Detection and Enumeration of Heterotrophic Bacteria and Fecal Coliforms in Drinking Water from Selected Commercial Food Establishments in Dasmariñas, Cavite

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Abstract

Access to safe drinking water is a basic human right but 1/3 of the global population do not have access to safe drinking water. This study aimed to evaluate the potability of drinking water collected from commercial food establishments in Dasmariñas, Cavite using the Most Probable Number (MPN) index, Heterotrophic Plate Count (HPC), and detection of fecal coliforms from water samples. Fifty-five percent (n=11) of the samples were positive in MTT which is indicative of the presence of total coliforms. Forty percent (n=8) of the water samples had unacceptable HPC at >6,500 CFU/mL. Thirty percent (n=6) of the drinking water samples were confirmed positive for fecal coliforms (Escherichia coli) as manifested by dark pink colonies in VRBA. In summary, only 45% (n=9) of the collected drinking water samples were considered as potable.

keywords: water analysis; medical laboratory science; most probable number; drinking water quality assessment; total coliform

1. Introduction

Safe and reliable drinking water is a fundamental necessity for maintaining public health. Access to safe drinking water is a basic human right but 1/3 of the global population do not have access to safe drinking water (UNESCO, 2019). Between homes with money and those without, there is a difference in access to clean water. Families with low incomes are more likely to consume contaminated water that has coliforms in it (Alfonso et al., 2022). In addition, unclean water and poor sanitation can lead to a multitude of diseases that could endanger the community's health (UNESCO, 2019). Therefore, both developing and developed nations share the need to have access to clean drinking water.

Since they might reveal the presence of possible pathogens, coliform bacteria are frequently used as indicators to evaluate the microbiological quality of water sources (Momba et al., 2012). In the intestinal tracts of both humans and animals, coliforms are a category of bacteria that are naturally found in the environment (World Health Organization [WHO], 2011). Indicators of fecal contamination and the possible danger of waterborne infections include the presence of coliforms in drinking water (Sow et al., 2017).

Consequently, it is essential to monitor and comprehend the frequency and distribution of coliforms in drinking water in order to ensure the security of water sources.

Numerous investigations into the prevalence and traits of coliform bacteria in drinking water sources have been carried out globally. These investigations have identified a variety of coliform contamination sources, including soil and vegetation as well as human and animal waste (Brettar et al., 2012; Monteiro et al., 2015). Inadequate sanitation techniques, outdated infrastructure, and inadequate treatment procedures can also be blamed for the prevalence of coliforms in drinking water (Rajal et al., 2014; Xu et al., 2022). For the creation and implementation of efficient water management plans and public health interventions, it is crucial to comprehend the factors influencing coliform contamination in drinking water.

Coliforms and the heterotrophic plate count (HPC) of bacteria are important markers of hygiene and food safety in food operations. Escherichia coli and other coliforms are frequently utilized as a sign of fecal contamination and as a sign of pathogenic bacteria (Saxena et al., 2018). Contrarily, HPC bacteria cover a wide spectrum of environmental bacteria and may provide information on the overall microbial load and food and water quality (Sule et al., 2020). Fast food chains need to be closely watched because they are well-known eateries with lots of customers and frequent handling of food in order to maintain the quality and safety of their offerings.

There is little research on the identification of coliforms and counting of HPC bacteria in fast food establishments in the Dasmariñas, Cavite area. If suitable hygiene procedures and sanitation precautions are not followed, these facilities could serve as a source of foodborne illnesses, hence it is crucial to evaluate their microbiological quality. Previous studies have emphasized the necessity for ongoing monitoring and intervention due to the prevalence of coliforms and high HPC counts in a variety of food businesses (Karunasena et al., 2016; Ramrez-Castillo et al., 2016).

By looking into the detection of coliforms and the counting of HPC bacteria isolated from fast food chains in Dasmariñas, Cavite, this research piece seeks to close the existing knowledge gap. To conduct the study, drinking water samples from a few different fast-food chains will be collected, and their microbiological quality will be assessed in a lab. The results of this study will help to build useful food safety protocols and treatments by offering important new insights into the local fast-food chains' existing microbial status.

2. Objectives of the Study

This study specifically aimed to:

1. Determine the Most Probable Number (MPN) index of drinking water samples
2. Determine the Heterotrophic Plate Count (HPC) of drinking water samples
3. Detect and isolate fecal coliforms from drinking water samples

3. Scope and Limitations of the Study

This study involves the collection and testing of drinking water samples from selected fast-food chains in Dasmariñas, Cavite where the researchers have access to. Samples were limited to service water provided by the commercial establishments, and the water sources whether piped water connection, treated piped water, hand pumps, dug wells, rainwater, water vendors, or filtered water were not specified in this study. Other physico-chemical parameters such as pH, biological oxygen demand (BOD), electric conductivity (EC), total dissolved solids (TDS), free and total chlorine, color, turbidity, odor, and taste of drinking water were not determined, analyzed or corelated with the results of the bacteriological tests and the indicator organisms. Detection of pathogens such as Salmonella sp. or other Enterobacteriaceae, apart from Escherichia coli and other fecal coliforms, were not the focus of this study. This study is a preliminary study to the detection of the presence of antibiotic resistant bacteria in drinking water.

4. Methodology

Research Design

This study utilized experimental-quasi research design. Drinking water samples (n=20) were collected from commercial food establishments using sterile collecting bottles. Water samples were tested for total coliforms using the Multiple Tubes Techniques (MTT) with triple strength Lauryl Tryptose Broth. Heterotrophic plate counts (HPC) were determined using spread plate method with R2A Agar that had been incubated for 24 hours at 37°C. Fecal coliforms were confirmed by observing dark pink colonies in Violet Red Bile Agar (VRBA) through pour plate method. The study protocol was approved by the IRB and ERB of the institution.

Study Area: Dasmariñas, Cavite

Dasmariñas City is the second largest city in CALABARZON in the Philippines, with an area of 90.13 km^2. According to the 2020 population census it is the second most populous city in CALABARZON, with 703,141 inhabitants. Among the 20 municipalities and three cities comprising Cavite, the largest and most populous place was Dasmariñas with 18.4 percent share to the total population of the province. It is also the wealthiest Local Government Unit (LGU) in Cavite (PSA, 2021).

Selection of Commercial Establishment

As it is not possible to collect service and drinking water available in all commercial establishments in Dasmariñas, Cavite, 20 were randomly selected from different locations as part of the population enough to determine the possibility of determining the feasibility of the study.

Water sampling from various sources and water quality analysis

A total of 20 samples from various commercial food establishments using sterile collecting bottles with a minimum of 500 mL each location. Collected water samples were then brought to the laboratory for analyses and were analyzed immediately. MTT and HPC procedures and the discussion on the detection of pathogens using VRBA were described in Standard Methods for the Examination of Water and Wastewater (APHA, 2017).

Determination of Total Coliforms using MTT

Broth was prepared by suspending 106.8 mL Lauryl Sulphate Broth (triple strength) powder in 1L distilled water. It was dissolved by gently heating. Ten milliliter of broth was distributed in tubes containing inverted Durham's tubes. The media was sterilized by autoclaving at 121 °C for 15 minutes. Tubes were cooled down at room temperature prior to use. Twenty milliliter water sample was inoculated aseptically in each tube. Tubes were incubated at 37 °C for 24 days. Turbidity with gas production was noted as positive for coliforms. See Figure 1.

Figure 1. Determination of Total Coliforms (r-l: MTT, Incubation, Interpretation)

Enumeration of Heterotrophic Bacteria using R2A Agar

HPC, also known as standard plate count (SPC), is a procedure for estimating the number of live, culturable heterotrophic bacteria in water. Colonies may arise from pairs, chains, clusters, or single cells—all of which are included in the term colony-forming units (CFU). The final count also depends on interaction among developing colonies. R2A agar was prepared by dissolving 18.12 g R2A powder in 1L distilled water; and sterilized by autoclaving at 121 °C for 15 minutes. Molten R2A was pre-poured in Petri dishes and used for spread plating. The agar was inoculated with 0.1 mL water sample and spread plated in triplicates. Plates were incubated at 35 °C for 24 days at an inverted position. Colonies were counted and the absence of colonies was reported as <10 CFU/mL. See Figure 2.

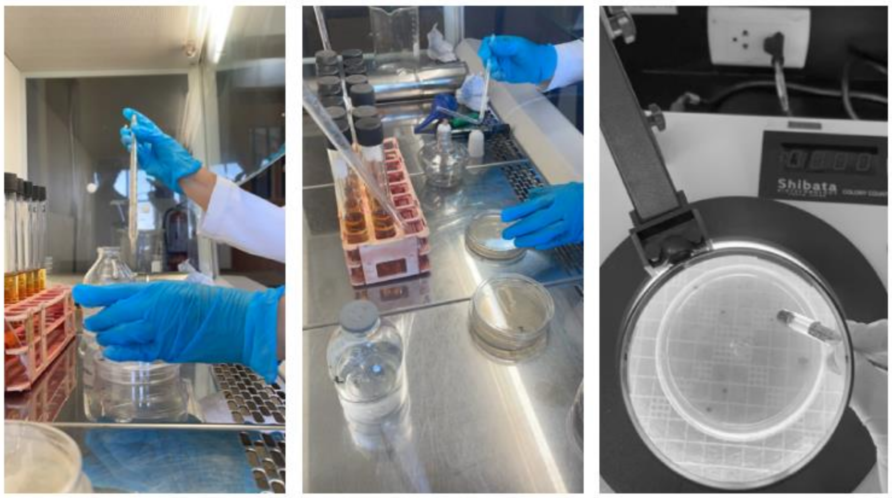

Figure 2. Enumeration of Heterotrophic Bacteria (r-l: Inoculation on R2A Agar, Spread Plating, Colony Counting)

Determination of Fecal Coliforms and Total Coliforms using VRBA

Fecal coliforms were determined using VRBA. VRBA was prepared by dissolving 41.5 g powder in 1L distilled water. Suspension was mixed and dissolved by heating with frequent agitation and brought to boil until all powder is completely dissolved. It was cooled to 45 °C prior to pour plating. One mL of water sample was inoculated first in Petri plate and 15 to 20 mL molten VRBA was poured. Samples were mixed by swirling and allowed to solidify. Pour plating was done in triplicates. Plates were incubated at 37 °C for 18 to 24 days at an inverted position. Colonies were counted and the absence of colonies was reported as <10 CFU/mL. Fecal coliforms were confirmed by observing dark pink colonies surrounded by reddish precipitation zones with 1-2 mm diameter. Colorless to pink pin-point colonies were lactose-negative Enterobacteriaceae. See Figure 3.

Figure 3. Determination of Fecal Coliforms and Total Coliforms (r-l: Preparation of VRBA, Pour plating, Interpretation)

5. Results

Table 1 MPN Index of the Drinking Water Samples (n=20)

MPN Index	Number of Positive Samples	Percentage
<1.1*	9	45.0%
1.1	3	27.3%
2.6	0	0
4.6	1	9
8.0	4	36.4%
>8.0	3	27.3

Table 1 shows consistency with the findings of Duressa and Assefa (2019) that coliforms may be present in countries with poor water delivery systems.

Table 2 Heterotrophic Bacteria of the Drinking Water Samples (n=20)

HPC (in CFU/mL)	Number of Samples	Percentage
>6,500	8	40%
500-6,500	0	0
<500*	8	40%
No Growth*	4	20%

Table 2 shows high heterotrophic plate counts are indicative of water contamination in processing system, even, from industrial sources (Rygala and Berlowska, 2020)

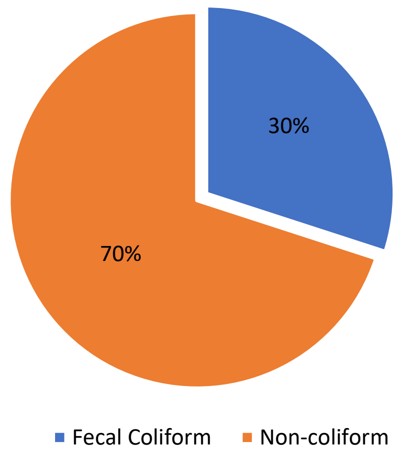

Figure 4. Positive Fecal Coliforms from Drinking Water Samples (n=5)

Figure 4 shows consistency with the findings of Castro and Obusan (2023), Sy et al. (2023), that coliforms may be present in Philippine drinking water.

6. Discussion

Only 45% (n=9) of the collected water samples were bacteriologically safe for drinking. Fifty-five percent (n=11) of the samples were positive in MTT which is indicative of the presence of total coliforms. Forty percent (n=8) of the water samples had unacceptable HPC at >6,500 CFU/mL. Thirty percent (n=6) of the drinking water samples were confirmed positive for fecal coliforms (Escherichia coli) as manifested by dark pink colonies in VRBA. In summary, only 45% (n=9) of the collected drinking water samples were considered potable.

MPN Index of the Drinking Water Samples

The most probable number (MPN) index used in this study to estimate the potability of the drinking water samples studied yields insightful findings. Only 9 of the 20 samples, or 45% of the total, had an MPN index of 1.1, it was discovered. This shows that these samples have low levels of microbial contamination and meet the requirements for potability.

It's interesting to note that three of the 20 samples, or 27.3% of them, also had an MPN index of 1.1. Given that these samples also comply with the permissible requirements for microbiological contamination, this discovery strengthens the case for the existence of potable water.

It is crucial to remember that one sample out of the 20 had an MPN index of 4.6, which represents 9% of the total. This greater score could indicate a bigger risk to the public health because it shows a higher microbial load in that specific sample. To locate the causes of contamination and put in place the necessary safeguards to guarantee the security of the water supply, more research is required.

Furthermore, the MPN index was 8.0 in four out of the 20 samples, or 36.4% of the total. This suggests a high level of microbial contamination in these samples, raising the possibility of waterborne diseases. To maintain the safety and potability of the water, it is critical to investigate potential sources of contamination and implement the necessary corrective measures.

Additionally, three of the 20 samples, or 27.3% of them, had MPN indices above 8.0. This suggests that there is a significantly larger microbial load in these specific samples, raising the possibility of waterborne diseases. To lower the microbiological count and ensure that customers have access to clean drinking water, it is crucial to address the causes of pollution and adopt efficient treatment and disinfection techniques.

Overall, the findings show that MPN indices are significantly higher than the permitted limits in a substantial majority of the drinking water samples examined in this study. These results highlight the significance of ongoing monitoring and quality control procedures to guarantee the safety of drinking water. Protecting the public's health requires locating and eliminating the sources of microbial contamination.

It is extremely concerning to find total coliforms and high MPN indices in drinking water samples. In order to assure the safety and quality of drinking water, it is crucial to monitor these characteristics (Ray, I., and Smith, K. R. 2021; Johnson & Brown, 2019).

A group of bacteria known as total coliforms operate as markers for potential fecal contamination in water sources. These bacteria's existence raises the possibility of the presence of other dangerous infections that could endanger the health of consumers (Ray, I., and Smith, K. R. 2021). Total coliforms were found in the drinking water samples used in this investigation, which indicates a possible violation of the water quality regulations.

The investigation also discovered high MPN indices in a few drinking water samples. The MPN index measures the amount of microorganisms in water and sheds light on any potential health problems brought on by microbial pollution. Increased MPN values imply a higher level of microorganisms in the water, including harmful bacteria (Johnson & Brown, 2019).

Drinking water samples with high MPN indices and total coliform counts raise questions about the security of the water supply. Total coliforms are a sign of potential fecal matter contamination in water, which may contain dangerous bacteria and viruses (Ray, I. and Smith, K. R. 2021). The large microbial load further suggested by the high MPN index raises the risk of waterborne illnesses.

These results underline how crucial it is to put in place efficient water treatment procedures and monitoring programs. The microbial load can be decreased, and hazardous pathogens can be eliminated or rendered inactive with the use of adequate treatment procedures, such as disinfection and filtration

(Johnson & Brown, 2019). Total coliforms and MPN indices must be routinely monitored in order to spot possible problems and take immediate corrective action.

Heterotrophic Plate Count

The findings of this investigation show that the HPC of the 20 drinking water samples examined varied. 40% of the samples had an HPC of more than 6,500 colony-forming units per milliliter (CFU/mL), according to the results (Rygala, 2020). This high count indicates a considerable bacterial load in those samples, which may be caused by several elements including the quality of the source water or insufficient treatment procedures.

It's interesting to see that 40% of the samples all had an HPC of under 500 CFU/mL. This lower count suggests that the heterotrophic bacterial population in those samples was relatively low, pointing to higher water quality and a more efficient water treatment system.

The study also discovered that 20% of the samples exhibited no bacterial growth (Rygala, 2020). This lack of bacterial growth in those samples may be due to the water treatment system's efficient disinfection or treatment procedures.

The disparities in HPC counts between samples demonstrate the diversity of the bacterial load in drinking water sources. These variances may be affected by elements including the quality of the source water, the treatment procedures, and the disinfection techniques.

High HPC counts that are above the permitted level can raise questions about the water's microbiological quality. Increased HPC counts could be a sign of opportunistic infections or ineffective treatment methods, both of which put customers' health at risk.

On the other hand, it is encouraging that some of the samples had low HPC numbers and no bacterial growth. It implies that efficient treatment and disinfection procedures produce drinking water that is safer and of greater quality.

For the purpose of guaranteeing the security and caliber of water supplies, regular monitoring and evaluation of HPC counts in drinking water are essential. High HPC counts can pose hazards, but they can be reduced and safe drinking water can be provided by regularly monitoring disinfection, implementing efficient treatment procedures, and continuously evaluating the quality of the source water.

The study's findings point to a worrying rise in HPC levels in samples of drinking water. A large proportion of samples with HPC counts that above the permitted limits were found in the study's analysis of a representative sample of drinking water sources (Rygala, 2020). High HPC counts may be a sign of opportunistic infections or insufficient treatment methods, which pose a risk to the public's health.

There are many reasons for the high HPC counts seen in this study. The caliber of the source water is one potential influencing element. Organic waste can act as a source of nutrients for bacterial development, making surface water sources like rivers and lakes more vulnerable to contamination (Jones & Johnson, 2018). Moreover, the existence of additional microorganisms

The effectiveness of water treatment procedures is another aspect that could contribute to high HPC concentrations. Remaining germs in the finished drinking water can result from inadequate disinfection or inefficient organic matter removal during treatment (Gupta et al., 2017). This emphasizes how critical it is to adopt reliable and effective treatment procedures to guarantee the microbiological safety of drinking water.

High HPC counts in drinking water samples have important consequences. In especially for susceptible groups like children, the elderly, or people with weakened immune systems, elevated bacterial burdens can raise the risk of waterborne diseases (Smith et al., 2020; Rygala, 2020). High HPC concentrations can also affect the water's flavor, odor, and overall aesthetic quality, which can decrease consumer satisfaction and Monitoring and evaluating the water quality on a regular basis is crucial to addressing the problem of excessive HPC numbers. High HPC counts in drinking water can be reduced with the implementation of strict control measures, such as better source water protection, optimized treatment methods, and routine disinfection monitoring (Williams et al., 2019).

In conclusion, this study emphasizes the alarming discovery of elevated HPC levels in drinking water samples. The findings highlight the need for ongoing surveillance and sensible management techniques to guarantee the drinking water's microbiological safety and purity. To protect the public's health, it is essential to address the causes of high HPC counts, such as source water quality and treatment procedures.

Fecal Coliform

The formation of dark pink colonies on VRBA agar revealed the presence of fecal coliforms in 30% (n=5) of the samples investigated in this investigation. This discovery highlights the requirement for ongoing evaluation and enhancement of the water treatment and disinfection procedures in order to guarantee the security of the drinking water supply.

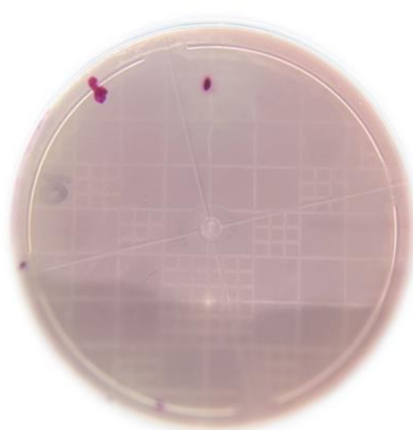

Figure 5. Dark Pink Colonies on VRBA

Coliforms seen in feces (Figure 5). are a subclass of coliform bacteria frequently discovered in the intestines of warm-blooded creatures, such as humans. Their presence in water samples suggests a possible weakness in the system's defenses that could let in microbial pollutants derived from feces. The dark pink colonies seen on the selective fecal coliforms' detection medium VRBA agar (see Figure 5) further support the presence of these bacteria in the tested samples.

A potential source of fecal contamination in the water supply is suggested by the discovery of fecal coliforms in 30% of the samples. Fecal coliforms are a sign of potential fecal-borne pathogens like Escherichia coli and Salmonella, which can infect the gastrointestinal tract when consumed. This result emphasizes the significance of ongoing surveillance to guarantee the microbiological safety of drinking water.

Contrarily, the research discovered that 70% of the samples tested negative for coliforms, meaning they were non-coliforms. A wide range of microorganisms known as non-coliform bacteria lack the distinctive traits of coliforms. The lack of coliforms indicates acceptable water quality, although it does not ensure it.

The necessity for more research into the potential sources of contamination is highlighted by the discovery of fecal coliforms in several of the samples. Inadequate sewage treatment, defective septic systems, agricultural runoff, or cross-contamination during distribution are a few potential sources. The safety of the drinking water supply and the prevention of waterborne illnesses depend on locating and eliminating these sources of pollution.

A probable fecal contamination in the drinking water supply is indicated by the presence of fecal coliforms in 30% of the samples that were examined. This finding emphasizes the significance of thorough monitoring methods for water quality and efficient treatment procedures. To protect public health and maintain the drinking water's microbiological safety, it is essential to identify and eliminate probable sources of contamination.

In order to keep ecosystems and people healthy and happy, high-water quality must be maintained. The type of bacteria known as fecal coliforms, which is often present in the intestines of warm-blooded animals, is frequently utilized as a sign of fecal contamination in water sources. We can learn more about potential dangers, environmental effects, and the demand for effective mitigation solutions by looking at these results.

Coliforms found in feces, such as E. coli, According to the USEPA (2012), are frequently used as indicators of fecal pollution in water sources. Fecal coliforms are indicators of the probable presence of dangerous enteric infections linked to fecal material (WHO, 2017). Although not necessarily harmful, these bacteria operate as fecal contamination markers since their presence suggests the possibility of pathogens that could cause disease.

The discovery of 70% non-coliforms and 30% fecal coliforms in the water sample has important ramifications for determining water quality. Fecal contamination from warm-blooded animals, including humans, is possible when fecal coliform levels are over tolerable limits, raising the risk of contracting waterborne infections (Smith and Carbone, 2019).

It is notable that there were more non-coliform bacteria in the sample since it shows how diverse the bacterial communities were in the water source. It is important to understand that other non-coliform bacteria may also be present in substantial numbers even though fecal coliforms are frequently utilized as markers. While some of these non-coliform bacteria may be benign, others might be harmful or point to sources of pollution (Olyphant and Ehrlich, 1999).

The presence of 30% fecal coliforms suggests that immediate action is required to ensure the safety of the water supply. To decrease fecal contamination and safeguard the public's health, effective water quality management and mitigation techniques should be put into place. This may entail locating and removing pollution sources, enhancing hygienic procedures, and putting in place the proper water treatment procedures to get rid of or render microorganisms inactive (Geldreich, 1990).

The prevalence of non-coliform bacteria further emphasizes the significance of thorough water quality monitoring systems that take a larger variety of microbial indicators and factors into account. To ensure a complete evaluation of water quality, monitoring programs should include a variety of bacterial, viral, and protozoan markers (Bonadonna and Briancesco, 2019).

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The status of drinking water in commercial establishments is important in determining the health risk of diarrheal diseases which is caused mainly by pathogenic E. coli and other fecal coliforms. Foodborne and waterborne transmission of diseases can lead to simple diarrhea to fatal hemolytic uremic syndrome and even death (Olsen et al., 2002; Wittler, 2023; Stephen, Kennedy and Anthony, 2021; Travert et al., 2021).

This study may be as simple as determining water quality by the presence of total coliforms and fecal coliforms but if follows the trend on how these water quality indicators can be health hazard once they achieve multidrug resistance in the environment. This ability of the microorganisms to acquire antibiotic resistance from biofilm (Salcedo et al., 2014), from transformation (Kim et al., 2014), from wastewater treatment (De Sotto et al., 2016) and from conjugation (Pak et al., 2016) were described previously and could be of consideration in terms of developing new strains of multidrug resistant bacteria coming from waterborne pathogens in drinking water.

7. Conclusion

Based on the findings of study, drinking water samples from selected commercial establishments could potentially be hazardous to its consumers. The presence of Escherichia coli from water samples is indicative of fecal contamination. The results of the study show that coliform contamination is the key problem with drinking water safety. Hence, strict regulation and educational awareness among food handlers and owners may be warranted.

8. Recommendations

The following are the researchers' recommendations:

1. It is recommended to determine the genotypic characterization of the different fecal coliforms using molecular methods and to understand whether the microbiological examination could reveal multidrug resistance in drinking water in commercial establishments.
2. It is recommended to study the physico-chemical properties of drinking water which can have underlying relationships with the presence of coliforms.
3. It is recommended to study the other components of service water in commercial establishments (i.e., disposable cups, ice, source, piping system) which could also be sources of contamination of the drinking water.
4. It is recommended to provide and foster educational awareness about the results of this study through various platforms in social media to reduce the high incidence of diarrheal diseases caused by unsafe drinking water.

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