

Quantitative and qualitative assessments of microbial contamination in some bottled and tap water with their drug resistant pattern

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Abstract

The present work focussed on the concerns of the existence of coliform, faecal coliform, and other pathogens in both tap water and commercially available bottled water, along with the drug resistant pattern of the isolates. The physico-chemical features of the bottled water samples were satisfactory, but most of the tap water exceeded the marginal limit. A total of 21 samples (10 of tap water and 11 of bottled water) were collected and processed for microbiological analysis. All the samples were found to be contaminated with total viable bacteria up to 10^8 CFU/mL. Among the 21 samples, seven samples were found to be contaminated with *E. coli* up to 10^6 CFU/mL, and six samples had *Klebsiella* spp. up to 10^2 CFU/mL. Faecal contamination was totally absent in all bottled water, but present in four tap water samples. Fungi was found in six samples within the range of 10^2 to 10^3 CFU/mL. Surprisingly, *Staphylococcus* spp. were observed in all bottled water. *Vibrio* spp. were detected in three samples. An elevated number of faecal coliforms, *Klebsiella* spp., *Salmonella* spp., *Shigella* spp., *Vibrio* spp., and *Pseudomonas* spp. were estimated among the tap water samples up to 10^5 CFU/mL. The water samples, especially tap water, collected from the different areas were microbiologically unsafe, as few pathogenic microorganisms were found in several samples. This indicated as public health threat. Most of the isolates from both tap and bottled water samples were found to be resistant against more than one antibiotic tested, which is extremely alarming for the consumers. Very few antibiotics were found to be effective against the bacterial isolates.

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Keywords

coliform and faecal coliform, drug resistance, waterborne diseases, water microbiology

Introduction

Safe drinking water is a basic human right, and an essential step to improve the living standards of people (Acharjee *et al.*, 2011; 2014; Tabassum *et al.*, 2019). Though there is sufficient freshwater to meet the needs of global human population, these resources are not evenly distributed. Besides, water bodies including river, lake, ponds, and wells are teeming with pathogenic and non-pathogenic bacteria, protozoa, fungi, and viruses. Among all the microbial contaminants, enteric pathogens are the most important to control (Acharjee *et al.*, 2014). Usually, *E. coli* and other enteropathogens are present in environmental water bodies at a very low concentration; it is an extremely time-consuming and

complex to detect them (Munshi *et al.*, 2012; Acharjee *et al.*, 2014). As coliforms are most abundant in intestinal flora of humans and warm-blooded animals, they are found plenty in faecal wastes (Rompré *et al.*, 2002; Acharjee *et al.*, 2014). As a consequence, coliforms, which are detected in higher concentration than pathogenic bacteria, are used as indicators for pathogenic bacteria in water environments (Acharjee *et al.*, 2011; Munshi *et al.*, 2012).

Though coliforms are routinely found in different natural environments, drinking water is not a natural environment for them (DiPaola, 1998; McLellan, 2004; Ahmed *et al.*, 2005). Their presence in drinking water is considered as a possible threat or indicative of microbiological water quality

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deterioration. Positive total coliform samples in treated water, which is supposed to be coliform-free, may imply loss of disinfectant, treatment ineffectiveness (McFeters *et al.*, 1986), the supply of polluted water into the potable water supply (Clark *et al.*, 1996), or regrowth problems (LeChevallier, 1990) in the distribution system, which should not be ignored. There is still dispute over using coliform group as an indicator of the possible presence of enteric pathogens in aquatic system as waterborne disease outbreaks were reported previously, despite authorities adhering to coliform regulations (Payment *et al.*, 1991; Moore *et al.*, 1994; MacKenzie *et al.*, 1994; Gofti *et al.*, 1999). However, different methods are now available to monitor the existence of coliform, faecal-coliform, and other pathogens in drinking water by which the quality of the water can be easily detected (Ahmad *et al.*, 2013; Acharjee *et al.*, 2014; Tabassum *et al.*, 2019). The present work was therefore undertaken to assess the presence and loads of coliform, faecal-coliform, and other waterborne bacteria in bottled and tap water, along with their resistance properties against antibiotics.

Materials and methods

Study area and sampling

The present work was conducted by including the community of Dhaka metropolis, where people generally consume water from the tap and commercially available bottled. In total, 21 water samples (10 tap water from different households, and 11 commercially available bottled water) were obtained from June to July 2019. The targeted community of the Dhaka city used the tap water directly (without any treatment) for their daily use. Samples were collected in properly labelled sterile screw-capped bottles, under aseptic condition, and placed in a thermal stabilising box of 25°C while transporting them to the laboratory for microbiological analysis (Munshi *et al.*, 2012; Acharjee *et al.*, 2011; 2014).

Physico-chemical parameters of water samples

All the water samples were subjected to evaluate their physico-chemical properties such as dissolved oxygen, temperature, pH, electrical conductivity (EC), salinity, total dissolved solid (TDS), and turbidity through the standard guidelines of American Public Health Association (APHA, 1995) and American Society for Testing and Materials (ASTM) using different calibrated standard instruments. The pH meter was used to

measure the pH of water samples (model HI 98130 Hanna, Mauritius) and the conductivity of the samples was measured using a conductivity meter (model HI 98130 Hanna, Mauritius). Turbidity meter was used to determine the turbidity of the water samples (model 2100P Turbidimeter HACH, Colombia, USA). TDS in water samples were determined following the standard methods of APHA (APHA, 1995) by the filtration process.

Microbiological quality of water samples

For the estimation of total viable bacteria (TVB), coliform (*E. coli*, *Klebsiella* spp.), and faecal coliform, an aliquot of 0.1 mL of each sample was spread onto nutrient agar (NA), MacConkey agar, and membrane faecal coliform (MFC) agar, respectively, using the spread plate technique (Cappuccino and Sherman, 1996). Inoculated plates were incubated at 37°C for 24 h, except for MFC agar plates, which were incubated at 44.5°C. Eosin methylene blue (EMB) agar was used for further confirmation of *E. coli* by observing distinctive green metallic sheen colonies. Mannitol salt agar (MSA) and thiosulfate citrate bile salts sucrose (TCBS) agar were used to determine the *Staphylococcus* spp. and *Vibrio* spp., respectively. For the final identification, all isolates were biochemically analysed by following the standard methods (Cappuccino and Sherman, 1996; Alfred, 2007).

Antibiotic susceptibility test

All the isolates identified through biochemical tests were subjected to antibiotic susceptibility test (either resistant or susceptible) against commonly used antibiotics on Mueller-Hinton agar (Difco, Detroit, USA) by following the standard protocol of disc diffusion assay (Bauer *et al.*, 1966; Munshi *et al.*, 2012). Antibiotics used were trimethoprim/sulfamethoxazole (25 µg), erythromycin (15 µg), amoxicillin (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), streptomycin (10 µg), ampicillin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), cefixime (5 µg), polymyxin B (300 units), kanamycin (30 µg), vancomycin (30 µg), gentamicin (10 µg), nalidixic acid (30 µg), azithromycin (15 µg), and penicillin G (10 µg).

Results and discussion

Drinking water is not sterile as it always carries different microorganisms from reservoir, distribution system, tap, and other sources. Most of them are considered innocuous, but the presence of

opportunistic pathogen might cause problems. More than 500 pathogens are listed for implication with various waterborne diseases in drinking water by the US Environmental Protection Agency (Fawell and Nieuwenhuijsen, 2003). The quality of drinking water in terms of microbial contamination is not the same around the globe. Considering the consumers' health safety, the present work attempted to explore the contamination level in some commercially available bottled drinking water as well as from different household tap water in Dhaka metropolis. The establishment of resistance or susceptibility of all isolates found in the samples against commonly used antibiotics was another focus of the present work.

Physico-chemical parameters of water samples

For tap water, the DO was from 4.7 to 7.7 mg/L, the pH was from 7.7 to 10.4, the EC was from 291 to 460 $\mu\text{s}/\text{cm}$, the salinity was from 0.14 to 0.27 ppt, the TDS was from 132 to 255 ppm, the turbidity was from 0.25 to 2.09 NTU, and the temperature was from 26 to 27°C (Table 1).

For bottled water, the DO was from 4.3 to 7.4 mg/L, the pH was from 6.5 to 6.9, the EC was from 286 to 314 $\mu\text{s}/\text{cm}$, the salinity was from 0.12 to

0.19 ppt, the TDS was from 117 to 163 ppm, the turbidity was from 0.43 to 2.54 NTU, and the temperature was constant at 26°C (Table 1).

Based on these results, most of the tap water samples exceeded the marginal limit of all parameters (DO, temperature, pH, EC, salinity, TDS, and turbidity). This may diminish the overall quality of drinking water such as taste, odour, smell, and colour.

Microbiological quality of tap water samples

All tap water samples were heavily contaminated with numerous bacteria. Total viable bacterial (TVB) count of the samples was in the range of 10^2 to 10^8 CFU/mL (Table 2). Samples 01, 04, 06, and 08 were contaminated with faecal coliform, which indicated the presence of faecal contamination and probable risk of other microbial pathogens. *E. coli* and *Klebsiella* spp. were also detected in these four samples in a range of 10^2 to 10^6 CFU/mL. *E. coli* is quite notorious in causing waterborne diseases, and disease outbreaks caused by pathogenic *E. coli* are well documented in previous studies (O'Connor, 2002; Olsen *et al.*, 2002; Park *et al.*, 2018). *Klebsiella* spp. are natural inhabitants of many water bodies, and can grow in

Table 1. Physico-chemical parameters of the water samples.

Sample type	Sample number	DO (mg/L)	Temperature (°C)	pH	EC ($\mu\text{s}/\text{cm}$)	Salinity (ppt)	TDS (ppm)	Turbidity (NTU)
Tap water	S-01	7.7	27	8.8	291	0.14	132	0.72
	S-02	7.5	26	8.6	460	0.23	200	1.53
	S-03	6.9	26	9.6	440	0.27	255	2.09
	S-04	4.7	26	9.0	390	0.18	174	1.72
	S-05	4.8	26	10.4	370	0.21	189	0.79
	S-06	6.8	26	8.8	452	0.23	190	0.50
	S-07	4.7	26	7.7	360	0.19	169	0.88
	S-09	5.7	26	8.5	445	0.20	197	0.79
	S-10	5.8	26	8.9	388	0.19	169	0.25
	Bottled water	Fresh	4.3	26	7.9	304	134	0.12
Spa		6.5	26	6.9	286	125	0.13	1.00
Shena		7.0	26	6.7	300	127	0.14	0.53
Aquafina		4.8	26	7.7	290	126	0.14	0.49
Mum		6.7	26	6.7	302	135	0.15	1.57
Kinley		4.9	26	7.9	298	130	0.14	1.18
Evian		4.8	26	7.8	314	139	0.15	1.34
Pran		4.5	26	6.6	284	124	0.13	2.54
Jibon		7.8	26	6.5	376	163	0.19	1.40
Eco		5.5	26	6.5	274	117	0.13	0.43
Nestle	4.5	25	5.5	264	116	0.11	0.40	

DO = dissolved oxygen; EC = electrical conductivity; TDS = total dissolved solid.

Table 2. Microbiological assessment of tap water (CFU/mL).

Sample number	HPC	FCC	<i>E. coli</i>	<i>Klebsiella</i> spp.	<i>Salmonella</i> spp.	<i>Shigella</i> spp.	<i>Vibrio</i> spp.	<i>Pseudomonas</i> spp.	<i>Staphylococcus</i> spp.
S-01	2.6×10^7	2.3×10^3	2×10^6	2×10^2	0	2×10^2	0	0	6.2×10^2
S-02	5.8×10^6	0	0	0	5.9×10^2	4.8×10^2	0	0	1.3×10^3
S-03	7.5×10^8	0	0	0	1.1×10^2	4.4×10^3	0	0	6.6×10^2
S-04	3.7×10^6	3.1×10^3	1.8×10^2	1.4×10^2	3.3×10^3	0	0	0	0
S-05	5.6×10^5	0	0	0	4.8×10^2	3×10^2	0	7×10^2	0
S-06	1.7×10^4	5.3×10^2	0	2×10^3	0	9.3×10^2	2.9×10^2	5×10^5	9.5×10^2
S-07	9.9×10^2	0	0	0	1.7×10^2	4.5×10^3	0	4.5×10^3	2×10^2
S-08	7.7×10^3	1×10^2	7.8×10^4	2×10^2	4.5×10^2	0	0	8.8×10^2	0
S-09	2.0×10^5	0	0	0	0	8.4×10^2	0	6×10^5	0
S-10	1.7×10^8	0	0	0	0	0	0	0	0

HPC = Heterotrophic Plate Count; FCC = Fecal Coliform Count

organic nutrient-rich environments. Most of the *Klebsiella* spp. detected in drinking water are biofilm-former and sensitive to disinfectants. Proper treatment with disinfectant readily eliminates them from water, and their presence indicates the improper and inadequate treatment of drinking water (WHO, 2003; 2004).

More than half of the samples (sample 02 to 05, 07, and 08) were contaminated with *Salmonella* spp. in a range of 10^2 to 10^3 CFU/mL (Table 2). *Salmonella typhi* and *S. paratyphi* cause typhoid fever, while other non-typhoidal *Salmonella* spp. cause salmonellosis. In 2014, an outbreak of gastroenteritis was reported in Croatia which was caused by *S. enterica* from ground water, and lasted for 12 days (Kovačić *et al.*, 2017).

Almost all the tap water samples were contaminated with *Shigella* spp. in a range of 10^2 to 10^3 CFU/mL, except for samples 04, 08, and 10. Only sample 06 harboured *Vibrio* spp. at an amount of 10^2 CFU/mL (Table 2). *Pseudomonas* spp., which may become opportunistic under favourable condition, were detected in a total of five tap water samples (05 to 09), where the highest range was 10^5 CFU/mL for samples 06 and 09. *Staphylococcus* spp. was present in samples 01, 02, 03, 06, and 07 in a range of 10^2 to 10^3 CFU/mL. It was evident that the microbiological quality of the tap water samples was poor for consumption, and might pose serious health risk for the consumers. Further treatment is recommended for the tap water before consumption (Acharjee *et al.*, 2014).

Microbiological quality of bottled water samples

In all commercially available bottled water samples, TVB was detected at 10^5 CFU/mL. Similar results were found in Iran and Bangladesh by Khaniki *et al.* (2010) and Majumder *et al.* (2011),

respectively, where the presence of heterotrophic bacteria were observed in all the commercially available bottled water samples. Another study conducted by El-Salam *et al.* (2008) showed that most of the bottled water samples were contaminated with heterotrophic bacteria. In the present work, four samples were contaminated with *E. coli* up to 10^2 CFU/mL, and two were contaminated with *Klebsiella* spp. up to 10^2 CFU/mL. Fungi was found in five samples in the range of 10^2 to 10^3 CFU/mL. *Staphylococcus* spp. were observed in all 11 bottled water samples, while *Vibrio* spp. were detected in two samples (Table 3). The presence of coliform in drinking water indicates faecal contamination and the probable presence of other pathogens, which may cause various waterborne diseases (Rompré *et al.*, 2002; Acharjee *et al.*, 2014). Overall, these results are beyond the acceptable microbiological limits in drinking water, thus making them unsuitable for human consumption (Acharjee *et al.*, 2014).

Biochemical identification

Eight biochemical tests were performed to further identify the isolates (Table 4). Colonies of *E. coli* and *Klebsiella* spp. on MacConkey agar were transferred onto EMB agar, and seven of 21 samples were found to be contaminated with *E. coli* by the presence of green metallic sheen. The presence of *Pseudomonas* spp., *Vibrio* spp., *Staphylococcus* spp., *Salmonella* spp., and *Shigella* spp. were confirmed by distinctive biochemical characteristics.

Drug resistance / susceptibility pattern of bacterial isolates

To evaluate the efficiency of commonly used antibiotics as well as the clinical significance of the bacterial isolates, antibiotic susceptibility test was performed. Both *E. coli* and *Klebsiella* spp. from tap

Table 3. Microbiological assessment of bottled water (CFU/mL).

Sample type	TVB	Fungi	Coliform		Faecal coliform	Staphylococcus spp.	Vibrio spp.
			<i>E. coli</i>	<i>Klebsiella</i> spp.			
Fresh	2.6×10^5	3.2×10^3	5.3×10^2	2.3×10^2	0	2.0×10^3	1.9×10^2
Spa	3.0×10^5	3.8×10^3	1.0×10^2	0	0	1.0×10^3	1.0×10^2
Shena	2.8×10^5	4.2×10^3	0	0	0	1.9×10^3	0
Aquafina	2.0×10^5	2.5×10^3	0	0	0	2.3×10^3	0
Mum	2.7×10^5	0	0	0	0	1.6×10^3	0
Kinley	2.3×10^5	0	1.6×10^2	2.0×10^2	0	3.0×10^3	0
Evian	2.6×10^5	0	0	0	0	2.7×10^3	0
Pran	3.5×10^5	0	3.3×10^2	0	0	1.9×10^3	0
Jibon	2.2×10^5	0	0	0	0	4.0×10^3	0
Eco	2.9×10^5	2.5×10^2	0	0	0	4.8×10^3	0
Nestle	2.8×10^5	2.9×10^2	0	0	0	1.9×10^3	0

TVB = total viable bacteria.

Table 4. Biochemical tests of different pathogens.

Assumed pathogenic microorganism	TSI				Motility	Indole production	MR	VP	Citrate utilization	Catalase	Oxidase
	Slant	Butt	Gas	H ₂ S							
<i>E. coli</i>	Y	Y	+	-	+	+	+	-	-	+	-
<i>Klebsiella</i> spp.	Y	Y	+	-	+	-	-	-	+	+	-
<i>Vibrio</i> spp.	R	Y	-	-	+	-	+	-	-	+	+
<i>Staphylococcus</i> spp.	Y	Y	-	-	+	-	+	-	-	+	-
<i>Pseudomonas</i> spp.	R	Y	-	-	+	-	+	-	-	+	+
<i>Shigella</i> spp.	R	Y	+	-	-	+	+	-	-	+	-
<i>Salmonella</i> spp.	R	Y	+	+	-	-	+	-	+	+	-

All experiments were repeated thrice, with reproducible results. Values are from representative data. + = positive; - = negative; TSI = triple sugar iron test; Y = yellow (acid); R = red (alkaline); MR = methyl red; and VP = Voges-Proskauer.

and bottled water samples showed similar response: 100% susceptibility against aminoglycoside antibiotics (kanamycin, streptomycin, and gentamicin) and 100% resistance towards amoxicillin and ceftriaxone. Surprisingly, both *E. coli* and *Klebsiella* spp. from tap and bottled water samples also showed 100% susceptibility against vancomycin (Table 5). *Shigella* spp. and *Pseudomonas* spp. from tap water samples, and *Vibrio* spp., both from tap and bottled water samples exhibited 100% resistance towards most of the antibiotics (13 out of 16 antibiotics), except streptomycin, gentamicin, and azithromycin. *Staphylococcus* spp. from both tap and bottled water

samples expressed identical traits against all the antibiotics, excluding polymyxin B and cefixime, where tap water isolates showed 100% resistance towards polymyxin B and cefixime, while reverse result (100% susceptible) was observed for bottled water isolates. Multidrug resistant trait of the isolates might occur due to horizontal gene transfer, point mutation, genetic disorders, and mechanistic factors or by epidemiological factors (Bennett, 2008; Canton, 2009; Hung and Kaufman, 2010; Acharjee *et al.*, 2014).

Finally, the present work reported that some of the drinking water samples both from tap and bottled were not recommended for drinking because

of the presence of indicator bacteria *E. coli* and *Klebsiella* spp., while the presence of opportunistic pathogen *Pseudomonas* spp. is another possible health threat to the young, old, and immunosuppressed people. Several factors including lack of education and training, environmental contamination, inadequate processing, and improper handling might be responsible for the contamination of drinking water. Besides, the presence of drug resistance traits in the identified isolates might be a hindrance to eradicate waterborne diseases.

Conclusion

Diseases transmitted through polluted water are the major problem in developing countries due to poor sanitation, unhygienic management of environment and water bodies, low level of hygiene practices, and lack of monitoring and healthcare awareness. The present work aimed to determine the microbiological quality of drinking water from taps of different household points, and commercially available bottled water. Coliforms and indicator microorganisms (*E. coli* and *Klebsiella* spp.) were detected in a number of water samples; both from tap and bottled water. Isolates were further tested against 16 commonly available antibiotics for resistance potential. Some of the samples were found grossly polluted with faecal strains, which implied that the water would be unsafe for consumption. Besides, the presence of opportunistic pathogen *Pseudomonas* spp. in several tap water samples posed a health threat to immunocompromised people. The present work raises concern about the microbiological quality and safety of the drinking water as well as emphasises the importance of routine microbiological study to monitor and prevent contamination of drinking water.

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