



Original Research Article

Antigenic and Molecular Properties of *Escherichia coli* O157:H7 Isolated from Diarrhoeic Patients in Ondo State, Nigeria

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Abstract	Keywords
<p>The antigenic and molecular properties of the <i>Escherichia coli</i> O157:H7 was carried out among diarrhoeic patients in Ondo State in this study. Samples were collected from primary, secondary and tertiary health care centres. The organism was isolated using standard conventional, microbiological methods. The organism DNA was extracted and subjected to polymerase chain reaction assay using two set of primers namely: <i>flicH7</i> and <i>rfbO157</i>. Randomly amplified polymorphic DNA-polymerase chain reaction assay (RAPD-PCR) was used to investigate clonal relatedness and diversity of the organism. Two operon primers; OPR-02: CACAGCTGCC and OPC-04: CCGCATCTAC were used for the PCR, the result showed clonal diversity among the isolates using both primers. Out of the 540 samples collected for the study, 50 were sorbitol negative, 32 were positive for both the <i>flicH7</i> and <i>rfb</i> O157 primers, and were therefore EHEC O157:H7. Seven were only positive for the primer <i>flicH7</i>, while the remaining isolates were positive for <i>rfb</i> O157. For the study data, one way ANOVA was done using statistical software SPSS. A 5.9% rate of infection was recorded. Out of the people sampled, the highest percentage rate of occurrence was found frequent in females (7.9%) than males 3.9%. In respect to location of the study, the primary health care centres showed the highest occurrence rate 6.6% while the lowest 5.5% was found in tertiary health care centre. This study has been able to show that 5.9% of diarrhoeic cases in Ondo State, Nigeria were caused by <i>E. coli</i> O157:H7 and that there were diversities in their strains. It is therefore suggested that effort should be geared at coming up with control measure to prevent the infection from attaining epidemiological status.</p>	<p>Antigenic <i>E. coli</i> O157:H7 Molecular properties Ondo State Water contamination</p>

Introduction

Since the first reported outbreak in the US in 1983 (Isibor et al., 2013), *E. coli* O157:H7 infections have been reported most frequently in developed

countries for example United Nation and Great Britain (Bonyadian et al., 2010). Since then, *E. coli* O157:H7 diarrhoeal illness has been reported from

multiple locations in South Africa, Central Africa, Kenya, Gabon, Nigeria and Ivory Coast (Olatoye, 2010). *E. coli* O157:H7 is considered an emerging disease pathogen (Nataro and Kaper, 1998). This organism has a unique cultural characteristic of inability to ferment sorbitol – a nutrient used for isolation of *E. coli* from stool specimen. This pathogen has become more significant than other well – recognised food borne pathogens for reasons including the severe consequences of infection that affect all age groups, their low infectious dose, their unusual acid tolerance and their apparent special but inexplicable association with ruminants that are used for food (Buchanan and Doyle, 1997).

The organism is transmitted primarily through the ingestion of faecal contaminated foods, particularly undercooked beef (Brazil et al., 2007, Szalanski et al., 2004). It can also be transmitted through the consumption of contaminated drinking water or contact with recreational water (Olatoye, 2010).

Although, a lot of works have been done on the infection rate of this organism in different part of the world, for instance (Ogunsanya et al., 1994; Akinyemi et al., 1998; Olorunshola et al., 2000; Okeke et al., 2000 and 2003), in Ondo State little or no data is available on the prevalence studies of EHEC O157:H7 infections. This study was therefore aimed at determining the antigenic and molecular properties of *E. coli* O157:H7 among diarrhoeic patients in Ondo State as well as those factors that influence infection, as this could help in strategies for the management of infections caused by the pathogen thus averting possible disease outbreaks.

Materials and methods

Five hundred and forty (540) diarrhoeic stool samples were collected from infected patients by cluster sampling method at primary, secondary and tertiary health care centres in the eighteen local governments of Ondo State, Nigeria (Fig. 1). Ethic consent form was administered and questionnaires were filled in respect to sex, age, marital status, occupation, type of water and toilet used by individual when the samples were being collected from the patients. The samples were immediately brought to the laboratory for analysis. The samples were first cultured on eosin methylene blue agar and the plates were incubated at 37°C for 24 h. Bluish

black with a metallic sheen colonies were transferred onto plates of sorbitol MacConkey agar (a differential medium used for culturing the organism) and also incubated at 37°C for 24 h. Colourless colonies were taken as *E. coli* O157:H7. The following biochemical tests were done to ascertain that the organism was *E. coli*: Gram stain, blood haemolysis, and methyl red Voges-Proskaur, Indole, citrate and motility test according to the method of Olutiola et al. (2000).

Sorbitol negative colonies were also tested by latex agglutination using Wellcolex *E. coli* O157:H7 diagnostic kit (Oxoid, TSMX7825, US). Agglutination positive isolates were confirmed by PCR assay for combination of O157 and H7 flagella genes. Random amplified polymorphic DNA-polymerase chain reaction assay was used to determine the clonal relatedness and diversity of the isolates.

Fig. 1: Map of Ondo State, showing 18 local Governments where samples of diarrhoeic patients were collected.



Results

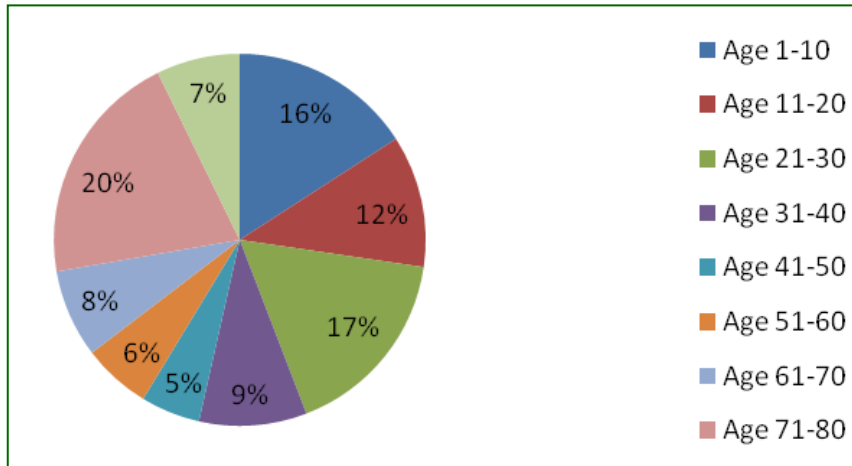
Out of 540 diarrhoeic patients sampled during the course of this research work, 32 (5.9%) were positive for *E. coli* O157:H7. Ten (3.9%) of the 260 male sampled were positive while twenty two (7.9%) of the 280 females sampled were positive for the organism. That is a higher proportion of the occurrence was found among the females (Table 1).

Table 1. Rate of occurrence of *E. coli* O157:H7 among diarrhoeic patients in 18 local Governments of Ondo State in 2014 based on sex.

Sex	No. of samples collected	No positive	% positive
Male	260	10 ^a	3.9
Female	280	22 ^b	7.9
Total	540	32	5.9

Mean with different alphabetical subscripts are significantly different $p < 0.05\%$

Fig. 2: Rate of occurrence of *E. coli* O157:H7 among diarrhoeic patients in the 18 local Governments of Ondo State based on age group.



The relationship between the rate of occurrence and age-group can be seen in Fig. 2. The occurrence was higher in age-group of 71-80 (20%), followed by age-group 21-30 (17%), then the age-group 1-10 (16%) and age-group 11-20 (12%). The rates of occurrence in the other age-group were as follow: 31-40 (9%), 61-70 (8%), 81-90 (7%), 51-60 (6%) and 41-50 (5%).

The relationship between the rate of occurrence and occupation is seen in Fig. 3. The rate of occurrence was highest among farmer (10%), followed by

student (7.5%), pupil and trader (6%), day-care (5.7%), teacher and public servant (5%) and the lowest was found among civil servant and commercial motor-circle rider (4%). The State senatorial districts which individual patient belonged also contributed to the rate of occurrence as seen in Fig. 4. Patients that lived in centre senatorial district of their States had the highest rate of occurrence of (6.6%), followed by the north senatorial district (6.1%). The least occurrence rate was found among those that lived in south senatorial district of their States (5%).

Fig. 3: Rate of occurrence of *E. coli* O157:H7 on 18 local Governments of Ondo State in 2014 based on occupations.

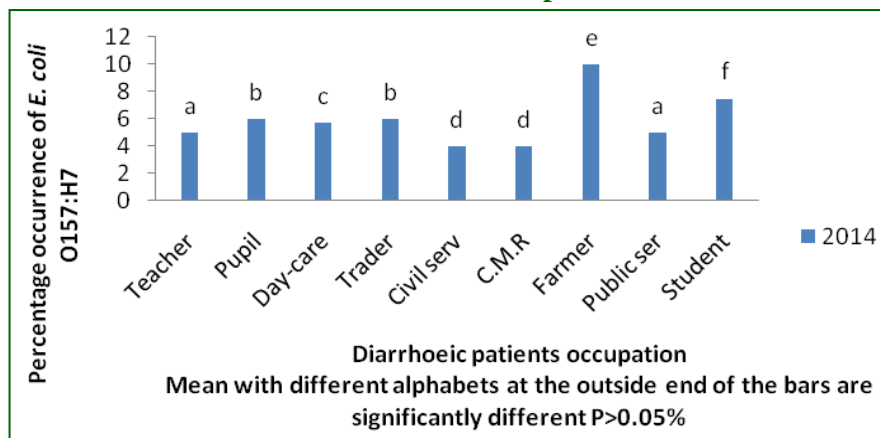


Fig. 4: Rate of occurrence of *E. coli* O157:H7 among diarrhoeic patients in the 18 local Governments of Ondo State based on senatorial districts.

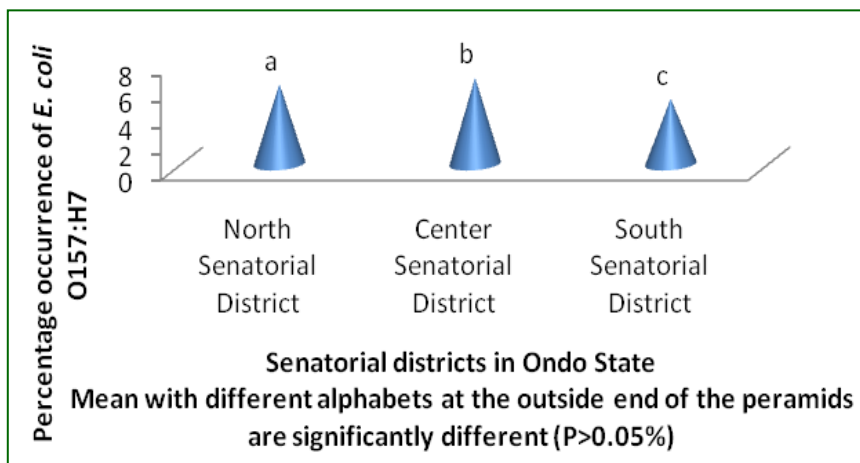


Table 2. Rate of occurrence of *E. coli* O157:H7 among diarrhoeic patients in 18 local Governments of Ondo State in 2014 based on water used.

Water used	No. of samples collected	No positive	% positive
Borehole	189	0	0.0
Well	170	28 ^a	16.5
Sachet	181	4 ^b	2.2
Total	540	32	5.9

Mean with different alphabetical subscripts are significantly different $p > 0.05\%$

Table 3. Rate of occurrence of *E. coli* O157:H7 among diarrhoeic patients in 18 local Governments of Ondo State in 2014 based on marital status.

Sex	No. of samples collected	No positive	% positive
Married	274	15 ^a	5.5
Single	266	17 ^b	3.2
Total	540	32	5.9

Mean with different alphabetical subscripts are significantly different $p > 0.05\%$

Table 4. Rate of occurrence of *E. coli* O157:H7 among diarrhoeic patients in 18 local Governments of Ondo State in 2014 based on health care centres.

Health care	No. of samples collected	No positive	% positive
Primary	180	12 ^a	6.6
Secondary	180	10 ^b	5.5
Tertiary	180	10 ^b	5.5
Total	540	32	5.9

Mean with similar alphabetical subscripts are not significantly different $p > 0.05\%$

The water used by individual patient also had influence on the rate of occurrence of the organism Table 2. Rate of occurrence was higher among those using deep well water (16.5%), followed by that using sachet water (2.2%). Out of 189 samples collected among the people using borehole water, nothing was found in them. Marital status of individual patient also influenced the rate of occurrence Table 3. The rate of occurrence was higher among the married individual patient (5.5%),

while the lowest was found among the single patient (3.2%).

The health care centre attended by individual patient also had influence on the rate of occurrence Table 4. The rate of occurrence was higher among the patients attending primary health care centres (6.6%), followed by that attending secondary and tertiary health care centres (5.5%). The toilet facility used by the patients had influence on the rate of

occurrence (Fig. 5). The rate of occurrence was higher among the patient using dunghill toilet (10.5%), followed by that using pit toilet (5.9%). The lowest rate of occurrence was found among the patients using water system (1.5%).

Local government of individual patient also contributed to the rate of occurrence as seen in Fig. 6. The rate of occurrence was highest among the

patients residence in Owo (16.6%), followed by that in Akoko South West and Ilaje West (13%), Akure North and Ilaje (10%), Okitipupa, Idanre and Akoko South East (6.6%). The lowest occurrence rate was found among Akure South, Ilaje West and Ondo East (3.3%). The rate of detection of *rfb* and *flic* in *E. coli* O157:H7 isolated from Ondo state showed 13, 25 and 62% presence *rfb*, *flic* and both *rfb*, *flic* genes (Figs. 7, 8 and 9).

Fig. 5: Rate of occurrence of *E. coli* O157:H7 on 18 local Governments of Ondo State in 2014 based on toilet facility used by diarrhoeic patients.

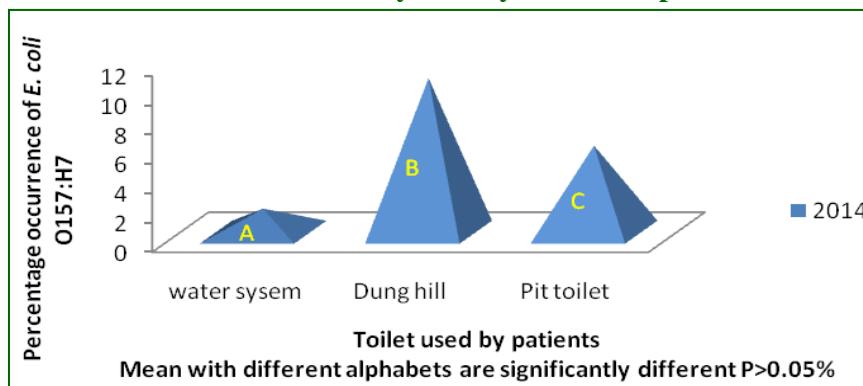
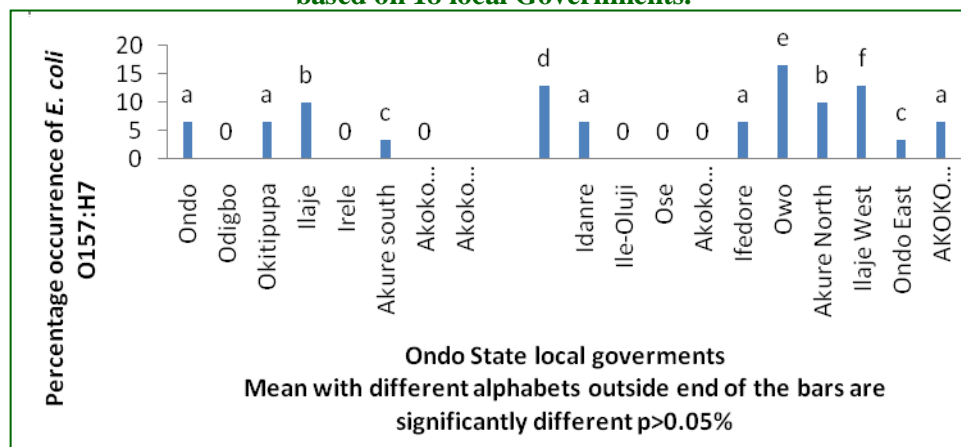


Fig. 6: Rate of occurrence of *E. coli* O157:H7 among diarrhoeic patients in Ondo state based on 18 local Governments.



Discussion

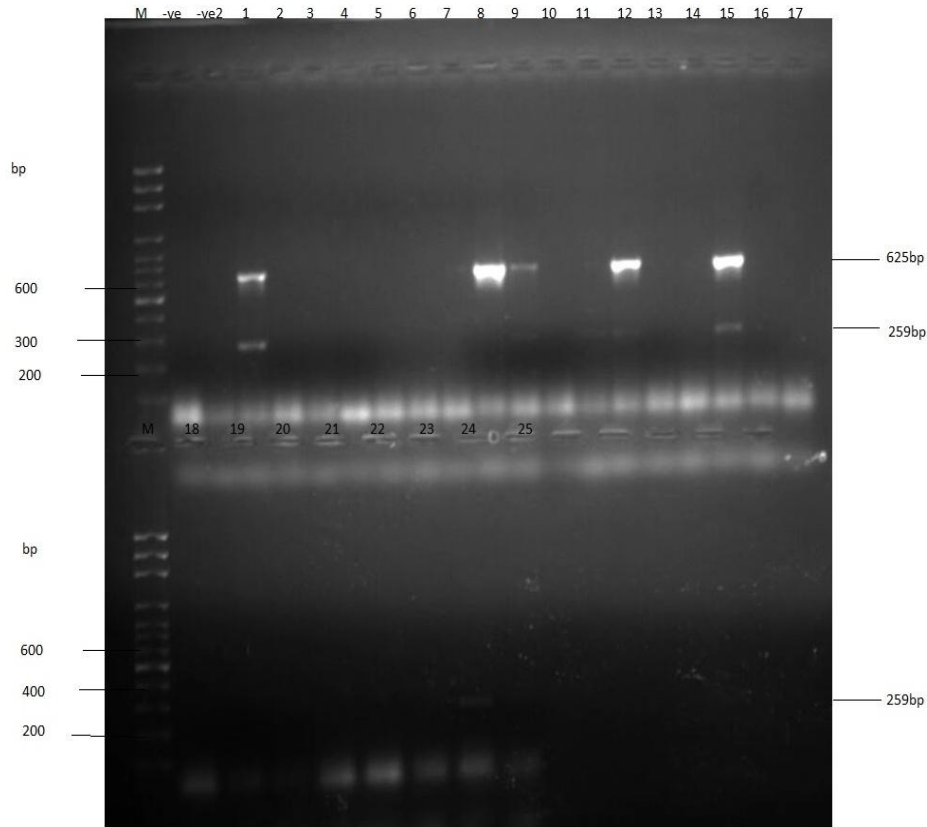
The antigenic and molecular properties of *E. coli* O157:H7 isolated among diarrhoeic patient in Ondo State was carried out in this study. It was found that 5.9% diarrhoeal cases were caused by *E. coli* O157:H7. This case was higher than that reported in Putra Malaysia, which was 5.2% by Chang et al. (2013) and the one reported in Edo State, Nigeria which was 2.7% by Isibor et al. (2013), but lower than that reported by Pennington (2010) in US

which was 40%. The discrepancy might probably be due to the time frame the studies were carried out, the level of hygiene standard of the individual countries and the origin of the samples examined. For example, this work was carried out on diarrhoeic human stool samples whereas that of Pennington (2010) and Chang et al. (2013) were carried out on calves and vegetable samples, besides, the largest outbreak recorded so far happened in England in August and September, 2009, with 93 infections, 78 patients had symptoms

and 17 developed haemolytic uremic syndrome (Pennington, 2010). The higher incidence of *E. coli* O157:H7 in Owo, Ilaje West and Akoko Local government area compared with Akure in this study

is in agreement with the report of Pennington (2010), that the incidence of human disease is greater in rural areas which have high densities of cattle and sheep than in urban area.

Fig. 7: Gel electrophoresis of the PCR assay amplifying 259 base pair segment of *rfb* O157 and 625 base pair of *flicH7* gene of *E. coli* O157:H7.



(Lane M: 100bp DNA marker; lane -v: master mix without DNA; lane -v2: *E. coli* K12; lane 1-25: isolates. Isolates 1, 9, 11, 12 and 15 were positive for both the *flicH7* and *rfb* O157 primers, and were therefore EHEC O157:H7. Sample 7 and 8 are only positive for the primer *flicH*, while sample 24 is positive for *rfb* O157)

Fig. 8: Gel electrophoresis of PCR assay on clonal divergence of *E. coli* O157:H7 isolated from Ondo State. This result showed clonal diversity among the isolates using both primers OPC 04 and OPR 02.

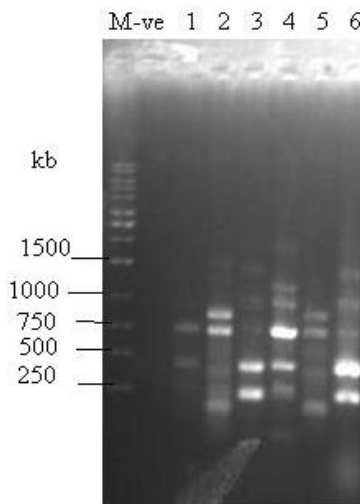
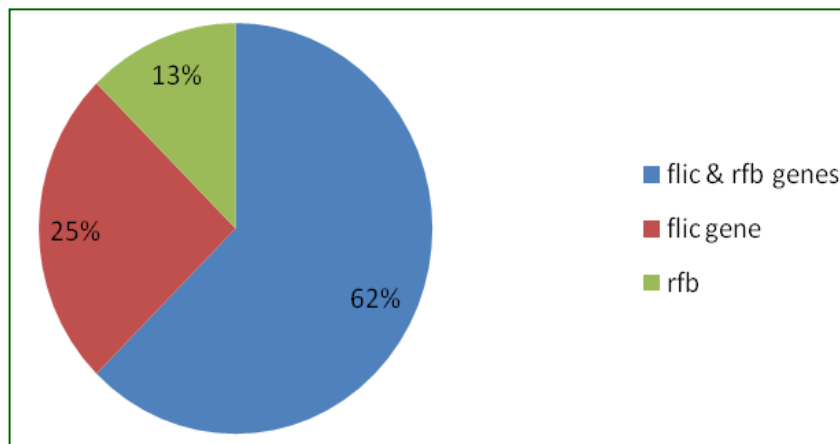


Fig. 9: Genomic comparison of the different strains of *E. coli* O157:H7 isolated in Ondo State.

The higher incidence observed among the people using well water than other sources of water in this study for instance sachet water and borehole water could probably be due to flooding and poorly located wells recorded in most of the local government areas during the cause of this study that brought about contamination of the water in the deep well used by the citizens. In the report of O'Sullivan et al. (2008), heavy rain is frequently associated with *E. coli* O157:H7 outbreaks: notable examples are the first outbreak recorded in Africa (a large one in Swaziland and South Africa in 1990; the large outbreak at Walkerton, ON, Canada, in 2000 and the smaller outbreak at the Glastonbury festival in England, in 1997). Heavy rain has been repeatedly associated with the occurrence of outbreaks linked to the consumption of water from private wells and springs. Concerning, the study location, the infection with this organism was more prevalence in primary health care centre (6.6%) than the secondary and tertiary health care centre. The slight discrepancy might be as a result people usually patronise local health care than the secondary or tertiary ones especially during minor health issues.

This study also showed that there is a significant relationship between persons' age group and the proportion of infection with *E. coli* O157:H7. Out of 9 age-groups sampled during the cause of this study, age-group 71-80 had the highest prevalence. This was in accordance with the report of Karch et al. (2005) and that of Smith et al. (2009) that infection with the organism often leads to hemorrhagic diarrhoea and occasionally to kidney failure, especially in young children and the elderly. The

higher proportion of prevalence of the organism in centre senatorial district compared with other districts in Ondo State could probably be due to the teaming population of people in this area which might invariably gave way to unhygienic environmental contamination of food and water being consumed by the people of this district.

This study has been able to show that 5.9% of diarrhoeic cases in Ondo State are caused by *E. coli* O157:H7. Moreover, it has been able to categorised the isolates into 3 groups; those with both *flic* and *rfbE* genes, those with only the *flic* gene and those with only the *rfbE* gene. The absence of the gene that is, the gene that encodes the H7 flagellum in most of the isolates showed that they lack a functional flagellum. This category of organism, according to Manning (1994) is referred to as *E. coli* O157: H* and was reported to be less pathogenic.

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