Accuracy of Conventional PCR Targeting the 16S rRNA Gene with the Ot-16sRF1 and Ot-16sRR1 Primers for Diagnosis of Scrub Typhus: a Case-Control Study

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We retrospectively evaluated the accuracy of conventional PCR targeting the 16S rRNA gene (16S C-PCR) using the Ot-16sRF1/Ot-16sRR1 primers for diagnosing scrub typhus. The diagnosis of Orientia tsutsugamushi infection by 16S C-PCR presented an increased sensitivity of 87.0% and specificity of 100% compared with those obtained with other targets and is thus a simple and clinically useful method with good diagnostic accuracy.

Scrub typhus is an acute febrile disease that is spread by the bite of a chigger infected with Orientia tsutsugamushi (1–3). In general, the course of scrub typhus is not serious, and the disease is easily treated with antibiotics. However, a delay in diagnosis can lead to the development of complications, which may result in death in some patients (4). Therefore, rapid and early diagnosis is critical.

In this case-control study, we compared the clinical usefulness and diagnostic accuracy of conventional PCR targeting the 16S rRNA gene (16S C-PCR) with those of the following methods: C-PCR targeting the 56-kDa, 47-kDa, and groEL genes; nested-PCR (N-PCR) targeting the 56-kDa and 47-kDa genes; and real-time PCR (qPCR) targeting the 47-kDa gene.

We collected and stored blood sample from adult patients who visited Chosun University Hospital, Gwangju, South Korea, from 2007 to 2008 due to acute febrile disease within 4 weeks of symptom onset. Blood specimens were randomly selected by an infectious disease specialist. Buffy coat samples from 115 scrub typhus patients (diagnosed with scrub typhus if their O. tsutsugamushi indirect immunofluorescence assay [IFA] IgM titer or IgG titer was increased by at least 4-fold), 52 clearly diagnosed non-scrub typhus patients, and 35 non-scrub typhus febrile patients who had neither scrub typhus nor any characterized illness (non-scrub typhus group with acute pyrexia of unknown origin) were sent to the laboratory and retrospectively tested using the various PCR target primers. A patient was considered uninfected with O. tsutsugamushi if the indirect IFA did not detect antibodies and if the results of diverse diagnostic tests, such as serology, culture, and peripheral blood smears, led the infectious disease specialists to conclude that the patient was clearly infected with a febrile disease other than scrub typhus or acute pyrexia of unknown origin. The researchers performed the PCR assays in a blinded manner (without any clinical information or clear knowledge of the diagnosis). This study was approved by the institutional review board of Chosun University Hospital.

Using a basic local alignment search tool database program and the Primer 3 program from the National Center for Biotechnology Information, we designed the Ot-16sRF1 and Ot-16sRR1 primers based on the 16S rRNA gene from the O. tsutsugamushi Boryong strain (BankIt 1356164, GenBank accession no. HM352765), which was amplified from a blood sample from a patient who visited the hospital (5).

To compare the diagnostic accuracy of each PCR method, we used the same primers, probe, and PCR conditions detailed in published articles (5–9). The diagnostic values of C-PCR targeting the 16S rRNA gene with the Ot-16sRF1/Ot-16sRR1 primers and the other PCR methods were compared using McNemar’s test.

The specificity of the various PCR methods was determined using DNA from three O. tsutsugamushi strains, various Rickettsia strains, and diverse bacterial strains (data not shown). The only strains that produced amplicons were the three O. tsutsugamushi strains (data not shown).

The diagnostic specificity and sensitivity of the various PCR assays were determined using DNA from 202 patients diagnosed with acute febrile disease (data not shown). Amplification of the 16S rRNA gene by C-PCR using the 16-OT1-F/16-OT1-R primers identified 76 of the 115 scrub typhus patients as positive (positive rate, 66.1%), and the C-PCR exhibited a specificity of 100%. 16S C-PCR using the Ot-16sRF1/Ot-16sRR1 primers designated 100 patients as positive (positive rate, 87.0%) and presented a specificity of 100%. A subanalysis revealed that 96 patients did not take antibiotics against O. tsutsugamushi before they visited the hospital. Of the 96 patients who were not administered antibiotics prior to their hospital visit, 86 were identified as positive by PCR, and the sensitivity increased to 90%. The 47-kDa C-PCR and 47-kDa N-PCR assays presented positive rates of 2.6% and 80.9%, respectively. If cross point cycle (Cp) of ≥38 was assumed to represent a...
negative result from the qPCR test (5, 10), 87 of the 115 patients were positive (positive rate, 75.7%), and the specificity was 97.7% (two false positives in the non-scrub typhus group) (Table 1). The 56-kDa C-PCR and 56-kDa N-PCR tests showed positive rates of 7.8% and 80.0%, respectively, and groEL C-PCR showed a positive rate of 66.1%. The diagnostic value of C-PCR targeting the 16S RNA gene with the Ot-16sRF1/Ot-16sRR1 primers was significantly different from that of other PCRs, including 16S C-PCR using the 16-OT1-F/16-OT1-R primers (P < 0.001), groEL C-PCR (P < 0.001), 47-kDa N-PCR (P = 0.039), and 47-kDa qPCR (P = 0.013) but was not significantly different from that of 56-kDa N-PCR (P = 0.057).

Parola et al. (11) reported that 56-kDa C-PCR using the OtsuF/OtsuR primers showed a positive rate of 17.5% in the analysis of 63 buffy coat samples. Mahajan et al. (12) reported that 56-kDa C-PCR with the same OtsuF/OtsuR primers gave positive results for two among 21 buffy coat samples. In our previous study, we found that 56-kDa C-PCR with the P10/P11 primers yielded negative results for all 41 blood samples tested and that conventional 47-kDa C-PCR presented a positivity rate of 7% (5). The comparison of 56-kDa C-PCR with the OtsuF/OtsuR primers and 16S C-PCR with the Ot-16sRF1/Ot-16sRR1 primers using the buffy coats from 20 randomly selected scrub typhus patient samples showed that the positivity rate of 56-kDa C-PCR with the OtsuF/OtsuR primers was 45%, and the positivity rate of 16S C-PCR with the Ot-16sRF1/Ot-16sRR1 primers was 95% (data not shown).

In this study, we proposed a method that is not only rapid but also inexpensive and easy to implement. Therefore, our study provides important information for clinicians, particularly those working in clinics or hospitals in underdeveloped countries. Our study had some limitations associated with the study design and potential overestimated sensitivities because it is not a prospective cohort study.

In conclusion, because C-PCR targeting the 16S rRNA gene with the Ot-16sRF1/Ot-16sRR1 primers exhibited higher diagnostic accuracy for clinical specimens than that of other PCR methods, 16S C-PCR with the Ot-16sRF1/Ot-16sRR1 primers may be a clinically useful method for the rapid and early diagnosis of patients with suspected scrub typhus infection.

ACKNOWLEDGMENTS

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**TABLE 1 Diagnostic sensitivity and specificity of various PCR methods using the clinical specimens**

<table>
<thead>
<tr>
<th>PCR method</th>
<th>Scrub typhus group</th>
<th></th>
<th></th>
<th>Non-scrub typhus group</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of</td>
<td>% patients</td>
<td>Sensitivity %</td>
<td>No. of</td>
<td>% patients</td>
<td>Specificity %</td>
</tr>
<tr>
<td></td>
<td>specimens</td>
<td>positive</td>
<td>(95% CI)</td>
<td></td>
<td></td>
<td>(95% CI)</td>
</tr>
<tr>
<td>16S rRNA C-PCR with Ot-16sRF1/Ot-16sRR1 primers</td>
<td>115</td>
<td>100</td>
<td>87.0 (79.1–92.3)</td>
<td>87</td>
<td>87</td>
<td>100 (94.7–100)</td>
</tr>
<tr>
<td>16S rRNA C-PCR with 16-OT1-F/16-OT1-R primers</td>
<td>115</td>
<td>76</td>
<td>66.1 (56.6–74.5)</td>
<td>87</td>
<td>87</td>
<td>100 (94.7–100)</td>
</tr>
<tr>
<td>groEL-STG C-PCR</td>
<td>115</td>
<td>76</td>
<td>66.1 (56.6–74.5)</td>
<td>87</td>
<td>87</td>
<td>100 (94.7–100)</td>
</tr>
<tr>
<td>56 kDa C-PCR</td>
<td>115</td>
<td>9</td>
<td>7.8 (3.9–14.7)</td>
<td>87</td>
<td>87</td>
<td>100 (94.7–100)</td>
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<tr>
<td>47 kDa C-PCR</td>
<td>115</td>
<td>3</td>
<td>2.6 (0.7–8.0)</td>
<td>87</td>
<td>87</td>
<td>100 (94.7–100)</td>
</tr>
<tr>
<td>56 kDa N-PCR</td>
<td>115</td>
<td>92</td>
<td>80.0 (71.3–86.7)</td>
<td>87</td>
<td>87</td>
<td>100 (94.7–100)</td>
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<tr>
<td>47 kDa N-PCR</td>
<td>115</td>
<td>93</td>
<td>80.9 (72.3–87.4)</td>
<td>87</td>
<td>87</td>
<td>100 (94.7–100)</td>
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<tr>
<td>47 kDa Q-PCR</td>
<td>115</td>
<td>87</td>
<td>75.7 (66.6–83.0)</td>
<td>87</td>
<td>85</td>
<td>97.7 (92.9–99.9)</td>
</tr>
</tbody>
</table>


d CI, confidence interval.

Two cases with positive 47-kDa qPCR and negative IFA result, a 36-year-old female with chickenpox and a 73-year-old female with acute pyelonephritis.

**FUNDING INFORMATION**

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**REFERENCES**