

ANTIBIOGRAM AND PLASMID PROFILE OF *E. coli* O157:H7 ISOLATED FROM APPARENTLY HEALTHY FUTA STUDENTS, NIGERIA.

J.F. John-Isa, and T.T. Adebolu,

Department of Microbiology, Federal University of Technology, Akure, Nigeria.

* E- mail of Corresponding Author: johnisa66@yahoo.com.

ABSTRACT

A total of 500 faecal samples were collected from apparently healthy FUTA students to determine the carrier rate of *Escherichia coli* O157:H7, the aetiological cause of enterohemorrhagic diarrhoea using standard microbiological and serological methods. The antibiogram and plasmid profiles were also determined using standard methods. Twenty-nine (5.8%) of the sampled examined were positive for *E. coli* with highest frequency of occurrence in males and age group, 20-24 years. The isolates were resistant to most of the convectional antibiotics used except Ofloxacin and Ciprofloxacin. Twelve (41.4%) of the isolates showed multi- drug resistance to three or more antibiotics group while the size of the plasmids isolated ranged from 23,130 - 34,637 bp. With the high incidence of carriage of *E. coli* O157:H7 observed in this study, there is need to set up national surveillance programs in order to prevent epidemiological outbreak of infections and complications caused by this organism, more so that the organism exhibited multiple resistance to common antibiotics.

Keywords: *E. coli* O157: H7, Antibiogram, Plasmid Profile, Apparently Healthy Students.

INTRODUCTION

Since there is no data available in Akure, Ondo State regarding the prevalence of *E. coli* O157:H7 in humans, this study aimed at determining the prevalence of *E. coli* O157:H7 in apparently healthy students of FUTA, Ondo State. *E. coli* is a gram negative facultative anaerobic rod, non-sporulating bacterium that is commonly found in the lower intestine of warm-blooded animals. Most strains of *E. coli* are harmless, but some such as serotype O157:H7, can cause serious food poisoning in humans, and are occasionally responsible for product recalls (Vogt &

Dippold, 2005). The harmless strains are part of the normal flora of the gut, and can benefit their host, by producing vitamin K₂ and also prevents the establishment of pathogenic bacteria within the intestine (Hundault *et al.*, 2001). Enteric *E. coli* (EC) are classified into five groups on the basis of ecological characteristics and virulence properties, these include, Enterotoxigenic *E.coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Enterohaemorrhagic *E. coli* (EHEC) and Enteroaggregative *E. coli* (EAEC) (Todar, 2007). Enterohaemorrhagic *E. coli* (EHEC)

belongs to a pathogenic subgroup of verotoxin (VTEC) or shiga-toxin producing *E. coli* (STEC) (Karch *et al.*, 2005). They are mainly found in humans, cattle and goat. The most famous member of their group is strain O157:H7, which causes bloody diarrhoea with no fever, hemorrhagic colitis and hemolytic uremic syndrome which is the important cause of sudden kidney failure especially in

MATERIALS AND METHODS

SAMPLES

Five hundred (500) stool samples were collected from apparently healthy students of FUTA at the university's health centre into sterilized bottles.

CULTURING AND ISOLATION OF *E. coli* O157: H7

Culturing and isolation of *E. coli* O157: H7 was done using the methods of Olutiola *et al.* (1991).

SEROLOGICAL IDENTIFICATION OF *E. coli* O157:H7

This was done using bacteria agglutination test of Walker and Taub (2004).

ANTIMICROBIAL SENSITIVITY TEST

The antibiotic sensitivity assay was done according to the method of Olutiola *et al.* (1991).

PLASMID PROFILE

The plasmid profile analysis was done using the method of Kraft *et al.* (1988).

RESULTS

Out of 500 faecal samples collected from apparently healthy individuals in FUTA, 29

young children and the elderly. Most illness has been associated with eating undercooked, contaminated ground beef, contaminated vegetables, drinking unpasteurized milk, contaminated water or swimming in it. In *E. coli* O157:H7, the "O" in the name refers to cell wall antigen number (157) whereas the "H" refers to flagella antigen (H7) (Chapman,1994).

(5.80%) were positive for *E. coli* O157:H7. Eighteen (6.29%) out of the male gender were positive for the organism while 11(5.14%) out of the female gender were positive for the organism (Table 1). That is, the carrier rate was higher in the male gender. In relation to age, the carrier rate was highest in age group, 20- 24years (7.60%) (Table 2). Concerning the antibiogram, the organism was resistant to all the antibiotics used except the fluoroquinolones (ofloxacin (O%) and ciprofloxacin (O%)). The highest resistance was against the β – lactams. (Cefuroxime (89.7%), Ceftazidime (58.6%) and Ampicillin (72.4%) (Table 3). Plate 1 shows a typical antibiotic sensitive pattern of the isolates to the antibiotics used. The plasmid profile of *E. coli* O157: H7 isolates examined in this investigation can be seen in Plate 2. Seventeen (58.6%) were observed to have only one plasmid i.e a single plasmid, four (13.8%) had multiple plasmids while eight (27.6%) had no plasmid. The plasmid size ranged from 23,130bp to 34,637bp. Six had a plasmid with molecular weight of 23,130bp, five with molecular weight of 24,055bp, seven with molecular weight of 24,980bp, one with molecular weight of 32,787bp, one with molecular weight of 33,712bp and two with molecular weight of 34,637bp.

Table 1: Carrier rate of *E. coli* O157:H7 in relation to gender among FUTA students

Sex	No of samples collected	No of positive	% of positive
Male	286	18	6.29
Female	214	11	5.14
Total	500	29	5.80

Table 2: Carrier rate of *E. coli* O157: H7 in relation to age among FUTA students.

Age group(yrs)	No of samples collected	No positive	% positive
15-19	212	8	3.77
20-24	263	20	7.60
25-29	25	1	4.00
Total	500	29	5.80

Table 3: Percentage of *E. coli* O157: H7 isolates that are resistant to differents groups of antibiotics

Class of antibiotics	Antibiotics	No Tested	No of isolates sensitive	% Sensitivity
Fluoroquinolones.	Ofloxacin	29	0	0.0
	Ciprofloxacin	29	0	0.0
Aminoglycoside.	Gentamicin	29	8	27.6
Furanes.	Nitrofurantoin	29	9	31.5
Beta- lactams (2nd Generation.	Cefuroxime	29	26	89.7
Cephalosporins) Beta - lactams (3rd Generation.	Ceftazidime	29	17	58.6
Cephalosporins) Beta - lactams (Penicillins).	Ampicillin	29	21	72.4
Beta – lactams (Penicillin + Beta – lactamase Inhibitors)	Augmentin	29	10	34.5

Table 4: Multiple antibiotics Resistance pattern of the isolated *E. coli* O157: H7 (n= 29).

Classes of antibiotics	No of isolates resistant	% of resistant isolates
B-lactams (penicillins) + Aminoglycoside + β -lactams (cephalosporins) + β -lactams (3 rd generation).	7	24.1
B-lactam (penicillin + β -lactamase inhibitors)B-lactams (penicillins) + β -lactams (cephalosporins) + β -lactams (3 rd generation).	2	6.9
B-lactam (penicillin + β -lactamase inhibitors) + B-lactams (penicillins) + β -lactams (cephalosporins) + furanes.	1	3.4
B-lactam (penicillin + β -lactamase inhibitors) + furanes + β -lactams (cephalosporins) + β -lactams (3 rd generation).	1	3.4
Furanes + Aminoglycoside +B-lactam (penicillin + β -lactamase inhibitors)+ B-lactams (penicillins) + β -lactams (3 rd generation).	1	3.4
B-lactams (penicillins) + β -lactams (cephalosporins) + β -lactams (3 rd generation).	5	17.2
B-lactam (penicillin + β -lactamase inhibitors) + Furanes + β -lactams (cephalosporins).	1	3.4
B-lactams (penicillins) + β -lactams (cephalosporins).	3	10.3
Furanes + β -lactams (cephalosporins)	1	3.4



Plate 1: Plate showing antibiotic sensitivity pattern of one of the isolated *E. coli* O157:H7.

KEY

OFL = Ofloxacin (5 μ g)

NIT = Nitrofurantoin (300 μ g)

CPR = Ciprofloxacin (5 μ g)

CRX = Cefuroxime (30 μ g)

(Abtek Biologicals Ltd, UK)

AUG = Augmentin (30 μ g)

AMP = Ampicillin (10 μ g)

GEN = Gentamicin (10 μ g)

CAZ = Ceftazidime (30 μ g)

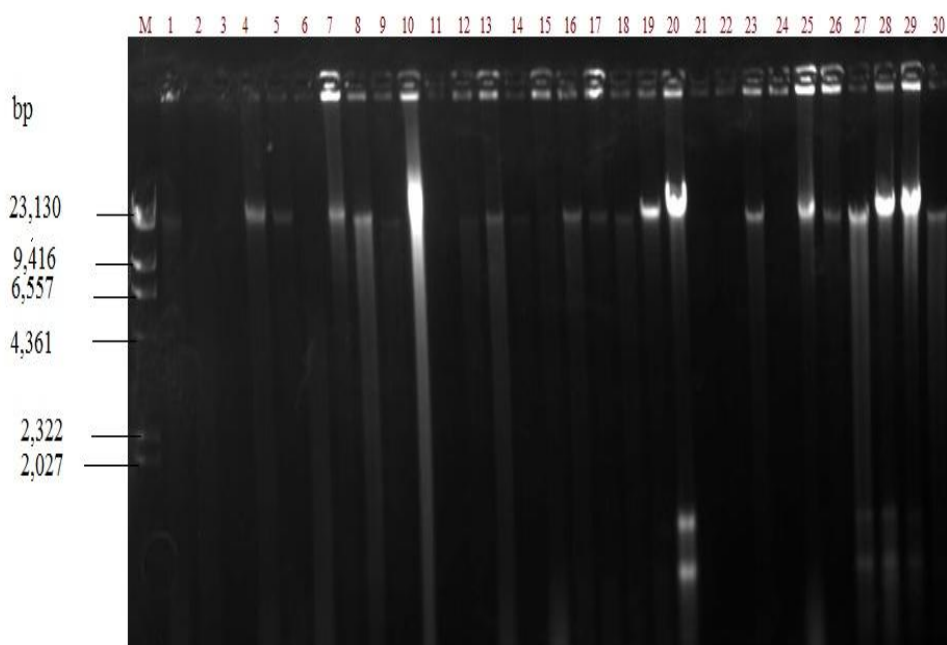


Plate 2 : Plasmid Profile Analysis of the isolated *E. coli* O157: H7

KEY : M = Marker, bp = base pair (unit of molecular weight), 1 - 29 = Isolated *E. coli* O157 :H7

DISCUSSION

In this study, the carrier rate of *E. coli* O157: H7 in the stool of apparently healthy FUTA students, the antibiogram and plasmid profiles were carried out. The 5.8% carrier rate observed in this investigation was lower than that reported by Abong and Momba (2008) though their own study was not on apparently healthy individuals but on water, vegetables and meat products that serve as vehicles of transfer of the organism. In their investigation, which was done in South Africa, the occurrence frequency of this organism in drinking water, meat and vegetables were 35.5%, 25.5% and 21.7% respectively. The higher incidence in males than in females observed in this study might probably be due to the fact that males are normally careless in their eating habit than the female gender. The observation that the organism is resistant to almost all the conventional antibiotics used except Ofloxacin and Ciprofloxacin agrees with the report of the study carried out by Smith *et al.* (2009) that the human isolates from Lagos and Zaria in their investigation were all sensitive to the fluoroquinolones (ofloxacin and ciprofloxacin). The highest resistance of *E. coli* O157: H7 isolates to cefuroxime (89.7%), followed by ampicillin (72.4%) and ceftazidime (58.6%) which are different generations of β -lactams, was similar to what was observed by Aibinu *et al.* (2004), who reported 0% sensitivity of their *E. coli* O157: H7 isolates to ampicillin, a β lactam. That is, the organism is highly resistant to β lactams. The reason for this high resistance to commonly used antibiotics may be due to widespread and indiscriminate use in our environment. The observation that 12 (41.4%) out of 29 isolates showed multi – drug resistance (i.e isolates resistance to four or more antibiotics) and harbour plasmids with sizes ranging from 24,130bp – 34,637bp is similar to what was observed by Smith *et al.* (2003). The reason may be due to the fact that

E. coli O157: H7 easily acquire drug resistance genes in natural habitat and transfer them to other organisms of clinical relevance (Smith *et al.*, 2003).

CONCLUSION

In conclusion, this study has shown that the carrier rate of *E. coli* O157: H7 among apparently healthy students was 5.8% and that the organism is resistant to almost all conventional antibiotics, it is therefore recommended that all episodes of diarrhoea should be reported to the Health centre for proper management to prevent epidemiological outbreak of this life threatening infection with its associated complications.

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