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Original Research Article

A proportional study on the existence of coliform and fecal coliform in the post-treatment (filtered and boiled) water samples

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ABSTRACT

Background: The elimination of pathogenic microorganisms from drinking water is the first and foremost requirement in terms of maintaining the quality of water as well as reducing the water- borne diseases. The presence of coliform in water acts as an indicator of the presence of others pathogens.
Methods: Present study attempted to focus on the existence of coliform and fecal coliform in drinking water along with their drug resistant pattern through conventional culture methods and Kirby-Bauer method (disk diffusion).
Results: A total of 30 samples (15 were boiled and 15 were filter) were collected and processed for microbiological action. Significant numbers of samples (both filter and boiled water) were found to be contaminated with coliform bacteria especially *E. coli* and *Klebsiella* spp. within the range of 10^2 to 10^4 cfu/ml. In addition, few samples exhibited fecal contamination. A total of 15 available drugs were used against the identified *E. coli* and *Klebsiella* spp. Most of the identified bacteria were found to be sensitive against commonly used antibiotics. Very few strains of *E. coli* and *Klebsiella* spp. in both filter and boiled water were found to be resistant to more than one antibiotic. Only *Klebsiella* spp. from boiled water showed 100% sensitivity against all the drugs.
Conclusions: The presence of drug resistant coliform and fecal coliform in drinking water is a clear indication of poor water quality which might be a threat for consumer's health, especially for the children.

Keywords: Drinking water, Coliform, Fecal coliform, Drug resistance

INTRODUCTION

Water-borne diseases are frequent among the communities due to the mismanagement in water distribution process, cross-contamination, man-made pollution, personal unsanitary condition and lack of proper education.[1-3] Though, consumption of safe drinking water is the main aspect to ensure public and environmental health safety.[1,2] Drinking water can be contaminated with pathogenic bacteria implicated with water-borne diseases. Among the pathogenic bacteria, enteric pathogens are the striking ones for the water microbiologist.[1] Therefore, sources of fecal pollution in waters devoted to human activity must be strictly controlled. Entero-pathogens, such as *Escherischia coli* O157:H7, are generally present at a very low concentration in environmental waters within a diversified microflora. Complex methods are required to detect them, however, these are extremely time-consuming.[1,4] In most of the cases, presence of fecal

contamination in drinking water can easily be determined by the existence of indicator organisms.[1,5]

The existence of coliforms in higher concentrations than pathogenic bacteria is considered as an index of the potential presence of entero-pathogens in water environments.[4,6] Among the coliform group, *E. coli* is the most significant one as its presence indicates the existence of other pathogens in the water sample.[7] Coliforms are also routinely found in diversified natural environments.[8-11]

Their presence in drinking water is not only an indicator of poor water quality but also an obstacle to ensuring sustainable health safety for people. Moreover, the presence of total coliform in treated water which is usually coliform-free may indicate treatment ineffectiveness, loss of disinfectant, breakthrough, interference of contaminated water into the potable water supply or regrowth problems in the distribution system.[12-15]

The use of the coliform group as an indicator of the possible presence of enteric pathogens in aquatic systems has been a subject of debate for many years. However, different methods are available to monitor the existence of coliform and fecal coliform in drinking water by which we can easily detect the quality of the water.[7,16-19] Along these lines, the present study was designed to chalk out the existence of coliform and fecal-coliform in drinking water along with their resistant properties against antibiotics.

METHODS

Study area and sampling

The study was designated only based on the community of Dhaka metropolis where inhabitants generally consume water after filtration and boiling. A total of 30 water samples (15 were boiled water and another 15 were from filter water) were collected from separate households during the time period of February, 2016 to March, 2016. Samples were collected aseptically in sterile screw capped bottles and stored in a thermal stabilization box with a constant temperature of 25°C, then transported to the laboratory within one hour, and immediately subjected to microbiological analysis.[1,4,6]

Microbiological quality analysis of water samples

An aliquot of 0.1 ml of each sample was introduced on to nutrient agar, MacConkey agar and Membrane fecal coliform (mFC) agar through spread plate technique for the isolation of total viable bacteria (TVB), coliform (E. coli, Klebsiella spp.) and fecal coliform consecutively. Plates were incubated at 37°C for 24 hours excluding mFC agar plates which were incubated at 44.5°C. Presence of green metallic sheen on EMB agar media further specified the presence of *E. coli*. For the final identification, all the isolates were biochemically analyzed following the standard methods.[1,20-23]

Biochemical identification of other gram negative coliform bacteria

In EMB media, there were some other colonies excepting the ones with green metallic sheen which were then identified by employing standard biochemical methods to find a complete microbiological profile of the drinking water samples.[22,23]

Antibiotic susceptibility test of the identified bacteria

The pathogenic isolates were examined for antibiotic susceptibility traits (either drug resistant or sensitive) by disc diffusion assay on Mueller-Hinton agar (Difco, Detroit, MI) against commonly used antibiotics following the standard protocoll.[4,24,25] Antibiotics used in the study included trimethoprim/sulfamethoxazole (25 µg), erythromycin (15 µg), amoxicillin (30 µg), ceftriaxon (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

Existence of pathogenic bacteria in drinking water

Among the 30 samples (both filter and boiled water), 11 samples (5 from filter and 6 from boiled water) were found to be contaminated with *E. coli* up to 10^4 cfu/ml and 8 samples (4 from filter and 4 from boiled water) exhibited the existence of *Klebsiella* spp. within the range of 10^2 to 10^4 cfu/ml. Sample F3, F4, F5, F7, F9, F10, F11, F12, F14, F15 and B1, B4, B5, B7, B8, B9, B12, B14 were totally free from the growth of *E. coli*. Likewise, the presence of *Klebsiella* spp. in samples F2, F3, F4, F5, F7, F8, F9, F10, F11, F13, F15 and B2, B3, B4, B5, B6, B8, B9, B11, B12, B14, B15 were not noticed (Table 1). In case of fecal coliform sample F1, F8, F13 and B3, B6, B11, B13 were found to be highly contaminated within the range of 10^2 to 10^3 cfu/ml (Table 1).

Biochemistry of the isolates

Total eight types of biochemical tests showed their result for the confirmation of *E. coli* and *Klebsiella* spp. (Table 2). The growth of *E. coli* and *Klebsiella* spp. on MacConkey agar plates were further transferred on to the EMB media and 11 samples among 30 were found as *E. coli* by observing green metallic sheen.

Proliferation of drug-resistant bacteria in drinking water

To evaluate the efficacy of commonly available antibiotics as well as the clinical significance of the bacterial isolates, present study introduced antibiotic susceptibility test. Identified bacterial isolates were experimented to determine the antibiotic susceptibility against the commonly therapeutically used antibiotics. Both *E. coli* and *Klebsiella* spp. from filter and boiled water were sensitive against most of the antibiotics (Table 3).

Table 1: Microbiological assessment of drinking water.

Samples	Coliform		Fecal coliform
	E. coli	*Klebsiella spp.*	
F1	5.3×10^3	2.3×10^2	2.8×10^3
F2	1.0×10^3	0	0
F3	0	0	0
F4	0	0	0
F5	0	0	0
F6	1.6×10^4	2.0×10^4	0
F7	0	0	0
F8	3.3×10^2	0	2.7×10^2
F9	0	0	0
10	0	0	0
F11	0	0	0
F12	0	2.8×10^2	0
F13	3.0×10^4	0	1.0×10^3
F14	0	1.0×10^4	0
F15	0	0	0
B1	0	1.9×10^3	0
B2	$2.2\times10^{3`}$	0	0
B3	1.0×10^3	0	2.0×10^3
B4	0	0	0
B5	0	0	0
B6	2.0×10^4	0	1.3×10^2
B7	0	3.3×10^3	0
B8	0	0	0
B9	0	0	0
B10	0	1.8×10^2	0
B11	1.0×10^3	0	2.3×10^2
B12	0	0	0
B13	2.1×10^2	5.3×10^3	3.3×10^3
B14	0	0	0
B15	2.3×10^4	0	0

F=filter water, B=boiled water

Table 2: Biochemical tests of the isolates found on EMB agar.

Isolated strain	TSI			H^2S reaction	Indole Test	MR test	VP test	Citrate Test	Motility test	Oxidase test
	Slant	Butt	Gas							
E. coli	Y	Y	+	-	-	+	-	-	+	-
***Klebsiella* spp.**	Y	Y	+	-	-	-	-	+	-	-

All the experiments have been done three times and the results were reproducible. One representative data have been shown. TSI=triple sugar iron test, Y=Yellow (Acid) , R=Red (Alkaline), MR=Methyl red, VP=Voges-Proskauer

Table 3: Antibacterial susceptibility test of the isolates.

Antibiotic	Filter water				Boiled water			
	Disc-content	*E. coli*, (n=5)		*Klebsiella* spp., (n=4)		*E. coli*, (n=6)		*Klebsiella* spp. (n=3)	
		R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Polymixin B	300 units	0	100	100	0	0	100	0	100
Kanamycine	30 µg	0	100	0	100	0	100	0	100
Streptomycine	10 µg	0	100	0	100	0	100	0	100

Continued.

Antibiotic	Filter water					Boiled water			
	Disc-content	*E. coli*, (n=5)		*Klebsiella* spp., (n=4)		*E. coli*, (n=6)		*Klebsiella* spp. (n=3)	
		R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Vancomycine	30 µg	0	100	0	100	0	100	0	100
Gentamycine	10 µg	0	100	50	50	0	100	0	100
Nalidixic acid	30 µg	0	100	0	100	0	100	0	100
Azythromycine	15 µg	0	100	0	100	0	100	0	100
Penicillin G	10 µg	100	0	100	0	0	100	0	100
Erythromycine	15 µg	100	0	100	0	0	100	0	100
Amoxicillin	30 µg	100	0	100	0	100	0	0	100
Ceftriaxone	30 µg	100	0	100	0	100	0	0	100
Ciprofloxacine	5 µg	0	100	0	100	50	50	0	100
Streptomycine	10 µg	40	60	0	100	0	100	0	100
Ampicillin	10 µg	100	0	100		100	0	0	100
Tetracycline	30 µg	100	0	50	50	100	0	0	100
Chloramphenicol	30 µg	0	100	0	100	50	50	0	100
Cefixime	5 µg	0	100	100	0	50	50	0	100

In case of resistance, both *E. coli* and *Klebsiella* spp. from filter water were showed 100% resistance against penicillin G, erythromycin, amoxicillin and ceftriaxone. *E. coli* isolated from filter water was found to be 100% resistant against ampicillin and Tetracycline while *Klebsiella* spp. exhibited their resistant pattern against polymixin B, ampicillin and cefixime. In case of boiled water *E. coli* was found to be resistant against amoxicillin, ceftriaxone, ampicillin and tetracycline whereas all antibiotics were 100% reduced the growth of *Klebsiella* spp. up to the sensitive range (Table 3).

DISCUSSION

Propagation of coliform and fecal coliform in drinking water due to poor sanitation and hygienic condition is the main causative agent of diarrhea and dysentery.[4,26] Such diseases are very common in developing countries like Bangladesh where the water distribution line and the sewerage line are present in the same way.[1,3,4] In these area, people rely more on the filtration and boiling methods to reduce the growth of water born infectious pathogens.[1] Considering the consumers' health safety, present study attempted to focus on the post contamination level of drinking water after boiling and filtration. Another important aspect of our study was to detect the resistant properties of all the isolates found in the samples. The presence of coliform and fecal coliform in drinking water indicates the presence of other pathogens which may lead the various water-born diseases1,5. Many authors have reported that the poor water distribution system and the improper water treatment strategy may open the route to get an entry of water borne pathogens.[1,16-19]

Finally, our study reported that some of the processed drinking water samples were not recommended for drinking because of the presence of indicator bacteria *E. coli* and *Klebsiella* spp, as coliform which also indicated the possible presence of other pathogenic bacteria. Several factors such as environmental contamination, inadequate processing and improper handling might be responsible for the contamination of drinking water. Resistance gene might be evolved due to point mutation, genetic disorders, and mechanistic factors or by epidemiological factors.[1,27-29] Besides, the presence of drug resistance traits in the identified isolates might be responsible to make serious obstacles to eradicate the water-associated diseases.

CONCLUSION

Polluted water associated diseases are the major problem for the communities of developing countries due to the lack of knowledge on sanitation, unhygienic management of environment and water bodies, lack of education and training, and the extraordinary burden of wastes saturated with pathogenic microorganisms. The main important aspect of this study is to evaluate the post contamination level and reasons behind the contamination of treated water after collecting from the direct supply. In the current research, we detected *E. coli* and *Klebsiella* spp. as coliform as well as indicators in drinking water samples and also identified their efficacy against 15 commonly used antibiotics. Some of the samples were found to be contaminated with fecal strains which signify the water is not safe for consumption. To ensure consumer health safety this sort of routine microbiological study of both pre-treated and post-treated drinking water is necessary which would also help to set a proper regulation of microbiological quality of water and proper sanitation practices in individuals.

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REFERENCES

1. Acharjee M, Rahman F, Jahan F, Noor R. Bacterial proliferation in municipal water supplied in mirpur locality of Dhaka city, Bangladesh. Clean–Soil, Air, Water. 2014;42(4):434-41.
2. Islam T, Acharjee M, Tabassum N, Acharjee MR. Bacterial Propagation in Municipal Water and Deep Tube-well Water in Kashipur Locality of Narayanganj City, Banglades. J Water Environm Tech. 2020;18(5):327-37.
3. Acharjee M, Rahman F, Beauty SA, Feroz F, Rahman MM, Noor R. Microbiological study on supply water and treated water in Dhaka city. Stamfor J Microbiol. 2011;1(1):42-5.
4. Munshi SK, Rahman MM, Noor R. Detection of virulence potential of diarrhoeagenic Escherichia coli isolated from surface water of rivers surrounding dhaka city. J Bangladesh Acad Sci. 2012;36(1):109-21.
5. Rompré A, Servais P, Baudart J, De-Roubin MR, Laurent P. Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. J Microbiol Methods. 2002;49(1):31-54.
6. Razzak SBA, Sweety MA, Lubna MA, Akter T, Ahmmed J, Hossaini F, Uddin SHM, Roy TS, Acharjee M. Quantitative and qualitative assessment of microbial contamination in some bottled water and tap water along with their drug resistant pattern. International Food Research Journal. 2021.
7. Tabassum N, Akter M, Acharjee M. Study on Microbiological Quality Analysis of Tap-Water and the Effects of Halo-tab Plus Tablet on Water Born Microorganisms. IOSR J Biote Bioch. 2019;5(3):6-10.
8. Parveen S, hmed MSU, Tania N. Microbial contamination of ater in around Dhaka city'. Bang J Sci Industr Res. 2008;43(2):273-6.
9. DiPaola TS. Biological and Chemical Renovation of Wastewater with a Soil Infiltrator Low-Pressure Distribution System (Doctoral dissertation, Virginia Tech).
10. McLellan SL. Genetic diversity of Escherichia coli isolated from urban rivers and beach water. Appl Environmen Microbiol. 2004;70(8):4658-65.
11. Ahmed W, Neller R, Katouli M. Host species-specific metabolic fingerprint database for enterococci and Escherichia coli and its application to identify sources of fecal contamination in surface waters. App Environmen Microbiol. 2005;71(8):4461-8.
12. McFeters GA, Kippin JS, LeChevallier MW. Injured coliforms in drinking water. App Environmen Microbiol. 1986;51(1):1-5.
13. Clark RM, Geldreich EE, Fox KR, Rice EW, Johnsonn CH, Goodrich JA, et al. Tracking a Salmonella serovar typhimurium outbreak in Gideon, Missouri: role of contaminant propagation modelling. aqua-london then oxford. J Int Water Supp Assoc. 1996;45:171-83.
14. American Public Health Association. Standard Methods for the Examination of Water and Wastewater. Washington DC: 1998.
15. LeChevallier MW. Coliform regrowth in drinking water: a review. J Am Water Works Assoc. 1990;82(11):74-86.
16. Payment P, Richardson L, Siemiatycki J, Dewar R, Edwardes M, Franco E. A randomized trial to evaluate the risk of gastrointestinal disease due to consumption of drinking water meeting current microbiological standards. Am J Pub Heal. 1991;81(6):703-8.
17. Moore AC, Herwaldt BL, Craun GF, Calderon RL, Highsmith AK, Juranek DD. Waterborne disease in the United States, 1991 and 1992. J Am Water Works Assoc. 1994;86(2):87-97.
18. Mac Kenzie WR, Hoxie NJ, Proctor ME, Gradus MS, Blair KA, Peterson DE, et al. A massive outbreak in Milwaukee of Cryptosporidium infection transmitted through the public water supply. N Engl J Med. 1994;331(3):161-7.
19. Gofti L, Zmirou D, Hartemann P, Potelon JL. Waterborne microbiological risk assessment: a state of the art and perspectives. Revue d'epidemiologie et de Sante Publique. 1999;47(1):61-73.
20. Colwell RR. Bacterial death revisited. InNonculturable microorganisms in the environment. Springer, Boston, MA. 2000;325-42
21. Oliver JD. Recent findings on the viable but nonculturable state in pathogenic bacteria. FEMS Microbiol Rev. 2010;34(4):415-25.
22. Cappuccino, J.G. and Sherman, N. (1996). Microbiology- A Laboratory Manual. The Benjamin/ Cummings publishing Co., Inc., Menlo Park, California.
23. Alfrad EB. Bensons Microbiological Applications. Mcgraw-Hill Book Company, New York. 2007.
24. Bauer AW, Kirby WMM, Sherris JC, Tierh M. Antibiotic susceptibility testing by a standardized single disc method. Ame J Clin Patholog. 996;45(4):493-6.
25. Ferraro MJ, Craig WA, Dudley MN. Performance standards for antimicrobial susceptibility testing. NCCLS, Pennsylvania, USA. 2001
26. 26. Mead, A.M., Helm, G., Callan, P. and Atlas, R. M. (1999). 'A prospective study of drinking water quality and gastrointestinal diseases'. New Eng.J. Med, Vol. 245, No. 9, pp. 224-248.
27. Bennett PM. Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. Brit J Pharmacol. 2008;153(S1):S347-57.
28. Canton R. Antibiotic resistance genes from the environment: a perspective through newly identified antibiotic resistance mechanisms in the clinical setting. Clinic Microbiol Infect. 2009;15:20-5.

29. Kaufmann BB, Hung DT. The fast track to multidrug resistance. Molecul cell. 2010;37(3):297-8.

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