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Detection of bacterial contamination Salmonella sp. on broiler chicken meat for sale at the traditional market of East Surabaya Indonesia

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Abstract

Objective: This research aimed to determine the presence of *Salmonella* sp. contamination on broiler chicken meat sold in traditional markets in East Surabaya Indonesia.

Method: The research sample consisted of 30 broiler chickens taken from six traditional East Surabaya markets: Manyar Market, Jojoran Market, Tempurejo Market, Semolowaru Market, Pucang Anom Market, and Soponyono Market. Each sample was inoculated in Lactose broth media, after incubation for 24 hours then inoculated in Tetrathionate Broth media. The samples were then inoculated on Salmonella Shigella Agar (SSA) media. If colonies grow on SSA media that appear round, transparent with a black spot (black center), then proceed with the Gram staining test and biochemical tests, including Triple Sugar Iron Agar (TSIA), urease test, Sulfide Indole Motility (SIM), and Simmons Citrate Agar (SCA). Gram staining of Salmonella sp. shows Gram-negative bacteria, and the TSIA (Triple Sugar Iron Agar) test shows Alkaline/Acid and produces H2S. The SCA (Simmons Citrate Agar) test showed positive results, while the urease test showed negative results. The SIM (Sulfide Indole Motility) test showed a negative indole test.

Results: The results of 30 chicken meat samples sold in traditional markets in East Surabaya showed that 1 sample (3.3%) was positively contaminated with *Salmonella sp*. bacteria.

Conclusion: Broiler chicken meat sold in traditional markets in East Surabaya was found to be one out of a total of 30 samples (3.3%) contaminated with *Salmonella sp*. Based on the study results obtained, through this research it is recommended to provide a clean water source for washing equipment and carcasses. It is also necessary to educate traders regarding the importance of implementing sanitation and personal hygiene. Supervision in the process of taking chickens also needs to be tightened and ensured that chickens are free from *Salmonella sp.*

Keywords: Broiler Chicken Meat; Contamination; *Salmonella sp*.; Traditional Market; East Surabaya Indonesia

1. Introduction

Human protein intake needs can be met from two sources, namely vegetable and animal. Eating chicken, beef, goat, and eggs can meet animal protein needs. Chicken meat is an animal protein that is popular with Indonesian people, because it is more affordable and has high nutritional value. Based on data from the Central Statistics Agency (BPS), in 2023 demand for chicken meat will increase by around 9.7% from the previous year [1].

The meat consumed by the public must be safe, healthy, intact and halal, which means that the meat must be free from dangerous contamination, have high nutrition, not be mixed with other ingredients, and be processed based on Islamic

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law [2]. Chicken meat contains high levels of protein and water, making it an ideal condition for the growth of microbial contaminants originating from the surrounding environment, causing the meat to spoil easily or perishable [3]. Some bacteria that can contaminate chicken meat are *Escherichia coli, Staphylococcus aureus, Salmonella sp., Pseudomonas sp., Clostridium perfringens*, and *Shigella flexneri*. Bacterial contamination can cause foodborne disease or diseases caused by consuming contaminated food [4].

Salmonella sp. is one of the bacteria that often contaminates chicken meat. According to SNI number 7388 in 2009 concerning the maximum limit of microbial contamination and residue limits in food ingredients of animal origin, states that chicken meat must be negative per 25 grams of bacteria *Salmonella sp*. This bacterium can have a detrimental impact on human health, namely causing salmonellosis. *Salmonella sp*. can contaminate chicken meat from the farm, slaughter location, cutting equipment, storage area, environment, personal hygiene and sanitation of traders [5]. Salmonellosis in Indonesia is estimated to occur in 60,000 to 1,300,000 cases with 20,000 deaths [6]. The high number of salmonellosis cases is caused by a lack of hygiene and sanitation in handling chicken meat and its products. Hygiene at traders and sanitation at sales places also influence food safety [7].

In traditional markets, chicken meat is sold in open condition at room temperature and does not pay attention to the hygiene aspects of the products being sold. This increases the chances of chicken meat being contaminated by pathogenic microbes in particular *Salmonella sp*., because this bacteria can grow optimally at a temperature of 37˚C and can grow at room temperature [8]. Market conditions in Surabaya are still unhygienic, and sanitation is poor, resulting in contamination by pathogenic bacteria Salmonella sp. still occurs frequently [4]. According to research by Salsabilla [9], chicken meat samples were taken from the East Surabaya market in 2021 still shows the prevalence of contamination Salmonella sp. by 10%. This is still not by the standards set by SNI.

Considering the potential contamination of *Salmonella sp*. in chicken meat, the author deemed it necessary to conduct this research. Based on this background, the study aims to investigate the presence of *Salmonella sp*. contamination in chicken meat sold in traditional markets in East Surabaya Indonesia.

2. Material and methods

The sample used in this research was broiler chicken meat weighing 25 grams purchased from 6 traditional markets in East Surabaya, namely Manyar Market, Jojoran Market, Tempurejo Market, Semolowaru Market, Pucang Anom Market, and Soponyono Market. Five samples of chicken meat were taken from each of the six markets, with a total of 30 samples. The selection of traditional markets is determined based on purposive which is based on (1) high frequency of buying, selling and public visits; (2) the number of chicken meat traders is a minimum of six stalls; (3) broiler chicken meat is sold by traders openly or without packaging. This research began to be carried out in April 2024. Sample testing was carried out at the Bacteriology and Mycology Laboratory, Veterinary Microbiology Division, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya Indonesia.

The tools used in this research are gloves, masks, digital balances, autoclave, mortar, test tube, petri dish, incubator, Erlenmeyer, dropper pipette, measuring cup, cotton, aluminum foil, tube, Bunsen, microscope, glass object, hot plate, tube rack, and 1 cc syringe. The Materials used are broiler chicken meat, distilled water, Lactose Broth (LB), Tetrathionate Broth (TTB), Salmonella Shigella Agar, lugol, crystal violet, 96% alcohol or acetone alcohol, safranin, reagents Kovacs, emersion oil, Triple Sugar Iron Agar, Urea Agar Base, Media Sulfide Indole Motility (SIM), Simmons Citrate Agar.

2.1. Sampling

Samples were taken from Manyar Market, Jojoran Market, Tempurejo Market, Semolowaru Market, Pucang Anom Market, and Soponyono Market. The number of samples used was 30 samples of chicken meat from the pectoralis muscle, weighing 25 grams. Samples that have been obtained from the market are labeled and put in a cool box and continued with examination at the Bacteriology and Mycology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya. The chicken meat sample was weighed at 25 grams, then the sample was put into an Erlenmeyer flask and the solution of Lactose Broth (LB) was added as much as 225ml. Then incubated at 37˚C for 24 hours [10]. The pre-enrichment culture was homogenized, then 1 ml was taken and transferred into 10 ml of Tetrathionate Broth (TTB). Then incubated at 37˚C for 24 hours (SNI, 2008).

2.2. Cultivation of SSA media

Bacteria that grow on TTB media were then taken using a sterile loop and inoculated on the Salmonella Shigella Agar (SSA), then incubated at 37˚C for 24 hours [11]. Suspected colony *Salmonella sp*., which is round, and transparent with a black spot (black center), then continued with microscopic examination and biochemical tests.

2.3. Gram stain

Suspected *Salmonella sp*. colonies followed by Gram staining by making smear preparations. The way to make a smear preparation was by dripping 1 drop of 0.96% NaCl onto a glass object. Next, the colonies on SSA media were taken with inoculating loop, then placed on a glass object that had been dripped with 0.96% NaCl and flattened, then fixed over a Bunsen flame. The smear was then dripped with 1-2 drops of crystal violet until it covers the smear and left for 1 minute then rinsed with running water. The smear was then dripped with 1-2 drops of Lugol, left for 1 minute, and rinsed with running water. The smear was then dripped with 96% alcohol or acetone, left for 30 seconds, and washed with running water. Then the smear was dripped with 1-2 drops of safranin and left for 1 minute, then rinsed with running water [12]. The smear was allowed to dry, and then observed under a microscope with magnification of 1000x using emersion oil. *Salmonella sp*. is a Gram-negative bacteria, it looks rod-shaped and red in color under the microscope observation.

2.4. Identification of bacteria with biochemical tests

Identification of suspected colony *Salmonella sp*. were carried out using biochemical tests [13]. The biochemical test includes the TSIA test (Triple Sugar Iron Agar), urease test, Sulfide Indole Motility (SIM), and Simmons Citrate Agar (SCA).

2.5. Triple Sugar Iron Agar Test

Suspected colony *Salmonella sp*. on SSA media, then inoculated into TSIA media using an inoculating needle. The inoculating needle is pierced into the bottom of the agar medium, then streaked onto the agar slant, and incubated at 37˚C for 24 hours. On Triple Sugar Iron Agar (TSIA) test, positive for *Salmonella sp*. characterized by a change in color on the TSIA media to red on slanted agar, yellow on upright agar, the formation of H2S, with the presence or absence of gas [14].

2.6. Urease Test

Suspected colonies *Salmonella sp*. on SSA media, then inoculated into the Urea Agar using a loop by streaking it on the surface and then incubating at a temperature of 37˚C for 24 hours [14]. The urease test of *Salmonella sp*. showed negative results, where the media will remain yellow.

2.7. Sulfide Indole Motility Test

Suspected colonies *Salmonella sp*. on SSA media, then inoculated into the media Sulfide Indole Motility (SIM) using a needle loop by piercing up to two-thirds of the bottom of the media, then incubating at 37˚C for 24 hours. Then, add 0.2 to 0.3 ml reagents Kovacs. The indole test of *Salmonella sp*. showed negative results, namely no red ring was formed after being added reagents Kovacs, while in the motility test of *Salmonella sp*. can show motile and non-motile (SNI, 2008) [14].

2.8. Simmons Citrate Agar Test

Suspected colony of *Salmonella sp*. on SSA media, then inoculated into the Simmons Citrate Agar (SCA) media using a needle loop, and then incubated at 37˚C for 24 hours. The SCA test of *Salmonella sp*. shows positive results where there is a change in the media from green to blue (SNI, 2008) [14].

2.9. Data analysis

The data obtained in this research is then presented descriptively and presented in table form.

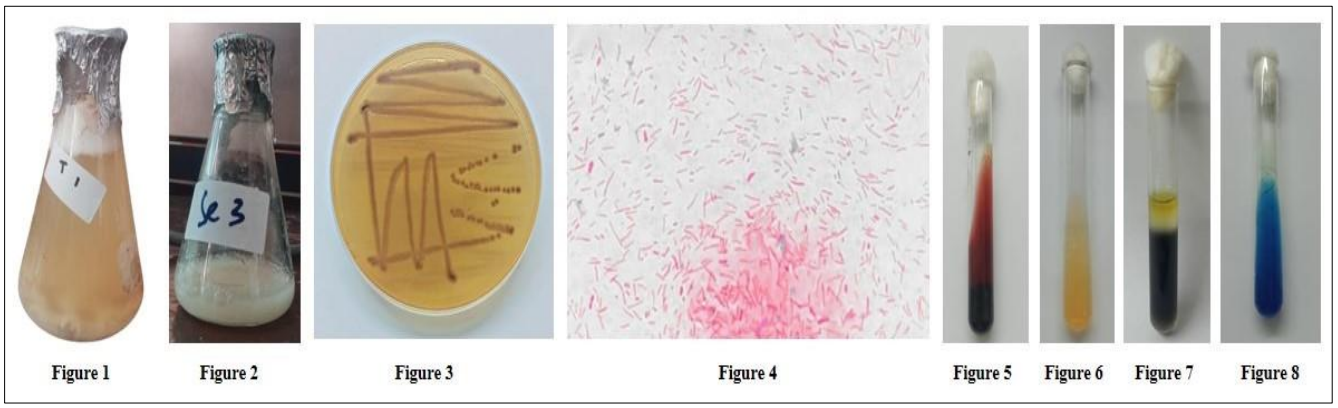
3. Results

Study is carried out to detect bacterial contamination *Salmonella sp*. on broiler chicken meat sold in traditional markets in East Surabaya Indonesia. Based on the study results, it showed that of the 30 samples of broiler chicken meat taken, one sample was contaminated with bacteria *Salmonella sp*., which can be seen in table 1.

Table 1 Detection results of *Salmonella sp.* on broiler chicken meat sold in traditional markets in East Surabaya

No.	Market	Number of Samples	Positive Amount *Salmonella sp.*
1	Manyar Market	5	-
2	Jojoran Market	5	-
3	Tempurejo Market	5	-
4	Semolowaru Market	5	-
5	Passer Pucang Anom	5	-
6	Soponyono Market	5	1
Total			1 (3.3%)

To detect the presence of bacteria *Salmonella sp*. need to carry out the isolation and identification stages. At the isolation stage, the media used is Salmonella Shigella Agar (SSA). The pre-enrichment stage uses media Lactose broth. Samples that have been inoculated into the media Lactose broth and incubated for 24 hours with a temperature of 37oC, indicates turbidity, can be seen in Figure 1.



Figures of the results of the examination of *Salmonella sp*. contamination on broiler chicken meat for sale at the traditional market of east Surabaya.

Figure 1 Sample results on media Lactose broth (LB) after incubation,

Figure 2 Sample results on Tetrathionate Broth (TTB) media after incubation,

Figure 3 Suspected colony of *Salmonella sp*. on SSA media,

Figure 4 Gram staining results with 1000x magnification**,**

Figure 5 TSIA media after bacterial inoculation,

Figure 6 Urea Agar media after bacterial inoculation,

Figure 7 Sulfide Indole Motility media after bacterial inoculation**,**

Figure 8 Simmons Citrate Agar media after bacterial inoculation

At the level of enrichment using media Tetrathionate Broth (TTB), showing turbidity in the media after incubation for 24 hours at a temperature of 37oC, can be seen in figure 2. Next, bacteria that grow on TTB media are inoculated into the media Salmonella Shigella Agar (SSA). Suspected colony *Salmonella sp*. on SSA media shows the characteristics of being round, transparent in color with a black spot (black center), can be seen in Figure 3. Out of total of 30 samples, 25 samples showed suspected colony growth *Salmonella sp*. on SSA media. Suspected Colony of *Salmonella sp*. on SSA media

then proceed with the steps identification, namely Gram staining and biochemical tests. Colony *Salmonella sp*. is a Gram-negative bacteria, rod-shaped and red in color, as seen in Figure 4. Based on the results of the Gram staining that was carried out, there were 25 samples that showed Gram-negative bacteria. After Gram staining, biochemical tests are then carried out. In this research, the biochemical test used are Triple Sugar Iron Agar (TSIA), Urease test, Sulfide Indole Motility (SIM), and Simmons Citrate Agar (SCA). Presumptive TSIA test of *Salmonella sp*. shows a red color change on the agar slant, yellow on upright agar (button), the formation of H2S, and no gas formation, can be seen in Figure 5. In the urease test, the suspected colonies of *Salmonella sp*. showed negative results, where there was no color change on the agar media, as seen in Figure 6. On Sulfide Indole Motility (SIM) test, suspected colonies of *Salmonella sp*. show the formation of H2S, non-motile, and indole negative as seen in Figure 7. On the test Simmons Citrate Agar (SCA), suspected colonies of *Salmonella sp*. show a positive result, namely a color change from green to blue, as seen in Figure 8.

4. Discussion

Based on research conducted on broiler chicken meat sold in traditional markets in East Surabaya Indonesia, one sample out of a total of 30 samples (3.3%) was contaminated with bacteria *Salmonella sp*. Even though the percentage of contamination by *Salmonella sp*. including low, but this is not by the standards set out in SNI 7388:2009 which requires that every 25 grams of chicken meat must be negative for bacteria *Salmonella sp*. Observations on the media Salmonella Shigella Agar (SSA) shows the results, the colonies are round, and transparent with a black spot (black center). Colonies that appear transparent with black spot on SSA media are due to ability *Salmonella sp*. which can produce thiosulfate reductase and H2S [15]. The color change of the SSA media from red to yellow is because SSA contains peptone which will be used as an energy source *Salmonella sp*. to form ammonia. Ammonia-produced *Salmonella sp*. can raise the pH in SSA media causing the color of the media to change from red to yellow [16].

Colonies that grow on SSA media and have characteristics *Salmonella sp*., followed by Gram staining and biochemical tests. The results of the Gram staining that was carried out showed that the bacteria belonged to the Gram-negative group, namely rod-shaped and red in color. The red color seen on Gram staining is due to the high lipid content in the cell walls. When given alcohol, acetone or 96% alcohol will dissolve the lipids contained in the cell walls, causing the cell walls to be porous and unable to maintain the crystal violet color. When given safranin, the cell walls will absorb the red color from the safranin, so that under a microscope the bacteria will appear red [17]. After Gram staining, proceed with biochemical tests. The biochemical test carried out are the Triple Sugar Iron Agar (TSIA), Urease Test, Sulfide Indole Motility (SIM), and Simmons Citrate Agar (SCA). The TSIA test was carried out to determine the ability of bacteria to produce H_2S and the ability to ferment certain carbohydrates, such as lactose, sucrose, and glucose. The results of the TSIA test are the formation of a black color, a change in the color of the media at the top to red, and a change in the color of the media at the bottom to yellow. This color change is due to *Salmonella sp*. cannot ferment lactose and sucrose, but can ferment glucose [18]. The black color that appears on the media is due to the formation of H_2S. *Salmonella sp*. use sodium thiosulfate contained in TSIA media as a source of sulfur to produce hydrogen sulfide (H_2S). This H_2S will react with iron citrate so produce ferrous sulfide which causes the black color of the agar [19].

Urease test to determine the ability of bacteria to produce the urease enzyme. Positive urease test results are indicated by a change in yellow color to pink [20]. This color change can occur because urease successfully catalyzes the hydrolysis of urea into ammonia and carbonate which are alkaline. This alkaline atmosphere can later change the yellow color to pink [21]. Bacteria *Salmonella sp*. in the urease test showed negative results, namely there was no change in color from yellow to pink. Sulfide Indole Motility test is to determine the ability of bacteria to produce indole, sulfide, and motility [22]. If the bacteria are motile, a white mist or turbidity will be visible on the media. In the media a black precipitate will form if the bacteria can produce sulfide [23]. The indole test is used to determine the presence of the tryptophanase enzyme in bacteria which will be used to hydrolyze the amino acid tryptophan into indole and pyruvic acid. The presence of indole can be determined by adding reagents Kovac's which will form a layer on the surface of the medium. A positive reaction is characterized by the formation of a red ring on the surface of the medium, while a negative reaction is characterized by the formation of a yellow ring [24]. In the indole test, *Salmonella sp*. gave negative results, which means this bacteria does not have the tryptophanase enzyme [25].

Simmons Citrate Agar (SCA) test is conducted to see the ability of bacteria to use citrate as a carbon source [26]. Test results on citrate test shows positive, namely there is a change in the color of the media from green to blue. This is because the use of citrate by bacteria causes an increase in pH which changes the color of the medium [27]. Based on research that has been carried out, there was one sample that was positively contaminated with bacteria *Salmonella sp*. namely samples originating from the Soponyono market. From observations of Manyar Market, Jojoran Market, Tempurejo Market, Semolowaru Market, Pucang Anom Market, and Soponyono Market, the conditions of these markets have different sanitation conditions. Tempurejo Market has better sanitation than other markets, because the market environment is supported by good air circulation and there is no rubbish scattered around. Manyar, Jojoran and

Puncang Anom markets have good air circulation even though there are lots of buyers, but there is still rubbish strewn around. At Soponyono market there is still a lot of rubbish scattered and piled up, air circulation is not good, so it feels hot and humid. In this market there is no clean water source used for washing hands and equipment, such as knives and cutting boards. According to Buckle [28], market conditions, such as poor environmental sanitation, support increased contamination and the development of Salmonella sp contamination. This can occur due to contact of chicken meat with contaminated soil, water, feces and insects *Salmonella sp*. [5]. At the Soponyono market, there are still many insects such as flies in the chicken meat.

According to Nisa et al. [28], contamination of *Salmonella sp*. on chicken meat would increase if the tools used for cutting, such as knives and cutting boards are dirty. The cutting boards used by traders at traditional markets in East Surabaya are made from wood which is more susceptible to contamination by bacteria. Wooden cutting boards easily absorb water, so even after washing they can leave water contaminated with bacteria. Apart from that, if the place for storing chicken meat and the scales used are not cleaned before use, they can also increase contamination *Salmonella sp*. [19].

According to Zelpina et al. [29], the water used in the chicken carcass production process also influences the occurrence of Salmonella sp. contamination. Water will be used for washing carcasses and cleaning equipment, if the water is contaminated with *Salmonella sp*. then the meat may be contaminated with these bacteria. At the Soponyono market, the water used to wash chicken meat is often used repeatedly until it is dirty, and the water is only replaced after it turns cloudy. According to the Decree of the Minister of Health Number 519/Menkes/SK/VI/ 2008 concerning Guidelines for Organizing Healthy Markets, the availability of storage places for food ingredients such as fish and meat uses appropriate refrigeration with a temperature of 4-10˚C. This is not in accordance with these regulations, because the chicken meat sold at the Soponyono market is only placed on the table without a cooling device and is open, this can also increase the level of bacterial contamination [30]. *Salmonella sp*. can contaminate chickens from the farm. Species of Salmonella which can attack poultry, namely Salmonella pullorum and Salmonella gallinarum which is non-motile (Berhanu and Fulasa, 2020) [31]. Contaminated chicken meat with *Salmonella sp*. found at the Soponyono market, probably came from infected chickens since from the farm, because based on the motility tests carried out it shows that the bacteria are non-motile.

Salmonella that contaminates chicken meat can cause salmonellosis [32]. Around 95% is caused by consuming contaminated animal products such as meat, poultry, eggs, milk, seafood and fresh products [33]. Symptoms include fever, diarrhea, nausea, vomiting and stomachache. According to Dewanti [34], based on the symptoms, salmonellosis is grouped into typhoid fever, enteric fever and gastroenteritis. Typhoid fever can last quite a long time, namely 1-8 weeks with a mortality rate of up to 10%. Enteric fever can last for 1-3 weeks. Gastroenteritis can last for 1-4 days with a mortality rate of 0.1-0.2%

5. Conclusion

Based on the study that has been carried out, it can be concluded that broiler chicken meat sold in traditional markets in East Surabaya was found to be one out of a total of 30 samples (3.3%) contaminated with *Salmonella sp*. This research it is recommended to provide a clean water source for washing equipment and carcasses. It is also necessary to educate traders regarding the importance of implementing sanitation and personal hygiene. Supervision in the process of taking chickens also needs to be tightened and ensured that chickens are free from *Salmonella sp*.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The study was approved by the Faculty of Veterinary Medicine Animal Ethics Committee of Universitas Airlangga. All variables were considered in accordance with the Ethics Committee related to the animal handling to ensure no discomfort or pain was caused to the animals during sampling (certificate registration number: 2024/11-KE).

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