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

Sunday Akidarju Mamza(1), Godwin Onyemaechi Egwu(2) & Gideon Dauda Mshelia(3)

**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**

**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 cosina 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 dodi 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**

**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-Proskauer (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 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 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), cephalaxin (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 (Mueller-Hinton) (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 × 10^4 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 × 10^4 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 χ² 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**

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of tissue samples tested*</th>
<th>No. of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>Broilers</td>
<td>552</td>
<td>33/313 (10.5)(a)</td>
</tr>
<tr>
<td>Healthy</td>
<td>421</td>
<td>9/226 (4.0)(c)</td>
</tr>
<tr>
<td>Sick</td>
<td>131</td>
<td>24/87 (27.6)(b)</td>
</tr>
<tr>
<td>Layers</td>
<td>464</td>
<td>27/263 (10.3)(a)</td>
</tr>
<tr>
<td>Healthy</td>
<td>321</td>
<td>11/190 (5.8)(w)</td>
</tr>
<tr>
<td>Sick</td>
<td>143</td>
<td>16/73 (21.9)(h)</td>
</tr>
<tr>
<td>Local chickens</td>
<td>284</td>
<td>29/229 (12.7)(h)</td>
</tr>
<tr>
<td>Healthy</td>
<td>150</td>
<td>5/165 (3.0)(d)</td>
</tr>
<tr>
<td>Sick</td>
<td>134</td>
<td>24/64 (38.0)(h)</td>
</tr>
<tr>
<td>Total</td>
<td>1 300</td>
<td>89/805 (11.1)</td>
</tr>
<tr>
<td>Healthy</td>
<td>892</td>
<td>25/582 (4.3)(k)</td>
</tr>
<tr>
<td>Sick</td>
<td>408</td>
<td>64/223 (28.7)(d)</td>
</tr>
</tbody>
</table>

* 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)

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

<table>
<thead>
<tr>
<th>Category of chicken and isolate</th>
<th>Age of chicken/no. of isolates (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-4 weeks</td>
<td>5-10 weeks</td>
</tr>
<tr>
<td>Broilers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samples screened*</td>
<td>127</td>
<td>159</td>
</tr>
<tr>
<td>Layers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samples screened*</td>
<td>56</td>
<td>94</td>
</tr>
<tr>
<td>E. coli isolates</td>
<td>6/52 [11.5]**</td>
<td>7/70 [10.0]**</td>
</tr>
<tr>
<td>S. aureus isolates</td>
<td>8/47 [17.0]**</td>
<td>11/72 [15.3]**</td>
</tr>
<tr>
<td>Local chickens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samples screened*</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>E. coli isolates</td>
<td>1/7 [14.3]**</td>
<td>4/27 [14.8]**</td>
</tr>
<tr>
<td>S. aureus isolates</td>
<td>0/6 [0.0]</td>
<td>1/21 [4.8]**</td>
</tr>
<tr>
<td>Total</td>
<td>199</td>
<td>285</td>
</tr>
<tr>
<td>S. aureus isolates</td>
<td>10/89 [11.2]**</td>
<td>15/161 [9.3]**</td>
</tr>
</tbody>
</table>

* 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 cefuroxime (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 susceptibility to ciprofloxacin (MIC range: 16-64 μg/ml).
Beta-lactamase Escherichia coli and Staphylococcus aureus
isolated from chickens in Nigeria

Sunday Akidarju Mamza, Godwin Onyemaechi Egwu
& Gideon Dauda Mshelia

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

<table>
<thead>
<tr>
<th>No. of tissue samples tested</th>
<th>No. of isolates screened</th>
<th>No. positive for β-lactamase (%)</th>
<th>No. negative for β-lactamase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli</td>
<td>S. aureus</td>
<td>E. coli</td>
</tr>
<tr>
<td>Trachea (196)</td>
<td>111</td>
<td>93</td>
<td>17 (15.3)</td>
</tr>
<tr>
<td>Lung (196)</td>
<td>123</td>
<td>103</td>
<td>19 (15.5)</td>
</tr>
<tr>
<td>Liver (196)</td>
<td>89</td>
<td>59</td>
<td>15 (16.9%)</td>
</tr>
<tr>
<td>Small intestine (196)</td>
<td>144</td>
<td>56</td>
<td>14 (9.7%)</td>
</tr>
<tr>
<td>Hock joint (158)</td>
<td>71</td>
<td>74</td>
<td>3 (4.2%)</td>
</tr>
<tr>
<td>Digital pad (157)</td>
<td>84</td>
<td>121</td>
<td>12 (14.3%)</td>
</tr>
<tr>
<td>Cloacal swab (201)</td>
<td>183</td>
<td>154</td>
<td>9 (4.9%)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Isolate</th>
<th>No.*</th>
<th>PenG</th>
<th>Amp</th>
<th>Amo</th>
<th>Minimum inhibitory concentration range (µg/ml)</th>
<th>Cef</th>
<th>Cip</th>
<th>Gen</th>
<th>Chlo</th>
<th>Tetra</th>
<th>Dox</th>
<th>Ery</th>
<th>Tyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>12</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>32±128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>S. aureus</td>
<td>14</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>16±128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
</tbody>
</table>

* 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

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 oxyminocephalosporins 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 cephalosporins, 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 cephalaxin 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 multidrug 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 (PBP2a) in S. aureus responsible for resistance to β-lactam antibiotics (30, 52). In our study, S. aureus isolates were highly multidrug resistant. These isolates may possibly possess 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
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Conflict of interest

The authors have no conflict of interest to disclose.

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