Beta-lactamase *Escherichia coli* and *Staphylococcus aureus* isolated from chickens in Nigeria

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Summary

The occurrence of beta-lactamase-producing *Escherichia coli* and *Staphylococcus aureus* in chickens was investigated. Specimens ($n = 1\,300$) were collected from 400 chickens and were streaked on MacConkey agar plates. From each plate, presumptive growths of organisms were picked and streaked on eosin methylene blue and Baird-Parker agars, respectively. Typical colonies of *E. coli* and *S. aureus* with similar morphologies were identified by biochemical tests. Isolates were tested for beta-lactamase production and antimicrobial susceptibilities. Results indicated that 805 *E. coli* isolates from which 89 (11%) were beta-lactamase-positive and 660 *S. aureus* from which 58 (8.8%) were beta-lactamase-positive. Both isolates showed a high level of resistance to all twelve antibiotics screened. The increased prevalence of antibiotic resistance amongst bacterial organisms is undoubtedly correlated with the discovery and characterisation of multiple, transferrable resistance determinants, such as beta-lactamases, corresponding to their respective phenotypes. The implications of this for humans when handling and/or consuming chickens and chicken products contaminated with strains of such isolates, is a risk of transferrable multi-drug resistance and a failure of treatment. The results of our study indicated that beta-lactamase-producing *E. coli* and *S. aureus* are prevalent in chickens in Nigeria.

Keywords

Antibiotic, Beta-lactamase, Chickens, Drug, *Escherichia coli*, *Staphylococcus aureus*, Public health, Preliminary survey, Resistance, Risk.

Escherichia coli e *Staphylococcus aureus* produttori di beta-lattamasi isolati da polli in Nigeria

Riassunto

E' stato condotto uno studio sulla presenza di Escherichia coli *e* Staphylococcus aureus *produttori di beta-lattamasi nei polli in Nigeria. Sono stati seminati, su piastre di agar MacConkey, 1300 campioni isolati da 400 polli. Da ogni piastra, sono state raccolte presunte crescite di organismi, in seguito, seminate su eosina blu di metilene e agar di Baird-Parker. Mediante prove biochimiche sono state isolate e testate colonie di* Escherichia coli *e* Staphylococcus aureus *per verificare la produzione di beta-lattamasi e la loro sensibilità a dodici antibiotici. I risultati hanno indicato, come produttori di beta-lattamasi, 89 (11%)* Escherichia coli *su 805 e 58 (8,8%)* Staphylococcus aureus *su 660. I campioni hanno evidenziato un alto livello di resistenza agli antibiotici impiegati nello studio. L'aumento dell'antibiotico resistenza è correlata alla scoperta e alla caratterizzazione degli agenti responsabili dell'antibiotico resistenza multipla trasferibile come, ad esempio, la beta-lattamasi. Tra i rischi per la specie umana, determinati dalla manipolazione e/o consumo di pollo contaminato e derivati, c'è l'antibiotico-resistenza connessa al fallimento della terapia. I risultati dello studio hanno indicato che* Escherichia coli *e*

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Staphylococcus aureus *produttori di beta lattamasi hanno un'elevata prevalenza nei polli in Nigeria.*

Parole chiave

Antibiotico, Beta-lattamasi, *Escherichia coli*, Farmaco, Pollo, Resistenza, Rischio, Sanità Pubblica, *Staphylococcus aureus*.

Introduction

Beta-lactamase production is an important mechanism of resistance to β-lactam antibiotics by both aerobic and anaerobic bacteria. *Escherichia coli* and *Staphylococcus aureus* species are amongst the most important bacterial disease agents that affect the poultry industry (26, 40) worldwide and that have been reported to produce β-lactamases (9, 12, 45, 54, 55). These β-lactamase-producing strains play a role in poly-microbial avian infections. They do this by having a direct pathogenic impact in causing the infections as well as an indirect effect through their ability to produce the β-lactamase enzymes (11). Some of these enzymes have been designated TEM β-lactamases (38), SHV β-lactamases (8), metallo-β-lactamases (4), CTX-M β-lactamases (10, 27), CMY β-lactamases first described in 2006 (54) and carbapenemases which include IMP, VIM and KPC β-lactamases (28, 32).

Expanded spectrum cephalosporins have been made specifically to resist hydrolysis by the common broad spectrum β-lactamases: TEM-1, TEM-2 and SHV-1 (39). The production of mutant forms of β-lactamases called extended-spectrum β-lactamases (ESBLs), by non-inducible β-lactamase-producing bacteria, such as *E. coli*, have given these bacteria the ability to resist the newer cephalosporins and aztreonam (16). Biochemical and molecular studies have revealed that many ESBLs are derivatives of TEM-1, TEM-2 and SHV-1 β-lactamases (16, 18, 54). Some of these enzymes only differ from the parent enzymes by one or two amino acids (16, 54).

Inducible β-lactamase producers, such as *Pseudomonas aeruginosa, Proteus vulgaris, S. aureus, Acinetobacter* spp., etc., produce enzymes that are capable of hydrolysing both extended-spectrum penicillins and cephalosporins (25, 50). *S. aureus*, which is resistant to penicillinase-resistant penicillins and which is referred to as methicillin-resistant *S. aureus* (MRSA), is not only resistant to cephalosporins and ESBL agents, but also to many of the commonly used antibiotics, including aminoglycosides, macrolides, chloramphenicol, tetracyclines and fluoroquinolones (31), as well as to recently effective antimicrobial agents, such as vancomycin and linezolid (20).

The emergence of resistance to the expanded-spectrum cephalosporins has been of major concern (54), both in human and veterinary medicine. The occurrence of organisms producing ESBLs varies widely across the world (39, 54): TEM and SHV β-lactamases have been reported in Africa (6, 41), but none has been reported in Nigeria, especially from animal sources. Various authors have reported β-lactamase production by *E. coli* and *S. aureus* isolated from humans and animals (8, 9, 12, 17, 27, 45, 50, 55). Little or no information on isolates from chickens is available in Nigeria. This study was therefore initiated to provide a preliminary investigation of the occurrence of β-lactamase-producing *E. coli* and *S. aureus* in chickens in Maiduguri, an arid zone of Nigeria, with a view to highlighting some possible public health implications.

Materials and methods

Collection and processing of specimens

The specimens ($n = 1\,300$) used in this study were obtained from 400 chickens (143 broilers, 130 layers and 127 local chickens) purchased from 100 randomly selected poultry farms in Maiduguri, between April 2005 and April 2007. A minimum of 4 chickens, apparently healthy or sick, were purchased from each farm and were taken to the post-mortem laboratory of the State Veterinary Hospital in Maiduguri where the specimens were collected in aseptic conditions. The specimens included trachea, lungs, liver, digital pads, hock joints, small intestines and cloacal swabs. Specimens were transported immediately on ice packs to the research laboratory of the Department of Veterinary Medicine, University of Maiduguri,

for processing and culturing. Specimens were processed according to the method described by Forbes and Granato (19).

Isolation and identification of organisms

Culture media

The culture media used in this study for the isolation of the organisms included MacConkey agar (LAB 2 (Idg®, Lancashire), eosin methylene blue (EMB) agar (LAB 61) (Idg®, Lancashire) and Baird Parker agar modified (CM 0961) (Oxoid®, Cambridge). Media were prepared according to the instructions provided by the manufacturers. The processed specimens were inoculated onto MacConkey agar and incubated at 37°C for 24 h. Some presumptive growths were sub-cultured onto EMB agar for *E. coli* isolation and Baird Parker agars for *S. aureus* isolation.

Identification methods

Methods of identification of the organisms included Gram stain reactions (5), colonial morphology, methyl red and Voges-Proskuaer (29), catalase (33), oxidase and indole tests, lactose fermentation, and slide and tube agglutination tests using freshly prepared rabbit plasma (29). *E. coli* American type culture collection (ATCC®) 25922 was used as a quality control strain. Staphytect plus (DR-0850M) (Oxoid®) was used for confirmatory identification of *S. aureus* in circumstances where results were doubtful; a latex slide agglutination was used to differentiate *S. aureus* by detection of the clumping factor, protein A and certain polysaccharides found in methicillin-resistant *S. aureus* from those that do not possess these properties. Isolates were stored on nutrient agar slants at 4°C until use for β-lactamase and minimum inhibitory concentration (MIC) testing.

Beta-lactamase determination

The production of β-lactamase was determined using the chromogenic cephalosporin method, with a cephalosporin solution (500 µg/ml) according to standard method (37), in a 96 well-U-shaped microtitre trays and commercially prepared nitrocephin–impregnated touch sticks (β-lactamase test sticks (BR0066A) (Oxoid®), in accordance with the instructions of the manufacturer. A represen-tative 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 small mass of pure cells and was observed for changes in colour at 10 min, in the case of *E. coli*, and at 1 h in the case of *S. aureus*. The colour changes from yellow to red (with the nitrocephin solution) and from yellow to pink-red (with the β-lactamase test sticks) were recorded positive for β-lactamase production, while the test is negative when the test solution or stick remains yellow (no colour change).

Determination of minimum inhibitory concentrations

The antimicrobial agents used in this study were ampicillin (10044), cephalexin (22238), penicillin G (PEN-B), chloramphenicol (23275), amoxicillin (10039), ciprofloxacin (17859), doxycycline (33429), cefuroxime (C4417), gentamicin (48757), tetracycline (87130), erythromycin (45673) and tylosin (93806), obtained from Sigma-Aldrich® (South Africa). The MIC for each antibiotic was determined using a standardised broth micro-dilution method according to standard techniques (15). The antibiotics were dissolved according to the manufacturer's instructions. Each drug was diluted to a concentration range of 0.25 µg/ml to 128 µg/ml. An inoculum of each test organism was prepared by making a direct suspension of colonies of the isolates cultured on Mueller-Hinton agar (MHA 225250) (Difco™) at 35°C for 48 h. The suspension was then adjusted to a 0.5 McFarland standard and was diluted at 1:100. All MIC plates (96 well-U-shaped microtitre trays) were inoculated with approximately 5×10^5 cfu/well in Mueller-Hinton broth (Oxoid®) using a multi-dropper pipette. Plates were sealed and incubated at 35°C for 16 to 18 h. Reference strains *E. coli* ATCC® 25922 and *S. aureus* ATCC® 25923 were used as quality control strains in each batch at approximately 5×10^5 cfu/well. Interpretative breakpoints were in accordance with the guidelines of the National Committee on Clinical Laboratory Standards (13). The MIC

was defined as the lowest concentration of the antibiotics that inhibited growth (visual turbidity) after 18-24 h (45), and the isolates were considered susceptible for each drug at MICs equal to or less than the critical concentration.

Statistical analysis

Results were compared by χ^2 test with Yates correction for continuity.

Results

Prevalence of *Escherichia coli* and *Staphylococcus aureus* in chickens

E. coli was isolated in 805 and *S. aureus* in 660 of the 1 300 specimens examined. Of the 805 *E. coli* isolates, 313 (38.9%) were isolated from broilers, 263 (32.7%) from layers and 229 (28.4%) from local chickens, whilst of the 660 *S. aureus* isolates, 211 (32%) were isolated from broilers, 231(35%) from layers and 218 (33%) from local chickens; whereas 582 (65.2%) and 223 (54.7%) *E. coli* and 380 (42.6%) and 280 (68.6%) *S. aureus* isolates were from healthy and sick chickens, respectively (Table I). Isolation based on age group showed that 111 (55.8%), 20 (11.4%) and 518 (63.5%) *E. coli* were isolated from chickens of age groups 0-4 weeks, 5-10 weeks and above 10 weeks, respectively, whilst 89 (44.7%), 161 (56.5%) and 410 (50.2%) were *S. aureus* isolates, respectively (Table II).

β-lactamase production in isolates of *Escherichia coli* and *Staphylococcus aureus* in chickens

Results of the β-lactamase test showed that 33 (10.5%), 27 (10.3%) and 29 (12.7%) *E. coli* and 9 (4.3%), 41 (17.7%) and 8 (3.7%) *S. aureus* isolates from broilers, layers and local chickens, respectively, were positive for β-lactamase production. In addition, 25 (4.3%) *E. coli* and 17 (4.5%) *S. aureus* isolates from healthy chickens were β-lactamase positive and 64 (28.7%) *E. coli* and 41 (14.6%) *S. aureus* isolates from sick chickens were β-lactamase-positive (Table I). There was no significant difference (p>0.05) between β-lactamase-positive *E. coli* and *S. aureus* isolates from healthy chickens. Similarly, there was no significant difference (p>0.05) between β-lactamase-positive isolates of *E. coli* amongst the various categories of chickens.

Table I
Prevalence of *Escherichia coli and Staphylococcus aureus* in chickens in Maiduguri (n = 1 300)

Category	No. of tissue samples tested*	No. of isolates(%)	
		Escherichia coli	*Staphylococcus aureus*
Broilers	552	33/313 (10.5)[a]	9/211 (4.3)[c]
Healthy	421	9/226 (4.0)[c]	3/121 (2.5)[d]
Sick	131	24/87 (27.6)[f]	6/90 (6.7)[e]
Layers	464	27/263 (10.3)[a]	41/231 (17.7)[i]
Healthy	321	11/190 (5.8)[e]	12/133 (9.0)[f]
Sick	143	16/73 (21.9)[j]	29/98 (29.6)[g]
Local chickens	284	29/229 (12.7[b]	8/218 (3.7)[d]
Healthy	150	5/165 (3.0)[d]	2/126 (3.0)[d]
Sick	134	24/64 (38.0)[k]	6/92 (6.5)[e]
Total	1 300	89/805 (11.1)	58/660 (8.8)
Healthy	892	25/582 (4.3)[c]	17/380 (4.5)[c]
Sick	408	64/223 (28.7)[g]	41/280 (14.6)[h]

* trachea, lung, liver, small intestine, hock joint, digital pad and cloacal swabs

Figures in brackets are the percentage of beta-lactamase positive isolates
Numerator: number of beta-lactamase positive isolates
Denominator: number of isolates screened
Mean values in a column with different superscripts differ significantly (p<0.05)

Table II
Distribution of beta-lactamase *Escherichia coli and Staphylococcus aureus* on the basis of age of chickens in Maiduguri
(*n* = 1 300)

Category of chicken and isolate	Age of chicken/no. of isolates(%) 0-4 weeks	5-10 weeks	>10 weeks	Total
Broilers				
Samples screened*	127	159	287	552
E. coli isolates	5 /52 (9.6)[a]	9/79 (11.4)[a]	19/ 182(10.4)[a]	33 / 313 (10.5)[a]
S. aureus isolates	2/36 (5.6)[b]	3/68 (4.4)[b]	4/107(3.7)[b]	9/ 211(4.3)[b]
Layers				
Samples screened*	56	94	321	464
E. coli isolates	6/ 52 (11.5)[a]	7/70 (10.0)[a]	14/141 (9.9)[a]	27/ 263 (10.3)[a]
S. aureus isolates	8/47 (17.0)[e]	11/ 72(15.3)[c]	22/112 (19.6)[f]	41/ 231 (17.7)[e]
Local chickens				
Samples screened*	16	32	208	284
E. coli isolates	1/ 7 (14.3)[c]	4/27 (14.8)[c]	24/ 195 (12.3)[d]	29/ 229 (12.7)[d]
S. aureus isolates	0/6 (0.0)	1/21 (4.8)[b]	7/191 (3.7)[b]	8/218 (3.7)[b]
Total				
Samples screened*	199	285	816	1 300
E. coli isolates	12/111 (10.8)[g]	20/ 176 (11.4)[g]	57/ 518 (11.0)[g]	89/805 (11.1)[g]
S. aureus isolates	10/89 (11.2)[g]	15/ 161 (9.3)[h]	33/410 (8.0)[h]	58/ 660 (8.8)[h]

* trachea, lung, liver, small intestine, hock joint, digital pad and cloacal swabs

Figures in brackets are the percentage of beta-lactamase positive isolates
Numerator: number of beta-lactamase positive isolates
Denominator: number of isolates screened
Mean values in a column and along the row with different superscripts differ significantly ($p<0.05$)

Furthermore, β-lactamase isolates of *S. aureus* from broilers and local chickens did not differ significantly ($p>0.05$). In addition, the distribution of isolates based on age groups of chickens showed that 12 (10.8%), 20 (11.4%) and 57 (11.0%) of *E. coli* and 10 (11.2%), 15 (9.3%) and 33 (8.0%) of *S. aureus* isolated from chickens of age groups 0 to 4 weeks, 5 to 10 weeks and above 10 weeks, respectively, were positive. More β-lactamase-positive *E. coli* were isolated from local chickens (12.7%), whilst more β-lactamase-positive *S. aureus* were isolated from layers (17.7%). β-lactamase isolates of *E. coli* from broilers and layers did not show any significant difference ($p>0.05$). Similar isolates of *S. aureus* isolated from all age groups in broilers, and those from age groups 5 to 10 weeks and above 10 weeks old in local chickens did not differ significantly ($p>0.05$).

β-lactamase-positive strains of *E. coli* were more prevalent in isolates from the liver (16.9%), lung (15.5%), trachea (15.3%) and digital pad (14.3%) than from small intestine (9.7%), cloacal swab (4.9%) and hock joint (4.2%) whilst positive strains of *S. aureus* were more prevalent in isolates from the small intestine (16.1%), liver (13.6%) and digital pad (10.7%) than from the trachea, lung and cloacal swabs (Table III).

Minimum inhibitory concentration

Table IV shows the result of antibiotic susceptibility tests of the isolates. The MICs of the antibiotics against the isolates revealed a high level of resistance in *E. coli* to ampicillin, amoxicillin, penicillin, chloramphenicol, tetracycline, doxycycline, erythromycin and tylosin (MIC >128 μg/ml), cephalexin and cefuroxine (MIC range: 32 μg-128 μg/ml), and decreased susceptibility to ciprofloxacin (MIC range: 4-128 μg/ml) and gentamicin (MIC range: 16-128 μg/ml). *S. aureus* isolates showed a high level of resistance to all the antibiotics with only 2 (14%) that showed decreased susceptibilityto ciprofloxacin (MIC range: 16-64 μg/ml).

Table III
Prevalence of β-lactamase *Escherichia coli and Staphylococcus aureus* in tissues of chickens *

No. of tissue samples tested	No. of isolates screened		No. positive for β-lactamase (%)		No. negative for β-lactamase (%)	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*
Trachea (196)	111	93	17 (15.3)[1]	9 (9.7%)[7]	94 (84.7)	84 (90.3)
Lung (196)	123	103	19 (15.5)[1]	10 (9.7%)[7]	104 (84.5)	93 (90.3)
Liver (196)	89	59	15 (16.9%)[2]	8 (13.6%[8]	74 (83.1)	51 (86.4)
Small intestine (196)	144	56	14 (9.7%)[3]	9 (16.1)[9]	130 (90.3)	47 983.9)
Hock joint (158)	71	74	3 (4.2)[4]	6 (8.1)[10]	68 (95.8)	68 (91.9)
Digital pad (157)	84	121	12 (14.3)[5]	13 (10.7)[11]	72 (85.7)	108 (89.3)
Cloacal swab (201)	183	154	9 (4.9)[6]	3 (1.9)[12]	174 (95.1)	151 (98.1)
Total (1 300)	805	660	89 (11.1)	58 (8.80)	716 (88.9)	602 (91.20)

Mean values in a column with different superscripts differ significantly ($p<0.05$)

Table IV
Minimum inhibitory concentrations for some representative isolates of β-lactamase *Escherichia coli and Staphylococcus aureus* from chickens in Maiduguri

Isolate	No.*	Minimum inhibitory concentration range (µg/ml)											
		PenG	Amp	Amo	Cep[a]	Cef[a]	Cip[b]	Gen[c]	Chlo	Tetra	Dox	Ery	Tyl
E. coli	12	>128	>128	>128	32≥128	32≥128	4≥128	16≥128	>128	>128	>128	>128	>128
S. aureus	14	>128	>128	>128	>128	>128	16≥128	>128	>128	>128	>128	>128	>128

* number of isolates screened

(a) 4 of the *E. coli* isolates had a MIC range of 32 µg/ml-64 µg/ml; the rest had ≥128 µg/ml
(b) 5 of the *E. coli* isolates had a MIC range of 4 µg/ml-32 µg/ml; 2 of the *S. aureus* isolates had a MIC range of 16 µg/ml-64 µg/ml; the rest had ≥128 µg/ml
(c) 6 of the *E. coli* isolates had a MIC range of 16 µg/ml-64 µg/ml

PenG penicillin G
Amp ampicillin
Amo amoxicillin
Cep cephalexin[a]
Cef cefuroxime[a]
Cip ciprofloxacin[b]
Gen gentamicin[c]
Chlor chloramphenicol
Tetra tetracycline
Dox doxycycline
Ery erythromycinm
Tylo tylosin

Discussion

E. coli and *S. aureus* infections have created major human and animal health problems globally (21, 42, 43, 52). *E. coli* and *S. aureus* are the most common bacterial organisms isolated and identified in clinical microbiology (36, 49) and have been described as indicator bacteria and opportunistic bacteria, respectively (24, 53). The high rate of isolation of these organisms in the present study in chickens is therefore not surprising.

The production of β-lactamases by different organisms has long been recognised as one of the mechanisms of conferring resistance against β-lactam antibiotics (1, 55). The emergence and the rising prevalence of β-lactamases in Gram-positive and Gram-negative bacteria has increased the possibility that antimicrobial agents may be ineffective in the empiric therapy of infections due to β-lactamase-producing bacteria in both humans and animals. Development and widespread use of oxymino-cephalosporins led to the emergence of ESBLs that hydrolyse penicillins, extended-spectrum cephalosporins and aztreonams (7). In the present study, the isolates demonstrated resistance against penicillins and second generation cephalo-sporins, and most probably they might

hydrolyse oxymino-cephalosporins. The frequency of occurrence of the β-lactamase-positive isolates in our study revealed that *E. coli* were more prevalent (12.7%) in local chickens whereas *S. aureus* was more prevalent (17.7%) in layers than in broilers and local chickens. Both organisms were observed to be more prevalent in diseased chickens than in apparently healthy ones. The isolation of β-lactamase-producing organisms in local chickens in this region of the country pose a serious danger of transferrable resistance to antibiotics in rural communities who are accustomed to local chickens.

Several studies have reported the isolation of β-lactamase *E. coli* and *S. aureus* from humans, animals and various organs or tissues in chickens (9, 21, 30, 32, 42, 44). The high prevalence of these organisms in tissue isolates from chickens in the present study is an indication that they are the most common microflora of both human and animal bodies and can revert to virulence, being opportunistic pathogens. Lack of data on β-lactamase-producing organisms in Nigeria could be a drawback in establishing the prevalence of the β-lactamase enzymes and the associated public health risk, thus making a basis for comparison in the present study very difficult.

The prevalence of ESBL-producing strains varies from country to country and from species to species, and even between the types of samples collected (1). Organisms producing ESBLs have been isolated and identified in several places across the world including South Africa (38), England (23), France (46), China, Thailand and countries in the Asia-Pacific region (22) and elsewhere. In the present study, 75% and 66.7% of the *E. coli* isolates tested were resistant to cephalexin and cefuroxime (cephalosporins), respectively. These isolates might possess gene encoding ESBLs, but this needs to be investigated.

β-lactam antibiotics are used widely in human and veterinary medicine in the treatment of human and animal infections. This widespread use of antibiotics could be associated with selection of antibiotic resistance mechanisms in pathogenic and non-pathogenic *E. coli* and *S. aureus* (9). Antibiotic resistance in bacterial organisms has increased significantly in recent years. Resistance rates vary amongst species and also from one region to another. Even within the same species, MICs to particular agents may vary significantly (14). *Salmonella* spp. was reported to be resistant to ampicillin, gentamicin, chloramphenicol, tetracycline and amoxicillin (3). Studies at Meriden and Putra (Malaysia) independently reported a multi-drug resistant *E. coli* which was highly resistant to ampicillin, amoxicillin, enrofloxacin, norfloxacin, apramycin, doxycycline, neomycin and colistin (48). The high rates of resistance by *E. coli* obtained in the present study against these antibiotics is not surprising as the efficacy of these drugs has even recently been doubted in the therapy of salmonellosis in humans in Tanzania (51) and in Nigeria (2). Despite the low use of macrolide antibiotics, erythromycin and tylosin and aminoglycosides, gentamicin and streptomycin, in this part of the country, high resistance still occurs amongst common laboratory isolates of *E. coli* and *S. aureus*. Resistance in these isolates to tylosin, erythromycin and gentamicin reported in the present study might be gene-mediated since overuse of these drugs has not been reported in poultry in this region.

Methicillin-resistant *S. aureus* (MRSA) has become a rapidly emerging clinical and epidemiological problem worldwide since its discovery in the 1960s (36). Since then, the strain has continued to be isolated in both humans and animals (9, 10, 35, 47). Multiple-drug resistant staphylococci and strains resistant to β-lactam antibiotics have been suspected of being methicillin-resistant strains (52), and may carry the *mecA* chromosomal gene responsible for encoding the penicillin-binding protein (PBP_{2a}) in *S. aureus* responsible for resistance to β-lactam antibiotics (30, 52). In our study, *S. aureus* isolates were highly multi-drug resistant. These isolates may possibly posses other resistant markers in addition to the production of β-lactamases.

The prevalence of penicillin and cephalosporin-resistant clinical isolates of *E. coli* and *S. aureus* has been on the increase and most of the isolates were β-lactamase

producers (12, 34). The present study supports the above reports. Resistance to penicillins and cephalosporins by these bacteria may pose a possible human risk of transferrable resistance and may portend further complications in the treatment of diseases caused by these organisms in animals, as well as in humans who consume chicken meats carrying resistant strains of these organisms.

Conclusion

In conclusion, the results from our study critically demonstrated the presence and prevalence of β-lactamase-producing *E. coli* and *S. aureus* isolates in chickens in Nigeria, to our knowledge for the first time, and that these isolates are multi-drug resistant, thus indicating a serious future threat to public health.

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Conflict of interest

The authors have no conflict of interest to disclose.

References

1. Aggarwal R., Chaudhary U. & Yadav A. 2006. Prevalence and antimicrobial susceptibility of extended-spectrum Beta-lactamase-producing Gram-negative bacilli in blood stream infection. *J Infect Dis Antimicrob Agents*, **23**, 111-114 (www.idthai.org/Publication/pdf/Vol23-3/Ch1-V23-3.pdf accessed on 12 May 2010).
2. Akinyemi K.O., Smith S.I., Oyefolu A.O. & Coker A.O. 2005. Multi-drug resistance in *Salmonella* enterica serovar typhi isolated from patients with typhoid fever complications in Lagos, Nigeria. *Public Health*, **119**, 321-327.
3. Akinyemi K.O., Philipp W. Beyer W. & Böhm R. 2007. *In vitro* antimicrobial susceptibility patterns of *Salmonella enterica* serovars and emergence of *S. typhimurium* phage DT071 in a suspected community-associated outbreak in Lagos, Nigeria. *J Infect Dev Ctries*, **1**, 48-54 (www.oloep.org/uploadedFiles/jidc/issues/2007_08/09_ms0507_akinyemi_F_AQ.pdf accessed on 12 May 2010).
4. Ambler R.P. 1980. The structure of beta-lactamases. *Philos Trans R Soc. Lond B Biol Sci*, **289**, 321-331.
5. Baker F.J., Silverton R.E. & Pallister C.J**.** 2001. Gram staining. *In* Baker and Silverton's introduction to medical laboratory technology, Nigerian Ed. Bounty Press Ltd, Ibadan, 275-277.
6. Ben Hassen A., Fournier G., Kechrid A., Fendri C., Ben Redjeb S. & Philippon A. 1990. Résistance enzymatique au cefotaxime chez cinquante-six souches de *Klebsiella* spp., *Escherichia coli* et *Salmonella* spp. dans un hôpital tunisien (1984-1988). *Path Biol*, **38**, 464-469.
7. Brad M.W. & Edward H.E. 2008. Current perspectives on extended-spectrum beta-lactamase-producing Gram-negative bacilli. *J Pharm Pract*, **21**, 338-345.
8. Bradford P.A. 2001. Extended-spectrum beta-lactamases in the 21st century, characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev*, **48**, 933 951.
9. Briñas L., Zarazaga M., Sáenz Y., Ruiz-Larrea F. & Torres C. 2002. Beta-lactamases in ampicillin-resistant *Escherichia coli* isolates from foods, humans and healthy animals. *Antimicrob Agents Chemother*, **46**, 3156-3163.
10. Briñas L., Moreno M.A., Zarazaga M., Porrero C., Sáenz Y., García M., Dominguez L., Torres C. 2003. Detection of CMY-2, CTX-M-14, and SHV-12 β-lactamases in *Escherichia coli* fecal sample isolates from healthy chickens. *Antimicrob Agents Chemother*, **47**, 2056-2058.
11. Brook I. 2005. Beta-lactamase-producing bacteria. *Rev Med Microbiol*, **16**, 91-99.

12. Chlebicki M.P. & Oh H.M.L. 2004. Extended-spectrum beta-lactamases in clinical isolates of *Escherichia coli* and *Klebsiella* spp. in Singapore Hospital clinical spectrum. *Ann Acad Med*, **33**, 302-306.
13. Clinical and Laboratory Standards Institute (CLSI) (previously: National Committee on Clinical Laboratory Standards: NCCLS) 2001. Performance standards for antimicrobial susceptibility testing, 11th Informational Supplement: Approved Standard, M2-A7 and M7-A5, NCCLS, Wayne, Pennsylvania, 4-5.
14. Clinical and Laboratory Standards Institute (CLSI) (previously: National Committee on Clinical Laboratory Standards: NCCLS) 2006. Methods for antibacterial susceptibility testing of anaerobic bacteria; Approved standard, Seventh Ed. CLSI, Wayne, Pennsylvania, Vol. 27, No. 2, CLSI Document M11-A7, 1 p (www.clsi.org/source/orders/free/m11a7.pdf accessed on 11 May 2010).
15. Clinical and Laboratory Standards Institute (CLSI) (previously: National Committee on Clinical Laboratory Standards: NCCLS) 2008. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: Approved Standard, Third Ed., M31-A3, **28** (8). CLSI, Wayne, Pennsylvania, 11 pp (www.clsi.org/source/orders/free/m31-a3.pdf accessed on 23 November 2009).
16. Dubois S.K., Marriot M.S. & Amyes S.G.B. 1995. TEM- and SHV-derived extended-spectrum beta-lactamases: relationship between selection, structure and function. *J Antimicrob Chemother*, **35**, 7-22.
17. Dyke K.G.H., Parker M.T. & Richmond M.H. 1970. Penicillinase production and metal-ion resistance in *Staphylococcus aureus* cultures isolated from hospital patients. *J Med Microbiol*, **3**, 125-136.
18. Farkosh M.S. 2009. Extended-spectrum beta-lactamase-producing Gram-negative bacilli. Johns Hopkins Medicine, Hospital Epidemiology/Infection Control, Baltimore, Maryland (www.hopkinsmedicine.org/heic/ID/esbl/ accessed on 12 May 2010).
19. Forbes B.A. & Granato P.A. 1995. Processing specimens for bacteria. *In* Manual of clinical microbiology, 6th Ed. (P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover & R.H. Yolken, eds). ASM Press, Washington, DC, 265-278.
20. Gold H.S & Moellering Jr R.C. 1996. Antimicrobial-drug resistance. *N Engl J Med*, **355**, 1445-1452.
21. Hanchun Y.S., David G.W., Shaohua Z., McDermott P., Walker R. & Jianghong M. 2004. Characterization of multiple antimicrobial-resistant *Escherichia coli* isolates from diseased chickens in China. *J Clin Microbiol*, **42**, 3484-3489.
22. Hawser SP., Bouchillon S.K., Hoban D.J., Badal R.E., Hsueh P.R. & Paterson D.L. 2009. Emergence of high levels of extended-spectrum-beta-lactamase-producing Gram-negative bacilli in the Asia-Pacific region: data from the study for monitoring antimicrobial resistance trends (SMART) Program, 2007. *Antimicrob Agents Chemother*, **53**, 3280-3284.
23. Hibbert-Rodgers L.C., Heritage J., Todd N. & Hawkey P.M. 1994. Convergent evolution of TEM-26, a beta-lactamase with extended-spectrum activity. *J Antimicrob Chemother*, **33**, 707-720.
24. Ishihara K., Kira T., Ogikubo K., Morioka A., Kojima A., Kijima-Tanaka M., Takahashi T. & Tamura Y. 2004. Antimicrobial susceptibilities of *Campylobacter* isolated from food-producing animals on farms (1999-2001): results from the Japanese Veterinary Antimicrobial Resistance Monitoring Program. *Int J Antimicrob Agents*, **24**, 261-267.
25. Jana M.S., Janet A.H & Peterson L.R. 1995. Special tests for detecting antibacterial resistance. *In* Manual of clinical microbiology, 6th Ed. (P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover & R.H. Yolken, eds). ASM Press, Washington, DC, 1356-1363.
26. Jensen E.L. & Miller C.L. 2001. *Staphylococcus* infections in broiler breeds. *AviaTech*, **1** (1), 1-6 (www.canadianpoultry.ca/cms_pdfs/AviaTech_Staph.pdf accessed on 12 May 2010).
27. Karisik E., Ellington M.J., Pike R., Warren R.E., Livermore D.M. & Woodford N. 2006. Molecular characterization of plasmids encoding CTX-M-15 β-lactamases from *Escherichia coli*. strains in the United Kingdom. *J Antimicrob Chemother*, **58**, 665-668.
28. Kim J.Y., Jung H.I., An Y.J., Lee J.H., Kim S.J., Jeong S.H., Lee K.J., Suh P.G., Lee H.S., Lee S.H. & Cha S.S. 2006. Structural basis for the extended substrate spectrum of CMY-10, a plasmid-encoded class C-beta-lactamase. *Mol Microbiol*, **60**, 907-916.
29. Kloos W.E. & Jorgensen J.H. 1985. *Staphylococci*. *In* Manual of clinical microbiology, 4th Ed. (E.H. Lennette, A. Balows, W.J. Hausler Jr & H.J. Shadomy, eds). American Society for Microbiology, Washington, DC, 143-153.
30. Kwon N.H., Park K.T., Jung W.K., Youn H.Y., Lee Y., Kim S.H., Bae W., Lim J.Y., Kim J.M., Hong S.K. & Park Y.H. 2006. Characteristics of methicillin-resistant *Staphylococcus aureus* isolated from from

chicken meat and hospitalized dogs in Korea and their epidemiological relatedness. *Vet Microbiol*, **117**, 304-312.

31. Lee J.H. 2006. Occurrence of methicillin-resistant *Staphylococcus aureus* strains from cattle and chicken, and analyses of their *mecA*, *mecRI* and *mecI* genes. *Vet Microbiol*, **114**, 155-159.
32. Lee J.H., Jung H.I., Jung J.H., Park J.S., Ahn J.B., Jeong S.H., Jeong B.C., Lee J.H. & Lee S.H. 2004. Dissemination of transferable Amp^C-type beta-lactamases (CMY-10) in a Korean hospital. *Microb Drug Resist*, **10**, 224-230.
33. MacFaddin J.F. 1980. Biochemical tests for identification of medical bacteria, 2nd Ed. Waverly Press Inc., Baltimore, Maryland, 51-74.
34. Mansouri S. & Khalegh M. 1997. Antibacterial resistance pattern and frequency of methicillin-resistant *Staphylococcus aureus* isolated from different sources in south-eastern Iran. *Iran J Med Sci*, **22**, 89-93.
35. Mazura F. 1990. Determination of beta-lactamase production in strains of *Staphylococcus aureus* isolated from the milk of cows. *Vet Med (Praha)*, **35**, 267-274.
36. Merlino J., Gill R. & Robertson G.J. 1996. Application of lipovitelin-salt-mannitol agar for screening, isolation, and presumptive identification of *Staphylococcus aureus* in a teaching hospital. *J Clin Microbiol*, **34**, 3012-3015.
37. Miles R.S. &. Amyes S.G.B. 1996. Laboratory control of antimicrobial therapy. *In* MacKie and McCartney's Practical medical microbiology, 14th Ed. 1996. (J.G. Collee, A.G. Fraser, P.B. Marmion & A. Simmons eds). Longman, Churchill Livingstone, Singapore, 151-157.
38. Paterson D.L., Hujer K.M., Hujer A.M. & Yeiser M.D.B. 2003. Extended spectrum beta-lactamases in *Klebsiella pneumoniae* blood stream isolates from seven countries: dominance and wide spread prevalence of SHV-and CTX-M-type beta-lactamases. *Antimicrob Agents Chemother*, **47**, 3545-3560.
39. Pitout J.D.D., Thomson K.S., Hanson N.D., Ehrhardt A.F., Moland E.S. & Sanders C.C. 1998. Beta-lactamases responsible for resistance to expanded-spectrum cephalosporins in *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabilis* isolates recovered in South Africa. *Antimicrob Agents Chemother*, **42**, 1350-1354.
40. Raji M.A., Adekeye J.O., Kwaga J.K.P. & Bale J.O.O. 2003. *In vitro* and *in vivo* pathogenicity studies of *Escherichia coli* isolated from chickens in Nigeria. *Israel J Vet Med*, **58**, 58-106.
41. Richard C., Philippon A., M'Boup S. & Vieu J.F. 1989. Épidemiologie des infections pédiatriques a *Klebsiella* dans deux hôpitaux de Dakar. Production de β-lactamases à spectre élargi (1987-1988). *Med Mal Infect*, **19**, 753-759.
42. Saed A.K., Mohammed S.N., Ashraf A.K. & Carl E.C. 2000. Transfer of erythromycin resistance from poultry to human clinical strains of *Staphylococcus aureus*. *J Clin Microbiol*, **38**, 1832-1838.
43. Sanjiv K., Kataria A.K., Sharma R. & Singh G. 2008. Epidemiological typing of *Staphylococcus aureus* by DNA restriction fragment polymorphism of *coa* gene. *Vet Arhiv*, **78**, 31-38 (hrcak.srce.hr/file/38238 accessed on 12 May 2010).
44. Schroeder C.M., Meng J., Zhao S. DebRoy C., Torcolini J. Zhao C. McDermott P.F., Wagner D.D., Walker R.D. & White D.G. 2002. Antimicrobial resistance of *Escherichia coli* O26, O103, O111, O128, and O145 from animals and humans. *Emerg Infect Dis*, **8**, 1409-1414.
45. Shakibaie M.R., Mansouri S. & Hakak S. 2002. Plasmid pattern of antibiotic resistance in beta-lactamase producing *Staphylococcus aureus* strains isolated from hospitals in Kerman, Iran. *Archiv Iran Med*, **2** (2) (www.ams.ac.ir/AIM/9922/shakibaei9922.html accessed on 12 May 2010).
46. Soilleux M.J., Morand A.M., Arlet G.J., Scavizzi M.R. & Labia R. 1996. Survey of *Klebsiella pneumoniae* producing extended-spectrum beta-lactamases: prevalence of TEM-3 and first identification of TEM-26 in France. *Antimicrob Agents Chemother*, **40**, 1027-1029.
47. Teshager T., Dominguez L., Moreno M.A., Sáenz Y., Torres C. & Cardnosa S. 2000. Isolation of an SHV-12 beta-lactamase-producing *Escherichia coli* strain from a dog with recurrent urinary tract infections. *Antimicrob Agents Chemother*, **44**, 3483-3484.
48. The Poultry Site 2009. Antimicrobial sensitivity testing and heat treatment on Orego-Stim powder (www.thepoultrysite.com/articles/1514/antimicrobial-sensitivity-testing-and-heat-treatment-on-oregostim-powder accessed on 12 May 2010).
49. Trepeta R.W. & Edberg S.C. 1983. Methylumbelliferyl-beta-D-glucuronide-based medium for rapid isolation and identification of *Escherichia coli*. *J Clin Microbiol*, **19**, 172-174.
50. Uma A., Mehta A., Ayyagari A., Kapil A., Shahnvi A., Rodrigues C., Pepa C., Pujol M., Ardanuy C., Ricart A., Pallares R. & Lipares J. 2004. Prevalence of beta-lactamase producing strains among

clinical isolates obtained from hospital in-patients across India and comparison of antibacterial susceptibility using disk diffusion method. Expert group on antibiotic susceptibility tests (EGAST) 2000. *Hosp Today*, **9**, 70-80.

51. Vaagland H., Blomberg B., Krüger C., Naman N., Jureen R. & Langeland N. 2004. Nosocomial outbreak of neonatal *Salmonella enterica* serotype *Enteritidis meningititis* in a rural hospital in northern Tanzania. *BMC Infect Dis*, **4**, 35.
52. Van Duijkeren E.V., Box A.T.A., Heck M.E. O.C., Wannet W.J.B. & Fluit A.C. 2004. Methicillin-resistant *Staphylococci* isolated from animals. *Vet Microbiol*, **103**, 91-97.
53. Wesley E.K & Tammy L.B. 1995. *Staphylococcus* and *Micrococcus*. *In* Manual of clinical microbiology, 6th Ed. (P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover & R.H. Yolken, eds). ASM Press, Washington, DC, 282-292.
54. Wikipedia 2009. Beta-lactam (en.wikipedia.org/wiki/beta-lactam accessed on 12 May 2010).
55. Yu C., Karr T., Turner D., Towns V. & Sinha J. 1999. Rapid detection of beta-lactamase production in penicillin sensitive *Staphylococcus aureus* by the Phoenix™ automated ID/AST system. Paper presented at the 9th European Congress of clinical microbiology and infectious diseases (ECCMID), 21-24 March 1999, Berlin. Becton, Dickinson and Company (BD) Biosciences, Sparks, Maryland, 2 pp (www.bd.com/ds/technicalCenter/whitepapers/LR642.pdf accessed on 23 November 2009).