



In Vitro Susceptibility Testing of Bedaquiline against *Mycobacterium avium* Complex

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ABSTRACT We performed bedaquiline broth microdilution susceptibility testing using Clinical and Laboratory Standards Institute (CLSI) guidelines on 103 respiratory isolates of *Mycobacterium avium* complex (MAC), including multidrug-resistant isolates. Approximately 90% of isolates had bedaquiline MICs of ≤ 0.008 $\mu\text{g/ml}$, and 102/103 isolates had MICs of ≤ 0.015 $\mu\text{g/ml}$. Bedaquiline has excellent potential for use in patients with MAC infections, although for reasons of its metabolism by the cytochrome P450 system, it should not be given with rifampin.

KEYWORDS *Mycobacterium avium* complex, bedaquiline, nontuberculous mycobacteria, susceptibility testing

Bedaquiline (formerly known as TMC207 or R207910, trade name Sirturo; Janssen Therapeutics, Inc.) is a new diarylquinolone that is being developed for the treatment of drug-susceptible (DS) and multidrug-resistant (MDR) tuberculosis (TB) (1, 2). Bedaquiline, as part of combination therapy, received accelerated approval in December 2012 from the U.S. Food and Drug Administration (FDA) as the first FDA-approved drug for treatment of pulmonary TB due to MDR-TB in adults in 40 years (3, 4).

The drug offers a unique target and mechanism of action (i.e., inhibition of ATP synthase) (5–8). The gene encoding the subunit of mycobacterial ATP is called *atpE*, and its amino acid sequence is known to be highly conserved in isolates of *Mycobacterium tuberculosis* (3). Studies by Segala and colleagues used multiple mutations in subunit C in the mycobacterial C ring to show its importance in the binding of bedaquiline (9). There appears to be no cross-resistance with standard antituberculous agents, although there is cross-resistance with clofazimine, a drug used primarily for leprosy but occasionally for MDR-TB, and with some nontuberculous mycobacteria (NTM) (10, 11).

Bedaquiline is metabolized by the cytochrome P-450 (CYP) 3A4 enzyme, which is greatly enhanced in the presence of rifampin (steady-state concentrations decreased by 75 to 80%) (12). Rifabutin, which is also used for the treatment of MAC infection, has less induction of the cytochrome P450 3A4 enzyme (20-fold versus 80-fold) than rifampin (13).

A total of 101 respiratory isolates of MAC were submitted to the Mycobacteria/Nocardia Laboratory at the University of Texas Health Science Center at Tyler from 2015 to 2016 for susceptibility testing; one patient each from October 2013 and November 2014 were also tested. No duplicate isolates were tested.

Isolates were identified as MAC by AccuProbe (Hologic-GenProbe, San Diego, CA) and/or sequence (partial 16S rRNA gene by MicroSeq Life Technologies, Carlsbad, CA).

Isolates were tested by broth microdilution in cation-adjusted Mueller-Hinton broth using customized frozen microtiter panels from Thermo Fisher (Cleveland, OH) with

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TABLE 1 MIC ranges and concentrations that inhibit the MIC₅₀ and MIC₉₀ of 103 strains of *Mycobacterium avium* complex for bedaquiline, amikacin, moxifloxacin, linezolid, and clarithromycin

Antimicrobial	MIC range ($\mu\text{g/ml}$)	MIC ₅₀ ($\mu\text{g/ml}$)	MIC ₉₀ ($\mu\text{g/ml}$)
Bedaquiline	≤ 0.008 –0.03	≤ 0.008	0.015
Amikacin	4–>2048	32	64
Clarithromycin	0.5–>128	4	8
Moxifloxacin	≤ 0.5 –64	4	8
Linezolid	4–>128	32	64

doubling dilution concentrations (0.008 to 32 $\mu\text{g/ml}$), following the Clinical and Laboratory Standards Institute (CLSI) recommended procedure, although there are no specific standardized guidelines for bedaquiline susceptibility testing (14). MICs were read using a mirrored light box after incubation at 35°C for 7 to 14 days when sufficient growth was evident. Comparator antimicrobials included amikacin, clarithromycin, moxifloxacin, and linezolid (Table 1).

There were no manufacturer guidelines for MIC quality control (QC) testing other than those for *M. tuberculosis*. This organism could not be tested as our laboratory is not a biosafety level 3 facility. Quality control against clarithromycin, amikacin, linezolid, moxifloxacin, and bedaquiline was performed using *M. avium* ATCC 700898 (14).

Antimicrobial susceptibility testing (AST) was performed on 103 nonduplicate isolates of MAC from 103 patients, of whom only one was known to have received prior clofazimine and none to have received bedaquiline treatment. Bedaquiline MICs ranged from ≤ 0.008 to 0.03 $\mu\text{g/ml}$. Ninety (87%) of the isolates showed bedaquiline MICs of ≤ 0.008 ; only 12 isolates (12%) showed MICs of 0.015 $\mu\text{g/ml}$ and only one isolate had an MIC of 0.03 $\mu\text{g/ml}$ (Table 1).

Six patients had amikacin resistance MICs of $\geq 1,024$ $\mu\text{g/ml}$, and four patients showed clarithromycin resistance MICs of > 128 $\mu\text{g/ml}$. Three of six isolates with amikacin resistance were also clarithromycin resistant with 2/3 bedaquiline MICs of ≤ 0.008 $\mu\text{g/ml}$ and 1/3 bedaquiline MIC of 0.015 $\mu\text{g/ml}$. For the remaining three amikacin-resistant isolates, the bedaquiline MICs were ≤ 0.008 , 0.015, and 0.03 $\mu\text{g/ml}$. One isolate was clarithromycin resistant only and had a bedaquiline MIC of ≤ 0.08 $\mu\text{g/ml}$.

Quality control for *M. avium* ATCC 700898 was within acceptable MIC ranges for clarithromycin (0.5 to 2 $\mu\text{g/ml}$), amikacin (4 to 16 $\mu\text{g/ml}$), moxifloxacin (0.5 to 4 $\mu\text{g/ml}$), and linezolid (8 to ≥ 32 $\mu\text{g/ml}$). The bedaquiline MIC values for *M. avium* ATCC 700898 were ≤ 0.008 to 0.06 $\mu\text{g/ml}$. The QC values for panel 1 (lowest bedaquiline value, 0.008 $\mu\text{g/ml}$) revealed that 25/28 test values were ≤ 0.008 $\mu\text{g/ml}$. In a second panel, not used for this study, 37/37 values for this reference isolate were ≤ 0.008 $\mu\text{g/ml}$.

The results of this current study are similar to previously reported findings of *in vitro* AST in MAC isolates using agar dilution rather than the currently recommended broth microdilution. Huitric and colleagues found 100% of 22 strains of MAC to have bedaquiline MICs of ≤ 0.25 $\mu\text{g/ml}$ (MIC₉₀) and 50% to have MICs of 0.03 $\mu\text{g/ml}$ (MIC₅₀) (7). Similar MICs were reported by Andries et al. for seven isolates of MAC using the Bactec culture system and showed a median bedaquiline MIC of 0.010 $\mu\text{g/ml}$ (range, 0.007 to 0.010 $\mu\text{g/ml}$) (6).

In an early intraperitoneal infection murine model (treatment started on the day after infection), Lounis et al. (15) showed that 1 month of five times weekly monotherapy with clarithromycin and bedaquiline decreased the CFU counts of MAC by 1.99 and 2.56 log₁₀ compared to those of untreated controls ($P = 0.005$ and $P = 0.002$, respectively). In a late intraperitoneal infection murine model (mice untreated for 1 month and then treated for 4 months), bactericidal activity was achieved following 3 months of triple treatment with clarithromycin plus amikacin plus bedaquiline ($P = 0.001$). The minimal bactericidal concentration (MBC) was also much higher (MBC of > 128 $\mu\text{g/ml}$) than the MIC (0.015 $\mu\text{g/ml}$). However, bedaquiline may still be a poten-

tially useful companion drug for treatment of MAC as not all drugs active against MAC (e.g., rifampin or ethambutol) are bactericidal (15).

Although bedaquiline is not approved by the FDA for the treatment of MAC infection, Philley et al. reported on six patients with AFB smear-positive refractory MAC lung disease treated with a bedaquiline-containing regimen for a minimum of 24 weeks (16).

After 2 months on bedaquiline, 5/6 patients showed decreases in their semiquantitative sputum cultures (four patients had negative cultures or countable colonies). Two patients who had shown intermittent decreases in counts previously reverted back to their baseline counts. Three patients were judged to be clinically improved, two were unchanged, and one patient was evaluated as worse (16).

Finally, the decline in bedaquiline blood levels when given in combination with rifampin may prevent the two drugs from being given together. Rifampin is a frequently used drug in the initial treatment of MAC infection (17). For bedaquiline-containing regimens, if a rifamycin is needed, we recommend that rifabutin replace rifampin.

Current quality control values for the *M. avium* reference strain suggest the need for lower bedaquiline test concentrations to determine its validity for quality control.

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