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Modification of Results of Drug Susceptibility Tests by Coexistence of *Mycobacterium avium* Complex with *Mycobacterium tuberculosis* in a Sputum Sample: Case Report and Experimental Considerations

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We report on a patient whose sputum contained both *Mycobacterium tuberculosis* and *Mycobacterium avium* complex (MAC). The MAC failed to be detected by the PCR-based AMPLICOR test. The unrecognized coexistence of MAC in the sample modified the results of drug susceptibility tests. Experiments revealed that the presence of both *M. tuberculosis* and MAC was not detected by the AMPLICOR test under certain conditions.

Two direct-amplification tests, the Gen-Probe (San Diego, Calif.) *Mycobacterium* tuberculosis Direct Test (MTD) and the PCR-based AMPLICOR MYCOBACTERIUM (Roche, Basel, Switzerland) tests, have been approved in the United States for direct detection of tubercle bacilli in respiratory samples that are smear positive for acid-fast bacilli (AFB) (3). The two tests have been widely used in Japan as well since 1995 (1, 9). We have routinely performed the rapid detection of *Mycobacterium tuberculosis* and *Mycobacterium avium* complex (MAC) in respiratory specimens by using the AMPLICOR test. The direct tests, however, have some drawbacks (2, 3, 7), such as a low sensitivity for AFB-smear-negative samples, and thus conventional smear and culture techniques are still needed.

We present here a case of a patient with pulmonary tuberculosis who was coinfected with MAC and whose sputum contained both of the mycobacteria. We detected *M. tuberculosis* alone in the sputum by the AMPLICOR test. The unrecognized coexistence of MAC seriously modified the results of subsequent drug susceptibility tests. We also performed experiments to investigate the effect of the coexistence of MAC with *M. tuberculosis* on the results of AMPLICOR, AccuProbe, and drug susceptibility tests.

Case report. A 64-year-old man was admitted to our hospital in April 1996 because of persistent cough. A chest roentgenogram revealed diffuse cavitary infiltrates in both lung fields. The Mantoux test with purified protein derivative (2.5 tuberculin units) was strongly positive (dimensions of redness, 32 by 20 mm). Because microscopic examination of the sputum sample revealed numerous AFB, we performed the AMPLICOR test, which showed that the sample contained *M. tuberculosis* without MAC (optical density at 450 nm [OD] = 1.954 for *M. tuberculosis*, OD = 0.044 for *M. avium*, and OD = 0.037 for *Mycobacterium intracellulare*). Isoniazid (INH), rifampin (RFP), and ethambutol (EB) were administered based on the diagnosis of pulmonary tuberculosis. The sample was cultured both on a slant of Ogawa egg medium and in the Mycobacterium Growth Indicator Tube (MGIT; Becton Dickinson, Sparks, Md.) system (4, 10, 12, 14, 16). After 10 days, a positive fluorescent signal was detected in the MGIT system. The growing bacterium was identified as *M. tuberculosis* by the acid fastness and probe analysis determined by using the AccuProbe test. Based on drug susceptibility test results of the MGIT system, the recovered mycobacteria were determined to be resistant to all drugs tested (INH, RFP, EB, and streptomycin [SM]). Thus, a new regimen was started. After 4 weeks of culture, heavy growth of homogeneous rough colonies had developed on the Ogawa slant; these colonies were again identified as *M. tuberculosis* by using the AccuProbe test. The drug susceptibility testing for the mycobacteria grown on the slant with 1% Ogawa egg medium (8) also revealed resistance to all drugs tested. However, the fact that colonies had also grown on medium containing 500 μg of *p*-nitrobenzoic acid (PNB) per ml, which inhibits the growth of *M. tuberculosis* (5, 13), led us

TABLE 1. Results of smear and culture of sputum samples

Sample collection date (mo/day)	Smear results[a]	Culture results (CFU)[b]	Identification[c]
2/5[d]		2+	*M. tuberculosis*
2/16[d]		2+	Both *M. tuberculosis* and MAC
4/17 (on admission)	3+	4+	Both *M. tuberculosis* and MAC
5/1	1+	2+	*M. tuberculosis*
5/2	2+	4+	Both *M. tuberculosis* and MAC
5/7	2+	4+	MAC
6/10	1+	30	*M. tuberculosis*
7/1	1+	20	*M. tuberculosis*
7/29	1+	1	*M. tuberculosis*
8/20	1+	1	*M. tuberculosis*
9/17			

[a] Number of AFB with Ziehl-Neelsen staining. Scores: 1+, 1 to 4 AFB/slide or 1 AFB/several fields (500×); 3+, more than 12 AFB/field; 2+, score between 1+ and 3+.
[b] Number of CFU on Ogawa egg medium. 4+, more than 2,000 CFU; 2+, 200 to 500 CFU.
[c] Identification by probe analysis by using the AccuProbe test.
[d] Sputum samples from another hospital.

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TABLE 2. Results of AMPLICOR, MGIT, and AccuProbe tests for samples containing known CFU of both *M. tuberculosis* and *M. avium*[a]

Sample	No. of CFU in sample		AMPLICOR test results for:		Susceptibility to indicated drug by MGIT[d]				AccuProbe test results[e] for:	
	M. tuberculosis[b]	*M. avium*[c]	*M. tuberculosis*	*M. avium*	INH	RFP	EB	SM	*M. tuberculosis*	MAC
A	2×10^2	2×10^5	−	+	R	R	R	R	+	+
B	2×10^3	2×10^5	+	+	R	R	R	R	+	+
C	2×10^4	2×10^5	+	+	R	R	R	R	+	+
D	2×10^5	2×10^5	+	+	R	R	R	R	+	+
E	2×10^5	2×10^4	+	−	R	R	R	R	+	+
F	2×10^5	2×10^3	+	−	R	S	R	R	+	+
G	2×10^5	2×10^2	+	−	S	S	S	S	+	−
H	2×10^5	2×10^1	+	−	S	S	S	S	+	−

[a] Representative data of four experiments with the same result.
[b] *M. tuberculosis* H37Rv.
[c] *M. avium* Mino.
[d] S, susceptible; R, resistant.
[e] Identification of the grown bacteria in the tube.

to suspect that some colonies of nontuberculous mycobacteria had been recovered from the sputum sample collected at the time of patient admission. Thus, we identified all colonies recovered from the patient's sputum samples that had been stored in our laboratory by using probes for both *M. tuberculosis* and MAC. As shown in Table 1, the recovered mycobacteria consisted of *M. tuberculosis* alone, MAC alone, and both mycobacteria, on different occasions. Testing for colonies containing *M. tuberculosis* alone by using the MGIT system and the Ogawa method revealed susceptibility to INH, RFP, and SM. The patient was put on a new regimen containing those drugs starting in July. The patient's sputum was found to be both smear and culture negative in September.

Experiment 1. Separation of *M. tuberculosis* colonies from MAC colonies in the mycobacteria recovered from the sputum sample collected upon patient admission. To confirm the mixed recovery of *M. tuberculosis* and MAC, we tried to separate the two mycobacterial colonies from each other. Colonies were lifted from the Ogawa slant and cultured in modified Dubos Tween albumin liquid medium (11) for 14 days. The bacterial suspensions were diluted a millionfold, and the dilutions were plated on a Middlebrook 7H10 agar plate (Difco, Detroit, Mich.). Various types of colonies were grown on the agar, six of which were selected and cultured in Dubos medium again. After 14 days, the bacteria in each suspension were identified by using the AccuProbe test and drug susceptibility testing was done with the MGIT system. Two *M. tuberculosis* strains obtained were resistant to EB alone. Two MAC strains were resistant to INH, RFP, and EB, while the other MAC strain was resistant to INH, RFP, EB, and SM. One mixed strain was resistant to INH, RFP, and EB. These results indicated that the recovery of a mixture of MAC and *M. tuberculosis* from the sputum sample collected upon admission modified the results of the drug susceptibility testing.

Experiment 2. Rapid identification of the samples containing both *M. tuberculosis* and *M. avium* by the AMPLICOR test. The case described herein encouraged us to investigate the effect of the coexistence of MAC with *M. tuberculosis* on the results of the AMPLICOR test. Various numbers of CFU of *M. tuberculosis* H37Rv and *M. avium* Mino (15) were added to sample tubes containing 5 ml of saline, and each sample was analyzed by the AMPLICOR test. As shown in Table 2, both mycobacteria were detected only when the numbers of CFU of *M. avium* were the same, 10 or 100 times higher than those of *M. tuberculosis* (samples B to D). The same samples were cultured, and the drug susceptibilities of the grown bacteria were tested by using the MGIT system. Samples A to E showed resistance to all drugs tested, and sample F showed resistance to INH, EB, and SM. The grown bacteria were identified by the AccuProbe test as well. Samples A to F were identified as both *M. tuberculosis* and MAC, while samples G and H were identified as *M. tuberculosis* alone. These results demonstrated that coexistence of certain numbers of MAC with *M. tuberculosis* (samples E and F) could not be detected by the AMPLICOR test but could have a serious effect on the subsequent drug susceptibility testing.

A patient with pulmonary tuberculosis is sometimes transiently coinfected with nontuberculous mycobacteria, but antituberculosis chemotherapy alone is sufficient to eradicate both of the mycobacteria, as described previously (6). This coinfection can usually be recognized by the presence of different types of colony formation on the solid medium, agar-based culture plate in particular, in which case each colony can be separately identified. In the case reported herein, however, homogeneous rough colonies on the Ogawa slant prevented the recognition of the coexistence. Moreover, the result of the AMPLICOR test led us to neglect to rule out the coexistence of MAC in the grown bacteria by using the AccuProbe test. Finally, colonies grown on the Ogawa medium with PNB demonstrated to us the coexistence of nontuberculous mycobacteria (5, 13). The present experimental data also documented the limitations of the AMPLICOR test to rule out the coexistence of MAC with *M. tuberculosis* in a sample. Clinical specimens must be cultured not only in a broth but also on both egg-based and agar-based media for visual inspection. In addition, medium with PNB or *p*-nitro-α-acetylamino-β-hydroxy-propiophenone (NAP) should be used to rule out the coexistence of nontuberculous mycobacteria. Moreover, if unusual susceptibility test results occur, an intensive search for a second species should be performed as well.

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