JOURNAL OF CLINICAL MICROBIOLOGY, May 1993, p. 1376–1378
0095-1137/93/051376-03$02.00/0

Vol. 31, No. 5

Conventional Identification Characteristics, Mycolate and Fatty Acid Composition, and Clinical Significance of MAIX AccuProbe-Positive Isolates of *Mycobacterium avium* Complex

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A total of 145 isolates belonging to the *Mycobacterium avium* complex (MAC) were tested with commercial acridinium ester-labeled DNA probes (AccuProbe, Gen-Probe). *M. avium* and *M. intracellulare* probes reacted with 102 and 36 isolates, respectively. The remaining seven isolates were clearly positive with the new probe, designated MAIX. Thus, the combined sensitivity of *M. avium* and *M. intracellulare* probes was 95.2%. The MAIX probe improved the sensitivity up to 100%. The MAIX probe also reacted with all *M. avium* (n = 20) and *M. intracellulare* (n = 20) isolates tested. Three of the seven MAIX-positive isolates were considered clinically significant. We conclude that the new MAIX probe should be used, in addition to *M. avium* and *M. intracellulare* probes, for the identification of MAC isolates. Our results also suggest that the probe may be used alone to cover the whole MAC when species differentiation is not required.

Infections caused by organisms belonging to the *Mycobacterium avium* complex (MAC) have become a major clinical problem, especially when associated with AIDS (2, 4). Rapid and accurate identification of MAC strains is therefore crucial. The usefulness of conventional biochemical identification tests is limited because of the slow growth rate of MAC organisms. Further, *M. avium* and *M. intracellulare* cannot be differentiated from each other by simple biochemical tests. This differentiation might be important, because the prognosis of pulmonary diseases caused by *M. avium* appears to be worse than that of pulmonary diseases caused by *M. intracellulare* (14). Recently developed acridinium ester-labeled DNA probes (AccuProbe; Gen-Probe, San Diego, Calif.) offer a rapid and reproducible, nonradioactive means of identifying MAC strains and of differentiating *M. avium* from *M. intracellulare*. The use of these probes to replace tedious and often inconsistent biochemical tests, however, requires confirmation that they do not miss any strains belonging to the MAC. Several reports have shown excellent specificities for the probes; however, the reported sensitivities have varied from 89 to 100% for the radioactive probes (3, 6, 8, 10, 12, 13) and from 95.2 to 97.2% for the nonradioactive probes (3, 7). Thus, it is evident that some MAC isolates would not be detected if only the probes were used for identification. With this complication in mind, the Gen-Probe company has developed a third probe for the MAC. This probe, designated MAIX, should theoretically detect the strains nonreactive with the original *M. avium* and *M. intracellulare* probes.

In this study, the sensitivities and specificities of these three probes were compared with those of conventional methods in identifying 145 single-patient isolates. These isolates represent all MAC strains sent to our laboratory (National Public Health Institute) for identification during 1991. All isolates were identified by conventional methods, including auramin staining for acid-fast bacteria, growth rate, and pigment production studies; biochemical tests included semiquantitative catalase, catalase at 68°C, Tween 80 hydrolysis, and tellurite reduction tests (11). The isolates negative with *M. avium* and *M. intracellulare* probes were further analyzed for fatty acid and alcohol composition by gas-liquid chromatography (5) and for mycolic acids by thin-layer chromatography (1).

AccuProbe culture identification tests were performed according to the manufacturer's specifications. The strains were grown for 2 to 4 weeks prior to being tested. One loopful of bacteria was sonicated in a tube containing glass beads and the lysing reagent for 15 min in a sonication bath (Finnsonic; Ultrasonic Oy., Lahti, Finland). Then, 100 μl of the lysate was incubated with the lyophilized DNA probe at 60°C for 15 min. After 300 μl of the selection reagent was added, the contents of the tube were mixed well, incubated further at 60°C for 5 min, and kept at room temperature for 5 min. Finally, the light signal emitted by the labeled probe was measured with a luminometer (Leader 50; Gen-Probe). Results were expressed as relative light units. Samples producing signals greater than 30,000 relative light units were considered positive.

Of the 145 isolates identified as MAC constituents by conventional tests, 102 reacted with the *M. avium* probe and 36 reacted with the *M. intracellulare* probe. Seven isolates were negative with the *M. avium* and *M. intracellulare* probes, even when tested repeatedly. In contrast, these seven isolates proved clearly positive with the MAIX probe (all seven had signals of more than 500,000 relative light units). Thus, the combined sensitivity of the *M. avium* and *M. intracellulare* probes was 95.2%. The MAIX probes improved the sensitivity up to 100%.

The MAIX probe reacted with all *M. avium* (n = 20) and *M. intracellulare* (n = 20) isolates tested (Table 1). The signals were of the same magnitude as those obtained from the seven isolates positive only with the MAIX probe. In contrast, the probe did not react with *M. gordonae* (n = 10) or *M. szulgai* (n = 2) strains (Table 1). Further, the MAIX probe-positive MAC isolates did not react with the *M. gordonae* AccuProbe.

The MAIX-positive isolates exhibited a fatty acid compo-

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TABLE 1. Specificity characteristics of MAIX probe and biochemical test profiles of MAC strains and two pigmented, slowly growing mycobacterial species[a]

Strain (total no. of strains)	No. of strains positive/ no. of strains tested with MAIX probe	Pigmentation results (n/p/s)	% Strains positive by:				
			Nitrate reduction	Semiquantitative catalase[b]	Catalase at 68°C	Tween 80 hydrolysis	Tellurite reduction
MAC (MA^+) (102)	20/20	102/0/0	0	0	57	0	82
MAC (MI^+) (36)	20/20	36/0/0	0	0	52	0	90
MAC (MA^- MI^-) (7)	7/7	3/3/1	0	0	29	0	86
M. gordonae[c] (10)	0/10	0/0/10	1	90	96	100	29
M. szulgai[c] (2)	0/2	0/0/2	100	98	93	49	53

[a] Abbreviations: n, number of nonchromogenic strains; p, number of photochromogenic strains; s, number of scotochromogenic strains; MA^+, positive with *M. avium* AccuProbe; MI^+, positive with *M. intracellulare* AccuProbe; MA^- MI^-, negative with both probes.
[b] Positive limit, 45 mm.
[c] See reference 11 for biochemical test data.

sition typical of the MAC. In addition to tuberculostearic acid (10-Me-18:0) and other markers of members of the genus *Mycobacterium*, they all contained significant amounts of 2-eicosanol (2-OH-20:Oalc) but lacked hexacosanoic acid (26:0). The mycolate patterns contained alpha-, keto-, and carboxymycolates. *M. terrae* is another slowly growing species with a mycolate and fatty acid composition similar to that of MAC strains (9). The two species can, however, be differentiated by biochemical tests; e.g., Tween 80 hydrolysis, in which MAC strains are negative and *M. terrae* strains are positive. The MAIX-positive isolates proved to be clearly negative in repeated Tween 80 hydrolysis tests (Table 1).

Neither the growth rates nor the conventional biochemical test results of the MAIX-positive isolates differed significantly from those for *M. avium* probe- or *M. intracellulare* probe-positive isolates (Table 1). Three of these seven strains were nonchromogenic, three were photochromogenic, and one was scotochromogenic (Table 1). All except one of the MAIX-positive strains were positive in tellurite reduction tests, and all of them were negative in nitrate reduction and semiquantitative catalase tests, whereas two isolates were positive in a catalase test at 68°C (Table 1). Nitrate reduction, semiquantitative catalase, and Tween 80 hydrolysis tests discriminated the MAIX-positive isolates clearly from *M. gordonae* and *M. szulgai* strains (Table 1).

Four of the MAIX-positive strains were recovered from sputum, one was recovered from a bronchial aspirate, and two were recovered from urine. One of the urine isolates was recovered from a Somali immigrant. The other isolates were recovered from Finnish patients living in various regions of Finland. Six of the patients were male. The patients ranged in age from 25 to 88 years. One of the patients was infected with human immunodeficiency virus type 1 and had AIDS.

Three of the seven MAIX-positive isolates were considered clinically significant. Two of them were isolated from the sputa of two patients with chronic lung infiltrations. One of the patients was treated with a combination of isoniazid, rifampin, ethambutol, and pyrazinamide for 2 years. The other patient was treated with a combination of rifampin, ethambutol, ethionamide, and cycloserine for 6 weeks, ethambutol, ethionamide, and cycloserine for 10 months, and finally, ethionamide and cycloserine for 11 months. With both patients, the therapy had no effect on either the lung infiltrations or the excretion of mycobacteria into sputum. The third clinically significant isolate was recovered from the urine of the AIDS patient. The patient had a disseminated MAC infection and other opportunistic infections. He was treated with a combination of isoniazid, rifampin, ethambutol, pyrazinamide, and ciprofloxacin without any favorable outcome. The patient died because of liver function failure. In two of the seven cases, the significance of the isolate was considered probable. One of these isolates was recovered from the sputum of a patient with hemoptysis and pulmonary emphysema. The patient received no mycobacterial chemotherapy; his clinical condition has remained stable. The other isolate of probable significance was recovered from the urine of the Somali immigrant. The main symptom of the patient was hematuria. The patient was treated with a combination of isoniazid, rifampin, and ethambutol for 6 months. Pyrazinamide was included in the drug combination for the first month. Despite the treatment, the hematuria of the patient continued. The remaining two isolates were apparently of no clinical significance.

This study demonstrates that the new MAIX probe considerably improves the sensitivity of the AccuProbe system in identifying mycobacteria belonging to the MAC. Our results also indicate that two slowly growing scotochromogens, *M. gordonae* and *M. szulgai*, are not misidentified with the MAIX probe. However, further studies may be needed to verify the specificity of the probe.

Six of the seven MAIX-positive isolates were recovered from Finnish patients; one isolate originated from an immigrant. In only one case, AIDS was associated with the recovery of these strains. The MAIX-positive isolates had no exceptional pathogenic properties and were not associated with the presentation of any single disease. The infections due to the MAIX-positive isolates proved to be very resistant to mycobacterial chemotherapy, as is also usually the case with the infections caused by other MAC strains.

We conclude that the new MAIX probe should be used, in addition to *M. avium* and *M. intracellulare* probes, for the identification of MAC isolates. The MAIX probe seems to also detect *M. avium* and *M. intracellulare* strains. Thus, it may be used alone to cover the whole MAC when species differentiation is not required.

We thank Marita Kirjonen for excellent technical assistance. The language of the manuscript was revised by Jeri L. Hill.

This study was supported by the Finnish Antituberculosis Foundation, the Tampere Tuberculosis Foundation, and the Foundation of Väinö and Laina Kivi.

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