IDENTIFICATION OF ANTIBIOTIC RESISTANCE PATTERNS IN *Escherichia coli* BACTERIA FROM CLOACAL SWAB SAMPLES OF BROILER CHICKENS FROM FARM THAT USE PROBIOTIC *Lactobacillus* sp.

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Abstract

This study aims to determine the resistance pattern of Escherichia coli in broiler chickens given Lactobacillus sp. during the maintenance period. A total of 48 chicken cloacal swab samples given Lactobacillus sp. and 48 samples of untreated chicken cloacal swabs were taken from farms in Cimarigi Village, Sukadana District, Ciamis Regency. E. coli was isolated and identified, followed by an antimicrobial susceptibility test using the disc diffusion method according to the Kirby Bauer method against the antibiotics amoxicillin (20 µg), erythromycin (15 µg), and ciprofloxacin (5 µg). Data on the diameter of the antibiotic inhibition zone were compared with standard bacterial sensitivity and classified as sensitive, intermediate, and resistant. The results showed that E.coli from both sample groups were 100% resistant to amoxicillin and erythromycin. The pattern of resistance to ciprofloxacin in the sample group given probiotics was 76% intermediate and 24% resistant, while the sample group that was not given probiotics was 96% resistant, 2% intermediate, and 2% sensitive.

Key words: *Escherichia coli, Lactobacillus sp., resistance pattern of antibiotics, broiler chicken*

INTRODUCTION

The use of AGP (Antibiotic Growth Promotor) in broiler chicken feed can result in the formation of resistant bacteria in the body of broiler chickens and continue in humans (Prasetyo, 2020). The formation of bacteria that are resistant to antibiotics due to high exposure to antibiotics so bacteria form a defense mechanism against antibiotics (Besung et al, 2018). Among the microorganisms carrying antibiotic-resistance genes with the highest clinical relevance are Extended Spectrum *β-lactamase*(ESBL)-producing *Enterobacteria*, especially *Escherichia coli* which has been listed among the twelve serious threats that are drug-resistant by the Centers for Disease Control and Prevention (CDC) (CDC, 2017). *Eschericia coli* is a classic indicator of fecal contamination that is routinely used to assess the microbiological quality of water and food and plays a major role in the spread of antibiotic resistance (Szmolka & Nagy, 2013). *Escherichia coli* is a bacterium that is an opportunistic pathogen that is commensal in the digestive tract of both humans and animals and is spread in the environment (Loncaric et al., 2013). The bacteria Escherichia coli, which originates from poultry farms, has the potential to disseminate into the environment, primarily through manure, serving as a means for the transmission of resistance from poultry farms (Wegener, 2012). Currently, the incidence of antibiotic resistance has become a global problem, based on data obtained in 2009, Indonesia is a country with the title of multi-drug resistance ranked 8th out of 27 countries with the highest rating in the world (Supriyantoro, 2011). An alternative solution of antibiotics is needed that can be used to prevent poultry disease and also improve the performance of chickens during rearing but does not have a negative impact on its use. One of them is the use of probiotics. Giving

Lactobacillus spp. to broiler chickens as a treatment can reduce the production of toxins by harmful microorganisms and minimize the negative effects caused by pathogenic bacteria. This can improve feed absorption by repairing the digestive organs, particularly the small intestine, boosting the production of digestive enzymes, increasing antibody production in the digestive tract, and generating vitamins and antimicrobial substances. These actions help achieve optimal digestive organ health (Sumarsih et al., 2012).The positive effects arising from the use of *Lactobacillus* spp. and maintaining the stability of the gut microbiota is also a mechanism by which probiotics can influence the spread of antibiotic resistance. A study conducted by Ouwehand (2016) showed that lactic acid produced by *lactobacilli* strains can increase the susceptibility of Gram-negative bacteria to antimicrobial agents. Lactic acid produced by *Lactobacillus* spp. can work as a permeabilizer on gram-negative bacterial cells. Permeabilizers do not need to possess bactericidal or bacteriostatic properties against gram-negative bacterial cells. Instead, their function is to facilitate the penetration of other compounds, thereby enhancing susceptibility to hydrophobic antibiotics, detergents, lysozyme, or bacteriocins (Alakomi et al., 2005 as cited in Hongmei et al., 2021). The mechanism of action of probiotics involves competition between probiotics and pathogenic microorganisms. The antagonistic competition mechanism among bacteria in the digestive tract serves as an ecological balance, preventing excessive growth of any specific species within the digestive tract.

MATERIALS AND METHODS

Research materials

The sample used in this study was a cloacal swab sample taken from 48 Cobb chickens given the probiotic Lactobacillus sp. and 48 broiler chickens that were not given probiotics according to the program from the broiler farm owned by PT. YAM. In this study, no intervention was performed on the sample population during maintenance. The sampling location was carried out in Cimarigi Village, Sukadana District, Ciamis Regency in June - July 2022.

Sampling method

To collect cloacal swab samples, chickens that met the research criteria were captured. A sterile cotton swab (Nesco) was then gently inserted into the cloaca, rotating it slowly to a depth of 1.5 to 2.5 cm. The swab was rotated 360° inside the cloaca before being carefully removed. Any excess sample (feces > 0.5 cm) was discarded. The swab sample was placed into a transport medium by opening the tube and inserting the swab tip until it reached about ¾ of the bottom of the tube. The excess swab tip was cut using sterilized scissors soaked in 70% alcohol, and the tube was tightly closed. A number label was assigned to the tube containing the sample. The sample was then placed in a cool box at a temperature of 2-8°C and sent to the laboratory within one day of sampling (BAVET Semarang, 2018).

Isolation, identification, and bacterial sensitivity test of *Eschericia coli*

All samples are then sent to the laboratory of the West Java Animal Health and Veterinary Public Health Center for sensitivity testing against antimicrobials. Each sample from both groups was grown on Nutrient Agar and Eosin Methylene Blue Agar (EMBA) media. Bacterial colonies that are metallic green in color with a dark center are suspected as *E. coli* colonies which will be followed by the identification of the bacteria. Identification was carried out by Gram staining and biochemical tests, using Tripple Sugar Iron Agar (TSIA), Simmons citrate Agar (SCA), Sulphide Indole Motility (SIM), and Methyl Red Voges Proskauer (MRPV) methods (Oxoid, Basingstoke, UK).

Escherichia coli bacteria that have been identified are followed by a sensitivity test to antibiotics Amoxicillin (20 µg), Erythromycin (10 µg), and Ciprofloxacin (10 µg). The sensitivity test was carried out by agar diffusion using the Kirby-Bauer method. Colonies of *E. coli* were then grown in liquid Mueller Hinton medium and incubated for 2 hours at 37ºC until a turbidity equivalent to 0.5 Mc Farland was obtained (containing 106 cells/ml). Then 0.5 ml of the culture was planted on Mueller Hinton Agar (MHA) media and spread evenly and incubated for about 30 minutes.

Data analysis

The data obtained from positive *Escherichia coli* cloacal swab samples will be subsequently analyzed both descriptively and quantitatively. This analysis will involve calculating the percentages of bacteria that exhibit sensitivity, intermediate resistance, and full resistance to antibiotics. The test results will be presented in tabular form. Furthermore, statistical analysis of the data will be conducted using the Mann-Whitney test, which is utilized for comparative analysis of two independent samples containing ordinal data (Siregar, 2013).

RESULTS AND DISCUSSIONS

Identification of bacteria

Identification results of broiler chicken cloacal swab samples from PT. YAM which shows positive isolates of *Eschericia coli* bacteria is shown in the Table 1.

Table 1. *Eschericia coli* identification

No.	Sample	Total Sample	*Eschericia coli* positive	*Eschericia coli* negative
1.	Non-probiotic Group	48 Samples	48 Samples	(-)
2.	Probiotic Group	48 Samples	48 Samples	(-)

The results in this study were obtained from two different groups, namely the group of chickens that were treated with antibiotics as many as 48 samples, and the group of chickens that were given antibiotics and probiotics as many as 48 samples.

Based on the data in Table 1. with a total sample of 48 samples from each group, positive results were obtained from the probiotic and non-probiotic sample groups, each of which was 48 positive samples of *Eschericia coli*.

Table 2. Zone of Inhibition Interpretation Standard

Antibiotics	Bacteria	Zone of Inhibition Interpretation Standard (mm)		
		Resistent	Intermediate	Sensitive
Amoxicillin	*E. coli*	≤ 13 mm	14-17 mm	≥ 18 mm
Ciprofloxacyn	*E. coli*	≤ 15 mm	16-20 mm	≥ 21 mm
Erythromycin	*E. coli*	≤ 12 mm	14-22 mm	≥ 23 mm

Resistance pattern of Eschericia coli

Antimicrobial susceptibility test (AST) is used to determine the pattern of resistance of bacteria to antibiotics. The potency of an antibiotic that was tested for sensitivity to *Escherichia coli* bacteria was classified into three criteria according to the guidelines of the Clinical and Laboratory Standard Institute (CLSI), shown in the Table 2.

Resistance pattern to amoxicillin

The results of sample testing for amoxicillin antibiotics are presented in the Tables 3 and 4.

Table 3. Pattern of amoxicillin resistance from probiotic group samples

Amoxicillin			
Probiotic Group	Diameter of Inhibition Zone		
	0-13 mm	14-17 mm	≥ 18 mm
Interpretation	Resistent	Intermediate	Sensitive
Jumlah	48 Samples	0 Sample	0 Sample

Table 4. Amoxicillin resistance patterns from non-probiotic group samples

Amoxicillin			
Non-Probiotic Group	Diameter of Inhibition Zone		
	0-13 mm	14-17 mm	≥ 18 mm
Interpretation	Resistent	Intermediate	Sensitive
Jumlah	48 Samples	0 Sample	0 Sample

Table 5. Mann-Whitney U Test for amoxicillin resistance

Statistic	Value	Information
Mann-Whitney U	1152,000	H_0 accepted
Sig. (2-tailed)	1,000	

Based on the data above, it can be seen the distribution of data for the Probiotic and Non-Probiotic groups in testing the antibiotic amoxicillin. The results of testing the samples from the group of chickens that were given probiotics showed resistant results in all samples. AST testing on samples from chickens that were not given probiotics also showed resistant results in all samples. Next, hypothesis testing is conducted to examine the difference between the probiotic and non-probiotic groups in the testing of erythromycin as follows:

H0: (η1 = η2), there is no difference between the group of chickens given *Lactobacillus* sp. probiotics and the group of chickens not given *Lactobacillus* sp. probiotics.

H1: (η1 ≠ η2), there is a difference between the group of chickens given *Lactobacillus* sp. probiotics and the group of chickens not given *Lactobacillus* sp. probiotics.

With α = 5%, the results of the analysis are as follows. Statistical test results were not significantly different ($P > 0.05$) between cloacal swab samples of chickens given probiotics and not given probiotics (Table 5).

Resistance pattern to erythromycin

The results of sample testing for erythromycin antibiotics are presented in the Table 6.

Table 6. Patterns of erythromycin resistance from probiotic group samples

Erythromycin			
Probiotic Group	Diameter of Inhibition Zone		
	0-13 mm	14-22 mm	≥ 23 mm
Interpretation	Resistant	Intermediate	Sensitive
Total	48 Samples (100%)	0 Sample	0 Sample

Table 7. Patterns of erythromycin resistance from non-probiotic group samples

Eritromisin			
Non-Probiotic Group	Diameter of Inhibition Zone		
	0-13 mm	14-22 mm	≥ 23 mm
Interpretation	Resistant	Intermediate	Sensitive
Total	48 Samples (100%)	0 Sample	0 Sample

Table 8. Mann-Whitney U test for erythromycin resistance

Statistic	Value	Information
Mann-Whitney U	1152,000	H_0 accepted
Sig. (2-tailed)	1,000	

The table above shows the results of the distribution of AST data for the antibiotic erythromycin in both groups. The probiotic and non-probiotic groups had the same results, that is, all samples obtained resistant results with a percentage of 100%. Statistical test results were not significantly different ($P > 0.05$) between cloacal swab samples of chickens given probiotics and not. The pattern of *Escherichia coli* resistance did not change from the cloacal swab samples of chickens given *Lactobacillus* sp. to erythromycin can also be due to the antibacterial properties of the lactic acid component produced by *Lactobacillus* sp. not persistent in *Eschericia coli* that has been cultured from the sample.

Resistance pattern to ciprofloxacin

The results of sample testing for ciprofloxacin antibiotics are presented in the following tables.

Table 9. Pattern of Ciprofloxacin Resistance from Probiotic Group Samples

Ciprofloxacin			
Probiotic Group	Diameter of Inhibition Zone		
	0-15 mm	16-20 mm	≥ 21 mm
Interpretation	Resistant	Intermediate	Sensitive
Total	13 Samples (27%)	35 Samples (73%)	0 Sample

Table 10. Pattern of ciprofloxacin resistance from non-probiotic group samples

Ciprofloxacin			
Non-Probiotik Group	Diameter of Inhibition Zone		
	0-15 mm	16-20 mm	≥ 21 mm
Interpretation	Resistant	Intermediate	Sensitive
Total	46 Samples (95%)	1 Sample (2%)	1 Sample (2%)

Table 11. Mann-Whitney U Test for ciprofloxacin resistance

Statistic	Value	Information
Mann-Whitney U	377,500	H_0 denied
Sig. (2-tailed)	0,000	

All samples from the probiotic group showed an intermediate resistance pattern with a percentage of 73%, 27% of the samples showed resistant results, and none of the samples had sensitive results. Furthermore, for the non-probiotic group, sample testing obtained resistant results with a percentage of 96%, intermediate by 2%, and sensitive by 2%. The statistical test results showed a significant difference (P < 0.05). This shows that there is a significant difference between the resistance patterns of *Eschericia coli* from the sample groups that were not given probiotics and those that were given probiotics, whereas in the samples given probiotics, 35 out of 48 samples had intermediate resistance patterns.

DISCUSSIONS

Amoxicillin and erythromycin resistance patterns

There was no difference in the pattern of resistance between chicken samples given *Lactobacillus* and not given *Lactobacillus* to the pattern of amoxicillin resistance. Dust scattered in chicken coops can contain 105-106 *Eschericia coli* cells/gram and may spread into the cloaca of chickens (De Carli et al., 2015; Sayad et al., 2018). In addition, the more aerobic condition of the cloaca can also cause changes in the metabolic pathways of *Lactobacillus* sp. Research conducted by Quatravaux et al. (2006) in Zotta et al. (2017) found that under aerobic conditions, the presence of oxygen can interfere with the transcription of the nLDH operon and that NADH-dependent oxidase NOX can compete with nLDH for the NADH pool, diverting pyruvate from lactate production. Acetate accumulation has been found in aerobically grown cultures of homofermentative and heterofermentative *Lactobacillus* species. This allows *Eschericia coli* from the environment and digestive tract to grow better in the cloaca compared to *Lactobacillus* sp. Based on this, it can be concluded that the use of *Lactobacillus* sp. did not change the resistance pattern of *Eschericia coli* strains taken from cloacal swab samples of broiler chickens to amoxicillin.

Based on the findings by Alakomi et al. (2005), lactic acid works as a strong disintegrating agent against the outer membrane of gram-negative bacteria which can cause the release of lipopolysaccharide bonds, making bacteria more susceptible to antimicrobial agents when interacting directly with bacteria. Lactic acid which interacts directly with lipopolysaccharide cell membranes can increase the susceptibility of gram-negative bacteria to hydrophobic antibiotics, one of which is erythromycin. The effect of taking samples from a cloacal swab also affects the test results because, under aerobic conditions, *Lactobacillus* sp. diverts the pathway of pyruvate metabolism away from lactate production. The results of this study indicate that Lactobacillus sp. did not affect the susceptibility of *Eschericia coli* taken from cloacal swabs of broiler chickens to the genetic stage so the pattern of resistance to erythromycin did not change.

Ciprofloxacin resistance pattern

All samples from the probiotic group showed an intermediate resistance pattern with a percentage of 73%, 27% of the samples showed resistant results, and none of the samples had sensitive results. Furthermore, for the non-probiotic group, sample testing obtained resistant results with a percentage of 96%, intermediate by 2%, and sensitive by 2%. The statistical test results showed a significant difference ($P < 0.05$). This shows that there is a significant difference between the resistance patterns of *Eschericia coli* from the sample groups that were not given probiotics and those that were given probiotics, whereas in the samples given probiotics, 35 out of 48 samples had intermediate resistance patterns. The difference in the pattern of resistance from testing the two samples is thought to be due to the probiotic *Lactobacillus* sp. has an anti-adhesion effect that can prevent the adhesion of 80% of *ciprofloxacin-resistant Eschericia coli* strains studied in Caco-2 cell cultures (Abedi et al., 2013). The result indicate that *Lactobacillus* sp. supplementation can increase *Escherichia coli* sensitivity to ciprofloxacin.

Research conducted by Abedi et al. (2013) on Caco-2 cell culture, *Lactobacillus* sp. is more successful at binding to cellular receptors and preventing the adhesion of pathogenic bacteria by pre-attaching to those sites. The anti-adhesion effect produced by *Lactobacillus* sp. through the mechanism of production of antimicrobial materials including bacteriocins, lactic acid, and biosurfactants can effectively prevent the formation of biofilms from *Eschericia coli*. The biofilm produced by *Eschericia coli* can make it resistant to many antibiotics compared to *Eschericia coli* in a free (planktonic) state and almost resistant to ciprofloxacin, carbenicillin, cloxacillin, cephaloridine, novobiocin, and vancomycin (Yeganeh et al., 2017). Based on the findings from a study conducted by Yeganeh et al. (2017), it was found that *Lactobacillus* sp. has an inhibitory effect on Ciprofloxacin Resistant Uropathogenic *Eschericia coli* (UPEC) strains in body tissues. The results of this study confirm the hypothesis of Yeganeh et al. (2017) that *Lactobacillus* sp. can inhibit the growth of *Eschericia coli* strains that are resistant to ciprofloxacin by preventing the formation of biofilms so that different patterns of resistance are obtained in the research results. The decrease in the percentage of resistant *Eschericia coli* strains in samples originating from chickens given probiotics compared to the unexpected was due to *Eschericia coli* strains that were resistant to ciprofloxacin derived from breeding so that after being given *Lactobacillus* sp., the growth of the *Eschericia coli* strain was inhibited. Further research is needed to determine the *Eschericia coli* strain obtained from cloacal swabs of broiler chickens given *Lactobacillus* sp. to confirm the findings of this study.

CONCLUSIONS

Based on the findings in this study, it can be concluded that the administration of *Lactobacillus sp*. in broiler chickens affected the resistance pattern of *Eschericia coli* to ciprofloxacin from 96% resistant, 2% sensitive and 2% intermediate to 27% resistant, 96% intermediate, and 0% sensitive. There was no change in the pattern of resistance to amoxicillin and erythromycin.

SUGGESTIONS

Further research is needed to see the effect of *Lactobacillus* sp. on the pattern of resistance of *Eschericia coli* in other segments of the digestive tract of broiler chickens to the class of antibiotics commonly used for broiler therapy.

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