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Mycobacterium avium Complex, an Emerging Pathogen in Massachusetts

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We report a study of 1,953 patients whose laboratory records from 1972 through 1983 at the Massachusetts Mycobacteria Reference Laboratory indicated the isolation of *Mycobacterium avium* complex (MAC) organisms. At least one clinical specimen from each patient during this period exhibited the organism. The incidence of isolation of MAC has increased fivefold since 1972, with a doubling of the number of patients with positive MAC specimens from normally sterile sites occurring since 1980. A concomitant increase of more than fourfold in other nontuberculous mycobacteria has occurred. Most isolates came from high-density population centers. Communities whose drinking water comes from a distant rather than a local source were more likely to have patients with MAC.

The decreasing prevalence of tuberculosis in the United States has led to an increasing awareness of nontuberculous mycobacterial disease (1). Wolinsky reviewed the subject of nontuberculous mycobacterial disease in 1979 and emphasized that two species or complexes, *Mycobacterium kansasii* and *Mycobacterium avium* complex (MAC), were the predominant cause of this disease in the United States (26). The rate of occurrence of MAC, which has been identified as the most frequently isolated mycobacterium other than *M. tuberculosis*, has increased markedly in Massachusetts in recent years (11, 12, 18, 23).

We report a study of 1,953 patients whose laboratory records from 1972 through 1983 at the Massachusetts Mycobacteria Reference Laboratory indicated the isolation of MAC organisms.

MATERIALS AND METHODS

All laboratory reports on record at the Massachusetts Mycobacteria Reference Laboratory from 1972 to 1983 were examined for the isolation of *M. tuberculosis* and mycobacteria other than *M. tuberculosis*, especially MAC. Data collected from cases in which both *M. tuberculosis* and MAC were isolated from the same specimen were not used, because it has been our policy to consider *M. tuberculosis* as the true and overriding pathogen found in mixed cultures and such cases are designated tuberculosis rather than mycobacteriosis. A total of 1,953 patients exhibited MAC as the only acid-fast bacillus from at least one clinical specimen during the 12-year period. During this period, there were no procedural changes in the isolation or identification of mycobacteria by Centers for Disease Control-recommended methods that would explain increases in frequency of isolation (25). MAC is recognized by the inability to produce urease or nitrate reductase or to hydrolyze polysorbate. In addition, the vast number of MAC isolates encountered convert potassium tellurite in 3 days to its metallic form.

We identified 55% of the patients from specimens initially sent to the Massachusetts Mycobacteria Reference Laboratory. A total of 45% of the patients were identified at hospital laboratories; however, the identity of each isolate was confirmed by subcultures of the original isolate sent to Massachusetts Mycobacteria Reference Laboratory. Data collected from patient records included sex and age, month and year of isolation, residence at time of isolation, source of the isolate identified by the Massachusetts Mycobacteria Reference Laboratory, and treatment with antimycobacterial agents, including number and type of drugs used. Clinical sources were divided into three categories: category 1, single isolates from sputum, bronchial washings, urine, and gastric fluid; category 2, multiple isolates from any combination of sputum and bronchial washings, urine, and gastric fluid; and category 3, isolates obtained by invasive procedures from normally sterile sites.

Because *M. avium* cannot be distinguished in vitro from *Mycobacterium intracellulare* by cultural characteristics or biochemical tests, seroagglutination gives the most useful separation (26). Serovars 1, 2, and 3 are classical *M. avium* strains, serovars 4 to 11 are intermediate between the two species, and higher-numbered serovars are *M. intracellulare*. Fifty representative MAC strains isolated in the period 1978 to 1979 were grouped by the Schaeffer seroagglutination method through the cooperation of Robert Good and Edward R. Beam, Centers for Disease Control, Atlanta, Ga.

In an epidemiologic analysis, populations with different water supplies were compared statistically by using a binomial test for the equality of rates underlying two Poisson-distributed observations.

RESULTS

Since 1972 Massachusetts has experienced a fivefold increase in isolation of MAC. In 1972 the statewide rate of isolation was 0.87 people per 100,000 population. By 1977, the number of people with positive MAC cultures had increased to over 4.1 per 100,000 population. In 1983, isolation of MAC was 4.6 per 100,000 population. The isolation rate of all other nontuberculous mycobacteria, including *M. kansasii*, *M. terrae* complex, *M. fortuitum* complex, and *M. gordonae*, increased fourfold. *M. tuberculosis* decreased from 12.79 cases per 100,000 in 1972 to 6.78

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TABLE 1. Isolation of mycobacteria in Massachusetts from 1972 to 1983

Calendar yr	No. of specimens	*M. tuberculosis*	MAC	All other mycobacteria
		% of specimens (people)	% of specimens (people)	% of specimens (people)
1983	28,967	1.07 (310)	0.91 (263)	1.58 (457)
1982	31,668	1.24 (392)	0.75 (236)	1.34 (423)
1981	30,974	1.40 (433)	0.71 (220)	1.29 (399)
1980	30,219	1.27 (384)	0.71 (216)	1.33 (402)
1979	31,168	2.04 (636)	0.70 (219)	1.22 (379)
1975	27,654	3.06 (846)	0.27 (75)	0.36 (99)
1972	25,730	4.05 (1,043)	0.19 (50)	0.38 (97)

cases per 100,000 in 1983. Table 1 summarizes key data collected by the Massachusetts State Laboratory Institute.

In this study, 1,121 patients were male and 832 patients were female. Predominance of male to female patients was 1.35 to 1. The ages of the patient at the time of first isolation ranged from 2 to 97 years. The age at which most individuals were colonized was 60 years. Of patients with cultures positive for MAC, 4% were 20 years of age or younger.

From a total of 1,953 patients there were 2,716 MAC isolates. A total of 1,606 patients (82.2%) had a single isolate, and 347 patients (17.8%) had MAC recovered from more than one specimen. The number of patients with a single culture positive for MAC increased fivefold from 1972, and the number of patients with multiple isolates increased eightfold. To fully appreciate this phenomenon from a clinically significant vantage point, we examined separately specimens recovered from normally sterile anatomic sites. Of 1,953 patients, 208 (10.7%) such cases were identified. A breakdown of sources is shown in Table 2. Since 1972 there has been a fivefold increase of such specimens positive for MAC. Since 1980, there has been a doubling of the number of patients whose specimens reveal MAC.

Information regarding antimycobacterial chemotherapy was examined from the records of 613 patients. Of these, 442 patients (72.1%) were receiving no antimycobacterial therapy regardless of culture positivity; 171 (27.9%) were receiving some form of antimycobacterial therapy. Treatment with one drug was indicated in 24% of patients, 46% received two-drug combinations, and 25% were treated with three drugs. Only 5% had a regimen consisting of four agents. The most popular agents used were isoniazid (87%) and ethambutol hydrochloride (54%), usually in combination, and then rifampin (29%) and streptomycin sulfate (8%). Patients were treated with second-line antituberculous drugs, including D-cycloserine (9.0%), *para*-aminosalicylic acid (5.6%), ethionamide (4.8%), pyrazinamide (7.3%), and kanamycin sulfate (1.6%).

TABLE 2. Isolation and distribution of MAC from sterile sites from 1972 to 1983

Source of isolates	No. of isolates
Pulmonary	
Lung (including empyema and bronchial lymph nodes) ..	70
Pleural (fluid, lymph nodes, and mediastinum)	26
Cervical lymph nodes	61
Disseminated disease	
Tissue (bone, marrow, brain and liver)	16
Fluids (cerebrospinal, peritoneal, etc.)	12
Soft tissue, joints, peripheral lymph nodes	23

Fifty isolates were serotyped. Irrespective of the anatomical site or frequency of isolation, serovar 4 was the most prevalent and accounted for almost half of the isolates in each of the three categories of specimens (single, multiple, and surgical). We found 10 unidentified serotypes for which no antiserum is yet available or which were autoagglutinating. Others (serovars 3, 7, 8, 9, 16, 17, and 28) were occasionally observed.

The residences of 1,454 patients at time of isolation during the 10-year period 1972 to 1981 were identified, and the number of patients residing in a given community was correlated with the 1980 population of that community. The 351 Massachusetts communities involved were broken down into five groups of similar population sizes: below 15,000, 15,000 to 25,000, 25,000 to 50,000, 50,000 to 100,000, and greater than 100,000. Approximately 1,000,000 people made up each of the five groups. The incidence of isolation per 100,000 population is shown in Fig. 1. The larger municipalities had a higher rate of MAC isolation. No two patients were members of the same family.

Urban communities (>25,000 population according to the 1980 U.S. census) were subdivided, and population densities were matched according to source of water supply. The average annual rate of MAC per 100,000 population was then examined. The water supply of the Metropolitan District Commission (MDC), which supplies Boston and 44 other communities, is unique in that the source is a series of watersheds located as far as 65 miles from most consumers and requires extensive transport through an aging distribution system. Boston and the other urban communities with this source had an average annual incidence of 3.93 cases of

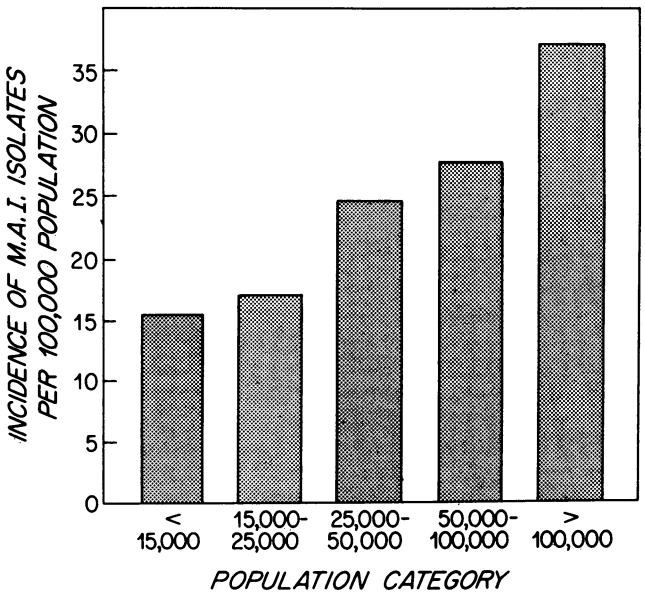

FIG. 1. Incidence of MAC isolation per 100,000 by population group of patient community. Approximately 1,000,000 people were in each of the groups.

MAC per 100,000 population (773 isolates), compared with 2.12 cases per 100,000 (324 isolates) in urban communities with other (non-MDC) water sources ($P < 0.001$) (Fig. 2).

DISCUSSION

Mycobacteria other than *M. tuberculosis*, particularly MAC, have never been clearly shown to be transmitted among humans (24). The diseases caused by these mycobacteria range from cervical lymphadenitis to chronic pulmonary mycobacteriosis (G. C. du Moulin, D. Jaciow, D. Teres, and K. D. Stottmeier, Program Abstr. 23rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1048, 1983) (7, 9, 17, 21, 24, 27). Disseminated disease caused by MAC can occur in patients with acquired immune deficiency syndrome or in patients with underlying hematologic disorders, and it has a fatality rate of up to 73% (1, 28). One hundred cases of MAC infection were studied over a 3.5-year period in Wisconsin (21). Thirty-two patients had chronic obstructive pulmonary disease associated with their MAC infections, 28 had previous *M. tuberculosis* infection, 20 were alcoholics, and 15 had silicosis. The remaining patients had diabetes, atherosclerosis, cancer, gastrectomy, or schizophrenia. No combination of chemotherapy or surgery was particularly effective in the treatment of these infections (5, 7, 21, 28). The majority of MAC strains isolated in Massachusetts are resistant in vitro to nearly all currently licensed antituberculous agents. Susceptibility to cycloserine in concentrations of ≥50 μg/ml is observed.

Because of its increasing isolation, MAC, once confined to the Southeastern United States, is now challenging *M. tuberculosis* as the preponderant acid-fast isolate in the Northeastern United States, including Massachusetts (3, 6, 8, 13, 22). It is suspected that the increasing isolation of MAC has resulted from both contamination of some specimens from water and other environmental sources and increasing frequency of disease. The isolation of a few colonies of MAC from only a single urine or sputum specimen may often result from contamination. In contrast to the rare isolation of MAC from surgical specimens in Massachusetts before 1976, the increasing isolation of MAC from sterile sites underscores the increasing importance of this mycobacterium as a pathogen. We made great efforts in this study to show that isolation of MAC from surgical materials or from specimens obtained by invasive procedures from normally sterile sites in the absence of other pathogens, notably *M. tuberculosis*, can be indicative of MAC infection. It is improbable that dozens of surgical specimens in Massachusetts were contaminated with MAC, nor does the number of colonies on primary cultures matter in this context (in contrast to respiratory specimens), because only a few colonies were isolated, often from minute punch or needle biopsies, although the pathologic processes were as severe as and comparable to much larger granulomata removed from parenchyma of large organs showing an abundance of growth. Additionally, primary growth was sometimes obtained in broth medium to enhance recovery of acid-fast bacilli from tissue, as we have demonstrated (20). The disease is being observed in increasingly susceptible populations, such as patients with acquired immune deficiency syndrome. In 1983, positive surgical specimens were recovered from two patients with acquired immune deficiency syndrome (bone marrow and lung). In 1984 data examined to date, 20 additional patients with acquired immune deficiency syndrome were identified with MAC-positive surgical specimens. Isolations from these patients were made from bone marrow, blood, liver, and lymph node.

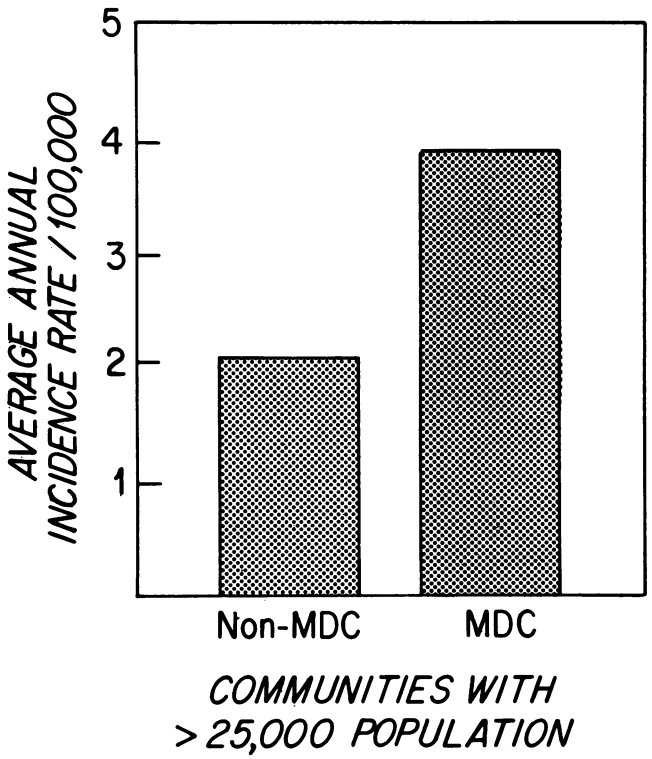

FIG. 2. Comparison of average annual rate MAC isolation per 100,000 people in urban settings (population, >25,000) with MDC sources (n = 29 communities) and non-MDC sources (n = 32 communities).

A link between drinking water and acquisition of organisms was suggested in a hospital-acquired outbreak of MAC contamination of clinical specimens, with subsequent isolation of MAC from hospital water supplies (G. C. du Moulin, G. Friedland, K. Indorato, and K. D. Stottmeier, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, Q70, p. 206). Disseminated mycobacteriosis in rhesus monkeys in a Massachusetts primate center, with subsequent isolation of MAC from water supplies in the colony, has also been reported, although a temporal relationship could not be established (9). We isolated MAC, as well as *M. gordonae*, *Mycobacterium flavescens*, *M. fortuitum* complex, and *Mycobacterium chelonei*, from the water distribution system supplying Boston. These isolates were recovered from urban areas. *Mycobacterium xenopi* was recently isolated from water taps in a Veterans Administration hospital in Connecticut and implicated in 19 cases of pulmonary disease in hospitalized patients (4). These observations correlate with an increase in clinical isolation of this mycobacterium. In addition to *M. xenopi*, an extensive study from the Regional Centre for Tuberculosis Bacteriology, London, England, incriminated the water system as a common source of *M. kansasii* (19). People living in communities whose water is supplied by the MDC are almost twice as likely, on the average, to have cultures positive for MAC. Infection due to waterborne nontuberculous mycobacteria has been postulated by others (2, 10, 14–16). Although we do not know why there is an increasing trend toward MAC isolation in more densely populated communities, we believe a number of factors probably contribute to waterborne dissemination. These include resistance of mycobacteria to chlorination (16), optimal growth temperatures, nutrient availability, and

stagnant or interrupted water flow, all of which might be linked to protracted time in man-made distribution systems. We are now investigating these factors.

The literature contains little evidence of airborne infection, but this source cannot be ruled out in light of the high incidence of pulmonary involvement. It is unrealistic to suppose that aspiration of acid-fast bacilli is responsible for all cases. Acquisition of the bacteria from aerosolized water may be a common occurrence, particularly during summer months.

The data collected and examined in this study offer evidence of increasing exposure to MAC in the Commonwealth of Massachusetts. Water may provide the vehicle responsible for transmission, with propagation of mycobacteria in the water distribution system encouraged by as yet uninvestigated ecological factors.

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