Detection of the CTX-M Gene Associated with Extended-Spectrum β-Lactamase (ESBL) in Broiler Chickens in Surabaya Traditional Markets

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Abstract

A common indicator used to examine the frequency and distribution of antibiotic resistance against other enteric bacteria in humans and animals is the commensal enteric bacterium, *Escherichia coli*. The transmission of plasmids harboring ESBL enzymes, primarily generated by *E. coli*, is the cause of this resistance. The purpose of this study was to identify the CTX-M gene in ESBL-producing *E. coli* from broiler chicken cloacal swabs in traditional Surabaya markets. The samples used were 96 cloacal swabs from broiler chickens in the traditional markets of Dukuh Kupang, Keputran, Pacar Keling, and Pucang. The antibiotic disks used in this study belonged to five different antibiotic classes; they are aztreonam (monobactam), chloramphenicol (phenicol), kanamycin (aminoglycoside), ciprofloxacin (fluoroquinolone), and tetracycline (tetracycline). Presumptive ESBL strains were then molecularly screened for the presence of CTX-M gene. Results revealed that out of the 96 chicken cloacal swab samples collected, 58 (60.42%) were positive for *E. coli* based on morphological culture, Gram staining, and biochemical tests. Additionally, 15 out of the 58 *E. coli* isolates recovered from broiler chicken cloacal swabs were multidrug-resistant (MDR) while 7 of *E. coli* isolates harbored CTX-M gene. Conclusively, this study has shown that broiler chickens sold in traditional Surabaya markets harbor MDR *E. coli* which possess CTX-M gene. Conditions in traditional markets with low levels of cleanliness and chickens placed close together can spread resistance genes with serious public health consequences. Therefore, it is imperative to observe good hygienic practices in Surabaya traditional markets in order to curtail the spread of MDR bacterial pathogens in the food chain.

Keywords: chickens, CTX gene, *Escherichia coli*, Extended-Spectrum β-Lactamase, public health

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INTRODUCTION

Antibiotic resistance is one of the problems faced by the poultry industry which causes increased morbidity and mortality of birds, decreased effectiveness of treatment, and increased healthcare costs (Zou *et al.*, 2021; Mustika *et al.*, 2024). Pathogenic bacteria that can cause foodborne disease are *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter* spp., and *Staphylococcus aureus* (Abebe *et al.*, 2020; Khairullah *et al.*, 2022). Unwise use of antibiotics can cause antibiotic resistance, such as drug-resistant *Escherichia coli* (Kallau *et al.*, 2018). Several incidents of antibiotic-resistant *E. coli* in broiler chickens have been reported in Indonesia, including in Sleman, Yogyakarta (Untari *et al.*, 2021). In Bali, *E. coli* exhibited resistance to streptomycin (62.5%) and doxycycline (50%) (Dwipayana *et al*., 2022) while in Bogor, *E. coli* was resistant to cefpodoxime (100%), cefoxitin (58.82%), ceftizoxime (100%), aztreonam (94.12%), oxacillin (100%), nitrofurantoin (58.82%), and sulfamethoxazole–trimethoprim (82.35%) (Putri *et al*., 2020).

A common indicator used to examine the frequency and distribution of antibiotic resistance against other enteric bacteria in humans and animals is the commensal enteric bacterium *E. coli* (Faridah *et al*., 2023; Rugumisa *et al*., 2016). In addition, *E. coli* is a potential source of Antimicrobial Resistance (AMR) genes which can be transmitted to humans in various ways (Kheiri and Akhtari, 2016; Overdevest *et al*., 2011). *E. coli* is carried through feces or livestock waste, thereby polluting the environment (Berthe *et al*., 2013; Kartikasari *et al*., 2019). Two types of pathogenic *E. coli* are recognized: diarrheagenic *Escherichia coli* (DEC), which causes enteric infections and extraintestinal pathogenic *Escherichia coli* (ExPEC), which causes infections outside the digestive tract (Gomes *et al*., 2016).

Multidrug-resistant (MDR) bacteria, which are resistant to three or more different classes of antibiotics, arise as a result of *E. coli* bacteria's resistance to multiple classes of antibiotics (Wibisono *et al*., 2020). Extended-spectrum β-lactamase (ESBL)-producing bacteria frequently exhibit multidrug resistance traits (Effendi *et al*., 2021). ESBL synthesis is a defense mechanism used by Gram-negative bacteria belonging to the Enterobacteriaceae family, particularly *E. coli* and *Klebsiella pneumoniae* (Biutifasari, 2018). The transmission of plasmids harboring ESBL enzymes, primarily generated by *E. coli*, is the cause of this resistance (Athanasakopoulou *et al*., 2021). This enzyme is a group of β-lactamase enzymes that are capable of hydrolyzing penicillin antibiotics, third-generation cephalosporins, and monobactam (aztreonam), thereby causing resistance to these antibiotics in ESBL-producing bacteria (Yanestria *et al*., 2022).

There are three main genes encoding ESBL, namely temoneira (TEM), variable sulfhydryl (SHV), and cefotaximase (CTX-M) (Castanheira *et al*., 2021). CTX-M can hydrolyze the third-generation cephalosporin antibiotic, cefotaxime (Maryam and Khan, 2017). In Bogor City Chicken Slaughter Center, 12 (6%) out of 200 samples of broiler chicken excrements were reported to be positive for ESBL-producing *E. coli* that harbored CTX-M gene (Lukman *et al*., 2016). Additionally, the CTX-M gene was discovered in 45 (28.13%) out of the 160 cloacal swab samples of broiler chicks in the city of Blitar that contained ESBL-producing *E. coli* (Wibisono *et al*., 2020). In India, 42% of laying hens have *E. coli* that produces ESBLs (Brower *et al*., 2017).

Molecular detection is a form of genotypic testing to determine the genes carried by ESBL in *E. coli* using PCR (Saka *et al*., 2020). Although there is a sample of studies that have reported the contamination of chicken by MDR ESBL-producing bacterial pathogens; however, there is still a paucity of information concerning the molecular characterization of MDR ESBL-producing *E. coli* implicated in broiler chicken in traditional Surabaya markets. It is also imperative to continuously monitor the current antimicrobial resistance trends in the food chain in order to devise effective treatment strategies and good control measures to guide against the increasing spread of antimicrobial resistance locally, nationally, and globally. Hence, this study was designed to identify the CTX-M gene in ESBL-producing *E. coli* from broiler chicken cloacal swabs in traditional Surabaya markets.

MATERIALS AND METHODS

Ethical Approval

The ethical clearance committee of the Faculty of Veterinary Medicine at Universitas Wijaya Kusuma Surabaya, Indonesia, provided consent for the use of animals (Ethics No: 109D-KKE).

Study Period and Location

A total of 96 cloacal swabs were aseptically collected from broiler chickens in 4 traditional markets of Dukuh Kupang, Keputran, Pacal Keling, and Pucang in Surabaya, Indonesia using sterile cotton swabs (Onemed, Indonesia) from January to March, 2024. Collected cloacal swabs were enriched in test tubes containing buffered peptone water (HiMedia) and then transported in a thermobox at 4°C to the laboratory for bacteriological analysis.

Isolation and Identification of *E. coli*

Loopfuls from positive tubes (turbid appearance of bacterial growth from cloacal swabs) of the enriched buffered peptone water (HiMedia) were then streaked on Eosin Methylene Blue Agar (EMBA) (HiMedia M317) and incubated for 18 to 24 hours at 37°C for the isolation of *E. coli*. Identified *E. coli* (green metallic sheen colonies) were then further characterized by other physiological and biochemical tests such as Gram staining, IMVIC (Indole-motility, methyl red, Voges-Proskauer), and TSIA (Triple sugar iron agar) (Anisharieta *et al*., 2021).

Antibiotic Sensitivity Test

This was carried out using the disk diffusion method on Mueller-Hinton Agar (MHA) media (HiMedia M173) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2020). The following antibiotics belonging to 5 different antibiotic classes were used: aztreonam (monobactam), chloramphenicol (phenicol), kanamycin (aminoglycoside), ciprofloxacin (fluoroquinolone), and tetracycline (tetracycline). Test bacterial isolates were firstly standardized to 0.5 McFarland standard (1.5×10^8 CFU/mL) using physiological saline (NaCl). Next, sterile cotton swabs were dipped into tubes containing the standardized isolates, drained on the side of the tube to remove excess inoculum, and then streaked on the whole surface of the Mueller Hinton Agar (MHA). Thereafter, the antibiotic-impregnated disks were carefully placed on the inoculated MHA plates and allowed to dry for a few minutes before incubating aerobically for 16–18 hours at 35°C. After incubation, inhibition zone diameters (IZDs) were then measured with the aid of a meter rule and results were interpreted as resistant or susceptible according to the CLSI criteria (CLSI, 2020; Wibisono *et al*., 2020). Bacterial isolates that exhibited resistance to at least three or more different classes of antibiotics were deemed to be multidrug-resistant (MDR). Presumptive ESBL is known if there is bacterial resistance to ≥ 3 types of antibiotics from different groups including aztreonam (Effendi *et al*., 2021).

CTX-M Gene Detection

Presumptive ESBL strains were then molecularly characterized for the presence of CTX-M gene. DNA extraction was done using QIAamp® DNA kit (QIAGEN, Germany). CTX-M primers used in this study were presented in Table 1. PCR denaturation temperature conditions are 94°C, 1 minute, annealing 54°C, 30 minutes; extension 72°C, 45 seconds, extended extension 72°C, 5 minutes, this reaction was carried out for 30 cycles of amplification by PCR. After that, the amplicons were visualized by electrophoresis using 2% agarose gel.

Statistical Analysis

Data was represented in tables and figures and then analyzed descriptively.

RESULTS AND DISCUSSION

Isolation of *E. coli*

The results of the sample inspection revealed that, out of the 96 chicken cloacal swab samples taken, 58 (60.42%) were positive for *E. coli* based on morphological culture, Gram staining, and biochemical tests (Table 2). The morphological culture of *E. coli* is successful when metallic green bacterial colonies emerge on EMBA media (Figure 1). Indicators of a negative Gram staining outcome include the presence of red colonies and short rods (Figure 2). An indication that the IMViC test finds *E. coli* is the indole ring in the SIM test (indole positive), the inverted spruce formation in the SIM test (motil), the red color change in the methyl red (MR) test (MR positive), the yellow color in the Voges–Proskauer (VP) test (VP negative), and the green color in the citrate test (negative citrate) (Figure 3).

There were 58 (60.42%) positive samples for *E. coli* after the bacterium from 96 broiler chicken cloacal swab samples were isolated and identified from four traditional markets in Surabaya. Samples were taken from the traditional markets of Dukuh Kupang, Keputran, Pucang, and Pacar Keling.

Poultry trading methods in Indonesia are still traditional. This makes the spread of disease very risky. Apart from that, the implementation of sanitation and biosecurity in traditional markets is still not given enough attention by market workers (Wardhana *et al*., 2021; Bardosh *et al*., 2023). The poultry slaughtering place is located in the market area which shows a dirty market environment, such as lots of rubbish, piled up waste disposal, muddy market floors, and poor sanitation. This is in line with research by Mpundu *et al*. (2019) which states that the high level of *E. coli* contamination in traditional markets in chicken meat, intestines, and water left over from cleaning chickens can come from dirty slaughtering places, water, and slaughtering equipment.

Extraintestinal pathogenic *Escherichia coli* (ExPEC), which causes several diseases in humans, is frequently linked to chicken and chicken products that have colibacillosis (Zou *et al*., 2021). One of the ExPEC substrains is Avian Pathogenic *Escherichia coli* (APEC), which is an agent that causes colibacillosis and septicemia in poultry in the poultry industry (Kathayat *et al*., 2021). Acute septicemia, pericarditis, airsaculitis, salpingitis, and peritonitis are the hallmarks of colibacillosis and are thought to be the primary factors contributing to lower production and higher mortality rates (Kabir, 2010; Pradika *et al*., 2019). The incidence of colibacillosis has been reported in chickens in Sukabumi and Bogor districts at 22.2% (Wiedosari and Wahyuwardani, 2015). The incidence of colibacillosis in broiler chicken farms in Bali with a prevalence of 5% and is characterized by symptoms of respiratory problems, intestinal distension, and white feces (Suryani *et al*., 2014). This is in accordance with chicken samples obtained from traditional market extracts which showed white feces.

Apart from that, chickens distributed from farms to markets are generally transported using pickup cars or trucks which are overcrowded and crowded. This is in line with the study of Abalaka *et al*. (2017) where it was reported that colibacillosis often occurs in chickens under certain stressful conditions, such as overcrowding, poor sanitation, malnutrition, extreme temperatures, and immune suppression (immunosuppression). This disease suppresses the immune system where there is a change in the balance between *E. coli* and the body's defense system which causes the bacteria to become pathogenic and infect chickens. Good husbandry and strict biosecurity can reduce the risk of colibacilosis (Wiedosari and Wahyuwardani, 2015; Purnamasari and Tyasningsih, 2023).

Table 1. Primers used in detecting CTX-M

Gene	Primer	Sequence (5–3)	Base pair	Reference
CTX-M	CTX-M–Forward	5-CGC TTT GCG ATG TGC AG-3	550 bp	(Prayudi *et al*., 2023)
	CTX-M–Reverse	5-ACC GCG ATA TCG TTG GT-3		

Table 2. Isolation and identification of *E. coli*

Location	n	Identification test							Positive *E. coli* (%)
		EMBA	Gram stain	IMViC test					
				Indol	Motile	MR	VP	Citrate	
Dukuh Kupang	24	20	17	12	12	12	12	12	12 (50)
Keputran	24	19	15	14	14	14	14	14	14 (58.33)
Pucang	24	22	20	19	19	19	19	19	19 (79.17)
Pacar Keling	24	19	17	13	13	13	13	13	13 (54.17)
Total	96	80	69	58	58	58	58	58	58 (60.42)

n= total samples, % (Positive percentage).

Table 3. Zone of inhibition in the sensitivity test using the disk diffusion test method

Group of antibiotics	Resistance profile	Number of isolates (n=58) Resistant isolates (%)	Total number of isolates (%)
0	No one is resistant	2 (3.45)	2 (3.45)
1	ATM	3 (5.17)	27 (46.55)
	T	14 (24.14)	
	K	1 (1.72)	
	C	9 (15.52)	
2	ATM – T	2 (3.45)	14 (24.14)
	ATM – K	1 (1.72)	
	ATM – C	6 (10.34)	
	T – K	1 (1.72)	
	T – C	3 (5.17)	
	C – CL	1 (1.72)	
≥3	ATM – T – C	1 (1.72)	15 (25.86)
	ATM – K – C	1 (1.72)	
	T – K – C	3 (5.17)	
	T – K – CL	2 (3.45)	
	T – C – CL	3 (5.17)	
	ATM – T – C – CL	2 (3.45)	
	T – K – C – CL	3 (5.17)	

ATM= Aztreonam, T= Tetracycline, K= Kanamycin, C= Ciprofloxacin, CL= Chloramphenicol.

Table 4. Identification of multidrug-resistant *E. coli*

Sample code	Resistance profile	Antibiotic				
		AML	TE	S	TS	CAZ
TRA 64	AML – TE – TS	✓	✓	–	✓	–
TRA 65	AML – S – TS – CAZ	✓	–	✓	✓	✓
TRA 69	AML – S – CAZ	✓	–	✓	–	✓
TRA 98	AML – TS – CAZ	✓	–	–	✓	✓
TRA 113	AML – S – CAZ	✓	–	✓	–	✓
TRA 120	AML – S – CAZ	✓	–	✓	–	✓
TRA 129	AML – S – CAZ	✓	–	✓	–	✓
TRA 133	AML – TE – S	✓	✓	✓	–	–

✓= Resistant, S= Streptomicin, CAZ= Ceftazidime, TS= Trimethoprim-sulfamethoxazole, TE= Tetracycline, AML= Amoxicilin.

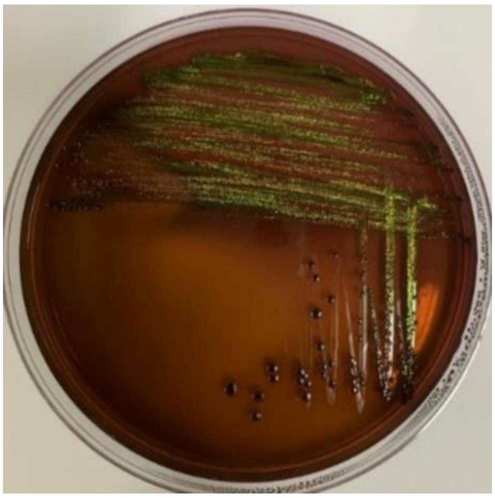

Figure 1. Metallic green colonies on EMBA media are suspected to be *E. coli* colonies.

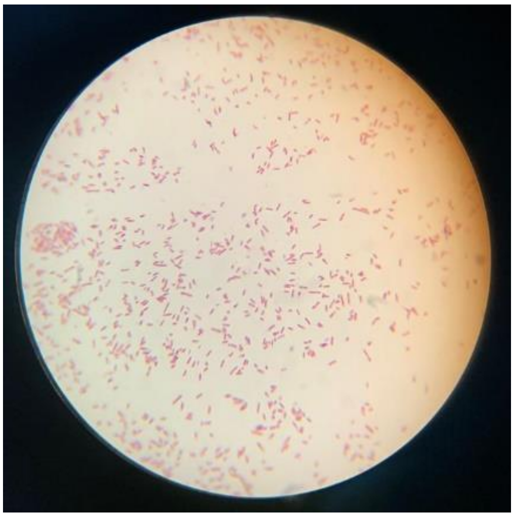

Figure 2. Negative gram staining results at 1000× magnification.

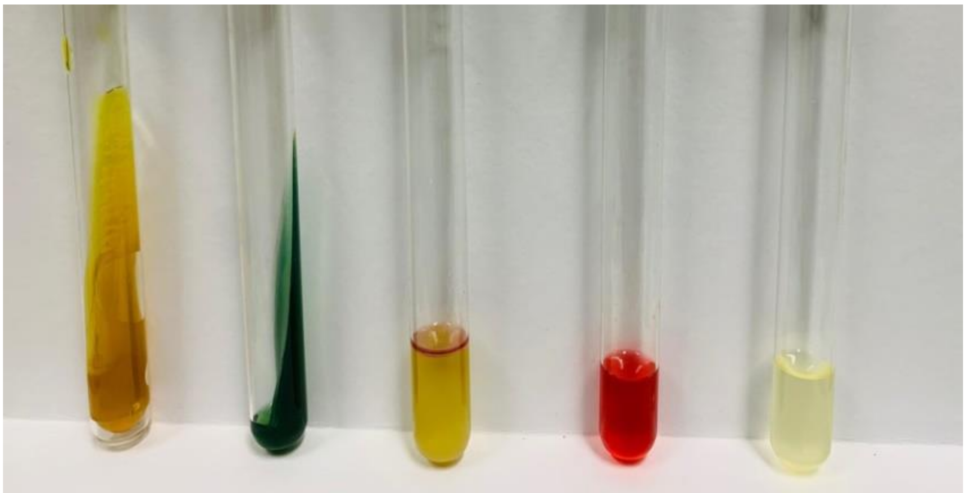

Figure 3. Positive results for *E. coli* in the IMViC test.

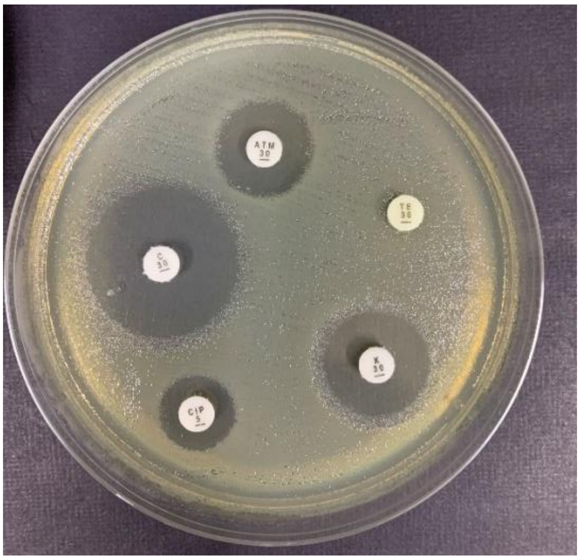

Figure 4. Antibiotic sensitivity test results using agar disk diffusion test method.

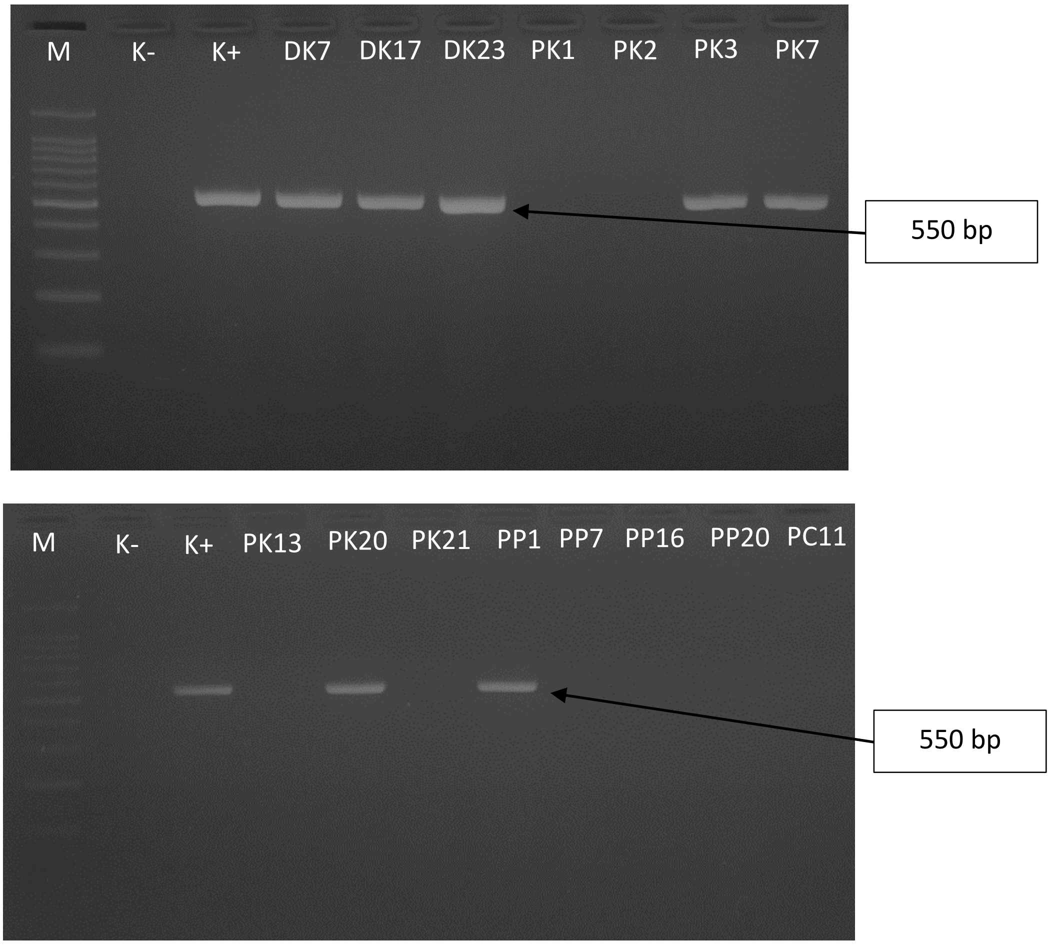

Figure 5. The results of the detection of the CTX-M gene encoding ESBL isolates from *E. coli* MDR.

Antimicrobial Sensitivity Test

Antimicrobial sensitivity results showed that out of the recovered 58 *E. coli* isolates, 27 (46.55%) were resistant to one class of antibiotic, 14 isolates (24.14%) were resistant to two classes, while 15 isolates (25.86%) exhibited resistance to three or more different antibiotic classes (Table 3 and figure 4). The isolates that exhibited resistance to at least 3 antibiotics were classified as multidrug-resistant (MDR) strains. The dominant antibiotic resistance patterns were T–K–C, T–C–CL, and T–K–C–CL, and were expressed by three isolates each (Table 4).

In this study, 15 *E. coli* isolates were found to be multidrug-resistant (MDR) from 58 *E. coli* isolates obtained from broiler chicken cloaya swabs at Surabaya traditional markets. The results of our study, including the antimicrobial sensitivity results are in agreement with a similar study carried out in Surabaya, Indonesia by Effendi *et al*. (2021) who also reported MDR and ESBL-producing *E. coli* in the cloacal swabs of broiler chickens. However, we reported a higher frequency of MDR *E. coli* (15/58) with 7 isolates harboring the CTX-M gene, unlike the previous report of Effendi *et al*. (2021) which reported a lower frequency of MDR (12) and ESBL-producing *E. coli* (2/12). This difference in *E. coli* frequency might be due to the difference in cloacal sample size as our study analyzed 96 cloacal swabs while the study of Effendi *et al*. (2021) analyzed 60 cloacal swab samples. Elsewhere, MDR *E. coli* has been implicated in bacterial contamination of dressed chickens in abattoirs (Mpundu *et al*., 2019), chicken colisepticaemia (Abalaka *et al*., 2017), and among healthy chickens (Zou *et al*., 2021) in Zambia, Nigeria, and China, respectively. The level of antibiotic use by farmers to increase livestock productivity, without referring to appropriate antibiotic use standards, triggers MDR events. This is consistent with a study conducted in Zimbabwe, which found a substantial correlation between the amount of resistance in *E. coli* isolates from cattle, pigs, poultry, and antibiotics (Chantziaras *et al*., 2014; Fadlilah *et al*., 2021). The normal flora in the intestine is generally resistant to commonly used antibiotics, such as gentamicin, tetracycline, ampicillin, cotrimoxazole, erythromycin, and third-generation cephalosporins and many experience MDR (Perdikouri *et al*., 2019).

Since farmers often only use one kind of antibiotic at a time, a rotation program is required when giving antibiotics to avoid the development of antibiotic resistance. As a result, it is important to teach farmers how to utilize a variety of antibiotics or alternate medicines when treating illnesses (Manyi-Loh *et al*., 2018; Dameanti *et al*., 2022). In addition, veterinarians play a critical role in overseeing the use of antibiotics on farms. The proper dosage and administration of antibiotics should be observed, and they should not be used to prevent illness. Another factor contributing to the development and spread of antibiotic resistance is the chicken industry's high demand (Hedman *et al*., 2020; Abdurrahman *et al*., 2022). The widespread incidence of antibiotic resistance, especially multidrug resistance (MDR) in pathogenic bacteria, can pose challenges in disease treatment therapy. According to the one health concept, it is critical to investigate environmental resistance since it contributes to the bacterial transmission that affects humans, animals, and the environment (Veloo *et al*., 2020; Yunita *et al*., 2020).

The highest multidrug resistance (MDR) pattern in *E. coli* obtained from cloacal swabs of broiler chickens in Surabaya traditional markets is T–K–C–CL from four classes of antibiotics, namely tetracycline (tetracycline), aminoglycoside (kanamycin), fluoroquinolone (ciprofloxacin), and phenicol (chloramphenicol) were present in three of the 15 isolates. Another pattern observed for two out of the 15 MDR isolates was ATM–T–C–CL; namely antibiotics belonging to the monobactams (aztreonam), tetracyclines (tetracycline), fluoroquinolones (ciprofloxacin), and phenicols (chloramphenicol) groups. Differences in resistance patterns arise due to differences in the combination of antibiotic types given by breeders. This is following Hardiati *et al*. (2021) who stated that resistance patterns show diversity which was attributed to the use of various antibiotics, geographical differences, and different poultry production systems causing differences in resistance patterns. The usage of broad-spectrum antibiotics can impact patterns of resistance to shared drugs (Kim *et al*., 2018; Khairullah *et al*., 2020).

Detection of ESBL-encoding CTX-M Gene

Out of the 15 MDR *E. coli* isolates analyzed in this investigation, seven (7) tested positive for CTX-M gene based on the PCR results. The seven isolates were samples DK7, DK17, DK23, PK3, PK7, PK20, and PP1 (Figure 5).

Antibiotic resistance in *E. coli* can produce extended-spectrum β-lactamase (Halabi *et al*., 2021). ESBL production causes resistance to cephalosporins as third-generation antibiotics and monobactam where genes between plasmids can be transferred to different bacteria (Shaikh *et al*., 2015; Wibisono *et al*., 2021). This enzyme is found in the Enterobacteriaceae family, such as *E. coli*, *Klebsiella pneumonia*, and *Salmonella enterica* serovar (Bialvaei *et al*., 2016). Humans and food-producing fowl, especially hens, have been found to harbor ESBL-producing *E. coli*, which has a significant negative influence on both human health and poultry productivity (Castanheira *et al*., 2021; Wijaya *et al*., 2021).

Several antibiotics, including aminoglycosides, fluoroquinolones, trimethoprim, tetracycline, aminoglycosides, and sulfonamides are frequently resistant to ESBL-producing *E. coli*, which can lead to a shortage of viable antibiotic alternatives (Parvin *et al*., 2020). According to this study, 17 of the 58 *E. coli* isolates that were looked at were resistant to β-lactam antibiotics, particularly aztreonam. Overuse of β-lactam antibiotics has made it possible for bacteria to produce genes encoding β-lactamase, which makes treating bacterial infections more difficult because of resistance (Ansharieta *et al*., 2021; Tyasningsih *et al*., 2022). In Ghana, reports of ESBL-producing *E. coli* have come from both people and animals (Falgenhauer *et al*., 2019). In addition, additional research indicates that 86% of frozen chicken meat in

Bangladesh and Surabaya's broiler chickens had significant prevalences of ESBL-producing *E. coli* (Effendi *et al*., 2021; Parvin *et al*., 2020).

There is currently little knowledge regarding ESBL-producing *E. coli* in Indonesian chicken farms (Yanestria *et al*., 2022). The primary genes that produce ESBL are the SHV (bla-SHV), TEM (bla-TEM), and CTX-M genes (Pishtiwan and Khadija, 2019). The most common form of ESBL-producing *E. coli* detected worldwide is CTX-M, and poultry is thought to be a possible source of these bacteria (Liebana *et al*., 2013; Widodo *et al*., 2024). The distribution of CTX-M throughout the world continues to develop. Bacteria that are resistant to antibiotics are caused by horizontal gene transfer and gene mutation (Faridah *et al*., 2023). Genes that code for resistant *E. coli* can come from humans and animals (Xia *et al*., 2015; Wardhana *et al*., 2021). ESBL-producing *E. coli* bacteria can be transmitted in several ways, such as by consuming contaminated meat, feces in the environment, and exposure to people infected with ESBL transmitted from humans through animals (Gay *et al*., 2023).

Interactions with chicken sold in wet markets, which is the source of MDR and ESBL bacteria, can expose humans to antibiotic-resistant bacteria (14). Several previous studies showed that *E. coli* harboring the CTX-M gene was found in cloacal swabs of broiler chickens and laying chickens in Tanzania, the Philippines, and Malaysia with occurrence frequency of 4.7%, 44.23%, and 80% respectively (Gundran *et al*., 2019; Lemlem *et al*., 2023; Mgaya *et al*., 2021). Besides, the CTX-M gene has been found in other animals and animal products, such as pork in Vietnam (66.6%) and beef in China (9.5%) (Hoang *et al*., 2017; Zhang *et al*., 2021).

Conditions in traditional markets with low levels of cleanliness and chickens placed close together can spread resistance genes. This is in line with research on food-producing animal production by Effendi *et al*. (2021) who reported that microorganisms can survive in aerosol form while in the air and transmit through the air. This is influenced by a bad environment so that the spread of ESBL-producing *E. coli* can easily occur. The incidence of resistance in *E. coli* producing extended-spectrum β-lactamase (ESBL) to several types of antibiotics is related to antibiotic usage when mixed in feed or drinking water. The lowest dose that can stimulate resistance to pathogenic and commensal bacteria in the digestive tract is added to antibiotics in animal feed (Manyi-Loh *et al*., 2018).

CONCLUSION

This present study showed that broiler chickens sold in different traditional markets in Surabaya, Indonesia harbor multidrug-resistant *E. coli* which also possess the ESBL-encoding CTX-M gene. To curb the increasing spread of MDR bacterial pathogens in the food chain, especially in poultry meat and their by-products, regulatory agencies need to encourage prudent use of antimicrobials and also enforce strict hygienic practices in Surabaya traditional markets. This will greatly avert potential public health consequences in both veterinary and human medicine. More studies that will focus on other bacterial pathogens implicated in the food chain are however needed to comprehensively decipher their current trend of resistance, epidemiology, and possible effective therapeutic options.

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AUTHORS' CONTRIBUTIONS

ARK and IBM: Conceived, designed, and coordinated the study. AH and RS: Designed data collections tools, supervised the field sample and data collection, and laboratory work as well as data entry. MFRP and IF: Validation, supervision, and formal analysis. MKJK and FJW: Contributed reagents, materials, and analysis tools. SMY and MHE: 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.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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