

## ASSESSMENT OF BACTERIAL CONTAMINANTS ASSOCIATED WITH HYGIENE BEHAVIOR IN THAI TEA SOLD ON THE ROADSIDE AROUND EDUCATIONAL AREA, LAMPUNG, INDONESIA

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### Abstract

**Introduction:** Thai tea is one of the aromatic drinks widely sold around the Institut Teknologi Sumatera (ITERA), Lampung, Indonesia. Bacteria often contaminate this drink due to unhygienic handling. The number and types of contaminating bacteria have yet to be widely reported, especially in Lampung province. This study aimed to detect various bacterial contaminants in Thai tea beverages sold on the road in the ITERA region and their relationship with the hygiene behavior of Thai tea sellers. **Methods:** The sampling technique used in this study involved accidental sampling by detecting microbes using the most probable number method and the specific medium. The number and types of bacteria were analyzed for diversity and correlated with the behavior of Thai tea sellers. **Results and Discussion:** Of the 50 Thai tea samples, coliform bacteria, and *Pseudomonas sp.* in all samples (100%), *Aeromonas sp.* (36%), *Shigella sp.* (68%), *Escherichia coli* (76%), and *Salmonella sp.* (8%). The highest concentration of pollution occurred in Sukarame District (SK). SK10 had the highest number of contaminants, namely, *Pseudomonas sp.* ( $2.96 \times 10^3 \pm 165$  CFU ml<sup>-1</sup>), *E. coli* ( $7.2 \times 10^3 \pm 190$  CFU ml<sup>-1</sup>), *Shigella sp.* ( $3.35 \times 10^3 \pm 350$  CFU ml<sup>-1</sup>) and *Salmonella sp.* ( $9.65 \times 10^3 \pm 50$  CFU ml<sup>-1</sup>). The poor quality of Thai tea is caused by unhygienic tea raw materials and the habits of the seller, who does not perform hygienic tasks during the preparation and use of Thai tea. **Conclusion:** All samples did not meet the requirements for the presence of bacteria in drinking water, based on regulation No.492/MENKES/Per/IV/2010 and World Health Organization.

## INTRODUCTION

Tea is one of the most widely consumed aromatic beverages worldwide and has been tested for many health benefits (1). Tea drinks have various presentation innovations, so different variations appear, including Thai tea. Thai tea is a popular beverage in Indonesia. This drink is often sold on the road around educational areas, such as the Institut Teknologi Sumatera (ITERA), Lampung, Indonesia. ITERA is in the education area and administratively located in four districts: Sukarame, Jati Agung, Tanjung Senang, and Way Halim. Students dominate the four villages, so they are strategic locations for selling Thai tea. Therefore, This location has many Thai tea sellers (2).

However, attention should be paid to the hygiene and safety of Thai tea products against microbial contamination. Most tea bacteria belong to the Bacillaceae family, including *Pseudomonas psychrotolerans*, *Staphylococcus warneri*, *Pantoea gaviniae*, and *Clostridium perfringens*, most of which are pathogenic to humans (3). This is because tea beverages contain raw materials often contaminated by various factors. For example, water at temperatures below boiling does not eliminate all microbes. Tea is often contaminated with bacterial, yeast, and mold pathogens during processing and storage, posing a potential health hazard to consumers. Millions worldwide drink at least one cup of tea daily, making an overall analysis of the

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microbial contamination profile of different types of Thai tea imperative (1). In addition, using ice in making that tea is one of the sources of microbial contamination in Thai tea drinks (4). Ice from tea drinks is the leading cause of cholera outbreaks and the spread of *V. cholerae* O1, an El Tor strain, in Vietnam (5).

In general, contaminant bacteria such as *Enterobacteriaceae* can cause diarrheal diseases and pyogenic hepatic abscesses, which have continued to increase over the past few decades (6). According to data from the Bandar Lampung City Health Office, the incidence of diarrhea until July 2013 was 8,375 cases (7). The prevalence of diarrhea in 2018 increased to 5% of the total adult population and 10% of the incidence in children under five (8). This bacterial contamination can be obtained from the water used to produce Thai tea, namely refill water. The quality of refilling drinking water in Bandar Lampung and South Lampung needs to meet the requirements of Regulation of Ministry of Health Republic Indonesia No. 492/MENKES/2010 about Quality Requirements of Drinking Water (9-10). Unsafe drinking water is one of the leading causes of childhood morbidity, especially among adolescents with middle-to-lower economic conditions. Safe water consumption depends on the source and process of use (11).

Several previous studies have reported bacterial contamination in Thai tea drink products in Indonesia, including bacterial coliform contamination in samples sold in Yogyakarta (12) and contamination with *Alcaligenes faecalis*, *Enterobacter* spp., *Pseudomonas* spp., and *Klebsiella pneumoniae* in Thai tea samples sold along Jalan Jatinangor, Bandung (13). Meanwhile,

in Bandar Lampung, the types of bacteria found included *Escherichia coli*, *P. aeruginosa*, *S. aureus*, *Klebsiella* sp., *Enterococcus* sp., *Salmonella* sp., and *Proteus* sp. However, the samples used were still limited and used minimal detection media, namely Desoxycholate Lactose Sucrose (DCLS) medium and Cystine-Lactose-Electrolyte-Deficient (CLED) Medium (2), it is necessary to evaluate the presence of bacteria in Thai tea drinks sold throughout the campus area of the ITERA using a variety of more comprehensive detection media.

To determine whether a drink is free of various contaminant bacteria, it is necessary to test the distribution of bacterial contamination using the Most Probable Number (MPN) method to detect coliform bacteria. Furthermore, it is necessary to detect other bacteria such as *P. aeruginosa*, *Aeromonas* sp., *Salmonella* sp., *Shigella* sp., and *E. coli* using a specific medium so that the number and type of microbes found are diverse. Based on the description above, this study aimed to detect various bacterial contaminants found in Thai tea drinks sold on the roadside in the village around the ITERA and their relationship with the hygiene behavior of Thai tea sellers.

## METHODS

### Research Design and Study Site

This research design was cross-sectional with a descriptive type of research aimed at describing the condition of bacterial contaminant contamination in drinks sold on the roadside around the Institut Teknologi Sumatera (ITERA) area. The sampling was conducted in December 2019.

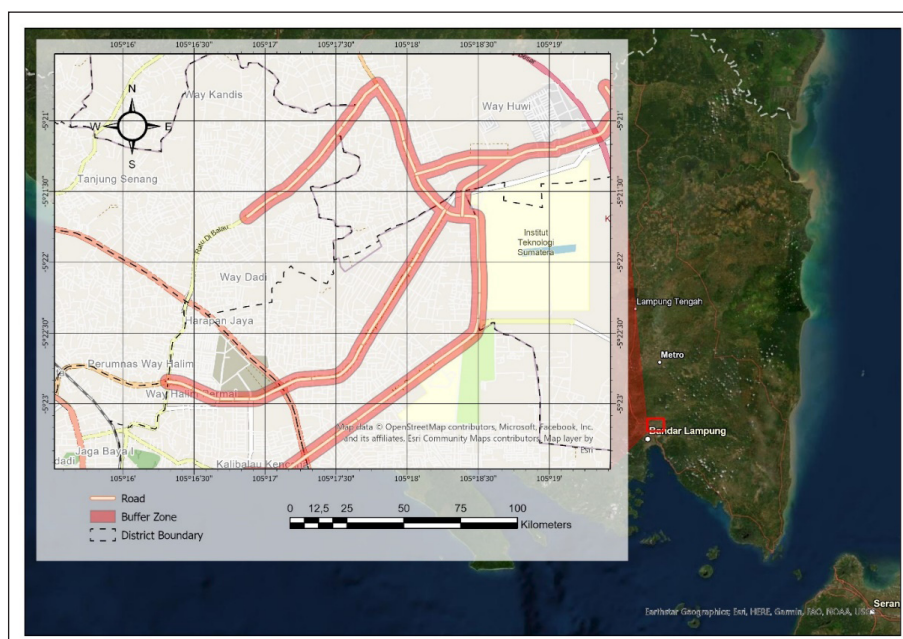


Figure 1. Map of Sampling Location (buffer zone is a sampling path)

The research object used was a sample of iced Thai tea drinks sold around the ITERA campus area, spread across four districts: Sukarame, Jati Agung, and. These four villages are located at the main point of activity of the community/students at the Institut Teknologi Sumatera (Figure 1). The sampling technique in the study was accidental sampling, that is, taking samples that existed at the time of the investigation according to the existence of the model, that is, based on the presence of tea drink sellers along the path of the sampling point. The technique used to take samples in this study is accidental sampling. Because the population in this study is homogeneous, namely Thai tea sellers. In addition, the use of this sampling technique is because Thai tea sellers do not have a fixed and the same time every day for selling Thai tea.

### Collecting Samples and Seller Behaviour Observation

Thai tea drink samples were collected by purchasing from 50 merchants specializing in selling Thai tea drinks along the ITERA area road with the condition of the selling place in a roadside kiosk. During the purchase process, the hygienic behavior of the seller, such as washing hands before making Thai tea, using gloves, using an apron, cleaning the apron, covering the head (head cover/hijab), long/short fingernails, using jewelry on hands, smoking, spitting, sneezing, or coughing near food/during serving, and serving money transactions during the process of making and serving Thai tea drinks. These observations are the final consideration for analyzing the potential spread of bacteria in the samples. The samples were neatly packed in boxes to maintain their pieces in good condition. The samples were taken to the ITERA Biology Laboratory, Lampung, Indonesia, to analyse the number and type of bacteria present in the drink. The bacterial contamination test was performed directly without sample storage or incubation. This was done to prevent cell division of contaminating bacteria from becoming more numerous.

### Enumeration and Detection of Various Bacterial Contaminants

Serial dilutions of Thai tea samples were subjected to total bacterial count (TB) analysis of up to  $10^{-6}$ . Samples (100  $\mu$ L) from dilutions were spread on the surface of a Petri dish containing Nutrient Agar (NA) medium (Merck, Germany). Petri dishes containing NA and the samples were incubated in an incubator (Memmert, Germany) at  $30 \pm 1$  °C for 24 h. Whole colonies growing on the surface of the medium were counted using a colony counter (3). The total coliform counts

were calculated using the most probable number (MPN) method (14). Lactose broth (LB) (Merck, Germany) was used for the presumptive coliform test. The presumptive test used nine test tubes into which Durham tubes had been inserted. Tubes containing the samples were incubated for 24 h at 35°C. The most probable number of coliforms per 100 ml of each Thai tea sample was calculated from the number of tubes showing gas formation and compared with the MPN index table.

*Pseudomonas* sp. and *Aeromonas* sp. were detected and enumerated using Glutamate Starch Phenol (GSP) agar medium (Merck, Germany) (15). This medium is selective for growing *Pseudomonas* and *Aeromonas* bacterial groups. The Petri dishes containing the samples were incubated at  $35 \pm 2$ °C for 24 h. After incubation, the colonies that grew on the surface of the medium were observed. Red bacterial colonies are classified as *Pseudomonas*, while yellow colonies are classified as *Aeromonas* (16-17). Detection and Enumeration of *Salmonella* sp., *Shigella* sp., and *E. coli* using 100  $\mu$ l of dilutions were spread on Salmonella-Shigella (SS) agar medium (Merck, Germany). This is a selective and differential medium for *Salmonella* spp. and *Shigella* spp. growth. The Petri dishes containing the samples were incubated at  $35 \pm 2$ °C for 24 h. After incubation, colonies that had developed on the surface of the medium were observed. Black bacterial colonies are classified into the *Salmonella* genus, whereas colorless colonies are classified into the *Shigella* genus. *E. coli* can grow in this medium with limited growth and form pink colonies.

### Data analysis

The data obtained in microbial counts, microbial types, and personal hygiene of Thai tea sellers were analyzed and presented using Microsoft Excel 2017.

## RESULTS

The successfully analyzed Thai tea samples were from four urban villages spread across four sub-districts in Lampung Province, Indonesia. Korpri Village (Sukarame Subdistrict (SK)) is the location that sells most Thai tea drinks. 68% of Thai tea samples came from this location (Table 1).

**Table 1. Total Sample Each Sampling Location**

Districts	$\Sigma$ Sample	%
Sukarame (SK)	34	68
Way Halim (WH)	4	8
Jati Agung (JA)	2	4
Tanjung Senang (TS)	10	20
<b>Total</b>	<b>50</b>	<b>100</b>



Coliform bacteria were detected in all 50 tea samples (100%). *Pseudomonas* sp. and *Aeromonas* sp. were also found in GSP media. All Thai tea samples (100 %) were contaminated with *Pseudomonas* sp., and 18 samples (36%) were contaminated with *Aeromonas* sp. Only four samples (8%) were contaminated with *Salmonella* spp. on SS agar medium. The highest percentage was observed in the presence of *E. coli* in 38 samples (76%) and *Shigella* spp. in 34 samples (68%) (Table 2).

**Table 2. Percentage of the Sample with Contaminant Bacteria**

Bacteria Type	Number of Samples Detected	%
Total Coliform	50	100
<i>Pseudomonas</i> sp.	50	100
<i>Aeromonas</i> sp.	18	36
<i>Salmonella</i> sp.	4	8
<i>Shigella</i> spp.	34	68
<i>E. coli</i>	38	76

Based on the sampling location, the type of bacteria *Pseudomonas* sp. was found in all samples at the four locations. In addition to *Pseudomonas* sp., some bacteria dominate all other locations, namely, *Shigella* sp. (58.82-100%) and *E. coli* with 50-100% percentage. *Aeromonas* sp. were found in all locations. *Salmonella* sp. was the type of bacteria with the lowest level of contamination among the four sampling locations. The TS location had the highest *Salmonella* sp., rate found in three samples (30%) of Thai tea (Table 3).

**Table 3. Percentage of Total Bacteria at Each Sampling Location**

Bacteria Type	Sampling Location			
	SK (n=34)	WH (n=4)	TS (n=10)	JA (n=2)
Total Coliform	34 (100%)	4 (100%)	10 (100%)	2 (100%)
<i>Pseudomonas</i> sp.	34 (100%)	4 (100%)	10 (100%)	2 (100%)
<i>Aeromonas</i> sp.	13 (38.23%)	2 (50%)	2 (20%)	1 (50%)
<i>Salmonella</i> sp.	1 (2.94%)	0 (0%)	3 (30%)	0 (0%)
<i>Shigella</i> sp.	20 (58.82%)	3 (75%)	9 (90%)	2 (100%)
<i>E. coli</i>	26 (76.47%)	4 (100%)	7 (70%)	1 (50%)

Contaminating bacteria were detected in the 50 Thai tea samples. The total was in the range of  $1 \times 10^3$  -  $3.3 \times 10^6$  CFU ml<sup>-1</sup> (Figure 2). The coliform test results showed that all the samples were contaminated with coliform bacteria. There were three samples with the highest amount of contamination by coliform bacteria,

namely samples coded SK 21, SK 22, and SK 26, with a total of 1100 MPN 100 ml<sup>-1</sup> each (Figure 2). *Pseudomonas* sp. was found in all bacterial samples. Based on location, the Sukarame (SK) became the location with the highest amount of *Pseudomonas* sp. contamination with a total of  $4.82 \times 10^3 \pm 80.0$  CFU ml<sup>-1</sup> from sample SK32, followed by SK13, SK19, and SK34 (Table 4). *Aeromonas* spp. were only found in 18 out of the 50 Thai tea samples. Samples from SK were the highest locations where the genus *Aeromonas* sp. Thai tea with sample code SK14 had the highest number of *Aeromonas* sp., amounting to  $2.24 \times 10^3 \pm 65.0$  CFU ml<sup>-1</sup>.

In almost all the samples and locations, *E. coli* was found in large numbers. The TS site had the highest amount of *E. coli* contamination in sample TS01 at  $5.22 \times 10^4 \pm 52.0$  CFU ml<sup>-1</sup>, followed by SK15, SK12, SK13, and SK21. The highest *Shigella* sp. count from all sites was found in sample SK34 at  $3.52 \times 10^4 \pm 100$  CFU ml<sup>-1</sup>, followed by SK29 and SK16. Furthermore, *Salmonella* spp. was only found in four Thai tea samples, namely SK10, TS04, TS06, and TS08. Sample SK10 had the highest number of *Salmonella* spp., which amounted to  $9.65 \times 10^3 \pm 350$  CFU ml<sup>-1</sup> (Table 4). Sample SK10 was the Thai tea sample with the highest number of contaminants in each type compared to the other two samples, namely *Pseudomonas* sp. ( $2.96 \times 10^3 \pm 165$  CFU ml<sup>-1</sup>), *E. coli* ( $7.2 \times 10^3 \pm 190$  CFU ml<sup>-1</sup>), *Shigella* sp. ( $3.35 \times 10^3 \pm 350$  CFU ml<sup>-1</sup>) and *Salmonella* sp. ( $9.6 \times 10^3 \pm 50.0$  CFU ml<sup>-1</sup>). While samples SK16, SK18, SK21, SK22, and WH01 were contaminated by the types of bacteria *Pseudomonas* sp., *Aeromonas* sp., *E. coli*, and *Shigella* sp. Sample SK16 was a Thai tea sample with the highest number of contaminants in each type compared to the other four samples, namely *Pseudomonas* sp. ( $1.445 \times 10^3 \pm 35.0$  CFU ml<sup>-1</sup>), *Aeromonas* sp. ( $8.9 \times 10^2 \pm 30.0$  CFU ml<sup>-1</sup>), *E. coli* ( $6.75 \times 10^3 \pm 75.0$  CFU ml<sup>-1</sup>) and *Shigella* sp. ( $1.23 \times 10^4 \pm 200$  CFU ml<sup>-1</sup>). However, the three samples with the lowest contaminant types, SK03, SK19, and SK20, were only contaminated with *Pseudomonas* sp. bacteria with low amounts of  $1.18 \times 10^3$  CFU ml<sup>-1</sup>,  $3.23 \times 10^3$  CFU ml<sup>-1</sup>, and  $1.5 \times 10^2$  CFU ml<sup>-1</sup>, respectively (Figure 3).

The presence of bacteria in Thai tea drink samples correlates with the cleanliness of Thai tea sellers. Thai tea sellers' behaviour is deplorable because most Thai tea sellers ignore hygiene and sanitation practices (Figure 4). Tea sellers neglect hand hygiene before serving Thai tea drinks to buyers, such as not washing hands before preparing drinks, not using gloves,

accepting money from buyers while serving drinks, and not washing hands after holding money. Only 30% of tea sellers use aprons to serve drinks. Of the total number of apron users, only 53.3% used clean aprons. None of the tea sellers in this study used gloves while serving drinks.

In addition, 46% of the sellers' hands used jewellery in rings and watches, and as many as 20% of the sellers' fingers had long nails. Seven male tea sellers smoked while serving Thai tea drinks. However, none of the tea sellers spit, cleaned, or cough near food.

**Table 4. Amount of Contaminant Bacteria in Each Sample**

Sample Code	<i>Pseudomonas</i> sp. (CFU ml <sup>-1</sup> ) ± SD	<i>Aeromonas</i> sp. (CFU ml <sup>-1</sup> ) ± SD	<i>E. coli</i> (CFU ml <sup>-1</sup> ) ± SD	<i>Shigella</i> sp. (CFU ml <sup>-1</sup> ) ± SD	<i>Salmonella</i> sp. (CFU ml <sup>-1</sup> ) ± SD
SK01	1x10 <sup>1</sup> ±0.00	0±0.00	9.6x10 <sup>3</sup> ±100	0±0.00	0±0.00
SK02	1.315x10 <sup>3</sup> ±130	2x10 <sup>2</sup> ±10.0	3.5x10 <sup>2</sup> ±20.0	0±0.00	0±0.00
SK03	1.18x10 <sup>3</sup> ±0.00	0±0.00	0±0.00	0±0.00	0±0.00
SK04	6.95x10 <sup>2</sup> ±15.0	5±0.00	0±0.00	0±0.00	0±0.00
SK05	3.8x10 <sup>2</sup> ±10.0	6.5x10 <sup>1</sup> ±5.00	5.1x10 <sup>3</sup> ±100	0±0.00	0±0.00
SK06	9.95x10 <sup>2</sup> ±50.0	5x10 <sup>1</sup> ±4.00	0±0.00	7x10 <sup>2</sup> ±150	0±0.00
SK07	4.2x10 <sup>2</sup> ±40.0	6.5x10 <sup>1</sup> ±2.00	7.65x10 <sup>3</sup> ±350	0±0.00	0±0.00
SK08	9.95x10 <sup>2</sup> ±15.0	5x10 <sup>1</sup> ±0.00	4.7x10 <sup>3</sup> ±200	5.6x10 <sup>1</sup> ±2.00	0±0.00
SK09	2x10 <sup>3</sup> ±100	0±0.00	3.9x10 <sup>1</sup> ±2.00	0±0.00	0±0.00
SK10	2.965x10 <sup>3</sup> ±165	0±0.00	7.2x10 <sup>2</sup> ±190	3.35x10 <sup>3</sup> ±350	9.65x10 <sup>3</sup> ±50.0
.SK11	5.35x10 <sup>2</sup> ±35.0	0±0.00	1.5x10 <sup>3</sup> ±100	1x10 <sup>3</sup> ±100	0±0.00
SK12	2.63x10 <sup>3</sup> ±150	0±0.00	1.32x10 <sup>4</sup> ±175	0±0.00	0±0.00
SK13	3.91x10 <sup>3</sup> ±110	0±0.00	1.275x10 <sup>4</sup> ±150	0±0.00	0±0.00
SK14	2.06x10 <sup>3</sup> ±60.0	2.24x10 <sup>3</sup> ±65.0	1x10 <sup>2</sup> ±15.0	0±0.00	0±0.00
SK15	1.2x10 <sup>3</sup> ±10.0	0±0.00	1.395x10 <sup>4</sup> ±150	6.4x10 <sup>3</sup> ±300	0±0.00
SK16	1.445x10 <sup>3</sup> ±35.0	8.9x10 <sup>2</sup> ±30.0	6.75x10 <sup>3</sup> ±75.0	1.23x10 <sup>4</sup> ±200	0±0.00
SK17	7.25x10 <sup>2</sup> ±25.0	2.1x10 <sup>2</sup> ±10.0	0±0.00	6.4x10 <sup>3</sup> ±50.0	0±0.00
SK18	9.2x10 <sup>2</sup> ±120	7.1x10 <sup>1</sup> ±1.00	4.3x10 <sup>3</sup> ±75.0	6.3x10 <sup>3</sup> ±100	0±0.00
SK19	3.23x10 <sup>3</sup> ±30.0	0±0.00	0±0.00	0±0.00	0±0.00
SK20	1.5x10 <sup>2</sup> ±10.0	0±0.00	0±0.00	0±0.00	0±0.00
SK21	1.06x10 <sup>3</sup> ±60.0	1x10 <sup>1</sup> ±0.00	1.27x10 <sup>4</sup> ±200	8.8x10 <sup>2</sup> ±100	0±0.00
SK22	3.75x10 <sup>2</sup> ±5.00	5±0.00	5x10 <sup>1</sup> ±5.00	1x10 <sup>2</sup> ±10.0	0±0.00
SK23	2x10 <sup>3</sup> ±5.00	0±0.00	7.05x10 <sup>3</sup> ±50.0	3.95x10 <sup>3</sup> ±87.0	0±0.00
SK24	6.05x10 <sup>2</sup> ±5.00	0±0.00	1.85x10 <sup>3</sup> ±25.0	4.4x10 <sup>3</sup> ±100	0±0.00
SK25	1.92x10 <sup>3</sup> ±120	0±0.00	5.7x10 <sup>3</sup> ±120	1.4x10 <sup>3</sup> ±50.0	0±0.00
SK26	1.2x10 <sup>2</sup> ±10.0	0±0.00	4x10 <sup>2</sup> ±50.0	2.5x10 <sup>2</sup> ±50.0	0±0.00
SK27	8.5x10 <sup>1</sup> ±5.00	0±0.00	3.45x10 <sup>3</sup> ±140	0±0.00	0±0.00
SK28	4.55x10 <sup>2</sup> ±55.0	0±0.00	1x10 <sup>2</sup> ±7.00	1x10 <sup>2</sup> ±10.0	0±0.00
SK29	1.8x10 <sup>2</sup> ±10.0	0±0.00	3.8x10 <sup>3</sup> ±170	1.355x10 <sup>4</sup> ±575	0±0.00
SK30	1x10 <sup>1</sup> ±0.00	0±0.00	4.5x10 <sup>3</sup> ±50.0	0±0.00	0±0.00
SK31	1x10 <sup>1</sup> ±0.00	1x10 <sup>1</sup> ±0.00	0±0.00	1x10 <sup>2</sup> ±5.00	0±0.00
SK32	4.82x10 <sup>3</sup> ±80.0	0±0.00	1x10 <sup>2</sup> ±0.00	8x10 <sup>2</sup> ±10.0	0±0.00
SK33	1.45x10 <sup>2</sup> ±15.0	0±0.00	0±0.00	1x10 <sup>2</sup> ±0.00	0±0.00
SK34	3.035x10 <sup>3</sup> ±35.0	0±0.00	1.95x10 <sup>3</sup> ±35.0	3.52x10 <sup>4</sup> ±100	0±0.00
WH01	1.68x10 <sup>3</sup> ±40.0	3x10 <sup>1</sup> ±5.00	7x10 <sup>2</sup> ±10.0	9.5x10 <sup>2</sup> ±50.0	0±0.00
WH02	3.15x10 <sup>2</sup> ±15.0	2.6x10 <sup>2</sup> ±35.0	950±65.0	0±0.00	0±0.00
WH03	3.35x10 <sup>2</sup> ±15.0	0±0.00	2.55x10 <sup>3</sup> ±250	5x10 <sup>1</sup> ±5.00	0±0.00
WH04	1.035x10 <sup>3</sup> ±70.0	0±0.00	6.8x10 <sup>3</sup> ±100	1x10 <sup>2</sup> ±15.0	0±0.00
TS01	1.235x10 <sup>3</sup> ±45.0	0±0.00	5.22x10 <sup>4</sup> ±52.0	2x10 <sup>2</sup> ±20.0	0±0.00
TS02	9.45x10 <sup>2</sup> ±20.0	9.5x10 <sup>1</sup> ±5.00	0±0.00	0±0.00	0±0.00
TS03	9.95x10 <sup>2</sup> ±35.0	0±0.00	8.5x10 <sup>2</sup> ±10.0	4.2x10 <sup>3</sup> ±130	0±0.00
TS04	1.44x10 <sup>3</sup> ±110	0±0.00	2.4x10 <sup>3</sup> ±80.0	5x10 <sup>2</sup> ±10.0	1x10 <sup>3</sup> ±100
TS05	1x10 <sup>1</sup> ±0.00	0±0.00	3.95x10 <sup>3</sup> ±20.0	1x10 <sup>3</sup> ±50.0	0±0.00
TS06	1x10 <sup>1</sup> ±0.00	0±0.00	1x10 <sup>3</sup> ±30.0	1x10 <sup>3</sup> ±75.0	5x10 <sup>2</sup> ±25.0
TS07	3.6x10 <sup>2</sup> ±20.0	3x10 <sup>1</sup> ±5.00	0±0.00	8.55x10 <sup>3</sup> ±127	0±0.00

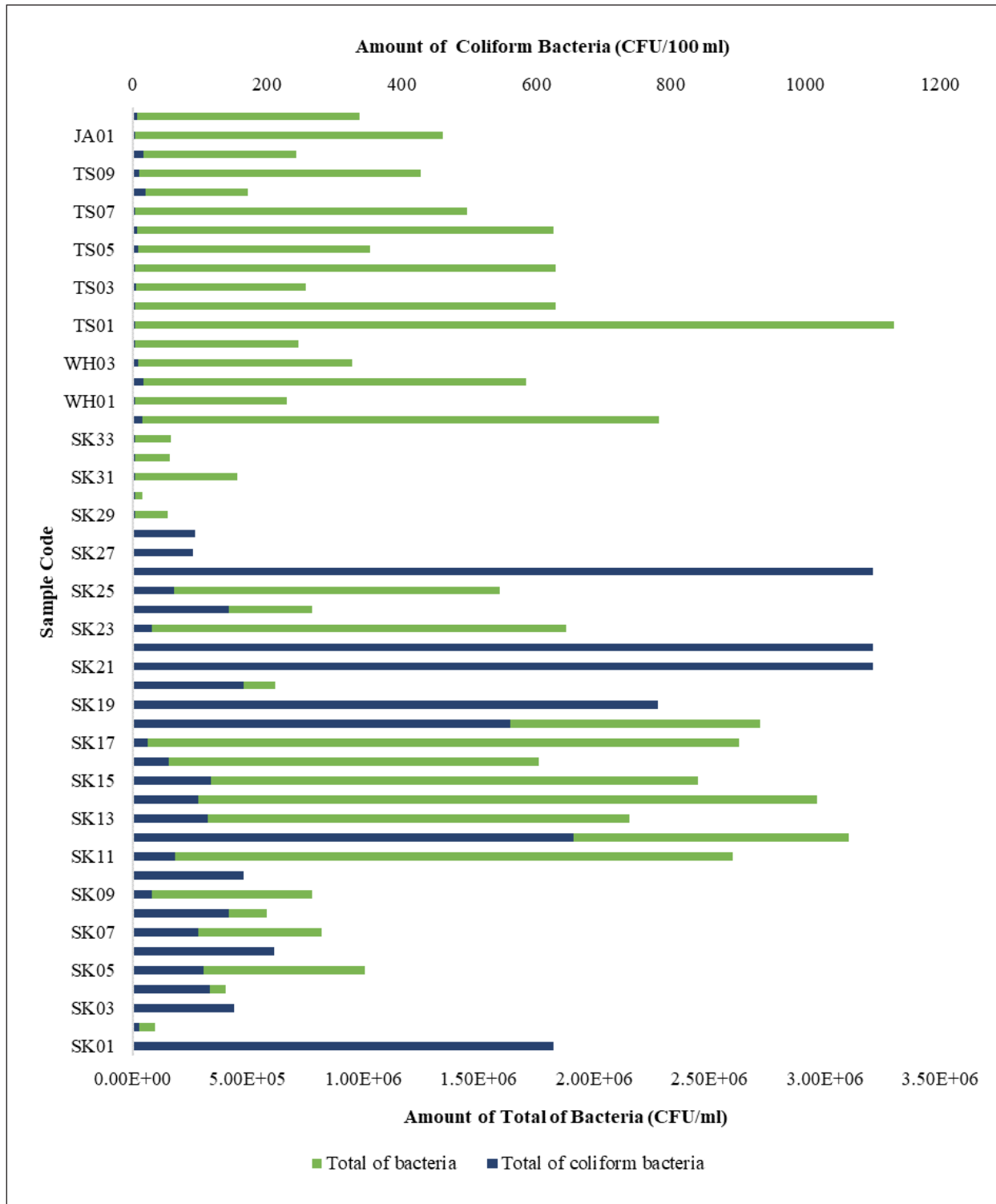


Figure 2. Total Bacteria and Coliform Bacteria Each Sample in All Locations

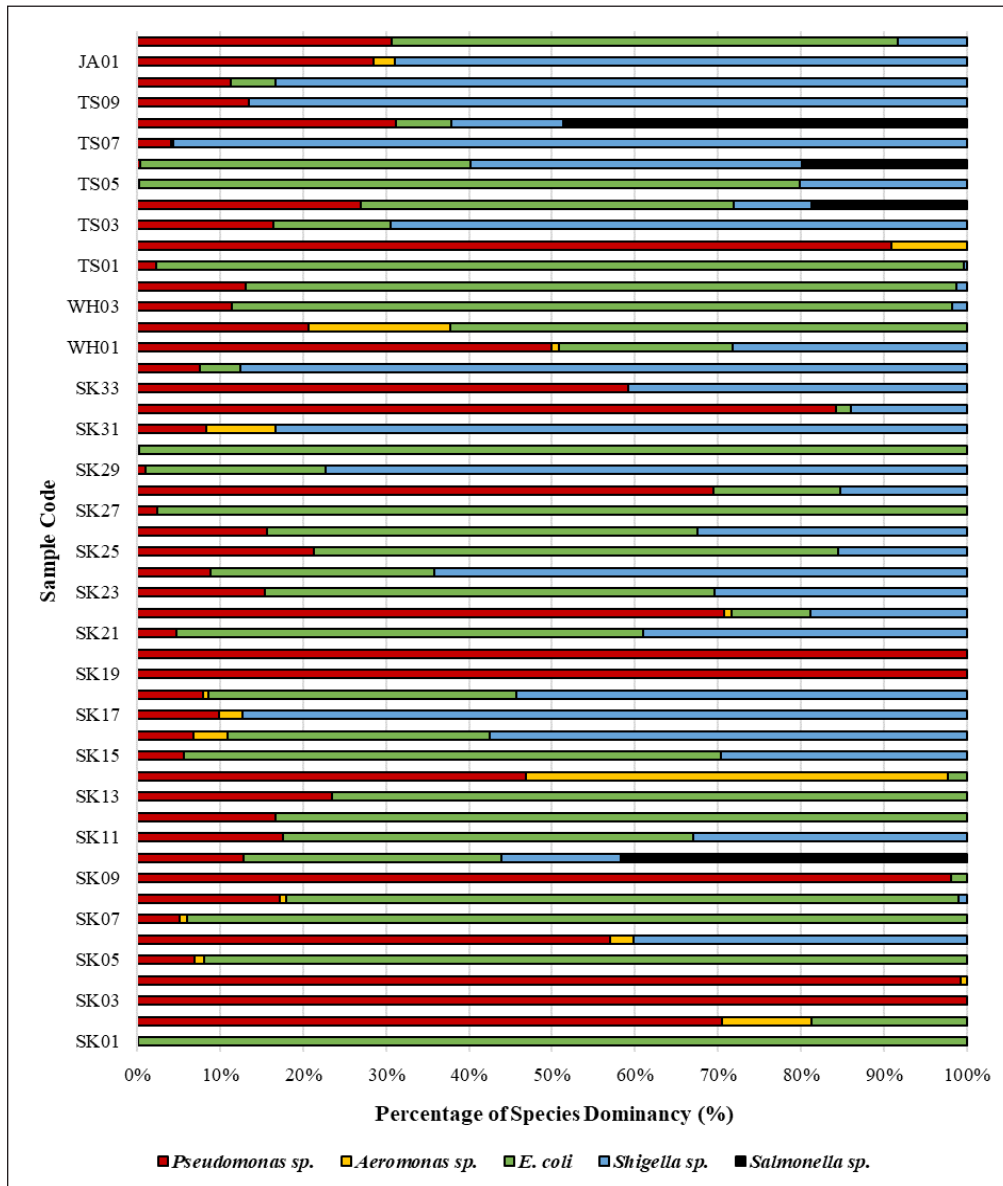


Figure 3. Percentage of Dominant Bacteria in Each Sample

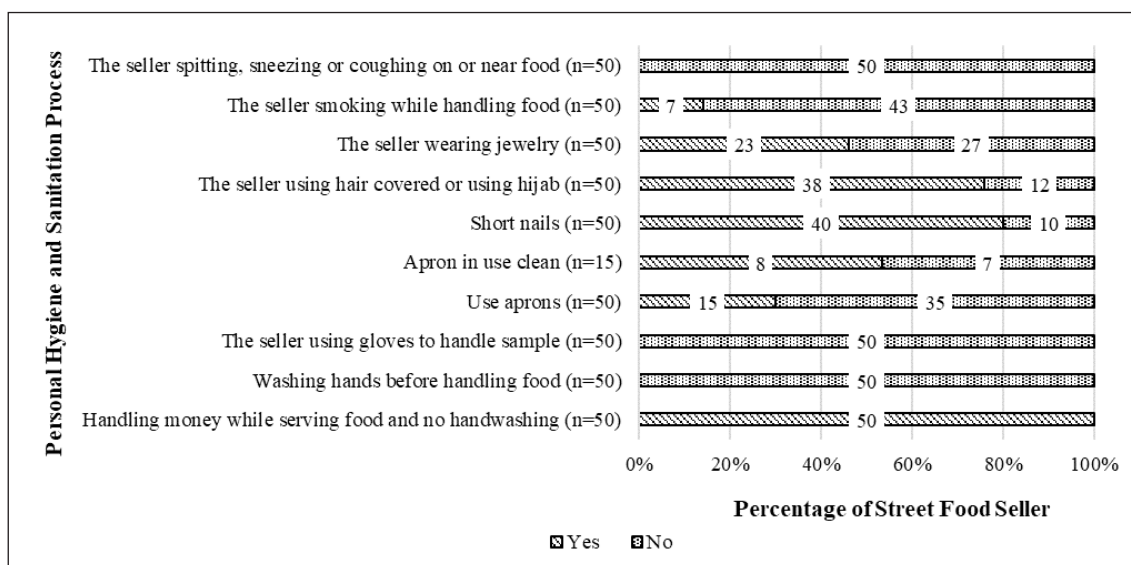


Figure 4. Observations on Personal Hygiene and Practice of Thai Tea Sellers

## DISCUSSION

Sukarame (SK) has the highest number of drink sellers because it is located closest to educational institutions. Educational institutions affect the number of street vendors. However, all collection locations were urban villages at the main point of community/student activities in ITERA (2). From the high total bacteria and coliforms in Thai tea samples, very diverse bacteria were found, namely, from the genera *Pseudomonas* sp., *Aeromonas* sp., and *Salmonella* sp. *Shigella* sp., *E. coli*. Bacterial contamination of this drink has a significant impact on health. Tea is the most widely consumed aromatic beverage in the world. However, tea is often subjected to pathogenic bacterial contamination during manufacture and storage (1). Tea is usually stored in various forms, such as tea bags, for example, used in black tea or green tea. Tea stored in bags has been identified as having multiple bacterial contaminants. Multiple types of contaminating bacteria were found, including *Pseudomonas psychrotolerans*, *Staphylococcus warneri*, *Pantoea gaviniae*, and *Clostridium perfringens*. Most of these species are pathogenic to humans (3). Despite drying, the number of bacteria in tea does not change during storage (18).

This paper showed that *E. coli* was found in large numbers in almost all the samples and locations. Faecal coliform bacteria, like *E. coli*, indicate drinking water quality and safety (19-20). The presence of these coliforms in water sources indicates contamination of drinking water by *Enterobacteriaceae*. Contamination of drinking water sources by these bacteria can increase the incidence of diarrheal diseases (21–23). *E. coli* in drinking water indicates faecal contamination of the water source or the surrounding environment around the water source (24). *E. coli* can be transmitted from the faeces of infected humans and animals to new hosts through environmental reservoirs such as hands, water, and soil (19,25). According to the WHO, the water quality of all samples is in the polluted category because they have been contaminated with  $\geq 1$  CFU 100 ml<sup>-1</sup> of total coliform (26-27). Based on the distribution of bacterial types in each sample, samples SK10, TS04, TS06, TS08, SK16, SK18, SK21, SK22, and WH01 were the most contaminated. Samples SK10, TS04, TS06, and TS08 were contaminated with *Pseudomonas* sp., *E. coli*, *Shigella* sp., and *Salmonella* sp. Similar to previous CLED media detection results, samples TS06 and TS08 were reportedly contaminated with *Klebsiella* sp., *E. coli*, *Salmonella* sp., and *Proteus* sp. (2).

In addition, *Pseudomonas* sp. has been found in various ecological niches, including soil, water, rhizosphere, plants, animals, and environmental waste

(28-29). This bacterium is a waterborne pathogen and a health hazard (30). *Pseudomonas* plays a role in cystic fibrosis, urinary tract infections, gastrointestinal infections, bacteremia, and respiratory system infections (31). *P. psychrotolerans* is also found in black and green tea and causes infections in immunocompromised patients (3). *Aeromonas* spp. are often found in drinking water and are indicators of microbial regrowth in non-chlorinated drinking water distribution systems (32). The *Aeromonas* genera associated with gastroenteritis are *A. caviae*, *A. veronii*, *A. dhakensis*, and *A. hydrophila*. The presence of these bacteria in food or beverages in high numbers should also be considered. This is because *Aeromonas* can be infectious and human susceptibility is high, similar to other dangerous enteropathogens such as *Campylobacter* or *Salmonella* (33). In addition, *Aeromonas* is significantly more common in patients with diarrhoea than in those with different types of bacteria commonly thought to cause diarrhoea (34).

The bacteria that pose a high risk are *Salmonella* sp. and *Shigella* sp. in the samples. These two types of bacteria are responsible for the incidence of death due to acute diarrhea, especially in children (35). Shigellosis is caused by *Shigella* spp. and is a global problem, although it is more common in developing countries (36-37). *Shigella* spp. are restricted to the human intestinal tract and cause bacillary dysentery, leading to watery or bloody diarrhea (35). A species that is a waterborne pathogen found in drinking water is *S. flexneri* (38-39). *Salmonella* is a foodborne pathogenic bacterium that is harmful to individuals with compromised immune systems (18). Unhygienic food preparation, storage, and repeated heating are closely associated with *Salmonella* contamination. Poor sanitation and hygienic status, the habit of not washing hands before and after meals, and latrines contribute to instances of typhoid *Salmonella* infection (40–44). The results showed that four samples contained *Salmonella* spp., namely SK10, TS04, TS06, and TS08. Sample SK10 had a very high number of *Salmonella* spp. This bacterium has survived in dried tea samples for 23 days (18) and is dangerous if found in beverages. *S. paratyphi* is a pathogenic bacterial species that can survive various pH changes in the stomach (45).

The presence of bacteria in Thai tea drinks is also due to the manufacturing process, which uses water at temperatures below the boiling point. The samples were brewed in warm water (not boiling) to dissolve the tea. Some sellers have dissolved tea with water, and when there are buyers, they only mix it with other ingredients (e.g., milk and ice). Hot water at its boiling point removes all microbes, including thermophilic and



bacterial endospores (1). Tea that uses boiling water at its boiling point or tea that is directly boiled can reduce the total population of bacteria and coliforms to  $10^5$  CFU  $g^{-1}$  (46). Drinking water as a raw material to prepare Thai tea plays an essential role in the presence of bacterial contaminants in Thai tea. Drinking water is the most efficient pathway for the spread of pathogenic microbes (47). Based on observations during sampling, all tea sellers used drinking water from the refill depots. Refill water is susceptible to coliform bacteria because of the equipment's lack of cleanliness and sanitation (48). Contaminated storage containers, hands, and other equipment in contact with stored drinking water cause a decrease in drinking water quality (49). This is supported by a report that the quality of refilled drinking water in Bandar Lampung, Indonesia, which is the sampling location of this study, does not meet the requirements based on Permenkes RI No. 492/Menkes/Per/IV/2010 regarding the presence of bacteria in drinking water. The 42% of refilled drinking water depots in Bandar Lampung were contaminated with coliform bacteria (50). The same was also reported in another city in Indonesia, Makassar, particularly in Barrang Lompo. *K. pneumoniae* and *P. aeruginosa* were found in the drinking water samples that had been analyzed (51).

Another raw material that causes contamination in Thai tea drinks is ice cubes. This causes the ice cube-making industry to increase both on a household scale and a larger scale. This condition causes a lack of quality control of the raw material for making ice cubes (water), a high chance of contamination during transportation from the production site to the seller's hands, and even a lack of hygiene among of the workers responsible for transporting the ice cubes to the hands of buyers (those tea sellers). Various reports of bacterial contamination in ice cubes in similar Thai tea drinks sold on the street in various cities in Indonesia have been widely reported (52-53). In addition, ice cubes can increase the populations of *E. coli* and coliform bacteria in milk tea. The number of *E. coli* bacteria was higher in milk tea that added ice than in those that did not (4). Ice can potentially be a source of microbial contamination in milk tea. The bacteria in ice cubes cannot be separated from raw drinking water as the raw material to form ice cubes. Bacteria from contaminated water do not die during the freezing process; therefore, bacteria reactivate when ice conditions melt and mix with drinks (54-55).

The presence of enteric pathogenic bacteria is associated with poor personal (sellers) and environmental hygiene. Money is an effective mediator of microbial

transfer. The continuous money exchange process between individuals with various activity backgrounds can potentially increase contamination, especially among pathogenic microbes. Encountering money when serving drinks can cause contamination (56). Bacterial contamination of banknotes is very high, with *E. coli*, *Salmonella* sp., and *Staphylococcus aureus* present on banknotes in food outlets from 10 countries (57). Public knowledge of the transmission of these enteric bacteria through hands, surfaces, and soil is more effective in preventing their spread (47), one of which is washing hands before starting activities, especially in making Thai tea drinks.

Clean aprons, gloves, hair covering, and hand washing before handling food or beverages are basic hygiene practices that food and beverage sellers should fulfil. The behavior of ignoring basic hygiene practices, such as not using a clean apron, gloves, or covering hair, was also reported (56). These data indicate that these vendors have violated and ignored the basic hygiene behaviours of street food and beverage vendor (58). This suggests that street food can be a source of public health problems, given the low level of food safety in street food and beverages. Practices, knowledge, and risk factors for the lack of awareness by food and beverage vendors of personal hygiene by food and beverage vendors, especially nails, were also found in Uganda, where 68.6% of vendors had uncut nails, and about 75.7% had unclean nails. Some vendors were located to smoke (2.0%), spit, sneeze, or cough near food (6.2%) (59). These practices increase the likelihood of food contamination owing to physical, chemical, or biological food safety hazards. They can also be an avenue for transferring infectious diseases from sick vendors to many street food consumers.

Based on the location of the sample, the sample with the highest contamination is in the Sukarame Subdistrict (SK). This location is a commercial area, and the road is used as an exit toll. This causes an increased flow of traffic and human activity in that location. The location of roadside sales significantly affects the hygiene of beverage products (4). Commercial establishments and heavy traffic are associated with high levels of *E. coli*, *Salmonella*, *Shigella*, *Campylobacter*, and *S. aureus* contamination in juice drinks sold on the side of the road (60). This condition is also exacerbated by the behavior of less hygienic sellers, thus adding a source of bacterial contamination of the environment to the drinks sold. Unhygienic environmental conditions cannot minimize contamination by the number of bacteria in the environment.

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## CONCLUSION

Of the 50 Thai tea samples, coliform bacteria and *Pseudomonas* sp. in all samples (100%), *Aeromonas* sp. (36%), *Shigella* sp. (68%), *E. coli* (76%), and total bacteria were in the range of  $1 \times 10^3$ - $3.3 \times 10^6$  CFU ml<sup>-1</sup>. Most coliform bacteria in the SK21, SK22, and SK26 samples were 1100 MPN/100 ml. The highest pollution samples were from the Sukarame district (SK). SK10 was a tea sample with the most pollutants, including *Pseudomonas* sp., *Escherichia coli*, *Shigella* sp., and *Salmonella* sp. *Pseudomonas* sp., *Aeromonas* sp., *E. coli*, and *Shigella* sp. contaminated SK 16. The lowest number of contaminants was found in samples SK20, SK03, and SK19, which were only contaminated by *Pseudomonas* sp. Samples SK10, TS04, TS06, and TS08 contained only *Salmonella* spp. The poor quality of Thai tea is caused by the habits of sellers who need to maintain cleanliness while serving thai tea such as not maintaining hand hygiene (source of raw material, handling money, not washing hands, not using gloves). All samples did not meet the requirements for the presence of bacteria in drinking water, based on PERMENKES RI No.492/Menkes/Per/IV/2010 and WHO on the presence of bacteria in drinking water. Quality raw materials and hygienic presentation can improve the quality of thai tea sold.

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