

Molecular characterization of methicillin resistance gene among *Staphylococcus aureus* isolated from poultry farms in Kaduna, Nigeria

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ABSTRACT: Zoonotic transfer of resistance genes has been reported as one of the major causes of increased community associated methicillin resistance *Staphylococcus aureus* (CAMRSA), which has contributed to high hospital visit, mortality and morbidity in clinic. This study evaluates the occurrence of *S. aureus* encoding *mecA* gene in poultry birds from Kaduna metropolis. Sample collection was carried out using standard epidemiological procedure. *S. aureus* isolation, identification and biochemical test were carried out using standard microbiological methods. Antibiotic susceptibility testing was carried out using agar diffusion method while molecular analysis was performed using PCR techniques. A total of 600 poultry samples from 300 layers and 300 broilers were randomly collected from 4 poultry farms for evaluation. Using Microgen biochemical kit, 24.5% of the samples collected yielded *S. aureus*. Using agar diffusion method, 37.2% (61) of the *S. aureus* were resistant to oxacillin. The resistance profile of the oxacillin resistant isolates showed that the isolates were highly resistant to tetracycline (88.5%), ciprofloxacin (80.3%), mildly resistant to cotrimoxazole (32.8%), vancomycin (31.1%) and susceptible to amoxiclav (93.4%), cefoxitin and gentamicin (97.7% respectively). High percentage of the isolates 34.4% (21) harbored the *mecA* gene that amplified at 162 base pair while none of the isolates harbor *mecA* gene with 500 bp. This study reports the presence of MDR *S. aureus* encoding *mecA* gene among *S. aureus* isolates evaluated from poultry farm in Kaduna metropolis, hence this calls for concern as poultry products serves as means to fast dissemination of livestock and community associated methicillin resistant *S. aureus* as high percentage of poultry farmers, abattoirs and meat vendors carry out their activity without veterinary nor government control.

Key words: Antibiotic resistance, *mecA* gene, poultry birds, *S. aureus*.

INTRODUCTION

In livestock, community and clinics; methicillin-resistant *Staphylococcus aureus* (MRSA) infections, are the major cause of illnesses and death, which has imposed serious economic costs on patients, hospitals, the community and management of livestock farms (Eili et al., 2007). Over time this superbug impact and effect tend to have a double fold increment in occurrence, making MRSA an endemic, and even epidemic in hospitals, long-term care facilities and communities, as a result of penicillin and newer narrow-spectrum β -lactamase misuse and

development of resistance (Strausbaugh et al., 1996; Crum et al., 2006; Eili et al., 2007). In clinics, MRSA has been highlighted as the major cause of lower respiratory tract infections, surgical site infections, nosocomial bacteremia, pneumonia and cardiovascular infections (Richards et al., 1999; Wisplinghoff et al., 2004). This same human strain of bacterium that causes significant morbidity and mortality in clinic has been detected in several species and animal-derived products, given a clue that there is the possibility of human contamination

of poultry carcasses by slaughterhouse employees (Davy et al., 2009). This livestock-associated MRSA strains may be spreading into the community and healthcare system, or the reverse might be the case (Carleton et al., 2004). Livestock such as pigs, cattle, calves, raw chicken meat or carcasses have been proved to be a major reservoir for MRSA clonal complex CC398 and multilocus sequence type 398 (ST398) (Lee, 2006; Leonard and Markey 2008; Spohr et al., 2011). MRSA has been isolated in countries such as Korea (Lee, 2006), Belgium (Davy et al., 2009), Japan (Kitai et al., 2007) and Malaysia (Neela et al., 2013). Report has shown that people with no exposure to livestock are becoming colonized and infected with this new strain of poultry-associated MRSA, as this might be possible through eating or handling of contaminated poultry meat (Jesper et al., 2016). Friese et al. (2013) had reported that the practice commonly observed in local poultry farms without proper monitoring by authorities encourage giving poultry birds low doses of antibiotics to spur their growth and compensate for overcrowding and unsanitary living conditions has led to the rising tide of new strain of MRSA identified globally. Jesper et al. (2016) also noted that food inspectors do not typically test poultry and other food products for MRSA contamination and instead are focused on *Salmonella* and other more typical food-borne pathogens. An understanding of the magnitude of the problem caused by MRSA through period surveillance and studies like this could enable accurate national estimates of incidence, development of government policies and management of such infection. Thus, the objective of this study is to evaluate the possibility of human exposure to MRSA through poultry birds in Kaduna metropolis as farmers, farm workers, veterinarians and others working directly with livestock are at risk of MRSA infections due to their potential for zoonotic transmission.

METHODOLOGY

Sample collection areas

Samples were collected from four geographical locations with the coordinate of 10° 31' 23" North and 7° 26' 25" East of Kaduna metropolis, Nigeria; Kaduna East, Kaduna South, Kaduna West and Kaduna North.

Sample size and collection

A total of 600 samples of 300 layers and 300 broilers were randomly collected from 4 poultry farms representing the 4 geographical zones of Kaduna metropolis using the method describe by Kadam and Bhalerao (2010). $n = Z_{\alpha}^2 PZ_{1-\beta} / L^2$. Where: n = Number of samples, Z_{α} = Standard normal deviation at 95% confidence limit = 1.96,

P = Prevalence = 50% (Nworie et al., 2016), $Z_{1-\beta} = 1 - 0.1584 = 0.8416$ and L = Allowable error of 5% = 0.05. Thus, $n = 1.96^2 \times 0.5 \times 0.8416 / 0.05^2 = 647$.

Four different sample sources from the poultry birds (broilers and layers) were collected: Nasal, Trachea, Droppings and Cloacae swabs of chicks were aseptically collected using a sterile cotton swabs. Samples were transported to the laboratory for bacteriological analysis in ice packs.

Isolation and purification of *Staphylococcus aureus*

Samples were inoculated into sterile nutrient broth and incubated at 37°C for 24 hours. The overnight cultures were then sub-cultured on the surface of sterile mannitol salt agar by streaking and incubating at 37°C for 24 hours. Cultural characteristics of the resulting colonies were noted. Isolates that produced colonies exhibiting characteristics deep golden yellow coloration were selected and sub-cultural onto increased salt concentrated mannitol salt agar and incubated again overnight. Isolates that grow and still maintained the deep golden yellow coloration were sub-cultured onto nutrient agar (NA) slants and incubated overnight at 37°C. After which the slants were stored in refrigerator until required (Chesbrough, 2000).

Biochemical and confirmatory tests

Catalase test

The ability of the isolates to produce an enzyme catalase was tested by the addition of 1 ml of 3% w/v hydrogen peroxide solution to 24 hour culture of the isolates. Rapid evolutions of gas bubbles indicated the breakdown of hydrogen peroxide into oxygen and water by catalase peroxidase enzyme present. This shows a positive result. $2H_2O_2 \rightarrow H_2O + O_2$

Staphylococcus agglutination test

Staphytest plus latex slide agglutination test (Oxoid Ltd England) was used for differentiation of *S. aureus* by detection of clumping factor, protein A, and certain polysaccharides found in *S. aureus* from those staphylococci that do not possess these properties.

Microgen Staph ID test

The test was carried out according to the manufacturer's instructions. A single colony from 18 to 24 hours culture was emulsified in the suspended medium supplied in the kit, and mixed thoroughly. The adhesive tape used to

seal the microwell test strip(s) was carefully peeled. Using a sterile Pasteur pipette, 3 to 4 drops (approximately 100 μ l) of the bacteria suspension was added to each well of the strip(s). After inoculation, 10th and 11th wells were overlaid with 3 to 4 drops of mineral oil. The top of the microwell test strips were sealed with the adhesive tape removed earlier and incubated at 35 to 37°C. The Microwell test strips were read after 18 to 24 hours incubation.

The adhesive tapes were removed and all positive reactions were recorded with the aid of the colour chart substrate reference table provided by the manufacturer. One drop of PYR reagent was added to well 12 and read after 10 minutes. Formation of a very deep pink/red colour indicated positive results. Nitrate reduction test was performed on well 9 after reading and recording the β -glucuronidase reaction, one drop of nitrate A reagent and one drop of nitrate B reagent was added to the well and read after 60 seconds. The development of a red colour indicated that nitrate has been reduced to nitrite. All reports were recorded. Using the software provided, the isolates were identified to species level.

Antibiotic susceptibility test

Antibiotic susceptibility testing was carried out to obtain the susceptibility pattern of the *S. aureus* isolates against 8 panels of antibiotics: Vancomycin (VA), Oxacillin (OX), Tetracycline (TE), Ciprofloxacin (CIP), Cotrimoxazole (SXT), Amoxicillin (AMC), Cefoxitin (FOX) and Gentamycin (CN). The isolates were tested against a panel of 8 antibiotics according to the Kirby-Bauer modified disc agar diffusion (DAD) technique (Cheesbrough, 2000; CLSI, 2014). Discrete colonies on NA plate were emulsified in 3 ml of normal saline and the turbidity were adjusted to 0.5 McFarland. Using sterile swab sticks, the surface of MHA in 90 mm diameter plates were inoculated with the bacteria suspension by streaking the surface of the agar in three directions, rotating the plate approximately to 60° to ensure even distribution. The inoculated plates were allowed to dry for 10 minutes after which the antibiotic discs were placed on the surface of the agar. The plates were left at room temperature for 5 minutes for pre-diffusion time before inverted and incubated aerobically at 37°C for 16 to 18 hours. The diameter of the zones of growth inhibition were measured to the nearest millimeter and isolates classified as; sensitive, intermediate or resistant based on CLSI interpretative chart zone size (Table 1) (CLSI, 2014).

Determination of Methicillin resistance using Oxacillin Disc

A direct colony suspension of each *S. aureus* isolate was prepared equivalent to 0.5 McFarland standards and plated on Mueller Hinton agar surfaces. Oxacillin (1 μ g/ml) and Cefoxitin 30 μ g disc were placed on the plate.

Plates were incubated at 35°C for 24 hours. The zones of inhibition were measured and compared to that of CLSI interpretative chart (CLSI, 2014). Inhibition zones diameter of 21 mm was considered as methicillin resistant and ≥ 22 mm as methicillin sensitive.

Molecular analysis

Molecular characterization of isolates selected on the basis of their being methicillin and vancomycin resistant phenotypically was carried out at International Institute of Tropical Africa (IITA) Ibadan, Oyo state, Nigeria. The isolates were analyzed for carriage of *mecA* gene; the genetic determinant of methicillin resistance.

DNA extraction

Genomic DNA extraction was carried out using the method described by Zymo Research Protocol (Lephoto and Gray, 2013). Three millilitres (3 ml) of an overnight Lauryl and Burette (LB) culture of each *S. aureus* isolate were centrifuged at 10,000 \times g for 1 minute and the supernatant discarded into a disinfecting Jar. The harvested cell pellet was dislodged and 200 μ l of deionized water was added and mixed thoroughly by vortexing. Exactly 400 μ l of the lysis solution was added to the mixture and mixed. The mixture was further incubated at 70°C for 15 minutes until the cells were completely lysed and appearing viscous to prevent clogging of the zymo-spin column. Exactly 400 μ l supernatant was transferred to a zymo-spin™ IV spin filter in a collection tube and centrifuged at 7000 rpm for 1 minute. About 1200 μ l of DNA binding buffer was added to the filtrate in the collection tube from the preceding step. Exactly 800 μ l of the mixture from step above was transferred to a zymo-spin IIC column in a new collection tube and centrifuge at 10000 \times g for 1 minute. The flow through step above in the collection tube was discarded and step above repeated. A measure of 200 μ l DNA prewash buffer was added to zymo-spin column in a new collection tube and centrifuge at 10000 \times g for 1 minute. About 500 μ l of DNA wash buffer was added to zymo-spin column and centrifuged at 10000 \times g for 1 minute. The zymo-spin was transferred to a clean 1.5 ml micro centrifuge tube and 100 μ l DNA elution buffer added directly to the column matrix and centrifuge at 10000 \times g for 1 minute to elude the DNA (Lephoto and Gray, 2013).

Polymerase chain reaction (PCR) and agarose gel electrophoresis for detection of *mecA* gene

PCR was performed using the thermal cycling conditions as stated by Zymo Research UK (Lephoto and Gray, 2013). A master mix was prepared in an Eppendorf Tube and the total volume was determined by the number of

Table 1. Clinical laboratory standard index for antibiotics tested.

S/N	Antibiotics	Resistance (mm)	Intermediate (mm)	Susceptibility (mm)
1	Vancomycin (5µg/ml)	-	-	≥ 15
2	Tetracycline (30µg/ml)	≤ 11	12 – 14	≥ 15
3	Oxacillin (1µg /ml)	≤ 10	11 – 12	≥ 13
4	SMX/TMP (1.25/23.75µg/ml)	≤ 10	11 – 15	≥ 16
5	Ciprofloxacin (5µg/ml)	≤ 15	16 – 20	≥ 21
6	Gentamycin (10µg/ml)	≤ 12	13 – 14	≥ 15
7	Amoxil/clavulenic (20/10µg/ml)	≤ 13	14 – 17	≥ 18
8	Cefoxitin (30µg/ml)	≤ 14	15 – 17	≥ 18

(CLSI, 2014).

Table 2. Primers for the detection of *mecA*.

S/N	Primer	Forward	Reverse	Base pair	Reference
1	<i>MecA1</i>	GGGATCATAGCGTCATTATTC	AACGATTGTGACACGATAGCC	500	Strommenger et al., 2003
2	<i>MecA2</i>	TCCAGATTACAACCTTCACCAGG	CCACTTCATATCTTGTAACG	162	

Table 3. Identification of *S. aureus*.

Farms (No of samples)	Isolates on Mannitol	Microscopy	Catalase Test	Latex Agglutination Test	Microgen S. ID	Percentage (%)
Farm 1 (136)	128	106	81	60	8	5.9
Farm 2 (154)	137	117	96	85	57	37.0
Farm 3 (156)	149	128	98	69	62	39.7
Farm 4 (154)	116	103	87	80	29	18.8
Total (600)	530	454	362	294	156	26

samples that were analysed. The total volume of the reactants per reaction was 10 µl multiplied by the number of samples to give the total volume of the master mix. The master mix was then vortexed to mix thoroughly; this was aliquated using repeater pipette into the PCR tubes numbered to correspond with the samples. The DNA template of 2.0 µl for each sample was added to the corresponding tubes. The PCR tubes were then vortexed to mix well. The temperatures and cycling times was optimized for each new DNA template target and primers (Table 2) to achieve maximum yield and specificity. PCR process was started with an initial denaturation step at 40°C for 5 minutes. This was followed by three steps cycling: denaturation at 94°C for 30 seconds, annealing at temperature of 56°C, and extension step for one minute at 72°C. The total number of cycles was 35 and then the final extension time was 7 minutes at a temperature of 72°C. Holding temperature was at 10°C for infinity (∞). After amplification of the gene, the PCR products were subjected to electrophoresis and viewed in the illumination system.

RESULTS

Isolation, identification and characterization of *S. aureus*

Using Microgen biochemical kit, 26% of the samples collected were identified as *S. aureus* (Table 3).

Distribution of *S. aureus* among broilers and layers in Kaduna metropolis

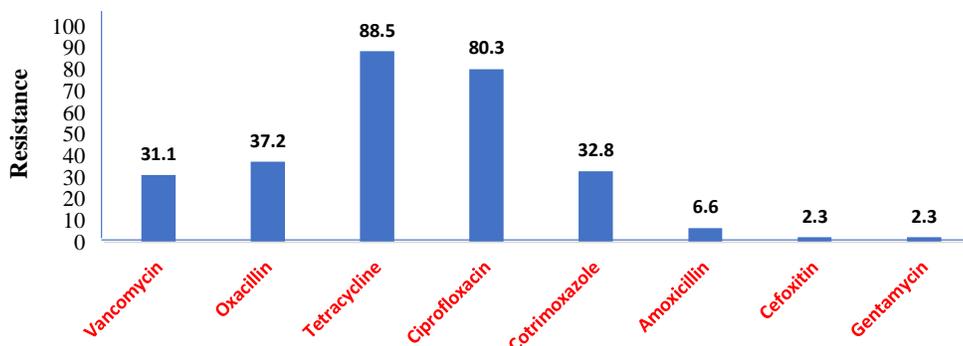
The results (Table 4) showed that *S. aureus* in Kaduna metropolis was more in Layer (49.4%) poultry birds than broilers (45.7%) poultry birds.

Antibiotics resistance profile of *S. aureus*

Figure 1 showed that significant number [37.2% (58)] of the *S. aureus* were resistant to oxacillin. The resistance

Table 4. Distribution of *S. aureus* in broiler and Layers in Kaduna metropolis.

Farms	Broiler	Layers	Total
Farm 1	3	5	8
Farm 2	21	36	57
Farm 3	28	34	62
Farm 4	23	6	29
Total (%)	75(48.1)	81 (51.9)	156 (100)

**Figure 1.** Antibiotics Resistance Profile of *S. aureus*.

profile of the oxacillin resistant isolates showed that the isolates were highly resistant to tetracycline (88.5%), ciprofloxacin (80.3%), mildly resistant to cotrimoxazole (32.8%), vancomycin (31.1%) and susceptible to amoxiclav (93.4%), cefoxitin and gentamicin (97.7%) respectively.

Evaluation of MARI among the isolates showed that 15.4% of the isolates have MARI ≤ 2 while 84.6% had MARI ≥ 3 as shown in Table 5.

Among the antibiotic resistance pattern observed, simultaneous resistance to TE, CIP and OX (32.8%) was the most occurring resistance pattern against the antibiotics tested followed by TE, CIP, SXT, OX (17.2%), TE, CIP, VA, OX (10.3%), OX and VA, OX (5.2%) respectively (Table 6).

On comparing the occurrence of MDR among the birds showed that layers had more occurrence of MDR than broilers (Figure 2).

Molecular characterization of *mecA* gene using polymerase chain reaction

High percentage of the isolates that were oxacillin resistant [34.4% (21)] harbored the *mecA* gene that amplified at 162 base pair while none of the isolates harbor *mecA* gene with 500 bp (Figure 3).

DISCUSSION

Poultry meat is considered the most commonly reported

foodborne pathogens vehicle, followed by the red meat, in which *S. aureus* related food poisoning is the third largest cause of food related illness worldwide (Hughes et al., 2007). *S. aureus* is responsible for causing a variety of animal diseases such as mastitis, arthritis and urinary tract infections and a prominent cause of food poisoning due to poor hygienic practices (Sasidharan et al., 2011). These infections are becoming untreatable due to antibiotics misuse and development of drug resistance strains, such as methicillin resistant *S. aureus* (MRSA) and vancomycin resistant *Staphylococcus aureus* (VRSA) (Saleha and Zunita, 2010). The identification of livestock-associated MRSA in food-producing animals has raised questions regarding the presence of MRSA in food of animal origin (Andrea et al., 2011). *Staphylococcus aureus* is among the most prevalent causes of clinical infections globally and has garnered substantial public attention due to increasing mortality associated with multidrug resistance (Andrew et al., 2011). Using Microgen biochemical kit, this study observed a 26% occurrence of *S. aureus* among the poultry samples collected in Kaduna metropolis (Table 3). The percentage occurrence of *S. aureus* observed in this study is in line with other findings, although higher than the reports of Kitai et al. (2005) in Japan and Lee (2006) in Korea, who sampled chicken carcasses from slaughter houses and did not find any livestock-associated *S. aureus* strains. The study conducted by Andrew et al. (2011) observed a 41% incidence of *S. aureus* in US. This indicates that the occurrence of *S. aureus* in most study depends on the geographical location and

Table 5. Multiple antibiotics resistance index of oxacillin Resistant *S. aureus* from poultry birds in Kaduna Metropolis.

MARI	Frequency	Percentage
0.1	3	5.17
0.2	6	10.3
0.3	24	41.4
0.4	20	34.5
0.5	4	6.9
0.6	0	0.0
0.7	0	0.0
0.8	1	1.7

Table 6. Antibiotic Pattern of Oxacillin and Cefoxitin Resistance *S. aureus* in Poultry Birds.

Antibiotic Pattern	Frequency (%)
TE, CIP, SXT, OX	10 (17.2)
TE, CIP, OX	19 (32.8)
TE, VA, FOX, CIP	1 (1.7)
TE, CIP, OX, AMC	1(1.7)
TE, CIP, VA, OX	6 (10.3)
TE, SXT, OX	1(1.7)
FOX, AMC, OX	1(1.7)
CN, FOX, VA, SXT, CIP, AMC, OX, TE	1(1.7)
VA, OX, CIP	1(1.7)
TE, VA, OX	1(1.7)
TE, OX	2 (3.4)
OX	3 (5.2)
VA, OX	3(5.2)
TE, CIP, VA, SXT, OX	1(1.7)
TE, CIP, VA, SXT, OX	1(1.7)
TE, CIP, OX, CN	1(1.7)
TE, CIP, SXT, AMC, OX	1(1.7)
TE, SXT, OX	1(1.7)
TE, OX	1(1.7)
TE, CIP, VA, SXT, OX	1(1.7)
VA, OX, SXT, CIP.	1(1.7)

VA, Vancomycin; OX, Oxacillin; TE, Tetracycline; CIP, Ciprofloxacin; SXT, Cotrimoxazole; AMC, Amoxicillin; FOX, Cefoxitin; CN, Gentamycin.

population study.

Reports have shown that contamination of retail meats by *Staphylococcus aureus* usually occur as a result of poor food safety practices during handling or directly from infected food-producing animals (Pu et al., 2009). On comparing the distribution of *S. aureus* among Broiler and Layers, *S. aureus* in Kaduna metropolis were more in Layer poultry birds (49.4%) than Broilers poultry birds (45.7%) (Table 4). This study concurs with the findings of Bala et al. (2016) in Kano and Nworie et al, (2016) in Abakaliki who also observed high incidence of *S. aureus* among Layer samples than Broilers.

On phenotypic evaluation for the presence of *mecA* gene, 37.2 % (58) of the *S. aureus* were resistant to oxacillin, which according to Feng-Jui et al. (2014), *mecA* promoter mutations play an important role in determining

the level of oxacillin resistance, hence, high resistance to oxacillin is an indication of MRSA. The study of Raghavendra et al. (2017) in Nepal who observed 31.82% resistance to oxacillin among MRSA *S. aureus* isolated from poultry samples is in line with this study. The resistance profile of the oxacillin resistant isolates showed that the isolates were highly resistant to tetracycline (88.5%), ciprofloxacin (80.3%), mildly resistant to cotrimoxazole (32.8%), vancomycin (31.1%) and susceptible to amoxiclav (93.4%), cefoxitin and gentamicin (97.7% respectively) (Figure 1). Similar patterns of antimicrobial susceptibility have been reported in Zaria, Nigeria by Olatu et al. (2011), Italy by Pesavento et al. (2007) and United States by Waters et al. (2011). This might be as a result of extensive usage of these antimicrobial agents in animal husbandry over time, which has contributed to the selection of drug-resistant strains (Mostafa et al., 2008). Evaluation of MARI among the isolates showed that 15.4% of the isolates have MARI ≤ 2 while 84.6% had MARI ≥ 3 (Table 5). Among the antibiotic resistance pattern observed, simultaneous resistance to TE, CIP and OX (32.8%) was the most occurring resistance pattern against the antibiotics tested followed by TE, CIP, SXT, OX (17.2%), TE, CIP, VA, OX (10.3%), OX and VA, OX (5.2%) respectively (Table 6). Suggesting that these resistant isolates originated from a high-risk source of contamination where antibiotics were often used or at a large proportion of the bacterial isolates have been pre-exposed to several antibiotics (Christopher et al., 2013). Also, the use of antibiotics as prophylaxes, growth promoters or inaccurate dosages given to sick flocks by unqualified personnel may likely have resulted in plasma concentrations that are inconsistent with the desired objectives in the poultry birds, which might have possibly influenced resistance profile of the birds (Suleiman et al., 2013). On comparing the occurrence of MDR among the birds showed that Layers had more occurrence of MDR than broilers (Figure 2). This study is in line with the finding of Nsofor and Ukachukwu (2014), who observed that all the tested strains of *E. coli* 0157 isolated from fresh droppings of old layers, new layers, main layers and cockrells in Madonna University poultry, Nigeria were found 100% resistant to tetracycline, nalidixic acid, chloramphenicol, cotrimoxazole, ceftriaxone, cefepime and cefoxitin while 10% were susceptible to ciprofloxacin. The study conducted by Bala et al. (2016) in Kano, Nigeria also correlated with the finds of this study. Classification of multidrug resistant (MDR) among the *S. aureus* isolates showed that isolates from layers exhibited high multidrug resistant profile of 81.5% followed by those from farm workers (66.7%), while 45.9% of isolate from broilers were multidrug resistant. This study concurred with the findings of Bala et al. (2016) and Hosny et al. (2016) who also observed that isolates from layers were more resistant to the antibiotics tested than those from broiler. Recently, domestic animals are described as carriers and

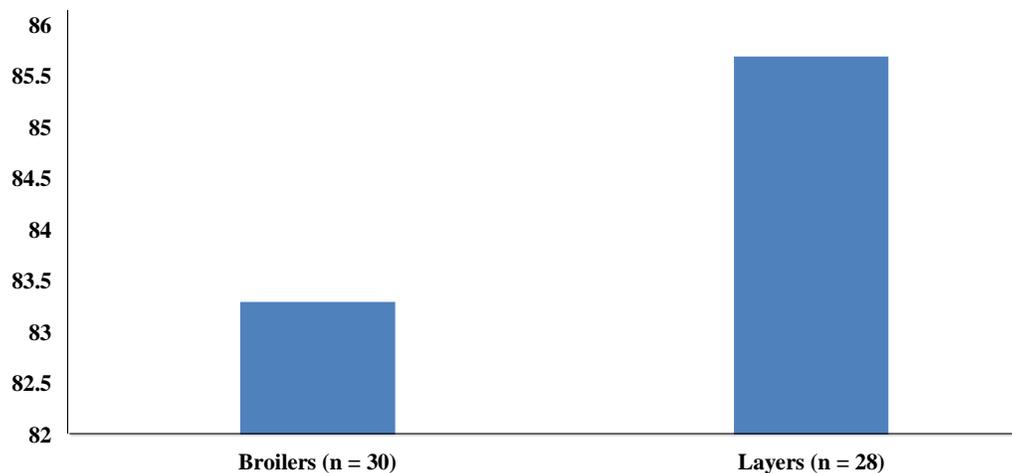
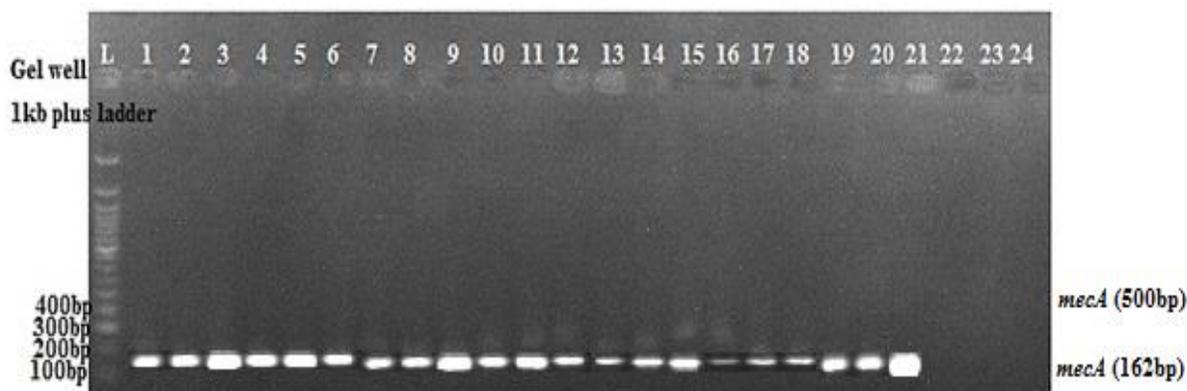


Figure 2. Percentage comparative evaluation of occurrence of multidrug resistance in broiler and layers in Kaduna, Nigeria.



Lane	Isolates	Lane	Isolates	Lane	Isolates	Lane	Isolates
1	F3BC18	7	F1LT14	13	HRCF2BN20	19	F4BC2
2	F1BN20	8	HRVF2BT3	14	F2BN5	20	F3WE6
3	F2BD14	9	HRCF4BN7	15	F2BT1	21	F3BN7
4	F3LT7	10	F3BN18	16	HRCF4BN5	22	F4LD20
5	F2LN6	11	F3BC11	17	F3BN16	23	F1LT8
6	F3LT9	12	F3BT11	18	F3BT1	24	F2BN4

Figure 3. Amplification of *mecA* Gene of 500bp and 162bp on Gel Electrophoresis. Lane L = 1kb plus DNA ladder.

possible reservoirs of MRSA (Hosny et al., 2016). This study evaluated the presence of *mecA* gene in poultry samples in Kaduna metropolis and observed that high percentage of the isolates that were oxacillin resistant [34.4% (21)] also harbored the *mecA* gene that amplified at 162 base pair while none of the isolates harbor *mecA* gene with 500 bp. This result was in accordance with the study of Saderi et al. (2014) who observed that 87 of 99 isolates that were oxacillin resistance encoded *mecA* gene. Methicillin-resistant *S. aureus* (MRSA) with this base pair has been reported to be capable of causing a

wide range of food poisoning due to the production of endotoxins, pneumonia, post-operative wound and nosocomial infections (Cuny et al., 2010). The report of Igbiosa et al. (2016) in Benin City, Edo State, who observed 52% *mecA* incidence in pork, 28% in beef and 20% in chicken samples substantiate the possibility of isolating *mecA* encoded MRSA from food sample and this correlated with the objective of this study. Report has shown that poultry associated MRSA have been linked to increased morbidity and mortality in patients and increased medical care costs as a result of zoonotic

transfer, particularly in developing countries where there are limited and generally unaffordable therapeutic options (Otalú et al., 2011).

Conclusion and recommendation

This study showed the possibility of isolating livestock associated MRSA with high antibiotics resistance profile from apparently healthy birds sampled in Kaduna metropolis. It showcases the potential health hazards associated with poultry consumption and it highlights the possibility of zoonotic transfer of this gene of clinical importance through this route. Hence, the need for high hygiene practices, control of antibiotics use in poultry management and periodic investigations of the genetic pathogenicity of microorganism in our local and global epidemiological studies is very important to prevent the possible escalation of MRSA from being endemic to pandemic in our localities.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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