MOLECULAR DETECTION AND PHYLOGENETIC ANALYSIS OF A SHIGA TOXIN-PRODUCING STRAIN ESCHERICHIA COLI (PARTIAL RFB AND FLIC<sub>H7</sub> GENE), SEROTYPE O157:H7 ISOLATED FROM A LIVIING CHICKEN OF A TRADITIONAL MARKET IN INDONESIA

S. G. NINGRUM<sup>1</sup>, I. KHAERUNNISA<sup>2</sup>, SUPRIYONO<sup>3</sup> & I. W. T. WIBAWAN<sup>4</sup>

<sup>1</sup>Faculty of Veterinary Medicine, Universitas Wijaya Kusuma Surabaya, Surabaya, Indonesia; <sup>2</sup>Faculty of Agriculture, Bandung Raya University, Bandung, Indonesia; <sup>3</sup>Laboratory of Medical Entomology, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia; <sup>4</sup>Department of Animal Disease and Veterinary Public Health, IPB University, Bogor, Indonesia

Summary


The objective of this study was to identify a Shiga toxin-producing strain Escherichia coli partial rfbE and fliC<sub>H7</sub> gene of O157:H7 isolated from a faeces sample collected from a live chicken in a traditional market in Bogor, Indonesia. The isolate was investigated using multiplex polymerase chain reaction (PCR) to detect stx1, stx2, rfbE, and fliC<sub>H7</sub> gene of STEC O157:H7. Then, sequencing was applied to identify the antigen markers, which are the rfbE and fliC<sub>H7</sub> genes. In the present study, the isolate which was obtained from a live chicken was successfully identified as STEC O157:H7 strain. This conclusion was based on multiplex PCR and a nucleotide sequence analysis. This pathogen was not only found in the live chicken, but it was further suggested that the rfbE and fliC<sub>H7</sub> genes can be used as alternative targets for molecular identification of this pathogen.

Key words: fliC<sub>H7</sub>, live chicken, multiplex PCR, rfbE

Shiga toxin-producing Escherichia coli (STEC) O157:H7 is an emerging human zoonotic infection that evolves annually (Sheng et al., 2016). This strain induces non-bloody diarrhoea, haemorrhagic colitis, and haemolytic uremic syndrome (HUS) gastrointestinal manifestations (Perera et al., 2015). STEC O157:H7 infection cases are generally rare in developing countries. Most outbreaks caused by this infection have occurred in developed countries (Yara et al., 2020). This
strain was isolated from animal and human faeces in Indonesia in 2011 (Suardana et al., 2011). Meanwhile, an outbreak of STEC O157:H7 infection has never been reported in Indonesia, despite significant livestock rearing involvement (Ozuruonye, 2017). It seems highly possible that STEC O157:H7 infections are present in Indonesia. However, this case is too weak to be reported in Indonesia.

Several authors have identified cattle as the primary source of STEC O157:H7 transmission to the environment (Worley et al., 2017; Swaggerty et al., 2018) and 80% of cattle population share this antigen between animals (Matthews et al., 2006). In Argentina, chicken burger contamination at some restaurants has been reported and studied (Chinen et al., 2009). However, the researchers did not found STEC O157:H7 strains in their poultry samples to determine that chickens were also a reservoir or carrier of this strain. For this reason, the present study provides a detailed characterisation of a STEC strain isolated from a live chicken. On the other hand, O and H serogroups have been recognised as STEC strains causing human disease for years (De Rauw et al., 1999; Ningrum et al., 2016). The specific nucleotide primers are listed in Table 1. DNA was amplified by PCR in a thermocycler (Labcyler, Sensoquest, Germany). PCR with 0.5 U 2G Fast Taq polymerase (Kapa Biosystems) was performed in a total volume of 50 µL, 31.9 µL aquadest (DNAse, RNAse free), 10 µL 5× PCR buffer (Kapa Biosystems), 200 µM dNTP (Kapa Biosystems), and 10 pmol primers. A 5-µL DNA template was applied to the PCR solution, which was initially denatured at 95 °C for 4 min until 35 cycles of 95 °C for 30 s, 56 °C (rfbE gene)/59 °C (fliC_H7 genes) for 30 s and 72 °C for 1 min, and then a final step...
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Table 1. Primer sequences used in the present study

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Size (bp)</th>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>rfbE</td>
<td>292</td>
<td>RfbF</td>
<td>5’-GTGTCCATTATACGGACATCCATG-3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RfbR</td>
<td>5’-CCTATAACGTACATGCAATTGCG-3’</td>
</tr>
<tr>
<td>fliC</td>
<td>625</td>
<td>FLICH7-F</td>
<td>5’-GCGCTGTCAGCTATCGAGC-3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FLICH7-R</td>
<td>5’-CAACGCTGACTTATCGCCAATCC-3’</td>
</tr>
<tr>
<td>stx1</td>
<td>210</td>
<td>SLT-IF</td>
<td>5’-TGTAACGTGAAAAGGTGAGTATA-3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SLT-IR</td>
<td>5’-GCTATTCTGAGTCACGAAAATAA-3’</td>
</tr>
<tr>
<td>stx2</td>
<td>484</td>
<td>SLT-IIF</td>
<td>5’-GTGTTTCCTCCTCATCATCGC-3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SLT-IIR</td>
<td>5’-GATGCATCTCTGTTGATTAC-3’</td>
</tr>
</tbody>
</table>

Fig. 1. Detection of rfbE (O157), stx1, stx2, fliC (H7) genes of E. coli by multiplex PCR. Lane M: Marker (100–3,000 bp); lane A: positive control (E. coli O157: H7 35150); lane B: E. coli O157: H7 (present strain).

of 72 °C for 5 min for the last cycle. DNA sequencing was carried out using the BigDye™ Terminator v3.1 cycle sequencing kit (Applied Biosystem, USA) based on the manufacturer’s instructions. The sequencing primers to determine PCR fragments are listed in Table 1 (Hu et al., 1999). Sequence reactions were analysed on the 310 genetic analyzers (Applied Biosystem, USA).

Analysis of the aligned and the construction of phylogenetic trees were performed by software MEGA7 (Kumar et al., 2016) under maximum-likelihood al-
The best-fit model of nucleotide sequences was found by the model selection analysis jModelTest 2 (Darriba et al., 2012). The statistical significance of the resulting trees was evaluated by a bootstrap test with 100 replications (Efron et al., 1996).

The phenotypic studies showed the characteristic biochemical properties of this strain. They indicated that the investigated strain exhibited the typical characteristics of this serotype.

The genes rfbE, fliC\textsubscript{h7}, stx1 and stx2 in this study could also be identified by a multiplex PCR. The rfbE, fliC\textsubscript{h7}, stx1 and stx2 products could be detected as specific bands using UV illuminator (Fig. 1).

Sequencing the rfbE and fliC\textsubscript{h7} genes revealed a sequence identity of 100% and 99% to the sequences of type strain \textit{E. coli} O157:H7 ATCC 35150, respectively. Using the rfbE and fliC\textsubscript{h7} specific oligonucleotide primer, the isolate investigated in this study could be sequenced yielding 100% and 99% identity of the rfbE and fliC\textsubscript{h7} sequences among each other. Typical dendrogram of the rfbE and fliC\textsubscript{h7} of \textit{E. coli} and other references from NCBI GenBank is shown in Fig. 2 and 3.

This study successfully confirmed the presence of STEC O157:H7 in the faeces of a live chicken. So far, STEC O157:H7 had only been found on the surface of chicken meat due to cross-contamination. However, this discovery proved that chickens can be hosts and carriers of this species. The species isolated from the live chicken has also been characterised by sequencing.

![Fig. 2. Dendrogram analysis of fliC\textsubscript{h7} gene of E. coli O157:H7 (present strain), type strain E. coli O157:H7 ATCC 35150 and other E. coli O157:H7 strain obtained from NCBI GenBank; *Accession number.](image1)

![Fig. 3. Dendrogram analysis of RfbE gene of E. coli O157:H7 (present strain), type strain E. coli O157:H7 ATCC 35150 and other E. coli O157:H7 strain obtained from NCBI GenBank; *Accession number.](image2)
Generally, the 16S rDNA gene has been a choice for the identification of bacteria. However, in this analysis, the genes rfbE and fliC were used for molecular identification (Fig. 1–3). These genes have been widely used as specific primers and probes on the serotype of E. coli O157:H7. Earlier studies (Geissler et al., 2015; Basli et al., 2016) used rfbE and fliC genes to detect E. coli O157:H7 by multiplex PCR. At the same time, the pathogenicity of E. coli O157:H7 was attributed to the production of Shiga toxins (Stx1 and Stx2) (Hessain et al., 2015). In our study, the isolate also harboured the Shiga toxin 1 encoding gene stx1 and Shiga toxin 2 encoding gene stx2 (Fig. 1). Hence, the present strain isolated from chicken could be classified as STEC O157:H7. This was unexpected because STEC O157 strains are mostly absent in non-ruminants (Mainil & Daube, 2005) and have not been found in live chickens, naturally (Beutin et al., 1993). Although we successfully isolated STEC O157:H7 strains from a live chicken in samples collected from the traditional market, the results suggested that chickens can be reservoirs in the transmission of STEC O157:H7 to the environment or between animals.

This study showed a high possibility that the isolated E. coli O157:H7 could cause an outbreak in Indonesia. However, no outbreak of cases has been observed since this strain was discovered. Because this strain can cause illnesses in developed countries, this lack of outbreak is surprising. Selim et al. (2014) also observed a general lack of reports from developing countries, mainly in North Africa and the Middle East.

Unfortunately, no supporting data on the medical record of kidney failure patients in Indonesia are available. In a developed country, doctors will suggest taking faecal samples from a patient who has kidney problems before prescribing an antibiotic since this strain can worsen the kidney function if exposed to an antibiotic. In future studies, we recommend collection of STEC O157:H7 strains in samples from kidney failure patients in Indonesia and their testing in order to estimate a more accurate rate of STEC O157:H7 infection in humans.

REFERENCES


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Correspondence:

S. G. Ningrum
Faculty of Veterinary Medicine,
Universitas Wijaya Kusuma Surabaya,
Surabaya, 60225, Indonesia
e-mail: sitiningrum@uwks.ac.id

Paper received 31.03.2020; accepted for publication 09.06.2020