The predominance of subtype I among Mycobacterium kansasii clinical isolates from Poland, as evidenced by PCR restriction-enzyme analysis of the hsp65 gene

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Objectives

Mycobacterium kansasii is a slow-growing non-tuberculous mycobacterium (NTM) which causes pulmonary and extrapulmonary infections. It is also one of the most frequent NTM pathogens isolated from clinical samples throughout the world [1,2]. In Poland, among the cases of NTM disease, whose number has been increasing remarkably in recent years, those attributable to M. kansasii are in majority [3]. One in three NTM species isolated from patients with pulmonary mycobacterial infections is M. kansasii [1,3].

The heterogeneity within the M. kansasii species, evidenced by several molecular analyses, may have important pathogenic, clinical, and epidemiological implications. PCR restriction-enzyme analysis (PCR-REA) of the hsp65 gene has shown the presence of seven subtypes among both environmental and clinical isolates of M. kansasii. The results of the so far performed studies have suggested that M. kansasii isolates that are involved in human disease almost exclusively belong to types I and II, with the former predominating [4,5].

The aim of this study was to investigate the distribution of M. kansasii subtypes among Polish patients suspected of having pulmonary NTM disease.

Methods

A total of 153 isolates recovered from 87 patients with suspected M. kansasii infection (55 women and 32 men; age range: 27-92 years; median age: 65.7±16 years) were included in the study. All isolates were collected at the Department of Internal Medicine, Pulmonology, and Allergology of the Warsaw Medical University between 2000 and 2013. Each isolate was identified as M. kansasii based on the high-performance liquid chromatography (HPLC) analysis of the mycicolic acids. For the amplification of a 441-bp fragment of the hsp65 gene Tbl1 and Tbl2 primers were used, as described by Telenti et al. [6]. The PCR mixes were prepared with a TopTaq Master Mix kit (Qiagen) in a final volume of 50 µL containing ca. 10 ng of genomic DNA. Amplified fragments were digested with HaeIII and EcoRII (BstEII restriction enzymes; FastDigest®, under conditions recommended by the manufacturer (ThermoScientific), separated by electrophoresis in 4% agarose gels, and visualized by staining with ethidium bromide (0.5 µg/mL) and exposure to UV light.

Sequences were classified into subtypes based on their PCR-REA patterns obtained in two separate PCR-REA assays.

Results

All M. kansasii isolates tested yielded, upon PCR amplification, a single product of the expected size (ca. 440 bp). All but one (99%) isolates had indistinguishable patterns (HaeIII bands at 140, 105 and 80 bp, and BstEII bands at 240 and 210 bp) characteristic of subtype I. One isolate exhibited the subtype II pattern (HaeIII band at 140 and 105 bp and BstEII bands at 240, 195, 80 bp).

This study demonstrates that M. kansasii clinical isolates from Poland are almost exclusively of the hsp65 PCR-REA subtype I. The high detection rate of M. kansasii subtype I in clinical samples may suggest that this genotype has a particular ability for colonization and/or infection of the human host.

Conclusions

References