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Full Length Research Paper

CTX-M-1 and CTX-M-55 Producing *Escherichia coli* Isolated from Broiler Feces in Poultry Slaughterhouse, Bogor, West Java Province

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This study was aimed to determine the occurrence of CTX-M-1 and CTX-M-55 producing *Escherichia coli* isolated from broiler feces in Bogor, West Java, Indonesia. A total of 200 broiler fecal samples were collected from poultry slaughterhouses from March to April 2016. Presence of extended-spectrum beta-lactamase (ESBL) producing *E. coli* was detected by disc diffusion test based on the recommendation from Clinical and Laboratory Standards Institute. Bacterial strains which were confirmed as producing ESBLs were further analyzed for the presence of *bla* genes of the ESBL by polymerase chain reaction (PCR). The results showed that CTX-M producing *E. coli* isolates were detected in 12 samples from 200 samples (6.0%). The β-lactamase genes detected included CTX-M-1 (n = 6) and CTX-M-55 (n = 6). Most (95.8%) of the CTX-M producing *E. coli* isolates showed multidrug resistance phenotypes to at least three antibiotics. The highest incidence of antibiotics resistance was against penicillin G (100.0%), followed by gentamicin (79.2%), tetracycline (79.2%), streptomycin (70.8%), enrofloxacin (70.8%), ciprofloxacin (62.5%), trimethoprim-sulfamethoxazole (45.8%), and polymyxin B (16.7%). The occurance of CTX-M ESBL among fecal isolates could be a potential risk factor to public health.

Keywords: broiler feces, CTX-M, *Escherichia coli*, poultry slaughterhouse

INTRODUCTION

Antimicrobial resistance bacterias have been known as a serious problems in the treatment of infectious diseases in humans and animals. One of the most important mechanism of antibiotic resistant in Enterobacteriaceae is the production of extended spectrum β-lactamases (ESBL) which will confer resistances to β-lactam, cephalosporins and monobactams antibiotics (Minh et al., 2016). Among these enzymes, CTX-M ESBL have become the most dominant in the world. Of particular concern is the emergence and dissemination of CTX-M family ESBLs among *E. coli* within the community. The CTX-M family is consist of more than 80 heterogeneous ESBLs and can be divided into five different groups (CTX-M-1, M-2, M-8, M-9 and M-25) based on amino acid sequence similarities. Within each group, ESBLs share greater than 90% sequence identity (Sun et al., 2010). Food producing animals could be roled as a reservoir to dissaminate CTX-M-1 (von Salviati et al., 2015). *Escherichia coli* can be used as a reference

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species to track the dissemination of antimicrobial resistance (AMR) genes. Besides being a genetic versatile commensal of humans and animals, it is also widely disseminated in the environment (water, soil and food) and can constitute an important reservoir of antibiotic resistance determinants that can be readily transferred to and from other pathogenic bacteria (Meireles et al., 2015).

The spreading of CTX-M ESBL bacteria through fecal carriage is an important factor for among human communities and animals and will be influenced by factors such as previous treatment with antimicrobials. The dissemination of CTX-M producing *E. coli* may occur through fecal cross-contamination in food production units between groups of animals (or individuals), and the contamination of food derived from animals may occur during processing in the slaughterhouse (Horton et al., 2011). Consequently, without good hygienic practices, foods may act as a vehicle of transferring of ESBL producing bacteria to the gastrointestinal tract of the consumers (Gundogan and Avci, 2013). This study was aimed to determine the occurrence of CTX-M producing *E. coli* from broiler feces in poultry slaughterhouses, Bogor, Indonesia.

MATERIALS AND METHODS

Isolation and Identification of ESBL Producing *Escherichia coli*

Isolation and identification of ESBL producing *E. coli* was done by refering to Sudarwanto et al. (2016). A total of 200 fecal samples from poultry slaughterhouses, Bogor, Indonesia were collected from March to April 2016. Each fecal sample was collected directly from cloaca. Fecal samples were put in sterile plastic bags and transported to the laboratory using cooling box. Fecal samples were rinsed in 0.1% buffered peptone water (OXOID CM1049, England). Cloaca contents (10 g) were diluted in 90 mL of 0.1% buffered peptone water. Rinsates (10 mL) were enriched for 24 h at 37 °C supplemented with 20 µL cefotaxime (1 µg/mL). There after the enrichment was streaked onto MacConkay agar (MERCK 1.05465.0500, Germany) containing 1 mg/L cefotaxime and incubated at 37 °C for 24 h under aerobic condition. The colonies which were presumed as *E. coli* will appear as red colonies in the media and surrounded by turbid zone. Further works were continued by KOH test, Gram staining, oxidase test (OXOID MB0266A, England), sulfide, indole, and motility (SIM) test, and biochemical test [indole, methyl red, Voges-Proskauer, and citrate (IMViC)]. The colonies that were presumed as *E. coli* were selected and subcultured onto tryptic soy broth (MERCK 1.05458.0500, Germany) at 37 °C for 24 h. The identification of the *E. coli*-like colonies were then confirmed using API 32E (BIOMERIEUX, United States). Isolates were stored in tryptic soy broth containing 20% glycerol at -20 °C until further workup.

ESBL Confirmation and Antibiotic Susceptibility Testing

All cefotaxime-resistant and KOH-positive isolates were confirmed for ESBL production by the disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2014). The inhibition zones were determined for each isolate, using antibiotic disks, each containing 30 µg of cefotaxime, ceftazidime, or cefpodoxime, either alone or in combination with 10 µg of clavulanic acid (MAST Group, Germany). *E. coli* isolates which produced ESBL were subjected to susceptibility testing against 8 antimicrobial agents (penicillin G, streptomycin, gentamycin, ciprofloxacin, enrofloxacine, tetracycline, trimethoprim-sulfamethoxazole, and polymyxin B) with disk diffusion method according to CLSI protocols and evaluated with CLSI criteria. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as a control strain.

Characterization of β-Lactamase by Polymerase Chain Reaction (PCR)

Bacterial strains which were confirmed as producing ESBLs were further analyzed for the presence of *bla* genes of the ESBL subtypes TEM, SHV, and CTX-M (group 1, 2, 8, 9, or 25) by PCR using primers and conditions as previously reported (Sudarwanto et al., 2016). Bacterial DNA was isolated with the DNeasy blood and tissue kit (QIAGEN, Germany) according to the manufacturer's recommendations. *K. pneumoniae* ATCC 700603 was used as standard of ESBL-positive strains and *E. coli* ATCC 25922 was used as a non-ESBL producing organism or as a negative control. PCR products were determined by electrophoresis in a 2% agarose gel (BIOZYM, Germany). The molecular marker GeneRuler 100-bp DNA ladder (MBI FERMENTAS, Germany) was used.

Sequencing of *bla* Genes

The ESBL-encoding genes bla_{TEM}, bla_{SHV}, and bla_{CTX-M} of the ESBL-positive isolates were amplified with primers and PCR conditions as described previously in Sudarwanto et al. (2016). Resulting amplicons were purified using the PCR purification kit (QIAGEN, Germany). Sequencing was performed at SeqLab (Goettingen, Germany). Results were ev
aluated using the BLAST algorithm available at http://blast.ncbi.nlm.nih.gov/Blast.cgi.

Data Analysis

Data were descriptively analyzed to describe occurrence of CTX-M producing *E. coli* isolated from broiler feces in poultry slaughterhouses.

Tabel 1. Molecular characterization and antibiotic susceptibilities of ESBL producing *E. coli* isolates

No.	Sample code	*bla* gene product		Antibiotic resistance[a]								Total[b]		
				β-lactam	Amino-glycosides		Fluoroqui-nolones		Tetra-cycline	Folic acid inhibitor	Lipopep-tide			
		CTX-M	Other	P	S	CN	CIP	ENR	TE	STX	PB	R	I	S
1	8	CTX-M-55		R	R	R	R	R	R	R	S	7	0	1
2	9	CTX-M-1		R	I	R	S	I	R	S	S	3	2	3
3	18		TEM-1	R	R	R	R	R	R	S	S	6	2	0
4	30	CTX-M-1		R	S	R	S	I	R	S	S	3	1	4
5	41		TEM-1	R	S	R	S	I	R	I	S	3	2	3
6	56	CTX-M-55	TEM-1	R	R	R	R	R	R	R	S	7	0	1
7	59		TEM-1	R	R	S	R	R	S	R	S	5	0	3
8	74	CTX-M-1		R	R	R	R	R	R	R	S	7	0	1
9	85		TEM-1	R	R	R	R	R	R	R	S	7	0	1
10	106	CTX-M-55		R	R	S	R	R	S	R	R	6	0	2
11	108	CTX-M-55		R	R	S	I	R	S	R	R	5	1	2
12	109	CTX-M-55		R	R	S	I	R	S	R	R	5	1	2
13	111		TEM-1	R	R	R	R	R	R	R	S	7	0	1
14	117	CTX-M-1		R	I	R	I	R	R	R	R	6	2	0
15	126	CTX-M-55	TEM-1b	R	R	R	S	S	R	S	S	4	0	4
16	138		TEM-1	R	R	R	R	S	R	R	S	6	0	2
17	155		TEM-1	R	I	S	S	I	S	S	S	1	2	5
18	157	CTX-M-1		R	I	R	S	I	R	S	S	3	2	3
19	161		TEM	R	I	R	R	R	R	S	S	5	1	2
20	167	CTX-M-1		R	R	R	R	R	R	S	S	6	0	2
21	170		TEM-1	R	R	R	R	R	R	S	S	6	0	2
22	173		TEM-1	R	R	R	R	R	R	S	S	6	0	2
23	176		TEM-1	R	R	R	R	R	R	S	S	6	0	2
24	177		TEM-1	R	R	R	R	R	R	S	S	6	0	2

a P: penicillin G; S: streptomycin; CN: gentamicin; CIP: ciprofloxacin; ENR: enrofloxacin; TE: tetracycline; STX: trimethoprim-sulfamethoxazole; PB: polymyxin B
b R: resistance; I: Intermediate; S: sensitive

RESULTS

In this present study, 12 ESBL-producing *E. coli* isolated from broiler fecal samples produced CTX-M type ESBL. The bla_{CTX-M} types were identified as CTX-M-1 (n = 6) and CTX-M-55 (n = 6). PCR analysis followed by partial sequencing revealed the presence of bla_{TEM-1} (n = 12), both $bla_{CTX-M-1}$ and bla_{TEM-1} (n = 1), and both $bla_{CTX-M-55}$ and bla_{TEM-1} (n = 2). All of CTX-M-1 and -55 producing *E. coli* isolates showed multidrug resistance phenotypes against at least three antibiotics. The highest incidence of antibiotics resistance was to penicillin G (100.0%), followed by gentamicin (79.2%),

tetracycline (79.2%), streptomycin (70.8%), enrofloxacin (70.8%), ciprofloxacin (62.5%), trimethoprim-sulfamethoxazole (45.8%), and polymyxin B (16.7%). Detail results on characteristics and antibiotic susceptibilities of multidrug resistant of 12 ESBL producing *E. coli* isolates were described in Table 1.

DISCUSSION

CTX-M producing *E. coli* isolates were detected in 12 of the 200 (6.0%) broiler fecal samples. In this work, the β-lactamase genes were detected in these isolates were $bla_{\text{CTX-M-1}}$ (50.0%) and $bla_{\text{CTX-M-55}}$ (50.0%). Some of them harbored an additional $bla_{\text{TEM-1}}$ gene but none of them was positive for the bla_{SHV} gene. This is the first report of CTX-M producing *E. coli* in broiler feces, in Indonesia.

Our result was similar to previous study in United Kingdom showed that $bla_{\text{CTX-M-1}}$ genes were detected in 5 of the 32 (15.6%) broiler fecal samples and indicated that the predominant sequence type in United Kingdom broiler feces was CTX-M-1 (Horton et al., 2011). Another study conducted in Korea reported that *E. coli* from 9 (9.0%) of the 100 chicken fecal samples examined produced CTX-M type ESBL namely CTX-M-14 (n = 4), CTX-M-15 (n = 4), and CTX-M-1 (n = 1) (Tamang et al., 2014).

Interistingly, in this study reported that CTX-M-55 was also predominant sequence type among CTX-M producing *E. coli* isolated from broiler feces. CTX-M-55 was first identified in *E. coli* and *Klebsiella pneumoniae* isolates in Thailand in 2007 (Sun et al., 2010). In Asia, CTX-M-55 producing bacterias are commonly reported from human and animals (Hasman et al., 2015). CTX-M-55 is a variant of CTX-M-15 by one amino acid substitution of Ala-77-Val (Xia et al., 2014). Another study reported that CTX-M-55 has similar hydrolytic activity as CTX-M-15 and to exhibit increased catalytic efficiency against ceftazidime as well as cefotaxime (Lee et al., 2013). The mutation and subsequent epidemiological change of major ESBL genotypes circulated (e.g. CTX-M-3 to CTX-M-15 and CTX-M-55) may result from the injudicious used of antimicrobial agents among livestock and hospitals (Xia et al., 2014). The rapid proliferation and worldwide spread of CTX-M in *E. coli* is a matter of concern both in human and veterinary medicine. Furthermore, CTX-M producing *E. coli* has been reported that could be transfered these determinants to other commensal Enterobacteriaceae, such as *K. pneumoniae*, or to pathogens like *Shigella* or *Salmonella* spp. (Sudarwanto et al., 2016).

Multidrug resistance (MDR) to other classes of antibiotics was also described in this study. Multidrug resistance was defined as resistance to 3 or more different classes of antimicrobials (Blaak et al., 2015). This study showed that 95.8% (23/24) CTX-M producing *E. coli* displayed MDR to at least three antibiotics. The production of ESBLs is often plasmid mediated that frequently carry genes encoding resistance to other drug classes, such as fluoroquinolones, aminoglycosides, sulfa-derivatives and trimethoprim (Dierikx et al., 2012). Since these drugs are frequently used in broiler production, co-selection through usage of these drugs may have played a role in the selection for ESBL-producing isolates (Dierikx et al., 2010).

The presence of CTX-M producing *E. coli* in Bogor poultry slaughterhouse could be important factors for environmental contamination and spread to the food chain. Contamination of ESBL producing *E. coli* to meat and meat products can be easily during animal evisceration after slaughter through contact with contaminated water or during meat handling (Skockova et al., 2015). Contaminated surface water with feces-borne bacteria has long been a water quality issue owing to the potential for disease transmission (Ma et al., 2012). The human population may be exposed to antimicrobial resistance bacteria through contaminated surface water because it is an important source for drinking water production and is used for recreational activities and irrigation of crops. Moreover, also animals (livestock, wild life) may be exposed to these bacteria by drinking from or foraging in contaminated water (Blaak et al., 2015). In addition, very high air contamination with coliforms, including ESBL/AmpC-producing strains, was described in the air of poultry slaughterhouses, especially during shackling, killing, and evisceration (Laube et al., 2014)

ESBL producers is estimated that will increase in future in both animals and humans (Sudarwanto et al., 2016). More research is however needed to understand how persistence and dissemination can be minimized. Dissemination through plasmids adds a second dimension to this problem and most probably prolongs the ability for resistance phenotypes to persist (Brolund, 2014).

In conclusion, CTX-M-1 and CTX-M-55 producing *E. coli* are the most prevalent type of our CTX-M ESBL-positive isolates and 95.8% of CTX-M producing *E. coli* isolates showed multidrug resistance phenotypes to at least three antibiotics. The dissemination of these enzymes among fecal isolates of healthy food-producing animals can be a problem of food safety, environment, human, animals, and other pathogen bacteria.

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REFERENCES

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.

Brolund A (2014). Overview of ESBL-producing Enterobacteriaceae from a Nordic perspective. Infect. Ecol. Epidemiol. 4: 24555 - 24564.

Clinical and Laboratory Standards Institute (CLSI) (2014). Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement. CLSI document M100–S24. Wayne: Clinical and Laboratory Standards Institute. [Online] Available: http://ncipd.org/control/images/NCIPD_docs/CLSI_M100-S24.pdf. Accessed on March 7th, 2016.

Dierikx C, van der Goot J, Fabri T, van Essen-Zandbergen A, Smith H, Mevius D (2012). Extended-spectrum-β-lactamase- and AmpC-β-lactamase-producing *Escherichia coli* in Dutch broilers and broiler farmers. J. Antimicrob. Chemother. 68: 60–67.

Dierikx C, van Essen-Zandbergen A, Veldman K, Smith H, Mevius D (2010). Increased detection of extended spectrum beta-lactamase producing *Salmonella enterica* and *Escherichia coli* isolates from poultry. Vet. Microbiol. 145: 273–278.

Gundogan N, Avci E (2013). Prevalence and antibiotic resistance of extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella* species isolated from foods of animal origin in Turkey. Afr. J. Microbiol. Res. 7(31): 4059-4064.

Hasman H, Hammerum A, Hansen F, Hendriksen R, Olesen B, Agerso Y, Zankari E, Leekitcharoenphon P, Stegger M, Kaas R, Cavaco L, Hansen D, Aarestrup F, Skov R. (2015). Detection of mcr-1 encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark. Euro Surveill. 20(49):pii=30085.

Horton RA, Randall LP, Snary EL, Cockrem H, Lotz S, Wearing H, Duncan D, Rabie A, McLaren I, Watson E, La Ragione RM, Coldham NG (2011). Fecal carriage and shedding density of CTX-M extended-spectrum-lactamase-producing *Escherichia coli* in cattle, chickens, and pigs: implications for environmental contamination and food production. Appl. Environ. Microbiol. 77(11): 3715-3719.

Laube H, Friese A, von Salviati C, Guerra B, Rösler U (2014). Transmission of ESBL/AmpC-producing *Escherichia coli* from broiler chicken farms to surrounding areas. Vet. Microbiol. 172: 519–527.

Lee W, Chung HS, Lee H, Yum JH, Yong D, Jeong SH, Lee K, Chong Y (2013). CTX-M-55-type extended-spectrum β-lactamase-producing *Shigella sonnei* isolated from a Korean patient who had travelled to China. Ann. Lab. Med. 33(2): 141–144.

Ma J, Liu JH, Lv L, Zong Z, Sun Y, Zheng H, Chen Z, Zeng ZL (2012). Characterization of extended spectrum β-lactamase genes found among *Escherichia coli* isolates from duck and environmental samples obtained on a duck farm. Appl. Environ. Microbiol. 78(10): 3668-3673.

Meireles D, Martins L, Bessa LJ, Cunha S, Fernandes R, de Matos A, Manaia CM, da Costa PM (2015). Molecular characterization of quinolone resistance mechanisms and extended-spectrum β-lactamase production in *Escherichia coli* isolated from dogs. Comp. Immunol. Microb. 41: 43–48.

Minh DH, Minh SH, Honjoh K, Miyamoto T (2016). Isolation and bio-control of extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* contamination in raw chicken meat by using lytic bacteriophages. LWT-Food Sci. Technol. 71: 339-346.

Skockova A, Kolackova I, Bogdanovicova K, Karpískov R (2015). Characteristic and antimicrobial resistance in *Escherichia coli* from retail meats purchased in the Czech Republic. Food Control. 47: 401-406.

Sudarwanto MB, Lukman DW, Latif H, Herwin Pisestyani H, Sukmawinata E, Akineden Ö, Usleber E (2016). CTX-M producing *Escherichia coli* isolated from cattle feces in Bogor slaughterhouse, Indonesia. Asian Pac. J. Trop. Biomed. 6(7): 605–608.

Sun Y, Zeng Z, Chen S, Ma J, He L, Liu Y, Deng Y, Lei T, Zhao J, Liu JH (2010). High prevalence of bla_{CTX-M} extended-spectrum β-lactamase genes in *Escherichia coli* isolates from pets and emergence of CTX-M-64 in China. Clin. Microbiol. Infec. 16(9): 1475–1481.

Tamang MD, Gurung M, Kang MS, Nam HM, Moon DC, Jang GC, Jung SC, Park YH, Lim SK (2014). Characterization of plasmids encoding CTX-M β-lactamase and their addiction systems in *Escherichia coli* isolates from animals. Vet Microbiol. 174:456-462.

von Salviati C, Laube H, Guerra B, Roesler U, and Friese A. 2015. Emission of ESBL/AmpC-producing *Escherichia coli* from pig fattening farms to surrounding areas. Vet Microbiol. 175: 77–84.

Xia S, Fan X, Huang Z, Xia L, Xiao M, Chen R, Xu Y, Zhuo C (2014). Dominance of CTX-M-type extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolated from patients with community-onset and hospital-onset infection in China. PLoS ONE. 9(7): e100707.