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Antimicrobial resistance characteristics of multidrug resistance and extended-spectrum beta-lactamase producing *Escherichia coli* from several dairy farms in Probolinggo, Indonesia

AGUS WIDODO[1], MIRNI LAMID[2], MUSTOFA HELMI EFFENDI[3,♥], ASWIN RAFIF KHAIRULLAH[1], SHENDY CANADYA KURNIAWAN[4], OTTO SAHAT MARTUA SILAEN[5], KATTY HENDRIANA PRISCILIA RIWU[1], LITA RAKHMA YUSTINASARI[6], DANIAH ASHRI AFNANI[7], FIDI NUR AINI EKA PUJI DAMEANTI[8], SANCAKA CHASYER RAMANDINIANTO[9]

[1]Doctoral Program in Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia
[2]Department of Animal Husbandry, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia
[3]Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia. Tel./Fax.: +62-31-5993016, ♥email: mhelmieffendi@gmail.com
[4]Master program of Animal Sciences, Department of Animal Sciences, Specialisation in Molecule, Cell and Organ Functioning, Wageningen University and Research. Wageningen 6708 PB, Netherlands
[5]Doctoral Program in Biomedical Science, Faculty of Medicine, Universitas Indonesia. Jl. Salemba Raya No. 6 Senen, Jakarta 10430, Indonesia
[6]Joint Graduate School, Veterinary Anatomy Laboratory, Faculty of Veterinary Medicine, Yamaguchi University. 1677-1 Yoshida, Yamaguchi 753-8511, Japan
[7]Graduate Program in Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia
[8]Microbiology and Immunology Laboratory, Faculty of Veterinary Medicine, Universitas Brawijaya. Jl. Veteran, Ketawanggede, Lowokwaru, Malang 65145, East Java, Indonesia
[9]Lingkar Satwa Animal Care Clinic. Jl. Sumatera No. 31L, Gubeng, Surabaya 60281, East Java, Indonesia

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Abstract. *Widodo A, Lamid M, Effendi MH, Khairullah AR, Kurniawan SC, Silaen OSM, Riwu KHP, Yustinasari LR, Afnani DA, Dameanti FNAEP, Ramandinianto SC. 2022. Antimicrobial resistance characteristics of multidrug resistance and extended spectrum beta-lactamase producing* Escherichia coli *from several dairy farms in Probolinggo, Indonesia. Biodiversitas 23: 215-221. Escherichia coli* bacteria initially reside in the digestive tract of humans and animals but are able to adapt to new environments that are different from their initial habitat. The pathogenicity of *E. coli* can occur when these bacteria grow more than normal limits, produce toxins, and are resistant to certain types of antibiotics. The purpose of this study was to investigate the antimicrobial resistance characteristics of MDR and ESBL-producing *E. coli* from several dairy farms in the Probolinggo district of East Java province, Indonesia. A total of 150 samples consisting of 109 milk and 41 environmental samples from 41 dairy farms were used for isolation. TSIA and IMViC biochemical tests were used to identify *E. coli* bacteria. *Escherichia coli* resistance profile was obtained through disc diffusion test on several antibiotics, namely tetracycline, streptomycin, trimethoprim, chloramphenicol and aztreonam. *Escherichia coli* that was resistant to 3 or more antibiotics was defined as MDR. The results of isolation and identification obtained 124 (82.6%) isolates characterizing *E. coli* bacteria. The antimicrobial susceptibility test of *E. coli* showed 9 (7.26%) MDR isolates and 2 (22.22%) ESBL isolates by double-disc synergy test (DDST). MDR *E. coli* was dominated by the pattern of antimicrobial drug resistance TE-S-W (tetracycline, streptomycin, trimethoprim) with a total of 8 (38.10%) isolates, followed by antimicrobial drug resistance pattern TE-S-W-ATM (tetracycline, streptomycin, trimethoprim, aztreonam) with one (4.76%) *E. coli* isolates. The pattern of antimicrobial drugs of ESBL *E. coli* showed in one (11.11%) sample of ESBL *E. coli* from a milk sample with the pattern of TE-S-W-ATM (tetracycline, streptomycin, trimethoprim, aztreonam) and one (11.11%) sample ESBL *E. coli* (AL 30) from the environmental sample with a pattern of TE-S-W pattern (tetracycline, streptomycin, trimethoprim). The discovery of MDR *E. coli* isolates and ESBL *E. coli* from milk and environmental samples at several dairy farms in Probolinggo district, East Java, Indonesia is a matter of concern and requires real action to reduce antibiotic resistance.

Keywords: Dairy farm, ESBL, *Escherichia coli*, MDR, public health

INTRODUCTION

Escherichia coli is an important bacterium that can cause public health problems, especially food hygiene issues (Abebe et al. 2020). *Escherichia coli* usually live well in the digestive tract of humans and animals, under certain circumstances moving to other habitats and causing disease (Disassa et al. 2017; Katouli et al. 2010). *Escherichia coli* is a motile bacterium with flagella (Sim et al. 2017), so it can grow and spread more quickly under optimum growth conditions. *Escherichia coli* ferment glucose including lactose to survive. Milk is rich in lactose for the growth of *E. coli* bacteria (Chaleshtori et al. 2017). Several studies have found that milk can be an important medium for *E. coli* which has an effect on public health problems (Widodo et al. 2020) and some even cause more

serious diseases in humans (Mueller and Tainer 2022). The presence of pathogenic *E. coli* in milk raises concerns about the emergence of a health problem known as Milk Borne Disease (Chaleshtori et al. 2017).

The presence of *E. coli* in food can be a public health problem, due to the presence of enteropathogenic and toxigenic properties that cause gastrointestinal disorders (Jang et al. 2017). *Escherichia coli* can grow in milk and dairy products (Chaleshtori et al. 2017) and is associated with mastitis (Aslam et al. 2021). Its contamination usually occurs, during the milking process and the application of poor environmental hygiene (Garbaj et al. 2016; Sharafati-chaleshtori and Rafiean-kopaei 2014). *Escherichia coli* can be used as an indicator of environmental sanitation assessment (Martin et al. 2016). The presence of *E. coli* in cow's milk is considered an indication of contamination by cow feces (Tanaro et al. 2010; Jaakkonen et al. 2019) but may also indicate that most of the contamination in milk is caused by contamination from the environment (Martin et al. 2016; Calahorrano-Moreno et al. 2022).

Antibiotics used in livestock and poultry farming for therapeutic health purposes have created new phenomena and problems, namely the emergence of bacterial resistance to certain antibiotics (Effendi et al. 2018; Effendi et al. 2021; Wibisono et al. 2020). *Escherichia coli* is opportunistic so it can live in various places and grow rapidly (Kristiawan et al. 2022), strains of *E. coli* bacteria become resistant and pathogenic and are able to infect animals and humans (Widodo et al. 2020). Manifestations of disease in animals caused by these bacteria result in mastitis (Aslam et al. 2021). Other diseases are digestive diseases in animals and humans which are often caused by *E. coli* (EPEC) strains (Eldesoukey et al. 2022), urinary traTable 1ct diseases and septic shock in humans (Mueller and Tainer 2022).

Escherichia coli resistance was reported on dairy farms in Ethiopia by Dejene et al. (2022) screened 27 isolates were 100% MDR *E. coli*. 60% of these urinary tract infections in Canada are MDR *E. coli*, which is resistant to more than three classes of antibiotics. The occurrence of ESBL *E. coli* in dairy farms has also been reported in Germany (Dahms et al. 2015), China (Zheng et al. 2019), New Zealand (Burgess et al. 2021) and Malaysia (Kamaruzzaman et al. 2020). In Indonesia, the first occurrence of ESBL *E. coli* was found in a milk sample from a dairy farm in West Java (Sudarwanto et al. 2017). Reported on several samples from dairy farms in Yogyakarta province in 2021 (Maulana et al. 2021), from dairy farm feces in East Java in 2019 (Putra et al. 2019), from raw milk samples in 2021 (Ansharieta et al. 2021), and milk samples from dairy cows in East Java in 2022 (Widodo et al. 2022), its was found that *E. coli* from the samples contained ESBL encoding gene.

Escherichia coli is a bacterium that needs attention in terms of its ability to spread inter-species and intra-species resistance genes (Effendi et al. 2018). MDR *E. coli* carried by cattle can be transmitted to humans through contact with carrier animals or through the consumption of contaminated food products (Rasheed et al. 2014). Bacteria that are MDR are very dangerous if they cause disease or pathogens in humans and animals. The choice of types of antibiotics for treatment is limited, the healing of the disease takes longer and the cost of treatment also increases (Handayani et al. 2017). The multidrug-resistant form of *E. coli* that is now a concern for researchers is ESBL. ESBL enzyme is a beta-lactamase enzyme that has an expanded ability to hydrolyze penicillins, third-generation cephalosporins and monobactam antibiotics including aztreonam (Mariana et al. 2021). The presence of the ESBL enzyme is encoded by the TEM, SHV and CTX genes in bacterial plasmids (Widodo et al. 2020). So, the aim of this study was to determine the antimicrobial resistance characteristics of MDR and ESBL *E. coli* from several dairy farms in the Probolinggo district of East Java province, Indonesia.

MATERIALS AND METHODS

Sample collection

A total of 150 samples included 109 milk and 41 environment samples from several dairy farms in Probolinggo district, East Java, Indonesia. The study was carried out between July and September of 2021. The samples were taken in a sterile screw-capped vial, transported in an icebox to the laboratory within two hours, and examined. Samples were processed at the Department of Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia.

Isolation and identification

The isolated *E. coli* was cultured at 37°C for 18 to 24 hours utilizing enrichment in Brilliant Green Lactose Broth (BGLB) media (Merck, 105454). *Escherichia coli* bacteria was grown using selective media in Eosin Methylene Blue Agar (EMBA) media (Merck, 101347), and then kept warm (35-37°C) for 20-24 hours. *Escherichia coli* colonies were identified on EMBA media and confirmed using Gram staining kit (HiMedia, K001-1KT). Pure colonies of *E. coli* were further verified by biochemical testing of IMVIC media including Sulifide Indol Motility (SIM) media (Merck, 105470), Methyl Red-Voges Proskauer (MR-VP) media (Merck; 105712), Simmons Citrate Agar (Oxoid, CM155) and Triple Sugar Iron Agar TSIA media (Merck; 103915) (Effendi et al. 2018; Tyasningsih et al. 2022).

Antimicrobial susceptibility test

Antimicrobial susceptibility test was performed on *E. coli* isolates using the Kirby-Bauer disc diffusion technique, as recommended by the Clinical and Laboratory Standards Institute (CLSI 2018). Mueller Hinton Agar (MHA) (Merck, 105437) was prepared as directed by the manufacturer and allowed to cool to 45-50°C before pouring into plates. Plates were allowed to dry after the agar had solidified. A broth culture of *E. coli* isolates aged 18-24 hours was standardized by diluting to 0.5 McFarland's standard. A sterile swab stick was inserted into the standardized *E. coli* inoculum, drained to remove excess inoculum load, and inoculated on the surface of prepared MHA plates by spreading. After that, inoculated MHA plate was allowed to dry at room temperature (29°C)

with the lid closed for a few minutes. After the agar surface was dried, antibiotic-impregnated discs of known concentrations for *E. coli*, including tetracycline (30 g), streptomycin (10 g), chloramphenicol (30 g), trimethoprim (5 g), and aztreonam (30 g), were carefully placed on the inoculated MHA plates with sterile forceps and incubated at 37°C for 18-24 hours. The diameters of inhibition zones were measured with a ruler to the nearest millimeter. Results were recorded and interpreted according to the CLSI (CLSI 2018). The presence of multidrug resistance, namely the sensitivity of *E. coli* bacteria to more than three classes of antibiotics, was one of the outcomes of resistance (Ansharieta et al. 2021)

ESBL confirmation test by DDST

Escherichia coli isolates were further investigated for the presence of ESBL using the double-disc synergy test (DDST). The antibiotic discs used for DDST were amoxicillin-clavulanate (20/10 μg), cefotaxime (30 μg), and ceftazidime (30 μg) (Tyasningsih et al. 2022). The results of evaluation after incubation showed synergy between cefotaxime/ceftazidime with the Amoxycillin-clavulanic combination in the form of an increase in inhibition zone ≥ 5 mm between the diameter of the cephalosporin disk and the cephalosporin-clavulanate combination showed *E. coli* positive ESBL bacteria (Putra et al. 2020; Tyasningsih et al. 2022). DDST was used to look for the presence of ESBL in *E. coli* isolates. Amoxicillin-clavulanate (20/10 g), cefotaxime (30 g), and ceftazidime (30 g) antibiotic discs were used for DDST (Anshariesta et al. 2021). The evaluation after incubation revealed a synergy between cefotaxime/ceftazidime and the Amoxycillin-clavulanic combination in the form of a 5 mm increase in inhibition zone between the diameter of the cephalosporin disc and the cephalosporin-clavulanate combination, according to *E. coli* positive ESBL bacteria (Putra et al. 2020; Tyasningsih et al. 2022).

RESULTS AND DISCUSSION

A total of 150 samples (109 milk samples and 41 environmental samples) were used in the study, and 124 samples (82.67%) were found positive for *E. coli* (Figure 1.A). Sample examination showed that 87 (70.16%) samples were positive for *E. coli* in milk and 37 (29.84%) samples were positive in environmental samples, based on morphological culture characteristics and biochemical tests (Table 1). The positive number of *E. coli*, which was 82.67% can be attributed to a variety of factors, the majority of which were related to milking hygiene and environmental sanitation.

The results of classification of *E. coli* based on resistance group of antibiotic (Table 2) showed that 9 *E. coli* isolates (7.26%) were resistant to 1 class of antibiotics tested, while 3 *E. coli* isolates (2.42%) were resistant to 2 classes of antibiotics, and 9 *E. coli* isolates (7.26%) were confirmed to be MDR because they were resistant to 3 or more classes of antibiotics (Figure 1.B) which were dominated by the pattern of antimicrobial drug resistance TE-S-W (tetracycline, streptomycin, trimethoprim) with a total of 8 (38.10%) *E. coli* isolates followed by antimicrobial drug resistance pattern TE-S-W-ATM (tetracycline, streptomycin, trimethoprim, aztreonam) with one (4.76%) *E. coli* isolates (Table 3).

The existence of MDR *E. coli* isolates was caused by the occurrence of integrated antimicrobial genetic transfer of the genome from certain sources. Table 4 showed that there were 9 isolates of MDR *E. coli*, 6 from milk and 3 from environmental samples. Only two (22.22%) isolates of positive ESBL *E. coli* were isolated from one milk sample and one sample from environment as shown in Table 5. The results of ESBL confirmation using DDST showed synergy between cefotaxime/ceftazidime and Amoxycillin-clavulanic combination in the form of an increase in the inhibition zone between the diameter of cephalosporin disc with the cephalosporin-clavulanate combination was expressed as positive ESBL *E. coli* (Figure 1.C). The pattern of antimicrobial drugs resistance of ESBL *E. coli* is presented in Table 6, one sample of ESBL *Escherichia coli* (AS 27) from a milk sample with the pattern of TE-S-W-ATM (tetracycline, streptomycin, trimethoprim, aztreonam) and one sample ESBL *E. coli* (AL 30) from the environmental sample with a pattern of TE-S-W pattern (tetracycline, streptomycin, trimethoprim).

Table 1. Isolation of *Escherichia coli* from various samples

Sample types	Samples code	Sample size	Positive *E. coli* (%)
Milk	AS	109	87 (70.16%)
Environment	AL	41	37 (29.84%)
Total		150	124 (82.67%)

Note: % (Percentage of positive *Escherichia coli*).

Table 2. Classification of *Escherichia coli* based on resistance of antibiotic class

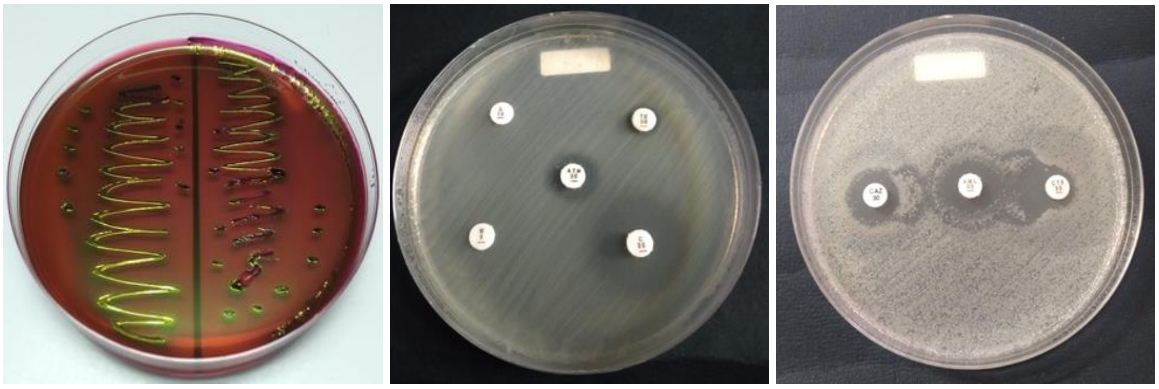
Resistance of antibiotics	Number of isolates	Percentage
3 or more antibiotics	9	7.26 %
2	3	2.42 %
1	9	7.26 %
No resistance	103	83.06 %
Total	124	100 %

Note: % = (Percentage of resistance on antibiotic class).

Table 3. Antimicrobial drug resistance pattern of *Escherichia coli*

Antibiotic pattern	Frequency (n = 21)	Percentage
TE	6	28.58%
S	2	9.52%
ATM	1	4.76%
TE - S	2	9.52%
S - W	1	4.76%
TE - S - W	8	38.10%
TE- S - W - ATM	1	4.76%
Total	21	100%

Note: TE: tetracycline 30 μg, S: streptomycin 10 μg, C: chloramphenicol 30 μg, W: trimethoprim 5 μg, ATM: aztreonam 30 μg; and %: (Percentage of antibiotic pattern).



A **B** **C**

Figure 1. A. *Escherichia coli* isolates in Eosin Methylene Blue Agar (EMBA**),** B. Antimicrobial susceptibility test of multidrug-resistant (MDR) producing *E. coli* isolate in MHA, C. Confirmation for extended spectrum beta-lactamase (ESBL) *Escherichia coli* isolates by DDST (AS-27)

Table 4. Antimicrobial susceptibility profile of multidrug resistance *Escherichia coli* isolates

Sample type	Sample codes	Antibiotic pattern of multidrug resistance				
		TE (30 µg)	S (10 µg)	C (30 µg)	W (5 µg)	ATM (10 µg)
Milk	AS 1	√	√	-	√	-
	AS 5	√	√	-	√	-
	AS 12	√	√	-	√	-
	AS 15	√	√	-	√	-
	AS 27	√	√	-	√	√
	AS 40	√	√	-	√	-
Environ-ment	AL 19	√	√	-	√	-
	AL 30	√	√	-	√	-
	AL 40	√	√	-	√	-

Note: √: *Resistant*, TE: *tetracycline*, S: *streptomycin*, C: *chloramphenicol*, W: *trimethoprim*, ATM: *aztreonam* 30.

Table 5. Total number confirmed extended spectrum beta-lactamase *Escherichia coli* isolates by DDST

Sample types	Positive *E. coli* (n= 124)	No. of isolates tested ESBL by DDST (n = 9)	No. of positive ESBL	Percentage
Milk	87	6	1	11.11%
Environment	37	3	1	11.11%
Total	124	9	2	22.22%

Note: %: Percentage of positive ESBL *Escherichia coli* isolates.

Table 6. Antimicrobial susceptibility profile of extended spectrum beta-lactamase *Escherichia coli* isolates

Sample types	Samples code	Antibiotic pattern of Extended-spectrum beta-lactamase				
		TE (30 µg)	S (10 µg)	C (30 µg)	W (5 µg)	ATM (10 µg)
Milk	AS 27	√	√	-	√	√
Environment	AL 30	√	√	-	√	-

Note: √: Resistant, TE: tetracycline, S: streptomycin, C: chloramphenicol, W: trimethoprim, ATM: aztreonam 30.

Discussion

The presence of *E. coli* in food is a public health problem, due to the presence of enteropathogenic and toxigenic properties that cause gastrointestinal disorders (Jang et al. 2017). *Escherichia coli* is one of the pathogenic bacteria that can grow in milk and dairy products (Chaleshtori et al. 2017) and is associated with mastitis on dairy cattle (Aslam et al. 2021). This research affirms that Multidrug resistance (MDR) and extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* can be found in several dairy farms in Probolinggo district, East Java, Indonesia. The results are similar to previous research, which identified MDR *E. coli* from dairy cow's milk (Widodo et al. 2022), raw milk samples from the shelter (Anshariesta et al. 2021) and ESBL-producing *E. coli* from fecal dairy cows sample in East Java, Indonesia (Putra et al. 2020).

The positive number of *E. coli* identified on 124 (82.67%) isolates in present study can be attributed to various factors, most of which are related to milking hygiene and environmental sanitation. Sample examination showed that 87 (70.16%) samples were positive for *E. coli* in milk and 37 (29.84%) samples were positive in environmental samples, based on morphological, culture characteristics and biochemical tests. This suggests that there are more *E. coli* in milk than in environmental samples, raising the possibility that milk acts as a vehicle for the transmission of diseases caused by *E. coli* bacteria. To survive, *E. coli* ferments glucose and lactose. This is supported by the statement of Chaleshtori et al. (2017) that milk is rich in lactose for the growth of *E. coli* bacteria. Milk contamination can come from the milk handling system, udder health from the udder surface to the teat, and milk storage equipment (Reta et al. 2016). The presence of *E. coli* in cow's milk has long been considered an indication of contamination by dairy cow fecal (Jaakkonen et al. 2019), but other findings show that most of the contamination in milk is caused by contamination from the environment (Calahorrano-Moreno et al. 2022).

Although the number of *E. coli* in environmental samples was lower than in milk samples in the present

study, the potential of spread of *E. coli* from environment dairy cows should not be underestimated. The rapid growth patterns of *E. coli* and the discovery of multidrug-resistant *E. coli* in the environment indicate a potential threat to public health (Jang et al. 2017). Nevertheless, the contamination of MDR and ESBL *E. coli* in dairy cows environment found in this study, illustrates the potential and highlights the possibility of disease spread from dairy cows environment to other environments. Milk contamination can come from the milk handling system, udder health from the udder surface to the teat, and milk storage equipment (Reta et al. 2016). *Escherichia coli*'s rapid growth patterns and the discovery of multidrug-resistant *E. coli* in the environment suggest a potential concern to public health (Jang et al. 2017). Contaminated milk can transfer *E. coli* to humans (Ribeiro et al. 2019). According to Dhanashekar et al. (2012), humans can contaminate milk during milking, but *E. coli* can also come from the environment and spread to milk and other surroundings (Arbab et al. 2021).

The results of the classification of *E. coli* based on the resistance of antibiotics classes showed that 9 *E. coli* isolates (7.26%) were resistant to 1 class of antibiotics tested, while 3 *E. coli* isolates (2.42%) were resistant to 2 classes of antibiotics, and 9 *E. coli* isolates (7.26%) were confirmed to be multidrug resistant because they were resistant to 3 or more classes of antibiotics. This study found a multidrug resistance pattern which was dominated by a combination pattern of the antibiotic tetracycline, streptomycin, and trimethoprim with a total of 8 (38.10%) *E. coli* isolate, followed by a combination pattern of the antibiotic tetracycline, streptomycin, trimethoprim and aztreonam with one (4.76%) *E. coli* was resistant to more than 3 groups of antibiotics. This percentage is lower than the research conducted by Ansharieta et al. (2021) who found 16 out of 250 milk samples (9.1%) contaminated with *E. coli* resistant to more than 3 groups of antibiotics. Another study in Ethiopia by Dejene et al. (2022) screened 27 milk samples, of which 27 isolates (100%) were *E. coli* (O157:H7) resistant to more than 3 groups of antibiotics. Multidrug resistance of *E. coli* isolates are quite prevalent in many countries and is the cause of a variety of serious infections that are challenging to treat. Studies on the prevalence of *E. coli* infections in the urinary tract in Canada have revealed that 60% of these infections are resistant to more than three classes of antibiotics (Kot 2019).

Multidrug resistance reported in this study is due to the highly irrational use of antimicrobials in individual cows to treat various diseases affecting milk quality. Instances of multidrug resistance in food or foodborne outbreaks are challenging to identify and treat, making the prevalence of multidrug resistance a public health concern. The presence of a multidrug of *E. coli* in food is a source of resistant genes that can spread rapidly (Founou et al. 2016). A total of 8 (38.10%) MDR isolates in this study showed a pattern of combination with antibiotics of tetracycline, streptomycin, and trimethoprim. One (4.76%) isolate was confirmed to contain MDR *E. coli* with resistance pattern of tetracycline, streptomycin, trimethoprim and aztreonam. Tetracyclines have the highest antibiotic resistance because they are often used in veterinary medicine (Ilbeigi et al. 2021). Similarly, tetracycline had the highest percentage values of antibiotic resistance in this study, with value of 28.58%, followed by streptomycin (9.52%) and aztreonam (4.76%) for other antibiotics. Ansharieta et al. (2021) discovered that *E. coli* has the highest percentage value in tetracycline (17.05%), followed by streptomycin (14.20%), and aztreonam (1.7%). Because of their effectiveness, broad-spectrum antibiotics such as tetracyclines and beta-lactams are more frequently used in cases of clinical mastitis in dairy cattle. The use of broad-spectrum antibiotics, such as tetracycline and β-lactams is more common in cases of clinical mastitis in dairy cattle, because of their effective results. Tetracycline and aminoglycoside groups are the primary choice of antibiotics for respiratory and digestive system issues, followed by a combination of sulfonamide-trimethoprim medications that have a considerable impact on rumen microbial activity, and third-generation cephalosporin antibiotics (Economou et al. 2015; Widodo et al. 2022). The percentage pattern of MDR *E. coli* isolates to several antibiotic agents can be caused by inappropriate antibiotic administration or excessive antibiotic administration in the treatment of infectious diseases in dairy cattle (Chowdhury et al. 2021) and can also be caused by contamination from the environment and farmers during the milking process or livestock activities that allow transmission of *E. coli* with multidrug resistant characteristics can occur (Ahmedsham et al. 2018).

The results of ESBL confirmation test revealed that 2 (22.22%) ESBL *E. coli* isolates consisting of one (11.11%) ESBL *E. coli* (AS 27) isolate originating from milk samples with a pattern of resistance to tetracycline, streptomycin, trimethoprim, aztreonam and one (11.11%) ESBL *E. coli* (AL 30) isolate from an environmental sample with a pattern of resistance to tetracycline, streptomycin and trimethoprim. Sudarwanto et al. (2017) found that out of 129 samples with resistance pattern to penicillin, tetracycline, streptomycin and trimethoprim, only 4 (3.1%) were ESBL *E. coli*. Another study by Maulana et al. (2021) on the incidence of ESBL *E. coli* from several samples of dairy farms in the province of Yogyakarta reported that 50 (54%) were ESBL *E coli*, dominated by a pattern of resistance to trimethoprim, tretracycline and gentamicin. However, the pattern of resistance to aztreonam found in ESBL *E. coli* isolates (AS 27) in this study is a new finding that needs attention, considering that aztreonam is rarely used in the treatment of dairy cows in Indonesia.

The incidence of ESBL *E. coli* in dairy farms is also found in other countries such as 70.6% in Germany (Dahms et al. 2015) and 43.6% in China (Zheng et al. 2019). Compared to New Zealand, 1.7% were found to be positive for ESBL *E. coli* from faecal samples and 6.7% from dairy effluent samples (Collis et al. 2022), while in Malaysia 18 (4.8%) were positive contains ESBL *E coli*. Of these, only one (0.27%) of the faecal samples of dairy cows were positive for ESBL *E. coli*, as much as 1.32% came from environmental samples and 3.18% of the milk

samples which were positive for ESBL *E. coli* (Kamaruzzaman et al. 2020).

In the present study, one isolate from milk (AS 27) and one isolate from environmental samples (AL 30) were identified showing the same pattern of resistance for tetracycline, streptomycin, and trimethoprim, except for milk isolates (AS 27) which was also resistant to aztreonam. Although the level of drug resistance of ESBL *E. coli* isolates to tetracycline, streptomycin, and trimethoprim remains low, antibiotic use in dairy cattle should be evaluated on a regular basis. Based on the findings of aztreonam antibiotic resistance and confirmation of ESBL *E. coli* from milk, it is necessary to pay attention to the potential of milk as a source of bacterial zoonotic infections that can manifest and attack humans. Zoonotic bacterial diseases can be transmitted to humans via a variety of routes, including contaminated animal products such as milk (Effendi et al. 2018; Tyasningsih et al. 2022), the direct fecal-oral route, improper food handling, and cooking (Harijani et al. 2020; Permatasari et al. 2020). Thus, according to one health concept, humans who are in close proximity to animals can contract zoonotic pathogenic bacteria, which can then spread to other humans in the community (Decline et al. 2020; Kristianingtyas et al. 2020). Most farmers in this study milk their cows by hand, which increases the milk's exposure to ESBL *E. coli* on the milker's hands. This study supports previous findings that milk can transmit ESBL to consumers through food consumption (Grami et al. 2014).

The environmental sample in this study, however, was not representative of the population, as in other prevalence studies. The presence of bacteria in the farm environment does not always indicate zoonotic transmission between cattle and workers because environmental contamination, such as wastewater, can pose an even greater risk (Friese et al. 2013). Transmission of ESBL *E. coli* to humans through wastewater from dairy farms needs further investigation.

In conclusion, Results showed the presence of MDR and ESBL *E. coli* from several dairy farms in the Probolinggo district of East Java Province, Indonesia. MDR and ESBL *E. coli* from milk and dairy farming environments can spread to the community and threaten public health. A novelty in this study was the discovery of MDR and ESBL *E. coli* from a dairy farm. In addition, preventing and controlling the spread of infection with MDR *E. coli* and ESBL *E. coli* in dairy farms in the Probolinggo district of East Java, Indonesia.

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