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Microbiological Quality of Drinking Water from Dispensers in Roadside Restaurants of Bangladesh

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Abstract: The microbiological status of water from dispensers in different roadside restaurants of Dhaka city and Savar area was analyzed in this study. Seven samples from Dhaka and 8 samples of Savar were checked. The heterotrophic plate count was in a range of 1.0×10^3 CFU mL^{-1} to 2.0×10^4 CFU mL^{-1} (from new bottles), 1.0×10^3 to 1.5×10^4 CFU mL^{-1} (after dispensation), and 1.5×10^3 CFU mL^{-1} to 1.0×10^5 CFU mL^{-1} (from serving glass). In several of the samples, the heterotrophic plate count was higher than the count in water from new bottle or after dispensation, suggesting added contamination from the serving glass. 80% of the samples were contaminated with total and fecal coliform bacteria, which render these waters unacceptable for human consumption. The samples were found to contain gram negative bacteria like E. *coli, Shigella* sp., *Klebsiella* sp., *Enterobacter* sp., *Pseudomonas* sp., and Salmonella sp., which are potential pathogens and thus pose a serious threat to public health. This study elucidates the importance of monitoring the bottling companies and the restaurants and put them under strict regulations to prevent future outbreak of any water borne diseases caused by consumption of dispensed water.

Key words: Water dispensers, microbial contamination, fecal coliforms, waterborne pathogens, roadside restaurants

INTRODUCTION

Safety and quality of drinking water is always an important public health concern (Hrudey and Hrudey, 2007; Reynolds *et al*., 2008) as contamination of potable water has been frequently found associated with transmission of diseases causing serious illness and mortality throughout the world (Marshall *et al.,* 2006; Jones *et al.,* 2007 ; O'Reilly *et al*., 2004). Although poor sanitation and food sources are integral to enteric pathogen exposure, drinking water is a major source of microbial pathogens in developing regions (Ashbolt, 2004).

In Bangladesh, a large number of people living in major cities and suburbs eat their meals in various roadside restaurants. In recent times, the microbiological safety of drinking water has become a burning issue and public awareness is gradually increasing regarding waterborne diseases. Several incidents of contamination of municipal water supply from various extraneous sources and poor maintenance of pipe line causing leakage of the water pipes forced the consumers to seek safer options regarding potable water. Although costly, bottled water from different companies has become an option. However, a popular, low cost alternative is the drinking water provided in large closed containers by various companies which are directly attached to dispensing machines. After dispensation, the water is provided to the consumers in small glasses.

As water from dispensers is widely popular among the consumers now a day, the microbiological quality should be analyzed to check the safety level of this water. Although several studies have been carried out on water from dispensers in different countries by research groups like Zamberlan da Silva *et al.* (2008) and Ligouri *et al*., (2010) there is no published data regarding the microbiological analysis of water from dispensers in Bangladesh yet. Our goal was to check the microbial aspect of drinking water from dispensers in different roadside restaurants of Dhaka city and Savar area. According to Tambekar *et al.* (2006), improper handling and serving practice in hotels and restaurants add microbial contaminants to the water provided to the consumers. In this study, we also checked if the dispensers and the serving glasses contributed any further contamination to the dispensed water.

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MATERIALS AND METHODS

Study site and time period: This study was carried out in Gono shasthaya Vaccine Research Laboratory (GVRL) in Gono Bishwabidyalay, Mirzanagar, Savar, and Dhaka-1344 during the time period of May, 2009 to September, 2009.

Collection of samples: Samples were collected from 7 restaurants in Dhaka city and 8 restaurants in Savar area near Dhaka. Each of the samples originated from one single bottling company, but was collected in three different steps:

Step 1: Directly from the new 20 L bottles provided by a specific company,
Step 2: Immediately after dispensation from the machine
Step 3: Glass in which the dispensed water was served to the consumers 250 mL of water from each step was collected in sterile sampling bottles and carried to the laboratory in ice box.

Heterotrophic Plate Count (HPC): For enumeration of total heterotrophic bacteria in each of the samples, water collected in three different steps was serially diluted with distilled water following the ten-fold dilution procedure. One hundred microliler of the original sample and different dilutions (10^{-1}, 10^{-2} and 10^{-3}) were spread on plates containing nutrient agar and were incubated overnight at 37° C. The colony forming units (CFU) on each plate were counted after incubation and CFU mL^{-1} was calculated to get Heterotrophic Plate Count (HPC).

Total (TC) and fecal coliforms (FC): Total coliform and thermotolerant fecal coliform counts were conductedby modified membrane filter counting technique (Eaton *et al*., 2005). From each bottle, 100 mL of water was passed through sterile Millipore filter papers (porosity of 0.45 μM) to isolate the microorganisms present in the water samples. The filter papers were then aseptically transferred to plates containing m-ENDO agar media (for detecting total coliforms) and mFC agar (for fecal coliforms) and the plates were incubated at 37° and 44.5°C up to 48 h for the growth of coliforms and thermotolerant-coliforms, respectively.

Identification of specific bacteria: Different dilutions of the samples were spread on Nutrient agar, MacConky agar and EMB agar medium. The plates were incubated overnight at 37°C. After incubation the plate cultures were examined for the presence of morphologically distinct colonies. Each isolated colony from the incubated plates was aseptically transferred and streaked in nutrient agar slants (1.5ΔmL vials). Then the agar slant cultures were incubated for 24 h at 30°C. After incubation the cultures were stocked. The staining and biochemical properties of the isolates were studied for identification of each isolate at genera level.

RESULTS

The Heterotrophic Plate Counts (HPC) from each of the 15 samples is presented in Table 1. The HPC varied in a range of 1.0×10^{3} to 2.0×10^{4} cfu m^{-1} (from new bottles), 1.0×103 to 1.5×10^{4} cfu mL^{-1} (after dispensation), and 1.5×10^{3} to 1.0×10^{5} (from serving glass). In terms of total heterotrophic bacterial count, the lowest number was found to be 1.0×10^{3} cfu mL^{-1} in step 1 (from the new bottle) of Sample S-8, whereas the highest number was 1.2×10^{5} cfu mL^{-1} found in step 3 (from serving glass) of Sample S-2. In a number of samples like S-2, S-3, S-4, D-1 and D-4, the microbial counts were found to be several folds higher in the serving glass (step -3) than that of the count directly from the dispenser (step-2), suggesting added contamination from the glass. The contaminations might come from the cleaning water or the personnel involved in washing the utensils. All the 15 samples were contaminated by heterotrophic bacteria at a level far above the limit set by WHO (World Health Organization) which is <100 cfu mL^{-1} when in cubated at 37°C. In Table 2, the presence or absence of Total and fecal contaminants in each of the samples is shown. Except for three samples (S-3, S-4 and S-6) from Savar, all of the samples were found to be contaminated with total and fecal coliforms, indicating the presence of other

Table 1: Heterotrophic plate count (HPC) of each of the water samples collected in three different steps

	Heterotrophic plate count (CFU mL^{-1})		
Sample code	From new bottle	After dispensation	From serving glass
Savar area			
S-1	7.0×10^{3}	5.0×10^{3}	8.0×10^{3}
S-2	1.6×10^{4}	1.5×10^{4}	1.2×10^{5}
S-3	8.0×10^{3}	5.0×10^{3}	1.3×10^{4}
S-4	1.3×10^{3}	2.0×10^{3}	8.0×10^{3}
S-5	4.0×10^{3}	3.0×10^{3}	2.1×10^{3}
S-6	3.0×10^{3}	5.0×10^{3}	6.0×10^{3}
S-7	2.0×10^{3}	8.0×10^{3}	5.0×10^{3}
S-8	1.0×10^{3}	1.4×10^{3}	4.0×10^{2}
Dhaka city			
D-1	2.0×10^{3}	5.0×10^{3}	1.0×10^{5}
D-2	5.0×10^{3}	1.0×10^{3}	2.0×10^{3}
D-3	5.0×10^{3}	2.0×10^{3}	3.0×10^{3}
D-4	8.0×10^{3}	6.0×10^{3}	1.2×10^{4}
D-5	1.6×10^{4}	6.0×10^{3}	1.5×10^{3}
D-6	5.0×10^{3}	1.0×10^{4}	1.0×10^{4}
D-7	2.0×10^{4}	4.0×10^{3}	1.5×10^{4}

Table 2: The presence or absence of Total (TC) and Fecal coliform (FC) bacteria in the collected water samples

	From new bottle		After dispensation		From serving glass	
Sample code	TC	FC	TC	FC	TC	FC
Savar area						
S-1	√	√	√	√	√	√
S-2	√	√	√	√	√	√
S-3	×	×	×	×	×	×
S-4	×	×	×	×	×	×
S-5	√	√	√	√	√	√
S-6	×	×	×	×	×	×
S-7	√	√	√	√	√	√
S-8	√	√	√	√	√	√
Dhaka city						
D-1	√	√	√	√	√	√
D-2	√	√	√	√	√	√
D-3	√	√	√	√	√	√
D-4	√	√	√	√	√	√
D-5	√	√	√	√	√	√
D-6	√	√	√	√	√	√
D-7	√	√	√	√	√	√

√: Presnce, ×: Absent

Table 3: Identified microorganisms from each of the samples using standard biochemical tests

Sample code	Isolated microorganisms
Savar area	
S-1	*E. coli., Shigella, Klebsiella*
S-2	*Enterobacter, Klebsiella*
S-3	*E. coli., Shigella, Klebsiella*
S-4	*E. coli., Shigella, Klebsiella*
S-5	*Klebsiella, Enterobacter*
S-6	*Klebsiella, Enterobacter*
S-7	*Shigella, Enterobacter, Klebsiella*
S-8	*Enterobacter, Klebsiella*
Dhaka city	
D-1	*Enterobacter, Klebsiella, Staphylococcus*
D-2	*E. coli, Shigella, Enterobacter*
D-3	*Enterobacter, E. coli, Staphylococcus*
D-4	*Klebsiella, Pseudomonas*
D-5	*Salmonella, Shigella, Klebsiella*
D-6	*Klebsiella, Pseudomonas*
D-7	*E. coli, Shigella, Klebsiella*

pathogenic bacteria. This result also indicated the possibility of fecal contamination and did not conform to the guideline of WHO, which limits the number of fecal coliforms to be zero per 100 mL of water. Table 3 contains the data regarding specific bacteria detected in each of the water samples. Biochemical tests revealed that all the samples were contaminated mainly with gram negative bacteria like *E. coli*, *Shigella* sp., *Klebsiella* sp., *Enterobacter* sp., *Pseudomonas* sp. and *Salmonella* sp., which are potential pathogens.

DISCUSSION

The main objective of this study was to analyze the microbiological quality of the water plumbed in dispensers of various roadside restaurants of Dhaka city and Savar area of Bangladesh. Another goal was to determine whether any microbial contamination was being added from the dispenser itself or the serving glass in which the water was provided to the consumer. Besides, the presence of any pathogenic or harmful bacteria was also checked.

All the samples showed presence of high number of bacteria as revealed by heterotrophic plate count, which is far beyond the limit set by WHO (World Health Organization) and USEPA (United States Environmental Protection Agency) for drinking water considered to be safe to public health. Even when sampled from new bottles prior to attachment to the dispensing machines, the minimum number of bacteria was found to be 1.0×10^3 in sample S-8, which was tenfold higher than the stipulated WHO limit (100 CFU mL^{-1}) and twofold higher than the USEPA limit (500 CFU mL^{-1}). Although presence of large number of HPC bacteria doesn't necessarily indicate a significant health risk (Allen *et al*., 2004) and a risk assessment analysis of HPC bacteria in water determined that the risk of colonization from oral ingestion of HPC bacteria was <1 10,000 CFU mL^{-1} for a single exposure (Rusin *et al*., 1997), it's important to note that pathogenic waterborne bacteria are generally heterotrophic (i.e., *Salmonella*, *Shigella*, *Vibrio*, etc.) and so the results from our experiment indicated the lack of qualit y assurance of the water provided in large bottles for dispensers by various companies.

The samples were collected in three steps to check whether new contamination was being added to the water from the dispenser itself or from the serving glass during cleaning procedure, and in case of several samples, this was found to be true. When dispensed water was collected from the glass, the microbial count was found to be several times higher than when collected from the new bottles or immediately after dispensing. For instance, in case of sample D-1, the water from cleaned glass showed a count of 1.0×10^5, whereas water directly collected just after dispensation gave a bacterial count of 5.0×10^3. Thus, the count in the serving glass was found to be about 20 times higher. This added contamination might be from the tap water supply used to clean the glasses, or from hands of the personnel of the restaurants involved in cleaning.

Except for 3 restaurants, all the samples were found to contain total and fecal coliform bacteria. Although coliform organisms may not always be directly related to the presence of fecal contamination, the presence of coliforms in drinking water suggested the potential presence of pathogenic enteric microorganisms such as Salmonella sp., Shigella sp., and Vibrio cholerae. (Zamberlan da Silva *et al*., 2008). The presence of coliform bacteria in 80% of the samples indicated the extremely low efficiency of the treatment system adopted by the bottling

companies. This result corresponded to the isolated bacterial strains like *E. coli*, *Shigella* sp., *Klebsiella* sp., *Enterobacter* sp., *Pseudomonas* sp. and *Salmonella* sp., which are pathogenic for human health. According to WHO (Anonymous, 2006), the bacteria that pose a serious disease risk whenever present in drinking water include *Salmonella* sp., *Shigella* sp., pathogenic *E. coli*, *Vibrio cholerae*, *Yersinia enterocolitica*, *Campylobacter jejuni* and *Campylobacter coli*. The presence of *E. coli* in water is nearly always associated with recent fecal pollution and it is the preferred ndicator organism for his purpose (Eaton *et al*., 2005).

From this study, it was clear that none of the water samples collected was suitable for human consumption in terms of TC, FC, HPC and due to the fact that potentially pathogenic microorganisms were present. While water from dispensers is gaining popularity day by day among the city dwellers, most of them are unaware about the lack of microbial safety of the water they are consuming at a cost. Immediate action from the government and raising awareness among the people is necessary to control the microbiological quality of water from the dispensers in the roadside restaurants. Thorough investigation of the bottling companies should be carried out to ensure proper water treatment. Besides, the tap water supply and the hygienic practice of the personnel involved in cleaning utensils in the restaurants should be monitored.

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