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Heterotrophic Plate Count (HPC) of the Commercially Available Bottled Water in Dhaka, Bangladesh

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Abstract: *Drinking water, also known as potable water, is water that is safe to drink or to use for food preparation, without risk of health problems. Water helps us to maintain the balance of body fluids, control calories, energize muscles, keep skin looking good, keep our kidneys good condition, maintain normal bowel function. Water should be free from all types of pathogens. Otherwise it may be harmful for our body. Our objective of this study was to assess the "Heterotrophic Plate Counts" of local bottled water samples obtained from different areas of Dhaka, Bangladesh. We took 12 different brands of bottled water. Nutrient agar method was used to count HPC bacteria at 37 ^oC for 24-48hours and maintained pH was 7.3±0.2. We found that W1, W2, W3, W5, W6, W7, W8, W9, W10, W11 and W12 samples HPC count were <500 cfu/ml but W4 sample's count was >500cfu/ml.*

Keywords: *Bottle water, Heterotrophic Plate Count, HPC, CFU.*

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I. Introduction

Heterotrophs are broadly defined as microorganisms that require organic carbon for growth. They include bacteria, yeasts and moulds. A variety of simple culture-based tests which are intended to recover a wide range of microorganisms from water are collectively referred to as "heterotrophic plate count" or "HPC test" procedures. Accordingly, the terms "heterotroph" and "HPC" are not synonymous [(1)].

There is no universal 'HPC measurement'. Although standardized methods have been formalized, HPC test methods involve a wide variety of test conditions that lead to a wide range of quantitative and qualitative results. Temperatures employed range from around 20-40 °C, incubation times from a few hours to 7 days or a few weeks, and nutrient conditions from low to high. The test itself does not specify the organisms that are detected [(1)].

Only a small proportion of the metabolically active microorganisms present in a water sample may grow and be detected under any given set of HPC test conditions, and the population recovered will differ significantly according to the method used. The actual organisms recovered in HPC testing can also vary widely between locations, seasons and between consecutive samples at a single location [(1)].

Microorganisms recovered through HPC tests generally include those that are part of the natural (typically non-hazardous) microbiota of water; in some instances they may also include organisms derived from diverse pollutant sources [(1)].

Heterotrophic bacteria includes all bacteria that use organic nutrients for growth. These bacteria are universally present in all types of water, food, soil, vegetation, and air. Under this broad definition, primary and secondary bacterial pathogens are included, as are coliforms (*Escherichia, Klebsiella, Enterobacter, Citrobacter, Serratia*). Heterotrophic plate count (HPC) bacteria represent those microbes isolated by a particular method, whose variables include media composition, time of incubation, temperature of incubation, and means of medium inoculation. Other terms that have been used to describe this group of bacteria in water include ''standard plate count'', ''total viable count'', ''total count'', ''plate count'', ''total bacterial count'', ''water plate count'', ''colony count'', ''aerobic mesophilic viable count'', and ''autochthonous flora''. All of

these terms describe the same general bacterial group, i.e., the population of bacterial colonies produced on an agar-based medium under defined incubation temperature and time. With the 16th edition of Standard Methods for the Examination of Water and Wastewater, ‘‘Heterotrophic Plate Count’’ was the term selected to designate this group of bacteria in water. It is important to understand that while the term ‘‘heterotrophic bacteria’’ denotes all bacteria requiring organic nutrients for growth, all HPC methods enumerate only a fraction or subpopulation of heterotrophic bacteria in any water, food, soil, vegetation, air, etc. Further, it is not possible to know which percentage of the subpopulation of heterotrophic bacteria is enumerated by any HPC method, and it is not possible to differentiate which of the subpopulation includes potential pathogens [(2)].

Drinking water quality is a worldwide concern and has the greatest impact on human health [(3)]. Consumption of contaminated drinking water was associated with 80 percent of disease and one third of death in developing countries [(4)]. Therefore, an essential basic requirement for health protection is to provide the public with adequate supply of drinking water that is safe [(5)]. Advances in water treatment have significantly increased the quality and specially the safety of water [(6)]. However, drinking water quality can deteriorate by microbial attack during transport, storage and handling before reaching the consumer [(7), (8)]. Distribution systems, service lines and home devices could influence the quality of drinking water [(7)]. HPC has been widely adopted as a standard and simple traditional technique for microbiological testing and safety management of drinking water [(8)]. Given the importance of drinking water safety and identification of potential microbial sources of drinking water this study was conducted to evaluate the microbial quality of water from bottled water available in Dhaka city, Bangladesh.

II. Materials and Methods

Materials

Study Setting and Design:

This study was carried out in Dhaka, the capital city of Bangladesh. Dhaka is divided administratively into 2 city corporations, namely, Dhaka north and Dhaka south. It covers a total area of 306.38 square kilometers with a population of 8,906,039. Available sources of drinking water in the city include bottled water, piped water, wells, boreholes, and rivers. Safe drinking water is a major issue in Bangladesh. There are over 20 bottled water brands in Bangladesh and around 15 of them are in Dhaka. However, the common brands in the market are 12.

III. Methods

Sample collection:

A total of 60 samples (60 bottled) of all the common brands of bottled water available on market during the month of June, 2017 were collected for this study. A total of 12 brands of bottled water were sampled. All samples were purchased randomly from several retail outlets and supermarkets in the selected trading centers of Dhaka. At the retail outlets, five samples (two) bottles of water of each brand and of the different batches were procured.

All the samples were retained in their original sealed containers and clearly marked for identification with sample 1 to 60 water samples. They were transported to IBN SINA Diagnostic Networks at Badda in Dhaka.

Water Sample Analysis in the Laboratory:

For total heterotrophic bacteria, water samples (collected different brands from different sources and brands are marked as W1, W2, W3, W4, W5, W6, W7, W8, W9, W10, W11, W12) were agitated by vortex mixture (Model VM-2000, Digi system Laboratory Instruments Inc., Taiwan) for 15sec at 1500 rpm and ten-fold serial dilutions were prepared for each sample using different types of pipettes (Dragon, China). From all, volumes of undiluted and diluted samples were spread over nutrient agar medium under class-II Laminar Airflow (NUVO Sanayi Malzemelzeni, Imalat Vc Ticaret A.S., Turkey). Nutrient agar medium is composed of Beef heart extract 500.0gm/L, Tryptose 10.0gm/L, Sodium Chloride 5.0gm/L, and Agar 150gm/L (IBN SINA, Bangladesh) for 1 liter distilled water. The pH maintained at 7.3±0.2 at 25 ^{o}C. Plates were incubated in the incubator (WTB blinder, Germany) at 37 ^{o}C for 24-48 hours as described in Standard Methods. Following incubation media plates exhibited 2-300 colonies and results were expressed as colony-forming units per milliliter (CFU/ml). The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the heterotrophic plate count.

III. Results

For HPC bacterial count samples from different batches of bottled water were obtained from different sources of Dhaka, Bangladesh. In this study, colony forming units of different water samples were obtained and presented in Table-1. It was also observed that only bottled water sample W4 produced more colony counts compare to others.

Table 1. Heterotrophic plate count of drinking bottled water samples

HPC	Bottled Water Sample											
	W1	**W2**	**W3**	**W4**	**W5**	**W6**	**W7**	**W8**	**W9**	**W10**	**W11**	**W12**
CFU/ml	10	20	80	3×10^3	10	70	10	20	65	27	112	55
	21	25	62	1×10^3	15	45	15	31	81	41	124	75
	14	24	55	2×10^3	22	39	18	36	87	42	154	47
	12	16	86	3×10^3	32	80	26	17	75	51	102	69
	18	20	77	1×10^3	36	66	31	11	65	24	88	71
Mean	**15**	**21**	**72**	**2×10^3**	**23**	**60**	**20**	**23**	**75**	**37**	**116**	**63**

In this study obtained geometric mean of HPC count of bottled water of different brands such as W1, W2, W3, W4, W5, W6, W7, W8, W9, W10, W11 and W12 were 15, 21, 72, $2x10^3$, 23, 60, 20, 23, 75, 37, 116 and 63 CFU/ml respectively. Geometric mean of HPC was found lowest in W1 sample but highest in W4 in this study. So we finally found that W1, W2, W3, W5, W6, W7, W8, W9, W10 and W12 samples HPC count were <100cfu/ml, W11 sample's count was >100cfu/ml and W4 count was >500cfu/ml.

Bottled ("packaged") water is considered drinking water under some regulatory schemes and as a food in others. Some authorities distinguish natural mineral water from other bottled waters. WHO Guidelines for Drinking-water Quality are referred to directly in international norms (Codex Alimentarius Commission) and are considered applicable to bottled waters [(1)]. HPC has no health effects; it is an analytic method used to measure the variety of bacteria that are common in water. The lower the concentration of bacteria in drinking water, the better maintained the water system is [(9)]. As a whole our result showed lower concentration of bacteria.

IV. Discussion

HPC bacteria count varies widely in drinking water. Bacterial count depends on the quality of the source water. This count also depends on the types and efficacy of treatment, the type and concentration of disinfection residuals, the age and the condition of the storage and distribution system, the concentration of organics in the treated drinking water, the ambient temperature of the raw and finished water, the elapsed time between the water treatment plant and sampling locations, and, of course, the HPC method and time and temperature of incubation. Mentioned examples of variables have a profound effect on the enumeration of HPC bacteria. With all these of variables, it is obvious that the range of HPC populations in drinking water is considerable, i.e., < 0.02 to 10^4cfu/ml or higher [(2)]. Our obtained result was satisfactory as per Martin *et al.* (2004). Drinking water must be aesthetically acceptable as well as safe. Aesthetic acceptability is directly relevant to health since rejection of safe, but unacceptable (undesirable) water, may lead users to consume acceptable but potentially unsafe alternative waters. HPC testing may be used in investigating aesthetic quality and it is used by some authorities as a marker for some of the underlying causes of some aesthetic problems [(1)].

Health-based justification is no available for setting an upper HPC limit in drinking water. A number of countries have established mandatory limits for HPC bacteria in drinking water. As would be expected, different countries use a variety of terms to describe their respective bacterial count method, specify different analytical procedures (media, temperature, time) that can be used, and establish different maximum acceptable counts, which can range from 20 to 1000cfu/ml. Some have argued that lower HPC bacterial populations in drinking water are more desirable than higher populations, but there is no epidemiological evidence that higher HPC populations have any public health significance. Typically, public water systems with conventional treatment are able to limit HPC bacterial populations to below 100cfu/ml in the distribution system, although many systems experience increased HPC populations (500-1000cfu/ml) during the summer months. Bottled water that has no disinfectant residual may have much higher HPC populations. While a maximum HPC population of 500cfu/ml in drinking water is often cited as a health-based standard, this perception is fallacious and not based on fact. As reviewed below, there is no health-based substantiation for HPC regulations [(2)]. We continued our study in summer and obtained most of the count <500cfu/ml except one. As per Martin *et al.* (2004) may be our bottled water samples contained disinfectant residual and for this reason we found 15 to 116cfu/ml for 11 of the 12 samples.

The commonly used ''level of concern'', 500cfu/ml, originated from studies that examined the effect of HPC populations on analytical recovery of total coliforms. It was never a health-based action level. Possibly the first evidence that high HPC populations may interfere with the detection of coliforms by the multiple-tube-fermentation method (MTF) or the membrane-filtration method (MF) [(10)]. In reviewing the bacteriological results from a 1969 survey of 969 public water systems in the US, the authors stated: ''While bacteria enumerated by plate count do not usually have a direct health significance, heavy growths of bacteria and other microorganisms do indicate the potential for contamination. Also, research findings suggest that high plate counts inhibit the growth of coliform bacteria on laboratory media, there by obscuring their presence'' [(11)]. They further examined the question of interference specifically and reported that the 1969 survey data found the

frequency of detecting total and fecal coliforms by the membrane filtration method increased as the standard palte count (SPC) levels increased to 500cfu/ml, but decreased in frequency when SPC levels exceeded 1000cfu/ml.

Drinking-water can contain a range of microorganisms, including *Pseudomonas aeruginosa*, non-tuberculous *Mycobacterium* spp., *Acinetobacter* spp., *Aeromonas* spp. and *Aspergillus* spp.. There is no evidence that these microorganisms represent a health concern through water consumption by the general population, including most patients in health care facilities. However, additional processing may be required to ensure safety for consumption by severely immunosuppressed persons, such as those with neutrophil counts below 500 per µl [(12)].

To further examine this interference phenomenon, researchers [(11)] collected 613 samples from 32 dead-end water main flushing sites in the Cincinnati, OH, distribution system. This study found 76 samples contained coliforms by the Multiple Tube Fermentation (MTF) procedure, but only 19 by the (Membrane Filter) MF procedure. Data analysis demonstrated a correlation between excess SPC densities and desensitization of the MF method when SPC bacteria exceeded 500cfu/ml. Other researchers [(13), (14), (15), (16), (17), (18)] have also reported method desensitization or coliform antagonism by HPC bacteria clustering in the 500-1000cfu/ml range. These investigations demonstrated that high SPC (HPC) densities can substantially interfere with both the MTF method and especially the MF method, but that this phenomenon may not occur consistently.HPC interferences on the recovery of coliforms, to 25 years later, the following have been demonstrated that there is no EPA, FDA, or WHO health-based HPC regulation [(11)].

HPC concentrations are mentioned only twice in EPA regulations: first, as a cause of false-negative coliform tests in which lactose-based media (i.e., MTF and MF) are employed and second, as a surrogate for chlorine residuals in distribution systems. Suppression of coliform recovery only occurs with lactose-based media formulations. Defined Substrate Technology methods (e.g., ColilertR, ColisureR) do not suffer from HPC suppression. HPC populations greater than 500cfu/ml in drinking water are significant because they desensitize membrane-based coliform methods that contain lactose. Given that routine analysis of drinking water for coliforms and *Escherichia coli* is the most common and the most important determination as to the microbiological safety of drinking water, desensitization by HPC bacteria may have grave public health consequences. For this reason, it is imperative that HPC analysis be performed in parallel with each MF coliform/*E.coli* determination. This quality assurance approach ensures that coliform/*E. coli* data, especially negative results, accurately reflect the true microbiological quality of drinking water [(2)].

In the late 1980s, the development of the Defined Substrate Technology [(19)] for the simultaneous enumeration of coliforms and *E. coli* provided a method that was not subject to HPC interferences, resulting in greater confidence that negative coliform/*E.coli* drinking water samples correctly reflect their microbiological quality. While there is no validated clinical evidence that the consumption of drinking water containing high levels of HPC bacteria poses increased health risks, HPC measurements do have value as a tool to ensure drinking water quality. The purpose of water treatment is to provide a safe water supply through the use of unit processes that reduce turbidity, and chemical, and microbiological contaminants to desired levels. Beyond the water quality gains as a result of treatment, there remains the challenge of maintaining water quality during storage and distribution prior to reaching consumers [(2)].

HPC is a useful tool for monitoring the efficiency of the water treatment process, including disinfection; help to obtain supplemental information on HPC levels that may interfere with coliform detection in water samples collected for regulatory compliance monitoring; able to assess changes in finished-water quality during distribution and storage and distribution system cleanliness; assessing microbial growth on materials used in the construction of potable water treatment and distribution systems; measuring bacterial regrowth or after growth potential in treated drinking water; monitoring bacterial population changes following treatment modifications such as a change in the type of disinfectant used [(20)].

V. Conclusion

In conclusion, the present results showed that 11 among 12 commercially available bottled water of Dhaka, Bangladesh contain <500 HPC bacteria per ml of water and only one sample showed HPC count >500cfu/ml. This result shows great impact that our bottled water plant is running properly as per presently established scientific evidences. Little more monitoring to control the HPC count <500cfu/ml with disinfectant residue measurement can make the drinking bottled water strongly satisfactory.

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