TESTING FOR BACTERIAL CONTAMINATION ON CHICKEN (Gallus gallus domesticus) IN THE TRADITIONAL MARKET OF SOUTH LANGOWAN DISTRICT

Delvino Stevy Sambeka*, Revolson A. Mege, Helen J. Lawalata, Iriani Setyawati, Christny F. E. Rompas

Biology Departement, Faculty of Matehematics and Natural Science, Universitas Negeri Manado, Indonesia.

*Corresponding author: Sambeka1991@gmail.com

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Abstract

Chicken meat (Gallus gallus domesticus) plays an important role in society with its role as one of the sources of fulfillment of animal protein in Indonesia. With the high consumption of chicken meat by the public, the hygiene and safety of chicken meat must be ensured so as not to incur losses to the people. This study aimed to determine the bacterial contamination based on the testing of Total Plate Counts, Coliform, Escherichia coli, and Salmonella sp. Meet the requirement of the Indonesian National Standard on chicken meat in the traditional market of Langowan Selatan District. The method used in this research is a descriptive method to analyze and describe the results of the study, the testing using several indicators to directly conclude bacterial contamination. The results of the total plate count bacterial contamination test of the colonies, TPC in sample $1 = 29.1 \times 10^6 \text{ kol/cm}^2$, sample $2 = 29.4 \times 10^6 \text{ kol/cm}^2$, sample 3= 27,7x10⁶ kol/cm². MPN of Coliform and Escherichia coli results obtained in samples S1, S2, and S3 are 11x10⁴ kol/gr. The results of microbact Salmonella sp. In samples S1 are positive, and S2 and S3 are negative. Based on observations, it is suspected that contamination occurs because sellers use equipment that is less sterile and there is no cover or barrier so the chicken meat is in direct contact with the air and customer. It is concluded that based on bacterial testing indicators, chicken meat in the traditional market of Langowan Selatan District has exceeded the limit of SNI 7388 2009 and is contaminated.

Keywords: Chicken Meat, Bacterial Contamination, Traditional Market

INTRODUCTION

Chicken meat (*Gallus gallus domesticus*) plays an important role in society with its role as one of the sources of fulfillment of animal protein in Indonesian society. The demand for chicken meat is increasing along with the increase in the level of consumption of chicken meat by the public. chicken meat is also a source of animal protein that is easily available and affordable. With the high consumption of chicken meat by the community, the hygiene and safety of chicken meat must be ensured so as not to

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cause harm to the community.

Angka Lempeng Total (ALT) or Total Plate Count (TPC) is a test used to determine the number of microorganisms both bacteria and fungi in food, cooking utensils, or cutlery (Muhammad *et al.*, 2022). Total Coliform is a group of bacteria used as an indicator of contamination (Himyatul *et al.*, 2022). *Escherichia coli* is a pathogenic bacteria, which acts as a major cause of morbidity and mortality worldwide (Taufik *et al.*, 2022). Salmonellosis is an infection caused by *Salmonella* sp. Which enters the body through contaminated food and drink (Ulinnuha *et al.*, 2022). It is expected that chicken meat sold by sellers in the Langowan Selatan District traditional market can meet the requirements of the Indonesian National Standard to ensure bacterial contamination.

Table 1. Maximum limit of microbial contamination in fresh, frozen meat (carcass and boneless) and minced meat.

Jenis Cemaran Mikroba	Batas Maksimum
Angka Lempeng Total (ALT) Coliform	1×10^6 koloni/gr 1×10^2 koloni/gr
Escherichia Coli Salmonella sp.	$5 imes 10^1$ koloni/gr negatif/25gr

Based on on-site observations, chicken meat sold by sellers in the Langowan District traditional market is cut manually by the sellers with simple equipment, there is no cover or glass barrier on the chicken meat and there is direct contact with customers and the surrounding air which can result in cross-contamination. Traditional markets are one of the places that have a high possibility of contamination and multiplication of microbes (Putu *et al.*, 2022). This study was conducted to look at bacterial contamination through the total plate count test, Coliform test, *Escherichia coli* test, and *Salmonella* sp. Test on chicken meat in the traditional market of Langowan Selatan District.

Broilers are a subspecies of the red partridge (*Gallus gallus*), broilers (*Gallus gallus doemsticus*) are mainly farmed for their meat which is a source of protein for the public. Chicken meat is one of the most popular foods in the public because, in addition to its delicious taste, it also has a high nutritional content, at an affordable price. In addition, broiler meat has a soft fiber texture that is easily digested, as a result, people generally prefer chicken meat as a source of animal protein compared to beef and goat meat (Isye et al., 2020). Traditional markets are one of the places that have a high possibility of contamination and multiplication of microbes. This is due to the lack of awareness of sellers regarding the health and hygiene of the meat that is being sold, where chicken meat sold can be contaminated by pathogenic microorganisms, when serving chicken meat. if not handled properly it will have adverse effects on human health (Putu et al., 2021). This study aimed to determine bacterial contamination in chicken meat in the traditional market of Langowan Selatan District, based on the total plate count test, Coliform test, *Escherichia coli* test, and *Salmonella* sp. Test, following the Indonesian National Standard.

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RESEARCH METHODS

Materials used in this study are Aquades, alcohol, plate count agar (PCA), buffered peptone water (BPW), brilliant green lactose broth (BGLB), lauryl typtose broth (LTB), *Escherichia coli* broth (ECB), and *Salmonella* shigella agar (SSA).

This study uses a descriptive method to analyze and describe the results of the study, testing uses several indicators to directly conclude bacterial contamination. Samples were collected from Langowan Selatan Subdistrict, Minahasa Regency, North Sulawesi, Indonesia. with bacterial contamination testing at the Biology laboratory of the Faculty of Mathematics, Natural and Earth Sciences, Universitas Negeri Manado, and the laboratory Balai Teknik Kesehatan Lingkungan Dan Pengendalian Penyakit (BTKLPP) Kelas 1 Manado. The samples used were chicken carcasses sold by sellers and often consumed by people in the traditional market of Langowan Selatan District. There were 3 chicken meat samples taken from 3 different chicken meat sellers in the traditional market of Langowan Selatan Subdistrict. Bacterial contamination test indicators were Total Plate Numbers, Coliform, Escherichia coli, and Salmonella sp.

Sample preparation, the sample was weighed as much as 25 grams of chicken carcass parts and minced, 225 ml of sterile buffer solution was put into a glass bottle with the sample, the suspense was then homogenized for 2 minutes. This solution included the first dilution 10^{-1} .

Total Plate Count (TPC) method, 1 ml of the suspense of sample dilution 10^{-1} was transferred with a sterile pipette into a test tube with 9 ml of buffered peptone water to obtain dilution 10^{-2} . Dilutions 10^{-3} 10^{-4} 10^{-5} were made in the same way as before. 1 ml of suspense was taken from each dilution and put into Petri dishes in duplicate. 15 ml of plate count agar that had been cooled to 45°C was added to each petri dish that contained the suspense. To mix the sample and media, the petri dish was rotated back and forth in a figure-of-eight shape and left to cool until it became solid. The suspense was incubated at 37°C for 24 hours by placing the dish upside down (National Standardization Agency, 2008).

Coliform and *Escherichia coli* Most Probable Number (MPN) method, in the estimation test, 1 ml of dilution solution 10^{-1} is transferred with a sterile pipette into a tube containing 9 ml buffered peptone water to obtain dilution 10^{-2} , Dilution 10^{-3} is made in the same way. 1 ml of each dilution was pipetted into 3 series of 9 ml lauryl typtose broth tubes containing durham. The suspension was incubated at 37°C for 48 hours. The test result is positive if gas is formed. In the confirmation test, positive tubes from each lauryl typtose broth media were transferred into brilliant green lactose broth (BGLB) tubes for Coliform testing and tubes containing *Escherichia coli* broth (ECB) media for testing *Escherichia coli* bacteria that had been filled with Durham. The suspense was incubated at 37°C for 48 hours. The test result is positive if gas is formed. The Most Probable Number (MPN) table is used to determine the MPN value based on the number of positive BGLB and ECB tubes as the number of Coliform and Escherichia coli per milliliter or gram. With the calculation formula: MPN sample = MPN table value/100 x dilution factor in the middle (National Standardization Agency, 2008).

Identification of Salmonella sp. For bacterial enrichment, 1 ml of dilution solution 10⁻¹ was

transferred with a sterile pipette into 9 ml buffered peptone water solution to obtain dilution 10^{-2} , 1 ml of dilution solution 10^{-2} was transferred with a sterile pipette into 9 ml buffered peptone water solution to obtain dilution 10^{-3} , in the same way, dilution 10^{-4} , 10^{-5} , 10^{-6} were made. Each suspense from dilutions 10^{-4} , 10^{-5} , and 10^{-6} is taken 1 ml and put into 3 tubes series containing 9 ml lauryl typtose broth with inverted Durham. The tubes were incubated at 37° C for 48 hours (Kartika *et al.*, 2014). Selective test, the tube from the positive presumptive test (gas formed) was stirred with a vortex. Then the suspense solution was spread on the surface of the specific medium Salmonella shigella agar (SSA) and flattened using an inoculation needle, each dilution series was made 3 times. SSA medium was incubated at 37° C for 24 hours. If *Salmonella* sp. Colonies grow, the colonies will be colorless with a large black core in the middle. Then *Salmonella* sp. Colonies that grow on SSA media are taken with a sterile inoculation needle and then put into the buffer and then mixed. After that, 2-3 drops of solution were poured into each microbact hole and incubated for 1 day for calculation (Narumi et al, 2009).

RESULTS AND DISCUSSION

Total Plate Count Testing

The results of total plate count testing on chicken meat samples obtained, TPC results in samples S1, S2, and S3 dilutions 10^{-3} , 10^{-4} there are more than 300 colonies, and bacterial colonies at dilution 10^{-5} in each sample are S1 = 291, S2 = 294, S3 = 277. The counting was done on agar media with microbial populations between 30-300 to avoid calculation or testing errors. The media is plate count agar that incubated for 24 hours, all colonies that grow will be counted with a colony counter.

Table 2. Test results using the ALT method

Sampel	Pengenceran _	Jumlah Koloni		ALT (kol/ _{cm²})
Campoi	i chigoriocian =	Cawan 1	Cawan 2	, , , , , , , , , , , , , , , , , , ,
S1	10^{-3}	TBUD	TBUD	
	10^{-4}	TBUD	TBUD	
	10^{-5}	295	287	29,1 x 10 ⁶
S2	10^{-3}	TBUD	TBUD	
	10^{-4}	TBUD	TBUD	
	10^{-5}	297	291	29,4 x 10 ⁶
S3	10^{-3}	TBUD	TBUD	
	10^{-4}	TBUD	TBUD	
	10^{-5}	284	270	27,7 x 10 ⁶

Information:

ALT: Angka Lempeng Total

TBUD : Terlalu banyak untuk dihitung

The safety and hygiene of food products must be ensured and maintained so as not to cause harm to the community. One way to determine the presence of microbial contamination in food products is by conducting microbiological examinations. This examination is an indicator of bacterial contamination that exceeds the maximum limit standard in food products. The Total Plate Count (ALT) method is one way to

calculate the number of bacterial colonies contained in the test sample.

Based on the study data of bacterial contamination tests on chicken meat in the traditional market of Langowan Selatan sub-district by Total Plate Count obtained. From the colonies counted with a colony counter, the TPC results in sample $1 = 29.1 \times 10^6$ kol/gr, in sample $2 = 29.4 \times 10^6$ kol/gr, in sample $3 = 27.7 \times 10^6$ kol/gr. Based on the research data, the three samples did not meet the maximum TPC value of 1×10^6 kol/gr in the requirements of SNI 7388 of 2009 regarding the maximum limit of microbial contamination in meat.

Bacterial contamination can cause damage to meat in the form of changes in the color and texture of chicken meat or chicken meat that looks slimy. Bacteria can cause food to smell bad and mucus to appear, the longer the handling time, the more contamination by bacteria occurs (Isye, 2020). High levels of bacterial contamination can be caused by contaminated equipment and the lack of hygiene standards in the marketplace. Traditional markets are one of the places where buying and selling transactions take place that are vulnerable to contamination and bacterial proliferation. The lack of awareness of traders regarding the cleanliness of meat processing equipment and selling places can make meat contaminated by pathogenic microorganisms that if not properly treated will have a negative impact on human health (Marantika and Yuliandi, 2022). Based on observations made in the traditional market of Langowan Selatan Subdistrict, it is suspected that contamination occurs because sellers use less sterile equipment and there is no cover or barrier so that chicken meat is in direct contact with the air and customers.

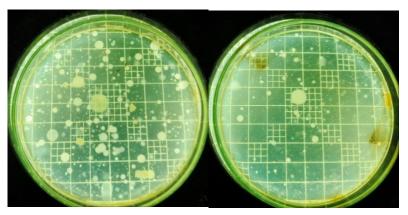


Figure 1. counting bacterial colonies that grow on plate count agar media, using a colony counter.

Most Probable Number Coliform Testing

The MPN method is used to detect and estimate the number of Coliform bacteria found in chicken meat. The MPN test is carried out through 2 stages, the presumptive test and the confirmed test. The presumptive test and confirmation test are conducted to check for the presence of lactose fermenter bacteria that can produce gas. The formation of gas bubbles that occur in the Durham tube is caused by the carbohydrate fermentation reaction.

The presumptive test is used as an initial step to detect the presence of Coliform bacteria, positive results are characterized by the formation of gas in the Durham tube. The confirmation test (completed test) is then used to further confirm that the bacteria tested are Coliform bacteria. the media used is BGLB

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which is a selective media that can ensure that the bacteria found in chicken meat samples are correct Coliform bacteria. Testing is conducted using a series of 3 tubes.

Table 3. MPN Coliform test results

Kode		Media Bakteri		MDNI/
Sampel	Pengenceran	LTB	BGLB	MPN/gr
S1	10 ⁻¹	Positif	Positif	>1100
	10^{-2}	Positif	Positif	>1100
	10^{-3}	Positif	Positif	>1100
S2	10^{-1}	Positif	Positif	>1100
	10^{-2}	Positif	Positif	>1100
	10^{-3}	Positif	Positif	>1100
S3	10^{-1}	Positif	Positif	>1100
	10^{-2}	Positif	Positif	>1100
	10^{-3}	Positif	Positif	>1100

Information:

LTB : Lauryl typtose broth

BGLB : Brilliant green lactose broth MPN : Most probable number

Coliform is a group of bacteria used as an indicator of pollution and unsanitary conditions in water or food. The presence of Coliform bacteria in food or drink indicates the possibility of enteropathogenic and or toxigenic microbes that are harmful to human health. Based on the MPN results of the Coliform bacteria contamination test on chicken meat in the traditional market of Langowan Selatan Subdistrict, the results of the MPN calculation on samples S1, S2, and S3 were 11x10⁴ kol/gr. Based on the study result, the three samples did not meet the maximum limit requirements for the MPN Coliform value of 10² kol/gr based on the standard by SNI 7388 of 2009 regarding the quality requirements for the maximum limit of microbial contamination in meat.

Traditional markets are one of the meat marketing places that are vulnerable and at high risk of pathogenic bacterial contamination. Contamination can be caused by poor equipment hygiene, postcutting meat handling, meat storage, storage time, equipment cleanliness, and market environment (Apriyanti et al., 2020). Based on the observations made, chicken meat is cut in the same place with the same cutting equipment, allowing contamination between chicken meat. The processing such as feather plucking and cleaning of chicken meat can also be a contributing factor to the contamination of chicken meat.

26 Sambeka et al., 2024

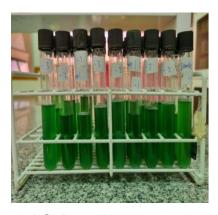


Figure 2. the results of incubation with BGLB, positive results were indicated by the formation of gas in the Durham tube

Most Probable Number Escherichia Coli Testing

The MPN method is used to detect and estimate the number of *Escherichia coli* bacteria found in chicken meat. The MPN test is carried out through 2 stages, the presumptive test and the confirmed test. The presumptive test and confirmation test are conducted to check for the presence of lactose fermenter bacteria that can produce gas. The forming of gas bubbles in the Durham tube is caused by the carbohydrate fermentation reaction.

The presumptive test is used as an initial step to multiply and detect the presence of *Escherichia coli* bacteria where positive results are signified by the presence of gas in the Durham tube. The confirmation test (completed test) is used to confirm that the bacteria tested are *Escherichia coli* bacteria. the media used is *Escherichia coli* broth, which is a selective media that can ensure that the bacteria found in chicken meat samples are correct *Escherichia coli* bacteria. Testing was carried out using a series of 3 tubes.

Table 4. MPN Escherichia coli results

Kode	Dongonooron	Media Bakteri		MDN/ar
Sampel	Pengenceran -	LTB	ECB	MPN/gr
S1	10^{-1}	Positif	Positif	>1100
	10^{-2}	Positif	Positif	>1100
	10^{-3}	Positif	Positif	>1100
S2	10^{-1}	Positif	Positif	>1100
	10^{-2}	Positif	Positif	>1100
	10^{-3}	Positif	Positif	>1100
S3	10^{-1}	Positif	Positif	>1100
	10^{-2}	Positif	Positif	>1100
	10^{-3}	Positif	Positif	>1100

Information:

LTB: Lauryl typtose broth ECB: Escherichia coli broth MPN: Most probable number

Escherichia coli is a common bacteria in contaminated or unclean water, usually, contamination occurs because water is contaminated with human or animal feces directly or indirectly. Escherichia coli is reported as a bacteria that intensively contaminates chicken meat, including broiler chickens. Not only contaminating fresh chicken meat but this bacteria was also found to contaminate frozen chicken meat (Zamra *et al.*, 2022). Based on the test results of Escherichia coli contamination test on chicken meat in the traditional market of Langowan Selatan sub-district by MPN, the results of MPN calculation on samples S1, S2, S3 were 11x10⁴ kol/gr. Based on the test results, the three samples did not meet the requirements of Escherichia coli MPN value of 1x10¹ kol/gr following the standard by SNI 7388 of 2009. regarding the quality requirements of the maximum limit of microbial contamination in meat.

The high percentage of the behavior of not cleaning equipment can cause cross-contamination because the knives used are not washed regularly, the more *Escherichia coli* attached to the knife that can be transferred to chicken meat (Putu *et al.*, 2022). Based on observations made, cutting chicken meat in the same place allows contamination between chicken meat. *Escherichia coli* itself is a common bacterium in the digestive system of living things, improper handling of chicken meat can make *Escherichia coli* in the chicken's digestive system contaminate chicken meat.

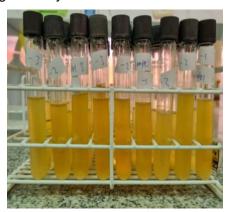


Figure 3. the results of incubation with ECB, positive results were indicated by the formation of gas in the Durham tube

Salmonella sp. Identification Testing

Tests were carried out on 3 samples of chicken carcass parts, which were taken from the traditional market of Langowan Selatan District, the samples were put into Lauryl typtose broth enrichment media for preparation for the next stage. At this stage, the bacteria will be multiplied so that it is easier to test and count the bacteria.

Selective testing is done to detect the presence or absence of *Salmonella* sp Bacteria in chicken meat samples. From selective testing of chicken meat samples with SSA media, sample S1 dilutions 10^{-4} and 10^{-6} contained *Salmonella* sp. Colonies and sample S3 dilution 10^{-4} also contained *Salmonella* sp. Colonies. Based on observations, positive samples are characterized by the formation of bacterial colonies with a black core in the middle, Salmonella sp colonies will then be taken to be tested with a microbact kit.

The test of *Salmonella* sp. From chicken meat samples using microbact is to ensure that the bacteria tested are correct *Salmonella* sp bacteria. From the next test with the microbact kit, the results of bacterial colonies in sample S1 dilution 10^{-4} are negative and for dilution 10^{-6} is positive, for sample S3 dilution 10^{-4} is negative.

Table 5. Salmonella sp. identification test results

Kode sampel	pengenceran	media	
	•	SSA	microbact
1	10^{-4}	positif	negatif
	10^{-5}	negatif	-
	10^{-6}	positif	positif
2	10^{-4}	negatif	-
	10^{-5}	negatif	-
	10^{-6}	negatif	negatif
3	10^{-4}	positif	-
	10^{-5}	negatif	-
	10^{-6}	negatif	-

Information:

SSA: Salmonella Shigella Agar

Salmonella sp. Is one of the main bacteria that cause major foodborne diseases, when consumed Salmonella sp. Can cause digestive system disorders (colitis), systemic infections, decreased absorption in the intestines, and fever. The risk of disease due to Salmonella sp infection is very large due to the lack of investigation of Salmonellosis in developing countries and limited laboratory studies. (Rosdiyanah *et al.*, 2021).

Based on the results of the study, *Salmonella* sp. Contamination test on chicken meat in the traditional market of Langowan Selatan District, the microbact results on sample S1 were positive, and samples S2 and S3 were negative. Based on the results of the study, one of the samples proved positive for Salmonella sp. and has exceeded the standard set by SNI, which is negative/25gr, which can endanger health if consumed.

Hygienic meat handling practices for *Salmonella* sp. Raw meat should be stored in separate bags apart from ready-to-eat foods to avoid cross-contamination. Storing meat is an important step. Raw meat/poultry should be stored in a sealed bag at the bottom of the refrigerator as early as possible. This limits the time for *Salmonella* sp. to grow and avoids dripping onto other foods. Freezing the meat before use will stop the growth of bacteria. Defrosting can be done in a tray at the bottom of the fridge. It is recommended to defrost 2.5 kg of meat or chicken after 24 hours. However, if defrosting is done in the microwave, it should be consumed immediately. Hands should be washed before and after handling raw meat. All types of meat need to be properly cooked before consumption to avoid the introduction of bacteria. For whole chicken, cooking should be at 180°C for 20 minutes (Olugbenga *et al.*, 2021). Based on observations, the handling of chicken meat is not following standards which can allow contamination. Chicken meat that is sold is only placed on the table, not provided with a cover, and in an open state.

Contamination of chicken meat will also increase if the equipment used for cutting such as knives and cutting boards is dirty (Siti *et al.*, 2018). In addition to the cleanliness of the chicken meat, the cleanliness of the place and equipment also needs to be maintained, and use good and correct procedures in processing chicken meat to prevent cross-contamination.

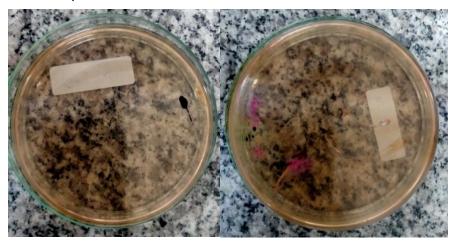


Figure 4. the results of incubation with BGLB media, positive results were indicated by the formation of colonies with black core.



Figure 5. positive results are stated with the microbact kit calculation

CONCLUSION

Based on the results of the study, it can be concluded that chicken meat in the Langowan Selatan Subdistrict from the Total Plate Counts test of the three samples is contaminated and exceeds the standard. The Coliform and *Escherichia coli* MPN tests of the three samples are contaminated and exceed the standard. The *Salmonella* sp. The test found that chicken meat in sample 1 was contaminated with *Salmonella* sp. This means chicken meat does not meet the requirements of the maximum limit of microbial contamination in meat following Indonesian national standard 7388 2009 and is contaminated.

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