Clinical and Epidemiological Importance of Typing of *Mycobacterium avium* Complex Isolates

ANNA Y. TSANG,^{1†*} JAMES C. DENNER,¹ PATRICK J. BRENNAN,² AND J. KENNETH McCLATCHY^{1‡}

Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206,¹
and Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523²

Received 23 May 1991/Accepted 14 November 1991

The results of the application of a range of typing procedures to the identification and classification of 6,264 cultures of nontuberculous mycobacteria from human sources and the environment are reported. Seroagglutination, an enzyme-linked immunosorbent assay applied to whole bacteria or the glycolipid typing antigens and based on serovar-specific polyclonal or monoclonal antibodies, thin-layer chromatography of these antigens, and gas chromatography of their specific sugar determinants were used to arrive at identifications. As a result of this comprehensive approach, 4,452 (71%) of all cultures and 88% of those of samples from patients with AIDS proved to be typeable. The rank order of frequency of occurrence of individual organisms within the entire group of isolates was *Mycobacterium avium* complex serovar 4 > serovar 8 > serovar 1 > serovar 9 > serovar 6 > serovar 14 > serovar 2 > *M. fortuitum* > *M. kansasii* > *M. xenopi* > an apparent mixture of serovar 4 and *M. xenopi* > a mixture of serovar 4 and serovar 8. These results were similar but not identical to the pattern observed for isolates obtained from patients with AIDS; the order was *M. avium* complex serovar 4 > serovar 8 > serovar 1 > a mixture of serovar 4 and *M. xenopi*, a mixture of serovar 4 and serovar 8 > serovar 9 > serovar 2 > serovar 6. Serotyping was also used to demonstrate the possible clinical significance of nontuberculous mycobacteria recovered from different body sites. Other information on the distribution of *M. avium* serovars in patients from different geographical environments is provided.

Mycobacterium avium complex organisms are considered one of the most common causes of disseminated bacterial infections in patients with AIDS (1, 4, 23, 33, 34). Recent reviews of AIDS cases within the United States have reported an overall 5.5% incidence of disseminated nontuberculous mycobacterioses, with over 95% attributable to organisms of the *M. avium* complex (4, 17). Significant geographical and racial differences were observed; Caucasian and Afro-American patients had an incidence of disseminated nontuberculous mycobacterioses higher than that in Hispanics, and the incidence was lower in the mid- and south Atlantic states than elsewhere in the United States. Much of this important information was based on identification of species. However, the *M. avium* complex (*M. avium* and *M. intracellulare*), as we now know it, consists of 28 well-characterized serovars (27), and *M. scrofulaceum*, which is often classified within the *M. avium* complex, consists of 3 serovars. A full comprehension of the antigenic basis of the serological activity of members of the *M. avium* complex is now known and, with it, the molecular basis of the serospecificity of each serovar (22). With these developments, a variety of chemical and immunological tools has emerged; these tools are capable of distinguishing *M. avium* complex serovars with great accuracy (8). In the present study, these protocols were applied to help answer clinical and epidemiological questions such as the relevance of nontuberculous bacterial isolates from nonsterile body sites, the distribution patterns of individual serovars in the environment and within patient populations, and the consequent epidemiological implications.

MATERIALS AND METHODS

Sources of mycobacterial strains. During the period 1 May 1982 through 31 May 1991, 6,264 cultures were received at the National Jewish Center for Immunology and Respiratory Medicine for purposes of serotyping under the auspices of a program sponsored by the National Institute of Allergy and Infectious Diseases (see Table 1). Of these, 1,265 cultures from 759 patients with AIDS were submitted by the following U.S. investigators: Mary Jo Bacon, Methodist Hospital of Indiana, Indianapolis; Paolo deGirolami and Carol Sisson, New England Deaconess Hospital, Boston, Mass.; Gary du Moulin, Beth Israel Hospital, Boston, Mass.; Gilda Forseter, Westchester County Department of Laboratories, Valhalla, N.Y.; Norman Glover, Olive View Medical Center, Sylmar, Calif.; Leroy Graham, Medical College of Virginia, Richmond; the late Howard Gruft, New York State Department of Health, Albany; W. Keith Hadley and David Yajko, San Francisco General Hospital, San Francisco, Calif.; Charles Horsburgh and Michael Iseman, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colo.; Donald Jungkind, Thomas Jefferson Hospital, Philadelphia, Pa.; Ray Kaplan and Melodie Beard, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Ill.; Timothy Kiehn, Memorial Sloan-Kettering Institute, New York, N.Y.; Joseph Libonati, State of Maryland Laboratories Division, Baltimore; Bonnie Lutz, Orange County Public Health Department, Santa Ana, Calif.; Stephen Moser, Washington University Medical Center, St. Louis, Mo.; Joseph Plouffe, Ohio State University, Columbus; Richard B. Porwancher, St. Francis Medical Center, Trenton, N.J.; Linda Schultz, Hospital of the University of Pennsylvania, Philadelphia; Irene Sherman, State Laboratory Institute, Jamaica Plain, Mass.; Rodney Swartz, Georgetown University Hospital, Washington, D.C.; Richard Wallace and David Murphy, University of Texas Health Center at Tyler, Tyler; Nancy G. Warren, Department of General Services,

* Corresponding author.

† Present address: Department of Microbiology, Colorado State University, Fort Collins, CO 80523.

‡ Present address: National Health Laboratories, Inc., Englewood, CO 80111.

Richmond, Va.; and Marlene Willner and Jan Pennington, University Hospital, Cincinnati, Ohio.

Cultures of samples from almost every body site were obtained. In addition to the usual respiratory sites, many samples were from otherwise sterile body sites such as blood, bone marrow, cerebrospinal fluid, kidney, liver, lung, lymph node, and spleen.

Growth and harvesting of mycobacteria. As cultures were received, the patient's name, source of specimen, and species identification, as provided by the contributing laboratory, were recorded. Cultures were grown and harvested (28) with modifications as described in the accompanying report (8) for purposes of seroagglutination, thin-layer chromatography (TLC), enzyme-linked immunosorbent assay (ELISA), and gas chromatography (GC).

Seroagglutination, ELISA, TLC, and GC. The seroagglutination procedure of Schaefer (27), with slight modifications (21, 28), was performed as described in the accompanying report (8). Rabbit antisera were those raised against individual members of the *M. avium* complex (serovars 1 to 28), the *M. scrofulaceum* complex (serovars 41 to 43), *M. szulgai*, *M. xenopi*, *M. kansasii*, *M. marinum*, *M. simiae* I and II, and *M. malmoense*, as described previously (27). Extraction and preparation of the alkali-stable glycopeptidolipid antigens from dried cells were conducted as described previously (8, 28). TLC was conducted in the two solvent systems described in the accompanying report (8). Whole cells were subjected to ELISA by using monoclonal antibodies. The alditol acetate derivatives of the sugars from the serovar-specific polar glycopeptidolipids were subjected to GC as described in the accompanying report (8).

RESULTS

A total of 6,264 cultures were received for purposes of identification during the period from 1 May 1982 to 31 May 1991 (Table 1). Of these, 1,265 cultures were from patients with AIDS and 2,669 were from patients without AIDS. Of the remaining 2,161 cultures, most were from the environment. Some were from outside the United States or Canada, and these included isolates from patients with and without AIDS. A few cultures were either laboratory derived or reference strains sent for confirmatory identification.

The rank order of the organisms most frequently encountered among the 6,266 cultures examined were *M. avium* complex serovar 4 > serovar 8 > serovar 1 > serovar 9 > serovar 6 > serovar 14 > serovar 2 > *M. fortuitum* > *M. kansasii* (Table 1). This order differed from that which emerged from a previous study (21). The rank order at that time was *M. avium* complex serovar 8 > serovar 16 > serovar 4 > serovar 19 > serovar 9 > *M. scrofulaceum* serovar 42 > *M. avium* complex serovar 1. Most striking was the evidence for a mixture of serovars within 362 of the cultures, representing 6% of the total number examined. Of these, the most frequently encountered combinations were *M. avium* complex serovar 4 and *M. xenopi*, and *M. avium* complex serovars 4 and 8. Other combinations such as serovars 1 and 13, and serovars 8 and 10 were infrequently encountered (Table 1). To determine whether these cultures were a mixture of two or more organisms, single colonies were picked and streaked onto plates for purity. Although, as expected, colonies with different morphologies emerged from a single colony, repeat serotyping of pure colonies again demonstrated dual reactivity. Serovars cannot be readily separated by colony picking.

A notable outcome of the present survey was the obser-

TABLE 1. Results of analysis of 6,266 cultures received for serotyping between 1 May 1982 and 31 May 1991

Species and serovar	No. of cultures from the following sources ^a :		Others	Total
	United States and Canada			
	Non-AIDS	AIDS		
<i>M. avium</i> complex				
1	147	110	141	398
2	34	39	94	167
3	7	5	22	34
4	179	332	258	769
5	8	1	3	12
6	50	32	163	245
7	14	2	9	25
8	222	216	22	460
9	94	45	120	259
10	9	13	8	30
11	5	0	23	28
12	78	11	25	114
13	10	3	4	17
14	63	5	119	187
15	3	0	14	17
16	43	1	31	75
17	57	3	20	80
18	39	2	15	56
19	51	4	0	55
20	11	0	12	23
21	3	2	12	17
22	3	0	6	9
23	8	2	4	14
24	5	0	2	7
25	26	0	8	34
26	4	0	12	16
27	2	0	1	3
28	4	0	4	8
<i>M. scrofulaceum</i> complex				
41	2	0	27	29
42	46	6	14	66
43	44	6	32	82
Mixtures				
1 and 8	10	19	0	29
4 and 8	17	40	27	84
4 and <i>M. xenopi</i> ^b	22	80	2	104
8 and 42	0	14	8	22
Other mixtures ^c	8	51	64	123
<i>M. kansasii</i>	68	16	29	113
<i>M. szulgai</i>	4	2	13	19
<i>M. xenopi</i> ^b	40	41	25	106
<i>M. simiae</i> I	16	4	58	78
<i>M. simiae</i> II	10	4	10	24
<i>M. malmoense</i>	1	0	42	43
<i>M. fortuitum</i>	45	0	69	114
<i>M. peregrinum</i>	37	0	44	81
<i>M. chelonae</i>	42	0	49	91
<i>M. abscessus</i>	2	0	3	5
<i>M. marinum</i>	12	1	28	41
<i>M. gordonae</i>	0	0	39	39
Untypeable	725	153	934	1,812
Total	2,330	1,265	2,669	6,264

^a Cultures are categorized into those from patients without AIDS (non-AIDS), patients with AIDS, and other sources. Other sources include environmental cultures, cultures from patients without and with AIDS from outside the United States and Canada, and cultures used for "research purposes."

^b Cultures received after 1 June 1989 were not evaluated for the presence of *M. xenopi*.

^c Other mixtures were those which occurred from one to three times.

vation that of the 6,264 cultures received, 4,452 (71%) were typeable by agglutination, TLC, ELISA, or GC or by a combination of all of these procedures. These values should be compared with those of McClatchy obtained in 1981 (21); McClatchy reported that of 1,661 strains subjected to serotyping, only 690 (41.5%) belonged to one of the 31 known serovars. Among the 1,812 cultures not amenable to typing through the combined approach were those that were rough and thus devoid of the typing antigens, those that were not established organisms and hence not identifiable, those that belonged to species or genera outside the typing system (e.g., *M. tuberculosis*, *Nocardia* spp., *Rhodococcus* spp.), or those that were not viable.

Results from serotyping of 1,265 cultures of samples from patients with AIDS. Of the 6,264 cultures received, 1,265 were of samples from 759 patients with AIDS in the United States. Of these, 1,112 (88%) were typeable. These came from 659 patients. Thus, compared with the total group of isolates, those recovered from patients with AIDS were more likely to be an established serovar. The rank order of identification of these isolates was as follows: serovar 4 > serovar 8 > serovar 1 > a mixture of serovar 4 and *M. xenopi* > serovar 9 > *M. xenopi* > a mixture of serovars 4 and 8 > serovar 2 > serovar 6 (Table 2). When serovars were ranked by patient rather than by culture, the results were as follows: serovar 4 > serovar 8 > serovar 1 > a mixture of serovar 4 and *M. xenopi* > a mixture of serovars 4 and 8 > serovar 9 > serovar 2 > serovar 6. Thus, evaluation of the frequency of serovars on the basis of the numbers of cultures instead of the numbers of patients can be misleading, in that many patients had multiple cultures of a particular serovar.

Disease sites in patients with AIDS compared with those in patients without AIDS. In the previous study (21), the majority of isolates (84%) arose in pulmonary sites. In the present study, only 28% of the isolates from patients with AIDS stemmed from pulmonary areas. Conversely, only 13% of the isolates from the previous study (21) were from extrapulmonary sites, whereas 64% of the isolates in the present study were from extrapulmonary sites. Of these, about 75% of the extrapulmonary sites represented otherwise sterile body areas such as blood, bone marrow, sterile biopsy specimens, and sterile body fluids. This fact undoubtedly

TABLE 3. Use of information on isolates from different body sites to determine clinical significance

No. of isolates/ patient	Total no. of patients	No. (%) of patients with multiple isolates			
		From all sites (sterile and nonsterile)		From normally sterile sites only	
		With the same serovar	With different serovars	With the same serovar	With different serovars
2	106	56	50	46 (29)	36 (34)
3	40	25	15	25 (63)	10 (25)
4	19	14	5	14 (74)	5 (26)
5	11	11	0	11 (100)	0 (0)
>6	23	22	1	22 (96)	1 (4)

reflects the disseminated nature of *M. avium* complex infections in patients with AIDS.

Identification of multiple isolates from different body sites to help establish those which are of clinical significance. One clinical application of serotyping was to allow a comparison of isolates from multiple body sites, which included those from sterile as well as those from nonsterile body sites (Table 3).

For 199 patients there were two or more isolates submitted for serotyping identification. When cultures of samples from normally sterile sites were taken and compared with those of samples from normally nonsterile sites, the following results were observed. When two isolates from each patient were submitted, with one each from sterile and nonsterile sites, 29% of the time the isolate from the sterile body site was of the same serovar as that from the nonsterile site. Also, 34% of the time, the isolate from the sterile body site had a serovar different from that of the isolate from the nonsterile body site. In other words, with two isolates, there was very little predictive value in identifying the serovars of isolates recovered from nonsterile body sites compared with those recovered from sterile body sites.

However, when three or more isolates were submitted, with at least one isolate being from a sterile body site, the isolate from the sterile body site proved to be the same as at

TABLE 2. Distribution of 1,112 typeable *M. avium* complex and other isolates from 659 patients with AIDS^a

Rank	Serovar or species	No. (%) [approx.] of isolates	Serovar or species	No. (%) [approx.] of patients
1	4	332 (30)	4	189 (29)
2	8	216 (19)	8	94 (14)
3	1	110 (10)	1	68 (10)
4	4 and <i>M. xenopi</i> mixture	80 (7)	4 and <i>M. xenopi</i> mixture	56 (9)
5	9	45 (4)	4 and 8 mixture	30 (5)
	<i>M. xenopi</i>	41 (4)		
	4 and 8 mixture	40 (4)		
	2	39 (4)		
6	6	32 (3)	9	22 (3)
			2	21 (3)
			6	20 (3)
			<i>M. xenopi</i>	20 (3)
7	1 and 8 mixture	19 (2)	10	10 (2)
8	8 and 42 mixture	14 (1)	8 and 42 mixture	9 (1)
	<i>M. kansasii</i>	16 (1)	12	8 (1)
	10	13 (1)	1 and 8 mixture	6 (1)
	12	11 (1)		
	Other species or serovars, each <1%	109 (9)	Other species or serovars, each <1%	106 (16)

^a Of 1,265 cultures, 153 were untypeable. Of 759 patients, 100 had cultures which were untypeable.

TABLE 4. Geographical distribution of the predominant *M. avium* serovars

<i>M. avium</i> complex serovar	% of total no. of isolates from ^a :								
	Calif. (n = 76)	Colo. (n = 60)	Ill. (n = 15)	Ind. (n = 26)	Mass. (n = 103)	Md. (n = 20)	N.Y. (n = 196)	Ohio (n = 137)	Tex. (n = 45)
1	3	12	0	31	16	10	9	9	9
2	3	5	0	15	9	0	2	5	0
3	0	3	0	12	0	5	0	1	0
4	16	15	67	54	17	35	37	23	7
6	3	2	0	12	3	0	3	1	7
8	11	3	7	38	7	10	9	23	16
9	8	5	0	12	1	0	2	4	2
10	3	3	0	8	2	0	0	4	0
12	1	0	0	4	1	0	0	1	0

^a Values in parentheses are total number of isolates.

least one of the isolates from a nonsterile body site, making it more predictive (63 to 100%). Thus, an isolate from stool specimens or respiratory secretions which matched those from blood or bone marrow might indicate gastrointestinal or respiratory involvement, respectively. Conversely, isolates from stool specimens or respiratory secretions which did not match those from otherwise sterile body sites might indicate that those from stool specimens or the respiratory tract are merely environmental contaminants.

Observations on the geographical distribution of *M. avium* serovars. Limited information about serovar distribution in patients with AIDS from different states in the United States was derived from the present analysis. Data from 678 patients with AIDS having the nine most predominant *M. avium* serovars from nine different states are provided in Table 4. Serovar 4 was the most common serovar encountered in Colorado, Illinois, Indiana, Massachusetts, Maryland, and New York, whereas serovar 8 was more common in Texas. In California and Ohio, there seemed to be about an equal distribution of serovars 4 and 8.

DISCUSSION

This study addressed the clinical and epidemiological worth of serotyping nontuberculous mycobacteria from patients with AIDS. The application of a comprehensive approach to serotyping (8), in particular, the use of TLC, ELISA, and GC, revealed much more information than hitherto has been available. By using this combined approach, 71% of isolates were identified, including many from environmental sources, whereas 41.5% of isolates were identified by seroagglutination alone in a previous study (21). More impressively, about 88% of the 1,265 isolates from 759 patients with AIDS produced clear-cut results, again indicating that the majority of these isolates conform to the well-characterized collection of *M. avium* complex serovars (27, 28). The focus of attention for future studies will be the group of cultures that were of the *M. avium* complex but that did not conform to a known serovar. These cultures apparently represent new, heretofore unidentified serovars.

A striking observation was the frequency with which *M. avium* serovars (serovars 1 to 10) were encountered compared with the frequency with which *M. intracellulare* serovars (serovars 11 to 28) were encountered. Laboratories that only perform seroagglutination for such purposes reported a similar general trend (12, 31). For instance, Yakus and Good (31) reported the rank order for isolates from 727 patients with AIDS to be *M. avium* serovar 4, serovar 8,

serovar 1, a mixture of serovars 4 and 8, serovar 10, serovar 9, and serovar 6.

The findings of *M. xenopi* and *M. xenopi* mixed with *M. avium* serovars are of particular interest. *M. xenopi* and *M. avium* are closely related in terms of their biochemical properties and colonial morphologies. Thus, *M. xenopi* might easily be mistaken for an isolate of the *M. avium* complex. However, serotyping by agglutination and TLC can easily distinguish these two organisms on the basis of specific antisera prepared to *M. xenopi* and the different chemical compositions of the type-specific antigens.

Also striking was the presence of apparently mixed serovars in 6% of all cultures that were submitted. In an attempt to determine whether cultures were mixed, one colony was picked and streaked onto a fresh plate of 7H11. In some cases, different colonial morphology types emerged, even with repeat colony pickings. In some cases, pure cultures were obtained. Repeated serotyping of the pure cultures and of the segregated colony types produced the same results in many cases: a mixture of serovars. Thus, cultures with a combination of two or more serovars probably represented a mixture of two or more different bacteria that were inseparable by the technique of colony picking.

Since the beginning of the AIDS epidemic, the incidence of infections with the *M. avium* complex, especially disseminated infections, has increased dramatically (1, 6, 18). This phenomenon can be attributed to several factors, some of which were addressed in this study. One explanation may be that certain serovars are more virulent; i.e., they persist longer in the human host. The results of the comparative serotyping of strains that were considered virulent and those that were considered avirulent have indicated that this notion is probably not correct (5). Instead, it seems likely that individuals acquire whatever serovar predominates in the immediate environment. Results of the present study indicate that certain serovars are found more frequently in patients, and this frequency varies from state to state in the nine states from which cultures were obtained for this study. For example, serovar 4 was the most commonly isolated serovar from patients with AIDS in Illinois, Indiana, Maryland, and New York, whereas serovar 8 was the most common in Texas, serovars 4 and 8 occurred equally in California and Ohio, and serovars 1 and 4 had similar frequencies in Colorado and Massachusetts. One prevalent theory is that drinking water contaminated with *M. avium* complex bacteria is the likely source of infection (9, 10, 29). This concept is supported by the evidence presented here, which shows that 11% of the *M. avium* complex cultures

from patients with AIDS were from the gastrointestinal tract. Serotyping also demonstrated that the majority of the *M. avium* organisms isolated from a Boston hospital water supply system and from municipal chlorinated and unchlorinated water supplies in several Massachusetts communities were serovars 1 and 4, which correlated with the nature of the preponderant serovar found in patients with AIDS in Massachusetts (Table 4). Among the cultures examined in this study, there were also some that arose from another hospital water supply system (13). Serotyping demonstrated that serovar 4 predominated among a series of isolates and that a reagent used to process specimens was probably the point source of contamination of serovar 4 in the urine of 29 patients. In addition, a recent study by Hoffner et al. (16), who used a multiple approach to serotyping, demonstrated that serovar 6 predominated among isolates from 25 Swedish patients, followed by serovars 4 and 9 and *M. scrofulaceum* serovar 43. There was no evidence of serovar 8 in that study (16). Thus, while the exact mechanism of transmission of *M. avium* complex organisms to patients with AIDS is not certain, despite intensive examination and speculation (4, 6, 7), the most plausible scenario seems to be that the predominant serovars within individuals simply reflect those in their immediate environment. The organisms are presumably ingested in the water, colonize the gastrointestinal tract, and, if or when the conditions permit, invade the intestinal wall and disseminate throughout the body (6, 13). Thus, serotyping of mycobacteria recovered from water is necessary in order to study their epidemiology in the context of the pathogenesis of mycobacterial infections.

In addition to epidemiological considerations, serotyping has provided important clinical information. Casual isolates from respiratory specimens, which usually appear in very low colony numbers, can be a diagnostic dilemma. However, serotyping of cultures obtained from multiple body sites, especially including those from sites which are otherwise sterile, can establish whether the organisms from the nonsterile sites are the same or different from those from sterile sites. If, for instance, some or all of the organisms are of different serovars, it could be concluded that the organisms were not the same and that those from the nonsterile sites were probably casual isolates from the environment.

Serotyping identification might also be useful in establishing whether the patient's current mycobacterial disease is new or recrudescent. The nature of a current isolate can be compared with those of previous isolates, and if the isolates obtained from each time period are different, it can be concluded that the organisms did not arise from the previous infection and that the source of these organisms is exogenous. However, if the isolates obtained from each time period are the same, then the current infection might be considered recrudescent and the source of the organisms might be endogenous, arising from sites of the previous infection. This latter conclusion, however, needs more study and may be difficult to substantiate, since it seems reasonable to assume that if the serovar is one of the dominant ones in that geographical region, even an exogenous reactivation would most likely be of the same serovar. Further testing might be warranted to establish that the current disease is new, because in many situations, the isolation of a few colonies from a single specimen again represents a casual isolate (11). Thus, the establishment of nontuberculous mycobacteria as the causative agent in pulmonary disease requires multiple isolations taken over a period of several days with the recovery of large colony numbers (30). Even when these conditions were met, there was still the question

of whether these bacteria colonized the respiratory tract or whether they had actually invaded tissue to cause progressive disease. The fact that emerged from the present study was that 64% of isolates from patients with AIDS arose in extrapulmonary sites, with the majority of them being otherwise sterile sites. This undoubtedly reflects the disseminated nature of *M. avium* infection in patients with AIDS (1, 12, 15, 17, 18, 23, 33, 34) and is in clear contrast to the situation in which disease in patients without AIDS is localized primarily in the lungs of adults (4, 14, 25, 30) or in the lymph nodes of children (30). This conclusion also arose from the studies reported by Lucas (20) and Horsburgh and Selik (18).

Thus, serotyping of nontuberculous mycobacteria has provided an important tool for clinical and epidemiological applications. It can be used to establish the clinical significance of specific isolates from nonsterile body sites by comparing them with those from sterile body sites, it can be used to decide whether there is a point source of contamination of organisms recovered from a series of clinical specimens, it can be used to determine the epidemiology of nontuberculous mycobacteria organisms in both patients and the environment, and it might be used to determine whether the current disease in a patient is recrudescent or new. To date, only the immunogenic markers involved in serotyping are able to discern differences and similarities among strains of the same species; DNA probes, to date, have not been able to identify different serovars within one species. Although the procedures described here are lengthy and are not recommended for routine use in the clinical laboratory, they can be readily performed in certain reference laboratories.

ACKNOWLEDGMENT

This work was supported by contract NO1-AI 52574 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

REFERENCES

1. Armstrong, D., J. W. M. Gold, J. Dryanski, E. Whimbey, B. Polsky, C. Hawkins, A. E. Brown, E. Bernard, and T. E. Kiehn. 1985. Treatment of infection in patients with the acquired immunodeficiency syndrome. *Ann. Intern. Med.* 103:738-743.
2. Baess, I. 1983. Deoxyribonucleic acid relationships between different serovars of *Mycobacterium avium*, *Mycobacterium intracellulare*, *Mycobacterium scrofulaceum*. *Acta Pathol. Microbiol. Immunol. Scand. Sect. B* 91:201-203.
3. Brennan, P. J., M. Souhrada, B. Ullom, J. K. McClatchy, and M. B. Goren. 1978. Identification of atypical mycobacteria by thin-layer chromatography of their surface antigens. *J. Clin. Microbiol.* 8:374-379.
4. Collins, F. M. 1989. Mycobacterial disease, immunosuppression, and acquired immunodeficiency syndrome. *Clin. Microbiol. Rev.* 2:360-377.
5. Crowle, A. J., A. Y. Tsang, A. Vatter, and M. H. May. 1986. Comparison of 15 laboratory and patient-derived strains of *Mycobacterium avium* for ability to infect and multiply in cultured human macrophages. *J. Clin. Microbiol.* 24:812-821.
6. Damsker, B., and E. J. Bottone. 1985. *Mycobacterium avium-Mycobacterium intracellulare* from the intestinal tracts of patients with the acquired immunodeficiency syndrome: concepts regarding acquisition and pathogenesis. *J. Infect. Dis.* 151:179-181.
7. de Caprariis, P. J., J. A. Giron, J. A. Goldstein, V. J. LaBombardi, J. J. Guarneri, and H. Laufer. 1984. *Mycobacterium avium-intracellulare* infection and possibly venereal transmission. *Ann. Intern. Med.* 101:721.
8. Denner, J. C., A. Y. Tsang, D. Chatterjee, and P. J. Brennan. 1992. Comprehensive approach to identification of serovars of

- Mycobacterium avium* complex. J. Clin. Microbiol. 30:473-478.
9. du Moulin, G. C., and K. D. Stottmeier. 1986. Waterborne mycobacteria: an increasing threat to health. ASM News 52: 525-529.
  10. du Moulin, G. C., K. D. Stottmeier, P. A. Pelletier, A. Y. Tsang, and J. Heddy-Whyte. 1988. Concentration of *Mycobacterium avium* by hospital hot water systems. JAMA 260:1599-1601.
  11. Edwards, L. B., and C. E. Palmer. 1959. Isolation of "atypical" mycobacteria from healthy persons. Am. Rev. Respir. Dis. 80:747.
  12. Good, R. C. 1985. Opportunistic pathogens in the genus *Mycobacterium*. Annu. Rev. Microbiol. 39:347-369.
  13. Graham, L., Jr., N. G. Warren, A. Y. Tsang, and H. P. Dalton. 1988. *Mycobacterium avium* complex pseudobacteriuria from a hospital water supply. J. Clin. Microbiol. 26:1034-1036.
  14. Guthertz, L. S., B. Damsker, E. J. Bottone, E. G. Ford, T. F. Midura, and J. M. Janda. 1989. *Mycobacterium avium* and *Mycobacterium intracellulare* infections in patients with and without AIDS. J. Infect. Dis. 160:1037-1041.
  15. Helbert, M., D. Robinson, D. Buchanan, T. Hellyer, M. McCarthy, I. Brown, A. J. Pinching, and D. M. Mitchell. 1990. Mycobacterial infection in patients infected with the human immunodeficiency virus. Thorax 45:45-48.
  16. Hoffner, S., G. Källénus, B. Petrini, P. J. Brennan, and A. Y. Tsang. 1990. Serovars of *Mycobacterium avium* complex isolated from patients in Sweden. J. Clin. Microbiol. 28:1604-1620.
  17. Horsburgh, C. R., Jr., U. G. Mason III, D. C. Farhi, and M. D. Iseman. 1985. Disseminated infection with *Mycobacterium avium-intracellulare*. A report of 13 cases and a review of the literature. Medicine 64:36-48.
  18. Horsburgh, C. R., Jr., and R. M. Selik. 1989. The epidemiology of disseminated nontuberculous mycobacterial infection in the acquired immunodeficiency syndrome (AIDS). Am. Rev. Respir. Dis. 139:4-7.
  19. Iseman, M. D. 1989. *Mycobacterium avium* complex and the normal host: the other side of the coin. N. Engl. J. Med. 321:896-898.
  20. Lucas, S. B. 1990. Missing infections in AIDS. Trans. R. Soc. Trop. Med. Hyg. 84(Suppl. 1):34-38.
  21. McClatchy, J. K. 1981. The seroagglutination test in the study of nontuberculous mycobacteria. Rev. Infect. Dis. 3:867-870.
  22. McNeil, M., D. Chatterjee, S. W. Hunter, and P. J. Brennan. 1989. Mycobacterial glycolipids: isolation, structures, antigenicity, and synthesis of neoantigens. Methods Enzymol. 179:215-242.
  23. Modilevsky, T., F. R. Sattler, and P. R. Barnes. 1989. Mycobacterial disease in patients with human immunodeficiency virus infection. Arch. Intern. Med. 149:2201-2205.
  24. Parker, B. C., M. A. Ford, H. Gruft, and J. O. Falkinham III. 1983. Epidemiology of infection by nontuberculous mycobacteria. IV. Preferential aerosolization of *Mycobacterium intracellulare* from natural waters. Am. Rev. Respir. Dis. 128:652-656.
  25. Prince, D. S., D. D. Peterson, R. M. Steiner, J. E. Gottlieb, R. Scott, H. L. Israel, W. G. Figueroa, and J. E. Fish. 1989. Infection with *Mycobacterium avium* complex in patients without predisposing conditions. N. Engl. J. Med. 321:863-868.
  26. Rivoire, B., B. J. Ranchoff, D. Chatterjee, H. Gaylord, A. Y. Tsang, A. H. J. Kolk, G. O. Aspinall, and P. J. Brennan. 1989. Generation of monoclonal antibodies to the specific sugar epitopes of *Mycobacterium avium* complex serovars. Infect. Immun. 57:3147-3158.
  27. Schaefer, W. B. 1980. Serological identification of atypical mycobacteria. Methods Microbiol. 13:323-344.
  28. Tsang, A. Y., I. Drupa, M. Goldberg, J. K. McClatchy, and P. J. Brennan. 1983. Use of serology and thin-layer chromatography for the assembly of an authenticated collection of serovars within the *Mycobacterium avium-Mycobacterium intracellulare-Mycobacterium scrofulaceum* complex. Int. J. Syst. Bacteriol. 33:285-292.
  29. Wallace, R. J., Jr. 1987. Nontuberculous mycobacteria and water: a love affair with increasing clinical importance. Infect. Dis. Clin. N. Am. 1:677-686.
  30. Wolinsky, E. 1979. Nontuberculous mycobacteria and associated diseases. Am. Rev. Respir. Dis. 119:107-159.
  31. Yakus, M. A., and R. C. Good. 1990. Geographic distribution, frequency, and specimen source of *Mycobacterium avium* complex serotypes isolated from patients with acquired immunodeficiency syndrome. J. Clin. Microbiol. 28:926-929.
  32. Yanagihara, D. L., V. L. Barr, C. V. Knisley, A. Y. Tsang, J. K. McClatchy, and P. J. Brennan. 1985. Enzyme-linked immunosorbent assay of glycolipid antigens for identification of mycobacteria. J. Clin. Microbiol. 21:569-574.
  33. Young, L. S. 1988. *Mycobacterium avium* complex infection. J. Infect. Dis. 157:863-867.
  34. Zakowski, P., S. Fligel, O. G. Berlin, and B. L. Johnson, Jr. 1982. Disseminated *Mycobacterium avium-intracellulare* infection in homosexual men dying of acquired immunodeficiency. JAMA 248:2980-2982.