A RETROSPECTIVE STUDY ON *E. COLI* ENTERITIS INCIDENCE IN BULGARIA FOR A PERIOD OF TEN YEARS (2011-2020)

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ABSTRACT

Intestinal diseases caused by diarrheagenic *E. coli* account for a significant proportion of infections, especially in children. *E. coli* enteritis can occur as sporadic cases but often assumes an epidemic nature. This study aims to determine the significance and prevalence of infections caused by diarrheagenic *E. coli* in Bulgaria for a period of ten years 2011-2020. **MATERIALS AND METHODS.** The etiological role and distribution of diarrheagenic *E. coli* among Bulgarian population for the last decade were determined by a retrospective analysis of microbiological and epidemiological data. **RESULTS.** Data from the studied period reveal the role of enterotoxigenic *E. coli* (ETEC) O6 as a leading etiological cause of epidemic and sporadic enteritis in the country, followed by enteropathogenic *E. coli* (EPEC) O126 and O127. There are only two reported cases of lethal hemorrhagic uremic syndrome (HUS) caused by enterohemorrhagic *E. coli* (EHEC) that happened in 2011. Most affected by diarrheagenic *E. coli* (DEC) infections are infants and young children. Neonatal meningitis in Bulgaria is rare, the etiological agents are *E. coli* O18 and O25. The most common registered DEC infections are during the summer months of May - September. Several epidemics have been registered in young children, and the etiological cause is ETEC O6. **CONCLUSION.** The main etiological agent of *E. coli* infections in Bulgaria is ETEC O6, and infants and young children are at risk. Recent DEC epidemics have not been reported. The ethiologic diagnosis of infections is underestimated, which is a risk factor for increasing the number of healthy carriers, outbreaks and unlimited agent spreading through the food chain.

Keywords: *diarrheagenic E. coli, intestinal diseases, pediatric diarrhoea*

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INTRODUCTION

Escherichia coli (E. coli) is the predominant facultative anaerobe of human colonic flora, which has gained importance due to its association with diarrheal diseases. Diarrheagenic *Escherichia coli* (DEC) strains are the main etiologic agent of moderate to severe diarrhoea in humans that is transmitted through the consumption of contaminated foods. They are classified into six pathogenic types based on specific virulence traits- Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enterohemorrhagic *E. coli* (EHEC), which constitute the subgroup of Shiga-like toxin-producing *E. coli* (STEC), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC), and Diffusely adherent *E. coli* (DAEC) (1,2).

ETEC are the most important cause of dehydration infant diarrhoea, mainly in developing countries. In addition, ETEC are a frequent cause of travelers' diarrhoea in developed countries (3).

EPEC are an important category of DEC, which adhere to the mucosal cells of the small intestine producing pedestal-like structures (4). This results in watery diarrhoea, which is usually self-limited but can become chronic.

EAEC are an important cause of diarrhoea worldwide and are frequently observed as the aetiological agent of persistent paediatric diarrhoea in sporadic and outbreak situations (5).

EHEC cause intestinal and renal diseases. These strains express one or two Shiga toxins Stx1 and Stx2. Shiga toxin-producing *E. coli* (STEC) are also known as Vero toxin-producing *E. coli* (VTEC). The disease starts with severe crampy abdominal pain, watery diarrhoea followed by grossly bloody diarrhoea, and little or no fever. It may present in the form of sporadic cases or outbreaks. The illness is often designated as hemorrhagic colitis (1, 6).

EAEC are defined by their bricklike aggregative adherence patterns to cultured HEp-2 cells. At first, EAEC colonize the intestinal mucosa. Then, EAEC produce a mucus-mediated biofilm on the enterocyte surface. Finally, the toxins are released by EAEC, causing the inflammatory response, intestinal secretion, and mucosal toxicity (1, 7).

DAEC strains are defined based on the presence of a diffuse adherence pattern on HeLa and HEp-2 epithelial cells, where bacteria uniformly cover the cell monolayer. These strains are age-dependently involved in children's diarrhea, and can also be asymptomatic intestinal microbiota strains both in children and adults. The pathogenicity and clinical pertinence of DAEC in urinary tract infections and pregnancy complications are well established (8, 9).

The present retrospective epidemiological study aimed to evaluate the contribution of the different DEC categories to infectious diarrhoea in Bulgaria for a ten year period 2011 – 2020, to update the epidemiological data for the country and to justify the implementation of future measures for prevention and control, and for rapid and accurate diagnosis of infections caused by diarrhoeagenic *E. coli.*

MATERIALS AND METHODS

The etiological role and distribution of DEC among Bulgarian population for the last decade were determined by a retrospective analysis of microbiological and epidemiological data. Official statistics data from the National Center for Public Health and Analysis (NCPHA), data from the annual analyses of communicable diseases made by the Regional Health Inspectorates (RHI), and data from own epidemiological studies at the National Reference Laboratory, NCIPD were used.

According to Ordinance № 21/2005 on the Procedure for Registration, Communication and Reporting of Infectious Diseases of Bulgarian Ministry of Health , all DEC isolates must be sent together with clinical and epidemiological data to the NRL for Enteric Infections, Pathogenic Cocci and Diphteria at NCIPD for identification and confirmation of the bacterial agents. All data on *E. coli* isolates throughout the country are stored for a minimum of 10 years. Data on all DEC isolates reported to NRL during the studied period, were aggregated.

RESULTS

For the studied ten-year period 2011-2020, laboratory-confirmed intestinal infections by DEC, accounted for ***3 633*** *(3 633/ 530 870),* ***7,26%*** of the reported cases of acute infectious diseases (AID), influenza and acute respiratory diseases, tuberculosis, AIDS, sexually transmitted infections, and COVID-19 excluded. The greatest number of *E. coli* infections was reported in 2011 with ***514*** cases, and the lowest in 2017 with only ***240*** cases (Table 1). The seasonal distribution of DEC infections is characterized with an increased frequency during the summer months of May - September. The territorial analysis reveals the highest morbidity in the North-Eastern administrative-territorial units of Bulgaria: Varna *(48, 93%000)*, Silistra *(24, 47%000)*, Dobrich *(23, 37%000)*, Shumen *(19, 33%000)* and in the South-Eastern administrative-territorial units: Yambol *(33, 63%000)*, Sliven *(21, 35%000)* and Burgas *(8, 49%000)*. The summarized data determine the role of ETEC O6 as a leading etiological cause of epidemic and sporadic enteritis in the country, followed by ETEC O78; EPEC O126; O127, O128; O 44 (Fig.1). Except for one case in 2011, all isolates belonging to the EHEC O157 were negative for H:7 phase according to the data from primary bacteriological tests performed in the microbiological laboratories throughout the country. The characteristic complications of HUS have not been registered. In 2011, a case of enterohemorrhagic infection caused by ***E. coli*** **O157 H:7** was reported (morbidity *0.01% 000*). It refers to a 55-year-old woman from Yambol, without data of contact with a sick person or animal, or association with consumed food. No epidemiological association with the outbreak of O157 H:7 in Germany during the same year was established, either.

During the studied ten-year period, several neonatal meningitides were reported from different areas in Bulgaria. Five cases caused by *E. coli* O6 were reported in 2013 and two more caused by *E. coli* O18, in 2012. The last cases caused by *E. coli* O25 were reported in June 2020. These were three 0-20 days old infants from the same neonatal ward in the University hospital in North Bulgaria. A nosocomial infection could be suspected based on the BOX and ERIC1/2 PCR profile results obtained in the NRL. However, an epidemiological investigation report was missing, due to Covid-19 pandemics.

The affected by DEC infections were mostly infants and young children with morbidity by age groups, as follows: 0-11 months- ***141, 86%000***; 1-4 years- ***57, 59%000***; 5-9 years- ***8, 50%000*** (Fig. 2 and 3.) Out of all ***3 633*** DEC cases, ***1 805*** *(49, 68%)* were reported in females, and ***1 828*** *(50, 32%)* in males.

In the studied period 2011-2020, a total of seven outbreaks were registered. For all of them the infection source was contaminated food. The etiological agents of the outbreaks were *E. coli* O6 (more than once), O18, O168, O59 and O44 (Figure 5).

In the studied decade, **2** deaths were registered (***0, 26%*** of all AID), which determines a total lethality for the DEC intestinal infections of ***0, 46%*** **(**Table 2**).** One of the lethal cases was a 66 years old man diagnosed

with hemorrhagic *E. coli* enteritis caused by serotypes O27 and O139 in 2011, and the other - a one-year-old child from Plovdiv district, hospitalized in infectious ward in 2014. The child suffered from hydrocephalus and spina bifida. The premorbid condition has most probably contributed for the exitus Until now, there has been no reported death case of HUS caused by diarrheagenic *E. coli* in Bulgaria.

Table 1. *DEC infections morbidity in relation to reported cases of acute infectious diseases for the period 2011-2020 in Bulgaria.*

YEAR	NUMBER OF ACUTE INFECTIOUS DISEASES (without Influenza and Acute Respiratory Diseases, Tuberculosis, AIDS and Sexually Transmitted Infections, and COVID-19)	NUMBER OF DEC	DEC MORBIDITY (per 100,000)	% relative share of all ACUTE INFECTIOUS DISEASES
2011	58 259	514	6,98%000	0,88
2012	60 998	446	6,09%000	0,73
2013	67 916	333	4,57%000	0,49
2014	50 800	368	5,08%000	0,72
2015	54 471	382	5,30%000	0,7
2016	61 283	360	5,03%000	0,59
2017	52 393	240	3,38%000	0,46
2018	48 092	307	4,35%000	0,64
2019	54 397	385	5,50%000	0,71
2020	22 261	298	4,29%000	1,34
SUM	530 870	3 633	5,1%000	7,26

Table 2. *DEC infections mortality in relation to reported cases of acute infectious diseases for the period 2011-2020 in Bulgaria.*

YEAR	NUMBER of death cases of acute infectious diseases without Influenza and Acute Respiratory Diseases, Tuberculosis, AIDS and Sexually Transmitted Infections, and COVID-19	AID mortality (per 100,000)	AID total lethality (%)	NUMBER of death cases of DEC	DEC mortality (per 100,00)	DEC total letality (%)
2011	64	0,87% 000	0,11	1	0,01	0,19
2012	84	1,15% 000	0,14	0	0,00	0,00
2013	79	1,08% 000	0,12	0	0,00	0,00
2014	77	1,06% 000	0,15	1	0,01	0,27
2015	71	0,99% 000	0,13	0	0,00	0,00
2016	82	1,15% 000	0,13	0	0,00	0,00
2017	93	1,31% 000	0,18	0	0,00	0,00
2018	90	1,28% 000	0,19	0	0,00	0,00
2019	87	1,24% 000	0,16	0	0,00	0,00
2020	52	0,75% 000	0,23	0	0,00	0,00
SUM	779	1,19%000	1,54	2	0,002	0,46

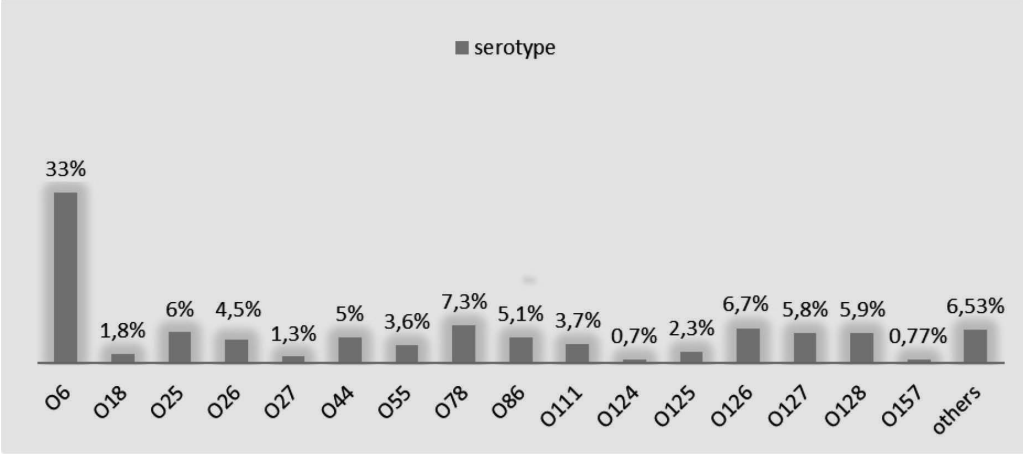

Fig. 1. Distribution of diarogenic E. coli according to the O-group for the period 2011-2020 in Bulgaria.

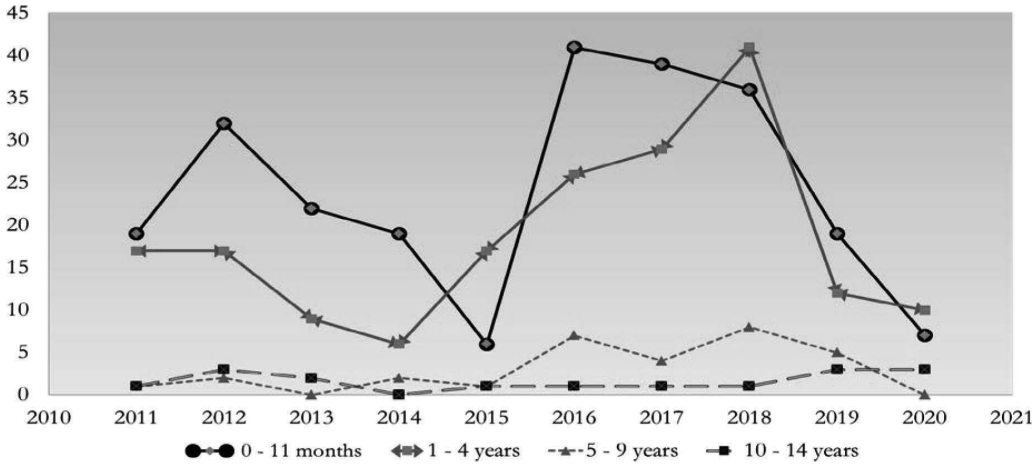

Fig 2. Distribution of DEC infections among children (0–14 years) according to age structure for the period 2011-2020 in Bulgaria.

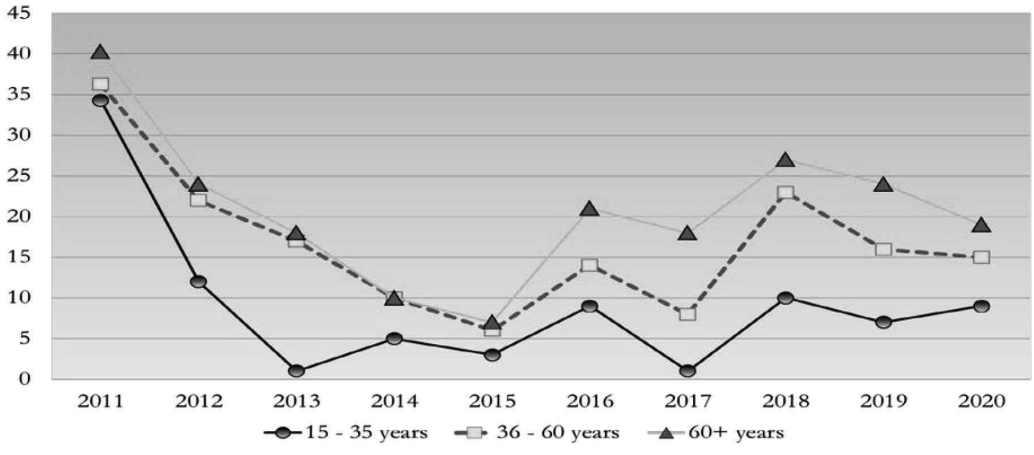

Figure 3. Distribution of DEC infections according to age structure 15–60 + years for the period 2011-2020 in Bulgaria

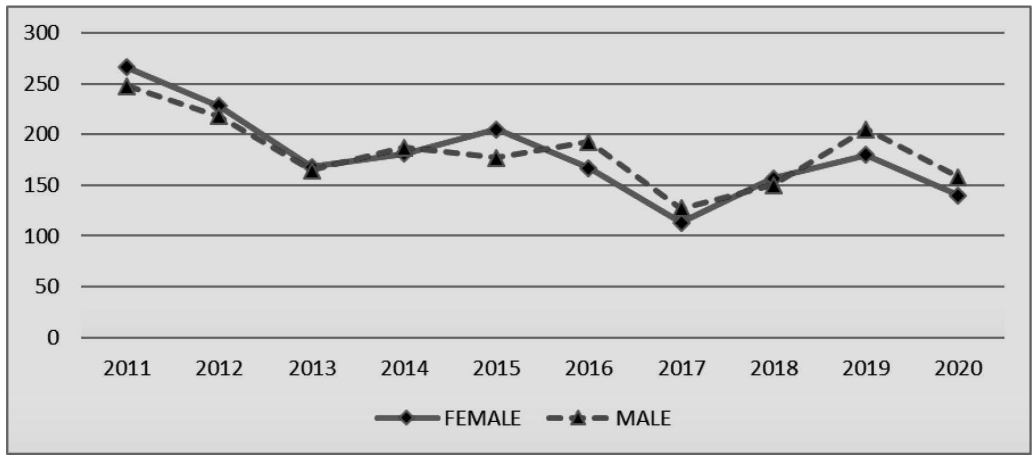

Fig. 4. Distribution of DEC cases (3 633) according to sex for the period 2011-2020 in Bulgaria.

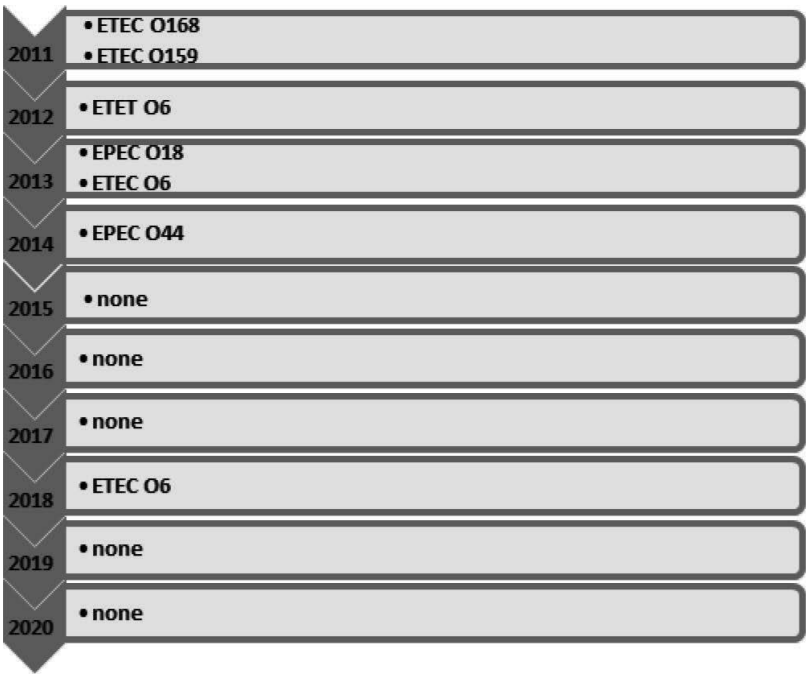

Figure 5. Annual distribution of reported DEC outbreaks for the period 2011-2020 in Bulgaria.

DISCUSSION

Escherichia coli are bacteria found in the environment, foods, and intestines of people and animals. DEC are the most common cause of a number of foodborne outbreaks, travellers' diarrhoea, chronic diarrhoea in HIV-infected patients and the main reason, neonatal meningitis and, last but not least, pediatric diarrhoea (10, 11). ETEC contribute substantially to overall morbidity, may contribute to delayed growth of infected children, and above all account for hundreds of thousands of children' deaths each year. ETEC is the predominant pathogen in DEC. Because many travellers arrive from regions with poor sanitation had been at high risk for acquisition of ubiquitously distributed ETEC infection (12 -15). The prevalence of ETEC in Bulgaria is not different from that in Europe, Asia or America. ETEC (*E.coli* O6, followed by O78) were the leading cause of pediatric diarrhoea and DEC outbreaks during the period 2011-2020. The second most frequently isolated were EPEC (*E. coli* O126, O127, O128), typical for adult diarrhoea (25-45 years) and asymptomatic carriers. EHEC and EIEC infections have a small share among Bulgarian population. Laboratory-proven cases of O157 are H: (-). Fortunately, the clinical manifestations were not related to the characteristic complications of HUS with the exception of one case of enterohemorrhagic infection in 55-years old woman without epidemiological data. At the same time, dozens of O157 H:7 cases, an epidemic that took lives, have been registered in Germany, but we do not have data that could relate the Bulgarian case with the German epidemic (16).

Neonates are at high risk of meningitis, which might lead to neurologic complications. *Escherichia coli* is the second most frequent cause of neonatal meningitis and a major cause of neonatal mortality (rates vary between 10% and 15%). Among *E. coli* strains causing extraintestinal infections in adults and children worldwide, the serotypes O1, O2, O4, O6, O7, O8, O16, O18 are predominant (17, 18, 19). Similarly, in recent years, serotypes O6 and O18 have been the most frequent cause of neonatal meningitis in Bulgaria . However, in 2020, four cases of meningitis caused by E. coli O25 were registered in one neonatal ward for a month. A molecular study using BOX and ERIC1/2 PCR was performed to prove a possibly nosocomial infection though the epidemic situation caused by Covid-19 hindered the study.

The seasonal distribution of STEC infection (May-September), as well as the most frequently affected age groups, do not stand out from the general data for Europe and the world (10, 20). The most affected age groups are infants followed by young children. Both sexes were almost equally affected at any age. The underdeveloped immune system in children and poor mastered hygiene habits are major epidemic factors in children's groups - kindergartens, training centers and others. Outbreaks reported among children's groups accounted for 90% of all cases.

Another important factor is the asymptomatic carrier state in adults involved in food preparation.

In general, the share of DEC infections in the country (*7, 26%* of the total number of AID without Influenza and Acute Respiratory Diseases, Tuberculosis, AIDS and Sexually Transmitted Infections, and COVID-19) are few as compared with reported cases from other European countries (20). Most likely this is due to reduced diagnostics in Bulgarian microbiology laboratories. Hospital microbiology laboratories perform serotyping of diarrheagenic *E. coli* mainly for pediatric and immunocompromised patients. This practice of neglecting other patients' groups leads to inaccurate data on diarrhea infections in the country, as well as increased risk of spreading the pathogens especially among asymptomatic carriers, who are a reservoir for future infections and/or epidemic outbreaks.

CONCLUSION

The diarrheal disease continues to be a health problem worldwide with a wide range of etiological agents. Among the bacterial pathogens, E. coli plays an important role (5). During the period 2011-2020, the registered cases of E. coli enteritis in Bulgaria decreased, alongside with increased number of etiologically undeciphered enterocolitis cases. Moreover, there was a significant decrease in the number of registered cases in 2020 as compared to previous years (2019 - 54,397 years, morbidity 777.10% 000; 2018 - 48,092SL., morbidity 682.15% 000; 2017 - 52 393 ff., morbidity 737.74% 000), as a result of the complex impact of factors associated with COVID-19 epidemic.

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