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Full Length Research Paper

Occurrence and Antimicrobial resistance of ESBL-producing Escherichia coli in Indigenous Chickens and retailed Table-Eggs in Sokoto Metropolis, Nigeria

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Extended-Spectrum beta-Lactamases (ESBL) producing Escherichia coli has become a serious global problem, with their occurrence varying based on the use of antibiotics and environmental settings. This study was aimed at determining the frequency of occurrence of ESBL-producing E. coli and their antibiotic resistance profile from indigenous chickens and table-eggs on retail in Sokoto Metropolis, Nigeria. Between March and September 2015 (6 months), cloacal swab samples obtained from 246 indigenous chickens and 85 table-eggs samples were analyzed for ESBL-producing E. coli, using double-disk synergy test on Mueller Hinton agar. The identified ESBL strains were further subjected to antimicrobial susceptibility test to nine antimicrobial agents, using Kirby-Bauer disc-diffusion method. The overall prevalence of ESBL-producing E. coli in indigenous chickens and table-eggs samples were 8.9% and 5.7% respectively. Statistically significant difference was noted in the rates of ESBL-producing E. coli detection between indigenous chicken and retailed table-eggs (P <0.05). Over 25% of the ESBL-producing E. coli isolates are resistant to beta-lactam and other antimicrobial agents, with over 50% multi-drug resistant strains. ESBL-producing E. coli strains showed higher resistance to Tetracycline, Sulphamethoxazole and Ampicillin. These results indicate that indigenous chickens are potential reservoir for multi-drug resistant ESBL-producing E. coli, which is of public and animal health concern.

Key words: ESBL-producing E. coli, Antimicrobial-resistance, Indigenous-Chickens, Table Eggs.

INTRODUCTION

Antibiotics such as third generation cephalosporins. Extended spectrum β-lactamase-producing (ESBL) strains of bacteria are emerging worldwide, particularly amongst the Enterobacteriaceae where the exchange of multidrug-resistant (MDR) plasmids between members of the family is common (Owen et al., 2015). ESBLs comprise rapidly evolving groups of β-lactamases, capable of inactivating the third-generation cephalosporins, penicillins and monobactams (Aarestrup 2006), which are inhibited by clavulanic acid (the β-lactamase inhibitor). They are frequently resistant to many antimicrobial agents usually recommended for the treatment of infections such as gentamicin, fluoroquinolones, and trimethoprim-sulfamethoxazole (Rodrigues et al., 2005). An increased trend of ESBLs resistance to commonly used antibiotics, namely, ampicillin, cotrimoxazole, gentamicin, erythromycin, tetracycline, and third-generation cephalosporins, has been observed (Smet et al., 2010). ESBL-producing Escherichia coli strains have emerged as a potential health hazard, as they have not only been identified from human clinical samples, but also from food producing animals. Food-producing animals carrying extended-spectrum β-lactamase-producing E. coli (ESBL-EC) have posed a potential threat to human and animal health. Asymptomatic carriage of these pathogens in food animals and/or their products may represent a reservoirs and a potential threat for spread to the community.

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Several studies have shown that the prevalence of ESBL producing \textit{E. coli} varies with respect to geographical difference and antimicrobial usage patterns (Owen et al., 2015). ESBL-E. coli have been detected in different ecological niches in the environment, including poultry and poultry-products on retail (Randall et al., 2011).

Table eggs are consumed worldwide and are considered the most nutritious; inexpensive source of protein, that can be part of a healthy diet. Eggs also act as substrate for the growth and multiplication of microbial agents (Adesiyun et al., 2006). Microbial contamination of egg has important outcome to the poultry industry and illness from contaminated egg is a serious public health problem around the world. It has been suggested that, poultry and their eggs among other poultry products might be implicated in the transmission of harmful pathogens such as pathogenic \textit{E. coli} strains to humans (Adesiyun et al., 2007, Obi et al., 2009). Investigation for the prevalence of occurrence of ESBL-producing \textit{E. coli} in poultry-eggs is important, in order to know the risk of spreading the organisms in community settings.

In Nigeria, enterobacteriacea including the ESBL-producing strains has been isolated from different poultry species (Chah and Oboegbulem 2007, Mamza et al., 2010, Emmanuel et al., 2013), and also from table-eggs (Obi et al., 2009, Okolocha et al., 2010, Salihu et al., 2015). However, little is known on ESBL-producing \textit{E. coli} from indigenous chickens and poultry-products in Sokoto state of Nigeria. In Sokoto state, indigenous chickens are extensively reared with limited or no veterinary supervision, and they live in close proximity to human dwelling thereby may play an important role in environmental contamination. In addition, they may serve as significant vehicles for the transfer of antibiotic-resistant pathogens to humans, by way of handling of live birds or consumption of contaminated meat and other poultry products (Otalu et al., 2011). This study was therefore, conducted to determine the prevalence of occurrence of ESBL-producing \textit{E. coli} in cloacal sample of indigenous chickens and retailed table-eggs in Sokoto metropolis, Nigeria. Also to determine the antimicrobial resistance levels of the isolates against commonly prescribed antimicrobial agents in the study area.

**MATERIALS AND METHODS**

**Description of the study area**

The study was conducted in and around Sokoto Township, the capital city of Sokoto State, Nigeria. The state is semi-arid, located to the extreme North-western Nigeria (between longitudes 4° 8’ E and 6° 54’ E and latitudes 12° N and 13° 58’ N). It covered a total land area of about 32,000 square km, with an estimated human population of about 2.4 million (NPC 2006) and an estimated livestock population of; 1.4 million cattle, 2.2 million sheep, 2.8 million goats, 46,000 camels and variable species of poultry (RIMS1991, MAHF 2012). Sokoto township housed a major livestock market and a metropolitan central market where a large number of animal species, including different species of poultry are marketed daily. The birds are mostly indigenous/mixed breeds raised locally by extensive system of management, from with-in the state and neighboring villages, towns and countries such as Niger Republic.

**Sample collection and Processing**

The target population for this study was indigenous chickens brought for sale at Sokoto Central Market. Between March and September 2015 (6 month period), Cloacal-swab samples obtained from 200 indigenous chickens were analyzed for the detection of ESBL-producing \textit{E. coli}. For sampling purpose, a weekly visit to the poultry unit of Sokoto Central Market was done, and on each week-visit, Cloacal swab samples were collected from indigenous chicken chosen at random based on systematic sampling technique of 1: 5 respectively. The cloacal-swab samples were collected by swabbing using a sterile cotton-tipped swab. Each cloacal swab was then transferred to a tube containing 2.5 ml Buffered Peptone Water (BPW). In addition, a total of 70 Table-eggs were purchased from different selling-stalls situated in and around Sokoto Township. Each batch of 3-4 eggs purchased from a single source was placed in a sterile polythene bag containing 25ml Buffered Peptone Water (BPW). The collected cloacal-swab and table egg samples were immediately transported in an ice-cooled container to the veterinary microbiology laboratory of Usmanu Danfodiyo University Sokoto, for bacteriologic analysis.

The eggs were first processed using a shell-rinse method described by Musgrove et al., (2005). For the egg-contents (yolk and albumen) the surface of each of the eggs was first disinfected with 70% ethanol and then broken after the contents were thoroughly mixed, a swab of the content was then taken and placed in a tube of BPW. Each cloacal-swab in BPW, 1ml portion of BPW-rinsate from each egg-shell and the swab of each egg-content in BPW were pre-enriched at 37°C for 18-24 hours aerobically. A loop-full of the pre-enriched culture was then transferred to MacConkey agar plate and incubated at 37°C for 24 hours. Colonies exhibiting morphological features of \textit{E. coli} were purified on Tryptic Soy Agar (TSA), and identification of \textit{E. coli} was done using routine bacteriological methods as detailed by McFaddin (2000). This involved IMVIC and H2S production tests.

**Detection of ESBL-producing \textit{Escherichia coli}**

The \textit{E. coli} strains were screened phenotypically for the production of ESBL with the double disk synergy test.
Table 1: antimicrobial resistance profile of ESBL-producing E. coli from Indigenous Chickens and Table eggs

<table>
<thead>
<tr>
<th>ANTIMICROBIALS</th>
<th>Chickens n = 20</th>
<th>% Resist</th>
<th>Eggs n = 4</th>
<th>% Resist</th>
<th>TOTAL n = 24</th>
<th>% Resist</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>7</td>
<td>35.0</td>
<td>1</td>
<td>25.0</td>
<td>8</td>
<td>33.3</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>3</td>
<td>15.0</td>
<td>0</td>
<td>0.0</td>
<td>3</td>
<td>12.5</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>1</td>
<td>5.0</td>
<td>2</td>
<td>50.0</td>
<td>3</td>
<td>12.5</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1</td>
<td>5.0</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>4.2</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Sulphamethoxazole</td>
<td>4</td>
<td>20.0</td>
<td>2</td>
<td>50.0</td>
<td>6</td>
<td>25.0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>8</td>
<td>40.0</td>
<td>2</td>
<td>50.0</td>
<td>10</td>
<td>41.7</td>
</tr>
<tr>
<td>Trimethoprim-Sulfamethoxazole</td>
<td>1</td>
<td>5.0</td>
<td>1</td>
<td>25.0</td>
<td>2</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Disks (Oxoid) containing cefotaxime, ceftazidime, and equivalent disks with clavulanic acid were used according to NCCLS (2012) guidelines. Any isolate with a ≥ 5 mm increase in zone diameter for either antibiotic tested in combination with clavulanic acid, versus without clavulanic acid, was considered to be an ESBL producer.

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion method on Mueller Hinton agar. The antimicrobials tested were, ampicillin (10 μg), amoxicillin (10 μg), streptomycin (30 μg), sulphamethoxazole (30 μg), trimethoprim-sulphamethoxazole (1.25/23.75 μg), tetracycline (30 μg), chloramphenicol (30 μg), gentamicin (10 μg) and ciprofloxacin (15 μg). A suspension of approximately 0.5 McFarland standard was prepared for each isolate in Mueller-Hinton agar supplemented with 5% v/v sheep blood agar, and incubated at 37°C for 24-48 hours. The inhibition zone were recorded and interpreted according to the guidelines of NCCL (2012).

**Data analysis**

All data were entered into Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for calculation of the isolation frequency rates of Antimicrobial-resistance among the isolates. The Chi-square test was employed to compare the association of categorical variables. P-value of less than 0.05 was considered as statistically significant.

**RESULTS**

Of the total 246 cloacal-swab samples tested, 138 (56.1%) were E. coli positive, and 25 (35.7%) of the 70 table egg samples were also contaminated with the organism. Extended-spectrum beta-lactamase-producing E. coli (ESBL-E.coli) were identified from 22 (8.9%) and 4 (5.7%) from chicken and egg samples respectively. The rate of ESBL-EC detection was significantly higher in chickens (P < 0.05) compared to table eggs. Of the 4 ESBL-producing E. coli isolated from egg samples, 3 (75.0%) were from egg-shell and only one (25.0%) were identified from egg-contents. Table 1 presented the prevalence of ESBL-producing E. coli in chicken and table egg samples.

Antimicrobial sensitivity testing of the ESBL- E. coli isolates revealed 13 (65.0%) from indigenous chickens and 4 (100.0%) of the table egg isolates were resistant to at least one of the 9 antimicrobial agents tested. Multiple-drug resistance (MDR) to two or more antimicrobial agents was expressed by 8 (42.1%) and 2 (50.0%) of the indigenous chicken and table egg isolate respectively. The resistance profile of the ESBL-E. coli strains is presented in Table 1. There was a high resistance to tetracycline (41.7%), followed by ampicillin (33.3%), Trimethoprim-sulphamethoxazole (25.0%) and chloramphenicol (7.2%), and sulphamethoxazole (5%). No resistance was observed for ciprofloxacin and gentamicin and streptomycin. Six patterns of multidrug resistance, involving mostly tetracycline, ampicillin and sulphamethoxazole were observed (Table 2).

**DISCUSSION AND CONCLUSIONS**

Strains of E. coli exhibiting resistance to cephalosporins present a major challenge, especially in resource-poor settings where drugs for treatments are costly or not readily available. The transfer of ESBL strains between food-producing animals and humans via direct contact or food-animals products has been reported (Mesa et al., 2006, OverDevest et al., 2011, Graveland et al., 2011). In the present study, 8.9% of the indigenous chickens examined were ESBL-E. coli carriers. The result is consistent with the report of Mamza et al., (2010), who reported a prevalence of 11% among chickens in Maiduguri, north-eastern Nigeria. The area is similar to very similar to Sokoto in climatic conditions. However, a higher prevalence rates ranging from 18-22% had been recorded in humid, south-eastern part of the country among broiler and layer chickens (Emmanuel et al., 2013, Carissa et al., 2013). The source(s) of ESBL-E.coli in...
indigenous chicken could be from; the environment and/or cross-transmission from other species of animals in close contact or reared in the same settlements. In some communities, backyard poultry houses at residential premises may disseminate antimicrobial-resistant ESBL producers in the environment as previously observed (Obi et al., 2009, Graveland et al., 2011). ESBL-E. coli was detected in 5.7% of the eggs tested, which is higher than 3% reported for salmonella by Okolocha et al.,(2010) from Zaria. The detection rate was however, lower than what has been obtained elsewhere (Musgrove et al., 2006, Adesiyun et al.,2007). The prevalence of ESBL-E.coli on egg shells was generally higher than in egg-contents, which is in contrast to the findings of Adesiyun et al., (2007). Contamination of eggs with ESBL- E. coli could be due to contamination of egg-shell with fecal matter, which may subsequently contaminate the egg-content when the egg is broken (Humphrey et al., 1991; Marshell et al., 1996).

Extended-spectrum beta-lactamase-producing bacteria are frequently resistant to many antimicrobial agents usually recommended for the treatment of infections such as gentamicin, fluoroquinolones, and trimethoprim-sulfamethoxazole (Rodrigues et al., 2005). Data from the present study showed that majority of the ESBL strains of E. coli were resistant to tetracyclines, ampicillin and sulphonamethoxazole. Our study was in agreement to that of Emmanuel et al., (2013) in south-eastern Nigeria and Eids et al., (2015) from Egypt. However, in disagreement with the report of Musgrove et al.,(2006), who reported multi-drug resistance in only 1% of the isolates from chickens in Athens. However, an increased trend of resistance to commonly used antibiotics, namely, ampicillin, cotrimoxazole, gentamicin, erythromycin, tetracycline, and third-generation cephalosporins, has been observed (Smet et al.,2008, Machado et al.,2008), and this leads to challenges in the treatment of ESBL-Enterobacteraeae infections, as the bacterial plasmid may harbor several antibiotic resistance determinants (Aarestrup, 2006).

This study reveals the presence of antimicrobial-resistant ESBL-producing E. coliisolates in the indigenous chickens on sale in poultry-selling unit of Sokoto Central market, and in table-eggs sold for human consumption within the township and environs. The obtained prevalence of occurrence of the pathogen and the related drug-resistance suggest an emerging problem that could impact negatively on poultry health and a threat to public health. Asymptomatic carriage of these pathogens in poultry specie and their products may represent a reservoirs and a potential threat for spread to the community. We recommend further studies on molecular epidemiology of ESBL producing E. coli and other enterobacteriaceae and their impacts on our local/indigenous poultry specie, which have not been receiving regular health care in the study area.

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