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# Antimicrobial resistant coagulase positive *Staphylococcus aureus* from chickens in Maiduguri,Nigeria

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**Abstract**

*Staphylococcus aureus* is an Important agent of food poisoning. In many countries, it is the main bacterial organism responsible for diseases caused by exotoxin production and direct invasion with systemic dissemination. In poultry, *S. aureus* is associated with many clinical syndromes including tenosynovitis, omphalitis, femoral head necrosis, infected hock and stifle joints secondary to coccidiosis and "bumblefoot". A total of 100 tracheal swabs from 100 apparently healthy chickens from small holder flocks in Maiduguri, Nigeria were analysed for the presence of *S. aureus*. Isolates of coagulase positive *S. aureus* resistance to 10 antimicrobials was determined by disc diffusion method. *Staphylococcus aureus* was isolated from 83 (83.0%) and coagulase positive *S. aureus* from 54 (54%) of all samples. Isolates of coagulase positive *S. aureus* were completely (100%) susceptible to cephalexin, ciprofloxacin and gentamycin but showed varying degrees of resistance to ampicillin (100%), erythromycin (100%), ceftriazone (57.4%), ofloxacin (57.4%), clindamycin (51.9%), cotrimoxazole (50.0%) and augmentin (33.3%).

*Keywords*: Antimicrobial, Coagulase Positive, Nigeria, Poultry, *Staphylococcus aureus*.

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**Introduction**

Bacterial organisms of the genus *Staphylococcus* are one of the most prevalent pathogens in both humans and animals (Casey *et al.,* 2007). *Staphylococcus aureus* is an important agent of food poisoning all over the world (Aarestrup *et al*., 1995; Balaban & Rasooly, 2000). It is the most important bacterial agent implicated in toxin production (toxic shock and staphylococcal scalded-skin syndromes), direct invasion and systemic disseminating (bacteremia, septic shock syndrome, skin infections and abscesses) (Hatakka *et al*., 2000; Martineau *et al*., 2000).

Although it is found in water, dust, and air, *S. aureus* primarily colonises the mucosa of nasopharynx and skin of humans and animals (Songer & Post, 2005). The bacterium is considered to be a normal flora of the chicken, isolated from the skin and feathers as well as in the respiratory and intestinal tracts (Sleekes, 1997; Casey *et al.,* 2007). However, some of the common forms of *S. aureus* associated poultry infections include tenosynovitis (Butterworth, 1999), omphalitis (Hill *et al*., 1989), femoral head necrosis, infected hock and stifle joints secondary to coccidiosis or vaccine reactions (McNamee & Smyth, 2000) and "bumblefoot" (Skeeles, 1997).

In Nigeria and many other parts of the developing world, chickens are extensively reared in close proximity to human dwellings and therefore play an important role in environmental contamination, in addition to serving as significant vehicles for the transfer of such pathogens to humans by way of handling of live birds or consumption of contaminated meat and other poultry products.

Bacterial antimicrobial resistance is a global concern that has been aggravated by the fact that only few new antimicrobial agents come out from the pharmaceutical channels in recent times (Talbot *et al*., 2006; Okonko *et al*., 2009). Selective increase in resistant bacteria has been reported to be as a result of widespread use of antibiotics in animals for growth promotion and prevention of colonization by pathogenic bacteria (Munoz *et al*., 1993; Davies, 1994).

Even though poultry industry in Nigeria contributes substantially to the Nation's Food Security and Gross Domestic Product (FAO, 2008), most of the flocks are reared by small scale farmers under limited or no veterinary supervision. In such production systems, antimicrobials are freely used as feed or water additives (Kabir *et al*., 2004; Otalu *et al*., 2011). These practices can facilitate the emergence and spread of antibiotic resistant pathogens among birds with possible transmission to humans. The present study was therefore conducted to determine the resistance of *S. aureus* isolates from small holder flocks in Maiduguri, Nigeria to some of the commonly used antimicrobials.

**Materials and methods**

*Study area*

The study was carried out in Maiduguri, the capital of Borno state, North-eastern Nigeria. The state has an estimated area of 69, 435 kilometre square and lies between latitude 10°N and 13°N and longitude 12°E and 15°E. The state is located between the Sahel and Sudan savannah vegetation zones with daily temperatures ranging from 32-40°C and generally low relative humidity throughout the year (Arku *et al*., 2012).

*Isolation and identification of Staphylococcus aureus*

A total of 100 tracheal swabs collected from 100 apparently healthy free ranging and semi intensively managed chickens raised by small holder farmers in Maiduguri were tested for the presence of *S. aureus*. *Staphylococcus aureus* was isolated and identified from the samples using conventional methods which included growth on mannitol salt agar (Oxoid, UK), Gram staining and microscopy, catalase, oxidase, coagulase and sugar fermentation tests as described by Geidam *et al.* (2012b).

*Antimicrobial susceptibility test*

Antimicrobial susceptibility Test (AST) was carried out for the coagulase positive *S. aureus* isolates using disc diffusion method according to Clinical and Laboratory Standards Institute methods (CLSI, 2010). Briefly, each of the *S. aureus* isolates was inoculated into nutrient broth (Oxoid, UK) and incubated at 37°C for 24 hours before they were tested. Turbidity of the growing culture was adjusted to correspond with that of a barium sulphate (0.5 MacFarland) standard. About 0.1 ml of the nutrient broth culture was subsequently inoculated onto Mueller Hinton agar plates and spread over the surface with sterile cotton swabs. Antimicrobial discs were then placed on the surface of each plate by means of antibiotic disc dispenser and incubated at 37°C for 24 hours. Diameters of inhibition zone were measured using a transparent ruler and the interpretative breakpoints for resistance were determined by comparing zone diameters as recommended by Clinical and Laboratory Standards Institute (CLSI, 2010). The coagulase positive *S. aureus* isolates were tested against a panel of 10 antimicrobials namely; ampicillin (30µg), augmentin (amoxicillin clavulanate) (30 µg), cephalexin (30 µg), ceftriazone (30 µg), ciprofloxacin (5 µg), clindamycin (10 µg), cotrimoxazole (50 µg), erythromycin (10 µg), gentamycin (10 µg) and ofloxacin (5 µg).

**Results**

*Staphylococcus aureus* was isolated from 83 (83%) of the tracheal swabs collected. Coagulase positive *Staphylococcus aureus* was isolated from 54(54%) of the samples. All the coagulase positive *S. aureus* isolated in the study were resistant to ampicillin and erythromycin but susceptible to ciprofloxacin, gentamycin, and cephalexin. Thirty one (57.4%) of the coagulase positive *S. aureus* isolates were resistant to both ceftriazone and ofloxacin. This was followed by clindamycin (51.9%), cotrimoxazole (50.0%) and augmentin (33.3%) in decreasing order of resistance (Table 1). All of the coagulase positive *S. aureus* isolates were resistant to at least three antimicrobials with most being resistant to multiple clinically important antimicrobial classes (Table 2).

**Table 1**: Antimicrobial resistance of coagulase positive *Staphylococcus. aureus* isolates from chickens in Maiduguri, Nigeria

| Antimicrobials | Number resistant (%) |
|---|---|
| Ampicillin (AK) | 54 (100) |
| Erythromycin (E) | 54 (100) |
| Ceftriazone (CRO) | 31 (57.4) |
| Ofloxacin (OFX) | 31 (57.4) |
| Clindamycin (DA) | 28 (51.9) |
| Cotrimoxazole (STX) | 27 (50.0) |
| Augmentin (amoxicillin clavulanate) (AMC) | 18 (33.3) |
| Cephalexin (CE) | 0 (0) |
| Ciprofloxacin (CIP) | 0 (0) |
| Gentamycin (CN) | 0 (0) |

**Table 2**: Antimicrobial resistance patterns of coagulase positive *Staphylococcus. aureus* isolates from chickens in Maiduguri, Nigeria

| Resistance Patterns | Number of Isolates |
|---|---|
| AK, AMC, CRO, DA, E, OFX | 1 |
| AK, AMC, CRO, DA, E, STX | 2 |
| AK, CRO, DA, E, OFX, STX | 2 |
| AK, AMC, CRO, DA, E | 2 |
| AK, AMC, CRO, E, OFX | 2 |
| AK, AMC, DA, E, OFX | 2 |
| AK, AMC, DA, E, STX | 1 |
| AK, CRO, DA, E, OFX | 2 |
| AK, CRO, DA, E, STX | 4 |
| AK, CRO, E, OFX, STX | 2 |
| AK, DA, E, OFX, STX | 2 |
| AK, AMC, CRO, E | 4 |
| AK, AMC, E, OFX | 1 |
| AK, AMC, E, STX | 1 |
| AK, CRO, DA, E | 4 |
| AK, CRO, E, STX | 5 |
| AK, DA, E, OFX | 4 |
| AK, DA, E, STX | 2 |
| AK, E, OFX, STX | 3 |
| AK, AMC, E | 2 |
| AK, CRO, E | 1 |
| AK, E, OFX | 2 |
| AK, E, STX | 3 |

**Discussion**

The extensive antimicrobial usage in Nigerian poultry industry has no doubt enhanced the capacity of *S. aureus* to acquire various resistance genes and thus become more virulent (Otalu *et al*., 2011). All the 54 coagulase positive *S. aureus* isolates recovered were resistant to ampicillin and erythromycin and all isolates were susceptible to ciprofloxacin, gentamycin, and cephalexin. Similar patterns of antimicrobial susceptibility have been reported in Nigeria (Otalu *et al*., 2011) and such other countries as Italy (Pesavento *et al*., 2007), United States (Waters *et al*., 2011) and Ireland (Leonard and Markey, 2008) where occurrence of multidrug resistant *S. aureus* in poultry is rather frequent. However, Geidam *et al*. (2012b) reported a lower resistance of 53% and 85% for ampicillin and erythromycin respectively in *S. aureus* isolates from commercial poultry farms within Selangor area of Malaysia. This may be connected to the fact that antimicrobial use in commercial poultry settings is more likely to be regulated than in small holder farms. In addition, veterinarians and poultry farmers generally use these antimicrobials for specific treatment of staphylococcal infections in Nigeria (Otalu *et al*., 2011). Thus, sub-therapeutic use of such drugs as prophylaxes, growth promoters or inaccurate dosages given to sick flocks by unqualified personnel may likely result in plasma concentrations that are inconsistent with the desired objectives which might possibly have led to the level of resistance observed in the present study.

All of the isolates were resistant to at least three of the antimicrobials tested. This finding may be reflective of the extent of poor drug control and legislations concerning sales of antimicobials in Nigeria (Geidam *et al*., 2012a). This is in agreement with findings of Olonitola *et al*. (2007) and Taiwo *et al*. (2008) in which resistance to methicillin and other clinically important antibiotics occur rather frequently in human *S. aureus* isolates. Beta-lactam antibiotic resistant *Staphylococci* have also been suspected of being methicillin-resistant strains (Van Duijkeren *et al*., 2004) and may carry the *mec*A chromosomal gene responsible for production of the altered penicillin binding protein PBP-2a (Martineau *at al*., 2000). This is of major public health concern since methicilin is not reportedly used in poultry (Bager *et al*., 1997) and is mostly the drug of choice in the treatment of human Staphylococcal infections. The findings of this study therefore emphasize the need to legislate and enforce laws that will limit the prescription and dispensation of antimicrobials to only qualified professionals. In addition, sensitivity of the isolates to ciprofloxacin, gentamycin, and cephalexin may be a pointer that these antimicrobials are less abused in the studied area and may possibly be recommended for treatment of Staphylococcal infections in poultry. The level of antimicrobial resistance shown by poultry isolates of Coaglase positive *S. aureus* reported here might have been underestimated as the present work laid emphasis only on live birds in small size flocks. Commercial flocks, live bird markets, poultry litter and manure may also play vital roles in propagation of resistant bacteria.

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