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Journal of Microbiological Methods 55 (2003) 797–800

Journal
of Microbiological
Methods

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Note

A real-time PCR assay for detection of isoniazid resistance in *Mycobacterium tuberculosis* clinical isolates

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Received 25 June 2003; received in revised form 16 July 2003; accepted 21 July 2003

Abstract

A real-time PCR genotypic assay was developed for the detection of isoniazid (INH) resistance in *Mycobacterium tuberculosis*. The assay detects mutations C(–15)T and, possibly, G(–24)T in the regulatory region of the *inhA* gene and proved as sensitive and specific as nucleotide sequencing in all the clinical isolates tested. Our assays mapped the mutations efficiently in 10 out of 35 resistant isolates, thereby covering 29% of all resistant strains.

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Keywords: Real-time PCR assay; Isoniazid resistance; *Mycobacterium tuberculosis*

Isoniazid (INH) is a critical component of the first-line multidrug therapy of tuberculosis (TB). Mutations in several genes and genomic regions of *Mycobacterium tuberculosis* are involved in the occurrence of resistance to INH (Piatek et al., 2000; Slayden and Barry, 2000). The *katG* gene is the most commonly targeted, with the majority of mutations occurring at codon 315 in 30–90% of INH-resistant strains depending on geographical areas (Haas et al., 1997; Kiepiela et al., 2000; Mokrousov et al., 2002; Piatek et al., 2000). Another frequent target is the regulatory region of the *inhA* gene, where mutations have been reported in up to 32% of INH-resistant isolates (Kie-

piela et al., 2000; Lee et al., 1999; Morris et al., 1995; Telenti et al., 1997). Mutations in other genomic regions, such as the promoter of *ahpC* gene and in the *kasA* gene, have been reported in 12–24% and 10–14% of INH-resistant strains, respectively (Kiepiela et al., 2000; Lee et al., 1999; Mdluli et al., 1998; Telenti et al., 1997). Genotypic assays that detect mutations within such regions are predictive of clinical drug resistance and have the potential to provide rapid detection of resistance in mycobacterial isolates. During the past few years, in fact, several genotypic assays have been developed for detection of the mutations responsible for INH resistance, particularly those at codon 315 of the *katG* gene (Telenti et al., 1997; Nachamkin et al., 1997; Garcia de Viedma et al., 2002; Piatek et al., 2000; Torres et al., 2000; Mokrousov et al., 2002). With regard to the regulatory

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Table 1
Characteristic melting temperatures for *inhA* mutation detection probe

No. of isolates	INH Phenotypic test ^a	Nucleotide sequence	Real-time PCR ($T_m \pm S.D.$)
21 ^b	susceptible	wild type	62.6 \pm 0.6
9	resistant	C(–15)T	58.2 \pm 0.2
1	resistant	G(–24)T	61.0 \pm 0.2 ^c
35	resistant	wild type	62.7 \pm 0.5

^a Determined by radiometric BACTEC system.

^b *M. tuberculosis* H37Rv is included.

^c Data derived from four determinations.

showed melting curves with peak T_m of 58.2 ± 0.2 °C, while 1 isolate yielded a melting curve with peak T_m of 61 ± 0.2 °C; nucleotide sequencing showed mutation C(–15)T for all the former isolates and mutation G(–24)T for the latter. The other 35 isolates phenotypically resistant to INH yielded basically the same T_m (62.7 ± 0.5 °C) as INH-susceptible isolates, thus indicating a wild-type genotype for the studied region. Indeed, nucleotide sequencing confirmed the absence of mutations in the entire regulatory region of the *inhA* gene. The melting curves of representative isolates with a wild-type genotype, and C(–15)T and G(–24)T mutations are given in Fig. 2.

Taken altogether, our results show that the real-time PCR assay described here is 100% concordant with the more laborious nucleotide sequencing method. The assay allows to detect the mutation C(–15)T with maximal sensitivity and specificity. A similar assay, which employs a different set of sensor/anchor hybridization probes that allows detection of C(–15)T mutation, has recently been published by Torres et al. (2003). Our assay also proved able to detect the mutation G(–24)T, adjacent to the anchor probe. However, as this mutation was harbored by only 1 of our 45 INH-resistant isolates, the result needs to be validated on a suitable number of isolates.

In conclusion, our LightCycler real-time PCR assay, covering 29% of all resistant isolates, appears to be excellent for rapid and reliable prediction of C(–15)T- and, possibly, G(–24)T-based phenotypical resistance to INH of *M. tuberculosis* isolates. The assay, used in combination with other available real-time PCR-based assays for *katG* gene mutations (Garcia de Viedma et al., 2002; Torres et al., 2000), might provide a rapid and accurate tool to identify most of the known mutations conferring resistance to INH in clinical isolates, as well as directly in clinical samples, which would minimize the time needed for initiation of a specific anti-TB chemotherapy.

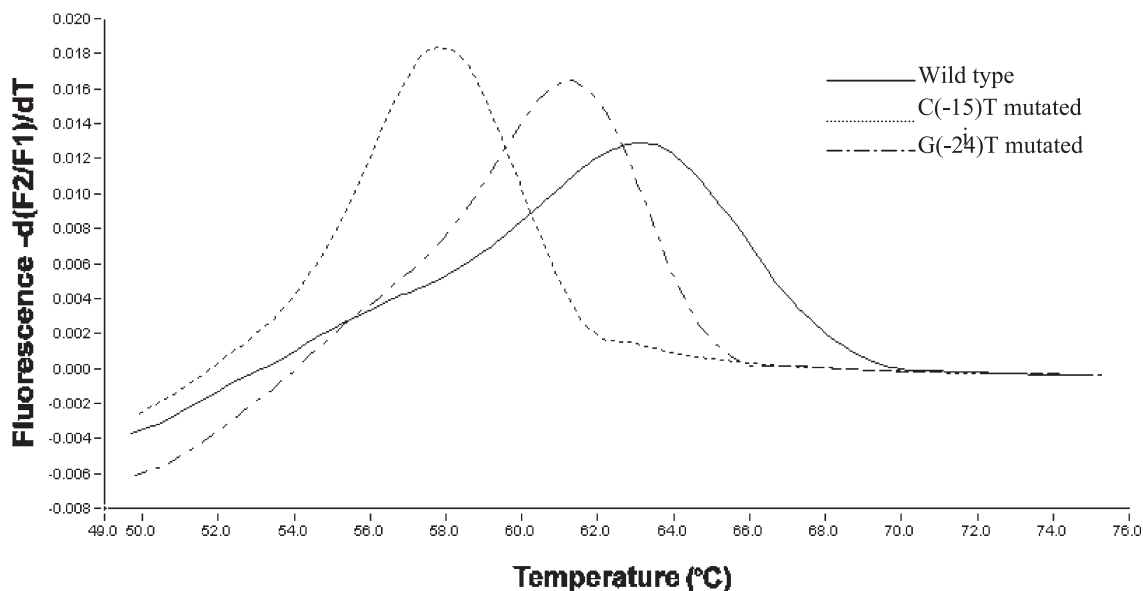


Fig. 2. Representative experimental melting patterns for wild type, C(–15)T and G(–24)T isolates, as measured in fluorimetric channel F2 by LightCycler.

Acknowledgements

This work was supported by grant Cofin-2002 from “Ministero della Istruzione, Università e Ricerca”, and partly by National Research Program on AIDS grant no. 50D.11.

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