

Analysis of DNA *gyrA* Gene Mutation in Clinical and Environmental Ciprofloxacin-Resistant Isolates of Non-Tuberculous Mycobacteria Using Molecular Methods

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Abstract

Background

During the past several years, nontuberculous mycobacteria (NTM) have been reported as some of the most important agents of infection in immunocompromised patients.

Objectives

The aim of this study was to evaluate the ciprofloxacin susceptibility of clinical and environmental NTM species isolated from Isfahan province, Iran, using the agar dilution method, and to perform an analysis of *gyrA* gene-related ciprofloxacin resistance.

Materials and Methods

A total of 41 clinical and environmental isolates of NTM were identified by conventional and multiplex PCR techniques. The isolates were separated out of water, blood, abscess, and bronchial samples. The susceptibility of the isolates to 1 µg/mL, 2 µg/mL and 4 µg/mL of ciprofloxacin concentrations was determined by the agar dilution method according to CLSI guidelines. A 120-bp area of the *gyrA* gene was amplified, and PCR-SSCP templates were defined using polyacrylamide gel electrophoresis. The 120-bp of *gyrA* amplicons with different PCR-SSCP patterns were sequenced.

Results

The frequency of the identified isolates was as follows: *Mycobacterium fortuitum*, 27 cases; *M. goodnae*, 10 cases; *M. smegmatis*, one case; *M. conceptionense*, one case; and *M. abscessus*, two cases. All isolates except for *M. abscessus* were sensitive to all three concentrations of ciprofloxacin. The PCR-SSCP pattern of the *gyrA* gene of resistant *M. abscessus* isolates showed four different bands. The *gyrA* sequencing of resistant *M. abscessus* isolates showed 12 alterations in nucleotides compared to the *M. abscessus* ATCC 19977 resistant strain; however, the amino acid sequences were similar.

Conclusions

This study demonstrated the specificity and sensitivity of the PCR-SSCP method for finding mutations in the *gyrA* gene. Due to the sensitivity of most isolates to ciprofloxacin, this antibiotic should be considered an appropriate drug for the treatment of related diseases.

Keywords: Ciprofloxacin, DNA Gyrase A, PCR-SSCP