Halophilic, Lactose-Positive *Vibrio* in a Case of Fatal Septicemia

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A halophilic *Vibrio* species was isolated from blood cultures from a 59-year-old male with enteritis. The strain differed from *Vibrio parahaemolyticus* and *Vibrio alginolyticus* by its ability to ferment lactose, its production of β -galactosidase, and its lower NaCl tolerance. A report of this infection and a description of the isolate is presented.

Halophilic vibrios have often been isolated from seawater and other marine sources, especially seafood (1, 4, 10). The best known species are *Vibrio parahaemolyticus* and *Vibrio alginolyticus*. The former is a common human pathogen causing gastroenteritis and, less often, tissue infection (1, 4, 8, 11, 16). The latter has been associated with otitis and also with wound infections after exposure to ocean water (4, 7, 12, 13, 16). A third halophilic *Vibrio*, isolated from blood cultures, spinal fluid, and soft tissue infections (5, 6, 15), has been defined by Hollis et al. (6). This halophilic organism is similar or identical to *Beneckea vulnifica* described by Reichelt et al. (9) and has been referred to as lactose-positive (Lac⁺) *Vibrio* because the ability to ferment lactose is the main characteristic that distinguishes this species from *V. parahaemolyticus* and *V. alginolyticus*.

Only a few reports with full clinical information correlated with full bacteriological identification of halophilic lactose-fermenting *Vibrio* septicemia are listed in the literature. We report on such a case.

CASE REPORT

A 59-year-old male patient was admitted to the medical intensive care unit on 18 September 1977 for septic shock. Five years previously, he underwent prosthetic mitral valve replacement. He was well until 3 days before admission, when he became acutely ill, presenting chills, nausea, abdominal cramps, and watery diarrhea. The next day the abdominal pains persisted and became even more intense, necessitating the administration of analgetics. No antibiotics were given. The situation remained unchanged until the night before admission when the patient suddenly became confused and developed muscle weakness on the right side. He was then

transferred to our hospital. Neither a history of seafood consumption nor information on any contact with seawater was available; the patient's town of residence is situated 45 miles (ca. 72.4 km) from coastal waters. On admission his temperature was 39.6°C (103.3°F), and his blood pressure was 110/80 but degraded soon thereafter; his heart rate was 130 beats per min. His right upper and lower limbs were paralyzed. His skin felt wet and cold. Both forearms were edematous, indurated, and cyanotic up to the elbows. Bluish inflammatory cocardiform lesions (diameter, 5 to 10 cm) were noticed on his legs and buttocks. Heart rhythm was irregular and accelerated. The sounds of the prosthetic valve were heard, but no murmur was observed. Examination of the lungs was normal. No enlargement of the liver or spleen was found. The abdomen was tender and painless by palpation. At 2 h after admission the patient died in shock with acute left heart failure, resisting all therapeutic means.

At the autopsy, segmental necrosis of a 20-cm portion of the small intestine was found without abnormalities in the intestinal blood vessels. Microscopically, these lesions corresponded with massive abscess formation in the intestinal wall. However, the role of Lac⁺ *Vibrio* as the cause of necrotizing enteritis could not be determined since no stool cultures or autopsy cultures of the small intestine were taken. The skin lesions consisted of significant fibrinous exudate and hemorrhage and contained massive numbers of bacteria and leukocytes. A thrombosis of the left femoral vein was found. No abnormalities were seen at the prosthetic mitral valve.

RESULTS

Six blood cultures were taken. From 11 bottles we obtained a gram-negative slightly curved rod which later was identified as a Lac⁺ *Vibrio* spe-

cies as defined by Hollis et al. (6).

The strain grew readily on horse blood agar after overnight incubation, forming smooth colonies approximately 2 mm in diameter. There was a clear zone of hemolysis around the colonies and a greenish discoloration of the blood cells in the area of confluent growth, which became more prominent after 48 h of incubation.

Table 1 shows the biochemical reaction of our strain (strain H 1447) and the percentage of positive reactions for *Lac*⁺ *Vibrio* as listed by Hollis et al. (6). Most of the media and procedures used in testing our strain have been described previously (3, 4, 6, 10, 14). All media were supplemented with a 10% NaCl solution to obtain a final concentration of 1%. NaCl tolerance was determined by using nutrient broth (Difco Laboratories) containing different percentages of NaCl. Indole production was detected by using tryptophan broth (Difco), SIM medium (Difco), and urea-indole medium (Bio-Mérieux) as substrates; Kovacs reagent was added after extraction of the culture fluids with toluene.

The results of the susceptibility tests by the method of Bauer et al. (2) revealed sensitivity to penicillin (22 mm), ampicillin (25 mm), carbenicillin (28 mm), cephalothin (23 mm), kanamycin (22 mm), gentamicin (26 mm), tobramycin (21 mm), tetracycline (32 mm), chloramphenicol (34 mm), co-trimoxazole (30 mm), and sulfonamide (25 mm), but resistance to colistin. The identification of our strain has been confirmed by R. E. Weaver (Center for Disease Control, Atlanta, Ga.; strain 78-034820). No stool or autopsy cultures were performed.

DISCUSSION

The key reactions that differentiate *Lac*⁺ *Vibrio* species from *V. parahaemolyticus* and *V. alginolyticus* are fermentation of lactose and production of β -galactosidase (6). The other distinctive biochemical reactions are listed in Table 2. Our strain seems to be closely related to those studied by Hollis et al. (6) but not exactly identical, since it does not produce indole and is somewhat less NaCl tolerant. The susceptibility to antimicrobial agents also distinguishes this *Lac*⁺ *Vibrio* from the other halophilic *Vibrio* species. *Lac*⁺ *Vibrio* is sensitive to penicillin, ampicillin, and carbenicillin and resistant to colistin, whereas *V. parahaemolyticus* and *V. alginolyticus* are resistant to penicillin, carbenicillin, and ampicillin; susceptibility to colistin varies in these species (6).

Hollis et al. feel that *Lac*⁺ *Vibrio* is taxonomically distinct from *V. alginolyticus* and *V. para-*

TABLE 1. Biochemical characteristics of strain H 1447 and comparison with other *Lac*⁺ vibrios

Test or substrate	Reaction with strain H 1447 ^b	% Positive predicted ^a
Oxidase	+	100
Fermentation of glucose	+	100
Growth on:		
MacConkey agar	+	100
SS agar	—	39 (8)
Nitrate reduction	+	100
Citrate (Simmons) alkaline	+	76 (11)
Urease (Christensen)	—	0
Gelatinase	+	97
Moeller decarboxylase medium		
Lysine	+	97 (3)
Arginine	—	0
Ornithine	+	66 (26)
Indole	—	97 (3)
ONPG	+	100
Voges-Proskauer	—	0
Growth in nutrient broth:		
Without NaCl	—	0
With 4% NaCl	+	ND ^c
With 5% NaCl	—	ND
With 6% NaCl	—	100
Motility	+	100
Acid from:		
D-Glucose	+	100
D-Mannitol	—	66
Lactose	(+)	8 (16)
Sucrose	—	3
Maltose	+	100
Salicin	+	100
D-Galactose	+	100
D-Fructose	+	100
D-Mannose	+	100
Trehalose	+	100
Cellobiose	+	100
Starch	+	100
Inositol	—	0
L-Rhamnose	—	0
D-Xylose	—	0
Raffinose	—	0
L-Arabinose	—	0
Melibiose	(+)	26 (53)
Adonitol	—	0
Dulcitol	—	0
Esculin hydrolysis	+	ND
Tetrathionate reduction	—	ND
Malonate	—	ND
Phenylalanine deaminase	+	ND
Sensitivity to vibrio-static compound 0/129	+	ND

^a Percentage of positive reactions for *Lac*⁺ *Vibrio* as listed by Hollis et al. (6).

^b +, Positive reaction; —, negative reaction; (+), positive delayed reactions (delayed for 3 or more days).

^c ND, Not done.

TABLE 2. Differentiation of *Lac*⁺ *Vibrio* species, *V. parahaemolyticus*, and *V. alginolyticus*

Test or substrate	Reaction with <i>Lac</i> ⁺ <i>Vibrio</i>	Reaction with <i>V. parahaemolyticus</i>	Reaction with <i>V. alginolyticus</i>
Lactose	+	—	—
ONPG	+	—	—
Mannitol	+ or —	+	+
Sucrose	—	—	+
Voges-Proskauer	—	—	+
NaCl tolerance at:			
8% NaCl	—	+	+
10% NaCl	—	—	+

ahaemolyticus (unpublished data). This is supported by their unique antibiogram as listed above. Deoxyribonucleic acid relatedness studies also confirm these findings (9). Different clinical entities seem to be related to different halophilic *Vibrio* species. *V. parahaemolyticus* is known as a cause of gastroenteritis (1, 4). *V. alginolyticus* has been isolated from cases of otitis (7, 13, 16). Both species have been found to be a cause of wound infection (4, 7, 8, 11–13, 16), but septicemia occurs very rarely (17, 18). On the other hand, *Lac*⁺ *Vibrio* causes fulminating septicemia with a fatal outcome in most instances (5, 6, 15). Some of the fulminant septicemias due to *V. parahaemolyticus* reported in the literature (5, 15) turned out to be *Lac*⁺ *Vibrio* as demonstrated by Hollis et al. (6). Septicemia resulted from skin infections or gastroenteritis after contact with seawater or ingestion of contaminated seafood, although this bacterium has never been isolated from stools. However, because of the fermentation of lactose and the production of β -galactosidase, it can easily be missed by routine stool culture procedures. We therefore suggest that oxidase tests always be performed on isolates from Kligler iron agar (or TSI agar) that show anaerogenic fermentation of glucose, even when there is acidification of the slant.

In our case, the patient had a history of acute gastroenteritis and autopsy showed necrotizing enteritis, although *Lac*⁺ *Vibrio* as the cause of the infection could not be determined because no stool cultures or autopsy cultures of small intestine were taken.

In many reports of bacteremia with *Lac*⁺ *Vibrio*, there is an underlying disease, such as diabetes, alcohol abuse, or liver disease (5, 15, 18). In our case, no such condition was present. Most impressive were the skin lesions which microscopically correspond to bacterial invasion;

a thrombosis of the left femoral vein was found at the post mortem examination. These findings have been reported by others (5, 15). We think that this case presents the typical features of *Lac*⁺ *Vibrio* species infection. The presence of septic shock with fatal outcome and impressive inflammatory skin lesions evokes the possibility of *Lac*⁺ *Vibrio* infection.

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