

Molecular typing of *Mycobacterium kansasii* clinical isolates from Europe

Zofia Bakula¹, Elizabeta Bachiyiska², Emanuele Borroni³, Daniela Maria Cirillo³, Joanna Humięcka⁴, Jakko van Ingen⁵, Panayotis Ioannidis⁶, Rafał Krenke⁷, Uros Kuzmič⁸, Klavdia Levina⁹, Igor Mokrousov¹⁰, Vlad Nikolayevskyy¹¹, Dimitrios Papaventsis⁶, Małgorzata Proboszcz⁷, Aleksandra Safianowska⁷, Vit Ulmann¹², Viacheslav Zhuravlev¹³, Manca Žolnir Dovč⁸, Tomasz Jagielski¹

¹Department of Applied Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw, Poland

²National Reference Laboratory for Tuberculosis, Sofia, Bulgaria

³Emerging Bacterial Pathogens Unit, San Raffaele Scientific Institute (HSR), Milano, Italy

⁴Hospital of Infectious Diseases in Warsaw, Warsaw, Poland

⁵Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands

⁶National Reference Laboratory for Mycobacteria, "Sotiria" Chest Diseases Hospital of Athens, Greece

⁷Department of Internal Medicine, Pulmonary Diseases & Allergy, Warsaw Medical University, Poland

⁸Laboratory for Mycobacteria, University Clinic of Respiratory and Allergic Diseases Golnik, Slovenia

⁹Mycobacteriology Section Laboratory, North Estonia Medical Centre, Tallinn, Estonia

¹⁰Laboratory of Molecular Microbiology, St. Petersburg Pasteur Institute, Russia

¹¹Public Health England (PHE) National Mycobacterium Reference Laboratory, London, United Kingdom

¹²Institute of Public Health, Ostrava, Czech Republic

¹³Research Institute of Phthiopulmonology, St. Petersburg, Russia

The study was financed by the National Centre for Research and Development «LIDER» Programme (LIDER/044/457/L-4/12/NCBR/2013) and the Faculty of Biology “DSM” grant (501-D114-86-0115000-01)

Background: *Mycobacterium kansasii* is an established opportunistic pathogen and one of the six most frequently isolated non-tuberculous mycobacterial (NTM) species worldwide. The genetic heterogeneity of *M. kansasii* is defined by the presence of seven genetic subtypes. Types I and II are associated with disease in humans, whereas type III-VII are commonly isolated from the environment and rarely cause disease. Therefore, subtyping of *M. kansasii* isolates from human samples is helpful for clinical diagnosis.

The aim of this study was to determine the distribution of *M. kansasii* subtypes among clinical isolates in different European countries.

Materials/methods: A total of 279 isolates recovered between 2000 and 2017 from as many patients with suspected *M. kansasii* infection were analyzed. The isolates were collected from different European countries (i.e. Bulgaria, Czech Republic, Estonia, Greece, Italy, Poland, Russia, Slovenia, and the UK). For the PCR restriction-enzyme analysis genotyping, protocols described by Telenti et al. (J. Clin. Microbiol., 1993, 31:175-178) and Bakula et al. (Diagn. Microbiol. Infect. Dis., 2016; 84:318-321) were used. The patients were categorized as having *M. kansasii* disease following the American Thoracic Society 2007 diagnostic criteria.

Results: The vast majority of isolates (250; 89.6%) had indistinguishable patterns characteristic of subtype I. Fifteen isolates (15; 5.4%) exhibited the subtype II pattern. There were 8 (2.9%), 1 (0.35%), 1 (0.35%) and 4 (1.4%) isolates of type III, IV, V, and VI, respectively. The isolates of type I, II, and III were isolated from both disease-associated and non-disease associated cases, whereas the isolates of type IV and VI from non-disease associated cases only. For one type V isolate, data concerning *M. kansasii* disease was unavailable. The highest frequency of *M. kansasii* type I isolates was observed for Poland (140/142; 98.6%), and the lowest for Estonia (2/7; 28.6%).

Conclusions: This study demonstrated that subtype I predominates among *M. kansasii* isolates in Europe and it has been isolated from both disease-related and non-disease-related clinical cases. Therefore, genotyping in a format currently available does not permit differentiation between isolates of clinical relevance and those representing merely isolation, without causing a disease. Furthermore, the genetic diversity of the European *M. kansasii* population show important regional variation.