A PRELIMINARY REPORT ON ANTIBIOTIC RESISTANT ESCHERICHIA COLI NON-O157 ISOLATED FROM CATTLE IN KADUNA STATE, NIGERIA

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ABSTRACT
A total of two hundred and forty (240) faecal samples were obtained from apparently healthy (233) and diarrhoeic (7) cattle in 8 randomly selected commercial farms in Kaduna State, Nigeria. Presumptive *E. coli* colonies from 76 (31.2 %) faecal samples were confirmed based on standard procedure. Characterization of isolates revealed three heterogeneous serogroups (O111, O118 and O126) from 6 apparently healthy cattle, while no *E. coli* serogroup was isolated from diarrhoeic cattle. Six (6) non-O157 serogroups obtained from cattle faeces were tested for antimicrobial susceptibility. The antimicrobial susceptibility test indicated that isolates from cattle faeces were 100 % resistant to nitrofurantoin, amoxicillin and cefuroxime, and 100 % sensitive to ciprofloxacin and ofloxacin. The study confirmed cattle as important source of antibiotic-resistant enterohaemorrhagic *Escherichia coli* in Kaduna state, Nigeria.

Key words: Antimicrobial sensitivity, Escherichia coli, cattle, Nigeria

INTRODUCTION
Antimicrobial sensitivities have been determined on Mueller-Hinton agar by disk diffusion method using certain antimicrobial agents for Gram negative bacteria, including *Escherichia coli* (Cheesbrough, 2000). The relatively high frequency of antimicrobial-resistant *E. coli* of cattle may be due to the use of antimicrobial drugs in cattle production (Schroeder et al., 2002). Bovine O118:H16 strain showed resistance to antimicrobial agents such as ampicillin and tetracycline. This indicates that drug resistance genes accumulated over time in O118: H16 strains of *E. coli* (Pestana de Castro et al., 2003). Cattle may thus be an important source of new emerging antibiotic-resistant *E. coli* strains of non-O157 serogroups (Blanco et al., 2000; Clarke, 2001). *E. coli* O111 are the most frequently implicated non-O157 strains causing gastroenteritis with haemolytic uraemic syndrome (HUS), particularly in the United States of America and Europe (Bettelheim, 2000; Pearce et al., 2006). Previous studies show that majority of O111 serogroups were recovered from individuals with haemorrhagic colitis (HC) and HUS (Nataro and Kaper, 1998) than from cattle (Bettelheim, 2003). Cattle and human O118 serogroups represent the same clones and are similar in virulence attributes and antimicrobial drug resistance, labeling them as possible zoonotic pathogens or threat to human beings (Wieler et al., 2000; Maidhoff et al., 2002).

*E. coli* O126 has been reportedly isolated from the faecal samples of human beings (Bettelheim, 2000). The serogroup O126 has not been implicated in cases of HUS (Buchanan and Doyle, 1997; Bettelheim, 2000). Some non-O157 serogroups were among the major EHEC implicated in an outbreak of diarrhoea, HC and HUS in human beings elsewhere (Bettelheim, 2003). In this study, we report for the first time, the sensitivity pattern of *E. coli* non-O157 isolated from cattle in Kaduna state, Nigeria to some antimicrobials.

MATERIALS AND METHODS

Study design
The study was designed as a cross-sectional (prevalence) study and sample size was determined using the method described by Mahajan (1997).

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Study area

The study area was Kaduna State, which is located between latitude 10° and 11°N and longitude 7° and 8°E, North-Western Nigeria (Figure 1).
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Sample collection
A total of two hundred and forty (240) faecal samples from apparently healthy (233) and diarrhoeic (7) cattle were collected from 8 randomly selected commercial farms in Kaduna state, Nigeria using stratified sampling technique (Field and Graham, 2003). The farms were designated as farms A (FA), B (FB), C (FC), D (FD), E (FE), F (FF), G (FG) and H (FH) located in five different local government areas of Kaduna State, Nigeria (Fig. 1). Faecal material (1-2 g) was aseptically collected from the rectum of each animal using clean disposable hand gloves. The samples were placed in separate sterile bottles containing 8-9 mL of tryptone soya broth (TSB), kept in a cold box at 4 °C and then transported to the Bacteriology Laboratory, Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria where they were processed immediately.

Isolation and identification of suspected colonies
Bacterial isolation, identification and biochemical tests were carried out using standard procedures described elsewhere (Cheesbrough, 2000).

Biochemical characterization
Colonies growing on EMB agar plates suspected to be E. coli were subjected to biochemical tests (indole, methyl red, Voges Proskauser, citrate (IMViC), motility and triple sugar iron, TSI) (Cheesbrough, 2000).

Identification of somatic O isolates
The confirmed E. coli isolates were sub-cultured onto nutrient agar slants and stored at 4 °C for serogrouping (Blanco et al., 2006). Determination of somatic O antigens for EHEC O111, O118 and O126 was performed using specific antisera (SIFIN Berlin Germany) (Blanco, 2006).

Sensitivity to antimicrobials
The sensitivity pattern of the isolates to antimicrobials was determined on nutrient agar (NA) by disk diffusion method (Sozmen et al., 2011). These include nitrofurantoin (N, 100 µg), ciprofloxacin (Cip, 5 µg), tetracycline (Te, 50 µg), norfloxacin (nobilacin- NB, 10 µg), amoxicillin (AX, 20 µg), ofloxacin (OF, 5 µg), chloramphenicol (C, 10 µg), cefuroxime (zinnat- CF, 30 µg), ampicillin (AM, 10 µg) and gentamicin (GN, 10 µg) (Poly-Test Med. Laboratories®, Pune, India). The non-O157 positive E. coli isolates from commercial cattle farms were inoculated onto NA plates and the antimicrobial disks were placed on the plates using sterile forceps followed by incubation at 37 °C for 18- 24 h.

Statistical analysis
The results were analyzed using chi-square two by two contingency table with statistical package for social sciences (SPSS) (Petrie and Watson, 1999) 14.0 version and Microsoft Excel version 2007.

RESULTS
Spatial distribution of enterohaemorrhagie E. coli
The specific prevalence of the farms investigated ranged between 0.0 % (FA, FB, FC, FD and FG) and 10.0 % (FF). A total of 6 (2.5 %) E. coli serogroups from apparently healthy cattle were found, of which 1 (3.0 %) isolated from FE and FH was O111, 1 (4.4 %) from FF was O118, 1 (3.0 %) from FH and 2 (8.7 %) from FF were O126 serogroups respectively. E. coli isolates O126 occurred more frequently, followed by O111 and O118. Majority of the cattle farms had no E. coli serogroups. The P-value was statistically significant (P< 0.05) (Table 1).
Table 1. Specific prevalence of *E. coli* serogroups in commercial cattle farms in Kaduna State, Nigeria

<table>
<thead>
<tr>
<th>Farm</th>
<th>Specific prevalence (%)</th>
<th>Positive <em>E. coli</em> serogroup (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>O111</td>
</tr>
<tr>
<td>A</td>
<td>0.0</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>B</td>
<td>0.0</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>C</td>
<td>0.0</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>D</td>
<td>0.0</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>E</td>
<td>3.3</td>
<td>1 (3.0)</td>
</tr>
<tr>
<td>F</td>
<td>10.0</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>G</td>
<td>0.0</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>H</td>
<td>6.7</td>
<td>1 (3.0)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>2.5</td>
</tr>
</tbody>
</table>

$\chi^2 = 0.04, P(< 0.05)$

**Sensitivity pattern of *E. coli* isolates to antimicrobials**

All 6 isolates tested were resistant to nitrofurantoin, amoxicillin and cefuroxime or zinnat and sensitive to ofloxacin and ciprofloxacin (Table 2). Resistance to chloramphenicol (83.3 %), ampicillin (66.7 %), norfloxacin (33.3 %), gentamicin (33.3 %) and tetracycline (16.7 %) were also observed.

Table 2. Antimicrobial sensitivity pattern of *E. coli* non-O157 isolated from commercial cattle farms in Kaduna State, Nigeria

<table>
<thead>
<tr>
<th>Isolate no</th>
<th>N</th>
<th>CIP</th>
<th>TE</th>
<th>NB</th>
<th>AX</th>
<th>OF</th>
<th>C</th>
<th>CF</th>
<th>AM</th>
<th>GN</th>
</tr>
</thead>
<tbody>
<tr>
<td>E 3</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>F 5</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>F 14</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>F 18</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
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<tr>
<td>H 10</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>H 25</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
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<td>S</td>
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</tbody>
</table>

S = Sensitive (17-21 mm) R = Resistant (10-15 mm) N = Nitrofurantoin CIP = Ciprofloxacin TE = Tetracycline NB = Norfloxacin (nobarin) AX = Amoxicillin OF = Ofloxacin C = Chloramphenicol CF = Cefuroxime (zinnat) AM = Ampicillin GN = Gentamicin

**DISCUSSION**

The isolates from cattle farms investigated in this study were 100 % resistant to nitrofurantoin, amoxicillin and cefuroxime. There are growing concerns by consumers and health officials regarding antibiotic resistance of food borne pathogens that may be associated with the practice of adding growth promoting antibiotics to animal feeds. This suggests that antimicrobial resistance is widespread among *E. coli* O111, O118 and O126 inhabiting cattle in commercial farms. Thus, cattle could be an important source of new emerging zoonotic antibiotic-resistant *E. coli* that may present a risk of spreading antibiotic resistance to human beings in Kaduna state, Nigeria. This is because isolates of *E. coli* have been implicated in human disease, leading to severe outbreaks, affecting a good number of populations. An example was that of *E. coli* O111 in America as reported by Belnap and O'Donnell (1955). Our findings are consistent with the works of Schröder *et al.* (2002) and Pestana de Castro *et al.* (2003), who reported resistance among *E. coli* O111 and O118 isolates that showed multi-resistance to about 8 different antimicrobial drugs, predominated by *E. coli* O118 strains. Thus, it may be suggestive that drug resistance genes may have accumulated over time in O111, O118 and could possibly occur in other non-O157 serogroups.
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This is the first report on E. coli non-O157 resistance in Kaduna state, Nigeria and therefore, there is no knowledge about the possible effect of drug resistant E. coli serotypes on the human populations in the study area. It is concluded that research should be carried out to document the presence and role of antimicrobial resistance genes in animal and human populations in the study area.

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REFERENCES

