Full Length Research Article
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 11/03/2023;
Date Revised: 17/07/2024;
Available Online: 15/10/2024;

Author's Affiliation:
1. Master Program in Veterinary Public Health, Faculty of Veterinary Medicine, Airlangga University - Indonesia
2. Department of Veterinary Public Health, Faculty of Veterinary Medicine, Airlangga University – Indonesia
3. Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Airlangga University – Indonesia
4. Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki – Nigeria

***Corresponding Author:**
Mustofa Helmi Effendi
Email: mheffendi@yahoo.com

How to Cite:
Maghfiroh L, Effendi MH, Tyasningsih W, Witaningrum AM, Bernard AN (2024). Identification of *Bla*TEM Gene Encode Extended Spectrum Beta Lactamase (ESBL) Producing *Escherichia coli* Isolated from Fresh Beef. Adv. Life Sci. 11(4): 741-747.

Keywords:
Escherichia coli; *bla*TEM; ESBL; Fresh beef; Public health

Open Access

Identification of *Bla*TEM Gene Encode Extended Spectrum Beta Lactamase (ESBL) Producing *Escherichia coli* Isolated from Fresh Beef

Lailatul Maghfiroh[1], Mustofa Helmi Effendi[2*], Wiwiek Tyasningsih[3], Adiana Mutamsari Witaningrum[2], Agumah Nnabuife Bernard[4]

Abstract

Background: The community needs fresh beef of good quality. Dirty sanitation causes *Escherichia coli* to easily contaminate fresh meat and *E. coli* is one of the bacteria that can produce ESBL enzymes that impact human health. This study aimed to isolate *Escherichia coli* from fresh meat at the Surabaya Traditional Markets to identify the presence of the ESBL-encoding *bla*TEM gene.

Methods: One hundred fifty fresh beef samples were collected from six traditional markets located in Surabaya city. Then, a polymerase chain reaction (PCR) test was performed to detect the blaTEM gene which codes for ESBL in isolates that unveiled positive results for MDR (resistant to three antibiotics) and suspected beta-lactamase enzyme (resistant to ampicillin).

Results: The research showed that from 150 samples of fresh beef at the Surabaya Traditional Market, 68 *Escherichia coli* isolates were found (45.3%). *Escherichia coli* isolates were shown to be susceptible to the antibiotic's ampicillin (73.5%), gentamicin (95%), tetracycline (7.5%), aztreonam (100%), and ciprofloxacin (95%). The highest percentage of resistance of *Escherichia coli* isolates to the antibiotic ampicillin (19.1%). PCR analysis of ampicillin-resistant isolates showed that the *bla*TEM gene encoding ESBL was present in 7 (53.8%) of the 13 *Escherichia coli* isolates.

Conclusion: The research results show that there is a risk of ESBL transmission to the community through Escherichia coli which was identified in fresh beef at the Surabaya Traditional Market. Therefore, it is hoped that public awareness can increase regarding food safety issues which can have an impact on public health.

Introduction

In the twenty-first century, antimicrobial resistance (AMR) is one of the greatest public health problems due to its widespread impact on humans, animals, and the environment [1,2]. Antimicrobial resistance (AMR) is a worldwide issue that poses a threat to human health because of inadequate research, dedication, infection control, illogical use of antibiotics, low-quality antibiotics, and inadequate supervision [3,4]. The emergence of pathogenic bacteria resistant to antibiotics causes an increase in patients infected with more difficult bacteria, takes longer to heal, and requires new types of antibiotics [5,6].

Beef is one of the important sources of animal origin for the community, especially to meet the needs of animal protein. Meat that contains complete nutrients makes it a good medium for the growth of bacteria, one of which is *Escherichia coli*. The presence of *Escherichia coli* in meat exacerbates the incidence of infection in humans because it can transmit infection and disease during processing, preparation or when consumed by consumers [7]. Fresh meat sold in retail markets can transmit Extended Spectrum Beta-Lactamase (ESBL) from animals to humans [8]. Research by Ansharieta et al., [9] showed that the TEM gene encoding ESBL was found in foodstuffs of animal origin. This is also confirmed by Imasari [10] that the gene encoding ESBL has spread between humans and animals. Of the ESBL-positive isolates, 48.1% had the TEM gene identified [11]. *bla*TEM is one of the most frequently confirmed ESBL coding genes in a study [12]. The research of Naelasari et al., [13] also showed that the TEM gene was found in isolates of patients from Dr. Soetomo Hospital. As per the findings of Bajpal et al., [14], the TEM gene accounted for 48.7% of the ESBL coding genes detected in isolates obtained from individuals suffering from urinary tract infections.

The increasing incidence of ESBL has become a public health focus. However, data on the incidence of ESBL produced by *Escherichia coli* in food of animal origin, especially fresh meat in the city of Surabaya, is still limited. No molecular research has detected the spread of the gene encoding ESBL resistance (*bla*TEM) produced by *Escherichia coli* isolated from fresh beef sold in traditional markets in Surabaya. Given the foregoing context, it is critical to carry out investigations to ascertain whether *Escherichia coli* isolates from fresh beef offered in Surabaya's traditional markets carry the *bla*TEM gene, which codes for ESBL.

Methods

Ethical approval

Since this investigation used fresh beef, ethical approval was not required. In Surabaya, Indonesia, six traditional markets provided samples of fresh meat.

Isolation and Identification of *Escherichia coli*

This research was conducted from February to April 2022. As many as 150 fresh beef samples were obtained from six traditional markets in the city of Surabaya, namely Wonokromo Market, Keputran Market, PacarKeling Market, Pabean Market, Manukan Market, and Jagir Market. The samples were isolated through a swab using a sterile cotton bud and planted in Buffered Peptone Water (BPW) media which functions as a resuscitation medium. They were transported using a cool box to the Division of Bacteriology and Mycology Laboratory, Department of Microbiology, Faculty of Veterinary Medicine, Airlangga University. Furthermore, 1 ml of each of the samples was planted into 9 ml of selective media enrichment Brilliant Green-bile Lactose Broth (BGLB), and coliform positive results were grown onto selective media of Eosin Methylene Blue Agar (EMBA) (Figure 1). The presumptive results of *Escherichia coli* were indicated by the growth of metallic green colonies with black midpoints. Furthermore, identification was carried out using Gram stain and IMViC biochemical test. Samples that yielded *Escherichia coli* were subjected to antibiotic sensitivity tests to determine their antibiotic resistance profile.

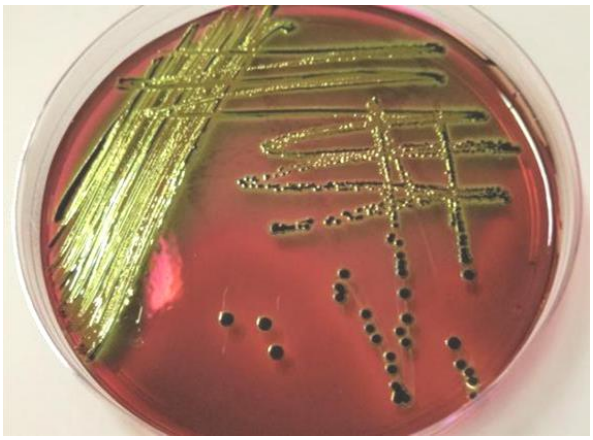

Figure 1: Metallic green colonies on EMBA media show the growth of *Escherichia coli* bacteria.

Antibiotic Sensitivity Test

Antibiotic sensitivity tests were performed on *Escherichia coli* isolates following the National Committee for Clinical Laboratory Standards [15]. To find out which five classes of antibiotics could stop the isolated *Escherichia coli* isolates from growing, the isolates were grown on Muller Hinton Agar (OXOID CM0337) medium and were given 30 µg of Aztreonam, 10 µg of Ampicillin, 30 µg of Tetracycline, 10 µg of Gentamycin, and 5 µg of Ciprofloxacin. The suspension of *Escherichia coli* isolate adapted to Mac Farland 0.5

standard was spread evenly on the surface of MHA media using sterile cotton buds and 5 different antibiotic groups were planted with a distance of 30 mm. There are three criteria, namely Susceptible (S), Intermediate (I), and Resistant (R). Furthermore, the sample isolates that showed positive results of MDR (resistance to three antibiotics) and presumptive Beta Lactamase enzyme (resistant to Ampicillin) were analyzed for the presence of *bla*TEM gene encoding ESBL using Polymerase Chain Reaction (PCR).

Molecular detection

The objective of the DNA extraction procedure is to remove the DNA from the bacterial cell before it is amplified in the PCR reaction. The DNA extraction used in this study followed the procedure for Extracting Gram-negative DNA from QIAGEN KIT.

PCR amplification of the *bla*TEM gene with 21 L of PCR reagents contained 12.5 L of PCR Master Mix, 1 L of forward primer, 1 L of reverse primer, 1 L of DNA template, and 5.5 L of RNAse free water. The mixing of PCR reagents was carried out using a thermocycler. The primers used and the PCR conditions are shown in Table 1 below.

Target Gene	Primers	*Amplicon size* (bp)	Thermo cycling	Reference
*bla*TEM	F': 5'-ATA AAA TTC TTG AAG ACG AAA 3'	1086 bp	Initial incubation at 94°C was followed by 30 cycles of denaturation at 94°C for 2 minutes, annealing at 52°C for 30 seconds, elongation at 72°C for 45 seconds, and final elongation at 72°C for 5 minutes.	[6]
	R': 5' GAC AGT TAC CAA TGC TTA ATC-3'			

Table 1: Primers used in PCR amplification.

5 L of PCR product was put into a 2% agarose gel well and electrophoresed at 100 V for 40 minutes in tris-acetate buffer. Afterward, the image was produced using ethidium bromide staining, UV illumination, and gel documentation equipment. A 100 bp DNA ladder served as the PCR marker. The results obtained in the form of a DNA band pattern with a size of 1086 bp were declared positive for the ESBL-encoding *bla*TEM gene.

Statistical Analysis

Following qualitative processing, data from molecular analyses, bacterial sensitivity tests, and isolation results are presented descriptively.

Results

The Presence of *Escherichia coli* in Fresh Beef

The results of this study indicate the presence of *Escherichia coli* bacteria in fresh beef at the Surabaya City Traditional Market by 45.3% (68/150) as shown in Table 2.

Antibiotic resistance pattern

Sixty-eight isolates of *Escherichia coli* tested with antibiotic sensitivity showed that all *Escherichia coli* isolates were still sensitive to the antibiotic Aztreonam, namely 68 isolates (100%). Then, there were 13 resistant isolates, 5 intermediate isolates, and 50 sensitive isolates on the antibiotic ampicillin. In tetracycline antibiotics, there were 51 sensitive isolates, five intermediate isolates, and 12 resistant isolates. Furthermore, on the antibiotic Gentamycin, there were 61 sensitive isolates, three intermediate isolates and four resistant isolates. Ciprofloxacin isolates contained 61 sensitive isolates, three intermediate isolates and four resistant isolates (Table 3).

Molecular detection

PCR testing was used to find the ESBL-encoding *bla*TEM gene in isolates of *Escherichia coli* bacteria that were resistant to the antibiotic ampicillin. The results showed that of the 13 isolates of *Escherichia coli* isolated from fresh beef at the Surabaya Traditional Market, seven isolates (53.8%) had the *bla*TEM gene encoding Extended Spectrum Beta Lactamase (ESBL). The results of electrophoresis of PCR products, the *bla*TEM gene will show a DNA band of 1086 base-pair (bp) as shown in Figure 2.

Figure 2: Electrophoresis results of the *bla*TEM gene PCR product in the 1086 base-pair band. K+: positive control *Escherichia coli* ATCC 35218; K-: negative control *Escherichia coli* ATCC 25922; W7-A1: *Escherichia coli* isolate resistant to ampicillin.

Discussion

One of the bacteria that play a role in AMR cases is *Escherichia coli. Escherichia coli* plays an important role in ecology and is used as a bioindicator of antimicrobial resistance [22]. This study showed higher results than the research of Eyi [16] which showed the prevalence of *Escherichia coli* isolated from beef was 25% (14/56) and research by Langgar et al., [17] showed a positive result of 9.75% *Escherichia coli* (4/21).

Market	Sample size	Isolation			Identification		Number of *E. Coli* (Percentage)
		BPW	BGLB	EMBA	Pewarnaan Gram	IMViC	
Wonokromo	30	30	30	24	24	12	12 (40%)
Keputran	30	30	30	24	24	19	19 (63,3%)
Pacarkeling	30	30	30	17	17	11	11 (36,6%)
Manukan	15	15	15	11	11	10	10 (66,6%)
Pabean	15	15	15	7	7	7	7 (46,6%)
Jagir	30	30	30	14	14	9	9 (30%)
TOTAL	150	150	150	97	97	68	68 (45,3%)

Table 2: The isolation and identification of *Escherichia coli.*

Location	Antibiotics				
	Aztreonam	*Ampicillin*	Tetracycline	*Gentamycin*	*Ciprofloxacin*
Wonokromo (12 isolates)	R: 0 (0%) I: 0 (0%) S: 12 (100%)	R: 3 (25%) I: 1 (8,3%) S: 8 (66,6%)	R: 2 (16,6%) I: 0 (0%) S: 10 (83,3%)	R: 1 (8,3%) I: 0 (0%) S: 11 (91,6%)	R: 0 (0%) I: 0 (0%) S: 12 (100%)
Keputran (19 isolates)	R: 0 (0%) I: 0 (0%) S: 19 (100%)	R: 2 (10,5%) I: 1 (5,2%) S: 16 (84,2%)	R: 3 (15,7%) I: 4 (21%) S: 12 (63,1%)	R: 0 (0%) I: 0 (0%) S: 19 (100%)	R: 2 (10,5%) I: 0 (0%) S: 17 (89,4%)
PacarKeling (11 isolates)	R: 0 (0%) I: 0 (0%) S: 11 (100%)	R: 1 (9%) I: 2 (18,1%) S: 8 (72,7%)	R: 1 (9%) I: 1 (9%) S: 9 (81,8%	R: 0 (0%) I: 0 (0%) S: 11 (100%)	R: 0 (0%) I: 2 (18,1%) S: 9 (81,8%)
Manukan (10 isolates)	R: 0 (0%) I: 0 (0%) S: (100%)	R: 3 (30%) I: 0 (0%) S: 7 (70%)	R: 4 (40%) I: 0 (0%) S: 6 (60%)	R: 3 (30%) I: 1 (10%) S: 6 (60%)	R: 1 (10%) I: 0 (0%) S: 9 (90%)
Pabean (7 isolates)	R: 0 (0%) I: 0 (0%) S: (100%)	R: 1 (14,2%) I: 1 (14,2%) S: 5 (71,4%)	R: 2 (28,5%) I: 0 (0%) S: 5 (71,4%)	R: 1 (14,2%) I: 2 (28,5%) S: 4 (57,1%)	R: 1 (14,2%) I: 0 (0%) S: 6 (85,7%)
Jagir (9 isolates)	R: 0 (0%) I: 0 (0%) S: (100%)	R: 3 (33,3%) I: 0 (0%) S: 6 (66,6%)	R: 0 (0%) I: 0 (0%) S: 9 (100%)	R: 1 (11,1%) I: 0 (0%) S: 8 (88,8%)	R: 0 (0%) I: 1 (11,1%) S: 8 (88,8%)
Total (68 isolates)	R: 0 (0%) I: 0 (0%) S: 68 (100%)	R: 13 (19,1%) I: 5 (7,3%) S: 50 (73,5%)	R: 12 (17,6%) I: 5 (7,3%) S: 51 (75%)	R: 4 (5,8%) I: 3 (4,4%) S: 61 (89,7%)	R: 4 (5,8%) I: 3 (4,4%) S: 61 (89,7%)

Table 3: Antibiotic Sensitivity Test Results against *Escherichia coli* isolates.

The prevalence of ESBL-producing *Escherichia coli* in fresh beef in Singapore is 7.3% and in Australia 5.3% [18]. However, previous studies have also shown higher results, such as the Sulaxono study [19]; namely from 189 fresh meat samples in the Maros Veterinary Centre Work Area, it was found that 85.19% (161/189) were contaminated with *Escherichia coli*. Research by Indraswari et al., [20] found 84.69% (83/98) of *Escherichia coli* in beef in the Yogyakarta. In Adzitey's research [21] *Escherichia coli* was successfully isolated from beef in abattoirs by 88%.

In order to acquire resistance genes from other bacteria and transfer their resistance genes to other bacteria, *Escherichia coli* functions as both a donor and a recipient of resistance genes. Therefore, in both human and veterinary medicine, Escherichia coli is considered to be the primary reservoir of resistance genes that cause treatment failure [23]. Given that it generates the Enzyme Extended Spectrum Beta-Lactamase (ESBL), *Escherichia coli* has the potential to be the primary source of AMR and is crucial to the development and spread of AMR pathways [1]. of except for cephamycin and carbapenems, the enzyme Extended Spectrum Beta-Lactamase (ESBL) may hydrolyze penicillin antibiotics as well as third- and fourth-generation cephalosporins and monobactams [24]. The global spread of *Escherichia coli* producing ESBL enzymes poses a significant threat to the effectiveness of antimicrobials, especially third and fourth-generation cephalosporins [25].

Where the difference in the prevalence of *Escherichia coli* according to Abayneh et al., [26] can be caused by differences in sanitation standards from the kiosks of fresh meat traders, low sanitation standards, and poor hygiene practices when handling meat contribute to cross-contamination of *Escherichia coli* isolates in meat. According to Haile et al., [27] contamination of beef or beef carcasses can occur during the preparation process such as skinning, removal of fat, and distribution. Furthermore, the area in traditional markets where beef is sold is still mixed with vendors selling other necessities; the meat that is placed on tables is not sterile, is left exposed without a cover, and is left to hang at room temperature; additionally, the traditional market setting, with its environmental conditions and all buying and selling activities carrying some risk of contamination. Fecal infection is one possible cause of ESBL-producing *Escherichia coli* in fresh meat. This is evidenced by the research of Palmeira et al., [28] that found *Escherichia coli* producing ESBL in the feces of beef cattle in Brazil. In Germany, 11.2% of beef cattle also have ESBL-producing *Escherichia coli* [29]. In addition, research by Montso et al., [30] revealed that there was ESBL-producing *Escherichia coli* in isolates from fresh meat and beef cattle in South Africa.

Aztreonam is a monobactam antibiotic that is used as an index for suspected ESBL. The study results showed that all *Escherichia coli* isolates were still sensitive to the antibiotic Aztreonam and was in line with exploration by Nadira et al., [31] who set up that

Escherichia coli isolates from cow feces samples were still 100% sensitive to Aztreonam. The rare use of the antibiotic Aztreonam in beast is the reason that aztreonam perceptivity is still high. Still, these results are different from the previous study by Ansharieta et al., [32] which showed that Escherichia coli isolates from fresh cow's milk in East Java endured resistance to Aztreonam of 2.16%.

Ampicillin is a penicillin-class antibiotic that's used as an indicator of the presence of beta-lactam enzymes. The results of the study showing that 19 Escherichia *coli* isolates were resistant to Ampicillin are substantiation of a fairly high position of resistance and are in line with former exploration that more than 50 of beta- lactam antibiotics are resistant to bacterial isolates forming from food of beast origin. Exploration by Dsani et al., [33] set up that 57 of *Escherichia coli* isolates from beef were resistant to Ampicillin.

Antibiotics made from Ampicillin and Penicillin are constantly used to treat bacterial infections in livestock raised for food, like cattle. It's known that the antibiotics Ampicillin and Penicillin are frequently used to treat bacterial infections in animals similar to cattle. In addition, penicillin antibiotics in humans are also frequently used in the treatment of infections similar to urinary tract infections. Thus, while choosing a course of remedy for ails brought on by *Escherichia coli* that has been linked to beef, this needs to be taken into consideration. Ampicillin is a beta-lactam drug that works by inhibiting bacterial wall synthesis. Beta-lactam antibiotics bind to Penicillin-Binding Proteins (PBPs) in bacteria, thereby inhibiting bacterial cell wall synthesis because the trans-peptidase process between peptidoglycan chains is disrupted, then proteolytic enzyme activation occurs in the cell wall. Ampicillin resistance can occur by several factors, one of which is the formation of the beta Lactamase enzyme which is excreted by Gram-negative bacteria into the periplasmic cavity between the cytoplasmic membrane and the bacterial cell wall so that it can reach the right antibiotic target to interfere with the way the antibiotic works [34].

Inappropriate and excessive use of antibiotics causes selective pressure that favours the growth of resistant bacteria. Through conjugative plasmid-mediated horizontal gene transfer, the colonization of resistant bacteria in the intestines of people and animals result in the transmission of bacterial resistance genes in the intestinal flora. The majority of plasmid genes encode ESBLs. It was discovered that Enterobacteriaceae possessed plasmids containing resistance genes. The *bla*TEM gene is located on a plasmid [35]. The IncFII plasmid group is known as the group encoding the ESBL gene and is widely distributed in Enterobacteriaceae and is called the epidemic-resistant plasmid group. *bla*TEM is one of the dominant ESBL genes in the world and was found in human, animal, and environmental isolates. The *bla*TEM gene was found mainly in isolates of *Escherichia coli* and *Klebsiella pneumoniae* bacteria [36]. This result is higher than research by Dsani et al., [33] which detected the *bla*TEM gene by 4% (4/98) in *Escherichia coli* isolates isolated from fresh beef in Ghana.

The most prevalent beta-lactamase seen in Gram-negative bacteria is the *bla*TEM gene. 90% of *Escherichia coli*'s resistance to ampicillin is brought on by the *bla*TEM gene production [37]. The presence of resistant bacteria in food-producing animals and food products of animal origin is caused by the continuous use of antibiotics for therapeutic and non-therapeutic purposes in the production system [38–40]. In addition, fresh beef can be contaminated with antibiotic-resistant pathogens at the point of unhygienic slaughter and sale. Although selective pressure is the main cause while, sanitary conditions at slaughter, sale, and processing sites can influence the intensity pattern of distribution along the food chain [33].

Fresh beef containing ESBL-producing *Escherichia coli* poses a major risk to public health, and people need to be made aware of the risks these pathogenic bacteria represent. The evidence by molecular identification using PCR in this study showed the presence of the *bla*TEM gene in fresh beef sold at Traditional markets in Surabaya. This proves that there is a risk of transmission of AMR caused by *Escherichia coli* producing ESBL from food of animal origin to humans.

Acknowledgements

This study was supported in part by the Lembaga Pengelola Dana Pendidikan (LPDP) Kementerian Keuangan Republik Indonesia with grant number: KET-3889/LPDP.4/2020.

Author Contributions

Lailatul Maghfiroh and Mustofa Helmi Effendi: Conceived, designed, and coordinated the study.

Wiwiek Tyasningsih: Designed data collection tools, supervised the field sample and data collection, and laboratory work as well as data entry.

Adiana Mutamsari Witaningrum: Contributed reagents, materials, and analysis tools.

Agumah Nnabuife Bernard: Carried out the statistical analysis and interpretation and participated in the preparation of the manuscript.

All authors have read, reviewed, and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

1. Nüesch-Inderbinen M, Kindle P, Baschera M, Liljander A, Jores J, et al.. Antimicrobial resistant and extended-spectrum β-lactamase (ESBL) producing Escherichia coli isolated from fecal samples of African dromedary camels. Scientific African, (2020); 7: e00274 .
2. Yanestria SM, Dameanti FNAEP, Musayannah BG, Pratama JWA, Witaningrum AM, et al. Antibiotic resistance pattern of Extended-Spectrum β-Lactamase (ESBL) producing Escherichia coli isolated from broiler farm environment in Pasuruan district, Indonesia. Biodiversitas: Journal of Biological Diversity, (2022); 23(9): 4460–4465.
3. Effendi MH, Tyasningsih W, Yurianti YA, Rahmahani J, Harijani N, et al. Presence of multidrug resistance (MDR) and extended-spectrum beta-lactamase (ESBL) of Escherichia coli isolated from cloacal swabs of broilers in several wet markets in Surabaya, Indonesia. Biodiversitas, (2021); 22(1): 304–310.
4. Wibisono FJ, Sumiarto B, Untari T, Effendi MH, Permatasari DA, et al. Molecular identification of CTX gene of extended spectrum beta-lactamases (ESBL) producing Escherichia coli on layer chicken in Blitar, Indonesia. Journal of Animal and Plant Sciences, (2021); 31(4): 954–959.
5. Effendi MH, Faridah HD, Wibisono FM, Wibisono FJ, Nisa N, et al. Detection of virulence factor encoding genes on Escherichia coli isolated from broiler chicken in Blitar District, Indonesia. Biodiversitas: Journal of Biological Diversity, (2022); 23(7): 3437-3442.
6. Tyasningsih W, Ramandinianto SC, Ansharieta R, Witaningrum AM, Permatasari DA, et al. Prevalence and antibiotic resistance of Staphylococcus aureus and Escherichia coli isolated from raw milk in East Java, Indonesia. Veterinary World, (2022); 15(8): 2021–2028.
7. Shabana II, Al-Enazi AT. Investigation of plasmid-mediated resistance in Escherichia coli isolated from healthy and diarrheic sheep and goats. Saudi Journal of Biological Sciences, (2020); 27(3): 788–796.
8. Karacan Sever N, Sahan Yapicier O, Akan M. Distribution of Serotypes and Antibiotic Resistance of Avian Pathogenic Escherichia coli Strains Isolated from Chickens. Kafkas Universitesi Veteriner Fakultesi Dergisi, (2022); 28(6): 767-772.
9. Ansharieta R, Ramandinianto SC, Effendi MH, Plumeriastuti H. Molecular identification of blaCTX-M and blaTEM genes encoding extended spectrum beta-lactamase (ESBL) producing Escherichia coli isolated from raw cow's milk in East Java Indonesia. Biodiversitas, (2021); 22(4): 1600–1605.
10. Imasari T. Prevalence and Genotypic Patterns of SHV, TEM, CTX-M Bacteria Enterobacteriaceae Produce Extended Spectrum β-Lactamase from Dairy Cow Feces and Residents Around Livestock in Surabaya. Thesis. (2017).
11. Mensah DO, Noah ON, Evelyn YB, Ebenezer OM, Kingsley ID, et al. (2016) Genetic characterization of TEM-type ESBL-associated antibacterial resistance in Enterobacteriaceae in a tertiary hospital in Ghana. Annals of Clinical Microbiology and Antimicrobials, (2016); 15(29): 1–9.
12. Dirar MH, Bilal NE, Ibrahim ME, Hamid ME. Prevalence of extended-spectrum Beta-lactamase (ESBL) and molecular detection of blaTEM, blaSHV, and blaCTX-M genotypes among Enterobacteriaceae isolates from patients in Khartoum, Sudan. Pan African Medical Journal, (2020); 37(213): Page numbers not for citation purposes.
13. Naelasari DN, Koendhori EB, Dewanti L, Sulistiawati Sarassari R, Kuntaman, K. The Prevalence of Extended Spectrum Beta Lactamase (ESBL) Producing Gut Bacterial Flora among Patients in Dr. Soetomo Hospital and Primary Health Center in Surabaya. Folia Medica Indonesiana, (2018); 54(4): 256–262.
14. Bajpai T, Pandey M, Varma M, Bhatambare GS. Prevalence of TEM, SHV, and CTX M Beta Lactamase genes in the urinary isolates of a tertiary care hospital. The Avicenna Medical Journal, (2017); 7(1): 12–16.
15. Wayne PA, Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically, https://clsi.org/media/1928/m07ed11_sample.pdf, (Accessed: 2.9.2024).
16. Eyi A, Arslan S. Prevalence of Escherichia coli in retail poultry meat, ground beef and beef. Med. Weter, (2012); 68(4): 237–240.
17. Langgar SMC, Sanam MUE, Annitha IR, Detha D. Prevalence of Escherichia coli in Beef in Oeba Slaughterhouse, Kupang City. Jurnal Veteriner Nusantara, (2021); 4(1): 1–10.
18. Guo S, Aung KT, Lee P, Tay MYF, Seow KLG, et al. Prevalence and genomic analysis of ESBL-producing Escherichia coli in retail raw meats in Singapore. Journal of Antimicrobial Chemotherapy, (2020); 76(3): 601–605.
19. Sulaxono H. Escherichia coli Contamination on Fresh Meat in the Working Area of the Maros Veterinary Center. Proceedings of the National Seminar on Animal and Veterinary Technology, (2020); 906–913.
20. Indraswari A, Dyah AW, Aris H, I Wayan S. Escherichia coli diarrhea in Beef in the Special Region of Yogyakarta and the Pattern of Resistance to Antibiotics. (2022).
21. Adzitey, F. Incidence and antimicrobial susceptibility of Escherichia coli isolated from beef (meat muscle, liver, and kidney) samples in Wa Abattoir, Ghana. Cogent Food & Agriculture, (2020); 6(1): 1718269.
22. Ramos S, Silva V, Dapkevicius MLE, Caniça M, Junco MTT, et al. Escherichia coli as Commensal and Pathogenic Bacteria among Food-Producing Animals: Health Implications of Extended Spectrum β-Lactamase (ESBL) Production. Animals, (2020); 10(12): 2239.
23. Poirel L, Madec JY, Lupo A, Schink AK, Kieffer N, et al. Antimicrobial Resistance in Escherichia coli. Microbiology Spectrum, (2018); 6(4): ARBA-0026-2017.
24. Hassuna NA, Khairalla AS, Farahat EM, Hammad AM, Fattah MA. Molecular characterization of Extended-spectrum β-lactamase producing Escherichia coli recovered from community-acquired urinary tract infections in Upper Egypt. Scientific Reports, (2020); 10 (1): 2772.
25. Geser N, Stephan R, Hachler H. Occurrence and characteristics of extended-spectrum-β-lactamase (ESBL) producing Enterobacteriaceae in Food-producing animals, minced meat and raw milk. BMC Veterinary Research, (2012); 8(21): 1–9.
26. Abayneh M, Tesfaw G, Woldemichael K, Yohannis M, Abdissa A. Assessment of extended-spectrum β-lactamase (ESBLs) – producing Escherichia coli from minced meat of cattle and swab samples and hygienic status of meat retailer shops in Jimma town, Southwest Ethiopia. BMC Infectious Diseases, (2019); 19: 1–8.
27. Haile AF, Alonso S, Berhe N, Atoma TB, Boyaka PN, et al. Prevalence, Antibiogram, and Multidrug-Resistant Profile of E. coli O157: H7 in Retail Raw Beef in Addis Ababa, Ethiopia. Frontiers in Veterinary Science, (2022); 9: 734896.
28. Palmeira JD, Haenni M, Matayer V, Madec JY, Ferreira HMN. Epidemic spread of IncI1/pST113 plasmid carrying the Extended-Spectrum Beta-Lactamase (ESBL) blaCTX-M-8 gene in Escherichia coli of Brazilian cattle. Veterinary Microbiology, (2020); 243: 108629.
29. Michael GB, Kaspar H, Siqueira AK, Costa EF, Corbellini LG, et al. Extended-spectrum b-lactamase (ESBL)-producing Escherichia coli isolates collected from diseased food-producing animals in the GERM-Vet monitoring program 2008–2014. Veterinary Microbiology, (2017); 200: 142–150.
30. Montso KP, Dlamini SB, Kumar A, Ateba CN. Antimicrobial Resistance Factors of Extended-Spectrum Beta-Lactamases Producing Escherichia coli and Klebsiella pneumoniae isolated from Cattle Farms and Raw Beef in North-West Province, South Africa. BioMed Research International, (2019); 2019(1): 1–13.

31. Syahmifariza, Indrawati N, Supriatna A, Faith, Sensitivity Test of Several Antibiotics against Escherichia coli Isolates from Australian Cattle Feces Samples, http://repository.ipb.ac.id/handle/123456789/94731, (Accessed: 2.9.2024).
32. Ansharieta R, Effendi MH, Plumeriastuti H. Genetic Identification of Shiga Toxin Encoding Gene from Cases of Multidrug Resistance (MDR) Escherichia coli Isolated from Raw Milk. Interciencia Journal, (2021); 46 (3): 1-9.
33. Dsani E, Afari EA, Appiah AD, Kenu E, Kaburi BB, et al. Antimicrobial resistance and molecular detection of extended spectrum Beta-lactamase producing Escherichia coli isolates from raw meat in Greater Accra region, Ghana. BMC Microbiology, (2020); 20: 1–8
34. Arivo D, Dwiningtyas AW. Antibiotic Sensitivity Test against Escherichia coli Causes Urinary Tract Infections. Journal of Medical Sciences and Health , (2017); 4(4): 216–225.
35. Wibisono FJ, Sumiarto B, Untari T, Effendi MH, Permatasari DA, et al. Pattern of antibiotic resistance on extended-spectrum beta-lactamases genes producing Escherichia coli on laying hens in Blitar, Indonesia. Biodiversitas, (2020); 21(10): 4631–4635.
36. Soekoyo AR, Sulistiawati, Wahyu S, Kuntaman K. The Epidemiological Pattern and Risk Factor of ESBL (Extended Spectrum β-Lactamase) Producing Enterobacteriaceae in Gut Bacterial Flora of Dairy Cows and People Surrounding in Rural Area, Indonesia. Indonesian Journal of Tropical and Infectious Disease, (2020); 8(3): 144–151.
37. Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, et al. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Research, (2020); 48(D1): D517–D525.
38. Wibisono FM, Wibisono FJ, Effendi MH, Plumeriastuti H, Hidayatullah AR, et al. A review of salmonellosis on poultry farms: Public health importance. Systematic Reviews in Pharmacy, (2020); 11(9): 481–486.
39. Permatasari DA, Witaningrum AM, Wibisono FJ, Effendi MH. Detection and prevalence of multidrug-resistant Klebsiella pneumoniae strains isolated from poultry farms in Blitar, Indonesia. Biodiversitas Journal of Biological Diversity, (2020); 21(10): 4642–4647.
40. Effendi MH, Oktavianto A, Hastutiek P. Tetracycline Resistance Gene In Streptococcus agalactiae Isolated From Bovine Subclinical Mastitis In Surabaya, Indonesia. Jurnal International The Philippine Journal of Veterinary Medicine, (2018); 55(SI): 115–120.