JOURNAL OF ADVANCED VETERINARY AND ANIMAL RESEARCH
ISSN 2311-7710 (Electronic)
http://doi.org/10.5455/javar.2021.h489
A periodical of the Network for the Veterinarians of Bangladesh (BDvetNET)

March 2021
VOL 8, NO. 1, PAGES 84–90

ORIGINAL ARTICLE

Isolation and detection of antibiotics resistance genes of *Escherichia coli* from broiler farms in Sukabumi, Indonesia

Aprilia Hardiati, Safika Safika, I Wayan Teguh Wibawan, Agustin Indrawati, Fachriyan Hasmi Pasaribu
Department of Animal Infectious Diseases and Veterinary Public Health, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia

ABSTRACT

Objective: This study aimed to isolate and identify *Escherichia coli* from broiler samples from Sukabumi, Indonesia. Also, antibiogram studies of the isolated bacteria were carried out considering the detection of the antibiotic resistance genes.

Materials and Methods: Cloaca swabs (n = 45) were collected from broilers in Sukabumi, Indonesia. Isolation and identification of *E. coli* were carried out according to standard bacteriological techniques and biochemical tests, followed by confirmation of the polymerase chain reaction targeting the *uspA* gene. Antibiotic sensitivity test, using several antibiotics [tetracycline (TE), oxytetracycline (OT), ampicillin (AMP), gentamicin (CN), nalidixic acid (NA), ciprofloxacin (CIP), enrofloxacin (ENR), chloramphenicol, and erythromycin] was carried out following the Kirby–Bauer disk diffusion method. Detection of antibiotic resistance coding genes was carried out by PCR using specific oligonucleotide primers. Statistical analysis was carried out with one-way analysis of variance.

Results: The results showed that 55.6% (25/45) of the samples were associated with the presence of *E. coli*. Antibiotic sensitivity test showed that the *E. coli* isolates were resistant to TE (88%; 22/25), OT (88%; 22/25), AMP (100%; 25/25), CN (64%; 16/25), NA (100%; 22/25), CIP (88%; 22/25), ENR (72%; 18/25), chloramphenicol (0%; 0/25), and erythromycin (92%; 23/25). On the other hand, the antibiotic resistance coding genes were *tetA* (86.4%; 19/22), *blaTEM* (100%; 25/25), *aac(3)-IV* (0%; 0/16), *gyrA* (100%; 25/25), and *ermB* (13%; 3/23). It was found that chloramphenicol is markedly different from other antibiotic treatment groups.

Conclusion: *Escherichia coli* was successfully isolated from cloacal swabs of broiler in Sukabumi, Indonesia. The bacteria were resistant to TE, OT, AMP, CN, NA, CIP, ENR, and erythromycin. Chloramphenicol was more sensitive and effective than other antibiotics in inhibiting the growth of *E. coli.* The antibiotic resistance genes detected were *tetA*, *blaTEM*, *gyrA*, and *ermB*.

ARTICLE HISTORY

Received July 29, 2020
Revised December 06, 2020
Accepted December 18, 2020
Published March 05, 2021

KEYWORDS

Antibiotics; Broiler; Escherichia coli; Gene; Resistance

Introduction

Sukabumi district of Indonesia has many chicken farms, mostly broiler farms, having an estimation of 10.096.368 broiler chickens [1]. A high population without adequate management can cause an increase in the incidence of disease or even emerging microbial diseases. Antibiotics are randomly used to treat bacterial infections nowadays [2].

Antibiotics are mostly produced by microorganisms (bacteria and/or fungi), functioning as inhibitors of bacterial growth or killers of other microorganisms [3]. In both humans and livestock, antibiotics have an essential role in the minimizing disease [4]. Antibiotics are generally given to treat diseases caused by bacterial infections [5]. However, on chicken farms, antibiotics are used for treatment, disease prevention, and growth triggers [6]. The unauthorized use of antibiotics is one of the risk factors influencing growing antibiotic resistance [5].

Escherichia coli acts as normal flora present in the digestive tract of humans, animals, and birds [7]. *Escherichia coli* resistance to various antibiotics was recorded at 88.2% in chickens. It is a massive concern for animal health and the veterinary communities [8]. *Escherichia coli* resistance

Correspondence Fachriyan Hasmi Pasaribu ✉ fhpasaribu@gmail.com Department of Animal Infectious Diseases and Veterinary Public Health, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia.

How to cite: Hardiati A, Safika S, Wibawan IWT, Indrawati A, Pasaribu FH. Isolation and detection of antibiotics resistance genes of *Escherichia coli* from broiler farms in Sukabumi, Indonesia. J Adv Vet Anim Res 2021; 8(1):84–90.

to antibiotics in chickens can be transferred directly or indirectly through foods and other chickens or animals. Resistant bacteria colonize and can share their resistance properties to normal flora. Resistance genes are transferred vertically between genera and family or horizontally between bacteria in the genus and family [9]. Therefore, this research aims to isolate and identify *E. coli* and assessing antibiotic sensitivity patterns considering the antibiotic resistance genes.

Materials and methods

Ethical statement

Written or verbal permission was taken from the farm owner/manager/chicken handler before sample collection. A professional veterinarian did the sample collection. No animals were used for many experiments in this study.

Sample collection

Sample collection used a random sampling method. Cloacal swab samples (n = 45) were collected in January 2019 from healthy broilers in Sukabumi, Indonesia. The samples were stored in buffer peptone water in an ice box at 4°C during transportation. The samples were stored in a refrigerator at 4°C until further analysis.

Microbiological analysis

Samples were cultured by direct streaking method on Eosin Methylene Blue and MacConkey agars, and then incubated for 18–24 h at 37°C [10]. The tests were carried out on triple sugar iron agar media, urea, and indole, methyl-red, Voges–Proskauer, and citrate (IMViC) media–. Bacterial incubation in the triple sugar iron agar and urea media was carried out at 37°C for 18–24 h. IMViC test was carried out at 37°C for 48 h. Test results referring to *E. coli* were then stained with Gram stain to determine bacterial cell morphology [7].

According to the manufacturer's instructions, the PrestoTM Mini gDNA bacteria kit (Geneaid) was used to extract bacterial DNA. Confirmation of *E. coli* isolates was carried out using the PCR method with MyTaq™ HS Red Mix (Bioline). The *uspA* gene amplification was carried out using primers reported by Mishra et al. [11]. A volume of 25 µl was prepared for conducting PCR reactions containing 4 µl template, 2 µl forward primer (20 µM), 2 µl reverse primer (20 µM), 12 µl MyTaq™ Red Mix (2×), and added ddH_2O to 25 µl. Thermal Cycler T100™ (Bio-Rad) was used to carry out PCR amplification.

Predenaturation was carried out at 95°C for 1 min. Amplification of 30 cycles consisted of denaturation at 95°C for 15 sec, annealing at 58°C for 15 sec, and extension at 72°C for 10 sec. A final extension step was carried out at 72°C for 5 min. Electrophoresis for PCR products was carried out on 1% agarose gel in TAE buffer (1×). The staining for the amplified DNA fragments was carried out by using 2 µl FloroSafe DNA Stain (1st BASE). A DNA marker (100 bp) was used as a standard. The positive samples for the *uspA* gene will show DNA bands at an amplicon length of 884 bp. The positive control bacterium was *E. coli* ATCC 25922.

Antibiotics sensitivity testing

The Kirby–Bauer's disk diffusion method was used in this study to identify antibiotic resistance patterns in *E. coli* [9]. Bacterial suspension from Tryptic Soy agar was diluted with sterile physiological NaCl to make the standard equivalent of 1.5×10^8 colony forming units/ml with the 0.5 McFarland standard. The suspension was cultured on Mueller–Hinton agar media using a sterile cotton bud then antibiotic disks were put on the agar surface. Incubation was carried out for 16–18 h at 35°C. Antibiotics used in this study included tetracycline (TE) 30 µg/disk, oxytetracycline (OT) 30 µg/disk, ampicillin (AMP) 10 µg/disk, gentamicin (CN) 10 µg/disk, nalidixic acid (NA) 30 µg/disk, ciprofloxacin (CIP) 5 µg/disk, enrofloxacin (ENR) 5 µg/disk, chloramphenicol (C) 15 µg/disk, and erythromycin (E) 30 µg/disk. Antibiotic inhibition zones formed on the Mueller–Hinton agar medium were then measured and adjusted to the standards set by Clinical and Laboratory Standards Institute [12].

Detection of antibiotic resistance genes

According to the manufacturing procedures, the PCR detection of antibiotic resistance genes using the MyTaq™ HS Red Mix (Bioline) was carried out. The specific primer pairs were prepared for *tetA* [13], *blaTEM* [14], *aac(3)-IV* [15], *gyrA* [16], and *ermB* gene [17]. The 25 µl PCR component consisted of 4 µl template, 2 µl forward primer (20 µM), 2 µl reverse primer (20 µM), 12 µl MyTaq™ Red Mix (2×), and then added ddH_2O to 25 µl. The PCR process was carried out with Thermal Cycler T100™ (Bio-Rad) and then visualized by electrophoresis.

Statistical analysis

Statistical analysis for each antibiotic group was carried out using the one-way analysis of variance method using Statistical Package for the Social Sciences 21 software to see its difference. Follow-up tests were carried out to see which group was different. First, the homogeneity test of variance; if the homogeneous variety is continued with Scheffe's test, if the variance is not homogeneous, then the Dunnet C test is used. A p-value < 0.05 was considered significant.

Results and Discussion

Overall, the prevalence of *E. coli* was 55.6% (25/45). Confirmation of *E. coli* was carried out by targeting the *uspA* gene. The *uspA* gene plays an essential role for *E. coli* to survive during growth and helps in adhesion and motility. As long as the bacteria are growing normally, *uspA* does not affect, but when the conditions are not sufficient, for example, lack of carbon, the *uspA* will be expressed [18]. In this study, the *uspA* gene in *E. coli* could be amplified using specific primers. The amplification product by PCR was 884-bp (Fig. 2a).

The antibiotic sensitivity of *E. coli* isolates was conducted against nine antibiotics. The antibiotics used in this study were often used in Indonesian chicken farms. Based on the data from the Directorate General and Animal Health of Indonesia [6], the widely used antibiotics included: ENR (49.4%), CIP (5.1%), OT (4.8%), AMP (3.9%), and erythromycin (3.1%). Zalizar et al. [19] stated that TE (1.83%) and aminoglycoside (0.46%) antibiotics were also widely used in Indonesian chicken farms. Table 1 shows the patterns of antibiotic resistance in *E. coli*; eight antibiotic resistance patterns were recorded. Each pattern shows *E. coli* resistance to AMP and NA. All isolates tested showed multidrug-resistant (MDR) properties. Multidrug resistance is a condition of resistance to three or more antibiotics groups in an isolate [20]. Two main mechanisms cause the nature of MDR in bacteria; they are (1) accumulation of several genes in plasmid R (resistance), each of which encodes the resistance to one antibiotic in a single cell, and (2) due to the increased gene expression that encodes the efflux pump against several antibiotics [21].

Table 1. Antibiotics resistance patterns in *E. coli*.

No	Antibiotic groups	Resistance Patterns	Resistant isolates
1	6	AMP[1]-NA[2]-E[3]-TE[4]-OT[4]-CIP[5]-ENR[5]-CN[6]	10
2	5	AMP[1]-NA[2]-E[3]-TE[4]-OT[4]-CIP[5]-ENR[5]	6
3	5	AMP[1]-NA[2]-TE[4]-OT[4]-CIP[5]-ENR[5]-CN[6]	3
4	5	AMP[1]-NA[2]-E[3]-CIP[5]-ENR[5]-CN[6]	1
5	5	AMP[1]-NA[2]-E[3]-CIP[5]-CN[6]	1
6	4	AMP[1]-NA[2]-TE[4]-OT[4]-CIP[5]-ENR[5]	1
7	4	AMP[1]-NA[2]-E[3]-TE[4]-OT[4]	2
8	3	AMP[1]-NA[2] -CN[6]	1

[1]β-lactamase group, [2]Quinolone group, [3]Macrolide group, [4]Tetracycline group, [5]Fluoroquinolone group, [6]Phenocol group.
AMP = amphicilin, NA = nalidixic acid, E = erythromycin, TE = tetracycline, OT = oxytetracycline, CIP = ciprofloxacin, ENR = enrofloxacin, CN = gentamicin.

Antibiotic resistance in this study (Fig. 1) followed several studies conducted in Indonesia. According to Edityandari [22], *E. coli* from chickens were resistant to erythromycin (100%), AMP (100%), ENR (80%), OT (20%), and still sensitive to CN. The utilization of antibiotics, while not an oversight, will cause cases of antibiotic resistance. According to Arief et al. [23], 72.3% of the farmers used antibiotics without veterinary supervision. Antibiotics are recommended to treat bacterial infections. However, only 30.2% of the farmers in Indonesia used antibiotics for treatment purposes. The majority of breeders in Indonesia (81.4%) use antibiotics as disease prevention. Besides, a small proportion of farmers (0.3%) use antibiotics as a growth promoter [6].

Antibiotics added to feed as antibiotics growth promoters are considered to trigger livestock growth significantly in a relatively short time. Furthermore, it increases feed

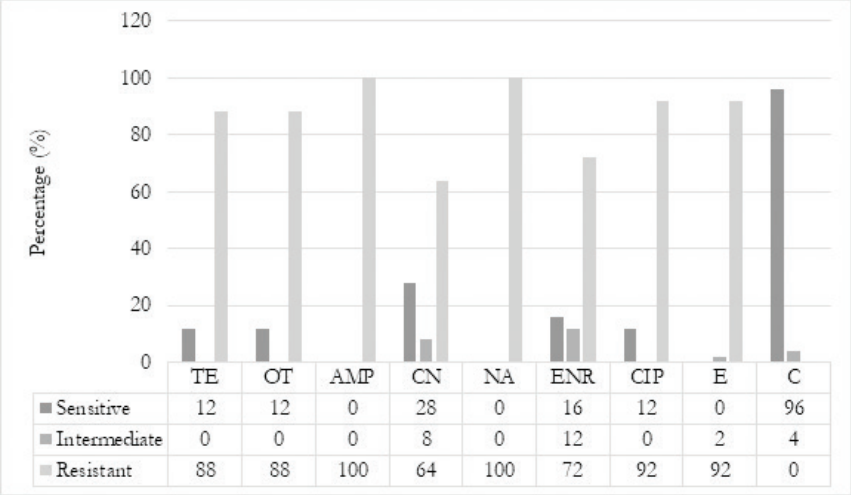

Figure 1. Antibiogram profile of *E. coli* from the poultry farm in Sukabumi. TE = tetracycline, OT = oxytetracycline, AMP = amphicilin, CN = gentamicin, NA = nalidixic acid, ENR = enrofloxacin, CIP = ciprofloxacin, E = erythromycin, C = chloramphenicol.

efficiency. The addition of antibiotics to feed in Indonesia has been carried out since 1970 when broiler chicken farms began to develop [24]. Since the 1990s, some countries have been using antibiotics as food additives, like Sweden (1986), Denmark (1995), German (1996), and Switzerland (1996) [25]. Prohibition of the addition of antibiotics in feed in Indonesia has existed since 2009, which is regulated by Law Number 18 of 2009 Article 22 Paragraph 4c. However, the law has not been effectively implemented. The Indonesian Ministry of Agriculture has reiterated the ban since January 2018, with Permentan Number 14 of 2017 [26].

The efflux pump activity in antibiotic-resistant Gram-negative bacteria was encoded by *tet*, one of which is *tetA*. Efflux pump activity removes TE from cells using protons as an energy source [27]. The results of *tetA* gene amplification (Fig. 2b) are shown with 965-bp amplification products. Isolates of *E. coli* are TE and OT-resistant, which has 86.4% *tetA* gene (19/22). These findings supported the previous report of Ibrahim et al. [28], who described that 78.4% of *E. coli* isolates possessed the *tetA* gene. According to van Hoek et al. [29], in addition to the *tetA* gene, other *tet* genes could encode bacterial resistance to the TE group. The other *tet* genes included *tetB*, *tetC*, *tetD*, and *tetE* [30].

The β-lactamase enzymes induce the β-lactam group's resistance in many bacteria, especially the enzyme extended-spectrum β-lactamases (ESBLs). ESBL will hydrolyze the AMP β-lactam ring in the periplasm of bacteria. Broken bonds cause antibiotics to fail, so antibiotic reactions do not occur with Penicillin Binding Proteins PBPs [31]. One of the ESBLs encoding genes is *blaTEM*. The TEM β-lactamase produced by clinical *E. coli* strains was firstly reported in 1965 [32]. In this research, the *blaTEM* gene was present in all isolates (100%; 25/25) with a 516-bp PCR product (Fig. 3a), as reported by Hayati et al. [33].

Bacterial resistance to aminoglycosides is primarily occurred by modifying the chemical composition of antibiotics by aminoglycoside modifying enzymes. The enzyme consists of three subclasses based on chemical modifications made to aminoglycosides, namely AG N-acetyltransferases, AG O-nucleotidyltransferases, and AG O-phosphotransferases. Every subclass modifies certain specific positions [34]. The *aac(3)-IV* gene was not present (0%, 0/16) in this study (Fig. 3b). In contrast to the research by Amer et al. [35], eight (40%; 8/20) isolates of *E. coli* were detected having the *aac(3)-IV* gene. *E. coli* might have other genes related to CN resistance, namely *aac(3)-I*, *aac(3)-II*, *aac(3)-III*, *aac(3)-VIII*, *aac(6)-I*, and *aac(6)-II* [36].

In this study, all the isolates showed (100%; 25/25) the presence of the *gyrA* gene. The *gyrA* gene amplification product was 626-bp (Fig. 4a). A high percentage (98.3%) of *gyrA* gene detection was also reported by Ogbolu et al. [37]. The resistance to CIP, NA, and ENR found in this study probably resulted from mutations in the *gyrA*. According to Hopkins et al. [38], quinolone and fluoroquinolone resistance are mostly caused by changes in the *gyrA* gene. The target protein change occurs in the terminal amino acid domain, the quinolone resistance determining region. The amino acid substitution will change the target protein structure, thus decreasing the affinity by enzyme [39]. Based on the report of Ogbolu et al. [37], Gram-negative bacteria having resistance property to CIP and NA undergo amino acid substitution in *gyrA* gene codon 83 protein

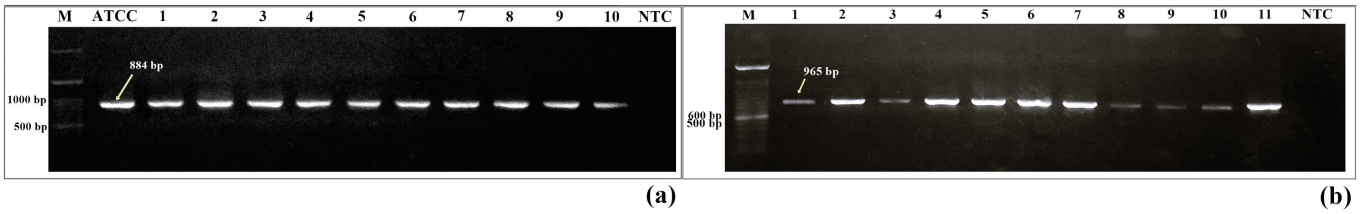

Figure 2. Amplification *uspA* gene 884-bp (a) and *tetA* gene 965-bp (b) from *E. coli*. M = DNA marker 100 bp, ATCC = *E. coli* ATCC 25922 as a positive control, 1–11 = *E. coli* isolates, NTC = non-template control.

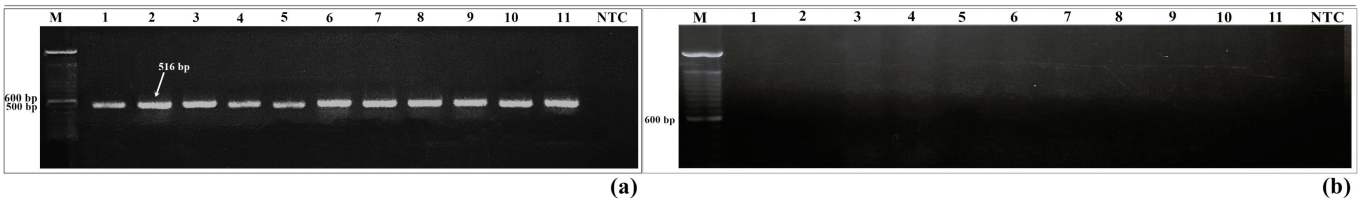

Figure 3. Amplification *blaTEM* gene 516-bp (a) and *aac(3)-IV* gene 286-bp (b) from *E. coli*. The *aac(3)-IV* gene was not detected. M = DNA marker 100 bp, 1–11 = *E. coli* isolates, NTC = non-template control.

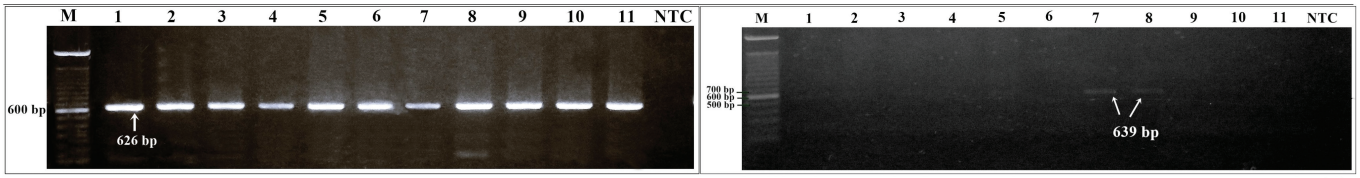

Figure 4. Amplification *gyrA* gene 626-bp (a) and *ermB* gene 639-bp (b) from *E. coli*. M = DNA marker 100 bp, 1–11 = *E. coli* isolates, NTC = non-template control.

(Serin → Leucine). The *gyrA* gene of ENR-resistant *E. coli* changed the codon 83 (Serin → Leucine) and 87 (Aspartate → Glycine) [40].

The *ermB* gene amplification results in erythromycin-resistant *E. coli* isolates showed 13% (3/23) positive isolates of the *ermB* gene with 639-bp amplification products (Fig. 4b). According to Cesur and Demiroz [41], erythromycin-resistant isolates are caused by Gram-negative bacteria's impermeable nature to macrolide that is hydrophobic. The action of macrolide antibiotics is to inhibit protein synthesis. Erythromycin binds 50s ribosome subunit in bacteria so that protein synthesis is inhibited [42]. The *erm* gene (erythromycin ribosome methylase) encodes the Erm methyltransferase enzyme group. The Erm methyltransferation enzyme modifies a single 23s rRNA in the 50s ribosome subunit. For this reason, it causes a decrease in the affinity of the antibiotics bond [43].

The mean test results for group differences showed a mean difference in each antibiotic group with a value of $p = 0.00$ ($p < 0.05$). The Dunnet C test was chosen for the follow-up test because there were differences in each group's mean. Chloramphenicol had a significant difference in mean difference with all groups. The TE treatment group had a significantly different mean difference with CN, ENR, and CIP, in addition to the chloramphenicol group. In addition to having differences with the chloramphenicol treatment group, AMP had a significantly different mean difference with CN, ENR, CIP, erythromycin, and chloramphenicol. The CN treatment group also had a significantly different mean difference with NA and erythromycin. The NA treatment group had a significantly different mean difference with ENR, CIP, and erythromycin. AMP and TE antibiotics are indeed the antibiotics that are often used in the world of chicken farming [6,14].

The findings of this study enrich the data of current antibiotic resistance conditions. Antibiotic-resistant bacteria in animals multiply and become the dominant bacterial population. They transmit their antibiotics resistance genes to offspring via vertical gene transfer, called innate or natural or intrinsic resistance. Antibiotic-resistant bacteria, called acquired resistance, can horizontally transfer their resistance genes within and between bacterial species [44].

Veterinarians, farmers, abattoir workers, and food handlers can be contaminated by resistant bacteria directly, where the exchange and acquisition of resistance mechanisms occur [45]. The indirect transmission along the food chain is a complex pathway. By contact or ingestion of infected food items, humans may be exposed to resistant bacteria. The presence of resistant bacteria in various food products from different animal sources (poultry, cattle, pigs, goats, and sheep) and diverse food production stages has been identified [46,47]. Many antibiotic resistance consequences include inadequate treatment of pathogenic bacterial infection, increased patient-level morbidity and mortality, increased resource use, higher costs, and decreased hospital operation at the healthcare level [48].

Conclusion

MDR *E. coli* have been isolated from poultry farms in Sukabumi, Indonesia. The *E. coli* were resistant to TE, OT, AMP, CN, NA, CIP, ENR, and erythromycin. The genes, namely *tetA*, *blaTEM*, *gyrA*, and *ermB*, were present in the *E. coli* isolates.

List of abbreviations

°C, degree CelciusCelsius; PCR, polymerase chain reaction; TAE, Tris Acetic EDTA; ATCC, American Type Culture Collection; ml, milliliter; µl, microliter; h, hours; min, minutes; sec, seconds; bp, base pair.

Acknowledgments

The authors thank the broiler farmer in Sukabumi who supplied the samples. The study's funder was the Ministry of Research and Technology/National Agency Republic of Indonesia for Research and Innovation for the scholarship of the Masters to Doctorate Program for Superior Bachelor (PMDSU) Batch III.

Conflict of interest

The authors declare that they have no conflict of interests.

Authors' contribution

FHP and S designed the study. AH was involved in laboratory work, interpreted the data, and drafted the manuscript. S, IWTW, and AI took part in the critical checking of this manuscript. All the authors read and approved the publication of this article.

References

[1] West Java Central Statistics Agency. Poultry population by Regency/City and poultry type in West Java Province. 2018; Available via https://jabar.bps.go.id/statictable (Accessed July 5, 2018).

[2] Barton MD. Antibiotic use in animal feed and its impact on human health. Nutr Res Rev 2000; 13(2):279–99; https://doi.org/10.1079/095442200108729106

[3] Madigan MT, John MM, Kelly SB, Daniel HB, David AS. Brock biology of microorgansms, global edition. Pearson Education Limited, London, UK, pp 387–5, 2015.

[4] Ayatollahi J, Shahcheraghi SH, Akhondi R, Soluti SS. Antibiotic resistance patterns of *Escherichia coli* isolated from children in Shahid Sadoughi Hospital of Yazd. Iran J Pediatr Hematol Oncol 2013; 3(2):78–82.

[5] George DF, Gbedema SY, Agyare C, Adu F, Boamah FE, Tawiah AA, et al. Antibiotic resistance patterns of *Escherichia coli* isolates from Hospitals in Kumasi, Ghana. Int Sch Res Notices 2012; 10:1–5; https://doi.org/10.5402/2012/658470

[6] Directorate General and Animal Health. Current Situation and Government Policy on Antimicrobial Resistance (AMR) in the Livestock and Animal Health Sector. Presented at the AMR Studium Generale at Menara 165 Jakarta, Indonesia. 2019.

[7] Quinn PJ, Markey BK, Leonard FC, FitzPatrick ES, Fanning S. Concise review of veterinary microbiology. 2nd edition, Wiley Blackwell, Hoboken, NJ, pp 58–60, 2015.

[8] Yassin AK, Jansen G, Patrick K, Guangwu L, Luca G, Lanjing W, et al. Antimicrobial resistance in clinical *Escherichia coli* isolates from poultry and livestock, China. Plos One 2017; 12(9):e0185326; https://doi.org/10.1371/journal.pone.0185326

[9] Talebiyan R, Mehdi K, Faham K, Mohammad R. Multiple antimicrobial resistance of *Eschericia coli* isolated from chickens in Iran. Vet Med Int 2014; 49:14–8; https://doi.org/10.1155/2014/491418

[10] Leboffe MJ, Burton EP. A photographic atlas for the microbiology laboratory. 4th edition, Morton Publishing, Englewood, CO, pp 96–7, 2011.

[11] Mishra AK, Desh DS, Gururaj K, Geetika G, Nitika S, Naveen K, et al. *UspA* gene based characterization of *Escherichia coli* isolated from different disease condition in goats. J Anim Res 2017; 7(6):1–6; https://doi.org/10.5958/2277-940X.2017.00168.1

[12] CLSI. Performance standards for antimicrobial susceptibility testing. 28th edition, Clinical and Laboratory Standards Institute, Westminster, MD, p M100, 2018.

[13] Chuah L, Ahamed-Kamal SS, Ismail MS, Tajudin FH, Gulam R. Data on antibiogram and resistance genes harboured by *Salmonella* strains and their pulsed-field gel electrophoresis clusters. Data Brief 2018; 17:698–702; https://doi.org/10.1016/j.dib.2018.01.098

[14] Colom K1, Perez J, Alonso R, Fernández-Aranguiz A, Lariño E, Cisterna R. Simple and reliable multiplex PCR assay for detection of *blaTEM*, *bla(SHV)* and *blaOXA-1* genes in *Enterobacteriaceae*. Fed Eur Microbiol Soc Microb Lett 2003; 223(2):147–51; https://doi.org/10.1016/S0378-1097(03)00306-9

[15] Van TTH, Chin J, Chapman T, Tran LT, Coloe PJ. Safety of raw meat and shellfish in Vietnam: an analysis of *Eschericia coli* isolations for antibiotic resistance and virulence genes. Int J Food Microbiol 2008; 124(3):217–23; https://doi.org/10.1016/j.ijfoodmicro.2008.03.029

[16] Nawaz M, Khan SA, Tran Q, Sung K, Khan AA, Adamu I, et al. Isolatioan and characterization of mutidrug-resistant *Klebsiella* spp. isolated from shrimp imported from Thailand. Int J Food Microbiol 2012; 155(3):179–84; https://doi.org/10.1016/j.ijfoodmicro.2012.02.002

[17] Song JH, Chang HH, Suh JY, Ko KS, Jung SI, Oh WS, et al. Macrolide resistance and genotyping characterization of *Streptococcus pneumoniae* in Asian countries: a study of the Asian network for surveillance of resistant pathogens (ANSORP). J Antimicrob Chemother 2004; 53(3):457–63; https://doi.org/10.1093/jac/dkh118

[18] Nachin L, Nannmark U, Nystrom T. Differential roles of the universal stress proteins of *Escherichia coli* in oxidative stress resistance, adhesion, and motility. J Bacteriol 2005; 187(18):6265–72; https://doi.org/10.1128/JB.187.18.6265-6272.2005

[19] Zalizar L, Relawati R, Pancapalaga W. Usage of antibiotic on chicken in district of Malang, East Java, Indonesia. Proc. Int. Sem. November 3–5 2015, Kendari, Indonesia. Proceeding of International "Improving Tropical Animal Production for Food Security" 3–5 November 2015, Halu Oleo University, Kendari, Indonesia.

[20] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012; 18:268–81; https://doi.org/10.1111/j.1469-0691.2011.03570.x

[21] Nikaido H. Multidrug resistance in bacteria. Ann Rev Biochem 2009; 78:119–46; https://doi.org/10.1146/annurev.biochem.78.082907.145923

[22] Edityandari CP. Antibiotic resistance profiles of *Escherihia coli* from feces of australian imported cattle. Thesis. IPB University, Bogor, Indonesia, pp 8–11, 2017.

[23] Arief RA, Darmawan RD, Sunandar, Widyastuti MDW, Nugroho E, Jatikusumah A, et al. 2016. Penggunaan antibiotika pada peternakan ayam petelur di Propinsi Jawa Tengah, Indonesia. *Konferensi Ilmiah Veteriner Nasional 14*; Serpong, Indonesia: PDHI, pp 163–5, 2016.

[24] Soeharsono. Probiotics: scientific basis of application and practical aspects. Widya Padjajaran, Bandung, Indonesia, pp 23–9, 2010.

[25] Midili M, Alp M, Kocabagli N, Muglali OH, Turan N, Yılmaz H, et al. Effects of dietary probiotic and prebiotic supplementation on growth performance and serum IgG concentration of broilers. S Afr J Anim Sci 2008; 38:21–7; https://doi.org/10.4314/sajas.v38i1.4104

[26] Sumanto D. Awareness of the antibiotics growth promoters (AGP) and its application in chicken feed. Proc Int Sem Live Stock Prod Vet Technol 2016; 2016:462–8; https://doi.org/10.14334/Proc.Intsem.LPVT-2016-p.462-468

[27] Tuckman M, Petersen PJ, Projan SJ. Mutations in the interdomain loop region of the *tetA* tetracycline resistance gene increase efflux of minocycline and glycylcyclines. Microb Drug Resist 2000; 6:277–82; https://doi.org/10.1089/mdr.2000.6.277

[28] Ibrahim RA, Cryer TL, Lafi SQ, Basha EA, Good L, Tarazi YH. Identification of *Escherichia coli* from broiler chickens in Jordan, their antimicrobial resistance, gene characterization and the associated risk factors. BMC Vet Res 2019; 15(1):159; https://doi.org/10.1186/s12917-019-1901-1

[29] van Hoek AH, Mevius D, Guerra B, Mullany P, Roberts AP, Aarts HJ. Acquired antibiotic resistance genes: an overview. Front Microbiol 2011; 2:203; https://doi.org/10.3389/fmicb.2011.00203

[30] Nguyen F, Starosta AL, Arenz S, Sohmen D, Donhofer A, Wilson DN. Tetracycline antibiotics and resistance mechanism. Biol Chem 2014; 395(5):559–75; https://doi.org/10.1515/hsz-2013-0292

[31] Munita JM, Arias C. Mechanism of antibiotic resistance. Microbiol Spectr 2016; 4(2):1–37; https://doi.org/10.1128/microbiolspec.VMBF-0016-2015

[32] Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 2005; 18:657–86; https://doi.org/10.1128/CMR.18.4.657-686.2005

[33] Hayati M, Indrawati A, Mayasari NLPI. Molecular detection of extended spectrum β-lactamase-producing *Klebsiella pneumoniae* isolates of chicken origin from East Java, Indonesia. Vet World 2019; 12(4):578–83; https://doi.org/10.14202/vetworld.2019.578-583

[34] Ramirez MS, Tolmasky ME. Aminoglycoside modifying enzyme. Drug Resist Updat 2010; 13(6):151–71; https://doi.org/10.1016/j.drup.2010.08.003

[35] Amer MM, Hoda MM, Aziza MA, Hanaa SF. Antimicrobial resistance genes in pathogenic *Escherichia coli* isolated from diseased broiler chickens in Egypt and their relationship with the phenotypic resistance characteristics. Vet World 2018; 11(8):1082–8; https://doi.org/10.14202/vetworld.2018.1082-1088

[36] Zarate SG, Luisa DCC, Raul B, Julia R, Anrdreas GS, Agatha B. Overcoming aminoglycoside enzymatic resistance: design of novel antibiotics and inhibitors. Molecules 2018; 23(2):284; https://doi.org/10.3390/molecules23020284

[37] Ogbolu DO, Daini OA, Ogunledun A, Terry AAO. Effects of *gyrA* and *parC* mutation in quinolones resistant clinical gram-negative bacteria from Nigeria. Afr J Biomed Res 2012; 15:97–104.

[38] Hopkins KL, Davies RH, Threlfall EJ. Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: recent developments. Int J Antimicrob Agents 2005; 25(5):358–73; https://doi.org/10.1016/j.ijantimicag.2005.02.006

[39] Hooper, DC. Mechanisms of action and resistance of older and newer fluoroquinolones. Clin Infect Dis 2000; 31(2):S24–8; https://doi.org/10.1086/314056

[40] Jurado S, Orden JA, Harcajo P. Characterization of fluoroquinolone resistance in *Escherichia coli* strains from ruminants. J Vet Diagn Invest 2008; 20(3):342–5; https://doi.org/10.1177/104063870802000314

[41] Cesur S, Demiroz AP. Antibiotics and the mechanism of resistance to antibiotics. Med J Islamic World Acad Sci 2013; 21(4):138–42; https://doi.org/10.12816/0002645

[42] Riviere JE, Papich MG. Veterinary pharmacology and therapeutics. 10th edition. Wiley-Blackwell, Hoboken, New Jersey, pp 903–18, 2017.

[43] Wendlandt S, Shen J, Kadlec K, Wang Y, Li B, Zhang WJ, et al. Multidrug resistance genes in *Staphylococci* from animals that confer resistance to critically and highly important antimicrobial agents in human medicine. Trends Microbiol 2015; 23(1):44–54; https://doi.org/10.1016/j.tim.2014.10.002

[44] Holmes AH, Moore LS, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, et al. Understanding the mechanisms and drivers of antimicrobial resistance. Lancet. 2016; 387(10014):176–87; https://doi.org/10.1016/S0140-6736(15)00473-0

[45] Marshall BM, Levy SB. Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev 2011; 24(4):718–33; https://doi.org/10.1128/CMR.00002-11

[46] Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, et al. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. mBio 2012; 3(1):e00305–11; https://doi.org/10.1128/mBio.00305-11

[47] Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 2016;16(2):161–8; https://doi.org/10.1016/S1473-3099(15)00424-7

[48] World Health Organization. Critically Important Antimicrobials for Human Medicine. 2nd Revision. World Health Organization, Geneva, Switzerland, 2009. Available via http://www.who.int/foodsafety/foodbornedisease/CIA2ndrev2009.pdf. (Accessed 01 December 2020).