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Evaluation of Streptomycin and Ethambutol Concentrations for Susceptibility Testing of *Mycobacterium tuberculosis* by Radiometric and Conventional Procedures

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Clinical isolates of *Mycobacterium tuberculosis* were used to compare various concentrations of streptomycin and ethambutol in the BACTEC 460 (Johnston Laboratories, Inc., Towson, Md.) radiometric method for drug susceptibility testing with those in the conventional method. Streptomycin used at 2.0 μg/ml for both methods showed a 0.99 agreement with susceptible strains and a 0.97 agreement with resistant strains. Ethambutol used at 2.5 μg/ml for the radiometric method showed 1.00 agreement with both susceptible and resistant strains when compared with ethambutol at 5.0 μg/ml for the conventional method.

Increasing numbers of laboratories use the rapid radiometric method for testing the susceptibility of *Mycobacterium tuberculosis* to antimicrobial agents. Strains of tubercle bacilli are tested in 12A liquid medium containing radiolabeled substrate (4). Labeled CO_2 produced is detected and quantitated by the BACTEC 460 (Johnston Laboratories, Inc., Towson, Md.). Previous studies have shown that the radiometric method is comparable to the conventional method for testing the susceptibility of *M. tuberculosis* strains to drugs (2, 3, 5–7, 9). However, streptomycin (STR) and ethambutol (EMB) susceptibility tests showed lower agreement values than those for isoniazid and rifampin. In the conventional method, STR resistance is usually measured at two levels, 2.0 and 10.0 μg/ml. The suggested STR concentration for the radiometric method is 4.0 μg/ml (6). EMB is usually tested at 5.0 μg/ml for the conventional method and 10.0 μg/ml for the radiometric method (6). Laszlo et al. (3) suggested that EMB should be tested at a concentration other than 10.0 μg/ml, since 10 μg/ml almost always failed to detect resistance. In an attempt to improve agreement between the two methods, a number of resistant strains was used to compare the results of several concentrations of STR and EMB used for the radiometric procedure with those of the standard concentration of these drugs used for the conventional method.

Two hundred *M. tuberculosis* strains were clinical isolates received by the Mycobacteriology Section, Centers for Disease Control, Atlanta, Ga. The strains were subcultured on Lowenstein-Jensen medium for both the radiometric (12A medium) and conventional (7H10 agar) methods of testing for drug susceptibility. The indirect drug susceptibility test with 7H10 agar was a modified version of the proportion method of Canetti and co-workers (1). The modification, including the preparation and concentration of drugs, was described by Vestal (8). The liquid medium used was Middlebrook and Cohn 7H9 (Difco Laboratories, Detroit, Mich.) containing albumin, dextrose, catalase enrichment (Difco), and 0.05% Tween 80. The drug-containing solid medium was Middlebrook and Cohn 7H10 agar (BBL Microbiology Systems Cockeysville, Md.) containing oleic acid, albumin, dextrose, catalase enrichment (GIBCO Laboratories, Grand Island, N.Y.), and 0.5% glycerol. Isoniazid (Eli Lilly & Co., Indianapolis, Ind.), STR (Merck & Co., Inc., Rahway, N.J.), rifampin (CIBA-GEIGY Corp., Summit, N.J.), and EMB (Lederle Laboratories, Pearl River, N.Y.) were all incorporated into 7H10 agar as recommended by Vestal (8). The rapid radiometric drug susceptibility test was the indirect method described by Siddiqi et al. (6) with the following modifications. A suspension of each culture was made from a Lowenstein-Jensen slant by mixing it with 3 ml of diluting fluid (Johnston Laboratories) and 1.5 g of glass beads (diameter, 3 mm). This suspension was adjusted with diluting fluid to approximate a McFarland turbidity standard of 0.5. Vials of 12A medium containing the test drug were inoculated with 0.1 ml of the suspension and tested daily for emission of radiolabeled CO_2 with the BACTEC 460 instrument with TB hood (Johnston Laboratories). Drug concentrations for radiometric tests were prepared from the antituberculosis drug kit available from Johnston Laboratories.

A summary of the comparison of the two methods with

TABLE 1. Comparison of drug susceptibility tests of 100 *M. tuberculosis* strains with recommended concentrations for BACTEC and conventional agar methods

Drug[a]	Concn (μg/ml) in[b]:		Proportion of agreement for the following strains:		
	7H10 agar	12A medium	Total[c]	Susceptible[d]	Resistant[e]
STR	2.0	4.0	0.93	0.98	0.86
	4.0	4.0	0.90	0.88	0.94
	10.0	4.0	0.82	0.78	1.00
INH	0.2	0.2	0.96	1.00	0.91
RIF	1.0	2.0	0.98	0.98	0.94
	2.0	2.0	0.99	1.00	0.95
EMB	5.0	10.0	0.86	1.00	0.11
	10.0	10.0	0.96	1.00	0.33

[a] INH, Isoniazid; RIF, rifampin.
[b] 7H10 agar was used for the conventional method, and 12A medium was used for the BACTEC method.
[c] Total agreement = (A + D)/(A + B + C + D), where A is number of strains susceptible in 7H10)/(number susceptible in 12A), B is (number of strains susceptible in 7H10)/(number of strains resistant in 12A), C is (number of strains resistant in 7H10)/(number of strains susceptible in 12A), D is (number of strains resistant in 7H10)/(number of strains resistant in 12A).
[d] Agreement for susceptible strains (specificity) = A/(A + B), where A and B are as defined in footnote *b*.
[e] Agreement for resistant strains (sensitivity) = D/(C + D), where C and D are as defined in footnote *b*.

TABLE 2. Comparison of drug susceptibility tests of 100 *M. tuberculosis* strains with recommended drug concentrations for 7H10 agar and 12A medium

Drug[a]	Concn (μg/ml) in:		Proportion of agreement for the following strains:		
	7H10 agar	12A medium	Total[b]	Susceptible[c]	Resistant[d]
STR	2.0	2.0	0.96	0.99	0.97
	2.0	6.0	0.89	0.99	0.63
	10.0	2.0	0.83	0.81	1.00
	10.0	6.0	0.94	0.93	1.00
INH	0.2	0.2	0.97	1.00	0.94
RIF	1.0	2.0	1.00	1.00	1.00
EMB	5.0	2.5	1.00	1.00	1.00
	5.0	5.0	0.98	1.00	0.88
	5.0	7.5	0.91	1.00	0.44
	7.5	2.5	0.94	0.93	1.00
	7.5	5.0	0.97	0.97	1.00
	7.5	7.5	0.96	0.99	0.70

[a] INH, Isoniazid; RIF, rifampin.

[b] Total agreement = (A + D)/(A + B + C + D), where A is (number of strains susceptible in 7H10)/(number of strains susceptible in 12A), B is (number of strains susceptible in 7H10)/(number of strains resistant in 12A), C is (number of strains resistant in 7H10)/(number of strains susceptible in 12A), D is (number of strains resistant in 7H10)/(number of strains resistant in 12A).

[c] Agreement for susceptible strains (specificity) = A/(A + B), where A and B are as defined in footnote *c*.

[d] Agreement for resistant strains (sensitivity) = D/(C + D), where C and D are as defined in footnote *c*.

drug concentrations recommended by BACTEC and the Centers for Disease Control (conventional) is presented in Table 1. By the BACTEC procedure, 4.0 μg of STR per ml correlated best with 4.0 μg of STR per ml by the conventional method, but less correlation was observed with the standard 2.0 and 10.0 μg/ml concentrations. When tested by the BACTEC procedure, 10.0 μg of ethambutol per ml inhibited most strains resistant to 5.0 μg of EMB per ml by the conventional procedure, and the sensitivity by the conventional procedure was only 0.11. With another set of *M. tuberculosis* strains, the two procedures were compared with a different series of STR and EMB concentrations (Table 2). Greater agreement was obtained with STR concentrations of 2.0 and 6.0 μg/ml in 12A medium by the BACTEC procedure than with 2.0 and 10.0 μg/ml in 7H10 agar by the conventional procedure. However, 2.0 μg/ml used for both methods correlated best, showing 0.99 agreement with susceptible strains and 0.97 agreement with resistant strains. When 6.0 μg/ml was used as the single concentration, 0.63 agreement was shown for resistant strains with 2.0 μg of STR per ml in 7H10 agar and 1.0 agreement was shown for resistant strains with 10.0 μg of STR per ml in 7H10 agar. Ethambutol results were best when 5.0 μg/ml in 7H10 agar was compared with 2.5 μg/ml in 12A medium. When 7.5 μg/ml used in 7H10 agar was compared with 5.0 μg/ml in 12A medium, the overall agreement was 0.97. However, I found six strains susceptible to 7.5 μg/ml in 7H10 agar that were resistant to 5.0 μg/ml in 7H10 agar. These changes in STR and EMB concentrations to 2.0 and 2.5 μg/ml, respectively, suggest that the accuracy of the radiometric method of susceptibility testing can be improved to the level obtained with isoniazid and rifampin if the drug concentrations are adjusted according to the concentrations used in the conventional procedure.

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