Supplementary methods

Phenotypic antimicrobial susceptibility testing

Cultures from Lowenstein-Jensen medium, homogenized in 4.5 ml sterile water, and adjusted to McFarland standard 1.0 using optical density measurement, were transferred and grown in MGIT960 according to manufactured instructions (BD Microbiology systems, USA). The purity of the MGIT culture was checked by Ziehl–Neelsen stain, blood agar plate and MPT64 antigen rapid test (BD Microbiology systems, USA). A positive MGIT tube was used for DST up to and including the fifth day after incubation. 1.0 ml of the culture suspension was transferred to a container with 4.0 ml of sterile saline. 0.5 ml of 1:5 dilution of the test culture was used to inoculate in tubes containing the anti-TB agent 0.5-fold, 1-fold, and 2-fold of the current critical concentration of bedaquiline, clofazimine and linezolide, i.e. 1mg/L (WHO). The growth control in drug free medium was inoculated at 1:500. DST results were determined at the time the growth control tube displays >400 Growth Units (GU) between day 4 and day 13. The interpretation of the DST results was as follows: no growth in the drug vial "susceptible", 1-399 GU "intermediate", and >400 GU "resistant".

Isolation of genomic DNA

M. tuberculosis complex strains were inoculated on Löwenstein Jensen medium at 37 °C, until growth was clearly visible. Colonies were transferred to a microcentrifuge tube (2.0 ml) containing 400 µl TE buffer and heated for 20 min at 80°C to kill the bacteria. After 3 min centrifugation at 13,000 g we discarded the supernatant and added 400 µl TE-buffer, followed by vortexing to separate cells. We then added 50 µl lysozyme (10 mg/ml) vortexed briefly and incubated the solution overnight at 37 °C. The next day, we added 70 µl 10 % SDS, 5 µl proteinase K (10 mg/ml), vortexed softly and incubated the solution 10 min at 65 °C. Subsequently, we added 100 µl 5M NaCl, 100 µl CTAB/NaCl (pre-warmed at 65 °C), followed by vortexing and incubation for 10 min at 65° C. we then added 750 µl Chloroform/Isoamyalcohol mix (24:1?), inverted the tube few times and centrifuged at room temp for 15 min at 13,000 g. The aqueous supernatant was carefully transferred to a new microcentrifuge tube, and 0.6 volume isopropanol was added to precipitate the nucleic acids for 30 min at -20 °C (or longer). We then centrifuged for 10 min at room temperature at 13,000 g, discarded the supernatant, and washed the DNA in 0.5 ml of cold 75 % Ethanol while inverting the tube few times, followed by 5 min centrifugation at room temperature and discarding the supernatant cautiously. The DNA-pellet was dried at 60 °C for about 10 min, and DNA was eventually dissolved in 100 µl TE-buffer at 37 °C for 30 min or at room temperature until DNA was completely dissolved.

Rifampicin resistant tuberculosis treatment guidelines in the Republic of Moldova (2016-2018)

During the study, the treatment for MDR-TB patients in the Republic of Moldova was provided according to the National TB Treatment Protocol in accordance with the MDR-TB treatment guidelines of the WHO [1,2]. All patients initially started with a standardized regimen of five second-line TB drugs including a fluroquinolone (levofloxacin or moxifloxacin), a second-line injectable (capreomycin or amikacin), ethionamide, cycloserine and pyrazinamide). The standardized regimen was then adjusted, when necessary, once results of phenotypic drug susceptibility testing (DST) became available. The treatment duration was guided by the time of sputum culture conversion and consisted of an initial 6-8 months intensive phase followed by a continuation phase with a duration of 12-16 months.

Statistics

Patients with available baseline isolates and treatment outcomes were divided into two groups: positive treatment outcome (considered as cure, i.e. no signs of disease relapse (clinical or microbiological) up to 6 months after treatment completion) and negative treatment outcome (including death caused by TB or other causes, and treatment failure, i.e. no negative culture within 8 months of treatment). The following predictors for negative treatment outcome were analyzed: MTBC lineage, gender, case classification (new case, and previously treated), XDR (i.e. MDR with additional resistance against a fluoroquinolone and a second-line injectable drug; WHO classification until 12/2020), presence of cavities, HIV status, age, and number of drugs with predicted resistance at baseline that were included in the bedaquiline-based regimen, i.e. inactive drugs. Pairwise Fisher Exact tests were employed to compare differences between predictors with categorical variables and odds ratios were calculated for 2x2 contingency tables. For contingency tables with zero cell counts we used the Haldane-Anscombe correction by adding 0.5 to each cell. To compare predictors with continuous variables, i.e. age and number of inactive drugs, we employed logistic regression analysis. The difference between number of inactive drugs between patients with positive and negative treatment outcomes as well as patient age were compared with a Mann-Whitney-U test, as we did not assume a normal distribution ($P<0.001$, Shapiro-Wilk normality test). Pairwise comparisons of patients characteristics (included vs excluded patients) were performed with Fisher Exact tests.

References

[1] Ministerul Sănătăţii al Republicii Moldova Tuberculoza la adult Protocol clinic naţional PCN-123. https://msmps.gov.md/wp-content/uploads/2021/02/PCN-12-Tuberculoza_la_adult.pdf Date last accessed July 12, 2021

[2] Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis. https://apps.who.int/iris/bitstream/handle/10665/130918/9789241548809_eng.pdf?sequence=1 Date last accessed July 12, 2021