A Pediatric Cluster of *Shigella dysenteriae* Serotype 1 Diarrhea with Hemolytic Uremic Syndrome in 2 Families from France

Véronique Houdouin,†‡ Catherine Doit,§ P. Mariani,¶ Naïma Brahimi,† Chantal Loirat,† Antoine Bourrillon,§ and Edouard Bingen†,‡

Services de †Microbiologie, ‡Néphrologie, and § Pédiatrie Générale, and ¶Laboratoire associé au Centre National de Référence E. coli-Shigella, Hôpital Robert Debré, Paris, France

We report a cluster of pediatric diarrhea due to *Shigella dysenteriae* serotype 1 involving 11 children in France, including the index case, who had returned from Senegal. Child-to-child transmission was documented by ribotyping. Five children developed hemolytic uremic syndrome (HUS). On the basis of our findings, the choice of antimicrobial treatment for infections with *S. dysenteriae* serotype 1 should take into account widespread drug resistance and the risk of HUS.

*Shigella dysenteriae* serotype 1 infection is associated with serious morbidity and mortality in developing countries [1]. It can cause lethal, bloody diarrhea in all age groups, but especially in children. Shiga toxin, produced by *S. dysenteriae* serotype 1, has been linked to hemolytic uremic syndrome (HUS) in developing countries [2, 3] but rarely in industrialized countries [4]. We investigated a cluster of diarrhea due to *S. dysenteriae* with HUS in France involving 11 children belonging to 2 families.

**Patients and methods.** During a 2-week period in late 1997, eleven children developed bloody diarrhea due to *S. dysenteriae* serotype 1. Five children belonged to one family, and the other 6 children belonged to another family living in close proximity to the first. The children lived in Paris, France, and were close contacts of the index case, who had recently returned from a visit to Dakar, Senegal. All of the children were admitted to our hospital with urgency. At admission, each of the children underwent a differential blood cell count; blood urea, electrolyte, serum creatinine, and C-reactive protein assays; and a blood culture. All of the children received antibacterial chemotherapy.

Five of the children developed HUS, which was diagnosed on the basis of a platelet count of <140 × 10^9/μL, erythrocyte fragmentation on a peripheral blood smear (≥5 cells per high-powered field), and kidney damage (hematuria, defined as grade >1+ by dipstick analysis; and/or proteinuria, defined as grade >1+ by dipstick analysis; and/or azotemia, defined as serum creatinine concentration >95th percentile for age and sex).

Fresh stool specimens were examined microscopically and cultured for *Salmonella, Shigella, Campylobacter*, and *Vibrio* species, by use of standard methods. *S. dysenteriae* was identified using standard biochemical methods (API 20E test; BioMérieux). Serological typing was done by means of the slide agglutination test with specific antiserum (Serum anti–*S. dysenteriae* monovalent, Bio-Rad). Susceptibility to ampicillin, ceftriaxone, ciprofloxacin, trimethoprim-sulfamethoxazole, and azithromycin was determined by the disk diffusion method on Mueller-Hinton agar and interpreted according to NCCLS guidelines [5]. The MIC of azithromycin was also assessed by the E-test method, according to the manufacturer’s guidelines (AB Biodisk).

Ribotyping was used to determine the relatedness of the *S. dysenteriae* isolates. Total *S. dysenteriae* DNA was prepared as described elsewhere [6], then digested with EcoRI and subjected to Southern blotting with *Escherichia coli* 16S rRNA plus 23S rRNA as the probe, after random oligopriming with a mixture of hexanucleotides (Pharmacia) and cloned Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories) in the presence of 0.35 mmol/L digoxigenin-11-deoxyuridine-5′-triphosphate (DIG-11dUTP; Boehringer). The chemiluminescence detection procedures have been described in detail elsewhere [7].

The stx gene was detected by direct PCR analysis of stool samples, as described elsewhere [8]. We used Fisher’s exact test and a *t* test for matched continuous data. *P* values of <.05 were considered statistically significant.

**Results.** The patients were 6 boys and 5 girls aged 1–12 years (mean ± SE, 54 ± 42 months). They belonged to 2 families who lived in the same block in Paris. The index case, who had originated from Senegal, was hospitalized immediately after his return from a visit to Dakar, Senegal. The 4 siblings of the index case and the 6 children belonging to another close family,
who had originated from Mali, were hospitalized within 2 weeks after the index case.

All of the children were well-nourished and had no relevant medical history. All presented with bloody diarrhea. The interval between symptom onset and emergency admission ranged from 1–5 days. The demographic and clinical characteristics of the patients are described in table 1.

_S. dysenteriae_ serotype 1 was isolated from the stool samples of 8 children. All of the isolates had the same resistance pattern, namely resistance to ampicillin and trimethoprim-sulfamethoxazole and susceptibility to ceftriaxone and ciprofloxacin. The azithromycin MIC\textsubscript{90} for the 8 isolates was 2 μg/mL. None of the children was bacteremic.

Five children developed HUS while in the hospital, 5–10 days after beginning antibiotic therapy. The presenting signs and symptoms did not differ between patients according to subsequent onset of HUS. The most noteworthy differences at admission between patients with and patients without HUS were marked leukocytosis (5 of 5 patients vs. 2 of 6, \( P = 0.045 \)) and hyponatremia (mean sodium concentration ± SD, 124 ± 1.8 vs. 134 ± 2.6 mmol/L; \( P < 0.001 \)). Three children were treated with ceftriaxone at a dosage of 50 mg/kg/day, and all 3 developed HUS. Three children were treated with ciprofloxacin at a dosage of 30 mg/kg/day, and 1 developed HUS. The other 5 children were treated with ceftriaxone for 5 days and then with ciprofloxacin for 5 days, and 1 of these children developed HUS. Antibiotic therapy lasted 10 days in every case. The risk of HUS did not appear to be influenced by the type of antibiotic therapy received. After the completion of antibiotic therapy, all children had stool cultures negative for _S. dysenteriae_ serotype 1.

The outcome for the 6 children who did not develop HUS was good. None developed chronic diarrhea, and the median hospital stay was 5 days. Four of the 5 children with HUS received blood transfusions. One had severe HUS with colonic perforation and required hemodialysis and mechanical ventilation. Five years later, none of the 5 children with HUS had developed chronic renal failure.

Results of _stx_ PCR of stool samples were positive for all 8 children with culture-positive stool samples and for 1 child with culture-negative stool samples. A stool sample for one of the other 2 children was negative for _stx_ by PCR, and samples were lacking for the other child. Both of these children developed HUS.

Eleven _S. dysenteriae_ isolates were selected for molecular studies. Eight isolates were from the 8 children with culture-positive stool samples. Three epidemiologically unrelated _S. dysenteriae_ isolates were studied comparatively. The 11 isolates belonged to 4 ribotypes. The 8 clinical isolates belonged to the same ribotype, which clearly differed from the 3 ribotypes of the 3 control strains (figure 1).

### Discussion

HUS due to _Shiga_ toxin–producing _S. dysenteriae_ serotype 1 is a major potential complication of dysentery in developing countries [2, 9], but rarely in developed nations. Two cases were recently reported in Oregon [4]. HUS occurs most frequently in children, whose renal glomeruli strongly express _Shiga-toxin_ receptors [10]. In our study, all 5 children with HUS were aged <5 years. As in previous reports [2, 3, 11], our patients with HUS had significant leukocytosis and severe hyponatremia at admission, compared with the 6 patients who did not develop HUS. Empirical antibiotic treatment of traveler’s diarrhea is recommended before obtaining fecal samples [12], and this strategy was applied for our index case. The other children were treated as contacts of the index case. Child-to-child transmission was shown by ribotyping, which demonstrated the genetic relatedness of the isolates obtained from 8 children. To our knowledge, this is the largest reported family cluster of _S. dysenteriae_ infection in an industrialized country. Results of _stx_ PCR of stool samples were positive for all of the children with _Shigella_-positive stool cultures, and also for 1 child from whom _S. dysenteriae_ was not recovered by culture. This confirms that PCR is able to detect _Shiga_ toxin–producing organisms in stool samples, even when culture fails to do so [13]. One child negative for _stx_ by PCR developed HUS, although the first stool sample was obtained 4 days after the beginning of treatment.

Because of widespread resistance to amoxicillin and cotrimoxazole, 3 children received ceftriaxone, 5 received ceftriaxone followed by ciprofloxacin, and 3 received only ciprofloxacin. High-level, multidrug resistance among _Shigella_ species

### Table 1. Demographics and clinical characteristics of 11 children with and without hemolytic uremic syndrome (HUS) who had _Shigella dysenteriae_ serotype 1 diarrhea.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Developed HUS (( n = 5 ))</th>
<th>Did not develop HUS (( n = 6 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Age, median months</td>
<td>50</td>
<td>58</td>
</tr>
<tr>
<td>Duration of illness, median days</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Abdominal tenderness</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Vomiting</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Bloody diarrhea</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;38.5°C</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>&lt;36.5°C</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Dehydration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Moderate</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Shock</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Altered consciousness</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

NOTE. For all characteristics, \( P \) was not statistically significant.
isolates has been reported previously in industrialized countries [4]. Severe shigellosis is one of the only diseases for which first-line use of fluoroquinolones may be justified in children, because these drugs can reduce the case-fatality rate among children infected with S. dysenteriae serotype 1 [1]. One of the 3 children who received fluoroquinolone monotherapy developed HUS. Zhang et al. [14] reported that fluoroquinolones may be inappropriate for treatment of Shiga toxin-producing E. coli infection. Indeed, fluoroquinolones, which inhibit DNA gyrase, can induce a bacterial SOS response, which increases toxin-phage levels in Shiga toxin–producing E. coli [15]. This phenomenon has not been reported with the chromosomally encoded Shigella Shiga toxin. In addition to fluoroquinolones, cephalosporins have also been implicated in the pathogenesis of HUS [16]. The 3 children treated with ceftriaxone alone all developed HUS. Excessive release of Shiga toxin during treatment with β-lactam agents may be a contributory factor in the development of HUS in patients with S. dysenteriae infection. Unlike other β-lactam agents, imipenem induces only a low level of Shiga toxin release in vitro [17], but imipenem is not excreted in the gut. Because of the small number of cases in this cluster, the risk of HUS could not be linked to the treatment strategy, although the risk of HUS with antibiotic treatment is still discussed in connection with Shiga toxin–producing E. coli [18]. The emergence and dissemination of Shigella strains resistant to several antibiotics, including fluoroquinolones [4], may require new treatments. Recent reports have shown that azithromycin yields a high bacterial eradication rate in children with shigellosis [19], probably because it reaches high concentrations in stool. Median fecal concentrations of azithromycin exceeded by 100 times the MIC90 values of our Shigella isolates [20]. Macrolides bind selectively to the 50S subunit of the bacterial ribosome and inhibit protein synthesis by blocking transpeptidation. This mechanism of action has been linked to low-level release of Shiga toxin and lower lethality in a murine model of E. coli 0157:H7 infection [21].

According to World Health Organization and pediatrician consensus statements [12], antimicrobial treatment is recommended for Shigella diarrhea. Additional clinical studies of approved treatments for S. dysenteriae serotype 1 infection in children are needed, taking into account the risk of HUS.

References
