Nova Biotechnol Chim (2022) 21(1): e935
DOI: 10.36547/nbc.e935

Nova Biotechnologica *et* Chimica

Prevalence and molecular characteristics of extended spectrum β-lactamase producing *Escherichia coli* isolated from retail chicken meat sold at the modern and traditional markets in Jakarta, Indonesia

Yulia Tanti Narwati[1, 2, ✉], Abu Tholib Aman[3], Lutfan Lazuardi[4], Tri Wibawa[3]

[1]*Doctoral Program, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Jl. Farmako Sekip Utara, Yogyakarta 55281, Indonesia*
[2]*Department Microbiology, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jl. Pluit Raya no. 2, Jakarta 14440, Indonesia*
[3]*Department Microbiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Jl. Farmako Sekip Utara, Yogyakarta 55281, Indonesia*
[4]*Department of Health Policy and Management, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Jl. Farmako Sekip Utara, Yogyakarta 55281, Indonesia*

✉ Corresponding author: *yulia.tnarwati@gmail.com*

Article info

Article history:
Received: 1st April 2021
Accepted: 8th November 2021

Keywords:
bla$_{CTX-M}$
bla$_{SHV}$
bla$_{TEM}$
ESBL
Escherichia coli

Abstract

The use of antibiotics in veterinary and human treatment can cause the development of antibiotic resistance in bacteria for β-lactam antibiotics. Worldwide, this resistance has become a growing concern in public health. Limited data are currently available regarding Extended Spectrum β-Lactamase (ESBL) *Escherichia coli* in Indonesia. The current study determined the prevalence and characteristics of ESBL genes of *E. coli* in retail chicken meat and humans in Indonesia. Two hundred eighty retail chicken meat were randomly collected from various modern and traditional markets in Jakarta (70 retail sourced chicken meat from modern markets and 210 from traditional markets). The prevalence of *E. coli* from the chicken meat sold at traditional markets was 97.14%, which was significantly higher than those of the modern markets with 78.57 % ($P < 0.05$). The prevalence of ESBL-producing *E. coli* isolated from chicken meat sold at traditional market was 40.47% and the modern market was 35.71 % and the prevalence of ESBL-producing *E. coli* isolated from chicken meat was 38.09 %, which is significantly higher than those of the clinical sample (with average 5.57 %). The most predominant gene is *bla*$_{TEM}$ in 54.54 % as a single gene or mixed with other genes followed by *bla*$_{CTX-M}$ in 44.31 % and *bla*$_{SHV}$ gene was only found in three isolates in 1.13 %. This study found that isolates from both the broiler chicken meat and clinical samples were having the same molecular characteristics. It is speculated that there is a relationship between them. However, this needs to be substantiated further.

Introduction

One of the health problems occurring all over the world is the bacterial antibiotic resistance phenomenon, which causes a serious decline in the quality of health service. According to a recent study, thousands of patients die each year from bacterial infections resistant to more than one type of antibiotic (Li and Webster 2018). Veterinary and human treatments of infections often use antibiotics

not only for control but also prevention of infectious diseases. The major cause of Gram-negative bacteria resistance to β-lactam antibiotics is the production of β-Lactamases (Eiamphungporn *et al*. 2018).

Gram-negative bacteria demonstrate increased resistance against newly developed β-lactam antibiotics, which are the most commonly used treatment for bacterial infections. These variant enzymes are known as extended-spectrum β-Lactamases (ESBLs) (Shaik *et al*. 2015). Bacteria can resist penicillin, cephalosporins (first. Second, and third generations) as well as aztreonam due to ESBL enzymes through hydrolysis of these antibiotics (Chishimba *et al*. 2015). In the past, infections with ESBL-producing bacteria tended to be exclusively hospital-associated, also known as nosocomial, but today such infections are commonly occurring among community-dwelling patients without a history of hospitalization or antimicrobial use (Eiamphungporn *et al*. 2018). *E. coli* is a commensal microbiota present in human and animal intestines and is also known as one of the most important pathogens (Conway and Cohen 2015). Some *E. coli* pathotypes cause intestinal and extraintestinal diseases such as diarrhea, urinary tract infections, septicemia, and neonatal meningitis (Safitri *et al*. 2017; Rojas-Lopez *et al*. 2018). Recent research concerning ESBL-producing *E. coli* in human subjects and animals has demonstrated a significant threat to public health. Those studies show that many foods can be sources of contamination from ESBL-producing *E. coli* and represent a growing global health problem (Le *et al*. 2015a, 2015b; Bhoomika *et al*. 2016; Hoang *et al*. 2017; Falgenhauer *et al*. 2019). Public health is directly affected by the presence of ESBL-producing bacteria in poultry-based food because *E. coli* can contribute to the spreading of resistance genes in animals and humans. Such bacteria are known to be able to exchange resistant genetic material among strains (da Costa *et al*. 2013). Resistant gene transfer generally can occur through three mechanisms: conjugation, transduction, and transformation. Horizontal gene transfer among bacterial species is the most common cause of antibiotic resistance. Bacteria are becoming more efficient in adapting to environmental changes using a variety of mechanisms, compared to random mutations. The most commonly emerging transfer routes for gene transfer between bacterial cells are through transformation and conjugation. The antibiotic-resistant gene transfer from commensal bacteria to pathogenic bacteria relies on the transfer mechanisms of availability, selective pressure, and the density of the donor, nutrients, and the recipient bacteria (Marshall *et al*. 2009).

Indonesia is currently undergoing significant development and has become the world's fourth most populous country. Unfortunately, limited data are currently available on the prevalence and molecular characteristics of ESBL-producing *E. coli* in Indonesia. In the present study, we investigated the prevalence and molecular characteristics of ESBL-producing *E. coli* isolated from chicken meat and clinical isolates from three hospitals in Jakarta, Indonesia.

Experimental

Identification and bacterial isolation

Between October 2018 and March 2019, a total of 280 chicken carcasses were randomly collected from various modern and traditional markets in Jakarta (70 retail-sourced chicken carcasses samples from modern markets and 210 from traditional markets). A total of 280 chicken meat samples were collected. Among these, 70 samples were collected from 70 modern markets sales points (supermarkets) while another 210 samples were collected from 53 traditional markets (at each traditional market, we collected 4 samples each from a different seller). Because in traditional markets in Indonesia there are many sellers of chicken meat in one market, so we chose 4 samples of chicken meat each from different sellers in one market. So, 52 × 4 = 208, and only 2 samples were taken from the last market. All samples were collected and placed into sterile sampling bags, then immediately taken to the laboratory in a thermobox container maintained at 4 °C. 25 g chicken meat samples were homogenized in Buffered Peptone Water (BPW) (Product code: 100908, Merck, Germany) from each sample then 0.1 mL sample aliquots were swabbed on

Brilliance™ *E. coli*/coliform Selective Agar (Product code: CM1046, Oxoid™, England) then incubated 24 h at 37 ºC. Colonies with a typical red color were collected from each plate. Identification of *E. coli* was done using MICROBACT™ Gram-negative Identification System 12A (Product code: MB1073, Oxoid™, England). Human clinical *E coli* isolates were obtained from a hospital clinical microbiology laboratory in three hospitals (Royal Taruma Hospital, Atmajaya Hospital, and Husada Hospital) in the Jakarta area. The clinical specimens comprised sputum, blood, pus, urine, and feces.

Antimicrobial susceptibility testing and phenotypic detection of ESBL-producing E. coli

The disc diffusion test by Kirby-Bauer's method was performed using Muller-Hinton agar plates (Product code: 103872, Merck, Germany) according to the Clinical and Laboratory Standards Institute Standards (CLSI) (Clinical Laboratory Standard Institute 2017). Antibiotics used for this study were: tobramycin (TOB), gentamicin (GEN), cefazolin (KZ), cefuroxime (CXM), aztreonam (ATM), amoxicillin (AML), levofloxacin (LEV), ciprofloxacin (CIP), trimethoprim-sulfamethoxazole (SXT), amoxicillin-clavulanate (AMC), cefoxitin (FOX), fosfomycin (FOS), meropenem (MEM), and amikacin (AMK) (Oxoid™, England). Suspected ESBL-producing isolates were tested for ESBL-production by double-disc synergy test (DDST), 5 antibiotics were used for DDST namely ceftriaxone (30 µg), ceftazidime (CAZ) (30 µg), and cefotaxime (CXM) (30 µg), amoxicillin-Clavulanic acid (20/10 µg), aztreonam (ATM) (30 µg). The amoxicillin-clavulanic acid disc was placed at the center and these discs were placed at a distance of 30 mm apart (center to center). The enhancement of the zone of inhibition of any one of the three discs toward the disc containing clavulanic acid at 37 °C after 24 h incubation was indicative of a potential ESBL-positive organism (Drieux *et al.* 2008).

Genetic characterization of ESBLs producing isolates

DNA extraction was performed using the boiling method as follows: isolated bacteria were cultured in Luria Bertani Broth (Product code: M1245, HiMedia®, India) overnight, then 2 mL of the culture were centrifuged for 10 min at 8,000 rpm. The pellet was used after the supernatant was discarded and mixed with 200 µL of sterile distilled water. Samples were then boiled in a dry bath for ten minutes and immediately chilled on ice for 5 min. The samples were then centrifuged at 6,000 rpm, and the supernatant which contained the nucleic acid material was pipetted off and stored for genetic testing (Kaur and Aggarwal 2013). Multiplex PCR analysis was performed for the simultaneous detection of $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} genes that are responsible for ESBL activity, using primers as described in Table 1 (Lim *et al.* 2009; Le *et al.* 2015b). Multiplex PCR Plus Kit (Product code: 206152, Qiagen®, USA) was used to detect ESBL genes, using primers as described in Table 1. PCR amplification products were visualized by gel electrophoresis in a 2 % agarose gel for 30 min.

Table 1. *Multiplex PCR* (Le *et al.* 2015b; Lim *et al.* 2009).

Primers	Sequences 5' 3'	PCR Conditions	Product [bp]
TEM-410F	GGTCGCCGCATACACTATTCTC	Initial denaturation at 95 ºC for 5 min, followed by 35 cycles of denaturation at 95 ºC for 30 s, annealing at 90 °C for 90 s, extension at 72 ºC for 90 s, and a final cycle of amplification at 68 °C for 10 min	372
TEM-781R	TTTATCCGCCTCCATCCAGTC		
SHVF-287F	CCAGCAGGATCTGGTGGACTAC		231
SHVR-517R	CCGGGAAGCGCCTCAT		
CTX-M F	ATGTGCAGYACCAGTAARGT		593
CTX-M R	TGGGTRAARTARGTSACCAGA		

Statistical analysis

The statistical analysis was performed using the Chi-Square test with the level of significance, $P = 0.05$.

Results

A total of 259 *E. coli* isolates were collected from 280 chicken meat sold in traditional and modern markets in Jakarta. The prevalence of *E. coli* from the chicken meats sold at traditional markets was 97.14 % (Table 2) which was significantly higher than those of the modern markets with 78.57 % ($P < 0.05$). Phenotypic detection showed that the average prevalence of ESBL-producing *E. coli* isolated from chicken meat was 35.71 % from the modern markets and 40.47 % from the traditional markets.

Table 2. Isolated bacteria *E. coli* and ESBL-producing *E. coli* from chicken meat and clinical samples.

	% (numbers)	
	E. coli	**ESBL-producing *E. coli***
Broiler chicken meat		
Traditional market	97.14 % (204/210)	40.47 % (85/210)
Modern market	78.57 % (55/70)	35.71 % (25/70)
Clinical isolates		
RSA	17.90 % (43/239)	3.35 % (8/239)
RSRT	13 % (39/300)	7.33 % (22/300)
RSH	17.4 % (101/579)	6.05 % (35/579)

RSA – Rumah Sakit Atmajaya/Atmajaya Hospital; RSRT – Rumah Sakit Royal Taruma/Royal Taruma Hospital; RSH – Rumah Sakit Husada/Husada Hospital.

Table 3. Antibiotic susceptibility profiles of ESBL-producing *E. coli* isolates.

	% (numbers)		
	Traditional (n = 85)	**Modern (n = 25)**	**Clinical (n = 65)**
AML	1.18 % (1)	8.00 % (2)	3.08 % (2)
AMC	65.88 % (56)	68.0 % (17)	58.46 % (38)
ATM	17.65 % (15)	44.00 % (11)	6.16 % (4)
CAZ	45.88 % (39)	28.00 % (28)	1.54 % (1)
CXM	4.71 % (4)	12.00 % (3)	1.54 % (1)
KZ	2.35 % (2)	8.00 % (2)	0 % (0)
FOX	88.24 % (75)	72.00 % (18)	95.38 % (62)
LEV	48.24 % (41)	48.00 % (12)	3.08 % (2)
CIP	3.53 % (3)	0 % (0)	0 % (0)
SXT	16.47 % (14)	40.00 % (10)	33.85 % (22)
AMK	100 % (85)	100 % (25)	100 % (65)
TOB	54.94 % (45)	48.00 % (12)	27.69 % (18)
GEN	24.71 % (21)	28.00 % (7)	50.77 % (33)
FOS	9412 % (80)	92.00 % (23)	96.92 % (63)
MEM	100 % (85)	100 % (25)	100 % (65)

AML – amoxicillin, AMC – amoxicillin-clavulanate, ATM – aztreonam, CAZ – ceftazidime, CXM – cefuroxime, KZ – cefazolin, FOX – cefoxitin, LEV – levofloxacin, CIP – ciprofloxacin, SXT – trimethoprim-sulfamethoxazole, AMK – amikacin, TOB – tobramycin, GEN – gentamicin, FOS – fosfomycin, MEM – meropenem.

The antibiotic resistance profiles of all 175 ESBL-producing *E. coli* isolates are shown in Table 3. Low percentages of susceptibility to AML (1.18 – 8 %), KZ (0 – 8 %), CIP (0 – 3.53 %) and high rate of susceptibility to FOS (92 – 96 %). Most of the clinical isolates showed a lower susceptibility percentage to almost all the tested antibiotics than those isolates from chicken meat, except for GEN, which was significantly higher than those isolates from the chicken meat ($P < 0.05$). Most notably, all the isolates exhibited susceptibility to MEM and AMK.

All of the 175 *E. coli* isolates (110 isolates from the chicken meat and 65 isolates from the clinical samples) which were phenotypically confirmed as ESBL were genetically characterized using Multiplex Polymerase Chain Reaction (PCR) (Fig. 1). The results showed that the most predominant gene was bla_{TEM} (54.54 %) as a single gene or mixed with other genes, followed by bla_{CTX-M} (44.31 %) while the bla_{SHV} gene which was only found in three isolates (1.13 %) (Table 4). In five of the isolates, the process of multiplex PCR did not detect any genes responsible for the ESBL phenotype. Since in this study only three genes were investigated, the results suggested that those five isolates harbored other genes responsible for ESBL production.

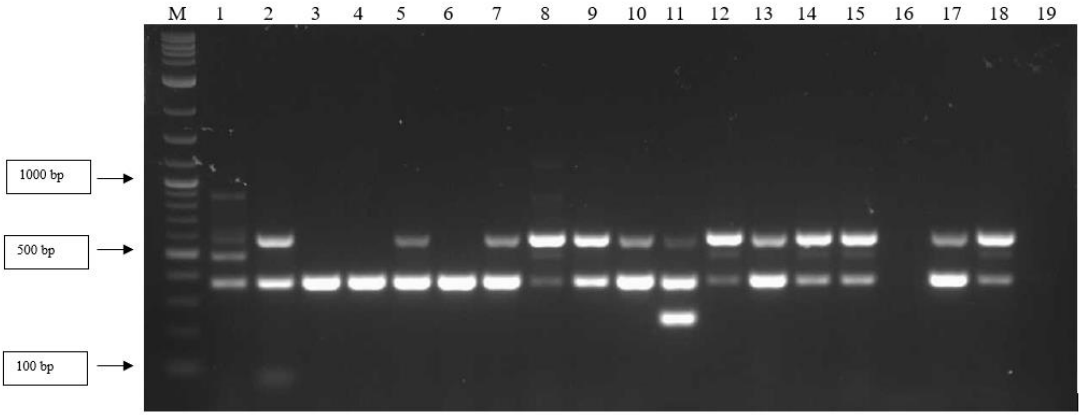

Fig. 1. The electrophoresis result of PCR products indicates genes bla_{CTX-M} with a band of 593 bp, bla_{TEM} 372 bp, and bla_{SHV} 231 bp.

Table 4. Distribution of ESBL types among *E. coli* isolates.

	Number [%]		
	Traditional	**Modern**	**Clinical**
CTX-M	13 (16 %)	3 (12 %)	8 (13 %)
TEM	25 (31%)	12 (48 %)	14 (22 %)
SHV	0 (0 %)	1 (4 %)	1 (2 %)
CTX-M/TEM	44 (54 %)	8 (32 %)	40 (63 %)
CTX-M/TEM/SHV	0 (0 %)	1 (4 %)	0 (0 %)

Discussion

The worldwide prevalence of ESBL-producing *E. coli* is rapidly increasing both in hospitals and in the communities. A possible connection between ESBL-producing bacteria in retail meat products and humans has been suggested (Leverstein-van Hall *et al*. 2011; Odwar *et al*. 2014). This study successfully isolated *E. coli* in almost all chicken meat bought from traditional markets (97.1 %) and in a significant proportion from modern markets (78.6 %). In another study, the level of *E. coli* contamination in chicken meat had a comparable result with 78 % (Odwar *et al*. 2014). Other result from traditional markets was also high as reported in Greece is 91 % (Dakic *et al*. 2014). In Indonesia, *E coli* found in chicken meat varied. *E. coli* was reported in 62 % of samples of chicken meat from traditional markets in Surabaya (Safitri *et al.* 2017), while 90.03 % of *E. coli* were found in chicken meat also from traditional markets in

Surabaya (Wardhana *et al*. 2020). *E. coli* was found in the clinical samples in this study in 13 – 17 % of samples, while 12 % of clinical samples from a hospital in Manado were reported positive for *E. coli* (Palit *et al*. 2018).

The significant difference in the results of E. *coli* percentage isolated from chicken meat bought from the traditional versus the modern markets can be caused by several conditions. Generally, modern markets tend to be more hygienic, and the broiler chicken meat sold in modern markets is usually wrapped in plastics. Meanwhile, in traditional markets, the chicken meat is left exposed to the air where it is easier to become contaminated through contact with the buyers or the sellers themselves. Personal hygiene of the sellers in the modern market tends to differ from traditional markets, where the modern market seller usually uses a uniform, gloves, and apron. Stall conditions in traditional markets are generally not well-maintained, where the sellers often use a big open table shared with other chicken meat sellers and sometimes with sellers of other products. The presence of chickens that are still alive, where the chicken meat is sold is common in traditional markets. Market sanitation in traditional markets tends to be poor due to the accumulation of garbage in many places, lack of adequate wash facility, and usage of the wooden cutting board. Frequently, the meat products are placed at room temperature due to not having a refrigerator to store the meat (Mukti *et al*. 2013). When measuring the sensitivity of the isolates, it was especially notable that all the isolates exhibited susceptibility to MEM and AMK. The same result was found in Nigeria, indicating that the sensitivity of ESBL-producing *E. coli* to AMK was 100 % (Alo *et al.* 2012). In the study by Uyanik *et al.* (2021), the authors reported that all ESBL-producing *E. coli* isolates were resistant to AMK and MEM, which is parallel with the outcome of the current study (Uyanik *et al.* 2021). It was further reported that the sensitivity of ESBL-producing *E. coli* isolates to MEM from urinary infection samples was 100 % (Soltani *et al.* 2014). In this study, nearly half (40.47 %) of the *E. coli* isolated from broiler chicken meat sold in traditional markets were positive for ESBL-producing *E. coli*. The research conducted in Belgia (Smet *et al.* 2008) had the same result (45 %). Also, more than 40 % of samples were found positive for ESBL-producing *E. coli* isolated from chicken meat (Le *et al.* 2015a). In this study, phenotypic detection of ESBL-producing *E. coli* from a clinical sample of the three selected hospitals varied between 3.35 – 7.33 %.

Genetic characteristics analysis showed that the most predominant gene was bla_{TEM} as a single gene or mixed with other genes and the bla_{SHV} gene was found in only three isolates. A study in Erbil, Iraq found that the bla_{TEM} gene was the most frequent in ESBL-producing *E. coli* isolated from thalassemia patients followed by the $bla_{\text{CTX-M}}$ gene (Pishtiwan and Khadija 2019). Other studies in India, Iraq, and Iran reported bla_{TEM} as predominantly found in isolates of ESBL-producing *E. coli* from patients with urinary tract infections (Yazdi *et al*. 2012; Jena *et al.* 2017; Michael and Saadi 2019). Research in Korea, had similar results to our study, wherein the bla_{TEM} gene was found as the most common ESBL gene in ESBL-producing *E. coli* isolated from chicken meat (Lim *et al*. 2015).

Chicken as one of the common food-producing animals has the potential as a reservoir for ESBL-producing bacteria. The ESBL-producing bacteria can be transmitted from animals to humans, potentially causing the spread of zoonotic diseases. ESBL-producing bacterial infections from consuming food-producing animals can lead to limited options in the treatment of patients. Such conditions can cause an increase in the occurrence of the diseases, increase the cost of treatment, and extend the treatment period and possibly cause death (Khosbayar *et al*. 2014). Research in the Netherlands suggested a relationship between contamination of chicken meat with drug-resistant bacteria and appearance of ESBL genes in humans, isolates from both the chicken meat and human samples showed that most *E. coli* harboring $bla_{\text{CTX-M-1}}$ or bla_{TEM52} and belong to clusters containing strains from both sources (Overdevest *et al.* 2011). For these reasons, the poultry industry has been considered a potential reservoir for transmission of ESBL-producing *E. coli* from poultry to humans are most likely through the food chain (Leverstein-van Hall *et al*. 2011). This study found that isolates from both the broiler chicken meat and clinical samples were having the same molecular

characteristics. Accordingly, the most frequent ESBL gene was bla_{TEM} followed by bla_{CTX-M} and bla_{SHV}. It is speculated that there is a relationship between them. However, this needs to be substantiated further. For instance, the genetic relations between bacteria are investigated by methods such as pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST). In our study, we investigated the presence of blaTEM, blaCTX-M, and blaSHV genes. However, the subgroups of these genes were not investigated, which is a limitation of this study. Detection of these genes alone may not indicate an association between hospital-acquired and chicken-derived isolates. Therefore, further investigation is needed to strengthen the preliminary findings of this study.

Conclusion

The result of this study affirmed the presence of ESBL-producing *E. coli* in chicken meats sold in traditional and modern markets in Jakarta. This study found that isolates from both the broiler chicken meat and clinical samples were having the same molecular characteristics, the most predominant ESBL gene was bla_{TEM} followed by bla_{CTX-M} and bla_{SHV}. It is speculated that there is a relationship between them. However, this needs to be substantiated further. These findings emphasize the need for continuous surveillance to detect ESBL-producing *E coli* from food and clinical samples, strict guidelines for the antibiotics used in veterinary and human medicines for treatment, control, and prevention of infectious diseases. Further research is needed to determine the relationship between contamination of chicken meat with drug-resistant bacteria and the appearance of ESBL genes in human isolates.

Acknowledgments

We sincerely thank The Head of Atma Jaya Hospital, Royal Taruma Hospital, Husada Hospital, and The Head of Clinical Laboratory, Atma Jaya Hospital, Royal Taruma Hospital, and Husada Hospital, Jakarta, Indonesia, and Dr. Enty Tjoa, Department of Microbiology, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia for the necessary experimental support for this study.

Conflict of Interest

The authors declare that there is no conflict of interest.

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