Extended spectrum β-lactamase (ESBL) OF *Escherichia coli* resistance from the broiler cloaca in the traditional market banda aceh

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Abstract. Extended Spectrum β-Lactamase (ESBL)-producing *Escherichia coli* is a type of bacteria that can cause several infectious diseases in various animal and human tissues. ESBL bacteria have been reported to be resistant to various classes of antibiotics including penicillin, third-generation cephalosporins (cefotaxime, ceftriaxone and ceftazidime), and monobactam (aztreonam). This study focused on the presence of *Escherichia coli* as an ESBL producer in the cloaca of broiler. The purpose of this study was to determine the presence or absence of ESBL-producing *Escherichia coli* in the cloaca of broiler in the traditional market, Banda Aceh. This study used 47 samples of broiler cloacal swabs. The study used Cross-Sectional Observation and the disc diffusion methods. The presence of ESBL-producing *Escherichia coli* in cloacal swabs was identified using Eosin Methylene Blue Agar (EMBA) media. Detection of ESBL production using β-lactam antibiotic discs, namely cefotaxime, ceftazidime and ampicillin. The results showed the presence of ESBL-producing *Escherichia coli* bacteria isolated from cloacal swabs of broiler in the traditional market, Banda Aceh, which reached 21.27% (10/47). Sensitivity test results showed high resistance of *Escherichia coli* to cefotaxime 100% (10/10), ceftazidime 90% (9/10), and ampicillin 70% (7/10). Referring to CLSI, all isolates (100%) were ESBL-producing *Escherichia coli*. Based on the results of the study, it can be concluded that there is Extended Spectrum β-lactamase (ESBL) producing *Escherichia coli* in the cloaca of broiler in the traditional market, Banda Aceh.

1 Introduction

Broiler is one of the sources of animal protein that is often consumed by the public [1]. Broilers have been specifically designed to produce meat, with a relatively fast growth period

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of about 4-5 weeks [2]. Broilers are usually obtained from traditional markets that are identical to dirty, disorganized places, and the meat of broiler sold is simply placed without a supportive mat, making it easier for bacterial contamination to occur [1]. Bacteria can be in the body of the broiler even though it does not show any disease symptoms due to the presence of these bacteria [3]. Broilers contain various pathogenic enteric microorganisms in their digestive tract, including *Escherichia coli* [4].

Escherichia coli is one of the normal floras in animals and humans but has the potential to cause disease under certain conditions. The negative impact of *Escherichia coli* which is pathogenic is the occurrence of Colibacillosis in the meat of broilers [5]. Colibacillosis in the meat of broilers can cause very high economic losses for farmers. Colibacillosis can be treated using antibiotics [6]. However, the continuous and uncontrolled use of antibiotics will result in increased resistance to antibiotics [7]. One of the impacts of the unwise use of antibiotics is the emergence of multidrug resistance bacteria including Extended Spectrum β-Lactamase (ESBL)-producing bacteria [8].

Extended Spectrum β-Lactamase (ESBL) is an enzyme derived from mutation of the β-lactamase enzyme, which results in increased enzymatic activity of beta-lactamase [9]. Extended Spectrum β-Lactamase is found in bacterial plasmids that can hydrolyze penicillin group antibiotics, third-generation cephalosporins (cefotaxime, ceftriaxone and ceftazidime), and monobactam group (aztreonam) [10]. Extended Spectrum β-Lactamase is produced by many Enterobacteriaceae group bacteria, especially *Escherichia coli* [11]. *Escherichia coli* bacteria that produce ESBL can be found in the digestive tract and urinary system of humans, livestock, and infected wild animals [12].

Research conducted by [13], revealed the presence of ESBL-producing *Escherichia coli* resistant to several drugs in broilers in West Benggala, India, with the dominance of the CTX-M gene. Research conducted by [3], successfully identified the CTX gene in ESBL-producing *Escherichia coli* from cloacal swabs of broilers in Blitar Regency. The study found 97.8% of ESBL-producing *Escherichia coli* from 46 isolates.

2 Methods

2.1 In Study area

The method used in this study is the Cross-Sectional Obsrvation method, while the sampling was carried out using th proportional sampling method. ESBL confirmation was performed using the disc diffusion method based on the recommendations of the Clinical and Laboratory Standards Institute (2023).

2.2 Isolation and identification of *Escherichia coli*

A total of 50 cloacal swabs of broiler isolate samples were collected from traditional markets in Banda Aceh. After sample collection, *E. coli* bacteria were isolated on Eosin Methylene Blue Agar (EMBA) media. The nutrient broth (NB) containing the swab samples was incubated at 37°C for 24 hours. Subsequently, the suspension from the NB media was cultured on EMBA media using the streak quadrant method. The media were then incubated at 37°C for 24 hours. *Escherichia coli* colonies were identified on EMBA media and confirmed using a Gram-staining kit. The pure colonies of *E. coli* were further verified by biochemical testing using IMViC media, including Sulfide Indole Motility (SIM) media,

Methyl Red-Voges Proskauer (MR-VP) media, Simmons Citrate Agar, and Triple Sugar Iron Agar (TSIA) media.

2.3 Antibiotic sensitivity testing

The *E. coli* bacteria were rejuvenated by culturing the bacteria on NB media for 24 hours. Then they were made into a suspension by inserting several drop of bacteria into sterile physiological NaCl with a turbidity level corresponding to 0.5 McFarland (1.5×10^8 CFU/ml). The suspension was evenly streaked on MHA media using a cotton bud and allowed to stand for a while. Next, antibiotic discs (ampicillin, cefotaxime, ceftazidime, kanamycin, erythromycin, and sulfamethoxazole) were placed on the agar surface and incubated at 37°C for 24 hours in an incubator. After 24 hours, the results of the resistance testing of *E. coli* bacteria to antibiotics were observed by measuring the diameter of the inhibition zone formed. The resistance test was repeated three times, and then the average of the results was calculated. Furthermore, the results of these measurements were interpreted with reference to the Clinical and Laboratory Standards Institute (CLSI) 2023. An isolate is defined as multidrug-resistant if it is not sensitive to one or more antimicrobials in 3 different antimicrobial classes (Varga et al.2019). For the confirmation of ESBL using Double Disc Synergy Test (DDST), isolates that were positive for multidrug-resistant (MDR) were subjected to the Double Disc Synergy Test (DDST). The assay was performed on Mueller Hinton Agar with three antibiotic discs (Oxoid, England) including ceftazidime (CAZ; 30 µg), cefotaxime (CTX; 30 µg), and amoxycillin-clavulanic (AMC; 30 µg) placed on the media in parallel. The aztreonam antibiotic disc (ATM, 30 g) was placed next to the three parallel discs. The cultures were incubated at 35 °C±2 °C for 16-18 hours. The test was considered positive when there was synergy and an increase in zone diameter of 5 mm for any of the antimicrobial agents tested in combination with clavulanate [14, 3].

2.4 Detlection of Extended Spectrum β-Lactamase-producing *E. coli*

ESBL confirmation was conducted to ensure that the *E. coli* found were indeed ESBL-producing *E. coli*. The ESBL confirmation test used MASTDISCSTM D68C. The sensitivity of *Escherichia coli* to various antibiotics was tested using the disc diffusion method. Pure cultures were prepared in a suspension matching 0.5 McFarland turbidity. Cultures were sampled using a sterile cotton swab and evenly spread on the surface of Mueller-Hinton Agar (MHA). Antibiotic discs were placed on the MHA surface, and the plates were incubated for 24 hours at 37 °C. The inhibition zones for ESBL-producing *Escherichia coli* were determined based on CLSI (Clinical Laboratory Standards Institute) guidelines: cefotaxime ≤ 26 mm, ceftazidime ≤ 21 mm, and ampicillin ≤ 17 mm.

3 Results and Discussion

Eosin Methylene Blue Agar (EMBA) is a selective and differential medium. This medium contains eosin and methylene blue, which inhibit the growth of Gram-positive bacteria, so it can be used to grow Gram-negative bacteria. The colour of the media before planting bacteria is purplish red. The metallic green colour changes on EMBA media because *Escherichia coli* can ferment lactose which results in increased acid levels in the media. High acid levels can precipitate methylene blue in EMBA media [15]. *Escherichia coli* growth is indicated by the growth of separate colonies on EMBA media that are green with metallic flashes and the centre has a black dot taken for purification. *Escherichia coli* bacteria have a morphology of

3-6 mm in size and there is a turbid zone around the colony [16]. Isolates that are positive for *Escherichia coli* are purified with EMBA media as shown in Figure 1.

Fig. 1. Growth of *Escherichia coli* with green metallic colour on EMBA media

Escherichia coli isolates that were positive, then continued with the ESBL production detection test using the disc diffusion method on Mueller Hinton Agar (MHA) media. This test was carried out to determine that the *Escherichia coli* that had been isolated was ESBL-producing *Escherichia coli*. The results of this test show the measurement of the antibiotic inhibition zone on MHA media. The size of the inhibition zone diameter formed on each antibiotic, namely cefotaxime (30 µg), ceftazidime (30 µg), and ampicillin (10µg) can determine the sensitivity of an antibiotic to bacteria, where the larger the inhibition zone formed, the more inhibited bacterial growth. The determination of the inhibition zone can be guided by [14] as a reference to determine the nature of whether bacteria are sensitive, intermediate, and resistant. Then, when the inhibition zone formed on cefotaxime < 26 mm, cefotaxime < 21 mm and ampicillin < 17 mm is declared *Escherichia coli* ESBL producer. The resistance test results showed different levels of resistance from each antibiotic, with the results presented in Table 1.

Table 1. The results of measuring the zone of antibiotic inhibition of positive ESBL-producing *Escherichia coli* at MHA

Samples code	Zone of Inhibition Size of Antibiotic(mm)						Interpretation (ESBL)
	CTX		CAZ		AMP		
K1P1	21.3	R	15.8	R	16.1	R	+
K5P1	7.7	R	4.8	R	15.1	R	+
K7P1	9.2	R	0	R	20.6	S	+
K12P2	10.3	R	16.2	R	0	R	+
K14P2	11.1	R	13.2	R	0	R	+
K20P2	12.1	R	22.2	S	0	R	+
K25P3	9.3	R	19.1	R	0	R	+
K32P4	6.6	R	13.7	R	0	R	+
K41P5	2.6	R	11.1	R	18.2	S	+
K47P5	5.4	R	13.8	R	20.1	S	+

Description:
(K1P1) = Cloaca 1 Trader 1, (K5P1) = Cloaca 5 Trader 1, (K7P1) = Cloaca 7 Trader 1,
(K12P2) = Cloaca 12 Trader 2, (K14P2) = Cloaca 14 Trader 2, (K20P2) = Cloaca 20 Trader 2,
(K25P3) = Cloaca 25 Merchant 3, (K32P4) = Cloaca 32 Merchant 4, (K41P5) = Cloaca 41

Merchant 5, (K47P5) = Cloaca 47 Merchant 5, (CTX) = Cefotaxime, (CAZ) = Ceftazidime, (AMP) = Ampicillin, (+) = Positive E. ESBL-producing coli, (R) = Resistant, (S) = Sensitive

The results of the analysis in Table 1 obtained 21.27% positive ESBL-producing *Escherichia coli* where of the three antibiotics, namely cefotaxime which reached 100% (10/10), while the results of the resistance test against cefotaxime showed 90% (9/10) isolates had experienced resistance, and only 10% (1/10) isolates were still sensitive to cefotaxime antibiotics. In the sensitivity test with ampicillin antibiotics only showed 70% (7/10) resistance and only 30% (3/10) isolates were still sensitive to ampicillin antibiotics.

Based on the results (Table 1) obtained the highest percentage of resistance is CTX. This is in line with research conducted by [16], which states that Extended Spectrum β-Lactamase tends to be resistant to cefotaxime, so these bacteria are thought to carry the CTX gene. Cefotaxime β-lactamase was the most common type of ESBL among broiler samples (96%) [17]. The CTX-M β-lactamase was named because of its efficiency in hydrolyzing cefotaxime compared to ceftazidime. Conjugative plasmids are considered to be one of the main factors causing the successful spread of CTX-M type ESBLs [18].

Based on the results of research conducted by [19], it was found that some ESBL-producing bacteria were still sensitive to ceftazidime. This seems to be because the type of ESBL produced by these bacteria is the TEM-1 type, which is a type of ESBL that can hydrolyze penicillin-type antibiotics and first-generation cephalosporins but is unable to attack oxyimino cephalosporins (oxyimino cephalosporin). Oxyimino-type cephalosporin antibiotics can block TEM-1 ESBL due to the structural form of these antibiotics which can protect the beta-lactam group on cephalosporin antibiotics on TEM-1. However, the results of this study show that ceftazidime is a resistant antibiotic after cefotaxime.

Extended Spectrum β-Lactamase (ESBL) is a β-lactamase enzyme contained in bacterial plasmids that can hydrolyze penicillin, third-generation cephalosporin (cefotaxime, ceftriaxone and ceftazidime) and monobactam (aztreonam) antibiotics, causing antibiotic resistance in ESBL-producing bacteria [20]. Extended Spectrum β-Lactamase contains a number of mutations that cause hydrolysis of broad-spectrum β-lactam antibiotics [18]. Extended Spectrum β-Lactamase (ESBL) bacteria experience resistance to several antibiotics due to mutations or genetic changes caused by excessive antibiotic therapy [9].

The presence of ESBL-producing bacteria has been reported in food-producing animals [21]. Broilers are a potential reservoir for Escherichia coli ESBL that can cause health problems. ESBL-producing Escherichia coli is transmitted through several ways, namely consuming contaminated meat and an environment contaminated with feces containing ESBL-producing Escherichia coli. ESBL-producing Escherichia coli can be detected in the digestive system and urine in infected farm animals [12].

[22] stated that 30 to 90% of antibiotics will be excreted through urine and feces of animals and become a source of antibiotic resistance to bacteria in the environment. Rahayu (2008) stated that feces can contaminate the muscle tissue of meat during the slaughtering process. Considering the condition of the traditional market, which is run down and dirty like this, it causes the successful spread of resistance from ESBL-producing Escherichia coli.

Extended Spectrum β-Lactamase (ESBL)-producing bacteria in food-producing animals are currently a public health concern [6]. These bacteria can spread from animals to humans and potentially cause zoonotic diseases [23]. said that Escherichia coli plays a role in the spread of resistant genes to bacterial populations between animals and humans through foodborne. This can contaminate meat or other products of animal origin resulting in infection in humans who consume them and if the bacteria are resistant to antibiotics it can lead to serious illness due to failure of antibiotic treatment [24].

Carbapenems are antibiotics that can be used to treat ESBL infections. However, there has been an increase in the prevalence of infections caused by carbapenem-resistant Enterobacteriaceae as reported [25]. The World Health Organization (WHO) states that

Escherichia coli is one of the bacteria most in need of new types of antibiotics in medicine worldwide [26].

4 Conclusion

Based on the results of the study, it is concluded that extended spectrum β-lactamase (ESBL)-producing *Escherichia coli* which is 21.27% in the cloaca of broiler in the traditional market, Banda Aceh.

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