Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 4, 683-686

Multidrug resistance to antibiotics in *Escherichia coli* bacteria isolated from bats on Lombok Island, Indonesia

Alfiana L.D. Agustin[1], Fidi N.A.E.P. Dameanti[2], Mustofa H. Effendi[3*], Wiwiek Tyasningsih[4], Aswin R. Khairullah[5], Shendy C. Kurniawan[6], Ikechukwu B. Moses[7], Abdullah Hasib[8], Yolla R. Mustika[9], Kurnia N. Kinasih[9]

[1]*Doctoral Program in Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia.*
[2]*Microbiology and Immunology Laboratory, Faculty of Veterinary Medicine, Universitas Brawijaya, Jl. Puncak Dieng, Kalisongo, Malang 65151, East Java, Indonesia.*
[3]*Division of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia.*
[4]*Division of Veterinary Microbiology, Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia.*
[5]*Research Center for Veterinary Science, National Research and Innovation Agency (BRIN), Jl. Raya Bogor Km. 46 Cibinong, Bogor 16911, West Java, Indonesia.*
[6]*Master Program of Animal Sciences, Department of Animal Sciences, Specialisation in Molecule, Cell and Organ Functioning, Wageningen University and Research, Wageningen 6708 PB, Netherlands.*
[7]*Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki 480211, Nigeria*
[8]*School of Agriculture and Food Sustainability, The University of Queensland, Gatton, QLD 4343, Queensland, Australia.*
[9]*Master Program in Veterinary Science and Public Health, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia.*

ARTICLE INFO

Recieved: 04 January 2024

Accepted: 19 March 2024

***Correspondence:**

Corresponding author: Mustofa H. Effendi
E-mail address: mhelmieffendi@gmail.com

Keywords:

Bats
Multidrug resistance
E. coli
Antibiotic
Public health

ABSTRACT

Wild animals such as bats usually do not receive antibiotic therapy, however bats can have bacteria that have developed resistance to antibiotics due to water contamination from pharmaceutical factory waste disposal, expired medicines that are thrown away carelessly, feces and urine that pollute the environment. This research aimed to look at cases of multidrug resistance (MDR) in bats on the island of Lombok, Indonesia. A total of 135 bat rectal swab samples were taken using sterile cotton buds. Isolation of *E. coli* bacteria using Eosin Methylene Blue Agar media then looking at morphology and biochemical tests. The bacterial sensitivity test used the disk diffusion method using seven antibiotics, namely the antibiotics amoxicillin, ciprofloxacine, trimethoprim/sulfamethoxazole, tetracycline, gentamicin, cefotaxime and azithromycin. The research results showed that from 135 samples tested, 97 samples were positive for *E. coli*. Samples that were positive for *E. coli* were tested for sensitivity, showing that 41 (41/97, 42.26%) samples were still sensitive to all tested antibiotics and 56 samples experienced resistance with the results of samples experiencing resistance to the antibiotic azithromycin (AZM) as many as 37 samples (38.1%), amoxicillin (AML) 24 samples (24.7%), tetracycline (TE) 24 samples (24.7%), sulfamethoxazole/trimethoprim (SXT) 22 samples (22.6%), ciprofloxacine (CIP) 14 samples (14.4%), gentamicin (CN) 1 sample (1%), and for cefotaxime (CTX) 0 samples experienced resistance (0%), and 14 samples experienced multidrug resistance. These results explain that bats in caves on the Indonesian island of Lombok act as transmitters of MDR *E. coli* to public health.

Introduction

Antimicrobial-resistant (AMR) is not only a problem that occurs in Indonesia, but is a global health problem, the increasing incidence of AMR has an impact on the global economic decline and increases the number of deaths due to non-healing of patients who are infected with bacteria (Anshariet*a et al*., 2021; Siahaan *et al*., 2022). Irrational use of antibiotics in animals and humans leads to an increase in the incidence of AMR which later develops into multidrug resistance (MDR) (Khairullah *et al*., 2020; Sweileh, 2021). The potential for transmission of antibiotic resistance between humans, animals, and wildlife is a global concentration that is studied by multidisciplinary sciences in collaboration through a "One Health" approach (Velazquez-Meza *et al*., 2022).

Recent incidents of AMR *Escherichia coli* have not only been reported detected in domesticated animals, but have also been reported detected in wild animals, one of which is bats (Lagerstrom and Hadly, 2021). The ability to fly long distances, long lives, come from colonies, and can occupy various habitats make bats have the potential to acquire resistant bacteria and spread them (Rainho and Palmeirim, 2011). Wild animals that can move long distances are used as bioindicators or sentinels for the spread of antibiotic resistance in the environment (Plaza-Rodríguez *et al*., 2021). *E. coli* bacteria is a digestive tract microbiota that is often studied to see antibiotic resistance profiles (Bong *et al*., 2022). This bacterium reflects the use of antibiotics in humans, livestock, and pets.

Wild animals usually do not receive antibiotics for treatment, only wild animals in zoos and captivity receive antibiotics for therapy (da Costa and Diana, 2022). Bacterial AMR in bats is believed to be acquired from an environment contaminated with bacteria when they eat and drink (Devnath *et al*., 2022). Wild animals are contaminated with bacteria that are resistant to antimicrobials due to contact with humans, animals and the environment, anthropogenic activities carried out by humans such as hospitals, pharmaceutical industries, agricultural and fishing activities, water sources contaminated by feces are significant contamination vectors (Radhouani *et al*., 2014). Resistance genes from one bacterium can be transferred horizontally to other bacteria through mobile genetic element (MGE) mechanisms such as transponses, integrons, and plasmids (Khairullah *et al*., 2019; Riwu *et al*., 2022).

Extended Spectrum β-Lactamase (ESBL) is a serious problem faced by global health, widespread use of β-Lactam antibiotics such as third generation cephalosporins has resulted in the ineffectiveness of these drugs which can result in complications in the treatment of infections (Farizqi *et al*., 2023). The ESBL enzyme can hydrolyze higher β-Lactam antibiotics such as monobactam and cephalosporin but can be inhibited by β-Lactam inhibitors, one of which is clavulanate, this enzyme can

ISSN: 2090-6277/2090-6269/

be tested phenotypically (Prayudi *et al*., 2023). ESBL has been detected in communities, water environments, wildlife, pets, livestock, and food products (Kawamura *et al*., 2017).

Monitoring MDR and ESBL *E. coli* is important because it can be used as an indicator of the wildlife environment. Monitoring results can be compared to determine the transfer of resistance genes from humans to animals and vice versa. Apart from that, it can also be used to monitor the spread of antibiotic resistance in different ecosystems. This research was carried out to determine whether the *E. coli* bacteria in bats on the Indonesian island of Lombok are resistant to any antibiotics and to see the potential for ESBL genes in bats to obtain an overview of the situation in Lombok. The results of this research can later be used as a preventive measure for *E. coli* bacterial infections to reduce the use of antibiotics and as epidemiological surveillance.

Materials and methods

Ethical approval

Animal ethics approval was obtained via the ethical clearance committee of the Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia (Ethics no: 1.KEH.046.03.2023).

Research design

Samples were obtained by swabbing the rectum of live bats caught from caves on the island of Lombok, namely Gua Lawah in West Lombok and Pengembar cave in Central Lombok. This research was carried out from September 2023 to December 2023. Bats were caught using mist nets 20x20 mm mesh from 4 pm to 10 pm. Jaring selalu dimonitor dan apabila terdapat kelelawar yang terjaring diambil secara berkala dan siletakan ada kandang. Caught bats are handled for physical examination (sex and arm length) and rectal swab. Sampling in this study was approved by Balai Konservasi and Sumber Daya Alam Nusa Tenggara Barat.

Sample collection was carried out by cleaning the hair around the anus using 70% isopropyl alcohol to avoid bacterial contamination from the hair. A cotton swab was inserted into the rectum and rotated for a few moments to ensure that bacteria stick to the cotton swab. The swab were placed on transport media (Oxoid, UK) to be taken to the laboratory.

Isolation and identification of bacteria

A total of 135 samples on Blair carry media were planted in Brain Heart Infusion (BHI) fertilizing media (Oxoid, UK) and incubated at 37ºC for 24 hours in the Bacteriology and mycology Laboratory, Faculty of Veterinary Medicine, Airlangga University, Surabaya. The samples were then planted on Eosin Methylene Blue Agar (EMBA) media (Oxoid, UK) by stretching and then incubated at 37ºC for 24 hours. Colonies that grow metallic green are replanted to purify the bacteria on EMBA media and incubated at 37ºC for 24 hours. Gram staining tests and biochemical tests of Indol-motility (Oxoid, UK), Methyl Red and Voges Proskauer (Oxoid, UK) and Citrate (Oxoid, UK) (IMViC) were carried out at a temperature of 37ºC for 24 hours and Gram staining of bacteria growing from purification. Gram staining test was carried out by placing the bacteria on a glass object and fixing it, dripping with crystal violet for 2 minutes and washing with running water, then dripping with iodine, acetone alcohol for 1 minute, dripping with safranin for 2 minutes. The stained bacteria were examined using a microscope at 1000X magnification. Bacterial isolates confirmed to be *E. coli* were grown on Nutrient Agar (NA) media (Oxoid, UK).

Test the sensitivity of E. coli to antibiotics

Bacterial isolates confirmed to be *E. coli* were tested for antibiotic sensitivity using the Kirby-Bauer agar diffusion method on Mueller Hinton Agar (MHA) media (Oxoid, UK). Bacterial isolates in NA media were taken and a suspension was made in a tube containing NaCl and homogenized to the McFarland 0.5 standard. Suspension adjusted with McFarland 0.5 was swabbed on a plate containing MHA media, 7 antibiotics were tested to determine the sensitivity of the bacteria, namely the antibiotics amoxicillin 25 µg (AML) (Oxoid, UK), ciprofloxacine 5 µg (CIP) (Oxoid, UK), sulfamethoxazole-trimethoprim 25 µg (SXT) (Oxoid, UK), tetracycline 30 µg (TE) (Oxoid, UK), gentamicin 10 µg (CN) (Oxoid, UK), cefotaxime 30 µg (CTX) (Oxoid, UK), azithromycin 15 µg (AZM) (Oxoid, UK). Antibiotics that have been placed in MHA media are incubated at 37ºC for 24 hours. Interpretation of results is equated with Clinical Laboratory Standard Institute (CLSI) standards, results that show resistance to three or more families of antibiotics are said to be multidrug resistant (MDR).

Results

A total of 97 *E. coli* (53%) from 135 bat rectal swab samples were successfully isolated and identified. *E. coli* bacteria on EMBA media showed a metallic green metachromatic luster (Figure 1), in Gram stain test results showed a rod-shaped morphology and a reddish-pink color (Figure 2). The IMViC biochemical test results showed the presence of a pink ring, no color change in the MR medium after the methyl red reagent was dropped and there was no color change in the VP medium when the alpha naphthol reagent and 40% KOH were dropped, indicating positive *E. coli*. The green color of citrate media indicates a positive result for *E. coli*.

Fig. 1. Bacterial colonies growing on EMBA media glow metallic green metachromatic.

Table 1. Resistance to several antibiotics in *E. coli* bacteria from cave bats on the island of Lombok.

Group of antibiotics	Resistance pattern	Number of resistant isolates	Total number of isolates
0	No one is resistant	41	41
3	TE – SXT – CIP AZM – SXT – AML	2 1	3
4	TE – AZM – SXT – AML TE – AZM – SXT – CIP TE – SXT – CIP – AML	3 1 1	5
5	TE– AZM– SXT– CIP– AML	6	6

Note: TE = tetracycline, CIP = Ciprofloxacin, AML = Amoxicillin, AZM = Azithromycin, SXT = sulfamethoxazole-trimethoprim

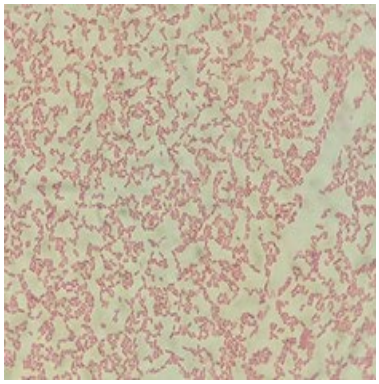

Fig. 2. The morphology of the bacteria is rod-shaped and pink-reddish in color

Bacteria identified as positive for *E. coli* were tested for sensitivity (Figure 3) with the results showing that 41 samples were still sensitive to all the antibiotics tested, 56 samples were resistant. Various resistances were shown by the test samples, where 437 samples (38.1%) were resistant to the antibiotic azithromycin (AZM), 24 samples (24.7%) of amoxicillin (AML), 24 samples (24.7%) of tetracycline (TE), sulfamethoxazole-trimethoprim (SXT) 24 samples (24.7%), ciprofloxacine (CIP) 14 samples (14.4%), gentamicin (CN) 1 sample (1%), and for cefotaxime (CTX) 0 samples experienced resistance (0%).

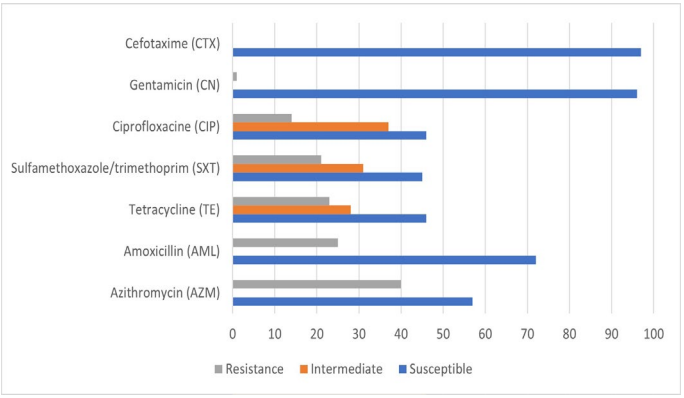

Fig. 3. Results of the sensitivity test for *E. coli* bacteria from cave bats on the island of Lombok

Discussion

The existence of antibiotic resistance against *E. coli* bacteria originating from bats indicates that the environment in which bats live, or the environment used for foraging for food has been contaminated with antibiotics. *E. coli* bacteria in wild animals are used as a parameter for antibiotic resistance of livestock, differences in geographic location and taxonomy of wild animals interpret different antibiotic exposure, areas that have high human and animal populations and intensive agricultural activities show higher antibiotic resistance results than with areas with lower populations (van den Honert *et al*., 2021). The emergence of antibiotic resistance in Enterobacteriaceae can cause problems in public health (Aslam *et al*., 2018).

The spread of genetic elements such as transpon, intergon and insertion in Enterobacteriaceae can cause the rapid spread of antibiotic resistance between humans and animals or vice versa, this spread can occur due to low levels of hygiene in humans, pollution of water and soil, as well as watering in agriculture (Sabbagh *et al*., 2021). Of the 97 samples of *E. coli* bacteria that were resistant to antibiotics, it was found that 14 (14.4%) samples had multidrug resistance, 3 (3.09%) samples had resistance to three classes of antibiotics, 6 (6.18%) samples resistant to four classes of antibiotics and 5 (5.15%) samples were resistant to five classes of antibiotics, azithromycin 38.1%, amoxicillin 24.7%, tetracycline 23.7%, sulfamethoxazole-trimethoprim 22.6%, and ciprofloxacine 14.4 % are antibiotics that often appear as antibiotics that have experienced resistance (Table 1).

There is still little research on antibiotic resistance in wild animals, especially bats in Indonesia. In previous research, *E. coli* in the bats studied was resistant to trimethoprim, sulphametoxazole, and cephalotin (Devnath *et al*., 2022). Cephalotin antibiotic showed the greatest resistance during the test, namely 21.42%. In contrast to this study, the β-lactam antibiotic was in second place with a total resistance of 24.7%. The macrolide class of antibiotics in this study showed the highest resistance results, namely 38.1%. The antibiotic azhithromycin is an antibiotic that has great antibacterial activity for Gram-positive and Gram-negative, this macrolide class antibiotic is used for treatment in humans and prevention of disease in animals because this antibiotic has low toxicity (McMullan and Mostaghim, 2015).

The results of third generation cephalosporin antibiotic tests on *E. coli* bacteria from bats from Lombok showed that all samples were still sensitive to this antibiotic. Third generation cephalosporin antibiotics such as cefotaxime are antibiotics that often appear as resistant antibiotics in test samples (Menkem *et al*., 2023). The results of the sample sensitivity test to tetracycline antibiotics showed that 24.7% had experienced resistance. In Indonesia, tetracycline antibiotics and the macrolide group are antibiotics that are often used on dairy farms. This antibiotic is used as a treatment for the digestive tract, respiratory tract and mastitis (Ramandinianto *et al*., 2020). The results of research on antibiotic sensitivity tests on *E. coli* bacteria in cattle and chickens in Lombok show a correlation with the results that occur in bats, the results of resistance tests against macrolide and tetracycline antibiotics show quite high levels of resistance as in this study, namely erythromycin (macrolide group) 94.4% in bats, tetracycline 61.1% in cattle and tetracycline 66.6% in chickens (Agustin *et al*., 2022; Kholik, 2022).

Tetracycline and sulfamethoxazole class antibiotics are used to increase production in chicken, pig, and fish farms (Chowdhury *et al*., 2021). The antibiotics tetracycline, chlortetracycline, bacitracin, neomycin, tylosin, virginiamycin, and avopracin are antibiotics that are often chosen for disease prevention and growth promoters at doses that are lower than doses for therapy, the use of antibiotics that do not reach the drinking dose to kill the bacteria can cause resistance bacteria in animals (Chopra and Roberts, 2001). The gentamicin antibiotic in this study showed a very low level of resistance, namely only one sample showed resistance, but in contrast to research conducted by Nowakiewicz *et al*. (2020) aminoglycoside antibiotics showed the highest level of resistance, namely 84%.

Based on this research, it can be assumed that the bats that were the object of the research looked for food in the form of insects in the livestock environment around the cave. Bats can fly away from the nest to a location where the insects come out every night (Bhalla *et al*., 2023). Higher deforestation activities or in places with fewer food sources will also affect bat numbers (Appel *et al*., 2023). Most of the antibiotics given to livestock are excreted in feces and urine, only small amounts of effective antibiotics are metabolized by livestock (Zubair *et al*., 2020). The results of the report show that 80% of antibiotics sold in the United States are used to increase animal growth and protect livestock from infection (Tian *et al*., 2021). The use of feed additives containing antibiotics can form antibiotic resistance in bacteria (Riwu *et al*., 2022).

The excretion of antibiotics given to livestock along with urine and feces can pollute the environment where the insects that feed bats live (Tian *et al*., 2021). Insects which are a food source for bats on farms consume organic product waste for food and development, especially feces from livestock experiencing diarrhea or soft feces which provide a place to breed (Paliy *et al*., 2021). Apart from contamination from livestock near the cave, we also assume that bats can be contaminated by antibiotics in the soil and water environment when they are looking for water (Mpakosi and Mironidou-Tzouveleki, 2023).

There are antibiotics in the soil and water environment because human feces and urine treated with antibiotics are not disposed of properly in storage tanks and there is poor wastewater treatment, disposal of unused medicines and careless disposal of expired medicines (Polianciuc *et al*., 2020). Bats are wild animals that are difficult to control. Preventing the spread of multidrug resistance in wild animals needs to be done to reduce risks to human health and animal health (da Costa *et al*., 2013). Farmers need to create good livestock programs and improve biosecurity to minimize the use of antibiotics. Communities need to maintain sanitation hygiene, prevent antibiotic contamination of soil and water so that wild animals are not contaminated (Wibisono *et al*., 2021).

Conclusion

The presence of bats showing multidrug resistance results explains that bats have the potential to spread MDR and can endanger animal and human health.

Acknowledgments

Researchers would like to thank the Faculty of Veterinary Medicine, Airlangga University for all the facilities provided during conducting research. This research was supported by funding for Doctoral Dissertation research from the Center for Higher Education Fund (BBPT) and Indonesia Endowment Funds for Education (LPDP), with a grant number from

Keputusan Kepala Pusat Layanan Pembiayaan Pendidikan Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi. Number: 00087/J5.2.3./ BPI.06/9/2022. BPI ID Number: 202209090573.

Conflict of interest

The authors have declared no conflict of interest.

References

Agustin, A.L.D., Ningtyas, N.S.I., Tirtasari, K., 2022. Resistance of *Escherichia coli* Isolated from Layer Chicken in Sesaot Village, West Lombok District. Vet. Med. J. 33, 87–95. doi:10.20473/mkh.v33i2.2022.87-95.

Ansharieta, R., Ramandinianto, S.C., Effendi, M.H., Plumeriastuti, H., 2021. Molecular identification of blaCTX-M and blaTEM genes encoding extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* isolated from raw cow's milk in East Java, Indonesia. Biodiversitas 22, 1600–1605. doi:10.13057/biodiv/d220402.

Appel, G., Meyer, C.F.J., Bobrowiec, P.E.D., 2023. Fear or food? Prey availability is more important than predation risk in determing aerial insectivorous bat responses across a disturbed tropical forest landscape. Biodiv. Conserv. 32, 3217–3235. doi:10.1007/s10531-023-02647-2

Aslam, B., Wang, W., Arshad, M.I., Khurshid, M., Muzammil, S., Rasool, M.H., Nisar, M.A., Alvi, R.F., Aslam, M.A., Qamar, M.U., Salamat, M.K.F., Baloch, Z., 2018. Antibiotic resistance: a rundown of a global crisis. Infect. Drug Resist. 11, 1645–1658. doi:10.2147/IDR.S173867.

Bhalla, I.S., Razgour, O., Rigal, F., Whittaker, R.J., 2023. Insectivorous bats in Indian rice fields respond to moonlight, temperature and insect activity. Lands Ecol. 38, 2947–2963. doi:10.1007/s10980-023-01764-1.

Bong, C.W., Low, K.Y., Chai, L.C., Lee, C.W., 2022. Prevalence and Diversity of Antibiotic Resistant *Escherichia coli* From Anthropogenic-Impacted Larut River. Front. Public Health 10, 794513. doi:10.3389/fpubh.2022.794513.

Chowdhury, S., Ghosh, S., Aleem, M.A., Parveen, S., Islam, M.A., Rashid, M.M., Akhtar, Z., Chowdhury, F., 2021. Antibiotic Usage and Resistance in Food Animal Production: What Have We Learned from Bangladesh? Antibiotics (Basel) 10, 1032. doi:10.3390/antibiotics10091032.

Chopra, I., Roberts, M., 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol. Mol. Biol. Rev. 65, 232–260. doi:10.1128/MMBR.65.2.232-260.2001.

da Costa, M.R., Diana, A., 2022. A Systematic Review on the Link between Animal Welfare and Antimicrobial Use in Captive Animals. Animals (Basel) 12, 1025. doi:10.3390/ani12081025.

da Costa, P.M., Loureiro, L., Matos, A.J., 2013. Transfer of multidrug-resistant bacteria between intermingled ecological niches: the interface between humans, animals and the environment. Int. J. Environ. Res. Public Health 10, 278–294. doi:10.3390/ijerph10010278.

Devnath, P., Karah, N., Graham, J.P., Rose, E.S., Asaduzzaman, M., 2022. Evidence of Antimicrobial Resistance in Bats and Its Planetary Health Impact for Surveillance of Zoonotic Spillover Events: A Scoping Review. Int. J. Environ. Res. Public Health 20, 243. doi:10.3390/ijerph20010243.

Farizqi, M.T.I., Effendi, M.H., Adikara, R.T.S., Yudaniayanti, I.S., Putra, G.D.S., Khairullah, A.R., Kurniawan, S.C., Silaen, O.S.M., Ramadhani, S., Millannia, S.K., Kaben, S.E., Waruwu, Y.K.K., 2023. Detection of extended-spectrum β-lactamase-producing *Escherichia coli* genes isolated from cat rectal swabs at Surabaya Veterinary Hospital, Indonesia. Vet. World 16, 1917–1925. doi:10.14202/vetworld.2023.1917-1925.

Kawamura, K., Nagano, N., Suzuki, M., Wachino, J.I., Kimura, K., Arakawa, Y., 2017. ESBL-producing *Escherichia coli* and Its Rapid Rise among Healthy People. Food Saf. (Tokyo) 5, 122–150. doi:10.14252/foodsafetyfscj.2017011.

Khairullah, A.R., Raharjo, D., Rahmahani, J., Suwarno, Tyasningsih, W., Harijani, N., 2019. Antibiotics resistant at Staphylococcus aureus and Streptococcus sp. isolated from bovine mastitis in Karangploso, East Java, Indonesia. Indian J. Forensic Med. Toxicol. 13, 439–444. doi:10.5958/0973-9130.2019.00329.3.

Khairullah, A.R., Ramandinianto, S.C., Effendi, M.H., 2020. A Review of Livestock-Associated Methicillin-Resistant Staphylococcus aureus (LA-MRSA) on Bovine Mastitis. Syst. Rev. Pharm. 11, 172–183. doi:10.31838/srp.2020.7.28.

Kholik, K., 2022. Detection of antibiotic resistant in *Escherichia coli* from the reproductive tract of Bali cattle on smallholder farm. J. Biosains Pascasarjana 24, 44–53. doi:10.20473/jbp.v24i1SP.2022.44-53

Lagerstrom, K.M., Hadly, E.A., 2021. The under-investigated wild side of *Escherichia coli*: genetic diversity, pathogenicity and antimicrobial resistance in wild animals. Proc. Biol. Sci. 288, 20210399. doi:10.1098/rspb.2021.0399.

McMullan, B.J., Mostaghim, M., 2015. Prescribing azithromycin. Aust. Prescr. 38, 87–89. doi:10.18773/austprescr.2015.030.

Menkem, E.Z., Nanfah, A.L., Takang, T., Awah, L.R., Achua, K.A., Akume S.E., Fekam Boyom, F.F., 2023. Attitudes and Practices of the Use of Third-Generation Cephalosporins among Medical Doctors Practicing in Cameroon. Int. J. Clin. Pract. 2023, 8074413. doi:10.1155/2023/8074413.

Mpakosi, A., Mironidou-Tzouveleki, M., 2023. The Cave Ecosystem in the Research of New Antibiotic Discovery and Development. Environ. Sci. Proc. 26, 116. doi:10.3390/environscproc2023026116

Nowakiewicz, A., Zięba, P., Gnat, S., Trościańczyk, A., Osińska, M., Łagowski, D., Kosior-Korzecka, U., Puzio, I., 2020. Bats as a reservoir of resistant *Escherichia coli*: A methodical view. Can we fully estimate the scale of resistance in the reservoirs of free-living animals? Res. Vet. Sci. 128, 49–58. doi:10.1016/j.rvsc.2019.10.017.

Paliy, A.P., Mashkey, A.N., Faly, L.I., Kysterna, O.S., Rebenko, H.I., Palii, A.P., 2021. Ecology of zoophilic flies in livestock biocenoses of Ukraine. Biosyst. Divers. 29, 258–263. doi:10.15421/012132.

Plaza-Rodríguez, C., Alt, K., Grobbel, M., Hammerl, J.A., Irrgang, A., Szabo, I., Stingl, K., Schuh, E., Wiehle, L., Pfefferkorn, B., Naumann, S., Kaesbohrer, A., Tenhagen, B.A., 2021. Wildlife as Sentinels of Antimicrobial Resistance in Germany? Front. Vet. Sci. 7, 627821. doi:10.3389/fvets.2020.627821.

Polianciuc, S.I., Gurzău, A.E., Kiss, B., Ştefan, M.G., Loghin, F., 2020. Antibiotics in the environment: causes and consequences. Med. Pharm. Rep. 93, 231–240. doi:10.15386/mpr-1742.

Prayudi, S.K.A., Effendi, M.H., Lukiswanto, B.S., Az Zah-ra, R.L., Benjamin, M.I., Kurniawan, S.C., Khairullah, A.R., Silaen, O.S.M., Lisnanti, E.F., Baihaqi, Z.A., Widodo, A., Riwu, K.H.P., 2023. Detection of Genes on *Escherichia coli* Producing Extended Spectrum β-lactamase Isolated from the Small Intestine of Ducks in Traditional Markets Surabaya City, Indonesia. J. Adv. Vet. Res. 13, 1600–1608.

Radhouani, H., Silva, N., Poeta, P., Torres, C., Correia, S., Igrejas, G., 2014. Potential impact of antimicrobial resistance in wildlife, environment and human health. Front. Microbiol., 5, 23. doi:10.3389/fmicb.2014.00023.

Rainho, A., Palmeirim, J.M., 2011. The importance of distance to resources in the spatial modelling of bat foraging habitat. PLoS One 6, e19227. doi:10.1371/journal.pone.0019227.

Ramandinianto, S.C., Khairullah, A.R., Effendi, M.H., Hestiana, E.P., 2020. Profile of multidrug resistance (MDR) and methicillin resistant Staphylococcus aureus (MRSA) on dairy farms in east Java province, Indonesia. Indian J. Forensic Med. Toxicol. 14, 3439–3445. doi:10.37506/ijfmt.v14i4.12157.

Riwu, K.H.P., Rantam, F.A., Effendi, M.H., Khairullah, A.R., Widodo, A., Kurniawan, S.C., Ugbo, E.N., 2022. Molecular detection of extended spectrum β-lactamase producing Klebsiella pneumoniae from wild deer. Biodiversitas 23, 4256–4262. doi:10.13057/biodiv/d230849.

Sabbagh, P., Rajabnia, M., Maali, A., Ferdosi-Shahandashti, E., 2021. Integron and its role in antimicrobial resistance: A literature review on some bacterial pathogens. Iran J. Basic Med. Sci. 24, 136–142. doi:10.22038/ijbms.2020.48905.11208.

Siahaan, S., Herman, M.J., Fitri, N., 2022. Antimicrobial Resistance Situation in Indonesia: A Challenge of Multisector and Global Coordination. J. Trop. Med. 2022, 2783300. doi:10.1155/2022/2783300.

Sweileh, W.M., 2021. Global research publications on irrational use of antimicrobials: call for more research to contain antimicrobial resistance. Global Health 17, 94. doi:10.1186/s12992-021-00754-9.

Tian, M., He, X., Feng, Y., Wang, W., Chen, H., Gong, M., Liu, D., Clarke, J.L., van Eerde, A., 2021. Pollution by Antibiotics and Antimicrobial Resistance in Livestock and Poultry Manure in China, and Countermeasures. Antibiotics (Basel) 10, 539. doi:10.3390/antibiotics10050539.

Velazquez-Meza, M.E., Galarde-López, M., Carrillo-Quiróz, B., Alpuche-Aranda, C.M., 2022. Antimicrobial resistance: One Health approach. Vet. World. 15, 743–749. doi:10.14202/vetworld.2022.743-749.

van den Honert, M.S., Gouws, P.A., Hoffman, L.C., 2021. *Escherichia coli* Antibiotic Resistance Patterns from Co-Grazing and Non-Co-Grazing Livestock and Wildlife Species from Two Farms in the Western Cape, South Africa. Antibiotics (Basel) 10, 618. doi:10.3390/antibiotics10060618.

Wibisono, F.J., Sumiarto, B., Untari, T., Effendi, M.H., Permatasari, D.A., Witaningrum, A.M., 2021. Molecular identification of CTX gene of extended spectrum beta-lactamases (ESBL) producing *Escherichia coli* on layer chicken in Blitar, Indonesia. J. Anim. Plant Sci. 31, 954–959. doi:10.36899/JAPS.2021.4.0289.

Zubair, M., Li, Z., Zhu, R., Wang, J., Liu, X., Liu, X., 2020. The Antibiotics Degradation and Its Mechanisms during the Livestock Manure Anaerobic Digestion. Molecules 28, 4090. doi:10.3390/molecules28104090.