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Resistance of Ampicillin, Ceftazidime, and Cefotaxime in Poultry's *Escherichia coli*

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Abstract

Beta-lactam antibiotics are important antibiotics that are widely used in the field of human and animal health. Ampicillin resistance has been widely reported. Another increase in resistance is 3rd generation cephalosporins. The purpose of this study was to compare the ampicillin resistance profiles in 2019 and 2021 in the same E. coli isolates and to determine the resistance profiles of ampicillin, ceftazidime, and cefotaxime in live chicken E. coli. The research stages were the preparation of isolates; culture on differential selective media and checking the uniformity of bacterial cell morphology; biochemical test; bacterial DNA extraction; uspA gene amplification; visualization of amplification results; manufacture of bacterial suspensions; Kirby-Bauer disk diffusion resistance test; measurement of inhibition zones and determination of isolate status; and compared the ampicillin resistance test data. All isolates were confirmed positive for E. coli. The uspA gene (884 bp) was detected in all isolates. Ampicillin resistance in 2019 and 2021 in the same E. coli isolates when compared, there was no difference. Resistance test showed E. coli was resistant to ampicillin (100%), ceftazidime (15.4%), and cefotaxime (64.5%). The conclusion of the study was that there was no difference between the ampicillin resistance in 2019 and 2021 in E. coli isolates. Escherichia coli in this study had the highest resistance profile to ampicillin, followed by cefotaxime, and the lowest was ceftazidime.

***Key words**: Escherichia coli*, resistance, ampicillin, ceftazidime, cefotaxime

Introduction

During the late 1950s and early 1960s, antibiotic resistance was detected for the first time, in enteric bacteria including *Salmonella*, *Shigella*, and *Escherichia coli*. These resistant strains cause great clinical, economic and mortality losses, especially in developing countries. However, in developed countries, the incidence of antibiotic resistance is considered a minor health problem because it is limited to enteric microbes. Understanding changed in the 1970s when *Neisseria gonorrhoeae* and *Haemophilus influenzae* were found to be resistant to ampicillin, whereas in the case of *Haemophilus* it was further reported to be resistant to tetracycline and chloramphenicol. The increasing use of antibiotics causes the incidence of resistance to accelerate, especially in developing countries because antibiotics are freely accessible without any prescription (Rossolini *et al.*, 2014).

Beta-lactam antibiotics are important antibiotics that are widely used in the field of human and animal health (World Health Organization, 2021). Ampicillin belongs to the penicillin class of beta-lactam antibiotics. According to Roth *et al.* (2019) The penicillin class is used to treat diseases caused by *E. coli* in chickens in several countries. The World Health Organization has identified several classes of beta-lactams, namely 3rd, 4th, and 5th generation cephalosporins, and carbapenems for use in humans (WHO, 2022). Several beta-lactam antibiotics are exclusively used in the field of veterinary medicine, including ceftiofur and cefquinome, which consist of 3rd and 4th generation cephalosporins (Cameron-Veas *et al.*, 2015). The alarming increase in 3rd generation cephalosporin-resistant bacteria reinforces suspicions of possible "unauthorized" use in chicks (Dutil *et al.*, 2010). In the United States, the use of cephalosporins in poultry and other species is prohibited by the Food and Drug Administration (FDA) (Food and Drug Administration, 2012).

Ceftazidime and cefotaxime belong to the 3rd generation cephalosporins. Both antibiotics will be used in this study, along with ampicillin. Chicken farms do not use ceftazidime and cefotaxime but there are many reports abroad (Vinueza-Burgos, 2019) and few domestically (Witaningrum *et al.*, 2020) regarding antibiotic resistance in chickens. Chen *et al.* (2014) also stated that ceftazidime resistance increased from 1993–2003 (18–27.2%). Cadena *et al.* (2007) said that bacteria with environmental conditions without exposure to antibiotics increased their sensitivity to certain antibiotics. Resistance to ampicillin, ceftazidime, and cefotaxime in this study was detected in *E. coli*. This study aimed to compare the resistance profile of ampicillin in 2019 and 2021 in the same *E. coli* isolates and determine the resistance profile of ampicillin, ceftazidime, and cefotaxime *in E. coli* from chicken.

Materials and Methods

Isolate

The identified *E. coli* isolates were archive isolates from the Medical Microbiology Laboratory, School of Veterinary Medicine and Biomedical Science, IPB University, Bogor, Indonesia. The number of *E. coli* isolates was 52 isolates (25 isolates from Sukabumi, 17 isolates from Bogor, and 10 isolates from Cianjur) derived from chicken cloacal swab samples. All from chicken cloacal swab samples. All isolates were stored at -20 ºC in tryptic soy broth (TSB) + 15% glycerol.

Microbiological Analysis

All *E. coli* isolates from TSB + 15% glycerol were grown on tryptic soy MacConkey agar (MCA) and eosin methylene blue agar (EMBA) media. Incubate for 18–24 hours at 37 ºC. The uniformity of bacterial cell morphology was seen by Gram staining (Markey *et al.*, 2013).

Biochemical tests were carried out on triple sugar iron agar (TSIA), urea, and indol-methyl red-Voges Proskauer-citrate (IMViC) media. Bacterial incubation for the TSIA test, urease production, indole test and citrate test were carried out for 18–24 hours at 37 ºC. Bacterial incubation for methyl red and Voges-Proskauer assays was 24–48 hours at 37 ºC (Markey *et al.*, 2013). Isolates identified as pure *E. coli* were phenotypically grown in TSA and stored at -4 C for further testing.

Isolate Confirmation

Bacterial DNA extraction using Presto™ Mini gDNA Bacteria Kit (Geneaid) according to manufacturing procedures. Polymerase chain reaction using forward 5´-CCG ATA CGC TGC CAA TCA GT-3´ and reverse 5´-ACG CAG ACC GTA GGC CAG AT-3´ (Mishra *et al.*, 2017) with a PCR product of 884 bp. The PCR and visualization of PCR result using a previously described method (Hardiati *et al.*, 2020).

Antibiotics Resistance Test

A total of 52 isolates of *E. coli* stored in TSA at 4 ºC were rejuvenated by re-culturing them in new TSA. The bacteria were incubated at 37 ºC for 18–24 hours. The rejuvenated bacterial colonies were suspended in sterile physiological NaCl until they reached the standard of 0.5 McFarland (1.5×10^8 CFU/ml).

Resistance testing using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (MHA) media refers to the Clinical Laboratory and Standards Institute (CLSI) year 2020. The antibiotics used in the test were ampicillin (AMP) 10 g, ceftazidime (CAZ) 30 g, and cefotaxime (CTX) 30 g. The bacterial suspension that has been made is cultured evenly on MHA media using a sterile cotton bud. For 10–15 minutes, the cultured MHA was left until the surface of the medium dries. The three discs containing antibiotics were placed on the surface of the MHA using sterile tweezers. Bacteria were incubated at 35 ºC for 16–18 hours. This test was carried out in triples.

The zone of inhibition is a clear area around the antibiotic disc. The zone of inhibition was measured in millimeters (mm). The results of the inhibition zone measurements were averaged and matched with the standard antibiotic inhibition zone in the CLSI 2020 guidelines.

Results and Discussion

All of the archived *E. coli* isolates (100%) showed conventional test results according to the literature (Markey *et al.*, 2013). The resistance of *E. coli* isolates when stored in TSB + 15% glycerol at -20 C in this study showed a 100% match with the initial observations before the bacteria were stored. The statement of Setiaji *et al.* (2015) support the results of this study. Setiaji *et al.* (2015) storage of *Aeromonas hydrophilla* in TSB + 15% glycerol media at -20 C provides growth resistance and does not change the characteristics of bacteria. TSB + 15% glycerol is the medium recommended by Handbooks Clinical Microbiology Procedures for maintaining bacterial cultures (Isenberg, 2004).

Universal stress proteins are proteins that are significantly expressed under unfavorable environmental stresses, such as nutrient starvation (lack of carbon, nitrogen, phosphate, sulfate, and amino acids), heat/cold stress, oxidative stress, heavy metal toxicity, transport chain release. electrons, exposure to polymyxin, cycloserine, ethanol and antibiotics and others (Kvint *et al.*, 2003). *Escherichia coli* has six different USPs namely USPA, USPC, USPD, USPE, USPF and USPG. Each protein is encoded by a different gene. The *uspA*, *uspC*, *uspD*, *uspE*, *uspF* and *uspG* genes are genes encoding universal stress proteins (USP) A, C, D, E, F and G, respectively. Each USP in *E. coli* has its own specific function under certain environmental stresses (Nachin *et al.*, 2005).

A total of 52 DNA samples were amplified against the *uspA* gene. The *uspA* gene was successfully amplified with an amplification product of 884 bp (Figure 1). According to Chen and Griffiths (1998) performing PCR amplification using the flank region primer of the *uspA* gene is a fast and effective method for screening non-pathogenic and pathogenic *E. coli*. Detection of *E. coli* by Mirzarazi *et al.* (2015) using primers from the *uspA* gene showed that all UPEC (Uropathogenic *E. coli*) isolates were positive. According to Godambe *et al.* (2017) and Bhowmik *et al.* (2022) the *uspA* gene is a genetic marker for the identification of *E. coli* by PCR method.

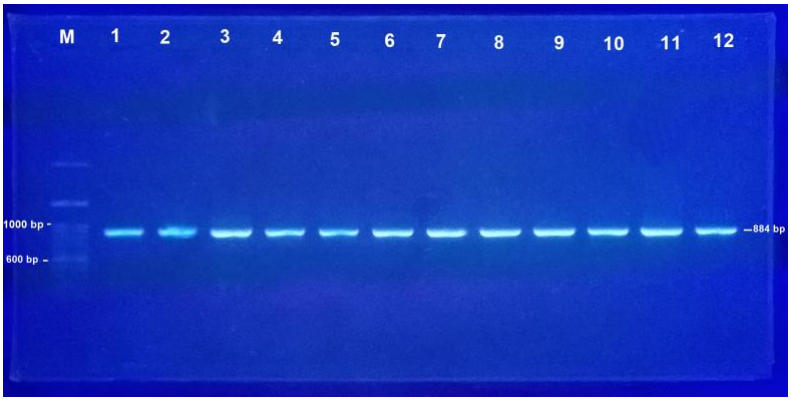


Figure 1. The results of *uspA* gene amplification with a product of 884 bp. M: 100 bp marker, 1–12: sample number 1–12

Ampicillin, ceftazidime, and cefotaxime used in this study are beta-lactam antibiotics. The condition of antibiotic resistance in *E. coli* from the three locations (Sukabumi, Bogor, and Cianjur) was dominated by ampicillin resistance. This is in line with research conducted by Khoirani *et al.* (2019) in chickens in West Java that 100% of *E. coli* isolates were resistant to ampicillin. Apart from chickens, *E. coli* resistance in various samples, animals and regions in Indonesia is considered quite high. Data on ampicillin resistance in *E. coli* isolated from cat samples at the Depok City veterinary clinic was 66% (Yaddi *et al.*, 2020). *Escherichia coli* originating from Bali cattle was recorded as 80% ampicillin resistant (Mustika *et al.*, 2015). *Escherichia coli* is considered as a reservoir bacteria and disseminator of antibiotic resistance (Tawfick *et al.*, 2022). Ampicillin is a broad-spectrum antibiotic that has long been used in both humans and animals so it is not surprising that the level of bacterial resistance to ampicillin is high.

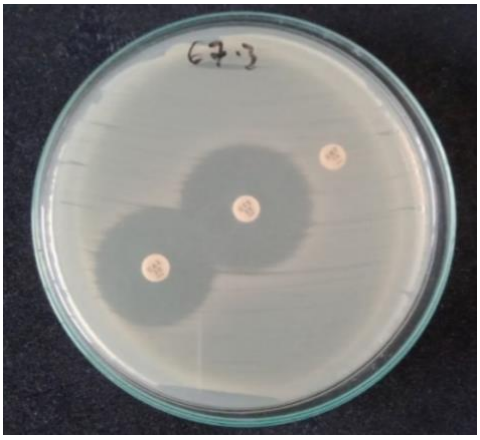


Figure 2. Antibiotics resistance tests on *E. coli*

Figure 3 presents ampicillin resistance profiles in *E. coli* isolates from three locations at different

testing times. The first test was carried out immediately after *E. coli* was isolated and identified from chicken cloacal swab samples. The second test was conducted 2 years later. The results of the first test showed that all isolates of *E. coli* were resistant to ampicillin. Resistance properties of *E. coli* still showed resistance after 2 years stored in TSB + 15% glycerol media at -20 °C. Dunai *et al.* (2019) cultured bacteria on media without exposure to antibiotics for 60 days by transferring to new media every day. Antibiotic resistance in bacteria can be reduced in potency within 480 generations during exposure to an antibiotic-free environment. Therefore, the rotation of the use of antibiotics is a very good policy and needs to be adhered to. Restricting the use of certain antibiotics will reduce the exposure of bacteria to certain antibiotics.

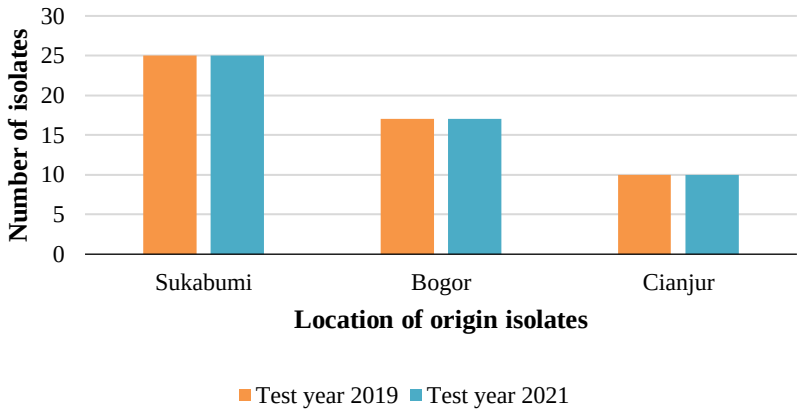


Figure 3. Comparison of ampicillin resistance profiles in *E. coli* tested in 2019 and 2021

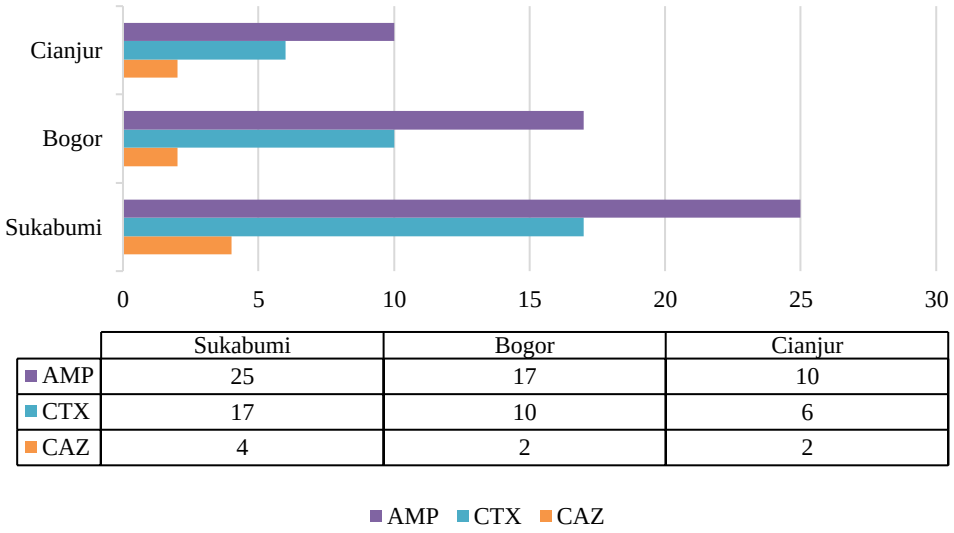
Ceftazidime is a 3rd generation cephalosporin with activity against many Gram-negative bacteria that are resistant to other antibiotics. Ceftazidime is not an antibiotic that is approved for use in food-breeding animals by the FDA but is often used in zoos, exotics and pets. Ceftazidime has been used to treat infections from enteric Gram-negative bacteria in dogs and cats. It is also used to treat skin infections, tissue wounds, and before surgery (Papich, 2016).

Resistance of *E. coli* to ceftazidime (Figure 4). Ceftazidime resistance in this study was 15,4%, still quite low. This is in line with research conducted by Davis *et al.* (2018) that ceftazidime resistance from *E. coli* from chicken meat did not reach 20%. However, one study related to ceftazidime resistance in *E. coli* in laying hens in Blitar showed the prevalence of ceftazidime resistance was 94% (Witaningrum *et al.*, 2020). Nguyen *et al.* (2015) found the prevalence of ceftazidime resistance in *E. coli* was higher at 44,2%. *Escherichia coli* was isolated from chicken feces on farms. In livestock, differences in bacterial resistance to antibiotics are caused by many factors including treatment procedures, management systems, breeding environments and so on (Manyi-Loh *et al.*, 2018).

The use of ceftazidime in chickens has not been found. However, cephalosporins are one of the most commonly used classes of antibiotics in human medicine (Nguyen *et al.*, 2013). Therefore, there may be transmission of resistance traits from humans or other species (eg pigs) to chickens. A finding related to possible transmission of antibiotic resistance is the presence of third-generation cephalosporin resistance in ESBL-producing *E. coli* from fish ponds in integrated farms. This relationship is related to the contact of chickens with fish pond water. The relevance of human activity can also be correlated with antibiotic resistance in poultry (Van Minh *et al.*, 2013). Early attention to ceftazidime antibiotic resistance is needed because ceftazidime has the ability to fight bacteria that are resistant to other antibiotics (Papich, 2016).

Cefotaxime is a broad-spectrum antibiotic and belongs to the 3rd generation of cephalosporin

antibiotics. Cefotaxime is most commonly used to treat birds with bacterial infections of the brain but is also useful for other serious infections. Cefotaxime is not adequately absorbed after oral administration and must be administered intramuscularly or intravenously to be effective (Flammer, 2006). Although cefotaxime was not used in chickens, data on cefotaxime resistance in E. coli from the three sites (Sukabumi, Bogor, and Cianjur) ranked second after ampicillin. The prevalence of cefotaxime resistance in this study was 63.4% (Figure 4). Cefotaxime resistance in E. coli studied by Hering *et al.* (2016) from various samples (chicken feces, shoe swabs of coop officers, and cage dust) also showed a fairly high number, namely 77.6%. Even Vinueza-Burgos *et al.* (2019) found very high cefotaxime resistance results (98.3%) in chickens in Ecuador. However, Januari *et al.* (2019) stated that cefotaxime resistance to *E. coli* from chicken meat was still low (12%). Nevertheless, these conditions cannot be ignored and must remain a concern. According to Van Minh *et al.* (2013) there is a relevance between human activities and antibiotic resistance in poultry. Significant genetic similarities between strains of resistant *E. coli* from poultry and those found in humans were found in the study of Kluytmans *et al.* (2013).



	Sukabumi	Bogor	Cianjur
AMP	25	17	10
CTX	17	10	6
CAZ	4	2	2

Figure 4. Resistance profile of ampicillin (AMP), ceftazidime (CAZ), and cefotaxime (CTX) in *E. coli*

Conclusion

The properties of ampicillin resistance in 2019 and 2021 in the same *E. coli* isolates showed no difference. *Escherichia coli* in this study had the highest resistance profile to ampicillin, followed by cefotaxime, and the lowest was ceftazidime.

References

Bhowmik, A., Goswami, S., Sirajee, AS., Ahsan S. 2022. Phylotyping, Pathotyping and Phenotypic Characteristics of *Escherichia coli* Isolated from Various Street Foods in Bangladesh. Journal of Microbiology, Biotechnology and Food Sciences, *12*(2): e4619. https://10.55251/jmbfs.4619.

Cadena, J., Taboada, CA., Burgess, DS., Ma, JZ., Lewis, JS., Freytes, CO., Patterson, JE. 2007. Antibiotic Cycling to Decrease Bacterial Antibiotic Resistance: A 5-Year Experience on A Bone Marrow Transplant Unit. Bone Marrow Transplantation, *40*(2): 151–155. https://doi:10.1038/sj.bmt.1705704.

Cameron-Veas, K., Solà-Ginés, M., Moreno, MA., Fraile, L., Migura-Garcia, L. 2015. Impact of the Use Of B-Lactam Antimicrobials on the Emergence of *Escherichia coli* Isolates Resistant to Cephalosporins Under Standard Pig-Rearing Conditions. Applied and Environmental Microbiology, *81*(5): 1782–1787 https://doi:10.1128/AEM.03916-14.

Chen, J., Griffiths, MW. (1998). PCR Differentiation of *Escherichia coli* from other Gram-Negative Bacteria Using Primers Derived from Thenucleotide Sequences Flanking the Gene Encoding Theuniversal Stress Protein. Letters in Applied Microbiology, *27*(6): 369–371. https://doi.org/10.1046/j.1472-765x.1998.00445.x.

Chen, X., Zhang, W., Yin, J., Zhang, N., Geng, S., Zhou, X., Wang, Y., Gao, S., Jiao X. 2014. *Escherichia coli* isolates From Sick Chickens in China: Changes in Antimicrobial Resistance Between 1993 and 2013. The Veterinary Journal, *202*(1): 112–115. https://10.1016/j.tvjl.2014.06.016.

Clinical and Laboratory Standards Institute. 2020. Performance Standards for Antimicrobial Susceptibility Testing, 30 th edition. New Jersey. Clinical and Laboratory Standards Institute.

Davis, GS., Waits, K., Nordstrom, L., Grande, H., Weaver, B., Papp, K., Horwinski, J., Koch, B., Hungate, BA., Liu, CM., Price, LB. 2018. Antibiotic-Resistant *Escherichia coli* from Retail Poultry Meat with Different Antibiotic Use Claims. BMC Microbiology, *18*(174): 1–7. https://10.1186/s12866-018-1322-5.

Dunai, A., Spohn, R., Farkas, Z., Lázár, V., Györkei, Á., Apjok, G., Boross, G., Szappanos, B., Grézal, G., Faragó, A., *et al.* 2019. Rapid Decline of Bacterial Drug-Resistance in an Antibiotic-Free Environment Through Phenotypic Reversion. eLife, 8: e47088. https://10.7554/eLife.47088.

Dutil, L., Irwin, R., Finley, R., Ng, LK., Avery, B., Boerlin, P., Bourgault, AM., Cole, L., Daignault, D., Desruisseau, A., *et al.* 2010. Ceftiofur Resistance in *Salmonella enterica* Serovar Heidelberg from Chicken Meat and Humans, Canada. Emerging Infectious Disease: *16*(1): 48–54. https://10/320/eid1601.090729.

Food and Drug Administration. 2012. New Animal Drugs; Cephalosporin Drugs; Extralabel Animal Drug Use; Order Of Prohibition. Food and Drug Administration, *77*(4): 735–745.

Flammer, K. 2006. Antibiotic Drug Selection in Companion Birds. Journal of Exotic Pet Medicine, *15*(3): 166–176. https://10.1053/j.jepm.2006.06.003.

Godambe, LP., Bandekar, J., Shashidhar, R. 2017. Species Specific PCR Based Detection of *Escherichia coli* from Indian Foods. 3 Biotech, *7*(2): 1–5. https://10.1007/s13205-017-0784-8.

Hardiati, A., Safika, S., Pasaribu, FH., Wibawan, IWT., Indrawati, A., Afiff, U. 2020. Detection of Antibiotic Resistance Genes of Escherichia coli Isolated from Layer Farm in Bogor Districts of Indonesia. Veterinary Practitioner, *21*(2): 324–328.

Hering, J., Frömke, C., von Münchhausen, C., Hartmann, M., Schneider, B., Friese, A., Rösler, U., Kreienbrock, L., Hille, K. 2016. Cefotaxime-Resistant *Escherichia coli* in Broiler Farms-A Cross-Sectional Investigation in Germany. Preventive Veterinary Medicine, *125*: 154–157. https://doi.org/10.1016/j.prevetmed.2016.01.003.

Isenberg, HD. 2004. Clinical Microbiology Procedure Handbook, 2 nd edition. USA. American Society for Microbiology Press.

Januari, C., Sudarwanto, MB., Purnawarman T. 2019. Resistensi Antibiotik pada *Escherichia coli* yang Diisolasi dari Daging Ayam pada Pasar Tradisional di Kota Bogor. Jurnal Veteriner, *20*(1): 125–131. https://10.19087/jveteriner.2019.20.1.125.

Khoirani, K., Indrawati, A., Setiyaningsih, S. 2019. Detection of Ampicillin Resistance Encoding Gene of *Escherichia coli* from Chickens in Bandung and Purwakarta. Jurnal Riset Veteriner Indonesia, *3*(1): 42–46. https://10.20956/jrvi.v3i1.6134.

Kluytmans, JAJW., Overdevest, ITMA., Willemsen, I., Kluytmans-van den Bergh, MFQ., van der Zwaluw, K., Heck, M., *et al*. 2013. Extended-Spectrum β-lactamase-producing *Escherichia coli* from Retail Chicken Meat and Humans: Comparison of Strains, Plasmids, Resistance Genes, and Virulence Factors. Clinical Infectious Diseases: *56*(4): 478–487. https://10.1093/cid/cis929.

Kvint, K., Nachin, L., Diez, A., & Nyström, T. (2003). The Bacterial Universal Stress Protein: Function and Regulation. Current Opinion in Microbiology, *6*(2): 140–145. https://10.1016/s1369-5274(03)00025-0.

Manyi-Loh, C., Mamphweli, S., Meyer, E., Okoh, A. (2018). Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources: Potential Public Health Implications. Molecules, *23*(4): 795. https://10.3390/molecules23040795.

Markey, B., Finola, L., Marie, A., Ann, C., Dores, M. 2013. Clinical Veterinary Microbiology, 2 nd edition. Skotlandia: Elsevier Ltd.

Mirzarazi, M., Rezatofighi, SE., Pourmahdi, M., Mohajeri, MR. 2015. Occurrence of Genes Encoding Enterotoxins in Uropathogenic *Escherichia coli* Isolates. Brazilian Journal of Microbiology, *46*(1): 155–159. https://10.1590/S1517-838246120130860.

Mishra, AK., Desh, DS., Gururaj, K., Geetika, G., Nitika, S., Naveen, K., Shivasharanappa, N., Sauvik, P. 2017. *UspA* Gene-Based Characterization of *Escherichia coli* Isolated from Different Disease Condition in Goats. Journal of Animal Research, *7*(6): 1–6. https://10.5958/2277-940X.2017.00168.1.

Mustika, OC., Pinatih, PKJ., Suardana, IW. 2015. Uji Kepekaan *Escherichia coli* O157:H7 Feses Sapi di Kecamatan Kuta Selatan Badung Bali terhadap Antibiotik.

Indonesia Mediscus Veterinus, *4*(4): 342–350.
Nachin, L., Nannmark, U., Nyström, T. 2005. Differential Roles of the Universal Stress Proteins of *Escherichia coli* in Oxidative Stress Resistance, Adhesion, and Motility. Journal of Bacteriology, *187*(18): 6265–6272. https://10.1128/JB.187.18.6265-6272.2005.
Nguyen, KV., Thi Do, NT., Chandna, A., Nguyen, TV., Pham, CV., Doan, PM., Nguyen, AQ., Thi Nguyen, CK., Larsson, M., Escalante, S., *et al.* 2013. Antibiotic Use and Resistance in Emerging Economies: A Situation Analysis for Vietnam. BMC Public Health: *13*(1158): 1–10. https://10.1186/1471-2458-13-1158.
Nguyen, VT., Carrique-Mas, JJ., Ngo, TH., Ho, HM., Ha, TT., Campbell, JI., Nguyen, TN., Hoang, NN., Pham, VM., & Wagenaar, JA., *et al.* 2015. Prevalence and Risk Factors for Carriage of Antimicrobial-Resistant *Escherichia coli* on Household and Small-Scale Chicken Farms in the Mekong Delta of Vietnam. Journal of Antimicrobial Chemotherapy, *70*(7): 2144–2152. https://10.1093/jac/dkv053.
Papich, MG. 2016. Saunders Handbook of Veterinary Drugs, 4 th edition. Missouri. Elsevier, Inc.
Rossolini, GM., Arena, F., Pecile, P., Pollini, S. 2014. Update on the Antibiotic Resistance Crisis. Current Opinion in Pharmacology, *18*: 56–60. https://doi:10.1016/j.coph.2014.09.006.
Roth, N., Käsbohrer, A., Mayrhofer, S., Zitz, U., Hofacre, C., Domig, KJ. 2019. The Application of Antibiotics in Broiler Production and the Resulting Antibiotic Resistance in *Escherichia coli*: A Global Overview. *Poultry Science. 98*(4), 1791–1804. https://doi.org/10.3382/ps/pey539.
Setiaji, J., Johan, TI., & Widantari, M. 2015. Pengaruh Gliserol pada Media *Tryptic Soy Broth* (TSB) terhadap Viabilitas Bakteri *Aeromonas hydrophila*. Dinamika Pertanian, *30*(1): 83–91.
Tawfick, MM., Elshamy, AA., Mohamed, KT., El Menofy, NG. 2022. Gut Commensal *Escherichia coli*, A High-Risk Reservoir of Transferable Plasmid-Mediated Antimicrobial Resistance Traits. Infection and Drug Resistance, *15*: 1077–1091. https://doi.org/10.2147/IDR.S354884.
Van Minh, H., Nguyen-Viet, H., Thanh, NH., Yang, JC. 2013. Assessing willingness to pay for improved sanitation in rural Vietnam. Environmental Health and Preventive Medicine, *18*(4): 275–284. https://doi.org/10.1007/s12199-012-0317-3.
Vinueza-Burgos, C., Ortega-Paredes, D., Narváez, C., De Zutter, L., Zurita, J. 2019. Characterization of cefotaxime resistant *Escherichia coli* isolated from broiler farms in Ecuador. PloS One, *14*(4): e0207567. https://doi.org/10.1371/journal.pone.0207567.
World Health Organization. 2021. Antimicrobial resistance. Accessed on Juny 1, 2022. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance
Witaningrum, AM., Wibisono, FJ., Permatasari, DA., Tyasningsih, W., Effendi, MH., Kurniawan, F. 2020. Potential Hazards of Antibiotics Resistance on *Escherichia coli* Isolated from Cloacal Swab in Several Layer Poultry Farms, Blitar, Indonesia. Indian Indian Journal of Public Health Research and Development, *11*(03): 2429–2435.
Yaddi, Y., Safika, S., Pasaribu, HP. 2020. Uji Resistensi terhadap Beberapa Antibiotika pada *Escherichia coli* yang Diisolasi dari Kucing di Klinik Hewan Kota Bogor. Jurnal Ilmu dan Teknologi Peterrnakan Tropis, *7*(3): 203–210. https://dx.doi.org/10.33772/jitro.v7i3.13442.