

## VIRULENT GENE DETECTION AND ANTIBIOGRAMMIC PROFILE OF METHICILLIN RESISTANT *Staphylococcus aureus* ISOLATED FROM BIRDS OF A POULTRY FARM

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### ABSTRACT

Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) are gaining global attention, largely due to their potential public health significance. In the current study, we investigated the prevalence of MRSA in poultry birds from Benson Idahosa University farm. Twenty-five samples each from poultry droppings, cloacae and nostrils were collected aseptically and screened for total heterotrophic and *Staphylococcus aureus* counts using standard culture-based methods. Phenotypic identification of MRSA was carried out using mannitol-oxacillin agar, while the presence of virulence genes (*mecA*, *entA*, *entB*, *entC*, *SCCmec* (I, II, III) and *tsstI*) was investigated by polymerase chain reaction using specific primers. Also, the antibiogram activities and multi drug-resistant index were investigated against 6 clinically relevant antibiotics (oxacillin (1 µg), vancomycin (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), amikacin (30 µg) and ofloxacin (5 µg)). The results showed that total heterotrophic bacterial and *S. aureus* are prevalent in poultry birds, with mean heterotrophic counts of  $243.08 \pm 21.01 \times 10^5$  cfu,  $169.37 \pm 25.30 \times 10^5$  cfu and  $216 \pm 15.89 \times 10^5$  cfu for poultry droppings, cloacae and nostrils respectively and mean *S. aureus* counts of  $176.30 \pm 28.85 \times 10^5$  cfu for poultry droppings,  $16.52 \pm 11.92 \times 10^5$  cfu for cloacae and  $2.72 \pm 0.41 \times 10^5$  cfu for nostrils. Interestingly, 93.33%, 25% and 10.5% of *S. aureus* isolated from the droppings, cloacae and nostrils respectively were methicillin resistant. The antibiogram activities showed the majority of isolates to be multi drug-resistant, while only one MRSA (from poultry droppings) had the *mecA* gene. The presence of these isolates in poultry birds is of a potential public health concern as they may travel through the food chain and efforts should be made by poultry owners to avoid the indiscriminate use of antibiotics.

**Keywords:** Methicillin-Resistant *Staphylococcus aureus*; Poultry; Public Health; Virulence Gene

### INTRODUCTION

*Staphylococcus aureus* is a Gram-positive coccus and belongs to the family *Staphylococcaceae*. It is a normal flora of the skin and mucous membrane of humans and animals and is often referred to as an opportunistic pathogen which can cause a variety of infections in both healthy individuals and subjects with compromised immune system (Onaolapo *et al.*, 2017). It is the causative agent of diseases such as abscesses, bacteremia, endocarditis, osteomyelitis and respiratory tract infections in humans (Kaźmierczak *et al.*, 2014), as well as mastitis, infected hock, and septicemia in animals (Fluit, 2012).

*Staphylococcus aureus* is becoming of major public health concern, principally because many strains of *Staphylococcus aureus* have developed resistance to antibiotics especially methicillin. Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) have been implicated in hospital infections

(Hospital-acquired MRSA), infection in healthy subjects (Community-acquired MRSA) and livestock (Livestock-acquired MRSA) (Persoons *et al.*, 2009; Ferreira *et al.*, 2012; Nworie *et al.*, 2017). These characteristics supposedly reflect an organism well-equipped to survive in diverse environments and adjust to different environmental conditions (Nworie *et al.*, 2017). As part of the normal flora of poultry birds, *Staphylococcus aureus* can cause lethal infections in the host under conditions such as wounds and mucosal damage (Zaheer *et al.*, 2017). *Staphylococcus aureus*, as well as the methicillin-resistant strains of *Staphylococcus aureus*, have previously been reported in poultry and poultry products, particularly in the nostril and cloacae (Feßler *et al.*, 2011; Zaheer *et al.*, 2017), and could be a serious threat to public health (Nworie *et al.*, 2017; Kwoji *et al.*, 2017), thereby causing infection of different organs when it finds its way into the bloodstream (Nworie *et al.*, 2017; Onaolapo *et al.*, 2017).

Most research in Nigeria had a focus on the prevalence of MRSA in the hospital environment, thus, downplaying the possible occurrence of MRSA infection through livestock. Additionally, there are no known data on the prevalence of this isolate as well as the distribution of virulence genes in poultry farm owned by a tertiary institution in Nigeria, as the assumption would be that such farms are better managed with low chances of antibiotics abuse. The current study was therefore aimed at investigating the prevalence of virulent genes in methicillin-resistant strains of *Staphylococcus aureus* isolated from poultry birds from Benson Idahosa University farm, and to determine their antibiotic susceptibility pattern.

## MATERIALS AND METHODS

### Sample Collection

Twenty-five samples each were collected from the cloacae, droppings and nostrils of poultry birds (broilers) from Benson Idahosa University Poultry Farm located in Benin City, Edo State, Nigeria. The samples from the cloacae and nostrils were collected using sterile swab sticks, while the droppings were collected using a sterile universal container. The samples were kept in an ice pack and transported to the laboratory for immediate analysis.

### Microbiological Analysis

Ten-fold serial dilution were done for all samples and the appropriate dilution (fifth) was cultured on the media of interest before incubating at 37 °C for 24 h.

### Total Heterotrophic Bacterial Counts

The total heterotrophic bacterial counts were determined by culturing the third dilutions into already prepared nutrient agar plates (in triplicate) by the spread plate method. The agar plates were incubated at 37 °C for 24 h and the number of colonies counted, using a colony counter.

### Total *Staphylococcus aureus* Counts

The total *Staphylococcus aureus* counts were determined as previously described by Igbinsosa *et al.* (2018). Briefly, 1.0 ml each of the appropriate dilution was cultured by the spread plate technique into an already prepared mannitol salt agar plates (in triplicate). Plates were incubated at 37 °C for 24

h and the number of colonies counted using a colony counter.

### Subculturing of *Staphylococcus aureus* Isolates

Discrete colonies on mannitol salt agar with the characteristic yellowish colony, indicative of mannitol fermentation were picked with a sterile wire loop and sub-cultured on nutrient agar plates and incubated at 37 °C for 24 h. Pure isolates were kept on agar slants at 4 °C for further studies.

### Presumptive Identification of *Staphylococcus aureus*

Colonies with the characteristic yellowish colour on mannitol salt agar were further identified as *Staphylococcus aureus* by their Gram reaction, catalase test, coagulase test and citrate test as described by Fooladi *et al.* (2015). Only one anatomically distinct isolate was screened from each plate.

### Phenotypic Identification of MRSA and MSSA

Organisms presumptively identified as *Staphylococcus aureus* from the biochemical test were further screened for their resistance or sensitivity to methicillin antibiotics as described by Fooladi *et al.* (2015). Isolates were cultured onto freshly prepared mannitol-oxacillin medium (mannitol salt agar (1 L) supplemented with 0.4 g of oxacillin) and incubated at 37 °C for 24 h. The growth of *Staphylococcus aureus* on the medium (mannitol-oxacillin medium) was indicative of methicillin-resistance while inhibition of *Staphylococcus aureus* growth indicated methicillin-sensitive *Staphylococcus aureus*.

### Antibiotic Susceptibility Test

All *Staphylococcus aureus* strains isolated were tested for their resistance and sensitivity to six commonly used antibiotics; oxacillin (1 µg), vancomycin (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), amikacin (30 µg) and ofloxacin (5 µg). The antibiogram profile was carried out according to the procedure described by the Clinical and Laboratory Science Institute (2015).

### Multiple Antibiotic Resistance (MAR) Index

The multiple antibiotic resistance (MAR) index was calculated as described by Blasco *et al.* (2008),

using the formula,

$$\text{MAR index} = \frac{\text{Number of antibiotics isolate is resistant to}}{\text{Total number of antibiotic tested}}$$

### DNA Extraction

Pure DNA was extracted from all MRSA isolates using the commercial DNA extraction kit (ZR fungal/bacterial DNA MiniPrep™, Zymo Research Corporation, USA). The DNA extraction was carried out according to the manufacturer's instructions. Pure template DNA was kept at 4 °C before use.

### Detection of Virulence Genes

Polymerase chain reaction (PCR) was used to amplify target genes in the template DNA using primers (forward and reverse) specific for each gene (*mecA*, *entA*, *entB*, *entC*, *SCCmec* (I, II, III) and *tssI*). The PCR conditions were done as described

by Fooladi *et al.* (2015). Each PCR reaction contained 0.5 µl of forward primer, 0.5 µl of the reverse primer, 1.5 µl of template DNA, and 12.5 µl of One Taq Quick-load purple (New England Biolab, UK). The volumes were then made up to 25 µl using nuclease-free water (AMRESCO, USA). The PCR tubes were loaded into the PCR machine (Hangzhou Bioer Technology Co. Ltd, Polo, USA) using an initial denaturation of 94 °C for 5 min, a denaturation step of 1 min at 94 °C, an annealing step of 2 min, with varying temperature depending on the gene of interest, extension step of 1 min at 72 °C and a final extension step of 5 min at 72 °C. The denaturation, annealing and extension were done over 35 cycles; while the holding temperature for all PCR runs was 4 °C. The primer sequence, annealing temperature and amplicon size (bp) are shown in table 1.

Table 1: Primers used, their Annealing Temperature and Product Size.

| Gene Name         | Forward Sequence        | Reverse Sequence        | AT (°C) | PS (bp) |
|-------------------|-------------------------|-------------------------|---------|---------|
| <i>mec A</i>      | TGAGTTGAACCTGGTGAAGTT   | TGGTATGTGGAAAGTTAGATTGG | 57      | 857     |
| <i>ent A</i>      | TTGGAACGGTTAAAAACGAA    | GAACCTTCCCATCAAAAACA    | 50      | 121     |
| <i>ent B</i>      | TTCGATCAAACCTGACAAACG   | GCAGGTACTCTATAAGTGCC    | 55      | 478     |
| <i>ent C</i>      | GGAGGAATAACAAAACATGAAGG | AAAGGCAAGCACCGAAGTAC    | 59      | 459     |
| <i>SCCmec I</i>   | TTTAGGAGGTAATCTCCTTGATG | TTTTGCGTTTGCATCTCTACC   | 52      | 154     |
| <i>SCCmec II</i>  | CGTTGAAGATGATGAAGCG     | CGAAATCAATGGTTAATGGACC  | 53      | 398     |
| <i>SCCmec III</i> | CCATATTGTGTACGATGCG     | CCTTAGTTGTCTGTAACAGATCG | 49      | 280     |
| <i>tssI</i>       | CTGGTATAGTAGTGGGTCTG    | AGGTAGTTCTATTGGAGTAGG   | 54      | 271     |

**KEY:** AT: Annealing Temperature; PS: Product Size

Source: Fooladi *et al.* (2015)

### Gel Electrophoresis

Gel electrophoresis was used to separate DNA fragments following polymerase chain reaction, using 1.5% agarose in 100 ml of 1 × TAE buffer. The agarose was stained with 3 µl of ethidium bromide (Madison, WI, USA) and poured into the gel container to cast. Ten microliters of each PCR product mixed with 2.5 µl of loading dye (New England Biolab, UK) was lowered into each well with DNA ladder (100 bp) being added into the first well. The amplified DNA was kept in the electrophoretic tank for 30 – 45 min at 100 Amp. Following electrophoresis, gels were viewed using a UV transilluminator (Nyx Technik, USA).

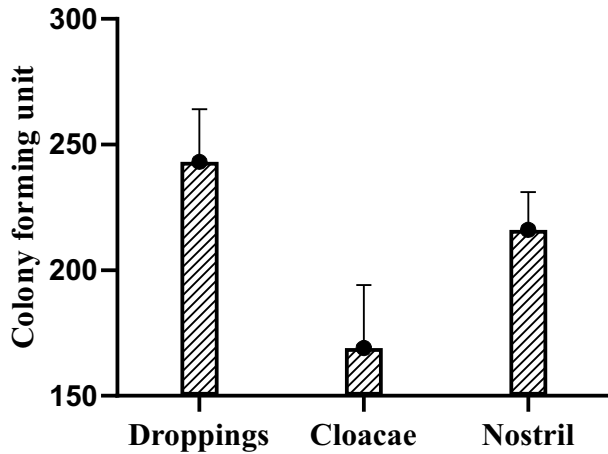
### Statistical Analysis

The mean and standard deviation of all samples were determined using the GraphPad Prism 8 software (GraphPad Software, Inc., San Diego, CA, USA).

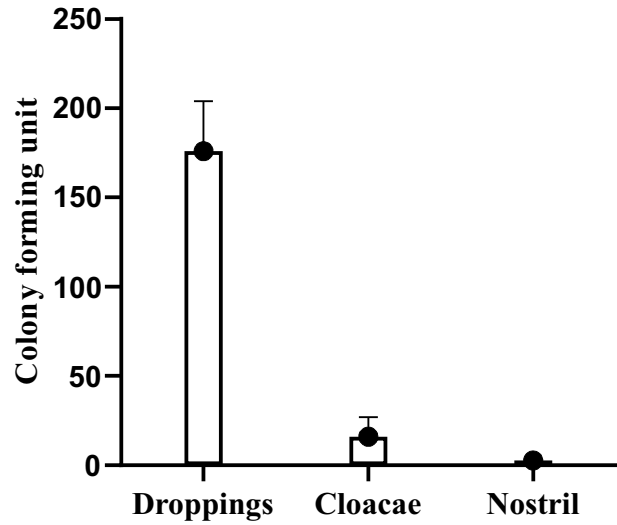
### RESULTS

The result of the current study shows that heterotrophic bacterial and *Staphylococcus aureus* are prevalent in poultry birds from Benson Idahosa University Farm. The total heterotrophic bacterial counts ranged from 27 to 300 × 10<sup>5</sup> cfu (mean = 243.08 ± 21.01 × 10<sup>5</sup> cfu) in poultry droppings; 4 to 200 × 10<sup>5</sup> cfu (mean = 169.37 ± 25.30 × 10<sup>5</sup> cfu) in the cloacae and 75 to 250 × 10<sup>5</sup> cfu (mean = 216 ± 15.89 × 10<sup>5</sup> cfu) from the nostrils of poultry birds investigated in the current study (Figure 1).

The total *Staphylococcus aureus* counts ranged from 0 to 200 × 10<sup>5</sup> cfu; 0 to 30 × 10<sup>5</sup> cfu and 0.33 to 9 × 10<sup>5</sup> cfu in poultry droppings, cloacae and nostrils respectively, with a mean of 176.30 ± 28.85 × 10<sup>5</sup> cfu for poultry droppings, 16.52 ± 11.92 × 10<sup>5</sup> cfu for cloacae and 2.72 ± 0.41 × 10<sup>5</sup> cfu for nostrils (Figure 2).



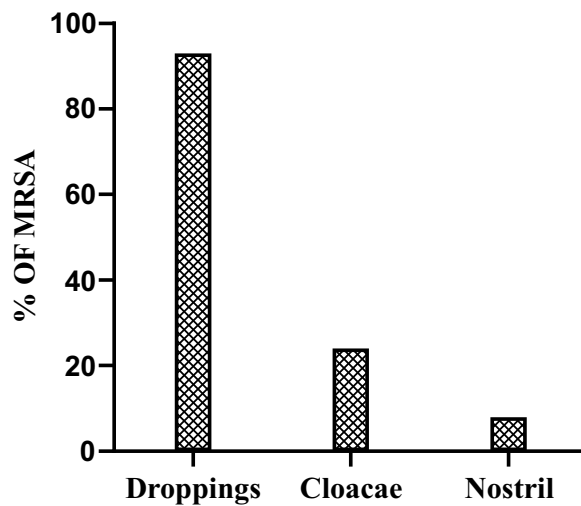
**Figure 1:** Mean Heterotrophic Bacterial Counts Obtained from Poultry Birds.



**Figure 2:** Mean *Staphylococcus aureus* Counts Obtained from Poultry Birds.

Interestingly, all *Staphylococcus aureus* isolates from poultry droppings except one were reported to be methicillin-resistant (MRSA), while 25% and 10.5% of *Staphylococcus aureus* isolated from the cloacae and nostrils respectively were reported to be methicillin-resistant (Figure 3). The antibiotic susceptibility profile shows that MRSA isolates were mostly multidrug-resistant when compared to MSSA. In the poultry droppings, 93.3% of

*Staphylococcus aureus* isolates were resistant to oxacillin, 73.3% were resistant to both ceftriaxone and ofloxacin. All the isolates were sensitive to vancomycin and amikacin. The multiple antibiotic resistant (MAR) index revealed that 14 out of the 15 *S. aureus* isolated from poultry droppings were multidrug-resistant. Interestingly, they were all MRSA (Table 2).



**Figure 3:** Distribution of MRSA in the Droppings, Cloacae and Nostril of Poultry Birds

Table 2: Antimicrobial Susceptibility Pattern of Selected *S. aureus* Isolates Present in the Droppings of Poultry Birds

| Sample Code  | OX (mm) | CAZ (mm) | VA (mm) | CRO (mm) | OFX (mm) | AMK (mm) | MAR Index | MRSA/MSSA |
|--------------|---------|----------|---------|----------|----------|----------|-----------|-----------|
| D1           | R       | R        | 13      | R        | 27       | 32       | 0.5       | MRSA      |
| D2           | R       | R        | 15      | 23       | 28       | 30       | 0.3       | MRSA      |
| D3           | R       | R        | 17      | R        | R        | 27       | 0.7       | MRSA      |
| D4           | R       | R        | 13      | R        | 32       | 22       | 0.5       | MRSA      |
| D5           | R       | R        | 11      | R        | R        | 28       | 0.7       | MRSA      |
| D6           | 14      | 11       | 13      | 24       | R        | 26       | 0.2       | MSSA      |
| D7           | R       | R        | 15      | 10       | R        | 26       | 0.5       | MRSA      |
| D8           | R       | R        | 11      | R        | R        | 30       | 0.7       | MRSA      |
| D9           | R       | R        | 20      | R        | R        | 27       | 0.7       | MRSA      |
| D10          | R       | R        | 20      | 12       | 14       | 40       | 0.3       | MRSA      |
| D11          | R       | R        | 12      | R        | R        | 24       | 0.7       | MRSA      |
| D12          | R       | R        | 12      | R        | R        | 23       | 0.7       | MRSA      |
| D13          | R       | R        | 12      | R        | R        | 40       | 0.7       | MRSA      |
| D14          | R       | R        | 13      | R        | R        | 34       | 0.7       | MRSA      |
| D15          | R       | R        | 14      | R        | R        | 24       | 0.7       | MRSA      |
| % Resistance | 93.3    | 93.3     | 0.0     | 73.3     | 73.3     | 0.0      |           |           |

**KEY:** OX = Oxacillin (1 µg); VA = Vancomycin (30 µg); CRO = Ceftriaxone (30 µg); AMK = Amikacin (30 µg); CAZ = Ceftriaxime (30 µg), OFX = Ofloxacin (5 µg).

Table 3: Antimicrobial Susceptibility Pattern of Selected *S. aureus* Isolates Present in the Cloacae of Poultry Birds

| Sample Code  | OX (mm) | VA (mm) | CRO (mm) | AMK (mm) | CAZ (mm) | OFX (mm) | MAR Index | MRSA/MSSA |
|--------------|---------|---------|----------|----------|----------|----------|-----------|-----------|
| C1           | 11      | R       | R        | R        | R        | R        | 0.8       | MSSA      |
| C2           | R       | R       | R        | R        | R        | R        | 1.0       | MRSA      |
| C3           | R       | 12      | R        | 23       | R        | R        | 0.7       | MRSA      |
| C4           | 15      | 15      | 10       | 22       | 14       | 19       | 0.0       | MSSA      |
| C5           | 12      | 14      | 14       | 20       | 20       | 19       | 0.0       | MSSA      |
| C6           | 14      | 12      | 12       | 20       | 7        | 15       | 0.0       | MSSA      |
| C7           | R       | 11      | R        | 13       | R        | 13       | 0.5       | MRSA      |
| C8           | 11      | 11      | 11       | 21       | R        | 19       | 0.2       | MSSA      |
| C9           | 11      | 12      | 10       | 22       | R        | 16       | 0.2       | MSSA      |
| C10          | 13      | 10      | 13       | 24       | 9        | 17       | 0.0       | MSSA      |
| C11          | R       | 10      | R        | 20       | R        | 18       | 0.5       | MRSA      |
| C12          | 19      | 17      | 15       | 21       | R        | 25       | 0.2       | MSSA      |
| C13          | 19      | 15      | 14       | 25       | R        | 26       | 0.2       | MSSA      |
| C14          | R       | 11      | R        | 18       | R        | 19       | 0.5       | MRSA      |
| C15          | 10      | 13      | 9        | 19       | R        | 17       | 0.2       | MSSA      |
| C16          | 10      | 14      | 14       | 24       | R        | 20       | 0.2       | MSSA      |
| C17          | 10      | 10      | R        | 18       | R        | 20       | 0.3       | MSSA      |
| C18          | 22      | 19      | 26       | 24       | 22       | 27       | 0.0       | MSSA      |
| C19          | 20      | 17      | 15       | 26       | R        | 29       | 0.2       | MSSA      |
| C20          | 14      | 12      | 14       | 21       | 10       | 16       | 0.0       | MSSA      |
| % Resistance | 40      | 10      | 35       | 10       | 70       | 15       |           |           |

**KEY:** OX = Oxacillin (1 µg); VA = Vancomycin (30 µg); CRO = Ceftriaxone (30 µg); AMK = Amikacin (30 µg); CAZ = Ceftazidime (30 µg), OFX = Ofloxacin (5 µg).

Table 4: Antimicrobial Susceptibility Pattern of Selected *S. aureus* Isolates Present in the Nostrils of Poultry Birds

| Sample Code  | OX (mm) | CRO (mm) | VA (mm) | CAZ (mm) | AMK (mm) | OFX (mm) | MAR Index | MRSA/MSSA |
|--------------|---------|----------|---------|----------|----------|----------|-----------|-----------|
| N1           | 15      | 22       | 16      | 13       | 20       | 10       | 0.0       | MSSA      |
| N2           | 20      | 14       | 10      | 10       | 25       | 11       | 0.0       | MSSA      |
| N3           | 25      | 30       | 19      | 13       | 20       | 9        | 0.0       | MSSA      |
| N4           | 20      | 22       | 15      | 19       | 25       | 11       | 0.0       | MSSA      |
| N5           | 18      | 19       | 22      | 24       | 19       | 13       | 0.0       | MSSA      |
| N6           | 22      | 10       | 14      | 17       | 23       | 10       | 0.0       | MSSA      |
| N7           | 16      | 21       | 19      | 20       | 20       | R        | 0.2       | MSSA      |
| N8           | 14      | 20       | 22      | 24       | 16       | 14       | 0.0       | MSSA      |
| N9           | 22      | 16       | 14      | 22       | 19       | 11       | 0.0       | MSSA      |
| N10          | 14      | 19       | 20      | 19       | 21       | 10       | 0.0       | MSSA      |
| N11          | 19      | 22       | 25      | 21       | 16       | 13       | 0.0       | MSSA      |
| N12          | R       | 15       | 13      | 19       | 19       | 11       | 0.2       | MRSA      |
| N13          | 22      | 10       | 15      | R        | 21       | 22       | 0.2       | MSSA      |
| N14          | 19      | 19       | 17      | 15       | 16       | 19       | 0.0       | MSSA      |
| N15          | 24      | 14       | 20      | 22       | 22       | 20       | 0.0       | MSSA      |
| N16          | 20      | 22       | 14      | 25       | 17       | 16       | 0.0       | MSSA      |
| N17          | 19      | 18       | 15      | 19       | 21       | 23       | 0.0       | MSSA      |
| N18          | R       | 15       | 25      | 15       | 17       | 20       | 0.2       | MRSA      |
| N19          | 24      | 17       | 19      | 11       | 18       | 23       | 0.0       | MSSA      |
| % Resistance | 10.5    | 0.0      | 0.0     | 5.3      | 0.0      | 5.3      |           |           |

**KEY:** OX = Oxacillin (1 µg); VA = Vancomycin (30 µg); CRO = Ceftriaxone (30 µg); AMK = Amikacin (30 µg); CAZ = Ceftriaxime (30 µg), OFX = Ofloxacin (5 µg).

Twenty anatomically distinct *S. aureus* isolates were screened from the cloacae of poultry birds and only 40% of the isolates were resistant to oxacillin (Table 3). Ceftazidime had the highest resistance (70%), while vancomycin, amikacin and ofloxacin were resistant on 10%, 10% and 15% of the isolates respectively. Only one isolate (C2) showed complete resistance to all the antibiotics used, while the MAR index reported 7 isolates from the cloacae of poultry birds, out of which, 5 were MRSA (Table 3). Six (6) isolates did not show resistance to any of the antibiotics tested. On the other hand, the majority of *S. aureus* isolates from the nostrils of poultry birds did not show any resistance to the antibiotics used. Ceftriaxone, vancomycin and amikacin completely inhibited the growth of all 19 isolates, while ceftazidime and ofloxacin were both sensitive to all isolates except 1 (Table 4). Interestingly, none of the isolates (including the 4 MRSA) was multidrug-resistant.

Polymerase chain reaction for the detection of virulence gene from all MRSA isolates showed that only one methicillin resistant *Staphylococcus aureus* isolate (from poultry droppings) harbored the *mecA* gene, while other genes (*entA*, *entB*, *entC*, *SCCmecI*, *SCCmecII*, *SCCmecIII* and *tsst1*) were absent.

## DISCUSSION

Methicillin-resistant strains of *Staphylococcus aureus* have been reported as an emerging pathogen in animals globally (Oguttu *et al.* 2014). However, there is paucity of data on the prevalence of this organism in poultry birds from poultry farms in southern Nigeria. Hence, the current study was aimed at investigating the prevalence of virulent gene in methicillin resistant *Staphylococcus aureus* (MRSA) in poultry droppings, cloacae and nostrils from Benson Idahosa University farm, located in Benin City, Edo State, Nigeria and to determine their antibiotic susceptibility pattern.

*Staphylococcus aureus* had previously been reported to be prevalent in poultry birds (broilers and layers) globally (Persoons *et al.*, 2009; Olatu Jr. *et al.*, 2015; Onaolapo *et al.*, 2017; Bakheet *et al.* 2018; Bounar-Kechih *et al.*, 2018). However, there are divergent views on which birds are more colonized by *S. aureus*. While Persoons *et al.* (2009) reported this organism to be more prevalent in

broiler birds from Belgium; Bounar-Kechih *et al.* (2018) reported layer birds to be more colonized by *S. aureus* (42%), when compared with broiler birds (12%) from Algeria. Similarly, Olatu Jr. *et al.* (2015) reported a comparable outcome from North Central Nigeria, with broiler birds harbouring 31% *S. aureus*, against 15% in layer birds. In the current study, only broiler birds were investigated, not because of this bias, but because only broilers were reared in the Benson Idahosa University farm.

The result of this study shows that heterotrophic bacteria and *Staphylococcus aureus* are prevalent in both the droppings, cloacae and nostrils of poultry birds from Benson Idahosa University farm. As expected, the population of *Staphylococcus aureus* and heterotrophic bacterial counts in the poultry droppings were significantly higher than those of the cloacae and nostrils. In a similar study, Bakheet *et al.* (2018) reported *S. aureus* and other *Staphylococcus* species from the nostril and cloacae of 50 healthy broiler birds in Egypt, while Onaolapo *et al.* (2017) also isolated *S. aureus* from the nostril, trachea and droppings of both broiler and layer birds from different farms in Kaduna State, North Central Nigeria. This organism is also increasingly more prevalent in poultry carcass (Capita *et al.* 2002; Bakheet *et al.* 2018), indicating the potential challenge of *S. aureus* to move through the food chain. This view was previously expressed by Oguttu *et al.* (2014) when they reported that the food value chain of ready-to-eat chicken was associated with staphylococcal food poisoning.

Although Staphylococci are considered to be a normal flora of chicken, it can be associated with many clinical syndromes including dermatitis, osteomyelitis, arthritis, synovitis, tenosynovitis, omphalitis, femoral head necrosis and “bumblefoot” (McNamee and Smyth, 2000; Olsen *et al.*, 2006; Moon *et al.*, 2007; Abd El-Tawab *et al.*, 2017). Even more worrisome, is the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in livestock, which was also reported in large numbers in the current study (especially poultry droppings). This finding may indicate that MRSA is present in small/medium scale broiler farms because of the high use of antimicrobial drugs in these animals without



prescription. Use of certain antimicrobial drugs in human hospitals had previously been reported to be a risk factor for acquiring MRSA infection, especially when the chosen treatment is inappropriate or insufficient (Dewaele *et al.* 2008). MRSA is also reported to persist on a poultry farm and colonize future flocks (Persoons *et al.* 2009), thereby posing a serious threat to public health.

The report on the prevalence of MRSA in poultry birds obtained in the current study is in keeping with that of previous studies (Persoons *et al.* 2009; Oke and Oke, 2013; Kwoji *et al.* 2017; Bounar-Kechih *et al.* 2018), and especially in poultry droppings (Bala *et al.* 2016), but contrary to the study of Neela *et al.* (2013) who reported no MRSA among chicken flocks and the poultry farmers in Malaysia. The challenge, however, with the report of Neela *et al.* (2013) could be the small sample size (30) as well as the sampling source (only nostrils). Judging from our study, it is clear that MRSA is sparsely distributed in the nostrils of poultry birds. In other studies, MRSA was reported to be prevalent in farmworkers /personnel (Oke and Oke, 2013; Kwoji *et al.* 2017) as well as in poultry meat and poultry products (Karmi, 2013), indicating that this variant can spread from farm to fork, thus, making the treatment of MRSA infections in poultry seemingly difficult. (Onaolapo *et al.*, 2017)

MRSA is well known for their resistance to multiple antibiotics (Bounar-Kechih *et al.* 2018). This was also the case with the current study. Of the MRSA isolated, only one carried the *mecA* gene for both cloacae samples and droppings while *mecA* gene was absent in the isolate from nostrils. Additionally, no pathogenic or toxigenic genes were reported in both the MRSA and MSSA strains. This is in keeping with the results obtained in Sudan by Elhassan *et al.* (2015) which reported that not all test isolates resistant to methicillin carried the *mecA* gene, an indication that the studied area is not as problematic as presumed. This result is also similar to that reported by Otalú Jr. *et al.* (2015) where out of 1400 samples tested, only one isolate possessed the *mecA* gene. In another study carried out by Bakheet *et al.*, (2018) in Egypt, only 64.5% of MRSA isolates possessed the *mecA* gene, which was somewhat at odds with the results gotten

from the antibiotic susceptibility testing where there was 100% resistance to oxacillin.

## CONCLUSION

Methicillin-resistant strains of *Staphylococcus aureus* are prevalent in the droppings, cloacae and nostrils of poultry birds from the Benson Idahosa University farm. These organisms showed varying degree of resistance to the 6 commonly used antibiotics that were tested against the strains, with the majority of MRSA being multi-drug-resistant. However, only one of the MRSA isolate had the *mecA* gene, while other pathogenic and toxigenic genes were absent in all isolates. Efforts must, therefore, be made by poultry owners to sidestep the indiscriminate use of antibiotics.

## CONFLICT OF INTEREST

The authors declare that there are no potential conflicts of interest.

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