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Conventional and Radiometric Drug Susceptibility Testing of *Mycobacterium tuberculosis* Complex

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A recently developed method of drug susceptibility testing of *Mycobacterium tuberculosis* which measures the evolution of labeled CO_2 from [1-^{14}C]palmitic acid (BACTEC 460 system) was compared to three conventional methods. The proportion method of drug susceptibility testing was the standard against which all test results were compared. Indirect drug susceptibility to isoniazid, streptomycin, rifampin, and ethambutol of 245 isolates belonging to the *M. tuberculosis* complex was determined. In 95% of the cases, results obtained by the radiometric method were available within 1 week, as opposed to 3 to 6 weeks needed in conventional methodology. Overall agreement was 96.4%. Specificity values ranged from 0.98 to 1.0; sensitivity values of 1.0 for rifampin, 0.96 for streptomycin, 0.91 for isoniazid, and 0.18 for ethambutol were obtained. The specificity of the absolute concentration and resistance ratio drug susceptibility testing methods were 0.99 and 1.0, respectively. The sensitivity of the former was higher than that of the radiometric method (0.99 verus 0.92), whereas that of the latter was lower (0.88 verus 0.96). Further testing indicated that the low sensitivity determined for ethambutol may be due to the choice of the critical concentration used, rather than to a shortcoming of the procedure. The radiometric method thus does not significantly differ in reliability from conventional methods of drug susceptibility testing of *M. tuberculosis*.

Current conventional methods of antimicrobial susceptibility testing of *Mycobacterium tuberculosis* require 3 to 6 weeks for completion (1, 7). The value of rapid susceptibility testing is obvious for the physician and the patient in terms of shorter delays required for an eventual correction of a chemotherapy regimen. In recent years a rapid, semi-automated radiometric drug susceptibility testing (RAD) method has been developed that measures the $^{14}CO_2$ produced by metabolic breakdown of (1-^{14}C) palmitic acid (BACTEC 460 system).

In a large scale comparative study, Snider and co-workers (5) evaluated this RAD method for indirect drug susceptibility testing of tubercle bacilli against the conventional 7H10 plate method. This RAD method yields high specificity values for the drugs tested, but somewhat lower sensitivity values for all drugs except rifampin (RIF); values of 0.83, 0.90, and 0.80 were found for streptomycin (SM), isoniazid (INH), and ethambutol (EB), respectively. In a smaller study, Siddiqi and co-workers (4) compared this RAD procedure to the conventional 7H11 plate method. As in the former study, specificity values are high (0.99 to 1.0) but sensitivity is again somewhat lower for all drugs except EB; values of 0.77, 0.88, and 0.83 were recorded for SM, INH, and RIF, respectively. Both studies show that the RAD method is significantly faster than conventional techniques in yielding results. Each of these studies uses a variant of the proportion (PR) method of conventional drug susceptibility testing of *M. tuberculosis*, differing by the choice of culture media and some of the critical drug concentrations.

In the present study, the standard PR method was performed on Loewenstein-Jensen medium and drug resistance was evaluated by the description of Canetti and co-workers (1). Although slower than the above-mentioned variants, this method equates directly with solid clinical evidence and remains, to date, the most widely used method of drug susceptibility testing of *M. tuberculosis* throughout the world. It seemed obvious that an evaluation against this World Health Organization-recommended methodology would not only complete the picture,

but might also reconcile the discrepancies evident in the previous studies. Moreover, the medium vials used in the previous studies were fitted with rubber septa that have since been shown to exert an inhibitory effect on the growth of *M. tuberculosis*. This inhibitory effect could have interfered with the results, especially in view of the fact that in almost all cases of disagreement the RAD method reported drug susceptibility and the conventional method reported drug resistance.

In the present study, two additional conventional drug susceptibility testing methods, i.e., the absolute concentration (AC) and the resistance ratio (RR) methods, were compared to standard PR method. The ensuing overall comparison provides a convenient frame of reference for the reliability of this rapid RAD drug susceptibility procedure in the routine of the mycobacteriology laboratory.

MATERIALS AND METHODS

Cultures. Clinical mycobacterial isolates referred to the National Reference Centre for Tuberculosis, Laboratory Centre for Disease Control, Health and Welfare, Canada, from laboratories across the country for routine drug susceptibility testing were used in this study. The collected strains were subcultured on Loewenstein-Jensen medium and the RAD and conventional procedures performed in parallel. A total of 245 cultures of *M. tuberculosis* complex were tested for drug susceptibility to SM, INH, RIF, and EB.

Conventional indirect drug susceptibility tests. Three conventional procedures of indirect drug susceptibility testing of *M. tuberculosis*, namely the AC method, the resistance ratio (RR) method, and the PR method were performed as described by Canetti and co-workers (1). The AC method determines the minimal inhibitory concentration of the test strain, in this case the critical concentration used was 0.2 μg of INH per ml. The RR method determines the ratio between the minimal inhibitory concentration of the test strain and that of the standard strain H37Rv; in this study a resistance ratio of 4 or more defined resistance to SM. The critical concentrations used in the standard PR were INH, 0.2 μg/ml; SM, 4 μg/ml; RIF, 40 μg/ml; and EB, 2 μg/ml.

RAD indirect drug susceptibility test. The RAD $^{14}CO_2$ method (BACTEC 460 system) used, has been described by Siddiqi and co-workers (4). Briefly, this method measures the metabolic breakdown of [1-^{14}C]palmitic acid in 7H12 medium, both in the presence and absence of antituberculous drugs. The drug concentrations used were INH, 0.2 μg/ml; SM, 4 μg/ml; RIF, 2 μg/ml; and EB, 10 μg/ml.

Analysis of the data. The following characteristics were determined for each test: sensitivity, the capacity of the test method to distinguish correctly resistant strains = D/(C + D); specificity, the capacity of the test method to distinguish correctly susceptible strains = A/(A + B); predictive value (resistance), the ratio of strains classified as resistant by the test method which were truly resistant = D/(B + D); and predictive value (susceptibility), the ratio of strains classified as susceptible by the test method which were truly susceptible = A/(A + C). Where A is the number of strains found to be susceptible by both methods, B is the number of strains found to be susceptible by the standard proportion method and resistant by the test method, C is the number of strains found to be resistant by the standard proportion method and susceptible by the test method, and D is the number of strains found to be resistant by both methods.

RESULTS

Of the 245 mycobacterial cultures tested, 216 were *M. tuberculosis*, 5 were *M. bovis*, and 24 were *M. bovis* biovar BCG. By the standard PR method, 134 (55%) of the total number of isolates were resistant to at least one drug, 88 (36%) of the cultures were resistant to INH, 76 (31%) were resistant to SM, 22 (9%) were resistant to RIF, and 21 (8.5%) were resistant to EB.

The time required for final evaluation of the radiometric drug susceptibility test is shown in Table 1; 91% of the results were reportable within 6 days, and 98% were reportable within 9 days. In contrast, both the AC and the RR methods require a minimum of 21 days, whereas the PR method requires a minimum of 28 days.

Table 2 presents the overall drug susceptibility results of the comparison study, RAD versus standard PR method. The RAD method identified more cultures as susceptible than the standard PR method in all drugs except RIF. The overall agreement between results, shown in Table 3, varies from a low of 91.4% for EB to a high of 100% for RIF. The RAD method reported as susceptible 3 cultures that were resistant to SM, 8 cultures that were resistant to INH, 18 cultures that were resistant to EB and reported as resistant 3 cultures that were susceptible to INH and 3 cultures that were susceptible to EB.

Sensitivity, specificity, and predictive values for resistance and susceptibility have been derived from the preceding data and are shown in

TABLE 1. Time required for final evaluation of drug susceptibility testing of the *M. tuberculosis* complex

No. of days	No. of cultures evaluated	Cumulative %
3	74	30.2
4	87	65.7
5	43	83.3
6	19	91.0
7	12	95.9
8	4	97.6
9	1	98.0
10	3	99.2
≥11	2	100.00

TABLE 2. Total number of susceptible and resistant cultures found by the RAD and the PR methods

Drug	Concn (μg/ml)		No. of cultures found by			
	RAD	PR	RAD		PR	
			S[a]	R[b]	S	R
SM	4	4	172	73	169	76
INH	0.2	0.2	161	84	157	88
RIF	2	40	223	22	223	22
EB	10	2	241	4	224	21

[a] S, Susceptible.
[b] R, Resistant.

Table 4. The specificity of the RAD method is high for all drugs tested, and its sensitivity is high for all except EB.

To give a different perspective, the RAD method as well as two additional conventional drug susceptibility testing methods were compared to the standard PR method. Table 5 shows the overall drug susceptibility results of this comparison. In this portion of the study, 207 of the 245 total cultures used were tested for susceptibility to INH by the AC method and to SM by the RR method. The RR method reported as susceptible 9 cultures resistant to SM, whereas the AC method mislabeled one culture resistant to INH and one susceptible to INH. Overall agreement in both cases was higher than 95% (Table 6). When sensitivity and specificity values are calculated (Table 7), it becomes obvious that the RAD method is comparable to the AC method and to the RR method.

DISCUSSION

In comparing results obtained with new laboratory procedures to those obtained with conventional methodology, there are various ways of expressing the degree of agreement. Wayne and Krasnow (8), comparing a plate method and a disk method of drug susceptibility testing of *M. tuberculosis*, used the overall agreement for three drugs and 10 drug levels as a means of evaluation and found complete agreement in 92% of the tests. Similarly, Griffith and co-workers (2) found an overall agreement of 93% for three drugs and five drug levels tested.

A more refined evaluation was performed by Montalbine and Collins (3), who in a comparison study of the Steeken minimal inhibitory concentration test and the Canetti PR method, determined overall agreements to each individual drug tested, i.e., 82% for SM, 95% for INH, and 89% for *p*-aminosalicylic acid.

Snider et al. (5), in a large-scale comparative study of drug susceptibility testing of *M. tuberculosis*, stated that a level of agreement of 90 to 95% between two tests must be considered good. Since two sets of experimental data agreed with results of the reference laboratory in 92 and 95% of the tests, the results of their study can be considered acceptable based on overall agreement. However, using this global criterion for evaluation, good overall agreement could conceal, as was the case in their study, poorer agreement on resistant strains.

However good the intrinsic value of a test might be, the extrinsic value, i.e., the value of the test as modified by the context in which it is applied, depends on a very important variable, namely prevalence. The relative frequency or prevalence of resistant and susceptible strains

TABLE 3. Analysis of 35 disagreements between the RAD and PR methods in 245 cultures tested

Drug	PR-S, RAD-R[a]	PR-R, RAD-S[b]	% Agreement
SM	0	3	98.8
INH	3	8	95.5
RIF	0	0	100.0
EB	3	18	91.4

[a] Susceptible by PR but resistant by RAD.
[b] Resistant by PR but susceptible by RAD.

TABLE 4. Sensitivity, specificity, and predictive values of the RAD method as compared with the PR method

Drug	Concn (μg/ml)		RAD/PR[a]			
	RAD	PR	Sensitivity	Specificity	PVR[b]	PVS[c]
SM	4	4	0.96	1.0	1.0	0.98
INH	0.2	0.2	0.91	0.98	0.96	0.95
RIF	2	40	1.0	1.0	1.0	1.0
EB	10	2	0.18	0.99	0.57	0.92

[a] Comparison of RAD method results to PR method results.
[b] PVR, Predictive value (resistance).
[c] PVS, Predictive value (susceptibility).

TABLE 5. Total number of susceptible and resistant cultures found by RAD, AC, and RR as compared with PR

Drug	No. of cultures found by					
	AC-RR		PR		RAD	
	S[a]	R[b]	S	R	S	R
SM[c]	144	63	135	72	138	69
INH[d]	130	77	130	77	133	74

[a] S, Susceptible.
[b] R, Resistant.
[c] SM tested by RR.
[d] INH tested by AC.

TABLE 6. Analysis of disagreements between the AC method for INH, the RR method for SM, and the RAD method for both, as compared with the PR method

Drug	No. of cultures			No. of cultures		
	PR-S, (AC/RR)-R[a]	PR-R, (AC/RR)-S[b]	% Agreement	PR-S, RAD-R[c]	PR-R, RAD-S[d]	% Agreement
SM[e]	0	9	95.6	0	3	98.5
INH[f]	1	1	99.0	4	6	95.2

[a] PR susceptible but AC or RR resistant.
[b] PR resistant but AC or RR susceptible.
[c] PR susceptible but RAD resistant.
[d] PR resistant but RAD susceptible.
[e] SM tested by the RR method.
[f] INH tested by the AC method.

included in the study has a determining influence on the predictive values of the test. It is well known that at very low prevalence rates (up to 1%) even tests with high sensitivity or specificity yield poor predictive values; conversely, tests with low sensitivity or specificity yield high-predictive values at prevalence rates of 5% or more (6). It is therefore generally accepted that the inherent value of a test, i.e., its reliability in discriminating between two states, in this case resistance and susceptibility, rests on two characteristics, namely the sensitivity or the capacity to identify true resistance and the specificity or the capacity to identify true susceptibility. Neither of these characteristics are affected by the relative frequency of resistant and susceptible strains. For these reasons, only these two criteria were used for the evaluation of this study.

The results of our study show that RAD testing is rapid; 83% of the strains can be reported within 5 days compared to 4 to 6 weeks for the conventional method. These results also show that the RAD method agrees overall with conventional results in 96.4% of the tests.

The inherent accuracy of the RAD method, as measured by the sensitivity and specificity parameters is high, in most cases well above the previously mentioned threshold value of 90% for SM, INH, and RIF. These results compare favorably with those of Siddiqi and co-workers (4) and Snider and co-workers (5) especially when one compares sensitivity values which so far constituted the most severe criticism of the RAD procedure. The case of EB is an interesting one, since in the present study the sensitivity or the capacity of the test method to predict resistance is very poor (0.57). In view of the fact that the RAD method almost always failed to detect resistance, in a subsequent study we lowered the critical concentration of EB in 7H12 to 2 μg/ml instead of the 10 μg/ml concentration that is commonly recommended in Middlebrook 7H10 medium. The correlation with the conventional method was considerably improved at this concentration, since 13 strains which were false susceptible at 10 μg of EB per ml became true resistant when retested at 2 μg of EB per ml. It seems probable, therefore, that the discrepancies were due to an improper choice of EB concentration in the medium rather than to an inherent flaw in the test method. This observation deserves further study, because it implies that the critical concentration of EB used in the standard PR method (Loewenstein-Jensen medium), cannot be equated to the critical concentration recommended in the 7H10 variant. This in turn implies, that the 7H10 variant may not always detect clinically significant resistance to EB.

TABLE 7. Sensitivity, specificity, and predictive value of the AC method for INH, the RR method for SM, and the RAD method for both, as compared with the PR method

Drug	(AC-RR)/PR[a]					RAD/PR[b]				
	Method	Sensitivity	Specificity	PVR[c]	PVS[d]	Method	Sensitivity	Specificity	PVR	PVS
SM	RR	0.88	1.0	1.0	0.94	RAD	0.96	1.0	1.0	0.98
INH	AC	0.99	0.99	0.99	0.99	RAD	0.92	0.97	0.95	0.92

[a] Comparison of AC or RR method results to PR method results.
[b] Comparison of RAD method results to PR methods results.
[c] PVR, Predictive value (resistance).
[d] PVS, Predictive value (susceptibility).

The predictive values for specificity and for sensitivity in at least three of the drugs tested, and very probably for EB as well, are high, as expected, since the study included a minimum of 8% drug resistance markers.

By comparing the RR drug susceptibility method for SM and the AC method for INH to the standard PR method we have shown that even conventional methods when compared to one another show a certain degree of disagreement which in the case of the RR method is greater than that shown by the RAD method (Table 7). The specificity and sensitivity of this RAD procedure compare favorably with conventional drug susceptibility testing methods.

Therefore, this rapid semi-automated method can be adopted without reservation in the specialty laboratory routine, especially if the expected number of *M. tuberculosis* resistant strains is or exceeds 5%.

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LITERATURE CITED

1. **Canetti, G., W. Fox, A. Kohmenko, H. Mahler, N. K. Menon, D. A. Mitchison, N. Rist, and N. A. Smelev.** 1969. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. Bull. W.H.O. **41**:21–43.
2. **Griffith, M., M. L. Barrett, H. L. Bodily, and R. M. Wood.** 1967. Drug susceptibility tests for tuberculosis using drug impregnated disks. Am. J. Clin. Pathol. **47**:812–817.
3. **Montalbine, V., and F. M. Collins.** 1971. Determination of drug resistance of *Mycobacterium tuberculosis* cultures. Appl. Microbiol. **21**:732–738.
4. **Siddiqi, S. H., J. P. Libonati, and G. Middlebrook.** 1981. Evaluation of a rapid radiometric method for drug susceptibility testing of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **13**:908–912.
5. **Snider, D. E., Jr., R. G. Good, J. O. Kilburn, L. F. Laskowski, Jr., R. H. Lusk, J. J. Marr, Z. Reggiardo, and G. Middlebrook.** 1981. Rapid susceptibility testing of *Mycobacterium tuberculosis*. Am. Rev. Respir. Dis. **123**:402–406.
6. **Toman, K.** 1981. Sensitivity, specificity and predictive value of diagnostic tests. Bull. Int. Union Tuberc. **56**:18–28.
7. **Vestal, A. L.** 1975. Procedures for the isolation and identification of mycobacteria. p. 97–115. Center for Disease Control (DHEW publication No. [CDC] 76-8230).
8. **Wayne, L. G., and J. Krasnow.** 1966. Preparation of tuberculosis susceptibility testing mediums by means of impregnated disk. Am. J. Clin. Pathol. **45**:769–771.