ISSN 1682-8356
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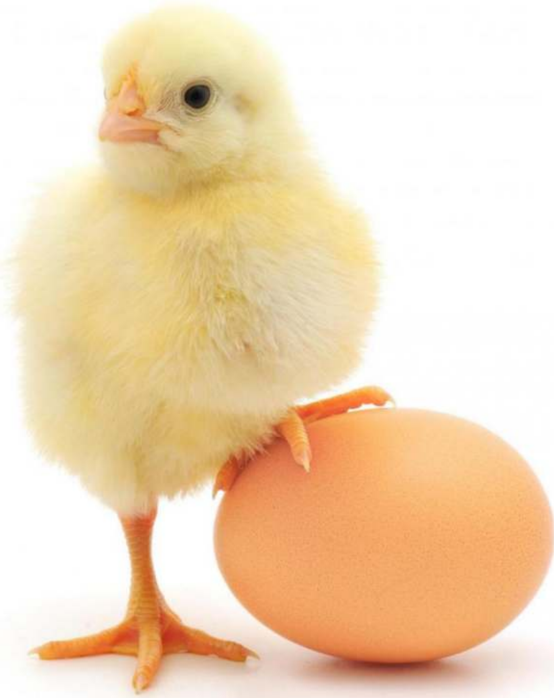

INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

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International Journal of Poultry Science 10 (11): 871-875, 2011
ISSN 1682-8356

Multi-drug Resistant Coagulase Positive *Staphylococcus aureus* from Live and Slaughtered Chickens in Zaria, Nigeria

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Abstract: A total of 400 samples were collected from 200 live chickens and 200 slaughtered chickens and examined for the presence of *S. aureus*. The susceptibility of 13 coagulase positive *Staphylococcus aureus* isolates from chickens in Zaria, Nigeria to 12 antimicrobials was determined by disk diffusion method according to CLSI standards. Coagulase positive *S. aureus* isolates had 100% resistance to tetracycline, penicillin and erythromycin and were in addition resistant to other antibiotics including vancomycin (46.2%).

Key words: *Staphylococcus aureus*, antibiotic resistance, Class 1 integron

INTRODUCTION

Bacterial antimicrobial drug resistance is a worldwide problem that is exacerbated by the diminishing number of new antimicrobial drugs in the pharmaceutical pipeline (Talbot *et al*., 2006; Okonko *et al*., 2009) and the effectiveness of currently available antibiotics is decreasing due to the increasing number of resistant strains causing infections (Nawaz *et al*., 2009). The poultry industry in Nigeria with a population of 140 million birds produces an estimated one billion table eggs and 500,000 metric tons of poultry meat annually contributing substantially to the nation's Gross Domestic Product and Food Security (FAO, 2008). Majority of the poultry flocks are raised by small holder farmers with limited veterinary supervision. Under these production conditions, antibiotics are liberally used (Kabir *et al*., 2004). Similarly, antibiotics are permitted as additives to feed or water in the country. The global use of antibiotics in humans, to treat infections and in animals, to promote growth and prevent colonization by pathogenic bacteria, has led to selective increase in resistant bacteria (Munoz *et al*., 1993; Davies, 1994). Several mechanisms involving mobile genetic elements such as plasmids and transposons, have been shown to contribute to the wide spread distribution of antibiotic resistant genes among bacteria. The role of integrons in horizontal transfer of antibiotic resistance through carriage of multiple resistance gene cassettes is well established (Hall and Stokes, 1993; Hall and Collis, 1995; Xu *et al*., 2007). Simultaneous carriage of more than 60 gene cassettes have been described, including cassettes conferring resistance to aminoglycosides, penicillins, cephalosporins, carbapenems, trimethoprim, chloramphenicol, rifampin, erythromycin, sulphonamide and quartenary ammonium compounds (Recchia and Hall, 1995; Mciver *et al*., 2002; Yan *et al*., 2010). Class 1 integrons on transferable elements like conjugative plasmids or transposons, play an important role in the dissemination of antimicrobial resistance genes (Fluit and Schmitz, 1999).

Chickens are extensively reared in close proxy to human habitation in the country and can thus play an important role in the contamination of the environment with pathogens as well as serving as an important vehicle for the transfer of these pathogens to humans through the handling and consumption of its meat. Live bird markets serve as collection points for birds from various sources in Nigeria and conditions under which these birds are kept coupled with the fact that some of the birds stay for long periods of time before sale can facilitate the emergence and spread of pathogenic organisms among birds and possible transmission to humans.

Bacteria of the genus *Staphylococcus* is among the most significant and widespread bacterial pathogens in humans and animals. *Staphylococcus aureus* is responsible for diseases caused by exotoxin production (toxic shock and staphylococcal scalded-skin syndromes) and by direct invasion and systemic dissemination (bacteremia, septic shock syndrome, skin infections and abscesses) (Martineau *et al*., 2000).

A noted property of Staphylococci is their ability to become resistant to several antimicrobials. The spread of resistance in *S. aureus* is largely due to the acquisition of plasmids and/or transposons (Lyon and Skurray, 1987). The emergence of antimicrobial-resistant pathogens has been associated with increased morbidity and mortality in patients (Holmberg *et al*., 1987; Romero-Vivas *et al*., 1995) and increased

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cost of medical care particularly in developing countries, where there are limited, generally unaffordable therapeutic options. The increased cost attributed to treating resistant infections also include the need for prolonged hospitalization, implementation of isolation or barrier precautions and increased laboratory utilization for more extensive surveillance or screening (Kim *et al*., 2001). The present study was conducted to determine the susceptibility of poultry isolates to commonly used antimicrobials.

MATERIALS AND METHODS

Identification of *Staphylococcus aureus*: A sample of 400 birds comprising 200 live birds from major live bird markets and 200 chicken carcasses collected from slaughter slabs and carcasses submitted for post mortem at the Avian Unit of the Ahmadu Bello University Veterinary Teaching Hospital in Zaria over a 6 month period was tested for the presence of *S. aureus*. Twenty-three *S. aureus* isolates were recovered using Baird Parker agar (Oxoid, Bakinstoke, UK) and characterized using conventional methods that included Gram staining, catalase and coagulase test, sugar fermentation and test for DNase, out of which 13 were found to be coagulase positive.

Antibiotic susceptibility test: Each of the 13 *S. aureus* isolate was inoculated into nutrient broth (Oxoid) at 37°C for 24 h before testing. The turbidity of the actively growing culture was adjusted to correspond with that of a barium sulphate (0.5 MacFarland) standard. Subsequently 0.1 ml of the nutrient broth culture was inoculated onto Mueller Hinton agar plates (90mm diameter disposable petri dishes) and spread over the surface with sterile cotton swabs. Six antimicrobial disks were then placed on the surface of each plate by means of antibiotic disk dispenser and incubated at 37°C for 18 hrs. Inhibition zone diameters were measured using a transparent ruler and the interpretative breakpoints for resistance determined by comparing zone diameters according to the Clinical Laboratory Standards Institute (CLSI, 2002) guidelines. The reference strain used for quality control was *S. aureus* ATCC 25923. Thirteen coagulase positive *S. aureus* strains were tested using a panel of twelve antimicrobials namely, sulphamethoxazole/trimethoprim, tetracycline, gentamicin, methicillin, amikacin, amoxycillin/clavulanic acid, penicillin, chloramphenicol, erythromycin, vancomycin, oxacillin and ciprofloxacin.

RESULTS

The antibiotic susceptibility of 13 coagulase positive *Staphylococcus aureus* isolates were tested; of these, (100%) were susceptible to amikacin while 13 (100%) isolates were resistant to tetracycline, penicillin and erythromycin. This was followed in decreasing order of resistance by methicillin, 8 (61.5%); vancomycin and chloramphenicol, 6 (46.2%) each; sulphamethoxazole/ trimethoprim and gentamicin, 5 (38.5%) each; oxacillin and amoxicillin/clavulanic acid, 3 (23%) each and ciprofloxacin, 2 (15.4%) (Table 1). All of the coagulase positive *S. aureus* isolates were resistant to at least three antimicrobials. Thirteen unique susceptibility profiles were identified among the *S. aureus* isolates with all resistant to multiple clinically important antimicrobial classes (Table 2). Resistance to penicillin, tetracycline and erythromycin was highly prevalent, but resistance to other important antimicrobials was also observed.

DISCUSSION

The widespread use of antibiotics has undoubtedly accelerated the virulence of *S. aureus*, by acquiring multiple resistance genes, has become able to survive almost all antibiotic families (Stefani and Goglio, 2010). Several workers have reported the occurrence of multidrug resistant *S. aureus* in poultry (Pesavento *et al*., 2007; Waters *et al*., 2011). All the 13 coagulase positive *S. aureus* isolates were resistant to penicillin, tetracycline and erythromycin and were all susceptible to amikacin. Such profiles of antibiotic resistance occur rather frequently in many of the *S. aureus* isolates from other countries (Kitai *et al*., 2005; Leonard and Markey, 2008; Lee, 2003). Achi and Madubuike (2007) reported a lower incidence of 59.2% and 0.83% for tetracycline and erythromycin respectively in *S. aureus* isolates from

Table 1: Susceptibility of coagulase positive *S. aureus* isolates from chickens in Zaria to 12 antimicrobial agents

Antibiotic	Disk potency (µg)	Number sensitive (%)	Number resistant (%)
Amikacin	30	13 (100.0)	0 (0.0)
Amoxycillin/Clavulanic acid	30	10 (77.0)	3 (23.0)
Chloramphenicol	30	7 (53.9)	6 (46.1)
Ciprofloxacillin	5	11 (84.6)	2 (15.4)
Erythromycin	5	0 (0.0)	13 (100.0)
Gentamycin	10	8 (61.5)	5 (38.5)
Methicillin	10	5 (38.5)	8 (61.5)
Oxacillin	5	10 (77.0)	3 (23.0)
Penicillin	10	0 (0.00)	13 (100.0)
Sulphamethoxazole/Trimethoprim	25	8 (61.5)	5 (38.5)
Tetracycline	30	0 (0.00)	13 (100.0)
Vancomycin	5	7 (53.8)	6 (46.2)

Table 2: Multidrug resistance profiles of coagulase positive *S. aureus* from chicken carcasses and live birds in Zaria

Isolate	Resistance profile
SGTB 69	C, E, CN, MET, P, SXT, TE
SMTC 9	AMC, C, E, MET, P, TE, VA
SMSB 9	C, E, P, SXT, TE
SMTL 6	C, E, P, TE
SMTB 2	AMC, E, MET, OX, P, TE, VA
XCB 11	C, E, CN, MET, OX, P, SXT, TE, VA
SMCL 3	AMC, E, MET, P, TE, VA
XCB 2	AMC, E, CN, P, TE,VA
XTL 15	E, CN, MET, P, TE
SGCB 30	AMC, C, E, CN, P, SXT, TE
XTL 4	E, P, TE
SGTB 18	AMC, C, E, CN, MET, OX, P, SXT, TE, VA
XTL 14	C, E, P, TE

ready to eat foods. For specific treatment of staphylococcal infections in poultry, veterinarians generally use penicillin, erythromycin and tetracycline (Tanner, 2000) and the sub-therapeutic use of these drugs either as prophylaxis or for growth promotion as well as imprecise dosages given to sick or healthy animals may have led to the level of resistance of our *S. aureus* isolates to these antibiotics. Susceptibility to chloramphenicol recorded in this study was 46.1%. The WHO recommended the prohibition of use of chloramphenicol in all food producing animals, particularly lactating cows and laying birds (Settepani, 1984; WHO, 1988). Susceptibility to chloramphenicol (46.1%) recorded in this study may reflect the extent of drug use control and poor legislation concerning drug use in Nigeria. All of our isolates were resistant to three or more of the antibiotics tested and susceptibilities of (61.54%) to methicillin and (46.2%) to vancomycin were recorded. Such rates of resistance to methicillin occur rather frequently in human *S. aureus* isolates in Nigeria. (Rotimi *et al*., 1987; Olonitola *et al*., 2007; Taiwo *et al*., 2008). Multiple-drug resistant Staphylococci that are resistant to beta-lactam antibiotics have 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 (Mamza *et al*., 2010). The occurrence of vancomycin resistance (46.2%) in this study is higher than those observed for poultry isolates in previous studies (Manie *et al*., 1998; Waters *et al*., 2011). The presence of vancomycin- resistant staphylococci associated with poultry products is of major concern since the drug is not reportedly used in poultry and other food-producing animal industries (Bates *et al*., 1994; Bager *et al*., 1997) and is most frequently the drug of choice in the treatment of MRSA infections. The susceptibility to sulphamethoxazole/ trimethoprim (38.5%) in this study was higher than that reported by Ghebremedhin *et al*. (2009) in Ibadan (3%) probably due to the widespread and indiscriminate use of the drug in human medicine. It is therefore less likely that the mechanism of multiple drug resistance seen in these isolates may be due to an integron based system, since the most common integron class i.e. Class 1 integrons are always related to sulphonamide resistance due to the carriage of *sul* gene (Livermore, 2003). Resistances to most of the drugs tested in this study may not be unconnected with the fact that these inexpensive drugs are widely available from distributors and can be purchased easily from certain vendors without a prescription in Nigeria. There is therefore the need to legislate and enforce laws to limit the prescription and dispensation of antibiotics to only qualified professionals. Also, the susceptibility of the isolates to amikacin (100%) and ciprofloxacin (15.38%) may be suggestive of fact that they are less abused in this environment and may be recommended for the treatment of *Staphylococcal* infections.

The prevalence of multidrug resistance might have been underestimated because this study only focused on live birds and carcasses, even though poultry litter and manure are important in the dissemination of resistant bacteria. Future studies may further elucidate genetic determinants of multidrug resistance in coagulase positive *Staphylococcus aureus* in Nigeria.

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