Preliminary Identification of Mycobacteria Isolated from Clinical Specimens

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From March 1979 to June 1980, we preliminarily identified mycobacteria at the time of their initial isolation by colonial morphology, pigmentation, and growth rate. The definitive identifications were predicted accurately for 92% of the mycobacteria isolated from 108 patients. For laboratories that isolate relatively few different species of mycobacteria, the advantages of this approach are its simplicity and the speed with which presumptive identifications can be made available for clinical use.

Although certain Mycobacterium species are typically recognized as pathogens (e.g., M. tuberculosis, M. kansasii, and M. avium-intracellulare), other species (e.g., M. gordonae and M. fortuitum) are infrequently responsible for disease, and their isolation usually represents colonization (9). Because the clinical presentations of tuberculous and nontuberculous mycobacterial infections are often similar, rapid and accurate identification of the mycobacteria isolated from clinical specimens should aid in selection of the most effective therapy. Furthermore, it should also minimize unnecessary therapy with potentially toxic drugs, such as those used for M. avium-intracellulare infections (9).

Although acid-fast stains detect approximately 40% of patients with mycobacterial infections at the time the cultures are received (3), positive stains cannot identify the Mycobacterium species involved. Thus, an additional 2 to 8 weeks are usually required for isolation and definitive identification (2). From 2 to 4 weeks are required for the initial isolation on culture media, and another 2 to 4 weeks are required for the definitive identification by biochemical testing if the isolate must be subcultured to produce an inoculum large enough for biochemical testing.

In 1959, Runyon (4) grouped mycobacteria by their colonial morphology, pigmentation, and growth rate. Because relatively few Mycobacterium species are isolated in our clinical microbiology laboratory, we found that the Runyon grouping was useful for preliminary identification of mycobacteria. In this study, we determined which Mycobacterium species were isolated in our hospital. Then, we prospectively evaluated the accuracy of preliminary identifications based on knowledge of the most frequent isolates and parameters such as pigmentation, colonial morphology, and growth rate.

MATERIALS AND METHODS

Specimen processing. Specimens received in the Barnes Hospital Mycobacteriology Laboratory were processed as previously described (3). Specimens from normally contaminated sources were treated with N-acetyl cysteine–2% sodium hydroxide and concentrated by centrifugation. Normally sterile body fluids and minced tissues were concentrated without decontamination. The concentrated sediment was used to prepare fluorechrome acid-fast smears (5) and to inoculate one tube each of Middlebrook 7H-10, Lowenstein-Jensen, and the Gruft modification of Lowenstein-Jensen media. All specimens were routinely incubated at 35°C for 8 weeks in an atmosphere of 10% CO₂ and 70% humidity. Specimens with positive acid-fast smears and other selected specimens were incubated for 12 weeks. Specimens from superficial sites (e.g., cutaneous lesions) were incubated at both 30 and 35°C.

Preliminary identification. From March 1979 to June 1980, we reported preliminary mycobacterial identifications when growth was initially detected. These identifications were based on colonial morphology, pigmentation, growth rate, and knowledge of the mycobacteria previously isolated at this institution. In addition, the report also indicated the number of mycobacteria isolated (few, moderate, or abundant) and stated that the organisms would be definitively identified. The growth rate was determined by subculturing an isolated colony onto Lowenstein-Jensen medium. Rapidly growing mycobacteria (Runyon group IV) grew within 3 to 4 days after subculture.

Final identification. Acid-fast isolates were definitively identified by the procedures described in the Manual of Clinical Microbiology (5). In addition to colonial morphology, pigmentation, and growth rate, the following biochemical tests were used routinely: niacin production, nitrate reduction, semiquantitative
catalase, arylsulfatase, tellurite reduction, Tween 80 hydrolysis, urease, salt tolerance, growth on MacConkey agar, and iron uptake.

Accuracy of preliminary identifications. The preliminary identifications reported from March 1979 to June 1980 were classified as accurate or inaccurate based on the final identifications. An accurate preliminary report was one which specified the organism definitively identified: e.g., M. kansasi for "acid-fast photochromogen, most likely M. kansasi," and M. fortuitum or M. chelonei for "rapid grower; M. fortuitum and M. chelonei are the most frequently isolated rapid growers at this hospital."

RESULTS

Mycobacteria isolated at this institution. From January 1977 through June 1980, mycobacteria were isolated from 3.5% of 21,250 specimens. During this period, eight Mycobacterium species were isolated: M. tuberculosis; two photochromogens, M. kansasi and M. marinum (Runyon group I); two scotochromogens, M. gordoneae and M. scrofulaceum (Runyon group II); one nonchromogen, M. avium-intracellulare (Runyon group III); and two rapid growers, M. fortuitum and M. chelonei (Runyon group IV) (Table 1).

Preliminary identifications. From March 1979 through June 1980, mycobacteria were isolated from 108 patients. The Mycobacterium species isolated were M. tuberculosis (34 patients), M. kansasi (35 patients), M. marinum (2 patients), M. avium-intracellulare (14 patients), M. gordoneae (10 patients), M. scrofulaceum (3 patients), M. fortuitum (5 patients), and M. chelonei (5 patients). The preliminary identifications of these isolates, based on the Runyon grouping and colonial morphology (Table 2), were accurate for 31 (91%) of 34 patients with M. tuberculosis and for 68 (92%) of 74 patients with nontuberculous mycobacteria. The preliminary identifications were inaccurate for nine isolates. One patient with M. tuberculosis had a mixture of both smooth and rough colonies on Lowenstein-Jensen medium and was preliminar-

<table>
<thead>
<tr>
<th>Organism</th>
<th>% of positive patients*</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. tuberculosis</td>
<td>37.3</td>
</tr>
<tr>
<td>M. kansasi</td>
<td>24.2</td>
</tr>
<tr>
<td>M. gordoneae</td>
<td>13.1</td>
</tr>
<tr>
<td>M. avium-intracellulare</td>
<td>12.5</td>
</tr>
<tr>
<td>M. fortuitum</td>
<td>6.1</td>
</tr>
<tr>
<td>M. scrofulaceum</td>
<td>3.7</td>
</tr>
<tr>
<td>M. chelonei</td>
<td>2.5</td>
</tr>
<tr>
<td>M. marinum</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*Positive cultures from 327 patients.

DISCUSSION

Most microbiology laboratories rely on biochemical testing for the definitive identification of mycobacterial isolates, which usually requires an additional 2- to 4-week delay after the initial isolation of the organism. Although gas-liquid chromatography has been used to preliminarily identify mycobacterial isolates (6), this technique is generally restricted to regional reference laboratories and is no more accurate than the results reported here.

During the past few years, we have emphasized to our laboratory staff that knowledge based on laboratory observations (such as pigmentation and colonial morphology) may be invaluable for clinical decision making. We have frequently discussed with our medical staff the cultural characteristics and probable identifications of mycobacteria isolated from their patients. Because of the success of these predictions, we decided to analyze prospectively this practice. In the experience reported here, the preliminary identifications were accurate for 91 and 92% of patients with M. tuberculosis and nontuberculous mycobacteria, respectively.

The accurate preliminary identification of 34 of 35 M. kansasi isolates was unexpected because no precautions had been taken to protect the cultures from light. These cultures were held (unprotected from light) in an incubator with a glass door and were examined weekly. However, a 1-h exposure to a 60-W light (6 to 12 in. from the culture) was necessary to induce pigmentation. Therefore, this experience suggests that the common laboratory practice of incubating one culture tube in the complete absence of light (i.e., wrapped in aluminum foil) may not be necessary for M. kansasi. The misidentified M. kansasi isolate did not form pigment initially after exposure to light. However, it was isolated after 6 weeks of incubation, and Wayne and Doubek have reported that some isolates will
TABLE 2. Initial characterization of mycobacteria and preliminary report*

<table>
<thead>
<tr>
<th>Runyon group</th>
<th>Colonial morphology and growth pattern</th>
<th>Preliminary report to physician</th>
<th>Acceptable final identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Photochromogens)</td>
<td>Dry, crumbly, buff-colored, and slow-growing</td>
<td>Acid-fast bacilli resembling <em>M. tuberculosis</em></td>
<td><em>M. tuberculosis</em></td>
</tr>
<tr>
<td>II (Scotochromogens)</td>
<td>Slow-growing, pigmented after exposure to light</td>
<td>Acid-fast photochromogen; <em>M. kansasii</em> is the most commonly isolated photochromogen at this hospital</td>
<td><em>M. kansasii</em></td>
</tr>
<tr>
<td>III (Nonchromogens)</td>
<td>Slow-growing, moist, smooth, and nonpigmented</td>
<td>Chromogenic acid-fast bacilli; <em>M. gordonae</em> and <em>M. scrofulaceum</em> are the most commonly isolated scotochromogens at this hospital</td>
<td><em>M. gordonae</em> or <em>M. scrofulaceum</em></td>
</tr>
<tr>
<td>IV (Rapid growers)</td>
<td>Rapid growth</td>
<td>Rapidly growing acid-fast bacilli; <em>M. fortuitum</em> and <em>M. chelonei</em> are the most commonly isolated rapid growers at this hospital</td>
<td><em>M. fortuitum</em> or <em>M. chelonei</em></td>
</tr>
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* Accurate preliminary identifications were those which specifically mentioned the species finally identified (see the text).

not pigment if the cultures are old or deprived of oxygen (8).

Another observation made during these studies was that the morphological features of mycobacterial colonies on media containing egg base (Lowenstein-Jensen) or glycerol (Lowenstein-Jensen and Middlebrook 7H-10) were sufficiently characteristic to permit their identification. This had been a matter of concern because Fregnan and Smith (1) had suggested that colonial morphology was less characteristic on egg base or glycerol-supplemented medium. Therefore, our results are similar to those of Vestal and Kubic (7) and suggest that the characteristic differences in morphology among mycobacteria are detectable on both Lowenstein-Jensen and Middlebrook 7H-10 media.

Although these results demonstrate the accuracy of preliminary identification based on laboratory criteria alone, it is important to emphasize that invaluable information may often be obtained from relatively simple clinical correlation. For instance, an acid-fast photochromogen isolated from cutaneous lesions in a patient with a history of contact with tropical fish tanks would suggest *M. marinum*, rather than the more frequently isolated photochromogen *M. kansasii*. Likewise, although *M. gordonae* is the most frequently isolated scotochromogen at our institution, a history of lymphadenopathy would suggest that the organism was *M. scrofulaceum*.

The delays required for the isolation and identification of pathogenic mycobacteria remain one of the major problems in clinical microbiology. We have presented a scheme for the preliminary identification of mycobacteria at the time of their initial isolation (based on the Runyon groupings) that shortens the time required for identification. Based on our data, this approach can be both accurate and faster than the methods currently in use. We suggest that other laboratories should review their previous experience in isolating mycobacteria and consider using that information together with Runyon grouping of the mycobacteria to report the most likely preliminary identification of individual clinical isolates. However, this approach may be less helpful in regions where multiple *Mycobacterium* species are frequently isolated from clinical material. Thus, careful consideration of previous experience with mycobacteria in each laboratory is essential before implementing such a system.

ACKNOWLEDGMENTS

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LITERATURE CITED