Original Paper

Isolation of pathogenic leptospires from blood samples of patients by PCR- RFLP method

Honarmand HR (PhD)*[1], Khayat L (MSc)[2]
Mansour Ghanaei F (PhD)[3], Rahbar Taromsari M (MD)[4]

[1]Assistant Professor, Department of Microbiology, Gastrointestinal and Liver Research Center, Guilan University of Medical Sciences, Rasht, Iran. [2]Department of Microbiology, Azad University, Lahijan, Iran. [3]Professor, Department of Internal Diseases and Gastrentrology, Gastrointestinal and Liver Research Center, Guilan University of Medical Sciences, Rasht, Iran. [4]Assistant Professor, Department of Legal Diseases, Razi Hospital, Guilan University of Medical Sciences, Rasht, Iran.

Abstract

Background and Objective: Leptospirosis is a worldwide zoonosis that is more common in tropical and semitropical regions and in flat area of Guilan province, nothern Iran, caused by pathogenic leptospires.Serotyping by MAT is complicated, expensive and time cosuming. Molecular typing can be useful alternative. This study was performed to identify the typing of endemic pathogenic leptospires, isolated in Guilan province, by PCR-RFLP.

Materials and Methods: In this discriptive study, specimens were taken from patients in Razi hospital, Rasht, Iran ,from April to August 2008. DNA of all positive cultures were extracted by Phenol-Chloroform method. PCR was performed by using two primer sets: B64-I, B64-II for Kirschnery serovars whose PCR product were digested by HinfI for RFLP, and G1,G2 for all other species whose PCR products were digested by DdeI. Band profiles of digested PCR products were compared to band profiles of standard servers to determine species and subspecies.

Results: 65 of totally 107 blood cultures were positive. 56 Samples were amplified by G1, G2 included Interrogans and Borgpeterseni and 9 samples were amplified by B64-I, B64-II including Kirschneri species.

Conclusion: This study showed that the majority of leptospires species are Interrogans and Borgpeterseni. Regarding to several existing problems in Seortyping of leptospires by MAT, PCR-RFLP can be useful for identifying isolating and studying the serovars in different certain species. It seems that PCR-RFLP can be performable for clinical samples for both early diagnosis and characterization.

Keywords: Leptospirosis, Molecular diagnosis, Molecular typing

*** Corresponding Author:** *Honarmand HR (PhD), E-mail: honarmand_36@yahoo.com*

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