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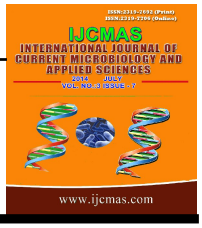

Original Research Article

Investigating potential of *Escherichia coli* in drinking water at a medical college and hospital

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Drinking water, MPN count, *Escherichia coli*

A B S T R A C T

Safe drinking water is a basic human requirement and is essential to all. A total of 116 drinking water samples were collected aseptically in sterilized container from different sources over a period of nine month September to May 2012. Most probable number (MPN) test was done to detect the *Escherichia coli* in drinking water. The MPN number was very high (≥ 180) of positive water samples. Analysis was performed using culture and biochemical methods. The organism was identified as *Escherichia coli* (28%) in total positive samples. To conclude, bacteriological assessment of all water sources for drinking should be planned and conducted on regular basis.

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). Diarrheal 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). The human pathogens that present serious risk of disease whenever present in drinking water include *Salmonella* species, *Shigella* species, pathogenic *Escherichia coli*, *Vibrio cholerae*, *Yersinia enterocolitica*, *Campylobacter* species, various viruses such as Hepatitis A, Hepatitis E, Rota virus and parasites such as *Entamoeba histolytica* and *Giardia* species and so on (Emde, 1992; Geldreich , 1992; Joklik *et al.*, 1992).

Escherichia coli is the most common coliform among the intestinal flora of warm-blooded animals and its presence might be principally associated with fecal contamination. No *Escherichia coli* are therefore allowed in drinking water.

Multiple-tube fermentation technique

The technique of enumerating coliforms by means of multiple-tube fermentation (MTF) has been used for over 80 years as a water quality monitoring method. The method consists of inoculating a series of tubes with appropriate decimal dilutions of the water sample.

Principle: Fermentation tubes (other suitable vessels containing lactose broth (MacConkey) are inoculated with measured volumes of water samples; the coliform bacteria present in the water sample multiply and are detected by formation of acid and gas. From the number with a positive reaction, the most probable number (MPN) of bacteria present in the original water sample can be determined statistically (Guideline manual for drinking water quality monitoring and assessment). This study was undertaken to identify and quantify *Escherichia coli* from drinking water sample in a medical college and hospital.

Materials and Methods

A total of 116 drinking water samples were collected from each source according WHO guidelines for drinking water quality assessment (Guideline manual for drinking water quality monitoring and assessment), over a period of nine months from September to May 2012.

Sample collection: About 200ml water samples from Government hand pump, water cooler and Municipal tap water were collected, labeled and transported to the laboratory for bacteriological analysis.

Bacteriolgical analysis: Bacteriological analysis was carried out for indicator organisms i.e. total and fecal coliform (*Escherichia coli*) by most probable number (MPN) method (Britton and Greeson, 1987; APHA, 1998). Ten tubes of MacConkey broth (Hi media Pvt. Ltd Mumbai) arranged in two rows with a 100ml blood culture bottle. First row containing 10ml double strength MacConkey broth was inoculated with 10ml of water sample and 50ml double strength MacConkey broth was inoculated with 50ml of water sample. Second row containing 1ml single strength MacConkey broth medium was inoculated with 1ml water sample respectively were incubated in an incubator at 44°C for 24 h.

After incubation, the number of bottles in which lactose fermentation with acid and gas production has occurred was counted. Finally, by referring to probability table (Macrady table-2) the MPN of coliform in 100 ml water sample was been estimated (Cheesbrough, 2006). Analysis is usually performed using culture and biochemical test also.

Results and Discussion

Total drinking water sample collected from Municipal tap water 58 (50%), from Government hand pump 32 (28%) and 26 (22%) collected from Water cooler. After determination of MPN number of positive sample, the culture & biochemical tests were performed for organism identification.

Culture: From the 1ml tube (single strength) of positive test, a loop full specimen was taken and streaked into the MacConkey agar plate and incubated at 37°C overnight.

Interpretation: A mixed growth of either dry lactose fermenting, mucoid lactose fermenting and non-lactose fermenting growth appeared after the incubation (Fig. 1). For specification, the colonies were subcultured on other individual MacConkey agar plate from primary plate and incubated at 37°C overnight. After the incubation a pure, heavy growth was appeared on MacConkey agar plate (Fig. 2).

Biochemical Test:

For species identification the biochemical (IMViC) tests also done as per WHO guideline (Fig. 3).

Conformational test of *Escherichia coli* was done by growth on Eoisin methylen blue (EMB) agar medium and incubated at 37°C for 24 to 48 hours, a bright metallic golden growth was appeared (Fig. 4).

Control of microbiological water quality (drinking and domestic water) is a key issue because of health impact 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 *Escherichia 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 *Escherichia coli* in 100 ml of water (Moon, 2010). Various researchers use lactose fermentation broth for presumptive test for coliform and EMB agar media to isolate *Escherichia coli* (Ebrahimi and Lotfalian, 2005; Hossain *et al.,* 2012).

Traditional methods to culture *Escherichia coli* are based on chromogenic and fluorogenic media designed to enumerate commensal *Escherichia coli* (ComEC). In the context of this study, these media have limitations because these media cannot enumerate diarrhoeagenic *Escherichia coli* (DEC). DEC consists mainly of the enteropathogenic *Escherichia coli* (EPEC), enterotoxigenic *Escherichia coli* (ETEC), enteroinvasive *Escherichia coli* (EIEC), enteroaggregative *Escherichia coli* (EAEC), diffusely adhering *Escherichia coli* (DAEC) and enterohaemorrhagic *Escherichia coli* (EHEC) (Wells, 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 Escherichia coli (STEC) or verocytotoxin (VT)-producing *Escherichia 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 *Escherichia coli* (both ComEC and DEC) in what is termed the viable but non-culturable (VNBC) state (Mahmoud, 2012; Mezule, 2012). After exposure to adverse environmental condition, *Escherichia 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, 2010). It is also independent of culturability of bacteria, and requires no additional confirmation steps.

In conclusion, we would like to recommend the proper sanitary survey, design and implementation of water and sanitation projects; regular disinfections, maintenances and supervisions of water sources and regular bacteriological assessment of all water sources for drinking should be planned and conducted.

1. The Government hand pump water is safe for drinking, bathing and food preparation as compared to municipal tap water and water cooler.
2. The water cooler revealed the high number of *Pseudomonas* sp. means required the proper maintenance by change the filter and washing the filter time to time as per guidelines.
3. The MPN number method is reliable and effective but time consuming to perform; use for all type of water.

Table.1 Biochemical characterization

Organism	Methyl Red	Voges - Proskauer	Indole	Urease	Citrate
Escherichia coli	+	-	+	-	-
Klebsiella	+	-	-	+	+
Pseudomonas sp.	-	-	-	-	+

Table.2 Profile of Positive Sample (n=116)

Source of Water ⟶	Municipal Tap Water	Government Hand Pump	Water Cooler	Total No.
Positivity	58	32	26	116
Organism				
Escherichia coli	20	-	1	21
Klebsiella Sp.	8	-	1	9
Pseudomonas Sp.	-	5	15	20
Mix Sample	12	-	1	13

Table.3 Profile of Mix Sample (n=13) with reference to sample no.

No. of Sample	Organism			
	Escherichia coli, Klebsiella Sp., *Pseudomonas* Sp.	*Escherichia coli, Klebsiella* Sp.	*Escherichia coli, Pseudomonas* Sp.	*Klebsiella* Sp., *Pseudomonas* Sp.
3	+	-	-	-
5	-	-	+	-
4	-	+	-	-
1	-	-	-	+

Table.4 Profile of total positive sample (n=63)

Source of water sample	No. of Sample collected	No. of Unsatisfactory sample (%)	Organism grown		
			Escherichia coli	*Pseudomonas* Sp.	*Klebsiella* Sp.
Municipal Tap Water	58(50%)	40(69%)	32	8	15
Government Hand Pump	32(27.59%)	5(15.62%)	-	5	-
Water Cooler	26(22.42%)	18(70%)	1	16	2
Total	116	63(54.31%)	33(28.44%)	29(25%)	17(14.65%)

Fig. 1 Mix growth on MacConkey Agar Plate

Fig. 2 Dry growth on MacConkey Agar plate

Fig.3 Confirmatory test of *Escherichia coli* On EMB Agar

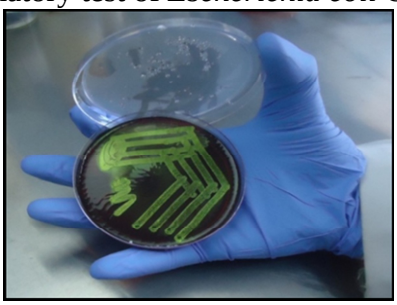

Fig.4 Biochemical test of *Escherichia coli*

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