Microbiological analysis of water through MPN

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Abstract---Water borne disease outbreaks associated with the drinking of unsafe water containing pathogenic bacteria of fecal origin is common in perspective of densely populated countries including Bangladesh. Present study employed a laboratory scale qualitative analysis of drinking water for the detection of indicator bacteria especially the fecal contamination that is responsible for health associated problems. Total Six water samples were collected from separate household and commercial points in Dhaka city Bangladesh. *Escherichia coli* was found as indicator bacteria in 1 water sample out of 6 samples as revealed through most probable number (MPN) method

Keyword: MPN Method, presumptive test results, confirmed test results, Antibiotic sensitivity.

1. Introduction:

The scenario of water pollution imparted by the existence of microorganisms in drinking water is not unusual and the resultant disease endemics or epidemics are common vastly in the developing countries where sanitation, water purification and the practice of hygiene are ignored [3]. Dhaka, the capital of Bangladesh, is an over populated city having more than 2.5 million people with the increasing rate of about 5% each year [10]. However, events of microbial contamination are frequent along this city resulting in various categories of enteric diseases heading from the consumption of water supplied by Dhaka Water Supply Authority (DWSA) without appropriate purification or processing [15,12,22,13,9] Mainly water supply in Dhaka metropolis is generated from the underground water (aquifer under 50 and 120 meters) sources without any treatment because of the lack of chance to microbial contamination from the outside environment. Some water supplies may also serve water from surface water sources which are treated to remove contaminations before supplying in the distribution system. General people in Dhaka use water for drinking purposes either directly consuming or after some processing of the water by means of filtration or boiling [3]. The major health risk from drinking water is caused by the presence or introduction of fecal coliforms in the drinking water supply which may come from the non-treated sewage systems sited nearly the water source or distribution system as well as overflow from them [19,8,14,2,20] Coliform bacteria may account for public health associated diseases including diarrhea, dysentery, typhoid, salmonellosis, listeriosis, parasitic worm infestations, and viral infections are introduced from the diseased or carrier stage patients shedding such pathogens which by chance come into contact with the potable water systems [7,21,17,16,18]. The quality of drinking water can be tested by the processes determining the presence of different contaminating bacteria in the water sample which is usually time consuming as well as costly [13]. Moreover, often the pathogens present are very low in number so that they could be missed during the testing procedures [11]. A comparatively easy way to examine the water quality is to determine the presence of coli form bacteria serving as indicator, and hence the presence of such bacteria indicates the risk of pathogenic contamination from fecal origin [5, 23] The most common test for indicator organism is to determine the presence of *Escherichia coli* which only indicates the possibility of fecal contamination, not the actual presence of fecal pathogens as well as the occurrence of diseases [13]. However, for rapid detection of indicator organisms in drinking water, the most probable number (MPN) method, which is not that common in usage, might be considered [1]. It is actually a qualitative test rather than quantitative indicating only the presence of coliforms, not their numerical presentation. This test is carried out in three continuous stages: presumptive test, confirmed test and completed test through which the presence of indicator organism *E. coli* is detected and confirmed [6]. The test is also called multiple tube test which involves the use of multiple tubes to determine the

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presence of indicator organism using some key characteristics of them including – lactose fermentation producing gas, green metallic sheen on eosin methylene blue (EMB) agar and by Gram reaction observed in the mentioned three stages, respectively. Overall, with a shortfall of microbial enumeration, the MPN method of water quality detection is cost effective and rapid as well to determine the water quality. Along these lines, the present study employed MPN method to determine the microbiological quality of drinking water.

2. MATERIALS AND METHODS:

2.1 Study area and sampling: The drinking water samples used by the residents of Dhaka metropolis were tested in the current study. Six water samples were collected from separate household and commercial points which were used to consume after pre-treatments during the time period of February, 2018 to March, 2018. Samples were collected aseptically in sterile screw capped bottles maintaining in a thermal stabilizing box with a constant temperature of 25°C, transported to the laboratory within one hour, and immediately subjected to microbiological analysis [3].

2.1 MPN test protocol

2.1.1 Presumptive test: Presumptive test involves the primary presumption for the presence of Gram negative coli form bacteria in the samples demonstrated by the appearance of gas in the lactose fermentation broth. For the presumptive test procedure 15 sets of test tubes containing lactose fermentation broth required for each sample under analysis. Each test tube contained 10 ml of fermentation broth and inoculated with the water sample in a sequential order of 10 ml in five of each 2X lactose fermentation broth, 1ml in five of each 1x lactose fermentation broth and lastly 0.1 ml in five of each 10ml 1X lactose fermentation broth. All the test tubes were incorporated with Durham tubes for detection of gas formation by Gram negative coli form bacteria. Test tubes were incubated with half circled screw caps at 37°C for 48 hours. This procedure was followed for all of the 6 samples individually.

2.1.2 Confirmed test: Positive samples with the production of gas in the lactose fermentation broth were selected for the confirmed test procedures to detect the indicator bacteria of fecal origin *Escherichia coli.*EMB media was used to differentiate other Gram negative coliform bacteria from the *Escherichia coli* by the production of green metallic sheen in the media. The presence of green metallic sheen in EMB confirms the presence the indicator bacteria *E. coli*. One loop full sample from the positive test tubes was inoculated on EMB by streaking and incubated at 37°C for 24 hours and then observed for the production of green metallic sheen.

1. Table: Presumptive test results:

Sample	5 of 10 ml Each	5 of 1 ml Each	5 of 0.1 ml each	MPN index Per 100 ml	95% Confidence Limits	
					Lower	Upper
01	2	0	0	5	<0.5	13
02	4	4	0	34	11	93
03	5	5	3	920	220	2600
04	5	3	0	79	22	220
05	5	5	3	920	220	2600
06	5	5	5	≥2400	700	…

2. Table: Confirmed test results:

Sample	Growth on EMB	Green metallic Sheen	Result
01	+	-	Potable
02	+	+	Non potable
03	+	-	Potable
04	+	-	Potable
05	+	-	Potable
06	+	-	Potable

3. Result and discussion:

Poor water quality, sanitation and hygiene account for about 2 million deaths a year world-wide (3.1% of all deaths and 3.7% of daily), mainly through infectious diarrhea [15,16].Such diseases are more common in developing countries like Bangladesh due to poor quality of drinking water [4,16] Present experiment was conducted for the determination of the processed drinking water quality to be either potable or non-potable on the basis of the presence of indicator bacteria which indicates the chance of fecal contamination as well as the health associated risks. This identification procedure was

done by applying the MPN method which is rather cheap and less time consuming method in the context of developing countries.

3.1 Bacteriological quality of the drinking water samples tested: The potable waters samples used in the current study were heavily contaminated with lactose fermentation positive bacteria which were determined by the formation of gas in the Durham tube after 48 hours of incubation at 37□C. Sample no. 3, 4, 5 and 6 showed maximum counts of positive results for each of the 15 test tubes by observing the formation of gas resulting 920, 79 ≥2400 MPN/100 ml of sample. Sample no. 1 showed lowest count as 5 MPN/100 ml of sample. Sample no. 2 exhibited green metallic sheen on EMB media indicating the presence of fecal coli form i.e. *E. coli* making the water sample non-potable. The presence of the indicator bacteria indicated the possible occurrence of fecal contamination. Processing drinking water by more sophisticated ways and also by detecting the ways of contamination, the degree of such health problems might be lessened. Finally, our study reported the non-portability of some processed drinking water samples (sample no.2) by observing the indicator bacteria *E. coli*. Which also indicated the possible presence of other pathogenic bacteria. Several factors such as environmental contamination, inadequate processing and improper handling etc. might be responsible for contamination of drinking water.

4. Conclusion:

Being a densely populated developing country, Bangladesh has long been suffering from the disease complications of her subjects due to poor, unhygienic management of environment and water bodies. The present data referred the microbiological test of water in various places in Dhaka city, Bangladesh. Due to the poor water distribution system the quality of supplied water samples were not satisfactory and were also indicative of the possibilities to impart various enteric diseases. The situation may aggravate in near future if the authority does not pay attention and take immediate actions to restore water quality in the distribution system.

5. Acklonowagement:

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