Pseudomonas putrefaciens as a cause of infection in humans

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SYNOPSIS *Pseudomonas putrefaciens*, a strongly H₂S-producing pseudomonad, was isolated from 10 human infections over a two-year period. In one patient the organism was repeatedly isolated from a phlegmone developing in the depth of a varicose leg ulcer. This is the first report on the occurrence of *Ps. putrefaciens* in humans outside the USA and the first to provide the detailed account of a clinical observation where the opportunistic pathogenic role of this unfamiliar organism has been sufficiently documented.

Data are presented on the bacteriological properties and on the antibiotic sensitivity of *Ps. putrefaciens*.

Although *Pseudomonas aeruginosa* is by far the most common pathogenic species of the genus *Pseudomonas*, several other species have recently attracted the attention of the clinical bacteriologist.

Recognition of these species has long been hampered by the lack of a clear classification system for nonfermentative Gram-negative bacteria and by the considerable confusion in their nomenclature. Improvements in bacteriological methods and the increased incidence of opportunistic and nosocomial infections have stimulated interest in *Pseudomonas* species other than *Ps. aeruginosa*. Simple criteria for characterization of unusual *Pseudomonas* species have been presented in a series of well-documented papers (Gilardi, 1971, 1972; Von Graevenitz, 1973).

Although easy to recognize because of its abundant hydrogen sulphide production, *Ps. putrefaciens* is one of the least well-known pseudomonads in medical bacteriology, and we have seen no mention of the isolation of this species in the European literature.

The first human isolates of a nonfermenting, H₂S-producing Gram-negative rod with polar flagellation were described by King in 1964, who gave them the provisional label 'lb'. In 1970, von Graevenitz and Simon reported 13 isolations of the same microorganism from different clinical speci-

mens. It was recognized by Hugh (1970) that the 'lb' bacterium, isolated by the previous observers, was identical with *Ps. putrefaciens*. This bacterium, first isolated from butter, was originally described under the epithet of *Achromobacter putrefaciens* (Derby and Hammer, 1931) and later transferred to the genus *Pseudomonas* (Long and Hammer, 1941). Similar bacteria were described by other investigators under different names—*Pseudomonas rubescens* (Pivnick, 1955) and *Flavobacterium* group 4 (Pedersen *et al*, 1970). These epithets are now believed to be synonymous with *Ps. putrefaciens*. More recently, a subdivision of the species into two biotypes on the basis of some growth and metabolic properties has been proposed (Levin, 1972) and independently supported (Riley *et al*, 1972).

This report presents data on the isolation and the clinical significance of this unusual pseudomonad in a large clinical laboratory. The history of the single patient, in whom the pathogenicity of this bacterium could be unequivocally demonstrated, is given in greater detail.

Material and Methods

The strains were recovered from clinical specimens submitted to the laboratory for routine bacteriological examination. All strains were isolated on MacConkey agar and attracted attention by the combination of blackening on Kligler iron agar and a positive oxidase reaction. Final identification was based on routine biochemical tests (Edwards and

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Received for publication 7 July 1975.

Ewing, 1972). Sensitivity testing was done using the Bauer-Kirby single disk method (Bauer *et al*, 1966).

Results

From February 1972 to December 1974, four strains (excluding repeated isolations from the same patient) of *Ps. putrefaciens* were obtained from clinical specimens submitted to our laboratory. An additional six cultures were received for identification or further investigation from four other Belgian hospitals. Clinical data on these isolates are summarized in table I.

In cases 1 and 3, repeated isolations from the sputum over a relatively long interval proved that the bacterium was not an accidental laboratory contaminant nor a transient saprophyte, but that the respiratory tract of both patients was actually colonized with this microorganism. However, its aetiological role in the causation of infection could not be established. In cases 5 and 7 subsequent urine cultures were negative for *Ps. putrefaciens*. Only in patient 9 was there convincing evidence that *Ps. putrefaciens* had invaded the tissues and that it acted as an opportunistic human pathogen. The history of this patient is therefore presented in full below.

Case history (patient 9)

On 19 September 1974 an 82-year-old woman was admitted as an emergency case to our hospital after an undefined form of syncope or transient attack. The patient was in a poor general condition and physical examination showed bilateral oedema of the legs. Laterally on the right leg confluent ulcerations

were seen surrounded by a warm erythematous infiltrated zone. The ulcerations covered an area of approximately 10 cm diameter. On the supramalleolar side of the left leg there were also many small ulcerations forming a confluent plaque of about 4 cm diameter. These ulcers were covered with a fibrinoid necrotic purulent exudate, in part showing a tendency towards granulation. The aspect of the surrounding skin was definitely erysipeloid, with wrinkled and scaly skin as typically seen in elephantiasis. The dermatological condition of the patient was classified as an 'erysipelas-like complication of varicose ulcers of the leg'.

Interrogation was difficult but revealed that the patient had suffered for more than 16 months from open wounds on both legs for which she had repeatedly refused hospitalization. She lived alone, in a state of severe domestic squalor, in extreme self-neglect, surrounded by cats, pigeons, and rabbits, and was cared for from time to time by a visiting home help. Her physician prescribed intermittent courses of oral penicillin and chloramphenicol for treatment of the infected leg ulcers.

On admission a swab of the exudate taken from the deep ulcer on the right leg was brought to the laboratory. Culture of this material gave a 'profuse growth of *Pseudomonas* sp. and coagulase-negative staphylococci'. Urine culture revealed a significant count (more than 10^5 organisms per ml) of *Escherichia coli* and *Proteus vulgaris* in the absence of pyuria. Other relevant laboratory findings are summarized in table II.

Treatment consisted of oral nitrofurantoin tablets (Furadantin MC, 4 × 100 mg per day) for the urinary infection, later replaced by co-trimoxazole (Eusaprim 2 tablets twice a day), which showed good

Case	Date	Age	Sex	Primary Diagnosis	Culture Source	Originating Laboratory
1	Feb 1972	48	F	Liver cirrhosis and lung tuberculosis	Sputum ¹	Leuven
2	Mar 1972	20	F	Amygdalitis	Tonsillar pus	Bruges (Dr. E. Callewaert)
3	Jan 1973	69	M	Tuberculosis and chronic bronchitis	Sputum ²	Bonheiden (Dr. R. Van Noyen)
4	Feb 1973	2	M	Urinary infection after indwelling catheter postop. for phimosis	Urine (> 10^4 bacteria/ml)	Bruges (Dr. H. Van Landuyt)
5	Apr 1974	51	F	Chronic pyelonephritis (phenacetin abuse?)	Urine (20 000 b/ml)	Bruges (Dr. E. Callewaert)
6	May 1974	?	F		Sputum	Aalst (J. Hoegaerts)
7	July 1974	30	F	Pregnancy; asymptomatic bacteriuria	Urine (> 10^4 b/ml) associated with <i>E. coli</i> and <i>Acinetobacter</i>	Leuven
8	Aug 1974	25	F	Cystitis	Urine (> 10^4 bacteria/ml) associated with <i>Enterobacter</i>	Bruges (Dr. E. Callewaert)
9	Sept 1974	82	F	Lymphoedema and varicose phlegmone of leg	Wound exudate ³	Leuven
10	Dec 1974	40	F	Acute bronchitis	Sputum	Leuven

Table I Clinical data on human *Ps. putrefaciens* infections

¹This strain was again isolated seven weeks later

²Three successive isolations with one week and two week intervals

³Six isolations over a period of one month

	Sept 19	Sept 25	Oct 15	Oct 24
Erythrocyte sedimentation rate (mm l ⁻¹ h ⁻¹)		70		15
White cell count/mm ³	16,200			5,500
Serum sodium (mEq/l)	131.5			140.5
Serum potassium (mEq/l)	3.2			4.5
Total plasma proteins (g/dl)		7	6.7	
Albumin		39.7%	51.2%	
Globulins				
α_1		6.0%	2.3%	
α_2		15.9%	10.5%	
β		16.8%	16.9%	
γ		21.6%	19.2%	
SGOT (IU/l)		42.5		7.5
SGPT (IU/l)		51.5		8
Serum bilirubin (mg/dl)		2.7		0.6
C. Reactive protein (mm)		4.5	0	0

Table II Selected laboratory data of case 9

in vitro activity against the different microorganisms grown from the urine and the exudate. The ulcers were dressed with chloramine cream. The patient was further given a high-protein diet supplemented with a mixture of vitamins and minerals.

On 27 September the deepest ulcer on the right leg was incised and abundant pus oozing from an underlying phlegmone was obtained. Culture showed a profuse and pure growth of a pseudomonad which was now fully identified as *Ps. putrefaciens*, susceptible to co-trimoxazole. Two days later, control swabs were again cultured with exactly identical results.

Under the combined local and general treatment, the ulceration on the left leg healed completely within three weeks. On the right leg, however, a rather superficial ulceration with a regenerating base persisted. Swabs taken from this site on 9, 10, and 11 October gave no growth.

Antibiotic treatment was stopped on 18 October. A week later a new swab was taken from the small residual ulcer left on the right leg. Culture was again positive for *Ps. putrefaciens*. Although no further general antibiotic treatment was given, cultures on 30 October and 11 November were again sterile.

Under the effect of further local treatment the lesion progressively healed and the patient was discharged on 22 November with slight oedema of the legs and a marked improvement of her general condition and biochemical serum values (table II).

Bacteriology

The growth characteristics and the biochemical reactions of the 10 strains isolated from our patients showed a high degree of similarity. All were composed of Gram-negative rods of variable length, growing abundantly on plain bacteriological culture media. Colonies on blood agar were convex, circular, smooth, and opaque with a tan-yellow to brown

pigment. A green discolouration of the medium was seen in the zone of heavy growth. On MacConkey agar the organism grew as mucoid, tan, lactose non-fermenting colonies. All strains grew readily on tubes of Kligler iron agar without production of acid. The most salient feature of *Ps. putrefaciens*, grown on this medium, was the heavy blackening of the stabbed butt due to production of H₂S. This character, generally evident after overnight incubation, masked the red colour of the butt and explains why this growth pattern was generally first mistaken for *Proteus*, *Salmonella* or *Citrobacter*.

With one exception, all strains were actively motile by means of one polar flagellum. Other biochemical reactions of our cultures are given in table III.

On the basis of their salt tolerance, assessed by growth in Trypticase soy broth containing 6% NaCl, and their growth on *Salmonella-Shigella* agar, our 10 isolates could be equally distributed between the two groups postulated by Levin (1972).

Only five strains, including the isolate from our case 9, were submitted to a full susceptibility test. The results are shown in table IV.

Oxidase	+	Phenyl-alanine deaminase	0
Catalase	+	Malonate	0
Growth on citrate	0	Lysine decarboxylase	0
Nitrate reduction	+	Arginine dihydrolase	0
Indole production	0	Ornithine decarboxylase	+
Gelatinase	+	Beta-galactosidase	0
Urea hydrolysis	0 (weak)	OF medium: oxidation of	
Aesculin hydrolysis	0	glucose, maltose, lactose,	
DNase	+	xylose	Variable
	(0)	Mannitol	0

Table III Biochemical reactions of *Ps. putrefaciens*

() exceptional reaction

Sensitive	Variable	Resistant
erythromycin	ampicillin	penicillin G
tetracyclines	cephalosporins	methicillin
chloramphenicol	carbenicillin	lincomycin
colistin	sulphonamides	
nitrofurane	streptomycin	
co-trimoxazole		
kanamycin		
gentamicin		

Table IV Antibiotic susceptibility of five strains of *Ps. putrefaciens*

Discussion

Ps. putrefaciens is a saprophyte with a wide distribution in nature. It has been isolated from butter, milk, fish, eggs, stream and lake water, sewage, natural gas, and petroleum brines and has been reported as a spoilage organism in butter and fish (Long and Hammer, 1941; Hugh, 1970).

Among the 22 isolates, collected at the Center for Disease Control, Atlanta, Georgia and listed by King (1964), several came from human sources—stored blood, spinal fluid, urine, faeces, wounds, sputum, and ear discharge. Among the 13 strains, described by von Graevenitz and Simon (1970), five were from patients with chronic otitis, four originated from chronic leg ulcers, three from sputum, and one from urine. Two of the strains described by Pedersen and associates (1970) were recovered from the faeces of premature infants and one from a foot wound. Although 12 clinical isolates were mentioned by Gilardi, only two strains were documented: one was associated with otitis media and a second was recovered from the wound of an infected open fracture of the tibia.

From all the published isolations, only one strain, repeatedly isolated from a chronic otitis media, was said to be aetiologically linked to the disease state in which it was cultivated (von Graevenitz and Simon, 1970).

In all the literature reports and in the majority of our own observations, the clinical information is so poor that no general conclusion can be drawn on the clinical significance of *Ps. putrefaciens*. In most cases it can be judged that the organism was a contaminant or, at most a saprophyte, thriving as a secondary invader on previously damaged tissues.

The present report contains the first known documented case of an infectious process where the aetiological significance of *Ps. putrefaciens* could be clearly demonstrated by its presence, over a long period of time, in a deep phlegmone, underlying a varicose ulcer of the leg.

Although widely distributed in nature, *Ps. aeruginosa* is best known as a highly resistant, hospital-acquired, opportunistic pathogen responsible for life-threatening infections in predisposed patients after major surgery, extensive burns, and steroid and antibiotic treatment. The disease spectrum associated with *Ps. putrefaciens* is clearly different. Recorded infections were generally benign and commonly limited to the superficial mucosae, the damaged skin, and the bladder urine. Most of our cases seem to have acquired their infection in the community, a point that had already been stressed by von Graevenitz and Simon (1970). Antibiotic resistance was not considered a problem and most of our strains, as were those of other observers (Pedersen *et al.*, 1970; von Graevenitz, 1973), were highly susceptible to common antibiotics, even to erythromycin, which in general is not active against Gram-negative bacilli.

Varicose leg ulcers are frequently infected with *Ps. aeruginosa* even outside the hospital (Verburg-van der Zwan and Siem, 1973). There is evidence

from our observation and from the experience of others (von Graevenitz and Simon, 1970) that *Ps. putrefaciens* shares the same affinity for necrotic tissue with poor circulation. The psychrophilic nature (von Graevenitz, 1973) of the latter organism makes it particularly well adapted to multiply in the colder, devitalized tissues of a varicose ulcer on the lower extremities. In our patient, the lack of personal hygiene and the intimate contact with pet animals may in turn have contributed to the contamination of the leg ulcer with soil saprophytes.

The authors are deeply indebted to Dr. R. E. Weaver (CDC, Atlanta), who kindly confirmed the identity of three isolates. They are also grateful to their Belgian colleagues who were responsible for the isolation of six of the strains reviewed in this article.

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