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Evaluating PCR-RFLP Technique in Identifying Genetic Diversity *Clostridium perfringens* Biotype A

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Abstract

BACKGROUND: *Clostridium perfringens* (*C. perfringens*) is an anaerobic Gram-positive bacillus with spores, whose biotype A is responsible for a variety of diseases, including intestinal inflammation, bloody diarrhea, and gas gangrene, and hemorrhagic bowel syndrome. Genetic variety can explain the bacteria's phenotypic diversity, geographic distribution, host specificity, pathogenicity, antibiotic resistance, and virulence. A molecular method using the pattern of DNA bands classifies bacteria based on the size of fragments produced by enzymatic digestion of the genome.

OBJECTIVES: This study aims to standardize the polymerase chain reaction (PCR)- restriction fragment length polymorphism (RFLP) method in identifying the genetic diversity of *C. perfringens* biotype A isolates.

METHODS: The genomic DNA of the investigated strains was extracted, and the complete sequence of the alpha toxin gene locus was synthesized using specific primers designed by PCR technique. Enzymatic cleavage of the synthesized amplicons was performed with the Mse l restriction enzyme, and the resulting fragments were separated by electrophoresis and analyzed by ImageJ and NTSYSPC software.

RESULTS: The findings showed that the alpha toxin gene locus sequence may change and is not conserved. In this research, 4 different patterns were identified based on enzymatic cleavage. Mutations in this locus can lead to diversity in *C. perfringens* biotype A and the creation of new strains.

CONCLUSIONS: The results of this research showed that the alpha toxin gene locus could be considered a DNA molecular marker in *C. perfringens,* and the PCR-RFLP technique can be used as a tool for typing this bacterium and estimating the phylogenetic relationships through comparative studies of nucleotide sequences.

Keywords: Alpha Toxin, *Clostridium perfringens*, Genome, Hemorrhagic Bowel Syndrome, Typing

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