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PHENOTYPIC DETECTION OF Escherichia coli PRODUCING Extended Spectrum Beta Lactamases (ESBLs) IN THE REPRODUCTIVE TRACT BALI COW

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Abstrak

Bakteri Escherichia coli yang resisten terhadap antibiotik golongan β-lactam dan dimungkin dapat memproduksi Extended-spectrum β-lactamase (ESBLs) telah ditemukan dari saluran reproduksi sapi Bali. Escherichia coli yang memproduksi ESBL akan menyebabkan timbulnya antimicrobial resistance (AMR) yang telah menjadi masalah kesehatan global. Tujuan penilitian ini untuk mendeteksi secara fenotip adanya Escherichia coli yang memproduksi ESBL dari saluran reproduksi Sapi Bali. Penelitian ini dilakukan pada Bulan Februari 2022 di desa Lando Kecamatan Terara, Kabupaten Lombok Timur dengan menggunakan 8 ekor sapi Bali betina pada peternakan rakyat. Sampel cairan reproduksi dikoleksi menggunakan plastic sheet gun yang dimasukkan pada media Brain Infusion Heart (BHI) dan diinkubasi di Balai Laboratorium Kesehatan Masyarakat dan Kalibrasi Provinsi Nusa Tenggara Barat. Sampel kemudian dikultur di media EMBA untuk isolasi Escherichia coli. Escherichia coli yang diisolasi kemudian diidentifikasi dengan pewarnaan gram dan uji biokimia berdasarkan Bergey's manual of determinative bacteriology. Escherichia coli yang teridentifikasi diuji skrining dengan metode difusi cakram menggunakan antibiotik Penisilin G 10U, Ceftazidime 30 µg, dan Cefotaxime 30 µg yang dilanjutkan dengan uji konfirmasi dengan metode double disk synergy test (DDST). Hasil uji skrining menunjukkan hanya 12,5% Escherichia coli (1 dari 8 sampel) yang resisten terhadap Penisilin G, namun belum terdeteksi memproduksi ESBL secara fenotipik.

***Kata Kunci**: Antibiotik, ESBLs, Escherichia coli, Fenotipik, Resistensi*

Abstract

Escherichia coli bacteria that are resistant to *β*-lactam antibiotics and may be able to produce Extended-spectrum *β*-lactamase (ESBLs) have been found in the reproductive tract of Bali cattle. *Escherichia coli* that produce ESBL will cause antimicrobial resistance (AMR) which has become a global health problem. The purpose of this study was to detect phenotypically the presence of *Escherichia coli* that produces ESBL from the reproductive tract of Bali cattle. This research was conducted in February 2022 in Lando Village, Terara District, East Lombok Regency using 8 female Bali cattle on smallholder farms. Reproductive fluid samples were collected using a plastic sheet gun which was inserted into the Brain Infusion Heart (BHI) media and incubated at the Public Health and Calibration Laboratory of West Nusa Tenggara Province. The samples were then cultured in EMBA media for the isolation of *Escherichia coli*. The isolated *Escherichia coli* were then identified by gram staining and biochemical tests based on Bergey's manual of determinative bacteriology. The identified *Escherichia coli* were screened using the disc diffusion method using the antibiotics Penicillin G10U, Ceftazidime 30 µg, and Cefotaxime 30 µg followed by a confirmation test using the double disk synergy test (DDST) method. The results of the screening test showed that only 12.5% of *Escherichia coli* (1 out of 8 samples) were resistant to Penicillin G, but they had not been detected to produce ESBL

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phenotypically.

***Keywords**: Antibiotic, ESBLs, Escherichia coli, Phenotypic, Resistance*

1. INTRODUCTION

resistance(AMR) which is a global health problem has been found in both animals and humans. O'Neill (2016) states that AMR has caused the death of around 700,000 people, and by 2050 this number is expected to increase to around 10 million deaths each year. The spread of AMR can be caused by bacteria encoding antibiotic-resistant genes that infect animals and humans or in the environment.

Escherichia coli (E. coli) bacteria are bacteria that can encode Extended-spectrum β-lactamase (ESBLs) genes. Extended-spectrum beta-lactamase is an enzyme produced by gram-negative bacteria including Escherichia coli which can hydrolyze β-lactam class antibiotics, especially in oxyimino-cephalosporins (Bradford, 2001).*E. Coli which* are resistant to antibiotics*β-lactam*likePenicillin and Cefotaxime have been documented in Bali cattle in several studies. E. coli isolated from Bali cattle faeces was also found to be resistant to Penicillin and Cefotaxime (Kholik et al. 2021). Aminuddi et al. (2020) found that Escherichia coli was resistant to Penicillin in Bali cattle which experienced reproductive disorders.

*Escherichia coli*Those who are resistant to β-lactam class antibiotics in Bali cattle, especially in the reproductive tract, are likely to produce Extended-spectrum β-lactamases (ESBLs) because the treatment of reproductive disorders cannot be separated from the use of intra-uterine antibiotics. Escherichia coli which is resistant to antibiotics will be able to spread in the human food chain either directly or indirectly. EFSA (2011) states that the AMR profile of Escherichia coli almost reflects the use of antimicrobials in animals for food production (EFSA, 2011). Escherichia coli that produce ESBL in the reproductive tract of cattle is very likely to be excreted from the reproductive tract fluid which can be disseminated into the environment and horizontally transfer resistance genes to other bacteria.

This research aims toto detect*Escherichia coil which* produces ESBL from the reproductive tract of female Bali cattle phenotypically carried out in cattle herds in West Nusa Tenggara Province which is the national cattle barn where most of them are Balinese cattle. Data from the NTB Central Statistics Agency (BPS) until 2019 states that the total cattle population in NTB is 1,234,357 heads (BPS, 2020). This research will be the initial data in the early anticipation of the spread of bacteria escherichia *coli*who manufactures extended *spectrum beta-lactamase*(ESBLs)in animals, humans, and the environment that can trigger AMR.

2. METHODS

The research method was a cross-sectional study conducted in February 2022. The target population for this study was Bali cattle in the stables of the Pade Angen II Livestock Farmers Group, Lando Village, Terara District, East Lombok Regency, West Nusa Tenggara Province (NTB), totaling 30 heads.

The sample size used in this study was 8 (eight) Bali cattle for their reproductive fluids to be taken. The sample size was determined based on the detect disease to estimate proportion formula set by Thrusfield (2005), with a population of 30 individuals, a 95% confidence level, and a minimum prevalence expectancy of 30%, so a minimum sample taken is 8 (eight).

A sampling of the reproductive fluids of Bali cattle used the purposive sampling method with criteria including female Bali cattle having normal estrus cycles without

interruption and having given birth at least once. Samples of reproductive fluid for Bali cattle were taken using an artificial insemination plastic sheet which was taken and cut approximately 2 cm and placed into the media brain *Heart Infusion*(BHI).

2.1 Culture of Escherichia coli

Reproductive fluid samples in media brain *Heart Infusion*(BHI) were cultured in Eosin Methylene Blue Agar (EMBA) and incubated for 24 hours. The metallic green colonies that form are an indication of the presence of Escherichia coli in the sample. Identification of Escherichia coli was carried out through Gram staining and biochemical tests referring to the Bergeys Manual of Determinative Bacteriology. (Holt et al., 1994).

2.2 Identification of Escherichia coli

Gram staining for the identification of Escherichia coli was carried out by taking one ose of bacterial isolates and placing them on an object glass measuring ± 1 cm2 and fixing them. The fixed bacterial isolate was dripped with 2 drops of crystal violet dye and allowed to stand for 1 minute, then washed with water and dried. The preparation was then dripped with Lugol's iodine solution and left for 1 minute and dried, then washed with a bleach solution (96% alcohol) for ± 30 seconds, after which it was washed with running water and then dried.

After drying, the preparations were given a fuchsin dye solution for 1 minute and washed with water and dried and observed under a microscope, Gram-positive bacteria appeared purplish blue while Gram-negative ones were red (Holt et al., 1994).

Biochemical tests were carried out to identify E. coli bacteria according to Bergey's manual of determinative bacteriology (Holt et al., 1994), including catalase test, glucose, sorbitol, arabinose, lactose, sucrose, mannitol, urea, maltose, Triple Sugar Iron Agar (TSIA), Kovac's reagent produced by Indole (I), and Test Citrate (C), Glucose Phosphate (GP), Alkali Phosphate (AP).

2.3 Screening Test for Extended Spectrum Beta Lactamases (ESBLs)

Escherichia cold those identified were then screened using the disk diffusion method according to the criteria of the National Committee for Clinical Laboratory Standards (NCCLS) and also referred to manual on Antimicrobial Susceptibility Testing. Department of Microbiology Christian Medical College Vellore (Lalita, 2004).

E. coli grown using the swab technique on Mueller Hinton Agar (MHA) medium and then treated with antibiotic paper discs containing antibiotics. The 3 (three) antibiotics used included: Penicillin G10U, Ceftazidime (CAZ) 30 µg, and Cefotaxime (CTX) 30 µg.

The zone of inhibition produced by antibiotics is said to be positive extended-*spectrum β-lactamase*(ESBLs) on what screening test if the inhibition zone produced by Penicillin G ≤ 17 mm, CAZ ≤ 22 mm, and CTX ≤ 21 mm. E. coli that is positive in the screening test is followed by a confirmation test (Hemeg, 2010).

2.4 Confirmation Test of Extended Spectrum Beta Lactamases (ESBLs)

Confirmation of Escherichia coli producing Extended Spectrum Beta lactamases (ESBLs)performed using the double disk synergy test (DDST) method. The double disk synergy test was carried out on Escherichia coli which was positive for the screening test which was grown on MHA media with the swab technique by placing three paper discs of antibiotics with a distance of 20 mm in parallel, where the middle disc was given the antibiotic amoxicillin and clavulanic acid (20 µg: 10 µg) while on the side placed cefotaxime and ceftazidime 30 µg each. The inhibition zones that were connected around the antibiotic discs to each other showed positive

Escherichia coli producing Extended Spectrum Beta Lactamases (ESBLs) (Hemeg, 2010).

The results of the study also stated that the results of testing with the disk diffusion method increased by 5 mm in the diameter of the zone for the antimicrobial agent tested with clavulanic acid versus the zone when tested alone confirmed that the microorganism is a producer of Extended-spectrum β-lactamases (ESBLs). (Igwe et al. 2014).

Other literature states that positive bacteria produce extended-*spectrum β-lactamase*(ESBLs) if there is an increase in the inhibition zone and the antibiotic disks of cefotaxime 30 µg and ceftazidime 30 µg towards the disc containing amoxicillin clavulanate (Dwina et al., 2016).

3. RESULTS

The results of isolation of Escherichia coli from 8 samples of reproductive fluids for Bali cattle from the cut end of a plastic sheet gun for artificial insemination in Lando Village, Terara District, East Lombok Regency, West Nusa Tenggara Province (NTB) found 1 positive sample with an indication of Escherichia coli bacteria based on identification results by Gram staining and biochemical test.

The results of Gram staining on Escherichia coli which were successfully isolated by macroscopic morphological observations showed spherical colony shapes, metallic green colony color, and shiny surfaces, with convex colonial surfaces. The results of these observations indicated that there was an indication of the presence of Escherichia coli which was then followed by biochemical tests to identify Escherichia coli isolates from the reproductive fluids of the Bali cattle. Culture results for Escherichia coli isolation on EMBA media can be seen in Figure 1.

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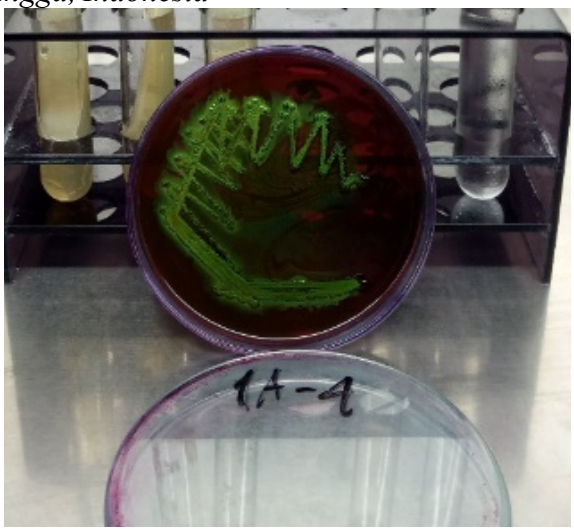


Figure 1. Isolate*Escherichia coliform* fluids originating from the reproductive tract of Bali cattle on EMBA Media

The results of the research on identifying the morphology of Escherichia coli microscopically using Gram staining showed that the morphology of Escherichia coli was faded red, in the form of cells in the form of red bacilli or red rods. This Gram stain will differentiate between Gram-positive and negative bacteria. Bacteria belonging to the Gram-positive appear purplish-blue while bacteria belonging to the Gram-negative will appear red.

The results of Gram staining of Escherichia coli isolates from the reproductive fluids of Bali cattle taken using a plastic sheet gun for artificial insemination can be seen in Figure 2.

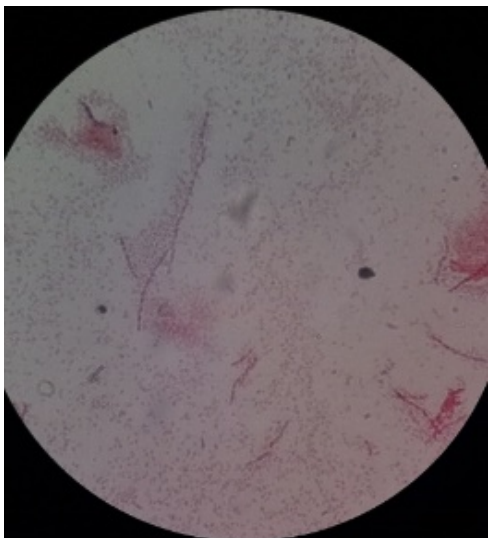


Figure 2. Morphology of Escherichia coli with Gram stain

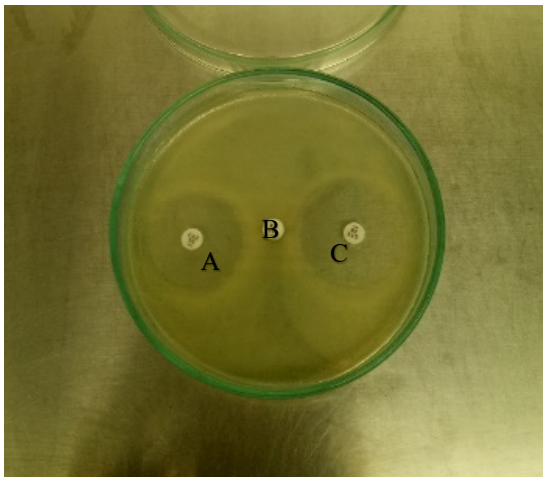
Biochemical test results of Escherichia coli isolates from the reproductive fluids of Bali cattle include tests for catalase, glucose, sorbitol, arabinose, lactose, sucrose, mannitol, urea, maltose, Tripel Sugar Iron Agar (TSIA), Kovac's reagent produced by Indole (I), and Citrate Test (C), Glucose Phosphate (GP), Alkaline Phosphate (AP) can be seen in Table 1.

Table 1. Isolate Biochemical Test Results*Escherichia coli*

Biochemical Test	Interpretation
Catalase	+
Oxidase	-
Glucose	+ (+) Gases
Lactose	+
arabinose	+
Sucrose	+
Alkaline Phosphatase	+
Maltose	+
mannitol	+
Indole	+
Sorbitol	+
Glucose Phosphate	+
Malonate	-
Motility	+
Urea	-
Cimon Citrate	-
Triple Sugar Iron Agar (TSIA)	acid/acid (+) gas

The biochemical test results in Table 1 show that isolated Escherichia coli had a positive catalase test, positive oxidase, fermented glucose, lactose, sucrose, and mannitol and did not ferment dulcitol. Escherichia coli isolates also produced positive indole, and positive motility, and did not produce urea and in the TSIA test, a yellow slope and a yellow agar base showed acid and did not produce sulfur.

The results of the Extended Spectrum Beta Lactamases (ESBLs) screening test on Escherichia coli isolates from the reproductive fluids of Bali cattle against the antibiotics Penicillin G µg, Ceftazidime (CAZ) 30 µg, and Cefotaxime (CTX) 30 µg using the antibiotic disc diffusion method on Mueller Hinton Agar can be seen in Figure 3.



Description: A: Ceftazidime, B: Penicillin G, C: Cefotaxime

Figure 3. Screening Test Observation ResultsBacteria*Escherichia coli* on Mueller Hinton Agar Media

Figure 3. Screening test on Mueller Hinton Agar (MHA) media shows only*Escherichia coli* which was successfully isolated from the reproductive fluids of Bali cattle were only resistant to penicillin G without the formation of an inhibition zone around the Penicillin G disc, whereas in the Ceftazidime and Cefotaxime discs an inhibition zone was formed. The results of the measurement of the inhibition zone on the growth of isolates Escherichia *coli* from the reproductive fluids of Bali cattle on MHA media produced by each antibiotic can be seen in Table 2.

Table 2. Isolate Screening Test Results*Escherichia coli*

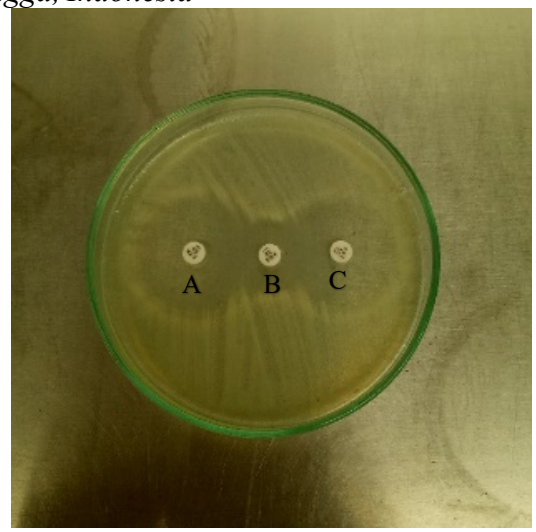
Types of Antibiotics	Obstacles zone	Information
Ceftazidime	29mm	Bacteria sensitive toCeftazidie
Penicillin G	0 mm	Bacteria resistant to penicillin G

Cefotaxime	34mm	Bacteria sensitive to Cefotaxime

Table 2 states that the inhibition zone of the Escherichia coli isolates screening test results of the reproductive fluids of Bali cattle against antibiotics Penicillin G µg, Ceftazidime (CAZ) 30 µg, and Cefotaxime (CTX) 30 µg using the disc diffusion method were 29 mm, 0 mm, and 34 mm, respectively.

The test results showed that the screening of Escherichia coli isolatesBali cattle reproductive fluids of the 8 samples tested, only Penicillin had no inhibition zone diameter, while the 3rd generation cephalosporin class antibiotics (Ceftazidime and Cefotaxime) had a high inhibition zone exceeding the requirements for a positive ESBL screening test, but to ensure the presence of Escherichia coil which produces Extended Spectrum Beta Lactamases(ESBLs) will continue the confirmation test

Laboratory test results Confirmation of Extended Spectrum Beta Lactamases (ESBLs) on Escherichia coli isolatesBali cattle reproductive fluids to the antibiotic ceftazidime30 µg, Amoxicillin Clavulanic Acid (20 µg:10 µg), and Cefotaxime 30 µg using the double disk synergy test (DDST) method on Mueller Hinton Agar which is 20 mm apart for each antibiotic can be seen in Figure 4.



Description: A: Ceftazidime; B: Amoxicillin-Clavulanic Acid; C: Cefotaxime

Figure 4. Observation Results of Escherichia coli Bacterial Confirmation Test on Mueller Hinton Agar Media

Figure 4 states that there is no synergy formed in the Confirmation Test of Extended Spectrum Beta Lactamases (ESBLs) on Escherichia coli bacteria.

4. DISCUSSION

The results of the study which succeeded in isolating 1 (one) Escherichia coli from 8 samples of the reproductive fluids of Bali cattle indicated that contamination occurred due to escherichia *coli* are normal bacteria in the environment. Molina and Lucy (2018) stated that Escherichia coli is a non-specific bacteria that normally exist in nature. E. coli can enter the reproductive tract of the cow, and this bacteria will become a pathogen (Molina and Lucy, 2018). Escherichia coli contamination can also be the result of an unfavorable environment, an especially postpartum. environment that is less well postpartum will facilitate the entry of microbes into the lumen uterus, contaminate the uterine lumen environment, interfere with the life of a viable embryo cause early embryonic death (Arthur et al., 2001).

Research results that get that Escherichia coli with shiny surface metallic green color, with a convex colony surface on Methylene Blue Agar (EMBA). These results are the same as the results of the studyCornelissen et al. (2013) who stated that Escherichia coli on EMBA medium had a shiny metallic green color like metal. The green color that appears shows what happened here is a reaction between bacteria and Methylene blue.

The results of identification of the morphology of Escherichia coli with gram staining showed the morphology of Escherichia coli in the form of red bacilli or red stems. These results thus is because Escherichia coli is a gram-negative bacterium that has a thinner wall. Gram-negative bacteria are marked with a pink color which indicates that these bacteria are unable to bind crystal violet color and are only stained by safranin (Hadioetomo, 1993).

The results of the biochemical test and gram staining were rod-shaped cells and were gram-negative, with negative oxidase, positive catalase, positive lactose, negative Simon citrate, and positive mannitol. These results were almost the same as a study conducted by Gunawan et al. (2018) that*Escherichia Coli isolated* from cow faeces ferment Glucose, sucrose, and indole positive and do not produce urea. These results indicate that there is a high probability of contamination in the reproductive tract of female Bali cattle. Based on the results of the biochemical test of Escherichia coli bacteria, it is positive on the catalase test and negative on the oxidase test, indicating that the cell cannot produce oxidase enzymes. These results are consistent with the results of the documentation by Cornelissen et al., (2013) which stated that Escherichia coli in the indole test showed positive results and negative reactions for the citrate test.

Results of Extended Beta Lactamases Screening on the antibiotics Ceftazidime, Penicillin G, and Cefotaxime against escherichia *coli* using the disc diffusion method respectively 29 mm, 0 mm, and 34 mm, while according to the CDC (2010), Ceftazidime is said to be sensitive ≥ 22 mm and resistant if ≤ 17 mm, Penicillin G is said to be sensitive ≥17 mm and resistant if ≤ 13 mm, Cefotaxime is said to be sensitive ≥26 mm and resistant if ≤ 22 mm. Test results screening by the disc diffusion method stated that one sample was obtained*Escherichia coli*(12.5%) resistant to Penicillin G from 8 samples tested. These results were almost the same as Aminuddi et al, (2019) who obtained 100% Escherichia coli which were found to be resistant to Penicillin G. This might have happened because Penicillin was often used. Penicillin, a β-lactam class of antibiotics, is one of the most frequently used antibiotics in animal husbandry and has caused resistance genes (Huang, L. et al., 2019; Qian, X. et al., 2019).

This fact indicates that Escherichia coli resistance to Penicillin G has occurred which can be caused by the use of Penicillin G that is less controlled.FAO (2016) states that the most significant factor in the emergence of AMR is Antimicrobial use (AMU) in humans and also in animals for the treatment and prevention of disease.

Poorly controlled administration of antibiotics will cause Escherichia coli bacteria to adapt and mutate, including the Extended-Spectrum β-Lactamases (ESBL) encoding genes found in chromosomes and plasmids. These results are in line with the question about the AMR profile of Escherichia coli almost reflecting the use of antimicrobials in animals for production (EFSA, 2011).

The results of this study state that the existence*Escherichia coil resistance* to Beta-Lactam antibiotics was quite low in the reproductive tract of female Bali cattle in Lando Village, East Lombok District. These results are in line with Putra et al.'s research. (2019) managed to find Escherichia coli as an ESBL producer in 3 of 65 samples

(4.61%) samples taken in the Surabaya livestock area. This fact indicates that the number of samples is crucial to the number of isolated ESBL-producing Escherichia coli.

Confirmation test results extended *Spectrum Beta Lactamases*(ESBLs) in Escherichia coli against the antibiotics Ceftazidime, Amoxicillin Clavulanic Acid, and Cefotaxime using the double disk synergy test (DDST) method in this study found no synergy formed on discs of Ceftazidime, Amoxicillin Clavulanic Acid, and Cefotaxime. Igwe et al. (2014)the results of the test using the disk diffusion method are said to be positive if there is a 5 mm increase in the diameter of the zone for the antimicrobial agent tested with clavulanic acid versus the zone when tested alone, then it can be believed that the samples isolated from the reproductive tract of female Bali cattle in Lando Village, District Terara, East Lombok Regency has not found Escherichia coli as a producer of Extended Spectrum Beta Lactamases (ESBLs).

As confirmation of a positive sample*Escherichia coli*There is no expansion of the inhibitory zone leading to beta-lactam inhibitors. It can be hypothesized that no ESBL-producing Escherichia coli was found because this study only detected ESBL using a phenotypic method that did not test genotypically isolated Escherichia coli. Kazemian et al. (2019) found positive genes related to ESBL did not show phenotypic resistance. Chatrou et al. (2012) stated that the level of accuracy of a gene is far better than the best morphology. The differences observed in the detection of ESBL-positive isolates by the two different methods can be justified by the lower sensitivity of the phenotypic method and the influence of environmental factors on the occurrence of resistance. (Yazdi et al. 2012)

5. CONCLUSIONS

The research results show that Escherichia coli bacteria can be isolated from the reproductive tract of female Bali cattle without reproductive disorders in Lando Village, Terara District, East Lombok Regency andonly 12.5% of Escherichia coli are resistant to Penicillin G, but have not been detected producing it*Extended Spectrum Beta Lactamases*(ESBLs) phenotypically.

Genotypic detection of the gene encoding Extended Spectrum Beta Lactamases (ESBLs)in Escherichia coli with a larger number of samples needs to be done to determine the ESBLs gene because different ESBLs genes also show different resistance to antibiotics,

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REFERENCES

Aminuddi S P, Alpian S B, Dita P and Kholik. 2019. Identification of gram-negative bacteria of Bali cattle with repeat breeding cases on East Lombok, West Nusa Tenggara Province J. Phys.: Conf. Ser. 1430 012013. https://iopscience.iop.org/article/10.1088/1742-6596/1430/1/012013

Azawi, O.I., Omran, S.N., Hadad, J.J., 2008. A study of endometritis causing repeat breeding of cycling Iraqi Buffalo cow. Reprod. Dom. Anim. 43(6): 735-743. CDC (2010) Healthcare Associated Infection: Laboratory Detection of Extended-Spectrum βLactamases (ESBLs). Centers For Disease Control

and Prevention, Atlanta. https://doi.org/10.1111/j.1439-0531.2007.00981.x

Arthur, G.H., Noakes, R.J., Pierson, H.W., 2001. Veterinary Reproduction and Obstetrics. W.B. Saunders Co., Philadelphia.

Badan Pusat Statisika (BPS). 2020. Kabupaten Propinsi NTB Dalam Anagka. BPS Propinsi NTB.

Chatrou, L. W., Pirie, M. D., Erkens, R. H., Couvreur, T. L., Neubig, K. M., Abbott, J. R., ... & Chase, M. W. 2012. A new subfamilial and tribal classification of the pantropical flowering plant family Annonaceae informed by molecular phylogenetics. *Botanical Journal of the Linnean Society*, *169*(1), 5-40. https://doi.org/10.1111/j.1095-8339.2012.01235.x

Cheaito K. and G M Matar . 2014, The Mediterranean region: a reservoir for CTX-M-ESBL-producing Enterobacteriaceae. Jordon J. Biologic. Sci. 7(1), 1-6. https://platform.almanhal.com/Files/Articles/56252

Cornelissen, C. N., Fisher, B. D. & Harvey R.A. 2013. Lippincott's Illustrated Reviews: Microbiology.3th. Philadelphia : *Lippincott Williams & Wilkins, a Wolters Kluwer business*.p. 111-5.

Dwina, Rika, Siska, M. 2016. Identifikasi Bakteri Aeromonas Hydrophila Dengan Uji Mikrobiologi Pada Ikan Lele Dumbo (Clarias Gariepinus) Yang Dibudidayakan Di Kecamatan Baitussalam Kabupaten Aceh. Jurnal Ilmiah Universitas syiah Kuala . https://www.neliti.com/publications/187086/identifikasi-bakteri-aeromonas-hydrophila-dengan-uji-mikrobiologi-pada-ikan-lele

European Food Safety Authority (EFSA). Scientific opinion on the public health risks of bacterial strains producing extended-spectrum-lactamases and/or AmpC-lactamases in food and food-producing animals. EFSA Journal 2011;9(8):2322.DOI: 10.2903/j.efsa.2011.2322

Food and Agriculture Organization of the United Nations (FAO). 2016. Drivers, dynamics, and epidemiology of antimicrobial resistance in animal production. *FAO*. https://agris.fao.org/agris-search/search.do?recordID=XF2017002096

European Food Safety Authority (EFSA). 2011. Scientific opinion on the public health risks of bacterial strains producing extended-spectrum-lactamases and/or AmpClactamases in food and food-producing animals. EFSA J. 9:1–95. https://doi.org/10.2903/j.efsa.2011.232

Gunawan, Nurmayani S, Mandrasari J, Tirtasari K, Kholik. Antimicrobial resistance of Escherichia coli that Isolated from Fecal samples of Bali Cattle at Local Farm on Lombok Island. Jurnal Sangkareang. 2018; 4(3): 10-13. https://sangkareang.org/index.php/SANGKAREANG/%20article/view/121

Hadioetomo, R.S.1993. 'Mikrobiologi Dasar Dalam Praktek Teknik dan Prosedur Dasar Laboratorium', Gramedia, Jakarta.

Hemeg, H.A. 2018. Molecular characterization of antibiotic resistant Escherichia coli isolates recovered from food samples and outpatient Clinics, KSA. Saudi Journal of Biological Science, 25(1), 928-931. https://doi.org/10.1016/j.sjbs.2018.01.016

Huang, L., Xu, Y., Xu, J., Ling, J., Zheng. L., Zhou, X, Xie, G. 2019. Dissemination of antibiotic resistance genes (ARGs) by rainfall on a cyclic economic breeding livestock farm. Int. Biodeter. Biodeger. 138, 114–121.

Holt, J.G., Krieg, N.R., Sneath, P., Staley, J.T. and Williams, S.T. 1998. Family Enterobacteriaceae; in Bergey's manual of determinative bacteriology (9th edition.), edited by W.R. Hensyl. Lippincott Williams & Wilkins, Baltimore, Maryland.

Igwe, J., Onaolapo, J., Kachallah, M., Nworie, A., Oladipo, H., Ojiego, B., Enose, O., Adeboye, S., Durowaiye, M., Akpa, A. and Ibanga, I. 2014. Molecular Characterization of Extended Spectrum *β*-Lactamase Genes in Clinical *E. coli* Isolates. *Journal of Biomedical Science and Engineering*, **7**, 276-285. http://dx.doi.org/10.4236/jbise.2014.75030

Kazemian, H., Heidari, H. 2019. Phenotypic and Genotypic Characterization of ESBL-, AmpC-, and Carbapenemase - Producing Klebsiella pneumoniae and Escherichia coli Isolates. https://doi.org/10.1159/000500311

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

Le Roux, F., & Blokesch, M. 2018. Eco-evolutionary dynamics linked to horizontal gene transfer in Vibrios. *Annual review of microbiology*, *72*, 89-110. https://doi.org/10.1146/annurev-micro-090817-062148

Lalitha, M. 2004. Manual on antimicrobial susceptibility testing. *Performance standards for antimicrobial testing: Twelfth Informational Supplement*, *56238*, 454-456.

Molina-Coto, R., & Lucy, M. C. 2018. Uterine inflammation affects the reproductive performance of dairy cows: A review. *Agronomía Mesoamericana*, *29*(2), 449-468. https://doi.org/10.15517/ma.v29i2.29852

O'Neill, J. 2016. Tackling Drug-resistant Infections Globally: Final Report and Recommendations. London: HM Government and Welcome Trust. https://apo.org.au/node/63983

Putra A. R. S . 2020. Investigation of Extended Spectrum Beta-Lactamase (ESBL) Producing Escherichia coli by Vitek-2 On Dairy Cows in Surabaya, Indonesia. https://connectjournals.com/03896.2020.20.6773

Qian X, Gu J, Sun W, Wang X, Su J, Stedfeld R. 2018. Diversity, abundance, and persistence of antibiotic resistance genes in various types of animal manure following industrial composting. J.Hazard. Mater. 344, 716–722. https://doi.org/10.1016/j.jhazmat.2017.11.020

Thrusfield M. 2005. Veterinary Epidemiology. 3rd ed. Blackwell Science Ltd. Oxford, UK.

Vandepitte, J., Verhaegen, J., Engbaek, K., Rohner, P., Piot, P., Heuck, C.C. 2003. Basic laboratory procedures in clinical bacteriology. 2nd ed. Geneva: World Health Organization.

Yazdi M, Nazemi A, Mirinargasi M, Jafarpour M, Sharifi SH. 2012. Genotypic versus Phenotypic methods to detect Extended-Spectrum Beta-Lactamases (ESBLs) in Uropathogenic Escherichia coli. Annals of Biological Research, 3 (5):24542458, http://scholarsresearchlibrary.com/archive.html