The occurrence of ESBL Producing *Escherichia coli* and *Klebsiella* species in Selected Broiler Farms in Jalingo, Taraba State Nigeria

1Mbah, M. I., 2Anyamene, C. O.
1Department of Medical Laboratory Science, Taraba State University, Jalingo Taraba State Nigeria
2Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka Anambra State Nigeria

**ABSTRACT**

Food-producing animals, including poultry, have been considered as potential sources of extended-spectrum ß-lactamase (ESBL) - producing *Escherichia coli*. This study investigates the occurrence and distribution of ESBL-producing *E. coli* and *Klebsiella* species among three broiler poultry farms in Jalingo, Taraba State Nigeria. One hundred and thirty-eight isolates which comprised of *E. coli*, *K. pneumoniae* and *K. oxytoca* were isolated and successfully identified from the broilers farms. The isolates were tested for beta-lactamase producing ability as well as screened for the antibiotic susceptibility profile and the curing rate of the ESBL positive bacteria using standard procedures. While the results obtained revealed the occurrence of 94.90%, 3.00% and 2.20% for the *E. coli*, *K. pneumoniae* and *K. oxytoca* in the farms respectively, the prevalence of ESBL producing bacteria isolates was 34.10%, 0.70% and 0% respectively for the *E. coli*, *K. pneumoniae* and *K. oxytoca*. It was also revealed that clavulanic acid inhibited the ESBL enzyme. The antibiotic susceptibility profile of the ESBL producing *E. coli* showed a 100% resistance to ampicillin, ceftazidime and ceftriaxone as well as a 46.80%, 70.20%, 2.10%, 25.50% and 59.60% resistance to chloramphenicol, ciprofloxacin, nitrofurantoin, gentamicin and tetracycline respectively. The curing rate of the ESBL positive producing *E. coli* was 16.70%. This study revealed the presence of ESBL-producing *E. coli* in chickens from small-scale commercial poultry farms in Jalingo, thus indicating that chickens may serve as important reservoirs for the transmission of antimicrobial-resistant pathogens to humans through the food chain.

**INTRODUCTION**

A high percentage of hospital-acquired infections are caused by drug-resistant bacteria such as extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* (Paterson and Bonomo 2005). Patients with infections caused by drug-resistant bacteria are generally at high risk of worse clinical outcomes and death and hence require more healthcare resources than patients infected with the same bacteria that are not resistant (Tumbarello, 2006).
The evolution of resistant strains is a natural phenomenon that occurs when microorganisms themselves erroneously mutate or when resistant traits are exchanged between them. The use and misuse of drugs accelerate the emergence of drug-resistant strains (Cheesbrough, 2010). Poor infection control and inappropriate food-handling encourage the further spread of antimicrobial resistance. New resistance mechanisms emerge and spread globally threatening our ability to treat common infectious diseases, resulting in the death and disability of individuals (Tumbarello, 2006). The achievements of modern medicine are put at risk by antimicrobial resistance. Without effective antimicrobial agents for the prevention and treatment of infectious diseases, the success of organ transplantation, cancer chemotherapy and major surgery would be compromised (WHO, 2014).

ESBL producing bacteria destroy the beta-lactam ring of penicillins and cephalosporins thereby rendering the antimicrobials inactive. ESBLs hydrolyze oxyimino-cephalosporins such as cefuroxime, cefotaxime, ceftriaxomine, ceftizoxime, cefazidime, cepirome and cefepime, aztreonam as well as penicillins and another cephalosporin except for cephamycin (cefoxitin and ceftetean) (Susic, 2004). ESBL producing gram-negative organisms limit the therapeutic options as well as their multidrug resistance (Anago et al., 2015). Drug resistance in animals is caused mainly by the large number of antimicrobial drugs used in food production. Grave et al., (2010) reported that the Netherlands is one of the highest users of antimicrobial agents in food production for animals which results in a high rate of drug resistance. This study was therefore aimed at evaluating the occurrence of ESBL producing E.coli and Klebsiella species isolated from selected broilers' farms in Jalingo, Taraba State.

MATERIALS AND METHODS

Study Area

Jalingo Local Government Area, the capital of Taraba State located in the North-East geopolitical zone and situated between latitude 8° 47’ North and 90°1’ North and longitude 11°9’ East and 11°30’ East was the study location.

Sample Collection

Samples were collected from three poultry farms which comprised of 2 broiler chicken farms with chickens 7 weeks old and one broiler chicken farm with chickens more than 7 weeks old. The broilers chickens used for this study were all healthy and bred specifically for meat production. The samples were cloaca, stool and floor swabs. Swab sticks were soaked in sterile distilled water and inserted into the cloaca of randomly selected chickens in the selected poultry farms. While in the cloaca, the swab stick was rotated three times before inserting it into its container. Sterile distilled water swab sticks were used to swab strategic areas on the floor of the poultry. A wide-mouthed sterile container was used to collect the stool of each of the chicken rearers.

Bacteria Isolation Procedures and Identification

Samples collected were cultured within 2 hours of collection on MacConkey agar and Eosin methylene blue (EMB) agar (Oxoid CM 516, UK) before being incubated at 37°C for 18–24 hours. Distinct colonies were examined for their morphology and ability to ferment lactose. Lactose fermenting colonies were further subcultured on the EMB agar, incubated for 18 h at 37°C and then examined for the appearance of metallic greenish colonies. All isolates (both lactose and non-lactose fermenters) were individually gram stained and subjected to biochemical tests. DNA sequencing using the sangar sequencing
technique was used for the molecular characterization of the isolates

**ESBL Detection**

Isolates were tested for beta-lactamase production using the paper acidimetric method as previously outlined by Cheesbrough, 2010. All the positive β-lactamase isolates were screened for ESBL production by double-disk synergy test (DDST) according to the procedures outlined by Liofichem (2014). Briefly, four millilitres of 0.5 McFarland equivalent standard of the test organisms were spread on the surface of a sterile Mueller Hinton agar plate using a sterile swab stick. After 20 minutes, the combination of augmentin disc of concentration 30 µg, amoxicillin of concentration 20 µg and clavulanic acid of concentration 10 µg was placed 15 mm apart from the centre of ceftiraxone disc of 30 µg concentration and ceftazidime disc of concentration 30 µg. This was incubated for 18 h at 37°C.

**Antimicrobial Susceptibility Testing**

The modified Kirby-Bauer disc diffusion method as described by the Clinical and Laboratory Standards Institute guidelines was adopted (CLSI, 2012). Briefly, suspensions were made from the 24 h growth of the organism maintained in saline to match the 0.5 McFarland turbidity standards. This was spread on the entire surface of Mueller-Hinton agar plates containing ceftazidime (30µg), ceftiraxone (30 µg), ampicillin (10 µg), nitrofurantoin (300 µg), gentamicin (10 µg), chloramphenicol (30 µg), tetracycline (30 µg) and ciprofloxacin (5 µg) using a sterile swab stick. The agar plates were incubated at 35°C for 18–24 h, after which the diameter of the zones of growth inhibition around the discs was measured using a measuring ruler. The results were further interpreted using the Performance Standards for Antimicrobial Susceptibility Testing (CLSI, 2012).

**Plasmid Profile of ESBL Positive Isolates**

The plasmid profile of the ESBL positive isolates was investigated according to the modified alkaline lysis plasmid extraction protocols described by Dagan et al. (2006) and Charles et al. (2010) respectively. A 1% Agarose gel was prepared and loaded into an electrophoresis chamber containing 8 wells; buffered with 40 mM Tris, 20 mM, 2 mM EDTA and adjusted to pH 7.8 with acetic acid. Electrophoresis was allowed to proceed at room temperature for 20 min after which the gels were stained with ethidium bromide (1 µL/mL) and viewed under UV transillumination. The molecular marker that was used was the Hind Phi III digest and extrapolations were made from the Electropherogram obtained (Piglansky et al., 2003).

**Plasmid Curing**

ESBL positive isolates were selected and subjected to acridine orange as described by Stanisich (1988). Each tested organism was grown in a solution of 5ml double strength nutrient broth supplemented with 0.1 ng/ml acridine orange and incubated at 37°C for 24 h. After incubation, the test organisms were retested for ESBL production using the double-disk synergy test (DDST).

**RESULTS AND DISCUSSION**

The result of the 138 microbial isolates obtained from the 3 broiler chicken farms shows that one hundred and thirty-one were *E. coli*, four were *K. pneumoniae* and three were *K. oxytoca* constituting 94.90%, 2.90% and 2.20% respectively. This result is presented in Figures 1 (a) – (c).
Figure 1 (a): Distribution of Bacterial Isolates obtained from the chicken cloaca, rearer stool and floor from the farm I containing 7 weeks old broiler chickens

Figure 1 (b): Distribution of Bacterial Isolates obtained from the chicken cloaca, rearer stool and floor from farm II containing 7 weeks old broiler chickens
Figure 1 (c): Distribution of Bacterial Isolates obtained from the chicken cloacae, rearer stool and floor from farm III containing over 7 weeks old broiler chickens

The number of the isolated bacteria was higher in the cloaca and stools of the chicken in all the farms investigated compared to the rearers stool and pen floors because the cloaca swabs and the stools were the contaminated samples with the floors merely contaminated as a result of the chicken’s droppings. The high occurrence of \(E. \text{coli}\) (94.9%) obtained in this study is higher than the 44% reported by Ashraf \textit{et al.} (2015) upon an investigation of the prevalence of \(E. \text{coli}\) on imported chickens. Nwakaeze \textit{et al.} (2013) and Dadheech \textit{et al.} (2016) also reported isolated 70.50% and 100% respectively of \(E. \text{coli}\) isolates from faecal and cloacal swab samples. The 3.0% \(K. \text{pneumoniae}\) occurrence rate in this study is lower than the 5.8% reported by Hassan \textit{et al.} (2015). A literature search by the researchers revealed that there has never been any report of the occurrence of \(K. \text{oxytoca}\) in chickens.

The result of the synergy between the clavulanic acid-containing disk with ceftazidime and ceftriaxone in the Double Disk Synergy Test (DDST) as well as the frequency exhibited by the ESBL positive \(E. \text{coli}\) among the 138 microbial isolates is presented in plate 1 and figure 2 (a – c).

Plate 1: Disc showing the synergy exhibited by the ESBL positive \(E.\text{coli}\) of clavulanic acid with ceftazidime and ceftriaxone in the Double Disk Synergy Test. Key: 1 - ceftazidime; 2 - ceftriaxone; 4 – augmentin.
Figure 2 (a): Distribution of ESBL positive microbial isolates obtained from the chicken cloaca, rearer stool and floor from the farm I containing 7 weeks old broiler chickens

Figure 2 (b): Distribution of ESBL positive microbial isolates obtained from the chicken cloaca, rearer stool and floor from farm II containing 7 weeks old broiler chickens
Figures 2 (c): Distribution of ESBL positive microbial isolates obtained from the chicken cloacae, reaper stool and floor from farm III containing over 7 weeks old broiler chickens

The result obtained from the DDST test showed that the zone of growth inhibition around ceftazidime (disc 1) and ceftriaxone (disc 2) augmented towards the antibiotic disc 4 which contained clavulanic acid (augmentin). This is because clavulanic acid inhibited the action of ESBL. The synergy of clavulanic acid with third-generation cephalosporin was previously reported by Oyinloye and Ezekiel (2011). The occurrence rates of ESBL producing E. coli, K. pneumoniae and K. oxytoca as reported in figures 2 (a-c) showed that while was E. coli has a prevalence rate of 34.1% higher than 14.2% reported by Nwakaese et al. (2013) with K. pneumoniae having a prevalence rate of 0.7% in agreement with the findings of Hiroi et al. (2012) who isolated one ESBL producing K. pneumoniae from the faecal samples of broilers. No occurrence was however observed with the K. oxytoca bacteria.

The susceptibility profile of the ESBL positive producing E. coli of the microbial isolates as well as their curing rate when challenged with acridine orange is presented in tables 1 as 2 as presented below.

Table 1: Antibiotic susceptibility profile of the ESBL positive E. coli

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>(µg/disc)</th>
<th>S</th>
<th>%</th>
<th>I</th>
<th>%</th>
<th>R</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>47</td>
<td>100</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30</td>
<td>14</td>
<td>29.8</td>
<td>11</td>
<td>23.4</td>
<td>22</td>
<td>46.8</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5</td>
<td>12</td>
<td>25.5</td>
<td>2</td>
<td>4.3</td>
<td>33</td>
<td>70.2</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>300</td>
<td>42</td>
<td>89.4</td>
<td>4</td>
<td>8.5</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10</td>
<td>32</td>
<td>60.1</td>
<td>3</td>
<td>14.4</td>
<td>12</td>
<td>25.5</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30</td>
<td>11</td>
<td>23.4</td>
<td>8</td>
<td>17</td>
<td>28</td>
<td>59.6</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>47</td>
<td>100</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>47</td>
<td>100</td>
</tr>
</tbody>
</table>

Key: S – sensitive; I – Intermediate; R – Resistant; % - Percentage

Corresponding author: Mbah, M. I. mdduruoha@gmail.com. Department of Medical Laboratory Science, Taraba State University, Jalingo. © 2021, Faculty of Tech. Edu, ATBU Bauchi. All rights reserved
Table 2: Curing rate of the ESBL positive E. coli isolates

<table>
<thead>
<tr>
<th>Farm</th>
<th>Bacteria</th>
<th>No of ESBL +ve Isolates</th>
<th>No of Isolates without plasmid after curing</th>
<th>% of isolates cured</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>E. coli</td>
<td>11</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>K. pneumoniae</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>K. oxytoca</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>E. coli</td>
<td>17</td>
<td>3</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>K. pneumoniae</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>K. oxytoca</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>E. coli</td>
<td>19</td>
<td>4</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>K. pneumoniae</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>K. oxytoca</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Key: No – Number; ESBL – Extended Spectrum Beta Lactamase; +ve – Positive; % - Percentage

The antibiotic susceptibility profile of ESBL positive E. coli presented in table 1 revealed that the bacteria showed 100% resistant to ampicillin, ceftriaxone and ceftazidime. The isolates were however 46.80%, 70.20%, 2.10%, 25.50% and 59.60% resistant to chloramphenicol, ciprofloxacin, nitrofurantoin, gentamicin and tetracycline respectively. The multidrug resistance observed in this study agrees with the report by Motayo et al. (2013) and Afunwa et al. (2011).

The curing rate of the ESBL positive isolates revealed that 16.70% of the ESBL positive isolates were cured while 69.20% were not (Table 2). This supports previous reports by Iroha et al. (2010) and Folasage et al. (2014).

CONCLUSION
The findings from this study confirm broilers’ farms as reservoirs of ESBL producing E. coli and K. pneumoniae. The occurrence of ESBL producing E. coli was higher than that reported for K. pneumonia.

REFERENCES


Clinical Laboratory Standards Institute (CLSI) (2012). Methods for dilution of antimicrobial susceptibility testing for bacteria that grows aerobically. 9th Edition (M07- A9), Clinical Laboratory Standards Institute, Wayne, PA, USA.


