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.

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