

## Prevalence and profile of antimicrobial resistance in *Escherichia coli* isolated from broiler meat in East Java, Indonesia

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### Abstract

**Background and Aim:** Antibiotic resistance occurs when bacteria can avoid the mechanisms of action of antibiotic drugs, resulting in a reduced antibiotic activity. This is dangerous for animals and humans because treatment of infectious diseases can take longer and may even lead to treatment failure. Bacteria in meat can be the cause of meat-borne diseases for consumers. This study aimed to determine the resistance profile of *Escherichia coli* from broiler meat slaughtered in several local government poultry slaughterhouses in East Java.

**Materials and Methods:** The 122 samples studied were from the pectoralis muscle of broilers from local government poultry slaughterhouses. The isolation and identification of *E. coli* from broiler meat were confirmed using MacConkey agar and eosin methylene blue agar, followed by Gram-staining, and an indole methyl red, Voges–Proskauer, and citrate test. The *E. coli* isolates were then tested for antibiotic resistance using the Kirby–Bauer method, and the results were interpreted using Clinical and Laboratory Standards Institute guidelines.

**Results:** The isolation and identification tests for *E. coli* indicated that 44.26% (54) of the samples were positive for *E. coli*. The results of the antibiotic resistance tests demonstrated that the resistance, from highest to lowest, was to erythromycin, trimethoprim, ampicillin, ciprofloxacin, streptomycin, cephalothin, tetracycline, and chloramphenicol antibiotics with resistance of 66.7%, 61.1%, 59.3%, 35.2%, 33.3%, 27.8%, 24.1%, and 24.1% respectively. Of the 54 isolates, 32 (59.26%) were resistant to  $\geq 3$  antimicrobials.

**Conclusion:** The study found that the prevalence of *E. coli* in broiler meat in East Java, Indonesia was 44.26%. These bacteria were resistant to all of the antibiotics that were examined with high to very high resistance levels and are associated with multi-drug resistance (MDR) (59.26%). The presence of *E. coli* in broiler meat for human consumption can cause meat-borne illness, and the discovery of MDR is a matter of concern in the One Health approach because apart from having an impact on human health as meat consumers, it can also have an impact on animal health and the environment.

**Keywords:** antibiotic resistance, broiler meat, *Escherichia coli*, public health.

### Introduction

The antimicrobial resistance (AMR) that Indonesia faces is similar to that of many other developing countries due to the misuse and over-use of antibiotics in humans, livestock, poultry, and aquaculture [1]. As low-income countries shift to higher incomes and are accompanied by population growth, there will be an increase in demand for quality sources of animal protein, further resulting in poultry production systems shifting from subsistence farming

practices to intensive food production, which implies routine antimicrobial usage [2].

The production of broiler meat in East Java Province was 506,731.16 tons in 2019, 424,942.68 tons in 2020, and 442,478.71 tons in 2021 [3]. The average broiler meat consumption in East Java in 2021 was 0.12 kg/capita/week [4]. Due to the large amount of production and number of consumers of broiler meat, there is a significant AMR risk from the misuse of antimicrobials. Antimicrobial resistance has led to various complex problems affecting many countries around the world, affecting patients, health care, and the economy [5].

Meat can serve as a vehicle for many pathogenic organisms due to its nutritive value. The presence of *Staphylococcus aureus*, *Salmonella* spp., and *Escherichia coli* has been demonstrated in chicken meat in Surabaya city, Indonesia [6]. *Escherichia*

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*coli* is a known indicator of the sanitary quality of foods [7]. Extraintestinal pathogenic *E. coli* (ExPEC), the most common cause of community and hospital-acquired extraintestinal infections, originates from the same bacterial lineage or has the same evolutionary roots as pathogenic *E. coli* in poultry, thus enabling poultry to be a reservoir for ExPEC in humans [8]. Therefore, this bacterium should be included in active AMR monitoring programs.

Meat-borne pathogens can be detected at various points in the supply chain. It is important to determine the source from which they arise and how they behave during meat production and processing. During the slaughtering process, meat can be exposed to many sources of contamination [9].

This study aimed to detect *E. coli* pathogens in fresh broiler meat slaughtered in poultry slaughterhouses in East Java, Indonesia, and to determine the resistance profiles of this bacterium against several antibiotics to ensure the safety of meat consumed by the public.

## Materials and Methods

### Ethical approval

Ethical approval for animal research was not required as live animals were not used in this study. Broiler chicken samples were purchased after the broilers were slaughtered at the local government poultry slaughterhouses.

### Study period and location

This study was conducted from February to June 2022. The study areas were Blitar City, Lamongan Regency, Probolinggo City and Nganjuk Regency, East Java, Indonesia. Identification of *E. coli* and its AMR test was carried out at the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga.

### Broiler meat sampling

Samples in the form of broiler meat were obtained from local government poultry slaughterhouses in four districts in East Java. The sample size was determined using the cross-sectional sampling method based on an assumption of 50% prevalence and a 5% error at a 95% confidence level. Sampling was performed by simple random sampling. Samples were obtained from the musculus pectoralis section and placed in a sterile polypropylene tube and then stored in an ice box containing ice gel at a temperature of 4°C. It was then transported to the laboratory for bacterial isolation, which was conducted immediately after arrival at the laboratory.

### Isolation and identification of *E. coli*

The test was comprised of several stages: isolation was confirmed using MacConkey agar and eosin methylene blue agar (EMBA, Merck, Germany) followed by Gram-staining, and then identified using biochemical tests. The biochemical tests included the indole production test (I), the Voges–Proskauer

test, the methyl red test, and the Citrate test (C). This series of tests are referred to as the indole, methyl red, Voges–Proskauer, and citrate. The identification of *E. coli* bacteria in this study is based on a microbiological testing method for *E. coli* bacteria and other coliforms [10].

### Resistance tests

The sensitivity of *E. coli* bacteria to antibiotics was determined using the Kirby–Bauer method, which uses a disk diffusion method to produce qualitative categories with assessments of resistant, intermediate, or susceptible. The antibiotic disks (Oxoid, UK) used were ampicillin 10 µg; erythromycin 15 µg; chloramphenicol 30 µg; tetracycline 30 µg; trimethoprim 5 µg; cephalothin 30 µg; ciprofloxacin 5 µg; and streptomycin 10 µg. The *E. coli* suspension used in this method had a turbidity number of 0.5, which was in accordance with the McFarland standard. A positive disk diffusion method test is indicated by the presence of a transparent or clear area around the paper disk as an area of inhibition (zone of inhibition) for bacterial growth. The diameter of the zone of inhibition was measured using a caliper by measuring the vertical diagonal plus the horizontal diagonal and then dividing their sum by two. The results were analyzed based on the Kirby–Bauer standard zone inhibition method according to the Clinical and Laboratory Standards Institute (CLSI) [11].

### Statistical analysis

The data that are listed in the tables are presented as percentages.

## Results

### Prevalence of *E. coli* in broiler meat

The appearance of colonies of *E. coli* grown on EMBA media was metallic green (Figure-1). The results from 122 samples of broiler meat from East Java indicated that 54 (44.26%) were positive for *E. coli*. The results of the *E. coli* identification tests are summarized in Table-1.

### Prevalence of antibiotic resistance and multi-drug resistance (MDR) in *E. coli* isolates

Of the 54 *E. coli* isolates identified from meat samples, high resistance was observed against eight antibiotics, namely, erythromycin (66.7%), trimethoprim (61.1%), ampicillin (59.3%), ciprofloxacin (35.2%), streptomycin (33.3%), cephalothin (27.8%), tetracycline (24.1%), and chloramphenicol (24.1%). The results of *E. coli* resistance to antibiotics are summarized in Table-2. Furthermore, in the *E. coli* isolates, the prevalence of MDR (59.26%) was common (Table-3).

## Discussion

Food safety is important because it causes suffering and economic problems. It causes consumers to be more aware of what they eat and their demands for safe and quality foods [12]. Meat-borne diseases are a central problem in the meat industry [13].

**Table-1:** Prevalence of *E. coli* in the examined sample.

| Poultry slaughterhouses distribution | n   | Suspected isolated (%) | Gram-negative | Biochemical test IMViC* | Confirmed <i>E. coli</i> (%) |
|--------------------------------------|-----|------------------------|---------------|-------------------------|------------------------------|
| Lamongan                             | 26  | 6                      | 6             | 3                       | 3 (11.54)                    |
| Blitar                               | 46  | 38                     | 31            | 24                      | 24 (52.17)                   |
| Probolinggo                          | 25  | 19                     | 17            | 16                      | 16 (64.00)                   |
| Nganjuk                              | 25  | 16                     | 13            | 11                      | 11 (44.00)                   |
| Total                                | 122 | 79                     | 67            | 54                      | 54 (44.26)                   |

\**Escherichia coli* classification is IMViC (Indol, MR, VP, Citrate) reaction with a pattern of + + - - or - + - -, IMViC=Indole methyl red, Voges-Proskauer, and citrate, *E. coli*=*Escherichia coli*

**Table-2:** Antimicrobial resistance profile of *E. coli* isolates (n=54).

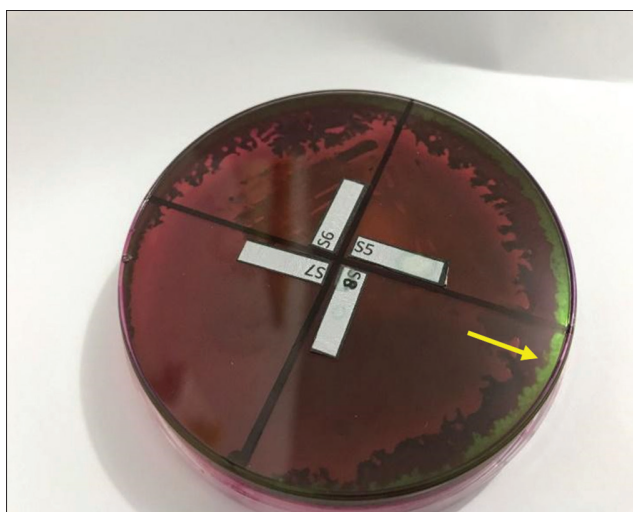
| Antimicrobials ( $\mu$ g) | Resistant (%) | Intermediate (%) | Sensitive (%) |
|---------------------------|---------------|------------------|---------------|
| Ampicillin 10             | 32 (59.3)**   | 3 (5.6)          | 19 (35.2)     |
| Erythromycin 15           | 36 (66.7)**   | 9 (16.7)         | 9 (16.7)      |
| Chloramphenicol 30        | 13 (24.1)*    | 30 (55.6)        | 11 (20.4)     |
| Tetracycline 30           | 13 (24.1)*    | 12 (22.2)        | 29 (53.7)     |
| Trimetoprim 5             | 33 (61.1)**   | 3 (5.6)          | 18 (33.3)     |
| Cephalothin 30            | 15 (27.8)*    | 7 (13.0)         | 32 (59.3)     |
| Ciprofloxacin 5           | 19 (35.2)*    | 4 (7.4)          | 31 (57.4)     |
| Streptomycin 10           | 18 (33.3)*    | 13 (24.1)        | 23 (42.6)     |

Low:  $\leq 10\%$  Moderate:  $>10\%$  to  $20\%$ ; \*High:  $>20\%$  to  $50\%$ ; \*\*Very High:  $>50\%$  to  $70\%$ , Extremely high:  $>70\%$ , *E. coli*=*Escherichia coli*

**Table-3:** Distribution of the prevalence MDR in the *E. coli* isolated (n=54).

| Poultry slaughterhouses distribution | n   | Susceptible to all antibiotics tested | Resistance to one antibiotic | Resistance to two antibiotics | Resistance to three antibiotics (MDR) |
|--------------------------------------|-----|---------------------------------------|------------------------------|-------------------------------|---------------------------------------|
| Lamongan                             | 3   | 0                                     | 0                            | 0                             | 3                                     |
| Blitar                               | 24  | 1                                     | 9                            | 3                             | 11                                    |
| Probolinggo                          | 16  | 0                                     | 2                            | 2                             | 12                                    |
| Nganjuk                              | 11  | 1                                     | 2                            | 2                             | 6                                     |
| Total                                | 54  | 2                                     | 13                           | 7                             | 32                                    |
| (%)                                  | 100 | 3.7                                   | 24.07                        | 12.97                         | 59.26                                 |

MDR=Multi-drug resistant, *E. coli*=*Escherichia coli*

**Figure-1:** Specific *Escherichia coli* colonies on eosin methylene blue agar are metallic green (yellow arrow).

*Escherichia coli* is a common inhabitant of the digestive tract of mammals and birds. *Escherichia coli* can be transmitted through feces that contaminate meat at the time of slaughter. It can enter drinking water sources and also adhere to fruits and vegetables. This study found high levels of *E. coli* contamination

in 44.26% (54/122) of broiler meat samples. In other studies, *E. coli* contamination was reported to be 100% (34/34) in raw goat milk samples in Blitar Regency, East Java, Indonesia [14]. Most strains of *E. coli* are harmless, but some, such as pathogenic enteropathogenic *E. coli* and strains of *E. coli* Shiga toxin-producing *E. coli* that produce Shiga toxin, have evolved to become pathogenic and can cause diseases in humans and animals [15], resulting in many waterborne outbreaks [16]. In chickens, these bacteria can cause Hjarre's disease, omphalitis, peritonitis, and Colibacillosis [17]. Therefore, chickens are significant reservoirs of *E. coli* bacteria that demand awareness.

Many antimicrobials are used in human medicine, for treating sick animals, added to food animals to promote growth, or for routine disease prevention in unsanitary industrial conditions [1]. Coyne *et al.* [18] determined that the use of antimicrobials in small commercial broiler systems in Indonesia was high, because farmers are able to access antimicrobials easily through local animal medicine sources at relatively low costs; however, farmers have difficulty accessing veterinary advice regarding responsible antimicrobial use. Based on a study of the use of antibiotics in 360 small and medium-scale commercial

broiler farms in three provinces in Indonesia, the most frequently used antibiotics were enrofloxacin (49.4%), amoxicillin/colistin (35.3%), trimethoprim/sulfadiazine (14.1%), and doxycycline (13.3%), and 81.4% of the farmers routinely gave antibiotics for prophylaxis [19]. Based on another study, a number of antibiotics have been registered and are often used in the treatment of poultry, including streptomycin, ampicillin, tetracycline, ciprofloxacin, cephalothin, erythromycin, trimethoprim, and chloramphenicol [20]. The overuse and widespread misuse of antibiotics have led to unintended consequences, namely their contribution to antibiotic resistance and pollution, which can directly affect human health and ecosystems [21].

This study demonstrated resistance to all antibiotics that were examined with high resistance levels (20%–50%) to those that were very high (50%–70%). The resistance to erythromycin (66.7%), trimethoprim (61.1%), and ampicillin (59.3%) was dominant in *E. coli* isolates (Table-2). These antibiotics are commonly used in livestock and poultry and have been known for a long time; consequently, resistance can occur due to the long-term use of these antibiotics in poultry production. These antibiotics should be used very carefully because elevated levels of resistance can result. Afayibo *et al.* [22] reported that in avian pathogenic *E. coli*, 98.7% exhibited erythromycin resistance [22]. Another high erythromycin resistance rate (>60%) was reported in poultry isolates from Bangladesh [23]. In Iran, moderate to high levels of resistance have been reported to erythromycin (47.36%), sulfonamides (47.36%), and tetracycline (53.63%) in *E. coli* isolated from chickens [24]. However, in Tunisia, poultry isolates exhibited the highest rates of antibiotic resistance to tetracycline (74.7%), trimethoprim (57%), and amoxicillin (57%) [25].

Bacteria can become resistant to antibiotics by three mechanisms: first, the drug cannot reach its site of action in the microbial cell, second, by inactivation of the antibiotic, and third, the microbe can alter the binding site of the antibiotic [26]. This is in agreement with the work of Munita and Arias' who determined that antibiotic resistance in bacteria can develop through mutation, adaptation, acquisition of genetic material, or changes in gene expression [27]. In another study, a high incidence of multi-drug *E. coli* resistance in poultry isolates was observed that possessed a common mediator of AMR, namely integrons among commensal multi-drug resistant *E. coli*, and this should be considered a serious health risk, since multi-drug resistant isolates may contaminate food products, and subsequently be transferred to humans [28]. This agrees with the research by Van den Bogaard *et al.* [29], who demonstrated that antibiotic-resistant bacteria (*E. coli*) can be transmitted from chickens to humans (poultry farm workers).

## Conclusion

This study demonstrated that the prevalence of *E. coli* from broiler meat in East Java, Indonesia was 44.26%. These bacteria are resistant to all the antibiotics evaluated with high to very high resistance levels and are associated with MDR (59.26%). The presence of *E. coli* in broiler meat for human consumption can cause meat-borne illnesses, and the discovery of MDR is a matter of concern in the One Health approach because apart from having an impact on human health as meat consumers, it can also have an impact on animal and environmental health. Therefore, to avoid this outcome, good hygienic practices are necessary in the abattoirs to prevent contamination of poultry products with intestinal content.

## Authors' Contributions

PAW and EBH: Conceptualization and design of the study. EBH and AMK: Collected samples. EBH, AMK, and DKW: Performed the laboratory procedures. PAW and AA: Interpreted the data. DKW and AA: Writing-original draft. PAW: Edited the final manuscript. All authors have read, reviewed, and approved the final manuscript.

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## Competing Interests

The authors declare that they have no competing interests.

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