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CHARACTERIZATION OF DIFFERENT MOLECULAR MARKERS FOR IDENTIFICATION OF *SALMONELLA ENTERICA* SEROVAR TYPHI IN PAKISTANI POPULATION

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ABSTRACT

Typhoid is caused by *Salmonella enterica* serovar Typhi that is usually diagnosed by using serologic and immuno-chromatographic techniques in developing counties including Pakistan, which is thought to be an unreliable diagnostic method. For accurate diagnosis we used molecular techniques to amplify 204 bp *StyR-36* and 498 bp flagellin gene for the identification of *Salmonella enterica* serovar Typhi. This study was done on 58 individuals diagnosed positive of typhoid via serologic tests and 50 healthy individuals as a control group. Success rate of amplification for flagellin gene was 77.58% while that for *StyR-36* gene was 68.97% showing that flagellin gene primer is characterized by higher value of percentage of amplification than that of *StyR-36* gene primer. For precise and accurate diagnosis of *Salmonella* infection, both genes can be targeted using specific molecular markers.

Key words: Molecular markers, *Salmonella enterica*, typhi, flagellin, *StyR-36*

INTRODUCTION

Water pollution is a serious problem in today's life that is caused due to the expanding population and industrialization thus, causing the shortage of clean and pure drinking water to the people (Vorosmarty et al., 2000; Carmo et al., 2015). As a result, many life threatening diseases like malaria, typhoid, gastrointestinal disorders, jaundice and other bacterial, viral and protozoal infections have become common in individuals causing serious health problems (Sele et al., 2015). Contaminated drinking water is a significant cause of many microbial infections and gastrointestinal disorders in developing countries of Asia and Africa. The sources of water contamination include unresolved and poor sanitation system, impure domestic water supplies and poor hygienic conditions, causing 1.7 million losses of lives per year (John, 2004). About 21.6 million individuals are affected every year, and this victimization rate fluctuates among 100 to 1000 typhoid patients per 100000 populations per annum (Crump and Mintz, 2010).

Typhoid, which predominates in less advanced areas of the world, is a highly contagious disease that is transmitted from person to person because of water or food contaminated with the feces of an infected person. The disease causes serious gastrointestinal problems like bleeding and perforation of intestine if it remains untreated (Atamanalp et al., 2015). Typhoid causes over 20 million deaths annually (90% mortality in Asia) due to contaminated food or water (Garcia et al., 2015).

Diagnosis of typhoid is quite problematic because it shows similar symptoms with other common febrile diseases, also showing multi-symptomatic characteristics that make the diagnosis quite complex (Hurley et al., 2014). In developing countries like Pakistan, the infectious agents

are serologically diagnosed which is usually considered as an unreliable method. Molecular techniques are the sole resolving methods to accurately diagnose *Salmonella enterica* serovar Typhi by targeting the specific genes i-e, flagellin and/or *StyR-36* genes (Lamkanfi et al., 2007; Nithya et al., 2015).

MATERIALS AND METHODS

Sample Collection

A total of 58 blood samples serologically diagnosed with typhoid were collected from Federal Government Polyclinic, Islamabad. Fifty control samples were obtained from healthy individuals.

DNA Extraction and Polymerase Chain Reaction (PCR)

Genomic DNA (gDNA) was extracted by following phenol-chloroform protocol described by Sambrook and Russel (2001) with slight modifications. Extracted gDNA was confirmed on 1% agarose gel and qualitatively and quantitatively analyzed using spectrophotometer (HP 8451A Diode Array Spectrophotometer, USA). Two pair of primers, i-e, ST1/ST2 (5'-TATGCCGCT ACATATGATGAG-3', 5'-TTAACGCAGT AAAGAGAG-3') and *StyR-36F*/*StyR-36R* (5'-TGCCATGTAATCGGACGCCGAC-3', 5'-AGCCAACAAACGCGGTTGCG-3') were used to amplify 498 bp of flagellin and 204 bp of *StyR-36* gene (Haque et al., 1999; Nithya et al., 2015), respectively. Amplification of respective genes was carried out using PCR Sprint Thermal Cycler (Thermo Electron Corporation, USA). The reaction mixtures for both genes contained 10x reaction buffer, 25mM $MgCl_2$, 10mM dNTPs, 5 U/µL Taq polymerase and 5pmol of each forward and reverse primer.

Amplification conditions for target genes include; single cycle of initial denaturation at 95° C for five min, 35 cycles of denaturation for 1 min at 95° C, followed by annealing for 1 min at optimized annealing temperatures for each primer (59° C for ST1/ST2 primers, 52° C for *StyR-36F*/*StyR-36R* priers) and elongation at 72° C for 2 minutes. Post extension was carried out at 72° C for five minutes. The amplified product was then analyzed by 1.5% agarose gel electrophoresis.

RESULTS AND DISCUSSION

DNA extracted from samples of both patients and control group was confirmed by agarose gel (1%) electrophoresis and the quality and quantity of gDNA was analyzed on spectrophotometer (Fig. 1 and Fig. 2).

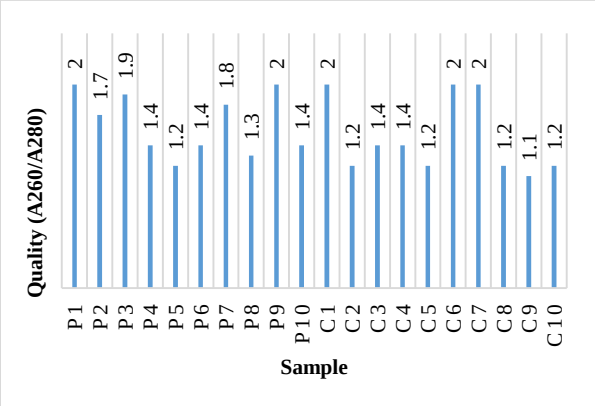

Figure 1: Graph showing quality of genomic DNA (A260/A280); P series shows patient samples; C series shows control samples.

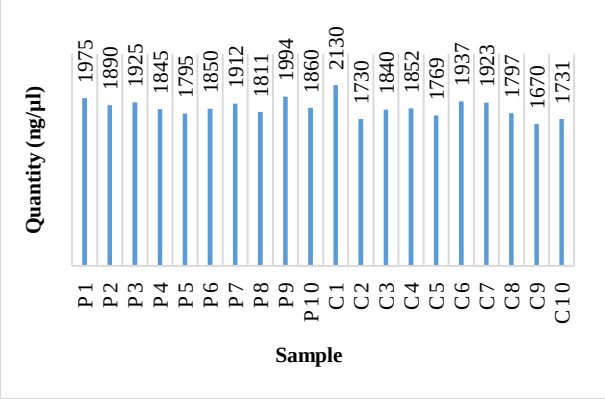

Figure 2: Graph presenting quantity of genomic DNA (ng/µL); P series shows patient samples; C series shows control samples.

Flagellin gene of 498 bp (Figure 3) and *StyR-36* gene of 204 bp (Figure 4) of *Salmonella enterica* serovar Typhi were amplified with the help of two primer pairs of ST1/ST2 and *StyR-36F*/*StyR-36R*, respectively. Under optimized conditions of PCR, no control sample showed any amplification for either gene, thus showing the accuracy of molecular markers for the specific amplification of target gene and the exclusiveness of *StyR-36F*/*StyR-36R* primers to *StyR-36* and ST1/ST2 primers to flagellin gene of *Salmonella enterica* serovar Typhi.

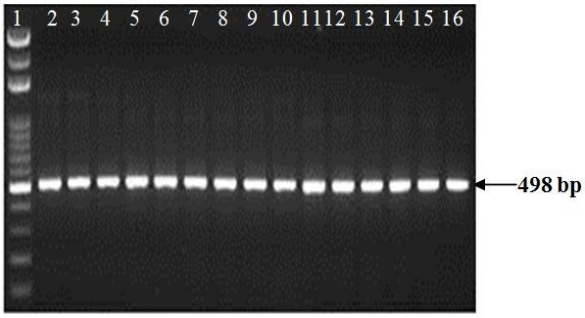

Figure 3: Amplified PCR product; Lane 1: 100bp DNA ladder; Lane 2-16: 498bp of flagellin gene.

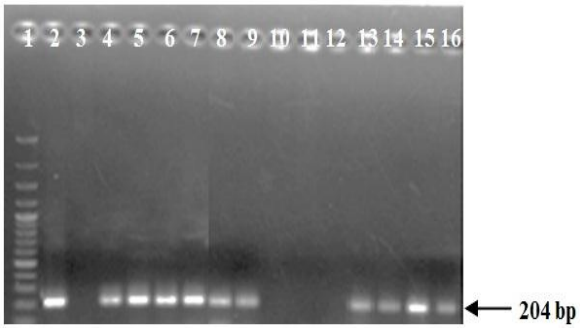

Figure 4: Amplified PCR product; Lane 1: 100bp DNA ladder; Lane 2-16: 204bp of *StyR-36* gene.

The unamplified samples represent PCR-diagnosed negative typhoid individuals divulging the false diagnosis by serological methods, because these methods only rely on the presence of antibodies against specific pathogen in an individual. The antibodies present in the serum may persist for a long time, even after the pathogen leaves its host victim. Therefore, the positive results of a serological diagnosis may not be the indicator of current infection (Ni et al., 2000; Morrow and Friedrich, 2003; Moreno et al., 2009; Cota et al., 2012; Paul et al., 2015).

Flagellin gene was amplified in 45 samples showing 77.58% amplification results. This gene has also been amplified in previous studies for molecular based identification of *Salmonella* infection in blood (Khan et al., 2012). A similar set of primers was used by Haque et al. (2001) to amplify flagellin gene in 55 typhoid suspected and 20 healthy individuals in order to compare the results of molecular technique with Widal-Test and blood culture. Results of the study for febrile patients to healthy individuals were 58.2/0, 14.5/0 and 52.7/45 percent for PCR, blood culture and Widal-Test, respectively. Results of serologic and culture methods in this study showed unsatisfactory and inaccurate results while that of PCR showed more precise and reliable results as no amplification was done in case of control group.

In current study, *StyR-36* gene was amplified in 40 samples showing 68.97% amplification results. Nithya et al. (2015) conducted a study on *Salmonella* species by targeting *StyR-3*, *StyR-36* and *StyR-143* genes in order to evaluate the specificity of genes in *S. paratyphi* and *S. typhi*. *StyR-3* and *StyR-46* was found in both the species while *StyR-36* was amplified only in *S. typhi*. The current study revealed that the targeted *StyR-36* gene exclusively belonged to *Salmonella* serovar Typhi, helping in the accurate identification and diagnosis of the respective species.

CONCLUSION

From current study it can be concluded that molecular techniques are more reliable and accurate methods of diagnosis. Moreover, both flagellin and *StyR-36* genes can be

targeted for diagnosis of *Salmonella* infection.

REFERENCES

Atamanalp S, Ozogul S, Kisaoglu A, Arslan S, Korkut E, Karadeniz E (2015). Typhoid intestinal perforations: has the clinical importance decreased in Eastern Anatolia for 36 years?. Eurasian J Med. 47: 135-137.

Carmo RF, Bevilacqua PD, Barletto M (2015). Social representations of drinking water: subsidies for water quality surveillance programmes. J Water Health. 13:671-679.

Cota GF, deSousa MR, Demarqui FN, Rabello A (2012). The diagnostic accuracy of serologic and molecular methods for detecting visceral Leishmaniasis in HIV infected patients: meta-analysis. PLoS Negl Trop Dis. 6: e1665.

Crump JA, Mintz ED (2010). Global trends in typhoid and paratyphoid fever. Clin Infect Dis. 50: 241-246.

Garcia A, Gallina S, Owczarek S, Dionisi A, Benedetti I, Decastelli L, Luzzi I (2015). Emergence of ciprofloxacin resistant *S. enterica* serovar Typhi in Italy. PLoS One.10: e0132065.

Haque A, Ahmed J, Qureshi J (1999). Early detection of typhoid by polymerase chain reaction. Ann Saudi Med. 19: 337-340.

Haque A, Ahmed N, Peerzada A, Raza A, Bashir S, Abbas G (2001). Utility of PCR in diagnosis of problematic cases of typhoid. Jpn J Infect Dis. 54: 237-239.

Hurley D, McCusker MP, Fanning S, Martin M (2014). Salmonella-host interactions- modulation of the host innate immune system. Front Immunol. 5: 1-11.

John NA (2004). Microbial contamination of drinking water and disease outcomes in developing regions. Toxicol. 198: 229–238.

Khan S, Harish BN, Menezes GA, Acharya NS, Parija SC (2012). Early diagnosis of typhoid fever by nested PCR for flagellin gene of *S. enterica* serotype Typhi. Ind J Med Res. 136: 850-904.

Lamkanfi M, Kanneganti TD, Franchi L, Núñez G (2007). Caspase-1 inflammasomes in infection and inflammation. J Leuk Biol. 82: 220-225.

Moreno EC, Gonçalves AV, Chaves AV, Melo MN, Lambertucci JR, Andrade ASR, Negrão CD, deFigueiredo CM, Carneiro M (2009). Inaccuracy of enzyme-linked immunosorbent assay using soluble and recombinant antigens to detect asymptomatic infection by Leishmania infantum. PLoS Neg Trop Dis. 3:e536.

Morrow RA, Friedrich D (2003). Inaccuracy of certain commercial enzyme immunoassays in diagnosing genital infections with herpes simplex virus types 1 or 2. Amer J Clin Path. 120: 839-844.

Ni YH, Lin JT, Huang SF, Yang JC, Chang MH (2000). Accurate diagnosis of Helicobacter pylori infection by stool antigen test and 6 other currently available tests in children. J Ped. 136: 823-827.

Nithya R, Ahmed AS, Hoe HC, Gopinath CS, Citartan M, Chinni VS, Lee PL, Rozhdestvensky ST, Tang HT (2015). Non-protein coding RNA genes as the novel diagnostic markers for the discrimination of salmonella species using PCR. PloS One. 10: e0118668.

Paul HY, Cross MB, Moric M, Levine BR, Sporer SM, Paprosky WG, Jacobs JJ, Della-Valle CJ (2015). Do serologic and synovial tests help diagnose

infection in revision hip arthroplasty with metal-on-metal bearings or corrosion?. Clin Ortho Rel Res. 473: 498-505.

Sambrook J, Russell DW (2001). Commonly used techniques in molecular cloning. In: *Molecular Cloning: A laboratory manual*. 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY: Appendix 8.

Sele LM, Balasubramaniam K, Elnegaard S, Søndergaard J, Jarbøl DE **(**2015). Lifestyle factors and experience of respiratory alarm symptoms in the general population. BMJ Open Resp Res. 2: 1-11.

Vorosmarty CJ, Green P, Salisbury J, Lammers RB (2000). Global water resources: Vulnerability from climate change and population growth. Science. 289: 284-288.