Global subtyping of *Mycobacterium kansasii* clinical isolates

K. CHOSZCZYK[1], Z. BAKUŁA[1], F. ARIAS[2], E. BACHIYSKA[3], E. BORRONI[4], D.M. CIRILLO[4], C. COULTER[5], C. GISKE[6], J. HUMIĘCKA[7], J. VAN INGEN[8], P. IOANNISDIS[9], K. KRANZER[10], U. KUZMIĈ[11], K. LEVINA[12], T. LILLERBAEK[13], I. MOKROUSOV[14], K. MORIMOTO[15], V. NIKOLAYEVSKYY[16], A. NORMAN[13], D. PAPAVENTSIS[9], O. PEUCHANT[17], A. SAFIANOWSKA[18], V. ULMANN[19], E. VASILIAUSKIENE[20], K. WON-JUNG[21], V. ZHURAVLEV[22], M.Ž. DOVĈ[11], R. KRENKE[18], T. JAGIELSKI[1]

[1]Department of Applied Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw, Poland
[2]Mycobacteria Laboratory, Public Health Institute of Chile, Santiago, Chile
[3]National Reference Laboratory for Tuberculosis, Sofia, Bulgaria
[4]Emerging Bacterial Pathogens Unit, San Raffaele Scientific Institute, Milano, Italy
[5]Queensland Mycobacterial Reference Laboratory, Queensland, Brisbane, Australia
[6]Department of Clinical Microbiology, Karolinska University Hospital, Solna, Sweden
[7]Hospital of Infectious Diseases in Warsaw, Warsaw, Poland
[8]Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands
[9]National Reference Laboratory for Mycobacteria, "Sotiria" Chest Diseases Hospital of Athens, Greece
[10]National Reference Center for Mycobacteria, Forschungszentrum Borstel, Leibniz-Zentrum für Medizin und Biowissenschaften, Borstel, Germany
[11]Laboratory for Mycobacteria, University Clinic of Respiratory and Allergic Diseases Golnik, Slovenia
[12]Mycobacteriology Section Laboratory, North Estonia Medical Centre, Tallinn, Estonia
[13]International Reference Laboratory of Mycobacteriology, Statens Serum Institut, Copenhagen, Denmark
[14]Laboratory of Molecular Microbiology, St. Petersburg Pasteur Institute, Russia
[15]Respiratory Disease Center, Fukujuji Hospital, Japan Anti-Tuberculosis Association, Tokyo, Japan
[16]Public Health England, National Mycobacterium Reference Laboratory, London, United Kingdom
[17]Laboratoire de Bactériologie, Centre Hospitalier Universitaire de Bordeaux, Bordeaux, France
[18]Department of Internal Medicine, Pulmonary Diseases & Allergy, Warsaw Medical University, Poland
[19]Institute of Public Health, Ostrava, Czech Republic
[20]Infectious Diseases and Tuberculosis Hospital, Vilnius University Hospital Santariskiu Klinikos, Lithuania
[21]Division of Pulmonary and Critical Care Medicine; Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, South Korea
[22]Research Institute of Phthisiopulmonology, St. Petersburg, Russia

Introduction and Aim. *Mycobacterium kansasii* is a slow growing non-tuberculous mycobacterium (NTM) which causes pulmonary and extrapulmonary infections. Although the incidence of *M. kansasii* shows significant geographical variability, it is, on average, the sixth most frequent NTM isolated from clinical samples throughout the world [1]. To date, seven (I-VII) subtypes of this species have been identified by PCR restriction-enzyme analysis (PCR-REA) of the *hsp65* (65-kDa heat shock protein), *rpoB* (β-subunit of RNA polymerase) and *tuf* genes [2-4]. Most of the disease-related strains belong to subtype I and II, while the other subtypes (III-VII) are considered nonpathogenic. The aim of this study was to investigate the global distribution of *M. kansasii* subtypes from clinical samples.

Materials and Methods. A total of 491 isolates of *M. kansasii* recovered from as many patients were used in this study. The isolates were collected from 18 countries (across 4 continents) between 2000 and 2017. Genomic DNA was extracted using GenoLyse kit (Roche, Basel, Switzerland). Subtyping was done according to isolates' PCR-REA patterns obtained in two PCR-REA assays i.e. described by Telenti et al. (*hsp65* gene) [4] and Bakuła et. al. (*tuf* gene) [1]. Briefly, PCR-amplified DNA fragments were digested with restriction

enzymes (FastDigest®), under conditions recommended by the manufacturer (ThermoScientific, Waltham, USA), separated by electrophoresis in 4% agarose gels, and visualized by staining with ethidium bromide (0.5 μg/mL) and exposure to UV light (λ = 320 nm). Patients were classified as having or not having a disease, following the criteria of the American Thoracic Society (ATS).

Results. Among 476 isolates, the most frequent were subtypes I (n=395; 82.98%) and II (n=43; 9.03 %). There were 18 (3.7%), 4 (0.84%), 3 (0.63%) and 13 (2.73%) isolates of subtype III, IV, V, and VI, respectively (**Table 1**). The largest number of *M. kansasii* subtype I isolates was observed for Poland (n=140; 98.6%). *M. kansasii* subtype II was the most frequent in Italy (n=7; 41.2%) (**Figure 1**). Isolates from patients meeting the ATS case definition criteria belonged to subtype I (90.4%), II (8.4%), III (0.6%) and IV (0.6%).

Table 1: Global distribution of *M. kansasii* subtypes.

Country	*M. kansasii* subtypes						TOTAL
	I	II	III	IV	V	VI	
Australia	18 (62.1%)	2 (6.9%)	3 (10.3%)	0	0	6 (20.7%)	29 (6.1%)
Bulgaria	2 (50%)	0	1 (25%)	0	0	1 (25%)	4 (0.8%)
Chile	13 (100%)	0	0	0	0	0	13 (2.7%)
Czech Republic	15 (68.2%)	0	2 (11.1%)	1 (5.6%)	0	0	18 (3.8%)
Denmark	18 (56.3%)	13 (40.6%)	0	0	0	1 (3.1%)	32 (6.7%)
Estonia	2 (50%)	0	0	0	0	2 (50%)	4 (0.8%)
France	10 (90.9%)	0	0	0	0	1 (9.1%)	11 (2.3%)
Germany	52 (52.1%)	9 (11.7%)	10 (13.9%)	3 (3.9%)	2 (2.6%)	1 (1.3%)	77 (16.2%)
Greece	4 (100%)	0	0	0	0	0	4 (0.8%)
Italy	9 (52.9%)	7 (41.2%)	1 (5.9%)	0	0	0	17 (3.6%)
Japan	9 (90%)	1 (10%)	0	0	0	0	10 (2.1%)
Lithuania	3 (60%)	1 (20%)	0	0	0	1 (20%)	5 (1.1%)
Poland	140 (98.6%)	1 (0.7%)	0	0	1 (0.7%)	0	142 (29.8%)
Russia	3 (42.9%)	4 (51.1%)	0	0	0	0	7 (1.5%)
Slovenia	30 (90.9%)	3 (9.1%)	0	0	0	0	33 (6.9%)
South Korea	17 (100%)	0	0	0	0	0	17 (3.6%)
Sweden	5 (71.4%)	2 (28.6%)	0	0	0	0	7 (1.5%)
UK	45 (97.8%)	0	1 (2.2%)	0	0	0	46 (9.7%)
TOTAL	395 (82.98%)	43 (9.03%)	18 (3.78%)	4 (0.84%)	3 (0.63%)	13 (2.73%)	476 (100%)

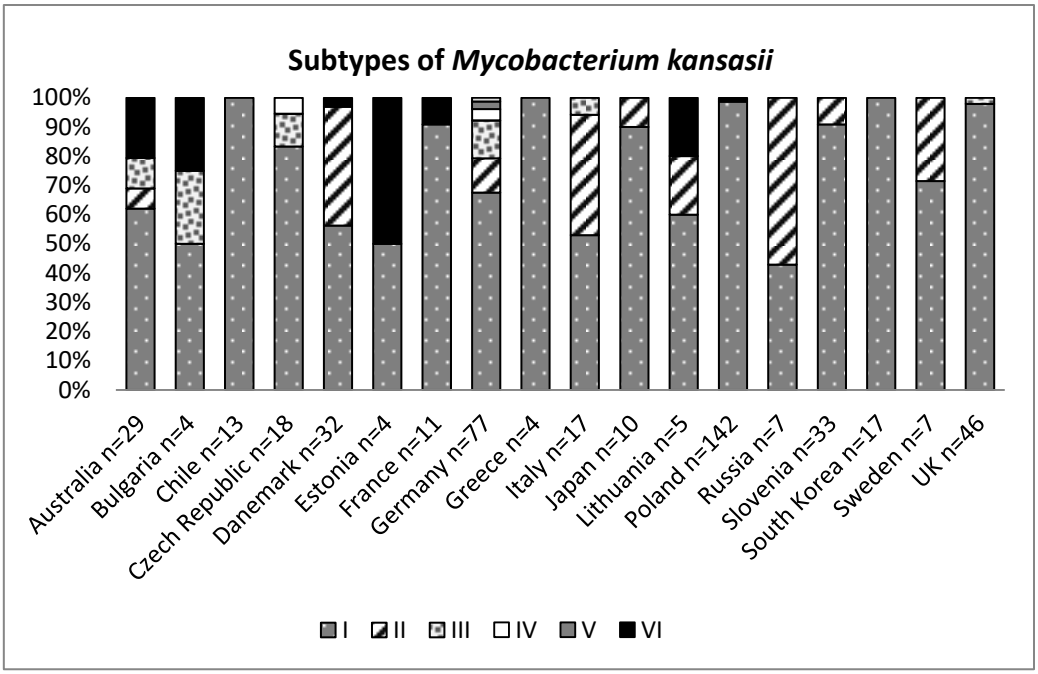

Figure 1: Worldwide distribution of *M. kansasii* subtypes.

Conclusion. The results of this study show, that subtype I and II predominate among *M. kansasii* isolates worldwide. However, important variation in the global distribution of *M. kansasii* subtypes was noted. Furthermore, not only *M. kansasii* subtype I and II, but also subtype III and IV were associated with the NTM disease.

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References

1. Hoefsloot W et. al. (2013): The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples, Eur Respir J 42: 1604-1613
2. Bakuła Z, et al. (2016): Proposal of a new method for subtyping of *Mycobacterium kansasii* based upon PCR restriction enzyme analysis of the *tuf* gene, Diagn Microbiol Infect Dis 84: 318-321
3. Picardeau M, et al. (1997): Genotypic characterization of five subspecies of *Mycobacterium kansasii*, J Clin Microbiol 35: 5-32
4. Telenti A, et al. (1993): Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis, J Clin Microbiol 31: 175-117