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Molecular characterisation of methicillin-resistant *Staphylococcus aureus* associated with livestock in selected poultry farms in port-harcourt, Nigeria

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Abstract
Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a strain of Gram-positive bacteria that is distinct from other *Staphylococcus aureus* due to its ability to develop or acquire multiple drug resistant to Beta-lactam (β-lactam) antibiotics. It is an opportunistic pathogen capable of colonizing humans, companion animals and has had increased mention in disease causation in livestock. This research was to evaluate the presence of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) particularly in poultry in the Greater Port Harcourt City, Rivers State - Nigeria. A total of 195 swab (Choanal cleft and cloaca) samples were collected from 5 selected poultry farms locations in Port Harcourt namely: Elelenwo, Akpajo, Eliminiqwe, Eliozu and Umuebule – Etche. Using aseptic standard microbiological procedures, 92 (47.2%) isolates of *Staphylococcus aureus* were identified and these were subjected to antimicrobial susceptibility testing. Significant number of the isolates were resistant to cefoxitin and oxacillin. Out of the 92 *Staphylococcus aureus* isolates tested, 46 (50%) were Methicillin-Resistant *Staphylococcus aureus* (MRSA). About 20 representative MRSA isolates were assayed for the genes of interest (*mec*A and *CC398* genes). Seven (7) isolates (35%) were identified as harboring the CC398 genes, signifying the prevalence of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) in Port Harcourt. It is evident that individuals who rear poultries are in higher risk of becoming LA-MRSA carriers. There is need for increased MRSA and LA-MRSA surveillance in both human and animals in our communities, in order to plan for effective control of these strain.

Keywords: *Staphylococcus aureus*, methicillin-resistance, livestock, community-acquired

1. Introduction
Staphylococcus aureus is a well-known commensal of man. About one third of healthy individuals carry the organism on their skin and nose [1]. *S. aureus* has established itself as a leading and most frequent cause of a wide range of both hospital and community-acquired infections [2-4] and bacteremia associated with it can be fatal [5]. Some health issues associated with *S. aureus* include osteomyelitis, pneumonia, meningitis, arthritis, endocarditis, septicemia, deep tissue abscesses, skin and soft -tissue infections (SSTIs) as well as toxic shock syndrome amongst others [6]. It is also a common cause of wound and urinary tract infections [7, 8].

The first penicillin-resistant *S. aureus* strain was identified in 1942 [9]. The semi synthetic antibiotic, methicillin, was designed in 1950s, and methicillin-resistant *S. aureus* (MRSA) was then clinically detected in 1960s

Shortly after the introduction of methicillin, a β-lactamase stable antibiotic in the early 1960s, Methicillin-resistant *S. aureus* (MRSA) emerged, predominantly associated with infections in clinics and health-care settings. Methicillin-resistant *S. aureus* infections in hospitals and clinical settings are referred to as hospital associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) infections. However, in the 1990s, the epidemiology of MRSA changed with the emergence of MRSA infection in those without the risk factors of HA-MRSA [10].

This new group of MRSA is more invasive and causes mainly skin and soft tissue infections in healthy individuals especially amongst the young and has been designated as community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) [11].

Methicillin-resistant *S. aureus* is the leading cause of hospital and community acquired infections worldwide [8]. It remains the most common agents of nosocomial infections, increasing the cost and mortality associated with staphylococcal infections as compared to methicillin sensitive *Staphylococcus aureus* (MSSA) infections. *Staphylococcus aureus* as a pathogen is not limited to man. Infections and colonization of livestock have been documented [12]. It has now been established that livestock can harbor and constitute a reservoir and source of a novel and rapidly emerging type of MRSA. This new type of MRSA belonging to clonal complex (CC) 398 is predominant in livestock. It is associated with diseases in livestock but has also been implicated in human infections [13]. Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is an emerging problem in many parts of the world. Although animal-adapted LA-MRSA has been known for many years, recent reports suggest a possible increasing trend in the zoonotic transmission of LA-MRSA in Europe and other parts of the world [14].

The LA-MRSA CC398 lineage, which apparently emerged in European pigs between 2003 and 2005, has since been detected in other animal species in many European countries and also in North America, where it can colonize the animal but only rarely cause infections.

Livestock-associated methicillin-resistant *Staphylococcus aureus* has been reported as an emerging problem in many parts of the world [14]. Despite the enormous reports of LA-MRSA both in animals and food sampled in continental Europe, and some parts of Africa, there have been limited reports of its discovery in south-south area of Nigeria. The World Health Organisation (WHO) has included MRSA on their list of priority bacterial resistant pathogens and that made it a high priority target for further research and treatment development.

Livestock and companion animals are particularly important in this regard considering the relatively high usage of antimicrobials in these species. There is a risk to humans who come into direct contact with animals acquiring MRSA but there is also the risk of animals acquiring MRSA from colonized humans.

There is evidence of recent emergence of Livestock-Associated Methicillin *Staphylococcus aureus* (LA-MRSA) in some western and Northern part of Nigeria [15] so determining the prevalence of MRSA in poultry in the Greater Port Harcourt area will enhance proper surveillance and treatment. This Study, therefore seeks to investigate the prevalence of *mec*A gene and *CC398* gene in the Greater Port Harcourt city, Nigeria.

2. Materials and Methods

2.1 Study Area: The study was carried out in Port Harcourt metropolis, a coastal city in the Niger Delta region of Nigeria, located some 64 kilometres from the Atlantic Ocean. Port Harcourt was developed on a bend of the Bonny River, where a bluff of solid land lay close to deep water. It was laid out in response to a need on the part of the colonial government of Nigeria for effective control of the hinterland, in order to tap the vast oil palm resources of southeastern Nigeria [16]. It lies along the Bonny River (an eastern distributary of the Niger river, 41 miles (66 km) upstream from the Gulf of Guinea Port Harcourt was founded in 1912 in an area traditionally inhabited by the Ijo and Ikwerre people and it began to serve as a port (Named for Lewis Harcourt, then Colonial Secretary) after the opening of the rail link to the Enugu coalfields in 1916 [17]. Agricultural activities, including Poultry farming, are a major occupation in this region. The study was carried out across the Metropolis (Greater Port Harcourt City) in 5 selected Poultry farms.

2.2 Study Population: The population studied was birds (mainly broilers and layers) from selected poultry farms in Greater Port Harcourt.

2.3 Obtained Consent: Consent was duly given by the owners of the poultry farms before sample collection.

2.4 Study Design

This was an observational cross-sectional study across 5 selected poultry farms in Greater Port Harcourt region, specifically in ObioAkpor, Eleme and Etche Local Government Areas. The towns within these Local Government Areas where samples were collected are Elelenwo (31), Akpajo (52), Eliminigwe (45), Eliozu (37), and Umuebule - Etche (30).

2.5 Sample Size Calculation: Sample size was calculated using the formula by Sharma *et al*. [18].

$$N = \frac{Z^2pq}{d^2}$$

Where:

N = sample size

Z = Z scores at 95% level of confidence, Z value is 1.96

P = Expected prevalence of Livestock Associated Methicillin-Resistant *Staphylococcus aureus* (CC398 gene) in Nigeria = 9% = 0.09 [18]

d = precision = 0.5

q = l-p = 1-0.09 = 0.91

$$\text{Therefore } N = \frac{(1.96)^2 x\ 0.09\ (0.91)}{(0.05)}$$

$$= \frac{0.315}{0.0025}$$

= 126

2.6 Sample Collection: A total of one hundred and ninety-five (195) swab samples were collected, which comprised 31 samples from a poultry farm in Elelenwo, 52 samples from a poultry farm in Akpajo, 45 samples from a poultry farm in Eliminiqwe, 37 samples from a poultry farm in Eliozu, and 30 samples from a poultry farm in Umuebule – Etche. The samples were collected from Choanal (palatine) cleft and cloaca of the birds, using sterile Swab sticks moistened with sterile normal saline. Appropriate personal protective equipment (PPEs) were worn in order to reduce cross contamination. These samples were transported in cold chain immediately to the Microbiology Laboratory in Rivers State University, Port Harcourt.

2.7 Inclusion Criteria

Samples were collected from both cocks and hens of varying ages ranging from 4 to 48 weeks old.

2.8 Exclusion criteria
Newly hatched birds were excluded from the research. Birds on special treatment including antibiotics were also excluded from the research.

2.9 Processing and Culture of the Samples
The choanal (Palatine) cleft and cloaca swabs were inoculated on Mannitol Salt Agar, CLED and Mac Conkey agar and incubated aerobically at 37 ^{0}C for 24-48 hours [19].

2.10 Culture, Isolation and Storage of suspected *Staphylococcus aureus* isolates
Isolates were purified on fresh nutrient agar (Oxoid, UK) and later identified by Gram reaction and biochemical tests such as the Catalase, Coagulase, haemolysis of blood and sugar fermentation tests [20]. Confirmed isolates were preserved frozen in Brain Heart Infusion Broth (Oxoid, UK) supplemented with 10% glycerol and in Nutrient agar (Oxoid, UK) slants.

2.11 Antimicrobial Susceptibility Testing of *Staphylococcus aureus* isolates
All isolates of *S. aureus* were subjected to antimicrobial susceptibility testing using the Kirby-Bauer disc diffusion method. A bacterial suspension of the *Staphylococcus aureus* was prepared to match a 0.5 Mcfarland turbidity standard and later lawn unto Muellar-Hinton agar plate. Using a sterile forceps, the antibiotic discs were then placed on the surface of the pre-inoculated agar and incubated aerobically for 24 hours at 35 ºC [21]. After incubation, the zones of inhibition around the discs were measured with a metre rule to the nearest whole millimeter (mm) and interpreted as sensitive, and resistant according to the standards set for interpretation by the Clinical and Laboratory Standards Institute [22]. Multiple drug resistance was defined as resistance to at least three different classes of antibiotics including one of the two markers – Oxacillin and Cefoxitin [23].
MRSA were screened using oxacillin (lμg) and cefoxitin (30μg) disc (Oxoid, UK) according to Kirby Bauer disc diffusion method. The Zone sizes were measured and interpreted according to set standards [22]. For Oxacillin,- Susceptible = zone diameter of $\geq$11mm, Resistance = zone diameter of $\leq$10mm: for Cefoxitin- Susceptible = zone diameter of $\geq$22 mm, Resistance = zone diameter of $\leq$21 mm.

2.12 Molecular Analysis and Identification
The confirmed isolates that were preserved frozen in Brain Heart Infusion Broth (Oxoid) supplemented with 10% glycerol and in Nutrient agar (Oxoid) were taken in a cold chain to a Nucleometrix Research Facility in Niger Delta University, Bayelsa State for molecular detection of selected resistance genes.

2.13 DNA Extraction
Using the boiling method, 5μl of overnight broth cultures of test bacteria in Luria Bertani (LB) were dispensed into eppendorf tubes and spun at 12000 rpm for 3 minutes. The cells were re-suspended in 500μl of normal saline and heated at 95 °C for 15 minutes. The heated bacterial suspension was fast-cooled on ice and spun for 3 minutes at 12000 rpm. The supernatant containing the DNA was transferred to a 1.5 ml micro-centrifuge tube and stored at -20 °C for other molecular analysis.

2.14 Quantification of DNA
The extracted genomic DNA was quantified using the Nano-drop 1000 spectrophotometer. The software of the equipment was lunched by double clicking on the Nano-drop icon. The equipment was initialized with 2μl of sterile distilled water and blanked using normal saline. Two microliter of the extracted DNA was loaded onto the lower pedestal of the Nano-drop; the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure" button, and the quantity of the nucleic acid obtained from the extraction was measured in ng/ml.

2.15 Amplification of *MecA* Gene
The *mec*A genes from the isolates were amplified using the *mec*A F: 5' TGGCTATCGTGTCACAATCG and *mec*A R: 5' CTGGAACTTGTTGAGCAGAG primers on an ABI 9700 Applied Biosystems thermal cycler, at a final volume of 40 μl for 35 cycles. The PCR mix contained the X2 Dream taq master mix, supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.5 μM and 50 ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95 ºC for 5 minutes; denaturation, 95 ºC for 30 seconds; annealing, 52 ºC for 30 seconds; extension, 72 ºC for 30 seconds for 35 cycles and final extension, 72 ºC for 5 minutes. The amplified product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a UV trans-illuminator for a 1500 bp amplicons.

2.16 *CC398* Gene Amplification
The clonal complex 398 (CC398) genes of the isolates were amplified using the CC398F: 5'-AGGGTTTGAAGGCGAATGGG and CC398R: CAATTTGTCGGTCGAGTTTGCTG primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 μl for 35 cycles. The PCR mix contained the X2 Dream Taq master mix supplied by Inqaba Biotec, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.5μM and 50 ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95 ºC for 5 minutes; denaturation, 95 ºC for 30 seconds; annealing, 51 ºC for 30 seconds; extension, 68 ºC for 30 seconds for 35 cycles and final extension, 68 ºC for 3 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light trans-illuminator for a 530 bp amplicons.

2.17 Statistical Analysis
Data obtained were statistically analyzed to aid interpretation of results and discussion, using GraphPad Prism. Values are presented as mean and standard deviation, while comparisons were made using ANOVA. Values were considered significant at $p \leq 0.05$.

3. Results
3.1 Distribution of Isolates from different Poultry Farms
A total of 195 swab samples collected from Birds from 5 different poultry farms were cultivated for isolation of *Staphylococcus aureus*. Out of the 195 (100%) samples

collected, 31 (15.9%) were from Elelenwo farm, 52 (26.7%) were from Akpajo farm, 45 (23.1%) were from Eliminigwe farm, 37 (18.9%) were from Eliozu farm and 30 (15.4%) were from Umuebule farm. Of the 195 samples cultured, a total of 92 *Staphylococcus aureus* (47.2%) were isolated. This comprised of 16 (51.6%) from Elelenwo farm, 24 (46.1%) from Akpajo farm, 14 (31.1%) from Eliminigwe farm, 17 (45.9%) from Eliozu farm, and 21 (70%) from Umuebule farm. Out of the 92 *Staphylococcus aureus* isolated, 46(50%) were Methicillin-Resistant *Staphylococcus aureus* (MRSA), 6 (37.5%) were from Elelenwo farm, 12 (50%) were from Akpajo farm, 6 (42.8%) were from Eliminigwe farm, 10 (58.8%) were from Eliozu farm and 12 (57.1%) were from Umuebule farm. (Table 1).

Table 1: Distribution of Isolates from different Poultry Farms.

Poultry Farms	Number of samples	Number of isolates	Number of MRSA
Elelenwo Farm	31 (15.9%)	16 (51.6%)	6 (37.5%)
Akpajo Farm	52 (26.7%)	24 (46.1%)	12 (50%)
Eliminigwe Farm	45 (23.1%)	14 (31.1%)	6 (42.8%)
Eliozu Farm	37 (18.9%)	17 (45.9%)	10 (58.8%)
Umuebule Farm	30 (15.4%)	21 (70%)	12 (57.1%)
Total	195 (100.0%)	92 (47.2%)	46 (50%)

3.2 Antimicrobial Susceptibility Pattern

The overall sensitivity pattern is shown in the Table 2. Amikacin showed the highest level of susceptibility of 82 (89.1%), followed by Ceftriaxone 80 (86.9%), while Erythromycin was the least sensitive 27 (29.3%).

Table 2: Antimicrobial Susceptibility Pattern using the 92 *Staphylococcus aureus* isolates

Antibiotics	Susceptibility n (%)	Resistance n %)
FOX (30 µg)	70 (76.1%)	22 (23.9%)
OX (1 µg)	46 (50%)	46 (50%)
E (15 µg)	27 (29.3%)	65 (70.7%)
DA (2 µg)	39 (42.4%)	53 (57.6%)
OFX (5 µg)	70 (76.1%)	22 (23.9%)
AZN (15 µg)	35 (38.0%)	57 (62.0%)
AMC (30 µg)	66 (71.7%)	26 (28.3%)
LEV (5 µg)	71 (77.2%)	21 (22.8%)
CRO (30 µg)	80 (86.9%)	12 (13.1%)
CXM (30 µg)	75 (81.5%)	17 (18.5%)
AK (30 µg)	82 (89.1%)	10 (10.9%)

FOX – Cefoxitin, OX- Oxacillin, E- Erythromycin, DA- Clindamycin, OFX- Ofloxacin, AZN- Azithromycin, AMC- Amoxycillin/Clavulanic acid, LEV- Levofloxacin, CRO- Ceftriaxone, CXM - Cefuroxime, AK - Amikacin.

3.3 Distribution of *mec*A *gene* in MRSA Isolates

Table 3 shows the distribution of *mec*A *genes* in MRSA isolates. 5 (25%) out of the 20 representative isolates were identified to have *mec*A *genes*. Isolates from Elelenwo Farm had about 3 (50%) *mec*A *genes*, isolates from Eliozu had 1(25%) and isolates from Umuebule Farms had 1 (50%) *mec*A *gene* respectively while no target was detected in isolates from Akpajo and Eliminigwe Farms.

Table 3: Distribution of *mec*A *gene* in MRSA Isolates

Poultry Farms	Number of MRSA	Selected MRSA	mecA gene
Elelenwo Farm	8 (17.4%)	6 (75%)	3 (50%)
Akpajo Farm	13 (38.3%)	5 (38.5%)	0 (0.0%)
Eliminigwe Farm	6 (13.0%)	2 (33.3%)	0 (0.0%)
Eliozu Farm	10 (21.7%)	4 (40%)	1 (25%)
Umuebule Farm	9 (19.6%)	3 (33.3%)	1 (50%)
X^2	46 (50%)	20 (43.3%)	5 (25%)

3.4 Distribution of *clonal complex 398 (CC398) gene* in MRSA Isolates

The presence of *clonal complex 398 (CC398) gene* in MRSA isolates was also determine. A total of 7 (35%) out of 20 representative isolates were identified to harbour the clonal complex 398 (CC398) gene. Isolates from Akpajo Farm had about 3 (60%), while isolates from Elelenwo and Eliozu Farms had about 2 (50%) *clonal complex 398 (CC398) gene* respectively. No target was detected in isolates from Eliminigwe and Umuebule Farms (Table 4).

Table 4: Distribution of *clonal complex 398 (CC398) gene* in MRSA Isolates

Poultry Farms	Number of MRSA	Selected MRSA	*CC398 gene*
Elelenwo Farm	8 (17.4%)	6 (75.0%)	2 (33.3%)
Akpajo Farm	13 (38.3%)	5 (38.5%)	3 (60.0%)
Eliminigwe Farm	6 (13.0%)	2 (33.3%)	0 (0.0%)
Eliozu Farm	10 (21.7%)	4 (40%)	2 (50%)
Umuebule Farm	9 (19.6%)	3 (33.3%)	0(0.0%)
X^2	46(50%)	20(43.5%)	7 (35.0%)

3.5 Distribution of *mec*A and *clonal complex 398 (CC398) genes* with their MAR Index

This section explains the distribution of *mecA* and *clonal* complex 398 (CC398) genes with their multiple antibiotics resistant (MAR) Index. The MAR Index for a single isolate is calculated by dividing the number of antibiotics which the isolate is resistant to by the number of antibiotics tested. From Table 5, the isolates from the birds had the following MAR indexes: Eliozu Farm 0.8, Eliminigwe Farm 0.75, Elelenwo farm 0.7, Akpajo Farm 0.68 and Umuebule Farm 0.45 respectively.

Table 5: Distribution of *mec*A and *clonal complex 398 (CC398) gene* with their MAR Index

Poultry Farms	*mec*A gene	*CC398 gene*	MARI
Elelenwo Farm	0.1± 3 (50%)	0.1± 2 (33.3%)	0.7
Akpajo Farm	0.1± 0 (0.0%)	0.1± 3 (60.0%)	0.68
Eliminigwe Farm	0.1± 0 (0.0%)	0.1± 0 (0.0%)	0.75
Eliozu Farm	0.1± 1 (25%)	0.1± 2 (50%)	0.8
Umuebule Farm	0.1± 1 (50%)	0.1± 0(0.0%)	0.45
X^2	0.1± 5(25%)	0.1±7 (35.0%)	0.0

3.6 Agarose Gel Electrophoresis of *mec*A *gene* of the Representative Isolates

Plate 1 shows the agarose gel electrophoresis of 10 of the representative isolates. Lane V represents the 100bp Molecular ladder as a control. Lanes 3, 4 and 6 show positive *mec*A gene bands with 310bp.

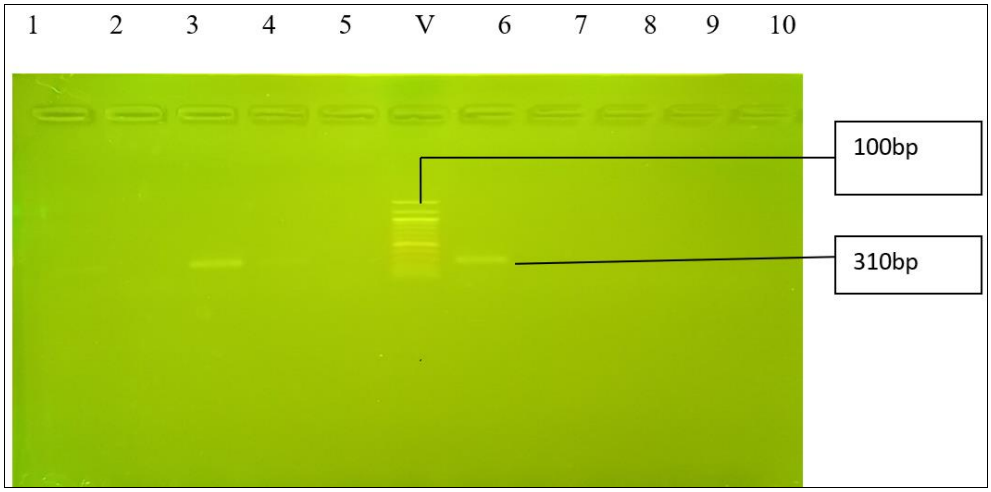

Plate 1: Agarose gel electrophoresis of *mec*A genes of representative isolates.

3.7 Agarose Gel Electrophoresis of *CC398 gene* of the Representative Isolates

Plate 2 below shows the agarose gel electrophoresis of detected *CC398* genes. Lane V represent the 500bp Molecular ladder, while lanes 2, 6, 7, 8, 11, 15 and 16 represent the *CC398 gene* bands at 200 bp.

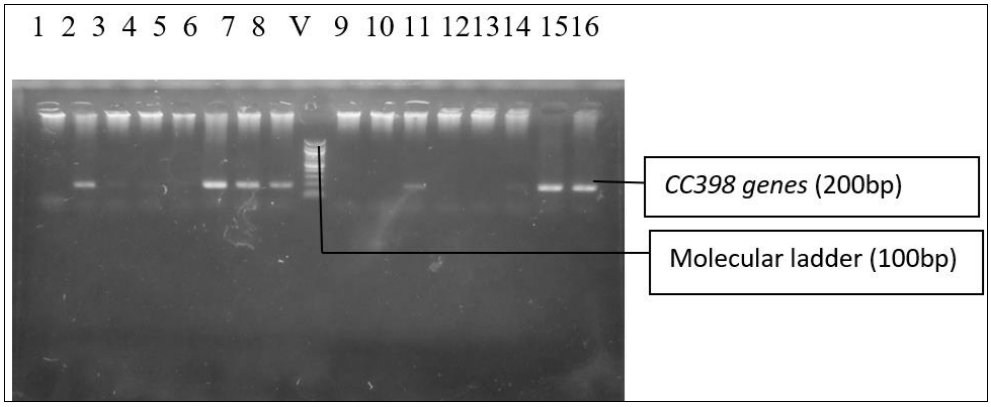

Plate 2: Agarose gel electrophoresis showing the amplified *CC398* gene bands.

4. Discussion

This Study investigated the prevalence of Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) and some genetic markers of resistance in the Greater Port Harcourt city, Nigeria. The 35% prevalence of LA-MRSA in this study shows a slight decrease in the prevalence of LA-MRSA compared to the study by Peter *et al*. [24] which showed the prevalence of 44.3%. This study also shows that the overall prevalence of MRSA in Nigeria is less than 50% which is in line with the report from Abubakar and Sulaiman [25]. Livestock-associated methicillin-resistant *Staphylococcus aureus* has been reported as an emerging problem in many parts of the world [15]. The World Health Organization (WHO) classifies Methicillin Resistant *Staphylococcus aureus* (MRSA) as a priority bacterial pathogen, which underscores appropriate infection prevention and control strategies.

The result from Table1 shows the distribution of isolates from different poultry farms. A total of 195 swab samples were cultured, 92 *Staphylococcus aureus* representing 47.2% were isolated and 50% of this were Methicillin Resistant *Staphylococcus aureus* (MRSA).

Table 2 shows the overall antimicrobial susceptibility pattern of the 92 *Staphylococcus aureus* isolates. The Macrolides – Erythromycin and Azithromycin showed the highest resistant pattern of 70.7% and 62% respectively followed by Clindamycin with 57.6% resistant pattern. The Beta-lactam antibiotics – Oxacillin, Cefoxitin and Amoxycillin/Clavulanic acid which serves as markers in this studies showed an average resistant pattern of 50%, 23.9% and 28.3% respectively. The Aminoglycosides – Amikacin demonstrated the highest susceptibility pattern of 89.1% followed by the Cephalospin - Ceftriaxone with the susceptibility pattern 86.9% and Cefuroxime with 81.5% susceptibility pattern.

Tables 3 and 4 shows the distribution of *mec*A and *clonal complex 398 (CC398)* gene in MRSA Isolates. A total of 5 (25%) and 7 (35%) isolates were identified to have the *mec*A gene and clonal complex 398 *(CC398)* gene respectively which are the major genetic markers this study aimed to target. Methicillin resistance is conferred by the expression of the *mec*A gene, which encodes PBP2a, a protein with low affinity for β-lactam antibiotics, conferring resistance to methicillin [26]. It was expected that all the representative MRSA assayed in this study should have *mec*A gene but this was not the case as only 25% were positive for *mec*A gene. Nwokah *et al.*, (2016) [27]. Reported a similar finding of 8 *mec*A-negative MRSA. This discordance could be as a result of some gene mutation that may have led to the loss of some genetic properties, and consequent non expression.

Table 5 shows representative MRSA Isolates used for

Molecular Studies and their Antibiotic Resistant Pattern with their Multiple Antibiotics Resistant (MAR) Index showing presence or absence of *mec*A genes and *CC398* genes. 20 isolates of MRSA were selected for the molecular characterization base on their resistant patterns. From the results, all the Multiple Antibiotics Resistant (MAR) Index values were greater than 0.2 suggesting that MRSA from poultry source can contribute significantly to the spread of multi-antibiotic resistant organisms due to high usage of antibiotics and this calls for more surveillance and appropriate use of antibiotics in both human and animals. This is in line with the work done by Afunwa *et al*. [28]. This could arise from the indiscriminate use of antibiotics in bird feeds in poultries. The result also showed that only 2 isolates (Each from Elelenwo and Eliozu farm) possessed both *mec*A and *CC398* genes. It was observed that the two isolates were resistant to all the antibiotics used in the study including the multiple drug resistant makers (Oxacillin and Cefoxitin) used in this work.

As stated earlier, *mec*A gene encodes for low-affinity penicillin-binding protein, which is responsible for MRSA. However, it is observed from this study that some of the isolates were negative to *mec*A gene while positive to CC398 gene. Again, this could be due to some mutations acquired by the isolates. The major reason some isolates are CC398 positive but *mec*A gene negative is still largely unknown and beyond the scope of this study. Livestock-associated and human-associated CC398 strains share some virulence factors, but there are also distinct virulence factors that appear to be important in host adaptation. Exchange of genes encoding these virulence factors between strains may expand the host range and thereby threaten public health [29].

5. Conclusion

This study revealed that Methicillin-Resistant *Staphylococcus aureus* (MRSA) associated with Livestock is present in poultries in Port Harcourt City following the identification and characterization of CC398 gene. 47.2% of *Staphylococcus aureus* and 50% of Methicillin-resistant *Staphylococcus aureus* (MRSA) were isolated. *CC398* genes prevalence was 35%. This study suggests that poultry handlers are potential carriers of LA-MRSA and this may result in community spread of this strain to susceptible individuals. Furthermore, multiple resistant strains of organisms are also reported. It is therefore recommended that: Poultry farmers and other farmers should observe proper standard hygienic measures in order to control and prevent the spread of LA-MRSA in Nigeria.

There is need of LA-MRSA surveillance in the state and the country at large to ascertain the level of antibiotic resistant and prevent further spread of the resistant genes.

6. Conflict of Interest: Not available

7. Financial Support: Not available

8. References

1. Menyhárt-Laub K. The epidemiology of *Staphylococcus aureus* nasal carriage in preschool children and university students in Hungary. Doctoral School of Pathological Sciences, Semmelweis University; c2017.
2. Bale MI. Molecular characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from blood samples of patients attending selected hospitals in Ilorin, Nigeria (Doctoral dissertation). Kwara State University (Nigeria); c2021.
3. Mbogori CK. Methicillin-resistant *Staphylococcus aureus* in selected secondary schools in Nairobi County, Kenya (Doctoral dissertation); c2016.
4. Nwokah EG, Abbey SD. Inducible-clindamycin resistance in *Staphylococcus aureus* isolates in Rivers State, Nigeria. American Journal of Clinical and Experimental Medicine. 2016;4(3):50-55.
5. Nabera CK. *Staphylococcus aureus* bacteremia: Epidemiology, pathophysiology, and management strategies. Clinical Infectious Diseases. 2009;48(4):231-237.
6. Jensen SO, Lyon BR. Genetics of antimicrobial resistance in *Staphylococcus aureus*. Future Microbiology. 2009;4(5):565-582.
7. Sani H, Baba-Moussa F, Ahoyo TA, Mousse W, Anagonou S, Gbenou JD, et ak. Antibiotic susceptibility and toxins production of *Staphylococcus aureus* isolated from clinical samples from Benin. African Journal of Microbiology Research. 2011;5(18):2797-2803.
8. Shittu AO, Okon K, Adesida S, Oyedara O, Witte W, Strommenger B, *et al*. Antibiotic resistance and molecular epidemiology of *Staphylococcus aureus* in Nigeria. BioMed Central Microbiology. 2011;11(92):1-8.
9. Rammelkamp CH. Tyrothricin therapy of experimental hemolytic streptococcal empyema. The Journal of Infectious Diseases; c1942. p. 40-46.
10. Chen LF. The changing epidemiology of methicillin-resistant *Staphylococcus aureus*: 50 years of a superbug. American Journal of Infection Control. 2012;41:448-451.
11. Skov R, Christiansen K, Dancer SJ, Daumd RS, Drydene M, Huang Y, *et al*. Update on the prevention and control of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA). International Journal of Antimicrobial Agents. 2012;39:193-200.
12. Spanu V, Spanu C, Virdis S, Cossu F, Scarano C, De Santis EP. Virulence factors and genetic variability of *Staphylococcus aureus* strains isolated from raw sheep's milk cheese. International Journal of Food Microbiology. 2012;153:53-57.
13. Hunter PA, Dawson S, French GL, Goossens H, Hawkey PM, Kuijper EJ, *et al*. Antimicrobial-resistant pathogens in animals and man: Prescribing, practices and policies. Journal of Antimicrobial Chemotherapy. 2011;66:3-17.
14. Anjum MF, Marco-Jimenez F, Duncan D, Marín C, Smith RP, Evans SJ. Livestock-associated methicillin-resistant *Staphylococcus aureus* from animals and animal products in the UK. Frontiers in Microbiology. 2019;10:2136.
15. Omoshaba EO, Ojo OE, Sofela O, Onifade OI. Prevalence and antibiotic resistance patterns of methicillin-resistant *Staphylococcus aureus* in raw milk and soft cheese (wara) sold in Abeokuta, Nigeria. Sokoto Journal of Veterinary Sciences. 2018;16(1):1-8.
16. Izeogu CV, Salau AT. Port Harcourt. Cities. 1985;2(1):14-23.
17. George-Ukpong I. Nature under siege: Portrait of environmental crisis in the Niger Delta. Author House;

2012.
18. Sharma SK, Mudgal SK, Thakur K, Gaur R. How to calculate sample size for observational and experimental nursing research studies. National Journal of Physiology, Pharmacy and Pharmacology. 2020;10(1):1-8.
19. Okareh OT, Erhahon OO. Microbiological assessment of food and hand-swab samples of school food vendors in Benin City, Nigeria. Food and Public Health. 2015;5(1):23-28.
20. Cheesbrough M. District Laboratory Practice in Tropical Countries Part II; c2000.
21. Salie F, Eagles PFK, Leng HMJ. Preliminary antimicrobial screening of four South African Asteraceae species. Journal of Ethnopharmacology. 1996;52(1):27-33.
22. Lubbers BV, Diaz-Campos D, Schwarz S. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. 5th edition. Clinical and Laboratory Standards Institute, VET01S supplement; c2020.
23. Kosikowska U, Andrzejczuk S, Grywalska E, Chwiejczak E, Winiarczyk S, Pietras-Ożga D, *et al*. Prevalence of susceptibility patterns of opportunistic bacteria in line with CLSI or EUCAST among Haemophilus parainfluenzae isolated from respiratory microbiota. Scientific Reports. 2020;10(1):1-11.
24. Peter IU, Ngwu JN, Edemekong CI, Ugwueke IV, Uzoeto HO, Joseph OV, *et al*. First report prevalence of livestock-acquired methicillin-resistant *Staphylococcus aureus* (LA-MRSA) strain in South Eastern Nigeria. IOSR Journal of Nursing Health Science. 2022;11(1):50-56.
25. Abubakar U, Sulaiman SA. Prevalence, trend and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* in Nigeria: A systematic review. Journal of Infection and Public Health. 2018;11(6):763-770.
26. Carretto E, Visiello R, Nardini P. Methicillin resistance in *Staphylococcus aureus*. In: Pet-to-Man Travelling Staphylococci. Academic Press; c2018. p. 225-235.
27. Nwokah EG, Abbey SD, Wachukwu CK. mecA gene profile of methicillin-resistant *Staphylococcus aureus* isolates from clinical sources in Port Harcourt, Nigeria. American Journal of Biomedical and Life Sciences. 2016;4(3):41-48.
28. Afunwa RA, Ezeanyinka J, Afunwa EC, Udeh AS, Oli AN, Unachukwu M. Multiple antibiotic resistant index of gram-negative bacteria from bird droppings in two commercial poultries in Enugu, Nigeria. Open Journal of Medical Microbiology. 2020;10(4):171-181.
29. Verkade E, Kluytmans J. Livestock-associated *Staphylococcus aureus* CC398: Animal reservoirs and human infections. Infection, Genetics and Evolution. 2014;21:523-530. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel: An investigation of several outbreaks. Veterinary Microbiology. 2014;141(1):96-102.

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