

# Prevalence of Extended-Spectrum Beta-Lactamases in producing *Escherichia coli* in beef sold in traditional markets in Surabaya, Indonesia

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Manuscript received: 29 January 2021. Revision accepted: 23 April 2021.

**Abstract.** Wardhana DK, Safitri DA, Anisa S, Harijani N, Estoepangestie S, Maghfiroh L. 2021. Prevalence of Extended-Spectrum Beta-Lactamases in producing *Escherichia coli* in beef sold in traditional markets in Surabaya, Indonesia. *Biodiversitas* 22: 2789-2793. Meat is a source of protein, but it can be contaminated by microorganisms such as *Escherichia coli* (*E. coli*). Some species of *E. coli* become resistant to antibiotics and produce Extended-Spectrum Beta-Lactamase (ESBL) that limit the choice of antibiotics for treatment of *E. coli* infection. The purpose of the study was to investigate the prevalence of ESBLs leading to the growth of *Escherichia coli* (*E. coli*) in beef sold in local markets in Surabaya City, Indonesia. A total of 60 samples from 10 traditional markets were tested. Isolated and identified *E. coli* strains were examined to detect the ESBL production through four-disc diffusion tests using cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), and aztreonam (30 µg). Then, the minimum inhibitory concentration was measured. The study showed the presence of ESBL-producing *E. coli* in beef sold in the traditional markets in Surabaya. The average *E. coli* concentration in the beef was at 43.3%, and the highest resistance of the isolates was observed for cefotaxime (46.1%), ceftazidime (23.1%) ceftriaxone (19.2%), and aztreonam (38.4%). Data of ESBL-producing *E. coli* in broiler chicken meat in the traditional markets showed all of the *E. coli* isolates were resistant to ampicillin, while 48.4% of them were resistant to, cephalosporin. Only few (13%) were resistant to ceftazidime, and few others (9.6%) showed resistance to cefotaxime. The least number of them (6.4%) were resistant to ceftriaxone, and most of them were resistant to tetracycline (87.2%). Therefore, ESBLs were not reported producing *E. coli* in the beef. The presence of ESBL-producing *E. coli* in the beef gives an alert that good hygiene practices should be applied to handle meat safety and control the use of antibiotics.

**Keywords:** Beef, *Escherichia coli*, ESBL, Surabaya, traditional markets

## INTRODUCTION

Meat is an important human diet that strongly influences health, economics, and culture around the world. Many factors affecting meat production include domestic species, religious beliefs, culture, convenience, and availability (Paredi et al. 2013). Although meat has complete nutrition, it can become a growth medium for bacteria causing zoonotic diseases due to its potential to contaminate meat. Food products, especially meat ones, can be contaminated along with the levels of food chain, for example during production, distribution, preparation, and final consumption (Hemalata and Virupakshaiah 2016). As bacteria contaminate food, one of the zoonotic diseases is popularly known as food-borne disease.

Although developed countries have improved the hygienist of all meat production processes, food-borne diseases still threaten human and animal health. Food-borne diseases infect people as contaminants enter the body of humans through the consumption of contaminated food. This becomes one of the main public health problems worldwide (Tan et al. 2013). It will affect social welfare

and impact economic stability (Akbar et al. 2013). Food-borne diseases also create an enormous social and economic burden on health systems and communities (Ajayi et al. 2011). Specifically, this study brings about Extended Spectrum-Beta-Lactamase (ESBL) producing *E. coli* as a food-borne disease that is commonly observed.

*Escherichia coli* is a commensal bacterium in the intestines of animals and humans. It is a species of the Enterobacteriaceae family that most of its species are not pathogenic. It has a mutualism with its hosts and rarely causes diseases in the hosts (Ramos et al. 2020). Unlike other species of Enterobacteriaceae family, this very complex species can be grouped into pathogenic strains. Based on its virulence factors and clinical symptoms in the hosts, *E. coli* are classified into zoonotic intestinal pathogenic *E. coli* (IPEC) and extraintestinal pathogenic *E. coli* (EPEC) (Lindstedt et al. 2018).

Nowadays, some species of *E. coli* have become resistant to antibiotics. Mobile genetic elements such as a plasmid, insertion sequences, and transposons contribute to the plasticity of genome. The diffusion of antibiotics resistance has been promoted by horizontal gene transfer

between this species and other commensal bacteria (Raimondi et al. 2019), especially in environments such as the digestive tract, where the diversity of species and number of microorganisms are large. Therefore, surveillance of antimicrobial resistance is significant to detect the resistance of *E. coli* as sentinel bacteria, especially related to beta-lactam antibiotics (Nyirabahizi et al. 2020).

Extended-Spectrum Beta-Lactamase (ESBL) can hydrolyze a broad spectrum of cephalosporins, including cefotaxime, ceftriaxone, ceftazidime, or cefepime, and monobactam. More than 1,000 types of ESBLs, such as SHV, TEM, OXA, and CTXM types, have been discovered, and many more of those can be identified in the future (Allen et al. 2010; Jia et al. 2017).

The issues of ESBLs have recently been allied to health care systems, even more, food safety and environmental exposure. To estimate the transmission of the bacteria along the food chain and the exposure to the human population, information about the resistance of bacteria in the main production is an important factor needed (Valentin et al. 2014).

It has been observed that 14.84% of ESBL-producing bacteria in processed animal products is a public health problem involving the environmental source of ESBL producers for human and animal health (Niasono et al. 2019; Yusha'u and Umar 2016). The spread of ESBLs-producing *E. coli* is frequently reported in livestock (Kaesbohrer et al. 2019). Safitri et al. (2017) have found the sensitivity test of broiler chicken meat in traditional markets in Surabaya indicated *E. coli* isolates were resistant to ampicillin (100%), cephalazolin (48.4%), ceftazidime (13%), cefotaxime (9.6%), ceftriaxone (6.4%), and tetracycline (87.2%). However, studies on the prevalence of ESBL-producing *E. coli* in beef sold in traditional markets in Surabaya are still limited.

Thus, this study aimed to find out the presence of ESBL-producing *E. coli* in beef samples obtained from traditional markets in Surabaya.

## MATERIALS AND METHODS

### Isolation and Identification of *E. coli*

This study sampled beef through purposive sampling method based on some criteria (Acharya et al. 2013). The samples were taken from the surface of beef. Samples of fresh beef (n= 60) were collected from 10 different traditional markets in Surabaya City from August to November 2020. Six samples were obtained from each market. For the isolation of *E. coli*, each sample (25g) was homogenized in buffered peptone water. One ml of the suspension was filled into 9 ml of brilliant green bile broth media (Merck 1.05454.0500) with Durham tube inside and then incubated at 45.5°C for 24 - 48 hours. When the tube was filled with gas, the isolates were suspected to be *E. coli*. One loop of suspected *E. coli* was taken and inoculated on Eosin Methylene Blue Agar (Merck 1.01347.0500) and then incubated at 35°C for 24 hours. One colony showing typical *E. coli* morphology was

picked, and the identification of the colony was verified by Gram staining and classical biochemical test (IMViC) including tests for the utilization of indole, methyl red, and citrate, and Voges-Proskauer test, as well as sugar fermentation test on triple sugar iron agar following the standard protocol (Hensyl 1994).

### Susceptibility test of ESBL-producing *E. coli*

The susceptibility test of ESBL-producing *E. coli* was done by the disc diffusion method as Bauer did according to the guidelines of Clinical Laboratory Standards Institute (CLSI) (CLSI 2018). The colonies of *E. coli* (1-2 colonies) were taken by inoculating the loop and suspending it in 5ml of sterilized Sodium Chloride (NaCl) and incubating it at 37°C for 24 hours. The suspension was adjusted to achieve turbidity equivalent to 0.5 McFarland standards. Then, the Mueller-Hinton Agar (MHA) (Merck 1.05435.0500) plate surface was inoculated by spreading 0.2 ml of the bacterial suspension over the entire agar surface using a sterile glass spreader. The sterile glass spreader was rotated at an angle of 45° and waited up to 15 min till the bacterial suspension was absorbed.

The ESBL-producing *E. coli* was tested for four beta-lactam antibiotics which included cefotaxime Oxoid (30 µg), ceftazidime Oxoid (30 µg), ceftriaxone Oxoid (30 µg), and aztreonam Oxoid (30 µg). The diameters of inhibition zones were measured and interpreted according to the guidelines of the Clinical and Laboratory Standards Institute after 24-hour incubation. For a control strain, of the *E. coli* isolate (ATCC® 25922™) was used for susceptibility testing. This organism is a CLSI control strain for antimicrobial susceptibility testing (CLSI 2018) (Table 1). The data were presented descriptively in percentages displayed in tables.

## RESULTS AND DISCUSSION

### Isolation and identification of *E. coli*

The results of isolation and identification of *E. coli* are shown in Table 2. A total of 60 meat samples were obtained from 10 traditional markets in Surabaya. Twenty-six samples (43,3%) were found to be positive for *E. coli*. The positive isolates of *E. coli* showed positive results as observed from glucose, maltose, mannitol, lactose, sucrose, indole, motility, and triple sugar iron agar. Meanwhile, Simmon citrate and urea showed negative results.

**Table 1.** Inhibition Zone of ESBL-producing *E. coli* based on Guidelines of Clinical and Laboratory Standards Institute (CLSI 2018)

Antibiotics disc		Inhibition zones
Cefotaxime	CTX 30 µg	Inhibition zone ≤ 27 mm
Ceftazidime	CAZ 30 µg	Inhibition zone ≤ 22 mm
Ceftriaxone	CRO 30 µg	Inhibition zone ≤ 25 mm
Aztreonam	ATM 30 µg	Inhibition zone ≤ 27 mm

### ESBL-producing *E. coli* in beef obtained from local markets in Surabaya

Regarding the resistant bacteria in the food chain, recent studies show that there are significant differences in the prevalence of *E. coli* in beef. In this study, all twenty-six isolates of *E. coli* were tested for susceptibility against four beta-lactam antibiotics including cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), and aztreonam (30 µg). The antimicrobial resistance profiles of the *E. coli* isolates were presented in Table 3. Briefly, 12 isolates (46.1%) were found to have the highest resistance to cefotaxime. A total of 23.1% of the isolates were resistant to ceftazidime. While 19.2% and 38.4% of the isolates were resistant to ceftriaxone and aztreonam, respectively. ESBLs are resistant to all penicillins, third-generation cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone), and aztreonam, except cephamycin (cefoxitin and cefotetan) and carbapenems (Shaikh et al. 2015).

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**Table 3.** Resistance profiles of ESBL-producing *Escherichia coli* in beef obtained from the traditional markets in Surabaya

Antibiotics	ESBL Production (n = 26)		Positive percentages (%)
	Positive	Negative	
Cefotaxime	12	14	46.1
Ceftazidime	6	20	23.1
Ceftriaxone	5	21	19.2
Aztreonam	10	16	38.4

**Table 2.** *Escherichia coli* isolation of 60 beef samples obtained from the traditional markets in Surabaya, Indonesia

Markets	<i>E. coli</i> (n=60)		Positive percentages (%)
	Negative	Positive	
A	2	4	66.7
B	6	0	0
C	3	3	50
D	3	3	50
E	4	2	33.3
F	0	6	100
G	5	1	20
H	6	0	0
I	2	4	66.7
J	3	3	50
Total	34	26	-
Mean of positive <i>E. coli</i>			43.3

### Discussion

#### *Escherichia coli* in beef

This study discovers that 43.3% of the *E. coli* isolates were positive (see Table 2), and this finding is relevant to the research finding of Rahman et al. (2017) which showed a higher prevalence. It was reported the prevalence of *E. coli* in beef in Bangladesh was detected in 7 of 10 samples (70%). Soepranianondo et al. (2019) supported the finding that *E. coli* was found in 32.5% of beef collected from East Java regions, Indonesia. Research conducted by Kassem et al. (2020) also suggested a similar result that *E. coli* was detected in 38 samples (76%) of 50 samples in Beirut, Lebanon.

The presence of *E. coli* in beef is usually associated with the improper implementation of sanitary hygiene (Eyi and Arslan 2012). The contamination of pathogenic bacteria such as *E. coli* occurs because of unhygienic processing, storage, cutting, slaughtering area, and market area (Zafar et al. 2016; Bahri et al. 2019). Packing also becomes one of the key factors allied to the presence of *E. coli* in meat. Yang et al. (2017) state that *E. coli* contamination in beef occurs because of contaminated cutting table, conveyor belt, and mesh gloves in the packing process. The temperature of meat during storing process must be set properly to avoid the growth of coliform bacteria including *E. coli* (Harlia 2017).

Beef is sold by retailers in the traditional markets in Surabaya, thereby giving more probability of *E. coli* contamination. The research conducted by Eyi and Arslan (2012) highlighted that retail beef was dangerous for human health because of insufficient sanitation and hygiene in the processing and handling. The contamination of *E. coli* in beef is likely spread by the personnel and equipment used for processing such as knives and cutting boards in addition to poor hygiene sanitation (Martínez-Chávez et al. 2015). The contamination during the slaughtering process can be transmitted from the slaughtermen's hands, water, and equipment used (Nyamakwere et al. 2016). A particular strain of *E. coli* in beef will lead to foodborne diseases that have become a public health concern (Bantawa et al. 2018).

#### *ESBL-producing Escherichia coli* in beef

In this study, beef samples were tested to identify the presence of ESBL-producing *E. coli*. The current results were compared to those of previous studies. The results showed lower numbers of *E. coli* than those of previous studies that observed the bacteria in minced meat collected from the regions of Southwest Ethiopia (Abayneh et al. 2019). The previous study found the resistance levels of the bacteria against cefotaxime, ceftazidime, ceftriaxone were at 85.7%, 71.4%, and 85.7%, respectively. Similarly, another earlier study done by Montso et al. (2019) showed 45% of the bacteria were resistant to aztreonam. However, the percentage is higher than what Effendi et al. (2020) found. In their study, the resistance levels of *E. coli* to cefotaxime, ceftazidime, and ceftriaxone were at 3%, 6%, and 3% respectively. In comparison to the research locations, it means that the traditional markets in Surabaya

have increasing cases of ESBL-producing *E. coli* but lower cases than in Ethiopia.

ESBLs are enzymes that hydrolyze the oxyimino-beta-lactams which contribute to the damage of antibiotics structure and thus lead to the resistance of bacteria (Widodo et al. 2020). Resistant genes in integrons exist because of the ESBL-producing resistant *E. coli* to antibiotics (Chen et al. 2017). Furthermore, Class I integrons and resistant genes have been captured to spread ESBL-producing *E. coli* in meat (Moawad et al. 2017).

The use of non-therapeutic and therapeutic antibiotics can be a contributing factor to the spread of antibiotic-resistant bacteria (Marshall and Levy 2011). Cattle beef becomes a good reservoir of ESBL-producing *E. coli*, and thus the use of antibiotics in livestock should be considered (Aslantaş et al. 2017). The use of antibiotics in humans and animals must be regulated and monitored properly to detain the spread of antibiotic-resistant bacteria (Chaisatit et al. 2012).

Unhygienic processing and production are the factors causing the high prevalence of multi-resistant *E. coli* (Rahman et al. 2017). Environmental factors are assumed to contribute to the presence of ESBL-producing *E. coli* as well. Research conducted by Blaak et al. (2015) shows that urban wastewater significantly could facilitate the spread of ESBL-producing *E. coli*.

Livestock excrement also may be media for the spread of ESBL-producing *E. coli*. This statement is supported by Montso et al. (2019) who found ESBL-producing *E. coli* in beef and livestock excrement. Whereas, Saleem et al. (2017) also observed ESBL-producing *E. coli* in beef and livestock excrement. The ESBL-producing Enterobacteriaceae in livestock excrement raises the risk of raw product contamination (Geser et al. 2012). Strict supervision on imported meat should be indispensable as imported meat can also be one of the carrier factors of ESBL-producing *E. coli* (Kim et al. 2018). Meat sold in local markets can potentially spread ESBL-producing *E. coli*. To control the transmission, it takes public awareness to sort out and assure meat sold (Effendi et al. 2020).

Nowadays, the ESBL-producing *E. coli* has widely affected the health system, food safety, and environmental exposure. Therefore, understanding the prevalence of antibiotic-resistant bacteria in the food chain is essential in predicting ESBL exposure to the human population (Kaesbohrer et al. 2019). It is also inevitable that ESBL-producing *E. coli* has gradually increased in animal food productions worldwide (Geser et al. 2012).

Indiscriminate use of antibiotics, poor hygiene and sanitation, types of antibiotics used for veterinary medicine, inadequate infection control programs in health centers and hospitals, and lack of proper managerial procedures on animal farms can all lead to antibiotics resistance, even more the high prevalence of ESBL-producing *E. coli* in animals and humans. Since *E. coli* is a microorganism that spreads widely in humans, animals, and the environment, the handling of antibiotics resistance requires several health programs, such as translational research, epidemiology, and surveillance in human health and veterinary drugs. It is believed that strategies to

overcome the resistance of *E. coli* to antibiotics must be adopted on an immense and comprehensive scale (Poirel et al. 2018).

It can be concluded the average prevalence of *E. coli* in 60 beef samples obtained from traditional markets in Surabaya was at 43.3%. *E. coli* isolates showed the highest resistance to the third-generation cephalosporins which are cefotaxime (46.1%), ceftazidime (23.1%) ceftriaxone (19.2%), and aztreonam (38.4%). From these findings, good hygiene and sanitation of the food processing can anticipate the cross-contamination and horizontal gene transfer. The prevalence of ESBL genes awaits further study.

## ACKNOWLEDGMENTS

The authors would like to thank to Rector of Universitas Airlangga, Surabaya, Indonesia and Dean of Faculty of Veterinary Medicine, Universitas Airlangga for funding this study. This study was supported by *Penelitian Dosen Pemula* from Universitas Airlangga (Contract no.: 346/UN3/2020).

## REFERENCES

- Abayneh M, Tesfaw G, Woldemichael K, Yohannis M, Abdissa A. 2019. Assessment of extended-spectrum  $\beta$ -lactamase (ESBLs)-producing *Escherichia coli* from minced meat of cattle and swab samples and hygienic status of meat retailer shops in Jimma town, Southwest Ethiopia. *BMC Infect Dis* 19 (1): 897. DOI: 10.1186/s12879-019-4554-6.
- Acharya AS, Prakash A, Saxena P, Nigam A. 2013. Sampling: Why and how of it. *Indian J Med Spec* 4 (2): 330-333.
- Ajayi O, Williams LL, Oluwoye J, Johnson JU, Okafor F, Sanders O-G, Wilson T. 2011. Epidemiological approaches to food safety. *Food Prot Tends* 31 (9): 560-568.
- Akbar A, Anal KA. 2013. Prevalence and antibiogram study of *Salmonella* and *Staphylococcus aureus* in poultry meat. *Asian Pac J Trop Biomed* 3 (2): 163-168. DOI: 10.1016/S2221-1691(13)60043-X.
- Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. 2010. Call of the wild: antibiotic resistance genes in natural environments. *Nat Rev Microbiol* 8 (4): 251-259. DOI: 10.1038/nrmicro2312.
- Aslantaş O, Elmacıoğlu S, Yılmaz ES. 2017. Prevalence and characterization of ESBL-and AmpC-producing *Escherichia coli* from cattle. *Kafkas Univ Vet Fak Derg* 23: 63-67.
- Bahri S, Rokhim S, Prasiska YS. 2019. Kontaminasi bakteri *Escherichia coli* pada sampel daging. *J Health Sci Prev* 3 (1): 62-67. DOI: 10.29080/jhsp.v3i1.195.
- Bantawa K, Rai K, Limbu DS, Khanal H. 2018. Food-borne bacterial pathogens in marketed raw meat of Dharan, eastern Nepal. *BMC Res Notes* 11 (1): 1-5. DOI: 10.1186/s13104-018-3722-x.
- Blaak H, Lynch, G, Italiaander R, Hamidjaja RA, Schets FM, de Roda Husman AM. 2015. Multidrug-resistant and extended-spectrum beta-lactamase-producing *Escherichia coli* in Dutch surface water and wastewater. *PLoS One* 10 (6): e0127752. DOI: 10.1371/journal.pone.0127752.
- Chaisatit C, Tribuddharat C, Pulsrikarn C, Dejsirilert S. 2012. Molecular characterization of antibiotic-resistant bacteria in contaminated chicken meat sold at supermarkets in Bangkok, Thailand. *Jpn J Infect Dis* 65 (6): 527-534. DOI: 10.7883/yoken.65.527.
- Chen CM, Ke SC, Li CR, Wu YC, Chen TH, Lai CH, Wu LT. 2017. High Diversity of Antimicrobial Resistance Genes, Class 1 Integrons, and Genotypes of Multidrug-Resistant *Escherichia coli* in Beef Carcasses. *Microb Drug Resist* 23 (7): 915-924. DOI: 10.1089/mdr.2016.0223.
- Effendi MH, Cicilia R, Rahmahani J, Tyasningsih W. 2020. Public Awareness for Antimicrobial Resistance from *Escherichia coli*

- Isolated from Beef Sold on Several Wet Market in Surabaya, Indonesia. *Indian J Public Health Res Dev* 11 (9): 295-300.
- Eyi A, Arslan S. 2012. Prevalence of *Escherichia coli* in retail poultry meat, ground beef and beef. *Med Weter* 68 (4): 237-240.
- Geser N, Stephan R, Hächler H. 2012. Occurrence and characteristics of extended-spectrum  $\beta$ -lactamase (ESBL) producing *Enterobacteriaceae* in food-producing animals, minced meat and raw milk. *BMC Vet Res* 8 (1): 1-9. DOI: 10.1186/1746-6148-8-21.
- Hemalata VB, Virupakshiah DBM. 2016. Isolation and identification of food-borne pathogens from spoiled food samples. *Intl J Curr Microbiol Appl Sci* 5 (6): 1017-1025. DOI: 10.20546/ijcmas.2016.506.108.
- Hensyl WR. 1994. *Bergey's Manual of Systematic Bacteriology*. 9th ed, Williams and Wilkins, Baltimore.
- Jia B, Raphenya AR, Alcock B, Wagglechner N, Guo P, Tsang KK, Lago BA, Dave BM, Pereira S, Sharma AN. 2017. Expansion and model-centric curation of the comprehensive antibiotic resistance database. *Nucleic Acids Res* 45 (D1): D566-D573. DOI: 10.1093/nar/gkw1004.
- Kaesbohrer A, Bakran-Lebl K, Irrgang A, Fischer J, Kämpf P, Schiffmann A, Werckenthin C, Busch M., Kreienbrock L, Hille, K. 2019. Diversity in prevalence and characteristics of ESBL/pAmpC producing *E. coli* in food in Germany. *Vet Microbiol* 233: 52-60. DOI: 10.1016/j.vetmic.2019.03.025.
- Kassem II, Nasser NA, Salibi J. 2020. Prevalence and loads of fecal pollution indicators and the antibiotic resistance phenotypes of *Escherichia coli* in raw minced beef in Lebanon. *Foods* 9 (11): 1543. DOI: 10.3390/foods9111543.
- Kim YJ, Moon JS, Oh DH, Chon JW, Song BR, Lim JS, Sung K. 2018. Genotypic characterization of ESBL-producing *E. coli* from imported meat in South Korea. *Food Res Intl* 107: 158-164. DOI: 10.1016/j.foodres.2017.12.023.
- Lindstedt BA, Finton MD, Porcellato D, Brandal LT. 2018. High frequency of hybrid *Escherichia coli* strains with combined Intestinal Pathogenic *Escherichia coli* (IPEC) and Extraintestinal Pathogenic *Escherichia coli* (ExPEC) virulence factors isolated from human faecal samples. *BMC Infect Dis* 18: 544. DOI: 10.1186/s12879-018-3449-2.
- Marshall BM, Levy SB. 2011. Food animals and antimicrobials: impacts on human health. *Clin Microbiol Rev* 24 (4): 718-733. DOI: 10.1128/CMR.00002-11.
- Martínez-Chávez L, Cabrera-Díaz E, Pérez-Montañón JA, Garay-Martínez LE, Varela-Hernández JJ, Castillo A, Martínez-González NE. 2015. Quantitative distribution of *Salmonella spp.* and *Escherichia coli* on beef carcasses and raw beef at retail establishments. *Intl J Food Microbiol* 210: 149-155. DOI: 10.1016/j.ijfoodmicro.2015.06.016.
- Melvin P, Weinstein, MD. 2018. M100 Performance Standards for Antimicrobial. Clinical and Laboratory Standards Institute, USA.
- Moawad AA, Hotzel H, Awad O, Tomaso H, Neubauer H, Hafez HM, El-Adawy, H. 2017. Occurrence of *Salmonella enterica* and *Escherichia coli* in raw chicken and beef meat in northern Egypt and dissemination of their antibiotic resistance markers. *Gut Pathog* 9 (1): 57. DOI: 10.1186/s13099-017-0206-9.
- Montso KP, Dlamini SB, Kumar A, Ateba CN. 2019. Antimicrobial Resistance Factors of Extended-Spectrum-Beta-Lactamases Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolated from Cattle Farms and Raw Beef in North-West Province, South Africa. *Biomed Res Intl* 2019: 4318306. DOI: 10.1155/2019/4318306.
- Niasono AB, Latif H, Purnawarman T. 2019. Antibiotic resistance to *Escherichia coli* bacteria isolated from broiler farms in Subang Regency, West Java. *J Veteriner* 20: 187-195. DOI: 10.19087/jveteriner.2019.20.2.187.
- Nyamakwere F, Muchenje V, Mushonga B, Makepe M, Mutero G. 2016. Assessment of *Salmonella*, *Escherichia coli*, *Enterobacteriaceae* and Aerobic Colony Counts contamination levels during the beef slaughter process. *J Food Saf* 36 (4): 548-556. DOI: 10.1111/jfs.12275.
- Nyirabahizi E, Tyson GH, Dessai U, Zhao S, Kabera C, Crarey E, Womack N, Crews MK, Strain E, Tate H. 2020. Evaluation of *Escherichia coli* as an indicator for antimicrobial resistance in *Salmonella* recovered from the same food or animal ceca samples. *Food Control* 115: 107280. DOI: 10.1016/j.foodcont.2020.107280.
- Paredi G, Sentandreu MA, Mozzarelli A, Fadda S, Hollung K, de Almeida AM. 2013. Muscle and meat: new horizons and applications for proteomics on a farm to fork perspective. *J Proteomics* 88: 58-82. DOI: 10.1016/j.jprot.2013.01.029.
- Poirer L, Madec JY, Lupo A, Schink AK, Kieffer N, Nordmann P, Schwarz S. 2018. Antimicrobial resistance in *Escherichia coli*. Antimicrobial resistance in bacteria from livestock and companion animals. In: Schwarz S, Cavaco LM, Shen J (eds.). *Antimicrobial Resistance in Bacteria from Livestock and Companion Animals*. Wiley-Blackwell, New York.
- Rahman MA, Rahman AK, Islam MA, Alam MM. 2017. Antimicrobial resistance of *Escherichia coli* isolated from milk, beef and chicken meat in Bangladesh. *Bangladesh J Vet Med* 15 2: 141-146. DOI: 10.3329/bjvm.v15i2.35525.
- Raimondi S, Righini L, Candelieri F, Musmeci E, Bonvicini F, Gentilomi G, Erjavec MS, Amaretti A, Rossi M. 2019. Antibiotic resistance, virulence factors, phenotyping, and genotyping of *E. coli* isolated from the feces of healthy subjects. *Microorganisms* 7: 251. DOI: 10.3390/microorganisms7080251.
- Ramos S, Silva V, Dapkevicius MdLE, Canica M, Tejedor-Junco MT, Igrejas G, Poeta P. 2020. *Escherichia coli* as commensal and pathogenic bacteria among food-producing animals: health implications of Extended-Spectrum Beta-Lactamase (ESBL) production. *Animals* 10: 2239. DOI: 10.3390/ani10122239.
- Safitri RD, Cicilia R, Bintari IG, Hermawan IP, Effendi MH, Ernawati R, Rahmahani J. 2017. Detection of encoding gene Extended-Spectrum Beta-Lactamase on *Escherichia coli* isolated from broiler chicken meat in traditional market Surabaya. *Intl J Dev Res* 7 (11): 17354-17357.
- Shaikh S, Fatima J, Shakil S, Rizvi SMD, Kamal MA. 2015. Antibiotic resistance and extended-spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi J Biol Sci* 22: 90-101. DOI: 10.1016/j.sjbs.2014.08.002.
- Soepranionondo K, Wardhana DK, Budiarto, Diyanoro. 2019. Analysis of bacterial contamination and antibiotic residue of beef meat from city slaughterhouses in East Java Province, Indonesia. *Vet World* 12(2): 243-248. DOI: 10.14202/vetworld.2019.243-248.
- Tan SL, Lee HY, Abu BF, Abdul KMS, Rukayadi Y, Mahyudin NA. 2013. Microbiological quality on food handlers hands at primary schools in Hulu Langat District, Malaysia. *Intl Food Res J* 20 (5): 2973-2977.
- Valentin L, Sharp H, Hille K, Seibt U, Fischer J, Pfeifer Y, Michael GB, Nickel S, Schmiedel J, Falgenhauer L, Frieze A, Bauerfeind R, Roesler U, Imirzalioglu C, Chakraborty T, Helmuth R, Valenza G, Werner G, Schwarz S, Guerra B, Appel B, Kreienbrock L, Käsbohrer A. 2014. Subgrouping of ESBL-producing *Escherichia coli* from animal and human sources: an approach to quantify the distribution of ESBL types between different reservoirs. *Intl J Med Microbiol* 304: 805-816. DOI: 10.1016/j.ijmm.2014.07.015.
- Widodo A, Effendi MH, Khairullah AR. 2020. Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* from livestock. *Sys Rev Pharm* 11 (7): 382-392.
- Yang X, He A, Badoni M, Tran F, Wang H. 2017. Mapping sources of contamination of *Escherichia coli* on beef in the fabrication facility of a commercial beef packing plant. *Food Control* 75: 153-159. DOI: 10.1016/j.foodcont.2016.12.004.
- Zafar A, Ahmed E, Wajiha, H, Khan A. 2016. Microbiological evaluation of raw meat products available in local markets of Karachi, Pakistan. *Proc Pakistan Acad Sci B Life Environ Sci* 53 (2): 103-109.