

## **Clinical relevance of new and rare species of non-tuberculous mycobacteria**

Matúš Dohál<sup>1\*</sup>, Michaela Horníková<sup>2</sup>, Margo Diricks<sup>3,4</sup>, Nils Wetzstein<sup>3,4,5</sup>, Simona Mäsiarová<sup>6</sup>, Erik M Rasmussen<sup>7</sup>, Mária Škereňová<sup>1</sup>, Igor Porvazník<sup>8,9</sup>, Ivan Solovič<sup>8,9</sup>, Jarmila Hnilicová<sup>10,11</sup>, Věra Dvořáková<sup>2</sup>, Juraj Morký<sup>6</sup>

<sup>1</sup>Biomedical Centre Martin, Jessenius Faculty of Medicine in Martin, Comenius University, Bratislava, Slovakia;

<sup>2</sup>National Institute of Public Health, Prague, Czech Republic; <sup>3</sup>Molecular and Experimental Mycobacteriology, Research Center Borstel, Borstel, Germany; <sup>4</sup>German Center for Infection Research (DZIF), Partner Site Hamburg-Lübeck-Borstel-Riems, Borstel, Germany; <sup>5</sup>Department of Internal Medicine, Infectious Diseases, Goethe University, University Hospital, Theodor-Stern-Kai; <sup>6</sup>Department of Pharmacology, Jessenius Faculty of Medicine in Martin, Comenius University, Bratislava, Slovakia; <sup>7</sup>International Reference Laboratory of Mycobacteriology, Statens Serum Institut, Copenhagen, Denmark; <sup>8</sup>National Institute of Tuberculosis, Lung Diseases and Thoracic Surgery, Vyšné Hágy, Slovakia; <sup>9</sup>Faculty of Health, Catholic University, Ružomberok, Slovakia; <sup>10</sup>Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic; <sup>11</sup>Department of Genetics and Microbiology, Faculty of Science, Charles University, Prague, Czech Republic

The number of reported infections caused by nontuberculous mycobacteria (NTM) is increasing, but the true prevalence remains uncertain. The emergence of rare and emerging NTM species further complicates the management and control of these infections. However, characterizing individual NTM species is crucial for proper patient management.

In the study, we performed targeted sequencing and whole genome sequencing (WGS) on all NTM isolates that were not identified to the species level using line-probe assays between 2019 and 2023 in the Czech Republic and Slovakia. Minimal inhibitory concentrations of amikacin, ciprofloxacin, moxifloxacin, clarithromycin, and linezolid were determined. Clinical data were collected for all patients and treatment outcome was evaluated in patients with confirmed infection.

Twenty-eight cultures from different patients were included, of which 9 met ATS/IDSA criteria (7/9 with confirmed pulmonary infection). Analysis of targeted sequencing data allowed classification of 26 (92.8%) isolates to species level. Based on WGS data available for 19 isolates, the concordance rate with tNGS was 63.2%, with the main difference observed in the identification of mixed NTM species in a single sample.

Our study demonstrates the effectiveness of targeted sequencing and WGS in identifying rare and novel NTM species and unexpectedly high rate of clinically relevant NTM disease.

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