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Reliability of the MB/BacT System for Testing Susceptibility of *Mycobacterium tuberculosis* Complex Isolates to Antituberculous Drugs

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The susceptibility of 115 *Mycobacterium tuberculosis* complex clinical isolates to isoniazid, streptomycin, ethambutol, and rifampin was assessed by the MB/BacT and BACTEC 460TB systems. The correlation between the two tests was 98.3% for isoniazid, 100% for streptomycin and rifampin, and 95.8% for ethambutol. Turnaround times for antimicrobial susceptibility testing ranged from 5 to 11 days (median, 8.5 days) for MB/BacT and from 4 to 8 days (median, 6 days) for BACTEC 460TB.

One aspect of tuberculosis control is rapid diagnosis and treatment with effective antituberculous drugs. The methods used in mycobacteriology laboratories should be consistently capable of meeting the Centers for Disease Control and Prevention recommendation that susceptibility test results for *Mycobacterium tuberculosis* complex isolates be available, on average, 28 to 30 days from receipt of a specimen in the laboratory (10).

The most rapid method of antimicrobial susceptibility testing (AST) is currently the radiometric BACTEC 460TB system, which requires 4 to 12 days of incubation before results are available (7). Recently, new strategies for the rapid detection of mycobacterial growth not based on radiometric methods have been developed and their reliability for *M. tuberculosis* complex AST has been evaluated (3, 4, 9). The MB/BacT system (Organon Teknika) has been reported to be a rapid, sensitive method for the growth and detection of mycobacteria from clinical specimens (5, 8): in this system, a colorimetric CO_2 detection device indicates mycobacterial growth in a closed system and a fully automated technology ensures almost continuous monitoring of bacterial growth. This avoids the cumbersome handling of vials during incubation, which is an additional drawback of the radiometric system.

To evaluate the potential of MB/BacT for the AST of *M. tuberculosis* complex isolates, we conducted a comparative study of the MB/BacT and BACTEC 460TB systems by using clinical isolates tested against the first-line antituberculous agents isoniazid (INH), streptomycin (STR), ethambutol (ETH), and rifampin (RIF).

A total of 120 strains identified as belonging to the *M. tuberculosis* complex by DNA probe (Gen-Probe, Inc.) were analyzed in this study. All strains were niacin positive, and no further testing was performed to identify the members of the *M. tuberculosis* complex. Duplicate strains from the same patient were excluded. One hundred fifteen strains were obtained by primary isolation, and five were reference strains from the American Type Culture Collection (ATCC) (ATCC 27294, susceptible to all drugs; ATCC 35822, INH resistant; ATCC 35820, STR resistant; ATCC 35837, ETH resistant; ATCC 35838, RIF resistant). All isolates were maintained as stock cultures in glycerol at −70°C and inoculated onto a Löwenstein-Jensen medium slant prior to AST.

For inoculum preparation, one BACTEC 12B bottle and one MB/BacT Alert Process Bottle were inoculated with the growth on the Löwestein-Jensen medium and incubated in the respective instrument. On the day the BACTEC 12B bottle reached a growth index of 500 to 799 (corresponding to a 0.5 to 1 McFarland standard), 0.1 ml of the culture was inoculated into a 12B bottle containing each of the antibiotics (4-ml final volume). The following antibiotics and concentrations were tested: INH, 0.1 μg/ml; STR, 2.0 μg/ml; ETH, 2.5 μg/ml; RIF, 2.0 μg/ml. Drug-free controls consisted of medium inoculated with 0.1 ml of the inoculum (control 1) and with 0.1 ml of a 1:100 dilution of the bacterial inoculum (control 2). Bottles were incubated a 37°C, and growth was monitored daily in the BACTEC instrument.

MB/BacT susceptibility testing was performed as described by Beer et al. (2). A 0.5-ml volume of the culture that was positive by the instrument (corresponding to a turbidity between a 0.5 and a 1 McFarland standard [W. G. Barron, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother, abstr. D-102, 1998]) was delivered on the same day or within 2 days in an MB/BacT bottle containing an antibiotic (11.5-ml final volume) at a concentration of 1.0 μg/ml (INH and RIF) or 2.0 μg/ml (STR). The concentrations used were based on results of experiments carried out to determine what final drug concentrations in the MB/BacT system correlated with the critical concentrations used with the proportion method and with the BACTEC 460TB system (1, 2). Drug-free control vials were prepared by adding 0.5 ml of water and 0.5 ml of a bacterial inoculum (control 1) or 0.5 ml of an inoculum diluted 1:100 (control 2). Bottles were incubated at 37°C. The result was recorded as "resistant" when the drug-containing vial was positive prior to, or at the same time as, corresponding control 2 and "susceptible" when the drug-containing vial remained negative or became positive later than control 1.

The proportion method was applied to strains showing discrepant results according to a standard protocol using Middlebrook 7H10 agar (6). The following concentrations were used: INH, 0.2 and 1.0 μg/ml; STR, 2 and 10 μg/ml; ETH, 5 and 10 μg/ml; RIF, 1 μg/ml.

Susceptibility test results are summarized in Table 1. In all cases, ATCC control strains performed as expected. Full

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TABLE 1. Susceptibilities of *M. tuberculosis* complex isolates as determined by the MB/BacT and BACTEC 460TB systems

Drug	Total no. of strains tested	No. of isolates with the following result(s):			
		Both S[a]	BACTEC, S; MB/BacT, R[b]	BACTEC, R; MB/BacT, S	Both R
INH	120	103		2	15
RZIF	120	116			4
ETH	120	109	5		6
STR	120	115			5

[a] S, susceptible.
[b] R, resistant.

agreement of STR and RIF results was found for all isolates. INH results agreed for 118 (98.3%) of the 120 isolates. Two isolates were susceptible by MB/BacT and resistant by BACTEC 460 and the proportion method. ETH results agreed for 115 (95.8%) of the 120 isolates. Five isolates were resistant by MB/BacT and susceptible by BACTEC 460 and the proportion method. In all, there were 480 test combinations with a total of seven discrepancies between the BACTEC and MB/BacT results (1.4%).

Turnaround times for AST ranged from 5 to 11 days (median, 8.5 days) for MB/BacT and from 4 to 8 days (median, 6 days) for BACTEC 460TB. With the latter system, the turnaround times previously reported were 4.2 to 6.9 days (7). The mean time ($\pm$ the standard error of the mean) to a resistant result (positive growth in the antibiotic-containing vial before or at the same time as control 2) for INH was 3.46 $\pm$ 1.30 days (range, 3 to 4 days) with BACTEC and 5.59 $\pm$ 2.54 days (range, 2.5 to 12.2 days) with MB/BacT; for RIF, it was 3.25 $\pm$ 0.5 days (range, 3 to 4 days) with BACTEC and 4.9 $\pm$ 1.72 days (range, 2.5 to 6.3 days) with MB/BacT; for ETH, it was 3 days with BACTEC and 5.03 $\pm$ 1.65 days (range, 2.8 to 7.5 days) with MB/BacT; for STR, it was 3 days with BACTEC and 4.6 $\pm$ 1.49 days (range, 2.8 to 7.5 days) with MB/BacT.

The false-resistance and false-susceptibility rates for all four drugs with MB/BacT compared with those with BACTEC 460TB are shown in Table 2. Specificity, i.e., the ability to detect true susceptibility, was 95.6% for ETH and 100% for INH, RIF, and STR. Sensitivity, i.e., the ability to detect true resistance, ranged from 88% for INH to 100% for RIF, ETH, and STR. The predictive value of a resistant result (PPV) was low for ETH (54.5%) but high for the other drugs (100%). The predictive value of a susceptible result (NPV) was lower for INH (98%) than for the other drugs (100%).

The very low PPV with ETH might depend on both the bacteriostatic mechanism of action of this drug and the working principle of the MB/BacT system, which is based on detection of CO_2 produced by metabolic processes. Metabolic reactions which are not fully inhibited by ETH should produce CO_2 amounts comparable to those produced by the untreated controls, in particular if the MIC for the strain is near the concentration used in the test and/or the inoculum is larger than wished. However, due to the low incidence of ETH-resistant strains in our country, the disadvantage that one might encounter when using MB/BacT for ETH is that this drug may not be used in a low percentage (5%) of infections due to ETH-susceptible *M. tuberculosis*. Moreover, it appears that to ensure detection of resistance to ETH, two concentrations should be tested. If this is done, the agreement with ETH could increase.

TABLE 2. Accuracy and reliability of the MB/BacT system compared with the BACTEC 460TB system for the four drugs tested

Drug	Specificity (%)	Sensitivity (%)	NPV (%)	PPV (%)	Accuracy (%)
INH	88	98	100	98	98
RIF	100	100	100	100	100
ETH	100	95.6	54.5	100	95.8
STR	100	100	100	100	100

Studies on *M. tuberculosis* complex AST using nonradioactive liquid media in comparison with BACTEC 460TB have been carried out, to the best of our knowledge, only with the nonautomated MGIT system (Becton Dickinson). The specificity, sensitivity, PPV, and NPV of MB/BacT are similar to or higher than those of MIGIT for RIF and STR, but the sensitivity is lower for INH (88 versus 97.6%) and the PPV is lower for ETH (54.5 versus 96.3%) (8).

In conclusion, these results suggest that the MB/BacT system is a valid alternative to the BACTEC 460TB system for *M. tuberculosis* complex AST. The inclusion of a relatively low number of resistant strains does not allow a conclusion to be drawn about how well this method will perform with resistant strains. Additional studies with the MB/BacT system are required in order to improve ETH susceptibility testing.

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