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Microbial quality and public health risks of filtered drinking water served in restaurants: A case study from the Dhaka division of Bangladesh

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Abstract: Considering the ongoing drinking water crisis, a small-scale study was conducted to evaluate the microbial quality of filtered water commonly served in restaurants across the Dhaka division of Bangladesh. This research focused on samples collected from restaurants in Manikganj, Savar, and Dhaka city of Bangladesh to assess the hygiene standards of this widely consumed water. A total of 15 water samples were analyzed, including five from each location. The findings revealed that 11 samples, four from Manikganj, four from Savar, and three from Dhaka contained both total and fecal coliforms. Conversely, only four samples, consisting of one from Manikganj, one from Savar, and two from Dhaka, were found free of these contaminants. The detection of coliforms highlights the potential risk of contamination with pathogenic microorganisms. Additionally, most of the samples exceeded the acceptable limits for total heterotrophic count as prescribed by the World Health Organization (WHO) and the Bangladesh Standards and Testing Institution (BSTI). Biochemical analysis suggested the possible presence of pathogens such as Shigella, Salmonella, Enterobacter, and Klebsiella species. Although typically offered at an affordable price of BDT 1 or 2 in most restaurants, this filtered water does not meet the microbiological standards for safe drinking. It is imperative that supply companies undergo rigorous inspections to enforce quality control measures. Furthermore, restaurant practices require scrutiny, including the hygiene standards of workers and the cleanliness of water supply systems. Contamination risks may also arise from the glasses used to serve water, which can become tainted if washed with untreated water from restaurant supplies.
Keywords: Microbial contamination, Coliform bacteria, Public health risks, Water quality standards, Dhaka division restaurants

1. Introduction

Access to clean water is vital for the health and well-being of both humans and animals (Pitkänen et al., 2020). Contaminated water is a significant factor in the spread of numerous diseases affecting humans and livestock (Fida et al., 2023). The introduction of fecal matter from sewage, unwashed hands, and other sources into natural water supplies poses an increased risk of disease transmission to humans (Mattos et al., 2021). In developing countries, diarrheal diseases resulting from unsafe water is a severe public health concern, while they remain a persistent issue in some developed regions. Waterborne pathogens, including bacteria such as *Enterobacteriaceae* and *Vibrio* species, viruses like Hepatitis A, and protozoa such as Giardia, are common culprits (Rahman et al., 2021). These microorganisms typically thrive in the human intestinal tract and are excreted through feces (Yamamura et al., 2023). Fecal coliforms are widely recognized as indicators of fecal contamination and are frequently used to assess the microbiological quality of water and estimate associated health risks (Holcomb and Stewart, 2020). The Most Probable Number (MPN) test is a common method for detecting fecal coliforms, relying on lactose fermentation at elevated temperatures and specific medium formulations (Xu, 2021).

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Ensuring the provision of safe and quality drinking water is a pressing public health priority (Gunnarsdottir et al., 2020). Contaminated water is a well-documented source of disease transmission, contributing to significant morbidity and mortality worldwide (Fida et al., 2023). In developing nations, drinking water is often a major source of microbial contamination, alongside inadequate sanitation, and unsafe food, which are well-known pathways for exposure to enteric pathogens (Kumar et al., 2021). In Bangladesh, many individuals in areas such as Manikganj, Savar, and Dhaka depend on roadside restaurants for their daily meals (Initiative, 2023). Concerns over the microbiological safety of drinking water have grown significantly, raising public awareness of waterborne illnesses (Kumar et al., 2021). Frequent contamination of municipal water supplies, often due to external pollutants or leaks in deteriorating pipelines, has driven consumers to explore safer options (Taiwo et al., 2023). Although bottled water is considered a reliable choice, its high cost makes it inaccessible to many (Grebitus et al., 2020). A cost-effective alternative is water supplied in large, sealed 20-liter containers by various companies (Stutz, 2024). These containers are typically attached to dispensing machines, and the water is served to customers in small glasses after dispensing (Faisal et al., 2023).

With the growing reliance on water dispensed from machines, assessing its microbiological quality has become crucial to ensure consumer safety (Venuti et al., 2024). Although studies on the microbiological quality of dispenser water have been conducted in various countries, published data on its condition in Bangladesh remains scarce. This research focused on evaluating the microbial quality of water dispensed in roadside restaurants in Dhaka, Manikganj, and Savar. Since improper handling and serving practices in restaurants and hotels are known to contribute significantly to water contamination (Karim et al., 2023), the study also investigated whether the dispensers and serving glasses used in these establishments contributed to additional microbial contamination.

2. Materials and methods

2.1. Study area and sample collection: Fifteen water samples were collected from restaurants located in Manikganj, Savar, and Dhaka, Bangladesh. The filtered water samples were collected using the following methods: a) directly from the supply bottle, b) from the dispensing machine, and c) from the drinking glass. Approximately 300 mL of water was collected in 500 mL sterile plastic bottles. Prior to collection, the bottles were sterilized using an autoclave and thoroughly rinsed with distilled water. Each bottle was labeled with details such as sample number, source, date, and time of collection. The samples were transported to the laboratory within six hours for further analysis.

2.2. Microbial analysis

2.2.1. Total viable count: The spread plate technique was employed to determine the total viable count of the water samples (Guo et al., 2021). A ten-fold serial dilution was performed for each raw water sample. Nutrient Agar was used as the culture medium to enumerate the total viable bacteria. A 100 µL aliquot of each diluted sample was transferred onto agar plates using a micropipette and spread evenly with a sterile bent glass rod. The plates were then incubated at 37 °C for 18 to 24 hours. The total bacterial count was recorded as colony-forming units per 100 mL (cfu/mL).

2.2.2. Total coliform count (TCC) and fecal coliform count (FCC): The presence of coliforms in drinking water samples was determined using the membrane filtration technique. Membrane filters with a pore size of 0.45 µm, capable of retaining microorganisms larger than this size, were used for the analysis. A volume of 100 mL from each water sample was filtered through these membranes (Pall Corporation, Michigan, USA). The filters were then placed onto MacConkey agar for total coliforms (TC) and m-FC agar for fecal coliforms (FC). For TC detection, the plates were incubated at 37 °C for 24 hours, and for FC estimation, the plates were incubated at 44.5 °C for 24 hours. The number of coliforms was expressed as the most probable number (MPN) of coliforms per 100 mL of water. After incubation, colonies were enumerated using the standard plate count method (Costa et al., 2021).

2.2.3. Cultural and biochemical examination of samples: The bacteriological analysis of the water samples was conducted following the standard methods outlined by ICMSF (Ahmad et al., 2023). The analysis included detailed observation of colony characteristics as well as morphological and biochemical properties. To identify different microorganisms, present in the water, bacterial colonies were isolated in pure culture from the agar plates and identified using methods described by Krieg. Further confirmation of presumptive bacterial identification was performed through Gram staining and biochemical tests, following guidelines in Bergey's Manual of Determinative Bacteriology (Warpala et al., 2020). A total of 12 biochemical tests were

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carried out on the 15 samples, including Motility Indole Ornithine (MIO) Test, Citrate, Voges-Proskauer (VP), Oxidase, Catalase, Methyl Red (MR), Triple Sugar Iron (TSI) agar Test, and Eosin Methylene Blue (EMB) agar Test.

3. Results and discussion

3.1. Determination of total viable bacteria

The total viable count (TVC) reflects the overall microbial load and viable bacterial population present in water. According to the United States Environmental Protection Agency (USEPA), the acceptable limit for TVC in drinking water is <500 CFU/mL. The TVC of all 15 water samples was analyzed, with results ranging from 1.0×10^3 to 8.0×10^6 CFU/mL (Table 1). Among these, 28% of the samples (n = 23) exceeded the acceptable limit of 500 CFU/mL, while 72% of the samples remained within the acceptable range (<500 CFU/mL). This study aimed to assess the microbiological quality of water dispensed from units installed in various restaurants across Dhaka, Bangladesh. It also investigated whether the dispenser systems or the serving glasses contributed to microbial contamination. Additionally, the study evaluated the presence of potentially harmful or pathogenic bacteria in the water. The heterotrophic plate counts (HPC) of all tested samples revealed a high bacterial load, exceeding the safe drinking water limits set by the World Health Organization (WHO) and the United States Environmental Protection Agency (USEPA). While elevated HPC levels do not necessarily indicate an immediate health risk (Carabin et al., 2024), risk assessments suggest that the chance of colonization from a single exposure at levels below 10,000 CFU/mL is minimal (Mabeo, 2020). Nevertheless, many pathogenic waterborne bacteria, including *Salmonella, Shigella*, and *Vibrio* species (Richiardi et al., 2023), are heterotrophic in nature (Chowdhury et al., 2021). These findings underscore deficiencies in quality assurance protocols for the bottled water distributed to dispensers by various suppliers.

3.2. Determination of total coliform and fecal coliform count

Total Coliform Count (TCC) includes aerobic and facultative anaerobic, Gram-negative, non-spore-forming bacilli capable of fermenting lactose and producing acid or aldehyde within 24 hours at 35-37 °C in the presence of high concentrations of bile salts. In this study, 72% of the 15 water samples tested positive for the presence of coliform and fecal coliform bacteria, while 28% showed no presence of these bacteria. The presence or absence of total and fecal coliforms in each sample is detailed in Table 1. All samples, except for four (M2, S5, D3, and D4), were contaminated with coliform and fecal coliform bacteria, suggesting potential contamination with other pathogenic microorganisms. The study involved collecting water samples in three stages to determine whether contamination was being introduced through the dispenser or the serving glass during the cleaning process. In several cases, contamination was indeed detected. When water was dispensed into glasses, the microbial count was significantly higher compared to water collected directly from new bottles or immediately after dispensing. This increase in microbial load was likely due to the use of tap water for cleaning glasses or the unhygienic practices of restaurant staff responsible for cleaning (Some et al., 2021).

Table 1. Result of Heterotrophic plate count (HPC), Fecal & Total Coliform

Sample No		Total Count(cf√u/ml)	Fecal coliform(Fc)	Total coliform(Tc)
M1	1A	$3X10^5$	√	√
	1B	$5X10^6$	√	√
	1C	$6X10^4$	√	√
M2	2A	$5X10^3$	X	X
	2B	$8X10^4$	X	X
	2C	$7X10^5$	X	X
M3	3A	$4X10^6$	√	√
	3B	$3X10^4$	√	√
	3C	$2X10^5$	√	√
M4	4A	$2X10^7$	√	√
	4B	$5X10^6$	√	√
	4C	$8X10^6$	√	√
M5	5A	$2X10^6$	√	√
	5B	$5X10^4$	√	√
	5C	$2X10^5$	√	√

	1A	$1X10^7$	√	√
S1	1B	$2X10^7$	√	√
	1C	$3X10^6$	√	√
	2A	$1.6X10^3$	√	√
S2	2B	$6X10^4$	√	√
	2C	$7X10^4$	√	√
	3A	$2X10^3$	√	√
S3	3B	$4X10^3$	√	√
	3C	$1.5X10^4$	√	√
	4A	$1X10^3$	√	√
S4	4B	$1.4X10^4$	√	√
	4C	$4X10^4$	√	√
	5A	$5X10^7$	X	X
S5	5B	$1X10^4$	X	X
	5C	$2X10^8$	X	X
	1A	$2X10^7$	√	√
D1	1B	$2X10^7$	√	√
	1C	$1.5X10^5$	√	√
	2A	$1X10^8$	√	√
D2	2B	$6X10^3$	√	√
	2C	$2X10^8$	√	√
	3A	$1.5X10^3$	X	X
D3	3B	$2.3X10^3$	X	X
	3C	$1X10^4$	X	X
	4A	$2X10^9$	X	X
D4	4B	$5X10^6$	X	X
	4C	$4X10^4$	X	X
	5A	$1X10^7$	√	√
D5	5B	$3X10^4$	√	√
	5C	$8X10^7$	√	√

3.3. Isolation and identification of the bacterial isolates

Different types of bacterial contaminants were identified from water samples of different restaurants of Dhaka city. The isolates were further confirmed by gram staining and their morphological characteristics. Based on their microscopic observation and morphological characteristics the isolates were listed as *E. coli, Klebsiella*, *Shigella, Salmonella, Enterobacter, Staphylococcus, Pseudomonas* (Table 3). Of the restaurants sampled, only four provided waters free of both total and fecal coliform bacteria. While the presence of coliforms does not necessarily indicate fecal contamination, it serves as an indicator of potential contamination by pathogenic microorganisms, such as *Salmonella, Shigella*, and *Vibrio cholerae* (Organization, 2022).

Coliform bacteria were found in 72% of the samples, reflecting the inadequate water treatment processes used by bottling companies. Pathogenic bacteria isolated from the samples included *Escherichia coli, Shigella, Klebsiella, Enterobacter, Pseudomonas*, and *Salmonella*, all of which present significant health risks.

The World Health Organization (WHO, 2022) has categorized bacteria like *Salmonella, Shigella*, pathogenic *E. coli*, *Vibrio cholerae*, *Yersinia enterocolitica*, and *Campylobacter*. as serious health threats when found in drinking water (Khan and Gupta, 2020). Among these, *E. coli* is a well-established indicator of recent fecal contamination (Khan and Gupta, 2020).

Table 2. Gram Staining and Morphological Characteristics

Sample	Isolates Code	Gram Reaction	Cell Morphology
	1	-	Rod
M1	2	+	Cocci
	3	+	Cocci
	4	-	Rod
	5	-	Rod
	1	+	Cocci
M2	2	+	Cocci
	3	+	Cocci
	4	-	Rod

	5	+	Cocci
	1	-	Rod
	2	+	Cocci
M3	3	-	Rod
	4	-	Rod
	5	-	Rod
	6	-	Rod
	7	-	Rod
	1	+	Cocci
	2	-	Rod
M4	3	+	Cocci
	4	-	Rod
	5	+	Cocci
	6	+	Cocci
	1	-	Rod
	2	+	Cocci
	3	-	Rod
M5	4	-	Rod
	5	-	Rod
	6	+	Cocci
	7	+	Cocci
	8	-	Rod
	1	+	Cocci
	2	-	Rod
S1	3	-	Rod
	4	-	Rod
	5	+	Cocci
	1	-	Rod
	2	-	Rod
S2	3	-	Rod
	4	+	Cocci
	5	+	Cocci
	1	+	Cocci
	2	-	Rod
S3	3	-	Rod
	4	-	Rod
	5	+	Cocci
	6	-	Rod
	1	-	Rod
	2	+	Cocci
	3	-	Rod
S4	4	+	Cocci
	5	-	Rod
	6	+	Cocci
	7	+	Cocci
	8	-	Rod
	1	+	Cocci
	2	+	Cocci
S5	3	-	Rod
	4	-	Rod

	5	-	Rod
	1	+	Cocci
	2	-	Rod
D1	3	+	Cocci
	4	+	Cocci
	5	+	Cocci
	1	-	Rod
	2	-	Rod
D2	3	-	Rod
	4	+	Cocci
	5	-	Rod
	6	+	Cocci
	1	-	Rod
	2	+	Cocci
D3	3	-	Rod
	4	-	Rod
	5	+	Cocci
	1	+	Cocci
	2	-	Rod
D4	3	+	Cocci
	4	-	Rod
	5	-	Rod
	1	-	Rod
D5	2	+	Cocci
	3	-	Rod

Table 3. Identification of isolates

Sample Number	Microorganisms
M1	*E. coli, Shigella spp., Klebsiella spp.*
M2	*E. coli, Shigella spp., Klebsiella spp.*
M3	*Klebsiella spp., Enterobacter spp.*
M4	*Shigella spp., Enterobacter spp., Klebsiella spp.*
M5	*Enterobacter spp., Klebsiella spp., Staphylococcus spp.*
S1	*Enterobacter spp., E coli, Staphylococcus spp.*
S2	*Salmonella spp., Shigella spp., Klebsiella spp.*
S3	*E. coli spp., Shigella spp., Klebsiella spp.*
S4	*Enterobacter spp., Klebsiella spp.*

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S5	*E. coli, Shigella spp., Enterobacter spp.*
D1	*Enterobacter spp., Klebsiella spp.*
D2	*Klebsiella spp., Pseudomonas spp.*
D3	*E. coli, Klebsiella spp., Pseudomonas spp.*
D4	*Klebsiella spp., Pseudomonas spp.*
D5	*E.coli, Pseudomonas spp.*

3.4. Biochemical test for presumptive conformation of bacterial species

Total eleven types of biochemical test showed their result for the confirmation of *E. coli, Shigella, Klebsiella*, *Enterobacter*, *Pseudomonas* and *Salmonella* which are potential pathogens. Each of the samples showed the presence of these organisms by biochemical test (Table 4). The biochemical tests provide the confirmation of bacterial species isolated from water samples in different area of Dhaka city. The presence of *Salmonella, Pseudomonas, E. coli, Klebsiella*, and *Staphylococcus* species in water samples indicates microbial contamination and poor sanitation. A significant risk of waterborne illnesses is indicated by pathogenic organisms like *Salmonella* and *E. coli*, but the presence of *Pseudomonas* and *Klebsiella* suggests the possibility of opportunistic infections. The results of this study clearly indicate that none of the sampled water met the safety standards for human consumption, based on TC, FC, heterotrophic plate count (HPC), and the presence of potentially pathogenic microorganisms. While water from dispensers is becoming increasingly popular among urban populations, many consumers are unaware of the microbial safety concerns associated with it. Immediate government action is necessary to enforce quality control measures and raise public awareness regarding the potential health risks. Bottling companies must undergo thorough investigations to ensure proper water treatment practices are in place. Additionally, the quality of tap water used for cleaning and the hygiene standards of restaurant staff handling utensils should be closely monitored.

Table 4: Biochemical characteristics

	Oxidase	Catalase	TSI	MSA	HT	MR	VP	MIO	Indole	amylase	Citrate
Sample: M1											
1	-	+	Y/Y	Y	γ	+	+	+	-	-	+
2	-	+	R/R	Y	γ	-	-	-	-	-	+
3	-	+	R/R	R	A	-	-	-	-	-	+
4	-	+	R/Y	R	B	+	-	+	-	-	+
Sample: M2											
1	-	+	R/R	Y	γ	+	-	+	-	-	-
2	-	+	R/R	R	γ	-	-	-	-	-	+
3	-	+	R/R	R	B	-	-	-	-	-	+
4	-	+	R/R	Y	γ	+	-	+	-	-	+
5	-	+	R/Y	R	B	+	-	+	-	-	-
Sample: M3											
1	-	+	R/Y	Y	γ	+	-	-	-	-	+
2	-	+	R/R	Y	γ	-	-	-	-	-	-
3	-	+	R/R	Y	γ	-	-	-	-	-	+
4	-	+	Y/Y	Y	γ	+	-	+	-	-	+

5	-	+	Y/Y	Y	γ	+	-	+	-	-	+
6	-	+	Y/Y	Y	γ	+	-	+	-	-	+
7	-	+	Y/Y	Y	γ	+	-	-	-	-	+
Sample: M4											
1	+	+	Y/Y	Y	γ	+	_	+	-	_	_
2	-	_	Y/Y	Y	γ	+	+	+	-	_	+
3	-	_	Y/Y	R	γ	+	_	+	-	+	_
4	-	_	Y/Y	Y	γ	+	_	+	-	+	_
5	-	_	Y/Y	Y	γ	+	_	+	_	_	_
6	_	+	Y/Y	Y	γ	+	_	+	_	_	+
Sample: M5											
1	-	+	R/Y	Y	γ	+	-		-		+
2	-	+	R/R	R	γ				-		
3	-	+	R/R	R	γ				-		
4	-	+	R/Y	Y	γ				-		+
Sample: S1											
1	-	+	Y/Y	Y	γ	+	-	+	-	-	+
2	-	+	Y/Y	R	γ	+	-	+	-	-	_
3	-	+	R/R	R	γ	+	-	-	-	-	_
4	-	+	Y/Y	Y	γ	+	-	+	-	-	+
5	_	+	R/Y	Y	γ	+	_	+	_	_	+
Sample: S2											
1	-	+	R/R	R	γ	-	-	-	-	-	+
2	-	+	R/R	Y	γ	+	-	+	-	-	+
3	-	+	R/R	Y	B	+	-	+	-	-	+
4	-	+	R/R	R	B	+	-	-	-	-	+
5	-	+	R/R	R	γ	+	-	+	-	-	-
Sample: S3											
1	+	+	R/R	R	B	-	-	-	-	-	+
2	-	-	R/R	R	γ	-	-	-	-	-	+
3	-	-	Y/Y	Y	γ	+	-	+	-	-	+
4	-	-	R/R	R	B	-	-	-	-	-	-
5	-	+	R/R	R	γ	-	-		-	-	+
6	-	+	R/R	R	γ	-	-		-	-	-
Sample: S4											
1	-	+	R/Y	Y	B	+	-	-	-	-	-
2	-	+	R/Y	Y	γ	+	-	+	-	-	+
3	-		R/Y		γ	+	-		-		
4	-	+	R/Y	Y	γ	+	-	-	-	+	+
5	-	-	R/Y	Y	γ	+	-	+	-	-	+
6	-	+	R/Y	Y	γ	+	-	+	-	+	+
7	-	-	R/Y	Y	γ	+	-	+	-	-	+
8			R/Y		γ	+	-	+	-	+	+
Sample: S5											
1	-	+	R/Y	Y	B			+	-	+	-
2	-	+	Y/Y	Y	γ		-	+	-	-	+
3	-	+	Y/Y		γ		-	+	-	-	+

4	-	-		Y		γ	-	+	-	-	+
5	-	+	Y/Y	Y		γ	-	+	-	-	+
Sample: D1											
1	-	+	R/Y	R	γ	+	-	-	-	-	+
2	-	+	R/Y	Y	γ	+	-	+	-	+	-
3	-	+	R/Y	Y	B	+	-	+	-	+	-
4	-	+	R/R	R	B	+	-	-	-	-	+
5	-	+	R/Y	R	γ	+	-	+	-	+	-
Sample:D2											
1	-	+	R/R	R	γ	-	-	-	-	-	+
2	-	+	R/Y	Y	γ	+	-	-	-	-	+
3	-	+	R/R	R	γ	-	-	-	-	-	+
4	-	+	R/R	R	B	-	-	-	-	-	+
5	-	+	R/R	R	B	-	-	-	-	-	+
6	-	+	R/R	R	B	-	-	-	-	-	+
Sample:D3											
1	-	+	R/R	R	γ	-	-	-	-	-	+
2	-	+	R/R	R	γ	-	-	-	-	-	+
3	-	+	R/R	R	γ	-	-	-	-	-	+
4	-	+	R/R	Y	γ	+	-	-	-	-	+
5	-	+	R/Y	Y	γ	-	-	-	-	-	-
Sample:D4											
1	-	_	Y/Y	Y	γ	_	_	+	-	+	+
2	-	+	Y/Y	Y	γ	+	_	_	-	+	+
3	-	+	Y/Y	Y	γ	+	+	+	-	_	+
4	-	+	Y/Y	Y	γ	+	+	+	-	_	+
Sample:D5											
1	-	+	Y/Y	Y	γ	+	_	_	_	_	+

2	-	_	R/R	R	B	_	_	_	_	_	+
3	-	+	R/R	R	B	_	_	_	_	_	+

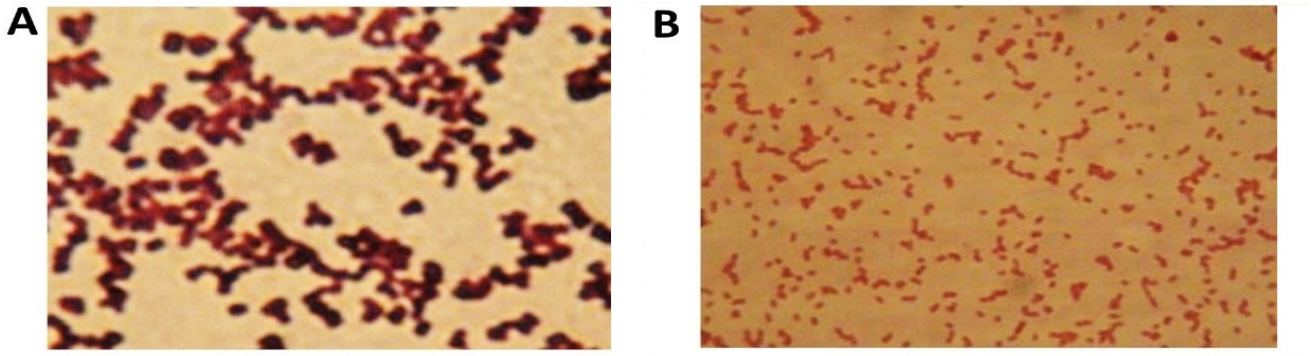

Figure 1: Gram staining results (A) Gram positive bacteria and (B) Gram negative bacteria.

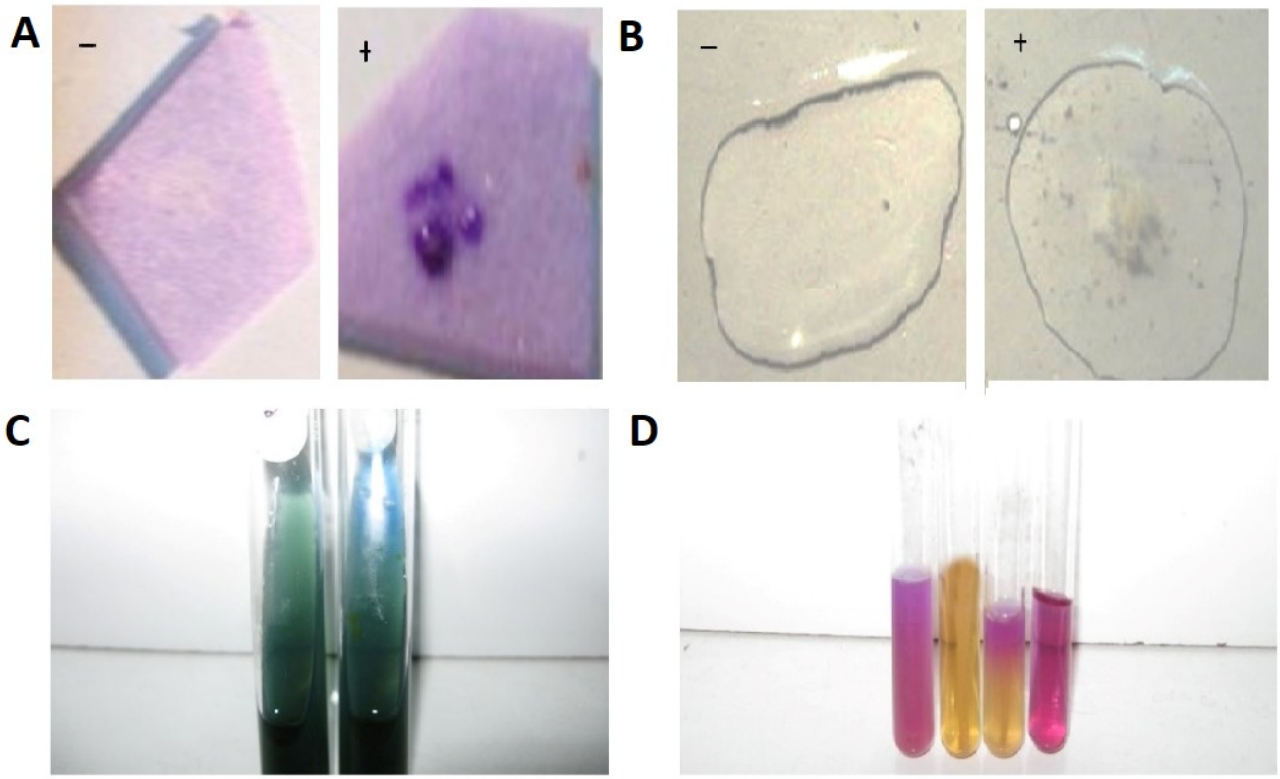

Figure 2: Biochemical test of isolated bacteria for confirmation of pathogens. (A) Oxidase Test, (B) Catalase Test, (C) Citrate test and (D) Motility Indole Ornithine (MIO).

4. Conclusion

This study assessed the microbiological quality of "filtered water" served for a fee in various restaurants and food shops in Manikganj, Savar, and Dhaka. Except for four samples, all others tested positive for Total Coliform and Fecal Coliform bacteria, indicating possible contamination by pathogenic microorganisms. Most samples exceeded the acceptable limits for heterotrophic plate count (HPC) set by the Bangladesh Standards and Testing Institution (BSTI) and the World Health Organization (WHO). Biochemical analysis of the isolated microorganisms revealed the presence of potentially harmful pathogens, such as *Shigella, Salmonella*, *Enterobacter, Pseudomonas*, and *Klebsiella*, which are associated with waterborne and foodborne illnesses. These results underscore the urgent need for regulatory agencies to enforce stringent quality control measures for supply companies. Additionally, the hygiene practices of restaurant staff and the quality of water used for handwashing and utensil cleaning need to be regularly assessed, as these factors significantly contribute to

waterborne contamination. Immediate action and ongoing monitoring are crucial to protect public health and ensure the microbiological safety of drinking water in restaurants.

Author's contribution

Md. Sharifull Islam conceptualized, data curation, methodology and wrote the manuscript and revised the manuscript.

Ethics approval and consent to participate

Not applicable.

Competing Interest

The authors declared no conflict of interest.

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