DRF-376
[1-8]

RESEARCH ARTICLE Asian Journal of Dairy and Food Research

Identification of the Genotypes of Extended-spectrum β-lactamase Producing *Escherichia coli* in the Small Intestine of Broiler Chickens at a Traditional Market in Surabaya, Indonesia

Reichan Lisa Az Zahra[1], Sasqia Khairunnisa Aulia Prayudi[1], Tri Wahyu Suprayogi[2], Dhandy Koesoemo Wardhana[3], Mustofa Helmi Effendi[3], Aswin Rafif Khairullah[4], Shendy Canadya Kurniawan[5] **10.18805/ajdfr.DRF-376**

ABSTRACT

Background: *Escherichia coli* infection from inside the chicken's body may be present if the previously slaughtered chicken was contaminated with pathogens or the cage's hygiene is inadequate. One of the resistance mechanisms against antibiotics used by Gram-negative bacteria of the Enterobacteriaceae family is the production of extended-spectrum β-lactamases (ESBL). Research is required to determine which *E. coli* genotypes in broiler chicken samples collected from Surabaya's traditional marketplaces produce ESBL.

Methods: A total of 100 small intestine samples collected from broiler chickens were used in this study. After initial processing all the samples were separately injected into Eosin Methylene Blue Agar (EMBA) and Indole, Methyl Red, Voges Proskauer and Citrate (IMViC) tests were used to confirm the results. A test for antibiotic sensitivity based on the Kirby-Bauer method called the Double Disk Synergy Test (DDST) was used to analyze isolates that showed resistance to aztreonam and multidrug resistance (MDR). PCR testing was performed on ESBL-verified isolates to detect the TEM and CTX-M genes.

Result: The results of the test for *E. coli* antibiotic resistance revealed that 14 isolates were proven to be multidrug-resistant (MDR) and six of them were found to be *E. coli* that produced ESBL. Four isolates were found to carry the TEM and CTX-M genes after genotyping analysis. Understanding the dangers of using antibiotics as feed additives and growth promoters is crucial for farmers to address the problem of ESBL-producing *E. coli* bacteria in chickens. Furthermore, the government needs to take action to monitor and enforce stronger controls on the use of antibiotics, which are even readily available on the market.

Key words: Antibiotics, Broiler chicken, *E. coli*, ESBL, Public health.

INTRODUCTION

The presence of *E. coli* contamination which is from inside the chicken's body can occur if the previously slaughtered chicken has been infected by bacteria, or the sanitary conditions of the cages are poor (Adeyanju and Ishola, 2014). While the slaughtering, handling, air quality and long-term storage of chicken can result in environmental contamination; the small intestine of chicken, in particular, is frequently observed to have this bacterial invasion (Pan and Yu, 2014). Due to the current lack of effective treatments, the survival of bacteria that produce ESBL in food of animal origin may create public health problems like pneumonia, respiratory tract infections, cramping in the stomach, diarrhoea and urinary tract infections in man (Sivakumar *et al.,* 2021).

Antibiotic-resistant bacteria can travel through the small intestine of food-producing animals and subsequently be eliminated via feces (Xu *et al.,* 2022). Animal manure-borne resistant bacteria can spread across farms, slaughterhouses, or poultry slaughterhouses, as well as during the meat-processing process (Jeżak and Kozajda, 2022). The environment around farms and slaughterhouses or chicken abattoirs may also be contaminated even though they are far from the source of contamination. This is significant because human-animal disease transmission is possible at any point in the production chain and at any moment (Ramos *et al.,* 2020; Tyasningsih *et al.,* 2022).

[1]Profession Program in Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia.
[2]Division of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia.
[3]Division of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia.
[4]Research Center for Veterinary Science, National Research and Innovation Agency, Jl. Raya Bogor Km. 46 Cibinong, Bogor 16911, West Java, Indonesia.
[5]Master Program of Animal Sciences, Department of Animal Sciences, Specialisation in Molecule Cell and Organ Functioning, Wageningen University and Research, Wageningen 6708 PB, Netherlands.

Corresponding Author: Mustofa Helmi Effendi, Division of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia. Email: mhelmieffendi@gmail.com

How to cite this article: Zahra, R.L.A., Prayudi, S.K.A., Suprayogi, T.W., Wardhana, D.K. Effendi, M.H., Khairullah, A.R. and Kurniawan, S.C. (2024). Identification of the Genotypes of Extended-spectrum β-lactamase Producing *Escherichia coli* in the Small Intestine of Broiler Chickens at a Traditional Market in Surabaya, Indonesia. Asian Journal of Dairy and Food Research. doi: 10.18805/ajdfr.DRF-376.

Submitted: 28-11-2023 **Accepted:** 13-07-2024 **Online:** 09-08-2024

In Indonesia, people usually purchase chicken at traditional marketplaces because these are the areas where buying and selling activities take place face-to-face and where there is typically a negotiation process (Alfani *et al.,* 2021). Traditional markets are typically associated with filthy, chaotic locations and the chicken that is sold there is typically arranged in a way without any sort of supporting mat to prevent bacterial infection (Siddiky *et al.,* 2022). Surabaya, one of the biggest cities in Indonesia, has several such markets. One of the causes contributing to *E. coli* contamination is the placement of chicken meat combined with innards in the form of the small intestine (Hussain *et al.,* 2017).

Antibiotics can lose their ability to combat *E. coli* germs over time, leading to uncontrolled infections (Khairullah *et al.,* 2024; Nalband *et al.,* 2020). One of the reasons for this is the inappropriate use of antibiotics to stop the spread of *E. coli* infection (Aslam *et al.,* 2018; Widodo *et al.,* 2022). Bacterial resistance is more prevalent, making it challenging to treat illnesses, particularly moderate or severe infections (Yunita *et al.,* 2020; Khairullah *et al.,* 2020; Nwobodo *et al.,* 2022). Extended-spectrum β-lactamase (ESBL) synthesis is one of the resistance mechanisms used by Gram-negative bacteria in the Enterobacteriaceae family (Shaikh *et al.,* 2015; Durairajan *et al.,* 2021).

This resistance results from acquiring a plasmid with the gene for the ESBL enzyme, which is mostly generated by *E. coli* (Lemlem *et al.,* 2023). The ability of the enzyme to break down antibiotics like penicillins, cephalosporins and monocyclic amides is crucial for understanding how *E. coli* develops its antimicrobial resistance, but β-lactamase inhibitors like sulbactam, tazobactam and clavulanic acid typically prevent ESBL production from occurring (Miao *et al.,* 2017). The two primary coding genes for ESBL are TEM and CTX-M (Castanheira *et al.,* 2021). Both of these genes generate ESBL, which hydrolyze β-lactam antibiotics.

A particularly severe issue in the field of medicine is bacterial resistance to antibiotics (Khairullah *et al.,* 2023). However, case information on the prevalence of *E. coli* contamination that produces ESBLs in conventional market contexts is infrequently reported. Research is required to identify the *E. coli* genotypes that produce ESBL in Surabaya's traditional marketplaces.

MATERIALS AND METHODS

Study area and sample collection

In this investigation, 100 small intestines of broiler chickens from various vendors were collected aseptically from Surabaya City's traditional markets. This study was carried out from April 2023 to May 2023. This research was conducted at the Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia.

Bacterial isolation of *E. coli*

One gram is the weight of the contents that have been taken out of each chicken's small intestine. The sample is homogenized using a 0.1% buffered peptone water solution at a ratio of 1:10 as the initial step in isolating *E. coli* bacteria. The samples were then streaked over sterile loop-adjusted Eosin Methylene Blue Agar (EMBA) at a distance sufficient to divide colony development and incubated for a full day at 37°C.

Gram staining was used to identify the colonies and the morphology of *E. coli* was examined under a 1000x magnification oil immersion microscope. The IMViC tests, which consist of the Indole test, methyl red (MR), Voges Proskauer (VP) test and citrate utilization test, were then used to confirm colonies suspected of being *E. coli*.

Antibiotic resistance of *E. coli*

The isolated and identified pure cultures from physiological saline as a suspension with 0.5 McFarland turbidity (1-2 x 108 CFU/ml) was used for the antibiotic susceptibility test. Using a clean cotton swab, the culture was transferred to Mueller Hinton Agar (MHA), where it was spread out and let to stand for around five minutes. The antibiotic disks were then, using the Kirby-Bauer technique, positioned above the MHA, which had been smeared with pure culture, at a distance of 25-30 mm. After that, the culture was kept at 35°C for 16-18 hours. Five different antibiotics ampicillin (10 µg), gentamicin (10 µg), aztreonam (30 µg), ciprofloxacin (5 µg) and chloramphenicol (30 µg) were used for sensitivity tests and resistance profiling.

Tests for antibiotic sensitivity, multidrug resistance (MDR) and resistance to the antibiotic aztreonam were performed on isolates using the Double disk synergy test (DDST). The data were interpreted with the help of the Clinical and Laboratory Standards Institute (CLSI) 2022 guidelines. An antibiotic disk was used to confirm the phenotype. Ceftazidime (30 µg), amoxicillin-clavulanic acid (20 µg) and cefotaxime (30 µg) are among the cephalosporins in this group.

Molecular detection of *E. coli*

Five microliters of DNA are utilized as a template. The specific primers utilized were designed to target the TEM and CTX-M genes (Table 1). Mixture for the PCR reaction with a volume of 25 µl consisting of 12.5 µl PCR Mastermix

Table 1: Primers used in the present study.

Primers	Sequences (5′ to 3′)	Target gene	Amplicons size	References
TEM forward	ATA-AAA-TTC-TTG-AAG-ACG-AAA	blaTEM	1086 bp	(Ansharieta *et al.,* 2021)
TEM reverse	GAC-AGT-TAC-CAA-TGC-TTA-ATC	blaTEM		
CTX-M forward	CGC-TTT-GCG-ATG-TGC-AG	blaCTX-M	550 bp	(Faridah *et al.,* 2023)
CTX-M reverse	ACC-GCG-ATA-TCG-TTG-GT	blaCTX-M		

(0.5 U Taq Polymerase, 0.2 mM dNTP, 1.5 mM $MgCl_2$ and Buffer 1x), 1.25 µl for each primer and 5 µl DNA template. The PCR was conducted under the following conditions: initial denaturation at 94°C for 7 minutes, 35 cycles of 96°C for 50 seconds of denaturation, 50°C for 40 seconds of annealing and 72°C for 1 minute of extension and finally 10 minutes of extension at 72°C.

1.5% agarose was used to visualize the PCR result. Five microliters of the PCR product and one microliter of the marker were mixed together, put on parafilm and left in an ethium bromide solution for ten minutes in the dark. A UV transilluminator with a 360 nm wavelength was used to observe the PCR product that has been produced.

RESULTS AND DISCUSSION

Following morphological culture, Gram staining and biochemical testing, it was shown that 63 (63%) of the 100 intestinal samples collected from broiler chickens tested positive for *E. coli* (Table 2). The appearance of metallic green bacterial colonies on EMBA media indicated that the morphological culture of *E. coli* was successfully achieved (Fig 1). A Gram-negative staining result indicated organisms are either pink or red in color and short rods in Gram staining (Fig 2). The appearance of an inverted spruce formation on the SIM test (Motility), an indole ring on the SIM test (Indol positive), a yellow color on the VP test (negative VP), a red color change on the MR test (positive MR) and green on the citrate utilization test (citrate negative) all indicated that the IMViC test had produced positive indications for *E. coli* (Fig 3).

The interaction between bacteria and the environment, which infects the chicken's body through the feed, contributes to the presence of bacteria in the digestive system of chickens (Hossain *et al.,* 2023). Environmental contamination was one of numerous causes of the rise of *E. coli* bacteria in specific chickens. It is suspected that the chickens that were given a drink before being slaughtered and kept in a place with moist husks containing lots of chicken feces could be a potent source of such infections (Biesek *et al.,* 2023).

The *E. coli* isolates examined in this study showed that 56 showed the highest level of resistance to ampicillin, 19 isolates were resistant to ciprofloxacin, 17 isolates were resistant to gentamicin, 11 isolates were resistant to chloramphenicol and 4 isolates were resistant to aztreonam (Table 3). The *E. coli* antibiotic resistance test results revealed that of the 14 isolates, 22.22% were confirmed to be multidrug resistant (MDR) due to their resistance to three to five different classes of antibiotics (Fig 4). The most prevalent pattern of antibiotic resistance was found in the isolates that fell into the ampicillin, chloramphenicol, ciprofloxacin and gentamicin) group, which consists of 5 isolates (Table 4).

Six of the 14 *E. coli* isolates with the greatest documented resistance pattern to the four antibiotics ampicillin, chloramphenicol, ciprofloxacin and gentamicin were confirmed to be MDR. The development of resistant bacteria demonstrates that these drugs do not stop them

Fig 1: Colonies of *E. coli* on EMBA.

Table 2: Identification of *E. coli* isolatedfrom faecal samples collected from small intestines of broiler birds.

Location	Markets	Sample size	Identification test							Positive *E. coli* (%)
			EMBA	Gram stain	IMViC test					
					Indole	Motility	MR	VP	Citrate	
Central Surabaya	Keputran	10	10	10	10	6	5	5	5	5 (50%)
	Kembang	10	10	10	10	10	10	10	10	10 (100%)
East Surabaya	Pucang	10	10	10	9	7	7	7	7	7 (70%)
	Pahing	10	10	10	10	10	10	10	10	10 (100%)
South Surabaya	Wonokromo	10	10	10	10	5	5	5	5	5 (50%)
	Pagesangan	10	8	8	8	8	8	8	8	8 (80%)
West Surabaya	Darmo	10	10	10	10	10	5	5	5	5 (50%)
	Manukan	10	9	9	9	9	9	9	9	9 (90%)
North Surabaya	Pabean	10	10	10	9	5	4	4	4	4 (40%)
	Pegirian	10	10	10	8	8	0	0	0	0 (0%)
Total		100	97	97	93	78	63	63	63	63 (63%)

Note: % (Percentage of positive).

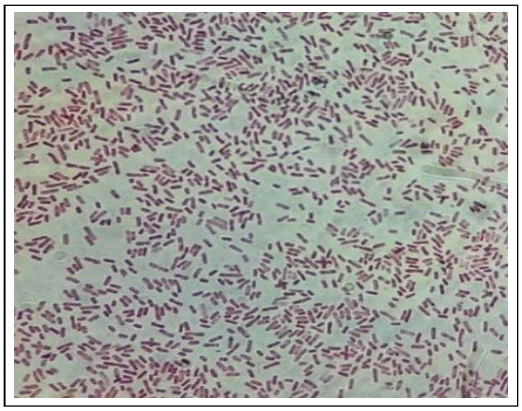

Fig 2: *E. coli* stained with Gram stain by using normal microscopy.

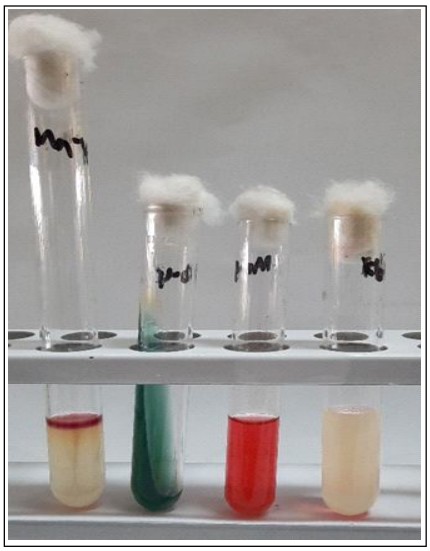

Fig 3: Results of the IMViC tests showed the above positive results indicative for *E. coli.*

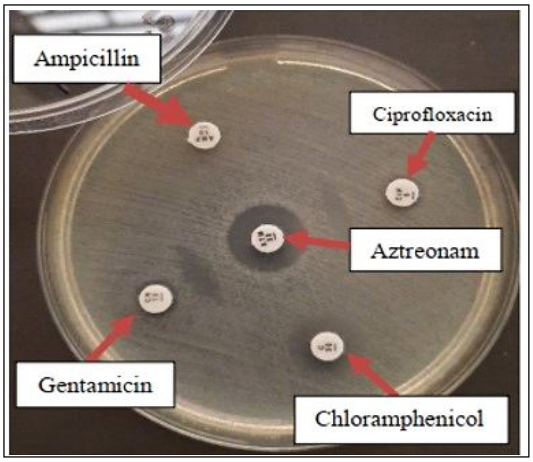

Fig 4: The isolates of *E. coli* showed multidrug resistance in their sensitivity test findings on MHA media.

from growing and they cannot be treated with them since they have no therapeutic effect (Sandegren, 2014). Bacteria that are classed as intermediates to an antibiotic suggest that the antibiotic has an unreliable therapeutic effect and that its use has started to decline (Breijyeh *et al.,* 2020). The presence of bacteria that are still sensitive to antibiotics indicates that these drugs can still stop bacterial development and have a good chance of having therapeutic effects (Łapińska *et al.,* 2022).

The Double Disk Synergy Test was performed on 14 isolates of MDR *E. coli* and those that had ESBLs identified and subsequently detected using three different antibiotics. Six of the 14 isolates tested by the DDST test were determined to be *E. coli* strains that produce ESBL (Fig 5). A hazard to the public's health is posed by the existence of ESBL bacteria that produce MDR (Igbinosa *et al.,* 2023). There may be few effective treatments for certain disorders. Because antibiotics are misused in terms of dosage, diagnosis and infection-causing bacteria, there is a significant incidence of bacterial resistance (Aslam *et al.,* 2021). The use of antibiotics above the recommended dose places a stronger selection pressure on bacteria, causing them to mutate and eventually develop resistance. Antibiotics also have a minimum dose to produce therapeutic effect (Raymond, 2019). Underdosing on antibiotics prevents the bacteria from being entirely eliminated, which forces the remaining germs to adapt and develop antibiotic resistance (Munita and Arias, 2016).

The presence of the TEM and CTX-M genes in six isolates of *E. coli* that were confirmed to produce ESBLs was subsequently determined by genotyping. Four of the six isolates carried both the TEM and CTX-M genes in the electrophoresis results of the six isolates studied, while the other two isolates contained just the CTX-M gene (Table 5). The emergence of a single band on the electrophoresis findings indicates a favorable outcome (Fig 6).

The results of the ESBL resistance gene detection from the six isolates under examination revealed that four of the isolates had both the TEM and CTX-M genes, while the other two isolates had only the CTX-M gene. The β-lactamase enzyme is typically to blame for the presence of these two genes in Gram-negative bacteria (Tooke *et al.,* 2019). The plasmids that contain the antibiotic resistance genes for aminoglycosides, trimethoprim, sulfonamides, tetracyclines and chloramphenicol have ESBL that can hydrolyze antibiotics (Wibisono *et al.,* 2021). The plasmid also harbors the TEM and CTX-M genes, which are descended from the bacterial chromosome (Faridah *et al.,* 2023; Widodo *et al.,* 2023). Integrins and transposons influence gene activity.

The two primary categories of β-lactam antibiotics are TEM and CTX-M genes (Bajpai *et al.,* 2017). However, certain studies have reported a higher prevalence of the CTX-M gene (Leão *et al.,* 2021; Abo-Almagd *et al.,* 2023; Seo and Lee, 2021). The ability of the bacteria to resist antibiotics is due to the presence of a detectable antibiotic resistance gene (Kunhikannan *et al.,* 2021). This is

Table 3: Antibiotic group-specific resistance profile of isolated *E. coli.*

Group of antibiotics	Resistance profile	Number of isolates (n=63) / Resistant isolates (%)	Total number of isolates (%)
0	No one is resistant	7 (11.11%)	7 (11.11%)
1	AMP	28 (44.44%)	28 (44.44%)
2	AMP – ATM	1 (1.59%)	14 (22.22%)
	AMP – C	2 (3.17%)	
	AMP – CIP	6 (9.52%)	
	AMP – GM	5 (7.94%)	
≥3	AMP– C –CIP	1 (1.59%)	14 (22.22%)
	AMP– C – GM	1 (1.59%)	
	AMP– CIP – GM	4 (6.35%)	
	AMP–ATM–CIP–GM	1 (1.59%)	
	AMP–C–CIP–GM	5 (7.94%)	
	AMP–ATM–C–CIP–GM	2 (3.17%)	

Note: GM = Gentamicin, AMP = Ampicillin, C = Chloramphenicol, CIP = Ciprofloxacin, ATM = Aztreonam.

Table 4: Isolates of *E. coli* with a multidrug resistance profile.

Location	Markets	Sample code	Resistance profile	Antibiotic				
				AMP	ATM	C	CIP	GM
Central Surabaya	Keputran	KP 7	AMP–C–CIP–GM	✓	–	✓	✓	✓
		KP 9	AMP–C–CIP–GM	✓	–	✓	✓	✓
East Surabaya	Pucang	PC 10	AMP–C–CIP–GM	✓	–	✓	✓	✓
	Pahing	PH 1	AMP– CIP – GM	✓	–	✓	✓	✓
		PH 2	AMP–C–CIP–GM	✓	–	✓	✓	✓
		PH 7	AMP– CIP – GM	✓	–	–	✓	✓
		PH 10	AMP–ATM–C–CIP–GM	✓	✓	✓	✓	✓
South Surabaya	Wonokromo	WO 6	AMP– C –CIP– GM	✓	–	✓	✓	✓
		WO 7	AMP– CIP – GM	✓	–	–	✓	✓
		WO 8	AMP– CIP – GM	✓	–	–	✓	✓
	Pagesangan	PS 5	AMP–ATM–CIP–GM	✓	✓	–	✓	✓
		PS 8	AMP–ATM–C–CIP–GM	✓	✓	✓	✓	✓
West Surabaya	Darmo	DP 1	AMP–C–CIP	✓	–	✓	✓	–
		DP 6	AMP–C– GM	✓	–	✓	–	✓

Note: ✓= Resistant, GM= Gentamicin, AMP= Ampicillin, C= Chloramphenicol, CIP= Ciprofloxacin, ATM= Aztreonam.

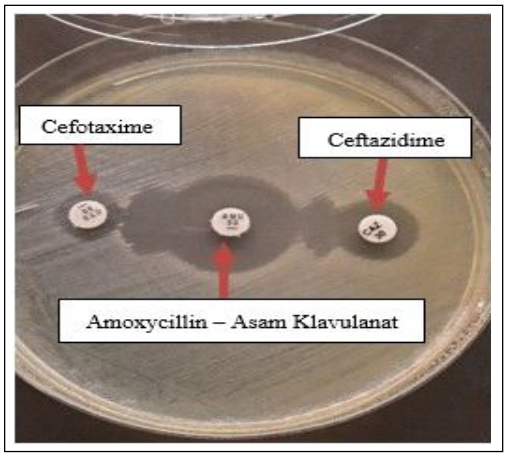

Fig 5: Results of the DDST test on isolates of ESBL-producing *E. coli.*

suggested by the existence of genes found in isolates that show resistance in the sensitivity test. The phenomena where Gram-negative bacteria can transmit horizontal or lateral gene transfer from plasmids and clone ESBL-producing *E. coli* present in animal bodies is linked to the high prevalence of CTX-M and TEM in creating *E. coli* resistance to third-generation cephalosporin antibiotics (Chukwu *et al.,* 2022; Mondal *et al.,* 2022).

The key to resolving the issue of ESBL-producing *E. coli* bacteria in chickens is for farmers to be aware of the risks of using antibiotics as feed additives and growth promoters (Saliu *et al.,* 2020; Sakthikarthikeyan *et al.,* 2023). In addition, constant use of disinfectants to keep the cage clean is necessary (White *et al.,* 2018). Furthermore, the government needs to take action to monitor and enforce stronger controls on the use of antibiotics, which are even readily available on the market.

Table 5: TEM and CTX-M isolate *E. coli* gene molecular identification.

Sample code	TEM genes	CTX-M genes
PC10	Positive	Positive
PH 1	Positive	Positive
PH 2	Positive	Positive
PH 10	Positive	Positive
PS 5	Negative	Positive
PS8	Negative	Positive

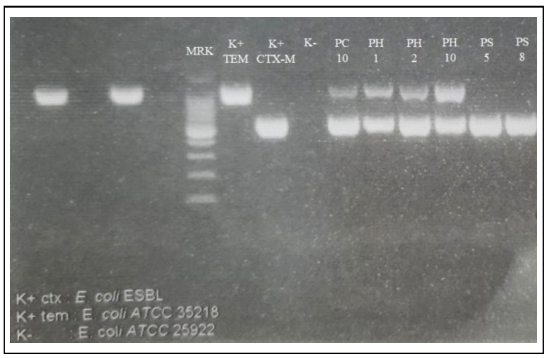

Fig 6: TEM and CTX-M gene detection electrophoresis results.

Maintaining a clean environment and washing hands before eating are two ways to prevent foodborne illness (Khairullah *et al.,* 2022; Ramandinianto *et al.,* 2020). Because many workers in traditional markets are unaware of the risks associated with slaughtering poultry without personal protective equipment, they must wear work safety equipment like masks, gloves and boots as a preventative measure to avoid being exposed to bacteria that are resistant to multiple antibiotics (Verbeek *et al.,* 2020).

CONCLUSION

To conclude the study it can be said that 14 MDR isolates were isolated from 100 chicken intestine samples, 6 of which produced ESBL and 4 of which had the TEM and CTX-M genes. The key to resolving the issue of ESBL-producing *E. coli* bacteria in chickens is for farmers to be aware of the risks of using antibiotics as feed additives and growth promoters.

ACKNOWLEDGEMENT

This study was supported in part with the Penelitian Unggulan Airlangga (PUA) Universitas Airlangga, Indonesia, in the fiscal year 2023, with grant number: 1710/UN3.LPPM/PT.01.03/2023·

Conflict of Interest

The authors have declared no conflict of interest.

REFERENCES

Abo-Almagd, E.E., Sabala, R.F., Abd-Elghany, S.M., Jackson, C.R., Ramadan, H., Imre, K., Morar, A., Herman, V. and Sallam, K.I. (2023). β-lactamase producing *Escherichia coli* encoding *bla*CTX-M and *bla*CMY genes in chicken carcasses from Egypt. Foods. 12(3): 598. doi: 10.3390/foods12030598.

Adeyanju, G.T. and Ishola, O. (2014). Salmonella and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State. Nigeria. Springerplus. 3(1): 139. doi: 10.1186/2193-1801-3-139.

Alfani, F.R., Sukarno and Haryono, A. (2021). Selling-and-buying transaction patterns in a traditional market: A generic structure potential approach. Leksema. 6(1): 47-60. doi: 10.22515/ljbs.v6i1.3466.

Ansharieta, R., Ramandinianto, S.C., Effendi, M.H. and Plumeriastuti, H. (2021). Molecular identification of *bla*CTX-M and *bla*TEM genes encoding extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* isolated from raw cow's milk in East Java, Indonesia. Biodiversitas. 22(1): 1600-1605. doi: 10.13057/biodiv/d220402.

Aslam, B., Khurshid, M., Arshad, M.I., Muzammil, S., Rasool, M., Yasmeen, N., Shah, T., Chaudhry, T.H., Rasool, M.H., Shahid, A., Xueshan, X. and Baloch, Z. (2021). Antibiotic resistance: One health one world outlook. Frontiers in Cellular and Infection Microbiology. 11(1): 771510. doi: 10.3389/fcimb.2021.771510.

Aslam, B., Wang, W., Arshad, M.I., Khurshid, M., Muzammil, S., Rasool, M.H., Nisar, M.A., Alvi, R.F., Aslam, M.A., Qamar, M.U., Salamat, M.K.F. and Baloch, Z. (2018). Antibiotic resistance: A rundown of a global crisis. Infection and Drug Resistance. 11(1): 1645-1658. doi: 10.2147/IDR.S173867.

Bajpai, T., Pandey, M., Varma, M. and Bhatambare, G.S. (2017). Prevalence of TEM, SHV and CTX-M Beta-Lactamase genes in the urinary isolates of a tertiary care hospital. The Avicenna Medical Journal. 7(1): 12-16. doi: 10.4103/2231-0770.197508.

Biesek, J., Banaszak, M., Grabowicz, M. and Wlaźlak, S. (2023). Chopped straw and coffee husks affect bedding chemical composition and the performance and foot pad condition of broiler chickens. Scientific Reports. 13(1): 6600. doi: 10.1038/s41598-023-33859-9.

Breijyeh, Z., Jubeh, B. and Karaman, R. (2020). Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve It. Molecules. 25(6): 1340. doi: 10.3390/molecules25061340.

Castanheira, M., Simner, P.J. and Bradford, P.A. (2021). Extended-spectrum β-lactamases: An update on their characteristics, epidemiology and detection. JAC-Antimicrobal Resistance. 3(3): dlab092. doi: 10.1093/jacamr/dlab092.

Chukwu, E.E., Awoderu, O.B., Enwuru, C.A., Afocha, E.E., Lawal, R.G., Ahmed, R.A., Olanrewaju, I., Onwuamah, C.K., Audu, R.A. and Ogunsola, F.T. (2022). High prevalence of resistance to third-generation cephalosporins detected among clinical isolates from sentinel healthcare facilities in Lagos, Nigeria. Antimicrobial Resistance and Infection Control. 11(1): 134. doi: 10.1186/s13756-022-01171-2.

Durairajan, R., Murugan, M., Karthik, K. and Porteen, K. (2021). Farmer's Stance on Antibiotic Resistance to *E. coli* and Extended Spectrum-β-lactamase Producing (ESBL) *E. coli* Isolated from Poultry Droppings. Asian Journal of Dairy and Food Research. 40(1): 88-93. doi: 10.18805/ajdfr.DR-1574.

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

Hossain, E., Nesha, M., Chowdhury, M.A.Z. and Rahman, S.H. (2023). Human health risk assessment of edible body parts of chicken through heavy metals and trace elements quantitative analysis. PLoS One. 18(3): e0279043. doi: 10.1371/journal.pone.0279043.

Hussain, A., Shaik, S., Ranjan, A., Nandanwar, N., Tiwari, S.K., Majid, M., Baddam, R., Qureshi, I.A., Semmler, T., Wieler, L.H., Islam, M.A., Chakravortty, D. and Ahmed, N. (2017). Risk of transmission of antimicrobial resistant *Escherichia coli* from commercial broiler and free-range retail chicken in India. Frontiers in Microbiology. 8(1): 2120. doi: 10.3389/fmicb.2017.02120.

Igbinosa, E.O., Beshiru, A., Igbinosa, I.H., Cho, G.S. and Franz, C.M.A.P. (2023). Multidrug-resistant extended spectrum β-lactamase (ESBL)-producing *Escherichia coli* from farm produce and agricultural environments in Edo State, Nigeria. PLoS One. 18(3): e0282835. doi: 10.1371/journal.pone.0282835.

Jeżak, K. and Kozajda, A. (2022). Occurrence and spread of antibiotic-resistant bacteria on animal farms and in their vicinity in Poland and Ukraine-Review. Environmental Science and Pollution Research. 29(2): 9533-9559. doi: 10.1007/s11356-021-17773-z.

Khairullah, A.R., Kurniawan, S.C., Silaen, O.S.M., Effendi, M.H., Sudjarwo, S.A., Ramandinianto, S.C., Gelolodo, M.A., Widodo, A., Riwu, K.H.P., Kurniawati, D.A. and Rehman, S. (2023). Methicillin Resistant *Staphylococcus aureus* (MRSA) Isolation and *mec*A Gene Detection from Milk and farmer hand swab in tulungagung, Indonesia. Tropical Animal Science Journal. 46(2): 231-238. doi: 10.5398/tasj.2023.46.2.231.

Khairullah, A.R., Ramandinianto, S.C. and Effendi, M.H. (2020). A Review of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) on Bovine Mastitis. Systematic Reviews in Pharmacy. 11(7): 172-183. doi: 10.31838/srp.2020.7.28.

Khairullah, A.R., Sudjarwo, S.A., Effendi, M.H., Kurniawan, S.C., Widodo, A., Silaen, O.S.M., Ramandinianto, S.C. (2024). Identification of Methicillin-resistant *Staphylococcus aureus* Isolated from dairy cow's milk in tulungagung district, Indonesia. Asian Journal of Dairy and Food Research. doi: 10.18805/ajdfr.DRF-341.

Khairullah, A.R., Sudjarwo, S.A., Effendi, M.H., Ramandininto, S.C., Gelolodo, M.A., Widodo, A., Riwu, K.H.P., Kurniawati, D.A. and Rehman, S. (2022). Profile of Multidrug Resistance and Methicillin-resistant *Staphylococcus aureus* (MRSA) on dairy cows and risk factors from farmer. Biodiversitas. 23(6): 2853-2858. doi: 10.13057/biodiv/d230610.

Kunhikannan, S., Thomas, C.J., Franks, A.E., Mahadevaiah, S., Kumar, S. and Petrovski, S. (2021). Environmental hotspots for antibiotic resistance genes. Microbiology Open. 10(3): e1197. doi: 10.1002/mbo3.1197.

Łapińska, U., Voliotis, M., Lee, K.K., Campey, A., Stone, M.R.L., Tuck, B., Phetsang, W., Zhang, B., Tsaneva-Atanasova, K., Blaskovich, M.A.T. and Pagliara, S. (2022). Fast bacterial growth reduces antibiotic accumulation and efficacy. eLife. 11(1): e74062.

Leão, C., Clemente, L., Moura, L., Seyfarth, A.M., Hansen, I.M., Hendriksen, R.S. and Amaro, A. (2021). Emergence and Clonal Spread of CTX-M-65-Producing *Escherichia coli* From Retail Meat in Portugal. Frontiers in Microbiology. 12(1): 653595. doi: 10.3389/fmicb.2021.653595.

Lemlem, M., Aklilu, E., Mohammed, M., Kamaruzzaman, F., Zakaria, Z., Harun, A. and Devan, S.S. (2023). Molecular detection and antimicrobial resistance profiles of extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* in broiler chicken farms in Malaysia. PLoS One. 18(5): e0285743. doi: 10.1371/journal.pone.0285743.

Miao, Z., Li, S., Wang, L., Song, W. and Zhou, Y. (2017). Antimicrobial resistance and molecular epidemiology of ESBL-producing *Escherichia coli* Isolated from Outpatients in town hospitals of Shandong province, China. Frontiers in Microbiology. 8(1): 63. doi: 10.3389/fmicb.2017.00063.

Mondal, T., Batabyal, K., Dey, S., Joardar, S.N., Samanta, I., Isore, D.P., Samanta, S. and Paul, A. (2022). Detection and Characterization of β-Lactamases and Biofilm-producing *Escherichia coli* and *Klebsiella pneumoniae* from Ducks and Duck Environments in West Bengal. Indian Journal of Animal Research. doi: 10.18805/IJAR.B-4915.

Munita, J.M. and Arias, C.A. (2016). Mechanisms of Antibiotic Resistance. Microbiology Spectrum. 4(2): 10.1128/microbiolspec.VMBF-0016-2015. doi: 10.1128/microbiolspec.VMBF-0016-2015.

Nalband, S.M., Kolhe, R.P., Deshpande, P.D., Jadhav, S.N., Gandhale, D.G., Muglikar, D.M., Kolhe, S.R., Bhave, S.S., Jagtap, U.V. and Dhandore, C.V. (2020). Characterization of *Escherichia coli* Isolated from bovine subclinical mastitis for virulence genes, phylogenetic groups and ESBL production. Indian Journal of Animal Research. 54(10): 1265-1271. doi: 10.18805/ijar.B-3883.

Nwobodo, D.C., Ugwu, M.C., Anie, C.O., Al-Ouqaili, M.T.S., Ikem, J.C., Chigozie, U.V. and Saki, M. (2022). Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. Journal of Clinical Laboratory Analysis. 36(9): e24655. doi: 10.1002/jcla.24655.

Pan, D. and Yu, Z. (2014). Intestinal microbiome of poultry and its interaction with host and diet. Gut Microbes. 5(1): 108-119. doi: 10.4161/gmic.26945.

Ramandinianto, S.C., Khairullah, A.R., Effendi, M.H. and Hestiana, E.P. (2020). Profile of multidrug resistance (MDR) and methicillin resistant *Staphylococcus aureus* (MRSA) on dairy farms in East Java province, Indonesia. Indian Journal of Forensic Medicine and Toxicology. 14(4): 3439-3445. doi: 10.37506/ijfmt.v14i4.12157.

Ramos, S., Silva, V., Dapkevicius, M.L.E., Caniça, M., Tejedor-Junco, M.T., Igrejas, G. and Poeta, P. (2020). *Escherichia coli* as commensal and pathogenic bacteria among food-producing animals: Health Implications of Extended Spectrum β-lactamase (ESBL) Production. Animals (Basel). 10(12): 2239. doi: 10.3390/ani10122239.

Raymond, B. (2019). Five rules for resistance management in the antibiotic apocalypse, a road map for integrated microbial management. Evolutionary Applications.12(6): 1079-1091. doi: 10.1111/eva.12808.

Sakthikarthikeyan, S., Sivakumar, M., Manikandan, R., Senthilkumar, P., Sureshkumar, V., Malmarugan, S., Prabhu, M. and Ramakrishnan, V. (2023). Prevalence and Molecular Characterization of Multidrug-resistant ESBL-producing *E. coli* in Commercial Poultry. Indian Journal of Animal Research. doi: 10.18805/IJAR.B-5166.

Saliu, E.M., Ren, H., Boroojeni, F.G., Zentek, J. and Vahjen, W. (2020). The Impact of direct-fed microbials and phytogenic feed additives on prevalence and transfer of extended-spectrum beta-lactamase genes in broiler chicken. Microorganisms. 8(3): 322. doi: 10.3390/microorganisms 8030322.

Sandegren, L. (2014). Selection of antibiotic resistance at very low antibiotic concentrations. Upsala Journal of Medical Sciences. 119(2): 103-107. doi: 10.3109/03009734.2014. 904457.

Seo, K.E. and Lee, Y.J. (2021). The occurrence of CTX-M-producing *E. coli* in the broiler parent stock in Korea. Poultry Science. 100(2): 1008-1015. doi: 10.1016/j.psj.2020.09.005.

Shaikh, S., Fatima, J., Shakil, S., Rizvi, S.M. and Kamal, M.A. (2015). Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi Journal of Biological Sciences. 22(1): 90-101. doi: 10.1016/j.sjbs.2014.08.002.

Siddiky, N.A., Khan, M.S.R., Sarker, M.S., Bhuiyan, M.K.J., Mahmud, A., Rahman, M.T., Ahmed, M.M. and Samad, M.A. (2022). Knowledge, attitude and practice of chicken vendors on food safety and foodborne pathogens at wet markets in Dhaka, Bangladesh. Food Control. 131(1): 108-456.

Sivakumar, M., Abass, G., Vivekanandhan, R., Anukampa, Singh, D.K., Bhilegaonkar, K., Kumar, S., Grace, M.R. and Dubal, Z. (2021). Extended-spectrum beta-lactamase (ESBL) producing and multidrug-resistant *Escherichia coli* in street foods: a public health concern. Journal of Food Science and Technology. 58(4): 1247-1261. doi: 10.1007/s13197-020-04634-9.

Tooke, C.L., Hinchliffe, P., Bragginton, E.C., Colenso, C.K., Hirvonen, V.H.A., Takebayashi, Y. and Spencer, J. (2019). β-Lactamases and β-Lactamase Inhibitors in the 21st Century. Journal of Molecular Biology. 431(18): 3472-3500. doi: 10.1016/j.jmb.2019.04.002.

Tyasningsih, W., Ramandinianto, S.C., Ansharieta, R., Witaningrum, A.M., Permatasari, D.A., Wardhana, D.K., Effendi, M.H. and Ugbo, E.N. (2022). Prevalence and antibiotic resistance of *Staphylococcus aureus* and *Escherichia coli* isolated from raw milk in East Java, Indonesia. Veterinary World. 15(8): 2021-2028. doi: 10.14202/vetworld.2022. 2021-2028.

Verbeek, J.H., Rajamaki, B., Ijaz, S., Sauni, R., Toomey, E., Blackwood, B., Tikka, C., Ruotsalainen, J.H. and Balci, F.S.K. (2020). Personal protective equipment for preventing highly infectious diseases due to exposure to contaminated body fluids in healthcare staff. Cochrane Database of Systematic Reviews. 4(1): CD011621. doi: 10.1002/14651858.CD011621.pub4.

White, D., Gurung, S., Zhao, D., Farnell, Y., Byrd, J., McKenzie, S., Styles, D. and Farnell, M. (2018). Evaluation of layer cage cleaning and disinfection regimens. Journal of Applied Poultry Research. 27(2): 180-187. doi: 10.3382/ japr/pfx056.

Wibisono, F.J., Sumiarto, B., Untari, T., Effendi, M.H., Permatasari, D.A. and Witaningrum, A.M. (2021). Molecular identification of CTX gene of extended spectrum betalactamases (ESBL) producing *Escherichia coli* on layer chicken in Blitar, Indonesia. Journal of Animal and Plant Sciences. 31(4): 954-959. doi: 10.36899/JAPS.2021.4.0289.

Widodo, A. Lamid, M., Effendi, M.H., Tyasningsih, W., Raharjo, D., Khairullah, A.R., Kurniawan, S.C., Yustinasari, L.R., Riwu, K.HP., Silaen, O.S.M. 2023. Molecular identification of blaTEM and blaCTX-M genes in multidrug-resistant Escherichia coli found in milk samples from dairy cattle farms in Tulungagung, Indonesia. Journal of Veterinary Research. 67(1): 381-388. doi: 10.2478/jvetres-2023-0052

Widodo, A., Lamid, M., Effendi, M.H., Khairullah, A.R., Riwu, K.H.P., Yustinasari, L.R., Kurniawan, S.C., Ansori, A.N.M., Silaen, O.S.M. and Dameanti, F.N.A.E.P. (2022). Antibiotic sensitivity profile of multidrug-resistant (MDR) *Escherichia coli* isolated from dairy cow's milk in Probolinggo, Indonesia. Biodiversitas. 23(10): 4971-4976. doi: 10.13057/ biodiv/d231002.

Xu, C., Kong, L., Gao, H., Cheng, X. and Wang, X. (2022). A review of current bacterial resistance to antibiotics in food animals. Frontiers in Microbiology. 13(1): 822689. doi: 10.3389/fmicb.2022.822689.

Yunita, M.N., Effendi, M.H., Rahmaniar, R.P., Arifah, S. and Yanestria, S.M. (2020). Identification of *spa* gene for strain typing of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from nasal swab of dogs. Biochemical and Cellular Archives. 20(1): 2999-3004. doi: 10.35124/bca.2020.20.S1.2999.