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## **TOTAL PLATE COUNT (TPC) TEST AND CONTAMINATION OF *Coliform Escherichia coli* AND *Salmonella* sp. BACTERIA IN CUCUMULAWAK JAMU IN TRADITIONAL MARKETS IN MEDAN CITY**

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### **ABSTRACT**

Jamu is a traditional Indonesian drink derived from plants that are efficacious for use in efforts to maintain health and cure diseases. However, because the manufacturing process is still simple, jamu is at high risk of contamination by microorganisms such as fungi and pathogenic bacteria such as *Escherichia coli* and *Salmonella* sp. This study aims to determine the presence of *Escherichia coli* and *Salmonella* sp. Coliform contamination in Javanese turmeric herbal medicine sold in traditional markets. This study is based on the simple process of making jamu using hands, unclean clothes from jamu makers, and unclean processing areas, this will increase the risk of contamination and result in jamu being contaminated by microorganisms that can endanger consumer health. Data collection techniques use testing and counting of bacterial colonies with the TPC (Total Plate Count) method and *Escherichia coli* bacteria with the MPN (Most Probable Number) method which consists of Presumptive Test, Confirmatory Test and Complementary Test. The results of the analysis showed that in the TPC (Total Plate Count) test, three of the five samples, namely Sp 1, Sp 3 and Sp 4, showed results that were suitable for consumption because the TPC (Total Plate Count) value did not exceed the maximum limit of BPOM 2019 and two of the five samples, namely Sp 2 and Sp 5, showed results that were not suitable for consumption because they exceeded the maximum limit of BPOM 2019.

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## Introduction

Jamu is a traditional Indonesian beverage passed down through generations and known for its medicinal properties. Made from natural ingredients such as medicinal plants, jamu is used to maintain health and cure illnesses. However, due to its simple manufacturing process, jamu is at high risk of contamination by microorganisms such as fungi and pathogenic bacteria (Utami *et al.*, 2018). Unhygienic processing practices, such as the use of raw materials, dirty hands, dirty clothing, and unclean sales locations and environments are the main factors contributing to bacterial contamination in jamu (Fhityani *et al.*, 2017).

*Coliforms* like *Escherichia coli* and *Salmonella* sp. are microbes that can contaminate herbal medicine. These bacteria are the most common cause of serious conditions, such as poisoning, in humans. *E.coli* is an indicator of water and food quality because its presence indicates fecal contamination. *Coliform* is an indicator microbe in food and beverage products, indicating contamination, while the presence of *Salmonella* sp. in beverages indicates poor sanitation (Jiwintarum *et al.*, 2017).

Microbiological testing is an important test because it can be used as an indicator of food and beverage sanitation or as an indicator of food safety. Microbiological testing includes quantitative testing of pathogenic bacteria to determine their safety level (Yunita *et al.*, 2015). Therefore, this study is crucial to determine the number of colonies in Javanese turmeric herbal medicine sold in traditional markets.

to determine the presence of *Coliform* contamination *E.coli* and *Salmonella* sp. in Javanese turmeric herbal medicine sold in traditional markets and to determine the relationship between the cleanliness of the production house and the environment where the herbal medicine is sold and bacterial contamination in the herbal medicine.

## Materials and Methods

The study was conducted from February to April 2025 at five traditional markets in Medan City and at the Microbiology Laboratory of the Biology Department of Medan State University. The study population consisted of Javanese turmeric herbal medicine collected from various vendors at different sales locations in traditional markets in Medan Marelan and Medan Deli Districts. A 50 ml sample was collected aseptically, then placed in a sterile plastic bag, tightly sealed, and immediately transported for testing.

The equipment to be used must be thoroughly washed and dried beforehand. The mouths of test tubes and Erlenmeyer flasks should be covered with aluminum foil, while petri dishes should be tightly wrapped in newspaper. Equipment to be used in the test must be sterilized beforehand to prevent possible contamination by microorganisms. Heat-resistant glassware should be wrapped in newspaper and placed in an autoclave, where the sterilization process is carried out at 121°C for 20 minutes. After that, the media preparation process continues.

(*Lactose Broth*) medium, 3.7 grams of ECB (*Escherichia coli* Broth) and EMBA (*Eosin Methylene Blue Agar*) Brands were dissolved in 100 ml of distilled water, stirred until dissolved and homogeneous. The medium was heated until well dissolved, and sterilized using an autoclave at 121°C for 15 minutes (Cartas *et al.*, 2022).

The MPN (*Most Probable Number*) test uses 3 dilution series of  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ . The MPN method is a method that utilizes the fermentation process in a Durham tube to determine the number of *Coliform* bacteria. The MPN method consists of a presumptive test, a confirmatory test, and a complementary test, then gram staining of bacteria is carried out. Testing for *Salmonella* sp. is carried out by enriching the sample by putting 9 ml of LB (*Lactose Broth*) solution into a test tube, then adding 1 ml of herbal medicine sample, then incubating at 37 °C for 2 x 24 hours. Then the sample is taken

using an ose needle, then streaking is carried out using the 4- *way streak method* onto SSA (*Salmonella Shigella Agar*) medium. After incubation for 2 x 24 hours, observations are made.

Data analysis was performed with TPC calculations reported in CFU/ml. Colonies below 30 were unacceptable, and colonies exceeding 300 on a plate were considered too close together to be counted as distinct colony units. Data analysis for the MPN test was performed qualitatively and quantitatively.

## Results and Discussion

### Total Plate Count (TPC) Test

Table 1. Number of Bacterial Colonies in Herbal Medicine *Total Plate Count* (TPC) Test

Parameter	Results	BPOM Maximum Limit 2019	Quality Status
		< 1 x 10 <sup>5</sup> (100,000)	
Sp 1	9 x 10 <sup>4</sup>	90,000	Suitable for consumption
Sp 2	1.9 x 10 <sup>5</sup>	190,000	Not fit for consumption
Sp 3	8.0 x 10 <sup>4</sup>	80,000	Suitable for consumption
Sp 4	5.3 x 10 <sup>4</sup>	53,000	Suitable for consumption
Sp 5	5.3 x 10 <sup>7</sup>	53,000,000	Not fit for consumption

Based on Table 1 above, the results of the microbial contamination test show that of the five sample locations, two locations have TPC values that are still above the maximum microbial contamination (TPC) threshold in traditional herbal medicine according to the requirements of BPOM Number 32 of 2019, namely the normal limit for the maximum number of TPC in traditional herbal medicine

is <105 . The high TPC value of herbal medicine indicates poor sanitation and *hygiene* , but does not indicate the presence of pathogenic bacteria, so further testing is needed to determine the type of pathogenic bacteria in the temulawak herbal medicine sample.

In accordance with the results of research conducted by Sari *et al.*, (2024), the number of colonies in the turmeric and tamarind herbal drink carried out on four samples taken in RT 004 RW 012 Kayuringin Jaya, Bekasi City, showed that no bacterial colonies were found growing in the three samples, while one sample of turmeric and tamarind herbal drink exceeded the threshold, namely 1.2 x 10<sup>7</sup> CFU/ml.

The hygiene and sanitation of turmeric herbal medicine production at the five sample locations did not meet the criteria, starting from the simple method. The process of making turmeric herbal medicine is passed down through generations, so poor *hygiene* and sanitation practices persist due to a lack of knowledge regarding proper turmeric herbal medicine production. The personal *hygiene* of the herbal medicine makers at the five sample locations still did not meet the criteria for good turmeric herbal medicine production.

### Most Probable Number (MPN) Test

#### a. Presumptive Test

Table 2. Results of the Presumptive Test

Sample Code	Dilution	Test	Change		Mark MPN /ml	Mark MPN SNI	Note
			Gas Formation	Turbidity			
Sp 1	10 <sup>-1</sup>	A	-	3 clear tubes	0	<20 MPN /ml	MS
		B	-				
		C	-				
	10 <sup>-2</sup>	A	-	3 clear tubes			
		B	-				
		C	-				
	10 <sup>-3</sup>	A	-	3 clear tubes			
		B	-				
		C	-				

Sp 2	10 <sup>-1</sup>	A	+	3 cloudy tubes	>100 0
		B	+		
		C	+		
	10 <sup>-2</sup>	A	+	3 cloudy tubes	
		B	+		
		C	+		
	10 <sup>-3</sup>	A	+	3 cloudy tubes	
		B	+		
		C	+		
Sp 3	10 <sup>-1</sup>	A	-	3 clear tubes	0
		B	-		
		C	-		
	10 <sup>-2</sup>	A	-	3 clear tubes	
		B	-		
		C	-		
	10 <sup>-3</sup>	A	-	3 clear tubes	
		B	-		
		C	-		
Sp 4	10 <sup>-1</sup>	A	-	3 clear tubes	0
		B	-		
		C	-		
	10 <sup>-2</sup>	A	-	3 clear tubes	
		B	-		
		C	-		
	10 <sup>-3</sup>	A	-	3 clear tubes	
		B	-		
		C	-		
Sp 5	10 <sup>-1</sup>	A	+	3 cloudy tubes	>100 0
		B	+		
		C	+		
	10 <sup>-2</sup>	A	+	3 cloudy tubes	
		B	+		
		C	+		
	10 <sup>-3</sup>	A	+	3 cloudy tubes	
		B	+		
		C	+		

Description:

A: First tube

B: Second tube

C: Third tube

(-): No gas in the Durham tube

(+): There is gas in the Durham tube

MS: Meets standards

TMS: Does not meet standards

Based on Table 2 above, it can be seen that Sp 2 at the 2nd location and Sp 5 at the 5th location showed positive results (+) having an abundant number of *Coliform*

*bacteria* which can be seen from the high MPN value exceeding the standard limit set by the SNI standard which is indicated by the emergence of gas and turbidity in the Durham tube. MPN is used to determine the total number of *Coliform bacteria* in drinks. According to SNI 7388: 2009, the maximum limit for MPN *Coliform* for traditional herbal medicine is <20 MPN/ml.

b. Confirmatory Test

Table 3. Confirmatory Test Results

Sample Code	MS	Dilution	Test	Change		Mark MPN /ml	Mark MPN SNI	Note
				Gas Formation	Turbidity			
Sp 2 MS	10 <sup>-1</sup>	A	+	3 cloudy tubes	>100 0	<3 MPN /ml	TMS	
		B	+					
		C	+					
	10 <sup>-2</sup>	A	+	3 cloudy tubes				
		B	+					
		C	+					
	10 <sup>-3</sup>	A	+	3 cloudy tubes				
		B	+					
		C	+					
Sp 5 TMS	10 <sup>-1</sup>	A	+	3 cloudy tubes	>100 0		TMS	
		B	+					
		C	+					
	10 <sup>-2</sup>	A	+	3 cloudy tubes				
		B	+					
		C	+					
	10 <sup>-3</sup>	A	+	3 cloudy tubes				
		B	+					
		C	+					

Description:

A: First tube

B: Second tube

C: Third tube

(-): No gas in the Durham tube

(+): There is gas in the Durham tube

MS: Meets standards

TMS: Does not meet standards

Based on Table 3 above, it can be seen that both samples, namely Sp 2 and Sp 5 positive (+), contained *Coliform* and *E-coli bacterial activity*, indicated by the appearance of gas and turbidity in the

Durham tube. These bacteria can be calculated by looking at the MPN value, and the results showed that the MPN value of both samples exceeded the SNI MPN standard, which is <3 MPN/ml.

#### c. Complementary Test

Table 4. Supplementary Test Results

No	Sample Code	Results
1	Sp 2	There is growth of red to pink colored bacteria
2	Sp 5	There is growth of red to pink colored bacteria

Based on Table 4 above, it can be seen that the two samples did not have a metallic green color, but rather a red to pink color. This indicates that only *Coliform bacteria were present*, and no *E.coli bacteria were present* in the two herbal medicine samples.

The results of this study are similar to those of Nugrahan *et al.* (2022), where four of the twelve herbal medicine samples were negative for *Escherichia coli* and eight of the twelve samples were positive for *Escherichia coli*. This could be due to several factors that influence the hygiene of herbal medicine products, thus allowing for the occurrence of pollution microbes on sample. Wrong the only one environmental factors that is building, location And equipment Which used moment processing process herbal medicine. Besides That factor next can caused by Because Human factors include physical conditions, knowledge, behavior and outlook on life, as well as herbal medicine ingredients such as selection of raw materials, herbal medicine management, storage of finished products, distribution and presentation medicine.

#### *Salmonella sp.* Test

Table 5. Results of *Salmonella sp.* Test

Sample Code	Results
Sp 1	-
Sp 2	+
Sp 3	-
Sp 4	-
Sp 5	+

#### Description:

(-): Not contaminated with *Salmonella sp. bacteria*.

(+): Contaminated with *Salmonella sp. bacteria*.

Based on Table 5 above, Sp 2 and Sp 5 are contaminated with *Salmonella sp* bacteria. In SSA (*Salmonella Shigella Agar*) media A and C, no bacteria grew after bacterial cultivation and incubation, while in SSA media B and E, *Salmonella sp* bacteria grew. The sample is said to be positive if it grows transparent white bacterial colonies accompanied by black spots, if it does not grow transparent white bacterial colonies accompanied by black spots, it can be said that the sample is negative for *Salmonella sp* bacteria.

The results of samples 2 and 5 are in accordance with the results of research by Salsabila *et al.* (2024), that 4 of 8 herbal medicine samples were positive for *Salmonella sp.* and 4 other samples in different locations were found to be negative for *Salmonella sp.* according to samples 1, 3 and 4. This can occur if the herbal medicine is processed properly or the ingredients used are not contaminated by these pathogenic bacteria. The presence of *Salmonella sp.* bacterial contamination in herbal medicine samples sold in traditional markets can be caused by the herbal medicine manufacturing process that does not pay attention to cleanliness in the form of raw material selection, production process, packaging or personal hygiene as well as cleanliness during distribution to consumers.

#### The Relationship Between Trader Cleanliness and Herbal Medicine Contamination

The relationship between trader hygiene and bacterial contamination of herbal medicines sold in traditional markets is very important to note. Poor hygiene practices can increase the risk of contamination with pathogenic bacteria. The sampling location where herbal medicine is sold is located near sources of pollution, such as garbage or waste disposal and sewers. All



herbal medicine sellers did not wash their hands before and after processing, nor did they wear masks, gloves, aprons, or head coverings. There was direct contact between the herbal medicine maker and their product during the filtering process to separate the turmeric water from the dregs, which was carried out using a filter cloth and then squeezing the product directly using their hands without gloves. This direct contact between the herbal medicine maker's hands and the herbal product could potentially transmit bacteria from their hands. Furthermore, the water used by the sellers appeared cloudy, as it was used to clean ingredients, equipment, and as an ingredient in herbal medicine production. The clean water source used by herbal medicine makers still does not meet clean water criteria. Water quality that does not meet these criteria can lead to bacterial contamination of equipment, raw materials, and the resulting herbal medicine product.

Factors causing contamination of *Coliform* and *Salmonella* sp. bacteria in sp 2 at location 2 and sp 5 at location 5 due to lack of cleanliness of tools and sales locations, the herbal medicine seller uses herbal medicine containers or equipment that have been used repeatedly and not washed thoroughly, this is evidenced by the condition of the herbal medicine container which is slightly black and crusty. The water used to wash the glass becomes cloudy or dirty because it has been used repeatedly. Then the washed equipment is simply placed on the cart so that it can be contaminated with dust or other dirt as well as bacteria carried by dust and dirt from the air which then sticks to the herbal medicine glass and spoon, where the sales location is on the edge of the market close to the ditch. Most do not use soap in washing. Serving bottles that are not washed thoroughly using soap can cause dirt or microbes to remain stuck in the bottle which contaminates the temulawak herbal product.

According to Putri *et al.* (2023), the hygiene of herbal medicine vendors at the herbal medicine sampling locations still does

not meet the criteria for good herbal medicine manufacturing practices, and is still rudimentary. The personal hygiene of herbal medicine makers does not meet the criteria for good herbal medicine manufacturing practices. According to Isnawati (2012), hygiene is a health effort by maintaining and protecting the hygiene of the individual subject. Sanitation is a preventative effort that emphasizes the activities and actions necessary to free food and beverages from all hazards that can disrupt or damage health, starting from before production, during processing, storage, and transportation until ready for public or consumer consumption. According to Juhaina (2020), sellers are people who are directly in contact with beverage products and equipment from the preparation, cleaning, processing, transportation, and serving stages. In the beverage processing process, the role of food vendors is very important. Many infections are transmitted through food handlers. Good beverage processing practices are those that do not cause damage to the beverage as a result of incorrect processing methods and follow the rules or principles of good hygiene and sanitation or what is called GMP.

According to Prabandari's (2023) research, the process of making and serving herbal medicine (jamu gendong) is highly susceptible to bacterial contamination, primarily due to a lack of attention to the cleanliness of the equipment and ingredients used. The seller's hygiene is also an important factor in preventing bacterial contamination. Personal hygiene is the first step in maintaining the physical hygiene of individuals who are the primary source of disease-causing microbes in food. Implementing good personal hygiene practices and handling food and drinks safely, such as using clean equipment and maintaining cleanliness during serving, can reduce the risk of bacterial contamination if supported by adequate knowledge. Traditional herbal medicine manufacturing methods also pose a risk of bacterial contamination, especially If process

processing like boiling no carried out perfectly so that it has the potential to increase microorganism contamination.

## Conclusion

The conclusion of this study is that from 5 samples of temulawak herbal medicine, sp 1 was at location 1, sp 3 was at location 2, 3 and sp 4 at location 4 no microbial contamination was found and shows that the herbal medicine is fit for consumption, while SP 2 is at location 2 and SP 5 at location 5 is not suitable for consumption because *Coliform* was found and *Salmonella* sp. Factors causing contamination by *Coliform* and *Salmonella* sp. bacteria include inadequate equipment and sales locations.

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