

Prevalence of Enterobacteriaceae Producing Extended Spectrum Beta-Lactamase (ESBL) in Broiler Meat (*Gallus domesticus*) Sold in Regional Markets of West Surabaya

Kadek Rio Risnanda¹, Masfufatun^{2,*}, Agusniar Furkani Listyawati³,
Kuntaman Kuntaman³, Akhmad Sudibya³

¹Faculty of Medicine; ²Department of Biochemistry; ³Department of Microbiology,
Faculty of Medicine, Universitas Wijaya Kusuma Surabaya
Jl. Dukuh Kupang XXV No. 54, Dukuh Kupang, Kec. Dukuh Pakis, Kota Surabaya, Jawa Timur, Indonesia.

Corresponding author*
masfufatun@uwks.ac.id

Manuscript received: 23 August, 2023. Revision accepted: 03 November, 2023. Published: 09 November, 2023.

Abstract

Broiler meat is a foodstuff that is easily damaged by bacterial growth. Several pathogenic bacteria from the *Enterobacteriaceae* family have also been found as contaminants in chicken meat. While *Extended Spectrum Beta-Lactamases* (ESBL) are enzymes that have the ability to hydrolyze antibiotics of the penicillin class, first, second, and third generation *cephalosporins* as well as the *monobactam* group. Based on this phenomenon, the researchers wanted to examine the prevalence of the ESBL-producing *Enterobacteriaceae* family in broiler chicken (*Gallus domesticus*) meat sold in the West Surabaya market. This research is a descriptive observational type using a cross-sectional approach. The population in this study was broiler chicken meat taken from broiler traders in several markets in West Surabaya which had met the inclusion and exclusion criteria with a large sample of 50 samples. Then the data were analyzed using the chi-square test. The results of the analysis showed that 13 samples produced ESBL. The prevalence of *Enterobacteriaceae* bacteria producing ESBL in broiler chicken (*Gallus domesticus*) meat in several markets in West Surabaya is 26% of the 50 samples used. The results of the chi-square test showed that there was no significant difference between locations where ESBL was found.

Keywords: *Enterobacteriaceae*; *Extended Spectrum Beta-Lactamase*; Broiler Chicken Meat.

Abbreviations: ESBL *Extended Spectrum Beta-Lactamase*; MDR: *Multiple Drug Resistance*.

INTRODUCTION

Poultry meat, especially chicken meat, is a source of animal protein that is in great demand in Indonesia (Region, 2019). Most of Indonesia's demand for meat comes from poultry, especially chickens. Based on 2018 data, broiler production increased by 480,309 heads. According to statistical data for 2019, the consumption of chicken meat is 4.94 kg per person per year (Direktorat Jenderal Peternakan dan Kesehatan Hewan, 2019). Broiler meat is a food ingredient that is easily damaged by bacterial growth. Marketed chicken meat can be a breeding ground for microbes that cause food poisoning. Several pathogenic bacteria from the *Enterobacteriaceae* family have also been found as contaminants in chicken meat, including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella sp.*, *Pseudomonas sp.*, *Clostridium perfringens* and *Shigella flexneri* (Ray, 2014).

Enterobacteriaceae contamination of food occurs through soil, air, water, dust, digestive tract, human hand contact, and the influence of animals such as cockroaches

and flies (Mu'arofah et al. 2020). Laying the carcass or body parts of chickens directly on the ground after slaughtering a chicken that has been washed can result in contamination with carcass remnants, such as blood, feathers, excrement, and its own stomach contents (Ramadhani et al., 2020).

A study by Mashak revealed that 16.25% of chicken meat from Alborz, Iran, was *E.coli* positive. Previous research also stated that several samples of chicken meat from local markets in Indonesia presented microbial contamination, which included *S. aureus* (6.7%), *Salmonella sp.* (85%), and *E. coli* (90.03%). Contamination of poultry products including raw broiler meat by pathogenic microorganisms, especially bacteria, has become one of the most challenging problems in the food industry worldwide (Wardhana et al. 2021). *Extended Spectrum Beta-Lactamases* are enzymes that have the ability to hydrolyze penicillin class antibiotics, first, second and third generation *cephalosporins* as well as *monobactam* groups and cause resistance to all of these antibiotics (Biutifasari, 2018).

Epidemiologically, the prevalence of ESBL varies in various countries. The prevalence of ESBL produced by *Enterobacteriaceae* families such as *E. coli* and *Klebsiella pneumoniae* varies, ranging from 42.7% in Latin America, 5.8% in North America, 2% to 31% in Europe, and 4.8% to 12% in isolates. in Asia (Yessy, 2021). Research in five hospitals in Indonesia in 2013 showed the prevalence of ESBL-producing bacteria was 32-68% (Kuntaman, 2013). The results of the 2016 AMR study by the Department of Health Control on Antimicrobial Resistance in 8 hospitals with ESBL indicator bacteria *E. coli* and *K. pneumoniae* showed an average prevalence of 60%.

Beta-lactams are used against gram-positive and gram-negative bacteria. Bacterial resistance to lactams are using three pathways: Disruption of the antibiotic lactamase enzyme, alteration of the target of the antibiotic and decreased cellular uptake of the antibiotic. All of these pathways play an important role in antibiotic resistance. However, lactam-producing and lactam-destroying bacteria are the main causes of resistance (Masruroh et al. 2016).

Antibiotics are chemical substances produced by fungi or bacteria that have the ability to kill or prevent the growth of pathogenic microorganisms, while their toxicity to humans is relatively low. Irrational use of antibiotics causes negative effects, such as the immunity of microorganisms to some antibiotics, increased drug side effects and even death. Bacterial resistance to antibiotics is a global health problem that threatens the treatment of infectious diseases and causes serious disability and death in the healthcare community. Bacteria that naturally carry antibiotic resistance genes can transfer these genes to other bacteria. In addition, bacteria can produce enzymes that inhibit the action of antibiotics (Aidara-Kane et al., 2013). One of the antibiotics used is a beta-lactam class of antibiotics which works by inhibiting the cell wall. Improper use of antibiotics, including selection, dosage, and patient non-adherence to treatment, play a role in the development of antibiotic resistance. One of the resistance mechanisms of Gram-negative bacteria belonging to the *Enterobacteriaceae* family is the production of ESBL.

According to the results of a study on 781 hospital patients throughout Indonesia, 81% of *Escherichia coli* were found to be resistant to various antibiotics, namely ampicillin (73%), cotrimoxazole (56%), chloramphenicol (43%), ciprofloxacin (22%), and gentamicin (18%) (Kementerian Kesehatan Republik Indonesia, 2013). Based on the data and research results obtained above, the researchers wanted to examine the prevalence of *Enterobacteriaceae* family bacteria such as *E. coli* producing ESBL in broiler chicken meat (*Gallus Domesticus*) sold in West Surabaya market in the hope of knowing whether the consumption of broiler chickens contaminated with bacteria can affect the percentage of

Indonesian people who are resistant to antibiotics due to the high *E. coli* bacteria in broiler chicken meat.

MATERIALS AND METHODS

Research Design

This research is a descriptive observational type using a cross-sectional approach.

Population and Research Sample

The population in this study was broiler chicken meat taken from broiler traders in several markets in West Surabaya with a sample size of 50 samples that met the inclusion and exclusion criteria. This sampling used reference to the Basic and Clinical Biostatistics book.

Data Analysis Techniques

The method used in this study was univariate analysis to determine the prevalence rate of ESBL-producing bacteria in meat sold in markets in West Surabaya and bivariate analysis using the chi-square test.

RESULTS AND DISCUSSION

Results

Table 1. Identification Results of Enterobacteriaceae Producing Extended Spectrum Beta-Lactamase (ESBL) in Broiler Chicken (*Gallus domesticus*) Meat in Regional Markets of West Surabaya.

Location	Number of Samples	Extended Spectrum Beta-Lactamase (ESBL)
I	10	2
II	10	4
III	10	2
IV	10	2
V	10	3
Total	50	13 (26%)

Notes: I: Darmo Market Location, II: Darmo Market Location, III: Kupang Dukuh Market Location. IV: Kupang Gunung Market Location, V: Simo Gunung Market Location

Identification of Enterobacteriaceae producing Extended Spectrum Beta-Lactamase (ESBL) in broiler chicken (*Gallus domesticus*) meat in West Surabaya Regional Market

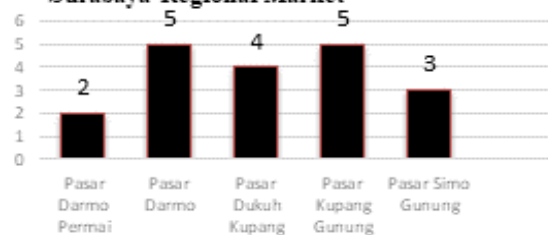


Figure 1. Lactose fermenter bacteria isolated from broiler chicken meat.

Based on the table and figure above, it can be seen that from 50 samples of broiler chickens in several markets in West Surabaya, 19 samples were positive for *Enterobacteriaceae* and 13 of them produced *Extended Spectrum Beta-Lactamase* (ESBL). At location I there were 2 positive samples for ESBL. Of the 10 samples at location II, 5 samples were positive for *Enterobacteriaceae* and 4 of them produced ESBL. Of the 10 samples at location III, there were 4 positive samples for *Enterobacteriaceae* and 3 of them produce ESBL. At location IV, 5 positive samples were found for *Enterobacteriaceae* and 2 of them produced ESBL and at location V, out of the 10 samples used, 3 samples were found positive for *Enterobacteriaceae* and all of them ESBL.

Based on Table 1, it can be calculated the prevalence of *Enterobacteriaceae* bacteria producing ESBL in broiler chicken (*Gallus domesticus*) meat in several markets in West Surabaya, namely 26% of the 50 samples used.

Table 2. Crosstab Places with Enterobacteriaceae Bacteria.

Location	Enterobacteriaceae		Total
	Positif	Negatif	
Pasar Darmo Permai	2 (4%)	8 (16%)	10 (20%)
Pasar Darmo	5 (10%)	5 (10%)	10 (20%)
Dukuh Kupang	4 (8%)	6 (12%)	10 (20%)
Pasar Kupang Gunung	5 (10%)	5 (10%)	10 (20%)
Pasar Simo Gunung	3 (6%)	7 (14%)	10 (20%)
Total	19 (38%)	31 (62%)	50 (100%)

Based on Table 2, it shows that most of the *Enterobacteriaceae* were found in the Darmo market and Kupang Gunung market, namely each (10%).

Table 3. Relationship of Place with Extended Spectrum Beta-Lactamase (ESBL) Bacteria.

Location	ESBL		Total	p-value
	Positif	Negatif		
Darmo	6 (30%)	14 (70%)	20 (100%)	0,599
Kupang	7 (23%)	23 (76%)	30 (100%)	
Total	13 (26%)	37 (74%)	50 (100%)	

The results of the chi-square test showed that there was no significant difference between locations where *Extended Spectrum Beta-Lactamase* (ESBL) was found.

Discussion

The results of the analysis showed that from 50 samples of broiler chickens in several markets in West Surabaya, 19 samples were positive for *Enterobacteriaceae* and 13

of them produced *Extended Spectrum Beta-Lactamase* (ESBL). The prevalence of *Enterobacteriaceae* bacteria producing Extended Spectrum Beta-Lactamase (ESBL) in broiler chicken (*Gallus domesticus*) meat in several markets in West Surabaya is 26% of the 50 samples used.

Research by Puspendari, Sunarno, et al. was conducted in four markets and two slaughterhouses in the Jakarta area. Research with a total of 240 chicken meat samples showed that ESBL-producing *E. coli* was isolated from 161 (67.1%) of 240 broiler samples, 161 (84.3%) of 191 suspected colonies on MacConkey agar supplemented with cefotaxime 0 media .4% confirmed as ESBL producer *E. coli* (Puspendari et al., 2021).

The results of this study provide information about ESBL-producing *Enterobacteriaceae* associated with antibiotic resistance found in broiler chickens in several markets in West Surabaya. The problem of Multiple-Drug Resistance (MDR) is exacerbated by the ability of bacteria to transfer genetic material carrying resistance properties from one bacterium to another vertically through genetic mutation and horizontally through conjugation, transduction and transformation. MDR occurs when more and more antibiotics are used, the greater the selective pressure on the process of evolution and proliferation of resistant bacterial strains to defend themselves so that vertical resistance arises from genetic mutations and horizontal resistance from the exchange of resistant gene material against various types of different antibiotic resistance mechanisms. (Gregova et al. 2012). Multidrug resistance is a common occurrence in ESBL producing bacteria. Aminoglycoside modifying enzyme (AME) and ESBL are genes that encode resistance enzymes commonly found in bacterial plasmids. That is, gene transfer occurs in genetic factors such as transposons, integrons, and plasmids (Alocati et al., 2013). The ESBL gene that appears in *E. coli* is caused by a plasmid-mediated genetic mutation, especially the ESBL gene with TEM and SHV types, then a new ESBL group appears, namely CTXM (Hasibuan, 2017).

This study is in accordance with research conducted (Wibisono et al. 2020) the results obtained from 185 samples of cloacal swabs which tested positive for ESBL-producing *E. coli* contained 7.03% ESBL (DDTS). Food products of animal origin contaminated with the *Enterobacteriaceae* family such as ESBL-producing *E. coli* can pose a health risk, although the level of risk is difficult to measure. Broiler chickens can serve as a reservoir for ESBL *E. coli*. ESBL bacteria are transmitted in several ways, namely: consuming contaminated meat, faecal contaminated environment containing ESBL-producing *E. coli*, exposure to patients or people infected with ESBL and transmitted to humans via animals and possibly causing zoonoses. Contamination of the broiler chickens under study can also come from the water used to wash the chickens, the equipment used by traders to cut the chickens and other equipment that has direct contact with the meat, as well as the hygiene of the chicken slaughterhouse itself.

Placing chicken meat directly on the sales counter without the need for a special place and handling such as packaging or temperature control which is also commonly found in the market can affect the level of bacterial contamination, one of which is *E. coli* bacteria. Flies can infect meat as vectors for *E. coli*. In addition to flies, the hands of buyers and sellers who are not clean can cause the meat to be exposed to *E. coli* bacteria. It is known that *E. coli* is harmful to health because it produces a toxin (shiga toxin) (Reich et al., 2013).

CONCLUSIONS

The results of the analysis showed that out of 50 samples of broiler chickens in several markets in West Surabaya, 13 samples produced *Extended Spectrum Beta-Lactamase* (ESBL). The prevalence of *Enterobacteriaceae* bacteria producing Extended Spectrum Beta-Lactamase (ESBL) in broiler chicken (*Gallus domesticus*) meat in several markets in West Surabaya, namely 26% of the 50 samples used, most of the *Enterobacteriaceae* were found in Darmo market and Kupang Gunung market, respectively each by (10%). The results of the chi square test showed that there was no significant difference between locations where Extended Spectrum Beta-Lactamase (ESBL) was found.

Acknowledgements: This research is supported by Prof. Kuntaman, dr., MS., Sp.MK(K), and also Institute Research and Community Service that has provided research funding assistance.

Authors' Contributions: Kadek Rio Risnanda & Kuntaman designed the study. Kadek Rio Risnanda, Masfufatun, and Agusniar Furkani Listyawati carried out the laboratory work. Kadek Rio Risnanda analyzed the data and wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

Funding: Funding for this research is Institute of Research and Community Service

REFERENCES

- Aidara-Kane, A., Andremont, A., Collignon, P. (2013) Antimicrobial resistance in the food chain and the AGISAR initiative. *J Infect Pub Hea*. 6. 162-165.
- Biutifasari, V. (2018). Extended Spectrum Beta-Lactamase (ESBL). 1(1), 1–11.
- Direktorat Jenderal Peternakan dan Kesehatan Hewan. (2019). statistik peternakan dan kesehatan hewan. Jakarta: Direktorat Jenderal Peternakan dan Kesehatan Hewan Kementerian Pertanian RI.
- Gregova, Gabriela et al. 2012. "Antibiotic Resistance of Escherichia Coli Isolated from a Poultry Slaughterhouse." *Annals of Agricultural and Environmental Medicine* 19(1): 75–77
- Hasibuan, Malayu. (2017). Manajemen Sumber Daya Manusia. Jakarta: Bumi Aksara
- Kemenkes RI. 2013. Riset Kesehatan Dasar. Jakarta: Badan Penelitian dan Pengembangan Kesehatan Kementerian Kesehatan Republik Indonesia
- Kuntaman K, U Hadi, H Paraton, M Qibtiyah et al. 2013. Surveilans multi senter *Klebsiella pneumoniae* dan *Escherichia coli* penghasil ESBL di Indonesia.
- Masruroh, Cholilia Abadiatul et al. 2016. "Tingkat Kejadian Escherichia Coli Penghasil Extended Spectrum B -Lactamase Yang Diisolasi Dari Feses Broiler Di Kota Bogor." 34(1): 42–49.
- Mu'arofah, Binti, Radita Yuniar Arizandy, Budi Utomo, and Kuntaman Kuntaman. 2020. "Detection Of Extended Spectrum B-Lactamase (ESBL) Gene Patterns Of Enterobacteriaceae In Broiler Chicken Meat Sold In Traditional Markets In The East Surabaya." *STRADA Jurnal Ilmiah Kesehatan* 9(1): 12–19.
- Puspandari, N. et al. (2021) 'Memproduksi beta-laktamase spektrum luas *Escherichia coli* surveilans pada sektor manusia , rantai makanan , dan lingkungan : Tricycle project (pilot) di Indonesia', 13(September)
- Ramadhani, W.M., Rukmi, I. and Jannah, S.N. (2020) 'Kualitas mikrobiologi daging ayam broiler di pasar tradisional Banyumanik Semarang Microbiological quality of broiler chicken meat sold at Banyumanik traditional markets of Semarang', 3(1), pp. 8–16
- Ray, B. dan A. Bhunia. 2014. *Fundamental Food Microbiology*. Boca Raton: CRC Press.
- Region, T. (2019) 'Morfometrik Ayam Broiler dengan Pemeliharaan Intensif dan Akses Free Range di Daerah Tropis', 07(2), pp. 75–80
- Reich, Felix, Viktoria Atanassova, and Günter Klein. 2013. "Extended-followed by punctuation "&" the second author's last name Spectrum β -Lactamase- and Ampc-Producing Enterobacteria in Healthy Broiler Chickens, Germany." *Emerging Infectious Diseases* 19(8): 1253–59
- Wardhana, Dhandy Koesoemo et al. 2021. "Detection of Microbial Contamination in Chicken Meat from Local Markets in Surabaya , East Java , Indonesia." 14: 3138–43
- Wibisono F, Sumiarto B, Untari T. Short Communication: The Presence of Extended- Spectrum Beta-Lactamase (ESBL) Producing *Escherichia coli* on Layer Chicken Farms in Blitar Area, Indonesia. *Biodiversitas* 2020, 21(6)
- Yessy Resi Maharani, Nunung Yuniarti, Ika Puspitasari. 2021. Prevalensi Bakteri Extended-Spectrum Beta-Lactamase dan Evaluasi Kesesuaian Antibiotik Definitif pada Pasien Rawat Inap di RSUP Dr Soeradji Tirtonegoro Klaten. *Majalah Farmaseutik* Vol. 17 No. 2: 157-165