BIODIVERSITAS
Volume 23, Number 9, September 2022
Pages: 4460-4465

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230911

Antibiotic resistance pattern of Extended-Spectrum β-Lactamase (ESBL) producing *Escherichia coli* isolated from broiler farm environment in Pasuruan district, Indonesia

SHEILA MARTY YANESTRIA[1], FIDI NUR AINI EKA PUJI DAMEANTI[1], BETI GISTAWATI MUSAYANNAH[2], JUNIANTO WIKA ADI PRATAMA[3], ADIANA MUTAMSARI WITANINGRUM[4], MUSTOFA HELMI EFFENDI[4,♥], EMMANUEL NNABUIKE UGBO[5]

[1]Doctoral Program in Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Mulyorejo, Kampus C FKH-UNAIR, Surabaya 60115, East Java, Indonesia
[2]Undergraduate Student in Faculty of Veterinary Medicine, Universitas Wijaya Kusuma. Jl. Dukuh Kupang XXV No.54, Surabaya 60225, East Java, Indonesia
[3]Department of Basic Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Wijaya Kusuma. Jl. Dukuh Kupang XXV No.54, Surabaya 60225, East Java, Indonesia
[4]Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Mulyorejo, Kampus C FKH-UNAIR, Surabaya 60115, East Java, Indonesia. Tel./fax.: +62-31-5992785, ♥email: mhelmieffendi@gmail.com
[5]Department of Applied Microbiology, Faculty of Science, Ebonyi State University. 480211, Abakaliki, Nigeria

Manuscript received: 7 July 2022. Revision accepted: 25 August 2022.

Abstract. *Yanestria SM, Dameanti FNAEP, Musayannah BG, Pratama JWA, Witaningrum AM, Effendi MH, Ugbo EN. 2022. Antibiotic resistance pattern of Extended-Spectrum β-Lactamase (ESBL) producing* Escherichia coli *isolated from broiler farm environment in Pasuruan district, Indonesia. Biodiversitas 23: 4460-4465. Escherichia coli* is one of the bacteria that can be used as an indicator of environmental pollution. This bacterium has the ability to become an antimicrobial-resistant bacterium that impacts public health. The antimicrobial ability can develop into a bacterium that produces extended-spectrum β-lactamase (ESBL). The purpose of this study was to reveal the occurrence of extended-spectrum β-lactamase (ESBL) producing *E. coli* from a broiler farm environment in Pasuruan district and to describe the phenotypic pattern of *E. coli* producing ESBL that has been detected. A total of 175 samples were used in this study consisting of 115 samples of coop swabs and 65 samples of wastewater around the farm. The samples were isolated and identified to find *E. coli* by using different culture media *viz.* McConkey agar (MCA), eosin methylene blue agar (EMBA), Gram staining, indole test, methyl red Voges Proskauer (MR-VP), citrate, and triple sugar iron agar (TSIA). Detection of ESBL using the double disc synergy test (DDST) according to standard Clinical and Laboratory Standards Institute procedures and the VITEK®2 compact apparatus. The results of ESBL confirmation with DDST and VITEK®2 showed that 16 (9.14%) of the 175 environmental samples confirmed *E. coli* produced ESBL. The results of the VITEK®2 test also produced a phenotypic pattern of resistance properties of ESBL-producing *E. coli* and found 12 types of resistance patterns. The combination of "AM AMP ATM KZ CTX CRO CIP" and "AM AMP ATM KZ CTX CRO GM CIP SXT" are the 2 most common resistance patterns (18.75%), while the other 10 resistance patterns occur at the same level (6.25%). The data presented here confirmed the presence of ESBL-producing *E. coli* in the farm environment, which can contribute to the dissemination of MDR bacteria in the environment if not monitored. Therefore, the presence of ESBL-producing *E. coli* in Pasuruan is worrisome since it can lead to an impact on human health.

Keywords: *Escherichia coli*, ESBL, public health, resistance patterns, VITEK®2 test

INTRODUCTION

Resistance is the ability of any bacteria to adapt to antibiotic exposure (Spellberg et al. 2013). This trait is a natural mechanism for survival. The main cause of resistance is the overuse of antibiotics in humans and animals (Niasono et al. 2019; Rahmahani et al. 2020). Bacterium show resistance to antibiotics if their growth cannot be inhibited antibiotics, even at maximum doses. A bacterium that carries antimicrobial resistance has a fatal effect on the patient. Deshpande et al. (2011) stated that antimicrobial resistance resulted in a prolonged illness, and increased risk of a longer period of hospitalization and death.

One form of antimicrobial resistance (AMR) is extended spectrum β-lactamase (ESBL). ESBL is a β-lactamase enzyme that can hydrolyze penicillins, first, second, and third-generation cephalosporins and aztreonam (except cephamycin and carbapenem) where their activity can be inhibited by clavulanic acid. The gene encoding ESBL is located on a plasmid that is easily transferred to another bacterium, resulting in the spread of resistance (Paterson and Banomo 2005). Various studies have shown that ESBL bacterium causes higher morbidity and mortality than non-ESBL bacterium (Nathisuwan et al. 2001; Paterson and Banomo 2005).

Resistance to ESBL-producing bacterium has been widely reported in Indonesia, including ESBL-producing

Escherichia coli originating from broiler farms (Effendi et al. 2021). *Escherichia coli* producing ESBL was found by detecting the CTX-M gene in broiler chicken feces samples at the Bogor City Chicken Slaughter Center in as many as 12 of 200 samples (6.0%) (Lukman et al. 2016). ESBL-producing *E. coli* which was also found by detecting the CTX gene in broiler cloacal swab samples in Blitar City were 45 of the 160 samples examined (28.13%) (Wibisono et al. 2020a).

The presence of ESBL-producing *E. coli* in food-producing animals is associated with public health problems because spread from animals to humans can occur at any time (Masruroh et al. 2016). Resistant bacterium contained in feces can migrate around farms, poultry slaughterhouses and during meat processing (Santos et al. 2013). There are several studies that show a relationship between the transfer of the ESBL-producing *E. coli* gene from birds to humans who come into direct contact with these animals. In addition to direct zoonotic transfer, food of animal origin has the potential to be a risk factor for bacterium colonization or infection in humans (Widodo et al. 2020).

The occurrence of *E. coli* producing ESBL in broiler chickens, apart from being caused by the unwise and overuse of antibiotics on farms, can also occur due to the transfer of resistant material from the environment outside the cage into the cage environment. The environment around the cage can store various resistant materials that can transfer between bacterium (Niasono et al. 2016, Widodo et al. 2020). The environment is the main source of resistance, there is a relationship between humans, animals and the environment that allows the transfer of bacterium including mobile genetic elements (MGEs) between bacterium species (Woolhouse et al. 2015; Riwu et al. 2020). The spread of resistance to other organisms through MGEs (plasmids and transposons) is being accelerated and strengthened by agricultural and livestock activities as well as human waste that pollutes the environment (FAO 2018). Therefore, the purpose of this study was to reveal the occurrence of *E. coli* producing ESBL from a broiler farm environment in Pasuruan district and to describe the phenotypic profile of *E. coli* producing ESBL that has been detected.

MATERIALS AND METHODS

Sampling collection

Sampling was carried out in three sub-districts (Sukorejo Pandaan, and Kejayan) in Pasuruan district, Indonesia. A total of 175 samples were taken in this study which consisted of 115 samples of swab cages and 65 samples of wastewater around the farm. The swab sample of the cage is a swab from the wall of the chicken coop which was taken aseptically using a sterile cotton swab (Onemed, Indonesia). All swab samples were put into sterile tubes containing sterile buffered peptone water (HiMedia, India) during transportation. A sampling of wastewater is carried out in rivers or ditches closest to the farm. A sample of 50 mL of wastewater was put into sterile plastic bottles for transportation (Ibrahim et al. 2013; Samanta et al. 2014). All samples were then taken using a thermobox at a temperature of 4°C to the laboratory for further analysis (Yanestria et al. 2019).

Isolation and identification of *Escherichia coli*

The *E. coli* enrichment stage was carried out by inserting 1 mL of wastewater or a swab sample into 9 mL of Mac Conkey broth (HiMedia, India) in a test tube, then incubated at 43°C for 24 h (Damayanti and Purwantisari, 2020). *Escherichia coli* was isolated using selective eosin methylene blue agar (EMBA) media (Oxoid, England) and incubated at 37°C for 18-24 h (Putra et al. 2020). Identification of *E. co*li using Gram staining and biochemical tests IMVIC (Indol-motility, methyl red, Voges Proskauer, citrate) and TSIA (Triple sugar iron agar) (Effendi et al. 2019; Wibisono et al. 2021).

Antibiotic sensitivity testing

Antibiotic Sensitivity Testing assay was carried out by using disc diffusion antibiotic sensitivity test using Mueller Hinton agar (MHA) (HiMedia, India) in accordance with the Clinical and Laboratory Standards Institute standard procedures. The disc (Oxoid, England) used in this study consisted of 5 antimicrobial classes (AMC), namely fluoroquinolones (ciprofloxacin, nalidixic acid), macrolides (erythromycin), tetracyclines (tetracyclines), aminoglycosides (streptomycin, gentamicin), β-lactams (ampicillin, amoxicillin-clavulanate, aztreonam). Planting in plates was done by taking 1-2 colonies of *E. coli* isolates on EMBA media using loop inoculation, then put into physiological NaCl using 0.5 Mc Farland (1.5×10^8 CFU/ml). By using a sterile swab, the suspension that has been made is then flattened on the surface of the MHA media. The plates were incubated at 35°C±2°C for 16-18 h under aerobic conditions. Interpretation of inhibitory results by measuring the diameter of the inhibition zone formed according to CLSI (CLSI 2020; Musa 2020; Putra et al. 2020). An isolate is defined as multidrug resistant if it is not sensitive to one or more antimicrobials in 3 different antimicrobial classes (Varga et al. 2019).

Confirmation of ESBL using Double Disc Synergy Test (DDST)

Isolates that were positive for multidrug resistance (MDR) were forwarded to the double disc synergy test (DDST). The assay was performed on Mueller Hinton Agar with three antibiotic discs (Oxoid, England) including ceftazidime (CAZ; 30 g), cefotaxime (CTX; 30 g), and Amoxycillin-clavulanic (AMC; 30 g) placed on the media in parallel. The aztreonam antibiotic disc (ATM, 30 g) was placed next to the three parallel discs. The cultures were incubated at 35°C±2°C for 16-18 hours. The test was considered positive when there was synergy and an increase in zone diameter of 5 mm for any of the antimicrobial agents tested in combination with clavulanate (Guo et al. 2019; CLSI 2020; Wibisono et al. 2021).

Confirmation of ESBL using VITEK®2

Isolates that were positive for ESBL in the DDST assay were forwarded to testing using the VITEK®2 compact (Biomerieux, USA) to confirm ESBL-producing *E. coli* and its resistance phenotypic profile. After isolation of the primary organism, handling is minimized in a simple standard inoculum. The inoculum is placed into the VITEK®2 cassette at the SMART CARRIER STATION™, where the VITEK® 2 card and the sample are virtually connected. After the cassette is loaded, the incubation and read system regulates the incubation and reading of each card (Putra et al. 2020).

RESULTS AND DISCUSSION

The results of the isolation and identification of *E. coli* showed that 35.65% were positive for *E. coli* from 115 samples of swab cages and 64.62% were positive for *E.coli* from 65 samples of wastewater. About 47.43% of the total 175 samples were detected with *E. coli* (Figure 1). AMR testing using the diffusion method showed that 33.04% of 115 coop swab samples and 36.92% of 65 wastewater samples, a total of 34.86% of 175 environmental samples, detected the presence of multidrug resistance *E. coli*. The results of ESBL confirmation with DDST and VITEK®2 showed that 16 (9.14%) of the 175 environmental samples confirmed to be *E. coli* producing ESBL (Figures 2-3, Table 1). The VITEK®2 test results also produced a phenotypic pattern of resistance properties of ESBL-producing *E. coli* (Table 2).

All ESBL-producing *E. coli* isolates (100%) were resistant to amoxicillin, ampicillin, cefazolin, cefotaxime and ceftriaxone, and very high resistance to aztreonam (87.5%) and ciprofloxacin (81.25%). To a lesser extent, resistance to gentamicin was about 62.5% and trimethoprim/sulfamethoxazole was about 56.25%. Low resistance rates were similar to the antibiotics ceftazidime (31.25%), ampicillin-sulbactam (25%) and lowest to cefepime (18.75%).

Multidrug resistance to three antimicrobial classes (AMC) was detected in all ESBL-producing *E. coli* isolates. The frequency profile of resistance to 4 and 5 AMC is 37.5% and 31.25%, respectively.

Based on the results of the study, 12 types of resistance patterns were found in *E. coli* producing ESBL (Table 2). The combination of "AM AMP ATM KZ CTX CRO CIP" and "AM AMP ATM KZ CTX CRO GM CIP SXT" were the 2 most common resistance profiles (18.75%), while the other 10 resistance profiles occurred at the same level (6.25%).

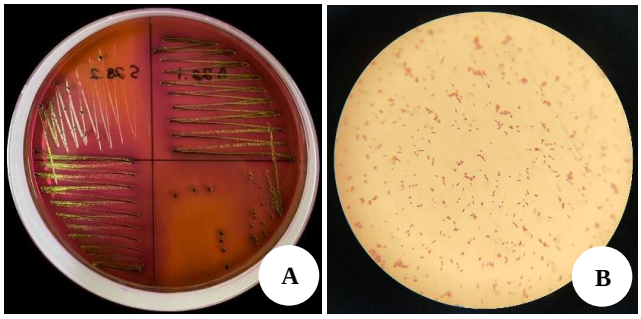

Figure 1. A. *Escherichia coli* on eosin methylene blue agar plate, B. Microscopic of *Escherichia coli*

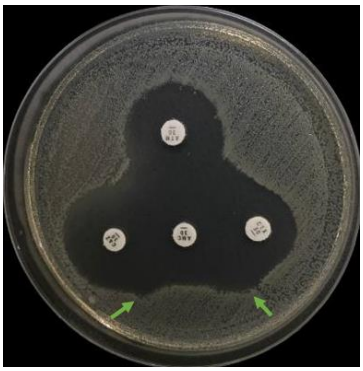

Figure 2. Positive result of double disc synergy test. Note: Green arrow indicates synergy of antibiotics

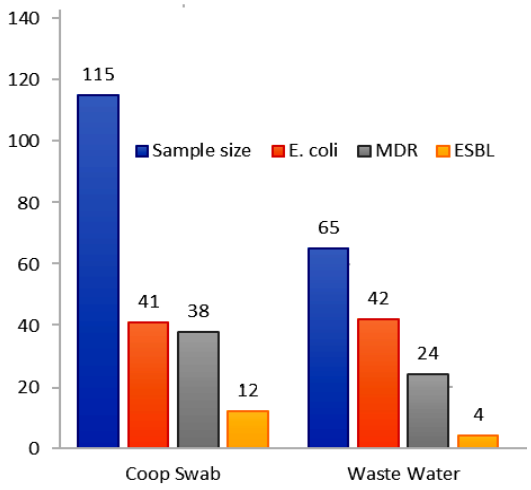

Figure 3. Results of detecting the amount of Extended Spectrum β-Lactamase (ESBL) producing *Escherichia coli* from environmental samples

Table 1. Detection Results of Extended Spectrum β-Lactamase (ESBL) producing *Escherichia coli* from Environmental Samples

Samples type	Sample size	*Escherichia coli*	MDR	ESBL	
				DDST	VITEK®2
Coop Swabs	115	41	38	12	12
Waste water	65	42	24	4	4
Total	175	83	62	16	16

Note: MDR: Multidrug Resistance, DDST: Double Disc Synergy Test

Table 2. Results of ESBL-producing *Escherichia coli* Phenotypic Patterns using VITEK®2

Antibiotics resistance pattern	No of AMC	*Escherichia coli* ESBL		Sample code
		No	%	
AM AMP ATM KZ CTX CRO CIP	3	3	18.75	S 26.1; S 42.2; A 15.1
AM AMP ATM SAM KZ CTX CRO GM	3	1	6.25	S 1.3
AM AMP ATM KZ CTX CRO FEP GM	3	1	6.25	A 10.3
AM AMP KZ CTX CRO GM CIP	4	1	6.25	A 38.1
AM AMP ATM KZ CTX CRO CIP SXT	4	1	6.25	S 10.2
AM AMP ATM KZ CTX CAZ CRO CIP SXT	4	1	6.25	S 2.3
AM AMP ATM SAM KZ CTX CAZ CRO GM CIP	4	1	6.25	S 19.1
AM AMP ATM SAM KZ CTX CAZ CRO CIP SXT	4	1	6.25	A 1.1
AM AMP SAM KZ CTX CAZ CRO GM SXT	4	1	6.25	S 1.2
AM AMP ATM KZ CTX CRO GM CIP SXT	5	3	18.75	S 10.2; S 12.1; S 44.2
AM AMP ATM KZ CTX CRO FEP GM CIP SXT	5	1	6.25	S 18.4
AM AMP ATM KZ CTX CAZ CRO FEP GM CIP SXT	5	1	6.25	S 52.2

Note: AMC: Antimicrobial classes, AM: Amoxicillin, AMP: Ampicillin, SAM: Ampicillin/Sulbactam, KZ: Cefazolin, CTX: Cefotaxime, CAZ: Ceftazidime, CRO: Ceftriaxone, FEP: Cefepime, ATM: Aztreonam, GM: Gentamicin, CIP: Ciprofloxacin, SXT: Trimethoprim/ sulfamethoxazole

Discussion

In Indonesia, information about *E. coli* producing ESBL in chicken farms is still limited. This study showed that the prevalence of ESBL-producing *E. coli* in broiler farms is around 9.14%. This prevalence can be considered low as compared to that reported by researchers from other countries. Research in Thailand, Vietnam, and Pakistan, both in Asia, showed the prevalence of *E. coli* ESBL in the poultry environment was 43.8%, 42.6%, and 14% (Ueda et al. 2015; Rahman et al. 2018; Rodroo et al. 2015). In other countries, Washington State has a prevalence of 11% and Spain has a prevalence of 20.7% (Shah et al. 2019; Alvarez et al. 2022). However, the prevalence in this study was considered higher when compared to studies from Nigeria with a prevalence of 4.6% (Aworh et al. 2020).

All ESBL-producing *E. coli* isolates (100%) were MDR. This is consistent with other studies in Thailand and Nigeria that all ESBL-producing *E. coli* originating from the polling environment have MDR properties (Tansawai et al. 2019; Aworh et al. 2020). The problem of MDR is exacerbated by the ability of the bacterium to transfer genetic material that carries resistance traits from one bacterium to another vertically through genetic mutations and horizontally through conjugation, transduction and transformation. MDR occurs when over antibiotics are used, the greater the selective pressure on the evolution and proliferation of resistant bacterium strains to defend themselves, resulting in vertical resistance from genetic mutations and horizontal resistance from the exchange of resistant gene material against various types of different antibiotic resistance mechanisms. Multidrug resistance is a common occurrence in ESBL-producing bacterium (Gregova et al. 2012; Effendi et al. 2021).

In this study, the resistance pattern "AM AMP ATM KZ CTX CRO GM CIP SXT" is one of the most frequent resistance phenotype profiles of ESBL-producing *E. coli* (3 out of 16 ESBL-producing *E. coli* isolates). This incident is similar to the results of another study in Indonesia (Blitar District) which showed this resistance pattern was also the most common (3 out of 10 ESBL-producing *E. coli* isolates), but the sample used was a cloacal swab (Wibisono et al. 2020b). There is a similar pattern of resistance although different types of samples, this study used environmental samples of poultry, while the other used a poultry cloacal swab. This is due to the high use of this antibiotic in the poultry sector, besides it can be related that *E. coli* that produces ESBL that is spread in the chicken farm environment comes from chicken feces.

The presence of ESBL bacterium in the poultry sector occurs due to the use of antibiotics as treatment, metaphylactic, prophylactic and growth promoters. The prohibition on the use of antibiotics as growth promoters has been compensated by the increasing use as metaphylactic and prophylactic in commercial livestock (Woolhouse et al. 2015; Wongsuvan et al. 2018, Permatasari et al. 2020). Antibiotics are often added to water or animal feed including broilers as a pragmatic solution because they are raised in groups making it difficult to isolate and treat only the infected. In addition, efforts to isolate broiler chickens can stress animals (Singer et al. 2016).

The percentage of the drug dose metabolized or absorbed by individual animals ranges from 10-80%, depending on the species and the drug used, the rest is excreted as the active compound through urine and feces into the environment. Waste from animals treated with antibiotics contains antibiotics and resistant bacterium (FAO 2018). Several studies in Indonesia have shown that *E. coli* producing ESBL is detected in broiler chicken feces, referring to the studies of Lukman et al. (2016) and Masruroh et al. (2016).

The release of antibiotics into the environment also coincides with the release of antibiotic resistance genes (ARGs) (Hu et al. 2013; Chang et al. 2015; Newton et al. 2015). Co-location of antibiotics and ARGs in the environment can lead to novel combinations of AMR that can be shared between microorganisms by horizontal gene transfer (HGT) on mobile genetic elements (MGEs), such as plasmids, thereby increasing the prevalence and combination of multiple drug resistances in the microbial

community (Xu et al. al., 2015). The competitive and chemically challenging environment provides favorable conditions for amplifying resistance genes and creating new resistance genes or genome sets (Zhang and Zhang 2011).

ESBL-producing bacterium in the environment can spread from animals to humans and have the potential to cause zoonotic diseases. Spread can be through direct contact with a polluted environment and consumption of contaminated meat. Chickens that have *E. coli* that produce ESBL in their digestive tract or come from a cage environment that is contaminated with ESBL-producing *E. coli*, it is possible that their meat is contaminated with this bacterium if during slaughter and handling of meat it is not hygienic. In Indonesia, there have been studies have shown ESBL-producing *E. coli* contamination in broiler chicken meat. Mu'arofah et al. (2020) reported a prevalence of 30% in samples from traditional markets in East Surabaya. Widhi and Saputra (2021) reported a prevalence of 71.4% in a sample from the Purwokerto City Market. Wardhana et al. (2020) reported a prevalence of 90.03% in a sample from the Surabaya Traditional Market.

Chicken meat contaminated with ESBL bacterium has the potential to spread the bacterium to humans (Harijani et al. 2020). Foods of animal origin have a higher risk making them difficult to handle and control. In the era of global trade, the presence of ESBL in products of animal origin is a threat that must be taken seriously (Calistri et el. 2013). Health problems caused by these resistant bacterium infections can include medical costs, limited treatment options for patients, longer hospital stays, and even death. It is known that *E. coli* is harmful to health because it produces a toxin, like the Shiga toxin (Ansharieta et al. 2021). In addition, ESBL-producing *E. coli* is at risk of spreading resistance genes, especially in susceptible individuals such as pregnant women, infants, children, the elderly, and immunosuppressed patients as well as in postoperative and chemotherapy patients (Franz et al. 2015).

In conclusion, present studies indicated the presence of 16 (9.14%) of the 175 environmental samples that confirmed ESBL-producing *E. coli* in Pasuruan, Indonesia. The results of the VITEK®2 test also produced a phenotypic pattern of resistance properties of ESBL-producing *E. coli* and found 12 types of resistance patterns. VITEK®2 test results further explain the resistance pattern of ESBL producing *E. coli*. The presence of ESBL-producing *E. coli* in Pasuruan is worrisome since it can lead to an impact on human health due to eating contaminated chicken meat and the use of water contaminated by wastewater from poultry farms. There is an urgent need for constant and proper monitoring of the use of antimicrobial agents on chicken farms by veterinarians.

ACKNOWLEDGMENTS

This article was supported in part by the Penelitian Pasca Sarjana - Penelitian Disertasi Doktor funding from PENELITIAN DRTPM KEMENDIKBUDRISTEK TAHUN 2022, with grant number from Universitas Airlangga: 905/UN3.15/PT/2022.

REFERENCES

Alvarez SM, Sanz S, Olarte C, Sanz RH, Carvalho I, Fernandez RF, Burguet AC, Fernandez JL, Zarazaga M, Torres C. 2022. Antimicrobial resistance in *Escherichia coli* from broiler farm environment, with detection of SHV-12-Producing isolates. Antibiotics 11: 444. DOI: 10.3390/antibiotics11040444.

Ansharieta R, Effendi MH, Plumeriastuti H. 2021. Genetic identification of Shiga toxin encoding gene from cases of multidrug resistance (MDR) *Escherichia coli* isolated from raw milk. Trop Anim Sci J 44 (1): 10-15. DOI: 10.5398/tasj.2021.44.1.10.

Aworh MK, Kwaga J, Okolocha E, Harden L, Hull D, Hendriksen RS, Thakur S. 2020. Extended-spectrum β-lactamase-producing *Escherichia coli* among humans, chickens and poultry environments in Abuja, Nigeria. One Health Outlook 2: 8. DOI: 10.1186/s42522-020-00014-7.

Biomerieux. 2021. Vitex 2 Microbiology with Confidence. https://www.biomerieuxusa.com/sites/subsidiary_us/files/18-vitek2-systembrochure_v2_0.pdf . [20 April 2021]

Calistri P, Iannetti S, Danzetta ML, Narcisi V, Cito F, Sabatino DD, Bruno R, Sauro F, Atzeni M, Carvelli A, Giovannini A. 2013. The components of 'One World-One Health' approach. Transbound Emerg Dis 60: 4-13. DOI: 10.1111/tbed.12145.

Chang HH, Cohen T, Grad YH, Hanage WP, O'Brien TF, Lipsitch M. 2015. Origin and proliferation of multiple-drug resistance in bacterial pathogens. Microbiol Mol Biol Rev 79: 101-116. DOI: 10.1128/MMBR.00039-14.

CLSI. 2020. Performance standards for antimicrobial susceptibility testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

Damayanti T, Purwantisari S. 2020. Deteksi *Escherichia coli* dalam sampel obat tradisional jenis jamu bubuk di Balai Besar Pengawasan Obat dan Makanan (BBPOM) Semarang. Jurnal Akademika Biologi 9 (2): 15-19. [Indonesian]

Deshpande PR, Rajan S, Sudeepthi BL, Nasir CPA. 2011. Patient-reported outcomes: A new era in clinical research. Perspect Clin Res 2 (4): 137-144. DOI: 0.4103/2229-3485.86879.

Effendi MH, Harijani N, Budiarto, Triningtya NP, Tyasningsih W, Plumeriastuti H. 2019. Prevalence of pathogenic *Escherichia coli* isolated from Subclinical Mastitis in East Java Province, Indonesia. Indian Vet J 96 (3): 22-25. DOI: 10.13057/biodiv/d220137.

Effendi MH, Tyasningsih W, Yurianti YA, Rahmahani J, Harijani N, Plumeriastuti H. 2021. Presence of multidrug resistance (MDR) and extended beta-spectrum beta-lactamase (ESBL) of *Escherichia coli* isolated from cloacal swabs of broilers in several wet markets in Surabaya, Indonesia. Biodiversitas 22 (1): 304-310. DOI: 10.13057/biodiv/d220137.

Food and Agriculture Organization (FAO). 2018. Antimicrobial Resistance in the Environment. http:/www.fao.org/3/BU656en/bu656en.pdf. [28 April 2021].

Franz E, Veenman C, van HA, Husman AR, Blaak H. 2015. Pathogenic *Escherichia coli* producing extended-spectrum β-lactamases isolated from surface water and wastewater. Sci Rep 5 (1): 1-9. DOI: 10.1038/srep14372.

Gregova G, Kmetova M, Kmet V, Venglovsky J, Feher. 2012. Antibiotic resistance of *Escherichia coli* isolated from poultry a Slaughterhouse. Ann Agric Environ Med 19 (1): 75-77.

Guo S, Tay MYF, Aung KT, Seow KLG, Ng LC, Purbojati RW, Drautz-Moses DI, Schuster SC, Schlundt J. 2019. Phenotypic and genotypic characterization of antimicrobial resistant *Escherichia coli* isolated from ready-to-eat food in Singapore using disk diffusion, broth microdilution and whole genome sequencing methods. Food Control 99: 89-97. DOI: 10.1016/j.foodcont.2018.12.043.

Harijani N, Oetama SJT, Soepranianondo K, Effendi MH, Tyasningsih W. 2020. Biological hazard on multidrug resistance (MDR) of *Escherichia Coli* collected from cloacal swab of broiler chicken on wet markets Surabaya. Indian J Forensic Med Toxicol 14 (4): 3239-3244. DOI: 10.37506/ijfmt.v14i4.12125.

Hu Y, Yang X, Qin J, Lu N, Cheng G, Wu N, Pan Y, Li J, Zhu L, Wang X, Meng Z, Zhao F, Liu D, Ma J, Qin N, Xiang C, Xiao Y, Li L, Yang H, Wang J, Yang R, Gao GF, Wang J, Zhu B. 2013. Metagenome-wide analysis of antibiotic resistance genes in a large cohort of human gut microbiota. Nat Commun 4: 2151. DOI: 10.1038/ncomms3151.

Ibrahim MA, Emeash HH, Ghoneim NH, Abdel-Halim MA. 2013. Seroepidemiology studies on poultry salmonellosis and its public health importance. J World Poult Res 3 (1): 18-23.

Lukman DW, Sudarwanto M, Purnawarman T, Latif H, Pisestyani H, Sukmawinata E, Akineden O. 2016. CTX-M-1 dan CTX-M-55 Producing *Escherichia coli* isolated from broiler feses in poultry slaughterhouse, Bogor, West Java Province. Glob Adv Res J Med Med Sci 5 (12): 287-291.

Masruroh CA, Sudarwanto MB, Latif H. 2016. The occurance of extended spectrum b-lactamase-producing *Escherichia coli* from broiler feces in Bogor. J Sain Vet 34 (1): 42-49. [Indonesia]

Mu'arofah B, Arizandy RY, Utomo B, Kuntaman K. 2020. Detection of extended spectrum β-lactamase (ESBL) gene pattern of enterobacteriaceae in broiler chicken meat sold in traditional market in the East Surabaya. STRADA J Ilmiah Kesehatan 9 (1): 12-19. DOI: 10.30994/sjik.v9i1.264. [Indonesia]

Musa L, Proietti PC, Branciari R, Menchetti L, Bellucci S, Ranucci D, Marenzoni ML, Franciosini MP. 2020. Antimicrobial susceptibility of *Escherichia coli* and ESBL-producing *Escherichia coli* diffusion in conventional, organic and antibiotic-free meat chickens at Slaughter. Animals 10 (7): 1215. DOI: 10.3390/ani10071215.

Nathisuwan S, Burgess DS, Lewis IIJS. 2001. Extended-spectrum β-lactamases: Epidemiology, detection and treatment. Pharmacotherapy 21 (8): 920-928. DOI: 10.1592/phco.21.11.920.34529.

Newton RJ, McLellan SL, Dila DK, Vineis JH, Morrison HG, Eren AM, Sogin ML. 2015. Sewage reflects the microbiomes of human populations. MBio 6 (2): e02574. DOI: 10.1128/mBio.02574-14.

Niasono AB, Latif H, Purnawarman T. 2019. Resistensi antibiotik terhadap bakteri *Escherichia coli* yang diisolasi dari peternakan Ayam Pedaging di Kabupaten Subang, Jawa Barat. Jurnal Veteriner 20 (2): 187-195. [Indonesian]

Paterson DL, Bonomo RA. 2005. Extended-Spectrum β- Lactamases: A clinical update. Clin Microbiol Rev 18 (4): 657-686. DOI: 10.1128/CMR.18.4.657-686.2005.

Permatasari DA, Witaningrum AM, Wibisono FJ, Effendi MH. 2020. Detection and prevalence of multidrug-resistant *Klebsiella pneumoniae* strains isolated from poultry farms in Blitar, Indonesia. Biodiversitas 21 (10): 4642-4647. DOI: 10.13057/biodiv/d211024.

Putra AR, Effendi MH, Kurniawan F. 2020. Investigation of extended spectrum Beta-Lactamase (ESBL) producing *Escherichia coli* by Vitek-2 on Dairy Cows in Surabaya, Indonesia. Biochem Cell Arch 20 (2): 6773-6777. DOI: 10.03896/bca.2020.20.6773.

Rahmahani J, Salamah, Mufasirin, Tyasningsih W, Effendi MH. 2020. Antimicrobial resistance profile of *Escherichia coli* from cloacal swab of domestic chicken in surabaya traditional market. Biochem Cell Arch 20 (1): 2993-2997. DOI: 10.35124/bca.2020.20. S1.2993.

Rahman S, Ahmad S, Khan I. 2018. Incidence of ESBL-producing-*Escherichia coli* in poultry farm environment and retail poultry meat. Pak Vet L 39 (1): 116-120. DOI:10.29261/PAKVETJ/2018.091.

Riwu KHP, Effendi MH, Rantam FA**.** 2020. A review of extended-spectrum β-Lactamase (ESBL) producing *Klebsiella pneumoniae* and Multidrug-Resistant (MDR) on companion animals. Syst Rev Pharm 11 (7): 270-277. DOI:10.31838/srp.2020.7.43.

Rodroo J, Intanon M, Kreausukon K, Kongkaew A, Bender J, Awainanont N. 2021. Occurance of extended-spectrum beta-bactamase producing *E. coli* in broiler farm workers and the farm environment in Chiang Mai-Lumphun, Thiland. Vet Integr Sci 19 (1): 23-35. DOI: 10.12982/VIS.2021.002.

Samanta I, Joardar SN, Das PK, Sar TK, Bandyopadhyay S, Dutta TK, Sarkar U. 2014. Prevalence and antibiotic resistance profiles of *Salmonella* serotypes isolated from backyard poultry flocks in West Bengal, India. J Poult Res 23: 536-545. DOI: 10.3382/japr.2013-00929.

Santos LL, Moura RA, Agilar-Ramires P, Castro AP, Lincopan N. 2013. Current status of extended-spectrum β-lactamase (ESBL)-producing enterobacteriaceae in animals. FORMATEX 3: 1600-1607.

Shah DH, Board MM, Crespo R, Guard J, Paul NC, Faux C. 2020. The occurance of *Salmonella*, extended-spectrum β-lactamase producing *Escherichia coli* and carbapenem resistant non-fermenting Gram-negative bacterium in a backyard poultry flock environment. Zoonoses Publ Health 67 (6): 742-753. DOI: 10.1111/zph.12756.

Singer AC, Shaw H, Rhodes V, Hart A. 2016. Review of antimicrobial resistance in the environment and its relevance to environmental regulators. Front Microbiol 7: 1728. DOI: 10.3389/fmicb.2016.01728

Spellberg B, Barlett JG, Gilbert DN. 2013. The future of antibiotics and resistance. N Engl J Med 368: 299-302. DOI: 10.1056/NEJMp1215093.

Tansawai U, Walsh TR, Niumsup PR. 2019. Extended spectrum β-lactamase-producing *Escherichia coli* among backyard poultry farms, farmers, and environments in Thailand. Poult Sci 98: 2622-2631. DOI: 10.3382/ps/pez009.

Ueda S, Ngan BT, Huong BT, Hirai I, Tuyen le D, Yamamoto Y. 2015. Limited transmission of BlaCTX-M-9-type-positive *Escherichia coli* between humans and poultry in Vietnam. Antimicrob Agents Chemother 59: 3574-3577. DOI: 10.1128/AAC.00517-15.

Varga C, Guerin MT, Brash ML, Siavic D, Boerlin P, Susta L. 2019. Antimicrobial resistance in *Campylobacter jejuni* and *Campylobacter coli* isolated from small poultry flocks in Ontario, Canada: a two-year surveillance study. PLoS One 14 (8): e0221429. DOI: 10.1371/journal.pone.0221429.

Wardhana DK, Effendi MH, Harijani N, Ooi H. 2020. Detection of extended-spectrum-beta-lactamase (ESBL) producing *Escherichia coli* in meat chicken from traditional market in Surabaya, East Java, Indonesia. Indian J Publ Health Res Dev 11 (1): 1353-1357. DOI: 10.37506/v11/i1/2020/ijphrd/194032.

Wibisono FJ, Sumiarto B, Untari T, Effendi MH, Permatasari DA, Witaningrum AM. 2020a. CTX gene of extended spectrum beta-lactamase (ESBL) Producing *Escherichia coli* on broilers in Blitar, Indonesia. Syst Rev Pharm 11 (7) : 396-403. DOI: 10.31838/srp.2020.7.59.

Wibisono FJ, Sumiarto B, Untari T, Effendi MH, Permatasari DA, Witaningrum AM. 2020b. Short communication: pattern of antibiotic resistance on extended-spectrum beta-lactamases genes producing *Escherichia coli* on laying hens in Blitar, Indonesia. Biodiversitas 21 (10): 4631-4635. DOI: 10.13057/biodiv/d211022.

Wibisono FJ, Sumiarto B, Untari T, Effendi MH, Permatasari DA, Witaningrum AM. 2021. Molecular identification of CTX Gene of extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* on layer chicken in blitar, Indonesia. J Anim Plant Sci 31 (4): 954-959. DOI: 10.36899/JAPS.2021.4.0289.

Widhi APK, Saputra INY. 2021. Residu antibiotik serta Keberadaan *Escherichia coli* penghasil ESBL pada Daging Ayam Broiler di Pasar Kota Purwokerto. Jurnal Kesehatan Lingkungan Indonesia 20 (2): 137-142. DOI: 10.14710/jkli.20.2.137-142. [Indonesian]

Widodo A, Effendi MH, Khairullah AR. 2020. Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* from livestock. Syst Rev Pharm 11 (7): 382-392. DOI: 10.31838/srp.2020.7.57.

Wongsuvan G, Wuthiekanun V, Hinjoy S, Day NP, Limmathurotsakul D. 2018. Antibiotic use in poultry: A survey of eight farms in Thailand. Bull World Health Organ 96 (2): 94-100. DOI: 10.2471/BLT.17.195834.

Woolhouse M, Ward M, van Bunnik B, Farrar J. 2015. Antimicrobial resistance in humans, livestock and the wider environment. Phil Trans R Soc B 370: 1-7. DOI: 10.1098/rstb.2014.0083.

Xu J, Xu Y, Wang H, Guo C, Qiu H, He Y, Zhang Y, Li X, Meng W. 2015. Occurrence of Antibiotics and antibiotic resistance genes in a sewage treatment plant and its effluent-receiving river. Chemosphere 119: 1379-1385. DOI: 10.1016/j.chemosphere.2014.02.040.

Yanestria SM, Rahmaniar RP, Wibisono FJ, Effendi MH. 2019. Detection of *inv*A gene of *Salmonella* from milkfish (*Chanos chanos*) at sidoarjo wet fish market, Indonesia, using polymerase chain reaction technique. Vet World 12 (1): 170-175. DOI: 10.14202/vetworld.2019.170-175.

Zhang XX, Zhang T. 2011. Occurrence, abundance, and diversity of tetracycline resistance genes in 15 sewage treatment plants across China and other global locations. Environ Sci Technol 45: 2598-2604. DOI: 10.1021/es103672x.