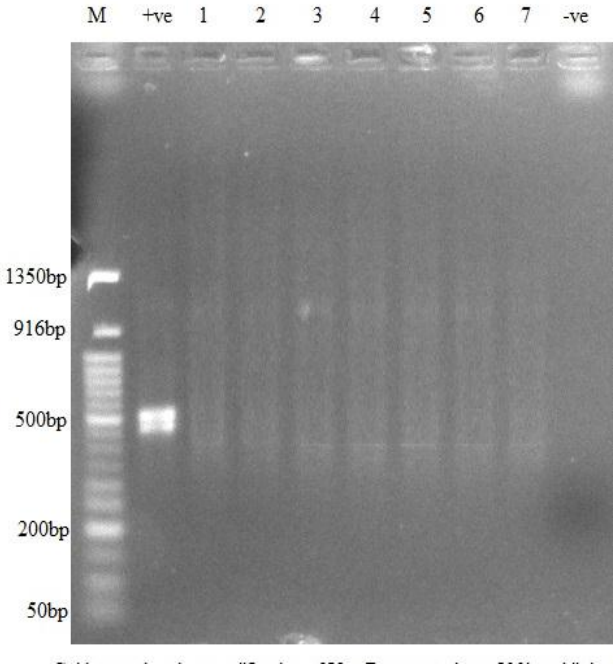

Plate 2b. Agarose gel electrophoresis of PCR-amplified vancomycin resistance gene (vanB)

Lanes: M= 50-bp ladder; Lane 1: *S. aureus* isolates showing 500 bp vanB amplicon.

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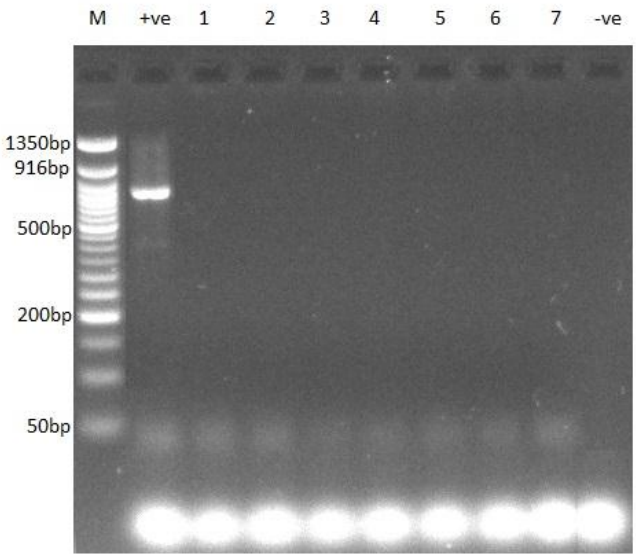

Plate 2c: Agarose gel electrophoresis of PCR-amplified vancomycin resistance gene (*vanXY*)
Lanes: M= 50-bp ladder; Lanes 1: *S. aureus* isolates showing 800 bp *vanXY* amplicon

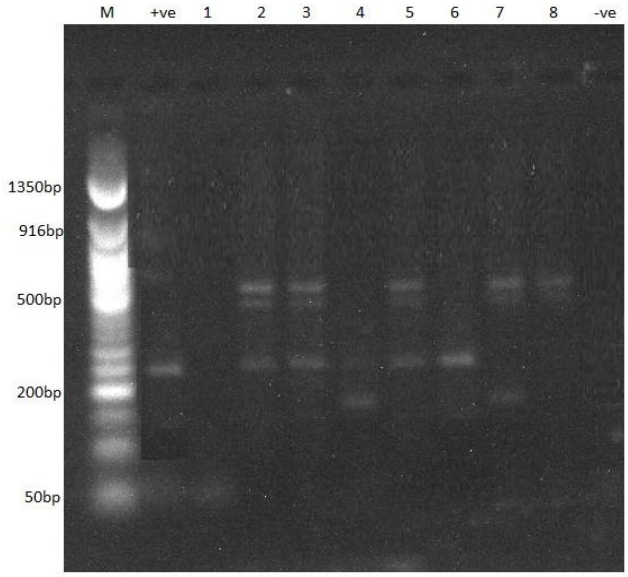

Plate 3: Agarose gel electrophoresis of PCR-amplified biofilm forming determinant (*icaA*)
Lanes: M= 50-bp ladder; Lanes 1-: *S. aureus* isolates showing 250 bp *icaA* amplicon.

DISCUSSION

Staphylococcus aureus incidence rates of 50% in chicken meat suggest that chicken meat are highly contaminated with *S. aureus* and therefore constitute a higher risk in the transmission of *S. aureus* to the public in Niger state, Nigeria. This could be attributed to possible human transmission of *S. aureus* during packaging, depending on environmental conditions, hygiene, handling practices and cross-contamination during processing (27). This could also serve as route for dissemination of community acquired *Staphylococcus aureus* (CA-SA) (28), as the emergence of this clonal species has become important human pathogen in various parts of the world particularly in developing nation such as Nigeria in recent years (27). Therefore, the highest incidence of *Staphylococcus aureus* (63.8%) in Suleja suggest that Sulejas' environmental conditions, hygiene, handling practices aided the proliferation of this *S. aureus*, leading to a higher incidence in chicken meat in that area. This finding is in agreement with several studies in literatures which had reported the presence of *S. aureus* in frozen meat samples. Namir *et al.,* (29) reported that 76.7% of frozen retail pork outlets in India were contaminated with *S. aureus*. Higher incidence of *S. aureus* has also been reported from frozen meat and fresh meat samples in Karbala province (Iraq) where 64% of the frozen meat samples were contaminated with *S. aureus* (30).

The percentage of antibiotics resistance observed in this study is low. The lower percentage of resistance observed in this study may be due to the fact that most frozen chicken were broilers rather than layers, as layers are more exposed to high antimicrobial doses and time compared to broiler. Our findings also concurred with the reports of Bala *et al.,* (2) and Geidam *et al.,* (31), which

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observed high resistance to tetracycline. This could be associated with the vast use of these antibiotics for the treatment of animal diseases (32). This calls for concern, as tetracycline is commonly used in both human and veterinary medicine.

Unfortunately, *S. aureus* isolates in our study showed high resistance to linezolid (39.1–52.0%), azithromycin (18.3–47.9%), and augmentin (30.4–64.9%), contrary to the previous reports by Okorie-Kanu, *et al.,* (27) and Igbinosa, *et al.,* (33). The high raising resistant profile by *S. aureus* calls for serious periodic surveillance as these are the priority and commonly prescribed antibiotics in human medicine (34). The reason for the observed high resistance is not apparent from this study because these drugs lack veterinary preparations and aren't routinely used in a clinical setting. The high levels of resistance to multiple antibiotics raise concerns about the potential transmission of antibiotic-resistant bacteria to humans through the handling and consumption of contaminated poultry products. This underscores the importance of responsible antibiotic use in both human and veterinary medicine, as well as proper food safety practices to minimize the risk of antibiotic-resistant infections.

Vancomycin resistance is a major public health concern due to its impact on treatment options for bacterial infections. In this study, 1 (1.1%) of the *Staphylococcus aureus* were resistant to vancomycin. This is in line with the reports of Bamigboye *et al.,* (35), where 1 (1.4%) VRSA out of 73 *S. aureus* clinical isolates was detected. However, in another report from Nigeria, Taiwo *et al.,* (36) documented 6 (12.2%) VRSA. Although our findings show a low incidence of VRSA compared with the report from Zaria study, which reported 46.2% of VRSA (37), it still calls for caution in the use of antibiotics for meat production. Furthermore, the VRSA could also be of origins other than food animal production. It could even be one of the repercussions of poor hygiene.

The phenotypic biofilm occurrence observed in this study showed that the *Staphylococcus aureus* isolates have adhesive potentials to attach its host's extracellular matrix; these likely favour zoonotic potential, persistence, and colonization (38, 39). In Nigeria, where a significant proportion of the population is non-vegetarian, suggests that a large population is at risk of meat-borne hazards. In line with our findings, previous studies have reported the ability of biofilm formation by *S. aureus* isolates from raw meat, several food-processing facilities and from food of animal origin (40, 41, 42, 43).

Molecular analysis showed that the vancomycin-resistant *S. aureus* encode the vanA gene in its genome, which confirmed its exhibition in its resistant profile. VRSA isolates have been reported to be resistant to the majority of the prescribed and accessible antibiotics (44), implying that human infection resulting from the VRSA strains will have fewer or no treatment options available to them, and there is also a high risk of the emergence of clinical vanA-type VRSA in such environment, since the *van operon* can be horizontally spread to other bacteria in humans and the environment (40). Therefore, VRSA strain are particularly a threat to chicken farmers, handlers, and consumers in Niger state, Nigeria. The detection of these strains in chicken meats may not be unrelated to the increased use of glycopeptides (vancomycin) in recent times in poultry production (45). This study shows the important of using both phenotypic and genetic method in characterizing VRSA. Both method are in conformity in this study. Several reports show varied levels of correlation between gene detection and phenotypic method of identifying vancomycin-resistant *Staphylococcus aureus* (44, 45). Furthermore, the lack of expression

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of vanB and vanXY by the isolates that were phenotypically identified as VRSA in this study could be attributed to the possibility of carriage of other vancomycin-resistant determinant genes not investigated in this study. The *S. aureus* were also observed to exhibit biofilm formation ability phenotypically the genes encoding for adherence determinant (icaA and icaD genes respectively) were absent when they were molecularly characteristics. This study therefore highlight the importance of monitoring antibiotic resistance in foodborne pathogens and its implications for public health

CONCLUSION

This study reported incidences of 50% *Staphylococcus aureus* with high resistant profile to commonly prescribed antibiotics, 9.5% VISA and 1.1% VRSA in frozen chicken sold in Niger state. The *S. aureus* were also observed to exhibit biofilm formation ability phenotypically the genes encoding for adherence determinant (icaA and icaD genes respectively) were absent when they were molecularly characteristics

Conflict of Interest: Author BM conducted the experiment and computed the results, KFA did the literature review, KFA, AUN and BJD supervised the work and reviewed the methods and discussion. All the authors agreed that there is no conflict of interest among them

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