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Resistance of *Escherichia coli* Isolated from Water Sources on West Lombok Farms to Antibiotics

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ABSTRACT

Antibiotics are frequently used arbitrarily in livestock industry without regards for dosage or norms use. The administration of antibiotics to livestock can lead to a decline in their efficacy and contribute to the emergence of antibiotic resistance, hence posing challenges to the quality and safety of livestock products. *Escherichia coli* (*E.coli*) is recognized as a bacterial indicator pollution. This study aims to determine the resistance of *E. coli* to the antibiotic's penicillin G and tetracycline. The study used a survey method with cross-sectional study design in July, 2023 on farms located in West Lombok. Testing was carried out on 10 water samples taken from water sources on farms that showed 2 negative samples and 8 positive samples of *E. coli* on EMBA media, then continued with resistance tests using Penicillin G and Tetracycline antibiotics by measuring the inhibitory zone that formed. The results of this study showed that *E.coli* samples experienced resistance to Penicillin G as many as 8 samples and tetracycline as many as 6 samples. E.coli bacteria isolated from drinking water have experienced resistance to antibiotics Penicillin G by 100% and Tetracycline 75%.
Keyword: *Escherichia coli,* Penicillin G, Resistance, Tetracycline

INTRODUCTION

The biological quality of water criteria requires that it be free from pathogenic microorganisms such as coliform bacteria, one of which is *Escherichia coli* (*E. coli*). The presence of these bacteria indicates pollution of water sources (Kadek and Konsukartha, 2007). Groundwater pollution factors can be caused by the construction and disposal of industrial waste, be it from the livestock industry or the household industry (Mahyudin *et al*., 2015). Livestock farms in the West Nusa Tenggara region are mostly traditional community farms. The location of the cage adjacent to the settlement, the traditional treatment of livestock fecal waste, and the distance between the

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cage and the water source that is too close have the potential to cause bacteria in the cage to pollute the water source. According to Yustiani *et al*. (2017), the distance of the well < 15 meters from the cage can cause well water to be polluted by various microorganisms in feces. Because groundwater runoff eventually enters the well, bacteria can also be introduced into the drinking water supply when it rains. Bacteria that enter this water can cause disease. Water contaminated by pathogenic bacteria can cause diseases including diarrhea.

Based on the survey results, water sources on farms are used by farmers as drinking water and for daily life. The water on this farm comes from dug wells, which means utilizing groundwater. The distance between dug wells and farms is <10 km. The close distance between the chicken coop and the water source has made the bacteria in the cage pollute the water source. Gunawan (2022) determined that the drinking water source had been contaminated by *E. coli* bacteria on a cattle farm in Central Lombok with a distance of 10 meters between the pen and the drinking water source. Four *E. coli* positive well water samples were isolated during research on the isolation of *E. coli* bacteria in well water at Narmada poultry farms (Ningtyas *et al*., 2022). Although *E. coli* bacteria are part of the normal flora in the digestive tract, too much of it can cause problems such as diarrhea (Lopez-Lozaro, 2000).

Many resistant *E. coli* bacteria isolated from livestock have been isolated in several areas of West Nusa Tenggara. Research conducted by Agustin *et al*. (2022) in West Lombok showed a resistance level of 100% in penicillin G antibiotics, 37.5% in tetracycline antibiotics, and 75% in oxytetracycline antibiotics. Agustin and Kholik (2018) research conducted on farms in North Lombok showed that 89% of *E. coli* bacteria were resistant to the antibiotic penicillin, 85% resistant to ciprofloxacin, 63% resistant to erythromycin and trimethoprim, and 59% resistant to ampicillin antibiotics. The results of the resistance data were obtained from 27 *E. coli* samples.

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only for other animals in the cage but can also infect humans.

Based on the research above, it can be found that antibiotic resistance, especially penicillin and tetracycline groups, has occurred in other regions in Lombok. The level of knowledge of farmers is still minimal, and patterns of maintenance and improper use of antibiotics in animals can be factors supporting high levels of resistance. It is important to prevent the spread of bacteria that have been resistant to antibiotics by being aware of the increase in antibiotic resistance (Wibisono *et al*., 2020). This study aimed to detect the resistance of *E. coli* bacteria isolated in drinking water sources in West Lombok farms that have a cage distance of less than 10 meters.

MATERIALS AND METHODS

The study used a survey method with cross sectional study design in July, 2023 on farms located in West Lombok. There were 10 samples from water sources used for livestock needs, drinking water for residents, and water sources within <10 meters from the farm site. Isolation and resistance tests were carried out at the Center for Health Testing and Calibration Laboratory (BLKPK) West Nusa Tenggara Province. The well water to be sampled is placed in a sterile tube of 200 ml which is then placed in an ice box to be taken to the laboratory.

The water sample is first homogenized by stirring, then as much as one ml of the sample is diluted with 9 ml of sterile water in the first tube, then homogenized by shaking. The sample in the first tube is taken 1 ml and diluted again with 9 ml of sterile aquadest in the second tube, then homogenized. From the second tube, the sample is taken 1 ml for dilution in the third tube in the same way as in the dilution of the first and second tubes, and then from the third tube, the sample is diluted again in the fourth dilution tube in the same way as in the dilution of the first, second, and third tubes. In the fourth dilution tube of inoculum, as much as 0.1 ml was dripped into EMBA media. After that, it was incubated for 24 hours at a temperature of 37 °C.

In EMBA media, colonies will be observed, and if there are bacterial colonies, they will be cultured on Nutrient Agar slant (NA Slant) media. Colonies that grow on nutrient agar are planted and then incubated for 24 hours. The growing bacteria will be stained with gram staining and characterized by biochemical tests consisting of Sulfite Indol Motility (SIM), Methyl Red (MR), Voges Proskauer (VP), Triple Sugar Iron Agar (TSIA), simmon citrate, urease, and fermentation of carbohydrates (confectionery). The tubes are then incubated in the incubator at 37°C for 24 hours. Biochemical test results will be analyzed based on Bergey's manual of determinative bacteriology (Holt *et*

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al. 1994). Furthermore, resistance testing was carried out using Muller-Hinton agar media with penicillin G and tetracycline antibiotics. Based on the results of surveys and interviews, this antibiotic is often used because it is cheap and easy to get by the public. Data analysis in this study is presented descriptively by presenting resistance results in the form of tables based on inhibitory zones, referring to the Clinical and Laboratory Standards Institute (CLSI, 2020).

RESULTS AND DISCUSSION

The results of the study of 10 water samples obtained 2 samples negative and 8 samples that were positive for *E. coli* by showing metallic green growth in EMBA media; this showed bacteria growing on the media were able to ferment lactose. Colonies grown in EMBA were cultured using NA, followed by biochemical tests and Gram staining. Staining is done to determine the morphological shape of bacteria that have grown in agar media by giving dyes to bacterial scratches on glass objects. *E. coli* bacteria on a microscope with a magnification of 400x, showing the morphological shape of short red bacilli-shaped bacteria after being observed under a microscope. This is due to the concentration of lipids and the thickness of the peptidoglycan layer on the bacterial cell wall. The red color indicates that the bacteria absorb the given dye.

The detection of *E. coli* in well water samples at chicken farms in the Narmada region indicates that well water, which is a source of water that is also used for human needs, is contaminated. The source of this contamination can be obtained from the presence of contaminants, one of which comes from feces (WHO, 2008). The results of the study can be used as a reference for administering and controlling the administration of antibiotics to minimize the widespread resistance of *E. coli* bacteria to antibiotics. Similar research was also conducted in farms in the Central Lombok region, which detected the presence of *E. coli* bacterial contamination (Gunawan *et al*., 2022).

Table 1. Standard inhibitory zones on Penicillin G and Tetracycline antibiotics, Susceptible (S), Intermediate (I), Resistant (R).

Antibiotics	**S**	**I**	**R**
Penicilin G	**≥17 mm**	**14-16 mm**	**≤13 mm**
Tetracycline	**≥15 mm**	**12-14 mm**	**≤11 mm**

(Source: CLSI, 2020)

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The use of antibiotics Penicillin G and tetracycline are classes of antibiotics that are widely used in animals and humans. Tests conducted on 8 positive *E. coli* samples in Table 2 showed resistance to penicillin G in 8 samples (100%) and resistance to tetracycline in 6 samples (75%).

Table 2. Results of the Inhibition Zone Test of Penicillin G and Tetracycline Antibiotics.

Sampel	Penicilin G (mm)	Tetracycline (mm)
A1	0	5
A2	0	22
A3	0	7
A4	0	9
A5	0	0
A6	0	18
A7	0	0
A8	0	0

The results of this study are in line with the research of Agustin and Kholik (2018) conducted on farms in North Lombok, showing that 89% of *E. coli* bacteria have been resistant to the antibiotic penicillin by 85%. Many resistant *E. coli* bacteria isolated from livestock have been isolated in several areas of West Nusa Tenggara. Research conducted by Agustin *et al.* (2022) in West Lombok showed the resistance level of *E. coli* isolates from layer chicken cloaca swabs was 100% in penicillin G antibiotics and 37.5% in tetracycline antibiotics. Penicillin G antibiotic resistance of 91.7% in *E. coli* and tetracycline bacteria of 58.3% isolated in patients at the Teaching Animal Hospital of Mandalika University of Education (Agustin and Ningtyas, 2022).

Penicillin antibiotics are -lactam class antibiotics, and it is known that Gram-negative bacteria such as *E. coli* have beta-lactamase enzyme, which is an enzyme that is able to inactivate beta lactam antibiotics (Siswandomo, 2008). Penicillin is an antibiotic derived from

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the -lactam group, so penicillin works by inhibiting the formation of mucopeptides needed for the synthesis of bacterial cell walls. The enzyme -lactam has the ability to hydrolyse penicillin G, ampicillin, amoxicillin, and penicillin carboxy. The bactericidal effect of penicillin is due to inhibition of synthesis in the bacterial cell wall. Penicillin is widely known and used in developing countries. Penicillin is the antibiotic most often used to treat sick livestock or animals (Lopez-Lozaro *et al*., 2000).

The results of this study did not form an inhibitory zone for the antibiotic penicillin G. It stated that *E. coli* bacteria were resistant to the antibiotic penicillin G. Treatment with the antibiotic penicillin G in chickens was used only for the treatment of disorders of the digestive tract, routinely resulting in resistance. The mechanism of resistance to the penicillin group is due to: (1) inactivation of antibiotics by beta-lactamase; (2) modification of target PBPs; (3) damage to drug penetration into target PBPs; and (4) the presence of an outflow pump for beta-lactamase production. Therefore, the use of penicillin G antibiotics is no longer effective in inhibiting the growth of *E. coli*.

In this study, resistance to tetracycline was 75%. This can occur due to changes in the envelope permeability of microbial cells. In sensitive cells, the drug will be in the environment and will not leave the cell, while in drug-resistant cells, it cannot be actively transported into the cell or will disappear rapidly so that minimal inhibitory concentrations cannot be maintained; the mechanism is controlled by plasmids (Meles *et al*., 2011).

Based on the results of resistance tests in research on drinking water sources on this farm, it can be a consideration about the use of antibiotics, the dose of antibiotics, and the time of use of antibiotics in livestock. The use of antibiotics that have been resistant to bacteria will have an impact on animals on farms. *E. coli* bacteria resistant to some antibiotics are also found in feces and cloacal swabs in poultry farms (Agustin *et al*., 2022). Antibiotic-resistant genetic material can be transferred between *Escherichia coli* through the transfer of genetic elements. Enterobacteriaceae can transfer these genes between animals and owners through horizontal transfer (Yao *et al*., 2016).

CONCLUSION

Based on 10 waater samples taken from water sources on the farm, 8 positive E. coli samples were obtained. Antibiotic resistance tests of penicillin G and tetracycline showed results of 100% resistance to penicillin G and 75% to tetracycline. There needs to be supervision of the use of antibiotics, especially penicillin G and tetracycline.

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