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# Multidrug Resistance in Stray Cats of The North Surabaya Region, East Java, Indonesia

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## **ABSTRACT**

Stray cats survive by getting food or drink that is available in the environment, correspondingly, stray cats have relatively high exposure to antibiotic resistance obtained from resistant bacteria found in the environment. The present study was conducted to determine patterns of multidrug resistance and *Escherichia coli* resistance in stray cats. A total of 50 stray cat anal swab samples were taken randomly from the previously recorded stray cat population in the Surabaya area, East Java, Indonesia. Samples were brought using buffered peptone water. They were cultured on MacConkay Agar differential selective media, and all suspicious colonies of *Escherichia coli* were examined by biochemical tests. Isolates were then identified, and susceptibility testing was performed according to the Clinical and Laboratory Standards Institute. The results of the resistance test indicated that the multidrug resistance in *Escherichia coli* bacteria taken from cats was 14.6% (7/48). The high antibiotic resistance of *Escherichia coli* bacteria in stray cats, which were resistant to many drugs, provides an early warning of environmental health. Environmental health is closely related to animal and human health, especially antibiotic resistance.

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# INTRODUCTION

Stray cats which inhabit unregulated and open environments, demonstrate a remarkable adaptability, enabling them to thrive in diverse settings despite lacking human ownership (Vasileva and McCulloch, 2023). Cats are potentially exposed to pathogen infection from the environment (Agustin and Ningtyas, 2022). Previous research by Mauwalan et al. (2022) showed the existence of *Escherichia coli* resistance in an environment in Indonesia. The level of antibiotic resistance in the ampicillin 100%, streptomycin 73.3%-86.7%, gentamicin 100%, and kanamycin 33.3%. Pathogen contamination is generally caused by the entry of feces, animal waste, and garbage into water bodies, such as the return of wastewater into wells and leaky water pipes in dirty areas. Stray cats have a significant potential to interact with such environments, triggering the spread of pathogens such as *Escherichia coli* bacteria (Gargano et al., 2022). *Escherichia coli* is a gram-negative known as a normal flora found naturally in the digestive tract of humans and animals. *Escherichia coli* is considered pathogenic when its concentration surpasses typical levels (Mueller and Tainter, 2023).

Antibiotic drug use in the animal sector reached 80% (Hosain et al., 2021). The effectiveness of antibiotics against some animals, such as cats has decreased currently, this is indicated by an increase in multidrug resistance observed in 9% of isolated cats (Li et al., 2021). Improper dosage, indications, and antibiotic use can lead to animal resistance (Rahman et al., 2022). Antibiotic resistance occurs when bacteria adapt and thrive in an environment exposed to antimicrobials, posing a severe threat to public health globally (Salam et al., 2023). Multidrug resistance refers to resistance to three or more different groups of antibiotics (Mandal et al., 2022). High levels of multidrug resistance in *Escherichia coli* were detected in healthy and diseased cats, raising concerns about transmission to humans and supporting the need for a one-health approach to address the potential threat of cats as a reservoir for AMR (Fayez et al., 2023).

Since cats interact with humans, studying antibiotic resistance is crucial whether domestic or stray, facilitating the transfer of genes for resistance to the common bacterium *Escherichia coli* (Li et al., 2021; Fayez et al., 2023). It is crucial to determine the resistance levels of *Escherichia coli* in stray cats to ampicillin, streptomycin, and tetracycline due to the significant antibiotic resistance present in the environment. Stray cats are exposed to the environment, which

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puts them at risk. The present study aimed to determine multidrug resistance *Escherichia coli* isolated from anal samples of stray cats in the North Surabaya region of Indonesia.

# MATERIALS AND METHODS

# **Ethical approval**

All research methods and procedures, along with the utilization of animals, have been approved by the Research Ethics Committee, Faculty of Veterinary Medicine, Wijaya Kusuma Surabaya University, Indonesia, with certificate number 123-KKE/III/FKH-UWKS/2023.

# Sample collection

In the present cross-sectional study, 50 stray cats, both male and female, were used randomly. Samples were collected from North Surabaya, East Java, Indonesia, from March to May 2023. The sample size was taken proportionally based on the stray cat population in that district. The samples were collected from five districts in North Surabaya, including Bulak (12 samples), Kenjeran (12 samples), Semampir (9 samples), Pabean (11 samples), and Krembangan (6 samples). Samples were collected directly by anal swab and incubated buffered peptone water (BPW) in cold conditions (4°C) to transport to the laboratory for detecting *Escherichia coli* at the Faculty of Veterinary Medicine Laboratory, Wijaya Kusuma University Surabaya, Indonesia.

#### Isolation and identification

The isolation of *Escherichia coli* was carried out following the Indonesian National Standard, SNI 7388:2009. Anal swab samples were incubated in buffered peptone water broth (BPW, Merck, Germany) to enrich *Escherichia coli* in samples. After incubation at 37°C for 24 hours, each loop of enrichment broth samples was cultured on MacConkey agar (MCA, HiMedia MH081, India) for further incubation at 37°C for 24 hours. *Escherichia coli* colonies were subcultured on nutrient agar (NA, Merck, Germany) for further incubation at 37°C for 24 hours to check biochemical tests. The confirmed *Escherichia coli* strains are cultured on Triple Sugar Iron Agar (TSIA, HiMedia M021, India) and incubated at 37°C for 24 hours.

# Antimicrobial susceptibility testing

After identifying *Escherichia coli*, the Kirby-Bauer agar plate diffusion method was used to test the sensitivity of bacteria to antibiotics on Muller Hinton agar (MHA, HiMedia, India). The sensitivity of bacteria to antibiotics was evaluated by comparing the results with the CLSI standard (2022). *Escherichia coli* ATCC 25922 was utilized as a quality control, and the findings were then compared with the CLSI standards (2022). The antibiotic discs used in the present study included ampicillin (AMP 10μg, Oxoid<sup>TM</sup>), streptomycin (S 10μg, Oxoid<sup>TM</sup>), and tetracycline (TE 30μg, Oxoid<sup>TM</sup>).

# Data analysis

According to the evaluation's post-incubation results, the presence of inhibition zones, namely susceptible, intermediate, and resistant, was interpreted according to CLSI guidelines. Data analysis of the current study was carried out descriptively (Effendi et al., 2018).

# RESULTS AND DISCUSSION

The results of the present study indicated that 48/50 (96%) of the samples tested positive for *Escherichia coli* from stray cats in North Surabaya. The *Escherichia coli* was isolated from Bulak, Semampir, and Pabean districts at 100%, Kenjeran at 91.7%, and Kembangan at 83.3%. Although *Escherichia coli* bacteria are not pathogenic, these bacteria are opportunistic bacteria that can cause pathogens in cats (Núñez-Samudio et al., 2024). The pathogenicity of *Escherichia coli* can be influenced by several predisposing factors, including feed, environment, and the intensity of the pathogen strain (EFSA AHAW Panel, et al., 2022; Damborg et al., 2023). *Escherichia coli* has several pathogenic strains that cause diarrhea, namely diarrheagenic *Escherichia coli* strains, which can produce toxins (Das et al., 2023). Humans and cats can be infected by *Escherichia coli* (Li et al., 2021). Farizqi et al. (2023) have indicated that cats and their owners could be infected with dangerous *Escherichia coli* strains. Despite the absence of symptoms in the cat, it has the potential to act as a reservoir or carrier of enterohemorrhagic *Escherichia coli* in humans (Gargano et al., 2022).

Antibiotic sensitivity test on positive *Escherichia coli* isolates was conducted using three different antibiotic groups, including beta-lactam (ampicillin), aminoglycoside (streptomycin), and tetracycline group (Tetracycline; Table

1). The percentage of isolates exhibiting multidrug resistance in this test was 14.6% (7/48). Based on the tested isolates, resistance was observed in all used antibiotic disks, indicating multidrug resistance, as shown in Figure 1. The high results of antibiotic resistance were for ampicillin (35.41%), streptomycin (18.75%), and tetracycline (22.92%), respectively in North Surabaya that are following previous research which showed resistance to ampicillin (31%) and tetracycline (21%) (Gargano et al., 2022). However, the results of this study are higher than previous studies, which show in stray cat ampicillin resistance (19%), tetracycline (15%), and streptomycin (8%) (Wibisono et al., 2024). Stray cats can develop antibiotic resistance through the environment. The water contaminated with resistant *Escherichia coli* bacteria can be a bridge for the spread of antibiotic resistance in stray cats (Janke et al., 2023). Resistance genes could be exchanged between animals and humans, or environmental bacteria (Saraiva et al., 2022). A previous study by Chen et al. (2019), reported a significant risk of the spread of resistant *Escherichia coli* bacteria from pets to humans, and consequently using antibiotic in pets should be strictly regulated.

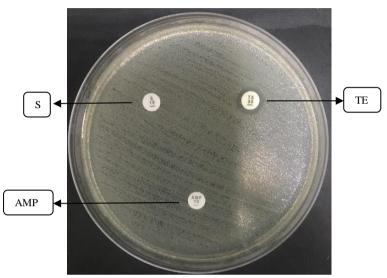


Figure 1. Multidrug resistance of Escherichia coli in stray cats. S: Streptomycin, TE: Tetracycline, AMP: Ampicillin

Ampicillin, a beta-lactam antibiotic, has encountered the most significant resistance. Ampicillin is a versatile antibiotic that is utilized in the treatment of various infections, including gastrointestinal infections (Kim et al., 2019), and urinary tract infections (Kang et al., 2018) is a crucial antibiotic that has been most widely used in humans and animals health (Hardiati et al., 2021). Ampicillin, belonging to the aminopenicillin group, is a semisynthetic antibiotic that is widely known for its effectiveness against Escherichia coli, the bacterium commonly associated with resistance (Monaghan et al., 2021). The agricultural use or contamination of antibiotics directly or indirectly exposes soil, water, livestock, and plant foods to these substances (Skandalis et al., 2021). A recent revealed that 57.1% of Escherichia coli strains isolated from the wastewater of a hospital in Banda Aceh, Indonesia, exhibited resistance to ampicillin (Anelia et al., 2023). The results of sensitivity testing for antibiotics from the tetracycline group (tetracycline) indicated 22.92% (11/48). The obtained result was higher than previous research on stray cats in Poland (53%) (Rzewuska et al., 2015). The percentage of resistance to the aminoglycoside group in stray cats in the North Surabaya region showed a resistance result of 18.75% (9/48). Cats can encounter resistance to the antibiotic streptomycin, as well as resistance to ampicillin and tetracycline, from their environment (Fayez et al., 2023). According to Mandal et al. (2022), beta-lactam, tetracycline, and aminoglycoside antibiotics are widely used to treat bacterial infections in humans and the livestock industry, therefore, an environment polluted by resistant bacteria can spread resistance genes in stray cats. The resistance of stray cats to various antibiotics may be attributed to the food they consume or the environment they inhabit. This is evident in the high levels of resistance found in the environment and traditional markets where stray cats reside in the Northern Surabaya area (Dewi et al., 2023).

As can be seen in Table 2, according to the resistance pattern, the percentage of positive isolates of *Escherichia coli* exhibiting the highest resistance at a level of 14.6% (7/48), specifically against the antibiotics ampicillin, tetracycline, and streptomycin (AMP–TE-S). The resistance pattern of *Escherichia coli* indicates multidrug resistance. High dosages of antibiotics used in the medical field, particularly in the fisheries, for the treatment of humans and animals might lead to the emergence of antibiotic-resistant harmful microorganisms. One of the two pathways mentioned below has the potential to result in multidrug resistance. Initially, within a single cell, bacteria can accumulate numerous genes responsible for drug resistance. Resistance plasmids are often the site of this buildup. Additionally, increased expression of genes producing multidrug efflux pumps, which enable them to secrete a range of medicines, can lead to a second

mechanism of multidrug resistance (Das et al., 2023; Fayez et al., 2023). Animals, such as cats, dogs, calves, pigs, turkeys, chickens, and rodents have all been found to harbor multidrug-resistant bacteria (Harijani et al., 2020; Wibisono et al., 2021; Zhao et al., 2021). A multidrug-resistant *Escherichia coli* strain with an AMP–TE–S resistance pattern to ampicillin, tetracycline, and streptomycin was found in 14.6% (7/48) of the isolates based on the findings of the antibiotic sensitivity test. *Escherichia coli* often exhibits multidrug resistance when exposed to beta-lactam, tetracycline, and aminoglycoside antibiotics (Nguyen et al., 2021; Saraiva et al., 2022).

Table 1. Antibiotic sensitivity test of Escherichia coli in stray cats at North Surabaya, Indonesia

Sub-district	Escherichia coli (%)	Antibiotic Sensitivity Test (%)									
		Ampicillin			Streptomycin			Tetracycline			MDR
		R	I	S	R	I	S	R	I	S	
Kenjeran	11/12	36.4%	0	63.6%	18.2%	54.5%	27.3%	9.1%	0	90.9%	0 (0/11)
	(91.66 %)	4/11	(0/11)	(7/11)	(2/11)	(6/11)	(3/11)	(1/11)	(0/11)	(10/11)	
Semampir	(9/9)	22.2%	0	77.8%	22.2%	22.2%	55.6%	33.3%	0	66.7%	22.2%
	(100 %)	(2/9)	(0/9)	(7/9)	(2/9)	(2/9)	(5/9)	(3/9)	(0/9)	(6/9)	(2/9)
Krembangan	(5/6)	20%	0	80%	20%	0	80%	20%	0	80%	20 (1/5)
	(83.3 %)	(1/5)	(0/5)	(4/5)	(1/5)	(0/5)	(4/5)	(1/5)	(0/5)	(4/5)	
Bulak	(12/12)	50%	0	50%	25%	8.3%	66.7%	33.3%	25	41.7%	25%
	(100 %)	(6/12)	(0/12)	(6/12)	(3/12)	(1/12)	(8/12)	(4/12)	(3/12)	(5/12)	(3/12)
Pabean	(11/11)	36.4%	0	63.6%	9.1%	9.1%	81.8%	18.2%	9.1%	72.7%	9.1%
	(100 %)	(4/11)	(0/11)	(7/11)	(1/11)	(1/11)	(9/11)	(2/11)	(1/11)	(8/11)	(1/11)
Total	(48/50)	35.4%	0	64.6%	18.7%	20.8%	60.4%	22.9%	8.3%	68.7%	14.6%
	(96 %)	(17/48)	(0/48)	(31/48)	(9/48)	(10/48)	(29/48)	(11/48)	(4/48)	(33/48)	(7/48)

R: Resistant, I: Intermediate, S: Susceptible, MDR: Multidrug resistance

**Table 2.** Resistance pattern of *Escherichia coli* in stray cats at North Surabaya, Indonesia

Number	Resistance pattern	Percentage
1	AMP-TE-S	14.6 (7/48)
2	AMP-TE	6.2 (3/48)
3	AMP-S	4.2 (2/48)
4	TE-S	0 (0/48)

S: Streptomycin, TE: Tetracycline, AMP: Ampicilline

Antibiotic-resistant bacteria can be transmitted through various means, including feces, food, and animal products (Eltai et al., 2020). The livestock environment, the slaughterhouse environment, and the environment during meat processing can all be contaminated by resistant bacteria found in the feces of food animals (Indrawati et al., 2019; Osman et al., 2021). Test obtained results indicate that multidrug resistance can be dangerous and need to be handled. Cats can contaminate their surroundings and act as a reservoir for the propagation of resistant genes (Gargano et al., 2022). Escherichia coli bacteria can transfer antibiotic-resistance genes between animals and owners through horizontal transfer (Fayez et al., 2023). Stray cats can serve as reservoirs by consuming vectors, such as rats and cockroaches, as well as consuming raw meat (Koutsoumanis et al., 2021; Rafiq et al., 2022). Proper use of antibiotics and awareness of their side effects are essential in preventing antibiotic resistance, as excessive use can lead to the presence of antibiotic residues (Handayani et al., 2017). Moreover, the issue of antibiotic resistance can be addressed by educating the public to enhance awareness and knowledge about antibiotic resistance, influence public behaviour toward the responsible use of antibiotics, and adopt appropriate sanitation and hygiene measures (Suherman et al., 2023). Three distinct antibiotic groups were used in this study: ampicillin (penicillin), streptomycin (aminoglycoside), and tetracycline. To determine if Escherichia coli is sensitive to antibiotics or resistant, it would be ideal if testing on a variety of drugs could be conducted. This would reveal any potential resistance of Escherichia coli germs in stray cats. This can surely help to improve the management of antibiotic use. The Kirby-Bauer disk diffusion method, which is employed in this work, is the gold standard for confirming bacterial susceptibility. More information would be available if the resistance genes of the bacterium were identified.

# **CONCLUSION**

According to the present study, 96% of anal swab samples originate from stray cats in the Northern region of Surabaya, Indonesia. Sensitivity testing revealed 14.6% multidrug resistance in *Escherichia coli* from anal swab samples of stray cats in North Surabaya. The most significant antibiotic resistance was observed with ampicillin, then tetracycline, and

finally streptomycin. It is crucial to recognize the significance of understanding the utilization of antibiotics in animals and humans to effectively combat antibiotic resistance in the environment and prevent it from posing a threat to stray cats.

#### **DECLARATIONS**

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#### **Authors' contributions**

Freshinta Jellia Wibisono and Dyah Ayu Widiasih conceived and designed the study. Mutia Isnaeni, Afif Fajrul Islam, Andi Oktaviana Mentari, Ismul Jalal, Seryna Hasna Qurratu'ain, and Al Fardiansyah contributed to antimicrobial agent and susceptibility testing. Freshinta Jellia Wibisono, Dyah Ayu Widiasih, and Hung Nguyen-Viet contributed to the interpretation of the results, and AOM led the writing of the manuscript. All authors provided critical feedback and helped shape the research, analysis, and writing of the manuscript.

## **Competing interests**

The authors declare that they have no conflict of interest.

#### **Ethical considerations**

This paper was written originally by the authors. The authors were not submitting this paper to the other journal or publisher.

# Availability of data and materials

All data of the current study are available upon reasonable request from the authors.

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