



Prevalence of Multi-Drug Resistance *Escherichia coli* in Broiler Chicken Meat in Jember, Indonesia

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ABSTRACT

Antibiotic resistance has become one of the global health problems nowadays. Chicken meat is one of the largest food commodities in the world. *Escherichia coli* (*E. coli*) is one of the bacteria that is often found in chicken meat. These bacteria are capable of being pathogenic in both animals and humans. This study aimed to determine the prevalence of multidrug-resistant *E. coli* isolated from broiler chicken meat in the study location. The *E. coli* utilized in this study were derived from 25 grams of chicken meat obtained from 30 samples procured from six markets within the Jember district. The resistance test method used was Kirby-Bauer with Mueller-Hinton media. The results of the study showed that 100% of chicken meat was contaminated with *E. coli*. All isolated *E. coli* from samples in the study were multidrug-resistant. *E. coli* was 100% resistant to cotrimoxazole and cefixime, 96.67% resistant to chloramphenicol and amoxicillin-clavulanic, 93.3% resistant to tetracycline, 90% resistant to ceftriaxone, and 80% resistant to azithromycin and ciprofloxacin. The minimum resistance profile to 5 types of antibiotics with a multiple antibiotic resistance (MAR) index was between 0.625-1. Thus, the study revealed a high risk of infection associated with the consumption of uncontrolled chicken meat.

Keywords: Antibacterial agent, Chicken, *Escherichia coli*, Multi-drug resistance

INTRODUCTION

Antibiotic resistance is one of the global health problems nowadays. This is because antibiotic resistance has a reasonably high morbidity and mortality rate (Akova, 2016). The 2022 report of the Global Antimicrobial Resistance and Use Surveillance System (GLASS) draws attention to the worrying prevalence of antibiotic resistance among common bacterial pathogens. The average reported rates of resistance across 76 countries are 42% for third-generation cephalosporin-resistant *Escherichia coli* (*E. coli*) and 35% for methicillin-resistant *Staphylococcus aureus*, which is a matter of serious concern (WHO, 2022). Antibiotic resistance is also one of the health problems faced by the Indonesian state. A study proved that 43% of *E. coli* bacteria taken from 2,494 Indonesians are resistant to various types of antibiotics, including ampicillin, cotrimoxazole, chloramphenicol, ciprofloxacin, and gentamicin (Dirga et al., 2021). Antibiotic resistance makes antibiotics less effective in the fight against infection. One of the leading causes of the onset of antibiotic resistance in the community is the inappropriate use of antibiotics. The incidence of antibiotic resistance is rapidly increasing, and this is because antibiotics are not only used directly by humans but are also commonly given to animal food sources (WHO, 2014). The use of antibiotics in animal food sources increases livestock production. Doses of antibiotics given to farm animals to increase growth tend to be given under the recommended therapeutic dose. Antibiotic resistance arising from the inappropriate use of antibiotics in farm animals can cause the onset of antibiotic resistance in humans (Van et al., 2020).

Chicken is one of the largest food commodities in the world. Chicken meat is a dietary necessity for humans due to its high protein content (Wahyono and Utami, 2018). *Escherichia coli* is one of the most common bacteria in chicken meat. These bacteria are capable of being pathogenic both in chickens and in humans. Bacterial contamination in chicken meat can be transmitted to humans during production, packaging, sales, and when consumed (FAO, 2013). The high need for chicken meat makes this commodity one of the causes that have the potential to cause antibiotic resistance in the community (Uddin et al., 2019). Several studies conducted in Indonesia have demonstrated the presence of high levels of multidrug resistance (MDR) in *E. coli* isolated from poultry. The MDR rates observed ranged from 45% to 92.7% across all samples tested (Wibawati, 2023; Febriliani et al., 2024; Sirindon et al., 2024).

Jember Regency has become one of the areas with a considerable poultry population in East Java, Indonesia. According to data from the Central Statistics Agency of East Java, in 2016, the poultry population in Jember Regency reached more than 14 million. The poultry population in Jember Regency is dominated by broilers, with a population of

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more than 11 million (JATIM, 2016). Multidrug resistance is a condition in which a bacterium is resistant to at least one type of antibiotic from ≥ 3 antibiotic groups (Sweeney *et al.*, 2018). *Escherichia coli* can become resistant to antibiotics by producing enzymes to damage antibiotics and modify their metabolic processes. *Escherichia coli*, which has been resistant, can transfer resistant genes to other groups of enterobacteria (Poirel *et al.*, 2018).

To determine further policies in dealing with the incidence of antibiotic resistance, monitoring and supervising the incidence of bacterial resistance in the food chain should be conducted (WHO, 2014). The objective of the study was to investigate the prevalence of multidrug-resistant *E. coli* in broiler chicken meat isolates in the Jember Regency.

MATERIALS AND METHODS

Ethical approval

The Medical Faculty of Ethics Commission at the University of Jember authorized this study with no objections (1562/H25.1.11/KE/2022).

Sample collection and transportation

The sample comprised 30 units of 25 grams of broiler raw chicken meat obtained from six traditional markets in the Jember Regency. The sampling locations were selected from a list of chicken markets supplied by the Indonesian Department of Veterinary Services. The multistage random selection method was employed to identify markets. Samples were taken from the market to the microbiology laboratory of the medical faculty of Jember University using sterile polythene bags to prevent contamination during transport.

Isolation and identification of *Escherichia coli*

A total of 25 grams of chicken meat from the upper thighs was extracted from each sample and soaked in 225 milliliters of sterile aquadest for 15 minutes. Once the soaking process was complete, the aquadest and chicken meat were blended until a homogenous mixture was achieved (Kaihena and Ferdinandus, 2009). A suspension of broiler chicken meat was plated onto Salmonella Chromogenic Agar media (Chromagar, Paris, France). By a streaking method. Suspension that has been planted on the medium is incubated for 24 hours at 37°C. The blue colony that grows in the media could be *E. coli* (Oktavianto *et al.*, 2016). Blue colonies in *Salmonella* chromogenic agar media suspected to be *E. coli* bacteria are then implanted in Eosyn methylene blue (EMB) media (Merck KGaA, Darmstadt, Germany) for confirmation that the bacteria are accurate to be *E. coli*. Colonies are planted on EMB media using the streaking method and incubated for 24 hours at 37°C. In EMB media, *E. coli* grows as colonies with a green metallic sheen color (Lal and Cheeptham, 2016).

Bacterial culture

Before being planted on Mueller-Hinton media, colonies of *E. coli* were first cultured on nutrient agar media (Oxoid, UK). Planting on a densely tilted nutrient agar (NA) medium using the continuous strike method. After being planted on NA media, the bacterial colony is incubated for 24 hours at 37°C.

Antibiotic susceptibility testing

Antibiotic susceptibility tests are performed using the Kirby-Bauer method using Mueller-Hinton media (Oxoid, UK). Before planting on Mueller-Hinton (MH) media, colonies of *E. coli* in NA media are diluted first using aquadest with a concentration equal to a standard solution of 0.5 McFarland. The suspension of *E. coli* is further implanted on MH media using a sterile cotton swab. Mueller-Hinton media that contained *E. coli* were then implanted with antibiotic discs. Antibiotics to be tested in this study are tetracycline, amoxicillin-clavulanic, ceftriaxone, cefixime, cotrimoxazole, azithromycin, ciprofloxacin, and chloramphenicol. All antibiotics used are Oxoid (Oxoid, UK) brands that are under Clinical and Laboratory Standards Institute (CLSI) guidelines. In accordance with the CLSI guidelines, the Kirby-Bauer method for the detection of antibiotic inhibition zones is categorized into three distinct groups: sensitive, resistant, and intermediate (CLSI, 2020). Mueller-Hinton media is then incubated for 24 hours at a temperature of 37°C.

Observation

After incubation for 24 hours, the diameters of inhibition growth zones on MH media were observed and measured using a vernier caliper with an accuracy of 0.05 mm. The results of the diameters of inhibition growth zones are then interpreted based on guidance from CLSI. The data obtained on antibiotic resistance will then be collated to create the Multiple Antibiotic Resistance (MAR) index. The multiple antibiotic resistance index is calculated by dividing the number of resistant antibiotics in samples by the entire number of antibiotics tested in the study (Adzitey, 2018). Data obtained from subsequent resistance tests are categorized into MDR criteria.

Statistical analysis

The evaluation's post-incubation results indicated the presence of inhibition zones, categorized as susceptible, intermediate, and resistant, in accordance with CLSI guidelines. The data analysis in the current study was conducted descriptively (Febrilianti et al., 2024).

RESULTS

The findings of the current investigation revealed that 30 out of 30 samples (100%) tested positive for *E. coli* from six traditional markets in Jember. The antimicrobial resistance and susceptibility profiles of the examined *E. coli* isolates against eight chosen antimicrobial drugs were assessed using the agar disc diffusion method, with the findings presented in Table 1. All isolates (100%) have shown resistance to two antimicrobial agents: cotrimoxazole and cefixime, 96.67% resistant to chloramphenicol and amoxicillin-clavulanic, 93.3% resistant to tetracycline, 90% resistant to ceftriaxone, and 80% resistant to azithromycin and ciprofloxacin. Most *E. coli* isolates exhibited numerous drug profiles (≥ 3 antimicrobial classes) and a MAR index of ≥ 0.2 (Table 2). Moreover, all *E. coli* isolates exhibited resistance to four and five distinct classes of antibiotics, respectively (Table 3).

Table 1. Antibiotic resistance profile of 30 isolated *E. coli* from meat samples in Jember, Indonesia, 2022

Antibiotic	Sensitive		Intermediates		Resistant	
	n	%	n	%	n	%
Azithromycin	6	20%	0	0.00%	24	80%
Cotrimoxazole	0	0.00%	0	0.00%	30	100.00%
Ciprofloxacin	3	10%	3	10%	24	80%
Chloramphenicol	1	3.33%	0	2.22%	29	96.67%
Cefixime	0	0.00%	0	0.00%	30	100,00%
Tetracycline	0	0.00%	2	6.67%	28	93.33%
Amoxicillin-clavulanic	1	3.33%	0	0.00%	29	96.67%
Ceftriaxone	2	6.67%	1	3.33%	27	90%

Table 2. Multiple antibiotic resistance index of isolated *E. coli* from meat samples in Jember, Indonesia, 2022

Sample code	Resistant antibiotics	Number of resistant antibiotics	MAR index
D1	AZMCXTCIPCCFMTAMCCRO	8	1
D2	AZMCXTCIPCCFMTAMCCRO	8	1
D3	AZMCXTCIPCCFMTAMCCRO	8	1
D4	AZMCXTCIPCCFMTAMCCRO	8	1
D5	AZMCXTCIPCCFMTAMCCRO	8	1
E1	AZMCXTCCFMTAMCCRO	6	0.75
E2	AZMCXTCIPCCFMTAMCCRO	8	1
E3	AZMCXTCIPCCFMTAMCCRO	8	1
E4	AZMCXTCIPCCFMTAMCCRO	8	1
E5	AZMCXTCIPCCFMT	6	0.75
F1	AZMCXTCCFMTAMCCRO	7	0.875
F2	AZMCXTCIPCCFMTAMCCRO	8	1
F3	AZMCXTCIPCCFMTAMCCRO	8	1
F4	AZMCXTCIPCCFMTAMCCRO	8	1
F5	CXTCIPCCFMTAMCCRO	7	0.875
G1	AZMCXTCIPCCFMTAMCCRO	8	1
G2	CXTCIPCCFMTAMCCRO	7	0.875
G3	AZMCXTCIPCCFMTAMCCRO	8	1
G4	AZMCXTCCFMTAMC	6	0.75
G5	AZMCXTCIPCCFMTAMCCRO	8	1
H1	CXTCIPCCFMTAMCCRO	7	0.875
H2	CXTCIPCCFMTAMCCRO	7	0.875
H3	AZMCXTCCFMTAMCCRO	7	0.875
H4	AZMCXTCIPCCFMTAMCCRO	8	1
H5	CXTCIPCCFMTAMCCRO	7	0.875
I1	AZMCXTCIPCCFMTAMC	6	0.75
I2	AZMCXTCIPCCFMTAMCCRO	8	1
I3	CXTSFMTAMCCRO	5	0.625
I4	AZMCXTCIPCCFMTAMCCRO	8	1
I5	AZMCXTCIPCCFMTAMCCRO	8	1

MAR: Multiple antibiotic resistance, AZM: Azithromycin, CXT: Cotrimoxazole, CIP: Ciprofloxacin, C: Chloramphenicol, CFM: Cefixime, T: Tetracycline, AMC: Amoxicillin-clavulanic, CRO: Ceftriaxone.

Table 3. Classification of isolated *E. coli* from chicken meat samples by multi-drug resistance category

Sample code	Number of resistant antibiotics	MDR category
D1	8	MDR
D2	8	MDR
D3	8	MDR
D4	8	MDR
D5	8	MDR
E1	6	MDR
E2	8	MDR
E3	8	MDR
E4	8	MDR
E5	6	MDR
F1	7	MDR
F2	8	MDR
F3	8	MDR
F4	8	MDR
F5	7	MDR
G1	8	MDR
G2	7	MDR
G3	8	MDR
G4	6	MDR
G5	8	MDR
H1	7	MDR
H2	7	MDR
H3	7	MDR
H4	8	MDR
H5	7	MDR
I1	6	MDR
I2	8	MDR
I3	6	MDR
I4	8	MDR
I5	8	MDR

MDR: Multiple-drug resistance.

DISCUSSION

This study found that 100% of the 30 samples tested positive were contaminated by *E. coli*. The contamination of *E. coli* in this study tended to be higher than previous studies also conducted in Jember. In that study, *E. coli* contamination from 6 tested was 66.67% (Putri et al., 2018). The results of *E. coli* contamination in this study are aligned with other studies conducted in Pringsewu Regency in 2015. The study revealed that bacterial contamination was present in 100% of the samples tested, despite the colony-forming unit remaining low (Utari et al., 2016). *Escherichia coli* bacteria are generally present in the digestive tract of chickens (Mourand et al., 2020). Contamination of *E. coli* in chicken meat occurs most during the chicken slaughter process. During the slaughter process, the ruptured digestive tract causes *E. coli* initially present in the digestive tract to contaminate chicken meat (FAO, 2013). The variability in *E. coli* recovery rates noted in this study may be associated with farm biosecurity protocols and the hygiene practices of personnel. Farm hygiene is essential for minimizing pathogen infection. This study corroborates the report by Olopade et al. (2022) which indicated that animal handlers and equipment contributed to the ongoing contamination of the farm environment (Olopade et al., 2022).

In chicken meat, contamination of *E. coli* can also occur during transportation or processing. *Escherichia coli* contained in chicken meat has the potential to be able to transmit to the human body and cause serious public health problems. The emergence of drug-resistant *E. coli* has led to the accumulation of resistance genes in both human and animal populations. This phenomenon has the potential to complicate the management of infections caused by *E. coli*, which are often amenable to antibiotic treatment. The resultant challenges can impose a significant financial burden on patients (Mensah et al., 2022). The significant prevalence of potentially clinically important *E. coli* identified in this study is unsurprising, given *E. coli* is a prominent member of the Enterobacteriaceae family known to inhabit the gastrointestinal tract of chickens. Moreover, *E. coli* serves as a recognized indicator organism for investigating the dissemination of antibiotic-resistance genes (Al Azad et al., 2019).

In this study, the level of resistance of *E. coli* to macrolide (azithromycin), tetracycline, and cotrimoxazole was higher than in previous studies conducted in Blitar City, Indonesia. That study found resistance levels at 73.9% in macrolide, 45.8% in tetracycline, and 67% in cotrimoxazole (Wibisono et al., 2020). Alternative investigations presented disparate data, with the research conducted in Bangladesh. The resistance levels to tetracycline, cotrimoxazole, and ciprofloxacin were 100% (Al Azad et al., 2019). The level of resistance to the antibiotic chloramphenicol found in this study was lower than in other studies conducted in India. In that study, *E. coli* resistance to chloramphenicol was 100% (Joshi et al., 2012). The level of resistance to ceftriaxone in this study was also higher when compared to previous studies conducted in Bangladesh in 2019. The study obtained a level of resistance to chloramphenicol of 56.67% (Sarker et al., 2019). The level of resistance to amoxicillin-clavulanic antibiotics in this study was also higher when compared to previous studies conducted in Algeria by 43.3% (Halfaoui et al., 2017). The difference in the prevalence of *E. coli* resistance to antibiotics between regions and countries can be attributed to a multitude of factors. The educational background of farmers is a factor that influences the prevalence of antibiotic resistance in *E. coli* strains isolated from broiler chickens. *Escherichia coli* isolates from livestock owned by farmers with higher levels of education tend to demonstrate lower levels of antibiotic resistance (Wibisono et al., 2020). Poor sanitation in livestock areas is also one factor that affects the level of resistance of *E. coli* to antibiotics. Other factors that can affect the level of antibiotic resistance include the absence of antibiotic delivery programs, the type of feed used, and the absence of support from veterinarians during the process of animal management (Wibisono et al., 2020).

The prevalence of MDR-*E. coli* obtained in this study was 100%. These results tend to be higher than other studies that have been done before in Blitar City in 2019. In that study, the prevalence of MDR in broiler chickens was 83.75% (Wibisono et al., 2020). The prevalence of MDR *E. coli* from broiler chickens in Jember Regency had similar results as a study conducted in Bangladesh in 2019. In the study, the prevalence of *E. coli* is MDR by 100% (Al Azad et al., 2019). A series of studies conducted in Portugal between 2014 and 2019 revealed a prevalence of MDR *E. coli* from food-producing animals in the country ranging from 70% to 90% (Costa et al., 2022). The prevalence of MDR-*E. coli* in broiler chickens varies considerably across different regions and countries, and this variation is shaped by several different factors. Factors that can affect the prevalence rate of MDR in a region include the diversity of poultry production systems, geographical conditions, the type of antibiotics used, and government policies against the use of antibiotics in the livestock sector (Bywater et al., 2004; WHO, 2014).

Bacterial resistance in broiler chicken meat can occur due to improper antibiotics in the livestock sector. The use of antibiotics on livestock is used not only to prevent the spread of infection in livestock animals but also as a growth trigger. Antibiotics used to trigger growth are usually given at doses below the therapeutic dose (Mehdi et al., 2018). Administration with doses under therapy continuously will cause the accumulation of antibiotic residue in chicken meat. This causes bacteria that contaminate chicken meat to be exposed to low doses of antibiotics for a long time, which will

cause bacterial resistance to antibiotics (Mund et al., 2017).

Resistance to *E. coli* arising from improper use of antibiotics on livestock can affect general health conditions. Elevated resistance to antibiotics may result from the frequent consumption of animal products containing substantial antibiotic residues. Antibiotic resistance arises from the transfer of plasmids from resistant bacteria to susceptible bacteria, occurring when initially susceptible bacteria are exposed to antibiotics. This antibiotic is frequently utilized within the community and as an addition to animal feed to stimulate growth. This is a contributing factor to antibiotic resistance in Indonesia (Wibisono et al., 2020).

Infections in humans caused by resistant *E. coli* will be harder to cure (FAO, 2013). Antibiotics commonly used as therapeutic options against bacteria become less efficient (Mund et al., 2017). The inappropriate use of antibiotics in animal livestock has been identified as a contributing factor to the rise in antibiotic resistance. The utilization of antibiotics as food additives, particularly in low and middle-income countries, has been documented as a prevalent practice. To prevent the increasing bacterial resistance to antibiotics, it is necessary to establish policies from the government to regulate and monitor the inappropriate use of antibiotics in society. In addition to preventing the transmission of *E. coli* that have been antibiotic-resistant to the human body, broiler chicken meat should be cooked first at a temperature above 100°C before consumption (Adeyanju and Ishola, 2014; Ajayi et al., 2024).

CONCLUSION

The findings of this study indicate the presence of antibiotic resistance in *E. coli* isolates from broiler chicken meat in Jember City. All isolates exhibited 100% resistance to cotrimoxazole and cefixime, with high levels of resistance to other antibiotics. The minimum resistance profile was to 5 types of antibiotics with a MAR index between 0.625 and 1. All isolated *E. coli* from broiler chickens in the study were multidrug-resistant. Thus, the study revealed a high risk of infection associated with the consumption of chicken meat. Consequently, more molecular investigations employing random amplified polymorphic DNA analysis are advised to elucidate the clonal relationships of MDR-*E. coli* isolates from both animal and human sources.

DECLARATIONS

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Authors' contributions

Enny Suswati served as the lead investigator and was the catalyst for the study's conceptualization. Dava Rizky Pratama engaged in data collection, conducted the analysis of the data, and composed and finished the text for publication. Bagus Hermansyah contributed to the data analysis and played a crucial role in developing and finalizing the text for publication. All authors have reviewed and endorsed the final version of the study for publication in the current journal.

Competing interests

The authors declare that there is no conflict of interest.

Ethical consideration

This document was initially authored by the individuals responsible. The authors were not submitting this paper to any other journal or publisher.

Availability of data and materials

All data from the current study are available upon reasonable request from the authors.

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