Journal of Clinical Microbiology, Oct. 1995, p. 2757–2758 Vol. 33, No. 10
0095-1137/95/$04.00+0

NOTES

Recovery of *Mycobacterium avium* from Cigarettes

TWILLA EATON,[1] JOSEPH O. FALKINHAM III,[1]* AND C. FORDHAM VON REYN[2]

Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0406,[1] and Infectious Disease Section, Dartmouth-Hitchcock Medical Center, Lebanon, New Hampshire 03756

Received 21 February 1995/Returned for modification 31 March 1995/Accepted 14 July 1995

***Mycobacterium avium* was recovered from tobacco, cigarette paper, and cigarette filters. *M. avium* could also be recovered from cigarette filters after the cigarettes had been smoked.**

Disseminated *Mycobacterium avium* infection is prevalent in patients with AIDS, affecting as many as 40% of patients with advanced disease (4). Pulmonary *M. avium* infection occurs in patients with chronic lung disease (9). Recently it was shown that DNA fingerprint patterns of *M. avium* isolates recovered from AIDS patients were identical to patterns from isolates recovered from water samples to which the patients were exposed (6). Although water and soil are thought to be likely sources of *M. avium* in AIDS patients (6) and patients with chronic lung disease (9), other possible sources of *M. avium* have not been excluded.

In a study of 19 patients with pulmonary alveolar proteinosis, *M. avium* was recovered from bronchoalveolar lavage samples in 8 (57%) of 14 smokers and 0 (0%) of 5 nonsmokers (8). Accordingly, we sought to determine whether *M. avium* could be isolated from cigarettes.

Four brands of cigarettes were sampled: Benson and Hedges 100 Lights (Benson and Hedges, Richmond, Va.), Marlboro Light 100s (Philip Morris, Inc., Richmond, Va.), Winston (R.J. Reynolds Tobacco Co., Winston-Salem, N.C.), and Camel Lights (R.J. Reynolds Tobacco Co.). Packs of cigarettes were purchased at three different times over a 3-month period, twice in Blacksburg, Va., and once in New Market, Va., to ensure that different batches of cigarettes were sampled. Two unsmoked cigarettes from each pack were analyzed. In addition, two cigarettes from each pack were smoked and the filters and paper surrounding the filter were analyzed. Measures were taken to ensure that the only source of mycobacteria was the cigarettes themselves. Packages were opened with sterile gloves in a sterilized laminar flow hood. Cigarettes were smoked by employing a sterilized vacuum apparatus. With a sterilized razor blade, the cigarettes were dissected in a sterile field and separated into the following fractions: (1) tobacco, (2) paper enclosing tobacco, (3) filter, and (4) paper surrounding filter. The filter and both paper fractions were each weighed following transfer into preweighed sterilized containers and suspended in 5 ml of sterile 0.0068 M phosphate buffer containing 0.85% (wt/vol) NaCl, 0.01% (wt/vol) gelatin, and 0.05% Tween 80. The tobacco was transferred to a preweighed sterile container, weighed, and suspended in 10 ml of the same buffer. By using a sterile glass rod in the sterile field, the fractions were pulverized and then shaken at 120 oscillations per min for 2 h at room temperature. The material was separated from the liquid extract by filtration through sterilized sintered glass filter holders (Millipore Corporation, Bedford, Mass.) and collected in plastic centrifuge tubes, and the volume was measured. The filtered extract was centrifuged at $5{,}000 \times g$ for 20 min at 4°C, the supernatant liquid was discarded, and the pellet was suspended in 1 ml of the sterile extraction buffer. A 0.1-ml sample of each extract was spread to dryness on each of five TTC agar medium plates (3). To demonstrate that there was no contamination of the samples and to confirm the sterility of all solutions and tubes, cigarette-free negative controls were analyzed. To measure the possible inhibition of *M. avium* colony formation by cigarette extracts, 0.1 ml of each extract was spread with 0.1 ml of a suspension of *M. avium* LR25 diluted to yield 280 CFU/0.1 ml. All plates were wrapped in Parafilm (American Can Co., Greenwich, Conn.), incubated at 37°C, and examined for colony formation for up to 30 days.

Acid-fast colonies were identified by Ziehl-Neelson staining, acid-fast colonies with the same morphology were counted, and representative single colonies were streaked on Middlebrook 7H10 agar medium slants (BBL Microbiology Systems, Cockeysville, Md.) and incubated at 37°C for 2 weeks. Isolates which took more than 7 days to form colonies were identified by DNA probe according to the manufacturer's instructions (Gen-Probe, Inc., San Diego, Calif.).

M. avium was recovered from the tobacco, tobacco paper, filter, and paper surrounding the filter of three of the four cigarette brands sampled (Table 1). All of the slowly growing acid-fast isolates were identified as *M. avium* by DNA probe, first with the *M. avium* complex combination probe and then with the *M. avium*-specific probe (Gen-Probe). *M. avium* concentrations (i.e., CFU per gram of sample) ranged from 13 to 19,000 (Table 1). *M. avium* was not recovered consistently from all cigarettes, though within a single pack there was uniformity. For example, *M. avium* was recovered from the tobacco and both paper samples from one brand A cigarette and from the tobacco in a second cigarette from the same pack. *M. avium* was also recovered from the tobacco of a cigarette from a second pack of the same brand, purchased in Blacksburg at a different time from the first. A second example of the consistency of *M. avium* recovery within a pack was demonstrated by high numbers in the tobacco of smoked and unsmoked cigarettes from the same pack of brand C. Some of the isolates were recovered from TTC agar medium spread with *M. avium* LR25. Those isolates were distinguished from strain LR25 on the basis of differences in colony pigmentation and morphol-

* Corresponding author. Mailing address: Department of Biology, Virginia Tech, Blacksburg, VA 24061-0406. Phone: (540) 231-5931. Fax: (540) 231-9307. Electronic mail address: JOFIII@VT.edu.

TABLE 1. Recovery of *M. avium* from cigarettes

Fraction	Brand A		Brand B		Brand C		Brand D		Total	
	Recovery rate[a]	CFU/g	Recovery rate	CFU/g	Recovery rate	CFU/g	Recovery rate	CFU/g	Recovery rate	% Positive
Unsmoked										
Tobacco	3/6	470	0/6	<2	1/6	1,100	0/2	<2	4/20	20
Tobacco paper	2/6	37	1/6	27	0/6	<2	0/2	<2	3/20	15
Filter	1/6	13	0/6	<2	1/6	9,400	0/6	<2	2/20	10
Filter paper	2/6	55	1/6	36	1/6	100	0/6	<2	4/20	20
Smoked										
Filter	0/2	<2	0/2	<2	1/2	19,000			1/6	17
Filter paper	0/2	<2	0/2	<2	0/2	<2			0/6	0

[a] Number of cigarettes yielding *M. avium*/total number tested.

ogy and confirmed as distinct isolates on the basis of biochemical and cultural characteristics and plasmid DNA profiles (data not shown). In addition to *M. avium*, rapidly growing mycobacteria and non-acid-fast bacteria were recovered from cigarettes (data not shown). Those isolates were not identified.

Exact values for survival of strain LR25 after exposure to the extracts were impossible to obtain, because the extracts were not sterilized and other acid-fast colonies with the same morphology appeared on the medium. However, exposure of *M. avium* LR25 cells to some extracts on the TTC agar medium resulted in loss of CFU. For the tobacco extract of brand A, only 5 strain LR25 colonies appeared on plates that should have had 280. For brand C, there were more than 280 colonies on the TTC agar medium spread with tobacco, filter, or paper extracts. Thus, it is likely that the numbers of *M. avium* in the different extracts (especially tobacco) were underestimated.

Isolation of *M. avium* from the cellulose acetate-based filters is not surprising in light of the observation of Ridgway et al. (5) that mycobacteria adhere to cellulose acetate reverse osmosis membranes used for municipal water treatment. If cigarette filter material is washed during preparation, *M. avium* could adhere to the filters because municipal (1, 7) and natural (2, 7) waters contain *M. avium*. The presence of *M. avium* in the tobacco is not surprising, since these organisms are found throughout the environment (1, 2, 7, 9). Further, these hydrophobic bacteria are likely to adhere to tobacco leaves in the field or during processing.

Recovery of *M. avium* from the smoked filters demonstrates that these organisms can survive exposure to gases and high temperatures generated by smoking. We did not collect cigarette smoke to determine whether *M. avium* passes through the filter. The present study demonstrates that cigarettes, cigarette paper, and cigarette filters are frequently contaminated with *M. avium* and that these organisms often survive the smoking process.

This work was supported by grant AI-30373 from the National Institute of Allergy and Infectious Disease.

REFERENCES

1. **duMoulin, G. C., and K. D. Stottmeier.** 1986. Waterborne mycobacteria: an increasing threat to health. ASM News **52:**525–529.
2. **Falkinham, J. O., III, B. C. Parker, and H. Gruft.** 1980. Epidemiology of infection by nontuberculous mycobacteria. I. Geographic distribution in the eastern United States. Am. Rev. Respir. Dis. **121:**931–937.
3. **George, K. L., and J. O. Falkinham III.** 1986. Selective medium for the isolation and enumeration of *Mycobacterium avium-intracellulare* and *M. scrofulaceum*. Can. J. Microbiol. **32:**10–14.
4. **Nightingale, S. D., L. T. Byrd, P. M. Southern, J. D. Jockusch, S. X. Cal, and B. A. Wynne.** 1992. *Mycobacterium avium-intracellulare* complex bacteremia in human immunodeficiency virus positive patients. J. Infect. Dis. **165:**1082–1085.
5. **Ridgway, H. F., M. G. Rigby, and D. G. Argo.** 1984. Adhesion of a *Mycobacterium* sp. to cellulose diacetate membranes used in reverse osmosis. Appl. Environ. Microbiol. **47:**61–67.
6. **von Reyn, C. F., J. N. Maslow, T. W. Barber, J. O. Falkinham III, and R. D. Arbeit.** 1994. Persistent colonisation of potable water as a source of *Mycobacterium avium* infection in AIDS. Lancet **343:**1137–1141.
7. **von Reyn, C. F., R. D. Waddell, T. Eaton, R. D. Arbeit, J. N. Maslow, T. W. Barber, R. J. Brindle, C. F. Gilks, J. Lumio, J. Lähdevirta, A. Ranki, D. Dawson, and J. O. Falkinham III.** 1993. Isolation of *Mycobacterium avium* complex from water in the United States, Finland, Zaire, and Kenya. J. Clin. Microbiol. **31:**3227–3230.
8. **Witty, L. A., V. F. Tapson, and C. A. Piantadosi.** 1994. Isolation of mycobacteria in patients with pulmonary alveolar proteinosis. Medicine **73:**103–109.
9. **Wolinsky, E.** 1979. Nontuberculous mycobacteria and associated diseases. Am. Rev. Respir. Dis. **119:**107–159.