

Antibiotic Resistance of *Escherichia coli* Produced Extended Spectrum Beta-Lactamase In Broiler Chicken Meat of Banda Aceh City Markets

(RESISTENSI ANTIBIOTIK *ESCHERICHIA COLI*
PENGHASIL EXTENDED SPECTRUM BETA-LACTAMASE
PADA DAGING AYAM BROILER DI PASAR KOTA BANDA ACEH)

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ABSTRACT

Broiler chicken meat is one of the animal proteins commonly consumed by the public due to its good nutritional content. Like other animal-based foods, broiler chicken meat is also highly perishable due to bacterial growth, including *Escherichia coli*. This bacterium has developed resistance to β -lactam antibiotics and other classes of antibiotics, leading to multidrug resistance. Extended spectrum β -lactamase (ESBL) is an enzyme capable of breaking down penicillin, first, second, and third generation cephalosporins, and monobactams. The main objective of this study was to determine the resistance of ESBL-producing *E. coli* in broiler chicken meat to several groups of antibiotics, namely ampicillin, kanamycin, cefotaxime, ceftazidime, and trimethoprim-sulfamethoxazole. This study used 47 samples of broiler chicken meat taken using random sampling. Broiler chicken meat samples were isolated to obtain ESBL-producing *E. coli* bacterial colonies. The detection of *E. coli* was performed using Eosin Methylene Blue Agar (EMBA) media mixed with cefotaxime antibiotic (2 μ g/mL) and followed by testing the sensitivity to several antibiotics using the disc diffusion test. The results of the antibiotic testing were observed by measuring the diameter of the inhibition zone formed and interpreted with reference to the Clinical and Laboratory Standards Institute (CLSI) standards. Directly positive multidrug-resistant bacteria are defined as bacteria that are resistant to ≥ 3 types of antibiotics. The results showed that 10 samples (21.27%) were positive for *E. coli* bacteria with a resistance rate of

90% for cefotaxime, 80% for ceftazidime, 80% for ampicillin, 30% for kanamycin, and 80% for trimethoprim-sulfamethoxazole. Overall, ESBL-producing *E. coli* isolates from broiler chicken meat in Banda Aceh City Market showed resistance to more than three classes of antibiotics, thus categorized as multidrug resistance.

Keywords: Antibiotics; Chicken Meat; *Escherichia coli*; ESBL; Multidrug Resistant

ABSTRAK

Daging ayam broiler merupakan salah satu protein hewani yang umumnya banyak dikonsumsi oleh masyarakat karena kandungan gizi yang baik. Seperti bahan pangan asal hewan lainnya, daging ayam broiler juga sangat mudah busuk akibat pertumbuhan bakteri, termasuk *Escherichia coli*. Bakteri ini telah mengembangkan resistensi terhadap antibiotik β -laktam dan kelas antibiotik lainnya, yang menyebabkan resistensi multidrug. *Extended spectrum β -lactamase* (ESBL) adalah enzim yang mampu memecah penisilin, sefalosporin generasi pertama, kedua, dan ketiga, serta monobaktam. Tujuan utama studi ini adalah untuk mengetahui resistensi *E. coli* yang memproduksi ESBL pada daging ayam broiler terhadap beberapa kelompok antibiotik, yaitu ampicilin, kanamisin, cefotaxim, ceftazidim, dan trimethoprim-sulfametoksazol. Studi ini menggunakan 47 sampel dari daging ayam broiler yang diambil dengan metode *random sampling*. Sampel daging ayam broiler diisolasi untuk mendapatkan koloni bakteri *E. coli* penghasil ESBL. Deteksi keberadaan *E. coli* dilakukan menggunakan media *Eosin Methylene Blue Agar* (EMBA) yang telah dicampurkan dengan antibiotik cefotaxime (2 μ g/mL) dan dilanjutkan dengan menguji sensitivitas terhadap beberapa antibiotik dengan uji difusi cakram. Hasil dari pengujian terhadap antibiotik diamati dengan mengukur diameter zona hambat yang terbentuk dan diinterpretasikan dengan mengacu pada standar *Clinical and Laboratory Standards Institute* (CLSI). Bakteri resisten multidrug yang langsung positif didefinisikan sebagai bakteri yang resisten terhadap ≥ 3 jenis antibiotik. Hasil menunjukkan terdapat 10 sampel (21,27%) yang positif bakteri *E. coli* dengan tingkat resistensi sebesar 90% untuk cefotaxime, 80% untuk ceftazidime, 80% untuk ampicillin, 30% untuk kanamycin, dan 80% untuk trimethoprim-sulfamethoxazole. Secara keseluruhan, isolat *E. coli* penghasil ESBL dari daging ayam broiler di Pasar Kota Banda Aceh menunjukkan resistensi terhadap lebih dari tiga kelas antibiotik, sehingga dikategorikan sebagai resistensi multidrug.

Kata-kata kunci: antibiotik; daging ayam; *Escherichia coli*; ESBL; resisten multidrug

INTRODUCTION

Global public health risks are now increasingly significant along with the spread of foodborne pathogens, especially bacterial strains that have the ability to resist the effects of antibiotics (Kuntaman *et al.*, 2025). Among them, the emergence and spread of *Escherichia coli* (*E. coli*) that produces Extended Spectrum beta-Lactamase (ESBL) in the food chain is of critical concern, as these bacteria often exhibit multidrug-resis-

tant (MDR) phenotypes that limit effective treatment options for human infections (Ferasyi *et al.*, 2025). Poultry farms serve as critical reservoirs for antibiotic-resistant bacteria, particularly *E. coli* producing Extended Spectrum *beta-Lactamase*, due to intensive production practices and high animal density. The emergence and spread of these resistant strains is primarily driven by the extensive and indiscriminate use of antimicrobials for non therapeutic purposes, such as growth promoter and prevention of prophylactic di-

seases (Aliyu *et al.*, 2024).

In Indonesia, broiler chicken meat is one of the most important sources of animal food due to its affordability, high production volume, and wide availability (Ariningsih *et al.*, 2023). However, the increasing demand for meat has prompted the extensive use of antibiotics in farming, particularly in poultry, increasing the prevalence of resistant bacteria including *E. coli* in meat and meat products (Mulchandani *et al.*, 2020). In *E. coli*, *extended spectrum beta-lactamase* production and carbapem and fluoroquinolone resistance result in a decreased efficiency of critically important antibiotics, such as third and fourth generation cephalosporins, meropenem and ciprofloxacin (Adams *et al.*, 2018)

Beta-lactam antibiotics, including penicillin and its derivatives-cephalosporins, and carbapenem, are widely used against bacterial infections (Zabizsak *et al.*, 2023). However, *E. coli* often produce the enzyme β -lactamase as the main defense, which inactivates these antibiotics (Cag *et al.*, 2016). The β -lactamase dominant in Gram-negative bacteria include *Temoneira* (TEM), *Sulphydryl Variable* (SHV), *Oxacillinase* (OXA), and *Cefotaxime-Munich* (CTX-M), with CTX-M now recognized as the most widespread enzyme (Jena *et al.*, 2017). Initially, *blaTEM* and *blaSHV* are narrow-spectrum β -lactamase genes, conferring resistance to penicillin. However, mutations have caused the broad-spectrum β -lactamase, which hydrolyzes third-generation cephalosporins (Bush *et al.*, 2016). The *blaOXA* gene encodes oxylinase-type β -lactamase, some of which, such as *blaOXA-48*, have evolved into carbapenemas, posing significant treatment challenges (Evans *et al.*, 2014). The *blaCTX-M* gene, derived from the *Kluyvera ascorbata*, has now surpassed *blaTEM* and *blaSHV* as the most common ESBL genes globally, encoding enzymes with high activity against cefotaxime and ceftazidime (Cantón *et al.*, 2014). Resistance genes for the β -lactamase enzyme are often plasmid-encoded, facilitating their spread across bacterial populations by horizontal transfer (Böhm *et al.*, 2020). The spread of β -lactamase-producing

organisms has emphasized the need for a "One Health" approach to Antimicrobial Resistance (AMR) management (Ramatla *et al.*, 2023).

Traditional wet markets in cities such as Banda Aceh (including markets such as Al-Mahirah and Seutui) are important nodes in AMR transmission. Unlike modern retail environments, traditional markets often feature limited hygiene infrastructure, high human animal interactions, and practices that facilitate cross-contamination between raw meat, surfaces and consumers. Various microorganisms, including *E. coli* and other pathogenic microbes, frequently contaminate chicken meat (Rouger, 2017). *Escherichia coli* contamination can occur during chicken processing, from slaughtering to marketing, and can originate from feces, traders, water, and the market environment. Currently, the increasing resistance in Gram negative enteric bacilli receives special attention because of the potential horizontal spread to pathogens (Collignon *et al.*, 2018).

Research in the Aceh region has shown that *E. coli* contamination in this market can be as high as 31% with isolates showing 100% resistance to ampicillin and high resistance to other common antibiotics (Ferasyi *et al.*, 2025).

Despite the known risks, most AMR research in Southeast Asia has focused on the agricultural level, often ignoring the post-farmgate environment in which products are handled and sold. Recent findings in Banda Aceh have identified ESBL-producing *E. coli* not only on the meat itself but also on the traders' utensils, such as tables, knives, and cutting boards, posing a direct danger to public health. Therefore, this study was aimed to investigate the prevalence and resistance patterns of ESBL-producing *E. coli* in the Banda Aceh markets. It is essential to provide local data for integrated surveillance strategies and to maintain food safety for the community.

RESEARCH METHODS

Study Period and Location

This study was conducted from September to November 2023 in Banda Aceh, Aceh, Indonesia. The population of Banda Aceh city, is around 270,000 people. Banda Aceh has a humid tropical climate, characterized by high annual rainfall (2,000–3,000 mm) and average temperatures ranging from 26°C to 32°C. This region have a high demand for poultry products, which also serve as the main distribution point for broiler meat. The traditional poultry markets in Banda Aceh are located in a public market complex. Poultry trade is carried out in semi-permanent stalls, often with limited hygiene infrastructure. The markets usually operates from morning to noon, with peak sales before noon. More than 75% of the broiler chickens brought to this traditional markets are sourced from small farms in other districts. Some of them are supplied from agriculture in the neighboring province of North Sumatra.

Study Design

This study was designed as an initial cross-sectional study to investigate the occurrence and pattern of ESBL-producing *E. coli* AMR throughout the poultry supply chain. A total of 47 samples were collected from two traditional poultry markets. Steps from sample collection, bacterial isolation and antibiotic susceptibility testing.

Sample Collection

The target sample size is determined based on logistical feasibility, taking into account the availability of resources, time constraints, and the need to represent the sample. Therefore, this study should be considered a preliminary surveillance study. Samples were collected from two traditional markets in Banda Aceh City. A total of 47 samples from chicken meat swabs. The decision to use 47 samples per market was based on a balance between logistical feasibility, resource availability, and the need for adequate representation of the animal-human-environment interface in each market. Sampling was carried out during morning market operating hours (7:00–10:00 AM),

when poultry transactions are most active. Collection is conducted on three separate market days, both weekdays and weekends, per location within the same month, to reduce temporal bias. Samples are randomly selected from the sellers available in each market to avoid systematic bias in sample collection. All swabs are immediately placed into sterile tubes containing nutrient broth as a transport medium, which supports the recovery of stressed enteric bacteria. All collected samples are transported to the laboratory in an icebox container and maintained at a temperature of 4°-10°C during transport to prevent excessive bacterial growth or degradation.

Isolation and Identification of *E. coli*

The isolation and identification of *E. coli* were performed by growth on different culture media Nutrient Broth and Eosin Methylene Blue (EMB) agar media containing Cefotaxime (2 µg/mL), different biochemical tests (sugar fermentation test, along with indole and methyl red (MR) tests, Voges-Proskauer (VP), and citrate utilization tests) and Gram-staining as previously describe.

Antimicrobial susceptibility test (AST) isolates were tested for AST using the Kirby-Bauer disc diffusion method on Mueller–Hinton (MH) agar plates, following the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines. The bacterial concentrations were standardized to a 0.5 McFarland standard and spread on the agar. Isolates were tested against five commonly used antibiotic, such as: ampicillin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), trimethoprim-sulfamethoxazole (25 µg), canamycin (30 µg) and incubated at 35°C for 24 hours. The results of the sensitivity test are interpreted based on the guidelines from the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines. The *E. coli* strain ATCC 25922 is used as an ESBL negative control and sensitivity test control. Isolates that exhibited resistance to at least three antibiotic classes were considered multi-drug resistant (MDR).

Detection of ESBL by DDST

The isolates resistant to third-generation cephalosporins (ceftriaxone, cefpodoxime and ceftazidime) were tested for ESBL production using the Double Disc Synergy Test (DDST) method. The test employed discs of amoxicillin/clavulanate (20 µg/10 µg), ceftazidime (30 µg), and cefotaxime (30 µg). These discs were placed 30 mm apart from the amoxicillin/clavulanate disc (center to center) and incubated at 37°C for 24 h. A positive result was indicated by reduced susceptibility to ceftazidime, or cefotaxime, accompanied by a clear enhancement of the inhibition zone toward the amoxiclav disc European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2017)

Data Analysis

The research data obtained was analyzed descriptively. The collected of the data were presented in the form of a table on the proportion of resistance of ESBL-producing *E. coli* to antibiotics from broiler chicken meat swab samples in the Banda Aceh city traditional markets.

RESULTS AND DISCUSSION

The results of the isolation and identification of *E. coli* based on the research conducted on broiler chicken meat samples that collected from the traditional markets in Banda Aceh, it were found that the meat was 100% contaminated with *E. coli* (47/47 samples). From 21.2% (10/47) were confirmed to be ESBL producers, while 78.7% (37/47) were not. The contamination of chicken meat with *E. coli* was indicated by growth on Eosin Methylene Blue Agar (EMBA) media. Suspected *E. coli* colonies exhibited a metallic green color with black dot in the center of colony (Jang *et al.*, 2017). *Escherichia coli* positive isolates on EMBA were presented in Figure 1a. The phenotype of ESBL-producing *E. coli* with the formation of a characteristic zone of "keyhole" is shown in Figure 1b. Resistance of ESBL-producing *E. coli* is also shown against cefo-

taxime (9/10, 90% of samples), ceftazidime (8/10, 80% of samples), ampicillin (8/10, 80% of samples), trimethoprim-sulfamethoxazole (8/10, 80% of samples) and canamycin (3/10, 30% of samples). The proportions and patterns of resistance of ESBL-producing *E. coli* isolates to antibiotics are presented in detail in Tables 1.

According to Blaak *et al.* (2015), the percentage of ESBL-producing *E. coli* in various places can vary based on several factors, including the region or region where the sample was taken, the level of fecal contamination, the time of sampling, the speed of the effluent flow and the condition of the sewer. Normaliska *et al.* (2019) in her research found that ESBL-producing *E. coli* was found in baskets for heads and feet, workers' boots, iron hanging tools, transportation tools/trucks, floors and knife sheaths with an average of 12.5% from the Chicken Slaughter House environment of Bogor city.

There are three main genes encoding ESBLs, namely TEM, SHV and CTX-M. These three genes are the genes responsible for producing ESBLs that are able to hydrolyze β -lactam antibiotics. These ESBL genes are located in plasmids that can be spread easily between bacterial species (Ima-sari *et al.*, 2018). The ESBL CTX-M group is the most widely found group in bacterial resistance. This is because the plasmid encoding the CTX-M gene is a type of large plasmid that also encodes genes that are resistant to other classes of antibiotics. Conjugative plasmids are also considered to be one of the factors in the spread of CTX-M type ESBL (Kang *et al.*, 2017).

The trait of multi-drug resistance by bacteria to antibiotics including the β -lactam group is often found in every case of bacterial resistance to antibiotics, especially *E. coli* (Normaliska *et al.*, 2019). Resistance is the ability of bacteria to adapt to antibiotic exposure. This trait is a natural mechanism for bacteria to survive. Bacteria that produce β -lactamase and use this enzyme to destroy β -lactams are the main factors in the development of resistance to antibiotics. The results of this study provide information

Tabel 1. Hasil pemeriksaan status praesens kucing Lucy

No	Jenis Pemeriksaan	Hasil	Nilai Rujukan*)	Keterangan
1.	Frekuensi jantung (kali/menit)	156	140-220	Normal
2.	Frekuensi pulsus (kali/menit)	152	140-220	Normal
3.	Capillary Refill Time (CRT) (detik)	> 2	< 2	Tidak Normal
4.	Frekuensi respirasi (kali/menit)	40	24-42	Normal
5.	Suhu tubuh (oC)	38,4	38,1-39,2	Normal

Keterangan: *) Sumber: Tilley dan Smith (2015)

Table 1. Results of antibiotic inhibition zone measurement of positive Extended-Spectrum Beta-Lactamase (ESBL) producing *Escherichia coli* on Mueller Hinton Agar (MHA)

Sample code	Antibiotic Inhibition Zone Sizes (mm)									
	Antibiotics of the β -Lactam Class						Antibiotics of Other Classes			
	CTX		CAZ		AMP		SXT		K	
D1	8.30	(R)	19.00	(I)	8.90	(R)	0.00	(R)	0.00	(R)
D2	9.25	(R)	0.00	(R)	9.60	(R)	0.00	(R)	8.35	(R)
D9	0.00	(R)	0.00	(R)	7.50	(R)	9.08	(R)	13.92	(I)
D10	0.00	(R)	0.00	(R)	7.80	(R)	7.40	(R)	11.80	(R)
D15	4.70	(R)	11.80	(R)	16.10	(I)	26.20	(S)	17.30	(I)
D28	7.70	(R)	18.80	(I)	15.10	(I)	25.95	(S)	16.55	(I)
D30	5.30	(R)	8.00	(R)	0.00	(R)	0.00	(R)	16.75	(I)
D33	6.80	(R)	12.00	(R)	0.00	(R)	0.00	(R)	15.90	(I)
D42	25.30	(S)	15.50	(R)	0.00	(R)	0.00	(R)	15.50	(I)
D46	8.70	(R)	11.80	(R)	0.00	(R)	0.00	(R)	16.65	(I)

Description: Meat 1 (D1), Meat 2 (D2), Meat 9 (D9), Meat 10 (D10), Meat 15 (D15), Meat 28 (D28), Meat 30 (D30), Meat 33 (D33), Meat 42 (D42), Meat 46 (D46), Sensitive (S), Intermediet (I), Resistant (R), Cefotaxime (CTX), Ceftazidime (CAZ), Amlicilin (AMP), Trimethoprim-sulfametoxazole (SXT), Kanamycin (K).

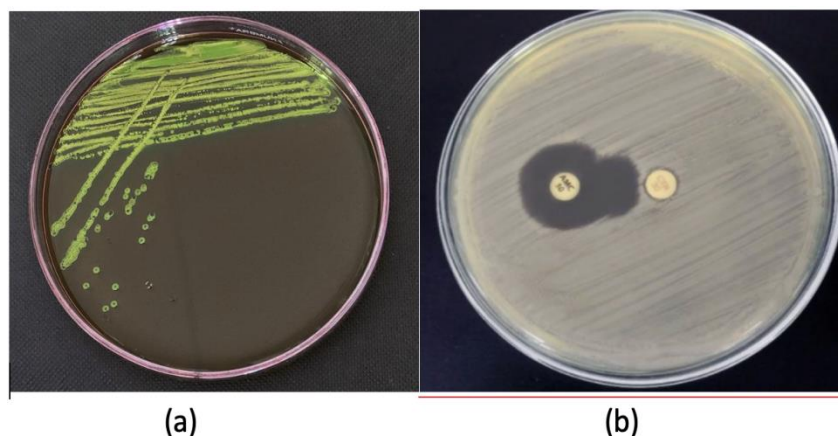


Figure 1. ESBL-producing *E. coli* colonies . 1a: *E. coli* colonies in EMBA, 1b: ESBL-producing *E. coli* positive results are indicated by the formation of a characteristic "keyhole" zone.

about ESBL-producing *E. coli* associated with antibiotic resistance. This refers to the Clinical Laboratory Standards Institute (CLSI, 2023) showing anti-biotic resistance testing against *E. coli* shows a fairly high level of resistance to cefotaxime (90%), ceftazidim (80%) and ampicillin (80%).

Generally, the formation of resistance is related to the transfer of genetic material encoding resistance-determining genes. The genetic material can be obtained by bacteria from other bacteria in the environment through several mechanisms such as transformation, transduction and conjugation. After the nature of resistance is formed, microbes will respond to several resistance mechanisms, one of them is by forming an enzyme. Modification of antibiotic target molecules through the help of enzymes is a mechanism that often occurs because this mechanism is the easiest and fastest to be carried out by bacteria, for example in the ability of Gram-negative bacteria to produce β -lactamase enzymes that hydrolyze the β -lactam ring of β -lactam antibiotics (Kurnianto and Syahbanu, 2023). The occurrence of resistance in ESBL-producing *E. coli* to several types of antibiotics can also be associated with the use of antibiotics mixed in feed or drinking water. The concentration of antibiotics added in animal feed is in a low dose that has been shown to spur resistance of pathogenic bacteria and commensal bacteria in the digestive tract and results in limited treatment options (Ngangguk *et al.*, 2014).

The ESBL-producing *E. coli* detected in effluents are at risk of contaminating the environment through the spread of resistance to other bacteria of the same or different species horizontally and vertically through Mobile Genetic Elements (MGE) consisting of plasmids, transposons and bacteriophage (Zhang *et al.*, 2019). Several other studies have shown that ESBL-producing *E. coli* can indirectly transmit to animals and humans through hygiene and sanitation and contaminated food chains (Franz *et al.*, 2015). The ESBL-producing *E. coli* polluted in the environment can be a risk factor for causing

disease in humans and animals, and can even become zoonotic. Research report of Wade-pohl *et al.* (2020) corroborates some of these studies by stating that the spread of ESBL-producing *E. coli* can occur between humans, chickens both alive and in the form of carcasses or feces, and the environment within the abattoir or landfill area.

Consumption of chicken meat food containing antibiotic residues will cause health problems. The hazards of residual veterinary drugs can be in the form of short-term direct hazards such as allergies, digestive disorders, skin disorders, anaphylaxis and hypersensitivity, as well as long-term indirect hazards such as microbiological resistance, carcinogenic, mutagenic, teratogenic and reproductive disorders (Ruegg *et al.*, 2013). This research is strengthened by Reich *et al.* (2013) which states that food products of animal origin contaminated with the Enterobacteriaceae family such as ESBL-producing *E. coli* can pose a health risk, although the level of risk is difficult to measure. Broiler chickens can serve as a reservoir for *E. coli* ESBL. The ESBL bacteria are transmitted in several ways, including consuming contaminated meat, environment contaminated with feces containing ESBL-producing *E. coli*, exposure to ESBL-infected patients or people and transmitted to humans through animals and possibly causing zoonosis.

CONCLUSION

Based on the results of the study, it can be concluded from 47 swab samples of broiler chicken meat, 10 samples are *E. coli* that produces ESBL. Resistance levels were 9/10 (90%) for cefotaxime, 80% (8/10) for ceftazidime, 80% (8/10) for ampicillin, 30% (3/10) for kanamycin, and 80% (8/10) for trimethoprim-sulfamethoxazole. Overall, ESBL-producing *E. coli* isolates from poultry meat show resistance to more than three classes of antibiotics, so they are categorized as multidrug resistance.

SUGGESTION

Based on the results of the study, in future time it can be carried out a research on ESBL-producing *E. coli* molecularly on resistance genes.

ACKNOWLEDGEMENT

This article was supported in part by the Lecturer Research (*Penelitian Lektor*) funding from Universitas Syiah Kuala, Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia for the 2023 Budget Year, Number: 250/UN112.1/PT.01.03/PNBP/2023.

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