Extended-spectrum β-lactamase Producing *Escherichia coli* in Broiler Farms in Ciampea Bogor

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Abstract. Long-term use of antibiotics in broiler chicken farming causes the emergence of resistant bacteria and increases the potential for multidrug-resistant bacteria. This study aims to investigate the presence and characteristics of extended-spectrum β-lactamase (ESBL) producing Escherichia coli (*E. coli*) in broiler farms located in Ciampea, Bogor. A total of 90 samples were taken from three broiler chicken farms located in Ciampea, Bogor. Sampling was carried out by swabbing the cloaca of 1% of the population on each farm. Gram staining and IMVIC biochemical tests were performed on colonies suspected of being *E. coli*. Confirmation of ESBL-producing *E. coli* using a double disc diffusion test with MHA. The results showed that ESBL-producing *E. coli* was present in 28,9% of broiler chicken cloaca swab samples (26/90). Antibiotic resistance tests of ESBL-producing *E. coli* isolates showed resistance to four antibiotics, namely chloramphenicol, amoxicillin, ciprofloxacin, and ampicillin. In this test, 22 isolates showed multidrug-resistant (MDR).

Keywords: Escherichia coli, ESBL, Broiler, Antibiotics, Bacteria.

Abstrak. Penggunaan antibiotik dalam waktu yang panjang selama pemeliharaan pada ayam pedaging menyebabkan munculnya bakteri resisten dan menimbulkan potensi adanya bakteri yang resisten terhadap banyak obat. Penelitian ini bertujuan untuk mengetahui keberadaan dan karakteristik bakteri *E. coli* penghasil ESBL pada peternakan ayam pedaging di wilayah Ciampea, Bogor. Sebanyak 90 sampel dikumpulkan dari tiga peternakan ayam pedaging yang berlokasi di Ciampea, Bogor. Pengambilan sampel dilakukan secara swab kloaka sebanyak 1% dari jumlah populasi pada setiap peternakannya. Pada koloni yang diduga *E. coli* dilakukan pewarnaan Gram dan uji biokimia IMVIC. Konfirmasi *E. coli* penghasil ESBL menggunakan uji difusi cakram ganda dengan MHA. Hasil penelitian menunjukkan bahwa *E. coli* penghasil ESBL terdapat pada 28,9% sampel usap kloaka ayam pedaging (26/90). Pengujian resistensi antibiotik terhadap isolat *E. coli* penghasil ESBL menunjukkan resistensi terhadap empat antibiotik: kloramfenikol, amoksisilin, ciprofloxacin, dan ampisilin. Pada pengujian ini 22 isolat menunjukkan multidrug-resisten (MDR).

Keywords: Escherichia coli, ESBL, Broiler, Antibakteri, Bakteri.

Introduction

Bacterial resistance to antibiotics is a major problem for both animal and human health, as well as global food security. It has become a serious threat to public health due to its emergence and spread. If antibiotic resistance is not controlled, many bacterial pathogens could become much more deadly in the future. The World Health Organization (WHO) and many other groups and researchers agree that the spread of antimicrobial resistance is an urgent problem that requires a coordinated global action plan to address (Castanheira et al., 2021).

The repeated use of antibiotics in chickens can lead to the emergence of antibiotic-resistant bacteria. This can include new strains of bacteria that are resistant to multiple drugs. Even though chickens may not show any symptoms of disease (Ayinla et al., 2023; Bavadharani et al., 2023). Extended-spectrum β-lactamases (ESBL) are enzymes that provide resistance to many different classes of antibiotics. These enzymes are mainly produced by Enterobacteriaceae, particularly *E. coli* and Klebsiella pneumoniae (Rawat and Nair, 2010). The overuse of beta-lactam antibiotics and the selective pressure from naturally occurring beta-lactams have led to the emergence of beta-lactamases (Lopatkin et al., 2017). Recent studies have shown that ESBL-producing *E. coli* has been increasingly detected in poultry farms worldwide (Or et al. 2025), including in countries with strict antibiotic regulations, indicating the persistence of this issue despite control efforts. Furthermore, some

studies have reported that ESBL genes are not only present in *E. coli* but also in other bacterial species, highlighting the potential for horizontal gene transfer between different bacterial populations (Kpoda et al. 2018).

Research conducted in several countries including Indonesia has found *E. coli* ESBL in healthy poultry. The presence of ESBL-producing *E. coli* in broiler chickens has been reported in many countries including in the countries of Malaysia (Aklilu et al., 2022) and India (Singh et al. 2020). Likewise in Indonesia it was reported that ESBL-producing *E. coli* was found in broiler chicken meat sold at Purwokerto City Market (Widhi and Saputra, 2021), in broiler chicken meat sold at traditional markets in Surabaya (Mu'arofah et al, 2020) and in broiler chicken farms in Blitar Regency (Wibisono et al., 2020)

Based on observations and interviews in the field, the farm uses beta-lactam antibiotics as prevention and treatment of diseases in chickens. Excessive use of beta-lactam antibiotics and selective pressure from beta-lactams found in nature have led to the emergence of beta-lactamase (Lopatkin et al., 2017). The condition of the farm has also not implemented good biosecurity. Antibiotic resistance is more likely to occur in crowded cage conditions and poor sanitation. Furthermore, it has the potential to contaminate the next stage of production and have an impact on antibiotic resistance in both animals and humans through the food chain. Humans and animals have the potential to share resistant bacterial genes (Rousham et al., 2018). Therefore, it is necessary to conduct research on ESBL-producing *E. coli* on poultry farms in Bogor.

This study aims to investigate the presence and characteristics of ESBL-producing *Escherichia coli* in broiler farms located in Ciampea, Bogor. Unlike previous studies that mainly focused on retail meat samples, this research will directly assess bacterial resistance in live broilers, their environment, and farm management practices. The high prevalence of antibiotic-resistant bacteria in Bogor (Hermana et al. 2020), particularly against beta-lactam antibiotics, warrants further investigation and consideration as a key factor in this study for antimicrobial resistance management strategies. The results of this study can serve as a reference for relevant agencies, particularly the government, in formulating strategies to prevent and control the spread of ESBL-producing *E. coli* bacteria in the food chain and environment

Materials and Methods

Study Area and Farm Selection

This study aimed to investigate the presence of ESBL-producing *Escherichia coli* in broiler farms located in Ciampea District, Bogor Regency, Indonesia. Sampling was conducted at three selected broiler farms: Cihideung Hilir, Cihideung Udik, and Cinangka. These farms were chosen based on their production scale, accessibility, and their contribution to the regional broiler supply chain. A total of 90 samples were obtained, with 30 samples collected from each farm. To ensure unbiased sampling and population representativeness, individual chickens were selected randomly. The selected farms operated as contract farms under agreements with poultry companies that regulate management aspects such as feeding, medication, and biosecurity practices. Given that Ciampea District is key poultry production center, selected farms provide an appropriate setting for assessing antibiotic resistance patterns in commercial broiler production systems.

Sample Size and Collection Method

A total of 90 cloacal swab samples were collected, representing 1% of the broiler population at each farm. This proportion was selected to balance representativeness with logistical feasibility. Each swab sample was placed in sterile test tubes containing 10 mL of 0.1% buffered peptone water (BPW) solution

and transported under controlled conditions to the Microbiology Laboratory of the Animal Husbandry Department at the Bogor Agricultural Development Polytechnic. During transportation, samples were maintained at a temperature range of 4–8°C by using a cool box with ice packs and a thermometer to preserve bacterial viability.

Sampling Considerations

Several factors were considered when selecting sampling sites, including production volume, logistical accessibility, zoonotic disease risks, and geographical representation. The cloacal swabbing technique was chosen due to its widespread acceptance as a reliable method for detecting enteric bacterial pathogens in poultry. To enhance representativeness, individual broilers were randomly sampled from each flock.

Isolation and Identification of *E. coli*

The isolation and identification of *E. coli* were conducted following the Global Tricycle Surveillance ESBL *E. coli* protocol (WHO, 2021). Initially, each sample was homogenized by mixing with 0.1% BPW solution at a 1:10 ratio, followed by homogenization using a tube shaker. The homogenized samples were then inoculated into *Escherichia coli* broth (Oxoid, UK) and incubated at 45.5°C for 48 h. The presence of turbidity & gas production in Durham tubes was considered indicate of positive *E. coli* growth.

Subsequent isolation involved inoculation onto Tryptone Bile X-Glucuronide (TBX) agar, with bluish-green colonies selected for further analysis. Five presumptive *E. coli* colonies were subculture onto MacConkey Agar (MCA) supplemented with 1 µg/mL cefotaxime (Oxoid, UK) and incubated at 36 ± 1°C for 18–24 hours. Presumptive *E. coli* isolates were confirmed through Gram staining and biochemical characterization using the IMViC (Indole, Methyl Red, Voges-Proskauer, and Citrate) tests.

Confirmation of ESBL-Producing *E. coli*

The presence of ESBL-producing *E. coli* was confirmed using the disc diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI, 2023) guidelines. Bacterial suspensions were streaked onto Mueller Hinton Agar (MHA) plates using sterile cotton swabs. The Kirby-Bauer disk diffusion assay was performed using antibiotic discs containing Cefotaxime (30 µg), Cefotaxime-Clavulanic Acid (30 µg), Ceftazidime (30 µg), and Ceftazidime-Clavulanic Acid (30 µg), which are commonly used antibiotics in poultry farms. The inoculated plates were incubated at 35°C for 24 hours, and ESBL production was confirmed if the inhibition zone diameters for Cefotaxime versus Cefotaxime-Clavulanic Acid and Ceftazidime versus Ceftazidime-Clavulanic Acid differed by at least 5 mm.

Antibiotic Susceptibility Testing

The antibiotic susceptibility profiles of the isolated *E. coli* strains were determined using the Kirby-Bauer disk diffusion method in triplicate. Bacterial suspensions were adjusted to a 0.5 McFarland standard and evenly spread onto MHA plates using sterile cotton swabs. Antibiotic discs containing Amoxicillin (10 µg), Ciprofloxacin (10 µg), Chloramphenicol (10 µg), and Ampicillin (10 µg) were applied to the agar surface. Plates were incubated at 35–37°C for 18–24 hours, and antimicrobial susceptibility was evaluated based on inhibition zone diameters, interpreted CLSI (2023) criteria.

Data Analysis

The prevalence of ESBL-producing *E. coli* in broiler fecal samples collected from farms in Ciampea, Bogor, was analyzed using descriptive statistical methods.

Results and Discussion

Characteristics of Respondents

Samples were taken randomly from three broiler farms from farms in Ciampea, Bogor. The chickens looked healthy/did not show any

symptoms of disease. A total of 90 samples were obtained from cloacal swabs. From the results of macroscopic, microscopic, and biochemical morphological tests. Of the 90 samples, 46 isolates were successfully identified as *E. coli*. *E. coli* colonies on TBX agar appeared as round, blue-green colonies (Figure 1a). TBX agar is a selective medium that has the highest sensitivity (>95%) for detecting *E. coli*. However, there may still be some false positive results, so the positive colonies on TBX media are grown again on Mac Conkey Agar (MCA) media for purification purposes. MCA inhibits the growth of Gram-positive bacteria containing crystal violet and bile salts, and it can differentiate between Gram-negative bacteria that can ferment lactose and those that cannot (Wanger 2017). *E. coli* colonies that grow on MCA are characterized by large, mucoid, and pink colonies (Figure 1b).

1a 1b

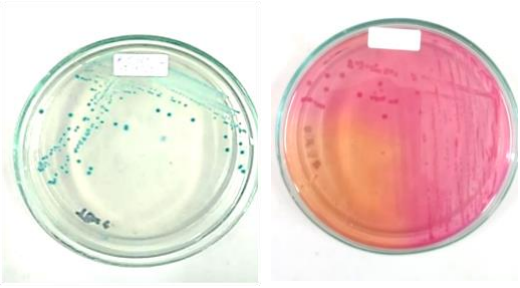

Figure 1. Colony of suspected *E. coli* producing ESBL (1a. colonies on TBX Agar and 1b. colonies on MacConkey Agar)

Following isolation of *E. coli* colonies, a Gram staining test was conducted, which revealed the presence of rod-shaped bacteria with a red color. To further confirm the identification, a biochemical test called IMVIC was performed. The test results indicated that the bacteria showed positive results for indole and MR tests, but tested negative for the VP test.

E. coli is Gram-negative rod-shaped bacterium with a size ranging from 1.0-1.5 µm x 2.0-6.0 µm, non-motile or motile with flagella and can grow with or without oxygen. Other biochemical characteristics of *E. coli* are its ability to produce indole, less able to ferment citrate, negative in urease analysis. *E. coli* commonly lives in the digestive tract of animals (Mueller and Tainter, 2022). The complete results of the isolation and identification process of *E. coli* as seen in Table 1.

From the results of the IMVIC test, 46 positive isolates of *E. coli* were obtained, and after carrying out the ESBL confirmation test using ceftazidime, cefotaxime, Cefotaxime Clavulanic Acid, and Ceftazidime-Clavulanic Acid, 26 were identified as having a diameter difference of ≥ 5 mm in the acid zone. Clavulanate compared with the zone without clavulanic acid. ESBL-producing *E. coli* was identified in 26 isolates.

ESBL is a type of beta-lactamase enzyme that can cause resistance to various antibiotics, including penicillin, 1st, 2nd, and 3rd generation cephalosporins, as well as aztreonam, except for cephalin and carbapenems (Naelasari et al., 2018). Organisms that produce ESBL also tend to be resistant to many other classes of antibiotics. This enzyme is most commonly produced by Enterobacteriaceae, particularly by *E. coli* and Klebsiella pneumoniae (Rawat and Nair, 2010).

The gene responsible for producing the ESBL enzyme is centered on a plasmid and develops into a point mutation, which results in a change in the configuration of the active part of the original gene and is known as β-lactamase (Umadevi et al., 2011). This ESBL enzyme has spread to various organisms. *E. coli* ESBL can be found in humans, livestock, and wildlife (Schaufler et al. ,2015).

Table 1. Isolation and identification results of *Escherichia coli*

Specimen	Sample	*Escherichia coli* Positive (%)	ESBL-producing *E. coli* (%)
Farm A	35	21 (60)	12 (57,14)
Farm B	35	15 (42.85)	8 (53,33)
Farm C	20	10 (50)	6 (60)
Total	90	46 (51.11)	26 (56,52)

Colonies with positive biochemical test results were followed by a double disk diffusion test to confirm ESBL-producing *E. coli*. The description of the *E. coli* ESBL confirmation test results was interpreted by referring to the Clinical and Laboratory Standards Institute (CLSI 2023), by looking at the diameter of the inhibition zone formed (Figure 2).

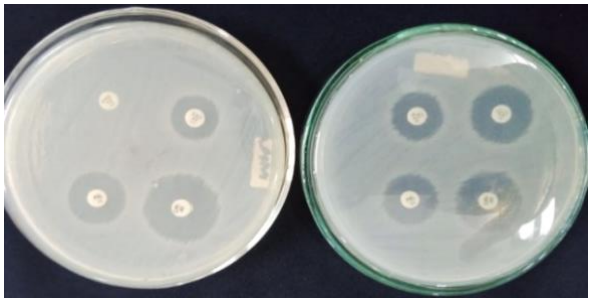

Figure 2. ESBL confirmation test using ceftazidime, cefotaxime, Cefotaxime Clavulanic Acid, and Ceftazidime-Clavulanic Acid on *E. coli*

This study examined resistance to four antibiotics amoxicillin, ciprofloxacin, chloramphenicol, and ampicillin belonging to three classes: penicillin, quinolone, and chloramphenicol. Where these antibiotics are often used for prevention and treatment in the three farms. Standards for determining resistance classification. Intermediate and sensitive based on guidelines from the Clinical and Laboratory Standards Institute (CLSI, 2023). Twenty-six positive isolates of *E. coli* ESBL showed resistance to Amoxicillin 100%, Ampicillin 100%, Ciprofloxacin 73.08% and Chloramphenicol 35% (Table 2).

Based on observations and interviews, these farmers partner with companies, so that farmers have a program for the use of antibiotics during chicken farming for prevention and treatment regulated by the company that partners with the farmers. The same antibiotics with inappropriate doses and given continuously will trigger the emergence of antibiotic resistance (Bahri et al., 2005).

In this study, broiler farms used commercial feed from large manufacturers, and the use of antibiotic growth promoters in feed has been officially banned by regulations. However, antibiotic administration for prevention and treatment is still practiced, particularly beta-lactam antibiotics, which are commonly used in these farms. The resistance findings in this study, especially to amoxicillin (100%) and ampicillin (100%), indicate a possible link to the frequent use of beta-lactam antibiotics in the farms. Additionally, high resistance to ciprofloxacin (73.08%) and chloramphenicol (35%) suggests that antibiotic usage practices in these farms may contribute to the development of antibiotic resistance. The findings confirm the presence of multidrug-resistant isolates, as all ESBL-producing *E. coli* isolates exhibited resistance to at least two antibiotic classes, with some also resistant to chloramphenicol. MDR's emergence isolates poses a significant challenge in both veterinary and human medicine, as it restricts available treatment options and increases risk of therapeutic failure.

Therefore, enhanced antimicrobial stewardship, stricter regulations on antibiotic use in poultry farming, and alternative disease control strategies such as improved biosecurity measures and vaccination programs are urgently required to mitigate the risk of antibiotic resistance. ESBL enzyme has been distributed in various organisms. These enzymes can not only hydrolyze penicillin, but also the newest antibiotics, namely group 3 cephalosporins and monobactams. *E. coli* ESBL can be found in humans and livestock (Schaufler et al., 2015).

Table 2. Results of antibiotic resistance tests

Antibiotic disk	Σ isolat *E. coli* ESBL	Resistant		Intermediate		Sensitive	
		Σ	%	Σ	%	Σ	%
Chloramphenicol	26	9	35	13	50	4	15
Amoxicillin	26	26	100	0	0	0	0
Ciprofloxacin	26	19	73,08	5	19,23	2	7,69
Ampicillin	26	26	100	0	0	0	0

Most antibiotic resistance commonly arises from mutations or horizontal transfer of genes that carry resistance traits. Resistance genes can be inherited or aquired from plasmids which are mobile genetic element that can transfer between bacteria (Read and Woods 2014). Low doses (subtherapeutic) of antibiotics can increase the development of antibiotic resistance by inducing genetic changes (Ventola, 2015).

The results of a study conducted by Müller et al (2015), ESBL-producing *E. coli* was found in poultry feces samples on laying hen farms (65%) and broiler chickens (81%). The study also found *E. coli* ESBL in wastewater, other livestock, dust, surface water near livestock, soil, flies, and in the barn air. The highest prevalence and concentration were found in outdoor environments, especially in the soil of laying hen farms (100%). The presence of ESBL-producing *E. coli* in poultry farms and their environment can pose a health risk if the bacteria reach places that can come into contact with humans.

Conclusions

ESBL-producing *E. coli* were detected in 28,9% of 90 cloacal swab samples from three broiler farms in Ciampea, Bogor. Antibiotic resistance testing of ESBL-producing *E. coli* isolates showed resistance to four antibiotics, namely chloramphenicol, amoxicillin, ciprofloxacin, and ampicillin. In this test, 22 ESBL-producing *E. coli* isolates showed multidrug-resistant (MDR).

References

Ayinla AO, and ALP Mateus. 2023. Extended-spectrum beta-lactamases in poultry in Africa: a systematic review. Frontiers in Antibiotics. 2: 1140750.

Bavadharani M, ST Rai, AK Arora, and S Singh. 2023. Antimicrobial sensitivity pattern and detection of antimicrobial resistance genes of *E. coli* isolated from respiratory tract infections in poultry. The Indian Journal of Animal Sciences., 93(3): 251–255.

Castanheira M , PJ Simner, and PA Bradford. 2021. Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. JAC-Antimicrobial Resistance. 3(3)

Clinical and Laboratory Standards Institute (CLSI). 2021. CLSI M100-ED31:2021 Performance Standards for Antimicrobial Susceptibility Testing, 31st Edition.

Hermana NSP, Z Nilasari, E Yuniarti, A Riyanto, Safika, A Indrawati, FH Pasaribu. Antibiotic resistance profile of Staphylococcus aureus, Streptococcus spp. and Klebsiella spp. isolated form chicken farm in Bogor, Sukabumi, and Cianjur. Journal of Physics: Conference Series. West Java. 1430: 1-6

Kpoda DS, A Ajayi, M Somda, O Traore, N Guessennd. 2018. Distribution of resistance genes encoding ESBLs in Enterobacteriaceae isolated from biological samples in health centers in Ouagadougou, Burkina Faso. BMC Research Notes. 11:471.

Lopatkin AJ, HR Meredith, JK Srimani, P Pfeiffer, R Durrett, and L You . 2022. Persistence and reversal of plasmid-mediated antibiotic resistance. Nature Communications. 8(1):1689.

Mu'arofah B, RY Arizandy,B Utomo, and Kuntaman K. 2020. Detection Of Extended Spectrum B-Lactamase (ESBL) Gene Patterns Of Enterobacteriaceae In Broiler Chicken Meat Sold In Traditional Markets In The East Surabaya. Jurnal Ilmiah Kesehatan. 9 (1); 12-19. doi: 10.30994/sjik.v9i1.264

Mueller M, and CR Tainter. 2022. Escherichia coli infection. StatPearls. Treasure Island (FL): StatPearls Publishing. PMID: 33231968.

Müller A, R Stephan, Magdalena, and Nüesch-Inderbinen . 2015. Distribution of virulence factors in ESBL-producing Escherichia coli isolated from the environment, livestock, food and humans. Science of The Total Environment. 541:667-672.

Naelasari DN, EB Koendhori, L Dewanti, R Sarassari, and K Kuntaman. 2018. The prevalence of extended spectrum beta-lactamase (ESBL) producing gut bacterial flora among patients in Dr. Soetomo hospital and primary health centre in Surabaya. Folia Medica Indonesiana. 54(4): 256-262.

Rawat D, and D Nair. 2010. Extended-spectrum β-lactamases in gram negative bacteria. Journal of Global Infectious Diseases. . 2(3):263-74.

Rousham EK, L Unicomb, and MA Islam. 2018. Human, animal and environmental contributors to antibiotic resistance in low-resource settings: integrating behavioural, epidemiological and one health approaches. Proceedings: Biological Sciences. . 285(1876): 20180332.

Rumokoy, L, and WL Toar. 2022. Application of Thoracic Immunogen of Musca domestica on Immunoglobulin-G Level of Goats Detected Through a Single Radial Immuno-Diffusion Test. Animal Production. 24(1):1–5.

Schaufler K, Bethe A, Lübke-Becker A, Ewers C, Kohn B, Wieler L. H, Geunther S. 2015. Putative connection between zoonotic multi resistant extended-spectrum β-lactamase (ESBL)-producing Escherichia coli in dog feces from a veterinary campus and clinical isolates from dogs. Infection Ecology & Epidemiology. 5(4):25334-25339.

Umadevi S, Kandhakumari G, Joseph NM, Kumar S, Easow JM, Stephen S .2011. Prevalence and antimicrobial susceptibility pattern of ESBL-producing gram-negative bacilli. Journal of Clinical and Diagnostic Research. 5:236-239.

van Hoek AHAM, C Dierikx, T Bosch, L Schouls , E van Duijkeren, and M Visser. 2020. Transmission of ESBL-producing Escherichia coli between broilers and humans on broiler farms. Journal of Antimicrobial Chemotherapy. 75: 543–549.

Ventola CL. 2015. The antibiotic resistance crisis: part 1-causes and threats. Pharmacy Therapeutics. 40(4):277-83. PMCID: PMC4378521

[WHO] World Health Organization. 2018. Antimicrobial resistance. In: facts sheet

[WHO] World Health Organization. 2021. WHO integrated global surveillance on ESBL-producing *E. coli* using a “One health” approach.

Wibisono FJ, B Sumiarto, T Untari, MH Effendi, DA Permatasari, and AM Witaningrum. 2020. CTX Gene of Extended Spectrum Beta-Lactamase (ESBL) Producing Escherichia coli on Broilers in Blitar, Indonesia. Scientific Research Publishing. . 11(7): 396-403.

Widhi APKN, and INY Saputra. 2021. Broiler Chicken Meat Sold at Purwokerto's Market Has Antibiotic Residues and Escherichia coli That Produces ESBL. J. Kesehatan Lingkungan Indonesia. 20(2):137-142