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A New Biotype of *Legionella dumoffii*

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A new biotype of *L. dumoffii* was isolated from lung and sputum samples of an immunosuppressed patient with pneumonia. This strain differs from other described strains of *L. dumoffii* in that it fails to produce browning of tyrosine-containing buffered yeast extract medium.

The phenotypic description of *Legionella dumoffii* was originally based on the characteristics of two isolates, one clinical and the other environmental (2, 7). We report the isolation and characterization of an *L. dumoffii* strain which differs phenotypically from those previously described.

Case report. A 36-year-old woman with alpha-1 antitrypsin deficiency and consequent severe emphysema developed pneumonia in a Los Angeles-area hospital. She had been treated for emphysema for 3 days at one hospital and then transferred to another hospital, where pneumonia was diagnosed 2 days later. She had been taking methylprednisolone (30 to 40 mg/day) for several months before her initial admission.

Penicillin and gentamicin therapy was given for her pneumonia without response. A transtracheal aspirate was then taken; this yielded oral flora on routine culture. Because of failure to respond to therapy, which included extension and cavitation of her roentgenographic infiltrate, a lung biopsy was performed. She was then started empirically on erythromycin (2 g/day intravenously) and rifampin (600 mg/day orally). She responded slowly but was able to go home after 3 weeks of hospitalization.

Histopathology of the lung biopsy revealed necrotizing pneumonia. A Gimenez stain of an imprint of the biopsy revealed full fields of small coccobacilli, whereas a Gram stain showed no organisms. Direct immunofluorescent staining of the lung was negative for *L. pneumophila* serogroups 1 through 4 but was positive for *L. dumoffii*. The lung and subsequent sputum samples were cultured on buffered charcoal-yeast extract medium supplemented with alpha-ketoglutarate (BCYEa) and with and without antimicrobial agents (4).

Culture for *Legionella* spp. was performed by using BCYEa medium, BCYEa medium supplemented with cefamandole, polymyxin B, and anisomycin (BMPAa), and BCYEa medium supplemented with glycine, vancomycin, polymyxin B, and anisomycin (MWY). Incubation was performed at 35°C in a humidified-air incubator. Immunofluorescence, biochemical, growth, and DNA homology studies were performed as previously described (2–4).

A legionella-like organism (Wadsworth 81-782A; deposited with the American Type Culture Collection as ATCC 35850) was recovered in pure and heavy growth from the lung biopsy specimen plated on the BCYEa, BMPAa, and MWY plates after 4 days of incubation. Also, a single colony of a similar-appearing organism was isolated from sputum taken 4 days after the lung biopsy specimen (direct immunofluorescence analysis of sputum was negative for *L. dumoffii*). The sputum isolate was detected only on the MWY plate after 14 days of incubation (Wadsworth 81-825A); failure to isolate this organism on the other media could not be ascribed to overgrowth of other organisms. Since 81-825A and 81-782A appeared identical on preliminary testing, only 81-782A was extensively investigated.

The legionella-like organism was a short, gram-negative bacillus with a monopolar flagellum. It did not grow on BCYEa medium made without L-cysteine, nor did it grow on tryptic soy–5% sheep blood agar after 10 days of incubation. The organism autofluoresced an electric blue-white color under long-wave UV light. It was catalase, oxidase, gelatinase, and beta-lactamase positive. It failed to hydrolyze sodium hippurate. Although it grew well on tyrosine-supplemented yeast extract medium, no soluble brown pigment was produced after prolonged observation (10 days). Simultaneous testing of strain TEX-KL (*L. dumoffii*) produced identical results except that TEX-KL produced browning of the tyrosine-supplemented yeast extract medium. This was true even after monthly passage of 81-782A on BCYEa medium for 1.5 years. Both 81-782A and 81-825A stained brightly with *L. dumoffii* antibody direct immunofluorescent conjugate but failed to react with anti-*L. pneumophila* antibody conjugates. Both TEX-KL and 81-782A had nearly identical cellular fatty acid compositions, with over 80% of the fatty acid being methyl-12-methyl-tetradecanoate (anteiso15:0). There was 90% DNA relatedness between TEX-KL and 81-725A.

Serum samples taken 4 days after the lung biopsy and 18 days later both demonstrated antibody titer levels $\geq$1,024 for multiple *Legionella* antigens on indirect fluorescent-antibody testing. These antigens included *L. pneumophila* serogroups 1 and 4, *L. longbeachae* serogroups 1 and 2, *L. bozemanii*, and *L. dumoffii*. The antibody titer levels were $\leq$32 in both specimens for *L. micdadei*, *L. jordanis*, *L. gormanii*, *L. wadsworthii*, and *L. pneumophila* serogroup 5. Serum antibody titer levels for serogroups 2 and 3 of *L. pneumophila* were intermediate between the two extremes; antibody titer levels directed against serogroup 6 of *L. pneumophila* rose from 64 to 256.

Pigment production by *L. pneumophila* was first described by Feeley et al. (6); Baine and Rasheed established that the pigment was a tyrosine derivative (1). Of the known 22 *Legionella* species, 20 produce pigment, the exceptions being *L. micdadei* and *L. wadsworthii* (2, 7); this report establishes that at least one *L. dumoffii* strain also fails to produce pigment. Pigment production is not required for pathogenicity, as demonstrated by the case reported herein

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and by the known inability of other pathogenic *Legionella* species to produce pigment.

The finding of a new biotype of *L. dumoffii* is apparently of taxonomic interest only and should be expected with such a diverse group of organisms. The case reported here appears to differ little from other reported cases of *Legionella* pneumonia in terms of host susceptibility, clinical presentation, roentgenographic characteristics, or response to therapy.

Isolation of *L. dumoffii* from sputum and its growth on selective media have not been reported previously, to our knowledge. Its isolation from sputum despite negative direct immunofluorescence examination for the homologous serotype is not unusual, as the sensitivity of direct immunofluorescence examination for *Legionella* spp. in respiratory tract samples is relatively low (9). This emphasizes, as do several other case reports of infections with rarely diagnosed *Legionella* spp., that culture diagnosis should be a primary means of detecting these infections (5, 8).

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