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Analysis of Coliform Contamination and Detection of *Escherichia coli*, *Salmonella* sp. Bacteria in Lettuce Vegetables (*Lactuca Sativa* L.) at Banyuwangi Traditional Market

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ABSTRACT

Lettuce (Lactuca sativa L.) is one of the most popular vegetables. The unchanged nutritional content is the reason for choosing lettuce to consume immediately. Coliform contamination exceeding the maximum human tolerance limit can cause various diseases; therefore, vegetable hygiene should be considered. This study aims to analyze the presence of Coliforms in lettuce from the Banyuwangi traditional market, with and without washing, and to detect E. coli and Salmonella spp. The method used was Most Probable Number (MPN) 5-5-5, and E. coli was detected on selective Eosin Methylene Blue Agar (EMBA) medium. In contrast, Salmonella sp. was detected on Salmonella Shigella Agar (SSA). The result of the Coliform test was obtained. MPN values on lettuce without washing (TC1 and TC2) were>2400 MPN/g, whereas those on lettuce with washing (C1 and C2) were 350 MPN/g and 46 MPN/g, respectively. Based on the results, it can be interpreted that each test sample does not meet the standard MPN value of >3 MPN/g. Detection of E. coli and Salmonella sp. yielded positive results in all test samples.

INTRODUCTION

Vegetables are high-fiber foods that are beneficial for human nutrition. Vegetable consumption is generally processed first. However, some types of vegetables can be eaten raw, a practice commonly known as lalapan. Vegetables are types of vegetables that have a crispy texture and taste that allows them to be eaten raw (Anggreni & Harlita, 2023). Vegetables have a fixed nutritional content that does not disappear when consumed, whereas cooking dissolves nutrients. Some nutrients that are good for the body's health include protein, calcium, and phosphorus (Sari et al., 2019). One raw vegetable people like to use is lettuce.

Lettuce, with the scientific name *Lactuca sativa* L., is a plant widely cultivated by farmers using hydroponic methods. This vegetable has a fairly high market demand. Apart from being a leaf, lettuce leaves are also widely used in salads and as vegetables in Burgers, Sandwiches, and other foods. Lettuce is widely favored as a dish consumed directly because, when cooked, it has a slightly clayey taste (Prameswarie et al., 2019). However, when consuming raw vegetables, one needs to ensure they are clean. Less clean vegetables can lead to microbial contamination, which can cause various diseases. According to Krainara et al. (2023), who examined several samples of fresh vegetables, lettuce has a higher prevalence of Coliforms and *E. coli* than other vegetables. The risk of microbial contamination in raw vegetables is a serious concern because these foods are generally consumed without heat treatment, allowing pathogenic microbes that adhere to their surfaces to persist and enter the human digestive tract (Alreshoodi et al., 2024; Kowalska, 2023).

Foodborne disease is a disease caused by eating food contaminated with pathogenic microbes. Microbes that are often found contaminating fruits and vegetables include bacteria *Escherichia coli* and *Salmonella* sp. Both bacteria are often found in the digestive tract and can cause various diseases. The bacterium *E. coli* can cause gastrointestinal tract infections, diarrhea, and meningitis, while *Salmonella* spp. may cause typhoid (Rudin et al., 2021). Contamination by these microbes is often the main cause of diarrheal diseases from food consumption. Based on the Regulation of the Food and Drug Control Agency, or *Badan Pengawas Obat dan Makanan Republik Indonesia* (BPOM) in 2019, the maximum limit of microbial contamination in fresh vegetables for direct consumption is < 3 MPN/g.

The Banyuwangi traditional market is one of the places where lettuce is sold. The market's cleanliness and hygiene also need to be considered to avoid bacterial contamination. One preventive measure to reduce microbial contamination in vegetables is washing. Washing with running water has been reported to reduce microbial contamination (Anggreni & Harlita, 2023). In a study, Wulandari et al. (2023) examined coliform contamination in lettuce at the Banyuwangi traditional market. However, the study did not test coliform contamination using the MPN method of the 5-5-5 variety. It did not compare lettuce conditions with and without washing as a variable in this study. The washing process is an important indicator of food sanitation quality and is commonly performed by the community before consuming raw vegetables. In addition, according to Blodgett (2023), the use of the MPN method with the 5-5-5 variety in this study has advantages over the 3-3-3 variety, namely, the number of test tubes, so it is expected to detect the presence of coliform bacteria more effectively. Therefore, it is hoped that this study can provide a clearer picture of coliform contamination in lettuce (*Lactuca sativa* L.), with and without washing, using the Most Probable Number (MPN) 5-5-5 variety, and detect bacteria *E. coli* and *Salmonella* using selective media.

RESEARCH METHODS

Study Design

This is descriptive research using a cross-sectional approach. The results obtained are presented in a table and described. The test method used the Most Probable Number (MPN) and bacterial detection using Eosin Methylen Blue Agar (EMBA) selective media for *E. coli* and Salmonella Shigella Agar (SSA) for *Salmonella* sp.

This research was carried out in July - August 2023. Lettuce samples were collected from the Banyuwangi Regional Traditional Market (8°12'37.70"S 114°22'30.99" E), as shown on the location map in Figure 1. Sample examination and bacterial detection were carried out at the Microbiology Laboratory, STIKES Banyuwangi.

Tools and Materials

The tools used in this study are an autoclave, a laminar airflow, an analytical balance, an incubator, a micropipette, a measuring pipette, a Petri dish, a test tube, a tube rack, an Erlenmeyer flask, a beaker, an ose, and a stirring rod. The ingredients used are lettuce (*Lactuca sativa* L.), Lactose Broth (LB), Brilliant Green Lactose Broth (BGLB), aquades, NaCl, Eosin Methylen Blue Agar (EMBA), and Salmonella Shigella Agar (SSA).

Figure 1. Research location

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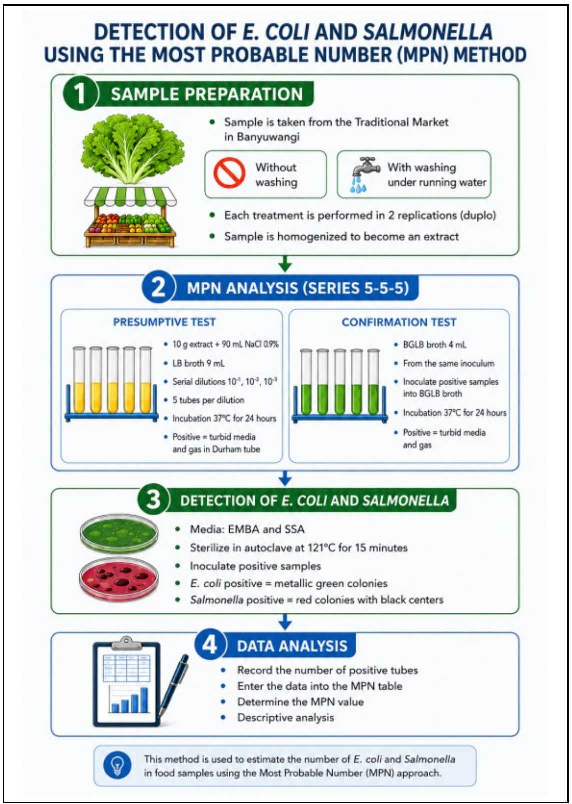

Figure 2. Procedure of research

Procedure

The overall procedure for sample preparation, MPN analysis, detection of *Escherichia coli* and *Salmonella*, and data analysis is summarized in **Figure 2**. The workflow includes sample preparation, presumptive and confirmation tests using the Most Probable Number (MPN) method, bacterial identification on selective media, and calculation of MPN values for descriptive analysis.

Sample Preparation

Lettuce vegetable samples were taken from vegetable traders at the Banyuwangi Traditional Market. The sample was divided into two treatments: without washing and with running water washing. Each treatment was carried out in two repetitions (*duplo*). Lettuce vegetable samples are mashed using a blender. The finely ground vegetable extract is ready for further analysis.

Most Likely Number (MPN) Analysis

The MPN method used in this study is a variant of 5-5-5, consisting of a conjecture test and an affirmation test. A total of 10 g of mashed lettuce extract is added to 90 mL of sterile 0.9% NaCl solution and homogenized. Furthermore, the sample was tested by preparing 9 mL of *Lactose Broth* (LB) in a test tube. Samples were grown on LB medium at dilutions of 10-1, 10-2, and 10-3, each with 5 replicates. Furthermore, the sample was incubated at 37°C for 24 hours. A positive result is characterized by the presence of gas or bubbles in the Durham tube.

The affirmation test began by preparing 4 mL of Brilliant Green Lactose Broth (BGLB) in a test tube. Dilution is made the same as the conjecture test. A positive sample from the presumptive test was taken, 1 ose, and inoculated into dilutions of 10-1 and so on. All samples were incubated in a 37°C temperature incubator for 24 hours. Positive results showed cloudy media and the presence of gas formed in Durham.

Detection of E. coli and Salmonella Bacteria

E. coli and *Salmonella* sp. bacteria are detected by creating a growth medium.

Eosin Methylen Blue Agar (EMBA) and Salmonella Shigella Agar (SSA) media are manufactured and sterilized by autoclaving at 121°C and 1 ATM for 15 minutes. Both media are poured into a petri dish. After the media solidified, the positive samples in the affirmation test were inoculated as much as one ose in the two selective media. Positive *E. coli* growth is characterized by a metallic green color in the bacterial colony, while positive *Salmonella* growth is blackish-pink.

Data Analysis

The resulting data were then analyzed descriptively as the number of positive tubes in each dilution. The data is displayed as tables of predictor and affirmation test results. Next, the MPN value is calculated using the MPN variety table as a reference.

RESULTS AND DISCUSSION

This study was conducted on four lettuce samples, consisting of two without a washing treatment (TC 1; TC 2) and two with a running-water washing treatment. MPN analysis, also known as the Most Likely Number, or *Angka Paling Mungkin* (APM), is carried out in two stages: the guessing test and the affirmation test. According to Ningsih et al (2018), a presumptive test is an initial or preliminary test to detect the presence of Coliform bacteria in the media Lactose Broth (LB), and the affirmation test is a follow-up test to confirm the truth of the existence of Coliform in the media Brilliant Green Lactose Broth (BGLB).

Based on the results of the suspect test presented in Table 1, all Coliform-positive tubes were obtained in TC 1, TC 2, and C2 samples, while in C1, in dilutions 10^2 and 103, one tube was negative. Positive results are characterized by the formation of Gas bubbles in the Durham tube; in negative results, no gas bubbles form. This is also stated by Jufri & Rahman (2022): positive results for Coliform contamination were characterized by the presence of gas bubbles and cloudy media, while negative results were marked by the absence of gas bubbles and a clear medium. The air bubbles in the Durham tube indicate the presence of acids produced by bacterial metabolic activity, which cause the LB medium to become cloudy. According to Sari et al. (2019), the media Lactose Broth (LB) contains lactose, a carbohydrate used by bacteria for fermentation.

Table 1. Results of the predictive test

Sample Code	Positive Tube Count		
	10^{-1}	**10^{-2}**	**10^{-3}**
TC 1	5	5	5
TC 2	5	5	5
C 1	5	4	4
C 2	5	5	5

Table 2. Affirmation test results

Sample Code	Positive Tube Count		
	10^{-1}	**10^{-2}**	**10^{-3}**
TC 1	5	5	5
TC 2	5	5	5
C 1	5	4	4
C 2	5	1	1

The affirmation test was obtained from the positive results of the suspect test inoculated on BGLB media. The results of the confirmation test (Table 2) show that all Coliform positive tubes in each dilution of lettuce samples without washing, namely TC 1 and TC 2. Samples with C1 washing obtained positive results of 4 tubes, namely dilution of 10^{-2} and 10^{-3}, as well as 1 negative tube on C2 with the same dilution. Positive results are indicated by gas formation in the Durham tube and a turbid BGLB medium.

Jufri & Rahman (2022) stated that the BGLB medium contains lactose and bile salts for the optimal growth of Coliform bacteria; the bile salt content inhibits Gram-positive bacteria, allowing only Gram-negative bacteria to grow on the medium. Coliform bacteria can ferment lactose in this medium into fumaric acid, succinic acid, oxygen (O_2), and carbon dioxide (CO_2). The presence of this gas indicates the presence of Coliform bacteria in the sample.

Based on MPN calculations, lettuce (*Lactuca sativa* L.) purchased from vegetable traders at the Banyuwangi Traditional Market tested positive for Coliform in both treatment groups, namely without washing and with washing. Lettuce vegetables without washing, namely TC 1 and TC 2, showed Coliform bacteria counts>2400 MPN/g, while lettuce vegetables with running-water washing, C 1 and C 2, were 350 MPN/g and 46 MPN/g, respectively (Table 3).

Similar research by Wulandari et al. (2023) eported coliform contamination in unwashed lettuce sold at a traditional market in Banyuwangi, with an MPN index exceeding 1,100. Consistent with these findings, unwashed lettuce in the present study was confirmed to contain Coliform bacteria. In contrast, lettuce washed under running water exhibited lower MPN values.

However, neither treatment group met the requirements set by the Food and Drug Supervisory Agency (BPOM, 2019) for the maximum microbial contamination limit in fresh vegetables, which is < 3 MPN/g. It is also regulated by the Indonesian National Standard (SNI 7388:2009), which sets the safety standard for fresh vegetables for direct consumption, with *E. coli* < 3 MPN/g. Similar research by Brandão et al. (2014) also reported MPN test results for fresh lettuce from supermarkets that exceeded the specified safety standard of 22 MPN/g.

Table 3. Results of MPN analysis of coliform bacteria in lettuce vegetables at Banyuwangi traditional market

Sample Code	Number of Coliform Bacteria (MPN/g)	BPOM Standard (MPN/g)	Remarks
TC 1	>2400	< 3	TMS
TC 2	>2400	< 3	TMS
C 1	350	< 3	TMS
C 2	46	< 3	TMS

Description: TC (Lettuce Without Washing); c (lettuce with washing); TMS (Not Qualified)

Various factors can contribute to the high MPN values in lettuce samples. According to Ramadhani (2017), the environmental conditions of vegetables in Traditional Markets can affect vegetable quality, sanitation, and the condition of the lettuce storage area. It is known that lettuce samples from the Banyuwangi Traditional Market are placed in a row alongside other vegetables, thereby increasing the risk of contamination. Storage of lettuce is recommended in a room at a low temperature of 0-7°C to inhibit respiration, contamination, and transpiration and maintain lettuce quality.

In addition, the washing technique and the water used can affect lettuce quality. It can be seen in samples without washing, and with washing, there is a difference in the number of coliform microbes. However, all samples are still below the specified standards. Samples C 1 and C 2 are suspected to have been washed in running water that is neither clean nor thorough, so the water does not completely remove coliform bacteria that have contaminated the vegetables. In addition, the tap water used may still contain coliform bacteria. It is also mentioned in the study by Sari et al. (2019) that the presence of *E. coli* can come from water that is used in less hygienic conditions and is raw. Cooking water first can more effectively minimize bacterial contamination. Anggreni & Harlita (2023) reported that the washing technique with running water and saltwater immersion reduced bacterial contamination in basil, cabbage, and lettuce.

Similar findings were also reported in the study by Widinugroho & Asri (2022). That is, treating lettuce with fermented bacteria can reduce *Coliform* and *E. coli* levels, but the results still exceed the safe limits for fresh vegetables. Research by Alreshoodi et al. (2024) also shows that the washing process reduces E. coli contamination in raw vegetables, including lettuce. The characteristics of the vegetable surface also influence the contamination, as lettuce has folded, slit leaves, making bacteria easier to pat and harder to remove. Therefore, the results of this study are consistent with the finding that washing with running water can reduce coliform bacteria counts. However, it is not entirely effective at removing this microbial contamination, especially if it originates from soil, water, tools, traders' hands, or unhygienic storage practices.

Table 4. Results of detection of *E. coli* and *Salmonella* sp bacteria in selective media

Sample Code	*E. coli*	*Salmonella* sp.
TC 1	+	+
TC 2	+	+
C 1	+	+
C 2	+	+

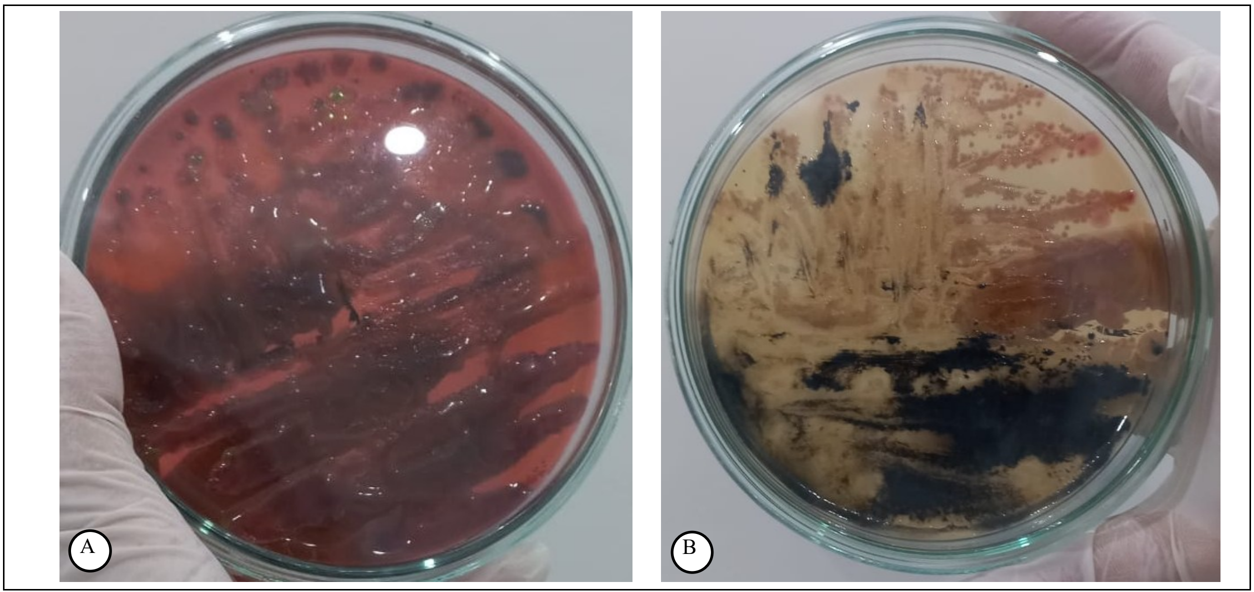

Figure 3. Growth bacteria. A. *E.coli* in EMBA, and B. *Salmonella* sp. in SSA

Detection of *E. coli* and *Salmonella sp.* on the selective media EMBA and SSA was positive in all test samples (Table 4). Positive results for *E. coli* in EMBA media, characterized by the presence of bacterial growth in metallic green media (Figure 3). According to Jamilatun & Aminah (2016), the color change is due to *E. coli* fermenting lactose, which increases acid levels in the medium; increased acid can precipitate methylene blue, giving the bacteria a metallic green appearance. EMBA media is a differential media or selective media that contains lactose, eosin, and methylene blue. Eosin and methylene blue can inhibit the growth of Gram-positive bacteria, and only Gram-negative bacteria can grow in media containing lactose fermentation. Characteristics of colonies *E. coli* in the media, so that according to Fatiqin et al (2019), i.e., spherical, convex, and smooth edges, while the cell *E. coli* is bacilli-shaped, Gram-negative, and is facultative anaerobic and does not form spores. In a study, Olianovi & R. Pasaribu (2017) found that *E. coli* (100%) was also present in lettuce samples at the Grogol District Regional Market. According to Wibowo et al. (2017), there is bacterial contamination *by E. coli* on fresh lettuce around campus A of Universitas Airlangga at 57%.

Positive results *Salmonella sp.* characterized by the growth of red bacteria accompanied by black spots in the middle of the SSA media (Figure 3). Detection results *for Salmonella sp.* from lettuce vegetables in traditional markets in Ramadhani's (2017) study also tested positive. According to him, the contamination can come from spraying contaminated water with *Salmonella sp.* or from fertilizing with animal manure.

In addition, contamination can also come from the processes of production, harvesting, packing, processing, distribution, and marketing. Fatiqin et al. (2019) state that *Salmonella* is a motile, Gram-negative bacterium that does not form spores. According to Sari et al (2019), sporic or non-spore bacteria, viruses, and parasites are microorganisms that can contaminate fresh vegetable and fruit products. The results of the case study showed that the majority of contamination came from non-spore bacteria, accounting for up to 55%, including *E. coli* and *Salmonella.*

Based on research by Raja et al. (2024), EMBA and SSA media were also used to detect microbial contamination in vegetables in the Medan traditional market, where 5 samples showed E. coli contamination on temporary EMBA media, and *Salmonella* sp. was found in 1 sample on SSA media. These two bacteria are often used as indicators of food safety. Rudin et al. (2021) mention that the bacteria *E. coli* and *Salmonella* spp. are indicators of fecal coliform contamination, which is a disease-causing condition related to food sanitation, such as diarrhea, vomiting, typhoid, and other gastrointestinal infections. According to Sandita et al. (2025), *Salmonella*, a pathogenic bacterium, is known to contaminate foodstuffs.

This research needs to be continued for several reasons, such as the limited sample size and the washing method, which uses only running water. Therefore, it is unknown whether other washing methods are more effective at reducing microbial contamination. Detection *of E. coli* and *Salmonella* sp. requires further identification for greater specificity. In addition, other environmental factors have not been analyzed either. Further research may add a larger sample count, a variety of washing methods, and more specific methods of bacterial identification.

CONCLUSION

Lettuce vegetable samples with and without washing did not meet BPOM requirements, namely exceeding the maximum limit of microbial contamination in fresh vegetables < 3 MPN/g, with the MPN value of lettuce vegetables without washing (TC 1 and TC 2) >2400 MPN/g, respectively, while lettuce vegetables with washing (C 1 and C 2) were 350 MPN/g and 46 MPN/g—detection of the presence of *E. coli and Salmonella sp.* Positive results were obtained in all samples tested. The washing process can reduce the number of coliform bacteria. However, the results show that the washing process is not optimal, so coliform bacteria are still found that exceed the safe limit. Consumption of raw lettuce requires a more hygienic, optimal washing method.

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