Antimicrobial Resistance of *Escherichia Coli* Isolated from Cecum and Carcasses Samples of Broiler Chickens

Susan M. Noor(✉), Suhaemi Suhaemi, Sumirah Sumirah, S. Mulyati, T. Ariyanti, F. Rachmawati, and Andriani Andriani

Research Organization for Health, Centre for Veterinary Research, The National Research and Innovation Agency, Bogor, Indonesia
susan.maphilindawati.noor@brin.go.id

Abstract. Poultry production is an important part of the agricultural industry in many countries, including Indonesia. Antimicrobial agents are widely used in the poultry industry to reduce the significant losses caused by *Escherichia coli* infections and welfare of animals. However, utilizing antibiotics in food production in excess and/or inappropriately animal infections have also evolved antibiotic resistance. This study aims to determine the resistance patterns of *Escherichia coli* in broiler chickens to antimicrobial agents. In this study, 50 *E. coli* strains isolated from the cecum and carcass samples of broiler chickens from markets in Bogor West Java, Indonesia were tested for antimicrobials of both veterinary and human significance (sulfamethoxazole, meropenem, ciprofloxacin, colistin sulphate, and chloramphenicol). The antimicrobial susceptibility of isolated *E. coli* was determined using the standard disc diffusion procedure and the Clinical and Laboratory Standards Institute (CLSI) Performance Standards. The results showed that 66% of *E. coli* isolates were resistant to colistin sulphate, 52% were resistant to Sulfamethoxazole, 30% were resistant to meropenem, and 15% were resistant to ciprofloxacin, and 8% were resistant to chloramphenicol. The presence of multiple drug-resistant *E. coli* strains in broiler chickens is highlighted in this study. It showed that there was multiple resistance of *E. coli* to four types of antibiotics (4%), three types of antibiotics (20%), and two types of antibiotics (18%). In this study, *Escherichia coli* has developed resistance to colistin sulphate and chloramphenicol, both of which have been banned from use in livestock. This may be due to the spreading of contamination of resistant bacteria from the environment. The data revealed the relative risks associated with the use of antimicrobials in the poultry industry, therefore antibiotic usage in poultry farms should be restricted.

Keywords: Antimicrobial · *Escherichia* coli · cecum · carcass · broiler chickens

1 Introduction

An international concern known as antimicrobial resistance (AMR) has evolved, and developing nations like Indonesia are likely to be more susceptible to the spread of

I. Nurlaila et al. (Eds.): ICHR 2022, AHSR 56, pp. 29–37, 2023.
https://doi.org/10.2991/978-94-6463-112-8_4

resistant bacteria at human-animal interfaces [1]. Antimicrobial resistance (AMR) has an impact on public health, especially food contamination and infectious diseases [2–4]. AMR in bacteria, primarily caused by foodborne pathogens, is on the rise in most parts of the world, including ASEAN countries, with serious consequences for the economy, human and animal health, and the international food trade. Pathogenic bacteria's proclivity to become multidrug-resistant (MDR) has received the most serious attention in AMR [3, 4].

Antimicrobials have been widely used in poultry farming for three purposes: therapy, prevention, growth promoters, and additional feed to increase chicken productivity. This is the root cause of AMR's emergence and persistence [2, 5, 6]. Antimicrobial agents were first used to treat disease in livestock in the mid-1940s, and antimicrobial agents were first used in commercial feed for cattle, pigs, and poultry in the early 1950s [7]. Waste management on farms and production line processes is a determining factor in the risk of AMR emergence and the level of food contamination. Antimicrobials have been widely used in poultry farming for three purposes: therapy, prevention, growth promoters, and additional feed to increase chicken productivity. This is the root cause of AMR's emergence and persistence [2, 8].

The standard bacterial species used to identify AMR-associated infections are *Enterobacteriaceae* such as *Escherichia coli* (*E. coli*) and *Klebsiella pneumonia* [2]. There are significant health concerns for humans from a number of virulence genes that are possibly zoonotic in *E. coli* strains [9]. Meat contaminates with antimicrobial-resistant *E. coli* due to the widespread use of antimicrobial agents in the manufacture of animal products [7]. Antibiotic-resistant *E. coli* in animal products can endanger public health [10]. According to [11], The primary source of *E. coli* with MDR is animal-derived food, and antibiotics are a critical component of treatment. Furthermore, animal-derived *E. coli* can serve as a source of antimicrobial resistance genes for other bacteria. As a result, the widespread use of antimicrobial agents in food-producing animals may contribute to an increase in the burden of antimicrobial resistance in humans. Bacteria from animal reservoirs are of particular interest due to the fact that they are resistant to antimicrobials that are crucial for human therapy (e.g., aminoglycosides, fluoroquinolones, and third and fourth-generation cephalosporins) due to the fact that they are resistant to antimicrobials that are crucial for human therapy (e.g., aminoglycosides, fluoroquinolones, and third and fourth-generation cephalosporins) [7].

A number of Indonesian regions have been reported to have *E. coli* resistant to antibiotics in chicken, with varying incidence rates for AMR [12–14]. This study aims to determine the pattern of resistance of *E. coli* to antimicrobial agents isolated from broiler chickens in Bogor, West Java, Indonesia.

2 Materials and Methods

In this study, 50 local isolates of *E. coli* isolated from broiler chicken cecum (21 isolates) and carcass samples (29 isolates) obtained from Bogor's traditional markets used in this study. A fresh cecum sample was taken aseptically and put in a sample pot, and the chicken carcasses were placed in sterile plastic bags. All samples brought to the laboratory using an ice box. *Escherichia coli* ATCC 25922 was used as a positive control strain. Each sample was first planted on Eosin Methylene Blue Agar (EMBA) media with an overnight incubation at 35 °C before being planted in an enrichment solution of Buffer Pepton Water. Colonies having a predominant metallic green and a dark center were suspected to contain *E. coli*, and the process of identifying it continued. Gram staining and biochemical tests such as Simmons citrate Agar (SCA), Sulphide Indole Motility (SIM), Methyl Red Voges Proskauer (MRPV), and Urea Agar are used to identify samples (SNI 2897:2008 with modification).

The 5 types of antibiotics (Oxoids) in disc form, namely sulphamethoxazole/SXT (30 μg/disc), meropenem/Mem (10 μg /disc), ciprofloxacin/ CIP (5 μg/disc), colistin sulphate/ CT (10 μg /disc), and chloramphenicol/ C(30 μg/disc) were selected based on their clinical or epidemiological significance to human and animal health. The antimicrobial susceptibility of all *E. coli* isolates was determined using the Kirby-Bauer disk diffusion method with Mueller-Hinton (MH) agar (Thermo Fisher Scientific, USA) according to the Clinical and Laboratory Standards Institute standard procedure (CLSI) [15]. Isolates of *E. coli* were grown for 24 h in BHI broth (Oxoid CM0225) media before being incubated at 37 °C. Furthermore, the bacteria were grown on Nutrient Agar (OXOID) medium and incubated at 37 °C for 24 h. The bacteria were then reintroduced into Brain Heart Infusion (BHI) broth to form a suspension that was adjusted to match the turbidity of 0.5 McFarland standard using McFarland Densitometer (Biosan). Mueller-Hinton Agar (OXOID CM0337) medium in 3 and 1 mL volumes were used. Each antibiotic disc is placed on the agar surface after it has dried. The agar medium was then incubated at 37 °C for 24 h. Furthermore, based on the diameter of zone inhibition on the plate, antibiotic test results are classified as sensitive, intermediate, or resistant.

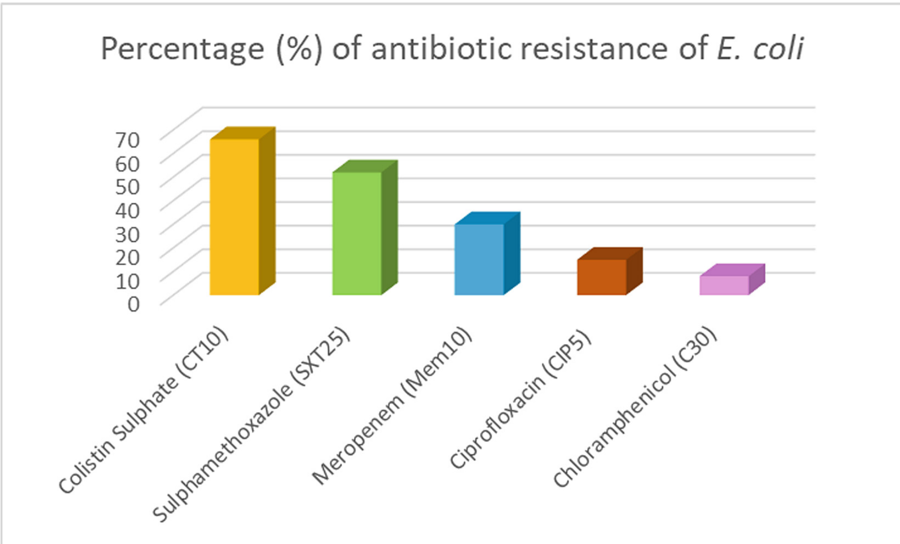

Fig. 1. Prevalence of resistance among *E. coli* isolates from broiler chickens to antibiotics.

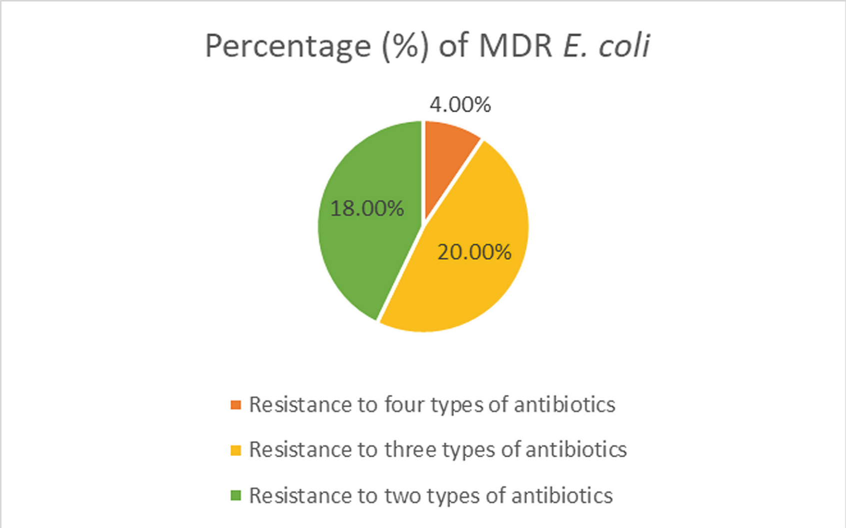

Fig. 2. Multiple resistance drug (MDR) of *E. coli* towards antibiotics

3 Results

A total of 50 *E. coli* isolates from cecum and chicken carcasses obtained from traditional markets in the Bogor area were tested by disc diffusion using the Kirby-Bauer method to determine the profile of their resistance to five different antibiotics. We found that 66% of *E.coli* isolates were resistant to colistin sulphate, 52% were resistant to sulfamethoxazole, 30% were resistant to meropenem, 15% were resistant to ciprofloxacin, and 8% were resistant to chloramphenicol (Fig. 1) (Fig. 2).

Furthermore, *E. coli* was found to have multiple resistances to four types of antibiotics (4%), three types of antibiotics (20%), as well as two different antibiotics (18%).

4 Discussion

The inappropriate use of antibiotics in human medicine is considered the main driver of the phenomenon, but the overuse of antibiotics in livestock farming also contributes to the threat of antibiotic resistance. According to the Organization for Economic Cooperation and Development (OECD), there will be a 67% increase in the amount of antimicrobials used in food animals worldwide from 63,151 tons in 2010 to 105,596 tons in 2030 [16]. One of the five nations with the highest expected percentage increases in antimicrobial consumption by 2030 is Indonesia. According to a Ministry of Agriculture study, the most often used antibiotics were enrofloxacin (49.4%), amoxicillin/colistin (35.3%), trimethoprim/sulfadiazine (14.1%), and doxycycline (13.3%), in 360 small and medium-sized commercial broiler farms in three provinces: West Java, East Java, and South Sulawesi [17].

The study of antibiotic resistance against *E. coli* in the cecum and chicken carcasses from Bogor's traditional market discovered that 66% of *E. coli* isolates were resistant to colistin sulphate, 52% were resistant to sulfamethoxazole, 30% were resistant to meropenem, 15% were resistant to ciprofloxacin, and 8% were resistant to Chloramphenicol. Furthermore, *E. coli* was found to have multiple antibiotic resistances to four types of antibiotics (4%), three types of antibiotics (20%), and two types of antibiotics (18%). Surprisingly, *E. coli* has developed resistance to colistin sulphate and chloramphenicol, despite the fact that both of these antibiotics are prohibited for use in livestock. The possibility caused by colistin and chloramphenicol resistance might be due to the inappropriate use of antimicrobials, including abuse and overuse in humans, and agricultural production. Resistant bacteria can contaminate environment e.g. soil and plants. Through contact with the environment, animals can contract resistant bacteria. Furthermore, eventhough wild animals are not typically treated with antimicrobials; however, they can transport antimicrobial-resistant bacteria from the farm's contaminated surroundings [18]. These findings are similar to those of [6], who discovered resistance to colistin, streptomycin, erythromycin, oxytetracycline, tetracycline, and cefotaxime. The resistance pattern to several antibiotics was 99 percent in these isolates. According to [5], the high prevalence of *E. coli* isolates with the mcr-1 gene, a marker of colistin sulfate resistance, was quite concerning in Argentina. Commercial broiler farms can be a significant source of *E. coli* strains containing the mcr-1 gene.

This study revealed that 52% of *E. coli* was resistant to sulfamethoxazole. Antimicrobial agents are widely used in the poultry industry to reduce the significant losses caused by *E. coli* infections, but this has a negative impact because it can increase the resistance of digestive tract organisms to antibiotics [19, 20]. Antibiotic use in poultry farms must be restricted in order to reduce antibiotic resistance because it possess great threat to public health when the resistant *E. coli* spread from food animals to humans through chicken carcasses.

Meanwhile, the resistance of *E. coli* to meropenem in this study reached 30%. Numerous routes, both connected to and unrelated to agriculture, including wastewater, soils, manure applications, direct contact between people and animals, and food intake, can result in the transmission of AMR bacteria and genes across systems. Meropenem is a carbapenem antibiotic that is used as a last-resort antibiotic in the treatment of human infections [21, 22]. WHO classifies carbapenem-resistant *Enterobacteriaceae* as the most critical priority group of pathogens that pose a threat to human health [22]. There is no utility for carbapenem in veterinary medicine. This is most likely a result of humans using antibiotics unwisely, which has led to the spread of meropenem-resistant *E. coli* into the environment. It is unknown whether these resistant qualities can be passed from animals to people or the other way around. Because the prevalence of carbapenem resistance was found to be higher in humans than in animals, it is likely that the resistance acquired in animal isolates was acquired through environmental spread [23]. Larbi *et.al* 2021 [24] discovered that in Ghana's Greater Kumasi Metropolis 100% meropenem resistance in *E. coli* isolates from broiler chickens, pigs, and cattle. The presence of carbapenem resistance in *E. coli* of animal origin highlights the need for effective surveillance and intervention to prevent infection.

According to [25], ciprofloxacin antibiotics from the fluoroquinolone group and chloramphenicol antibiotics from the phenicol group are still sensitive, which means they are still quite effective for the treatment of *E. coli* infection because they can still inhibit bacterial growth. Several other studies, on the other hand, have found that the antibiotics ciprofloxacin and chloramphenicol have a low level of resistance to *E. coli* isolates when compared to other antibiotics [26–28]. Resistance rates to ciprofloxacin and chloramphenicol were similar to those found in this study. The fluoroquinolone group is an important antimicrobial agent for treating various types of infections in both humans and animals because it is known to be bactericidal against almost all bacteria. Resistance to these antimicrobial agents is usually due to mutations in the drug target, i.e., the genes for DNA gyrase and topoisomerase IV, but other mechanisms such as decreased outer membrane permeability, protection of target structures, or upregulated efflux pumps may also play a role [23].

The percentage of multidrug-resistant (MDR) E.coli isolated from the cecum and carcass of chickens found dual resistance to four antibiotics (4%), three antibiotics (20%), and two antibiotics (18%). The MDR profile was also reported in the [29] study. MDR properties were found in 76 percent of *E. coli* isolates, with 31.1 percent resistant to 4 antibiotic classes and 2.9 percent resistant to 7 antibiotic classes. According to [30], *E.coli* isolated from Se'i meat in Kupang showed resistance to two or more classes of antibiotics, with 11 different resistance patterns, with the highest MDR pattern observed against nine classes of antibiotics.

Antimicrobial resistance can develop as a result of the use of antibiotics in animals and humans, as well as the subsequent transfer of bacterial resistance genes between animals, humans, animal products, and the environment [31]. High antibiotic resistance in *E. coli* can lead to resistance to other pathogenic bacteria, endangering the health of animals, humans, and the environment [6]. Temperature, solar radiation exposure, pH, soil type, and microbial bio complexity can all affect resistant bacteria's ability to survive, reproduce, die, and exchange/spread resistance genes [32]. Antibiotic resistance can be managed by limiting the spread of resistant bacteria or by limiting antibiotic use [33]. Using an alcohol-based hand sanitizer or often washing your hands with soap and water are two ways to prevent AMR. Be sure to handle food properly by keeping raw and cooked ingredients apart, completely cooking meals, and using clean water. Avoid getting too near to sick people.

5 Conclusion

From this study, it can be concluded that *Escherichia coli* isolated from broiler chickens collected from Bogor's traditional markets has resistance to antibiotic colistin sulphate, sulfamethoxazole, meropenem, ciprofloxacin, and, chloramphenicol. There was found multidrug-resistance of *E. coli* to more than two antibiotics.

Acknowledgments. The authors would like to express their gratitude to the technicians of the *Enterobacteriaceae* Bacteriology laboratory as well as the librarians of the Indonesian Center for Veterinary Research for their assistance.

References

1. Palma E, Tilocca B, Roncada P. Antimicrobial resistance in veterinary medicine: An overview. *Int. J. Mol. Sci.* 2020, *Volume 21(6)*, pp. 1–21, doi: https://doi.org/10.3390/ijms21061914.
2. Sirichokchatchawan W, Apiwatsiri P, Pupa P, Saenkankam I, Khine N.O, Lekagul A, Lugsomya, K.Hampson D.J., Prapasarakul N. Reducing the Risk of Transmission of Critical Antimicrobial Resistance Determinants From Contaminated Pork Products to Humans in South-East Asia. *Front. Microbiol.* 2021, *Volume 12*, pp. 1–19, doi: https://doi.org/10.3389/fmicb.2021.689015.
3. Roth N, Käsbohrer A, Mayrhofer S, Zitz U, Hofacre C, Domig K.J. The application of antibiotics in broiler production and the resulting antibiotic resistance in *Escherichia coli*: A global overview. *Poult. Sci.* 2019, *Volume 98(4)*, pp. 1791–1804, doi: https://doi.org/10.3382/ps/pey539.
4. Archawakulathep A, Kim C.T.T, Meunsene D, Handijatno D, Hassim H.A, Rovira H.R.G, Myint K.S, Baldrias L.R, Sothy M, Aung M, Wahyu N.H, Chea R, Boonmasawai S, Vannamahaxay S, Angkititrakul, S, Collantes T.M.A, Van T.N, Punyapornwithaya V, Zakaria Z, Chuanchuen R. Perspectives on antimicrobial resistance in livestock and livestock products in ASEAN countries. *Thai J. Vet. Med.* 2014, *Volume 44(1)*, pp. 5–13.
5. Dominguez J.E, Redondo L.M, Espinosa R.A.F, Cejas D, Gutkind G.O, Chacana P.A, Conza J.A.D, Miyakawa M.E.F. Simultaneous carriage of mcr-1 and other antimicrobial resistance determinants in *Escherichia coli* from poultry. *Front. Microbiol.* 2018, *Volume 9*, pp. 1–10, doi: https://doi.org/10.3389/fmicb.2018.01679.
6. Januari C, Sudarwanto M.B, Purnawarman, T. Resistensi Antibiotik pada *Escherichia coli* yang Diisolasi dari Daging Ayam pada Pasar Tradisional di Kota Bogor. *J. Vet.* 2019, *Volume 20(1)*, pp. 125–131, doi: https://doi.org/10.19087/jveteriner.2019.20.1.125.
7. Hammerum A.M, Heuer O.E. Human health hazards from antimicrobial-resistant *Escherichia coli* of animal origin. *Clin. Infect. Dis.* 2009, *Volume 48(7)*, pp. 916–921, doi: https://doi.org/10.1086/597292.
8. Agustin A.L.D, Ningtyas N.S.I, Tirtasari K. Resistensi Antibiotik terhadap Bakteri *Escherichia coli* yang Diisolasi dari Ayam Layer di Desa Sesaot Kabupaten Lombok Barat. *Media Kedokt. Hewan* 2022, *Volume 33(2)*, pp. 87–95, doi: https://doi.org/10.20473/mkh.v33i2.2022.87-95.
9. Gregova G, Kmet V. Antibiotic resistance and virulence of *Escherichia coli* strains isolated from animal rendering plant. *Sci. Rep.* 2020, *Volume 10(1)*, pp. 1–7, doi: https://doi.org/10.1038/s41598-020-72851-5.
10. Rahman M.A, Rahman A.K.M.A, Islam A.A, Alam M.M. Antimicrobial Resistance of *Escherichia coli* Isolated From Milk, Beef and Chicken Meat in Bangladesh. *Bangl. J. Vet. Med.* 2017, *Volume 15(2)*, pp. 473–482, doi: https://doi.org/10.3329/bjvm.v15i2.35525.
11. Ho P.L, Chow K.H, Lai E.L, Lo W.U, Yeung M.K, Chan J, Chan P.Y, Yuen K.Y. Extensive dissemination of CTX-M-producing *Escherichia coli* with multidrug resistance to ‘critically important’ antibiotics among food animals in Hong Kong, 2008-10. *J. Antimicrob. Chemother.* 2011, *Volume 66(4)*, pp. 765–768, doi: https://doi.org/10.1093/jac/dkq539.
12. Susilo Setyaningsih, M Mulyawati D. *Escherichia coli* Strains of Chicken Intestines: Characterization of Ciprofloxacin and Erythromycin Antibiotic Resistance Profiles. JPBN 2022, Volume 8(1), pp. 103–113, doi: https://doi.org/10.36987/jpbn.v8i1.2484.
13. Handayani N.M.S, Erni P, Frimananda P.B, Riti N, Surya A.K. Resistensi Bakteri *E. coli* Terhadap Beberapa Antibiotika dari Isolat *Caecum* Ayam Broiller di Provinsi Bali, Nusa Tenggara Barat dan Nusa Tenggara Timur. In Prosiding Penyidikan Penyakit Hewan Rapat Teknis dan Pertemuan Ilmiah Kesehatan Hewan (RATEKPIL) dan Surveilans tahun 2020. Jakarta, Indonesia, 24 November 2020.

14. Besung I.N.K, Suarjana I.G.K, Gelgel K.T.P. Resistensi Antibiotik pada *Escherichia coli* yang Diisolasi dari Ayam Petelur. *Bul. Vet. Udayana* 2019, Volume 11(1), pp. 28–32, doi: https://doi.org/10.24843/bulvet.2018.v11.i01.p05.
15. Gargita I.G, Besung I.N.K, Rompis A.L.T. Escherichia Coli on Bali Cattle According To Maturity Levels in Different Geographicies Area and It's Pattern of Antibiotics Resistance. *Bul. Vet. Udayana* 2018, *Volume 10(2)*, pp. 169–175, doi: https://doi.org/10.24843/bulvet.2018.v10.i02.p10.
16. Organisation for Economic Co-operation and Development. Global antimicrobial use in the livestock sector. *Work. Party Agric. Policies Mark* 2015*,* 1–43. Available online: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=TAD/CA/APM/WP(2014)34/FINAL&docLanguage=En
17. Sivaraman S, Parady V. Antibiotic Use in Food Animals: India Overview. *ReAct Asia-Pacific* 2018, pp. 1–31. Available online: https://www.reactgroup.org/wp-content/uploads/2018/11/Antibiotic_Use_in_Food_Animals_India_LIGHT_2018_web.pdforg
18. Carroll D, Wang J, Fanning S, McMahon B.J. Antimicrobial Resistance in Wildlife: Implications for Public Health. *Zoonoses Public Health* 2015, *Volume 62(7)*, pp. 534–542, doi: https://doi.org/10.1111/zph.12182.
19. Luhung Y.G.A, Suarjana I.G.K, Gelgel K.T.P. Sensitivitas Isolat *Escherichia coli* Patogen dari Organ Ayam Pedaging Terinfeksi Koliseptikemia terhadap Oksitetrasiklin, Ampisilin dan Sulfametoksazol. *Bul. Vet. Udayana* 2017, *Volume 9(1)*, pp. 60–66, doi: https://doi.org/10.21531/bulvet.2017.9.1.60.
20. Andriyani M, Afiff U, Tiuria, R. Gambaran kepekaan *Escherichia coli* dari peternakan ayam broiler di Desa Bojongkerta Kabupaten Sukabumi terhadap antibiotik. *ARSHI Vet. Lett.* 2020, *Volume 4(1)*, pp. 19–20, doi: https://doi.org/10.29244/avl.4.1.19-20.
21. Elmanama A.A, Al-Reefi M.R, Shamali M.A, Hemaid H.I. Carbapenems Resistance among Gram Negative Bacteria Isolated from Poultry Samples in Gaza - Palestine. *Int. Arab. J. Antimicrob. Agents* 2019, *Volume 8(3:3)*, pp. 1–9, doi: https://doi.org/10.3823/826.
22. Yasmin S, Karim A.M, Lee S.H, Zahra R. Temporal Variation of Meropenem Resistance in *E. coli* Isolated from Sewage Water in Islamabad, Pakistan. *Antibiotics* 2022, *Volume 11(5)*, p. 635, doi: https://doi.org/10.3390/antibiotics11050635.
23. Poirel L, Madec J.Y, Lupo A, Schink A.K, Kieffer N, Nordmann P, Schwarz S. Antimicrobial Resistance in *Escherichia coli*. Microbiol. Spectrum 2018, Volume 6(4), doi: https://doi.org/10.1128/microbiolspec.ARBA-0026-2017.
24. Larbi R.O, Ofori L.A, Sylverken A.A, Ayim-Akonor M, Obiri-Danso K. Antimicrobial Resistance of *Escherichia coli* from Broilers, Pigs, and Cattle in the Greater Kumasi Metropolis, Ghana. *Int. J. Microbiol.* 2021, 7 pp, doi: https://doi.org/10.1155/2021/5158185.
25. Hilda Berliana. Pola Resistensi Bakteri *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa* Terhadap Berbagai Antibiotik. *J. Mahakam Husada* 2015, *Volume IV(1)*, pp. 11–17.
26. Sasongko H. Uji Resistensi Bakteri *Escherichia coli* dari Sungai Boyong Kabupaten Sleman terhadap Antibiotik Amoksisilin, Kloramfenikol, Sulfametoxasol, dan Streptomisin. *J. Bioedukatika* 2014, *Volume 2(1),* pp. 25–29.
27. Sumampouw O.J. Uji Sensitivitas Antibiotik Terhadap Bakteri *Escherichia coli* Penyebab Diare Balita Di Kota Manado (The Sensitivity Test Of Antibiotics To Escherichia Coli Was Caused The Diarhhea On Underfive Children In Manado City). *J. Curr. Pharm. Sci.* 2018, *Volume 2(1)*, ISSN 2598–2095.
28. Rosyidi A, Sriasih M, Sukartajaya I.N. Deteksi *Escherichia coli* Sumber Ayam Kampung dan Resistensinya Terhadap Berbagai Antibiotik. *MADURANCH J. Ilmu Peternak.* 2008, *Volume 3(1)*, pp. 17–22.

29. Kiiti R.W, Komba E.V, Msoffe P.L, Mshana S.E, Rweyemamu M, Matee M.I.N. Antimicrobial Resistance Profiles of *Escherichia coli* Isolated from Broiler and Layer Chickens in Arusha and Mwanza, Tanzania. *Int. J. Microbiol.* 2021, pp. 1–9, doi: https://doi.org/10.1155/2021/6759046.
30. Amalo G.F, Purnawarman T, Pisestyani H. *Escherichia coli* Contamination and Its Resistance to Antibiotics in Se'i Meat. *J. Vet. Sci.* 2021, *Volume 15(1)*, pp. 27–30, doi: https://doi.org/10.21157/j.ked.hewan.v15i1.18204.
31. Tadesse H.A, Gidey N.B, Workelule K, Hailu H, Gidey S, Bsrat A, Taddele H. Antimicrobial Resistance Profile of *E. coli* Isolated from Raw Cow Milk and Fresh Fruit Juice in Mekelle, Tigray, Ethiopia. *Vet. Med. Int.* 2018, 7 pp, doi: https://doi.org/10.1155/2018/8903142.
32. [FAO] Food and Agriculture Organization 2018. Antimicrobial Resistance in the Environment. Available online: [http://www.fao.org/3/BU656en/bu656en.pdf. (accessed on 18 June 2022).
33. Wegener H.C. Antibiotik Resistance-Linking Human and Animal Health. In Improving Food Safety Through a One Health Approach. Editor Choffnes, E.R., Relman, D.A., Olsen, L., Hutton, R., Mack, A., Eds.; National Academies Pr.: Washington, US, 2012; pp. 331–348.