Evaluation of Delamanid Susceptibility in Mycobacterium tuberculosis: An insight into emerging resistance

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Abstract

Tuberculosis diagnoses dropped 18% from 2019 to 2020 according to the WHO. Further, 15% fewer people worldwide started treatment regimens designed to treat first-line resistant Mycobacterium tuberculosis (Mtb). Without notification and proper treatment, the COVID-19 pandemic has reversed years of progress in reducing global TB burden and exacerbated the spread of drug-resistant disease. This puts further strain on newly developed antibiotics and their ability to treat susceptible disease. Once such antibiotic is Delamanid, which was approved for medical use in 2014, and has rapidly been added to treatment regimens. It is expected that antibiotic resistance to Delamanid will develop over the coming years, and research has begun to study resistance mechanisms that will prevent proper dosing and treatment. Here, we present the minimum inhibitory concentration of Delamanid to strains in MRIGlobal's NIH Tuberculosis Quality Assurance project repository. Paired with full genomic characterization, this data serves to provide preliminary insight into both susceptible and elevated MIC thresholds that are currently being surveyed in labs around the world.

Introduction

Delamanid, developed by Otsuka and available in Europe since 2014, is an antimycobacterial drug. Approved shortly after Bedaquiline, both are the first drugs of new classes registered for tuberculosis treatment in 40 years. Unlike other recently developed antibiotics, both drugs have quickly been added to clinical studies and treatment regimens. While naturally occurring resistance to Bedaquiline is already being observed, data on resistance to Delamanid is currently sparse. Initial studies have disagreed on where the critical concentration should be set, with some suggesting as high as 0.2μg/mL [1] while others point to a lower value. With the advent of whole genome sequencing, genome analysis can also add understanding in real-time and separate naturally occurring phylogenetic mutations from those that confer true resistance. By creating a catalog of known variants linked to phenotypic resistance, clinicians can be empowered to assess drug suitability prior to treatment initiation.

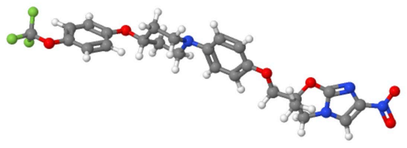

Figure 1: A 3D model of Delamanid.

Delamanid is a nitroimidazole that blocks the manufacture of mycolic acids in Mtb, which destabilizes the bacterial cell wall.

Methods

Phenotypic testing was conducted using TREK Sensititre plates with concentrations ranging from 0.0005 to 0.25 μg/mL. Plates were inoculated with a 0.5 McFarland standard and incubated at 37ºC for 14 days. Testing was performed in duplicate with H37Rv used as the standard control.

Whole genome sequencing was performed on cultured isolate material using both Oxford Nanopore and Illumina platforms. This hybrid data was used to construct *de novo* chromosome assemblies that were assayed for variants against the H37Rv reference genome. Using an in-house variant database as well as 3rd-party software, genes and promoter regions denoted in literature as conferring resistance were queried. The resulting variants were then checked against literature and WHO databases.

Results

Phenotypic

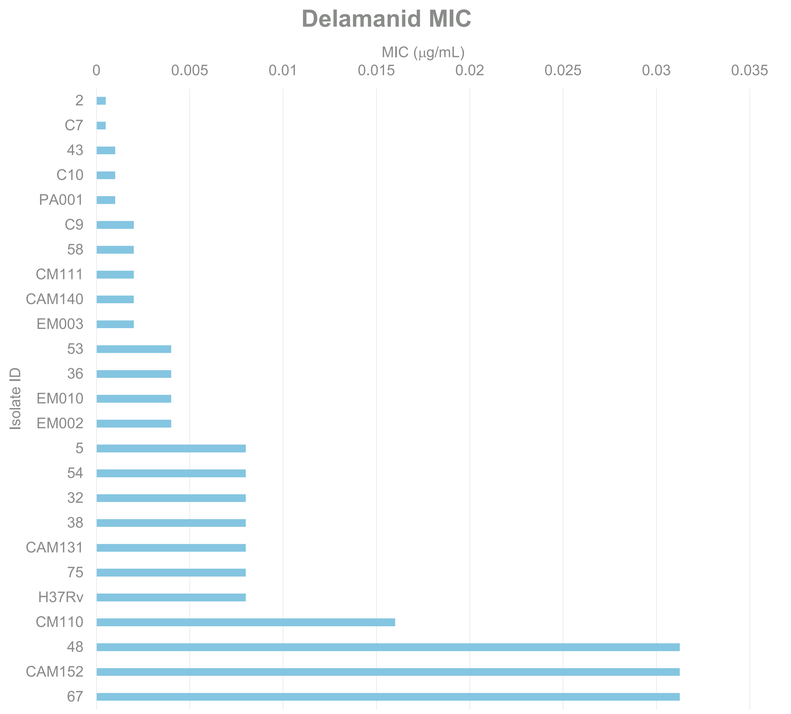

Table 1: Shown here are the minimum inhibitory concentrations observed for a subset of isolates within the MRIGlobal TBQA isolate repository

Discussion

While antibiotic susceptibility is sometimes conceptualized within a binary paradigm, MICs along with epidemiological cutoff values represent a range of real-world factors that play into treatment decisions. The lack of alternative antibiotics in cases of extremely resistant disease, combined with the increasing prevalence of genomic sequencing to inform treatment dosing further accelerate the necessity of this on-going research. Here we show preliminary phenotypic and genotypic information for 25 distinct, globally diverse isolates. While our phenotypic data represent a spectrum of MIC values, little to no correlation is observed in the genotypic data, from known or unknown variants in regions of interest. As such, our data point toward a higher MIC value, above 0.001 or 0.012 μg/mL, than has been proposed by some studies. While the genomic data is limited by our current understanding of mechanisms of resistance and gene targets, we find no data that point toward a lower MIC value. While no final breakpoints have been set by CLSI, FDA, or the WHO, data like this from labs around the world continues to be generated to inform eventual testing targets.

Genotypic

Isolate	MIC (μg/mL)	DR Status	Genome Position	Locus Tag	Gene	Variant
2	0.0005	Sensitive	491591	Rv0407	fgd1	p.Lys270Met
			3640372	Rv3261	fbiA	c.-171A>G
C7	0.0005	Other	491591	Rv0407	fgd1	p.Lys270Met
43	0.001	Other	491742	Rv0407	fgd1	c.960T>C
C10	0.001	MDR	1303095	Rv1173	fbiC	c.165G>A
PA001	0.001	XDR	No variants of interest found			
C9	0.002	MDR	1303095	Rv1173	fbiC	c.165G>A
58	0.002	MDR	491591	Rv0407	fgd1	p.Lys270Met
			3641104	Rv3261	fbiA	p.Val188Phe
CAM111	0.002	Sensitive	491591	Rv0407	fgd1	p.Lys270Met
CAM140	0.002	MDR	491591	Rv0407	fgd1	p.Lys270Met
EM003	0.002	Other	No variants of interest found			
53	0.004	MDR	No variants of interest found			
36	0.004	HR-MDR	491591	Rv0407	fgd1	p.Lys270Met
			1302920	Rv1173	fbiC	c.-11G>A
EM010	0.004	MDR	491591	Rv0407	fgd1	p.Lys270Met
EM002	0.004	MDR	491591	Rv0407	fgd1	p.Lys270Met
5	0.008	Sensitive	491742	Rv0407	fgd1	c.960T>C
54	0.008	MDR	491194	Rv0407	fgd1	c.412C>T
			3641164	Rv3261	fbiA	p.Ile269Val
32	0.008	HR-MDR	No variants of interest found			
38	0.008	MDR				
CAM131	0.008	HR	3987011	Rv3547	ddn	c.168C>T
75	0.008	MDR	No variants of interest found			
Rv	0.008	Sensitive				
CAM110	0.016	Sensitive	491784	Rv0407	fgd1	c.1002A>C
48	0.03125	MDR	No variants of interest found			
CAM152	0.03125	MDR				
67	0.03125	Pre-XDR	3641447	Rv3261	fbiA	p.Thr302Met
			3986666	Rv3547	ddn	c.-178C>T

Table 2: Shown here are the variants of interest related to Delamanid resistance within the TBQA Mtb isolate repository. **Green** represents known mutations not conferring resistance, **Red** shows known resistance mutations, and **Blue** represents currently unknown mutations.

References

1. Stinson, K. *et al.* Antimicrob Agents Chemother., 2016, 6; DOI: 10.1128/AAC.03014-15

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