The use of E-test for the drug susceptibility testing of *Mycobacterium tuberculosis* – A solution or an illusion?

JS Verma, D Rawat, A Hasan, MR Capoor, K Gupta, M Deb, P Aggarwal, *D Nair

Abstract

**Aim:** To evaluate E-test as a tool for rapid determination of drug susceptibility against the conventional LJ method focusing on reliability, expense, ease of standardization and performance of the technique in low resource settings. **Materials and Methods:** A total of 74 clinical isolates (2004-2005) of *Mycobacterium tuberculosis* were tested using E-test for susceptibility to streptomycin (STM), isoniazid (INH), rifampicin (RIF) and ethambutol (EMB) by E-strip and LJ (LJPM) proportion methods. **Results:** The LJPM method, the gold standard, detected resistance against STM in 16.2%, INH in 40.5%, RIF in 18.9% and EMB in 27% cases. In comparison, the resistance values showed by E-test was 66.67% for STM, 57.14% for INH 71.43% for RIF and 80% for EMB. The susceptible correlation was 90.32% for STM, 73.91% for INH, 93.33% for RIF and 59.26% for EMB. E-test correctly identified only eight of the 12 (66.6%) MDR isolates and wrongly identified four isolates which were not MDR. The overall agreement between the two methods was only 48.6%. Resistant isolates showed false positive resistance observed while using E-strip towards all the drugs. **Conclusion:** E-strips are not quite feasible as a replacement for LJ-proportion method on a large scale due to high risk of cross contamination, laboratory infection, expense associated with it and high false positive resistance observed to all first line drugs. However, the good correlation observed for RIF between the two methods indicates that E-test could contribute to the role in rapid screening of MDR TB isolates as rifampicin mutations are invariably observed in MDR TB isolates.

**Key words:** E-test, *Mycobacterium tuberculosis*, drug susceptibility testing

Introduction

Tuberculosis (TB) has been and continues to be one of the most significant causes of human morbidity and mortality. With 1.8 million cases occurring annually, India accounts for a fifth of the world’s new TB cases and two-third of the cases in South East Asia.[1-2] The spread of HIV during the last two decades and the emergence of multidrug resistant (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB), has greatly exacerbated an already grave situation in the developing world.[7] Resistance of tubercle bacilli to anti-tubercular drugs is the result of a spontaneous genetic event made worse as “a manmade amplification of the natural phenomenon”.[8] In the light of the worsening global situation, rapid and reliable drug susceptibility testing (DST) in the laboratory is paramount for proper management of patients, especially those with MDR or XDR-TB.

Different methods for testing drug susceptibility of the tubercle bacilli have been used in the past. The most widely accepted gold standards are the proportion method using Lowenstein-Jensen (LJ) medium and The BACTEC MGIT 960 system.[9] The long turnaround time of the LJ proportion method deters physicians in patient management although it is suitable for drug resistant surveillance (DRS). BACTEC 460 usually gives results within 10 days but expense and disposal of radioactive waste are important issues associated with its use. To shorten the turnaround time, numerous new techniques have been introduced aiming to detect drug resistance as early as possible. The most common methods are agar based E-test,[10] Middlebrook 7H11 agar proportion method,[11] automation based tests (MB/BacT[12] and MGIT[13]), A new molecular test; Line Probe Assay (LiPA)[14] is a highly sensitive and specific test for the detection of rifampicin resistance in culture isolates. The limitations in the adequate assessment of drug resistance are due to nonuniformity and lack of standardization of laboratory procedures, which is also an important factor, resulting in misleading clinical reports. A rapid and standardized method for DST, which is easy to perform and does not require expensive equipment, is a pressing need.

The E-test for susceptibility testing of mycobacteria was initially evaluated by Wanger and Mills as a rapid method for drug susceptibility testing of *Mycobacterium tuberculosis* (*M. tuberculosis*).[9] The advantages of this method are relative ease of performance, rapid turnaround time and possibility of testing as many as five drugs at the same time in a single plate of 150 mm diameter. There have been studies which attempted to evaluate the role of E-test in DST of *M. tuberculosis*. However, its reliability as an alternative to the conventional methods remains ambiguous.
The present study focuses on the evaluation of E-test as a tool for rapid determination of drug susceptibility against the conventional LJ proportion method focusing on the reliability, expense, ease of standardization and performance of the technique in low resource settings.

Materials and Methods

This study was conducted in the department of Microbiology in a tertiary care centre (approx. 1600 bed tertiary care centre), New Delhi. A total of 74 clinical isolates of *M. tuberculosis*, archived from 2004-2005 were speciated using standard methods including growth parameters, biochemical tests and Accuprobe (GenProbe Incorporated, San Diego, CA 92121 USA).[10]

All isolates were E-tested for susceptibility to streptomycin (STM), isoniazid (INH), rifampcin (RIF) and ethambutol (EMB) by E-strip and LJ proportion (LJPM) methods. Antibiotic powders were obtained from Oxoid, Cambridge, UK. The anti tuberculosis drugs critical concentrations used for LJPM were as per Tuberculosis Research Centre (TRC), Chennai guidelines[21] and those given in the manufacturers booklet for the E-test (AB Biodisk, Solna, Sweden)

*M. tuberculosis* H37RV, the reference strain sensitive to all antitubercular agents was used as the control strain in every batch of testing. The proportion method using LJ medium (prepared inhouse) were performed as per established standard procedures and calculations for resistance also made as recommended by TRC, Chennai.[21]

Susceptibility by E-test method was performed on Middle brook 7H11 agar (Difco, Becton, Dickinson and Company, Sparks, MD 21152 USA) with oleic acid-albumin-dextrose-catalase (OADC) supplement, Difco, BD, USA. E-strips containing gradient concentrations were recorded and reported as sensitive or resistant.[19] Isolates with discordant results between LJPM and E-test were retested thrice by both methods and the average of consistent result was recorded.

Results

A total of 74 clinical isolates of *M. tuberculosis* were identified and tested for susceptibility to STM, INH, RIF and EMB by both LJPM and E-test method. Forty 6 (62.2%) of the isolates were from sputum samples, 18 (24.3%) were from pus, 6 (81%) from pleural fluid and 4 (5.4%) were from synovial fluid.

LJPM, the gold standard, detected resistance against STM in 16.2%, INH in 40.5%, RIF in 18.9% and EMB in 27% cases. In comparison, the resistance correlation showed by E-test was 66.67% for STM, 57.14% for INH 71.43% for RIF and 80% for EMB. The false positive (FP) resistance by E-strip was 33.33% for STM, 42.86% for INH, 28.57% for RIF and 57.89% for ETM. The false negative (FN) resistance was 22.22% for STM and no false negative resistance was observed for INH, RIF and ETM [Table 1].

The susceptibility correlation was 90.32% for STM, 73.91% for INH, 93.33% for RIF and 59.26% for EMB. The FP sensitive by E-strip was 3.23% for STM, 26.09% for INH, 6.67% for RIF and 11.11% for ETM. The FN sensitive was 6.45% for STM, 33.33% for ETM and no false negative sensitive isolate was reported for INH and RIF [Table 1].

Thirty Six (48.6%) were sensitive to all the four drugs by the LJPM, while 20(27%) were sensitive by E-test. Both the methods gave the same results for 28(37.8%) of the isolates tested. In the remaining 46 isolates (62.1%), there were discrepancies in results between the two methods for one or more of the drugs tested. LJ proportion method and E-test identified 12 isolates each as multidrug resistant (MDR). However, only eight isolates were identified by both the methods. Four isolates that were MDR by LJPM were sensitive by E-test, while four (33.3%) isolates that were sensitive by LJPM were shown to be MDR by E-test. The agreement between two methods was found to be 89.2% in case of RIF although false resistance was also found (28.6%). E-strip also gave a high percentage of false resistance in EMB (57.89%) and the agreement between the two methods was only 45.9%.

Discussion

This study was initiated as an attempt to standardize E-strip method as a rapid DST in the department of Microbiology and DOTS referral centre with a large sample size.

The best correlation of results between the two methods was seen in the case of RIF. This observation is in accordance of earlier studies which have found excellent

| Antibiotic    | Sensitivity (%) | Specificity (%) | Positive predictive value (%) | Negative predictive value (%) | % False resistant | % False sensitive | % Resistance correlation | % Sensitive correlation |
|---------------|-----------------|-----------------|-------------------------------|-------------------------------|-------------------|-------------------|--------------------------|------------------------|
| Streptomycin  | 90.6            | 60              | 93.5                          | 50                            | 9.4               | 40                | 66.67                    | 90.32                  |
| Isoniazid     | 73.9            | 64.2            | 77.2                          | 60                            | 13.5              | 16.2              | 57.14                    | 73.91                  |
| Rifampcin     | 93.3            | 71.4            | 93.3                          | 83.3                          | 6.6               | 28.6              | 71.43                    | 93.33                  |
| Ethambutol    | 59.3            | 70              | 84.2                          | 38.9                          | 42.6              | 30                | 80                       | 59.26                  |

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correlation of the RIF E-strip results with proportion method. However, the performance of E-test for MDR was poor. Similar findings have been observed for EMB by Hausdorfer et al. In the present study, E-test did not perform as well for INH and STM as has been previously reported. It gave a substantial number of false resistant results for INH as well as EMB. This may be because of difficulties in achieving uniform inocula in terms of the true numbers of bacilli plated prior to the placement of E-test strips and the subsequent misinterpretation of the susceptibility profile. This inability to achieve uniform inoculum may have affected the proportion of resistant bacilli. Growth phase status can also contribute to the error observed as proportion of resistant bacilli may differ in log phase versus stationary phase cultures. Hazbon et al. also suggest that this event may relate to in vitro selection of a resistant subpopulation during subculturing.

E-test correctly identified only eight of the 12 (66.6%) MDR isolates and wrongly identified four isolates which were not MDR. The overall agreement between the two methods was only 48.6% in contrast to more than 80%, as has been reported earlier. However, Freixo et al. and Hausdorfer et al., have similar disappointing results of concordance between LJ and E-strip. Thus, although E-test has the advantage of being rapid and simple, its actual utility in DST towards DST for M. tuberculosis is limited.

Foremost among its disadvantages are-high rate of contamination, difficulty in standardizing the technique and obtaining uniform results, high risk of accidental infection to the worker and the potential hazard arising from the high inoculum needed for the E-test. The cost per sensitivity of E-test is approx USD 4-5 $/strip × four strips per strain plus approx USD 5 $/plate (Middlebrook with OADC) × four plates. So the total cost would be USD 40-50 $/per strain/test while LJ with drug costs approx USD1 $/bottle × four amounting to USD 4-5 $/per strain. Thus the cost of LJPM comes to 1/10th the cost of doing E-test. The high cost of the E-strips is further increased by the frequent need for repetition due to contamination or difficulty in interpreting results because of insufficient growth. Also there is need for specialized equipment like a CO₂ incubator.

This study did not find E-test to be a suitable alternative for routine DST of M. tuberculosis isolates. These findings are corroborated by the conclusion of Freixo et al. that for developing countries where financial constraints couple with larger workloads of M. tuberculosis isolates making quality control and standardization of the E-test very difficult to achieve. However, it could have an important role to play in the rapid screening for rifampcin resistance. As mono rifampcin resistance is very rarely encountered, the results of rapid E-strip resistance of rifampcin can be possibly equated to MDR TB. This could be used as a screen before the results of LJ or any other proportion method make themselves available. This statement, however, would need larger studies to validate it and in vivo studies to support it.

In conclusion, E-strips inspite of their stated advantages, are not quite feasible as a rapid DST method for use on a large scale. However, the excellent correlation observed for RIF between the two methods indicates that E-test may have a role in rapid screening of MDR TB isolates. As of now, the cost factor and elaborate laboratory specifications remains a hurdle in the routine use of E-strips.

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