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In vitro starvation model for assessing phenotypic drug tolerance on *Mycobacterium tuberculosis* lineages in Ethiopia

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Background: *Mycobacterium tuberculosis* persist in the human host for decades and reactivation can occur at any point. Becomes dormant and phenotypically drug tolerant when exposed adverse conditions. Understanding of the signals and processes which allow the bacteria to achieve this feat could potentially be used as a baseline to design new types of drugs or modify old drug regimens for improved cure and avert development of drug resistance.

Objective: To use *in vitro* starvation model in assessing if nutrient deprivation affects phenotypic drug tolerance in *Mycobacterium tuberculosis* lineages circulating in Ethiopia.

Methods: Three MTB lineages and one standard susceptible reference strain (H37Rv) were tested by different test methods at different time point from March to September 2017. All lineages tested to be sensitive to first line anti Tb drugs. Log phase (highest colony count on week 3-4) culture from Lowenstein Jenson medium was sub cultured to Middle-brook7H9 with 10% oleic acid albumin dextrose catalase as a normal, Phosphate Buffer Solution (PBS) (pH 7.2) and Sterile Distilled Water (SDW) as starvation media were used. Each week we performed culture growth reading, acid fast stain (AFS) by Ziehl Neelson (ZN), Lipid Bodies (LB) by Sudan black stain and viability by Fluorecin Diacetate (FDA) staining. On week 0, 3 and 6 drug susceptibility test was done by colorimetric MTT assay. Graph pad prism 6 and SPSS V20 used for data analysis.

Results: A total of 576 experiments were performed using4 strains of *Mycobacterium Tuberculosis* sub-cultured on SDW, PBS and 7H9. Of these, 324 microscopic tests using (108 (ZN) acid fastness, 108 (FDA) viability and 108 (Sudan black stain) lipid bodies), 108 culture growth reading done. After week 6 acid fastness, viability and culture growth decreased. 144 phenotypic DST done using MTT assay. A higher inhibitory drug concentration was required at the 6th week compared to the baseline and C50 (RMP=0.5; INH=0.1; STM=2.0 and for EMB=4.0), yet the proportion of lipid body containing bacilli increased continuously in all lineages.

Conclusion: Our study showed that the mycobacteria lineages behaved similarly in all media systems and reached stationary phase at similar time. The increased drug concentration observed at the 6th week coincided with the decline in viable *Bacilli* in all media systems, thus attributing this phenomena to lipid body accumulation alone was difficult.

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