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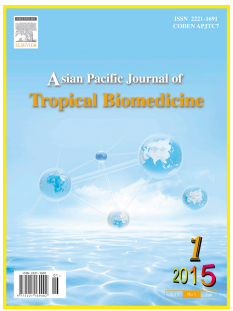

CTX-M producing Escherichia coli isolated from cattle feces in Bogor slaughterhouse, Indonesia

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Title: CTX-M producing *Escherichia coli* isolated from cattle feces in Bogor slaughterhouse, Indonesia

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ABSTRACT

Objective: To determine the occurrence of CTX-M producing *Escherichia coli* (*E. coli*) from cattle feces in Bogor slaughterhouse, Indonesia.

Methods: A total of 220 cattle feces samples were collected from Bogor slaughterhouse from March to April 2015. 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 (2014). Bacterial strains which were confirmed as producing ESBLs were further analyzed for the presence of *bla* genes of the ESBL by PCR.

Results: The results showed that CTX-M producing *E. coli* isolates were detected in 19 samples from 220 samples (8.6%). The β-lactamase genes detected were CTX-M-1 (n = 10) and CTX-M-9 (n = 9). All of the CTX-M producing *E. coli* isolates showed multidrug resistance phenotypes to at least four antibiotics. The highest incidence of antibiotics resistance was showed to ampicillin (100.0%), cefotaxime (100.0%), and cefpodoxime (100.0%), followed by streptomycin (84.3%), trimethoprim-sulfamethoxazole (73.7%), erythromycin (52.6%), kanamycin (26.3%), doxycycline (10.5%), and ceftazidime (0.0%).

Conclusions: Detection of CTX-M-producing *E. coli* in cattle feces raises important questions as they can represent a potential risk factor to public health.

1. Introduction

Escherichia coli (*E. coli*) belongs to the family of Enterobacteriaceae and is common in the gastrointestinal as normal microflora in human and animals[1,2]. These bacteria have capability to get and disseminate the resistant genes for antibiotics[3-5]. One of the currently most important resistance mechanisms is based on the plasmid-mediated production of extended spectrum β-lactamases (ESBL) that inactivate these compounds by hydrolyzing their β-lactam ring[6,7]. Until now, more than 600 ESBL variants are known. Among them, over 100 CTX-M enzymes so far reported may be grouped into five main subgroups[8]. The CTX-M types of β-lactamases are dominant family of ESBLs in *E. coli*, with particular subtypes associated with different geographic regions[2]. As a matter of growing concern, resistance caused by ESBLs is often associated with resistance to other classes of antibiotics such as fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole[8].

The dissemination of CTX-M *E. coli* in food production units may occur via fecal cross-contamination between groups of animals (or individuals), and the contamination of food derived from animals may occur during processing in the slaughterhouse[9]. Consequently, without good hygienic practices, foods may act as a vehicle of transferring of β-lactam-resistant bacteria to the gastrointestinal tract of the consumers[10]. This study was aimed to determine the occurrence of CTX-M producing *E. coli* from cattle feces in Bogor slaughterhouse, Indonesia.

2. Material and methods

2.1. Isolation and identification of ESBL producing E. coli

A total of 220 fecal samples from Bogor slaughterhouse, Indonesia were collected from March to April 2015. Each fecal sample was collected directly from rectum. 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). Rectum contents (25 g) were diluted in 225 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 that ware 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), and biochemical test [indole, methyl red, Voges-Proskauer, and citrate (IMViC)]. The colonies that were presumed as *E. coli* were selected and sub cultured 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 20E (Biomerieux). Isolates were stored in tryptic soy broth containing 20% glycerol at -20 ˚C until further workup.

2.2. ESBL confirmation and antibiotic susceptibility testing

All cefotaxime-resistant, and oxidase-negative, isolates were confirmed for ESBL production by the disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines[11]. The inhibition zones were determined for each isolate, using antibiotic disks, each containing 30 mg of cefotaxime, ceftazidime, or cefpodoxime, either alone or in combination with 10 mg of clavulanic acid (MAST Group Ltd., Reinfeld, Germany).

E. coli isolates which produced ESBL were subjected to susceptibility testing against 9 antimicrobial agents (ampicillin, cefotaxime, cefpodoxime, ceftazidime, streptomycin, trimethoprim-sulfamethoxazole, erythromycin, kanamycin, and doxycycline) with disk diffusion method according to CLSI protocols and evaluated with CLSI criteria[11]. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 (*K. pneumoniae*) were used as a control strain.

2.3. Characterization of β-lactamase by 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[12]. Bacterial DNA was isolated with the DNeasy blood and tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. Two strains, *K. pneumoniae* ATCC 700603 was used as standard ESBL-positive strains and a non-ESBL-producing organism (*E. coli* ATCC 25922) was used as negative control. PCR products were determined by electrophoresis in a 2% agarose gel (Biozym, Hessisch-Oldendorf, Germany). The molecular marker GeneRuler 100-bp DNA ladder (MBI Fermentas, St. Leon-Roth, Germany) was used.

2.4. Sequencing of bla genes

The ESBL-encoding genes bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ of the ESBL-positive isolates were amplified with primers and PCR conditions as described previously[12]. Resulting amplicons were purified using the PCR purification kit (Qiagen). Sequencing was performed at SeqLab (Goettingen, Germany). Results were evaluated using the BLAST algorithm available at http://blast.ncbi.nlm.nih.gov/Blast.cgi.

2.5. Data analysis

Data were descriptively analyzed to describe occurrence of CTX-M producing *E. coli* isolated from cattle feces in Bogor slaughterhouse.

3. Results

In this present study, CTX-M producing *E. coli* was detected in 19 samples from 220 samples (8.6%). The β-lactamase genes detected were CTX-M-1 (*n* = 10) and CTX-M-9 (*n* = 9). All of CTX-M producing *E. coli* isolates showed multidrug resistance phenotypes to at least four antibiotics.The highest incidence of antibiotics resistance was to ampicillin (100.0%), cefotaxime (100.0%), and cefpodoxime (100.0%), followed by streptomycin (84.3%), trimethoprim-sulfamethoxazole (73.7%), erythromycin (52.6%), kanamycin (26.3%), doxycycline (10.5%), and ceftazidime (0.0%). Detail results on antibiotic susceptibilities of multidrug resistant ESBL producing *E. coli* was described in Table 1.

4. Discussion

The results showed that the occrurance of 8.6% of CTX-M-producers in cattle feces was identified and $bla_{\text{CTX-M}}$ genes were detected. In this study the CTX-M-1 (52.6%) and CTX-M-9 (47.4%) groups were reported as the most prevalent in ESBL-producing *E. coli* isolates recovered from cattle feces samples. This is the first report of CTX-M producing *E. coli* in cattle feces, in Indonesia.

A similar study in Portugal showed that CTX-resistant *E. coli* isolates were detected in 5 of the 54 (9.3%) cattle feces samples and CTX-M-1 enzyme was the most dominant ESBL type found in *E. coli* cattle feces isolates[13]. Another study conducted in Germany sought that the presence of ESBL producing *E. coli* were detected in 17 of the 90 (18.9%) in cattle feces samples and showed a high prevalence of CTX-M-1, CTX-M-2 and CTX-M-9 ESBLs[14].

CTX-M β-lactamases are now the most prevalent type of ESBL in most areas of the world, where the significant increase in the incidence of ESBL in Enterobacteriaceae has been attributed to the dissemination of members of CTX-M-1 and CTX-M-9 families of CTX-M enzymes[15]. The over 100 CTX-M enzymes so far reported may be grouped into five main subgroups[8]. The rapid proliferation and worldwide spread of CTX-M-type ESBL in *E. coli* is a matter of concern both in human and veterinary medicine[15]. Furthermore, it has been reported that plasmids carrying CTX-M enzymes can transfer these determinants to other commensal Enterobacteriaceae, such as *K. pneumoniae*, or to pathogens like *Shigella* or *Salmonella* spp.[7].

As a matter of growing concern, resistance caused by CTX-M producer is often associated with resistance to

other classes of antibiotics like fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole[6,8]. This study showed that all of CTX-M producing *E. coli* exhibited multidrug resistance phenotypes to at least four antibiotics. It is important to emphasise that, often, some ESBL genes are located within mobile genetic elements, associated with other resistance genes, conferring resistance to antimicrobials that could be extensively used among animals and humans (*e.g.*, trimethoprim, sulfamethoxazole and streptomycin) and which could play an important role in the co-selection of these ESBL genes[4].

CTX-M producing *E. coli* that was found in Bogor slaughterhouse could release resistance genes to environment. Bacterial contamination of the surface water, particularly contamination with feces-borne bacteria, has long been a water quality issue owing to the potential for disease transmission[16]. Slaughterhouse biosecurity must be increased to prevent dissemination of ESBL producing *E. coli* to environment. Moreover, vectors such as mice and flies may also play a part in the spread of antimicrobial resistance in farms[17].

It is estimated that ESBL producers will increase in future, in both animals and humans[4]. Guidelines for alternative therapies, monitoring programs, and development of preventive medicine will decrease the spread of ESBLs in veterinary medicine. Together, these strategies could certainly reduce the impact of several multidrugs resistance microorganisms on animal health[18].

In conclusion, CTX-M β-lactamases (8.6%) are the most prevalent type of our ESBL-positive isolates and all of CTX-M producing *E. coli* isolates showed multidrug resistance phenotypes to at least four antibiotics. It could be threat for public health because CTX-M genes can be easily disseminate into the environment, food, human, animals, and other pathogen bacteria.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1]

Ryu SH, Lee JH, Park SH, Song MO, Park SH, Jung HW, et al. Antimicrobial resistance profiles among *Escherichia coli* strains isolated from commercial and cooked foods. *Int J Food Microbiol* 2012; 159: 263-6.

[2]

Wu G, Day MJ, Mafura MT, Nunez-Garcia J, Fenner JJ, Sharma M, et al. Comparative analysis of ESBL-positive *Escherichia coli* isolates from animals and humans from the UK, the Netherlands and Germany. *PLos One* 2013; 8(9): e75392.

[3]

Blanc V, Mesa R, Saco M, Lavilla S, Prats G, Miró E, et al. ESBL- and plasmidic class C β-lactamase-producing *E. coli* strains isolated from poultry, pig and rabbit farms. *Vet Microbiol* 2006; 118: 299-304.

[4]

Carattoli A. Animal reservoirs for extended spectrum β-lactamase producers. *Clin Microbiol Infect* 2008; 14(Suppl 1): 117-23.

[5]

Butaye P, van Duijkeren E, Prescott JF, Schwarz S. Antimicrobial resistance in bacteria from animals and the environment. *Vet Microbiol* 2014; 171: 269-72.

[6]

Zurfluh K, Hächler H, Nüesch-Inderbinen M, Stephan R. Characteristics of extended spectrum β-lactamase- and carbapenemase-producing Enterobacteriaceae isolates from rivers and lakes in Switzerland. *Appl Environ Microbiol* 2013; 79: 3021-6.

[7]

Njage PM, Buys EM. Pathogenic and commensal *Escherichia coli* from irrigation water show potential in transmission of extended spectrum and AmpC β-lactamases determinants to isolates from lettuce. *Microb Biotechnol* 2015; 8: 462-73.

[8]

Geser N, Stephan R, Korczak BM, Beutin L, Hächler H. Molecular identification of extended spectrum β-lactamase genes from Enterobacteriaceae isolated from healthy human carriers in Switzerland. *Antimicrob Agents Chemother*. 2012;56(3): 1609-1612.

[9]

Horton RA, Randall LP, Snary EL, Cockrem H, Lotz S, Wearing H, et al. Fecal carriage and shedding density of CTX-M extended-spectrum β-lactamase-producing *Escherichia coli* in cattle, chickens, and pigs: implications for environmental contamination and food production. *Appl Environ Microbiol* 2011; 77(11): 3715-9.

[10]

Gundogan N, Avci E. 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* 2013; 7(31): 4059-64.

[11]

Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement. CLSI document M100-S24. Wayne: Clinical and Laboratory Standards Institute; 2014. [Online] Available from: http://ncipd.org/control/images/NCIPD_docs/CLSI_M100-S24.pdf [Accessed on 3rd Febuary, 2016]

[12]

Sudarwanto M, Akineden Ö, Odenthal S, Gross M, Usleber E. Extended-spectrum β-lactamase (ESBL)-producing *Klebsiella*

pneumoniae in bulk tank milk from dairy farms in Indonesia. *Foodborne Pathog Dis* 2015; 12(7): 585-90.

[13]

Ramos S, Igrejas G, Silva N, Jones-Dias D, Capelo-Martinez JL, Caniça M, et al. First report of CTX-M producing *Escherichia coli*, including the new ST2526, isolated from beef cattle and sheep in Portugal. *Food Control* 2014; 31: 208-10.

[14]

Schmid A, Hörmansdorfer S, Messelhäusser U, Käsbohrer A, Sauter-Louis C, Mansfeld R. Prevalence of extended-spectrum β-lactamase producing *Escherichia coli* on Bavarian dairy and beef cattle farms. *Appl Environ Microbiol* 2013; 79(9): 3027-3.

[15]

Tamang MD, Gurung M, Kang MS, Nam HM, Moon DC, Jang GC, et al. Characterization of plasmids encoding CTX-M β-lactamase and their addiction systems in *Escherichia coli* isolates from animals. *Vet Microbiol* 2014; 174: 456-62.

[16]

Ma J, Liu JH, Lv L, Zong Z, Sun Y, Zheng H, et al. 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* 2012; 78(10): 3668-73.

[17]

von Salviati C, Laube H, Guerra B, Roesler U, Friese A. Emission of ESBL/AmpC-producing *Escherichia coli* from pig fattening farms to surrounding areas. *Vet Microbiol* 2015; 17: 77-84.

[18]

Nóbrega DB, Brocchi M. An overview of extended-spectrum beta-lactamases in veterinary medicine and their public health consequences. *J Infect Dev Ctries* 2014; 8(8): 954-60.

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

Sample code	*bla* genes	Antibiotic resistance									Total		
		CAZ	CPD	CTX	STX	AMP	DO	K	SPT	E	R	I	S
44	CTX-M-9	S	R	R	R	R	I	I	R	R	6	2	1
45	CTX-M-1	S	R	R	R	R	S	R	R	R	7	0	2
62	CTX-M-1	S	R	R	R	R	I	S	R	S	5	1	3
65	CTX-M-1	S	R	R	R	R	S	S	R	S	5	0	4
66	CTX-M-1	S	R	R	R	R	S	S	R	S	5	0	4
67	CTX-M-9	S	R	R	R	R	S	S	R	S	5	0	4

69	CTX-M-1	S	R	R	R	R	S	R	R	R	7	0	2
79	CTX-M-1	S	R	R	R	R	S	S	R	R	6	0	3
80	CTX-M-1	S	R	R	R	R	S	I	R	S	5	1	3
87	CTX-M-1	S	R	R	R	R	S	I	R	R	6	1	2
88	CTX-M-1	S	R	R	S	R	R	S	I	S	4	1	4
89	CTX-M-9	S	R	R	R	R	I	R	R	R	7	1	1
91	CTX-M-9	S	R	R	S	R	R	I	R	R	6	1	2
100	CTX-M-9	S	R	R	S	R	I	S	R	R	5	1	3
101	CTX-M-9	S	R	R	R	R	S	I	R	S	5	1	3
104	CTX-M-9	S	R	R	S	R	I	S	I	R	4	2	3
107	CTX-M-1	S	R	R	S	R	S	I	I	R	4	2	3
115	CTX-M-9	S	R	R	R	R	S	R	R	S	6	0	3
119	CTX-M-9	S	R	R	R	R	S	R	R	S	6	0	3

CAZ: Ceftazidime; CPD: Cefpodoxime; CTX: Cefotaxime; STX: Trimethoprim-sulfamethoxazole; AMP: Ampicillin; DO: Doxycycline; K: Kanamycin; SPT: Streptomycin; E: Erythromycin. R: Resistant; I: Intermediate; S: Susceptible.

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