Microbial Contamination in Chicken Meat in Traditional Markets in Banyuwangi

Prima Ayu Wibawati[1]*, Yunia Chrismonica[1], Racca Biana Susanti[1], Mario Putra Himawan[1], Afaf Heny Sintya Devy[2], Juwita Ayu Nirmalasari[2], Abzal Abdramanov[3]

[1]Study Program of Veterinary Medicine, Faculty of Health, Medicine, and Life Sciences, Universitas Airlangga, Banyuwangi, Indonesia, [2]Study Program of Aquaculture, Department of Health and Life Sciences, Faculty of Health, Medicine, and Life Sciences, Universitas Airlangga, Banyuwangi, Indonesia, [3]Department of Veterinary Sanitary Expertise and Hygiene, Faculty of Veterinary Medicine, Kazakh National Agrarian Research University, Almaty, Kazakhstan.

*Corresponding author: primaayuwibawati@fkh.unair.ac.id

Abstract

This study aimed to analyze the microbial contamination of chicken meat and its pH value from traditional markets in Banyuwangi, East Java to ensure public health. A total of 30 raw chicken meat samples from three traditional markets were used in this study and examined for the presence of *Escherichia coli* and *Salmonella* spp. *E. coli* and *Salmonella* spp. were identified using Gram staining, culturing, and biochemical tests. The Most Probable Number (MPN) method was used for coliform and *E. coli*. Measuring the pH value of chicken meat was carried out after purchasing the meat and 24 hours after storage at cold temperatures. The results showed coliform contamination in all samples with an average of >1100 CFU/G with *E. coli* contamination of 20% (6/30), exceeding the maximum recommended limit. Furthermore, the identification of *Salmonella* spp. in all samples showed negative results. Chicken meat pH value decreased with the length of postmortem time; immediately after purchase (0 hours), the pH value was 5.78 ± 0.26, and after 24 hours of storage, it raised 6.02 ± 0.28. In conclusion, there was high coliform contamination out of 30 raw chicken meat samples taken from some traditional markets in Banyuwangi. Furthermore, *E. coli* contamination was as much as 20%, and *Salmonella* spp. was negative.

Keywords: *Escherichia coli*, MPN, pH value, public health, *Salmonella* spp.

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INTRODUCTION

Food is a basic need for life, therefore, the fulfillment of food needs, especially food of animal origin, needs to be considered to ensure the needs of the customer and the safety of the food consumed. Based on the Center for Diet and Activity Research (CEDAR) (2014), the increased income and education of the community make it more critical to choose and assess the food ingredients needed. There are several causes of hazards in food, namely physical, chemical, and biological. One of the biological hazards in food is bacteria (Hidanah *et al*., 2023). Bacteria in food can cause foodborne diseases (BC Cook Articulation Committee, 2015).

Foodborne disease is both toxic and infectious, caused by disease agents that enter the body through the consumption of contaminated food. This disease is susceptible to infants, children, and the elderly whose immune system is compromised (WHO, 2022). Bacteria, parasites, toxins, and viruses can cause foodborne diseases (Wijaya *et al*., 2021; Haskito *et al*., 2023). Bacterial contamination is only 30% of cases of foodborne disease. However, several studies have shown that the highest outbreaks and mortality rates for foodborne diseases are caused by bacterial infections (Heredia and García, 2018). Foodborne disease transmission is generally through the mouth; ingesting and entering the digestive tract will cause clinical symptoms, including nausea, vomiting, and diarrhea (Syahrul, *et al*., 2020).

Chicken meat is highly consumed in Indonesia. The average consumption of broiler chicken in the national household will reach 6,048

kg per capita per year in 2021 (BPS, 2022). Chicken meat is an affordable source of animal protein, yet it is also easily digestible. Meat is the best source of essential nutritional elements and various vitamins that the body needs. Many of these minerals are not found in grains and vegetables. Meat contains high protein and micronutrients essential for health (Klurfeld, 2018). Based on Wardhana *et al*., 2021, chicken meat in Surabaya, East Java, identified *Salmonella* spp. and *E. coli* contamination. The presence of bacteria in the meat can affect the physical quality of meat, among others, the acidity of the meat (pH), the ability to bind water, and the value of cooking shrinkage (Soleha *et al*., 2018; Kartikasari *et al*., 2019). This study was carried out to investigate the number of contamination coliform, *E.coli*, and *Salmonella* spp. in chicken meat and its pH value from traditional markets in Banyuwangi.

MATERIALS AND METHODS

Ethical Approval

This study did not require ethical approval because there was no treatment of animals.

Study Period and Location

The study was carried out from March to July 2022 at the Laboratory of Veterinary Public Health, Faculty of Health, Medicine, and Life Sciences, Universitas Airlangga.

Isolation of Coliform

Each sample (25 g) was homogenized in Buffered Peptone Water (BPW, Merck, Germany). Samples were diluted 10^{-1}, 10^{-2}, and 10^{-3}, then 1 mL of each dilution was taken and put into a tube containing 5 mL of Lauryl Sulfuril tryptose Broth (LSTB, Merck) which contained an inverted Durham tube. Then incubated at 37°C for 48 hours. Followed by taking one loop of LB media using inoculation loops and transferring it to a test tube containing Brilliant Green Blue Broth (BGLB, Merck) media which contained an inverted Durham tube. Then incubated at 37°C for 48 hours. The test is positive if gas or bubbles form in the Durham tube. The number of cylinders that formed gas during the affirmation test was recorded and then adjusted to the MPN table (SNI 2897:2008).

Most Probable Number (MPN) Methods

The positive tube in the test for suspected coliform bacteria, 1 loop was taken and put into a test tube containing 9 mL of BGLB medium in which there was an inverted Durham tube. Then incubated at 37°C for 24–48 hours. The number of cylinders formed by gas was recorded and adjusted to the MPN table (SNI 2897:2008). The test tube which was suspected to contain *E. coli* (if gas was formed), was selected and taken 1 loop, then inoculated on Eosin Methylene Blue Agar (EMBA, Merck, Germany) medium. Followed by incubation at 37°C for 24 hours. A positive test for the presence of *E. coli* was indicated by a metallic green colony (Figure 1). Then, followed by gram staining and biochemical tests, namely, the IMViC test, consisting of indole production test, methyl red (MR) test, Voges-Proskauer (VP) test, and citrate test (Agatha *et al*., 2023).

Isolation and Identification of *Salmonella* spp.

Detection of *Salmonella* spp. in samples of raw chicken meat requires 25 g per sample, then put in 225 mL LB (Merck). then incubated at 35°C for 24 hours. 1 mL of the suspension sample was inoculated into 9 mL of Tetrathionate Broth (TB) (Merck, Germany) and then incubated at 35°C for 24 hours. Followed by taking one loop of TB media using inoculation loops and streaking on Bismuth Sulfite Agar media (Merck, Germany) and then incubating at 35°C for 24 hours. Typical colonies of *Salmonella* spp. analyzed by Gram staining, Lysine Iron Agar (LIA) media (Merck, Germany), and followed by biochemical tests (Triple Sugar Iron Agar (TSIA) (Merck, Germany), Simmons Citrate and urease test) (BSN, 2008).

Data Analysis

The data were presented descriptively in percentages and displayed in tables.

RESULTS AND DISCUSSION

The test results presented in Table 1 show Coliform contamination in all samples of >1100 CFU/G. This value exceeds the maximum recommended limit of 1×10^2 CFU/G, likewise with the results of the identification of *E. coli* in the sample showing that 20% of the sample exceeded the standard limit of SNI 7388:2009. Meanwhile, the results of identifying *Salmonella* spp. in all samples showed negative results. *E. coli* contamination in products of animal origin is widespread. In Blitar Regency, East Java, contamination of *E. coli* was identified from a raw goat milk sample of 100% (34/34) (Agatha *et al*., 2023). In Surabaya, East Java, high microbial contamination was identified in chicken meat from local markets, namely *S. aureus* 58.3%, *Salmonella* spp. 48.3% and *E. coli* 40% (Wardhana *et al*., 2021; Pisestyani *et al*., 2021).

Table 1. Number of contamination coliform, *E. coli*, and *Salmonella* spp. in chicken meat from traditional markets in Banyuwangi

Traditional market	N	Coliform		*E. coli*		*Salmonella* spp.
		Mean (CFU/G)	Positive Sample (%)	Mean (CFU/G)	Positive Sample (%)	
Banyuwangi	14	>1100*	14 (100)	< 3	0 (0)	Negative
Karangrejo Pujasera	8	>1100*	8 (100)	< 3	3 (37.5)	Negative
Blambangan	8	>1100*	8 (100)	< 3	3 (37.5)	Negative
Total	30	>1100*	30 (100)	< 3	6 (20)	Negative

Standard Limit Coliform: 1×10^2 CFU/G and *E. coli*: 1×10^1 CFU/G (SNI 7388:2009)
*exceeds the standard limit.

Table 2. pH value of chicken meat from traditional markets in Banyuwangi

Traditional market	N	Mean pH post storage	
		0 hours	24 hours
Banyuwangi	14	5.65 ± 0.15	5.83 ± 0.16
Karangrejo Pujasera	8	6.06 ± 0.30	6.32 ± 0.31
Blambangan	8	5.64 ± 0.04	5.90 ± 0.13
Total	30	5.78 ± 0.26	6.02 ± 0.28

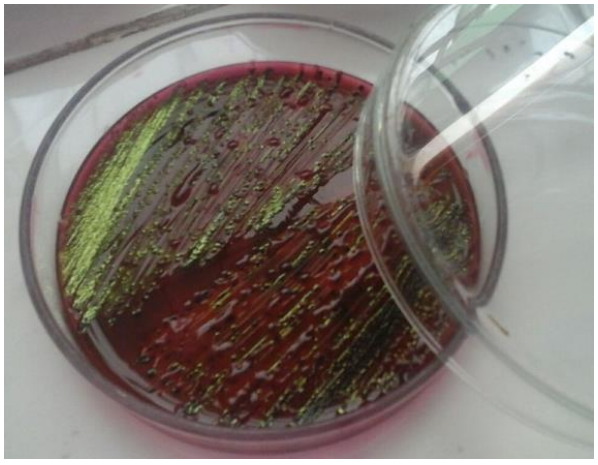

Figure 1. *E. coli* (metallic green color) on the EMBA growth media.

Coliform bacteria generally belong to four general of the Enterobacteriaceae: *Citrobacter freundii*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *E. coli*, and *Klebsiella pneumoniae* (Halkman and Halkman, 2014; Wardhana *et al*., 2019). In Indonesia, there are national quality standards for coliform and *E. coli* in foodstuffs, including poultry meat (Pradika *et al*., 2019). The results showed that the number of coliforms in all samples exceeded the maximum recommended

limit, while *E. coli* contamination exceeded the standard limit (1×10^1 CFU/G) is 20% of samples. Furthermore, these results indicate that 20% of broiler meat samples are not recommended and are unacceptable for public consumption (BSN, 2009). The presence of coliform and *E. coli* can cause health problems for consumers, especially if the meat is not cooked correctly (Fikri *et al*., 2022). Coliform bacteria contamination can be through the hands of the seller, unhygienic cutting so that bacteria from cutting tools can move to meat, from less sterile packaging, from water used to clean meat or cutlery which may be contaminated and from the meat itself (Collin and Huey, 2015; Dameanti *et al*., 2022).

E. coli is one of the indicator bacteria for sanitation (Price and Wildeboer, 2017). So the presence of *E. coli* in meat is greatly influenced by the process the meat is obtained or the treatment of the chicken after slaughter; in this case, it can be affected by, among other things: unclean water for washing, chicken holding tanks after depilation, means of transporting meat from the slaughterhouse to the market, cutting knives or tables where they sell in the market or from hand hygiene and clothes of chicken sellers and all objects that come in direct contact with meat (Fikri and Purnama, 2020). Pathogenic *E. coli* that causes diarrhea is classified into five groups: the pathogenic *E. coli* group, namely enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), hemorrhagic *E. coli* (EHEC), and Enteroaggregative *E. coli* (Nataro and Velarde, 2012; Wardhana *et al*., 2021). *E. coli* in the food consumed can cause digestive disorders in consumers (causing foodborne disease). *E. coli* is a facultative anaerobic species most commonly found in the human digestive tract (10^9 CFU/g feces), so the presence of this bacterium at a certain amount can be used as an indicator of fecal contamination in food and beverages (Abdurrahman *et al*., 2022). Some strains of *E. coli* produce enterotoxins or other virulence factors. The serotypes and pathogenic groups of *E. coli* are made based on their lipopolysaccharide (O) and flagella antigen (H) (Tham, 2012).

Pathogenic bacteria are a significant cause of the disease (foodborne disease) and death in less developed countries, killing around 500 thousand people per year and the world's third leading cause of death. In developed countries, pathogens that contaminate food are responsible for millions of cases of gastrointestinal infectious diseases each year and cost billions of dollars in medical care (WHO, 2022).

The study showed negative results for *Salmonella* spp. contamination; these results follow SNI 7388:2009. In contrast to the results of a study in Medan City at one slaughterhouse and three markets, which showed a positive result of *Salmonella* spp. contamination in one meat sample from Brayan Market and negative results from samples at three other locations (Purnama *et al*., 2019; Manurung *et al*., 2023).

Based on the results of the study showed a decrease in pH from 0 hours with a pH value of 5.96 to 5.78 after 24 hours of storage (Table 2). These results are consistent with the study of Mbaga *et al*. (2014), which stated that chicken breast meat had lower pH values compared to other parts of chicken meat, further, the pH decreased more rapidly, and thereafter, the decline was gradual at 4, 6, 12, and 24 hours of storage have pH values of 5.96, 5.90, 5.91, and 5.83 respectively. The pH value of chicken meat 24 hours of storage in the refrigerator was 5.71 ± 0.09, and the meat pH value decreased with the length of postmortem time (Wibawati *et al*., 2019). Two abnormal conditions of fowl meat known as Pale Soft Exudative (PSE) and Dark Firm Dry (DFD) meat might occur on meat as a result of short-term and long-term stress or poor animal welfare. The pH value of chicken meat can determine meat quality (PSE, normal or DFD), furthermore ranges to be considered are ≤ 5.8 (PSE), 5.9–6.2 (standard meat properties) and ≥ 6.3 (DFD) (Ristic and Damme, 2013).

CONCLUSION

It can be concluded that there was high coliform contamination out of 30 raw chicken meat samples taken from some traditional markets in Banyuwangi. Furthermore, *E. coli*

contamination was as much as 20%, and *Salmonella* spp. was negative. The pH value of the meat still meets the standards. These results are very important from the public health point of view. Moreover, these results have recommended the application of hygienic handling of chickens in slaughter, transportation of chicken carcasses, and tables and equipment that come into direct contact with chicken meat at points of sale.

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AUTHORS' CONTRIBUTIONS

PAW: Conceptualization and drafted the manuscript. AA and PAW: Validation, supervision, and formal analysis. YC, RBS, MPH, AHS, and JAN: Performed sample evaluation. AA and PAW: Prepared tables and figure. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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