

ISOLATION OF POTENTIALLY PATHOGENIC *ESCHERICHIA COLI* O157:H7 FROM THE WATER SOURCES

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ABSTRACT

Water a universal solvent and a basic requirement for all, if becomes prone to contaminants and unfit for use, would cause hazardous health effects on humans and animals. Most of the diseases outbreaks are reported due to exposure to contaminated water. Both solid waste and chemical mixture prove contaminant to water. Many drinking water and recreational water sources are reported nowadays with contamination of a particular strain of *E.coli* known as *E.coli* O157:H7. Cattle or humans with O157:H7 infection can contaminate streams, lakes, irrigation ditches or swimming pools with faeces. We conducted a preliminary study to know the frequency of this pathogenic strain and studied characteristics in drinking water sources, collected from different areas in Namakkal district.

KEYWORDS

Water sample, *E.coli* , *E.coli* O157:H7, pond water, rocky water.

INTRODUCTION

E.coli is usually considered as an indicator organism for faecal contamination and is an important parameter in food and water hygiene. These organisms transmitted by direct contact or through contaminated food and water. While generic *E.coli* is considered as an intestinal pathogen, many strains of these species can be pathogenic leading to diarrhoeal diseases. Many drinking water and recreational water sources are reported nowadays with contamination of a particular strain of *E.coli* known as *E.coli* O157:H7. Cattle or humans with O157:H7 infection can contaminate streams, lakes, irrigation ditches or swimming pools with faeces.

Enterohemorrhagic *Escherichia coli* O157:H7 was identified in 1982 as an important

human pathogen causing hemorrhagic colitis and hemolytic uremic syndrome (HUS) and has been reported with increased frequency during the past decade as a cause of human illness^{1,2,3}. The number of serotypes of verotoxin-producing *E. coli* causing human disease is increasing, but *E. coli* O157:H7 continues to be the dominant cause of hemorrhagic colitis and HUS⁴.

Escherichia coli O157, which is a strain of the enterohemorrhagic *E. coli* group, is recognized as an organism whose presence in any food material can lead to serious disease outbreak⁵. The growth of this strain in the human intestine is known to produce large quantity of toxins, which can cause severe damage to the lining of the intestine and other organs of the body⁶. These toxins are very similar to the toxins produced by *Shigella dysenteriae*^{7,8}. The organism is particularly

associated with the development of hemolytic uremic syndrome, known to result in a mortality rate of 2 - 10%⁹. The potentially high mortality associated with *E. coli* 0157 infection, therefore make its presence in any food material worrisome and of serious public health concern as most of the outbreaks recorded has been traced to consumption of beef contaminated with the *E. coli* 0157:H7 strain and water^{10,11}.

Escherichia coli 0157:H7 is an emerging cause of food-borne illness, with over 20,000 cases of infection occurring each year in the United States alone. In humans infections with this serotype may cause bloody diarrhea, and in children infections with this serotype may lead to hemolytic-uremic syndrome². Illness is often linked to the consumption of contaminated and undercooked ground beef and unpasteurized fruit juices, but transmission by other means such as person-to person transmission in child care centers and in families and by swimming in feces-contaminated water is also possible¹². *E. coli* 0157:H7 is 1 of over 200 serotypes that are recovered from humans and that produce Shiga-like toxins, i.e., are verocytotoxigenic *E. coli* (VTEC); over 50 of these VTEC serotypes produce bloody diarrhea or hemolytic-uremic syndrome in humans and are thus classified as enterohemorrhagic *E. coli*⁶.

E. coli 0157:H7 does not ferment sorbitol, and this fact is used in its isolation on sorbitol-containing bacteriological media. Many non-0157:H7 VTEC strains of bovine and other origins have been isolated from humans and have been associated with disease but are not sought in human clinical microbiology laboratories, which generally screen only for 0157:H7; these non-0157:H7 VTEC strains ferment sorbitol, and there are no convenient culture means to screen for them. The significance of non-0157:H7 VTEC strains in human disease is of research interest in a number of laboratories^{13,14,15}.

0157:H7 was first associated with disease outbreaks in the United States in 1982. The virulence properties and genetic diversity of 0157:H7 isolates have been widely studied in the United States and other developed countries¹⁶.

Far less is known about 0157:H7 prevalence in developing countries, where diarrheal disease and associated mortality are much more pervasive. The first major outbreak of bloody diarrhea in the developing world associated with 0157:H7 occurred in Swaziland in 1992¹⁷. 0157:H7 infection may have accounted for tens of thousands of cases during this epidemic.

In India, the status of STEC and 0157:H7 prevalence and contribution to disease is uncertain¹⁸. In 2002, researchers in Calcutta, India, reported finding non-0157:H7 STEC isolates in 1.4% of stool samples from humans suffering from bloody diarrhea¹⁹. They concluded that STEC was not an important cause of diarrhea in India.

However, the sensitivity and specificity of the methods used to isolate *E. coli* 0157:H7 are continually improving, and hence the rates of finding *E. coli* 0157:H7 will increase. This study was aimed at determining the prevalence of *E. coli* 0157:H7 in drinking water sources, collected from different areas in Namakkal district.

MATERIALS AND METHODS

Collection of Samples

Five samples (Sample 1- Cauvery water, Sample 2- Water from rocky area, Sample 3- Pond water, Sample 4- Well Water, Sample 5- Tap water) were collected in properly washed and sterilized bottles, directly from the source, leaving an air space at the top, contamination from other sources have to be avoided. The top can be allowed to run for five minutes before collection. Samples are stored below 30°C for maximum time of 6 hours. All the samples were screened initially for *E. coli* from which all positive isolates were further screened for *E. coli* 0157:H7.

Isolation and Identification of *E. coli* isolates

Each of the water samples of 1 ml in 9 ml buffered peptone water. Serial dilutions of up to 10⁻⁷ were then made and 1 ml of each was plated on Eosin methylene blue (EMB)

agar. They were then incubated at 37°C for 24 hours. Pure cultures of all colonies exhibiting typical dark to purple red colonies with metallic sheen which is characteristic of *E. coli* on EMB were then made ready for biochemical tests²⁰.

Identification of *E. coli* 0157:H7

Pure cultures of all positive *E. coli* were cultured on Cefixim Tellurite Sorbitol-MacConkey (CT-SMAC) agar using the recommended method²¹ and incubated at 37°C for 18 - 24 h. Suspected colonies of *E. coli* 0157:H7 were confirmed using slide agglutination test with *E. coli* 0157: H7 antiserum.

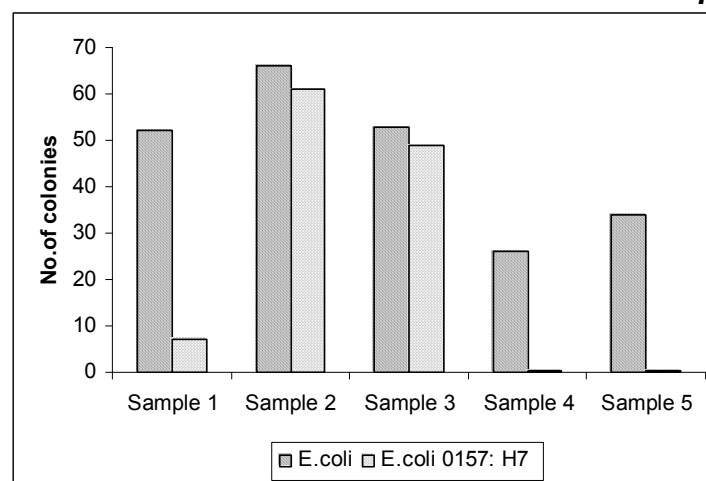
RESULTS AND DISCUSSION

E. coli, which are normal flora of the human and animal intestine, have been identified as a leading cause of water borne illness all over the world. *E. coli*²² and the *E. coli* 0157: H7 strain¹⁰ has previously been isolated from meat samples and has also been implicated in the contamination of vegetables such as lettuce¹¹.

A preliminary study was conducted to observe the frequency of *E. coli* 0157: H7 strain as a precautionary measure to prevent sudden outbreak. Humans in the age group of 1- 4 were the main victims of this particular strain. The study was conducted on various water samples which showed the occurrence of this particular strain. The number of *E. coli* colonies from the stagnant water on the rocks was found to be higher than the pond and river Cauvery respectively. The water sample from well and tap water showed a lesser occurrence of *E. coli*.

On examination of the *E. coli* colonies for the particular strain *E. coli* 0157: H7, showed positive identification for most of the *E. coli* colonies in pond and rocky water samples compared to the other water samples. This particular strain was found to be higher in stagnant water on the rocks (Figure 1). This result is consistent with the work of Suhalim *et al.* (2008) who showed high prevalence rate of *E. coli* 0157: H7 in pond water sample.

Figure 1
***E.coli* and *E.coli* 0157:H7 in different water sample.**



Sample 1- Cauvery water
Sample 2- Water from rocky area
Sample 3- Pond water
Sample 4- Well Water
Sample 5- Tap water

We have billions of *E.coli* bacteria in our bodies. Most strains of these bacteria are completely, harmless and many are beneficial, as they can

supply us with vitamins essential for our bodies to function some strains however are less benign. The probability that all *E.coli* colonies

giving positive identification could be by transfer of a large 'R' plasmid from each isolate in to plasmid less *E.coli* recipient strain. The R plasmids from *E.coli* O157: H7 strain was shown to encode for resistance to ampicillin, streptomycin and for the production of colicine. These results demonstrate that the virulence of these verocytotoxigenic isolates. Studies conducted have indicated higher frequency of water borne antibiotics resistance microorganism in nature, which is a serious threat to chemotherapy.

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CONCLUSION

Conclusively it is to be noted that with the low level of sanitary practices observed and lack of adequate data on infections. Consequent upon this, it is recommended that consumers take boiled water properly before they are consumed. This study reveals the organisms found in water samples are multiplying at a rapid rate as well as connecting non-pathogenic *E.coli* into pathogenic strain by conjugation. Large scale screening and prevention steps could be under taken to prevent serious outbreaks in future.

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