

Detection of Unculturable Bacteria in the Bile Juice of Patients with Biliary Diseases by PCR-DGGE Methods

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Background & Objectives: Bile in healthy people is sterile and presence of each bacterium in the bile could be a marker of a disorder, like cholelithiasis. Nucleic acid “fingerprinting” techniques using universal polymerase chain reaction (PCR) primers targeting 16S rRNA gene, such as denaturing gradient gel electrophoresis (DGGE), could be a powerful technique for detection of all culturable and unculturable bacteria responsible for these infections. The aim of this study is survey about distribution and similarity of unculturable bacterial agents in the bile juice of patients with biliary diseases to search about the relation between their presence and bile duct infections.

Methods: 102 bile samples were collected during the 6 month in 2011 from patients subjected to ERCP in Taleghani hospital, in Tehran, Iran. All the samples were analyzed both by bacteriological and molecular Methods. Total DNA was extracted by phenol-chloroform Methods from all culture positive and negative samples. DGGE patterns were analyzed for each 16s rRNA PCR product had been obtained from every one of the extracted DNA sample in compare to control strains.

Results: Comparison of results for bacteriological and molecular Methods showed presence of unculturable bacteria among both of the culture positive and culture negative samples in a frequency of 70 to 80%. The results also showed that more than 70% of a bacterial population in each sample is related to unculturable bacteria with a similarity rate of 50% among the samples. There was no significant relation between the DGGE pattern and kind of diseases.

Conclusion: Results of this study showed sensitivity of PCR-DGGE as a powerful technique for detection of unculturable bacteria in the bile samples. High frequency of similar banding patterns among different samples proposed their colonization or probable involvements in the disease occurrence that needs further studies.

Keywords: Bile Duct; Unculturable Bacteria; ERCP; PCR-DGGE