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Pseudomonas paucimobilis from a Leg Ulcer on a Japanese Seaman

M. M. PEEL,[1]* J. M. DAVIS,[1] W. L. H. ARMSTRONG,[2] J. R. WILSON,[2] AND B. HOLMES[3]

Microbiological Diagnostic Unit, University of Melbourne, Parkville, Victoria, 3052,[1] and Geelong District Hospital, Geelong, Victoria, 3220,[2] Australia, and National Collection of Type Cultures, Central Public Health Laboratory, London, NW9 5HT, England[3]

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Pseudomonas paucimobilis was isolated in pure culture from an ulcer on the leg of a Japanese seaman while in an Australian port. A description of the isolate is given. This may be the first report of a human infection in which this recently characterized species is implicated as a pathogen.

A yellow-pigmented, nonfermentative, gram-negative, rod-shaped bacterium conforming in its characteristics to the recently described species *Pseudomonas paucimobilis* (5) was isolated from a leg ulcer. This species corresponds to Weaver group IIk biovar 1, formerly classified as a "*Pseudomonas*-like" bacterium (10). Although *P. paucimobilis* has been isolated from a variety of human clinical specimens and the hospital environment, it does not appear to have been incriminated previously as an agent of human infection (5).

CASE REPORT

About 6 weeks before he attended hospital, a 46-year-old Japanese seaman suffered a rope burn injury to his right shin. The abrasion failed to heal, and the area became infected. On examination at presentation, a shallow ulcer was seen immediately above the midshin. The ulcer was about 2 cm^2 in size and had regular edges and a serous discharge. The area surrounding the ulcer was swollen and dull red in color for a distance of approximately 5 cm from the ulcer margin and contained a few serous blisters which were discharging. The right superficial inguinal glands were enlarged and tender, but the patient was afebrile and feeling well. Upon admission to hospital, he was given a single dose of penicillin (1,000,000 U intramuscularly) and started on a regimen of amoxicillin (500 mg, three times daily). However, 1 day after admission, the patient developed an itchy rash over the face, arms, and chest, indicative of a hypersensitivity reaction. Antibiotic therapy was therefore discontinued, and the patient was treated with oral antihistamine while calamine lotion and hydrocortisone were applied to the rash. Eusol and paraffin dressings were applied to the ulcer with the affected leg rested in an elevated position.

The ulcer and associated cellulitis remained unchanged for 1 week and then resolved rapidly. The patient was discharged with the ulcer healed but with some discoloration of the surrounding area as a residuum of the cellulitis.

MATERIALS AND METHODS

Swabs of the ulcer were taken before the administration of antibiotics or the application of antibacterial dressings. Smears were made for Gram staining, and the exudate from the ulcer was cultured aerobically and anaerobically for pathogens. The bacteriological examination was repeated 1 week later, when the ulcer began to subside.

Colonial morphology, including pigmentation, was described from nutrient agar (25 g of Oxoid nutrient broth powder CM67, 3 g of Oxoid yeast extract L21, and 12 g of Davis agar per liter of distilled water), and hemolysis was described from 5% (vol/vol) horse blood agar. Ability to grow anaerobically was tested on nutrient agar by incubation for 2 days in a McIntosh and Fildes jar containing 90% H_2 and 10% CO_2. Motility was determined by the hanging-drop method on 6- and 24-h cultures in nutrient broth (25 g of Oxoid nutrient broth powder CM67 and 3 g of Oxoid yeast extract L21 per liter of distilled water) at 22 and 37°C. The presence and arrangement of flagella were determined by electron microscopy.

Biochemical characteristics were investigated by the methods and media of Cowan (3) unless otherwise stated (see below). Where alternative methods or media are listed in Cowan (3), the procedures used are given in parentheses as follows: acid and gas production from glucose-peptone-water (bromocresol purple indicator; final concentration of carbohydrate, 1 g/100 ml); Thornley arginine hydrolysis (method 2); catalase activity (method 1); esculin hydrolysis (agar); gelatin liquefaction (method 2); indole production (method 2); nitrate reduction (method 1); cytochrome oxidase production (method 1); Tween 80 hydrolysis (method 1); urease production (method 1).

In addition, certain biochemical characteristics were determined according to methods described in references given in parentheses as follows: growth on cetrimide (cetyltrimethylamine bromide) agar (1); growth in the presence of triphenyltetrazolium chlo-

ride (4); formation of poly-β-hydroxybutyrate inclusion granules from basal medium containing β-hydroxybutyric acid as sole carbon source (9), detected by staining with Sudan black B (2).

Tests for antimicrobial agent susceptibility were performed by the Bauer-Kirby standardized single disk method (8).

RESULTS

Examination of the initial Gram-stained smear revealed the presence of pus cells and gram-negative, rod-shaped bacteria in moderate numbers. A strictly aerobic gram-negative, rod-shaped bacterium was isolated in pure culture. No pus cells or bacteria were seen in the specimen taken 1 week later, and cultures were sterile. As only gram-negative rods and pus cells were present and prominent in the original smear, there was no indication for mycological investigation at that time. The original plates were kept for 1 week before being discarded, and this would probably have allowed any fungus to grow, should one have been present.

Colonies on nutrient agar after 48 h were 1 mm in diameter, circular, low convex, opaque, smooth, and butyrous in consistency. A nondiffusible yellow pigment was produced. The isolate grew optimally at 30°C, so all biochemical tests were carried out at this temperature. The isolate also grew at 22 and 37°C, but not at 5 or 42°C. Colonies were nonhemolytic on horse blood agar.

Motility was detected after culture for 6 and 24 h in nutrient broth at 22°C, but was not seen in cultures incubated at 37°C. Bacteria with single polar flagella could be seen by electron microscopy, but only a low percentage (between 5 and 10%) of the bacteria were flagellated in a 24-h culture at 22°C. However, at least twice as many bacterial cells were motile after 6 h of incubation at 22°C, compared with 24 h at that temperature.

The isolate was identified as *P. paucimobilis* on its morphological, physiological and biochemical characteristics. Table 1 shows that the characteristics of the isolate agree with those for which all strains of *P. paucimobilis* give positive or negative reactions, respectively (5). In addition, the isolate gave concordant results for characteristics which were positive in 85% or more of the 29 strains of *P. paucimobilis* examined by Holmes et al. (5) (i.e., Tween 80 hydrolysis, lack of motility at 37°C, acid from ammonium salt sugar-salicin, acid from ammonium salt sugar-ethanol, and formation of poly-β-hydroxybutyrate inclusion granules) as well as for those characteristics which were positive for 70% or more of the strains (i.e., tyrosine hydrolysis, motility at 22°C, and no change in Hugh and Leifson oxidation/fermentation medium). The identity of the isolate as *P. paucimobilis* was confirmed at the National Collection of Type Cultures, Central Public Health Laboratory, London, England, on the results of 68 phenotypic tests in conjunction with an unpublished probability matrix.

TABLE 1. *Comparison of the characteristics of the isolate with those of P. paucimobilis*

For isolate and all strains of *P. paucimobilis*:	Characteristic
Positive	Acid from ASS[a]-arabinose
	Acid from ASS-glucose
	Acid from ASS-lactose
	Acid from ASS-maltose
	Acid from ASS-sucrose
	Acid from ASS-xylose
	Catalase activity
	Cytochrome oxidase production
	Deoxyribonuclease production
	Esculin hydrolysis
	β-Galactosidase production
	Growth at 22°C
	Growth at 37°C
	Growth on triphenyltetrazolium chloride
	Production of yellow pigment
Negative	Acid from PWS[b]-glucose
	Acid from ASS-inositol
	Acid from ASS-mannitol
	Anaerobic growth
	Arginine hydrolysis
	Casein digestion
	Fluorescence on King medium B
	Gas from PWS-glucose
	Gelatin liquefaction
	Growth at 5°C
	Growth at 42°C
	Growth on cetrimide agar
	Growth on MacConkey agar
	Growth on Simmons citrate
	Hydrogen sulfide production[c]
	Indole production
	KCN tolerance
	Nitrate reduction
	Opalescence on lecithovitellin agar
	Phenylalanine deaminase
	Urease production

[a] ASS, Ammonium salt sugar medium.
[b] PWS, Peptone water sugar medium.
[c] Triple sugar iron agar.

The isolate was susceptible to tetracycline, kanamycin, gentamicin, sulfamethoxazole, chloramphenicol, carbenicillin, and tobramycin. It was resistant to ampicillin, cephalothin, streptomycin, and colistin.

DISCUSSION

A characterization of 47 strains of yellow-pig-

TABLE 2. *Major characteristics for differentiation of P. paucimobilis from other yellow-pigmented, gram-negative, rod-shaped bacteria which may be isolated from clinical material*

Bacterium	Differential characteristic										
	Acid from trehalose ammonium salt sugar medium[a]	Casein digestion[a]	Cytochrome oxidase production[a]	Esculin hydrolysis[a]	Growth on MacConkey agar[a]	Growth on Simmons citrate[a]	Hugh and Leifson oxidation/fermentation test[b]	Motility at room temp[a]	Nitrite reduction[a]	Poly-β-hydroxybutyrate inclusion granules[a]	Urease production[a]
Pseudomonas paucimobilis	+	−	+	+	−	−	−	+	−	+	−
Pseudomonas cepacia	+	+	+	+ or −	+	+	O	+	−	+	+ or −
Pseudomonas stutzeri	−	−	+	−	+	+	O	+	+	−	+ or −
Flavobacterium breve (7)[c]	−	+	+	−	+	−	O or −	−	−	−	−
Flavobacterium meningosepticum/group IIb (10)	+	+	+	+	+	−	O	−	−	−	−
Flavobacterium odoratum (6)	−	+	+	−	+	−	Alk	−	+	−	+
Enterobacter agglomerans[d]			−		+	+ or −	F	+			−
Group IIk biovar 2 (10)[c]	+	−	+	+	+	−	O	−	−	−	+
Group Ve (10)[c]	+	+ or −	−	+ or −	+	+	O	+	−	−	+ or −

[a] +, ≥85% of strains positive; −, ≤15% of strains positive.
[b] Alk, Alkali producton; F, fermentative reaction; O, oxidative reaction; −, no change to medium.
[c] These taxa appear to be encountered only infrequently in clinical material.
[d] Synonym: *Erwinia herbicola*.

mented, nonfermentative, gram-negative, rod-shaped bacteria from clinical specimens, the hospital environment, and other sources, in comparison with 51 reference strains of *Pseudomonas* and six other genera of gram-negative bacteria by Holmes et al. (5) showed that 29 of the 47 strains formed a homogeneous phenetic group. It was concluded (5) that this group should be considered a new species, for which the name *P. paucimobilis* was proposed. The 29 strains included 2 strains of Weaver group IIk biovar 1 (10), indicating that *P. paucimobilis* and Weaver group IIk biovar 1 are probably identical taxa.

The characteristics of the isolate described here conform to those of *P. paucimobilis*. Salient features that differentiate *P. paucimobilis* from other yellow-pigmented, gram-negative, rod-shaped bacteria which may be isolated from clinical material are given in Table 2.

The isolation of *P. paucimobilis* under the circumstances described above is of particular interest because its presence in pure culture indicates a probable pathogenic role. Moreover, failure to recover the same strain from the ulcer after it began to resolve is further evidence that the original isolate was responsible for the infection. Although specific mycological investigations were not undertaken, we found no evidence to suggest that the infection could be of fungal origin. Hitherto, there has been no evidence to incriminate the strains placed in *P. paucimobilis* by Holmes et al. as agents of infection in humans, despite their isolation from clinical material and the hospital environment (5). The occurrence of this organism in respirators and similar equipment suggests that water is its primary source (5).

A water source of contamination is probable in the case of the Japanese seaman, and it is likely that the trauma associated with the rope burn predisposed the injured leg to infection by this opportunistic pathogen.

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