World Academy of Science, Engineering and Technology
International Journal of Medical and Health Sciences
Vol:13, No:04, 2019

Evaluation of Four Different DNA Targets in Polymerase Chain Reaction for Detection and Genotyping of Helicobacter pylori

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Abstract : Polymerase chain reaction (PCR) assays targeting genomic DNA segments have been established for the detection of Helicobacter pylori in clinical specimens. However, the data on comparative evaluations of various targets in detection of H. pylori are limited. Furthermore, the frequencies of vacA (s1 and s2) and cagA genotypes, which are suggested to be involved in the pathogenesis of H. pylori in other parts of the world, are not well studied in Kuwait. The aim of this study was to evaluate PCR assays for the detection and genotyping of H. pylori by targeting the amplification of DNA targets from four genomic segments. The genomic DNA were isolated from 72 clinical isolates of H. pylori and tested in PCR with four pairs of oligonucleotides primers, i.e. ECH-U/ECH-L, ET-5U/ET-5L, CagAF/CagAR and Vac1F/Vac1XR, which were expected to amplify targets of various sizes (471 bp, 230 bp, 183 bp and 176/203 bp, respectively) from the genomic DNA of H. pylori. The PCR-amplified DNA were analyzed by agarose gel electrophoresis. PCR products of expected size were obtained with all primer pairs by using genomic DNA isolated from H. pylori. DNA dilution experiments showed that the most sensitive PCR target was 471 bp DNA amplified by the primers ECH-U/ECH-L, followed by the targets of Vac1F/Vac1XR (176 bp/203 DNA), CagAF/CagAR (183 bp DNA) and ET-5U/ET-5L (230 bp DNA). However, when tested with undiluted genomic DNA isolated from single colonies of all isolates, the Vac1F/Vac1XR target provided the maximum positive results (71/72 (99% positives)), followed by ECH-U/ECH-L (69/72 (93% positives)), ET-5U/ET-5L (51/72 (71% positives)) and CagAF/CagAR (26/72 (46% positives)). The results of genotyping experiments showed that vacA s1 (46% positive) and vacA s2 (54% positive) genotypes were almost equally associated with VaCA+/CagA- isolates (P > 0.05), but with VacA+/CagA+ isolates, S1 genotype (92% positive) was more frequently detected than S2 genotype (8% positive) (P< 0.0001). In conclusion, among the primer pairs tested, Vac1F/Vac1XR provided the best results for detection of H. pylori. The genotyping experiments showed that vacA s1 and vacA s2 genotypes were almost equally associated with vaCA⁺/cagA⁻isolates, but vacA s1 genotype had a significantly increased association with vacA⁺/cagA⁺isolates.

Keywords : H. pylori, PCR, detection, genotyping
Conference Title : ICIDH 2019 : International Conference on Infectious Diseases and Health
Conference Location : Boston, United States
Conference Dates : April 24-25, 2019