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COMPARISON OF THE SUSCEPTIBILITY OF SIX ANTIBIOTICS AGAINST ESCHERICHIA COLI ISOLATED FROM THE CHICKEN CECUM USING THE KIRBY-BAUER DISK DIFFUSION METHOD

Perbandingan Kerentanan Enam Antibiotik terhadap *Escherichia coli* yang Diisolasi dari Sekum Ayam Menggunakan Metode Difusi Cakram Kirby-Bauer

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Abstract

Chicken farms often face bacterial infections, one of which is *Escherichia coli*. Treatment of *Escherichia coli* bacterial infections in chickens generally uses antibacterial agents in the form of antibiotics, to suppress the growth and spread of bacteria. Intensive use of antibiotics to prevent and treat infections can trigger resistance, which reduces the effectiveness of therapy. This study aims to evaluate the sensitivity of six types of antibiotics against *E. coli* isolates from 5 chicken cecum samples using the Kirby-Bauer Disk Diffusion method. Samples were taken from Chicken Farm X in Kulon Progo Regency, with the cecum collected into labeled plastic and stored in a Styrofoam box containing ice packs. Cecum samples were isolated on MacConkey agar and Nutrient Agar media. Identification was carried out based on colony morphology on MacConkey agar and bacterial colony purification on Nutrient Agar media, further confirmed by Indole testing. E. coli bacterial isolates were grown on Mueller-Hinton agar media, treated with antibiotic disks, and incubated. The inhibition zone was measured after 18 to 24 hours of incubation to assess antibiotic effectiveness. The results of the resistance test were as follows: ciprofloxacin (100%), ampicillin (80%), gentamicin (80%), tetracycline (60%), and sulfamethoxazole and trimethoprim (20%) were resistant. Factors causing resistance include the dose and duration of antibiotic administration, farm management, and regulations, supervision, and education of farmers.

Keywords: antibiotics, chickens, *Escherichia coli*, kirby-bauer, antibiotic resistance.

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Abstrak

Peternakan ayam sering menghadapi penyakit infeksi bakteri, salah satunya *Escherichia coli*. Pengobatan infeksi bakteri *Escherichia coli* pada ayam umumnya menggunakan agen antibakteri berupa antibiotik, untuk menekan pertumbuhan dan penyebaran bakteri. Penggunaan antibiotik yang intensif untuk mencegah dan mengobati infeksi dapat memicu resistensi, yang mengurangi efektivitas terapi. Penelitian ini bertujuan mengevaluasi sensitivitas enam jenis antibiotik terhadap isolat *Escherichia coli* dari 5 sampel sekum ayam menggunakan metode *Kirby-Bauer Disk Diffusion*. Sampel diambil dari Peternakan ayam X di Kabupaten Kulon Progo, dengan bagian sekum dikoleksi ke dalam plastik berlabel dan disimpan pada boks sterofoam berisi *icepack*. Sampel sekum diisolasi pada media *MacConkey agar* dan media *Nutrient Agar*. Identifikasi dilakukan berdasarkan morfologi koloni pada *MacConkey agar* dan pemurnian koloni bakteri di media *Nutrient Agar*, selanjutnya dikonfirmasi dengan uji *Indole*. Isolat bakteri *E. coli* ditanam pada media *Mueller-Hinton agar*, diberi cakram antibiotik, dan diinkubasi. Zona hambat diukur setelah 18 hingga 24 jam inkubasi untuk menilai efektivitas antibiotik. Hasil uji resistensi yang diperoleh sebagai berikut, *ciprofloxacin* (100%), *ampicillin* (80%), *gentamicin* (80%), *tetracycline* (60%), dan *sulfamethoxazole* dan *trimethoprim* (20%) resisten. Faktor penyebab resistensi meliputi dosis dan durasi pemberian antibiotik, manajemen peternakan, dan regulasi, pengawasan, dan edukasi peternak.

Kata kunci: antibiotik, ayam, *Escherichia coli*, *kirby-bauer*, resistensi antibiotik.

INTRODUCTION

Chickens are one of the important sources of animal protein for people in Indonesia. Poultry farming not only plays a role in providing food but also supports the community's economy (Andriani *et al.*, 2024). One of the health problems commonly found in poultry farms is infection caused by *Escherichia coli*. *Escherichia coli* (*E. coli*) is a Gram-negative bacterium that is naturally found in the digestive tract of animals; however, certain strains are pathogenic and can cause disease (Langgar *et al.*, 2021). Pathogenic bacteria can also infect humans. Infections caused by *E. coli* can be treated using antibacterial agents known as antibiotics. Antibiotics that are widely used in broiler farms include ampicillin, as it can be obtained without a prescription and is relatively affordable (Putri *et al.*, 2023). This condition encourages intensive use of antibiotics, which has the potential to trigger resistance in *E. coli* bacteria (Wulandari *et al.*, 2025).

Antibiotic resistance is a condition in which bacterial growth is not inhibited by the administration of certain antibiotics, resulting in ineffective treatment of diseases in livestock (Audiya *et al.*, 2024). A study by Koju *et al.* (2022) stated that *E. coli* isolates from the chicken cecum were resistant to tetracycline (86%), ciprofloxacin (66%), and showed multidrug resistance to three types of antibiotics (71%). Research by Putri *et al.* (2024) showed a high level of ciprofloxacin resistance in broiler chickens in Surabaya, reaching 85%. Susilo *et al.* (2022) reported a similar resistance rate of 71.42% in broiler chickens in Yogyakarta. The cecum is part of the digestive tract that serves as the main site for *E. coli* colonization. Sampling bacterial isolates from the cecum is considered relevant for testing because the presence of *E. coli* in this organ reflects the bacterial population within the chicken's body (Ananda *et al.*, 2023). Monitoring antibiotic resistance in *E. coli* isolates is very important as a basis for decision-making in treatment, one of which is through the Kirby-Bauer Disk Diffusion method. The Kirby-Bauer Disk Diffusion method is used to assess bacterial sensitivity to antibiotics by measuring the diameter of the inhibition zone formed (Sari & Febriawan, 2021).

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Studies evaluating the sensitivity of *E. coli* bacteria have previously been reported using isolates from the chicken cecum, but with different methods. The study by Koju *et al.* (2022), for example, used ciprofloxacin and tetracycline antibiotics. Sensitivity testing of *E. coli* using isolates from the chicken cecum with the Kirby-Bauer Disk Diffusion method has been reported, but data are still limited in the Yogyakarta region. Based on this, it is necessary to conduct research on antibiotic sensitivity using isolates from the chicken cecum with the Kirby-Bauer Disk Diffusion method. The results of this study are expected to support the One Health approach, serve as a reference for prudent antibiotic use in the livestock sector, and strengthen antibiotic resistance monitoring data by Balai Besar Veteriner Wates. This study is expected not only to provide academic contributions but also to support policy-making in controlling antibiotic resistance.

RESEARCH METHODS

Research Object

The object of this study was *E. coli* bacterial isolates obtained from the cecum of broiler chickens from a poultry farm in Kulon Progo Regency, Special Region of Yogyakarta.

Research Design

This study used a quantitative descriptive research design with laboratory testing. The testing was carried out using the disk diffusion method (Kirby-Bauer Disk Diffusion) to determine the level of sensitivity of *Escherichia coli* isolates to several types of antibiotics.

Research Variables

The independent variable in this study was the type of antibiotic used, namely ciprofloxacin, chloramphenicol, ampicillin, gentamicin, tetracycline, and sulfamethoxazole and trimethoprim (SXT), while the dependent variable was the diameter of the inhibition zone (mm) formed around the antibiotic disk on Mueller-Hinton Agar (MHA) as an indicator of the sensitivity level of *Escherichia coli*. The control variables included the concentration of the bacterial suspension, the type of agar medium, incubation temperature, incubation time, distance between antibiotic disks, and bacterial inoculation technique. In addition, the standard control strain *E. coli* ATCC 25922 was used as a positive control.

Data Collection Method

Data were collected using the disk diffusion test (Kirby-Bauer Disk Diffusion). Antibiotic susceptibility testing was carried out through several stages, including bacterial isolation and identification, preparation of bacterial suspension, inoculation onto the medium, placement of antibiotic disks, and observation of results. Five samples of broiler chicken cecum were obtained from one poultry farm. This study has limitations in terms of the number and source of samples, as it only used five samples from a single farm; therefore, the results cannot be generalized to represent broader conditions, particularly in the Kulon Progo area. The procedure for isolating *E. coli* began with inoculation on MacConkey agar and incubation at 37°C for 18 to 24 hours. Colonies showing characteristics of *E. coli*, namely round, smooth, and pink colonies, were purified on Nutrient Agar and confirmed using the Indole test (Harsono *et al.* 2015 in Dewi *et al.*, 2021). Pure isolates were suspended in 0.9% physiological NaCl, and the turbidity was adjusted to the 0.5 McFarland standard (Bhakti *et al.*, 2023). The suspension was then evenly inoculated onto the surface of Mueller-Hinton Agar using a cotton swab, allowed to stand for 5 to 10 minutes, and six antibiotic disks, ciprofloxacin (5 µg), chloramphenicol (30 µg), ampicillin (10 µg), gentamicin (10 µg), tetracycline (30 µg), and sulfamethoxazole-trimethoprim (25 µg) were placed on the agar medium (Nassar *et al.*, 2019). The plates were incubated in an inverted position at 37°C for

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18 to 24 hours, after which the inhibition zone diameters were observed and measured as the test results (Syukur & Permana, 2022).

Data Analysis

The data obtained in the form of inhibition zone diameters were analyzed using quantitative descriptive methods. The inhibition zone diameters were compared with the standard interpretation table of the Clinical and Laboratory Standards Institute (CLSI) M100 (2023) to determine the bacterial susceptibility categories for each antibiotic, namely sensitive (S), intermediate (I), or resistant (R). The results were presented in tables and as percentages of isolate susceptibility to each antibiotic, allowing the pattern of antibiotic resistance in the tested *E. coli* isolates to be identified.

RESULTS AND DISCUSSION

Results

The antibiotic susceptibility testing of ciprofloxacin, ampicillin, gentamicin, tetracycline, and sulfamethoxazole and trimethoprim (SXT) was conducted on *Escherichia coli* isolates obtained from the cecum of broiler chickens from a poultry farm in Kulon Progo Regency, Special Region of Yogyakarta. Isolation of *E. coli* on MacConkey Agar (MCA) showed colony growth with lactose fermentation characteristics (indicated by a color change in the colonies), and the indole test yielded positive results for all isolates, as indicated by the formation of a red ring. The results of the Kirby-Bauer Disk Diffusion test showed that *E. coli* isolates from the broiler chicken cecum exhibited the following resistance levels: ciprofloxacin (100%), ampicillin (80%), gentamicin (80%), tetracycline (60%), and sulfamethoxazole and trimethoprim (SXT) (20%). Ciprofloxacin showed complete resistance (100%) with an average inhibition diameter of 10.57 mm, while chloramphenicol was the most effective, with only 20% resistance and the highest average inhibition zone of 16.63 mm. Ampicillin and gentamicin showed very small inhibition diameters, indicating low effectiveness against the isolates. Tetracycline showed an inhibition zone of 10 mm, while sulfamethoxazole-trimethoprim showed an inhibition diameter of 8.57 mm. Chloramphenicol still showed effectiveness or sensitivity against *E. coli* isolates, while tetracycline and sulfamethoxazole-trimethoprim (SXT) were effective against some isolates.

Discussion

Principles and Results of *Escherichia coli* Isolation

Isolation of *Escherichia coli* began with sampling the contents of the broiler chicken cecum for inoculation onto MacConkey Agar (MCA). This study used MacConkey Agar (MCA) as a selective medium specifically for growing Gram-negative bacteria and differentiating bacteria based on their ability to ferment lactose (Odeh *et al.*, 2017). MCA contains bile salts and crystal violet, which can inhibit the growth of certain Gram-positive bacteria (Seck *et al.*, 2018). MCA also contains neutral red, which functions as a pH indicator to distinguish lactose fermenters from non-lactose fermenters (Supriatin *et al.*, 2021). Bacteria that ferment lactose produce acid and lower the pH, resulting in red to pink-colored colonies.

The isolation and identification test of *E. coli* bacteria from broiler chicken cecum samples cultured on MacConkey Agar (MCA) and incubated at 36-37°C for 18 to 24 hours produced round, bright pink colonies, as shown in Figure 1. Typical *E. coli* bacteria are lactose fermenters; therefore, on MCA they form bright pink colonies, while non-fermenting bacteria remain colorless or pale (Bria *et al.*, 2022). *E. coli* ferments lactose in the agar medium, producing acid that lowers the pH around the colony and causes the pH indicator to change to pink. This pink color indicates presumptive *E. coli* colonies, distinguishing them from non-

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fermenting bacteria that produce pale colonies (Supriatin *et al.*, 2021). Furthermore, an indole test is required to confirm the bacterial colonies formed. Isolation of *E. coli* is generally carried out by inoculation on selective media such as MacConkey agar to allow optimal colony growth, followed by identification. The indole test is one of the important tests in the identification of *E. coli*, as this bacterium typically shows a positive indole result and is therefore often used as an initial indicator in the confirmation process (Zhang *et al.*, 2019).

Confirmation of *Escherichia coli* Using the Indole Test

The indole test is one of the biochemical methods used to confirm whether a bacterial isolate of *E. coli* possesses the enzyme tryptophanase, which is capable of breaking down tryptophan into indole, ammonia, and pyruvate (Zhang *et al.*, 2019). The bacterial isolate is inoculated into a medium containing tryptophan (tryptophan broth), then incubated for 24 to 48 hours at 35-37°C. After incubation, five drops of Kovac's reagent (hydrochloric acid, p-dimethylaminobenzaldehyde, butanol) are added. If the reagent reacts to form a red layer (pink to dark red) on the surface of the medium, the bacteria are indole-positive. This process is rapid and easy to perform in diagnostic laboratories and is commonly used as a confirmatory test for *E. coli* (Khoirunnisak *et al.*, 2024). Positive indole test results can be seen in Figure 2. Based on Figure 2, all five bacterial isolates grown on MCA showed positive results, indicated by the formation of a pink indole ring after the addition of Kovac's reagent.

Results of Antibiotic Susceptibility Testing

The results of antibiotic susceptibility testing in Table 1 show a high level of resistance of *E. coli* to ciprofloxacin, with all samples being resistant, indicating that the bacteria possess genetic resistance to fluoroquinolone antibiotics. This result is supported by the occurrence of ciprofloxacin resistance of 69.1% in broiler chickens in Bangladesh, where resistance genes are carried via plasmids (Das *et al.*, 2020). Ciprofloxacin resistance is usually caused by mutations in genes that reduce the effectiveness of this drug against its target bacteria (Das *et al.*, 2023). A surveillance study in poultry farms by Das *et al.* (2023) found that resistance to ciprofloxacin is associated with continuous use of fluoroquinolone antibiotics. As a consequence of this resistance, therapeutic doses of ciprofloxacin are no longer sufficient to eliminate bacteria, leading to treatment failure and the spread of resistant bacterial isolates (Truswell *et al.*, 2023). Based on this, the use of ciprofloxacin in livestock must be strictly monitored to prevent the acceleration of resistance spread.

The results in Table 1 show that several *E. coli* isolates still had sufficiently large inhibition zones for chloramphenicol. This indicates that chloramphenicol remains effective against 4 out of 5 (80%) *E. coli* isolates. Resistance to chloramphenicol usually occurs due to drug inactivation by the enzyme acetyltransferase (Rahmaniar *et al.*, 2022). The relatively high effectiveness of chloramphenicol in some isolates may be due to its less intensive use compared to other antibiotics in poultry farms (Martínez-Álvarez *et al.*, 2022). The use of chloramphenicol in food-producing animals has been restricted in several countries due to concerns about residues and side effects; therefore, despite its sensitivity, its use in farms is limited (Asni & Sianita, 2020).

Table 1 also shows resistance of *Escherichia coli* to ampicillin in 4 isolates. Ampicillin resistance generally occurs through the production of β-lactamase enzymes that break down the β-lactam ring of the drug, preventing inhibition of bacterial cell wall formation (Indana *et al.*, 2020). A study on Multiple Antimicrobial Resistance of *E. coli* in Iran reported that ampicillin resistance is among the highest, with more than 95% of samples from each farm showing resistance (Talebiyan *et al.*, 2014 in Mudenda *et al.*, 2023). High resistance to

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ampicillin in *E. coli* isolates indicates that treatment with this class of antibiotics is ineffective (Truswell *et al.*, 2023). This complicates the use of penicillin-derived antibiotics in treating bacterial infections in poultry farms. Therefore, their use needs to be monitored and replaced with other antibiotics if resistance is shown to be widespread (Ballo *et al.*, 2023).

The data in Table 1 show that some isolates had very small or even no inhibition zones against gentamicin and tetracycline. This indicates resistance or low sensitivity. Resistance to gentamicin in poultry farms should be of concern because gentamicin is one of the aminoglycoside antibiotics commonly used via injection or topical application (Abraham *et al.*, 2023). The presence of isolates resistant to gentamicin indicates that the bacteria have accumulated resistance genes that can spread to other bacterial populations. Another antibiotic with widespread resistance is tetracycline, which has been reported in several poultry farms. A study by Niasono *et al.* (2019) on antibiotic resistance testing in *E. coli* isolates showed the highest resistance in tetracycline at 97.3%. The frequent and prolonged use of tetracycline over several decades can lead to resistance, so its use as an antibiotic therapy in the field needs to be limited (Subagyo *et al.*, 2021).

The results of antibiotic susceptibility testing in Table 1 show that 2 out of 5 bacterial isolates were still sensitive to sulfamethoxazole-trimethoprim (SXT). Resistance to SXT reached 39.6% based on a Multiple Antimicrobial Resistance study in Iran (Talebiyan *et al.*, 2014 in Mudenda *et al.*, 2023). Resistance to this combination is concerning because SXT is a relatively inexpensive and widely used antibiotic option. The presence of isolates resistant to SXT also indicates that these bacteria may be multidrug-resistant (resistant to other antibiotics) and could serve as a source of resistance genes for other pathogenic bacteria (Martínez-Álvarez *et al.*, 2022).

The factors contributing to antibiotic resistance in poultry are multifactorial and therefore need to be analyzed from multiple perspectives. The following discussion is divided into several interconnected aspects. Routine use of antibiotics in poultry farms, either as growth promoters or for disease prevention (prophylaxis) without specific diagnosis, creates high selective pressure on microorganisms (Rafiq *et al.*, 2022). Continuous use of antibiotics at low doses allows sensitive bacteria to die while bacteria with tolerant mutations survive and proliferate, leading to an increase in resistant populations. This condition is exacerbated when antibiotic use is not based on veterinary recommendations or susceptibility testing results, resulting in inappropriate use in terms of dosage, duration, and antibiotic selection. Resistant bacteria can emerge rapidly in environments with intensive contact among chickens (Wibawati *et al.*, 2023).

Management-related factors such as high stocking density, poor ventilation, inadequate air circulation, poor cage sanitation, and improper waste management increase the risk of disease infection. A case study in Binjai Village found that poor cage hygiene and the administration of antibiotics through drinking water without proper dosage measurement were significantly associated with resistance in *Escherichia coli* (Silalahi *et al.*, 2024). Poor management systems allow resistant bacteria to develop and spread more easily among poultry or into the surrounding environment, thereby reinforcing the cycle of resistance (Ramanda & Agustina, 2024).

Regulation of antibiotic use as feed additives, as well as monitoring and enforcement in Indonesia, remains suboptimal. This is reflected in the continued unrestricted use of antibiotics (Wibisono *et al.*, 2022). Another contributing factor is that many farmers lack adequate training or information regarding the risks of antibiotic resistance and proper

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antibiotic use practices. A study in Tabanan showed that low farmer knowledge regarding antibiotic use and Antimicrobial Resistance (AMR) worsens resistance conditions (Ardiana *et al.*, 2024). A study by Adnyana *et al.* (2025) reported that farmers' knowledge of Antimicrobial Resistance (AMR) is still limited, with only 15% of farmers having sufficient understanding.

CONSCLUSION AND SUGGESTIONS

Conclusion

This study shows that *Escherichia coli* isolates from the cecum of broiler chickens exhibit high resistance to several antibiotics, including ciprofloxacin, ampicillin, and gentamicin. The resistance results obtained were as follows: ciprofloxacin (100%), ampicillin (80%), gentamicin (80%), tetracycline (60%), and sulfamethoxazole and trimethoprim (SXT) (20%), based on testing of five cecal isolates.

Suggestions

Farmers are advised to follow antibiotic use protocols based on the results of bacterial susceptibility testing and veterinary recommendations. Improvements in farm management, sanitation, and stocking density need to be implemented to reduce the selective pressure for resistant bacteria. Education on antibiotic use and resistance should be enhanced among farmers. The government needs to strengthen regulations and supervision of antibiotic use in livestock farming. Further studies are recommended to evaluate antibiotic resistance periodically and to include a wider range of antibiotics.

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Table

Table 1. Results of the Antimicrobial Susceptibility Test (AST) of chicken cecum samples using the disk diffusion method.

Type of Antibiotic	*Reference values Disk Diffusion (mm)			Diameter of Inhibition Zone (mm)					
	S	I	R	C	S1	S2	S3	S4	S5
Ciprofloxacin (CIP 5)	>26	22-25	<21	27.59	16.51 (R)	0.00 (R)	9.74 (R)	9.36 (R)	17.25 (R)
Chloramphenicol (C 30)	>18	13-17	<12	19.89	19.17 (S)	21.50 (S)	19.29 (S)	0.00 (R)	23.18 (S)
Ampicillin (AMP 10)	>17	14-16	<13	13.41	0.00 (R)	16.86 (1)	0.00 (R)	0.00 (R)	0.00 (R)
Gentamicin (CN 10)	>18	15-17	<14	17.08	15.34 (I)	9.02 (R)	8.64 (R)	0.00 (R)	0.00 (R)
Tetracycline (TE 30)	>15	12-14	<11	22.78	0.00 (R)	9.72 (R)	20.04 (S)	0.00 (R)	20.43 (S)
Sulfamethoxazole-Trimetoprim (SXT 25)	>16	11-15	<10	23.94	16.24 (S)	17.85 (S)	0.00 (R)	0.00 (R)	8.78 (R)

*Testing guidelines refer to the Clinical and Laboratory Standards Institute (CLSI) M100, 2023. Note: Susceptible (S), Intermediate (I), Resistant (R), Control (C), Sample (S1-S5)

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Figures

Figure 1. Isolation results of suspected *Escherichia coli* bacteria from chicken cecum samples on MacConkey Agar (MCA).

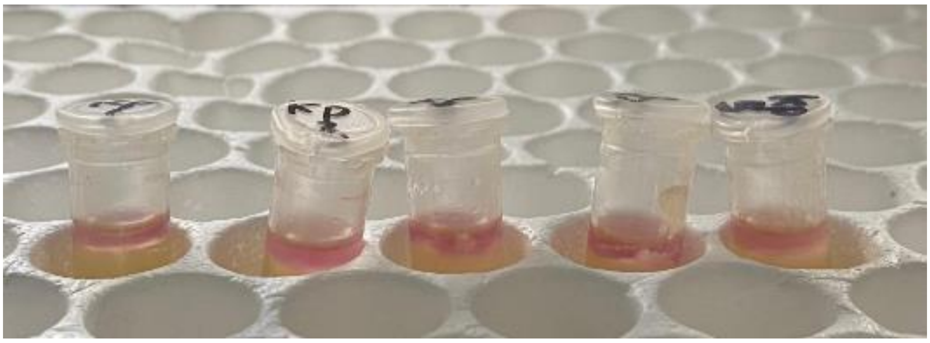

Figure 2. Biochemical test results using the indole test for *Escherichia coli.*

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