**Emergence of low level delamanid and bedaquiline resistance during extremely drug resistant tuberculosis treatment**

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**Abstract**

The two new drugs delamanid and bedaquiline have recently been approved for treatment of multi (MDR) and extensively drug resistant (XDR) tuberculosis. Here we report a case of clofazimine, bedaquiline, and low level delamanid resistances acquired during treatment of a patient with XDR tuberculosis.

According to the World Health Organization, 6.2% of the 490,000 multi-drug resistant (MDR, defined by resistance to isoniazid and rifampicin) tuberculosis (TB) cases met the criteria for extensively drug resistant (XDR)-TB in 2016.[1] XDR-TB is defined as MDR-TB plus resistance to at least one fluoroquinolone and a second-line injectable agent (amikacin, capreomycin, or kanamycin). Treatment of XDR-TB is extremely challenging due to limited therapeutic options, poor drug tolerability associated with frequent adverse events and high treatment failure and mortality rates.[2] The recent approval of two new anti-tubercular drugs bedaquiline and delamanid has expanded treatment options for patients with XDR-TB. These drugs have been shown to improve treatment success rates in MDR/XDR-TB patients.[3] So far, only few cases of acquired resistance to these drugs have been described in the literature.[4]

Here, we report a case of acquired delamanid resistance with a moderately increased minimal inhibitory concentration (MIC) due to a novel resistance mechanism in a patient with pulmonary XDR-TB and bedaquiline resistance acquired during treatment with clofazimine. The patient, a 50 year-old man, was initially treated for pulmonary TB in the Republic of Moldova in 1993. The patient was diagnosed with a second episode of pulmonary TB in 2012 in the Ukraine. There, he received a second line drug regimen including linezolid, capreomycin, pyrazinamide and amoxicillin/clavulanic acid over a period of three years. Additionally, weekly endobronchial amikacin was administered for three months. Results of drug susceptibility testing (DST) performed at the time were not available, but the treatment regimen suggests the patient was diagnosed with pre-XDR or XDR-TB.

In December 2016, the patient presented to a hospital in Berlin (Germany) and was initially started on an empirical regimen with *p*-aminosalicylic acid, clofazimine, linezolid, cycloserine, trimethoprim/sulfamethoxazole, and delamanid informed by previous drug exposures (figure 1). At presentation, the patient was found to have significant hearing loss caused by previous treatment with aminoglycosides.

DST of the first *Mycobacterium tuberculosis* isolate (baseline isolate) cultured in December 2016 confirmed XDR-TB (figure 1). It tested resistant to isoniazid, moxifloxacin, prothionamide, capreomycin, ethambutol, linezolid, pyrazinamide and susceptible to amikacin using agar dilution method on Middlebrook 7H10 (7H10) and/or the mycobacterium growth indicator tubes (MGIT™, Becton Dickinson, Sparks, Md., USA).[5 6] Rifampicin was resistant at the critical concentration (1.0 mg/L) on 7H10 but tested susceptible at the critical concentration (1.0 mg/L) in MGIT. Rifabutin tested susceptible both on 7H10 and in MGIT. Whole genome sequencing (WGS) revealed several resistance associated polymorphisms and identified *rpoB* L452P which is known to be associated with increased rifampicin MICs.[7] The baseline isolate was susceptible in MGIT to delamanid, bedaquiline, and clofazimine at their respective critical concentrations recently recommended by WHO.[6] In addition low MICs were obtained using the colorimetric resazurin microtiter assay (REMA) (figure 1).[8]

Once DST results were available, the regimen was adjusted accordingly (figure 1). Subsequent changes had to be made because of side effects. DST and WGS were repeated at weeks 22, 32, 42, and 64 of treatment and did not reveal any differences compared to the baseline isolate except for new mutations in genes associated with resistance to delamanid, bedaquiline and clofazimine.

Both, baseline and week 22 isolates tested susceptible to delamanid in MGIT and revealed low MIC in REMA. WGS data of these two isolates showed wildtype for *ddn* and a silent mutation in *fbiB* (acg/accC, T92T). The isolates of weeks 32, 42 and 64 tested delamanid resistant in MGIT and showed an increased MIC value of 0.25 mg/L in REMA. WGS of those three isolates revealed mutation *ddn* G53D in 78% (172/220), 99% (304/306) and 100% (398/400) of reads respectively, indicating the presence of heteroresistance in the week 32 isolate.

When delamanid resistance was detected bedaquline was added to the drug regimen. Isolates of weeks 22, 32, 42 and 64 were retrospectively investigated for bedaquiline and clofazimine susceptibility using phenotypic DST and WGS. WGS sequencing revealed an insertion in *Rv0678* (185ins\_CAG) in 48% (119/248), 87% (136/156), 87% (194/223) and 92% (263/286) of reads in week 22, 32, 42 and 64 isolates, respectively. DST in MGIT using the WHO recommended critical concentrations showed bedaquiline and clofazimine resistance for the isolates of weeks 22, 32, 42 and 64. We also found increased bedaquiline and clofazimine MICs in REMA system; up to 8-fold compared to the baseline isolate.

Due to limited drug treatment options a lobectomy was performed on week 79 of treatment to remove the diseased lung. Since then the patient has been well and culture conversion has been achieved.

*Ddn* (Rv3547) encodes for the F420-dependent nitroreductase Ddn that metabolizes the inactive prodrug delamanid to its active form. The cofactor F420 is synthesized and reactivated by a group of enzymes encoded by genes *fgd1, fbiA, fbiB* and *fbiC*. Polymorphisms in some of these genes have been observed to lead to *in vitro* resistance to delamanid.[4 8] However, to the best of our knowledge, the particular *ddn* G53D mutation has not yet been reported as a putative resistance conferring mutation. NCBI database search (<https://www.ncbi.nlm.nih.gov/protein>) revealed that the amino acid G53 is located in the conserved domain of the Ddn protein; its exchange may affect enzymatic function.

The delamanid resistant isolate recovered from our patient had an increased MIC of just three dilution steps above the established ECOFF of 0.03 mg/L. In contrast the study by Schena et al reported 1000-times higher MICs (>32 mg/L) in *M. tuberculosis complex (Mtbc)* isolates carrying stop mutations in the *ddn* gene resulting in truncated Ddn proteins.[8] We speculate that the *ddn* mutation G53Donly partly limits the enzyme activity causing low level but clinically relevant resistance. At the current licensed dosage (100 mg twice daily) delamanid plasma concentrations are just above the MIC of our isolate (0.372-0.562 mg/L).[9] The patient experienced treatment failure of a delamanid based regimen without further emergence of mutations conferring higher-level delamanid resistance. This underlines the clinical relevance of the *ddn* G53D variant despite an only slightly increased MIC. This information is crucial when designing treatment regimens and monitoring treatment success in MDR/XDR-TB patients with extremely limited treatment options.

Our patient also developed polymorphisms in *Rv0678*, a transcriptional repressor of the genes encoding the MmpS5-MmpL5 efflux pump and conferring low-level resistance to clofazimine and bedaquiline.[10] The resistance was acquired while receiving a regimen including clofazimine and delamanid. Countries who have extensively used clofazimine for the treatment of MDR-TB need to be aware of the risk of acquired resistance not just to clofazimine, but also bedaquiline. This is particularly important in view of the new WHO treatment recommendations categorizing bedaquiline as a group A drug together with levofloxacin/moxifloxacin and linezolid to be prioritized in MDR-TB regimens. Resistance associated polymorphisms in *Rv0678* may also be caused and selected by other factors suggested by a recent study showing unexpectedly high prevalence of *Rv0678* polymorphisms associated with elevated clofazimine and bedaquiline MICs among MDR-TB patients without prior exposure to these drugs.[11]

Combination therapy with delamanid and bedaquiline together with other drugs with proven antimycobacterial activity using phenotypic and molecular methods may be the best alternative for XDR-TB patients with no other treatment options.[12] However, it is likely that more frequent use of these relatively new drugs will lead to emergence and transmission of resistant *Mtbc* strains. It is essential that phenotypic DST including measurement of MICs and possibly sequencing are performed to determine background resistance levels before initiating treatment with these new drugs. Furthermore, our patient highlights the necessity of regular and repeated, quality controlled DST for both delamanid and bedaquiline before and during treatment. WGS analysis during treatment may allow to detect hetero-resistance by identifying relevant mutations present in at least 10% of the reads ensuring >100-fold average genome wide coverage. Our patient also provides evidence, that much lower MIC levels than previously reported may lead to clinically relevant delamanid resistance and treatment failure. Future interpretation of molecular and phenotypic delamanid DST results will need to take this into consideration.

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**Figure 1: Treatment history, phenotypic drug susceptibility testing and whole genome sequencing results**

Smear result, – negative for acid fast bacilli; + positive for acid fast bacilli; Culture result, - culture negative; + culture positive; c, culture contaminated;

Orange line, drug administered; grey column, mutation *ddn* G53D % of WGS-reads; grey line, delamanid MIC; blue column, insertion in *Rv0678* % of WGS-reads; light blue line, bedaquiline MIC; dark blue line, clofazimine MIC;

Abbreviations: DST, drug susceptibility testing; MGIT, mycobacterium growth indicator tubes; 7H10, Middlebrook 7H10 Agar; R, resistant; S, susceptible; nd, not determined; CC, critical concentration; MIC, minimal inhibitory concentration; REMA, resazurin microtiter assay; INH, isoniazid; RIF, rifampicin; RFB, rifabutin; MXF, moxifloxacin; PTO, prothionamide; AMK, amikacin; CM, capreomycin; KAN, kanamycin, CS, cycloserine; LZD, linezolid; EMB, ethambutol; PZA, pyrazinamide; PAS, *p*-aminosalicylic acid; CLR, clarithromycin; THIO, thioridazine; SXT, trimethoprim-sulfamethoxazole; ERT/AUG, ertapenem/amoxicillin clavulanic acid; CFZ, clofazimine; BDQ, bedaquiline; DLM, delamanid; WGS, whole genome sequencing;

