Bangladesh Pharmaceutical Journal ***19****(2): 206-210, 2016*

Detection of *Escherichia coli* in Drinking Water Sources of Filter Units and Supply Water

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Received: July 01, 2016; Accepted: July 20, 2016; Published (Web): July 31, 2016

Abstract

The identification of coliforms has been extensively used as an indicator of drinking water quality. The study was aimed to identify the presence or absence of *E. coli* from drinking water. Samples were collected from different filtration units and tap water sources of an institution in Dhaka city. All the samples were tested through presumptive, confirmed and completed bacteriological test through MPN (most probable number) using 3 sets of lactose broth (LB) media and MF (membrane filtration) technique using bacteriological filter and nutrient agar media to find out the CFU (colony forming unit). About 10% of samples showed positive results for *E. coli* in completed test, and also gave positive results in confirmed test and gram staining; having no more than 1100 MPN per 100 ml for samples of filter taps but the source water remained negative.

Key words: Lactose broth, Most probable number, Membrane filtration

Introduction

Drinking water is one of the most essential components in our life. It is very closely associated with human health. About 33% of morbidity occurs due to the intake of poor quality water worldwide and most of the other diseases are also linked with water (UNCED, 1992). Around 4.6 billions of diarrheal cases were estimated over the world by WHO, and that caused death of 2.2 millions people of whom majorities were children below five years (WHO, 2010). Physicochemical, radiological and biological contaminations are usually checked to determine the quality of water (Diersing, 2009). The presence of pathogens in water is most vulnerable and it is quite impossible to test water for all known waterborne pathogens to address whether it is safe for drinking or not (WHO, 2011). Most common and omnipresent testing is the detection of coliform bacteria, which has been used for almost a century, as the indicator of the bacteriological safety of drinking water (Table 1) (Leclerc *et al.*, 2001; Malhotra *et al.*, 2015; Rajesh and Rattan, 2004). Coliform is a facultative anaerobic bacteria that ferments lactose to produce gas and is a gram- negative, non-spore-forming rod (Benson, 2002). *Escherichia coli*, a member of the coliform group, is generally considered the most vulnerable coliform bacteria since its presence indicates fecal contamination as well as possibility of enteric disease (Rice *et al*., 1991). *E. coli*. are regularly found in various natural environment as well as in the intestine of human being but not in drinking water, because the survivability of *E. coli* in drinking water at around 16 ºC is only 4-12 weeks (Rompre *et al.*, 2002; Edberg *et al*., 2000; Filip *et al.*, 1987; Kudryavtseva, 1972). Two widely used standard enumeration methods for fecal coliform in drinking water are multiple tube fermentation technique, and the membrane filtration technique that provides the count of colony forming unit per 100 ml

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when placing on the surface of agar after filtration of liquid medium (APHA, 1995). Presumptive test, confirmed test and completed test are the regular approaches (Kromoredjo and Fujioka, 1991). The maximum acceptable number of *E. coli* in portable drinking water should be none per 100 ml (Health Canada, 2014).

Table 1. Guidelines for determination of fecal contamination of water.

Class	Grade of water sample	Presumptive coliform count/ 100 ml	*E. coli* count per 100 ml
I	Excellent	0	0
II	Satisfactory	1–3	0
III	Suspicious	4–10	0
IV	Unsatisfactory	> 10	0, 1 or more

This study was targeted to identify and confirm the presence of coliform bacteria specifically the *E. coli* in the drinking water of different types of water filter taps and the sources of water in an educational institute in Dhaka city.

Material and Methods

Sampling: A total of 31 water samples from different sources of drinking water were collected. Most of the samples were collected from taps of filter units used in common spaces of the institute. Few of them were collected from deep tube-well as well as municipality water supply. While collecting, the filter taps were opened fully and water was let to run waste for 1 minute or for a time sufficient to permit cleaning the taps opening. About 200 ml of water was collected for analysis from a single site in the 250 ml sterilized duran bottle. Then the samples were transported to the laboratory immediately. Microbiological examination was started promptly to avoid unpredictable changes preferably within 2 hrs.

Media for growth and identification: Three different types of media were used for the growth, isolation and detection of enterobacteriaceae. Among them lactose broth medium was used for the detection of lactose fermentation by the *E. coli* in water samples, nutrient agar medium was used as a growth medium to culture bacterial cells and MacConkey agar was used for the isolation, enumeration, and differentiation of *E. coli* from other bacteria.

Preparation of lactose broths (LBx's): Three different marked lactose broth media, on the basis of their concentration and addition of sample volumes, were prepared as LB-2x, LB-1x and LB-0.1x in accordance with the standard procedure (Cappuccino and Sherman, 2013).

Bacteriological analysis

Presumptive, confirmed and completed tests were done for the qualitative analysis of water samples. In the presumptive test all samples were tested in a series of 9 sterile tubes of lactose broth for each, one control (C_1 with *E. coli* positive) and a blank (C_2), were inoculated with measured amounts of water to see if the water contains any lactose-fermenting bacteria. After incubation for 24 hrs at 37 °C, each test tube was examined for gas production. This test was also used to determine the most probable number of *E. coli* per 100 ml of water through 95% confidence limits for various combinations of positive results (Benson, 2002). In the confirmed test, MacConkey agar medium which inhibit the growth of gram-positive bacteria was used to differentiate the gram negative bacteria from gram positive. In the completed test, water samples from the positive confirmed test were incubated on nutrient agar slant and lactose broth for over 24 hours at 37°C temperature. Also, a gram staining slide was prepared

from the slant that were previously found positive in the presumptive test and examined under oil immersion optics for non-spore forming rod that ferments lactose.

Membrane filter method was acquired for quantitative analysis in which 100 ml of all the samples were passed through 0.45 micrometer filter papers and then aseptically placed on the MacConkey agar media and then incubated for 24 hours for identification and counting of bacteria (Filip *et al.*, 1987).

Results and Discussion

According to WHO, the presence of *E. coli.* in drinking water provides conclusive evidence of very recent fecal pollution (WHO, 2011). The most recommended technique used to determine the fecal contamination of water by *E. coli.* are multiple tube fermentation and membrane filtration method (Lin, 1974).

Figure 1. LB-2x, LB-1x and LB-0.1x after 24 hrs incubation.

Multiple tube fermentation: Among the tap water samples, 3 produced gas in lactose broth (Figure 1). The positive tubes with gas production were counted and MPN was determined from standard chart (Benson, 2002). The results are presented in Table 2. Samples that produced gases in the lactose broths were tested on MacConkey agar and after incubation over 24 hrs at 37 ^{o}C temperature they produced small colonies with dark centers (nucleated colonies) on MacConkey agar (Figure 2). The presence of dark centered colonies confirmed the presence of lactose fermenting gram-negative *E. coli*. In the completed test, all 3 samples producing gas in the lactose tube as well as grown on agar slant indicated the presence of *E. coli* in water sample.

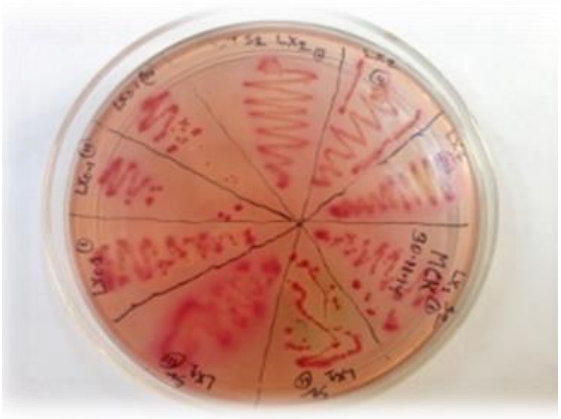

Figure 2. Positive samples after spreading on MacConkey agar.

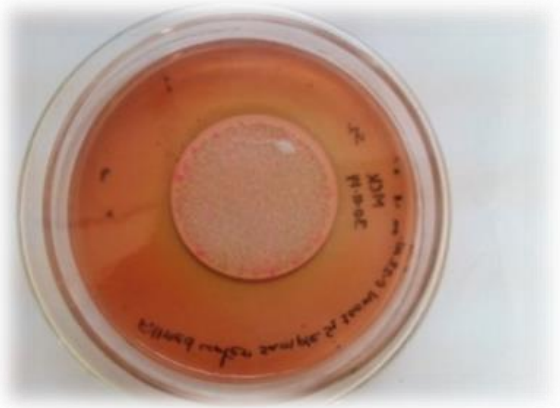

Figure 3. Positive samples after passing through the filter paper.

Gram staining also showed the pink color which confirmed presence of the gram negative *E. coli*

Membrane filtration: After incubation at 37 ^{o}C temperature for 24 hrs, filter papers that were used in membrane filtration method showed the pink color colonies on the MacConkey agar (Figure 3) for sample 1, sample 2 and sample 3, respectively (Table 3).

Table 2. MPN index and 95% confidence limits for various combinations of positive results when three tubes are used per dilution (10 ml, 1 ml, 0.1 ml portion of sample).

Sl. No.	GAS									(+) Control	Blank	MPN Per 100 ml	Range 95% probability	Coliform (+/-)
	LBX-2x			LBX-1x			LBX-0.1x							
	Test tube			Test tube			Test tube							
	1	2	3	1	2	3	1	2	3					
1	+	+	+	-	-	-	-	-	-	+	_	23	4-120	+
2	+	+	+	+	+	+	-	-	-	+	_	240	36-1300	+
3	+	+	+	+	+	+	+	+	+	+	_	1100	150-4800	+
4	-	-	-	-	-	-	-	+	-	+	-	3	<0.5- 9	-
5	-	-	-	+	-	-	-	-	-	+	-	3	<0.5- 13	-
6	-	-	-	-	-	-	-	+	-	+	-	3	<0.5- 9	-
7	+	-	-	-	-	-	-	-	-	+	-	4	<0.5- 20	-
8	-	+	-	-	-	-	+	-	-	+	-	7	1-21	-
9	-	-	-	-	+	-	-	-	-	+	-	3	<0.5- 13	-
10	-	-	-	+	-	-	-	-	-	+	-	3	<0.5- 13	-
11	-	-	-	-	+	-	-	-	-	+	-	3	<0.5- 13	-
12	-	-	-	-	-	-	+	-	-	+	-	3	<0.5- 9	-
13	-	-	-	-	-	-	-	-	+	+	-	3	<0.5- 9	-
14	+	-	-	-	-	-	-	-	+	+	-	4	<0.5- 20	-
15	-	-	-	-	-	-	-	+	-	+	-	3	<0.5- 9	-
16	+	-	-	-	-	-	-	-	-	+	-	4	<0.5- 20	-
17	-	-	+	-	-	-	-	-	-	+	-	4	<0.5- 20	-
18	-	-	-	-	-	+	-	-	-	+	-	3	<0.5- 13	-
19	-	-	-	-	-	-	-	+	-	+	-	3	<0.5- 9	-
20	-	-	-	-	-	-	-	-	-	+	-	3	<0.5- 9	-
21	-	-	-	-	+	-	-	-	-	+	-	3	<0.5- 13	-
22	-	-	-	-	-	-	-	-	-	+	-	3	<0.5- 9	-
23	+	-	-	-	-	-	-	-	-	+	-	4	<0.5- 20	-
24	-	-	-	-	-	-	-	+	-	+	-	3	<0.5- 9	-
25	-	-	-	-	-	-	-	-	+	+	-	3	<0.5- 9	-
26	-	-	+	-	+	-	-	-	-	+	-	7	1- 23	-
27	-	-	-	-	-	-	-	+	-	+	-	3	<0.5- 9	-
28	-	-	-	-	-	+	-	-	-	+	-	3	<0.5- 13	-
29	-	-	+	-	-	-	-	-	-	+	-	4	<0.5- 20	-
30	-	-	-	-	-	-	-	+	-	+	-	3	<0.5- 9	-
31	-	-	-	-	+	-	-	-	-	+	-	3	<0.5- 13	-

+ (positive), – (negative), MPN (most probable number).

Table 3. Number of colonies found after filtration of 100 ml water and overnight placing on the MacConkey agar media.

Sample No.	No. of colonies
1	20
2	195
3	>300

Conclusion

The presence of *E. coli* in few samples of filter units is a serious concern. Regular cleaning and monitoring of filter taps are recommended to use the filter units for drinking water. However, it was found that the samples collected from a specific site of municipality water and deep tube-well water were completely free from this bacterium during the time of analysis. It is believed that the filter units showing positive results for *E. coli* were somehow contaminated during transfer of source water to filter units. Those filter taps having *E. coli* contamination were given immediate attention and corrective measures were taken by the authorities.

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