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Mycobacterium avium Complex Pseudobacteriuria from a Hospital Water Supply

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From July 1983 through November 1985, organisms belonging to *Mycobacterium avium* complex were isolated from the urine of 29 patients. Strains recovered from the urine of nine patients from July 1983 through August 1984 were serotyped. Eight of the nine samples belonged to serovar 4. *M. avium* complex was isolated from the urine of 21 patients during the period from November 1984 through November 1985. While the possibility of a point source contamination was investigated, *M. avium* complex was recovered from the phenol red solution used for processing urine specimens in the mycobacteriology laboratory and the deionized tap water of that laboratory that is used to make the reagent. *M. avium* complex serovar 4 was subsequently recovered from the tap water of the laboratory and four hospital wards. During the year following the installation of a microbiological filter for the mycobacteriology laboratory deionized tap water, 2 urine isolates were recovered, compared to 26 the previous year. This study demonstrates the importance of filtration devices at tap water sites that are used to make laboratory reagents and the value of serotyping as a marker for the detection of a specific source of *M. avium* complex contamination.

The *Mycobacterium avium* complex contains 28 serovars which cannot be reliably distinguished from one another by common laboratory biochemical tests (10; R. C. Good, Clin. Microbiol. Newsl. 1:1–4, 1979). These organisms have been found in soil and in water associated with piggeries and have been recovered from avian, bovine, porcine, and amphibian sources (4, 5, 7, 8). Extensive studies have been conducted which establish their presence in marine and fresh waters in the eastern United States (2, 3). Although often regarded as environmental contaminants, these organisms are increasingly being associated with human illness. *M. avium* complex causes a tuberculosislike disease (primarily in adults) and cervical adenitis (primarily in children). Bacteremia is common in individuals with acquired immunodeficiency syndrome, resulting in the recovery of these organisms from various foci. No evidence exists documenting animal-to-human or human-to-human transmission. This leaves the environment as the probable source of acquisition.

The increased recovery of *M. avium* complex from urine specimens by a hospital laboratory was the reason for initiating this study. A systematic study of the environmental water sources within the hospital, using serovar identification, focused attention on the most probable source of contamination which led to its subsequent elimination.

Clinical specimens were processed by using previously described methods (6). Briefly, voided urine specimens were treated with 4% sulfuric acid and neutralized with 4% sodium hydroxide, using phenol red as an indicator. All resulting sediments were used to prepare smears for acid-fact microscopy and to inoculate two tubes each of Middlebrook and Cohn 7H-10 agar (BBL Microbiology Systems, Cockeysville, Md.) and Lowenstein-Jensen media (BBL). The inoculated media were incubated in a horizontal position at 35°C in an atmosphere of 10% CO_2. The cultures were examined for growth at weekly intervals for 8 weeks.

Water samples (1 liter) from each of four hospital wards and two laboratory areas were collected in sterile bottles on three consecutive days. Selection of water-sampling areas corresponded to the sections of the hospital where the majority of the patients from whom *M. avium* complex urine isolates were recovered were housed. Within 2 h of collection, the water samples were shaken vigorously and filtered through a sterile 0.45-μm (pore size) membrane filter (Millipore Corp., Bedford, Mass.). The filters were placed on plates of Middlebrook and Cohn 7H-11 agar (Difco Laboratories, Detroit, Mich.) containing a final concentration of 50 U of polymyxin B per ml, 5 μg of amphotericin B per ml, 25 μg of carbenicillin per ml, and 2.5 μg of trimethoprim per ml (PACT solution; Johnston Laboratories, Inc., Towson, Md.). The plates were sealed in polyethylene bags, incubated at 35°C in an atmosphere of 10% CO_2, and examined in the same manner as the clinical specimens.

Samples of the phenol red reagent used as an indicator during the urine decontamination procedure were inoculated directly onto Middlebrook and Cohn 7H-11 agar plates and incubated as previously described.

Mycobacterial isolates were identified by standard methods (6). Procedures included determinations of growth rate, photochromogenicity, niacin accumulation, reduction of nitrate and potassium tellurite, hydrolysis of Tween 80, catalase activities, and the presence of arylsulfatase and urease. Seroagglutination studies were performed at the National Jewish Center for Immunology and Respiratory Medicine by the method of Schaefer (9). When seroagglutination results were inconclusive, thin-layer chromatography was done by the method of Brennan et al. (1).

M. avium complex was isolated from the urine of nine patients between July 1983 and August 1984 and serotyped

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(Table 1). Seroagglutination tests revealed that eight of the isolates were of serovar 4. One strain was untypeable. All but two of the patients were located on different hospital wards at the time the urine specimen was submitted to the laboratory. The *M. avium* complex colonies grown from each specimen were few. According to the information available in the patient records, no clinical diagnosis of *M. avium* complex infection was made in any of these patients.

From November 1984 to November 1985, *M. avium* complex was isolated in small numbers from the urine of 21 patients. The serovars of these isolates were not determined. The possibility of a point source contamination led to an investigation of laboratory procedures. The phenol red solution used in the processing of urine specimens in the mycobacteriology laboratory was found to be contaminated with *M. avium* complex serovar 4. The deionized tap water in that laboratory which is used to make the phenol red solution was also found to harbor *M. avium* complex serovar 4 and was thus a suspected source. A 0.2-μm capsule microbiological filter (Gelman Sciences Inc., Ann Arbor, Mich.) was installed in December 1985 and subsequently replaced at monthly intervals. The number of *M. avium* complex urine isolates decreased from the 26 of the previous year to 2 from December 1985 to December 1986.

The results derived from the water samples taken from two laboratory areas and four hospital wards, which, in the past, were the sites which most frequently housed patients from whom *M. avium* complex was isolated, are found in Table 2. *M. avium* complex was isolated from each of the six sites. Serotyping was performed on all isolates. Thin-layer chromatography was done to clear existing discrepancies. Serovar 4 was the most common strain, being isolated from the laboratory and each of the wards sampled. A strain not typeable by seroagglutination which gave a thin-layer chromatography pattern which could have been that of either serovar 4 or serovar 6 was isolated from ward 10. A strain cross-reacting with serovars 1 and *Mycobacterium xenopi* was isolated from ward 4. Serovars 11 and 42 were isolated from the laboratory medium kitchen and ward 10, respectively.

Because of its high prevalence in the environment, it can be assumed that random isolation of *M. avium* complex serovar 4 will continue. An increased incidence most likely indicates point source contamination, and corrective action should be taken. Filtration, rather than autoclaving, is probably the most appropriate action to take because of the possibility of false-positive acid-fast stains occurring. It must be stressed that multiple positive cultures from separately collected urine specimens are needed for the laboratory confirmation of the diagnosis of bacteriuria.

In this study, we have demonstrated the following: (i) the occurrence of 29 cases of pseudobacteriuria with *M. avium* complex, with 8 of 9 serotyped strains being serovar 4; (ii) that environmental acid-fast bacillus isolates commonly occur in the hospital setting in both the ward and the laboratory; (iii) the value of serotyping as a marker for the detection of a specific source of *M. avium* complex contamination; and (iv) that the incidence of *M. avium* complex isolates should be monitored in every laboratory as a quality assurance factor. We recommend that filtration devices be installedat all tap water sites used to make laboratory reagents to prevent contamination and the inevitable misleading results.

TABLE 1. Clinical information and serovars for patients from whom *M. avium* complex was isolated

Patient no.	*M. avium* complex serovar	Hospital ward	Underlying clinical diagnosis
1	4	1	Chronic airway obstruction, congestive heart failure, chronic hepatitis
2	4	2	Urinary tract infection (*Klebsiella* spp.), chronic enteritis, bladder abscess
3	4	3	Diabetes
4	4	3	Dysuria
5	4	4	Hematuria, upper gastrointestinal bleedings, ongoing heart disease
6	4	5	Granuloma in lung
7	4	6	Active tuberculosis, alcohol abuse, anorexia, urinary tract infection (*Escherichia coli*)
8	4	7	Unknown
9	Untypeable	8	Unknown

TABLE 2. Serovars of *M. avium* complex isolated from hospital water sources

Hospital site	*M. avium* complex serovar(s)	TLC[a] serovar results
Medium kitchen	11, untypeable	ND[b]
Tuberculosis laboratory	4, *M. xenopi*, untypeable	4
		4
Ward 9	Probably *M. simiae*, similar to 4	ND
		4
Ward 10	42, 4, *M. xenopi*, similar to 4, untypeable, 1, 4	ND
		4
		4
		4 or 6
		1
		ND
Ward 6	4, untypeable	ND
		ND
Ward 4	4, untypeable 1, *M. xenopi*	ND
		1
		ND

[a] TLC, Thin-layer chromatography.
[b] ND, Not done.

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