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Table of Contents

Shiga Like Toxin 1 (STX-1) Detection From Escherichia coli O157:H7 Local Isolates 1-3
I W. Suardana, K. J. P. Pinatih, and D. A. Widiasih

Optimization Of Isolation Method Of Carrageenan From Kappaphycus Alvarezii Doty Using Factorial Experimental Design 4-7
Ketut Widyani Astuti, Ni Putu Ayu Dewi Wijayanti, I Gusti Ngurah Agung Dewantara Putra, Ni Putu Linda Laksmiani

Rapid Detection Of Methicillin Resistant Staphylococci Using Multiplex PCR With Boiling Method For DNA Isolation 8-10
Ida Bagus Gede Adiguna Wibawa, Agus Eka Darwinata, Ni Nengah Dwi Fatmawati, Nyoman Sri Budayanti

Occurrence of Hypoglycemia, Hypokalemia and Hyperkalemia in Diabetic Hypertensive Patients Using Insulin and Diuretics (Research Conducted in Outpatient and Hospitalized Patient in Sanglah General Hospital Denpasar) 11-15
Sarasmita, M.A., Setyawan, E.I, Hendra Jaya, A.

The Determinants of Ethical Principle Implementation in Nursing 16-22
Ni Putu Emy Darma Yanti, Hanny Handiyani, Kuntarti

In Vitro Evaluation Of Antioxidant Activity Of Flavonoid Compounds From Terong Belanda (Solanum Betaceum, cav.) 23-26
Ida Ayu Raka Astiti Asih, Ni Made Puspawati and Wiwik Susanah Rita

Perception Of Contraception Access And Utilization In Teenagers In Senior High School No 8 Denpasar, Bali 27-30
R Listyowati, NMS Nopiyani, PA Indrayathi

DNA Probe Design for Detection Mutation at Codon 315 In katG Gene of Mycobacterium Tuberculosis to Real-Time Polymerase Chain Reaction 31-41
I Gusti A. A. Santhi Rahmaryani, Ni Kadek Ariani, Dyah Subadrika Warma Dewi, Ni Komang Sasi Ani, Ade Ari Sundari, Kadek Widya Yuli Hartati, and Sagung Chandra Yowani
Shiga Like Toxin 1 (STX-1) Detection From
Escherichia coli O157:H7 Local Isolates

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Abstract
Shiga-like toxin (Stx) otherwise known as verotoxin and verocyctotoxin is a toxin produced by some strains of Escherichia coli particularly by strain O157: H7. This toxin is an Aβ5 toxin type which is known to have similarities with the toxins produced by Shigella dysenteriae. Stx from E. coli O157: H7 can be distinguished into stx1 and stx2. Stx1 is usually associated with most outbreaks and detrimental sporadic cases of illness in humans. In this research, we observed the titer of Shiga toxin (Stx1 / VT1) from local isolates isolated from cattle and human feces by using vero toxin Escherichia coli-reverse passive latex agglutination test (VTEC-RPLA) method. The results showed local isolates KL-48 (2) of human origin, SM-25 (1) of cattle feces origin and DS 21 (4) of beef origin positively produced VT1 2 units of titer, meanwhile the isolate SM 7 (1) was negative. Titer of toxin Stx1 produced from local isolates was known to be lower when compared to the control isolate ATCC 43894 with 8 units of titer.

Key words: E.coli O157:H7, VT1, local isolates, zoonoses.

I. INTRODUCTION
Infection through food due to Shiga toxin from Escherichia coli (STEC) was reported as the most prominent cause of several outbreak cases [1,2]. One characteristic of STEC is the existence of a kind of cytotoxin known as Verotoxin or Shiga like toxin (Stx) [3]. Escherichia coli O157:H7 is one of the main serotypes behind hemolytic uremic syndrome (HUS) cases, aside of other contributing serotypes like O26:H11, O103:H2 and O111:H- serotypes that range from as much as 20% until 25% [4]. Cattle are essential reservoir of Shiga toxin Escherichia coli (STEC) that includes E.coli O157:H7 serotype as the producer of STEC bacteria. Surveys showed that around 1-5% of the number of cattle would release E.coli O157:H7 in their feces with a contamination level of < 10^2 cfu/g until 10^5 cfu/g [5].

Generally, E.coli is considered as normal flora inside animal’s digestive system (cattle) that can contaminate both the meat and the surrounding environment of the slaughterhouse during the butchery process. Cattle meat that had initially been contaminated along with improper cooking process became the source of infections for a number of food poisoning cases, including the ones caused by STEC [3]. Although there are other mediums for transmitting STEC outbreak cases into humans, cattle feces are still regarded as the most common source of contamination [3]. Research development in order to detect STEC rapidly has initiated since 1987 [6]. Mohammad et al., (1985 in Samadpour et al., 2002) with his direct counting technique towards produced toxin for instance, managed to detect 28 out of 172 samples (16%) of cattle feces as STEC positive. Detection by using probe Stx-I and II DNA by Samadpour et al in the year of 1990, was also successful in detecting 9 out of 28 (32%) of calves in feces as STEC positive [7].

After considering the fact of how there is no information about Shiga toxin produced by local isolate E.coli O157:H7, particularly Shiga like toxin 1 (Stx-1), research about how to detect Stx-1 from E.coli O157:H7 toxin especially the one from local isolates isolated from cattle feces and meat is deemed as necessary.

II. METHODS
A. Isolate Preparation
As many as 5 E.coli O157:H7 isolates that include KL-48(2), SM-25(1), SM-7(1) and DS-21(4) along with control
isolate ATCC 43894, part of the researcher’s collection, were taken from glycerol stock to be replanted in Brain Heart Infusion (BHI) gelatine medium. Grown isolates were subsequently analysed.

B. E. coli O157 Serotype

Grown isolates in BHI medium were later planted at selective sorbitol MacConkey gelatine media (SMAC) (Oxoid CM 0813). The research also used E. coli O157:H7 ATCC 43894 as positive control. After incubated at 37°C for 24 hours, colonies of E. coli were identified as E. coli O157. Their characteristics were clear colonies, colourless, or negative sorbitol [8].

C. Agglutination Test with E. coli O157 Latex Agglutination Test

In order to confirm that the positive colonies from SMAC media were E. coli O157, they were then tested again along with the positive control isolates by using E. coli O157 latex agglutination test (Oxoid DR620 M), with the following methods: as much as 2-3 µl of E. coli positive isolates from isolate stocks and presumptive E. coli O157 from SMAC media were put into 1 ml of physiological NaCl and heated at 100°C for 1-2 hours. After the heating, as much as 1 drop of isolates was reacted with 1 drop of reacted latex. Result of the positive test was indicated by the occurrence of precipitation, according to the available positive controls [8].

D. Shiga Toxin-1 Test with VTEC-RPLA

Shiga toxin Stx-1 test was carried out by mixing 0.5 ml of solvent into each prepared vial kit. Next, a plate consisted of 3 columns with each column consisted of 8 pits. In each pit, 25 µl of solvent was added. Starting from pit 1, 25 µl of tested sample was added and then diluted in series until pit 7. Meanwhile, pit 8 contained only solvent. Next step, 25 µl of VT 1 test latex was added into every pit column 1. The mixture on the plate was then mixed by shaking it. Then, the plate was sealed and incubated in room temperature for 24 hours. Positive result was indicated by with the occurrence of sediment/precipitation at the bottom of the plate.

E. Data Analysis

Data obtained from the research would be presented descriptively in the form of Tables [9].

III. RESULTS AND DISCUSSION

A. Isolation Results and E. coli O157:H7 Isolate Identification

Testing result of 5 E. coli O157 isolates on sorbitol MacConkey gelatine media (SMAC), showed the shape of colourless colonies and this indicated that the grown colonies did not ferment the sorbitol. Further test with Latex Agglutination Test as confirmation showed positive agglutination reaction towards O157 anti serum test. Based on the identification result, the whole isolates were then considered as E. coli O157:H7 and were then deemed as decent to be used in further tests.

B. Shiga Like Toxin-1 Production (VT-1) with VTEC-RPLA Agglutination Test

Test result of reverse passive latex agglutination test (VTEC-RPLA) towards VT-1 from E. coli O157:H7 local isolates was shown on Table I.

<table>
<thead>
<tr>
<th>Samples Codes</th>
<th>Isolate Origins</th>
<th>VT-1 Titer VT-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 43894</td>
<td>Control</td>
<td>+++ 8</td>
</tr>
<tr>
<td>KL-48(2)</td>
<td>Human</td>
<td>+ 2</td>
</tr>
<tr>
<td>SM-25(1)</td>
<td>Cattle</td>
<td>+ 2</td>
</tr>
<tr>
<td>SM-7(1)</td>
<td>Cattle</td>
<td>-</td>
</tr>
<tr>
<td>DS-21(4)</td>
<td>Meat</td>
<td>+ 2</td>
</tr>
</tbody>
</table>

Notes:
- : Agglutination didn’t take place
+ : 25% agglutination from isolate volume
++ : 50% agglutination from isolate volume
+++ : 75% agglutination from isolate volume

Table 1 presented how the identification towards 4 E. coli O157:H7 local isolates showed that only 3 local isolates were positively identified to produce Shiga like toxin-1 (VT-1). The existence of E. coli O157:H7 that did not produce Stx was also reported by Avery et al., (2002). Out of 24 tested isolates, 19 isolates showed positive results towards Stx2, 2 positive isolates towards Stx 1 and 2, as well as 3 negative isolates to both Stx 1 and Stx 2 [10]. Foley et al., (2004) also discovered that not all E. coli O157:H7 isolates could produce both types of verocytotoxin. There were chances that 1 isolate would produce both (Stx 1 and Stx 2). However, there would be a number of strains that produced only 1 type of toxin which would be either Stx 1 or Stx 2 [2].

LeJeune et al., (2004) mentioned that main virulent factor of E. coli O157:H7 was the existence of prophage that coded Shiga like toxin. Besides that, it was further explained that the bigger the production of Shiga like toxin for the bacteria, the more severe the illness would be when infected into humans [11]. The same conclusion was also mentioned in Fey et al., (2000) that stated how Shiga like toxin 1 and 2 were main virulent factors of E. coli O157:H7 and were directly correlated to the cases of hemorrhagic colitis and hemolytic uremic syndrome (HUS). This was particularly due to their interactions with endothelial cells in infected areas, including the glomerulus, artery, and kidney. Based on the research result along with the existing theoretical perspectives, it could be concluded that the 3 local isolates were most likely pathogen and would be interesting enough to be further researched [12].
IV. CONCLUSION

Based on the detection result of Shiga like toxin-1 (Stx-1) with VTEC-RPLA method, it was shown that 3 out of 4 tested isolates which were KL-48(2), SM 25(1), dan DS-21(4) were proven to produce Stx-1 toxin with 2 unit titer individually. As a result, they could potentially be further researched.

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