Evaluation of Molecular Tests With Different Complexities for Detection of Multi-Drug Resistant Tuberculosis at the Time of Russia's Invasion of Ukraine: Results From TBpreparedness4Ukraine Study

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RATIONALE: High burden of multidrug- and rifampicin-resistant tuberculosis (MDR/RR-TB) combined with ongoing war remain the main challenges to control TB in Ukraine. In 2023, Ukraine reported 2944 MDR/RR-TB cases - in 2.7 times (1108) more than all 30 European Economic Area countries and USA together (WHO, 2024). However, only every second TB patient in the world was initially diagnosed with a WHO-recommended rapid test. The aim of this study was to evaluate diagnostic accuracy of mfloDx MDR-TB assay (EMPE Diagnostics AB, Sweden), TB Resistance DNA-array (GenID GmbH, Germany) and targeted next generation sequencing (tNGS) Deeplex Myc-TB (GenoScreen, France) in MDR-TB detection compared with culture-based drug susceptibility test (pDST). **METHODS:** This was a prospective cohort study. Sputum samples from 226 TB patients (mean age 43.2 (95%CI 41.3-45.1) years; 32 (16.8%) individuals were female) from Ukraine (n=76, 33.6%), Moldova (n=38, 16.8%) and Spain (n=112, 49.6%) with bacteriologically confirmed TB were consecutively tested at the Germans Trias i Pujol Research Institute, Badalona, Spain from September 2021 to August 2024. Each clinical site received ethical approval from independent Ethical Review Board. All patients gave their informed consent before providing sputum sample. **RESULTS:** A total of 143/226 (63.3%), 104/215 (48.4%) and 24/30 (80.0%) sputum specimens tested by mfloDx MDR-TB assay, TB Resistance DNA-array and Deeplex Myc-TB, respectively, had a conclusive result with certain interpretation of resistance. The mfloDx™ MDR-TB assay and TB Resistance DNA-array were capable of simultaneous detection of resistance to INH and RIF showing sensitivity 100 (95%CI 93.0-100) and 94.1 (95%CI 80.3-99.3) while specificity was 98.7 (95%CI 92.8-100) and 96.3 (95%CI 87.3-99.6), respectively (kappa=0.94 (95%CI 0.88-0.99) and kappa=0.90 (95%CI 0.81-1) respectively, p<0.01). We observed 100 (95%CI 82.4-100) sensitivity for Deeplex Myc-TB in detection of MDR-TB. However, there were

documented 4 episodes of false MDR-TB detection. All 4 strains of *Mycobacterium tuberculosis complex* belonged to Beijin clade phylogenetic Lineage 2 harboring high-confidence INH and RIF mutations in *katG* S315T and rpoB S450L gene loci. Considering the fact that all 4 TB patients were successfully treated with 1st line regimen, we could explain the observed discordance between tNGS and pDST either by heteroresistance (both susceptible and resistant populations coexist within a sample) or suspicion of cross-contamination. **CONCLUSIONS:** Rapid detection of MDR-TB in sputum samples using new molecular tests could reduce the time to appropriate treatment initiation minimizing impact of attacks on Ukraine's energy infrastructure and fossil fuels electricity generation for long-lasting culture-based DST.

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