African Journal of Biotechnology Vol. 2 (9), pp. 322-324, September 2003
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315

Short Communication

Plasmid profile of *Escherichia coli* 0157:H7 from apparently healthy animals

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Accepted 22 August 2003

One hundred samples from healthy animals were screened for the presence of enterohaemorrhagic *Escherichia coli* 0157: H7 and 17 were positive for EHEC 0157:H7 after confirmation using serology kits. Antibiotic susceptibility patterns showed the isolates to be highly susceptible to the various antibiotics screened with a few showing multiple antibiotic resistance. The plasmid profiles revealed that 8/17 (47%) of the animal isolates harboured detectable plasmids ranging in size from 0.564 kb to >23 kb.

Key words: *Escherichia coli*, EHEC, animals, plasmid profile.

INTRODUCTION

Infection caused by *Escherichia coli* 0157:H7 has become a significant public health problem world-wide (Armstrong et al., 1996). The forms of transmission are animal-to-person, waterborne and person to person (Armstrong et al., 1996; Coia, 1998). Cattle faeces has been recognised as the principal reservoir of the microorganism in waterborne and food-borne *E. coli* 0157:H7 outbreaks and sporadic infections (Armstrong et al., 1996; Coia, 1998). Enterohaemorrhagic *E. coli* (EHEC) 0157:H7 is the dorminant shiga toxin producing strain that is known to be associated with both outbreak and sporadic cases of human diseases ranging from uncomplicated diarrhoea to haemorrhagic colitis and haemolytic uraemic syndrome (HUS). Their ability to cause severe diseases is related to their capacity to secrete shiga toxins also called verotoxins.

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The prevalence of EHEC in animals has not been studied in Nigeria to the best of our knowledge. In this report, the commonly consumed animals in Nigeria were investigated for the prevalence of EHEC 0157:H7.

MATERIALS AND METHODS

Faeces from cattle, pigs, rams and goats observed defaecating were collected from Ventata farms, Gbagada Cattle Ranch, and Ajayi Farms in Lagos State and Pakoto farm in Ifo, Ogun State in universal sterile container and immediately transported to the laboratory for processing and culture. Briefly, 1 g suspension of faeces was placed in 9 ml of peptone water and vortexed. After which, 0.1 ml of the same buffer was equally spread onto the surface of sorbitol MacConkey agar and incubated for 24 h at 37^0C.

Non-sorbitol fermenting colonies were picked and characterized using biochemical systems. The sorbitol negative colonies were serologically typed for 0157:H7 antigens by slide agglutination with polyvalent and

monovalent anti-*E. coli* O and H sera (Biogenetics Diagnostics, Padua, Italy). Haemolytic activity of the isolates were tested by culturing the isolates in BHI containing 7% human blood and incubating at 37^0C for 24 h.

Susceptibility of the organisms to antibiotics was tested by the disk diffusion method on BHI according to Bauer et al. (1966). The following antibiotics were used: ampicillin, colistin, gentamicin, nitrofurantoin, cotrimoxazole, streptomycin and tetracycline. 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.

Table 1. Distribution of serologically confirmed *E. coli* isolates and their sources.

Animal type	No. of samples	Non-sorbitol fermenters	Serologically confirmed
Cow	40	27	7
Ram	30	13	2
Pig	17	12	5
Goat	13	6	3
Total	100	58	17

RESULTS AND DISCUSSION

EHEC 0157:H7 was isolated from 17% of animals in this study (Table 1). The E. coli isolates showed haemolytic activity on blood agar plates. Our results show that 18% of the animal isolates showed multiple antibiotic resistance while when tetracycline resistance was taken alone it was 35% (Table 2). There has been increasing concern of the possible development of resistance to antimicrobial agents in the Enterobacteriaceae, especially *E. coli* as a result of the use of such agents in animal feed (Willis, 2000). This resistance is quite high and it could be as a result of the widespread use of such agent in animal feed in Nigeria. Plasmid profile analyses revealed that there are detectable plasmids in 47% of the isolates. Plasmids were detected from eight (47%) animal isolates, three from cows (>23.13 and 4.361 kb; 0.564 kb), one from goat (>23.13 kb), two from pigs (>23.13, 2.322 and 2 kb; >23.13 kb. The last two from ram had sizes of >23.13 kb each (Figure 1).

All the isolates had large molecular weight plasmids >23.130 kb. Large molecular weight plasmids (90 kb) have commonly been associated with toxigenic strains (Bopp et al., 2003). Further virulence tests on these isolates are required as these animals are commonly

Table 2. Antibiotic susceptibility patterns of the *E. coli* isolates.

Code no.	Antibiotic susceptibility patterns							
	Ap	Col	Gen	Nal	Nit	Cot	Str	Tet
P1	**R**	**R**	S	S	S	S	S	**R**
P2	S	**R**	S	S	S	S	S	**R**
P3	S	**R**	S	S	S	S	**R**	S
P4	S	S	S	S	S	S	S	**R**
G1	S	S	S	S	S	**R**	S	**R**
G2	S	**R**	S	S	S	S	S	S
P5	S	**R**	S	S	S	S	S	S
G3	S	**R**	S	S	S	S	S	S
R1	S	**R**	**R**	**R**	**R**	**R**	**R**	**R**
R2	S	**R**	S	S	S	S	S	S
C1	**R**	**R**	S	**R**	**R**	**R**	**R**	**R**
C2	**R**	**R**	S	**R**	**R**	**R**	**R**	S
C3	**R**	S	S	S	S	S	S	S
C4	S	S	S	S	S	S	S	S
C5	S	**R**	S	S	S	S	S	S
C6	S	**R**	S	S	S	**R**	S	S
C7	S	**R**	S	S	S	S	S	S

Ap, ampicillin; Col, colistin; Gen, gentamicin; Nal, nalidixic acid; Nit, nitrofurantoin; Cot, cotrimoxazole; Str, streptomycin; Tet, tetracycline. R, resistant; S, sensitive.
P, pig; G, goat; R, ram; C, cow.

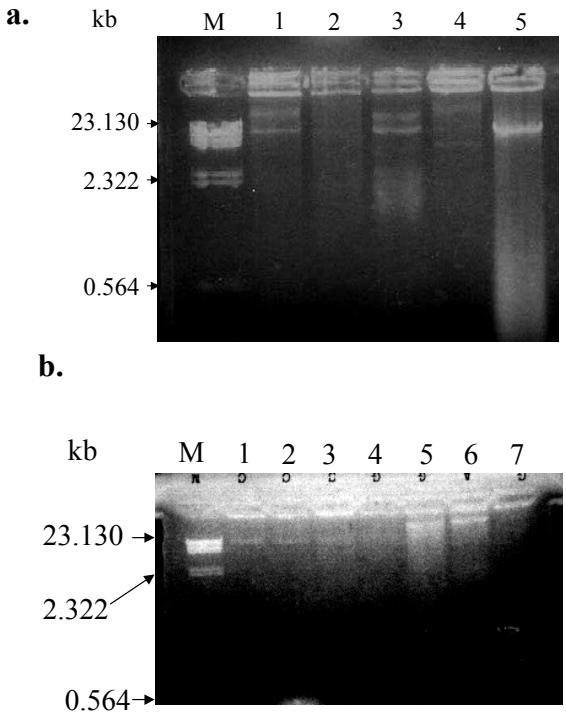

Figure 1. Agarose gel electrophoresis showing plasmid profile of EHEC from the isolates. (**a**) Lanes: M, molecular weight marker (λ, *Hind*III digest); 1, ram isolate; 2, pig isolate; 3, pig isolate; 4, cow isolate (>23.13 kb); 5, cow isolate (9.416 kb). (**b**) Lanes: M, molecular weight marker (λ, *Hind*III digest), 1, cow isolate (no plasmid); 2, cow isolate (0.564 kb); 3, cow isolate (no plasmid); 4, goat isolate (no plasmid); 5, goat isolate (>23.13 kb); 6, ram isolate (>23.13 kb); 7, goat isolate (no plasmid).

consumed animals in Nigeria. The goat and ram isolates shared the same plasmid size (>23.13 kb) and were from the same farm, showing that the EHEC isolates may be epidemiologically related. In conclusion, EHEC 0157:H7 strains from animals in Nigeria have shown a high prevalence. These isolates have shown high resistance especially to tetracycline and so farm workers and health workers should greatly consider the health risk to humans associated with using such agents in animal feed. This is due to development of resistance amongst these animals and the possible transfer to humans.

ACKNOWLEDGEMENT

SIS is grateful to Alexander von Humboldt for the provision of the Gel Cam camera used for taking the pictures in this article.

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