

Detection of *mecA* Gene in Methicillin resistance *Staphylococcus aureus* (MRSA) from rural chickens, slaughter environment and personnel in Maiduguri, Borno State, Nigeria

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Abstract

Cross sectional study was carried out from Monday and Gamboru markets in Maiduguri, Nigeria to detect the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) and determine the interplay between rural chickens, slaughter environment and personnel in the spread of MRSA. Samples were taken from skin of rural chickens, dressing tables, containers, knives and from the skin and nostrils of slaughter personnel. A total of 135 presumptive *S. aureus* isolates were analyzed and subjected to ORSAB test to identify MRSA using standard techniques. The test detected 57 (42.2%) MRSA isolates out of which 25 were randomly selected and subjected to PCR to detect *S. aureus* specific sequence gene and *mecA* gene. The isolates showed evidence of expression of *S. aureus* specific sequence gene with 107 bp targeted band whilst only 1 isolate was positive for *mecA* gene with a targeted band of 532 bp. It is therefore evident from this result that *mecA* gene is present in MRSA isolate from rural chicken which means transmission between human and animals is possible. Awareness programs should be implemented to educate the slaughter personnel and general public on its veterinary and public health importance. Therefore, these emphasize the need for further research to be carried out on the phylogenesis of MRSA in poultry and other livestock in Nigeria.

Keywords: MRSA, PCR, *mecA* gene, Sequence, Maiduguri, Nigeria.

Introduction

Rural poultry production occupies a vital position as a source of income and protein to economically less privileged rural communities in Nigeria¹. Nevertheless, rural poultry production suffers drawback associated with management practices, diseases and predators^{1,2}. The birds are kept under extensive management and as such there is very little care which make them prone to predators, accident and infectious diseases leading to death or poor productivity in terms of eggs and meat and consequently low income to the farmers³. Zoonotic diseases caused by viral, bacterial, fungal and other infectious agents play important role in the quality and safety of eggs and meat produced by these birds and have raised some public health concerns. For instance, the emergence of methicillin resistant *Staphylococcus aureus* (MRSA) in the early 1960s, when some strains of *Staphylococcus aureus* showed resistance to methicillin soon after its introduction⁴.

Historically, infections caused by MRSA were predominantly nosocomial in immune-compromised patients and those with foreign objects in their body⁵. *MecA* gene conferred resistance in methicillin and other β -lactam antibiotics by altering the penicillin-binding protein located within the cell wall⁶. This

alteration by penicillin-binding protein renders β -lactam antibiotics which act by interfering with bacterial cell wall synthesis ineffective against MRSA⁶. *Staphylococcal cassette chromosome (SCCmec)* is a large heterogeneous mobile genetic element that encompassed both the *mec* gene complex (the *mecA* gene and its regulators) and the cassette chromosome recombinases (*ccr*) gene complex that encodes the site-specific recombinases responsible for the mobility of *SCCmec*^{6,7}.

This gene complex also allows cross resistance to non-beta lactam antibiotics such as clindamycin, ciprofloxacin, cotrimoxazole, erythromycin and gentamycin because of the presence of insertion sites for plasmids and transposons⁸.

Recently, MRSA have been isolated with increasing tendencies from other non-human animate and inanimate sources such as animals and foodstuff⁷.

However, the newly emerged clonal complex 398 (CC398) in livestock has been reported to cross infect humans^{9,10}. Initially, MRSA CC398 was suspected to be livestock-associated pathogen but whole genome sequence typing (WGST) - based phylogeny strongly suggests that its originated from humans as methicillin-sensitive *Staphylococcus aureus* (MSSA) and then

spread to livestock, it then acquired the staphylococcal cassette chromosome (SCC_{mec}) cassette and methicillin resistance in the livestock¹¹. Thus, human infection with the livestock-associated CC398 could be a reintroduction to the original host¹¹. Indiscriminate use of antibiotics in livestock production could be responsible for antibiotic resistance in *S. aureus* and other bacteria¹¹. Livestock colonized by MRSA (especially CC398) could be good sources of infections to their human handlers¹²⁻¹⁵.

In Netherlands, there were reports of higher incidences of MRSA amongst abattoirs workers who handle livestock compared to those who don't^{15,16}.

In this study, we ascertain the presence of MRSA from rural chickens, slaughter environment and personnel using genotypic techniques, sequencing of the *mecA* gene from the MRSA isolates and comparison of the gene sequences against other isolates from around the world.

Materials and methods

A total of 135 presumptive *S. aureus* isolates were identified and methicillin resistant *S. aureus* (MRSA) isolates were detected using Oxacillin Resistance Screening Agar Base (ORSAB) test. Presumptive MRSA isolates obtained were 57 (42.2%). The antibiotic sensitivity pattern of *S. aureus* isolates was determined initially showing resistance to Cefoxitin (83%) and oxacillin (72.6%) according to the method of Bauer-Kirby¹⁷ by using commercially prepared discs (Oxoid® England) with known concentrations of antibiotics. PCR assay was carried out for detection of *S. aureus* specific sequence gene and *mecA* gene from 25 presumptive MRSA isolates out of the total number of the 57 presumptive MRSA isolates.

Molecular identification: DNA extraction: The genomic DNA extraction of MRSA isolates used in this study was performed according to the DNA laboratory protocol. Sample collection was done by using sterile cotton swab which was rubbed and twisted around in an overnight grown culture of MRSA isolates. 400µl of lyses buffer and 4µl of proteinase K were dispense into 1.5ml tube with cotton swab cut end. The tubes were closed and thoroughly vortexed. The tubes were placed on heat block at 50°C-55°C for 3 hours. The lysate was carefully transferred as much as possible to a new tube.

To every 300µl recovered lysate, 75µl of 5M sodium chloride were added and mixed by flicking the tube. The tubes were centrifuged at (5,000xg–12,000xg) for 10 minutes at 4°C in refrigerated centrifuge. Carefully, the lysate was transferred into a new tube and the pellets were harvested. To a total of 300µl lysate, 600µl of -20°C cold ethanol was added and mixed by inverting the tube several times. The tubes were spun at 12,000 x g for 10-20 minutes at 4°C. The ethanol was discarded; 400 µl of another 70% ethanol was added and spun at 12,000xg for 5 minutes at 4°C. The above procedure was repeated to remove

the buffer. Ethanol was removed by spinning the tube for additional 30 seconds. The DNA was air dried by leaving the tube open for 3-5 minutes. The pellets were re-suspended in 20-50µl sterile distilled water.

Five microlitre of the extracted DNA was mix with a loading dye and run on agarose gel electrophoresis to estimate the quantity and integrity of the DNA at 100 volts for 1hour. The bands were visualized using Ultraviolet light box with camera (Gel Doc 2000, BIO RAD).

Primers: Primers corresponding to the *S. aureus* specific sequence gene and *mecA* gene were obtained from Bioneer Inc., Alameda, CA 94501, USA. These primers were re- suspended in 107.5µl (reverse primer) and 158µl (forward primer) of sterile distilled water from which 20 pmol/µl were aliquoted into eppendorf tubes. The diluted primers were stored at -20°C.

Polymerase Chain Reaction Premix (PCR Premix): Hotstart PCR Premix (10µl) (Accupower®, Bioneer Inc) was employed for PCR amplification. It is a mixture prepared in lyophilized form and it contains Taq DNA polymerase, reaction buffer, dNTPs (dATP, dGTP, dCTP, and dTTP), loading dye, MgCl₂ and tracking dye for efficient PCR amplification.

Amplification of *S. aureus* specific sequence gene and *mecA* gene: The amplification of *S. aureus* specific sequence gene and *mecA* gene was employed using PCR machine (GeneAmp PCR System 2400).

Detection of PCR Products and sequencing: The PCR product for *S. aureus* specific sequence gene was run on 2% agarose gel and for *mecA* gene on 1.5% agarose gel at 110 volts for 1 hour. The DNA bands were visualized using Ultraviolet light box with camera (Gel Doc 2000, BIO RAD) and photographed.

The expected size of the PCR products for *S. aureus* specific sequence and *mecA* genes were estimated in relation to 100 bp DNA ladder and 1kb DNA ladder (Bioneer Inc., USA) respectively.

DNA sequencing of *mecA* was carried out using Dye termination sequencing cycle Quick start master mix (CEQ™2000 Dye Terminator Cycle Sequencing Chemistry Protocol).

Results and discussion

Staphylococcus aureus specific sequence gene and *mecA* gene were screened by PCR in 25 out of the 57 presumptive MRSA isolates. All the 25 isolates assayed showed evidence of expression of *S. aureus* specific sequence gene with a targeted band of 107 bp (Figure-1) which confirmed the assumption that all the strains were *S. aureus*. The result of the PCR based on targeted *mecA* gene revealed that 1 out of the 25 isolates showed

evidence of expression of *mecA* gene with a corresponding band of 532 bp (Figure-2). This finding which showed reduction in the sensitivity and specificity of the PCR method could be explained by absence of or reduced expression of *mecA*-encoded protein, PBP2a as reported by García-Álvarez *et al.*²⁰. It also concurs with García-Álvarez *et al.*²⁰ who reported the emergence of novel divergent *mecA* gene designated *mecA*_{LGA251} which is 70% similar to some strains of *S. aureus*²⁰. These strains which are recently discovered in Europe are negative for *mecA* gene and resistant to methicillin²⁰. Discovery of *S. aureus* isolates encoding *mecA*_{LGA251} as methicillin-resistant by conventional laboratory

culture and antimicrobial susceptibility testing, may produce false positive results if currently available confirmatory tests are used²¹. Furthermore, this finding highlights the possibility of emergence of new MRSA strains through the acquisition of additional *mecA* alleles circulating in the environment by *S. aureus*²². The *mecA* gene was sequenced and 470 bases were found (Figure-3). The bases were aligned using BLAST (Basic Local Alignment Search Tool) from the National Centre for Biotechnology Information (NCBI) gene bank and 82 sequences producing significant alignments with the highest similarity score (99%) to the field isolate were considered the specie identity.

Table-1: Primers used to amplify *S. aureus* specific sequence gene and *mecA* gene.

Gene	Primers	Position	Product length	Sequence (5'-3')	Reference
SSSG	MecA ₂ forward	5-34	107 bp	5'-AAT CTT TGT CGG TAC ACG ATA TTC TTC ACG-3'	18
	MecA ₂ reverse	112-83		5'-CGT AAT GAG ATT TCA GTA GAT AAT ACA ACA-3'	18
<i>MecA</i>	MecA forward	1282-1301	532 bp	5'-AAA ATC GAT GGT AAA GGT TGG C-3'	19
	MecA reverse	1814-1793		5'-AGT TCT GCA GTA CCG GAT TTG C-3'	19

Table-2: Reaction set up for amplification of *S. aureus* specific sequence gene and *mecA* gene using Hot Start PCR Premix (10 µl)

Component	Volume
PCR Premix	10 µl
DNA Template	5 µl
Forward Primer	1 µl
Reverse Primer	1 µl
Distilled H ₂ O	13 µl
Total	30 µl

Table-3: Thermal cycling program of PCR for amplification of *S. aureus* specific sequence gene and *mecA* gene.

Steps	Temperature (°C)
Pre-denaturation	94°C for 5 min.
Denaturation	94°C for 30sec.
Annealing	55°C for 30sec.
Extension	72°C for 60sec.
Repeat step 2,3 and 4 for 40 cycles	
Final extension	72°C for 4 min.

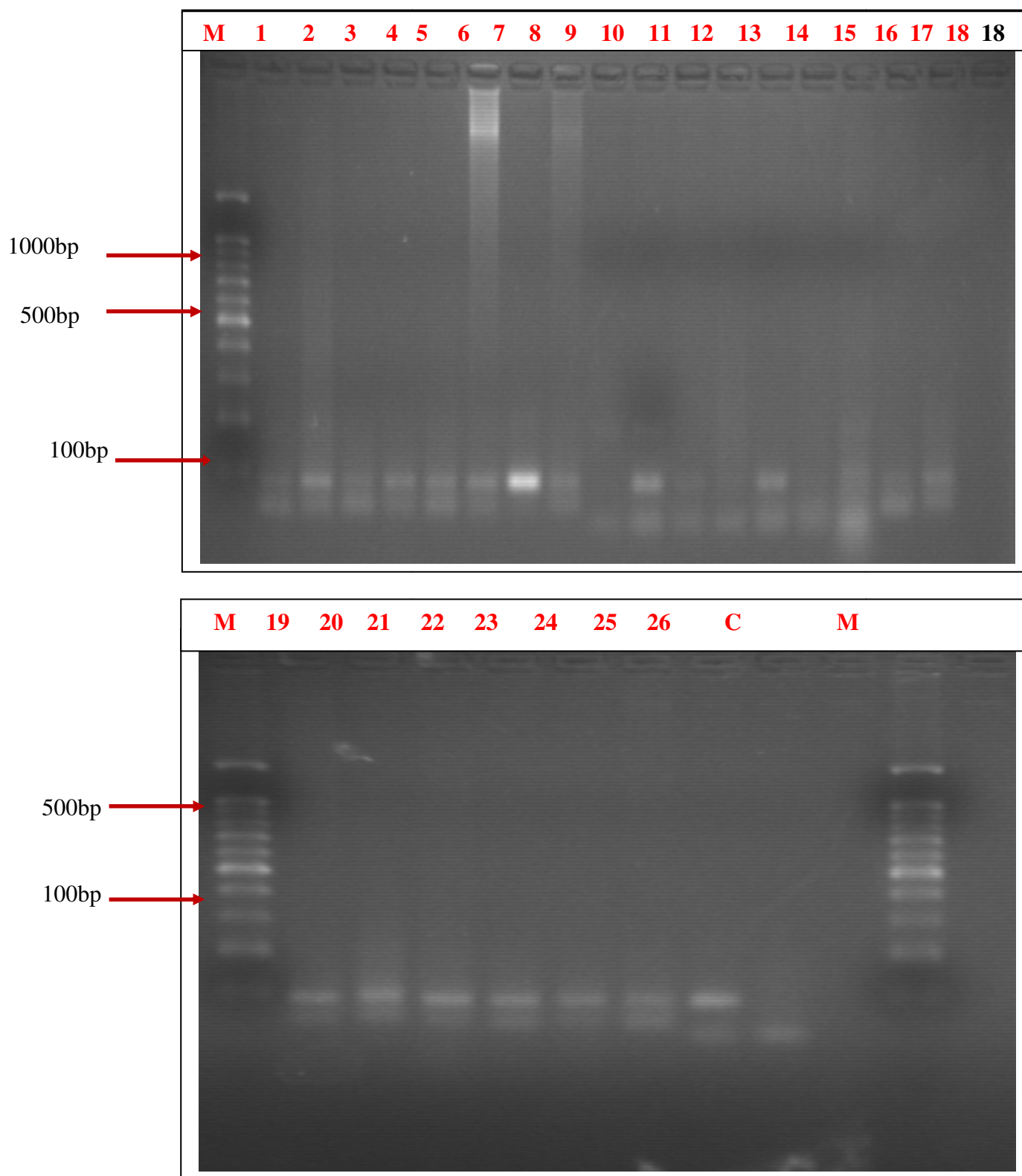


Figure-1: Agarose gel electrophoresis of PCR of *S. aureus* isolates using primers set of *mecA*₂ for and *mecA*₂ rev. Lane M: 100bp molecular weight marker (fermentas®). Lane 1-25: very weakly positive samples. Lane 7: Strongly positive (+++) *S. aureus* specific gene at 107bp. Lane C: Negative control. N.B: The band pointed by the arrow corresponds to the 107bp PCR product.

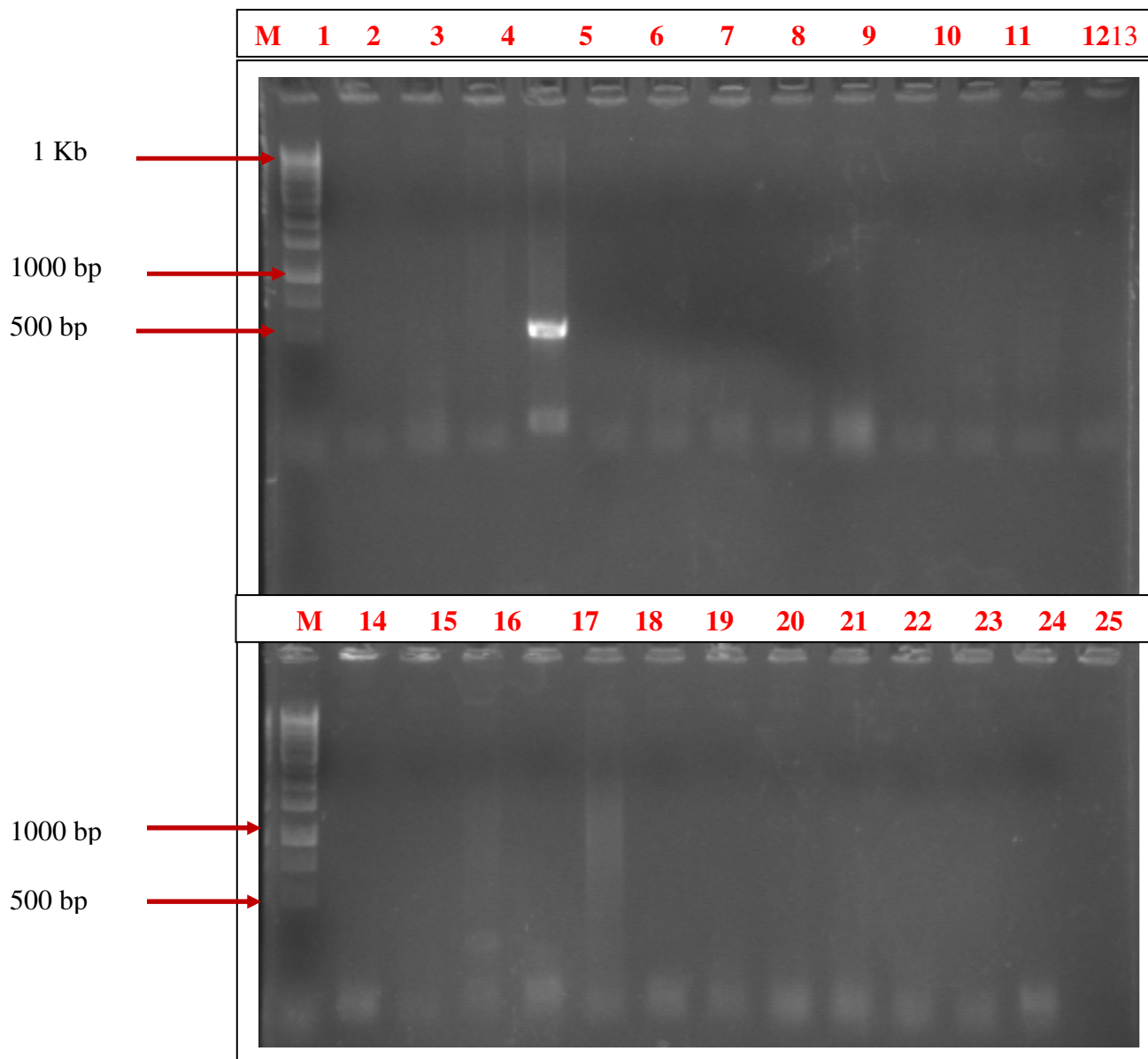


Figure-2: Agarose gel electrophoresis of PCR of *S. aureus* isolates using primers set of *mecA* for and *mecA*rev. Lane M: 1kb molecular weight marker (fermentas®). Lane 1-3: Showing no band. Lane 4: Very clear band corresponding to the 537bp PCR Product indicating positive result. Lane 5-25: indicate no band (Negative results). Lane C: Negative control. N.B: The band pointed by the arrow corresponds to the 537bp PCR product.

1-ACGTTACAGATATGAAGTGGTAAATGGTAATATCGACTTAAAACAAGCAATAGAAT=56
57-CATCAGATAACATTTTCTTTGCTAGAGTAGCACTCGAATTAGGCAGTAAGAAATTTG=113
114-AAAAAGGCATGAAAAAACTAGGTGTTGGTGAAGATATACCAAGTGATTATCCATTT=169
170-TATAATGCTCAAATTTCAAACAAAAATTTAGATAATGAAATATTATTAGCTGATTCA=226
227-GGTTACGGACAAGGTGAAATACTGATTAACCCAGTACAGATCCTTTCAATCTATAGC=283
284-GCATTAGAAAATAATGGCAATATTAACGCACCTCACTTATTTAAAGACACGAAAAA=339
340-CAAAGTTTGAAGAAAAATATTATTTCCAAAGAAAATATCAATCTATTAAGTATGG=396
397-TATGCAACAAGTCGTAAATAAAACACATAAAGAAGATATTTATAGATCTTATGCAA=452
453-ACTTAATTGGCAAATCCG=470

Figure-3: Transcription of Sequenced *mecA*

Conclusion

Based on this study, genotypic identification and sequencing of *mecA* from MRSA strains will provide meaningful data on the importance of molecular characterization of MRSA as a confirmatory tool. To the best of my knowledge; this is the first time *mecA* has been detected from rural chickens in the study area, therefore, these emphasize the need for further research to be carried out on the phylogenesis of MRSA in poultry and other livestock in Nigeria. Awareness programs should be implemented to educate the personnel and community on the economic importance of MRSA. Improved sanitary measures during handling and processing of chickens should be encouraged in order to minimize the risk of MRSA colonization and spread. A modern poultry abattoir with proper facilities should be constructed by government and private organization to reduce the risk of MRSA spread.

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