Standardisation of polymerase chain reaction for the detection of Salmonella typhi in typhoid fever

Rama Chaudhry, B V Laxmi, Nazima Nisar, Koninika Ray, Dinesh Kumar

Abstract

To improve the diagnosis of Salmonella typhi infection, a polymerase chain reaction (PCR) assay was developed for the amplification of the dh flagellin gene of S typhi. Primers were designed from dh flagellin gene sequence which will give an amplification product of 486 base pairs. In tests to study the specificity of the assay, no amplification was seen in non-salmonella strains or salmonella strains with flagellar gene other than “dh”. Sensitivity tests determined that 28 pg of S typhi target DNA or 3 x 10^6 target bacteria could be detected by the PCR assay. Subsequently, the PCR technique was used for detection of S typhi in blood or clot cultures from 84 patients clinically suspected of having typhoid fever, and from 20 healthy control subjects. Twenty five of 84 samples from clinically suspected cases were positive by PCR; four of which were culture negative. No amplification was seen in samples from patients who were culture positive for organisms other than S typhi or from controls. The time taken for each sample for PCR analysis was less than 48 hours compared with three to five days for blood or clot culture. PCR appeared to be a promising diagnostic test for typhoid fever.

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Keywords: Salmonella typhi; polymerase chain reaction; typhoid fever

Typhoid fever, a septicaemic disease caused by Salmonella typhi, is a serious health problem in developing countries. Diagnosis of typhoid fever currently relies on blood culture and Widal’s test. Blood cultures are negative in 30-65% of cases with typhoid fever because of...
prior administration of antibiotics or a low number of organisms. \(^5\) Negative blood culture reports in patients with typhoid fever underestimates the actual incidence of disease. Widal’s test has been found to be non-specific and difficult to interpret in areas where typhoid fever is endemic. \(^6\) No non-cultural test for typhoid fever has been consistently shown to be sufficiently sensitive and specific. \(^7\) There is, therefore, a need to develop a highly sensitive and specific method for the diagnosis of patients with negative blood cultures. We report the development and evaluation of a polymerase chain reaction (PCR) assay to detect S typhi from peripheral blood of patients with typhoid fever by amplification of dH flagellin gene.

### Methods

Six S typhi strains and 10 non-salmonella strains were grown overnight in Luria broth (table 1) and tested to study the specificity of the PCR assay. S901 (motile), a standard strain of S typhi was used as positive control. The primers were designed based on published dH flagellin gene sequence\(^8\) using Oligo Computer Program. To investigate the sensitivity of the system, overnight culture of S901 was titrated by counting colonies on nutrient agar plates after 10-fold serial dilution of organisms ranging from 10\(^7\) to 10\(^3\); DNA extracted from S typhi was serially diluted to determine the minimum amount of DNA detectable by PCR.

Blood samples were collected from 84 patients with clinically suspected typhoid fever attending our institution as well as from 20 normal healthy individuals to be used as negative controls. Serum was removed from 5 ml of blood collected in a sterile tube. The clot was then added to 5 ml of 10% bile broth. Clots were broken by vortexing with sterile glass beads for five minutes and incubated overnight at 37°C. The following morning DNA was extracted by boiling method.\(^8\) For 36 samples, DNA was extracted from citrated blood by lysis method using Proteinase K and Triton X-100. The target for amplification was the dH flagellin gene. A 486 base pair region was amplified with specific primers RK1 (5’ TGG GCG ACG ATT TCT ATG CC 3’) and RK2 (5’ TTT CGC GAA CCT GGT TAG CC 3’). Amplification was carried out with 50 pmol of each primer in 25 µl of the PCR solution containing 0.625 units of Taq DNA polymerase, 2.5 mM MgCl\(_2\), 200 µM each of dATP, dCTP, dTTP, and dGTP, and 1 × PCR buffer (Perkin Elmer, Rotkreuz, Switzerland). The reactions were performed in a DNA thermocycler (MJ Research Inc, Massachusetts, USA) as follows: 40 cycles of denaturation at 94°C for one minute, annealing at 57°C for one minute 15 seconds, elongation at 72°C for three minutes, and final extension of five minutes at 72°C. PCR products were visualised on a 1.2% agarose gel stained with ethidium bromide.

### Results

PCR with RK1 and RK2 primers was found to be specific for dH flagellin gene, amplifying the S typhi specific band of 486 base pairs. No amplification was seen in any non-salmonella strain or the other seven Salmonella spp with flagellar gene other than “d”, or in DNA extracted from humans (fig 1). We also investigated other Salmonella spp containing dH flagellin gene—S stanley, S livingstone, and S Schwarzangrund—and amplification of the 486 base pair segment was seen.

The minimum number of organisms detected by PCR was 3 x 10\(^3\). The amount of DNA which could be detected after serial dilution was 28 pg (fig 2).

Of 84 patients with clinically suspected typhoid fever, 21 were both culture and PCR positive for S typhi. Four additional cases were positive by PCR but culture negative after 48 hours (table 2). No amplification was seen in patients that were culture positive for organisms other than S typhi or in controls.

### Discussion

Isolation of S typhi from blood is the most satisfactory method of diagnosis in the early stage of typhoid fever as bacteraemia is present during the early stage of the disease.
Other Salmonella spp containing DH flagellin do not infect humans. In addition, these primers were designed to amplify a region of S typhi that is not affected by the deletion mutation reported by Song et al. 11 Therefore, it seems to be a more promising diagnostic approach compared with the conventional procedure of culturing and identifying S typhi strains with DH antisera that may miss S typhi strains with mutated flagellin gene, that is, jH rather than DH.

The specificity of PCR was 93.7% and sensitivity was 100%. The predictive positive value was 84% and predictive negative value was 100%. Four culture false positive cases were actually additional cases of typhoid fever detected by PCR. There were no false negative cases.

PCR detection of S typhi took less than 48 hours, compared with five to seven days for blood or clot culture. Therefore, PCR was a rapid, sensitive, and specific test for the diagnosis of typhoid fever, especially where blood culture was negative because of prior antibiotic treatment, low level of bacteraemia, and culture done in late stages of disease, thus enabling the clinician to use appropriate treatment and to avoid diagnostic delay.

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Table 2: Results of culture and PCR assay

<table>
<thead>
<tr>
<th>Blood/clot culture</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR positive</td>
<td>21</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>PCR negative</td>
<td>0</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>63</td>
<td>84</td>
</tr>
</tbody>
</table>
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