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J Vet Res 67, 381-388, 2023
DOI: 10.2478/jvetres-2023-0052

Molecular identification of *bla*TEM and *bla*CTX-M genes in multidrug-resistant *Escherichia coli* found in milk samples from dairy cattle farms in Tulungagung, Indonesia

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Received: March 30, 2023 *Accepted: September 13, 2023*

Abstract

Introduction: *Escherichia coli* is an opportunistic bacteria that can grow easily, produce toxins, and resist antibiotics. The phenomenon of *E. coli* developing multidrug resistance is currently the subject of extensive research. The objective of this study was to molecularly identify *bla*TEM and *bla*CTX-M genes in multidrug-resistant *E. coli* found in milk samples from dairy cattle farms in Tulungagung, Indonesia. **Material and Methods:** One hundred and ten milk samples were collected from 45 dairy cattle farms in Tulungagung, Indonesia. Indole, methyl red, Voges–Proskauer and in citrate tests and triple iron sugar agar tests were used to identify *E. coli*. Multidrug resistance was determined in isolates through antibiotic sensitivity tests using tetracycline, streptomycin, trimethoprim, chloramphenicol and aztreonam. Extended-spectrum beta lactamase enzyme production was confirmed by double-disc synergy test (DDST). Molecular identification was performed to confirm the *bla*TEM and *bla*CTX-M genes. **Results:** One hundred and one (91.82%) *E. coli* strains were isolated from the samples. The antibiotic sensitivity test showed four (3.96%) multidrug-resistant (MDR) and one (0.99%) ESBL-positive *E. coli* by DDST confirmation. There were three (77.78%) *bla*TEM genes and one (0.99%) *bla*CTX-M gene discovered in the MDR *E. coli* isolates using PCR for molecular identification. **Conclusion:** The findings of the *bla*TEM and *bla*CTX-M genes encoding ESBL *E. coli* in dairy cattle milk in Tulungagung, Indonesia is concerning and argues for prompt action to stop the emergence of antibiotic resistance which has an impact on public health.

Keywords: *E. coli*, *bla*TEM, *bla*CTX-M, MDR, dairy farm, public health.

Introduction

Escherichia coli is a bacterial species that typically lives in both human and animal digestive tracts. However, under some conditions, *E. coli* can spread outside of the digestive system (21). Numerous studies have shown that milk is an advantageous medium for the growth of *E. coli* and that an overabundance of these bacteria in this foodstuff can be harmful to the general public's health (18, 28). Antibiotic resistance has become a major issue on a global scale as a result of their use to treat diseases in humans and animals. Long-term antibiotic use will have an effect on the normal bacterial ecology, where pathogens adapt and change to survive. *Escherichia coli* is a bacterium that can easily gain an antibiotic deactivation enzyme expressed by an antimicrobial resistance gene (24). Penicillin, third generation cephalosporins, and monobactams are known to be hydrolysed by enzymes termed extended-spectrum beta-lactamases (ESBLs) from *E. coli* bacteria, these enzymes being the subject of extensive research at the moment.

The spread and transfer of *E. coli* with ESBLs can take place through food supply chains, contaminated faeces, contaminated water, and hazardous waste. Clinical symptoms or disorders caused by *E. coli* can appear as urinary tract infections, septic shock, and diarrhoeal illnesses (9, 18). Mastitis is a condition that can affect lactating animals and has been linked to *E. coli* infections (3, 12). According to research from 2019, 5.21% of samples of *E. coli* in dairy cattle faeces in Indonesia were ESBL-producing strains (19). Data from dairy cattle milk samples showed an incidence of ESBL *E. coli* of up to 2.15% in 2021 (2). Additional research into the prevalence of ESBL *E. coli* in 2021 on dairy farm samples indicated the rate to be up to 54% (17). However, it was estimated that by 2022 up to 0.18% of milk samples and the area around dairy cattle farms would have ESBL *E. coli* (28). The surroundings of dairy cattle sheds may contain various bacterial elements of antimicrobial resistance (27, 30). Mobile genetic elements can move between bacterial species, and transmission of resistance elements to other bacteria through plasmids and transposons can be accelerated and increased by animal activities and agricultural as well as human waste that pollutes the environment (11). An environment which is optimal in the aspect of providing bacterial resistance elements can be the main source from which these elements transfer to bacteria which could potentially infect humans, animals, or other environments (20).

Escherichia coli requires careful attention because of its strong ability to transmit resistance genes both within and between species (8). When *E. coli* with the capacity to produce ESBL enzymes infects people and other animals, it poses a serious threat. The ESBL enzyme in *E. coli* is encoded by several different ESBL genes, including the *bla*TEM, *bla*SHV, and *bla*CTX-M genes found in bacterial plasmids (29). Of these three, the *bla*CTX-M gene predominates in *E. coli* bacteria, and it can be co-expressed with the *bla*TEM gene to create the ESBL enzyme (15). A previous study discovered the phenomenon of the majority of the *bla*SHV and *bla*TEM genes becoming inactivated, making the *bla*CTX-M gene more widespread in *E. coli* bacteria (26). As noted above, the ESBL enzyme hydrolyses penicillin, third generation cephalosporins, and monobactams, meaning that three classes of antibiotic are resisted by bacteria producing the enzyme. Three antibiotic classes is the threshold for classification of a bacterial strain as multidrug resistant (MDR). Therefore, the objective of this study was to molecularly identify *bla*TEM and *bla*CTX-M genes among MDR *Escherichia coli* found in milk samples from dairy cattle farms in Tulungagung, Indonesia.

Material and Methods

In total, 110 milk samples were taken from 45 dairy farms in the Indonesian region of Tulungagung. The research was carried out from August to October of 2021. Samples were acquired from all four quarters and then placed into sterile sample vials, carefully capped, and chilled in the refrigerator for approximately 2 h before being taken to the laboratory for research. Samples were treated in the Veterinary Public Health Laboratory at the Faculty of Veterinary Medicine, Airlangga University, Indonesia. The isolated *E. coli* was incubated in GranuCult BRILA brilliant green lactose broth medium (cat. No. 105454; Merck, Darmstadt, Germany) medium at 37°C for 18 to 24 h. Eosin methylene blue agar (EMBA) selective was used to cultivate *E. coli* bacteria, which were then allowed to stand at warm temperatures (35–37°C) for 20–24 h. Colonies were verified using a Gram stain kit (cat. No. K001-1KT; HiMedia, Maharashtra, India) (9, 27). Biochemical analysis confirmed the presence of pure *E. coli* colonies by means of triple sugar iron agar (TSIA) (cat. No. 103915; Merck) and indole, methyl red, Voges–Proskauer and in citrate (IMViC) tests, the latter using sulphide indole motility (cat. No. 105470; Merck) and methyl red and Voges–Proskauer media (cat. No. 105712; Merck) and Simmons citrate agar (cat. No. CM155; Oxoid, Basingstoke, UK) (23, 28).

Antibiotic sensitivity testing on *E. coli* isolates was carried out using the disc diffusion method, as advised by the Clinical and Laboratory Standards Institute (5). After being prepared as instructed by the manufacturer, Mueller–Hinton agar (MHA) (cat. No. 105437; Merck) was cooled to 45–50°C and poured into plates. The medium was then allowed to solidify. The EMBA medium culture of *E. coli* isolates was grown for 18–24 h and standardised by dilution to 0.5 McFarland turbidity equivalence. A sterile swab stick was immersed in the standard *E. coli* dilution, dried to remove the excess load of inoculum, and smeared

across the surface of the ready MHA plate. The MHA plates were given some time to dry with the lid closed at ambient temperature (29°C). The antibiotic discs for *E. coli* susceptibility testing (Oxoid, Basingstoke, UK) with a panel of tetracycline (30 µg), streptomycin (10 µg), chloramphenicol (30 µg), trimethoprim (5 µg) and aztreonam (30 µg) were carefully placed on MHA plates using sterile forceps.

To investigate ESBL production by *E. coli* isolates, the double-disc synergy test (DDST) was employed. Cefotaxime (30 µg), ceftazidime (30 µg) and amoxicillin-clavulanate (30 µg) were the antibiotic discs used in the DDST (Oxoid). After phenotypic confirmation with the DDST, MDR and ESBL *E. coli* were genotypically validated by further examining the presence of the ESBL enzyme–coding *bla*TEM and *bla*CTX-M genes using multiplex PCR as detailed in Table 1 (2, 19). The QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) was used to extract bacterial DNA (23). As positive controls, *Escherichia coli* ATCC 35218 was utilised for the *bla*TEM gene and ESBL *E. coli* EQASIA 2021/E 21 for the *bla*CTX-M gene. *Escherichia coli* ATCC 25922 was the negative control. After amplification, the amplicons were subjected to UV light for the PCR product to be read on gel electrophoresis using a documentation system (Promega, Madison, WI, USA).

Results

The study examined 110 milk samples total from 45 dairy cattle farms, of which 101 (91.82%) tested positive for *E. coli* based on the characteristics of the EMBA culture (Fig. 1), on Gram staining and on the TSIA and IMViC tests. The antibiotic susceptibility test conducted on the 101 isolates positive for *E. coli* showed that four (3.96%) were resistant to three or more antibiotics, as shown in Table 2. The *E. coli* isolates shown in Fig. 2 to have had resistance to three or more antibiotics were MDR. In this study, aztreonam resistance was discovered in two (1.98%) of the isolates. The evaluation of the DDST following incubation revealed synergy between cefotaxime/ceftazidime and the amoxicillin-clavulanate combination, as evidenced by an expansion of the inhibition zone by 5 mm between the disc diameters of the two antibiotics, which was indicative of ESBL-positive *E. coli* bacteria (23, 28). One (0.99%) ESBL-producing isolate was obtained through being confirmed positive on the DDST, which is presented in Fig. 3.

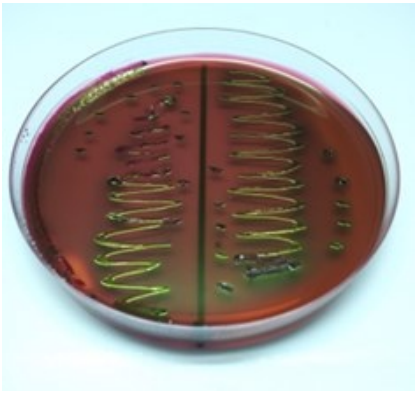

Fig. 1. *E. coli* strains growing on eosin methylene blue agar after isolation from from dairy cattle in Tulungagung, Indonesia

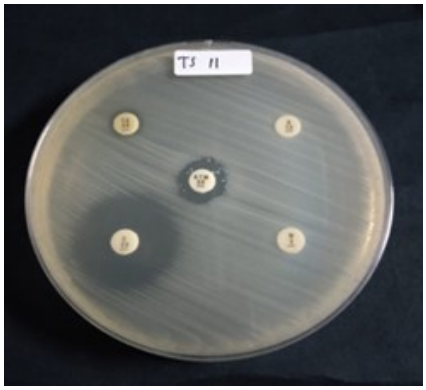

Fig. 2. Antibiotic sensitivity test in Mueller–Hinton agar of a multidrug-resistant *E. coli* strain isolated from dairy cattle in Tulungagung, Indonesia

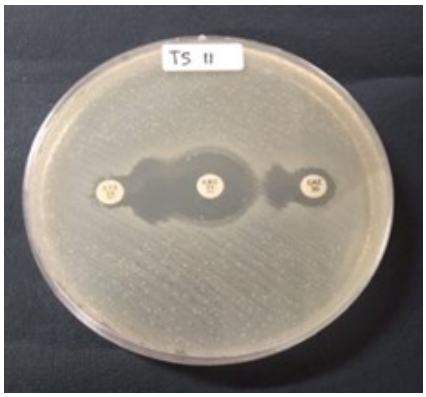

Fig. 3. Confirmation by double-disc synergy test of production of extended spectrum beta-lactamase by an E. coli strain isolated from dairy cattle in Tulungagung, Indonesia

Table 1. Primers used in this study

Primers	Sequences (5′ to 3′)	Target gene	Amplicon size	Reference
TEM-F TEM-R	ATA AAA TTC TTG AAG ACG AAA GAC AGT TAC CAA TGC TTA ATC	TEM	1.086 bp	(2, 19)
CTX-F CTX-R	CGC TTT GCG ATG TGC AG ACC GCG ATA TCG TTG GT	CTX	550 bp	(2, 19)

bp – base pairs; F – forward; R – reverse

Table 2. Characteristics of multidrug-resistant (MDR) and extended-spectrum beta-lactamase (ESBL)–producing *E. coli* strains isolated from milk samples collected from dairy cattle in Tulungagung, Indonesia

	Sample code	*E. coli* positivity	Resistance					MDR	ESBL-positivity	Identified gene	
			TE	ST	C	W	ATM			*bla*TEM	*bla*CTX-M
Farm 1	TS1	+	−	−	−	−	−	−	−	−	−
Farm 2	TS2	+	−	−	−	−	−	−	−	−	−
	TS3	+	−	−	−	−	−	−	−	−	−
	TS4	+	−	−	−	−	−	−	−	−	−
	TS5	+	−	−	−	−	−	−	−	−	−
	TS6	+	−	−	−	−	−	−	−	−	−
Farm 3	TS7	+	+	+	+	−	−	+	−	+	−
	TS8	+	−	−	−	−	−	−	−	−	−
Farm 4	TS9	+	−	−	−	−	−	−	−	−	−
Farm 5	TS10	+	−	−	−	−	−	−	−	−	−
Farm 6	TS11	+	+	+	−	+	+	+	+	−	+
	TS12	+	−	−	−	−	−	−	−	−	−
	TS13	−	−	−	−	−	−	−	−	−	−
Farm 7	TS14	−	−	−	−	−	−	−	−	−	−
	TS15	+	−	−	−	−	−	−	−	−	−
	TS16	+	−	−	−	−	−	−	−	−	−
	TS17	−	−	−	−	−	−	−	−	−	−
	TS18	+	−	+	−	−	−	−	−	−	−
	TS19	+	−	+	−	−	+	−	−	−	−
	TS20	+	−	−	−	−	−	−	−	−	−
Farm 8	TS21	+	−	−	−	−	−	−	−	−	−
	TS22	+	−	−	−	−	−	−	−	−	−
Farm 9	TS23	+	−	−	−	−	−	−	−	−	−
	TS24	+	−	−	−	−	−	−	−	−	−
	TS25	+	−	−	−	−	−	−	−	−	−
Farm 10	TS26	+	−	−	−	−	−	−	−	−	−
Farm 11	TS27	+	+	+	+	−	−	+	−	+	−
	TS28	+	−	−	−	−	−	−	−	−	−
Farm 12	TS29	+	−	−	−	−	−	−	−	−	−
	TS30	+	−	−	−	−	−	−	−	−	−
Farm 13	TS31	+	−	−	−	−	−	−	−	−	−
	TS32	+	−	−	−	−	−	−	−	−	−
Farm 14	TS33	+	−	−	−	−	−	−	−	−	−
	TS34	+	−	−	−	−	−	−	−	−	−
	TS35	+	−	+	−	−	−	−	−	−	−
Farm 15	TS36	+	−	−	−	−	−	−	−	−	−
	TS37	+	−	−	−	−	−	−	−	−	−
	TS38	+	−	−	−	−	−	−	−	−	−
Farm 16	TS39	+	−	−	−	−	−	−	−	−	−
	TS40	+	−	−	−	−	−	−	−	−	−
	TS41	+	−	−	−	−	−	−	−	−	−
Farm 17	TS42	+	+	+	−	+	−	+	−	+	−
	TS43	+	−	−	−	−	−	−	−	−	−
	TS44	+	−	−	−	−	−	−	−	−	−
	TS45	+	−	−	−	−	−	−	−	−	−
	TS46	+	−	−	−	−	−	−	−	−	−
Farm 18	TS47	+	−	−	−	−	−	−	−	−	−
Farm 19	TS48	+	−	−	−	−	−	−	−	−	−
	TS49	+	−	−	−	−	−	−	−	−	−
Farm 20	TS50	+	−	−	−	−	−	−	−	−	−
	TS51	+	−	−	−	−	−	−	−	−	−
Farm 21	TS52	+	−	−	−	−	−	−	−	−	−
	TS53	+	−	−	−	−	−	−	−	−	−
	TS54	+	−	−	−	−	−	−	−	−	−
	TS55	+	−	−	−	−	−	−	−	−	−
	TS56	−	−	−	−	−	−	−	−	−	−
	TS57	+	−	−	−	−	−	−	−	−	−
Farm 22	TS58	+	−	−	−	−	−	−	−	−	−

	TS59	+	−	−	−	−	−	−	−	−	−
	TS60	+	−	−	−	−	−	−	−	−	−
Farm 23	TS61	+	−	−	−	−	−	−	−	−	−
Farm 24	TS62	+	+	−	−	−	−	−	−	−	−
	TS63	+	+	−	−	−	−	−	−	−	−
Farm 25	TS64	+	−	−	−	−	−	−	−	−	−
	TS65	+	−	−	−	−	−	−	−	−	−
	TS66	+	−	−	−	−	−	−	−	−	−
	TS67	+	−	−	−	−	−	−	−	−	−
Farm 26	TS68	+	−	−	−	−	−	−	−	−	−
	TS69	+	−	−	−	−	−	−	−	−	−
Farm 27	TS70	+	−	−	−	−	−	−	−	−	−
	TS71	+	−	−	−	−	−	−	−	−	−
	TS72	+	−	−	−	−	−	−	−	−	−
Farm 28	TS73	+	−	−	−	−	−	−	−	−	−
Farm 29	TS74	+	−	−	−	−	−	−	−	−	−
	TS75	+	−	−	−	−	−	−	−	−	−
Farm 30	TS76	+	−	−	−	−	−	−	−	−	−
Farm 31	TS77	+	−	−	−	−	−	−	−	−	−
	TS78	+	−	−	−	−	−	−	−	−	−
Farm 32	TS79	+	−	−	−	−	−	−	−	−	−
	TS80	+	−	−	−	−	−	−	−	−	−
	TS81	+	+	−	−	−	−	−	−	−	−
	TS82	+	−	−	−	−	−	−	−	−	−
Farm 33	TS83	+	−	−	−	−	−	−	−	−	−
	TS84	+	−	−	−	−	−	−	−	−	−
Farm 34	TS85	−	−	−	−	−	−	−	−	−	−
	TS86	+	−	−	−	−	−	−	−	−	−
Farm 35	TS87	+	−	−	−	−	−	−	−	−	−
	TS88	+	−	−	−	−	−	−	−	−	−
Farm 36	TS89	+	−	−	−	−	−	−	−	−	−
	TS90	−	−	−	−	−	−	−	−	−	−
Farm 37	TS91	+	−	−	−	−	−	−	−	−	−
	TS92	+	−	−	−	−	−	−	−	−	−
Farm 38	TS93	+	−	−	−	−	−	−	−	−	−
	TS94	+	−	−	−	−	−	−	−	−	−
	TS95	+	−	−	−	−	−	−	−	−	−
Farm 39	TS96	+	−	−	−	−	−	−	−	−	−
	TS97	+	−	−	−	−	−	−	−	−	−
	TS98	+	−	−	−	−	−	−	−	−	−
Farm 40	TS99	+	−	−	−	−	−	−	−	−	−
Farm 41	TS100	+	−	−	−	−	−	−	−	−	−
Farm 42	TS101	−	−	−	−	−	−	−	−	−	−
	TS102	−	−	−	−	−	−	−	−	−	−
Farm 43	TS103	+	−	−	−	−	−	−	−	−	−
	TS104	+	−	−	−	−	−	−	−	−	−
Farm 44	TS105	+	−	−	−	−	−	−	−	−	−
	TS106	−	−	−	−	−	−	−	−	−	−
	TS107	+	−	−	−	−	−	−	−	−	−
Farm 45	TS108	+	−	−	−	−	−	−	−	−	−
	TS109	+	−	−	−	−	−	−	−	−	−
	TS110	−	−	−	−	−	−	−	−	−	−
Total		101	7	7	2	2	2	4	1	3	1
Percentage			6.93	6.93	1.98	1.98	1.98	3.96	0.99	2.97	0.99

TE – tetracycline; ST – streptomycin; C – chloramphenicol; W – trimethoprim; ATM – aztreonam

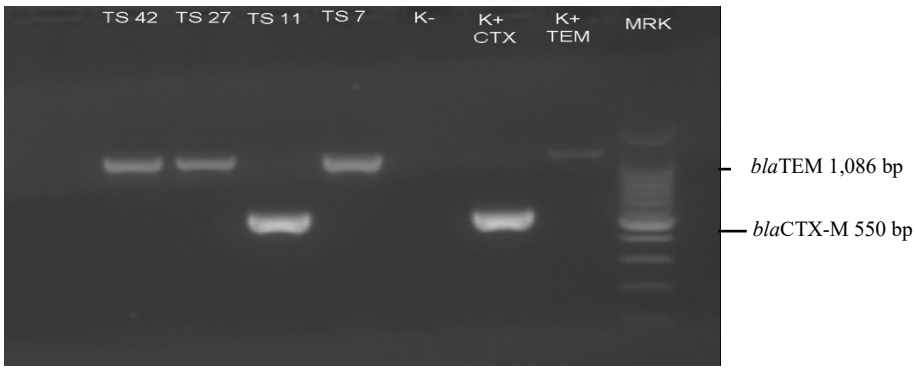

Fig. 4. Electrophoresis result for multidrug-resistant and extended-spectrum beta-lactamase–producing E. coli strains isolated from dairy cattle in Tulungagung, Indonesia. MRK – M-marker; K + TEM – positive control for blaTEM gene with E. coli ATCC35218); K + CTX – positive control for blaCTX-M gene with E. coli WHO 21.4.; K – negative control with E. coli ATCC 25922; TS7, TS27 and TS42 – multidrug-resistant E. coli confirmed double-disc synergy test–negative isolates containing the blaTEM gene (indicated by a single band of 1.086 base pairs); TS11 – multidrug-resistant E. coli confirmed double-disc test–positive isolate containing the blaCTX-M gene (indicated by a single band of 550 base pairs)

The four MDR isolates showed three patterns of antibiotic resistance, which are described in more detail in Table 3. The patterns were tetracycline (TE)-streptomycin (ST)-chloramphenicol (C) antibiotic resistance proven for two *E. coli* isolates, TE-ST-trimethoprim (W) antibiotic resistance observed for one *E. coli* isolate and TE-ST-W-aztreonam (ATM) resistance displayed by the last MDR isolate. The single ESBL-positive isolate of *E. coli* had resistance as TE-ST-W-ATM.

Molecular identification results showed that three (2.97%) MDR isolates confirmed DDST negative were detected to have the *bla*TEM gene and one (0.99%) isolate confirmed positive DDST was detected to have the *bla*CTX-M gene. Visualisation of the bands of the *bla*TEM and *bla*CTX-M gene fragments in this study is presented in Fig. 4. The electrophoresis results of positive isolates showed the same fragments as the positive controls, with gene lengths of 1,086 bp for the *bla*TEM gene and 550 bp for the *bla*CTX-M gene, while the results of isolates negative for the *bla*TEM and *bla*CTX-M genes did not represent the same fragments as the positive controls.

Discussion

In many nations, MDR *E. coli* is highly prevalent and is the cause of serious and hard-to-treat infections (28). The risk of MDR *E. coli* infection is increased by milk contamination, which can be caused by improper sanitation practices during milking and milk processing, as well as by dairy cow contact with reservoir animals. Four (3.96%) of the 101 *E. coli* isolates in this study were confirmed to be MDR *E. coli*, which means they were resistant to three or more antibiotics. This percentage is lower than that noted in a previous study which found 9 (7.26%) MDR *E. coli* isolates out of 150 isolates (28). Another study of faecal samples from cattle suffering diarrhoea found 21 (77.8%) and 28 (63.6%) MDR isolates of *E. coli* from samples of dairy cows and beef cattle, respectively (31). *Escherichia coli* isolates were tested in Ethiopia and 27 (100%) were MDR, 188 (100%) *E. coli* isolates in Nigeria were reported to be resistant to three to seven different classes of antibiotics, and 76 (53%) *E. coli* isolates demonstrated multidrug resistance in Vietnam (4, 7, 14). A discovery of MDR *E. coli* was made in milk, perhaps because of improper or excessive antibiotic use in treating infectious diseases in dairy cattle, environmental and farm personnel contamination transferred during the milking process, or the free movement of the animal (25). In this study, only one (0.99%) MDR isolate of *E. coli* was found to be positive in a DDST.

Escherichia coli with ESBLs being present in milk is dangerous enough to warrant special attention, as this bacterium can harm human consumers and calves. When dairy calves are lactating, ESBL *E. coli* can sometimes be found in their milk, whether or not they are exhibiting mastitis symptoms. This suggests that inadequate cleanliness of the milking pens also poses a risk of ESBL *E. coli* contamination of cow's milk products (25). It can be difficult to find alternative medicines to treat mastitis brought on by infection with ESBL *E. coli*, because many antibiotics (third-generation cephalosporins and aztreonam) are ineffective against such *E. coli* after the ESBL enzyme hydrolyses them (26). Animals can be responsible for human intestinal illnesses, and humans can contract them either directly from the animals (by eating food of animal origin, for example), or indirectly (*via* drinking water tainted with animal waste) (29). The animal-hosted pathogens causing these diseases in humans may transfer to people along similar exposure routes to those which have been described for various samples regarded as potential carriers of ESBL *E. coli* (17). The many potential pathways of *E. coli* ESBL transmission make epidemiological investigation very difficult (8). The expansion of ESBL-coding genes among different bacterial species will be facilitated through interactions

at the microbial level in humans, animals, and the environment through horizontal gene transfer (2).

Molecular identification results showed that three (2.97%) MDR *E. coli* isolates confirmed ESBL-negative by DDST were detected to have the *bla*TEM gene and one (0.99%) MDR *E. coli* isolate confirmed ESBL-positive by the same test was detected to have the *bla*CTX-M gene. Bacteria that are positive in the DDST are ascertained to be ESBL enzyme–producing bacteria. The ESBL bacteria were all cephalosporin-resistant, and inhibitory zone interactions with beta-lactamase inhibitor medicines such as clavulanate were discovered (Fig. 3). Moreover, the carriage of ESBL genes by the isolates implied that they could act as reservoirs of antibiotic resistance. Foods of animal origin have regularly been reported to contain *E. coli* with the *bla*TEM and *bla*CTX-M genes (22). In this study, the findings of *E. coli* isolates containing the *bla*TEM and *bla*CTX-M genes are in accord with those of other reports, which showed the same prevalence of ESBL coding genes detected in milk samples (10) and environmental samples (6). It was found that *E. coli* with ESBL encoded by the *bla*CTX-M gene and also exposed to antibiotics may under certain circumstances be able to spread the gene to other pathogenic bacteria (9).

A study has shown that CTX-M gene–bearing *E. coli* is the dominant genotype, the gene often being seen singly or in combination in strains of this genotype (15). Other research has investigated *K. pneumoniae* which produces ESBL encoded by the *bla*CTX-M gene (20). According to a study, the *bla*CTX-M-15 gene was discovered in ten clinical isolates, the *bla*CTX-M-1 gene in two clinical isolates, the *bla*CTX-M-14 gene in two clinical isolates, and the *bla*CTX-M-9 gene in two food isolates (1). The *bla*CTX-M gene–bearing *E. coli* genotype is one of the most common ESBL genotypes that cause human infections in various countries (29). Since there is a strong correlation between the presence of ESBL *E. coli* in food and the development of infections in humans, it can be inferred that food of animal origin may contain resistant bacteria, aiding in their spread among humans.

The presence of ESBL *E. coli* in milk can be associated with the milking process and inadequate environmental sanitation (16). A major portion of milk contamination is caused by improper and unclean handling of milk, particularly during the milking process. Understanding and identifying the potential for limiting the spread of ESBL-coding genes and infection in people requires an integrated approach. Global cooperation in suppressing the ecology and thus the development of ESBL *E. coli* for the protection of public health can be achieved through a multisectoral approach to healthcare in the fields of veterinary medicine and animal food production (13). The implementation of the One Health integration idea is anticipated to hasten disease prevention and prediction in the fight against ESBL *E. coli*.

The discovery of the *bla*TEM and *bla*CTX-M genes in milk samples from dairy cattle farms in Tulungagung, Indonesia is concerning and requires prompt action to prevent antibiotic resistance from developing. Furthermore, this is a new potential threat of multidrug resistance which can spread and endanger public health. Multidrug-resistant bacteria can be encouraged to colonise milk by proximate sources of pollutants from dairy cattle urine and faeces. It is possible to keep the environment clean to prevent contamination from spreading extensively, and it is particularly important to do so in areas close to dairy farms. Additional risk factors for MDR *E. coli* dissemination, such as the usage of antibiotics and general dairy farm management, should be investigated in future studies. To inhibit the spread and rise in prevalence of MDR *E. coli*, in particular impeccable hygiene in milking processes must be guaranteed and more thorough wastewater treatment methods must be devised urgently. In order to prevent a large increase in the incidence of ESBL *E. coli*, it is essential to raise public awareness of the importance of sanitation and hygiene, and suitable initiatives should be state-directed. The One Health integrative approach might alternatively be applied as a prevention strategy if its implementation is continuous.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: This research was supported in part by the Penelitian Pascasarjana - Penelitian Disertasi Doktor funding from PENELITIAN DRTPM KEMENDIKBUDRISTEK Year 2023, with a grant from Universitas Airlangga, Surabaya, Indonesia under No.: 740/UN3.LPPM/PT.01.03/2023.

Animal Rights Statement: In Indonesia, ethical committee approval is needed for research conducted on pets, laboratory animals, and wild animals which subjects them to veterinary procedures, but it is not needed for research on milk collected from dairy cattle.

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