Jurnal Sain Peternakan Indonesia
Available at https://ejournal.unib.ac.id/index.php/jspi/index
DOI: https://doi.org/10.31186/jspi.id.18.4.235-241
P-ISSN 1978-3000
E-ISSN 2528-7109
Volume 18 Issue 4 October-December 2023

Antibiotic Resistance in *Escherichia Coli* Bacteria Isolated from Water Sources and Waste Disposal in Livestock Farms in East Lombok

S. N. Sa'diyah[1], K. Kholik[1]*, M. Munawaroh[1], A. T. D. Aprianti[2], S. E. Rahmawati[1], and K. H. P. Riwu[1]

[1] Faculty of Veterinary Medicine, Universitas Pendidikan Mandalika,
Jl. Pemuda No.59A, Mataram City 83125, Indonesia
[2] Master Degree of Biology Program, Faculty of Sains and Technology, Universitas Airlangga,
Jl. Dr. Ir. H. Soekarno, Mulyorejo, Surabaya, Jawa Timur 60115, Indonesia
Corresponding Author: kholiqvet@gmail.com
Revised: 2023-12-25, Accepted: 2023-12-28, Publish: 2023-12-30

ABSTRACT

Poor antimicrobial stewardship in livestock farms will lead to the emergence of bacterial resistance to antibiotics. Dug wells as a water source and waste disposal on livestock farms have a close distance, allowing transmission of *Escherichia coli*-resistant bacteria through soil absorption and fecal contamination. This study aims to isolate *Escherichia coli* from water sources and livestock waste disposal in East Lombok Regency and determine their sensitivity to several antibiotics. The type of this research is a descriptive cross-sectional survey using four water wells and four waste disposals with a criterion of < 10m range. Escherichia coli bacteria were isolated using culture techniques on Eosin Methylene Blue Agar, and identification was carried out using gram staining and biochemical tests. Determining sensitivity to antibiotics was performed using the Kirby-Bauer disk-diffusion method. The results showed that *Escherichia coli* bacteria have been isolated from water sources and waste disposal on livestock farms in East Lombok Regency. 100 % *Escherichia coli* isolates sensitive to Gentamicin, Ciprofloxacin, and Cefotaxime. 87.5% Escherichia coli isolates sensitive to Oxytetracycline, 12.5% *Escherichia coli* isolates resistant to Oxytetracycline, 100% *Escherichia coli* isolates resistant to Penicillin G. *Escherichia coli* bacteria isolated from water sources and waste disposal at a livestock farm in East Lombok.

Keywords: antimicrobials, environment, farming, Lombok Island

INTRODUCTION

Antimicrobial resistance (AMR) is one of the biggest threats to global health connected with the misuse of antibiotics in humans and animals (WHO, 2021). AMR will be related to antibiotics in the water around the farm due to their use in both animals and humans. Antimicrobials are used in livestock farms to improve animal health, welfare, and productivity (Woolhouse et al., 2015). Cordero et al. (2019) reported that farmed antibiotics have been used for treatment and feed mixtures to enhance growth. Antibiotics used in livestock farms have the potential to contaminate water sources or be in sewage disposal so that they can encourage the emergence of bacterial resistance in the water. A multisectoral approach is necessary to overcome the complexity of controlling antimicrobial resistance, which can also be transmitted through the environment. If there is no global control effort, it is estimated that by 2050, AMR will become the most common killer in the world, with the death rate reaching 10 million people each year (O'Neill, 2016).

The contribution of the livestock sector as a source of AMR transmission is still being debated. West Nusa Tenggara Province is one of the central provinces for cattle production to supply beef needs in Indonesia, with the 1000 head of cattle program in pilot villages (Mashur et al., 2022). Smallholder farms dominate Bali cattle farming in Lombok. Still, poor antimicrobial stewardship will lead to bacterial resistance to antibiotics on farms, allowing the spread of AMR, which threatens the health of livestock, humans, and the environment around the farm.

Escherichia coli is a bacteria reportedly resistant to antibiotics in community farming on Lombok Island. *Escherichia coli* on Lombok Island has been isolated from Bali cattle on community farms (Aminuddi et al., 2020). *Escherichia coli,* as an indicator bacterium of animal origin, was found to be resistant to several antibiotics such as Penicillin, Oxytetracycline, and Cefotaxime from 4 samples of *Escherichia coli* which had been isolated (Kholik et al., 2021). *Escherichia coli* bacteria are used as indicators of animal origin because the AMR profile of *Escherichia coli* almost reflects the use

of antimicrobials in animals for food production (EFSA, 2011). Previous research in Peru reported that 31.3% of Escherichia coli isolated from water in a rural environment were resistant to tetracycline antibiotics in 266 samples collected (Hartinger et al., 2021). Its presence is used as an indicator of poor water quality and food quality (Odonkor and Ampofo, 2013). *Escherichia coli* successfully isolated from livestock and water in the livestock environment will be a source of AMR because *Escherichia coli* can produce Extended Spectrum Beta-lactamase (ESBL) and encode the ESBL gene. A survey in the Netherlands showed that pathogenic ESBL *Escherichia coli* was isolated from river water and wastewater (Franz et al., 2015).

Escherichia coli carrying the Extended Spectrum Beta-lactamase (ESBL) gene will be able to cause AMR and increase the incidence of AMR by horizontal transfer of the AMR gene to other bacteria in the environment and their colonies. Van Duin and Doi (2017) reported multiple genes contributing to ESBL and *Klebsiella pneumoniae* carbapenemase resistance, including *blaCTX-M*, *blaOXA*, *blaSHV* and *blaTEM*, *blaKPC*, *blaVIM*, *blaIMP*, and *blaNDM.* These genes can be transferred horizontally to other species on the plasmid (Peterson and Bonomo, 2005). The previous study documented that *Escherichia coli*-producing ESBL also encodes the TEM and CTX-M genes found in cow feces and the environment in Peninsular Malaysia (Kamaruzzaman et al., 2020).

The research on isolating *Escherichia coli* in water sources and waste disposal in livestock pens to determine antibiotic susceptibility can be used as initial data in studying AMR on smallholder farms with minimal biosecurity implementation on Lombok Island. Haberecht et al. (2019) stated that waterborne *Escherichia coli* are a significant reservoir of antimicrobial resistance (AMR). The existence of resistant *Escherichia coli* from water and waste sources can be used as a reference in handling AMR because water sources on livestock farms on the island of Lombok are generally close to kennels and residents' houses, so *Escherichia coli,* which is resistant to antibiotics from human and animal feces will be able to contaminate contaminated water sources used on farms.

MATERIALS AND METHODS

Study site

This research was conducted in Lando Village, Terara District, East Lombok Island Regency, Indonesia, in March 2022. A sampling of water sources and waste disposal at the Pade Angen II livestock farm, Lando Village, Terara District, East Lombok Regency. Examining Escherichia coli samples from dug wells as water sources and waste disposal is conducted at the West Nusa Tenggara Province Testing and Calibration Health Laboratory. 2008, ISO 15189; 2012 and accredited by the Health Laboratory Accreditation Commission (KALK).

Study design

This type of research is a cross-sectional survey study on the presence of *Escherichia coli* in water sources and waste disposal and their susceptibility to antibiotics. The target population for this study was all dug wells as water and waste disposal sources in the Pade Angen II livestock farm, Lando Village, Terara District, East Lombok Regency, a total of 30 cattle farms.

Sample Size and Sampling Method

The sample size was calculated based on the detection disease to estimate proportion formula set by Martin et al. (1987), with a confidence level of 95% and a minimum expected prevalence of 30%. Eight samples were obtained. Sampling was taken using the purposive sampling method, namely by selecting samples based on the criteria for the distance between the water source and the waste disposal, which is less than 10 meters. The water samples were taken from 4 dug wells and four waste disposals. Samples of water from dug well and waste disposal were taken as much as 250 ml each, which were placed into sterile tubes and taken to West Nusa Tenggara Province Health Laboratory for Testing and Calibration (BLPK) using a cool box for isolation of Escherichia coli bacteria and testing the sensitivity of several antibiotics.

Isolation and Identification of *Escherichia coli*

Water samples from dug wells and waste disposal were taken 2 ml from a sterile bottle, placed in a Triple Strength Lactose (TSL) medium, and incubated for 24 hours at 37°C in the laboratory. Then 1 ml was taken and planted in *Escherichia coli* Broth (ECB) incubated for 24 hours at 37°C. The samples were then embedded

in Eosin Methylene Blue Agar (EMBA) and incubated for 24 hours. Bacteria that grow will be stained with Gram stain characterized by biochemical tests and analyzed based on Bergey's manual of determinative bacteriology. The biochemical tests conducted in this study include catalase test, glucose, sorbitol, arabinose, lactose, sucrose, mannitol, urea, maltose, Triple Sugar Iron Agar (TSIA), Kovac reagents produced by Indole (I), and Citrate Test (C), Glucose Phosphate (GP), Alkali Phosphate (AP) (Brenner al., 2007).

Antibiotic Sensitivity Test

The sensitivity test of *Escherichia coli* isolates to antibiotics used the disk diffusion method. It was carried out by taking *Escherichia coli* colonies, then putting them into a test tube containing 0.9% NaCl and homogenizing it to reach the McFarland standard of 0.5. *Escherichia coli* suspension that has reached the Mc Farland standard of 0.5 is swabbed on MHA (Mueller Hinton Agar), and then the antibiotic discs are placed on MHA media. The antibiotics used in this study included Gentamicin 10 µg, Ciprofloxacin 10 µg, Oxytetracycline 30 µg, Penicillin G 10U, and Cefotaxime 30 µg. The zone of inhibition (clear area) describes Escherichia coli's sensitivity to antibiotics or materials expressed by the width of the diameter of the inhibition zone (Vandeppitte et al. 1991). The interpretation of the sensitivity test results refers to the Clinical and Laboratory Standards Institute (CLSI, 2015).

RESULTS AND DISCUSSION

Isolation and Identification of *Escherichia coli*

Escherichia coli bacterial culture results from 8 samples collected, namely four samples of dug wells as water sources with sample codes A1, B1, C1, and D1, and four from waste disposal with sample codes A2, B2, C2, and D2 in livestock farm, Lando Village on East Lombok. The results of *Escherichia coli* bacteria culture on EMBA are illustrated in Figure 1. Figure 1 illustrates that the *Escherichia coli* bacteria macroscopically form round colonies, the edges of the colonies are convex, the elevations are flat-sided, and the color of the colonies is metallic green.

Bacteria grown on EMBA media are then identified by carrying out gram staining to microscopically determine the morphology of the bacteria. The results of Gram staining of *Escherichia coli* isolates can be seen in Figure 2. Figure 2 is the result of observing *Escherichia coli* with gram staining using a microscope with 1000x magnification, finding that *Escherichia coli* is red, in the form of bacilli (rods), and is gram-negative. The bacteria form metallic green colonies in Figure 1 due to the reaction between the bacteria and methylene blue. *Escherichia coli* bacteria can ferment lactose quickly and produce a lot of acids to produce shiny metallic colonies and metallic green pigment deposits. *Escherichia coli* on Eosin Methylene Blue Agar had a metallic green color that sparkled like metal. Observing *Escherichia coli* with gram staining documented that *Escherichia coli* is red, in the form of bacilli in Figure 2, because the cells of *Escherichia coli* have a thinner peptidoglycan layer. *Escherichia coli,* as a Gram-negative bacteria, have cell walls with a thinner peptidoglycan layer, so they cannot retain the Crystal Violet dye during staining (Brenner et al., 2007).

Figure 1. *Escherichia coli* Colonies in Eosin Methylene Blue Agar (head arrow)

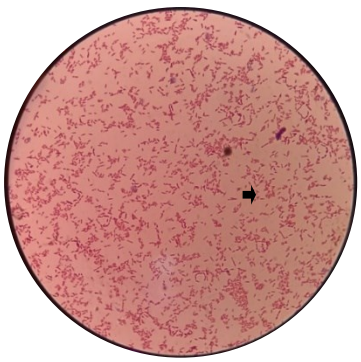

Figure 2. Morphology of *Escherichia coli* by Gram Staining (head arrow)

Escherichia coli was successfully cultured on EMBA media and gram staining. Then, biochemical tests were conducted to identify and determine the biochemical properties

of the isolated bacteria to confirm that the bacteria were *Escherichia coli*. The results of biochemical tests on *Escherichia coli* isolates, namely four isolates from water sources with sample codes (A1, B1, C1, and D1) and four isolates from waste disposal with sample codes (A2, B2, C2, and D2) can be seen in Table 1.

The results of the biochemical test in Table 1 showed that *Escherichia coli* isolated from dug wells as water sources with sample codes (A1, B1, C1, and D1) and waste disposal with sample codes (A2, B2, C2, and D2) had positive catalase test results. The *Escherichia coli* in this study fermented carbohydrates on TSIA media by changing the red colour of the media to yellow and producing gas. The *Escherichia coli* showed Sulfide Indole Motility (SIM) positive and fermenting Maltose, Glucose, Lactose, and Mannitol. In this research, *Escherichia coli* showed negative Cimon Citrate test results.

The biochemical test results of *Escherichia coli* isolates from water sources and waste disposal that fermented carbohydrates on TSIA, positive Sulfide Indole Motility (SIM), fermenting sugars, did not use citrate to produce carbon, and negative Cimon Citrate test results. The results of this study are similar to biochemical tests on *Escherichia coli* from the Bali cattle reproductive tract, which state that *Escherichia coli* in the indole test showed positive results and an adverse reaction for the citrate test (Kholik, 2022). The biochemical test result of *Escherichia coli* with positive indole and negative catalase can be used to distinguish it from other digestive tract bacteria (Cornelissen et al., 2012).

Table 1. Biochemical Test Results of Isolated *Escherichia coli*

Code of samples	Biochemical test														
	TSIA	**SIM**	**SC**	**G**	**L**	**Mn**	**Mt**	**S**	**Sr**	**A**	**GP**	**M**	**U**	**C**	**AP**
A1	A/A +gas	+	-	+gas	+	+	+	-	+	+	+	-	-	+	+
A2	A/A +gas	+	-	+gas	+	+	+	-	+	+	+	-	-	+	+
B1	A/A +gas	+	-	+gas	+	+	+	-	+	+	+	-	-	+	+
B2	A/A +gas	+	-	+gas	+	+	+	-	+	+	+	-	-	+	+
C1	A/A +gas	+	-	+gas	+	+	+	-	+	+	+	-	-	+	+
C2	A/A +gas	+	-	+gas	+	+	+	-	+	+	+	-	-	+	+
D1	A/A +gas	+	-	+gas	+	+	+	+	+	+	+	-	-	+	+
D2	A/A +gas	+	-	+gas	+	+	+	+	+	+	+	-	-	+	+
Control	A/A +gas	+	-	+gas	+	+	+	-	+	+	+	-	-	+	+

TSIA: Triple Sugar Iron Agar, SIM: Sulfur Indole Motility, SC: Simmon Citrate, G: Glucose, L: Lactose, Mn: Mannitol, Mt: Maltose, S: Sucrose, Sr: Sorbitol, A: Arabinose, M: Malonate, U: Urea, (C): Catalase, AP: Alkali phosphate, AA: Acid/Acid: Control using E. coli ATCC 29922; Code (A1, B1, C1, D1): Water source samples, Code (A2, B2, C2, D2): Waste disposal source samples.

Antibiotic Sensitivity Test

The results of the *Escherichia coli* isolates sensitivity test to the antibiotics Gentamicin 10 µg, Ciprofloxacin 10 µg, Oxytetracycline 30 µg, Penicillin G 10U, and Cefotaxime 30 µg using the Kirby Bauer disc diffusion method on MHA media (Mueller Hinton Agar) are shown by the formation of clear zones from antibiotics can be seen in Figure 3.

The susceptibility of *Escherichia coli* to antibiotics is grouped into resistant, intermediate, and susceptible to antibiotics based on the size of the inhibition zone formed. The results of the *Escherichia coli* susceptibility test for antibiotics the inhibition diameter zone formed in this study according to Clinical and Laboratory Standards Institute (CLSI, 2015) in Table 2.

Table 2 shows that 100% of *Escherichia coli* from the eight samples collected (4 samples of dug wells as water sources with sample codes (A1, B1, C1, and D1) and four from waste disposal with sample codes (A2, B2, C2, and D2) are susceptible to antibiotics Gentamicin, Ciprofloxacin, and Cefotaxime. Table 2 also shows that 100% of *Escherichia coli* is resistant to Penicillin G, and only one *Escherichia coli* (12.5%) is resistant to Oxytetracycline, namely *Escherichia coli* from samples with code C1. Samples with code C1 are samples originating from water sources.

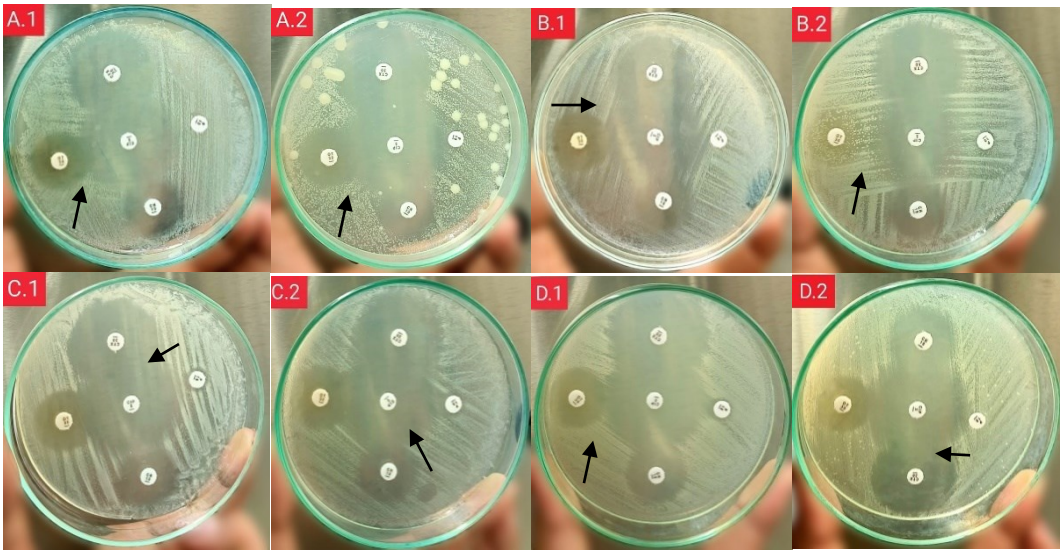

Figure 3. Antibiotic Susceptibility Test of *Escherichia coli* with Disc Diffusion Method: (A1, B1, C1, D1) = Water source samples; (A2, B2, C2, D2)= Waste disposal source samples; (arrow) = inhibition zone.

The susceptibility test result of *Escherichia coli* bacterial isolates to the antibiotics Gentamicin 10 µg, Ciprofloxacin 10 µg, Oxytetracycline 30 µg, Penicillin G 10U, and Cefotaxime 30 µg. The results of this study aligned with Kholik et al. (2021), stated that 100% of *Escherichia coli* bacteria were resistant to Penicillin G and 25% of *Escherichia coli* were resistant to Oxytetracycline from 4 samples isolated from feces of Bali cattle at Balinese cattle farms on Lombok Island. Tasyakusuma et al. (2022) also reported that 100% of *Escherichia coli* bacteria isolated from eight fluids of reproductive Bali cattle on smallholder farms in East Lombok were resistant to Penicillin G.

Table 2. The result of The Antibiotics Sensitivity Test of Isolated *Escherichia coli*

Code of samples	Inhibition Zone (mm)				
	Gentamicin	Ciprofloxacin	Oxytetracycline	Penicillin G	Cefotaxime
A1	20 (S)	40 (S)	17 (S)	0 (R)	33 (S)
A2	19 (S)	32 (S)	18 (S)	0 (R)	37 (S)
B1	22 (S)	32 (S)	22 (S)	0 (R)	31 (S)
B2	20 (S)	28 (S)	0 (R)	0 (R)	32 (S)
C1	20 (S)	30 (S)	19 (S)	8 (R)	33 (S)
C2	21 (S)	34 (S)	22 (S)	8 (R)	33 (S)
D1	21 (S)	38 (S)	21 (S)	7 (R)	36 (S)
D2	21 (S)	32 (S)	20 (S)	0 (R)	33 (S)

S= susceptible, I= Intermediate, R= Resistant, Code (A1, B1, C1, D1)= Water source samples, Code (A2, B2, C2, D2)= Waste disposal source samples

The results of the study found that *Escherichia coli* originating from water sources and sewage sources were resistant to Penicillin G, indicating that resistant *Escherichia coli* could originate from feces or animal waste from cattle pens and also from feces or secretions of humans

who had used Penicillin class antibiotics. Resistance of *Escherichia coli* to Penicillin occurs because Penicillin has often been used in the treatment of livestock on farms, so Escherichia coli has adapted and produced genes that are resistant to Penicillin. Huang et al. (2019) stated that penicillin is the antibiotic often used in animal husbandry that has generated resistant genes. Other studies also support that penicillin is often used in animal husbandry, evidenced by the discovery of Penicillin residues in beef 1.66% from 60 samples collected from 5 markets in Bali (Siswanto and Sulabda, 2018).

Resistance of *Escherichia coli* to penicillin can also be caused by frequent use in humans, so it can contaminate water sources when excreted. Penicillin antibiotics have been declared frequently used for treating urinary tract infections. Norafika et al. (2020) reported that *Escherichia coli* isolated from urinary tract infection of humans developed resistance to Cefpodoxime (47%) which is a beta-lactam antibiotic in the cephalosporin class from 61 patients at Haji Hospital, Surabaya, Indonesia. This incident proves that *Escherichia coli* can potentially increase the incidence of AMR in both animals and humans. Kholik et al. (2023) reported that *Escherichia coli,* which encodes the blaTEM gene from Bali cattle, has a genetic relationship with Escherichia coli strain U-10 from human urine and feces; this incident will lead to a broader spread of antibiotic resistance genes by giving their genetic material to other bacteria in other animals, the environment, and humans.

CONCLUSION

Based on the study results, *Escherichia coli* bacteria had been isolated from water sources and waste disposal in the livestock farm on East Lombok Island. Isolated *Escherichia coli* shows resistance to Penicillin G and Oxytetracycline. Antimicrobial stewardship in livestock farms needs to be a concern to prevent *Escherichia coli* from being resistant to spread into the water to water sources as well as waste disposal. This data can be used as initial data in studying AMR on smallholder farms on Lombok Island.

REFERENCES

Aminuddi, S.P., S.B. Alpian., P. Dita. 2020. Identification of Gram-negative Bacteria of Bali Cattle with Repeat Breeding Cases on East Lombok, West Nusa Tenggara Province. *Journal of Physics: Conference Series* 1430, 1, 012013. DOI: https://doi.org/10.1088/1742-6596/1430/1/012013.

Brenner, Don J., N,R. Krieg., and J.R. Staley. 2007. *Bergey*. Springer Science & Business Media.

Cornelissen, C.N., R.A. Harvey., and B.D. Fisher. 2012. *Microbiology*. Lippincott Williams & Wilkins.

Clinical and Laboratory Standards Institute (CLSI). 2015. M100-S24: Performance Standards for Antimicrobial Susceptibility. Retrieved October 25, 2023.

Cordero, J, C. Alonso-Calleja., C. García-Fernández, and R. Capita. 2019. Microbial Load and Antibiotic Resistance Patterns of Escherichia coli and Enterococcus faecalis Isolates from the Meat of Wild and Domestic Pigeons. *Foods* 8(11): 536. DOI: https://doi.org/10.3390/foods8110536

EFSA Panel on Biological Hazards (BIOHAZ). 2011. Scientific Opinion on the Public Health Risks of Bacterial Strains Producing Extended-Spectrum β-Lactamases and AmpC β-Lactamases in Food and Food-Producing Animals. EFSA Journal 9(8): 2322. https://doi.org/10.2903/j.efsa.2011.2322

Franz, E., C. Veenman., Angela H. A. M. Van Hoek, Ana de Roda Husman, and H. Blaak. 2015. Pathogenic Escherichia coli producing Extended-Spectrum β-Lactamases isolated from surface water and wastewater. *Scientific Reports* 5(1). DOI: https://doi.org/10.1038/srep14372

Haberecht, H.B., N.J. Nealon., J.R. Gilliland., A.V. Holder., C. Runyan., R.C. Oppel., H.M. Ibrahim., L. Mueller., F.Schrupp., S. Vilchez., L. Antony., J. Scaria., and E.P. Ryan. 2019. Antimicrobial-Resistant *Escherichia coli* from Environmental Waters in Northern Colorado. *Journal of Environmental and Public Health* 2019, 1-13. DOI: https://doi.org/10.1155/2019/3862949

Hartinger, S. M., M. L. Medina-Pizzali., G. Salmon-Mulanovich., A.J. Larson., M. Pinedo-Bardales., H. Verastegui., M. Riberos, and D. Mäusezahl. 2021. Antimicrobial Resistance in Humans, Animals, Water and Household Environs in Rural Andean Peru: Exploring Dissemination Pathways through the One Health Lens. *International Journal of Environmental Research and Public Health* 18:(9): 4604. DOI: https://doi.org/10.3390/ijerph18094604

Huang, Lu., Y. Xu., J. Xu., J. Ling., L. Zheng., X. Zhou, and G. Xie. 2019. Dissemination of

antibiotic resistance genes (ARGs) by rainfall on a cyclic economic breeding livestock farm. *International Biodeterioration & Biodegradation* 138: 114-121. DOI: https://doi.org/10.1016/j.ibiod.2019.01.009

Kamaruzzaman, E,A., S. A. Aziz., A. A. Bitrus., Z. Zakaria., and L. Hassan. 2020. Occurrence and Characteristics of Extended-Spectrum β-Lactamase-Producing Escherichia coli from Dairy Cattle, Milk, and Farm Environments in Peninsular Malaysia. *Pathogens* 9 (12):1007. DOI: https://doi.org/10.3390/pathogens9121007

Kholik, K. 2023. Detection of Antibiotic Resistant in Escherichia coli from The Reproductive Tract of Bali Cattle on Smallholder Farm. *Jurnal Biosains Pascasarjana* 24(1): 44-53. DOI: https://doi.org/10.20473/jbp.v24i1sp.2022.44-53

Kholik, K., M. Munawaroh., M. R. I. Saputra., R. Rahmawati., and P. Srianto. 2021. Antibiotic Resistance in Escherichia coli Isolated from Feces of Bali Cattle With Reproductive Disorders. *Jurnal Biodjati* 6(2): 303-311. DOI: https://doi.org/10.15575/biodjati.v6i2.13925

Kholik K., P. Srianto., A. Aulanniam., F. A. Rantam., S.P. Madyawati. 2023. Characterization and phylogenetics of beta-lactamase Temoneira gene in Escherichia coli of the Bali cattle on Lombok Island, Indonesia. Iraqi Journal of Veterinary Sciences 37(2): 487-93. DOI: https://doi.org/10.33899/ijvs.2022.135062.2441

Martin, S. W., A. H. Meek., and P. Willeberg. 1987. *Veterinary Epidemiology*. Iowa State Press.

Mashur, M., M. R. Bilad., K. Kholik., M. Munawaroh., Q.Cheok., N. Huda., and R. Kobun. 2022. The Sustainability and Development Strategy of a Cattle Feed Bank: A Case Study. *Sustainability* 14 (13): 7989. DOI: https://doi.org/10.3390/su14137989

Norafika., N. Arbianti., S. Prihatiningsih., D.W. Indriani., and D.W. Indriati. 2020. A retrospective cross-sectional study of urinary tract infections and prevalence of antibiotic-resistant pathogens in patients with diabetes mellitus from a public hospital in Surabaya, Indonesia. *Germs* 10(3):157-166. DOI: https://doi.org/10.18683/germs.2020.1201

Odonkor. S.T. and J. K. Ampofo. 2013. Escherichia coli as an indicator of bacteriological quality of water: an overview. *Microbiology Research* 4(1): 2. DOI: https://doi.org/10.4081/mr.2013.e2

O'Neill, J. 2016. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. Retrieved from https://apo.org.au/node/63983.

Paterson, D.L., R.A. Bonomo. 2005. Extended-spectrum Beta-Lactamases: A Clinical Update. *Clinical Microbiology Review*, 18(4): 657-686. https://doi.org/10.1128/cmr.18.4.657-686.2005

Siswanto, S., I.N. Sulabda. (2018). Tetracycline and Penicillin Antibiotic Residues in Bali Beef Which Are Traded in Several Markets in Bali. Jurnal Veteriner, 19(4) 497-501. https://ojs.unud.ac.id/index.php/jvet/article/view/29616.

Tasyakusuma, L.P., K. Kholik., M. Janah., A.L.D. Agustin, and S.E. Rahmawati. 2023. Phenotypic Detection of Escherichia coli Producing Extended Spectrum Beta Lactamases (ESBLs) In The Reproductive Tract Bali Cow. *Jurnal Biosains Pascasarjana* 24(1):64-73. DOI: https://doi.org/10.20473/jbp.v24i1sp.2022.64-73

Van Duin, D., Y, Doi. 2017. The Global Epidemiology of Carbapenemase-Producing Enterobacteriaceae. *Virulence*, 8(4): 460-469. https://doi.org/10.1080/21505594.2016.1222343

Vandeppitte, J., J. Verhaegen., K. Engbaek., P. Rohner., P. Piot., C. Heuck. 1991. Basic Laboratory Procedures in Clinical Bacteriology. WHO. Geneva, Switzerland.

World Health Organization (WHO). 2021. Antimicrobial Resistance. Retrieved from https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance.

Woolhouse, M., M. Ward., B. van Bunnik., J. Farrar. 2015. Antimicrobial Resistance in Humans, Livestock and the Wider Environment. *Philos Trans R Soc Lond B Biol Sci*, 370(1670): 20140083. https://doi.org/10.1098/rstb.2014.0083