Original Article

Mutation in *atpE* and *Rv0678* Genes Associated with Bedaquline Resistance among Drug-resistant Tuberculosis Patients: A Pilot Study from a High-burden Setting in Northern India

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Abstract

Background: Mutations in *atpE* gene or transcriptional repressor *Rv0678* gene associated with inhibition of adenosine 5′-triphosphate synthase and upregulation of efflux pumps, respectively, may potentially lead to *in vitro* resistance to bedaquiline. This is the first study from India, which looks at mutations associated with this novel drug. **Methods:** In 2019 (January to June), a total of 68 laboratory-confirmed pre-extensively drug-resistant tuberculosis (XDR-TB) (fluoroquinolone resistant [n = 52] and second-line injectables resistant [n = 12]) and 4 × DR-TB culture specimens were included. All specimens were evaluated for genetic analysis using predesigned primers of *atpE* and *Rv0678* genes. **Results:** Among the pre-XDR-TB isolates (n = 64), there were no mutations found in either *atpE* or *Rv0678*. However, among the XDR-TB isolates (n = 4), one specimen (25%) was found to be associated with a mutation in *atpE* gene at position 49, resulting in the amino acid leucine replaced by proline (L-49-P). No mutations were observed with the *Rv0678* gene. **Conclusion:** In this study, genetic analysis showed that only one-fourth XDR-TB isolates had a mutation in the *atpE* gene; there were no other mutations found in the *Rv0678* gene. To the best of our knowledge, this novel mutation (L-49-P) in *atpE* gene is being reported for the first time in northern India.

Keywords: Bedaquline, drug susceptibility testing, *Mycobacterium tuberculosis* complex

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Introduction

The incidence of multi-drug resistant tuberculosis (MDR-TB) and extensively drug-resistant TB (XDR-TB) has been increasing substantially. India (24%), China (13%), and the Russian Federation (10%) account almost half of the MDR/rifampicin (RIF)-resistant-TB cases.[1] In our effort to fight against TB and plan and vision to End TB by 2035, drug-resistant TB is a major obstacle. The novel drug bedaquiline (BDQ) was introduced as a hope in this gloomy scenario to manage drug-resistant (DR) TB. It was approved in 2012 by the US Food and Drug Administration (FDA) for the treatment of multidrug-resistant (MDR) TB.[2,3] The drug is presently available in India as a part of the condition access program for BDQ for the treatment of drug-resistant TB under the Revised National TB Control Programme Central TB Division Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India.[4]

BDQ represents the diarylquinolines group of antimycobacterial agents, with significant bactericidal activity in both

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replication and nonreplicating mycobacteria.[5] The activity of ATP-synthase inhibited by BDQ results in the reduction of bacterial ATP synthesis.[6] However, different mutation patterns already found in the *atpE* gene leads to resistance against BDQ.[7] A low-level resistance associated with transcriptional receptor *Rv0678* gene, an efflux pump-related MmpS5-MmpL5 genes have been associated with cross-resistant between clofazimine (CFZ) and BDQ *in vitro*.[8] Although BDQ has shown the least cross-resistance with most pre-existing first- and second-line anti-TB drugs, mutations associated with *Rv0678* were majorly linked with the mechanism of CFZ resistance.[9,10] A recent study has reported the association of low-level BDQ resistance with *pepQ* gene with unknown mode of action.[11]

In India, BDQ is available free of cost under the National TB program under condition access. It is administered along with the standard DR-TB regimen. Recommended dose is 400 mg once daily for 2 weeks, followed by 200 mg thrice weekly for 22 weeks. After 24 weeks of BDQ therapy, the MDR-TB regimen should be continued as per the national TB treatment guidelines.[12,13] No study from India has looked into the resistance pattern of this drug. To the best of our knowledge, this is the first study from India, which looks at mutations in *atpE* and *Rv0678* genes associated with BDQ drug and has important implications in the management of DR TB.

Methods

Ethics approval

This study was conducted between January 2019 and June 2019 on 68 culture-positive *Mycobacterium tuberculosis* specimens (64 were pre-XDR-TB and four were XDR-TB) at Intermediate Reference Laboratory, All India Institute of Medical Sciences (AIIMS) Hospital, New Delhi, India and was approved by Ethics Committee of the AIIMS, New Delhi, India (Ref No. IEC-256/05.04.2019, RP-15/2019, Date of approval: 22.04.2019). In this study, we have selected patients who were not yet exposed to BDQ in their regimen.

Specimen processing

All investigations associated with culture-positive specimens were processed in biosafety cabinet Class-II in a biosafety level III laboratory. The samples were processed according to the WHO guidelines by using the NALC-NaOH decontamination procedure (final NaOH concentration, 1%). The decontaminated and digested samples were neutralized using (phosphate-buffered saline; pH 6.8) and mixed properly.[14]

Line probe assay

Line probe assay (LPA) methods ware applied to find out initial investigations. A GenoLyse kit (Hain Lifescience, Nehren, Germany) was used for DNA extraction from all specimens. The interpretation was performed for both Genotype MTBDRplus V.2[15] and Genotype MTBDRsl V.2[16] according to the manufacturer's instructions (Hain LifeScience GmbH, Nehren, Germany).

Conventional drug susceptibility testing on mycobacteria growth indicator tube-960

Indirect drug susceptibility testing (DST) was performed by using the final critical concentration of anti-TB drugs, i.e., 1.0 μg/ml of amikacin (AMK), 2.5 μg/ml of kanamycin (KAM) and capreomycin (CAP), 0.5 μg/ml and 2.0 μg/ml of moxifloxacin (MFX), 2.0 μg/ml ofloxacin (OFX) and 1.5 μg/ml levofloxacin (LFX).[17] H37Rv strain was used as the reference standard for the phenotypic DST test. Due to the unavailability of BDQ and CFZ drugs, no DST was performed for the same in this study. However, a recent study has been successfully validated the role of mycobacteria growth indicator tube-960 for the DST of BDQ drug powder.[18]

Gene sequencing

Extracted DNA was amplified using specific primers *atpE* and *Rv0678* genes [Table 1].[7,19] Sequencing was performed on ABI Prism 3130 × l genetic analyzer (Applied Biosystem, Carlsbad, CA, USA) according to the manufacturer's instructions.

Sequence analysis

Sequence results were analyzed using BioEdit Software (Thomas Hall, Raleigh, NC, USA) and ClustalW 2.0.

Results

A total of 68 including culture isolates of pre-XDR (n = 64) and XDR-TB (n = 4) were selected for this study. Among pre-XDR-TB, 52 isolates were confirmed as fluoroquinolone (FLQ) resistant, while 12 isolates were found to have second-line injectables (SLI) resistant using as LPA as an initial method to identify resistance patterns in the present study. Four isolates were identified as XDR, i.e., resistant to both FLQ and SLI on LPA. In phenotypic DST, all 52 FLQ-resistant isolates were observed resistant to RIF, INH, LFX, OFX, MFX (0.5 μg/ml) drugs, while 12 SLI-resistant isolates were found to be resistant to RIF, INH, AMK, KAM drugs. In the case of XDR, all four isolates were found to be resistant to RIF, INH, LFX, OFX, MFX (0.5 μg/ml), AMK, KM, CAP drugs [Table 2].

On performing DNA sequencing using targeted *atpE* and *RV0678* gene, no mutations were observed in either FLQ mono-resistant (n = 52) or SLI mono-resistant (n = 12) isolates. In case of XDR-TB (n = 4) isolates, one (25%) isolate was associated with mutation in *atpE* gene at position 145 (CTG-145-CCA) which resulted in the change of the amino acid from leucine to proline (L-49-P); however, no mutation was observed with RV0678 gene [Table 2].

Discussion

In the global epidemic of DR-TB, the role of novel drugs like BDQ remains crucial. Recent trials also look into the potential role of the drug in shorter treatment regimens.[20] The safety and side effect profile is currently being investigated as a part of the Phase III STREAM (Standardized Treatment Regimen of Anti-TB drugs for patients with MDR-TB) trial.[20] While it is important to closely look at the clinical effects of the drug,

Table 1: Primers used for polymerase chain reaction amplification

Gene	Primer	Primer sequence (5′-3′)	PCR product size (bp)
Rv0678[19]	CV010	ATGGCGACCACAACCAGG	649
	CV017	TTTTACGCGTGTTGCTCATCAGTCGTCCTC	
	30F22	AGCCGGAAACTTCGTACTCCAC	906
	916R20	GCTGGACAACACGGTCACCT	
atpE[7]	*atpE* forward	TGTACTTCAGCCAAGCGATGG	454
	atpE reverse	CCGTTGGGAATGAGGAAGTTG	

PCR: Polymerase chain reaction, bp: Base pair

Table 2: Drug susceptibility pattern with mutation in *atpE* and *Rv0678* (*n*=68)

Drug resistance pattern	Number of isolates	Mutation in *atpE*	Mutation in *Rv0678*
MDR and fluoroquinolone resistant RIF, INH, LFX, OFX, MFX (0.5 µg/ml)	52	No mutation	No mutation
MDR and second-line injectables resistant RIF, INH, AMK, KAM	12	No mutation	No mutation
XDR RIF, INH, LFX, OFX, MFX (0.5 µg/ml), AMK, KM, CAP	4	CTG-145-CCA; L-49-P (*n*=1)	No mutation
		No mutation (*n*=3)	

MDR-TB: Multidrug-resistant, RIF: Rifampicin, INH: Isoniazid, AMK: Amikacin, KAM: Kanamycin, CAP: Capreomycin, OFX: Ofloxacin, LFX: Levofloxacin, MFX: Moxifloxacin, L: Leucine, P: Proline, XDR: Extensively drug resistant

it is equally important to identify mutations and keep a close watch to determine the future of this wonder drug.

In recent years, clinical failure cases of BDQ based treatment regimen has been reported form worldwide[21,22] and most of them were found to be resistant due to the mutation in the *Rv0678* gene associated with efflux pump pathways.[8,23] A study conducted in Belgium by Villellas *et al*., 2018 showed a high prevalence of mutation in the *Rv0678* gene among CFZ or BDQ unexposed MDR-TB patients.[19] In contrast, the present study did not find any mutations in the *Rv0678* gene region of pre-XDR or XDR-TB isolates. However, mutations associated with *atpE* gene linked with BDQ drug target site were found in one sample. In sequencing, we have found a new type of mutation in *atpE* gene at position L-49-P, which has not been reported in previous studies. The clinical significance of this new mutation needs to be determined.

A relatively low burden of BDQ resistance in Indian scenario as in this study needs clinical correlation and a larger cohort to confirm the same. However, if the same is confirmed it may be an encouraging ray of light in efforts toward managing DR-TB epidemic. Close monitoring of this drug is important to decide its future and is an important step toward our commitment to eliminate TB.

A study of Tiberi *et al*., 2018 suggested that only one-third of CFZ-resistant strains were also found to be BDQ resistant, while all BDQ resistant were found to be resistant to CFZ.[24] This could have important implications as BDQ resistance may act as a "surrogate marker" for CFZ resistance. However, in the present study, we did not perform CFZ DST to confirm the same.

Conclusion

This study shows a novel mutation in BDQ-associated *atpE* gene in XDR-TB isolates. No other mutations were found in the *RV0678* gene either in XDR or pre-XDR-TB isolates. Although mutations in *atpE* and *Rv0678* genes were found to be uncommon in this pilot study from a high disease burden country a larger sample set comprising diverse strains along with phenotypic correlation is the need of the hour to know the resistance pattern of this new drug.

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Conflicts of interest

There are no conflicts of interest.

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