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# 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.

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## 2. Materials 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.

**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.