# *Clostridium difficile* in Children: A Review of Existing and Recently Uncovered Evidence

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**Abstract**

The clinical significance of the presence of *Clostridium difficile* in children's faeces remains uncertain using current diagnostic procedures. *Clostridium difficile* is a relatively common finding in infants with no symptoms of gastrointestinal disease, suggesting it may be an incidental finding and form part of the normal gut micro-flora in this age group. On the other hand, particularly in older children or those with significant co-morbidity, there are examples where *C. difficile* causes disease and exerts considerable morbidity and even mortality (*C. difficile* infection, CDI). Between these extremes lie a substantial group of children who have both diarrhoea and *C. difficile* in their stools but where the nature of the association is not clear: *Clostridium difficile* associated disease (CDAD). We review the significance of *C. difficile* in children presenting recently uncovered paediatric data from a large UK epidemiological study that informs some key unanswered questions.

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## 4.1 Introduction

The clinical significance of *Clostridium difficile* colonisation in children is crucial in determining testing and treatment policies. *C. difficile* is the leading cause of nosocomial diarrhoea in adults and one of the Healthcare Acquired Infections (HAI) specifically targeted by the National Health Service (NHS) in England [1]. Mandatory monitoring in the UK currently requires reporting of all diarrhoeal samples positive for *C. difficile* in those over the age of 2 years [2, 3]. *C. difficile* infection (CDI) in adults has been extensively studied and there are clear guidelines for its diagnosis and management [3–8]. The situation is much less clear in children because [9, 10] of the following issues:

a. ***Clostridium difficile* carriage rates are high in young children, especially infants:** Asymptomatic carriage of *C. difficile* in children is much more frequent than in adults. Carriage can be found in up to 64 % of neonates (under 4 weeks old), who appear to acquire *C. difficile* environmentally in the first few weeks of life [10]. Asymptomatic carriage is so high in infants under 1 year of age that testing for *C. difficile* is of questionable benefit [11]. Carriage declines with age, [12, 13] whilst the proportion with symptomatic disease increases to approach levels in adults (5–8 %, [10]).
b. ***Clostridium difficile* may be an incidental finding:** Acute diarrhoeal illness is common in young children, and due to a wide range of potential pathogens [12, 14–17], although in a substantial proportion no pathogen can be identified even after extensive testing for a range of bacteria, viruses and parasites. Given that so many children suffer diarrhoea without an identifiable cause, it is possible that even in those cases where *C. difficile is* identified, it is in fact not the causative agent but an incidental finding. It is even possible that colonisation with *C. difficile* may be protective [18, 19] against CDI.
c. **The relationship between prior antibiotic usage and *C. difficile* is considerably weaker in younger children:** Not only do many children with *C. difficile* in their stool sample not have a recent history of antibiotic exposure [10, 20, 21], but a significant proportion of them have concurrent infection with other pathogenic organisms [14, 20, 21]. Thus, two of the original diagnostic criteria for CDI in adults [6] would exclude a large proportion of symptomatic children with *C. difficile* positive stools. Although there are recent guidelines for diagnosing CDI in adults [7, 8], no equivalent guidelines exist for children, where the evidence base is much more limited.

Despite this, there is a consensus that *C. difficile* can cause serious disease in children [10, 22]. It is helpful to maintain a distinction between examples of CDI, where *C. difficile* is the accepted cause of children's symptoms, and "*C. difficile associated* disease" (CDAD) where the exact nature of the association between *C. difficile* and gastrointestinal symptoms remains unclear. Although variably defined, for example "gastrointestinal symptoms in a child with *C. difficile* toxin positive stools" [23], or "children with a clinical diagnosis of *C. difficile* infection on discharge who had *C. difficile* testing and antibiotic therapy for CDAD" [24], the incidence of CDAD and/or CDI in children appears to be on the increase, particularly in cases arising from the community [25]. Recent reviews have highlighted the importance of considering CDAD/CDI in children and outside the hospital setting [26–28]. Difficult questions are raised for clinicians faced with children suffering from diarrhoea [9, 10]:

1. When should *C. difficile* be considered as a cause for the child's symptoms and what is the appropriate testing strategy?
2. Should the presence of *C. difficile* in diarrhoea in children be treated, a*nd if so how*?

With current technologies, testing for *C. difficile* in children is controversial [11]. This is especially true for children under the age of 1, who are the most likely to suffer from diarrhoea, have the highest prevalence of asymptomatic carriage of *C. difficile*, and also form a group for whom the incidence of CDAD is increasing [23]. Devising the most appropriate policy has significant implications for all acute NHS Hospital Trusts in England, who face financial penalties if their

**Table 4.1** Key features of different testing methods for *C. difficile*

| | Tests requiring organism culture | | Tests used directly on stool specimens | | | |
|---|---|---|---|---|---|---|
| | Selective culture | TGC | GDH EIA | PCRCDT | Toxin A/B EIA | CCA |
| Test target | Whole organism | Products/genes of isolate | Enzyme product | Toxin gene (s) | Toxin (s) | Toxin (s) |
| Detects | | | | | | |
| Non-toxigenic *C. difficile* | + | – | + | – | – | – |
| Toxigenic *C. difficile* | + | + | + | + | ±[a] | ±[a] |
| Turnaround time | 2–5 days | 2–5 days | Minutes | 1–3 h | Minutes | 1–3 days |
| Performance in adults[c] | Sensitivity | cf. CCA | 0.80–0.97 | 0.87–1.00 | 0.31–0.99 | |
| | | cf. TGC | 0.60–0.74 | 0.86–0.94 | 0.32–0.79 | 0.55–0.67 |
| | Specificity | cf. CCA | 0.75–0.97 | 0.94–1.00 | 0.65–1.00 | |
| | | cf. TGC | 0.76–0.95 | 0.94–0.97 | 0.84–1.00 | 0.98 |
| Performance in children[d] | Sensitivity[b] | 0.75 (by EIA) 0.90 (by PCR) | | 0.95 | 0.35 | |
| | Specificity[b] | 1.00 | | 1.00 | 1.00 | |

[a] Tests are only positive if sufficient toxin present in faeces
[b] The "reference standard" was any stool specimen where at least four of the six tests used were positive (stool EIA, postculture EIA, stool PCRCDT for *tcdA*, postculture PCRCDT for *tcdA*, stool PCRCDT for *tcdB*, postculture PCRCDT for *tcdA*)
[c] Crobach et al. [7]; Tenover et al. [39]; Carroll [40]; Stamper [100]
[d] Luna [42]

rates of *C. difficile* infection fail to meet national targets under a system that has been criticised as unfair due to arbitrary thresholds [29]. This is a potential disincentive to test in populations expected to have higher prevalence of colonisation with *C. difficile*, or to improve the accuracy of testing strategies in ways that increase reportable rates of CDI [30]. Such conflicts can only be resolved by a better understanding of the significance of *C. difficile* colonisation and infection in children that can inform new guidelines.

## 4.2 The Complexity of Testing for *C. difficile* and Diagnosing *C. difficile* Infection

Conventional detection of *C. difficile* relies on selective culture of the organism from faecal samples or detection of toxin by cell culture cytotoxicity assay (CCA) or enzyme immunoassay (EIA) [4]. *Clostridium difficile*'s pathogenicity is associated primarily with expression of toxin B (cytotoxin), with the role of toxin A (enterotoxin) being less certain [31, 32]. Not all strains are capable of producing toxin A [33, 34] so tests directed against this alone may miss CDI. Molecular assays based on amplification of *C. difficile* toxin gene sequences (PCRCDT) provide rapid and sensitive results but may not be as specific for CDI as phenotypic testing methods (e.g. CCA) which detect toxin production [35]. *Clostridium difficile* exhibits considerable genomic variation [36], allowing development of genetic typing schemes using techniques such as PCR ribotyping [37]. Genetic variation within the toxin genes may affect the performance of both phenotypic and genetic detection methods [38, 39]. Detection of *C. difficile* will therefore depend on the strain present and the test used (Table 4.1) [4, 7, 40]. A two-stage testing strategy has recently been implemented in the UK, [3] based on an observational diagnostic study in four UK laboratories [41] that confirmed that *C. difficile* toxin EIAs are not suitable as stand alone tests for the diagnosis of CDI or detection of *C. difficile.* The new UK recommendation contains a two test screening protocol comprising a

GDH EIA (or toxin gene PCR) followed by a sensitive toxin EIA. If the first test (GDH or toxin gene test) is negative, the second test (sensitive toxin EIA) does not need to be performed.

Unfortunately, nearly all of the studies comparing the performance of different testing strategies have been conducted in adults and the clinical significance of the results will be subject to different interpretations in paediatric and neonatal populations. One study that investigated both children and young adults is a recent prospective study from Texas that compared direct stool EIA toxin A/B and direct stool PCRCDT with toxigenic culture (TGC, testing for toxin with either EIA or PCRCDT after the organism has been cultured selectively from faeces) [42]. Stools from 96 patients (age 15 days–25 years, median 4 years) suspected of having CDAD were tested using EIA toxin A/B and PCRCDT, both before (direct) and after (TGC) isolation and selective culture. Although lacking an independent gold standard, the "reference standard" for calculating sensitivities and specificities was any stool specimen where at least four of the six tests used (stool EIA, postculture EIA, stool PCRCDT (*tcdA*), stool PCRCDT (*tcdB*), postculture PCRCDT (*tcdA*), postculture PCRCDT (*tcdB*)) were positive. Direct stool PCRCDT had the greatest sensitivity of all methods used (95 %, compared to only 35 % for EIA; specificity of both PCRCDT and EIA: 100 %). Interestingly, positivity rates for *C. difficile* have doubled (from ~8 to 16 %) since the introduction of PCRCDT as the standard testing strategy at this US institution (while the number of samples sent for testing has stayed the same).

## 4.3 Epidemiology of *C. difficile* in Children

Few published studies are well placed to establish when *C. difficile* should be considered the cause of a child's diarrhoea, or when it should be treated. We therefore reanalysed paediatric data from the English community-based Infectious Intestinal Disease Study (IIDS) [12, 43] which have not previously been reported within the peer reviewed literature. The IIDS data is informative as the study attempted to: (a) test each sample for a range of viral, bacterial and parasitic pathogens to identify co-infection and the possibility that *C. difficile* is an "innocent bystander"; (b) use an appropriately sensitive testing strategy for *C. difficile* (including selective culture); (c) test both symptomatic and asymptomatic children to assess the association between *C. difficile* and symptoms; and (d) present data within clinically relevant age ranges. A second study (IID2) is currently in progress [44].

### 4.3.1 *Clostridium difficile* in Children in the Community

Several community-based studies have demonstrated that detection of *C. difficile* and/or *C. difficile* toxin is as common in asymptomatic children as in those suffering with diarrhoea (Table 4.2). Conducted between 1993 and 1996, the IIDS collected data and stools from over 6,000 cases of infectious intestinal diseases in children and adults, as well as from controls [12, 43]. The IIDS included two components: a community cohort recruited randomly from 70 General Practitioner (GP) practices across England and a GP-based case-control study which included subjects presenting spontaneously with symptoms of diarrhoea to one of 34 practices. All faecal samples were subject to extensive testing for a range of potential pathogens including *C. difficile*. Asymptomatic carriage of *C. difficile* was highest in infants under 1 year of age: 21 % in those recruited as part of the community cohort and 16.6 % in the age and sex-matched controls for those presenting to their GP with diarrhoea. Carriage rates in infants *with* diarrhoea were not much greater (28.6 % in the community cohort) or even less (7.2 % in infants presenting to their GP). 1 % of children aged 1–4 years of age had *C. difficile* in their faeces, and *C. difficile* was rarely found in children 5 years or older. There was no significant association of *C. difficile* with diarrhoeal symptoms for children under the age of 5 in either the GP study (derived odds ratio for diarrhoea if *C. difficile* positive 0.59; 95 % confi-

**Table 4.2** Prevalence of *C. difficile* in children in the community: asymptomatic children compared to those with diarrhoea for different groups*

| Group | Country | Dates | Age, years (median) | Prevalence (n) | Test Methods | Setting |
|---|---|---|---|---|---|---|
| Community Non-diarrhoea | U.K. | **1993-6** | **<1<br>1-4<br>5-14** | **21.0% (19)<br>1.0% (98)<br>0% (72)** | CCA (also selective culture (CCFA), TGC via Toxin A EIA, and PCR ribotyping) | Age- and sex-matched controls to community diarrhoea cohort (12) |
| | U.K. | **1993-6** | **<1<br>1-4<br>5-14** | **16.6% (199)<br>1.0% (510)<br>0.6% (177)** | | Age- and sex-matched controls to GP presentations of diarrhoea, families not part of cohort (12) |
| | U.S.A. | **1981** | **0.04 - 16** | **14.8% (135)** | Selective culture (CCFA) | Outpatient attendees (45) |
| | | **2001-2** | **(1.27)** | **3.5% (484)** | Toxin A/B EIA | Community (17) |
| | Japan | **2004** | **<1<br>1<br>2<br>3<br>4<br>5** | **100% (12)<br>75% (20)<br>45.5% (11)<br>24% (25)<br>38.5% (13)<br>23.5% (17)** | Selective culture, toxin gene typing (PCR), PCR ribotyping and pulsed-field gel electrophoresis | 2 day nurseries and a kindergarten (13) |
| Community Diarrhoea | U.K. | **1993-6** | **<1<br>1-4<br>5-14** | **28.6% (21)<br>0.9% (116)<br>0% (94)** | CCA (also selective culture (CCFA), TGC via Toxin A EIA, and PCR ribotyping) | Diarrhoeal episode within community (asked to provide samples when have diarrhoea), recruited to cohort via GP practice lists (12) |
| | U.K. | **1993-6** | **<1<br>1-4<br>5-14** | **7.2% (180)<br>1.7% (468)<br>0.6% (167)** | | Presentations to GP with diarrhoea, families not part of cohort (12) |
| | U.S.A. | **1981** | **0.04 - 16** | **7% (171)** | Selective culture (CCFA) | Outpatient attendees (45) |
| | | **2001-2** | **(1.27)** | **1.9% (431)** | Toxin A/B EIA | Community (17) |
| | | **1998-2001** | **0.01-28.6 (1.3)** | **6.7% (688)** | CCA | Paediatric emergency admissions (14) |
| | | **1998-01<br>2000-01** | **0.05-18.75 (1.61)** | **9.0% (89)<br>0% (15)** | CCA | Private paediatric outpatient clinic<br>Clinic serving mainly immigrants (15) |
| | Austria | **2007** | **<5<br>5-19** | **5% (20)<br>6.5% (46)** | Toxin A/B EIA and TGC (Toxin A/B EIA) | Presentations to GP with acute gastroenteritis (16) |

*** Where available, matching shades show data from the same study to facilitate comparison**

dence interval (CI) 0.34–1.02) or the community cohort (odds ratio 1.21; 95 % CI 0.37–3.9).

Two other studies comparing *C. difficile* in symptomatic children with asymptomatic controls showed similar results. Boenning et al. [45] showed that the prevalence of *C. difficile* amongst paediatric outpatients was actually higher in those with non-diarrhoeal illnesses (14.8 %) than those with diarrhoea (7.0 %; Odds ratio 0.43, 95 % CI 0.20–0.92), and found no association with antibiotic exposure in the preceding month. More recently, Vernacchio et al. [17] carried out a prospective cohort study of healthy children in the community in which baseline normal stools were cultured and compared to those from the same children who subsequently developed diarrhoea during the study period. Of healthy baseline stools, 3.5 % contained *C. difficile* compared to just 1.9 % in diarrhoeal specimens (matched relative risk 0.54, 95 % CI 0.20–1.50). Contrasting results were obtained from an earlier study of diarrhoeal outbreaks in community day centres [46], where rates of *C. difficile* isolation were greater amongst children with diarrhoea (57 %) than in their healthy classmates (9 %; OR 13.3, 95 % CI 3.5–51).

Other community-based studies have either tested only asymptomatic children [13] or children with diarrhoea [14–16], and therefore cannot provide comparative data regarding the association of *C. difficile* with symptoms. They nevertheless demonstrate that *C. difficile* is a relatively common finding (5–9 %, Table 4.2) in children presenting from the community.

Although the prevalence in different age ranges have varied between studies, a high prevalence of *C. difficile* has largely been found in young children and infants, with an average age in these studies typically 18 months or less. For example, in the recent US study [24] the mean

**Table 4.3** Prevalence (%) of identified microorganisms in diarrhoeal samples from children in the community (IIDS GP data shown, cohort data not shown)

| Putative pathogen | | Setting | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Class | Organism | GP presentations [12] 1993–1996 (UK) | | | Paediatric outpatients[a] [15] 1998–2001 (USA) | Emergency department [14] 1998–2001 (USA) | Community diarrhoea [17] 2001–2002 (USA) | GP presentations [16] 2007 (Austria) | |
| | | <1 year | 1–4 years | 5–14 years | 0.05–18.75 years (1.6 years) | 1.3 years | 0.5–3 years (1.25 years) | <5 years | 5–19 years |
| Bacteria | *C. difficile* (toxin) | 7.2 | 1.7 | 0.6 | 9.0 | 6.7 | 1.9 | 5 | 6.5 |
| | *C. perfringens* | 4.0 | 5.6 | 4.5 | | | 2.3 | | |
| | *Aeromonas* spp. | 10.3 | 4.3 | 7.2 | | | 1.0 | | |
| | *Campylobacter* spp. | 2 | 5.4 | 11.3 | 4.1 | 1.5 | 0.7 | 0 | 4.3 |
| | Pathogenic *E. Coli* | | | | | | | | |
| | DAEC | 3.6 | 2.9 | 2.3 | | | | | |
| | AEEC | 6.1 | 9.8 | 3.3 | | | | | |
| | EAggEC | 4.0 | 5.5 | 4.7 | | | 4.1 | | |
| | EPEC | 0 | 0.2 | 0.5 | | | 12.9[b] | | |
| | ETEC | 0 | 0.5 | 0.5 | | | 0.2 | | |
| | EHEC/STEC | 0 | 0.3 | 0.5 | 0 | 2.4 | 0.2 | 0 | 0 |
| | *Salmonella* spp. | 2.3 | 2.5 | 5.9 | 0 | 2.4 | 0 | 0 | 0 |
| | *Staphylococcus* aureus | 1.3 | 0 | 0 | | | | | |
| | *Yersinia* | 0.3 | 2.0 | 1.8 | 0.4 | 0.1 | 0.2 | 0 | 0 |
| | *Shigella* spp. | 0 | 0.3 | 0.4 | 0 | 0.9 | 0 | | |
| Viruses | Rotavirus | 21.3 | 17.6 | 8.1 | 14.2 | 20.4 | 5.2 | 5 | 6.5 |
| | Norovirus/SRSV | 8.9 | 11.0 | 5.5 | | | 1.9 | 20 | 8.7 |
| | Adenovirus | 6.9 | 10.3 | 1.0 | 6.3 | 4.3 | 5.7 | 10 | 0 |
| | Astrovirus | 1.9 | 6.7 | 3.0 | 3.1 | 6.5 | 3.5 | 0 | 0 |
| | Sapovirus/Calicivirus | 5.8 | 3.8 | 0.5 | | | 3.0 | | |
| Parasites | *Giardia* | 1.0 | 1.2 | 1.8 | | 0.6 | 0.2 | 0 | 0 |
| | *Cryptosporidium* | 0.7 | 2.8 | 5.0 | | 0.3 | 0.5 | 0 | 0 |
| *No pathogen identified* | | *42.0* | *30.9* | *45.7* | *52* | *53* | *57.5* | *60* | *74* |

[a] Data from Site A only (private clinic)
[b] 'Atypical' EPEC

age was 15.2 (±7.5) months, with most children under the age of 3 years. In studies that report prevalence for infants separately this is universally higher than for older children.

A small study in Japan was notable for the particularly high prevalence of *C. difficile* in asymptomatic children at two day nurseries and a kindergarten [13]. All the infants tested had *C. difficile* in their faeces but carriage rates declined steadily with increasing age to less than 25 % by 5 years of age. Nearly all (21/22) of the *C. difficile* strains (by PCR ribotype and PFGE type) isolated from environmental swabs at one nursery were identical to those found in the faeces of infants in that nursery, suggesting acquisition from the day-care environment.

Community onset diarrhoea is relatively common in young children and infants, with a range of potential pathogens other than *C. difficile*. Table 4.3 summarises data from five studies which carried out extensive testing for pathogens in children with community-acquired diarrhoea. A number of trends are revealed across the different settings, methodologies and even countries

where the studies were based. Despite the investigators' best efforts to test for a large range of pathogens, none could be identified in 30–75 % of the cases of diarrhoea. Viruses were the commonest identified pathogen in all studies, especially in infants and pre-school age children. Rotavirus was most frequently encountered (~14–20 %, except in an Austrian study, where there is routine vaccination against rotavirus [16]), followed by norovirus (where tested, ~9–11 %), adenovirus (~4–10 %) and astrovirus (~2–7 %). *Clostridium difficile* was the commonest identified bacterium in most studies. Other common isolates were *C. perfringens*, pathogenic *E. coli*, *Aeromonas* spp., *Salmonella* spp. and *Campylobacter* spp. (notably the latter two were more commonly isolated in older children).

A proportion of *C. difficile* positive children were co-infected with other known pathogens. In Klein et al.'s [14] study, 25 (6.7 %) of the 372 specimens that underwent extensive testing were positive for *C. difficile* toxin B, but nine of these had concomitant viral or bacterial pathogens. Only 4.3 % of children with diarrhoea had *C. difficile* as the only identifiable pathogen [14] and some common causes (e.g. norovirus) were not tested for, so it is possible that there may have been underestimation of co-infection with *C. difficile*. Denno et al. tested for the same range of organisms, and found one case of co-infection out of 75 children undergoing 'complete' analysis (one of the eight stools positive for *C. difficile* was also positive for adenovirus) [15]. Co-infection is also common in hospitalised children, with other pathogens reported in 36 % [21], 44 % [20] 27 % [47] and 23–38 % of *C. difficile* culture positive stools [48]. In three recently reported cases of severe CDAD in children, it was noted that two had co-infection with viruses (rotavirus and calicivirus), although these two also had underlying chronic medical conditions (Hirschsprung's disease and Down's syndrome) [49].

#### 4.3.1.1 Explanations for High Levels of Asymptomatic Colonisation

The high rates of carriage of *C. difficile* may be explained by a lack of 'colonisation resistance' in infants as the intestinal micro-flora is in the process of becoming established [50], but this cannot explain the absence of symptomatic disease (CDI). Asymptomatic carriage does not simply occur because the strains of *C. difficile* present are non-toxigenic, for most of the studies discussed previously demonstrated the presence of toxin in faeces from asymptomatic children. One hypothesis is that the strains typically found in children have reduced virulence compared to those in adults. Factors other than toxin expression, such as those influencing adherence and intestinal colonisation, are also recognised contributors to virulence and may play a part in childhood disease [51].

The hypervirulent strain of *C. difficile* associated with outbreaks of severe CDI in adults (PCR ribotype 027) has greatly increased *in vitro* levels of expression of *tcdA* (16-fold) and *tcdB* (23-fold) [51]. This strain was responsible for a high proportion (19.4 %) of *C. difficile* toxin positive stools in symptomatic children in a recent study in the USA [52]. A study of hospitalised but asymptomatic children in Thailand demonstrated relatively high carriage rates for *C. difficile* (11.0 % of infants, and 21.1 % of 1–11 year olds) but low rates of toxin A gene detection (0.9 % of infants and none of the older children; toxin B not tested) [53]. 87.2 % of the strains isolated in asymptomatic children in the Japanese day-care study were also non-toxigenic (*tcdA*−/*tcdB*−) [13]. Unfortunately neither study examined rates of carriage or toxin expression in symptomatic children for comparison. A small study in Brazil attempted to compare strains of *C. difficile* in hospitalised children with acute diarrhoea with asymptomatic children recruited from day-care centres, but none of the controls were culture positive for *C. difficile.* Nine out of the ten strains isolated from symptomatic patients were toxigenic: six were *tcdA*+/*tcdB*+ and three were *tcdA*−/*tcdB*+ [54].

Only the IID study provides direct data comparing *C. difficile* colonisation and toxin B detection for cases of diarrhoea and asymptomatic controls [12]. Below 2 years of age, children presenting to their GP with diarrhoea had slightly higher rates of *C. difficile* colonisation (24 % of 374 cf. 19.5 % of 385 cultured) but lower rates of toxin B detection than asymptomatic age- and sex-matched controls (4.3 % of 391 cf. 9.0 % of 423 tested). For children 2 years or older, con-

**Table 4.4** Prevalence of *C. difficile* in hospitalised children for different groups*

| Group | Country | Year | Age Years (median) | Prevalence (n) | Test Method | Setting |
|---|---|---|---|---|---|---|
| Hospital Diarrhoea | Germany | 1989 | **0-1**<br>**>1** | **~30%**<br>**~5%**<br>**(418)** | Culture and Y-1 cell culture (Toxin B) and rabbit ileal loop test (Toxin A) | Hospitalised children (55) |
| | Canada | 1991-99 | **0.03-17 (1.3)** | **18.0%** | Toxin A EIA | Paediatric and Women's Hospital (56) |
| | USA | 1998-99<br><br>2001-03 | **(1.3)**<br><br>**(2.4)** | **26% (676)**<br>**(Toxigenic: 15%)**<br>**49% (301)**<br>**(Toxigenic: 14%)** | Selective culture (CCFA) and TGC (PCRCDT), PCR fingerprinting | Stand alone paediatric hospital<br><br>Paediatric department within general hospital (48) |
| | Turkey | 2001 | **0.08-13**<br>**(Mean 3.2)** | **22% (100)** | Toxin A/B EIA | Inpatients treated with antibiotics with diarrhoea (47) |
| Hospital Non-diarrhoea | Germany | 1989 | **0-1**<br>**>1** | **~30%**<br>**~5%**<br>**(348)** | Culture and Y-1 cell culture (Toxin B) and rabbit ileal loop test (Toxin A) | Hospitalised children (55) |
| | Thailand | 1998-99 | **0-1**<br>**1-11yrs** | **11.9% (235)**<br>**21.1% (76)** | CCFA culture, PCR *tcdA* and PFGE analysis for type | Hospitalised children (53) |
| | Turkey | 2001 | **0.08-13**<br>**(Mean 4.1)** | **10% (50)** | Selective culture (CCFA) and Toxin A/B EIA | Inpatients treated with antibiotics but asymptomatic for diarrhoea (47) |

*** Matching shading shows data from the same study to facilitate comparison**

trols had lower rates of both toxin B detection (0.2 % of 1,616 cf. 1.1 % of 1,866 tested) and *C. difficile* colonisation (0.4 % of 1,613 cf. 1.0 % of 1,845 cultured). These data suggest that above the age of 2 years, rates of colonisation drop dramatically, and toxin production is more likely to be associated with disease.

### 4.3.2 *Clostridium difficile* in Hospitalised Children

The prevalence of *C. difficile* in hospitalised children is higher than in the community but appears similar in children with and without diarrhoea (Table 4.4). Karsch et al. [55] found high rates of carriage, particularly in infants (30 %), with most isolates producing toxin (82 % toxin A and 43 % toxin B), but no significant difference between symptomatic children and controls, and no clear association with previous antibiotic therapy. A prospective study in Denmark also showed significantly higher isolation of *C. difficile* in infants and no relationship to antibiotic exposure ($p<0.001$), but it was the only identified pathogen in 12 % of children of all ages with acute gastroenteritis, and this was significantly ($p<0.01$) higher than for asymptomatic controls [21]. As 78 % of the positive cultures were obtained within 2 days of admission this suggests acquisition in the community rather than nosocomial infection. A retrospective cohort study in the US reported that 26 % of cases of CDAD occured in infants [24], but it remains unclear whether *C. difficile* was truly the underlying cause. Of the 56 % of specimens from paediatric inpatients in Canada with nosocomial diarrhoea where a pathogen was identified, most were viruses (38 % of episodes, viral diagnosis typically in younger children with mean age 0.8 years) [56]. *Clostridium difficile* was identified in 18 % of all episodes (mainly in older children, mean age 3.9 years). A retrospective case control study in Canada was unable to demonstrate any difference in clinical characteristics between infants with *C. difficile* toxin in their stool and those without toxin present, nor could it identify a significant treatment effect of metronidazole [11]. Colonisation with *C. difficile* was relatively high at two US institutions (49 % and 26 %), but toxigenic colonisation was less common (14 % and 15 %), and many isolates were unique (92 % within the general hospital), indicating that they did not arise from a common source [48]. Underlying medical conditions and exposure to two or more antibiotics were associated with increased toxigenic strain colonisation. In Turkey, although *C. difficile* toxin was found more frequently in

hospitalised children with nosocomial diarrhoea (22 %) than asymptomatic controls (10 %), this was not significant (odds ratio 2.54, 95 % CI 0.90–7.17) and co-infection was found in six cases (27 %) of CDAD (rotavirus was the only viral pathogen tested) [47]. Children with CDAD were older (mean 5.4 years) than those asymptomatic controls with *C. difficile* toxin in their stools (all under 2 years).

### 4.3.3 *Clostridium difficile* in Neonates

Neonates acquire *C. difficile* from the environment, resulting in high rates of carriage within the first few weeks of birth [57–60]. In one study, no neonatal faeces cultured *C. difficile* on day 1 after birth but 17 % did by day 4, and most strains were toxigenic (58–65 %) [60]. None of the maternal rectal swabs and only one vaginal swab cultured *C. difficile,* whereas 13 % of environmental samples did and these were all of the same strain (matching 11 of the 31 neonatal strains typed). On one neonatal intensive care unit (NICU), *C. difficile* acquisition reached 33 % after 2 weeks with all cultures toxigenic [61]. In common with previous studies [58, 59], there was no association between *C. difficile* acquisition and gastrointestinal symptoms. In another NICU, 90 % of samples cultured *C. difficile* after only 6 days, and although toxin was detected directly in only 36 % of these, 94 % of the isolates were found to be toxigenic *in vitro* [57]. One small study suggested that neonates with toxin A positive stools are likely to experience increased numbers of days with frequent and abnormal stools [62], but most demonstrate asymptomatic carriage of *C. difficile* in neonates, even when a high proportion of stools test positive for toxin.

## 4.4 Burden of CDAD in Children

The high asymptomatic prevalence of *C. difficile* in young children initially led to the conclusion that CDI was not a problem in this age group [45, 63]. However, several studies have suggested that CDAD (and by implication CDI) is an increasing problem in children. A retrospective cohort study in the USA showed that from 2001 to 2006 the incidence rate of CDAD (defined as "clinical symptoms, such as diarrhoea or bloody stools, in a patient whose stool specimen tested positive for *C. difficile* toxin") amongst outpatients increased by 11 % (from 1.24 to 1.38 cases per 1,000 visits) [23]. The incidence of CDAD in patients attending the emergency department increased 2.5 fold, largely due to an increase in community-associated CDAD (from 0.84 to 2.04 cases per 1,000 visits), while inpatient incidence of CDAD decreased over the same period [23]. In another study the annual incidence of CDAD (identified by the combination of discharge diagnosis of CDI, positive test assay for CDI and treated with antibiotics against CDI) increased from 2.6 to 4.0 cases/1,000 admissions over the same time period [24]. Hospitalisation rates for children with CDAD almost doubled between 1997 and 2006 [64]. Complications of CDAD can be severe, including pseudomembranous colitis [22, 49, 65, 66], rectal prolapse [67], osteomyelitis [68] and reactive arthritis [69, 70].

## 4.5 When to Consider CDAD and How to Test for it

The new UK Department of Health testing algorithm [41] contains a two test screening protocol comprising a GDH EIA (or toxin gene PCR) followed by a sensitive toxin EIA. If the first test (GDH or toxin gene test) is negative, the second test (sensitive toxin EIA) does not need to be performed.

### 4.5.1 Children Without Co-morbidities

Because the prevalence of *C. difficile* in children is so dependent on age it might be more appropriate to tailor the testing and management strategy accordingly (Table 4.5). Given the very limited data on the performance of different testing strategies in children (Table 4.1), adult guidelines may eventually be shown to perform poorly in children, where PCRCDT might be

**Table 4.5** Suggested investigation and management of children with suspected *C. difficile* infection based on current evidence

| Age | Rationale | Management |
|---|---|---|
| Neonates (0–4 weeks) | High incidence of carriage, true CDI very rare/ indeterminate | Do not test for *C. difficile.* If very unwell, treatment for necrotising enterocolitis will include cover for *C. difficile* |
| Infants (4 weeks–1 year) | Relatively high prevalence of asymptomatic carriage of *C. difficile* and diarrhoeal illness common (likely CDAD rather than CDI) | UK 2012: GDH EIA (or toxin gene) followed by a sensitive toxin EIA[a]. If strong clinical suspicion of CDI, test faeces for *C. difficile* and for common viral and bacterial pathogens. Where community acquired and treatment *not considered urgent*, treat for CDI only if GDH EIA (or toxin gene) *and* sensitive toxin EIA positive *and* viral and other bacterial testing negative. If hospital acquired or urgent treatment considered necessary, treat for CDI if GDH EIA (or toxin gene) *and* sensitive toxin EIA positive |
| Pre-school (1–4 years) | Incidence of community acquired CDAD declines with age; incidence of CDI increases above the age of 2 years. Significant risk of viral pathogens causing disease | |
| School age (5–18 years) | Community acquired diarrhoea; risk of CDI increases with increasing age, also risk of other bacterial and viral pathogens. Hospital acquired diarrhoea: CDI incidence similar to adults: investigate and manage as for adults | |

For a child presenting with a mild or recent onset diarrhoeal illness then this is likely to be viral and self-limiting: *no testing is indicated* and the child should be managed supportively according to recent NICE guidance [71]
For a child with a significant chronic disease (IBD, cystic fibrosis, cancer, HIV) and significant diarrhoea there is a higher risk of CDI requiring treatment. PCRCDT is the most appropriate first line or only test for paediatric samples where available. *Treat for CDI if PCRCDT positive.* (see footnote[a] )
Secondary care management *where diarrhoea is severe or protracted* (*>7 days*)
[a] Current paediatric evidence suggests a more prominent role of PCRCDT may be justified in future testing strategies

considered a more appropriate formal second line test after GDH EIA due to its rapid turnaround time and good correlation with toxigenic culture (Table 4.1).

Infants have high asymptomatic prevalence, so testing stools for *C. difficile* is not recommended unless illness is severe and there is a high level of clinical suspicion [71]. Serious cases of CDI (pseudomembranous colitis) have been reported in infants, but have generally been associated with other conditions such as prematurity, Hirschprung's disease, obstruction or necrotising enterocolitis [65, 72, 73], and the role of *C. difficile* in the pathogenesis has not been confirmed. These reports suggest symptomatic infants should only be tested for *C. difficile* in carefully selected circumstances. Other organisms may also be responsible for disease and viral testing in particular should be undertaken alongside testing for *C. difficile*.

It would be helpful to identify reliable predictors for development of CDI in children. Despite the conflicting evidence for an association between antibiotic usage and CDI in children (against association: [11, 20, 21, 45, 55, 74, 75]; for association: [14, 47, 48, 63, 76–79]), severe diarrhoea in the context of recent antibiotic therapy is likely to remain one such predictor that clinicians will use.

### 4.5.2 Children with Co-morbidities

Certain co-morbitities are associated with higher rates of *C. difficile* colonisation, and some of these children may be at particular risk of CDI: inflammatory bowel disease (IBD), Hirschprung's disease, cystic fibrosis, cancer patients and organ transplant recipients.

The prevalence of *C. difficile* is significantly greater in children with IBD compared to controls (indicating an increased risk of colonisation) and also greater in children with IBD experiencing active disease compared to those with inactive disease (indicating a potential role of the organism in the symptom exacerbation) [80]. It is important to use an appropriate testing strategy to prevent missing CDI in the context of inflammatory bowel disease, as the diagnosis may be missed in up to 41 % of IBD patients if a single-toxin

assay is used [81]. Such errors could lead to misattribution of symptoms to an exacerbation of underlying IBD and result in inappropriate treatment, potentially even colectomy. Steroids given to treat IBD without appropriate antibiotics to treat CDI are likely to exacerbate symptoms rather than resolve them [82].

Case reports of severe CDI associated with Hirschsprung's disease suggest that this may form another group of children at increased risk [66, 83]. Increased *C. difficile* was found in Hirschsprung's patients with enterocolitis compared with asymptomatic Hirschsprung's patients or healthy controls [84].

Carriage rates of *C. difficile* in patients with cystic fibrosis have been reported to be 22–46 %, double that of control patients receiving antibiotics [85–87]. These high carriage rates were described in older patients (median age 18.5, youngest 15 years) in the absence of symptoms of diarrhoea or abdominal pain, despite the presence of toxigenic strains [87]. Nevertheless, severe cases of CDI do occur in children with cystic fibrosis, emphasizing the importance of considering the diagnosis [88]. Patients may present atypically, without watery diarrhoea but rather abdominal distension and reduced bowel motions, risking confusion with faecal impaction or meconium ileus equivalent [89, 90]. There also appears to be a greater risk of CDI following lung transplantation for cystic fibrosis, with patients experiencing a fulminant course resulting in high mortality [91, 92].

Cancer patients are thought to have increased carriage of *C. difficile* due to their chemotherapy treatment and increased exposure to antimicrobials [74, 77]. Most reported cases have been associated with haematological malignancies, but a recent Italian study demonstrated that 6 % of children with solid tumours had *C. difficile* toxin A in their stools and gastrointestinal symptoms, with three out of nine of these being under 1 year old [93]. An earlier prospective study of oncology patients demonstrated higher rates of toxin detection in asymptomatic (19 %) than in symptomatic (8.7 %) children, questioning the significance of *C. difficile* as a pathogen in this patient group [74].

## 4.6 Treatment Strategies

If *C. difficile* is the only pathogen identified and seems the most likely cause of disease in a child over the age of 1 then treatment should be considered [10]. In adults, where exposure to broad spectrum antibiotics is a recognised trigger for CDI, cessation of the antibiotic, if clinically possible, is an important step. As previously discussed, the association between antibiotic use and CDAD in children is much weaker, but it is sensible to discontinue broad spectrum antibiotics where possible. Oral metronidazole and, if this fails, vancomycin are the standard treatments for adults with severe CDI requiring treatment and are also used in children. Up to 25 % of patients experience a further episode of CDI within 2 months, and 50–65 % of these will suffer from further recurrences [94].

More recently, interest has moved to alternative treatment strategies for CDI, in particular to prevent recurrence of disease [10, 95]. Alternative strategies may be divided into those that attempt to re-establish colonisation resistance (use of probiotics, faecotherapy), direct chemical neutralisation of the toxins (toxin binding by ion exchange resins and polymers) and immunotherapy (passive immunoglobulin therapy) [94, 95]. Unfortunately, few randomised controlled trials have been conducted in adult populations, and none have been done in children. A recent Cochrane review [96] found little evidence that probiotics were useful in adults, identifying only one study that showed a beneficial effect when added to antibiotic therapy [97]. Extrapolation of such data to children is problematic given that in a child's early years the natural intestinal flora is different from adults and evolves over time. None of the alternative strategies explored so far has demonstrated sufficient success to recommend incorporation into general clinical practice [95].

**Table 4.6** Outstanding questions for research

| |
|---|
| Are children suffering with CDAD colonised with different strains of *C. difficile* from those found in asymptomatic, age-matched controls? |
| What are the virulence factors other than toxin expression that influence pathogenicity in *C. difficile* and how do these explain the varying prevalence of symptoms with the age of the host? |
| Are there markers that correlate more closely with disease than toxin expression? Such markers might be specific to the strain of *C. difficile* present (e.g. other virulence genes) or the host specific response to infection (e.g. cytokine profile) |
| How do the new 'gold standard' tests for diagnosing *C. difficile* infection in adults perform in children at different ages? |
| What relationships exist between *C. difficile* and other members of the gut micro-flora, in both health and disease? |
| How common is co-infection in CDAD in children? |
| Are other bacterial or viral pathogens a risk factor for the development of CDI or *vice versa*? |
| Is colonisation with specific strains of *C. difficile* protective against other pathogens in children? |
| What factors underlie geographical variations in the prevalence of *C. difficile* colonisation and infection in children? |

## 4.7 Conclusions

*Clostridium difficile* is a relatively common finding in the faeces of infants under 1 year of age, and is very unlikely to signify disease, even when toxins are produced. Children with diarrhoea may have *C. difficile* in their stools, but viruses are a more likely cause of symptoms. It is not possible to identify the aetiological agent in a large proportion of childhood diarrhoea, but the disease is typically self-limiting and requires only supportive care [71].

CDAD appears to be increasing in children and should be considered as adult-type CDI whenever symptoms are particularly severe, protracted or the child belongs to a known at risk group such as inflammatory bowel disease or cystic fibrosis following lung transplantation. The use of predictors for CDAD such as recent antibiotic usage or prior hospitalisation is generally unhelpful in children, where these associations are much weaker and colonisation appears to occur in the community.

Few diagnostic tests in routine clinical use for *C. difficile* infection have been evaluated in children, where the true sensitivities and specificities are likely to be different from adult populations. The evidence on which to base decisions of when and how to treat CDAD in children remains limited and many important, interrelated research questions are yet unanswered (Table 4.6).

## 4.8 Search Strategy and Selection Criteria

This review was prompted by re-appraisal of data contained in a large, UK government-funded investigation report (The Infectious Intestinal Diseases (IID) Study in England [12]) in the light of a growing appreciation that *C. difficile* in children differs substantially from adults [9, 10, 98]. The IID study produced substantial data on the incidence of *C. difficile* in children in the community not contained in the original journal article [43], which combined data for children and adults. Other data and references were obtained by searching PubMed using "infant", "child(ren)", "p(a)ediatric" and "*C. difficile*" as search terms. Retrieved titles and abstracts were screened and full text versions obtained of suitable articles in English. Where necessary, odds ratios and confidence intervals were derived from original data presented in the manuscript by a standard method [99].

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