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

DHANDY KOESOEMO WARDHANA1,2, DEVI AYU SAFITRI1, SUWAIBATUL ANNISA2, NENNY HARIJANI3, AGNES THERESIA SOELIH ESTOEangepastie1, LAILATUL MAGHIROH2

1Veterinary Public Health Research Group, Division of Veterinary Public Health, Department of Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C, Mulyorejo, Surabaya 60115, East Java, Indonesia. Tel.: +62-31-5992785, 5993016, Fax.: +62-31-5993015. *email: dhandy.koesoemo.wardhana@fkh.unair.ac.id*

2Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C, Mulyorejo, Surabaya 60115, East Java, Indonesia


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), cefazidime (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%), cefazidime (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, cephalozin. Only few (13%) were resistant to cefazidime, 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, ceftiraxone, 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%), cephalzin (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 NaTrum 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, 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)

<table>
<thead>
<tr>
<th>Antibiotics disc</th>
<th>Inhibition zones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxime</td>
<td>CTX 30 μg</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>CAZ 30 μg</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>CRO 30 μg</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>ATM 30 μg</td>
</tr>
</tbody>
</table>

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 ceftazidime. 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 cephemycin (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

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>ESBL Production (n = 26)</th>
<th>Positive percentages (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxime</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>10</td>
<td>16</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Markets</th>
<th>E. coli (n=60)</th>
<th>Positive percentages (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>A</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>E</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>G</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>H</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>J</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>I</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>26</td>
</tr>
</tbody>
</table>

Mean of positive E. coli: 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 (Martinez-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.

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