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Pseudomonas mesophilica Infections in Humans

SHARON M. SMITH,[1,2]* ROBERT H. K. ENG,[3,4] AND CATHERINE FORRESTER[3,4]

Microbiology Section, Laboratory Service,[1] and Medical Service,[3] Veterans Administration Medical Center, East Orange, New Jersey 07019, and Departments of Microbiology[2] and Medicine,[4] University of Medicine and Dentistry of New Jersey, Newark, New Jersey 07103*

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Reported here is the case of a patient with metastatic adenocarcinoma of the lung who had bacteremia involving *Pseudomonas mesophilica*. Of the common laboratory media tested at 35°C, buffered charcoal yeast extract agar and nutrient agar provided the best growth; however, other media supported growth at lower temperatures. Since blood cultures are routinely subcultured onto chocolate agar and then incubated at 35°C, awareness of the characteristics of *P. mesophilica* and of the isolation techniques as outlined may enhance the recovery of this and related bacterial species.

Pseudomonas mesophilica has been called *Beijerinckia* sp. (16), *Chromobacterium* sp. (6), *Protaminobacter* sp. (3, 5), *Mycoplana rubra* (5), *Pseudomonas methanica* (12, 17), and *Vibrio extorquens* (1). It is one of several related methane-producing bacteria which can be isolated from leaf surfaces (1). Human infection caused by this organism has been reported with certainty only once (11). Additional isolates of *P. mesophilica* and a related organism have been recovered from humans; however, little clinical information was reported (10). We report a case of a severely immunocompromised patient who had bacteremia involving this organism. The procedures used to recover this organism from blood cultures are discussed.

CASE REPORT

A 38-year-old man was admitted to the hospital with complaints of lower-back pain, a 30-lb (ca. 13.6 kg) weight loss, and night sweats of a 2-month duration. He had smoked two packs of cigarettes per day for over 20 years and had four previous hospital admissions for alcohol detoxification. The initial physical examination showed a cachectic man with a rectal temperature of 98.4°F, a pulse rate of 72/min, a respiratory rate of 16/min, and blood pressure of 130/80 mmHg. No lymphadenopathy was detected. The chest was clear to auscultation and percussion. A firm, fixed, and tender 3-cm mass was noted adjacent to the dorsal spines at the level of T_{10}. The neurological examination was unremarkable. Blood chemistries, which included serum glutamic oxalacetic acid, serum glutamic pyruvic transanimase, lactate dehydrogenase, and alkaline phosphatase, were normal. Hemoglobin was 15.1 g/dl, and the leukocyte count was 10,000/mm^3. Initial chest X ray showed a prominent right hilar shadow. Bronchoscopy showed a hilar mass in the right mainstem bronchus, and histology of this biopsied mass was consistent with adenocarcinoma.

Soon after admission new soft tissue masses appeared in the right axilla, over the left frontal bone, and in the right mandible. He received 2,400 rads of radiation to the T_{12} area and 10,000 rads to the mandible. After completion of radiation therapy (3 months after hospital admission), the patient developed a fever of 102°F with a peripheral leukocyte elevation to 14,700 cells per mm^3 and differential count of 86% neutrophils, 3% lymphocytes, 10% monocytes, and 1% eosinophils. A repeat chest X ray was interpretated as consistent with postobstructive pneumonia. Two sets of blood cultures were obtained and were negative after 7 days of incubation. He was treated empirically with 2 g per day of ampicillin orally for 2 days and then with 2 g of cefamandole intravenously every 6 h. Temperature elevations to 103°F persisted, and two more sets of blood cultures were obtained. When these blood cultures remained negative, the patient was placed on an antitumor chemotherapy regimen which included vinblastine, mitomicin C, and *cis*-platinum. Despite the fact that the patient still received cefamandole for the next 3 weeks, evening temperature elevations were up to 102°F. Cefamandole was discontinued, and he received a second course of the same antitumor chemotherapy. The peripheral leukocyte count increased to 26,000 cells per mm^3 with no band forms. One set of blood cultures was obtained during one of the temperature elevations, and cefamandole was restarted. The aerobic bottle of the blood culture set was reported to be growing a gram-negative rod on the sixth day of incubation. Although the patient did not appear toxic, his fever of 102 to 103°F continued, and he died 1 week later.

MATERIALS AND METHODS

Initial isolation of the organism was by the BACTEC (Johnston Laboratories, Towson, Md.). The routine blood culture set consisted of aerobic (6B) and anaerobic (7D) supplemented tryptic soy broths containing radiolabeled nutrients. The bottles were incubated in a 35°C warm room, and the aerobic bottles were rotated at 200 cycles per min, whereas the anaerobic bottles were incubated in a stationary position. The aerobic bottles were radiometrically monitored twice daily for the first 3 days and then daily thereafter, whereas the anaerobic bottles were monitored daily. The radiometric index of the aerobic bottle was noted to increase steadily from a base line of 25 starting with day 5 of incubation and was 50 by day 7. The radiometric index of the anaerobic bottle remained at the base line of 10. Because of an elevated index of the aerobic bottle, a Gram stain of the contents was performed, but no recognizable bacterial forms were noted. However, a Wayson stain showed rod-shaped bacteria. A ×1,000 microscopic examination of a wet preparation showed vacuole-filled rods of ca. 1 by 3 to 4 μm (Fig. 1A). Subsequent stains of the blood culture bottle contents showed that the rods did not take up the Gram stains (Fig. 1B), did not stain acid-fast by the Ziehl-Neelsen stain, and stained only mildly acid-fast by the modified acid-fast stain. Initial subcultures of the contents onto sheep blood, choco-

* Corresponding author.

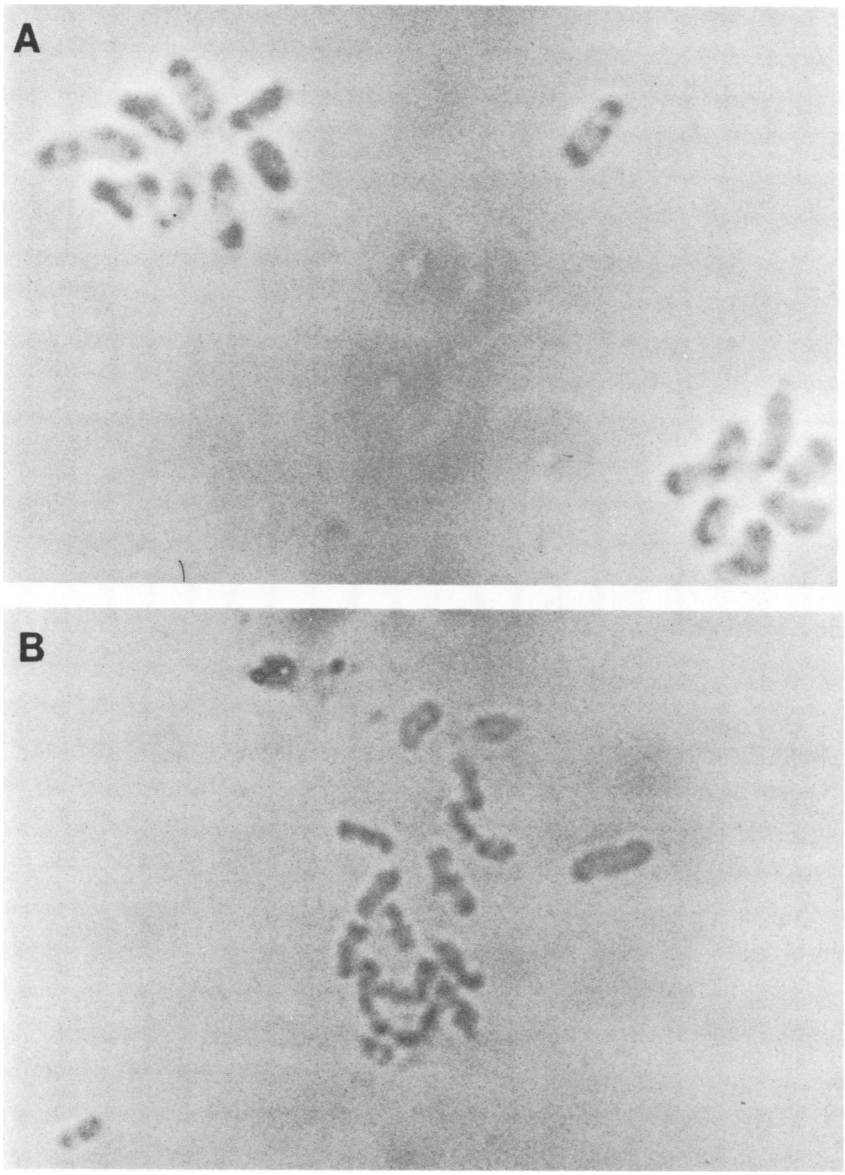

FIG. 1. (A) *P. mesophilica* in a wet preparation with phase-contrast microscopy at ×1,000. Note the many vacuoles within the organism. (B) Gram stain of *P. mesophilica* at the same magnification. Note that most bacilli did not stain well and appear amorphous.

late, and MacConkey agar plates (BBL Microbiology Systems, Cockeysville, Md.) showed no growth after 5 days of incubation at 35°C. The organism, however, grew slowly and was maintained in the aerobic blood culture bottle. After five passages in 6B bottles, the organism was subcultured onto commonly used laboratory media and incubated at various temperatures. A summary of the results of the growth on artificial media at various temperatures is shown in Table 1. Growth was generally poor, if at all, at 35°C on most media tested, including sheep Trypticase soy agar (BBL) or chocolate agar, which could in part explain the initial failure to isolate the organism on solid media. The best overall growth occurred at 25°C; however, good growth was noted on buffered charcoal yeast extract agar (GIBCO Laboratories, Fairfield, N.J.) after as little as 48 h at all temperatures tested (25, 30, and 35°C). Colonies were round, pink, smooth, and glabrous. The pink pigment was associated with the cells and did not diffuse into the medium. In broth medium the organism grew near the surface, forming a web-like film. The isolate was referred to the Special Bacterial Pathogens Laboratory of the Centers for Disease Control (Robert Weaver) for identification. Characteristics of this isolate of *P. mesophilica* are listed in Table 2 and are consistent with those reported in the literature for this organism (10, 14). Tests were performed at both institutions unless otherwise noted in the table.

Antimicrobial susceptibility testing was performed by the disk-diffusion method on nutrient agar (BBL) with an inoculum size of 10^8 CFU/ml and by macro-broth dilution in 10% yeast extract broth (Difco Laboratories, Detroit, Mich.) with an inoculum size of 5×10^5 CFU/ml. Antibiotic powders were obtained from their respective manufacturers and were diluted and stored according to their instructions. The nutrient agar plates and macro-broth dilution tubes were incubated at 25°C for 48 h before reading. The MICs are shown in Table 3. A good correlation existed between the results of the disk-diffusion test and the macro-broth dilution test. The isolate was susceptible to the aminoglycosides, erythromy-

TABLE 1. Growth of the isolated *P. mesophilica* on artificial media

Medium	Growth[a] at:		
	25°C	30°C	35°C
Brain heart infusion broth	7 D;P	—	—
Buffered charcoal yeast extract agar	2 D;G	2 D;G	2 D;G
Chocolate agar	5 D;P	—	—
Lowenstein-Jensen agar	—	—	—
MacConkey agar	4 D;P	—	—
Mueller-Hinton agar	—	—	—
Nutrient agar	3 D;G	3 D;G	3 D;G
Schaedler broth + K_1	3 D;P	3 D;P	3 D;P
Schaedler agar + K_1 + 5% sheep blood	3 D;G	3 D;G	4 D;P
Trypticase soy broth	4 D;G	5 D;P	7 D;P
Trypticase soy agar + + 5% sheep blood	3 D;G	4 D;P	—

[a] D, Day; G, good growth; P, poor growth; —, no growth in 7 days.

cin, and tetracycline by the macro-broth dilution test, and on the disk-diffusion test it had zones of growth inhibition of at least 10 mm for the aminoglycosides and of at least 30 mm for erythromycin and tetracycline. The isolate was resistant to the majority of penicillins and cephalosporins.

RESULTS AND DISCUSSION

P. mesophilica was originally described as a gram-negative rod which could be found on leaf surfaces (1) and has been called by several names in the past (3, 5, 6, 12, 16, 17). Much work has been devoted to classifying this and related organisms. Recent descriptions of this organism indicate that it is a polar monotrichous gram-negative rod with fewer than three flagella per pole and is characteristically vacuolated and can utilize methanol as a sole carbon source (10, 14). Reports on this organism have been primarily from environmental microbiologists or bacterial taxonomists (1–3, 16, 17).

The patient reported here had severe immunological defects. Both his cellular and humoral immunity were depressed from malnutrition, recent radiation therapy, and two courses of cytotoxic antitumor chemotherapy. However, he was not neutropenic, but had leukocytosis, during the *P. mesophilica* bacteremia. The traceable sources of this organism and the site of infection which lead to bacteremia with *P. mesophilica* in this patient remain unknown. Since the organism can be found on tree leaves and in the soil, the possibility exists that the organism could have been brought into the hospital on plants by his family members or friends. Also the organisms may have been ingested as part of uncooked leafy vegetables and subsequently became locally invasive in the intestinal tract. This organism could have been present in his sputum and pleural fluid samples before death but was not detected by conventional stains or recovered by the conventional microbiological culture methods.

Lambert et al. (11) described a patient with a skin ulcer infected by *P. mesophilica*. Their isolation procedure for this organism was not described in detail; however, it appeared that the organism grew on Penassay agar containing tryptic soy and glycerol at 37°C. Louria et al. (13) reported an "unknown" organism which caused sepsis in three patients, and the description of the organism was consistent with the characteristics of *P. mesophilica*. The sites from which the organism was isolated included blood (two of three patients), lymph node (two of three patients), liver (three of three patients), bone marrow (three of three patients), and several organs at autopsy (one of three patients). Nineteen additional isolates of pink-pigmented oxidative bacteria (11, blood; 3, respiratory tract; 1, cerebrospinal fluid; 1, ascitic fluid; and 3, other sources) have been isolated from humans (10).

There is a parallel between *P. mesophilica* and *Legionella* spp. Both organisms are common in the environment and appear to infect predominently the immunosuppressed patient (9). The isolate of *P. mesophilica* appeared also to grow well on buffered charcoal yeast extract agar, a routine isolation medium for *Legionella* organisms, and in the BACTEC culture media, which have been reported to support the growth of *Legionella pneumophila* (4). The susceptibility pattern of the one clinical isolate of *P. mesophilica* also resembled the pattern for *Legionella* spp., with resistance to penicillins and cephalosporins and susceptibility to erythromycin and the tetracyclines (7, 8, 15). *P. mesophilica*, like *Legionella* spp., may be one of many closely related species of environmental organisms which can occasionally infect the immunocompromised host. Thus far, only the present

TABLE 2. Characteristics of this isolate of *P. mesophilica*

Characteristic or test	Result
Vacuolated rod	+
Flagella: Single polar	+[a]
Growth at:	
5°C	−[b]
25°C	+
30°C	+
35°C	+
42°C	−
Rose or coral pigment	+
Catalase production	+
Indophenol oxidase production	+
Indole production	−
Methyl red reaction (Clark and Lubs)	−[b]
Voges-Proskauer reaction	−[b]
Citrate utilization (Simmons)	+
H_2S production (TSI Agar [BBL])	−
Phenylalanine deaminase (Ewing, Davis, and Reavis)	−
Urease production (Christensen)	−[b]
L-Arginine dihydrolase (Moeller)	−
L-Lysine decarboxylase (Moeller)	−
L-Ornithine decarboxylase (Moeller)	−
Nitrate reduction	−
Nutrient broth:	
0% NaCl	+[a]
6% NaCl	−[a]
Esculin hydrolysis	−[a]
Acid from:	
Glucose (1% OFBM)[c]	−[a]
Lactose	−[a]
Maltose	−[a]
Mannitol	−[a]
Sucrose	−[a]
D-Xylose	−[a]
Susceptible to:	
Ampicillin	−[b]
Amikacin	+[b]
Chloramphenicol	−[b]
Erythromycin	+[b]
Gentamicin	+[b]
Penicillin	−[b]
Tetracycline	+[b]
Tobramycin	+[b]

[a] Test performed only at the Centers for Disease Control.
[b] Test performed only at the Veterans Administration Medical Center.
[c] OFBM, Oxidative-fermentative basal medium.

TABLE 3. MICs for the isolate of *P. mesophilica* performed in yeast extract broth at 25°C

Antibiotic	MIC (μg/ml)
Amikacin	0.25
Ampicillin	>32.0
Cefoperazone	32.0
Cefotaxime	8.0
Cefoxitin	16.0
Cefamandole	32.0
Chloramphenicol	8.0
Clindamycin	2.0
Erythromycin	0.25
Gentamicin	2.0
Oxacillin	>32.0
Penicillin	>32.0
Piperacillin	>64.0
Sulfamethoxazole	512.0
Tetracycline	0.12
Ticarcillin	>64.0
Trimethoprim	>25.6
Trimethoprim/sulfamethoxazole	25.6/512.0
Tobramycin	1.0
Vancomycin	>32.0

case and one other (11) and three probable cases (13) of infections caused by this organism have been described. Additional isolates of pink-pigmented bacteria have been primarily recovered from patients with chronic diseases; however, little other clinical information was available (10). As a result little information is known about the actual diseases caused by this organism.

For the clinical microbiologist, the use of the BACTEC system for blood cultures may give the initial clue to the presence of this or another fastidious organisms by a high radiometric index reading. If a microscopic examination of a Gram stain is negative, then a Wayson stain or a wet preparation of the blood culture medium should confirm the presence of a bacterial organism. Attempts should then be made to isolate the organism so that identification and susceptibilities can be performed. Any organism which is detected microscopically but shows no growth on blood or chocolate agar after 3 days of incubation at 35°C should be subcultured onto additional media, such as buffered charcoal yeast extract agar, Schaedler with K_1, and nutrient agar, and should be incubated at both 25 and 35°C. If this or a similar organism is suspected in an immunocompromised host, a trial of antibiotics to include erythromycin, tetracycline, amikacin, gentamicin, or tobramycin should be attempted.

Alertness of the microbiology personnel to the presence of this organism in blood cultures may be the only way in which this organism can be recovered and identified and the full spectrum of human disease caused by this organism can be defined.

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