SCIENTIFIC DAYS 10-12 May 2022

MEDECINS SANS FRONTIERES

Clinical utility of target-based next-generation sequencing for drug-resistant tuberculosis: a pilot from Mumbai, India

*Homa Mansoor[1], Nilma Hirani[2], Vijay Chavan[1], Ameeta Joshi[2], D. Vikas Oswal [3]Jaishankar Sharma[1], D. Aparna Iyer[1], Kathleen England[4], Zibisiso Ndlovu[5], Arunima Silsarma[1], Petros Isaakidis[5], Mabel Morales[1]

*MSFOCB-Delhi-MED@brussels.msf.org

[1]Médecins Sans Frontières (MSF), Mumbai, India; [2]Grant Medical College & Sir J J Group of Hospitals, Mumbai, India; [3]National Tuberculosis Elimination Program, Mumbai, India; [4]Independent Consultant, Honolulu, USA; [5]MSF, Cape Town, South Africa

Introduction

In countries with a high tuberculosis (TB) burden, poor access to drug susceptibility testing is a major bottleneck in diagnosing drug-resistant (DR) TB. India is estimated to account for a quarter of multidrug-resistant (MDR)-TB patients globally, with around

124,000 cases in 2020. Mumbai, a densely populated city in Maharashtra State, is a DR-TB hotspot with 24% of treatment-naïve cases, and 41% of previously-treated cases, having MDR-TB, and a high frequency of fluoroquinolone resistance occurring among these MDR-TB cases. Targeted next-generation sequencing (tNGS) is a promising technology for rapid detection of resistance. We assessed the role of tNGS for diagnosis of DR-TB.

Methods

We performed a laboratory-based study involving *Mycobacterium tuberculosis* (MTB)-positive samples from patients with presumptive TB or DR-TB identified by GeneXpert in Shatabdi Hospital, Mumbai. A total of 161 sputum samples from bacteriologically-confirmed TB cases were included in the study. The study was conducted at Sir JJ Hospital's TB lab, with sample collection occurring from patients living in M-East Ward (MEW), Mumbai. Two sputum samples were collected from each presumptive TB patient at MEW. Spot samples with a positive result on Xpert MTB/Rif were sent for tNGS and conventional testing (phenotypic drug sensitivity testing (pDST), line probe assays (LPA), and mycobacteria growth indicator tubes (MGIT)) at Sir JJ Hospital's TB lab. tNGS samples were processed using Deeplex MycTB-kit (GenoScreen, France) and sequenced on a MiSeq platform (Illumina, USA). These samples were also processed for pDST using 16 drugs on MGIT (Becton Dickinson, USA) and LPA (MTBDRplus and MTBDRsl, Hain Lifesciences, Germany). To ensure sequence quality, Xpert results with cycle threshold values <20 or direct smear results >2+ were prepared for tNGS using direct sputum sediments. Primary cultures were prepared for samples with lower bacterial loads.

Ethics

This study was approved by the ethics committee of the Grant Medical College & Sir J J Group of Hospitals, Mumbai, India. Permission was granted by the Medical Director of MSF, Operational Centre Brussels.

Results

The median age of patients with samples included was 24 years (interquartile range, 20-40), and 57% were female. Approximately 70% of cases had no previous history of TB. Of 161 samples evaluated, 15 (9.3%) were rifampicin-sensitive and 146 (90.7%) were rifampicin-resistant (RR). 161 samples with completed pDST, tNGS and LPA were analysed. Of these, 88.2% had RR/MDR-TB resistance per WHO definitions, 58.5% had additional fluoroquinolone-resistance (pre-XDR) and 9.2% had fluoroquinolone resistance plus resistance to either linezolid or bedaquiline (extensively drug-resistant (XDR). Thirteen of 161 samples (8%) were culture-negative, yet resistance to one or more drugs was demonstrated in 8/13 samples with tNGS. Resistance frequency was similar across methods, with discordance in drugs less reliable in pDST or limited mutational representation within databases. Sensitivities aligned with the WHO catalogue for most drugs. 10% of the sample showed hetero-resistance and 75% of strains were of lineages 2 and 3.

Conclusion

In countries with a high burden of DR-TB, and high transmission rates, tNGS can provide information to rapidly design individualised regimens for early initiation and effective case management. It also gives information regarding lineages, uncharacterized mutations, hetero-resistance and mixed infection status of TB cases. Potentially tNGS could provide a diagnostic tool for rapid initiation of treatment in high DR-TB settings.

Conflicts of interest

None declared.

Dr. Homa Mansoor is a clinician with a background in tropical medicine. She joined MSF as a national doctor in Mumbai in 2007 and worked in the domains of HIV 2nd and 3rd line treatment, drug-resistant tuberculosis, and hepatitis C virus disease. Presently she is associated with MSF, Operational Centre Brussels, as mission technical referent. She has been working in the field of tuberculosis for over 14 years, focussing on diagnosis and treatment as well as managing co-morbidities and co-infections, and responsible for research, policy, and advocacy. She was involved in a joint monitoring mission in 2015 to review India's Revised National TB Control Programme, and has recently contributed to the development of WHO's consolidated guidelines on tuberculosis, Module 5: Management of Tuberculosis in Children and Adolescents. She has co-authored numerous operational research papers in national as well as international journals.