



## Occurrences *Salmonella* sp. and *Escherichia Coli* in Bulk and Packaged Chicken Sausages in Surabaya, Indonesia

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### A B S T R A C T

*Salmonella* sp. and *Escherichia coli* are pathogenic bacteria that cause foodborne diseases that often contaminate food and are harmful to human health. Sales of unpackaged (bulk) sausages accompanied by poor hygiene and environmental sanitation conditions have a high potential for *Salmonella* and *Escherichia coli* contamination. The purpose of this study was to compare the contamination level of bacterial, *Salmonella* sp. and *Escherichia coli* in bulk and packaged chicken sausages as well as to determine the relationship between the hygiene and sanitation of traders and the level of bacterial contamination, *Salmonella* sp. and *Escherichia coli* contamination in chicken sausages sold at traditional markets in Surabaya, Indonesia. This research is a cross-sectional study and purposive sampling method. The results showed that the level of bacterial contamination in the bulk chicken sausage was higher (5.98 Log CFU/g) than in packaged chicken sausage (4.83 Log CFU/g). *Salmonella* sp. contamination in the bulk chicken sausage was higher (44.44%) than in packaged chicken sausage (10.00%) and *Escherichia coli* contamination in the bulk chicken sausage was higher (22.22%) than in packaged chicken sausage (20.00%). There is a significant relationship between the hygiene and sanitation of traders with the contamination level of bacteria, *Salmonella* sp., and *Escherichia coli* in bulk and packaged chicken sausages.

## 1. INTRODUCTION

### 1.1. Research Background

Sausage is a processed food product made from a mixture of fine meat and flour with the addition of spices or food additives. Sausage products are very easily contaminated or overgrown with bacteria from the surrounding environment due to inadequate storage facilities. Sausages sold in traditional markets are not stored in the refrigerator, there are even sausages that are not packaged properly (bulk) which have a high risk of bacterial contamination.

The sale of unpackaged processed food (bulk) accompanied by poor hygiene and environmental sanitation conditions has a high potential for contamination by pathogenic bacteria. Unpackaged food has a higher risk of bacterial contamination either from the equipment or from the environment around the sale. Poor sanitation conditions and high humidity in traditional markets pose a risk of direct and indirect contamination of processed food.

*Salmonella* sp. and *Escherichia coli* are pathogenic bacteria that cause foodborne diseases that often contaminate food [1]. The incidence of foodborne disease due to consumption of food contaminated with pathogenic bacteria is 4 billion people and 2.2 million of them died [2]. In Surabaya in 2018, the incidence of poisoning due to consuming food that has been contaminated with bacteria is 2,115 cases [3].

From 2015 to 2019, the identification of risk factors for *Salmonella* sp. in samples of processed products originating from the chicken have been carried out, namely 51 cases in 2015 [4] and since May 2017 found 12 outbreaks and 285 cases [5]. *Salmonella* and *Escherichia coli* are commonly found in raw meat, poultry, and poultry products due to environmental pollution [6]. Chicken sausage samples obtained from the Flamboyan Pontianak market which were stored at room temperature (28°C-30°C) were positive for aerobic bacteria, *Coliform* bacteria, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* sp. [7]. Sausages sold in Jatinagor District were detected by *Escherichia coli* in 7 samples (29.17%) with a value range of 9.2-240 APM/g and *Salmonella* sp. was detected in 1 sample (4.16%) [8].

The maximum limit of microbial contamination in processed meat in sausage is  $1 \times 10^5$  colonies/gram, for *Salmonella* it must be negative/25gram sample, and for *Escherichia coli*  $<3$  APM/gram. Microbiological quality is very important in food safety and maintaining food quality [9].

## 1.2. Literature Review

### 1.2.1. Food Contamination

Food contaminants are materials that unintentionally contaminate and are unwanted in food originating from the environment or as a result of processes along the food chain, in the form of biological contaminants, chemical contaminants, mycotoxins, radioactive substances, residues of veterinary drugs, and pesticides as well as other objects that can interfere with, harm, and endanger human health. Food safety can be realized by paying attention to food sanitation, regulation of food additives, regulation of genetically engineered food products, regulation of food irradiation, determination of food packaging standards, provision of food safety and food quality assurance, and guarantee of halal products for those who are required [10].

Factors that affect food contamination are food processing, food handler, hygiene and sanitation practice, temperature, storage time, and packaging type of food. The packaging used for sausage packaging is LDPE (Low-Density Polyethylene) plastic which is then vacuum packed. Vacuum packaging aims to extend the shelf life of sausages because of its airtight structure, while the use of polyethylene can maintain the aroma and taste of a product even though it is stored at the freezing point [11]. Bulk sausages are sausages that are not packaged and stored properly. Bulk sausages have a higher risk of contamination than packaged sausages. Bulk sausages are in great demand by the public because they are cheaper than branded packaged sausages [12].

### 1.2.2. *Salmonella* sp.

*Salmonella* is a rod-shaped bacterium or bacillus grouped in the *Enterobacteriaceae* family [13]. *Salmonella* is a gram-negative bacteria that has a length of 1-2  $\mu\text{m}$ , rod-shaped that does not form spores, and is motile with peritrichous flagella. *Salmonella* is a facultative anaerobic bacterium that can ferment glucose, and produce acid, and gas but is unable to use lactose and sucrose. *Salmonella* has the optimum temperature for growth at 38°C and can grow at low  $a_w$  ( $a_w$  0.93) and is active at pH 3.6-9.5 [14].

*Salmonella* is usually found in raw poultry meat, where poultry meat is the main ingredient in the manufacture of several processed foods. In processed chicken, *Salmonella* can survive for 16 weeks in frozen storage at -20°C [15]. *Salmonella* sp. can survive at a temperature of 67°C and has an optimum growth temperature of 20-45°C.

*Salmonella* usually attacks the human intestine through food that has been contaminated and which is not properly prepared. Symptoms caused when infected with *Salmonella* are symptoms of fever, abdominal cramps, and pain [16].

### 1.2.3. *Escherichia coli*

*Escherichia coli* is a gram-negative, facultative anaerobic bacterium in the form of a short rod with a length of about 2  $\mu\text{m}$ , a diameter of 0.7  $\mu\text{m}$ , a width of 0.4-0.7  $\mu\text{m}$  which usually infects the digestive tract in humans within a few hours after consuming food contaminated with these bacteria. Pathogenic *Escherichia*

*coli* is a disease that causes large outbreaks of infant diarrhea, bloody diarrhea, cystitis, pyelonephritis, meningitis, and so on [17]. *Escherichia coli* grows in colonies with round, convex, smooth, with pronounced edges.

*Escherichia coli* can grow quickly at a temperature of 30-42°C, grow slowly at a temperature of 44-45°C, and cannot grow at a temperature of 10°C or lower. This strain is resistant to pH 4.5 or lower. Bacteria will die at a pasteurization temperature of 64.3°C for 9.6 seconds, but cells can survive in food at a temperature of -20°C. *Escherichia coli* is an indicator of contaminants with faecal sources. The natural habitat of *Escherichia coli* is the lower digestive tract of animals and humans [18].

## 1.3. Research Objective

The purpose of this study was to compare the contamination level of bacterial, *Salmonella* sp. and *Escherichia coli* in bulk and packaged chicken sausages as well as to determine the relationship between the hygiene and sanitation of traders and the level of bacterial contamination, *Salmonella* sp. and *Escherichia coli* contamination in chicken sausages sold at traditional markets in Surabaya, Indonesia.

## 2. MATERIALS AND METHODS

### 2.1. Materials and Tools

The main raw materials are used are sausage samples consisting of 18 bulk chicken sausage samples and 10 packaged chicken sausage samples obtained by 23 sausage traders in the East Surabaya traditional market. The materials used are Buffered Pepton Water (Oxoid), Selenite Cystine Broth (Oxoid), Xylose Lysine Deoxycholate (Oxoid), Eosin Methylene Blue Agar (Oxoid), Plate Count Agar (Merck), Triple Sugar Iron Agar (Oxoid), Sulfide Indole Motility Medium (Oxoid), Methyl Red Voges Proskauer Medium (Oxoid), Simmons Citrate Medium (Oxoid), Urease Agar, Kovac's Indole Reagent (Merck), Methyl Red Indicator (Merck), Alpha Naphthol Reagent (Merck), 40% KOH (Merck), 70% alcohol, and sterile distilled water.

The tools used include autoclave, incubator, laminar flow, microwave, analytical balance, vortex, refrigerator, Erlenmeyer, measuring cup, petri dish, test tube, ose needle, 1.5 ml Eppendorf tube, micropipette, blue tip, yellow tip, bunsen, dropper, test tube rack, Eppendorf tube rack, and test tube cap.

### 2.2. Design Experiment and Analysis

This research is a cross sectional study and purposive sampling method. Sampling at the traditional markets in East Surabaya area and bacterial testing were carried out at the BSL - 2 (Bio Safety Level 2) Laboratory, Airlangga University. Laboratory analysis includes testing for total bacteria, detection of *Salmonella* sp. and *Escherichia coli* in sausage products.

### 2.3. Implementation of Research

#### 2.3.1. Total Bacteria Test with Drop Plate Method [19].

One gram of sample was weighed and homogenized in 9 ml of 0.85% NaCl solution to make  $10^{-1}$  to  $10^{-6}$ . A total of 50  $\mu$ l of samples from each dilution was dripped onto the surface of a sufficiently dry sterile PCA agar medium and incubated at 37°C for 18-24 hours. Growing colonies were calculated in Colony Forming Units per gram (CFU/g) of a sample using the formula:

$$\text{CFU} = \text{number of colonies} \times 1000/50 \times \text{dilution factor}$$

#### 2.3.2. Isolation and Identification of *Salmonella* sp. [20] [1].

Isolation of *Salmonella* sp. of sausage products through three stages are Pre-enrichment, Selective enrichment, and Selective plating. *Pre-enrichment*, 25 grams of chicken sausage samples were aseptically ground and homogenized with 225 ml of Buffered Pepton Water (BPW), then incubated at 37°C for 24 hours. *For selective enrichment*, one ml of Buffered Pepton Water (BPW) was added aseptically to 10 ml of *Selenite Cystine Broth* (SCB) and incubated at 37°C for 24 hours. For selective plating, 1 ose of SCB was taken and scratched on the surface of sterile *Xylose Lysine Deoxycholate* (XLD) media and incubated at 37°C for 24 hours. Criteria for confirmation of *Salmonella* sp. obtained from the isolation stage are based on the following biochemical characteristics:

##### 2.3.2.1. Triple Sugar Iron Agar (TSIA) Test

Colonies taken from positive (+) XLD were transferred using an ose needle to a sterile TSIA agar medium (in test tubes) by scratching on the oblique and piercing the upright, then incubated at 37°C for 24-48 hours. The results of the identification of the presence of *Salmonella* sp. indicated by a yellow color change with or without black color ( $\text{H}_2\text{S}$ ), and the oblique part remains red (unchanged).

##### 2.3.2.2. Indole Test

Colonies were taken from positive (+) XLD with ose transferred to sterile *Sulfide Indole Motility* (SIM) media by piercing to the bottom of the agar medium and incubated at 37°C for 24 hours. Then 0.2 - 0.3 ml of Kovacs reagent was added. Specific test results for *Salmonella* sp. were negative indole test, positive motile, and the formation of black  $\text{H}_2\text{S}$ .

##### 2.3.2.3. Methyl Red - Voges Proskauer (MR-VP) Test

Colonies were taken from positive (+) XLD with ose inoculated into 6 ml of sterile MR-VP media, incubated for 48 hours. The MR-VP medium was divided into 2 portions of 3 ml each. Each part was added with a methyl red reagent for the methyl red test. Alpha naphthol reagents and 40% KOH were added for the Voges Proskauer test. The red color on the MR-VP test shows a positive result, and the yellow color on the MR-VP test shows a negative result.

##### 2.3.2.4. Citrate Test

Colonies were taken from positive (+) XLD with ose and then inoculated onto sterile Simmon's Citrate Agar (SCA) media by scratching on the agar slanted media and then incubated at 37°C for 24 hours. A positive test result is indicated by the growth of colonies followed by a color change from green to blue. A negative test result is indicated by no color change. *Salmonella* sp. gave a positive result on the citrate test.

##### 2.3.2.5. Urease Test

Colonies were taken from positive (+) XLD with ose then inoculated into urea medium, then incubated at 37°C for 24 hours. Specific test results for *Salmonella* sp. are negative for urease test indicated by the color remains yellow on the media.

#### 2.3.3. Isolation and Identification of *Escherichia coli* [20] [1].

Isolation of *Escherichia coli* colonies from sausage products went through two stages, namely Pre-enrichment and Selective plating. *Pre-Enrichment*, 25 grams of chicken sausage samples were aseptically ground and homogenized with 225 ml of Buffered Pepton Water (BPW), then incubated at 37°C for 24 hours. *For selective plating*, 1 ose of BPW was taken and inscribed on the surface of sterile Eosyn Methylene Blue Agar (EMBA) media and incubated at 37°C for 24 hours. Samples considered positive for *Escherichia coli* were indicated by the formation of metallic green colonies. The criteria for confirmation of *Escherichia coli* isolates obtained from the isolation stage are based on the following biochemical characteristics:

##### 2.3.3.1. Triple Sugar Iron Agar (TSIA) Test

Colonies taken from positive (+) EMBA were transferred using an ose needle to a sterile TSIA agar medium (in test tubes) by scratching on the oblique and piercing the upright, then incubated at 37°C for 24-48 hours. The results of the identification of the presence of *Escherichia coli* were indicated by a yellow color change in the slant and butt (A/A) or (A/A) and the formation of gas without black color ( $\text{H}_2\text{S}$ ).

##### 2.3.3.2. Indole Test

Colonies taken from positive (+) EMBA with ose were transferred to sterile Sulfide Indole Motility (SIM) media by piercing to the bottom of the agar medium and incubated at 37°C for 24 hours. Then 0.2-0.3 ml of Kovacs reagent was added. The specific test results for *Escherichia coli* were positive for indole and positive for motile.

##### 2.3.3.3. Methyl Red - Voges Proskauer (MR-VP) Test

Colonies were taken from positive (+) EMBA with ose inoculated into 6 ml sterile MR-VP media and incubated for 48 hours. The MR-VP medium was divided into 2 portions of 3 ml each. Each part was added with a methyl red reagent for the methyl red test. Alpha naphthol reagents and 40% KOH were added for the Voges Proskauer test. *Escherichia coli* specific test results were positive for the MR test and negative for the VP test.

##### 2.3.3.4. Citrate Test

Colonies were taken from positive (+) EMBA with ose then inoculated onto sterile Simmon's Citrate Agar (SCA) media by scratching on the agar slanted media and then incubated at 37°C for 24 hours. A positive test result was indicated by the presence of colony growth without a change in the color of the medium (which remains green). *Escherichia coli* specific test results gave negative results on the citrate test.

### 2.4. Analytical methods

The total bacteria were converted into log form before being analyzed. Data on the level of bacterial contamination, and contamination of *Salmonella* sp. and *Escherichia coli* were

expressed in absolute values and percentages using Microsoft Office Excel 2016. The relationship between hygiene and sanitation of traders with the level of bacterial contamination, *Salmonella* sp. and *Escherichia coli* were analyzed by Chi-square test at  $P < 0.05$  using SPSS software (version 25). Statistical significance was defined as  $P < 0.05$  at an error rate of 5%.

### 3. RESULT AND DISCUSSION

#### 3.1. Total Bacterial

Total bacteria (Table 1) showed that 14 (77.78%) of the 18 bulk sausage samples had bacterial contamination levels exceeding the SNI 3820:2015 standard ( $>5.00$  log CFU/g), while 4 (22.22%) met the SNI 3820 standard. :2015 ( $<5.00$  log CFU/g). Packaged sausages (Table 2) showed that 2 (20%) of the 10 samples had a level of bacterial contamination exceeding the maximum standard of SNI 3820:2015 ( $>5.00$  log CFU/g), while 8 (80%) met the standard of SNI 3820:2015 ( $< 5.00$  log CFU/g).

Table 1. Observations of Total Bacteria in Bulk Chicken Sausage

Code	TPC (Log CFU/g)	Description	Code	TPC (Log CFU/g)	Description
A <sub>1</sub>	5.81	NE	F <sub>1</sub>	5.99	NE
A <sub>2</sub>	7.54	NE	G <sub>1</sub>	6.40	NE
B <sub>1</sub>	4.87	E	H <sub>1</sub>	7.05	NE
B <sub>2</sub>	6.01	NE	I <sub>1</sub>	7.93	NE
C <sub>1</sub>	4.02	E	J <sub>1</sub>	5.69	NE
C <sub>2</sub>	5.74	NE	K <sub>1</sub>	4.08	E
D <sub>1</sub>	3.91	E	L <sub>1</sub>	6.51	NE
D <sub>2</sub>	5.36	NE	L <sub>2</sub>	5.45	NE
E <sub>1</sub>	6.81	NE	M <sub>1</sub>	6.27	NE

Note :

E : (Eligible): Total bacteria does not exceed the standard of SNI Number 3820:2015 ( $<5.00$  Log CFU/g)

NE : (Not Eligible): Total bacteria exceeds the standard of SNI Number 3820:2015 ( $>5.00$  Log CFU/g)

Table 2. Results of Observation of Total Bacteria in Packaged Chicken Sausage

Code	TPC (Log CFU/g)	Description	Code	TPC (Log CFU/g)	Description
N <sub>1</sub>	7.29	NE	S <sub>1</sub>	4.11	E
O <sub>1</sub>	4.36	E	T <sub>1</sub>	4.23	E
P <sub>1</sub>	4.80	E	U <sub>1</sub>	4.36	E
Q <sub>1</sub>	5.57	NE	V <sub>1</sub>	4.36	E
R <sub>1</sub>	4.90	E	W <sub>1</sub>	4.36	E

Note :

E : (Eligible): Total bacteria does not exceed the standard of SNI Number 3820:2015 ( $<5.00$  Log CFU/g)

NE : (Not Eligible): Total bacteria exceeds the standard of SNI Number 3820:2015 ( $>5.00$  Log CFU/g)

Bulk chicken sausages that are sold in open conditions have a higher total bacteria. Sausages served open can increase the chance of contamination in sausages because of the large surface area of the food that is exposed, so the total bacteria in bulk chicken sausages are higher than in packaged chicken sausages [21]. This is following Ref. [12], that the total bacteria in bulk chicken sausage is higher ( $1.9 \times 10^5$  CFU/g) than in packaged chicken sausage, which is  $7.5 \times 10^3$  CFU/g.

Table 3. Relationship of Trader's Sanitary Hygiene to Total Bacteria of Chicken Sausage

Hygiene and Sanitation Conditions	Total Bacterial				Total	$\alpha$	p-Value	
	Qualify*		Not Eligible**					
	N	%	N	%				
Good	8	80.00	2	20.00	10	100	.05	.007
Poor	3	23.10	10	76.90	13	100		
Total	11	47.80	12	52.20	23	100		

Note:

Eligible\*: total bacteria does not exceed the standard of SNI Number 3820:2015 ( $<5.00$  Log CFU/g)

Does not eligible\*\*: total bacteria exceeds the standard of SNI Number 3820:2015 ( $>5.00$  Log CFU/g)

Based on the Chi-Square test (Table 3). it was obtained (p-value  $0.007 < 0.05$ ) indicating that there was a significant relationship between the trader's sanitation hygiene and the level of bacterial contamination in bulk chicken sausage and packaged chicken sausage. Sausage packaging conditions, as well as processing and storage processes, can also affect the level of bacterial contamination. This is following Ref. [22]. which states that the condition of the packaging of processed food products affects the level of microbial contamination.

#### 3.2. Isolation and Identification *Salmonella* sp.

##### 3.2.1. Pre-Enrichment



Fig.1. Pre-enrichment stage observation results

Isolation and identification of *Salmonella* sp. begin with doing enrichment (pre-enrichment) using *Buffered Pepton Water* (BPW) media. Bacterial growth was characterized by turbidity on BPW media as shown in Fig. 1.

##### 3.2.2. Selective Enrichment

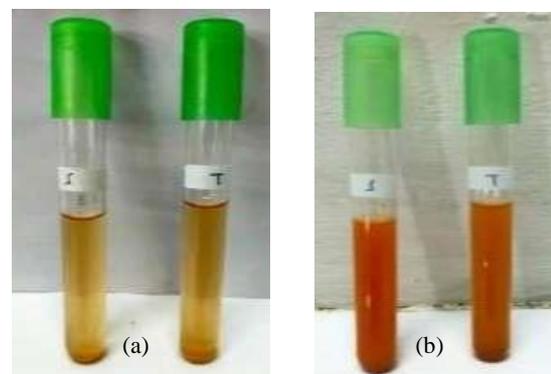


Fig. 2(a). SCB media before bacterial inoculation  
2(b). SCB media after bacterial growth

Selective enrichment is aimed at increasing the growth of *Salmonella* sp. and inhibiting other bacteria from growing.

Changes in *Selenite Cystine Broth* (SCB) to red due to the acid formed due to bacterial growth. These changes occur because the media contains sodium selenite inhibitor which is reduced to selenium and a reaction occurs with sulfur-containing amino acids so that it can prevent the growth of other bacteria. The results of the growth of *Salmonella* sp.. on SCB media can be seen in Fig. 2(a) and 2(b).

3.2.3. Selective Plating

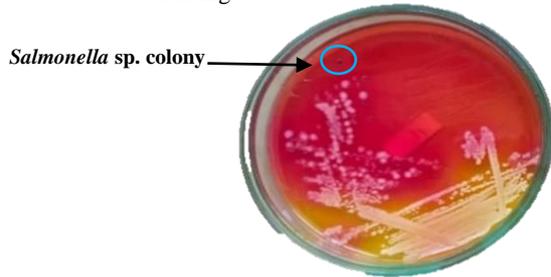


Fig. 3. Observation of *Salmonella* sp. on XLD media

Selective plating using *Xylose Lysine Deoxycholate* (XLD) media for the growth of *Salmonella* sp.. shown a change from clear transparent color (colorless) to pink (pink) after incubation for 24 hours. Colony characteristics of *Salmonella* sp.. red and no / there is a black spot in the middle. The results of observations of colonies of *Salmonella* sp.. on XLD media can be seen in Fig. 3.

3.2.4. Biochemical Characteristics of *Salmonella* sp.. isolates.

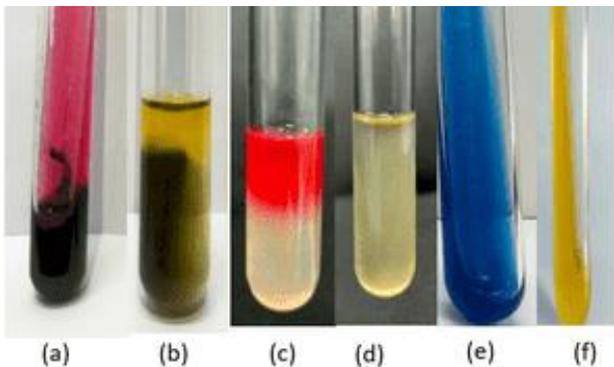


Fig. 4. (a) TSIA test; (b) SIM test; (c) Methyl Red (MR) test; (d) Voges-Proskauer (VP) test; (e) Citrate tes; (f) Urease test.

*Salmonella* bacteria in TSIA media were characterized by the presence of alkali (red color) in the slant and the presence of acid (yellow color) on the butt with or without gas and H<sub>2</sub>S. The media changes to red in the slant because the bacteria are alkaline and do not ferment lactose and sucrose. The color change to yellow on the butt indicates that the bacteria are fermenting glucose. The production of H<sub>2</sub>S is indicated by the presence of a black precipitate [20] [1].

SIM media showed motility in the media with growth spreading in the punctured area and the indole test showed a negative result without the formation of an indole ring (red color) [1]

Methyl red test for *Salmonella* sp. showed positive results which were indicated by a change in color to red and the Voges-Proskauer *Salmonella* sp. shows a negative result in yellow [1].

Citrate test. a positive result for *Salmonella* sp. indicated by a change in the color of the medium from green to blue indicating that bacteria can use citrate as a carbon source and inorganic ammonium salt (NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>) as a nitrogen source [1].

The urease test showed negative results indicated by no change in the media. which remained yellow because the urease enzyme could not break the carbon and nitrogen bonds to form ammonia and changed the color of the media. Observation of the biochemical characteristics of *Salmonella* sp. can be seen in Fig 4 [20] [1].

The contamination level of *Salmonella* sp. in bulk chicken sausage (44.44%) was higher than in packaged chicken sausage (10.00%). The high contamination of *Salmonella* sp. in bulk sausages. apart from being caused by poor sanitary hygiene conditions. bulk sausages are not packaged properly and sausage storage conditions are at room temperature to accelerate bacterial growth.

3.2.5. The Relationship of Trader's Sanitary Hygiene Conditions to *Salmonella* sp. Contamination

Table 4. The Relationship of Trader's Sanitary Hygiene Conditions to *Salmonella* sp. Contamination in Bulk and Packaged Chicken Sausage

Traders' Hygiene and Sanitation Conditions	<i>Salmonella</i> sp. Contamination				Total		α	p-Value
	Positive		Negative		N	%		
	N	%	N	%				
Good	9	90.00	1	10.00	10	100	0.05	0.029
Poor	6	46.20	7	53.80	13	100		
Total	11	65.20	12	34.80	23	100		

Based on the *Chi-Square* test (Table 4). it was obtained (p-value 0.029 < 0.05) that there was a significant relationship between the sanitary hygiene of traders and contamination of *Salmonella* sp. in bulk sausages and packaged sausages. This is following Ref. [23]. that there is a significant relationship (p-value 0.022 < 0.05) between the sanitation hygiene of traders and the level of contamination of *Salmonella* sp. on processed chicken sold by traveling traders in Malang City.

3.3. Isolation and Identification *Escherichia coli*.

3.3.1. Pre-Enrichment



Fig. 5. Pre-enrichment stage observation results

The main stage of isolation and identification of *Escherichia coli* is by pre-enrichment or pre-enrichment using BPW media. Bacterial growth in BPW is characterized by cloudy BPW conditions which can be seen in Fig 5.

### 3.3.2. Selective Plating

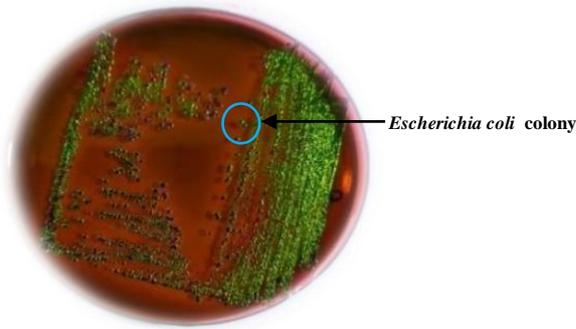


Fig. 6. Observation of *Escherichia coli* on EMBA media

Eosin Methylene Blue Agar (EMBA) media is a differential medium for *Escherichia coli* and a positive result will be metallic green which indicates *Escherichia coli* can ferment lactose to produce a strong acid final product. The results of observations of *Escherichia coli* colonies on EMBA media can be seen in Fig 6.

### 3.3.3. Biochemical Characteristics of *Escherichia coli*.

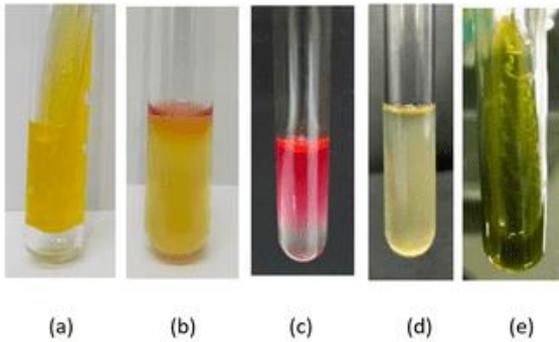


Fig. 7. (a) TSIA test; (b) SIM test; (c) Methyl Red (MR) test; (d) Voges-Proskauer (VP) test; (e) Citrate test.

The biochemical characteristics of *Escherichia coli* on TSIA media showed that there was a change in the medium to acid (yellow) in the slant and butt. This change is because bacteria can ferment glucose, not producing H<sub>2</sub>S but producing gas which is the result of H<sub>2</sub> and CO<sub>2</sub> fermentation [20] [1].

Sulfide Indole Motility (SIM) showed the presence of motility, which was indicated by the growth spread over the puncture area on the media and the indole test showed a positive result of the formation of an indole ring (red color) [1].

The methyl red test showed positive results, indicated by a change in color to red after adding methyl red reagent, while the Voges-Proskauer test showed a negative result in yellow [1].

The citrate test showed negative results, namely, the media remained green (no change in the color of the media) [1]. Observation of the biochemical characteristics of *Salmonella* sp. can be seen in Fig 7.

*Escherichia coli* contamination in the bulk chicken sausage was higher (22.22%) compared to packaged chicken sausage (20.00%). This shows that the risk of *Escherichia coli* contamination in bulk chicken sausages sold in traditional markets is higher than in packaged chicken sausages so it is dangerous for consumers' health if not handled properly.

### 3.3.4. The Relationship of Trader's Sanitary Hygiene Conditions to *Escherichia coli* Contamination

Based on the Chi-Square test (Table 5), it was obtained (p-value 0.043 < 0.05) that there was a significant relationship between the sanitation hygiene of traders and *Escherichia coli* contamination in bulk and packaged sausages. This is following Ref. [24], that there is a relationship between the hygiene practices of traders and the level of *Escherichia coli* contamination in food sold around the Semarang State University campus (p-value 0.021 < 0.05).

Table 5. The Relationship of Trader's Sanitary Hygiene Conditions to *Escherichia coli* Contamination in Bulk and Packaged Chicken Sausage

Trader's Hygiene and Sanitation Conditions	<i>Escherichia coli</i> contamination				Total		α	p-Value
	Positive		Negative		N	%		
	N	%	N	%				
Good	11	91.70	1	8.30	12	100	0.05	0.043
Poor	6	54.50	5	45.50	11	100		
Total	17	73.90	6	26.10	23	100		

## 4. CONCLUSION

The average level of bacterial contamination in the bulk chicken sausage was higher (5.98 Log CFU/g) than in packaged chicken sausage (4.83 Log CFU/g) and exceeded the maximum limit of SNI standards (greater than 5.00 Log CFU/g). *Salmonella* sp. contamination in the bulk chicken sausage was higher (44.44%) than packaged chicken sausage (10.00%). *Escherichia coli* contamination in the bulk chicken sausage was higher (22.22%) compared to packaged chicken sausage (20.00%). There is a significant relationship between the sanitary conditions of traders and the level of bacterial contamination, contamination of *Salmonella* sp. and *Escherichia coli* in bulk chicken sausage and packaged chicken sausage. The worse the hygiene and sanitation of traders, the higher the bacterial contamination. *Salmonella* sp. and *Escherichia coli*.

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