Proteus penneri Isolated from the Pus of a Patient with Epidural Abscess

Zhongxing LI, Xiuhua WANG, Zhanshui BIAN, Shumin LI, Heping ZHENG,
Baozhen ZHAO and Jingpo CHEN

Bacteriological Laboratory, The Second Affiliated Hospital, Hebei Medical College, Shijiazhuang, China

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Abstract

P. mirabilis and *P. vulgaris* are the two wellknown species in the genus *Proteus*. *P. myxofaciens* and *P. penneri* are recent additions to the genus. We isolated *P. penneri* from the pus of a patient with suppurative otitis media and an epidural abscess. The characteristics of the organism, including morphology, staining, physiology and biochemistry, were studied. Clinical microbiological laboratories should suspect *P. penneri* in the case of as *Proteus* strain that is negative for indole, salicin and esculin, but otherwise resembles *P. vulgaris*.

Proteus penneri, formerly known as *Proteus vulgaris* indole-negative or as *Proteus vulgaris* biogroup 1, was named by Hickman et al in 1932. Little information about human infection by this organism is available. In 1982, Hickman and co-workers studied 20 strain of *P. penneri* which were isolated from clinical specimns (urine, stool, etc.) in the USA. However, its clinical significance, until recently, was unknown¹⁾. We isolated a strain of *P. penneri* from the pus of a patient with suppurative otitis media and an epidural abscess on June 10 and 15, 1989. This paper concerns the problems encountered in identifying this organism and its clinical significance.

Case Report

A 17-year-old male was admitted to our hospital on June 10, 1989, with a 2-week history of headache, high fever and occasional vomiting. The patient had taken antibiotics with some improvement in symptoms, but headache was not relieved. There was a rather ill-defined 8-year history of left suppurative otitis media with acute episodes as well as epidural abscess.

Examination: T. 37.5°C, P. 84/min, R. 20/min, BP. 110/70 mmHg. The patient was well-developed and moderately well-nourished. The skin and sclera showed no xanthochromia. The lungs and heart were normal. The liver and spleen were not palpable. Neurological examination showed left incomplete facial paralysis. The left mastoid region was mildly swollen a purulent secretion was evident on the surface of the drum membrane. Diagnosis: suppurative otitis media with epidural abscess and otogenic meningitis.

Radical mastoidectomy was carried out on June 10, 1989. 150 ml of yellowish-green putrid pus was drained from the epidural space. The pus was sent immediately for bacteriological examination and a second specimen of the purulent secretion was cultures on the fifth day after the operation. Both cultures grew *P. penneri*. Based on susceptibility testing, the patient was given co-trimoxazole and gentamicin and discharged after recovery.

Fig. 1 Colonies of *P. penneri* on blood agar

Fig. 2 Flagellum of *P. penneri*

Isolation and Identification

The pus was inoculated directly on to blood agar according to standard methods. After 24 hours of incubation at 35°C, it produced moderate (2 mm), convex, circular colonies with smooth edges (Fig. 1). Swarming appeared after the third generation of subculturing. Gram staining of the isolate revealed Gram-negative bacilli. Motility was determined by direct microscopic examination of a wet mount and by a flagella stain, which showed active motile rods with peritrichous flagella (Fig. 2).

The isolate was oxide negative, and produced phenylalanine deaminase and urease. On Kligler agar, there was acidification of glucose with a small bubble of gas, lactose was not present and a slant culture turned dark red, with blaking of the medium due to the abundant production of H₂S. We assigned the isolate to the genus *Proteus*, and performed additional biochemical tests for further identification. The results were positive for ornithine, sucrose and maltose, but still did not permit unequivocal identification

Table 1 Characteristics differentiating three biogroups of *P. vulgaris*

	Biogroup 1	Biogroup 2	Biogroup 3
Indole	—	+	+
Salicin	—	+	—
Esculin	—	+	—

Table 2 Differential characteristics of *P. penneri* and other *Proteus*⁴⁾

	<i>P. mirabilis</i>	<i>P. myxofaciens</i>	<i>P. vulgaris</i>		
			<i>P. penneri</i>	Biogroup 2	Biogroup 3
Indole production	—	—	—	+	+
Ornithine decarboxylase	+	—	—	—	—
Maltose fermentation	—	+	+	+	+
Salicin fermentation	—	—	—	+	—
D-Xylose fermentation	+	—	+	+	+
Esculin hydrolysis	—	—	—	+	—
Occurs in human clinical specimens	+	—	+	+	+
Occurs as pathogen of gypsy moth larvae	—	+	—	—	—
Chloramphenicol disk	S	S	R	V	S

S: susceptible; R: resistant; V: variable

Table 3 Biochemical reaction of *P. penneri*

Test	Reaction	Test	Reaction
Indole	—	Inositol	—
MR	+	Lactose	—
VP	—	Maltose	+
Citrate (Simmons)	—	D-Mannitol	—
H ₂ S	+	D-Mannose	—
Urea	+	Melibiose	—
Phenylalanine	+	Raffinose	—
L-Lysine	—	L-Rhamnose	—
L-Ornithine	—	Sorbitol	—
L-Arginine	—	Salicin	—
Motility	+	Sucrose	+
Gelatin	+	Trehalose	+
KCN	+	Xylose	+
Malonate	—	Esculin	—
Glucose: Acid	+	Mucate	—
Gas	+	Acetate	—
Acid from:		NO ₃ -NO ₂	+
Adonitol	—	DNase (25°C)	+
Arabinose	—	35°C	+
D-Arabitol	—	Oxidase	—
Cellobiose	—	ONPG	—
Dulcitol	—	Swarming on blood agar	+
Galactose	+	C+O content of DNA	38.3mol%
Glycerol	+		

of the isolate as either *P. vulgaris* or *P. mirabilis*. We suspected that this organism represented a new species of *Proteus*.

In Bergey's Manual of Systematic Bacteriology (Vol. 1 1984), the genus *Proteus* includes three species, *P. vulgaris*, *P. mirabilis* and *P. myxofaciens*. The latter, which does not produce H₂S, has not been isolated from human clinical specimens.^{2,3)}

Hickman et al studied DNA hybridization of *P. vulgaris* and found three biogroups within the species, one of which was indole-, salicin- and esculin-negative. They indicated that this group probably represented a new species of *Proteus* and referred to it as *P. vulgaris* biogroup 1. Differentiation among the 3 biogroups shown in Table 1. Our isolate was similar to *P. vulgaris* biogroup 1. *P. penneri* sp. nov was proposed as the name for this biogroup by Hickman in 1982.

We performed a series of biochemical tests, listed in Table 2, to further identify the present isolate. The results showed that this strain and *P. penneri* had identical characters. In view of the original isolation of this organism in China, we performed further biochemical tests and confirmed the isolate as *P. penneri* (Table 3).

Antibiotic susceptibility was tested by the Kirby-Bauer disk method, showing that this organism was susceptible to gentamicin, co-trimoxazole and kanamycin, intermediate to carbenicillin and neomycin, and resistant to ampicillin, etc.

Discussion

P. mirabilis and *P. vulgaris* are the two well-known species in the genus *Proteus*. Two other species, *P. myxofaciens* and *P. penneri* are more recent addition to the genus. *P. penneri* was not included in Bergey's Manual of Systematic Bacteriology (Vol. 1, 1984), because it was described after the submission date for the

chapter on *Proteus*. However, it has recently been recognized that the genus *Proteus* is composed of four species⁴⁾.

In 1981, Hickman et al studied the DNA hybridization groups of *P. vulgaris* and indicated that one group (indole-, salicin-, and esculin-negative) probably represented a new species of *Proteus*. They did not give the species a scientific name, referring to it instead as *P. vulgaris* biogroup 1. In addition, there are two other biogroups: *P. vulgaris* biogroups 2 which is indole-, salicin-, and esculin-positive, and biogroups 3, which is indole positive, and salicin-, and esculin-negative.

In 1982, the DNA hybridization of the three biogroups of *P. vulgaris* was once again studied by Hickman et al. A highly related group was formed (88 to 99% relatedness at 60°C and 67 to 96% relatedness at 75°C) when DNA from *P. penneri* strain 1808-73 was labeled and tested against unlabeled DNA from 13 other *P. penneri* strains. The relatedness of unlabeled DNA from the 14 *P. penneri* strains to labeled DNA from the type strain of *P. vulgaris* and from biogroups 2 strains was determined. These strains were not related to the *P. penneri* strains at the species level, and strains of biogroups 2 were not highly related to the type strain of *P. vulgaris*. For this reason, Hickman proposed that the strains that had previously been designated *P. vulgaris* biogroups 1 should be elevated to the species level within the genus *Proteus*. and emphasized that low relatedness by DNA hybridization and phenotypic differences were the main criteria for proposing the new species. The name *Proteus penneri* was coined to honour John L. Penner, a Canadian microbiologist who made many contributions to the study of the three genera in the tribe *Proteus*.

The type strain of *P. penneri* was CDC 1803-73 (ATCC33519). The G + C content was 38 mol% (our strain, 38.3 mol%).

P. penneri has the following common characteristics of the genus *Proteus*: positive tests for urea hydrolysis, phenylalanine deaminase, gelatin hydrolysis, H₂S production, and swarming on blood agar (our strain, after the third generation of subculturing); and negative results for D-mannose fermentation. The following tests are positive for most strains: methyl red, motility, growth in the presence of KCN, and fermentation of D-galactose, maltose scurose and D-xylose; the others are negative for all or most *P. penneri* strains. Clinical laboratories should suspect *P. penneri* in the case of a *Proteus* strain that is indole-, esculin- and salicin-negative, and resistant to chloramphenical (zone size, 14 mm).

The clinical significance of *P. penneri* is unknown, although the 20 strains reported by Hickman et al included 9 isolated from urine, 4 from stool, 1 each from blood, abdominal wound, and respiratory tract, and 4 from unspecified sources. The ecology of this organism and its role in human disease will be substantiated as clinical information is accumulated. The present case involved a patient who had suffered from chronic suppurative otitismedia for eight years. Some inappropriate antimicrobial agents were administered, so that the patient's condition worsened with epidural abscess and otogenic meningitis. Therapy with surgery and effective antibiotics following bacteriological susceptibility testing resulted in rapid resolution of the infection.

References

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Proteus penneri による硬膜外膿瘍の一例

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症例は17歳男性，2週間来持続する頭痛および高熱，悪心により入院す。入院時現症は体格中等度，栄養良好，皮膚および眼球結膜に黄疸を認めず，体温37.5℃，脈拍84/分整，血圧110/70mmHg，聴打診上では異常なく，腹部所見では肝，脾触知せず，その他の異常は認めなかった。神経学的には左側の不完全顔面神経麻痺を認めた。局所所見としては，左乳様突起部位に軽度の腫張を認め，鼓膜表面に化膿性分泌物を認めた。既往歴に8年

来の左化膿性中耳炎を罹患していることより，硬膜外膿瘍および耳原性髄膜炎を合併した化膿性中耳炎と診断，根治的乳様突起切除術を施行した。硬膜下腔より150mlの膿汁を採取，細菌検査により *P. penneri* を検出，感受性試験により co-trimoxazole と gentamicin を使用し軽快，退院した。*P. penneri* は *P. vulgaris* として分類されていたが，1982年 Hickman らにより尿，糞便より検出されたがその臨床的意義は不明である。