Esherichia coli O157:H7 Serotypes Isolation from Children in Stool Samples

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Escherichia coli **serotype O157:H7 is a recently recognized human pathogen associated with hemorrhagic colitis. This work was therefore aimed at isolating** *Esherichia coli* **O157 from human stool. A total of 100 stool samples were collected from patients with age ranging from (0-05) 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** *Esherichia coli* **isolates that appeared as pink flat lactose fermenting on MacConkey Agar and green metallic sheen on Eosin Methylene Blue agar were picked and confirmed biochemically as** *Esherichia coli* **using biochemical test kit. The confirmed** *E. coli* **isolates were then cultured on Sorbitol MacConkey Agar which shows non sorbitol fermenting. Detection of** *E. coli* **O157:H7 on SMAC medium had a sensitivity of 100%, a specificity of 85%, and an accuracy of 86%. SMAC medium stool culture is a simple, inexpensive, rapid, and reliable means of detecting** *E. coli* **O157:H7. Although 1.39% prevalence rate of** *Esherichia coli* **O157 was obtained it is pertinent to note that,** *Esherichia 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: H7, SMAC Medium, Low infectivity dose.

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 second most common cause of infant deaths worldwide and is a common cause of mortality in developing countries[1]. 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[2].

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. Pathogenic strain of *Escherichia coli that is Escherichia coli* O157:H7 is responsible to cause diarrhoea and other severe complications such as haemolytic colitis, haemolytic uraemic

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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[3-4]. 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 causes approximately 70,000 illnesses and 60 deaths annually in the United States and is a cause of several outbreaks of gastroenteritis around the world[5]. In developing countries where diarrhoeal disease and associated mortality are much more pervasive there is very limited information about *E. coli* 0157:H7 prevalence[6]. The first major outbreak of bloody diarrhoea in the developing world associated with *E. coli* 0157:H7 occurred in Swaziland in 1992[7] and infection with this pathogenic strain may have accounted for tens of thousands of cases during this epidemic[8-10]

Due to the low infective dose of *E. coli* O157:H7, the potential severity of the infection and the possibility of laboratory-acquired infections[11], 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[12]. Their ability to survive in the environment and the environmental contamination with *Escherichia coli* O157:H7 may be an important public health problem[13-14]. 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[15] This work therefore, sought to isolate and characterise *Escherichia coli* O157 from human stool.

MATERIAL AND METHOD

Study Area: Rama Medical College Hospital, Kanpur.
Sampling: A total of 100 stool samples were collected from Rama Medical College Hospital, Kanpur.
Isolation of and Identification of *E. coli* Isolates

The colony appeared as green with black metallic sheen of the stool samples which were cultured on Eosin Methylene Blue agar , and again were picked and sub cultured on fresh EMB agar plates to obtain presumptive *E. coli* isolates. These presumptive *E. coli* isolates were confirmed by the conventional biochemical test for *E. coli* (IMViC)
Isolation of *E. coli* O157:H7

The confirmed *E. coli* isolates were cultured on Sorbital MacConkey Agar plates (SMAC), the colonies that appeared colourless on SMAC were tagged as presumptive *E. coli* O157.
Ethical consideration

The study was ethically approved by the institutional ethics review board of Rama Medical College, Kanpur. Written consent was obtained from parents/guardians of the children before enrolment into the study.

RESULTS

In 100 fecal samples, *E. coli* O157:H7 was isolated and collected in Rama Medical College Hospital, Kanpur which is summarized in Table 1. The *E. coli* O157:H7 appears as sorbitol-nonfermented colonies on MacConkey agar (white-gray) as shown in Figure 1. The confirmed *E. coli*

Table 1. Occurrence of *E. coli* in Stool Samples

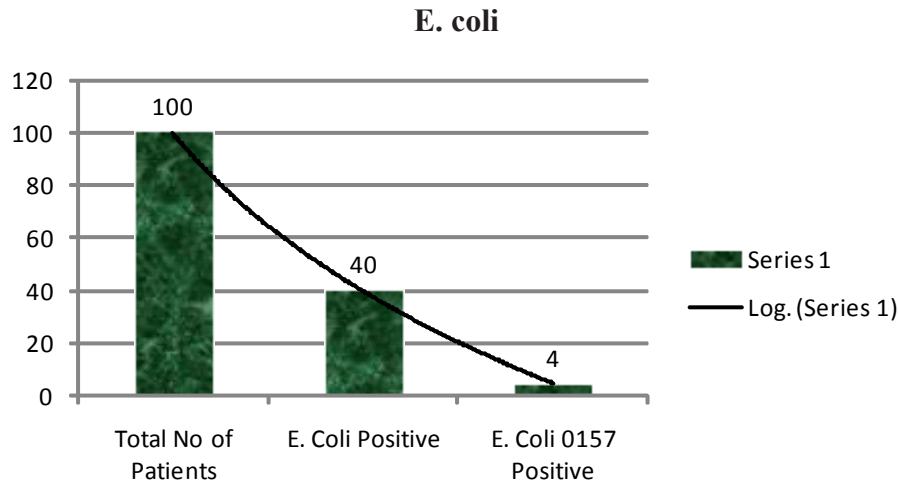| S.NO	Total No of Patients	*E. coli* Positive	*E. coli* 0157 Positive	p-value
1	100	40	4	0.012

E. coli

Fig. 1. Show the frequency Occurrence of *E. coli* in Stool Samples

isolates were cultured on Sorbital MacConkey Agar plates (SMAC), the colonies that appeared colourless on SMAC were tagged as presumptive *E. coli* O157.

Analysis of the risk factors associated with diarrhea 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 analyzed had p values greater than 0.05 which means they had no statistical significant difference.

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.*[18] 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.*[19].

The 1.39% prevalence rate of *E. coli* O157 in this study is lower than the 6% prevalence by Olorunshola *et al.*[10] in Lagos and the 20% prevalence recorded by Esumeh *et al.*[16] in Benin. *Escherichia coli* O157 remains an aetiological agent for diarrhoea in Nigeria, 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 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.[4]

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 are 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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