Anaerobic Vibrio-Like Organisms Cultured from Blood: *Desulfovibrio desulfuricans* and *Succinivibrio* Species

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Two unusual anaerobic vibrio-like organisms were recovered from blood cultures of two patients. One isolate was identified as *Desulfovibrio desulfuricans*. It appeared to be the cause of a 24-h episode of fever, chills, and profuse perspiration. This is apparently the first documented report of human infection due to this organism. The second isolate was a *Succinivibrio* species. It has rarely been described as a cause of bacteremia. The clinical significance of the organism remains unclear.

Anaerobic vibrio-like organisms have rarely been reported from human clinical specimens. In particular, only one case of *Succinivibrio* infection has apparently been reported (10), and no cases of *Desulfovibrio* infection have been described in the literature. The role of such anaerobic vibrios in human bacteriology is unknown. *Succinivibrio* have been considered rumen bacteria (1), whereas *Desulfovibrio* are sulfate-reducing bacteria that exist in practically every type of soil and water (2). This report describes the clinical histories of two patients with bacteremia caused by anaerobic vibrios. The morphological and biochemical properties of the organisms are presented.

CASE REPORTS

Case 1. The patient was a 67-year-old white male who had had several admissions to the Long Beach Veterans Administration Hospital for diffuse epigastric and chest pain. In mid-February, the patient was admitted for chest pain and a syncopal episode. Laboratory studies showed elevated liver function tests (total bilirubin, 5.0 mg; alkaline phosphatase, 450 U; serum glutamic-oxalacetic transaminase and serum glutamic-pyruvic transaminase, 195 U). Clinical impressions at that time were abnormal liver function probably secondary to hepatitis. The patient was discharged after 8 hospital days, at which time his liver function tests approached a normal range. The patient was readmitted on 21 March 1976 after 3 days of diffuse epigastric and chest pain. The physical examination was unrevealing, and laboratory studies showed an elevated total bilirubin and alkaline phosphatase. On hospital day 3, the patient developed a fever of 102°F (ca. 39°C) and experienced chills, nausea, and profuse perspiration. Anaerobic vibrio-like organisms grew on two sets of blood cultures taken at that time. A urine culture was negative. A complete blood count showed a leukocyte count of 9,700/mm³ with 91% polymorpholeukocytes, 8% bands, and 1% monocytes. The symptoms defer-

vesced in 24 h without therapy. Thereafter, the patient received an upper gastrointestinal series, which showed a scarred duodenum. An intravenous cholangiogram revealed no visualization of the gallbladder and biliary system, and the impression was a calculus. An oral cholecystogram showed poor visualization of the gallbladder and a calcified stone. Therefore, the patient received a cholecystectomy and common bile duct exploration. Postoperatively, the patient has done well, and liver function tests are normal.

Case 2. The patient was a 45-year-old male admitted on 27 May 1976 to Long Beach Veterans Administration Hospital after he began vomiting bright red blood and passing red blood per rectum. He claimed to have had "passing-out" spells. In the emergency room, his hematocrit was 23%, and a nasogastric passage was performed. Endoscopy was performed and showed a moderate-sized esophageal hiatal hernia. There was evidence of esophageal varices, gastritis, or a duodenal ulcer. The source of the gastrointestinal bleeding was not located, and the bleeding failed to respond to light saline lavage, vitamin K, and fresh, frozen plasma. During surgery on 28 May, marked esophagitis was found. Several small eroded areas were oversown, a vagotomy and pyloroplasty were performed, and a frontal plication was performed to try to reduce his hiatal hernia. Postoperatively the patient did well except for several days of low-grade fever. On 28 May an unusual gram-negative, anaerobic, spirilliform organism grew on a set of blood cultures. The patient was not treated with antibiotics. Additional cultures were negative, and his fever gradually resolved.

RESULTS

Initial isolation of the anaerobic vibrios was obtained in blood culture bottles (Pfizer E-vac) containing 50 ml of tryptic soy broth (vented) and Columbia broth (unvented). Subcultures were performed anaerobically on brucella-mendadione blood agar (BMB). Biochemical proper-

ties and acid products of the organisms were analyzed by conventional anaerobic procedures (3, 4). Additional tests included detection of the pigment desulfoviridin (7) and special growth studies for classifying sulfate-reducing bacteria (6, 9). Antimicrobial susceptibility tests were performed by a modified broth-disk method described previously (4, 11).

The organism identified in case 1 was *Desulfovibrio desulfuricans*. Agreement with our identification of the isolate was obtained from J. R. Postgate (University of Sussex, Sussex, England) and L. V. Holdeman (Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg). Important morphological and biochemical characteristics are as follows. The isolate was a gram-negative curved rod showing occasional sigmoid or spiriloid forms (Fig. 1). Colonies on BMB after 72 h were nonhemolytic, pinpoint, slightly buff, smooth, circular, convex, entire, semitranslucent, and butyrous.

An outstanding feature of *Desulfovibrio* bacteria is the production of a great abundance of H_2S . This was demonstrated in SIM and Kligler iron agar media. The detection of the pigment desulfoviridin is characteristic for *Desulfovibrio* (7). A strong red fluorescence was demonstrated with a cellular suspension made alkaline with 2.0 N NaOH and observed at once under ultraviolet light at 365 nm. The identification of *Desulfovibrio* as to species was determined by growth tests (6). Our isolate grew in Postgate basal medium with pyruvate as a carbon source in the absence of sulfate. Bile growth was 4+.

The organism failed to attack carbohydrates (4). Other negative reactions were hemolysis, oxidase, indole, nitrate (slight, but considered negative), milk (no change), starch hydrolysis, esculin hydrolysis, gelatin liquefaction, urease, lecithinase, and lipase. A characteristic feature of *D. desulfuricans* was motility by a single, polar flagellum (9). The catalase test produced a moderately positive reaction. Gas chromatographic analysis detected acetic acid as the major (and only) acid product from pyruvate cultures.

The second anaerobic vibrio isolated from blood (case 2) was identified as a *Succinivibrio* species. The identification was confirmed by the Anaerobe Laboratory at Virginia Polytechnic Institute and State University.

The characteristics of the *Succinivibrio* species isolate are as follows. It stained gram negative and displayed helical cells usually with one to four coils (Fig. 2); occasionally, "comma" forms appeared. The cells displayed varying widths, and many appeared swollen. The colo-

nies on BMB after 72 h were nonhemolytic, pinpoint, slightly buff, smooth, circular, convex, entire, semitranslucent, and butyrous. The motile organism was lophotrichous, with a bipolar flagellum. Carbohydrates that gave strong acid reactions were fructose, galactose, glucose, lactose, maltose, melezitose, raffinose, and sucrose. Mannose gave a weak acid reaction. Negative reactions were obtained for nitrate, indole, oxidase, catalase, esculin hydrolysis, starch hydrolysis, gelatin liquefaction, milk (no change), lecithinase, lipase, urease, H_2S , adonitol, amygdalin, arabinose, cellobiose, erythritol, dulcitol, glycogen, inositol, mannitol, rhamnose, ribose, salicin, sorbitol, and xylose. Bile growth was 4+.

The results of antimicrobial susceptibility

FIG. 1. Typical cellular morphology of *D. desulfuricans*.

FIG. 2. Typical cellular morphology of *Succinivibrio* species.

tests are as follows. *D. desulfuricans* was sensitive to penicillin (2 U/ml), clindamycin (1.6 µg/ml), chloramphenicol (12 µg/ml), tetracycline (6 µg/ml), and erythromycin (3 µg/ml). *Succinivibrio* was resistant to clindamycin (1.6 µg/ml) but sensitive to penicillin (2 U/ml), chloramphenicol (12 µg/ml), tetracycline (6 µg/ml), and erythromycin (3 µg/ml).

DISCUSSION

Anaerobic vibrios are rarely encountered in clinical specimens, and there is little information available regarding their significance. We encountered two different anaerobic vibrios in blood cultures. The clinical histories of the patients were obtained, and the morphological and biochemical characteristics of the organisms were thoroughly analyzed.

The *Desulfovibrio* was first detected in two of four blood cultures (unvented Columbia broth) on day 4 by the Gram stain and growth on subcultures. Additionally, the organism was recovered from one of two bottles of vented tryptic soy broth only on a 5-day subculture. Unfortunately, the negative blood culture was discarded prematurely, thus eliminating further attempts for recovery. A strong H₂S odor was described by the technologist performing the blood subcultures. Initial studies revealed that the isolate grew poorly unless sulfates were present in the medium. In broths containing additional sulfates, abundant growth occurred, and a black precipitate developed, probably due to formation of iron salts (2).

It is somewhat surprising that an organism as ubiquitous in nature as *Desulfovibrio* is essentially unencountered in human bacteriology. Its known habitat is fresh water, particularly polluted waters, seawaters, marine mud, and soil (2). *Desulfovibrio* is a sulfate-reducing bacterium of considerable industrial and ecological importance (8). It has never been isolated from clinical material, although sulfate reducers have been reported from human feces (5). The bacteria use sulfate as terminal electron acceptors for their respiration (a process called dissimilatory sulfate reduction). In contrast to other microbes that generate H₂S metabolically, sulfate-reducing bacteria generate it directly, and greater turnovers of sulfur are involved.

In case report 1, *D. desulfuricans* was isolated from blood of a patient experiencing chills, fever, nausea, and profuse perspiration. The symptoms subsided within 24 h, and no similar episodes occurred. The major clinical problem of the patient was abnormal liver function secondary to choledocholithiasis. It can be

surmised that the patient acquired the organism from some water source and carried it in his gastrointestinal tract.

The second anaerobic vibrio was isolated from two of two blood cultures (one vented tryptic soy broth and one unvented Columbia broth). It was detected by Gram smear after 48 h. Laboratory tests indicated that the organism was most like a *Succinivibrio* species. Communication with L. V. Holdeman revealed that the Anaerobe Laboratory has encountered several *Succinivibrio* species from blood cultures; however, clinical histories of the patients were not available. Bacteremia due to a species of *Succinivibrio* was described by Southern (10). Prior to this recent report, *Succinivibrio* species were recognized primarily in ruminants (1).

Our patient was operated on for uncontrolled gastrointestinal bleeding. On the same day after the procedure, anaerobic vibrios grew on blood cultures. This suggests that the source of the organism may have been the gastrointestinal tract. However, no signs of sepsis were observed, and no antibiotic therapy was administered.

We have much more to learn in anaerobic bacteriology, particularly about the anaerobic flora of the gastrointestinal tract. The isolation of new organisms from clinical specimens warrants complete studies regarding taxonomy, antimicrobial susceptibility, and physiological and biochemical properties, as well as medical histories of the patients.

In conclusion, the clinical microbiologist must be able to recognize anaerobic vibrio-like organisms that may be rarely encountered from human specimens. The *Desulfovibrio* species are motile, gram-negative, nonfermentative rods that produce copious H₂S, have polar flagella, produce desulfoviridin, use sulfate as a terminal electron acceptor, and produce acetate from pyruvate. The *Succinivibrio* species are motile, gram-negative, fermentative rods that have polar flagella and produce succinic acid, usually with acetic acid, from fermentation of carbohydrates.

LITERATURE CITED

1. Bryant, M. P., and N. Small. 1956. Characteristics of two new genera of anaerobic curved rods isolated from the rumen of cattle. *J. Bacteriol.* 72:22-26.
2. Butlin, K. R., M. E. Adams, and M. Thomas. 1949. The isolation and cultivation of sulfate-reducing bacteria. *J. Gen. Microbiol.* 3:46-59.
3. Dowell, V. R., Jr., and T. M. Hawkins. 1974. Laboratory methods in anaerobic bacteriology. Publication no. 74-8272 (CDC). Department of Health, Education and Welfare. Government Printing Office, Washington, D.C.

4. Holdeman, L. V., and W. E. C. Moore (ed.). 1975. Anaerobe laboratory manual, 3rd ed. Virginia Polytechnic Institute and State University, Blacksburg.
5. Moore, W. E. C., J. L. Johnson, and L. V. Holdeman. 1976. Emendation of *Bacteroidaceae* and *Butyrivibrio* and descriptions of *Desulfomonas* gen. nov. and ten new species in the genera *Desulfomonas*, *Butyrivibrio*, *Eubacterium*, *Clostridium*, and *Ruminococcus*. Int. J. Syst. Bacteriol. 26:238-252.
6. Postgate, J. R. 1952. Growth of sulphate-reducing bacteria in sulphate-free media. Research (London) 5:189-190.
7. Postgate, J. R. 1959. A diagnostic reaction of *Desulphovibrio desulphuricans*. Nature (London) 183:481.
8. Postgate, J. R. 1965. Recent advances in the study of the sulfate-reducing bacteria. Bacteriol. Rev. 29:425-441. -
9. Postgate, J. R., and L. L. Campbell. 1966. Classification of *Desulfovibrio* species, the nonsporulating sulfate-reducing bacteria. Bacteriol. Rev. 29:359-363.
10. Southern, Jr., P. M. 1975. Bacteremia due to *Succinivibrio* species. Am. J. Clin. Pathol. 64:540-543.
11. Wilkins, T. D., and T. Thiel. 1973. Modified broth-disk method for testing the antibiotic susceptibility of anaerobic bacteria. Antimicrob. Agents Chemother. 3:350-356.