

Original Research Article

Water-Supply Potability Status of Bangladesh University of Health Sciences (BUHS)

ABSTRACT

Water that is free of disease-producing microorganisms and chemical substances harmful to health is called potable water. Drinking water quality has a microbiological and a physicochemical dimension. In public water supply systems, water should be disinfected. This study will be conducted through a multiple tube method. And the water supply channel of BUHS (Bangladesh University of Health Sciences) served as the study's sample. Using an aseptic procedure, 105 ml of water from various sources is collected for each sample on a conical flask. The selected sample was from the four different water supply point at BUHS institution. From sample 1, the MPN index we found the combination of positive sets (0-1-0). The MPN value per 100ml water is (1). From sample 2, the MPN index we found the combination of positive sets (1-1-1). The MPN value per 100ml water is (5). From sample 3, the MPN index we found the combination of positive sets (1-2-1). The MPN value per 100ml water is (7). From sample 4, the MPN index we found the combination of positive sets (0-1-0). The MPN value per 100ml water is (1). In Dhaka city, there is a possibility of contamination of water. Bangladesh University of Health Sciences is a renowned institution with a big number of staff. So, it is important to examine the water condition in this institution. Our work and research reflect a little about it. Gladly and hopefully, we found that there is no pathogenic organism in the water of this institution which is used every day and everybody drinking.

Keywords: BUHS, E.Coli, Coliforms, Macconkey, Potability

1. INTRODUCTION

Water supply is a channel by which we have water that we do drink or use. According to the WHO¹ water has got two distinctive characteristics, either it is safe for use and drink that termed “potable” and which is not safe is termed as “non-potable”. The classification is based on the presence or absence of some selective indicator microorganisms in water. This research will be conducted on Bangladesh University of Health Sciences (BUHS) water supply to assess the potability status of this institute’s water. After this study, we will have an assessment of our institute’s water supply, which will tell us whether we are using safe and pure water or using unsafe and impure water.

It is impractical to attempt the routine isolation of pathogens because they are present in relatively small numbers compared with other types of microorganism. Moreover, there are many types of pathogens and each requires a unique microbiological isolation technique stated by International Standard organization². The approach that has been adopted is to analyze for indicator organisms that inhabit the gut in large numbers and are excreted in human feces. One study³ suggested that, the presence of these indicator organisms in water is evidence of fecal contamination and, therefore of a risk that pathogens are present. If indicator organisms are present in large numbers, the contamination is considered to be recent and/or severe. Bacteria in water are, in general, not present individually, but as clumps or in association with particulate matter. Department of the U.S. environment⁴ reported that when enumerating bacteria in water it is not the number of individual bacteria present which are counted, but the number of clumps of bacteria or the particles and their associated bacteria. Each clump or particle may have many bacteria associated with it. Historically, most of our concern about water purity has been related to the transmission of diseases that are mostly waterborne diseases like shigellosis, salmonellosis, etc. Therefore, tests have been developed to determine the safety of the water.

The tests for water purity in use today are aimed instead at detecting particular indicator organisms. This can be seen that there are several criteria for an indicator organism. The most important criterion is that the microbe be consistently present in human feces in substantial numbers so that its detection is a good indication that human waste is entering the water⁵. In the United States, the usual indicator organism in freshwater is “*coliform bacteria*”. Coliforms are defined as aerobic or

facultative anaerobic, gram-negative, non-endospore forming, rod-shaped bacteria that ferment lactose to form gas within 48 hours of being placed in lactose broth at 35°C, but there is another thermotolerant coliform named fecal coliform has got the capability to grow on >44°C⁶

2. METHODS and MATERIALS

This study will be conducted through a multiple tube method. And the sample for this study is the water supply channel of BUHS (Bangladesh University of Health Sciences). From different sources, 105 ml water for each sample is been collected on a conical flask with aseptic technique. Observation on the procedure will be that, either the sample containing fecal coliform or not.

2.1 The multiple-tube method

The technique has been used for the analysis of drinking water for many years with satisfactory results. It is the only procedure that can be used if water samples are very turbid or if semi-solids such as sediments or sludge's are to be analyzed. The procedure followed is fundamental to bacteriological analyses and the test is used in many countries. It is customary to report the results of the multiple fermentation tube test for coliforms as a most probable number (MPN) index⁷. This is an index of the number of coliform bacteria that, more probably than any other number, would give the results shown by the test. It is not a count of the actual number of indicator bacteria present in the sample.

2.2 Qualitative analysis through MPN method

2.2.1 Presumptive test

Presumptive test is the very first portion of the MPN test method. This is primarily done for detection of the Gram negative coliform bacteria in the water samples. For this task, 15 series of test tubes containing 10 mL of lactose fermentation broth were needed for each sample. Ten mL, 1 mL

and 0.1 mL samples were added sequentially in 5 test tubes containing 10 mL lactose fermentation broth 2X, 5 tubes containing 10 ml lactose fermentation broth 1X and 5 test tubes containing 10 ml 1X lactose fermentation broth. Each tube was incorporated with a Durham tube indicating gas formation after lactose fermentation by coliform bacteria⁸.

2.2.2 Confirmed test

The test tubes showing positive results by the accumulation of gas in the Durham tubes were selected for the confirmed test to determine the presence of *E. coli* in the respective water samples. The loopful sample from the broths which gave positive result in the presumptive test, were inoculated on EMB agar to detect as well as differentiate *E. coli* and other Gram negative coliform bacteria. The plates were incubated at 37°C for 24 hours⁸.

2.2.3 Completed test

This is the final part of the MPN test procedure which was completed after the confirmation of the indicator bacteria *Escherichia coli* found in the EMB agar medium. The suspected *E. coli* from a single colony of green metallic sheen was introduced into a lactose fermentation broth 1X again for the assurance of the gas production after fermentation of lactose. Gram staining was also performed for the confirmation of *E. coli* isolates⁸.

Table 1: Reactions over different media.

Medium	Reactions	
1. Macconkey broth. 2. Macconkey agar. 3. EMB agar. 4. MIU agar	Total coliforms at 35 or 37 °C	Thermotolerant coliforms at 44 or 44.5 °C
	1. Gas visible in the inverted fermentation (Durham) tube plus turbidity of the medium 2. Lactose fermenting pink colony. 3. Greenish Metallic sheen colony will appear. 4. Indole positive with gas production and non-motile in nature.	1. Same as total coliforms at 35 or 37 °C. 2. Not applicable. 3. Same as total coliform at 35 or 37°. 4. Same as 35 or 37° C

3. RESULTS

The selected sample was from the four different water supply point at BUHS institution. And the sampling technique is random sampling. They are enlisted here.

Table 2: The four samples from different water supply point at BUHS.

Sample no.	Collection point. (105 ml/sample)
1	Filter water
2	Tap water at washroom
3	Canteen water supply
4	Vice Chancellor office.

Following reactions, we will find the media we used after observing and these results will be considered positive results, if these characteristics will not be found then the result will be considered negative.

From the result of different testing procedure here we have one double strength set positive with coliform bacteria (E.Coli). From MPN index we found the combination of positive sets (0-1-0). The MPN value per 100ml water is (1)

Table 3: Sample 1 Report Status

Date	8 th June 2018. Sample placed at broth.		Sample no.	1	Location	Bangladesh University of Health Sciences campus					
Date	10 th June 2018	Date	11 th June 2018		Date	11 th June 2018	Date	12 th June 2018		Date	12 th June 2018
Tube Name	MacConkey Broth purple 37 °c/48h		Mac agar	EMB agar		EMB agar 44°c/24h	MIU				Result
			37°c/24h				Indole 37°c/24h	Indole 44°c/24h			
BUHS											----
D-50m	No growth.		----	----		----		----	----		----
D-1	No growth		----	----		----		----	----		----
D-2	No growth		----	----		----		----	----		----
D-3	No growth		----	----		----		----	----		----
D-4	No growth		----	----		----		----	----		----
D-5	Growth positive		Positive	Positive		Negative		Positive	----		Total Coliform identified.
S-1	No growth		----	----		----		----	----		----
S-2	No growth		----	----		----		----	----		----
S-3	No growth		----	----		----		----	----		----
S-4	No growth		----	----		----		----	----		----
S-5	No growth		----	----		----		----	----		----

Table 4: Sample 2 Report Status

Date	8 th June 2018. Sample placed at broth.	Sample no.	2	Location	Bangladesh University of Health Sciences campus						
Date	10 th June 2018	Date	11 th June 2018		Date	11 th June 2018	Date	12 th June 2018		Date	12 th June 2018
	MacConkey Broth purple 37 °c/48h		Mac agar	EMB agar		EMB agar 44°c/24h	MIU			Result	
		37°c/24h			Indole 37°c/24h		Indole 44°c/24h				
Tube Name	BUHS										----
D-50m	Growth positive		positive	positive		positive	negative	positive			Thermotolerant coliform
D-1	Growth positive		positive	positive		positive	positive	positive			Thermotolerant coliform
D-2	No growth		----	----		----	----	----			----
D-3	No growth		----	----		----	----	----			----
D-4	No growth		----	----		----	----	----			----
D-5	No growth		----	----		----	----	----			----
S-1	No growth		----	----		----	----	----			----
S-2	No growth		----	----		----	----	----			----
S-3	No growth		----	----		----	----	----			----
S-4	No growth		----	----		----	----	----			----
S-5	Growth positive		positive	positive		negative	positive	positive			Thermotolerant coliform

From MPN index we found the combination of positive sets (1-1-1). The MPN value per 100ml water is (5)

Date	13 th June 2018.sample placed at broth.	Sample no.	3	Location	Bangladesh University of Health Sciences campus
------	--	------------	---	----------	---

Table 5: Sample 3 Report Status

Date	15 th June 2018	Date	16 th June 2018		Date	16 th June 2018	Date	17 th June 2018		Date	17 th June 2018
	MacConkey Broth purple 37 °c/48h		Mac agar	EMB agar		EMB agar 44°c/24h		MIU			Result
			37°c/24h					Indole 37°c/24h	Indole 44°c/24h		
Tube Name		-----									
D-50m	Growth positive		positive	positive		positive		negative	positive		Thermotolerant coliform
D-1	No growth		----	----		----		----	----		----
D-2	No growth		----	----		----		----	----		----
D-3	Growth positive		positive	positive		positive		positive	positive		Thermotolerant coliform
D-4	Growth positive		positive	positive		positive		negative	positive		Do
D-5	No growth		----	----		----		----	----		----
S-1	No growth		----	----		----		----	----		----
S-2	No growth		----	----		----		----	----		----
S-3	Growth positive		positive	positive		negative		positive	----		Total coliform
S-4	No growth		----	----		----		----	----		----
S-5	No growth		----	----		----		----	----		----

From MPN index we found the combination of positive sets (1-2-1). The MPN value per 100ml water is (7)

Table 6: Sample 4 Report Status

Date	13 th June 2018.sample placed at broth.	Sample no.	4	Location	Bangladesh University of Health Sciences campus
------	--	------------	---	----------	---

Date	15 th June 2018	Date	16 th June 2018		Date	16 th June 2018	Date	17 th June 2018		Date	17 th June 2018
	MacConkey Broth purple 37 °c/48h		Mac agar	EMB agar		EMB agar 44°c/24h		MIU			Result
			37°c/24h					Indole 37°c/24h	Indole 44°c/24h		
Tube Name	-----										
D-50m	No growth		----	----		----		----	----		----
D-1	No growth		----	----		----		----	----		----
D-2	No growth		----	----		----		----	----		----
D-3	No growth		----	----		----		----	----		----
D-4	No growth		----	----		----		----	----		----
D-5	Growth positive		positive	positive		negative		positive	----		Total Coliform detected
S-1	No growth		----	----		----		----	----		----
S-2	No growth		----	----		----		----	----		----
S-3	No growth		----	----		----		----	----		----
S-4	No growth		----	----		----		----	----		----
S-5	No growth		----	----		----		----	----		----

From the result of different testing procedure here we have one double strength set positive with coliform bacteria (E. coli). From the MPN index we found the combination of positive sets (0-1-0). The MPN value per 100ml water is (1).

Table 7: Compare the combination of positive result, from the most probable number index that is values/100ml of sample for a tests of one 50ml, five 10ml, and five 1 ml volumes⁷.

No of tubes giving a positive reaction			MPN/100ml
1×50 ml	5×10 ml	5×1 ml	
0	0	0	<1
0	0	1	1
0	0	2	2
0	1	0	1
0	1	1	2
0	1	2	3
0	2	0	2
0	2	1	3
0	2	2	4
0	3	0	3
0	3	1	5
0	4	0	5
1	0	0	1
1	0	1	3
1	0	2	4
1	0	3	6
1	1	0	3
1	1	1	5
1	1	2	7
1	1	3	9
1	2	0	5
1	2	1	7
1	2	2	10
1	2	3	12
1	3	0	8
1	3	1	11

1	3	2	14
1	3	3	18
1	3	4	21
1	4	0	13
1	4	1	17
1	4	2	22

Now the sample is being placed on the MacConkey broth for their further investigation and the reporting of each individual testing is also sorted out along with the dates. The following chart will tell us the interpretation of each particular sample analysis. From this reporting will be able to know the status of water quality.

From each sample, we have a particular result for them.

Table 8: The result of the samples.

Sample no.	Isolated organism	MPN value /100 ml
1	Total coliform	1
2	Thermotolerant coliform	5
3	Thermotolerant coliform	7
4	Total coliform	1

Here's a typical classification is been presented that is based on increasing orders of magnitude of fecal contamination.

Table 9: Example of classification and color-code scheme for thermotolerant (fecal) coliforms or E. coli in water supplies⁹.

Count per 100ml	Category and colour code	Remarks
0	A (blue)	In conformity with WHO guidelines
1–10	B (green)	Low risk
10–100	C (yellow)	Intermediate risk
100–1000	D (orange)	High risk
>.1000	E (red)	Very high risk

4. DISCUSSION

U.S Environmental Protection Agency⁴ stated that the term “total coliforms” refers to a large group of Gram-negative, rod-shaped bacteria that share several characteristics. The group includes thermotolerant coliforms and bacteria of fecal origin, as well as some bacteria that may be isolated from environmental sources. Thus, the presence of total coliforms may or may not indicate fecal contamination. In extreme cases, a high count for the total coliform group may be associated with a low, or even zero, count for thermotolerant coliforms. Such a result would not necessarily indicate the presence of fecal contamination. It might be caused by the entry of soil or organic matter into the water or by conditions suitable for the growth of other types of coliforms. In the laboratory, total coliforms are grown in or on a medium containing lactose, at a temperature of 35 or 37 °C. They are provisionally identified by the production of acid and gas from the fermentation of lactose. One reported that thermotolerant (fecal) coliforms the term “fecal coliform” has been used in water microbiology to denote coliform organisms that grow at 44 or 44.5 C and ferment lactose to produce acid and gas. In practice, some organisms with these characteristics may not be of fecal origin and the term “thermotolerant coliform” is, therefore, more correct and is becoming more commonly used¹⁰. Nevertheless, the presence of thermotolerant coliforms nearly always indicates fecal contamination. Usually, more than 95 percent of thermotolerant coliforms isolated from water are the gut organism *Escherichia coli*. The presence of which is definitive proof of fecal contamination.

As a result, it is often unnecessary to undertake further testing to confirm the specific presence of *E. coli*. In the laboratory, thermotolerant coliforms are grown on media containing lactose, at a temperature of 44 or 44.5 °C. They are provisionally identified by the production of acid and gas from the fermentation of lactose¹¹ Nutrient-rich environments may encourage the growth or persistence of some species of thermotolerant coliform other than *E. coli*. This possibility should be considered when, for example, an unusually high result is obtained from water that was thought to be relatively clean. In such a case, the advice of a microbiology laboratory should be sought for the determination of the more specific indicator, *E. coli*.

WHO¹² reported that Fecal streptococci the presence of fecal streptococci is evidence of fecal contamination. Fecal streptococci tend to persist longer in the environment than thermotolerant or total coliforms and are highly resistant to drying. It is, therefore, possible to isolate fecal

streptococci from water that contains few or no thermotolerant coliforms, for example, when the source of contamination is distant in either time or space from the sampling point. Fecal streptococci grow in or on a medium containing sodium azide, at a temperature of 37-44 °C. They are usually detected by the reduction of a dye (generally a tetrazolium-containing compound) or the hydrolysis of aesculin. Routine methods may give “false positives” and additional confirmatory tests may be required. Based on this data we have a clear indication that the BUHS water supply is potable and safe to drink.

5. CONCLUSION

However, where piped small-community water supplies are being analyzed and samples are taken at various points in the system, water quality may differ in different parts of the system at any one time. Again, the reasons for this may become obvious during the sanitary inspection or—if these differences are the result of cross-contamination or contamination caused by leaks in pipework—after resampling.

A microbiological analysis of water quality from the institute, Bangladesh University of Health Sciences (BUHS) shows that its water supply is potable. In addition, contamination through fecal coliform is the least. The drinking water here is safe to drink and the most probable number of fecal coliform bacteria in per 100 ml of water is less than the risk limit. Nevertheless, regular inspection is necessary here to provide safe water to drink for all the personnel here

6. REFERENCES

1. World Health Organization. Guidelines for drinking-water quality. Vol. 3. Drinking-water quality control in small-community supplies. World Health Organization; 1985.
2. ISO Water Quality C Enumeration of Viable Microorganisms. Colony Count by Inoculation in or on a Solid Medium. International Standard ISO 6222, International Organization for Standardization, Geneva 1988.
3. World Health Organization. Aluminium in drinking-water: background document for development of WHO Guidelines for drinking-water quality. World Health Organization; 2003.
4. U.S. Environmental Protection Agency (EPA), Washington, D.C. "Method 1680: Fecal Coliforms in Biosolids by Multiple-Tube Fermentation Procedures." Draft. Document no. EPA-821-R-02-026. October 2002.
5. Greenberg AE, Clesceri LS, Eaton AD, American Public Health Association, American Water Works Association. Standard methods for the examination of water and wastewater. (No Title). 1992.
6. Clasen T, Schmidt WP, Rabie T, Roberts I, Cairncross S. Interventions to improve water quality for preventing diarrhoea: systematic review and meta-analysis. *Bmj*. 2007 Apr 12;334(7597):782.
7. Oblinger JL, Koburger JA. Understanding and teaching the most probable number technique. *Journal of Milk and Food technology*. 1975 Sep;38(9):540-5.
8. Cappuccino, J.G. and Sherman, N. *Microbiology- A Laboratory Manual*. The Benjamin/Cummings publishing Co., Inc., Menlo Park, California.1996.
9. Todar, K. "Pathogenic E. coli". Online Textbook of Bacteriology. University of Wisconsin–Madison Department of Bacteriology. Retrieved 2007-11-30.
10. Fresno CA. Fresno county department of public health. E. coli. 2009.

11. U.S. Geological Survey. Ohio Water Microbiology Laboratory, Columbus, OH. "mFC agar method for fecal coliforms." Analytical Methods. January 2007.

12. World Health Organization. Aluminium in drinking-water: background document for development of WHO Guidelines for drinking-water quality. World Health Organization; 2003.

UNDER PEER REVIEW