Aeromonas Primary Wound Infection of a Diver in Polluted Waters

S. W. JOSEPH,1* O. P. DAILY,1 W. S. HUNT,2 R. J. SEIDLER,3 D. A. ALLEN,3 and R. R. COLWELL3

Department of Microbiology, Naval Medical Research Institute, Bethesda, Maryland 20014; Naval School, Diving and Salvage, Washington, D.C. 20374; and University of Maryland, College Park, Maryland 20740

Received for publication 18 April 1979

Two separate species of Aeromonas, A. sobria (not listed as a species in Bergey’s Manual of Determinative Bacteriology, 8th ed.) and A. hydrophila, were primary pathogens isolated from the leg wound of a diver conducting operations in polluted waters. This is the first recorded instance of a primary infection of soft tissue in a human caused by two species of Aeromonas, one of which was resistant to tetracycline. Because of the very rapid development of this wound infection, cytotoxicity of these organisms was examined in several biological systems. A. sobria was hemolytic for sheep erythrocytes, cytotoxic for Y-1 adrenal cells, and enterotoxic in rabbit ligated intestinal loops, whereas A. hydrophila was hemolytic and cytotoxic. Pertinent clinical, bacteriological, and environmental features of the case are presented.

Aeromonas spp. (usually Aeromonas hydrophila) have been associated with a wide range of human infections, indicating that these organisms may act as primary pathogens. Aeromonas spp. comprise gram-negative, facultatively anaerobic, asporogenous, polarly flagellated, oxidase-positive, fermentative rods with an overall deoxyribonucleic acid composition in the range of 57 to 63 mol% guanine plus cytosine. Aeromonads are frequently isolated from soil, water, and human feces. Aeromonas spp. occurring solely or in combination with other organisms are now considered to be agents of human disease under certain conditions, as reviewed by von Gravenitz and Mensch (27) and, more recently, by Davis et al. (6).

Infections caused by Aeromonas spp. have been infrequently observed in healthy, immunologically competent individuals, with few cases described where Aeromonas has been the sole agent of infection (6, 9, 13, 19, 21, 22, 25–27). Aeromonas spp. are usually considered to be secondary or opportunistic pathogens. Most detailed reports of Aeromonas infections describe patients who were either immunologically compromised (1, 5, 7, 12, 15, 16, 18, 24, 25) or suffering from chronic disease (5, 6, 7, 25, 27).

A unique occurrence of a primary soft tissue infection caused by two species of Aeromonas is reported herein. A penetrating wound of the lower leg infected with A. hydrophila and A. sobria (not listed as a species in Bergey’s Manual of Determinative Bacteriology, 8th ed. [4]) was sustained by a student diver conducting scuba operations in the Anacostia River. This report discusses the unusual circumstances of the case, as well as the bacteriological and cytotoxic characteristics of the isolates.

MATERIALS AND METHODS

Case report. During June 1978, a 19-year-old white male was seen for complaint of a severe left temporal headache approximately 0.5 h after surfacing from a 130-foot (ca. 39.6-m) seawater (FSW) scuba qualification dive which lasted less than 10 min. Prior history was uncomplicated, with the exception of a 10-min deep puncture wound to his left lower leg lateral to the tibia sustained the previous evening while scuba diving without a wetsuit in the Anacostia River tidal basin, Washington, D. C., an area of extremely low salinity. Although the wound showed no drainage, there was a 4- to 6-cm area of erythema and swelling and considerable pain in the swollen area, the medial knee, and the left groin.

At that time there was also marked tenderness to palpation along the proximal tibia, medial knee, and in the left inguinal area, where four to six swollen lymph nodes were detected. The medial aspect of the left knee and thigh was warmer than that of the right side, but there was no erythematous streaking. Neurological examination revealed decreased sensation to pin and light touch along the lateral and superior aspect and on the distal sole and toes of the right foot. There was adequate strength in the left extremity, considering the pain from the wound, but notable weakness in dorsiflexion of the right foot and great toe. The patient was immediately taken to the recompression chamber, his weakness requiring almost full support, where he was placed on oxygen and taken to a pressure equivalent to 60 FSW. During compression he had difficulty clearing his left ear. At 40 FSW he became unresponsive to voice command for about 40 s, but had a regular, strong pulse and normal...
respiration. At 60 FSW the patient seemed fully alert and stated that his headache and left leg pain had fully resolved. Neurological examination revealed continued improvement from the sensory deficit and previously described weakness. The patient was fully recovered after 8 min at 60 FSW. Treatment consisted of treatment table 6 with a 60-min extension at 30 FSW, dexamethasone (10 mg given intravenously, followed by 4 mg 5 h later), and oral fluids given as required.

He was hospitalized on the same day for further observation and was asymptomatic after treatment except for local tenderness of his leg wound. The results of a neurological examination were normal, and routine hematological and biochemical results were within normal limits. Microscopic evaluation of the leg wound aspirate revealed gram-negative rods which, upon initial culture, yielded A. sobria and A. hydrophila in a ratio of approximately 5 to 1. Tetracycline was administered for 10 days (250 mg, four times per day), and recovery was complete.

Isolation and identification. An aspirate of the leg wound was cultured both upon admission (specimen 1) and 3 days later (specimen 2). Trypticase soy agar with 5% sheep blood added, MacConkey agar, thioglycollate-citrate-bile salts-sucrose agar (Baltimore Biological Laboratory, Cockeysville, Md.), brain heart infusion (BHI) agar, and BHI agar containing 2% (final concentration) NaCl were inoculated and incubated aerobically for 24 h at 37°C. Enriched thioglycollate broth, supplemented with heme and vitamin K but without indicator, was also inoculated and incubated at 37°C.

Identification of the isolates was performed at 37°C with the API 20E system (Analytab Products, Plainview, N.Y.), previously found suitable by Ljungh et al. (14) for identifying Aeromonas isolates from human infections. Further characterization and differentiation of the isolates was based on esculin hydrolysis, growth in KCN broth, elastase production (judged by zones of clearing in agar), and gas production from glucose at 30 and 37°C, as described by Popoff and Véron (20).

Antibiotic susceptibility. The isolates were submitted to disk antibiotic susceptibility assays as previously described (10, 17).

Toxin assays. Assays for toxin production were performed as described elsewhere (11, 23). Briefly, the organisms were grown in stationary culture in BHI broth incubated at 37°C for 18 to 24 h. The cultures were centrifuged at 37°C for 10 min, and the supernatant was filtered through 0.45-μm membrane filters (Millipore Corp.). The filtrates were inoculated on Y-1 adrenal cells, which were examined for cytotoxicity or enterotoxin production or both.

Rabbit ligated loop assays were performed, as previously described, to determine fluid-accumulating response to the separately tested, agar-grown organisms and to cell-free filtrates prepared from broth (11).

RESULTS

Bacteriological identification. The Gram-stained aspirate from the leg wound contained numerous inflammatory cells and many thick, curved, loosely arranged, gram-negative rods approximately 0.5 to 2.0 μm long.

Two species of Aeromonas were isolated from specimen 1. A. sobria produced colonies which were approximately 2 mm in diameter on blood agar, convex, with soft entire edges, grayish-white pigmentation, and large zones of beta hemolysis; this species was present in about a five-fold-higher concentration than the second species. A. hydrophila was similar but produced larger colonies (3 mm) which were only slightly raised.

Because of the patient’s exposure to brackish water, the aspirate was inoculated onto thiosulfate-citrate-bile salts-sucrose agar and BHI agar containing 2.0% NaCl to screen for halophilic vibrios, i.e., V. parahaemolyticus, V. alginolyticus, and lactose-positive vibrios. No halophilic vibrios were isolated. Colonies of both species of Aeromonas, however, were found on media containing 2% NaCl, but were approximately one-half their normal size. Although anaerobes were not isolated from thioglycollate broth, both species of Aeromonas were recovered from this medium on anaerobic subculture in prerduced BHI agar. Both organisms grew on MacConkey agar but did not ferment lactose. Only A. hydrophila was isolated from specimen 2.

Both isolates were positive for β-D-galactosidase, oxidase, gelatinase, arginine dihydrolase, lysine decarboxylase, indole, Voges-Proskauer, and growth in KCN broth. Carbohydrates fermented by both species were glucose and mannitol, and those not fermented included inositol, sorbitol, and amygdalin. Citrate was not utilized, tryptophan was not deaminated, urea was not hydrolyzed, H₂S was not produced, and ornithine was not decarboxylated (Table 1).

Antibiotic susceptibility. The isolates were susceptible to chloramphenicol, tobramycin, gentamicin, kanamycin, nalidixic acid, neomycin, and erythromycin. Both were resistant to ampicillin, carbenicillin, and cephalothin, but only A. hydrophila was resistant to tetracycline.

Toxin assay. Undiluted filtrates of both organisms showed cytotoxic activity on Y-1 adrenal cells (Table 2). Unheated A. hydrophila filtrate was cytotoxic when diluted fourfold. This filtrate at a 1:4 dilution, when heated at 56°C for 10 min, showed cholera enterotoxin-like activity, as reported by Ljungh et al. (14).

Beta-hemolytic activity for sheep erythrocytes was noted for both species, but was not quantified further, as done by Bernheimer and Avigad (2) and Ljungh et al. (14). A. sobria grown on BHI agar and resuspended in BHI broth at a concentration of 10⁶ to 10⁸
lancer et al. (3) reported recently that eight A. sobria strains of eight tested from fish elicited positive rabbit loop reactions. A. hydrophila cells and cell-free filtrate produced no reaction, confirming the findings recently reported by Donta and Haddow for this species (8).

**DISCUSSION**

The clinical and bacteriological features of this case are unique. Current concepts of decompression sickness made it difficult to reconcile a 130-FSW, no decompression, air-scuba dive with decompression sickness, even though the striking neurological signs and symptoms and their rapid response to pressure and oxygen supported that diagnosis. The dive duration (less than 10 min) required only a normal ascent rate of 60 feet (ca. 18.3 m) per min for adequate decompression.

The hyperbaric oxygen therapy, initiated for the possible decompression sickness, may have contributed significantly to the rapid, successful wound healing without further complications developing from the Aeromonas infection. The degree to which the patient’s leg wound may have contributed to his neurological condition cannot be assessed. Furthermore, a primary infection involving two separate species of Aeromonas, i.e. A. hydrophila and A. sobria, is indeed unusual.

A. hydrophila is the most frequently isolated species of the aeromonads associated with infections, although in a few instances A. shigelloides has been implicated (6). Popoff and Véron (20) concluded that there are distinct and significant differences between A. hydrophila and A. sobria. To our knowledge, the case we report here represents the first in which A. sobria was isolated from an active human infection. Both isolates examined in this study were hemolytic and cytotoxic. A. sobria, when tested against Y-1 tissue culture cells, demonstrated cytotoxicity but did not elicit a cholera enterotoxin-like effect, whereas A. hydrophila demonstrated cytotoxicity and a cholera enterotoxin-like effect. Although A. sobria did cause fluid accumulation in ligated rabbit ileum, A. hydrophila did not.

The antibiograms were similar to those reported previously (6, 25, 27), except for the resistance to tetracycline exhibited by the two separate isolates of A. hydrophila. The data point out potential treatment difficulties, especially in view of the characteristic resistance of the organisms to penicillin and its congeners. Upon initial isolation from the first culture, A. sobria was found to be present at approximately a fivefold-higher concentration than A. hydrophila. Although A. hydrophila was judged to be resistant to tetracycline, based on in vitro tests,
the patient’s progressive recovery after 3 days encouraged continued tetracycline therapy.

An ongoing survey (to be published elsewhere) at the site of the diver’s exposure to polluted water has yielded 193 isolates of Aeromonas, of which 25 to 30% are cytotoxic. Of these, 172 were classified as biotypes of A. hydrophila. Eight showed reactions identical to those of the A. hydrophila isolate described herein. Of 21 A. sobria isolates, 12 had biotype patterns identical to those of the A. sobria obtained from the wound. Because the A. hydrophila isolated from the diver was tetracycline resistant, environmental strains of Aeromonas obtained by standard sampling procedures from water at the exposure area were examined for antibiotic susceptibility. Resistance to tetracycline was noted for 17 of the 193 isolates tested. Sixteen of these were identified as A. hydrophila, and one was identified as A. sobria. These findings are consistent with reports describing the occurrence of Aeromonas spp. in the environment and describe the contaminating milieu to which the diver was exposed (19, 22, 27). As Hanson et al. (9) have suggested, Aeromonas may indeed be a more frequent pathogen than has been appreciated, and with increasing activities in aquatic environments, greater awareness of these organisms is recommended.

ACKNOWLEDGMENTS

We thank C. Adkins, R. Gates, L. Petrone, and J. W. Demming for expert technical assistance and C. Lissner for valuable discussions.

This work was performed under Naval Medical Research and Development Command Work Unit number ZF51524009.0557, National Oceanic and Atmospheric Administration grant 04-8-M01-71, and National Oceanic and Atmospheric Administration contract 01-8-M01-2027.

LITERATURE CITED