Original Article

Validation of Bedaquiline Drug-Susceptibility Testing by BACTEC MGIT 960 System for *Mycobacterium tuberculosis*

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Abstract

Background: Bedaquiline (BDQ) is a new antituberculosis (TB) drug effectively used for the treatment of multidrug-resistant and extensively drug-resistant TB. However, the reports on drug-susceptibility testing (DST) for BDQ are scarce. The study aimed to validate and standardize BDQ DST by BACTEC MGIT 960 system for *Mycobacterium tuberculosis*. **Methods:** A panel of ten *M. tuberculosis* isolates comprising 8 BDQ sensitive and 2 BDQ resistant strains were used to test accuracy, repeatability, and reproducibility of BDQDST by MGIT 960. BDQ DST by Middlebrook 7H11 agar method using polystyrene tubes was used as a standard method to calculate the accuracy of the validation. **Results:** DST by MGIT for BDQ showed 100% accuracy, repeatability, and reproducibility, although variations were observed in the growth units of the "test" MGIT tubes between technologist and drug stocks while testing for reproducibility. **Conclusion:** BDQ DST by MGIT 960 system is accurate, repeatable, and reproducible and hence can be implemented in certified laboratories routinely performing DST by MGIT 960 system.

Keywords: Bedaquiline, MGIT 960, *Mycobacterium tuberculosis*

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Introduction

Bedaquiline (BDQ) belonging to the group of a diarylquinoline is the first novel anti-tuberculosis (TB) drug approved for human use in more than 40 years.[1] The drug has received conditional/accelerated approval for use against drug-resistant TB. Following the approval of BDQ by the US Food and Drug Administration to treat drug-resistant TB, centers for diseases control[2] issued federal guidelines on its use for the treatment of TB in children, pregnant women, and individuals with other health complications in addition to multidrug-resistant TB (MDR-TB). The World Health Organization (WHO) issued interim guidelines on the use of BDQ in combination with other TB drugs for MDR-TB and extensively drug-resistant TB (XDR-TB).[3] In 2015, BDQ was approved for use in the United States, the EU, South Korea, South Africa, India, the Russian Federation, and Peru.[4]

In India, BDQ is in use since 2016 for the treatment of MDR-TB and XDR-TB under the conditional access program of Revised National TB Control Program.[5] In the current WHO DRTB treatment guidelines,[6] the use of BDQ is prioritized over other drugs, especially injectables for the treatment of DRTB. With this, the use of BDQ is likely to be multiple times higher. However, information on existing or emerging resistance to BDQ is currently very limited as a clear guideline on a standard drug-susceptibility testing (DST) method is awaited. Few premier reference laboratories have adopted different methodologies, including the minimum inhibitory concentration (MIC) using solid Middlebrook 7H11 medium or Middlebrook 7H9 liquid broth-based methods.[7]

DST at a critical concentration of 1 mg/L using the commercial BACTEC MGIT960 liquid culture system has been recently recommended by the WHO.[8] DST to BDQ with a single concentration of the drug using MGIT960 system earlier was hampered due to adsorption of the drug to the polypropylene

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plastic tubes.[9] With the recent introduction of polystyrene tubes by the manufacturer, this issue has been resolved.

In the present study, validation following the standard protocol described in the current GCLP guidelines[10] for BDQ DST by BACTEC MGIT 960 was carried out.

Methods

Setting

The present study was carried out at the NABL accredited ICMR-National Institute for Research in Tuberculosis, Chennai, a Supranational Reference Laboratory under WHO and National Reference Laboratory under the Revised National Tuberculosis Control Program of India. This validation was done as part of continued efforts toward capacity building of the laboratory to be on par with international laboratories to meet crucial demands for TB control.

Ethical statement

As the study neither involve human clinical specimens nor clinical isolates, ethical clearance was not necessary.

Bedaquiline drug

The pure form of BDQ drug with 100% purity was a kind gift from Cipla Pharmaceuticals, Mumbai, India.

Mycobacterium tuberculosis strains

A panel of ten *M. tuberculosis* isolates comprising 8 BDQ susceptible and 2 BDQ-resistant strains were used for the study. All the strains were obtained from the Institute of Tropical Medicine (ITM), Antwerp, Belgium. MIC protocol in Middlebrook 7H11 method using polystyrene tubes[7] approved by EUCAST was used as the gold standard.[11] Only one BDQ-resistant strain was received and the same was duplicated. All isolates were coded for each validation parameter, and the results were compared after decoding.

Drug stock preparation

Drug stock was prepared at par with WHO recommended concentration.[8] Accordingly, 1 µg/mL concentration was prepared from the lyophilized BDQ powder using the following formula. Three stocks of 1 ml each were prepared.

Weight (mg) = Volume (mL) × Concentration (µg/mL) × dilution factor/assay potency (µg/mg)

- Volume (in milliliters) is the desired volume of stock solution
- Concentration (micrograms per milliliter) is the desired concentration of stock solution
- Dilution factor is the number of times the drug added to the tube (100 µl) is getting diluted by the total volume of the medium in the tube (8.3 ml) = 83
- Assay potency (micrograms per milligram) is the activity or potency specified by the manufacturer of the reference standard powder. This value usually appears on the label or the certificate of analysis
- Weight (milligrams) is the weight of powder needed to prepare the desired volume of stock solution at the desired concentration.

Accordingly weight (mg) =1 × 1 × 83/1000 = 0.083 mg.

As it was difficult to weigh 0.083 mg, 100 times the weight, i.e., 8.3 mg was weighed and dissolved in 1 ml of dimethyl sulphoxide. The stocks were aliquoted at 120 µl each and stored at −80°C. At the time of MGIT DST, the stock was diluted 1:100 (100 µl of stock + 9.9 ml sterile DW) to get the desired working stock.

BACTEC MGIT 960

The isolates were freshly sub-cultured and DST was done at 1 µg/ml using the manufacturer's protocol for "undefined drug." The duration of the protocol is 3–13 days.

Validation parameters

Accuracy

BDQ DST by MGIT 960 was done for the panel of ten cultures was performed by a technologist, and the results were compared with standard method (Middlebrook 7H11 agar tested using polystyrene tubes) to calculate the accuracy of the method.

Repeatability

BDQ DST by MGIT 960 for the panel of ten cultures was performed in duplicates by a technologist on the same day (morning and evening) to calculate repeatability of the method.

Reproducibility

BDQ DST by MGIT 960 for the panel was performed by two technologists on three different days using three different batches of the drug stocks to calculate reproducibility.

Results

Accuracy

DST by MGIT 960 demonstrated 100% accuracy with two known BDQ-resistant strains identified as resistant and all eight BDQ susceptible strains identified as susceptible in comparison with DST on Middlebrook 7H11 agar [Table 1].

Repeatability

DST by MGIT960 for the panel of cultures performed by a technologist on the same day in duplicates demonstrated 100% repeatability [Table 2].

Reproducibility

The results of BDQDST by MGIT 960 performed by two technologists on 3 days using 3 different batches of drug stocks demonstrated 100% reproducibility between the users and days and between the batches [Table 3]. However, the growth unit of MGIT showed mild variations but was well within the cutoff.

Discussion

Treatment of DRTB till recently comprised less effective but more toxic drugs for prolonged durations. BDQ has been in

Table 1: Accuracy testing of bedaquiline drug-susceptibility testing by MGIT 960 in comparison with Middlebrook 7H11

MGIT 960	Middlebrook 7H11		
	Resistant	Sensitive	Total
Resistant	2	0	2
Sensitive	0	8	8
Total	2	8	10

Table 2: Repeatability testing of bedaquiline drug-susceptibility testing by MGIT 960

Time point 2	Time point 1		
	Resistant	Sensitive	Total
Resistant	2	0	2
Sensitive	0	8	8
Total	2	8	10

Table 3: Reproducibility testing of bedaquiline drug susceptibility testing by MGIT 960

Technologist 2	Technologist 1		
	Resistant	Sensitive	Total
Day 1			
Resistant	2	0	2
Sensitive	0	8	8
Total	2	8	10
Day 2			
Resistant	2	0	2
Sensitive	0	8	8
Total	2	8	10
Day 3			
Resistant	2	0	2
Sensitive	0	8	8
Total	2	8	10

use in national TB control programs with conditional access.[5] Being an oral drug with less toxic side effects, the WHO in its recent recommendations has prioritized BDQ over other injectables for the treatment of DRTB.[6]

Any DST not only guides treatment but also forms the tool for monitoring the emergence of drug resistance through surveillance studies. With the anticipated widespread use of BDQ, availability of a standard method of DST for BDQ becomes paramount, especially in a country like India with high-DRTB burden. With cross resistance between clofazimine and BDQ being reported,[12] it is all the more important to establish a standard DST methodology for BDQ independently.

While stressing the need for developing an accurate and reliable DST method for BDQ, the WHO had earlier recommended the MIC method. BDQ DST by MIC using Middlebrook 7H11 agar and BACTEC MGIT960[7] and Middlebrook 7H9 broth[13] have been reported earlier. A presumptive MIC of 0.25 mg/L for Middlebrook 7H11 and rezazurin microtiter assay (REMA) was suggested by (ITM: Antwerp, Belgium) and MIC of 1 mg/L was reported for MGIT960.[7] However, reports on BDQ DST using a single concentration are scarce.

Against a background of scarce reports on BDQ DST using a single concentration of the drug, the current work is the first report from India on the validation and feasibility of a much-needed protocol on BDQ DST by BACTEC MGIT 960. The commercial liquid culture-based system is currently considered the gold standard for phenotypic DST due to its rapidity, reliability, and reproducibility and can be subjected to quality assurance protocols. Evidence on the feasibility of a standard methodology on BDQ DST would expand the scope of DST-guided treatment for drug-resistant TB patients.

Conclusion

Bedaquiline drug susceptibility testing using BACTEC MGIT960 system has been successfully validated and may be adopted for use with clinical isolates of Mycobacterium tuberculosis.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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