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Detection of *Pseudomonas mesophilica* as a Source of Nosocomial Infections in a Bone Marrow Transplant Unit

MARY J. R. GILCHRIST,[1,2]* JEFFREY A. KRAFT,[1] JEANNE G. HAMMOND,[3] BEVERLY L. CONNELLY,[2] AND MARTIN G. MYERS[2,3]

Clinical Microbiology Laboratory,[1] Program in Infection Control,[3] and Division of Infectious Diseases,[2] The Children's Hospital Medical Center, Cincinnati, Ohio 45229

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Pseudomonas mesophilica **was isolated from fungal blood cultures of two bone marrow transplant recipients who consecutively occupied the same room. The isolation of *P. mesophilica* was temporally associated with febrile illnesses in these two granulocytopenic patients at 1 and 3 weeks posttransplant. A third patient, housed separately on the same bone marrow transplant unit, had nasopharyngeal colonization by this organism. Epidemiologic risk factors in common included staff, medications, and oral and perineal irrigations with tap water. Surveillance cultures detected *P. mesophilica* in none of 24 pharmaceutical preparations and in 10 of 40 tap water samples (100 to 600 CFU/ml) from implicated and control rooms on the same floor. Antimicrobial susceptibility testing of 14 patient and environmental isolates by agar dilution revealed similar profiles; some environmental isolates exhibited higher MICs. Because of restrictive nutritional and temperature requirements, *P. mesophilica* is undetected by many clinical laboratory protocols and may represent a previously undetected source of febrile illness in neutropenic patients.**

The organism *Pseudomonas mesophilica* has been described as a pink-pigmented bacterium isolated from leaf surfaces (1). The organism has been reported to occur in clinical specimens (5), in a patient's skin ulcers (4), in blood cultures of debilitated patients from whom there was insufficient clinical information to determine the significance of the observation (2), and in a case of bacteremia in a patient with metastatic adenocarcinoma of the lung (6).

The source of these organisms in the hospital environment is not well defined. Respiratory therapy equipment has been documented to be colonized with *P. mesophilica* (D. Reinhardt, personal communication) but without a known clinical outcome.

In our hospital, a cluster of patients experienced colonization or bacteremia due to this organism, detected as a result of ongoing longitudinal surveillance. Our investigation revealed an environmental source for the organism (tap water), and prevention of exposure to this source has resulted in a 9-month period without recovery of the organism from clinical specimens despite continued microbiologic monitoring.

MATERIALS AND METHODS

Initial clinical isolation of *P. mesophilica* was accomplished by the use of biphasic brain heart infusion medium incubated at 30°C and permanently vented to ambient atmospheric conditions. The time to first detection of isolates ranged from 3 to 29 days of primary culture incubation. The organisms were subcultured to Trypticase soy agar (BBL Microbiology Systems) with 5% sheep blood, chocolate agar, brain heart infusion agar with 5% sheep blood, and anaerobic blood agar and incubated at 30°C for 4 to 5 days without the appearance of visible growth; successful subculture was first achieved on Middlebrook and Cohn 7H11 agar incubated at 30°C for 3 to 5 days. Subsequent transfers were to nutrient agar; all strains grew adequately on the medium at 30°C. The isolates were identified by the Minitek (BBL) system employing the biochemical parameters described by the Centers for Disease Control (7).

Media for the isolation of *P. mesophilica* from clinical and environmental specimens were assessed by using 4 clinical and 10 environmental isolates. Overnight growth from a plate of nutrient agar incubated at 30°C was dispersed in Mueller-Hinton broth to a turbidity equivalent to a McFarland standard of 0.5, diluted 1:10 in Mueller-Hinton broth, and replicated onto the surface of the following media: chocolate agar, Trypticase soy agar with 5% sheep blood, Mueller-Hinton agar, brain heart infusion agar, brain heart infusion agar with 5% sheep blood, Bordet-Gengou agar, Sabouraud's dextrose agar, Middlebrook and Cohn 7H11 agar, nutrient agar, legionella isolation agar (buffered charcoal-yeast extract [BCYE]), buffered yeast extract agar (BYE; equivalent to BCYE but without the charcoal), and MacConkey agar. After incubation at 25, 30, and 35°C for 48 h, growth was graded as pronounced, sparse, or absent.

Antimicrobial susceptibility testing was performed by an agar dilution technique. Organisms (48 h of growth on nutrient agar) were suspended in Mueller-Hinton broth to a turbidity equivalent to a 0.5 McFarland standard, diluted 1:10, and replica plated onto the surface of BYE agar containing 1% yeast extract, ACES (*N*-2-acetamido-2-aminoethanesulfonic acid) buffer (pH 6.9), 0.04% L-cysteine, 0.1% alpha-ketoglutarate, and 0.025% ferric pyrophosphate, without charcoal (3). Control strains for antibiotic activity were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213. Control plates containing no antibiotics were used as positive controls, and media unable to support the growth of *P. mesophilica* (Trypticase soy agar with 5% sheep blood, Mueller-Hinton agar) were stamped as contamination controls. All plates were incubated at 30°C without added CO_2. Control results were read at 18 and 48 h. *P. mesophilica* results were read at 48 h. The MIC was read as the lowest dilution of antimicrobial agent (micrograms per milliliter) yielding no growth or a slight haze of growth.

Environmental surveillance cultures were conducted as

* Corresponding author.

follows. Povidone-iodine bottles removed from patient care units were filtered through membrane (0.45-μm pore size) filters. The filters were then rinsed with 1.5% sodium thiosulfate (three 100-ml rinses) and placed onto the surface of nutrient agar plates. Heparinized saline and saline solutions were similarly filtered, but no rinse was done. Used respiratory equipment was cultured by swabbing the interior moist surfaces and plating the swabs onto the surface of nutrient agar. Tap water samples (50 ml) were collected into sterile centrifuge tubes containing 1.0 ml of 1.5% sodium thiosulfate. The tubes were centrifuged for 15 min at 2,000 × *g*. The supernatant was removed by aspiration, the pellet was suspended, and known volumes were plated onto the surface of nutrient agar, 7H11, and BCYE containing polymyxin, anisomycin, and vancomycin or dye, glycine, vancomycin, and polymyxin (Remel, Lenexa, Kans.). The plates were incubated at 30°C for 10 days. Pink and coral colonies were picked for identification.

RESULTS

Case 1. A 21-year-old male received a bone marrow transplant as treatment for acute lymphocytic leukemia. His preparative regimen included total body irradiation and cyclophosphamide. Immediately before the transplantation, the patient developed severe Stevens-Johnson syndrome, thought to be drug induced, with severe exfoliative skin and mucous membrane involvement and profound secretory diarrhea. Skin and mucous membrane care included oral irrigation and sitz baths with tap water. After the transplant, the patient received oral ketoconazole. Because of the extensive dermal and mucosal involvement in association with fever, he was begun early after the transplant on parenteral mezlocillin and cefotaxime. Because of persistent spiking fevers and shaking chills, the antimicrobial therapy was changed to vancomycin and gentamicin. A blood culture obtained on the day antibiotics were changed grew *P. mesophilica*. While receiving vancomycin and gentamicin, his fevers resolved, coincident with his resolving granulocytopenia.

Case 2. An 18-year-old female received a bone marrow transplant as treatment for acute myelocytic leukemia. Before the transplantation, she had total body irradiation, received cyclophosphamide, and was begun on oral trimethoprim-sulfamethoxazole and ketoconazole prophylaxis. One week posttransplant, the patient developed fever in association with her profound granulocytopenia. Although cultures were negative, she was treated with mezlocillin and cefotaxime. Because of persistent fever and granulocytopenia, methicillin and amphotericin B were added. Severe oropharyngeal mucositis and esophagitis developed and were treated with tap water irrigation. The fever and granulocytopenia resolved 5 days before the antibiotics were discontinued. A surveillance blood culture obtained on the last day of antimicrobial therapy grew *P. mesophilica*, for which the patient received no additional therapy; oral trimethoprim-sulfamethoxazole was continued.

Case 3. A 14-month-old female received a second autologous bone marrow transplant for mucopolysaccharidosis I. Her preparative regimen for the second transplant included cyclophosphamide, busulfan, antithyomcyte globulin, and cyclosporine A. The patient received oral polymyxin, trimethoprim-sulfamethoxazole, and ketoconazole prophylaxis. After the transplantation, her course was complicated by fever, prolonged (> 4 weeks) and profound granulocytopenia, pneumonitis, and *Streptococcus mitis* catheter-related bacteremia for which she received parenteral mezlocillin, cefotaxime, and vancomycin at various times. The patient had extensive mucositis but no known exposure to tap water irrigation. Aerobic surveillance cultures detected colonization by *P. aeruginosa* of the nasopharynx and rectum and *P. mesophilica* from nasopharyngeal culture alone. Fever resolved following the institution of vancomycin as treatment for *S. mitis* bacteremia, coincident with recovery from granulocytopenia.

When it was noted that three patients in class A isolation rooms had experienced colonization or infection with the same unusual organism, an epidemiologic investigation was conducted. Risk factors in common included recent receipt of a bone marrow transplant, housing on the same nursing unit, exposure to the same pharmaceutical preparations, and saline-tap water irrigation of disrupted mucous membranes. Environmental surveillance cultures yielded no isolation of *P. mesophilica* from seven povidone-iodine preparations, five heparinized saline preparations, three liquid antipyretic preparations, three respiratory apparatus, and eight miscellaneous pharmaceutical preparations. However, when tap water was sampled from implicated and control rooms on the

TABLE 1. Permissive and restrictive media for 14 isolates of *P. mesophilica*[a]

Medium	Temp (°C)	Growth (no. of strains)		
		Pronounced	Sparse	Absent
Bordet-Gengou agar with 20% sheep blood	25		14	
	30		14	
	35		14	
BCYE	25		14	
	30	14		
	35		14	
BYE	25		14	
	30	14		
	35		14	
Brucella agar with hemin, vitamin K, and 5% sheep blood	25		14	
	30		14	
	35		14	
Chocolate agar	25			14
	30			14
	35			14
Middlebrook and Cohn 7H11 agar	25		14	
	30	14		
	35		14	
Mueller-Hinton agar	25			14
	30		4	10
	35			14
Sabouraud's dextrose agar	25		14	
	30		14	
	35		14	
Schaedler's agar	25		14	
	30		14	
	35		14	
Trypticase soy agar with 5% sheep blood	25			14
	30		8	6
	35			14

[a] Isolates were suspended to 10^7/ml in Mueller-Hinton broth and replicated onto the surface of the medium. Incubation was for 48 h at the temperature indicated.

TABLE 2. Biochemical properties[a] of 14 *P. mesophilica* isolates

Property	Result	% Isolates positive
Pigment	Pink	100
Gram stain	Gram negative	100
Kligler's iron agar	N or K/N	100
Catalase	+	100
Oxidase	+	100
Motility	v[b]	43
Esculin	−	0
Indole	−	0
Urea	+	100
Xylose	+	100
Maltose	−	0
Mannose	−	0
Ornithine decarboxylase	−	0
Lysine decarboxylase	−	0
Arginine dihydrolase	−	0
Nitrate reductase	v	36
ONPG[c]	−	0
Sucrose	−	0
Citrate	v	93
Glucose (anaerobic)	−	0
Glucose (aerobic)	−	0

[a] Determined by Minitek (BBL) methodology.
[b] v, Variable.
[c] ONPG, *o*-Nitrophenyl-β-D-galactopyranoside.

unit, 10 of 40 samples yielded growth of the organism (100 to 600 CFU/ml). Because tap water used for the irrigation of disrupted mucous membranes was implicated as a source of colonization of these patients, subsequent patients have been managed with sterile solutions during the period of profound granulocytopenia. Despite extensive surveillance of subsequent patients, colonization of *P. mesophilica* has not been observed over a period of 9 months.

The *P. mesophilica* appeared to be nonviable when it was first isolated from a clinical specimen. Subculture to any of the routine clinical subculture media failed to result in growth, even when incubation was done at 30°C. Difficulties in subculturing the organisms upon first encounter were a result of their restrictive nutrient and temperature requirements. So that routine clinical microbiology laboratories might readily work with these organisms, a survey was conducted of media routinely available in such laboratories (Table 1). Permissive media included nutrient agar, BCYE, and 7H11. Nonpermissive media included Mueller-Hinton agar, Schaedler's agar, Trypticase soy agar with 5% sheep blood, and chocolate agar. Some environmental isolates exhibited less restrictive nutritional requirements than those of the clinical isolates, exhibiting some growth on Mueller-Hinton agar and Trypticase soy agar with 5% sheep blood. Because the permissive media for *P. mesophilica* may not be routinely available in many laboratories, this organism may be infrequently detected unless exceptional efforts are made.

The biochemical properties of the isolates are shown in Table 2. Inconstant characteristics of the organisms, motility, nitrate reductase, and citrate utilization, were equally distributed among the clinical and environmental isolates. The identities of the clinical isolates were confirmed by the Ohio Department of Health Laboratories. The Gram stain of the original clinical isolates showed a range from swollen, vacuolated gram-negative bacillary organisms to coccoid-to-coccobacillary organisms. Previous publications have emphasized the common occurrence of vacuolation in *P. mesophilica* (1, 2, 6). After repeated passage on laboratory media, most organisms appeared similar on Gram stain; in many Gram stain preparations, the organisms retained some of the crystal violet and appeared partially gram positive. Gram stain appearance is shown in Fig. 1a. Colony morphology exhibited by these organisms began as colorless and progressed over several days to increasingly pink. When

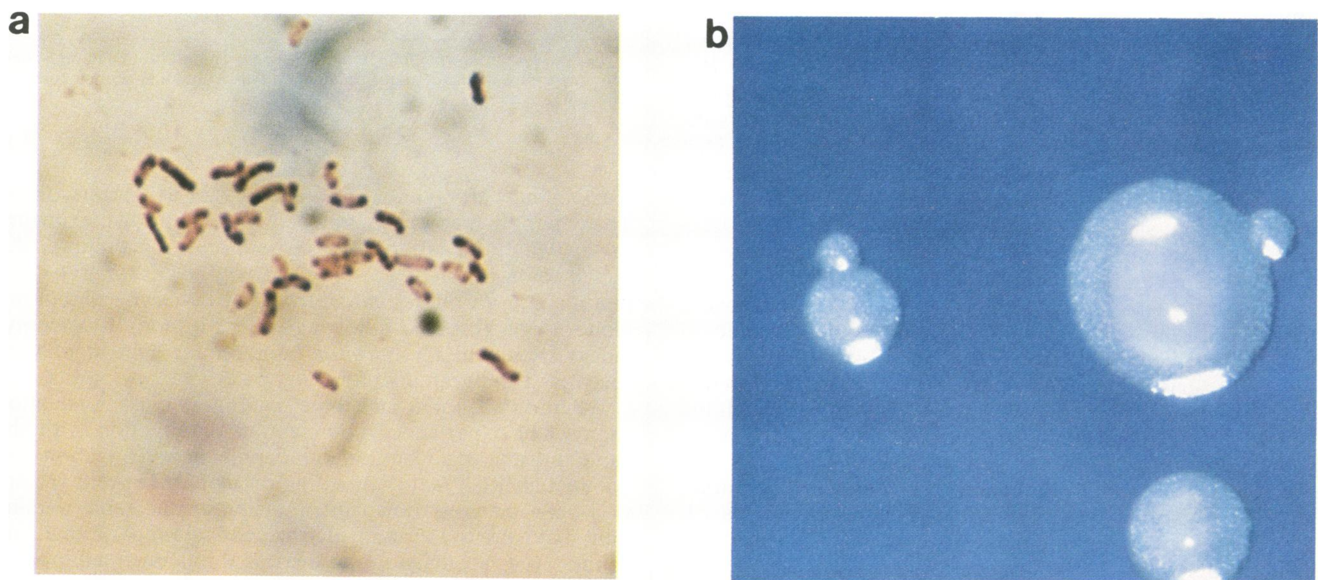

FIG. 1. (a) Appearance of *P. mesophilica* in Gram stain. Upon first isolation from patients, the organisms appeared as swollen, vacuolated gram-negative structures (not shown). Upon passage on laboratory media, the organisms assumed the appearance shown here. In many Gram stain preparations, the organisms appeared partially gram positive, as shown here. (b) Appearance of *P. mesophilica* colonies. Colonies of the organism growing on nutrient agar were intensely pink after several days of growth. When examined by transmitted light under low magnification, young colonies were transparent and colorless with a ground glass appearance. As colonies matured, they developed a pink pigmentation in the center.

TABLE 3. Antibiotic susceptibility of *P. mesophilica* isolates from clinical and enviromental sources[a]

Antibiotic	MIC (μg/ml [range]) for:		MIC_{50}/MIC_{90}[b] (μg/ml) for 14 isolates
	Patient isolates (*n* = 4)	Environmental isolates (*n* = 10)	
Tobramycin	2–8	<2–8	4/8
Gentamicin	<2–8	<2–32	4/8
Amikacin	<8–16	<8–32	8/32
Cefotaxime	<32–64	<32–128	64/128
Mezlocillin	<32–64	<32–64	32/64
Ampicillin	>32	>32	>32/>32
Cephalexin	>64	>64	>64/>64
Chloramphenicol	>32	>32	>32/>32

[a] Overnight growth from nutrient agar was suspended in Mueller-Hinton broth to a McFarland turbidity standard of 0.5 and diluted 1:10 for replication onto BYE agar. Incubation was at 30°C for 48 h.

[b] MIC_{50}/MIC_{90}, MIC for 50 and 90% of isolates tested, respectively.

examined with transmitted light at low power, the colonies had a ground glass appearance when young, and over a period of several days of observation, the centers of the colonies acquired pink pigmentation. These characteristics are useful in screening appropriate colonies for subsequent identification. This appearance is shown in Fig. 1b.

Agar dilution MIC determinations on *P. mesophilica* isolates are shown in Table 3. Broth dilution MIC determinations were attempted, but the organisms grew poorly or not at all in Mueller-Hinton broth. Three of the environmental isolates exhibited greater antimicrobial resistance than isolates from patients, but these isolates were subjected to fewer passages before susceptibility testing. The organisms showed resistance to ampicillin, cephalexin, and chloramphenicol. Most of the isolates were relatively susceptible to aminoglycosides, mezlocillin, and cefotaxime.

DISCUSSION

Patients undergoing bone marrow transplantation at our institution are housed in private laminar air flow rooms or in private class A isolation rooms maintained at positive pressure relative to the hallways from the beginning of preparative irradiation and chemotherapy through the period of profound neutropenia following transplantation. As a consequence of the chemotherapy, radiation therapy, and later graft versus host disease, virtually all patients experience various degrees of mucous membrane disruption. Therapy for this problem has included irrigation of the affected areas with copious quantities of saline. Before this investigation, sterile water was used in the laminar air flow rooms for preparation of the saline, but in the class A isolation rooms saline for irrigation was prepared with tap water, which was implicated as the source of colonization. Of the three patients whose cases are described here, one had nasopharyngeal colonization and two were bacteremic. In at least one of these cases, bacteremia was transient and untreated. In another, bacteremia was symptomatic but cannot conclusively be said to have been causal. All three patients eradicated the organism coincident with recovery from granulocytopenia.

Because of the epidemiologic association of these patients with mucous membrane disruption during exposure to tap water containing *P. mesophilica*, it was recommended that all patients receive minimal exposure to tap water during periods of profound granulocytopenia. In the subsequent months, there have been no additional cases of colonization or infection due to this organism, even though culture frequency and techniques are similar to those used during the time in which the problem was detected.

It is difficult to assess the impact of *P. mesophilica* on nosocomial infections, because most laboratory protocols do not include media, temperatures, or incubation periods appropriate to detect this organism in routine bacterial blood (or miscellaneous) cultures. Indeed, we initially detected *P. mesophilica* in our patients from cultures submitted for the detection of fungi; thus, the culture medium (biphasic brain heart infusion agar), temperature (30°C), and incubation period were sufficient for detection. Few other routine media are adequately sensitive to detect the organism. Laboratories stocking nutrient agar, 7H11, or BCYE can readily isolate the organism. Middlebrook and Cohn 7H11 agar and BCYE with polymyxin, anisomycin, and vancomycin are relatively efficient for selectively isolating the organism in the presence of environmental contaminants, as these media suppress the growth of other floras. Laboratories serving immunocompromised patients may wish to address the need to survey for organisms preferring lower temperatures and unenriched media.

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