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Reliability of Mycobacterial Growth Indicator Tube (MGIT) 960 for the detection of isoniazid resistance in a tuberculosis endemic setting

Sir,

Isoniazid (INH) is one of the forerunners in the treatment of tuberculosis (TB). Resistance to INH along with that of rifampicin (RIF) is considered multidrug resistance (MDR)[1]. Several methods have been validated for detection of MDR-TB. One of the promising methods well evaluated and accepted in varying settings is the fluorimetry based liquid culture detection system, Mycobacterial Growth Indicator Tube (MGIT 960) (Becton and Dickinson, USA)[2-4]. However, reports of "false resistance" to INH at a concentration of 0.1 µg/ml recommended by the manufacturer have been documented[5-7]. Studies suggest that use of a higher concentration (0.4 µg/ml) provides better distinction of the "false resistant" (FR) isolates[5,8,9]. Being a vital drug in the treatment of tuberculosis, such discrepancies might render treatment ineffective. In the current study, we assessed the reliability of the lone use of the manufacturer recommended drug concentration of INH (0.1 µg/ml) with respect to conventional minimum inhibitory concentration (MIC) method on Lowenstein-Jensen (LJ) medium.

The study conducted in the department of Bacteriology, National Institute for Research in Tuberculosis, Chennai, India, included two batches of cultures. The first batch consisted of 101 *Mycobacterium tuberculosis* isolates obtained from new (n=30) and 71 previously treated pulmonary TB patients (inclusive of 45 Category I and 26 Category IV failure). These isolates were tested for drug susceptibility (DST) to INH at 0.1µg/ml concentration by MGIT 960 system. Conventional DST by MIC method on L-J medium at concentrations 0.2, 1.0, and 5.0 µg/ml was performed for all the isolates[10].

The second batch had 60 *M. tuberculosis* isolates received from the Institute of Tropical Medicine, Belgium, as a part of routine external quality assurance (EQA). The isolates were tested for susceptibility to INH by MIC method and proportion susceptibility test (PST) at a concentration of 0.2 µg/ml[10], BACTEC 460 and MGIT 960 at a concentration of 0.1 µg/ml. DST by liquid culture systems was performed according to the manufacturer's protocol. Appropriate numbers of duplicates were included in MGIT 960 and BACTEC 460 as internal quality controls. Statistical methods using Chi square testing to assess the performance parameters of MGIT 960 were performed using SPSS software version 14.0, USA.

The results indicated high sensitivity (>91%), specificity (>95%) and accuracy (>92%) for detection of INH resistance by MGIT 960 in comparison with conventional MIC method (Table I) for the 101 isolates tested. Four isolates exhibited discordant results by MGIT 960 of which two were categorized as false resistant (FR) and two as false susceptible (FS).

Comparison of MGIT 960 with phenotypic methods (MIC and PST) and BACTEC 460 for EQA isolates showed a good concordance in sensitivity and specificity (Table II). MGIT 960 indicated three FR and

Table I. Comparison of INH susceptibility between MGIT 960 and conventional MIC method among clinical isolates (n=101)

MGIT 960	MIC method		
	R	S	Total
R	61	2	63
S	2	36	38
Total	63	38	101

Sensitivity 97%, Specificity 95%,
PPR 97%, PPS 95% and Accuracy 96%
Kappa 0.916, CI 0.825- 0.997
PPR/S, positive predictive values for resistance/susceptible

FS isolates in comparison with MIC and PST. One of the three FS isolates in MGIT 960 showed intermediate resistance (IR) phenotype by conventional MIC method. According to Van Deun[11] reporting IR strain as susceptible is acceptable in case of INH. Similar case of FS isolate was observed by Abe *et al*[12] with phenotypic resistance at 1.0 µg/ml. Two of the FS isolates showed MIC of 5 µg/ml by conventional MIC method. MGIT 960 in comparison with BACTEC 460 demonstrated three FS isolates and a single FR isolate. Results of the latter were resolved in accordance with MGIT 960 when compared with MIC and PST methods. The susceptibility pattern of duplicates was concordant with that of original.

Use of MGIT 960 in routine mycobacteriology has dramatically reduced the turn around time for detection of resistance thus paving way for early and accurate intervention[13]. Sensitivity and specificity of MGIT 960 for detection of INH resistance observed in our study was in accordance with earlier reports[6,7,9]. Studies indicate that MGIT 960 has a tendency to indicate more INH resistance rates than BACTEC 460 with increased chance for FR[4,8,14]. False resistant and FS isolates were observed to a limited extent in the present study. The uneven distribution of the heterogeneous population in subculture might have resulted in a false susceptible result[2]. In addition, varying growth indices in MGIT 960 while preparing inocula could also contribute to such discrepancies at higher frequencies. This warrants further evaluation with a larger number of isolates with intermediate resistances and different ranges of growth indices.

Presence of micro clumps in the inoculum carrying uneven distribution of mycobacteria, seeding the inoculum using pipette which allows large clumps and difference in the DST procedure when performed at different time points may contribute to false resistance in MGIT 960[14]. Despite the discrepancies observed, the accuracy of MGIT 960 was acceptable (≥92%). Agreement between the methods was found to be high (>0.8).

One limitation in this study was that the status of discrepant isolates was not reconfirmed by INH at 0.4µg/ml concentration. Validation of FR using a higher drug concentration was thought to be superfluous with high accuracy of MGIT 960 and limited discrepancy between phenotypic methods. In an unrelated study by the authors[15], existence of intermediate resistance (IR) to INH (data not shown) that could lead to discrepancy between MGIT 960 and conventional methods was found to be minimal in our setting. High level isoniazid resistance (≥5µg/ml) due to mutation in *kat*G gene was observed within the subcontinent[15]. Hence, with less number of IR to INH, DST by MGIT 960 can be performed at manufacturer's recommended concentration of 0.1 µg/ml in the present clinical setting.

Currently, MGIT 960 being considered the gold standard in liquid culture system and introduced for

Table II. Comparison of INH susceptibility by MGIT 960, BACTEC 460 and phenotypic methods among external quality assurance (EQA) isolates (n = 60*)

MGIT 960	Phenotypic methods			BACTEC 460		
	R	S	Total	R	S	Total
R	36	1	37	29	1	30
S	1	19	20	3	18	21
Total	37	20	57	32	19	51
Sensitivity %		97			91	
Specificity %		95			95	
PPR %		97			97	
PPS %		95			86	
Accuracy %		97			92	
Kappa (CI)		0.923 (0.818- 1.028)			0.836 (0.682- 0.990)	

R, resistant; S, susceptible; PPR/S, positive predictive values for resistance/susceptible
Phenotypic methods include MIC and PST. CI, Confidence interval at 95%. There was complete concordance between MIC and PST methods for quality assurance isolates
*Three and nine of the 60 EQA isolates were contaminated in MGIT and BACTEC, respectively and excluded from analysis

diagnosis and DST of *M. tuberculosis* under Revised National TB Control Programme[16], laboratory specific assessment of the recommended drug concentrations at regular time intervals is required to eliminate inconsistency and improve reliability.

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References

1. WHO Fact sheet on tuberculosis. October 2012. Available from: *http://www.who.int/mediacentre/factsheets/fs104/en/index.html*, accessed on November 8, 2012.
2. Garrigo M, Aragon ML, Alcaide F, Borrell S, Cardenosa E, Galan JJ, *et al*. Multicenter laboratory evaluation of the MB/BacT *Mycobacterium* Detection System and the BACTEC MGIT 960 system in comparison with the BACTEC 460TB system for susceptibility testing of *Mycobacterium tuberculosis*. *J Clin Microbiol* 2007; *45* : 1766-70.
3. Balabanova Y, Drobniewski F, Nikolayevskyy V, Kruuner A, Malomanova N, Simak T, *et al*. An integrated approach to rapid diagnosis of tuberculosis and multidrug resistance using liquid culture and molecular methods in Russia. *PLoS One* 2009; *4* : e7129.
4. Tortoli E, Benedetti M, Fontanelli A, Simonetti MT. Evaluation of automated BACTEC MGIT 960 system for testing susceptibility of *Mycobacterium tuberculosis* to four major antituberculous drugs: Comparison with the radiometric BACTEC 460TB method and the agar plate method of proportion. *J Clin Microbiol* 2002; *40* : 607-10.
5. Avendaño D, López B, Símboli N, Masciotra N, Barrera L. The Mycobacteria Growth Indicator Tube (MGIT) is useful for anticipating the resistance of *Mycobacterium tuberculosis* to rifampicin but not to isoniazid. *Rev Argent Microbiol* 2001; *33* : 197-202.
6. Ardito F, Posteraro B, Sanguinetti M, Zanetti S, Fadda G. Evaluation of BACTEC Mycobacteria Growth Indicator Tube (MGIT 960) automated system for drug susceptibility testing of *Mycobacterium tuberculosis*. *J Clin Microbiol* 2001; *39* : 4440-4.
7. Scarparo MC, Ricordi P, Ruggiero G, Piccoli P. Evaluation of the fully automated BACTEC MGIT 960 system for testing susceptibility of *Mycobacterium tuberculosis* to pyrazinamide, streptomycin, isoniazid, rifampin, and ethambutol and comparison with the radiometric BACTEC 460TB method. *J Clin Microbiol* 2004; *42* : 1109-14.
8. Bergmann JS, Fish G, Woods GL. Evaluation of the BBL MGIT (Mycobacterial growth indicator tube) AST SIRE system for antimycobacterial susceptibility testing of *Mycobacterium tuberculosis* to 4 primary antituberculous drugs. *Arch Pathol Lab Med* 2000; *124* : 82-6.
9. Abe C, Kobayashi I, Mitarai S, Wada M, Kawabe Y, Takashima T, *et al*. Biological and molecular characteristics of *Mycobacterium tuberculosis* clinical isolates with low-level resistance to isoniazid in Japan. *J Clin Microbiol* 2008; *46* : 2263-8.
10. Standard Operating Protocol for Mycobacteriology laboratory. Version 1.0 June 2010. National Institute for Research in Tuberculosis, Chennai. Available from: *http://www.trc-chennai.org/pdf/sop.pdf*.
11. Van Deun A, The Union & Antwerp Coordinating SRL. DST proficiency testing rounds in the Supranational Reference Laboratory Network. Available from: *http://www.stoptb.org/wg/gli/assets/html/day%201/Van%20Deun%20-, %20SRL%20DST%20profficiency%20testing.pdf*.
12. Abe C, Aono A, Hirano K. Evaluation of the BACTEC MGIT 960 system for drug susceptibility testing of *Mycobacterium tuberculosis* isolates compared with the proportion method on solid media. *Kekkaku* 2001; *76* : 657-62.
13. Somoskovi A, Clobridge A, Larsen SC, Sinyavskiy O, Surucuoglu S, Parsons ML, *et al*. Does the MGIT 960 system improve the turnaround times for growth detection and susceptibility testing of the *Mycobacterium tuberculosis* complex? *J Clin Microbiol* 2006; *44* : 2314-5.
14. Piersimoni C, Olivieri A, Benacchio L, Scarparo C. Current perspectives on drug susceptibility testing of *Mycobacterium tuberculosis* complex: the automated nonradiometric systems. *J Clin Microbiol* 2006; *44* : 20-8.
15. Nusrath Unissa A, Selvakumar N, Narayanan S, Narayanan PR. Molecular analysis of isoniazid-resistant clinical isolates of *Mycobacterium tuberculosis* from India. *Int J Antimicrob Agents* 2008; *31* : 71-5.
16. RNTCP response to challenges of drug resistant TB in India. January 2012 (update). Available from: *http://www.tbcindia.nic.in/pdfs/RNTCP%20Response%20DR%20TB%20in%20India%20-%20Jan%202012%20update.pdf*, accessed on November 8, 2012.