The Journal of Animal and Plant Sciences

Home
About the Journal
Instructions to Authors
Accepted Papers
Latest Issue
Journal Archives
Supplementary Series
Supplementary Articles
Abstracting and Indexing Agencies
Publication Ethics
Editorial Board
Advisory Board
Managing Board
Contact us

ISSN (Print):
1018-7081

ISSN (Electronic):
2309-8694

Online Submission

EDITORIAL BOARD

Prof. Dr. Abrar Hussain Gilani
Chief Editor

Prof. Dr. Khalid Javed
Managing Editor

SUBJECT EDITORS

Crop Production

Prof. Dr. Fayyaz-ul-Hassan Sahi,
PMAS University of Arid Agriculture, Rawalpindi P akistan

Plant Protection

Dr. Khawar Jabran
Faculty of Agricultural Sciences, Nigde Omer Halis demir University, Turkey

Environmental Sciences & Climate Change

Dr. Saeed Ahmad Asad
Centre for Climate Research and Development (C CRD), COMSAT University Islamabad

Agronomy

Dr. Mubashir Hussain
Baha ud Din Zakriya University, Multan, Pakistan

Soil Science

Dr. Muhammad Sanaullah
Institute of Soil and Environmental Sciences, Uni versity of Agriculture Faisalabad

Plant Breeding and Agri. Biotechnology

Dr. Khurram Bashir
Syed Babar Ali School of Science and Engineering

Lahore University of Management Sciences (LUMS), Pakistan

Animal Sciences

Dr. Tanveer Ahmad
PMAS University of Arid Agriculture, Rawalpindi

Veterinary Sciences

Prof. Dr. Muhammad Ijaz
University of Veterinary and Animal Sciences, Lah ore

EDITORIAL BOARD MEMBERS

Prof. Dr. Muhammad Abdullah
University of Veterinary & Animal Sciences, Lahor e

Dr. A.S. Chaudhry,
School of Agri., Food &. Rural Development, Univ ersity of Newcastle Upon Tyne, UK.

Prof. Dr. Hussain Farid,
Haley Institute, Deptt. of Plant and Animal Scienc es, Nova Scotia Agriculture College B2N 5E3, Trur o, Nova Scotia Canada

Dr. Pamela C. Rasmussen,
Michigan State University West Circle Drive, East Iancing MI 48824-1045

Prof. Dr. Mirza Barjees Baig,
King Saud University, Riyadh KSA

Prof. Dr. Fahrul Zaman Huyop
Universiti Teknologi Malaysia.

Dr. Harpinder Singh Randhawa
Lethbridge Research & Development Center, Cana da

Prof. Dr. M. Ihsan Soysal,
Namik Kamal University Tikerdag, Turkey

Dr. Hafiz Zahid Mahmood

COMSATS University Islamabad Lahore Campus-L ahore

Prof. Dr. Weijun Guan,
Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing 100193, China

Prof. Dr. Phil Harris,
Deptt. of Plant Sciences, School of Science & The Environment, Coventry University, UK

Dr. Ali Olfati
Department of Animal Science, Faculty of Agricult ure, Tabriz, IRAN

Prof. Dr. Zeliha Selamoglu
Faculty of Medicine Nigde Ömer Halisdemir Unive rsity Campus 512-Turkey

Prof. Dr. Tessema (Tess) Astatkie,
Professor, Faculty of Agriculture, Dalhousie Univer sity, Truro, NS, B2N 5E3, Canada

Prof. Dr. Shagufta Naz,
Lahore College for Women University, Lahore Pakistan

Prof. Dr. Yang Li,
Shihezi University Xinjiang, China

Prof. Dr. Sohail Ahmed
University of Agriculture, Faisalabad

Prof. Dr. Sun Ming-Zhong,
Dalian Medical University Dalian, China

Dr. Tugay Ayasan,
Osmaniye Korkut Ata University, Kadirli Academy of Applied Sciences, Osmaniye, Turkey

Prof. Dr. Abdur Rab,
The University of Agriculture Peshawer KPK, Pakistan

Dr. Ghulam Bilal
PMAS Uni. Arid Agri., Rawalpindi, Pakistan

Tariq Aziz,
H. # 385-B Street 23 Rawal Town, Islamabad.

Dr. Effa, Emmanuel Bassey
Department of Crop Science, University of Calabar, P.M.B. 1115 Calabar, Nigeria

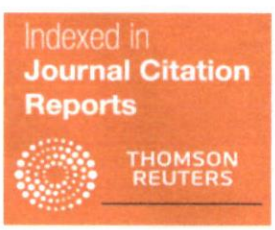
Dr. Sardar Alam Cheema
Department of Agronomy, University of Agriculture Faisalabad. Pakistan

Prof. Dr. Mushtaq Ahmad
Quaid-i-Azam University, Islamabad, Pakistan

Prof. Dr. Zia-ul-hassan Shah
Sindh Agri University, Tando Jam, Sindh-Pakistan

Dr. Umer Farooq
Deptt. of Physiology, Islamia University Bahawalpur, Pakistan

Dr. Muhammad Sohail Sajid
University of Agri., Faisalabad



Designed and Managed by: HiSoft Solutions

The Journal of Animal & Plant Sciences, 31(4): 2021, Page: 954-959
ISSN (print): 1018-7081; ISSN (online): 2309-8694

MOLECULAR IDENTIFICATION OF CTX GENE OF EXTENDED SPECTRUM BETA-LACTAMASES (ESBL) PRODUCING *ESCHERICHIA COLI* ON LAYER CHICKEN IN BLITAR, INDONESIA

F. J. Wibisono[1], B. Sumiarto[2], T. Untari[3], M. H. Effendi[4*], D. A. Permatasari[4] and A. M. Witaningrum[4]

[1]Doctoral Program in Veterinary Science, [2]Department of Veterinary Public Health, Faculty of Veterinary Medicine, [3]Department of Microbiology, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia; [4]Department of Veterinary Public Health, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia
Corresponding author's email: mheffendi@yahoo.com

ABSTRACT

Escherichia coli is one of the ESBL-producing bacteria responsible for the rise of antibiotic resistance. The most common type of ESBL-encoding gene is cefotaximase (CTX). The aim of study was to identify CTX-encoding gene among *Escherichia coli* on Layer Chicken in Blitar, Indonesia by using DNA-based techniques. To do this, a total of 130 swab cloacal samples were randomly collected from layer chicken in 4 districts of Blitar. Samples was initially cultured on MacConkey agar and Eosin Methyllen blue agar. Subsequently, the suspected isolates were identified in TSIA test and IMVIC biochemistry test. The method of Confirmation test ESBL-producing *Escherichia coli* was performed by Double Disc Synergy Test (DDST), and the final characterization of the isolates were conducted using VITEK® 2 Compact. Following that, the identified isolates were exposed to PCR amplification for the presence of CTX gene in ESBL-producing *Escherichia coli*, followed by the visualization of the amplicons in the electrophoresis. Overall, the results was showed that 80% of the ESBL-positive isolates contained CTX gene. In conclusion, this report the high frequency of CTX gene in ESBL producing *Escherichia coli*, and thereby posing a significant threat for the animal and human health.

Key words: CTX gene; ESBL; *Escherichia coli*; Human health; PCR; VITEK®

https://doi.org/10.36899/JAPS.2021.4.0289 *Published online December 15, 2020*

INTRODUCTION

Antibiotic resistance is a serious problem worldwide, especially center of layer poultry in Blitar, Indonesia (Wibisono *et al.,* 2020a). *Escherichia coli* is one of the causes responsible for infection and antibiotic resistance in humans and animals through ESBL-mechanism (Paterson and Bonomo, 2005; Amelia *et al.,* 2016). The presence of pathogenic *Escherichia coli* infections in poultry can adversely affect economy (Wibisono *et al.,* 2018). The usage antibiotics can pose a risk of antibiotic resistance in poultry (Santos *et al.,* 2013). There is restricted data related to the prevalence of antibiotic resistance in developing countries, in particular, Indonesia. Surveillance of antibiotic resistance is needed to monitor the emergence of antibiotic resistance (Kurniawati *et al.,* 2015).

Antibiotic resistance which produced from *Escherichia coli* is Extended Spectrum β- lactamase (ESBL) (Santos *et al.,* 2013; Hammerum *et al.,* 2014). Extended-spectrum beta lactamase is an enzyme that is characterized by the ability to hydrolyze third generation cephalosporins and aztreonam but are inhibited by clavulanic acid (Paterson and Bonomo, 2005; Public Health England, 2014). Exposure large amounts of beta-lactam antibiotics can induces production and mutation of beta-lactamase type enzyme.

This mutation causes an increase in the enzymatic activity of beta-lactamase so that this enzyme can hydrolyze third generation cephalosporins and aztreonam (Paterson and Bonomo, 2005; Lim *et al.,* 2013). ESBL-producing bacteria can also be resistant to antibiotics from aminoglycoside, fluoroquinolone, tetracycline, chloramphenicol, and sulfamethoxazole-trimethoprim (Brower *et al.,* 2017; Sudarwanto *et al.,* 2017). Extended Spectrum Beta-Lactamase genes are often found in mutated genes, namely cefotaximase (CTX-M), temoneira (TEM) and variable sulfhydryl (SHV). The CTX-M gene in ESBL-producing *Escherichia coli* is a gene that codes and produces enzymes that can hydrolyze beta lactam rings from third-generation beta lactam antibiotics and cephalosporins (Biutifasari, 2018). The molecular detection is a genotypic confirmatory test to screen the ESBL encoding gene in *Escherichia coli* bacteria using PCR (Bradford, 2001). The highest prevalence of ESBL-producing bacteria with CTX-M-1 is the most common type of ESBL in poultry (Saliu *et al*., 2017; Upadhyay *et al.,* 2015; Rao *et al.,* 2014).

ESBL-producing *Escherichia coli* in India was about 42% in layer chicken (Brower *et al.,* 2017). In this study, we aimed to identify CTX gene for encoding ESBL producing *Escherichia coli* from cloacal swabs of layer chicken in Blitar, Indonesia.

MATERIALS AND METHODS

Isolation and Identification of Escherichia coli: A total of 130 cloacal swab samples was randomly taken from 4 districts in Blitar. The samples were kept in Amies Swab Viscosa (deltalab, Spain) transport medium at 4 ºC, and immediately taken to the laboratory for further analyses (Seni *et al.,* 2016). Isolation of *Escherichia coli* bacteria using selective media Mac Conkey Agar no. 3 CM0115 (Oxoid, England) and differential media Eosin Methylene Blue Agar CM0069 (Oxoid, England), incubated at of Escherichia coli were identified by biochemical testing of IMVIC (Indol-Motility, Methyl Red, Voges Proskauer, Citrate) and TSIA (Triple Sugar Iron Agar) (Effendi *et al.,* 2018a; Effendi *et al.,* 2019). The identification of *Escherichia coli* bacteria was confirmed by VITEK® 2 compact using a VITEK® 2 GN card (Biomerieux, 2017).

Confirmation for ESBL producing *Escherichia coli*: ESBL-producing *Escherichia coli* bacteria isolated from cloacal swabs of layer chicken were confirmed by the Double Disc Synergy Test (DDST) and VITEK® 2 compact. This confirmation test with DDST was conducted to evaluate the presence of inhibitory zones of ESBL activity with clavulanic acid using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Merck, Germany). Double Disc Synergy Test uses the antibiotic disc Amoxycillin-clavulanic 30µg (Oxoid, England), Cefotaxime 30µg (Oxoid, England), Ceftazidime 30µg (Becton Dickinson, USA), and Aztreonam 30µg (Oxoid, England).

Culture was incubated at 35-37 ºC for 18-24 hours (CLSI, 2017; Effendi *et al.,* 2018b). The results of the evaluation after incubation showed that the inhibition zone that appeared in the plate was measured based on CLSI 2017 guidelines (CLSI, 2017) as shown on Figure 2.

The antibiotic sensitivity 102 test of ESBL-producing Escherichia coli bacteria by the DDST was then confirmed by the VITEK® 2 compact. The bacterial suspension was homogenized and a bacterial turbidity of 0.50 to 0.63 Mc Farland was made using VITEK® 2 DensiCHEK (Biomerieux, 2017). Antimicrobial susceptibility and phenotypic detection of ESBL producers using AST N280 cards (bioMérieux, Marcy-L'Étoile, France). These results are analyzed automatically by the system and interpreted as sensitive, intermediate, and resistant (Biomerieux, 2017; Brower *et al.,* 2017).

Characterization of CTX gene by Polymerase Chain Reaction (PCR): The ESBL-positive strains were then subjected to molecular screening of CTX-gene. To do this, DNAs were extracted according to the instructions by a mini QIAamp® DNA kit (QIAGEN, Germany). *Escherichia coli* ATCC™ 35218 was used as positive control standard for strains of ESBL-producing bacteria, and *Escherichia coli* ATCC™ 25922 is used as negative control or non-ESBL-producing bacteria. The primers designed for screening CTX-M gene were used to encode CTX encoding genes using CTX-MA primers (CGCTTTGCGATGTGCAG), CTX-MB (ACCGCGATATCGTTGGT), respectively and the amplicon is 550-bp. The PCR conditions were given as with denaturation temperatures 94ºC, 2 minutes; extended denaturation 94ºC, 1 minute; annealing 54 ºC, 30 seconds; extension 72ºC), 45 seconds; extended extension 72 ºC, 5 minutes, this reaction is carried out for 30 cycles (Ali *et al.,* 2016), and the amplification was carried out by PCR (Blue-Ray Biotech Turbo Cycler, TST-9620). After that, the amplicons were visualized by electrophoresis using 2% agarose gel (Invitrogen, USA) (Yanestria *et al.,* 2019).

RESULTS

This study was conducted for the characterization of CTX gene among ESBL-positive E. oli strains isolated from 130 cloacal swab samples in layer poultry. The results were 8.69% (10) confirmation positive of ESBL-producing *Escherichia coli* on layer chicken cloacal swab by the Double Disc Synergy Test (DDST), shown on Figure 2. The presence of ESBLs-producing bacteria by double discs synergy test (DDST) to detect ESBL producing bacteria and then confirmed by the automatic VITEK® 2 compact and indicated 100% ESBL producing Escherichia coli as shown in Table 1. For the identification of CTX-encoding gene present in ESBL producing *E. coli* PCR was used (Putra *et al,* 2019), as shown on Figure 3.

Tabel 1. Data ESBL producing *Escherichia coli* from cloacal swabs layer chicken in Blitar.

Subdistric Location	Sample size	No of *Escherichia coli* isolates	No of ESBL positive strain		PCR
			DDST	VITEK®2 compact	No of CTX gene positive strain
Ponggok	40	31	-	-	-
Srengat	45	43	6	6	6
Talun	20	18	1	1	-
Kademangan	25	23	3	3	2
Total in Blitar	**130**	**115 (115/130; 88,5%)**	**10 (10/115; 8.69%)**	**10 (10/10; 100%)**	**8 (8/10; 80%)**

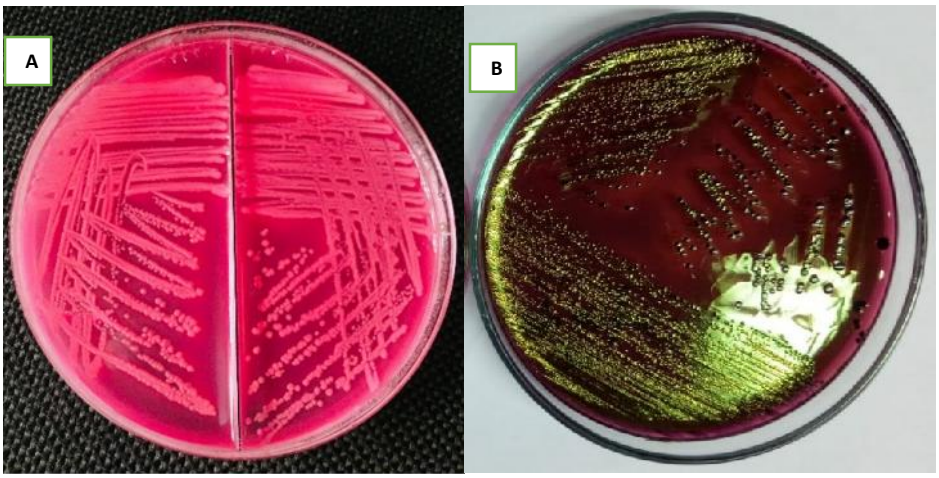


Figure 1. *Escherichia coli* on MacConkey Agar (A) and on Eosin Methylen Blue Agar (B)

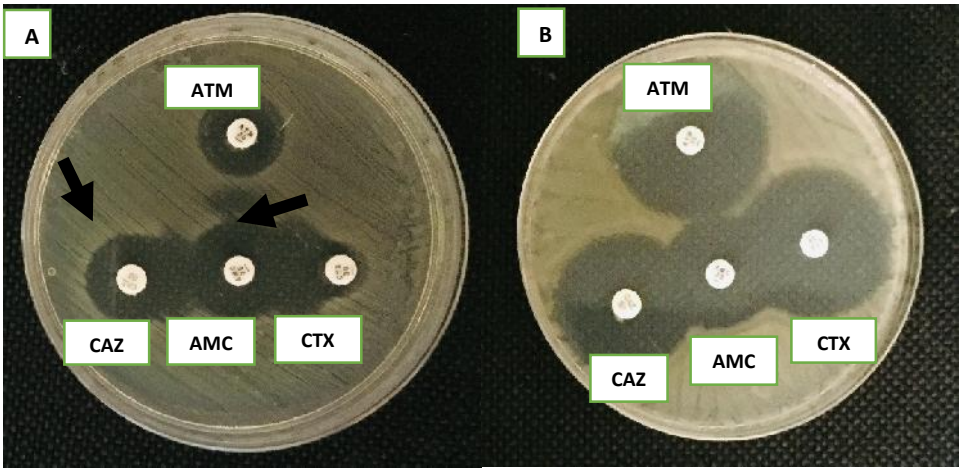


Figure 2. ESBL-producing *Escherichia coli* confirmation test with Double Disc Synergy Test (DDST) (A) positive result (B) negative result. Note: ATM: Aztreonam, CAZ: Ceftasidime, AMC: Amoxycillin clavulanic, and CTX: Cefotaxime.

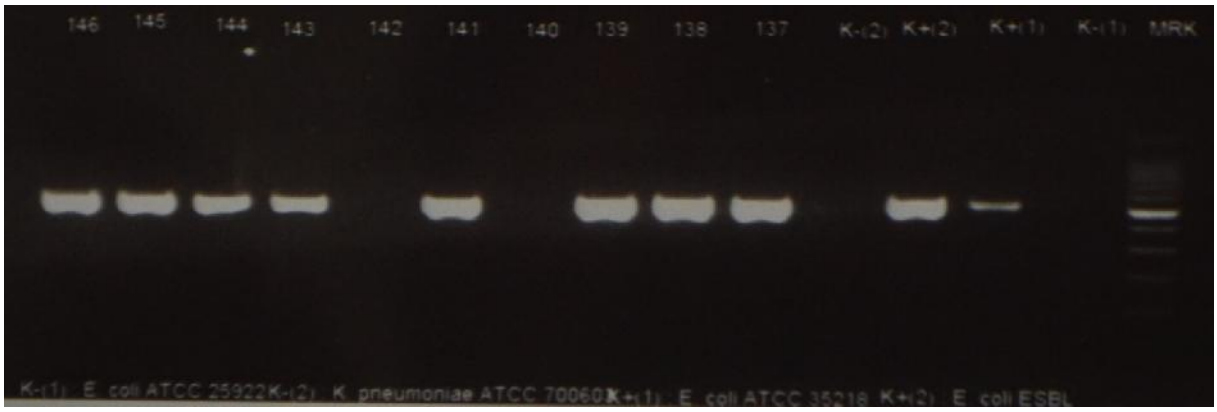


Figure 3. Molecular identification CTX gene of ESBL-producing *Escherichia coli* by PCR on 550 bp.
Legend: Code sample 140 and 142 there were no CTX gene.

DISCUSSION

Several other studies have examined the number of *E. coli* isolates that isolated from animal and animal products, showing concordance results between studies as shown on Table 1 (Saliu *et al.,* 2017; Upadhyay *et al.,* 2015; Rao *et al.,* 2014). The relative abundance of the ESBL producing *E. coli* in samples from cattle and dogs has been shown to vary with geographic location (Putra *et al.,* 2019; Kristianingtyas *et al.,* 2020). In this study,

isolates including ESBL producing *E. coli* were dominated by encoding CTX gene.

Table 1 showed the spread of ESBL-producing *Escherichia coli* in 3 districts from 4 districts. Srengat District has 6 samples, one sample from Talun district and Kademangan district was 3 samples from 10 ESBL-producing *Escherichia coli* samples, while Ponggok district was not found any ESBL producing *Escherichia coli*.

Cefotaxime synergy with the combination of amoxilin-clavulanate in the form of an expansion inhibition zone between the two discs represent that the bacteria is positive ESBL, synergy of third generation cephalosporins with combination of cephalosporin-clavulanic acid in the form of an expansion inhibition zone between the two discs. Positive results on ESBL-producing bacteria confirmed that there was increase in inhibition zone ≥ 5 mm between diameter of cephalosporin disc and cephalosporin-clavulanate disc combination revealed an ESBL positive (CLSI, 2017). The incidence of ESBL producing *Escherichia coli* from cloaca swabs on layer chicken was consistent with the incidence of *Escherichia coli* on slaughterhouses in Bogor by 8.6% (Sudarwanto *et al.,* 2016), but smaller compared to the incidence of *Escherichia coli* as ESBL producing *Escherichia coli* from feces of broiler chickens in Bogor ESBL by 25% (Masruroh *et al.,* 2016) and the incidence of ESBL producing *Escherichia coli* in India on layer chicken was around 42% (Brower *et al.,* 2017).

Molecular identification as shown in Table 1 that 80% (8/10) samples of ESBL producing *Escherichia coli* encoding CTX gene. The CTX encoding gene is most commonly found in *Escherichia coli*. CTX enzymes have hydrophilic ability against cephalosporins, especially cefotaxime, so called CTX (Bradford, 2001). Molecular identification shown in Figure 3 that visualization of the CTX gene fragment band. Electrophoresis results of CTX gene represent samples describing the same fragments as positive controls with a gene length of 550 bp (Ali *et al.,* 2016) as shown on Figure 3.

ESBL bacteria can be identified by detecting the presence of ESBL encoding genes (Bhoomika *et al.,* 2016, Surgers *et al.,* 2019). This research showed that the CTX gene was found in 80% ESBL samples. Other studies have been carried out mainly ESBL producing Kleibsiella pneumoniae (Hayati *et al.,* 2019). Some investigations show that the dominant genotype found was CTX gene (Zarfel *et al.,* 2014; Ibrahim and Hameed, 2015). This type of ESBL is often seen as single or combination. In this study, the ESBL encoding CTX gene was detected dominance of ESBL producing *Escherichia coli* samples from layer chicken. The CTX beta lactamase is the most prevalent ESBL type among chicken (96%) samples (Valentin *et al,* 2014). In many countries CTX gene is one of the most frequent ESBL types in ESBL-producing bacteria, causing human infections (Alonso *et al.,* 2017), therefore the evidence of CTX gene in this study should be used as reference in controlling the spread of ESBL encoding gene in poultry farms (Wibisono *et al.,* 2020b).

The spreading of genetic elements such as transposons, insertion and integrons in the bacteria cause ESBL genes move quickly from animals to humans or vice versa. Genetic factors can also spread the virus nature of resistance to other bacteria in animals digestive tract. The bacteria then spread from cage to the surrounding environment through facilitated waste by poor hygiene and sanitation, which pollutes land and water around agriculture. ESBL bacteria are also detected in vegetables, soil and surrounding water agriculture and markets (Wu *et al.,* 2016).

The presence of ESBL producing *Escherichia coli* is threat to the public health and animal health (Kristianingtyas *et al.,* 2020). This condition can occur in limited maintenance options. The steps that can be done is to build supervision program, supervising feed and poultry. Farmers also need to improve biosecurity practice. Garbage and laying chicken manure must be correct managed in an intensive production system, to prevent air, soil and water contamination, as well negative consequences for human health (Thyagarajan *et al.,* 2014).

Conclusion: One hundred and fifteen *Escherichia coli* samples were isolated from cloacal swabs layer chicken in layer farms Blitar, East Java, Indonesia. Ten E. coli were classified as ESBL bacteria. Through PCR testing, ESBL encoding gene of CTX gene was identified in eight samples. The presence of ESBL encoding gene in bacteria has potential to spread its resistance to the other bacteria in the gastrointestinal tract of layer chickens as well as in the poultry environment.

Conflict of interest: We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

Acknowledgements: The authors would like to thank the Rector of Airlangga University for providing HIBAH MANDAT research funds with grant numbers; 371/UN3.14/LT/2019 on the research title: PENANGGULANGAN *VIRULENCE FACTOR* ISOLAT *GRAM NEGATIVE BACTERIA* DARI SUMBER PANGAN ASAL HEWAN DENGAN PENGGUNAAN *POLYMERASE CHAIN REACTION*. This article is part of the research.

REFERENCES

Ali, T., S. Rahman, L.Zhang, M.Shahid, S.Zhang, G.Liu, J.Gao, and B. Han (2016). ESBL-Producing Escherichia coli from Cows Suffering Mastitis in China Contain Clinical Class 1 Integrons with

CTX-M Linked to ISCR1. Front. Cell. Infect. Microbiol. 7:1–11.

Alonso, C.A., M. Zarazaga, R.Ben Sallem, A. Jouini, K.Ben Slama, and C.Torres (2017). Antibiotic resistance in *Escherichia coli* in husbandry animals. the African perspective. Lett. Appl. Microbiol. 64:318–334.

Amelia, A., A. Nugroho, and P. N. Harijanto. (2016). Diagnosis and Management of Infections Caused by Enterobacteriaceae Producing Extended Spectrum Beta-Lactamase. Acta Medica Indones. The Indones. J. Intern. Med. 48:156–166.

Bhoomika, S. Shakya, A.Patyal, NE.Gade (2016). Occurrence and characteristics of extended-spectrum β-lactamases producing *Escherichia coli* in foods of animal origin and human clinical samples in Chhattisgarh, India. Vet. World, 9(9): 996-1000.

Biomerieux. (2017). AST and Resistance Detection, Antibiotic Suceptibility Testing bioMérieux Industry website.

Biutifasari, V. (2018). Extended Spectrum Beta – Lactamase (ESBL). *Ocean. Biomed*. J. 1:1–6.

Bradford, P. (2001). Extended spectrum betalactamase in the 21 century: characterization, epidemiology, and detection of this important resistant threat. Clin. Microbiol Rev. 14:933–951.

Brower, C. H., S. Mandal, S. Hayer, M. Sran, A. Zehra, S. J. Patel, R. Kaur, L. Chatterjee, S. Mishra, B. R. Das, P. Singh, R. Singh, J. P. S. Gill, and R. Laxminarayan (2017). The prevalence of extended-spectrum beta-lactamase-producing multidrug-resistant Escherichia coli in poultry chickens and variation according to farming practices in Punjab, India. Environ. Health Perspect. 125:1–10.

CLSI. (2017). M100 Performance Standards for Antimicrobial. 27th ed. Clinical and Laboratory Standards Institute, USA.

Effendi, M. H., I. G. Bintari, E. B. Aksoro, and I. P. Hermawan. (2018a). Detection of blaTEM Gene of Klebsiella pneumoniae Isolated from swab of food-producing animals in East Java. Trop. Anim. Sci. J. 41:174–178.

Effendi, M.H, N. Harijani, S.M. Yanestria, and P. Hastutiek. (2018b). Identification of shiga toxin-producing Escherichia coli in raw milk samples from dairy cows in Surabaya, Indonesia. Philippine J Vet Med.55(SI):109-114.

Effendi, M.H., N.Harijani, Budiarto, N.P. Triningtya, W.Tyasningsih, and H. Plumeriastuti (2019). Prevalence of Pathogenic *Escherichia Coli* Isolated from Subclinical Mastitis in East Java Province, Indonesia. *Indian Vet. J*., 96 (03) : 22 - 25

Hammerum, A. M., J. Larsen, V. D. Andersen, C. H. Lester, T. S. S. Skytte, F. Hansen, S. S. Olsen, H. Mordhorst, R. L. Skov, F. M. Aarestrup, and Y. Agerso (2014). Characterization of extended-spectrum β-lactamase (ESBL)-producing Escherichia coli obtained from Danish pigs, pig farmers and their families from farms with high or no consumption of third- or fourth-generation cephalosporins. J. Antimicrob. Chemother. 69:2650–2657.

Hayati M, A. Indrawati, NLPI Mayasari, I. Istiyaningsih, N. Atikah (2019). Molecular detection of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolates of chicken origin from East Java, Indonesia. Vet. World, 12(4): 578-583.

Ibrahim, I.A.J. and T.A.K Hameed (2015). Isolation, Characterization and antimicrobial resistance patterns of lactose-fermenter *Enterobacteriaceae* isolates from clinical and environmental samples. J. Med. Microbiol., 5 : 169-176.

Kristianingtyas, L., M. H.Effendi, W.Tyasningsih, and F. Kurniawan (2020). Genetic Identification of blactx-M Gene and blatem Gene on Extended Spectrum Beta Lactamase (ESBL) Producing *Escherichia Coli* from Dogs. Indian Vet. J. 97 (01) : 17 - 21

Kurniawati, A. F., P. Satyabakti, and N. Arbianti (2015). Differences in Risk of Multidrug Resistance Organisms (MDROS) According to Hand Hygiene Risk Factors and Compliance. J. Berk. Epidemiol. 3:277–289.

Lim, C. L. L., W. Lee, A. L. C. Lee, L. T. T. Liew, S. C. Nah, C. N. Wan, M. P. Chlebicki, and A. L. H. Kwa. (2013). Evaluation of ertapenem use with impact assessment on extended-spectrum beta-lactamases (ESBL) production and gram-negative resistance in Singapore general hospital (SGH). BMC Infect. Dis. 13:1–10.

Masruroh, C. A., M. B. Sudarwanto, and H. Latif. (2016). Incidence Rate of Escherichia coli Producing Extended Spectrum B-Lactamase Isolated from Broiler Faeces in Bogor City. J. Sain Vet. 34:42–49.

Paterson, D. L., and R. A. Bonomo. (2005). Extended Spectrum Beta-Lactamases : a Clinical Update. Clin. Microbiol. Rev. 18:657–686.

Public Health England. 2014. Extended-spectrum beta-lactamases (ESBLs)_ guidance, data, analysis - GOV.

Putra, A. R. S., M.H. Effendi, S. Koesdarto, and W. Tyasningsih (2019). Molecular Identification of Extended Spectrum Beta-Lactamase (ESBL) Producing *Escherichia coli* Isolated From Dairy Cows in East Java Province, Indonesia. Indian Vet. J. 96 (10) : 26 – 30.

Rao L, L. Lv, Z. Zeng, S. Chen, D. He, X. Chen, C. Wu, Y. Wang, T. Yang, P. Wu, Y. Liu, and JH Liu. (2014) Increasing prevalence of extended-spectrum cephalosporin-resistant *Escherichia coli* in food animals and the diversity of CTX-M genotypes during 2003-2012. Vet. Microbiol. 172:534–541.

Saliu, E. M., W. Vahjen, and J. Zentek. (2017). Types and prevalence of extended-spectrum beta-lactamase producing Enterobacteriaceae in poultry. Anim. Heal. Res. Rev. 18:46–57.

Santos, L. L., R. A Dos,. P. Moura, A. P. Aguilar-Ramires,. De Castro, and N. Lincopan. (2013). Current status of extended-spectrum β -lactamase (ESBL) -producing Enterobacteriaceae in animals. Microb. Pathog. Strateg. Combat. them: Sci. Technol. Educ. 1600-07.

Seni, J., L. Falgenhauer, N. Simeo, M. M. Mirambo, C. Imirzalioglu, M. Matee, M. Rweyemamu, T. Chakraborty, and S. E. Mshana (2016). Multiple ESBL-producing Escherichia coli sequence types carrying quinolone and aminoglycoside resistance genes circulating in companion and domestic farm animals in Mwanza, Tanzania, harbor commonly occurring plasmids. Front. Microbiol. 7:1–8.

Sudarwanto, M. B., D. W. Lukman, H. Latif, H. Pisestyani, E. Sukmawinata, Ö. Akineden, and E. Usleber. (2016). CTX-M producing Escherichia coli isolated from cattle feces in Bogor slaughterhouse, Indonesia. Asian Pac. J. Trop. Biomed. 6:605–608.

Sudarwanto, M. B., D. W. Lukman, T. Purnawarman, H. Latif, H. Pisestyani, and E. Sukmawinata. (2017). Multidrug resistance extended spectrum β-lactamase and AmpC producing Escherichia coli isolated from the environment of Bogor Slaughterhouse, Indonesia. Asian Pac. J. Trop. Biomed. 7:708–711.

Surgers L, P. Boersma, PM. Girard, A. Homor, D. Geneste, G. Arlet, D. Decré, and A. Boyd (2019). Molecular epidemiology of ESBL-producing *E. coli* and *K. pneumoniae*: establishing virulence clusters. Infection and Drug Resistance, 12: 119–127

Thyagarajan, D., M. Barathi, and R. Sakthivadivu, (2014) Risk mitigation of poultry industry pollutants and waste for environmental safety. *Glob. J. Sci. Front. Res. Agric. Vet*., 14(1): 1-10.

Upadhyay, S., A. Hussain, S.Mishra, A.P. Maurya, A.Bhattacharjee, and S.R Joshi (2015) Genetic Environment of Plasmid Mediated CTX-M-15 Extended Spectrum Beta-Lactamases from Clinical and Food Borne Bacteria in North-Eastern India. PloS ONE **10**: e0138056.

Valentin, L., H Sharp, K.Hille, U. Seibt, J. Fischer, and Y. Pfeifer (2014). Subgrouping of ESBL-producing *Escherichia coli* from animal and human sources: an approach to quantify the distribution of ESBL types between different reservoirs. *Int. J. Med. Microbiol.* 304, 805–816.

Wibisono, F. J, B. Sumiarto, and TA. Kusumastuti (2018). Economic losses estimation of pathogenic Escherichia coli infection in Indonesian Poultry Farming. Bul. Peternak. 42:341–346.

Wibisono, F.J., B.Sumiarto, T.Untari, M.H. Effendi, D.A. Permatasari, and A.M. Witaningrum. (2020a). The Presence Of Extended Spectrum Beta-Lactamase (ESBL) Producing Escherichia Coli On Layer Chicken Farms In Blitar Area, Indonesia. Biodiversitas**, 21** (6): 2667-2671

Wibisono, F.J., B.Sumiarto, T.Untari, M.H. Effendi, D.A. Permatasari, and A.M. Witaningrum. (2020b). CTX Gene of Extended Spectrum Beta-Lactamase (ESBL) Producing Escherichia coli on Broilers in Blitar, Indonesia. Sys Rev Pharm, 11(7): 396-403.

Wu, H., M. Wang, Y Liu,., X. Wang, Y. Wang, J. Lu, and H. Xu (2016). Data on the phylogenetic typing, integrin gene cassette array analysis, multi-drug resistance analysis and correlation between antimicrobial resistance determinants in *Klebsiella* strains. Data Brief, 8 : 1289-1294.

Yanestria, S.M., R.P. Rahmaniar, F.J. Wibisono, and M.H. Effendi, (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.

Zarfel, G., H. Galler, J., Luxner, C. Petternel, , F.F. Reinthaler, D. Haas, C. Kittinger, A.J. Grisold, P. Pless, and G. Feierl, (2014). Multiresistant bacterial isolated from chicken meat in Austria. Int. J. Environ. Res. Public Health, 11(12): 12582-12593.