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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 ^{0} and 11 ^{0}N and longitude 7 ^{0} and 8 ^{0}E, North-Western Nigeria (Figure 1).

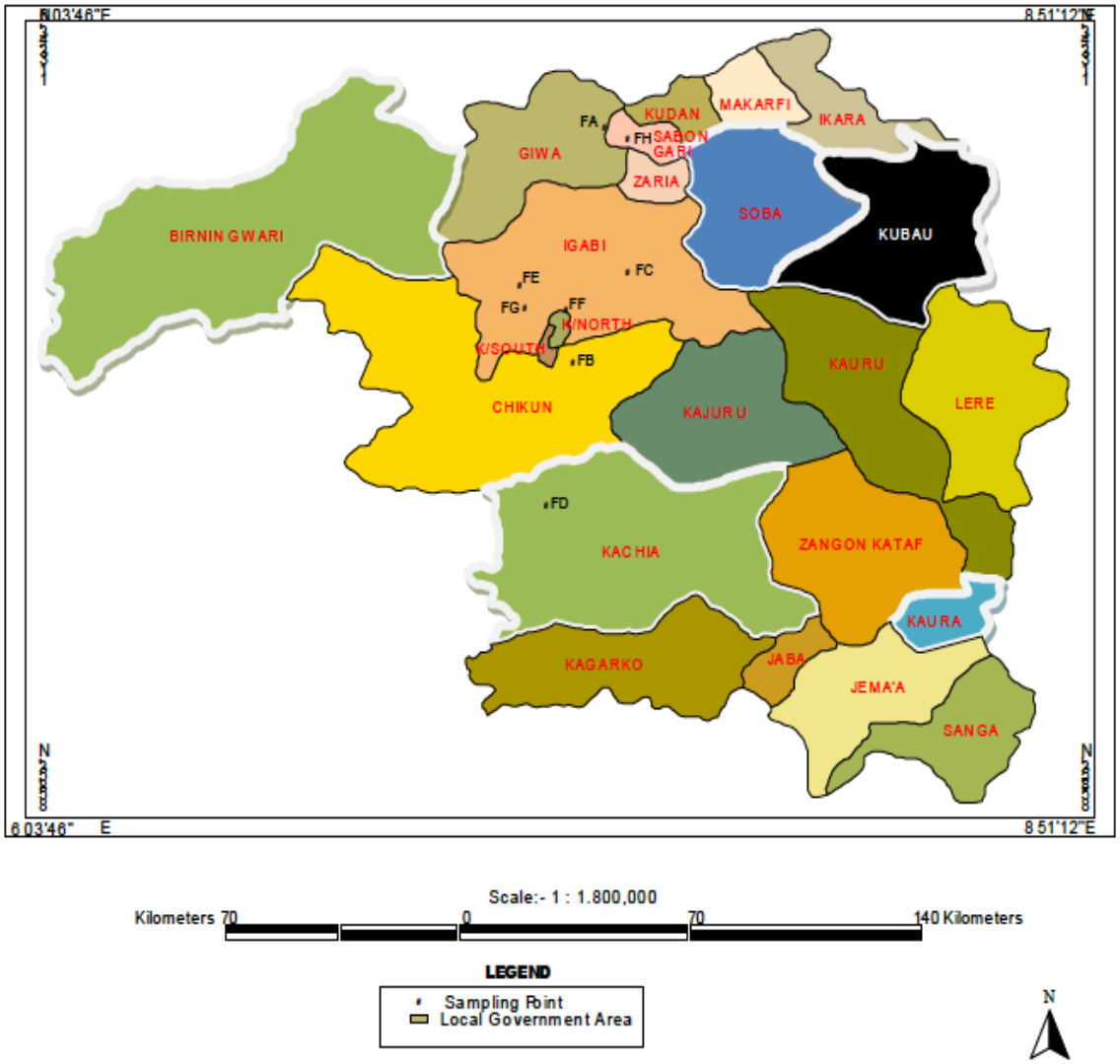

Fig.1: SAMPLING POINTS DISTRIBUTION IN KADUNA STATE

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 ^{o}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 Proskauer, 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 ^{o}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 (nobactin- 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 ^{o}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 enterohaemorrhagic *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

Farm	Specific prevalence (%)	Positive *E. coli* serogroup (%)		
		O111	O118	O126
A	0.0	0 (0.0)	0 (0.0)	0 (0.0)
B	0.0	0 (0.0)	0 (0.0)	0 (0.0)
C	0.0	0 (0.0)	0 (0.0)	0 (0.0)
D	0.0	0 (0.0)	0 (0.0)	0 (0.0)
E	3.3	1 (3.0)	0 (0.0)	0 (0.0)
F	10.0	0 (0.0)	1 (4.4)	2 (8.7)
G	0.0	0 (0.0)	0 (0.0)	0 (0.0)
H	6.7	1 (3.0)	0 (0.0)	1 (3.0)
Total	2.5	3 (0.8)	1 (0.4)	3 (1.3)

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

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

Isolate no	Antimicrobial agent									
	N	CIP	TE	NB	AX	OF	C	CF	AM	GN
E 3	R	S	S	S	R	S	R	R	R	S
F 5	R	S	S	R	R	S	R	R	R	S
F 14	R	S	S	S	R	S	R	R	R	S
F 18	R	S	R	S	R	S	R	R	S	S
H 10	R	S	S	R	R	S	R	R	R	R
H 25	R	S	S	S	R	S	S	R	S	R

S = Sensitive (17-21 mm) R = Resistant (10-15 mm) N = Nitrofurantoin CIP =Ciprofloxacin TE = Tetracycline NB = Norfloxacin (nobactin) 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.

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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