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Antibiotic Resistance Patterns of *Escherichia coli* Isolates in Broiler Chickens at Slaughterhouse and Retails

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ABSTRACT

This study aims to identify the antibiotic resistance pattern of *Escherichia coli* in the broiler food chain at poultry slaughterhouses and outlets. The sampling method used purposive sampling. Each of the 30 cecum and carcass samples was obtained from a Poultry Slaughterhouse, and 40 samples from 1 retail (consisting of 5 outlets), which received supplies from the same Poultry Slaughterhouse, were isolated and identified as *E. coli*. The results showed that 57 isolates were positively identified as *E. coli*, and the sensitivity was tested for the Antibiotic Susceptibility Test using the disc diffusion method. Statistical analysis using the Chi-Square method and Fisher's Exact Test (p-value=0.01) showed a pattern of sensitivity of *Ciprofloxacin* (p=0.89), *Colistin* (p=0.07), *Sulfamethoxazole* (p=1.00), *Chloramphenicol* (p=0.67) and *Meropenem* (p= <0.001). The highest resistance was found in frozen carcass isolates at the outlet to *Colistin* (69%), and *E. coli* isolates from cecum at the poultry slaughterhouse showed resistance to *Meropenem* (67%). Furthermore, it was discovered that 35% of isolates were only resistant to one type of antibiotic. Resistance bacteria may spread and threaten consumers' health while they get carried from farm to retail.

Keywords: Antimicrobial resistance, Antimicrobial resistance pattern, Broiler chicken, *Escherichia coli*

INTRODUCTION

Bacterial antibiotic resistance poses risks not only to human health but also to animals, plants, and the environment. Resistance is the primary cause of bacterial adaptability to aeons of antibiotic exposure (Spellberg et al., 2013). The problem of antimicrobial resistance (AMR) is a global health threat. Antimicrobial resistance (AMR) happens when microorganisms like bacteria, viruses, and fungi become immune to medicines used to kill them. Drug resistance increases the risk of disease transmission, serious illness, disability, and death by making antibiotics and other antimicrobial medications ineffective, which makes it harder or impossible to treat infections. (World Health Organization, 2023).

Antibiotics are widely used for treatment in both humans and animals. Nevertheless, improper use of antibiotics can lead to dangerous bacteria developing resistance to specific antibiotics, endangering the health of humans and animals. Antimicrobial resistance genes can be passed from farm animals to humans, resulting in bacterial antibiotic resistance (Kusumaningsih and Sudarwanto, 2011). Antimicrobial resistance is a natural process that happens over time, but it is being sped up by how we use antibiotics and other medicines. People are using too many of these medicines and are not always using them correctly, which helps resistant bacteria spread.

Microorganisms are widespread and can be found in food, water, soil, air, and the human body. These microorganisms require isolation from the environment and another microorganism to be observed. This can be done with isolation techniques. A variety of methods are often used in microbial isolation. The techniques employed are additionally adapted to the kind of microorganisms that will be observed. (Jufri, 2020).

The principle of microbial isolation is to separate a specific type of microbe from other microbes that originated from a mixture of different bacteria. Microbial isolation aims to cultivate microorganisms that are away from their native habitat. Separating microorganisms away from the environment, called pure cultures, are bacterial cultures that no longer mix with other bacteria. This can be performed by cultivating it on solid media, where the microbial cells will establish a colony that remains. (Lestari and Hartati, 2017).

The use of antimicrobials in the livestock sector has increased in the past few years.

Antimicrobials are used in animals for various purposes, such as treatment, prevention, disease Antimicrobials are used in animals for various purposes, such as treatment, prevention, disease control, growth promotion, and feed efficiency. (Marshall and Levy, 2011). Antimicrobials used in animals reared for food production are estimated to account for 73% of all antimicrobial usage worldwide, with an increase of 11.5% projected in animal use overall by 2030, mainly in Asia. Indonesia is the fourth most populated country in the world. The Indonesian broiler sector accounts for 87% of the consumed meat, and empirical studies indicate that the broiler industry accounts for around 60% of the antimicrobial use in livestock. (Sani et al., 2023).

The connection between antibiotic-resistant bacteria and livestock farming with AMR is a significant threat since the antibiotic classes applied to veterinary and human treatment are the same (Magnusson et al., 2021). Either through the food chain or animal excrement, pathogenic bacteria in animal intestinal tracts can spread to humans. (Kousar et al., 2021). The antibiotic resistance pattern shown by *Escherichia coli* (*E. coli*) bacteria in the food chain can be ascertained by obtaining information on antimicrobial resistance in poultry meat from slaughterhouses and retail outlets.

Chicken meat is frequently contaminated by microorganisms such as *Escherichia coli* (Bhunia, 2008). To explain the possible spread of contamination along the food chain from the slaughterhouses to the outlets, it is necessary to consider the possibility that the E. coli bacteria could contaminate chicken meat. This suggests that the source farms of broiler chickens may also experience resistance.

MATERIALS AND METHODS

Using target *E. coli* bacterial isolates, this study intends to determine the resistance pattern in the broiler food chain from farms to retail outlets. Bacterial isolation, identification, and identification tests and antibiotic sensitivity testing were conducted at the Bogor Veterinary Research Center (BBLitvet). The antibiotics used were *Ciprofloxacin, Colistin, Meropenem, Sulfamethoxazole, and Chloramphenicol.* The selection of antibiotics is based on considering the priority classification of antibiotics that are very important for humans and animals. (Food and Agriculture Organization of the United Nations, 2019; World Health Organization, 2019), antibiotics that are very important for the production of animals (World Organisation for Animal Health, 2019), antibiotics that are not permitted for use in animal husbandry (Indonesian Ministry of Agriculture, 2017), and the availability of antibiotic types in the testing laboratory.

Sampling Method and Data Analysis

The method used for the sampling was purposive sampling. A total of 100 samples were collected: 30 samples from carcasses, 30 samples from fresh broiler cecum at slaughterhouses in Bogor Regency, and 40 samples from frozen broiler carcasses from 5 locations of the same retail company that sourced its supplies from the slaughterhouse that had been sampled beforehand. In this study, a cross-sectional statistical research design was used. Chi-Square and Fisher's exact test were used in the statistical analysis, with a p-value of 0.01. The antibiotic resistance patterns were examined based on bacteria resistant to several antibiotic classes.

Antimicrobial Susceptibility Test

Isolation and identification of *E. coli* bacteria from all samples was carried out using the indole test with Sulfide Indole Motility (SIM) media. Isolates confirmed as *E. coli* were then subjected to Antimicrobial susceptibility testing using the Kirby-Bauer disk diffusion methods on Mueller-Hinton Agar (MHA) media, following guidelines provided by the Clinical and Laboratory Standards Institute (CLSI).

RESULTS AND DISCUSSION

Isolation and Identification of *E. coli*

Given that the majority of asymptomatic farm animals have commensal *E. coli* bacteria in their guts, these bacteria are frequently used as an indicator of the degree of resistance in Gram-negative bacteria. Conjugative plasmids containing resistance genes are also commonly acquired by *E. coli*, resulting in the dissemination of resistance to additional intestinal bacteria. (Cheney et al., 2015). Out of 100 samples, 57 isolates were identified as positive for *E. coli*. In the samples from the slaughterhouse, 21 positive isolates were identified as *E. coli* from chicken cecum samples and 20 isolates from fresh chicken carcass samples. Of the 40 frozen chicken carcass samples from retail, 16 isolates were positively identified as *E. coli*. The results of identifying *E. coli* bacteria isolates that tested positive are shown in Table 1.

Table 1. *E. coli* Isolation and Identification

No.	Business Unit	Sample Type	Number of Samples	Percentage of *E. coli* Positive Isolates (%)
1.	Slaughterhouse	Cecum	30	21 (70%)
2.	Slaughterhouse	Fresh carcass	30	20 (67%)
3.	Retail 1 (5 Outlets)	Frozen carcass	40	16 (40%)
Total			100	57 (57%)

Antibiotic resistance of *E. coli* Isolates

Based on testing through five different antibiotic classes, it was revealed that frozen carcass isolates from outlets had the highest resistance against *Colistin* (69%). In slaughterhouses, *E. coli* isolates from chicken cecum showed high resistance to *Meropenem* (67%) and good sensitivity to *Chloramphenicol* (95%). *E. coli* isolates from fresh chicken carcass samples at the slaughterhouse showed strong resistance to *Colistin* (60%) and good sensitivity to *Meropenem* (100%). On the other hand, all of the *E. coli* isolates from frozen chicken carcass samples obtained from retail outlets had no resistance to *Meropenem* (100%). In contrast, most isolates had a significant resistance level to *Colistin* (69%).

Based on the results of statistical analysis using Chi-Square and Fisher's Exact Test, the sensitivity pattern of *E. coli* bacteria was phenotypically found to have similar resistance patterns to four types of antibiotics, namely *Ciprofloxacin* (p = 0.89), *Colistin* (p = 0.07), *Sulfamethoxazole* (p = 1.00), and *Chloramphenicol* (p = 0.67). However, there was a significant difference in the resistance pattern of *E. coli* bacteria to the *Meropenem* antibiotic (p = 0.001), as seen in Table 2. These results indicate that there is an association between the use of *Meropenem* antibiotics and resistance patterns in *E. coli* bacteria isolated from chicken cecum in slaughterhouses with chicken carcasses in slaughterhouses and chicken carcasses in retail outlets. *Meropenem* is an antibiotic used for human treatment; thus, it is not listed in the Indonesian Veterinary Drug Index. (Indonesian Ministry of Agriculture, 2023) Because it is not commonly used for veterinary medicine or on farms.

Table 2. *E. coli* Resistance to 5 Antibiotics

Type of Antibiotics	Sensitive		Intermediate		Resistant		*p-value*
	n	%	n	%	n	%	
Ciprofloxacin (CIP)	33	58	12	21	12	21	
E. coli Isolate in cecum from Slaughterhouse	11	52	5	24	5	24	0,89
E. coli Isolate in fresh carcass from Slaughterhouse	11	55	5	25	4	20	
E. coli Isolate in frozen carcass from Outlet	11	69	2	12	3	19	
Colistin (COL)	0	0	27	47	30	53	
E. coli Isolate in cecum from Slaughterhouse	0	0	14	67	7	33	0,07
E. coli Isolate in fresh carcass from Slaughterhouse	0	0	8	40	12	60	
E. coli Isolate in frozen carcass from Outlet	0	0	5	31	11	69	
Meropenem (MEM)	43	75	0	0	14	25	
E. coli Isolate in cecum from Slaughterhouse	7	33	0	0	14	67	<0,001*
E. coli Isolate in fresh carcass from Slaughterhouse	20	100	0	0	0	0	
E. coli Isolate in frozen carcass from Outlet	16	100	0	0	0	0	
Sulfamethoxazole (SMX)	31	54	0	0	26	45	
E. coli Isolate in cecum from Slaughterhouse	11	52	0	0	10	48	1,00
E. coli Isolate in fresh carcass from Slaughterhouse	11	55	0	0	9	45	
E. coli Isolate in frozen carcass from Outlet	9	56	0	0	7	44	
Chloramphenicol (CHL)	52	96	1	2	1	2	
E. coli Isolate in cecum from Slaughterhouse	20	95	0	0	1	5	0,67
E. coli Isolate in fresh carcass from Slaughterhouse	18	90	0	0	2	10	
E. coli Isolate in frozen carcass from Outlet	14	88	1	6	1	6	

Note: * Significant at p-value <0.01 (Chi-square method and Fisher's Exact Test)

This resistance may occur due to the cross-resistance, co-resistance, or co-selection of one or more resistant bacteria to antibiotics in one or more classes (Murray et al., 2024). In addition, *Colistin* has also been banned from circulation and use in animals because it is an essential antibiotic and a last resort for human treatment.

Resistance can also occur due to the transfer of resistant material from the environment outside the cage that enters the cage environment. Since the environment is a crucial source of resistant bacteria from antibiotics in humans and animals, the environment surrounding the cage may contain various substances resistant to being passed between bacteria. (Niasono et al., 2019). These findings indicate the existence of contaminating bacteria that are transported from the farm to the food production chain, which causes antibiotic resistance.

Antibiotic Resistance Pattern of *E. coli* Bacteria

Out of the 57 *E. coli* isolates that tested positive (refer to Table 3), the majority (35%) were found to be resistant to one type of antibiotic, with the most significant percentage occurring in the slaughterhouse unit (fresh carcass) at 14%. In addition, thirty per cent of the isolates were resistant to two different antibiotic classes; the highest percentage of these isolates were discovered in the cecum samples and fresh carcass samples from slaughterhouses (11% each). The cecum samples from the slaughterhouse had the highest percentage of isolates resistant to three antibiotics (11%), whereas the fresh carcass samples and retail samples had the highest percentage of isolates resistant to four antibiotics (2% each). Additionally, isolates that showed no resistance were predominantly obtained at 7% each in the slaughterhouse (fresh carcasses) and retail (frozen carcasses) units.

Table 3. Resistance Pattern of *E. coli* to Specific Antibiotics

Antibiotic Pattern of Resistance	**Slaughterhouse (Cecum)**		**Slaughterhouse (Fresh Carcass)**		**Retail (Fresh Carcass)**		**Total**	
	n	**%**	**n**	**%**	**n**	**%**	n	%
Non-resistant	2	4	4	7	4	7	10	18
Resistant to 1 antibiotic	7	12	8	14	5	9	20	35
Resistant to 2 antibiotics	6	11	6	11	5	9	17	30
Resistant to 3 antibiotics	6	11	1	2	1	2	8	14
Resistant to 4 antibiotics	0	0	1	2	1	2	2	4
Resistant to 5 antibiotics	0	0	0	0	0	0	0	0
Total	21	37	20	35	16	28	57	100

Large-scale antibiotic use and inadequate dosages can lead to the growth of pathogenic bacteria resistant to multiple antibiotics, which might render livestock disease cases unresponsive to treatment. It is typical for bacteria to simultaneously acquire various resistance mechanisms to distinct classes of antibiotics because a single resistance mechanism does not ensure the survival of the bacterium (Jeżak and Kozajda, 2022; Nurjanah et al., 2020). The regular and continuous use of low-dose antibiotics in animal feed for the purpose of preventing disease may cause the natural balance of microflora to be upset, leading to a decrease in sensitive bacterial groups and an increase in resistant bacterial groups (Normaliska et al., 2019).

Bacteria that are frequently exposed to antibiotics develop a resistance against them because they are able to protect themselves against the effects of the drugs by developing cell membranes that block the entry of antibiotics into bacterial cells. The use of uncontrolled antibacterials will lead to changes like resistance in these bacteria, such as incorrect medicine selection, duration of injection, and dose. This will lead to failure in the livestock treatment process. (Besung et al., 2019).

Bacteria that resist three or more different classes of antimicrobials are called multidrug-resistant (MDR) bacteria. It's also important to keep a watch out for the possible spread of gene changes in MDR bacteria (Brooks et al., 2007). The significant pattern of resistance bacterial spread concentrated in E. coli and multidrug-resistant E. coli can lead to antibiotic-resistant bacteria in the surrounding animals and humans, posing a risk for higher disease disorders and a wider spread. (Kallau et al., 2019).

The research results support the idea that the *E. coli* isolated from broiler chicken samples shows a pattern of simultaneous resistance to multiple antibiotics.

Potential Risks of Resistant Bacteria to the Environment

Various factors, such as user behaviour and antibiotic accessibility, can influence this possibility of risk. Furthermore, cross-resistance between naturally occurring *E. coli* strains is a possibility, particularly between strains of *E. coli* that carry resistance traits against susceptible strains. *E. coli* can conjugate between strains naturally to exchange some of its adaptive traits (Sasongko, 2014). The use of antimicrobials in livestock and companion animals, as well as farm management and veterinary medicine, contributes to the spread of antimicrobial resistance in animal reservoirs and the environment, which may increase the resistance of human colonising bacteria to antimicrobials (Nurjanah et al., 2020).

These factors fall into two categories: microbes carried by former cage occupants due to improper cage cleaning methods and cross-contamination or cross-infection of animals in the cage simultaneously. Antimicrobial resistance pathogens may be released during the animal's stay at the shelter if the animal develops new diseases or if pre-existing infections reappear in carrier animals. (EFSA BIOHAZ Panel et al., 2022).

Potential Risks of Resistant Bacteria to Consumers

Both humans and animals are at risk from resistant microorganisms. (Indonesia Ministry of Health, 2017). Antibiotic resistance in pathogenic bacteria that may infect people is one of the implications of the improper overuse of antibiotics in poultry farming. (Nurjanah et al., 2020). The transmission of resistance in farm animals and animal-based food products can occur directly through direct contact and indirectly through the food chain, water, air, soil, fertilisers, and mud. It is possible for consumers to come into direct touch with resistant bacteria or to consume animal products with a longer and more complicated transmission path. There is some evidence that a significant amount of resistant bacteria and their resistance genes are present in foods derived from different animal sources, regardless of the level of processing. (Marshall and Levy, 2011).

It is well known that *E. coli* is a pathogenic bacteria that can harm human health. Enterotoxigenic *E. coli* is a type of bacteria that causes diarrhoea. Enterohemorrhagic *E. coli* 0157:H7 contaminate food and are also known to be pathogenic bacteria that cause foodborne diseases (Rorong and Wilar, 2020). Every interaction throughout the animal-origin food chain, including farms, slaughterhouses, retail establishments, and markets, can transmit resistant bacteria from livestock to humans. Therefore, it is up to consumers to take precautions against resistant bacteria contaminating food that will be consumed by preparing food hygienically and cooking it perfectly.

Products made from poultry in slaughterhouses may get contaminated by unclean equipment and unhygienic workers (slaughterers) (Novianti et al., 2021). Multidrug-resistant *E. coli* bacteria from the intestines can contaminate other poultry and chicken products in butcheries or slaughterhouses. If hygiene standards are unmet, poultry products, including chicken meat, packed in plastic can quickly become contaminated with harmful pathogenic bacteria and *E. coli.* (Akond et al., 2009). Therefore, improving hygiene and sanitation in the retail and slaughterhouse areas is also necessary.

Patients who are resistant to antibiotics may become more challenging to treat, which could lead to higher treatment expenses, more prolonged hospital admissions, and a rise in mortality rates. (Indonesia Ministry of Health, 2017).

CONCLUSION

The study's findings on bacterial resistance to five different antibiotic classes indicate the potential for resistant bacteria to migrate up the food chain from farms and poultry slaughterhouses to retail outlets. Out of 100 samples, 57 isolates were identified as positive for *E. coli*. The sensitivity pattern of *E. coli* bacteria to five types of antibiotics showed no significant difference in *Ciprofloxacin* ($p = 0.89$), *Colistin* ($p = 0.07$), *Sulfamethoxazole* ($p = 0.1$), or *Chloramphenicol* ($p = 0.67$). While in *Meropenem* ($p = 0.001$), there is a significant difference in the resistance pattern of *E. coli* bacteria. Bacteria isolated from slaughterhouses and retail outlets showed similar phenotypic antibiotic resistance patterns. Furthermore, it was discovered that 35% of isolates were only resistant to one type of antibiotic.

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