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

Deana Fyra Adi Nur¹, Ratna Yulistiani¹*, Dedin F. Rosida¹, and Dadik Raharjo²

¹ Food Science and Technology Department, Faculty of Engineering. Universitas Pembangunan Nasional "Veteran" Jawa Timur. Surabaya. Indonesia.
² Faculty of Veterinary Medicine. Airlangga University, Surabaya. Indonesia

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CORRESPONDING AUTHOR

*E-mail: ratna.tp@upnjatim.ac.id

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.

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 Jatinagar 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].

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.

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The maximum limit of microbial contamination in processed meat in sausage is 1x10³ 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 μm, 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 μm, a diameter of 0.7μ m, a width of 0.4-0.7μm 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), Sulhide 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.

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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 µl 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:
CFU = number of colonies x 1000/50 x 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 ml 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 the yellow color change
with or without black color (H₂S), 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 H₂S.

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 Simmons’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]
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 ml of BPW was taken and
inscribed on the surface of a 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 the
confirmation of Escherichia coli isolates were obtained from the
isolation stage are based on the following biochemical
characteristics:

2.3.3.1. Tripe 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 (H₂S).

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 Simmons’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

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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

<table>
<thead>
<tr>
<th>Code</th>
<th>TPC (Log CFU/g)</th>
<th>Description</th>
<th>Code</th>
<th>TPC (Log CFU/g)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>5.81</td>
<td>NE</td>
<td>F1</td>
<td>5.99</td>
<td>NE</td>
</tr>
<tr>
<td>A2</td>
<td>7.54</td>
<td>NE</td>
<td>G1</td>
<td>6.40</td>
<td>NE</td>
</tr>
<tr>
<td>B1</td>
<td>4.87</td>
<td>E</td>
<td>H1</td>
<td>7.05</td>
<td>NE</td>
</tr>
<tr>
<td>B2</td>
<td>6.01</td>
<td>NE</td>
<td>I1</td>
<td>7.93</td>
<td>NE</td>
</tr>
<tr>
<td>C1</td>
<td>4.92</td>
<td>E</td>
<td>J1</td>
<td>5.69</td>
<td>NE</td>
</tr>
<tr>
<td>C2</td>
<td>5.74</td>
<td>NE</td>
<td>K1</td>
<td>4.08</td>
<td>E</td>
</tr>
<tr>
<td>D1</td>
<td>3.91</td>
<td>E</td>
<td>L1</td>
<td>6.51</td>
<td>NE</td>
</tr>
<tr>
<td>D2</td>
<td>5.36</td>
<td>NE</td>
<td>L2</td>
<td>5.45</td>
<td>NE</td>
</tr>
<tr>
<td>E1</td>
<td>6.81</td>
<td>NE</td>
<td>M1</td>
<td>6.27</td>
<td>NE</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Code</th>
<th>TPC (Log CFU/g)</th>
<th>Description</th>
<th>Code</th>
<th>TPC (Log CFU/g)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>7.29</td>
<td>NE</td>
<td>S1</td>
<td>4.11</td>
<td>E</td>
</tr>
<tr>
<td>O1</td>
<td>4.36</td>
<td>E</td>
<td>T1</td>
<td>4.23</td>
<td>E</td>
</tr>
<tr>
<td>P1</td>
<td>4.80</td>
<td>E</td>
<td>U1</td>
<td>4.36</td>
<td>E</td>
</tr>
<tr>
<td>Q1</td>
<td>5.57</td>
<td>NE</td>
<td>V1</td>
<td>4.36</td>
<td>E</td>
</tr>
<tr>
<td>R1</td>
<td>4.90</td>
<td>E</td>
<td>W1</td>
<td>4.36</td>
<td>E</td>
</tr>
</tbody>
</table>

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 x 10³ CFU/g) than in packaged chicken sausage, which is 7.5 x 10³ CFU/g.

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

<table>
<thead>
<tr>
<th>Hygiene and Sanitation</th>
<th>Total Bacterial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Qualify</strong>*</td>
<td><strong>Not Eligible</strong></td>
</tr>
<tr>
<td>Conditions</td>
<td>N</td>
</tr>
<tr>
<td>Good</td>
<td>8</td>
</tr>
<tr>
<td>Poor</td>
<td>3</td>
</tr>
</tbody>
</table>

Note:
Refer to 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

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

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

![Salmonella sp. colony](image)

**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 l 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.

![Image of biochemical test results](image)

**Fig. 4.** (a) TSIA test; (b) SIM test; (c) Methyl Red (MR) test; (d) Voges-Proskauer (VP) test; (e) Citrate test; (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$_2$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$_2$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].

### 3.2.5. The Relationship of Trader’s Sanitary Hygiene Conditions to Salmonella sp. Contamination

<table>
<thead>
<tr>
<th>Traders’ Sanitation Conditions</th>
<th>Salmonella sp. Contamination</th>
<th>Total</th>
<th>α</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>9</td>
<td>1</td>
<td>10</td>
<td>0.05</td>
</tr>
<tr>
<td>Poor</td>
<td>6</td>
<td>7</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>8</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

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

![Image of pre-enrichment stage observation results](image)

**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.

![Escherichia coli colony](image)

Fig. 7. (a) TSIA test; 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 H2S but producing gas which is the result of H2 and CO2 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

<table>
<thead>
<tr>
<th>Trader's Hygiene and Sanitation Conditions</th>
<th>Escherichia coli contamination</th>
<th>Total</th>
<th>a</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Good</td>
<td>11</td>
<td>91.70</td>
<td>1</td>
<td>8.30</td>
</tr>
<tr>
<td>Poor</td>
<td>6</td>
<td>54.50</td>
<td>5</td>
<td>45.50</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>73.90</td>
<td>6</td>
<td>26.10</td>
</tr>
</tbody>
</table>

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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