Primary Isolation Of *Mycobacterium chelonei* Subspecies *abscessus* from Pus Inoculated into Peptone Broth

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We have described the isolation of *Mycobacterium chelonei* subspecies *abscessus* which on two occasions grew out within 4 days in routine cultures after inoculation with purulent material from injection abscesses in a patient with diabetes mellitus. Media isolation and culture characteristics of this organism in comparison to others in the *Mycobacterium fortuitum* complex are described. Mycobacteria in routine laboratory media can be mistaken for diphtheroids or other "contaminants" or can be overlooked entirely. The possibility of clinical infection of mycobacterial species should be kept in mind when confronted with "sterile" abscesses at injection sites.

Special isolation techniques are usually desirable and/or necessary in obtaining positive cultures of mycobacteria from clinical material. It is possible that acid-fast organisms may be overlooked when mycobacterial infection is not suspected and ordinary culture media are used for isolation. This paper describes the primary isolation of an acid-fast organism from peptone broth and also from thioglycolate broth.

**EXPERIMENTAL**

A 36-year-old female was admitted to the hospital for treatment of diabetic ketoacidosis. She had a history of diabetes mellitus for the last 8 years, and during the past year subcutaneous abscesses had developed near the sites of insulin self-injections. The abscesses had healed, either spontaneously or after surgical drainage. Before this admission, all attempts to isolate an organism from the purulent material had failed repeatedly, with one exception. Six months previously, a "diphtheroid-like" organism was first observed in thioglycolate broth 2 days after inoculation of bloody material from an aspirate of a leg abscess and also at the same time from an arm abscess. (No bacteria had been seen on the direct smear of the aspirate.) The organism at first appeared gram negative and was subcultured to MacConkey and 5% sheep blood agar plates, which showed no growth after 24 h. Four days after inoculation the thioglycolate broth showed gram-variable branching rods that grew out on blood agar plates in 48 h. The organism was identified by the Georgia Department of Human Resources as one of the *Mycobacterium fortuitum* complex of acid-fast organisms.

On this admission the patient had one 3-cm open abscess on the left anterior thigh and an abscess below the right clavicle that was thought not to be associated with an insulin injection site. However, there was no evidence of generalized infection. The subclavicular abscess yielded dirty-yellow purulent material, which was inoculated into peptone broth (Becton-Dickinson Co., Rutherford, N.J.) in a 1:10 dilution. (The physician in charge was unaware of the isolation of acid-fast organisms 6 months previously.) An acid-fast organism was isolated from the peptone broth and was identified as one of the *M. fortuitum* complex.

The organism appeared in the peptone broth 4 days after inoculation. It was a faintly staining, beaded, gram-variable organism resembling over-decolorized chains of streptococci. Kinyoun and auramine O stains showed numerous bacilli. Subculture was made to MacConkey and 5% sheep blood agars, Lowenstein-Jensen slants, and Middlebrook 7H11 plates. Within 5 days grayish-cream, smooth, entire, nonhemolytic colonies 2 to 4 mm in diameter appeared on the blood agar plate. Reddish colonies 2 mm in diameter appeared on MacConkey agar, and smooth, white, yeastlike colonies 2 mm in diameter appeared on the 7H11 plate. There was no filamentous growth on 7H11 agar typical of *M. fortuitum*. On the Lowenstein-Jensen slants the colonies were a dirty cream color, rounded, and easily moved about on the media with a needle. In 7 days they became umbonate in shape.

By using an inoculum from the Lowenstein-Jensen slant, various media were inoculated. The organism grew at room temperature and 37 C on Thayer-Martin, chocolate, and colimycin-nalidixic acid agars. Good growth appeared in 24 h at 37 C and 48 h at room temperature. Ten thousand organisms inoculated into 19 ml of peptone broth produced a pellicle in 2 days. Pus concentrated by the *N*-acetyl-l-cysteine method grew out the organism in 2 days on Lowenstein-Jensen slants and also on Sabouraud agar slants. There was no growth on Mycosel agar with cycloheximide after 21 days.

Arylsulfatase was 1(+) positive in 3 days and 4(+) positive in 2 weeks. The organism was nitrate reductase negative when tested with nitrate reductase test...
Table 1. Comparison of reactions of three mycobacteria and the test organism

| Determination                  | M. chelonei subsp. chelonei | M. fortuitum | M. chelonei subsp. abscessus | Test organism |
|--------------------------------|-----------------------------|--------------|-----------------------------|---------------|
| Arylsulfatase (3rd day)        | Positive                    | Positive     | Positive                    | Positive      |
| NaCl (5%)                     | No growth                   | Growth       | Growth                      | Growth        |
| Nitrate reductase              | Negative                    | Growth       | Negative                    | Negative      |
| Citrate                       | Growth                      | Growth       | No growth                   | No growth     |
| Deoxycholate (1%)             | No growth                   | Growth       | Growth                      | Growth        |
| Iron uptake                   | Negative                    | Positive     | Negative                    | Negative      |

Drug sensitivities run by the Center for Disease Control Mycobacteriology section showed the organism to be resistant to 5.0 μg of isoniazid (INH), 10.0 μg of streptomycin, 2.0 μg of PAS, 5.0 μg of ethionamide, 5.0 μg of kanamycin, 50.0 μg of pyrazinamide, 20.0 μg of D-cycloserine, and 1.0 μg of rifampin per ml. We have described the isolation of an acid-fast organism that on two occasions grew out within 4 days in routine broth cultures after inoculation with purulent material. We feel the biochemical and colonial characteristics of this isolate identify it as M. chelonei subsp. abscessus as described by Kubica et al. (8, 9).

**DISCUSSION**

Many atypical mycobacteria may be isolated from the soil and other natural sources (10). It is not surprising that they would appear in simple laboratory media on primary isolation. Many cases of injection abscess due to mycobacteria have been reported, but the media of isolation specified were usually Lowenstein-Jensen or other special media for the growth of mycobacteria (1, 2, 5-7). The first report of infection with M. abscessus described isolation on Sabouraud glucose agar (11). In another case a mycobacterium from a subcutaneous abscess was isolated on "cycloheximide media for fungi" at room temperature (4). An organism identified as M. chelonei grew out as tiny white colonies on blood agar 4 days after direct inoculation of pus from an injection abscess (3).

Mycobacteria can appear in simple laboratory media more often than is realized and be mistaken for diphtheroids or other contaminating organisms. Since growth may be sparse in broth the first few days and many mycobacteria Gram stain poorly, they may be overlooked entirely. An acid-fast stain and subculture to Lowenstein-Jensen media allowed us to confirm the presence of mycobacteria under these circumstances. We have described the isolation of an acid-fast organism that on two occasions grew out within 4 days in routine broth cultures after inoculation with purulent material. We feel the biochemical and colonial characteristics of this isolate identify it as M. chelonei subsp. abscessus as described by Kubica et al. (8, 9).

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