

The Detection of Resistant *Escherichia coli* Isolated From Cats In Dukuh Kupang Sub-District, Surabaya

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ABSTRAK

Resistensi antimikrob merupakan isu global yang menjadi pusat perhatian dunia. Penularan pada manusia melalui hewan peliharaan penting untuk diwaspadai. Penelitian ini dilakukan untuk mengetahui adanya resistensi antimikrob bakteri *Escherichia coli* pada kucing. Sebanyak 60 sampel swab diambil, yang terdiri atas 30 sampel kucing liar dan 30 sampel kucing peliharaan. Sampel diuji di Laboratorium Kesmavet Fakultas Kedokteran Hewan Universitas Wijaya Kusuma Surabaya. Sampel kemudian di isolasi dan identifikasi dengan media selektif diferensial *Eosin Methylene Blue Agar*. Isolat *Escherichia coli* kemudian dilakukan uji sensitivitas untuk mengetahui adanya resistensi pada antibiotik ampicillin, tetracycline, dan streptomycin. Hasil penelitian menunjukkan 83% sampel terdapat bakteri *Escherichia coli* pada kucing, dengan resistensi antibiotik sebesar 16% terhadap antibiotika ampisilin, pada kucing liar 19%, dan kucing peliharaan 12%. Resistensi pada antibiotik tetrasiklin menunjukkan 12%, dimana kucing liar 15%, dan kucing peliharaan 8%. Sedangkan resistensi pada antibiotik streptomisin menunjukkan 4% (2/50), dengan resistensi pada kucing liar 8% dan pada kucing peliharaan tidak ditemukan adanya resistensi (0%). Kucing sebagai hewan yang memiliki kedekatan dengan kehidupan manusia, mampu menjadi faktor penular resistensi antimikroba, hal ini menjadi kewaspadaan dini sebagai tindakan pencegahan penularan resistensi antimikrobia pada manusia.

Kata Kunci: *Escherichia coli*, kucing, *multidrug resistance*, resistensi antimikrob

ABSTRACT

Antimicrobial resistance is a global issue that has become the center of world attention. Transmission to humans through pets is important to be aware of. This research was conducted to determine the presence of antimicrobial resistance in *Escherichia coli* bacteria in cats. A total of 60 swab samples were taken, consisting of 30 stray cats samples and 30 domestic cats samples. Samples were tested at the Veterinary Health Laboratory, Faculty of Veterinary Medicine, Wijaya Kusuma University, Surabaya. The samples were then isolated and identified using differential selective media *Eosin Methylene Blue Agar*. *Escherichia coli* isolates were then subjected to a sensitivity test to determine resistance to the antibiotics ampicillin, tetracycline and streptomycin. The results showed that 83% of the samples contained *Escherichia coli* bacteria in cats, with 16% antibiotic resistance to the antibiotic ampicillin, 19% in stray cats, and 12% in domestic cats. Resistance to tetracycline antibiotics shows 12%, where stray cats are 15%, and domestic cats are 8%. Meanwhile resistance to the antibiotic streptomycin showed 4% (2/50), with resistance in stray cats 8% and in domestic cats no resistance was found (0%). Cats, as animals that have a close relationship with human life, can be a factor in transmitting antimicrobial resistance, this is an early precaution as a measure to prevent the transmission of antimicrobial resistance to humans.

Keywords: antimicrobial resistance, cat, *Escherichia coli*, *multidrug resistance*

INTRODUCTION

The bacterium *Escherichia coli*, also referred to as *E. coli*, resides in the lower intestine of a typical, healthy cat. The majority of warm-blooded mammals, including humans, also have it. In addition to the gastrointestinal tract, *E. Coli* can also infect cats and cause illness (Bourély *et al.*, 2020; Don Bamunusinghage *et al.*, 2019). The name "colibacillosis" refers to the illness that *E. coli* produces in cats, and it frequently results in a bloodstream infection that can cause sepsis or septicemia, which is a dangerous condition. Through the bacteria they excrete in their feces, cats transmit *E. coli*. If someone eats or licks their fingers after coming into contact with cat excrement on their skin and fails to properly wash their hands, they may unintentionally contract this bacterial infection. Environmental contamination with antibiotic-resistant bacteria appears to contribute significantly to the transmission of antibiotic-resistant *E. coli* in humans. Animals can carry *E. coli* without exhibiting any symptoms; they do not necessarily need to be sick to transmit the infection (Gargano *et al.*, 2022; Osman *et al.*, 2021).

Stray cats are different from domestic cats. Stray cats are generally used to eating food that comes from waste and drinking from stagnant water. Bacterial infections that cause disease in cats can originate from stagnant water and waste (Ngitung, 2021). According to Zikra *et al.*, (2018), microbiologically, the quality of drinking water is poor. This is due to bacterial contamination in the water. Stray cats can be a reservoir for the emergence of resistant bacteria that can be transferred through food and the environment (Agustin & Ningtyas, 2022). Bacteria that are resistant to antibiotics can migrate in the environment through animal feces. The environment will be polluted even though it is far from the pollutant source (Doosti *et al.*, 2015). Apart from insects and parasites, rodents such as mice and rats are the most likely source of transmission of antimicrobial resistance. Rodents have resistant microorganisms; transmission to humans is possible through various routes, such as direct interaction or through the food chain, where rodents can come into direct contact with food and surfaces (Gwenzi *et al.*, 2021).

In domestic cats, exposure to antibiotics can come from medical therapy when the cat is sick. The treatment that is still the main choice for treating infections caused by *E. coli* bacteria is the use of antibiotics. The use of antibiotics in the animal sector reaches 80%. The use of antibiotics in several animals has been reported to experience a decrease in effectiveness, including in cats. The continuous use of antibiotics for a long time can lead to antibiotic

resistance (Awosile *et al.*, 2018). Resistance to three or more different antibiotic classes can be expressed as multidrug resistance. *E. coli* is a commensal bacterium that is multidrug resistant, which causes health problems because *E. coli* can transfer resistant genes to other pathogenic bacteria in the digestive tract (Masruroh *et al.*, 2016). Many cases of antimicrobial resistance have been reported in humans, animals or the environment. This research is important to do to determine the existence of resistant *E. coli* in cats, which can be transmitted to humans.

MATERIAL AND METHODS

Ethical approval was approved by the Experimental Animal Ethics Committee Team, Faculty of Veterinary Medicine, Wijaya Kusuma Surabaya University, no 123-KKE. The research was conducted from January to February 2023. This study uses 60 cat anal swab samples to diagnose antimicrobial resistance. Domestic cats are taken from owned cats in Dukuh Kupang sub-district and stray cats are taken from cats that come from the street or around traditional markets in Dukuh Kupang sub-district. Samples were isolated and identified with differential selective Eosin Methylene Blue Agar media. Isolates identified as *E. coli* were then subjected to Gram staining tests, biochemical tests, and sensitivity tests to determine the presence of antimicrobial resistance. The data were then analyzed descriptively to determine the incidence of antimicrobial resistance of *Escherichia coli* isolates to the antibiotic ampicillin 10µg (Oxoid™ catalog number: CT0003B), tetracycline 30µg (Oxoid™ catalog Number: CT0054B), and streptomycin 10µg (Oxoid™ catalog number: CT0047B). This antibiotic is an antibiotic that is often used in the fields of human and animal health, so it has quite high potential in the environment (CLSI, 2022; Effendi *et al.*, 2021; Witaningrum *et al.*, 2020).

RESULTS

The results of the isolation and identification of *E. coli* (Figure-1) in cats in Dukuh Kupang, Surabaya (Table 1) as a whole showed 83% (50/60) positive, domestic cats had 80% (24/30) positive samples, and stray cats, as much as 87% (26/30), were positive isolates with *E. coli* characteristics. Macroscopically, the isolated colonies are convex-spherical in shape. Colonies identified as positive for *E. coli* (Figure-1), grown on Eosin Methylene Blue Agar (EMBA) media, had a distinctive color, namely metallic green.

The results of the sensitivity test (Figure-1) showed a clear zone or an inhibition zone formed around

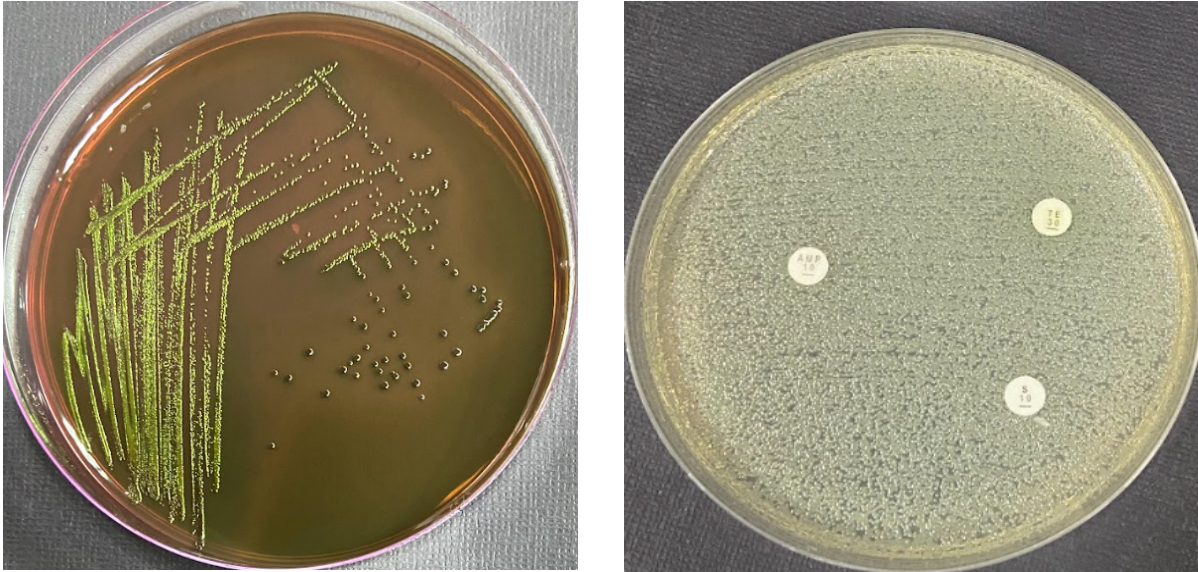


Figure-1. *E. coli* on EMBA (a); Multidrug resistance of *E. coli* (b)

the antibiotic disk. The results of the sensitivity test were based on measuring the diameter of the inhibition zone formed on Mueller Hinton Agar (MHA) media, then compared the level of sensitivity with the standard diameter of the inhibition zone in the *Enterobacteriaceae* group in the 2020 Clinical and Laboratory Standards Institute literature. An inhibition zone that does not form is an indication of no inhibition of bacterial growth by antibiotics or resistance. Based on the test results, it was found that the isolates experienced antimicrobial resistance (Table 1) to the antibiotics ampicillin by 16%, tetracycline by 12%, and streptomycin by 4%.

The prevalence of *E. coli* resistance to ampicillin in stray cats (19%) was higher than in domestic cats (12%), resistance to tetracycline antibiotics was more elevated in stray cats (15%) than in domestic cats (8%). In contrast, in streptomycin antibiotics the incidence was in stray cats by 8% and no resistance was found in domestic cats. The overall prevalence of resistance in feral cats is higher than in domestic cats. This could be due to exposure to high-resistance antimicrobial transmission from the environment.

The most common pattern of resistance (Table 2) found was ampicillin and tetracycline resistance pattern of 8%, while the resistance pattern of ampicillin – tetracycline – streptomycin was 2% (1/5). The resistance pattern of these three antibiotic classes is multidrug. The pattern of resistance between ampicillin – streptomycin and the pattern of resistance between tetracycline – streptomycin was not found in *E. coli* isolates from cat anal swabs in Dukuh Kupang, Surabaya. The single pattern of antibiotic resistance to ampicillin was 6% (3/50), while the single pattern

of resistance to tetracycline and streptomycin was 2% (1/50), respectively.

DISCUSSION

The result showed that 83% (50/60) of the samples were positive for *E. coli*. This can happen because most of them are in the intestinal tract of animals as normal flora (Conway & Cohen, 2015; Dewandaru *et al.*, 2019; Kittana *et al.*, 2018). *E. coli* can be pathogens in cats. According to Dewandaru *et al.*, (2019) pathogenicity of *E. coli* can be influenced by predisposing factors, including feed, environment, and the intensity of the pathogenic strain. Pathogenic strains that cause diarrhea are diarrheagenic *E. coli* (DEC) strains carrying plasmids that can produce toxins and facilitate the attachment of bacteria to the surface of intestinal cells in the host (Blum *et al.*, 2018; O'Reilly *et al.*, 2017). Cats can transmit *E. coli* bacteria to humans and vice versa. This is supported by research conducted by Busch *et al.*, (2007) that cats can be reservoirs and carriers of enterohemorrhagic *E. coli* (EHEC) in humans even though these cats show no symptoms similar to what was stated by Davies *et al.*, (2019), Pathogenic *E. coli* strains can be transmitted between cats and their owners.

This study was found that the percentage of *E. coli* was greater in stray cats, namely 87% (26/30). In comparison, it was 80% (24/30) in domestic cats. The percentage of isolates in stray cats identified as *E. coli* was greater than in domestic cats, this could happen because stray cats are used to eating food that comes from waste and drinking from stagnant water.

Table 1. Sensitivity test *Escherichia coli* isolates in cat anus swabs

	Sample	<i>Escherichia coli</i>	Sensitivity test								
			Ampicillin			Tetracycline			Streptomycin		
			S	I	R	S	I	R	S	I	R
Domestic cats	30	80% (24/30)	88% (21/24)	0% (0/24)	12% (3/24)	92% (22/24)	0% (0/24)	8% (2/24)	92% (22/24)	8% (2/24)	0% (0/24)
Stray cats	30	87% (26/30)	77% (20/26)	4% (1/26)	19% (5/26)	73% (19/26)	12% (3/26)	15% (4/26)	92% (24/26)	0% (0/26)	8% (2/26)
Total	60	83% (50/60)	82% (41/50)	2% (1/50)	16% (8/50)	82% (41/50)	6% (3/50)	12% (6/50)	92% (46/50)	4% (2/50)	4% (2/50)

Table 2. Pattern of Resistance *Escherichia coli* isolates in cat anus swabs

No	Pattern of antimicrobial resistance	%
1	AMP – TE – S	2% (1/50)
2	AMP – TE	8% (4/50)
3	AMP – S	0% (0/50)
4	TE – S	0% (0/50)
5	AMP	6% (3/50)
6	TE	2% (1/50)
7	S	2% (1/50)

AMP = ampicillin, TE = tetracycline, S = streptomycin.

Diseases can arise due to bacterial contamination found in stagnant water and waste consumed by cats (Agustin & Ningtyas, 2022)

The antibiotic disks used were ampicillin, tetracycline, and streptomycin. Based on the Clinical and Laboratory Standards Institute literature (2020), there are three other groups, namely, susceptible (S), intermediate (I), and resistant (R). The larger the diameter of the inhibition zone formed, the more inhibited the growth of bacteria (CLSI, 2020; Effendi *et al.*, 2018). Resistance to the antibiotic ampicillin has a higher percentage compared to the antibiotics tetracycline and streptomycin. This can happen because the antibiotic ampicillin is the antibiotic chosen as first-line therapy or first line for the treatment of *Escherichia coli* bacterial infections in both cats and dogs (Awosile *et al.*, 2018).

Based on the results, the percentage of resistance to the antibiotic ampicillin in stray cats was higher, namely 19% (5/26), compared to domestic cats, which was 12% (3/24). Domestic cats can develop resistance to the antibiotic ampicillin because ampicillin is the first line of treatment given by a veterinarian. Resistance can occur due to inappropriate use of ampicillin antibiotics. That is, Cat owners occasionally treat their cats with ampicillin antibiotics for less than three days. Stray cats can be exposed to ampicillin antibiotic resistance through polluted environments and foodborne (Agustin & Ningtyas, 2022).

The overall results were sensitive to tetracycline antibiotics by 82% (41/50) and 12% (6/50) resistant to tetracycline antibiotics. Unlike the research conducted by Rzewuska *et al.*, (2015), tetracycline antibiotic resistance reached 53%. The percentage of resistance to tetracycline antibiotics in stray cats was 15% (4/26), higher than in domestic cats, which was 8% (2/24). The high incidence of resistance to tetracycline antibiotics in stray cats can be caused by foodborne in which stray cats eat meat such as poultry. Some poultry farms use tetracycline antibiotics as a therapy and efficiency of animal feed to accelerate growth or antibiotic growth promoter (AGP). Poultry farms provide antibiotics through several routes, including drinking, feeding, and parenteral (Nurjanah *et al.*, 2020). The spread of resistance genes in stray cats and domestic cats can occur through the environment, especially in dirty environments. The results of research conducted by Ho *et al.*, (2012) showed that rats can be a reservoir for the spread of *Escherichia coli* resistance genes to tetracycline antibiotics by 5%.

Based on the results of the sensitivity test, it was found that 92% (46/50) were sensitive to streptomycin antibiotics and 4% (2/50) were resistant to streptomycin antibiotics. The results of the sensitivity test conducted by (2015) show that streptomycin antibiotic resistance reached 96.6%. The percentage of resistance to streptomycin in stray cats was higher, which was 8% (2/26), compared to domestic cats,

which was 0% (0/24), but there were intermediates of 8% (2/24). Cats can develop streptomycin antibiotic resistance due to inappropriate use of antibiotics as well as resistance to the antibiotics ampicillin and tetracycline. Streptomycin is an aminoglycoside class of antibiotics that is widely used to treat Gram-negative bacterial infections. Streptomycin antibiotics are also commonly used in the livestock world because they are widespread in the market and can be purchased without a doctor's prescription (Nurjanah *et al.*, 2020). Based on the results of the sensitivity test, it was found that the isolates experienced resistance to the three tested antibiotic disks or multidrug resistance of 2% (1/50). *E. coli* was found to be resistant to multidrug, namely beta-lactam, tetracycline, and streptomycin antibiotics (Wibisono *et al.*, 2020). In contrast to the results of the sensitivity test conducted by Beltrán *et al.*, (2020), *E. coli* in the tested cats has a multidrug resistance percentage of 30,8%.

The pattern of resistance that occurs in the distribution of resistance to several categories of antibiotics is shown in Table 2. The pattern of resistance is divided into four categories, namely AMP – TE – S, AMP – TE, AMP – S, and TE – S. Some isolates are susceptible to all or none. Any of the three antibiotics tested. Based on the pattern of resistance, the highest percentage of isolates that experienced resistance showed 8% (4/50) to the antibiotics ampicillin and tetracycline. Isolates that encountered resistance to the antibiotics ampicillin, tetracycline, and streptomycin were 2% (1/50). Isolates that underwent multidrug resistance were the isolates with sample code L DK 04. In contrast, the research results of sensitivity tests on chickens in Blitar District showed that 95.9% experienced multidrug resistance with the AMP – TE – S pattern (Wibisono *et al.*, 2020).

The occurrence of multidrug resistance in cats can be caused by inappropriate administration of antibiotics. Prevalence of multidrug resistance in cats can be caused by the administration of antibiotics alone, in combination with other antibiotics or inappropriate chemotherapeutics. Previous studies have shown that 85% of cat owners do not understand how to use antibiotics properly. Antibiotics must be given in the right dosage, diagnosis, and timeframe for use (Agustin & Ningtyas, 2022; Utami, 2011; Yaddi *et al.*, 2020). Bacteria that have antibiotic-resistant genes can spread in various ways, one of which is through the feces and products of food-producing animals. Resistant bacteria contained in the faeces of food-producing animals can migrate around the livestock environment, in the environment around the slaughterhouse and also around the environment during meat processing, even though they have a great

distance from the source of bacterial contamination (Masruroh *et al.*, 2016).

The occurrence of multidrug resistance in the test results can be a serious problem and must be addressed immediately. Cats can be a reservoir for the spread of resistance genes and pollute the surrounding environment. *E. coli* bacteria can transfer antibiotic resistance genetic material between animals and owners through horizontal transfer. Cat owners will contract resistant gene bacteria through cat feces if cat owners do not clean cat feces properly (Agustin & Ningtyas, 2022).

Cats become reservoirs by eating vectors such as rats and cockroaches and eating raw meat. Not only stray cats, domestic cats can also be exposed to resistant genes through raw food diets, commonly called raw meat-based diets (RMBDs). Giving a raw food diet to cats can be a source of spread of bacteria, both pathogenic bacteria and commensal bacteria that have resistant genes from animal products (Davies *et al.*, 2019). Cats can be vectors in the spread of resistant genes from non-pathogenic bacteria to pathogenic bacteria. Cat owners can contract resistant genes from cats through polluted air, contaminated food, cat feces, cat urine, saliva and direct contact with cats. (Gwenzi *et al.*, 2021). Antibiotic resistance can be prevented by carrying out appropriate therapy and being aware of the side effects of antibiotics. Antibiotic resistance can also be controlled by providing education to increase public awareness and understanding of antibiotic resistance to change people's behaviour in using antibiotics wisely and implementing good sanitation and hygiene (Utami, 2011).

The prevalence of antibiotic resistance in cats around Dukuh Kupang, Surabaya, showed higher results in stray cats than in domestic cats. This shows the high level of exposure in the environment to the existence of antibiotic resistance in *Escherichia coli* isolates. The increased exposure to antibiotic resistance in the environment in cats also has the potential for high exposure to antibiotic resistance in humans. So, it becomes important to be aware of the transmission of antimicrobial resistance in humans, animals, and the environment.

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“All authors declare that there are no conflicts of interest.”

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