E-Test: An Alternative Method for Susceptibility Testing of *Mycobacterium tuberculosis*

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Key Words

Tuberculosis · Susceptibility · E-test · Agar proportion

Abstract

Objective: The purpose of this study was to compare the agar proportion method with the E-test method for susceptibility testing of *Mycobacterium tuberculosis*. **Materials and Methods:** A total of 100 isolates were tested for isoniazid, rifampin, streptomycin and ethambutol susceptibility using an indirect-proportion method as well as the E-test method. **Results:** Categorical agreement between the methods was 100% for isoniazid, rifampin, streptomycin, and ethambutol. **Conclusion:** The E-test method appears to be an alternative method to agar proportion for testing the susceptibility of *M. tuberculosis* isolates to the first-line antituberculous agents.

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Introduction

Due to the recent increase in the incidence of tuberculosis (TB) and the variable susceptibility of isolated strains in certain parts of the world, the need for rapid diagnosis of TB has become paramount. According to the latest fig-

ures of the World Health Organization, one third of the world's population is infected with *Mycobacterium tuberculosis*, and there are 8–10 million new TB cases every year. Because of the emerging resistance to the two most important antituberculosis drugs, isoniazid (INH) and rifampin (RIF), laboratories are challenged to provide rapid identification and antimicrobial susceptibility testing for effective treatment of the disease [1].

Current methods for the susceptibility testing of *M. tuberculosis*, as described in the tentative standard (M24-T) of the National Committee of Clinical Laboratory Standards, include the agar proportion and BACTEC (Becton-Dickinson, Sparks, Md., USA) radiometric methods [2]. Unfortunately, both methods suffer from limitations, such as standardization for only the four first-line antituberculous agents [INH, RIF, ethambutol (EMB), and streptomycin (STR)] and reliance on a single critical concentration of an antimicrobial agent for susceptibility categorization. Both methods also require technical expertise for the interpretation of results. The agar proportion method has the additional disadvantage of requiring 3 weeks of incubation [3], and although the BACTEC broth method is more rapid, it requires expensive equipment and supplies that are currently too expensive for less developed countries where the prevalence of TB is high [4]. Although molecular techniques such as

PCR and DNA hybridization assays provide results within 24 h, they also require specialized equipment and highly skilled personnel, and equally important, these techniques have not yet been developed for all known mutations and antimycobacterial drugs [5].

E-test (AB Biodisk, Solna, Sweden) is a new method for determining minimum inhibitory concentrations (MICs) of antimicrobial agents. It has been shown to compare well with conventional dilution techniques for numerous bacterial pathogens [6–8]. To further evaluate the E-test method for susceptibility testing of *M. tuberculosis*, we compared the E-test with the agar proportion method using four first-line antituberculous agents.

Materials and Methods

A total of 100 isolates maintained as stock cultures on egg yolk medium agar slants (Löwenstein-Jensen) at +4°C were used in this study. We included the quality-control strains *M. tuberculosis* H37RV, which is susceptible to all first-line drugs; ATCC 35838, which is resistant to RIF; ATCC 35837, which is resistant to EMB; ATCC 35822, which is resistant to INH, and ATCC 35820, which is resistant to STR. The strains were subcultured on Löwenstein-Jensen slants and their species was reconfirmed on the basis of results of standard biochemical tests [9].

All strains were tested for INH, RIF, STR and EMB (Sigma Chemical Co., USA) susceptibility using an indirect-proportion method on Middlebrook 7H10 medium at the critical concentrations shown in table 1 [8]. Colonies from a Löwenstein-Jensen slant were homogenized in phosphate-buffered saline (pH 7.0) to achieve turbidity equal to a McFarland 1.0 standard, corresponding to approximately 10^9 cfu/ml. This bacterial suspension was used for agar dilution by inoculating plates with a Steers replicator. In the agar proportion methods, an isolate was classified as susceptible to a drug if the number of colonies that grew on a control plate was <1% of the number of colonies that grew on a control plate without the drug, partially resistant if the number was between 1 and 10%, and resistant if the number was >10%.

The E-test was performed as previously described [10]. E-test strips (AB Biodisk, Solna, Sweden) contained gradients of RIF (0.002–32 µg/ml), INH (0.016–256 µg/ml), STR (0.016–256 µg/ml), and EMB (0.016–256 µg/ml). Colonies from a Löwenstein-Jensen agar culture that was 4–6 weeks old were homogenized in 3 ml of 7H9 broth with 0.5% glycerol and four sterile glass beads. The cap was screwed firmly and vortexed for 3 min. The clumps were allowed to settle out for 20 min. The turbidity of the bacterial suspension was adjusted by the addition of sterile distilled water to equal a McFarland 3 turbidity standard. The 90-mm plates containing Middlebrook 7H11 agar with 10% oleic acid albumin dextrose complex were inoculated and incubated at 37°C in 5% CO₂ for 24 h, after which time the E-test strip was placed on the agar surface. The plates were then incubated under the same conditions until an inhibition ellipse was visible (5–10 days). The MIC was interpreted as the point at which the ellipse intersected the E-test strip.

Table 1. Critical concentrations used for the agar proportion method on Middlebrook 7H10 agar

Agent	Critical concentration µg/ml
INH	0.2
RIF	1.0
STR	2.0
EMB	5.0

Table 2. Comparison of susceptible (S) and resistant (R) *M. tuberculosis* strains by the E-test and agar proportion methods

Agent	Number of isolates with indicated result by				% agreement
	agar proportion method		E-test method		
	S	R	S	R	
INH	88	12	88	12	100
RIF	91	9	91	9	100
STR	85	15	85	15	100
EMB	90	10	90	10	100

Results

All quality-control strains produced expected results by both methods. Comparative results of drug susceptibility testing of test isolates are shown in table 2.

Both methods identified 19% of strains as resistant to at least 1 of the 4 antibiotics. Resistance to STR was the highest at 15%, while resistance to INH, RIF, and EMB were 12, 9, and 10%, respectively. Resistance to 1, 2, 3 and 4 antibiotics was observed in 6, 5, 2, and 6% of the isolates, respectively. Multidrug resistance (MDR) was found in 8% of the isolates. The MICs at which 50% (MIC₅₀) and 90% (MIC₉₀) of the isolates were inhibited for susceptible and resistant strains are shown in table 3.

It was possible to read the results at 6–10 days by E-test and 21 days by agar incorporation. Using the critical concentrations recommended for the agar proportion method as the breakpoint MICs for the E-test method (table 1), there was 100% agreement by kappa statistical analysis in susceptibility categories between the E-test and agar proportion methods for RIF, INH, EMB, and STR ($p = 0.000$).

Table 3. MIC₅₀ and MIC₉₀ values for susceptible and resistant strains by the E-test method

Agent	Susceptible strains			Resistant strains		
	n	MIC ₅₀ μg/ml	MIC ₉₀ μg/ml	n	MIC ₅₀ μg/ml	MIC ₉₀ μg/ml
INH	88	<0.016	<0.016	12	8	>256
RIF	91	<0.002	<0.002	9	>32	>32
STR	85	<0.016	<0.016	15	>256	>256
EMB	90	<0.016	<0.047	10	>256	>256

Discussion

The good agreement between the E-test and agar proportion methods for determining MICs for the four first-line antituberculous agents in this study indicates that E-test is a viable alternative to the agar proportion method currently used to test susceptibility of *M. tuberculosis*. Wanger and Mills [10] have also found good correlations between E-test and agar proportion: 100, 94, 93, and 90% for RIF, STR, INH and EMB, respectively; E-test and BACTEC: 100, 96.8 and 90% for RIF, EMB, and INH, respectively. Joloba et al. [4] found that the categorical agreement between E-test and agar proportion methods was 100% for RIF, EMB, and STR, and 98% for INH. Sanchez et al. [11] demonstrated excellent agreement (100% for RIF, 96.8% for EMB, and 90% for INH) between the E-test MIC distributions and the BACTEC interpretive criteria for all clinical isolates of *M. tuberculosis* tested.

On the other hand, Hausdorfer et al. [12] and Freixo et al. [13] noted that the E-test gave a significant number of false sensitive and false resistant results when compared with agar proportion. They also observed that variations in the inoculum size of *M. tuberculosis* isolates affected the MICs to a substantial degree. They suggested that these discrepancies, along with the expense of the media, the E-test strips, and the specialized equipment required (CO₂ incubator), all make this method less useful in developing countries. However, we did not find false sensitive and false resistant results by the E-test. Since more rapid visual growth was achieved with the heavier inoculum, a density of McFarland 3 was preferred. In optimal conditions, the same subculture would have been used to perform the drug susceptibility testing by both methods. This can be interpreted for better performance of E-test in our study.

Early recognition and appropriate treatment have been proven to be one of the most effective strategies to control MDR TB even in human immunodeficiency virus-infected populations. Knowledge of the drug susceptibility pattern of the MDR clinical isolate is necessary to design and prescribe an appropriate treatment for the patient. Susceptibility testing can prevent treatment failures and thereby diminish the number of secondary cases of MDR TB. Hazbon et al. [5] compared the performance of E-test to that of the reference proportion method, using 95 *M. tuberculosis* clinical isolates of which 42.1% (40 of 95) were resistant to at least one antibiotic by the reference method. In their study, overall agreement between E-test and the reference method was 98.9% (94 of 95) for detection of MDR; for resistance to individual drugs, agreement was 97.9, 96.0, 94.7, and 85.3% for RIF, EMB, INH, and STR, respectively.

E-test is an accurate and precise MIC method covering 15 twofold dilutions and has emerged as the method of choice for the susceptibility testing of fastidious organisms, including rapidly growing mycobacteria. Various studies have shown that E-test is a promising new method for the susceptibility testing of slowly growing mycobacteria as well [14–16].

Conclusion

In our evaluation of 100 clinical isolates of *M. tuberculosis*, the E-test was found to be a reasonably fast, accurate, reproducible, and easy method to determine the isolates' susceptibility to 4 antituberculous agents, when compared to the agar proportion method.

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