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Multi-Drug Resistant (MDR) Detection in *Escherichia coli* in Canary Birds (*Serinus canaria*) Imported from Malaysia

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Abstract

The frequency of canary imports continues to increase every year. Antibiotic resistance is a global problem that threatens human and animal health worldwide. Human interaction with birds as pets is a public health concern because it has the potential to increase zoonotic diseases. This study was conducted to identify the antibiotic resistance of *E. coli* using fecal swab samples of imported canaries from Malaysia. Samples were grown on an EMBA medium for the isolation test, Gram staining test, and IMViC test were performed to continue the identification test -Kirby-Bauer diffusion test - to determine antibiotic sensitivity. Based on morphological culture, Gram staining, and biochemical tests, the sample examination results showed 18% (27/150) were positive for *E. coli*, 16% (24/150) showed the highest resistance to tetracycline, 15,33% (23/150) amoxicillin, 12,66% (19/150) trimethoprim-sulfamethoxazole, 6% (9/150) ciprofloxacin, and 14,66% (22/150) isolates were confirmed MDR because they were resistant to three to four antibiotics. Further efforts are needed to understand and address the factors that lead to antibiotic resistance in the context of animal and public health. Prudent management of antibiotic use and monitoring of antibiotic resistance needs to be improved to maintain animal health and prevent the risk of transmission of resistant bacteria to humans.

Keywords

Communicable disease, *Escherichia coli*, multi-drug resistance, public health concern.

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Introduction

The high public interest in keeping pet birds has led to an increase in the bird trade in Indonesia, especially in East Java. The frequency of canary imports from Malaysia continues to increase every year. According to data from the East Java Animal, Fish, and Plant Quarantine Center in 2021, there were four imports of canaries totaling 4,250 birds. In 2022, the frequency of imports increased to 14 times with a total of 11,807 birds. It should be noted that bird trade traffic during travel can allow the transmission of antibiotic-resistant bacteria or antimicrobial resistance (AMR) genes (Oteo *et al*., 2018; Bottery *et al*., 2021). Pet birds are considered a source of multi-drug resistance bacteria (MDR) (Diren Sigirci *et al*., 2020).

Antibiotic resistance is a global problem that threatens human and animal health worldwide (Zawack *et al*., 2016; WHO, 2023). The World Health Organization (WHO) states that antibiotic resistance poses a serious challenge to human health, causing more than 700,000 deaths from bacterial infections each year worldwide (Zarei-Baygi *et al*., 2021). Research by O'Neill (2016) stated that the threat of antibiotic resistance, if uncontrolled, could lead to an increase in mortality. Human interaction with birds as pets is a public health concern because it has the potential to increase zoonotic diseases (Ahmed *et al*., 2021; Mohamed *et al*., 2022). Poultry, especially birds as pets, have been identified as an environment where antibiotic-resistant bacteria can thrive (Dalazen *et al*., 2023; Islam et al., 2023). This statement was reinforced by Fuentes-Castillo *et al*. (2019) and Melo *et al*. (2019) who state that there are antibiotics resistant to *E. coli* bacteria. Aklilu *et al*. (2022) suggested that poultry can be a reservoir of MDR *E. coli*, which has the potential to cause human infections. Pathogenic *E. coli* can cause gastroenteritis, bacteremia, and urinary tract infections in humans (Ong *et al*., 2020). In poultry, *E. coli* causes aerosacculitis, polyserositis, septicemia, and other extraintestinal diseases (Varriale *et al*., 2020). Resistant pathogenic bacteria can proliferate in humans, animals, and the environment, threatening public health (Landecker, 2016).

Antibiotic resistance refers to changes in bacteria that make them immune to the effects of antibiotics (Apriliani and Pinatih, 2017). *E. coli* is a reservoir of antibiotic-resistance genes and can transfer genes encoding antibiotic-resistance traits to other bacterial species (O'Neill, 2016; Harijani *et al*., 2020). *E. coli* can be resistant to more than one type of antibiotic (Xia *et al*., 2016). In the study conducted by Diren Sigirci *et al*. (2020), it was found that the majority of *E. coli* isolates exhibited resistance to tetracycline (84%), sulfamethoxazole (46%), streptomycin (34%), and kanamycin (25%). The results of research by Mohamed *et al*. (2022) stated that *E. coli* isolates showed multi-drug resistance to all antibiotics. One of the signs and characteristics of *E. coli* can spread resistance genes because of its ability to form MDR (Effendi *et al*., 2022).

The administration of antibiotics without medical control is common and contributes to the increase in antibiotic resistance (Varriale *et al*., 2020). Antibiotic resistance of *E. coli* bacteria in humans, animals, and the environment is considered a public health issue (Yılmaz and Dolar, 2017). However, research data indicating MDR resistance to *E. coli* bacteria in imported bird traffic are limited. With the increasing frequency of imported birds from Malaysia, research is needed to detect MDR resistance in *E. coli*. This study is expected to provide an overview of the potential of imported canary bird traffic as a reservoir for the spread of *E. coli*

capable of producing multi-drug resistance in the community.

Materials and Methods

Study Period and Location

The study was conducted from October to December 2023. Fecal swab samples of imported canaries from Malaysia were taken at the Animal Quarantine Installation in Malang City, and bacterial isolation and sensitivity tests were conducted at the East Java Animal, Fish, and Plant Quarantine Center Laboratory.

Sample

As many as 150 fecal swab samples of imported canaries from Malaysia with fresh feces criteria were collected. Swabs were labeled, and sampling was performed aseptically. Sterile swab sticks (Oxoid, Bangistoke, UK) were placed in tubes containing 2.5% Buffered Peptone Water (BPW). Then, the samples were stored in a cooler box and brought to the laboratory.

Isolation and Identification

Canary fecal swab samples (n=150) were grown on Eosin Methylene Blue Agar (EMBA) media in streaks and incubated at 37^0C for 24 hours to select and isolate *E. coli*. One colony with typical *E. coli* morphology was selected and identified using conventional methods (Yilmaz and Guvensen, 2016).

Sensitivity Test

The antibiotic sensitivity test using the Kirby-Baurer method with the disk diffusion test produces qualitative categories with sensitive, intermediate, and resistant assessments (Indana *et al*., 2020). Bacterial cultures obtained from colonies found on EMBA media were grown in test tubes containing 8 ml of physiological NaCl, homogenized using a vortex until a turbidity equal to the McFarland standard of 0.5 was obtained, equivalent to $1.5x10^8$ cfu/ml. Then, 0.2 ml was taken and applied to the entire surface of Mueller Hinton Agar (MHA) media. Germs were allowed to stick to the media for 15 minutes, then antibiotic disks were placed on MHA media. The disc was slightly pressed on the surface of the agar and then the bacterial culture incubated at 37^0C for 24 hours.

Results and Discussion

Isolation and identification

The results of morphological culture, Gram staining, and biochemical testing, showed that 19, 33% (29/150) presented *E. coli* (Table 1). The presence of metallic green bacterial colonies on EMBA media signifies a successful morphological culture of *E. coli* (Figure 1). The presence of red colonies and short rods on the Gram stain indicates a negative Gram stain result (Figure 2). Indole ring on SIM test (positive Indole), inverted fir tree formation on SIM test (Motile), red color change on methyl red (MR) test (positive MR), yellow color on Voges-Proskauer (VP) test (negative VP), and green color on citrate test (negative citrate) indicate that the IMViC test results are positive for *E. coli* (Figure 3). IMViC test results showed 18% (27/150) were positive for *E. coli* (Table 1).

Table 1. IMViC test.

Test	*Eosin Methylene Blue Agar*	Indole	Motile	Citrate	*Methyl Red*	*Voges Proskauer*
Total	29	27	27	27	27	27

Figure 1. Escherichia coli colonies in Eosin Methylene Blue Agar.

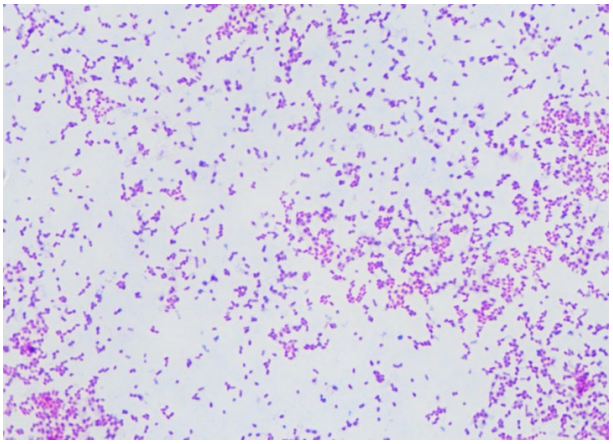

Figure 2. Gram-stained Escherichia coli colonies under the microscope.

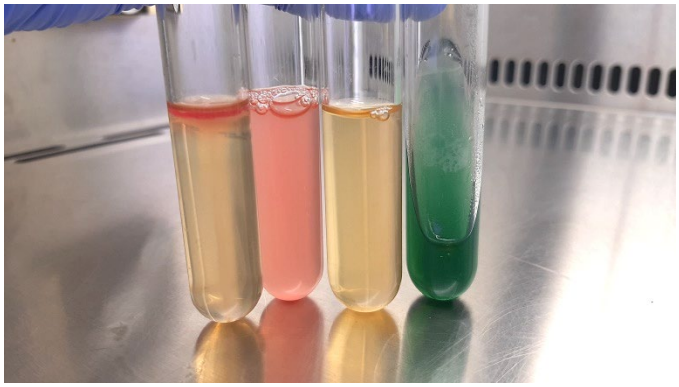

Figure 3. IMViC test results indicate Escherichia coli

EMBA media culture contains *E. coli* bacteria; the acid produced during lactose fermentation will produce green colonies with a metallic sheen (Ansharieta *et al*., 2021). With microscopic identification at 1000x magnification, Gram-stained *E. coli* bacteria

showed a short rod-shaped morphology and a red color. This is because the thin nature of the Gram-negative bacterial cell wall, which consists of lipoproteins and peptidoglycan, prevents *E. coli* from retaining crystal violet dye during the Gram-staining procedure (Rahmahani *et al*., 2020).

Positive indole test results are evidenced by a red ring at the top after the addition of Kovac's reagent, and there is turbidity that resembles an upside-down fir tree in the area of the needle prick. The observed turbidity serves as evidence of *E. coli's* capability to move on semisolid substrates (Yanestria *et al*., 2022). The enzyme tryptophanase, synthesized by *E. coli*, catalyzes the breakdown of tryptophan into indole and pyruvic acid, resulting in the development of a red ring (Jayan and Sun, 2022).

A positive methyl-red test result is indicated by a yellow-to-red color change. This is because bacteria oxidize glucose by producing acid, the methyl-red test results turn red (Bren *et al*., 2016). A methyl red pH indicator was added after the media was incubated for 48 hours. The pH of the media can drop to ≤4.4 as a consequence of glucose fermentation and the production of various acidic chemicals by *E. coli* (Rombouts *et al*., 2020). After being diluted with α-naphthol and a 40% KOH solution, the Voges-Proskauer test showed a negative result marked by a change in color to brownish yellow. This can be attributed to *E. coli's* capacity to convert carbohydrates into acidic compounds instead of neutral products like acetoin (Förster and Gescher, 2014).

The results of the citrate test performed with Simmons Citrate Agar (SCA) medium (CM0155 Oxoid™) after a 24-hour incubation period at 37°C were characterized by a green color. However, no color change was observed on the citrate test media, indicating a negative result for *E. coli*. This is because *E. coli* bacteria do not utilize citrate as a carbon source in the environment (Zlatkov and Unlin, 2019).

Antibiotic Sensitivity Test

The Kirby-Bauer diffusion method was used to determine the sensitivity of bacteria on MHA media using a 0.5 McFarland standard bacterial suspension. The zone of inhibition was measured with a caliper and then compared with the CLSI 2020 standard (Table 2) after the media was incubated for 24 hours at 37°C. 22 *E. coli* isolates showed resistance to three to four different antibiotics, with an MDR of 14.66% (22/150).

Table 2. Interpretation of inhibition zone measurements based on the provisions of the Clinical and Laboratory Standard Institute (CLSI, 2020).

Antibiotics	Disk Content	Resistant ≤ mm	Intermediate	Sensitive ≥ mm
Ceftazidime	30 µg	17	18-20	21
Amoxicillin	20 µg	13	14-17	18
Ciprofloxacin	5 µg	21	22-25	26
Tetracycline	30 µg	11	12-14	15
Trimethoprim sulfamethoxazole	23.75 µg	10	11-15	16

The results of the *E. coli* antibiotic resistance test showed the highest level of resistance to tetracycline 16% (24/150). This study determined the level of resistance of *E. coli* to various antibiotics, including 15,33% (23/150) to amoxicillin, 12, 66% (19/150) to trimethoprim-sulfamethoxazole, 6% (9/150) to ciprofloxacin, and no antibiotic resistance to ceftazidime, as shown in Table 3.

Table 3. Resistance Test.

Antibiotics	Resistant	Intermediate	Sensitive
CAZ	-	-	27
AML	23	-	4
CIP	9	-	18
TE	24	1	2
SXT	19	-	8

Notes: CAZ = Ceftazidime, AML = Amoxicillin, CIP = Ciprofloxacin, TE = Tetracycline, SXT = Trimethoprim sulfamethoxazole.

The antibiotic resistance profile based on the results of the *E. coli* resistance test showed that of the 18% (27/150) positive *E. coli*, one isolate was resistant to one class of antibiotics, while two isolates were resistant to two classes of antibiotics, and 14% (22/150) isolates were confirmed MDR because they were resistant to three to four classes of antibiotics (Figure 4). The pattern of antibiotic resistance is as follows: AML-TE-SXT, AML-CIP-TE, and AML-CIP-TE-SXT, each with one isolate (Table 4).

Table 4. Resistance Pattern.

Resistance Pattern	Total
AML-TE-SXT	14
AML-CIP-TE	4
AML-CIP-TE-SXT	4
MDR	**22**

Notes: CAZ = Ceftazidime, AML = Amoxicillin, CIP = Ciprofloxacin, TE = Tetracycline, SXT = Trimethoprim sulfamethoxazole.

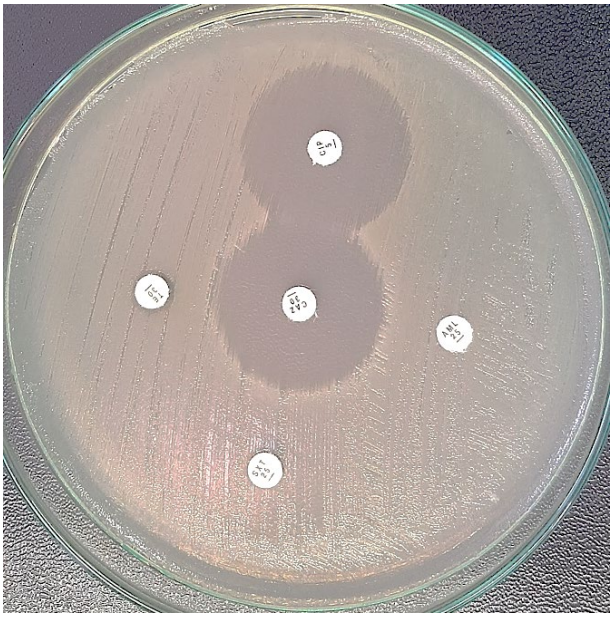

Figure 4. Analysis of the susceptibility to antibiotics of an Escherichia coli

Antibiotic resistance is one of the public health problems facing the world. Organisms resistant to antibiotics can spread rapidly, posing a danger to the population in the form of more difficult-to-cure and treat types of infectious diseases (Li and Webster, 2018). There were 24 isolates (16%) that showed the highest resistance to tetracycline. Tetracycline is a bacteriostatic antibiotic (Simanjuntak *et al*., 2022). Tetracycline works by inhibiting protein synthesis in the 70s and 80s subunits of the ribosome. The mechanism of action of tetracyclines involves suppression of protein synthesis in bacterial cells, which can be achieved through direct inhibition as well as structural modifications to inhibit the protein synthesis process. Resistance to tetracycline is due to the presence of genes encoding resistance, namely extrachromosomal genes that can replicate and synthesize proteins for plasmid needs (Agustanty and Andre, 2022).

Amoxicillin includes antibiotics in the form of β-lactam with a broad spectrum that is bactericidal, and effective in inhibiting Gram-positive and Gram-negative bacteria (Maida and Kinanti, 2019). There were 23 isolates (15,33%) that showed antibiotic resistance to amoxicillin. Amoxicillin works by inhibiting bacterial cell wall synthesis through binding to penicillin-binding proteins (PBPs). This results in the inhibition of the final stage in the transpeptidase of peptidoglycan synthesis in the bacterial cell wall. As a result, the cell wall biosynthesis process is inhibited, and the bacterial cell is lysed because the β-lactam compound binds to one or more of the penicillin-protein bonds (Anggita *et al*., 2022).

There were 19 isolates (12, 66%) that showed the maximum level of antibiotic resistance to trimethoprim-sulfamethoxazole. Trimethoprim is considered a sulfonamide potentiator used in combination with sulfonamides. This drug combination has a synergistic effect, blocking folic acid metabolism in bacteria through two different mechanisms. However, misuse of trimethoprim leads to drug residues in animal foods and bacterial resistance in farm animals.

The effects of trimethoprim residues in humans can cause serious problems, such as antibiotic resistance, nausea, emesis, headache, pruritus, and rash (Chen *et al*., 2017).

Treatment failure may occur due to microbial resistance to effective broad-spectrum antibiotics. Treatment failure and difficult-to-treat infections can lead to high mortality rates. Drug target mutations (DNA gyrase and DNA topoisomerase IV), are mutations that limit drug accumulation, and plasmids that protect cells from lethal effects (Aslam *et al*., 2018). Three mechanisms of ciprofloxacin resistance that have been found are changes in target enzymes, changes in drug permeation, and plasmid-mediated quinolone resistance (Syari'ati *et al*., 2022). There were nine isolates (6%) that showed the maximum level of antibiotic resistance to ciprofloxacin.

The occurrence of MDR *E. coli* in imported birds can be caused by several factors, including environmental factors related to the feeding habits of the birds. Different feeding habits affect the presence of *E. coli* pathogens in birds, as reported in several surveys (Hughes *et al*., 2009; Kobayashi *et al.,* 2009). In addition, high humidity and low temperature are a good atmosphere for *E. coli* to survive (Calero-Cáceres *et al*., 2017). Research results from Al-Mustapha *et al.* (2023) reported the occurrence of phenotypic resistance to 1-7 classes of antibiotics (MDR) other than beta-lactam antibiotics. The most common resistance was to fluoroquinolones (especially ciprofloxacin, nalidixic acid, levofloxacin, and moxifloxacin), tetracyclines (oxytetracycline and tigecycline), aminoglycosides (gentamicin, streptomycin, kanamycin, and tobramycin) and phenicols (florfenicol and chloramphenicol). These findings align with other studies conducted in Nigeria, which have also reported multi-drug resistance (MDR) among extended-spectrum beta-lactamase (ESBL) *E. coli* isolates of poultry origin (Ayandiran *et al*., 2018; Ayeni *et al*., 2020; Aworh *et al*., 2021; Tama *et al*., 2021; Al-Mustapha *et al*., 2022). Mohamed *et al*. (2022) reported all *E. coli* isolates showed resistance to all antibiotics tested, with 100% MDR (196/196) from wild bird, chicken, and environmental samples in Malaysia.

Antibiotic resistance can be caused by factors originating from the bacteria themselves. Genetic changes that occur in bacteria can cause bacteria that are initially sensitive to an antibiotic to become less sensitive or even completely insensitive or resistant (Islam *et al*., 2023). Genetic changes can occur through multi-drug resistance mutations, transposons, and integrons. This mechanism causes the carrier gene (plasmid) to fuse into the bacterial chromosome so that the resistance that occurs persists and can even be passed down to the next generation (Sari *et al*., 2015).

In Nigeria, several studies have reported the overuse and prescription of antibiotics in humans and animals, particularly, ESBL *E. coli* (Ojo et al., 2016; Ayandiran *et al*., 2018; Al-Mustapha et al., 2020). To date, it remains unclear whether this is a major driver of resistance. In low- and middle-income countries (LMICs), other contextual factors such as poor diagnostic capacity, a lack of antibiotic stewardship programs, poor animal disease surveillance systems, and biosecurity measures, may be involved. The use of unconfirmed infection-spectrum antimicrobials may contribute to the emergence and dissemination of multi-drug resistance bacteria, including extended-spectrum beta-lactamase (ESBL) *E. coli*, in both humans and

animals (Ikhimiukor *et al*., 2022). Despite the widespread belief that illicit antimicrobial use is the primary cause of antimicrobial resistance (AMR) (Holmes *et al*., 2016), research has shown that several other determinants impact the emergence and spread of AMR pathogens (Holmes *et al*., 2016; Silva *et al*., 2021). In general, there is no single solution, and multiple synergistic, overlapping, and complementary approaches are required, with the overarching common goal of reducing bacterial zoonotic transmission and associated economic losses.

Conclusion

Identification results showed that 18% (27/150) were positive for *E. coli*. Antibiotic sensitivity tests showed 14, 66% (22/150) of *E. coli* isolates were confirmed MDR as they were resistant to two to three antibiotics, with the highest level of resistance to tetracycline. This study provides important insights into the level of antibiotic resistance of *E. coli* in imported canaries from Malaysia. Further efforts need to be made to understand and address the factors causing resistance. Prudent management of antibiotic use and resistance monitoring need to be improved to maintain animal health and prevent the risk of transmission of resistant bacteria to humans.

Approval of Ethical Commission

All tests were conducted without harming or sacrificing any live animals. Therefore, ethical clearance for animal experiments was deemed unnecessary. Field investigations were executed with official approval from the Quarantine Agency in Indonesia, ensuring no animals were harmed for the duration of the study. Additionally, no human participants were engaged in this research.

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Author's Contribution

II, MHE, and M'AK: Conceived, designed, and coordinated the study. FKS, TEP, and DAR: Designed data collection tools and supervised the field sample and data collection, laboratory work, and data entry. M and BS: Validation, supervision, and formal analysis. DR: Carried out the statistical analysis and interpretation and participated in preparing the manuscript. All authors have read, reviewed, and approved the final manuscript.

Conflict of Interest

The authors declare no relevant conflicts of interest related to this research, whether financial, personal, or professional.

Data Availability Statement

The data used in this study are the result of our research and have been included in this manuscript, including tables, graphs, and appendices.

References

Agustanty, A., and Andre, B., 2022. Resistance Pattern of *Vibrio cholerae* Bacteria to

Ciprofloxacin and Tetracycline Antibiotics. *J. Heal. Sci.*, 6(1), pp.73-78. https://doi.org/10.35971/gojhes.v5i3.13611

Ahmed, H.A., Awad, N.F.S., Abd El-Hamid, M.I., Shaker, A., Mohamed, R.E., and Elsohaby, I., 2021. Pet birds as potential reservoirs of virulent and antibiotic-resistant zoonotic bacteria. *J. Comp. Immunol.. Microbiol. Infect. Dis.*, 75(1), pp.55. https://doi.org/10.1016/j.cimid.2020.101606

Aklilu, E., Harun, A., and Singh, K.K.B., 2022. Molecular characterization of blaNDM, blaOXA-48 , mcr-1, and blaTEM-52 positive and concurrently carbapenem and colistin-resistant and extended-spectrum beta-lactamase-producing *Escherichia coli* in chicken in Malaysia. *J. Bmc. Vet. Res.*, 18(1), pp.1–10. https://doi.org/10.1186/s12917-022-03292-7

Al-Mustapha, A., Adetunji, V., and Heikinheimo, A., 2020. Risk perceptions of antibiotic usage and resistance: a cross-sectional survey of poultry farmers in Kwara state Nigeria. *J. Ant.*, 9(7), pp.378. https://doi.org/10.3390/antibiotics9070378

Al-Mustapha, A.I., Raufu, I.A., Ogundijo, O.A., Odetokun, I.A., Tiwari, A., Brouwer, M.S.M., Adetunji, V., and Heikinheimo, A., 2023. Antibiotic resistance genes, mobile elements, virulence genes, and phages in cultivated ESBL-producing *Escherichia coli* of poultry origin in Kwara State, North Central Nigeria. *J. Food. Mic.*, 389(1), pp. 25. https://doi.org/10.1016/j.ijfoodmicro.2023.110086

Anggita, D., Siti, N., and Edward, P.W., 2022. Mechanism of Action of Antibiotics. *J. Med.*, 7(1), pp.46-58.

Ansharieta, R., Ramandinianto, S.C., Effendi, M.H., and Plumeriastuti, H., 2021. Molecular identification of blaCTX-M and blaTEM genes encoding extended-spectrum ß-lactamase (ESBL) producing *Escherichia coli* isolated from raw cow milk in East Java, Indonesia. *Biodiversitas*, 22(4), pp.1600–1605. https://doi.org/10.13057/biodiv/d220402

Apriliani, N.P.E.U., and Pinatih, K.J.P., 2017. Prevalence of blaCTX-M-1 Gene Group in *Klebsiella pneumoniae* at Sanglah Central General Hospital, Denpasar. *J. Med.*, 6(2), pp.1-7.

Aslam, B., Wang, W., Arshad, M.I., Khurshid, M., Muzammil, S., and Rasool, M.H., 2018. Antibiotic resistance: an overview of the global crisis. *J. Infect. Drug. Resist.*, 11(1), pp.1645-1658. DOI https://doi.org/10.2147/IDR.S173867

Aworh, M.K., Kwaga, J.K.P., Hendriksen, R. S, Okolocha, E. C, and Thakur., S., 2021. Genetic relatedness of multidrug-resistant *Escherichia coli* isolated from humans, chickens, and poultry environments. *Antimicrob. Resist. Infect. Control.*, 10 (58), pp.1-13. https://doi.org/10.1186/s13756-021-00930-x

Ayandiran, T., Falgenhauer, L., Schmiedel, J., Chakraborty, T., and Ayeni, F., 2018. High resistance to tetracycline and ciprofloxacin in bacteria isolated from poultry farms in Ibadan, Nigeria. *J. Infect. Dev. Ctries.*, 12 (6), pp.462–470. DOI: https://doi.org/10.3855/jidc.9862

Ayeni, F., Falgenhauer, J., Schmiedel, J., Schwengers, O., Chakraborty, T., and Falgenhauer, L., 2020. Detection of

blaCTX-M-27-encoding *Escherichia coli* ST206 in Nigerian poultry stocks. *J. Ant. Chem.*, 75(10), pp.3070–3072. https://doi.org/10.1093/jac/dkaa293

Bottery, M.J., Pitchford, J.W., and Friman, V.P., 2021. Ecology and evolution of antimicrobial resistance in bacterial communities. *J. ISME.*, 15(4), pp.939-948. https://doi.org/10.1038/s41396-020-00832-7

Bren, A., Park, J.O., Towbin, B.D., Dekel, E., Rabinowitz, J.D., and Alon, U., 2016. Glucose being one of the worst carbon sources for *Escherichia coli* on a poor nitrogen source due to its suboptimal levels. *J. Camp. Sci.*, 6(1), pp.24834. https://doi.org/10.1038/srep24834

Calero-Cáceres, W., Méndez, J., Martín-Díaz, J., and Muniesa, M., 2017. The occurrence of antibiotic resistance genes in a Mediterranean river and their persistence in the riverbed sediment. *J. Env. Pol.*, 223, pp.384-394. https://doi.org/10.1016/j.envpol.2017.01.035

Chen, C.H., Hsieh, C.H., and Hwang, D.F., 2017. PCR-RFLP analysis using capillary electrophoresis for species identification of Cyprinidae-related products. *J. Food. Cont.*, 33(2), pp.477–483. https://doi.org/10.1016/j.foodcont.2013.03.036

Dalazen, G., Fuentes-Castillo, D., Pedroso, L.G., Fontana, H., Sano, E., Cardoso, B., and Lincopan, N., 2023. CTX-M-producing Escherichia coli ST602 carrying a wide resistome in South American wild birds: Another pandemic clone of one health concern. *One Health,* 6(2), pp.100586. https://doi.org/10.1016/j.onehlt.2023.100586

Diren Sigirci, B., Celik, B., Halac, B., Adiguzel, M.C., Kekec, I., Metiner, K., Ikiz, S., Bagcigil, A.F., Ozgur, N.Y., and Kahraman., B.B., 2020. Antimicrobial resistance profiles of *Escherichia coli* isolated from companion birds. *J. Kin. Saud. Univ. Sci.*, 32(1), pp.1069–1073. https://doi.org/10.1155/2021/6759046

Effendi, M.H., Hartadi, E.B., Witaningrum, A.M., Permatasari, D.A., and Ugbo, E.N., 2022. Molecular identification of blaTEM gen of extended-spectrum beta-lactamase-producing *Escherichia coli* from healthy pigs in Malang district, East Java, Indonesia. *J. Adv. Vet. Anim. Res.*, 9(3), pp.447-457. https://doi.org/10.5455/javar.2022.i613

Förster, A.H., and Gescher, J., 2014. Metabolic engineering of *Escherichia coli* for production of mixed acid fermentation end products. *Front Bioeng Biotechnol.*, 2(1), pp.16. https://doi.org/10.3389/fbioe.2014.00016

Fuentes-Castillo, D., Farfán-López, M., Esposito, F., Moura, Q., Fernandes, M.R., Lopes, R., Cardoso, B., Muñoz, M.E., Cerdeira, L., Najle, I., Muñoz, P.M., Catão-Dias, J.S., and González-Acuña, D., Lincopan, N., 2019., Wild owls colonized by international clones of a broad-spectrum β-lactamase (CTX-M) produce *Escherichia coli* and *Salmonella infantis* in the American southern cone. *Sci. Total. Environ.*, 674, pp. 554-562. https://doi.org/10.1016/j.scitotenv.2019.04.149

Harijani, N., Oetama, S.J.T., Soepranianondo, K., Effendi, M.H., and Tyasningsih, W., 2020. Biological hazard on multidrug resistance (MDR) of *Escherichia coli* collected from a cloacal swab of broiler chicken on wet

markets Surabaya. *Indian Journal of Forensic Medicine & Toxicology*, 14(4), pp.3239– 3244. https://doi.org/10.37506/ijfmt.v14i4.12125

Holmes, A.H., Moore, L.S., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., and Piddock, L.J., 2016. Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet*, 387(10014), pp.176–187. https://doi.org/10.1016/s0140-6736(15)00473-0

Hughes, D.T., Clarke, M.B., Yamamoto, K., Rasko, D.A., and Sperandio, V., 2009. The QseC adrenergic signaling cascade in enterohemorrhagic *E. coli* (EHEC). *PLoS. Pathog.*, 5(8), pp.1000553. https://doi.org/10.1371/journal.ppat.1000553

Ikhimiukor, O.O., Odih, E. E., Donado-Godoy, P., and Okeke, I., 2022. A bottom-up view of antimicrobial resistance transmission in developing countries. *Nat. Microbiol.*, 7(6), pp.757–765. https://doi.org/10.1038/s41564-022-01124-w

Islam, M.S., Rahman, A.T., Hassan, J., and Rahman, M.T., 2023. Extended-spectrum beta-lactamase *in Escherichia coli* isolated from humans, animals, and environments in Bangladesh: A One Health perspective systematic review and meta-analysis. *One Health*, 6(1), pp.10526. https://doi.org/10.1016/j.onehlt.2023.100526

Indana, K., Effendi, M.H., and Soeharsono, S., 2020. Uji Resistensi antibiotic Ampicillin Pada bakteri *Escherichia coli* Yang Diisolasi Dari Beberapa Peternakan di Surabaya. *Jurnal Peternakan Lingkungan Tropis*, 3(1), pp. 37-43.

Jayan, H., Pu, H., and Sun, D.W., 2022. Detection of bioactive metabolites in *Escherichia coli* cultures using surface-enhanced Raman spectroscopy. *Applied Spectroscopy*, 76(7), pp.812-822.

Kobayashi, H., Kanazaki, M., Hata, E., and Kubo., M., 2009. Prevalence and characteristics of eae-and stx-positive strains of *Escherichia coli* from wild birds in the immediate environment of Tokyo Bay. *J. Appl. Environ. Microbiol.*, 75(1), pp.292-295. https://doi.org/10.1128/aem.01534-08

Landecker, H. 2016. Antibiotic resistance and the biology of history. Body & Society, 22(4), pp.19-52.

Li, B., and Webster, T.J., 2018. Bacterial antibiotic resistance: new challenges and opportunities for implant-related orthopedic infections. *J. Orthop. Res.*, 36(1), pp.22-32. https://doi.org/10.1002/jor.23656

Maida, S., and Kinanti, A.P.L., 2019. Aktivitas Antibakteri Amoksisilin terhadap bakteri Gram Positif dan Gram Negatif. *Jurnal Pijar MIPA*, 14(3), pp.189-191.

Mohamed, M.Y.I., Abu, J., Zakaria, Z., Khan, A.R., Abdul Aziz, S., Bitrus, A.A., and Habib, I., 2022. Multi-Drug Resistant Pathogenic *Escherichia coli* Isolated from Wild Birds, Chicken, and the Environment in Malaysia. *Antibiotics*, 11(10), pp.1-14. https://doi.org/10.3390/antibiotics11101275

Melo, L.C., Haenni, M., Saras, E., Cerdeira, L., Moura, Q., Boulouis, H.J., and Madec, J.Y., Lincopan, N., 2019. Genomic characterization of multidrug-resistant extended-spectrum β-lactamase-positive TEM-52b *Escherichia coli* ST219 isolated from a cat in France. *J. Glob. Antimicrob.*

Resist., 18(1), pp.223–224. https://doi.org/10.3390/microorganisms11020525

Ojo, O. E., Schwarz, S., and Michael, G.B., 2016. Detection and characterization of extended-spectrum β-lactamase-producing *Escherichia coli* from chicken production chains in Nigeria. *Vet. Microbiol*., 194(2), pp.62–68. https://doi.org/10.1016/j.vetmic.2016.04.022

O'Nei, J., 2016. Tackling drug-resistant infections globally: final report and recommendations. The review on Antimicrobial Resistance. https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf

Ong, K.H., Khor, W.C., Quek, J.Y., Low, Z.X., Arivalan, S., Humaidi, M., Chua, C., Seow, KL.G., Guo, S., Tay, M.Y.F., Schlundt, J., Ng, L.C., and Aung, K.T., 2020. Occurrence and antimicrobial resistance traits of *Escherichia coli* from wild birds and rodents in Singapore. *Int. J. Environ. Res. Public. Health.*, 17(15), pp.1-17. https://doi.org/10.3390/ijerph17155606

Oteo, J., Menciá, A., Bautista, V., Pastor, N., Lara, N., González-González, F., Garciá-Penã, F.J., and Campos, J., 2018. Colonization with Enterobacteriaceae-producing ESBLs, AmpCs, and OXA-48 in wild avian species, Spain 2015-2016. *Microb. Drug. Resist.*, 24(7), pp.932–938. https://doi.org/10.1089/mdr.2018.0004

Rahmahani, J., Salamah, Mufasirin, Tyasningsih, W., and Effendi, M.H., 2020. Antimicrobial resistance profile of *Escherichia coli* from cloacal swabs of native chicken in Surabaya traditional market. *Biochemical and Cellular Archives*, 20(1), pp.2993-2997. http://dx.doi.org/10.35124/bca.2020.20.S1.2993

Rombouts, J.L., Kranendonk, E.M.M., Regueira, A., Weissbrodt, D.G., Kleerebezem, R., and Van Loosdrecht, M.C.M., 2020. Selecting bacteria that produce and utilize lactic acid in anaerobic enrichment cultures. *Biotechnol. Bioeng*., 117(5), pp.1281-1293. https://doi.org/10.1002/bit.27301

Sari, P.A., Erly, E., and Arisanty. D. 2015. Perbandingan Efektivitas Daya Hambat Kotrimoksazol Generik dan Paten Terhadap Pertumbuhan Bakteri *Escherichia coli* Sebagai Penyebab Infeksi Saluran Kemih Secara in Vitro. *Andalas Journal of Health*, 4(1), pp.227-232.

Syari'ati, A., Arshadi, M., Khosrojerdi, M.A., Abedinzadeh, M., Ganjalishahi, M., Maleki, A., Heidary, M., and Khoshnood, S., 2022. The resistance mechanisms of bacteria against ciprofloxacin and new approaches for enhancing the efficacy of this antibiotic. *Front Public Health*, 10, pp.1025633 https://doi.org/10.3389/fpubh.2022.1025633

Simanjuntak, H.A., Simanjuntak, H., Maimunah, S., Rahmiati, R., and Situmorang, T.S., 2022. Zone of Inhibition Diameter of Amoxicillin and Tetracycline Antibiotics against *Escherichia coli*. *Herbal Medicine Journal*, 5(2), pp.55-59. https://doi.org/10.58996/hmj.v5i2.52

Silva, A.C., Nogueira, P.J., and Paiva, J.A., 2021. Determinants of antimicrobial resistance among the different European countries: more than human and animal antimicrobial consumption. *Antibiotics (Basel)*, 10(7), pp.834. https://doi.org/10.3390/antibiotics10070834

Tama, S.C., Ngwai, Y.B., Pennap, G.R.I., Nkene, I.H., and Abimiku, R.H, 2021. Molecular detection of extended Spectrum Beta-lactamase resistance in *Escherichia coli* from poultry droppings in Karu, Nasarawa State Nigeria. *Int. J. Pathog. Res.*, 6(4), pp.31–42. https://doi.org/10.9734/ijpr/2021/v6i430169

Varriale, L., Dipineto, L., Russo, T.P., Borrelli, L., Romano, V., D'orazio, S., Pace, A., Menna, L.F., Fioretti, A., and Santaniello, A., 2020. Antimicrobial resistance of *Escherichia coli* and Pseudomonas aeruginosa from companion birds. *Antibiotics (Basel)*, 9(11), pp.780. https://doi.org/10.3390/antibiotics9110780

Word Health Organization. 2023. Antimicrobial resistance. https://www-who-int.translate.goog/news-room/fact-sheets/detail/antimicrobial-resistance?_x_tr_sl=en&_x_tr_tl=id&_x_tr_hl=id&_x_tr_pto=tc

Xia, J., Sun, J., Cheng, K., Li, L., Fang, L.X., and Zou, M.T., Liao, X.P., Liu, Y.H., 2016. Persistent spread of the rmtB 16S rRNA methyltransferase gene among *Escherichia coli* isolates from diseased food-producing animals in China. *Veterinary Microbiology*, 188(2), pp.41–46. https://doi.org/10.1016/j.vetmic.2016.03.018

Yanestria, S.M., Dameanti, F.N.A.E.P., Musayannah, B.G., Pratama, J.W.A., Witaningrum, A.M., Effendi, M.H., and Ugbo, E.N., 2022. Antibiotic resistance patterns of extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* isolated from environmental broiler farms in Pasuruan District, Indonesia. *Biodiversitas*, 23(9), pp.4460-4465. https://doi.org/10.13057/biodiv/d230911

Yılmaz, E.Ş., and Dolar, A., 2017. Detection of Extended-Spectrum β-Lactamases in *Escherichia coli* From Cage Birds. *J. Exot. Pet. Med.*, 26(1), pp.13–18. https://doi.org/10.1053/j.jepm.2016.10.008

Yilmaz, E.S., and Guvensen, N.C., 2016. In vitro, biofilm formation in ESBL-producing *Escherichia coli* isolates from cage birds. *Asian Pac J Trop Med.*, 9(11), pp.1069-1074.

Zarei-Baygi, A., and Smith, A.L., 2021. Intracellular versus extracellular antibiotic resistance genes in the environment: prevalence, horizontal transfer, and mitigation strategies. *Bioresour Technol.*, 319, pp.124181. https://doi.org/10.1016/j.biortech.2020.124181

Zlatkov, N., and Uhlin, B.E., 2019. Absence of global stress regulation in *Escherichia coli* drives pathoadaptation and c-di-GMP-dependent metabolic capabilities. *Sci Rep.*, 9(1), pp.2600. https://doi.org/10.1038/s41598-019-39580-w

Zawack, K., Li, M., Booth, J.G., Love, W., Lanzas, C., and Grohn, Y.T., 2016. Monitoring antimicrobial resistance in the food supply chain and its implications for FDA policy initiatives. *Antimicrob Agents Chemother*, 60(9), pp.5302-5311. https://doi.org/10.1128/aac.00688-16