Comparison of three PCR-based methods with high-pressure liquid chromatography (HPLC) analysis for the identification of *Mycobacterium kansasii* clinical isolates

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Objectives

Mycobacterium kansasii is among the commonest causes of pulmonary and extrapulmonary infections due to nontuberculous mycobacteria (NTM). Until recently, the reference method for species identification of NTM was high-pressure liquid chromatography (HPLC) analysis of mycolic acids, whose applicability is limited by the high cost of the instrumentation and the use of organic solvents. For rapid identification of mycobacteria, molecular techniques are gaining increasing importance.

The aim of this study was to compare three different PCR-based methodologies, i.e. the reverse hybridization line probe assay (GenoType Mycobacterium AS) (**i**), the *hsp65* (**ii**), and *rpoB* PCR restriction-enzyme analysis (PCR-REA) (**iii**) with the reference HPLC technique for the identification of *M. kansasii*. No such comparative study has been undertaken before.

Methods

A sample of 105 isolates recovered from as many patients with suspected *M. kansasii* infection were analyzed. All isolates were collected at the Department of Internal Medicine, Pulmonology, and Allergology of the Warsaw Medical University over a 13-year period (2000 to 2013) and identified as *M. kansasii* with the HPLC analysis, in accordance with the Centers for Disease Control and Prevention (CDC) guidelines. The GenoType Mycobacterium AS assay (Hain Lifescience, Germany) was done following the manufacturer's instructions. PCR-REA assays of the *hsp65* and *rpoB* genes were performed as described by Telenti *et al*. [1] and Kim *et al*. [2].

Results

For 98 (93.3%) isolates, the results from all the methods were consistent (Table 1). All but two (103/105; 98.1%) isolates produced the *M. kansasii*-specific pattern with the GenoType Mycobacterium AS assay. All but three (102/105; 97.1) and four (101/105; 96.2%) isolates were classified as *M. kansasii* by *hsp65* and *rpoB* PCR-REA, respectively. One strain was classified as *M. xenopii* by *rpoB* PCR-REA, though it was identified as *M. kansasii* with the remaining three methods.

Table I. Identification of *M. kansasii* by four different methods.

Number of strains	Method: HPLC	GenoType Mycobacterium CM/AS	*hsp65* PCR-REA	*rpoB* PCR-REA
98	*M. kansasii*	*M. kansasii*	*M. kansasii*	*M. kansasii*
2	*M. kansasii*	*M. kansasii*	*M. kansasii*	no PCR product
2	*M. kansasii*	*M. kansasii*	no PCR product	no PCR product
1	*M. kansasii*	not determined	no PCR product	*M. kansasii*
1	*M. kansasii*	*M. kansasii*	*M. kansasii*	*M. xenopii*
1	*M. kansasii*	other than *Mycobacterium* sp.	*M. kansasii*	*M. kansasii*

Conclusions

The results of the study revealed a high (96.2-98.1%) concordance of the HPLC analysis with the GenoType Mycobacterium AS assay and both of the PCR-REA assays. Of the three molecular methods evaluated, none could be considered superior over the other two. In the absence of the result, by one of the PCR-based methods, HPLC should be performed.

References

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