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BACTERIAL LOAD IN DRINKING WATER SUPPLIED AT DIFFERENT PRIVATE HOSPITALS IN DHAKA, BANGLADESH

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Abstract

Water samples supplied in different private hospitals of Dhaka city, Bangladesh were collected for microbiological analysis. The hospitals were Ibrahim Memorial Hospital (BIRDEM), Lab Aid Hospital, Gonoshasthaya Nagor Hospital, Ibn Sina Hospital and Japan Bangladesh Friendship Hospital. Aerobic heterotrophic bacterial count ranged between 1.8×10^4 and 4.6×10^4 cfu/100 ml. Enteric and related bacterial abundance in MacConkey, SS and Cetrimide agar media ranged from 0 to 7.6×10^4, 0 to 3.4×10^4 and 0 to 2.2×10^4 cfu/100 ml, respectively. A total of 27 bacteria was isolated during the study period. Among them 19 were heterotrophic and 8 were enteric and related bacteria. Among the heterotrophic isolates 14 were gram-positive, of which 13 were rod shaped, spore former under the genus *Bacillus viz. Bacillus circulans, B. fastidiosus, B. badius, B. alcalophilus, B. brevis, B. lentus, B. firmus, B. subtilis* and another one was coccus as *Planococcus*. Five heterotrophic gram-negative isolates were under the genus *Pseudomonas* and *Aeromonas*. All enteric and related isolates were gram-negative, short rod and non-spore former and belong to genera *Salmonella, Shigella, Klebsiella Pseudomonas* and *Escherichia*.

Key words: Bacteria, Drinking water, Private hospitals

Introduction

Access to safe drinking water is one of the basic human rights and is enormously crucial to health. For a nation to maintain optimal health and development there has to be continual supply of safe drinking water to its population. According to the presence of thermo tolerant (fecal) coliform or *E. coli* could be classified on color code scheme. A count of 0 *E. coli*/100 ml of sample in conformity with the WHO guidelines is as category A (blue), 0-10 *E. coli* as category B (green) with low risk, 10-100 *E. coli* as category C (yellow) with intermediate risk, 100-1000 *E. coli* as category D (orange) with high risk, and >1000 *E. coli* as category E (red) with very high risk for drinking water (Drechsel *et al.* 2008). Coliform bacteria are indicator organisms used to determine the efficiency of treatment and the integrity of the water work system. *E. coli* bacterium is a sub-group of total coliform bacteria and is used as a screen for fecal contamination. Thus,

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the presence of coliform organisms in treated water indicates that barriers are ineffective and there is an increased chance for waterborne pathogens to follow the same pathway.

However, the absence of coliforms does not guarantee safe water and the presence of coliforms does not necessarily indicate an immediate hazard. Generally, the presence of total coliform indicates that treatment is not effective or that there is a secondary contamination in the water works system such as from a cross connection or biofilm growth or accumulation (Hart and Kariuki 1998). The presence of *E. coli* indicates ineffective treatment and recent fecal contamination (Rompre´*et al.* 2002). Pathogenic bacteria that may be associated with faecal contamination include pathogenic strains of *E. coli*, *Campylobacter* sp., *Salmonella* sp., *Shigella* sp. and *Vibrio cholerae*. In addition to these organisms causing human diseases, resistance to antibiotics has made treatment of the diseases they cause more difficult (Okeke *et al.* 2007). According to the UN, diarrhoea accounts for 80% of all diseases and over one third of death in developing countries, which are caused by the patients' consumption of contaminated water (Al-Khatib *et al.* 2003). In a medical environment, the presence of pigmented bacteria in tap water which is used to supply through humidifiers or other water-using therapy machines or instruments, poses a potential health problem that includes gastrointestinal upsets, pyrogenic reactions (Quarles *et al.* 1974). Among the pathogens disseminated in water sources, enteric pathogens are the ones most frequently encountered. As a consequence, sources of fecal pollution in waters devoted to human activity must be strictly controlled. Entero-pathogens, such as *E. coli* O157:H7, are generally present at very low concentrations in environmental waters within a diversified micro flora. Complex methods are required to detect them and these are time consuming (Rompre´ *et al.* 2002). The aim of this study was to compare the bacteriological contamination and enumeration of both heterotrophic and enteric bacteriological abundance in 5 hospitals, situated in Dhaka, Bangladesh.

Materials and Methods

Study site and sampling: In the present study water samples were collected from five private hospitals in Dhaka city, namely Ibrahim Memorial Hospital or BIRDEM, Lab Aid Hospital, Japan Bangladesh Friendship Hospital, The Ibn Sina Hospital and Gonoshasthaya Nagor Hospital which are currently the leading and well equipped private hospitals in Dhaka city. Sterile plastic bottles were used for sample collection. Tap and filter water supplied at these hospitals were collected twice during sampling period (Plate. 1).

Isolation of bacteria: Nutrient agar (NA) medium was used for the enumeration and isolation of aerobic heterotrophic bacteria, while MacConkey agar (Difco), SS agar (Diagnostic Pasteur) and Cetrimide agar (Difco) media were used for the determination and isolation of enteric bacteria present in water samples. The pH of the medium was adjusted to 6.5 since most of the samples were within the range of 6.2-6.5. Three different

Plate. 1. Map showing the close up view of sampling sites. A). 1. Ibrahim Memorial Hospital, B). 2. Lab Aid hospital, 3. Gonoshasthaya Nagor Hospital, 4. Japan Bangladesh Friendship Hospital and 5. Ibn Sina Hospital.

techniques, *viz.* Serial dilution plate technique (Greenberg *et al.* 1998), Spread plate (Sharp and Lyles 1969) and Membrane filtration techniques (Atlas *et al.* 1995) were used for the enumeration and isolation of bacteria. The pH was adjusted before the addition of agar and sterilization. All the culture plates were marked with sample name and incubated at 37°C in the dark for 48 hours. Bacterial colony counting was made with the help of a digital colony counter (DC-8 OSK 100086, Kayagaki, Japan). Discrete bacterial colonies were isolated immediately after counting. In case of MacConkey agar medium, pink or brick red colonies were considered as coliform bacteria, while white colonies were considered as non-lactose fermenter, whereas in SS agar medium, black colonies were considered as highly pathogenic. In Cetrimide agar medium, green colonies were considered *Pseudomonas* and pathogenic. During this investigation, of the total 40 isolates from nutrient agar medium, 27 were randomly selected and purified for provisional identification on the basis of their morphological characters, gram reaction and necessary biochemical tests.

Physico-chemical properties of water: Temperature of water samples was measured by a mercury centigrade thermometer. pH was measured in the laboratory after collection of samples by a electric pH meter (Jenway 3310 pH meter, U.K) immediately after the collection of the samples.

Identification of bacteria: Important physiological and biochemical characteristics were studied for the identification of the selected isolates. Bergey's Manual of Systematic Bacteriology (Sneath *et al.* 1986) was followed for the identification of aerobic heterotrophic bacteria while, Manual for laboratory investigations of acute enteric

infections (WHO 1987) and Bergey's manual of systematic bacteriology (Krieg and Holt 1984) were consulted for gram negative, enteric and related bacteria.

Results and Discussion

The physico-chemical properties of the water samples are given in Table 1. Temperature and pH of the collected samples varied in different hospitals. The water temperature ranged between 28.1 and 31.5 °C. Minimum water temperature 28.1°C was recorded in the Ibrahim Memorial Hospital, while maximum 31.5°C was in Gonoshasthaya Nagor Hospital. The pH of the water samples ranged between 6.2 and 6.5. The maximum pH (6.5) was found in Lab Aid Hospital, while the minimum (6.2) was in the Gonoshasthaya Nagor Hospital.

Table 1. Water temperature and pH of different samples.

Serial no.	Sampling sites	Water temperature (°C)	pH
1	Ibrahim Memorial Hospital	28.1	6.3
2	Lab Aid Hospital	30.6	6.5
3	Gonoshasthaya Nagor Hospital	31.5	6.2
4	Ibn Sina Hospital	30.0	6.5
5	Japan Bangladesh Friendship Hospital	30.2	6.2

Aerobic heterotrophic bacterial count was higher in comparison to bacterial count of enteric and related bacteria. Aerobic heterotrophic bacterial count ranged between 1.8×10^4 cfu/100 ml and 4.6×10^4 cfu/100 ml. In SS agar average bacterial counts varied from 0 to 3.4×10^4 cfu/100 ml and the count showed significant difference in different hospitals and no bacterial colony was observed in Ibrahim Memorial Hospital and Lab Aid Hospital. Bacterial count on MacConkey agar ranged between 0 and 7.6×10^4 cfu/100 ml, but no colony was observed in water collected from Ibrahim Memorial Hospital. In MacConkey agar medium the count also showed significant difference among hospitals. In cetrimide agar medium bacterial count was within the range of 0 to 2.2×10^4 cfu/100 and among the five hospitals, no bacterial count was observed in three hospitals (Table 2).

A total of 27 bacteria was isolated of which, 19 were heterotrophic and 8 were enteric and related bacteria (Table 3). Among the heterotrophic isolates 14 were gram-positive, of which, 13 were rod shaped, spore former and member of the genus *Bacillus* and the other was coccus shaped of *Planococcus*. The five heterotrophic gram-negative isolates were under the genus *Pseudomonas and Aeromonas*. In the genus *Bacillus*, there were eight distinct species, *viz. Bacillus circulans* (2), *B. fastidiosus* (2), *B. badius* (2), *B. alcalophilus* (2), *B. brevis*, *B. lentus*, *B. firmus* and *B. subtilis* (2). All enteric and related

isolates were gram negative, short rod, non-spore former and belonged to genera *Salmonella*, *Shigella*, *Klebsiella*, *Pseudomonas* and *Escherichia* (Table 4). The World Health Organization (WHO) allows less than 10 coliform per 100 ml as maximum for

Table 2. Bacterial count (cfu/100 ml) of the water samples of different private hospitals.

Sampling sites	Aerobic heterotrophic bacteria	Enteric and related bacteria on		
		MacConkey agar	SS agar	Cetrimide agar
Ibrahim Memorial Hospital	2.1×10^4	0	0	0
Lab Aid Hospital	4.6×10^4	2.7×10^4	0	0
Gonoshasthaya Nagor Hospital	1.8×10^4	7.6×10^4	3.4×10^4	0
Ibn Sina Hospital	3.5×10^4	8.7×10^3	2.8×10^4	2.2×10^4
Japan Bangladesh Friendship Hospital	4.0×10^4	3.4×10^4	3.3×10^4	1.2×10^4

Table 3. Biochemical characteristics and provisional identification of the selected heterotrophic bacterial isolates.

Isolate No.	Oxidase	Catalase	Starch	Casein	Tyrosine	VP	MR	Nitrate reduction	Citrate utilization	H_2S	Gelatin	Provisionally identified name
IS-3	–	+	–	+	+	–	–	+	–	+	+	*Pseudomonas syringae*
GN-4	–	+	+	+	–	+	–	+	–	–	+	*B. brevis*
BA-11	–	+	+	+	–	+	–	+	+	–	+	*B. lentus*
LA-16	+	+	+	+	–	+	–	+	+	–	–	*B. firmus*
LA-22	+	+	–	+	–	+	–	+	+	–	+	*B. fastidiosus*
BA-18	–	+	+	+	–	+	+	+	–	–	+	*B. fastidiosus*
IS-9	+	+	–	+	+	–	–	+	–	+	+	*P. putida*
BA-29	+	+	+	+	–	+	–	+	+	–	+	*B. circulans*
BA-34	–	+	–	+	+	–	–	+	+	+	+	*P. aeruginosa*
OP-23	+	+	+	+	–	+	–	+	–	–	+	*B. subtilis*
JB-33	+	+	–	–	–	–	–	+	–	–	+	*Aeromonas* sp.
GN-17	–	+	+	+	–	+	–	+	–	–	+	*B. circulans*
OP-57	–	+	+	+	–	+	+	+	+	–	+	*B. badius*
GN-25	–	+	–	+	–	+	+	+	–	–	+	*Planococcus citreus*
BA-48	–	+	+	+	–	+	–	+	–	–	–	*B. subtilis*
IS-19	–	+	+	+	–	+	+	+	–	–	+	*B. alcalophilus*
IS-36	+	+	–	+	+	–	–	+	+	+	+	*P. aeruginosa*
BA-53	+	+	+	+	–	+	–	+	+	–	+	*B. alcalophilus*
BA-67	–	+	+	+	–	+	+	+	–	–	+	*B. badius*

"+" indicates the positive result and "–" indicates the negative result.

Table 4. KIA test for enteric and related isolates.

Isolate No.	KIA test				Provisionally identified name
	Slant	Butt	H_2S	Gas	
OP-3	A	A	+	–	*Salmonella* sp.
GN-13	A	A	+	–	*Klebsiella pneumoniae*
JB-9	A	A	+	+	*P. aeruginosa*
JB-15	K	A	+	–	*P. aeruginosa*
JB-28	A	A	+	–	*E. coli*
OP-9	K	A	+	–	*K. pneumoniae*
GN-11	A	A	–	–	*Shigella* sp.
GN-19	A	A	–	–	*Shigella* sp.

"+" sign indicates positive results, "–" sign indicates negative results, K = Alkaline red and A = Acid.

small community supplies. According to WHO, European and International Standards for drinking water, no coliform should be present in 90% samples (WHO 1970). Yusen *et al.* (2011) reported that hospital-acquired Legionnaires' disease is directly linked to the presence of *Legionella* in hospital drinking water. LeChevallier and seidler (1980) reported that *Staphylococcus aureus* may be contaminating the well or has colonized the storage tank or house lines. In such cases, the faucet screen surfaces could have provided the necessary nutritional conditions to promote re-growth or provided the focal point. Hashmi and Shahab (1999) advocated for the strong need to establish standards and guidelines for quality drinking water. Preventive measures must be taken at all levels to prevent water contamination. Protection of water sources should be the first line of defense and all water distributing agencies must be supplied copies of the finalized standards. This study revealed that drinking water supplied in the different public hospitals was contaminated with fecal and non-fecal coliforms with other pathogenic bacteria. Abundance of heterotrophic bacteria was also significantly harmful for human consumption. In the present observation the total and faecal coliform count in all the sampling stations were higher than the recommended standards.

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