JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1991, p. 1777–1779
0095-1137/91/091777-03$02.00/0

Vol. 29, No. 9

# Pneumonia and Empyema Infection Associated with a *Bacillus* Species That Resembles *B. alvei*

PHILIP E. COUDRON,[1]* JANE M. PAYNE,[1] AND SHELDON M. MARKOWITZ[2]

*Departments of Pathology[1] and Medicine,[2] McGuire Veterans Affairs Medical Center, Richmond, Virginia 23249*

Received 14 February 1991/Accepted 27 May 1991

**An organism resembling *Bacillus alvei* was isolated from the lung and pleural fluid of an immunocompetent patient. The isolate differed from the type strain of *B. alvei* in its ability to reduce nitrate and its inability to produce dihydroxyacetone and acetylmethylcarbinol. The isolate was resistant to ciprofloxacin and showed intermediate susceptibility to vancomycin.**

*Bacillus* species are ubiquitous in nature, and when they are isolated in a clinical microbiology laboratory, they are frequently dismissed as contaminants. Historically, *B. anthracis* has been recognized as the only pathogenic member of the genus. However, *B. cereus* and *B. subtilis* have also been described as pathogens (8, 10, 11).

*B. alvei* and organisms resembling *B. alvei* have been reported as the cause of human infection in only four cases. *B. alvei* was recovered from the blood of a 26-year-old woman with sickle cell disease (9) and from a culture of a foreign body that was removed from an eye of a 22-year-old man who sustained a traumatic eye injury (1). In the remaining cases, a *Bacillus* sp. was isolated from the cerebrospinal fluid of two neonates with meningitis (7, 13). Both isolates were reported as *B. alvei*, although some atypical biochemical reactions were noted. No details of these reactions were given.

We report a case of lung and pleural fluid infection in an immunocompetent 62-year-old man that was caused by a *Bacillus* species which resembled *B. alvei*. The biochemical properties of our isolate are described.

## CASE REPORT

A 62-year-old black male with a history of organic heart disease and congestive heart failure was admitted to the Hunter Holmes McGuire Department of Veterans Affairs Hospital complaining of shortness of breath. He was a retired tobacco and small-crop farmer and pipeline construction worker. He had no history of diabetes or alcohol abuse and had smoked one pack of cigarettes a day for 50 years. Physical examination revealed a thin male with an irregular pulse, a temperature of 37.6°C, blood pressure of 110/80 mm Hg, respiration rate of 18/min, dullness to percussion and decreased breath sounds over the right hemithorax, and a palpable liver edge extending 6 cm below the right costal margin. Diagnostic evaluation revealed a large multiloculated right pleural effusion. Aerobic and anaerobic cultures of a pleural aspirate yielded no growth, and cytology was negative for malignancy. A purified protein derivative test was negative, and a positive reaction was seen with the mumps antigen test. Human immunodeficiency virus testing was not performed because the patient was not in a high-risk group. A pleural biopsy specimen revealed an acute and chronic inflammatory infiltrate; stains and cultures for mycobacteria and fungi were negative. The patient was discharged and empirically treated for presumed anaerobic or pneumococcal empyema with 750 mg of penicillin V four times a day (QID) for 6 weeks. A repeat chest X ray 4 months later showed a decreasing right pleural effusion; the patient was clinically stable.

Fifteen months later the patient was readmitted complaining of shortness of breath, a productive cough, generalized weakness, and anorexia. Repeat X rays and computerized tomography of the chest revealed an increased loculated right pleural effusion and a right lower lobe pneumonia. Thoracentesis on two occasions each yielded approximately 10 ml of a purulent fluid. Fluid samples, as well as lung and pleural tissue obtained by transbronchial biopsy, were sent for culture. A *Bacillus* species which was susceptible to penicillin was recovered from all cultures. The patient was discharged and treated with 500 mg of penicillin V QID and was followed as an outpatient.

One month later, the patient was admitted a third time with pulmonary symptoms after discontinuation of penicillin. A large loculated right pleural effusion that was believed to be an empyema was seen on X rays. The patient underwent a right thoracotomy with pleural space drainage and decortication of the right lung. At the time of surgery, a thick right pleural "peel" was removed, together with a "moderate amount" of empyema fluid. A culture of the fluid grew a *Bacillus* species, and histopathologic examination revealed chronic fibrosing pleuritis and chronic empyema. The patient was discharged, was treated with 500 mg of penicillin V QID for 8 weeks, and had an uneventful postoperative course. Four months following discharge, the patient was asymptomatic except for mild shortness of breath with exertion.

## MATERIALS AND METHODS

Unless indicated otherwise, biochemical tests were performed by conventional methods (2). Nitrate reduction and production of dihydroxyacetone and acetylmethylcarbinol were also performed as described by Gordon et al. (3). The nitrate broth medium consisted of peptone (5 mg/ml), beef extract (3 mg/ml), and potassium nitrate (1 mg/ml). The dihydroxyacetone medium consisted of 1 g of yeast extract and 2 ml of glycerol in 100 ml of nutrient agar. The medium was inoculated by streaking and was incubated at 37°C for 10 days. It was then flooded with Fehling solution, which consisted of a 1:1 mixture of the following two solutions: (i) $CuSO_4 \cdot 5H_2O$ (6.9%) and (ii) $KNaC_4H_4O_6 \cdot 4H_2O$ (34.6%) in 2.5 N NaOH. If a red halo appeared around the bacterial growth after 2 h, the dihydroxyacetone test was read as

* Corresponding author.

TABLE 1. Phenotypic characteristics of *Bacillus* sp., possibly *B. alvei*

| Test | % of strains positive[a] | Reaction of VA strain[b] |
|---|---|---|
| Motility | 100 | + |
| Acid production from: | | |
| Glucose | 100 | + |
| Xylose | 0 | − |
| Mannitol | 0 | − |
| Lactose | 58 | − |
| Sucrose | 100 | + |
| Maltose | 100 | + |
| Fructose | ND | + |
| Galactose | ND | − |
| Catalase | 94 | + |
| Oxidase | 100 | + |
| Growth on MacConkey agar | 5 | − |
| Citrate (Simmons) | 0 | − |
| Urea (Christensens) | 67 | + |
| Nitrate reduction | 47 | + |
| Indole | 63 | + |
| Triple sugar iron, acid (slant/butt) | 100/100 | +/+ |
| $H_2S$ (triple sugar iron butt) | 100 | + |
| Methyl red | 67 | + |
| Voges-Proskauer | 0 | − |
| Gelatin hydrolysis | 78 | + |
| Growth at 25/35/42°C | 79/100/74 | +/+/+ |
| Esculin hydrolysis | 85 | + |

[a] Data are from reference 5. The number of strains tested was 19. ND, not done.
[b] VA, Veterans Affairs medical center; +, positive; −, negative.

positive. The Voges-Proskauer medium contained sodium chloride (0.5%) in lieu of dipotassium phosphate (conventional), was filter sterilized, and was dispensed at 5 ml per tube. The medium was inoculated; and on days 2, 6, and 9, 3 ml of 40% KOH and then 1 mg of creatine powder were added. The development of a red color after 30 to 60 min indicated a positive Voges-Proskauer reaction.

In vitro susceptibility testing was performed by the broth macrodilution method as described previously (6). *Staphylococcus aureus* ATCC 29213 was tested as the standard control organism. Cefinase (BBL Microbiology Systems, Cockeysville, Md.) was used to detect β-lactamase.

## RESULTS

Routine cultures of pleural fluids collected on three different occasions during the patient's second and third hospital admissions all yielded moderate to large numbers of gram-positive bacilli on primary media. Cultures of lung and pleural tissue also yielded gram-positive bacilli. No other aerobic organisms were isolated. None of the three cultures for fungi and mycobacteria or the three anaerobic cultures yielded additional microorganisms. Isolates from all five cultures yielded identical biochemical test results (Table 1). The organism was tentatively identified as *B. alvei* and was sent to the Virginia State Reference Laboratory and the Centers for Disease Control, where it was identified as a *Bacillus* species, possibly *B. alvei*.

Additional biochemical tests described by Gordon et al. (3) were performed to differentiate our strain from the type strain of *B. alvei* (ATCC 6344). Test results showed that the American Type Culture Collection (ATCC) and Veterans Affairs Medical Center (VA) strains belonged to the assigned and unassigned catagories of strains of *B. alvei*, respectively (Table 2).

TABLE 2. Characteristics differentiating strains assigned to *B. alvei* and unassigned strains[a]

| Test | No. of strains tested (% positive)[b] | | Test result | |
|---|---|---|---|---|
| | Assigned[c] | Unassigned | ATCC 6344 | VA |
| Nitrate reduction | 13 (0) | 3 (100) | − | + |
| Dihydroxyacetone production | 12 (92) | 3 (0) | + | − |
| Voges-Proskauer | 12 (92) | 3 (0) | + | − |

[a] Tests were performed as described by Gordon et al. (3).
[b] Data are from reference 4.
[c] Includes five ATCC strains and eight N. R. Smith collection (NRS) strains. Results of dihydroxyacetone and Voges-Proskauer tests for one strain each were equivocal and were therefore omitted.

The VA isolate showed the following susceptibility pattern (MIC): susceptible to penicillin (0.12 μg/ml), ampicillin (0.12 μg/ml), erythromycin (0.12 μg/ml), clindamycin (0.5 μg/ml), and gentamicin (1.0 μg/ml); intermediate to tetracycline (8.0 μg/ml) and vancomycin (8.0 μg/ml); and resistant to ciprofloxacin (8.0 μg/ml). The isolate was negative for β-lactamase production.

## DISCUSSION

*B. alvei* was first isolated from diseased honeybee larvae (Latin *alvei*, "from a beehive"). Our patient had no known exposure to bees, nor was he immunocompromised. The portal of entry of the organism was unclear, although spore inhalation was possible because of the patient's occupation (tenant farmer and construction worker). Other reported cases of human infection caused by *B. alvei* or organisms resembling *B. alvei* involved an immunocompromised woman (9), a man with a traumatic eye injury (1), and two neonates (7, 13).

Only one of the strains isolated in these four cases was well characterized (9). The authors of one report indicated that their isolate could have been a contaminant (1). The two strains isolated from neonates gave some biochemical test results that were atypical for *B. alvei*, but no details were given. One of these strains reduced nitrate and failed to produce acetylmethylcarbinol (13, 14). It was possible, therefore, that this strain was an unassigned strain, as described by Gordon and colleagues (Table 2). The strain isolated from the second neonate also failed to produce acetylmethylcarbinol, but it did not reduce nitrate (7). It is unclear, therefore, whether this was an unassigned strain or whether test results were due to other phenomena, such as the use of different test reagents or procedures. Gordon et al. (3) noted that the designation of strains as unassigned merely indicated that they were unable to assign these strains to a definite species because the small number in each group precluded them from establishing species descriptions. Phenotypically, the VA strain clearly falls in the unassigned group of strains that resemble *B. alvei*.

Weber and colleagues (12) performed microdilution susceptibility testing of 54 *B. cereus* and 35 non-*B. cereus* isolates to selected drugs. Vancomycin MICs ranged from less than 0.25 to 4.0 μg/ml. These authors concluded that the drug of choice for *Bacillus* infections appeared to be vancomycin. Reboli et al. (9) reported that for their strain of *B. alvei*, the vancomycin MIC was 0.2 μg/ml. For the VA

strain, the vancomycin MIC was relatively high 8.0 μg/ml (intermediate susceptibility). In addition, Weber et al. (12) reported that the MICs of ciprofloxacin for the non-*B. cereus* strains ranged from less than 0.25 to 1.0 μg/ml (all susceptible [6]) and that 71% of these strains were also susceptible to tetracycline when they were tested by the disk diffusion method. For the VA strain, the ciprofloxacin MIC was 8.0 μg/ml (resistant), and the strain showed intermediate susceptibility to tetracycline. Sliman et al. (11) reviewed the records of 38 patients with serious infections caused by *Bacillus* species that occurred at five Cleveland hospitals between 1981 and 1986. They reported that the most effective antimicrobial agents in vitro appeared to be gentamicin, vancomycin, and clindamycin. The interpretation of the clindamycin MIC for the VA strain was borderline susceptible (6). These data underscore the need for in vitro susceptibility testing of each clinical isolate.

In summary, we isolated a *Bacillus* species resembling *B. alvei* from the pleural fluid of an apparently immunocompetent patient. The isolate differed from typical *B. alvei* strains on the basis of three biochemical reactions. It was also more resistant to vancomycin and ciprofloxacin than the other bacilli reported in the literature were.

## ACKNOWLEDGMENTS

We thank Annette Reboli for providing an English translation of reference 7 and Dannie Hollis for helpful comments about identification of the isolate.

## REFERENCES

1. **Antonello, A., and G. W. Weinstein.** 1989. Successful treatment of *Bacillus alvei* endophthalmitis. Am. J. Ophthalmol. **108**:454–455.
2. **Clark, W. A., D. G. Hollis, R. E. Weaver, and P. Riley.** 1984. Identification of unusual pathogenic gram-negative aerobic and facultatively anaerobic bacteria, p. 8–25. Centers for Disease Control, Atlanta.
3. **Gordon, R. E., W. C. Haynes, and C. H. N. Pang.** 1973. The genus *Bacillus*, p. 6–7, 12, 62–66, 109–116. U.S. Government Printing Office, Washington, D.C.
4. **Gordon, R. E., W. C. Haynes, and C. H. N. Pang.** 1973. The genus *Bacillus*, p. 218–219. U.S. Government Printing Office, Washington, D.C.
5. **Hollis, D. G., and R. E. Weaver.** 1986. Gram-positive organisms: a guide to identification, p. 15. Centers for Disease Control, Atlanta.
6. **National Committee for Clinical Laboratory Standards.** 1990. Approved standard M7-A2. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 2nd ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.
7. **Park, S. J., Y. Chong, and S. Y. Lee.** 1976. Bacterial and fungal species from cerebrospinal fluid in the past five years. Korean J. Pathol. **10**:137–142.
8. **Patrick, C. C., C. Langston, and C. J. Baker.** 1989. *Bacillus* species infections in neonates. Rev. Infect. Dis. **11**:612–615.
9. **Reboli, A. C., C. S. Bryan, and W. E. Farrar.** 1989. Bacteremia and infection of a hip prosthesis caused by *Bacillus alvei*. J. Clin. Microbiol. **27**:1395–1396.
10. **Richard, V., P. Van der Auwera, R. Snoeck, D. Daneau, and F. Meunier.** 1988. Nosocomial bacteremia caused by *Bacillus* species. Eur. J. Clin. Microbiol. Infect. Dis. **7**:783–785.
11. **Sliman, R., S. Rehm, and D. M. Shlaes.** 1987. Serious infections caused by *Bacillus* species. Medicine (Baltimore) **66**:218–223.
12. **Weber, D. J., S. M. Saviteer, W. A. Rutala, and C. A. Thomann.** 1988. In vitro susceptibility of *Bacillus* spp. to selected antimicrobial agents. Antimicrob. Agents Chemother. **32**:642–645.
13. **Wiedermann, B. L.** 1987. Non-anthrax *Bacillus* infections in children. Pediatr. Infect. Dis. J. **6**:218–220.
14. **Wiedermann, B. L. (George Washington University Medical Center).** 1990. Personal communication.