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Antibiotic Susceptibility Profile of Methicillin Resistant *Staphylococci aureus* in Poultry Farm, in Zaria, Nigeria

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Abstract

The wide spread of Methicillin resistance gene calls for concern even in livestock. The epidemiological and antibiotic susceptibility of *S. aureus* in poultry farms in Zaria, Nigeria was carried out in this study, due to the increasing resistance to antibiotics associated with *S. aureus* in poultry birds. In this study, 250 samples of chicken droplets were collected from five different poultry farms (50 samples from each farm) within Zaria metropolis. Eighty eight (88) isolates of *S. aureus* were confirmed using standard microbiological methods. The antibiotic susceptibility profile of the isolates against 8 antibiotics showed that the isolates have varying antibiotics susceptibility pattern. The isolates (41.2%) were observed to be resistant to Methicillin and produced β-lactamase while 44.3% were classified as multidrug resistant. The result also showed high MIC value of Oxacillin (≥64 µg/ml) and Vancomycine (4 µg/ml) against the Methicillin resistant isolates. The MARI result showed that 60% of the isolates had MAR index of ≥0.4; indicating that these isolates have been pre-exposed to the antibiotics used in this study. This study showed high incidence of *S. aureus* with antibiotics resistance among poultry birds in Zaria, Nigeria, and calls for antibiotic surveillance and education of the poultry farm workers to curb the wide spread of resistance gene, which could be transferred in zoonotic diseases.

Keywords: *S. aureus;* Antibiotic susceptibility profile; Poultry birds; Zoonotic disease; Resistance

Introduction

Methicillin resistant *S. aureus* is a notorious pathogenic microorganism. Though, antimicrobials such as Penicillin and other β-lactams are commonly used in livestock production for the treatment of disease associated with *S. aureus*, and to improve production; the development of resistance through the production of β-lactamase enzyme against β-lactam antibiotics by *S. aureus* creates a huge some of economic burden [1]. This limitation had prompted the development and use of methicillin antibiotics; a new synthetic β-lactam drug that resisted β-lactamase producing *S. aureus* [2]. The use of methicillin was stopped due to its toxicity, development of *MecA* resistance gene and wide spread of resistance after a few years of production [3]. These accruing problems call for concern as resistance to methicillin is not mediated through the production of β-lactamase, but rather acquisition of mobile genetic element known as staphylococcal cassette chromosome mec (SCCmec) [4]. This staphylococcal cassette chromosome is majorly possessed by coagulase-negative staphylococci (CNS) that carries MecA gene, which encodes for an altered penicillin-binding protein (PBP2a or PBP2') [5]. The PBP2a according to Sarah and Robert, [6] has a lower affinity for β-lactam antimicrobials than the normal PBP such that these antimicrobials are deactivated. The wide spread of this gene might be attributed to the ability of some of the organisms to acquire a competitive advantage property that could aid inherent resistance, leading to proliferation in bacteria population [7]. Community acquisition of *MecA* gene as found in dairy, pig, cat, poultry, cattle and poultry farm worker with phenotypical and genotypical indistinguishable *MecA* gene, suggests a cross-species transmission (livestock-associated MRSA (LA-MRSA)) either by contact or indirectly via the food chain, water, air, manure and sludge-fertilized soils [1,5,8,9], which could be endemic in rural areas with low medical facilities in zoonotic disease outbreak [10]. Furthermore, the staphylococcal cassette chromosome contains additional insertional DNA sequences that allow for incorporation of additional antimicrobial resistance markers [11]. These insertional sequences explain why many methicillin-resistant staphylococci are resistant to non-β-lactam antimicrobials that act through mechanisms other than interference with bacterial cell wall synthesis (e.g., macrolides, fluoroquinolones) and thus why methicillin-resistant strains can be multi-drug resistant. In both humans and animals, in-apparent colonization is far more common than outright infection, and colonization is more often transient than chronic [12].

Till date, there are no comprehensive data on the situation of LA-MRSA in Zaria, Nigeria and although infections with MRSA are much less reported than carriage. Hence the need to evaluate the occurrence and antibiotics susceptibility profile of MRSA in poultry farms in Zaria, Nigeria in other to investigate into the pathogenicity potential of MRSA in our environment and to curb resistance spread through the provision of information for surveillance purpose becomes imperative.

Methodology

Sample collection

Fifty (50) samples of fresh chicken droplets were collected aseptically in 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 pack to the laboratory for bacteriological examination.

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Staph. species identification, isolation and microscopy

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 3 (17.5%) of 12.5 g NaCl to 1 L of Mannitol salt agar in other to inhibit the growth of other organism was carried out using the method described by Onaolapo" Split the sentence in two: "Gram staining and microscopy was also carried out to identify Gram positive organisms." Followed by "Further morphological characterization of the colonies isolated from concentrated Mannitol salt agar (17.5%; 12.5 g NaCl to 1 L of agar in order to inhibit the growth of other organism) was carried out using the method described by Onaolapo [12].

Biochemical test and β-lactamase production test

The following conventional biochemical tests; catalase, coagulase and deoxyribonuclease (DNase) tests as described by Cheesbrough [13] were also adopted to distinguish *S. aureus* from other forms of Staph. spp. The test tube method according to Lennette et al., [14] and Plateacidimetric method according to Cheesbrough [13] were also used to determine the ability of the identified *S. aureus* to produce β-lactamase.

Antibiotic susceptibility test and multiple antibiotic resistance index (MARI) evaluation

The susceptibility profiles of the identified *S. aureus* was tested against eight selected antibiotics (ampicillin, ciprofloxacillin, methicillin, tetracycline, Vancomycin, gentamicin, pefloxacin and oxacillin) using disc diffusion method as described by Cheesbrough [13] and the corresponding results interpreted using CLSI [15]. 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 [16,17].

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 128 µg/ml was prepared. This working solution (2 ml) was then serially diluted in nutrient broth (2 ml) up to the last tube. Eighteen hours cultures of the isolates were standardized to contain about 106 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°C for 18 hrs 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 4 µg/ml and 6 µg/ml of Vancomycin were prepared.

Five milli litre (5 ml) of the stock solution (4 µg/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°C for 24-48 hrs. 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 106 cfu/ml. The plates were allowed to dry at room temperature and then incubated at 37°C for 24-48 hrs. This was repeated for all the resistant isolates.

Result

Sample collection and identification of *S. aureus* isolates

Out of the 250 chicken droplets collected, isolates that produced round smooth, glistening deep golden to white colonies on nutrient agar were selected for microscopy. The microscopy result showed that 157 isolates were Gram positive *Staphylococcus* spp. with uniform sized cocci appearance, occurring predominantly in characteristic clusters or bunches and retained the purple colour of crystal violet. On mannitol salt agar, 98 isolates of *Staphylococcus* spp. fermented mannitol to acid and produced golden yellow colouration within 24 hrs of incubation.

The biochemical characterization of the isolates showed that all the 98 *S. aureus* were catalase positive, 88 (89.8%) were coagulase positive, 10(10.2%) were coagulase negative while 87 and 88 of the isolates were deoxyribonuclease and β-lactamase producing *S. aureus* respectively (Table 1).

Antibiotic susceptibility profile of *S. aureus* from poultry farms in Zaria, Nigeria

The antibiotic susceptibility profile of the 88 isolates of *S. aureus* that were β-lactamase producers were evaluated against the 8 antibiotics commonly used in poultry farm in Zaria metropolis. Figure 1 depicts the susceptibility and the resistance of the isolates to the 8 antibiotics. The result showed that the isolates were 90.8% susceptible to Ciprofloxacin, 76.2% to Vancomycin, 72.2% to Pefloxacin, 65.6% to Gentamicin, 58.8% to Methicillin, 57.6% to Oxacillin, 49.6% to Ampicillin and 25.3% to Tetracycline. Their percentage resistance varied from 9.2, 23.9, 27.8, 34.4 and 42.4 for Ciprofloxacin, Vancomycin, Pefloxacin, Gentamycin and Oxacillin, respectively (Figure 1). The results showed that most of the *S. aureus* from poultry farms in Zaria, Nigeria are resistant to β-lactam (Methicillin, Ampicillin, Oxacillin) and tetracyclines, and the pattern of antibiotic resistance also varies from one isolate to another. The isolates were also found to be 44.3% (39) multidrug resistant (44.3%), 40.9% XDR (40.9%) 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

S/No	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 *S. aureus* (n=98)	98	0	88	10	88	10	88	10
The result showed the biochemical characteristics of the identified *S. aureus* from different farm sources. N=number of farms, n=number of *S. aureus* from various farms in Zaria, metropolis.									

Table 1: Biochemical characterization and β-Lactamase production.

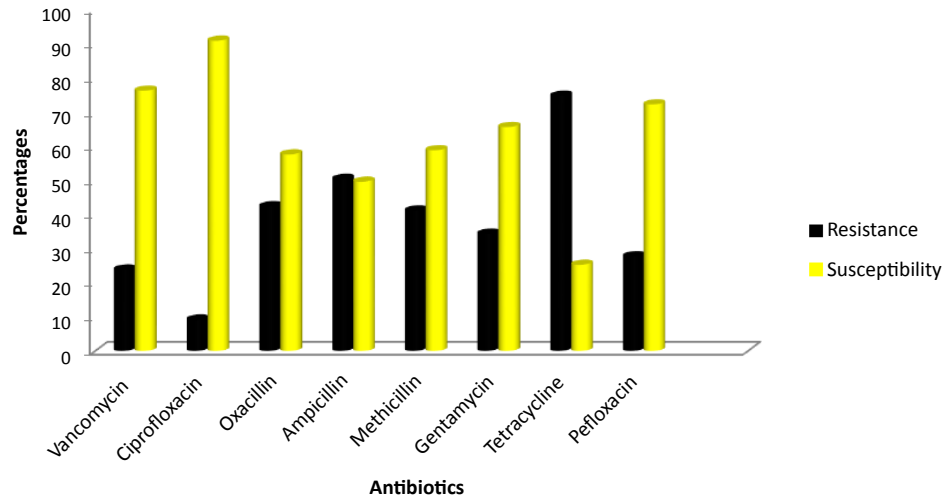

Figure 1: Percentage antibiotic susceptibility of *S. aureus* poultry forms in Zaria, Nigeria.

antibiotics used in this study. From all the farms evaluated 40% (35) of the *S. aureus* were observed to be resistant to methicillin antibiotics. The isolates resistant pattern, MARI, and multidrug classification according to the International Expert Proposal for Interim Standard Definitions for acquired resistance adapted from Magiorakos *et al.,* [17] is shown in Table 2.

Minimum inhibitory concentration (MIC) to oxacillin

The result of the MIC of oxacillin against the 35 isolates that were was resistant to methicillin showed that 74.3% of the isolates had high MIC ≥64 µg/ml and the remaining 25.7% had MIC of 2 µg/ml (Table 3). This is as shown in Table 3. The MIC break points for oxacillin are MIC of ≤2 µg/ml is susceptible while that of ≥4 µg/ml is resistant [18].

Determination of vancomycin resistance

Table 4 shows that 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 48 hrs incubation on mannitol salt agar impregnated with 4 µg/ml Vancomycin. The isolates were also grown on Brain heart infusion agar impregnated with 6 µg/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.

Discussion

Previous studies on poultry farms have recognized *S. aureus* as an important pathogenic organism [19], and based on geographical locations and site of sample collection, different percentages of *S. aureus* from poultry farms have been reported. In Nigeria, Suleiman *et al.,* [20] had reported 84% (84) *S. aureus* from tracheal swabs in Maiduguri, Adeyeye and Adewale [21] in Ogbomoso reported 100% (30) from nasal swabs while in Zaria, from chicken droplets, 39.2% (98) of the total samples (250) evaluated in this study were observed to be *S. aureus*. This showed that the percentages of *S. aureus* from different samples sources varies. The most commonly isolated of this specie is coagulase positive (89.8% (88)) while coagulase negative were 10.2% (10) (Table 1). The report of Roda and Fatema [22] concurred with the high percentages of coagulase positive *S. aureus* (42.4% (14)) in poultry farms compared with the coagulase negative (30.3%) *S. aureus*. The susceptibility profile of the *S. aureus* to commonly used antibiotics in the hospital and poultry management showed that the isolates were highly susceptible to Ciprofloxacin, Vancomycin, Pefloxacin and Gentamycin. The isolates showed high resistance (74.7% and 50.4%) to Tetracycline and Ampicillin respectively while 41.2% of the isolates were resistant to Methicillin and produced β-lactamase enzyme (Figure 1). The observed variation in the percentage susceptibility profile expressed by *S. aureus* in this study also concurred with the report of Roda and Fatema [22] who observed a variation of 3 to 66.66%. The study of Roda and Fatema [22] and the work of Yaqub *et al.,* [23,24] also observed high resistance to penicillin and tetracycline. This could be associated with the vast used of these antibiotics for the treatment of animal diseases [25]. Among these, 44.3% were found to be multidrug resistant, 40.9% XDR while 14.8% were neither MDR nor XDR (Table 2). This high occurrence of multidrug resistance in this study might be due to the production of beta-lactamase enzyme and horizontal gene transfer of antibiotic resistant gene [5,9]. The MAR index result showed that 40% of the isolates had MAR index of ≤0.3 while 60% had MARI of ≥0.4; indicating that the *S. aureus* tested were pre-exposed to the antibiotics used in this study. 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 value of ≥64 µg/ml and the remaining 25.7% had MIC of 2 µg/ml (Table 3). This form of high resistance according to Cohn and Middleton, [3] and Sarah and Robert, [6] could be linked with an altered penicillin-binding protein, PBP2a, which is encoded by the **mec**A gene. 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 48 hrs incubation on mannitol salt agar impregnated with 4 µg/ml Vancomycin. The isolates were also grown on Brain heart infusion agar impregnated with 6 µg/ml Vancomycin. The result showed that 88.5% (23) of the isolates were still resistant while 21.5% (3) were sensitive (Table 4). Though the mechanism of Vancomycin resistance in *S. aureus* still remain unclear, the report of Natasha *et al.,* [25], had suggested that there is a likelihood of clinical treatment failure when Vancomycin is used to treat infections where the MIC is above 2 µg/ml. Howden *et al.,* [26] suggested that resistance to Vancomycin (the principal drug used to treat MRSA infections) might have emerged as a result of point mutation (six nucleotide substitutions), which occurred in graRS, encoding a putative two-component regulatory sensor, leading to a change from a polar to a nonpolar amino acid (T136I) in the conserved histidine region of

S/No	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
S/N	**Lab Code**	**Antibiotic Resistant Pattern**	**NAR**	**GAR**	**MDR**	**MARI**
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
75	Z199	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

Key: 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.

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

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			

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

the predicted protein, while the work of Hiramatsu *et al.,* [27] had suggested that this persistent resistance may be due to the acquisition of the vanA resistance determinant from enterococci which is plasmid mediated. Other inferences like the work of Cui *et al.,* [28], and Sieradzki and Tomasz [29] ascribed the thickened cell wall containing dipeptides capable of binding Vancomycin as the major cause of the resistance to Vancomycin. This thickened cell wall according to Susana and Alexander [30] is caused by transposon Tn*1546,* acquired from Vancomycin-resistant *Enterococcus faecalis*, which is known to alter cell wall structure and metabolism. An observation of this high resistance in our environment should been indeed a major concern to clinicians, veterinarians, pharmaceutical companies, and consumers worldwide due to the ability of acquired Methicillin and Vancomycin resistant gene to render diseases associated with *S. aureus* such as dermatitis, pneumonia, septicaemia to osteomyelitis and meningitis in humans and swine as well as bovine mastitis in cattle and bumblefoot disease in poultry untreatable [31]. Although no clinical trials suggest superiority of Vancomycin over any comparator antibiotics, some studies have provided evidence of its inferiority [32]. Therefore strong consideration

S/N	Isolates	2 µg/ml Vancomycin	4 µg/ml Vancomycin	6 µg/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.

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

like the use of Ciprofloxacin as observed in this study, or a combination of Vancomycin and Piperacillin-Tazobactam or Oxacillin or Cefepime [33] should be used as alternative agents in the treatment of serious *S. aureus* infections from poultry farms in Zaria, Nigeria.

Conclusion and Recommendations

Methicillin-resistant *S. 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 *S. 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. 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.

References

1. Huber H, Koller S, Giezendanner N, Stephan R, Zweifel C (2010) Prevalence and characteristics of meticillin-resistant Staphylococcus aureus in humans in contact with farm animals, in livestock, and in food of animal origin, Switzerland. Euro Surveill 15: 19542.
2. Llarrull LI, Fisher JF, Mobashery S (2009) Molecular Basis and Phenotype of Methicillin Resistance in Staphylococcus aureus and Insights into New β-Lactams That Meet the Challenge. Antimicrob Agents Chemother 53: 4051-4063.
3. Middleton JR (2013) Antimicrobial Resistance: Implications for Human and Animal Health on Dairy Farms. Extension America's research based learning network.
4. Garcia-Alverez L, Holden MTG, Lindsey H, Webb CR, Brown DFG, et al. (2011) Methicillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis.
5. Cohn LA, Middleton JR (2010) A veterinary perspective on methicillin-resistant staphylococci. J Vet Emerg Crit Care 20: 31-45.
6. Sarah MD, Bonomo RA (2010) Three Decades of β-Lactamase Inhibitors. Clin Microbiol Rev 23: 160-201.
7. Beceiro A, Tomás M, Bou G (2013) Antimicrobial Resistance and Virulence: a Successful or Deleterious Association in the Bacterial World. Clin Microbiol Rev 26: 185-230.
8. Juhasz-Kaszanyitzky E, Janosi S, Somogyi P, Dan A, van der Graaf-av Bloois L, et al. (2007) MRSA transmission between cows and humans. Emerg Infect Dis 13: 630-632.
9. Hasman H, Moodley A, Guardabassi L, Stegger M, Skov RL, et al. (2010) spa type distribution in Staphylococcus aureus originating from pigs, cattle and poultry. Veterinary Microbiology 141: 326-331.
10. Vanderhaeghen W, Hermans K, Haesebrouck F, Butaye P (2010) Methicillin-resistant Staphylococcus aureus (MRSA) in food production animals. Epidemiology and Infection 138: 606-625.
11. Liu GY (2009) Molecular Pathogenesis of Staphylococcus aureus Infection. Pediatr Res 65: 71-77.
12. Onaolapo JA (2005) Isolation and identification of Staph aureus from other microbial contaminants using increased salt concentration media. In: Bacterial Genetics Lecture Note. Ahmadu Bello University, Zaria.
13. Cheesbrough M (2000) District Laboratory Practice in Tropical Countries Part 2. Cambridge University Press: 135-142, 158-159.
14. Lennette EH, Balones P, Hausa WJ, Shadonmu HJ (1990) Manual of Clinical Microbiology, Washington DC. pp: 10-20.
15. Clinical Laboratory Standard Institute (CLSI) (2014) Performance Standards for Antimicrobial Susceptibility Testing.
16. Paul S, Bezbarauh RL, Roy MK, Ghosh AC (1997) Multiple antibiotics resistance (MAR) index and its reversion in Pseudomomas aeruginosa. Letters in Applied Microbiology 24: 169-171.
17. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, et al. (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbial Infection 18: 268-281.
18. Clinical Laboratory Standard Institute (CLSI) (2006) Performance standards of antimicrobial disc and dilution susceptibility tests for bacteria isolated from animal, approved standard.
19. Skeeles JK (1997) Staphylococcosis. In: Calnek BW, Barnes HJ, Beard CW, McDougald LR, Saif YM (eds.) Diseases of Poultry (11thedn) Ames IA, Iowa State University Press: 247-253.
20. Suleiman A, Zaria LT, Grema HA, Ahmadu P (2013) Antimicrobial resistant coagulase positive Staphylococcus aureus from chickens in Maiduguri, Nigeria. Sokoto Journal of Veterinary Sciences 11: 51-55.
21. Adeyeye JOKE, Adewale AOKE (2013) Incidence of Methicillin-Resistant Staphylococcus aureus (MRSA) In a Small Poultry in South West, Nigeria. Journal of Agriculture and Veterinary Science 5: 53-55.
22. Al Thani RF, Al-Ali F (2012) Incidences and antimicrobial susceptibility profile of Staphylococcus species isolated from animals in different Qatari farms. African Journal of Microbiology Research 6: 7454-7458.
23. Geidam YA, Zakaria Z, Aziz SA, Bejo SK, Abu J (2012) High Prevalence of Multi-drug Resistant Bacteria in Selected Poultry Farms in Selangor, Malaysia. Asian Journal of Animal and Veterinary Advances 7: 891-897.
24. Alekshun MN, Levy SB (2007). Molecular mechanisms of antibacterial multidrug resistance. Cell 128: 1037-1050.
25. Holmes NE, Johnson PDR, Howden BP (2012) Relationship between Vancomycin-Resistant Staphylococcus aureus, Vancomycin-Intermediate S. aureus, High Vancomycin MIC, and Outcome in Serious S. aureus Infections. J Clin Microbiol 50: 2548-2552.
26. Howden BP, Stinear TP, Allen DL, Johnson PD, Ward PB, et al. (2008) Genomic analysis reveals a point mutation in the two-component sensor gene gras that leads to intermediate Vancomycin resistance in clinical Staphylococcus aureus. Antimicrob Agents Chemother 52: 3755-62.
27. Hiramatsu K, Aritaka N, Hanaki H, Kawasaki S, Hosoda Y, et al. (1997) Dissemination in Japanese hospitals of strains of Staphylococcus aureus heterogeneously resistant to Vancomycin Lancet 350: 1670-1673.
28. Cui L, Ma X, Sato K, Okuma K, Tenover FC, et al. (2003) Cell wall thickening is a common feature of Vancomycin resistance in Staphylococcus aureus. J Clin Microbiol 41: 5.
29. Sieradzki K, Tomasz A (1999) Gradual alterations in cell wall structure and metabolism in Vancomycin-resistant mutants of Staphylococcus aureus. J Bacteriol 181: 7566.
30. Gardete S, Tomasz A (2014) Mechanisms of Vancomycin resistance in Staphylococcus aureus. Journal of Clinical Investgation 124: 2836-2840.
31. Quinn PJ, Carter ME, Markey BK, Carter GR (2000) Staphylococcus species. Clinical Veterinary Microbiology Mosby, Edinburgh. pp: 118-126.
32. Deresinski S (2007) Counterpoint: Vancomycin and Staphylococcus aureus-An Antibiotic Enters Obsolescence. Clinical Infectious Diseases 44: 1543-1548.
33. Dilworth TJ, Sliwinski J, Ryan K, Dodd M, Renée-Claude M (2014) Evaluation of Vancomycin in Combination with Piperacillin-Tazobactam or Oxacillin against Clinical Methicillin-Resistant Staphylococcus aureus Isolates and Vancomycin-Intermediate S. aureus Isolates In Vitro. Antimicrob Agents Chemother 58: 1028-1033.