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Fimbriae-C Salmonella typhi Primers as Clinical Detection of Typhoid Disease by PCR Methods

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Abstract. Salmonella typhi is bacteria causes typhoid disease in human. The mortality rate of typhoid disease in Indonesia is increasing. The most common detection method currently used is serological test. However, this method is often resulted in less accurate, less sensitive and specific detection. The previous research has successfully discovered fim-C-S. typhi gene which is able to code protein to contribute in adherent or colonization in human epithelial cell. The information of fim-C-S. typhi gene is used to develop more specific and more accurate detection method for S. typhi bacteria by amplification, characterization, specificity and sensitivity assay, and continue with clinical assay. The PCR result was the amplified 0.2 kilo base (kb) DNA fragment using the fimbriae-C-S. typhi primer pairs. The specificity assay was conducted by comparing the amplicon in size of 0.2 kb S. typhi with Gram negative bacteria genome with similar characteristics. The results indicated the fimbriae-C-S. typhi primers can differentiate amplicon in size of 0.2 kb S. typhi against Enterobacter aerogenes, Klebsiella pneumonia, Vibrio cholera, Escherichia coli, and Pseudomonas aeruginosa. However, they cannot differentiate S. typhimurium which have higher homology in their genome. Sensitivity assay was conducted by determining the detection level of fim-C-S. typhi primers to S. typhi genome. The sensitivity assay showed the detected fimbrae-C primers S. typhi with 1.295 x 10^-9 µg/mL DNA chromosome. The clinical assay was evaluated by comparing the PCR product of suspected patient blood, the isolated bacteria S. typhi genome served as positive control, and uninfected human blood as negative control. The PCR product from 10 suspected patient bloods showed the band size 0.2 kb equals to the size of band produced by the PCR product from isolated S. typhi bacteria genome. Meanwhile, the band was not detected in the uninfected blood. Band size 0.2 kb from the suspected patient blood sample indicated positive infection of S. typhi bacteria. The above results demonstrated the developed clinical detection for typhoid disease using fimbriae-C-S. typhi primer pairs.

INTRODUCTION

Salmonella typhi (S. typhi) is bacteria causes typhoid diseases in human. A number of 358-810 person/100.000 Indonesian people were infected by typhoid diseases in 2007. Increasing cases was observed in poor sanitation areas. The disease is not only infected adults, but babies, children and teenagers as well. Therefore, an alternative method is required to handle and prevent the typhoid disease [1, 2]. Clinical diagnosis of typhoid disease was often inaccurate since specific clinical manifestation was not available. Common detection methods used nowadays is serology methods. However, the results showed different sensitivity and specificity against S. typhi antigen. Based on that reason, development of rapid, economic and easy diagnostic methods with high specificity and sensitivity for typhoid diseases is required [3]. Genotypic diagnosis using Polymerase Chain Reaction (PCR) method as an
alternative for typhoid diseases diagnosis has been developed through research in many laboratories. This method is considered to be more economical, specific, sensitive, and faster than the conventional method [4-6].

Previous research discovered that the fimbriae-C S. typhi primers would give an amplification product of 0.2 kilo base (kb) [7-9]. As a molecular detection, the primers have also been tested for its specificity and sensitivity. The specificity result indicated that the primers can differentiate amplicon in size of 0.2 kb S. typhi from Enterobacter aerogenes, Klebsiella pneumonia, Vibrio cholera, Escherichia coli, and Pseudomonas aeruginosa. Nevertheless, they cannot differentiate S. typhimurium which have higher homology in their genome (98%) [10-18]. However, the fim-C S. typhi primers is still very potential to detect S. typhi in human with the PCR method. These conditions occur because the host of S. typhimurium is animal, so if the sample was taken from human, the possible positive result detection was only S. typhi. The sensitivity assay using dilution methods indicated that the ability of the fimbriae-C S. typhi primers to detect S. typhi genome as a template was very high, which is 1.295 x 10^39 µg/mL DNA chromosome. Hence, it was very potential detection tool for typhoid disease [9]. Aside from sensitivity and specificity, another important information in the molecular detection tool is their ability to detect S. typhi bacteria in human body. Based on the results and information on the necessity to develop detection tool for S. typhi in human body, this research is aimed to test the ability of the fimbriae-C S. typhi primers to be the potential detection tool for S. typhi bacteria using clinical assay in human blood samples.

MATERIALS AND METHODS

Following are the order to test the ability of the fimbriae-C S. typhi primers for clinical assay in human blood samples, (1) Blood sample collection from a healthy and suspected typhoid fever patient. (2) Detection S. typhi from blood sample of suspected patient by Widal assay. This assay followed Swemed Diagnostic’s Kit. (3) Blood sample preparation for PCR methods which consists of (a) washing red color with Tris-EDTA buffer pH 8, (b) lysis of blood sample with Proteinase K (10 mg/ml) [6, 8, 9, 10, 11], (c) isolation of Genomic bacteria by Wizard® Genomic DNA Purification Kit by Promega [12], and characterization by electrophoresis gel agarosa at 80 volts for 1 hour and visualization under 260 nm wavelengths UV lamp [13]. (4) The next step was amplification of S. typhi genome from a healthy and suspected blood sample. The amplification process was performed using thermal cycle machine (BioRad), fimbriae-C-S. typhi primer pairs and Master Mix PCR (BioRad). Total reaction was involving 25 µL: Master Mix PCR 12.5µL, primer FW-Int2 1.25µL (12.5 pmol), primer Rev-1a New 1.25µL (12.5 pmol), nuclease free water 9µL, and 1µL of sample. The mixture was loaded at thermal cycle machine with the initial temperature of 95 °C for 5 minutes, denaturated at 95 °C for 1 minutes, annealed at 57.5 °C for 1 minutes, elongation at 72 °C for 1 minute, and product stabilization at 72 °C for 7 minutes for 35 cycles. [8, 9, 14].

RESULTS AND DISCUSSION

The amplification of S. typhi culture from healthy and suspected patient blood by PCR with fimbriae-S. typhi primers have been completed. The results are displayed in Fig. 1(a) and Fig. 1(b).

Figure 1 shows DNA genome template of S. typhi bacteria, and DNA from suspected blood sample of typhoid indicated by amplicon of 193 base pairs or (± 0.2 kb). The band size of S. typhi genome from pure culture was similar with the size of DNA from suspected patient blood template. The negative control did not indicate amplicon of 0.2 kb. However, the appearance of DNA smear on normal sample blood template is still a question, therefore further research should be conducted. DNA band for all amplification results with DNA template from the patient blood sample was observed at 0.2 kb, thus indicated that fimbriae-S. typhi primers have successfully amplified the fim-C gene fragment in the size of 0.2 kb from the sample. This result also indicated that the blood sample of the patient contains S. typhi bacteria, and the patient is infected with typhoid disease. Furthermore, the DNA band from PCR amplification with primer pairs also showed specific and sensitive result.

Comparative data of detection results with fimbriae-S. typhi primers using PCR and Widal assay from some patients is displayed in Table 1.

Table 1 shows that the suspected blood from sample C3, F3, G3, and J5 give positive results, and the other sample, which using Widal assay have negative results, however PCR methods show the positive results. Based on those results, PCR method is indicated to have higher specificity and sensitivity results compared to Widal test. The advantages of PCR methods are as follows: (1) The PCR is highly sensitive due to only small amount of sample is required, i.e only few copy of DNA molecule was applied in this research in concentration of 1.295 x 10^{-39} µg/mL.
FIGURE 1. Amplifications results of fim-C Gene with fimbriae-S. typhi primers stage 1 (a): (1) DNA Marker (Biorad), (2) Positive control (DNA Genome S. typhi), (3) Negative control (healthy blood), (4) Suspected blood sample A6, (5) Suspected blood sample B3, (6) Suspected blood sample C3, (7) Suspected blood sample D5, (8) Suspected blood sample E6. Stage 2 (b): (1) DNA Marker (Biorad), (2) Positive control (DNA Genome S. typhi), (3) Negative (healthy blood), (4) Suspected blood sample F3, (5) Suspected blood sample G3, (6) Suspected blood Sample H3, (7) Suspected blood Sample L5, (8) Suspected blood sample J5

TABLE 1. Comparison of Widal Test and PCR Amplification Results with Fimbriae-S. typhi Primers

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample code</th>
<th>Assayed Widal Test</th>
<th>Assayed PCR Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>blood patient</td>
<td>Antigen O</td>
<td>Antigen H</td>
</tr>
<tr>
<td>1.</td>
<td>A6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>B3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>C3</td>
<td>0</td>
<td>1/80</td>
</tr>
<tr>
<td>4.</td>
<td>D5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>E6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>F3</td>
<td>1/80</td>
<td>0</td>
</tr>
<tr>
<td>7.</td>
<td>G3</td>
<td>0</td>
<td>1/160</td>
</tr>
<tr>
<td>8.</td>
<td>H3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9.</td>
<td>I5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10.</td>
<td>J5</td>
<td>1/320</td>
<td>-</td>
</tr>
</tbody>
</table>

DNA chromosome, yet high intensity of DNA band was observed [9]. (2) Blood sample used in the experiment can be in a very small amount compared to the serological test. The sample used for PCR were from various specimens consist of S. typhi bacterial, due to this reason, it is suitable for a wide range applications (3) Specific characteristic, because PCR method will only amplified DNA fragment on the primer length. In this research, fimbriae-S. typhi primers was able to differentiate the S. typhi and S. typhi murium against another Gram negative strains [9], (4) High accuracy, because the primer was designed based on DNA sequence of tested bacteria. (5) Faster detection time compared to the serological test.

The weaknesses of PCR detection are: (1) Requires a well trained expert, (2) Expensive test equipments and tools. Many advantages offered by the PCR method has made this method highly potential to be developed to enhance the health quality of the society. The sooner a patient can be diagnosed, the earliest a physician can take the proper action to handle the disease. Although human resources and costs are still an issue, many solutions can be promoted in terms of investment in human resources and equipments to resolve these problems. It can be concluded that development of PCR method for typhoid diseases detection using fimbriae-S. typhi primers might be applied as a basic principle to improve the previous detection method.
CONCLUSIONS

The PCR method with fim-C-S. typhi primer pairs that produced 0.2 kb amplicon is potential detection tool for typhoid disease. The amplicon was produced from the lysis result template of suspected typhoid patient and bacteria culture S. typhi genome as positive control. DNA band size of 0.2 kb fim-C Styphi gene in the electrophoresis result showed that this research has succeeded in developing detection tool for typhoid disease by using PCR method. This result can be used as a basic principle for further development of detection tool with more specific, more sensitive, and faster properties.

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