Case Report

Clostridium sordellii as a Cause of Fatal Septic Shock in a Child with Hemolytic Uremic Syndrome

Rebekah Beyers,1 Michael Baldwin,2 Sevilay Dalabih,1 and Abdallah Dalabih1

1 Department of Child Health, University of Missouri, 400 Keene Street, Columbia, MO 65201, USA
2 Department of Molecular Microbiology and Immunology, University of Missouri, Columbia, MO 65201, USA

Correspondence should be addressed to Abdallah Dalabih; dalabiha@missouri.edu

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Clostridium sordellii is a toxin producing ubiquitous gram-positive anaerobe, mainly associated with trauma, soft tissue skin infections, and gynecologic infection. We report a unique case of a new strain of Clostridium sordellii (not present in the Center for Disease Control (CDC) database) infection induced toxic shock syndrome in a previously healthy two-year-old male with colitis-related hemolytic uremic syndrome (HUS). The patient presented with dehydration, vomiting, and bloody diarrhea. He was transferred to the pediatric critical care unit (PICU) for initiation of peritoneal dialysis (PD). Due to increased edema and intolerance of PD, he was transitioned to hemodialysis through a femoral vascular catheter. He subsequently developed severe septic shock with persistent leukocytosis and hypotension, resulting in subsequent death. Stool culture confirmed Shiga toxin producing Escherichia coli 0157:H7. A blood culture was positively identified for Clostridium sordellii. Clostridium sordellii is rarely reported in children; to our knowledge this is the first case described in a pediatric patient with HUS.

1. Introduction

Clostridium sordellii is a toxin producing ubiquitous gram-positive rod anaerobe, mainly associated with trauma, soft tissue skin infections, and gynecologic infection [1]. It is known to cause a severe shock syndrome characterized by a leukemoid reaction and significant capillary leak [2]. It has a characteristically high mortality rate, approaching 70% in previously healthy individuals [3]. It is rarely described in children and rarely isolated from the blood. It has been previously reported to cause sepsis and omphalitis in children [4], but never in association with other infections. We describe the case of a toxic shock syndrome caused by a new strain of Clostridium sordellii in a previously healthy two-year-old male, with acute Escherichia coli 0157:H7 positive hemolytic uremic syndrome.

2. Clinical Presentation

A previously healthy two-year-old male was admitted to the general pediatric floor with a two-day history of abdominal pain, vomiting, and diarrhea. The diarrhea was initially watery and became bloody at the time of admission. Vital signs and laboratory values on admission were normal except for white blood cells (WBCs) of 25.90 × 10^3 /μL (83% neutrophils and 7% bands). His hemoglobin was 14.8 g/dL and platelets were 427 × 10^3 /μL, and initial BUN and creatinine were 17 and 0.3 mg/dL, respectively. Abdominal ultrasound showed no abnormal findings.

The following day he had decreased urine output, elevated blood pressure, lower extremity and eyelid edema, and fever of 38.5°C. Vital signs and laboratory values at that time revealed tachycardia and elevated WBCs 34.60 × 10^3 /μL (61% neutrophils and 20% bands); hemoglobin dropped to 10.8 g/dL and platelets to 43 × 10^3 /μL. LDH level was 1319 unit/L and C3 and C4 were 76 and <9 mg/dL, respectively (normal values for a 2-year-old male are as follows: C3 = 80–170 mg/dL and C4 = 14–44 mg/dL). The peripheral smear showed toxic granulations and megakaryocytes and schistocytes. Abnormal electrolytes at that time were serum sodium of 131 mmol/L, potassium of 4.8 mmol/L, and elevated serum creatinine (0.9 mg/dL). Urine output had decreased from 3 cc/kg/hour to 1.3 cc/kg/hour. A presumptive diagnosis of
hemolytic uremic syndrome (HUS) was made and he was transferred to the pediatric critical care unit (PCCU) for further management. Repeated stool studies and blood cultures were obtained upon transfer to the PCCU. A chest radiograph showed hazy bilateral airspace opacities and small right pleural effusion. Upon admission to the PCCU a peritoneal dialysis (PD) catheter (Covidien, Dublin) was inserted, and a 3 lumen (5 French/12 cm) central line (Cook Medical, IN) was placed in the left femoral vein. Next day in the PCCU the fever recurred and the peritoneal dialysis catheter became nonfunctional. The blood culture from the day before became positive for gram-positive rods, growing in anaerobic media. A new culture was repeated before starting antibiotics and the patient was started on cefepime and piperacillin-tazobactam for empiric coverage. At that time vancomycin was avoided due to impaired renal function. The stool culture and toxin testing from the day of admission was also reported positive for Shiga toxin producing Escherichia coli 0157:H7.

In the following few hours the patient became progressively oliguric, with increasing serum creatinine level to 1.6 mg/dL and persistent hypotension. Laboratory values at day 2 in the PCCU showed WBC $56.7 \times 10^3 /\mu L$ (29% neutrophils, 28% bands), Hgb 13.2 g/dL, and platelets of $26.10^5 /\mu L$. Due to altered mental status he was emergently intubated and after insertion of a right side 11.5 French femoral hemo-dialysis catheter hemodialysis was started. Due to the hypotension and acidosis (lactate level of 15 mmol/L) the patient was started on epinephrine and dopamine infusions (peak infusion rates of 15 mcg/kg/minute of dopamine and 0.1 mcg/kg/minute of epinephrine). He continued to be hemodynamically unstable, so a stress dose of hydrocortisone was administered. Clindamycin was added for empiric treatment of toxin production. During the whole stay in the PCCU, the patient continued to be hypotensive not responsive to fluid resuscitation or inotropic medications. Within 36 hours of admission to the PCCU transfusion was also noted to be positive for Shiga toxin producing Escherichia coli 0157:H7.

The repeated stool culture was also noted to be positive for E. coli 0157:H7 and tested positive for Shiga toxin. Abdominal and pleural fluid cultures did not grow any organisms. Final blood culture identification was Clostridium sordellii. This identification was confirmed from 2 blood cultures 24 hours apart in the hospital laboratory and a plated sample was forwarded to an expert at the state laboratory. Repeated manual identification confirmed Clostridium sordellii (Table 1). When the samples of the bacteria were sent to the CDC, the center examined the samples and reported that this was a new strain of Clostridium sordellii not currently present in the CDC database.

### 3. Materials and Methods

#### 3.1. Cell Cytotoxicity of Culture Supernatants

Vero cells were either left untreated (control) or incubated with various dilutions of sterile culture filtrates in medium containing fetal cell serum (FCS) for 2 hours at 37°C. Morphological changes of intoxicated cells were directly analyzed in wells using an inverted microscope equipped with a DIC prism (Figure 1).

#### 3.2. PCR Analysis of Sordellilysin Expression in C. sordellii

Oligonucleotides (Integrated DNA Technologies Inc.) (Forward primer = 5'-GTACATATCCAGGAGCATTACAAC-3'; Reverse primer = 5'-CCACCATTTCCAGCAAGACCTGT-3') were designed to amplify sordellilysin based on the reported sequence of perfringolysin O [5]. PCR was performed using Pfu high fidelity polymerase (Agilent Genomics) and appropriate cycling conditions. Products were separated via agarose gel electrophoresis and compared with corresponding products from C. sordellii ATCC9714 (Figure 2).

#### 3.3. Detection of Large Clostridial Toxins via Inhibitory Antibodies

Certain toxigenic isolates of C. sordellii produce toxins that are very similar to the toxins of C. difficile.

### Table 1: The list of the biochemical tests on the panel.

| Substrates                                | Abbreviations | Organism reaction |
|-------------------------------------------|---------------|-------------------|
| p-Nitrophenyl-β-D-galactopyranoside       | BGAL          | Negative          |
| p-Nitrophenyl-α-D-galactopyranoside       | AGAL          | Negative          |
| bis-p-Nitrophenyl-phosphate               | BPO4          | Negative          |
| p-Nitrophenyl-N-acetyl-β-D-glucosaminide  | NGLU          | Negative          |
| p-Nitrophenyl-α-D-glucopyranoside         | AGL           | Positive          |
| o-Nitrophenyl-β-D-glucopyranoside         | BGL           | Negative          |
| p-Nitrophenyl-phosphate                   | PO4           | Negative          |
| p-Nitrophenyl-α-L-fucopyranoside          | AFU           | Positive          |
| p-Nitrophenyl-α-D-mannopyranoside         | MNP           | Negative          |
| L-Leucine-β-naphthylamide                 | LEU           | Negative          |
| DL-Methionine-β-naphthylamide             | MET           | Negative          |
| L-Lysine-β-naphthylamide (alkaline)       | LYB           | Negative          |
| L-Lysine-β-naphthylamide (acid)           | LYA           | Negative          |
| Glycylglycine-β-naphthylamide             | GGLY          | Negative          |
| Glycine-β-naphthylamide                   | GLY           | Negative          |
| L-Proline-β-naphthylamide                 | PRO           | Positive          |
| L-Arginine-β-naphthylamide                | ARG           | Negative          |
| L-Pyrrolidinol-β-naphthylamide            | PYR           | Negative          |
| L-Tryptophan-β-naphthylamide              | TRY           | Negative          |
| 3-Indoxyl phosphate                       | IDX           | Negative          |
| Trehalose                                 | TRE           | Negative          |
| Urea                                      | URE           | Positive          |
| Indole                                    | IND           | Positive          |
| Nitrate                                   | NIT           | Negative          |

This set of biochemical reactions was compared to the MicroScan Database for Clostridia species. The result of this set of positive and negative biochemical reactions was consistent with a 99.99% probability of Clostridium sordellii.
The hemorrhagic toxin (toxin HT) of *Clostridium sordellii* is very similar to toxin A whereas the lethal toxin (toxin LT) is very similar to toxin B. Antibodies against the *Clostridium difficile* toxins neutralize the toxins HT and LT of *C. sordellii*, and antibodies against toxins HT and LT neutralize toxins A and B. To confirm the absence of HT or LT expression in our *C. sordellii* isolate a *Clostridium difficile* Toxin/Antitoxin Kit (Techlab, VA) was used in conjunction with a Vero cell culture cytotoxicity assay in accordance with the manufacturer’s instructions. In brief, serial dilutions of culture supernatants from *C. difficile* 630, *C. sordellii* ATCC9714, and *C. sordellii* isolate XXX (our study) were mixed with either control or antitoxin antibodies prior to incubation within monolayers of Vero cells. Vero cells were monitored for up to 24 hr for cell rounding and visually scored. As expected, antitoxin antibodies effectively neutralized the toxin activity present in culture supernatants from *C. difficile* and *C. sordellii* ATCC9714, but not from *C. sordellii* isolate XXX.

4. Discussion

*Clostridium sordellii* is a spore forming gram-positive rod anaerobe found commonly in soil and the intestinal tract of mammals. Infections with *Clostridium sordellii* are mainly associated with trauma and medically induced abortions. It has been noted to be highly lethal in previously healthy individuals. It has been more recently described in normal term deliveries and heroin users [6]. There are over 40 different strains of *C. sordellii* and not all are toxin producing. Seven different exotoxins have been identified to be produced by *C. sordellii*, the two most virulent being lethal toxin and hemorrhagic toxin. These toxins are related to the large clostridial cytotoxin family, which affect cell signaling and are responsible for the characteristic leukemoid reaction and severe capillary leakage associated with *C. sordellii* infection [6]. Lethal toxin causes cell necrosis and edema secondary to increased vascular permeability. Hemorrhagic toxin has
onset as in our case [4]. The rapid progression of the sepsis hypotension and shock occurred within hours of symptom with death occurring most commonly within 2–6 days of is also associated with very rapid progression of the disease, damycin may have the added effect of decreasing toxin effect have been tested had already died. Penicillin, tetracycline, and clindamycin blood culture identification was only available after the child makesthe infection difficult to identify and treat; our patient's sordellii leakage and profound hypoalbuminemia [4]. pulmonary edema are common and associated with capillary and systemic vascular resistance [6].

Figure 2: Analysis of sordellilysin in Clostridium sordellii isolates. Primers designed to amplify cholesterol dependent cytolysins (cdc) based on the reported sequence of perfringolysin O (pfo) were included in PCR reactions for each C. sordellii isolate. Amplification products were separated by 0.8% agarose gel electrophoresis and visualized via ethidium bromide staining. Lanes are designated by the strain names of each isolate in the figure. C. sordellii ATCC9714 was used as a positive control from sordellilysin expression.

been noted to be directly cytotoxic in vivo studies [7]. The toxins have been known to directly depress cardiac output and systemic vascular resistance [6].

The initial absence of fever is a characteristic finding and often persists throughout the course of the illness. Severe symptoms of resistant hypotension and tachycardia follow quickly. Laboratory findings include leukocytosis, thrombocytosis, elevated hematocrit, and significant hypoalbuminemia. The leukocytosis is usually profound, often exceeding 75,000 and with a remarkable left shift, which we observed in our patient. Leukocytosis is also known to be a poor prognostic factor in HUS and highly associated with mortality in C. sordellii infection [6]. Pleural effusion and pulmonary edema are common and associated with capillary leakage and profound hypoalbuminemia [4]. Clostridium sordellii infection not only carries a high mortality rate but is also associated with very rapid progression of the disease, with death occurring most commonly within 2–6 days of initial infection. In many reported cases, onset of severe hypotension and shock occurred within hours of symptom onset as in our case [4]. The rapid progression of the sepsis makes the infection difficult to identify and treat; our patient's blood culture identification was only available after the child had already died. Penicillin, tetracycline, and clindamycin have been tested in vivo for their efficacy in treatment. Clindamycin may have the added effect of decreasing toxin effect [5]. Treatment is mainly supportive in regard to hypotension and cardiovascular strain.

Although C. sordellii infection is relatively rare, there is suspicion that it is underreported, particularly in critically ill patients, due to its rapid progression and fastidious nature [6, 7]. A high index of suspicion, rapid initiation of treatment, and increased early identification would be key to decrease mortality associated with this organism. This case is unique in that C. sordellii infection has not been extensively described in children; isolation of C. sordellii from the blood is rare and to our knowledge has not been described in association with HUS. Although the exact source of Clostridium sordellii infection in this case is unknown, development of C. sordellii sepsis caused by translocation through the GI tract has been suspected in adult patients with comorbidities [6]. Hunley et al. had suggested that diarrhea associated HUS comprises a clinical entity which appears to predispose to a traumatic C. septicum infection, where acidic and anaerobic conditions in the diseased colon favor C. septicum invasion [8].

This could explain the mechanism of the suggested bacterial translocation in our patient. Clinicians should be aware of the possibility of clostridial infection in the presence of gastrointestinal pathology and hemodynamic instability, considering Clostridia and broadening the antibiotic coverage early on in the course might be lifesaving.

Abbreviations
HR: Heart rate
WBCs: White blood cells
PCCU: Pediatric critical care unit
PD: Peritoneal dialysis
CDC: Center for Disease Control
FCS: Fetal cell serum
CPE: Cytopathic effects
HUS: Hemolytic uremic syndrome.

Conflict of Interests
The authors declare that there is no conflict of interests regarding the publication of this paper.

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