# ANTIBIOTIC SUSCEPTIBILITY PROFILE OF METHICILLIN RESISTANT *Staphylococci aureus* IN POULTRY FARM, IN ZARIA, NIGERIA.

**BY**

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# PRESENTATION OUTLINE

- Introduction
- Methodology
- Results and Discussion
- Conclusion
- Recommendations
- References

# INTRODUCTION

- Methicillin resistant *Staph. aureus* (MRSA) is a notorious pathogenic microorganism even in poultry.

- This strain of *Staph. aureus* do not just produce β-lactamases but also posses mobile genetic element known as staphylococcal cassette chromosome *mec* (SCC*mec*) (Garcia-Alverez *et al.*, 2011).

- That are predominantly present in coagulase-negative staphylococci (CNS), which carries *mecA* gene and encodes for an altered penicillin-binding protein (PBP2a or PBP2') (Cohn and Middleton, 2010).

- The PBP2a according to Sarah and Robert, (2010) has a lower affinity for β-lactam antimicrobials than the normal PBP such that these antimicrobials are deactivated.

- MRSA also contains additional insertional DNA sequences that allow for incorporation of additional antimicrobial resistance markers (George, 2009), which enables it to develop resistance to non-β-lactam.

- *Staph. aureus* with this characteristics could also produce virulent toxins and acquire antibiotics resistant genes to create a huge economic burden (Huber *et al.*, 2010), influence livestock management, treatment of diseases and reduce productivity.

# RESEARCH AIM

- This study evaluate the impact of Live stock Associated Methicillin Resistant *Staph. aureus* on the commonly prescribed antibiotics in Zaria, Nigeria in other to curb resistance spread through the provision of information for surveillance purpose.

# JUSTIFICATION OF RESEARCH PROBLEM

- Phenotypic and genotypic indistinguishable *MecA* gene found in dairy, pig, cat, poultry, cattle and even in poultry farm workers suggests a cross-species transmission and Community acquisition of *MecA* gene of livestock-associated MRSA (LA-MRSA) (Juhasz-Kaszanyitzky, 2007 and Hasman *et al*., 2010).

- This is possible either by contact or indirectly via the food chain; water, air, manure and sludge-fertilized soils (Cohn and Middleton, 2010; Huber *et al*., 2010), which could be endemic in rural area with low medical facilities in zoonotic disease outbreak (Vanderhaeghen *et al*., 2010).

# METHODOLOGY

- **Sample Collection**
- Fifty (50) samples of fresh chicken droplets were collected aseptically into a clean sterile universal bottle from five poultry farms (Hanwa new extension, Kongo, Zangon, A.B.U staff quarters Samaru, Dakace quarters) located in Zaria metropolis and were transported on an ice park to the laboratory for bacteriological examination.

# *Staph.* Species Identification, Isolation and Microscopy

- Collected chicken droplets were suspended in sterile normal saline for 24hrs and then inoculated on the surface of sterile nutrient agar (NA), and incubated at $37^{O}C$ for 18hrs. Gram staining and microscopy was also carried out to identify Gram positive organisms while further morphological characterization of the colonies isolated from concentrated Mannitol salt agar organism was carried out using the method described by Cheesbrough (2000).

# Biochemical Test and β-Lactamase Production Test.

- The following conventional biochemical tests; catalase, coagulase and deoxyribonuclease (DNase) tests as described by Cheesbrough (2000) were also adopted to distinguish *Staph*. *aureus* from other forms of *Staph*. spp. Test tube method according to Lennette *et al*., (1990) and Plate-acidimetric method according to Cheesbrough (2000) were also used to determine the ability of the identified *Staph. aureus* to produce β-lactamase

# Antibiotic Susceptibility Test and Multiple Antibiotic Resistance Index (MARI) Evaluation

- The susceptibility profiles of the identified *Staph. aureus* was tested against eight selected antibiotics (ampicillin, ciprofloxacillin, methicillin, tetracycline, Vancomycin, gentamicin, pefloxacin and oxacillin) using disc diffusion method as described by Cheesbrough (2000) and the corresponding results interpreted using CLSI (2014). The multiple antibiotic resistant (MAR) index was determined for each isolate. This is defined as the number of antibiotics to which the organism is resistant to, divided by the total number of antibiotics tested (Paul *et al*., 1997).

# Minimum Inhibitory Concentration (MIC) to Oxacillin

- Resistance to methicillin was confirmed by the determination of the MIC of Oxacillin to the isolates. A working stock solution of 128µg/ml was prepared. This working solution (2ml) was then serially diluted in nutrient broth (2ml) up to the last tube. Eighteen hours cultures of the isolates were standardized to contain about $10^6$cfu/ml inoculum size. The diluted antibiotic was aseptically inoculated with 1-2 drops of the standardized inoculum. The test tubes were inoculated at 35$^o$C for 18hrs and this was repeated for all the resistant isolates

## Determination of Vancomycin Resistance

- Isolates that were resistant to oxacillin from the minimum inhibitory concentration results were picked for this test.

- Fresh stock solution of 4µg/ml and 6µg/ml of Vancomycin were prepared.

- Five millilitre (5ml) of the stock solution (4µg/ml) were aseptically mixed with sterilized mannitol salt agar and distribute into petri-dish and allowed to solidify.

- The dried agar surface was inoculated with the standard inoculum of the test isolates by streaking and incubated at $37^{O}C$ for 24-48hrs.

- This was repeated for all the isolates. Brain heart infusion agar (BHI) was mixed with 6 µg/ml of Vancomycin and distributed into petri-dishes and allowed to solidify.

- Overnight culture of the test isolates were standardized to an inoculum size of $10^{6}$cfu/ml.

- The plates were allowed to dry at room temperature and then incubated at $37^{O}C$ for 24-48hrs. This was repeated for all the resistant isolates

# RESULT AND DISCUSSION

- **Sample collection and Identification of *Staph. aureus* Isolates**

- Out of the 250 chicken droplets collected,157 isolates showed the Gram positive characteristics of *Staph.* spp. while 98 of the isolates fermented mannitol to acid and produced golden yellow colouration within 24hrs of incubation.

# Table 1: Biochemical Characterization and β-Lactamase Production in isolated *Staph. aureus*

The result showed the biochemical characteristics of the identified *Staph. aureus* from different farm sources.

| S/N | Sample Source (N = 5 Farms) | Catalase | | Coagulase | | DNase | | β-lactamase Production | |
|---|---|---|---|---|---|---|---|---|---|
| | | +ve | -ve | +ve | -ve | +ve | -ve | +ve | -ve |
| 1 | Hanwa New Extension (n=15) | 15 | 0 | 11 | 4 | 13 | 2 | 11 | 4 |
| 2 | Kongo Quarters (n=21) | 21 | 0 | 20 | 1 | 20 | 1 | 20 | 1 |
| 3 | Dakace Quarters (n=22) | 22 | 0 | 21 | 1 | 19 | 3 | 21 | 1 |
| 4 | Zangon Shanu Behind Aviation (n=25) | 25 | 0 | 23 | 2 | 23 | 2 | 23 | 2 |
| 5 | ABU Staff Quarters, Samaru (n=15) | 15 | 0 | 13 | 2 | 13 | 2 | 13 | 2 |
| | **Total *Staph. aureus* (n = 98)** | **98** | **0** | **88** | **10** | **88** | **10** | **88** | **10** |

N= number of farms, n = number of *Staph. aureus* from various farms in Zaria, metropolis

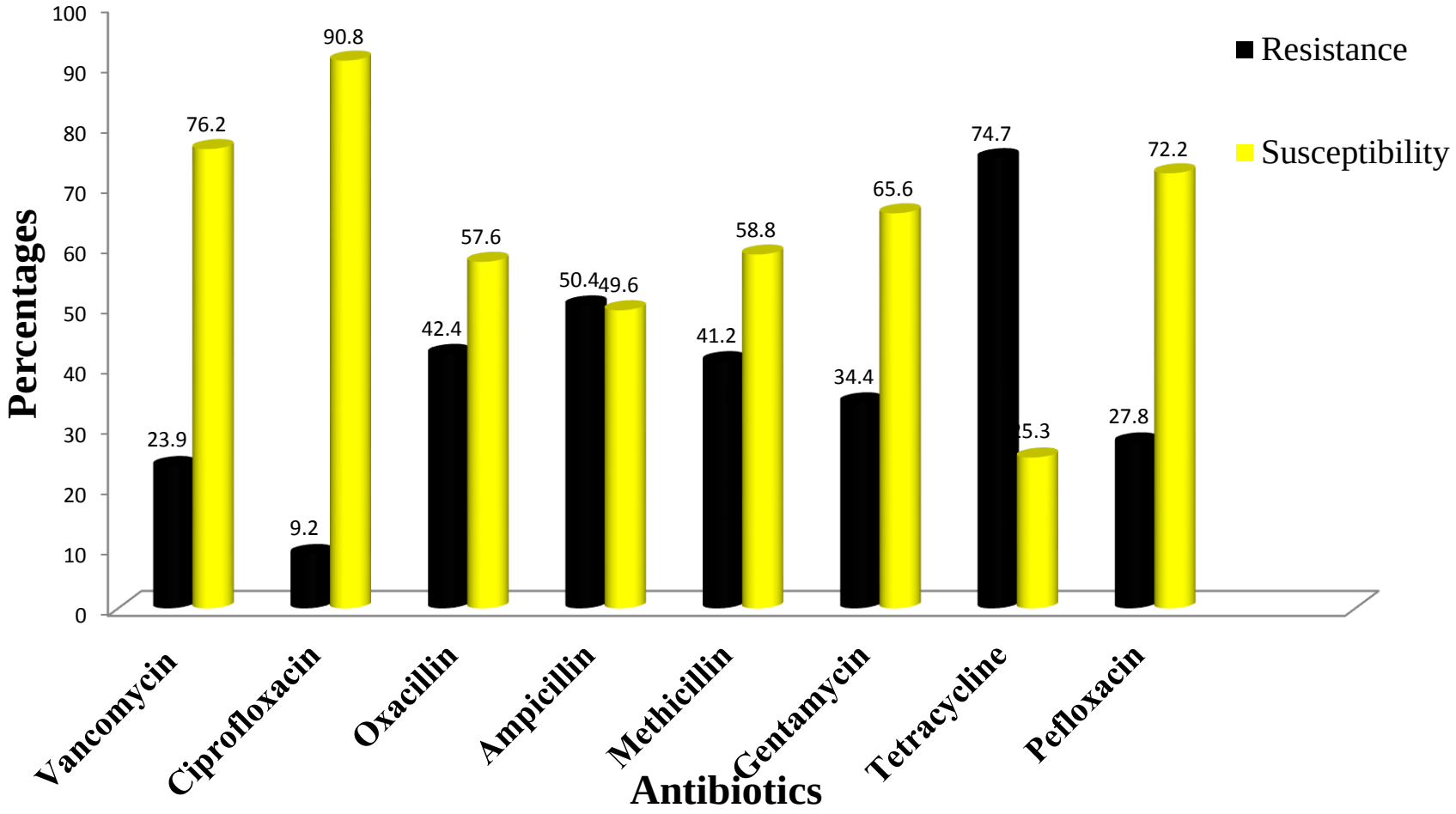


**Figure 1: Antibiotic Susceptibility Profile of *Staph. aureus* from Poultry Farms in Zaria, Nigeria**

# Antibiotic Resistance Pattern

- This study showed that the pattern of antibiotic resistance of *Staph. aureus* from poultry farms in Zaria, Nigeria varies from one isolate to another.

- Most of the isolates were resistant to β-lactam and tetracyclines.

- The isolates were also found to be 44.3% (39) multidrug resistant, 40.9% XDR while 14.8% were neither MDR nor XDR.

- The multiple antibiotic resistance index (MARI) at ≥0.4 was observed to be high (60%), indicating an environment with pre-exposure to the antibiotics used in this study.

- From all the farms evaluated 40% (35) of the *Staph. aureus* were observed to be resistant to methicillin antibiotics. This is shown in Table 2.

**Table 2: Antibiotic Resistant Pattern and MARI of *Staph. aureus* from Poultry Farms in Zaria, Nigeria**

| S/N | Lab Code | Antibiotic Resistant Pattern | NAR | GAR | MDR | MARI |
|---|---|---|---|---|---|---|
| **Farm 1 (Hanwa New Extension)** | | | | | | |
| 1 | H7 | Amp, **Met**, Ox, Pef, Tcn, Van | 6 | Bt, Flu, Tet, Gp | MDR | 0.8 |
| 2 | H9 | Amp, CN, **Met**, Ox, Tcn, Van | 6 | Bt, Ami, Tet, Gp | MDR | 0.8 |
| 3 | H10 | Amp, CN | 2 | Bt, Ami | XDR | 0.3 |
| 4 | H18 | Amp, Tcn | 2 | Bt, Tet | XDR | 0.3 |
| 5 | H19 | **Met**, Ox, Tcn, Van | 4 | Bt, Tet, Gp | MDR | 0.5 |
| 6 | H25 | CN, **Met**, Tcn | 3 | Bt, Ami, Tet | MDR | 0.4 |
| 7 | H32 | Cip, **Met**, Pef, Tcn | 4 | Bt, Flu, Tet | MDR | 0.5 |
| 8 | H40 | Amp, Ox, Tcn | 3 | Bt, Tet | XDR | 0.4 |
| 9 | H45 | Amp, Tcn | 2 | Bt, Tet | XDR | 0.3 |
| 10 | H49 | CN, **Met**, Tcn | 3 | Bt, Ami, Tet | MDR | 0.4 |
| 11 | H50 | Ox, Tcn | 2 | Bt, Tet | XDR | 0.3 |

**Farm 2 (Kongo Quarters)**

| | | | | | | |
|---|---|---|---|---|---|---|
| 12 | K53 | Amp, CN, **Met**, Ox, Pef, Tcn. | 6 | Bt, Ami, Flu, Tet | MDR | 0.8 |
| 13 | K55 | Amp, Pef. | 2 | Bt, Flu | XDR | 0.3 |
| 14 | K58 | Amp, Ox, Tcn | 3 | Bt, Tet | XDR | 0.4 |
| 15 | K59 | Amp, **Met**, Ox | 3 | Bt | Nil | 0.4 |
| 16 | K60 | Amp, **Met**, Ox | 3 | Bt | Nil | 0.4 |
| 17 | K61 | CN, Ox, Tcn, Van | 4 | Bt, Ami, Tet, Gp | MDR | 0.5 |
| 18 | K62 | **Met**, Ox, Tcn, Van | 4 | Bt, Tet, Gp | MDR | 0.5 |
| 19 | K63 | Cip, Pef. | 2 | Flu | Nil | 0.3 |
| 20 | K64 | **Met** | 1 | Bt | Nil | 0.1 |
| 21 | K68 | **Met**, Pef, Tcn | 3 | Bt, Flu, Tet | MDR | 0.4 |
| 22 | K70 | Cip, CN, Tcn, Van | 4 | Flu, Ami, Tet, Gp | MDR | 0.5 |
| 23 | K71 | Amp, CN, **Met**, Pef, Tcn | 5 | Bt, Ami, Flu, Tet | MDR | 0.6 |
| 24 | K72 | CN | 1 | Ami | Nil | 0.1 |
| 25 | K75 | Amp, **Met**, Pef, Tcn | 4 | Bt, Flu, Tet | MDR | 0.5 |
| 26 | K77 | Tcn | 1 | Tet | Nil | 0.1 |
| 27 | K78 | CN, Tcn, Van | 3 | Ami, Tet, Gp | MDR | 0.4 |
| 28 | K79 | CN, Tcn, Van | 3 | Ami, Tet, Gp | MDR | 0.4 |
| 29 | K82 | Amp, **Met**, Ox, Tcn, Van | 5 | Bt, Tet, Gp | MDR | 0.6 |
| 30 | K84 | Amp, Tcn | 2 | Bt, Tet | XDR | 0.3 |
| 31 | K97 | Amp, Tcn | 2 | Bt, Tet | XDR | 0.3 |

**Farm 3 (D = Dakace Quarters)**

| | | | | | | |
|---|---|---|---|---|---|---|
| 32 | D105 | **Met**, Ox, Pef, Tcn, Van | 5 | Bt, Flu, Tet, Gp | MDR | 0.6 |
| 33 | D108 | Ox, Tcn | 2 | Bt, Tet | XDR | 0.3 |
| 34 | D109 | CN, **Met**, Ox, Tcn, Van | 5 | Bt, Ami, Tet, Gp | MDR | 0.6 |
| 35 | D115 | **Met**, Ox, Tcn, Van | 4 | Bt, Tet, Gp | MDR | 0.5 |
| 36 | D117 | Cip, **Met**, Tcn, Van | 4 | Bt, Flu, Tet, Gp | MDR | 0.5 |
| 37 | D119 | Amp, Cip, **Met**, Pef, Tcn | 5 | Bt, Flu, Tet | MDR | 0.6 |
| 38 | D124 | Amp, Ox, Tcn, Van | 4 | Bt, Tet, Gp | MDR | 0.5 |
| 39 | D127 | Amp | 1 | Bt | Nil | 0.1 |
| 40 | D129 | CN, Pef, Tcn | 3 | Ami, Flu, Tet | MDR | 0.4 |
| 41 | D130 | CN, | 1 | Ami | Nil | 0.1 |
| 42 | D131 | Amp, CN | 2 | Bt, Ami | XDR | 0.3 |
| 43 | D132 | CN, Pef, | 2 | Ami, Flu | XDR | 0.3 |
| 44 | D133 | **Met**, Ox, Tcn | 3 | Bt, Tet | XDR | 0.4 |
| 45 | D134 | Amp | 1 | Bt | Nil | 0.1 |
| 46 | D136 | Amp, CN, **Met**, Van | 4 | Bt, Ami, Gp | MDR | 0.5 |
| 47 | D139 | Amp, CN, Pef, | 3 | Bt, Ami, Flu | MDR | 0.4 |
| 48 | D141 | Amp, Pef, | 2 | Bt, Flu | XDR | 0.3 |
| 49 | D143 | Amp, Tcn | 2 | Bt, Tet | XDR | 0.3 |
| 50 | D144 | Amp, CN, Ox, Pef, Tcn, | 5 | Bt, Ami, Flu, Tet | MDR | 0.6 |
| 51 | D149 | Amp, Cip, CN, Ox, Tcn, Van | 6 | Bt, Flu, Ami, Tet, Gp | MDR | 0.8 |

**Farm 4 (Z = Zangon Shanu Behind Aviation)**

| | | | | | | |
|---|---|---|---|---|---|---|
| 52 | Z151 | Amp, **Met**, Ox, Tcn, Van | 5 | Bt, Tet, Gp | MDR | 0.6 |
| 53 | Z152 | Tcn, Van | 2 | Tet, Gp | XDR | 0.3 |
| 54 | Z153 | Amp, Ox, Tcn, | 3 | Bt, Tet | XDR | 0.4 |
| 55 | Z158 | Amp, **Met**, Pef, | 3 | Bt, Flu | XDR | 0.4 |
| 56 | Z161 | Amp, **Met**, Pef, Tcn | 4 | Bt, Flu, Tet | MDR | 0.5 |
| 57 | Z162 | Amp, Tcn, | 2 | Bt, Tet | XDR | 0.3 |
| 58 | Z163 | **Met**, Ox, Tcn | 3 | Bt, Tet | XDR | 0.4 |
| 59 | Z164 | Amp, CN, **Met**, Tcn | 4 | Bt, Ami, Tet | MDR | 0.5 |
| 60 | Z165 | Amp, **Met**, Ox, Tcn | 4 | Bt, Tet | XDR | 0.5 |
| 61 | Z169 | Pef, Tcn | 2 | Flu, Tet | XDR | 0.3 |
| 62 | Z170 | Cip, Pef, Tcn | 3 | Flu, Tet | XDR | 0.4 |
| 63 | Z173 | Amp, CN, Ox, Pef, Tcn, Van | 6 | Bt, Ami, Flu, Tet, Gp | MDR | 0.8 |
| 64 | Z178 | CN, Ox, Tcn, Van | 4 | Bt, Ami, Tet, Gp | MDR | 0.5 |
| 65 | Z180 | Amp, Ox, Tcn | 3 | Bt, Tet | XDR | 0.4 |
| 66 | Z182 | CN, Ox, Tcn, | 3 | Bt, Ami, Tet | MDR | 0.4 |
| 67 | Z185 | CN, Pef | 2 | Ami, Flu | XDR | 0.3 |
| 68 | Z187 | Amp | 1 | Bt | Nil | 0.1 |
| 69 | Z188 | Cip, **Met**, Tcn | 3 | Bt, Flu, Tet | MDR | 0.4 |
| 70 | Z191 | CN, Ox | 2 | Bt, Ami | XDR | 0.3 |
| 71 | Z192 | Cip, Tcn | 2 | Flu, Tet | XDR | 0.3 |
| 72 | Z193 | **Met**, Tcn | 2 | Bt, Tet | XDR | 0.3 |
| 73 | Z196 | Amp, Tcn | 2 | Bt, Tet | XDR | 0.3 |
| 74 | Z198 | Amp, Tcn | 2 | Bt, Tet | XDR | 0.3 |

**Farm 5 (A = ABU Staff Quarters, Samaru)**

| | | | | | | |
|---|---|---|---|---|---|---|
| 76 | A201 | Amp, CN, **Met**, Ox, Tcn | 5 | Bt, Ami, Tet | MDR | 0.6 |
| 77 | A202 | Amp, **Met**, Ox, Pef, Tcn | 5 | Bt, Flu, Tet | MDR | 0.6 |
| 78 | A205 | Amp, Ox, Tcn | 3 | Bt, Tet | XDR | 0.4 |
| 79 | A209 | Amp, Pef, | 2 | Bt, Flu | XDR | 0.3 |
| 80 | A211 | Tcn | 1 | Tet | Nil | 0.1 |
| 81 | A215 | **Met**, Ox, Tcn | 3 | Bt, Tet | XDR | 0.4 |
| 82 | A220 | CN, **Met**, Ox, Tcn, Van | 5 | Bt, Ami, Tet, Gp | MDR | 0.6 |
| 83 | A222 | CN, Tcn | 2 | Ami, Tet | XDRz | 0.3 |
| 84 | A230 | Amp, CN, Pef | 3 | Bt, Ami, Flu | MDR | 0.4 |
| 85 | A234 | Amp, **Met** | 2 | Bt | Nil | 0.3 |
| 86 | A235 | Ox, Pef, Tcn | 3 | Bt, Flu, Tet | MDR | 0.4 |
| 87 | A240 | Amp, Tcn | 2 | Bt, Tet | XDR | 0.3 |
| 88 | A245 | Amp, Ox, | 2 | Bt | Nil | 0.3 |

Keys: Amp = ampicillin, Cip = Ciprofloxacillin, Met = Methicillin, Tcn = tetracycline, Van = Vancomycin, CN = gentamicin, Pef = pefloxacin and Ox = oxacillin, Bt = β-lactams, Gp = Glycopeptides, Ami = Aminoglycoside, Tet = Teteracycline, Flu = Fluoroquinolone, NAR = Number of antibiotics resistant to, GAR = Groups of antibiotics resistant to, MDR = Multidrug resistant, MARI = Multiple antibiotics resistant index. MDR: Multidrug-resistant, XDR: Extensively drug-resistant NIL: neither MDR nor XDR. MDR: non-susceptible to ≥1 agent in ≥3 antimicrobial categories. XDR: non-susceptible to ≥1 agent in all but ≥2 categories. PDR: non-susceptible to all antimicrobial agents listed. PDR was not considered because not all the antibiotics contained in the proposal of Magiorakos *et al.,* (2012) are used in poultry management in Zaria, Nigeria.

# Minimum Inhibitory Concentration (MIC) to Oxacillin

- The result of the MIC of oxacillin against the 35 isolates that were resistant to methicillin showed that 74.3% of the isolates had high MIC ≥ 64µg/ml and the remaining 25.7% had MIC of 2µg/ml.

- This is as shown in Table 3. The MIC break points for oxacillin are MIC of ≤ 2µg/ml is susceptible while that of ≥ 4 µg/ml is resistant.

**Table 3: Minimum Inhibitory Concentration (MIC) of Methicillin Resistant *Staph. aureaus* from Poultry Farm in Zaria, Nigeria to Oxacillin**

| S/N | Isolates | MIC | S/N | Isolates | MIC |
|---|---|---|---|---|---|
| 1 | 19 | ≥ 64 | 19 | 115 | ≥ 64 |
| 2 | 25 | ≥ 64 | 20 | 117 | ≥ 64 |
| 3 | 32 | ≥ 64 | 21 | 119 | ≥ 64 |
| 4 | 40 | ≥ 64 | 22 | 124 | ≤ 2 |
| 5 | 49 | ≥ 64 | 23 | 133 | ≥ 64 |
| 6 | 50 | ≥ 64 | 24 | 136 | ≤ 2 |
| 7 | 53 | ≤ 2 | 25 | 151 | ≥ 64 |
| 8 | 58 | ≥ 64 | 26 | 153 | ≥ 64 |
| 9 | 59 | ≤ 2 | 27 | 158 | ≥ 64 |
| 10 | 60 | ≤ 2 | 28 | 161 | ≥ 64 |
| 11 | 61 | ≥ 64 | 29 | 163 | ≥ 64 |
| 12 | 62 | ≥ 64 | 30 | 164 | ≥ 64 |
| 13 | 64 | ≥ 64 | 31 | 165 | ≥ 64 |
| 14 | 68 | ≥ 64 | 32 | 188 | ≥ 64 |
| 15 | 71 | ≥ 64 | 33 | 193 | ≥ 64 |
| 16 | 75 | ≥ 64 | 34 | 201 | ≤ 2 |
| 17 | 78 | ≤ 2 | 35 | 205 | ≤ 2 |
| 18 | 82 | ≤ 2 | | | |

# Determination of Vancomycin Resistance

- The 74.3% (26) isolates that showed high MIC value against Oxacillin were tested against Vancomycin.

- The result showed that 80.8% (21) of the isolates were resistant to Vancomycin while 19.2% (5) were sensitive even after 48hrs incubation on mannitol salt agar impregnated with 4µg/ml Vancomycin.

- The isolates were also grown on Brain heart infusion agar impregnated with 6µg/ml Vancomycin. The result showed that 88.5% (23) of the isolates were resistant while 21.5% (3) were sensitive. This is shown in Table 4

**Table 4: Vancomycin Resistance in *Staph. aureus* from Poultry Farms in Zaria, Nigeria**

| S/N | Isolates | 2µg/ml Vancomycin | 4µg/ml Vancomycin | 6µg/ml Vancomycin |
|---|---|---|---|---|
| 1 | 19 | + | + | + |
| 2 | 25 | + | + | + |
| 3 | 32 | + | + | + |
| 4 | 40 | + | + | + |
| 5 | 49 | + | + | + |
| 6 | 50 | + | - | + |
| 7 | 58 | + | - | - |
| 8 | 61 | + | + | + |
| 9 | 62 | + | + | + |
| 10 | 64 | + | + | + |
| 11 | 68 | + | - | - |
| 12 | 71 | + | + | + |
| 13 | 75 | + | + | + |
| 14 | 115 | + | + | + |
| 15 | 117 | + | + | + |
| 16 | 119 | + | + | + |
| 17 | 124 | + | - | + |
| 18 | 133 | + | + | + |
| 19 | 151 | + | + | + |
| 20 | 153 | + | + | + |
| 21 | 158 | + | + | - |
| 22 | 161 | + | + | + |
| 23 | 163 | + | - | + |
| 24 | 164 | + | + | + |
| 25 | 165 | + | + | + |
| 26 | 193 | + | + | + |

**Key:** + = resistance - = susceptible

# CONCLUSION

- Methicillin-resistant *Staph. aureus* (MRSA), once restricted to hospitals is spreading rapidly in poultry farms in Zaria, Nigeria and this could play a potential role in disseminating pathogens between animal and human resulting into community acquired MRSA.

- This study established the first complete *Staph. aureus* isolates to be Vancomycin resistanct with an elevated Vancomycin MIC within the susceptible range in Zaria, Nigeria among poultry farms.

- It also showed that MRSA is able to develop Vancomycin resistance, in which the spread of this resistant trait might influence untreatable diseases in zoonotic outbreak.

# RECOMMENDATIONS

- To improve the efficacy of Vancomycin therapy we suggest a further study on the combination of Vancomycin with Ciprofloxacin or Gentamicin, or Pefloxacin to infections associated with highly resistant MRSA.

- Also antibiotic surveillance and control on the use of beta-lactam antibiotics including other classes of antibiotics in our community should be emphasized.

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# THANKS FOR LISTENING