

# Prolonged carriage of *Clostridium difficile* in Hirschsprung's disease

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## Abstract

**The role of *Clostridium difficile* in the aetiology of diarrhoea in children with Hirschsprung's disease was investigated in a prospective longitudinal study. In 64 children with Hirschsprung's disease no significant difference was found in the isolation rate of *C difficile* in patients with diarrhoea (32%) and without diarrhoea (26%).**

**Comparable isolation rates were found in 47 control children with and without diarrhoea (27% and 16% respectively). The number of strains producing toxin B was similar in the four groups of children.**

**In contrast to the disappearance of *C difficile* by 12 months of age in the control groups of children, *C difficile* could be repeatedly isolated from a proportion of children with Hirschsprung's disease over 12 months of age. These findings help to reconcile the existing contradictory reports on the incidence of *C difficile* in Hirschsprung's disease associated enterocolitis.**

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Diarrhoea, varying in severity from loose stools to life threatening enterocolitis, is a common complication in children with Hirschsprung's disease.<sup>1-5</sup> The enterocolitis is associated with a significant morbidity and mortality in children.

Thomas *et al* reported an increased isolation rate of *Clostridium difficile* and production of cytopathic toxin from the stools of children with Hirschsprung's disease enterocolitis (defined as onset of loose stools) compared with children whose Hirschsprung's disease was not complicated by enterocolitis.<sup>6,7</sup> Wilson-Storey *et al*, in contrast, found no association between *C difficile* and enterocolitis in newly diagnosed patients with Hirschsprung's disease up to 2 years of age.<sup>8</sup>

Toxigenic *C difficile* can be isolated from the stools of a high proportion of healthy children up to 18 months of age without any apparent disease<sup>9-12</sup> yet fatal pseudomembranous colitis has been described in children with Hirschsprung's disease.<sup>13,14</sup> Consequently, the significance of *C difficile* in the aetiology of diarrhoea in children with Hirschsprung's disease remains uncertain.

We have examined the causes of diarrhoea and the isolation rate and toxigenicity of *C difficile* in a longitudinal study of children with Hirschsprung's disease treated at the Hospitals for Sick Children, London during the period from 1988-91.

## Patients and methods

### PATIENTS

A total of 122 stool samples were obtained from 64 children with Hirschsprung's disease and 47 control children (children without any primary intestinal disease) admitted to hospital or seen as outpatients. Each visit was considered as a separate episode. If more than one specimen was obtained from a child during the stay in hospital it was considered as a separate episode only if obtained four weeks after the previous specimen.

The samples were categorised into four groups with the number of episodes from the number of children as follows. Group I: Hirschsprung's disease without diarrhoea; 31 episodes from 27 children. Group II: Hirschsprung's disease with diarrhoea; 44 episodes from 37 children. Group III: controls without diarrhoea; 25 episodes from 25 children. Group IV: controls with diarrhoea; 22 episodes from 22 children. Episodes of diarrhoea were considered either as sporadic (one recorded episode of loose stools lasting for less than two weeks) or chronic/recurrent (lasting over two weeks and presenting with loose stools more than twice in a year).

### BACTERIOLOGICAL CULTURE

In addition to routine microbiological investigations, stool specimens were examined for the presence of *C difficile* using cycloserine-cefoxitin-fructose agar (Oxoid-Unipath Ltd) and incubated for 48 hours.

### *C DIFFICILE* TOXIN B TESTING

The isolates of *C difficile* were tested for the production of the cytotoxin toxin B using a 48 hour culture supernatant tested on human embryonic lung cells and cytopathic effect was identified by neutralisation with *Clostridium sordellii* antitoxin (Wellcome Ltd).

### EDTA-SOLUBLE PROTEIN PATTERNS

The EDTA-soluble protein pattern of *C difficile* was used to type the isolates.<sup>15</sup> The EDTA-soluble antigens were prepared using the method of Poxton and Byrne<sup>16</sup> and run on 12.5% gels using the PhastSystem. Bands were visualised using the manufacturers' silver stain method.

### STATISTICAL ANALYSIS

Differences between groups were tested for using the  $\chi^2$  test with Yates's correction. Significance was taken at 95%.

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*Incidence of C difficile in children with Hirschsprung's disease and controls*

|                           | Hirschsprung's disease    |        |                              |        | Controls                    |        |                              |       |
|---------------------------|---------------------------|--------|------------------------------|--------|-----------------------------|--------|------------------------------|-------|
|                           | Group I<br>(no diarrhoea) |        | Group II<br>(with diarrhoea) |        | Group III<br>(no diarrhoea) |        | Group IV<br>(with diarrhoea) |       |
|                           | CD+                       | CD-    | CD+                          | CD-    | CD+                         | CD-    | CD+                          | CD-   |
| No of episodes            | 7                         | 24     | 16                           | 28     | 4                           | 21     | 6                            | 16    |
| No of patients            | 7                         | 20     | 12                           | 25     | 4                           | 21     | 6                            | 16    |
| Mean age (months)         | 3.7                       | 29.3   | 40.5                         | 50.4   | 3.3                         | 27.3   | 7.7                          | 26.2  |
| Range                     | 2-8                       | <1-132 | <1-180                       | <1-162 | 1-7                         | <1-156 | 4-12                         | 2-156 |
| No (%) >18 months*        | 0                         |        | 7 (44)                       |        | 0                           |        | 0                            |       |
| No (%) producing toxin B† | 4 (57)                    |        | 8 (50)                       |        | 2 (50)                      |        | 2 (33)                       |       |

CD+: *C difficile* isolated; CD-: *C difficile* not isolated.

\* Number of episodes in children over 18 months old. † Production of toxin B by strains of *C difficile*.

**Results**

ISOLATION RATE OF *C DIFFICILE*

The isolation rate of *C difficile* from the four groups of children is given in the table. No significant difference was found between the four groups of children when analysed either by the number of episodes (group I: 23%; group II: 36%; group III: 16% group IV: 27%;  $p > 0.05$ ) or by the number of children (group

I: 26%; group II: 32%; group III: 16%; group IV: 27%;  $p > 0.2$ ).

Of the children with Hirschsprung's disease and diarrhoea, the isolation rate of *C difficile* was not significantly different between those with sporadic diarrhoea (six out of 18 children: 33%) and those with chronic or recurrent diarrhoea (six out of 19 children: 32%;  $p > 0.1$ ).

In the children with sporadic diarrhoea microbial enteropathogens were identified in 13 of the 18 children (adenovirus four, rotavirus one, small round structured virus one, enteropathogenic *Escherichia coli* two, enterotoxigenic *E coli* one, *Campylobacter jejuni* one, *Aeromonas hydrophila* two, and cysts of *Cryptosporidium* sp one). Other than *C difficile* and one adenovirus (see below), no enteropathogens were identified in the children with recurrent/chronic diarrhoea.

PRODUCTION OF TOXIN B

There was no significant difference in the proportion of strains of *C difficile* that produced toxin B between the four groups of children (table).

AGE RELATED CARRIAGE OF *C DIFFICILE*

The age distribution of the isolation rates of *C difficile* was examined. *C difficile* was isolated with similar frequency in all four groups of children up to 12 months of age, whereas in children over 12 months *C difficile* could be isolated only in children from group II. The pattern of isolation is shown in fig 1 and the details of the age distribution are given in the table.

EDTA-SOLUBLE ANTIGEN TYPING

Repeated isolation of *C difficile* was seen in two children with Hirschsprung's disease in group II who had recurrent episodes of diarrhoea. One child (P1) had undergone total colonic resection for long segment Hirschsprung's disease. Stool samples were obtained during four separate episodes of diarrhoea over a two year period from which *C difficile* was isolated in each case. The strains were all non-toxicogenic and had identical EDTA-soluble antigen profiles on analysis with sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (fig 2). No other enteric pathogens were isolated.

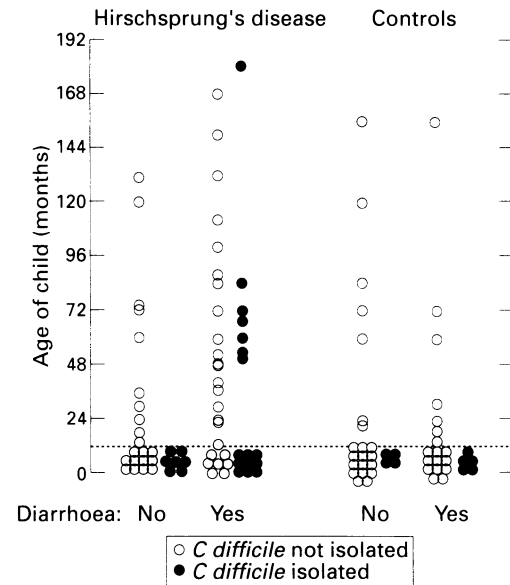


Figure 1 Age range of children from whom stool samples were cultured. Note the prolonged carriage of *C difficile* in children with Hirschsprung's disease with diarrhoea.



Figure 2 EDTA-soluble antigen profiles of *C difficile* in two children with Hirschsprung's disease with recurrent episodes of diarrhoea (P1, P2). Antigens were run through 12.5% SDS homogeneous gel and visualised using a silver stain. The first column from the left shows molecular weight markers ( $M_r=29K, 45K, 66K, \text{ and } 116K$ ); Y=yes, N=no.

The second child (P2) had four episodes of diarrhoea in which stool samples were collected. *C. difficile* was isolated during two of these episodes. Both strains were toxigenic and had identical EDTA antigen profiles (fig 2). In the other two samples adenovirus was seen on electron microscopic analysis in one and no enteric pathogen was identified in the other. A toxigenic strain of *C. difficile* was also isolated when the child was not suffering from diarrhoea (P2c; fig 2). In both children treatment with oral vancomycin resulted in the disappearance of *C. difficile* from the faeces within two weeks.

### Discussion

A significant incidence of enterocolitis both before and after pullthrough surgery has been reported in several large series.<sup>2 17 18</sup> The differentiation between enterocolitis and gastroenteritis is difficult but important if enterocolitis is to be promptly treated.

Diarrhoea of short duration (lasting less than one week) resembling gastroenteritis was associated with microbial enteropathogens whereas chronic/recurrent diarrhoea was mostly not. Although microbial enteropathogens have not been found in most studies on Hirschsprung's disease associated enterocolitis, rotavirus<sup>8</sup> and enteropathogenic *E. coli*<sup>19</sup> have been described in more recent studies. Our findings of virus identification in a high proportion of children with Hirschsprung's disease and diarrhoea suggest that microbial enteropathogens, particularly enteric viruses, are an important cause of diarrhoea in these children.

A high incidence of colonisation with *C. difficile* in children with Hirschsprung's disease was found but no significant difference existed between the isolation rate or toxigenicity of isolates of *C. difficile* from those children with or without diarrhoea. *C. difficile* is a common isolate from the stools of children. The isolation rate falls quickly to adult rates (approximately 3%) after 12 months of age.<sup>20</sup>

The isolation of *C. difficile* in children over 12 months of age could explain the apparently contradictory results of the previous studies by Thomas *et al.*<sup>6 7</sup> and Wilson-Storey *et al.*<sup>8</sup> In children up to 12 months of age no difference will exist in the incidence of colonisation with *C. difficile*, whereas prolonged carriage in children with Hirschsprung's disease and diarrhoea will occur after 12 months of age and thereby create an association between diarrhoea and *C. difficile*.

The prolonged carriage of *C. difficile* seen in the older children with Hirschsprung's disease and diarrhoea might result from repeated antibiotic treatment during hospital admissions. The staged course of treatment of Hirschsprung's disease usually involves three admissions for surgery with appropriate antibiotic prophylaxis. Previous studies have indicated that repeated exposure to antibiotics permits colonisation with *C. difficile* in adults with inflammatory bowel disease<sup>9</sup> and children with cystic fibrosis.<sup>21</sup> However, no evidence for

this phenomenon was found in a study of children up to 18 months old by Tullus *et al.*<sup>22</sup>

The two cases of repeated isolation of *C. difficile* illustrate the difficulty in ascribing a pathogenic role to this organism in children over 12 months of age. The first child (P1) had repeated isolation of *C. difficile*, which had identical EDTA-soluble antigen profiles suggesting that the child was colonised with the same strain. However, all four isolates were non-toxicogenic.

In the second child (P2) all the *C. difficile* isolates were toxigenic and disappeared after vancomycin treatment suggesting a causal role in the diarrhoea. However, toxigenic *C. difficile* was isolated again when the child was asymptomatic (P2c; fig 2) and further episodes of diarrhoea responded to vancomycin despite the fact that *C. difficile* was not isolated.

The significance of *C. difficile* in this age group remains difficult to interpret but treatment with vancomycin may still be appropriate.

In summary, although the overall incidence and toxigenicity of *C. difficile* was similar in children with Hirschsprung's disease and diarrhoea, an association was found between prolonged intestinal carriage of *C. difficile* in children with Hirschsprung's disease and diarrhoea over 12 months of age, which was not seen in children with Hirschsprung's disease without diarrhoea or in control children. Although a causal role cannot be established, these findings help to reconcile previous contradictory studies on the association of toxigenic *C. difficile* with children with Hirschsprung's disease.

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### Cryptosporidium

The first case of human cryptosporidial infection was described in 1976. *Cryptosporidium* is a protozoan parasite with a worldwide distribution, which infects birds, fish, reptiles, and other mammals as well as people. It is recognised as a common cause of acute diarrhoea and as a cause of chronic diarrhoea in the immunocompromised. Recently workers at the Queen Elizabeth Hospital for Children in London reviewed their experience over a 6.5 year period (A D Phillips and colleagues, *Gut* 1992; 33: 1057-61).

Cryptosporidial oocysts are identified in the stools using a modified Ziehl-Nielsen stain and this has been done as a routine on stool specimens at the Queen Elizabeth Hospital since 1983. Between October 1983 and April 1990 cryptosporidium was identified in the stools of 123 children and the notes of 109 of these were reviewed.

*Cryptosporidium* identifications apparently followed an annual cycle with a peak in spring and a trough in autumn. One hundred of the 109 patients presented with diarrhoea which was usually watery and offensive and occasionally the stools contained mucus or blood. Non-diarrhoeal presenting symptoms were abdominal pain (n=4), vomiting (n=4), fever (n=3), and nausea (n=1). Fifty two (58%) of 90 children for whom the duration of diarrhoea was known had diarrhoea lasting for 14 days or more. It lasted for three weeks or more in 35 and for over six months in seven. Eventual spontaneous resolution occurred in all the children. None had evidence of immunodeficiency. Chronic diarrhoea was more common in younger children and in those with mixed infections. Other pathogens detected included *Giardia lamblia*, aeromonas, enteropathogenic *Escherichia coli*, shigella, salmonella, campylobacter, rotavirus, adenovirus, calicivirus, and astrovirus.

There were 18 children who had recently been abroad, mostly on the Indian subcontinent, and 17 who led an itinerant life living usually on caravan sites. Chronic diarrhoea was more common in both of these groups.

Nine patients with chronic diarrhoea and severe failure to thrive had jejunal biopsies which showed an enteropathy in all nine. In seven the organism was seen adhering to the epithelium of the villi but not of the crypts. (In immunodeficiency it is also seen on crypt epithelium.)

The number of children who had stool specimens examined was known for only one year (1989) and in that year cryptosporidium was identified in 1.6% of cases. The authors point out that their finding that half of the children with only cryptosporidium in their stools had chronic diarrhoea (14 days or more) indicates that this may be a more important cause of chronic diarrhoea than has been realised in the past. It is uncertain whether the chronic diarrhoea is due to persisting infection or whether it is a postinfective phenomenon.