Multidrug Resistance (MDR) Patterns of *E. coli* Isolated From *Macaca fascicularis* in IPB University Breeding Facility, Bogor

Muhammad Farid Rizal[1,8*], I Wayan T Wibawan[2], Joko Pamungkas[3], Roostita Lobo Balia[4], Huda Salahudin Darusman[5], Maryati Surya[6], Yusuf Farid Achmad[5], Betty Sundari[5], Yovi Alwi Soedarso[5], Denniswara Sibit[5], Fhady Riskhy Loe[6,] Suseno Amien[7], Itfa Alya[4], Reinier Melvern[4]

[1]Doctoral Program of Primatology, School of Graduate, IPB University, Bogor, Indonesia;
[2]School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia;
[3]Primate Research Center, IPB University, Bogor, Indonesia;
[4]Veterinary Study Program, Faculty of Medicine, Universitas Padjadjaran, Sumedang, Indonesia;
[5] Department Research and Development, PT. Biomol Nusantara Mandiri, Bandung, Indonesia;
[6] Department of Animal Diseases and Veterinary Public Health, Veterinary Study Program, Faculty of Medicine and Veterinary Medicine, Universitas Nusa Cendana, Kupang, Indonesia;
[7]Biotechnology Study Program, Graduate School, Universitas Padjadjaran, Bandung, Indonesia.
[8]Urban Animal Pet Care Clinic, Bandung, Indonesia

**Correspondence: Muhammad Farid Rizal, Doctorate Student of Primatology, School of Graduate, IPB University, Bogor, Indonesia; E-mail: faridrizal46@gmail.com*

Abstract

Background: Diarrhea is a common health problem in captive long-tailed macaques (*Macaca fascicularis*), often associated with *Escherichia coli* infection. Antimicrobial use in captive breeding facilities may contribute to the emergence of antimicrobial-resistant bacteria, posing risks to both animal and public health through potential zoonotic transmission. However, information regarding antimicrobial resistance in *E. coli* isolated from captive long-tailed macaques remains limited.

Objective: This study aimed to determine the antimicrobial resistance profiles and multidrug-resistant (MDR) patterns of *E. coli* isolated from diarrheic macaques in a breeding facility.

Material and Methods: A total of 30 previously characterized *E. coli* isolates were tested using the Kirby–Bauer disk diffusion method against seven antibiotics: ceftazidime (CAZ), sulfamethoxazole–trimethoprim (SXT), doxycycline (DO), meropenem (MEM),

streptomycin (S), chloramphenicol (C), and nalidixic acid (NA). **Results:** 10 isolates (33.3%) were sensitive to all tested antibiotics, while 20 isolates (66.7%) were resistant to at least one antibiotic. The highest resistance was observed against streptomycin (56.7%), followed by sulfamethoxazole–trimethoprim (30%), doxycycline (26.7%), chloramphenicol (13.3%), ceftazidime (10.0%), meropenem (6.7%), and nalidixic acid (6.7%). Multidrug resistance, defined as resistance to three or more antibiotic classes, was identified in 6 isolates (20%).

Conclusion: These findings indicate the presence of MDR *E. coli* in captive long-tailed macaques and highlight the importance of antimicrobial resistance surveillance and prudent antibiotic use in captive primate facilities.

***Keywords*:** Antibiotic resistance, Breeding facility, Diarrhea, *E. coli*, Long-tailed macaque.

1. Introduction

Long-tailed macaques are among the most frequently used animals in biomedical research. Their genome shares 92.83% similarity with that of humans, making them more suitable than other animal models [1]. These macaques are mainly sourced through captive breeding. One major health management challenge in captivity is diarrhea.

Diarrhea in long-tailed macaques is commonly caused by *E. coli*, Salmonella, Shigella, or other enteropathogens [2]. It can lead to malnutrition, dehydration, growth retardation, weakened immunity, weight loss, and even death, significantly affecting their reproductive potential. One commonly used treatment is antibiotics that are effective in eradicating the causative bacteria [3].

Bacteria exhibit remarkable genetic adaptability, allowing them to counter various environmental stressors, including exposure to antibiotics that threaten their survival [4]. Through evolution, bacteria coexisting with antimicrobial-producing organisms have developed sophisticated mechanisms to withstand harmful compounds, thus enabling intrinsic resistance. Evolutionarily, bacteria adopt two main genetic strategies to survive antibiotic pressure: (i) genetic mutations that alter antibiotic targets or mechanisms, and (ii) acquisition of exogenous DNA encoding resistance determinants via horizontal gene transfer (HGT), facilitating the spread of resistance traits [5].

The development of multidrug resistance in bacteria involves acquiring genetic mutations that provide survival advantages under antimicrobial pressure. Some bacterial cells in a susceptible population undergo mutations altering drug mechanisms, allowing these cells to survive and proliferate despite antibiotic intervention. When resistant mutants emerge, susceptible populations are eliminated, enabling resistant bacteria to dominate. This raises treatment costs significantly. Molecular mechanisms underlying MDR are multifaceted, involving strategies such as target site modification, decreased

drug uptake, efflux pump activation, and regulatory network modulation [6,7].

Bacterial evolution and antimicrobial resistance development are significantly influenced by HGT, a process facilitating the acquisition of foreign DNA. Environmental reservoirs, comprising genetic resistance determinants, are a productive source of resistance genes in clinically relevant bacteria. Mobile genetic elements (MGEs) such as plasmids and transposons play crucial roles in the development and spread of resistance [8], while integrons, site-specific recombination systems, facilitate antimicrobial resistance gene accumulation [8,9].

Antibiotic resistance is a major global health concern due to increasing treatment failures and mortality, as well as rising therapy costs [10]. Antibiotic resistance in captive long-tailed macaques poses a potential public health threat. Thus, understanding pathogenic bacteria and associated antibiotic resistance is crucial for managing and treating disease outbreaks [11]. Although numerous studies have reported antibiotic resistance profiles in livestock and poultry [12], data on antibiotic resistance in captive long-tailed macaques remain limited. Significant resistance may exist. Therefore, this study aims to evaluate antibiotic resistance and identify effective treatments for diarrhea in captive long-tailed macaques.

2. Materials and Methods

This study used 30 *E. coli* isolates obtained from the collection of the Primate Research Center (PSSP), IPB University, originally isolated from 30 diarrheic long-tailed macaques out of approximately 150 individuals maintained in the breeding facility. The isolates were re-identified using EMB agar, Gram staining, and microscopic examination. The isolates used in this study were previously characterized for virulence factors, including hemagglutination, hemolytic activity, and adhesion profiles, in our previous study [12], which served as the basis for the present antimicrobial resistance investigation. The seven antibiotics selected for susceptibility testing represented different antimicrobial classes commonly used in human and veterinary medicine and frequently included in antimicrobial resistance surveillance programs. Several of these antibiotics had not been administered to the macaques, allowing assessment of resistance patterns that may be associated with environmental exposure or other transmission pathways.

2.1 Procedures Antibiotic Susceptibility Testing

Escherichia coli susceptibility to antibiotics was tested using the Kirby-Bauer disk diffusion method on MHA (Oxoid, UK) media. McFarland-standard suspensions were applied to MHA plates and tested with seven antibiotics to determine bacterial susceptibility: ceftazidime 30µg (CAZ), streptomycin (S) 10µg, sulfamethoxazole-trimethoprim 25µg (SXT), chloramphenicol (C) 30µg, doxycycline (DO) 30µg, meropenem (MEM) 10µg, and nalidixic acid (NA) 30µg (all Oxoid, UK). The plates were incubated at 37℃ for 24 hours. Results were compared with the Clinical Laboratory

Standards Institute [20] guidelines. Bacteria resistant to three or more antibiotic classes were classified as multidrug-resistant (MDR).

2.2 Data analysis

Data on MDR *E. coli* resistance in *M. fascicularis* with diarrhea were tabulated and analyzed descriptively.

3. Results

A total of 30 *E. coli* isolates re-identified on EMB agar showed characteristic metallic green sheen colonies (**Figure** 1), and Gram staining confirmed rod-shaped bacterial morphology (**Figure 2**). Antimicrobial susceptibility testing demonstrated marked variation in resistance across isolates. Of the 30 isolates, 10 (33.3%) were fully susceptible to all seven antibiotics tested, while 20 isolates (66.7%) exhibited resistance to at least one antibiotic.

Figure 1. Bacteria colony grown in EMB medium showed a characteristic metallic green sheen

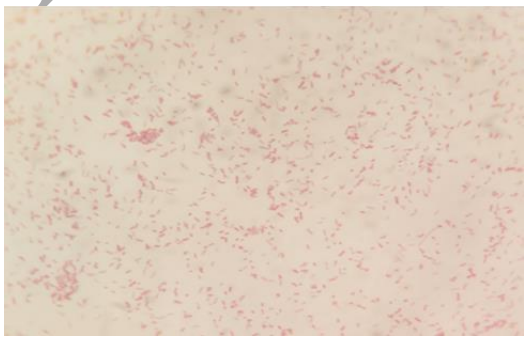

Figure 2. The morphology of the bacteria is rod-shaped and pink in color

The highest resistance rate was observed against streptomycin, with 17 isolates (56.7%) classified as resistant. Resistance to sulfamethoxazole–trimethoprim (SXT)

occurred in 9 isolates (30%), followed by doxycycline, with 8 resistant isolates (26.7%). Resistance to chloramphenicol was identified in 4 isolates (13.3%), while meropenem and nalidixic acid each showed resistance in 2 isolates (6.7%). Three isolates (10%) exhibited resistance to ceftazidime. Several isolates also showed intermediate susceptibility, particularly to streptomycin, nalidixic acid, and doxycycline, The overall resistance distribution is summarized in Table 1, while the sensitivity patterns are illustrated in Figure 3.

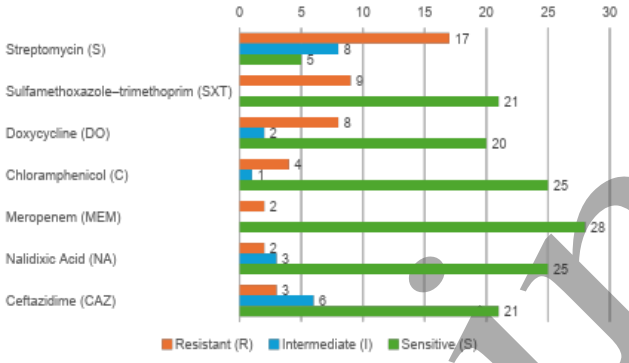

Figure 3. Sensitivity patterns of *E. coli* causing diarrhea in *M. fascicularis* to various types of antibiotics

Table 1. Resistance to several antibiotics in *E. coli* isolated from captive macaques

Antibiotic Class Group	Resistance Pattern	Number of Resistant Isolates	Total Isolates
0	No resistance detected	10	10
1	MEM	1	
	S	6	7
2	CAZ–S	1	
	CAZ–SXT	1	
	DO–S	1	
	S–C	1	
	SXT–DO	1	
	SXT–S	2	7
3	SXT–DO–S	2	2
4	SXT–DO–S–C	2	
	SXT–DO–S–NA	1	3
6	CAZ–DO–MEM–S–C–NA	1	1
Total MDR (≥3 classes)	—	6	6

Note: DO = Doxycycline, SXT = Sulfamethoxazole–Trimethoprim, MEM = Meropenem, C = Chloramphenicol, NA = Nalidixic Acid, S = Streptomycin, CAZ = Ceftazidime

Analysis of resistance patterns revealed variability in the number of antibiotic classes resisted by each isolate. Seven isolates (23.3%) were resistant to a single antibiotic class, while another seven isolates (23.3%) were resistant to two classes. Two isolates (6.7%) exhibited resistance to three antibiotic classes, three isolates (10%) to four classes, and one isolate (3.3%) to six classes. Based on the definition of multidrug resistance (MDR), characterized by resistance to three or more antibiotic classes, six isolates (20%) were identified as MDR.

The MDR profiles observed included SXT–DO–S (three classes), SXT–DO–S–C and SXT–DO–S–NA (four classes), as well as a highly resistant isolate exhibiting CAZ–DO–MEM–S–C–NA (six classes). These findings highlight the presence of varied antimicrobial resistance and multiple MDR patterns among *E. coli* isolates from diarrheic long-tailed macaques, suggesting persistent antimicrobial selection pressure within the captive environment. These MDR profiles correspond to the patterns summarized in Table 1.

4. Discussion

This study provides new insights into the antimicrobial resistance patterns of Escherichia coli isolated from diarrheic long-tailed macaques in captivity. Although antimicrobial resistance has been extensively studied in livestock, poultry, and companion animals, antimicrobial resistance surveillance in nonhuman primates especially long-tailed macaques remains limited and is often overlooked. As a result, data on antimicrobial resistance in captive macaque populations are scarce, despite their close genetic similarity to humans and their widespread use as important biomedical research models. The present study therefore contributes valuable baseline information to an area of primate health management that has received insufficient scientific attention.

The high proportion of isolates resistant to at least one antibiotic (66.7%) reflects possible environmental, management, or antimicrobial-related pressures within the breeding facility. The highest resistance rate was observed against streptomycin (56.7%), consistent with previous findings that aminoglycosides often show substantial resistance due to their long-standing use in human and veterinary medicine [15,16]. Environmental contamination from agricultural or human sources may also contribute to aminoglycoside-resistant strains [17].

Resistance to sulfamethoxazole–trimethoprim (30%) and doxycycline (26.7%) aligns with reports indicating frequent resistance in Enterobacteriaceae due to extensive use of these antibiotics in poultry and livestock. Long-term tetracycline use contributes to a high environmental load of tetracycline resistance genes [18], which may explain the observed doxycycline resistance.

Detection of meropenem resistance (6.7%), despite carbapenems not being used in primate husbandry, is noteworthy and warrants further investigation. Because molecular characterization was not performed in this study, the underlying resistance mechanisms could not be determined. However, carbapenem-resistant *Enterobacterales* and carbapenem resistance determinants have been increasingly reported in domestic animals, wildlife, and environmental sources worldwide [19–21]. Future studies incorporating molecular approaches are needed to identify the genetic determinants associated with carbapenem resistance in these isolates.

Nalidixic acid resistance was relatively low (6.7%), although quinolone resistance in E. coli is increasingly reported worldwide. Environmental and anthropogenic factors may play a substantial role in shaping resistance patterns in captive primates. Captive facilities often harbor higher abundances of antimicrobial resistance genes, and human–primate interactions may further facilitate transmission pathways [22]. Prior work in the same facility reported contamination of water and feed sources exceeding permissible thresholds [2], reinforcing the likelihood of environmental contributions to resistance.

The presence of MDR phenotypes—particularly isolates resistant to three, four, and six antibiotic classes—highlights the potential for resistant E. coli to serve as reservoirs for resistance genes within captive environments. *E. coli* is well recognized as an indicator organism for resistance due to its ability to acquire and disseminate genetic elements rapidly [13]. Similar patterns of chloramphenicol resistance have been described in other primates and animal species [23,24], emphasizing the broader ecological relevance.

Multidrug-resistant bacteria represent a major public health concern due to their ability to spread across species boundaries [22]. Given the close interactions between humans and captive macaques, monitoring resistance trends is essential for both animal health and zoonotic risk mitigation. Although molecular analyses were not repeated in this study because these isolates had been previously characterized for virulence factors, future research should incorporate genomic approaches to better elucidate mechanisms and pathways of resistance dissemination.

This study has several limitations. The sample size was relatively small and originated from a subset of macaques within the breeding population, which may limit the generalizability of the findings. In addition, molecular analyses of antimicrobial resistance genes were not performed; therefore, the genetic mechanisms underlying the observed resistance patterns could not be determined.

5. **Conclusion**

This study revealed that *Escherichia coli* isolated from diarrheic long-tailed macaques in captivity exhibited diverse antimicrobial resistance patterns, with 66.7% of isolates showing resistance to at least one antibiotic and 20% classified as multidrug-resistant (MDR). Streptomycin, sulfamethoxazole–trimethoprim, and doxycycline presented the highest resistance levels, indicating notable antimicrobial pressure within

the captive environment. The presence of MDR isolates highlights the potential risk of resistance transmission and emphasizes the need for continuous monitoring, responsible antibiotic use, and improved health management practices in captive macaque populations.

Acknowledgments

The authors sincerely acknowledge the Primate Research Center, IPB University, for providing laboratory facilities and research support. Appreciation is also extended to PT. Biomol Nusantara Mandiri for providing access to laboratory facilities, equipment, and research materials used in this study. The authors also thank drh. Fadhy R. Loe for providing the original bacterial isolates used in this research.

Authors' Contribution

MFR contributed to manuscript writing and drafting. IWTW, JP, and RLB contributed to methodology and supervision. HSD and SA contributed to the article review and manuscript revision. MS contributed to laboratory work assistance. YFA contributed to laboratory work assistance. BS and YAS contributed to laboratory work assistance. DS contributed to supervision and translation. FRL contributed as the provider of the original isolates used in this study. IA and RM contributed to templating and drafting assistance. All authors have read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

The study was approved by the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, IPB University (Approval No. 260/KEH/SKE/X/2024).

Study area

Funding/Suport

The authors received no financial support, funding, or grant for the conduct of this study.

Artificial Intelligence (AI) Statement

Artificial intelligence tools (ChatGPT and Grammarly) were used only for language editing, grammar correction, and manuscript refinement. All data, scientific interpretation, results, and conclusions presented in this manuscript are original and were independently conducted, analyzed, and verified by the authors.

Data Availability

All data supporting the findings of this study are available within this article

References

1. Hansen MF, Gill M, Nawangsari VA, Sanchez KL, Cheyne SM, Nijman V, et al. Conservation of long-tailed macaques: Implications of the updated IUCN status and the COVID-19 pandemic. Primate Conserv. 2021;35:1-11.

2. Loe FR, Tomongo S, Saepuloh U, Sajuthi D, Suparto IH. Prevalence and sensitivity of enteropathogenic bacteria in diarrheagenic long-tail macaque in the breeding facilities of IPB University at Dramaga, Bogor. J Vet. 2021;22(4):564-73. https://doi.org/10.19087/jveteriner.2021.22.4.523

3. Taylor K. Clinical veterinarian's perspective of non-human primate (NHP) use in drug safety studies. J Immunotoxicol. 2010;7(2):114-9. https://doi.org/10.1080/15476910903213539

4. Muteeb G, Rehman MT, Shahwan M, Aatif M. Origin of antibiotics and antibiotic resistance, and their impacts on drug development: A narrative review. Pharmaceuticals (Basel). 2023;16(11):1615. https://doi.org/10.3390/ph16111615

5. Munita JM, Arias CA. Mechanisms of antibiotic resistance. Microbiol Spectr. 2016;4(2):VMBF-0016-2015. https://doi.org/10.1128/microbiolspec.vmbf-0016-2015

6. Melnyk AH, Wong A, Kassen R. The fitness costs of antibiotic resistance mutations. Evol Appl. 2015;8(3):273-83. https://doi.org/10.1111/eva.12196

7. Belay WY, Gestachew M, Tegegnew BA, Tefferra ZH, Dagne A, Zeleke TK, et al. Mechanism of antibacterial resistance, strategies and next-generation antimicrobials to contain antimicrobial resistance: a review. Front Pharmacol. 2024;15:1444781. doi:10.3389/fphar.2024.1444781

8. Bhat BA, Mir RA, Qadri H, Dhiman R, Almilaibary A, Alkhanani M, et al. Integrons in the development of antimicrobial resistance: critical review and perspectives. Front Microbiol. 2023;14:1231938. https://doi.org/10.3389/fmicb.2023.1231938

9. Yang S, Liu Y, Yang N, Lan Y, Lan W, Feng J, et al. The gut microbiome and antibiotic resistome of chronic diarrhea rhesus macaques (*Macaca mulatta*) and its similarity to the human gut microbiome. Microbiome. 2022;10:29. https://doi.org/10.1186/s40168-021-01218-3

10. Boonkusol D, Thongyuan S, Jangsuwan N, Sanyathitiseree P. Antimicrobial resistance profiles in bacterial species isolated from fecal samples of free-ranging long-

tailed macaques (*Macaca fascicularis*) living in Lopburi Old Town, Thailand. Vet World. 2020;13(7):1397. https://doi.org/10.14202/vetworld.2020.1397-1403

11. Kasimanickam V, Kasimanickam M, Kasimanickam R. Antibiotics use in food animal production: escalation of antimicrobial resistance: where are we now in combating AMR? Med Sci. 2021;9(1):14. doi:10.3390/medsci9010014

12. Rizal MF, Pamungkas J, Wibawan IWT, Balia RL, Darusman HS, Surya M, et al. Characterisation of *E. coli* virulence factors causing diarrhea in Macaca fascicularis at IPB University captivity, Bogor, Jawa Barat, Indonesia. J Kajian Vet. 2025;13(2). https://doi.org/10.35508/jkv.v13i2.20778

13. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 31st ed. Clinical and Laboratory Standards Institute; 2021. Available from: https://clsi.org/

14. Lees P, Pelligand L, Giraud E, Toutain PL. A history of antimicrobial drugs in animals: Evolution and revolution. J Vet Pharmacol Ther. 2021;44(2):137-71. https://doi.org/10.1111/jvp.12895

15. Wibisono FJ, Widiasih DA, Nguyen-Viet H. Multidrug resistance of Escherichia coli in cats and the level of understanding of cat owners on antimicrobial resistance. Res J Pharm Technol. 2024;17(8):3855-62. https://doi.org/10.52711/0974-360X.2024.00598

16. Aworh MK, Kwaga JKP, Hendriksen RS, Okolocha EC, Thakur S. Genetic relatedness of multidrug resistant Escherichia coli isolated from humans, chickens and poultry environments. Antimicrob Resist Infect Control. 2021;10:1-13. https://doi.org/10.1186/s13756-021-00930-x

17. Pazra DF, Latif H, Basri C, Wibawan IWT, Rahayu P. Detection of tetracycline resistance genes and their diversity in Escherichia coli isolated from pig farm waste in Banten province, Indonesia. Vet World. 2023;16(9):1907. doi:10.14202/vetworld.2023.1907-1916

18. Roscetto E, Varriale C, Galdiero U, Esposito C, Catania MR. Extended-spectrum beta-lactamase-producing and carbapenem-resistant Enterobacterales in companion and animal-assisted interventions dogs. Int J Environ Res Public Health. 2021;18(24):12952. https://doi.org/10.3390/ijerph182412952

19. Tseng CH, Liu CW, Liu PY. Extended-spectrum β-lactamases (ESBL) producing bacteria in animals. Antibiotics (Basel). 2023;12(4):661.

https://doi.org/10.3390/antibiotics12040661

20. Yasmin S, Karim AM, Lee SH, Zahra R. Temporal variation of Meropenem resistance in *E. coli* isolated from sewage water in Islamabad, Pakistan. Antibiotics (Basel). 2022;11(5):635. https://doi.org/10.3390/antibiotics11050635

21. Ramírez-Castillo FY, Guerrero-Barrera AL, Alvelar-González FJ. An overview of carbapenem-resistant organisms from food-producing animals, seafood, aquaculture, companion animals, and wildlife. Front Vet Sci. 2023;10:1-18. https://doi.org/10.3389/fvets.2023.1158588

22. Chong CW, Alkatheeri AHH, Ali N, Tay ZH, Lee YL, Paramasivam SJ, et al. Association of antimicrobial resistance and gut microbiota composition in human and non-human primates at an urban ecotourism site. Gut Pathog. 2020;12:1-12. doi:10.1186/s13099-020-00352-x

23. Grassotti TT, de Angelis Zvoboda D, da Fontoura Xavier Costa L, de Araújo AJG, Pereira RI, Soares RO, et al. Antimicrobial resistance profiles in *Enterococcus spp.* isolates from fecal samples of wild and captive black capuchin monkeys (Sapajus nigritus) in South Brazil. Front Microbiol. 2018;9:2366. https://doi.org/10.3389/fmicb.2018.02366

24. Amini A, Jamin F, Rosmaidar, Admi M, Armansyah TR, Amirudin. Isolation and antibiotic sensitivity test against Salmonella and Shigella in feces of Sumatran orangutan (*Pongo abelii*) and Bornean orangutan (*Pongo pygmaeus*) at North Sumatra Zoo. J Ilm Mhs Vet. 2023;7(4):101-13.