Identification of *Escherichia coli* Multidrug Resistance in Cattle in Abattoirs

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Abstract

Escherichia coli is a typical flora of an animals' digestive tract. Based on these details, this study was done on the detection and identification of multidrug-resistant *E. coli* in cattle in Surabaya abattoirs. Each cattle rectum swab sample was streaked into EMB agar media and followed by Gram staining. The IMViC test was used to confirm the presence of *E. coli*. Based on morphological culture features, Gram staining, and biochemical testing, the sample examination results revealed that 41 samples (41%) of the 100 cattle rectal swab samples that were isolated were confirmed to be positive for *E. coli*. The profile of antibiotic resistance from the results of the *E. coli* resistance test to antibiotics showed that out of a total of 41 isolates of *E. coli*, 12 isolates (29.27%) were detected as resistant to 1 class of antibiotics tested, while 2 isolates (4.88%) were resistant to 2 classes of antibiotics, and 3 isolates (7.32%) were confirmed to be multidrug resistance (MDR).

Keywords: *Escherichia coli*, multidrug resistance, rectal swabs

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INTRODUCTION

The most essential human need is food, which encompasses all products from agriculture, forestry, fisheries, animal husbandry, water use, and water-related activities, both processed and unprocessed, that are used to produce food or beverages for human use (Gebre and Gebremedhin, 2019). Depending on the source, food ingredients are separated into plant-based and animal-based categories (McClements and Grossmann, 2021). Beef is a key product in satisfying the demand for animal food that is rich in nutrients in people and is a significant nutritional component in determining nutrition, which can impact the quality of human resources (Smith *et al*., 2018).

Beef is a good medium for the growth of numerous microorganisms, including bacteria and fungi, as an animal food source (Sohaib *et al*., 2016). Bacteria in beef can alter the texture of food, resulting in health issues for the body or a condition known as foodborne illness (Bintsis, 2017). *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Salmonella* spp. are a few of the bacteria that frequently result in foodborne illness (Azinheiro *et al*., 2021; Wibisono *et al*., 2020). Beef is contaminated with bacteria from the digestive tract before and after the animal is slaughtered in the abattoirs, as well as from sanitary variables such as tools, workers, exposure to the floor surface, water use, and equipment (Nkosi *et al*., 2021; Wardhana *et al*., 2021).

E. coli is a typical flora of an animal's digestive tract, and it can contaminate meat and the environment around the abattoirs when cattle are being slaughtered (Ramos *et al*., 2020; Kartikasari *et al*., 2019). These microbes are prevalent in cattle's digestive tracts (Stein and Katz, 2017; Purnama *et al*., 2019). *E. coli* strains that are pathogenic can infect both humans and

animals and cause gastroenteritis, cystitis, pneumonia, and septicemia (Sarowska *et al*., 2019; Fikri and Purnama, 2020). The rectal canal is the primary colonization site for the human intestinal disease-causing Enterohaemorrhagic *E. coli* (EHEC) 0157:H7 in cattle (Lara-Duran *et al*., 2019). Due to their ease in spreading resistance genes to other bacteria, these bacteria can also serve as reservoirs for the development of antibiotic resistance (Puvača and de Llanos Frutos, 2021).

The development of microorganisms that are resistant to antibiotics is known as antibiotic resistance (Uddin *et al*., 2021). A major health concern is antibiotic resistance (Riwu *et al*., 2020). Antibiotic use in animals is currently double that in humans (Effendi *et al*., 2021). In food-producing animals, antibiotic use reached 63,151 tons in 2010 and is anticipated to rise by 67% by 2030 (Ma *et al*., 2021; Fikri *et al*., 2022). Some bacteria have developed self-defense mechanisms to combat exposure to different types of antibiotics as a result of the inappropriate use of antibiotics for the treatment of infectious disorders and supplementary feed (Khairullah *et al*., 2022). Multidrug resistance (MDR) is currently the most prevalent mechanism of antibiotic resistance (Ansharieta *et al*., 2021). A bacteria known as MDR is resistant to three different types of antibiotic classes (Widodo *et al*., 2022).

A high incidence of MDR *E. coli* bacteria, including those resistant to tetracycline (86.67%), gentamycin (86.67%), sulphamethoxazole-trimethoprim (20%), and amoxicillin (100%) (Dutta *et al*., 2017). This was further supported by the findings of a study carried out in East Algeria, which revealed the identification of 45 MDR-positive *E. coli* isolates from 198 isolates obtained by swabbing cattle rectal (Barour *et al*., 2019). According to a study conducted in Ethiopia, food of animal origin may be a vehicle for the spread of antibiotic-resistant *E. coli*, as evidenced by the similarities between MDR *E. coli* isolated from food of animal origin and *E. coli* that causes human urinary tract infections (Pradika *et al*., 2019; Geresu and Regassa, 2021; Dameanti *et al*., 2022).

According to a study performed by Rahmahani *et al*. (2020), 17 *E. coli* isolates from beef discovered in the wet market in Surabaya were shown to be resistant to Amoxicillin by 35%, Ampicillin by 35%, Ampicillin-sulbactam by 5.8%, Gentamicin by 5.8%, Ciprofloxacin by 35%, and Trimethoprim-sulfamethoxazole by 35%. These findings are in line with a study by Adenepekun *et al*. (2015) that found that *E. coli* isolated from animal-sourced food was resistant to several antibiotics, including those belonging to the tetracycline, aminoglycoside, sulfonamide, β-lactam, cephalosporin, trimethoprim, and fluoroquinolone groups. Based on these details, a study was done on the detection and identification of multidrug-resistant *E. coli* in cattle in Surabaya abattoirs. To take preventive action, it is envisaged that the study's findings would be able to characterize the possibility of and provide information on the contamination of humans with *E. coli* multidrug resistance through cattle that will be butchered in abattoirs.

MATERIALS AND METHODS

Ethical Approval

This study did not require ethical approval because there was no treatment in animals.

Study Period and Location

This study was performed from August to October 2021. Fecal sampling was collected at the Surabaya abattoirs, whereas bacterial isolation and sensitivity tests were done at the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Airlangga University.

Study Area and Sample Collection

100 cattle rectum swabs were used to collect the samples. The results of the swab were labeled, and aseptic handling of the swab sample was required. Sample-contained cotton swabs were put in tubes containing Buffer Pepton Water (BPW) (E. Merck, Darmstadt, Germany). The sample was then transported to the lab while being kept in a cooler box.

Isolation and Identification of *E. coli*

Each cattle rectum swab sample was streaked into Eosin Methylene Blue Agar (EMBA) (E. Merck, Darmstadt, Germany) media and incubated at 37°C for 18–24 hours to isolate and identify *E. coli*. On EMBA media, *E. coli* colonies typically have a metallic green hue.

Gram staining was applied to isolates from EMBA medium that were thought to be *E. coli* to examine the bacteria's cell structure and Gram characteristics. Physiological NaCl was injected into a syringe and dropped onto an object glass to perform the first stage of the Gram staining procedure. Utilizing a loop, apply the bacterial isolate to the glass object. Make the application as thin as possible, let it dry, and then fix it over a Bunsen flame. The second procedure involves applying Lugol drops, drizzling crystal violet coloring, and waiting two minutes before washing with running water. In the third stage, add safranin (leave it on for 20 to 30 seconds), wash it with running water, and then drop 95% alcohol (leave it on for 10 to 20 seconds). The final step involves drying with absorbent paper, adding immersion oil to an object glass, and examining under a 1000× magnification microscope.

The IMViC test, which included the indole test, the methyl red (MR) test, the Voges Proskauer (VP) test, and the citrate test, was used to confirm the presence of *E. coli*. After adding 2 to 3 drops of Kovach's reagent, the indole test on SIM media (E. Merck, Darmstadt, Germany) incubated at 37°C for 24 hours yields a positive result indicated by the creation of a pink indole ring on the surface of the media (Widodo *et al*., 2023). To perform the MR-VP test, colonies from the EMBA medium were inoculated into a 10 mL tube of MR-VP media. For 48 hours, the inoculum was incubated at 37°C. The media is then split between two test tubes. Two to five drops of the MR indicator are drip-tested into the first tube for the MR test. MR test results are positive for *E. coli* and produce a red color. The second tube is for the VP test, and it is filled with 0.2 mL of 40% KOH and 0.6 mL of naphthol solution. The VP test for *E. coli* produced negative results with no color change (yellow-brown) (Yanestria *et al*., 2022). Colonies from the EMBA medium are inoculated into Simon Citrate Agar (SCA) (E. Merck, Darmstadt, Germany) media for the citrate test. The absence of a color change in the citrate test medium after the inoculum was incubated at 37°C for approximately 24 hours also indicated that the inoculum was free of *E. coli* (Effendi *et al*., 2022).

Antibiotic Sensitivity Test

The antibiotic sensitivity test employed the Kirby-Baurer technique, which utilized the Disk diffusion test to generate qualitative categories with sensitive, intermediate, and resistant assessments. In test tubes containing 8 mL of physiological NaCl and homogenized using a vortex, bacterial cultures from colonies found in EMBA medium were cultivated until turbidity matching the Mc Farland standard of 0.5 was achieved (Waruwu *et al*., 2023).

The procedure for planting on the agar plate involved taking 1–2 colonies of *E. coli* isolates on EMBA media using ose, placing it in physiological NaCl that had been tested for turbidity with the Mc Farland standard 0.5, taking 0.2 mL, and slowly wiping it over the media Mueller Hinton Agar (MHA) (E. Merck, Darmstadt, Germany). The disc diffusion method was used to conduct sensitivity testing on five different antibiotic kinds using the following dosages: amoxicillin 10 µg (Oxoid, CT0161B), gentamicin 10 µg (Oxoid, CT0024B), ceftazidime 30 µg (Oxoid, CT0412B), ciprofloxacin 5 µg (Oxoid, CT0425B), and tetracycline 30 µg (Oxoid, CT0054B). Bacterial cultures were cultured for 24 hours at 37°C. A caliper with mm-based measurement units was used to calculate the antibiotic's area of inhibition against bacterial growth (Riwu *et al*., 2022).

RESULTS AND DISCUSSION

Bacterial Isolates

Based on morphological culture features, Gram staining, and biochemical testing, the sample examination results revealed that 41 isolated samples (41%) of the 100 cattle rectal swab samples were confirmed to be positive for *E. coli* (Table 1). The emergence of metallic green

Table 1. Isolation of *E. coli* from a sample of a cattles' rectum swab in abattoir

n Samples	Identification test							Positive *E. coli* (%)
	EMBA	Gram stain	IMViC test					
			Indol	Motile	MR	VP	Citrate	
100	66	66	41	41	41	41	41	41 (41%)

% (Percentage of positive).

Table 2. Resistance status of *E. coli* to several antibiotics

Type of antibiotic	*E. coli* sensitivity status (n=71)		
	Resistant	Intermediate	Sensitive
Gentamicin	1 (1.41%)	8 (11.27%)	62 (87.32%)
Ceftazidime	1 (1.41%)	1 (1.41%)	69 (97.18%)
Ciprofloxacin	3 (4.22%)	5 (7.04%)	63 (88.73%)
Tetracycline	10 (14.08%)	0 (0%)	61 (85.91%)
Amoxicillin	12 (16.9%)	0 (0%)	59 (83.1%)

Table 3. Isolated *E. coli* resistance profile by antibiotic group

Group of antibiotics	Resistance profile	Number of isolates (n=41)	Total number of isolates (%)
		Resistant isolates (%)	
0	No one is resistant	24 (58.54%)	24 (58.54%)
1	TE	5 (12.19%)	12 (29.27%)
	AML	7 (17.07%)	
2	TE–AML	2 (4.88%)	2 (4.88%)
≥3	CIP–TE–AML	1 (2.44%)	3 (7.32%)
	CAZ–CIP–TE–AML	1 (2.44%)	
	GM–CIP–TE–AML	1 (2.44%)	

GM = Gentamicin, CAZ = Ceftazidime, CIP = Ciprofloxacin, TE = Tetracycline, AML = Amoxicillin.

Table 4. *E. coli* isolates with a profile MDR

Location	Sample code	Resistance profile	Antibiotic				
			GM	CAZ	CIP	TE	AML
Abattoirs	SR 18	CAZ–CIP–TE–AML	–	✓	✓	✓	✓
	SR 70	CIP–TE–AML	–	–	✓	✓	✓
	SR 99	GM–CIP–TE–AML	✓	–	✓	✓	✓

✓= Resistant, GM = Gentamicin, CAZ = Ceftazidime, CIP = Ciprofloxacin, TE = Tetracycline, AML = Amoxicillin.

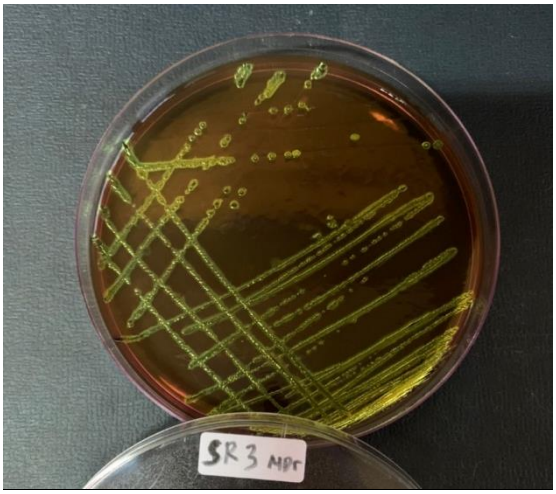

Figure 1. *E. coli* colonies in EMBA.

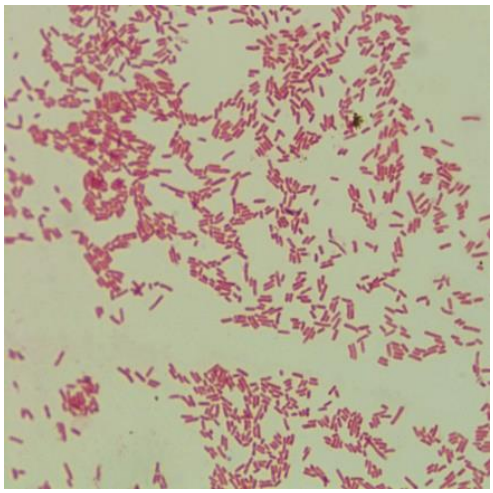

Figure 2. Gram-stained *E. coli* colonies under a microscope.

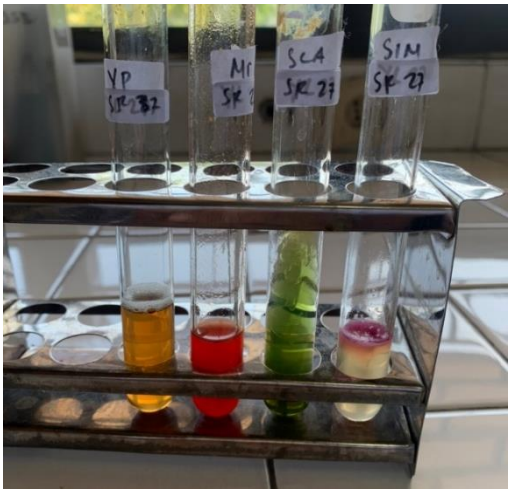

Figure 3. IMViC test results indicate *E. coli* positivity.

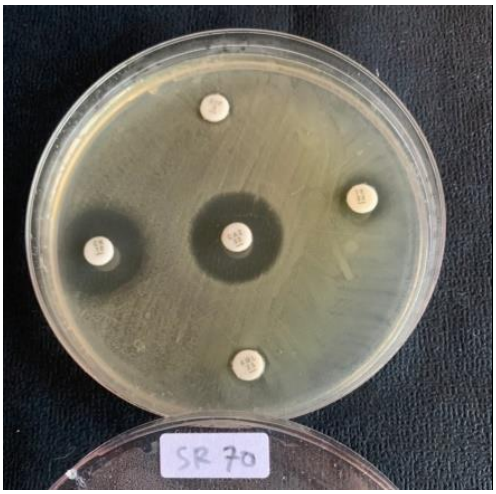

Figure 4. Analyze the susceptibility to antibiotics of *E. coli* isolate cultured on MHA.

bacterial colonies on EMBA medium suggested a positive morphological culture of *E. coli* (Figure 1). The presence of red colonies and short rods in the Gram stain indicates a negative Gram result (Figure 2). An indole ring on the SIM test (Indol positive), an inverted spruce formation on the SIM test (Motil), a red color change on the MR test (positive MR), a yellow color on the VP test

(negative VP), and green in the citrate test (citrate negative) were all signs that the IMViC test had detected *E. coli* (Figure 3).

Antibiotic Resistance of *E. coli*

The highest level of resistance of *E. coli* in this study was to the antibiotic amoxicillin, namely 12 isolates. While the level of resistance of *E. coli* to several other antibiotics, namely 10 isolates of tetracycline antibiotics, 3 isolates of ciprofloxacin antibiotics, 1 isolate of ceftazidime antibiotics, and 1 isolate of gentamicin antibiotics (Table 2).

The profile of antibiotic resistance from the results of the *E. coli* resistance test to antibiotics showed that out of a total of 41 isolates of *E. coli*, 12 isolates (29.27%) were detected as resistant to 1 class of antibiotics tested, while 2 isolates (4.88%) were resistant to 2 classes of antibiotics, and 3 isolates (7.32%) were confirmed to be multidrug resistance (MDR) because they were resistant to 3 to 4 antibiotic classes (Figure 4) with the pattern of antibiotic resistance CIP–TE–AML (Ciprofloxacin, tetracycline, amoxicillin), CAZ–CIP–TE–AML (ceftazidime, ciprofloxacin, tetracycline, amoxicillin), and GM–CIP–TE–AML (Gentamicin, ciprofloxacin, tetracycline, amoxicillin) (Table 3). This study found that 12 isolates of *E. coli* had the highest level of amoxicillin resistance.

Three cattle rectum swab samples from the Surabaya abattoirs contained MDR isolates of *E. coli* (Table 4). This could account for the 3 isolates from 100 samples of cattle rectum swabs that were investigated, representing the city of Surabaya's remaining low MDR *E. coli* infections.

Bacterial Isolates

Following all isolation and identification tests, it was determined that up to 41 out of 100 samples obtained from the Surabaya abattoirs contained *E. coli*. This finding supports studies by Mir *et al*. (2020), which discovered *E. coli* bacteria in samples from cattle rectal swabs. The most common anaerobic bacterium found in the large intestine of humans and animals is *E. coli*, which is also widely dispersed (Gomes *et al*., 2016). Even though *E. coli* bacteria are generally dangerous, they rarely cause illness in healthy people (Torres, 2017). Human infection can result from eating improperly prepared meat tainted with *E. coli* bacteria (Chagnot *et al*., 2017). For harmful germs like *E. coli* to grow in the digestive tract and cause disease, bacteria first must enter the human digestive tract through food and then be digested and absorbed by the body (Martinson and Walk, 2020).

BPW was used as a transport medium during the sample collection process. Previous studies frequently utilize BPW media, a liquid medium, to transport swab samples to the lab. Even though their viability may diminish after several hours of storage, pathogenic bacteria can live longer when kept in BPW media (Bourassa *et al*., 2019).

Following sample collection, the samples are isolated on EMBA media. The color of the *E. coli* colonies was discovered to be metallic green based on the isolation results on EMBA media. Due to the selective and differential nature of EMBA media, which contains lactose carbohydrates, EMBA media exhibits a metallic green color change (Adityawardhana *et al*., 2021). Lactose can be fermented by *E. coli*, which raises the media's acidity levels (Shahzadi., *et al*., 2021). In EMBA media, methylene blue can precipitate at high acidity levels (Sophian, 2022).

Gram staining was used to study *E. coli* bacterium colonies on EMBA medium under a microscope. Gram staining is used to distinguish between Gram-positive and Gram-negative bacteria based on the elements of the cell wall (Silhavy *et al*., 2010). The Gram stain test findings revealed that some bacteria isolates were Gram-negative and had short rod-shaped and red-stained results. Gram-negative bacteria will lose the color of crystal violet after being cleaned with alcohol, making them appear red when stained with safranin (Thairu *et al*., 2014).

A biochemical test called the IMViC test was used to confirm the presence of *E. coli*. The IMViC test is used to determine between bacteria from the Enterobacteriaceae family. An indole test, a methyl red (MR) test, a Voges Proskauer (VP) test, and a citrate test make up the IMViC test. These four become the standard in

determining the biochemical properties of coliform bacteria (Cappuccino and Welsh, 2017).

The indole test will result in a positive result, and why a red ring appears on the SIM media because *E. coli* can create indole by breaking down the amino acid tryptophan using the enzyme trytophanase (Li and Young, 2013). The formation of a red ring, which denotes positive indole, resulted from the indole reaction with the aldehyde in Kovach's reagent (Almeida *et al*., 2020). Since Methyl Red (MR) is a pH indicator and turns red when the pH is acidic, it may be used to assess bacteria's capacity to produce and maintain acid stability from the fermentation of glucose (Chairunisa and Imawan, 2019). This allowed *E. coli* to produce a positive result for the Methyl Red test. The results of the Voges Proskauer (VP) test for *E. coli* will be negative after adding alpha naphthol and KOH. This test is negative because *E. coli* can ferment carbohydrates into acidic products and does not produce neutral products. The presence of neutral products such as acetoin will cause a red color change (Khasanah *et al*., 2021). The *E. coli* Citrate test will produce negative findings because *E. coli* cannot use citrate as a carbon source (Van Hofwegen *et al*., 2016).

Antibiotic Resistance of *E. coli*

The Kirby-Bauer method was used to conduct sensitivity tests on *E. coli* isolates to identify the pattern of antibiotic resistance to gentamicin, ceftazidime, ciprofloxacin, tetracycline, and amoxicillin. In this test, bacteria are grown on MHA media around an antibiotic-impregnated disk. The diameter of the clean zone on the media is then measured. An indirect indicator of an antibiotic's capacity to prevent harmful germs from growing around the disc is its presence or absence (Nassar *et al*., 2019). The sensitive, moderate, and resistant categories from The Clinical & Laboratory Standards Institute 2020 are used to evaluate the qualitative categories produced by this measurement (CLSI, 2020).

Based on the results of the study, as many as 41 isolates of *E. coli*, which had been tested for sensitivity using the agar disk diffusion method, were found to be resistant to amoxicillin 29.27% (12/41), tetracycline 24.39% (10/41), ciprofloxacin 7.32% (3/41), gentamicin 2.44% (1/41), and ceftazidime 2.44% (1/41) as seen from inhibition zone measurements. These findings support a study by Haulisah *et al*. (2021) that found *E. coli* isolates had a high level of resistance to penicillin, amoxicillin, streptomycin, trimethoprim-sulfamethoxazole, and tetracycline among other antibiotics. The use of antibiotics as feed additives in livestock and routine administration of antibiotics in animal feed to avoid disease in livestock are two examples of factors that impact the emergence of resistance (Chattopadhyay, 2014).

Several processes, including enzymatic inactivation of antibiotics, alteration of antibiotic targets, augmentation of efflux pumps, and restriction of drug absorption, can lead to the development of resistance mechanisms in *E. coli* (Reygaert, 2018). Given that bacteria can employ one or more resistance mechanisms, the antibiotic utilized will determine the mechanism of resistance (Putra *et al*., 2023). Irrational exposure to antibiotics causes the process of bacterial resistance to antibiotics and transfer occurs from one type of bacteria to another (Ohene *et al*., 2021). The use of antibiotics in earlier treatments has a direct correlation to the prevalence of *E. coli* resistance in livestock, such as cattle (Suzuki *et al*., 2022).

The MDR of *E. coli* in this investigation was 7.32% (3/41). Bacteria resistant to three or more classes of antibiotics are said to have multidrug resistance (MDR) (Wibisono *et al*., 2021). MDR events can happen for several reasons, including administering antibiotics in the incorrect dosage, an incorrect diagnosis, and the incorrect bacteria (Llor and Bjerrum, 2014). One of two molecular pathways can result in MDR in bacteria. First, each of these bacteria's cells can acquire numerous genes that individually code for drug resistance (Tyasningsih *et al*., 2022). Resistance plasmids typically have this buildup (Bennett, 2008). Secondly, the expression of genes that code for efflux multidrug pumps, which expel several medications, can also increase, leading to

the development of multidrug resistance (Yasufuku *et al*., 2011).

In situations of bacterial resistance, particularly in *E. coli* and *Salmonella* sp., multidrug resistance by bacteria to antibiotics, β-lactam groups, streptomycin, and tetracycline is frequently identified (Wardhana *et al*., 2019; Lauteri *et al*., 2021). The findings of a study by Bourély *et al*. (2019) revealed that *E. coli* isolates were resistant to the antibiotics tetracycline and amoxicillin, with cattle having the highest resistance (65%), despite these two antibiotics being often used in the field of veterinary care. Antibiotics are typically administered to animals for therapeutic purposes (Chattopadhyay, 2014). The most used antibiotic for treating infections brought on by Enterobacteriaceae is the β-lactam class (Ni *et al*., 2016). Tetracycline is an antibiotic frequently administered to cattle to treat digestive tract diseases (Granados-Chinchilla and Rodríguez, 2017).

The pathogenic bacteria *E. coli* is a common one in the digestive tract (Katouli 2010). *E. coli* contamination in beef strongly correlates with inadequate sanitation issues during the meat-handling process (Diyantoro and Wardhana, 2019). Due to widespread antibiotic overuse, particularly in developing countries like Indonesia, bacterial resistance to antibiotics poses a threat to global health (Manyi-Loh *et al*., 2018). *E. coli* MDR is placed on the plasmid, promoting the transmission of this resistance gene to society and posing a severe threat to human health when food is contaminated by antibiotic-resistant bacteria (Rozwadowski and Gawel, 2022).

CONCLUSION

A total of 100 samples of bovine rectum swabs were collected at the Surabaya abattoirs, and 41 of those samples underwent isolation and identification testing and were found to be positive for *E. coli*. Out of the 41 isolates analyzed for the sensitivity test, 3 isolates of MDR *E. coli* were discovered. Antibiotic resistance and the possibility of *E. coli* MDR infection in animal-derived foods, particularly beef that will be served to the general public, require routine monitoring and oversight.

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AUTHORS' CONTRIBUTIONS

MHE: Conceptualization and drafted the manuscript. YRM, MHE, YP, HP, and ARK: Collected and evaluated samples. ARK, KNK, and MHE: Validation, supervision, and formal analysis. YP and MHE: Performed the statistical analysis and the preparation of tables and figures. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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