International Food Research Journal 21(1): 131-134 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

Incidence of multiple potentially pathogenic bacteria in tap water from different restaurants in Dhaka city, Bangladesh

[1*]Md. Shahidul, K., [1]Mehadee, H. and [2]Sunjukta, A.

[1]*Department of Microbiology, Stamford University Bangladesh, Dhaka, Post code1217, Bangladesh*
[2]*Department of Microbiology, Dhaka University, Dhaka, Post code 1000, Bangladesh*

Article history

Received: 17 July 2013
Received in revised form: 15 August 2013
Accepted: 16 August 2013

Keywords

Coliforms
Faecal coliforms
Tap water

Abstract

This study was conducted to determine the presence of potentially pathogenic bacteria in 20 tap water sources used in different restaurants in Dhaka city. A questionnaire was used to determine the aesthetic quality and extent of use of these sources. In the microbiological examination, all samples were found to be contaminated with coliforms. Although fecal coliforms could not be detected in samples 5, 12 and 16, these samples were found to be contaminated with coliforms and pathogenic bacteria such as, *Vibrio* spp., *Shigella* spp. and *Salmonella* spp. Concentrations of total heterotrophic bacteria were beyond the recommendation suggested by the World Health Organization (1.2×10^4 and 5.4×10^4 cfu/ml). These contaminated waters pose threats to the health of the consumers. It is possible that deep tube well water is cross contaminated from underground sewerage lines which require repair and amendment of water supply system.

Introduction

Clean and safe water is essential for healthy living though many people do not get clean and safe water for drinking and household use (WHO, 2008). Waterborne diseases are very common in the developing countries and still pose major threats to those who cannot afford clean water. Waterborne diseases like cholera, typhoid fever and bacillary dysentery are reported more frequently during any drinking water associated outbreaks than it was reported before (Fenwick, 2006). According to the World Health Organization (WHO), more than 5 million people die each year due to water related diseases of which more than 50% deaths are due to cholera alone (Fenwick, 2006). Fresh waters and coastal sea water bodies are frequently contaminated with human and animal feces through discharge of untreated wastewater (Grabow, 1996; George, 2001; Fenwick, 2006). Many people in developed countries and children <5 years old in developing countries suffer from water related diseases due to contaminated water supply and poor hygienic conditions (Seas, 2000; Medema, 2003).

High incidence of waterborne diseases is increasingly reported in developing countries like India (Khera, 1996). Spreads of waterborne diseases are often found to be associated with the consumption of contaminated water possibly due to their ignorance, poverty and unavailability of clean water. There are several studies conducted on bottled water, DWASA water and surface water of Bangladesh but there is not enough study on tap water sources especially from restaurants in Dhaka city. In this study we surveyed the level of contamination in the water distribution network of Dhaka Water Supply and Sewerage Authority (DWASA). DWASA draws more than 80% of its water from the underground below Dhaka city and the remaining amount from surface water treatment plants.

Materials and Methods

Sampling

Twenty tap water samples were collected randomly from different restaurants from Moghbazar and Malibag area of Dhaka City. Specific locations of the sampling sites are shown in Table 1. Samples were collected between March 2011 and May 2012. Samples were collected in sterile 250 ml plastic bottles and preserved at 4-8°C temperature before analysis. All samples were collected and analyzed following the standard methods in the Department of Microbiology, Stamford University Bangladesh (APHA, 1995).

Questionnaire survey

A short interview was taken from both the authority of every restaurant and the customers regarding the aesthetic condition of water and the use of water available at the respective taps.

Heterotrophic plate count (HPC)

Water samples were serially diluted ten-fold in sterile normal saline (0.9 % NaCl w/v) up to 10^{-4} and

*Corresponding author.
Email: *mskabir@yahoo.com*
Tel: +88 028354577 (Ext-472); Fax: +88 02 9143531

Table 1. Description of sampling sites

Sample No.	Sampling area
1	Wireless railgate, Moghbazar
2	Chamelibagh
3	Outer Circular Road, Moghbazar
4	Modhubagh, Moghbazar
5	Doctor lane, Moghbazar
6	Zahabox lane, Moghbazar
7	Green way, Moghbazar
8	Sonalibag, Moghbazar
9	Pirpagla goli, Moghbazar
10	Noyatola, Moghbazar
11	Inner circular road, Moghbazar
12	Mouchak
13	Baily Road
14	Shantinogor
15	New Baily Road
16	Siddeshwari
17	Siddeshwari
18	Mouchak market
19	Bapari goli, Moghbazar
20	Malibag

0.1 ml sample was spread over nutrient agar. Plates were incubated at 37°C for 18-24 hours and the total number of colonies was enumerated to determine the presence of total heterotrophic bacteria per ml.

Total coliform count (TCC)

Hundred ml of each sample was passed through Millipore membrane filter (0.45 µm) (Millipore, Massachusetts, USA) housed in a special filter apparatus contained in a suction flask. Filters containing the trapped microorganisms were aseptically transferred onto membrane fecal coliform (mFC) agar (Oxoid, Hampshire, UK) plates. Culture plates were then incubated at 37°C for 18-24 hours. After incubation only lactose fermenting blue colonies were enumerated as total coliforms.

Fecal coliform count (FCC)

After filtration of 100 ml of the samples as stated above, the membrane filter containing the trapped microorganisms was aseptically transferred onto mFC agar (Oxoid, Hampshire, UK). These plates were incubated at 44°C for 18-24 hours. Following incubation characteristic blue colored colonies were counted as fecal coliforms.

Isolation of Vibrio spp.

Ten ml of water sample was added to 10 ml (2X) alkaline peptone water (APW) (Oxoid, Hampshire, UK) and incubated at 37°C for 4-6 hours. Enriched samples were inoculated on to thiosulphate citrate bile salts sucrose (TCBS) agar (Oxoid, Hampshire, UK) and incubated at 37°C for 18-24 hours. Both sucrose fermenting and non-fermenting colonies were further identified for the presence of *Vibrio* spp. through standard biochemical tests (Cappuccino, 1989).

Isolation of Salmonella and Shigella like organisms

Ten ml of water sample was added to 10 ml of Selenite F broth (2X) and incubated at 37°C for 6 hours. Enriched samples were streaked onto Salmonella Shigella (SS) agar (Oxoid, Hampshire, UK) and incubated at 37°C for 18-24 hours. Characteristic colonies were further identified through standard biochemical tests (Cappuccino, 1989).

Table 2. Presence of heterotrophic bacteria, total coliforms and fecal coliforms in tap water samples

Sample	HPC[a] (cfu/ml)	TCC[b] (cfu per 100 ml)	FCC[c] (cfu per 100 ml)
01	$5.4x10^4$	25	11
02	$3.2x10^4$	37	17
03	$4.4x10^4$	15	09
04	$3.2x10^4$	25	12
05	$2.2x10^4$	17	0
06	$1.7x10^4$	27	13
07	$4.6x10^4$	40	18
08	$3.0x10^4$	29	17
09	$3.4x10^4$	28	15
10	$2.2x10^4$	15	12
11	$5.1x10^4$	33	29
12	$3.2x10^4$	22	0
13	$3.5x10^4$	25	15
14	$4.5x10^4$	36	24
15	$4.5x10^4$	30	17
16	$3.2x10^4$	36	0
17	$3.8x10^4$	33	13
18	$4.3x10^4$	30	19
19	$1.2x10^4$	11	06
20	$2.8x10^4$	35	18

[a]Heterotrophic plate count (HPC)
[b]Total coliform count (TCC)
[c]Fecal Coliform count (FCC)

Statistical analysis

The correlation between the quantitative data of heterotrophic plate count (HPC), total coliform count (TCC) and faecal coliform (FCC) were determined using Microsoft Office Excel 2010 software (Table 4).

Results

Questionnaire survey

Restaurant owners as well as customers using these restaurants were asked regarding the quality and use of DWASA water supplied through different taps. On an average 65 people used each of the tap water sources every day. The user groups were mostly poor people, rickshaw puller from Dhaka city and visitors from the other parts of Bangladesh. All of the participants reported about the unacceptable taste and odor of the supplied tap water. However, these water sources were mainly used for drinking, washing utensils, fresh vegetables and ready-to-eat salad items. Bottled water and filtered water were served in the restaurants as alternative and safe drinking water for those who could afford to pay.

Microbiological examination

The concentrations of the total heterotrophic bacterial loads in all (20) tap water samples were shown to be high and fall beyond the acceptable limit recommended by WHO (Table 2). The total count of

Table 3. Isolation and biochemical identification of *Vibrio* spp., *Salmonella* spp. and *Shigella* spp. in tap water samples

No. of sample	*Vibrio* spp.	*Salmonella* spp.	*Shigella* spp.
1	+[a]	-[b]	-
2	+	+	+
3	-	+	+
4	-	+	+
5	+	+	+
6	-	-	+
7	+	-	+
8	+	-	-
9	+	-	-
10	-	-	+
11	-	-	+
12	-	-	+
13	-	-	-
14	-	+	-
15	+	+	+
16	-	-	+
17	+	-	+
18	+	-	-
19	-	-	-
20	+	+	-
Sample contamination (%)	50%	35%	60%

[a]indicates positive growth
[b]indicates negative growth.

heterotrophic bacteria was found between 1.2 x 10^4 and 5.4 x 10^4 cfu/ml.

Two indicator bacteria, total and faecal coliforms were enumerated in this study to determine the potability of tap water from the restaurants. It was found that all of the samples were contaminated with total coliforms and ranged between 15 and 40 cfu per 100 ml. Amongst these 20 tap water samples, samples 5, 12 and 16 were found to be free from faecal coliforms.

All water samples (20) were investigated for the presence of pathogenic bacteria: *Vibrio* spp., *Salmonella* spp. and *Shigella* spp. Biochemical identification of these pathogenic bacteria showed that 50%, 35% and 60% samples were contaminated with *Vibrio* spp., *Salmonella* spp. and *Shigella* spp., respectively (Table 3).

Discussion

We assessed microbiological quality of tap water supplied by DWASA in Dhaka city because of its importance in public health. To determine the total bacterial load and level of contamination, heterotrophic bacteria were quantitated in the water samples tested. Based on the heterotrophic plate count (HPC) it was found that all tap water sources were highly contaminated (HPC between 1.2 x 10^4 and 5.4 x 10^4 cfu/ml). The presence of high bacterial load indicates contamination and subsequent survival of the bacterial population in the water supply line. However, the concentration of residual chlorine was not measured in this study which could give a better picture of optimal growth condition of total bacteria. According to World Health Organization (WHO) recommendation of HPC in tap water is 100-500

Table 4. Correlation between the abundance of HPC, TCC and FCC

	HPC	TCC	FCC
HPC	1		
TCC	0.46	1	
FCC	0.48	0.53	1

Legend: HPC, Heterotrophic plate count; TCC, Total coliform count; FCC, Faecal coliform count

cfu/ml (WHO, 2008). Although high prevalence of heterotrophic bacteria was not found to be directly related to the presence of total and fecal coliform bacteria, 90% of the samples were contaminated with one or more of the three potential pathogenic species, *Vibrio*, *Shigella* and *Salmonella* (Table 3). Non-toxigenic forms of *Salmonella* and *V. cholerae* spp. are widely distributed in water environments which are relatively sensitive to disinfection (WHO, 2008). The presence of pathogenic forms therefore indicates fecal contamination of surface waters or alternatively contamination of deep tube well water from sewerage systems that warrants the need for disinfection. *Salmonella* and *Vibrio*, *Shigella* spp. are not particularly stable in water environments and their presence generally indicates recent fecal contamination (WHO 2008). Moreover, *Shigella* spp. are relatively sensitive to disinfection and they are unlikely to be present in the DWASA water samples. Accordingly, the presence of *Shigella* in some of the tested samples in this study may indicate post-treatment contamination during the water distribution process following a recent infection in the community. Similar results of contamination in the drinking water have previously been reported by other workers (Khera, 1996; Faechem, 1980). In this study, a lack of correlation between the presence of fecal pollution and presence of potential pathogens belonging to *Salmonella*, *Shigella* and *Vibrio* was also found. This was demonstrated by sample 11, which showed the presence of members of all three genera in the absence of fecal contamination. Samples 12 and 16 also contained *Shigella* spp. in the absence of any fecal indicators. This finding is in contrast to the generalization by the WHO that fecal indicator is a reliable indicator of the presence of *Salmonella* and *Shigella* spp. (WHO, 2008). However, this conclusion can only be drawn with confidence following further characterization of the isolates.

It appears from the study that consumers were not aware of the level of contamination in the DWASA tap water and its impact on public health, otherwise they would not use this water for drinking, washing utensils and salads. One of the limitations of this study was that the users were not followed up to detect their subsequent illness due to consumption of the contaminated tap water or eat salads washed

with such water. However, it may be concluded that the DWASA water somehow gets contaminated after entering the distribution chain although they are treated adequately (Mrityunjoy, 2011). Further study will be required to determine the exact point and sources of contamination. This study will create awareness amongst the consumers, shopkeepers and DWASA people. This finding suggests the necessity of regular monitoring the quality of the DWASA water at the user end and to take necessary steps to fix the problem immediately. Bacteriological contamination in the DWASA water also indicates the presence of other pathogenic microorganisms such as protozoa, fungi and viruses. Presence of diversified microorganisms can lead to the formation of biofilms and enhance the growth of contaminating microorganism and increase resistance to disinfection (Siqueira, 2011).

The limitation of this study is that it does not reflect the impact of seasonal variation which could be related to rainfall, sunshine, change in temperature etc. However, a broader study can be done to determine any such impact on the water supply system. This study could be expanded through sampling and subsequent follow-up in different parts of Dhaka city to determine the overall microbiological quality of DWASA water.

Conclusion

None of the water samples tested was found to be potable based on total, fecal coliform counts and the presence of pathogenic bacteria. It is a general practice to use tap water for cooking and washing in restaurants. Hence, it is possible that potentially pathogenic bacteria can remain on washed food or utensils if not cooked or treated properly and lead to disease in the consumers. A future direction for the present investigation would be to characterize the isolates and to determine the actual source of the pathogen. A follow-up study on the consumers of those restaurants as to the subsequent development of disease can help in determining the potential risk to public health.

Acknowledgements

This study was supported by the Department of Microbiology, Stamford University, Bangladesh. We declare that there is no conflict of interest in the publication of this work.

References

American Public Health Association (APHA). 1995. Standard methods for examination of water and wastewater. 19th edn. American Public Health Association, Washington, D. C. USA.

Cappuccino, J. G. and Sherman, N. 1996. Microbiology - A Laboratory Manual. 4th edn. California: The Benjamin/Cummings Publishing Co., Inc.

Faechem, R. G. 1980. Bacterial standards for drinking water quality in developing countries. Lancet 2: 255-56.

Fenwick, A. 2006. Waterborne Diseases - Could they be Consigned to History? Science 313: 1077–1081.

George, I., Crop, P. and Servais, P. 2001. Use of β-D-Galactosidase and β-D-Glucuronidase Activities for Quantitative Detection of Total and Faecal Coliforms in Wastewater. Canadian Journal of Microbiology 47: 670–675.

Grabow, W. O. K. 1996. Waterborne Diseases: Update on Water Quality Assessment and Control. Water SA. 22: 193–202.

Khera, A. K., Jain, D. C. and Dutta, K. K. 1996. Microbial contamination of various water sources in Delhi. Journal of Communicable Diseases 28: 129-138.

Medema, G. J., Payment, P., Dufour, A., Robertson, W., Waite, M., Hunter, P., Kirby, R. and Andersson. Y. 2003. Safe drinking water: an ongoing challenge. In Assessing Microbial Safety of Drinking Water. Improving Approaches and Method; WHO & OECD, pp. 11–45, IWA Publishing: London, UK.

Mrityunjoy, A., Rahman, F., Beauty, S.A., Feroz, F., Rahman, M.M. and Rashed, N. 2011. Microbiological study on supply water and treated water in Dhaka city. Stamford Journal of Microbiology 1(1¬): 42-45.

Seas, C., Alarcon, M., Aragon, J.C., Beneit, S., Quinone, M., Guerra, H. and Gotuzzo, E. 2000. Surveillance of Bacterial Pathogens Associated with Acute Diarrhea in Lima, Peru. International Journal of Infectious Diseases 4: 96–99.

Siqueira, V. M., Oliveira, H. M., Santos, C., Paterson, R. R., Gusmão, N. B. and Lima, N. 2011. Filamentous fungi in drinking water, particularly in relation to biofilm formation. International Journal of Environmental Research and Public Health 8(2): 456-469.

World Health Organization (WHO). 2008. Guidelines for Drinking-water Quality, Incorporating 1st and 2nd Addenda, Volume 1, Recommendations, 3rd edn.; WHO: Geneva, Switzerland.