

Genomic epidemiology and clinical management of nontuberculous mycobacteria isolated from patients in Ukraine, 2022-2024

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Background. Nontuberculous mycobacteria (NTM) are increasingly recognised as important opportunistic pathogens causing chronic pulmonary disease, particularly in patients with structural lung damage, chronic respiratory disorders, or immunosuppression. In Eastern Europe, however, the epidemiology of NTM remains poorly defined. In Ukraine, where the diagnostic pathway is largely focused on tuberculosis, NTM strains are likely under detected, misclassified, or treated empirically without species-level confirmation. We therefore aimed to characterise the spectrum of NTM strains isolated from Ukrainian patients with suspected mycobacterial disease, assess whether clustering patterns support ongoing transmission, and evaluate current clinical management in a resource-constrained setting.

Materials and methods. We performed a prospective observational cohort study at two major mycobacterial referral centres in the Kharkiv and Zaporizhzhia regions between December 2022 and February 2024. During the study period, 19,529 clinical specimens from approximately 8,200 patients were submitted for mycobacterial culture. Adult patients with respiratory and/or systemic symptoms, radiographic pulmonary abnormalities, at least one positive NTM culture, and a negative Xpert MTB/RIF result were eligible. Species identification was performed locally with line probe assays in Kharkiv and all strains were analyzed by whole-genome sequencing (WGS). Bioinformatic analysis included taxonomic classification, resistance prediction, and phylogenetic comparison with more than 4,200 publicly available NTM genomes. For *Mycobacterium kansasii*, *Mycobacterium avium*, and *Mycobacterium abscessus*

strains, a cluster analysis was performed using core genome Multi Locus Sequence Typing (cgMLST) with a threshold of maximum 10 allelic differences. Clinical records were reviewed for demographics, comorbidities, radiographic findings, treatment, and outcomes.

Results. After exclusion of contaminated and low-quality samples, 100 NTM strains from 99 patients were available for analysis. WGS identified 21 different species and two closely related strains representing a potential novel species most closely related to *Mycobacterium paragordoniae*. The NTM population was dominated by *M. kansasii* strains (43 %), followed by *M. avium* (16 %), and *M. abscessus* (5 %) strains, and strains of several less frequent or uncommon species. WGS also resolved multiple discrepancies with line probe assay results, mainly involving rare taxa or closely related species.

Among 41 evaluable *M. kansasii* isolates, 16 (39.0 %) formed six small cgMLST clusters, each confined to a single participating centre, suggesting geographically restricted circulation without evidence of a large outbreak. In a global comparison with 668 publicly available genomes, all Ukrainian *M. kansasii* isolates remained more than 10 alleles distant from international strains, supporting predominantly local rather than recent cross-border transmission. Five *M. abscessus* strains belonged to globally recognised dominant circulating clonal complexes, but no evidence of recent local spread was observed; the minimum allelic distance between Ukrainian strains was 25.

The median age of the patients was 50 years, 68.7 % were male, and all presented with pulmonary symptoms and radiographic abnormalities. Frequent comorbidities

included chronic obstructive pulmonary disease (n=20), anaemia (n=12), HIV infection (n=6), and previous tuberculosis (n=4). Cavitory disease was mainly associated with *M. kansasii* strain isolation. All patients received antimycobacterial therapy, but regimens often diverged from international guideline-based, species-adapted treatment and were commonly initiated empirically. Clinical improvement was documented in 89 of 99 patients, whereas eight patients died during follow-up. At the same time, confirmatory investigations required for a definitive diagnosis of NTM pulmonary disease, particularly repeat species confirmation and advanced chest imaging, were frequently unavailable.

Conclusions. This first systematic genomic study of NTM strains in Ukraine demonstrates a highly diverse

NTM population dominated by *M. kansasii* and *M. avium* strains, with no evidence of large-scale transmission or nationwide outbreaks. The observed genomic structure is more consistent with sporadic acquisition and small local clusters than with sustained epidemic spread. The study also reveals major diagnostic and therapeutic gaps, including limited access to species-level confirmation, incomplete adherence to international diagnostic criteria, and widespread empirical treatment without microbiological guidance. These findings support the urgent need to strengthen laboratory capacity, establish national guidance for NTM disease confirmation and management, and integrate WGS-based surveillance into routine clinical practice to improve diagnostic precision and rational antimicrobial use.