Escherichia coli O157:H7- Prevalence and Risk Factors of Infection in Edo State, Nigeria

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Abstract

We determined the prevalence of *Escherichia coli* O157:H7 as well as risks factors of infection in Edo State, Nigeria. One thousand (1000) fecal specimens from consenting persons from both sexes and of all age groups within the 3 Senatorial districts, reporting with cases of diarrhea and other gastrointestinal complaints as well as apparently healthy individuals, were inoculated onto MacConkey, Sorbitol MacConkey, Eosin Methylene Blue and Blood agar. Media were incubated aerobically at 37^{o}C for 24 hr. Isolates were identified using standard microbiological techniques. For the study data, Chi-square (χ^2) or Fisher's exact test as appropriate and odd ratio analysis were done using the statistical software INSTAT®. A 2.7% rate of infection was recorded. Most of the isolates were recovered from subjects living within the bustling commercial city of the state where access to fast-food and road side restaurants is common. Other favorable factors found to be associated with infection included diarrhea as clinical symptoms, the wetter seasons of the year and asymptomatic carriage of infection. Gender and age were not statistically significant ($p > 0.05$) risk factors of infection in this study.

Keywords: *Escherichia coli* O157:H7, Prevalence, Risk Factors, Infection, Edo State, Nigeria

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Introduction

Since the first reported outbreak in the US in 1982 (Riley, 1983), *E. coli* O157:H7 infections have been reported most frequently in developed countries, although illness due to *E.coli* O157:H7 has been reported in over 30 countries on six continents (Raji *et al*., 2006). *E.coli* O157:H7 is considered an emerging disease pathogen (Nataro and Kaper, 1998), which has eluded the skillful eyes of microbiologists because of its peculiar biochemical characteristics, its inability to ferment Sorbitol - a nutrient not incorporated in the growth medium routinely used for the isolation of *E. coli* from stool specimens. Infection with this organism causes hemorrhagic gastrointestinal disease and Hemolytic Uremic Syndrome (HUS), a renal complication mostly affecting children. This pathogen has become more significant than other well-recognized foodborne 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).

Human to human and animal to human contact have been implicated in the transmission of the disease (Duffy, 2003). The majority transmission is through eating of undercooked contaminated ground meat and consumption of raw milk, raw vegetables, fruits contaminated by water, cheese, curd and also through consumption of sprouts, lettuce and juice (Padhye and Doyle, 1991). *E. coli* O157:H7 has been isolated from a wide variety of hosts, specially cattle, sheep, goat, pig, poultry, dog, horses, deer, wild birds, flies (Hancock *et al*., 1998; Keen *et al.*, 2006). In developing countries of the world, where there is still an alarming rate of insanitary conditions, malnutrition and poor health facilities, there is an urgent need to study this organism and its characteristics with an aim to reduce the human hazard caused by this emerging pathogen. Few studies have been carried out in the South Western States of Nigeria (Ogunsanya *et al.,* 1994; Akinyemi *et al.,* 1998: Olorunshola *et al.,* 2000; Okeke *et al.*, 2000, 2003). From available literature, no concerted prevalence studies on EHEC O157:H7 infections have been carried out in Edo State.

This study is therefore aimed at determining the presence and prevalence rate of *E. coli* O157:H7 among people in Edo 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.

Subjects, Materials and Methods

Study Area:

Edo state lies roughly between longitude 06^0 04' E and 06^0 43' E and Latitude 05^0 44' N and 07^0 34' N. It is bounded in the south by Delta State, in the west by Ondo

State, in the north by Kogi State and in the east by Kogi and Anambra States. It occupies a land area of about 17,802 sq km, and has a population of 3,265,947**.** The state comprises of 18 local government areas, grouped into three Senatorial Districts, with Benin City as capital.

The wet season occurs between April and October, with a break in August, and an average rainfall ranging from 150cm (59") in the extreme north of the state to 250cm (98") in the South. The dry season lasts from November to April with a cold harmattan spell between December and January. The temperature averages about 25^0 C (77^0F) in the rainy season and about 28^0C (82^0F) in the dry season. The climate is humid tropical in the south and sub-humid in the north.

Informed Consent

Ethical clearance to use human subjects for this study was got from the Edo State Ministry of Health after the study proposal was considered and approved by the Research and Ethics Committee. Subjects enrolled for this study were those who gave their consent after the purpose of study was explained to them.

Specimen Collection

The sample size was determined using the formula of Dean *et al.* (1995). Between May 2006 and April 2011, one thousand (1000) fecal specimens were collected from persons of both sexes and of all age groups within the Senatorial districts. Those reporting with cases of diarrhea and other gastrointestinal complaints as well as apparently healthy individuals (control population) were included for the study, while those who had been on any type of antibiotic treatment for the preceding two weeks were excluded. Fresh fecal samples were collected by each person into sterile plastic universal containers (Sterilin, UK), and processed within 2 hours of collection. Samples from distant places were placed in Cary-Blair transport media (Oxoid CM 0519) and taken to the laboratory.

Microbiological Procedures

Gross examination of the fecal samples was performed to note the characteristics such as color, consistency, whether feces were formed, and semi-formed, unformed or watery, presence of blood / mucus. The specimens were then inoculated onto MacConkey Agar (Oxoid CM7) (for easy identification of lactose fermenting organisms), Eosin Methylene Blue Agar (Oxoid CM OO69) (for easy identification of the green metallic sheen appearance characteristic of *E. coli* colonies, Sorbitol MacConkey Agar (Oxoid, CM813) enriched with Cefixime-Tellurite supplement (Oxoid SR 172) (used to selectively differentiate the non-sorbitol-fermenting *E. coli* O157:H7 strains from other *E. coli* strains), and Blood

agar (Oxoid CM55) to differentiate hemolytic organisms from non-hemolytic ones; Each stool specimen was streaked onto the media and incubated aerobically at 37^{o}C for 24 hr to isolate *E. coli*. Each colonial morphology and reactions on agar media, like colony size, consistency, shape and pigmentation, lactose and sorbitol fermentation, were noted while the colonies were gram stained, and their motility tested. Biochemical tests were performed on presumptive *E. coli* colonies and other isolates using standard techniques (Cowan, 1993; Cheesbrough, 2006).

Serotyping of *E.coli* Isolates

All non-sorbitol-fermenting *E.coli* colonies were tested for agglutination with O157 latex reagents (Oxoid DR620) to determine if the isolates belonged to the O157 serogroup (Nataro and Kaper, 1998). This was done according to manufacturer's instructions.

Biochemical Diagnosis for *E. coli* O157: H7

Biochemical identification tests were performed on presumptive *E. coli* colonies. For definitive biochemical diagnosis, strains of *E. coli* that agglutinated with O157 latex reagents were further tested for β -glucuronidase activity by inoculating the colonies on Sorbitol MacConkey agar with BCIG (Oxoid CM O981), and incubated at 37^{o}C for 24 hours. The suspect colonies were also tested for cellubiose, dulcitol and raffinose fermentation. *E. coli* strains that appeared pale or colorless and lacked the enzyme β-glucuronidase were presumed to be *E. coli* serotype O157:H7. Strains that agglutinated with latex reagents and were β-glucuronidase negative, and could not ferment cellubiose but fermented dulcitol and raffinose were confirmed as *E. coli* O157: H7 serotype (Thompson *et al.*, 1990).

Data Analysis

Data obtained were analyzed using Chi-square (χ^2) or Fisher's exact test as appropriate and odd ratio analysis using the statistical software INSTAT®. A P-value of less than 0.05 (i.e. $P < 0.05$) was considered to be statistically significant, while P -value more than 0.05 ($P > 0.05$) was considered to be statistically not significant.

Results

Table 1 shows the different types of microorganism isolated from stool specimens of subjects screened in this study. On the whole, nine hundred and sixty (960) stool specimens yielded growth of microbial colonies. Forty (40) specimens yielded no microbial growth. The highest prevalence of bacterial growth was recorded for *E. coli* [316 (32.9%)].

Table 1: Distribution of Microorganisms Isolated from Stool Specimens of Persons in Edo State

(n=960; number showing no growth = 40)

Microorganisms	Number (%) of Isolates
Aeromonas sp	10(1.04)
Coliforms	67 (6.97)
Enterobacter sp	23 (2.39)
Enterococcus sp	64 (6.66)
Escherichia coli	316(32.9)
Klebsiella sp	143(14.8)
Proteus sp	103(10.7)
Pseudomonas sp	56(5.83)
Staphylococcus aureus	35(3.64)
Staphylococcus epidermidis	36(3.75)
Yeast cells	57(5.93)
Mixed bacterial growth	50(5.20)
Total	**960(100)**

Table 2: Contingency Table of Observed Frequency of Infection with *E. coli* O157:H7 and Person's Senatorial District (n= 1000)

Senatorial District	*E. coli* O157:H7 Infection		Total	Chi-Square	P-Value
	No.(%) Infected	Not Infected			
Edo North	0(0.00)	192	192	16.048	0.000
Edo Central	8(1.8)	442	450		
Edo South	19(5.3)	339	358		
Total	**27(2.7)**	**73(97.3)**	**1000**		

$\chi^2 = 16.048$ $df = 2$ $p < 0.05$

Table 3: Contingency Table of Observed Frequencies of Age of Persons and Infection with *E. coli* O157:H7

Age Group (Years)	*E. coli* O157:H7 Infection		Total No. of Persons	Chi-Square	P-Value
	No(%)Infected	Not Infected			
0 – 9	12(4.2)	277	289	11.261	0.128
10 - 19	2(0.8)	256	258		
20 – 29	10(3.4)	280	290		
30 – 39	2(2.1)	95	97		
40 – 49	0(0.0)	51	51		
50 – 59	1(12.5)	7	8		
60 – 69	0(0.0)	6	6		
70 – 79	0(0.0)	1	1		
Total	**27(2.7)**	**973(97.3)**	**1000**		

$\chi^2 = 11.261$ $df = 7$ $p > 0.05$

Table 4: Observed Frequencies of *E. coli* O157:H7 in Relation to Gender Of Persons Screened

Gender	Number Screened	Number (%) Infected	Odd Ratio (OR)	95% Confidence Limit	P-Value
Male	441	9(2 .04)	0.6262	0.2785 to 1.408	0.327
Female	559	18(3.22)			

P > 0.05

Table 5: Contingency Table of Observed Frequencies of Clinical Symptoms and Persons Infected with *E.coli* O157:H7

Clinical Symptoms	*E.coli* O157:H7 Infection		Total	Chi-Square	P-Value
	No (%) of Persons Infected	Not Infected			
Diarrhea	10(25.6)	29	39	1.073	0.000
Abdominal Cramps	5(2.7)	179	184		
Fever	3(1.8)	160	163		
Frequent Stool	3(3.7)	79	82		
Vomiting	2(6.4)	29	31		
Nil	2(0.4)	447	449		
Bloody Stool	1(50)	1	2		
Headache	0(0.0)	28	28		
Others	1(0.0)	21	22		
Total	**27**	**973**	**1000**		

$\chi^2 =$ **1.073** df **= 8** $P <$ **0.05**

The prevalence of infection with *E. coli* O157:H7 among subjects studied within the three senatorial districts of Edo State is shown in Table 2. Out of the 1000 persons screened, 19 (5.3%) from Edo South district showed the highest infection rate, while no infection was found among subjects from Edo North.

In Table 3 the observed frequencies of infection with *E. coli* O157:H7 are compared with the various age groups of patients. A cursory look at the table reveals that 12 (4.2%) persons in the 0-9 age group were infected with the pathogen, while 2 (0.8%) persons within the 10-19 age bracket, 10 (3.4%) in age group 20-29, 2 (2.1%) in age group 30-39 and 1 (12.5%) person whose age is between 50 and 59 were infected with the organism. The percentage distribution of persons infected shows that there is no much difference in the proportion of people living with *E.coli* O157:H7 by person's age.

The observed frequencies of *E. coli* O157: H7 in relation to gender of persons are given in Table 4, which reflects the Fisher's exact test values for the two nominal variables ("sex of persons" and "*E.coli* O157H7 infection") as well as the P-value and Odds ratio and 95% confidence interval. Since the 2-sided P-value (P

= 0.327) is greater than 0.05 cut off, there is not a significant association between the two tested variables.

Table 5 shows observed frequencies of clinical symptoms and persons infected with the pathogen. Here, the significance level (0.000) for the Chi-Square value (1.073) is less than the 0.05 cut off, and hence, there is a significant relationship between the clinical symptoms presented by persons screened and infection with the pathogen.

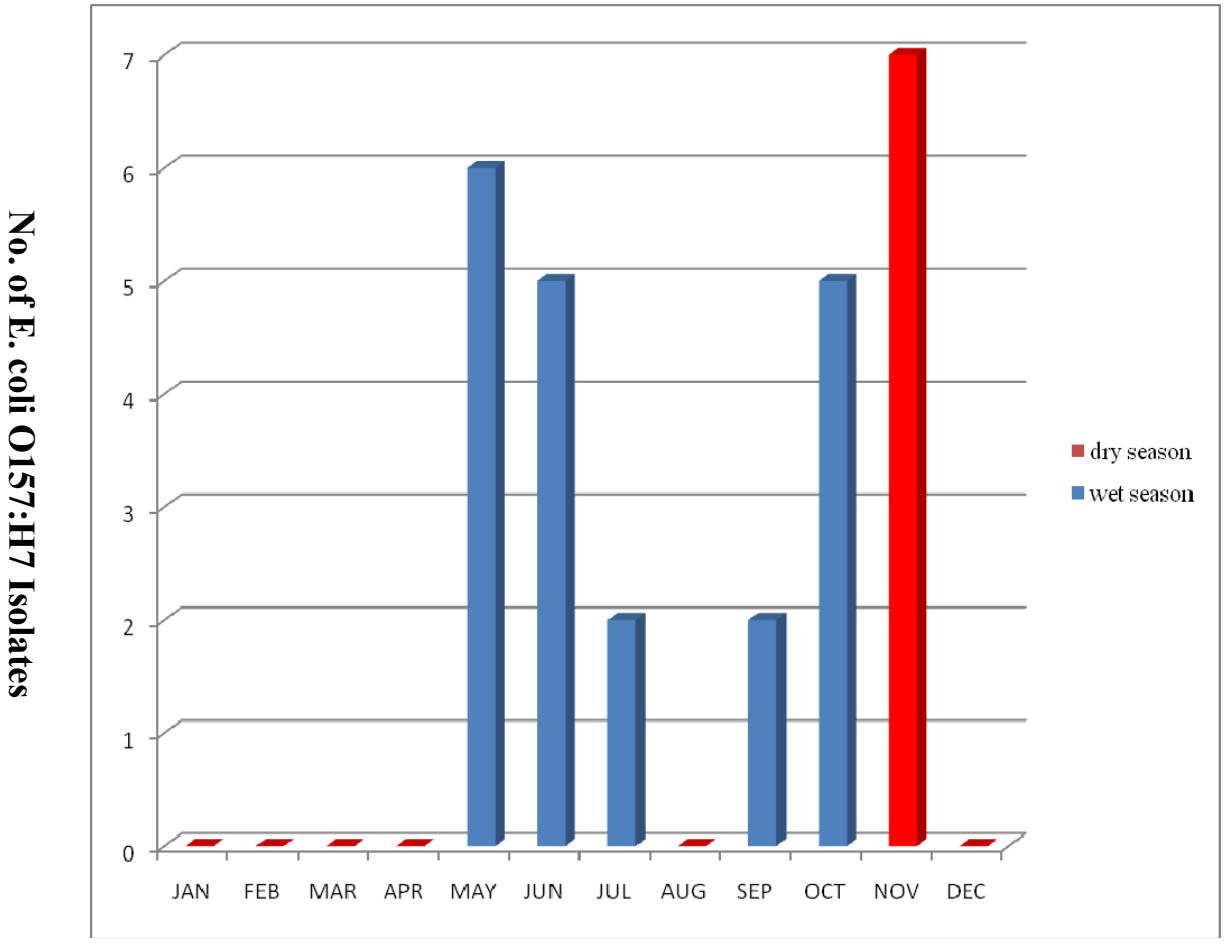

Figure 1: Seasonal Pattern of Isolation of *E. coli* O157:H7.

Figure 1 indicates the seasonal pattern of isolation of *E. coli* O157:H7 from persons screened. The pattern reveals that there were more isolations of *E.coli* O157:H7 during the wet season of the year.

Discussion

The first objective of this study was to determine the presence and prevalence rate of *E. coli* O157:H7 infection among people in Edo State. The study has established the presence of human infection in the state with a prevalence rate of 2.7%. This is the first recorded state-wide investigation of *E.coli* infection due to O157: H7 serotype. Statistical analysis of Table 2 shows that the actual result of the Chi-Square test is 16.048, with a significance level of 0.000 which is less than 0.05 cut-off. This shows that there is a significant relationship between persons' district of residence and the proportion of persons infected with *E.coli* O157:H7. Out of a total of four hundred and fifty (450) persons who reside in the Central district of Edo state, only 8(1.8%) were found infected with the organism. No person was found infected in the Northern district. Majority of persons residing in the cities in this zone (Auchi, Agenebode, Ibillo and Uzairue), still enjoy the benefit of rural settlements and cultural traditions, without the luxury of fast food restaurants which have been implicated in cases of infections.

Traditional modes of cooking food at high temperatures, unfavorable for microbial growth, could be responsible for the zero prevalence of infection. Of the three hundred and fifty-eight (358) persons living in the Southern district, 19(5.3%) were infected, with 16 (84.2%) persons residing in Benin City, while the other 3 persons (15.8%) were food vendors working in Ehor, another local government headquarter within the district. Benin City, the Edo State capital, covers the largest land area among the cities in the South senatorial district, having a large population as well as bustling human and commercial activities. Numerous hotels and fast- food restaurants provide ready- to- eat food to so many residents and travelers within the city. These foods, which may not meet with expected sanitary standards of preparation, for example adequate cooking, may be ready sources of infection with *E.coli* O157:H7.

Enabulele and Uriah (2009) isolated *E. coli* O157:H7 from meat sold at abattoirs and ready-to-eat grilled "Suya" meat (4.29%) sold in Benin City. Yah *et al*. (2009) have also observed a high level of *S. aureus* and *E. coli* contamination of meat-pie sold in Benin City, attributing this to poor hygienic and sanitary practices employed in the processing and packaging of these food products. Also, Wogu *et al.* (2010) recently investigated the microbial load of ready- to- eat rice from both local fast food centers and standard fast food centers within Benin City, and found that most of the ready- to-eat rice samples examined did not meet bacteriological quality standards. Other workers, Oluwafemi and Simisaye (2006) and Omoruyi *et al*., 2011, have respectively reported high contamination rates for ready-to- eat sausages and abattoir meats sold in Benin City. In Jos Nigeria, Ngbede *et al.* (2006) screened one thousand and fifty (1050) fecal samples, and out of eight

hundred and fifty (850) specimens from diarrhoegenic patients, 26 (3.1%) had *E. coli* O157:H7. In an Adamawa State population sampled by Dunah et *al.* (2010), 3.7% infection rate was recorded. These prevalence rates do not differ much from the results of this present study. In studies carried out on patients in Lagos, Nigeria, more than a decade ago, Akinyemi *et al.*, 1998 and Olorunshola *et al.*, 2000 respectively found prevalence rates of 8.4 % and 6.0% EHEC infection. These appear much higher than the 2.7% prevalence rate recorded in this study and this could be due to the more cosmopolitan and populated nature of Lagos environment.

In Table 3 the observed frequencies of infection with *E.coli* O157:H7 were compared with the various age groups of persons screened. Since the significance level of 0.128 for the Chi - Square value of 11.261 is greater than the 0.05 cut off, it can be concluded that *E.coli* O157:H7 infection is independent of person's age. According to Slutsker *et al.,* 1997, age-specific isolation proportions from fecal specimens in adults are not equivalent to population based incidence rates, although their findings suggest that infections were seen more in adults 50 years and above. In another study, however, the age specific annual incidence rate was highest for children younger than 5 years of age (Ostroff *et al.,* 1989). It can be inferred from the result obtained in Table 4 that since the P- value of 0.327 is greater than 0.05 cut off, there is no significant relationship between the sex of persons and their infection with the organism. Su and Brandt (1995) have also reported in their studies that *E.coli* O157:H7 generally affects both sexes equally. Thus it can be deduced that persons' gender does not constitute a risk factor for *E. coli* O157:H7 infection.

Of the one thousand persons enrolled for this study, five hundred and fifty one (551) presented with various symptoms while four hundred and forty nine (449) persons complained of no symptoms of disease. Statistical analysis of Table 5 showed that there was a significant relationship between infection with the pathogen and presence of clinical symptoms. In all, the highest proportion of persons (25.6%) infected with *E.coli* O157:H7 presented with diarrhea. Diarrhea is a predominant cause of childhood illness and death in developing countries. Bryce *et al.* (2005) estimated that between 2000 and 2003, sixteen percent of deaths of children under five in Africa were due to diarrhea.

It has also been estimated that 0.6% to 2.4% of cases of diarrhea are associated with *E. coli* O157:H7 (Cahoon and Thompson, 1987). In Mozambique, diarrheagenic *E. coli* were recovered from 41.8% of children with diarrhea and 18.2% of controls (Rappelli, 2005).

Also, this study has revealed that 2 persons (0.4%), although asymptomatic, had *E.coli* O157:H7 isolated from their stool specimens. According to Okeke *et al*., 2000 and Opintan *et al*. (2010) healthy carriage of enteric pathogens in general and diarrhoegenic *E.coli* in particular is high in many African studies. It is not surprising, therefore, to isolate this organism from apparently healthy individuals, as human infection caused by *E.coli* O157:H7 can present a broad clinical spectrum ranging from asymptomatic cases to death (Lim *et al*., 2010). Because asymptomatic cases can occur in outbreaks, there has been concern that persons with such infections could unwittingly spread infection to others. The existence of such asymptomatic cases during outbreaks has been well documented (Pavia *et al*., 1990; Belongia *et al.*, 1993). Asymptomatic infections have also been demonstrated in family members and other close contacts of persons with hemolytic uremic syndrome or symptomatic *E. coli* O157:H7 (Marsh *et al.*, 1992).

The rate of infection in relation to the seasons of the year is summarized in Figure 1. From the figure it can be seen that the total numbers of seasonal isolation of *E. coli* O157:H7 was highest during the wet seasons of the year i.e. May through October of the four-year period of study. A total of twenty (20) confirmed *E.coli* O157:H7 isolations were made during wet seasons while seven (7) isolations were made during the dry seasons. These findings agree with other studies (Boyce *et al.,* 1995, Chapman, 2000) which show that human infections associated with *E. coli* O157:H7 appear to be more common during the summer months, with the majority of cases occurring during the months of May through September. Ostroff *et al.* (1989) suggested that the increased shedding of *E.coli* O157:H7 by cattle during summer months may contribute to a similar seasonal pattern of *E.coli* O157:H7- associated food borne illness in humans. Thus it can be inferred that most food- borne and water- borne infections are commonest during the wet seasons of the year. Especially in Benin City, insanitary market places, over-flooded drainages and living environment as well as contaminated abattoir-work environment, could serve as sources of several bacterial infections.

Clearly, the data from this present study indicate that certain predisposing factors such as the presence of commercial communities where fast -food and roadside restaurants thrive, diarrhea, the wet seasons of the year and asymptomatic carriage of infection, can conduce to *E.coli* O157:H7 infection. Personal and environmental hygiene and adherence to good food handling practices represent the last line of defense for assuring prevention of *E. coli* O157:H7 infection. Since the infection primarily occurs via fecal-oral route, food hygiene measures like proper cooking of meat, consumption of pasteurized milk, washing fruits and vegetables especially those to be eaten raw and drinking chlorine- treated water could be very useful.

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