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# PREVALENCE OF DIARRHEAGENIC *E. COLI* AMONG HOSPITALIZED CHILDREN IN A CLINICAL CENTRE

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***Abstract. Introduction.*** *Escherichia coli is a common cause of acute diarrhea mainly in young children and, less frequently, in elderly or immunosuppressed patients. Many types of E. coli are part of the normal enteric flora, but can cause urinary tract or nervous system infections.* ***Objective.*** *To study the prevalence of the main types and serogroups of diarrheagenic E. coli among hospitalized children with enteric infections.* ***Material and methods.*** *Over a period of 5 years, 1,160 hospitalized children with acute diarrhea syndrome were studied. Fecal samples underwent culturing, biochemical and phenotypic identification.* ***Results.*** *Among the studied patients, 112/1,160 children (9.7%) had diarrhea caused by E. coli, and only 4 of the isolates were lactose-negative. The most common was diarrhea caused by ETEC – 65/112 (58.0%), followed by EPEC – 38/112 (33.9%), and in third place – EHES 9/112 (8.0%). We did not isolate EIEC types. Depending on the group of E. coli, we observed some differences in the clinical presentation and specifics in the distribution of patients by age.* ***Conclusion.*** *The study shows that this causative agent is common among Bulgarian children with diarrhea. Unfortunately, in Bulgaria the microbiological network is still not able to adequately respond to the challenges of the extended serodiagnosis for detection of diarrheagenic E. coli, which is performed in Western Europe and North America.*

***Key words:*** *E. coli, diarrhea, serogroups, fecal samples*

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## INTRODUCTION

Diarrhea is a global health problem in paediatrics. Globally, it is the second leading cause of death in children after inflammatory lung disease. The leading causes of acute enteric infections are usually viruses, followed by bacteria and much less often parasites and fungi [1, 2]. Escherichia coli is a common cause of acute diarrhea mainly in young children and, in some cases, in elderly or immunosuppressed patients. In most cases, *E. coli* is part of the non-pathogenic facultative flora in the human intestine. They colonize the newborn hours after birth and most often a symbiosis is established between them and the macroorganism [3, 4]. Outside the enteric tract, *E. coli* has been associated with urinary tract infections or neonatal meningitis. However, some members of the genus Escherichia are pathogenic and cause mild to severe enteric infections [5-7]. In some cases of immunosuppression, non-pathogenic *E. coli* may

also cause disease. Enteric infections caused by diarrheagenic *E. coli* (DEC) are most often transmitted by fecal-oral mechanism through contaminated food and water, as well as by household contact, and often, besides sporadic cases, epidemic outbreaks can occur [4, 8, 9]. The classification of diarrheagenic strains of *E. coli* is based on their genetic and biochemical differences, which are responsible for their virulence. Five main groups can be distinguished: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC). The different groups are associated with certain specifics of the clinical course, the age distribution and with some severe complications such as hemolytic uremic syndrome (HUS) in children. Each pathotype consists of serogroups determined by differences in O (lipopolysaccharide) antigen [6, 10, 11].

## MATERIAL AND METHODS

### *Patients and fecal samples*

Over a 5-year period (January 2016 – December 2020), 1,160 children with acute diarrhea syndrome, who were admitted to Specialized Hospital for Active Treatment of Infectious and Parasitic Diseases, "Prof. Ivan Kirov" were studied. The children were at 0 to 5 years of age, mean age 2.4 years. One sample of deposited feces was taken from each child and was tested at the hospital's microbiological laboratory and at the National Reference Laboratory (NRL) of Enteric Infections and Pathogenic Cocci at the National Center of Infectious and Parasitic Diseases.

## DIAGNOSTIC METHODS

### *Culturing*

All fecal samples were cultured on Levine, Deoxycholate Citrate and MacConkey Agar, as well as on enrichment media – selenite broth and/or Rappaport-Vassiliadis.

### *Biochemical identification and phenotyping*

Biochemical identification was performed using KIA, and lactose-negative *E. coli* were also confirmed using API20E according to the manufacturer's instructions. Phenotyping of isolates was performed using the anti-coli sera BulBio (BulBio-NCIPD) and Sifin (Germany) in order to fully cover all possible *E. coli* serotypes. DNA was isolated from all isolates for subsequent genotypic studies.

## RESULTS

Clinical fecal samples from 1,160 children up to 5 years of age with acute diarrhea were examined. Tests showed that 112/1,160 children (9.7%) had diarrhea caused by *E. coli*, and only 4 of the isolates were lactose-negative. Among the others, viral agents (rotaviruses), other bacterial pathogens (mainly Salmonella sp., and Campylobacter sp.), as well as some protozoa predominated, and 33% of the samples remained unspecified. Diarrhea caused by ETEC was the most common – 65/112 (58.0%). EPEC was ranked second – 38/112 (33.9%), and EHES – third 9/112 (8.0%) In our study, we did not isolate EIEC. The different serogroups are presenred in Table 1.

**Table 1.** Number of hospitalized children by *E. coli* serogroup

| Escherichia coli isolates serotype | number of patients |
|---|---|
| O6 | 43 |
| O25 | 14 |
| O78 | 5 |
| O15 | 4 |
| O44 | 17 |
| O55 | 10 |
| O111 | 3 |
| O125 | 13 |
| O127 | 3 |

Depending on the group of *E. coli*, we observed some differences in the clinical presentation and specifics in the distribution of patients by age. All children had a different severity of diarrhea syndrome, with or without blood admixtures, some of them had vomiting, fever, and the older ones also complained of abdominal pain. All children had varying degrees of dehydration and intoxication syndrome. Watery diarrhea, without blood admixtures, was common in children infected with ETEC, and most of them also had vomiting and fever. In patients infected with EPEC, diarrhea was also watery, with no pathological admixtures, but the diarrhea syndrome was less severe. Vomiting and abdominal pain were common symptoms, but fever was rare. Only one of the children infected with EHEC had bloody diarrhea (Table 2).

**Table 2.** Clinical characteristics of *E. coli* infection in children depending on the isolated pathogroup

| Pathogroup | Clinical features |
|---|---|
| ETEC | watery diarrhea without blood, 5-9 bowel movements daily, vomiting, fever |
| EPEC | watery diarrhea without blood, 2-5 bowel movements daily, vomiting, rarely fever |
| EHEC | watery diarrhea, sometimes bloody, 4-8 bowel movements daily, vomiting, abdominal pain, fever |

## DISCUSSION

Diarrheal diseases remain among the leading causes of hospitalization and mortality in childhood, and many of them are caused by diarrheagenic *E. coli* (DEC). Their frequency varies greatly in different studies – usually between 10 and 50%, and the most common agents are from the EPEC and ETEC groups [7, 8, 11]. In our study, these were also the two most common groups of DEC – 58% and 33.9%, respectively. The reported incidence of sporadic cases of diarrhea caused by ETEC in infants is about 10-25% [7, 11, 12]. In our study, the incidence in infants over 1 year of age was 33.8% (22 children) of all DEC cases. This shows a relatively high relative incidence, which is most likely related to poor hygiene standards in the upbringing of these children. The disease in such children was relatively severe – with more intense and long-lasting diarrhea syndrome, with a more severe degree of dehydration. Almost all other cases of diarrhea caused by ETEC – 37 (56.9%) were in children under 1 year of age who had recently discontinued breastfeeding. This is in line with numerous publications on the epidemiology of ETEC in early childhood [2, 11, 13]. The transition from breastfeeding to a varied diet facilitates the colonization of the enteric tract mainly with this pathogroup [10, 14].

The second most common pathogroup, EPEC, was isolated mainly in children under 1 year of age, regardless of their diet. Children up to 6 months of age infected with this group were 13 (34.2%). Such a clear age distribution of this pathogroup in the youngest is an exceptional characteristic of its epidemiology [3, 15]. Although young in age, the children had a less pronounced diarrhea syndrome and were less likely to have fever.

EHEC belongs to the group of *E. coli* called VTEC (verotoxigenic) due to the production of a specific verotoxin. Transmission of EHEC can occur through ingestion of contaminated foods such as meat, unpasteurized milk or water, human-to-human contact, or by zoonotic transmission. Cattle appears to be the main reservoir of EHEC and the germ has been isolated from seemingly healthy animals [16, 17]. EHEC can often cause bloody diarrhea, including diarrhea indistinguishable from bacterial dysentery. In rare cases, it can be a cause of severe complications in children – hemolytic uremic syndrome (HUS). The latter is defined as acute kidney failure with hemolytic anemia and thrombocytopenia against the background of bloody diarrhea. This condition is rare, but can be life-threatening. In the majority of cases, however, this pathotype causes watery non-bloody diarrhea and is indistinguishable from most of the acute enteric infections [18, 19]. In our study, only 1/9 (11.1%) of the children had bloody diarrhea, but the disease was self-limiting. In most cases, antimicrobial therapy for DEC infections is not necessary; the treatment is only symptomatic, and antibiotic therapy, as in many gastroenteric infections, may be contraindicated due to the possibility of provoking the release of toxins due to bacterial lysis and destruction of competing non-pathogenic enteric flora [7, 11, 12].

In our study, cases infected with EIEC were not confirmed. Most commonly, such infections feature severe clinical presentation in young children in developing countries due to their specific virulence, which is similar to that of Shigella dysenteriae [3, 20].

## CONCLUSION

DEC remains an important cause of disease worldwide. Our work shows that this causative agent is common among Bulgarian children with diarrhea. Although much has been learned in recent years about these organisms – their molecular epidemiology and virulence mechanisms are still to be further investigated. There are also other types of diarrheogenic *E. coli*, and subtypes of the current ones are likely to be found. In terms of prevention, high hygiene standards of food and water and the conditions in which young children, and particularly infants, are raised are most important. The microbiological network in Bulgaria is still not able to adequately respond to the challenges of the extended serodiagnosis for detection of DEC, which is performed in Western Europe and North America. This puts at risk both the individual health of Bulgarian children and public health in our country due to the danger of epidemic outbreaks.

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*Disclosure Summary*

The authors have nothing to disclose.

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