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Diagnosis of Typhoid Fever by Amplification of the Salmonella typhimurium's histidine transport operon in Egyptian Patients

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ABSTRACT

Background: Accurate diagnosis of typhoid fever at an early stage is important. The objective of this study was to diagnose typhoid fever by the amplification of hto gene.

Methods: This study comprised 115 patients from different ages experienced the clinical symptoms of typhoid fever. This study was carried out at Mansoura University Hospitals. The blood samples collected were subjected to PCR to target the hto gene of Salmonella Typhi.

Results: The PCR technique standardized for hto gene resulted in specific amplicon of 495-bp. In positive PCR cases of typhoid, a single band of 495bp corresponding to hisJ (hto) gene was obtained. The PCR assay was found to be highly specific and sensitive.

Conclusion: When exceptional sensitivity is obligatory, PCR technique utilized as a very sensitive and particular test for the presence of salmonella in blood samples. A typhoid fever diagnosis by PCR will provide crucial information on treatment and prevention.

Keywords

Typhoid fever, PCR, patients, hto gene.

1. INTRODUCTION

Typhoid fever is still a major public health issue in many regions of the world [1, 2]. The worldwide incidence of typhoid fever is between ~ 13.5–26.9 million cases annually [3].

Clinical diagnosis of enteric fever is difficult, as the symptoms associated with enteric fever overlap with those of other febrile illness. Conventional methods used for laboratory diagnosis include blood culture and serological testing are time consuming, costly and technically demanding.

The only diagnostic techniques for typhoid fever are the blood culture and Widal test. Widal test is quite sensitive but has become highly nonspecific and its value for early detection of the disease is very limited. Blood culture can detect only 40%-45% of cases [4].

Molecular diagnosis techniques target the pathogen itself so they are useful in early diagnosis of disease and for the diagnosis of a variety of infectious disorders; PCR is the gold standard approach. [5].

PCR is an efficient method for early typhoid diagnosis since it can be utilized even if antibiotic therapy was started or pathogen burden is low [1]. Histidine receptor binding protein (hisJ) can be used for differentiation of Typhi and Paratyphi A from other salmonellae [1].

Salmonella histidine transport operon oligonucleotide primers were chosen because it was thought to be highly preserved among Salmonella species [6].

The aim of this study is to diagnose typhoid fever by amplification of hto gene by PCR technique.

2. SUBJECTS AND METHODS

This study comprised 115 patients from different ages experienced the clinical symptoms of typhoid fever. This study was carried out at Mansoura University Hospitals in the period between January 2019 and September 2020. Patients signed a written consent form after the study was approved by the local ethics commission.

All the patients with typhoid fever were subjected to: complete history taking with special emphasis on clinical symptoms of typhoid fever; including fever, vomiting and diarrhea or constipation and abdominal pain.

Regardless of antibiotic dosing, all samples were collected in BacT/ALERT bottles (BIOMERIEUX, USA). An automated blood culture system was used to test blood samples. Sub-cultures on Blood agar, MacConkeys agar and Chocolate agar medium were used to identify all samples which are positive in culture (OXOID CO. UK). Blood agar and MacConkeys agar plates were inoculated with bacteria and incubated in aerobic conditions at 37 degrees Celsius. Chocolate agar plates were also incubated at 37 degrees Celsius under a CO2 atmosphere of 5-10% (Candle jar) and the growth was examined after about 18-24 incubation periods. The VITEK 2 Compact system (bioMérieux, France) was used to identify the type of growth [7, 8, 9].

QIAamp DNA blood Kit (Qiagen) was used to extract DNA, which was done according to the manufacturer's recommendations [10]. The cells (max 2 x 109 cells) were harvested by centrifuging for 10 minutes at 5000 x g (7500 rpm) in the microcentrifuge tube, and the supernatant was discarded. In 180 l Buffer ATL, the sediment was resuspended. The proteinase K (20 l) was added and well mixed by vortexing, after which the samples were incubated at 56°C and then placed in a thermomixer to disperse them. Vortexing was used to fully mix the buffer AL (200 l) into the sample. Then 200 l of ethanol (96–100%) was added, and the mixture was thoroughly mixed again by vortexing. The mixture was pipetted into the DNeasy Mini spin column which was placed in a 2 ml collection tube, and then was centrifuged at ≥ 6000 x g (8000 rpm) for 1 min. In a new 2 ml collection tube, the DNeasy Mini spin column was put, then 500 l Buffer AW1 was added and centrifuged for 1 minute at 6000 x g. (8000 rpm) for elution. The DNeasy Mini spin column was inserted in a new 2 ml collection tube (supplied), and then buffer AW2 (500 l) was added, and it was centrifuged for 3 minutes at 20,000 x g (14,000 rpm) to dry the membrane. The collecting tube and the flow-through tube were discarded. The DNeasy Mini spin column was inserted in a clean 2 ml microcentrifuge tube then 200 l Buffer AE was pipetted onto the DNeasy membrane directly, which was then incubated for 1 minute at ambient temperature before being centrifuged for 1 minute at 6000 x g (8000 rpm) to elute. In order to get the most DNA, the elution step was repeated once again.

For typhoid fever patients, the amplified portion of a 495-bp portion of Salmonella typhimurium's histidine transport operon was identified using 25-bp oligonucleotide primers (hto gene). This gene was chosen because it was thought to be highly preserved among Salmonella species. A software application was used to design the PCR primers. The forward and reverse oligonucleotide primer sequences, from 5' to 3', were the following: forward-looking strand, ACTGGCGTTATCCCTTTCTCTGGTG; reverse one, ATGTTGT- CCTGCCCCTGGTAAGAGA [11, 12].

The initial part of PCR optimisation was performed using conventional PCR. Each reaction in a total volume of 25 μL contained PCR Master Mix (12.5 μL) (Promega, USA), 5 μL of DNA template, 5.5

μL of molecular grade water and 1 μL of forward and reverse primers. The primer concentration used in the reaction was kept constant at 10 μM. Amplification was performed in Stepone thermal cycler. Annealing temperature optimisation was performed at 12 points using the following condition; Denaturation at 94 degrees Celsius for 2 minutes, then 35 cycles of 94 degrees Celsius denaturation for 1 minute, annealing at 49.8 degrees Celsius to 65.1 degrees Celsius for 1 minute, and extension at 72 degrees Celsius for 1 minute. [13, 14]. Products of PCR (5 μL) electrophoresed on agarose gel with a concentration of 2% in a 0.5× TBE buffer at 85 V for 60 minutes. Then the gel had been stained with GelRed™ (Biotium®, USA). A 50 bp DNA ladder (Fermentas, USA) was included as the molecular weight marker in every electrophoresis run. The PCR products were visualised under ultraviolet (UV) illumination using gel image documentation system (GelDoc 1000 system, Bio-Rad, USA).

A statistical software programmed has been used to analyse the data (SPSS for Windows, version 21, USA) [22]. The findings of all statistical tests were deemed significant at P-value ≤ 0.05.

3. RESULTS

On blood samples of 115 patients, PCR was carried out for S. typhi's histidine transport operon (hto gene) was positive in 15 patients. The amplified portion of a 495-bp fragment of S. typhi's histidine transport operon (hto gene) was identified using 25-bp oligonucleotide primers and at the end of the PCR, the amplified product was identified.

A total of 100 patients had negative PCR test results.

The PCR for the S. Typhi hto gene yielded a sensitivity of 92.86 % and yielded a specificity of 98.97 %. The PCR's positive predictive value (PPV) was shown to be 93.66 % with a precision and an accuracy of 96.52 %. The PCR findings were statistically significant (P= 0.001).

Table 1. Comparison of Blood culture and PCR test as a diagnostic tool for Typhoid fever

	Blood culture	**PCR**
AUC	0.010	0.005
Sensitivity (%)	87.5	92.86
Specificity (%)	96.90	98.97
PPV (%)	82.03	93.66
NPV (%)	95.31	97.01
Accuracy (%)	93.04	96.52
P - value	0.001	0.001

Table 1 showed the validity of Blood culture and PCR test as a diagnostic tool for Typhoid fever. PCR was sensitive than blood culture.

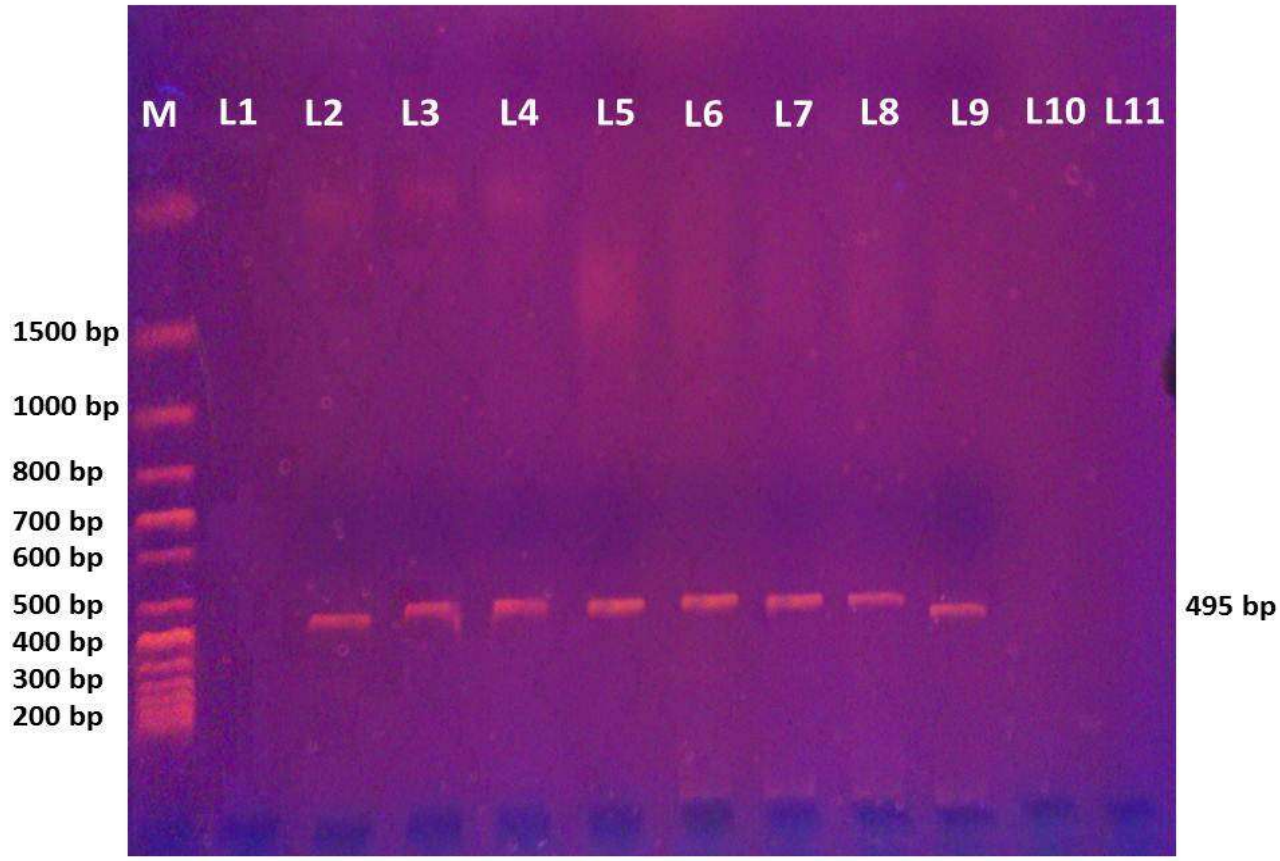

Figure 1. PCR amplicons electrophoresis of hto gene on an agarose gel after amplification of gene-specific DNA fragments from a blood sample for typhoid fever patients. Electrophoresis on 3 percent (wt /vol) agarose gels was used to examine the samples. DNA was seen under UV light after gels were stained with ethidium bromide. This results in amplicons 495 bp.

4. DISCUSSION

Typhoid fever is a major health problem throughout the world [20]. The clinical symptoms caused by typhoid fever overlaps with many other febrile illnesses.

Fluoroquinolone-resistant strains of Salmonella has been emerging and becoming major health problems throughout the world [1, 15].

Salmonella isolated from blood cultures were identified, followed by biochemical testing, is a problem in developing countries. Confirmation of typhoid fever can also be made by Widal test. Several PCR methods have been developed to detect Salmonella in clinical samples with the aim of improving diagnosis of typhoid infection.

In the present study, PCR standardized for hisJ (hto) gene result in specific amplicon of 495-bp. In positive PCR cases of typhoid (total 15), a single band of 495bp corresponding to hisJ (hto) gene was obtained. The PCR assay was found to be highly specific. PCR was sensitive than blood culture for typhoid fever diagnosis since the sensitivity and specificity of PCR are 92.86 % and 98.97% respectively and the sensitivity and specificity of blood culture are 87.5% and 96.90% respectively. The positive predictive value of PCR is 93.66% and the positive predictive value of blood culture is 82.03% respectively. The accuracy (%) of PCR and blood culture are 96.52% and 93.04% respectively.

We concluded that for the diagnosis of typhoid fever, PCR technique was more sensitive than blood culture, and it is especially useful when great sensitivity is essential. The results of molecular characterization are similar to which observed by some authors [2].

Three consecutive PCRs were used to identify the three major bacteria that cause enteric fever [17]. [16] developed a PCR method to detect S. Typhi. [1] aimed to develop a simple duplex PCR for diagnosis and differentiation of human Salmonella serovars Typhi and Paratyphi A from other salmonellae.

The sensitivity of blood culture is higher in the first week of the infection which can be reduced by use of antibiotics [18]. Diagnosis of typhoid fever is normally made in all developing countries by blood cultures and by serological testing. PCR was more effective than the Widal test, blood cultures in detecting typhoid fever. It was discovered that a cut off titer of TO > 1:80 and TH > 1:160 had superior diagnostic value [19].

When virus or bacteria concentrations are low, bacteria that are shed are nonviable, or isolation of an organism is difficult, amplification of DNA using the PCR technique can be achieved quickly and is of substantial benefit. More research is needed to determine how successful PCR is at detecting Salmonella species in blood samples. The use of PCR to amplify DNA may allow for the detection of bacteria at levels below those detectable by microbiologic culture.

Because the amount of organisms shed varies, identifying the species of Salmonella by culture typically necessitates the submission of numerous samples. The PCR methodology is easy and quick compared to other methods of gene amplification that do not use thermo stable DNA polymerases [21].

5. CONCLUSION

When great sensitivity is necessary, the PCR technique has been utilized as a highly sensitive and specific method for determination of pathogens in blood samples of patients. In terms of management, control, and reducing the risk, a genus-specific detection of typhoid fever would really be extremely useful. Because PCR detects specific sections of the genome of bacteria more sensitively than blood culture, only a single sample may be required for Salmonella identification.

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