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Comparative Study for Determination of *Mycobacterium tuberculosis* Susceptibility to First- and Second-Line Antituberculosis Drugs by the Etest Using 7H11, Blood, and Chocolate Agar▽

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We investigated the performance of blood and chocolate agar as alternatives to Middlebrook 7H11 agar for testing the susceptibility of *Mycobacterium tuberculosis* to first-and second-line drugs by the Etest method. A total of 39 strains of *M. tuberculosis* including 22 multidrug-resistant *M. tuberculosis* strains and 17 susceptible strains were tested. In conclusion, our results showed that chocolate agar gave insufficient growth, needing up to 21 days of incubation, while results on blood agar were comparable to those on Middlebrook 7H11 agar and can be further explored as an alternative for Etest-based susceptibility testing of *M. tuberculosis*.

Early clinical diagnosis of tuberculosis, identification of multidrug-resistant (MDR) *Mycobacterium tuberculosis* strains, and appropriate treatment are the most effective strategies to control the spread of MDR tuberculosis (18). Therefore, rapid and efficient methods are needed to accurately diagnose and control this disease efficiently. The CLSI reference method for susceptibility testing of *M. tuberculosis* is the agar proportion method (22) performed with Middlebrook 7H10 to -11 agar. On the other hand, there are semiautomated and automated systems (BACTEC 460 TB and BACTEC MGIT [mycobacterium growth indicator tube] 960; Becton Dickinson Diagnostic Systems, Sparks, MD) for susceptibility testing of *M. tuberculosis*, but they are available only in developed countries. Blood agar is a basic medium widely and routinely used in clinical microbiology laboratories worldwide. In addition, blood agar is suitable for isolation and culture of mycobacteria, including the causative agents of tuberculosis (11).

Etest (AB Biodisk, Solna, Sweden) is used for quantitative antibiotic susceptibility testing of numerous microorganisms, including various species of mycobacteria, including *M. tuberculosis* (1, 2, 12–15, 17–20). The current recommended Etest procedure for *M. tuberculosis* is based on Middlebrook 7H11 agar supplemented with oleic acid, albumin, dextrose, and catalase (OADC) (1). In recent years, it has been reported that *M. tuberculosis* isolates could be grown on blood agar in 1 to 2 weeks (4, 10, 11, 16). In addition, the results of our previous reports have shown that blood agar can be used as an alternative medium for testing *M. tuberculosis* with isoniazid (IZ), rifampin (RI), streptomycin (SM), and ethambutol (EB) (6–8, 31).

In this study, the performance of blood and chocolate agar was compared to that of Middlebrook 7H11 agar with the aim to evaluate them as possible alternatives for susceptibility testing of *M. tuberculosis* clinical isolates against IZ, RI, SM, EB, ofloxacin (OF), ciprofloxacin (CI), levofloxacin (LE), linezolid (LZ), and ethionamide (ET) by Etest.

A total of 39 strains of *M. tuberculosis* and H37Rv (control strain susceptible to all antituberculous drugs) were tested. While 22 MDR *M. tuberculosis* strains were isolated from sputum of chronic pulmonary tuberculosis patients in Istanbul, Turkey, 17 susceptible strains were isolated from sputum of pulmonary tuberculosis patients in Samsun, Turkey. All isolates were isolated using the BACTEC 460 TB system and Löwenstein-Jensen medium. Thirty of these 39 strains were also tested with all drugs, including IZ, RI, SM, EB, LZ, OF, CI, LE, and ET, whereas 9 of 39 strains were tested only with IZ, RI, SM, and EB.

Etest strips for nine drugs (IZ, RI, SM, EB, OF, CI, LE, LZ, and ET) were obtained from AB Biodisk, Solna, Sweden. Standard blood (supplemented with 5% sheep blood) and chocolate agar (chocolate agar plus PolyViteX) plates were purchased from Biomerieux, France, and stored at +4°C until use.

The Etest procedure was performed as described by AB Biodisk (1). The agar plates were sealed with a shrink seal and incubated at 37°C in 7 to 10% CO_2 for 5 to 10 days. The plates were visually examined and read after 5 and 10 days of incubation when the mycobacterial lawn of growth and inhibition ellipse became clearly visible (Fig. 1A and B).

All MIC results were interpreted into categories (susceptible or resistant) using the CLSI agar proportion breakpoints (22) for intermethod comparisons. Resistance was defined as a MIC of >0.2 μg/ml for IZ, >1 μg/ml for RI, >7.5 μg/ml for EB, >2 μg/ml for SM, >2 μg/ml for OF, >10 μg/ml for ET (22), >2 μg/ml for CI and LZ, and >1 μg/ml for LE (23, 28). Agreement of the Etest results for the nine antibiotics on blood agar and chocolate agar compared to the recommended Middle-

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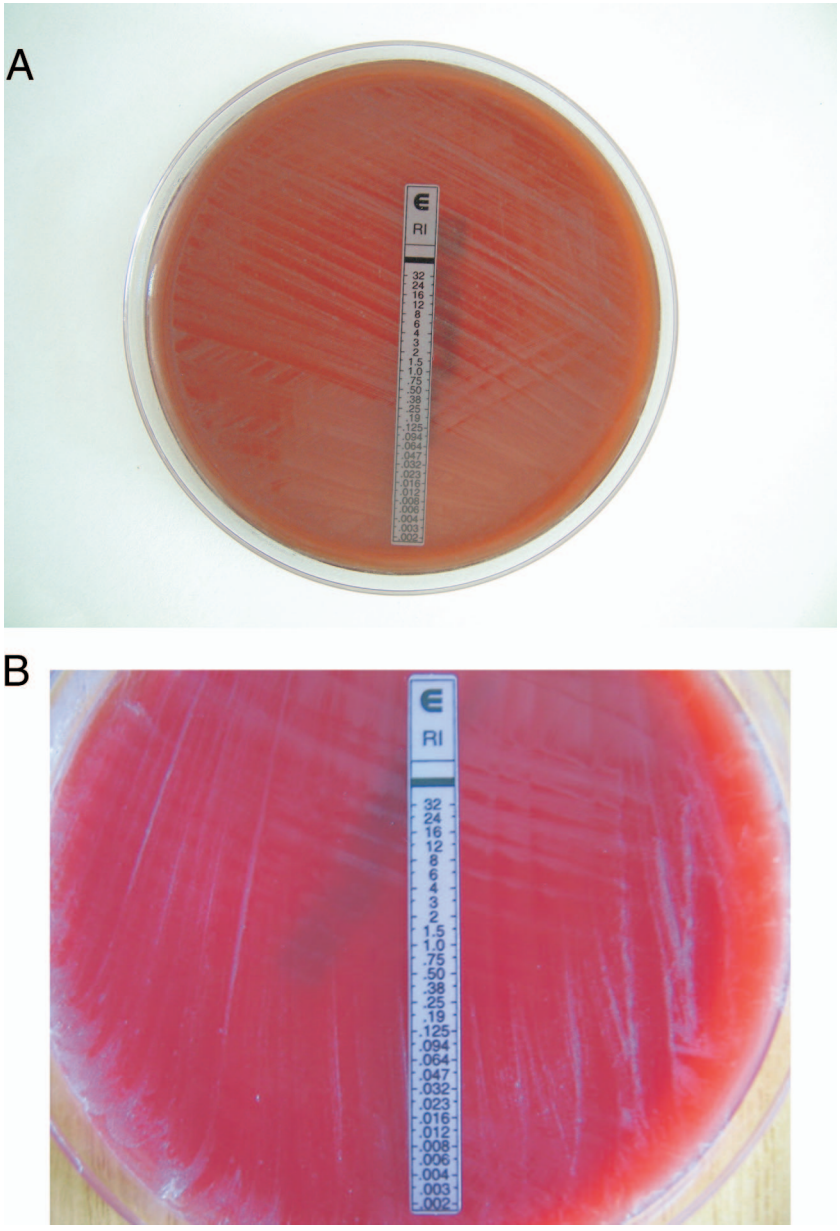

FIG. 1. Etest results for RI on blood agar after 10 days of incubation for resistant (MIC, >32 μg/ml) (A) and susceptible (MIC, 0.25 μg/ml) (B) *M. tuberculosis* isolates.

brook 7H11 agar and BACTEC 460 TB system are summarized in Tables 1, 2, and 3. All Etest plates were examined after the 10th, 14th, and 21st days of incubation, and Etest results (for all resistant or susceptible strains) were seen on the 10th day on Middlebrook 7H11 agar and blood agar, whereas results on chocolate agar were first obtained on the 21st day of incubation. When the incubation time was extended to 21 days on 7H11 and blood agar, MICs were not changed.

It has been reported recently that *M. tuberculosis* can be easily grown on blood agar and chocolate agar (10, 11). Moreover, other studies (6–8, 31) have noted that blood agar may be suitable for susceptibility testing of *M. tuberculosis*. We therefore evaluated the use of Etest for testing the susceptibility of *M. tuberculosis* to IZ, RI, SM, EB, OF, CL, LE, LZ, and ET using three different media: Middlebrook 7H11 agar (recommended by the manufacturer) and blood agar and chocolate agar. Etest has been evaluated for susceptibility testing of *M. tuberculosis*, especially for the first-line drugs. Wanger and Mills (29, 30) demonstrated an excellent correlation between Etest and agar dilution for *M. tuberculosis* tested with RI (1994) and suggested that the Etest may be considered as an alternative method for the first-line drugs (1996). Sanchez et al. (25) determined MICs of RI, IZ, and EB by the Etest and found very good agreements with the BACTEC 460 TB system: i.e., 100% for RI, 96.8% for EB, and 90% for IZ. Etest MICs could be read within 5 to 10 days. In the study of Sanic et al. (26), acceptable agreements found between the Etest and the proportion method (7H11 agar) were 83.1% for IZ, 78.8%

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TABLE 1. Comparison of categorical results between Etest on Middlebrook 7H11 and BACTEC 460 TB

Radiometric proportion method result[a]	No. with result by Etest on 7H11 agar[b]		% Agreement
	Resistant	Susceptible	
SM			
Resistant	8	1	94.9
Susceptible	1	29	
IZ			
Resistant	24	1	97.4
Susceptible	0	14	
RI			
Resistant	22	0	100
Susceptible	0	17	
EB			
Resistant	10	3	89.7
Susceptible	1	25	

[a] Day of detection, day 8.
[b] Day of detection, day 10.

for RI, 84.7% for SM, and 80.5% for EB. Kakkar et al. (20) also found excellent agreements between the Etest and the proportion method: 96% for IZ, 92% for RI, and 100% for SM and EB, and results were obtained after 7 to 10 days of incubation. Joloba et al. (19) also evaluated the Etest for *M. tuberculosis* and found excellent agreement for IZ (96%), RI (100%), SM (100%), EB (100%), and OF (100%), and results were obtained within 6 to 10 days of incubation. An overall agreement between Etest and the proportion method of 98.9% was found for the detection of MDR by Hazbon et al. (18). Varma et al. (27) found Etest to be a more rapid method (10 days) than agar proportion (3 weeks) for testing of *M. tuberculosis* with IZ and RI. Birinci et al. (5) suggested that the Etest could be routinely used in place of the proportion method. It has also been reported that the Etest has the advantage of being a rapid and simple method without the need for any additional equipment (24). Muralidhar and Srivastava (21) compared the proportion method using Löwenstein-Jensen (LJ) medium and 7H11 agar to Etest in testing *M. tuberculosis* with IZ, RI, SM, ET, and CI. They found an overall agreement of 87% between the proportion method (LJ medium) with Etest and 79% agreement between proportion method results performed with LJ and 7H11 media and concluded that the Etest was superior to the agar proportion methods. Akcali et al. (2) found excellent categorical agreements between the indirect proportion method and Etest (100% for IZ, RI, SM and ET. Fabry et al. (13) found the Etest to be suitable for MIC determinations of amikacin, SM, fusidic acid, RI, clarithromycin, CI, OF, and fleroxacin with *M. tuberculosis*.

TABLE 2. Comparison of categorical results for Etest on blood and chocolate agar to results with BACTEC 460 TB and Middlebrook 7H11 agar

Comparison	% Agreement				Day of detection
	SM	IZ	RI	EB	
BACTEC 460 TB vs[a]:					
Etest					
Blood agar	94.9	97.4	100	82.1	10
Chocolate agar	94.9	97.4	97.4	89.7	21
7H11 agar vs[b]:					
Etest					
Blood agar	94.9	100	100	92.3	10
Chocolate agar	94.9	100	97.4	89.7	21

[a] Day of detection, day 8.
[b] Day of detection, day 10.

TABLE 3. Comparison of categorical results of Etest on blood and chocolate agar versus results with Middlebrook 7H11 agar for second-line drugs

Etest parameter	% Agreement compared with 7H11 agar[a]					Day of detection
	ET	OF	LE	CI	LZ	
Blood agar	93.3	90	100	96.6	100	10
Chocolate agar	56.6	86.6	100	96.6	100	21

[a] Day of detection, day 10.

Freixo et al. (15) compared Etest to the standard proportion method using L-J medium in testing IZ, RI, and SM with *M. tuberculosis*. Although they achieved very good agreements between the methods (97% for RI, 96% for IZ, and 80% for SM), they reported the Etest to be less useful for developing countries because of the expense associated with media, Etest strips, and the need for a CO_2 incubator. Esteban et al. (12) did not recommend the use of Etest for detecting IZ resistance in *M. tuberculosis* complex strains due to the cost of Etest and the risk of contamination when streaking an agar plate with a high inoculum of viable cells. The manual MGIT and Etest were compared to the proportion method by Fegou et al. (14), and they concluded that the proportion method remains the method of choice. Delas et al. (9) found that the Etest provided low specificity for detection of resistance to SM and EB and suggested further investigations. In the *M. tuberculosis* study of Hausdorfer et al. (17), the Etest was not recommended by the authors for practical use in clinical laboratories due to false susceptibility results.

We investigated the performance of the Etest on three media for both first- and second-line drugs since only a few studies have reported Etest results for second-line drugs for *M. tuberculosis*. Alcala et al. (3) tested 117 isolates of *M. tuberculosis* with LZ using the proportion method and Etest and found high LZ in vitro activity against all isolates. We also found all *M. tuberculosis* clinical isolates to be susceptible to LZ by all methods. Muralidhar and Srivastava (21) tested CI by Etest and the proportion method using both LJ and Middlebrook 7H11 agar. Five isolates were found resistant to CI on the LJ medium, and no resistance was found by the Etest and proportion method on Middlebrook 7H11 agar. In the study by Joloba et al. (19), results for CI could not be read because of irregular growth along the Etest strip; however, the same investigators found an excellent categorical agreement of 100% for OF between the Etest and the proportion method for both media. In our study, all isolates were susceptible to CI by the Etest on Middlebrook 7H11 agar, while one strain was resistant on blood and chocolate agar. For OF, the agreement was 90% on blood agar and 86.6% on chocolate agar.

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In this study, the results with blood agar and 7H11 agar for Etest are concordant for time and result (susceptible or resistant). In the other study (8), the results were concordant, but in one center the results were obtained on the 14th day of incubation for 100 isolates, whereas in the other center, results were obtained on the 21st day of incubation for 47 isolates.

In our country, Middlebrook 7H11 agar is generally used for antituberculous susceptibility testing and research and it requires the OADC supplement. However, blood agar is routinely used. However, Drancourt et al. (11) reported that blood agar saves time, is cost-effective, and is at least as rapid as the automated systems.

In conclusion, Etest results on Middlebrook 7H11 agar and blood agar were equivalent and results could be obtained in 10 days. However, results on chocolate agar were available only after 21 days because of insufficient growth support. For laboratories with no access to Middlebrook 7H11 agar, we recommend blood agar as an alternative medium for the testing of *M. tuberculosis* susceptibility by Etest and encourage further studies and evaluation of improved versions of blood agar to attempt to shorten the incubation time.

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