

MIC testing of OSTB compounds

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Rationale

The model for measuring MIC of *Mycobacterium tuberculosis* (*M. tb*) in biofilms is difficult to scale up to provide repeat testing. We have adopted the strategy of growing a biofilm, then using that to seed a MGIT tube and then using that as the inoculum for MIC testing. Whilst this seems counter intuitive (the bacteria have been 'activated' and returned to planktonic phase) our experience is that the resulting culture retains a 'biofilm' phenotype to some degree. It is imperfect and needs further characterisation but has provided useful information in the past.

Materials and Methods

Preparation of Test Inoculum

Drug sensitive (DS) and drug resistant (DR) *M. tb* clinical isolates were recovered from -80°C, in addition to the reference laboratory strain H37Rv. From the frozen aliquots 50 µl was inoculated into Mycobacterium Growth Indicator Tubes (MGIT) (Becton Dickinson, BD) containing 800 µl of reconstituted MGIT PANTA (Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim, Azlocillin) with MGIT OADC (Oleic Acid Albumin Dextrose Catalase) growth supplement. The tubes were incubated within the BD BACTEC MGIT 960 machine (BD). Once positive the MGIT tubes were removed from the machine and were used for minimum inhibitory concentration (MIC) testing. A positive MGIT tube was only used for MIC testing between 1-5 days post positivity, such that the concentration of the inoculum enabled correct performance of susceptibility testing. The day the MGIT flags positive is referred to as day 0, if unloaded from the machine on day 0 the MGIT tubes were incubated further in a 37°C incubator until used for MIC testing. A minimum of 4 glass beads were added to a positive MGIT tube prior to testing, the MGIT was vortexed for 1-2 min and left to stand for 30 min prior to testing. A day 1-2 post positive MGIT tube was used neat, day 3-5 MGIT tubes were diluted 1:5 prior to MIC testing.

Preparation of Biofilms

Glass universals containing 10 mL Middlebrook 7H9 broth (no tween), supplemented with 10% OADC (BD) were inoculated with 100 µl of positive MGIT tubes cultures containing either DS, DR *M. tb* clinical isolates or H37Rv. The universals were kept at 37°C with no movement for 8 weeks. After 8 weeks a 10 µl loop was used to extract the top biofilm layer of each universal and placed into a PANTA containing MGIT tube. Once positive, this biofilm containing MGIT was used for the biofilm MIC testing of the compounds.

MIC Testing using the BD EpiCenter™ Software

The compounds were tested against the clinical and reference isolates with an MIC range of 40, 20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 µM, and diluted accordingly with dimethyl sulfoxide (DMSO, Sigma). 100 µl of each concentration was added to individual MGIT tubes, containing 800 µl OADC. A MGIT growth control (GC) containing 800 µl OADC only was also set up for each compound and isolate. 500µl of the positive *M. tb* MGIT tube was added to each compound containing tube, with the GC containing 500 µl of a 1:100 inoculum. The MIC testing tubes were registered on the BD EpiCenter™ software and placed inside the BD BACTEC MGIT 960 machine. The MIC results were obtained from the software, once the GC tube for each isolate had reached 400 growth units (GU). Once the GC of each isolate reached 400 GU the BD EpiCenter™ software registered the test as finished.

RESULTS

MIC results for DS, DR and H37Rv

MIC data extracted from the BD EpiCenter™ software showed resistance, with >40 µM, in clinical isolates and H37Rv. See Table 1.

Table 1. MIC results for targets tested against DS, DR and H37Rv

Targets Tested		MIC (µM)		
Sample ID	OSTB Code	H37Rv	Drug Sensitive	Drug Resistant
FI7-1	OSTBL5	> 40	> 40	> 40
FI12-2D	OSTBL2	> 40	> 40	> 40
FI16-1	OSTBL3	> 40	> 40	> 40
FI18-1	OSTBL1	> 40	> 40	> 40
FI19-1	OSTBL6	> 40	> 40	> 40
FI22-1	OSTBL4	> 40	> 40	> 40
FI10-2 Prep	OSTBS83	> 40	> 40	> 40
FI26-1	OSTBS98	> 40	> 40	> 40
FI31-2	OSTBL7	> 40	> 40	> 40

Resistance was also observed in the biofilms, with MIC values at > 40 µM. See Table 2.

Table 2. MIC results for targets tested against DS, DR and H37Rv biofilms

Targets Tested		MIC (µM)		
Sample ID	OSTB Code	H37Rv Biofilm*	Drug Sensitive Biofilm	Drug Resistant Biofilm
FI7-1	OSTBL5	> 40	> 40	> 40
FI12-2D	OSTBL2	> 40	> 40	> 40
FI16-1	OSTBL3	> 40	> 40	> 40
FI18-1	OSTBL1	> 40	> 40	> 40
FI19-1	OSTBL6	> 40	> 40	> 40
FI22-1	OSTBL4	> 40	> 40	> 40
FI10-2 Prep	OSTBS83	> 40	> 40	> 40
FI26-1	OSTBS98	> 40	> 40	> 40
FI31-2	OSTBL7	> 40	> 40	> 40

{The MIC results for the H37Rv biofilm should be repeated due to the BD EpiCenter™ software going offline. The GU of the GC was at approximately 10 000 when the test was stopped, as opposed to 400.}