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 For 98 (93.3%) isolates, the results from all the methods were consistent (Table 1). causes of pulmonary and extrapulmonary infections due All but two (103/105; 98.1%) isolates produced the M. kansasii-specific pattern to nontuberculous mycobacteria (NTM). Until recently, with the GenoType Mycobacterium AS assay. All but three (102/105; 97.1) and four the reference method for species identification of NTM (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 was high-pressure liquid chromatography (HPLC) analysis of mycolic acids, whose applicability is limited it was identified as *M. kansasii* with the remaining three methods. by the high cost of the instrumentation and the use of organic solvents. For rapid identification of Table I. Identification of *M. kansasii* by four different methods. mycobacteria, molecular techniques are gaining Mathad 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 Conclusions (2000 to 2013) and identified as *M. kansasii* with the HPLC analysis, in accordance with the Centers for The results of the study revealed a high (96.2-98.1%) concordance of the HPLC Disease Control and Prevention (CDC) guidelines. The analysis with the GenoType Mycobacterium AS assay and both of the PCR-REA GenoType Mycobacterium AS assay (Hain Lifescience, assays. Of the three molecular methods evaluated, none could be considered Germany) was done following the manufacturer's superior over the other two. In the absence of the result, by one of the PCR-based instructions. PCR-REA assays of the *hsp65* and *rpoB* methods, HPLC should be performed. genes were performed as described by Telenti *et al.* [1] and Kim *et al*. [2].

Results

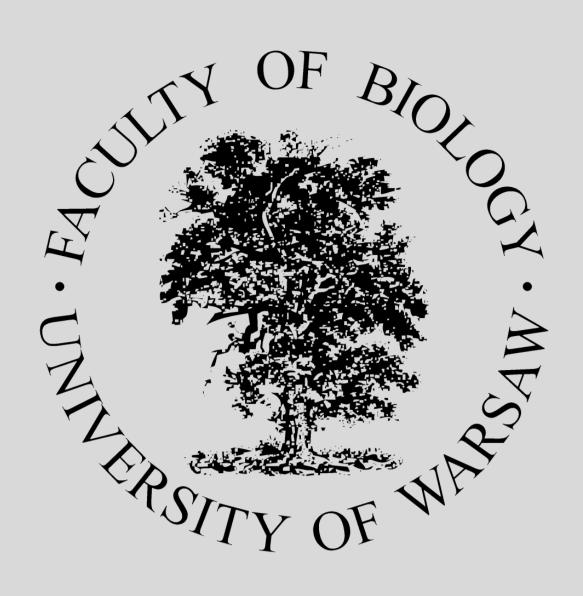
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|----------------------|-------------|--|----------------|---------------|
| Number of strains | HPLC | GenoType Mycobacterium CM/AS | hsp65 PCR-REA | rpoB PCR-REA |
| 98 | M. kansasii | M. kansasii | M. kansasii | M. kansasii |
| 2 | M. kansasii | M. kansasii | M. kansasii | no PCR produc |
| 2 | M. kansasii | M. kansasii | no PCR product | no PCR produc |
| 1 | M. kansasii | not determined | no PCR product | M. kansasii |
| 1 | M. kansasii | M. kansasii | M. kansasii | M. xenopii |
| 1 | M. kansasii | other than <i>Mycobacterium</i> sp. | M. kansasii | M. kansasii |

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[1] A. Telenti, F. Marchesi, M. Balz, F. Bally, E. C. Böttger, and T. Bodmer, "Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis," Journal of *Clinical Microbiology*, vol. 31, no. 2, pp. 175-178, 1993. [2] B. J. Kim, K. H. Lee, B. N. Park, S. J. Kim, G. H. Bal, S.J. Kim and Y. H. Kook, "Differentiation of mycobacterial species by PCR-restriction analysis of DNA (343 base pairs) of the RNA polymerase gene (rpoB)," Journal of Clinical Microbiology, vol. 39, no. 6, pp. 2102-2109, 2001.

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References

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