Jurnal Sain Veteriner, Vol. 43. No. 3. Desember 2025, Hal. 463-473
DOI: 10.22146/jsv.105866
ISSN 0126-0421 (Print), ISSN 2407-3733 (Online)
Tersedia online di https://jurnal.ugm.ac.id/jsv

Detection of ESBL-Encoding Genes in Antibiotic-Resistant *Escherichia coli* Isolated from Houseflies (*Musca domestica*) in Campus Food Court

Deteksi Gen Pengkode ESBL pada Escherichia coli Resistan Antibiotik yang Diisolasi dari Lalat Rumah (Musca domestica) di Food Court Kampus

Denny Widaya Lukman[1*], Herwin Pisestyani[1], Muhammad Rizki Aminudin[2], Supriyono[3], Eddy Sukmawinata[4]

[1]Division of Veterinary Public Health and Epidemiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, West Java, Indonesia
[2]Students of the Master of Veterinary Biomedical Science Study Program, School of Veterinary Medicine and Biomedical Sciences, IPB University, West Java, Indonesia
[3]Division of Parasitology and Medical Entomology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, West Java, Indonesia
[4]Research Centre for Veterinary Science, Research Organization for Health, National Research and Innovation Agency, Cibinong, Bogor, West Java, Indonesia
*Email: dennylukman@apps.ipb.ac.id

Article submitted: April 9, 2025, revised: May 6, 2025 accepted: July 8, 2025

Abstrak

Escherichia coli adalah patogen berbahaya yang dilaporkan dapat menyebabkan penyakit pada manusia maupun hewan. Bakteri *E. coli* umumnya dikaitkan dengan penyakit akibat kontaminasi makanan. Beberapa strain patogen telah menunjukkan resistansi terhadap berbagai antibiotik, salah satunya adalah *coli Extended-Spectrum Beta-Lactamases* ESBL-*E.* yang menyebabkan masalah kesehatan yang kompleks. Penularan bakteri patogen di lingkungan dapat diperantarai oleh lalat rumah (*Musca domestica*). Penelitian ini bertujuan untuk menganalisis keberadaan bakteri *E. coli* yang resistan terhadap antibiotik dan mendeteksi gen resistansi yang dibawa oleh lalat rumah (*M. domestica*) yang dikoleksi dari *food court* di lingkungan IPB University, Dramaga, Bogor. Penelitian ini meliputi pengambilan sampel lalat, identifikasi jenis lalat, isolasi dan identifikasi bakteri *E. coli* berdasarkan standar ISO 16649-2: 2001, pengujian sensitivitas antibiotik menggunakan metode difusi cakram *Kirby Bauer* berdasarkan CLSI tahun 2023, dan deteksi gen penyandi resistansi ESBL. Antibiotik yang diuji antara lain siprofloksasin, ampisilin, tetrasiklin, trimetoprim-sulfametoksazol, kloramfenikol, seftazidim, dan sefotaksim. Selanjutnya, dilakukan identifikasi gen pengkode ESBL menggunakan gen *bla*CTX, *bla*TEM, dan *bla*SHV. Hasil peneliti 5 isolat (5/40; 12,5%) bakteri *E. coli*. Sebanyak 4 isolat dari 5 isolat *E. coli* menunjukkan resistansi terhadap dua atau lebih antibiotik yang diuji. Deteksi gen penyandi resistansi ESBL pada 5 isolat *E. coli* menunjukkan bahwa 2 isolat (40%) positif terhadap gen *bla*TEM tetapi tidak menunjukkan hasil positif untuk gen *bla*CTX dan *bla*SHV. Keberadaan *E. coli* yang resistan terhadap antibiotik pada lalat rumah di kantin kampus harus diperhitungkan.

Kata kunci: ESBL; *multidrug resistance*; *Musca domestica*; resistansi antibiotik, vektor

Abstract

Escherichia coli is a dangerous pathogen that is reported to bring about disease in humans as well as animals. *E. coli* is commonly associated with diseases due to food contamination. Some strains of the pathogen have shown resistance to various antibiotics, one of them is ESBL-*E. coli*, leading to complex problems in health. The transmission of pathogenic bacteria in the environment can be mediated by houseflies

(*Musca domestica*). This study aimed to analyze the presence of antibiotic-resistant *E. coli* bacteria and detect resistance genes carried by houseflies (*M. domestica*) collected from food courts in IPB University, Dramaga Bogor, Indonesia. This study includes a collection of fly samples, identification of fly species, isolation and identification of *E. coli* bacteria based on ISO 16649-2: 2001 standard, antibiotic sensitivity testing using Kirby Bauer disc diffusion method based on CLSI in 2023, and detection of ESBL resistance encoding gene. Antibiotics tested included ciprofloxacin, ampicillin, tetracycline, trimethoprim-sulfamethoxazole, chloramphenicol, cefazidime, and cefotaxime. Then, the ESBL encoding genes were identified using the *bla*CTX, *bla*TEM, and *bla*SHV genes. The study found 5 *E. coli* isolates (5/40; 12.5%). A total of 4 isolates of 5 *E. coli* isolates indicated resistance against two or several antibiotics tested. Among the 5 *E. coli* isolates examined, two isolates (40%) harbored the *bla*TEM gene, while none carried *bla*CTX-M or *bla*SHV. The presence of antibiotic resistance *E. coli* in houseflies in the food court on campus should be taken into account.

Keywords: antibiotic resistance; ESBL; *multidrug resistance; musca domestica*; vector

Introduction

Vector-borne infectious diseases have turned into a global health concern that is directly related to public health. Based on WHO (2020), about 17% of infectious diseases are caused by vectors globally each year, with a high prevalence in both tropical and subtropical countries. Flies are one of the mechanical vectors that can transmit disease agents, including pathogenic bacteria (Isam-Elden *et al.,* 2022; Yin *et al.,* 2022). Mechanical transmission of diseases, especially those affecting the gastrointestinal tract through contaminated food, is primarily facilitated by flies. Pathogenic bacteria linked to these insects have been detected in diverse locations such as farms, hospitals, markets, garbage heaps, restaurants, and canteens (Geden *et al.,* 2021; Yin *et al.,* 2022). Food and beverages are frequently contaminated when these insects land on them (Yin *et al.,* 2022). In urban slums of Bangladesh, *E. coli* transmission to food is significantly influenced by the presence of flies. Consequently, food becomes a vehicle for disease-causing agents carried by these insects, posing health risks to humans (Lindeberg *et al.,* 2018). Among the various species, the house fly, *Musca domestica* (*M. domestica*), is notably recognized for its role in spreading pathogenic microorganisms. This species thrives in environments shared by humans and animals, including household kitchens, eateries, canteens, and unsanitary sites like garbage dumps. Such filthy locations serve as breeding grounds where the house fly completes its life cycle (Akter *et al.,* 2020).

The spread of antimicrobial resistance (AMR) is a substantial threat to health on a global scale. AMR does not only lead to increased healthcare costs but also increases morbidity and mortality. As reported by O'Neill (2016) the rate of mortality due to infection by antimicrobial-resistant pathogens is 700,000 per year and it is estimated that the infected population will reach 50 million people globally in 2050. Multiple drug-resistant bacteria are of great concern globally, one of which is the pathogenic bacteria *E. coli*. Therefore, *E. coli* has been commonly used as a biomarker to monitor AMR in the environment, including farms and hospitals. In addition, *E. coli* has been found to potentially contribute to the transmission of resistance genes. These resistance genes mediate drug resistance to carbapenems, colistin, and tigecycline in Gram-negative bacteria, which can lead to antimicrobial unavailability in humans and veterinary medicine. Extended-spectrum beta-lactamase *E. coli* (ESBL-*E. coli*) is one type of *E. coli* bacteria that can produce beta-lactamase enzymes that can inhibit a significant of beta-lactam antibiotics. In addition, ESBL-producing *E. coli* is also a global health problem (CDC, 2019).

Early detection and surveillance of antimicrobial resistance in bacteria is critical because it provides information needed to monitor and develop therapeutic guidelines, infection control policies, and public health interventions. Research on antibiotic-resistant bacteria carried by flies is still rare in Indonesia. IPB University Campus area represents various activities that can be a risk factor in the spread of antibiotic resistance, since the area consist of buildings for lectures, laboratories, administration, and food courts, teaching agriculture and farms, and teaching animal hospitals. Many food vendors in and around the IPB Campus provide food for students and faculty members. Poor hygiene and sanitation in food services can impact health problems. Reports of several studies showed that antibiotic-resistant *E.*

coli has been identified in ready-to-eat foods, such as kebabs, burgers, and chicken porridge, sold around campus (Asari *et al.,* 2021; Sari *et al.,* 2020; Saujana, 2023). This is undoubtedly very dangerous for health, especially the transmission of disease through food contaminated with pathogens or foodborne disease.

Materials and methods

The research has approved ethics by the Animal Ethics Commission School of Veterinary Medicine and Biomedical Sciences IPB University with certificate number 233/KEH/SKE/VII/2024. The research was from June to August 2024. Sampling of *M. domestica* flies was collected at food courts in IPB Dramaga Campus, Bogor Regency, West Java. Then sample observations were carried out including the identification of fly species, isolation and identification of *E. coli*, detection of resistance genes, and antibiotic resistance tests at the Laboratory of Veterinary Public Health, School of Veterinary Medicine and Biomedical Sciences, Bogor Agricultural University (IPB).

The fly-catching was collected in 10 food courts at the IPB Dramaga Campus. The food courts only chosen in this study should serve the dine-in food. Samples were taken four times in each food court so that the total number of samples was 40 pooled samples. Flies sampling in this study was collected at a frequency of one week and two times. The flies collection was conducted in the afternoon from 10:00 AM–2:00 PM. Flies were collected with the aid of a sweeping net. Flies caught were transferred to sterile plastic bags and stored in a cool box containing ice packs as a cooling substance. Fly identification was carried out by observing the morphological characteristics of flies and comparing them with the identification key of Nihei and De Carvalho (2009). Samples of *M. domestica* flies were collected into sterile petri dishes for bacterial isolation procedures.

Isolation of bacteria from flies based on Punyadi *et al.,* (2021) with modifying selective media (isolation of *E. coli* bacteria based on ISO 16649-2:2001 standard). The fly samples with *M. domestica* species were collected into a sterile petri dish to be isolated from the body of the flies. Bacterial isolation begins with the preparation of bacterial suspensions. Samples of flies obtained at one collection time were then separated using sterile scissors and tweezers into one sample of bacterial isolates (N=40). The collection of fly legs was put into 10 ml of 0.1% buffer peptone water (BPW) solution (Oxoid® CM0509), then incubated for 30 minutes at room temperature. 100 µl of bacterial suspension was placed on tryptone bile x-glucuronide (TBX) agar media (Himedia® M1591). The suspension media was then incubated under aerobic conditions at 37°C for 18–24 hours. Colonies suspected to be *E. coli* isolated from each sample were then purified using MacConkey agar (MCA) media (Oxoid® CM0007) and incubated for 18 –24 hours at 37°C. Isolates suspected of *E. coli* were subcultured on nutrient agar slant media (Oxoid® CM0003). The slant agar medium containing the isolates was then incubated at 37°C for 18–24 hours. Bacterial isolates were then subjected to Gram staining and IMViC biochemical tests consisting of indole test, methyl red (MR) test, Voges Proskauer (VP) test, and citrate test.

Kirby Bauer's disc diffusion method based on CLSI (2023) was used for resistance testing of *E. coli* against several antibiotics. *E. coli* isolates were re-cultured on tryptone soy agar (TSA) medium (Oxoid® CM0131), then incubated at 37°C for 18–24 hours. Colonies were suspended in 9 ml of physiological NaCl (Oxoid® LP0005) using a vortex mixer until the turbidity level was the same as the 0.5 McFarland 1 standard. The bacterial suspension was inoculated and leveled using a sterile cotton bud on Mueller-Hinton agar (MHA) media (Oxoid® CM0337). Antibiotic discs were placed on the media and incubated at 37°C for 18-24 hours. Antibiotic discs with each dose tested were ciprofloxacin 5 µg (Oxoid® CT0425B), ampicillin 10 µg (Oxoid® CT0003B), tetracycline 30 µg (Oxoid® CT0041B), trimethoprim-sulfamethoxazole 25 µg (Oxoid® CT0052B), chloramphenicol 30 µg (Oxoid® CT0013B), ceftazidime 30 µg (Oxoid® CT0412B), and cefotaxime 30 µg (Oxoid® CT0166B). The clear area or zone formed around the disk was then measured using a caliper to determine the zone of inhibition. Determination of the sensitivity level of *E. coli* to the tested antibiotics refers to the Clinical and Laboratory Standards Institute standard (CLSI, 2023).

E. coli isolates were screened for antibiotic resistance genes by polymerase chain reaction (PCR). The first procedure for *E. coli* isolates was DNA extraction. DNA amplification was performed using PCR. Amplification results were analyzed using the electrophoresis method. The primers used, and the results of the 5'-3' sequence for the detection of antibiotic resistance genes are *bla*CTX-M, *bla*TEM, and *bla*SHV (Table 1).

Amplification of the *bla*CTX-M gene was conducted by initial denaturation at 94°C for 5 min; 30 cycles of denaturation at 94°C for 25 s; annealing at 52°C for 40 s; elongation at 72°C for 50 s; and final elongation at 72°C for 6 min (Woodford *et al.,* 2006). Amplification of the *bla*TEM gene was conducted at 95°C for 5 min for initial denaturation; 30 cycles of denaturation, 95°C for 1 min; annealing at 56°C for 1 min; elongation, 72°C for 1 min; and final elongation, 72°C for 10 min. Amplification of the *bla*SHV gene was conducted at 95°C for 5 min for initial denaturation; 30 cycles of denaturation, 95°C for 1 min; annealing at 52°C for 1 min; elongation, 72°C for 1 min; and final elongation, 72°C for 10 min (Islam *et al.,* 2022).

Data results were analyzed descriptively using Microsoft Excel. The data obtained from the antibiotic resistance test measurements were then compared with the Clinical and Laboratory Standards Institute (CLSI) year of 2023 standard to determine the resistance category of *E. coli* bacteria. The result data were then presented in the form of tables and figures.

This study obtained 5 *E. coli* isolates from a total of 40 houseflies collection samples (5/40; 12.5%) successfully isolated from houseflies (*M. domestica*). None of the *E. coli* isolates showed ESBL positivity using DDST methods. The results of the antibiotic sensitivity analysis on *E. coli* isolates showed that 4 out of 5 isolates were found to be resistant against a minimum of 2 types of antibiotics tested. Most of *E. coli* isolates showed resistant to ampicillin and tetracycline. Multi-drug resistance (MDR) was found in 3 *E. coli* isolates with resistance patterns AMP-TE-SXT-CAZ, TE-SXT-C, and CIP-AMP-CTX, and 1 isolate with non-MDR category resistance was found with an AMP-TE pattern. Only one *E. coli* isolate is still classified as sensitive to the antibiotics tested (Table 2).

Two of the five (40%) *E. coli* isolates were confirmed positive for the *bla*TEM resistance encoding gene. Other genes, including *bla*CTX-M and *bla*SHV were not detected in *E. coli* isolated from flies (Table 3).

E. coli is commonly found in houseflies. This study proved that *E. coli* is found in houseflies. Bacterial isolation studies from flies have been reported by Akter *et al.,* (2020)

Table 1. Target genes encoding antibiotic resistance

Target genes	Sequence (5'-3')	Bp	Reference
*bla*CTX-M	F: AAAAATCACTGCGCCAGTTC R: AGCTTATTCATCGCCACGTT	415	(Woodford *et al.,* 2006)
blaTEM	F: CATTTCCGTGTCGCCCTTAT R: TCCATAGTTGCCTGACTCCC	793	(Islam *et al.,* 2022)
*bla*SHV	F: TCGCCTGTGTATTATCTCCC R: CGCAGATAAATCACCACAATG	206	(Islam *et al.,* 2022)

Table 2. Table of antibiotic resistance results on *E. coli* isolated from *M. domestica*

No	Isolat code	E. coli	ESBL	Antibiotic							Resistance patterns
				CIP	AMP	TE	SXT	C	CAZ	CTX	
1.	BC1	+	-	I	R	R	R	S	R	I	AMP-TE-SXT-CAZ
2.	PS1	+	-	S	S	S	S	S	S	I	-
3.	PS3	+	-	I	R	R	S	S	S	I	AMP-TE
4.	SP1	+	-	I	I	R	R	R	S	I	TE-SXT-C
5.	SV1	+	-	R	R	I	I	S	S	R	CIP-AMP-CTX

Notes: CIP = ciprofloxacin; AMP = ampicillin; TE = tetracycline; SXT = trimethoprim-sulfamethoxazole; C = chloramphenicol; CAZ = ceftazidime; CTX = cefotaxime; R = resistant; I = Intermediate; S = sensitive.

at Bangladesh Agricultural University who identified 51.4% *E. coli* from all isolates from houseflies. Isam-Eldeen *et al.,* (2022) also conducted the same study by isolating bacteria found in houseflies. They reported that bacterial species were successfully isolated with the most *E. coli* in Khartoum State, Sudan. *E. coli* can be found in houseflies (*M. domestica*) for several reasons including habitat and feeding behavior, houseflies are attracted to decaying organic matter, including animal feces, garbage, and other waste (Onwugamba *et al.,* 2020). A recent study by Soufiane *et al.,* (2024) reported that a total 15 *E. coli* were isolated from 26 Enterobacteriaceae collected from houseflies. Flies were collected from various collection areas including markets, restaurants, fish markets, poultry markets, health centers and abattoirs, with the highest prevalence found in health centers. The study showed that *E. coli* was the most common entero-bacterial species found in flies, especially houseflies.

Flies breed and finish their life cycle in the dirtiest places, especially in decaying vegetable waste (Akter *et al.,* 2020; Yin *et al.,* 2022). These environments are highly exposed to bacteria, including *E. coli*, which flies can acquire while feeding. Flies can transmit *E. coli* bacteria on the fly's body (mechanical transmission) even within the intestinal system. Houseflies can collect bacteria on the surface of the exoskeleton, when the fly lands on food or surfaces, the fly can transmit the bacteria. It is can potentially lead to contamination. The presence of *E. coli* in flies can also impacted by environmental factors such as sanitary conditions (Onwugamba *et al.,* 2020).

Ampicillin and tetracycline showed the highest resistance levels among the isolates (3/5, 60%). This finding aligns with previous studies, such as Saujana (2023), who reported 57% resistance to tetracycline in *E. coli* from chicken porridge samples near the same campus. However, resistance to ampicillin in this study (60%) was significantly higher than the 14.28% reported in Saujana's research, suggesting potential variations in bacterial reservoirs or selective pressures. Ampicillin is an antibiotic used to treat *Shigella* and *Salmonella* infections. Ampicillin is classified as aminopenicillin and was developed to overcome drug resistance problems and expand the antimicrobial scope of penicillin. Ampicillin belongs to the class of penicillin's commonly used for treatment and prophylaxis, but the use of these antibiotics without proper procedures can cause bacterial resistance, including *E. coli*. Tetracycline is an antibiotic that has bacteriostatic properties, namely the ability of antibiotics to inhibit protein synthesis in Gram-positive bacteria and Gram-negative bacteria (Aulia *et al.,* 2023). Research by Putra *et al.,* (2024) explained the finding of *E. coli* bacteria that have been resistant to tetracycline antibiotics by 70% taken from poultry cloacal swabs. The high level of *E. coli* resistance to ampicillin and tetracycline is a matter of concern regarding the use and spread of these antibiotics.

Sensitivity testing of *E. coli* to trimethoprim-sulfamethoxazole antibiotics was also quite high (2/5; 40%). *E. coli* bacteria that have been resistant to trimethoprim-sulfamethoxazole antibiotics are generally reported in livestock environments. Wibisono *et al.,* (2021) reported that 93.2% of *E. coli* isolates were resistant to trimethoprim-sulfamethoxazole antibiotics. This is inversely proportional to the report of Sari *et al.,* (2020), all of *E. coli* isolates from kebab meat sold around the IPB University campus, Dramaga, Bogor are still in the sensitive category to trimethoprim-sulfamethoxazole

Table 3. Detection of antibiotic resistance genes in *E. coli* isolated from houseflies

No	Isolat code	Target genes		
		blaCTX-M	blaTEM	blaSHV
1	BC1	-	+	-
2	PS1	-	-	-
3	PS3	-	+	-
4	SP1	-	-	-
5	SV1	-	-	-

antibiotics. The antibiotic is a combination of a pyrimidine analog and a sulfonamide group. Both components act sequentially in two consecutive steps in bacterial nucleic acid biosynthesis. Trimethoprim has bactericidal properties which can kill bacteria while sulfamethoxazole has bacteriostatic properties which means it can inhibit bacterial growth (Autmizguine *et al.,* 2018).

Other antibiotic sensitivity tests showed a low percentage compared to ampicillin, tetracycline and trimethoprim-sulfamethoxazole antibiotics. Each antibiotic there is only 1 isolate of *E. coli* that has been resistant. However, some of these antibiotics are included in the beta-lactamase group including ceftazidime and cefotaxime. Ceftazidime is a 3rd generation cephalosporin that is active against *Pseudomonas aeruginosa* (Matesanz & Mensa, 2021). Like ceftazidime, cefotaxime antibiotics are third-generation cephalosporin antibiotics that have a broad spectrum of action, strong antibacterial activity and have relatively lower side effects. Cefotaxime is one of the oxyimino-cephalosporin antibiotics. the mechanism of this antibiotic is enzymatic drug degradation through the production of broad-spectrum β-lactamase (ESBL) in Gram-negative pathogens, especially *Escherichia* pathogens (Halawani *et al.,* 2020). Bacteria have the ability to produce beta-lactamase enzymes that can inhibit a large number of beta-lactam antibiotics. This should also be a serious concern considering that ESBL-producing *E. coli* is also a global health problem today. A total of 1 *E. coli* isolate has also been resistant to the antibiotic ciprofloxacin. Ciprofloxacin is a fluroquinolone antibiotic that plays an important role in the treatment of several bacterial infections. The antibiotic is one of the potential antibiotics in the treatment of infections with drug-resistant bacterial pathogens with different levels of resistance, current therapeutic options such as *E. coli* and ESBL-producing Enterobacter (Zhang *et al.,* 2018).

Multi-drug resistance (MDR) was found in 3 *E. coli* isolates. Multi-drug resistance (MDR) is a condition where bacteria have been resistant against a minimum of 3 groups of antibiotics (F. J. Wibisono *et al.,* 2020). The results showed that MDR was found in 3 *E. coli* isolates with resistance patterns AMP-TE-SXT-CAZ, TE-SXT-C, and CIP-AMP-CTX. Multi-drug resistance findings were also reported by Asari *et al.,* (2021), and 3 out of 5 *E. coli* isolates isolated from burger meat around the IPB Dramaga Campus experienced multi-drug resistance. Sumampouw (2018) research reported that 62% of *E. coli* isolates causing diarrhea in children in Manado City had experienced MDR. A similar case was also found by Suhartono *et al.,* (2023), who found *E. coli* MDR of 30.9% in isolates of patient samples at RSUD Dr. Zainoel Abidin, Banda Aceh. Bacteria that are resistant to many antibiotics cause disease treatment to be more difficult, increase treatment costs, and can increase mortality rates because bacteria become resistant to three or several antibiotics (Rahman *et al.,* 2020). MDR in bacteria can occur through two mechanisms: first, the accumulation of many resistance-coding genes on the plasmid that causes the bacteria to be resistant to many antibiotics (Mustika *et al.,* 2024); second, the expression of genes that produce multi-drug efflux pumps and cause bacteria to eliminate antibiotics and other substances that are potentially harmful to bacteria (Nishino *et al.,* 2021).

The results showed that 2 out of 5 isolates (40%) of *E. coli* bacteria were positive for the *bla*TEM encoding gene but negative for the *bla*CTX-M and *bla*SHV genes. These results are different from the study by Soufiane *et al.,* (2024), which detected beta-lactamase resistance coding genes in Enterobacteriaceae isolates from houseflies resulted in the highest prevalence of the *bla*CTX-M gene, then *bla*TEM and *bla*SHV, each sequential percentage was 95.16%; 73.07%; 15.38%. Positive results are characterized by visible DNA bands aligned with the size marker (bp) according to the target gene with the *bla*TEM gene at 793 bp (Figure 1).

The *bla*TEM gene in *E. coli* is gene that can confer resistance to beta-lactam antibiotics. The *bla*TEM gene encodes the enzyme TEM-1 beta-lactamase, which can hydrolyze various beta-lactam antibiotics, including penicillins and cephalosporins (Effendi *et al.,* 2022; Tolenada & Dayrit, 2023). The results showed that one isolate of *E. coli* positive for the

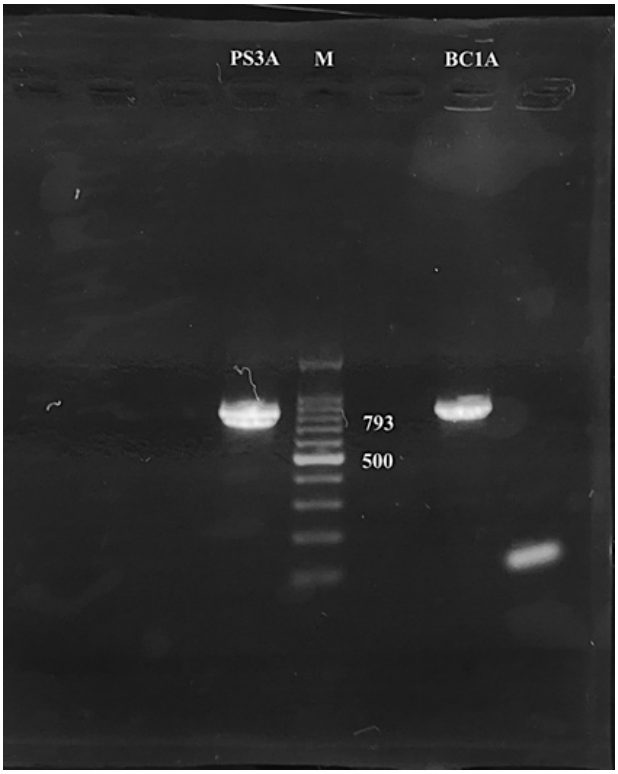

Figure 1. PCR results of *bla*TEM gene in *E. coli* isolates

*bla*TEM gene was resistant against penicillins and cephalosporins antibiotics. Still, the other isolate was only resistant against penicillin antibiotics. Although the *bla*TEM gene is present, not all *E. coli* isolates with this gene express enough enzymes to confer resistance against cephalosporins antibiotics. Some isolates may have low levels of gene expression or different regulatory mechanisms that inhibit the production of the enzyme required to inhibit cephalosporins (Effendi *et al.,* 2022; Tolenada & Dayrit, 2023). Another possibility is that the isolate may have other resistance genes that are more dominant or effective in conferring resistance only against penicillins. For example, the isolate may contain other genes that confer specific resistance against penicillins but not cephalosporins (Effendi *et al.,* 2022; Widodo *et al.,* 2023). Gundran *et al.,* (2019) reported that the *bla*TEM gene is commonly found in *E. coli* isolates from various sources, including livestock and human clinical samples. The *bla*TEM gene is one of the most common ESBL genes in *E. coli*, especially in samples from chickens and humans with ampicillin antibiotic-resistant *E. coli*. The distribution of the *bla*TEM gene in Indonesia has been reported in several studies. Among them is a study by Ansarietha *et al*. Ansharieta *et al.,* (2021) reported on ESBL in *E. coli* from raw cow milk samples in East Java. The study found that *E. coli* isolates from raw cow's milk in East Java contained the *bla*TEM gene. The *bla*TEM gene has a significantly higher prevalence than the *bla*CTX gene in the study. Another study by Faridah *et al.,* (2023) with cloacal swab samples of broiler chickens in East Java found that 38.2% of *E. coli* contained the *bla*TEM gene. These results indicate that the *bla*TEM gene is also found in ESBL bacteria isolated from broiler chickens.

Although *E. coli* has the *bla*TEM gene in the study, it does not mean it will produce beta-lactamase enzymes that are active as ESBL. The *bla*TEM gene does not express significant ESBL activity in the isolate. Another possibility is that the blaTEM gene is expressed in such small amounts that it cannot detect ESBL activity through the DDST test. The DDST test relies on high enough levels of beta-lactamase enzymes for synergistic interactions with antibiotics, so *E. coli* isolates with low gene expression would not be detected positively. The blaTEM gene is found on a plasmid within bacteria, and it can be shared between bacteria via horizontal gene transfer methods, including conjugation, transformation, and transduction. This mobility contributes to the widespread of resistance genes among bacterial populations. The presence of *bla*TEM genes in *E. coli* isolates can lead to the development of multidrug-resistant (MDR) strains. It represents a significant challenge in treating bacterial infections, as bacteria will become resistant to multiple antibiotics (Gundran *et al.,* 2019).

Unreasonable and overuse of antibiotics can accelerate the emergence of resistance in *E. coli* to multiple antibiotics (Soufiane *et al.,* 2024). The spread of resistant bacteria by flies can increase the risk of spreading resistant bacteria into the human environment, so it is important to take preventive measures to prevent the spread of resistant bacteria. The IPB Dramaga campus environment is highly complex; there are several sectors of activity, such as agriculture, hospitals, and livestock, that do not rule out the possibility of being a factor in the contamination of resistant bacteria. Another pathway in the contamination of bacterial resistance in the environment is

through water. Drainage channels, agricultural irrigation channels, and hospital waste are reported to be sources of antibiotic resistance in the water environment, as many microbial resistance genes accumulate (Efstratiou *et al.,* 2018). Lakes in the IPB Dramaga Campus area can be a source of antibiotic resistance contamination. As reported by Syafriana *et al.,* (2020), there are *E. coli* bacteria that have been resistant to antibiotics isolated from water in Lake ISTN Jakarta. It becomes more complex to find bacteria that have been resistant to several antibiotics carried by flies. Flies can act as vectors of disease spread in the campus environment. Houseflies are the most widespread vectors among all arthropod insects. Flies of the type *M. domestica* have great potential in spreading both pathogenic and commensal bacteria. This is because flies have the ability to move freely among various habitats and can fly freely over long distances of up to 5-7 kilometers (Sobur *et al.,* 2019). Prevention efforts must be carried out from multiple directions, namely by controlling the use of antibiotics themselves, controlling flies, and increasing sanitary hygiene in food providers in the IPB Dramaga Campus environment. These findings highlight the importance of implementing integrated hygiene and fly control measures at campus food courts. The detection of *bla*TEM-positive *E. coli* in flies suggests that AMR surveillance programs should not be limited to clinical or farm environments but should also target semi-public settings such as educational institutions. The study supports the inclusion of entomological vectors in One Health AMR monitoring frameworks.

This study has several limitations. The number of *E. coli i*solates was relatively small (n=5), which restricts the generalizability of resistance patterns. The pooled sample design may have obscured individual fly variability, and expression levels of resistance genes were not assessed. Additionally, phenotypic confirmation of ESBL activity yielded negative results despite the presence of *bla*TEM, likely due to low gene expression. Future research should incorporate larger sample sizes, metagenomic analysis, and quantitative PCR to assess resistance gene expression and diversity more robustly.

Conclusion

Flies can potentially serve as vectors for the spread of antibiotic-resistant *E. coli* in IPB Dramaga Campus environment. The presence of multi drug-resistant *E. coli* harboring the *bla*TEM gene in houseflies supports the role of *Musca domestica* as a mechanical vectors for antimicrobial resistance in environment. The distribution of bacteria by flies can increase the risk of spreading resistant bacteria into the human environment, especially canteens or food centers. It is hazardous because it can be transmitted between bacteria via horizontal gene transfer methods including conjugation, transformation, and transduction between microbes.

Acknowledgments

The research was funded by BIMA Ministry of Education, Culture, Research, and Technology Master Thesis Research program (PTM) based on Decree Number: 027/E5/PG.02.00.PL/2024 and Agreement/Contract Number: 22234/IT3. D10/PT.01.03/P/B/2024.

References

Akter, S., Sabuj, A. A. M., Haque, Z. F., Rahman, M. T., Kafi, M. A., & Saha, S. (2020). Detection of antibiotic-resistant bacteria and their resistance genes from houseflies. *Veterinary World*, *13*(2), 266–274. https://doi.org/10.14202/vetworld.2020.266-274

Ansharieta, R., Ramandinianto, S. C., Effendi, M. H., & Plumeriastuti, H. (2021). Molecular identification of *bla*CTX-M and *bla*TEM genes encoding extended-spectrum ß-lactamase (ESBL) producing *Escherichia coli* isolated from raw cow's milk in East Java, Indonesia. *Biodiversitas Journal of Biological Diversity*, *22*(4), 1600–1605. https://doi.org/10.13057/biodiv/d220402

Asari, K. A., Lukman, D. W., & Purnawarman, T. (2021). Identifikasi *Escherichia coli* yang Resistan Antibiotik pada Daging Burger yang Dijual di Sekitar Kampus IPB Dramaga Bogor. *Jurnal Veteriner*, *22*(4), 515–522. https://doi.org/10.19087/jveteriner.2021.22.4.515

Aulia, S. A., Sutiningsih, D., Setyawan, H., & Udiyono, A. (2023). Keberadaan Residu Tetrasiklin pada Daging Ayam Broiler di Kabupaten Kudus (Studi di Pasar Tradisional dan Pasar Modern Tahun 2019). *Jurnal Epidemiologi Kesehatan Komunitas*, *8*(1), 69–75. https://doi.org/10.14710/jekk.v8i1.6918

Autmizguine, J., Melloni, C., Hornik, C. P., Dallefeld, S., Harper, B., Yogev, R., Sullivan, J. E., Atz, A. M., Al-Uzri, A., Mendley, S., Poindexter, B., Mitchell, J., Lewandowski, A., Delmore, P., Cohen-Wolkowiez, M., & Gonzalez, D. (2018). Population Pharmacokinetics of Trimethoprim-Sulfamethoxazole in Infants and Children. *Antimicrobial Agents and Chemotherapy*, *62*(1), 1–19. https://doi.org/10.1128/AAC.01813-17

Centers for Disease Control and Prevention (CDC). (2019). *Antibiotic resistance threats in the United States, 2019*. https://doi.org/10.15620/cdc:82532

Clinical and Laboratory Standards Institute (CLSI). (2023). *Performance standards for antimicrobial susceptibility testing* (30th Edition, p. 282). Clinical and Laboratory Standards Institute.

Effendi, M. H., Hartadi, E. B., Witaningrum, A. M., Permatasari, D. A., & Ugbo, E. N. (2022). Molecular identification of *bla*TEM gene of extended-spectrum beta-lactamase-producing *Escherichia coli* from healthy pigs in Malang district, East Java, Indonesia. *Journal of Advanced Veterinary and Animal Research*, *9*(3), 447–452. https://doi.org/10.5455/javar.2022.i613

Efstratiou, M. A., Bountouni, M., & Kefalas, E. (2018). Spread of Antibiotic Resistance in Aquatic Environments: *E. coli* as a Case Study. *EWaS3 2018*, 693. https://doi.org/10.3390/proceedings2110693

Faridah, H. D., Wibisono, F. M., Wibisono, F. J., Nisa, N., Fatimah, F., Effendi, M. H., Ugbo, E. N., Khairullah, A. R., Kurniawan, S. C., & Silaen, O. S. M. (2023). Prevalence of the *bla*CTX-M and *bla*TEM genes among extended-spectrum beta lactamase–producing *Escherichia coli* isolated from broiler chickens in Indonesia. *Journal of Veterinary Research*, *67*(2), 179–186. https://doi.org/10.2478/jvetres-2023-0025

Geden, C. J., Nayduch, D., Scott, J. G., Burgess, E. R., Gerry, A. C., Kaufman, P. E., Thomson, J., Pickens, V., & Machtinger, E. T. (2021). House Fly (Diptera: Muscidae): Biology, Pest Status, Current Management Prospects, and Research Needs. *Journal of Integrated Pest Management*, *12*(1). https://doi.org/10.1093/jipm/pmaa021

Gundran, R. S., Cardenio, P. A., Villanueva, M. A., Sison, F. B., Benigno, C. C., Kreausukon, K., Pichpol, D., & Punyapornwithaya, V. (2019). Prevalence and distribution of *bla*CTX-M, *bla*SHV, *bla*TEM genes in extended- spectrum β- lactamase- producing *E. coli* isolates from broiler farms in the Philippines. *BMC Veterinary Research*, *15*(277), 1–8. https://doi.org/10.1186/s12917-019-1975-9

Halawani, E. M., Hassan, A. M., & Gad El-Rab, S. M. (2020). Nanoformulation of Biogenic Cefotaxime-Conjugated-Silver Nanoparticles for Enhanced Antibacterial Efficacy Against Multidrug-Resistant Bacteria and Anticancer Studies. *International Journal of Nanomedicine*, *Volume 15*, 1889–1901. https://doi.org/10.2147/IJN.S236182

Isam-Eldeen, I. I. B., AlaaEldin, Y. M. H., Mohamed, A. I. H., & Eltayib, H. A.-A. (2022). Isolation of potentially pathogenic bacteria from *Musca domestica* captured in hospitals and slaughterhouses, Khartoum state, Sudan. *African Journal of Microbiology Research*, *16*(2), 76–81. https://doi.org/10.5897/ajmr2021.9580

Islam, Md. S., Sobur, Md. A., Rahman, S., Ballah, F. M., Ievy, S., Siddique, M. P., Rahman, M., Kafi, Md. A., & Rahman, Md. T. (2022). Detection of *bla*TEM, *bla*CTX-M, *bla*CMY, and *bla*SHV Genes Among Extended-Spectrum Beta-Lactamase-Producing Escherichia coli

Isolated from Migratory Birds Travelling to Bangladesh. *Microbial Ecology*, *83*(4), 942–950. https://doi.org/10.1007/s00248-021-01803-x

Lindeberg, Y. L., Egedal, K., Hossain, Z. Z., Phelps, M., Tulsiani, S., Farhana, I., Begum, A., & Jensen, P. K. M. (2018). Can *Escherichia coli fly*? The role of flies as transmitters of *E. coli* to food in an urban slum in Bangladesh. *Tropical Medicine and International Health*, *23*(1), 2–9. https://doi.org/10.1111/tmi.13003

Matesanz, M., & Mensa, J. (2021). Ceftazidime-avibactam. *Revista Española de Quimioterapia*, *34*(Suppl 1), 38–40. https://doi.org/10.37201/req/s01.11.2021

Mustika, Y. R., Effendi, M. H., Puspitasari, Y., Plumeriastuti, H., Khairullah, A. R., & Kinasih, K. N. (2024). Identification of *Escherichia coli* Multidrug Resistance in Cattle in Abattoirs. *Jurnal Medik Veteriner*, *7*(1), 19–32. https://doi.org/10.20473/jmv.vol7.iss1.2024.19-32

Nihei, S. S., & De Carvalho, C. J. B. (2009). The Muscini flies of the world (Diptera, Muscidae): identification key and generic diagnoses. *Zootaxa*, *1976*(1). https://doi.org/10.11646/zootaxa.1976.1.1

Nishino, K., Yamasaki, S., Nakashima, R., Zwama, M., & Hayashi-Nishino, M. (2021). Function and Inhibitory Mechanisms of Multidrug Efflux Pumps. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.737288

O'Neill, J. (2016). *Tackling drug-resistant infections globally: final report and recommendations the review on antimicrobial resistance chaired by Jim O'neill*. https://apo.org.au/node/63983

Onwugamba, F. C., Mellmann, A., Nwaugo, V. O., Süselbeck, B., & Schaumburg, F. (2020). Antimicrobial resistant and enteropathogenic bacteria in 'filth flies': a cross-sectional study from Nigeria. *Scientific Reports*, *10*(1), 16990. https://doi.org/10.1038/s41598-020-74112-x

Punyadi, P., Thongngen, P., Kiddee, A., Assawatheptawee, K., Tansawai, U., Bunchu, N., & Niumsup, P. R. (2021). Prevalence of *bla*CTX-M and Emergence of *bla*CTX-M-5 Carrying *Escherichia coli* in Chrysomya megacephala (Diptera: Calliphoridae), Northern Thailand. *Microbial Drug Resistance*, *27*(5), 698–705. https://doi.org/10.1089/mdr.2020.0249

Putra, J. K., Wahyuni, A. E. T. H., & Untari, T. (2024). Sensitivitas *Escherichia coli* yang diisolasi dari unggas Phasianidae terhadap berbagai jenis antibiotika. *Resiliensi Industri Peternakan Tropis*, 247–262.

Rahman, M. M., Husna, A., Elshabrawy, H. A., Alam, J., Runa, N. Y., Badruzzaman, A. T. M., Banu, N. A., Al Mamun, M., Paul, B., Das, S., Rahman, Md. M., Mahbub-E-Elahi, A. T. M., Khairalla, A. S., & Ashour, H. M. (2020). Isolation and molecular characterization of multidrug-resistant *Escherichia coli* from chicken meat. *Scientific Reports*, *10*(1), 1–11. https://doi.org/10.1038/s41598-020-78367-2

Sari, D. Y., Pisestyani, H., & Lukman, D. W. (2020). Antibiotic resistance in *Escherichia coli* isolated from doner kebab sold in dramaga bogor, Indonesia. *Advances in Animal and Veterinary Sciences*, *8*(3), 278–284. https://doi.org/10.17582/journal.aavs/2020/8.3.278.284

Saujana, R. A. (2023). *Resistansi Isolat Escherichia coli dari Daging Ayam Suwir Bubur Ayam yang Dijual di Lingkar Kampus Institut Pertanian Bogor*. Sekolah Kedokteran Hewan dan Biomedis IPB University, Bogor.

Sobur, A., Haque, Z. F., Sabuj, A. A. M., Ievy, S., Rahman, A. M. M. T., El Zowalaty, M. E., & Rahman, T. (2019). Molecular Detection of Multidrug and Colistin-Resistant *Escherichia coli* isolated from house flies in various environmental settings. *Future Microbiology*, *14*(10), 847–858. https://doi.org/10.2217/fmb-2019-0053

Soufiane, S. D. M., Serge, Y. R., Tinlé, B., Sévérin, N., Firmin, K. N., Isidore, T., Jacques, Z., Inès, Y., Félicité, N.,

Aminata, F., Naomi, B., Jean-Bosco, O., Ibrahim, S., & Mahamoudou, S. (2024). Detection and molecular characterization of multiresistant Enterobacteriaceae carried by houseflies in the city of Bobo-Dioulasso, Burkina Faso. *International Journal of One Health*, *10*(1), 12–19. https://doi.org/10.14202/IJOH.2024.12-19

Suhartono, S., Hayati, Z., & Hayatunnida, R. (2023). Distribution of Multidrug Resistant *Escherichia coli* and *Klebsiella pneumoniae* Isolates among clinical specimens in the Zainoel Abidin General Hospital, Banda Aceh, Indonesia. *Research Journal of Pharmacy and Technology*, *16*(6), 2854–2858. https://doi.org/10.52711/0974-360X.2023.00470

Sumampouw, O. J. (2018). Uji Sensitivitas Antibiotik terhadap Bakteri *Escherichia coli* Penyebab Diare Balita di Kota Manado . *Journal of Current Pharmaceutical Sciences*, *2(1)*(2598–2095), 104–110.

Syafriana, V., Hamida, F., Sukamto, A. R., & Aliya, L. S. (2020). Resistensi *Escherichia coli* dari Air Danau ISTN Jakarta Terhadap Antibiotik Amoksisilin, Tetrasiklin, Kloramfenikol, dan Siprofloksasin. *Sainstech Farma*, *13*(3), 92–98. https://doi.org/doi.org/10.37277/sfj.v13i2.761

Tolenada, C. P. S., & Dayrit, G. B. (2023). Presence of *bla*CTXM-1, *bla*CTXM-9, and *bla*TEM-1 Genes in Extended-spectrum β-lactamase-producing *Escherichia coli* Isolates from Hospital Wastewater. *The Indonesian Biomedical Journal*, *15*(5), 278–287. https://doi.org/10.18585/inabj.v15i5.2531

WHO. (2020). *Vector-borne diseases*. https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases

Wibisono, F. J., Sumiarto, B., Untari, T., Effendi, M. H., Permatasari, D. A., & Witaningrum, A. M. (2020). Prevalensi dan Analisis Faktor Risiko *Multidrug Resistance* Bakteri *Escherichia coli* pada Ayam Komersial di Kabupaten Blitar. *Jurnal Ilmu Peternakan Dan Veteriner Tropis*, *10*(1), 15–22. https://doi.org/10.46549/jipvet.v10i1.74

Wibisono, F. M., Faridah, H. D., Wibisono, F. J., Tyasningsih, W., Effendi, M. H., Witaningrum, A. M., & Ugbo, E. N. (2021). Detection of invA virulence gene of multidrug-resistant Salmonella species isolated from the cloacal swab of broiler chickens in Blitar district, East Java, Indonesia. *Veterinary World*, *14*(12), 3126–3131. https://doi.org/10.14202/vetworld.2021.3126-3131

Widodo, A., Lamid, M., Effendi, M. H., Tyasningsih, W., Raharjo, D., Khairullah, A. R., Kurniawan, S. C., Yustinasari, L. R., Riwu, K. H. P., & Silaen, O. S. M. (2023). Molecular identification of *bla*TEM and *bla*CTX-M genes in multidrug-resistant *Escherichia coli* found in milk samples from dairy cattle farms in Tulungagung, Indonesia. *Journal of Veterinary Research*, *67*(3), 381–388. https://doi.org/10.2478/jvetres-2023-0052

Woodford, N., Fagan, E. J., & Ellington, M. J. (2006). Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum β-lactamases. *Journal of Antimicrobial Chemotherapy*, *57*(1), 154–155. https://doi.org/10.1093/jac/dki412

Yin, J. H., Kelly, P. J., & Wang, C. (2022). Flies as Vectors and Potential Sentinels for Bacterial Pathogens and Antimicrobial Resistance: A Review. *Veterinary Sciences*, *9*(300), 1–10. https://doi.org/10.3390/vetsci9060300

Zhang, G.-F., Liu, X., Zhang, S., Pan, B., & Liu, M.-L. (2018). Ciprofloxacin derivatives and their antibacterial activities. *European Journal of Medicinal Chemistry*, *146*, 599–612. https://doi.org/10.1016/j.ejmech.2018.01.078