



## Detection of Antibiotic Residues in Chicken Eggs from Traditional Markets in Maros Regency Using the Bioassay Method

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### Abstract

Chicken eggs stand as a cornerstone of food security and a vital source of affordable, high-quality animal protein for much of the Indonesian population. However, the food safety integrity of this strategic commodity is currently facing severe challenges due to the threat of antibiotic residues, which have been identified as a primary driver of the global crisis of Antimicrobial Resistance (AMR). The root of this issue often stems from suboptimal health management practices at the farm level, specifically the widespread negligence regarding the mandatory withdrawal period before harvest. This oversight results in the retention and accumulation of veterinary drug residues in animal products intended for human consumption. The presence of antibiotic residues in the food chain is not merely a technical livestock issue but a critical public health threat. Long-term exposure to low-dose residues can trigger bacterial resistance in the human body, leading to reduced efficacy of medical treatments (therapeutic failure) during infections. Furthermore, these residues pose potential risks of hypersensitivity reactions and toxicological effects on consumers. Addressing this urgency, this study was meticulously designed to identify the presence and calculate the prevalence of residues from four major antibiotic classes: Penicillin (PEN), Macrolide (MCR), Aminoglycoside (AMG), and Tetracycline (TCY). The research focused on commercial layer eggs circulated in traditional markets within Maros Regency, South Sulawesi, to analyze residue distribution patterns based on sampling locations. This study employed a cross-sectional design, collecting a total of 50 egg samples from four strategic districts: Turikale, Marusu, Lau, and Bantimurung. To ensure data validity, residue analysis was conducted at the Veterinary Public Health Laboratory of the Maros Veterinary Center (Balai Besar Veteriner Maros). The method utilized was a bioassay screening test strictly adhering to the Indonesian National Standard (SNI) 7424:2008. The laboratory investigation revealed critical findings: the overall prevalence of antibiotic residues was 12%, with 6 out of 50 samples confirmed positive. Specific results indicated that all positive samples were contaminated solely by Penicillin group residues. Meanwhile, residues from the Macrolide, Aminoglycoside, and Tetracycline groups were not detected in any of the tested samples. These findings highlight a specific dependency on beta-lactam antibiotics in the region and underscore the critical need for stricter monitoring and farmer education regarding responsible antibiotic usage to ensure consumer safety.

*Keywords: antibiotic residues, egg, bioassay*

## Introduction

Laying hens are a type of poultry farmed to produce eggs that have high nutritional value, are easy to process, and are widely available in the market. Chicken eggs are an important food source with a complete nutritional profile. However, it is important for consumers to pay attention to the quality of the eggs they consume to avoid negative impacts, such as diseases and damage that can endanger human health (Anton et al., 2020). Antibiotic residues in chicken eggs are detrimental to human health. These residues can arise from the improper use of antibiotics or incorrect treatment. Improper use of antibiotics can lead to the accumulation of large amounts of residues in livestock products, which can negatively affect human health, such as causing allergies. Antibiotic residues in eggs can occur if the antibiotic withdrawal period is not followed. This withdrawal period is the time when a substance is no longer found in a product, or the amount is within safe limits. Eggs from chickens undergoing treatment should be separated and not consumed until they are confirmed to be free of antibiotic residues (Aziz et al., 2021).

The World Health Organization (2014) states that antibiotic residues pose a serious threat to public health worldwide. Without immediate action, the continued rise in antibiotic residue cases will lead to antimicrobial resistance (AMR), which could cost the world more than \$3.4 trillion in annual gross domestic product (GDP) over the next 10 years. Antimicrobial resistance (AMR) is a growing global threat to human and animal health, significantly impacting food security, resilience, and the well-being of farming and livestock households (Food and Agriculture Organization, 2016). The magnitude of this crisis is evident in current mortality data; for instance, the European Union has reported that approximately 25,000 patients die annually from resistant microbial infections within its jurisdiction (European Centre for Disease Prevention and Control and European Medicines Agency, 2009). Looking ahead, the impact is projected to escalate drastically. It is estimated that AMR could result in 10 million deaths per year by 2050, with the highest burden of mortality expected to occur in Africa and Asia (O'Neill, 2014). To maintain food safety in livestock products, the Indonesian government banned the use of antibiotics as feed additives due to the risk of bacterial resistance through Minister of Agriculture Regulation No. 22 of 2017 concerning Feed Registration and Distribution.

In Indonesia, chicken eggs are an important source of animal protein and are widely consumed. Therefore, it is important to ensure the safety of chicken eggs for consumption and prevent their contamination with antibiotic residues. Previous studies have shown the presence of antibiotic residues in chicken eggs from several regions in Indonesia (Nurhidayah et al., 2015). However, data on the levels and types of antibiotic residues in chicken eggs sold in traditional markets, particularly in Maros Regency, are limited. Therefore, this study aimed to detect antibiotic residues in chicken eggs sold in traditional markets in Maros using a bioassay method. The results are expected to provide preliminary epidemiological data on the distribution of antibiotic residues in chicken eggs sold in Maros Regency and to inform policy recommendations for preventing antibiotic residues in animal-based food products, which will be formulated by the relevant government agencies.

### Antibiotic Use in Poultry Farming

The use of antibiotics is one method of controlling infections. The selection of appropriate antibiotics is crucial for the treatment of infectious diseases. A current global problem is

the inappropriate use of antibiotics. The irrational use of antibiotics in humans and animals has led to the emergence of antibiotic resistance. This poses a serious threat to human health and well-being and damages national economies worldwide, making treatment more difficult and requiring higher health-care costs (Noor et al., 2017). By 2030, antimicrobials are estimated to push as many as 24 million people into extreme poverty. Currently, at least 700,000 people die annually from drug-related conditions (Purnawarman and Efendi, 2020).

Antibiotics are not only used to treat and prevent bacterial infections but are also continuously added to animal feed to stimulate growth, increase feed efficiency, and reduce waste production. These are known as antibiotic growth promoters (AGPs). The use of AGPs in feed can accelerate growth but can also increase residues from livestock because livestock continuously consume feed containing antimicrobials until slaughter or laying eggs. The continued use of antimicrobials in chicken feed can increase the development of bacterial resistance (Noor et al., 2017). The potential dangers of antibiotic residues in laying hen farms are shown in Figure 1.

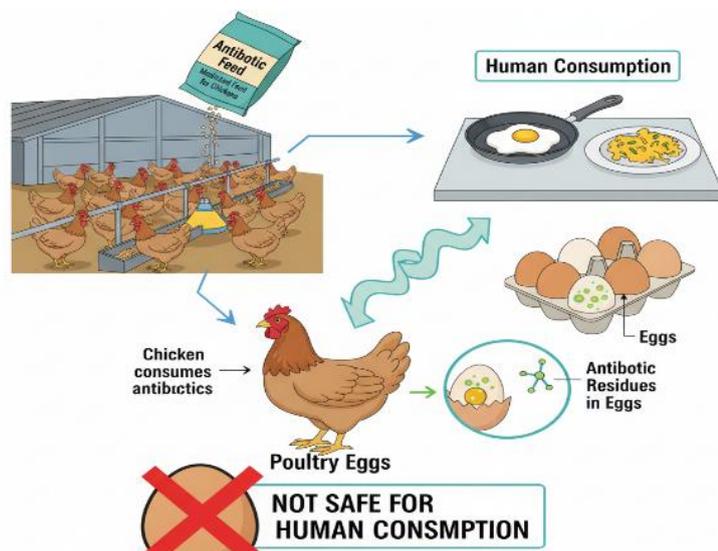


Fig. 1 Potential health risks from antibiotic residues

The practice of antibiotic use by farmers that does not comply with regulations is a factor contributing to the continued presence of antibiotic residues in broiler meat and eggs. A 2020 survey (Huong-Anh et al., 2020) on the knowledge, attitudes, and practices of 106 chicken meat samples showed that 27.4% of the samples tested positive for 11 types of antibiotics: fluoroquinolones, sulfonamides, tetracyclines, and macrolides. Fifty-nine percent of farmers needed to improve their knowledge of the proper antibiotic use. Although 54.7% of farmers had a positive attitude, only 17% had good practice. The survey results clearly indicate the lack of a harmonized approach to antibiotic monitoring per animal species and resistance evaluations. However, the official ban on AGP in feed in Indonesia began in January 2018, in accordance with Minister of Agriculture Regulation Number 22 of 2017 concerning Feed Registration and Distribution.

### Antibiotic Residues in Chicken Eggs

The use of antibiotics without regard for the drug's withdrawal period results in antibiotic residues in animal products. Antibiotic residues in chicken farms can have negative effects. This can lead to antibiotic resistance in humans, reduce the effectiveness of antibiotic treatment, and threaten public health. Furthermore, antibiotic residues in chicken eggs and meat can potentially cause side effects in consumers (Yani et al., 2022).

The dangers posed by the presence of antibiotic residues in animal-derived foods include increased resistance to several pathogenic microorganisms, mild-to-severe hypersensitivity reactions, and poisoning. Other impacts of antibiotic residues include allergic reactions, effects on the intestinal microflora, and immunopathology. Furthermore, residues can also cause carcinogenic, teratogenic, and mutagenic effects (Bayaou and Haile, 2017).

The effects of antibiotic residues in livestock feed initially received little attention. This is understandable because the concentrations of residues are generally so low that the effects are not immediately visible, and the resulting clinical symptoms are sometimes subtle. Monitoring residues and microbial contamination in animal-derived foods is crucial, especially for protecting consumer health and safety (Yani et al., 2022).

## **Materials and Methods**

### **Location and Time**

This study was conducted from March to July 2025 at the Integrated Veterinary Paramedic Laboratory of the Faculty of Vocational Studies, Hasanuddin University and the Veterinary Public Health Laboratory of the Maros Veterinary Center, South Sulawesi. Egg samples were collected from traditional markets in Maros Regency, covering four sub-districts (Turikale, Lau, Bantimurung, and Marusu).

### **Tools and Materials**

The tools used in this study were based on SNI 7424:2008 (BSN, 2008), namely 100 × 12 mm Petri dishes, 7, 20, and 50 ml test tubes, 50 ml centrifuge tubes, 50 and 100 ml volumetric flasks, 100 and 500 ml measuring cylinders, 250 and 500 ml Erlenmeyer flasks, 20 ml weighing bottles, 1, 2, 3, 5, 10, and 18 ml volumetric pipettes, 1, 5, 7, 10, 20 ml measuring pipettes, tube shaker, 3,000 rpm centrifuge, sterile cabinet, water bath, autoclave, refrigerator, analytical balance, three (3) types of incubators (30 °C ± 1 °C, 36 °C ± 1 °C, and 55 °C ± 1 °C), magnetic stirrer, pH meter, vernier caliper or other suitable tool for measuring the diameter of the inhibition area, 50 µL – 300 µL micro pipette, burner, loop, tweezers, and scissors. The materials used in this study were sodium penicillin as a reference standard for the penicillin group, oxytetracycline hydrochloride as a reference standard for the tetracycline group, kanamycin sulfate as a reference standard for the aminoglycoside group, tylosin-tartrate as a reference standard for the macrolide group, KH<sub>2</sub>PO<sub>4</sub> (potassium dihydrogen phosphate), Na<sub>2</sub>HPO<sub>4</sub> (disodium hydrogen phosphate), H<sub>3</sub>PO<sub>4</sub> (phosphoric acid), NaOH (sodium hydroxide), K<sub>2</sub>HPO<sub>4</sub> (dipotassium hydrogen phosphate), HCl (hydrochloric acid), NaCl (sodium chloride), and disc paper.

### **Sampling**

Sampling was conducted at the Bulu-bulu Market (Marusu District), Tramo Market (Turikale District), Bantimurung Market (Bantimurung District), and Barandasi Market (Lau District) using a purposive sampling method. Fifteen egg samples were collected from traditional markets in the Turikale and Marusu Districts and 10 from traditional markets in the Lau and Bantimurung Districts (each sample contained eight eggs). Sampling was performed aseptically. The samples were taken to the Integrated Veterinary Paramedic Laboratory for coding and then to the Maros Veterinary Center for testing for antibiotic residues using a bioassay method using *Bacillus stearothermophilus*, *Bacillus cereus*, *Bacillus subtilis*, and *Kocuria rhizophila*. The distribution of the number of samples per location is shown in Table 1.

<b>Districts</b>	<b>Number of Samples</b>
Turikale	15

Marusu	15
Lau	10
Bantimurung	10
<b>Total</b>	<b>50</b>

### Sample Testing

The collected egg samples were then tested for antibiotic residues in the Maros Veterinary Center laboratory using a bioassay test method, which is used to detect antibiotic residues in laboratories, based on SNI 7424:2008. The antibiotic residue testing procedure begins with the preparation of an agar medium by pouring approximately 15 ml of nutrient agar (NA) solution and bacteria into a Petri dish and allowing it to sit at room temperature until it solidifies. A 6 mm blank disc was inserted into the egg sample for approximately 10 seconds and then placed on the surface of the agar plate. The positive control, an egg sample treated with oxytetracycline, was dipped into the control solution for approximately 10 seconds. Incubation was performed at 54–60°C in a water-filled incubator for approximately 24 hours to prevent the medium from drying out due to evaporation. Changes were observed after 24 hours. A positive result was indicated by the formation of a zone around the disc, and a negative result was indicated by the absence of a zone (BSN, 2008). Antibiotic residue testing in the laboratory is performed by observing whether a zone of inhibition forms around the paper disc.

### Data Analysis

The analysis of the test results is presented in tabular form, and the antibiotic residue test results are presented descriptively in tables and figures.

### Results and Discussion

Paper discs with a diameter of 10 mm were used, and an inhibition zone of at least 14 mm ± 1 was considered a positive result. Figure 2 shows the results of penicillin residue detection in egg samples tested using a bioassay. Table 2 summarizes the findings on the antibacterial residues found in eggs sold in traditional markets in the Maros area.



Fig. 2 Positive result of penicillin residue

### Prevalence and Types of Antibiotic Residues

Of the 50 chicken egg samples tested from four sub-districts in Maros Regency, six samples (12%) tested positive for antibiotic residues. The remaining 44 samples (88%) tested negative. Further analysis of the residue types revealed that the six positive samples exclusively contained residues from the penicillin (PEN) group. None of the samples contained residues from the macrolide (MCR), aminoglycoside (AMG), or tetracycline (TCY) groups.

## Spatial Distribution and Statistical Analysis

The antibiotic residue findings showed uneven distribution across sampling locations. All positive samples were obtained from two districts, Turikale and Marusu. In Turikale, of the 15 samples tested, 4 (26.7%) tested positive for penicillin residues. In Marusu, two of the 15 samples (13.3%) tested positive for penicillin residues. In contrast, all samples from the Lau District (n=10) and Bantimurung District (n=10) tested negative for all antibiotic classes. A summary of the prevalence results is presented in Table 2.

Table 2. Antibiotic residues examination result

Districts	Number of Sampel (n)	PEN* Positive (%)	MCR* Positive (%)	AMG* Positive (%)	TCY* Positive (%)
Turikale	15	4 (26.7%)	0 (0%)	0 (0%)	0 (0%)
Marusu	15	2 (13.3%)	0 (0%)	0 (0%)	0 (0%)
Lau	10	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Bantimurung	10	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Total</b>	<b>50</b>	<b>6 (12,0%)</b>	<b>0 (0%)</b>	<b>0 (0%)</b>	<b>0 (0%)</b>

Information: \* PEN: penicillin; MCR: macrolide; AMG: aminoglycoside; TCY: tetracycline

The detection of elevated penicillin antibiotic residues in eggs is likely due to the prolonged and continuous administration of antibiotics via drinking water or feed at low concentrations. Penicillin disrupts the formation of bacterial cell walls, resulting in the rupture of the bacterial cell wall. However, excessive use of penicillin leads to resistance to antimicrobial agents. Certain bacterial groupings possess antibiotic-resistant characteristics, notably *Escherichia coli*, which can synthesize penicillinase, thereby degrading penicillin. Moreover, certain strains of *Staphylococcus aureus* and *S. pneumoniae* are resistant to penicillin. Numerous strains of *Enterobacteriaceae* exhibit intrinsic resistance to aminopenicillins, especially *E. coli* (Lobanovska and Giulia, 2017).

To determine whether the residual antibiotic content is still within the maximum standard residual limit or even surpasses it to the point where it can affect human health, the results of this study are still qualitative and need to be validated quantitatively by other methods, such as high-performance liquid chromatography-based techniques. Penicillin was found to be the predominant antibiotic in chicken liver found in Yogyakarta (Widiasih et al., 2019), according to the findings of this investigation, which also lends weight to the findings of other studies.

## Conclusion

Nevertheless, the results of our study are of interest since food animals, particularly eggs, were shown to be potent reservoirs of multidrug-resistant organisms. Thus, the high prevalence of antibiotic residues could be a source of resistance development among many bacterial strains. To decrease the resistance rate of bacteria, monitoring of resistance, surveillance, prudent use, research projects, awareness, and educational programs are recommended by the WHO (2017). The application of strict regulations on the use of antibiotics in the field of chicken farming and monitoring the presence of residues in livestock products before they are marketed is necessary to prevent the adverse effects of these residues on livestock-origin food products.

## Conflict of Interest

All authors declare that there are no conflicts of interest

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