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Detection of Genes on *Escherichia coli* Producing Extended Spectrum β-lactamase Isolated from the Small Intestine of Ducks in Traditional Markets Surabaya City, Indonesia

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Abstract

This research aimed to focus on the presence of ESBL-producing *E. coli* isolated from the small intestine of ducks in several traditional markets in Surabaya as there is still little information regarding cases of ESBL-producing *E. coli* in ducks in Indonesia. Samples from the small intestine of ducks were kept on Buffer Peptone Water (BPW) media and then streaked on Eosin Methylene Blue Agar (EMBA) media for isolation testing, while Gram staining and IMViC tests were used to continue the identification test. Kirby-bauer diffusion test for determining antibiotic sensitivity and confirmation of ESBL producing *E. coli* using the double disc synergy test (DDST). Molecular detection of TEM and CTX-M genes was carried out using Polymerase Chain Reaction (PCR). Based on morphological culture characteristics, Gram staining, and biochemical testing, the sample analysis results revealed that 32 samples (32%) of the 100 samples that were isolated were confirmed to be positive for *E. coli*. 10 from 32 *E. coli* isolates (31.25%) were confirmed to be multidrug resistance (MDR) because they were resistant to 3 to 4 antibiotic classes. Two out of ten *E. coli* isolates reported to be multidrug resistant were discovered to have positive DDST test findings after the ESBL identification of those isolates. Based on molecular examination, one isolate of *E. coli* was found containing TEM and CTX-M genes. Giving antibiotics to ducks must be considered in the dose or level so as not to cause antibiotic residues in the digestive tract of ducks. More farmer awareness of and public concern for the safety of food of animal origin is required, as is the use of antibiotics in poultry under the supervision of a veterinarian.

KEYWORDS
E. coli, ESBL, Duck, Small intestine, Public health.

INTRODUCTION

The demand for food with an animal origin grows every year due to population growth (Fukase and Martin, 2020). Poultry and beef cattle often make the largest contributions to the nation's meat supply (Effendi *et al*., 2021). To accommodate the need for meat and eggs, duck populations have expanded recently (Asiamah *et al*., 2020). In comparison to other animals, ducks typically have higher levels of disease resistance, making upkeep of them simple and risk-free (Babington and Campbell, 2022). Because they can produce as well, ducks are being developed for high and quick production in order to meet consumer demand (Fouad *et al*., 2018).

Escherichia coli bacteria have been linked to diseases brought on by bacterial infections in ducks (Li *et al*., 2022). This bacterium is always present in the digestive tract of animals, because naturally *E. coli* is one of the inhabitants of the digestive tract (Martinson and Walk, 2020). The surrounding environment and soil can make it easier for *E. coli* to exist (Jang *et al*., 2017). *E. coli* can emerge under specific circumstances, such as inadequate cage ventilation, overcrowding, and starvation (Anshariet*a et al*., 2021). All varieties of fowl, including turkeys and ducks, are susceptible to *E. coli* infection (Varga *et al*., 2019). The feces of ducks contained the highest percentage of *E. coli*, at 87.93%, followed by the intestines, at 81.25% (Assawatheptawee *et al*., 2022).

In poultry farming, feed expenses can account for 70% of the total costs (Mallick *et al*., 2020). The maintenance costs are higher since duck diets convert at a rapid rate (Sumiati *et al*., 2020). The overall use of antimicrobial agents has increased as a result of the rising demands for animal production (Tiseo *et al*., 2020; Khairullah *et al*., 2022). The ability of microorganisms (Bacteria, viruses, and parasites) to thwart the effects of antimicrobials such as antibiotics, antivirals, and antimalarials is known as antimicrobial resistance (Rahmahani *et al*., 2020). Antimicrobial resistance can develop because of the improper usage of antibiotics, rendering treatment ineffective (Waruwu *et al*., 2023). Moreover, antimicrobial resistance can lead to decreased productivity and financial loss (Widodo *et al*., 2022).

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Antibiotic resistance is mostly brought on by beta-lactamase enzymes, particularly in *E. coli* bacteria (Yanestria *et al*., 2022). In addition to *E. coli*, *Salmonella* and *Klebsiella pneumoniae* are two other Gram-negative bacteria that can manufacture extended-spectrum beta-lactamases (ESBL) enzymes (Riwu *et al*., 2020; Wibisono *et al*., 2020). There are around 33.3% of *Klebsiella pneumoniae* and 23% of *E. coli* ESBL-producing bacteria in Indonesia (Krisniawati and Widhi, 2021). Food-producing animals that are reservoirs for the ESBL-producing *E. coli* bacteria can be used to isolate ESBL-producing *E. coli* (Riwu *et al*., 2022). The environment is exposed to resistant microorganisms through animal excrement (Penakalapati *et al*., 2017; Putra *et al*., 2023). Animal excrement contains bacteria that can spread to nearby farms, slaughterhouses, contaminated water, and even via the air while transporting animals (Serwecińska, 2020). Gut flora can also act as a reservoir by transferring resistance genes to other bacteria (Pan *et al*., 2022).

To improve the effectiveness of feed use, feed additives like enzymes and antibiotics are added (Ayalew *et al*., 2022). This was commonly done before there were regulations governing the use of antibiotics in animals (Martin *et al*., 2015). Nevertheless, in 2017, the Indonesian Minister of Agriculture adopted a regulation that forbade the use of veterinary medications as feed additive, including both finished goods and raw ingredients in the form of antibiotics (Coyne *et al*., 2019). According to a study by Nadzifah *et al*. (2019), antibiotics are still utilized as feed additives in chicken feed, despite a ban on their use in feed being issued in 2017.

Resistance to cephalosporins third generation generally occurs in Gram-negative bacteria by producing Extended Spectrum β-lactamase (ESBL) enzymes (Rawat and Nair, 2010). In addition to people, ESBL-producing bacteria are also present in animals and the environment (Salgado-Caxito *et al*., 2021). TEM and CTX-M are the two primary ESBL coding genes (Bastidas-Caldes *et al*., 2022). The two genes together are in charge of creating ESBLs, which break down β-lactam antibiotics (Li *et al*., 2015). The majority of ESBL-producing bacteria display a phenotypic that is antibiotic-resistant (Eltai *et al*., 2018). Such an occurrence presents a difficulty for livestock practice infection management.

The use of antibiotics as a form of medication for livestock is still permitted, but it must be done so under strict control due to the numerous risks associated with antibiotic residues in food products from animals (Widodo *et al*., 2023). The most often found antibiotic residues in food products of poultry origin are those from tetracycline, oxytetracycline, sulfamethazine, ciprofloxacin, and amoxicillin (Treiber and Beranek-Knauer, 2021). Excessive amounts of antibiotic residues can affect the microflora in the intestine, leading to the development of antibiotic-resistant bacteria as well as hypersensitivity reactions (Arsène *et al*., 2022). Antimicrobial residues found in animal-derived foods and waste will contaminate the soil, water, and environment and help new strains of resistance arise and spread (Wibisono *et al*., 2021). Food of animal origin intended for human consumption faces a major danger to its quality and safety from antimicrobial resistance (Ritter *et al*., 2019).

Based on this background, it is necessary to conduct research that focuses on the presence of ESBL-producing genes in *E. coli* isolated from the small intestine of ducks in several traditional markets in Surabaya, as there is still little information regarding cases of ESBL-producing *E. coli* in ducks in Indonesia. In this study, the gene of the bacteria *E. coli* that produces the ESBL enzyme was tested for resistance and molecular testing to determine the level of spread of this bacterium in the traditional market of Surabaya, Indonesia.

MATERIALS AND METHODS

Ethical statement

Small intestine samples of ducks were used in this study taken from several traditional markets in Surabaya, Indonesia, hence ethical approval was not necessary. Samples were taken according to standard collection procedures.

Study area and sample collection

Throughout April and May 2022, this study was carried out. Bacterial isolation and molecular tests were carried out at the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Airlangga University, while fecal samples were collected from the small intestine of ducks at several traditional markets in Surabaya, namely Keputran, Kembang, Pucang, Pahing, Wonokromo, Pagesangan, Darmo, Manukan, Pabean, and Pegirian markets. A total of 100 samples of duck small intestine feces were collected (10 samples per market).

Bacterial isolation of E. coli

The feces that are still in the small intestine are weighed and labeled at up to one gram each item. Feces are combined in a 1:10 ratio with a 0.1% Buffered Peptone Water (BPW) solution, and then homogenized in a vortex.

E. coli colonies are intended to be isolated from other Coliform bacteria through planting on Eosin Methylene Blue Agar (EMBA) media. The media was autoclaved for 15 minutes at 121°C to sterilize it. The medium was then placed into a petri dish and left to stand at room temperature until it solidifies. Ose was used to take samples, after which the necessary sample was streaked on the EMBA medium. The incubation period was 18 to 24 hours at 37°C. Colonies that developed after incubation and had a metallic green appearance with a black dot in the center were regarded as *E. coli* colonies. The reaction between the bacteria and methylene blue causes the bacteria to form metallic colonies (Tyasningsih *et al*., 2022).

Gram staining was then done by placing a thin layer of metallic green colonies made from the culture medium on top of a sterilized glass object. After that, the thing was fixed by putting the bottom of the object's glass to the bunsen flame. The surface of the glass object was treated with Crystal Violet solution, left on for 3 to 5 minutes, washed under running water, then treated with Lugol's solution and left on for 1 to 2 minutes. The object glass was treated with Lugol's solution and 96% alcohol until the dye disappeared, then washed under running water. The final step involved letting the safranin solution sit on the object glass for 1-2 minutes before washing it off with running water once more. To confirm that the preparations contained Gram-negative, rod-shaped bacteria, they were examined under a microscope after drying at a magnification of 1000 times. *E. coli* bacteria cells appeared as short rods under a microscope and was red when stained with Gram stain (Effendi *et al*., 2022).

The IMViC test, which consisted of the Sulfide Indole Motility (SIM) Test, Methyl Red (MR), Voges Proskauer (VP) Test, and Citrate Test, was used to confirm bacterial colonies thought to be *E. coli*. SIM test for *E. coli* bacteria inoculated on SIM media and then incubated for 24 hours at 37°C will show a positive result if a pink indole ring forms on the surface of the media after dropping Kovac's reagent and the SIM media does not include any black colonies (Triadi *et al*., 2022). *E. coli* bacteria were placed on MR-VP media for the MR-VP test, which was subsequently incubated

for 48 hours at 37°C. Separating the MR-VP medium into two test tubes. Two to five drops of the methyl red indicator are added to the first tube before the MR test. If the color changes from blue to red, it is a sign that *E. coli* is present. The second tube is used for the VP test which was given 8-10 drops of alpha naphthol solution and 40% KOH, absence of change in color (Brownish yellow) indicated negative VP test is negative (Kartikasari *et al*., 2019). In the Citrate test, bacterial colonies were transplanted from EMBA media onto Simmons Citrate Agar (SCA) media and cultured at 37°C for 24 hours. If the color does not change, the outcome was considered to be unfavorable (Van Hofwegen *et al*., 2016).

Antibiotic resistance of E. coli

Kirby-Bauer diffusion test-based assessment of antibiotic sensitivity. In order to plant on agar media, a suspension of *E. coli* bacteria with a turbidity of 0.5 was used, which was then equally spread with a sterile brush over the Mueller Hinton Agar (MHA) media surface. Each antibiotic was positioned on the MHA at 25-30 mm and incubated at 35°C for 24 hours after being inserted into the agar media that had been planted with bacteria. Ampicillin (10 µg), aztreonam (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), and chloramphenicol (30 µg) antibiotics were cultivated on MHA media and were evenly distributed in each petri dish. In accordance with CLSI 2020 guidelines, the inhibition zone that emerged around the antibiotic disc was measured as the last step of the antibiotic sensitivity test (Khan *et al*., 2019).

E. coli isolates that had at least three antibiotic resistances were determined, and then the Double Disk Synergy Test (DDST) was used to determine whether the isolates have ESBL. The usual Mc Farland turbidity of 0.5 was used to create a suspension of the MDR *E. coli* isolates, which was then equally applied to the MHA media using a sterile swab. In the center of the MHA media that had been bacterially seeded, amoxicillin-clavulanate discs (20/10 µg) were positioned, and then cefotaxime and ceftadiazime discs were positioned 15 mm and 20 mm away. The ESBL positive test result suggested that a synergistic zone leading to the antibiotic disc composed of amoxicillin and clavulanate had formed (Wu *et al*., 2021).

Molecular detection of E. coli

The first phase of DNA extraction from bacterial culture was based on Kristianingtyas *et al*. (2020), with a few minor alterations to cycle conditions, before testing specific primers for the CTX-M and TEM genes (Table 1) as reported in Ali *et al*. (2016). Thermo Fisher Scientific Inc supplied the deoxyribonucleotide triphosphates, buffers, and Taq DNA polymerase enzyme for the PCR mixture. The thermocycling procedure was run in 30 cycles, with the first denaturation lasting 2 minutes at 94°C, then annealing for 30 seconds at 52°C, prolonging for 45 seconds at 72°C, and the last extension lasting 5 minutes at 72°C. The PCR products were seen using mini gel electrophoresis, and the outcomes were documented using the UV Reader / Gel Documentation System.

RESULTS

Bacterial isolation of E. coli

Based on morphological culture characteristics, Gram staining, and biochemical testing, the sample analysis results revealed that 32 samples (32%) of the 100 cat rectal swab samples that were isolated were confirmed to be positive for *E. coli* (Table 2). Metallic green bacterial colonies on EMBA media were a sign of a successful morphological culture of *E. coli* (Figure 1). Red colonies and short rods suggest the presence of a Gram stain with a negative Gram result (Figure 1). An indole ring on the SIM test (Indol positive), an inverted spruce formation on the SIM test (Motil) (Figure 2), a red color change on the MR test (Positive MR) (Figure 2), a yellow color on the VP test (Negative VP) (Figure 2), and green in the citrate test (Citrate negative) (Figure 2) were all signs that the IMViC test had detected *E. coli*.

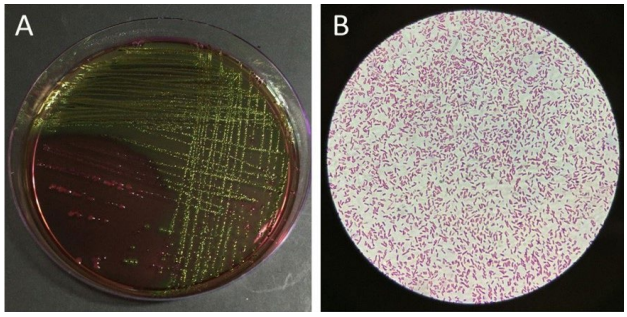

Fig. 1. (A) *E. coli* colonies in EMBA; (B) Gram-stained *E. coli* colonies under a microscope.

Table 1. Details of primers used in this study.

Primers	Sequences (5' to 3')	Target gene	Amplicons size	References
TEM *forward*	ATA-AAA-TTC-TTG-AAG-ACG-AAA	bla_{TEM}	1086 bp	Ansharieta *et al*. (2021)
TEM *reverse*	GAC-AGT-TAC-CAA-TGC-TTA-ATC	bla_{TEM}		
CTX-M *forward*	CGC-TTT-GCG-ATG-TGC-AG	bla_{CTX-M}	550 bp	Zeng *et al*. (2021)
CTX-M *reverse*	ACC-GCG-ATA-TCG-TTG-GT	bla_{CTX-M}		

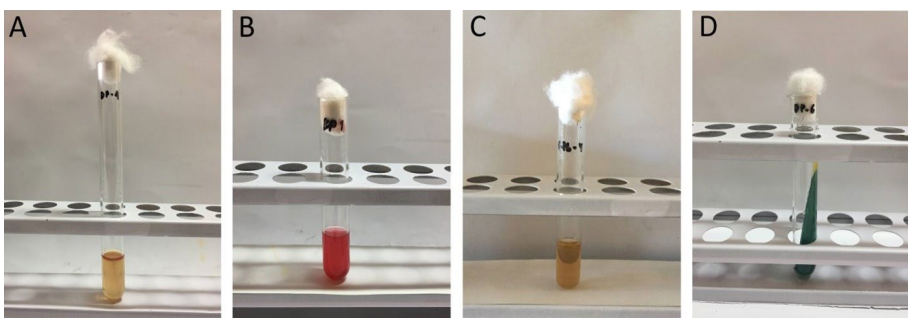

Fig. 2. (A) SIM test results showing positive *E. coli*; (B) MR test results showing positive *E. coli*; (C) VP test results showing positive *E. coli*; (D) Citrate test results showing positive *E. coli*.

Antibiotic resistance of E. coli

In this investigation, 25 isolates of *E. coli* had the highest level of resistance to the antibiotic ampicillin. While there were 14 isolates of *E. coli* resistant to ciprofloxacin, 10 isolates each of gentamicin and chloramphenicol, and 10 isolates of *E. coli* resistant to chloramphenicol, no isolates of *E. coli* were discovered to be resistant to the antibiotic aztreonam (Table 3).

The profile of antibiotic resistance from the results of the *E. coli* resistance test to antibiotics showed that out of a total of 32 *E. coli* isolates, 8 isolates (25%) were detected as resistant to 1 class of antibiotics tested, while 7 isolates (21.87%) were resistant to 2 classes of antibiotics, and 10 isolates (31.25%) were confirmed to be multidrug resistance (MDR) because they were resistant to 3 to 4 antibiotic classes (Figure 3) with an AMP – GM – C antibiotic resistance pattern (Ampicillin, gentamicin, chloramphenicol) of 1 isolate (3.12%), AMP – GM – CIP (Ampicillin, gentamicin, ciprofloxacin) 2 isolates (6.25%), AMP – CIP – C (Ampicillin, ciprofloxacin, chloramphenicol) 3 isolates (9.37%), and AMP – GM – CIP – C (Ampicillin, gentamicin, ciprofloxacin, chloramphenicol) as many as 4 isolates (12.5%) (Table 3).

In 10 samples of duck feces from various Surabaya City traditional markets, MDR isolates of *E. coli* were discovered in the small intestine (Table 4). This could account for the 10 isolates from 100 faecal samples in the small intestine of the ducks that were analyzed, which represent the city of Surabaya's remaining low MDR *E. coli* infections.

Two out of ten *E. coli* isolates reported to be multidrug resistant were discovered to have positive DDST test findings after the ESBL identification of those isolates (Table 4). This demonstrates that 2 ESBL isolates were discovered from 100 faecal samples in the small intestine of the ducks that were analyzed (Figure 3), indicating that there are still few instances of ESBL-producing *E. coli* infection in numerous traditional markets in Surabaya City.

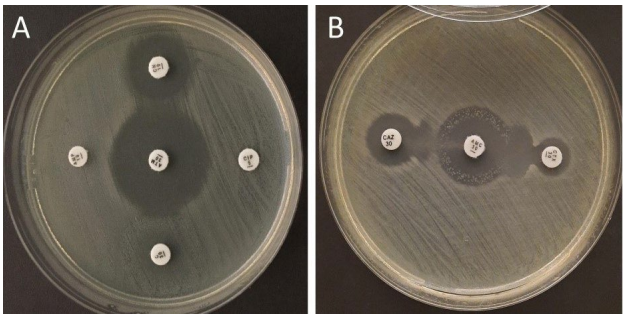

Fig. 3. (A) Sensitivity test results of *E. coli* isolates were MDR on MHA media; (B) DDST test results of ESBL-producing *E. coli* isolates.

Molecular detection of E. coli

Based on electrophoresis results, 2 positive *E. coli* isolates contained the TEM gene, while one positive isolate contained the CTX-M gene (Table 5). In addition, there was one positive *E. coli* isolates containing two genes at the same time as TEM and CTX-M, namely isolate PC 2. The results of the detection of the TEM and CTX-M genes were marked by the appearance of a sin-

Table 2. Isolation of *E. coli* from a sample of faeces found in the small intestine of ducks.

Location	Markets	Sample size	Identification test							Positive *E. coli* (%)
			EMBA	Gram stain	IMViC test					
					Indol	Motile	MR	VP	Citrate	
Central Surabaya	Keputran	10	10	10	4	4	4	4	4	4 (40%)
	Kembang	10	8	8	4	4	4	4	4	4 (40%)
East Surabaya	Pucang	10	10	10	6	6	6	6	6	6 (60%)
	Pahing	10	10	10	3	3	3	3	3	3 (30%)
South Surabaya	Wonokromo	10	9	9	4	4	4	4	4	4 (40%)
	Pagesangan	10	7	7	4	4	4	4	4	4 (40%)
West Surabaya	Darmo	10	5	5	3	3	3	3	3	3 (30%)
	Manukan	10	10	10	2	2	2	2	2	2 (20%)
North Surabaya	Pabean	10	4	4	2	2	2	2	2	2 (20%)
	Pegirian	10	6	6	0	0	0	0	0	0 (0%)
Total		100	79	79	32	32	32	32	32	32 (32%)

%: Percentage of positive

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

Group of antibiotics	Resistance profile	Number of isolates (n=32) Resistant isolates (%)	Total number of isolates (%)
0	No one is resistant	7 (21.87%)	7 (21.87%)
1	AMP	8 (25%)	8 (25%)
2	AMP – GM	3 (9.37%)	7 (21.87%)
	AMP – CIP	3 (9.37%)	
	AMP – C	1 (3.12%)	
≥3	AMP – GM – CIP	2 (6.25%)	10 (31.25%)
	AMP – GM – C	1 (3.12%)	
	AMP – CIP – C	3 (9.37%)	
	AMP – GM – CIP – C	4 (12.5%)	

ATM: Aztreonam; AMP: Ampicillin; GM: Gentamicin; CIP: Ciprofloxacin; C: Chloramphenicol.

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

Location	Markets	Sample code	Resistance profile	Antibiotic					DDST test
				ATM	AMP	GM	CIP	C	
Central Surabaya	Keputran	KP 1	AMP – GM – C	–	✓	✓	–	✓	Negative
East Surabaya	Pucang	PC 2	AMP – GM – CIP – C	–	✓	✓	✓	✓	Positive
	Pahing	PH 6	AMP – GM – CIP	–	✓	✓	✓	–	Positive
South Surabaya	Wonokromo	WO 3	AMP – GM – CIP – C	–	✓	✓	✓	✓	Negative
	Pagesangan	PS 3	AMP – CIP – C	–	✓	–	✓	✓	Negative
		PS 6	AMP – GM – CIP	–	✓	✓	✓	–	Negative
		PS 9	AMP – GM – CIP – C	–	✓	✓	✓	✓	Negative
West Surabaya	Manukan	DP 4	AMP – CIP – C	–	✓	–	✓	✓	Negative
North Surabaya	Pabean	PB 4	AMP – GM – CIP – C	–	✓	✓	✓	✓	Negative
		PB 8	AMP – CIP – C	–	✓	–	✓	✓	Negative

✓: Resistant; ATM: Aztreonam; AMP: Ampicillin; GM: Gentamicin; CIP: Ciprofloxacin; C: Chloramphenicol

gle band on the electrophoresis results (Figure 4).

Table 5. Molecular detection of TEM and CTX-M isolate *E. coli* genes

Sample code	TEM genes	CTX-M genes
PC 2	Positive	Positive
PH 6	Positive	Negative

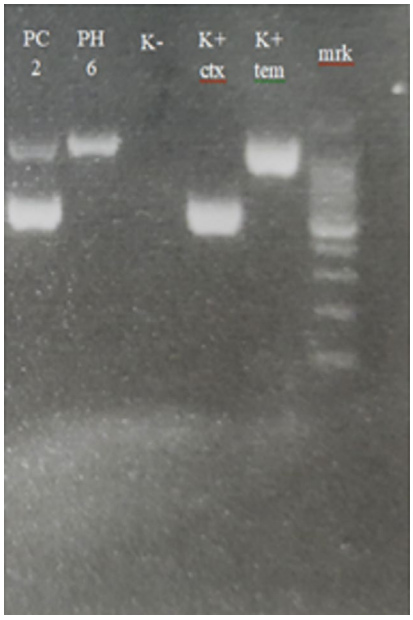

Fig. 4. Electrophoresis results of TEM and CTX-M gene detection.

DISCUSSION

The presence of nutrients, pH, the right temperature, and the presence of water all contribute to the possibility of microbial development (Shmeis, 2018). As a result, *E. coli* may thrive on lipids and proteins, which provide food for microorganisms (Christofi *et al*., 2019). On EMBA media, a primary isolation procedure was performed. If *E. coli* are present in the lactose-containing culture medium, the acid generated by lactose fermentation will result in a metallic-looking green colony hue (Gita *et al*., 2021). The bacteria are depicted by Gram stain as red and rod-shaped. *E. coli* bacteria turn red when exposed to alcohol because the crystal violet-iodine combination dissolves in Gram negative bacteria. The color obtained by safranin is the color that will be absorbed by the Gram-negative bacterial cells (Budin *et al*., 2012).

The sulfide test on the SIM media revealed no creation of the color black. As a result, hydrogen sulfide was not produced by *E. coli* bacterium (Li *et al*., 2019). The indole test results were positive with the formation of a red ring on the SIM media. This is because bacteria degrade the amino acid tryptophan and produce indole (Liu *et al*., 2022). The motility test yielded a favorable result, and the area around the puncture site began to develop. The citrate test will remain green on SCA media because the bacteria don't utilise citrate as a source of carbon (Jiang *et al*., 2021). The MR test appears red, and the VP test appears yellow on MR-VP media. The bacteria oxidized glucose in the MR test to produce acid, which resulted in the red hue (Akinterinwa and Cirino, 2011). However, no color change occurred in the VP test because acetonin was not found in the bacterial isolates (Vivijs *et al*., 2014).

The Kirby-Bauer method, which is the CLSI-recommended standard medium for antibiotic sensitivity tests, was employed in the antibiotic sensitivity test. The McFarland standard of 0.5 was used to regulate the turbidity of the bacterial suspension. This standard, which has been established for antibiotic susceptibility testing, is the most prevalent standard used in clinical microbiology laboratories (Gayathiri *et al*., 2018). The five kinds of antibiotics employed in this test are those that are frequently used to treat bacteria belonging to the Enterobacteriaceae phylum. Up to 10 of the 32 isolates were labeled as multidrug resistant because they exhibited resistance to at least three different antibiotic classes. This is consistent with the findings by Yassin *et al*. (2017), who discovered that 44 *E. coli* isolates from ducks were deemed 100% resistant to at least three different antibiotic classes.

The isolates of *E. coli* exhibited the highest levels of ampicillin resistance. Except for KP 1 isolate, all ten MDR *E. coli* isolates in this study displayed nearly identical patterns of antibiotic resistance, specifically resistance to antibiotics from the Penicillin (Ampicillin) and Fluoroquinolone (Ciprofloxacin) classes. No resistance to aztreonam was detected, or it can be said that aztreonam has the highest sensitivity of any antibiotic in this test. This resistance can be related to the five antibiotics success in treating the ducks that are circulating in Surabaya.

The results of Jamin *et al*. (2015) investigation indicated that ampicillin resistance had reached 100%. This is because ampicillin antibiotics are first-line, or antibiotics that are used to treat an infection for the first time (Hirai *et al*., 2022). Moreover, the high level of resistance to ampicillin antibiotics is due to their inappropriate use (Manyi-Loh *et al*., 2018). Ciprofloxacin is another antibiotic that is frequently utilized in chicken husbandry, according to Khan *et al*. (2015). Antibiotics containing ciprofloxacin are used to treat *E. coli*-related infections of the urinary tract, digestive system, and respiratory system (Reis *et al*., 2016). This study did not discover any *E. coli* isolates that were resistant to the antibiotic aztreonam. This could be attributed to the usage of monobactam class antibiotics, specifically aztreonam, which is today infrequently used in both human and veterinary medicine (Meletis, 2016).

Resistance can be acquired if a genetic mutation occurs during or after antibiotic therapy (Baym *et al*., 2016). Bacteria that were once susceptible to antibiotics develop resistance as a result

of mutations, making it impossible to treat them with therapeutic doses of antibiotics (Khairullah *et al*., 2023). In an antibiotic sensitivity test, a bacterium's resistance can be determined by the size of its inhibitory zone (Syal *et al*., 2017). The length of time the bacteria are impregnated in the agar medium, and the amount of antibiotics present are two factors that influence the diameter of the inhibition zone (Patel *et al*., 2021). For statistical and epidemiological purposes, the findings of sensitivity tests are classified into three categories: sensitive, intermediate, and resistant (Ramandinianto *et al*., 2020).

The synthesis of beta-lactamase enzymes is one of the mechanisms underlying the emergence of resistance to beta-lactam antibiotics, particularly in Gram-negative bacteria (Aurilio *et al*., 2022). This enzyme can degrade the beta-lactam ring, rendering the antibiotic inactive (Tooke *et al*., 2019). In this investigation, ESBL-producing *E. coli* isolates were obtained using the DDST test. Beta-lactam antibiotics are those utilized in this test. If a synergy results in antibiotic beta-lactam inhibitor disks, an isolate is considered to be an ESBL producer (Djuikoue *et al*., 2022). Three antibiotics were employed in the DDST test: cefotaxime (to identify ESBL type CTX-M), ceftazidime (to identify ESBL types TEM and SHV), and an antibiotic mix of amoxicillin and clavulanate.

In this investigation, *E. coli* isolates from the Pucang and Pahing markets contained 20% (2/10) of ESBL-producing *E. coli* isolates. ESBL production in phenotypic examination may be hidden by other types of beta-lactamases, which might result in falsely negative test findings. Negative results may be caused by the fact that *E. coli* isolates do not produce ESBL enzymes (Rawat and Nair, 2010). Genotyping is a different test that can be performed. The PCR technique was used for this examination to determine the isolate's ESBL type. Using DNA amplification, this method can find ESBL coding genes. Nevertheless, the PCR approach for genotype detection necessitates the purchase of specialized equipment, which prevents its usage due to inadequate infrastructure (Cheng *et al*., 2022).

This research led to the identification of the duck small intestine-derived TEM gene and CTX-M gene. According to a study on the results of research there, the epidemiological distribution of the CTX-M gene is comparable between animals and people in China (Zeng *et al*., 2021). It is common to find ESBL genes on plasmids that are easily transmissible within or between different bacterial species, and substantial findings on the transmission of genes from humans to animals or vice versa have been made over time (Rawat and Nair, 2010). There have even been claims of genetic similarities and the identification of plasmids in both humans and animals (Li *et al*., 2015). Numerous studies have shown that plasmids spread horizontally between species, causing humans who come into touch with animals to have the same strains or strains that share the same plasmids (Benz *et al*., 2021).

This analysis also identified a combination of the TEM and CTX-M genes in sample PC 2. The same study by Musa *et al*. (2019) found that 8% of individuals carried the CTX-M gene and that 77.4% carried both the TEM and CTX-M genes. This is because the huge IncFII plasmid that carries the CTX-M gene and a gene for antibiotic resistance to other classes is the same plasmid that encodes the CTX-M gene. Some of the discovered CTX-M genes are connected to IncFII or IncI1 plasmids (Zeng *et al*., 2021). The most prevalent IncFII plasmid type is F2:A-:B-, which has also been discovered to be linked to the CTXM bla gene in Enterobacteriaceae isolates from other nations (Mahérault *et al*., 2019). Due to the high transmissibility of other isolates carrying the CTX-M gene, resistance can spread quickly and effectively (Cantón *et al*., 2012). CTX-M-expressing bacteria are primarily multidrug resistant bacteria. Considering that plasmids, which are portable genetic elements, include TEM-forming genes, they can proliferate rapidly. The TEM, OXA, and aac-(6)Ibr genes on the IncFII plasmid, which carries many replicons, are also linked to the CTX-M gene, according to Li *et al*. (2015). Many clinical isolates include the CTX-M gene, which has a potent promoter in its insertion site (Yoon *et al*., 2020).

The intake of animal products that contain antibiotic residues has been linked in numerous studies to the emergence of resistance (Bacanlı and Başaran 2019; Treiber and Beranek-Knauer, 2021; Ghimpeţeanu *et al*., 2022). Hormones and antibiotics are prohibited as feed additives according to the Indonesian minister of agriculture's policy on the classification of veterinary medications (Siahaan *et al*., 2022). Antibiotic residue from improper antibiotic use can build up in animal meat (Qamar *et al*., 2023). Animal food residues can build up over time and make the body's microbes resistant to certain antibiotics if consumed regularly (Okocha *et al*., 2018). Maximum limit of antibiotic residues in food for the antibiotics' ampicillin (0.01 µg/g for meat and 0.01 µg/g for eggs), gentamicin (0.1 µg/g in meat and 0.1 µg/g in eggs), and chloramphenicol (0.01 µg/g for meat and 0.01 µg/g for eggs) (Sireli *et al*., 2006). Resistance to the microflora in the intestine might develop over time as a result of exposure to antibiotic residues in food (Kim *et al*., 2017).

Despite the samples coming from the same market, this investigation found that the isolates had varying levels of resistance. This may be because each animal's body reacts differently to the effects of antibiotics based on its capacity to acquire resistant genes through gene transfer or mutation, whereas no evidence of antibiotic resistance was found because breeders were already aware of how to use antibiotics as a treatment and stopped using them as feed additives (Lee *et al*., 2022).

Given that the goal is bacteria on the digestive tract's surface and that low doses or under treatment antibiotics levels are anticipated to not be dispersed deeply into the organs and leave no trace, it is possible that breeders are still adding antibiotics to feed at low doses or under treatment doses (Pan and Yu, 2014). Farmers are also starting to pay attention to the antibiotic withdrawal period for livestock, or the interval between the last administration of antibiotics and the consumption of livestock products, in terms of removing residues (Mutua *et al*., 2020). This may have an impact on the amounts of antibiotic residues found in animal-derived foods.

CONCLUSION

In the small intestine of ducks in multiple traditional markets in Surabaya, 100 faecal samples include two isolates of *E. coli* bacteria that produce ESBL and one isolates contain TEM and CTX-M genes. More farmer awareness of and public concern for the safety of food of animal origin is required, as is the use of antibiotics in poultry under the supervision of a veterinarian.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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