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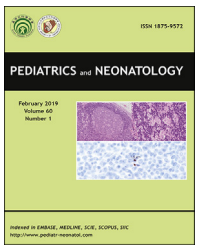

Original Article

An outbreak of hemolytic uremic syndrome in southern Romania during 2015—2016: Epidemiologic, clinical, laboratory, microbiologic, therapeutic and outcome characteristics

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Key Words
children;
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Background and aims: To describe the epidemiologic, clinical, microbiological, therapeutic and outcome characteristics of a HUS outbreak occurring in southern Romania from 2015 to 2016.
Methods: We retrospectively collected data from the medical records of all HUS cases hospitalized at the pediatric nephrology department of Marie Curie Children's Hospital of Bucharest, Romania.
Results: There were 32 HUS cases (19 girls/13 boys, 87.6% <2 years), all associated with diarrhea (bloody in 13, 40.6%). Thirteen (40.6%) and 4 (12.5%) patients had oliguria and anuria at admission. Extreme pallor, generalized edema, vomiting, dehydration, fever and seizures were found in 100%, 56.3%, 31.3%, 31.3%, 25% and 9.4% of patients, respectively. *E. coli* and STEC were identified in the stools of 6 and 8 patients, respectively; *E. coli* O_{26} and O_{157} infection were documented serologically in 10 and 3 children, respectively. There were 15/32 (46.9%) patients with confirmed HUS. Eighteen (56.3%) patients were hypertensive; other complications included infections, left ventricular hypertrophy, cardiopulmonary arrest, seizures and encephalopathy in 62.5%, 37.5%, 28.3%, 18.8% and 12.5%, respectively. Peritoneal dialysis and hemodialysis were performed in 23 (72%) and 2 patients, respectively. Three patients (9.4%) died early during hospitalization. A 6—12-month follow-up of 26 patients revealed that 65.4% had post-HUS sequelae (persistent hypertension and chronic renal failure in 34.6% and 30.8%, respectively).

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Conclusions: The principal STEC serotype involved was O_{26}:H_{11} and the number of confirmed HUS cases reached half of the patients. Compared with the medical literature, this outbreak had a higher rate of complications and renal sequelae and was associated with a high fatality rate.

1. Introduction

Hemolytic uremic syndrome (HUS) is a thrombotic microangiopathy characterized by three major symptoms: nonimmune hemolytic anemia with fragmentocytosis, low platelet count and acute kidney injury.[1–3] Shiga toxin-related HUS is the most common cause of acute kidney injury in childhood and constitutes around 90% of all HUS cases in this population.[1–3] The STEC strains produce 2 virulent types of Shiga-like toxin, Stx-1 and Stx-2; the latter is more toxic and is more frequently associated with progression to HUS, although both toxins may be associated with complicated STEC infections.[4,5]

Infections caused by STEC serotype O_{157}:H_7 are the most common cause of HUS in most countries worldwide.[3,6–9] However, other serotypes have also been described in the etiology of the diseases.[1–3,10] Gerber et al.[11] prospectively studied 394 pediatric patients diagnosed with HUS in Germany and Austria from 1997–2000 (disease incidences of 0.7/100,000 and 0.4/100,000 children <15 years old, respectively) and non-O_{157}:H_7 STEC serotypes were reported in 43% of stool cultures. In this study, patients with O_{157}:H_7 serotypes required dialysis for a longer period of time and had bloody diarrhea detected more frequently compared with patients with non-O_{157}:H_7 serotypes.[11] In a study describing 617 HUS patients <18 years of age admitted with HUS in the United States from 2000 to 2010, 436 patients (70.7%) had evidence with infection most likely caused by HUS-causing agents (401 STEC O_{157}, 21 non-O_{157} STEC, 1 O_{157} and non-O_{157} STEC, 11 *S. pneumoniae* and 2 other pathogens).[12] Among patients without microbiological evidence of STEC, 76.9% of those tested had serologic evidence of STEC infection.

E. coli O_{104}:H_4 recently caused a large outbreak of 3816 cases of gastroenteritis (54 deaths) in northern Germany in the months of May–July 2011, of them 845 (22%) developed HUS.[13–15] Most (88%) of the HUS patients were adults, the outbreak strain was typed as an enteroaggregative Shiga-toxin-producing *E. coli* O_{104}:H_4 and the consumption of sprouts was identified as the most likely vehicle of infection.[13,14] In summer 2013, a community-wide outbreak of HUS in Southern Italy prompted the investigation of a community-wide outbreak of Shiga toxin 2-producing *E. coli* (STEC) O_{26}:H_{11} infections.[15] STEC O_{26} infection was identified in 20 children (median age 17 months) with HUS, two of whom reported severe neurological sequelae. Molecular typing showed that two distinct STEC O_{26}:H_{11} strains were involved. An association between STEC O_{26} infection and consumption of dairy products from two local plants was reported.

During the period December 2015–February 2016, 15 cases of HUS were reported among children aged 5–38 months in southern Romania.[16] All the patients were hospitalized at the pediatric nephrology department of the "Marie Curie" Children's Hospital of Bucharest, Romania. Three patients died during this outbreak. In an environmental investigation, different cheese samples from a milk processing establishment in the Arges district tested positive for *stx* genes. Isolates of *E. coli* O_{26} were identified in a soft cheese from the same establishment which was closed in March 2016 and the suspected products were withdrawn from the Romanian market.

Following these initial 15 cases, 17 additional children were diagnosed with HUS and admitted during the period February–September 2016 at the same hospital. The present study describes the demographic, clinical, laboratory, microbiologic, therapeutic and outcome characteristics of this HUS outbreak in southern Romania.

2. Patients and methods

From December 29, 2015 through September 27, 2016, all children hospitalized with the clinical and laboratory diagnosis of HUS at the pediatric nephrology department of the Marie Curie Children's Hospital of Bucharest, Romania, were included in this study.

A hemolytic-uremic syndrome (HUS) case was defined as a case clinically compatible with all the following laboratory findings of:

- thrombocytopenia ($<150 \times 10^9$/L), and
- anemia (Hgb < 10.5 g/L) of hemolytic origin, and
- acutely reduced renal function (serum creatinine >35 μmol/L for patients < 1 years of age, >80 μmol/L for patients 1–15 years of age), and
- reported presence of fragmented red blood cells (schiztocytes) on peripheral blood smear,[1]

in the absence of septicemia, malignant hypertension, chronic renal failure or any primary vascular disease.

Case definition and case finding: ***A probable case*** was defined as a patient presenting with HUS, or with suspected HUS, and with diarrhea between December 2015 and September 2016. ***A confirmed case*** was defined as a patient with HUS and laboratory evidence of infection with STEC.

2.1. Variables collected

The following clinical variables were retrospectively collected: time from first symptom to admission; age at admission; duration of hospitalization; presence of prodromal diarrhea; presence of prodromal bloody diarrhea;

presence of hypertension at admission; development of oliguria/anuria, hypertension and/or proteinuria in the acute phase; extra-renal complications; death in the acute phase; and laboratory values at admission (hemoglobin, creatinine, platelet count, CRP, white blood cell count, sodium, peripheral blood smear).

2.1.1. Laboratory diagnosis of Shiga toxin-producing *Escherichia coli* (STEC) infection

The National Reference Laboratory from Cantacuzino Institute in Bucharest performed testing for stx (1 and 2) and eae (intimin) genes by PCR. Isolates were also tested with O antisera against the main STEC serogroups by slide agglutination. Twelve serum samples were examined at the Instituto Superiore di Sanita (ISS) in Rome, Italy, for the presence of antibodies to the lipopolysaccharide (LPS) of 6 major STEC serogroups (O_{157}, O_{26}, O_{103}, O_{111}, O_{145} and O_{55}) by Elisa.[15–17]

The following therapeutic intervention variables were collected: use of dialysis, type of dialysis used, duration of dialysis; red blood cell transfusions, platelet transfusions, plasma infusions/exchange, antibiotics (any indication); and use of other therapeutic modalities.

The following long-term/outcome variables were collected: presence of hypertension and/or proteinuria at first follow-up and at follow-up to 1 year following initial admission; and presence of renal sequelae/long-term complications.

3. Results

Thirty-two HUS patients (19 girls and 13 boys, median age 1.5 years, range 6 months—41 months) were hospitalized from 12/29/2015 untill 9/27/2016. One case was recorded in December 2015; the other 31 cases were recorded in 2016 (2, 13, 4, 1, 2, 1, 4, 1 and 3 cases in January, February, March, April, May, June, July, August and September, respectively)-(Fig. 1). Previous pathologies (conditions) were found in 6 (18.7%) patients: 1 with Downs syndrome and ventricular and atrial defect, 1 with gastroesophageal reflux, rectal prolapse and prior neonatal sepsis, 1 with prematurity, microcephaly and psychomotor retardation, 1with varicella one month before present admission, 1 with prior urinary tract infection and 1 after surgery for correction of anorectal agenesis. There were 15 (46.9%) patients under the age of 1 year and 28 (87.6) younger than 2 years. Twenty-eight (87.5%) patients had an urban residence. Fourteen (43.8%) were treated with antibiotics during the week preceding the hospitalization (ceftriaxone and ampicillin in 7 and 4 patients, respectively).

All HUS cases presented with diarrhea and 13 (40.6%) had bloody stools (Table 1); 13 (40.6%) and 4 (12.5%) cases had oliguria and anuria, respectively, at admission. The other main clinical manifestations included extreme pallor, generalized edema, vomiting, dehydration and fever (100%, 56.3%, 31.3%, 31.3% and 25%, respectively).

Median hemoglobin value at admission was 11.1 g/dL (range 4.3—13.1)-(Table 2). The mean serum creatinine value at admission was 1.84 ± 1.03 μmol/L (30/32 patients had abnormal values, calculated in relationship with their age). The platelet count at admission was $<150{,}000 \times 10^9$/L in 27 (87.5%) patients, with further decrease to values $< 150{,}000 \times 10^9$/L in the 4 other patients during the first 24 h of hospitalization. The mean white blood cells count was 12.756, 56 ± 7776, 87×10^9/L (values > 15,000 in 6 patients, 18.8%). Neutropenia was present at admission in 21 (65.6%) patients. Fourteen (43.8%) patients had CRP values > 20 at admission.

No data on stool cultures performed in the community were available. *E. coli* was found in the stool cultures (obtained at admission) of 6 patients (3 enteropathogenic and 3 enterohemorrhagic *E. coli*). *Clostridium difficile* toxin was identified in the stool of 2 additional patients.

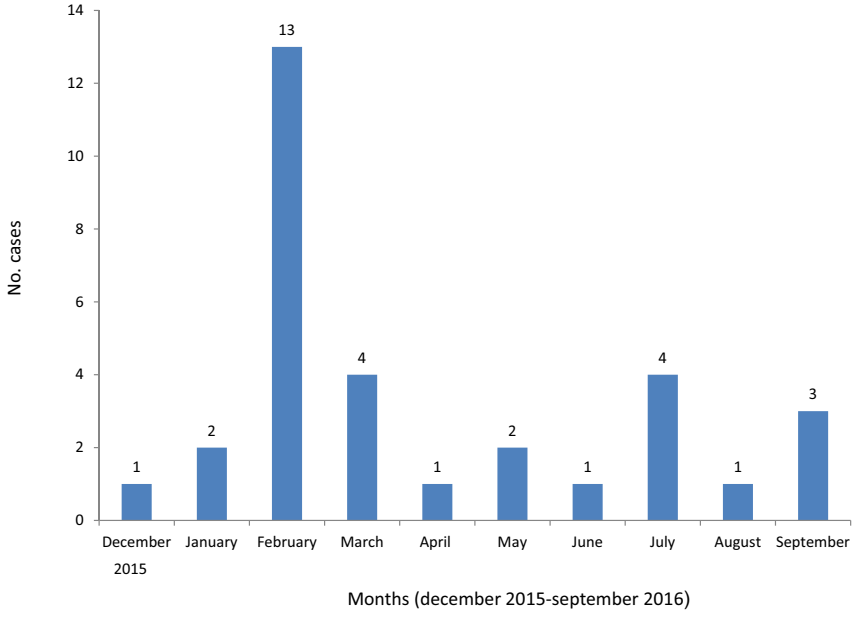

Figure 1 Distribution of HUS cases during the outbreak period.

Table 1 Clinical manifestations (at admission).

Clinical manifestation	n (%)
Prodromal diarrhea	32 (100)
- bloody diarrhea	13 (40.6)
Pallor	32 (100)
Generalized edema	18 (56.3)
Oliguria	13 (40.6)
- anuria	4 (12.5)
Vomiting	10 (31.3)
Dehydration	10 (31.3)
Fever	8 (25)
Somnolence	7 (21.9)
Petechial rash	5 (15.63)
Hematuria (macroscopic)	5 (15,63)
Restlessness (agitation)	3 (9.38)
Seizures	3 (9.38)

Table 2 Laboratory investigations (at admission).

Hemoglobin (g/dL)	
- Mean ± SD	9.54 ± 1.47
- Median	9.1
- Range	4.3–13.1
- ≤10.0	26
Creatinine (μmol/L)	
- Mean ± SD (all patients)	1.84 ± 1.03
- Range	0.3–5.06
- Median	1.6
- Normal	2 (6.3%)
Platelets (X 10^9/L)	
- Median	62
- Range	11–281
- Mean ± SD	80.93 ± 59.12
- <150.000	28 (87.5%)
- <100.000	24 (75.0%)
- <50.000	10 (31.3%)
WBC count (X 10^9/L)	
- Median	10.570
- Range	53,604–4.180
- Mean ± SD	12.756,56 ± 7776,87
- >15.000 (n, %)	6 (18.8%)
Sodium (μmol/L)	
- Median	132
- Range	115.7–139.1
- Mean ± SD	129.8 ± 6.85
- < 130	21 (65.6)
- < 120	2 (6.3)
C-Reactive Protein(mg/L)	
- Mean ± SD	28.32 ± 54.12
- Range	0.3–312.71
- Median	12.71
- <5	8 (25.0%)
- 5–10	8 (25.0%)
- 10–20	2 (6.3%)
- >20	14 (43.8%)

The serum of all 31 patients was tested with O antisera against the main STEC serogroups by slide agglutination and revealed 7 patients positive for *E. coli* O_{26}, 1 for *E. coli* O_{157} and 2 for *E. coli* O_{26}/O_{157}. Serotyping performed in Italy from the serum of 12 patients revealed 5 patients positive for *E. coli* O_{26} lipopolysaccharide (LPS) antibodies. Four serum samples were positive for *E. coli* O_{26} in the examinations performed in both countries and 1 serum positive for *E. coli* O_{26} in the Romanian institute was negative when examined in Italy. Four serum samples positive for *E. coli* O_{26} at the Cantacuzino Institute were not checked in Italy. The presence of the stx (1 and 2) and eae (intimin) genes was reported in 6 patients, of stx1+eae in one patient and of eae alone in an additional patient. Stx (1 and/or 2) and eae genes were identified in the stools of 3 HUS patients without any additional evidence of *E. coli* infection. In summary, *E. coli* was identified in the stools of 6 patients, *E. coli* O_{26} infection was documented in 9 children and *E. coli* O_{157} infection in 3 children with HUS. The presence of STEC was confirmed in 8 patients. Overall, 15/32 (46.9%) patients were defined as confirmed HUS cases.

Seizures were recorded in 3 (9.4%) patients. The median time from first registered symptom to admission was 5 days (range 1–11 days). All patients had venous central lines from the day of admission. Three (9.4%) patients died, all in the acute phase, two during the first 24 h after admission and one 5 days after admission.

Eighteen (56.3%) patients were hypertensive at admission or during hospitalization (Table 3). The main complications recorded (in descending order of frequency) during hospitalization included infections (present in 20 patients, 62.5%), cardiac complications (21, 65.5%), hypertension (18, 56.3%), neurological complications (7, 21.9%) and gastrointestinal complications (biliary sludge, 4, 12.5%).

Infectious complications included bacteremia (n = 2, 1 *Pseudomonas aeruginosa* and 1 bacteremia with *Klebsiella pneumoniae* + *S. warnerii*), candiduria (n = 9, 28.3%), peritonitis (n = 3, 2 *Candida albicans*, 1 *Enterococcus fecalis*) and urinary tract infection (n = 3, 1 *E. coli*, 1 *Acinetobacter baumanii* and 1 *K. oxytoca*). Three patients had stool examinations positive for Rotavirus antigen.

Cardiac complications were diagnosed by echocardiography in 21 (65.6%) patients and included (as single or mixed diagnosis) 12 cases of left ventricular hypertrophy, 8 of diastolic dysfunction, 6 of pericarditis, 3 of systolic dysfunction and 3 of mitral insufficiency. Pericardial fluid and aortic insufficiency (2 patients each) were additional cardiac complications diagnosed in the 21 patients. Ten patients had more than 1 cardiac finding diagnosed by echocardiography. Pulmonary edema was diagnosed in 2 patients during hospitalization.

Neurologic complications included seizures (7 patients), encephalopathy (6), cerebral hemorrhage (2) and coma (1).

Dialysis was performed in 23 (72%) patients (peritoneal dialysis in all 23 and hemodialysis in 2 patients following the peritoneal dialysis), for a median (±SD) duration 15.61 ± 6.95 days (median 15, range 3–33 days). Red blood cell transfusions were used in 31 (96.9%) cases, plasma infusions in 5 (15.6%) and thrombocytes infusion in 5 (15.6%)

Table 3 HUS complications recorded during hospitalization.

Complication	n (%)
Hypertension	18 (56.3)
Lt. Ventricular hypertrophy	12 (37.5)
Cardiopulmonary arrest requiring resuscitation	9 (28.1)
Infections	20 (62.5)
- Bacteremia	2 (6.3)
- Candiduria	9 (28.1)
- Peritonitis	3 (9.4)
- Urinary tract infection	3 (9.4)
- Rotavirus infection	3 (9.4)
Cardiac complications	21 (65.6)
- Lt. Ventricular hypertrophy	12 (37.5)
- Diastolic dysfunction	8 (25.0)
- Pericarditis	6 (18.8)
- Systolic dysfunction	3 (9.4)
- Mitral insufficiency	3 (9.4)
- Aortic insufficiency	2 (6.3)
- Pericardial fluid	2 (6.3)
Neurologic complications	
- Seizures	6 (18.8)
- Encephalopathy	4 (12.5)
- Coma	2 (6.3)
- Stroke	2 (6.3)
- Cerebral hemorrhage/Hematome	1 (3.1)
Biliary sludge	4 (12.5)
Deep vein thrombosis	3 (9.4)
Pulmonary edema	2 (6.3)
Nephrotic/nephritic syndrome	1 (3.1)

patients. All patients received broad spectrum antibiotics for various reasons; ceftriaxone, meropenem, vancomycin and piperacillin/tazobactam were used in 28 (87.5%), 10 (31.3%), 10 (31.3%) and 7 (21.9%) patients, respectively. All patients with candiduria were treated with a 7-day course of intravenous fluconazole.

Three patients (9.4%) died early during hospitalization (Table 4). All were very young (11, 12 and 16 months age) and all were females. One of them had bloody diarrhea; the duration of symptoms before admission was short (2—3 days in all cases). One patient was treated in the community with ceftriaxone prior to hospitalization. Two of them were febrile at admission. All three had leukocytosis and thrombocytopenia and 2/3 presented with anemia (Hb 10.2 and 8.7 g/L, respectively). Stool cultures were negative in all 3 infants; serology was positive to *E. coli* O_{26} in one patient. The stool examination was negative for stx1, stx2 and eae genes in all 3 patients. All 3 had severe acute renal failure at admission (creatinine 3.71, 1.23 1 and 1.6); one was anuric and another one oliguric. All 3 requested immediate dialysis (peritoneal dialysis in 3 and hemodialysis in one patient). All 3 were hypertensive at admission; one presented with convulsions, one with acute pulmonary edema and the third one with cerebral hemorrhage. All had severe metabolic acidosis, became comatose, developed cardiac arrhythmias and died after 48 h of hospitalization.

Follow-up during the period of 6 months—1 year after hospitalization was available in 26/29 (89.7%) discharged patients. Nine (34.6%) recovered completely. Seventeen

Table 4 Clinical, laboratory, microbiologic and therapeutic characteristics of 3 children with HUS and fatal outcome.

Patient	Age (mths)	Bloody diarrhea	Antibiotics before admission	Disease length till admission (days)	Fever*	WBC*	Platelets*	Sodium*	High blood pressure*	Oliguria/ anuria	Dialysis	Diagnoses at death
1	11	Yes	Ceftriaxone	2—3	38.2 °C	15,000	40,000	123.6	Yes	Anuria	Peritoneal + Hemodyalisis	Convulsions, Metabolic Acidosis, Melena, Coma, Hematuria, Bradycardia, Cardiac Resuscitation
2	15	No	No	2—3	38.6 °C	44,180	63,000	133.5	Yes	No	Peritoneal	Acidosis, Melena, Cerebral Hemorrhage, Bradycardia, Coma, Cardiac Resuscitation
3	12	No	No	2—3	No	18,400	61,000	131.3	Yes	Oliguria	Peritoneal	Pulmonary Edema (Acute), Severe Metabolic Acidosis, Bradycardia, Cardiac Resuscitation

*At admission.

(65.4%) patients were diagnosed with post-HUS sequelae. Persistent hypertension was seen in 9/26 (34.6%) and chronic kidney disease was diagnosed in 8 (30.8%) patients. Persistent minimal hematuria and proteinuria were found in 5 patients each. One patient developed nephritic/nephrotic syndrome. Left ventricular hypertrophy was diagnosed at follow-up in 3 patients.

4. Discussion

Since 2010, the number of large STEC outbreaks, often associated with HUS, reported annually in Europe, has steadily increased.[18] The most frequently identified serotype was O_{157}. In a study determining the incidence of HUS in 130 children in France during 1996 and assessing the role of STEC infection in the etiology of HUS, 86% had evidence of STEC infection and serum antibodies to *E. coli* O157 LPS were detected in 67% cases tested.[19] STEC $O_{26}$was the second most commonly reported serotype in the European Union (>400 cases per year).[20] STEC O_{26} patients were reported to be younger than O_{157} patients and have more severe diarrhea, with more aggressive forms and a higher proportion of the infected individuals reported to develop HUS.[22] Outbreaks caused by STEC O_{26} have been associated in the past with unpasteurized milk and dairy products in Italy, Austria and Belgium.[15,21,22]

In the present study, we have presented the epidemiologic, clinical and laboratory features, the therapeutic interventions and a one-year follow-up of the 32 children diagnosed with HUS during an outbreak occurring in southern Romania from December 2015 to September 2016. This report describes the largest outbreak of STEC-associated HUS ever observed in Romania. The principal STEC serotype involved was O_{26}:H_{11}. *E. coli*O_{26} infection was diagnosed in our study in 10 patients and *E. coli* O_{157} in 3, based on the detection of anti LPS antibodies, confirming the importance of LPS serology in identifying the specific STEC infections in HUS patients.[15–17] Stx2-producing *E. coli* O_{26} is considered a highly virulent STEC[23] and outbreaks with this pathogen have generally involved only young children.[10,11,19–22] In our study, the presence of STEC was confirmed in 8 patients. Overall, 15 (46.9%) of the patients described in our study patients were defined as confirmed HUS cases.

Several features about this southern Romania outbreak are noteworthy. The outbreak occurred over a large geographic area and included the highest number of HUS cases ever described in Romania. No obvious epidemiologic link among the cases was observed. The outbreak occurred over a long period of time (9 months) and all patients were very young (87.5% under two years of age). The male/female ratio was around 1.5 and no adult patients with HUS were reported during the outbreak period. All the HUS patients presented with diarrhea (bloody in 40.6%) and 43.8% were previously treated with antibiotics. The principal STEC serotype involved was O_{26}:H_{11}. Thirteen (40.6%) and 4 (12.5%) cases had oliguria and anuria, respectively, at admission. A considerable percentage of patients developed serious complications during the acute phase of the disease, including various infections and central nervous and cardiac systems pathologies and the fatality rate was higher than reported in the medical literature. Three patients died early during hospitalization. At a 6–12 month follow-up, 65.4% patients were diagnosed with post-HUS sequelae and persistent hypertension and chronic renal failure were seen in a high number of patients.

Coma, seizures, cognitive disturbances and visual problems are the most common acute neurologic complications of STEC-HUS.[23,24] Our data on the 32 HUS patients were similar to other studies concerning the rates of neurological complications, including seizures, encephalopathy and cerebral hemorrhage (7 patients, 21.9% of the cases).[11,25,26] In a study analyzing the neurological involvement and outcome in pediatric patients during the 2011 HUS epidemic caused by *E. coli* O_{104}:H_4, neurological involvement (stupor or coma, seizures, visual disturbances, paresis and myoclonus) was initially observed in 14/50 (28%) patients.[26] EEG abnormalities were more frequent in patients with neurological involvement than in those without and at the 3- and 6-month follow-ups were found in 14/40 (35%) and 7/36 (19%) patients, respectively.[26]

We recorded septic and cardiac complications in rates higher than reported in the literature. In a study reporting on the clinical features of HUS in Norwegian children in the period 1999–2008, 29% of cases were complicated by sepsis caused by various pathogens and 2 patients developed septic shock.[26] In the present study, infectious complications were reported in 20 (62.5%) of the patients, spanning a broad spectrum including bacteremia, peritonitis, urinary tract infections and candiduria, and all were treated with broad spectrum antimicrobials. Interestingly, 43.8% of our patients were treated with antibiotics during the week preceding hospitalization. In general, the use of antibiotics for the treatment of *E. coli* O_{157}/H_7 infections may increase the risk of HUS[27] although a meta-analysis study from 2002 did not confirm this association.[28] The current consensus advises against the use of antibiotics in STEC infections because of an assumed increased risk for HUS development as a consequence of increased toxin release, particularly following bactericidal antibiotic drugs.[29,30] We were not able to examine potential effects of antibiotics use in our study because the indications for antibiotics during the hospitalization were broad and variable.

Data on the cardiac involvement in HUS are limited and reported mainly as isolated case reports. The clinical manifestations of myocardial involvement in HUS are diverse, and they include myocardial dysfunction (poor peripheral perfusion and pulmonary edema), myocarditis, cardiac tamponade, dilated cardiomyopathy and even myocardial infarction.[31–34] In the outbreak in southern Romania, cardiac complication were diagnosed in a high number of patients (21, 65.6%) at admission and during hospitalization, when left ventricular hypertrophy, diastolic dysfunction and pericarditis were the main echocardiographic findings. However, these injuries were reversible, in their majority, and following a 6–12 months follow-up, residual left ventricular hypertrophy was present in only 3 patients. Knowledge of these frequent cardiac complications during the acute phase of HUS is of crucial importance and may be life saving. Therefore, routine evaluation of the myocardial function, appropriate hemodynamic support, use of inotropic agents, mechanical ventilation and, in extreme cases, extracorporeal support

are all recommended therapeutic measures requested until the cardiac function is restored.[35]

Three (9.4%) patients died early during hospitalization. The reported mortality rate from acute STEC-HUS is 2.9—5%.[36,37] In a case—control study of 17 deaths cases among children with HUS, 15 died during the acute phase of the disease.[38] Brain involvement was the most common cause of death (8 cases) while congestive heart failure, pulmonary hemorrhage and hyperkalemia were infrequent causes. The presence of oligoanuria, dehydration, WBC $>20X10^9$/L and hematocrit >23% represented a substantial risk for a fatal outcome.[38]

In the present study, among the cases where data on follow-up were available up to 1 year following initial admission, 34.6% had persistent hypertension and30.8% had chronic renal failure. These numbers are higher than those described in other studies, but the follow-up period was short. Follow-up data after a median follow-up of 3.0 years of 72 pediatric patients diagnosed with HUS during the 2011 outbreak associated with *E. coli* O_{104}:H_4 in Germany showed that hypertension and proteinuria were present in 19% and 28% of these patients, respectively.[38] Dialysis during the acute phase, dialysis duration and the duration of oligo/anuria were associated with the development of renal sequelae. In a metanalysis of 49 studies and 3476 diarrhea-associated HUS patients with a long-term follow-up (mean 4.4 years), death or end-stage renal disease occurred in 12% of patients and 25% of the survivors demonstrated long-term renal sequelae.[39] The severity of the acute illness, particularly central nervous system symptoms and the need for initial dialysis were strongly associated with a worse long-term prognosis. Spinale et al.[40] recently reviewed the data on long-term outcome after HUS in children and found an overall prevalence of sequelae of 20%—40%. Chronic kidney disease or end-stage renal disease occurred in 9%—18% and approximately 3% of the patients, respectively. Proteinuria (15%—30%) and hypertension (5%—15%) were frequently detected in patients after HUS, which might indicate a higher risk of renal or cardiovascular disease later in life. This had led to the recommendations of follow up controls for at least 5 years for diarrhea associated-HUS patients.[39,40]

There were limitations in this study, mainly related to its retrospective nature and the possibility that some information was missed or misunderstood. Stool cultures were not obtained in the community when the first disease symptoms occurred. The microbiological work-up and the characterization of the strains were incomplete in a number of study patients. However, we do not think that the outbreak reported here could have potentially had larger dimensions and that some cases with severe/bloody diarrhea would have escaped our attention, as a result of the major efforts made by our hospital (a tertiary care and the only referral center for HUS in southern Romania) and the overall collaboration and communication between several pediatric medical centers and the whole pediatric community during the outbreak.

In conclusion, the present outbreak supports the view that infections with *E. coli* O_{26} in children have a high probability to progress to HUS and represent an emerging public health problem. The described outbreak was characterized by a higher rate of infectious, cardiac and neurologic complications and of renal sequelae and was associated with a high fatality rate. We showed that VTEC infection is an emerging problem in Romania and epidemics with severe clinical manifestations may also occur in the future. An efficacious surveillance system for HUS may allow the rapid recognition of such outbreaks and establish their possible geographical or temporal clustering.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.pedneo.2018.04.011.