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Contamination of *Salmonella Sp*. in Broiler Meat Sold In Traditional Markets of Banjarbaru City

(*CEMARAN SALMONELLA SP. PADA DAGING BROILER YANG DIJUAL DI PASAR TRADISONAL KOTA BANJARBARU*)

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ABSTRACT

Meat is an important food commodity in meeting nutritional needs. Damage that leads to a decrease in the quality of fresh meat is mainly caused by microorganisms. One of the pathogenic bacteria that can contaminate broiler meat is Salmonella sp. This research aims to determine the food safety level of broiler meat sold in traditional markets in the city of Banjarbaru regarding contamination by Salmonella sp. bacteria and to prevent the circulation of poultry-origin products (broilers) that do not meet the required standards, which can threaten consumer health. The research method used in this study is a survey and laboratory analysis. This study utilized 56 chicken meat samples from 4 traditional markets using a random sampling method. The test results showed that 18 samples were contaminated with Salmonella sp. bacteria out of the 56 samples. This indicates that the quality of chicken meat sold in some traditional markets, 32.14%, does not meet the standards based on the Indonesian National Standard for chicken meat (SNI 7388:2009)

Keywords: Broiler meat, contamination, traditional market, and *Salmonella* sp.

ABSTRAK

Daging merupakan bahan pangan yang penting dalam memenuhi kebutuhan gizi. Kerusakan yang menyebabkan penurunan mutu daging segar, terutama disebabkan oleh mikroorganisme. Salah satu bakteri patogen yang dapat mengontaminasi daging *broiler* adalah *Salmonella* sp. Tujuan yang ingin dicapai dari penelitian ini mengetahui tingkat keamanan pangan daging broiler yang dijual di pasar tradisional yang ada di Kota Banjarbaru terhadap cemaran bakteri *Salmonella* sp. dan mencegah beredarnya produk

pangan asal ternak (broiler) yang tidak memenuhi syarat yang dapat mengancam kesehatan konsumen. Metode penelitian yang digunakan adalah survei dan analisis laboratorium. Penelitian ini menggunakan 56 sampel daging ayam berasal dari 4 pasar tradisional dengan metode random sampling. Hasil pengujian terdapat 18 sampel Positif tercemar bakteri *Salmonella* sp dari 56 sampel. Hal ini menujukkan bahwa kualitas daging ayam yang dijual di beberapa pasar tradisional 32.14% tidak memenuhi standar berdasarkan Standar Nasional Indonesia daging ayam (SNI 7388:2009).

Kata-kata kunci: Daging broiler, kontaminasi mikroba, pasar tradisional dan *Salmonella* sp.

INTRODUCTION

The Broiler meat plays a significant role in meeting the animal protein needs of the Indonesian population. According to available data, the average per capita consumption of broiler meat by the Indonesian population in 2021 was estimated to be around 0.14 kg per week, which is approximately 0.538 kg per capita per month or 6.456 kg per year. This consumption is higher than that of beef, which is 2.2 kg per capita per year (Badan Pusat Statistik, 2022).

Chicken meat is a favored food item among Indonesian people due to its ability to provide animal protein, as it contains a comprehensive range of essential nutrients, including protein, carbohydrates, fats, water, minerals and vitamins. Broiler chicken, at present time, is one of the leading contributors to animal protein, surpassing beef and remains a prominent commodity (Zelpina *et al*., 2020). Chicken meat is a food commodity rich in protein, fat, minerals and other essential nutrients required by the human body (Ken *et al*., 2016). The safety of animal food products for consumption relies on the absence of pathogenic microorganisms that could potentially harm the health of those who consume them. *Salmonella* sp. bacteria is one such pathogenic microbe commonly transmitted through food, *Salmonella* sp. outbreaks most commonly found in poultry products like broiler meat (Pires *et al.*, 2014)

Controlling microbial contamination in animal-derived foods is of utmost importance to prevent food product spoilage and reduce contamination risks. Therefore, the identification of microbial contamination, especially those that cause food borne illnesses, is crucial (Ken *et al*., 2016). Salmonellosis is a bacterial infection caused by *Salmonella* sp. bacteria. These bacteria can be found in animals, including chickens and cattle, and can be transmitted to humans through contaminated foods such as improperly processed meat, eggs and dairy products. Symptoms of salmonellosis in humans include diarrhea, nausea, vomiting, fever, and abdominal pain (Hedican *et al*., 2010; Bell *et al.*, 2016). In severe cases, *Salmonella* sp. infection to the elderly and immune-compromised individuals can lead to the disease becoming invasive and resulting in fatal outcomes due to occurrences such as bacteremia, sepsis, meningitis (Scallan *et al.*, 2011) dehydration, blood stream infections, and even death (Rosso *et al*., 2023). Hence, it is essential to ensure that consumed food has been properly processed and is free from *Salmonella* sp. bacterial contamination.

The prevalence of non-typhoidal Salmonella infections in Indonesia remains significantly concerning. Indonesia ranks among the nations with the highest occurrence of endemic food borne salmonellosis in Asia, trailing only China and India and surpassing Pakistan and Vietnam (Zelpina *et al.,* 2020). Contamination of broiler chicken meat by these bacteria usually occurs in traditional markets because microbial proliferation commonly takes place in these environments. Traditional market

settings are often associated with unclean and disorganized conditions, and chicken meat is often displayed without proper hygiene measures, making it susceptible to bacterial contamination (Zelpina *et al.*, 2018). Some of the bacteria that can lead to contamination in chicken meat include *Salmonella sp, Campylobacter,* and *Escherichia coli (E. coli)*. Aerita *et al*. (2014) prove that there is a correlation between the hygiene and sanitation levels of vendors and the contamination of *Salmonella* sp. in chicken meat. Therefore, it is crucial to ensure that chicken meat consumed has been processed correctly and is free from bacterial contamination. *Salmonella* sp. is a Gram-negative rod-shaped bacterium, classified within the Enterobacteriaceae family. It has a rod shape and does not form spores.

Based on the reason above, it is necessary to conduct research to detect the presence or absence of *Salmonella* sp. contamination in broiler chicken meat sold in the traditional markets of Banjarbaru. Information about the presence of *Salmonella* sp. contamination in broiler chicken products sold in the traditional markets of Banjarbaru can increase public awareness when buying and consuming such products.

RESEARCH METHODS

Materials

The material consists of 25 g of skinless broiler chicken meat taken from the thigh portion of each of the 56 chickens sold in four different traditional markets in Banjarbaru. The collected meat is placed in transparent plastic bags, labeled, and then stored in a cooling container. Subsequently, it is transported to the Banjarbaru Veterinary Investigation Center for microbiological examination.

Method

The research was conducted at the Banjarbaru Veterinary Investigation Center laboratories. Analysis of the meat samples involves several steps, including pre-enrichment, enrichment, isolation and identification, biochemical testing, and using the API 20 E kit.

Pre-enrichment. Weigh a 25 g sample of broiler chicken meat and put it in a sterile container. Add 225 mL Lactose Broth (LB) and homogenize using a bellyer for 1 minute. Incubate at 35ºC for 24 hours.

Enrichment. Take 0.1 mL of pre-enrichment culture then transfer it to 10 mL Rappaport Vassiliadis Broth (RV) medium. Homogenize using a vortex for 30 seconds. Incubate RV media at 42ºC for 24 hours.

Isolation and Identification. Colonies from the Rappaport Vassiliadis Broth (RV) are inoculated onto Hektoen Enteric Agar (HE) and Xylose Lysine Deoxycholate Agar (XLD) using the streaking method with an inoculating loop. The Petri dishes containing the streaked agar media are then incubated at a temperature of 35°C for duration of 24 hours. After the 24-hour incubation period, colonies on the HE and XLD media that are suspected to be *Salmonella* sp. will exhibit distinct characteristics. On HE, *Salmonella* colonies will appear bluish-green with black centers (indicating H2S production), while on XLD, most colonies will be pink, and nearly all will have black centers. These distinctive colony characteristics aid in the identification of *Salmonella* bacteria during the analysis process.

Biochemical Test. In the biochemical testing phase, colonies suspected of being *Salmonella* sp. from HE and XLD media are transferred to Nutrient Broth (NB) for 24-hour incubation. Subsequently, they are mo-ved to Bismuth Sulfite Agar (BSA) for another 24 hours at 37°C. Bismuth Sulfite Agar is used for further testing with the API 20E Kit, MacConkey and Oxidation-Fermentation (OF). Following the incubation, the colonies are

introduced to MacConkey (MC) and Oxidation-Fermentation (OF) media. Oxidation-Fermentation (OF) media has two variations, one with added paraffin oil (OF-F) and one without (OF-O), both incubated at 37°C for 24 hours. In MC media, positive Salmonella colonies become transparent, and the media turns yellow, indicating lactose nonfermentation. On OF-O, a yellow color signifies oxidative carbohydrate metabolism, and on OF-F, it indicates fermentative carbohydrate metabolism. A positive result on OF-F or OF-O shows a yellow color change, while negative results remain green or unaltered. The oxidase test involves taking a colony using an inoculating loop and placing it on an oxidase test paper. A color change to purple signifies a positive result. These biochemical tests are vital for confirming the presence of *Salmonella* bacteria in the samples.

KIT API 20 E. Create a suspension by taking suspected *Salmonella* sp. Colonies from Blood Agar (BA) media and introducing them into a 5 mL suspension medium with ose. Mix until homogeneous. Extract the suspension using a syringe and gently place it into each well of the strip, taking care to avoid bubbles. Fill the following wells halfway: O-nitrophenyl-beta-D-galactopyranoside (ONPG), Tryptophan Deaminase (TDA), Indole (IND), Glucose (GLU), Mannitol (MAN), Sorbitol (SOR), Rhamnose (RHA), Sucrose (SAC), Melibiose (MEL), Amygdalin (AMY) and Arabinose (ARA). For abbreviations with underlines, fill half the well and add paraffin oil, including Arginine Dihydrolase (ADH), Lysine Decarboxylase (LDC), Ornithine decarboxylase (ODC), Hydrogen Sulfide (H2S), and Urea (URE). Once all wells are filled, incubate for 24 hours at 37°C. After incubation, read the strip according to the API 20E reading table. For TDA, IND, VP, and GLU, additional reagents are needed. To perform an additional NO2 test, add one drop of NIT1 and NIT2 reagents into GLU and wait for 2 to 5 minutes. A red color indicates a positive result (Nitrogen Dioxide - NO_2). A negative reaction (yellow color) suggests nitrogen gas (N_2) reduction. For the oxidase test, use the API 20E oxidase test paper by placing BA media on the paper. A positive result is indicated by a purple color. Compare the obtained results with positive controls and refer to the API 20E reading table. Enter all positive and negative data into the software api-web™ for identification results. This detailed process ensures accurate detection and identification of *Salmo-nella* bacteria in the sample.

Data Analysis

Data analysis involves a descriptive approach to interpreting the test results of the samples. The reference used in this study is in accordance with SNI 7388:2009, which specifies the maximum permissible limits for microbial contamination in food. According to this standard, *Salmonella* sp. bacteria should be absent/negative per 25 g of the sample.

RESULTS AND DISCUSSION

The samples were collected from four different traditional markets in Banjarbaru with the condition shown in Table 2. The testing was conducted at the Laboratory of the Banjarbaru Veterinary Investigation Center. The results indicate that out of the 56 samples tested, 18 samples were found to be positive for *Salmonella* sp. After being tested through several stages, including isolation, identification, bio-chemical tests, and the API 20E KIT, it was observed that the positive results remained consistent across all test stages. The data were presented in Table 2.

Table 1. Overview of the markets, associated with sales area, drainage, good display and storage

Location	Condition			
	Sales Area	Drainage	Good Display	Storage
K	Dirty and wet	None	Open on table	Room temperature
S	Dirty and wet	None	Open on table	Room temperature
B	Moderately Clean	Available	Open on table	Room temperature
P	Dirty and wet	None	Open on table	Room temperature

Tabel 2. Sample test results obtain from four traditional markets in Banjarbru city

e	Location Code	Animal Sample	Code	Results
1	K1	Broiler Meat	STP1	Negative
2	K2		STP2	Negative
3	K3	Broiler Meat	STP3	Negative
4	K4	Broiler Meat	STP4	Positive
5	K5	Broiler Meat	STP5	Positive
6	K6	Broiler Meat	STP6	Negative
7	K7	Broiler Meat	STP7	Positive
8	K8	Broiler Meat	STP8	Positive
9	K9	Broiler Meat	STP9	Negative
10	S1	Broiler Meat	STP1	Negative
11	S2	Broiler Meat	STP2	Negative
12	S3	Broiler Meat	STP3	Negative
13	S4	Broiler Meat	STP4	Negative
14	S5	Broiler Meat	STP5	Negative
15	S6	Broiler Meat	STP6	Negative
16	S7	Broiler Meat	STP7	Negative
17	S8	Broiler Meat	STP8	Positive
18	S9	Broiler Meat	STP9	Negative
19	B1	Broiler Meat	STP1	Positive
20	B2	Broiler Meat	STP2	Negative
21	B3	Broiler Meat	STP3	Positive
22	B4	Broiler Meat	STP4	Positive
23	B5	Broiler Meat	STP5	Positive
24	B6	Broiler Meat	STP6	Positive
25	B7	Broiler Meat	STP7	Negative
26	B8	Broiler Meat	STP8	Negative
27	B9	Broiler Meat	STP9	Negative
28	B10	Broiler Meat	STP10	Negative
29	B11	Broiler Meat	STP11	Negative
30	B12	Broiler Meat	STP12	Negative
31	B13	Broiler Meat	STP13	Positive
32	B14	Broiler Meat	STP14	Negative
33	B15	Broiler Meat	STP1	Negative
34	P1	Broiler Meat	STP2	Negative
35	P2	Broiler Meat	STP3	Negative
36	P3	Broiler Meat	STP4	Negative
37	P4	Broiler Meat	STP5	Negative
38	P5	Broiler Meat	STP6	Positive
39	P6	Broiler Meat	STP7	Negative
40	P7	Broiler Meat	STP8	Negative
41	P8	Broiler Meat	STP9	Negative
42	P9	Broiler Meat	STP10	Positive
43	P10	Broiler Meat	STP11	Positive
44	P11	Broiler Meat	STP12	Negative
45	P12	Broiler Meat	STP13	Positive
46	P13	Broiler Meat	STP14	Positive
47	P14	Broiler Meat	STP15	Positive

48	P15	Broiler Meat	STP16	Negative
49	P16	Broiler Meat	STP17	Positive
50	P17	Broiler Meat	STP18	Negative
51	P18	Broiler Meat	STP19	Positive
52	P19	Broiler Meat	STP20	Negative
53	P20	Broiler Meat	STP21	Negative
54	P21	Broiler Meat	STP22	Negative
55	P22	Broiler Meat	STP23	Negative
56	P23	Broiler Meat	STP24	Negative

Notes : K, S, B, P: Location code for sampling; STP: Sample Code.

According to Table 2, the presence of *Salmonella* sp. in the sam-ples indicates that during sample collection at four different market loca-tions, the following findings were observed: In the market with code K, there were four positive *Salmonella* sp. samples out of nine tested. In the market with code S, one sample tested positive for *Salmonella* sp. out of nine. Market code B had five positive *Salmonella* sp. samples out of 15. Market code P had eight positive *Salmonella* sp. samples out of 24. Based on the calculation, out of the total 57 samples tested, 18 samples were found to be positive for *Salmonella* sp. with a prevalence of approximately 32.14%. Another research also showed that contamination is reported to be prevalent in chicken meat, constituting 46.1% in 168 sample (Pavelquesi *et al.,* 2023) and this aligns with the research conducted by Sartika *et al*. (2016), which stated that contamination of *Salmonella* sp. in traditional markets may occur due to the placement of clean chicken meat in open areas close to the internal organs, leading to potential contamination. Shah *et al.* (2012) also added that prevalence of *Salmonella* in chicken meat not only indicate poor sanitation but also indicates the health status of poultry as carriers of *Salmonella*. This is in line with the National Standard of Indonesia (SNI) ICS 67.120.20 from 2009, which sets the maximum allowable limit for bacterial contamination in fresh chicken meat at being negative for *Salmonella* sp. with a requirement that every 25g sample representing one fresh chicken should not contain *Salmonella* bacteria. However, according to the National Standard of Indonesia (2000), the maximum allowable limit for *Salmonella* sp. bacterial contamination in consumable meat is 1 x 10^4 CFU g^-1 (wherein 1 g of the sample should not contain more than 1,000 bacterial colonies).

The bacterial colony morphology on Bismuth Sulfite Agar can be observed in Figure 1, where the sizes vary, and they exhibit distinctive features such as a brown color surrounding the colonies that resembles a "rabbit's eye." This observation aligns with the results from Irianto (2006), who stated that Bismuth Sulfite Agar is a highly specific medium for isolating *Salmonella* sp. The presence of bismuth sulfite and brilliant green can inhibit the growth of Gram-positive bacteria. The Ferro Sulfite in the medium is transformed into H_2S, which plays a role in depositing iron, resulting in colonies that are brown, gray, or black, with a metallic sheen and the area around the colony typically appears brown, resembling a "rabbit's eye". The colonies identified as *Salmonella* sp. bacteria underwent further testing using Gram staining to determine the bacterial type. The test results revealed the presence of rod-shaped bacteria with a red or pink color, indicating that they belong to the group of Gram-negative bacteria. The Gram staining procedure began with the even application of crystal violet, followed by a 1 to 2-minute incubation period. Afterward, the samples were rinsed under running water, and iodine (lugol) was evenly added and left to incubate for 1 to 2 minutes. Following another rinse under running water,

safranin was evenly applied and incubated for 1 to 2 minutes. A 90% alcohol wash was then performed, and the excess water on the glass slide's surface was removed using a tissue. Finally, Oil Emulsion was added to enhance the light capture process, allowing the bacteria to become visible. Under the microscope, the Gram staining revealed that the colonies appeared red or pink and exhibited a rod-shaped morphology, indicating their Gram-negative nature.

To further ensure accuracy, additional testing was conducted using the API 20E kit. The choice of identification method using the API 20E kit was made due to its speed and ease of use. All the data obtained from the 20 mini-tubes or wells were entered into an identification table, allowing the bacterial species to be determined. After the incubation was completed, the strip was read based on the provided reading table in the API 20E kit. The addition of reagents was performed for the TDA, IND, VP, and GLU tests, and a positive result was indicated when the color turned purple. After all procedures were completed, a nine-digit numerical profile was obtained. The determination of the numerical profile values on the result sheet was divided into three groups of values: 1, 2 and 4 for each type of test, indicating specific outcomes. The assessment of each group was based on the positive reactions observed during the testing, resulting in a seven-digit profile number for 27 types of tests. The identification results were obtained using the apiweb^{TM} identification software.

In terms of the management and handling of Broiler Meat sales in traditional markets in Banjarbaru, it is generally subpar. Placing chicken on open tables in sales stalls carries a high risk of *Salmonella* sp. contamination. To prevent poor environmental conditions in the sale of fresh chicken meat, cleanliness in the selling areas must be maintained. According to Rortana *et al.* (2021) *Salmonella* sp contamination was more common in humid areas and was pointed out that dirty, humid, and odorous places can become breeding grounds for diseases caused by pathogenic bacteria.

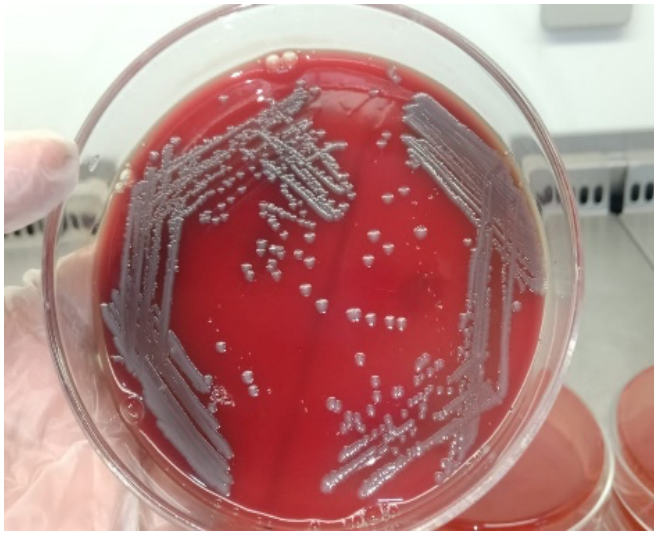

Figure 1. Bismuth Sulfite Agar (BSA) media with positive *Salmonella* sp

Thus, maintaining cleanliness in the selling areas is crucial. The condition of traditional markets can be observed in Figure 2. The placement of Broiler Meat for sale without any specific treatment, such as being left on sales tables, can affect the level of contamination and bacterial growth on the meat. Cross-contamination could also happen to the meat because unhygienic condition from the equipment like chopping board, knife and other contact surface which could

amplification the contamination of the carcass (Nidaullah *et al.,* 2017). The evisceration process can also lead to cross-contamination as it originates from fluids within the digestive tract of broiler chickens. During the evisceration stage, bacteria may contaminate the carcass, facilitating their easy spread to other parts of the carcass during the washing process or contaminating adjacent carcasses (Rivera-Perez *et al*., 2014). Sanitation could be improved by providing clean water for all activities, replacing used washing water, ensuring covered disposal areas, and maintaining the cleanliness of sales tools such as knives and cutting boards are essential practices (Aerita *et al.*, 2014). On the other hand, according to Trimoulinard *et al*. (2017), *Salmonella* sp. could survive up to 60°C. Therefore, for food items like chicken, if not processed immediately, it is advisable to store them in the freezer. During the cooking process, ensure that the chicken is thoroughly cooked at the appropriate temperature to eliminate any remaining bacteria, making the chicken safe for consumption

Figure 2. Banjarbaru Traditional Market Condition

Yulistiani *et al.* (2019) also reported that prevalence of *Salmonella* came from rinse water and intestinal contents. Broiler meat is a favorable medium for the growth of *Salmonella* sp., bacteria, because of the high water content, approximately 0.98-0.99 (Banerjee *et al.*, 2019), pH range of 5.7-6.7 (Putri *et al.*. 2022) and rich in nitrogen compounds, amino acids, proteins, minerals and vitamins, which serve as essential growth factors for bacteria (Bhaisare *et al*., 2014) . Afrianti *et al*. (2013) asserted that the duration of storage significantly influences the decrease in pH, whereby an extended storage period corresponds to a progressively declining pH level in the meat. Fresh chicken left without a cooling process will promote bacterial growth. The optimum growth temperature for *Salmonella* sp., is 20-45°C and exhibiting high resistance to desiccation, thriving at water activity (Aw) levels of 0.945-0.999, and capable of enduring for an extended period in dry products with a water activity of 0.200 (Trimoulinard *et al*., 2017) temperature control is important to prevent the growth of *Salmonella* sp., bacteria. Food should be stored under appropriate conditions because incorrect tempera-tures can allow *Salmonella* sp., bacteria to proliferate. Therefore, refrigerated fo-od products should be stored below 5°C, while heated or warm food products should be stored above 60°C.

Another factor that can contribute to the contamination of *Salmonella* sp., bacteria in the chicken meat could also be from the slaughterhouse. Contamination could occur at various stage of the food supply chain like distribution (Vest-

by *et al*., 2009). Poor transportation from the slaughterhouse to the market plays a role in microbial contamination (Wibi-sono *et al*., 2023). Muhaimi and Haifan (2019) advised that it should be transported using specialized meat transport designed with enclosed and refrigeration boxes to prevent external contamination.

Disease prevention in livestock can be achieved through good sanitation, transportation, providing a comfortable environment, vaccination programs, and biosecurity if practiced properly in the lengthy procedure of broiler meat process.

CONCLUSION

It can be concluded that chicken meat from several traditional markets in the city of Banjarbaru tested positive for *Salmonella* sp., bacteria, with a prevalence of approximately 32.14%. This result indicates that the quality of chicken meat sold in several traditional markets does not meet the standards outlined in the Indonesian National Standard for chicken meat.

SUGGESTION

It is hoped that the government will provide a policy regarding strict supervision of food sold to ensure food safety for consumers. As well as providing policies regarding improving good and healthy market infrastructure to mini-mize the amount of microbial conta-mination in chicken meat so that its safety is guaranteed. Further research needs to be carried out regarding *Salmonella* sp., bacterial contamination, but samples are taken from Chicken Slaughterhouses in order to find out where the source of contamination comes from

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