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Clostridium difficile and its toxin in healthy neonates

Clostridium difficile and its associated toxin are related to pseudomembranous colitis but their presence in the stools of healthy neonates has been intriguing and demanded further study.[1] This strictly anaerobic bacillus was first described in 1935 in the stools of four out of 10 normal breast fed infants in the first 10 days of life.[2] It is rarely found in healthy adults.[3] We reported a pilot study[4] showing a 30% incidence of *C difficile* in infants aged under 1 month. This present

the stools of healthy babies in the first 28 days of life. By the end of the fourth week nearly half were colonised. There were no significant differences between breast fed and bottle fed babies; between those born by normal delivery, instrumental delivery (forceps or breech), or caesarean section; or between those born after short rupture of membranes (0-4 hours) and long rupture of the membranes (4-20 hours). There was, however, a significant clustering of positive cases in one of the postnatal wards (ward A) compared with the other two (p < 0·05 by the Fisher exact probability test for samples 1 and 2, but no significant difference for sample 3).

Comment

The clustering we found may indicate that the organism is acquired from the environment rather than from the birth canal of the mother, or that one colonised baby may easily contaminate a ward with the spore forming organisms. Samples from the hands of staff and from equipment did not yield *C difficile*. Babies and nursing staff did not move from one ward to another. Only the medical staff attended all three wards. All the babies went home between the second and 10th day of life.

In none of the stool samples was the cytotoxin of *C difficile* found. This is in contrast to the results of Viscidi *et al*, who found toxin in about one quarter of cases.[3] In our study some strains of *C difficile* did, however, produce toxin in culture medium. Of the samples taken during the fourth week of life 26 yielded *C difficile* and, of those, eight produced toxin in culture. Probably the environment plays an important part in the production of *C difficile* toxin and this may be why the administration of antibiotics enhances the toxin producing ability of the organism in vivo. Further studies are necessary to elucidate the environmental factors which encourage toxin formation in vitro and in vivo.

The isolates of *C difficile* were indistinguishable by colonial morphology, antibiotic sensitivity, and fatty acid pattern on gas liquid chromatography. It has been suggested that analysis of stool specimens by gas liquid chromatography is a useful screening test for the presence of *C difficile*.[5] We examined stool specimens after preliminary incubation in cooked meat broth and of 177 samples screened for *C difficile* by gas liquid chromatography 52 were positive but only 42 could be confirmed as containing the organism by further culture. All 10 cases not so confirmed were from bottle fed babies. Organisms giving a positive trace for isocaproic acid included *C sporogenes*, *C sordellii*, and *C bifermentans*. This finding calls into question the use of gas liquid chromatography as the sole method of screening for the presence of *C difficile* in clinical practice.

Details of C difficile isolations from 59 healthy babies sampled on three occasions after birth

Sample	No (%) of stool samples with C difficile				No (%) with toxin in culture medium			
	Ward A (n = 27)	Ward B (n = 10)	Ward C (n = 22)	Total (n = 59)	Ward A (n = 27)	Ward B (n = 10)	Ward C (n = 22)	Total (n = 59)
1 (day 1 or 2)	4 (15)	0 (0)	0 (0)	4 (7)	0 (0)	0 (0)	0 (0)	0 (0)
2 (days 5 to 7)	9 (33)	1 (10)	2 (9)	12 (20)	2 (7)	0 (0)	1 (5)	3 (5)
3 (days 21 to 28)	15 (56)	3 (30)	8 (36)	26 (44)	3 (11)	2 (20)	3 (14)	8 (14)

study clarifies the time related colonisation of normal babies with this organism.

Methods and results

Stool samples from 59 healthy babies, none of whom had received antibiotics, were taken on three occasions during the neonatal period. All faecal samples were examined for neutralisable *C difficile* toxin by the use of Hep 2 cells at an initial dilution of 1/10. All samples were inoculated into cooked meat broth supplemented with cysteine, haemin, and menadione and incubated for 48 hours at 37°C. Gas liquid chromatography was performed on ether extracts of the culture medium supernatant. Those samples showing a peak for isocaproic acid were subcultured on to *C difficile* agar containing cycloserine and cefoxitin (Oxoid), and colonies of the *C difficile* were tested for toxin production. The results were analysed according to the method of feeding, delivery, length of ruptured membranes before delivery, and the ward in which the babies were nursed until their discharge home. A limited environmental survey was performed on one of the wards. The floor of the nurseries, pieces of equipment, and bed pan macerator were sampled with moistened swabs, which were then incubated in cooked meat broth and subcultured on to *C difficile* agar. Finger impressions from staff were taken directly on to *C difficile* agar.

The results (table) showed an increase in the carrier rate of *C difficile* in

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