MICROBIAL SAFETY OF STREET FOOD SAUCES : DETECTION OF ESCHERICHIA COLI AMONG VENDORS IN KLATEN REGENCY

Liss Dyah Dewi Arini[1*], Fredericus Pramonodjati[2], Azizah Zahra Nur Fitria[3]
Duta Bangsa University[1], Duta Bangsa University[2], Duta Bangsa University[3]
*Correspondence Email : liss_dyah@udb.ac.id

ABSTRACT

Egg rolls are one type of food that requires additional ingredients in the form of sauce. Many egg sellers use bicycles or two-wheeled vehicles, with merchandise placed behind the bicycle or motorbike. However, the cleanliness of egg rolls is often ignored, one of the causes is exposure to dust and air that enters the food. This study aims to test for contamination of Eschericia coli bacteria and identify the presence of these bacteria in the sauce used in egg rolls. The method used in this study was a field survey with random and descriptive sampling techniques to determine the presence or absence of Eschericia coli in several types of sauces sold with egg rolls. Sampling was carried out by selecting 10 types of sauces from egg roll food sold around in Klaten Regency. Tests for Eschericia coli bacteria were carried out at the Biology Laboratory of Sebelas Maret University, Surakarta, and one sample as a negative control using the most probable number (MPN) method, Escherichia coli test and gram staining. The results of the Most Probabe Number test and confirmation test showed that there was Eschericia coli, while from the results of gram staining, the detected bacteria were included in the gram-negative category. The results of the biochemical test showed that the bacteria in the sample included the Enterobacter and Escherichia coli species. The tests carried out showed that all results did not meet the standards set in SNI 01-3546-2004, which regulates the maximum limit of the Total Plate Count (TPC) in tomato sauce is 2 x 10^2 colonies/g, while according to the Regulation of the Head of the Food and Drug Supervisory Agency of the Republic of Indonesia number HK. 00. 06. 1. 52. 401, the maximum limit of the Most Probable Number (MPN) Coliform in tomato sauce is 100 colonies/g.

KEYWORDS

processed egg roll sauce; Most Probable Number; Biochemical Test; Gram Staining; Eschericia coli

INTRODUCTION

Food consists of ingredients consumed daily to meet the needs of maintenance, growth, activity, and replacement of damaged body cells (Launde, et al, 2020; Gisslevik, et al, 2025). However, food can also be a source of health problems for humans because it can be contaminated by physical, chemical, or microbiological substances. Food plays an important role in supporting body processes during growth or development, replacing damaged body tissue, providing energy for daily activities, and regulating metabolism and maintaining the balance of water, minerals and other body fluids. In addition, it also functions in the body's defense mechanism against disease (Kalangi, Sanggelorang and Malonda, 2024; Carlsen, et al, 2025). However, food is often contaminated by chemicals and biological organisms (Budiarti and Utami, 2021; Duan, et al, 2025).

One type of biological contaminant commonly found in food is bacteria from the *Coliform* group such as Escherichia coli (Amelia and Fayasari, 2020; Pakdel, Olsen and Bar, 2023). Escherichia coli comes from human and animal feces, which can contaminate food due to unclean food handlers, improper washing of equipment, the health of food processors and handlers, and the use of washing water contaminated by *coliform, E. Coli*, and faecal *Coliform* (Pasek, Budiman and Rismawati, 2020; Gupta, et al, 2022). Contamination of harmful bacteria in food and drinks can cause various diseases such as typhus, diarrhea, food poisoning, and others. These diseases are more susceptible to attacking individuals with weak immune systems, both due to internal and external factors. Therefore, in order to ensure the health and safety of consumers, routine microbiological laboratory examinations are needed (Amqam, et al, 2021).

Sauces that should be rich in nutrients and vitamins from ingredients such as tomatoes, chilies, and herbs and spices, must be processed in a healthy and correct way without adding harmful substances. However, sauces that are sold freely, both labeled and unlabeled, at various prices, sometimes contain harmful substances. The processing process is often very unhygienic, including the use of rotten papaya that has worms, rotting cassava skin, and the addition of preservatives, excessive MSG, textile dyes, borax, and formalin. In addition, sauces contaminated with microbes such as Coliform, S.aureus and fungi can endanger the health of people who consume them (Puspitasari, Noerhartati and Mujianto, 2024).

Egg rolls are a type of food that utilizes additional seasoning in the form of sauce. Many egg roll sellers use bicycles or motorbikes that load goods on the back. Often, the cleanliness of egg rolls sold around is not well considered, one of the causes is the large amount of air and dust that enters the food. This study aims to test for the presence of Escherichia coli bacteria and identify the content of these bacteria found in the sauce for egg roll food. The method used in this study was a field survey with random and descriptive sampling to determine the presence or absence of Escherichia coli bacteria in several sauce variants served with egg roll food (H, Ho, L., and S, Nurul, 2024).

RESEARCH METHOD

The Total Plate Count (TPC) Testing experiment was carried out in three repetitions and involved a petri dish containing media and a diluent solution without a sample as a control (blank). After the media hardened, the petri dish was incubated at

37°C for 24 hours in an inverted position. The colonies that grew on each petri dish were counted. The total number of bacteria in 1 gram of sample was calculated by multiplying the average number of colonies on the petri dish by the dilution factor used (Karlah, 2014; Arini and Wulandari, 2017; Susanti, et al, 2022). Identification of Escherichia coli was carried out by taking one loop from each positive culture in the Coliform bacteria confirmation test, then inoculating it into Eosin Methylene Blue Agar (EMBA) media and incubating it at 37°C for 24 hours. Colonies that were green with a metallic sheen and blue-green spots were selected from the EMBA media and smeared onto the Nutrient Agar (NA) slant media. After incubation at 37°C for 24 hours, a biochemical test was carried out to identify Escherichia coli through the IMViC test (Indol, Methyl Red, Voges-Proskauer, and Citrate) (Sugiah, et al, 2023).

Gram staining for bacteria was carried out using NA slant media culture in the Escherichia coli test and incubated for 24 hours at 37°C. Aseptically, one sample loop was taken and placed on each slide, then the sample was heated over a Bunsen flame until fixed. A drop of crystal violet was added to the slide and left for 30 seconds. Furthermore, the slide was rinsed with distilled water. Biochemical tests were carried out using KIA, SIM, LIA and Citrate media (Li, et al, 2021). The data obtained were analyzed through the Most Probable Number Test and the Presumptive Test, where positive results were indicated by the presence of bacterial cultures in test tubes in Durham tubes that had an empty space filled with air with a length of at least one third of the length of the Durham tube. In the confirmation test, positive results were also seen with bacterial cultures in test tubes in Durham tubes that had an empty space filled with air that was at least one third the length of the Durham tube (Aulya, Fadhliani, Mardina, 2022).

For the Escherichia coli test, positive results appear with metallic green bacterial cultures on EMBA media. Positive results in Gram staining can be seen from observations of preparations under a microscope with safranin and crystal violet staining, where the bacteria appear red (gram-negative bacteria). In the Biochemical test, positive Escherichia coli results are indicated through the KIA test with the appearance of a red color (indicating positive Indole) in the bacterial culture, and positive Coliform if there is no sulfide (no black color in the bacterial culture). Meanwhile, in the SIM test, there is at least motility or movement of the bacteria. Identification of bacteria is carried out through the biochemical tests KIA, SIM, LIA, and Citrate. The media that determines bacterial identification are mainly in the KIA and SIM tests, while the LIA and Citrate tests support the results of the KIA and SIM tests (Curticăpean, 2022).

RESULT AND DISCUSSION

The results of the study are presented in table 1 (MPN *Coliform* Results of the Presumptive Test on Egg Roll Sauce), table 2 (MPN *Coliform* Results of the Confirmatory Test on Egg Roll Sauce), table 3 (*Coliform* Identification Results) and table 4 (Biochemical Test Results on KIA, SIM, LIA and Citrate Tests)

Table 1. Results of MPN *Coliform* Presumptive Test on Egg Roll Sauce

Sample	*Coliform* (colony/ gr)	*Coliform* (10^3 colony/ gr)	Standart of *Coliform* (10^3 colony/ gr)	Information
1	36	36000	1000	TMS
2	37	37000	1000	TMS
3	37	37000	1000	TMS
4	36	36000	1000	TMS
5	36	36000	1000	TMS

6	36	36000	1000	TMS
7	37	37000	1000	TMS
8	37	37000	1000	TMS
9	36	36000	1000	TMS
10	37	37000	1000	TMS

Source: Regulation of the Food and Drug Supervisory Agency Number 13 of 2019 (Regulation of the Food and Drug Supervisory Agency Number 13 of 2019)

Information :
TMS : Not Eligible

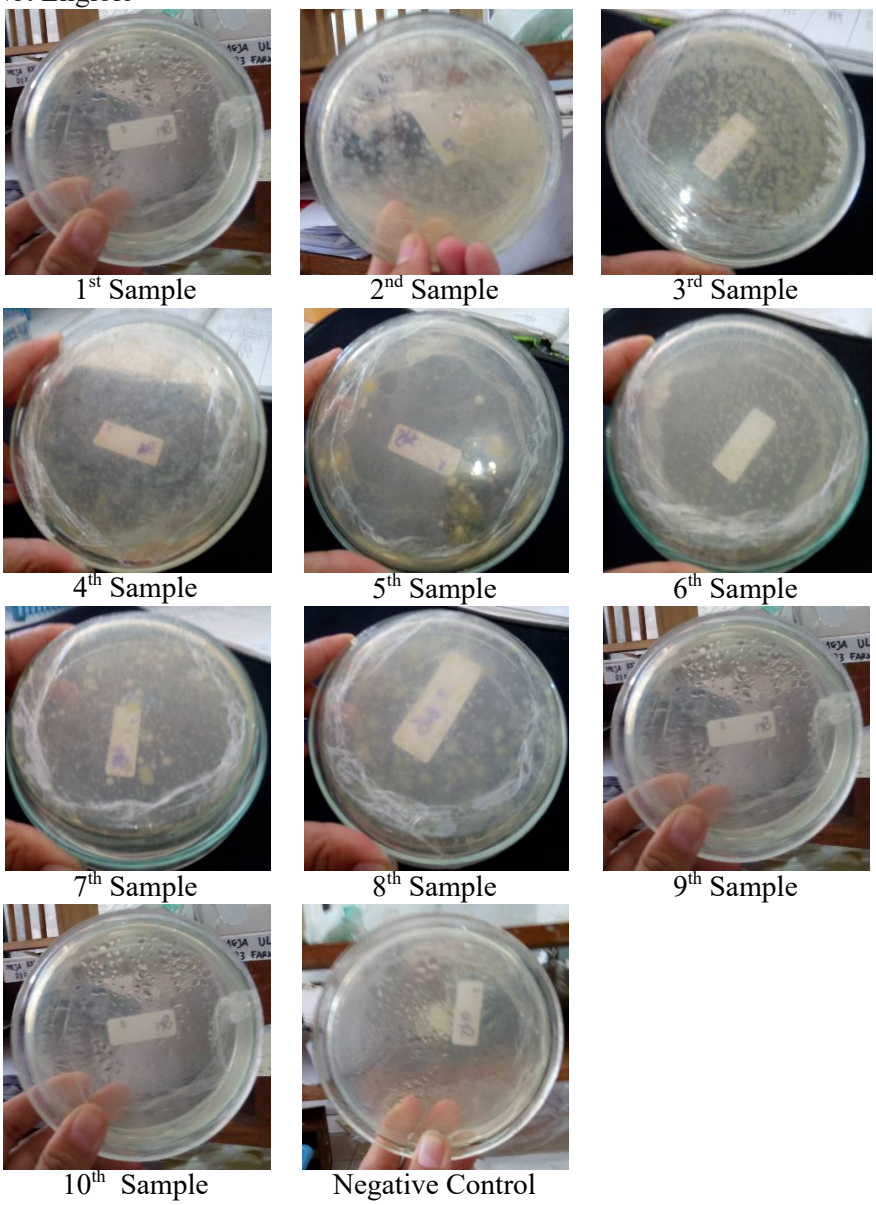

Figure 1. Total Plate Count Test Results for Siomai Sauce

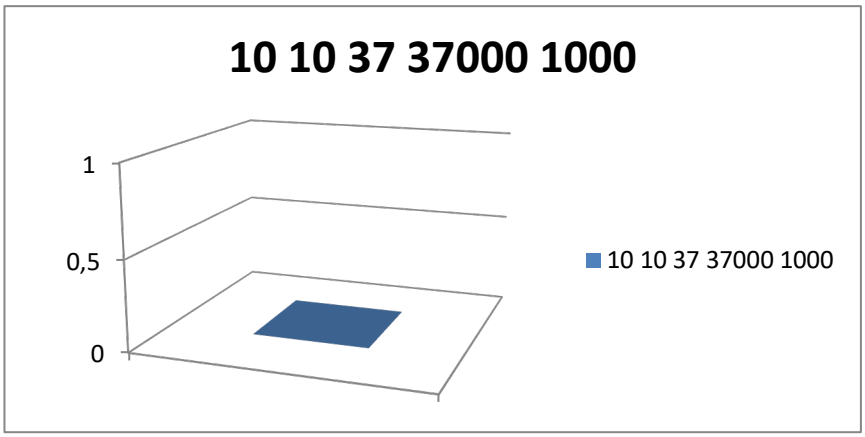

Figure 2. Results of MPN *Coliform* Presumptive Test on Egg Roll Sauce

Table 2. Results of MPN *Coliform* Confirmation Test on Rolled Eggs

Sample	*Coliform* (colony/ gr)	*Coliform* (10^3 colony/ gr)gr)	Standar *Coliform* (10^3 colony/ gr)	Information
1	>11000	>110000	1000	TMS
2	>11000	>110000	1000	TMS
3	9,4	9400	1000	TMS
4	>11000	>110000	1000	TMS
5	>11000	>110000	1000	TMS
6	>11000	>110000	1000	TMS
7	>11000	>110000	1000	TMS
8	>11000	>110000	1000	TMS
9	>11000	>110000	1000	TMS
10	>11000	>110000	1000	TMS

Source: Regulation of the Food and Drug Supervisory Agency Number 13 of 2019
(Regulation of the Food and Drug Supervisory Agency Number 13 of 2019)

Information :
TMS : Not Eligible

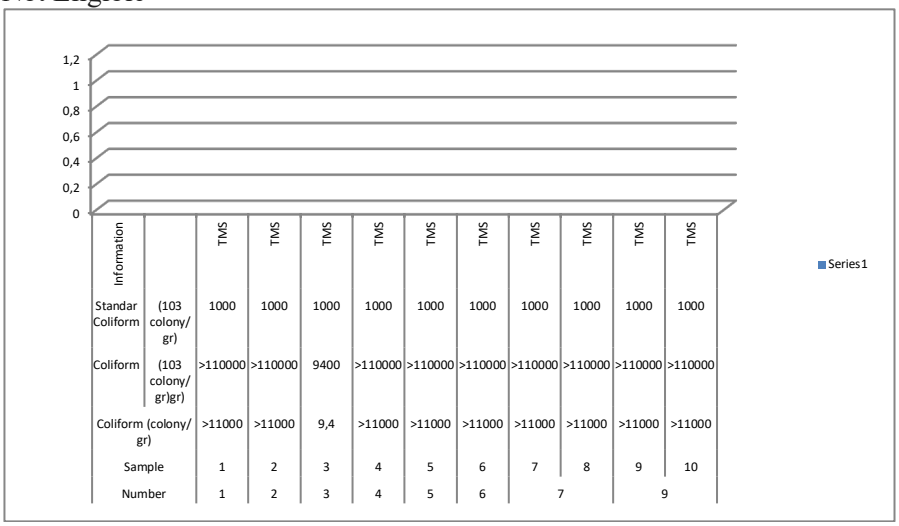

Figure 3. Results of MPN *Coliform* Confirmation Test on Rolled Eggs

Tabel 3. *Coliform* Identification Results

Sample	Cultural Color	Information
1	Blackish blue, metllic green flash	+
2	Blackish blue, metllic green flash	+
3	Blackish blue, metllic green flash	+
4	Blackish blue, metllic green flash	+
5	Blackish blue, metllic green flash	+
6	Blackish blue, metllic green flash	+
7	Blackish blue, metllic green flash	+
8	Blackish blue, metllic green flash	+
9	Blackish blue, metllic green flash	+
10	Blackish blue, metllic green flash	+

Keterangan : + : Positif *Coliform*

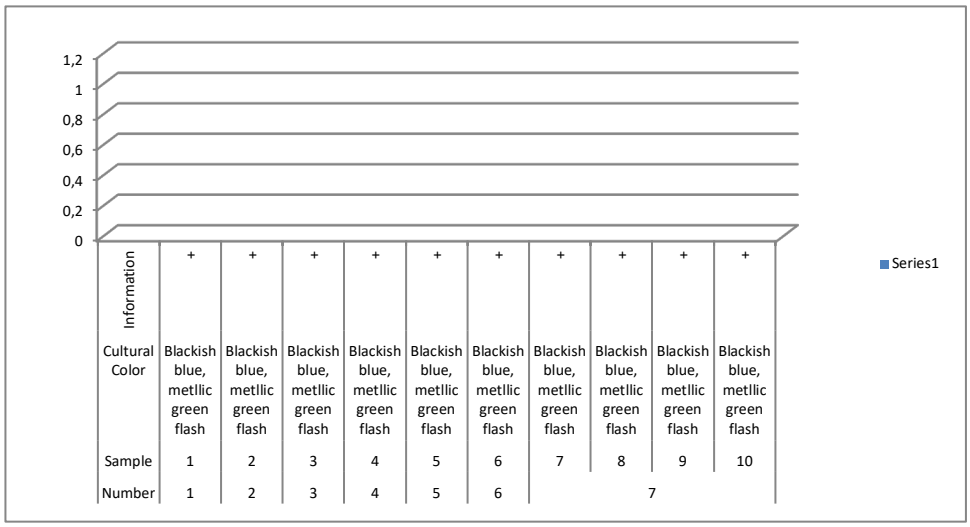

Figure 4. Coliform Identification Results

Table 4. Biochemical Test Results for KIA, SIM, LIA and Citrate Tests

Sample	KIA	SIM	LIA	Citrate	Information
1	A/AG S-	- - +	A/AG S-	+	*Enterobacteria*
2	A/AG S-	- + +	K/AG S-	+	*Enterobacteria*
3	A/AG S-	- + +	K/AG S-	+	*Escherichia coli*
4	A/AG S-	- -++	K/AG S-	+	*Enterobacteria*
5	A/AG S-	- + +	K/AG S-	+	*Enterobacteria*
6	A/AG S-	- - +	K/A S-	+	*Enterobacteria*
7	A/AG S-	- - +	K/A S-	+	*Enterobacteria*
8	A/AG S-	- - +	K/A S-	+	*Enterobacteria*
9	A/AG S-	- - +	K/A S-	+	*Enterobacteria*
10	A/AG S-	- - +	K/A S-	+	*Enterobacteria*

Information:
A/AG S- : above acid, below acid, gas present, no sulfide
- - + : sulfide absent, indole absent, motility present
- + + : sulfide absent, indole positive, motility present
- - - : sulfide, indole and motility are absent
A/AG S- : above acid, below acid, gas present, no sulfide
K/AG S- : above base, below base, gas present, no sulfide
K/A S- : top base, bottom acid, no sulfide
+ : positif of *Coliform*

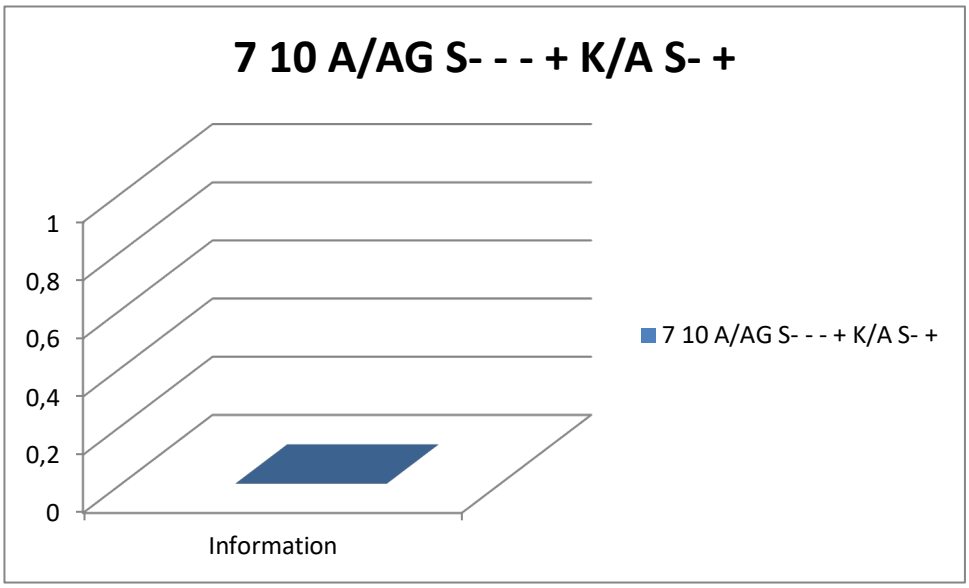

Figure 5. Biochemical Test Results for KIA, SIM, LIA and Citrate Tests

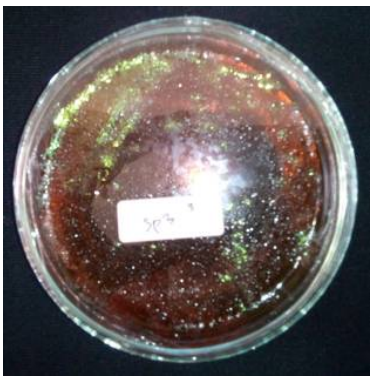

Figure 6. *Coliform* Identification Results

The media used to detect the presence of *Coliform* bacteria (Gram-negative bacteria) is based on the production of acid and gas that occurs due to lactose fermentation by bacteria from the coli group. Acid production can be seen through the turbidity in the lactose media, while the gas produced can be observed in the Durham tube in the form of air bubbles. The tube is considered positive for *Coliform* if there is gas formed as much as 10% or more of the total volume in the Durham tube. (Hunowu, Nurfadillah, and Lalu, 2023). The confirmation test uses a selective medium called Brilliant Green Lactose Bile 2% (BGLB). In addition to containing lactose, BGLB also

contains Brilliant Green which can inhibit the growth of Gram-positive bacteria. Based on Tables 1 and 2 above, it can be seen that the seven types of egg roll sauce tested contained *Coliform* bacteria in amounts that did not meet health standards based on the Regulation of the Food and Drug Supervisory Agency Number 13 of 2019, namely a maximum of (103 colonies/gr) (Regulation of the Food and Drug Supervisory Agency Number 13 of 2019). The presence of *Coliform* bacteria in food and drinks indicates the possibility of enteropathogenic and/or toxigenic microbes that can be harmful to health (Amaliyah, 2017; Isnawaida, et al, 2021).

EMBA is a solid medium used to identify the type of coli bacteria by giving positive results in a tube. EMBA media using eosin and methylene blue as indicators shows a striking difference between colonies that can ferment lactose and those that cannot. To calculate the number of E. coli bacteria, the Hopkins table is usually used, better known as the MPN (Most Probable Number) or the JPT (Number of Nearest Estimates) table; this table can help estimate the number of E. coli in 100 ml and 0.1 ml of water (Fatimah, et al, 2022; Lestari, Haliza and Setiawan, 2024). *Coliform* bacteria are seen as indicators of the presence of other pathogenic bacteria. More specifically, *Coliform* bacteria function as indicators of contamination by pathogenic bacteria. The determination of *Coliform* as an indicator of pollution because the number of colonies is positively related to the presence of pathogenic bacteria. In addition, detecting *Coliform* is cheaper, faster, and easier than searching for other pathogenic bacteria (Riyanto and Abdillah, 2012). Examples of *Coliform* bacteria include Escherichia coli and *Enterobacter aerogenes*. *Coliform* bacteria produce ethionine which has been shown in studies to cause cancer. These decomposing bacteria also produce various toxins such as indole and skatole which can cause disease if they accumulate too much in the body. The presence of *Coliform* bacteria in food indicates the possibility of microbes with enteropathogenic and toxigenic properties that are harmful to health (Sabaaturohma, Gelgel and Suada, 2020; Syamsussabri,, 2018).

Some diseases that often occur due to Escherichia coli bacteria are diarrhea, which is very common throughout the world. These bacteria are classified based on their virulence characteristics, and each group causes disease with different mechanisms, as explained. Symptoms that appear include diarrhea, which is a loose bowel movement with a frequency of 4 or more times a day, sometimes accompanied by vomiting, fatigue, fever, loss of appetite, and even blood and mucus in the stool. Diarrhea can cause loss of fluids and electrolytes, so that the baby becomes fussy or experiences heart rhythm disturbances and can cause brain hemorrhage (Wardani and Setianingrum, 2019; Manetu, M'masi and Recha, 2021). Urinary tract infections are the most common cause of urinary tract infections and occur in about 90% of young women. Symptoms include high frequency of urination, pain when urinating, blood in the urine, and pus in the urine. The majority of these infections are caused by Escherichia coli bacteria with various types of O antigens. If the mother's immune system is weak, Escherichia coli can enter the bloodstream and cause sepsis. For meningitis, Escherichia coli is one of the main causes in infants. Escherichia coli bacteria associated with meningitis cases have the KI antigen, but the virulence mechanism associated with the KI antigen is still not understood (Nasution, 2020; Lawati, Blair and Larnard, 2024).

The findings of this study indicate that the government and local governments, together with related institutions, still need to improve supervision of food quality, especially tomato sauce which is widely consumed by the public, based on article 68 of Law No. 18 of 2012 concerning Food. Supervision of foods such as tomato sauce aims to protect consumers from the possibility of food that does not meet standards and health requirements that can have a negative impact on health. This study is in line with

previous results (Sihaloho, 2025; Perez, et al, 2024), although samples were taken from a traditional market in Sleman, the tomato sauce brands tested were those circulating in Yogyakarta. These findings can provide information regarding bacterial contamination in tomato sauce.

The Role of the Environment in E. coli Contamination in Egg Roll Sauce from Street Vendors is 1. Cleanliness of the Water Used, for example street vendors often use water from unhygienic sources (unboiled tap water, open reservoir water, or used washing water) to wash equipment or dilute sauces and if the water is contaminated with E. coli from waste or dirt, then bacteria can enter the sauce; 2. Storage of Sauce in Open Containers, for example sauce is often stored in open containers or bottles without tight lids, which allows dust, flies, or other environmental particles that carry bacteria from dirt or open drains to enter; 3. Cross Contamination from the Environment, for example serving tables, rags, or unclean hands of traders can touch bottles or spoons of sauce, causing cross contamination from bacteria in the environment into the sauce; 4. Less Hygienic Trading Locations, for example traders often sell on the side of the road, near gutters, or dusty areas. Bacteria from the air, wild animals, and standing water can get into food or sauces and flies that land on waste and then on sauces can carry E. Coli; 5. No cooling or reheating, an example of which is that sauces are often left at room temperature all day, which is the ideal temperature for the growth of E. coli, especially if the sauce contains animal ingredients (e.g. eggs, milk or meat) as a flavor mixture; 6. Personal Hygiene of Traders, for example traders who do not wash their hands after touching money, sweating, or going to the toilet can transfer E. coli bacteria directly to spoons, sauces, or food.

The environmental influence on bacterial contamination in egg roll sauce at street vendors can be caused by unclean water used to wash equipment or dilute the sauce; dust and flies entering the sauce when the container is open in an open area; dirty tables or rags that cause cross contamination when touching the sauce bottle or spoon; the location of the sale is near a drain, trash can, or busy road; room temperature that can increase the risk of bacterial growth in the sauce and dirty hands of traders can be a direct medium for transmitting bacteria to the sauce/food

CONCLUSION

Samples 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 showed the presence of Coliform bacteria, with the number of MPN Coliform not meeting all requirements, which is more than 1100 APM/g. In addition, samples 1, 2, 3, 5, 6, 7, 8, 9 and 10 also showed the presence of Enterobacter bacteria, while sample 4 contained Escherichia coli bacteria. Based on the results of gram staining, all bacteria found in samples 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 are included in the gram-negative bacteria group. The results of all ALT and MPN Coliform tests showed that all of them did not meet the standards set out in the Regulation of the Food and Drug Supervisory Agency Number 13 of 2019, where the maximum limit of MPN Coliform for tomato sauce is 103 colonies/g. Based on these findings, researchers recommend that further studies conduct more detailed analyses of the presence of Escherichia coli bacteria in food to obtain more valid results.

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