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

Madhu Yadav1*, Ambika Bhatiani2, Ajay Bhagoliwal3, Anil Kumar4 and R. Sujatha2

1Department of Microbiology, G. S.V. M. Medical College, Kanpur, India.
2Department of Microbiology, Rama Medical College, Kanpur, India.
3Department of Community Medicine, Rama Medical College, Kanpur, India.
4Department of Biotechnology, Rama Medical College, Kanpur, India.

http://dx.doi.org/10.22207/JPAM.12.1.07

(Received: 10 February 2018; accepted: 20 March 2018)

Escherichia coli 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 countries1. It is estimated that 1.3 billion episodes of diarrhoea occur in children below five years of age with about 760,000 deaths occurring yearly2.

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
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. 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. In developing countries where diarrhoeal disease and associated mortality are much more pervasive there is very limited information about E. coli O157:H7 prevalence. The first major outbreak of bloody diarrhoea in the developing world associated with E. coli O157:H7 occurred in Swaziland in 1992 and infection with this pathogenic strain may have accounted for tens of thousands of cases during this epidemic. Due to the low infective dose of E. coli O157:H7, the potential severity of the infection and the possibility of laboratory-acquired infections, 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. Their ability to survive in the environment and the environmental contamination with Escherichia coli O157:H7 may be an important public health problem.

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. 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

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

Table 1. Occurrence of E. coli in Stool Samples

J PURE APPL MICROBIO, 12(1), MARCH 2018.
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,\(^1\) in Jos Plateau State and lower than the 43.1% obtained by Ifeanyi et al.\(^2\) 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.\(^3\).

The 1.39% prevalence rate of *E. coli* O157 in this study is lower than the 6% prevalence by Olorunshola et al.\(^4\) in Lagos and the 20% prevalence recorded by Esumeh et al.\(^5\) 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.\(^6\)
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.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the expertise of staff of the Department of Microbiology, Rama Medical College, Kanpur for their immense cooperation and assistance in the collection of specimen and processing.

REFERENCES

1. Victoria, C. G., Bryce, J., Fontaine, O., Monasch, R. Reducing deaths from Diarrhea through oral rehydration therapy. *Bulletin World Health Organization*, 2008; 73(10), 1246-1255.

2. World Health Organisation. 2013. Diarrhea Disease Fact sheet http://www.who.int/mediacentre/factsheets/fs330/en/.

3. Doyle, M.P. and Schoeni, J.L. Survival and Growth Characteristics of *Escherichia coli* O157:H7 Associated with Haemorrhagic Colitis. *Applied and Environmental Microbiology*, 1984; 48(4):855-856.

4. Hayes, P.S., Blom, K., Feng, P., Lewis, J., Stockbline, N.A., and Swaminathan, B. Isolation and Characterization of a Glucuronidase-Producing Strain of *Escherichia coli* Serotype O157:H7 in the United States. *Journal of Clinical Microbiology*, 1995; 33(12):3347-3348.

5. Mead, P.S., Slutsker, L., and Dietz, V. Food Related Illness and Death in The United States. *Emerging Infectious Disease*, 1999; 5(5): 607-625.

6. Nougang, M.E., Nola, M., Bessa, H.A., Kweyang, B.T.P., Olive, V., Ewoti, N. and Moungang, L.M. Prevalence of Pathogenic Strains of *Escherichia coli* in Urban Streams in The Equatorial Region of Cameroon, Central Africa. *Journal of Applied Biosciences*, 2011; 48: 3293-3305.

7. Effler, E.M., Isaacson, L., Arntzen, R., Heenan, P., Canter, T., Barrett, and Griffin, P.M. *Emerging Infectious Diseases*, 2001; 7: 812-819.

8. Ogunsanya, T.L, Rotimi, V.O. and Adenuga, A. Study of the Aetiological Agents of Childhood Diarrhoea in Lagos, Nigeria. *Journal of Medical Microbiology*, 1994; 40: 10-14.

9. Okeke, I.N., Lamikanra, A.H., Steinruck, and Kaper, J.B. Characterisation of *Escherichia coli* Strains from Cases of Childhood Diarrhoea in Provincial South Western Nigeria. *Journal of Clinical Microbiology*, 2000; 38(1):7-12.

10. Okeke, I.N., Ojo, O., Lamikanra, A., and Kaper, J.B. Aetiology of Acute Diarrhoea in Adults in Southwestern Nigeria. *Journal of Clinical Microbiology*, 2003; 41(10):4525-4530.

11. Pimbley D.W. Verotoxigenic *Escherichia coli* Detection by Commercial Enzyme Immunoassay. In, Encyclopedia of Food Microbiology. Academic press, London, 1999; 3: 2921-2231.

12. Greig, J.D., Todd, E.C.D., Bartleson, C. and Michaels, B. “Infective Doses and Pathen Carriage”, pp. 19-20, USDA2010 Food Safety Education Conference 2010.

13. Kudva, I.T., Blanch, K. and Hovde, C.J. Analysis of *Escherichia coli* O157:H7 Survival in Ovine or Bovine Manure and Manure Slurry. *Applied Environmental Microbiology*, 1998; 64(3): 3167-3174.

14. O’Brien, S.J., Adak, G.K. and Gilham, C. Contact with Farming Environment as a Major Risk Factor for Shiga Toxin (Verocytotoxin)-Producing *Escherichia coli* O157 Infection in Humans. *Emerging Infectious Disease*, 2001; 7:1049-1051.

15. Wittenberg, D.F. Emerging and re-emerging diseases—epidemic enterohaemorrhagic infections 100 years after Shiga. *South African Medical Journal*, 1999; 89: 750-752.

16. Esuneh, F.I, Isibor, J.O., Egbaibe, I.D.S. Screening For *Escherichia coli* O157:H7 In Diarrheic Patients InBenin City, Nigeria. *Journal of Microbiology and Biotechnology Research*, 2011; 1(4): 1-4.

17. Yilgwan, C.S. and Okolo, S.N. Prevalence of Diarrhoea Disease and Risk Factors in Jos University Teaching Hospital, Nigeria. *Annals of African Medicine*; 2012; 11(4):217-221.

18. Ifeanyi, C.I.C., Isu R. N., Akpa, A.C. and Ikeneche N.F. Enteric Bacteria Pathogens Associated With Diarrhoea of Children in the Federal Capital Territory Abuja, Nigeria. *New York Science Journal*, 2010; 3(1): 62-69.

19. Okeke, I.N., Ojo, O., Lamikanra, A., and Kaper, J.B. Aetiology of Acute Diarrhoea in Adults in Southwestern Nigeria. *Journal of Clinical Microbiology*, 2003; 41(10):4525-4530.