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Development and optimization of a duplex polymerase chain reaction to detect selected water-borne pathogenic bacteria

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Background: Water-borne pathogen diseases are a significant health problem worldwide. Consumption of polluted water may lead to common, widely distributed water-borne diseases like salmonellosis and shigellosis. Culture-based methods for detecting water-borne bacteria are laborious, time-consuming, and low in sensitivity and specificity. Duplex Polymerase Chain Reaction (PCR) is a rapid and highly sensitive method to detect pathogenic microorganisms in the aquatic environment.
Objective: To develop and optimize a duplex PCR to detect *Shigella* and *Salmonella* species in water.
Method: Ethical approval for the research was obtained from the Ethics Review Committee of KIU (KIU/ERC/19/11). *Salmonella typhimurium* and *Shigella sonnei* clinical isolates were obtained from the Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura. The boiling method was optimized, for DNA extraction from bacteria inoculated water samples. Previously published species-specific primers were used for the optimization of PCR. The annealing temperature was optimized using temperature gradient PCR.
Results: Boiling the sample at 95°C for 10 minutes and extracting to a final volume of 100µl of nuclease free water was identified as the optimum condition to extract bacterial DNA by boiling method. The optimum annealing temperature from the gradient PCR was 61.9°C. The duplex PCR gave bands at 429bp for *Salmonella* and 620bp for *Shigella* and two bands were clearly separated in 1.5% agarose gel.
Conclusion: Boiling method is a fast and cost-effective method for DNA extraction. The duplex PCR can be developed as a rapid and sensitive method for the diagnosis of selected water-borne pathogens.

Acknowledgment: KIU undergraduate research grant.