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Evaluation of Bacterial Population in Drinking Water Along with Hygiene and Sanitation Facilities of Different Schools in Port City, Bangladesh

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Abstract

In our daily life, drinking water is one of the most essential elements. However, safe and good-quality drinking water is a major concern for human health. Therefore, our study assessed the sanitation, hygiene conditions, and evaluation of bacterial population of drinking water in 40 schools in the port city, of Bangladesh. Total viable count (TVC), total coliform count (TCC), and survey questionnaire were used to complete our study. About 65% of water samples exceeded the acceptable limit (>500 cfu/ml) detected by TVC. The most probable number (MPN) method was used to see the presence of coliform bacteria where about 65% of samples were found contaminated with coliform, among them 12.5% of samples were at very high risk according to World Health Organization (WHO) guidelines. A total of 10 bacterial isolates like *Enterobacter* sp., *E. coli, Klebsiella* sp., *Salmonella* sp., *Proteus* sp., *Serratia* sp., *Citrobacter* sp., *Pseudomonas* sp., *Alcaligenes* sp., and *Micrococcus* sp. were detected in water samples by using four selective media and then identified by using cultural, morphological and biochemical tests. The disc diffusion method was used to detect antibiotic sensitivity with 10 commercially available antibiotics. About 80% of isolates were found completely resistant to erythromycin, amoxicillin, ampicillin, and rifampin and 90% were sensitive to ciprofloxacin, gentamicin, azithromycin, and tetracycline. Antibiotic resistance isolates might pose a serious threat to human health. In most schools, reservoirs of drinking water were found contaminated by various bacteria. Only 48.3% of schools used separate toilets for girls but of poor-quality hygiene and sanitation facilities. Due to poor hygiene, sanitation, and contaminated drinking water, students may suffer from various diseases. Therefore, this study emphasizes the need to raise awareness about hygiene and sanitation facilities by regularly monitoring school drinking water quality to ensure children are out of waterborne diseases.

Keywords: Total viable count (TVC), Most probable number (MPN), Total coliform count (TCC), Antibiotic sensitivity assay

Introduction

Safe drinking water is essential for all living things, the majority of ecological systems, human health, the development of children, food production, and economic growth [1, 2]. Fresh, clean water is more widely available and accessible, which lowers poverty and promotes sustainable development [3]. One-tenth of a person's productive time is lost to water-related diseases on average in underdeveloped nations, where drinking contaminated water is thought to be the cause of 80% of all ailments and over one-third of deaths [4]. Testing the water's appropriateness for drinking is therefore crucial. Water is thought to be the most conducive medium for the growth and spread of germs linked to humans [5]. It is a basic human right to have safe drinking water, and if it is tainted by harmful bacterial strains, people may have health issues [6, 7].

Many ailments, from mild gastrointestinal disorders that go away on their own to serious infections that can be fatal, can be brought on by contaminated water [8]. Water-borne bacteria are responsible for digestive tract diseases like cholera, dysentery, diarrhea, typhoid, etc [9]. Cholera and typhoid can spread across the population and can even cause epidemics.

One of the main public health problems is multidrug-resistant bacteria, which may be encountered in both the community and hospital settings. Changes in the drug target, blocking entry into the cell, drug inactivation, or removal through efflux pumps are the primary ways that bacteria become resistant to antibiotics. Currently, the rise of the antimicrobial resistance phenomena has a significant impact on the effectiveness of antibiotics, increasing morbidity and death globally [10].

A 2005 Bangladesh Health and Injury Report on children under 5 years, states that diarrhea claims the lives of 36,000 children annually [11]. Typhoid fever, bacillary dysentery, and diarrhea are frequent water-borne bacterial illnesses in Bangladesh [12]. As a result, it is crucial to guarantee the availability of clean drinking water since doing so may stop the spread of microbes, transmitted through the water [13]. The unclean and tainted potable water is directly connected to diseases like cholera, diarrhea, dysentery, hepatitis A, etc. Over 842,000 people worldwide are thought to perish from diarrhea each year [14,15].

Poor water, sanitation, and hygiene (WaSH) in schools causes serious health problems for kids, such as gastrointestinal tract infections, neurological disorders, and psychological illnesses [16]. It also harms kids' academic performance by raising absenteeism rates and impairing cognitive abilities [17-22].

Bangladesh's drinking water quality is seriously threatened. Regretfully, no thorough investigation has been carried up so far to ascertain the quality of the drinking water in Chattogram, Bangladeshi schools. Bangladesh's port city, Chattogram, is one of the heavily populated cities that has been plagued by a lack of clean drinking water, which is frequently linked to other issues with water quality. Only one-fourth of the city's water demand can be met by CWASA (Chattogram Water Supply Authority) due to inadequate distribution network systems [23].

This fact led to the current study's focus on the drinking water quality in Chattogram City, Bangladesh's various schools to improve the WaSH status of these schools and help school-age children achieve better health and educational results.

Materials and Methods

Sampling site

The study was conducted in port city Chattogram, the second-biggest city in Bangladesh with a population of over 2.5 million as per the 2011 census. Situated near the banks of the Karnaphuli River, the city is a popular destination for travelers and business enthusiasts due to its remarkable natural splendor and abundant resources. Samples of drinking water were taken from 40 separate schools spread over the city of Chattogram.

Sample collection

Water samples were collected from different schools in the Chattogram city area of Bangladesh. The schools were selected randomly and categorized into: combined high school and college, combined high school, combined primary school, girl's high school, and boy’s high school. Samples were collected from four different sources i.e. purified tap water, unpurified tap water, jar water, and bottled water. Forty water samples were collected aseptically and correctly labeled in sterile plastic vials. After that, the samples were transported in containers filled with ice to the laboratory [24]. Samples were kept cold at 4^{o}C for 24 hours before being subjected to microbiological analysis.

Total viable count

Using serial dilutions and pour plate technique on nutrient agar media (Hi Media, India), the microbial population in the collected drinking water sample was counted [24]. With this method, the number of colony forming units (cfu/ml) of the sample is represented by the count, which estimates the population of the living bacterial burden. The contaminated plates were incubated at 37°C for 24 hours, during which time the number of cfu on plates containing 30 to 300 colonies were counted.

Most probable number

The MPN method uses replicate lactose fermentation broth growth in ten-fold dilutions following normal procedure to assess the presence of coliform.[25] It is most frequently used to test water quality or determine whether coliform is present in the water and whether it is safe. The presence of the indicator organism *E. coli* was detected and confirmed using three continuous stages of the test: the presumptive test, the confirmed test, and the completed test. These stages included the use of lactose fermentation producing gas, the green metallic sheen on Eosin methylene blue agar, and the Gram reaction.

Isolation and identification of bacteria

To isolate bacteria from drinking water samples, four selective media were employed: Cetrimide agar, Xylose Lysine Deoxycholate (XLD) agar, Salmonella-Shigella (SS) agar, and Eosin Methylene Blue (EMB) agar (Hi Media, India). Bacteria were isolated by streaking test tubes containing TCC-positive samples of inocula on solidified agar plates (EMB, XLD, SS, and Cetrimide). After being developed on selective agar media, the colonies were moved to nutrient agar slants and kept there for additional microscopic and biochemical analysis [26]. After being selected from various selective media, the isolates underwent cultural, morphological and physicochemical tests to be identified.

Antimicrobial susceptibility test

Using the Kirby-Bauer technique, the susceptibility and resistance of bacterial isolates to several antibiotics were determined *in vitro* [27]. This technique made it possible to quickly ascertain a medication's effectiveness by measuring the width of the zone of inhibition that developed due to the agent diffusing into the medium around the disc. The test organism suspension was made in peptone broth by a 24-hour, 37ºC overnight culture. Using a sterile cotton swab, the culture broth was evenly dispersed across the surface of the solid medium. Using sterile forceps, antibiotic discs were aseptically positioned on Mueller-Hinton agar plates. Following a 30-minute refrigeration period to allow the antibiotic to permeate the medium, the plates were incubated for 24 hours at 37ºC. The following antibiotic discs were utilized: ciprofloxacin (5μg), rifampin (5μg), ceftriaxone (5μg), tetracycline (30μg), chloramphenicol (30μg), amoxicillin (10μg), ciprofloxacin (5μg), Ampicillin (10μg), gentamicin (10μg) (Hi Media, India). Based on the Kirby-Bauer disk diffusion susceptibility test technique, the plates were checked after incubation to determine the diameters of the zone of inhibition. The findings were reported as sensitive (S), resistant (R), and intermediate (I). The antibiotic susceptibility of tested isolates was interpreted using the guiding principles of the Clinical and Laboratory Standards Institute (CLSI) [28].

Inspection of drinking water, sanitation, and hygiene conditions of the schools

Through both on-site inspection and the completion of a questionnaire form, the water, sanitation, and hygiene conditions of the schools were examined. Questions on the schools' water, sanitation, and hygiene (WaSH) facilities were included in the questionnaire form. After obtaining permission from the school administration, we interviewed and observed a school representative in the field, recording his name and other pertinent details for the questionnaire.

Results and Discussion

Contamination of drinking water is one of the biggest global health issues, especially in impoverished nations. In this study, 40 distinct Chattogram City schools were chosen at random to evaluate the quality of their drinking water. In addition, field observations and the completion of a questionnaire Form were used to look at the water supply, sanitation, and hygiene conditions at these schools. The schools varied from one another in terms of the quantity of pupils, gender, and grade level, among other factors.

Microbiological analysis of drinking water samples

Determination of total viable bacteria

Total viable count is the total number of microbial burdens in drinking water samples. Samples' TVCs varied from 200 cfu/ml at the lowest to 80,000 cfu/ml at the highest. The USEPA states that the standard limit of the viable count in drinking water is around 500 cfu/ml. 26 (65%) of the 40 samples had TVC counts more than 500 cfu/ml, exceeding the allowable limit. This outcome is consistent with the research by Ahmed *et al*. which was conducted at several academic institutions in Abbottabad, Pakistan, and found that 63.5% of samples had drinking water quality levels above WHO guidelines[12,29,30]. In 14 (35%) samples, the TVC was less than 500 cfu/ml, falling within an acceptable range (Figure 1). This conclusion is based on Chowdhury *et al*. findings [31].

Eight samples (>30,000 cfu/ml) show very high TVC counts out of the 26 samples (65%). This result somewhat agrees with other results [32-35]. Therefore, a filtration system is necessary for these 26 schools (>500 cfu/ml) to improve the quality of their drinking water. If not, consuming polluted water will lead to the spread of infectious diseases, which might result in fatalities and other severe illnesses [36-38]. The CWASA distribution lines' microbiological water characteristics were shown to be nearly concerning for city people [39]. The drinking jar water of Chattogram city has a total bacterial count ranging from 1.5×102-1.6×104 cfu/ml, according to other findings [40].

Detection of coliform bacteria

Detection of coliform bacteria in the sample indicates bacterial contamination, potential risk of the presence of pathogenic organisms; and absence of coliform bacteria is evidence of bacteriologically safe drinking water [41]. MPN technique was used to estimate the total coliform bacteria and the range of values was found 0 to 2,400 MPN/100 ml. The MPN index per 100 ml, shown in Table 1, may be used to categorize drinking water into various risk groups following WHO criteria.

The WHO and Bangladesh's Environmental Quality Standard (EQS) state that drinking water's TCC should

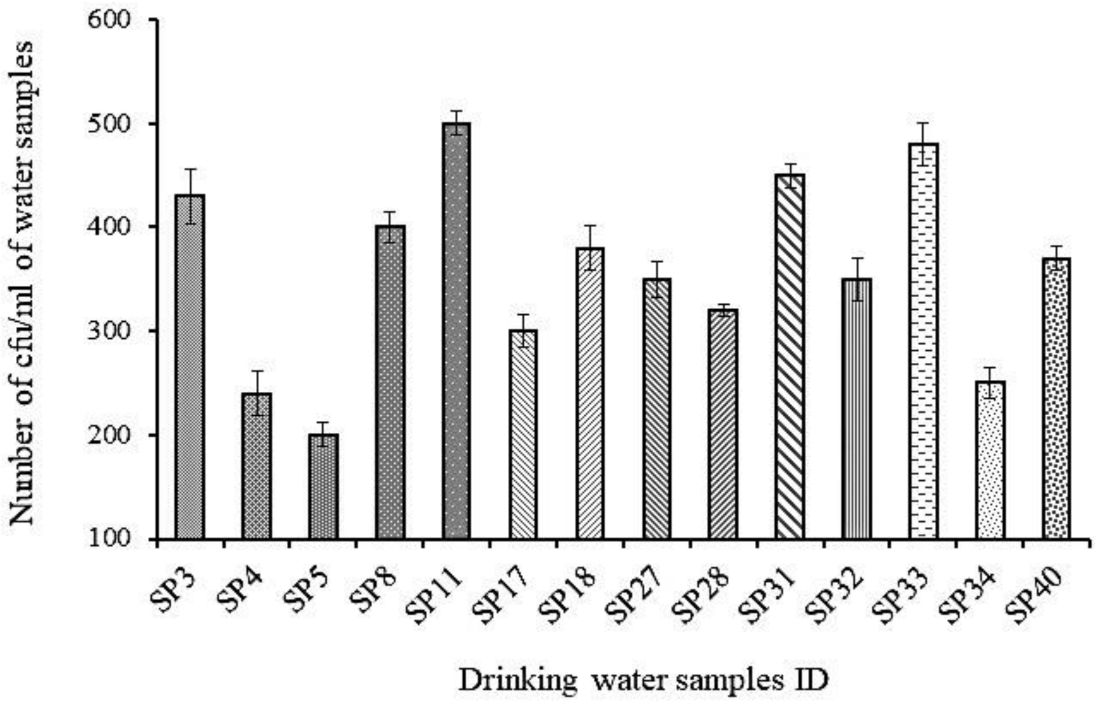

Figure 1 Total viable aerobic count (cfu/ml) of water samples.

be zero per 100 milliliters. According to this study, 65% of the drinking water samples from the schools were contaminated with coliform, indicating that the water quality at these institutions is subpar. 12.5% of coliform-contaminated water were at very high risk, 20% were at high danger, 30% were at intermediate risk, and 2.5% were at low risk. In accordance with WHO rules, only 35% of the water samples were deemed safe to drink (Table 1).

This result demonstrates the parallels with the findings of Parvez *et al.* whose investigation was conducted in 37 Bangladeshi regions where coliform was detected in 80% of the drinking water samples. Others have also made this discovery [30,35,42]. Mina *et al.* discovered that Chattogram City's plastic jar drinking water had 26.32% total and fecal coliform. In Gakulnagar village of Dhaka district, 60% of tube-well water is contaminated by coliform bacteria [40,43]. In the same way, 207 tube-well water samples were taken from the districts of Comilla, Brahmanbaria, and Sirajganj; of these, 41% had total coliform, 29% included fecal coliform, and 13% contained *E. coli* infection[44]. Another research indicated that the MPN/100mL of samples from rural shallow tube-wells included *E. coli* within ranges of 1 to 10, 10 to 100, and 100 to 2000 on 30, 9, and 4% of the samples, respectively [45]. The total coliform count is a useful metric for evaluating how clean storage facilities and distribution networks are. According to our research, thousands of pupils at the school are exposed to varying degrees of health danger.

Table 1 Categorization of MPN values of drinking water samples according to WHO guideline

MPN index / 100 ml	Category	Total samples	Percentage (%)	Remarks
0	A	14	35	No risk
1-10	B	1	2.5	Low risk
10-100	C	12	30	Intermediate risk
100-1000	D	8	20	High risk
>1000	E	5	12.5	Very high risk

Isolation and identification of the bacterial isolates

Using microscopic, cultural, and biochemical testing, contaminating bacteria were found and verified to assess the microbial contamination of the water samples. Green metallic-shaded dark colonies and those

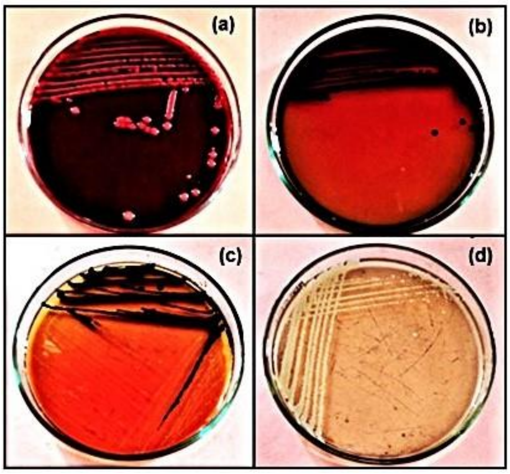

Figure 2 Bacterial isolates growing on selective culture media: a) Dark purple colonies on EMB agar b) With or without black centered colonies on SS agar c) Yellowish and grey with black centered colonies on XLD agar d) Yellowish colonies on Cetrimide agar.

without were selected after a 24-hour incubation period at 37°C on EMB agar. Colonies that were colorless, pink, and completely black on SS agar, yellow, pink, and red with a black core or wholly black on XLD agar, and colorless, yellowish, and blue-green colonies on Cetrimide agar were selected and purified. To identify the bacterial strain, the isolates were selected using various selective media and subsequently various morphological, cultural and physicochemical characteristics were studied (Table 2, Figure 2). The 8th edition of Bergey's Manual of Determinative Bacteriology, which contains standard descriptions, was compared to identify the bacterial isolates up to the genus level [46].

Percentage of different types of bacteria isolated from drinking water samples

Several kinds of bacteria were identified from samples of drinking water for this investigation. Figure 3 shows that the predominant bacteria in the majority of the samples were *Enterobacter* sp., *Citrobacter* sp., *Pseudomonas* sp., and *Micrococcus* sp. Just 10% of samples contained *Proteus* sp. Various percentages of other bacteria, including *Salmonella* sp., *Alcaligenes* sp., *Serratia* sp., and *E. coli*, were found in the various samples. This result somewhat agrees with previous study findings [30,31,35,39,45,47,49,50]. Islam *et al*. found that the WHO drinking water guideline value was surpassed in 50% of mineral water samples, 87.5% of filtered water tests, and 100% of tap water samples [12]. According to research by Acharjee *et al*. the microbes discovered in tap water were *Salmonella* sp. (0%), *E. coli* (60%), *Klebsiella* sp. (40%), *Enterobacter* sp. (20%), *Pseudomonas* sp. (70%), *Proteus* sp. (10%), and *Staphylococcus* sp. (40%) [48].

Determination of antibiotic sensitivity pattern of different bacterial isolates

Using the disc diffusion technique, ten distinct isolates were tested for antibiotic sensitivity by using ten different antibiotic discs. The findings of the antibiotic sensitivity patterns of the various isolates are shown in Table 3. The majority of the organisms were found to be extremely resistant to rifampin, erythromycin, amoxicillin, and ampicillin, according to the findings of the antibiotic sensitivity test. However, it was

Table 2 Morphological and biochemical properties of the bacteria isolated from drinking water samples of different schools in Chattogram port city area, Bangladesh.

Name of the isolates	Shape	Gram stain	MR test	VP test	Citrate test	TSI test				Indole test	Catalase test	Nitrate test	Urease test
						Slant	Butt	Gas	H_2S				
Enterobacter	Rod	-	-	+	+	A	A	+	-	-	+	+	-
E. coli	Rod	-	+	-	-	A	A	+	-	+	+	+	-
Citrobacter	Rod	-	+	-	+	A	A	+	+	-	+	+	+
Salmonella	Rod	-	+	-	+	K	A	+	+	-	+	+	-
Proteus	Rod	-	+	-	-	K	A	+	+	+	+	+	+
Pseudomonas	Rod	-	-	-	+	K	K	-	-	-	+	+	-
Micrococcus	Coccus	-	-	+	-	K	NC	-	-	-	+	-	-
Serratia	Rod	-	-	+	+	K	A	-	-	-	+	+	+
Alcaligenes	Rod	-	-	-	+	NC	NC	-	-	-	+	-	-
Klebsiella	Rod	-	-	+	+	A	A	+	-	-	+	+	+

TSI-Triple Sugar Iron test; A- acidic (yellow); K- alkaline (red); NC-no change; MR-methyl red; VP- Voges-Proskauer; (-) indicates negative; (+) indicates positive discovered that the organisms were extremely susceptible to tetracycline, azithromycin, gentamicin, and ciprofloxacin. This discovery partially aligns with previous research wherein isolated *Salmonella* was identified as the cause of many outbreaks and resistant to ampicillin, trimethoprim, and chloramphenicol [12,51,52]. *Staphylococcus* sp. was shown to be susceptible to ciprofloxacin, erythromycin, tetracycline, and norfloxacin by Faria *et al*. [53]. The indiscriminate use of antibiotics may be the cause of this high prevalence of multidrug resistance, as drug-resistant microbes from antibiotic-saturated settings may ultimately be displaced. For a long time, antibiotics that are freely accessible from any drugstore without a prescription have been used indiscriminately in Bangladesh. People are unaware that antibiotics are used on a scheduled basis. As a result, genetic recombination against one or more antimicrobial drugs may give rise to resistant organisms [54].

Water, sanitation, and hygiene facilities at the schools

Different sources of drinking water utilized in schools of Chattogram city area schools are listed and displayed in Figures 4 and 5. According to our research, 32.50% of schools get their drinking water from untreated tap water. Numerous bacteria, including *Klebsiella*, *Enterobacter*, *E. coli*, and *pseudomonas*, have been found in untreated water. These bacteria can cause a range of gastrointestinal disorders, such as dysentery, diarrhea, and typhoid [12,47,50,55]. Our investigation revealed the presence of *Micrococcus*, *Pseudomonas*, *Serratia*, *Salmonella*, *Citrobacter*, *E. coli*, *Enterobacter*, *Proteus*, *Klebsiella*, and *Alcaligenes* in unpurified water sources, which is consistent with previous findings.

During the on-site inspection, it was discovered that drinking water storage tanks were left filthy and exposed. Drinking water contamination might be a result of the storage tank's unsanitary state. Contaminants in water have the potential to have serious negative health impacts, such as neurological abnormalities, reproductive issues, and gastrointestinal illnesses. Certain pollutants may provide a particular risk of sickness to infants and young children [56]. Fourteen percent of the students at the school drank water purified from the tap. Purified water may still contain microorganisms because of ineffective filtration procedures at the source.

Table 3 Antibiotic sensitivity profile of different bacteria isolated from drinking water samples.

	Name of Antibiotics									
Bacterial Isolates	**CIP 5µg**	**GEN 10µg**	**AZM 15µg**	**TET 30µg**	**RMP 5µg**	**ERY 15µg**	**AMX 10µg**	**AMP 10µg**	**CAP 30µg**	**CRO 30µg**
Enterobacter sp.	S	S	S	S	R	R	R	R	R	R
Klebsiella sp.	S	S	S	S	R	R	R	R	S	S
E. coli	S	S	S	I	R	R	I	I	S	S
Citrobacter sp.	S	S	S	S	R	R	R	R	S	S
Salmonella sp.	S	S	I	R	R	R	R	R	R	R
Proteus sp.	I	S	S	R	R	R	R	R	S	R
Serratia sp.	S	S	S	S	R	S	S	S	R	R
Pseudomonas sp.	S	S	S	S	R	R	I	R	R	S
Micrococcus sp.	S	S	S	S	S	I	R	R	S	S
Alcaligenes sp.	S	S	S	S	R	R	R	R	R	R

S-sensitive; R-resistant; I-intermediate; CIP -ciprofloxacin, GEN-gentamicin, AZM-azithromycin, TET-tetracycline, RMP-rifampin, ERY-erythromycin, AMX-amoxicillin, AMP –ampicillin, CAP- chloramphenicol, CRO- ceftriaxon

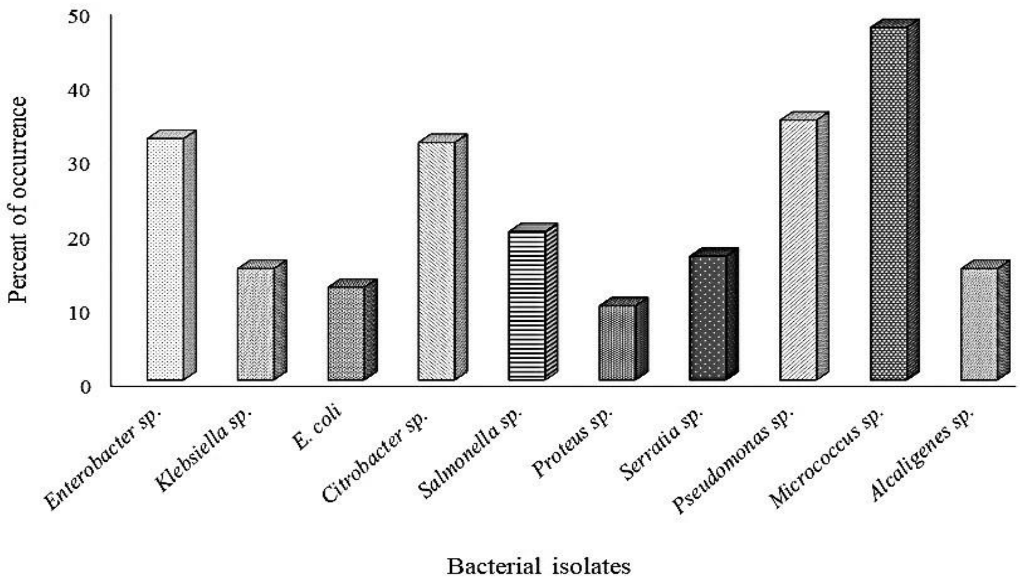

Figure 3 Percentage of bacteria isolated from drinking water samples of different school.

In this investigation, purified tap water was shown to include *Micrococcus*, *Pseudomonas*, *Serratia*, *Salmonella, Citrobacter, E. coli,* and *Enterobacter*. This conclusion is somewhat supported by the discovery of *E. coli* in the filter water by Chowdhury *et al*.[31]. About 12.5% of the schools utilized jar water as their main supply of drinking water. *Micrococcus, Pseudomonas, Salmonella, Citrobacter, E. coli*, and *Enterobacter* were found in the jar water used in this investigation. Poor quality jar water samples were discovered in a recent study, which might raise the risk of water-borne illneses. Jar water included the following bacteria: *Micrococcus, Pseudomonas, E. coli, Enterobacter, Aeromonas, Bacillus, Cardiobacterium, Corynebacterium, Lactobacillus, Clostridium,* and *Klebsiella* [40].

According to different research, 98% of the jar water samples taken from 24 locations across the metropolis of Dhaka had *E. coli* [57]. Jar water is therefore probably dangerous for the student's health. 7.5% of the schools got their drinking water from bottles. According to our investigation *Micrococcus*, *Pseudomonas*, *Klebsiella, Citrobacter, Serratia,* and *Alcaligenes* were found in

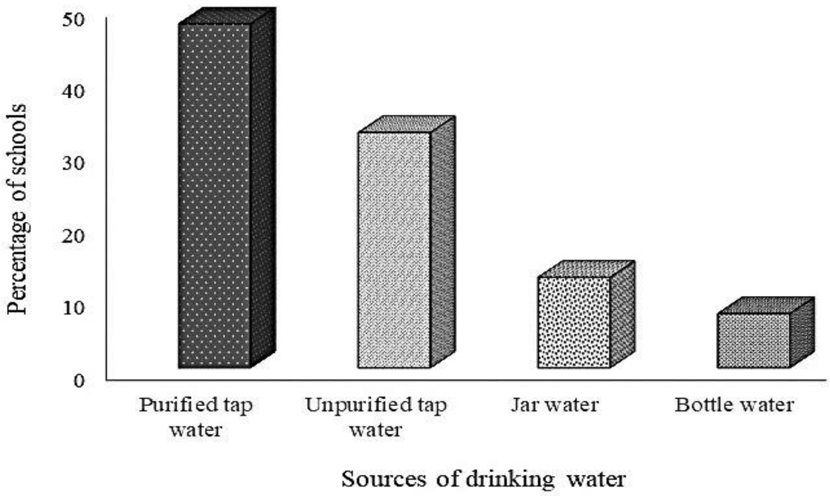

Figure 4 Percentage of use of drinking water sources at schools.

Figure 5 Sources of drinking water images in different schools of Chattogram city.

bottled water. The findings of this investigation support the discovery of *Staphylococcus* sp. in bottled water by Faria *et al*. [53]. As a result, the majority of drinking water sources were determined to be polluted and need to have the harmful substances removed [58].

Sanitation facilities at school

Every one of the 40 schools' sanitation facilities were observed and documented in the survey form (Figure 6). 22.5% of schools had two or less toilets, 17.5% with three, 25% with four, and 35% with five or more. In our survey, we also found the percentage of schools with separate restrooms for females (48.3%) and those without (51.7%). Globally, 66% of schools have access to basic sanitation services in 2016.[59] Most of the school toilets were discovered to be in poor and extremely unclean condition (Figure 7). A report on schools claims that a lot of kids stay away from school because of the unclean toilets [60]. Hepatitis A, the flu, gastrointestinal viruses, mildew, strep throat, and other illnesses can all be brought on by using unclean toilets [56]. Therefore, these institutions ought to upgrade their sanitation facilities to lower the many illnesses that result from inadequate sanitation systems and raise student attendance.

Hand washing facilities at schools

It's essential to wash your hands for optimum health. Inadequate access to soap and clean water for hand washing can contribute to the spread of illness among pupils. There is a 42 to 47% reduction in the incidence of diarrhea when hands are cleaned with soap [61]. It is

Figure 6 Percent distribution of the number of toilets in different schools.

observed that 50% of schools give soap in the sink for pupils to wash their hands after using the toilets, while 50% of schools do not. Numerous respiratory and gastrointestinal illnesses, including influenza and salmonellosis, can be brought on by contaminated hands. Hand hygiene is important in halting the transmission of the pathogens that cause these illnesses. Certain types of respiratory and gastrointestinal without soap, while 36% of schools offered no hand washing at all. An estimated 850 million children did not have access to basic services, and those who did have either a restricted or nonexistent hand-washing program at school [59]. Therefore, to lessen the issues that appropriate hand washing might entail, these institutions must provide proper hand-washing facilities (Figure 8).

Figure 7 Images of sanitation facilities of different schools.

Figure 8 Images of hand washing facilities of different schools.

infections can lead to life-threatening consequences, particularly in young children, the elderly, or people with compromised immune systems [62]. In 2016, 53% of schools worldwide offered a minimal hygiene service; just 11% of them provided hand washing stations

Conclusion

One of the most important things in our lives is water. Safe, high-quality drinking water is a constant public health problem. According to the findings, several schools' water had extremely high total viable and coliform counts, putting the pupils' health in danger. The study indicated that the research area's high TVC, coliform, and illness prevalence all point to a favorable relationship between health and water quality. The majority of the water samples had coliform, which suggests that the school administration is not very concerned about providing clean drinking water.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Postel, S.L., Daily, G.C. and Ehrlich P.R. 1996. Human appropriation of renewable fresh water. *Science* **271**(5250), 785-788.

2. Walters, K. and Cram, G. 2002. Drinking water in schools: hygiene standards at fountains. *Nutrition Food Science* **32**(1), 9-12.

3. Tekpor, M., Akrong, M., Asmah, M.H., Banu, R.A. and Ansa, E.D.O. 2017. Bacteriological quality of drinking water in the Atebubu-Amantin district of the Brong-Ahafo region of Ghana. *Applied Water Science* **7**, 2571–2576.

4. UNCED, 1992. Protection of the quality and supply of freshwater resources: Application of integrated approaches to the development, management and use of water resources. The United Nations Conference on Environment and Development. Chapter 18, Agenda 21

5. Faria, C., Vaz-Moreira, I., Serapicos, E., Nunes, O.C. and Manaia, C.M. 2009. Antibiotic resistance in coagulase negative staphylococci isolated from wastewater and drinking water. *Science of total environment* **407**(12), 3876-3882.

6. WHO, 2004. Guidelines for drinking water quality, 3rd edn. World Health Organization, Geneva

7. Fawell, J. and Nieuwenhuijsen, M.J. 2000. Contaminants in drinking water environmental pollution and health. *British Medical Bulletin*. **68**(1), 199-208.

8. Akoto, O. and Adiyiah, J. 2007. Chemical analysis of drinking water from some communities in the Brong Ahafo region. *International Journal of Environmental Science and Technology* **4**(2), 211–214.

9. Pelczar, M.J. and Reid, R.D. 1978. Microbiology. New-York: McGraw-Hill, p 500.

10. Chis, A.A., Rus, L.L., Morgovan, C., Arseniu, A.M., Frum, A., Vonica-T, incu, A.L., Gligor, F.G., Mures,an, M.L.,and Dobrea, C.M. 2022. Microbial resistance to antibiotics and effective antibiotherapy. *Biomedicines* **10**, 1121.

11. Rahman, A., Rahman, A.K.M.F., Shafinaz, S. and Linnan, M. 2005. Bangladesh health and injury report on children. DGHS, ICMH, UNICEF & TASC. Dhaka, Bangladesh

12. Islam, S., Begum, H.A. and Nili, N.Y. 2010. Bacteriological safety assessment of municipal tap water and quality of bottle water in Dhaka City: health hazard analysis. *Bangladesh journal of medical microbiology* **4**(1), 9-13.

13. Wilma, M.D.V. 2015. Microbiological and physico-chemical quality of deep well water in selected public elementary schools. *Asia Pacific Journal of Multidisciplinary Research* **3**(5), 105-109.

14. WHO, 2017a, July. Drinking-water Fact Sheets. Retrieved from World Health Organization.

15. WHO, 2017b. UN-water Global Analysis and Assessment of Sanitation and Drinking Water (GLAAS) 2017 Report: Financing Universal Water, Sanitation and hygiene under the Sustainable. World Health Organization, Geneva.

16. Alexander, K.T., Dreibelbis, R., Freeman, M.C. Ojeny, B. and Rheingans, R. 2013. Improving service delivery of water, sanitation, and hygiene in primary schools. A cluster-randomized trial in western Kenya. *Journal of Water and Health* **11**, 507–519.

17. Bar-David, Y., Urkin, J. and Kozminsky, E. 2005. The effect of voluntary dehydration on cognitive functions of elementary school children. *Acta Paediatrica* **94**, 1667–1673.

18. Blanton, E., Ombeki, S., Oluoch, G.O., Mwaki, A., Wannemuehler, K. and Quick, R. 2010. Evaluation of the role of school children in the promotion of point-of-use water treatment and handwashing in schools and households—Nyanza province, western Kenya. *American Journal of Tropical Medicine and Hygiene* **82**, 664– 671.

19. Freeman, M.C., Greene, L.E., Dreibelbis, R., Saboori, S., Muga, R., Brumback, B. and Rheingans, R. 2012. Assessing the impact of a school-based water treatment, hygiene and sanitation programme on pupil absence in Nyanza province, Kenya. A cluster-randomized trial. *Tropical Medicine & International Health* **17**, 380-391.

20. Bartlett, S. 2003. Water, sanitation and urban children: the need to go beyond "improved" provision. *Environment* and *Urbanization* **15**, 57–70.

21. Benton, D. and Burgess, N. 2009. The effect of the consumption of water on the memory and attention of children. *Appetite* **53**, 143–146.

22. Jasper, C., Le, T.T. and Bartram, J. 2012. Water and sanitation in schools. A systematic review of the health and educational outcomes. *International Journal of Environmental Research and Public Health* **9**, 2772–2787.

23. Amin, M. 2006. Status of water and sanitation services in Chittagong water supply and sewerage authority, Bangladesh. Capacity-building workshop on partnerships for improving the performance of water utilities in asia and the pacific region, organized by UNDESA and UNESCAP, 25-27.

24. Marzan, L.W., Hossain, M., Mina, S.A., Akter, Y. and Chowdhury, A.M.M.A. 2017. Isolation and biochemical characterization of heavy-metal resistant bacteria from tannery effluent in Chittagong City, Bangladesh: bioremediation viewpoint. *Egyptian Journal of Aquatic Research* **43**, 65-74.

25. Clesceri, L.S., Greenberg, A.E. and Eaton, A.D. 1996. Standard methods for the examination of water and wastewater. APHA, AWWA and WPCF, Washington DC, 43.

26. Rahman, I.M.M., Barua, S., Barua, R., Mutsuddi, R., Alamgir, M., Islam, F., Begum, Z.A. and Hasegawa, H. 2017. Quality assessment of the non-carbonated bottled drinking water marketed in Bangladesh and comparison with tap water. *Food Control.* **73**, 1149-1158.

27. Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. 1996. Antibiotic susceptibility testing by a standarized single disk method. *American Journal of Clinical Pathology* **45**(4), 493-496.

28. Waturu, G., Mutai, W., Kiiru, J., Musyoki, M,. Ochieng, L. 2017. Antimicrobial resistance encountered in garbage collection areas and dumpsites in Nairobi, Kenya using *Escherichia coli* and *Klebsiella* as indicator species. *Advances in Microbiology* **7,** 653-665.

29. Ahmed, T., Pervez, A., Mehtab, M. and Sherwani, S.K. 2015. Assessment of drinking water quality and its potential health impacts in academic institutions of Abbottabad, Pakistan. *Desalination Water Treatment* **54**, 1819-1828.

30. Moniruzzaman, M., Akter, S., Islam, M. and Mia, Z. 2011. Microbiological quality of drinking water from dispensers in roadside restaurants of Bangladesh. *Pakistan Journal of Biological Sciences* **14**, 142-145.

31.Chowdhory, A., Kabir, N., Chowdhury, M.M.I. and Chowdhury, J.A. 2016. Detection of Escherichia coli in drinking water sources of filter units and supply water. *Bangladesh Pharmaceutical Journal* **19**(2), 206-210.

32. Bartram, J., Cotruvo, J., Exner, M., Fricker, C. and Glasmacher, A. 2004. Heterotrophic plate count measurement in drinking water safety management: Report of an expert meeting Geneva. *International Journal of Food Microbiology* **92**(3), 241-247.

33. Kassenga, G.R. 2007. The health related microbiological quality of bottled drinking water sold in Dar es Salaam, Tanzania. *Journal of Water Health*. **5**(1), 179-185.

34.Svagzdiene, R., Lau, R. and Page, R.A. 2010. Microbiological quality of bottled water brands sold in retail outlets in New Zealand. *Water Science and Technology* **10**, 689-699.

35. Ahmed, T., Baidya, S., Acharjee, M. and Rahman, T. 2014. Qualitative analysis of drinking water through the most probable number (MPN) method. *Stamford Journal of Microbiology* **3**(1), 9-16.

36. Marshall, J., Thabane, M., Garg, A.X., Clark, W.F., Salvadori, M. and Collins, S.M. 2006. Incidence and epidemiology of irritable bowel syndrome after a large waterborne outbreak of bacterial dysentery. *Gastroenterology*. **131**(2), 445-450.

37. Jones, A.Q., Majowicz, S.E., Edge, V.L., Thomas, M.K., Mac, D.L., Fyfe, M., Atashband, S. and Kovacs, S.J. 2007. Drinking water consumption patterns in British Columbia: an investigation of associations with demographic factors and acute gastrointestinal illness. *Science of The Total Environment* **388**, 54-65.

38. Peace, T. and Mazumder, A. 2007. Tracking patterns of enteric illnesses in populations and communities. *Environ Health Perspectives* **115**: 58-64.

39. Zuthi, M.F.R., Biswas, M. and Bahar, M.N. 2009. Assessment of supply water quality in the Chittagong city of Bangladesh. *Journal of Engineering and Applied Science* **4**, 73-80.

40. Mina, S.A., Marzan, L.W., Sultana, T. and Akter, Y. 2018. Quality assessment of commercially supplied drinking jar water in Chittagong City, Bangladesh. *Applied Water Science*. **8**, 24.

41. Hunter, P.R., MacDonald, A.M. and Carter, R.C. 2010. Water supply and health. *PLOS medicine*. **7**(11), e1000361.

42. Parvez, A.K., Liza, S.M., Marzan, M., Ahmed, A. and Rahman, M.H. 2016. Bacteriological quality of drinking water samples across Bangladesh. *Archives of Clinical Microbiology* **7**, 1.

43. Rahman, S.H., Ahmed, S. and Jakariya, M. 2009. Investigation of shallow tube-well water quality considering the influence of nearby latrines in a rural village of Bangladesh. *IAHS pub*. 299-304.

44. Luby, S., Gupta, S., Sheikh, M., Johnston, R., Ram, P. and Islam, M. 2008. Tubewell water quality and predictors of

contamination in three flood-prone areas in Bangladesh. *Journal of Applied Microbiology* **105**(4), 1002-1008.

45. Geen, A.V., Ahmed, K.M., Akita, Y., Alam, M.J., Culligan, P.J., Emch, M., et al. 2011. Fecal contamination of shallow tube wells in Bangladesh inversely related to arsenic. *Environmental Science and Technology* **45** (4), 1199-1205.

46. Buchanan, R.E. and Gibbons, N.E. 1974. Bergey's Manual of determinative Bacteriology, 8th edn. Williams and Wilkins co. Baltimore.

47. Prasai, T., Lekhak, B., Joshi, D.R. and Baral, M.P. 2007. Microbiological analysis of drinking water of Kathmandu Valley. *Scientific World*. **5**(5), 112-114.

48. Acharjee, M., Rahman, F., Jahan, F. and Noor, R. 2014. Bacterial proliferation in municipal water supplied in mirpur locality of Dhaka city, Bangladesh. *Clean Soil Air Water*. **42**(4), 434-441.

49. Venkatesan, K.D., Balaji, M. and Victor, K. 2014. Microbiological analysis of packaged drinking water sold in Chennai. *International Journal of Medical Science and Public Health* **3**(4), 472-476.

50. Pant, N.D., Poudyal, N. and Bhattacharya, S.K. 2016. Bacteriological quality of bottled drinking water versus municipal tap water in Dharan municipality, Nepal. *Journal of Health, Population and Nutrition* **35**(1), 17.

51. Nazir, K.H.M.N.H., Rahman, M.B., Nasiruddin, K.M., Akter, F., Khan, M.F.R. and Islam, M.S. 2005. Antibiotic sensitivity of Escherichia coli isolated from water and its relation with plasmid profile analysis. *Pakistan Journal of Biological Sciences* **8**(11), 1610-1613.

52. Rowe, B., Ward, L.R. and Threlfall, E.J. 1997. Multidrug-resistant *Salmonella typhi*: a worldwide epidemic. *Clinical Infectious Diseases* **24**(l1),106-109.

53. Faria, Y.A., Rahman, S.S. and Hossain, M.M. 2017. A study on the microbiological status of mineral drinking water. *The Open Microbiology Journal* **11**, 31-44.

54. Buxton, A. and Fraser, G. 1977. Animal microbiology. Blackwell scientist publication, Oxford, London, Edinburg, Melbourne, 85-86.

55. Metcalf, Eddy 2000. Paper Presented at a seminar on ground water and waste water. Organized by Melamchi Water Supply Development Board, Kathmandu, Nepal.

56. Brunkard, J.M., Ailes, E., Roberts, V.A., Hill, V., Hilborn, E.D., Craun, G.F., Rajasingham, A., Kahler, A., Garrison, L. and Hicks, L. 2011. Surveillance for waterborne disease outbreaks associated with drinking water—United States. *MMWR Surveillance Summaries* **60,** 38-68.

57. Molla, M.A.M. 2017. Safe water in jar mostly unsafe. The Daily Star. Accessed 26 Dec.

58. Wallender, E.K., Ailes, E.C., Yoder, J.S., Roberts, V.A. and Brunkard, J.M. 2014. Contributing factors to disease outbreaks associated with untreated groundwater. *Groundwater* **52,** 886-897.

59. UNICEF, WHO 2018. Drinking water, sanitation and hygiene in schools: global baseline report. New York.

60. Adams, J., Bartram, J., Chartier, Y. and Sims, J. 2009. Water, sanitation and hygiene standards for schools in low-cost settings. *World Health Organization* 1-51.

61. Curtis, V. and Cairncross, S. 2003. Effect of washing hands with soap on diarrhea risk in the community: a systematic review. *Lancet Infectious Diseases* **3**(5), 275-281.

62. Aziz, K.M.A., Huttly, S.R.A., Minnatullah, K.M., et al. 1990. Water supply, sanitation and hygiene education: Report of a health impact study in Mirzapur, Bangladesh. UNDP – World Bank Water and Sanitation Program, The World Bank, Washington DC.