Isolation of *Legionella pneumophila* from a Transtracheal Aspirate

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Isolation of *Legionella pneumophila* from a transtracheal aspirate was achieved by using simple in vitro culture methods. Clinical microbiologists should routinely culture for this organism from appropriate body fluids obtained from normally sterile areas.

Legionella pneumophila, (Don Brenner, personal communication), the etiological agent of Legionnaires disease, has been recovered from a transtracheal aspirate (TTA) once previously (7). We wish to report an additional isolation from this source and to encourage clinical microbiologists to culture for this organism routinely.

The patient was a 79-year-old man admitted to the Veterans Administration Wadsworth Medical Center in September 1978 for metastatic prostatic carcinoma. He developed pneumonia which was diagnosed clinically as Legionnaires disease. He died of progressive pneumonia despite erythromycin therapy. Permission for autopsy was not granted; however, the family did permit postmortem lung aspiration.

A TTA obtained on the first day of illness was cultured on routine anaerobic and aerobic media. No media which would support the growth of *L. pneumophila* were used. Two days after incubation at 37°C, the inoculated thioglycolate broth (BBL 135C with vitamin K₁ and hemin; Baltimore Biological Laboratory, Cockeysville, Md.) was retrieved from the clinical microbiology laboratory. This was centrifuged for 30 min at approximately 1,200 × *g*, using a table top Dynac centrifuge (Clay Adams, Parsippany, N.J.). The sediment was pipetted from the bottom of the tube and plated onto charcoal yeast extract agar (4). This plate was incubated at 35 to 36°C in 3.5% CO₂ at 65 to 70% humidity.

The percutaneous lung aspirate was obtained aseptically 7 h after death from an affected area of the lung. Dilutions, 1:10 and 1:50, of this were cultured on conventional media aerobically, as well as on charcoal yeast extract media incubated in 3.5% CO₂.

Direct fluorescent-antibody (DFA) examinations of bacteriological isolates and clinical specimens were performed according to published methods (3) with a Leitz Dialux (E. Leitz, Rock-

leigh, N.J.) epi-illumination microscope. Knoxville antibody conjugate was supplied by the Biological Products Division, Center for Disease Control (CDC), Atlanta, Georgia. Los Angeles-1 and Togus antibody conjugates were kindly provided by Roger McKinney of the Bureau of Laboratories, CDC (R. M. McKinney, L. Thacker, P. P. Harris, K. R. Lewallen, G. A. Hebert, P. H. Edelstein, and B. M. Thomason, Ann. Intern. Med., in press).

Gelatinase production was tested by incubation of a cloudy suspension of organisms with Plus-X-Pan (Eastman Kodak Co., Rochester, N.Y.) undeveloped film strips in Trypticase soy broth at 37°C. Oxidase production was tested by using Kovac's reagent. Analysis of cellular fatty acids by gas-liquid chromatography was performed by Wayne Moss, CDC (9). Confirmation of the identity of the isolates was performed by Robert Weaver, CDC.

Examination of the TTA specimen by a DFA technique revealed >50 fluorescent bacilli per smear, using the Knoxville conjugate. The lung aspirate was also positive, showing about 25 organisms per smear with the same conjugate. Both specimens were DFA negative when either the Togus or Los Angeles-1 conjugates were used. Cultures of the lung aspirate grew *Citrobacter freundii* and *Enterobacter* sp., both of which were Knoxville-DFA negative. These organisms overgrew the charcoal yeast extract plate.

The TTA specimen, which had been retrieved from the thioglycolate broth, grew 20 colonies typical of *L. pneumophila* on day 12 of incubation (4). The plate had last been inspected on day 5, so it is impossible to know exactly when growth first appeared. Gram smear of the colonies revealed filamentous gram-negative bacilli up to 20 μm in length. Subculture onto 5% sheep blood agar plates, or onto Mueller Hinton agar,

with or without X and V factors, yielded no growth after 3 days of incubation at 36°C in 3.5% CO₂. The colonies were weakly catalase positive and weakly oxidase positive. The isolates were gelatinase positive at 3 days. Subculture onto FG agar resulted in fluorescence and browning after 4 and 10 days of incubation, respectively (5). Examination of the isolates by a DFA technique was positive only with the Knoxville conjugate. Analysis of cellular fatty acids by gas-liquid chromatography showed a pattern consistent with that of *L. pneumophila*.

Isolation of *L. pneumophila* from a TTA has been made only once before, although several isolations have been made from lung tissue or pleural fluid (1, 2, 8). The reason for this appears to be that coughed sputum is always contaminated with oral flora which readily overgrows fastidious organisms cultured on nonselective media. However, as demonstrated for other organisms, TTA is an excellent method to obtain uncontaminated lower respiratory tract secretions from most patients (10).

Both the previous report (7) and our case support the value of TTA in diagnosing Legionnaires disease. About 25% of patients with Legionnaires disease have organisms in the sputum demonstrated by a DFA technique (C. V. Broome, W. B. Cherry, W. C. Winn, and B. R. MacPherson, *Ann. Intern. Med.*, in press). Thus, it is likely that culture of TTA specimens would yield growth of *L. pneumophila* in cases of Legionnaires disease.

Routine culture of TTA specimens onto simple media may therefore be helpful in the diagnosis of Legionnaires disease. Since thioglycolate broth did not kill the organism, perhaps routine subculture of such broth onto charcoal yeast extract agar should be performed in every case of "culture-negative" pneumonia. It is possible that the dilution in thioglycolate broth was beneficial, because we have found that at least a 1:10 dilution of body fluids is essential for primary isolation of the organism (P. H. Edelstein, unpublished data).

We believe that the growth of enteric organisms from the lung aspirate represented post-

mortem colonization, although dual infection has been seen in Legionnaires disease (6).

The sensitivity of the method utilized in the study of our patient, as well as appropriate methods of specimen transport, need further investigation. It is possible that with improved media, culture of TTA specimens will yield a rapid bacteriological diagnosis.

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