Clostridioides (Clostridium) difficile carriage in asymptomatic children since 2010: a narrative review

Lyudmila Boyanova a, Nikolay Kalvatchev a, Daniel Yordanov a, Petyo Hadzhiyski a, Rumyana Markovska a, Galina Gergova a and Ivan Mitova a

aDepartment of Medical Microbiology, Faculty of Medicine, Medical University of Sofia, Sofia, Bulgaria; bSpecialized Hospital for Active Pediatric Treatment, Medical University of Sofia, Sofia, Bulgaria

ABSTRACT
Since 2010, there have been relatively scanty reports on Clostridioides (Clostridium) difficile asymptomatic colonization in children, especially in those aged >2 years. We evaluated asymptomatic C. difficile carriage in children according to literature data since 2010 and added our results for 61 asymptomatic Bulgarian children aged 2–17 years. Data analysis indicated that carriage is usually the most frequent in the youngest children aged ≤2 years, thereafter decreasing with intestinal microbiota establishment. However, the colonization rates are highly variable, ranging from 0–10% to 30–80%, according to the country, age group and underlying diseases and other concurrent conditions. In our study on older children (mean age, 6.9 years), the colonization rate was 6.6% and no toxigenic C. difficile were found. Among the reviewed reports, in many studies, toxigenic C. difficile carriage was low (0–13%), but much higher (15–54%) in children with inflammatory bowel disease (IBD) and oncological diseases. Binary positive carriage was rare or absent. The risk factors for C. difficile colonization included younger age, dysbiosis, present/recent hospitalization during previous 3 months, present/previous antibiotic or proton pump inhibitor use, underlying diseases such as IBD or oncological diseases as well as environmental contamination and tube feeding. Contamination control and breast feeding were protective factors. Probiotic plus prebiotic use and dietary changes may be beneficial. Briefly, since C. difficile carriage may be a potential source of infections, regular monitoring of colonization, especially of the toxigenic C. difficile is necessary to assess risks of clinically expressed diseases.

Introduction
Clostridioides (Clostridium) difficile infections (C. difficile-associated disease, CDAD) may range from asymptomatic colonization to post-antibiotic diarrhoea and the worst form of pseudomembranous colitis that is caused solely by these bacteria and may lead to a fatal outcome [1, 2]. C. difficile has been detected in the feces of healthy adults in about <3–15%, but more often (11–21%) in hospitalized patients with accompanying antibacterial treatment [3, 4]. Among Irish adults, C. difficile colonization prevalence was low (1.6%) in the community, higher (9.5%) in outpatients and reached 21% in hospitalized patients [5].

Subjects at highest risk of CDAD are: patients with prior antibiotic use, especially of broad spectrum beta-lactams, clindamycin or, for the hypervirulent strains, fluoroquinolones, as well as hospitalized patients, people aged ≥65 years and those with immune suppression, previous CDAD history, low serum albumin and mechanical ventilation, and possibly proton pump inhibitor (PPI) users [1, 2, 6]. CDAD develops as a complication of antibiotic therapy, suppressing normal intestinal microbiota with consequent C. difficile overgrowth. Recent studies showed that community-acquired CDADs were increasing, especially in younger subjects without a history of use of antibiotics in the past [7, 8].

Only toxigenic C. difficile strains cause disease after expression of two toxins: enterotoxin (toxin A) and cytotoxin (toxin B) encoded by tcdA and tcdB genes, respectively [9]. Since 2000, there has been broad spreading of hypervirulent strains such as PCR ribotypes 027 and 078, which can produce more toxins than classical strains, can have an additional third binary toxin and deletions in some genes, increasing toxin production, and fluoroquinolone resistance [10].
Infections with hypervirulent strains have been associated with a threefold higher mortality rate than those of classical strains [9]. Importantly, binary positive strains negative for toxins A and B have also been detected, including in pediatric patients [11]. On the other hand, the antibiotic resistance of anaerobic bacteria has increased in a number of studies [12].

Asymptomatic _C. difficile_ carriage is important since it may be a reservoir and source of infection, mainly in hospital settings, and, after the occurrence of hypervirulent strains, also in society [3, 13]. Alasmari et al. [3] found these clostridia in over 20% of hospitalized patients without diarrhoea, with 15% of all patients tested being carriers of toxigenic strains. Moreover, Curry et al. [14] demonstrated by multilocus variable number of tandem repeats analysis genotyping that 29% of CDAD cases were associated with asymptomatic carriers.

However, since 2010, studies on _C. difficile_ prevalence in asymptomatic children and especially in those aged >2 years are scanty, and data from Bulgaria are lacking. The aim of this study was to evaluate _C. difficile_ colonization in asymptomatic (non-diarrheal) children according to data from literature published since 2010 and our own data of 61 non-diarrheal children from a University pediatric hospital and day care centres (DDCs).

**Subjects and methods**

**Review of literature**

In this review, we assessed _C. difficile_ colonization rates in asymptomatic children, including older children aged ≥2 years based on recent literature data. Appropriate reports were identified in Medline, PubMed and Google Scholar with the following keywords or word combinations in the title or abstract: ‘Clostridioides difficile’, ‘Clostridium difficile’, ‘C. difficile’, ‘children’, ‘asymptomatic’, ‘carrier’, ‘carriers’ or ‘colonization’, ‘paediatric’, ‘pediatric’ and ‘prevalence’ or ‘rate’.

Only results from studies published since 2010 were included. Case reports (with small exceptions) and studies on less than 30 children were excluded. Studies in which evaluated children and adults could not be separated were also excluded. Data from studies on adults were used only to emphasize the similarities in and differences between _C. difficile_ colonization in children and adults.

**Sample collection**

We also included our data of 61 asymptomatic Bulgarian children aged 2–17 years (mean age, 6.9 years) from a University pediatric hospital (32 patients) and DDCs (29 children). _C. difficile_ was isolated by the methods described by Hink et al. [15] and Alasmari et al. [3] with slight modifications. Fecal suspensions in phosphate buffered saline (PBS) were treated with ethanol spore test, were enriched in selective (D-cycloserine, cefoxitin, Oxoid, UK) Brain heart infusion broth and incubated anaerobically (Anaerogen, Oxoid) at 37°C for 2–7 days. Dense suspensions were plated onto _C. difficile_ chromogenic agar plates (Oxoid) and _C. difficile_ selective medium with moxalactam and norfloxacin (Oxoid). They were incubated anaerobically at 37°C for 48–72 h. _C. difficile_ were identified with RapID ANA II (Oxoid). Antibiotic susceptibility of the isolates was tested by E tests (Liofilchem, Italy), and PCR for _C. difficile_ genes: _gluD_ gene, _tdcA_ gene for toxin A, _tdcB_ for toxin B, and _cdtA_ and _cdtB_ genes for binary toxin was performed as well [16–18].

**Ethics statement**

The study was approved by the Ethics Committee and adhered to the principles laid out in the Declaration of Helsinki.

**Results and discussion**

**Overall _C. difficile_ colonization characteristics and prevalence**

_C. difficile_ colonization results from ingestion of the clostridial spores and their germination in the anaerobic conditions of the intestinal tract [19]. The intestinal colonization with either nontoxicogen or toxicogen _C. difficile_ can be asymptomatic; therefore, there is interplay among bacterial virulence factors, the characteristics of the colonized subject and environmental factors for the progression of the colonization to CDAD [19].

Only toxicogen _C. difficile_ strains may have clinical and epidemiologic importance and carriage of nontoxicogen _C. difficile_ is deemed protective against possible following infections with toxicogen strains [20]. In the study of Jain et al. [21], colonization with nontoxicogen _C. difficile_ was protective against colonization with toxicogen _C. difficile_ strains and CDAD and diminished the risk of CDAD even in adult patients with hematopoietic stem cell transplantation. In their study, surprisingly, one patient (4% of all patients) colonized with nontoxicogen _C. difficile_ developed CDAD, probably due to his immunocompromised status, versus 61% in the
group of patents colonized by toxigenic strains over 90 days [21]. However, using nontoxigenic strains for prophylaxis or treatment of CDAD carries the risk of horizontal gene transfer of the pathogenicity locus of the bacteria and conversion of the nontoxigenic C. difficile to toxigenic strains [22].

C. difficile carriage can be temporary or long lasting. Among pediatric oncology patients, more than half of those with previous CDAD became either intermittent or persistent carriers [23]. However, since 2010 there have been relatively few studies on C. difficile colonization prevalence in asymptomatic children and especially in those aged >2 years. In some studies, the prevalence of both C. difficile carriage (0 to >45%) and toxigenic C. difficile colonization (0 to >54%) vary widely according to the country and years of the study, the diagnostic methods used, the age groups evaluated, and the lack or presence of hospitalization or underlying diseases (Table 1).

It is alarming that since 2000, there has been emergence and subsequent increase in infections caused by hypervirulent strains such as PCR ribotypes 027 and 078 [10, 24]. In asymptomatic children, however, carriage of hypervirulent C. difficile has been usually low (0.1%) or absent according to studies from China, France, Japan and the United Kingdom [25–31]. Similarly, no binary toxin positive C. difficile colonization was found among the 61 asymptomatic Bulgarian children in our study.

Diagnostic methods include glutamate dehydrogenase and toxin A and/or toxin B detection using enzyme immune or molecular (PCR based) techniques, toxigenic culture (isolation and toxin testing of the already isolated strain) and, more rarely, cytotoxicity neutralization assay in cell cultures [24]. Molecular methods can also detect the presence of binary toxin and deletions of the tcdC gene and ribotyping can determine the hypervirulent 027 and 078 ribotypes [24]. To assess non-colonized patients at hospital admission, enzyme-linked fluorescent assay for glutamate dehydrogenase and PCR for tcdB, and nucleic acid amplification test are appropriate screening methods [32].

Age-dependent prevalence

Overall, there has been a clear age dependent prevalence of asymptomatic C. difficile carriage. In children younger than 2 years, the asymptomatic carriage was the highest (2.5–90%), most often >25–37% in neonates aged ≤1 month, gradually dropping to that in adults (0–5%) after the age of 2 years [33–35]. Recent studies in neonates and children aged <2 years often show C. difficile colonization rates of >20% and that of toxigenic C. difficile carriage in ≥7% (Table 1). A longitudinal study on premature neonates in France revealed a fast C. difficile colonization after birth (from 20% at the age of 1 week to 61% at 1 month), showing low variety of non-toxigenic ribotypes, which increased following hospitalization as well as a low (5% of the strains) prevalence of toxigenic C. difficile [31].

Asymptomatic colonization in neonates and children aged <2 years usually has no clinical expression, while the older subjects colonized with toxigenic strains may develop CDAD [20]. However, the clinical importance of toxigenic C. difficile in the youngest children may be reconsidered because clinically expressed infections have been observed in the youngest age groups. Spigaglia et al. [11] reported a high (86%) proportion of symptomatic Italian children aged <3 years with C. difficile detected as the only possible pathogen. Other authors also suggest that the pathogenic C. difficile potential in children aged <2 years remains controversial and requires more evaluation [25].

High asymptomatic colonization rates have been found in studies in France, detecting C. difficile in >33% of children aged <3 years [25, 26]. In a recent Chinese study on community infants aged ≤36 months, the rate of C. difficile colonization was 22.8%, and, importantly, >55% of the strains were toxin-producers [30]. Children aged 25–36 months were the most frequent carriers of C. difficile (52.4%) and toxigenic isolates (23.8%) [30]. However, in another Chinese study, toxigenic C. difficile was PCR detected in all age groups: in children aged <1 year (7.5%), in those aged 1 to <3 years (8.0%), as well as in the age group of 3 to <6 years (2.0%) and in that of 6 to <14 years (12.0%) [28]. Therefore, monitoring of C. difficile colonization may vary according to the years and place of the study and it should be locally and regularly monitored.

Age-dependent prevalence was also detected in Ghana. In rural areas in Ghana, the C. difficile carriage rate among 21 asymptomatic children aged ≤5 years was 23.8% and that of toxigenic strains was 4.8%; however, none of the 20 older children aged 6–15 years carried C. difficile [36].

The high rate of asymptomatic colonization in neonates has been associated with competitive intestinal tract colonization by non-toxigenic C. difficile, lack of receptors for C. difficile toxins, immature intestinal
### Table 1. **Clostridioides** (Clostridium) difficile carriage rates in asymptomatic children according to some studies published since 2010.

| Country          | Children group and age                                                                 | Methods*                                                                 | Period     | Number of children | Number (%) of carriers of CD** | Number (%) of carriers of toxigenic CD | Number (%) of binary toxin positive | References |
|------------------|----------------------------------------------------------------------------------------|-------------------------------------------------------------------------|------------|-------------------|-------------------------------|----------------------------------------|-------------------------------------|------------|
| Bulgaria         | Asymptomatic, 2–17 yr.*, hospitalized (32 children) and from day care centres (29 children) | Culture, PCR                                                            | 2017–18    | 61                | 4 (6.6); hospitalized: 3 (9.4); day care centres: 1 (3.4) | 0 (0.0)                               | 0 (0.0)                             | This study |
| China            | Asymptomatic, hospitalized, ≤3 yr.                                                     | PCR                                                                     | 2014–15    | 103               | NA†                           | 8 (7.8)                                | 0 (0.0)                             | [28]       |
| China            | Asymptomatic, hospitalized, 3 to <14 yr.                                               | PCR                                                                     | 2014–15    | 100               | NA†                           | 7 (7.0)                                | 0 (0.0)                             | [28]       |
| China            | Healthy community, 0–3 yr.                                                              | Culture, PCR, ribotyping                                               | 2013–15    | 1098              | 250 (22.8)                    | 138 (12.6)                             | 1 (0.1)                             | [30]       |
| France           | Pediatric units outpatients, <2 yr.                                                     | Culture, PCR, ribotyping, MLST†                                         | 2008–09    | 294               | 99 (33.7)                     | 21 (7.1)                                | 0 (0.0)                             | [25]       |
| France           | Healthy nursery, 0–3 yr.                                                                | Culture, EIA, PCR, ribotyping                                          | 2010–11    | 85                | 38 (45.0)                     | 11 (13.0)                               | 0 (0.0)                             | [26]       |
| France           | Premature hospitalized, 1 week and longitudinal study                                   | Culture, PCR, ribotyping, MLVA                                         | 2008–09    | 121†              | 97 (80.0); 20.0 aged 1 week, 61.0 aged 1 month) | 8 (6.6)                                | 0 (0.0)                             | [31]       |
| Ghana (rural)    | Asymptomatic, 0–15 yr.                                                                  | RCA, culture, ribotyping                                               | 2013–14    | 41                | 5 (12.2, all in children aged ≤5 yr.) | 1 (2.4)                                | 0 (0.0)                             | [36]       |
| Japan            | Neonates <1 month                                                                      | Culture, RCA                                                           | 2012–13    | 95                | 0 (0.0)                      | 0 (0.0)                                | 0 (0.0)                             | [27]       |
| Japan            | Hospitalized without underlying disease, ≤15 yr.                                       | As above                                                                | 2012–13    | 199               | 43 (21.6)                     | 18 (9.0)                               | NA                                   | [27]       |
| Japan            | Hospitalized with underlying disease ≤15 yr.                                           | As above                                                                | 2012–13    | 52                | 16 (30.8)                     | 12 (23.1)                               | NA                                   | [27]       |
| Sweden           | Healthy preschool (day care centres), 1–5 yr.                                          | PCR                                                                     | 2010       | 438               | NA                           | 11 (2.5) toxin B positive, 3 (0.7) toxin A positive | NA                                   | [37]       |
| Netherlands      | Mostly (95.4%) asymptomatic, day care centres, 0–4 yr.                                 | PCR                                                                     | 2009–12    | 857               | NA                           | 141 (16.5)                             | NA                                   | [38]       |
| UK               | Mostly healthy infants, ≤2 yr.                                                          | EIA, PCR, WGS                                                           | 2010–12    | 338               | 58 (17.2)                    | 33 (9.8)                               | 0 (0.0)                             | [29]       |
| USA              | Neonatal intensive care unit, 23–42 weeks                                              | RCA, EIA, culture                                                      | NA         | 35                | 9 (25.7)                     | NA                                     | NA                                   | [39]       |
| USA              | Outpatient IBD children                                                                 | Culture, PCR                                                           | 2007–09    | 72                | NA                           | 12 (16.7)                              | NA                                   | [41]       |
| USA              | Control children                                                                       | As above                                                                | 2007–09    | 62                | NA                           | 2 (3.2)                                | NA                                   | [41]       |
| USA              | Pediatric oncology patients with no prior CDAD, median 7.6 yr.                          | PCR, culture, sequencing                                               | 2012       | 45                | NA                           | 10 (22.2)                              | NA                                   | [23]       |
| USA              | Pediatric oncology patients with prior CDAD, median 4 yr.                               | As above                                                                | 2012       | 33                | NA                           | 18 (54.5)                              | NA                                   | [23]       |
| USA              | IBD, 3–18 yr.                                                                           | Real-time PCR                                                          | 2006–12    | 145               | NA                           | 22 (15.2)                              | NA                                   | [42]       |
| USA              | Asymptomatic controls without IBD, 2–18 yr.                                            | As above                                                                | 2006–12    | 51                | NA                           | 6 (11.8)                               | NA                                   | [42]       |

*Methods: EIA: enzyme immune assay; MLST: multilocus sequence typing; MLVA: multi-locus variable-number tandem repeat analysis; PCR: PCR or PCR-based techniques; RCA: rapid cassette assay (C. DIFF QUIK CHEK COMPLETE†); WGS: Whole genome sequencing.

**CD: C. difficile
†yr: year/s.
‡NA: not available.
*IBD: inflammatory bowel disease.
Monitored during 16 months.
mucosa and protective effects of breast milk [25, 34]. However, asymptomatic carriers of toxigenic C. difficile can act as a reservoir of infectious strains and the presence of the same strains has been observed in infants and adults in a French study [25].

There are variations in the colonization rates with nontoxigenic and toxigenic C. difficile. However, although in French infants aged 0–3 years, the C. difficile colonization rate (45%) was higher than that (22.8%) in Chinese infants of the same age, the carriage of toxigenic isolates was about 13% in both studies [26, 30].

Childcare centres maintaining strict hygiene practices do not appear to carry risks of toxigenic C. difficile transmission, although children who attend DCCs are usually exposed to pathogens due to close contacts among them [37]. In the Netherlands, toxigenic C. difficile carriage was found in 16.5% of 857 children from DDCs in 2009–2012 [38]. However, in our study, only one (3.4%) of the 29 DDC Bulgarian children evaluated was C. difficile colonized and the strain was nontoxicogenic. Among all children evaluated in our study, the C. difficile colonization was 6.6% (4 of 61 children) and no toxigenic C. difficile carriage was detected. Very low colonization with toxigenic C. difficile was also observed in Sweden, with only 11 (2.5%) of 438 preschool children in DDCs being toxigenic C. difficile carriers [37]. Toxigenic C. difficile carriage was also low in rural Ghana (2.4%) and in a study on Japanese neonates (0%), [27, 36].

### Risk factors for colonization

The most frequent risk factors for C. difficile carriage are presented in Table 2. Present or prior hospitalization during the last year and antibiotic therapy during previous 3 months are important factors for the colonization (Table 2). Environmental contamination is a key factor for C. difficile dissemination. In a US study of neonates in a neonatal intensive care unit, 50% of the diaper scales were contaminated by C. difficile [39]. On the opposite, in Japan, Furuichi et al. [27] found no C. difficile in neonates versus a colonization rate of >21% in older children and explained the results with the lack of C. difficile contamination in the departments and frequent breast feeding. Likewise, in our study, only nontoxigenic carriage was detected in 3 (9.4%) of the 32 hospitalized children.

*Table 2. Risk factors for asymptomatic carriage of C. difficile in children.*

| Country         | Children (age, years) | Risk factors for CD* colonization                                                                 | Protective factors                                    | References |
|-----------------|-----------------------|--------------------------------------------------------------------------------------------------|------------------------------------------------------|------------|
| China           | Healthy community, 0–3 yr. | Age, hospitalization during previous 3 months, antibiotic use during previous 3 months          | Delivery term                                        | [30]       |
| Estonia         | In vitro and on animal model | NA**                                                                                             | Prebiotic/probiotic combination                      | [48]       |
| France          | Pediatric units outpatients <2 yr. | Age (1 month to 1 yr.), community acquisition in ≥67% cases                                      | Age (7–12 months); recent antibiotic use, intrapartum antibiotic prophylaxis for mothers, recent diversification of food | [25]       |
| France          | Healthy nursery, 0–3 yr. | Hospitalization; at 1 week; high gestational age and high birth weight                           | NA                                                   | [31]       |
| France          | Premature hospitalized, 1 week and longitudinal study | Age (<5 yr.) and antibiotic (ceftriaxone) use                                                   | NA                                                   | [36]       |
| Ghana (rural)   | Asymptomatic, 0–15 yr. | Lack of environmental contamination; common breast feeding                                       | NA                                                   | [27]       |
| Japan           | Neonates <1 yr. | For CD* colonization: age (12–23 months), tube feeding; for toxigenic CD: tube feeding         | NA                                                   | [27]       |
| Japan           | Hospitalized, ≤15 yr. | For severe CDAD*: age (>4 yr.), PPI# use                                                          | NA                                                   | [46]       |
| Taiwan          | 49 symptomatic and 75 asymptomatic hospitalized, 1–18 yr. | Age (older), Caesarean delivery, pet dogs                                                        | NA                                                   | [29]       |
| UK              | Mostly healthy infants, ≤2 yr. | Contaminated diaper scales                                                                      | NA                                                   | [39]       |
| USA             | Neonatal intensive care unit, 23–42 weeks | IBDo, PPI use                                                                                    | NA                                                   | [41]       |
| USA             | Outpatient IBD children and age-matched controls | Low intestinal microbiome biodiversity, dysbiosis                                                | NA                                                   | [49]       |
| USA             | Two children with IBD, 13 yr. | Underlying malignancy, prior CDAD                                                                | NA                                                   | [23]       |
| USA             | Pediatric oncology patients, median <8 yr. |                                                                                                   |                                                     |            |

*CD: C. difficile.  **NA: not available.  *IBD: inflammatory bowel disease.  #PPI: proton pump inhibitors.  &CDAD: C. difficile-associated disease.
Underlying diseases are associated with both *C. difficile* colonization and risks of CDAD. In the Japanese children aged $\leq 15$ years, the colonization rate with toxigenic *C. difficile* was 2.6-fold more common in the subgroup with underlying diseases compared with the subgroup without underlying pathology, and tube feeding was a risk factor for both *C. difficile* and toxigenic *C. difficile* colonization [27].

Inflammatory bowel disease (IBD) patients have predisposing factors for *C. difficile* colonization and/or infection such as previous hospitalizations and antibiotic and immunosuppressive drug treatments [40]. In a US study, asymptomatic toxigenic *C. difficile* carriage was 5.7-fold more prevalent among children with IBD (17%) compared with that in age-matched controls (3%) [41]. In another US study, the toxigenic (toxin B positive) *C. difficile* rates were 11.6% in children with Crohn disease, 18.4% in those with ulcerative colitis, and 11.8% in controls without IBD without significant differences among the groups [42]. According to a study by the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), *C. difficile* positive children are prone to a more severe IBD course [43]. In our study on 32 Bulgarian non-diarrheal children from a University pediatric hospital, one of the six IBD children was a *C. difficile* carrier and the strain was nontoxigenic. However, during the same time, we had a clinical case of multiple *C. difficile* recurrences caused by toxigenic strains in a boy with IBD (ulcerative colitis) [44].

There are some differences in colonization rates between children and adults. Children with either Crohn disease or ulcerative colitis can be colonized by toxigenic *C. difficile*, whereas in adult IBD patients, those with ulcerative colitis are more often colonized compared to Crohn disease patients [42, 45]. In US children, *C. difficile* colonization was 5.2-fold more frequent (16.7%) in outpatient IBD children compared with that (3.2%) in the controls [45]. PPI use increased the colonization rates. PPIs increase pH in the stomach, thus enhancing the colonization of the gastrointestinal tract by *C. difficile*. In the study of Hourigan et al. [41], PPI use was $\geq 2$-fold higher in *C. difficile* colonized children (54%) compared to that in age-matched controls (25%). The PPI use was detected as a risk factor for CDAD in children in a study in Taiwan as well [46]. Moreover, asymptomatic *C. difficile* colonization was about two-fold more frequent in IBD children compared with IBD adults and should be a topic of additional studies [41, 47]. In our study, one of the seven Bulgarian children taking PPIs was a nontoxigenic *C. difficile* carrier. Prevention of *C. difficile* spreading was suggested in the study of Hung et al. [20]. Stopping unnecessary antibiotic or PPI use, frequent hand washing and contact isolation are useful measures to impede *C. difficile* spreading in hospitals [20].

Pediatric patients with oncological disease and bone marrow transplants are prone to toxigenic *C. difficile* colonization. In the study of Dominguez et al. [23], when admitted to the hospitals, $\geq 22\%$ of asymptomatic pediatric oncology patients and, importantly, more than half (54.5%) of those with previous CDAD were PCR detected as *C. difficile* carriers. Contributing factors for the high colonization rates with *C. difficile* can be associated with numerous hospitalizations, immunosuppression caused by chemotherapy, and antibiotic or PPI use [23]. Interestingly, the presence of pet dogs was also found as a risk factor for colonization for infants in Oxfordshire, UK [29].

**Antibiotic susceptibility of *C. difficile* isolates**

No antibiotic therapy is recommended for asymptomatic *C. difficile* colonized subjects [20]. However, the risks of environmental contamination with toxigenic *C. difficile* or for progression of carriage to CDAD should not be underestimated.

In some studies, antibiotic susceptibility of isolates has been reported. In a French study [25], all isolates were metronidazole, vancomycin and linezolid susceptible and moxifloxacin resistance was low (3–10%). In a Chinese study on community infants aged $\leq 36$ months, toxigenic isolates were more often ceftriaxone resistant compared with nontoxigenic isolates and the nontoxigenic isolates were more frequently rifaximin and rifampicin resistant compared with the toxigenic isolates [30]. In our study, the four *C. difficile* isolates were levofloxacin and moxifloxacin resistant and vancomycin and metronidazole susceptible except for one strain showing single metronidazole resistant colonies as a subpopulation within the inhibitory zone of the E test strip.

**Control of *C. difficile* colonization**

Probiotics can have a beneficial role not only as adjuncts in treatment of CDAD but also may inhibit *C. difficile* colonization. In a study [48], a combination of the probiotic (*Lactobacillus plantarum* Inducia, DSM 21379) and prebiotic product (xylitol) suppressed the spore germination of toxigenic *C. difficile* and diminished gut colonization *in vitro* and on an animal model (hamsters). The authors explained the results
with both the antagonistic activities of the probiotic strain and the property of xylitol to reduce iron available for the spore germination of \textit{C. difficile} [48].

\textit{C. difficile} has been associated with reduced biodiversity of the intestinal microbiome and dysbiosis [25, 49]. In a recent study, dietary changes, including removal of grains, dairy foods except for hard cheeses, fermented yogurt, food additives and sweeteners except for honey, were used in two \textit{C. difficile} colonized children with Crohn disease [49]. The dietary modification resulted in a decrease in or elimination of the clostridia [49]. Therefore, some new and simple methods may be helpful to decrease the carriage and to prevent the progression of \textit{C. difficile} colonization to CDAD.

Breast feeding has been reported as a protective factor against \textit{C. difficile} colonization in several studies, probably due to anti-toxin antibodies in breast milk [27, 29, 35]. Other protective factors such as the control of environmental or staff hand contamination [27] should be strictly applied in all pediatric departments.

Conclusions

In conclusion, there was a wide variation in the prevalence of both \textit{C. difficile} and toxigenic \textit{C. difficile} colonization in children. The pathogenic potential of \textit{C. difficile} in youngest children needs additional evaluation. Risk factors for \textit{C. difficile} carriage have been age (≤2 years), comorbidity, present or prior hospitalization, antibiotic or PPI use, underlying diseases such as oncological diseases or IBD, tube feeding and environmental or hand contamination. Given that toxigenic \textit{C. difficile} carriage may be a potential reservoir and source of CDAD, especially in healthcare settings, surveillance studies for \textit{C. difficile} colonization rates, especially those of toxigenic \textit{C. difficile} can be valuable to analyze and predict possible upcoming risks of CDAD and outbreaks.

Disclosure statement

No conflict of interest was reported by the authors.

Funding

The study on the Bulgarian children was funded by the Grant/Contract 53/03.05.2018, project 7613/17.11.2017 of Council of Medical Science, Medical University of Sofia. This work was supported by the Bulgarian Ministry of Education and Science under the National Program for Research “Young Scientists and Postdoctoral Students”.

ORCID

Lyudmila Boyanova \( \text{http://orcid.org/0000-0001-9622-0873} \)

References

[1] Jones AM, Kuijper EJ, Wilcox MH. \textit{Clostridium difficile}: a European perspective. J Infect. 2013;66:115–128.
[2] Prechter F, Katzer K, Bauer M, et al. Sleeping with the enemy: \textit{Clostridium difficile} infection in the intensive care unit. Crit Care. 2017;21:260.
[3] Alasmari F, Seiler SM, Hink T, et al. Prevalence and risk factors for asymptomatic \textit{Clostridium difficile} carriage. Clin Infect Dis. 2014;59:216–222.
[4] Kazanowski M, Smolarek S, Kinnarney F, et al. \textit{Clostridium difficile}: epidemiology, diagnostic and therapeutic possibilities-a systematic review. Tech Coloproctol. 2014;18:223–232.
[5] Rea MC, O’Sullivan O, Shanahan F, et al. \textit{Clostridium difficile} carriage in elderly subjects and associated changes in the intestinal microbiota. J Clin Microbiol. 2012;50:867–875.
[6] Surawicz CM, Brandt LJ, Binion DG, et al. Guidelines for diagnosis, treatment, and prevention of \textit{Clostridium difficile} infections. Am J Gastroenterol. 2013;108:478–498. quiz 499.
[7] Weaver L, Michels HT, Keevil CW. Survival of \textit{Clostridium difficile} on copper and steel: futuristic options for hospital hygiene. J Hosp Infect. 2008;68:145–151.
[8] Khanna S, Pardi DS, Aronson SL, et al. The epidemiology of community-acquired \textit{Clostridium difficile} infection: a population-based study. Am J Gastroenterol. 2012;107:89–95.
[9] Leffler DA, Lamont JT. \textit{Clostridium difficile} infection. N Engl J Med. 2015;372:1539–1548.
[10] Martinez-Meléndez A, Camacho-Ortiz A, Morfin-Otero R, et al. Current knowledge on the laboratory diagnosis of \textit{Clostridium difficile} infection. WJG. 2017;23:1552–1567.
[11] Spigaglia P, Barbanti F, Castagnola E, et al. \textit{Clostridium difficile} causing pediatric infections: New findings from a hospital-based study in Italy. Anaerobe. 2017;48:262–268.
[12] Boyanova L, Kolarov R, Mitov I. Recent evolution of antibiotic resistance in the anaerobes as compared to previous decades. Anaerobe. 2015;31:4–10.
[13] Galdys AL, Nelson JS, Shutt KA, et al. Prevalence and duration of asymptomatic \textit{Clostridium difficile} carriage among healthy subjects in Pittsburgh, Pennsylvania. J Clin Microbiol. 2014;52:2406–2409.
[14] Curry SR, Muto CA, Schlackman JL, et al. Use of multilocus variable number of tandem repeats analysis genotyping to determine the role of asymptomatic carriers in \textit{Clostridium difficile} transmission. Clin Infect Dis. 2013;57:1094–1102.
[15] Hink T, Burnham CA, Dubberke ER. A systematic evaluation of methods to optimize culture-based recovery of \textit{Clostridium difficile} from stool specimens. Anaerobe. 2013;19:39–43.
[16] Terhes G, Urbán E, Soki J, et al. Community-acquired \textit{Clostridium difficile} diarrhea caused by binary toxin,
toxin A, and toxin B gene-positive isolates in Hungary. J Clin Microbiol. 2004;42:4316–4318.

[17] Persson S, Torpdahl M, Olsen KE. New multiplex PCR method for detection of Clostridium difficile toxin A (tcdA) and toxin B (tcdB) and the binary toxin (cdtA/cdtB) genes applied to a Danish strain collection. Clin Microbiol Infect. 2008;14:1057–1064.

[18] Persson S, Jensen JN, Olsen KE. Multiplex PCR method for detection of Clostridium difficile tcdA, tcdB, cdtA, and cdtB and internal in-frame deletion of tcdC. J Clin Microbiol. 2011;49:2300–2429.

[19] Furuya-Kanamori L, Marquess J, Yakob L, et al. Asymptomatic Clostridium difficile colonization: epidemiology and clinical implications. BMC Infect Dis. 2015;15:516. [cited 2019 Jun 24]

[20] Hung YP, Lee JC, Lin HJ, et al. Clinical impact of Clostridium difficile colonization. J Microbiol Immunol Infect. 2015;48:241–4824.

[21] Jain T, Croswell C, Urday-Cornejo V, et al. Clostridium difficile colonization in hematopoietic stem cell transplant recipients: a prospective study of the epidemiology and outcomes involving toxigenic and nontoxigenic strains. Biol Blood Marrow Transplant. 2016;22:157–163.

[22] Brouwer MS, Roberts AP, Hussain H, et al. Horizontal gene transfer converts non-toxigenic Clostridium difficile strains into toxin producers. Nat Commun. 2013;4:2601. [cited 2019 Jun 24]

[23] Dominguez SR, Dolan SA, West K, et al. High colonization rate and prolonged shedding of Clostridium difficile in pediatric oncology patients. Clin Infect Dis. 2014;59:401–403.

[24] Nagy E. What do we know about the diagnostics, treatment and epidemiology of Clostridioides (Clostridium) difficile infection in Europe? J Infect Chemother. 2018;24:164–170.

[25] Rousseau C, Lemée L, Le Monnier A, et al. Prevalence and diversity of Clostridium difficile strains in infants. J Med Microbiol. 2011;60:1112–1118.

[26] Rousseau C, Poilane I, Pontual L, et al. Clostridium difficile carriage in healthy infants in the community: a potential reservoir for pathogenic strains. CID. 2012;55:1209–1215.

[27] Furiuchi M, Imajo E, Sato Y, et al. Characteristics of Clostridium difficile colonization in Japanese children. J Infect Chemother. 2014;20:307–311.

[28] Wang Y, Guo S, Zhao CN, et al. [Colonization rate of Clostridium difficile in healthy children]. Zhonghua Er Ke Za Zhi. 2015;55:294–297.

[29] Stoesser N, Eyre DW, Quan TP, et al. Epidemiology of Clostridium difficile in infants in Oxfordshire, UK: Risk factors for colonization and carriage, and genetic overlap with regional C. difficile infection strains. PLoS One. 2017;12:e0182307. [cited 2019 Jun 24]

[30] Cui Q-Q, Yang J, Niu Y-N, et al. Epidemiological investigation of Clostridioides difficile colonization in Chinese community infants. Anaerobe. 2019;56:116–123.

[31] Ferraris L, Couturier J, Eckert C, et al. Carriage and colonization of C. difficile in preterm neonates: A longitudinal prospective study. PLoS One. 2019;14:e0212568. [cited 2019 Jun 24].

[32] Terveer EM, Crobach MJ, Sanders IM, et al. Detection of Clostridium difficile in feces of asymptomatic patients admitted to the hospital. J Clin Microbiol. 2017;55:403–411.

[33] Jangi S, Lamont JT. Asymptomatic colonization by Clostridium difficile in infants: implications for disease in later life. J Pediatr Gastroenterol Nutr. 2010;51:2–7.

[34] Borali E, De Giacomo C. Clostridium difficile infection in children: a review. J Pediatr Gastroenterol Nutr. 2016;63:e130–e140.

[35] Lees EA, Miyajima F, Pirmohamed M, et al. The role of Clostridium difficile in the paediatric and neonatal gut – a narrative review. Eur J Clin Microbiol Infect Dis. 2016;35:1047–1057.

[36] Janssen I, Cooper P, Gunka K, et al. High prevalence of nontoxigenic Clostridium difficile isolated from hospitalized and non-hospitalized individuals in rural Ghana. Int J Med Microbiol. 2016;306:652–656.

[37] Kaarme J, Hickman RA, Neveus T, et al. Reassuringly low carriage of enteropathogens among healthy Swedish children in day care centres. Public Health. 2016;140:221–227.

[38] Enserink R, Scholts R, Bruining-Verhagen P, et al. High detection rates of enteropathogens in asymptomatic children attending day care. PLoS One. 2014;9:e89946. [cited 2019 Jun 24]

[39] Faden HS, Dryja D. Importance of asymptomatic shedding of Clostridium difficile in environmental contamination of a neonatal intensive care unit. Am J Infect Control. 2015;43:887–888.

[40] Beniwal-Patel P, Stein D, Munoz-Price LS. The Juncture between Clostridium difficile infection and inflammatory bowel diseases. Clin Infect Dis. 2019;69:366–372.

[41] Hourigan SK, Chirumamilla SR, Ross T, et al. Clostridium difficile carriage and serum antitoxin responses in children with inflammatory bowel disease. Inflamm Bowel Dis. 2013;19:2744–2752.

[42] Lamousé-Smith ES, Weber S, Rossi RF, et al. Polymerase chain reaction test for Clostridium difficile toxin B gene reveals similar prevalence rates in children with and without inflammatory bowel disease. J Pediatr Gastroenterol Nutr. 2010;51:218(18)30183-X.

[43] Martinelli M, Strisciuglio C, Veres G, et al. Clostridium difficile and pediatric inflammatory bowel disease: a prospective, comparative, multicenter, ESPGHAN study. Inflamm Bowel Dis. 2014;20:2219–2225.

[44] Boyanova L, Markovska R, Hadzhiyski P, et al. Recurrent Clostridioides (Clostridium) difficile infection in a patient suffering from inflammatory bowel disease and benefits of resistotyping. Diagn Microbiol Infect Dis. 2019;94:336–339.

[45] Houngan SK, Chirumamilla SR, Ross T, et al. Clostridium difficile carriage and serum antitoxin responses in children with inflammatory bowel disease. Inflamm Bowel Dis. 2013;19:2744–2752.

[46] Boyanova L, Markovska R, Hadzhiyski P, et al. Recurrent Clostridioides (Clostridium) difficile infection in a patient suffering from inflammatory bowel disease and benefits of resistotyping. Diagn Microbiol Infect Dis. 2019;94:334–336.

[47] Hourigan SK, Chirumamilla SR, Ross T, et al. Clostridium difficile carriage and serum antitoxin responses in children with inflammatory bowel disease. Inflamm Bowel Dis. 2013;19:2744–2752.

[48] Lamousé-Smith ES, Weber S, Rossi RF, et al. Polymerase chain reaction test for Clostridium difficile toxin B gene reveals similar prevalence rates in children with and without inflammatory bowel disease. J Pediatr Gastroenterol Nutr. 2013;57:293–297.

[49] Martinelli M, Strisciuglio C, Veres G, et al. Clostridium difficile and pediatric inflammatory bowel disease: a prospective, comparative, multicenter, ESPGHAN study. Inflamm Bowel Dis. 2014;20:2219–2225.

[50] Boyanova L, Markovska R, Hadzhiyski P, et al. Recurrent Clostridioides (Clostridium) difficile infection in a patient suffering from inflammatory bowel disease and benefits of resistotyping. Diagn Microbiol Infect Dis. 2019;94:336–339.

[51] Hourigan SK, Chirumamilla SR, Ross T, et al. Clostridium difficile carriage and serum antitoxin responses in children with inflammatory bowel disease. Inflamm Bowel Dis. 2013;19:2744–2752.

[52] Boyanova L, Markovska R, Hadzhiyski P, et al. Recurrent Clostridioides (Clostridium) difficile infection in a patient suffering from inflammatory bowel disease and benefits of resistotyping. Diagn Microbiol Infect Dis. 2019;94:334–336.

[53] Hourigan SK, Chirumamilla SR, Ross T, et al. Clostridium difficile carriage and serum antitoxin responses in children with inflammatory bowel disease. Inflamm Bowel Dis. 2013;19:2744–2752.

[54] Boyanova L, Markovska R, Hadzhiyski P, et al. Recurrent Clostridioides (Clostridium) difficile infection in a patient suffering from inflammatory bowel disease and benefits of resistotyping. Diagn Microbiol Infect Dis. 2019;94:336–339.

[55] Hourigan SK, Chirumamilla SR, Ross T, et al. Clostridium difficile carriage and serum antitoxin responses in children with inflammatory bowel disease. Inflamm Bowel Dis. 2013;19:2744–2752.

[56] Boyanova L, Markovska R, Hadzhiyski P, et al. Recurrent Clostridioides (Clostridium) difficile infection in a patient suffering from inflammatory bowel disease and benefits of resistotyping. Diagn Microbiol Infect Dis. 2019;94:334–336.

[57] Hourigan SK, Chirumamilla SR, Ross T, et al. Clostridium difficile carriage and serum antitoxin responses in children with inflammatory bowel disease. Inflamm Bowel Dis. 2013;19:2744–2752.
[47] Kellermayer R. Burdening questions about *Clostridium difficile* in pediatric inflammatory bowel diseases. *J Pediatr Gastroenterol Nutr.* 2015;60:421–422.

[48] Rätsep M, Köljalg S, Sepp E, et al. A combination of the probiotic and prebiotic product can prevent the germination of *Clostridium difficile* spores and infection. *Anaerobe.* 2017;47:94–103.

[49] Suskind DL, Lee D, Solan P, et al. Dietary therapy for *Clostridium difficile* colonization: A case series. *Anaerobe.* 2019;57:1–3.