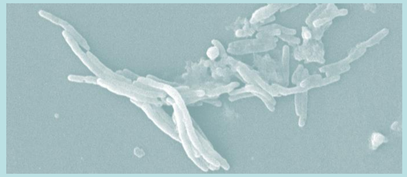

# The predominance of subtype I among *Mycobacterium kansasii* clinical isolates from Poland, as evidenced by PCR restriction-enzyme analysis of the *hsp65* gene

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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 mycolic acids.

For the amplification of a 441-bp fragment of the *hsp65* gene Tb11 and Tb12 primers were used, as described by Telenti et al. [6]. The PCR mixtures 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 Eco91I (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.

Strains 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, 135, 80 bp).

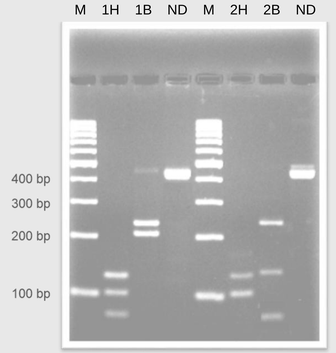


FIG.1. Differentiation of *M. kansasii* subtypes by PCR-REA of *hsp65*. Amplified *hsp65* fragments were digested with HaeIII (lanes 1H for type I and 2H for type II) and BstEII (lanes 1B for type I and 2B for type II). Lanes: M - GeneRuler 100 bp DNA Ladder (ThermoScienific), ND - non digested fragment of *hsp65*.

## Conclusions

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.

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