

Isolation and Characterisation of *Escherichia coli* O157 in Human Stool Samples from Parts of Kaduna Metropolis Nigeria

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Abstract *Escherichia coli* O157 is pathogenic strain of *Escherichia coli* that is known to cause diarrhoea leading to fluid loss, electrolyte imbalance and other severe complications like haemolytic uraemic syndrome. This work was therefore aimed at isolating and serologically characterising *Escherichia coli* O157 from human stool with the set objectives of identifying the risk factors associated with diarrhoea and determining the serological characteristics of *Escherichia coli* isolates. A total of one hundred and forty four (144) stool samples were collected from patients with age ranging from zero to sixty (0-60) years, statistical analysis of the risk factors showed that only zero to five years age range of the respondents had a significant statistical difference of 0.012 ($P < 0.05$). The presumptive *Escherichia coli* isolates that appeared as green metallic sheen on Eosin Methylene Blue agar were picked and confirmed biochemically as *Escherichia coli* using Microgen biochemical test kit. The confirmed *E. coli* isolates were then cultured on Sorbitol MacConkey Agar and the two isolates that appeared colourless on SMAC were confirmed serologically as *Escherichia coli* O157 using the latex agglutination test kit. Although 1.39% prevalence rate of *Escherichia coli* O157 was obtained it is pertinent to note that, *Escherichia coli* O157 is becoming a public health threat because of the debilitating effects it has on humans and also due to its low infectivity dose. There is therefore, the need for more public awareness to educate our citizens on ways of improving on the unsanitary environment.

Keywords: diarrhoea, risk factors, public health threat, *Escherichia coli* O157, low infectivity dose

Cite This Article: H.O. AbdulAziz, Maryam Aminu, and D. A. Machido, "Isolation and Characterisation of *Escherichia coli* O157 in Human Stool Samples from Parts of Kaduna Metropolis Nigeria." *American Journal of Food Science and Technology*, vol. 4, no. 5 (2016): 125-128. doi: 10.12691/ajfst-4-5-1.

1. Introduction

Enteric pathogens are gastrointestinal organisms known to cause gastrointestinal infection. Gastrointestinal infection also known as gastroenteritis is any infection caused by Viruses, Bacteria or Parasites and is characterised by excessive watery diarrhoea and stomach pain. Acute diarrhoea is a common cause of death in developing countries and the second most common cause of infant deaths worldwide [16]. It is estimated that 1.3 billion episodes of diarrhoea occur in children below five years of age with about 760,000 deaths occurring yearly [18].

Escherichia coli is a common inhabitant of the human and animal gut, but can also be found in the physical environment such as; water, soil and vegetation and are thus referred to as being ubiquitous. Many *Escherichia coli* strains are usually not harmful and act as commensals in the intestine of warm blooded animals, but some few strains have been found to cause mild to severe disease in man. *Escherichia coli* O157:H7 is a pathogenic strain of *Escherichia coli* that is known to cause diarrhoea and other severe complications such as haemolytic colitis, haemolytic uraemic syndrome and thrombotic thrombocytopenic

pupura in humans. The majority of *E. coli* O157:H7 strains can be distinguished from most *E. coli* by their inability to ferment sorbitol rapidly and by their lack of production of glucuronidase [1,5]. They also differ from other *E. coli* because of their ability to produce verocytotoxins (VT) or shiga toxins (ST).

Escherichia coli O157:H7 is a zoonotic food borne and waterborne pathogens with cattle serving as the main reservoir for this organism which they shed in their faeces and is often times used as manure by farmers. Transmission of this organism is usually through faecal oral route and Humans become infected with this pathogen through consumption of faecally contaminated fruits, vegetables and water or through person to person contact and direct contact with infected faeces.

Escherichia coli O157:H7 is responsible for several outbreaks of gastroenteritis around the world and causes approximately 70,000 illnesses and 60 deaths annually in the United States [8]. In developing countries where diarrhoeal disease and associated mortality are much more pervasive there is very limited information about *E. coli* O157:H7 prevalence [9]. The first major outbreak of bloody diarrhoea in the developing world associated with *E. coli* O157:H7 occurred in Swaziland in 1992 [3] and infection with this pathogenic strain may have accounted for tens of thousands of cases during this epidemic. There

have also been reported outbreaks in Democratic Republic of Congo. In Nigeria, some prevalence studies have been reported in some South Western cities [11,12,14].

Due to the low infective dose of *E. coli* O157:H7, the potential severity of the infection and the possibility of laboratory-acquired infections [15], an inoculation of fewer than 10 to 100 colony forming units (cfu) of *E. coli* O157:H7 is sufficient to cause infection, compared to over one-million cfu for other pathogenic *E. coli* strains [4]. Their ability to survive in the environment and the environmental contamination with *Escherichia coli* O157:H7 may be an important public health problem [7,10]. Also another major problem with *E. coli* O157:H7 is that it is not detected by the usual methods used to isolate and identify "traditional" enteric bacterial pathogens therefore, most microbiology laboratories in many countries of Africa do not routinely test for *E. coli* O157:H7, hence many infections may go unrecognized (Wittenberg, 1999). This work therefore, sought to isolate and characterise *Escherichia coli* O157 from human stool in parts of Kaduna Metropolis, Nigeria.

2. Material and Method

2.1. Study Area

Kaduna metropolis is located in Kaduna state Northern Nigeria, and with a global location of between longitude 30° east of Greenwich meridian and latitude 0900 and 11 30° North of the equator. Kaduna Metropolis is made up of 3 Local Government Areas namely Igabi, Kaduna South and Chikun. Therefore, samples were collected from 5 hospitals located within these Local governments.

2.2. Sampling

A total of 144 stool samples were collected from 5 different hospitals located within Kaduna state which are; Yusuf Dantsoho Memorial hospital Tudun wada, Gwamna Awan General Hospital Kakuri, Kawo General Hospital Kawo, Ungwar rimi primary health clinic Ungwar rimi and Shehu Idris School of Health Technology practice area Tudun Wada.

2.3. Isolation of and Identification of *E. coli* Isolates

The stool samples were cultured on Eosin Methylene Blue (EMB) agar and the colonies that appeared as green with black metallic sheen were pick and sub cultured on fresh EMB agar plates to obtain presumptive *E. coli* isolates. These presumptive *E. coli* isolates were subjected to the conventional biochemical test for *E. coli* (IMViC), isolates that had typical *E. coli* characteristics on IMViC test were then further confirmed by biochemically characterizing them using the MicrogenGnA+B-ID System test kit.

2.4. Isolation of *E. coli* O157

The confirmed *E. coli* isolates were cultured on SorbitalMacConkey Agar plates (SMAC), the colonies that appeared colourless on SMAC were tagged as presumptive *E. coli* O157 and were Serologically

confirmed as *E. coli* O157 using the latex agglutination test kit (Oxoid, Basingstoke, and Hampshire, England).

3. Results

A total of 17 (11.80%) isolates were biochemically confirmed as *Escherichia coli* isolates out of the 144 stool samples collected. Yusuf Dantsoho Memorial Hospital had the highest number of isolates while Kawo General Hospital had the lowest number of isolates. This result is presented on Table 1.

Table 1. Frequency of Occurrence of *E. coli* in Stool Samples Collected from 5 Hospitals in Kaduna Metropolis

Hospital	No. of Samples Analysed	No. Positive for <i>E. coli</i>	Prevalence (%)
YDMH	33	8	24.24
SISHT	30	4	13.33
GAGH	33	3	9.10
KGH	25	2	8.00
URPHC	23	1	4.35
TOTAL	144	18	12.50

KEY: YDMH = Yusuf Dantsoho

SISHT = Shehu Idris School Health Technology

GAGH = Gwamna Awan General Hospital

KGH = Kawo General Hospital

URPHC= Ungwar Rimi Primary Health Care Clinic.

Analysis of the risk factors associated with diarrhoea was carried out using the Fischer Exact Test and the result shows that only age of the respondents had a statistical significant difference of $p < 0.05$ ($p = 0.012$). the other risk factors that were analysed had p values greater than 0.05 which means they had no statistical significant difference. This result is highlighted on Table 2.

Table 2. Risk Factors Associated with Diarrhoea

Variables	No of Samples Analysed	No. Positive (%) For <i>E. coli</i>	P-Value
Age			
0-5	132	13 (9.85)	0.012
6-10	4	1 (25.0)	
11-15	2	1 (50.0)	
16-20	1	0 (0.0)	
21-25	3	1 (33.3)	
26-30	0	0 (0.0)	
31-35	1	1 (100)	
36-40	1	1 (100)	
Gender			
Male	64	6 (9)	0.448
Female	80	12 (15)	
Source of water			
Water board	62	7 (11.3)	0.691
Borehole	13	1 (7.7)	
Bottled water	20	3 (15.0)	
Well water	16	4 (25.0)	
Sachet water	31	3 (9.7)	
Breast milk	2	0 (0.0)	
Water treatment			
Yes	15	1 (6.7)	0.694
No	129	17 (10.9)	
Contact with diarrhoeic individual			
Yes	18	3 (16.7)	0.701
No	126	15 (11.9)	
Consumption of raw vegetables			
Yes	34	5 (14.7)	0.767
No	110	13 (11.8)	
Contact with animals			
Yes	31	5 (16.1)	0.541
No	113	13 (11.5)	
Total	144	18 (12.5)	

Serological characterisation was carried out and the result shows that only 2 isolates were positive for *E. coli* O157 and these 2 isolates were obtained from Yusuf

Dantsoho Memorial Hospital with a prevalence of 6.1%. These results are presented on Table 3 and Table 4.

Table 3. Identification of *Escherichia coli* O157:H7

Isolates	Growth on SMAC	Test Latex	Control Latex	Interpretation
Y26	Colourless colony	+	-	<i>E. coli</i> O157 Present
Y15	Colourless colony	+	-	<i>E. coli</i> O157 Present

Key: SMAC =Sorbitol MacConkey agar

- = No agglutination

+ = Agglutination

Y26, Y15=Stool samples collected from Yusuf Dantsoho Memorial Hospital (YDMH).

Table 4. Frequency of Occurrence of *E. coli* O157 in Stool Collected From Five Hospitals in Kaduna

Hospital	No. Of Samples Analysed	No. Positive for <i>E. coli</i> O157	%Frequency of <i>E. coli</i> O157
YDMH	33	2	6.1
SISHT	30	0	
GAGH	33	0	
KGH	25	0	
URPHC	23	0	
Total	144	2	1.39

4. Discussion

The findings in this study indicates that age remains a major risk factor in diarrhoea disease, children between the ages of 0-5 are highly vulnerable to diarrhoea as this study has shown. The prevalence of 9.8% diarrhoea in respondents 0-5 years in this work, is higher than the 2.6% obtained by Yilgwan and Okolo, [17] in Jos Plateau State and lower than the 43.1% obtained by Ifeanyi *et al.* [6] in Abuja. These differences might be due to breaches in sanitation and hygiene infrastructure of the respondents from these cities. The high occurrence rate of diarrhoea among children 0-5 years in this study may be due to the fact that children within this age group on their own cannot differentiate between what to eat and what not to eat; they have not learnt the rudiment of adherence to aseptic or hygienic practices. Another reason for their high vulnerability to diarrhoea may be due to weaker immunity as a result of them having lost their inborn immunity after being weaned from breast milk. Young children use more water over the course of a day given their higher metabolic rates, also their kidneys are less able to conserve water compared to older children and adults as such diarrhoea is usually prevalent and often life threatening too. In this study, it was observed that the number of diarrhoeic stool gotten from adults was quite small compared to that obtained from children and this might not be unrelated to the fact that, adults in the locality rarely visit health institutions when they have diarrhoea unless they perceive the diarrhoea as being serious, usually if blood is present as reported by Okeke *et al.* [13].

The 1.39% prevalence rate of *E. coli*O157in this study is lower than the 6% prevalence by Olorunshola *et al.* [14] in Lagos and the 20% prevalence recorded by Esumeh *et al.* [2] in Benin. Although there are differences in prevalence rate of *Escherichia coli* O157 in the stool samples in different parts of Nigeria, this result however shows that *Escherichia coli* O157 remains an aetiological agent for diarrhoea in Nigeria. The presence of *Escherichia coli* O157 in stool samples might not be unconnected to the fact that patients have been exposed to

unsanitary conditions such as consumption of contaminated water, food, fruits and vegetables.

5. Conclusion

This study has established that diarrhoea is higher among younger children than adults and also confirms the fact that *Escherichia coli* O157 even though is not part of the routine tests carried out for enteric pathogens in most laboratories visited is still an important aetiology for diarrhoea. It is pertinent to note that an exceptionally low dose of this organism is able to cause infection and once introduced into a closed group or family, it can spread by person-to-person transmission especially by children who are not toilet trained.

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