Enteroinvasive *Escherichia coli*: a Cause of Bacteremia in Patients with AIDS

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A strain of enteroinvasive *Escherichia coli* was isolated from the blood of a patient with advanced human immunodeficiency virus disease on repeated occasions, associated with severe diarrheal illness. The isolate was killed in vitro by control sera but not by sera collected from the patient before or after his bacterial illnesses.

Although *Escherichia coli* is the predominant aerobic organism of normal human fecal flora, there exist several pathogenic types (12). These include enteroinvasive *E. coli* (EIEC) strains that have the ability to attach to, invade, multiply within, and destroy colonic epithelial cells (12). The clinical manifestations of EIEC infections are similar to those caused by *Shigella* species, which are closely related to EIEC on a genetic level. In addition, the invasive potential of EIEC is associated with a plasmid which has significant sequence homology with the invasion plasmid of *Shigella* sp. (9, 16). Shigellosis has been described previously most commonly for malnourished infants, elderly persons, and other compromised hosts (10, 19), including patients with AIDS (13). Bacteremia caused by EIEC has not been described previously.

Diarrhea is a common problem in persons infected with the human immunodeficiency virus (HIV). The etiologic agent frequently cannot be identified, despite extensive investigation (18). We report a case of recurrent diarrhea and bacteremia due to an enteroinvasive strain of *E. coli*.

A 32-year-old man with a history of a positive serum antibody test for HIV presented with diarrhea and recurrent *E. coli* bacteremia in 1989. In February 1989 he complained of a 6-month history of 10 to 12 loose stools per day and weight loss of 25 pounds. Stool culture was negative for *Salmonella*, *Shigella*, and *Campylobacter* species, and examination for ova and parasites was negative. Three smears and cultures of stool for *Mycobacteria* spp. were negative, as were blood cultures for *Mycobacteria* spp. Routine blood cultures were negative. The CD4 T-lymphocyte count was 180/μl.

On 15 April 1989 the patient was admitted to the hospital for treatment of volume depletion. He reported 20 watery stools per day and crampy bilateral lower quadrant pain. Two samples of stool were cultured; both were negative for *Salmonella*, *Shigella*, and *Campylobacter* organisms, as was examination for ova and parasites. Two of five blood cultures yielded *E. coli*. Urinalysis was normal; urine culture was not performed. He was treated with cefapirin for 7 days. His diarrhea improved, and his fever resolved.

On 9 May 1989 he returned with an identical clinical syndrome. Microscopic examination of a stool sample revealed no leukocytes. Routine stool culture and examination for ova and parasites were again negative. Urinalysis was negative. One of four blood cultures yielded *E. coli*. A culture of blood for *Mycobacteria* spp. was negative. A sonogram of the right upper quadrant, computed tomography scan of the abdomen, and a barium enema did not reveal a source of the bacteremia. He was treated with intravenous cefotetan for 4 days and then with gentamicin for 4 days. His diarrhea improved and his fever resolved.

On 27 May 1989 he was readmitted with fever and diarrhea. Blood cultures again yielded *E. coli*. A hepato-imo-diabetic acid scan of the gall bladder and a Gallium-67 scan of the abdomen were normal. A computed tomography scan of the abdomen was unchanged from the previous exam. Flexible sigmoidoscopic exam was limited by the patient’s discomfort. Examination of the rectosigmoid colon under anesthesia revealed only internal hemorrhoids, which were resected. He was treated with intravenous cefotetan for 22 days. His fever resolved and his diarrhea improved.

On 27 July 1989 he returned with volume depletion and nearly constant watery stools. There was no fever. On the third hospital day his temperature rose to 38.9°C. Blood cultures again yielded *E. coli*. Upper gastrointestinal tract radiographs were normal. The patient was treated with intravenous cefapirin for 6 days. At that time a blood culture yielded a member of the *Mycobacterium avium* complex (MAC). Therapy was begun with ciprofloxacin (750 mg twice daily), clofazamine (100 mg daily), and rifampin (600 mg daily). The patient remained free of diarrhea and *E. coli* bacteremia on this regimen for 15 months. He died of progressive HIV encephalopathy. Biochemical and antimicrobial susceptibility testing of each blood isolate was performed with a Microscan automated testing panel (Baxter Health Care Corporation, West Sacramento, Calif.) according to the manufacturer’s recommendations. The antimicrobial susceptibility testing was confirmed by National Committee for Clinical Laboratory Standards-recommended broth microdilution on isolate 89-120, obtained from the July blood culture. Tests for lysine decarboxylase, ornithine decarboxylase, mucate, fermentation of gas from glucose, indole, and acetate were repeated by NCCLS recommended methods on the July blood isolate (strain 89-120). The isolate was examined for chromosomal DNA hybridization with

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DNA probes for EIEC, entero-effacing E. coli, and genes coding for Shiga-like toxin I and Shiga-like toxin II as previously described (6). Assays for production of heat-labile (15) and heat-stable (5) enterotoxin and the rabbit keratoconjunctivitis test (17, 22) were performed as previously described.

The E. coli isolate from the patient’s blood in July 1989 (strain E9-120) was cultured for 18 h at 37°C on Trypticase soy agar with 5% sheep erythrocytes. Cells were washed twice in sterile distilled water and then diluted in medium 199 (pH 7.4) to a final concentration of 10^9/mL. All sera were heated to 56°C for 30 min to inactivate HIV and then diluted in Hanks balanced salt solution. Because heating also inactivated complement, serum from a hypogammaglobulinemic patient was used as a standard complement source, as previously described (4). To remove any cross-reacting antibodies, this serum was absorbed 10 times for 30 min each time at 0°C with E. coli 89-120. Susceptibility of the E. coli strain to bactericidal activity in the serum specimens from the affected patient and nine homosexual controls (four HIV infected and five HIV uninfected) was tested as previously described (4).

Each of the patient’s blood isolates of E. coli had an identical biochemical profile, as follows: lysine decarboxylase negative, ornithine decarboxylase positive, mureate negative, glucose fermentation negative, indole positive, and acetate positive. Susceptibilities to antimicrobial agents also were identical for each of the strains. Strain 89-120 hybridized with the 17-kb EIEC probe and was Sereny test positive; its serotype was O28a:H-. The isolate did not hybridize with the enteropathogenic E. coli adherence factor probe, Shiga-like toxin I probe, or Shiga-like toxin II probe. It did not produce heat-labile or heat-stable enterotoxin. In an in vitro bactericidal assay, strain 89-120 was killed by pooled normal human serum and by serum from HIV-seronegative as well as HIV-seropositive homosexual controls (Table 1). Serum samples collected from our patient and stored prior to the onset of diarrheal disease (serum 1) and after several episodes of diarrhea with E. coli bacteremia (serum 2) did not kill the organism, whereas growth was inhibited in the presence of the antimicrobial agents (serum 3). Since this could have been due either to the acquisition of specific antibodies, resulting in complement-mediated bactericidal activity, or to levels of inhibitory antimicrobial agents in serum, we next studied the heat-inactivated serum without the addition of complement. Under these conditions, the two serum samples obtained prior to antimicrobial treatment showed no suppression (<0.05 log_10 decrease) whereas serum obtained during treatment (15 September 1989) produced a 0.88 log_10 decrease. Thus, the suppressive effect noted for serum 3 was at least partially due to the effect of the antimicrobial agents.

EIEC is isolated from the stools of 1.9 to 4% (7, 20) of children with diarrhea in the developing world, but its prevalence in the United States is difficult to ascertain because clinical laboratories do not screen for it routinely. EIEC was not sought in a large study of intestinal infections in AIDS (18), and a survey of fecal flora in 182 samples from 95 HIV-infected patients at the Department of Veterans Affairs Medical Center in Denver, Colo., has not detected any stool isolates of EIEC (1). A review of all blood isolates from HIV-infected patients at the Department of Veterans Affairs Medical Center in Denver from 1986 to 1990 revealed only two E. coli bacteremias. Both were associated with pyelonephritis. A recent review of 44 bacteremias in AIDS patients noted four E. coli bacteremias of unknown sources (18), but there was no identification of pathotypes. EIEC bacteremia has not been described previously, but it may occur sporadically in immunocompromised hosts and remain undiagnosed.

Most diarrheal illnesses of bacterial origin in normal hosts are self-limited, but in patients with AIDS recurrence of infection after a course of therapy is common (3, 14). Similarly, this patient had recurrence of disease after each course of antibiotics until chronic suppressive therapy was begun. Bacteremia due to diarrheal pathogens is uncommon, occurring mostly at the extremes of age or in compromised hosts (19). Bacteremia due to serum-sensitive organisms is uncommon except in immunocompromised individuals, such as our patient. Strain 89-120 was killed in vitro by pooled normal human serum and by sera from HIV-positive and HIV-negative homosexual controls but not by the patient’s serum obtained prior to his illness nor by sera collected between episodes. The lack of bactericidal activity correlates with the patient’s recurrent bacteremia. The demonstration of suppressive activity in the patient’s serum in the presence of antimicrobial agents is consistent with the lack of further episodes of bacteremia after therapy was begun.

None of the HIV-positive controls met the case definition for AIDS and therefore, they are likely to have less severe immune deficiency than did the case patient. It is likely that our patient was not unique in his inability to control EIEC infection. Rather, he was a man with severe immune depletion due to HIV, who encountered a pathogen that is relatively uncommon in the United States. In the developing world, where EIEC is more prevalent (20), this syndrome

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<th>Serum source and sample</th>
<th>Log_10 killing in 60 min</th>
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<td>Assay controls^a</td>
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<td>Medium alone</td>
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<td>PNHS</td>
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<td>HIPNHS</td>
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<td>Absorbed hypogammaglobulinemic serum + HIPNHS</td>
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<td>Homosexual controls^b</td>
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^a Results shown are means from duplicate assays on two different days, performed as described elsewhere (3).
^b PNHS, pooled normal human serum; HIPNHS, heat-inactivated (56°C for 30 min) PNHS.
^c Absorbed hypogammaglobulinemic serum was added as a complement source to heat-inactivated control or patient serum, as described elsewhere (3). The dates for the patient samples refer to dates serum was obtained.
may be more common. A recent study of bacteremia in HIV-1-seropositive adults in Kenya (8) reported two patients with *E. coli* bacteremia and one patient with *Shigella flexneri* bacteremia whose presentations with severe diarrhea and clinical courses were similar to those which we report. Perhaps these cases of *E. coli* bacteremia also were due to EIEC.

No attempt was made to isolate EIEC from the patient’s stool. It is likely that the *E. coli* bloodstream isolate originated from the gastrointestinal tract, since it was typical for EIEC, including a typical serotype, lack of lysine decarboxylase activity, hybridization with a 17-kb probe specific for EIEC, and Sereny test positivity. Furthermore, the patient’s clinical syndrome of diarrhea, fever, and prostration resolved each time antibacterial therapy was administered, and with his final episode, diarrhea clearly preceded the signs and symptoms of septicemia.

MAC was isolated from the patient’s blood at the time of his last admission for diarrhea. Previous attempts to isolate MAC from his stool and blood were unsuccessful. It is therefore unlikely that MAC was the etiologic agent of his recurrent diarrheal illnesses. Ciprofloxacin was chosen as one of his therapeutic agents for MAC bacteremia because it served the purpose of treating the EIEC as well. Although diarrhea has been noted for many patients with disseminated MAC infection, it is not clear that it causes diarrhea. In the study by Berry et al. of diarrheal disease in HIV-infected persons, the frequency of isolation of MAC was comparable for cases and controls (2).

Because *E. coli* is normal intestinal flora, evaluation of fecal *E. coli* isolates requires specialized testing. However, neither Sereny nor DNA probe testing for detection of EIEC are routinely available for testing clinical isolates. Although there is no simple screening test for EIEC, in one study all isolates were found to be lysine decarboxylase negative (21) whereas 75% of all *E. coli* isolates were positive. In the appropriate clinical setting, *E. coli* isolates from blood cultures which are lysine decarboxylase negative should be suspected of being EIEC.

In summary, we present a case of recurrent bacteremia due to EIEC in an immunocompromised man whose serum lacked the ability to kill the pathogen. Recognition of the pathophysiology of his recurrent bacteremias spared him further invasive evaluation for a more common anatomic source of *E. coli* bacteremia, such as biliary tract or urinary tract disease.

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REFERENCES


