Abstract

Background

Salmonella Typhi is still a public health issue in developing countries. The pathogen causes approximately 26.9 million typhoid fever cases and 217,000 deaths annually worldwide. As culture sensitivity is low, the diagnosis of typhoid fever relies on clinical syndrome, hence, there is a need of molecular diagnostic method for the diagnostic confirmation. Loop-Mediated Isothermal Amplification (LAMP) can be a quick, easy, cheap method that only requires a heat block, which can be available in resource limited settings.

Objectives

1. To establish a heat block LAMP assay for typhoid diagnosis in TMGH.
2. To validate the heat block LAMP assay against clinical bacterial isolates in Nepal.
3. To find a cost effective DNA extraction method for the heat block LAMP assay

Method

Primers for the LAMP assay for *Salmonella* Typhi were targeted to STY 2879 gene, which was selected from the already published research (Fan et. al, 2015). In the initial phase, the sensitivity and specificity of the common primers (F3 and B3) were tested in real-time PCR with purified *Salmonella* Typhi and six other bacterial DNA. The primers then used to develop LAMP assay along with other four primers. Loop primers (LP) were newly designed. Sensitivity (lower limit of detection) and specificity were determined. The heat block LAMP assay was performed in Siddhi Memorial Hospital to validate the assay. The validation was performed by checking against bacterial isolates collected over a period of one year from Jan 16 in 2017 to Mar 23 in 2018. These bacteria were isolated from blood culture of children in the hospital. Two

DNA extraction methods (CDC and Qiagen) were compared to find out the cost effective method for LAMP assay.

Result

Primers targeted to *S.* Typhi STY 2879 gene were found to be sensitive and specific in real-time PCR and LAMP assay. The lower limit of detection in heat block LAMP assay was 30 copies per 25 micro L reaction, and specific to *S.* Typhi. The LAMP assay had sensitivity and specificity of 100% and 91.6%, respectively with DNA extracted by CDC method, and had 100% and 95.8% by Qiagen method. The result of LAMP assay could be detected in 30 min and the cost was cheaper in CDC DNA extraction method.

Conclusion

A sensitive, specific, and cost effective endpoint LAMP assay was established and validated by using a heat block for the diagnosis of *S.* Typhi. This LAMP assay along with CDC DNA extraction method can be used for the confirmation of *S.* Typhi in resource limiting areas.