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Potential hazards of multidrug resistance *Escherichia coli* collected from wastewater on dairy farms in East Java, Indonesia

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Abstract. *Widodo A, Lamid M, Effendi MH, Tyasningsih W, Wurlina, Al-Arif MA, Raharjo D, Soeharsono, Khairullah AF, Riwu KHP, Yustinasari LR, Kurniawan SC, Silaen OSM, Benjamin MI, Afnani DA. 2023. Potential hazards of multidrug resistance* Escherichia coli *collected from wastewater on dairy farms in East Java, Indonesia. Biodiversitas 24: 1900-1907. Escherichia coli* bacteria initially reside in the digestive tract of humans and animals. This bacterium is treated with antibiotics, but this treatment may trigger antibiotic resistance. Antibiotic resistance genes found in *E. coli* can transfer to other bacteria, especially in the case of the emergence of multidrug resistance (MDR) bacteria. *E. coli* is a significant environmental contaminant on dairy farms and is frequently linked to genes that encode extended-spectrum beta-lactamase. This research focused on investigating the potential hazards of multidrug resistance *E. coli* collected from wastewater on dairy farms in East Java, Indonesia. The number of research samples used was 139 wastewater samples collected from 125 dairy farms in East Java, Indonesia. First, wastewater samples from dairy farms were isolated into BGLB media, then continued with culture on EMBA media, and identifying *E. coli* bacteria with TSIA and IMViC biochemical tests. The resistance patterns of *E. coli* were obtained through disk diffusion antibiotic tests on tetracycline, streptomycin, trimethoprim, chloramphenicol, and aztreonam. The results of the isolation and identification obtained 122 (87.77%) *E. coli* isolates. Antimicrobial susceptibility test to *E. coli* isolates showed 14 (11.47%) MDR *E. coli* isolates. The antimicrobial drug resistance pattern TE-ST-W (tetracycline, streptomycin, trimethoprim) accounted for most MDR *E. coli* isolates in this study, comprising 10 (36.26%); followed by TE-ST-C (tetracycline, streptomycin, chloramphenicol) with two (6.54%) MDR *E. coli* and two (6.54%) MDR *E.coli* isolates were resistant to four antibiotics, with the pattern of resistance to TE-ST-W-C (tetracycline, streptomycin, trimethoprim, chloramphenicol) antibiotic. The discovery of multidrug-resistant *E. coli* isolated from wastewater on dairy farms in East Java, Indonesia, is a new potential hazard that affects public health and needs solutions. One Health integration is anticipated to be used as a solution.

Keywords: ESBL, *Escherichia coli*, MDR, public health, wastewater

INTRODUCTION

Antimicrobial resistance to the environment is a potential public health hazard that appears globally. By 2050, it is predicted that the global cost of fatalities from illnesses caused by pathogens that are resistant to antibiotics will rise from 700,000 to 10 million per year (Murugaiyan et al. 2022). Bacteria resistant to antibiotics make it more difficult to treat serious diseases effectively and increase the risk of morbidity and mortality (Khairullah et al. 2022). Particularly, the rise of antibiotic resistance poses significant societal, economic, and health concerns (Dhingra et al. 2020). The environment has also been consistently recognized as a reservoir for infection-resistant genes (Bengtsson-Palme et al. 2018; Kunhikannan et al. 2021). The widespread development of antibiotic resistance, especially multidrug resistance (MDR), among bacterial infections is one of the most significant obstacles to therapeutic therapy (Catalano et al. 2022). In clinical pathogens, horizontal gene transfer is common for acquiring resistance genes (Sun et al. 2019). Environmental bacteria are a source of novel antibiotic-resistance genes that could be found in clinical infections (Larsson et al. 2022). Mobile genetic components, including plasmids, transposons, bacteriophages, integrons, insertion elements (IS), and genomic islands, may make it easier for genes to move horizontally between bacterial strains (Partridge et al. 2018).

Antimicrobial resistance research from wastewater was done by Ben et al. (2017) in China, Poland (Pazda et al. 2020), Pakistan (Ahsan et al. 2022), and Brazil (Machado et al. 2023). Antimicrobial resistance to environmental bacteria contained in wastewater is alarming because bacteria might contribute to transferring a resistant gene to other bacteria in the aquatic environment. Hospital sewage was long thought to be the main source of antibiotics in aquatic ecosystems, followed by municipal, aquacultural, and agricultural wastewater, including dairy farm wastewater, which has also been found to be a significant source of these substances and resistant bacteria (Guo et al. 2022). Multidrug resistance of *Escherichia coli* in rinse water from workers' hands demonstrates the risk of cross-contamination between dairy cattle and workers (Dahms et al. 2015). However, the presence of the bacteria does not automatically demonstrate zoonotic transfer between dairy cattle and workers, as environmental contamination, such as by wastewater, may represent an even greater risk (Maulana et al. 2021).

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). *Escherichia coli* impacts public health issues since it can cause diarrhea, and some strains are a prevalent source of urinary tract infections (Widodo et al. 2020; Ngene et al. 2021). Using *E. coli* is an environmental indicator because it has a high survival rate (Anjum et al. 2021). Recent studies have demonstrated that the antimicrobial-resistant profile of *E. coli* isolates from sewage correlates with antibiotic-resistance data from related populations (Hutinel et al. 2019; Huijbers et al. 2020). Additionally, surface waters appear to have a higher diversity of *E. coli* than wastewater, despite the latter's higher levels of antibiotic resistance (Delgado-Blas et al. 2021). *E. coli* has been extensively observed to exhibit antibiotic resistance, which makes the issue much worse (Chitanand et al. 2010). Moreover, *E. coli* can resist more antibiotics, including tetracycline, streptomycin, trimethoprim, chloramphenicol, and aztreonam. In addition, in slaughterhouse environments, *E. coli* is resistant to tetracycline (30%) and streptomycin (70%) (Normaliska et al. 2019). Likewise, it is resistant to other antibiotics such as trimethoprim (79%) and chloramphenicol (43%) (Harijani et al. 2020). The research on MDR *E. coli* in dairy farms proved resistant to tetracycline (17.05%) and streptomycin (14.2%); also resistant to the antibiotics trimethoprim (9.66%), chloramphenicol (7.95%) and aztreonam (1.7%) (Ansharieta et al. 2020). Although monobactam (aztreonam) has not been approved for use in veterinary medicine, the existence of resistance isolates increases the possibility of extended-spectrum beta-lactam (ESBL) isolates (Effendi et al. 2021). Therefore, ESBL *E. coli* isolates have enzymes that can hydrolyze penicillins, beta-lactam antibiotics, third-generation cephalosporins, and monobactam antibiotics or aztreonam (Mariana et al. 2021). Additionally, carbapenemase and ESBL enzyme production by *E. coli* was discovered in surface water (Mills and Lee 2019; Hooban et al. 2020).

Wastewater is the primary repository for antibiotics and bacteria resistant to antibiotics in the environment, and the environment is where microorganisms with the highest level of resistance to antibiotics are found (Maulana et al. 2021). Therefore, *E. coli* in the environment of dairy farms indicates the potential for disease transmission from the dairy farm environment to the outside environment. *Escherichia coli* in the farm environment does not always indicate zoonotic transmission; however, potential hazards such as wastewater can pose an even greater risk (Friese et al. 2013). Further research is required to confirm whether multidrug-resistant *E. coli* can be transmitted from the environment of dairy farms to humans through wastewater. So, this research was to investigate the potential hazards of multidrug-resistant *E. coli* collected from wastewater on dairy farms in East Java, Indonesia.

MATERIALS AND METHODS

Sample collection

A total sample of 139 wastewater samples was collected from 125 dairy farms in three regencies in East Java, Indonesia, namely Probolinggo, Tulungagung, and Blitar. The research was conducted in 2021 between July and September. About 20 mL of wastewater was taken from a ditch or sewage channel on each dairy farm using a disposable sterile plastic pipette, then put into a sterile sample bottle labeled by sample code. Samples were placed in a cool box containing ice packs and sent to the laboratory with a constant temperature of around 4°C. At the Faculty of Veterinary Medicine, Universitas Airlangga, the Department of Veterinary Public Health Laboratory processed the samples.

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). Next, *E. coli* bacteria were cultivated using selective media in eosin methylene blue agar (EMBA) media (Merck, 101347), and they were then kept warm (35-37°C) for 20-24 hours. A gram staining kit (HiMedia, K001-1KT) was used to present an image of pink rod-shaped Gram-negative bacteria criteria after identifying *E. coli* colonies in EMBA. Biochemical examination of triple sugar iron agar (TSIA) media (Merck; 103915) and IMVIC media, such as sulfide indol motility (SIM) media (Merck; 105470), methyl red-Voges Proskauer (MR-VP) media (Merck; 105712), and Simmons citrate agar (Oxoid, CM155), allowed for the identification and confirmation of pure colonies of *E. coli* (Effendi et al. 2018; Tyasningsih et al. 2022).

Antimicrobial susceptibility test

The Clinical and Laboratory Standards Institute's recommended Kirby-Bauer disk diffusion technique was used to determine the antimicrobial susceptibility of *E. coli* isolates (CLSI 2022). As instructed by the manufacturer, Mueller Hinton agar (MHA) (Merck, 105437) was produced and allowed to cool to 45-50°C before being poured into plates. Plates were allowed to dry after the agar had solidified. By diluting to 0.5 McFarland's standard, an *E. coli* isolation broth culture growing for 18 to 24 hours was standardized. First, the *E. coli* isolates were inserted into a standardized *E. coli* inoculum. Next, the culture was drained to remove excess inoculum load. Then, a sterile swab stick was used to inoculate the surface of the prepared MHA plates. The MHA plate with the inoculation was then allowed to dry briefly at room temperature (29°C) with the lid closed. Finally, the antibiotic-impregnated discs were carefully placed on the inoculated MHA plates using sterile forceps after the agar surface had dried for a few minutes. This study used tetracycline, streptomycin, chloramphenicol, trimethoprim, and aztreonam as antibiotic-impregnated discs.

MHA plates were incubated for 18 to 24 hours at 37 °C, and the widths of the inhibitory zones formed on the MHA plates were measured to the nearest millimeter using a ruler. Results were noted and interpreted following the CLSI (CLSI 2022). One result of resistance is the existence of multidrug resistance, which is the sensitivity of *E. coli* bacteria to more than three classes of antibiotics (Ansharieta et al. 2021)

RESULTS AND DISCUSSION

Isolation and identification of *Escherichia coli*

A total of 139 wastewater samples were isolated from 125 dairy farms, consisting of three locations with different research locations in East Java, Indonesia. After being incubated for 24 hours at 37°C on BGLB media, the *E. coli* bacterium colony color was murky green and began to generate gas in a Durham tube. In this investigation, 133 (95.68%) sample cultures on EMBA media revealed the formation of metallic green convex colonies with black spherical cores following incubation at 37°C for 24 hours (Figure 1A). Under a microscope at 1000x magnification, the *E. coli* identity with Gram stain showed pink rod-shaped bacteria. According to the biochemical test results, *E. coli* was a positive isolate on the TSIA medium and had yellow color in the slant and butt media, as well as a gas appearance but no black color (H2S). The results of the IMViC medium test are shown in Figure 2, which yielded positive results for indole, motile, and MR tests but negative results for sulfide, VP, and citrate tests.

In this study, 122 samples (87.77%) were positive for *E. coli*. Sample examination showed that 37 samples (26.62%) from the Probolinggo dairy farm, 41 samples (29.50%) from the Tulungagung dairy farm, and 44 samples (31.65%) from the Blitar dairy farm were positive for *E. coli* isolates based on biochemical testing and the parameters of morphological culture (Table 1). The positive number of *E. coli* in wastewater samples found in dairy farm environments can be attributed to various factors, mostly personal hygiene and dairy farm environment sanitation.

Antimicrobial susceptibility test of *Escherichia coli*

This study's antimicrobial susceptibility test results showed that 31 *E. coli* isolates (25.41%) were resistant to antibiotics, as shown in Table 2 and Figure 3. In addition, the isolates of *E. coli* showed resistance to several antibiotics, including: tetracycline (23.77%), streptomycin (15.57%), trimethoprim (23.77%), and chloramphenicol (4.10%), but not aztreonam. Therefore, the results of *E. coli* isolates by antibiotic resistance (Table 2) could be categorized as follows: 6 (six) *E. coli* isolates (4.92%) were resistant to only one class of antibiotics; 11 (eleven) *E. coli* isolates (9.02%) were resistant to two classes of antibiotics; and 14 (fourteen) *E. coli* isolates (11.47%) were confirmed to be MDR because they were resistant to three or more classes of antibiotics (Figure 1B). Furthermore, tetracycline, streptomycin, and trimethoprim (TE-ST-W) were antimicrobial drugs with resistance patterns predominated among MDR *E. coli* isolate. , accounting for 10 (32.26%) isolates. Its followed by TE-ST-C (tetracycline, streptomycin, chloramphenicol), which had two (6.54%) isolates. In addition, 2 (two) (6.54%) isolates of MDR *E. coli* were discovered to be resistant to four classes of antibiotics; with the resistance pattern to TE-ST-C-W (tetracycline, streptomycin, chloramphenicol, and trimethoprim) antimicrobial drugs, as shown in Table 3.

Incorporated antimicrobial genetic transfer of the genome from specific sources was the cause of the existence of MDR *E. coli* isolates. This study shows that there were 14 (11.47%) isolates of MDR *E. coli* consisting of 3 (2.46%) isolates from Probolinggo, 5 (4.09%) isolates from Tulungagung, and 6 (4.92%) isolates from Blitar (Table 4). The percentage pattern of MDR *E. coli* isolates to different antibiotic agents may be caused by inappropriate or excessive antibiotic use in treating infectious diseases in dairy cattle. It may also be due to environmental contamination and farmers during the milking process or livestock activities that allow the transmission of *E. coli* with multidrug resistance characteristics to occur.

Table 1. Isolation and identification of *Escherichia coli* from wastewater samples in dairy farm

Location	Sample code	Number of dairy farms	Number of samples	BGLB	EMBA	Gram stain	Biochemical test		Positive *E. coli* (%)
							TSIA	IMViC	
Probolinggo	AL	41	41	41	40	40	39	37	37 (26.62%)
Tulungagung	TL	45	45	45	45	45	41	41	41 (29.50%)
Blitar	SL	39	53	48	48	48	44	44	44 (31.65%)
Total		125	139	134	133	133	124	122	122
Percentage				96.40%	95.68%	95.68%	89.20%	87.77%	87.77%

Note: % (Percentage of positive *Escherichia coli*), BGLB: brilliant green lactose broth, EMBA: eosin methylene blue agar, TSIA: triple sugar iron agar, IMViC: indol-motility, methyl-red, Voges-Proskauer, and citrate

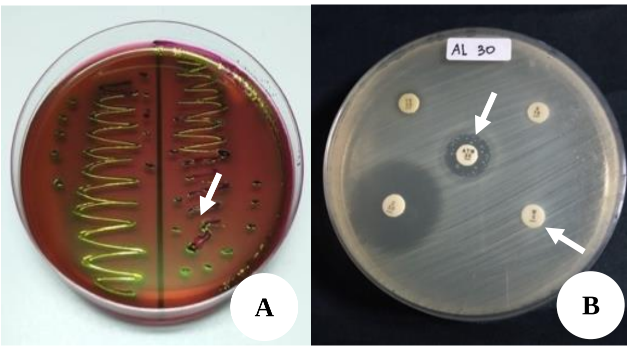

Figure 1. A. *Escherichia coli* isolates on eosin methylene blue agar (EMBA) could be seen in metallic green. B. Antimicrobial sensitivity test of multidrug-resistant (MDR) producing *Escherichia coli* isolates in Mueller Hinton agar (MHA) formed an inhibition zone around the antibiotic

Figure 2. *Escherichia coli* biochemical test results. A. The TSIA media on the slanted media is yellow and does not display black as an H_2S, B. Indol in SIM media appears as a pink ring, C. Negative citrate showed media's color is still green, D. Red media is seen on positive MR, E. Negative VP; the media remains yellow

Table 2. Classification of *Escherichia coli* based on the resistant to antibiotic class

Antimicrobial susceptibility tests	Resistant to antibiotics		Number of isolates (%)
No resistance			91 (74.59%)
Resistance	1 antibiotic	6 (4.92%)	
	2 antibiotics	11 (9.01%)	
	≥ 3 antibiotics	14 (11.47%)	
			31 (25.41%)
Total			122 (100%)

Note: % (Percentage of resistance on antibiotic class)

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

Number of antibiotics	Antibiotic pattern	Frequency (n =31)	Percentage (%)
1	TE	5	16.12 %
2	ST	1	3.23 %
6	TE-ST	3	9.68 %
7	TE-C	1	3.23 %
8	TE-W	6	19.35 %
9	ST-W	1	3.23 %
10	TE-ST-C	2	6.45 %
11	TE -ST-W	10	32.26 %
12	TE-ST-W-C	2	6.45 %
	Total	31	100%

Note: TE: tetracycline, ST: streptomycin, C: chloramphenicol, W: trimethoprim, ATM: aztreonam, (%): Percentage of the antibiotic pattern

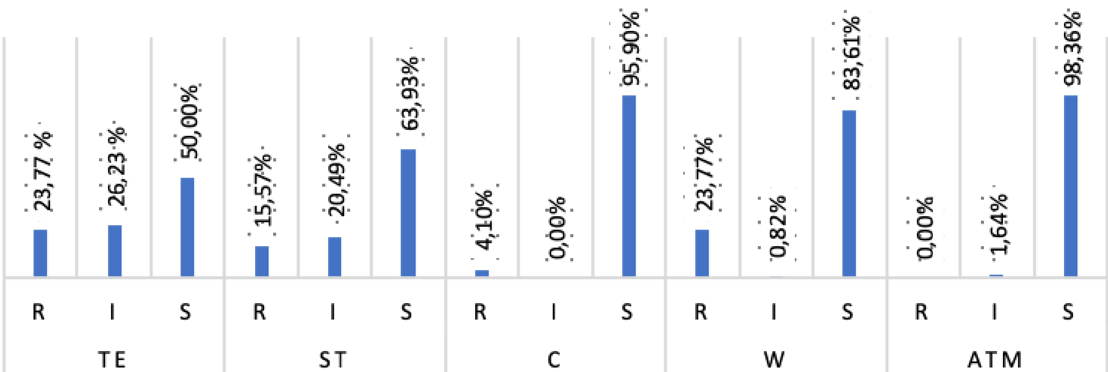

Figure 3. The proportion of antimicrobial resistance on *Escherichia coli* isolates. TE: tetracycline, ST: streptomycin, C: chloramphenicol, W: trimethoprim, ATM: aztreonam, R: resistant, I: intermediate, S: sensitive

Table 4. Antimicrobial susceptibility profile of MDR *Escherichia coli* isolates on tree location different research

Location	Number of MDR strains	Percentage of MDR (%) (n = 122)	Sample code	Antibiotic Pattern of MDR				
				TE	ST	C	W	ATM
Probolinggo	3	2.46%	AL (19)	+	+	-	+	-
			AL (30)	+	+	-	+	-
			AL (40)	+	+	-	+	-
Tulungagung	5	4.09%	TL (14)	+	+	+	+	-
			TL (18)	+	+	+	-	-
			TL (23)	+	+	+	-	-
			TL (31)	+	+	-	+	-
			TL (35)	+	+	-	+	-
Blitar	6	4.92%	SL (22)	+	+	-	+	-
			SL (30)	+	+	-	+	-
			SL (46)	+	+	-	+	-
			SL (47)	+	+	-	+	-
			SL (48)	+	+	+	+	-
			SL (51)	+	+	-	+	-
Total	14	11.47%						

Note: +: Resistant, -: not resistant , TE: tetracycline, ST: streptomycin, C: chloramphenicol, W: trimethoprim, ATM: aztreonam

Discussion

Escherichia coli is a Gram-negative bacteria that can grow in animal products and is associated with mastitis in dairy cattle (Aslam et al. 2021). *Escherichia coli* has long been thought to be a sign of contamination from dairy cow feces (Jaakkonen et al. 2019) and caused by environmental contamination (Calahorrano-Moreno et al. 2022). *E. coli* uses glucose and lactose from the environment to survive; Chaleshtori et al. (2017) state that *E. coli* bacteria can ferment lactose for growth quickly. This study's current is known for the potential hazards of multidrug-resistant *E.coli* isolated from wastewater on dairy farms in East Java, Indonesia. Sample examination showed that 122 samples (87.77%) were positive for *E. coli* from wastewater on dairy farms environment in East Java, consisting of 37 samples (26.62%) from Probolinggo dairy farm, 41 samples (29.50%) from Tulungagung dairy farm and 44 samples (31.65%) from Blitar dairy farm. That demonstrates more *E. coli* in wastewater in dairy farms as a potential hazard that can be transmitted to humans, affecting public health problems. The presence of *E. coli* in wastewater, especially strain enteropathogenic and toxigenic properties, cause gastrointestinal disorders in public health problems (Jang et al. 2017). In other research by Yanestria et al. (2022) on wastewater from broiler farm environments were 41 samples (64.62%) positive *E. coli* isolates and 24 samples (36.92%) positive *E. coli* with the ability resistant to 3 more antibiotics. Support research on several samples from dairy farm environments by Maulana et al. (2020) identified 127 samples (22.75%) of positive *E. coli* isolates, including 15 (16%) from 93 wastewater samples that were resistant to 3 more antibiotics.

In this study, the antimicrobial susceptibility test found 31 (25.41%) isolates were *E. coli* resistant to common antibiotics. For example, resistant to tetracycline (23.77%) and trimethoprim (23.77%). It's followed by streptomycin (15.57%) and chloramphenicol (4.10%) but not found resistant to aztreonam. Similarly, research in Japan by Suzuki et al. (2022), for eight months of the year, *E. coli* isolates from dairy cows and the environment around them on animal farms displayed an 8.3% (10/120) rate of antibiotic resistance, with tetracycline resistance being frequent in all strains. Other research on raw milk samples by Ansharieta et al. (2020) and dairy cow's milk samples by Widodo et al. (2022) show domination resistance to *E. coli* bacteria for tetracycline (17.05% and 13.71%). That was followed by resistance to streptomycin (14.20% and 9.68%), trimethoprim (9.66% and 8.87%), chloramphenicol (7.95% and 0.81%), and aztreonam (1.7% and 1.61%).

This study found no antimicrobial drug resistance to *E. coli* to aztreonam antibiotic, contrary to other research in broiler farm environments by Effendi et al. (2021), which revealed 3% of the *E. coli* isolates were aztreonam-resistant. Additionally, 70% and 38.4% of *E. coli* isolated from chicken meat and beef in traditional markets were resistant to aztreonam (Wardhana et al. 2020; Wardhana et al. 2021). In veterinary medicine, tetracyclines were frequently employed (Ilbeigi et al. 2021). The broad spectrum activity of tetracycline and the beta-lactam antibiotic class makes them more useful in clinical mastitis conditions in dairy calves. Tetracycline and aminoglycosides are the first choices of antibiotics in other cases involving respiratory and digestive tract issues in Europe. On the other hand, a combination of sulfonamide-trimethoprim is the second choice in cases involving rumen microbes and the last choice in the third cephalosporin group antibiotics (Economou et al. 2015).

The results of antibiotic resistance groups on *E. coli* isolates categorization revealed that 6 isolates (4.92%) were resistant to one antibiotic, 11 *E. coli* isolates (9.02%) were resistant to two antibiotics, and 14 isolates (11.47%) were resistant to three or more antibiotics. Potential hazard from multidrug-resistant *E. coli* isolates from wastewater on dairy farms in East Java are shown in Table 4. In this study, there were 14 (11.47%) isolates confirmed as MDR *E. coli* consisting of 3 (2.46%) isolates from Probolinggo, 5 (4.09%) isolates from Tulungagung, and 6 (4.92%) isolates from Blitar. This proportion is higher than that observed in an investigation of *E. coli* from milk samples located in Probolinggo, Pasuruan, Batu, and Blitar districts, of East

Java, which discovered 16 (9.1%) and 9 (7.26%) MDR *E. coli* isolates resistant to three or more antibiotics (Ansharieta et al. 2021, Widodo et al. 2022). Another study on the biological hazard of MDR *E. coli* from cloacal swabs of broiler chicken found 85.7% and 51.4% MDR *E. coli* isolates (Harijani et al. 2020). A supporting study by Witaningrum et al. (2020) found 47.1% of MDR *E. coli* isolates as a potential hazard of antibiotic resistance on *E. coli* from cloacal swabs in layer poultry farms.

Moreover, this investigation found 10 (32.26%) *E. coli* isolates on the TE-ST-W (tetracycline, streptomycin, trimethoprim) antimicrobial drug resistance patterns dominated the MDR *E. coli* isolates. Additionally, two (6.54%) isolates of MDR *E. coli* were discovered to be resistant to four antibiotics, with resistance patterns to TE-ST-W-C (tetracycline, streptomycin, trimethoprim, chloramphenicol) antimicrobial drugs. Similarly, another study of MDR *E. coli* isolates pattern from dairy cow's milk samples by Widodo et al. (2023) found 8 (38.10%) MDR *E. coli* isolates were resistant to TE-ST-W (tetracycline, streptomycin, trimethoprim) antimicrobial drugs, and one (4.76%) MDR *E. coli* isolates were resistant to TE-ST-W-ATM (tetracycline, streptomycin, trimethoprim, aztreonam) antimicrobial drugs. Moreover, tetracycline, streptomycin, and trimethoprim drug resistance levels remain low. However, still routine evaluations of the usage of antibiotics in dairy cows should be carried out. According to this study's antimicrobial susceptibility testing, aztreonam antibiotic resistance was not discovered in MDR *E. coli* isolates from wastewater. Still, according to a prior study by Widodo et al. (2022), it was discovered in dairy cow's milk samples. Therefore, the presence of antimicrobial resistance on aztreonam antibiotic was used for suspected ESBL *E. coli* but was not found in this study.

Another study by Maulana et al. (2021) reported 54% isolates of ESBL *E. coli* from several environment samples of dairy farms. The environment around the farms can store various resistant materials that can transfer between bacteria (Riwu et al. 2022; Widodo et al. 2020). The interaction between people, animals, and the environment allows bacteria, particularly mobile genetic elements (MGEs), to be transported between bacterial species. The environment is the main source of resistance (Niasono et al. 2019; Riwu et al. 2020). Resistance transmission to other organisms via MGEs (plasmids and transposons) is accelerated and enhanced by agricultural and animal activities and human waste contaminating the environment (FAO and VMD 2018). Therefore, according to one health idea, people close to possible hazards can get zoonotic pathogenic germs, which they can then disseminate to other individuals in their community (Decline et al. 2020). Therefore, the potential hazard of the spread of multidrug-resistant *E. coli* isolated from wastewater in dairy farms should not be underestimated. The multidrug-resistant *E. coli* found in the environment and the rapid growth patterns of *E. coli* point to a possible concern for public health (Jang et al. 2017). Nevertheless, the discovery of MDR *E. coli* in the environment of dairy farms in this study shows the potential for disease transmission from the dairy farm environment to the outside environment. Additionally, bacteria in the farm environment do not always indicate zoonotic transmission; potential hazards such as wastewater can pose an even greater risk (Friese et al. 2013).

Overall, the discovery of multidrug-resistant *E. coli* isolated from wastewater in dairy farms is a concern and requires real action so that it can be reduced and not spread. Furthermore, an integrated approach is required to comprehend and uncover the possibility of controlling the transmission of MDR bacteria and infection in humans (Sleeman et al. 2017). Therefore, to restrict the ecological spread of MDR *E. coli* for public health, a multi-sectoral strategy for medical treatment in veterinary medicine and animal food production can achieve worldwide cooperation (Iwu et al. 2020; Giufre et al. 2021). Furthermore, to manage MDR *E. coli*, adopting the One Health integration idea is anticipated to speed up illness prevention and prediction (Ghai et al. 2022).

In conclusion, this study found the illustration of potential hazards and highlights the possible spread of multidrug-resistant (MDR) *E. coli* isolates through wastewater from dairy farms to the outside environment. The emergence of the danger of MDR *E. coli* from wastewater in dairy farms requires all parties' attention. Controlling the spread of MDR *E. coli* can be done by maintaining the cleanliness and hygiene of dairy production. Elimination should also be done, especially for contaminants from waste products, dairy feces, and excretions, because poor hygiene handling can increase MDR bacterial infections in humans. Likewise, maintaining the cleanliness of the environment, especially locations close to dairy farms, must be done because the contaminants will reach humans through the environment and wastewater. Furthermore, this is worrying, and a new potential hazard needs a solution. Future studies must examine additional aspects, including the usage of antibiotics and the management of dairy farms in general. Wastewater treatment techniques must be developed immediately to stop the MDR from spreading and expanding. The government must raise public awareness of the value of sanitation and hygiene in preventative measures so that the prevalence of MDR *E. coli* does not increase significantly. The one-health integration preventive approach may be an alternative to be used continuously.

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