CASE REPORT

Aeromonas sobria sepsis complicated by rhabdomyolysis in an HIV-positive patient: case report and evaluation of traits associated with bacterial virulence

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Introduction

Aeromonas organisms are motile, Gram-negative small rods. They are facultatively anaerobic, with the ability to colonize both humans and animals. 1 Aeromonas is ubiquitous in water, 2 including chlorinated drinking water; in fact, these
organisms have the potential to grow within water distribution systems, where flagella facilitate biofilm formation. Although free cells of Aeromonas might be relatively susceptible to disinfection, populations associated with biofilms could survive high chlorine doses.  

Of all clinical isolates involved in human infections, 85% belong to three phenotypically defined species: A. hydrophila, A. caviae and A. sobria.  

Human infections are most often associated with open wounds, permitting exposure to contaminated fresh or brackish water or soil. As Aeromonas has also been isolated from drinking water, food, salad, ready-to-eat foods, smoked salmon and vegetables, fecal–oral transmission is another significant mode of infection.

Aeromonas species are the causative agents of a variety of illnesses; although gastrointestinal infections are the most common presentation, extraintestinal localizations, such as those involving the peritoneum, skin and soft tissues, meninges, hepatobiliary system, heart, eye and urinary tract, have also been increasingly reported. 

Aeromonas bacteremia, an uncommon event that usually affects immunocompromised subjects, including those with liver cirrhosis or malignancy, is a life-threatening condition that causes acute rhabdomyolysis.

Cytotoxicity assays and adhesion

Cytotoxic activity

Hep-2 cells were seeded in 96-well plates (initial inoculum of 5 × 10^3 cells ml^{-1}) and treated with bacterial supernatant filtrates after 24 hours; serial twofold dilutions of the supernatant (starting from 1:2) were added directly to the culture medium, incubated at 37°C in 5% CO₂ and monitored for 24 hours. Plates were read under an inverted microscope. The cytotoxic effect was noted by a rounding or detachment of the cells. The cytotoxic activity titre was expressed as the reciprocal of the highest dilution of the filtrates affecting at least 50% of the cells.

Adhesive capacity

The adhesion of the A. sobria isolates to Hep-2 cells was investigated. For this purpose, Hep-2 cells were seeded on glass coverslips in 24-well plates (initial inoculum of 5 × 10^4 cells ml^{-1}), supplemented with A. sobria at a multiplicity of infection (MOI) of 100 and incubated for 90 min at 37°C. Cells were then washed three times in serum-free MEM to remove nonadherent bacteria, fixed in methanol and stained with May-Grunwald-Giemsa. Adhesive capacity was assessed by light microscopy and expressed as the percentage of cells with more than ten adherent bacteria on the cell surface. In addition, samples were then fixed with 3.7% formaldehyde in PBS (pH 7.4) for 10 min at room temperature. After washing in the same buffer, cells were permeabilized with 0.5% Triton X-100 (Sigma, Milan, Italy) in PBS (pH 7.4) for 10 min at room temperature.

To analyze the nuclei, permeabilized cells were stained with Hoechst 33258 (Sigma; working dilution 1:1000) at 37°C for 30 min. After washing, coverslips were mounted with glycerol–PBS (2:1) and analyzed with a Nikon Optiphot fluorescence microscope.

Case report

A 43-year-old Italian male with a past history of intravenous drug abuse was diagnosed as HIV positive for the first time in
1985. Zidovudine monotherapy was initiated in 1990, when the CD4+ cell count was 239 cells/μL; thereafter, a number of combination antiretroviral regimens were prescribed according to current guidelines. This enabled the patient to maintain CD4+ cell count levels of >300 cells/μL and an undetectable plasma viral load (pVL) without developing AIDS-related opportunistic infections. As the patient was also HCV-coinfected (genotype 1a), leading to chronic hepatitis, interferon (IFN) therapy was prescribed on two occasions. However, both HCV treatment attempts were unsuccessful; in February 2005, the patient voluntarily discontinued IFN after a few administrations because of a sudden onset of jaundice.

In September 2006, the patient was hospitalized at the Clinic of Infectious Diseases, Bari with jaundice and severe weakness. At that time, saquinavir/ritonavir plus tenofovir plus emtricitabine were being administered; the CD4+ cell count was 224 cells/μL and pVL was undetectable. On admission, the patient presented with malaise and fatigue in addition to jaundice; both the pulse and respiration rate increased with only slight exertion, and his blood pressure was 110/70 mmHg. Hepatosplenomegaly was evident on abdominal examination. Laboratory values were as follows: hemoglobin 6.4 g/dl, white blood cell count 1.9 × 10^9/l, platelets 47 × 10^9/μL. The biochemical profile showed an elevated total (3.56 mg/dl) and unconjugated (indirect) (3.14 mg/dl) bilirubin, with moderately elevated alanine (ALT) and aspartate (AST) transaminases, and a reduction in the haptoglobin (7 mg/dl). An abdominal ultrasonography revealed a nodular liver in an advanced stage of cirrhosis, along with increased echogenicity with irregular-looking areas. As the direct Coombs test, antinuclear antibodies (ANAs) and anti-neutrophil cytoplasmic antibodies (ANCAs) were all positive, an autoimmune anemia was diagnosed. Therefore, corticosteroid therapy (methylprednisolone 3 mg/kg/day, total 125 mg) and blood transfusions were initiated, resulting in increased hemoglobin values (10 mg/dl) within a few days; however, the patient developed diabetes, thus requiring insulin administration. Ten days after beginning immunosuppressive therapy, the patient manifested ascites and edema of the ankles, and therefore diuretics were added to the therapy. Two weeks later, the patient complained of watery diarrhea without fever or any additional symptoms. Stool samples were repeatedly negative for protozoa such as Giardia, Cryptosporidium and Entamoeba. Acid-fast stains for Cyclospora, Isospora and Mycobacterium species were also negative, and other enteric bacterial pathogens, such as Salmonella, Shigella, Yersinia, Campylobacter jejuni, Vibrio and Escherichia coli, were also absent in multiple stool samples. Testing for Clostridium difficile toxins (A and B) was negative, and the presence of Candida spp. in fecal samples was also excluded. Lastly, no evidence of Cytomegalovirus infection was found. Rehydration, loperamide and octreotide were administered, without improvement.

Three days after the onset of diarrhea, the patient began to complain of abdominal pain; at this time, an abdominal CT scan demonstrated dilated loops, primarily in the transverse colon, with air levels that were compatible with the diarrheic almus. A perieal ulceration appeared on the left leg and the patient had a single peak of fever, during which two sets of blood cultures (Organon Teknika, Milan, Italy) were performed. Thereafter, empiric therapy with metronidazole and ceftriaxone was initiated. Blood chemistry (red and white blood cell counts, serum creatinine, urea and electrolytes, prothrombin and partial thromboplastin time, myoglobin, lactate dehydrogenase [LDH] and creatine phosphokinase [CPK], glycemia and transaminases) was within the normal range. Platelets were 58 000/μL, but previous platelet counts (since 2003) had always been <100 000/μL.

The following day, a rapid succession of events provoked an irreversible deterioration of the patient’s condition. The patient awoke with severe pain in both calves, which was initially disproportionate to physical findings, but continued to spread to the thighs; thereafter, an intense redness and induration of the lower extremities was followed by violaceous bullae formation. These circumscribed (>2 cm) tense lesions contained serous fluid and were initially localized on the right calf, but suddenly became generalized in both legs. A Doppler study of the legs was normal. His temperature was persistently normal, whereas the patient soon developed hypotension with tachypnea. At this time, laboratory values were as follows: white blood cell count 0.36 × 10^9/l, platelets 18 000/μL, PT (prothrombin) ratio 2.59, CPK 5570 U/l, myoglobin 4949 ng/ml. Acute renal failure, characterized by oliguria with retention of nitrogen (urea: 157 mg/dl and creatinine: 2.7 mg/dl), and metabolic acidosis, consistent with septic shock, developed. The patient’s condition rapidly worsened; bullae on the legs became hemorrhagic, the intense myalgia required morphine and a multiple organ dysfunction syndrome developed, leading to death.

The results of the blood cultures were available only postmortem. Positivity was noted after 24 hours of incubation; subcultures on blood agar produced colonies of a Gram-negative, oxidase-positive, β-hemolytic bacillus. The strain was identified as Aeromonas hydrophila group (MicroScan WalkAway, Dade Behring, Milan, Italy). The use of API test strips (API 20 NE, bioMérieux, Italy) performed at two different centers yielded a code (7177755/7176755) compatible with an optimum identification of A. sobria.

An antimicrobial susceptibility profile was performed by MicroScan WalkAway, Dade Behring, Milan, Italy. The strain was susceptible to amikacin, gentamicin, tobramycin, cefepime, ciprofloxacin, levofloxacin lomefloxacin, sulfamethoxazole-trimethoprim and chloramphenicol. The strain was resistant to β-lactam antibiotics because of production of an inducible β-lactamase. Extended β-lactamases were not detected by E Test (AB Biodisk, Biolife, Italy). Resistance to imipenem and meropenem were also observed.

In vitro experimental results on the expression of virulence traits of the clinical isolate of A. sobria were as follows: according to the measured OD values, the clinical isolate was found to be unable to form a biofilm. Fluorescence microscopy observation of control Hep-2 cells (Figure 1A) and cells treated with bacterial supernatant (Figure 1B) showed a marked reduction in the number of still-adhered Hep-2 cells. This effect, which was still detectable at the supernatant dilution of 1:2, presumably reflects the Hep-2 cell response to bacterial toxic agents released in the supernatant. The isolated strain did not show adhesive properties on Hep-2 cells and no evidence of significant morphological changes at the cytoskeletal level was revealed. Regarding the cell nuclei, no morphological alterations were observed.
Figure 1  Fluorescence micrographs of (A) untreated Hep-2 cells and (B) cells treated with A. hydrophila supernatant stained for detection of F-actin cytoskeleton (green) and nuclei (blue). Fluorescence microscopy observation of control Hep-2 cells and cells treated with bacterial supernatant showed a marked reduction in the number of still-adhered Hep-2 cells. This effect, which is still detectable at the supernatant dilution of 1:2, presumably reflects the Hep-2 cell response to bacterial toxic agents released in the supernatant. Regarding the cell nuclei, no morphological alterations were observed.

Discussion

Aeromonas is a significant human pathogen that causes a variety of intestinal and extraintestinal infections both in healthy hosts and in immunocompromised subjects. In vulnerable individuals with underlying diseases, such as cancer, leukemias, liver cirrhosis, hepatobiliary diseases and diabetes, Aeromonas infections that are either associated with wounds involving exposure to an aquatic environment or disseminated from the gastrointestinal tract can lead to bacteremia, resulting in a 30–70% fatality rate. To the best of our knowledge, little or no data exist regarding Aeromonas and HIV infection, thus indicating that HIV is not a major predisposing condition. Overall, three cases of Aeromonas infection have been reported in HIV-positive subjects: two patients with Aeromonas septicemia and concomitant leukaemia, and one patient with acute colitis. Interestingly, among HIV-infected persons, 40–80% of diarrheal illnesses, whose prevalence in Western countries ranges from 42 to 90%, are caused by infective agents, mainly bacteria. However, Sanchez et al. described trends in the occurrence of bacterial diarrhea among HIV-infected individuals during a ten-year period, demonstrating that the frequency of Aeromonas infection was 1.6% for all Aeromonas species compared to 54.4% for Clostridium species. Moreover, Suthienkul et al. reported that Aeromonas diarrhea was present in 4.6% of AIDS patients and 8.3% of non-AIDS patients in Thailand, thereby confirming that Aeromonas diarrhea is not of particular concern in HIV-infected individuals. Our patient was an HIV-positive subject, but also presented with HCV-related liver cirrhosis. Also, corticosteroids prescribed for the autoimmune anemia provoked the development of diabetes. Therefore, it can be presumed that the coexistence of these conditions played a crucial role in the onset and severity of the clinical course.

For patients with diarrhea and underlying diseases such as those in our patient, the translocation of Aeromonas across the bowel is the most likely source of bacteremia. Bacterial translocation is promoted by several factors, including overgrowth of intestinal bacteria, chemotherapy, suppression of the host immune defenses and conditions that affect the liver. However, only 5% of patients with a positive blood culture have diarrhea, possibly secondary to the ingestion of contaminated water or food. The source of bacteremia in our patient was unclear; although diarrhea was the first manifestation, multiple stool samples did not yield Aeromonas, and we cannot assert that bacteremia was the consequence of microbial translocation from the intestinal tract into the bloodstream. Likewise unclear was the mode of entry of the bacterium. The patient had no history of previous contact with potentially contaminated water or food products, and at the time when he manifested his symptoms, he had been hospitalized for about one month. Although we are aware that the possibility of a nosocomial infection due to Aeromonas does exist, as a consequence of intravascular catheter implant, medicinal leech therapy, ready-to-eat foods or water, no other infections due to the same bacterial strain of Aeromonas occurred in our hospital during this time and certainly our patient was not subjected to leeching.

Both host and microbial factors, such as infectious dose and expression of virulence properties, can affect the ability of Aeromonas to cause disease. The virulence of Aeromonas spp. is multifactorial; in fact, Aeromonas spp. possess a number of virulence properties considered responsible for intestinal and extraintestinal infections in human beings. Factors contributing to virulence include cytotoxic and cytotoxic enterotoxins, the translocation capacity (as demonstrated in a cellular culture model), cytotoxicity on Vero cells, proteases, hemolysins (which, in the case of A. sobria, are major virulence factors with both hemolytic and enterotoxic activity and cause diarrhea by activating HCO3 secretion), lipases, adhesins, agglutinins and various hydrolytic enzymes. More recently, a cytotoxin named vacuolating cytotoxic factor, which provokes the death of Vero cells, has been described in A. sobria.

In our case, the clinical isolate failed to demonstrate adherence, which is a crucial step for most microbial infections, including Aeromonas infection, because it protects the microorganism from elimination by host defense mechan-
isms. It has been observed that there is a close correlation between bacterial adherence in vitro and infectivity in vivo.27 Our isolate was also unable to form biofilms; this capacity represents an important mechanism of bacterial escape, as Aeromonas in biofilms could resist disinfection and persist for long periods of time. Lastly, when assayed on Hep-2 cells, the isolate did not exhibit any in vitro cytoytic activity. Overall, these results emphasize the limited virulence of the isolate obtained from our patient during bacteremia and do not seem to correlate the isolate with diarrhea, as the production of cytotoxins, enterotoxins and hemolysins by Aeromonas spp. is normally implicated in the pathogenesis of diarrhea. Alternatively, unrecognized host factors have a pivotal role in determining the conditions in which virulence factors are expressed and disease occurs.

Aeromonas spp. are generally susceptible to the majority of antibiotics active against Gram-negative bacilli; however, increasing antimicrobial resistance among Aeromonas spp. has been observed worldwide. In the case described herein, the strain was resistant to β-lactam antibiotics, including carbapenems. This finding is consistent with the described ability of Aeromonas spp. to produce three different inducible β-lactamases, including a carbapenemase,28 thereby indicating that β-lactams should be used with caution for the treatment of Aeromonas infections. As expected, our isolate retained its susceptibility to aminoglycosides,29 and also to treatment of Aeromonas infections. As expected, our isolate diarrheal stool. Recommended in order to increase the isolation rate from pain. The use of selective media for Aeromonas must be with enteritis and/or those presenting significant muscle rhabdomyolysis and myonecrosis complicating Aeromonas bacteremia in burn patients. Burn 2007; 33:255–7.


Conflict of interest: No conflict of interest to declare.

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References


