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Antibiotic Resistance in *Escherichia coli* Isolated from Chicken Meat in Traditional Markets of Yogyakarta

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ABSTRACT

The widespread use of antibiotics in the livestock industry serves both as a preventive measure against diseases and as a feed additive. However, excessive antibiotic use has led to antibiotic resistance, where bacterial exposure to antibiotics renders them resistant to one or more antibiotic agents. This study investigates the presence of antibiotic resistance in *Escherichia coli* isolated from chicken meat sold in traditional markets in Yogyakarta. An experimental method was employed, using ECB and EMBA media for bacterial isolation. Gram staining was performed to characterize the morphology of *E. coli*, followed by molecular testing using the *uspA* gene. Antibiotic susceptibility testing was conducted using the Kirby-Bauer disk diffusion method. A total of 10 chicken meat samples were collected from five traditional markets in Yogyakarta. The antibiotics tested included ampicillin, ciprofloxacin, and chloramphenicol. The results indicated resistance in all 10 isolates. Sample codes G1 and G2 were resistant to all three antibiotics. Samples P1, P2, and L1 exhibited resistance to ampicillin and chloramphenicol, with intermediate resistance to ciprofloxacin. Sample L2 showed resistance to ampicillin, intermediate resistance to ciprofloxacin, and sensitivity to chloramphenicol. Sample B1 exhibited resistance to all antibiotics tested. Sample B2 was resistant to ampicillin and ciprofloxacin, with intermediate resistance to chloramphenicol. Samples K1 and K2 were resistant to ampicillin and chloramphenicol but sensitive to ciprofloxacin. These findings highlight the growing concern of antibiotic resistance in bacterial strains isolated from chicken meat, emphasizing the need for stricter regulations on antibiotic use in the poultry industry.

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1. Introduction

Chicken meat is a widely consumed source of animal protein due to its affordability, delicious taste, and tender texture. It contains a balanced composition of essential amino acids and high nutritional protein value. However, chicken meat is also highly susceptible to biological deterioration due to its high water and protein content, which provides a favorable medium for microbial growth. Contamination can occur during production, distribution, or at the market, particularly in unhygienic environments. One of the most common bacteria contaminating chicken meat is *Escherichia coli*, which poses a significant health risk if not handled and processed properly (Kartikasari et al., 2019).

Excessive antibiotic exposure in the poultry industry has led to antibiotic resistance in bacteria such as *E. coli*. Antibiotic resistance is a global issue affecting human and animal health, where bacteria acquire resistance genes, allowing them to survive exposure to one or more antibiotics. A major contributor to this issue is the use of Antibiotic Growth Promoters (AGP) in animal feed, which, while improving productivity, also increases the risk of resistant bacterial strains (WHO, 2017). Studies have shown that *E. coli* isolates from chicken meat exhibit high resistance levels to multiple antibiotics, raising concerns about public health (Januari et al., 2019). For example, ESBL-producing *E. coli* found from traditional market chicken meat has a high level of resistance to β-lactam antibiotics and contains the blaCTX-M-1 and blaTEM genes (Hasib et al., 2024). Meanwhile, a study in India reported that *E. coli* from chicken meat labeled antibiotic-free showed resistance to tetracycline (80%), ciprofloxacin (67%), and imipenem (49%), and carried resistance genes such as tetA, qnrS, ampC, strB, and sul3 (Rawat et al., 2024). Moreover, improper slaughtering and handling practices in traditional markets contribute to the spread of resistant bacteria. Chicken meat exposed to microbes from equipment, water, and unclean market environments can serve as a source of *E. coli* contamination. Hartadi (2019) reported that chicken meat sold in traditional markets had higher bacterial contamination levels compared to supermarket meat, which is typically handled under stricter hygiene standards. Therefore, monitoring meat quality and enforcing hygiene practices during distribution are crucial in reducing the spread of resistant bacteria.

The increasing antibiotic resistance in *E. coli* isolates from chicken meat also affects the effectiveness of bacterial infection treatments in humans. Infections caused by resistant bacteria are more challenging to treat, often requiring stronger or combined antibiotics, which in turn increases medical costs and the risk of side effects (Holmes et al., 2016). As a result, monitoring antibiotic resistance in bacteria from chicken meat is essential for mitigating health risks in the community. The high prevalence of antibiotic-resistant *E. coli* in chicken meat has significant implications for food safety and public health. The presence of resistant bacteria in food products increases the risk of transmission to humans, either through direct consumption or cross-contamination in food preparation areas. Additionally, resistant *E. coli* can serve as a reservoir for resistance genes that may spread to other pathogenic bacteria, further complicating infection control measures (Van Boeckel et al., 2019). Addressing this issue requires integrated efforts in food safety regulations, surveillance programs, and antibiotic stewardship in poultry farming to minimize the spread of resistant bacteria. The level of antibiotic resistance in E. coli originating from chicken meat in traditional markets, especially in the Yogyakarta region, has not been widely reported until now. Thus, this study aims to determine the presence of *Escherichia coli* contamination in chicken meat from five traditional markets in Yogyakarta and assess the antibiotic resistance of *Escherichia coli* isolates obtained from chicken meat sold in traditional markets in Yogyakarta.

2. Methods

2.1. Sample collection

Chicken meat samples were collected from five traditional markets in Yogyakarta, including Beringharjo, Kranggan, Legi Kotagede, Prawirotaman, and Giwangan Market in June 2023. Two samples were taken from each market using purposive sampling from both the outer and inner sections of the market.

2.2. Sterilization of equipment

All laboratory equipment was washed with clean water and dried using a cloth or tissue. Test tubes and Erlenmeyer flasks were covered with cotton, while Petri dishes and measuring pipettes were wrapped in newspaper. All equipment was sterilized in an incubator at 170°C for 2 hours.

2.3. Preparation of culture media

Several types of culture media were prepared individually, including Eosin Methylene Blue Agar (EMBA), Nutrient Agar (NA), Nutrient Broth (NB), MacConkey Broth (ECB), and Mueller-Hinton Agar (MHA). Media components were weighed, mixed with distilled water, heated until homogeneous, and sterilized using an autoclave.

2.4. Preparation of 0.85% NaCl solution and McFarland standard 0.5

A 0.85% NaCl solution was prepared by dissolving 0,85 g of NaCl in 100 mL of distilled water and sterilized by autoclaving. McFarland standard 0.5 was prepared by mixing 0.05 mL of 1% BaCl2 with 9.95 mL of 1% H2SO4 and stored in a light-protected environment.

2.5. Isolation of *Escherichia coli*

Isolation of E. coli was carried out based on the method of Sudarmadi et. al. (2020) with modification. Ten test tubes containing 9 mL of ECB medium were prepared. One gram of chicken meat was added to each tube and incubated in a water bath at 44°C for 24 hours. A 1 mL aliquot from positive tubes (containing Durham tubes with gas bubbles) was streaked onto EMBA plates and incubated at 37°C for 24 hours. Colonies exhibiting a metallic green sheen were presumptively identified as *E. coli*.

2.6. Preparation of stock culture on slant NA medium

Metallic green colonies from EMBA plates were transferred to slant NA tubes aseptically and stored in a refrigerator as stock cultures.

2.7. Gram staining

Gram staining was performed to confirm bacterial morphology. Smears were prepared, heat-fixed, and stained sequentially with crystal violet, iodine, 70% alcohol, and safranin. The stained slides were examined under a microscope at 1000x magnification.

2.8. Molecular identification of *Escherichia coli*

Bacterial DNA was extracted using the colony PCR method with amplification of the *uspA* gene (Chen & Griffiths, 1998). Specific primer *uspA* target was used 5'-CCGATACGCCTGCCAATCAGT-'3 and 5'-ACGCAGACCGTAGGCCAGAT-3'. The PCR reaction was prepared using the following components: 6 µL of Dream Taq Green PCR Master Mix (2X), 0.5 µL of *uspA* forward primer (10 pmol/µL), 0.5 µL of *uspA* reverse primer (10 pmol/µL), 1 µL of DNA template, and 4.5 µL of ddH2O. The PCR was performed under the following conditions: initial denaturation at 95°C for 2 minutes, denaturation at 94°C for 30 seconds, annealing at 58°C for 1 minute, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes. Steps 2–4 were repeated for 30 cycles. The PCR products were analyzed using 1.5% agarose gel electrophoresis with DNA Nucleic Acid Dye from Promega for staining and visualized under a UV transilluminator.

2.9. Antibiotic susceptibility testing (Kirby-Bauer method)

E. coli cultures were grown in NB medium and incubated at 37°C for 16-18 hours. Bacterial suspensions were adjusted to match the McFarland 0.5 standard and spread onto MHA plates. Antibiotic discs (ampicillin, ciprofloxacin, and chloramphenicol) were placed on the agar surface and incubated at 37°C for 24 hours. Inhibition zone diameters were measured and classified according to CLSI guidelines as sensitive, intermediate, or resistant.

2.10. Data analysis

Quantitative data were obtained by measuring inhibition zone diameters (mm) with two times replication. Data were analyzed descriptively and presented in tables and figures. Antibiotic resistance profiles were compared to CLSI standards, and resistance percentages were calculated to determine the resistance patterns of *E. coli* isolates.

3. Results and Discussion

3.1. Bacterial isolation

All samples tested positive in ECB, and subsequent streaking on EMBA plates resulted in metallic green colonies with dark-centered formations, characteristic of *E. coli*. Gram staining confirmed the presence of Gram-negative bacilli, supporting the identification of *E. coli* (Rachmawati & Ariyanti, 2017). The role of EMBA as a selective medium for *E. coli* is crucial, as it contains lactose, which, upon fermentation, produces a characteristic metallic green sheen (Hidayah et al., 2022).

Table 1. Bacterial isolation results on ECB and EMBA media

Sample Code	ECB Turbidity	Gas in Durham Tube	EMBA Colony	Gram Staining
G1	Positive	Positive	Metallic Green	Gram-negative Bacillus
G2	Positive	Positive	Metallic Green	Gram-negative Bacillus
P1	Positive	Positive	Metallic Green	Gram-negative Bacillus
P2	Positive	Positive	Metallic Green	Gram-negative Bacillus

Sample Code	ECB Turbidity	Gas in Durham Tube	EMBA Colony	Gram Staining
L1	Positive	Positive	Metallic Green	Gram-negative Bacillus
L2	Positive	Positive	Metallic Green	Gram-negative Bacillus
B1	Positive	Positive	Metallic Green	Gram-negative Bacillus
B2	Positive	Positive	Metallic Green	Gram-negative Bacillus
K1	Positive	Positive	Metallic Green	Gram-negative Bacillus
K2	Positive	Positive	Metallic Green	Gram-negative Bacillus

Note: G1–K2 are chicken meat samples taken from the inner (1) and outer (2) parts of meats collected at Giwangan (G), Prawirotaman (P), Legi (L), Beringharjo (B), and Kranggan (K) markets.

Figure 1. Confirmation of *E. coli* growth on EMBA media

3.2. Microscopic observation

Microscopic observation using Gram staining further confirmed that all isolated bacteria exhibited characteristics of *E. coli*, appearing as red-colored bacilli under a 1000x magnification microscope. Gram-negative bacteria retain safranin after decolorization, resulting in a red coloration (Putri et al., 2023). The reaction differences are due to variations in bacterial cell wall structures, where Gram-negative bacteria possess only 5-20 layers of peptidoglycan, unlike Gram-positive bacteria, which contain up to 90 layers along with teichoic acids (Madigan et al., 2011).

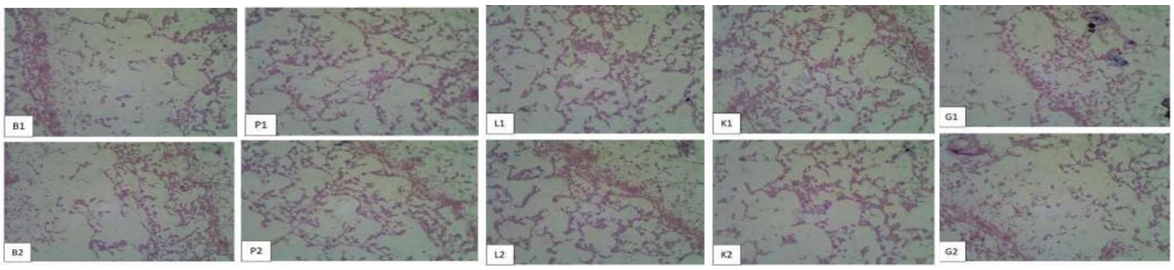

Figure 2. Gram staining of *E. coli* at 1000x magnification

3.3. Molecular identification

Molecular identification was conducted using PCR amplification of the *uspA* gene, a universal stress protein gene in *E. coli* that plays a role in bacterial survival under unfavorable environmental conditions. The presence of this gene serves as a specific molecular marker for identifying E. coli in various samples, including contaminated chicken meat (Chen & Griffiths, 1998). Polymerase Chain Reaction (PCR) is commonly used to detect the uspA gene, ensuring the accurate identification of *E. coli* isolates. The application of molecular techniques is crucial in antibiotic resistance research, as they provide more precise results than conventional methods. All isolates produced DNA bands of approximately 884 bp, corresponding to the uspA gene. This result confirms that all the bacterial isolates are *E. coli*. This method is effective for rapid screening of *E. coli* strains (Chen & Griffiths, 1998). The *uspA* gene serves as a genetic marker for *E. coli* identification due to its involvement in stress response (Dawes et al., 2010). Identification of bacteria using genes that are unique and specific to *E. coli*, such as *uspA*, has the advantage of being more accurate than culture-based methods or phenotypic traits, which can be affected by environmental conditions. In addition, this method is faster, cheaper, and more effective in detecting many isolates well.

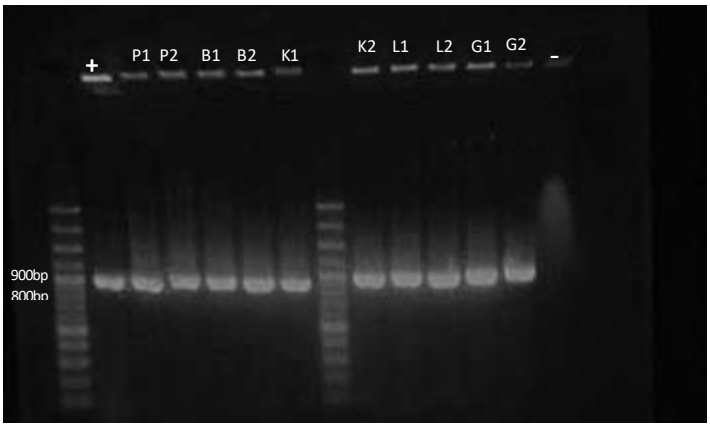

Figure 3. UV transilluminator visualization of *uspA* PCR products

3.4. Antibiotic resistance test

The antibiotic susceptibility test was performed using the disk diffusion method with ampicillin, ciprofloxacin, and chloramphenicol. The inhibition zone diameters were measured, and resistance was categorized as resistant (R), intermediate (I), or sensitive (S) based on CLSI guidelines.

Table 2. Antibiotic resistance profile of *E. coli* isolates

Isolate	Ampicillin		Ciprofloxacin		Chloramphenicol	
G1	6.20 ± 0.14	R	11.20 ± 0.14	R	9.05 ± 0.07	R
G2	6.35 ± 0.14	R	11.20 ± 0.28	R	8.00 ± 0.28	R
P1	6.00 ± 0.00	R	28.05 ± 0.21	I	6.10 ± 0.14	R
P2	6.11 ± 0.13	R	27.15 ± 0.21	I	6.10 ± 0.28	R
L1	6.00 ± 0.00	R	24.00 ± 0.00	I	12.00 ± 0.00	R
L2	6.35 ± 0.17	R	29.05 ± 0.07	I	12.10 ± 0.14	R
B1	6.15 ± 0.21	R	18.10 ± 0.14	R	12.00 ± 0.14	R
B2	6.00 ± 0.00	R	17.00 ± 0.00	R	15.20 ± 0.28	I
K1	6.35 ± 0.07	R	37.05 ± 0.21	S	12.00 ± 0.00	R
K2	6.10 ± 0.14	R	35.00 ± 0.00	S	9.05 ± 0.07	R

Note:
R (Resistant): (Ampicillin ≤ 13), (Ciprofloxacin ≤ 20), (Chloramphenicol ≤ 12)
I (Intermediate): (Ampicillin 14-16), (Ciprofloxacin 21-30), (Chloramphenicol 13-17)
S (Sensitive): (Ampicillin ≥ 17), (Ciprofloxacin ≥ 31), (Chloramphenicol ≥ 18)

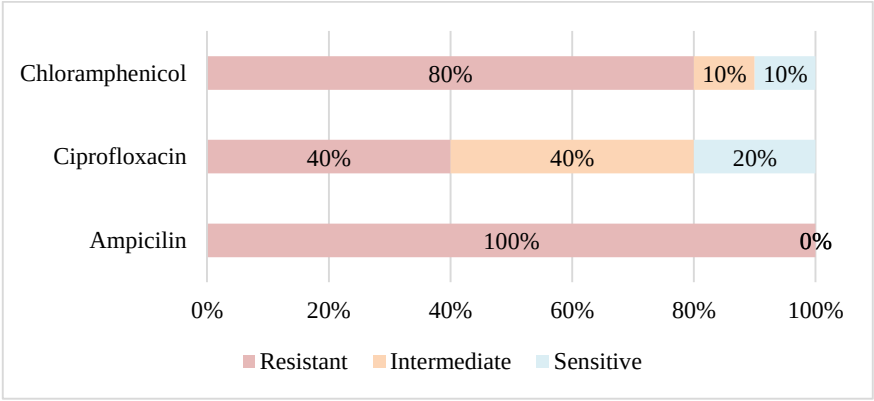

Figure 4. Antibiogram profile of *E. coli* from chicken meat samples

Based on the results, *E. coli* isolates exhibited 100% resistance to ampicillin, 40% resistance to ciprofloxacin (with 40% intermediate and 20% sensitivity), and 80% resistance to chloramphenicol (with 10% intermediate and 10% sensitivity). The results of this study are in line with the findings of Brătfelan et al. (2023), who also reported the prevalence of *E. coli* in chicken meat as well as high levels of resistance to various classes of antibiotics, including tetracycline, ampicillin, and third-generation cephalosporins. The highest resistance results in this study were against ampicillin antibiotics, similar to the results of the study by Hardiati et al. (2023) which also reported the highest

level of *E. coli* resistance to ampicillin (100%) in *E. coli* isolates from poultry isolated from Sukabumi, Bogor, and Cianjur. The high resistance to ampicillin is in line with the findings of Koju et al. (2022) in Nepal who reported higher resistance to ciprofloxacin (66%) compared to ampicillin (60%) and chloramphenicol (12%) in isolates from chicken cecum. A similar finding was found in a study in Lima, Peru showing that Escherichia coli isolates from non-organic market chickens had significantly higher levels of resistance than organic chickens, indicating that less controlled antibiotic use in non-organic chicken production contributes to increased resistance to antibiotics such as ciprofloxacin (Murray et al, 2021). The high resistance to chloramphenicol in this study (80%) is also supported by Javed et al. (2020) in Pakistan who found that isolates from animals showed significant resistance to chloramphenicol of 72%. In contrast, the results of the study by García-Béjar et al. (2021) in Spain showed much lower chloramphenicol resistance, which was only 7%, contrary to the results of this study. High resistance rates suggest frequent antibiotic use in poultry farming near Yogyakarta (Dita & Kholik, 2023). Improper antibiotic use in livestock can contribute to the emergence of multidrug-resistant *E. coli*, rendering treatments ineffective (Rozani et al., 2023). The findings of this study indicate antibiotic resistance in *E. coli* isolates from chicken meat, so comprehensive monitoring and control of the spread of antibiotic resistance are urgently needed. Further research can be conducted to confirm the identity of *E. coli* isolates using the 16S rRNA gene sequencing method to obtain more accurate identification. In addition, resistance tests can be conducted against other antibiotic groups that have not been tested, such as the carbapenem group, advanced cephalosporins, and aminoglycosides, to obtain a more comprehensive picture of the isolate resistance profile.

4. Conclusion

This study concluded that *Escherichia coli* contamination was found in chicken meat sold at five traditional markets in Yogyakarta (Beringharjo, Kranggan, Legi Kotagede, Prawirotaman, and Giwangan) based on the isolation of 10 bacterial strains confirmed as *E. coli* through molecular detection of the uspA gene. Antibiotic susceptibility testing showed that all isolates (100%) were resistant to ampicillin, 40% were resistant to ciprofloxacin, and 80% were resistant to chloramphenicol, indicating a high prevalence of multidrug-resistant *E. coli* in chicken meat from traditional markets in Yogyakarta.

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