Article

Ensuring safe drinking water: microbial evaluation of restaurants in Patuakhali district, Bangladesh

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Abstract: Drinking water (DW) from restaurants is a major source of microbial exposure among consumers that causes diseases. The evaluation of the microbiological quality of the drinking water in restaurants provides the foundation for appropriate action to minimize contamination and protect patrons from food-borne illnesses. The aim of this study was to determine the microbial quality of restaurant's drinking water. A total of 20 water samples were collected in sterile test tube from the restaurants in Dumki and Patuakhali Sadar Upazilla of Patuakhali district. Total viable count on Nutrient agar and total coliform count on MacConkey agar were done by standard plate count (SPC) technique and bacterial identification by different biochemical tests. The present study found all the water samples (100%) were contaminated with bacteria in the ranges of 4.50×10^4 to 7.00×10^5, and 95% of the samples were contaminated with *E. coli,* and the range was 0 to 2.8×10^2. A total of 26 distinct bacterial colony were isolated from various samples and morphologically characterized. Based on biochemical characteristics, we identified a total of 10 bacterial species. Among them, 6 (23.10%) were both for *Enterobacter aerogenes* and *Staphylococcus epidermidis* followed by *Escherichia coli* (*E. coli*) 5 (19.23%), *Pseudomonas* spp. 3(11.54%), and rest of the *Vibrio cholera, Klebsiella oxytoca, S. auerous*, *Bacillus* spp., *Aeromonas salmonicida* and *Salmonella* spp. were 1 (3.85%) for each. A diverse range of fecal coliform, enteropathogenic and pathogenic bacteria present in samples indicates the unsanitary handling and storage practices in restaurants; this could make customers more susceptible to illnesses and ailments linked to water.

Keywords: restaurant; drinking water; microbial quality; biochemical identification; Bangladesh

1. Introduction

Drinking water (DW) faces challenges in so many aspects based on its sources (WHO, 2023). Moreover, inadequate hygienic and sanitation practices shrinkage the possibility of getting safe drinking water at the point of use (POU) (Karim *et al*., 2023). An improved source of water enhances the possibility of having safe drinking water at the POU (Hasan *et al*., 2022). A nationwide survey in Bangladesh by UNICEF found 97.5% of people have access to improved drinking water but the bottleneck remains with safety (UNICEF, 2018a). As indicated in another report from the same organization, that even collecting water from improved sources 61%

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of household water had a medium to high risk of being contaminated by *E. coli*, suggesting contamination occurring during handling and storage (UNICEF, 2018b). In restaurants, the risk points for contamination are far greater in households, especially in low-investment settings.

Beside sources, storages facilities, handling, overall hygiene and sanitation etc. have the critical implications for the microbial quality of DW in Bangladesh. A study was conducted in Gazipur municipality, Bangladesh and found that the *E. coli* and total coliform in restaurants' water samples which exceed the world health organization (WHO) standards (Karim *et al*., 2023). Exceeding level of coliform and total viable count was also identified in all the study restaurants' DW in Sylhet city (Sarker *et al*., 2016) of Bangladesh. Unacceptable levels of fecal contamination were also reported in other studies conducted on water samples of restaurants, tea stalls and roadside vendors in Jashore, Dhaka and Chittagong districts of Bangladesh (Shaibur *et al*., 2021; Moniruzzaman *et al*., 2011; Nawas *et al*., 2012).

This study was conducted in Patukhali district, Bangladesh, a coastal area, where salinity and arsenic contamination is present in ground water, and drinkable ground water mainly accessible through deep tube well forcing people to use shared water facility at households and restaurants (Islam *et al*., 2017). Previous study found that the overall hygiene practices of the vendors are not satisfactory, and 72.22% restaurants use shared water facilities and collect DW in large drums, and nearly half of the restaurants don't own toilets for their workers and customers (Mali *et al*., 2020; Kundu *et al*., 2021). This study further identified that 60% of consumers have diarrhea indicating a high possibility of contamination of DW (Mali *et al*., 2020). Waterborne microbial contamination poses a significant threat to public health, and assessing the microbial quality of drinking water in restaurants is crucial for preventing waterborne diseases. Despite the importance of safe water consumption, there is a limited body of research specifically focusing on microbial contamination in restaurant water sources within this region. This study aims to identify the microbial quality specifically the presence of fecal coliform *E. coli* and total viable count (TVC) of restaurant drinking water in Patuakhali, Bangladesh. This study will suggest the extent of contaminated DW served in restaurants of the study area which possess immense public health significance.

2. Materials and Methods
2.1. Ethical approval
This study did not require ethical approval.

2.2. Place of work
All the investigations were carried out in the laboratory of Food Microbiology, Faculty of Nutrition and Food Science, Patuakhali Science and Technology University, Dumki, Patuakhali, Bangladesh from June to December, 2023.

2.3. Collection of sample
The water samples were compiled from 20 (twenty) different restaurants of Dumki and Patuakhali Sadar Upazilla in Patuakhali district with the help of test tube (Figure 1). Total 20 ml samples were collected from each restaurants. These test tubes were sterilized by autoclave under 15 lbs. pressure for 15 minutes at 121 ℃ temperature. Before sampling, test tubes were rinsed 3 to 4 times with water to be sampled. After collecting samples, the test tubes were sealed immediately to avoid contamination and labeled properly. To prevent a notable alteration in the quality of the water, every attempt was made to reduce the period of time that elapsed between the collection and analysis processes. Subsequently, every sample was brought to the laboratory in a separate foam box filled with ice to keep the temperature between 4-8 ℃.

2.4. Microbiological method
The quality of microbes was assessed following the standard method (Islam *et al*., 2022). Serial dilution was carried out from 10^{-1} to 10^{-3}. Nutrient agar (NA) was used for total viable count (TVC), and MacConkey agar for total *E. coli* count. 0.1 ml of the drinking water sample from the dilution was inoculated onto specific growth media and incubated at 37 °C for 24 to 48 hours for proper growth (Islam *et al*., 2022). Every analysis was performed in triplicate and reported as $\log_{10}$ (CFU/g) using the following equitation,

$$\text{Colony Forming Unit} = \frac{\text{Number of Colony} \times \text{Dilution Factor}}{\text{Sample taken}}$$

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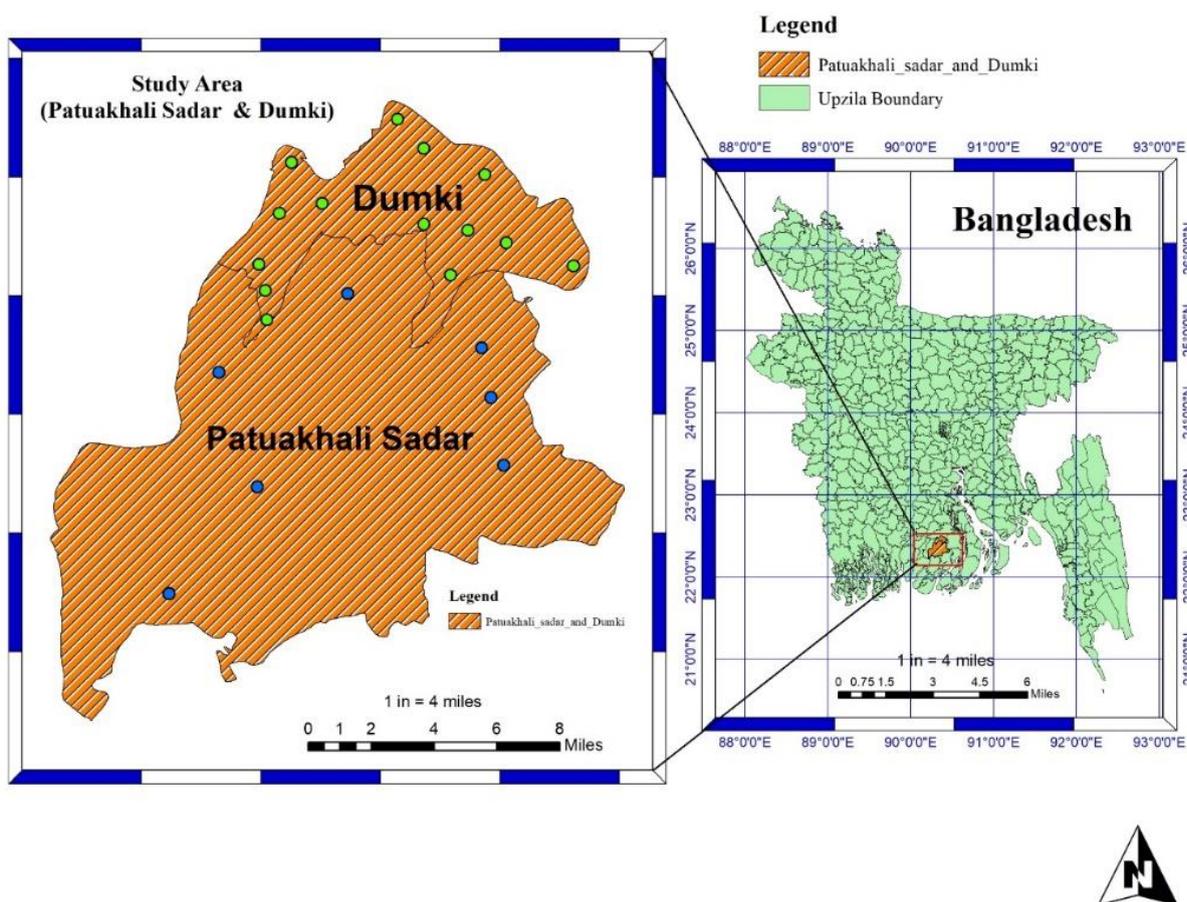
Figure 1. Map showing the location of the research area.

2.5. Purification and preservation of selected isolates

Careful observation was done of the colonies found in the various media. Several different types of colonies are present on a single plate. Therefore, in order to prevent confusion, each type of colony, which differs morphologically, was carefully chosen using a sterile loop, from different media and scattered throughout the same kind of media to obtain pure culture. A sterile loop was used to select the colonies aseptically once more because the results were identical to those of the master plate and streaked to obtain a pure culture onto a fresh nutrient agar plate. The purified isolates were then transferred to nutrient agar slants in one screw capped culture vial and preserved as stock culture (Islam *et al*., 2022).

2.6. Identification of bacterial isolates
2.6.1. Observation of colony morphology

A variety of colonies on different culture media were closely observed. Cultural characteristics of bacteria including size, pigmentation, form, margin, elevation, etc. of the colonies on various media were examined.

2.6.2. Physiological and biochemical studies

Following biochemical tests were performed to identify the bacterial isolates strains, catalase test, methyl red test, citrate utilization test, Indole test, carbohydrate fermentation test (lactose, sucrose and dextrose), hydrogen sulfide (H_2S) production tests were performed according to Cappuccino and Sherman (2014).

2.7. Data management and statistical analysis

The data were recorded in a Microsoft Excel 2010 spreadsheet (Microsoft Office 2010, Microsoft, Los Angeles, CA, USA) and analyzed using SPSS version 23. Descriptive statistics, including frequency and percentage, were computed for the analysis. The map was created by using ArcGIS software version 10.8.1.

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3. Results and Discussion

This study aimed to measure the amount of environmental bacteria in drinking water used in different restaurants. The average number of numerous species that were isolated from different areas is presented in Table 1. There was serious contamination of the area's drinking water supplies. In the present study, the number of microorganisms present in drinking water was in the ranges of 4.50×10^4 to 7.00×10^5 CFU/ml. All the samples found contaminated with bacteria. Different previous studies found variation in total viable counts in drinking water. Suthar *et al.* (2009) found the total microbial load in drinking water was in the ranges of 8.3×10^4– 2.83×10^5 in different localities of Rajasthan, India which supports the present study. Similar results observed in Nigeria that the microbial load after 24 hours and 48 hours of incubation, with the values 1.8×10^5 CFU/ml and 2.6×10^5 CFU/ml respectively, which is also higher than the recommended value (Sunday *et al.*, 2014). Additionally, Madni *et al.* (2022) found the microbial load 6.5×10^3 to 8.9×10^5 during winter and 5.5×10^3 to 7.3×10^5 during summer season in Swakin city, Sudan. According to guideline of WHO (1996), TVC value should be 1×10^3 CFU/ml. The US Environmental Protection Agency (EPA) also suggested that the TVC value should be 500 CFU/ml. But, compared to those figures, our TVC value was significantly higher. The distribution and occurrence patterns of microorganisms varied based on region, seasons and hygiene practices. Preventive strategies focusing the setting specific measures might appropriate to reduce the bacterial load in restaurants' drinking water.

Table 1. Bacterial counts in restaurants' drinking water in Patuakhali, Bangladesh (N=20).

Sample number	Total viable count (CFU/ml) $\log_{10}$ value	Total *E. coli* count (CFU/ml) $\log_{10}$ value
DW1	5.25×10^5	8.5×10^1
DW2	6.50×10^4	2.2×10^2
DW3	5.55×10^5	2.5×10^1
DW4	3.90×10^5	9.0×10^1
DW5	1.37×10^5	1.65×10^2
DW6	1.85×10^5	2.1×10^2
DW7	7.00×10^4	4.0×10^1
DW8	7.00×10^5	9.5×10^1
DW9	1.07×10^5	6.5×10^1
DW10	4.50×10^4	1.5×10^1
DW11	2.02×10^5	2.8×10^2
DW12	1.97×10^5	9.0×10^1
DW13	3.77×10^5	1.65×10^2
DW14	1.07×10^5	<1
DW15	1.42×10^5	4.0×10^1
DW16	1.12×10^5	5.0×10^0
DW17	1.82×10^5	7.0×10^1
DW18	1.05×10^5	8.5×10^1
DW19	2.10×10^5	6.0×10^1
DW20	1.52×10^5	1.1×10^2

*DW= drinking water, CFU= colony forming unit

The total *E. coli* content in drinking water was in the ranges of 0 to 2.8×10^2. A total of 95% of the sample was contaminated with *E. coli.* This finding was in line with the study conducted in Adama town, where all stored water was contaminated with total coliforms (UNEP, 2022). Bonso *et al.* (2023) found approximately 80% and 48% of the water samples were contaminated with total coliforms and *E. coli*. Likewise, a study done in Bangladesh on the water quality conditions of Sylhet city restaurants found that the drinking water of each restaurant was contaminated with total coliforms (Tawfick, 2022). Besides, Genet and Desta (2017) observed 100% water sample contaminated with *E. coli* in Farta district, Northern Ethiopia. In contrast, Bonso *et al.* (2023) found only 20% of tap water and 26% of the storage container water samples were contaminated with *E. coli*. Moreover Larson *et al.* (2019) found 37.3% in Cajamarca, Peru; Genet and Desta (2017) found 37% in Adama; Tambekar (2006) found 18% in Wageda, Northern Ethiopia. These findings were found lower compared to the present study. According to WHO recommendations, total coliforms or *E. coli* should not be present in drinking water. The drinking water standard value for faecal and total coliform is 0 (zero) in

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Bangladesh (BDS) (ECR, 1997) as wellas in the WHO standard (WHO, 2008). Fecal matter contamination of the water is indicated by the high levels of *E. coli* and total coliforms in the water samples. Consumers may be seriously at risk for major health problems as a result of this contamination. The high levels of contamination in the drinking water in this research area could be caused by a number of factors. One possibility is that the overall unhygienic practices of the vendors in the study area (Kundu *et al*., 2021). This level of contamination should be taken into concern in the study settings.

In this study, a total of 26 bacterial isolates were identified based in morphological characteristics. Based on biochemical characteristics, we identified total 10 bacterial species (Table 2). These include *Vibrio cholera*, *K. oxytoca*, *E. aerogenes*, *S. epidermidis*, *S. aureus*, *Bacillus* spp., *Aeromona salmonicida*, *Pseudomonas* spp., *E. coli*, and *Salmonella*.

Table 2. List of bacterial species isolated from drinking water samples.

Test organisms	Fermentation test			H_2S production	Indole production	MR production	Citrate use	Catalase activity
	Lactose	Dextrose	Sucrose					
Vibrio cholerae	-	+	+	-	+	+	+	+
Klebsiella oxytoca	A	-	A	-	+	-	+	+
Enterobacter aerogenes	A	A	A	-	-	-	+	+
Staphylococcus epidermidis	A	A	A	-	-	-	-	+
Staphylococcus auerous	A	A	A	-	-	+	-	+
Bacillus spp.	A	A	A	-	-	+	+	+
Aeromonas salmonicida	-	A	A	-	-	+	-	+
Pseudomonas spp.	A	A	A	-	-	+	+	+
E. coli	+	+	+	-	+	+	-	+
Salmonella	-	+	-	-	+	+	+	+

*+=present; - =absent; A= acid production

With regards to bacterial species, 6 (23.10%) were both for *E. aerogenes* and *S. epidermidis* followed by *E. coli* 5 (19.23%), *Pseudomonas* 3 (11.54%), and rest of the *Vibrio cholera*, *K. oxytoca*, *S. auerous*, *Bacillus* spp., *A. salmonicida*, *Salmonella* were 1 (3.85%) for each (Figure 2).
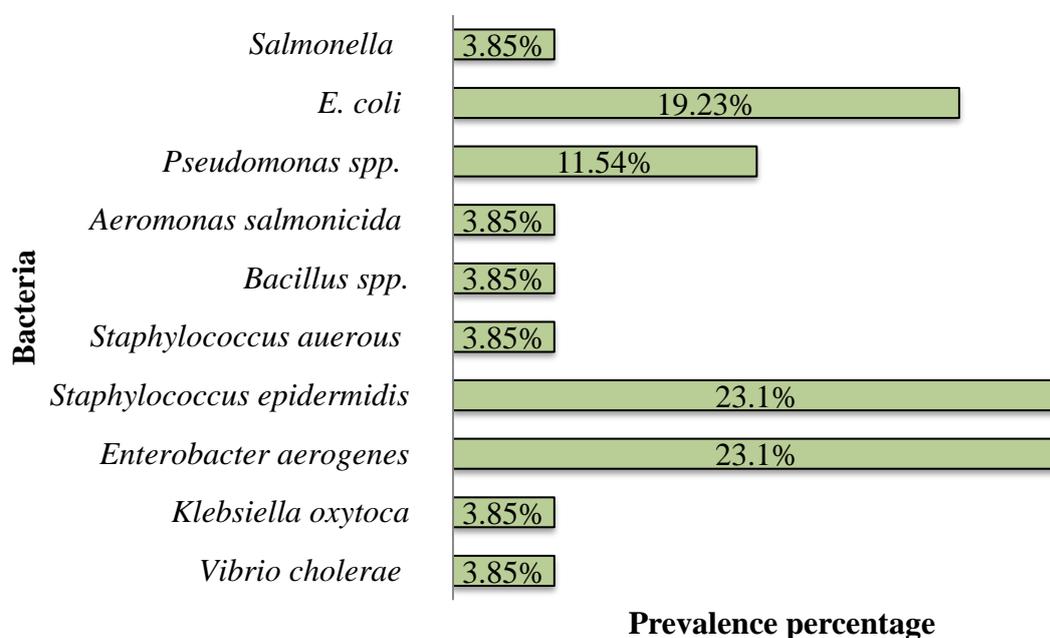
Figure 2. Prevalence of isolated bacteria.

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The predominant (23.10%) species isolated from water samples was *E. aerogenes* and *S. epidermidis*. The occurrences of *E. aerogenes* in drinking water is of primary importance because these make up the majority of the coliform organisms found in open water resources. Different studies found variety in *E. aerogenes* content. Such as Suthar *et al*. (2009) found 9.39% in drinking water from India which was lower than our findings. In contrast, Parvez *et al*. (2016) identified 44.45% *E. aerogenes* from drinking water which was higher than this study. In case of *S. epidermidis*, Santos *et al*. (2023) identified 40.6% (26/64) drinking water samples from 15 public fountains located in four urban parks of São Paulocity (Brazil). Alqahtani *et al*. (2015) found *S. epidermidis* (5.9%) in drinking water in Najran region, Saudi Arabia which is lower than present study. In the current investigation, of the total isolates, 19.23% was *E. coli*. Similar results was investigated by Bonso *et al*. (2023) which was detected *E. coli* in 10 (20%) and, 13 (26%) of tap water and storage containers, respectively. In contrast, Suthar *et al*. (2009) found bacterial species, for instance, *E. coli* showed the maximum occurrences recorded form 73.1% villages/towns drinking water which was much higher than the present study. *S. aureus* was found in 3.85% of the total isolates. Similar investigation observed by Bonso *et al*. (2023) in 3 (6%) of tap water and 7 (14%) of the water samples from storage containers. Sources of water and identification techniques may account for the discrepancy. The presence of the genus *Staphylococcus*, mostly *S. aureus*, is considered an indicator of poor hygienic status employed in the field of production or distribution of drinking water (Bencardino *et al*., 2021). *Klebsiella* species was found in 3.85%. Other studies observed that, 4% of tap water and 8% of storage container water samples was contaminated with *Klebsiella* (Bonso *et al*., 2023). Likewise, *Klebsiella* isolates from water containers were in line with the study in Cajamarca, Peru (8%), (Larson *et al*., 2019) and Andean, Peru (10.7%) (Hartinger *et al*., 2021). The prevalence of *Salmonella* species was 3.85%. The prevalence of the isolates of *Salmonella* in this study was lower than the study conducted in South Gondar (22.7%) and BuleHora (24%) (Dhengesu *et al*., 2022). *Pseudomonas* species was identified as 11.54% in this study. Vuki´c Luši´c *et al*. (2021) isolated 12.7% (7/55) of positive samples from a new water supply network which is closer to the values obtained in 2016 in a study conducted in Brazil in the São Paulo State, where the proportion of *P. aeruginosa* positive samples in the municipal public water supply was 7.6% (Anversa *et al*., 2019) and the present study. Contaminated DW from restaurants may cause enteric diseases among consumers. A three months recalled study in the area found that almost half of the respondents suffer from diarrheal symptoms (Khan *et al.,* 2018). Supply of safe drinking water in restaurants could minimize the burden of food-borne illnesses.

4. Conclusions
The present study found a diverse range of enteropathogenic microorganisms such as *E. aerogenes*, *E. coli*, *Salmonella* and *Klebsiella*. Additionally, some pathogenic bacteria like *Pseudomonas*, *Vibrio cholera*, *S. auerous*, and *Bacillus* spp. were found. Increased frequency of fecal coliform in water samples indicates the unsanitary handling and storage practices in restaurants. The information makes it abundantly evident that the health and safety of the residents of this area are seriously threatened by water-borne illnesses. Further study may be conducted to identify pathogenicity of isolated organisms. Implementing effective treatment methods and maintaining proper sanitation practices may be critical steps toward ensuring safe drinking water in restaurants.

Data availability
All relevant data are within the manuscript.

Conflict of interest
None to declare.

Authors' contribution
Abu Tareq participated in planning, coordinating, and preparing the methodology; Prosenjit Mondol carried out the lab work, did a formal analysis, and wrote the original draft. Md. Shajadul Islam wrote, reviewed, and edited the article; Md. Touheduzzaman Rifat carried out the lab work and formal analysis and wrote the original draft. Santo Shamol Das carried out the lab work and formal analysis, and Md Shafiqul Islam Khan supervised the study. All authors have read and approved the final manuscript.

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