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- **Short communication**

Microbial contamination in drinking water at restaurants in Savar, Dhaka, Bangladesh

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Out of 20 drinking water samples collected from Savar area, 17 samples (85%) were contaminated with a 1 to >18 MPN/100ml range. The indicator bacteria *Escherichia coli* was found in 7 out of 20 samples (35%) by confirmation on EMB medium that showed green metallic sheen, and the results of the tests were validated again by other biochemical reactions (IMViC). According to the findings, 85% of the tested water samples were microbiologically polluted, and drinking them might harm health.

Ensuring drinking water safety is a critical public health issue globally, with contaminated water being a major source of illness and disease transmission. In Bangladesh, water quality management remains a significant challenge due to inadequate infrastructure, pollution, and limited access to safe drinking water sources. Physical, chemical, and microbial parameters have been used to assess drinking water quality. Among them, water-related health illness is mainly caused by microbial contamination (WHO, 2008; Suthar *et al*., 2009). Access to bacteriologically safe water is a key component of public health, as unsafe drinking water can lead to outbreaks of waterborne diseases such as diarrhea, cholera, typhoid, and dysentery, responsible for millions of deaths worldwide annually (WHO, 2008). In Bangladesh, approximately 38 million people are exposed to unsafe water, and bacteriological contamination is a significant concern, particularly in urban and peri-urban areas (Hossain *et al*., 2024).

Restaurants and food service establishments in Bangladesh, particularly in rapidly urbanizing areas like Savar Upazila, serve a large portion of the population. However, they are often overlooked in water quality monitoring programs (Mujeri *et al*., 2021). Many establishments rely on untreated or poorly treated groundwater, which is highly susceptible to contamination by pathogens, heavy metals, and other pollutants due to improper waste disposal, agricultural runoff, and industrial activities (Nesha *et al*., 2023). Bacterial contamination, particularly with fecal coliforms such as *Escherichia coli*, is among the most common and concerning water quality issues in these settings. The presence of *E. coli* in water indicates fecal contamination and the potential presence of

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other harmful pathogens that can cause gastrointestinal infections (Zaric *et al*., 2023) Previous studies in Bangladesh have demonstrated alarming levels of microbial contamination in drinking water, particularly in food service establishments (Nesha *et al*., 2023). A study by Mou *et al*. (2023) found that over 70% of water samples from restaurants adjacent to Khulna University were contaminated with coliform bacteria, posing significant risks to public health. Similar research in other developing countries has revealed that water served in restaurants is frequently non-compliant with national and international water quality standards, necessitating regular monitoring and stricter regulations (Bain *et al*., 2014). In the context of Savar Upazila, an area with significant industrial activities and population growth, assessing the bacteriological quality of drinking water in restaurants is crucial, as many residents and workers rely on these establishments for their daily meals and hydration.

Given the potential health risks associated with bacteriological contamination of drinking water in restaurants, this study aims to assess the water quality served in selected food service establishments in Savar Upazila. The study focused on identifying the presence of coliform bacteria, particularly *E. coli*, and evaluating the water's compliance with the drinking water quality standards set by WHO. The findings of this research will provide recommendations for improving water quality management in local restaurants and reduce waterborne disease transmission and ensure public health protection in the region.

Sample Collection: Tap drinking water served in different restaurants of the Savar area was collected randomly from five different sites (Figure 1).

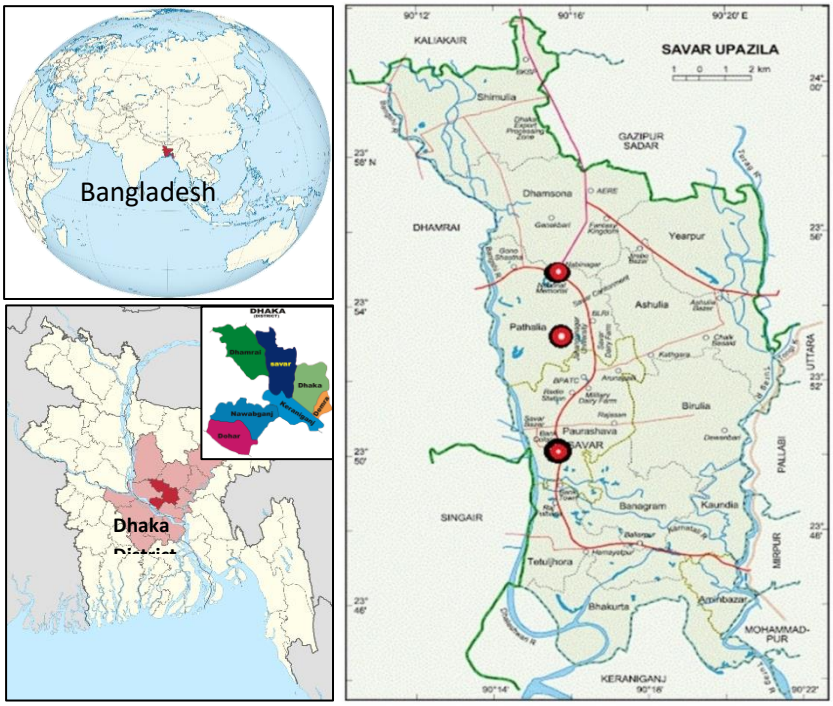

Fig. 1. Sampling location and sampling points

Four samples of each sampling site, total twenty samples were collected into 250 ml clean and sterilized glass bottles and immediately bring back to the laboratory for analysis.

MPN Method: MPN Method was carried out in three consecutive steps, such as: presumptive test, confirmed test and completed test (Alexander, 1983).

Presumptive test: The presumption for the presence of coliform bacteria in the samples, MacConkey broth is used with bromocresol purple as indicator. The upside-down Durham tube is used to estimate the production of gas. The change of media color into yellow and the presence of gas in Durham's tube can be presumed to be the presence of coliform bacteria in samples. Five sets of each 10 mL water samples were introduced into separate 10 mL double-strength presumptive broths, while one 50 mL water sample was mixed with 50 mL double-strength presumptive broth. Following 48-hour incubation at 37°C, the number of tubes showing positive results was noted for each set and then compared to a standard chart to determine the presumptive coliform count per 100 mL of the water sample (Alexander, 1983).

Confirmed Test: Samples that tested positive in the presumptive test were chosen to determine whether the presence of coliforms indicated the presence of *Escherichia coli*. Eosine Methylene Blue (EMB) agar medium was used to distinguish *Escherichia coli* from other Gram-negative coliform. This distinction was made based on observing a greenish metallic sheen, which confirms the presence of the indicator bacterium *E. coli* after 24 hours of incubation at 37ºC (Alexander, 1983).

Complete Test: The bacterial colonies developed on EMB medium were introduced into Luria-Bertani (LB) broth at a temperature of 44.5°C, along with Durham's tube, and a subculture of the colony was also placed on a MacConkey agar plate. The production of gas and color changes in media signifies the presence of *E. coli* according to Bergey's Manual of Systematic Bacteriology (Garrity, 2007) a satisfactory differentiation within the coliform group was done by indole, methyl red, Voges-Proskauer, and sodium citrate (IMViC) tests for further complete confirmation (Alexander, 1983).

Out of 20 samples, 17 had total coliform counts ranging from 1 to >18 MPN/100 mL, indicating the presence of lactose-fermenting bacteria (Table 1). In 3 samples, no coliform contamination was detected (<1 MPN/100 mL). Ten out of 17 coliform-contaminated samples showed maximum growth of lactose-fermenting coliform bacteria (>18 MPN/100 mL). The second-highest growth was observed in one sample, with 16 MPN/100 mL. One sample contained the smallest quantity of coliform bacteria capable of fermenting lactose (1 MPN/100 mL), while the remaining eight samples showed lactose-fermenting coliform bacteria ranging from 2 to 9 MPN/100 mL (Table 1).

Table 1. The coliform count by MPN method

Location	Sample name	Number of tubes giving a positive reaction		MPN Index per 100 mL	95 % confidence limits	
		1 × 50 mL	5 × 10 mL		Lower	Upper
Jahangirnagar University	P1	1	5	>18		
	P2	1	5	>18		
	D1	1	5	>18		
	D2	1	5	>18		
Savar	S1	1	5	>18		
	S2	1	4	16	4	40
	S3	0	2	2	0.5	6
	S4	1	5	>18		
Nobinagar	N1	1	5	>18		
	N2	0	0	<1		
	N3	1	3	9	2	21
	N4	0	2	2	0.5	6
Gerua	G1	0	1	1	0.5	4
	G2	1	5	>18		
	G3	0	0	<1		
	G4	0	2	2	0.5	6
Islamnagar	Is1	1	5	>18		
	Is2	0	0	<1		
	Is3	1	5	>18		
Control	C	0	4	5	1	13

The indicator organism *E. coli* was identified in 7 samples by the presence of a greenish metallic sheen on EMB media, while the remaining samples showed growth of coliform bacteria other than *E. coli*. The presence of *E. coli* in the water samples indicates fecal contamination (Table 2).

Table 2. Complete tests for *E. coli* isolates

Sample	Isolates	LB broth (+/-)	EMB agar	MacConkey's agar	Remarks
S1	S1C1	+	Deep pink	Fade pink (center yellow)	-
	S1C2	+	Whitish pink	Colorless	
	S1C3	+ (baby pink)	Greenish sheen	Deep pink	*Escherichia coli*
S2	S1C1	+	Deep pink	Fade pink (center yellow)	-
	S2C3	+ (baby pink)	Greenish sheen	Deep pink	*Escherichia coli*
S3	S1C2	+	Whitish pink	Colorless	
	S3C3	+ (baby	Greenish	Deep pink	*Escherichia coli*

		pink)	sheen		
S4	S3C4	+ (baby pink)	Greenish sheen	Deep pink	*Escherichia coli*
	S4C5	+	Pink	Fade pink (center yellow)	-
Is1	Is1C25	+	Deep pink	Fade pink (center yellow)	-
Is2	Is2C26	+	Deep pink	Colorless	-
	Is2C27	+	Fade pink	Colorless	-
Is3	S1C2	+	Whitish pink	Colorless	-
	S4C5	+	Pink	Fade pink (center yellow)	-
	Is3C3	+ (baby pink)	Greenish sheen	Deep pink	*Escherichia coli*
N1	N1C7	+	Whitish pink	Pink with yellow center (mucoid)	-
	N1C8	+	Deep pink	Yellowish pink	-
N2	N2C9	+	Whitish pink	Colorless	-
	N2C10	-	Deep pink	NG	-
N3	N3C11	-	Whitish pink	NG	-
	N2C9	-	Whitish pink	Colorless	-
	N2C10	-	Deep pink	NG	-
N4	N4C12	+	Whitish pink	Pink with yellow center (mucoid)	-
	N4C13	+	Deep pink	Pink with yellow center (mucoid)	-
D1	D1C3	+ (baby pink)	Greenish sheen	Deep pink	*Escherichia coli*
	D1C14	+	Deep pink	Colorless	-
D2	D2C15	-	Fade pink	NG	-
P1	P1C16	-	Whitish pink	NG	-
	P1C17	+	Whitish mucoid pink		-
P2	P2C3	+ (baby pink)	Greenish sheen	Deep pink	*Escherichia coli*
	P2C18	+	Deep pink	Deep pink (F)	-
G1	G1C19	+	Deep pink	Colorless	-
	G1C20	+	Pink	Fade pink (mucoid)	-
G2	G2C21	+	Pink	Colorless	-
	G2C22	+	Whitish pink	Deep pink (F)	-
G3	G3C23	-	Deep pink		-
G4	G4C23	+	Deep pink	Fade pink (center yellow)	-
C	CF24	+	Deep pink	Colorless	-

Biochemical tests (Indole, MR-VP, and Citrate) were conducted on the seven bacteria obtained from the contaminated samples to characterize *E. coli*. All isolates showed positive result for the Indole and MR test, negative for the VP test and citrate utilization test (Table 3).

Table 3. Biochemical test results of isolates

Isolates	Indole test	Methyl red test	Voges Proskauer test	Citrate test
S1C3	+	+	-	-
S2C3	+	+	-	-
S3C3	+	+	-	-
S3C4	+	+	-	-
IS3C3	+	+	-	-
D1C3	+	+	-	-
P2C3	+	+	-	-

Escherichia coli has reportedly been used as a biological indication of water treatment safety since 1890s (Edberg *et al*., 2000). The presence of *E. coli* bacteria determines the risk of pathogenic contamination from fecal origin as an indication (Tharannum *et al*., 2009; Bej *et al*., 1990). This investigation was done using the Multiple Fermentation Tube (MFT) or Most Probable Number (MPN) technique (WHO, 2008), which has been used successfully for many years to analyze drinking water. Instead of counting the actual quantity of indicator bacterial presence in the sample, the findings for the presence of coliform bacteria are displayed as a most probable number (MPN) index that would produce the results indicated by the experiment (Bartram and Ballance, 1996).

In this study, 85% of water samples (17 out of 20 samples) were found contaminated with coliform bacteria and rest 15% water sample (3 out of 20) was microbiologically safe. Out of 17 samples, *E. coli* was found in 7 samples, while the rest 10 samples were contaminated with other coliform. About 35% of samples were contaminated with *E. coli* that are detrimental to public health concern (White *et al*., 2021).

According to the WHO's recommendations, drinking water should maintain a standard of 0 Most Probable Number (MPN) per 100 ml of water (WHO, 2008). However, in this experiment, the coliform-contaminated 17 samples exceed the recommended value and have been considered unhealthy for drinking.

According to Phyo *et al*., 2019, nineteen bottled drinking water samples in Myanmar have been analyzed for coliform contamination where seven samples were microbiologically contaminated, while in the present study, the rate of contamination is lower. According to Tambekar *et al*., 2010, 225 water samples from different hotels and restaurants in Amravati City, India, were tested, and around 63.3% of samples were contaminated by coliform that is similar to present findings. The presence of coliform and fecal coliform (*E. coli*) have also been found in the Karnaphuli River water of Chittagong (Dey *et al*., 2017). The findings showed similar bacterial species to the contaminated samples of the present study. Nevertheless, Moniruzzaman *et al*. (2011) found 80% coliform contamination of collected samples from restaurants in Dhaka city and the Savar area and that also supports the present findings as well.

The findings of this study highlight a significant public health concern regarding the microbiological quality of drinking water in restaurants in the Savar area. The detection

of coliform bacteria in 85% of the water samples indicates widespread contamination, raising serious questions about the safety and sanitation practices surrounding water supply in local dining establishments. This level of contamination poses a potential health risk to the public, particularly to vulnerable populations such as children, adults, and individuals with compromised immune systems.

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