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MICROBIOLOGICAL QUALITY OF DRINKING WATER AND THEIR ANTIBIOGRAM AT ROADSIDE RESTAURANTS IN DHAKA CITY

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Abstract

The microbiological quality of water from dispensers in different roadside hotels and restaurants of Dhaka city was analyzed. Aerobic heterotrophic bacterial count ranged between 1.4×10^5 and 5.1×10^9 cfu/100 ml on PYG agar medium. Total coliform count was on MacConkey agar and ranged between 9.3×10^4 and 5.4×10^8cfu/100 ml. *Salmonella* and other bacteria grown on Salmonella-Shigella (SS) agar and the total count ranged between 3.1×10^4 and 7.0×10^5cfu/100 ml, while *Pseudomonas aeruginosa* was grown on cetrimide agar and the count ranged between 0 and 2.0×10^4cfu/100 ml. A total of 116 bacterial colonies were isolated of which 35 were selected for further study. Among them 18 isolates were heterotrophic and 17 were enteric and related bacteria. Among heterotrophic isolates, 15 were Gram-positive and 3 were Gram-negative bacteria. Out of 15 Gram-positive isolates 7 were *B. circulans*, *B. pumilus* (2), *B. subtilis* (3) and *B. coagulans*; 4 were *Micrococcus lylae*, *M. varians*, *M. nishinomiyaensis* and *M. roseus* and others were *Kurthiagibsoni, Listeria denitrificans* (2) and *Corynebacterium diptheriae*. Three Gram-negative isolates were *Pseudomonas aeruginosa* (2) and *Actinobacillus lignieresii*. All the 17 enteric and related isolates were Gram-negative, short rod and non-spore former and these were identified as *Escherichia*, *Klebsiella*, *Salmonella* and *Pseudomonas*.

Introduction

Safe and clean drinking water is the basic need for human good health. However, even in developed countries, sometime drinking water fails the quality and becomes considerable public health hazardous. In particular, microbiological quality failures can be a significant threat to the supply of drinking water. In public water supplies, inefficient water treatment of the source could result in unwanted microorganisms entering water distribution systems. The contamination of potable water has been frequently found associated with transmission of diseases causing serious illness and mortality throughout the world (Jones *et al*. 2007). The presence of *E. coli* in humans and animals as their natural hosts creates opportunities for contamination of drinking water if proper hygiene is not practiced (Echeverria *et al*. 1984).

The presence of coliforms, and faecal coliforms, is regarded as an index of bacteriological quality of water and food, though some indicator strains are pathogens, for example the toxigenic *E. coli* strains (Ohno *et al*. 1997). *E. coli* O157 : H7 has been responsible for several deaths have been documented through food- and waterborne outbreaks (Jones and Roworth 1996). Such toxigenic *E. coli* are also problematic to detect, as they may form viable but non-culturable cells in water (Kogure and Ikemoto 1997).

The faecal coliform along with some other members of the Enterobacteriaceae such as *Salmonella*, *Shigella*, *Klebsiella*, *Proteus*, *Serratia*, *Pasteurella*, *Yersinia* and *Erwina* as well as *Vibrio* and *Pseudomonas* are known to be involved in the transfer of antibiotic resistance by means of R-factors (Chatterjee and Starr 1972). Most strains of *E. coli* are generally harmless and certain of them are able to cause in human diseases, such as entero-pathogenic *E. coli* (EPEC), entero-invasive *E. coli* (EIEC), Shiga toxin-producing *E. coli* (STEC), entero-aggregative *E. coli*

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(EAEC), entero-toxigenic *E. coli* (ETEC) and diffusely adhering *E. coli* (DAEC) (Turner *et al*. 2006). Antimicrobial resistance among entero-pathogens, including *E. coli* has been reported to be increasing in recent years (Pitout and Laupland 2008) sometimes leading to point-break situations where no antibiotic treatment options remain. The burden of water-related disease varies according to context and is highest in low-income settings where diarrhea remains a leading cause of child deaths. According to the UN, diarrhea accounts for 80% of all diseases and over one third of deaths in developing countries, which are caused by the patients' consumption of contaminated water (Al-Khatib *et al*. 2003). The most common among these include the *Mycobacterium avium* complex (MAC), comprising *M. avium* and *M. intracellulare*, two clearly different species. An increase in the immunodeficient population and the prevalence of non-tuberculous mycobacteria in water systems contribute to an emerging problem of waterborne mycobacterial infections (Von Reyn *et al*. 1994) were among the first to document a relation between infections in HIV/AIDS patients and water as a source of MAC.

In Bangladesh, a large number of people live in Dhaka city and have their meals in various roadside hotels and restaurants and those hotels and restaurants provide low cost water in glass from a large closed container of various companies by dispensing machines. In recent times, the microbiological safety of drinking water has become a burning issue and public awareness is gradually increasing regarding waterborne diseases. Therefore, the present project was undertaken for enumeration of both heterotrophic and enteric bacteriological abundance and comparison microbial abundances among those hotels and restaurants situated in Dhaka city and lastly to find out a way to improve the quality of the drinking water.

Materials and Methods

Water samples were collected from Mama hotel and restaurant, Mayer Badhon Tehari Ghor, Mamun Biriyani house and Aftab hotel and restaurant in sterile plastic bottles and were kept in ice box before analysis.

Nutrient agar medium was used for the enumeration and isolation of aerobic heterotrophic bacteria, while MacConkey agar medium (Difco), SS agar medium (Diagnostic Pasteur), Cetrimide agar (Difco) media were used for the determination and isolation of enteric bacteria from water samples. The pH of the medium was adjusted at 7. Serial dilution plate technique (Greenberg *et al*. 1998), Spread plate technique (Sharp and Lyles 1969), and Membrane filtration technique (Atlas *et al*. 1995) were used for the enumeration and isolation of bacteria. All the culture plates were marked with sample name and incubated at 37°C for 48 hrs. Bacterial colonies were counted by a digital colony counter (DC-8 OSK 100086, Kayagaki, Japan). Discrete bacterial colonies were transferred onto nutrient agar slants. 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 as pathogenic *Pseudomonas* sp.

During this investigation, of the total 50 isolates from nutrient agar medium, finally a total 36 were randomly selected and purified for detailed identification.

Temperature of water samples was measured by a mercury centigrade thermometer. pH was measured in the laboratory after collection of samples by an electric pH meter (Jenway 3310 pH meter, U.K). 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 provisional 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.

Antibacterial sensitivity test was carried out with gentamycin (GEN-10), erytromycin (E-15), penicillin (P-10), doxycyclin (DO-30) and streptomycin (S-10) against the selected bacteria were tested for their ability to grow in the presence of different antibiotics at concentration selected for diagnostic value. The filter paper disks placed on the surface of Muller Hinton Agar (Atlas 1997) plates inoculated with 0.1 ml of bacterial suspension. Inoculated plates incubated at 37ºC for 24 hrs. The antibiotic disks gentamycin (GEN-10), erytromycin (E-15), penicillin (P-10), doxycyclin (DO-30) and streptomycin (S-10) were used. Development of a clear zone around the disk indicated sensitivity while antibiotic disk without clear zone indicated resistance to the antibiotic.

Results and Discussion

The physicochemical properties of the samples were studied. The water temperature ranged between 18 and 29ºC. Minimum water temperature was 18ºC recorded in the Mayer Badhon Tehari Ghor. Maximum was 29ºC recorded in the Aftab hotel and restaurant. The pH of the sample water ranged between 6.03 and 7.43. The maximum pH (7.43) was found in the sample of Mamun Biriyani house while the minimum (6.03) was recorded in sample of Aftab hotel and restaurant.

Aerobic heterotrophic bacterial count was higher in comparison to bacterial count of enteric and related bacteria. Aerobic heterotrophic bacterial count ranged between 1.4×10^5 and 5.1×10^9cfu/100 ml. In SS agar average bacterial count varied from 3.1×10^4 to 7.0×10^5cfu/100 ml and significant difference was found in different samples. Bacterial count on MacConkey agar ranged between 9.3×10^4 and 5.4×10^8cfu/100 ml. In cetrimide agar medium bacterial count was within the range of 0 to 2.0×10^4cfu/100 ml and no bacterial colony was observed in Mama hotel and restaurant and Mamun Biriyani house (Table 1).

Table 1. Bacterial count (cfu/100 ml) of the water samples of different hotels and restaurants.

Sampling sites	HPC	Enteric and related bacteria on		
		MacConkey agar	SS agar	Cetrimide agar
Mama hotel and restaurant	1.4×10^5	9.0×10^5	7.0×10^5	0
Mayer Badhon Tehari Ghor	1.5×10^6	1.0×10^5	4.9×10^4	2.0×10^4
Mamun Biriyani house	5.1×10^9	9.3×10^4	6.0×10^4	0
Aftab hotel and restaurant	8.6×10^6	5.4×10^8	3.1×10^4	2.0×10^4

Considering the physiological characteristics of the bacterial isolates, provisional identification was made. A total 35 bacteria were isolated, of them 18 were heterotrophic isolates and 17 were enteric and related bacteria (Table 2). From the 18 aerobic heterotrophic bacteria 15 were Gram-positive bacterial strains of which 7 belong to the genus *Bacillus* and rest 4 Gram-positive bacterial isolates were identified as *Micrococcus.* Under the genus *Bacillus* the provisionally identified species were *B. circulans*, *B. pumilus* (2), *B. coagulans*, *B. subtilis* (3) and other 4 were *Kurthia gibsoni, Listeria denitrificans* (2) and *Corynebacterium diptheriae*. The three heterotrophic Gram-negative bacterial isolates were *Pseudomonas aeruginosa* (2) and *Actinobacillus lignieresii*. All 17 enteric and related isolates were Gram-negative, short rod and non-spore former and belong to genera *Escherichia*, *Klebsiella*, *Salmonella* and *Pseudomonas*. The *E. coli* strains selected persisted beyond the 70-day experiment, with greater persistence

Table 2. Biochemical characteristics and provisional identification of the selected heterotrophic bacterial isolates.

Isolate no.	Oxidase	Catalase	Starch	Casein	Tyrosine	VP	MR	Nitrate reduction	H_2S	Gelatin	Provisionally identified name
MB-21	–	+	–	–	+	–	+	+	–	–	*Micrococcus lylae*
MB-22	–	+	–	–	–	–	+	+	+	–	*Micrococcus varians*
MB-23	–	+	+	+	–	+	+	+	–	+	*Bacillus coagulans*
MB-24	–	+	–	+	–	+	+	–	–	+	*Bacillus pumilus*
MB-33	–	+	–	+	–	+	–	–	–	+	*Micrococcus nishinomiyaensis*
MH-11	+	+	–	+	+	–	–	+	+	+	*Pseudomonas aeruginosa*
MH-12	–	+	+	+	–	+	+	+	–	+	*Bacillus subtilis*
MH-13	–	+	–	–	+	–	+	–	–	–	*Kurthia gibsoni*
MH-21	–	+	+	+	–	+	+	+	–	+	*Bacillus subtilis*
MH-22	–	+	+	+	–	+	+	+	–	+	*Bacillus subtilis*
MH-31	+	+	+	+	–	+	+	+	–	+	*Actinobacillus lignieresii*
MH-32	+	+	+	–	–	+	+	+	–	+	*Listeria denitrificans*
MH-35	–	+	+	+	–	+	+	+	–	+	*Bacillus pumilus*
MT-11	–	+	+	+	–	+	–	+	–	–	*Corynebacterium diptheriae*
AH-21	–	+	–	–	+	–	+	+	–	–	*Micrococcus roseus*
AH-22	–	+	+	+	–	+	+	+	–	+	*Bacillus circulans*
AH-31	+	+	–	+	+	–	–	+	+	+	*Pseudomonas aeruginosa*
AH-41	–	+	+	–	–	–	+	+	–	–	*Listeria denitrificans*

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

evident in the sterile microcosms in most cases and with *T*90 values indicating survival for considerably long periods in either drinking water or filter sterilized (0.22 μm) autoclaved drinking water (Abberton *et al*. 2016).

Bacterial isolates were tested for their antibiogram activities. Out of 16 tested isolates, 7 were susceptible to all antibiotics at different ranges, while isolate MH-32 and AH-41 were completely resistant to all five antibiotics (Table 3). Rest of the isolates was shown to be sensitive to some antibiotics and resistant to other antibiotics. Seven isolates were shown to resistant to penicillin only but sensitive to other antibiotics. However, 3 isolates (MB-21, MH-11 and AH-31) were resistant to both penicillin G and erythromycin.

Table 3. Antibiogram of the selected isolates.

Isolate No.	Inhibition zone measured in diameter (mm)				
	Name of the antibiotics				
	E-15	P-10	GEN-10	S-10	DO-30
MB-21	R	R	S (4)	S (3)	S (2)
MB-22	S (8)	R	S (6)	S (3)	S (7)
MB-23	S (9)	S (7)	S (14)	S (10)	S (19)
MB-24	S (15)	R	S (12)	S (9)	S (19)
MB-33	S (2)	S (24)	S (19)	S (16)	S (20)
MH-11	R	R	S (19)	S (9)	S (2)
MH-12	S (19)	S (6)	S (22)	S (6)	S (17)
MH-13	S (4)	R	S (12)	S (8)	S (6)
MH-21	S (24)	S (1)	S (21)	S (12)	S (19)
MH-22	S (23)	S (2)	S (13)	S (12)	S (20)
MH-31	S (22)	S (2)	S (14)	S (14)	S (18)
MH-32	R	R	R	R	R
MH-35	S (23)	R	S (12)	S (14)	S (21)
MT-11	S (4)	R	S (11)	S (13)	S (11)
AH-21	S (12.5)	R	S (9)	S (7)	S (6.5)
AH-22	S (24)	S (10)	S (16)	S (14)	S (20)
AH-31	R	R	S (21)	S (12)	S (5)
AH-41	R	R	R	R	R

S = Sensitive, R = Resistant, E-15 = Erythromycin, P 10 = Penicillin G, S-10 = Streptomycin, GEN-10 = Gentamycin, N30 = Doxycycline.

WHO, European and International standards for drinking water require that no coliform should be present in 90% samples (WHO 1971). From this study, it is clear that none of the water samples collected was suitable for human consumption. The samples were found to contain bacteria, like *Escherichia coli*, *Salmonella* sp., *Klebsiella* sp. and *Pseudomonas* sp. which are potential pathogens and thus pose a serious threat to public health. This study elucidates the importance of monitoring the hotels and restaurants and put them under strict regulations to prevent future outbreak of any water borne diseases caused by consumption of dispensed water.

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