

***Escherichia coli* Sensitivity Test on Meat and Liver of Broiler Chicken in Kupang City**

(Uji Sensitivitas *Escherichia coli* pada Daging dan Hati Ayam Broiler
di Kota Kupang)

Novianti N. Toelle*, Julita D. M. Yasa, Jois M. Jacob, Devi Y. J. A. Moenek

Study Program of Animal Health, Department of Animal Science,
Kupang State Agricultural Polytechnic

*Corresponding email: nelly_toelle@yahoo.com

ABSTRAK

Penelitian ini bertujuan untuk menguji sensitivitas *Escherichia coli* yang diisolasi dari daging dan hati ayam broiler yang dijual di Kota Kupang terhadap beberapa antibiotik. Sebanyak 30 isolat *E. coli* diidentifikasi dan diuji sensitivitasnya menggunakan media *Muller-Hinton Agar* (MHA) dengan empat jenis antibiotik, yaitu tetrasiklin (30µg), penisilin (10µg), gentamisin (10µg), dan oxasilin (10µg). Kekeruhan bakteri disesuaikan dengan standar *McFarland* No. 5 ($1,5 \times 10^8$ CFU/ml). Cakram antibiotik diletakkan pada permukaan media MHA yang telah diinokulasi dengan bakteri dan kemudian diinkubasi pada suhu 37°C selama 24 jam. Zona hambat yang terbentuk diukur dengan jangka sorong dan hasilnya diinterpretasikan sesuai dengan pedoman CLSI (2020). Hasil uji menunjukkan bahwa *E. coli* paling sensitif terhadap gentamisin (90%), diikuti tetrasiklin (60%), penisilin (30%), dan oxasilin (20%). Uji intermediat menunjukkan hasil pada tetrasiklin dan penisilin masing-masing sebesar 10%. Resistensi tertinggi ditemukan pada oxasilin (80%) dan penisilin (60%), sedangkan gentamisin menunjukkan resistensi terendah (10%). Data diuji statistik menggunakan ANOVA yang dilanjutkan dengan uji berganda Duncan untuk menentukan perbedaan antar kelompok. Hasil penelitian ini menunjukkan pentingnya pemantauan penggunaan antibiotik di peternakan ayam untuk mencegah perkembangan resistensi antibiotik yang lebih luas.

Kata Kunci : *Escherichia coli*; Kota Kupang; sensitivitas antibiotik

INTRODUCTION

According to Pelt et al. (2016), chicken meat is a popular animal-derived food in Indonesia. This is because chicken meat has a good nutritional value, a pleasant flavor, a soft texture, and is reasonably priced (Suradi, 2006). However, in order to produce hens with good body weight and quality,

farmers frequently utilize antibiotics extensively (Dewi et al., 2023a; Noor & Poeloengan, 2005a; Normaliska et al., 2019a). According to data, more than 70% of Indonesia's poultry farming sector employs antibiotics for prevention, disease therapy, and growth promotion (Dewi et al., 2023b; Noor

& Poeloengan, 2005b; Normaliska et al., 2019b; Widhi & Saputra, 2021b).

The unrestricted use of antibiotics in livestock husbandry frequently results in the development of resistance in both humans and animals. This is because both humans and animals absorb only around 80% of the antibiotics they consume, with the remainder released into the environment via urine and feces (Food and Agriculture Organization (FAO), 2018a). If this trend continues, antibiotic residues will accumulate, eventually causing antibiotic resistance in humans, animals, and the environment (Food and Agriculture Organization (FAO), 2018b; Widhi & Saputra, 2021b). Bacterial resistance to antibiotics is common in Enterobacteriaceae bacteria such as *Escherichia coli*. *E. coli* infections are one of the most commonly reported diseases and one of the leading causes of illness in commercial poultry farms (Pelt et al., 2016; Wibowo et al., 2011).

Research on antibiotic resistance in poultry meat has been extensively conducted. Anriani et al. (Widhi & Saputra, 2021b) reported that 7.14% of the meat contains tetracycline residues,

while ESBL-producing *E. coli* has a resistance of 71.4%. Pelt et al. (Pelt et al., 2016) discovered that 33 *E. coli* 0157 isolates are responsive to antibiotics such as tetracycline, ampicillin, cefoxitin, and ciprofloxacin. Furthermore, Marlina et al. (2015) discovered that macrolide antibiotic residues were present in 43.85% of chicken liver and meat. Ayudya et al. (Audya, 2023) discovered that 23.3% of chicken liver samples were contaminated with *E. coli*, and antibiotic resistance testing revealed that the *E. coli* isolates were resistant to ampicillin (100%), tetracycline (71%), and ciprofloxacin (14%).

Numerous research findings suggest that antibiotic resistance in chicken meat, liver, and other processed goods need urgent attention. Research about this resistance in East Nusa Tenggara (NTT) has been undertaken often; nevertheless, it has not yet been comprehensively integrated from agricultural producers to consumers. This research is essential as it examines chicken meat products and their processing, as well as the effects of antimicrobial resistance residues in environments frequented by humans.

MATERIALS AND METHODS

In this investigation, 30 *Escherichia coli* isolates were

isolated and identified from fresh chicken meat and liver that were

sold in traditional markets in Kupang City. Antibiotic susceptibility testing with Mueller-Hinton agar with tetracycline (30µg), penicillin (10µg), gentamicin (10µg), and oxacillin (10µg). The bacterial turbidity is calibrated to McFarland Standard No. 5 (1.5×10^8 CFU/ml). Inoculate 1 ml of *E. coli* bacteria onto MHA media with the spread method, then allow it to dry. Subsequently, antibiotic discs

are positioned over the MHA medium and incubated at 37°C for 24 hours. The inhibitory zone is measured in diameter (mm) with a caliper and interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2020). The outcomes of this procedure are denoted by a distinct area surrounding the paper disk, representing the zone of inhibition of bacterial proliferation.

RESULTS AND DISCUSSION

The data from the sensitivity test of *E. coli* against several antibiotics, interpreted using the Kirby-Bauer inhibition zone based on CLSI (2020), and the percentage of *E. coli* sensitivity test results against antibiotics categorized into three groups: sensitive, intermediate, and resistant (Table 1) (Figure 1) are presented. The most sensitive antibiotics to which *Escherichia coli* from this study responded were gentamicin (90%), tetracycline (60%), penicillin (30%), and oxacillin (20%). The intermediate test outcomes for tetracycline and penicillin were 10%. The resistance findings indicated oxacillin at 80%, penicillin at 60%, tetracycline at 30%, and gentamicin at 10% (Figure 2). Data were examined utilizing ANOVA, succeeded by Duncan's multiple range test (Table 2).

The Kirby on MHA medium is the gold standard for evaluating

the effectiveness of antibiotics against pathogenic bacteria such as *Escherichia coli*. The result of this test are interpreted based on the diameter of the inhibition zone formed around the antibiotic disc, referring to the *clinical and Laboratory Standards Institute* (CLSI, 2020) guidelines and the results of this antibiotic percentage can be seen in Figure 2.

Observations on the inhibition zone on MHA medium show figure 1 on the left and right there are black discs and blank disks and white are tetracycline, penicillin, gentamicin, and oxacillin antibiotics. The growth of *E.coli* bacteria around the clear zone, showing that the bacteria's sensitivity to the antibiotics is reflected in the expanding inhibition zone. Tetracycline, gentamicin, oxacillin, and penicillin exhibit antibacterial properties; however, gentamicin demonstrates

superior sensitivity relative to tetracycline. This signifies that *E. coli* bacteria exhibit more resistance to oxacillin and penicillin than to tetracycline and gentamicin. Furthermore, it suggests that a segment of the population is attributable to the existence of several antibiotic modes of action. According to the findings, bacteria acquire multidrug resistance via the mechanism of multidrug efflux

pumps, each capable of expelling multiple drug types (Li & Nikaido, 2009). Szmolka & Nagy (2013) assert that *E. coli* functions as a reservoir for resistance features, facilitating the dissemination of multidrug resistance genes to other bacteria. Studies indicate that the antibiotic gentamicin exhibits a comparatively low resistance level relative to three other antibiotics, resulting in its seldom use in poultry.

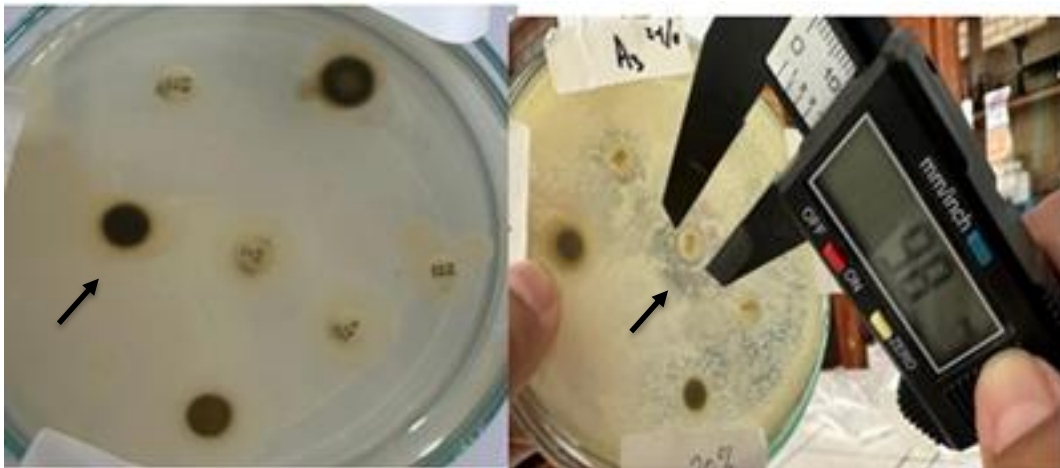


Figure 1. Diameter of the antibiotic inhibition zone against *E. coli*

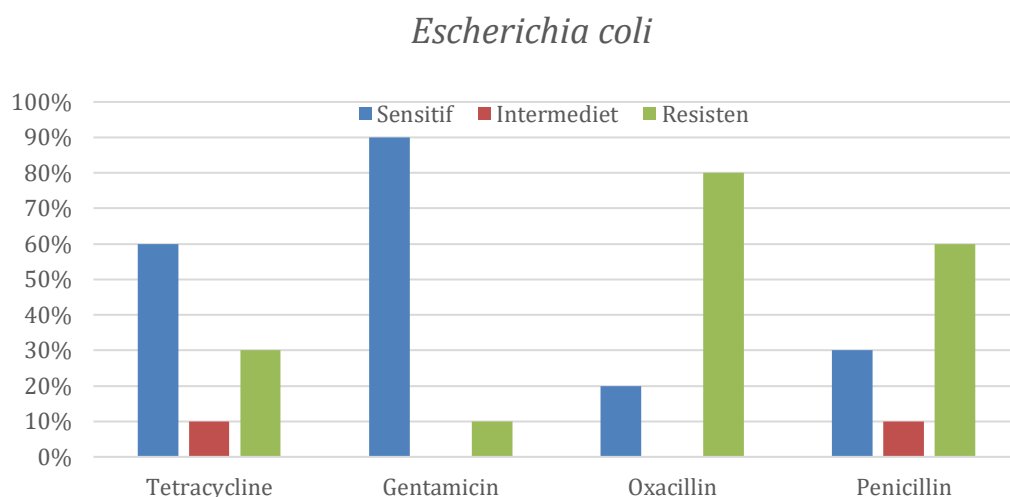


Figure 2. Percentage of sensitivity, intermediate, and resistant *Escherichia coli* to antibiotics

Table 1. *E. coli* antibiotic sensitivity test results

No	Bacteria	Antibiotics											
		Tetracycline			Gentamicin			Oxacillin			Penicillin		
		S	I	R	S	I	R	S	I	R	S	I	R
1	<i>E. coli</i>	40			33					5.7			6.1
2	<i>E. coli</i>	31			45					7.4			5.3
3	<i>E. coli</i>		12				10			6.3			9.8
4	<i>E. coli</i>	32			41					6.5			11
5	<i>E. coli</i>			10			12			7			12.6
6	<i>E. coli</i>	15.1			22					6.3			12
7	<i>E. coli</i>		12.9		20					7.3			8
8	<i>E. coli</i>			8.7	22					5.2			8
9	<i>E. coli</i>			3.4	26					8.3			8
10	<i>E. coli</i>		12		34					13			14
11	<i>E. coli</i>			10.1	41					7			10.1
12	<i>E. coli</i>	22.9			33			30					8.8
13	<i>E. coli</i>	20			25					6	20		
14	<i>E. coli</i>	51.6			31					10	47.1		
15	<i>E. coli</i>	32			35					8			10.2
16	<i>E. coli</i>	38.9			40.4					8.4	49.3		
17	<i>E. coli</i>			11	10.2					6.7			8.9
18	<i>E. coli</i>	36			30.1			18.3			31.6		
19	<i>E. coli</i>	23.9			31.8					8.2			8
20	<i>E. coli</i>	39.9			39.6			41.1			29.5		
21	<i>E. coli</i>	29			35.3			33			37		
22	<i>E. coli</i>	42.3			51					7			11
23	<i>E. coli</i>			6.9	43					12			10
24	<i>E. coli</i>			8	40					8			8
25	<i>E. coli</i>			7.3	31					8			9
26	<i>E. coli</i>			11	31					7.3			5.9

No	Bacteria	Antibiotics								
		Tetracycline			Gentamicin			Oxacillin		
		S	I	R	S	I	R	S	I	R
27	<i>E. coli</i>	30.5		31	38.3					8.4
28	<i>E. coli</i>	31.3		38.3	32.4			10		33.8
29	<i>E. coli</i>	30.3		32.4	33.9			8.3		53.3
30	<i>E. coli</i>	18.6		33.9	31.1			8.3		7.8

Description: *E. coli* isolate, Tetracycline (30 µg) (S: ≥15 mm, I: 12-14 mm, R: ≤ 11 mm), Gentamicin (10 µg) (S: ≥15 mm, I: 13-14 mm, R: ≤ 12 mm), Oxacillin (10 µg) (S: ≥18 mm, I: 15-17 mm; R: ≥ 14 mm), Penicillin (10 µg) (S: ≥17 mm, I: 14-16 mm; R ≤13 mm)

Table 2. Complete randomized design analysis with analysis of variance (ANOVA)

Types of Ab	Oeba		Naikoten		Oebobo	
	Daging	Hati	Daging	Hati	Daging	Hati
Tetracycline	25 ±0,5 aA	10±0,188bB	21±0,247bB	30±0,294aA	19±0,21bB	24 ±0,247bA
Gentamicin	28±0,402bA	25±0,471aB	33±0,388aB	30.42±0,298aA	40±0,45aA	33±0,340aB
Oxacillin	6±0,086cA	8±0,150cA	12±0,141dB	16.54±0,162cA	13±0,149dA	14.08±0,145cA
Penicillin	10±0,144dA	10±0,188cA	19±0,223cB	25.46±0,249bA	15±0,172cB	24.76±0,255bA

Description: Different superscripts in the same row indicate significant differences (P<0.05);
Different superscripts in the same column indicate significant differences (P<0.05)

There are growing apprehensions regarding the rise in antibiotic resistance in Indonesia, which is attributed to the improper use of antibiotics in poultry farms in terms of dosage, need, and treatment standards. The research conducted by Suandy (2011) revealed that the resistance of *Escherichia coli* in broiler chicken meat at traditional markets in Bogor was 97.3%, suggesting a very high level of bacterial resistance to antibiotics at the time. Diseases brought on by resistant bacteria provide health issues for both people and animals, including higher treatment expenses, fewer therapeutic alternatives for animals, longer hospital stays, and even mortality (Masrurroh 2016). Bacteria acquire multidrug resistance via multidrug efflux pumps, which can expel many drug types (Li & Nikaido, 2009).

The resistance findings for *Escherichia coli* indicate that oxacillin exhibits the highest resistance at 80%, followed by tetracycline at 60%. These findings align with Rahmانيar's (2021) research, which indicated that *Escherichia coli* exhibited an 85% resistance rate to the antibiotic tetracycline at 30 µg (17 out of 20 samples). Mgaya et al. (2021) report that the maximum resistance of *E. coli* to the antibiotic tetracycline is 91.9% in cloacal and broiler meat samples. Dita and Kholik (2023) reported varying results, indicating

that chicken meat exhibited resistance to penicillin at 80%, tetracycline at 40%, and gentamicin at 20%.

Alterations in the permeability of the microbial cell membrane result in tetracycline resistance. The drug cannot be actively internalized by the cell or is rapidly expelled in resistant cells. In sensitive cells, the medication will not exit the cell and will persist in the environment (Meles et al., 2011). Ribosomal alterations and the efflux of diverse medicines provide a mechanism of resistance to tetracycline and macrolides (Sen & Sarkar, 2018).

The diameter of the inhibition zone was insufficient to significantly impede the growth of *E. coli*, resulting in 25 of the 30 *E. coli* bacterial isolates exhibiting the highest resistance to oxacillin (80%). The zone of inhibition diameter of 12 mm was observed in four samples, which corresponds to the CLSI 2020 standard (Weinstein, 2019) of 12-14 mm. Consequently, this indicates intermediate resistance. Resistance in the inhibitory zone is attributed to the frequent use of antibiotics for therapy, as indicated by the measurement findings. Toelle's research (2014) demonstrates that the emergence of bacterial resistance to various antibiotics results from the misuse of these medications and the identification

of pathogenic agents, culminating in multi-drug resistance.

The sensitivity results for *Escherichia coli* indicate that the bacteria exhibit a 90% sensitivity to gentamicin. The application of this antibiotic in chicken farms is not widespread, so it remains effective against the bacterium. This signifies that gentamicin remains highly efficacious in addressing infections induced by *E. coli* in broiler chickens. Gentamicin functions by obstructing bacterial protein synthesis, and owing to its potent mechanism of action, resistance to this antibiotic remains comparatively low.

The effectiveness of four varieties of antibiotics against *E. coli* isolated from the meat and liver of broiler chickens in three locations (Oeba, Naikoten, Oebobo) is significantly different ($p < 0.05$) as indicated results in Table 2. Gentamicin exhibited the highest sensitivity, as evidenced by the largest inhibition zone (25–40 mm), particularly in the meat of Oebobo (40 ± 0.45 mm aA). These findings are consistent with the research conducted by Elfisa *et al.*, (2023), which asserts that gentamicin is a broad-spectrum antibiotic that is highly effective against Gram-negative bacteria, such as *E. coli*.

The efficacy of tetracycline varies significantly across different locations. It is effective in the liver of Naikoten (30 ± 0.294 mm aA), but it is less effective in the liver of Oeba (10 ± 0.188 mm bB). This may be attributable to the variations in the prevalence of the tet resistance gene at each location (Stokes *et al.*, 2019). Oxacillin and Penicillin exhibited the lowest inhibition zones (6–25.46 mm), which is in accordance with the CLSI (2020) report that *E. coli* is prone to resistance to β -lactam antibiotics as a result of β -lactamase production.

In general, meat is more responsive to gentamicin (e.g., 40 ± 0.45 mm aA in Oebobo), while the liver in Naikoten exhibits a higher sensitivity to tetracycline (30 ± 0.294 mm aA). The influence of sample type (Meat vs. Liver) is shown. This discrepancy may be associated with the metabolism of antibiotics and the fat content of hepatic tissue (Elfisa *et al.*, 2023). Significant differences between meat and liver are confirmed by the use of different uppercase letters (A vs. B) in tetracycline Oeba (aA vs. bB), which are likely the result of the accumulation of distinct antibiotic residues in both tissues (Bacanli & Bařaran, 2019).

CONCLUSION

A majority of *E. coli* bacteria extracted from broiler chicken

flesh and liver exhibit sensitivity to gentamicin (90%), tetracycline

(60%), penicillin (30%), and oxacillin (20%). They exhibit resistance to oxacillin at 80%,

penicillin at 60%, tetracycline at 30%, and gentamicin at 10%.

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