Prevalence and Characterization of Shiga Toxin-Producing *Escherichia coli* Isolated from Slaughtered Qurban Animal in Jakarta Province

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ABSTRACT

This study was conducted to investigate the presence of shiga toxin producing *Escherichia coli* (STEC) and the possibility of carrying *rfbE* gene and H7 flagellar on meat, liver, and stool samples collected from Jakarta Province of Indonesia. A total of 51 samples collected from meat, liver, and stool of slaughtered cattle from qurban festival were tested using conventional culture and multiplex PCR methods. STEC non O157 were detected in meat (5.3%) and stool (8.3%) with one isolate from stool carried H7 flagellar. However, all isolates were lacking of *rfbE* gene. In antimicrobial suscepti-bility tests, the STEC isolates showed antibiotic resistance to erythromycin and oxacillin. Overall, the result shows that meat and liver of this origin activity represents a potential risk to human health.

Key words: STEC, multiplex PCR, meat, feces, antimicrobial

ABSTRAK

Penelitian ini bertujuan untuk menginvestigasi keberadaan *Escherichia coli* penghasil racun *shiga* (STEC) dan kemungkinan adanya gen *rfbE* dan flagella H7 pada strain yang ditemukan di dalam sampel daging, hati dan feses yang diisolasi dari provinsi Jakarta, Indonesia. Sejumlah 51 sampel dikoleksi dari daging, hati dan feses sapi yang dipotong pada hari raya kurban diuji menggunakan metode konvensional kultur dan multiplex PCR. Hasil studi menunjukkan STEC non-O157 terde-teksi di daging (5,3%) dan feses (8,3%) sapi dengan satu isolat terdeteksi membawa flagela H7. Akan tetapi, semua isolat tidak membawa gen *rfbE.* Sensitivitas bakteri terhadap berbagai antibiotik juga diuji dalam penelitian ini. Isolat STEC menunjukkan sifat resisten terhadap eritromisin dan oxacilin. Secara keseluruhan, hasil penelitian ini menunjukkan adanya potensi cemaran STEC pada daging dan hati hewan kurban dalam studi ini yang dapat mengancam kesehatan masyarakat.

Kata kunci: STEC, multiplex PCR, daging, feses, antimikroba

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) are major food-borne pathogens, which are well known of producing Shiga toxin type 1 (Stx1), type 2 (Stx2), or both, encoded by the genes of *stx1* and *stx2*, respec-tively (Sharma, 2006; Torres *et al*., 2006). The Stxs are AB5 toxins that inhibit protein synthesis in the host cell. This process may lead to an apoptotic cell death, especially to a damage of renal glomerular endothelial cells (Melton-Celsa *et al*., 2012; Xicohtencatl-cortes *et al*., 2007). Stx2 is the toxin type most related to hemolytic uremic syndrome (HUS). Hemolytic uremic syndrome is characterized by acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia and is a poten-tially fatal cause of acute renal failure in children (Rahal *et al*., 2012). Deaths have been reported more frequently with O157:H7-induced HUS but might also occur with non-O157 STEC-induced HUS (Frank *et al*., 2011; Lim *et al*., 2010). Until now, there is no treatment for HUS and use of antimicrobial agents is contraindicative (Nguyen & Sperandio, 2012).

Qurban festival is a religious festival in which animals are sacrificed, including cattle, goat, and sheep. In developing countries such as Indonesia, these activi-ties are often constrained by the limitations of facilities and skilled human resources. In addition, most of the slaughter of sacrificial animals to date is done by people outside the abattoir, like in the courtyard of the mosque or on private or government field offices, generally at the center of settlements. Although all activities are al-ready under the supervision of a veterinarian or health workers appointed by the competent authority, nothing is known about a pathogenic microbial contamination in

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the slaughter process. Therefore, at present no information is available about pathogenic bacteria, especially STEC contamination in Qurban festival. Cattle are the main reservoir of STEC, where the organism typically colonizes the lower gastrointestinal tract and shed the bacteria through their feces (Inat & Siriken, 2010; Perera *et al.*, 2015). Also, cattle has a significant proportion of beef become contaminated during slaughter (Kundu *et al.*, 2014). In Indonesia, satay is a popular undercooked meat and will become a problem when the meat is contaminated by STEC. It is suggested that meat may be an important transmission vehicle for this food-borne pathogen. Previous study (Selim *et al.*, 2013) also indicated that an increasing number of strains are showing a multiresistance to a number of antibiotics. To address this, the present study was designed to investigate the presence of STEC in the meat, liver and bovine stools sample in Qurban festival in Jakarta Province of Indonesia by considering the possibility of STEC O157:H7 presence. The above isolates were additionally studied for virulence characteristics and antimicrobial susceptibility.

MATERIALS AND METHODS

Bacterial Strains

This study used 2 *E. coli* O157:H7 strains as control. Both strains, producing Stx1 and Stx2 were obtained from American Type Culture Collection (ATCC) 35150 and Bogor Agricultural University (BAU), respectively.

Study Design for Purposive Sampling

Purposive sampling was chosen in this study. A total of 51 samples including 19 meat, 20 liver and 12 fecal samples were collected from five different mosques in Jakarta in October 2014. The geographical distribution of the five mosques is depicted in Figure 1. At each mosque, all cattles were selected. It was made to ensure that fecal, liver and meat samples were from exactly the same animals. The investigations on this study are minor influenced by the sampling method adopted (Wang *et al.*, 1996).

Sampling and Bacterial Culture Methods for Feces, Liver and Meat

The procedure of culture method was based on a previous method with minor modification (Selim *et al.*, 2013). As many as 250 g of meat, liver and fecal samples respectively, were taken into an aseptic plastic bag and placed in an ice box. Upon arrival at the laboratory, the samples were held at 4°C, not longer than 24 h before analysis. About 25 g of each sample was placed aseptically in a stomacher bag with 225 mL phosphate buffered saline (PBS) 0.1% (Oxoid, UK) and mixed for 1 min. Each sample (1 mL) was transferred into 50 mL of brain heart infusion (BHI) broth (Oxoid, UK) for enrichment and incubated at 37°C for 24 h. After the enrichment procedure, one loop of the broth was inoculated onto eosin methylene blue agar (EMB) (Oxoid, UK) for selec-

tive and differential procedures. After 24 h- incubation at 37°C, up to two purple coloured colonies with green metallic sheen were transferred onto cefixime-tellurite sorbitol MacConkey (CT-SMAC) (Oxoid, UK) agar and incubated at 37°C for 24 h. SMAC agar is recommended for the isolation of pathogenic STEC O157. STEC O157 does not ferment sorbitol and therefore produces colourless colonies. In contrast, most of non STEC O157 ferment sorbitol and form pink colonies. Those identified as STEC O157 in CT-SMAC were subjected for the presence of virulence genes by multiplex PCR.

Characterization of STEC

The *E. coli* strains isolated in this study and the STEC O157 reference were cultured on 7% sheep blood agar (Oxoid, UK) for cultivation. After overnight culture, colonies from each plate were inoculated into BHI, and the broth was incubated at 37°C for 24 h prior to nucleic acid extraction. The broth cultures were centrifuged at 4,500 g for 15 min in a Biofuge *pico* (Heraeus, Hanau, Germany), the supernatant was discarded, the pellet was washed three times with 10 mL of phosphate buffered saline (PBS; pH 7.4). The cell pellet was resuspended in 200 μL of PBS. Total purified genomic bacterial DNA was extracted with a genomic DNA mini kit (QiAgen, DNeasy®, USA) according to manufacturer's instruction. All the extracted DNA of the standard strains and of the sample isolates were examined using multiplex PCR for molecular typing of the virulence genes (*rfbE*, *stx1*, *stx2*, *fliC*) using spesific nucleotide primers are listed in Table 1 (Hu *et al.*, 1999). Amplification condition was carried out according to Inat and Siriken (2010) with a modification. The reaction mixture consisted of 4.8 μL of aquadest (DNAse, RNAse free), 10 μL of Maxima hot start green PCR master mix (2x) (Thermo Scientific™), and 0.4 μL of each primer.

Figure 1. Geographical distribution of mosques A-E in Jakarta from which the samples were collected. Total number of samples= 51.

Temperature conditions consisted of an initial 95°C denaturation step for 4 min followed by 35 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 1 min. The final cycle was followed by 1 cycle at 72°C for 5 min in the thermal cycling system. Agarose gel electrophoresis was carried out according to Sanam (2015) with minor modification to evaluate the amplified fragments using standard PCR markers.

Antibiotic Susceptibility

It was determined by the standardized agar diffusion test on MüllerHinton agar (MHA, Difco, USA) using the following disks (Oxoid, UK): erythromycin (15 μg), cefepime (30 μg), oxacillin (30 μg), azithromycin (15 μg) and tetracyclines (30 μg). Isolates were categorized as susceptible, intermediate and resistant based upon interpretative criteria developed by the guideline of Clinical and Laboratory Standards Institute (CLSI, 2014).

RESULTS

Characterization of STEC Isolates by Multiplex PCR for *stx1*, *stx2*, *rfbE* and Flagellar H7 Gene

As shown in Table 2, two isolates (isolate number 11 and 14) were confirmed as STEC because they all carried *stx* genes. The isolates (n= 30) were also applied to a multiplex PCR assay to confirm the presence of flagellar gene. In flagellar H7 PCR assay, 1 isolate (isolate number 11) was found harboring H7 genes (Table 2). However, all isolates are lacking the *rfbE* gene (Table 2).

Antibiotic Resistance

The antibiotic resistance profiles of the STEC isolates against 5 antimicrobial agents are presented in Table 3, respectively. The results indicated that *E. coli* and STEC isolated from qurban festival in Jakarta were resistant to erythromycin (100%) and oxacillin (100%) (Figure 2). Resistance to cefepime, azithromycin, and tetracycline was rare.

DISCUSSION

In this study, 2 of STEC were detected in meat and feces samples as shown in Table 2. Meat sample (5.3%) and feces sample (8.3%) showed *stx1* gene and *stx2* gene

in multiplex PCR (Figure 3), respectively. No STEC was detected in the liver samples. Ruminants are considered as an important source of STEC and cattle were identified as the primary reservoir of STEC (Akanbi *et al.*, 2011). Intestinal carriages of STEC in ruminants cause fecal shedding and the release of STEC into the environment (Forano *et al.*, 2013).

The non-O157 populations of *E. coli* have been shown to vary in their distribution among bovine and clinical sources (Borgatta *et al.*, 2012). In the study, all isolates were lacking *rfbE* gene, which is well known as characteristic of O157 strains. On the other hand, gene *flic*, a characteristic of H7 strain was detected. From 51 isolates, 1 (8.3%) isolate was confirmed the presence of *fliC* genes i.e isolates no. 11 in feces sample. The pathogenic potential of STEC isolates has also been shown to be associated with the presence of Stx genotypes (Rahal

Table 2. Results of multiplex PCR

Samples	*rfbE* gene n (%)	*fliC* gene n (%)	*stx1* gene n (%)	*stx2* gene n (%)
Meat (n=19)	0 (0)	0 (0)	1 (5.3)	0 (0)
Liver (n=20)	0 (0)	0 (0)	0 (0)	0 (0)
Feces (n=12)	0 (0)	1 (8.3)	0 (0)	1 (8.3)
Total	0 (0)	1 (8.3)	1 (5.3)	1 (8.3)

Table 3. Antibiotic resistance profiles of the STEC non O157

Isolate number	Antimicrobial agent	Diameter of killing zone (mm)	Interpretative criteria
11	Erythromycin	0	R
	Cefepime	23	S
	Oxacillin	0	R
	Azithromycin	23	S
	Tetracycline	24	S
14	Erythromycin	0	R
	Cefepime	24	S
	Oxacillin	0	R
	Azithromycin	21	S
	Tetracycline	18	S

Note: R (Resistant), S (Susceptible)

Table 1. Primer sequences used in the multiplex PCR assay and the expected sizes of the products

| Target gene	Size (bp)	Primer	Sequence
rfbE	292	RfbF	5′-GTGTCCATTTATACGGACATCCATG-3′
		RfbR	5′-CCTATAACGTCATGCCAATATTGCC-3′
fliC	625	FLICh7-F	5′-GCGCTGTCGAGTTCTATCGAGC-3′
		FLICh7-R	5′-CAACGGTGACTTATCGCCATTCC-3′
stx1	210	SLT-IF	5′-TGTAACTGGAAAGGTGGAGTATAC-3′
		SLT-IR	5′-GCTATTCTGAGTCAACGAAAAATAAC-3′
stx2	484	SLT-IIF	5′-GTTTTTCTTCGGTATCCTATTCCG-3′
		SLT-IIR	5′-GATGCATCTCTGGTCATTGTATTAC-3′
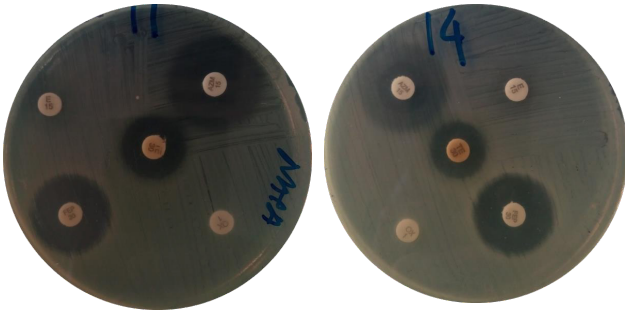
Figure 2. The killing zone performed by STEC non-O157 (left; isolate number 11) (right; isolate number 14)

et al., 2012). In this study, one STEC isolate produced Stx2 i.e isolate no. 11 in feces sample. Stx2 are known to be more toxic and are more often associated with HC or HUS in human infections than Stx1 (Etienne-Mesmin *et al.*, 2011; Lim *et al.*, 2010; Melton-Celsa *et al.*, 2012). One isolate obtained from meat of an animal investigated in the present study was positive for Stx1. In this study, STEC O⁻:H7 isolate carried Stx2 indicating the high pathogenic potential for human. Previous study (Koitabashi *et al.*; 2006) also suggested that Stx2 positive *E. coli* seem to be widely distributed in the Asian environment. This finding implies that humans were now exposed to STEC non-O157 from food of animal origin. Despite STEC non-O157 have lower virulence,

this strain may be capable of causing disease when a high inoculum is ingested or in particularly susceptible individuals.

According to Gould *et al.*, (2013); Luna-Gierke *et al.* (2014), the number of reported STEC non-O157 infections increased in the United States recently. In Indonesia, the case reports are not well established. However, nephrotic syndrome is one of the most frequent glomerular diseases seen in children. Approximately, there are six case of NS per year for every 100,000 child aged less than 14 years old in Indonesia (Mamesah *et al.*, 2016). Interestingly, the etiology is unknown.

In this study, the antibiotic resistance profiles of two STEC isolates against 5 antimicrobial agents are presented in Table 3. These findings showed the STEC isolates were 100% of resistance to erythromycin and oxacillin. Meanwhile, previous study found *E. coli* pathogen were resistant to streptomycin, chloramphenicol, sulfamethoxazole and gentamicin (Nugraha *et al.* 2013). Currently, the treatment of STEC infections with antibiotics is controversial (Pacheco & Sperandio 2012). However, many clinicians still used antimicrobial therapy as an option for the treatment of STEC infection. Thus, the use of oxacillin and erythromycin both in human and animals may select for resistance among STEC. The presest study provides initial data for the presence of STEC non-O157 in Qurban festival in Jakarta. These findings might help to lessen the risk of transmission of STEC non-O157 in public health.
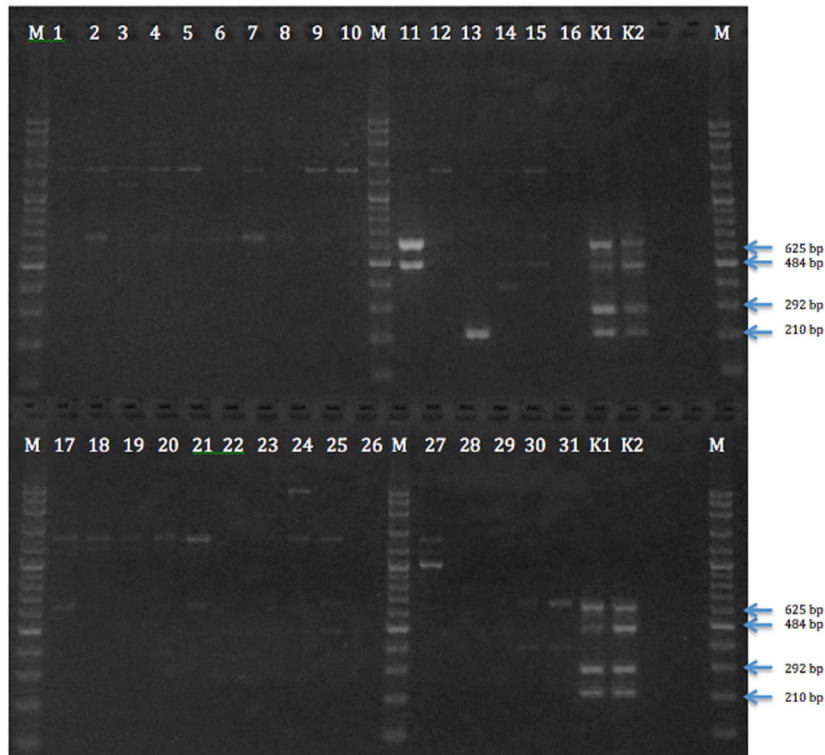

Figure 3. The determination of *rfbE* (O157), *stx1, stx2, fliC* (H7) genes of *E. coli* by multiplex PCR. Lane M: Marker (100~3,000 bp), Lane K1: positive control (*E. coli* O157:H7 35150), Lane K2: positive control (*E. coli* 1-AN), Lanes 1 to 31: samples isolated from qurban animal origin.

CONCLUSION

STEC non-O157 was detected in feces (8.3%) and meat (5.3%) both isolated from cattle slaughtered in Qurban festival. However, no STEC were found in the liver. In antimicrobial susceptibility tests, the STEC isolates showed antibiotic resistance to erythromycin and oxacillin. The result showed that meat and feces of this origin activity represents a potential risk to human health.

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