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Rapid diagnosis of bacterial infections has been considered as important criteria for a patient management and an appropriate therapy of bacteria-induced disease in the early phase. Among several techniques, capillary electrophoresis-based single-strand conformation polymorphism (CE-SSCP) combined with 16S rRNA gene-specific PCR has come into the spotlight due to its benefits such as high sensitivity, resolution and great reproducibility. In this study, we developed a novel technique for quantitative pathogen diagnosis by CE-SSCP coupled with multiplex PCR. PCR primers were designed for the optimal separation in CE-SSCP using a stochastic model. PCR conditions were then optimized in order to obtain linear correlation between peak area and the concentrations of the target pathogens. Consequently, we developed a two-step CE-SSCP diagnosis method; first step for identification and the second step for quantification. As a model system, 8 species of clinically important bacteria were simultaneously identified and individually quantified. The results illustrated the potentials of this method on pathogen diagnosis and high-throughput drug discovery.