Case 65

The patient was an 8-year-old male with a 2-day history of diarrhea. He presented with worsening diarrhea (14 movements that day) which had become bloody. He also complained of pain on defecation. He had vomited once. He had attended a cookout 6 days previously. He claimed that his mother made him eat a hamburger that was "pink inside" even though "he did not like it." His physical examination was benign except for obvious dehydration. His laboratory findings were significant for a white blood cell count of 13,100/μl with 9,700 neutrophils per μl, a methylene blue stain of feces that showed abundant polymorphonuclear cells, and a positive stool guaiac. He was treated with trimethoprim-sulfamethoxazole and intravenous fluid therapy for dehydration. He quickly improved and was discharged within 24 h. Culture of his stool specimen on MacConkey-sorbitol agar is shown in Fig. 1.

1. What is the most likely etiologic agent of his infection? What two important clues are found in this case that helped you determine the etiology of his infection?

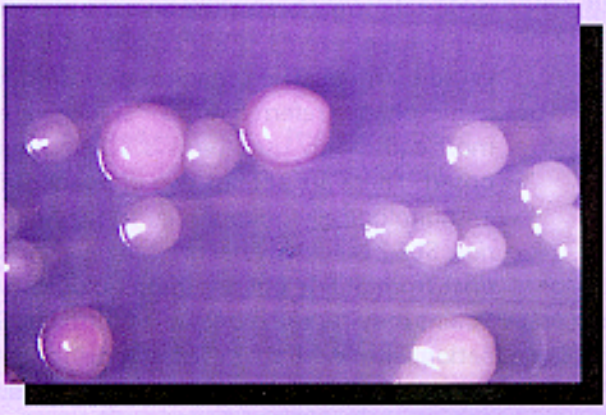

Figure 1

2. What are the major virulence factors produced by this organism? How do they act and what are their roles in the pathogenesis of disease?

3. Why are these organisms so difficult to detect in feces? Think about one of the major virulence factors produced by this organism and how it is encoded genetically.

4. Besides cultures, what other methods may prove useful for detecting this organism? Explain how these methods could be used to detect this organism.

5. How is the organism usually spread? How can infection with this organism be prevented?

6. What are sequelae associated with this infection? What organ and cell types are specifically targeted? What is the outcome of these sequelae?

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Case Discussion

1. This patient has bloody diarrhea due to enterohemorrhagic *Escherichia coli* or EHEC. The specific serotype of *E. coli* with which he is infected is O157:H7. This is the most common of the approximately 50 serotypes of *E. coli* which have been recognized as causing this disease. The two important clues in this case were bloody diarrhea, which is found in approximately 90% of patients with EHEC-associated diarrhea, and the eating of a hamburger which was "pink inside" (see answer 5 for further details). Abdominal pain, characterized as "pain on defecation" in this patient, would also be consistent with this infection.

2. EHEC possesses two major virulence factors which play a role in the diarrheal disease process. One, a transmembrane bacterial protein called intimin, mediates pedestal formation on the apical surface of enterocytes. Through a not-yet-defined sequence of events, this intimate binding results in changes in the enterocyte which cause diarrhea. The organism also produces a family of toxins, encoded by a lysogenic phage, referred to as "Shiga-like toxin" (SLT), verotoxin, or cytotoxin. The most commonly used term is SLT, although a new designation, Stx, has been proposed. There are at least two different SLTs. SLT-1 is functionally, biochemically, and immunologically quite similar to the Shiga toxin (Stx) produced by *Shigella dysenteriae*. SLT-2 is actually believed to be a family of toxins which are immunologically distinct from SLT-1, but are immunologically and biochemically related to each other. Strains may produce one or both toxins. Strains producing SLT-2 alone or in combination with SLT-1 are believed to be more virulent. SLT-1 and 2 have the same mechanism of action, i.e., inhibition of protein synthesis by enzymatic inactivation of the 80S ribosome. SLT-1 and 2 are A-B toxins. The B toxin binds to a specific receptor, a cell surface glycolipid called globotriaosylceramide. The toxin-glycolipid complex is internalized by cells, and the enzymatically active A subunit can then bind to and inactivate the 80S ribosome, inhibiting protein synthesis. It appears that these toxins target endothelial cells and can damage intestinal blood vessels, resulting in the bloody diarrhea seen in these patients. These toxins may also play a prominent role in the complications of this infection discussed in answer 6.

3. *E. coli* is very common in the gastrointestinal flora, and most individuals are colonized with nonpathogenic strains. Diarrheagenic strains of *E. coli* possess extrachromosomal genes which encode virulence factors. SLT-1 and 2 are encoded by genes contained in a lysogenic phage. Therefore, it is often difficult to distinguish pathogenic from nonpathogenic *E. coli* strains unless methods are used to detect virulence factors or the genes encoding them. Fortunately, *E. coli* O157:H7, the most common cause of EHEC, can be distinguished from over 90% of all other *E. coli* strains by its inability to ferment sorbitol. Almost all other *E. coli* isolates can ferment this sugar. To detect *E. coli* O157:H7 in the presence of nonpathogenic *E. coli* and other related enteric bacteria, stool specimens are plated on a differential and selective medium which contains sorbitol and a pH indicator to show whether sorbitol

has been fermented. The isolates that fail to ferment sorbitol are screened serologically to determine if they are *E. coli* O157:H7 (Fig. 1). This culture technique is widely available but may need to be specifically requested if this organism is being sought in feces.

4. Besides culture, EHEC can be detected either by demonstrating toxin production or by finding genes encoding the toxins. Detection of toxin production can be done directly on stool specimens or clinical isolates. Two approaches for toxin detection are widely used. One is based on the ability of SLT-1 and 2 to cause cytotoxic changes in a continuous cell line called Vero (thus the name verotoxin). Cytotoxicity assays are done as follows. Two aliquots of stool or culture filtrate are prepared. One is mixed with an equal volume of buffer and the other with an equal volume of antiserum specific for SLT-1 and 2. After a brief incubation period, the two samples are added to Vero cell monolayers and observed for up to 72 h. The following chart explains the interpretation of the test.

Filtrate and buffer	Filtrate and antiserum	Test interpretation
No cytotoxicity	No cytotoxicity	No toxin present
Cytotoxicity	No cytotoxicity	Toxin present
Cytotoxicity	Cytotoxicity	Uninterpretable
No cytotoxicity	Cytotoxicity	Technical error

Another approach to toxin detection is to use an enzyme immunoassay (EIA) for the immunologic detection of toxin. Tests for SLTs are commercially available. They are not as sensitive as the bioassay in cell monolayers but may be sensitive enough to detect clinical disease.

In addition to toxin detection, detection of genes coding for SLT-1 and 2 production may also be used. Detection of toxin genes can be done directly in stool or on isolates suspected of causing disease. The most common technique used to detect these genes coding for SLT-1 and SLT-2 is PCR (polymerase chain reaction).

5. The reservoir for *E. coli* O157:H7 is cattle. Ground beef, eaten either raw or as hamburgers which are pink inside, appears to be the major way in which this organism is spread. Several large outbreaks of this disease have been associated with the consumption of undercooked hamburgers prepared by fast-food restaurant chains. Why does ground beef but not other cuts cause outbreaks of this disease? The explanation is that the carcass of the meat animal may be contaminated with its own or other animals' fecal flora during rendering. The organisms will generally be on the surface of the meat and will be quickly killed by normal cooking. The exception is ground beef, where the process of grinding will introduce the organism throughout the meat. When beef patties are formed, organisms may be at the center of the patty. If the interior temperature of the patty fails to reach 150°F (ca. 67°C), the organism will survive cooking. Rare to medium-rare hamburgers ("pink inside") may not reach this temperature, resulting in the survival of some percentage of the EHEC.

As with *Shigella* spp., it appears that a low inoculum size can produce EHEC infection, so the small number of organisms that survive in the interior of undercooked hamburgers may be sufficient to be a hazard. Other sources of EHEC organisms have included unpasteurized cow's milk, water fecally contaminated by cattle, and unpasteurized cider made of apples which had fallen in a cattle grazing area and were not washed before cider preparation.

Preventive measures include avoiding the consumption of undercooked or raw hamburgers and unpasteurized milk. A simple rule is to cook hamburgers until the juices run clear and the meat is not pink inside. By law in several states, restaurants may not sell rare to medium-rare hamburgers; they must be thoroughly cooked.

6. The major sequela associated with this disease is the hemolytic uremic syndrome (HUS). The syndrome may also be called thrombotic thrombocytopenic purpura (TTP) in adults. HUS is seen primarily in children, and it is estimated to occur in approximately 10% of individuals following diarrheal disease with EHEC. The pathophysiology of HUS is due to the action of SLT on endothelial cells, particularly those in the kidney. HUS is characterized by thrombocytopenia, microangiopathic hemolytic anemia, and acute renal failure. Therapy for this disease is primarily supportive and may include erythrocyte transfusions and dialysis. The mortality of this disease is estimated at 5%; an additional 5 to 10% of patients have some level of chronic kidney failure.

References

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