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High-Catalase Strains of *Mycobacterium kansasii* Isolated from Water in Texas

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Isolation techniques with membrane-filtered potable water samples resulted in the isolation of potentially pathogenic high-catalase strains of *Mycobacterium kansasii* from 8 of 19 representative outlets in a small central Texas town. *Mycobacterium gordonae* was isolated from all samples, and *Mycobacterium fortuitum* was isolated from two samples. Data on chlorine levels are presented along with a possible explanation for the unusually high numbers of mycobacteria in these potable water samples. Findings suggest that water is a source of *M. kansasii* and may be an important link in the epidemiological picture of the disease.

Many *Mycobacterium* species have been isolated from environmental sources. Organisms such as *Mycobacterium fortuitum*, *Mycobacterium chelonei*, and *Mycobacterium avium* complex, which may cause disease in humans and animals, have been isolated repeatedly (3, 5, 7; H. Gruft, D. Blanchard, and J. Wheeler, Am. Rev. Respir. Dis. **113**:60, 1976), but *Mycobacterium kansasii* has been encountered only rarely. Froman isolated this organism from nature (cited in references 2 and 4), but his isolates were of the low-catalase variety, which has been shown to be nonpathogenic (12). In 1970, Bailey et al. reported the isolation of potentially pathogenic high-catalase strains of *M. kansasii* from tap water (2), but since that report additional isolates have not been reported in the United States; however, reports have been made in England (8) and Czechoslovakia (B. Medek, M. Kubin, V. Hudec, S. Chobot, Z. Olsovsky, M. Pelikan, S. Richtrova, E. Svandova, and J. Malis, J. Czech Physicians **118**:307-314, 1979).

In the United States the disease caused by *M. kansasii* has been observed most frequently in California, the midwestern United States, and Texas. Most of the cases in Texas involve persons living in a 150- to 200-mile-wide area from the Red River through the Dallas-Fort Worth metroplex southward to Austin (1). Due to an increased frequency of infections and peculiar skin test reactions, especially in this particular area of Texas (C. E. Alexander, unpublished data), and to the fact that the disease has not been shown to be communicable, investigations were undertaken by the Bureau of Laboratories and the Bureau of Tuberculosis Service of the Texas Department of Health to determine whether the presence mycobacteria in public water supplies was a factor in the situation described above.

MATERIALS AND METHODS

In the summer of 1978, water samples were collected in ethylene oxide-sterilized, 1-gallon polyethylene bottles from 19 representative outlets in a small central Texas town. This particular town was selected because of an unusually high number of peculiar skin test reactions. Samples were taken during a period from late morning to early afternoon; faucets were not flamed, but were flushed for 15 to 20 s before collection. All samples were immediately returned to the Texas Department of Health, Bureau of Laboratories, and were processed within 24 h of collection. Total chlorine levels were determined on each sample by the DPD colorimetric method (9) (see Table 1). Each sample was passed through a membrane filter (type HA; 0.45 μm; 47 mm; Millipore Corp., Bedford, Mass.). Filters were changed on each sample as necessary to allow the entire sample to be processed. The filters were then removed and placed in separate sterile bottles which contained approximately 5 ml of sterile physiological saline. If multiple filters were necessary on samples, all were placed in the same bottle. Each bottle was vigorously shaken on a Vortex mixer for 30 to 45 s, removing the organisms trapped on the filters.

The water from the washed filters was transferred to sterile screw-cap tubes (18 by 125 mm), and approximately 5 ml of sterile 4% sodium hydroxide (11) was added to each sample. The tubes were agitated for 30 s on the Vortex mixer and allowed to stand for 10 min to aid in decontamination. Each sample was diluted with approximately 10 ml of sterile distilled water, centrifuged for 15 min at 2,000 × *g*, and decanted. Smears were not made from the sediments. Approximately 1 ml of sterile 0.2% bovine albumin, fraction V, was added to each sediment, which was suspended on a Vortex mixer, and equal portions of each sediment were inoculated to one Middlebrook 7-H-10 medium plate (11) and three Lowenstein-Jensen

medium slants (11). The inoculated media were incubated at 35°C in approximately 10% carbon dioxide and were read weekly for growth.

RESULTS

All of the Lowenstein-Jensen medium slants on seven of the samples and at least one Lowenstein-Jensen medium slant on four additional samples were liquefied by proteolytic bacteria, rendering them totally unsatisfactory. Only one sample was completely overgrown on both types of media with a fungus which belonged to the *Aspergillus terreus* group. It was necessary to select colonies predominantly from the Middlebrook 7-H-10 medium plates for two reasons. First, as mentioned above, seven samples were unsatisfactory on Lowenstein-Jensen medium slants. Second, the growth on the Lowenstein-Jensen medium slants frequently was too confluent to allow selection of a single colony for subculture.

A variety of colony types from each sample were subcultured, and each type was subjected to the identification protocol for mycobacteria recommended by the Center for Disease Control, Atlanta, Ga. (11). Table 1 contains the results of the isolation and identification studies. All samples, except the one which was overgrown with fungus, contained *Mycobacterium gordonae*, two samples contained *M. fortuitum*, and eight samples contained the high-catalase strain of *M. kansasii*. Representative cultures of the *M. kansasii* isolates were submitted to the Center for Disease Control for confirmation. All were identified as *M. kansasii*, high-catalase strains.

TABLE 1. *Laboratory results from water samples*

Water sample no.	Total chlorine level (mg/liter)	Isolation results[a]			
		M. gordonae	*M. kansasii*[b]	*M. fortuitum*	Contaminated
1	0.1	+			
2	0.1	+			
3	1.3	+			
4	0.2	+	+		
5	0.0	+	+		
6	0.0				+ (fungus)
7	0.1	+			
8	0.1	+	+		
9	0.1	+			
10	0.1	+	+		
11	0.1	+	+		
12	0.1	+			
13	0.0	+	+		
14	0.2	+			
15	0.2	+			
16	0.1	+	+		
17	0.1	+	+		
18	0.0	+		+	
19	0.0	+		+	

[a] None was negative.
[b] High-catalase variety.

The 1975 United States Environmental Protection Agency Drinking Water Standards provide the criteria for the quality of public water supplies, including the guidelines for coliform organisms. The addition of chlorine to water is required only when necessary to reduce or eliminate coliform organisms which may be present. Also, the minimum level of chlorine is not specified, unless routine bacteriological studies are not performed, under which circumstances a minimum level of 0.2 mg of free chlorine per liter is required throughout the public water distribution system. Analyses for residual chlorine are to be made in accordance with the methods specified in *Standard Methods for the Examination of Water and Wastewater* (9). Table 1 indicates that 1 sample had a total chlorine level of 1.3 mg/liter, 3 samples contained 0.2 mg/liter, and 10 samples had readings of 0.1 mg/liter, which is the lowest measurable level by the DPD colorimetric method. Five samples had no measurable chlorine present. Coliform studies were not performed on these samples. It was noted that the number of colonies of mycobacteria decreased as the levels of chlorine increased, but there was no good correlation between the isolation of *M. kansasii* and chlorine levels. It is possible that other samples contained *M. kansasii* but were overgrown with the contaminating bacteria and fungi or the extremely high number of colonies of *M. gordonae*.

DISCUSSION

Investigations have been initiated to determine why this water supply contained unusually high numbers of mycobacteria, especially potentially pathogenic strains of *M. kansasii*. Studies of the water system of the town revealed that the water is obtained from deep ground wells, treated with chlorine, and stored in large enclosed steel and concrete reservoirs. Both above and below ground reservoirs are used. The Texas Department of Health, Water Hygiene Division, recommends that water storage reservoirs, "when possible, shall be constructed partially or wholly above ground" (10). All underground reservoirs must, of necessity, be constructed of concrete, which occasionally may allow seepage of water and soil into the reservoir. Even with no damage to the reservoir, soil and residue from the water from the wells will accumulate in the bottom and on the sides of both steel and concrete reservoirs and must be removed periodically (Floyd Williams, personal communication). Joynson demonstrated experimentally that *M.*

kansasii survived in a similar environment for up to 12 months (6). The mycobacteria could have entered the reservoir from either the deep water wells or the seepage and then passed into the water distribution system. Figure 1 shows a stylized schematic drawing of the collection sites throughout the water system. It is impossible to present the necessary details in this drawing, but careful examination of the city's water system revealed that all isolation sites for *M. kansasii*, with the exception of one, were dead-end 2-inch mains. Unless dead-end mains are flushed monthly (10), a buildup of debris may occur which will result in reduced chlorine levels and a possible multiplication of microorganisms which may include mycobacteria. As site no. 9 was a dead-end main, one may question why it was not positive. All of the Lowenstein-Jensen medium slants for this sample were overgrown with proteolytic bacteria, thus limiting the study of this site. As was suggested earlier, *M. kansasii* may not have been isolated from some samples due to contamination. The exception to the dead-end main theory was the positive isolation at site no. 4, which was the elevated reservoir. An explanation for the isolation of *M. kansasii* could be that the line was infrequently flushed, and a condition similar to that in the dead-end mains developed. The data gathered by McSwiggan and Collins appear to support this hypothesis of possible colonization of mycobacteria in the faucet area (8). Our findings, which corroborate the results of other investigators, demonstrate the probability that water is a source of *M. kansasii*, aiding in epidemiological studies of the disease.

Further investigations are being done to improve the isolation procedure, develop a technique for satisfactory smear preparation, and attempt to correlate data on chlorine levels and isolation of mycobacteria and coliform organisms from potable water supplies.

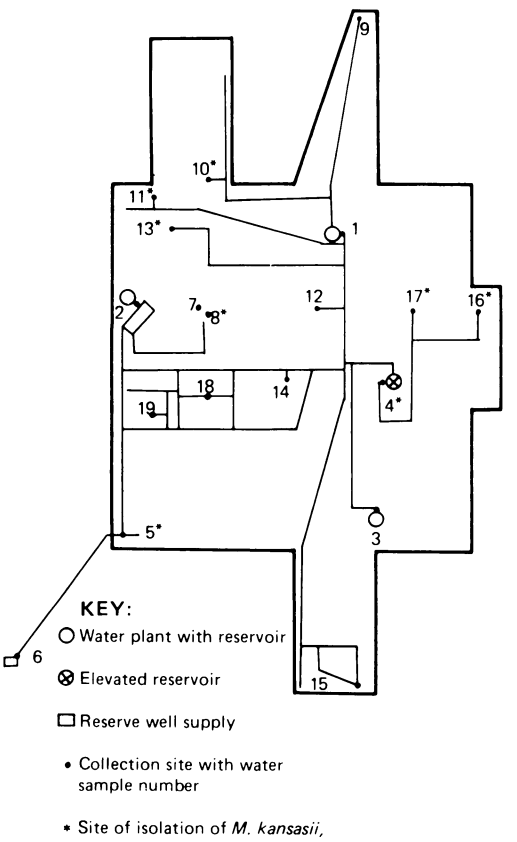

FIG. 1. *Stylized schematic drawing of the collection sites throughout the water distribution system of a small central Texas town.*

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