Comparison of Susceptibility Testing of *Mycobacterium tuberculosis* Using the ESP Culture System II with That Using the BACTEC Method

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The ESP Culture System II was evaluated for its capacity to test the susceptibility of 389 cultures of *Mycobacterium tuberculosis* to streptomycin, rifampin, ethambutol, and isoniazid. Good agreement with results with the BACTEC TB 460 was found. ESP II is a reliable, rapid, and automated method for performing susceptibility testing.

The reemergence of tuberculosis and the increasing number of drug-resistant strains of *Mycobacterium tuberculosis* pose a public health problem requiring rapid intervention (4).

To address the problems associated with current methods of susceptibility testing, such as radioactive disposal and culture contamination, new technologies need to be developed. The ESP Culture System II instrument (AccuMed International, Westlake, Ohio [formerly Difco]) is a fully automated, nonradioactive system providing noninvasive continuous monitoring of mycobacterial cultures by measuring changes in head space pressure due to gas production or consumption during microbial growth (1).

This study was performed to determine the feasibility of the ESP Culture System II as a test of the susceptibility of *M. tuberculosis* to rifampin, isoniazid, streptomycin, and ethambutol, in comparison with the BACTEC method.

The mycobacterial strains used in this study were reference organisms from the American Type Culture Collection (ATCC 27294, susceptible to all drugs tested; ATCC 35820, resistant to streptomycin; ATCC 35822, resistant to isoniazid; ATCC 35837, resistant to ethambutol; and ATCC 35838, resistant to rifampin) together with 389 clinical isolates of *M. tuberculosis* received in our Reference Center (Faculty of Medicine, Cordoba, Spain). All strains were identified by conventional methods, DNA probes and high-pressure liquid chromatography. The reference organisms were tested with each run.

For testing with the ESP Culture System II, a standardized inoculum, equivalent to a McFarland standard of 1.0, was obtained. A 1:10 dilution was prepared and used as the inoculum source for susceptibility testing.

The ESP Myco culture bottles used in the drug susceptibility testing contained 12.5 ml of enriched Middlebrook 7H9 broth with 0.2% glycerol, 0.1% casitone, and cellulose sponge disks. Middlebrook OADC enrichment (Myco growth supplement [GS]) was added to a final concentration of 10% before the inoculum was added. A subculture of the original inoculum to blood agar and Middlebrook agar was performed to verify culture purity.

For the inoculation of organisms into the ESP Myco bottles, 0.5 ml of a 1:10 dilution from a 1.0 McFarland standard of each strain was added to each of four drug-containing culture bottles and to one drug-free control culture bottle. A 1.0-ml volume of ESP Myco GS was also added to each bottle, giving a total volume of 15 ml per bottle. The bottles were mixed by inversion several times to ensure a proper distribution of GS, antibiotics, and inoculum throughout the Myco bottle. The bottles were placed inside the instrument once a bottle connector had been attached. A subculture of the original inoculum to blood agar and Middlebrook agar was performed to verify culture purity.

The instrument was checked periodically for positive bottles. When the control bottle signaled positive, the time of detection (TD) was calculated by rounding to the nearest whole number. Drug-containing bottles were monitored for a further 3 days after the control bottle showed positive (rounded to the nearest whole number), and the TD was obtained for all four antibiotics at all the concentrations tested. The results were interpreted according to the following formula: resistant was when the TD (drug bottle) was equal to or within (±3 days of the control TD; sensitive was when no growth occurred or when the drug bottle TD was >3 days after the control TD (M. S. Nagar, M. T. Sweezy, L. F. Srenkonski, R. A. McMillian, Abstr. 35th Intersci. Conf. Antimicrob. Agents Chemother., abstr. D35, 1995.)

Antimycobacterial susceptibility testing with the BACTEC TB 460 was performed and the results were interpreted according to NCCLS document M24-T (6). The agar proportion method was performed according to NCCLS procedures in order to distinguish strains (6).

Data analysis involved a standard two-by-two contingency table. The incubation times required to obtain results for ESP II versus BACTEC TB 460 were compared by a paired *t* test (2, 3, 5). The comparative results obtained for streptomycin, rifampin, ethambutol, and isoniazid are shown in Table 1.

With rifampin and ethambutol (each 7.5 µg/ml), similar results from ESP II and BACTEC TB testing were observed. When 2.5 µg of ethambutol/ml was used with the BACTEC method, two strains were found resistant by BACTEC TB 460 and susceptible by ESP II and the agar proportion method.

For streptomycin, susceptibility test results had a 99.7% agreement. Only one strain was found resistant by the ESP II method.
test and susceptible by BACTEC TB 460. When tested by the agar proportion method, this strain was found to be resistant. Agreement for the lower concentration of isoniazid tested by ESP II was 88.6%. A total of 44 strains were found susceptible by BACTEC TB 460 and resistant by ESP II. These isolates were shown to be susceptible by the agar proportion method. Sensitivity with BACTEC TB 460 and ESP II and the negative (susceptible) predictive value were 100%. Specificity was 82%, accuracy was 88.6%, and the positive (resistant) predictive value was 76.5%.

When the higher concentration of isoniazid (0.4 μg/ml) was used with ESP II, an agreement of 98.9% with BACTEC TB 460 was obtained. Two strains were found resistant by BACTEC TB 460 and susceptible by ESP II and were also found to be resistant by the agar proportion method. Another two strains were found susceptible by BACTEC TB 460 and resistant by ESP II and the agar proportion method.

The mean time (± the standard error) to obtain results with ESP II was 4.55 (± 0.26) days, with a range of 2 to 8 days. The mean time to obtain results with BACTEC TB 460 was 4.83 (± 0.83) days, with a range of 3 to 9 days. No differences were observed in these times between resistant strains and susceptible strains in either system tested.

Good agreement of the ESP Culture System II with the BACTEC TB 460 was found. The ESP system II is a reliable, rapid, and automated method for performing susceptibility testing.

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REFERENCES