

Escherichia Coli Contamination Test In Thai Tea Drinks In The Ayahanda Street Area In Medan Using The Mpn Method

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ABSTRACT

Thai tea is one of the drinks that are popular with urban people. However, the less hygienic processing and presentation process can be a source of microbiological contamination. This study aims to detect the presence of *Escherichia coli* in Thai tea drinks sold on Jalan Ayahanda, Medan, using the Most Probable Number (MPN) method. The research design was quantitative descriptive with a laboratory experimental approach, using ten samples from Thai tea sellers. The results showed that all samples were positive for coliform with an MPN value of 15 – 460 MPN/100 mL, eight samples were positive for the BGLB confirmatory test, and one sample (10%) was positive for *E. coli* in EMB media. Based on the SNI standard (0/100 mL), the positive sample was declared unfit for consumption. These findings confirm the importance of implementing good hygiene and sanitation in the processing and sale of beverages to ensure food safety.

Keywords: *Escherichia coli*; Thai tea; MPN; Microbiological contamination; food safety.

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INTRODUCTION

Food safety is a fundamental aspect of maintaining public health. In addition to serving as a source of energy, food and drink also play an important role in maintaining the body's physiological functions. Contamination of pathogenic microorganisms in food can be a means of spreading infectious diseases that have the potential to cause serious impacts, especially in densely populated areas. One of the parameters used in assessing the quality of food microbiology is the presence of *Escherichia coli* (*E. coli*), which is an indicator of fecal contamination due to unhygienic processing processes (Amyati, 2020; Nuryani et al., 2016; Rahmani & Handayani, 2016; Rohmah et al., 2018).

In recent years, people's consumption patterns have undergone significant changes, marked by the increasing popularity of ready-to-eat drinks such as *Thai tea*. This black tea-based drink mixed with sweetened condensed milk, sugar, and ice cubes is in great demand among teenagers and students (Rahmi, 2021). The sweet taste and distinctive aroma make it a popular drink and easy to find in various urban locations, including along Jalan Ayahanda, Medan City. However, such ease of access and popularity are not accompanied by adequate quality control of the cleanliness of raw materials and equipment used by street vendors.

One of the main risk factors for contamination in *Thai tea* drinks is the use of water and ice cubes that do not meet microbiological standards. Raw water used to make or cool drinks can be an ideal growing medium for *Escherichia coli* bacteria, especially if it does not go through a hygienic boiling or storage process (Braz et al., 2020; Jang et al., 2017; Ruiz & Silhavy, 2022). According to Nurjanah et al. (2021), the most critical stage in the safety of iced

drinks occurs during the mixing and serving process, where direct contact with non-sterile hands or containers is often the main cause of contamination.

E. coli bacteria are rod-shaped Gram-negative microorganisms that are normally found in the digestive tract of humans and animals. Most *E. coli* strains are non-pathogenic, but some types such as Enteropathogenic *E. coli* (EPEC) (Adrian et al., 2023), Enterotoxigenic *E. coli* (ETEC) (Scientific & Sandi, 2020), and Enterohemorrhagic *E. coli* (EHEC) (Wibisono, 2018) can cause serious diseases, such as diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (Karawang, 2023). *E. coli* contamination in beverages indicates exposure to fecal contaminated materials or environments, so it is an important indicator in the evaluation of food quality.

Several studies in Indonesia show that the level of *E. coli* contamination in snack drinks is still high. Hubaiba (2021) found that 12% of *Thai tea* samples in Kendari were positive for *E. coli*. Meanwhile, research by Yanni et al. (2024) reported that only 31.3% of household drinking water in Indonesia is free of *E. coli* contamination, indicating low implementation of sanitation practices at the community level and among small business actors. Globally, the World Health Organization (2023) estimates that foodborne diseases affect approximately 600 million people annually, with contaminated water and beverages being major transmission routes. These findings reinforce the urgent need for increased supervision of food safety to prevent foodborne diseases.

To detect the presence of *E. coli*, according to (Uliyanti¹ & Filemon², 2024) the Most Probable Number (MPN) method can be used because it has a high sensitivity to microbiological contamination. This method consists of three stages, namely the presumptive test, the confirmative test, and the completed test. WHO and BPOM recommend the MPN method for water and beverage quality testing because it is able to provide a quantitative estimate of the number of bacteria in the sample. This study aims to determine the presence and level of *Escherichia coli* contamination in Thai tea drinks sold in the Jalan Ayahanda area using the MPN method.

METHOD

This research is quantitative descriptive research that applies a laboratory experiment approach. The Most Probable Number (MPN) method was used to identify and estimate the concentration of *Escherichia coli* in samples of Thai tea drinks.

The entire series of laboratory tests were carried out at the Microbiology Laboratory of the University of North Sumatra (USU) from August to September 2025. During the study, each stage of the test was carried out with the principle of aseptic work to prevent cross-contamination.

This study used ten samples of Thai tea drinks obtained from all sellers operating in the Jalan Ayahanda area, Medan. Sampling was carried out directly using the total sampling technique, because the number of sellers at the research site was less than 100. Each sample was taken as much as ± 100 mL using a sterile container (glass or plastic bottle with a tightly closed lid) that was previously sterilized. The container is labeled with an identity tag in the form of a sample code, date, pick-up time, and seller's location.

The collected samples are immediately placed in a cooler box filled with ice to keep the temperature low ($\pm 4-8^{\circ}\text{C}$) during the transportation process to the laboratory. The examination

is carried out a maximum of 6 hours after collection, so that the bacteria in the sample do not experience overgrowth or decrease in number.

Laboratory equipment used includes test tubes, Durham tubes, Tube racks, Measuring pipettes, Beaker Glass, incubators, Autoclaves, Aquades, analytical balances, Spirit lamps, Hotplates, Sterile inculating loops, sterile petri cups, Microscopes, Sterile pipettes, Bunsen, Bunsen burners, Tube racks.

Some of the cultural media used in this study include:

- 1) Lactose Broth Double Strength (LBDS): Used for presumptive testing on 10 mL samples. The medium is made by dissolving 26 g of LBDS powder in 1000 mL of aquades, then heated until homogeneous. The media is sterilized at 121°C for 15 minutes using an autoclave.
- 2) Lactose Broth Single Strength (LBSS): It was prepared by dissolving 13 g of LBSS powder in 1000 mL of aquades, then sterilized in the same way.
- 3) Brilliant Green Lactose Broth (BGLB): It is a selective medium for coliform confirmation tests. The medium is made by dissolving 40 g of BGLB powder into 1000 mL of aquades, then sterilized at 121°C for 15 minutes.
- 4) Eosin Methylene Blue (EMB) Agar: Serves as a differential medium to identify typical colonies of *Escherichia coli*. The media is made by mixing ingredients according to the manufacturer's composition, then heated until dissolved and poured into a sterile petri dish at a temperature of 45–50°C.

Most Probable Number (MPN) Test Procedure

To ensure the accuracy of the results, each stage of testing on the Most Probable Number (MPN) method is carried out three times (triplo). The final value used is the average of the results of the three iterations, so as to minimize experimental errors and increase the validity of the research results. The MPN (Most Probable Number) method is carried out through three successive examination stages, namely the Presumptive Test, the Confirmative Test, and the Completed Test.

a. Presumptive Test

The initial stage aims to detect the possible presence of coliform bacteria. Each tube of LBDS and LBSS is filled with 10 mL, 0.1 mL and 0.01 mL of sample tubes, respectively. Each tube is equipped with a Durham tube to detect gas formation during lactose fermentation. The entire tube is incubated at 37°C for 24 – 48 hours. Positive results are characterized by the formation of gases in the Durham tubes and the turbidity of the media. A combination of positive tube counts (e.g. 3-2-0) is used to determine the MPN value of the standard table.

b. Confirmative Test

Tubes from the suspected stage showing positive results (gas formed) were taken 1 ose and inoculated into Brilliant Green Lactose Broth (BGLB) media. The tubes are re-incubated at 37°C for 24 – 48 hours. Positive results were characterized by turbidity of the media and gas formation in the Durham tube, indicating the presence of a group of true coliform bacteria.

c. Completed Test

From a positive BGLB tube, one ose culture is taken and then rubbed (streak) on the surface of the Eosin Methylene Blue (EMB) media. The media is incubated at 37°C for 24 hours. *E. coli* colonies are characterized by a shiny metallic green color, while other bacterial colonies are generally purplish-red with no luster. If a typical colony of *E. coli* is found,

microscopic observations are carried out to confirm the shape of the stem and the results of Gram-negative staining.

MPN Value Calculation

The MPN value is calculated based on the combination of the number of positive tubes at each dilution rate e.g. 3-2-0). The results were then matched with the standard table of MPN per 100 mL to get an estimate of the number of coliform bacteria or E.coli in each drink sample. The higher the MPN value, the greater the level of contamination that occurs. Data were analyzed quantitatively by comparing MPN values to the SNI 7388:2009 threshold for E. coli contamination, which stipulates that drinks are declared unfit for consumption if E. coli is detected in 100 mL of samples.

Ethical Compliance

All sampling and laboratory procedures followed biosafety guidelines and standard operating procedures established by the USU Microbiology Laboratory. The study did not involve human subjects or identifiable vendor information in data reporting, ensuring ethical integrity throughout the research process.

RESULTS AND DISCUSSION

The study was conducted on 10 samples of Thai tea drinks from sellers in the Jalan Ayahanda area of Medan. All samples are obtained directly from the seller in a ready-to-consume condition with the addition of ice cubes and served in plastic cups.

Most Probable Number (MPN) Test Results

Presumptive Test Results on LBSS and LBDS

The first test is a guesser test using Lactose Broth (LB) media, namely: LBSS and LBDS. Testing is carried out by the process of preparing media and tools, followed by an incubation process for 24 - 48 hours. After incubation at 37° C for 36 hours, the results are obtained in the following table:

Table 1 Presumptive Test Results (LBSS) of Thai Tea Beverages

No	Sample Code	Combinations (10/0,1/0,01)	MPN value/100 mL
1	Sample 1	3-2-0	93
2	Sample 2	2-1-1	20
3	Sample 3	3-2-1	150
4	Sample 4	3-2-0	93
5	Sample 5	3-1-1	75
6	Sample 6	3-3-0	240
7	Sample 7	3-3-1	460
8	Sample 8	3-1-2	120
9	Sample 9	2-1-0	15
10	Sample 10	3-1-0	43

Based on the results in Table 1, the highest MPN value was found in the 7th sample (460 MPN/100 mL) and the lowest in the 9th sample (15 MPN/100 mL). This difference illustrates

the variation in the level of cleanliness in the process of processing and serving Thai tea drinks at the research site

The test results were on the LBSS presumptive, followed by the LBDS presumptive test. The results are obtained in the following table:

Table 2 Presumptive Test Results (LBDS) of Thai Tea Beverages

No	Sample Code	Combinations (10/0,1/0,01)	MPN value/100 mL
1	Sample 1	3-0-1	39
2	Sample 2	2-2-1	28
3	Sample 3	2-1-0	15
4	Sample 4	3-0-0	23
5	Sample 5	2-2-1	28
6	Sample 6	2-1-0	15
7	Sample 7	2-1-0	15
8	Sample 8	3-3-1	460
9	Sample 9	2-2-0	21
10	Sample 10	3-2-0	93

The test results on LBSS media were consistent with the LBDS test, where most of the samples showed low to moderate MPN values. The 8th sample had the highest value of 460 MPN/100 mL, while some samples such as numbers 3, 6, and 7 showed the lowest value (15 MPN/100 mL). This confirms the presence of coliforms in all samples, with positivity rates varying between samples.

Confirmative test results at BGLB

Samples from tubes that were positive from the suspect test were inoculated on BGLB media. The initial process begins with the preparation of media and tools, until, then incubated for 24 hours. After incubating at 37° C for 24 hours, the results are obtained in the following table:

Table 3 Confirmation Test Results of EMB Thai Tea Drinks

Yes	Sample Code	Colony Growth in MPN	Status Escherichia coli
1	Sample 1	Not Typical	Negative
2	Sample 2	Not Typical	Negative
3	Sample 3	Not Typical	Negative
4	Sample 4	Not Typical	Negative
5	Sample 5	Not Typical	Negative
6	Sample 6	Not Typical	Negative
7	Sample 7	Not Typical	Negative
8	Sample 8	Metallic green colony	Positive
9	Sample 9	Not Typical	Negative
10	Sample 10	Not Typical	Negative

In Table 3 it can be seen that only one sample, namely Sample 8, shows a metallic green shiny colony typical of Escherichia coli, as shown in Figure 1 below. The other nine samples

showed non-typical colony growth. Thus, 10% of the samples were tested positive for *E. coli*, which means that they are not suitable for consumption based on the SNI standard (0/100 mL).

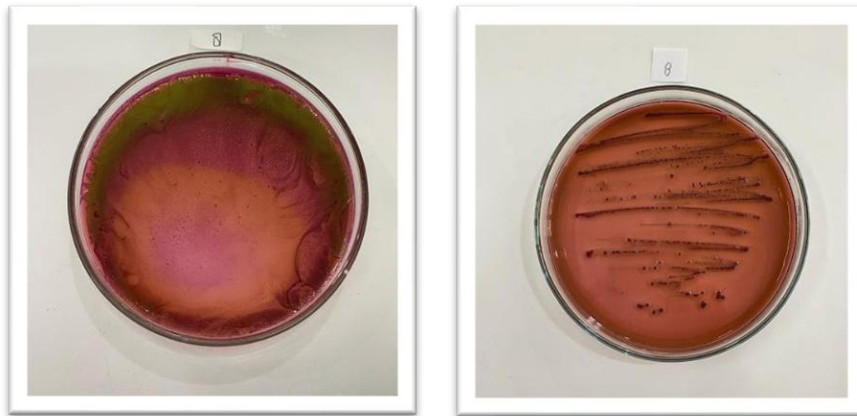


Figure 1 Colony of *Escherichia coli* on EMB media

The results indicated that all samples of Thai tea from the study area were contaminated with coliform bacteria, while only one sample (10%) was positive for *E. coli*. This shows that most of the contamination is sourced from non-fecal coliforms, but there is still a risk of fecal contamination that has the potential to cause health problems (Hubaiba, 2021).

These findings support the results of a study conducted by (Hubaiba, 2021) in Kendari City which reported that 12% of Thai tea samples were contaminated with *Escherichia coli*. This relatively small percentage indicates that although most products appear clean, there is still a risk of microbiological exposure due to poor sanitation and hygiene factors. In the context of this study, one *E. coli* positive sample (8th sample) had the highest MPN value, which was 460 MPN/100 mL, which far exceeded the maximum threshold of SNI 7388:2009 regarding the limit of microbial contamination in beverages, which is 0/100 mL. Thus, the drink is categorized as unfit for consumption.

Factors that can contribute to the high value of MPN include the use of immature water, ice cubes from unhygienic sources, and unclean serving equipment. (Nurjanah et al., 2021) stated that the serving stage is a critical point for bacterial contamination in iced drinks because many sellers do not pay attention to the hygiene of hands, containers, and utensils.

These results also support the view (Rahmani & Handayani, 2016), that the presence of *Escherichia coli* in food products is the main indicator of human or animal fecal contamination. The presence of these bacteria in the beverage indicates that the production chain is not sterile and can pose serious health risks such as diarrhea, gastroenteritis, and other gastrointestinal infections, especially in vulnerable groups such as children and the elderly.

In addition, it was found that differences in MPN values between samples could be due to variations in sanitation practices among sellers. Some sellers may have used boiled water and well-cleaned utensils, while others still use raw water or ice cubes from factories that do not have adequate hygiene standards. This shows that the level of knowledge and hygienic behavior of the seller has a direct effect on the microbiological quality of the drinks sold.

According to national food safety standards (Savitri et al., 2025), food and beverage products containing *Escherichia coli* are declared unsafe for consumption (SNI 7388:2009).

Therefore, although most of the samples in this study are considered suitable for consumption, the existence of one *E. coli* positive sample is enough to be an indicator that there is a potential health hazard in the community if there is no stricter supervision of food safety in snacks.

CONCLUSION

This study showed that all Thai tea samples examined contained coliform bacteria, and one sample (10%) of them was positive for *Escherichia coli*. Based on the SNI 7388:2009 standard, the sample was declared unfit for consumption. These results show the importance of the application of good sanitation and hygiene in the process of processing and serving beverages.

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