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Antibiotic susceptibility patterns of beta-lactamase-producing *Escherichia coli* and *Staphylococcus aureus* isolated from chickens in Maiduguri (Arid zone), Nigeria

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ABSTRACT

Escherichia coli and *Staphylococcus aureus* species though opportunist pathogens, are becoming a global clinical problem in both human and veterinary medicine. This study was designed to determine the antibiotic susceptibility patterns of β-lactamase-producing *E. coli* serotypes and *S. aureus strains* isolated from chickens in the Maiduguri Arid zone, Nigeria. Various tissue samples from apparently healthy and diseased chickens were collected and examined for the presence of *E. coli* and *S. aureus.* Isolates were identified by relevant biochemical tests. β-lactamase-producing strains of the isolates were determined by the chromogenic cephalosporin method, using nitrocefin-impregnated sticks and cephalosporin (nitrocefin) solution. The antibiotics susceptibility patterns of the isolates were determined for ten antibiotics (ampicillin, chloramphenicol, cephalexin, ciprofloxacin, lincomycin, doxycycline, tetracycline, tylosin, tylosin tartrate and penicillin) by the micro-broth dilution method. *E. coli* was isolated in 805 and *S. aureus* in 660 of 1300 tissue samples examined; from which 89 (11.1%) and 58 (8.8%) were β-lactamase-positive isolates respectively. Out of 540 *E. coli* isolates serotyped, 57 (10.6%) serogroups were identified from which 17 (29.8%) were serogroups O1, 5 (8.8%) were O2, and 2 (3.5%), 9 (15.8%), 6 (10.5%) and 18 (31.6%) were serogroups O26, O78, O86 and O141 respectively, whilst, 483 (89.4%) isolates were not typable with the available sera. Serogroups O141, O1 and O78 were more frequently isolated and serogroups O1 and O78 were more prevalent in sick chickens than in healthy chickens. *E. coli* exhibited high resistance to ampicillin, chloramphenicol, tetracycline, lincomycin, penicillin and tylosin with MIC values >8.0 μg/μL, as did *S. aureus* to all the antibiotics tested with MIC values >8.0 μg/μL. In conclusion, the study has demonstrated the presence of *E. coli* serotypes and *S. aureus* in various tissues of chickens and their antibiotic susceptibility patterns, clearly demonstrating multiple drug resistance.

Key words: beta-lactamase, *Escherichia coli*, *Staphylococcus aureus*, antibiotics susceptibility, chickens, Maiduguri

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Introduction

Escherichia coli (E. coli) and *Staphylococcus aureus (S. aureus*) infections constitute one of the most important bacterial diseases affecting the poultry industry (RAJI et al., 2003) in Nigeria. *E. coli* is the predominant facultative anaerobic normal bacterial flora in the avian intestine and plays an important role in maintaining intestinal homeostasis. The organism is a major pathogen of worldwide importance in commercially raised poultry, contributing significantly to economic losses in both chickens and turkeys (RAJI et al., 2003). *S. aureus* occurs among the normal flora of live poultry, and broiler chicks become contaminated with the organism within the first few days after being hatched (NORTTERMANS et al., 1982). *S. aureus* is important in relation to poultry and meat hygiene because of its ability to produce entero-toxins, which may cause food poisoning in humans. About 30% of all outbreaks of food borne diseases have been reported to be associated with poultry, and more than 25% of poultry disease outbreaks have been attributed to *S. aureus* globally (NORTTERMANS et al., 1982). Some of the poultry diseases ascribed to these agents such as coli septicaemia, coligranuloma or Hjarre's disease and omphalitis are incriminated with *E. coli* (RAJI et al., 2003); whilst joint infections (swollen hocks, arthritis) and plantar abscess (bumble foot) are associated with *S. aureus* (SAEED et al., 2000; JOHN, 2006).

The elaboration of entero-and invasive-toxins, cytotoxic necrotizing factor (CNF), attaching and effacing (eae) genes, fimbrial adhesins and production of haemolysins, siderophores, plasmids, integrons and haemolysins (GONZALEZ and BLANCO, 1996; PASS et al., 2000) by *E. coli* and the specific production of coagulase, DNase, hyaluronidase, staphylokinase, nuclease and penicillinase by *S. aureus* (JOHN, 2006) constitute some of the pathogenic-markers by which these agents produce diseases in their hosts. Some of these markers contribute to the emergence of multiple drug resistant strains (LAURA et al., 2002) in the two organisms and consequently to therapeutic failure in humans and animals (YOSHICHIKA et al., 2000; VAN DEN BOGAARD et al., 2001; HANCHUN et al., 2004) which is becoming a global health concern. Though opportunist pathogens, these organisms also provide a large reservoir of antibiotic resistance in tissues of the bodies of their hosts, and might transfer this to other organisms if the resistance genes concerned are translocated (SAEED et al., 2000; DEBORAH et al., 2005).

Both organisms are known to produce beta-lactamases, possibly contributing to the emergence of multiple drug resistant strains (LAURA et al., 2002).

Several studies have demonstrated that patterns of antibiotic usage greatly affect the number of resistant organisms which develop. Although the extent and speed to which bacteria develop resistance to antimicrobial drugs vary, indiscriminate broad-spectrum antibiotic therapy has been shown to result in increased antimicrobial resistance. So far, resistances by *E. coli* and *S. aureus* have been shown to occur to most antimicrobial

drugs (HOWARD et al., 1996) making this a serious setback in the treatment of bacterial infections (CHAH and OBOEGBULEM, 2005) in both humans and animals.

The emergence of bacterial species with extended spectrum beta-lactamases and acquired resistance to various broad spectrum beta-lactam antibiotics is becoming a clinical problem globally in both human and veterinary medicine (YOSHICHIKA et al., 2000; CARL et al., 2002). Field reports have shown evidence of emerging multiple drug resistant strains of these organisms in Nigeria following treatment of some bacterial diseases in poultry, coupled with the frequency of complaints of respiratory, diarrhoea, and joint problems.

There is paucity of information on the distribution of avian *E. coli* serotypes and *S. aureus* in the tissues of chickens in Nigeria, as well as the role of these disease agents in the elaboration of beta-lactamases responsible for multiple antimicrobial drug resistance and their likely public health implications in both animals and humans. This study was therefore undertaken to determine the antibiotic susceptibility patterns of β-lactamase-producing strains of *E. coli* and *S. aureus* isolated from chickens in Maiduguri, Nigeria.

Materials and methods

Sample collection and processing. Various tissue samples (196 each of the trachea, lungs, small intestines and liver, 135 hock joints, 136 digital pads and 201 cloacal swabs), totalling 1300, were collected aseptically from both apparently healthy and sick chickens (of which 892 samples were from 332 apparently healthy and 408 samples from 68 sick chickens) distributed across one hundred randomly selected poultry farms within Maiduguri Metropolis, Borno State, Nigeria between April 2005 and April 2007.

Samples were transported on ice packs within one hour of collection to the research and diagnostic laboratory of the Department of Veterinary Medicine, University of Maiduguri where they were processed.

Bacteriological isolation. Homogenized samples were inoculated onto MacConkey's agar (LAB 2, idg®, Lancashire) plates and incubated at 37 ^{O}C for 24 hrs. Some presumptive *E. coli* and *S. aureus* colonies were selected from each sample and sub-cultured onto EMB agar (LAB 61, idg®, Lancashire) for *E. coli* and modified Baird Parker agar (CM 0961, Oxoid®) for *S. aureus*.

Identification of isolates. Isolates were tested for Gram-staining, methyl red and Voges-Proskuaer (BAKER et al., 2001), catalase and coagulase (MacFADDIN, 1980) and then stored on nutrient agar slants until used for β-Lactamase production tests, *E. coli* serotyping and antimicrobial susceptibility tests. Isolates were routinely sub-cultured every 4 months to maintain purity and viability during the course of the work.

Beta-lactamase test. The production of β-lactamase enzymes was tested by the chromogenic cephalosporin method using commercially prepared nitrocephin-impregnated touch sticks (BR0066A, Oxoid® UK) according to the manufacture's instructions, and cephalosporin (500 mg/mL nitrocephin) solution as described by MILES and AMYES (1996).

The stick test was carried out according to the manufacturer's instructions. A representative pure colony from the growth medium was selected. This colony was touched with the impregnated end of the stick. The stick was then rotated to pick up a small mass of pure cells and was observed for 10 minutes for *E. coli* and up to 1hr for *S. aureus*. A colour change from yellow to pink-red indicated positive β-lactamase-producing organisms.

Cephalosporin C (22237, Sigma-Aldrich®, South-Africa) was used for the cephalosporin solution method, according to the method described by MILES and AMYES (1996). Cephalosporin C was said to exhibit resistance or stability to β-lactamases but was not sufficiently potent for clinical use. Therefore, its use as a β-lactamase test reagent is suitable for the detection of β-lactamase production in bacteria that can hydrolyze the β-lactam ring in its molecule. A colour change from yellow to red was recorded positive for β-lactamase-producing strains.

Serotyping of E. coli isolates. The kits for serotyping avian *E. coli* comprising serogroups O1, O2, O26, O78, O86 and O141 antisera were obtained from Prof. J.A. Blanco, Director of the *E. coli* Reference laboratory (LREC) in Spain. The serotyping was carried out according to the protocol (ANONYM., 2006).

The pure isolate of *E. coli* was inoculated onto a Tryptone Soya agar (TSA, 22091, Sigma-Aldrich, South Africa) plates and incubated for 48 hrs at 37 ^{O}C. Some of the growths from the agar were then suspended in 2 µL of 0.85% saline solution in sterile vaccuutainer tubes. The bacterial concentrations were adjusted by comparing the tubes to correspond with 1.8 x 10^9 MacFarland Barium sulfate turbidity standard. The tubes were heated in a boiling water bath for 1hr at 100 ^{O}C to inactivate K antigens. The suspensions were then allowed to cool and 2 µL of formalinized saline solution (0.5%, v/v) containing gentian violet (0.005%, w/v) were added.

The antisera were diluted (1x) to 1/80 using saline solution with sodium azide according to the protocol (ANONYM., 2006). The prepared antigen suspensions were tested with each of the diluted O antisera: 50 µL of each of the diluted antisera (1/80) were added to each corresponding well of a sterile V-shaped polystyrene micro-titre tray and equal volumes of the O antigen suspensions were then added. The trays were covered and incubated at 37 ^{O}C overnight and were then examined for agglutination. Negative reactions were indicated by a sharp point, whereas positive reactions by a carpet-like appearance. Positive and negative controls were also prepared alongside the test samples.

Serial dilutions of the O antisera were prepared in micro-titre dilution trays to give dilutions of 1/80, 1/160, 1/320 to 1/40960. Then 100 µL of each of the diluted O antisera (1/80) were added to the first well of the corresponding micro-titre tray and 50 µL of saline solution to the remaining 11 wells. Serial two-fold dilutions in 50 µL amounts were then carried out from well 1 up to well 10, while wells 11 and 12 served as negative controls. Then 50 µL of the corresponding O antigen suspension was added to each of the 12 wells of corresponding trays, starting from well 12 through to well 1, making the final dilution of the antiserum to 1/81920 in the last well (well 12). Then the trays were covered and incubated at 37 ^{O}C overnight and were examined for agglutination. Strains showing agglutination in dilutions of 1/160 or greater were considered to contain the same O antigen as the corresponding antiserum and this constituted the final identification of the somatic antigen or O antigen of the isolate.

Antibiotics susceptibility (MIC) test. The following ten antimicrobial agents (ampicillin (10044), cephalexin (22238), chloramphenicol (46109), ciprofloxacin (62143), doxycycline hydrochloride (44577), penicillin G benzathine (PEN-B), tetracycline hydrochloride (T3383), lincomycin hydrochloride (62143), tylosin tartrate (93806) and tylosin (T3397) obtained from Sigma Aldrich®, South-Africa, were tested. With the exception of ciprofloxacin, these antibiotics were found to be commonly used in the treatment of poultry infections in Maiduguri, Nigeria.

The minimum inhibitory concentrations (MICs) were determined by micro broth dilution according to standard method (GAIL and JOHN, 1995; MILES and AMYES, 1996), using inoculums of 5 x 10^4 CFU/well in a nutrient agar broth (No 1; 70122, Sigma-Aldrich®, South-Africa) with the ten antibiotics at concentrations ranging from 0.25 µg/µL to 8.0 µg/µL. The MICs of the test solutions were read as the lowest concentrations of the antibiotics that inhibited a colour change (EGWU et al., 1994) or inhibited growth in the wells of the micro-dilution trays after incubation for 18-24 hrs. Results were interpreted as susceptible and resistant based on the interpretive standards for dilution susceptibility testing (GAIL and JOHN, 1995).

Statistical analysis. Results were compared by X^2 test with Yates correction for continuity.

Results

Prevalence of E. coli and S. aureus in chickens in Maiduguri, Nigeria. Table 1 shows the prevalence of *E. coli* and *S. aureus* isolates from chickens in Maiduguri. Out of a total of 1300 tissue samples collected, 1,256 (96. 6%) yielded growth, of which 805 (64.1%) were *E. coli* and 660 (52.5%) were *S. aureus* isolates. 313, 263 and 229 representing 38.9%, 32.7% and 28.4% of the *E. coli* isolates were from broilers, layers and local chickens respectively. Similarly, 211, 231 and 218 representing 32%, 35%

and 33% of the *S. aureus* isolates were also from broilers, layers and local chickens respectively. Furthermore, 33 (10.5%), 27 (10.3%) and 29 (12.7%) of the *E. coli* and 9 (4.3%), 41(17.7%) and 8 (3.7%) of the *S. aureus* isolates were β-lactamase producing strains isolated from broilers, layers and local chickens, respectively. Of the isolates from healthy chickens, 25 (4.3%) *E. coli* and 17 (4.5%) *S. aureus* were β-lactamase positive strains, whilst 64 (28.7%) *E. coli* and 41 (14.6%) *S. aureus* were β-lactamase producing strains isolated from diseased chickens.

Table 1. Prevalence of *E. coli* and *S. aureus* strains in apparently healthy and diseased chickens in Maiduguri, Nigeria

Number (%) of β-lactamase positive isolates						
Type of chickens	Healthychickens		Sick chickens		Total	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*
Broilers	9/226 (4.0)^{n}	3/121 (2.5)^{l}	24/87 (27.6)^{f}	6/90 (6.7)^{j}	33/313 (10.5)^{g}	9/211 (4.3)^{h}
Layers	11/190 (5.8)^{m}	12/133 (9.0)^{4}	16/73 (21.9^{e}	29/98 (29.6)^{k}	27/263 (10.3)^{g}	41/231 (17.7)^{i}
Local/ chickens	5/165 (3.0)^{l}	2/126 (3.0)^{l}	24/64 (38.0)^{d}	6/92 (6.5)^{j}	29/229 (12.7)^{g}	8/218 (3.7)^{h}
Total	25/582 (4.3)^{a}	17/380 (4.5)^{a}	64/223 (28.7)^{b}	41/280 (14.6)^{c}	89/805 (11.1)^{k}	58/660 (8.8^{l}

Numerator = number of β-lactamase isolates; denominator = number of isolates screened; Means with different superscripts differ significantly (P<0.05)

Table 2. Number (%) of β-lactamase-positive *E. coli and S. aureus* isolates in tissues of chickens in Maiduguri

Tissue	*E. coli*	*S. aureus*
Trachea	17/111 (15.3) ^{a}	9/93 (9.7) ^{c}
Lung	19/123 (15.5) ^{a}	10/103 (9.7) ^{c}
Liver	15/89 (16.9) ^{a}	8/59 (13.6) ^{d}
Small/intestine	14/144 (9.7) ^{c}	9/56 (16.1) ^{a}
Hock joint	3/71 (4.2) ^{h}	6/74 (8.1) ^{e}
Digital pad	12/84 (14.3) ^{i}	13/121(10.7) ^{f}
Cloacal swab	9/183 (4.9)^{j}	3/154 (1.9) ^{g}
Total	89/805 (11.1) ^{1}	58/660(8.8)^{2}

Means with different superscripts differ significantly (P<0.05); Numerator = number of β-lactamase positive isolates; Denominator = number of isolates screened

Table 3. Distribution of *E. coli* serotypes isolates in tissues of chickens in Maiduguri, Nigeria

	Serogroups (%)						
Number of samples	O1	O2	O26	O78	O86	O141	NT
Trachea (82)	1	-	-	3	2	1	75
Lung (88)	2	1	-	1	-	3	81
Liver (72)	1	1	-	1	1	1	67
Small intestine (80)	1	-	1	-	-	1	77
Hoch joint (61)	2	-	-	-	-	-	59
Digital pad (61)	2	-	-	-	-	2	57
Cloacal swab (94)	8	3	1	4	3	1	65
Total (540)	17	5	2	9	6	18	483
	$(29.8)^1$	$(8.8)^2$	$(3.5)^3$	$(15.8)^4$	$(10.5)^2$	$(31.6)^1$	$(89.4)^5$

Means with different superscripts differ significantly ($P<0.05$); NT = not typable with available antisera

Table 4. O serogroups identified in *E. coli* strains isolated from healthy and sick chickens in Maiduguri

	Number of *E. coli* serogroups (%)		
O serogroup	Healthy chickens	Sick chickens	Total
	(n=392)	(n=148)	(n = 540)
O1	6 (1.5)	11 (7.4)	17 (29.8)
O2	2 (0.5)	3 (2.0)	5 (8.8)
O26	2 (0.5)	0 (0)	2 (3.5)
O78	3 (0.8)	6 (4.1)	9 (15.8)
O86	5 (1.3)	1 (0.7)	6 (10.6)
O141	16 (4.1)	2 (1.4)	18 (31.6)
NT	358 (91.3)	125 (84.5)	483 (89.4)
Totals	34 (8.8)	23 (15.5)	57 (10.6)

NT = not typable with available antisera

Distribution of E. coli and S. aureus isolates in tissues of chickens. Table 2 shows the tissue distribution of β-lactamase-producing strains of *E. coli* and *S. aureus* isolates from chickens in Maiduguri. Most strains of *E. coli* were isolated from the liver (16.9%), lung (15.5%), trachea (15.3%), digital pad (14.3%) and small intestine (9.7%), whilst most strains of *S. aureus* were similarly isolated from small intestines (16.1%), liver (13.6%) and digital pads (10.7%).

Table 5. Minimum inhibitory concentration values for some representative β-lactamase strains of *E. coli* (n = 12) and *S. aureus* (n = 14) isolated from tissues of chickens in Maiduguri, Nigeria

Antibiotic	isolate	Number of isolates with their MIC (µg/µL) values									
		Susceptible value µg/µL	0. 25	0. 5	1. 0	2. 0	4. 0	8. 0	>8. 0	Susceptible (%)	Resistant (%)
Ampicillin	*E. coli*	<0.25	2	-	-	2	-	-	8	2 (16.7)	8 (66.7)
	S. aureus		-	-	-	2	4	2	6	-	14 (100)
Cephalexin	*E. coli*	<8.0	2	-	2	2	-	-	6	6 (50)	6 (50)
	S. aureus		-	-	-	-	-	-	14	-	14 (100)
Chloramphenicol	*E. coli*	≤8.0	2	-	-	-	2	-	8	4 (33.3)	8 (66.7)
	S. aureus	-	-	-	-	-	-	4	-		14 (100)
Ciprofloxacin	*E. coli*	≤1.0	6	-	-	4	-	2	-	6 (50)	2 (16.7)
	S. aureus		-	-	-	8	-	6	-	-	14 (100)
Doxycycline	*E. coli*	≤2.0	-	-	-	2	-	4	6	2 (16.7)	6 (50)
	S. aureus		-	-	-	-	-	-	14	-	14 (100)
Lincomycin	*E. coli*	≤0.5	-	-	-	-	-	-	12	-	12 (100)
	S. aureus		-	-	-	-	-	-	14	-	14 (100)
PenicillinG	*E. coli*	≤0.12	-	-	-	-	-	-	12	-	12 (100)
	S. aureus		-	-	-	-	-	-	14	-	14 (100)
Tetracycline	*E. coli*	≤4.0	-	2	-	-	-	-	10	2 (16.7)	10 (83.3)
	S. aureus		-	-	-	-	-	-	14	-	14 (100)
Tylosintartrate	*E. coli*	≤0.7	-	-	-	-	-	-	12	-	12 (100)
	S. aureus		-	-	-	-	-	-	14	-	14 (100)
Tylosin	*E. coli*	≤0.7	4	-	-	-	-	-	8	4 (33.3)	8 (66.7)
	S. aureus		-	-	-	-	-	-	14	14 (100)	

Susceptible values were adapted from interpretive standards for dilution susceptibility test (GAIL and JOHN, 1995).

E. coli serotyping. Out of the total of 540 isolates serotyped 57 (10.6%) strains were identified to be associated to 6 serogroups from which 17 (29.8%) were of serogroup O1, 5 (8.8%) were O2, whilst, 2 (3.5%), 9 (15.8%), 6 (10.5%) and 18 (31.6%) were serogroups O26, O78 O86 and O141, respectively, whilst 483 (89.4%) strains were not typable with the available sera. With the exception of serogroup O26, each of the other serotypes occurred in the liver; whilst all serotypes occurred in the cloacal swab. Only serotypes O1 and O141 were isolated from the digital pad. Serogroup O141 was more frequently isolated from healthy, 16 (4.1%) than from sick, 2 (1.4%) chickens, whereas serogroups O1, 11 (7.4%), O2, 3 (2.0%), and O78, 6 (4.1%) were more frequently isolated from sick chickens than from healthy chickens, where 6 (1.5%), 2 (0.5%) and 3 (0.8%) were their respective ratios (Table 4). Serogroups O1 and O141 differed significantly ($P<0.05$) in prevalence from O78, whereas serogroups O1 and O141 did not show any significant difference ($P>0.05$).

Susceptibiliy to antibiotics. The minimum inhibitory concentrations of 12 *E. coli* and 14 *S. aureus* isolates to ten antibiotics are shown in Table 5. Ampicillin and cephalexin had a range of 0.25 µg/µL-2.0 µg/µL, chloramphenicol (0.25 µg/µL - 2.0 µg/µL), ciprofloxacin (0.25 µg/µL - 8.0 µg/µL), doxycycline (2.0 µg/µL - 8.0 µg/µL), tetracycline (0.5 µg/µL - >8.0 µg/µL), and tylosin (0.25 µg/µL - >8.0 µg/µL) respectively to *E. coli*. All the strains of *E. coli* tested were resistant to lincomycin, penicillin and tylosin tartrate, indicating a unimodal distribution. Two strains (16.7%) were susceptible to ampicillin, doxycycline and tetracycline, 4 (33.3%) to chloramphenicol, whilst resistance of 66.7% was observed in 8 strains to tylosin, chloramphenicol and ampicillin, respectively. Furthermore, variable resistances of 50% and 83.3% respectively were also observed against cephalexin and tetracycline. Ciprofloxacin and doxycycline showed variation in susceptibility to *E. coli* isolates. The tested strains of *S. aureus* demonstrated high levels of resistance to nine of the ten antibiotics, ampicillin, cephalexin, chloramphenicol, doxycycline, lincomycin, penicillin, tetracycline, and tylosin), considering the susceptible MIC values adapted. However, at higher concentrations between 2.0-8.0 µg/µL (i.e. above the adapted susceptible values) 57.1% of the *S. aureus* strains showed intermediate susceptibility to ciprofloxacin. Ampicillin had MIC values of 2.0 µg/µL to 2 (14.3%) isolates, 4.0 µg/µL to 4 (28.6%) and 2.0 µg/µL to 8 (57.1) of the *S. aureus* strains.

Discussion

In Nigeria, as in other countries in the world, epizootics of bacterial diseases occur frequently in poultry farms. These diseases occur in all age groups of chickens at any period of time, especially the early stages of life. The economic implications range from weight loss, high mortality rates, carcass downgrade and reduced production (BLANCO et al., 1994). Some of these diseases are associated with resident or ingested proliferation of

pathogenic *E. coli* and/or *S. aureus* in different organs or tissues of chickens, manifesting different signs.

In the present study the *E. coli* serogroup O141 strain was identified as the most widespread isolate amongst chickens in Maiduguri, Nigeria. The frequency of isolation showed that serogroups O1 and O78 were the most common serotypes isolated from sick chickens. This finding supports the reports of HANCHUN et al., (2004) and MISHRA (1994) in which they independently reported *E. coli* serogroups O1 and O78 as the most prevalent serotypes in chickens with colibacillosis. However, in a field study of clinical cases and dead-in-shell embryos in Zaria, Nigeria, RAJI et al., (2003) examined 86 *E. coli* strains isolated from colibacillosis and found that 17% of the strains belonged to only 2 serogroups, O8 and O9, whilst only 1.2% belonged to serogroups O26 and O78 respectively, and reported that serogroup O8 was the most frequently encountered strain. The findings in the present study did not agree with this report. This disagreement may likely arise from the use of specific antisera in the present study and /or probably from differences between the geo-climatic zones of Zaria and Maiduguri where the two studies were independently carried out. The occurrence of the *E. coli* serogroups O1, O2 and O78 in Nigeria as demonstrated by this study agrees with previous studies (ABDELKADER et al., 1995; HANCHUN et al., 2004).

Serogroup O26 was isolated in dairy products (raw cow milk) and water bodies (rivers) in Adamawa and Borno States of Nigeria (MOSES et al., 2005). It was also reported in chickens by RAJI et al. (2003) in Zaria, Nigeria. This serogroup was isolated in the present study from the small intestine and cloaca of local chickens. The isolation of this serogroup from local chickens in this study may suggest ingestion by the chickens of cattle faeces, since the serogroup was more frequently isolated from cattle (MOSES et al., 2005), and local chickens, being free range scavengers, may have had easy access to bovine faeces. There is also the likelihood that this serotype may cause food poisoning in humans from consuming chickens harbouring this strain, since the serotype belongs to the enterotoxigenic group of *E. coli.*

The main factor compromising the efficacy of drugs such as penicillins, cephalosporin and related compounds, is the production of β-lactamases (MILES and AMYES, 1996) by the affected bacteria. The present study shows that *E. coli* isolates from local chickens had higher (12.7%) β-Lactamase-producing strains, whilst β-lactamase-producing strains of *S. aureus* were higher (17.7%; $P<0.05$) in layers. The indiscriminate use of antibiotics by poultry farmers has also been linked to the emergence of bacteria with multiple-drug resistant strains (ABDELKADER et al., 1995) in poultry. Evidence of multiple resistant strains was most commonly encountered in this study. The frequency of resistance to antibiotics among enteric bacteria from domestic animals has increased markedly,

probably due to the excessive haphazard use of antibiotics by farmers (SAEED et al., 2000), which is a common phenomenon in Nigeria.

Previous studies (SAENZ et al., 2001; CARL et al., 2002; HANCHUN et al., 2004), reported resistances of clinical isolates of *E. coli* from chickens to tetracycline, chloramphenicol, ampicillin and amino glycosides. Similar observations were seen in this study. Such resistances could be ameliorated by the use of ethylene diamine tetra acetate (EDTA)-potentiated antibiotics *in vitro*, as this compound was reported to have significantly potentiated the activities of antibiotics such as tetracycline and ampicillin. It has also been reported to produce reversal of antibacterial resistant strains of Gram-negative bacteria (CHAH and OBOEGBULEM, 2005). This however, needs to be investigated *in vivo* during infections due to these organisms. The present study however, did not agree with the findings of WANG et al. (2001), SAENZ et al. (2001) and HANCHUN et al. (2004) in which they reported *E. coli* resistance of 79% and 90% to ciprofloxacin, since in the present study *E. coli* isolates from chickens were moderately sensitive to this antibiotic. This difference may arise from the lack of or low usage of this antibiotic in chickens in this part of Nigeria. Nevertheless, ciprofloxacin and other quinolones have been reported to be ineffective in the treatment of animal diseases in China (HANCHUN et al., 2004) and developed countries like Spain, where previous studies were conducted. In the developing countries like Nigeria, quinolones (enrofloxacins, ciprofloxacin, ofloxacin etc) are less commonly used in veterinary medicine, especially in the north-eastern part of the country and they account for 1.7% of all antibiotics used in poultry (MAMZA, 2008). However, the level of resistance to ciprofloxacin of poultry *E-coli* isolates observed in this study was similar to that found in The Netherlands, where approximately 10% resistance was reported (VAN DEN BOGAARD et al., 2001). This low resistance rate may also be associated with the low usage of this drug by poultry farmers in that country.

A most striking observation in this study was the wide-spread resistance of *S. aureus* strains to all the ten antibiotics. These strains may probably possess other resistant or genetic markers in addition to production of β-lactamases; or perhaps the isolates may be methicillin-resistant strains (MRSA): as methicillin-resistant *Staphylococcus aureus* has been reported to show resistance to almost all antibiotics, due to the presence of a novel penicillin-binding protein (PBP2a) which showed a decreased binding affinity for β-lactams (JOHN, 2006); hence, the popular reference to it as a "superbug". This finding may likely portend further complication in treatment of *E. coli* and *S. aureus* infections in both humans and animals from this region, as these organisms are becoming a major threat in both human and veterinary medicine. In a study in the US, SAEED et al. (2000) isolated *S. aureus* from the hock joints and internal organs of diseased chickens and reported that the isolates were sensitive to Ampicillin, Penicillin and ciprofloxacin. The finding in the

present study is in contrast with the above report. In this study *S. aureus* isolated from hock joints and internal organs of chickens were resistant to these drugs. The difference in the observations as presently reported could be linked to the production of β-Lactamases, which are capable of producing penicillinases and cephalosporinases that hydrolyze the β-lactam rings in these antibiotics (LAURA et al., 2002). The widespread resistance to antibiotics by *S. aureus* could be due to acquisition of plasmids and/or transposons (SAEED et al., 2000) by the bacteria. Some transposons (plasmids) have genetic elements called integrons that enable them to capture exogenous genes (HOWARD et al., 1996). A number of such genes may therefore be inserted into a given integron, resulting in multiple antimicrobial drug resistance (HOWARD et al., 1996), a typical case observed in the present study.

In conclusion, we have demonstrated the existence of various pathogenic strains of *E. coli* serogroups from various tissues of chickens; Phenotypically, *E. coli* and *S. aureus* have demonstrated multi-resistance patterns (based on the definition of multi-resistance by DUIJKEREN et al. (2004) to antibiotics such as ampicillin, doxycycline, tetracycline, penicillin, lincomycin, chloramphenicol and Tylosin that are commonly used in poultry in Nigeria. The resistance problem demands that a renewed effort be made to seek newer antibacterial agents effective against such pathogenic bacteria (*E. coli* and *S. aureus*) that are resistant to antibiotics, and the need to search for other compounds that could effectively inhibit the growth of these common members of organisms.

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SAŽETAK

Premda uvjetno patogene vrste *Escherichia coli* i *Staphylococcus aureus* predstavljaju globalni klinički problem u humanoj i veterinarskoj medicini. Ovo istraživanje provedeno je sa svrhom određivanja osjetljivosti prema antibioticima serovarova *E. coli* i izolata *S. aureus* izdvojenih iz pilića u sušnom području Maiduguri u Nigeriji. Različiti uzorci tkiva od naizgled zdravih te od bolesnih pilića bili su pretraženi na prisutnost bakterija *E. coli* i *S. aureus*. Izolati su bili identificirani različitim biokemijskim testovima. Sojevi što su proizvodili β-laktamazu bili su identificirani kromogen cefalosporinskom metodom uz uporabu nitrocefinom impregniranih stikova i otopine cefalosporina (nitrocefina). Osjetljivost spomenutih bakterija bila je određivana mikrodilucijskom metodom na 10 antibiotika (ampicilin, koramfenikol, cefaleksin, ciprofloksacin, linkomicin, doksiciklin, tetraciklin, tilozin, tilozin tartrat i penicilin). *E. coli* je bila izdvojena iz 805, a *S. aureus* iz 660 od 1300 pretraženih uzoraka tkiva od čega je 89 (11,1%) izolata *E. coli* i 58 (8,8%) izolata *S. aureus* bilo pozitivno na β-laktamazu. Od 540 serološki tipiziranih izolata *E. coli* identificirano je bilo 57 (10,6%) seroloških skupina od čega je 17 (29,8%) pripadalo serološkoj skupini O1, 5 (8,8%) serološkoj skupini O2, 2 (3,5%) skupini O26, 9 (15,8%) skupini O78, 6 (10,5%) skupini O86 i 18 (31,6%) serološkoj skupini O141, dok se 483 (89,4%) izolata nisu mogla tipizirati raspoloživim antiserumima. Najčešće su bile izdvojene serološke skupine O141, O1 i O78 s time da su serološke skupine O1 i O78 prevladavale u bolesnih pilića. *E. coli* je bila vrlo otporna na ampicilin, kloramfenikol, tetraciklin, linkomicin, penicilin i tilozin s vrijednostima minimalne inhibitorne koncentracije >8,0 μg/μL. I *S. aureus* je bio otporan prema svim pretraživanim antibioticima s minimalnim inhibitornim vrijednostima >8,0 μg/μL. U istraživanju je dokazana prisutnost serovarova *E. coli* i vrste *S. aureus* u različitim tkivima pilića te njihova osjetljivost na antibiotike s jasno dokazanom multiplom rezistencijom.

Ključne riječi: beta-laktamaza, *Escherichia coli*, *Staphylococcus aureus*, antibiotici, osjetljivost, pilići, Maiduguri