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Research Article

PREVALANCE OF SELECTED SEROTYPE OF ENTEROBACTERIACEAE PATHOGENS FROM SEWAGE AND DRINKING WATER ENVIRONMENT

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ABSTRACT

Enterobacteriaceae are a large family of bacteria that include pathogens such as *Salmonella and Escherichia coli.* They are facultative anaerobes which ferment sugar to produce lactic acid and are found in water and soil. E.coli of serotype O157:H7 causes food poisoning in humans and serious illness. It has the ability to transfer DNA via conjugation, transformation and allow genetic material to spread in existing population. Virulent strains of E.coli cause gastroenteritis, urinary tract infection and neonatal meningitis. Examples include Entero toxigenic *E.coli* which use fimbrial adhesins to bind to enterocyte cell in small intestine and produce LT and ST enterotoxin. On the other hand Entero pathogenic E.coli which lack fimbriae use adhesin to bind to intestinal cells which cause significant deformation. Entero invasive E.coli in humans use adhesin protein to bind and enter intestinal cells but do not produce toxin but damage cell. The present study is aimed at studying the Serotype of Enterobacteriaceae pathogens isolated from sewage and drinking water environments.

INTRODUCTION

E. coli is a type of fecal coliform bacteria commonly found in the intestines of animals and humans (Acheson *et.al.,* 1998). The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination (Ahmed *et.al.,* 2000). Sewage may contain many types of disease-causing organisms. Fecal coliforms are bacteria that are associated with human or animal waste (Bach *et.al.,* 2002). Presence of fecal coliforms in water may not be directly harmful, and does not necessarily indicate the presence of feces, however it does indicate an increased likelihood of harmful pathogens in the water (Beutin *et.al.,* 1993). *Escherichia coli* (abbreviated as *E. coli*) are bacteria found in the environment, foods, and intestines of people and animals. *E. coli* are a large and diverse group of bacteria (Chalmers *et.al.,* 2000). Although most strains of *E. coli* are harmless, others can make you sick (Gascon *et.al.*, 1998). Some kinds of *E. coli* can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses (Ingledew, W.J. and Poole, R.K. 1984).

E. coli O157:H7 is most commonly found on a small number of cattle farms where the bacteria can live in the intestines of healthy cattle (Meadand, P.S. and Griffin, P.M. (1998).

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Millions of germs can be released in a bowel movement from an infected human or animal. *E. coli* may also be found in water sources, such as private wells, that have been contaminated with feces from infected humans or animals (Muniesa *et.al.,* 2006). Waste can enter the water through different ways, including sewage overflows, sewage systems that are not working properly, polluted storm water runoff, and agricultural runoff (Rahn *et.al.,* 1998). Wells may be more vulnerable to such contamination after flooding, particularly if the wells are shallow, have been dug or bored, or have been submerged by floodwater for long periods of time (Tarr *et. al.,* 2002). Most strains are harmless and live in the intestines of healthy humans and animals. However, this strain, O157:H7, produces a powerful toxin that can cause severe illness (Varma *et.al.,* 2003). The present study is aimed at studying the Serotype of Enterobacteriaceae pathogens isolated from such sewage and drinking water environments.

MATERIALS AND METHODOLOGY

By plate count method 1 ml of the sample was prepared and transferred to 9 ml of saline and was maintained as master dilution. From this (10^{-1} to 10^{-6}) dilutions were prepared and 1 ml of sample was poured to cool sterilized agar count plate and incubated at 37°C for 24 hours. Colony was counted by colony counter. Morphological study was achieved by microscopic observation of Grams staining, Motility test, Catalase test and Oxidase test. A small portion of suspected colony was streaked on medias such as Nutrient Agar, MacConkey Agar and Eosin

Methylene Blue Agar. Biochemical tests were performed using Standard Protocol. Following this serological typing was done. Depression plates were taken and were marked as A, B and C. In A depression plate it was marked as negative control in which phenolized saline suspension was added. In B depression plate it was marked as test in which phenolized saline suspension and antiserum of respective organism was added and in C depression plate it was marked as positive control which contain phenolized saline suspension of known organism and antiserum.

RESULTS AND DISCUSSION

The total number of positive and negative samples obtained from sewage and drinking water for *E.coli* was found to be 30. In identification of bacterial isolate of morphological characteristics by Grams staining and motility for *E.coli,* it was found to be Gram negative small rods and motile. Cultural characteristics of *E.coli* on Nutrient Agar formed small colonies whereas on MacConkey Agar formed pink colour non mucoid colonies and on Blood Agar Non haemolytic colonies. Biochemical Test, Antibiotic Sensitivity Test and Serotype study results indicate the prevalence of *E. coli* to be dominant in the Sewage and Drinking water samples collected (Tables 1 – 4 and Figures 1 - 5).

Table 1 Biochemical Tests for *E.coli*

S.No.	Biochemical Test	Result
1.	Catalase	Positive
2.	Oxidase	Negative
3.	Triple Sugar	Acid
4.	Iron Agar	Positive
5.	Gas	Positive
6.	Hydrogen Sulphate	Positive
7.	Indole	Positive
8.	Methyl Red	Positive
9.	Voges Proskauer	Negative
10.	Citrate	Negative

Table 2 Antibiotic Sensitivity Test for *E.coli*

S.No.	Antibiotics	Zone of Inhibition (mm)
1.	Amikacin	18
2.	Chloramphenicol	22
3.	Co-trimoxazole	22
4.	Tetracycline	13
5.	Gentamycin	17
6.	Ceftriaxone	30
7.	Cephotaxime	19
8.	Norfloxacin	24
9.	Meropenem	10
10.	Imipenem	25

Zone of inhibition

Below 10mm – least active
Between 11-25mm – active
Above 26mm – very active

Table 3 Serotypying of E.coli from Sewage Sample

S. No.	Antiserum	Isolates	Result
1.	O157:H7	1,2,3,6,7,8,10,12,13,14,16,17,20,24,25,26,27,28	Positive
		4,5,9,11,15,18,19,21,23,29,30	Negative
2.	O104:H21	4,5,9,11,15,18,19,21,23,29,30	Positive
		1,2,3,6,7,8,10,12,13,14,16,17,20,22,24,25,26,27,28	Negative

Table 4 Serotypying of E.coli from Drinking Water Sample

S. No.	Antiserum	Isolates	Result
1.	O157:H7	1,2,3,4,5,6,7	positive
		6,7	negative
2.	O104:H21	6,7	positive
		1,2,3,4,5	negative

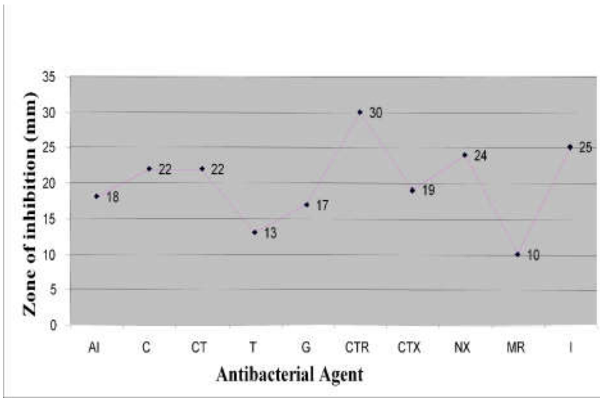


Figure 1 Antibacterial activity of *E.coli*

AI - Amikacin, C - Chloramphenicol, CT - Co-trimoxazole, T - Tetracycline, G - Gentamycin, CTR - Ceftriaxome, NX - Norfloxacin, MR - Meropenem, I – Imipenem

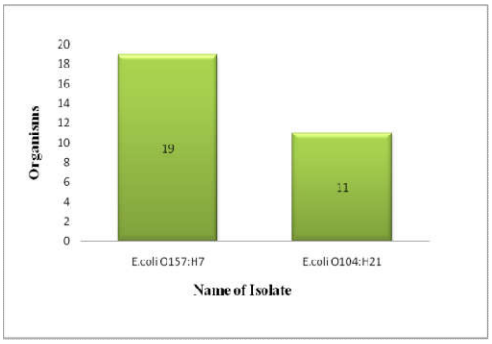


Figure 2 Serotyping of *E.coli* from sewage sample

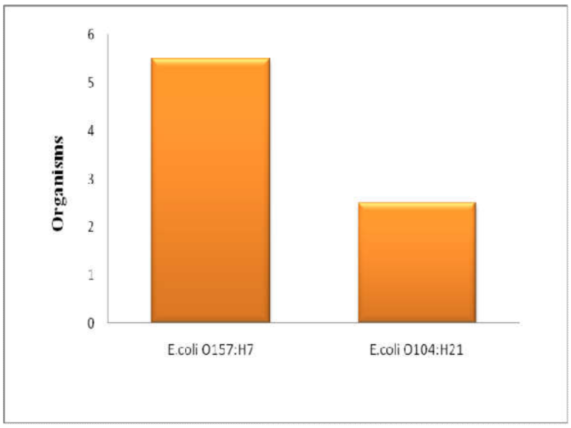
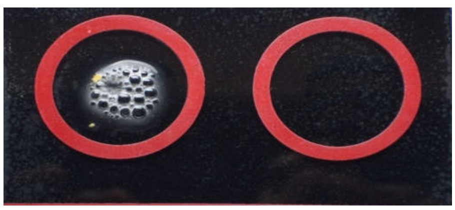


Figure 3 Serotyping of *E.coli* from water sample

Gram Staining

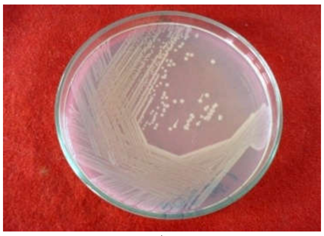
Gram Negative Rods Catalase Test



Positive Negative

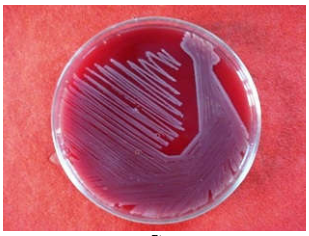
Oxidase Test

A = Negative Control
B = Positive Control
C = Test Sample

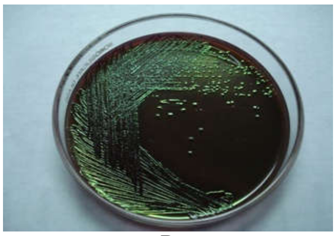


A

B



C



D

A - *E.coli* on Nutrient Agar
B - *E.coli* on MacConkey Agar
C - *E.coli* on Blood Agar
D - *E.coli* on EMB Agar

A = Indole
B = Methyl Red
C = Voges Proskaur
D = Citrate
E = TSI

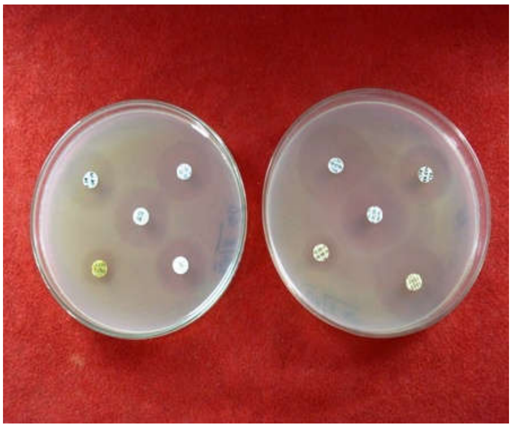


Figure 5 Antibiotic sensitivity test for *E.coli*

Environmental pollution by faecal contamination is a major threat to the community, which was highly ignored in the past. Drinking water can become contaminated with human or animal faeces by surface run-off and septic tank malfunction etc. When faecal contamination occurs, there is potential for disease causing germs to be present. Similar to ozone problem, green house effect and increase of atmospheric heat, this problem should also be dealt in seriously not only by environmentalists but also by every common man. It may be a true dream that India can emerge to be among the world's first four economic powers by 2020. Unless otherwise environmental pollution by faecal contamination is looked after genuinely, India cannot become a developed country in 2020.

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