JOURNAL OF CLINICAL MICROBIOLOGY, May 1990, p. 926–929
0095-1137/90/050926-04$02.00/0

Vol. 28, No. 5

Geographic Distribution, Frequency, and Specimen Source of *Mycobacterium avium* Complex Serotypes Isolated from Patients with Acquired Immunodeficiency Syndrome

MITCHELL A. YAKRUS* AND ROBERT C. GOOD

Division of Bacterial Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

Received 11 October 1989/Accepted 25 January 1990

Isolates of *Mycobacterium avium* complex from 727 patients with acquired immunodeficiency syndrome (AIDS) were submitted by medical centers across the United States to the Centers for Disease Control for serotyping. We were able to type 630 (87%) of these isolates by our seroagglutination procedure. Almost all typeable isolates were *M. avium* (serotypes 1 to 6 and 8 to 11). Blood was the major specimen source for both *M. avium* and the nontypeable isolates. *M. intracellulare* serotypes made up only 3% of all isolates from AIDS patients, with sputum being the major specimen source. More than 50% of the isolates originated from either New York or California, with serotype 4 being isolated most frequently in New York and serotype 8 appearing most frequently in California. AIDS patients in Los Angeles had a significantly higher isolation frequency for serotype 8 and a significantly lower one for serotype 4 in comparison with patients in either San Francisco or New York City.

Nontuberculous mycobacteria belonging to the *Mycobacterium avium* complex are often isolated from immunocompromised individuals. These opportunistic pathogens have been found to cause disseminated infection in up to 53% of patients with acquired immunodeficiency syndrome (AIDS) (7). The source of infection is presumed to be environmental (5), since the organisms are commonly isolated from soil, water, and house dusts (6). Infection might also be acquired from contact with fowl or domestic and wild animals (13).

Since the adaptation of the seroagglutination technique to nontuberculous mycobacteria (14), 28 serotypes have been established for members of the *M. avium* complex and 3 additional serotypes for *M. scrofulaceum*. Serotyping is based on the presence of specific oligosaccharide haptens located on the cell surface (2, 3, 12) of smooth-colony-forming strains (15). Rough-colony-forming strains do not form stable suspensions and therefore are unsuitable for serotyping.

The traditional designation of *M. avium* serotypes as 1 through 3 and *M. intracellulare* serotypes as 4 through 28 has been modified because of DNA homology (1) and T-catalase serology studies (18). The high correlation between these two methods plus evidence from pathogenicity studies (13) suggest that serotypes 1 through 6 and 8 through 11 should be considered *M. avium*, while serotypes 7 and 12 through 25 are *M. intracellulare*. The original designation of *M. scrofulaceum* as serotypes 41 through 43 remains unchanged.

In this survey, we report our findings concerning source, frequency, and geographic distribution of *M. avium* complex serotypes as a result of serotyping isolates from 727 AIDS patients.

MATERIALS AND METHODS

Isolates identified as *M. avium* complex from 727 AIDS patients were submitted from medical centers across the United States from January 1982 through July 1987. Identification was reconfirmed in our laboratories (9) when an isolate failed to serotype and did not autoagglutinate. Most isolates were initially received for drug susceptibility testing against rifabutin and were subsequently serotyped for this study. A smaller number of isolates were received for identification, and a few were directly solicited by us for serotyping. Specimen source information was obtained from data sheets submitted with all cultures. No particular specimen source was requested for any of our testing procedures. Large numbers of isolates were received from California and New York State, since both states contain large urban centers with high concentrations of risk group individuals, namely, homosexuals and intravenous drug abusers.

The preparation of type strains for antiserum production and the method for serotyping isolates have been described in detail (6). This method is basically a modification of the procedure of Schaefer (15). Briefly, strains were grown in complete Middlebrook 7H9 medium (Difco Laboratories, Detroit, Mich.) for 7 to 14 days at 35°C. Broth cultures (0.2 ml) were spread over petri plates (20 by 150 mm each) containing complete Middlebrook 7H10 medium (GIBCO Laboratories, Grand Island, N.Y.), sealed in plastic bags,

TABLE 1. Serotypes of *M. avium* complex isolates from AIDS patients

Serotype	No. of isolates (% of total)
1	64 (9)
2	11 (2)
4	290 (40)
6	13 (2)
8	124 (17)
9	23 (3)
10	27 (4)
4/8 cross	30 (4)
Other *M. avium*[a]	26 (4)
M. intracellulare[a]	22 (3)
Nontypeable[b]	97 (13)

[a] Serotypes and cross-reactions less than 1% of total.
[b] Cell suspensions either did not react with type-specific antisera or autoagglutinated.

* Corresponding author.

TABLE 2. Serotypes of *M. avium* complex associated with source of isolation

Serotype	No. of isolates from (% of total for serotype):					
	Blood	Tissue	Sputum	Stool	Other	Unknown
1	23 (36)	11 (17)	7 (11)	1 (2)	9 (14)	13 (20)
2	4 (36)	1 (9)	1 (9)	0 (0)	2 (18)	3 (27)
4	121 (42)	52 (18)	22 (8)	14 (5)	42 (14)	39 (13)
6	2 (15)	2 (15)	2 (15)	1 (7)	3 (20)	3 (20)
8	52 (42)	18 (15)	17 (14)	2 (2)	19 (15)	16 (13)
9	12 (52)	2 (9)	3 (13)	0 (0)	3 (13)	3 (13)
10	13 (48)	4 (15)	2 (7)	0 (0)	5 (18)	3 (11)
4/8 cross	12 (40)	7 (23)	3 (10)	2 (6)	4 (13)	2 (7)
Other *M. avium*	8 (31)	3 (12)	6 (23)	1 (4)	3 (12)	5 (8)
M. intracellulare	4 (18)	3 (14)	11 (50)	0 (0)	2 (9)	2 (9)
Nontypeable	40 (41)	18 (19)	11 (11)	3 (3)	15 (15)	10 (10)

and incubated at 35°C either for 3 weeks or until growth was confluent. Cells were harvested from plates with 0.01 M phosphate-buffered saline, pH 7.2, and heat killed by immersion in a water bath at 70°C for 70 min. Cell suspensions were standardized to 0.3 optical density units at 525 nm for either antiserum production or typing. Equal volumes (0.5 ml) of cell suspension and diluted antiserum were mixed in tubes (12 by 75 mm each). A positive test was indicated by complete agglutination of the cell suspension after incubation in a water bath at 37°C for 24 h. Proof of type was obtained by adsorbing reference antisera with cell suspensions of positively reacting isolates and subsequently testing the adsorbed antisera against their homologous strains. An absence of agglutination was considered a confirmation of identity, whereas a positive test indicated that the original isolate was a cross-reacting strain.

RESULTS

Frequency of serotypes. Of 727 *M. avium* complex isolates from AIDS patients, 630 (87%) were typeable by our seroagglutination procedure. Serotypes and frequency of isolation are listed in Table 1. *M. avium* serotype 4 accounted for 40% of the isolates, while *M. avium* serotype 8, the next most prevalent serotype, accounted for 17% of the isolates. Several isolates agglutinated in both serotype 4 and serotype 8 antisera and could not be identified as a single serotype by our adsorption technique. By single-colony selection it was shown that the serotypes were not mixed but gave a true cross in the seroagglutination reaction.

Only 22 isolates of *M. intracellulare* from AIDS patients were serotyped. *M. intracellulare* serotype 16 (three isolates) and *M. intracellulare* serotype 18 (four isolates) were most frequently encountered.

Isolates were considered nontypeable if they either did not react with our antisera or autoagglutinated. Nontypeable cultures accounted for 13% (97 isolates) of all our isolates.

Serotype and specimen source. *M. avium* complex isolated from AIDS patients was principally from blood, tissue, sputum, and stool specimens (Table 2). Blood was the major specimen source for the commonly isolated serotypes. Forty-two percent of both serotypes 4 and 8 isolates were from blood. No major *M. avium* serotype was significantly different from another in association with a particular specimen source.

Sputum appears to be the major source for *M. intracellulare*, since 11 of our 22 isolates originated from that specimen type. No *M. intracellulare* serotype isolate could be identified from stool samples.

Geographic distribution. Medical centers in California and New York State contributed over 50% of the *M. avium* complex isolates from AIDS patients. The serotypes found in these states were compared with each other and with those isolated in other western and eastern states (Table 3). *M. avium* serotypes 4 and 8 have a regional distribution. In California, serotype 8 was found slightly more often than serotype 4, and these two serotypes accounted for over 50% of cultures submitted from the state. In contrast, serotype 4 alone was identified in over 50% of the isolates from New York State AIDS patients. When isolates from western states other than California were examined, serotype 4 was found in slightly larger numbers than serotype 8. This again contrasts with isolates from eastern states other than New York, where the frequency of serotype 4 far exceeded that of serotype 8. Serotypes 1, 9, and 10 appear to have an even distribution in each region.

Serotypes of isolates from three cities that represent the largest centers of infection for *M. avium* complex in AIDS patients, New York City, Los Angeles, and San Francisco, were compared (Table 4). Los Angeles was found to have a high concentration of serotype 8 isolates, which accounted for 36% of the isolates from that city. Serotype 4 was found among only 14% of the isolates in Los Angeles. In contrast, the isolation frequency of serotype 4 was very high in New York City (49%) and San Francisco (42%), while the frequency of serotype 8 was low in New York City (8%) and San Francisco (16%). The numbers of serotype 4 and 8 isolates from Los Angeles were found to be significantly different from those from New York City and San Francisco when Fisher two-tailed exact *P* values were calculated.

DISCUSSION

M. avium complex serotypes 4 and 8 are the isolates encountered most often from AIDS patients in the United States, but their frequency of appearance can vary widely depending on geographic location.

When we examined 236 isolates from New York City AIDS patients, 49% (115 isolates) were serotype 4 and 8%

TABLE 3. Geographic distribution of *M. avium* complex serotypes from AIDS patients

Region	No. of isolates of serotype (% of total for region):									
	1	2	4	6	8	9	10	4/8 cross	Other	Nontypeable
California	11 (7)	6 (4)	37 (25)	1 (1)	43 (28)	6 (4)	10 (5)	4 (3)	15 (10)	21 (14)
Other western states[a]	8 (10)	0 (0)	22 (28)	0 (0)	16 (20)	3 (3)	5 (6)	0 (0)	9 (12)	15 (19)
New York	23 (9)	4 (2)	125 (51)	10 (4)	24 (10)	4 (2)	3 (1)	13 (5)	9 (4)	32 (13)
Other eastern states[b]	22 (9)	1 (9)	106 (42)	2 (1)	41 (17)	10 (4)	9 (4)	13 (5)	15 (6)	29 (12)

[a] States located west of Mississippi River including Alaska and Hawaii.
[b] States located east of Mississippi River including Commonwealth of Puerto Rico.

TABLE 4. Comparison of *M. avium* complex serotypes from AIDS patients in New York City, Los Angeles, and San Francisco

City	No. of isolates of serotype (% of total for city):									
	1	2	4	6	8	9	10	4/8 cross	Other	Nontypeable
New York	23 (10)	4 (2)	115 (49)	9 (4)	20 (8)	2 (1)	3 (1)	10 (4)	12 (5)	38 (16)
Los Angeles	8 (12)	3 (4)	10 (14)	0 (0)	25 (36)	3 (4)	6 (9)	2 (3)	4 (6)	8 (12)
San Francisco	3 (7)	0 (0)	19 (42)	0 (0)	7 (16)	2 (4)	4 (9)	1 (2)	2 (4)	7 (16)

(20 isolates) were serotype 8. Horsburgh et al. (8) serotyped 23 isolates from New York City AIDS patients and found that 61% (14 isolates) were serotype 4 and 9% (2 isolates) were serotype 8. They suggested that either the high incidence of serotype 4 in New York City represents a localized outbreak or this serotype is the most common pathogenic one in the New York City environment. Our data demonstrated that serotype 4 is the predominant serotype isolated from AIDS patients not only in New York City but also in all eastern states as a region. An argument can be made for localized outbreaks in California; Los Angeles had a high concentration of serotype 8 isolates (36%) and San Francisco had a high concentration of serotype 4 isolates (42%). Other western states as a region had a more even distribution of serotype 4 (28%) and serotype 8 (20%) isolates.

Blood was the major specimen source for isolation of *M. avium* serotypes from AIDS patients. High numbers of organisms are present in the blood of these patients with disseminated disease, and commercial equipment has been designed for their detection (19). The association of *M. avium* complex with the intestinal tracts of AIDS patients (4, 7, 10, 16) supports the conclusion that entry is gained either through ingestion or by some damage to the anorectal area (4).

M. intracellulare serotypes represented only 3% of our isolates from AIDS patients. Sputum was the only major source of isolation, and no isolates were recovered from stool samples. The respiratory tract may be the only major route of infection for these organisms. The low isolation rate of *M. intracellulare* from blood suggests that it is rarely associated with disseminated disease in AIDS patients.

Isolates from non-AIDS patients had a distribution of serotypes different from those from AIDS patients. We serotyped 59 isolates from non-AIDS patients over the same period as those from patients with AIDS. Our results revealed that 32% of the isolates were *M. intracellulare* serotypes and 30% were *M. avium* serotypes, and the remainder would not type with our antisera (unpublished data). The major serotypes found in non-AIDS patients were *M. avium* serotype 8 (five isolates) and *M. avium* serotype 1 (four isolates), with the rest of the isolates spread over a wide range of serotypes. Before the appearance of AIDS, our laboratories serotyped 415 *M. avium* complex strains between 1979 and 1982 (5). Serotype 8 appeared most often, followed by serotypes 1, 14, 4, 16, 9, 42, and 6. Horsburgh et al. (8) serotyped 75 isolates of *M. avium* complex collected between 1976 and 1982 from non-AIDS patients with pulmonary infection. As in our study, serotype 8 was identified most often but serotype 1 was not isolated. During a period from 1976 to 1978, McClatchy (11) serotyped 690 strains of *M. avium* complex, of which 84% were associated with pulmonary infection. By using the present criteria to distinguish species by serotype, 302 isolates were *M. intracellulare*, 304 isolates were *M. avium*, and 84 isolates were *M. scrofulaceum*. Again, the most frequent isolate was serotype 8, followed by 16, 4, 19, 42, and 1.

M. avium serotypes, particularly types 4 and 8, need to be better characterized for epidemiological studies to investigate environmental sources of infection and the possibility of direct transmission. Serotypes could be subdivided by numerous methods used individually or in combination, such as plasmid profiles, DNA restriction patterns, phage typing, antibiotic resistance patterns, and multilocus enzyme electrophoresis. Multilocus enzyme electrophoresis has been successful in identification of *M. fortuitum* strains involved in cardiac infections and in locating the same electrophoretic types in environmental sources (17). We are presently using this method to examine *M. avium* serotypes from AIDS patients and to attempt to find similar electrophoretic types in food and water.

LITERATURE CITED

1. **Baess, I.** 1982. Deoxyribonucleic acid relationships between serovars of *Mycobacterium avium*, *Mycobacterium intracellulare* and *Mycobacterium scrofulaceum*. Acta Pathol. Microbiol. Scand. Sect. B **91**:201–203.
2. **Brennan, P. J., G. O. Aspinall, and J. E. Nam Shin.** 1981. Structure of the specific oligosaccharides from the glycopeptidolipid antigens of serovars in the *Mycobacterium avium-Mycobacterium intracellulare-Mycobacterium scrofulaceum* complex. J. Biol. Chem. **256**:6817–6822.
3. **Brennan, P. J., and M. B. Goren.** 1979. Structural studies on the type-specific antigens and lipids of the *Mycobacterium avium-Mycobacterium intracellulare-Mycobacterium scrofulaceum* serocomplex. J. Biol. Chem. **254**:4205–4211.
4. **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.
5. **Good, R. C.** 1985. Opportunistic pathogens in the genus *Mycobacterium*. Annu. Rev. Microbiol. **39**:347–369.
6. **Good, R. C., and R. E. Beam.** 1984. Seroagglutination, p. 105–122. *In* G. P. Kubica and L. Wayne (ed.), The mycobacteria: a sourcebook. Microbiology series vol. 15. Marcel Dekker, Inc., New York.
7. **Hawkins, C. C., J. W. M. Gold, E. Whimbey, T. E. Kiehn, P. Brannon, R. Cammarata, A. E. Brown, and D. Armstrong.** 1986. *Mycobacterium avium* complex infections in patients with the acquired immunodeficiency syndrome. Ann. Intern. Med. **105**: 184–188.
8. **Horsburgh, C. R., Jr., D. L. Cohn, R. B. Roberts, H. Masur, R. A. Miller, A. Y. Tsang, and M. D. Iseman.** 1986. *Mycobacterium avium-M. intracellulare* isolates from patients with or without acquired immunodeficiency syndrome. Antimicrob. Agents Chemother. **30**:955–957.
9. **Kent, P. T., and G. P. Kubica.** 1985. Public health mycobacteriology. A guide for the level III laboratory. Centers for Disease Control, Atlanta.
10. **Kiehn, T. E., F. F. Edwards, P. Brannon, A. Y. Tsang, M. Maio, J. W. M. Gold, E. Whimbey, B. Wong, J. K. McClatchy, and D. Armstrong.** 1985. Infections caused by *Mycobacterium avium* complex in immunocompromised patients: diagnosis by blood culture and fecal examination, antimicrobial susceptibility tests, and morphological and seroagglutination characteristics. J. Clin. Microbiol. **21**:168–173.
11. **McClatchy, J. K.** 1981. The seroagglutination test in the study of nontuberculous mycobacteria. Rev. Infect. Dis. **3**:867–870.
12. **McNeil, M., A. Y. Tsang, and P. J. Brennan.** 1987. Structure and

antigenicity of the specific oligosaccharide hapten from the glycopeptidolipid antigen of *Mycobacterium avium* serotype 4, the dominant mycobacterium isolated from patients with acquired immunodeficiency syndrome. J. Biol. Chem. **262**:2630–2635.

13. **Meissner, G., and W. Anz.** 1977. Sources of *Mycobacterium avium* infection resulting in human diseases. Am. Rev. Respir. Dis. **116**:1057–1064.
14. **Schaefer, W. B.** 1965. Serologic identification and classification of the atypical mycobacteria by their agglutination. Am. Rev. Respir. Dis. **92**:85–93.
15. **Schaefer, W. B.** 1979. Serological identification of atypical mycobacteria, p. 323–344. *In* T. Bergan and J. R. Norris (ed.), Methods in microbiology. Academic Press, Inc. (London), Ltd., London.
16. **Stacey, A. R.** 1986. Isolation of *Mycobacterium avium-intracellulare-scrofulaceum* complex from faeces of patients with AIDS. Br. Med. J. **293**:1194.
17. **Wallace, R. J., Jr., J. M. Musser, S. I. Hull, V. A. Silcox, L. C. Steels, G. D. Forrester, A. Labidi, and R. K. Selander.** 1989. Diversity and sources of rapidly growing mycobacteria associated with infections following cardiac surgery. J. Infect. Dis. **159**:708–716.
18. **Wayne, L. G., and G. A. Diaz.** 1986. Differentiation between T-catalases derived from *Mycobacterium avium* and *Mycobacterium intracellulare* by a solid-phase immunosorbent assay. Int. J. Syst. Bacteriol. **26**:363–367.
19. **Young, L. S.** 1988. *Mycobacterium avium* complex infection. J. Infect. Dis. **157**:863–867.