Lactose-Positive *Vibrio* in Seawater: a Cause of Pneumonia and Septicemia in a Drowning Victim

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Lactose-positive *Vibrio* is a recently recognized marine organism that has pathogenic potential for humans. An organism was isolated from the sputum and blood of a man who was resuscitated after drowning in the sea. The isolates from both sources had the characteristics of lactose-positive *Vibrio*, which include positive oxidase, citrate, indole, and *o*-nitrophenyl-β-D-galactopyranoside reactions and negative Voges-Proskauer, urease, and sucrose reactions. Seawater samples from 21 sites around Galveston Island were cultured for lactose-positive *Vibrio* over a period of 4 weeks, and 36% of the samples yielded the organism. The environmental isolates were very similar to the clinical isolates in biochemical reactions and susceptibility to antimicrobial agents. The results indicate that lactose-positive *Vibrio* is a common organism in the marine environment and that it should be considered in the diagnosis of infections, including pneumonia, associated with exposure to the sea.

Marine vibrios of pathogenic potential are being increasingly recognized. *Vibrio parahaemolyticus* is an important cause of gastroenteritis (1) and an occasional cause of other types of infections (6). *V. alginolyticus* may be found in association with wound infections (7). A third *Vibrio* species has recently been described in association with septicemia acquired from the marine environment via the gastrointestinal tract or superficial wounds (3, 5). This marine *Vibrio* is distinguished by its ability to ferment lactose, and it is referred to as the lactose-positive (Lac⁺) *Vibrio* (4). This report describes a case of pneumonia and septicemia due to Lac⁺ *Vibrio* after inhalation of seawater. We also describe the results of a survey prompted by this case, which indicate that the Lac⁺ *Vibrio* is a common inhabitant of marine environments.

**MATERIALS AND METHODS**

**Case report.** On 28 May 1979, a 44-year-old black male was seen in the emergency room after being found floating face down in the sea near Galveston Island. The past history was unremarkable, and in particular, the patient had no history of previous liver or lung disease. Examination on admission revealed absent blood pressure, pulse, and respirations. Arterial blood gases showed marked hypoxia. After intubation and resuscitation, the blood pressure was 140/80 mmHg, and the pulse rate was 140/min. The patient was transferred to the intensive care unit and maintained on 50% inspired oxygen. The hypoxia was corrected, and the blood pressure was maintained at 106/66 with dopamine. Examination after resuscitation revealed diffuse pulmonary rales and wheezes and severe neurological impairment.

The patient’s condition remained stable until 2 June 1979, when he developed fever of 39.5°C. Blood and sputum cultures were obtained, and therapy with tobramycin was instituted. The following day, four of four blood cultures had growth of slightly curved gram-negative bacilli, and the sputum cultures demonstrated growth of a similar organism. The patient became afebrile after 3 days of tobramycin therapy, but suffered a fatal cardiac arrest on 7 June 1979.

**Culture, identification, and antimicrobial susceptibility.** Blood specimens were cultured in Columbia broth and thioglycolate broth (GIBCO Diagnostics, Madison, Wis.) at 37°C. Macroscopically positive cultures were transferred to Columbia agar with 5% sheep blood, and isolated colonies were identified with the API system (Analytab Products, Inc., Plainview, N.Y.). The API identification was confirmed using tubed biochemical media. Two or 3 drops of a sterile 3% NaCl solution was added to the tubed media to aid the growth of the Lac⁺ *Vibrio* isolates. Sputum specimens were cultured on Columbia agar with 5% sheep blood, eosin methylene blue agar, and chocolate agar. Supplemental NaCl was not added to any of the primary plating media. Isolated colonies were identified as described above. Susceptibility of the isolates to antimicrobial agents was determined by disk diffusion testing, as previously described (2).

**Environmental studies.** Twenty-one sites in the intertidal zone of the coastal region of Galveston Island were chosen for the sampling of seawater. The island is situated in a semitropical environment in the Gulf of Mexico. Water temperature during the study ranged from 29.5 to 31.5°C, and the salinity of the water varied from 6.5 ppt (mg/g) to 19.6 ppt at the different sampling sites. Each seawater sample was cultured for marine vibrios within 1 h of collection. For culture, 0.1 ml of each sample was diluted in 20 ml of sterile 3% saline. The diluted samples were then
passed through 0.45-μm-pore size membrane filters (Millipore Corp., Bedford, Mass.), and the filters were laid on the surface of thioulate-citrate-bile salts-sucrose agar plates. The plates were incubated at 37°C for 18 to 24 h, and the number of clear green to bluish-green colonies was recorded. These colonies were transferred to blood agar plates for further testing. Hemolysis was recorded, and the isolates were tested for cytochrome oxidase. Oxidase-positive organisms were screened by the o-nitrophenyl-β-D-galactopyranoside (ONPG) test (Pathotec ONPG) using a heavy inoculum. ONPG-positive isolates were tested on API strips and in tubed biochemical media for confirmation of identification as Lac⁺ Vibrio.

RESULTS
Clinical isolates. Gram-negative rods found in the blood cultures exhibited pleomorphism ranging from typical curved, gram-negative rods to large, bulbous, swollen forms. After 24 h, subcultures on blood agar produced smooth colonies of 2-mm diameter that showed slight greening of the medium. The predominant organism detected in sputum cultures had characteristics identical to the isolates from the blood. Subcultures of the isolates from blood and sputum on thiosulfate-citrate-bile salts-sucrose medium produced clear, bluish-green colonies. The biochemical characteristics of these isolates are presented in Table 1. The isolates from blood and sputum had the same biochemical reactions, and the reactions were consistent with those previously described for Lac⁺ Vibrio (4). These isolates also had similar antibiotic susceptibility profiles (Table 2). Both isolates were susceptible to ampicillin, chloramphenicol, gentamicin, tobramycin, tetracycline, and trimethoprim-sulfamethoxazole. They were indeterminate for carbenicillin, cephalothin, and kanamycin and resistant to colistin.

Environmental isolates. From 15 July to 15 August 1979, samples from 21 sites around Galveston Island were analyzed for the presence of Lac⁺ Vibrio. The sites included the popular swimming beaches as well as shellfish-gathering and fishing areas. Each site was sampled five to seven times, and a total of 129 samples was analyzed. Overall, 36% of the samples yielded Lac⁺ Vibrio in quantities of up to 500 bacteria per ml of seawater, and the organism was detected in 20 of the 21 sites. Five of the sampling sites repeatedly gave positive cultures. These sites represented rocky shoreline along a deep-water channel with heavy shipping traffic (three sites) or polluted, cloudy, calm water in a harbor (two sites).

The environmental isolates were all ONPG, citrate, indole, and oxidase positive, and they gave negative reactions for sucrose fermentation, urease, and Voges-Proskauer tests. Eight repre-
including positive oxidase, citrate, and indole tests and negative sucrose, Voges-Proskauer, and urease reactions. The Lac+ Vibrio is distinguished from *V. parahaemolyticus* by a positive ONPG reaction (4). The isolation of the Lac+ Vibrio from the sputum of our patient after inhalation of seawater suggests that the organism was acquired from the sea, and isolation of the same organism from the blood suggests that sepsis developed secondary to pulmonary invasion by the Lac+ Vibrio.

The apparent acquisition by this patient of a Lac+ Vibrio infection after inhalation of seawater prompted us to investigate the frequency of occurrence of the organism in the marine environment. We found that the organism was readily recovered from the waters around Galveston Island. Lac+ Vibrio was isolated at least once during the study from 20 of the 21 sampling stations, and 5 of the stations repeatedly gave positive cultures. The organisms isolated from seawater were biochemically identical to the clinical isolates, and they demonstrated similar antibiotic susceptibility as well. These results suggest that Lac+ Vibrio occurs frequently in the marine environment and that these organisms may be potentially pathogenic for humans.

A recent report described two types of infection associated with Lac+ Vibrio (3). One group of patients developed septicemia after consumption of raw oysters. These patients generally had a history of impaired liver function, and they developed severe, life-threatening infections. Another group of patients developed Lac+ Vibrio infections of superficial wounds after exposure to seawater, and some of these patients also had documented bacteremia. One other report described a case of fatal septicemia due to Lac+ Vibrio in a previously healthy patient, but the source of this infection was unknown (5). These findings indicate that Lac+ Vibrio infections may be acquired via the gastrointestinal tract or superficial wounds. The findings of the case described in the present report suggest that pneumonia due to Lac+ Vibrio may be a complication of near drowning and that septicemia may develop via the lung as well as via the gastrointestinal tract or superficial wounds. These findings also confirm the observation (5) that septicemia due to Lac+ Vibrio may develop in previously healthy individuals.

The association of Lac+ Vibrio infections with exposure to seawater or seafoods has prompted suggestions that this organism may be common in the marine environment (3). The results of the present study provide direct evidence that Lac+ Vibrio is a common organism in the waters around Galveston Island. The frequency of occurrence of this organism in seawater indicates that a significant potential for infection exists, and the case described here suggests that pneumonia and septicemia in resuscitated drowning victims may be one previously unrecognized manifestation of Lac+ Vibrio infection.

In conclusion, the results of the present study indicate that Lac+ Vibrio is a common organism in the marine environment and that this organism should be considered in the evaluation of patients with infections, including pneumonia, associated with exposure to seawater.

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**LITERATURE CITED**


