**Multidrug Resistance in E. coli 0157 Strains and the Public Health Implication**

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**ABSTRACT:** A survey of the antimicrobial resistant pattern of E.coli 0157 strains obtained from farm-animals and human infections in Lagos and Ogun state in Nigeria was carried out. A total of 350 fresh faecal droppings of animals (cattle, pig, chicken and sheep) and human stool comprising of diarrhoeac (150) and non diarrhoeac, (50) were cultured on Cefixime-Tellurite Sorbitol Mac-Conkey Agar.. Susceptibility testing, Double-disk diffusion, Conjugation and plasmid analysis tests were performed on strains isolated. Thirty nine (11.1%) of animal samples and 6 (3.0%) of human samples had non-sorbitol fermenting E.coli. Of these, 16 (4.6%) from animals and 2 (1.0%) from human were identified as E.coli 0157 serotypes by latex agglutination. Serotypes from humans exhibited resistance to 1 to 3 antibiotics while those from animals had resistance profile varying from one antibiotic to resistance to 13 antibiotics. Strains from Chicken had the highest multi-drug resistance, showing resistance to 2, 4, 5, 9 and 13 antibiotics to the quinolones. One of the strains from cattle expressed CTX-M like ESBL and transferred this trait by conjugation. All the strains isolated harbored plasmids ranging in size between 21,563 - 27,444kb. The multiple antimicrobial resistance exhibited by E. coli 0157 strains in this study, is an indication of spread of mobile genetic elements and suggests that these animals are important reservoir of antimicrobial-resistant organisms. An early identification and understanding of the epidemiology of this resistance will enable the development of preventive strategies which can limit or stop this emerging resistance, thereby facilitating a timely and appropriate public health response. [The Journal of American Science. 2007;3(3):22-33]. (ISSN: 1545-1003).

**Keywords:** E.coli 0157, Multidrug Resistance, ESBL

**INTRODUCTION**

In the last few decades, the frequency and spectrum of antimicrobial-resistant infections have increased in both the hospital and the community. Certain infections that are essentially untreatable have begun to occur as epidemics both in the developing world and in institutional settings in the United States and other developed regions. Antimicrobial resistance is resulting in increased morbidity, mortality, and health-care costs (Cohen, 1992). Of particular interest is the emergence of resistance to the beta-lactams and the fluoroquinolone antibiotics which has become more serious in recent decades. Broad use of fluoroquinolones has been followed by emergence of resistance (Hooper, 2001) and strains producing extended-spectrum beta-lactamases (ESBL) render many, if not all, penicillin and cephalosporin ineffective as therapy (Aibinu et al., 2003b). Many studies have demonstrated the great potential for the spread of ESBL-producing strains and also ESBL-encoding plasmids to different hospitals (Davin-Regli et al., 1996) and even different countries (De Gheldre, 2001). It is also reported that community-acquired strains possessing ESBLs might be selected from the existing gastrointestinal flora when it is exposed to broad-spectrum antimicrobial agents (Heseltine, 2000).

Escherichia coli, previously were thought to be a vanishing cause of diarrhea, but with the recognition of E. coli 0157, a serotype of Enterohaemorrhagic E. coli (EHEC), the situation has changed completely (Griffin and Tauxe, 1991). Although a variety of E. coli serotypes have been associated with human illness, the most important among these is O157:H7. EHEC 0157 is one of the six groups of E. coli recognized as
aetiological agents of diarrhoea (Aboaba et al., 2006). It was first identified as a cause of illness in 1982 (Riley et al., 1983) and the infections have now since been reported with increasing frequency (Fitzpatrick, 1999). Infection with this *Escherichia coli* serotype is associated with a spectrum of illnesses including watery diarrhoea, bloody diarrhoea, and the haemolytic uraemic syndrome, a potentially fatal condition characterized by acute renal failure (Griffin and Tauxe, 1991). Cattle are the principal reservoir for these organisms. Important sources of infection include consumption of undercooked hamburger and other contaminated food products and direct or indirect contact with infected persons (Wilson et al., 1997). It is of public health importance as it is readily isolated from human and animal wastes that pollute the environment (Smith et al., 2003). While therapeutic management of *E.coli* 0157 infection vary depending on the type of infection, the usefulness of antimicrobials in treating this Shiga-toxin-producing *E.coli* 0157 infection remains less clear (Griffin, 1995; Thielman and Guerrant, 1999).

With the emergence and dissemination of antimicrobial resistance in bacteria which is well documented worldwide, (Cohen, 2000). *E. coli*, an important gastrointestinal flora, known to be capable of accepting and transferring plasmids and which under stress readily transfers those plasmids to other species, is therefore considered an important reservoir of transferable antibiotic resistance (Enumeration of *Escherichia coli* and the Coliform Bacteria, 2002). Hence, active surveillance of the antimicrobial resistant pattern of EHEC *E.coli* 0157 serotype resident in animals that are resistant to its toxin is of public health importance. This study thus, investigates *E.coli* 0157 infection in human and apparently healthy animals; and the occurrence of multidrug resistance not only to commonly used antibiotics, but also to the broad spectrum drugs. The production of beta-lactamase enzyme as a mechanism of resistance employed by strains obtained in this study was also investigated.

**MATERIALS AND METHODS**

**Study population**

During a six-month period from June to November 2006, 200 human stool specimens submitted to the Medical Microbiology and Parasitology Departments of health institutions involved in this study, were analyzed. A hundred were from adults, age range 17-78 years while the remaining 100 were from children age range 4 months-12 years. Seventy-five of the specimens from adults were watery, diarrhoea stool out of which 40 were collected from out patients with diarrhea attending the Gastro Intestinal Tract Clinic of the Lagos University Teaching Hospital (LUTH). Thirty-five of the samples from adult were from patients attending the HIV Clinic of Nigerian Institute for Medical Research (NIMR). The remaining 25 stool samples collected from adult were non-diarrhoea stool and were used as control. Ten of these were collected from healthy individuals attending the diagnostic centre LUTH for routine medical check-up while 15 were from HIV patients (non-diarrhea) attending NIMR HIV clinic. The 100 samples from children included 25 non-diarrhoea stool from children attending pediatrics unit of LUTH for ailments other than gastro-enteritis (control), 15 diarrhoea stool, (comprising of one bloody diarrhoea stool and 14 non-bloody diarrhoea stool), from children at pediatrics unit of LUTH, and 60 diarrhoea stool from children attending Regina Mundi Children Hospital, Lagos. The diarrhoea stool specimens were those submitted to these health institutions for the examination of enteric pathogens. Three hundred and fifty faecal samples were collected from livestock from different parts of Lagos and Ogun state. The animal samples obtained were Cattle (200), pigs (50), chicken (50) and sheep (50).

**Specimen collection**

Fresh faecal specimen were obtained from human into sterile universal containers while for children, for those whose faecal samples could not be collected, rectal swabs were used. This was immediately inoculated into cold modified Stuart’s transport medium and kept on ice during transportation. Faeces from cattle, pigs, sheep and chickens observed defecating were collected in sterile universal bottles and transported to the laboratory within 1 hour of collection for processing and culture.

**Processing of samples**

All human faecal samples were cultured within 2 hours of collection on Cefixime Tellurite Sorbitol MacConkey agar (CT-SMAC, Oxoid CM 813, UK) plates and incubated at 37°C for 24 hours. All non-sorbitol fermenting isolates were characterized and species identification was carried out using standard diagnostic procedures (Farmer, 1999). Control strains used were NCTC 12900 0157:h7v7 (N) and *E. coli* ATCC 25922 obtained from the Research laboratory of the Department of Medical
Microbiology and Parasitology, College of Medicine, University of Lagos. Haemolytic activity of the isolates was tested for, by culturing the isolates on brain heart infusion agar (Oxoid, UK) containing 7% human blood and incubated at 37°C for 24 hours.

Serological analysis
All non-sorbitol fermenting identified E. coli colonies were tested with the latex dry spot agglutination kit for E. coli 0157 (Oxoid, DRO 120M, UK) according to the manufacturer’s instruction. A drop of saline was added to the small ring in both test and control reaction areas. A portion of the suspect colony was picked with a sterile stick and carefully emulsified in the saline drop. The suspension was spread to cover the reaction area. The same was done with the control latex and control organisms. The card was rocked for 60 seconds and observed for agglutination. Agglutination indicates the presence of E. coli serogroup 0157.

Susceptibility testing
Antibiotic susceptibilities were determined on Mueller-Hinton agar (Oxoid, Basingstoke, United Kingdom) by standard disk diffusion procedures (Bauer et al., 1966) to the following antibiotics: amoxicillin (30 µg), amoxicillin-clavulanic acid (30 µg), cefoxitin (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefuroxime (30 µg), ceftazidime (30 µg), meropenem (30 µg), gentamicin (10 µg), piperacillin (30 µg), ofloxacin (30 µg), levofloxacin (30 µg), ciprofloxacin (30 µg) (Oxoid, UK) tetracycline (25 µg), trimethoprim-sulfamethoxazole (25 µg) (Abbio disk), and pefloxacin (May and Baker, Nigeria). The control strains were run simultaneously with the test organisms. Results were interpreted with the National Committee for Clinical Laboratory Standards now known as Clinical Laboratory Standard Institute (CLSI) criteria for disk diffusion (NCCLS, 2000).

Double-Disk Synergy Test (DDST)
All isolates resistant to at least one of the extended-spectrum cephalosporins (ESCs) namely ceftazidime, ceftriaxone and cefotaxime were subjected to double-disk synergy tests (DDST) as described by Jarlier et al. (1988) with modifications suggested by Thomson and Sanders (1992) to detect the presence of ESBL enzyme.

Conjugation
The isolate resistant to at least one extended-spectrum cephalosporin and which was positive by the DDST testing, was subjected to conjugation test as previously described (Aibinu et al., 2003a). Transconjugants growing in the selection plates were subjected to DDST to confirm the presence of ESBL genes and were examined for the co transfer of other antibiotic resistance determinants present in the donor isolates.

Plasmid analysis
The method of Birnboim and Doly (1979) was employed for plasmid screening. The DNA were electrophoresed on 0.8% agarose gel, stained with ethidium bromide, visualized by UV transillumination and photographed. Molecular weights were calculated based on molecular weight standard.

Statistical analysis
Rate of isolation of E. coli 0157 in diarrhoeac stools from human and isolation rates amongst farm-animals were analyzed by Chi square (X2) test and a P value > 0.05 was considered as not statistically significant. The null hypothesis (Ho) states that the rate of isolation of E. coli 0157 in the diarrhoeac stools obtained from humans; and stool samples from farm-animals is not statistically significant while the alternate hypothesis states that isolation rate from the stool samples, is statistically significant.

RESULTS
Tables 1 and 2 show the sample categories and distribution.

In human, E. coli 0157 was isolated from the bloody faecal sample while the remaining diarrhoeac stools did not yield growth of E. coli 0157. One of the controls, a non-diarrhoeac stool from a 36 yrs old female who was HIV positive, also yielded the growth of E. coli 0157. In the animals, isolation of E. coli 0157 was in the following rate cattle 4 (2%), pigs 6 (12%), chicken 5 (10%) and sheep 1 (2%). Thus a total
of 16 (4.6%) \textit{E. coli} 0157 were isolated from animals and 2 (1%) from humans. All of these isolates were haemolytic on blood agar.

Antimicrobial susceptibility pattern of the isolates to various antimicrobial agents is shown on Table 3. All the 18 isolates were sensitive to meropenem. The \textit{E. coli} 0157 strains characterized in this study displayed resistance to one or more antimicrobials including penicillins, cephalosporins, quinolones, aminoglycosides, tetracycline and sulphonamides. Resistance was highest for tetracycline (94.4%), followed by cefuroxime and cotrimoxazole (50%) and by amoxicillin and piperacillin (33.3%) (Table 3). Resistance frequency was lowest for isolates in humans with the isolates from the HIV infected adult being resistant to only tetracycline but sensitive to all other antibiotics tested, while the other from the bloody diarrhoeac stool showed resistance to tetracycline, ceftriaxone and cefuroxime. None of the human strains was resistant to cotrimoxazole, augmentin, amoxicillin, piperacillin, gentamicin and the quinolones (Table 4).

The highest frequencies of antimicrobial-resistant phenotypes were observed for \textit{E. coli} 0157 strains from chicken, cattle and pigs (Tables 5). Isolates from Chicken faecal samples had the highest multi-drug resistance, showing resistance to 2, 4, 5, 9 and 13 antibiotics. Resistance to the quinolones (levofloxacin, pefloxacin, ciprofloxacin and ofloxacin) used was also highest amongst \textit{E.coli} 0157 strains from chickens. Five of the nine isolates of \textit{E.coli} 0157 found resistant to cotrimoxazole were found susceptible to ciprofloxacin. Of the five resistant to cefotaxime, 3 were resistant to ceftriaxone (an ESCs) but none of them showed the ESBL resistance phenotype. Resistance to ceftazidime (ESC) was observed in 2 strains; one from sheep and the other from a cow but ESBL resistance phenotype was also not observed in these strains. Resistance to both cefotaxime and ceftriaxone occurred in only 1 strain from a cattle faecal sample and it was only this strain that showed the ESBL resistance phenotype by the DDST. This strain was found to be sensitive to cefotaxim.

Only 3 strains (2 from chicken and 1 from cattle) were resistant to gentamicin. Comparison of the antibiotic resistant pattern of strains from each group is shown in fig 1. Plasmid analysis of isolates showed they all harbored detectable plasmids with size ranging between 21,771 – 27,444kb. Statistical analysis showed that we cannot reject the null hypothesis for the rate of isolation of \textit{E. coli} 0157 in diarrhoeac stool from humans and therefore conclude that for this study the rate of isolation of \textit{E. coli} 0157 from diarrhoeac stool of humans is not statistically significant as $P=0.9935$ ($P>0.05$). However, rate of isolation of \textit{E. coli} 0157 in stool samples from farm-animals was found to be statistically significant as $P$ value = 0.0079; ($P<0.05$) and thus the null hypothesis was rejected in the case of farm-animals and the alternate hypothesis accepted.

\begin{table}
\centering
\caption{Distribution of \textit{E.coli} 0157 Strains Isolated from Human Faecal Samples}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Categories of human stool & Population sampled & No of samples obtained & No of NSF \textit{E.coli} isolated & No of \textit{E.coli} 0157 isolated & Locations from which samples were collected \\
\hline
Watery/diarrhoeac stool & Adult & 75 & 3 & 0 & NIMR, LUTH and Regina Mundi Children Hospital Lagos \\
& Children & 74 & 0 & 0 & \\
\hline
Bloody diarrhoeac stool & Adult & 0 & 0 & 0 & LUTH, NIMR \\
& Children & 1 & 1 & 1 & LUTH \\
\hline
Non-diarrhoeac stool & Adult & 25 & 2 & 1 & NIMR, LUTH \\
& Children & 25 & 0 & 0 & \\
\hline
\textbf{TOTAL} & & \textbf{200} & \textbf{6 (3%)} & \textbf{2 (1%)} & \\
\hline
\end{tabular}
\end{table}

\textbf{Keys}

NSF= Non sorbitol fermenters, LUTH=Lagos University Teaching Hospital, NIMR=Nigerian Institute of Medical Research
TABLE 2. Distribution of *E. coli* 0157 Isolated From Farm-Animals’ Faecal Samples

<table>
<thead>
<tr>
<th>Animal type</th>
<th>Sample location</th>
<th>No. of samples</th>
<th>No of NSF</th>
<th>No. of <em>E. coli</em> 0157 isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>Berger</td>
<td>75</td>
<td>21 (10.5%)</td>
<td>4 (2%)</td>
</tr>
<tr>
<td></td>
<td>Ijesha</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ojo</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sub-total</td>
<td></td>
<td><strong>200</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigs</td>
<td>Agbara</td>
<td>50</td>
<td>8 (16%)</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>Chicken</td>
<td>Ojo</td>
<td>50</td>
<td>7 (14%)</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>Sheep</td>
<td>Ojo</td>
<td>50</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td><strong>350</strong></td>
<td><strong>39 (11.1%)</strong></td>
<td><strong>16 (4.6%)</strong></td>
</tr>
</tbody>
</table>

Keys: No=Number NSF= Non-Sorbitol Fermenters

TABLE 3. Antibiotic Resistant Pattern of *E. coli* 0157 Strains Isolated (N=18)

<table>
<thead>
<tr>
<th>Antimicrobial Agents</th>
<th>% Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meropenem</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>1 (5.6%)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>5 (27.8%)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>5 (27.8%)</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>9 (50%)</td>
</tr>
<tr>
<td>Amoxicillin-Clavulanate (Augmentin)</td>
<td>4 (22.2%)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>6 (33.3%)</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>6 (33.3%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>4 (22.2%)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>4 (22.2%)</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>4 (22.2%)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>4 (22.2%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>3 (16.67%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>17 (94.4%)</td>
</tr>
<tr>
<td>Cotrimoxazole (Trimethoprim/sulfamethoxazole)</td>
<td>9 (50%)</td>
</tr>
</tbody>
</table>

TABLE 4. Antibiotic Resistance Profile

<table>
<thead>
<tr>
<th>Resistance Profile</th>
<th>No of Strains Showing Profile</th>
<th>Animal/Human Showing Profile</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tet</td>
<td>4</td>
<td>CA 1</td>
<td>Berger</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 66</td>
<td>Ojo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PG 14</td>
<td>Agbara</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HA</td>
<td>NIMR</td>
</tr>
<tr>
<td>Tet, Cot</td>
<td>2</td>
<td>PG 9</td>
<td>Agbara</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH 8</td>
<td>Ojo</td>
</tr>
<tr>
<td>Tet, Cxm, Cro</td>
<td>1</td>
<td>H2B</td>
<td>LUTH</td>
</tr>
<tr>
<td>Tet, Cxm, Prl</td>
<td>1</td>
<td>PG 33</td>
<td>Agbara</td>
</tr>
<tr>
<td>Cot, Cxm, Caz</td>
<td>1</td>
<td>CA 110</td>
<td>Berger</td>
</tr>
<tr>
<td>Tet, Cot,fox, Cn</td>
<td>1</td>
<td>CH 13</td>
<td>Ojo</td>
</tr>
<tr>
<td>Tet, Cot, Cro, Fox, Ofx</td>
<td>1</td>
<td>PG 16</td>
<td>Agbara</td>
</tr>
<tr>
<td>Tet, Cxm, Cip, Lev, Pef</td>
<td>1</td>
<td>CH 16</td>
<td>Ojo</td>
</tr>
<tr>
<td>Tet, Amx, Aug, Caz, Prl</td>
<td>1</td>
<td>SH 5</td>
<td>Ojo</td>
</tr>
<tr>
<td>Tet, Cot, Amx</td>
<td>1</td>
<td>PG 13</td>
<td>Agbara</td>
</tr>
</tbody>
</table>
**Cxm, Fox, Prl**  
Tet, Amx, Cxm, Cro, Fox, Prl  
1  
PG 8  
Agbara

<table>
<thead>
<tr>
<th>Source of isolates and total number showing the resistance profile</th>
<th>Plasmid Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tet, Amx, Cxm, Cro, Fox, Prl, Lev, Pef, Ofx</td>
<td>CH 3</td>
</tr>
<tr>
<td>Tet, Cot, Amx, Aug, Cxm, Cro, Fox, Cn, Cip, Pef, Lev, Ofx, Prl</td>
<td>CH 5</td>
</tr>
<tr>
<td>Tet, Cot, Amx, Aug, Cxm, Cro, Ctx, Cn, Cip, Pef, Lev, Ofx, Prl</td>
<td>Ca 151 (ESBL-PRODUCER)</td>
</tr>
</tbody>
</table>

**Key:**  
CH=Chicken; SH=Sheep; CA=Cattle; PG=Pig; Tet=Tetracycline; Fox=Cefoxitin  
Cot=Cotrimoxazole (Trimethoprim/sulfamethoxazole); Cn=Gentamicin  
Amx=Amoxicillin, Aug=Augmentin (Amoxicillin/clavulanic acid); Cot=Cotrimoxazole, Cip=Ciprofloxacin, Pef=Pefloxacin; Lev=Levofloxacin; Ofx=Ofloxacin; Caz=Ceftazidime; Cro=Ceftriaxone; Ctx=Cefotaxime

| TABLE 5. Number of Antibiotics for Which Resistance Exist and their Plasmid Sizes |
|---------------------------------------------------------------|--------------|
| **Number of Antibiotics for Which there is resistance**      | **Source of isolates and total number showing the resistance profile** | **Plasmid Size** |
| One antibiotic                                               | HA CA 1 CA 66 PG 14 Total=4 | 23,222kb 27,444kb 27,444kb 28,881kb |
| Two antibiotics                                              | PG 9 CH 8 Total=2            | 24,881kb 24,881kb |
| Three antibiotics                                            | CA 110 PG33 H2B Total=3     | 23,222kb 24,881kb 21,771kb |
| Four antibiotics                                             | CH 13 Total=1               | 25,157kb |
| Five antibiotics                                             | CH 16 SH 5 PG 16 Total=3    | 25,157kb 21,563kb 23,222kb |
| Six antibiotics                                              | PG8 PG13 Total=2             | 24,881kb 21,771kb |
| Nine antibiotics                                             | CH3 Total=1                 | 27,444kb |
Thirteen antibiotics
CH5
CA151
Total=2

Key: CH=Chicken; SH=Sheep; CA=Cattle; PG=Pig; H2B =Human 2yrs baby; HA=Human adult

Comparison of Antimicrobial Resistance of E.coli 0157 Isolated from Animals and Humans in Nigeria

FIG 1    ANTIMICROBIALS

Keys
Cot=Cotrimoxazole (Trimethoprim/sulfamethoxazole)
Cro=Ceftriaxone    Cn=gentamicin
Tet=Tetracycline    Fox=Cefoxitin
Mem=Meropenem    Amx=Amoxicillin
Cxm=Cefuroxime    Aug=Augmentin (Amoxicillin/clavulanic acid)
Ofx=Ofloxacin    Prl=Piperacillin
Lev=Levofoxacin    Caz=Ceftazidime
Cip=Ciprofoxacin    Ctx=Cefotaxime
Pef=Pefloxacin

DISCUSSIONS
In recent years, enterohemorrhagic Escherichia coli 0157:H7 and 0157:H- have emerged as pathogens of significant clinical importance to public health. (Bitzana and Karch, 1992). Results from the present study showed a low incidence of E.coli 0157 infection in humans (1%) in the 3 health-care institutions used for this study in Lagos, Nigeria. A number of factors may be responsible for this. Firstly, studies have demonstrated a strong seasonal influence on occurrence of 0157 in cattle feces (Gansheroff and O’ Brien, 2000; Heuvelink et al., 1998). For instance in a study in England, E.coli O157 was isolated from the feces from 38% of cattle presented for slaughter in the spring, but only 4.8% during the winter (Chapmann et al., 1997). Reported incidents of 0157 food poisoning were correspondingly seasonal (CDC Divison of Bacterial and Mycotic Diseases 2000; Infectious Diseases and Immunization Committee, Canadian Pediatric Society, 1995) Secondly, different cultures have evolved a wide variety of practices to reduce the chances of food poisoning (Heritage et al., 1999)). In Nigeria for example, meat and most of the foods for consumption are usually cooked at ≥100°C, a temperature at which contaminating enteric
organisms will be destroyed. The heat sensitivity of these organisms is such that cases should not occur when foods are properly cooked (Ngede et al., 2006) Thirdly, the use of herbal remedies which are cheaper to obtain than conventional drugs, is on the increase in developing countries, as well as self medication using conventional drugs. In this respect, a lot of infections are treated without a record of information on what type of infection has occurred and what pathogen was responsible.

It is widely known that E. coli 0157 is widespread in nature, occurring naturally and sporadically in the gastrointestinal flora of humans, cattle, deer, sheep, dogs, horses, birds, and perhaps other species.” (Hancock and Besser, 1998) In this study animals sampled included cattle, sheep, pigs and chicken. Previous study by Smith et al. (2003) in this region had investigated this pathogen in the first 3 animals but not in chicken. The essence of inclusion of chicken in this study is that they are the most common animal protein source eaten by the Nigerian populace; they are readily reared at homes which means more people (particularly children and old adults in families) get to be exposed to them than the other animals which are reared in farms where only the care-takers come in contact with those animals. They are also easily and readily fed more than any other animals with antibiotic supplemented feeds to promote growth and for prophylaxis.

Result of this work showed that isolation rate of E. coli 0157 was highest in pigs (12%), closely followed by chicken and cattle (10%); and lastly by sheep (2%). This is contrary to result from some countries where cattle normally have the highest incidence rate for E. coli 0157. For example, a study in the UK found 0157 in 15.7% of cattle (dairy and beef), 2.2% of sheep, 0.4% of pigs and none in chickens (1000 of each examined) (Chapmann et al., 1997). A very high incidence of 0157 in cattle was also reported by Elder et al. (2000) in the United States. Chickens sampled in this study had an E. coli 0157 isolation rate of 10% ranking second amongst the group of animals from which E. coli 0157 was recovered. This work provides a maiden database for poultry infection by E. coli 0157 in Nigeria even though these chickens appeared healthy.

In this study, E. coli 0157 strains from Chicken faecal samples notoriously had the highest multidrug resistance, showing resistance to 2, 4, 5, 9 and 13 antibiotics. Resistance to the quinolones (levofloxacin, pefloxacin, ciprofloxacin and ofloxacin) was also highest amongst these strains from chickens. This result is in agreement with a study carried out in the United States by Jones and Schaffnner (2005) where more than 80% of the E. coli recovered from beef, pork, and poultry products were resistant to one or more antibiotics and greater than half of the samples of poultry bacteria were resistant to more than five drugs.

Of note is the occurrence of multidrug resistance to 13 antibiotics in 2 strains, one from chicken and the other from cattle. This included resistance to all the four quinolones used namely: ciprofloxacin, ofloxacin, levofloxacin and pefloxacin. In a report by The Humane Society of the United States (HSUS, 2007) it was stated that Quinolone antibiotics have been used in human medicine since the 1980s, but widespread antibiotic resistant Campylobacter didn’t arise until after quinolones were licensed for use in chickens in the early 1990s. In countries like Australia, which reserved quinolones exclusively for human use, resistant bacteria are practically unknown (Price et al., 2005). The FDA concluded that the use of Cipro-like antibiotics in chickens compromised the treatment of nearly 10,000 Americans a year. This high occurrence of resistance to the quinolones by E. coli 0157 strains from chicken and cattle in this region is quite worrisome. There is paucity of data on the use of antimicrobials in veterinary and agricultural setting in this region. However, it is noteworthy that use of levofloxacin is still new or even not yet in use in the country both in clinical and agricultural sector, yet there exist marked resistance to it by E. coli 0157 strains from this environment. This could be explained by the fact that most of the chickens reared in Lagos and some other parts of the country are imported from abroad in their large numbers where use of levofloxacin is already in existence. Any batch of chicken imported into the country harbouring micro-organisms exhibiting resistance to any antibiotics in use in such countries, will definitely have the resistance in circulation in the new region.

The multidrug resistant strain from cattle was found to be an ESBL-producer and was sensitive to cefoxitin. It was found to be resistant to cefotaxime, ceftriaxone and cefuroxime and according to Livermore and Brown (2001), isolate with this resistance phenotype is likely to be harbouring the CTX-M ESBL. This is the first study reporting the first isolation of an ESBL-producing E. coli 0157 strain from cattle in Nigeria. The ESBL resistance and resistance to the beta-lactam antibiotics were all transferred by this strain to a recipient E. coli by conjugation; while resistance to other antibiotics was not transferred. This indicates that resistance to these other antibiotics which were not transferred may probably be located on
the chromosomes while the transferable ones are located on plasmids. Plasmid analysis showed all the *E. coli* 0157 strains obtained in this study, harbored plasmids. Of note also in this study is the fact that some of the strains which were resistant to cefoxitin were also found to be resistant to at least one of the ESCs yet they did not show the ESBL phenotype. Amp-C enzyme production may be implicated in these cases and may have masked the expression of the ESBL phenotype (Thomson *et al*., 1999).

Indiscriminate use of antibiotics is the main factor resulting in this emergence, selection and dissemination of drug-resistant pathogens in both veterinary and human medicine. (Smith *et al*., 2005). The Centers for Disease Control and Prevention (CDC) reported that at least 17 classes of antimicrobials were approved for farm animal growth promotion in the United States (Anderson *et al*., 2003) In the report by HSUS (2007), it was stated that the world’s leading medical, agricultural, and veterinary authorities reached a consensus that antibiotic overuse in animal agriculture is contributing to human public health problems. Evidences were seen to support this when antibiotic-resistant infections in farm animal populations began to precede the emergence of the same resistance in humans, and microbial studies showed that antibiotic-resistant bacteria from farm animals not only infect humans, but may transfer that resistance to other bacteria that colonize the human gut. (WHO, 2003) The strongest evidence as reported by HSUS (2007) was the data from Europe’s experience, which showed that after antibiotics were banned for growth promotion, there was a subsequent decrease in the levels of antibiotic-resistant bacteria in farm animals, on meat, and within the general human population. (Smith *et al*., 2005). Veterinary drugs are sold and used without much control in Nigeria. This practice may have created a population of resistant bacteria in the meat animals. These resistant bacteria are sweeping aside the second and third line antibiotics and this may return us to the pre-antibiotic era (WHO, 2000).

There is paucity of data on resistance pattern of *E. coli* 0157 worldwide. One reason for this may be because treatment of hemorrhagic colitis, one of the spectrums of illnesses associated with *E. coli* 0157 infection, is usually supportive. Antibiotics are not typically used: as they do not seem to reduce symptoms, prevent complications or decrease shedding and do appear to increase the risk of Haemolytic Uraemic Syndrome (HUS) (Griffin, 1995). The emergence of multidrug resistance in the already notorious pathogen, *E. coli* 0157 has grave public health consequences. This is because as the bacteria become more resistant to the antibiotics fed to chickens and other animals raised for meat, they may become more resistant to the antibiotics needed to treat sick people. Resistance genes that emerge can then be swapped between bacteria (HSUS, 2007). Italian researchers recently published a DNA fingerprinting study in 2007 showing that these antibiotic-resistance genes could be detected directly in chicken meat and pork (Garofalo *et al*., 2007).

Some Scientists also suspect that by eating chicken and other animal products, women infect their lower intestinal tract with these antibiotic-resistant bacteria, which can then migrate into their bladder. (Brownlee, 2005).

This high rate of increase in multidrug resistance is quite alarming coupled with the fact that all these isolates harboured plasmids on which these genes may be located and which are highly transferable. The selection and spread of resistant organisms in developing countries, which can often be traced to complex socioeconomic and behavioral antecedents, has contributed to the escalating problem of antibiotic resistance worldwide (Okeke *et al*., 1999). In conclusion, this study shows that ESBL producers are present in the extra hospital setting and *E. coli* 0157 strains from Chicken and cattle in this region are multiply resistant to antibiotics. There is therefore a need for continuous surveillance of antimicrobial resistance trends worldwide particularly among organisms resident in the gastrointestinal tract of farm-animals which are implicated in infectious diseases in human. Implementing antibiotic use strategies at all levels of the cattle and other farm animals industry, will decrease the risk and the clinical threat posed by antimicrobial resistance due to use and misuse of antibiotics.

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