Asymptomatic neonatal colonisation by *Clostridium difficile*

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**SUMMARY** In a prospective survey of infants born in a single maternity unit, asymptomatic faecal colonisation by *Clostridium difficile* occurred in 31 (47%) of 66 babies who provided a faecal sample during week one of life and at age 14 and 28 days, and in 46 (30.7%) of the total of 150 babies for whom at least one faecal sample was obtained during the month of study. There was no evidence for acquisition of the organism from the mother during delivery and colonisation was unrelated to the means of delivery, infant sex, means of feeding, duration of hospital stay, or antibiotic treatment. New colonisation occurred throughout the month of the study and further evidence for environmental acquisition was obtained by the finding of a similar strain of *C difficile* in 7 babies from one ward together with positive environmental cultures. Colonisation was frequently transient and occasionally intermittent; most infants kept the same strain during their period of carriage. Twenty-two (47.8%) babies colonised by *C difficile* had low titres of cytopathic faecal toxin but none had symptomatic diarrhoea or features of necrotizing enterocolitis. The in vitro toxigenic potential of 57 toxigenic isolates from 36 babies was low and 12 babies carried non-toxigenic strains.

Transient colonisation by *C difficile* in early life is almost certainly more common than is generally recognised and the neonate provides an important reservoir of potential infection.

*Clostridium difficile* is now generally accepted as the major aetiological agent in most adults with antibiotic associated pseudomembranous colitis and in a variable percentage of patients with antibiotic associated diarrhoea.1 Faecal carriage of *C difficile* in healthy adults is around two per cent2 but although this may rise during antibiotic treatment,3 the presence of faecal cytotoxic toxin as detected by tissue culture assay is generally limited to those patients with diarrhoea.3,4 In neonates, however, asymptomatic faecal carriage of *C difficile*, often with positive cytotoxin, occurs in up to 40%.3,5,6 Whether this reflects a different susceptibility of the infant gut to the effects of the organism or a reduced pathogenicity of the organism itself is uncertain but it does seem that neonates provide an important reservoir for the organism. Most previous studies have reported results of single or infrequent specimens and the source of acquisition of *C difficile* and its natural history during the neonatal period are currently uncertain. The present prospective study was designed to follow all the babies born in a single maternity unit and to examine the questions of maternal or environmental acquisition together with a study of the natural history of *C difficile* in the neonatal period.

**Patients and methods**

**Patients.** All 174 women admitted in labour to a single maternity unit during an 18 day period in November 1981 were included in the study but in 13 no information was subsequently available and we therefore report the findings in the remaining 161 patients and their offspring.

Maternal rectal and vaginal swabs taken during early labour were put in Amies Charcoal Medium (Transwab, Medical Wire and Equipment, UK); specimens were maintained at 4°C and processed within 15 hours of collection. Both samples were available in 126 patients, a rectal swab only was available in 11, a vaginal swab only in two, and neither swab was available in the remaining 22 subjects.

One hundred and sixty-three babies were born to the 161 mothers; there were two sets of twins. Neonatal meconium and faecal samples were collected into dry specimen pots (Henley's Medical
Supplies, UK) on days 1, 4, 7, 14, and 28 of life where possible and were processed immediately. Outpatient samples were collected by visiting midwives and kept at 4°C where possible. All five faecal specimens were obtained from 35 babies, four specimens from 42, three from 27, two from 26, one from 20, and no faecal specimen was obtained from 13 babies. One hundred and fourteen samples were available from day 1, 99 from day 4, 101 from day 7, 100 from day 14, and 82 from day 28 giving a total of 496 faecal samples.

Wards. After delivery patients were nursed in three postnatal wards; wards 2 and 3 were joined by a common entrance while ward 4 was geographically separate but on the same floor. Although each ward was self contained and had its own nursing staff, wards 2 and 3 shared a common kitchen at the entrance to ward 3. Babies were nursed by the mother’s bed in the open ward during the day and often shared a common nursery at night. Two preterm infants were nursed in the special care unit which was self contained and on a separate floor.

Environmental swab cultures (Amies Charcoal Medium) were taken from the sluice, nursery, and ward areas of each ward at weekly intervals from the start of the study and were processed within one hour of collection.

Culture. Maternal and environmental swabs and neonatal faecal samples were plated on to a commercially available semiselective medium containing cefoxitin and cycloserine (Oxoid, UK) and were incubated anaerobically at 37°C for 48 to 72 hours in resealable jars (Becton Dickinson, UK) using gas generating kits.

All swabs and faecal samples were also incubated at 37°C in Shaedler meat broth (Oxoid, UK) containing 0-2% p-cresol (BDH Chemicals, UK) and subcultured on blood agar after 7 and 28 days incubation. Likely colonies of C difficile were subcultured on blood agar and identified by standard morphological and biochemical parameters and by gas liquid chromatography.

Cytopathic toxin assay. All toxin assays were performed using HEp2 cell monolayers (Flow Laboratories, UK) in microtitre plates (Flow Laboratories, UK), 50 μL of test suspension being added to 200 μL of medium in each well. After incubation at 37°C for 24 hours a positive cytopathic response was seen as a rounding up and detachment of cells from the monolayer, preventable by the prior addition of 50 μL of a 1 in 100 dilution of Clostridium sordelli antiserum (Wellcome Foundation, UK) to 150 μL of tissue culture medium in each test well.

Titration of the cytopathic toxin was performed using doubling dilutions from a starting dilution of 1 in 10 of test suspension in sterile phosphate buffered saline. The end point was recorded as the well showing at least 50% of cells affected and was expressed as the logarithm reciprocal of the final dilution.

Faecal supernatants
These were prepared from a 1 in 5 dilution of faeces in sterile phosphate buffered saline after centrifugation at 4000 rpm for 20 minutes and were stored at −20°C until tested in the cytopathic toxin assay. All samples were assayed within 14 days of collection.

Toxigenic potential of C difficile in vitro
This was taken as the cytopathic toxin titre (log10) of the supernatant taken after five days incubation of a pure culture in cooked meat broth (equal volumes of thioli broth (Difco, UK) and a solution containing 3% tryptone (Difco, UK) and 0-5% yeast extract (Difco, UK) in distilled water, with 1-5 g of meat granules (Lab M, UK) per 20 ml of final broth) and tested without prior storage.

Antibiotic sensitivity profiles
Antibiotic sensitivity profiles were obtained by the disc diffusion method using 6 mm impregnated discs (Mast Laboratories, UK) incorporating clindamycin (2 μg), tetracycline (25 μg), benzyl-penicillin (1 IU), cefoxilone (30 μg), erythromycin (5 μg), or neomycin (10 μg) on blood agar plates flooded with a 24 hour pure broth culture of the isolate under test. Each isolate was labelled resistant (R) if the diameter of the zone of growth inhibition was less than 10 mm. Strains were said to show intermediate resistance (I) if the zone of inhibition was between 10 and 20 mm, and to be fully sensitive (S) if the zone was greater than 20 mm.

Statistical analysis was performed using Student’s t test or Fisher’s exact test as appropriate.

Results

Maternal specimens. C difficile could not be cultured from any of the 128 maternal vaginal swabs and was isolated in only one of 137 maternal rectal swab cultures. In this patient C difficile was isolated only in broth culture after 28 days incubation. Vaginal culture was negative, and all five neonatal faecal specimens were negative on culture and in the toxin assay.

Neonatal specimens. Seventy five (15%) of the 496 neonatal faecal samples received contained either
organism (n=45) or toxin (n=4), or both (n=26) and the number of positive samples obtained on each collection day is shown in Table 1.

Forty six (30·7%) of the 150 babies from whom at least one faecal sample was obtained were positive for *C. difficile* or its cytotoxin at some stage during the study and in 22 of 46 (47·8%) faecal cytotoxin was found on one (n=15), two (n=6), or three (n=1) occasions. *C. difficile* was isolated in 45 infants but could not be identified in one further baby in whom specifically neutralisable faecal cytotoxin was present on one occasion. Faecal toxin titres were low, mean (SD) 1·43 (0·38) (range 1 to 2·2) (log10), and none of the 46 infants positive for *C. difficile* had symptomatic diarrhoea or features of necrotizing enterocolitis.

Complete faecal collections (that is, specimens from days 1, 4, 7, 14, and 28) were available in 35 babies and *C. difficile* was present at some stage in 16 (46%) of these. Fifteen (48%) of a further 31 babies with at least one faecal sample from the first week together with samples from both 14 and 28 days were found to be positive for *C. difficile* giving an overall carriage rate of 31 (47%) of 66 babies with representative faecal samples from throughout the month. Ten of these 31 babies (32%) had acquired the organism within the first week of life while a further 10 (32%) had become organism positive by age 14 days and the remaining 11 (36%) were first positive when tested at age 28 days (Fig. 1).

Faecal carriage of *C. difficile* in these 31 babies was often transient or intermittent; only four of the 10 babies who were culture positive during the first week were positive at age 14 and 28 days while two babies had apparently re-acquired *C. difficile* at 28 days having been negative at 14 days (Fig. 1). When all 46 babies who were positive for *C. difficile* are included, 16 (35%) had acquired the organism within the first week, 18 (39%) were organism positive when tested at age 14 days, and the remaining 12 (26%) were first positive at 28 days (Table 2).

There were no significant differences between infant sex or means of delivery and the overall incidence or the time *C. difficile* was first found (Table 2), and the duration of hospital stay was similar in the positive (mean (SD) 6·7 (4·3) days) and negative (5·8 (2·9) days) groups (P>0·1, NS). Nor was there any association between the maternal use of antibiotics in the month before delivery and subsequent neonatal carriage of *C. difficile*—antibiotics having been used by the mothers of three (6·5%) of the 46 positive babies compared with five (4·8%) of 104 *C. difficile* negative babies. Three infants were known to have received antibiotics during the period of study but none acquired *C
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Table 2  Relation between means of delivery, infant sex, and timing of first detection of C difficile in 150 neonates

<table>
<thead>
<tr>
<th>Means of delivery</th>
<th>Total studied</th>
<th>C difficile positive No (%)</th>
<th>Boys</th>
<th>Girls</th>
<th>Day on which toxin first found</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total No (%)</td>
<td>Positive No (%)</td>
<td>Total No (%)</td>
<td>Positive No (%)</td>
</tr>
<tr>
<td>SVD</td>
<td>96</td>
<td>29 (30)</td>
<td>58</td>
<td>18 (31)</td>
<td>38</td>
</tr>
<tr>
<td>LSCS</td>
<td>16</td>
<td>6 (37-5)</td>
<td>8</td>
<td>3 (37-5)</td>
<td>8</td>
</tr>
<tr>
<td>LF</td>
<td>5</td>
<td>2 (40)</td>
<td>3</td>
<td>2 (67)</td>
<td>2</td>
</tr>
<tr>
<td>BR</td>
<td>29</td>
<td>8 (27-5)</td>
<td>16</td>
<td>4 (25)</td>
<td>13</td>
</tr>
<tr>
<td>BR</td>
<td>4</td>
<td>1 (25)</td>
<td>2</td>
<td>1 (50)</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>46</td>
<td>87</td>
<td>28 (32)</td>
<td>63</td>
</tr>
</tbody>
</table>

SVD = spontaneous vaginal delivery; LSCS = caesarean section; KF = Keilland's forceps; LF = low forceps; BR = breech.

difficile. The method of infant feeding was unrelated to colonisation by C difficile with an overall positive rate of 21 (30-9%) of 68 breast fed infants compared with 25 (30-5%) of 82 bottle fed infants. Low titres of faecal cytopathic toxin were found in 8 (38%) of the 21 breast fed and in 14 (56%) of the 25 bottle fed babies (P>0-1, NS) but no infant had symptomatic diarrhoea or any features of necrotizing enterocolitis.

Eleven (23-9%) of the 46 positive babies first acquired C difficile during admission and the organism was present in a further 17 (37%) when first tested after leaving hospital. The remaining 18 (39-1%) babies who subsequently became organism or toxin positive had been negative when tested both during their stay and initially after discharge (Fig. 2).

The overall incidence of C difficile colonisation during the first month of life was similar for babies nursed in the various postnatal wards (Table 3), although significantly more babies in ward 3 than in ward 2 acquired the organism during admission (Table 3).

Antibiogram typing of C difficile. The isolates of C difficile cultured from the five babies who were organism positive during their stay in ward 3 all had the same unusual antibiogram when tested against a range of 6 antibiotics (resistant to clindamycin with intermediate resistance to cefuroxime) (Fig. 2) suggesting a single identity; two further babies (cases 058 and 150) who were culture positive when first tested after discharge also carried a strain of C difficile with the same antibiotic sensitivity profile (Fig. 2). One baby (case 055) had this same strain on three occasions and four babies had the same strain on two occasions during the month of follow up. A further two babies (cases 056 and 108) were colonised with this isolate initially but strains with different antibiograms were subsequently cultured at age 28 days (Fig. 2). Isolates of C difficile with the same antibiotic profile were also obtained from environmental swabs of the nursery and sluice of ward 3 on several occasions during the study period but C difficile was not cultured from environmental swabs of the other wards or the delivery suite and none of the babies from the other wards had strains of C difficile with this antibiogram. The antibiograms of the isolates cultured during admission to other wards or isolated subsequently showed considerable variation and are outlined in Fig. 2.

Twenty one (46%) of the 46 positive babies had C difficile positive cultures on more than one occasion and in 19 (41%) of these babies the C difficile antibiograms were the same on each occasion. Both the babies (cases 056 and 108) from whom an isolate with a different antibiogram was subsequently obtained had initially acquired C difficile during their stay in ward 3 (Fig. 2).

Toxigenic potential of C difficile. The toxigenic potential of 57 toxin producing isolates from 36 babies was generally low with mean (SD) 2·01 (0·34), (range 1·3 to 2·8) (log10). Non-toxigenic strains of C difficile were isolated on 14 occasions from 12 babies. In 8 babies these were the only strains found, in one baby (case 135) toxin (but not

Table 3  Incidence of colonisation by C difficile during hospital admission and after discharge of neonates nursed in various postnatal wards

<table>
<thead>
<tr>
<th>Ward no</th>
<th>Total babies</th>
<th>C difficile positives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total No (%)</td>
<td>During admission No (%)</td>
</tr>
<tr>
<td>2</td>
<td>52</td>
<td>15 (28-8)</td>
</tr>
<tr>
<td>3</td>
<td>43</td>
<td>10 (23-3)</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>19 (35-8)</td>
</tr>
<tr>
<td>SCU</td>
<td>2</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>46</td>
</tr>
</tbody>
</table>

* P<0·05 of ward 2.
organism) had been present previously, and in three babies toxigenic *C. difficile* was previously (cases 038 and 108) or subsequently (case 031) found (Fig. 2). Three additional faecal specimens contained cytopathic toxin but *C. difficile* could not be isolated on culture.

**Discussion**

The present finding of *C. difficile* in 46 (31%) of 150 asymptomatic, healthy neonates of whom 22 (48%) showed faecal cytopathic toxin confirms previous reports of a high carriage rate of both organism and
toxin in the neonatal period and also illustrates the frequently transient and occasionally intermittent nature of colonisation (Fig. 1). Single or infrequent faecal specimen analysis will underestimate the incidence of colonisation, which is likely to be even higher than the present overall finding of 47% (31 of 66) obtained when only those babies with at least one specimen from the first week plus samples at both 14 and 28 days are considered.

Sugestions that C difficile may be acquired by the neonate from the maternal birth canal during delivery, encouraged by the unconfirmed finding of 72% positive urogenital culture by Hafiz et al., are not supported by the present study which failed to find C difficile on vaginal culture of 128 mothers and found the organism in only one of 137 maternal rectal swabs, with subsequent negative cultures and toxin assays from the relevant neonate. This is further supported by the similar overall colonisation rate in babies born vaginally (30%) or by caesarean section (38%), with no increase in colonisation for those requiring instrumentation delivery with forceps (Table 2), although an increase in the incidence of faecal toxin after vaginal delivery has previously been reported.

Colonisation was unrelated to duration of hospital stay, pre- or postnatal antibiotic treatment, or means of infant feeding. The previously reported finding of an increased frequency of faecal toxin in breast fed infants has not been confirmed.

New cases of colonisation by C difficile occurred throughout the month of the study suggesting environmental acquisition, and a similar study of 59 neonates has recently reported an overall colonisation rate approaching 50% by age 28 days. The organism is widely dispersed in nature with an asymptomatic reservoir in domestic pets and in the hospital, environmental contamination and symptomatic cross infection by C difficile have been well described as has culture of the organism from the nursery environment and staff. C difficile, a spore forming anaerobe, is able to survive in adverse conditions for many months and it is perhaps surprising that in this study it was cultured from the environment of only one of the three postnatal wards.

Evidence for environmental acquisition with case clustering has previously been reported in a study of 451 newborn infants. Colonisation rates in different wards varied between two and 52% and on one occasion a potential common source of C difficile was isolated from the environment. In the present study, using the antibiotic sensitivity profile as a limited means of strain typing, isolates of C difficile with the same, unusual antibiogram (resistance to clindamycin; intermediate resistance to cephradine) were isolated from the environment of ward 3 and subsequently from 7 babies nursed on that ward—five who were organism positive during admission and two who were positive when first tested after leaving hospital—suggesting inpatient acquisition (Fig. 2). A further three babies nursed on ward 3 also became culture positive but with strains showing different antibiograms and all were acquired after leaving hospital (Fig. 2). The environmental strain of C difficile was found to predate its appearance in the neonates under study and was also isolated from the environment on several subsequent occasions up to 6 weeks after completion of the survey suggesting its persistence as a reservoir for infant colonisation. No similar pattern of organism acquisition was found in the other two postnatal wards, the organisms isolated having dissimilar or commonly occurring antibiograms, and although some infants on both wards acquired C difficile during their stay environmental cultures were negative (Fig. 2). Although significantly more babies acquired the organism during their stay on ward 3 than on ward 2, the overall incidence of colonisation during the month of follow up was similar for babies nursed on each of the three wards with a similar, though not significant, trend between wards 3 and 4 (Table 3). Such a pattern is in keeping with environmental acquisition from a wide variety of sources and although two of the infants with the 'ward 3 strain' subsequently acquired strains with different antibiograms (Fig. 2), study of the isolate antibiograms suggests that most infants keep the same strain during their period of colonisation.

None of the babies in this series had any features suggesting necrotizing enterocolitis and the role of C difficile in this condition must remain in doubt. Although Cashore et al. isolated C difficile toxin from the stools of several symptomatic neonates during a nursery epidemic of necrotizing enterocolitis, others have failed to identify C difficile cytotoxin in these patients and another study found toxin in both symptomatic and asymptomatic neonates with no correlation between toxin titre and disease. In the present study faecal cytopathic toxin titres were generally low (mean (SD) 1.43 (0.38) log₁₀) and well below the values usually found in symptomatic adults using the same assay (unpublished data). Some authors, however, have questioned the relation between toxin titre and disease severity. The role of cytotoxin (or toxin B) in the pathogenesis of disease is currently unknown and C difficile produces at least one other toxin, an enterotoxin (toxin A), which causes fluid accumulation in the rabbit ileal loop, although its role is also uncertain. Numbers of C difficile present in the asymptomatic infant gut are similar to those found in adults with...
symptomatic disease and although neonatal antibiotic associated pseudomembranous colitis has been recorded, it has been suggested that there may be an inherent insusceptibility of the neonatal mucosa to the effects of C. difficile.

Reduced pathogenicity of the colonising C. difficile has also been suggested to explain the lack of symptoms in the neonate and in the present study 12 (26%) of the 46 organism positive babies carried strains that failed to produce toxin in vitro under conditions previously shown to be optimal. These were the only strains found in 8 babies while toxigenic isolates were also found in four babies (Fig. 2). The toxigenic potential of those isolates of C. difficile producing toxin in vitro was low (mean (SD) 2.01 (0-34) log10) compared with isolates from symptomatic adults (mean greater than 3-0; personal observation) suggesting possible reduced pathogenicity. Viscoli et al have reported no difference, however, between the toxigenic potential of their isolates from neonates and symptomatic adults and as previously stated the pathogenic mechanisms of C. difficile induced disease remain unknown.

Although Holst et al found C. difficile in less than four per cent of their series of 49 neonates, the fact of a high incidence of asymptomatic neonatal colonisation by C. difficile now seems generally well established. Its relevance, however, remains uncertain. Most reports agree that after a colonisation incidence of between 40% and 90% in infants followed to one year of age the finding of faecal C. difficile falls rapidly thereafter to a frequency similar to that found in adults, possibly reflecting the change in the normal enteric flora. Pseudomembranous colitis is rare in childhood and it is possible that colonisation in infancy is desirable, perhaps inducing subsequent protection via some form of mucosal immunity, and this may explain the low incidence of childhood disease and the very variable course of the disease in adults.

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References

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31 Received 6 February 1984