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Research Article

Characterization of Methicillin-Resistant *Staphylococcus aureus* Using Multi-Locus Sequence Typing and SCCmec Typing from Poultry and Poultry Farm Workers in Kano, Nigeria

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ABSTRACT
Methicillin-resistant Staphylococcus aureus (MRSA) is an important zoonotic pathogen increasingly associated with livestock, particularly poultry, posing a significant public health threat. This necessitates molecular epidemiological surveillance using robust typing tools such as multi-locus sequence typing (MLST) and SCCmec typing. A cross-sectional study was conducted to characterize MRSA isolates recovered from poultry and poultry farm workers in Kano, Nigeria. S. aureus was identified using microbiological methods and by PCR targeting the nuc and mecA genes. Molecular characterization was performed using MLST based on amplification and sequencing of selected housekeeping genes, while SCCmec typing was conducted using multiplex PCR assays. Phylogenetic relationships were inferred to assess genetic diversity and clonal relatedness. A total of 13 MRSA isolates were confirmed by the presence of the mecA gene. The MLST analysis revealed multiple sequence types, indicating genetic heterogeneity among isolates, consistent with previous reports of diverse MRSA lineages in poultry environments. Three of the seven housekeeping genes were amplified. SCCmec typing identified types II and IV, with type IV being more prevalent. Phylogenetic analysis demonstrated clustering of isolates from both poultry and farm workers, indicating potential cross-species transmission. This study highlights the circulation of genetically diverse MRSA strains among poultry and poultry farm workers in Kano, with evidence of shared clonal lineages. The predominance of SCCmec type IV underscores the role of community and livestock-associated MRSA in this setting. Continuous surveillance using MLST and SCCmec typing is essential to understand transmission dynamics and to inform effective control strategies at the human–animal interface.

Keywords: Molecular characterization; MRSA; MLST; Poultry; SCC*mec*

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INTRODUCTION

Staphylococcus aureus, a Gram-positive coccus is a frequent cause of skin infections, such as boils and pimples. The emergence and global dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA) have significantly complicated treatment outcomes due to resistance to β-lactam antibiotics and frequent multidrug resistance profiles. The MRSA is now recognized as a major public health threat worldwide, particularly in low- and middle-income countries where surveillance and infection control measures are often limited (Emele *et al*., 2025; Akinduti *et al*., 2024). Molecular epidemiology plays a critical role in understanding the evolution, transmission dynamics, and genetic diversity of MRSA. About a third of healthy individuals carry *S. aureus* on their skin and nose (Goudarzi *et al*., 2016). Carriage of *S. aureus* Sequence Type (ST) 398 has primarily been reported as occurring among persons in contact with livestock, including swine, pigs and cattle. This association has

given rise to the characterization of this strain as livestock associated (Sinlapasorn *et al.,* 2015). This clonal complex associated with disease in livestock has also been implicated in human infection (Boswihi *et al*., 2020). It is known to cause diseases in poultry, feed and companions' animals (Konstantinovski *et al*., 2022). Methicillin-resistant *S. aureus* (MRSA) is becoming increasingly recognized among persons in the community without established risk factors (Chen and Wu, 2020).Occurrence of MRSA in animal disease have not been considered a source of infection to humans, although transmission appears to be primarily between animals, undistinguished isolates have been found in their human contacts, particularly those with occupational exposure (Narvaez-Bravo *et al*., 2016). Studies in Nigeria have demonstrated the presence of MRSA in humans, poultry, and environmental samples, highlighting the risk of cross-species transmission and the emergence of livestock-associated MRSA clones (Kwoji *et al*., 2019; Igbinosa *et al*., 2023). Staphylococcal cassette chromosomes (SCCs) are relatively large fragments of DNA that always insert into the *orfX* gene on the *S. aureus* chromosome and can encode antibiotic resistance and/or virulence determinants (Gu *et al*., 2020). It is a mobile genetic element that carries the central determinant for broad spectrum beta-lactam resistance encoded by the *mecA* gene. The emergence of methicillin resistant staphylococcal lineage is due to the acquisition and insertion of the SCC *mec* element into the chromosome of susceptible strains. The SCC*mec* types differ from one another by the number of genes in their architecture (Li *et al.,* 2023). The SCC*mec* type I, IV, V and VI encode resistance to β-lactam antibiotics only, while SCC*mec* type II and III carry multi-resistant genes some on plasmids and transposons (Tang *et al*., 2018). Eleven SCC*mec* elements are reported to date SSC *mec* I to XI. Among these, SCC*mec* type I-V are the most commonly reported (Sinlapasorn *et al*., 2015). Three types of SCC *mec* (I, II and III) are carried mostly by healthcare- associated MRSA strains throughout the world (Fasihi *et al*. 2017). While type IV and V are widely disseminated among community acquired MRSA infections (Akinduti *et al*., 2024). The SCC*mec* IV and V allotypes are smaller than the other SCC*mec* element I, II and III, more genetically mobile being readily transmissible between staphylococci and does not at present carry additional antimicrobial resistance genes (Igbinosa *et al*., 2023). Despite these findings, comprehensive studies integrating MLST, SCC*mec* typing, and phylogenetic analysis across human and animal sources remain limited. Phylogenetic analysis based on MLST data enables the identification of clonal relationships and evolutionary divergence among isolates, providing deeper insight into MRSA transmission pathways across ecological niches. Such approaches are essential for informing infection control strategies and antimicrobial stewardship programs. Therefore, this study aimed to characterize MRSA isolates obtained from chicken droppings and human nasal samples using MLST and SCC*mec* typing, and to investigate their genetic diversity and phylogenetic relationships. The findings are expected to contribute to the understanding of MRSA epidemiology in Nigeria and highlight the potential public health implications of zoonotic transmission.

MATERIALS AND METHODS

The study was conducted at the Microbiology laboratory of the Department of Microbiology, Bayero University, Kano, Nigeria. DNA extraction and PCR-amplifications were done in molecular laboratory of International Institute for Tropical Agriculture (IITA, Ibadan).

Bacterial isolates

Isolates were obtained from chicken droppings, cloacal swabs of chicks and nasal swab of farm workers. A total of 371 Gram positive, catalase positive cocci occurring in pairs, short chains or clusters were selected and subjected to growth on Mannitol Salt agar (MSA), coagulase and DNase tests.

Isolation and phenotypic identification of *S. aureus*

Each bacterial isolate was subjected to growth on MSA as described previously by Cheesbrough, (2020), incubated at 37^0C for 48 hours. All the isolates were tested for production of coagulase both free and bound and DNase tests (Cheesbrough, 2020). Agglutination test was carried out to further identify the isolates. Staphytect plus latex slide agglutination test kit (Oxoid Ltd England) was used for differentiation of *S. aureus* by detection of clumping factor, protein A. The test was carried out according to manufacturer's instructions.

Phenotypic identification for methicillin resistant *S. aureus* (MRSA)

Using disk diffusion method, cefoxitin was used to determine methicillin resistance in *S. aureus*. The test was carried out as described previously (CLSI, 2023). After incubation, strains that grew on the plates were selected for further studies. Diameter measured and compared with standard (CLSI, 2023). The cefoxitin disc was used as a surrogate for all penicillinase-stable penicillin and resistance was used to infer *mecA* - mediated methicillin resistance.

Molecular identification through PCR amplification

Extraction of *S. aureus* DNA

Genomic DNA was extracted using DNA extraction Mini Kit ((Oxoid Ltd England)). Pure cultures were grown overnight on Blood agar at 37^0C.The procedure was carried out according to the manufacturer`s instructions with an initial lysis step adapted for Gram-positive bacteria. The DNA concentration and purity were measured with a Nano Drop 2000 spectrophotometer (Thermo Fisher Scientific, USA) and standardized to 50–100 ng/µL for PCR. Extracted DNA was stored at 4°C until used for PCR. The isolates were analyzed for 16S rRNA, and spa typing. These primers amplify 228 bp region of 16S rRNA gene fragment of *S. aureus* (Goudarzi *et al*., 2016).

Preparation for agarose gel electrophoresis

Gel electrophoresis was used to separate DNA on the basis of their sizes by applying an electric field to move the DNA through an agarose matrix. The volume of the TAE and the weight of the agarose used were dependent on the number of wells or gene product. Thirty wells were used and so 3g agarose (10wells per gram) (Sigma -Aldrich, USA) was added to 200 mLx 1xTAE and warmed to dissolve in a microwave for 2min, it was removed from the microwave oven and allowed to stand for a few minutes. Ethidium bromide 8µL (Et Br) was added to stain the DNA product. After the addition of the ethidium bromide to the viscous agarose and shaken to mix, it was poured into the gel mold with 30well comb and allowed to stand for 30minutes to harden. The comb was carefully pulled out of the gel. The clamp of the mold was removed and the gel was transferred into the electrophoretic tank. The tank was filled with 1xTAE buffer to the maximum limit and the PCR products were loaded into the wells. The gel was run at 120V for 1hour. Following electrophoresis, the PCR product was viewed and the picture of the bands was taken (McDougal *et al*., 2003)

Detection of *mecA* gene and Staphylococcal Cassette Chromosome (SCC*mec*) Typing

The PCR was carried out to detect *mecA* gene and to determine the type of staphylococcal cassette chromosome. It was carried out as described previously by Liu *et al.* (2016). The *S. aureus* isolates were subjected to the detection of *mec*A genes and SCC*mec* using specific primer pairs. Primers used were as described and design by Bustin and Huggett (2017) and were for type 1 to type V SCC*mec* and *mecA* gene as shown in Table1. Master mix was prepared to include the *mecA* and the SCC*mec* types 1 -V primers according to the number of DNA templates. The master mix was vortexed to mix and aliquoted in volumes of 48 µL PCR tubes. The template DNA was added, vortexed to mix and then loaded in the thermocycler. The optimization process was carried out as described previously (Liu *et al.*, 2016). The amplified DNA/PCR products were loaded on the gel and run on electrophoretic machine at 120V for 1.5 hrs. Bands were viewed using transilluminator.

Multi-locus sequence typing (MLST)

The MLST was performed according to protocol described previously (Bustin and Huggett, 2017). It was carried out to detect the seven housekeeping Genes namely; *Arc, aroE, glpF, gmk, pta, tpi,* and *yqil*. A master mix was prepared for each of the seven-housekeeping gene. The total volume of the master mix was determined by the number of reactions of the DNA templates. Finally, the primers (forward and reverse) and the template DNA were added and agitated to mix and then loaded in the thermocycler. The amplification and gel electrophoresis were carried out as described by taken (McDougal *et al*. (2003) and Bustin and Huggett (2017). They were viewed in the GelDoc illumination system. The PCR products were purified as above before sequencing. After sequencing raw sequences were trimmed and assembled using BioEditv7.2.5 software. Alleles and sequence types (STs) were assigned by querying the PubMLST database (https://pubmlst.org) (Weiss *et al*., 2016). Primers used are shown in Table 2. An input sequence was prepared for building a phylogenetic tree as described previously (Weiss *et al*., 2016; Thieme *et al.,* 2016).

Phylogenetic tree construction

Phylogenetic relationships among *S. aureus* isolates were inferred using concatenated nucleotide sequences obtained from multilocus sequence typing (MLST). Internal fragments of the seven housekeeping genes (arcC, aroE, glpF, gmk, pta, tpi, and yqiL) were amplified, sequenced, and assembled as previously described for MLST characterization (William *et al*., 2015). Forward and reverse sequences were edited and assembled into consensus sequences using BioEdit software to remove low-quality regions and ambiguous bases. Individual gene sequences were concatenated in-frame to generate a single composite sequence representing each isolate. Corresponding reference sequences and sequence types (STs) were retrieved from the PubMLST *S. aureus* database for comparative analysis (Boyle and Adamowicz, 2015). Evolutionary distances were calculated using the Kimura two-parameter model. A representative non-related *Staphylococcus* species sequence was included as an out group to root the

tree. The final phylogenetic tree was visualized and annotated using MEGA software, and clustering patterns were interpreted to determine genetic relatedness and lineage distribution among the *S. aureus* isolates (Filipski *et al.*, 2015).

Table 1: Primers for SCC*mec* typing and mecA

Gene	Primer Sequence	Expected amplicon size
Type I Forward	GCTTTAAAGAGTGTCGTTACAGG	
Type I Reverse	GTTCTCTCATAGTATGACGTCC	613bp
Type II Forward	GATTACTTCAGAACCAGGTCAT	
Type II Reverse	TAAACTGTGTCACACGATCCAT	287bp
Type III Forward	CATTTGTGAAACACAGTACG	
Type III Reverse	GTTATTGAGACTCCTAAAGC	243bp
Type IVa Forward	GCCTTATTCGAAGAAACCG	
Type IVa Reverse	CTACTCTTCTGAAAAGCGTCG	776bp
Type IVb Forward	AGTACATTTTATCTTTGCGA	
Type IVb Reverse	AGTCATCTTCAATATGGAGAAAGTA	1000bp
Type IVc Forward	TCTATTCAATCGTTCTCGTATT	
Type IVc Reverse	TCGTTGTCATTTAATTCTGAACT	677bp
Type IVd Forward	AATTCACCCGTACCTGAGAA	
Type IVd Reverse	AGAATGTGGTTATAAGATAGCTA	1242bp
Type IVh Forward	TTCCTCGTTTTTTCTGAACG	
Type IVh Reverse	CAAACACTGATATTGTGTCG	663bp
Type V Forward	GAACATTGTTACTTAATGAGCG	
Type V Reverse	TGAAAGTTGTACCCTTGACACC	325bp
mecA Forward	TCCAGATTACAACTTCACCAGG	
mecA Reverse	CCACTTCATATCTTGTAACG	162bp
Sa442 Forward	AATCTTTGTCGGTACACGATATTCTTCACG	
Sa442 Reverse	CGTAATGAGATTTCAGTAGATAATACAACA	108bp

Liu *et al.* (2016)

Table 2: Primers used for Multilocus

(Weiss *et al*., 2016)

Gene	Primer Sequence	Expected Amplicon Size
arcC-Forward	TTG ATT CAC CAG CGC GTA TTG TC	
arcC-Reverse	AGG TAT CTG CTT CAA TCA GCG	456bp
AroE-Forward	ATC GGA AAT CCT ATT TCA CAT TC	
AroE-Reverse	GGT GTT GTA TTA ATA ACG ATA TC	456bp
glpF Forward	CTA GGA ACT GCA ATC TTA ATC	
glpF Reverse	TGG TAA AAT CGC ATG TCC AAT TC	465bp
*gmk,*Forward	ATC GTT TTA TCG GGA CCA TC	
*gmk,*Reverse	TCA TTA ACT ACA ACG TAA TCG TA	429bp
*pta,*Forward	GTT AAA ATC GTA TTA CCT GAA GG	
*pta,*Reverse	GAC CCT TTT GTT GAA AAG CTT AA	479bp
tpi Forward	TCG TTC ATT CTG AAC GTC GTG AA	
tpi Reverse	TTT GCA CCT TCT AAC AAT TGT AC	402bp
yqiL Forward	CAG CAT ACA GGA CAC CTA TTG GC	
yqiL Reverse	CGT TGA GGA ATC GAT ACT GGA AC	516bp

RESULTS

Isolation and identification of bacterial isolates

Out of the 371 bacterial isolates tested, only 241 were found to be DNAse, catalase, and coagulase positive and ferment mannitol. Ninety-eight (98), were confirmed as *S. aureus* by detection of clumping factor protein A. Phenotypically, 30 were found to be methicillin-resistant *S. aureus* using cefoxitin.

Detection of *mecA* gene

Out of the 22 selected cefoxitin resistant isolates tested, 6 of the isolates possessed *mecA* gene and the size of the amplicon corresponded to 162 bp, and seven possessed *mecA* gene and size of the amplicon corresponded to 500 bp. Altogether the result indicated that 13 isolates (59.0 %) amplified *mecA* gene coding for methicillin resistance confirmed by clear bands. The result is shown in Plate I.

Multi-locus sequence typing

Three of the seven multilocus housekeeping genes were amplified **{(***pta* (474bp)*, gmk* (429bp) and *yqil* (516bp)}, at 43.3 %, 20 % and 16.7 % respectively. Four genes were not amplified in all the isolates using the primers (Plate II).

DNA Sequencing

Sixteen of the isolates were selected for DNA sequencing of the PCR product. The phylogenetic tree comparing the nucleotide sequence of the selected isolates with the other *S. aureus* strain from the gene bank data base.

Phylogenetic tree

This result showed the inter relationship between the isolates. It revealed that MRSA isolates from poultry and poultry farm workers clustered into distinct clades corresponding to their sequence types. Isolates carrying SCC*mec* type IV formed a closely related cluster irrespective of host origin, suggesting possible transmission between poultry and poultry farm worker.

Variation analysis

The variation analysis revealed a substantial genetic diversity among the MRSA isolates recovered from poultry and poultry farm workers. This demonstrated close clustering of some poultry and human isolates suggesting shared evolutionary origins.

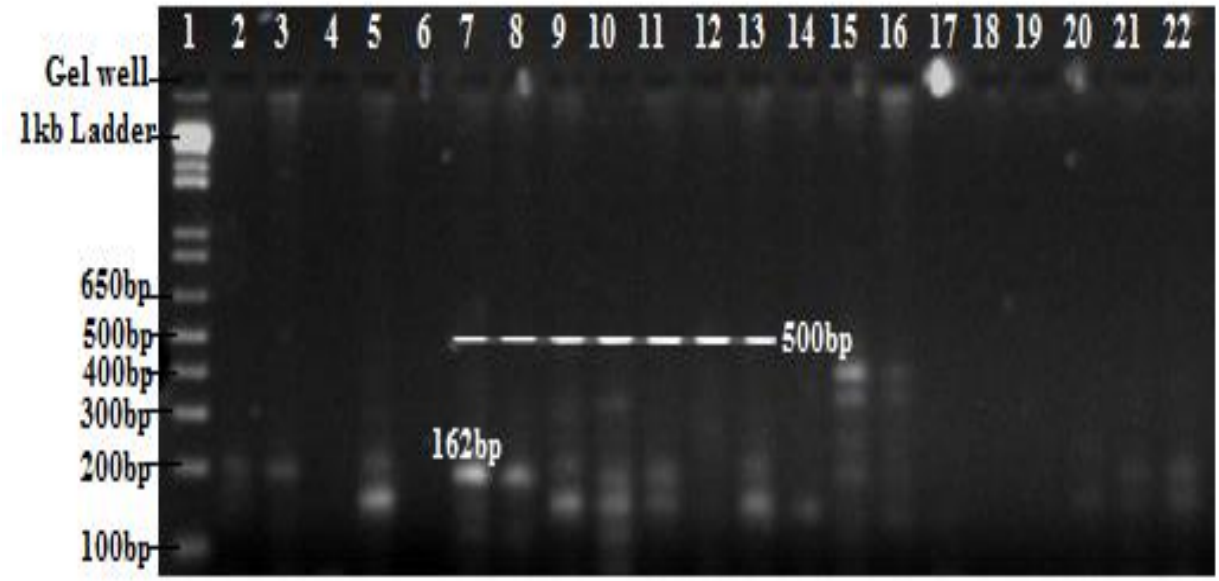

Plate I: Multiplex PCR product for detection of *mecA* gene in *S. aureus*

Lane 1 = 1 kb ladder,
Lane 2, 3 and 5 = *S. aureus* from cloacae *mecA* positive,
Lane 7 - 13 = *S. aureus* from cloacae *mecA* positive,
Lane 4 = *S. aureus* from cloacae *mecA* negative,
Lane17, 19 = *S. aureus* from cloacae *mecA* negative,
Lane 14 = *S. aureus* from cloacae *mecA* negative,
Lane 20 - 22 = *S.aureus* from nostril of farm worker *mecA* positive

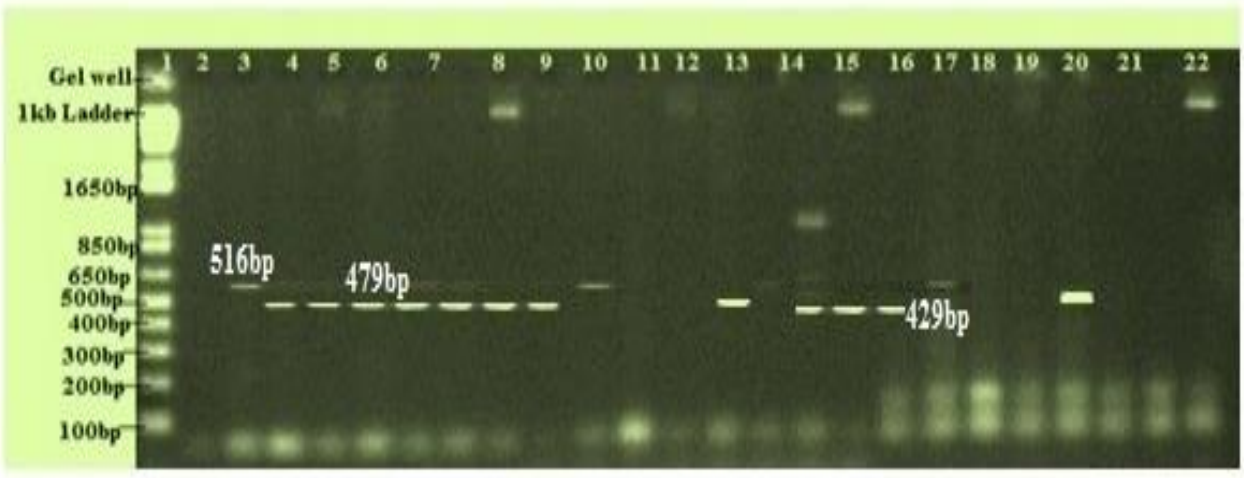

Plate II: Molecular characterization of the seven housekeeping genes {*pta* (479bp)*, gmk* (429bp) and *yqil* (516bp)}.
Lane 1: 1kb ladder
Lane 2,21 and 22: *S. aureus* from cloacae
Lane 3,10,: *S. aureus* from nostril of farm worker *yqil* positive
Lane 4- 9: *S. aureus* from cloacae *pta* positive
Lane13:*S. aureus* from nostril of farm worker *pta* positive
Lane 14-16: *S. aureus* from cloacae *gmk* positive

Lane 17 and 20: *S. aureus* from cloacae *yqil* positive

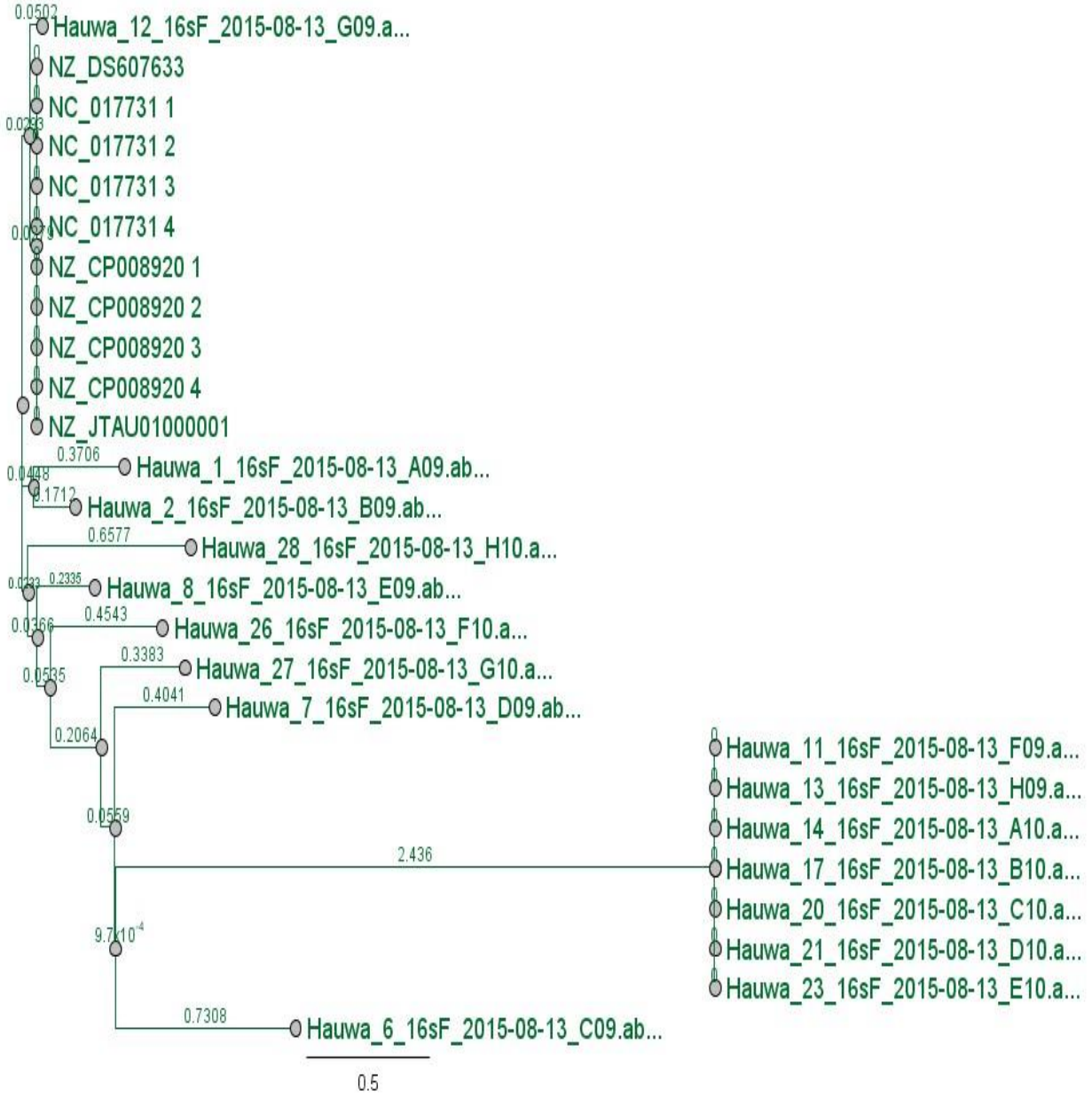

Plate III: Phylogenetic tree of 16 *S. aureus* isolates

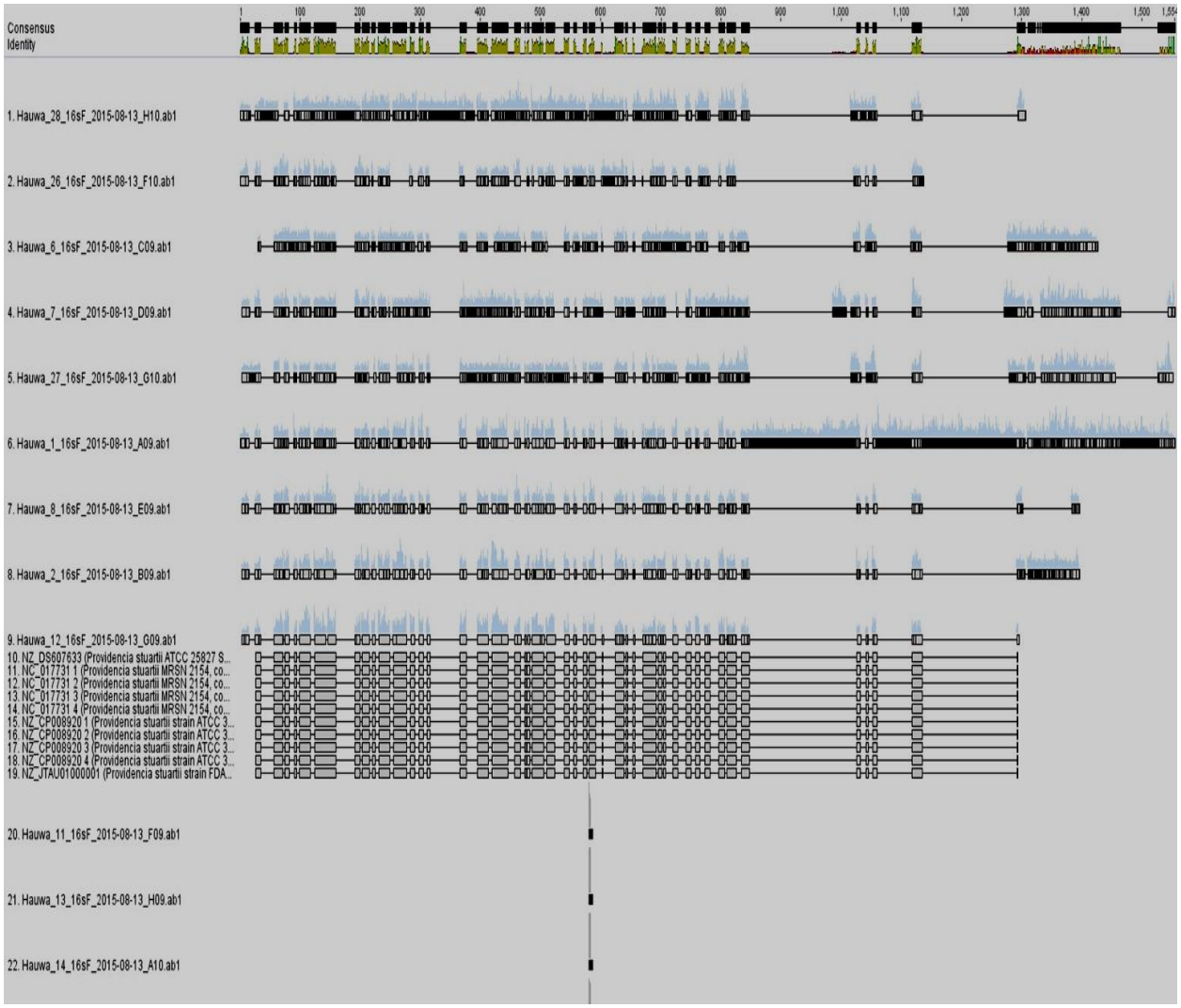

Plate IV: Variation analysis of *S. aureus isolates*

DISCUSSION

The isolation of *S. aureus* from poultry and human samples in this study further support the presence and ability of *S. aureus* to survive in various environment such as skin, nares and skin of animal carcasses and the possibility of zoonotic infections in human (Sinlapasorn *et al.,* 2015). The prevalence of *S. aureus* isolated in this study is consistent with several reports in both clinical and non-clinical settings suggesting widespread dissemination (Bale *et al*., 2018). The site of isolation of MRSA and specimen type has been found to be associated with prevalence of MRSA. The detection of MRSA across these reservoirs reinforces the growing concern regarding zoonotic transmission and the role of livestock as potential reservoirs of antimicrobial resistance (Kwoji *et al*., 2019). The prevalence of MRSA in this study was 30.6 %, this aligns with previous studies conducted in Nigeria on the prevalence of MRSA; For example, Okon *et al*., (2017) obtained 22.6 % among human population, while Odetokun *et al*. (2020) documented low prevalence across human, poultry and environment. Kwoji *et al*. (2017) reported a higher rate from chicken and farm personnel in Sokoto State, while Nworie *et al*. (2017) also reported high prevalence of MRSA in poultry farms in Ebonyi State. A common factor in all these studies is the method (phenotypic detection using cefoxitin) of detecting MRSA which is generally regarded as less sensitive in detecting classical methicillin resistance mediated by *mecA* gene that code for the production of additional penicillin binding protein PBP2a which confers resistance to all β-lactam antibiotic (Chen *et al*., 2020). The recovery of MRSA from chicken droppings in this study aligns with these findings and underscores the role of poor biosecurity and

indiscriminate antibiotic use in poultry farming. Only 5 isolates out of 60 sampled from human source were found to be MRSA positive yielding a prevalence estimate of 8.3 % of the total sample. Thirteen (59.0 %) out of the 22 cefoxitin resistant selected isolates carried *mecA* gene. Resistance has been known to be caused by a gene called *mecA,* which codes for resistance to methicillin in *S. aureus* (Gu *et al*., 2020), and the gene is located on the staphylococcal chromosomes' cassette *mec*. It has been recommended that oxacillin be replaced by cefoxitin, a more potent inducer of *mecA* expression that is less affected by test condition and hyper production of penicillinase (CLSI, 2020; Boswihi *et al*., 2020). The gold standard for the detection of MRSA is the polymerase chain reaction (PCR) that detects *mecA* gene or alternatively detecting the *mecA* gene product PBP2a by latex agglutination test (Chen *et al*., 2020). The staphylococcal cassette chromosome SCC*mec* is a genetic mobile element that contains the methicillin resistant mecA gene (Fasihi *et al*., 2017). Molecular characterization using MLST and SCC*mec* typing revealed the predominance of SCC*mec* types II and IV, which are commonly associated with both hospital-associated and community-associated MRSA strains. This dual presence suggests possible genetic exchange and adaptation between healthcare and community settings. Comparable findings have been reported in Nigerian isolates, where SCCmec type IV is frequently linked to community-acquired infections, while type II is more prevalent in hospital environments (Okon *et al*., 2017; Abdulgader *et al*., 2021). The sequenced SCCmec region of two strains did not match any known DNA sequence, presuming a non-human relation. Based on the finding that these strains were only positive in PCR when the probe harbouring this mutation was used, it was presumed that these two strains were most probably live-stock associated MRSA (Boswihi *et al*., 2020). The SCCmec typing revealed that some of the MRSA isolates were SCCmec type II (31.3%), and 18.8% carry SCCmec type IV. SCC*mec* type II carries various drug resistance genes, an integrated copy of staphylococcal plasmid and three of the isolates carries SCC*mec* type harbored *mecA* gene. The detection of SCC*mec* type II in this study concurred with the previous reports of Kondo *et al*., (2022) in Thailand and Liu *et al*., 2025 in China. The result contradicts the report from India where SCC*mec* type II dominate HA-MRSA and livestock-associated MRSA mainly carries SCC*mec* typeV (Rajkhowa *et al.,* 2016; Taskeen *et al*., 2024). The MRSA in Thailand have been diverse SCC*mec* types, including types II and IX, particularly among livestock associated strains (Sinlapasorn *et al.,* 2015) and Japan (Funaki *et al.,* 2019) which showed SCC*mec* type II, III and IV. In The Netherlands live-stock associated MRSA is increasingly isolated (Tamura *et al*., 2021). It has been shown that live-stock associated MRSA can be discriminated on the basis of a mutation in *orfX* (Petinaki and Spilliopoulou, 2015). The phylogenetic analysis of the 16 *S. aureus* isolates obtained from chicken droppings revealed distinct clustering patterns, indicating genetic diversity among the isolates. The sequences showed that the isolates formed several clades, suggesting the presence of multiple lineages circulating within the poultry environment. This aligns with previous studies reporting high heterogeneity of *S. aureus* strains in avian sources (El-Adawy *et al*., 2016; Khatoon *et al.,* 2024). Some isolates clustered closely with reference strains associated with both poultry and human infections, highlighting the zoonotic potential of these isolates. This suggests that poultry can serve as reservoirs for strains capable of crossing the species barrier, emphasizing the public health implications of poultry *S. aureus* colonization. The genetic distances observed between certain isolates indicate possible microevolution events occurring within the farm environment, which may be driven by selective pressures such as antimicrobial use, environmental stressors, and horizontal gene transfer. Interestingly, certain isolates from the same farm clustered together, suggesting clonal expansion and localized transmission. Conversely, isolates from different farms sometimes clustered within the same clade, indicating that common sources, such as feed, equipment, or personnel, may facilitate inter-farm dissemination of genetically related *S. aureus* strains. This observation supports findings from previous studies showing that poultry-associated *S. aureus* populations are shaped both by local farm management practices and broader environmental factors (El-Adawy *et al.,* 2016). Monitoring the phylogenetic relationships among poultry isolates is crucial for understanding the epidemiology of *S. aureus* in agricultural settings and for implementing targeted interventions to prevent the spread of potentially pathogenic or multidrug-resistant strains. This pattern is consistent with previous studies that have reported heterogeneous MRSA populations in Nigeria, driven by selective pressure from antibiotic misuse and horizontal gene transfer (Kolawole *et al.,* 2018; Iweriebor *et al.*, 2022).There is a relationship between isolate number 28 which was from farm worker and the members of the cluster which were from the birds and the difference in the time of

divergence is not wide. This showed that the organisms originated from the same source, indicating the possible transmission either from humans to birds or from birds to humans. Notably, one isolate positioned distantly on the phylogenetic tree suggests the presence of a genetically distinct lineage, which may have arisen from independent evolutionary events or external introduction. Comparable diversity has been documented in poultry-associated MRSA in Nigeria, where multiple sequence types and spa types, including novel variants, were identified (Nworie *et al.,* 2017). Such diversity highlights the adaptive capacity of MRSA and its ability to persist in different hosts and environments. MLST is a powerful tool for understanding the evolutionary dynamics of pathogens and to gain insight into their genetic diversity. Evolutionary analyses on the seven multilocus sequence typing (MLST) genes that encode for proteins of central metabolic functions which can only evolve through mutation and gene replacement, influencing genetic variation and causes phenotypic differences among population genetic was also carried out on the MRSA to analyze the genetic diversity present in the isolates (Petinaki and Spilliopoulou, 2015). The result showed that 3 of the seven housekeeping genes (*pta, gmk* and *yqil*) were also present in the isolates at 43.3 %, 20 % and 16.7 % respectively. Four genes were not amplified in all the isolates using the primers possibly due to alteration in the binding site of the primers. The findings of this study reinforce the role of poultry as a potential reservoir of MRSA and the urgent need for integrated surveillance systems that encompass human, animal, and environmental health sectors in line with the One Health approach. The detection of genetically related strains across different sources highlights the interconnectedness of these ecosystems and the necessity for coordinated antimicrobial stewardship programs. The integration of MLST and SCCmec typing provided a robust framework for understanding the genetic relationships among isolates and tracing possible transmission pathways. These results underscore the need for improved hygiene practices, rational antibiotic use, and coordinated surveillance strategies to mitigate the spread of MRSA in Nigeria.

CONCLUSION

The application of multilocus sequence typing (MLST) and SCC*mec* typing revealed the presence of genetically diverse MRSA lineages and demonstrated the occurrence of SCC*mec* types associated with livestock-associated strains. The detection of related sequence types among poultry and farm workers suggests possible bidirectional transmission at the human-animal interface, highlighting the zoonotic potential of MRSA within poultry production systems. These findings emphasize the importance of integrating molecular surveillance tools into routine monitoring programs to track the emergence and spread of high-risk MRSA clones. Strengthening antimicrobial stewardship, improving farm biosecurity practices, and implementing a One Health approach are essential to mitigate the transmission of MRSA between animals and humans. Future studies incorporating whole-genome sequencing and larger geographical sampling are recommended to further elucidate the evolutionary pathways and public health significance of these strains.

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