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Incidence of *Escherichia coli* producing Extended-spectrum beta-lactamase in wastewater of dairy farms in East Java, Indonesia

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Abstract. *Dameanti FNAP, Yanestria SM, Widodo A, Effendi MH, Plumeriastuti H, Tyasningsih W, Sutrisno R, Akramsyah MA. 2023. Incidence of* Escherichia coli *producing Extended-spectrum beta-lactamase (ESBL) in wastewater of dairy farms in East Java, Indonesia. Biodiversitas 24: 1143-1150.* Poor wastewater treatment on dairy farms can potentially become environmental contaminants (hazardous chemicals, organic matter, and pathogenic bacteria). Bacteria from dairy cattle that have been resistant can be found in wastewater. It can migrate around the farm and cause resistance to environmental microbiota. Over the years, cases of antimicrobial resistance (AMR) in *Escherichia coli* bacteria are increasing due to the uncontrolled use of antibiotics in dairy farms. The present study aimed to identify the potential occurrence of dairy farm wastewater as a reservoir for Extended-Spectrum Beta-Lactamase (ESBL)-producing *E. coli* in East Java. The number of research samples used was 342 and came from 6 cities/districts with the highest dairy cattle population in East Java (Pasuruan District, Malang District, Tulungagung District, Blitar District, Batu City, and Kediri District). The results showed that 69.30% (237/342) of dairy farm wastewater samples were positive for *E. coli*. The incidence of *Escherichia coli* AMR was 99.17% (235/237). The resistance conditions to each class of antibiotics were 76.4% ampicillin, 66.2% cefotaxime, 37.6% tetracycline, 15.6% ciprofloxacin, 96.2% streptomycin, 16.5% sulfamethoxazole-trimethoprim, and 84.0% chloramphenicol. The incidence of *E. coli* MDR was 84.25% (198/235), with the highest incidence (44.44%; 88/198) found in the four groups of antibiotics. The incidence of ESBL-producing *E. coli* from samples of dairy farm wastewater in East Java through DDST confirmation was 22.80% (78/342), with the highest incidence (20.51%; 16/78) in Pasuruan District and Batu City. In conclusion, dairy farm wastewater could be a reservoir for ESBL-producing *E. coli* which has the potential to impact human health in East Java Province.

Keywords: AMR-MDR, dairy farm, ESBL, *Escherichia coli,* human health, wastewater

INTRODUCTION

Dairy cattle in Indonesia use antibiotics as cattle need antibiotics during the dry and wet seasons while getting disease/sick to treat diseases, especially mastitis, endometritis, and diarrhea (Indriani et al. 2013). Uncontrolled use of antibiotics with inappropriate and effective concentrations can lead to antibiotic resistance (Astorga et al. 2019; Said et al. 2015). Traditional farms dominate dairy farming in Indonesia with small-scale ownership and disposal of the waste in rivers or the environment around farms. It leads to antibiotic resistance and harms public health (Pamungkasih and Febrianto 2021). Poor waste treatment causes resistant bacteria in livestock to migrate around the farm, so the environmental microbiota also experiences resistance (Sore 2020). The environment around the cage can store various resistant materials that can transfer between bacteria/mobile genetic elements (MGEs). Therefore, the environment is considered the primary source of resistance between humans and animals (Niasono et al. 2016; Woolhouse et al. 2015). Antibiotic resistance and environmental pollution in the last decade have increased and become a significant threat to global health (IMF 2014).

According to WHO (World Health Organization) (2019), the cases of death caused by AMR reach 700 thousands of people per year. The use of antibiotics globally in livestock is up to 80% higher than in humans on specific antibiotics. The use of antibiotics in the livestock sector in 2013 reached 131,109 tons and is expected to reach more than 200,000 tons in 2030 (WHO 2012; He et al. 2020). Beta-lactam antibiotics contribute to 50-70% of the antibiotics world's usage (Adekanmbi et al. 2020). The over and non-scientific use of antibiotics is unwise and causes the incidence of AMR in dairy cows. In general, the incidence of AMR in dairy cows is due to high doses of antibiotics to treat disease, increase milk and meat production and improve performance while raising dairy cows (Jayarao et al. 2019). Misuse of antibiotics causes difficulty in curing bacterial infections, increases the cost of animal health care, and even causes death (Overdevest et al. 2011). ESBL-producing *Escherichia coli* in dairy cattle causes various infectious diseases, especially mastitis, and transmits them to humans through direct contact with the food chain and the environment. In humans, ESBL-producing *E. coli* pathogenic strains are associated with urinary tract infections and bacteremia in communities and hospitals (Gelalcha and Dego 2022). Antimicrobial Resistance Collaborators reported cases of death associated with AMR in 2019, reaching 4.95 million and are predicted to continue to increase to 10 million per year in 2050, and 70% of these events are caused by fluoroquinolone and ESBL resistance (Antimicrobial Resistance Collaborators 2022).

Reports of incidents of ESBL-producing *E. coli* are of severe concern. Extended-spectrum beta-lactamase (ESBL) is an enzyme that can hydrolyze and deactivate beta-lactam antibiotics, penicillin, first-second-third generation cephalosporins, and aztreonam (Biutifasari 2018). Genes or types from the TEM, SHV, and CTX-M groups are the most frequently encountered ESBLs. The ESBL coding gene resides in MGEs and produces enzymes to hydrolyze beta-lactam antibiotics. ESBL genes are mediated mainly by plasmids making it easy to transmit to other bacteria (Adekanmbi et al. 2020; Huang et al. 2020). ESBL TEM and SHV have been reported for more than 100 variants, while CTX-M has 50 variants. That was probably caused by a mutation process- associated with bacterial infection, particularly *E. coli* in the community (Dhillon and Clark 2012). *Escherichia coli* is often used as an indicator of antibiotic resistance due to its high prevalence in the environment and healthy animals (Masruroh et al. 2016). Data on the incidence of ESBL-producing *E. coli* from rectal swab samples of dairy cows have been reported in various regions in Indonesia, namely, Surabaya at 72% and Sleman Yogyakarta at 25% (Imasari et al. 2018; Maulana et al. 2021). Livestock can transmit ESBL bacteria around the farm through a mixture of urine and feces with a liquid phase, commonly called liquid waste (Musruroh et al. 2016; Said et al. 2015). This situation can cause public health problems because livestock waste is the main reservoir of ESBL-producing *E. coli* in the environment, thus increasing the transmission of ESBL to humans (Normaliska et al. 2019). Although *E. coli* is considered a typical flora and rarely causes infection, it can transfer resistance genes horizontally to pathogenic bacteria, thereby contributing to the development of chronic infections and the spread of AMR (Weber et al. 2021).

Research on dairy farm wastewater as a reservoir for ESBL-producing *E. coli* in East Java Province is essential to report. East Java Province has the highest population number of dairy cattle in Indonesia. According to data from the East Java Livestock Service (2020), the population of dairy cows in East Java reaches 293,556 heads. It is spread over six districts, with the largest populations in Pasuruan District (94,101 heads), Malang District (86,986 heads), Tulungagung District (25,139 heads), Blitar District (19,258 individuals), Batu City (12,579 individuals) and Kediri District (10,786 individuals). An increase in the dairy cattle population produces waste pollution in the environment (Widiastuti et al. 2015). Dairy cattle farms in Indonesia are generally small-scale ownership that throws waste into rivers without prior processing, polluting the environment (Hidayatullah et al. 2005; Kasworo et al. 2013; Widiastuti et al. 2015). The data on the incidence of ESBL-producing *E. coli* from rectal swab samples of dairy cows have been reported in East Java like Tulungagung District at 6% and Pasuruan at 5.9% (Putra et al. 2020; Soekoyo et al. 2020). This study aimed to determine the potential for the occurrence of dairy cattle wastewater as a reservoir for *E. coli*-producing ESBL and monitoring AMR in East Java Province so that it can understand and overcome the global situation of ESBL-producing *E. coli*.

MATERIALS AND METHODS

Study area

This study used a purposive sampling method with inclusion criteria where samples came from six cities/districts with the largest dairy cattle population in East Java, and dairy farms had a record or history of antibiotic use. The sample was obtained from Pasuruan District, Malang District, Tulungagung District, Blitar District, Batu City, and Kediri District, with 342 samples of dairy farm wastewater in June 2022.

Sampling collections

A sample of 100 ml of wastewater was isolated from ditches/cages and stored in a centrifuge tube. The sample was stored in an icebox during sample transportation and brought to the laboratory for further testing (Yanestria et al. 2019).

Isolation and identification of *Escherichia coli*

The isolation stage of *Escherichia coli* was carried out by adding 5 ml of dairy farm wastewater to Buffer Pepton Water (BPW) media with 2% of concentration (1:1) (Oxoid, UK) and incubated at 37ºC for 24 hours. The isolates from BPW media were inoculated on Eosin Methylene Blue Agar (EMBA) media (Oxoid, UK) using a streak technique and incubated at 37ºC for 24 hours (Maulana et al. 2021). Colonies that appear as metallic green were identified using gram stain and biochemical assays, IMViC (Indol-motility (HiMedia, India), Methyl Red & Voges Proskauer (HiMedia, India), Citrate (HiMedia, India)), Triple Sugar Iron Agar (TSIA) (HiMedia, India) and Urease (HiMedia, India) (Imasari et al. 2018) to identifying as *E. coli* bacteria. Procedure for gram staining with crystal violet for 2 minutes and rinse with water, followed by iodine, acetone alcohol for 1 minute, and safranin for 2 minutes. The gram-stained preparations were then observed under a 1000x magnification microscope (Tripathi and Sapra 2022).

Antibiotic susceptibility test

Isolates with confirmed *E. coli* were tested for antibiotic sensitivity using the Kirby-Bauer disc diffusion method on Mueller Hinton Agar (MHA) media (Oxoid, UK). The isolates were inoculated on Brain Hearth Infusion Broth (BHIB) medium (GranuCult, Germany) and incubated for 24 hours at 37ºC. Isolates on BHIB media were suspended with turbidity equivalent to 0.5 McFarland. The bacterial suspension was grown evenly on the MHA medium. Then seven types of antibiotic classes were placed (penicillin/ampicillin (AMP) (Oxoid, UK), cefotaxime (CTX) (Oxoid, UK), tetracycline (TE) (Oxoid, UK), ciprofloxacin (CIP) (Oxoid, UK), streptomycin (S) (Oxoid, UK), sulfamethoxazole-trimethoprim (SXT) (Oxoid, UK) and chloramphenicol (C) (Oxoid, UK)) on the surface of the media (Blaak et al. 2015) then incubated at 37ºC for 24 hours. The class of antibiotics was selected based on the AMR testing literature and the use of antibiotics in dairy farms in Indonesia. In addition, the visual inhibition zone diameter-interpretation results were adjusted to the Clinical Laboratory Standard Institute (CLSI) standards (Table 1) (CLSI 2020). *Escherichia coli* isolates that show resistance to ≥ 3 groups of antibiotics are categorized as Multidrug Resistance Bacteria (MDR) (Yanestria et al. 2022).

Confirmation of ESBL using Double Disc Synergy Test (DDST)

DDST testing was carried out on bacterial isolates with MDR properties. The DDST method was used to confirm the phenotype of ESBL-producing bacteria. DDST testing was carried out using the Kirby-Bauer disc diffusion method on MHA media with three types of antibiotics ceftazidime (CAZ; 30g) (Oxoid, UK), cefotaxime (CTX; 30g) (Oxoid, UK), and amoxicillin-clavulanic (AMC; 30/10µg) (Oxoid, UK) (Zhang et al. 2016). The bacterial suspension was re-equated to 0.5 McFarland standard and grown evenly on MHA media. The AMC antibiotic disk was placed in the middle of the media and continued with CAZ and CTX disks at a 15 mm distance from AMC in parallel. The bacterial culture was incubated at 35-37ºC for 18-24 hours (Imasari et al. 2018). The sample was confirmed phenotypically positive for ESBL when a synergistic/increased diameter of the inhibition zone ≥5 mm between the cephalosporin inhibition zone (cefotaxime and ceftazidime) and amoxicillin-clavulanic acid (Chukwunwejim et al. 2018). ESBL confirmation was carried out with the control using *E. coli* ATCC 25922 as a negative control and *Klebsiella pneumoniae* ATCC 700603 as a positive control (Masruroh et al. 2016).

RESULTS AND DISCUSSION

The results of isolation and identification of *E. coli* from a total of 342 samples of dairy farm wastewater collected after testing were 69.30% (237/342) positive for *E. coli*. These results divided into Kediri District 13.45% (46/342), Blitar District 9.07% (31/342), Malang District 12.87% (44/342), Batu City 12.57% (43/342), Pasuruan District 13.45% (46/237) and Tulungagung District 7.89% (27/342) as shown in (Table 1). The positive *E. coli* sample showed a metallic green metachromatic sheen (Figure 1 (a)), which was caused by the end product of lactose fermentation (acid), resulting in the absorption of methylene blue color (Lal and Cheeptham 2007). The isolates from EMBA media were confirmed by gram staining and biochemical tests. The gram staining test results show a rod shape and pink-red (Figure 1 (b)) (Ulfah et al. 2017).

Confirmation of the IMViC, TSIA, and Urease biochemical tests (Figure 2) showed the results following the literature, where the TSIA media turns yellow and produces gas, indole, and several motile and non-motile. In addition, *E. coli* isolates were positive on the MR test and negative on VP, Citrate, and Urease tests (Cappucino and Welsh 2020; Chu et al. 2012; Leboffe and Pierce 2011).

Antimicrobial resistance (AMR) and multidrug resistance (MDR)

All *E. coli* isolates were continued to the antibiotic resistance test according to the CLSI standards. The antibiotics used in this test were based on the literature on antibiotic testing in wastewater (Blaak et al. 2015) and the types of antibiotics that are often used in dairy farms in Indonesia. This test showed that 99.17% of *E. coli* isolates (235/237) have AMR properties. They were resistant to each class of antibiotics; ampicillin (76.4%), cefotaxime (66.2%), tetracycline (37.6%), ciprofloxacin (15.6%), streptomycin (96.2%), sulfamethoxazole-trimethoprim (16.5%) and chloramphenicol (84.0%). The detailed AMR results can be seen in (Table 3).

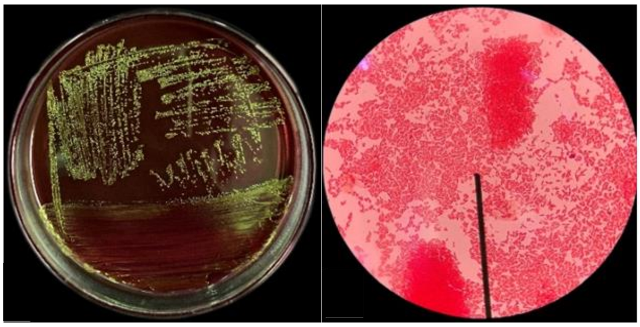
Escherichia coli isolates that show resistance to ≥ 3 are categorized as multidrug resistance bacteria (MDR). The results of the MDR incidence for 235 isolates of *E. coli* were 84.25% (198/235), presented in (Table 4). The MDR incidence showed 17.17% (34/198) for three groups of antibiotics, 44.44% (88/198) for four groups of antibiotics, 25.25% (50/198) for five antibiotic classes, 10.61% (21/198) for six antibiotic classes and 2.53% (5/198) for seven antibiotic classes.

Table 1. The standard interpretation of antibiotic sensitivity (CLSI 2020)

Antibiotics	Disc content	Inhibition zone diameter		
		S	I	R
Ampicillin	10µg	≥17	14-16	≤13
Cefotaxime	30µg	≥26	23-25	≤22
Tetracyiclin	30µg	≥15	12-14	≤11
Ciprofloxacin	5µg	≥31	21-30	≤20
Streptomycin	10µg	≥15	12-14	≤11
Trimethoprim-sulfamethoxazole	1.15/23.75µg	≥16	11-15	≤10
Cloramphenicol	30µg	≥18	13-17	≤12

Table 2. Results of the isolation and identification of *Escherichia coli* in the waste of dairy farms in East Java, Indonesia

Data isolation & identification	*Escherichia coli*		Other bacteria	
	n	%	n	%
Kediri	46	13.45	4	1.17
Blitar	31	9.07	24	7.01
Malang	44	12.87	19	5.56
Batu City	43	12.57	16	4.68
Pasuruan	46	13.45	15	4.39
Tulungangung	27	7.89	27	7.89
Total	237	69.30	105	30.70



A B

Figure 1. Results of isolation and identification of *Escherichia coli*. A. *Escherichia coli* on EMBA medium, B. *Escherichia coli* on gram staining in 1000x magnification

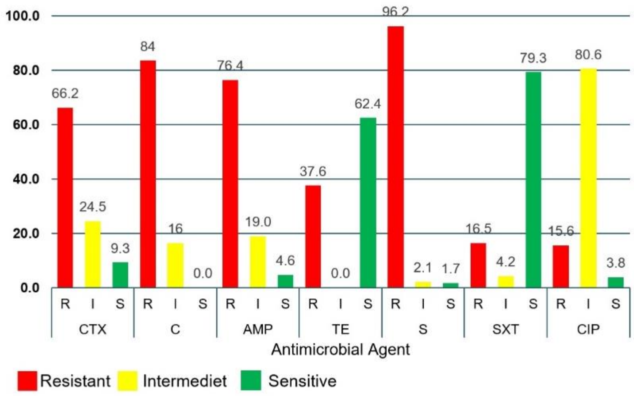
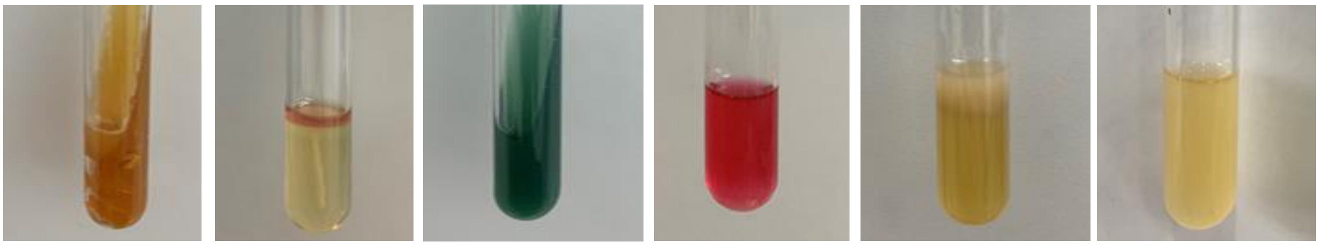


Figure 3. Results of *E. coli* antibiotic resistance to 7 classes of antibiotics. (CTX) cefotaxime, (C) chloramphenicol, (AMP) ampicillin, (TE) tetracycline, streptomycin (S), (SXT) sulfamethoxazole-trimethoprim and (CIP) ciprofloxacin, R: Resistant, I: Intermediate, S: Sensitive



A B C D E F

Figure 2. Identification results of *Escherichia coli* in the TSIA, IMViC, and Urease Biochemical tests. A. TSIA, B. SIM, C. Citrate, D. MR, E. VP, F. Urease

Table 3. Results of *Escherichia coli* antibiotic resistance in the wastewater of dairy farms in East Java, Indonesia

Data AMR		Kediri		Malang		Blitar		Batu City		Pasuruan		Tulungagung		Sub total	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%
CTX	R	24	10.1	15	6.3	19	8.0	32	13.5	41	17.3	26	11.0	157	66.2
	I	19	8.0	18	7.6	6	2.5	10	4.2	4	1.7	1	0.4	58	24.5
	S	3	1.3	11	4.6	6	2.5	1	0.4	1	0.4	0	0.0	22	9.3
C	R	38	16.0	33	13.9	25	10.5	37	15.6	41	17.3	25	10.5	199	84.0
	I	8	3.4	11	4.6	6	2.5	6	2.5	5	2.1	2	0.8	38	16.0
	S	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
AMP	R	39	16.5	30	12.7	20	8.4	32	13.5	36	15.2	24	10.1	181	76.4
	I	7	3.0	11	4.6	11	4.6	8	3.4	5	2.1	3	1.3	45	19.0
	S	0	0.0	3	1.3	0	0.0	3	1.3	5	2.1	0	0.0	11	4.6
TE	R	16	6.8	16	6.8	8	3.4	21	8.9	21	8.9	7	3.0	89	37.6
	I	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	S	30	12.7	28	11.8	23	9.7	22	9.3	25	10.5	20	8.4	148	62.4
S	R	45	19.0	42	17.7	31	13.1	43	18.1	43	18.1	24	10.1	228	96.2
	I	0	0.0	2	0.8	0	0.0	0	0.0	2	0.8	1	0.4	5	2.1
	S	1	0.4	0	0.0	0	0.0	0	0.0	1	0.4	2	0.8	4	1.7
SXT	R	11	4.6	9	3.8	4	1.7	8	3.4	3	1.3	4	1.7	39	16.5
	I	2	0.8	0	0.0	1	0.4	5	2.1	1	0.4	1	0.4	10	4.2
	S	33	13.9	35	14.8	26	11.0	30	12.7	42	17.7	22	9.3	188	79.3
CIP	R	5	2.1	2	0.8	5	2.1	9	3.8	11	4.6	5	2.1	37	15.6
	I	40	16.9	38	16.0	24	10.1	34	14.3	33	13.9	22	9.3	191	80.6
	S	1	0.4	4	1.7	2	0.8	0	0.0	2	0.8	0	0.0	9	3.8

Table 4. Results of MDR *Escherichia coli* occurrence in dairy farm wastewater in East Java, Indonesia

Antibiotic classes	Kediri		Malang		Blitar		Batu City		Pasuruan		Tulungangung		Sub total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
3	10	29.41	10	29.41	5	14.71	4	11.76	3	8.82	2	5.88	34	17.17
4	16	18.18	12	13.64	11	12.50	16	18.18	17	19.32	16	18.18	88	44.44
5	8	16.00	5	10.00	3	6.00	12	24.00	16	32.00	6	12.00	50	25.25
6	4	19.05	3	14.29	3	14.29	6	28.57	5	23.81	0	0.00	21	10.61
7	1	20.00	1	20.00	1	20.00	0	0.00	0	0.00	2	40.00	5	2.53

Table 5. The results of ESBL-producing *Escherichia coli* using the DDST test on the waste of dairy farms in East Java, Indonesia

Location	Positive ESBL	Negative ESBL
	n	n
Kediri	11	35
Blitar	12	19
Malang	9	35
Batu City	16	27
Pasuruan	16	30
Tulungagung	14	13
Total	78	159

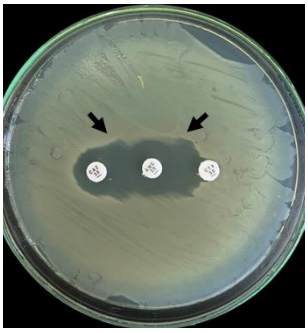


Figure 4. ESBL confirmation results for *Escherichia coli* using the DDST test. (AMC) amoxicillin-clavulanic acid, (CTX) Cefotaxime, (CAZ) Ceftazidime, arrows are the synergies formed

Confirmation of ESBL using *Double Disc Synergy Test* (DDST)

The DDST result was positive based on the synergy between cephalosporin antibiotics and amoxicillin-clavulanic acid. This synergy was observed with an increase in the diameter of the inhibition zone observed (Figure 4) (Chukwunwejim et al. 2018). The results of ESBL-producing *E. coli* from samples of dairy farm wastewater in East Java with DDST confirmation were 22.80% (78/342), presented in (Table 4). The confirmation results of ESBL-producing *E. coli* in Kediri District 14.10% (11/78), Blitar District 15.38% (12/78), Malang District 11.54% (9/78), Batu City and Pasuruan District 20.51% (16/78), 17.95% (14/78) Tulungangung District. The highest incidence occurred in Pasuruan District and Batu City at 20.51% (16/78).

Discussion

According to data from the East Java Livestock Service (2020), the population of dairy cows in East Java reaches 293,556 heads. It is spread over six districts, with the largest populations in Pasuruan District, Malang District, Tulungagung District, Blitar District, Batu City, and Kediri District. An increase in the dairy cattle population resulted in increasing environmental waste pollution (Widiastuti et al. 2015). Dairy cattle farms in Indonesia are generally small-scale ownership that throws waste into rivers without prior processing, polluting the environment (Hidayatullah et al. 2005; Kasworo et al. 2013; Widiastuti et al. 2015). In addition, waste from animals treated with antibiotics can contain antibiotic residues and resistant bacteria (FAO 2018; Loo et al. 2019). A study by Marshall and Levy (2011) stated that 75-90% of animal antibiotics are not metabolized in the intestine and are then excreted through waste. This incident can cause a problem because animal waste containing resistant bacteria and antibiotics can enter the sewage system and water sources, thus, helping the emergence of antimicrobial-resistant bacteria in the environment (Loo et al. 2019).

The results of this study indicated that of the 69.30% (237/342) samples that were positive for *E. coli*, the other 30.7% were for other pathogenic bacteria, like *Klebsiella sp.*, *Proteus sp.*, *Pseudomonas sp.*, and *Enterobacter sp.* based on the results of identification on EMBA media. Not only pathogenic, but these bacteria also resist most antibiotics and show a high Minimal Inhibitory Concentration (MIC) of 30 to 100 µg/ml (Atef and Idriss 2013). The *E. coli* isolates (99.17%; 235/237) had AMR properties. Based on Baker et al. (2022), the waste generated from dairy farms could be a way of discovering the presence of AMR bacteria. The prevalence value of AMR in this study was higher than the study conducted by Massé et al. (2021), which stated that 70% of the samples were resistant to the antibiotics tested. Bacteria with AMR properties that pollute the environment can indirectly affect humans (Landers 2012). AMR causes an increase in morbidity and mortality due to microorganisms in humans. The side effects of AMR are increased disease severity, increased risk of complications, increased case fatality rate, increased mortality, delayed treatment, treatment failure, use of expensive drugs, increased economic burden or costs, and more extended stay in hospital and intensive care units. Medical treatment to the higher costs associated with implementing AMR infection control measures in healthcare units (Friedman et al. 2015). The global cost of handling AMR is very high and varies in each country. The US Center for Disease Control and Prevention (CDC) estimates that the cost of losses due to antimicrobial resistance is $55 billion annually in the United States, of which $20 billion is spent on healthcare in the AMR sector and $35 billion due to decreased productivity (Prestinaci 2015).

The AMR *E. coli* showed significant antibiotic resistance, 76.7% ampicillin, 96.2% streptomycin, 66.2% cefotaxime, and 84.0% chloramphenicol. The antibiotics ampicillin (penicillin) and streptomycin show high resistance because these antibiotics are often used to treat mastitis and endometritis in dairy cows. This combination of antibiotics is used because it is a broad-spectrum antibiotic (Riyanto et al. 2016; Negasee 2020). The results of resistance to ampicillin and cefotaxime can indicate the ESBL enzyme's activity. Thus, the use of these antibiotics needs awareness of their side effects (Dhillon and Clark 2012). In addition, second and third-generation cephalosporin antibiotics have been reported to be a risk factor for infection with ESBL-producing bacteria (Amin et al. 2020; Wibisono et al. 2020).

Apart from penicillin and streptomycin, chloramphenicol, beta-lactam antibiotics, and the antibiotic sulfonamide group are often used in dairy cows (Ansharieta et al. 2021). Chloramphenicol also showed resistance results that were significantly different from the research conducted by Maulana et al. (2021) on samples on dairy farms in Sleman, Jogjakarta. Resistance to chloramphenicol is caused by pressure on bacteria through the co-selection of mobile resistance elements or unknown substrates against chloramphenicol (Tadesse et al. 2012). In different circummures, sulfamethoxazole-trimethoprim and tetracycline showed significant sensitivity in 79.3% and 62.4%. These results can be used as a basis for the recommended use of antibiotics to treat infectious diseases in dairy cattle in East Java.

The highest multidrug-resistant (MDR) incidence was 44.44% for four groups of antibiotics. In addition, 2.53% of isolates are resistant to 7 antibiotics that need awareness. Multidrug resistance is common in ESBL-producing bacteria (Gregova et al. 2012). Furthermore, MDR shows microbial resistance to three or more classes of antibiotics (Effendi et al. 2021). These results impact the selection of antibiotics for treating limited bacterial infections. Resistant bacterial infections cause prolonged illness, increase the risk of death, increase the length of stay in the hospital, slow treatment response failure, causing patients to be infectious for a long time (carriers) and significant opportunities for resistant bacteria to spread to other organisms in a population (Deshpande et al. 2011). In addition, MDR is caused by the uncontrolled use of antibiotics, causing selective pressure from resistant bacteria to the evolutionary process through genetic mutations, exchange of genetic material, and proliferation to defend themselves against various types of antibiotics (Yanestria et al. 2022).

The results of ESBL-producing *E. coli* from samples of dairy farm wastewater in East Java with a DDST confirmation of 22.80% (78/342). This result should be noticed because there is an increase in the incidence compared to the results of other studies in Sleman (Yogyakarta, Indonesia), which is 16% (15/93). Data on the incidence of ESBL-producing *E. coli* in dairy farm wastewater were also reported by several countries, like Chiang Mai (Thailand) 88.7% and Tunisia (North Africa) 15.8% (Maulana et al. 2021; Saekhow and Sriphannam 2021; Said et al. 2015). The incidence of ESBL-producing *E. coli* in Indonesia is also increasing, as evidenced by the data on the incidence of ESBL-producing *E. coli* bacteria in various samples. For example, pneumonia patients at Sanglah General Hospital (Bali) reached 93.3%; at Wahidin General Hospital (Makassar) 80%; Blitar from swabs broiler cloaca 28.75% and layer 7.03%; broiler stool samples in Bogor 25%; rectal swabs of dairy cows 72% and companion dogs 9.41% in Surabaya; environmental samples of broiler farms in Pasauruan 9.14% and samples of Sleman dairy farms (Yogyakarta) reached 54% (Manuaba et al. 2021; Pratama et al. 2019; Widhi and Saputra 2020; Masruroh et al. 2016; Wibisono et al. 2020; Kristianingtyas et al. 2021; Yanestria et al. 2022; Imasari et al. 2018; Maulana et al. 2021).

Some evidence shows increased ESBL-producing *E. coli*, especially in dairy cows. The increased ESBL organisms in dairy farms can be a public health risk because *E. coli* can be zoonotic and transmitted to humans by various routes. Environments contaminated with ESBL-producing *E. coli* bacteria can potentially spread these bacteria to humans. An environment polluted by ESBL-producing *E. coli* allows amino acid substitution or genetic mutations to horizontal gene transfer through transformation, transduction, and conjugation processes to the environmental microbiota. Environmental microbiota can be a reservoir of ESBL resistance genes (Amine, 2013). As a result, variants from the TEM, SHV, and CTX-M groups, through gradual mutations, which are identified, continue to increase (Gelalcha and Dego 2022). ESBL coding genes are mainly located on plasmids and chromosomes, transferred from one organism to another, impacting a higher prevalence epidemiologically (Biutifasari 2018; Canton et al. 2012). ESBL-producing *E. coli* has the risk of spreading resistant genes, especially to susceptible individuals such as pregnant women, infants, children, the elderly, and people with immunosuppression, as well as postoperative and chemotherapy patients (Franz et al. 2015). ESBL-producing *E. coli* causes mortality, morbidity, and increasing death incidences (Nóbrega and bronchi 2014).

This study concludes that ESBL-producing *E. coli* in the wastewater of dairy farms in East Java has the potential to become a reservoir in the livestock environment. These results are significant for creating public awareness of antibiotics and wastewater treatment used to create better public health and welfare. Research and collaboration within the scope of One health are needed to deal with the incidence of ESBL-producing *E. coli* from wastewater, and the government needs to supervise the treatment of dairy farm wastewater.

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