Analysis of beta-lactam antibiotic resistance genes in *Escherichia coli* isolated from dairy cattle manure in Bogor, Indonesia

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Abstract

The rapid growth of the dairy industry has led to increased antibiotic use in dairy cattle, causing a surge in antibiotic-resistant genes. This, in turn, has expedited bacterial resistance development. The objective of this research was to analyze the presence of beta-lactam antibiotic resistance genes in *E. coli* derived from dairy cattle manure in Bogor, Indonesia. In this study, twenty-five composite samples from different dairy farms in Bogor were collected. *E. coli* isolation and identification were performed following the Global Tricycle Surveillance extended-spectrum beta-lactamase (ESBL) *E. coli* set by WHO 2021. The real-time polymerase chain reaction (qPCR) was used for detecting beta-lactam resistance genes. Out of the total samples, 15 isolates (60%) exhibited one beta-lactam resistance gene. The prevalence of *blaTEM*, *blaCTX-M*, *blaCMY-2*, and *blaOXA* genes was found to be 36%, 24%, 16%, and 4%, respectively. For *blaSHV*, all samples were negative. Furthermore, it was observed that 20% of the isolates harbored two beta-lactam resistance genes. The high occurrence of beta-lactam resistance genes in the manure samples indicated that resistant bacteria and resistance genes have been transmitted from dairy cattle to the environment. This poses an alarming threat to public health, as the dissemination of these resistant bacteria and genes into the wider ecosystem could compromise the effectiveness of antibiotic treatments for human infections. Urgent action is needed to address this issue, including improving manure management practices on dairy farms and implementing stricter regulations on antibiotic use in livestock production. Failure to address this issue poses a significant threat to both animal and human health in the region.

Keywords: Beta-lactam resistance, *bla* genes, Manure, Dairy cattle, *E. coli*


Introduction

The social concern regarding environmental exposure to antibiotic resistance has constantly grown due to the excessive use of antibiotics in both veterinary and human medicine. Bacteria resistant to antibiotics can be transmitted through various ways, such as land application of animal manure, discharge of untreated wastewater, and food contamination. Among several potential sources for this influx, land application of animal manure containing antibiotic-resistant bacteria appeared to be one of the most dominant pathways for the release of antibiotic resistance into the environment because, unlike human waste, waste generated on farms does not undergo further treatment (Van Epps and Blaney, 2016).
Notably, AMR was directly accountable for 1.27 million fatalities that year. The burden was highest in Sub-Saharan Africa and South Asia. Six pathogens (Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa) were responsible for 73.4% of deaths attributable to bacterial AMR (Murray et al., 2022).

The direct impacts of AMR include increased healthcare costs, a higher risk of complications, greater rates of treatment failure, and longer recovery times for patients. In contrast, the indirect or secondary impacts of AMR are primarily productivity-related. These include reduced economic output and productivity when infected individuals are unable to work due to extended illness or hospitalization. It also encompasses lost wages and income for patients, as well as productivity losses for caregivers who have to take time off to care for family members with resistant infections. Unlike the more quantifiable direct healthcare expenditures, these indirect productivity losses are harder to measure and account for. However, they represent a significant component of the total societal impact of antimicrobial resistance. The failure to fully capture these secondary, productivity-related impacts has led to an underestimation of the true overall cost and burden of the AMR crisis globally (O’Neill, 2016).

Beta-lactam antibiotics (BLAs) have been declared the most successful antibiotic classes against most bacterial strains since the discovery of penicillin in 1929. This class of antibiotics accounts for up to 65% of all medical prescriptions for injectable antibiotics in some regions, including the USA, underscoring their widespread use in clinical practice. Unfortunately, the excess use and diversification of β-lactamases have posed indefinite health issues, which as bacterial resistance to the beta-lactamases, which resulted in the limitation of the clinical effectiveness of all current BLAs. Resistance to BLAs occurs through the production of beta-lactamase enzymes, such as plasmid-mediated AmpC enzymes, carbapenem-hydrolyzing beta-lactamas (carbapenems), and extended-spectrum β-lactamases (known as ESBL) (Bush and Bradford, 2016).

AmpC antibiotic resistance genes (ARGs) include variants such as blaCMY, which confer resistance to cephalosporin antibiotics. These AmpC-type beta-lactamases are an important mechanism of resistance, particularly in Gram-negative bacteria like E. coli. Similarly, carbapenem ARGs include variants such as blaOXA, which encode oxacillinases that can hydrolyze carbapenems. The ESBL resistance genes that are commonly reported include blaCTX-M, blaSHV, and blaTEM. These genes encode enzymes that can hydrolyze and confer resistance to a wide range of beta-lactam antibiotics, including extended-spectrum cephalosporins. ESBL is considered one of the highly important mechanisms of antibiotic resistance in various Gram-negative bacteria. ESBLs have become increasingly prevalent across numerous regions worldwide and currently comprise three hundred variants (Santos et al., 2020; Vrancianu et al., 2020).

E. coli acts both as an indicator of sanitation levels in cattle and as a reservoir for spreading resistance genes to other bacteria. E. coli can develop Multi-Drug Resistance (MDR) and create ESBL, allowing it to disseminate resistance genes. ESBL-producing E. coli is the most commonly found resistant bacteria in the Enterobacteriaceae family globally, particularly in food-producing animals, leading to a significant human mortality rate (Wibisono et al., 2020).

The most common genes encoding beta-lactamases are TEM and SHV, and because of the burden they inflicted on animal and human health, blaCTX-M, a new class of ESBL genes, seems to have gained global traction recently. The blaTEM gene is the most frequently found in livestock environments (Widodo et al., 2020). The blaCMY gene is the most commonly found AmpC-type beta-lactamase (ACBL) gene and is responsible for resistance to the antibiotic ceftriaxone. Recent reports showed that E. coli bacteria that produce the blaCMY gene can cause infections acquired from the environment (Zhao et al., 2001; Doi et al., 2010; Basbas et al., 2021).

Dairy farms represent an environment where antimicrobial use is prevalent to ensure animal health and productivity. The exposure of dairy cattle to beta-lactam antibiotics provides a selective pressure favoring the emergence and dissemination of beta-lactam resistance genes in E. coli strains. Manure can act as a repository of antimicrobial resistance genes, allowing for their dissemination into the soil, water bodies, and nearby ecosystems. This dissemination poses a risk of horizontal gene transfer, enabling
resistance genes to spread to other bacteria, including potential human pathogens (Ansharieta et al., 2020).

Indonesia has been reported to have the highest prevalence of ESBL-producing E. coli cases in clinical settings, according to a study by (Mendes et al., 2013). Bogor, located in West Java, is an area where dairy farming is a significant part of the local economy, with the dairy industry being an iconic part of the Kebon Pedes region (Gultom and Suharno, 2017).

Several studies have investigated the prevalence of E. coli, particularly ESBL-producing strains, in different environments within Bogor. One previous study found that 8.6% of E. coli isolates from cattle in Bogor were ESBL-producing (Sudarwanto et al., 2016). Another study reported a 14.3% prevalence of ESBL-producing E. coli in environmental samples collected from slaughterhouses in Bogor (Sudarwanto et al., 2017). Additionally, a more recent study aimed at detecting E. coli in local and broiler chickens sold at traditional markets in Bogor found that 76.47% of the sampled cloacal swabs were positive for E. coli (Rizal et al., 2024). These findings suggest that E. coli is widely present and potentially prevalent in the Bogor region. The current research focused on analyzing the presence of beta-lactam resistance genes in E. coli isolated from dairy cattle manure in Bogor. The results from this study can provide a basis for developing appropriate strategies to control antibiotic resistance in this area.

Materials and methods

Ethical approval
For the current study, ethical approval was deemed unnecessary. Samples were collected according to the established guidelines governing the collecting procedures (ISO 19458:2006 and SNI 6989.59-2008).

Time and location of study
This study was conducted from June 2023 to December 2023. Field sampling was carried out at listed dairy farms in the Kebon Pedes area of Bogor. E. coli detection and isolation as a means of sampling were conducted at the Laboratory of the Division of Veterinary Public Health and Epidemiology, School of Veterinary Medicine and Biomedical Sciences, IPB University. Followed by the detection of beta-lactamase resistance genes, namely blaTEM, blaCTX-M, blaCMY-2, blaOXA, and blaSHV genes, using real-time polymerase chain reaction (qPCR), performed at the Quality Control Laboratory and Certification of Animal Products, Ministry of Agriculture, Republic of Indonesia.

Samples collection
All of the dairy farms located in the Kebon Pedes area in Bogor (as many as 25 farms), as reported by DKPP of Bogor in 2023, were used in this study. The methodology for sampling involved creating a composite sample from each farm through the process of pooled pat sampling or manure composites, as advised by Widgren et al. (2013). Specifically, for each farm, three subsamples of manure were collected from different identified areas known for manure accumulation to ensure a representative sample. Each of these subsamples consisted of approximately 10 Grams of feces. The total number of collected subsamples was 75 (3 subsamples per farm x 25 farms). These subsamples were then securely transported in separate containers to the laboratory for pooling to enhance the accuracy of the composite samples. In the lab, all three subsamples from a single farm were weighed and meticulously combined into one 100 mL plastic container, creating a single composite sample per farm. Consequently, 25 composite samples representing all the dairy farms in the study area were systematically prepared. The timely collection of subsamples from the farms was completed within 2 hours during the morning, and subsequent pooling in the laboratory allowed for consistency across all samples.

E. coli isolation and identification
E. coli was identified and isolated using a standardized identification process, the Global Tricycle Surveillance Method. This method was designed by the WHO (WHO, 2021) to detect extended-spectrum beta-lactamase E. coli. However, this protocol can be modified and used for detecting other E. coli. This step aimed to confirm the isolates as E. coli using standard microbiological identification methods without evaluating their antimicrobial resistance profile or beta-lactamase production.

Initially, all samples were serially diluted at a ratio of 1:9 in sterile phosphate-buffered saline (PBS, pH ~7.4), resulting in a $10^{-5}$ dilution in duplicate. Then, 0.1 mL of each diluted sample was plated on the surface of tryptone bile X-
glucuronide agar (known as “TBX”; HIMEDIA®, Guangdong, China) using the spread plate technique. On TBX agar plates, bluish-green colonies were identified as E. coli colonies. All petri dishes whose colony count was ≤100 colony forming units (CFU)/mL were selected for further analysis. Five blue-green colonies from each TBX agar plate were loop-picked and transferred onto MacConkey agar (MCA) plates (Oxoid®, Hampshire, UK). On MCA plates, colonies suspected to be E. coli exhibited a dry, flat, pink morphology with nonmucoid morphology, with a surrounding dark pink area due to precipitated bile salts. These presumptive E. coli isolates were further confirmed by culturing on tryptic soy agar medium (TSA) (Oxoid®, Hampshire, UK). For E. coli confirmation, an indole test on sulfur indole motility (SIM) medium (Oxoid®, Hampshire, UK) was done. The E. coli isolate was positively confirmed when a pink-to-red color (cherry-red ring) was formed. The positive control used in this study was E. Coli ATCC 25922.

Detection of genes coding for β-lactamase resistance in E. coli

The DNA of the bacteria isolated was extracted using The Mericon DNA Bacteria Kit (Qiagen®, Hilden, Germany) in compliance with the manufacturer’s recommendations. The extracted DNA was of high quality and purity to ensure accurate results. The pure E. coli isolates were transferred using an inoculating loop from the culture medium to a microtube containing 1 mL of PBS. This was done to obtain a turbidity of 0.5 McFarland standard; this varies according to the isolate’s availability. To prepare the bacterial suspension, centrifugation was done for 5 min at 13,000×g. After discarding the supernatant, the bacterial pellet was mixed with 200 μL of sterile PBS, and then a tube shaker was used for mixture homogenization. The suspension was centrifuged again at 13,000×g for 5 min, and subsequent washing cycles were performed on the pellet till the suspension became colorless. Subsequently, 200 μL of Fast Lysis Buffer was added. The mixture was then subjected to controlled heating for 10 min in a ThermoMixer (Eppendorf®, Hamburg, Germany) at 100°C at a rotation speed of 122×g. After incubating the suspension at room temperature (RT) for 2 min, it was centrifuged once again at 13,000×g for 5 min. Subsequently, 100 μL of DNA-containing supernatant was put into a 2 mL microtube and stored at -20°C or -80°C until the following analysis.

The second step was quality control of the extracted DNA. To ensure the quality of the extracted DNA, a nanodrop spectrophotometer was used to test DNA concentration and purity. The DNA purity ratio, as measured by the nanodrop, was within the predetermined range of 1.8–2.0 (A260/A280), indicating the DNA was of high quality and purity. The DNA concentration required for qPCR testing was greater than 36 ng/μL.

The last step was qPCR amplification. In order to investigate the presence of beta-lactam resistance genes in E. coli, a probe method was employed using the primers in Table 1. These primers were subsequently amplified using the qPCR machine known as the thermal cycler rotor-gene Q. The qPCR procedure involves the following steps: Making the PCR reaction Master Mix, which has 5 μL of sample DNA, 1 μL of probe, 4.5 μL of nuclease-free water, and 1 μL of each of 10 μM reverse and forward primers. The mixture was added to microtubes according to the plate layout design, resulting in a 25 μL reaction volume. The microtubes are kept in a PCR plate cooler. The amplification conditions involve initial steps at 50°C for 2 min and 95°C for 10 min, then 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 60 seconds. A positive result is determined by a Ct value less than 40, accompanied by a well-defined amplification curve. On the other hand, a Ct value greater than 40 denotes a negative or undetectable result.

Results and discussion

E. coli isolation and identification

In all 25 composite samples examined, E. coli was detected through the entire identification process. The findings demonstrated that at every stage of testing (TBX, MCA, and Indole), the presence of E. coli was confirmed in 100% of the samples; this indicated consistent and comprehensive identification across all stages. Many researchers around the world documented this significant prevalence of E. coli in dairy farms reaching 100%. Putra et al. (2020) reported a high prevalence (100%) of E. coli in rectal swabs from dairy cattle in Surabaya, Indonesia. A study in Canadian dairy farms reported that among the 599 fecal composite samples obtained, 593 E. coli
isolates were recovered, which means 98.99% of the samples were positive (Massé et al., 2021). A high prevalence of *E. coli* (100%) was also observed in fresh cattle manure in Texas (Manishimwe et al., 2021). In 2019, conducted research in Bangladesh on different types of dairy farms, and the prevalence of *E. coli* varied accordingly, but the highest prevalence recorded was 86.67%.

In this study, the high occurrence of *E. coli* (100%) in manure samples from dairy farms can be mostly attributed to its fecal origin. Given that *E. coli* is a natural inhabitant of cattle’s intestinal flora, its presence in substantial quantities is unsurprising. These findings are consistent with a previous investigation conducted by Abdus Sobur et al. (2019), who stated that the high prevalence of *E. coli* in the manure samples originating from various farms was not unexpected since *E. coli* is ubiquitous in nature.

Other factors also contribute to *E. coli* high prevalence as poor hygiene practices in cattle farms; it can significantly contribute to *E. coli* high prevalence. Poor sanitation practices and improper handling/disposal of manure are well-known risk factors for *E. coli* contamination on dairy farms. Inadequate cleaning of pens, barns, and equipment can allow *E. coli* to proliferate. Moreover, it can result in the transmission of *E. coli* from animals to humans, as well as to the environment and ecosystems, through the dissemination of contaminated waste (Sarba et al., 2023).

During the sample collection process, it was observed that some farmers dispose of their farm waste directly into running water sources. This practice can have severe implications for the spread of *E. coli* and other bacteria. This contaminated water can then spread the bacteria to new locations as it flows downstream.

The risk is especially high as this water source was proven to be used for irrigation purposes. Consuming or coming into contact with contaminated water or contaminated crops can lead to *E. coli* infections in both humans and animals, posing a significant public health risk (Solomon et al., 2002).

One important factor is the structure and management of the farms in the Kebon Pedes. Even though the number of dairy cattle per farm is relatively low, ranging from 5 to 40, the farms themselves are small. This high-density environment can facilitate the spread of bacteria (Petersen and Hubbart, 2020). Additionally, some farms were mixed with neighboring farms, which could potentially lead to cross-contamination, exacerbating the spread of *E. coli*.

Moreover, the overuse and uncontrolled application of antibiotics in these farms could be contributing to the high prevalence of antibiotic-resistant *E. coli* strains. This is a significant concern, as it makes infections caused by these bacteria more difficult to treat, posing a risk to both animal and human health.

### Detection of beta-lactam resistance genes

In total, 15 isolates (60%) exhibited the presence of the beta-lactam antibiotic resistance genes. Among these, ten samples (40%) manifested a single beta-lactam ARGs, while five samples (20%) presented two ARGs. Conversely, ten samples (40%) displayed no detectable resistance gene. The amplification of beta-lactam resistance genes was assessed through real-time qPCR, and the results, portrayed in the form of curves, can

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer nucleotide</th>
<th>Nucleotide sequence (5'→3')</th>
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<td>blaTEM-79f</td>
<td>GATGCTGAAGATCGTGGGTTG</td>
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References:

- Basbas et al. (2021)
- Naas et al. (2013)
- Wei et al. (2016)
- Akomoneh et al. (2020)
- Jenifer and Sathiyamurthy (2020)
be observed in Figure S1. This graphical representation illustrated the dynamics of amplification for these genes, providing insights into the quantitative aspects of beta-lactam antibiotic resistance within the studied isolates. The amplification curves for \textit{blaTEM}, \textit{blaCTX}, \textit{blaCMY}, and \textit{blaOXA} showed notable differences. For \textit{blaTEM} (Figure S1A), the amplification curve grew until nine samples exceeded the threshold.

The amplification curves for \textit{blaCTX} (Figure S1B) and \textit{blaCMY} (Figure S1C) exceeded the threshold in six and four samples, respectively, whereas the amplification curve for \textit{blaOXA} (Figure S1D) increased in only one sample. On the other hand, all 25 manure samples and the negative control showed a flat curve in \textit{blaSHV} (Figure S1E), suggesting that the \textit{blaSHV} gene was absent.

The comprehensive results of qPCR analyses, specifically the Cycle Threshold (Ct) values for beta-lactam resistance genes across all samples, are briefly presented in Table 2.

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| Min         | 8.82           | 10.26          | 33.43          | 18.51          | -              |
| Max         | 32.87          | 35.67          | 33.43          | 26.24          | -              |

Ct=Cycle threshold; - = No detected DNA

The Ct value indicated the number of cycles required for the amplification of the target gene to reach a detectable level. Lower Ct values indicated a higher abundance of the gene. Lower Ct values indicated a higher abundance of the gene. The lowest Ct value recorded was 8.82 for the \textit{blaTEM} gene, and the highest was 35.67 for \textit{blaCTX}. The table showed that 40\% of \textit{E. coli} isolates (ten samples) carried only one beta-lactam antibiotic resistance gene, while 20\% of isolates (five samples) had two beta-lactam genes, and 40\% were negative. This structured presentation facilitates a clear understanding of the dynamics and distribution of beta-lactam resistance genes within the analyzed samples.

The presence of ARGs varies among samples, with some containing a single ARG and others harboring two. This variation established a distinct pattern in the distribution of ARGs within the studied dataset. The prevalence of these ARGs, along with the observed patterns of their occurrence, is presented in Figures 1 and 2.

The most dominant gene was \textit{blaTEM}, and it was detected in 36\% of all manure samples, followed by \textit{blaCTX} (24\%), \textit{blaCMY} (16\%), and \textit{blaOXA} (4\%), while the \textit{blaSHV} gene was not detected (Figure 1). Five patterns for \textit{bla} genes were identified among the samples. The dominant pattern was \textit{blaTEM} (33\%), followed by \textit{blaCMY}, \textit{blaTEM+blaCTX} (20\%), then \textit{blaCTX+blaOXA}, and \textit{blaTEM+blaCMY} (7\%) (Figure 2). These graphs give valuable insights into the distribution dynamics of antibiotic resistance within the samples, as they offer a comprehensive view of
both the prevalence of individual ARGs and the patterns emerging from their coexistence.

Figure 1: Prevalence percentage of beta-lactam resistance genes in manure samples from dairy farms. The total number of collected subsamples was 75 (three samples per farm × 25 farms).

Figure 2: The pattern of beta-lactam resistance genes in manure samples from dairy farms. The total number of collected subsamples was 75 (three samples per farm × 25 farms).

In the current study, we examined five types of bla genes that contribute to the development of resistance to beta-lactam antibiotics in E. coli through enzymatic inactivation. Among the five bla genes tested, all of them, except the blaSHV gene, were detected in the manure samples of dairy cattle. The blaTEM gene was the most dominant one (36%) in manure samples, followed by blaCTX (24%), blaCMY (16%), and blaOXA (4%), while the blaSHV gene was not detected. Yang et al. (2021) revealed that blaCMY-2, blaSHV, and blaOXA-48 were detected in dairy cattle feces in two Chinese intensive dairy farms, while in another study by Yang et al. (2022) in central China, blaCMY-2 and blaSHV were detected at a rate of 25%, 12.5%, respectively, while blaOXA-48 was not detected in cattle waste.

Anderson et al. (2023) conducted a study in Ontario, Canada, using PCR to assess the diversity and distribution of blaCTX-M, blaCMY, and blaSHV in E. coli in dairy cattle manure in both fresh state and after multiple treatment stages in six farms. The study reported the presence of blaCTX-M, blaCMY, or blaSHV in the fresh manure samples with a downward trend in the proportion of samples with resistant genes through the manure treatment stages. In Pakistan, a study conducted by Ejaz et al. (2021) revealed that among the ESBL-producing E. coli isolated from fecal specimens of dairy cows, 73.9% were found to carry the blaCTX-M gene, 26.1% carried the blaTEM gene, and 14.2% carried the blaSHV gene. The prevalence of these genes indicated a high presence of drug resistance in dairy cattle, highlighting the potential for transmission to humans. In a separate investigation conducted by Jalil et al. (2023), they focused on the notable prevalence of multidrug-resistant E. coli strains in fecal samples from healthy cows. Their findings revealed that during beta-lactamase genotyping of E. coli isolates, 47% of the isolates carried either blaCTX or blaTEM genes.

The absence of blaSHV genes in the study was in accordance with surveillance activities conducted in healthy animals worldwide, which have generated a vast amount of data on the distribution of ESBL. Notably, most SHV beta-lactamase producers are E. coli from swine and broiler fecal samples, as observed in China (Tian et al., 2012). In Spain, blaSHV has been associated with blaCTX-M in pigs and broilers (Blanc et al., 2006). Conversely, in Japan, blaSHV has been found in layers, cattle, and broilers but not in swine (Hiki et al., 2013; Kameyama et al., 2013). In the Netherlands, blaSHV has been identified in healthy broilers alongside blaTEM (Dierikx et al., 2010).

These genes play a crucial role in conferring resistance to beta-lactam antibiotics. They are often carried on mobile genetic elements such as plasmids, Integrons, and transposons. The presence of these genes in dairy cattle manure indicates the potential for the transfer of drug resistance to other bacteria, both within the animal population and potentially to humans. The blaTEM, blaCTX-M, and blaSHV are often carried on plasmids, enabling the spread of resistance to beta-lactam antibiotics, such as penicillins and early cephalosporins. The blaTEM and blaSHV have a broad host range of these plasmids, allowing the transfer among different
bacterial species. BlaCTX-M gene has gained attention due to its rapid dissemination and prevalence among Enterobacteriaceae, especially E. coli and Klebsiella pneumoniae. BlaCMY-2 is frequently associated with transposons, which are segments of DNA that can move within and between plasmids and chromosomes. The blaOXA gene family is often linked to both plasmids and integrons. Some strains of Acinetobacter baumannii, for instance, carry blaOXA genes on plasmids, facilitating their spread among Acinetobacter species. Integrons, especially class 1 integrons, also play a role in capturing and disseminating blaOXA genes among various Gram-negative bacteria (Partridge et al., 2018).

The high prevalence and the variety of the bla genes detected in manure samples from dairy farms in Bogor City indicated the occurrence of cattle-to-environment transmission. This transmission is a result of the overuse and uncontrolled application of beta-lactam antibiotics in these dairy farms. According to Casseri et al. (2022), antibiotics play a crucial role in dairy herd management. They are used to treat bacterial diseases, particularly mastitis, and to ensure optimal animal welfare. In the United States, approximately 16% of lactating dairy cows with mastitis infections receive antibiotic medication each year.

Additionally, 80% of dairy herds implement blanket dry cow therapy to prevent and treat intramammary infections. This therapy involves administering antibiotics via intramammary infusions to every cow in every udder quarter after each lactation. Penicillins, cephalosporins, and other beta-lactam antibiotics are the most often prescribed intramammary antibiotics (Landers et al., 2012; Hommels et al., 2021).

Considering the distinctive circumstances in the Kebon Pedes region, the transformation of the area into a dense urban area has probably made it an unsuitable location for sustainable dairy farming operations. Dairy farms are typically located far from urban areas to minimize any risks such as antibiotic resistance spread. However, this is not the case in Kebon Pedes, where dairy farms are in close proximity to urban areas. This proximity could accelerate the spread of E. coli carrying ARGs, as supported by a study by Xie et al. (2018), which found that the prevalence of ARGs was higher in environments closer to human activities.

The proximity of dairy farms to urban environments can accelerate the spread of ARGs. Kebon Pedes, previously a rural area, is experiencing rapid urbanization, transforming it into a densely populated urban area. This transformation has led to the encroachment of urban spaces into areas previously designated for dairy farming, resulting in close proximity of dairy farms to urban environments. Such proximity can exacerbate the spread of ARGs, as urban environments often have higher levels of antibiotic use and, therefore, a higher prevalence of resistant bacteria. This is supported by a study by Li et al. (2012), which found that urban environments could act as hotspots for the dissemination of ARGs.

Furthermore, the lack of stringent regulations and monitoring of antibiotic use in livestock farming can also contribute to the problem. In many regions, antibiotics can be easily obtained and used without a veterinary prescription, leading to their overuse and misuse (Van Boeckel et al., 2015). This not only selects for antibiotic-resistant bacteria but also promotes the horizontal gene transfer of ARGs among bacteria, further exacerbating the problem (Forsberg et al., 2012).

Apart from the proximity of dairy farms to urban environments and the misuse of antibiotics, several other factors may contribute to the high prevalence of ARGs. One such factor is the poor waste management practices prevalent in many dairy farms. Manure, a significant waste product from dairy farms, often contains high levels of antibiotics and antibiotic-resistant bacteria due to the widespread use of antibiotics in livestock (Heuer and Smalla, 2007). When this manure is improperly disposed of or used as fertilizer without appropriate treatment, it can contaminate the surrounding environment, including soil and water bodies, leading to the spread of ARGs.

The scarcity of data concerning ARGs, and specifically beta-lactam resistant genes in E. coli within dairy farms, is a notable issue in one of Bogor’s most bustling areas, Kebon Pedes. This gap in knowledge underscores the significance of this research. By focusing on this specific area, the study aims to fill this void and provide valuable insights into the prevalence of these resistant genes. This research is not only crucial for understanding the current situation but also carries significant connotations for both public
health and the management of dairy farms in the Bogor region. It can serve as a valuable reference point for shaping policies and introducing a comprehensive One Health approach to curb microbial contamination on dairy farms. To tackle this issue, a multi-faceted strategy should be put into action at the farm level. This includes the correct storage and processing of manure, such as composting or anaerobic digestion, to decrease or eliminate the survival of *E. coli*. Manure should not be directly applied to pastures or crops without undergoing this treatment.

Regular monitoring and surveillance of the manure are essential to detect ARGs and other harmful bacteria. It’s also crucial to elevate the awareness of dairy farmers in Bogor. This can be achieved by offering training and educational resources that highlight the risks of irresponsible antibiotic usage and the importance of proper manure management. These measures can serve as proactive steps to control the spread of *E. coli* and ARGs originating from dairy farms. Ultimately, these steps contribute to the protection of public health and the sustainability of Bogor’s local dairy industry.

**Conclusions**

The bla-TEM gene emerged as the most prevalent gene found in *E. coli* isolated from dairy cattle manure in Bogor, Indonesia. Among the various combinations of bla genes detected in these *E. coli* isolates, the blaTEM+blaCTX pattern was the most dominant. The high prevalence and diversity of the bla genes detected in manure samples from the dairy farms in the Kebon Pedes area, Bogor suggests that transmission of beta-lactam antibiotic resistance had occurred from dairy farms to the environment.

Poor hygiene, close proximity to urban areas, unregulated antibiotic use, and inadequate waste management practices in this region serve as a potential source for the dissemination of *E. coli*-carrying ARGs into the environment, posing a significant threat to public health. The study underscores the critical need for a comprehensive and multi-pronged approach to mitigate the spread of *E. coli* and ARGs in the dairy farming industry. This includes improved hygiene practices, proper waste disposal, responsible use of antibiotics, and continuous monitoring and surveillance. Implementing these measures is vital to safeguard public health and ensure the sustainability of the dairy industry in Bogor, particularly in the Kebon Pedes area.

**References**


