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Microbiological Study on Supply Water and Treated Water in Dhaka City

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Water borne diseases are the major cause of morbidity and mortality in developing countries like Bangladesh, where disease outbreaks occur due to unsafe drinking water, inadequate sanitation and poor hygienic practices. The objective of this study was to evaluate the quality of water used for drinking as well as household purposes in Dhaka city, Bangladesh. Eighteen water samples were collected from different rivers and canals. Among those, two samples were treated with chlorine. Physico-chemical parameters (pH, salinity, turbidity, conductivity and dissolved oxygen) were monitored during the time of sample collection using portable meters. For microbiological analysis, water samples were collected in sterile screw capped bottle. All Experiments were carried out within 24 hours after collecting the samples. All the water sources were found to be contaminated with total coliform, faecal coliform, *Escherichia coli*, *Klebsiella* spp., *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*, *Aeromonas* spp. and Fungi except treated water samples. Several biochemical tests were performed for confirmation of suspected organisms. According to these results, huge amount of total coliform and faecal coliform were observed in every sample and these amount were very much similar for other organisms. Treated water samples were found to be microbiologically accepted than untreated water samples.

Clean and healthy drinking water is important for life. Access to safe drinking water has been an important national goal in Bangladesh and in other developing countries. In Bangladesh, for daily usage, only 2-3% of the people use water from the central water supply system, 8-9% of the people are served primarily by hand-operated tube well, a small portion of the urban people rely on open water sources including ring well, ponds and rivers while 80-90% of the rural people mostly depend on open water sources (1).

While Bangladesh has almost achieved accepted bacteriological drinking water standards for water supply, high rates of diarrheal disease morbidity indicate that pathogen transmission continues through the water supply chain (and other modes). Aquatic flora and fauna of marine environment that are used as food (usually not thoroughly cooked or via drinking water), where salt concentration is negligible, is the cause of primary transmission (2).

Diarrheal disease is a major cause of morbidity and mortality in developing countries, including Bangladesh. Among 50 diseases prevalent in Bangladesh, 40 of them including diarrhea, dysentery, typhoid, parasitic worm infection etc. are related to the contaminated food and water. Different strains of *E. coli* are responsible for a variety of diseases including diarrhea, dysentery, hemolytic uremia syndrome (kidney failure), bladder infections, septicemia, pneumonia, meningitis, etc. (3).

Among the *E. coli* strains, the major pathotypes were enteropathogenic *E. coli* (EPEC) enterotoxigenic (ETEC) *E. coli* (4). EAggEC, on the other hand, is an enteric pathogen associated mainly with persistent diarrhea in developing countries (5).

Cholera is a water borne infectious disease caused by *V. cholerae* especially by epidemic strains of *V. cholerae* O1 and *V. cholerae* O139. Generally cholera is transmitted by fecal oral route and a person becomes infected only after ingestion of bacterium. Normally ingestion of 10^8 to 10^9 viable cells of *V. cholerae* O1 is required to cause cholera. Transmission and yearly seasonal outbreaks of cholera are associated with environmental aquatic reservoir (6). Aquatic flora and fauna may act as possible reservoirs of cholera in endemic areas (7). *V. cholerae* is a facultative human pathogenic bacterium endemic to aquatic environments whose ecology remains poorly understood (8).

The existence of a viable but non-culturable (VBNC) state, in which cells are apparently intact, but have lost the ability to form colonies on standard plate-count media, led to propose that the formation of VBNC cell is analogous to spore formation in differentiating bacteria. VBNC bacteria are a major concern in public-health risk assessments because many pathogenic Gram-negative bacteria, such as *V. cholerae*, *V. vulnificus* and *E. coli*, have been reported to enter a VBNC state, from which they are able to return to infectious state after passaging in animal hosts (9).

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MATERIALS AND METHODS

The study was carried out to understand the quality of Water and Sewerage Authority (WASA) water in Microbiology Laboratory of Stamford University Bangladesh during the period of November 2010 to January 2011. Total 18 water samples were collected from different areas of Dhaka city. The local people use all of these waters for drinking or house hold purposes. In the case of water sample collection, water was collected from at least 2 different spots of each river and canal and then the samples were aseptically taken with pre-sterilized screw capped bottle. The sampled bottles were marked properly and transported to the laboratory as early as possible. All Experiments were carried out within 24 hours after collecting the samples. All these samples were kept at 18 ^{o}C until these were analyzed.

Inoculation and incubation. MacConkey agar and media for fecal coliform (mFC) agar were routinely used to determine the total coliform and the fecal coliform, respectively by membrane filtration technique. Eosin Methylene Blue (EMB) agar was used for selective isolation of *E. coli*. Sabouraud Dextrose Agar (SDA) was used for fungal count. Spread plate technique was used to inoculate the samples on various culture media for enumeration of microbes. Six hours of enrichment in selenite broth and alkaline peptone water were done before inoculating the samples to the Xylose Lysine Deoxicolate Agar (XLD) and Thiosulfate Citrate Bile Salt (TCBS) agar in order to determine the presence of *Salmonella, Shigella* and *V. cholerae*, respectively.

Bacterial count. Plate count was restricted to 30-300 colonies and plates containing more than 300 colonies were designated as too numerous to count (TNTC) and plates containing fewer than 30 colonies were designated as too few to count (TFTC). The following formula was used for enumeration.

Number of cells per ml = number of colonies x dilution factor / volume of sample used (cfu/g)

Identification of microorganisms. Identification of bacterial isolates was carried out by different biochemical tests such as Triple Sugar Iron Test (TSI), Motility Indole Urease test (MIU), Citrate Utilization test, Methyl Red (MR) Test, Voges - Proskauer (VP) test, Oxidase test and salt tolarence test.

Treatment process of water. At first, water was transported from Shitolakhya River to DND canal and then from DND canal to Sydabad intake point. In this stage water was treated with chlorine and then conveyed to clarified stage (Fig. 1). Here Alum Sulfate was mixed with water and transported to the filter bed for filtration and at the last stage again water was treated with chlorine to examine the last count of organisms and transported to the treated water tank very safely.

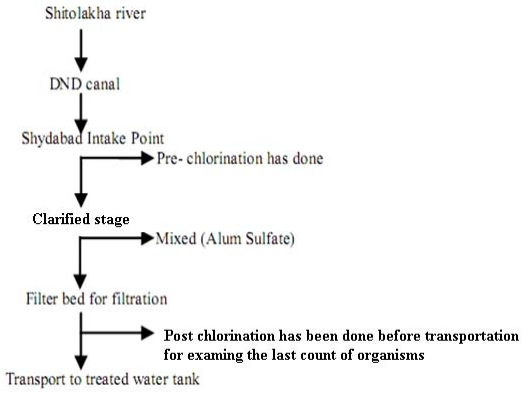

FIG 1: A schematic diagram showing the treatment procedure of supplied water.

RESULTS

Total coliform, and fecal coliform count in per 100ml is shown in table 1. According to the table, Sharulia river water (SW-1) contained the highest amount (TNTC colonies per 100ml) of total coliforms and Shitolakha river water (SR-2) and canal (DC-1) both contained the highest amount (3 colonies per 100ml) of fecal coliforms.

Among these 18 samples, DND canal (DC-2), Fresh water (FW-1& FW-2), clarified water (CW-1 & CW-2), and raw water (RW-1) were devoid of any fecal coliform but contained total coliform. Only treated water (TW -1 & TW-2) were free from both total & fecal coliform.

Table 1. Total coliforms, and fecal coliforms count of water samples per 100 ml

Sample ID	Total coliforms count/100 ml	Fecal coliforms Count/100 ml
SIP-1	02	01
SIP-2	02	01
SR-1	01	01
SR-2	03	03
DC-1	02	03
DC-2	02	0
FW-1	02	0
FW-2	02	0
CW-1	01	0
CW-2	01	0
SW-1	TNTC	01
SW-2	02	01
RW-1	01	0
RW-2	01	01
MB-1	02	01
MB-2	01	02
TW-1	0	0
TW-2	0	0

Note: TNTC-Too numerous to count

Frequency of other bacterial isolates of water sources in Dhaka city. The organisms consistently isolated as number of positive samples with their percentage frequency of occurrence. The result is given below in Table 2.

Table 2. Percentage of bacterial isolates.

Total sample	Group of bacteria	Number of positive sample	Percentage (%)
	Escherichia	03	16%
	Klebsiella	13	72%
18	*Salmonella*	09	50%
	Shigella	12	69%
	V. cholerae	03	16%
	Aeromonas	07	39%

Table 3. Comparison of treated and untreated water

Organisms	Treated water samples	Open water samples
E. coli	-	+
Klebsiella	-	+
Salmonella	-	+
Shigella	-	+
V.cholerae	-	+
Areomonas	-	+
Fungi	+ (very low amount)	+ (high amount)

+ : positive, - : negative

According to the comparison it was easily understood that the treated water samples were microbiologically acceptable.

Table 5. Summary of biochemical tests of *V. cholerae*

Suspected organism	Oxidase test	6.5% & 8% salt tolerant test
V. cholera	+	+

DISCUSSION

Life asks invariably for clean and healthy drinking water. In Bangladesh, water borne diseases are mainly related to the use of contaminated surface water (10). This is a frequent problem in rural area. Over 80% of the population of Bangladesh live in around 64,000 villages of this agrarian country. Villages lack good sanitation and clean drinking water. The sources of water in Bangladesh are surface water, groundwater and rainwater. The Buriganga, Brahmaputra, Meghna and Shitolakha river system discharges huge amount of surface water across Bangladesh, a part of which enters into ground to form groundwater. About 93% of the stream flow passing through the country originates from outside the Bangladesh. Rainfall within country contributes to the total water available in Bangladesh, a part of which infiltrates into ground to recharge existing groundwater, and the remaining rainwater flows as surface run-off.

The present study was designed to enumerate bacterial flora and identify pathogenic bacteria from river and canal water. In addition comparison between treated and untreated water supply from WASA in Dhaka was performed. People usually use water of these sources for their drinking purposes and various household activities such as washing utensils. As river and canal is open surface reservoir, it is very common to get huge amount of organisms. Fecal coliforms can contaminate these sources

Table 4. Summary of biochemical tests of *E. coli, Klebsiella, Salmonella, Shigella, and Aeromonas*

Assumed organism	TSI			H_2S creation	Indole test	MR test	VP test	Citrate test	Motility	Oxidase test
	Slant	Butt	Gas							
E. coli	Y	Y	+	-	+	+	-	-	+	ND
Klebsiella	Y	Y	+	-	-	-	+	+	-	ND
Salmonella	R	Y	-	+	-	+	-	-	+	ND
Shigella	R	Y	+	+	+/-	+	-	-	-	ND
Aeromonas	R	Y	-	-	ND	ND	ND	+	ND	-

ND: Not done; Y: yellow: R: red

discharge of waste from mammals and birds, from manure which are usually used as fertilizer in agriculture and storm runoff and from human sewage.

In this study a total of eighteen water samples were collected from different rivers and canals in Dhaka city. All of these samples were tested for the enumeration of total coliforms, faecal coliforms, *E. coli, Klebsiella* spp, *Salmonella* spp, *Shigella* spp, *V. cholerae and Aeromonas* spp. Physicochemical parameters of those water sources were detected to check the quality. Every sample contained coliform, faecal coliform and many pathogenic bacteria but treated water was free from microorganisms.

This study showed that, among all sources of water, the Shitolakha river, Sharulia water, DND canal and Clarified water contained higher amounts of total coliforms, fecal coliforms, have *E. coli and Klebsiella* than other sources. It might occur due to the presence of hanging latrines around the rivers and canals. Faecal materials are also drained to rivers and canals by water run off during rainy season.

In fine, it can be concluded that the treated waters are relatively free from microorganisms but the untreated ones are extremely polluted by bacteria and perilous pathogens. In addition, the quality of treated water samples from WASA were found to be microbiologicaly acceptable and hence it indicates that the treatment process of WASA is satisfactory.

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