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Detection of *Escherichia coli* Contamination Using *Most Probable Number* (MPN) Methods of Jamu Pahitan in Singaparna District, Tasikmalaya

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Abstract

Indonesians consume a lot of traditional jamu, including jamu pahitan. However, the hygiene aspect in the production and serving process is often neglected, so it has the potential to be contaminated with *Escherichia coli*. Using the MPN method, this study analyzed the presence of *E. coli* in jamu pahitan sold in Singaparna District. A total of 10 samples were tested, taken from jamu vendors who used plastic bottle packaging. The MPN test results showed that all samples contained *E. coli* with MPN values ranging from 6.0-1100 g/mL. None of the samples met food safety standards based on SNI 7388:2009, because the maximum limit of *E. coli* in drinks is <3 MPN/mL. The results of Gram staining of bacteria showed that the bacteria found were bacilli, red in color, including Gram-negative bacteria, indicating the presence of *E. coli* bacteria.

Keywords: *Escherichia coli*; food safety; Gram-negative; Jamu pahitan; MPN method.

INTRODUCTION

Jamu is one of the traditional forms of treatment traditional that has been used in a way passed down from generation to generation by the Indonesian people. Estimated that around 70–80% of people in developing countries still depend on traditional treatment, including jamu, as a main means of guarding health and treating various diseases. In development, jamu has experienced various innovation in formulation, packaging, and methods of its distribution along with increasing public awareness, which will return to nature (*back to nature*) (Biofarmaka, 2024).

One of the types of jamu consumed by the Indonesian people is jamu pahitan, which is known because its very bitter taste. Jamu is generally made from a mixture of jamu ingredients such as leaf sambiloto (*Andrographis paniculata Ness*) and stems (*Tinospora crispa*), two plant drugs that contain bioactive compounds such as andrographolide, saponins, flavonoids, and alkaloids. Leaves sambiloto are known to have antidiabetic, immunostimulant, and antibacterial activity (Paramitha & Rahmanisa, 2016), whereas brotowali is used as an antipyretic, antidiabetic, and detoxification agent (Malik, 2015).

However, behind its popularity, production and sales of jamu pahitan specifically in the form of jamu cradle still face a big challenge from the side of sanitation and safety. Many sellers of jamu cradle still use plastic packaging bottles that are used repeatedly without an adequate sterilization process. This is an open opportunity for the occurrence of contamination microorganisms, especially bacterial pathogenic as *Escherichia coli* (Ruwana *et al*., 2017). *E. coli* is a bacterial indicator of pollution commonly found in the environment that is not hygienic. The existence of *E. coli* in food or drink becomes a marker of existence the possibility of contamination from water, equipment, or contaminated raw materials (Rahayu, 2018).

Some strains of *E. coli* are natural pathogens and can cause digestive disturbance, such as diarrhea, vomiting, or even complications like uremic hemolytic syndrome (Manetu *et al*., 2021). Therefore, the existence of *E. coli* in jamu cannot be tolerated. Indonesian National Standard (SNI) No. 7388 of 2009 stipulates a limit maximum limit of contamination of *E. coli* in the product food liquid of <3 MPN/ mL. If the content exceeds the limit said, then the product is considered unworthy of consumption.

A study previously conducted by Arum *et al*. (2022) showed that of six sample jamu pahitan tested in East Karawang, five of them have an *E. coli* MPN value that exceeds the safe limit. This is associated with the habit of repeatedly using bottles without thoroughly cleaning

them. The surface of the bottle seen blackened and crusted, showing a lack of attention to cleanliness in tool production.

A similar situation was also found in the District of Singaparna, Regency Tasikmalaya. Based on data from the District Health Office Tasikmalaya (2024), recorded as many as 753 cases of diarrhea were recorded in year. Results of field observations show that there are still 10 sellers of jamu 10 sellers. It is known that part of the problem is that they mix jamu pahitan from mixture sambiloto and brotowali, which is then packed in bottles without a standard sterilization process.

To determine the For know existence and quantity of contamination by bacteria *Escherichia coli*, the MPN method was used. This method was chosen because it is quantitative and capable of estimating the population of bacteria based on results of fermentation in liquid media through three stage testing, namely the prediction, confirmation and complementary tests (Hafsan, 2014).

Based on the background behind said, research This done to analyze pollution bacteria *Escherichia coli* in jamu pahitan sold in the District area of Singaparna using the MPN method. Research This expected can give a description of quality microbiological jamu pahitan in the field as well as push effort to improve sanitation and safety in production jamu traditional, in order to protect the health of the public.

MATERIALS AND METHODS

Materials

This study was done from October 2024 to April 2025 in the Laboratory Biology of the University of Struggle Tasikmalaya. Tools used in the research this includes, among other tools glass (Pyrex) such as glass measure, glass chemistry, stem stirrer, tube reaction and erlenmeyer, incubator (Memmert), glass pipette , rack tube , scales, petri dish, tube round, wire ose, bunsen, Durham tube, autoclave (Gea Medical), microscope (Boeco) and LAF (Laminar Air Flow), Vortex (Oregon).

Materials used in the research: This is a jamu pahitan liquid that is sold by traders of jamu carry and use plastic packaging bottles. The chemicals used are LB (*Lactose Broth*) agar media (Himedia), BGLB (*Brilliant Green Lactose Broth*) (Merck), EMB (*Eosin Methylen*e *Blue*) (Oxoid), crystal violet (Merck), cotton (selection), lugol Rofa), paper umbrella (Asturo), safranin (O Merck), immersion oil (Rofa), and 95% alcohol.

Methods

This study used an experimental approach to analyze the presence of *Escherichia coli* bacteria contamination in jamu pahitan sold in the Singaparna District. The samples used were 10 liquid jamu pahitan packaged in plastic bottles from 10 different sellers, each 100 mL, which were then filtered using filter paper to separate the dregs from the liquid. Furthermore, the samples were tested using the *Most Probable Number* (MPN) method which consists of three stages: presumptive test, confirmatory test, and complementary test, and continued with Gram staining to ensure the morphology and classification of bacteria.

In the initial stage, the presumptive test was carried out by inoculating three sample dilutions (0.1 mL, 0.01 mL, and 0.001 mL) into *Lactose Broth* (LB) media containing Durham tubes, then incubated at 37°C for 24–48 hours. The presence of gas in the Durham tube indicates a positive result and is continued to the confirmatory test. The confirmatory test was carried out by transferring 1 loop from the positive tube to *Brilliant Green Lactose Bile Broth* (BGLB) media, then incubating again at 37°C for 24–48 hours. A positive result is indicated by the formation of gas in the Durham tube. The next complementary test uses Eosin *Methylene Blue Agar* (EMBA) media, where a positive result is indicated by the formation of metallic green or pink colonies which are characteristic of *Escherichia coli*.

After the three stages of the MPN test were completed, Gram staining was carried out to ensure that the bacteria found were Gram-negative in the form of bacilli, which is the typical morphology of *E. coli*. Staining was carried out using crystal violet, lugol's solution, 95% alcohol, and safranin, then observed under a microscope at 1000x magnification. Red bacteria indicated that they were Gram-negative. Finally, the MPN value was calculated based on the combination of positive tubes in each dilution by referring to the standard MPN Table, and compared with the microbial contamination standard based on SNI No. 7388 of 2009, where the maximum limit allowed for *E. coli* in drinks is <3 MPN/mL (Saridewi *et al*., 2017).

RESULTS AND DISCUSSION

In this study, samples of jamu pahitan packaged in plastic bottles were collected from ten different jamu sellers in Singaparna District. Each sample was diluted 3 times, each repeated 3 times (triple). Furthermore, the samples were tested using the MPN (*Most Probable Number*) method.

MPN Test Results

Results of the Presumptive Test

The initial stage of the study was the presumptive test, which aimed to determine the presence of *Coliform* bacteria in the jamu pahitan of samples. This test used LB media. *Coliform* bacteria such as *Escherichia coli* ferment sugar (lactose) in LB media. The lactose fermentation process produces acid and gas. The appearance of turbidity and gas produced by the activity of bacteria that convert lactose into lactic acid indicates a positive result of lactose fermentation. The presumptive test results can be seen in Figure 1.

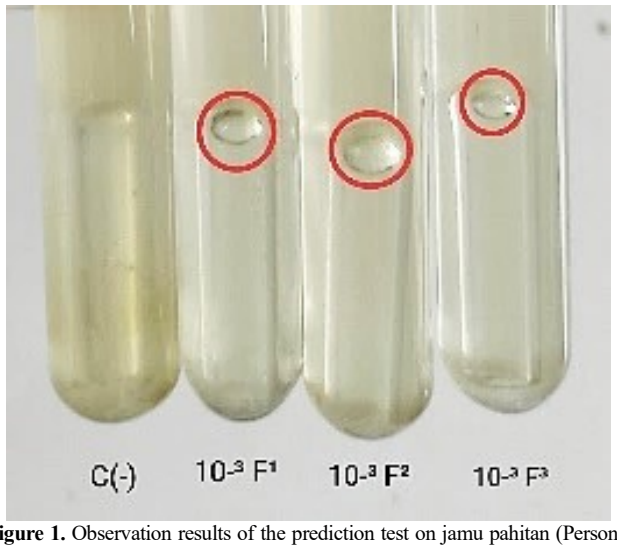

Figure 1. Observation results of the prediction test on jamu pahitan (Personal Documentation, 2025).

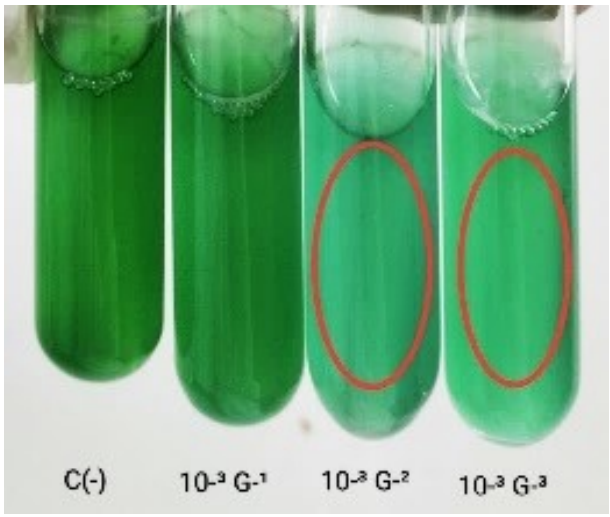

Figure 2. Observation results of the confirmed test on jamu pahitan (Personal Documentation, 2025).

Carbon gas will enter the Durham tube through a tightly closed test tube (Putri & Kurnia, 2018). Turbidity in the lactose medium and the gas produced in the Durham tube indicate the formation of acid. The results of the prediction test on jamu pahitan sold in the Singaparna District area can be seen in Table 1.

Table 1. Results of the presumptive test using LB media

Sample	Dilution Rate			MPN/g Value
	0.1 mL	0.01 mL	0.001 mL	
A	3	2	2	210
B	3	1	3	160
C	3	2	2	210
D	3	2	3	290
E	2	3	3	53
F	3	3	3	>1100
G	2	1	3	34
H	3	3	3	>1100
I	3	3	3	>1100
J	3	3	3	>1100

Note: values 1, 2, and 3 are the number of positive tubes from the sample.

The results of Table 1. show that the positive jamu pahitan samples have MPN values of 34-1100 g/mL for *Coliform* bacteria. Each sample has passed the presumptive test stage, which was continued to the second test, namely the confirmation test because all jamu pahitan samples showed positive results.

Result of the Confirmed Test

The test was confirmed using selective media for *Coliform* bacteria, namely BGLB. The purpose of the confirmation test is to strengthen the previous suspicion test regarding the presence of *Coliform* bacteria in the sample. The results of the confirmation test can be seen in Figure 2.

The results of the confirmation test on jamu pahitan sold in Singaparna District can be seen in Table 2.

Table 2. Results of the confirmed test using BGLB media

Sample	Dilution Rate			MPN/g Value
	0.1 mL	0.01 mL	0.001 mL	
A	2	2	2	35
B	2	1	2	27
C	0	1	1	6.1
D	1	0	1	7.2
E	1	1	0	7.4
F	0	2	0	6.2
G	0	0	2	6.0
H	2	2	0	21
I	2	2	1	28
J	2	1	1	20

Note: values 0, 1, 2 are the number of positive tubes from the sample.

The results of Table 2 show that the jamu pahitan samples have an MPN value of *Escherichia coli* bacteria of 6.0-35 g/mL, exceeding the maximum limit of *Escherichia coli* bacteria contamination in jamu, as specified in SNI No. 7388 of 2009, which is <3 MPN/mL.

Result of the Completed Test

The complete test was conducted using selective media *Eosin Methylene Blue Agar* (EMBA). EMBA is a selective medium for growing Gram-negative bacteria such as *Escherichia coli* and inhibiting the growth of Gram-positive bacteria. The results of the complementary test can be seen in Figure 3.

Figure 3. Observation results of the completed test on jamu pahitan (Personal Documentation, 2025)

The results of the complementary test on jamu pahitan sold in Singaparna District can be seen in Table 3.

Table 3. Results of the completed test using EMB media.

No	Sample Code	Observation Result
1	10^{-1} A $_1$	Pink
2	10^{-1} A $_3$	Pink
3	10^{-2} A $_1$	Metallic green
4	10^{-2} A $_3$	Metallic green
5	10^{-3} A $_1$	Metallic green
6	10^{-3} A $_2$	Metallic green and Pink
7	10^{-1} B $_1$	Metallic green and Pink
8	10^{-1} B $_3$	Pink
9	10^{-2} B $_1$	Pink
10	10^{-3} B $_1$	Metallic green
11	10^{-3} B $_3$	Pink
12	10^{-2} C $_2$	Pink
13	10^{-3} C $_2$	Metallic green and Pink
14	10^{-1} D $_3$	Pink
15	10^{-3} D $_2$	Metallic green and Pink
16	10^{-1} E $_2$	Pink
17	10^{-2} E $_1$	Metallic green
18	10^{-2} F $_1$	Pink
19	10^{-2} F $_2$	Pink
20	10^{-3} G $_2$	Metallic green and Pink
21	10^{-3} G $_3$	Pink
22	10^{-1} H $_1$	Metallic green and Pink
23	10^{-1} H $_2$	Pink
24	10^{-2} H $_1$	Metallic green and Pink
25	10^{-2} H $_2$	Pink
26	10^{-1} I $_1$	Metallic green and Pink
27	10^{-1} I $_2$	Metallic green and Pink
28	10^{-2} I $_1$	Pink
29	10^{-2} I $_2$	Metallic green and Pink
30	10^{-3} I $_1$	Pink
31	10^{-1} J $_1$	Pink
32	10^{-1} J $_2$	Metallic green and Pink
33	10^{-2} J $_3$	Pink
34	10^{-3} J $_2$	Pink

Note: P ink, metallic green color indicates the presence of *Escherichia coli* bacteria. 10^{-1} indicates sample dilution 1, A $_1$ indicates sample A first repeat.

Table 3. shows that all samples tested on EMBA media showed the growth of colonies with metallic green and pink colors. These colors indicate the presence of *Escherichia coli* bacteria which are indicators of fecal *coliform* bacteria.

Bacterial Gram Staining Test Results

Gram Staining Test Results*, In the field of bacteriology, Gram staining of bacteria differentiates groups of bacteria into gram-positive and gram-negative bacteria. The results of the Gram staining test of bacteria can be seen in Figure 4. Bacteria.

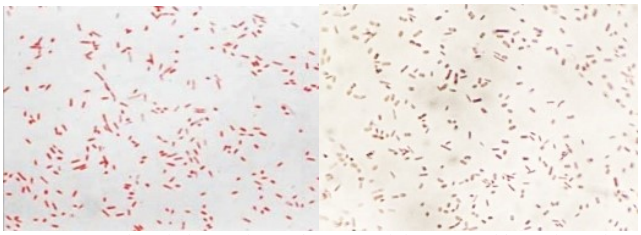

Figure 4. Results of observations of Gram staining tests of bacteria on jamu pahitan (Personal Documentation, 2025).

The samples examined were samples that in previous tests produced metallic green/pink colonies on EMBA media. The results of the Gram staining test on jamu pahitan sold in the Singaparna District area can be seen in Table 4.

Table 4. Results of bacterial Gram staining test.

No.	Sample Code	Results		Note
		Color	Form	
1	10^{-1} A $_1$	Red	Basil	Gram negative
2	10^{-1} A $_2$	Red	Basil	Gram negative
3	10^{-1} A $_3$	Red	Basil	Gram negative
4	10^{-2} A $_1$	Red	Basil	Gram negative
5	10^{-1} B $_1$	Red	Basil	Gram negative
6	10^{-2} B $_3$	Red	Basil	Gram negative
7	10^{-3} B $_1$	Red	Basil	Gram negative
8	10^{-1} C $_1$	Red	Basil	Gram negative
9	10^{-1} C $_2$	Red	Basil	Gram negative
10	10^{-1} C $_3$	Red	Basil	Gram negative
11	10^{-2} C $_1$	Red	Basil	Gram negative
12	10^{-2} C $_3$	Red	Basil	Gram negative
13	10^{-3} C $_3$	Red	Basil	Gram negative
14	10^{-1} D $_1$	Red	Basil	Gram negative
15	10^{-1} D $_2$	Red	Basil	Gram negative
16	10^{-2} D $_1$	Red	Basil	Gram negative
17	10^{-2} D $_3$	Red	Basil	Gram negative
18	10^{-1} E $_2$	Red	Basil	Gram negative
19	10^{-2} E $_1$	Red	Basil	Gram negative
20	10^{-2} E $_2$	Red	Basil	Gram negative
21	10^{-1} F $_1$	Red	Basil	Gram negative
22	10^{-1} F $_2$	Red	Basil	Gram negative
23	10^{-2} F $_1$	Red	Basil	Gram negative
24	10^{-3} F $_3$	Red	Basil	Gram negative
25	10^{-1} G $_1$	Red	Basil	Gram negative
26	10^{-1} G $_3$	Red	Basil	Gram negative
27	10^{-2} G $_2$	Red	Basil	Gram negative
28	10^{-3} G $_2$	Red	Basil	Gram negative
29	10^{-1} H $_1$	Red	Basil	Gram negative
30	10^{-1} H $_2$	Red	Basil	Gram negative
31	10^{-1} I $_1$	Red	Basil	Gram negative
32	10^{-1} J $_2$	Red	Basil	Gram negative

Note: Red indicates Gram-negative bacteria, purple indicates Gram-positive bacteria. 10^{-1} indicates sample dilution 1, A $_1$ indicates sample A first repeat.

Table 4.5 shows that the sample contains Gram-negative bacteria, which is indicated by the red staining test results, this is because Gram-negative bacteria have a thinner wall layer, so that when these bacteria are rinsed, the secondary red dye safranin replaces the purple color of crystal violet. (Ampou *et al*., 2015). In addition, the observed bacillus shape shows that the sample contains *E. coli* bacteria which are included in the group of Gram-negative bacteria in the form of bacillus.

Discussion

The results of the study showed that all samples of jamu pahitan obtained from Singaparna District were indicated to be contaminated by *E. coli* bacteria based on the *Most Probable Number* (MPN) method. This was shown through the results of the prediction test which showed gas formation and turbidity in LB media, the results of the confirmation test with BGLB media which also showed gas formation, and the results of the complementary test on EMBA media which showed metallic green and pink colonies characteristics of *E. coli*. This finding was reinforced by the results of Gram staining which showed that the bacterial isolates were Gram-negative in the form of bacilli, which was in accordance with the morphology of *E. coli* (Rahmatullah *et al*., 2021).

The MPN values obtained from most samples exceeded the threshold set in SNI No. 7388 of 2009, which is <3 MPN/mL, with some samples even reaching >1100 MPN/mL. This indicates that the jamu pahitan sold in the area does not meet food safety standards. This contamination is most likely caused by the lack of adequate sanitation in the production and serving of jamu, such as the repeated use of used plastic bottles without proper sterilization, as well as environmental conditions that allow the product to be contaminated by fecal bacteria (Fhitryani *et al*., 2017).

The high level of *E. coli* contamination in jamu pahitan products is a serious warning considering that this bacteria is an indicator of sanitation and its presence in consumer products indicates fecal contamination. Infection due to *E. coli* can cause digestive tract disorders such as diarrhea, which is potentially dangerous especially for vulnerable groups such as children and the elderly (Manetu *et al*., 2021). Therefore, jamu sellers need to implement good hygiene practices, including sterilization of equipment, selection of clean raw materials, and use of safe packaging containers that are not reused without adequate sanitation.

CONCLUSIONS

Based on the results of the study regarding "Detection Of *Escherichia coli* Contamination Using *Most Probable Number* (MPN) Methods Of Jamu Pahitan In Singaparna District Tasikmalaya", it can be concluded that all samples of jamu pahitan were polluted with *Escherichia coli* bacteria, with MPN values exceeding the maximum limit of <3 MPN/mL according to SNI No. 7388 of 2009. Contamination This is allegedly a consequence of low standard sanitation in the processing and use of packaging that is used without sterilization. Conditions This potentially endangers the health of consumers, so there is a need for education and supervision to ensure the security traditional jamu consumption.

Competing Interests: The authors declare that there are no competing interests.

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