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DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN CHICKEN CARCASSES AND LIVE BIRDS IN ZARIA, NIGERIA

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ABSTRACT

Methicillin resistant *Staphylococcus aureus* (MRSA) associated with livestock is an emerging threat to public health. Poultry production represents a key interface for the spread of such novel zoonotic and antibiotic resistant pathogens. The occurrence of MRSA in chickens in two live bird markets and in chickens presented for clinical purposes to the Avian Unit of Ahmadu Bello University Veterinary Teaching Hospital, Zaria, Nigeria was investigated. One thousand four hundred samples in the form of swabs of trachea (400), cloaca (400), skin surface (400) and joint (200) were collected from 200 live chickens and 200 chicken carcasses and examined for the presence of *Staphylococcus aureus*. Thirteen (0.9%) of the samples, 7 from carcasses of slaughtered chickens and 6 from live birds in the live bird markets contained coagulase positive *S. aureus*. None was isolated from chickens in the avian clinic. The 13 *S. aureus* isolates were subjected to polymerase chain reaction (PCR) to detect the presence of *mecA* gene that codes for methicillin resistance. A PCR product of 533bp size consistent with the expected PCR product size of *mecA* gene from a positive control strain was detected in 1 of the 13 *S. aureus* isolates. The presence of MRSA in chicken carcasses and live birds in Zaria, Nigeria was demonstrated, highlighting the potential role of poultry production in the spread of MRSA.

Keywords: Methicillin resistant *Staphylococcus aureus*, *mecA* gene, chickens

INTRODUCTION

The interaction between animal production and human health has been a subject of research and social debate for quite some time. Since the Swann report in Britain (Swan, 1969), a lot of attention has been given to the spread of antibiotic resistant pathogens of animal origin through the food chain to man. The resistant bacteria that have recently attracted attention include *Escherichia coli*, *Salmonella*, *Enterococcus*, and *Staphylococcus* (Foley *et al.*, 2008; Ruzauskas *et al.*, 2009; Karmi, 2013;

Zarfel *et al.*, 2014). The reservoirs of concern are related to intensively farmed animals including poultry. The use of antibiotics in poultry production brings about an increase in resistance to antibiotics not only in pathogenic bacterial strains, but also in commensal bacteria (Lukasova and Sustackova, 2003). Consequently, the extensive use and misuse of these antimicrobial agents either for treatment or growth promotion is bound to contribute to the selection of drug-resistant strains of microorganisms. Poor veterinary control over

administration of antibiotics common in countries like Nigeria exacerbates the problem of drug-resistant microorganisms in animal production. The emergence of resistant pathogenic bacteria has implications for both human and veterinary medicine. This problem exists within an interface between human and animal health making it more difficult to separate under prevailing medical and agricultural practices. It is within this interface that Livestock-Associated MRSA (LA-MRSA) emerged possibly from Community-Acquired MRSA (CA-MRSA). Methicillin resistant *Staphylococcus aureus* represents a major public health challenge as effective therapeutic options are becoming limited (Sandrine *et al.*, 2008). The major mechanism of resistance of MRSA to β -lactam antibiotics is due to the acquisition of the *mecA* gene encoding an additional penicillin-binding protein (PBP-2a) that can function as transpeptidase (Chambers, 1987). Presence of MRSA in chickens will invariably lead to an increased cost in medical care as well as promote the spread of MRSA due to the fact that birds are usually reared in large numbers and in close proximity to human habitation in Nigeria. It is essential to monitor the spread of pathogens such as MRSA and those that will impact heavily on public health. The present study was conducted to determine the presence of MRSA in chicken carcasses and live birds in Zaria, Nigeria and to determine the presence of *mecA* genes in coagulase positive *S. aureus* isolates.

MATERIALS AND METHODS

Sample collection

A total of 1400 samples consisting of 400 trachea, 400 cloaca, 400 skin surface and 200 joints were collected from 200 live birds and 200 chicken carcasses. Samples were collected from live chickens in live bird markets, from routinely slaughtered chickens and those from chickens during necropsy at the Avian Clinic of the ABU Veterinary Teaching Hospital (ABUVTH). Sampling from live birds was done using simple random sampling (marketers were assigned even and odd numbers, from which random selection was made) within the two major markets in Zaria. Tracheal, cloacal and skin surface swabs each were collected from the 200 live birds (comprising 125 broilers and 75

layers). Tracheal, cloacal, joint and skin surface swabs were also collected from 200 randomly slaughtered chickens (comprising 90 broilers, 80 layers, 20 cockerels and 10 local free range hens) at slaughter points in both markets. The third set of samples was collected from 50 chickens (layers) at necropsy in the Avian Clinic of the ABUVTH, consisting of tracheal, cloacal, joint and skin swab samples from each bird. Samples were obtained by inserting a sterile swab stick in the trachea or cloaca and gently rubbing the swab against the mucosa surface for approximately 5-10 seconds. For Skin surface swabbing, the feathers of bird were lifted up, and a moistened sterile swab stick was used to swab an area of approximately 2 by 2cm on either side of the keel.

Isolation of *Staphylococcus aureus*

Swab samples collected from the cloaca, trachea, joints, and surfaces of chicken carcasses as well as those from live birds were inoculated into 10ml trypticase soy broth containing 70mg/ml NaCl and incubated at 37 °C for 24h. A loopful of the inoculum from the enrichment broth above was streaked on Baird Parker agar and incubated at 37 °C for 24h.

Identification of isolates

Typically black, shiny, convex colonies with a narrow, white edge surrounded by a clear zone on Baird Parker agar were picked from the plate and inoculated into nutrient agar slants, incubated at 37 °C for 24hours, and stored in a refrigerator at 4°C as stock cultures. The isolates were later characterized using conventional methods that included Gram staining, catalase, coagulase, DNase test and sugar fermentation tests. Antibigram of the isolates was determined by the disk-diffusion method and reported previously (Otalù *et al.*, 2011).

DNA extraction

The genomic DNA from *S. aureus* isolates was extracted using a previously described method that employs the use of vancomycin (Japoni *et al.*, 2004). Pure DNA was prepared by growing bacteria in 5 ml Trypticase Soy broth for 18 h. The bacterial suspension was swabbed on Trypticase Soy agar while the surface of the medium was covered with standard vancomycin discs (3 discs for each plate) and incubated overnight. The bacterial colonies from edges of the zone of inhibition were then resuspended in

sterile distilled water and matched to 0.5 Mac Farland standard. The bacterial suspension was heated at 95°C for 15 min and cooled at room temperature. Crude lysate mixture (2.5 µl) was used as DNA template.

Amplification of the *mecA* gene

Amplification of the *mecA* gene was performed using the primers *mecA1* (5¹ AAAATCGATGGTAAAGGTTGGC3¹) and *mecA2* (5¹-AGTTCTGCAGTACCGGATTTGC 3¹), yielding a 533bp product (Murakami *et al.*, 1991). Polymerase chain reaction was performed in a 25µl volume with a PCR buffer containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, a 200µM concentration of each deoxynucleoside triphosphate (Promega, Madison, Wis), 1.25 U of Taq polymerase (Promega), and a 0.25µM concentration of each primer. DNA amplification was carried out for 40 cycles in 25µl of reaction mixture as follows: denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1 minute with a final extension at 72°C for 5 minutes. PCR products were resolved using electrophoresis on a 1% agarose gel at 80V for 45mins. A 100bp ladder molecular weight marker (Fermenters, UK) was included to determine band size. A positive result was inferred by detection of a 533 bp band, which represents the expected amplicon size of the *mecA* gene. Positive control

DNA was derived from a known MRSA strain (*S. aureus* ATCC 35591) bearing *mecA* gene.

RESULTS

Detection of *S. aureus* in chicken carcasses and live birds

Out of the 1400 samples examined bacteriologically on Baird Parker agar, 13 (0.93%) were found to contain coagulase positive *Staphylococcus aureus*. Of the 13 coagulase positive *S. aureus* isolates, 7 (53.85%) were recovered from slaughtered birds while 6 (46.15%) were recovered from live birds. Tracheal samples from both slaughtered and live birds yielded equal number of *S. aureus* isolates (Table 1). Broilers yielded more *S. aureus* isolates compared to other chicken types tested and was followed by layers (Table 2). One isolate was recovered from a cockerel while none was isolated from local free range chickens.

Detection of *mecA* in coagulase positive *S. aureus* isolates

PCR detection of the presence of *mecA* gene in DNA of 13 coagulase positive *S. aureus* isolates revealed the presence of ~533bp amplicon which is the expected PCR product size in an isolate that aligned with amplicon from a control MRSA strain known to possess the *mecA* gene (Plate 1). The *mecA* positive isolate was found in a tracheal sample obtained from a slaughtered broiler chicken.

Table 1: Coagulase positive and methicillin resistant *Staphylococcus aureus* from different sites of chicken carcasses and live birds in Zaria, Nigeria.

Site	Number of <i>S. aureus</i> from chicken carcasses		Number of <i>S. aureus</i> from Live chickens	
	Coagulase +ve	MRSA (%)*	Coagulase +ve	MRSA (%) ^a
Trachea	4	4 (100.0)	4	2 (50.0)
Cloaca	2	1 (50.0)	2	1 (50.0)
Skin surface	1	0 (0.0)	0	0 (0.0)
Joint	0	0 (0.0)	0	0 (0.0)
Total	7	5 (71.5)	6	3 (50.0)

^aExpressed as a percentage of coagulase positive isolates.

Table 2: Coagulase positive and methicillin resistant *Staphylococcus aureus* from different types of chicken carcasses and live birds in Zaria, Nigeria.

Chicken Type	Number of <i>S. aureus</i> from chicken carcasses		Number of <i>S. aureus</i> from Live chickens	
	Coagulase +ve	MRSA (%)*	Coagulase +ve	MRSA (%) ^a
Broiler	4	3 (75.0)	3	1 (33.3)
Layer	2	2 (100.0)	3	1 (33.3)
Cockerel	1	1 (100.0)	0	0 (0.0)
Local	0	0 (0.0)	0	0 (0.0)
Total	7	6 (85.7)	6	2 (33.3)

^aExpressed as a percentage of coagulase positive isolates.

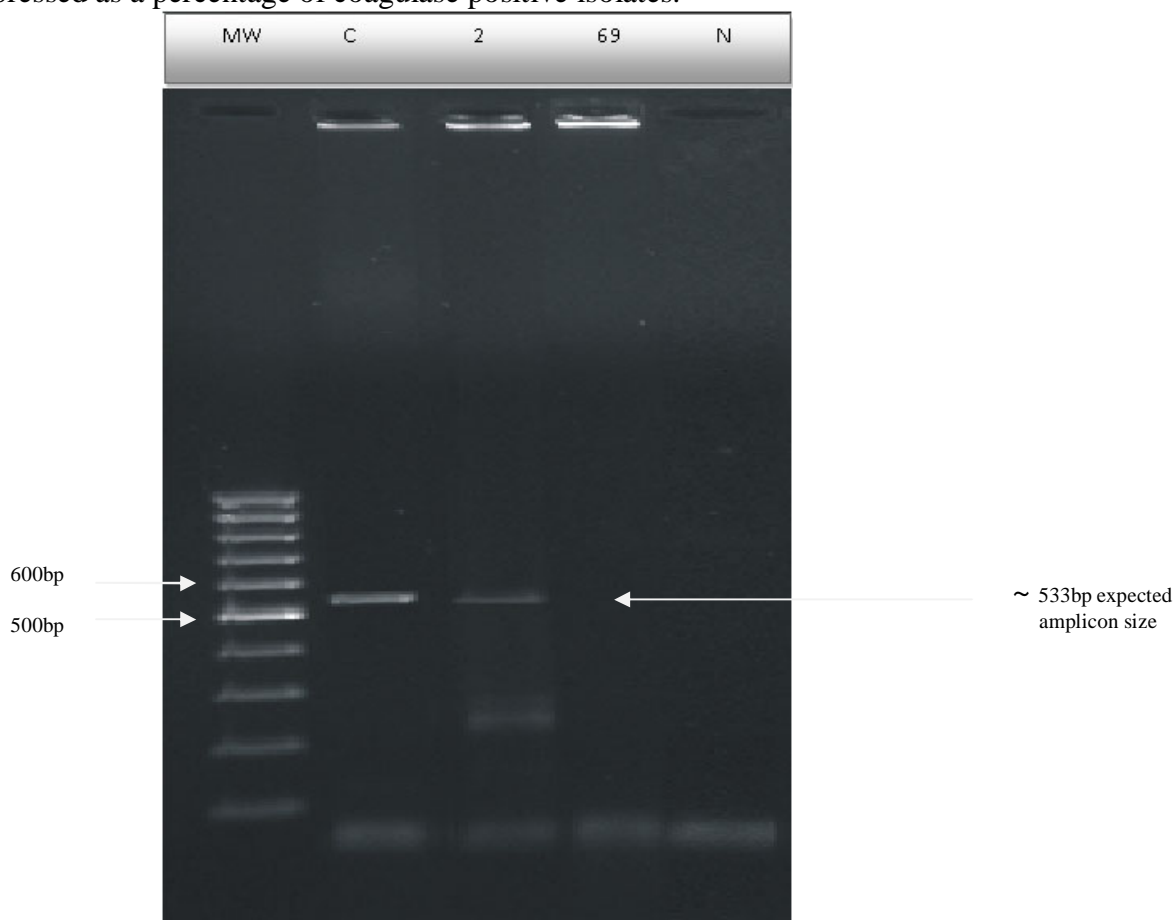


Plate1: PCR amplification of *mecA* gene demonstrating 533bp product in positive isolate

MW- 100bp molecular weight marker; C- positive control (ATCC 35591); 2- *mecA* positive isolate; 69- *mecA* negative isolate; N- negative control (Sterile distilled water).

DISCUSSION

The proliferation of antibiotic resistance genes on transposons and plasmids in animal populations that is a direct result of the widespread use of antibiotics is increased by the ease of dissemination of resistant strains between animals via faecal contact, especially among animals housed together in large groups, as with intensive farming units including poultry (Murray, 1994). The isolation of only 1 *mecA* positive isolate from 1400 (0.1%) samples in this study has demonstrated contamination of chickens with MRSA albeit at low level. This agrees with the findings of Lee (2003) who reported the isolation of 3 MRSA strains from 296 chicken samples in the Republic of Korea and Kitai *et al.* (2005), who reported 2 MRSA from 444 samples of chicken meat in Japan. Also the isolation of MRSA from broiler chicken agrees with the work of Persoons *et al.* (2009), who found *mecA* positive isolates only in broilers and none in layers. No MRSA isolate was recovered from local free range chickens. This may be as a result of non-administration of antimicrobial drugs in this category of chickens. The presence of MRSA in chickens confirms the fact that the administration of antimicrobials to food animals for growth promotion, prophylaxis, and treatment can lead either to the selection of resistant bacteria, which can be transmitted through the food chain (Witte, 2000; Mayrhofer *et al.*, 2004), or to the horizontal transfer of resistance genes to human pathogenic or commensal microflora (McDermott *et al.*, 2002). The epidemiologic factors responsible for the occurrence of MRSA in the samples tested were not determined within the scope of this work. However, the isolation of MRSA in this study may not be unconnected with category of bird, environmental, and personal hygiene by the butchers.

Several workers have reported the need for modifying DNA extraction protocols for successful release of DNA template for PCR of *Staphylococcus*. The method adopted in this study is one of such modifications that involved growing the organisms in the presence of vancomycin a well known and potent inhibitor of the cell wall synthesis (Suzuki *et al.*, 2003). The use of vancomycin is an inexpensive method. However, it has been reported that

vancomycin-induced stress could lead to the deletion of *mecA* in some strains of MRSA in which the deletion correlated with increased resistance to vancomycin (Adhikari *et al.*, 2004). It cannot be ruled out that the incidence of *mecA* could have been higher if another method that does not interfere with the *mecA* was utilized. A number of vancomycin resistant isolates were also noted in this study. It is not obvious whether the expression of the vancomycin resistance was related to *mecA* loss. Spontaneous loss of SCC*mec* has also been reported to occur in some lineages of *S. aureus* in vivo (Deplano *et al.*, 2000; Donnio *et al.*, 2002). The presence of MRSA in chickens as determined in this study raises serious public health issues as poultry provides rich source of protein and is widely consumed, hence the ease of spread of the pathogen in the community. There is also an enhanced possibility of horizontal spread within an infected flock owing to the compact nature in which birds are raised in intensive farming system. If MRSA becomes established in poultry populations, there is the possibility that free range birds which migrate great distances freely may interact with wildlife, thereby facilitating the dissemination of the pathogen. To the best of our knowledge, this study demonstrated for the first time the presence of *mecA* positive MRSA from chickens in Nigeria. Thus poultry and poultry products can be contaminated by MRSA and that poultry production is part of the transmission circle of MRSA. Future studies may further determine not just the presence of *mecA*, but other associated genes and typing so as to validate if the MRSA isolates are Livestock-Associated MRSA (LA-MRSA). Also, there is the need to carry out extensive investigation on poultry production and processing environment for microbial contamination especially with multidrug resistant organisms such as MRSA, so as to prevent the spread of pathogenic organisms to man via the food chain of poultry and poultry products.

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