

Epidemiological Characteristics of Shiga Toxin-Producing *Escherichia coli* Responsible for Infections in the Polish Pediatric Population

DOMINIKA SELIGA-GĄSIOR¹, BEATA SOKÓŁ-LESZCZYŃSKA^{1,2*}, JOLANTA KRZYSZTOŃ-RUSSJAN¹,
DIANA WIERZBICKA¹, KAROLINA STĘPIEŃ-HOŁUBCZAT³, PAULINA LEWANDOWSKA¹,
EWA FRANKIEWICZ⁴, ANDRZEJ CACKO⁴, BEATA LESZCZYŃSKA⁵,
URSZULA DEMKOW⁶ and EDYTA PODSIADŁY^{1,7}

¹ Microbiological Laboratory, University Center for Laboratory Medicine,
University Medical Center, Medical University of Warsaw, Warsaw, Poland

² Department of Medical Microbiology, Medical University of Warsaw, Warsaw, Poland

³ Department of Microbiology and Pharmaceutical Bioanalysis, Medical University of Warsaw, Warsaw, Poland

⁴ Department of Medical Informatics and Telemedicine, Medical University of Warsaw, Warsaw, Poland

⁵ Department of Pediatrics and Nephrology, Medical University of Warsaw, Warsaw, Poland

⁶ Department of Laboratory Medicine and Clinical Immunology of Developmental Age,
Medical University of Warsaw, Warsaw, Poland

⁷ Department of Dental Microbiology, Medical University of Warsaw, Warsaw, Poland

Submitted 27 January 2024, accepted 13 March 2024, published online 10 May 2024

Abstract

Shiga toxin-producing *Escherichia coli* (STEC) are zoonotic pathogens causing hemorrhagic colitis and hemolytic uremic syndrome (HUS) in children and the elderly. Stool samples were collected from 180 children hospitalized in five pediatric centers in Poland in 2018–2022. Direct *stx1/stx2* gene detection by PCR in feces and *E. coli* isolates was performed. Antibiotic susceptibility was tested according to EUCAST v.12. Randomly selected isolates were serotyped with O157 antiserum and genotyped by pulsed-field gel electrophoresis (PFGE). A total of 44 *E. coli* isolates were confirmed as STEC by PCR. Among them, 84.4% were positive for *stx2*, and equally 6.8% for only *stx1* and both *stx1* and *stx2* genes. The *stx1* gene was also found in one *Citrobacter freundii* isolate. *E. coli* serotype O157 was present in 97.6% of the isolates. STEC infections most often occurred between June–October with a peak in July and August (51%). The highest, 77.8% of STEC isolates were found in the 1–5 years old group. No extended-spectrum β -lactamases (ESBL) were found. Resistance only to amoxicillin/clavulanic acid (24.4%), piperacillin/tazobactam (3%), cefotaxime (6%), gentamicin (6%), ciprofloxacin (3%), azithromycin (3%), trimethoprim/sulfamethoxazole (24.2%) was detected. PFGE analysis showed 18 PFGE types with no clonal distribution. Eight isolates with A, B, and C PFGE types showed genetic relatedness in the type with no detection of transmission way of distribution. STEC strains pose a serious threat to human health, therefore demographic and epidemiological characteristics are crucial for their surveillance.

Key words: Shiga toxin-producing *Escherichia coli*, diarrhea, hemolytic uremic syndrome, epidemiology, Poland

Introduction

Shiga toxin-producing *Escherichia coli* (STEC), also known as verocytotoxin-producing *E. coli* (VTEC) strains are responsible for serious diseases such as hemorrhagic colitis and hemolytic uremic syndrome (HUS), especially among children and elderly. STEC are zoonotic pathogens, with ruminants considered the main reservoir. Enterohaemorrhagic *E. coli* (EHEC) is a subgroup of STEC serotypes strongly associated with bloody diarrhea and HUS in industrialized coun-

tries. HUS is defined as a combination of acute kidney injury, low platelet counts, and hemolytic anemia. After HUS, some children never recover kidney function and require long-term replacement therapy, while those who recover are at risk of developing chronic kidney disease and arterial hypertension. Some have residual non-renal problems, including neurological defects, insulin-dependent diabetes, pancreatic failure, or gastrointestinal complications. STEC associated with diarrhea may resolve without any long-term consequences (WHO 2018).

* Corresponding author: B. Sokół-Leszczynska, Microbiological Laboratory, University Center for Laboratory Medicine, University Medical Center, Medical University of Warsaw, Warsaw, Poland; e-mail: beata.sokol-leszczynska@wum.edu.pl

© 2024 Dominika Seliga-Gąsior et al.

This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 License (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

STEC serotypes produce high levels of various toxins in the large intestine which are closely related to the potent cytotoxins produced by *Shigella dysenteriae* type 1. They appear to directly damage mucosal and vascular endothelial cells in the gut wall. If absorbed, they exert toxic effects on another vascular endothelium, e.g., renal. The mainstay of treatment for STEC infection is supportive. Although *E. coli* is susceptible to most used antibiotics, they have not been shown to alleviate symptoms, reduce carriage of the organism, or prevent HUS.

The knowledge of how to reduce the incidence of STEC on cattle farms still needs to be elucidated. However, tests can be performed to determine whether a given animal carries the bacterium. If necessary, meat can be given bactericidal treatment, which involves heating or irradiation. Although these techniques are helpful, they do not guarantee the absence of STEC in food. Effective prevention requires the application of strict hygiene practices throughout the entire food chain, from producer to consumer. To prevent infection, children under the age of 5 should not come into contact with farm animals, especially cattle and sheep, and their environment as well as should avoid eating unpasteurized cheese or not washed fruits, vegetables, and herbs if they are eaten raw; nor drink water that has not undergone microbiological testing, e.g., from a well or spring (The Institut Pasteur 2023).

The aim of the study was to evaluate epidemiological characteristics of Shiga toxin-producing *E. coli* causing infection in the pediatric population in Poland.

Experimental

Materials and Methods

Clinical samples. The study was conducted from January 1st, 2018-December 31st, 2022. All consecutive samples delivered for STEC routine testing in the

Microbiology Laboratory of the University Medical Center of the Medical University of Warsaw were included in the study. A total of 180 stool samples from children suspected of HUS were included in the study. The samples came from five pediatric medical centers from five voivodeships: Mazovian, Podlaskie, Greater Poland, Lublin, and Warmian-Masurian.

Detection of STEC strains. Detection of the presence of *stx1/stx2* genes in stool samples was performed using the BD MAX[™] Enteric Bacterial Panel and the Viasure Real Time PCR Detection Kit (Becton, Dickinson and Company, Australia) according to the manufacturer’s instruction.

All examined samples were cultured on MacConkey agar to select *E. coli* isolates and on STEC-enriched broth (Axonbiotech GmbH, Germany). After overnight incubation at 37°C, the presence of *E. coli* was confirmed based on colony morphology and VITEK[®] 2 identification (bioMérieux, France). Total DNA was extracted from overnight cultures on Columbia Agar using the Genomic Micro AX Bacteria+ Gravity kit (A&A Biotechnology, Poland) according to the manufacturer’s protocol. Quality control and concentration of DNA samples were measured using a Thermo Scientific[™] NanoDrop[™] Spectrophotometer (Thermo Fisher Scientific, Inc., USA). The purity and concentration of the obtained DNA were determined by A₂₆₀/A₂₈₀ and A₂₆₀/A₂₃₀ absorbance measurements. Samples with a ratio between 1.8 ± 0.2 and a DNA concentration range between 25–50 ng were used for PCR analysis.

Primer and probe sequences applied in this study were previously reported and described by Perelle et al. (2004; 2005) (*stx1* and *stx2* genes) and Nielsen and Andersen (2003) (the *eae* gene) (Table I). Primer pair specificity was assessed by Kagkli et al. (2011). Additionally, a comparison of target sequences was performed with sequences found in the GenBank sequence database provided by NCBI, aligning them to all available bacterial, human, bovine, mouse, and rat

Table I
Characteristics of primers and probes used in the study.

Gene name	GenBank Access No.	Nucleotide sequences (5'→3')*	Amplicon size (bp)
<i>stx1</i>	M16625	Fw**:	132
		Rv**:	
		Probe:	
<i>stx2</i>	X07865	Fw:	128
		Rv:	
		Probe:	
<i>uh</i>	Z11541	Fw:	102
		Rv:	
		Poll:	

* – in the sequences, Y is (C/T), S is (C/G), W is (A/T), R is (A/G), and M is (A/C)
** – Fw – forward primer, Rev – reverse primer

genomes. RT-PCR reactions were performed according to the protocol described by Kagkli et al. (2011) with a slight modification. Applied GoTaq® Green Master Mix (Promega, USA) with Taq DNA polymerase was used. Applied Biosystems™ TaqMan® probes (Thermo Fisher Scientific, Inc., USA) were labeled with 6-carboxyfluorescein-6-carboxytetramethylrhodamine (FAM-TAMRA), the concentration of each primer was 0.6 µM and the probe 200 nM. The reaction mixtures were heated at 95°C for two minutes, of amplification at 95°C for 15 seconds and 60°C for a minute, followed by 40 cycles conducted in the CFX96 Touch™ Real-Time PCR System (Bio-Rad, USA).

Antibiotic susceptibility testing. Antimicrobial susceptibility testing (AST) was performed using a VITEK® 2 instrument (bioMérieux, France) for all antibiotics except azithromycin. For azithromycin susceptibility testing an MTS™ MIC Test Strip (Liofilchem S.r.l., Italy) was used and plated on Mueller-Hinton agar (Oxoid, Thermo Fisher Scientific, Inc., USA) according to the manufacturer's instructions. Results were interpreted according to EUCAST v.12 breakpoints (EUCAST 2022). Breakpoints for azithromycin were taken from the *Salmonella/Shigella* criteria.

Pulsed-field gel electrophoresis (PFGE). The analysis was performed using the CHEF-DR® II instrument (Bio-Rad, USA) according to the procedure described by Kawamori et al. (2008) with minor modifications.

E. coli from 24-hour cultures were suspended in cell suspension buffer (CBS) (100 mM Tris, 100 mM EDTA, pH 8.0) to a McFarland density of 2.0. Bacterial suspensions were mixed (1:1) with 1% agarose or agarose SeaKem® Gold Agarose (Lonza Rockland, Inc., USA) in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). The agarose plugs with the bacterial suspension were cooled to 4°C to solidify. Lysis was performed for two selected plugs using dissolved CSB (50 mM Tris, 50 mM EDTA, pH 8.0) and 1% *N*-lauroylsarcosine sodium (Sigma Aldrich®, Merck KGaA, Germany) for 2 hours of incubation at 54–55°C under constant and vigorous agitation. To prepare the genomic DNA, two plugs were digested for 2 hours at 37°C with the enzyme restrictive XbaI (50 units on a sample) (Thermo Scientific™, Thermo Fisher Scientific, Inc., USA) and 100 µg/ml bovine serum albumin (BSA) after washing 4 times with 10 ml TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). PFGE electrophoresis was performed using 1% PFGE agarose (Bio-Rad, USA) in 0.5 × Tris-Boran-EDTA (TBE) buffer under the following conditions: total duration 19 h, switch block included start time 2.2 s, end time 54.2 s, voltage 6 V/cm, switching angle 120° and temperature 14°C. After electrophoresis, the gel was stained with ethidium bromide solution and photographed using a Bio-Rad Gel Doc™ XR+ Imaging System (Bio-Rad, USA).

Agglutination with serotype O157 antiserum. *E. coli* O157 antigens were serotyped using the agglutination method on microtiter plates. A total of 3–5 colonies from Columbia agar with 5% sheep blood were selected for inoculation in the infusion broth and incubated overnight at 35–37°C. After incubation, 80 µl of O antiserum (SSI Diagnostica, Denmark) was mixed with 80 µl of boiled culture on a microtiter plate and incubated at 50–52°C overnight. The reaction was read under artificial light on a black background. In each run, a negative control was performed by mixing 80 µl of physiological saline at pH 7.4 with 80 µl of boiled culture.

Approval from the Ethics Committee of the Health Centers. Not applicable. Medical information was collected from medical records after the discharge of patients.

The patient's informed consent has not been requested to collect information. Medical information was collected from medical records after the discharge of patients.

Inclusion and exclusion criteria. A total of 180 stool samples from children suspected of HUS were included in the study. The samples came from five pediatric medical centers from five voivodeships: Mazovian, Podlaskie, Greater Poland, Lublin, and Warmian-Masurian.

Epidemiological analysis – risk factors for STEC infection. Medical documentation was analyzed in terms of contact with animals (e.g. domestic, farm, zoo), eating unwashed fruit, and vegetables, visiting a mini zoo, participating in scout camps.

Limitations of the study. The study was limited to children with HUS suspected who were hospitalized in tertiary hospitals.

Analysis of medical parameters of STEC-infected patients. Medical documentation was analyzed regarding diarrhea onset, platelet concentration, treatment results, recovery, and HUS complications.

Statistical analysis. Statistical analyses were performed in Statistica 13 (StatSoft, Inc., USA). The relationships between variables were evaluated using the following statistical tests: Shapiro-Wilk test for normality, Levene test for equality of variances, independent *t*-test for two samples, Mann-Whitney *U* test, chi-square goodness of fit test and chi-square test of independence, test of significance of Spearman's rank-order correlation coefficient. For all analyses, a *p*-value of <0,05 was considered statistically significant.

Results

Presence of verotoxin in the isolates. In 2018–2022, a total of 45 Shiga toxin-producing isolates were detected. One strain could not be cultured. The *E. coli*

Table II
Characteristics of patients infected with STEC as diagnosed in the Medical Center
of the Medical University of Warsaw, 2018–2022.

Characteristic of STEC patients	Value (No.)
Boys	26
Girls	19
Median age (range)	2 years 8 months (1 month–16 years and 6 months)
Clinical manifestation	Value (No.)
HUS	20
Only diarrhea	4
No data	21
Laboratory results	No. cases/No. total
Direct positive PCR for <i>stx1</i> and/or <i>stx2</i> from stool	45/45
Positive culture of STEC	44/45
PCR gene detection results	No. cases/No. total
<i>stx1</i> ¹ positive, <i>stx2</i> ² negative	3/45
<i>stx1</i> negative, <i>stx2</i> positive	38/45
<i>stx1</i> positive, <i>stx2</i> positive	3/45
<i>stx</i> – unknown <i>stx</i> type	1/45
<i>eaeA</i> ³	44/45
Serotypes	No. total
O157	40
other than O157	1

¹ – *stx1* – shiga toxine type 1 encoding gene; ² – *stx2* – shiga toxine type 2 encoding gene;
³ – *eaeA* – intimin encoding gene

species was confirmed for all isolates except one. One isolate carrying the genes *stx1* and *eae* belonged to the species *Citrobacter freundii*. The *stx2* gene was detected in 86.4% (38/44); three isolates presented only the *stx1* gene, and three had both genes, verotoxins *stx1* and *stx2*. Results of the detection of verotoxins genes *stx1*, and *stx2* are shown in Table II. The occurrence of *stx1* and *stx2* genes was not dependent on age ($p > 0.05$).

Serotyping. For 41 tested isolates, 97.6% (40/41) belonged to serotype O157, and 2.4 % (1/41) belonged to a serogroup other than O157 (Table II).

Sensitivity to antibiotics. Antimicrobial susceptibility tests were performed for 33 strains. The antimicrobial resistance was found to amoxicillin/clavulanic acid (24.4%), piperacillin/tazobactam (3%), cefotaxime (6%), gentamicin (6%), ciprofloxacin (3%), azithromycin (3%), trimethoprim/sulfamethoxazole (24.2%). None of the thirty-three strains produced ESBL. The sensitivity of the tested STEC strains is presented in Fig. 1. Due to the group size being too small, it was not possible to conduct a statistical analysis of the antibiotic sensitivity of the isolates in terms of the patient’s age and gender and the presence of significant or insignificant HUS risk factors.

Seasonal STEC distribution. The seasonality of infections caused by STEC has been demonstrated.

Infections most often occurred from June to October, with a peak in July and August (51%) (Fig. 2).

STEC infection and age. Most STEC isolates were detected among children aged 1–5 years – 77.8% (35/45). Lower percentages of 15.6% (7/45), 4.4% (2/45), and 2.2% (1/45) occurred among age groups below one year, 12–16 years, and over 16 years, respectively. In order to check whether the occurrence of genes is age-dependent, patients were divided into two groups. The first group included children up to 2 years of age (median age), and the second group included other patients. There were no statistical differences between groups ($p > 0.05$, chi-square test of independence Yates correction).

STEC infection and gender. There was no relationship between STEC infections and gender: 19/45 (42.2%) girls vs. 26/45 (57.8%) boys (Table II). The occurrence of the above *stx1* and *stx2* genes was not sex-dependent. No statistically significant difference was observed in the incidence of STEC in girls and boys ($p = 0.456$, chi-square test).

Country distribution of isolated STEC strains. Isolates were most often detected in patients hospitalized in the voivodeships Masovian and Podlaskie, 51.2% and 28.9%, respectively. In other voivodeships, this percentage was 12%.

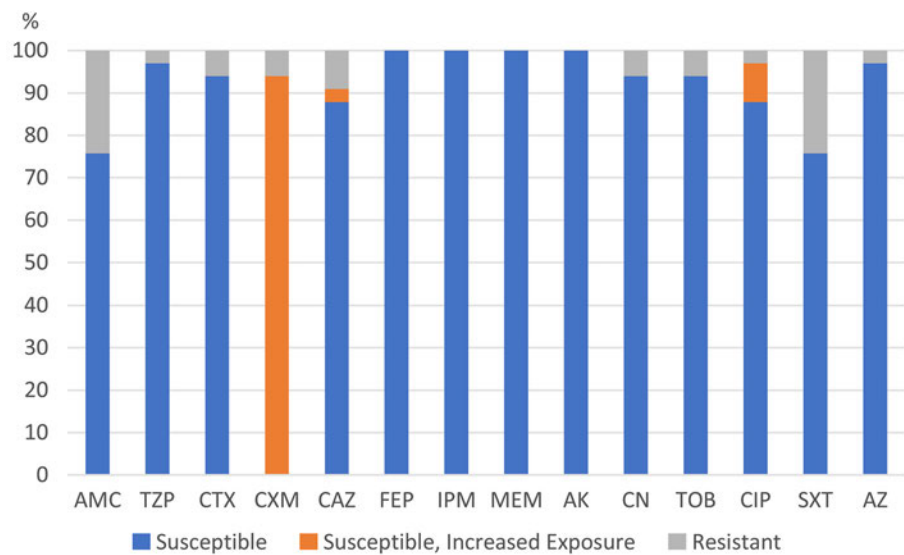


Fig. 1. Antibiotic susceptibility of STEC isolates (n = 33).
AMC – amoxicillin/clavulanic acid, TZP – piperacillin/tazobactam, CTX – cefotaxime, CXM – cefuroxime, CAZ – ceftazidime, FEP – cefepime, IPM – imipenem, MEM – meropenem, AK – amikacin, CN – gentamicin, TOB – tobramycin, CIP – ciprofloxacin, SXT – trimethoprim/sulfamethoxazole, AZT – azithromycin.

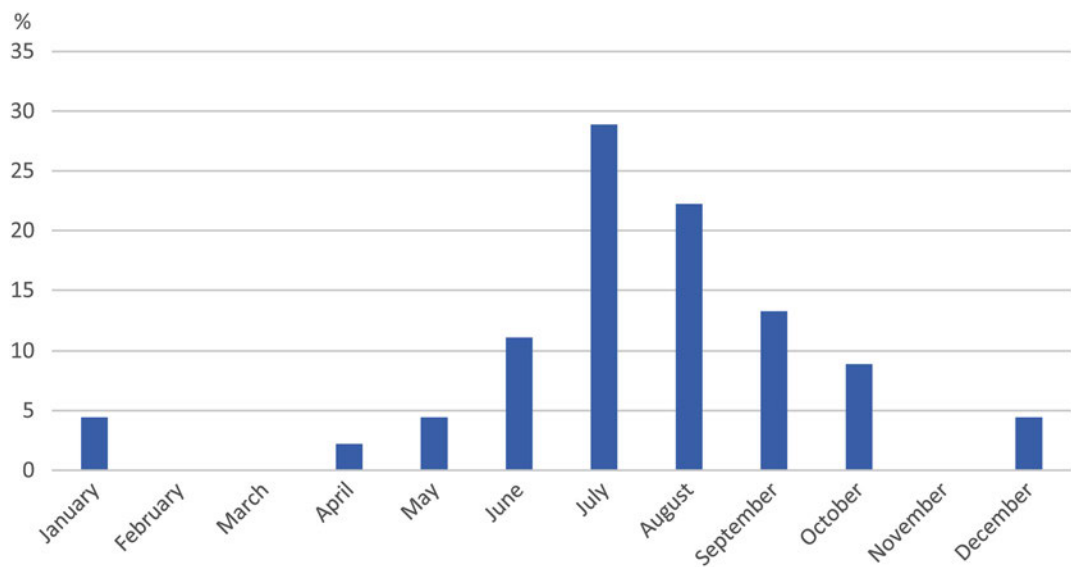


Fig. 2. Percentage of STEC isolates by month, 2018–2022 (n = 45).

Risk factor for STEC infection. The most common risk factors for HUS in our patients with STEC isolate included living on a farm (most often cattle were bred), eating unwashed fruit and vegetables, visiting a mini zoo, or participating in scout camps.

Medical parameters in patients infected with STEC. The time interval from the onset of diarrhea to HUS in patients ranged from 1 to 8 days. The range of platelet concentrations in patients with confirmed STEC infection was from $6\text{--}201 \times 10^3/\mu\text{l}$.

According to available data from 18 patients, the most common result was complete recovery – in 83.3% of patients (15/18). Reported complications of HUS in the studied children were neurological complications

and insulin-dependent diabetes in 11.1% (2/18) and 5.6% (1/18), respectively.

PFGE. All analyzed in PFGE strains presented STEC pathotypes with only the *stx2* gene. The genetic association was obtained by comparing bands in the analyzed PFGE patterns. Closely related strains were designed based on a difference of up to three bands, related up to eight bands, but unrelated to the difference between the above nine bands in the analyzed PFGE pattern. Unrelated PFGE types were marked with different letters from A to S. Among the 23 *E. coli* isolates tested, 18 PFGE types were distinguished. Among them, PFGE types A, B, and C were characterized by genetic relatedness and were represented by two

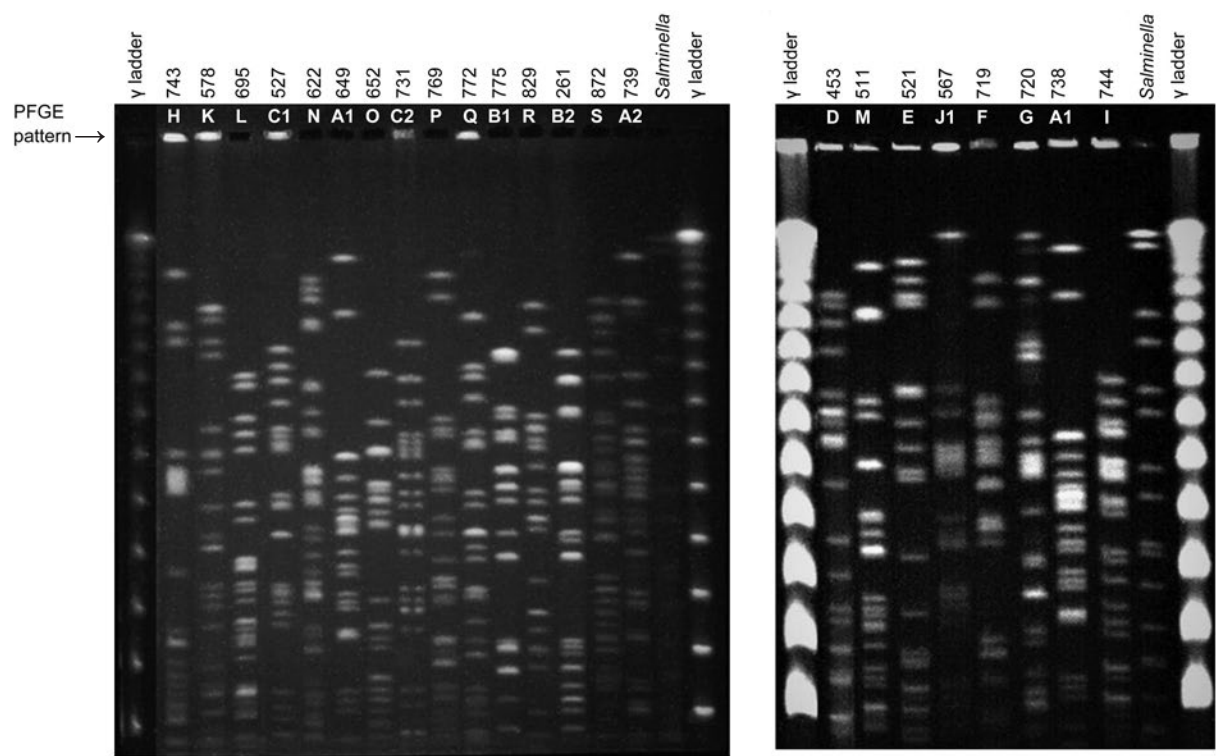


Fig. 3. PFGE patterns of selected *Escherichia coli* isolates. Original designations of the isolates and their PFGE types are shown above the corresponding lanes. *Salmonella Branderup* and γ ladder (New England Biolabs, USA) were used as PFGE molecular weight markers. Results of the analysis of genetic relatedness of STEC strains, 2018–2022.

following subtypes A1 (n=2), A2, (n=1); B1 (n=1), B2 (n=1) and C1 (n=2), C2 (n=1), respectively. The genetically related strains with A1, A2, C1, C2, and B2 PFGE subtypes originated from different children from Mazovian. The other single strains with genetic relatedness belonging to PFGE type B originated from Mazovian and Podlaskie, similar to those with PFGE type C originating from Mazovian and Podlaskie voivodeships. The majority of remaining isolates were sporadic strains, unrelated among others, and represented by a single *E. coli* isolate. Eleven PFGE types, including D, E, F, G, I, J, M, N, O, and Q types were found in Podlaskie, H and K types were found in Mazovian, P, R types found in Warmian-Masurian and finally, L PFGE type

was found in Greater Poland voivodeships. No PFGE type was determined for the tested *C. freundii* isolate. Table III and Fig. 3 shows the results of the analysis.

Discussion

Outbreaks and sporadic cases of STEC have been reported in several countries. Typical HUS caused by STEC presents with acute illness with symptoms of bloody diarrhea. About 25% of typical HUS do not show diarrhea. Almost 90% of individuals recover without sequelae, either spontaneously or after plasma infusion or replacement plasma (Noris et al. 2021). In

Table III
Molecular characteristic of selected isolates derived from gastrointestinal infections.

PFGE type/subtype	No. of isolates (Total No. 24)	<i>stx1</i> , <i>stx2</i> gene presence	Voivodeship
B1, D, E, F, G, I, J, M, N, O, Q	11	<i>stx2</i>	Podlaskie
A1, A2, B2, C1, C2, H, K	8	<i>stx2</i>	Mazovian
<i>Citrobacter freundii</i> (no PFGE type)	1	<i>stx1</i>	Mazovian
C1, P, R	3	<i>stx2</i>	Warmian-Masurian
L	1	<i>stx2</i>	Greater Poland

Genetic relatedness of PFGE types is marked by bold type.

our study, the most common outcome was complete recovery in 83.3% (15/18) of patients. However, we reported some complications of HUS, such as neurological complications and insulin-dependent diabetes, in 11.1% and 5.6%, respectively.

Our study showed that among the analyzed STEC patients, the *stx2* gene predominated with a frequency of 84.4%, and equally, 6.7% were identified with only the *stx1* gene and with both *stx1* and *stx2* genes. The *stx2* gene was reported as most frequently detected by others 49.1% – 83.3% (Jenssen et al. 2014). Januszkiewicz and Rastawicki (2016) described strains isolated in Poland from humans, cattle, and food between 1996 and 2010. The authors confirmed the presence of the *stx2* gene in 11.8% and the simultaneous occurrence of *stx1* and *stx2* in 88.2% of isolates but did not detect any *stx1* in human isolates. Our results confirm the circulation of *E. coli* producing verotoxin1 in the Polish pediatric population. Other researchers reported that the *stx1* gene is dominant in 57.7–83%, but the *stx2* gene in 11.7–17% of isolates (Bouzari et al. 2018).

Shiga toxins (ST) are a group of bacterial toxins involved in human and animal diseases. STs are produced by *E. coli* isolates. Other species reported as ST producers besides *S. dysenteriae* type 1 are occasionally *C. freundii*, *Enterobacter cloacae*, and *Shigella flexneri* (Herold et al. 2004). In our study, in one case, *C. freundii* was detected in the bloody stool of a patient suffering from diarrhoea having the *stx1* gene.

The fact that our study mainly includes high-risk STEC might support the serotyping results, as 40 (97.6%) of the strains tested had serotype O157 and encoded the Shiga toxin 2 gene. Reports of non-O157 STEC serotypes associated with severe disease vary among countries. According to the ECDC report, until 2021, the most frequently reported serogroup in human STEC cases was O157, followed by O26, but in recent years, there has been an increasing trend in number of non-O157 STEC infections. The last ECDC report on STEC infections (ECDC 2022) shows serogroup O26 as the most common (34%) among HUS cases, followed by O157 (19.8%). The results of our study concern the period 2018–2022, and sporadic cases of HUS in pediatric population. Detection of the O157 serogroup was not influenced by diagnosis as PCR amplification of Shiga toxin coding genes was applied for detection. Our results agree with some other studies suggesting that *E. coli* O157: H7 serotype causes in children with HUS more severe diseases than non-O157. In the USA, serotype O157:H7 was the most commonly associated with STEC and most often associated with HUS (Hermos et al. 2011). Similar results were described in a recent study from Sweden (Hua et al. 2021) and China (Zhu et al. 2023). These results are inconsistent with the currently described infections and HUS associated

with non-O157 *E. coli* serotypes, even from Poland (Rastawicki et al. 2020). This phenomenon requires further, more in-depth research.

ST production is necessary but not enough for STEC virulence. Shiga toxin production by O157:H7 is mediated by sensing quorum (Mühlen and Dersch 2020). Serotype O157:H7 harbors the locus of the pathogenic enterocyte efflux island, which encodes genes involved in removing intestinal epithelial cell microvilli and the tight adherence of bacteria to the epithelial cell membrane. The molecular mechanism underlying the pathogenicity of different STEC strains requires further elucidation. *Stx2d* has been described as a Shiga toxin whose cytotoxicity is activated 10- to 1,000-fold by elastase present in the intestinal mucus of mice or man (Matussek et al. 2023). Clinical STEC strains may have the following genes: *stx2a*, *stx2b*, *stx2c*, *stx2d*, *stx2e*, *stx2f*, *stx2g*, *stx2h*, *stx2i*, *stx2j*, *stx2k*, *stx2l*, *stx2m* (Bai et al. 2021). Bacterial genetic factors identified as molecular predictors of the development of HUS included *stx* subtype *stx2a*, *stx2a*, and *stx2c* and genes encoding intimin, toxins, secretory system proteins, and transcription regulators (Sallée et al. 2013). Rapid diagnostic targeting of these genes directly in stool samples could lead to improved care and infection control measures, reducing the number of patients suffering from severe STEC disease (Matussek et al. 2023).

The likelihood that a patient infected with *E. coli* O157:H7 will develop HUS varies with age. Fifteen to twenty percent of children less than 10 years of age with culture-confirmed *E. coli* O157:H7 infection develop HUS. Our study confirmed this because 77% of children with confirmed STEC infection and HUS were under 5 years of age.

Fatima and Aziz (2023) reported a seasonal pattern of STEC infections, with increased incidence during the summer months. Similarly, in the 2018–2022 analyzed period, we observed that infections most frequently occurred in June, July, August, and September, with a peak in July.

Because antibiotics are strong inducers of the bacterial SOS response, which initiates the production and release of phages from bacteria, antibiotic treatment is not recommended. It was mentioned that bacterial DNA damage due to antibiotic therapy may increase the expression of genes encoding verotoxins and increase the risk of HUS (Noris et al. 2021). It is believed that disease worsening after administration of antibiotics also occurs due to changes in the commensal intestinal flora, which allows STEC to attach to the intestinal wall (Harkins et al. 2020). There is no therapy against STEC infections, and current treatment is only supportive and includes hydration therapy and, if necessary, dialysis (Koyanagi et al. 2019). An *in vivo* mouse model using *E. coli* O86 demonstrated reduced Shiga

toxin production and mortality following azithromycin administration and reduced duration of STEC excretion in feces with a lower frequency of long-term STEC O104:H4 transfer (Nitschke et al. 2012). Cefotaxime, meropenem, ceftazidime, gentamicin, and kanamycin did not affect the *stx* gene expression or had little induction operation. Antibiotics affecting protein biosynthesis, such as polycyclic, do not affect the expression of the *stx2* gene (Chen et al. 2013). Patients treated with intravenous ciprofloxacin and meropenem significantly reduced the duration of ST excretion in stool, with a concomitant reduction in the incidence of seizures and death compared with patients who did not receive antibiotics (Prado et al. 1995). According to Prado et al. (1995) use of β -lactams during the first 3 days of illness was associated with an increased risk of STEC-HUS. Intravenous meropenem treatment promoted ST release by *E. coli* O157:H7 but not the O104:H subtype. Ciprofloxacin and norfloxacin increased *stx2* expression thousands of times compared to controls. Oral consumption of quinolones by *E. coli* patients infected with O157:H7 did not increase the possibility of progression to HUS (Chen et al. 2013). The use of trimethoprim did not show significant progression to STEC-HUS, symptomatic improvement or change in Shiga toxin excretion (Tarr et al. 2018). In a prospective study of 259 children below 10 years of age infected with *E. coli* O157:H7 (Wong et al. 2012) HUS was more common in children who received antibiotics, which included trimethoprim-sulfamethoxazole, β -lactams, metronidazole, and azithromycin (36 versus 12 percent for children who did not receive antibiotics). After controlling for other variables, the absolute increase in the risk of HUS associated with antibiotic therapy was 25 percent, equivalent to one case of HUS in every four children treated with antibiotics. Data in adults are sparser. In three adult studies that were included in the meta-analysis, antibiotics administered early in STEC infection were associated with the subsequent development of HUS (Wong et al. 2012). A multicenter study observing the application of fosfomycin in STEC infection showed a reduction/progression of STEC-HUS after administration within the first five days (Kurabayashi et al. 2015). Combination treatment with neutralizing antibodies against Stx1 and Stx2 and tigecycline reduced the toxicity levels of STEC isolates, *E. coli* cell counts, and the likelihood of transmission (Skinner et al. 2015). Bacterial DNA-interacting antibiotics, such as fluoroquinolones, trimethoprim/sulfamethoxazole, and β -lactams, induce bacteriophage lysogeny, leading to increased ST expression *in vitro* and increased transfer of toxin-encoding prophages to previously nontoxic strains *in vivo* (Bitzan 2009). Harkins et al. (2020) recommend that antibiotic therapy should be considered safe if the following criteria

are met: i) confirmed absence of STEC with a virulence profile associated with HUS in the stool sample; ii) absence of acute or chronic kidney disease; iii) in long-term carriers of STEC associated with HUS, antibiotic therapy should not be started earlier than 2 weeks after the first isolation of the pathogen, and identical types of STEC should be isolated in two separate samples. Although the usefulness of antibiotic treatment in patients with STEC infections is a subject of ongoing debate, knowledge of STEC antimicrobial susceptibility patterns in different geographic areas is important for therapeutic and strain characterization purposes. Prado et al. (1995) found that the strains were sensitive to furazolidone, ciprofloxacin, gentamicin, and amikacin. Resistance was detected to tetracycline at 4%, chloramphenicol – 5%, trimethoprim/sulfamethoxazole – 24%, and ampicillin – 25% (Kurabayashi et al. 2015). All our isolates were susceptible to cefepime, imipenem, meropenem, and amikacin. All strains were ESBL negative. Bielaszewska et al. (2011) reported a 2011 outbreak in Germany caused by *E. coli* O104:H4 *stx2*-positive with expanded CTX-M-1 β -lactamase. STEC serotype O121 ESBL-positive isolates were described in 2019 by Kikuchi et al. (2019). Our study showed that in justified cases, all permitted antibiotics can be used in the empirical therapy of STEC infections.

In our study, PFGE analysis of 23 selected *E. coli* and one *C. freundii* strain showed 18 PFGE types; however, types A, B, and C with two subtypes showed genetic relatedness. The remaining 15 PFGE types were derived from single patients and were not related to each other. All strains with genetic relatedness have been found in Mazovian voivodeships. However, strains with type B were also found in Podlasie and type C in Warmian-Masurian voivodeships. The STEC strains with A, B, and C PFGE types derived from different persons, times, and areas reflect no epidemic transmission of these strains and show their probable persistence in the environment. On the other hand, the occurrence of the *stx2* gene in the remaining sporadic STEC strains indicates other than clonal distribution of this Shiga toxin-producing gene. To evaluate the character of this distribution, further analysis should be performed. Contreras et al. (2011) described nineteen types of pulsed fields in 20 STEC isolates. Japanese scientists recovered STEC O157:H7 isolates from experimentally infected cattle and observed twelve new PFGE profiles. Changes in the PFGE profiles of STEC O157:H7 isolates occurred after passing through the gastrointestinal tract of livestock (Yoshii et al. 2009). Strain circulation is observed due to the persistence of dominant strains or repeated exposure of cattle to different strains, which can be demonstrated by PFGE analysis. No significant differences have been described between the fecal microflora of excretors and non-excretors (Bibbal et al. 2022).

Matussek et al. (2023) performed whole-genome multilocus sequence typing and genome-wide phylogeny analysis to assess the phylogenetic relatedness of STEC isolates from HUS and non-HUS patients and suggested genetic differences of pathogenic STEC strains in different geographic regions and populations. Human immunity and/or bacteria-host interaction may affect STEC-related disease progression. Accessory genes were differentially presented in HUS-STEC and non-HUS-STEC strains in a pangenome-wide association study. There was no significant difference in virulence genes between O157 strains from patients with and without HUS. These data suggest that the infection dose and differences in host innate and adaptive immunity, e.g., genetic variation in patients' complement genes, are predisposing factors to atypical HUS (Sallée et al. 2013) and may play a vital role in the pathogenesis and development of STEC-HUS. Susceptibility to STEC infection varies among people. Personalized medicine and sequencing of patient's DNA in the STEC-HUS diagnostic process may answer the question of what genetic variability in human genes is a critical factor and why STEC diarrhea in a patient resolves spontaneously while in others it leads to HUS. Whole-genome phylogeny and multiple pangenome correspondence analysis did not separate HUS-STEC strains from non-HUS-STEC strains, suggesting that STEC strains of different genetic origins may independently acquire genes determining their pathogenicity and that other non-bacterial factors may play a key role in the pathogenicity of HUS development (Matussek et al. 2023). Recent data suggest the involvement of the complement system in the pathogenesis of STEC-HUS (Mele et al. 2014). The gut microbiota plays a pivotal role in host defense against STEC. Precolonization of *Clostridium ramosum* in STEC-infected mice reduced the level of secreted STX2 and prevented kidney histopathological changes. *C. ramosum* inhibits growth and ST production by STEC more strongly than *C. perfringens* (Koyanagi et al. 2019).

Conclusion

STEC has been shown to be an important pathogen causing severe post-infectious complications and deaths among the youngest children in Poland. In our study, the genetic diversity of STEC strains was seen. Almost 86.4% of isolates carried the *stx2* gene, and 97.6% belonged to the serotype O157. The isolates were susceptible to antibiotics. The STEC infections were observed in the summer months, mainly in children 1–5 years (77.8%). Contact with animals was the most frequent HUS risk factor. Epidemiological analysis of STEC isolates is crucial for understanding the disease

and tracking outbreaks. Collaboration between physicians and microbiologists when diagnosing patients with diarrhea, especially in cases of contact with animals, is vital. Due to the occurrence of these infections as independent epidemic outbreaks in human-animal relationships, epidemiological surveillance should be conducted considering the One Health concept.

ORCID

Dominika Seliga-Gąsior <https://orcid.org/0009-0000-8301-5280>
 Beata Sokół-Leszczynska <https://orcid.org/0000-0001-7446-2222>
 Jolanta Krzysztoń-Russjan <https://orcid.org/0000-0001-7200-6178>
 Diana Wierzbicka <https://orcid.org/0009-0002-8027-2163>
 Karolina Stępień-Hołubczat <https://orcid.org/0000-0002-3974-7908>
 Paulina Lewandowska <https://orcid.org/0009-0004-2505-0351>
 Ewa Frankiewicz <https://orcid.org/0009-0008-2089-6816>
 Andrzej Cacko <https://orcid.org/0000-0001-5486-7732>
 Beata Leszczynska <https://orcid.org/0000-0001-6811-4934>
 Urszula Demkow <https://orcid.org/0000-0003-0674-9824>
 Edyta Podsiadły <https://orcid.org/0000-0002-1897-6568>

Author contributions

All authors contributed to the final version of the manuscript. E. Podsiadły, B. Leszczynska, J. Krzysztoń-Russjan, and B. Sokół-Leszczynska designed the study and prepared the manuscript. E. Frankiewicz and A. Cacko performed the statistical analysis. B. Sokół-Leszczynska and D. Wierzbicka carried out strain identification and sensitivity testing. J. Krzysztoń-Russjan, K. Stępień-Hołubczat, D. Seliga-Gąsior, P. Lewandowska, and D. Wierzbicka performed qPCR, PFGE, and agglutination analyses. D. Seliga-Gąsior and B. Sokół-Leszczynska collected the results. Demkow U, E. Podsiadły, J. Krzysztoń-Russjan, and B. Sokół-Leszczynska critically reviewed the final version of the manuscript.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

- Bai X, Scheutz F, Dahlgren HM, Hedenström I, Jernberg C. Characterization of clinical *Escherichia coli* strains producing a novel Shiga toxin 2 subtype in Sweden and Denmark. *Microorganisms*. 2021 Nov;9(11):2374. <https://doi.org/10.3390/microorganisms9112374>
- Bibbal D, Ruiz P, Sapountzis P, Mazuy-Cruchaudet C, Loukiadis E, Auvray F, Forano E, Brugère H. Persistent circulation of enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 in cattle farms: Characterization of enterohemorrhagic *Escherichia coli* O157:H7 strains and fecal microbial communities of bovine shedders and non-shedders. *Front Vet Sci*. 2022 Mar;9:852475. <https://doi.org/10.3389/fvets.2022.852475>
- Bielaszewska M, Mellmann A, Zhang W, Köck R, Fruth A, Bauwens A, Peters G, Karch H. Characterisation of the *Escherichia coli* strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: A microbiological study. *Lancet Infect Dis*. 2011 Sep;11(9):671–676. [https://doi.org/10.1016/S1473-3099\(11\)70165-7](https://doi.org/10.1016/S1473-3099(11)70165-7)

- Bitzan M.** Treatment options for HUS secondary to *Escherichia coli* O157:H7. *Kidney Int Suppl.* 2009 Feb;(112):62–66. <https://doi.org/10.1038/ki.2008.624>
- Bouzari S, Farhang E, Hosseini SM, Alikhani MY.** Prevalence and antimicrobial resistance of shiga toxin-producing *Escherichia coli* and enteropathogenic *Escherichia coli* isolated from patients with acute diarrhea. *Iran J Microbiol.* 2018 Jun;10(3):151–157.
- Chen ML, Hao Z, Tian Y, Zhang QY, Gao PJ, Jin JL.** Different effects of six antibiotics and ten traditional Chinese medicines on Shiga toxin expression by *Escherichia coli* O157:H7. *Evid Based Complement Alternat Med.* 2013;2013:121407. <https://doi.org/10.1155/2013/121407>
- Contreras CA, Ochoa TJ, Ruiz J, Lacher DW, Rivera FP, Saenz Y, Chea-Woo E, Zavaleta N, Gil AI, Lanata CF, et al.** Phylogenetic relationships of Shiga toxin-producing *Escherichia coli* isolated from Peruvian children. *J Med Microbiol.* 2011 May;60(Pt_5):639–646. <https://doi.org/10.1099/jmm.0.026666-0>
- ECDC.** STEC infection. In: ECDC. Annual epidemiological report for 2021. Stockholm (Sweden): European Centre for Disease Prevention and Control; 2022 [cited 2023 Oct 30]. Available from <https://www.ecdc.europa.eu/sites/default/files/documents/AER%20STEC%20-%202021.pdf>
- EUCAST.** The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0. Basel (Switzerland): The European Committee on Antimicrobial Susceptibility Testing; 2022.
- Fatima R, Aziz M.** Enterohemorrhagic *Escherichia coli*. [Internet, updated 2023 Jun 26]. Treasure Island (USA): StatPearls Publishing; 2023 [cited 2023 Oct 30]. Available from <https://www.ncbi.nlm.nih.gov/books/NBK519509>
- Harkins VJ, McAllister DA, Reynolds BC.** Shiga-toxin *E. coli* Hemolytic Uremic Syndrome: Review of management and long-term outcome. *Curr Pediatr Rep.* 2020;8:16–25. <https://doi.org/10.1007/s40124-020-00208-7>
- Hermos CR, Janineh M, Han LL, McAdam AJ.** Shiga toxin-producing *Escherichia coli* in children: Diagnosis and clinical manifestations of O157:H7 and non-O157:H7 infection. *J Clin Microbiol.* 2011 Mar;49(3):955–959. <https://doi.org/10.1128/jcm.02119-10>
- Herold S, Karch H, Schmidt H.** Shiga toxin-encoding bacteriophages – genomes in motion. *Int J Med Microbiol.* 2004 Sep; 294 (2–3):115–121. <https://doi.org/10.1016/j.ijmm.2004.06.023>
- Hua Y, Chromek M, Frykman A, Jernberg C, Georgieva V, Hansson S, Zhang J, Marits AK, Wan C, Matussek A, et al.** Whole-genome characterization of hemolytic uremic syndrome-causing Shiga toxin-producing *Escherichia coli* in Sweden. *Virulence.* 2021 Dec;12(1):1296–1305. <https://doi.org/10.1080/21505594.2021.1922010>
- Januszkiewicz A, Rastawicki W.** Molecular characterization of Shiga toxin-producing *Escherichia coli* strains isolated in Poland. *Pol J Microbiol.* 2016 Aug;65(3):261–269. <https://doi.org/10.5604/17331331.1215601>
- Jenssen GR, Hovland E, Bjerre A, Bangstad HJ, Nygard K, Vold L.** Incidence and etiology of hemolytic-uremic syndrome in children in Norway, 1999–2008 – A retrospective study of hospital records to assess the sensitivity of surveillance. *BMC Infect Dis.* 2014 May; 14:265. <https://doi.org/10.1186/1471-2334-14-265>
- Kagkli DM, Weber TP, Van den Bulcke M, Folloni S, Tozzoli R, Morabito S, Ermolli M, Gribaldo L, Van den Eede G.** Application of the modular approach to an in-house validation study of real-time PCR methods for the detection and serogroup determination of verocytotoxigenic *Escherichia coli*. *Appl Environ Microbiol.* 2011 Oct;77(19):6954–6963. <https://doi.org/10.1128/aem.05357-11>
- Kawamori F, Hiroi M, Harada T, Ohata K, Sugiyama K, Masuda T, Ohashi N.** Molecular typing of Japanese *Escherichia coli* O157: H7 isolates from clinical specimens by multilocus variable-number tandem repeat analysis and PFGE. *J Med Microbiol.* 2008 Jan; 57(Pt_1): 58–63. <https://doi.org/10.1099/jmm.0.47213-0>
- Kikuchi K, Lee K, Ueno H, Tomari K, Kobori S, Kaetsu A, Matsui M, Suzuki S, Sekizuka T, Kuroda M, et al.** Enterohaemorrhagic *Escherichia coli* O121:H19 acquired an extended-spectrum β -lactamase gene during the development of an outbreak in two nurseries. *Microb Genom.* 2019 Jul;5(7):e000278. <https://doi.org/10.1099/mgen.0.000278>
- Koyanagi Y, Suzuki R, Ihara K, Miyagi H, Isogai H, Yoneyama H, Isogai E.** Intestinal *Clostridium* species lower host susceptibility to enterohemorrhagic *Escherichia coli* O157:H7 infection. *Pathog Dis.* 2019 Jun;77(4):ftz036. <https://doi.org/10.1093/femspd/ftz036>
- Kurabayashi K, Tanimoto K, Fueki S, Tomita H, Hirakawa H.** Elevated expression of GlpT and UhpT via FNR activation contributes to increased fosfomycin susceptibility in *Escherichia coli* under anaerobic conditions. *Antimicrob Agents Chemother.* 2015 Oct;59(10):6352–6360. <https://doi.org/10.1128/aac.01176-15>
- Matussek A, Mernelius S, Chromek M, Zhang J, Frykman A, Hansson S, Georgieva V, Xiong Y, Bai X.** Genome-wide association study of hemolytic uremic syndrome causing Shiga toxin-producing *Escherichia coli* from Sweden, 1994–2018. *Eur J Clin Microbiol Infect Dis.* 2023 Jun;42(6):771–779. <https://doi.org/10.1007/s10096-023-04600-1>
- Mele C, Remuzzi G, Noris M.** Hemolytic uremic syndrome. *Semin Immunopathol.* 2014 Jul;36(4):399–420. <https://doi.org/10.1007/s00281-014-0416-x>
- Mühlen S, Dersch P.** Treatment strategies for infections with Shiga toxin-producing *Escherichia coli*. *Front Cell Infect Microbiol.* 2020 May;10:169. <https://doi.org/10.3389/fcimb.2020.00169>
- Nielsen EM, Andersen MT.** Detection and characterization of verocytotoxin-producing *Escherichia coli* by automated 5' nuclease PCR assay. *J Clin Microbiol.* 2003 Jul;41(7):2884–2893. <https://doi.org/10.1128/jcm.41.7.2884-2893.2003>
- Nitschke M, Sayk F, Härtel C, Roseland RT, Hauswaldt S, Steinhoff J, Fellermann K, Derad I, Wellhöner P, Büning J, et al.** Association between azithromycin therapy and duration of bacterial shedding among patients with Shiga toxin-producing enteroaggregative *Escherichia coli* O104:H4. *JAMA.* 2012 Mar;307(10):1046–1052. <https://doi.org/10.1001/jama.2012.264>
- Noris M, Bresin E, Mele C, Remuzzi G.** Genetic atypical hemolytic uremic syndrome. [Internet, update 2021 Sep 23]. In: Adam MP, Mirzaa GM, Pagon RA et al., editors. *GeneReviews*. Seattle (USA): University of Washington; 1993–2023 [cited 2023 Nov 30]. Available from <https://www.ncbi.nlm.nih.gov/books/NBK1367/>
- Perelle S, Dilasser F, Grout J, Fach P.** Detection by 5'-nuclease PCR of Shiga-toxin producing *Escherichia coli* O26, O55, O91, O103, O111, O113, O145 and O157:H7, associated with the world's most frequent clinical cases. *Mol Cell Probes.* 2004 Jun;18(3):185–192. <https://doi.org/10.1016/j.mcp.2003.12.004>
- Perelle S, Dilasser F, Grout J, Fach P.** Detection of *Escherichia coli* serogroup O103 by real-time polymerase chain reaction. *J Appl Microbiol.* 2005;98(5): 1162–1168. <https://doi.org/10.1111/j.1365-2672.2005.02545.x>
- Prado V, Basualdo W, Arellano C, Martínez J, Levine MM.** [In vitro susceptibility of enterohemorrhagic *Escherichia coli* to 11 antimicrobials. Relationship between antibiotic resistance and toxigenic genotype] (in Spanish). *Rev Med Chil.* 1995 Sep;123(9): 1085–1090.
- Rastawicki W, Śmietańska K, Rokosz-Chudziak N, Wołkowicz T.** Antibody response to lipopolysaccharides and recombinant proteins of Shiga toxin (STX)-producing *Escherichia coli* (STEC) in children with haemolytic uraemic syndrome in Poland. *Lett Appl Microbiol.* 2020 Jun;70(6):440–446. <https://doi.org/10.1111/lam.13295>

- Sallée M, Ismail K, Fakhouri F, Vacher-Coponat H, Moussi-Francés J, Frémaux-Bacchi V, Burtsey S. Thrombocytopenia is not mandatory to diagnose haemolytic and uremic syndrome. *BMC Nephrol*. 2013 Jan;14:3. <https://doi.org/10.1186/1471-2369-14-3>
- Skinner C, Zhang G, Patfield S, He X. An *in vitro* combined antibiotic-antibody treatment eliminates toxicity from Shiga toxin-producing *Escherichia coli*. *Antimicrob Agents Chemother*. 2015 Sep; 59(9):5435–5444. <https://doi.org/10.1128/aac.00763-15>
- Tarr GAM, Oltean HN, Phipps AI, Rabinowitz P, Tarr PI. Strength of the association between antibiotic use and hemolytic uremic syndrome following *Escherichia coli* O157:H7 infection varies with case definition. *Int J Med Microbiol*. 2018 Oct;308(7):921–926. <https://doi.org/10.1016/j.ijmm.2018.06.009>
- The Institut Pasteur. Enterohemorrhagic *Escherichia coli* (EHEC) [Internet]. Paris (France): Institut Pasteur; 2023 [cited 2023 Oct 30]. Available from <https://www.pasteur.fr/en/medical-center/disease-sheets/enterohemorrhagic-escherichia-coli-ehc>
- WHO. *E. coli*. [Internet]. Geneva (Switzerland): World Health Organization; 2018 [cited 2023 Oct 30]. Available from <https://www.who.int/news-room/fact-sheets/detail/e-coli>
- Wong CS, Mooney JC, Brandt JR, Staples AO, Jelacic S, Boster DR, Watkins SL, Tarr PI. Risk factors for the hemolytic uremic syndrome in children infected with *Escherichia coli* O157:H7: A multi-variable analysis. *Clin Infect Dis*. 2012 Jul;55(1):33–41. <https://doi.org/10.1093/cid/cis299>
- Yoshii N, Ogura Y, Hayashi T, Ajiro T, Sameshima T, Nakazawa M, Kusumoto M, Iwata T, Akiba M. Pulsed-field gel electrophoresis profile changes resulting from spontaneous chromosomal deletions in enterohemorrhagic *Escherichia coli* O157:H7 during passage in cattle. *Appl Environ Microbiol*. 2009 Sep;75(17):5719–5726. <https://doi.org/10.1128/aem.00558-09>
- Zhu W, Guo H, Xu J, Wu W, Yi Y, Wang J, Duan R, Tong J, Du Y. Enterohemorrhagic *Escherichia coli* O157:H7 – Xuzhou City, Jiangsu Province, China, 2001–2021. *China CDC Wkly*. 2023 Apr;5(14):311–314. <https://doi.org/10.46234/ccdcw2023.057>