Incidence and Origin Of Clostridium difficile in Neonates

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The stools of 65 of 92 (71%) infants in a special care nursery yielded Clostridium difficile on culture. Ninety percent of stools collected after 6 to 35 days in the unit were positive, and 36% of these also contained toxin. When tested in vitro, 94% of the isolates produced toxin. Of 110 swabs collected from the environment of the unit, 9% were positive for C. difficile, but the stools of 12 nurses working on the unit were negative. Thirty-five vaginal swabs collected from mothers just before delivery were negative for C. difficile on culture, but 16 of their infants had C. difficile in their stools. It was concluded that there is a high carriage rate in the stools of neonates of C. difficile acquired progressively during the course of their stay in the special care unit. Infection is mainly from environmental sources rather than maternal transmission.

Recent work has shown that Clostridium difficile and its toxin are the cause of a wide range of intestinal problems from asymptomatic (carrier) existence to fatal pseudomembranous colitis (3, 11) and antibiotic-associated colitis (2). C. difficile strains are commonly found in feces of healthy neonates and infants (8, 10, 12) but are rarely isolated from those of healthy adults (4, 10). The present study attempts to establish the prevalence of C. difficile in infants and in their environment and to discover the source of the infection.

MATERIALS AND METHODS

Over a 4-month period, stool samples from 92 infants aged 1 to 35 days in the Special Care Baby Unit at Princess Mary Maternity Hospital were investigated for the presence of C. difficile and its toxin. More than one specimen was received from 53 of these; from 39, only one specimen was investigated.

The stools were cultured on a selective medium originally developed by George et al. (5) and modified to consist of Columbia agar base (Oxoid Ltd.), 5% horse blood, 500 μg of cycloserine per ml, and 16 μg of cefoxitin per ml (1). Plates were incubated at 37°C for 48 h in an anaerobic jar with a gas-generating kit (Oxoid) and examined under UV light for the characteristic golden-yellow fluorescent colonies, which were then identified by the API ZYM system as described by Al-Jumaili and Bint (1).

Fecal filtrates were prepared by suspending stool specimens in normal saline at a dilution of 1:4. The suspensions were centrifuged at 10,000 × g for 30 min at 4°C, and the supernatant was filtered through 0.22-μm membrane filters (Millipore Corp., Bedford, Mass.) before cytotoxicity testing. For toxigenicity testing of C. difficile colonies isolated from neonatal stools, Robertson cooked meat broth medium (Southern Group Laboratories, Hither Green Hospital, Lewisham, England) was inoculated with each isolate and incubated at 37°C for 4 days under anaerobic conditions with a gas generating kit. The cultures were then centrifuged at 2,500 rpm for 20 min at 4°C. The supernatant was filtered as described above and used for cytotoxicity testing. Cytotoxicity testing for C. difficile toxin was performed on confluent monolayers of HeLa cells (Flow Laboratories, Inc., Rockville, Md.), 0.1 ml of each filtrate being added to 1 ml of culture medium. C. difficile toxin was considered to be present when cytopathic complete rounding of the cells occurred after 48 h of incubation at 37°C. In each case, neutralization with Clostridium sordellii antitoxin was also carried out.

The environment of the Special Care Baby Unit was tested for the presence of C. difficile. Using the V-PAK Transport Swab System (Exogen), 110 swabs were used to sample various sites, which included chart covers, dust mops, cubicle floors, air inlets, stethoscopes, thermometers, scales, wash basins, sinks, cot doors, and incubators. Vaginal swabs collected from 35 women just before delivery were also cultured. All swabs were inoculated onto selective medium as described above.

RESULTS

Of 92 infants in the Special Care Baby Unit, 65 (71%) were found to be harboring C. difficile in their stools and 29 (45%) contained the toxin as well at some time during their stay in the unit (Table 1).

Of the 50 infants tested aged between 1 and 5 days, C. difficile was isolated from 27 (54%), and the stools of 14 of these (28%) contained toxin, whereas 36 of 42 (90%) infants aged between 6 and 35 days were found to have C. difficile in their stools, and 15 (36%) of these also the toxin. Only 4 (6%) of the isolates were nontoxicogenic (Table 1).

Of the 110 swabs taken from the environment of the Special Care Baby Unit, 10 (9%) were positive for C. difficile, the sites for which included scales, chart covers, cubicle floors, and the air inlet and air duct of incubators. The cultures of the stools of 12 nurses working in the unit were all negative for C. difficile.

Vaginal swabs collected from 35 women just before delivery failed to yield C. difficile on culture. However, stool specimens from 16 of their infants, each collected within 3 days of delivery, were culture positive, and 8 were toxin positive. C. difficile-positive stools were obtained from 10 of 22 breast-fed babies and from 4 of 10 bottle-fed babies.

DISCUSSION

C. difficile was first identified in the feces of 4 of 10 healthy infants by Hall and O'Toole (8), and Snyder (12) found it in the stools of 10 of 22 infants, all aged between 2 weeks and 1 year. It has been suggested that the organism might be passed to the infant by the mother. Hafiz et al. (7), using a reinforced clostridium medium (Oxoid) with 0.2% p-
cresol added, reported the isolation of *C. difficile* from the urogenital tracts of 72% of 108 women attending a venereal disease clinic. It has since been found that the addition of 0.2% *p*-cresol is too inhibitory in a selective medium and that a medium containing cefoxitin and cycloserine was more sensitive for the isolation and differentiation of *C. difficile* (5); hence, the latter medium was modified for the work described here. Moreover, in the present study, the vaginal swabs were uniformly negative for *C. difficile*. Furthermore, as can be seen in Table 1, the present results show that infants appear to acquire infection during the course of their hospital stay several days after birth. This leads to the conclusion that *C. difficile* is not acquired from the mother during delivery.

It seems more likely, therefore, that the infection is acquired from the environment; indeed, 9% of the environmental cultures yielded *C. difficile*. Kim et al. (9) found that 2.5% of environmental swabs in a newborn intensive care unit yielded *C. difficile* compared to 2.8% in an adult unit. These figures rose to 7.7 and 11.1%, respectively, however, if *C. difficile*-positive patients were present in the units. *C. difficile* was also isolated from hands and stools of asymptomatic hospital personnel, and after inoculation of the organism onto a floor it persisted for 5 months (9). A small outbreak of pseudomembranous colitis in two adult hospital wards in an 11-day period was attributed by Greenfield et al. (6) to cross-infection.

Toxicogenicity testing showed that 94% of the isolates from the present study were capable of producing toxin in vitro. In the study of Kim et al. (9), all of 20 isolates produced toxin in vitro, and nontoxicogenic strains appear to be unusual. *C. difficile* is not normally isolated from the stools of healthy adults; George (4) reported its incidence as about 2%. However, it could be present in small numbers in the adult bowel as part of the normal flora without being detected by culture because of inhibitory action of the selective medium. But in the case of infants without complex colonic flora, conditions could be more favorable for *C. difficile* proliferation.

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**LITERATURE CITED**