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Prevalence and Characterization of Water Contamination Indicator Bacteria with Special Reference to Coliforms from Drinking Water Supply in Solan City of Himachal Pradesh

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ABSTRACT : Water becomes contaminated with enteric pathogens such as coliform group of bacteria viz *Salmonellae, Vibrio* and dysentery causing bacilli. The human faecal material carried along with domestic sewage is often dumped in rivers and lakes leading to water contamination. The present study was undertaken to detect the enteric pathogens and to measure the extent of drinking water contamination with the pathogenic microorganisms. Water samples were collected from different areas of Solan city in Himachal Pradesh. Most probable number (MPN) test was performed to detect the coliforms in water samples collected from surface water sources, hand pumps, taps, roof top storage tanks and aqua-guards. Chlorination of some water samples was done to see the effect of chlorine on bacteria. The indicator organisms isolated were *E. coli* and *Enterobacter aerogenes*. *E. coli* and *Enterobacter aerogenes* are the characteristic of intestinal tract of man and animals. The study reveals that surface water and roof top storage tank water was more liable to contamination whereas, ground water and aqua-guard water was safer for human consumption. Chlorine was effective in removing these bacteria from water.

Keywords : Water, Chlorination, MPN, MacConkey broth, *Escherichia coli, Enterobacter aerogenes*.

INTRODUCTION

Water is termed as "Elixir of life". The term elixir is derived from the Arabic Al Ikseer, which means a combination or a mixture (Needham *et al.*, 1976). Worldwide less than 900 million people lack reliable access to safe water that is free from disease and industrial waste and 90 % do not have access to adequate sanitation facilities This Results in one of the world's greatest health crises; 4,500 children die every day from water borne diseases (Bergey *et al.*, 1908). Water microbiology is concerned with the study of microorganisms that live in water, or can be transported from one habitat to other by water. Water supports the growth of many types of microorganisms (Chapelle, 2002). Water gets contaminated by enteric pathogens such as coliform group of bacteria, Salmonellae, Vibrio and dysentery causing agents. The human faecal material carried in sewage is often dumped in rivers and lakes. This increases water contamination. Therefore, water supply has to be regularly checked for microbial contamination. Coliform bacteria are the most reliable indicators of faecal contamination. However, presence of streptococci is strong evidence of faecal pollution (Park 2002). Worldwide, diarrheal diseases are second only to respiratory diseases. Every year around 5 million children (more than 13,600 a day) die from diarrheal diseases in Asia, Africa and South America. Developing countries carry a heavy load of water related diseases; the heaviest being the diarrheal diseases. Among which typhoid, paratyphoid fever, bacillary dysentery, *E. coli* associated diarrhea and cholera are very common. (Klein's *et al.*, 2002). According to the World Health Organization (WHO), diarrheal diseases account for 4.1% incidence of the total daily burden of disease and is responsible for the deaths of 1.8 million people every year (Nwachcuku *et al.*, 2004). WHO estimated that up to 80% of all sickness and diseases in world are caused by inadequate sanitation and polluted water (WHO, 1976). Chlorination plays an important role in removal of bacteria from water supplies. Chlorine kills pathogens such as bacteria and viruses by breaking the chemical bonds in their molecules. It is highly water soluble. Light chlorine (0.2-0.4 mg/L) is required to kill bacteria. Chlorine kills pathogenic bacteria, but it has no effect on certain viruses viz Polio and Hepatitis except when used in high doses (Ramesh, 2005). The main aim of the present study was the examination of bacteria from water samples collected from different sources at Solan city of Himachal Pradesh (India).

MATERIAL AND METHODS

COLLECTION OF WATER SAMPLES

Water samples were collected in clean sterilized bottles made of neutral glass of 230 ml capacity having ground glass stopper. Extreme care was taken to avoid any bacteriological contamination from environment during transportation of water samples to the laboratory (Park, 2000).

MOST PROBABLE NUMBER METHOD

Water was examined by MPN (Most probable number) test. This test was carried out in three stages namely presumptive test, confirmed test and completed test (Cruickshank *et al*., 1980).

PRESUMPTIVE TEST

A set of test tubes containing MacConkey broth (single and double strength) was inoculated with water samples and incubated at 37°C for 48 hours. Initial observations were made at the end of 24 hours. Incubation was prolonged for 24 hours more. All the test tubes were observed after 48 hours for any bubble formation in inverted Durham tube and colour change. Absence of air bubble in any test tube confirmed the absence of coliform group and do not need further confirmation or completed test. Tubes showing air bubbles were tested further for final confirmation of coliforms.

CONFIRMED TEST

This test is also called differential test or Eijkman test. After presumptive coliform test, subcultures were made from all the tubes showing acid and gas to fresh tubes containing single strength MacConkey broth (which was brought to 37°C in the water bath). All tubes were incubated at 37°C and examined after 24 hours for gas production. Tubes yielding gas at 37°C were regarded as containing *E. coli* (Typical coliforms) (Huang *et al.,* 1997). During Eijkman test, tubes containing peptone water were also inoculated from the presumptive positive tubes. All the tubes were incubated at 37°C. After incubation, Kovac's reagent was added along the sides of test tubes. Formation of red ring confirmed the presence of *E. coli*. Plates of Eosin Methylene Blue Agar (EMB) and MacConkey Agar were also streaked and incubated at 37°C for 24 hours. The results were then recorded. If no coliform colonies were present on petriplates and there was no gas production in MacConkey medium after 24 hours incubation at 37°C, then water was considered safe for drinking (Pelczar *et al.*, 2005).

COMPLETED TEST

An isolated colony was picked from the confirmatory test plate and inoculated into a tube of lactose broth and streaked on a nutrient agar slant to perform a Gram's stain. Tubes were incubated at 37°C. For differentiation between *E. coli* and *Enterobacter aerogenes*, biochemical tests were done. Results were then recorded. There was gas in lactose broth tubes, gram's negative bacilli on microscopic observation. Biochemical results were also recorded. This confirmed the presence of *E. coli* and *Enterobacter aerogenes* in different water samples (Cappucino *et al.,* 2006). Coliform Count and Most Probable Number of coliform were calculated by comparing the results of the presumptive test with the standard Table. 2., from; Standard methods for examination of water and wastewater, APHA, New York, (1971) (Dubey *et al.*, 2002).

RESULTS AND DISCUSSION

A large number of microorganisms including saprophytes and pathogenic organisms are found in drinking water (Stanier *et al*., 2007). Many bacteria provide an indication of faecal pollution in water. In the present study, a total of 17 water samples were collected from different areas of Solan and examined for microbial contamination. Water samples were collected from five different sources (Table 1). *E. coli* has been used as an indicator of faecal pollution in water for many decades. The bacterium is present in the intestinal tract of human and animals in large numbers and is more numerous than disease causing bacteria and viruses. *E. coli* has the advantage of not being capable of growing and multiplying in water (except warm and food laden waters). Therefore, the presence of this bacterium in water is indicator of recent faecal pollution (Madigan *et al*., 2000). Surface water sources are more liable to contamination as they are often loaded with various chemical and biological constituents. Tap water gets contaminated easily from its source or while passing through pipelines. Some other factors like soil and air can also act as source of water contamination. Ground water is considered more safe and reliable for consumption because sand filters remove most of bacteria from it (Borchardt *et al.,* 2004). In the present study the portability of water samples collected from drinking and natural water resources of Solan city (Himachal Pradesh) was determined on the basis of presumptive coliform test (MPN). On the basis of coliform test water samples were classified in four categories. Out of 17 samples tested, 9 were unsatisfactory, one sample was suspicious and only two were satisfactory whereas five were found excellent (Table 2 and Graph 2). The percentage distribution of water samples were like unsatisfactory 52.94%, Satisfactory 11.76%, Excellent 29.4% and Suspicious 5.88% (Graph 1). Unsatisfactory and suspicious samples were further tested for the presence of typical *E. coli* through Eijkman and lactose fermentation tests. Seven out of 10 unsatisfactory and suspicious samples were found positive in Eijkman test (Table 3). All the unsatisfactory and suspicious samples were found positive during lactose fermentation test (Table 4). Positive samples indicated the presence of coliforms. The positive samples were further confirmed through culturing on selective media (Fig. 1). The enteric bacteria were further identified on the basis of colony morphology, Gram staining and Biochemical tests. Two indicator organisms isolated from water samples during the present study were *E. coli* and *Enterobacter aerogenes*. *Enterobacter aerogenes* occur mostly on grains and plant surface, but may also inhabit the faeces of man and other animals. These bacteria are considered non pathogenic but presence of these in water indicates that faecal matter has entered in the water supply and is therefore, water is

contaminated (Mackie *et al.*, 2006). Among 10 isolated bacteria, seven were *E. coli* and three were *Enterobacter aerogenes* (Table 5). Both the enteric bacteria are considered as water pollution indicator organisms. However, some strains of *E. coli* are pathogenic and may cause enteric infections (Ananthanarayan *et al.*, 2005). The presence of these two indicator organisms in water samples indicates the extent of water contamination in the drinking water supply of the city. By applying MPN test, we can check the quality of drinking water. The contaminated water may be made good for use through filtration, sedimentation, disinfection by chlorination and some physical methods like osmosis, distillation and U.V. light. Such type of work is necessary in order to know the extent of water contamination.

Table 1: Sources of Water and Number of Samples Examined.

Sources	No. of samples
Natural sources of water	3
Handpump water	4
Tap water	6
Roof top storage tank water	2
Aquaguard water	2
Total	**17**

Table 2: Results of MPN test.

S.No.	Water samples	Results
1.	Natural source of water near Shoolini Temple	Unsatisfactory
2.	Natural source of water near Saproon	Unsatisfactory
3.	Natural source of water from Shilly road, Solan	Unsatisfactory
4.	Handpump water from Kotlanala	Unsatisfactory
5.	Handpump water from Ganj Bazaar	Satisfactory
6.	Handpump water from Jaunaji road near Shoolini Temple.	Suspicious
7.	Handpump water from Saproon.	Satisfactory
8.	Tap water from Public water supply, Ganj Bazaar	Unsatisfactory
9.	Tap water from Durga Temple, Lakkar Bazaar	Unsatisfactory
10.	Tap water from Microbiology laboratory II, SILB; before chlorination	Unsatisfactory
11.	Tap water from Microbiology laboratory II, SILB; after chlorination	Excellent
12.	Tap water from Girls Hostel Kotlanala, SILB; before chlorination	Unsatisfactory
13.	Tap water from Girls Hostel Kotlanala, SILB; after chlorination	Excellent
14.	Roof top storage tank water from Girls Hostel Kotlanala, SILB; before chlorination	Unsatisfactory
15.	Roof top storage tank water from Girls Hostel Kotlanala, SILB; after chlorination	Excellent
16.	Aquaguard water from Girls Hostel Kotlanala, SILB; before chlorination	Excellent
17.	Aquaguard water from Hostel Kotlanala, SILB; after chlorination	Excellent

contd.

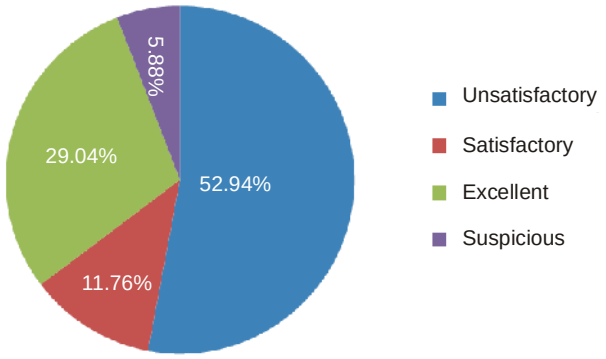

Fig. 1. The percentage of different classes of water samples found during the examination of water by MPN method.

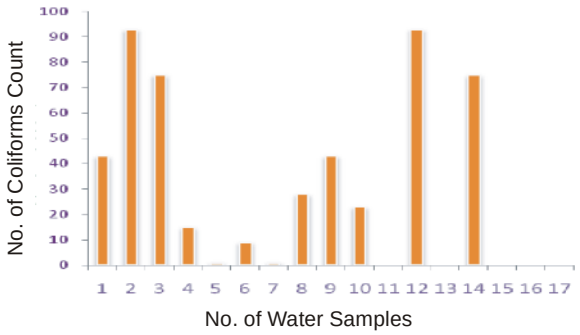

Fig. 2. Distribution of water samples on the basis of Colifroms count.

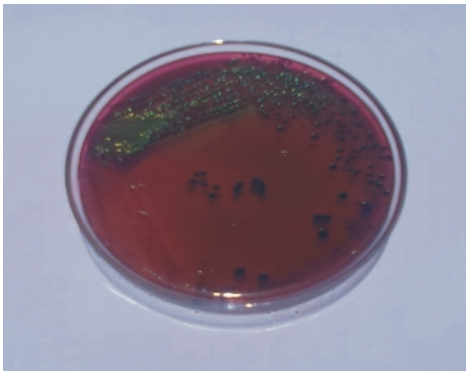

Fig. 3. *E. coli* on EMB Agar showing *sheen* colonies.

Table 3: Results of Eijkman test.

S.No.	Water samples	Results
1.	Natural source of water near Shoolini Temple	Positive
2.	Natural source of water near Saproon	Positive
3.	Natural source of water near Shilly road, Solan	Positive
4.	Handpump water from Kotlanala	Positive
5.	Handpump water from Ganj Bazaar	—
6.	Handpump water from Jaunaji road	Negative
7.	Handpump water from Saproon	—
8.	Tap water from public water supply, Ganj bazaar	Negative
9.	Tap water from Durga Temple, Lakkar Bazaar, Solan.	Negative
10	Tap water from Microbiology laboratory II, SILB; before chlorination	Positive
11.	Tap water from Microbiology Laboratory II, SILB; after chlorination	—
12.	Tap water from Girls Hostel Kotlanala, SILB; before chlorination	Positive
13.	Tap water from Girls Hostel Kotlanala, SILB; after chlorination	—
14.	Roof top storage tank water from Girls Hostel Kotlanala, SILB; before chlorination	Positive
15.	Roof top storage tank water from Girls Hostel Kotlanala, SILB; after chlorination	—
16.	Aquaguard water from Girls Hostel Kotlanala, SILB; before chlorination	—
17.	Aquaguard water from Girls Hostel Kotlanala, SILB; after chlorination	—

Table 4: Results of Lactose Fermentation test.

S.No.	Water samples	Lactose broth Test
1.	Natural source of water near Shoolini Temple	Positive
2.	Natural source of water near Saproon	Positive
3.	Natural source of water from Shilly road, Solan	Positive
4.	Handpump water from Kotlanala	Positive
5.	Handpump water from Ganj Bazaar	—
6.	Handpump water from Jaunaji road near Shoolini Temple	Positive
7.	Handpump water from Saproon	—
8.	Tap water from Public water supply, Ganj Bazaar	Positive
9.	Tap water from Durga Temple, Lakkar Bazaar	Positive
10.	Tap water from Microbiology lab. II, SILB; before chlorination	Positive
11.	Tap water from Microbiology laboratory II, SILB; after chlorination	—
12.	Tap water from Girls Hostel Kotlanala, SILB; before chlorination	Positive
13.	Tap water from Girls Hostel Kotlanala, SILB; after chlorination	—
14.	Roof top storage tank water from Girls Hostel Kotlanala, SILB; before chlorination	Positive
15.	Roof top storage tank water from Girls Hostel Kotlanala, SILB; after chlorination	—
16.	Aquaguard water from Girls Hostel Kotlanala, SILB; before chlorination	—
17.	Aquaguard water from Girls Hostel Kotlanala, SILB; after chlorination	—

Table 5: Results of Gram Staining and Biochemical Tests.

Sample no.	1	2	3	4	6	8	9	10	12	14
Gram	Gram positive bacilli	Gram positive bacilli	Gram positive bacilli	Gram positive bacilli	Gram positive bacilli	Gram positive bacilli	Gram positive bacilli	Gram positive bacilli	Gram positive bacilli	Gram positive bacilli
Indole	+	+	+	+	–	–	–	+	+	+
Methyl red	+	+	+	+	–	–	–	+	+	+
Voges Proskauer	–	–	–	–	+	+	+	–	–	–
Citrate	–	–	–	–	+	+	+	–	–	–
Glucose	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Lactose	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Sucrose	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Mannitol	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Urease	–	–	–	–	–	–	–	–	–	–
Nitrate Reduction	+	+	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+	+	+
Identified organisms	*E. coli*	*E. coli*	*E. coli*	*E. coli*	Ent. aerogenes	Ent. aerogenes	Ent. aerogenes	*E. coli*	*E. coli*	

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