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Full Length Research Paper

Identification and quantification of *Escherichia coli* from drinking water in Bangladesh

Shahedur Rahman[1]*, Anowar Khasru Parvez[2] and Rezwanul Islam[3]

[1]Department of Genetic Engineering and Biotechnology, Jessore Science and Technology University, Jessore -7408, Bangladesh.
[2]Department of Microbiology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.
[3]Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia-7003, Bangladesh.

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This study was undertaken to identify and quantify *Escherichia coli* from drinking water from the western part of Bangladesh. Fifty water samples were collected from different regions. The presence of *E. coli* was determined on the basis of gas formation in the test tubes after inoculating and incubating the water samples into the Lactose fermentation broth (LB). A certain amount of the accumulated gas was used to determine the quantity of the *E. coli* cell present in the water samples with the help of Minimum Probable Number (MPN) method. Then the confirmation test was done using Eosin Methylene Blue agar. Positive isolates were then subjected to the completed test (Gram staining). Among the 50 different water samples, 5 samples were found positive.

Key words: *Escherichia coli,* identification, minimum probable number (MPN), eosin methylene blue agar, metallic sheen, household water treatment.

INTRODUCTION

Safe drinking water is a basic human requirement and is essential to all. Contaminated drinking water has the greatest impact on human health worldwide, especially in developing countries (Momba et al., 2010). Outbreaks of human illness associated with the consumption of contaminated water have been reported from many countries (Cabral, 2010). A study in 2002 estimated that water, sanitation and hygiene were responsible for 4.0% of all deaths and 5.7% of the total disease burden occurring worldwide (Rahman et al., 2011). Diarrhoeal disease alone causes 2.2 million of the 3.4 million water-related deaths per year. Many of the deaths involve children less than five years of age. In developing countries, four-fifths of all the illness are caused by water-borne diseases, with diarrhea being the leading cause of childhood death (Choffnes and Mack, 2009; Noosorn and Niamkamnerd, 2009; Rahman et al., 2011). As a developing country, Bangladesh is not an exception. In Bangladesh, water-related diseases are responsible for 24% of all deaths. Every year, gastroenteritis and diarrhoeal diseases kill 110,000 children below the age of five. Waterborne diseases account for nearly a quarter of all illnesses in Bangladesh, while about 12% are accounted for by diarrhoea, and 10% by other gastro-intestinal illness including enteric fever. Thus water plays a major role in the overall disease profile of the country (Noosorn and Niamkamnerd, 2009). This study was undertaken to identify and quantify *Escherichia coli* from drinking water from the western part of Bangladesh.

MATERIALS AND METHODS

The experiment was carried out at the Biotechnology Laboratory, Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh. Microbiological study was done with 50 water samples (25 municipally supplied water samples and 25 tubewell water samples) which were collected from different parts of municipal and rural area of Kushtia district.

*Corresponding author. E-mail: shahed.rajib@gmail.com. Tel: +8801553116736.

Table 1. Presumptive test result after 24 h on the basis of gas formation in lactose broth.

Time	Sample number	LB(2X)+10ml			LB(1X)+1ml			LB(1X)+0.1ml			Positive control	Negative control
		Tube			Tube			Tube			Tube	Tube
		A	B	C	D	E	F	G	H	I	-	-
After 24 h	8	+	+	+	+	++	++	+	+	+	-	-
	15	+++	+++	-	-	-	-	-	-	-	-	-
	25	+	-	-	-	-	-	-	-	-	-	-
	40	+	++	+	-	+	-	-	-	-	-	-
	48	++	++	+	-	-	-	-	+	-	-	-
After 48 h	8	+	+++	+	+++	+++	+++	+++	+++	+++	-	-
	15	+++	+++	-	-	-	-	-	-	-	-	-
	25	++	-	-	-	-	-	-	-	-	-	-
	40	++	+++	+	-	++	-	-	-	-	-	-
	48	+++	+++	++	-	-	-	-	++	-	-	-

Note: "+" = Positive test („+++" = High gas formation, „++" = Moderate gas formation, „+" = Low gas formation), "-" = No gas formation, LB (2×) = Lactose broth double strength, LB (1×) = Lactose broth single strength.

These water samples were stored at appropriate condition before microbiological and molecular analysis was done in the laboratory.

Isolation of *E. coli*

The presumptive test for the presence of coliform was done on lactose fermentation broth (LB) media. The presence of lactose fermenting bacteria in the collected drinking water samples were determined by observing the presence of gas formed in darham tubes which were immerged in LB media.

To confirm the presence of *E. coli* in the drinking water, the bacteria isolated from positive test samples were inoculated in Eosin Methylene Blue (EMB) agar plates and incubated at 37°C. Following incubation for 24 h, the morphology of the growth of bacteria was recorded. Then completed test (Gram stain) was used to examine the *E. coli* colonies that appeared on the EMB agar plates (Harley, 2004).

Quantitative analysis

The accumulated gas in the presumptive tubes enabled the determination of the number of organisms present in the sample by means of the Most Probable Number (MPN) test. The MPN was estimated by determining the number of tubes in each group that show gas following the incubation period. The calculations were done on microcomputer based on Thomas" simple formula from standard methods (Thomas, 1942). The formula is as follows:

MPN/100 ml = number of positive tubes × 100
V ml sample in negative tubes × ml sample in all tubes

RESULTS

The results of the presumptive test are shown in the Table 1. The first results were recorded after 24 h of inoculation and the second results were recorded after 48 h of inoculation of water samples. Among the 50 different water samples, 5 samples were found to be presumptive test positive.

The bacteria isolated from positive test samples were inoculated in the Eosin Methylene Blue (EMB) agar plates and incubated at 37°C. Following incubation for 24 h, the morphology of the growth of bacteria was recorded (Table 2 Figure 1).

Colonies from the EMB agar plates 8, 15, 25, 40 and 48 which showed metallic sheen were used to perform gram staining (Table 3).

From Table 4, it has been observed that Sample number 8 contains 1100 cells per 100 ml. At 95% confidence level, the lower limits were 150 and the upper limits were 4800. In the case of Sample number 15, it contains 9 cells per 100 ml. At 95% confidence level, the lower limits were 1 and the upper limits were 36. Sample number 25 contains 4 cells per 100 ml and at 95% confidence level, the lower limits were <0.5 and the upper limits were 20. Sample number 40 contains 3 cells per 100 ml. At 95% confidence level, the lower limits for sample 40 were <0.5 and the upper limit were 9. In the case of sample number 48, it contains 39 cells per 100 ml. At 95% confidence level, the lower limits were 7 and the upper limits were 130.

DISCUSSION

Control of microbiological water quality (drinking and domestic water) is a key issue because of health impact

Table 2. The presence of light dark and metallic sheen colonies on EMB agar plates.

Sample number	Organisms	LB2X-10			LB1X-1			LB1X-0.1			Positive control	Negative control
		A	B	C	D	E	F	G	H	I		
8	Dark Colony	++	++	++	+	+	+++ Metallic sheen	+	+	+++ Metallic sheen	+	-
	Light Colony	+	+	+	-	-	-	++	++	-	+	-
15	Dark Colony	+++	+++ Metallic sheen	-	-	-	-	-	-	-	-	-
	Light Colony	-	-	+++	++	++	++	++	+	++	+	-
25	Dark Colony	+	++ Metallic sheen	++	-	-	-	-	+	-	-	-
	Light Colony	-	+++	-	-	-	-	-	+	-	-	-
40	Dark Colony	+	-	-	++ Metallic sheen	-	-	+	-	-	-	-
	Light Colony	-	+	-	-	+	-	-	-	-	-	-
48	Dark Colony	+++	+++	+++ Metallic sheen	+	-	-	-	+	-	-	-
	Light Colony	-	-	-	-	-	+	-	-	-	-	-

Note: "+++" = Heavy growth, "++" = Moderate growth, "+" = Low growth, "-" = No growth.

Table 3. Completed test results.

Sample number	Lactose broth	Gram stain	Potability	
		Reaction/Morphology	Potable	Non-potable
8 (*E. coli* strain-1)	+	Red coloured cell under microscope	No	Yes
15 (*E. coli* strain-2)	+	Red coloured cell under microscope	No	Yes
25 (*E. coli* strain-3)	+	Red coloured cell under microscope	No	Yes
40 (*E. coli* strain-4)	+	Red coloured cell under microscope	No	Yes
48 (*E. coli* strain-5)	+	Red coloured cell under microscope	No	Yes

Note: "+" = Growth, "No" = Suitable for drinking, "Yes" = Not suitable for drinking.

Table 4. Determination of bacterial quantity from multiple tube tests by Most Probable Number (MPN) Method.

Sample number	Number of tubes giving positive reaction			MPN Index per 100 ml	95% Confidence limits	
	3 of 10 ml each	3 of 1 ml each	3 of 0.1 ml each		Lower limit	Upper limit
8 (*E. coli* strain-1)	3	3	2	1100	150	4800
15 (*E. coli* strain-2)	2	0	0	9	1	36
25 (*E. coli* strain-3)	1	0	0	4	<0.5	20
40 (*E. coli* strain-4)	0	0	1	3	<0.5	9
48 (*E. coli* strain-5)	3	0	1	39	7	130

Note: From standard methods for the examination of water and waste water, 14th edition, American Public Health Association, American Water Works Association, Water Pollution Control Federation, Washington, D.C, 1975.

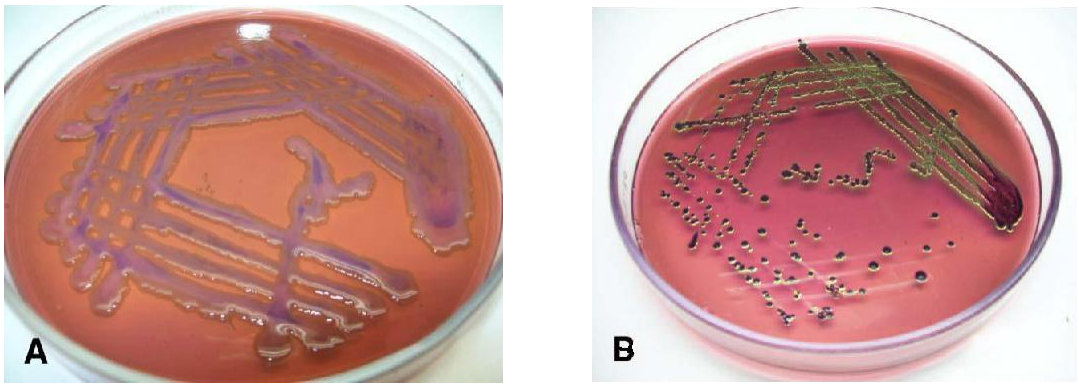

Figure 1: Types of colony found on EMB agar, A. light color colony, B. Green metallic sheen colony [Sample 8 (*E.coli* strain-1)].

and long-term sustainability. Nevertheless, there is a rather limited knowledge on the microbiological principles governing the prevalence and pathogenesis of emerging microbial pathogens in drinking water (Albinana-Gimenez et al., 2006). One of the main reasons for the lack of knowledge is that accurate detection, identification and quantification of microbial pathogens in water is difficult and only possible with a combination of conventional and molecular biology methods (Hossain et al., 2012).

The presence of *E. coli* in drinking water denotes that the water has been faecally contaminated and therefore presents a potential risk of excreta related diseases. Safe drinking water should have no *E. coli* in 100 ml of water (Moon, 2010). The findings of this study indicate that at least among the fifty samples, five samples are faecally contaminated.

Various researchers use lactose fermentation broth for presumptive test for coliform and EMB agar media to isolate *E. coli* (Ebrahimi and Lotfalian, 2005; Hossain et al., 2012). Traditional methods to culture *E. coli* are based on chromogenic and fluorogenic media designed to enumerate commensal *E. coli* (ComEC). In the context of this study, these media have limitations because these media cannot enumerate diarrhoeagenic *E. coli* (DEC). DEC consists mainly of the enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adhering *E. coli* (DAEC) and enterohaemorrhagic *E. coli* (EHEC) (Wells et al., 2010). EHEC are one of the most virulent types of bacteria that are now given significantly high importance among all food-borne pathogens. EHEC are also known as shiga-toxin producing *E. coli* (STEC) or verocytotoxin (VT)-producing *E. coli* (VTEC) (Hossain et al., 2012). Seropathotypes of EHEC are thought to be strongly associated with haemorrhagic colitis characterized by abdominal cramps, bloody diarrhea and dehydration. Other principal manifestations of illness in humans include heamolytic uraemic syndrome (HUS), which may lead to acute renal failure particularly among children and thrombotic thrombocytopeanic purpura (TTP) leading to various neurological disorders such as seizures, strokes and coma (Hossain et al., 2012; Trachtman et al., 2012).

Another important consideration is that culture-based methods are unable to detect *E. coli* (both ComEC and DEC) in what is termed the viable but non-culturable (VNBC) state (Mahmoud et al., 2012; Mezule et al., 2012). After exposure to adverse environmental condition, *E. coli* can enter the VBNC state (Wingender and Flemming, 2011). Therefore, it is highly urgent and significant to investigate other methods to determine the pathogenic contamination in the chlorinated water.

Molecular analyses can offer various advantages over culture-based methods, including detection of a wider range of target organisms with greater sensitivity and specificity (Matthews et al., 2010; Velusamy et al., 2010). It is also independent of culturability of bacteria, and requires no additional confirmation steps.

Conclusion

Unsafe drinking water continues to burden developing countries despite improvements in clean water delivery and sanitation, in response to Millennium Development Goal 7. Unsafe drinking water presents increased risk of opportunistic infections, and diarrhea-associated malabsorption of essential nutrients. So now is the time to widely promote household water treatment (HWT) systems in developing countries.

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