VAGINAL CARRIAGE AND NEONATAL ACQUISITION OF *CLOSTRIDIUM DIFFICILE*

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SUMMARY. The relationship between vaginal carriage and subsequent neonatal acquisition of *Clostridium difficile* was investigated. Vaginal carriage of *C. difficile* was detected in 11% of women attending the Department of Genital Medicine Clinic. *C. difficile* was isolated from the vagina in 18% of 50 mothers before delivery, and 8% after delivery; 62% of their babies had positive faecal cultures. Eight of nine of the babies whose mothers had positive cultures before delivery became colonised with *C. difficile*, while 23 of 41 babies whose mothers had negative cultures became colonised. This suggests that both the vagina and the environment may act as sources of neonatal acquisition of *C. difficile*. Broth enrichment culture proved a more sensitive method for isolating *C. difficile* from the vagina than direct plate culture and should be used in such investigations.

INTRODUCTION

The importance of *Clostridium difficile* in the pathogenesis of pseudomembranous colitis and antibiotic associated diarrhoea is well established (Bartlett *et al.*, 1978; George *et al.*, 1978; Larson *et al.*, 1978). This bacterium, however, may also be isolated from the faeces of 2–60% of asymptomatic infants and neonates (Hall and O'Toole, 1935; Holst, Helin and Mardh, 1981; Larson *et al.*, 1982; Nash *et al.*, 1982; Malamou-Ladas *et al.*, 1983). Neither the significance of this high isolation rate in neonates nor the source of the organism has yet been determined. Hafiz *et al.* (1975) reported a vaginal carriage rate of 72% in women attending the Sheffield Special Clinic and 18% in women attending a Family Planning Centre. These findings suggest the possibility that neonates may acquire *C. difficile* from the mother during birth rather than from the environment. On the other hand, Holst *et al.* (1981) and Larson *et al.* (1982) failed to isolate *C. difficile* from vaginal specimens obtained from mothers of babies who became colonised with *C. difficile*. An explanation for the discrepancy between these results might be differences in the methods used by the investigators for transport and culture of the specimens. Alternatively, other sources of *C. difficile*, such as the environment, may determine neonatal colonisation (Larson *et al.*, 1982; Malamou-Ladas *et al.*, 1983).

In view of these conflicting reports, we investigated the vaginal carriage of *C. difficile* in women attending the Department of Genital Medicine (DGM) clinic, the

vaginal carriage before and after delivery in parturient women and the occurrence of *C. difficile* in their babies' faeces, to determine the relationship between vaginal carriage and subsequent colonisation of the neonate by *C. difficile*. Two culture media, a conventional solid medium and an enrichment broth culture, were used throughout the study, to compare the recovery rates of *C. difficile*.

SUBJECTS, MATERIALS AND METHODS

Study population. Eighty-two consecutive female patients attending the DGM clinic were examined, 29 of whom were attending for the first time and 53 for a follow-up appointment. Fifty mothers attending the maternity unit, and their babies, were also studied. Forty-two babies were born by normal vaginal delivery and eight by Caesarian section.

Sampling methods. A pair of high vaginal swabs was taken from each of the 82 unselected patients attending the DGM clinic. In the maternity unit, the vagina was swabbed, with two plain cotton swabs, shortly after onset of labour. The integrity or otherwise of the 'membranes' was recorded. A further pair of swabs was obtained whenever possible at the final examination of the mother just before discharge from hospital. Two swabs were taken directly from each baby's soiled nappy on the 2nd and 4th or 5th days of life. When no faeces were available rectal swabs were obtained instead. Similar specimens were obtained from the eight babies delivered by Caesarian section and from their mothers. All swabs were placed directly into Cary Blair Transport Medium (Gibco Europe) and transported to the laboratory on the same day for processing, or refrigerated at 4°C until the following morning.

Culture methods. All vaginal and faecal swabs were treated in the same way. One of each pair of swabs was inoculated directly on to the selective medium, horse-blood agar containing cefoxitin, cycloserine and fructose (CCFA, Oxoid), and incubated in an anaerobic cabinet (N₂ 80%, H₂ 10%, CO₂ 10%) at 37°C for 7 days. Plates were examined for typical *C. difficile* colonies after incubation for 48 h and 7 days. Suspect colonies were subcultured on to blood agar to obtain a pure culture for further identification tests (see below).

The second of each pair of swabs was broken off into 20 ml of selective enrichment broth, containing proteose peptone 40 g/L, fructose 6 g/L, sodium chloride 2.0 g/L, magnesium sulphate 0.1 g/L, disodium hydrogen phosphate 5.0 g/L, potassium dihydrogen phosphate 1.0 g/L and sodium taurocholate 10 g/L. D-cycloserine and cefoxitin were added in concentrations of 500 mg/L and 16 mg/L, respectively, just before use (as Oxoid antibiotic supplement SR 96). The broths were incubated in the anaerobic cabinet at 37°C for a total of 7 days. Each broth was subcultured on to blood agar after 48 h and 7 days. Subculture plates were then treated in the same way as the direct culture plates.

Isolates were identified as *C. difficile* by their characteristic irregularly shaped colony, their distinctive odour of *p*-cresol and their typical pattern of volatile fatty acids detected by gas liquid chromatography (Holdeman, Cato and Moore, 1977).

RESULTS

Vaginal carriage of C. difficile in women attending the DGM clinic

Of 82 patients studied, nine (11%) had positive cultures; eight were positive from the enrichment medium only. Six (20.7%) of the 29 first attenders had positive cultures, and three (5.7%) of the 53 re-attenders.

Carriage by mothers and babies

The isolation rates from the vaginal swabs and the faecal specimens from the mothers and the babies are summarised in tables I and II.

TABLE I
Isolation of C. difficile from mothers and their babies

Source of <i>C. difficile</i> (n=)	Number (%) of subjects with positive cultures for <i>C. difficile</i>			
	Total	pre-delivery	post-delivery	both pre- and post delivery
Mothers (50)	11 (22)	9 (18)	4 (8)	2 (4)
Babies (50)	31 (62)	...	...	...

TABLE II
Isolation of C. difficile from vaginal (mothers) and faecal (babies) specimens

Source of specimen	Number of specimens examined	Number of specimens positive for <i>C. difficile</i>		
		Total	by direct CCFA culture	by broth enrichment only
Vagina (mothers)	95	13	2	11
Faeces (babies)	89	45	43	2

The *C. difficile* isolation rate from the vaginal swabs was 11 out of 50 (22%), and of these 11, only nine (18% of the total) were culture positive before delivery. Eight of these nine mothers delivered babies who became colonised with *C. difficile* during the first 4 days of life. Two mothers had positive cultures only after the third day post-delivery and two mothers had positive cultures both before and after delivery.

In all, 31 (62%) of 50 babies had one or more faecal cultures positive for *C. difficile* during the first 5 days of life. The isolation of *C. difficile* on different days of sampling from the babies is shown in table III. Approximately 50% of babies examined were colonised within the first 2 days of life. Eighteen babies had positive samples on two separate days. The relationship between positive and negative cultures from mothers and babies is shown in table IV. The isolation rate of *C. difficile* from babies whose mothers had a positive pre-delivery vaginal culture was 8 out of 9 (88.8%). Seven of these babies were colonised on the second day of life and one on the fourth day. Cultures were positive from two further babies whose mothers had positive cultures only after delivery. The isolation rate from babies whose mothers had negative pre-delivery vaginal cultures was 23 out of 41 (56%). Eight of these were colonised for the first time on the fourth or fifth day of life. The difference between the isolation rates in the groups of babies born to "positive" and "negative" mothers is not significant (Fisher's exact test).

The weekly isolation of *C. difficile* from mothers and their babies is shown in the figure. Babies acquired *C. difficile* throughout the period of study. Although the numbers are small, there was a trend to a greater number of positive cultures during the latter period of study.

TABLE III
Isolation of C. difficile from babies during the first 5 days of life

Day after birth	Number of babies examined	Number (%) of babies with	
		positive culture for <i>C. difficile</i>	negative culture for <i>C. difficile</i>
1	2	1 (50)	1
2	43	20 (46.5)	23
3	7	3 (42.8)	4
4	31	18 (58.1)	13
5	6	3 (50)	3

TABLE IV
Relationship between vaginal C. difficile isolation and subsequent neonatal colonisation

Result of babies' faecal cultures for <i>C. difficile</i>	Number of mothers with vaginal cultures for <i>C. difficile</i> that were	
	positive	negative
positive	8	23
negative	1	18

Eight of the 50 babies were delivered by Caesarian section and of these, four babies had positive stool cultures on the second or the fourth day of life. None of the mothers of these eight babies had a positive vaginal culture for *C. difficile*.

Comparison of direct and enrichment culture

The enrichment broth yielded increased isolations of *C. difficile* from the vaginal specimens: in nine compared with one by direct culture in the DGM clinic patients and in 13 compared with two in the mothers (table II). Only minimal increase—two additional isolations—was found in the neonatal faecal specimens.

DISCUSSION

In this investigation, the vaginal isolation rate of 11% from patients attending the DGM clinic is far lower than the isolation rate of 72% reported by Hafiz *et al.* (1975) in their "Special Clinic" patients. The possibility of cross-contamination in the latter group has not been ruled out. On the other hand, the pre-delivery carriage rate of 18% found in the mothers in our study is the same as that reported by Hafiz *et al.* (1975) in a Family Planning Clinic. These results are in marked contrast to those reported by Holst *et al.* (1981) and Larson *et al.* (1982) who found no positive cervical and high vaginal cultures in 15 and 16 mothers, respectively, whose infants were positive for *C. difficile*. An explanation for this difference may be that in both the investigation by Hafiz *et al.* (1975) and our own study a broth enrichment medium was used, whereas only solid medium was used by Larson *et al.* (1982) and Holst *et al.* (1981) did not add

sodium taurocholate to the medium. Sodium taurocholate was found to improve the recovery of clostridial spores from faeces (Raibaud *et al.*, 1974) and from environmental samples (Wilson, Kennedy and Fekety, 1982).

This study thus demonstrates the increased sensitivity of an enrichment broth for the isolation of *C. difficile*. Indeed, if we had used the solid medium (CCFA) only, the vaginal isolation rate would have fallen from 11 to two of 50 in the mothers and from nine to one of 82 in the DGM patients. When all the subjects are considered together 19 (86.4%) of the 22 vaginal *C. difficile* isolations were obtained by enrichment culture only and only three (13.6%) were positive by direct plating on CCFA (table II). Enrichment broth medium, however, seemed unnecessary in the neonatal faecal isolation of *C. difficile* for the increase was by two only (table II). This apparent difference between the vaginal and the neonates' faecal cultures of *C. difficile* may be related to the relative concentrations of the organism present in these two sites, the neonates usually yielding profuse growth, but could also be related to the presence of inhibitory factors in the vagina—such as other organisms with inhibitory effects on the

FIG.—Weekly isolation of *C. difficile* from mothers and their babies; □ total number of specimens tested, ▨ positive culture for *C. difficile* in babies and in pre-delivery vaginal specimens, ■ positive culture in post-delivery vaginal specimens.

growth of *C. difficile*, as was demonstrated during the isolation of *C. difficile* from the faecal flora both *in vivo* (Malamou-Ladas and Tabaqchali, 1982) and *in vitro* (Rolfe, Helebian and Finegold, 1981; Malamou-Ladas and Tabaqchali, 1982).

Furthermore both Holst *et al.* (1981) and Larson *et al.* (1982) examined, retrospectively post-natal vaginal or cervical specimens from the mothers whose babies had positive cultures for *C. difficile*. This may have contributed to their lack of isolation of *C. difficile*. In our study the post-delivery vaginal carriage rate was 8% compared with 18% pre-delivery; the reason for this is unclear.

The overall isolation rate of 62% found in the neonates is similar to previous reports (Hall and O'Toole, 1935; Viscidi, Willey and Bartlett, 1981; Larson *et al.*, 1982; Malamou-Ladas *et al.*, 1983). Yet there was a variation in the frequency of *C. difficile* isolation during the 5-month study period, in the neonates and in the mothers, suggestive of clustering of cases with possible cross-contamination (figure).

The results reported here are consistent with the acquisition of *C. difficile* from the mother in babies born to "positive" mothers. This is in contrast with the findings of Holst *et al.* (1981) and Larson *et al.* (1982), who stated that mothers were shown not to be the sources of their infants' organisms. There was still a considerable number of babies (56%) who acquired the organism from other sources, such as the environment; this is particularly well demonstrated in four of the eight babies delivered by Caesarian section who became colonised. Environmental surveys in maternity units and neonatal wards have produced evidence for the widespread presence of *C. difficile* on inanimate objects (Viscidi *et al.*, 1981, Larson *et al.*, 1982; Malamou-Ladas *et al.*, 1983), and also on nurses' fingers (Malamou-Ladas *et al.*, 1983).

In conclusion, our study has demonstrated that there is vaginal carriage of *C. difficile* and that the vagina is a possible but not exclusive source of neonatal faecal colonisation by *C. difficile*. Many questions, however, will remain unanswered until a comprehensive typing scheme for *C. difficile* is introduced.

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