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Detection of Extended-Spectrum-Beta-Lactamase (ESBL) Producing Escherichia Coli in Meat Chicken from Traditional Market in Surabaya, East Java, Indonesia

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Abstract

This study to detect Extended-Spectrum-Beta-Lactamase (ESBL) – producing *Escherichia coli* (*E. coli*) in chicken meats from traditional market in Surabaya. Total of 60 chicken meat samples from ten traditional markets, with each market contributing 6 samples. The ESBL-producing *E. coli* were tested for susceptibility using disk diffusion method as described by Bauer. The study affirmed the presence of ESBL producing *E. coli* in chicken meats from traditional market Surabaya. The highest resistance from *E. coli isolates* was recorded against ceftriaxone (87%) followed by cefotaxime (75%), aztreonam (70%) and ceftazidime (28,3%).The presence of ESBL producing *E. coli* in the chicken meats is alarming and requires adaptation of hygienic practices and controlling use of antibiotics.

Keywords: *Chicken meats, E. coli, ESBL, traditional market.*

Introduction

Poultry production is one of the most important parts of farm industry in Indonesia. One of the poultry production is chicken meat.In the poultry industry, enteric bacterial pathogens pose a threat to public health and can contribute to the transmission of zoonotic diseases[1][2]. One of the zoonotic diseases is that of the foodborne disease. Food-borne diseases, caused by agents that enter the body through the intake of contaminated food materials are one of the primary public health concerns[3].Food-borne disease remain a major public health problem across the globe. It affects the people's well-being, and imposes economic impacts[4]. ESBL-producing *E.coli* is included in a food-borne disease.

The high consumption of chicken meat requires great care to provide the safety of the industry against menacing factors[5]. Along with development of poultry farms and intensive culture, occurrence of the bacterial diseases and, consequently,overusing antibiotics have been increased in recent years[6].The inappropriate use of antibiotics, not only in human medicine but also in animal husbandry, has been considered a main driver leading to the increase of multidrug-resistant bacteria[7][8].Food-producing animals are known as an important reservoir of antimicrobial-resistant zoonotic bacteria[9].

Beta Lactamases are the most frequent source of resistance to beta lactam antibiotics, and the production of beta lactamaseis the primary mechanism of antibiotic resistance in *Enterobacteriaceae*. Various beta lactamases have been reported, including penicillinases, extended-spectrum beta-lactamases (ESBLs), cephalosporinases (AmpC), metallo-beta-lactamases (MBLs), and carbapenemases (KPCs)[10][11].

Among *Enterobacteriaceae, E. coli*is the species that causes the greatest number of infections and has become the main emergence of extended spectrum beta-lactamase (ESBL) producing bacteria[12]. *E.coli* is a common inhabitant of the vertebrate intestinal tract and a frequent microbial contaminant of retail meat products[13].

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The ESBL-producing *E.coli* in humans and animals has gained global notoriety during the past decade. This comes along with a fiery concern of foodanimals serving as potential reservoirs of ESBL genes [14].The purpose of this study to detect ESBL-producing *E.coli* in chicken meats from traditional market in Surabaya.

Materials and Method

Isolation and Identification of E. coli: A total of 60 chicken meat samples were obtained from 10 traditional markets in Surabaya. From each market, 6 carcasses were collected and from each carcass, 25 g chicken meat samples were homogenized in Buffered Peptone Water (BPW) media (Merck 1.07228.0500) for isolation of *E. coli*, then taking 1 ml suspension into 9 ml of Brilliant Green Bile Broth media (Merck 1.05454.0500) with Durham tube inside, and incubated at 45.5°C for 24-48 h. Gas-produced tube was positive and suspected to be *E. coli.* Taking 1 loop of suspected *E. coli* and inoculated on Eosin Methylene Blue Agar (EMBA) (Merck 1.01347.0500) and then incubated at 35°C for 24 h. All colonies with typical *E. coli* morphology were selected and confirmed by indole, methyl red, Voges–Proskauer, and citrate utilization biochemical tests.

ESBL-producing E. coli Susceptibility Test: The ESBL-producing *E. coli* were tested for susceptibility using disk diffusion method as described by Bauer. Making the bacterial suspension, one or two colonies of *E. coli* were cultured in 5 ml of sterilized NaCl (Natrium Chloride) at 37°C for 24 h and then homogenized using vortex until it was found to be similar to McFarland Standards. 0.2 ml bacterial suspension was inserted into a Petri dish containing Mueller-Hinton Agar (MHA) media (Merck1.05435.0500), then spread over the surface of agar using the sterile glass spreader carefully rotating the Petri dish at an angle of 45°C at the same time, and waited 15 min to absorb the bacterial suspension.

The ESBL-producing *E. coli* were tested against four beta-lactam antibiotics which included ceftazidime (30 µg), aztreonam (30 µg), cefotaxime (30 µg), and ceftriaxone (30 µg). The diameters of inhibition zones were measured and interpreted as per the guidelines of the Clinical and Laboratory Standards Institute [15]. The data were presented descriptively in the form of tables as percentages.

Findings: A total of 60 chicken meat samples were included in this study (mean weight 25 g). The results of *E. coli* isolation and identification from chicken meat samples revealed that 54 samples(90,03%) were positive. It is similar with Davis *et al*[16]where are chicken products (87.6%) were contaminated with *E. coli*in United States and similar with the findings of Patyal *et al*[17], who reported 68% prevalence rate of *E. coli* in Jaipur, Rajasthan, India. Sharma and Bist[18] also reported 70% prevalence rate *E. coli* in chicken meat in Mathura city of Uttar Pradesh, India.

Bacteria in food-producing animals are spread through the food chain[19].The high level of *E. coli* in meat might be caused by several factors including *E. coli* which is a normal flora in animal intestine so itis possible that the meat may come in contact with fecal contaminants [20][21].

Presence of pathogenic strains of *E. coli* in poultry meat is not only a potential threat of cross contamination but can also lead to become an infectious dose for handlers and consumers. *E. coli* presence in food materials are considered to be an indicator for the presence of other pathogenic bacteria in the respective food items[22].

The presumptive ESBL-producing *E. coli* isolates were tested for susceptibility against ceftriaxone (30 µg), cefotaxime (30 µg), aztreonam (30 µg)and ceftazidime (30 µg). According to our results from table 2, highest resistance from *E. coli* isolates from 60 chicken meat samples was recorded against ceftriaxone (87%) followed by cefotaxime (75%), aztreonam (70%) and ceftazidime (28,3%). These finding is agrees with Kwoji *et al*[23] where are similar higher resistance to ceftriaxone (96.9%) and aztreonam (98.5%) but contrast with ceftazidime (93.8%).For cefotaxime (75%) is contrast with Gundogan and Avci[24] where lower resistance to cefotaxime (33.3%) reported.

With regard to antibiotic resistance, methicillin-resistant staphylococci, in particular MRSA, and ESBL-producing *Enterobacteriaceae* are currently of special concern. In recent years, it has been widely recognized that the dissemination of MRSA and ESBL-producing bacteria is an issue no longer restricted to the medical/health care system [25–28].The emergence of ESBL producing *E. coli* in the food-producing animals and in foods of animal origin is a growing problem worldwide[29].

Wang *et al* found residues of antimicrobials in chicken meat and even detected some human antimicrobials, that are not usedas veterinary drugs. The spread of antimicrobial resistance genes in poultry may be associated with the prophylactic use of cephalosporins injected into eggs to control *E. coli* omphalitis in broiler chickens[30]. The production of extended-spectrum beta-lactamases (ESBLs) is the worldwide most important mechanism conferring resistance to 3rd generation cephalosporins in *E. coli*[31][32].

Doi *et al*. reported that 67% of retail meat samples in Seville, Spain, contained ESBL or ESBL-like resistance genes[33]. A survey of imported raw chicken in the United Kingdom reported ESBL genes in 10 of 27 samples[34]. In fact, Gregova showed that the occurrence of ESBLs in chicken meat could be related to the environmental microbes of the slaughterhouse,to the processes of scalding, defeathering, and evisceration, and to that the bacteria can be transferred from chickens because of the contact through water and incorrect cleaning and disinfecting[35].

Considering what is known about the epidemiology of *E. coli*, the abundance of ESBL genes in chicken meat is a likely explanation for current findings in humans. Although there are extensive campaigns promoting safe handling of chicken meat during processing, enteric pathogens are frequently transferred to humans and pose a continuous public health threat[36].The high antibiotic resistance among *E. coli* recorded in this present study might be due to uncontrolled administration of antibiotics to chickens in Indonesia farms.

Table 1. E. coli isolation from chicken meat obtained from traditional market in Surabaya

Market	E. coli		Positive Percentage (%)
	Positive	Negative	
A	6	0	100
B	6	0	100
C	5	1	83,3
D	3	3	50
E	4	2	67
F	6	0	100
G	6	0	100
H	6	0	100
I	6	0	100
J	6	0	100
Mean of E. coli Positive			90,03%

Table 2. Data of ESBL from chicken meat obtained from traditional market in Surabaya

Antibiotics	ESBL Production		Positive Percentage (%)
	Positive	Negative	
Ceftriaxone	53	7	87
Cefotaxime	45	15	75
Aztreonam	42	18	70
Ceftazidime	17	43	28,3

Conclusion

The study affirmed the presence of ESBL producing *E. coli* in chicken meats from traditional market in the study area.It can be concluded that highest resistance from *E. coli* isolates to third-generation cephalosporinswas recorded against ceftriaxone (87%), cefotaxime (75%), aztreonam (70%) and ceftazidime (28,3%) out of 60 samples.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Ethical Clearance: Ethical approval for animal research was not required as live animals were not used in this study. Meat samples were purchased from market.

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