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Recovery of Mycobacterium avium from Cigarettes

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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 from 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 × 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-Neelsen 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-
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.

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REFERENCES

### Table 1. Recovery of M. avium from cigarettes

<table>
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<tr>
<th>Fraction</th>
<th>Brand A</th>
<th>Brand B</th>
<th>Brand C</th>
<th>Brand D</th>
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<tbody>
<tr>
<td></td>
<td>Recovery rate</td>
<td>Recovery rate</td>
<td>Recovery rate</td>
<td>Recovery rate</td>
<td>Recovery rate</td>
</tr>
<tr>
<td>Tobacco</td>
<td>3/6 470</td>
<td>0/6 &lt;2</td>
<td>1/6 1,100</td>
<td>0/2 &lt;2</td>
<td>4/20 20</td>
</tr>
<tr>
<td>Tobacco paper</td>
<td>2/6 37</td>
<td>1/6 27</td>
<td>0/6 &lt;2</td>
<td>0/2 &lt;2</td>
<td>3/20 15</td>
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<tr>
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<td>1/6 13</td>
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<td>0/6 &lt;2</td>
<td>2/20 10</td>
</tr>
<tr>
<td>Filter paper</td>
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<td>1/6 36</td>
<td>1/6 100</td>
<td>0/6 &lt;2</td>
<td>4/20 20</td>
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<table>
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<th>Recovery rate</th>
<th>Recovery rate</th>
<th>Recovery rate</th>
<th>Recovery rate</th>
<th>Recovery rate</th>
<th>% Positive</th>
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<td>0/2 &lt;2</td>
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<td>0/2 &lt;2</td>
<td>1/6 17</td>
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<td>0/2 &lt;2</td>
<td>0/2 &lt;2</td>
<td>0/6 0</td>
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*Number of cigarettes yielding M. avium/total number tested.*