

Abstract

Mycobacterium tuberculosis (*M. tuberculosis*) is the causative agent of tuberculosis (TB) and remains the leading cause of death among various infectious agents. The disease is curable as it has both a vaccine and chemotherapy but due to the adaptive nature of *M. tuberculosis*, it is highly capable of evading host immunity by becoming phenotypically drug-tolerant or drug-resistant which negatively impacts the treatment outcomes and increases mortality rates. In addition, various studies have identified a non-replicating, drug-tolerant heterogeneous bacterial population termed differentially culturable tubercle bacilli (DCTB) in TB sputum samples that are most likely adding to the challenges involved in eradicating the disease. These DCTB are only resuscitated and detected in a liquid-based limiting dilutions most probable number assay and not by standard culture methods such as the colony forming unit and MGIT assays. Moreover, these DCTB are shown to be present at diagnosis and throughout the course of treatment indicating that the first-line drugs (rifampicin (RIF), isoniazid (INH), ethambutol (EMB) and pyrazinamide (PZA)) are unable to eliminate DCTB. Presently, it is unclear how these DCTB adapt and promote their survival during TB treatment therefore, determining whether these DCTB are phenotypically drug-tolerant or genetically resistant will aid in the development of novel drugs and improved treatment regimens in an attempt to lessen the TB burden. In this study, we purified and analyzed stored resuscitated DCTB samples from MPN assays performed in a previous study that quantified the DCTB population in sputa from TB patients on treatment. Once purified, the drug susceptibility of these samples to RIF, INH, EMB and PZA were determined using the broth microdilution assay. The data showed that 4 out of 9 patients had samples displaying drug resistance to one or more of the first-line drugs. The resuscitation of the bacteria in the MPN assay may have resulted in a loss of the DCTB phenotype thus, the resuscitated bacteria were forced back into a reversible non-replicating, drug-tolerant DCTB state using a carbon starvation model. Broth microdilution assays were performed on the DCTB samples to assess the drug tolerance of the bacteria to RIF, INH and EMB compared to the unstarved bacteria. The data showed that under stressful conditions, the bacteria revert to the DCTB state and have a higher drug tolerance than the unstarved bacteria. Lastly, the resistance-conferring regions of RIF, INH and EMB were amplified by polymerase chain reaction (PCR) for all patient samples displaying resistance to any of the drugs and sequenced to identify mutational changes. The sequencing data showed that these DCTB are phenotypically tolerant to RIF, INH and EMB. The data showed that TB bacterial populations are underestimated which may result in misdiagnosis and premature termination of treatment.