Phylogenetics of blaTEM Gene in *Escherichia coli* of the Bali Cattle from Small Family Farms on Lombok Island

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Abstract

Extended Spectrum β-Lactamase (ESBL), an enzyme produced by *E. coli* bacteria hydrolyses various β-lactam antibiotics, causing antibiotic resistance that will impact the occurrence of Antimicrobial Resistance (AMR). The occurrence of horizontal gene transfer to other bacteria from *E. coli* bacteria that encode ESBL genes in the microenvironment can potentially increase cases of AMR. *E. coli* encoding the blaTEM gene has been found in Bali cattle in traditional livestock groups on Lombok Island, but information on phylogenetic analysis is still minimal. This chapter will explain E. coli, β-Lactam antibiotics, pathways of *E. coli* resistance to β-lactam antibiotics, epidemiology of *E. coli*-producing ESBL, and the construction of phylogenetic analysis of *E. coli* encoding the blaTEM gene isolated from Bali cattle from family farms compared to several *E. coli* encoding the blaTEM gene in GenBank. Phylogenetic analysis of *E. coli* encoding the blaTEM gene isolated from Bali cattle is expected to provide information about the kinship and origin of *E. coli*-producing ESBL. *E. coli* of the Bali cattle from farms on Lombok Island showed the character of *E. coli* TEM-206 and related to the *E. coli* strain U-10. Data on the phylogenetic analysis of *E. coli* encoding the blaTEM gene is expected to be used to prevent transmission of *E. coli*-producing ESBL in animals, humans, and the environment.

Keywords: Kinship, *Escherichia coli*, Blatem, Bali cattle, Lombok

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Introduction

Escherichia coli is a bacterium that has attracted attention in the world of microbiology and epidemiology because it can secrete enzyme extended-spectrum β-lactamase (ESBL) and is also a major source of resistance genes that have the potential to impact treatment and the incidence of antimicrobial resistance (AMR) in humans and animals. Bacterial AMR is expected to have caused 1.27 million fatalities worldwide in 2019, contributing to 4.95 million human deaths (Murray et al., 2022).

Several studies have identified the β-lactamase antibiotic resistance gene from *E. coli* isolates in recent years from humans, the environment, and animals. Agrawal et al. (2021) stated that *E. coli* blaTEM has been discovered in the urine of Indian dairy cows with reproductive problems. *E. coli* with ESBL-type TEM genes has also been found in cow milk and the environment in Peninsular Malaysia (Kamaruzzaman et al., 2020). Fifty percent of the 59 *E. coli* isolated from cattle on Florida farms have two ESBL-encoding genes with the TEM and CTX-M kinds (Lee et al., 2020). On Lombok Island Indonesia, *E. coli* encoding the β-lactamase Temoneira (blaTEM) gene has been found in the Bali cattle reproductive tract at family farms (Kholik et al., 2023).

The presence of *E. coli* encoding the blaTEM gene in the reproductive tract of Bali cattle needs to be studied because *E. coli* is a normal flora that is generally found in the lower intestine of both animals and humans. The fact that *E. coli* is encoding the blaTEM gene in Bali cattle in farms on Lombok Island will raise questions about the origin of *E. coli*. *E. coli* can also come from humans or the environment due to gene transfer when *E. coli* is in the environment. Le Roux et al. (2018) documented that *E. coli* with extended-spectrum β-lactamase (ESBL) can spread horizontally by gene transfer in its surroundings, increasing AMR cases in humans and animals.

Based on the fact that *E. coli*-producing ESBL was isolated from animals, humans and the environment, then the occurrence of horizontal gene transfer of *E. coli*-producing ESBL in the microenvironment, it is necessary to take action to determine the origin of the bacteria that can spread the gene in livestock areas with beginner farmer groups and minimal biosecurity such as on the island of Lombok. Lombok Island is one of the islands rich in livestock and a national meat supplier. The data from the West Nusa Tenggara provincial statistics center until 2022 shows that 1,219,784 cattle spread across the islands of Lombok and Sumbawa. (Statistics of Nusa Tenggara Barat Province, 2024).

The phylogenetic analysis of *E. coli* encoding the β-lactamase Temoneira (blaTEM) gene isolated from Bali cattle will provide information about the kinship and origin of *E. coli* ESBL in livestock farming on the island of Lombok. Phylogenetic analysis of *E. coli* encoding the blaTEM gene can be used to prevent the transmission of *E. coli*-producing ESBL in animals, humans, and the environment. Wall et al. (2016) stated that it focuses on reducing the spreading of *E. coli* that encode resistance genes in the ecosystem.

Escherichia coli

E. coli is a normal flora bacteria of the large intestine in humans and animals. The name of this bacteria is taken from the name of the German bacteriologist, Theodor Von Escherich, who succeeded in isolating bacteria from human feces for the first time in 1885. Observations of *E. coli* under a microscope are in the form of short, slender rods with a length of 1–5 μm and a width of 0.3–0.4 μm, named Bacterium coli commune (Escherich, 1998). In 1919, the bacteria were named after their discoverers, namely Castellani and Chalmers, namely *E. coli* (LPSN, 2024).

E. coli can be isolated from animal feces such as cows, chickens, and other animals and the environment. *E. coli* can be cultured on several agar media, Eosin Methylene Blue Agar *E. coli* had a metallic green color that sparkled like metal (Fig. 1), *E. coli* is included in the gram-negative bacteria group when stained with gram staining it will appear red, in the form of a bacillus (Figure 2). *E. coli* has a cell wall with a thinner peptidoglycan layer, so it cannot be with crystal violet during staining (Holt et al., 1994).

Fig. 1: Colonies of *Escherichia coli* in Eosin Methylene Blue Agar

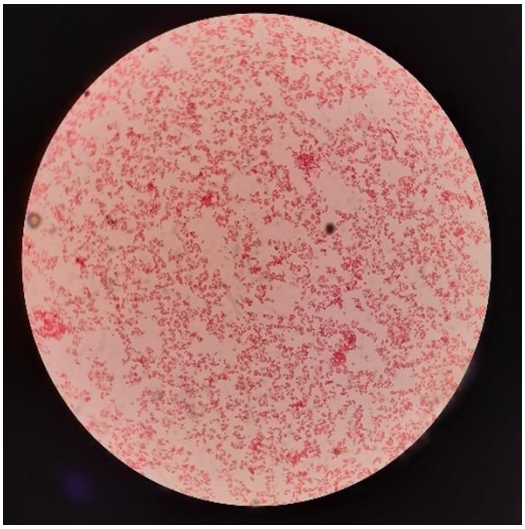

Fig. 2: Morphology of *Escherichia coli* by Gram Staining

β-Lactam Antibiotics

Antibiotics of the beta-lactam group are penicillins, cephalosporins, carbapenems, and monobactams. β-Lactam antibiotics have a cyclic amine structure of three carbons and one nitrogen known as a beta-lactam ring. A peptide link binds the variable group known as the beta-lactam ring to the core structure. "Lactam" refers to any cyclic amide, however, "β-lactam" indicates that the nitrogen is connected to the carbonyl's beta-carbon to form a ring that the beta-lactamase enzyme targets (Fig. 3) (Thenmozhi et al., 2014).

***Escherichia coli* Resistance to β-Lactam Antibiotics**

In general, bacteria are resistant to antibiotics through several pathways, including (a) degrading or degrading antibiotics, (b) bacteria actively transport antibiotics out of the cell or prevent their absorption, (c) bacteria produce special proteins to isolate antibiotics, or (d) bacteria modify, bypass, or protect their targets (Peterson et al., 2018). *E. coli* is resistant to beta-lactam antibiotics by producing beta-lactamase enzymes to hydrolyze the β-lactam ring so that they are degraded (Table 2).

Table 2: Common Resistance Genes to β-Lactam Antibiotics Encoded by *E. coli* (Tyson et al., 2015)

Gen resistance	β-Lactam antibiotics
blaTEM-1	Ampicillin
blaOXA-1	Ampicillin
blaCMY-2	amoxicillin/clavulanic acid (AMC), ampicillin, ceftriaxone, cefoxitin, ceftiofur

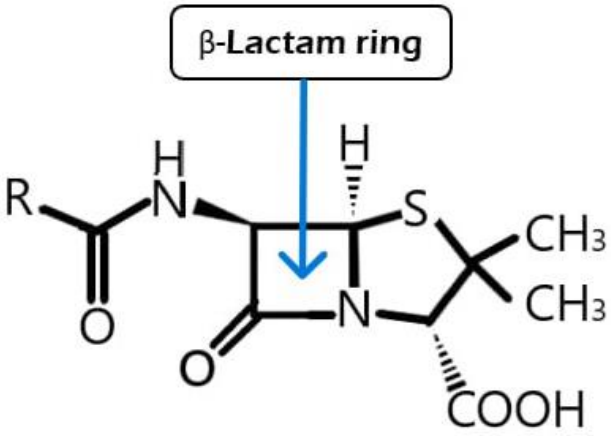

Fig. 3: Chemical Structure of Penicillin (β-Lactam Ring) which is the Target of Beta-lactamase Enzyme (Blue arrow) (Thenmozhi et al., 2014).

Extended Spectrum β-Lactamases

E. coli belonging to *Enterobacteriaceae* produce the enzyme extended-spectrum beta-lactamase, which has heightened activity in hydrolyzing β-lactam antibiotics, particularly oxyimino-cephalosporins (Bush & Bradford., 2016). This enzyme can hydrolyze β-lactam antibiotics such as penicillin, cephalosporin, and monobactam. β-lactam antibiotics with a β-lactam ring component consist of penicillin, cephalosporin, monobactam, and carbapenem. The β-lactam ring component inhibits bacterial cell wall synthesis (Reygaert, 2018).

Extended-spectrum beta-lactamase can induce resistance to penicillin, cephalosporin 1st, 2nd, 3rd generation, and aztreonam (except cephamycin and carbapenem) (Paterson & Bonomo, 2005). Resistance due to the production of β-lactamase enzymes has developed rapidly into extended-spectrum β-lactamases (ESBLs). The ability of ESBL-producing bacteria to hydrolyze β-lactam antibiotics is widely caused by several mutation genes such as SHV, TEM, and CTX-M (Bajpai et al., 2017). Variations in resistance genes such as blaCTX-M have been questioned to geographic location (Hawkey & Jones, 2009). The type of ESBL gene has genotypic variations including SHV, TEM, CTX-M, OXA, and VEB types (Bradford, 2001; Drieux et al., 2008).

Epidemiologi *Extended Spectrum β-Lactamases* (EBSL) *Escherichia coli*

The extended-spectrum *β-Lactamases* (ESBL) encoding genes of the TEM type consist of blaTEM-1 and blaTEM-2. blaTEM-1 was first discovered in Greece in 1965 from *E. coli* isolated from a patient named Temoneira in Greece, so the enzyme is called TEM (Guenther et al., 2011). The ESBL encoding genes in *E. coli* have undergone several mutations that allow it to hydrolyze a broad spectrum of beta-lactam antibiotics. Most ESBLs are derivatives of the Temoneira or Sulphydryl Variable enzymes (Bush et al., 1995).

Previous studies have shown the dominance of blaTEM and the presence of multi-gene combinations in *E. coli* originating from dairy farm wastewater in East Java (Dameanti et al., 2023). ESBL-producing E. coli, encoding the blaTEM gene also was found in 77.78% of one hundred and ten milk samples from 45 dairy farms in Tulungagung, Indonesia (Widodo et al., 2023). *E. coli*-producing ESBL has also been found in human blood with sequence type (ST) 131 in 64% of 293 isolates from some parts of the United Kingdom (Day et al., 2019). According to research conducted in Bangladesh, the results of a meta-analysis conducted on 36 showed that the frequency of *E. coli* that produces ESBL in humans, animals, and the environment was 17%, 22%, and 39% (Islam et al., 2023).

The results of other studies using the PCR method also showed the presence of the blaCTX-M gene and the double blaTEM gene in 2 *E. coli* produces ESBL isolates from 200 raw milk samples taken from dairy farms located in Probolinggo, Pasuruan, Batu, and Blitar Regencies, East Java Province, Indonesia (Ansharieta et al., 2021). *E. coli* encoding the blaTEM gene was discovered in the reproductive tract of Bali cattle on the Indonesian island of Lombok using the PCR technique which is similar to the *E. coli* blaTEM gene for TEM-206 (Kholik et al., 2023).

Bali Cattle

Bali cattle are superior local beef cattle breed widely raised by Lombok breeders. Bali cattle found in various regions in Indonesia have probably undergone crossbreeding with *Bos taurus, Bos indicus, Bos bibos*, and other cattle breeds, resulting in genetic diversity. Bali cattle have criteria such as red fur, legs from tarsus and carpus to white nails, and a black line on the back (Fig. 4). In female Bali cattle, the back of the thigh is white (white mirror) (Hardjosubroto, 1994). Bali cattle have high economic value, high fertility rates, low mortality, are easily adaptable to the environment, and have a high carcass percentage. The average live weight of Bali cattle at birth can reach 16.8 kg, while mature Bali cattle reach 303 kilograms (Talib et al., 2003).

Fig. 4: Morphology of Bali Cattle

Lombok Island

West Nusa Tenggara Province is one of the Indonesian provinces that includes the islands of Lombok and Sumbawa (Figure 5). The Earth of a Million Cattles program makes Lombok Island a national beef cattle supplier because of its abundance of communities and livestock. According to data from the West Nusa Tenggara Province's Central Statistics Agency, West Nusa Tenggara had 1,234,357 cattle in 2019, and in 2022 shows 1,219,784 cattle in the province (Statistics of Nusa Tenggara Barat Province, 2020; Statistics of Nusa Tenggara Barat Province, 2024). West Nusa Tenggara Province is also a

supplier of beef cattle to all countries in Indonesia with the 1000 cattle village program on the island of Lombok (Mashur et al., 2022).

The family Farms are part of livestock groups on Lombok Island, especially in East Lombok in there were livestock farmer groups consisting of beginner classes. Community livestock with no good sanitary management on Lombok Island can lead to the spread of *E. coli* encoded ESBL gene, in humans, animals, and the environment if antibiotics are used carelessly. Repeated uncontrolled administration of antibiotics in small amounts to livestock can cause selective pressure among bacteria in the animal's body which can increase the development of bacterial resistance to survive (Van et al., 2020).

Fig. 5: Lombok (L) and Sumbawa Island (S) in West Nusa Tenggara Province, Indonesia

Phylogenetics Analysis

Phylogenetics is a branch of biology that studies organisms' evolution and heredity patterns. Evolution is a gradual process of a species that has the potential to change in the next few generations of offspring. Offspring will have differences from their ancestors because they are changing in evolution (Dharyamanti, 2011).

The process of evolution will involve genetic mutations and gene recombination in species to form new species with different characteristics from their parents. The similarity of characters in species is the basis for analyzing the relationship of one species to another, so the phylogenetic tree is a logical approach to showing the evolutionary relationship between organisms. Phylogenetic or kinship analysis can be used as a model to represent the relationship of the ancestors of organisms, molecular sequences, proteins, or both (Brinkman and Leipe, 2001).

Phylogenetic tree construction is an interesting thing in the study of evolution, in addition to being able to see the kinship of organisms, phylogenetics will also be able to describe the estimated origin of organisms by looking at environmental conditions, animals, and humans that have the potential for an interaction that can cause transmission of organisms between species. Several methods have been used to construct phylogenetic trees but the Neighbor-joining Method (NJ) has been agreed to be the choice for building phylogenetic trees (Saitou and Imanishi, 1989).

Phylogenetics of blaTEM Gene in *Escherichia coli* of the Bali Cattle

Phylogenetic construction of the blaTEM gene in *E. coli* was preceded by isolation, antibiotic susceptibility testing, PCR, and sequencing of the blaTEM gene in *E. coli*. *E. coli* in this book chapter is *E. coli* originating from the reproductive fluid of Bali cattle. An insemination pistol covered with a plastic sheet was placed into the reproductive canal of Bali cattle to collect *E. coli* from reproductive fluid (Andriani et al. 2021). After cutting the end of the plastic sheet then incubated for 24 hours at 37°C in Brain Heart Infusion. To cultivate *E. coli*, the sample was placed in EMBA and incubated for 24 hours at 37˚C. The growing *E. coli* were then identified by Gram staining and biochemical tests referring to basic laboratory procedures in clinical bacteriology (Vandepitte et al. 2003).

The Kirby-Bauer disc diffusion test method was then used to evaluate the identified *E. coli* for antibiotic susceptibility and resistance using disc diffusion cultured on Mueller Hinton Agar. The double disk method is used to screen the presence of ESBL. The antibiotics used in Cefotaxime (CTX) 30 µg and amoxicillin-clavulanate (AMC) 30 µg were used in the double disc test method, with *E. coli* ATCC 25922 as a negative control (Kholik et al., 2023).

The diameter of the inhibition zone formed was measured to interpret the results of the *E. coli* antibiotic susceptibility test (Figure 6). The inhibition zone formed was based on the Clinical and Laboratory Standards Institute (CLSI, 2015). *E. coli* that produced ESBL using the double disk method will be subjected to PCR, sequencing, and phylogenetic analysis.

Polymerase chain reaction (PCR) against *E. coli* from Bali cattle samples was preceded by extraction of *E. coli* DNA. A total of 51 µL was used for the PCR, which included 3 µL of extracted *E. coli* DNA as a template, 1.5 µL of primers, 25 µL of Dream Taq HS PCR mix, and 20 µL of ddH2O. A Biorad i-cycler PCR equipment was used to process the mixture. The specific primers of the blaTEM forward gene and the blaTEM reverse gene are in Table 1, according to Von Salivati et al. (2014) after that, the blaTEM gen of PCR product continued to electrophoresis (Fig. 7).

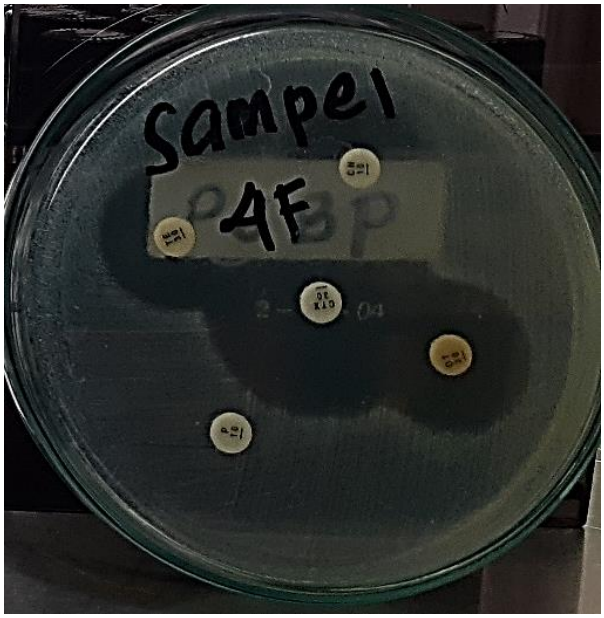

Fig. 6: Example of *E. coli* in the Double Disk Method

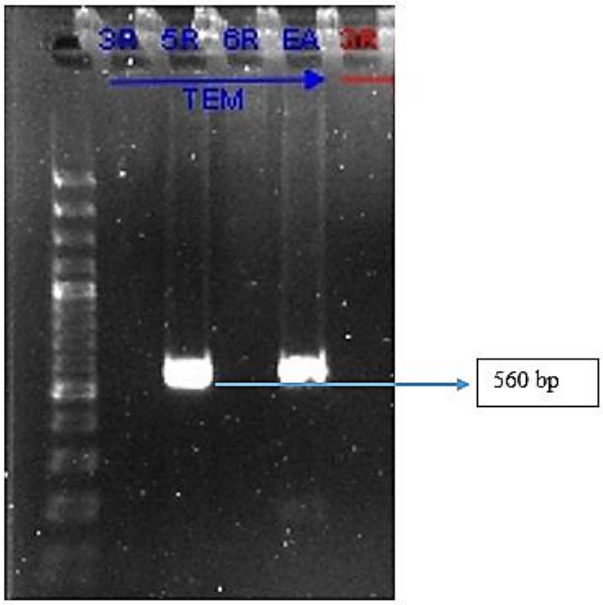

Fig. 7: *E. coli* Encoded blaTEM Gene in Gel Electrophoresis (Kholik et al., 2023)

Table 1: *E. coli* blaTEM Gene Primers

Gene	Primer	Sequence (5- 3)	Amplicon size [bp]	Reference
blaTEM	Primer Forward	5- TCCTTGAGTTTTCGCCCC -3	581	Von Saliviati et al., 2014
	Primer Reverse	5- CAGTGCTGCAATGATACCGC -3		

Fig. 7 documented that the blaTEM gene *E. coli* of Bali cattle was found on gel electrophoresis at 560 bp (Kholik et al. 2023). PCR amplification of *E. coli* from Bali cattle was sequenced to obtain nucleotide data for phylogenetic analysis. Phylogenetic tree construction of blaTEM gene sequence of *E. coli* from Bali cattle using the neighbor-joining (NJ) method (Tamura et al., 2011). The result of the phylogenetic tree of the blaTEM gene revealed a genetic link with GenBank references, specifically code NG_050238.1, as well as with codes MW183897.1 and MT789719.1 (Figure 8).

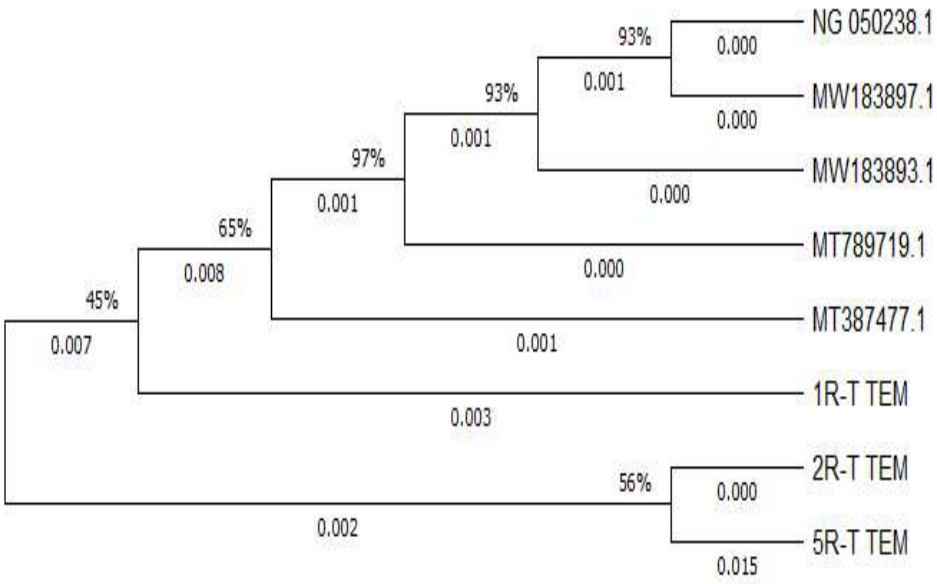

Fig. 8: The blaTEM Gene *E. coli* Isolated from Bali cattle by Phylogenetic tree. with data from GenBank. (1R-TTEM, 2R-TTEM, 5R-TTEM): E. Coli from Bali ; (NG_050238.1, MWI838897.1, MT387477.1, MW183893.1, MT789719.1, MT387477.1): Data reference in GenBank. (Kholik et al., 2023)

Based on the results of the phylogenetic tree analysis, the character of the ESBL encoding gene from *E. coli* isolated from Bali cattle can come from livestock shown by being related to the code NG_050238.1 (TEM-206) and MT789719.1 (blaTEM-1). *E. coli* ESBL from Bali cattle can also come from human feces or urine, which is shown to be related to the code MW183897.1, a beta-lactamase of the TEM family *E. coli* strain U-10. This can happen because the blaTEM gene has a wide distribution in various isolates. The beta-lactam resistance gene blaTEM-1

has been found in 43,339 genomes, which shows its wide distribution in various species and sources of isolates (Farooq et al., 2025)

These phylogenetic results can be a lesson that *E. coli* ESBL from Bali cattle can originate and spread from livestock, humans, and the environment which has the potential to continue to circulate in the cattle farming population (Figure 9).

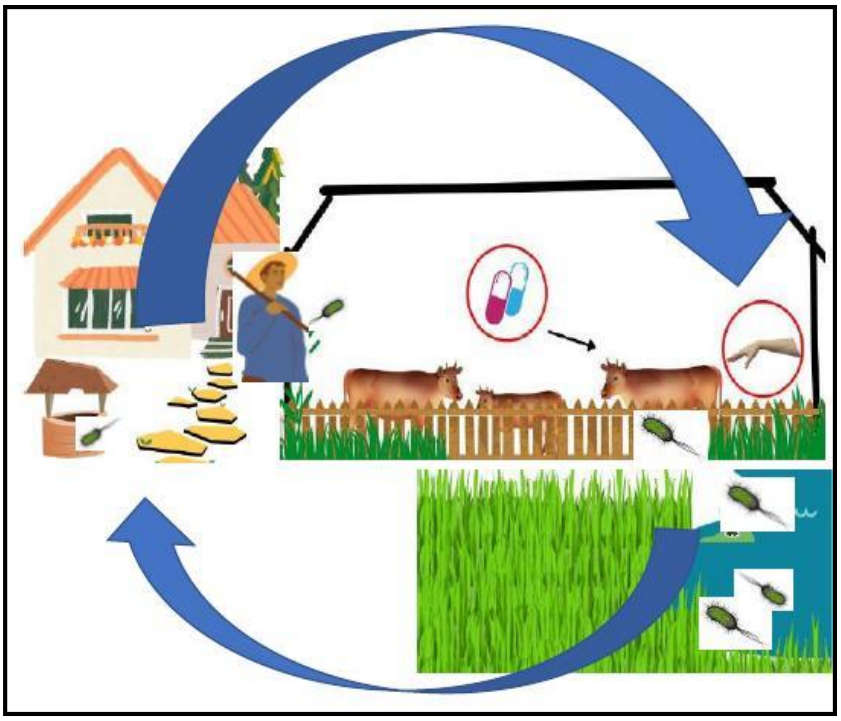

Fig. 9: Potential Circulation of ESBL *E. coli* from Balinese cattle on Small Family Farms on Lombok Island (Kholik et al., 2023)

Figure 9 illustrates that ESBL *E. coli* from Bali cattle can spread to the environment, including pastures as a source of feed, wells as a source of water, and humans who interact with Bali cattle, especially if livestock waste is not managed properly. Some losses from livestock and improper animal waste disposal practices result in the release of antibiotics and resistant bacteria into the environment, where natural microbes and pathogens exchange resistance genes (Kazim et al., 2024).

The potential for ESBL *E. coli* to continue to circulate in the cattle population is supported by the discovery of *E. coli* identified from Bali cattle experiencing reproductive abnormalities that are resistant to cefotaxime which have the potential for *E. coli* that manufacture the ESBL enzyme (Kholik et al., 2021). Recent horizontal transfer events involving the blaTEM-1 gene cluster and mobile genetic elements (MGEs) underscore the potential of MGEs in facilitating antibiotic resistance genes (ARGs) mobilization, making it essential to adopt a One Health approach to global genomic pathogen surveillance, aiming to uncover ARG transmission routes and formulate strategies to address the growing antibiotic resistance crisis (Farooq et al., 2025).

The blaTEM gene in *E. coli* of Bali cattle from small family farms on Lombok Island can be phylogenetically analyzed to at least provide an answer to the still-debatable question of how producing animals like Bali cattle on smallholder farms contributed to the AMR epidemic. The gene encoding β-lactamase Temoneira (blaTEM) *E. coli* in Bali cattle can come from livestock and human feces. This incident can occur because Bali cattle farms have Bali cattle and raise poultry and other animals with minimal sanitation and biosecurity. Kholik et al. (2024) stated that livestock groups on Lombok Island do not yet know biosafety and biosecurity in detail. Hence, they need to be introduced to the participatory rural appraisal (PRA) method.

Conclusion

This chapter explains that *E. coli* of the Bali cattle from small family farms on Lombok Island showed the character of the blaTEM gene of *E. coli* TEM-206 and related to the TEM family of *E. coli* strain U-10. *E. coli* that encodes the ESBL gene in Bali cattle can come from humans, animals, and the environment where the cattle are located. The potential for spreading *E. coli* encoding blaTEM through feces and urine in livestock, farmers, and the environment around people's farms on Lombok Island is possible because farms on Lombok Island generally have poor biosecurity. This incident requires cooperation between One Health domains to prevent antimicrobial resistance (AMR) in smallholder farms.

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