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Full Length Research

# Molecular characterization of methicillin resistant Staph. aureus from poultry farms in Kano State, Nigeria

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**ABSTRACT:** There is presently an increased rate of resistance by methicillin resistant *Staph. aureus* against commonly prescribed antibiotics for Staph aureus infection. This strain of Staph. aureus is not only a problem in clinical sector but also in livestock disease treatment and management as transferability of this resistant gene in zoonotic outbreak might be possible. This study was set out to evaluate the incidence of methicillin resistant Staph. aureus from poultry farms birds and the farm workers, and also to evaluate the likelihood of cross-infection between the birds and the farm workers in Kano State, Nigeria. Samples were collected using standard microbiological techniques. Antibiotic susceptibility was also carried out using disc diffusion method while genes that influence methicillin resistant were evaluated using PCR method. The results showed that out of the 1260 samples collected, 98 isolates were confirmed to be Staph. aureus. The antibiotics susceptibility test results showed that 30.6% (30) of the Staph. aureus isolates were resistant to cefoxitin (a phenotypic test for methicillin resistant) while 69.4% (68) were susceptible. The methicillin resistant isolates were observed to exhibit (100%) resistant to Ampicillin and Amoxicillin, 93.3% to Oxytetracycline, 90% to Chloramphenicol, 80% to Erythromycin, 76.7% to Oxacillin, 63.3% to Trimethroprim/Sulphamethozaxole, 30% to Ciprofloxacin and 26.7% to Gentamicin. The result also showed that 83.3% (25) of the isolates had multiple antibiotic resistance index (MARI) of > 0.3 and were also multidrug resistant (MDR) while 16.7% had MARI ≤ 0.3. The molecular analysis showed that all the isolates were Staph. aureus of 800bp, 66.7% of the MDR isolates harbored MecA gene (162bp), while 33.3% had MecA of 500bp. Further analysis showed that 3 of the 7 housekeeping genes (pta, gmk and yqil) were also present in the MDR isolates at 43.3, 20 and 16.7% respectively while 10% express spa typing. The results also showed that there is a correlation between phenotypic cefoxitin resistance and carriage of *MecA* gene.

**Key words:** Methicillin resistance, poultry farms, *Staph. Aureus*.

#### INTRODUCTION

In poultry management, antibiotics such as  $\beta$ -lactams are often used in animal food production for growth promotion and routine disease prevention without prescription or control measures. This encourage drug resistance superbug such as methicillin resistant *Staph. aureus (MRSA)*, which is now a major emerging public health problem (Klevens et al., 2007). With increase in population density within a particular geographical location, the incidence of both communities associated and hospital associated MRSA has been observed to increase with time, regardless of hospital size and control measures due to drug abuse and zoonotic transfer of

resistance gene through horizontal gene transfer (Adelisa et al., 1992; Igwe et al., 2013). In 2005, the standardized incidence rate of invasive MRSA in USA hospitals was 31.8 per 100 000 patients (interval estimate, 24.4-35.2) with standardized mortality rate of 6.3 per 100 000 (interval estimate, 3.3-7.5) (Klevens et al., 2007). This has indeed influenced high mortality and morbidity rates especially in immunocompromised patients and among persons of 65 years and older, as MRSA accounts for the most frequent cause of skin and soft tissue infections in USA (Sara et al., 2003; Moranet al., 2006; Klevens et al., 2007). Most community associated MRSA have been

reported to show susceptibility to non-β-lactam antimicrobial agents; carried staphylococcal cassette chromosome type IV, and frequently encoded the dermonecrotic cytotoxin known as Panton-Valentine leukocidin (Naimi et al., 2003; Ma et al., 2002). The transfer of nosocomial infections of MRSA majorly occur from patients who has recently visited the hospital or nursing home residence (Naimi et al., 2003). Vancomycin resistant Staph. aureus isolates that habour resistance genes against Glycopeptides especially Vancomycin with high MIC range have been reported (George et al., Glycopeptides especially Vancomycin antibiotics of choice for the treatment of MRSA infection. Surveys conducted by the National Antimicrobial Resistance Monitoring System (NARMS) indicate that retail meat and poultry products are frequently contaminated with multidrug-resistant Campylobacter species, Salmonella species, Enterococcus species, and Escherichia coli (FDA, 2007). This study therefore evaluates the pathogenicity of MRSA in poultry farms in Kano State, Nigeria due to lack of information in this area.

#### **METHODOLOGY**

#### Sample Collection

From the 3 geopolitical zones in Kano State, 4 farms each were randomly selected. Fifty (50) samples each from the chicken cloacae and nostril were aseptically collected. While 5 samples were collected from the poultry farm workers. A total of 1260 poultry chicken samples (consisting of 600 cloacae samples, 600 nostril samples and 60 samples from the poultry farm workers) were collected aseptically [using the method described by Adeyeye and Adewale (2013)] into a clean sterile universal bottle (Agary Pharmaceutical LTD, China) from the 12 poultry farms in Kano State and transported on an ice park to the laboratory for bacteriological examination.

## **Staph.** Species Identification, Isolation and Microscopy

Collected samples were suspended in sterile normal saline which was prepared according to Cheesbrough (2000), and incubated for 24 hrs at 37°C (National Appliance Co. Ltd, Oregon, USA: model 1630, 240V and 2340W), and there after inoculated on the surface of sterile nutrient agar (NA) for further 18 hrs incubation period at 37°C. Gram staining and microscopy (Wild Heerbrugg M11, Switzerland) were also carried out to Gram positive organisms identify according (2000)morphological Cheesbrough while further characteristics of Staph. aureus on Mannitol salt agar was also used to differentiate Staph. aureus from other microorganisms.

### Biochemical Test for the Identification of Staph. aureus

The following conventional biochemical tests; catalase, coagulase and deoxyribonuclease (DNase) tests as described by Cheesbrough (2002) were also adopted to distinguish *Staph. aureus* from other forms of *Staph.* spp. Further confirmatory tests for *Staph. aureus* i.e. STAPH Agglutination kit (Oxoid, UK) and Microgen STAPH kit (Microgene, UK) using manufacturers protocol were used to confirm the presumptive isolates to be *Staph. aureus*.

## Antibiotic Susceptibility Test, Multiple Antibiotic Resistance Index (MARI) Evaluation, and Classification of Drug Resistance

The susceptibility profiles of the identified Staph. aureus was tested against Cefoxitin, since the toxicity associated with methicillin as restricted its use in Hospitals. Also the ability of cefoxitin to induce mecA heteroresistance, long shelf life during storage and reproducibility of test in research has made it use better than oxacillin/methicillin (CLSI, 2014). Isolates that showed resistance to Cefoxitin were also evaluated for antibiotics resistance against other such Sulphamethoxazole/Trimetophrim (30µg), Vancomycin (15µg), Ciprofloxacin Erythromycin  $(5\mu g)$ ,  $(30\mu g)$ , Chloramphenicol (10µg), Oxacillin (1µg), Gentamicin Ampicillin (10µg), Oxytetracycline  $(10\mu q)$  $(30\mu q)$ . Augumentin (30µg) using disc diffusion method as described by Cheesbrough (2002) and the corresponding results interpreted according to CLSI (2014). The multiple antibiotic resistant (MAR) index was determined for each isolates. 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) while classification of drug resistance was determined according to the method described by Magiorakos et al. (2012). Multidrug resistance (MDR) is classified as nonsusceptible to ≥1 agent in ≥3 antimicrobial categories. extensive drug resistance (XDR) as non-susceptible to ≥1 agent in all but ≥2 categories, pandrug-resistance (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) were used in this study.

#### Molecular Analysis of Antibiotic Resistant and Pathogenic Genes Among *Staph. aureus* Isolated from Poultry Farms in Zaria, Nigeria

#### **DNA Extraction**

DNA extraction was carried out using Zymo research fungal/bacteria DNA miniprep<sup>TM</sup>kit protocol with catalog number D6005 (Ingaba, South Africa). The extracted

**Table 1.** Primers for Molecular Characterization of Antibiotic Resistant and Pathogenic Genes Among *Staph. aureus* Isolated from Poultry Farms in Zaria, Nigeria.

No.	Primer name	Forward	Metabolic Function	Base pair	Primer Source
	mecA <sub>1F</sub>	TCCAGATTACAACTTCACCAGG			- · · · · · · · · · · · · · · · · · · ·
1	mecA <sub>1R</sub>	CCACTTCATATCTTGTAACG		162	Oliveira and de Lencastre
	mecA <sub>2F</sub>	AAA ATC GAT GGT AAA GGT TGG C			(2011)
	$mecA_{2R}$	AGT TCT GCA GTA CCG GAT TTG C		500	(2011)
2	StaphF	AAT CTT TGT CGG TAC ACG ATA TTC ACG		800	de Lencastre and Duarte
	StaphR	CGTAATGAGATTTCAGTA GAT AAT ACA AC			(2002)
3	arcCF	TTG ATT CAC CAG CGC GTA TTG TC	Carbamate kinase	456	
	arcCR	AGG TAT CTG CTT CAA TCA GCG			
4	AroEF	ATC GGA AAT CCT ATT TCA CAT TC	Shikimate dehydrogenas	456	
	AroER	GGT GTT GTA TTA ATA ACG ATA TC			Contabt at al
5	glpF	CTA GGA ACT GCA ATC TTA ATC	Glycerol kinase	465	Enright et al., (2000)
	glpR	TGG TAA AAT CGC ATG TCC AAT TC			(2000)
6	GmkF	ATC GTT TTA TCG GGA CCA TC	Guanylate kinase	429	
	GmkR	TCA TTA ACT ACA ACG TAA TCG TA			
7	PtaF	GTT AAA ATC GTA TTA CCT GAA GG	Phosphate acetyltransferase	474	
	PtaR	GAC CCT TTT GTT GAA AAG CTT AA			
8	TpiF	TCG TTC ATT CTG AAC GTC GTG AA	Triosephosphate isomerase	402	
	TpiR	TTT GCA CCT TCT AAC AAT TGT AC			
9	yqiLF	CAG CAT ACA GGA CAC CTA TTG GC	Acetyl-CoA acetyltransferase	516	
	yqiLR	CGT TGA GGA ATC GAT ACT GGA AC	-		
10	spa 2F	GAACAACGTAACGGCTTCATCC		250-637	Shopsin et al., (1999)
11	spa 1514R	CAGCAGTAGTGCCGTTTGCCT		~425	Harmsen et al., (2003)

DNAs were run on 1% agarose gel electrophoresis (Schwarz/Mann, England) to confirm that the DNA was actually extracted while the purity of the extracted DNA was quantified using a Nano Drop Thermo machine (Eppendorf, UK).

#### Polymerase Chain Reaction

PCR was carried out using a cocktail mix of 3.5µl of 2X master mix from Promega, 0.5µl of 5pMol forward primer, 5pMol reverse primer, 3µl of 25ng/µl of the extracted DNA and 2.5µl nuclease free water to form a 10µl PCR cocktail. The following pathogenic genes were evaluated in this study, *MecA*, the staphylococcal protein A (*spa*), which codes for the polymorphic region of protein A (repeat polymorphism of the X-region of the *spa* gene) and the seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, *and yqiL*) using the under listed primers (Table 1) and PCR condition (Table 2).

#### Gel Electrophoresis

Agarose gel was prepared using 3 grams of agarose gradually dissolved in 200 ml 1X tris-acetate EDTA (TAE) (Sigma chemical Ltd.,England) at room temperature. The mixture was then brought to boil at 150°C for 2 mins using Microwave heater (HINARI Life Style 800watts model MX310TCSL, UK). Ethidium bromide (8µI) (Sigma chemical Ltd., England) was added to stain the amplicons. The PCR products (10µI) were loaded on the gel and run on a Bio-rad electrophorectic machine at 120 V for 1.5 hrs. At the end, the gel bands were viewed using the Bio-rad Gel Doc XR transilluminator Machine.

#### **RESULTS**

## Sample Collection and Distribution of *Staph. aureus* in Poultry Farms in Kano State

A total of 1260 samples were aseptically collected from

**Table 2.** Thermocycler Conditions for Antibiotic Resistant and Pathogenic Genes Amplification Among *Staph. aureus* Isolated from Poultry Farms in Zaria, Nigeria.

Initial Den.	Den.	Ann. Temp	Extension	No. of circles	Final Ext.	Hold Temp.
94°C	94°C	56°C	72°C	72°C	72°C	10 <sup>o</sup> C
5mins	30 Sec	30 Sec	45 Sec.	36	7mins	∞

Keys: Den. = Denaturation, Ann. Temp. = Annealing temperature, Ext. = Extension.

Table 3. Distribution of Staph. aureus in Poultry Farms in Kano State.

S/N	Sample Collection				GS	Presumptive Staph. aureus ID			Agglutina	Microgene
	NSB	CSB	NSW	_	GS	Catalase	DNase	Coagulase	tion Test	Kit
Kano	central (I	arms 1	-4)							
1	50	50	5	ր NSB	175	175	168	120		
2	50	50	5	≻ CSB	170	170	163	160	61	10
3	50	50	5	JNSW	11	11	9	8	_	19
4	50	50	5	Total	365	356	240	288	46 23	14 8
Kano I	North (Fa	arm 5 –	8)							
5	50 <sup>°</sup>	50	<sup>^</sup> 5	ነ NSB	160	160	145	100	00	40
6	50	50	5	ļ csв	120	120	80	50	29	12
7	50	50	5	NSW	15	15	10	5	20	9
8	50	50	5	Total	295	295	235	155	16	6
Kano	South (F	arm 9 –	12)						4.4	4.4
9	50 <sup>`</sup>	50	´5	n NSB	190	190	175	90	41	14
10	50	50	5	CSB	150	130	115	80	35	10
11	50	50	5	NSW	10	10	9	9	19	6
12	50	50	5	Total	350	330	299	179		
Total	600	600	60	<del>]-</del> 1260	1010	981	774	622	290	98

Keys: NSB = Nasal Swab of birds, CSB = Cloacae Swab of birds, NSW = Nasal Swab of Workers, GS = Gram staining, ID = Identification.

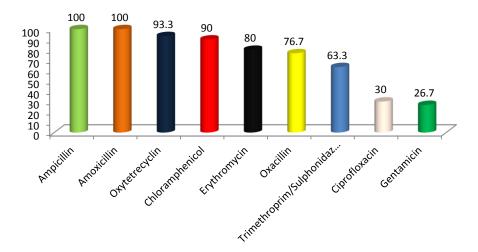
**Table 4.** Distribution of *Staph. aureus* in Cloacal and Nasal of Poultry Birds and Nasal Swab of Farm Workers Samples in Kano State.

	Zo	nes in Kano St		Percentage Total	
Sample Source	Kano central Kano Nor (Farms 1-4) (Farm 5 –		Kano South (Farm 9 – 12)		
Nasal Swab of birds	19	12	14	45	45.9
Cloacae Swab of birds	14	9	6	33	33.7
Nasal Swab of Workers	8	6	6	20	20.4
Total	41	27	26	98	100

12 farms in Kano State, of which conventional isolation and identification result showed that 562 of the samples had presumptive characteristics of *Staph. aureu*. Further evaluation using STAPH Agglutination kit reduced the number of the isolates to 290 while on identification using Microgen STAPH kit, 33.8% (98) of the presumptive isolates were confirmed as *Staph. aureus* (Table 3). The distribution of *Staph. aureus* among the sample sources showed that the birds nasal harbored more *Staph. aureus* (45.9%) than the birds cloacal (33.7%) and nasal of the farm workers in Kano State (Table 4).

#### **Antibiotics Susceptibility Result**

The antibiotics susceptibility profile of the isolates showed that all the isolates were (100%) resistant to Ampicillin and Amoxicillin, 93.3% to Oxytetracycline, 90% to Chloramphenicol, 80% to Erythromycin, 76.7% to Oxacillin, 63.3% to Trimethroprim/Sulphamethozaxole, 30% to Ciprofloxacin and 26.7% to Gentamicin (Figure 1). The multiple antibiotic resistance index showed that 83.3% of the isolates had MARI of > 0.3 and MDR respectively while 16.7% had MARI of  $\leq 0.3$  (Table 5).



**Figure 1.** Antibiotics Resistance Profile of Cefoxitin Resistance *Staph. aureus* from Poultry Farms in Kano State, Nigeria.

**Table 5.** Antibiotic Resistance Pattern, Classification and MARI of *Staph. aureus* from Poultry Farms in Kano State, Nigeria.

S/N	Isolate Code	Resistance Pattern	NART	ARC	MARI
1	12C	AMP,AMX,OT,	3	Nil	0.3
2	7N	AMP,AMX,OT,C,E,OX,SXT	7	MDR	0.7
3	83C	AMP,AMX,OT,C,E,OX,CN, SXT	8	MDR	0.7
4	88N	AMP,AMX,OT,C,E,OX,SXT	7	MDR	0.7
5	3C	AMP,AMX,OT,C,E,OX,SXT	7	MDR	0.7
6	90C	AMP,AMX,OT,C,E,OX,SXT	7	MDR	0.7
7	93C	AMP,AMX,OT,C,E,OX,CN	7	MDR	0.7
8	151C	AMP,AMX,OT,C,OX,SXT,E	7	MDR	0.7
9	67N	AMP,AMX,OT,C,OX,E,SXT,CIP,CN	9	MDR	0.9
10	73C	AMP,AMX,OT,C,E,OX,SXT	7	MDR	0.7
11	91N	AMP,AMX,OT,C,E,OX,SXT	7	MDR	0.7
12	86C	AMP,AMX,OT,C,E,SXT,CIP,CN	8	MDR	0.8
13	68C	AMP,AMX,OT,C,OX,SXT	6	MDR	0.6
14	14C	AMP,AMX,OT,C,E,OX,SXT,CIP	8	MDR	0.8
15	12N	AMP,AMX,C,	3	Nil	0.3
16	39C	AMP,AMX,OT, E,OX, SXT, C,	7	MDR	0.7
17	90C	AMP,AMX,OT,C,E,OX,CIP,CN	8	MDR	0.8
18	76C	AMP,AMX,OT,C,CIP,E,	6	MDR	0.6
19	31N	AMP,AMX,OT,C,E,OX,CN	7	MDR	0.7
20	145C	AMP,AMX,OT,C,E,OX,CN	7	MDR	0.7
21	69C	AMP,AMX,C,	3	Nil	0.3
22	64C	AMP,AMX,OT,C,E,OX,SXT,CIP	8	MDR	0.8
23	182C	AMP,AMX,OT,C,OX,SXT,E	7	MDR	0.7
24	184C	AMP,AMX,OT,C,E,SXT,CN,OX	8	MDR	8.0
25	95C	AMP,AMX,OT,C,E,OX,SXT,CIP	8	MDR	8.0
26	41C	AMP,AMX,OT,C,E,OX,SXT	7	MDR	0.7
27	35C	AMP,AMX,OT	3	Nil	0.3
28	39N	AMP,AMX,OT,	3	Nil	0.3
29	190N	AMP,AMX,OT,C,E,OX,CIP, SXT	8	MDR	0.8
30	199C	AMP,AMX,OT,C,E,CIP,OX,	7	MDR	0.7

Keys: NART = number of antibiotics resistance to, ARC = antibiotics resistance classification, MARI = multiple antibiotics resistance index, MDR = multiple antibiotic resistance.

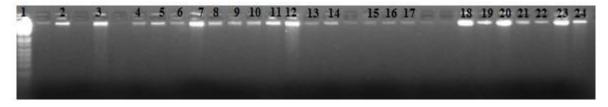
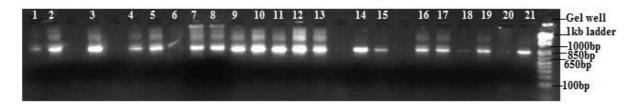
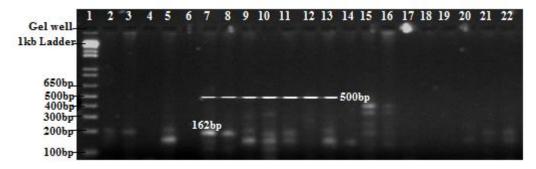


Figure 2. Extraction of DNA from Staph. aureus strains Isolated from Poultry Farms in Kano State.



**Figure 3.** PCR Confirmation of *Staph. aureus* among *Staph. aureus* Isolated from Poultry Farms in Kano State using *Staph. aureus* primer.



**Figure 4.** Molecular Characterization of *MecA* gene (162bp and 500bp respectively) among *Staph. aureus* Isolated from Poultry Farms in Kano State, Nigeria.

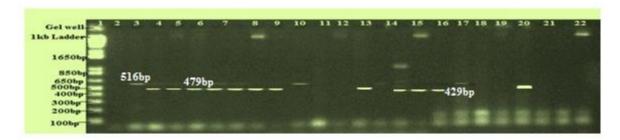
#### Molecular Analysis

Genomic DNA was extracted from the *Staph. aureus* isolates with multiple antibiotic resistance index ≥ 0.7 (Figure 2). Polymerase chain reaction showed that all the isolates tested were *Staph. aureus* of 800bp (Figure 3), 66.7% of the MDR isolates possess *MecA* gene (162bp) (Figure 4), while 33.3% had *MecA* of 500bp. Further analysis showed that 3 of the seven housekeeping genes (*pta, gmk* and *yqil*) were also present in the MDR isolates at 43.3, 20 and 16.7% respectively (Figure 5) while 10% express spa typing gene (Figure 6).

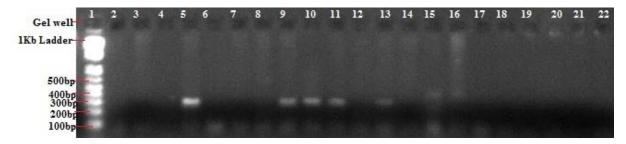
#### DISCUSSION

Staph. aureus remains one of the predominate health care emerging problem as a result of increase in pathogenicity and emergence of Methicillin-resistant Staphylococcus aureus (MRSA) with multidrug resistance

characteristics in hospitals, communities and in poultry (George, 2009; Michael and Robert, 2010; Jhalka et al., 2014). This rising problem calls for periodic surveillance, research and control measures in all areas suspected of Staph. aureus possible contamination especially in food industry (Trish and Emily, 2011). This study observed 33.8% (98/1260) occurrence rate of Staph. aureus from poultry farms and farm workers in Kano State, Nigeria (Table 3). This finding is lower than the report of Suleiman et al., (2013), who reported the occurrence of 83% Staph. aureus out of 100 tracheal swabs from 100 apparently healthy chickens from small house-holder flocks in Maiduguri, Nigeria, and Mohamed (2013) in Egypt who reported 40.8% (51/125) occurrence of Staph. Aureus but higher than the report of Neela et al., (2013) who reported 1.4% (7/503) in Malaysia. However, our result substantiates other reports that Staph. aureus could be isolated from animal trachea and nostril. High percentage of the isolates (30.6%) were observed to show resistance to cefoxitin; a presumptive identification



**Figure 5.** Molecular Characterization of the Seven Housekeeping genes (*pta* 474bp), *gmk* (429bp) and *yqil* (516bp) among *Staph. aureus* Isolated from Poultry Farms in Kano State.



**Figure 6.** Molecular Characterization of Spa gene (250-637bp) among *Staph. aureus* Isolated from Poultry Farms in Kano State.

for MRSA in Staph. aureus (Goran and Hong, 2005; Lisa et al., 2006; CLSI, 2013) and subsequently showed varying antibiotics resistance patterns (Table 4) and percentages resistance (Figure 1) from one isolate to another. This finding also concur with the report of Suleiman et al., (2013) who reported that coagulase positive S. aureus isolates were susceptible to ciprofloxacin and gentamycin but showed varying degrees of resistance to other antibiotics with most resistance to betalactam (Ampicillin and Amoxicillin). Eighty three point three percent (83.3%) of the isolates are MDR and have MARI > 0.3 (Table 4) showing that the isolates have been pre-exposed to antibiotics tested in this study with high multidrug resistance potential. This might be as a result of uncontrolled usage of antibiotics in poultry feeds for growth and diseases. Molecular characterization of mecA gene among these isolates showed that all the MDR isolates were Staph. aureus of 800bp (Figure 2 and 3), 66.7% of the MDR isolates also possess MecA gene (162bp), while 33.3% had MecA of 500bp (Figure 4). These findings concur with the report of Adeyeye and Adewale (2013) in South West Nigeria, who recorded 83.3% MRSA incidence in poultry attendants, and 95% in chickens from a poultry farm. MRSA of human-origin has been isolated from raw chicken meat or carcasses in Korea (Lee, 2006) and Japan (Kitai et al., 2005). Although, the pathogenicity of MRSA from different sources varies, those associated with poultry (livestock associated MRSA (LA-MRSA)) rarely cause disease (s). Also there are no known cases of people contracting MRSA from eating meat, if properly prepared (DH, 2013). However, LA-MRSA is a primary occupational risk for those in contact with affected livestock as it has been detected in the air of most barns (7 of 9, 77.8%), as well as in many samples originating from animals, with detections levels of 50 to 54% in broiler and 62 to 77% in turkey farms (Friese et al., 2013).

Reports have also highlighted the possibility of poultry products contamination by MRSA which when in contact with human nasal or skin lesion may lead to colonization and infection. Ingestion of poultry products contaminated with MRSA can cause gastro-intestinal colonization or food poisoning leading to staphylococcal enterotoxinassociated diarrhea and subsequent extra-intestinal infection or transmission (Feßler et al.,2010). Evolutionary analyses on the seven multilocus sequence typing (MLST) genes that encode for proteins of central metabolic functions which can only evolve through mutation and gene replacement, influencing genetic variation and causes phenotypic differences among population genetic (Enright et al., 2000; Lamers et al., 2011) was also carried out on the MDR MRSA to analyze the genetic diversity present in the isolates. The result showed that 3 of the seven housekeeping genes (pta, gmk and yail) were also present in the MDR isolates at 43.3, 20 and 16.7% respectively (Figure 5) while 10% express Spa typing (Figure 6). This implies that overtime, mutation and gene replacement, which influence genetic variation and causes phenotypic differences among population genetic of Staph. aureus had occurred in Kano

State, which could be the reason behind variation in antibiotic resistance profile expressed among the *Staph. aureus*. Also, there is a correlation between phenotypic cefoxitin resistance and carriage of *MecA* gene.

#### Conclusion and recommendation

This study isolated *Staph. aureus* with high resistance profile to commonly prescribed antibiotics in clinics from poultry farms in Kano State, Nigeria, which might be as a result of antibiotic usage in poultry farms, the presence of *MecA* gene and variation in population genetic induced by mutation among the isolated *Staph. aureus*. This study therefore recommends proper food hygiene and cooking in all abattoir and eateries, which is believed to reduce the risk of infection, transmission of resistance gene and possible colonization. Also strong awareness is advocated in other for consumers to understand the importance of proper handling of poultry products to avoid/reduce cross contamination of MRSA.

#### **CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

#### **REFERENCE**

- Adelisa L. P., David, H. C., Robert, P. G., Shailen, B., Tonya, S. H., James, S. T., & William, J. M. (1992). National Nosocomial Infections Surveillance System: Methicillin-Resistant Staphylococcus aureus in U.S. Hospitals, 1975–1991. Infection Control and Hospital Epidemiology, 13(10), 582-586.
- Adeyeye, J. O., & Adewale, A. O. (2013). Incidence of Methicillin-Resistant *Staphylococcus aureus* (MRSA)In a Small Poultry in South West, Nigeria. *Journal of Agriculture and Veterinary Science*, 5(3), 53-55.
- Cheesbrough, M. (2000). District laboratory practice in tropical countries (Part 11). Cambridge, University Press UK. Pp. 134-143.
- Cheesbrough, M. (2002). District Laboratory Practice in Tropical countries, Part 2. Cambridge University Press: Pp. 135-159.
- Clinical Laboratory Standard Institute (CLSI) (2014). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth. This document provides updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards M02-A11, M07-A9, and M11-A8. 30(1).
- de Lencastre, H., & Duarte, C. O. (2002) Multiplex PCR Strategy for Rapid Identification of Structural Types and Variants of the *mec* Element in Methicillin-Resistant Staphylococcus aureus. Antimicrob. Agents Chemother. 46(7), 2155-216.
- Department for Environment, Food & Rural Affairs, Animal Health and Veterinary Laboratories Agency, Department of Health, Public Health England and + others (DH) (2013). Livestock-associated MRSA found at a farm in East Anglia. https://www.gov.uk/government/news/livestock-associated-mrsa-found-at-a-farm-in-east-anglia.

- Enright, M. C., Day, N. P., Davies, C. E., Peacock, S. J., Spate, B. G. (2000). Multilocus sequence typing for characterization of methicillin resistant and methicillin susceptible clones of Staph aureus. Journal of Clinical Microbiology, 38(3), 1008-1015
- Feßler, A., Scott, C., Kadlec, K., Ehricht, R., Monecke, S. and Schwarz, S. (2010) Characterization of methicillin-resistant Staphylococcus aureus ST398 from cases of bovine mastitis. *Journal of Antimicrobial Chemotherapy* 65, 619-625.
- Food and Drug Administration (FDA) (2007). NARMS retail meat annual report, 2007. http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth /AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/UCM166132.pdf. Accessed 29 November 2010
- Friese, A., Schulz, J., Zimmermann, K., Tenhagen, B. A., Fetsch, A., Hartung, J., & Rösler, U. (2013). Occurrence of Livestock-Associated Methicillin-Resistant Staphylococcus aureus in Turkey and Broiler Barns and Contamination of Air and Soil Surfaces in Their Vicinity. *Appl. Environ. Microbiol.* 79(8), 2759-2766
- George, S, Pamela, A. M., Jerome S., Alan, F., Robert, C. M. J., & George, M. E. (2004). Relationship of MIC and Bactericidal Activity to Efficacy of Vancomycin for Treatment of Methicillin-Resistant Staphylococcus aureus Bacteremia. J. Clin. Microbiol. 42(6), 2398-2402
- George, Y. L. (2009). Molecular Pathogenesis of Staphylococcus aureus Infection. Pediatr Res. 65(5 Pt 2), 71R–77R.
- Goran, H., & Hong, F. (2005). Evaluation of Two New Chromogenic Media, CHROMagar MRSA and S. aureus ID, for Identifying Staphylococcus aureus and Screening Methicillin-Resistant S. aureus. J. Clin. Microbiol. 43(8), 4242-4244.
- Harmsen, D. C., Witte, H., Rothganger, W., Turnwald, D. J. (2003). Typing of methicillin-resistant *Staph. aureus* in a university hospital setting by using novel software for spa repeat determination and data base management. *Journal of Clinical Microbiology*, 41, 5442-5448.
- Igwe, J. C., Onaolapo, J. A., Dauda, E. O., & Oladipo, H. O. (2013). Plasmid Conjugation in *E. coli* and Drug Resistance. *Nigeria Journal of Biotechnology*; 26(1), 41-49
- Jhalka, K, Tara, C. S., & Dipendra, T. (2014). Staphylococcus aureus and Staphylococcal Food-Borne Disease: An Ongoing Challenge in Public Health. *BioMed Research International*, 2014 (2014), 9.
- Kitai, S., Shimizu, A., Kawano, J., Sato, E., Nakano, C., Uji, T., Kitagawa, H. (2005). Characterization of methicillin-resistant Staphylococcus aureus isolated from retail raw chicken meat in Japan. J. Vet. Med. Sci., 67(1),107-110.
- Klevens, R. M., Melissa, A. M., Joelle, N., Susan, P., Ken, G., Susan, R., Lee, H. H., Ruth, L., Ghinwa, D., John, M. T., Allen, S. C., Elizabeth, R. Z., Gregory, E. F., Linda, K. M., Roberta, B. C., & Scott, K. F. (2007). Invasive Methicillin-Resistant Staphylococcus aureus Infections in the United States. The Journal of the American Medical Association, 298 (15), 1763-1771.
- Lamers, R. P., Stinnett, J. W., Muthukrishnan, G., Parkinson, C. L., Cole, A. M. (2011). Evolutionary Analyses of *Staphylococcus aureus* Identify Genetic Relationships between Nasal Carriage and Clinical Isolates. *PLoS ONE*, 6(1), e16426. doi: 10.1371/journal.pone.0016426.
- Lee, J. H. (2006). Occurrence of methicillin-resistant Staphylococcus aureus strains from cattle and chicken, and

- analyses of their mecA, mecR1 and mecl genes. Vet. Microbiol, 114(1-2), 155-159.
- Lisa, L., Deirdre, S., Helen, M., Mary, V., & Andrew, E. S. (2006). Evaluation of a New Chromogenic Medium, MRSA *Select*, for Detection of Methicillin-Resistant *Staphylococcus aureus*. *J. Clin. Microbiol.*, 44(12), 4561-4563.
- Ma, X. X., Ito,T., Tiensasitorn, C., Jamklang, M., Chongtrakool,
  P., Boyle-Vavra, S., Daum, R. S., & Hiramatsu, K.
  (2002). Novel type of staphylococcal cassette chromosome
  mec identified in community-acquired methicillin-resistant
  Staphylococcus aureus strains. Antimicrob Agents
  Chemother., 46(4), 1147-1152
- Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, V, Weber, J. T., & Monnet, D. L. (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
- Michael, Z. D., & Robert, S. D. (2010). Community-Associated Methicillin-Resistant Staphylococcus aureus: Epidemiology and Clinical Consequences of an Emerging Epidemic. Clin Microbiol Rev., 23(3), 616-687.
- Mohamed, K. (2013). Prevalence of methicillin-resistant Staphylococcus aureus in poultry meat in Qena, Egypt. Veterinary World, 6(10), 711-715
- Moran, G. J., Krishnadasan, A., Gorwitz, R. J., Fosheim, G. E., McDougal, L. K., Carey, R. B., & Talan, D. A. (2006). Methicillin-resistant S. aureus infections among patients in the emergency department. N Engl. J. Med., 355(7), 666-674.
- Naimi, T. S., LeDell, K. H., Como-Sabetti, K., Borchardt, S. M., Boxrud, D. J., Etienne, J., Johnson, S. K., Vandenesch, F., Fridkin, S., O'Boyle, C., Danila, R. N., Lynfield, R., & Danila, R. N. (2003). Comparison of community-and health care associated methicillin-resistant Staphylococcus aureus infection. The Journal of the American Medical Association, 290(22), 2976-2984.
- Neela, V., Ghaznavi-Rad, E., Ghasemzadeh-Moghaddam, H., Nor-Shamsudin, M., van Belkum, A. & Karunanidhi, A. (2013). Frequency of methicillin resistant *Staphylococcus aureus* in the noses of Malaysian chicken farmers and their chicken. *Iranian Journal of Veterinary Research, Shiraz University*, 14(3), 226-231.

- Oliveira, D. C., de Lencastre, H. (2011). Methicillin-Resistance in *Staphylococcus aureus* Is Not Affected by the Overexpression in Trans of the *mecA* Gene Repressor: A Surprising Observation. *PLoS ONE*, 6(8), e23287.
- Onaolapo, J. A. (2005). Isolation and identification of *Staph. aureus* from other microbial contaminants using increased salt concentration media. In: Bacterial Genetics Lecture Note. Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria. Unpublished.
- Paul, S., Bezbarauh, R. L., Roy, M. K., & Ghosh, A. C. (1997).
  Multiple antibiotic resistance (MAR) index and its reversion in Pseudomonas aeruginosa. Letters in Applied Microbiology, 24,169-171.
- Sara, E. C., George S., Eli, N. P., Mitchell, J. S., Adolf, W. K., & Yehuda, C. (2003). Comparison of Mortality Associated with Methicillin-Resistant and Methicillin-Susceptible Staphylococcus aureus Bacteremia: A Meta-analysis. Clin Infect Dis., 36(1), 53-59.
- Shopsin, B., Gomez, M., Montgomery, S. O., Smith, D. H., Waddington, M., Dodge, E. E., Bost, A., Riehman, M., Naidich, S., & Kreiswirth, B. N. (1999). Evaluation of ProteinA Gene Polymorphic Region DNA Sequencing for Typing of Staph. aureus Strains. Journal of Clinical Microbiology, 37(11), 3556-356.
- Suleiman, A, Zaria, L. T, Grema, H. A., & Ahmad, P. (2013). Antimicrobial resistant coagulase positive *Staphylococcus aureus* from chickens in Maiduguri, Nigeria. *Sokoto Journal of Veterinary Sciences*, 11(1), 51-55.
- Trish, M. P., & Emily, R. M. S. (2011). Hospital Epidemiology and Infection Control in Acute-Care Settings. *Clin. Microbiol. Rev.*, 24(1), 141-173.