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Spontaneous Bacterial Peritonitis Due to a Group IIk-2 Strain

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This paper describes a patient with spontaneous bacterial peritonitis caused by a group IIk-2 strain. No other organism was isolated from the peritoneal fluid cultured aerobically and anaerobically.

Spontaneous bacterial peritonitis is a well-recognized entity (3). Organisms usually responsible for the disease are *Escherichia coli* and other enteric gram-negative rods (13, 18, 20) as well as *Streptococcus pneumoniae* (3, 5, 20). *Staphylococcus aureus* and *Pseudomonas aeruginosa* are less commonly associated with this syndrome (4, 22), and the finding of anaerobic bacteria as etiological agents is unusual (13, 18, 20, 22). Various individual case reports in the literature implicate several other organisms as being associated with this disease, including *Neisseria meningitidis* (1, 2), *Listeria monocytogenes* (17), and *Pasteurella multocida* (4, 7, 12).

This report describes a patient with spontaneous bacterial peritonitis caused by an as yet unnamed bacterium, Center for Disease Control group IIk-2. To our knowledge there has been no previous association of this bacterium to human disease. This report demonstrates the pathogenic potentialities of this organism.

Case Report. A 60-year-old white male with a history of alcoholic liver disease, diagnosed by liver biopsy in 1974, was hospitalized at Cook County Hospital on 19 April 1979, with complaints of nausea, vomiting, and vague abdominal discomfort of 2 weeks duration. He fell down twice after drinking heavily the night before admission which resulted in exacerbation of his abdominal pain and prompted him to come to the hospital. He denied history of fever, cough, constipation, diarrhea, hematemesis, or urinary symptoms. He had a past history of suicidal attempt by ingesting liquid Drano (a strong alkaline corrosive) in 1973. This resulted in esophageal stricture requiring dilation twice in 1974, with no subsequent dysphagia. He had been working as a watchman for the last 7 years.

Physical examination revealed the patient to be in no acute distress, with a temperature of 100.4°F (ca. 38.0°C), pulse rate of 110/min, respiratory rate of 22/min, and a blood pressure of 130/80 mmHg (ca. 10.664 kPa). His eyes were jaundiced. Abdominal examination revealed diffuse tenderness without guarding but with definite shifting dullness. No hepatosplenomegaly was noted and the rectal examination was normal with a negative stool guaiac test. Two small areas of ecchymosis were noted, one on the left anterior chest wall and the other on the skin over the left upper abdomen. The remainder of the physical examination was unremarkable.

On admission his white cell count was 14,400/mm^3, with 48% polymorphonuclear leukocytes and 38% band forms. The other laboratory data were as follows: blood glucose, 141 mg/100 ml; blood urea nitrogen, 15 mg/100 ml; serum sodium, 129 meq/liter; potassium, 4.1 meq/liter; chloride, 89 meq/liter; bicarbonate, 21.2 meq/liter; calcium, 7.5 mg/100 ml; amylase, 115 U/100 ml; total bilirubin of 5.8 mg/100 ml with 3.7 mg/100 ml being direct; albumin, 3.3 g/100 ml; globulin, 4.3 g/100 ml; alkaline phosphatase, 80 IU/ml; serum glutamic oxalacetic transaminase, 53 U/ml; and serum glutamic pyruvic transferase, 30 U/ml. The hemoglobin concentration was 13.9 g/100 ml, and the platelet count was 78,000/mm^3. Prothrombin time and partial thromboplastin time determinations were normal. Routine urinalysis showed only trace proteinuria and an occasional leukocyte per high-power field. Roentgenogram of the chest revealed borderline cardiomegaly, clear lung fields, and multiple old healed rib fractures on the right side. Four views of the abdomen were normal. Electrocardiogram showed sinus tachycardia with nonspecific ST-T changes.

A clinical diagnosis of spontaneous bacterial peritonitis was considered and abdominal paracentesis was performed, with aseptic precautions, soon after admission. The peritoneal fluid revealed a specific gravity of 1.027, a leukocyte count of 10,400/mm^3, an erythrocyte count of 20,000/mm^3, a protein content of 5.6 g/100 ml, and an amylase of 944 U/100 ml. The Gram stain revealed no organisms, and the fluid was cultured both aerobically and anaerobically. The patient was then placed on intravenous ampicillin, 2.0 g every 6 h. On the day after his admis-

sion, the patient developed delirium tremens and became tachypneic with bilateral chest rales. Arterial blood gases revealed a severe hypoxemia, necessitating mechanical ventilation. Roentgenogram of the chest taken subsequently showed an infiltrate in the left lower lobe, thought to represent an aspiration pneumonia. Intravenous gentamicin, 80.0 mg every 8 h, was added to the therapeutic regimen.

On April 23, the peritoneal fluid was reported to be growing an aerobic, gram-negative rod which was found by Kirby-Bauer methodology to be susceptible to tetracycline, carbenicillin, and trimethoprim-sulfamethoxazole but resistant to ampicillin, amikacin, gentamicin, chloramphenicol, cephalothin, and cefamandole. The bacterium was reported as an unidentified gram-negative, aerobic, nonfermentative rod and was forwarded to the Illinois Department of Public Health Laboratory for further identification. No other organism was recovered from the peritoneal fluid, and blood and urine cultures remained negative. In view of the patient's continuing fever and worsening abdominal pain, and the known resistance of the isolate to the antimicrobial agents being used, intravenous carbenicillin in a dosage of 20.0 g/day was substituted for ampicillin on April 24, while gentamicin was continued. The patient started to show improvement shortly thereafter, the hypoxemia resolved, and he was extubated on April 26. On April 27 he was noted to be afebrile for the first time, and his abdominal pain and tenderness had markedly diminished. Also, the abdominal girth decreased as he continued to show progressive clinical improvement. On May 5, the antibiotics were discontinued, and he remained afebrile until his discharge from the hospital on May 10. On follow-up examination 1 month later the patient was asymptomatic.

MATERIALS AND METHODS

Peritoneal fluid was collected aseptically before antimicrobial therapy. The fluid was sedimented and Gram stained. It was also cultured on 5% sheep blood agar plates (BBL Microbiology Systems) that were incubated in a candle extinction jar and on eosin methylene blue agar plates, and was inoculated into brain heart infusion broth as well as fresh thioglycolate broth. All cultures were incubated at 35°C.

Hemolysis and colonial morphology were determined from the blood agar plates. Pigment formation was studied on Mueller-Hinton agar plates (Difco Laboratories formulation) at both room temperature (20 to 22°C) and at 35°C. Growth on MacConkey agar was also determined at both temperatures. Growth at 42°C, 35°C, and room temperature was assessed on tryptose agar slants. Motility was studied by both wet preparations and by modified Fontana flagellar stains, both at 35°C and at room temperature. The oxidase test was performed by the Kovacs method and the catalase test with 3% H_2O_2.

Biochemical tests were performed with the API 20E system (Analytab Products, Inc.). Additional biochemical tests were production of acid from glucose (Corning O-F), production of deoxyribonuclease (DNase Test Agar W/Methyl Green; Difco), and esculin hydrolysis (Bile Esculin Agar, Difco).

Susceptibility tests were performed by the microbroth dilution method using microtiter plates (U-bottom, 8-by-12-well) (Cooke Engineering) and trypticase soy broth. An inoculum size of 5.5×10^5 organisms per ml was used. In addition, agar diffusion tests were performed by the Kirby-Bauer method.

RESULTS

Gram stain of the peritoneal fluid did not reveal organisms. On culture, a coccobacillary, aerobic, gram-negative, nonhemolytic bacterium was isolated after 48 h on sheep blood agar plates incubated at 35°C. The colonies were 1.0 mm in diameter and were smooth and discrete without obvious pigmentation. Also, in the same time span, small, colorless colonies were discernible on eosin methylene blue agar plates. On subculture, the strain grew well on Mueller-Hinton agar plates and produced, at both 35°C and at room temperature, a yellow, nondiffusible pigment after 48 h. No growth occurred on tryptose agar slants incubated at 42°C.

Motility tests were done on 6- to 8-h brain heart infusion broth cultures and from blood agar plates after incubation at both 35°C and room temperature. The initial impression was that motility was present at room temperature but not at 35°C, both on wet preparations and on flagellar stains. However, multiple subcultures on various media did not reveal motility at either temperature, a fact confirmed independently by both B. Holmes and R. E. Weaver (personal communications).

For biochemical characterization, the API 20E strip was incubated at 35°C and read after 48 h. Only the *o*-nitrophenyl-β-D-galactopyranoside and the urease tests were positive. Additional biochemical tests revealed that the following were positive: catalase, oxidase, esculin hydrolysis, and glucose oxidation. Results of all tests performed are shown in Table 1.

Results of susceptibility tests performed with 16 antimicrobial agents are given in Table 2. It is seen that the isolate was resistant to all aminoglycosides but, of the β-lactam antibiotics, it was susceptible to carbenicillin, piperacillin, and cefamandole. The strain was also susceptible to clindamycin, chloramphenicol, and trimethoprim-sulfamethoxazole.

As additional work was being performed in our laboratory, the Illinois Department of Public

TABLE 1. *Characteristics of group IIk organisms*

Test	Biotype 1[a,b]	Biotype 2[a]	Our isolate
Growth on tryptose agar at:			
42°C	−	−	−
35°C	NL	NL	+
Room temperature (20–22°C)	NL	NL	+
Growth on MacConkey agar	−	+	+
Motility (20–22°C)	V	−	−
Pigment (yellow) on:			
Blood agar	+	∓	−
Mueller-Hinton agar	NL	NL	+
Urease:			
Christensen agar	−	+	ND
API 20E	NL	NL	+
Oxidase	+	+	+
Catalase	+	+	+
β-Galactosidase	+	+	+
Esculin hydrolysis	+	+	+
Glucose oxidation	+	+	+
Indole	−	−	−
Citrate utilization	−	−	−
Gelatinase	−	−	−
Deoxyribonuclease	∓	−	−
Nitrite production	−	∓	−
Arginine dihydrolase	−	−	−
Lysine, ornithine decarboxylase	−	−	−

[a] Data derived from reference 8; also, see reference 21, p. 282.

[b] +, Most strains positive within 2 days; −, no reaction; ∓, most strains negative; V, variable; NL, not listed; ND, not done.

TABLE 2. *In vitro susceptibility of our strain of IIk-biotype 2 to 16 antimicrobial agents*

Antimicrobial agent	MIC[a] (μg/ml)	MBC[b] (μg/ml)
Penicillin	62.0	500.0
Ampicillin	31.0	250.0
Carbenicillin	40.0	40.0
Piperacillin	8.0	31.0
Cephalothin	31.0	500.0
Cefamandole	8.0	15.0
Cefoxitin	31.0	62.0
Gentamicin	31.0	125.0
Tobramycin	>500.0	>500.0
Amikacin	125.0	250.0
Kanamycin	>500.0	>500.0
Streptomycin	125.0	250.0
Clindamycin	<0.25	62.0
Chloramphenicol	4.0	>500.0
Trimethoprim-sulfamethoxazole	<0.06/1.16	4/74
Colistin	>500.0	>500.0

[a] MIC, Minimum inhibitory concentration.

[b] MBC, Minimum bactericidal concentration.

Health laboratory reported that the isolate belonged to Center for Disease Control group IIk. Because of a few key reactions that were somewhat troublesome and germane to definitive identification, such as the production of urease and of deoxyribonuclease, growth on MacConkey agar, and questionable motility, subcultures of the strain were forwarded for further study to B. Holmes and to R. E. Weaver. Multiple analyses by both and further tests by us confirmed the identity of the strain as group IIk-biotype 2. As yet, no specific name has been proposed for this organism.

DISCUSSION

The strains of bacteria belonging to group IIk were described as *Pseudomonas*-like bacteria and were subdivided into two biotypes (21), biotype 1 for which the name *Pseudomonas paucimobilis* has been recently proposed (11) and biotype 2 which is as yet unnamed. Both biotypes are aerobic, nonfermentative, gram-negative rods that grow on nonenriched media, often producing a nondiffusible yellow pigment. The principal characteristics of both biotypes and of the strain isolated from our patient are shown in Table 1. It is evident that the principal difference between biotypes 1 and 2 is the ability of biotype 2 to grow on MacConkey agar and to produce urease. In these respects our isolate conforms to biotype 2. As reported by Dees et al. (6), the cellular fatty acid compositions, as determined by gas-liquid chromatography, of *Pseudomonas paucimobilis* and of biotype 2 are distinctly different, as is the base composition of their deoxyribonucleic acid [11; E. Yabuuchi, I. Yano, E. Tanimura, and A. Ohyama, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C(H)32, p. 351]. The gas-liquid chromatography was performed on our isolate by S. Dees and was found to closely resemble the pattern of the biotype 2 group. The deoxyribonucleic acid base composition of our strain (38.4 mol% of G+C [guanine plus cytosine]) was determined by R. J. Owen and was found to be the same as the value reported for other strains of biotype 2 [11; Yabuuchi, et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C(H)32, p. 351].

Antimicrobial susceptibility data have not been published for group IIk-2 organisms, although Holmes (11), on the basis of unpublished results, states that strains are resistant to a wide range of antibiotics including amoxicillin, chloramphenicol, erythromycin, gentamicin, kanamycin, and novobiocin.

Group IIk strains have been isolated from various clinical specimens and from the environment (9–11, 14–16). *Pseudomonas paucimobilis*

(biotype 1) has been implicated in the production of an indolent leg ulcer in a Japanese seaman (16), as a cause of meningitis (10) and as a cause of septicemia (19). Although isolated from various clinical specimens, to our knowledge there have been no reports implicating biotype 2 as a causative agent of human disease.

The isolation of a strain belonging to group IIk biotype 2 in pure culture from a closed cavity (peritoneum), the lack of response clinically to the initial antimicrobial therapy to which the isolate was found to be resistant, and the subsequent response to carbenicillin, to which the strain was known to be susceptible, strongly suggest the etiological role of the isolate in this patient with spontaneous bacterial peritonitis and demonstrates the pathogenic potentialities of this organism.

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