Factors influencing the presence of faecal lactobacilli in early infancy

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Abstract

The faecal flora of 46 preterm infants and 52 born at full term was studied at 10 days of age; 46 born at full term and 37 preterm infants were also studied at 30 days. Viable counts of coliforms, lactobacilli, and bifidobacteria were made; gas liquid chromatography was used to identify the anaerobes. Lactobacilli, but not bifidobacteria, were found in high counts in the stools of most of the infants born at full term by 30 days of age. The mode of delivery, but not the method of feeding, had a significant influence on early colonisation. A selective deficiency of lactobacilli compared with coliform organisms was found in preterm infants. Previous treatment with antibiotics and being nursed in an incubator were also significantly associated with a lower rate of early colonisation with lactobacilli. Our findings indicate that lactobacilli may be an important part of the normal stool flora in early infancy, and that modern methods of neonatal care are associated with delayed or deficient colonisation.

There has recently been a resurgence of interest in the bacterial content of the stool of newborn infants, particularly that of preterm infants. It has been suggested that preterm neonates may benefit from being given lactobacilli orally,[1] though one recent study failed to show any advantage to infants so treated.[2] Other workers have used antibiotics given orally to adults in an attempt to induce a favourable bowel flora and thus promote 'colonisation resistance'.[3]

Although studies have been carried out in many parts of the world, the normal pattern of bacterial colonisation of the stool may be more complex than previously reported.[4] In particular, earlier studies almost unanimously reported an overwhelming predominance of bifidobacteria in the stool of infants born at full term who were breast fed,[5–7] but two recent studies using the more specific technique of gas liquid chromatography for anaerobe identification, indicated that neither bifidobacteria nor lactobacilli were predominant in most normal infants born at full term, whether they were breast fed or bottle fed.[8 9]

Before the possibility of influencing the bowel flora of preterm infants is seriously considered, it is advisable to determine—using modern techniques of identification of organisms—whether a characteristic pattern of colonisation with lactobacilli and bifidobacteria can be identified in infants born at full term, and in preterm infants.

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Subjects and methods

INFANTS BORN AT FULL TERM

Infants were recruited into the study within the first few days of life after informed parental consent had been obtained. In order to investigate the effect of mode of delivery and method of feeding, the study was designed to recruit roughly equal numbers of babies in the following four categories: vaginal delivery and breast fed, vaginal delivery and bottle fed, caesarean section and breast fed, and caesarean section and bottle fed.

The infants were either exclusively breast fed or bottle fed on cows' milk formula. Infants were excluded if they were treated with antibiotics or, in the case of infants who were breast fed, their mothers received antibiotic treatment. Of the 52 babies studied at 10 days, seven were excluded at 30 days: three had changed from breast to bottle feeding, two did not pass stools between 28 and 32 days, one had been prescribed antibiotics, and the mother of one infant being breast fed had been taking antibiotics. Forty five infants were studied at both 10 and 30 days of age. One infant was studied at 30 days only because no stool was passed between 8 and 12 days. None of the babies studied had been admitted to the special care baby unit. Clinical details are shown in table 1.

PRETERM INFANTS

All infants were born at 33 weeks' gestation or less, and all were in the special care baby unit when specimens were collected. None of the infants had received any antibiotics for at least 48 hours before collection. All infants were receiving enteral feeds, either expressed breast milk, pooled pasteurised breast milk, artificial milk, or a combination of breast and artificial milk. Infants were described as being nursed in a cot if they had been in open cots for at least 48 hours. Twenty infants were studied at 10 and 30 days. Clinical details of the preterm infants are shown in table 2.

Preliminary studies indicated that the faecal

Table 1 Clinical details of infants born at full term

	At 10 days of age (n=52)	*At 30 days of age (n=46)*
Median (range) birth weight (g)	3240 (2300–4320)	3350 (2300–4320)
Median (range) gestational age (weeks)	40 (38–42)	40 (38–42)
Born by vaginal delivery:		
Breast fed	15	13
Formula fed	12	12
Born by caesarean section:		
Breast fed	15	11
Formula fed	10	10

Table 2 Clinical details of preterm infants

	At 10 days of age (n=42)	At 30 days of age (n=37)
Median (range) birth weight (g)	1440 (620–2510)	1200 (620–2440)
Median (range) gestational age (weeks)	32 (25–33)	30 (25–33)
Vaginal delivery	18	18
Caesarean section	24	19
No (%) treated with antibiotics	30 (71)	32 (86)
No (%) nursed in incubator	36 (86)	20 (54)

flora is well established after the first week of life, and significant changes are unusual after 1 month of age. We decided, therefore, to collect our samples at 10 days (range 8–12) and at 30 days (range 28–32).

SAMPLE COLLECTION

A written explanation of how to collect the specimen was given to the parents of the infants born at full term. Approximately 1 g of stool was collected as soon as possible after voiding and immediately placed in a bottle of transport medium. This was then put in a domestic refrigerator or cool environment until processing.

Transport medium

Several criteria had to be met for the successful transport of infant faecal samples. Firstly, the medium had to be sufficiently viscous to prevent spillage when the bottle was opened, as it was weighed before collection of the sample and weighed again when the sample had been added. Secondly, it had to be sufficiently fluid to allow thorough mixing of the sample with the medium. Thirdly, the medium had to be clear so that the mothers could see how big a sample they had collected. Finally, the medium had to protect the bacteria being studied and keep them viable without proliferation between collection and processing. The medium selected was a buffered salt solution—Cary-Blair medium (Oxoid)—with only half the normal amount of agar. This allowed the sample to be kept for up to seven days at 4°C with little alteration to the components of the flora being studied. In practice samples were plated within three days of collection.

ISOLATION AND ENUMERATION OF BACTERIA

Coliforms were cultured using McConkey's agar (Oxoid No 3 CM115); for lactobacilli, acetate agar was used[10] and for bifidobacteria we developed a medium based on that of deMan *et al*[11] (without the acetate, as this interferes with the subsequent gas liquid chromatography), but with the addition of cysteine (0·05%); raffinose, fructose, and galactose (0·05% each); 70 μg/ml neomycin, and 0·05 μg/ml rifampicin. Serial dilutions of the above media were made in sterile 1/4 strength Ringer's salt solution, 1 ml of the appropriate dilution being incorporated in pour plates of the media. The viable count was expressed as the $\log_{10}$ of colony forming units/g wet weight of faeces. Other methods of differentiation of lactobacilli and bifidobacteria used included analytical profile identification strips, and gas liquid chromatography of bacterial metabolites.[12]

Previous work using known strains had established that there was a clear difference between the amount of acetic acid produced by lactobacilli and that produced by bifidobacteria.[12]

The methods allowed bacteria to be identified in counts of at least 10^3 g for coliform organisms, 10^4 g for lactobacilli, and 10^7 g for bifidobacteria.

STATISTICAL METHODS

The χ^2 test was used to assess the significance of differences between the numbers of infants colonised, and the Mann-Whitney U test was used to compare bacterial counts between groups. For analysis of smaller numbers Fisher's exact test was used.

The study received the approval of the joint ethics subcommittee of the Southampton and South West Area Health Authority, and the University of Southampton.

Results

INFANTS BORN AT FULL TERM

Tables 3 and 4 show the number of infants colonised and the median counts for the three organisms at 10 and 30 days, respectively, for mode of delivery and type of feeding.

Coliform organisms were present in most infants at both 10 and 30 days of age, and bifidobacteria were detected in a few, lactobacilli were present in most of the infants by 30 days of age. Colonisation with lactobacilli at 10 days was significantly associated with delivery by the vaginal route ($p=<0·03$), but there was no significant difference in the median counts of lactobacilli. Mode of delivery did not seem to have an appreciable influence on bacterial counts or on colonisation at 30 days. There was no significant difference between median counts or the proportion of infants colonised when breast and bottle fed infants were compared at either 10 or 30 days of age. Fifteen of the formula fed infants were receiving SMA

Table 3 Colonisation at 10 days in infants born at full term

	Coliforms		Lactobacilli		Bifidobacteria	
	No (%) colonised	Median $\log_{10}$ count/g wet weight of faeces	No (%) colonised	Median $\log_{10}$ count/g wet weight of faeces	No (%) colonised	Median $\log_{10}$ count/g wet weight of faeces
All infants born at full term (n=52)	46 (88)	8·82	23 (44)	8·56	11 (21)	10·09
Breast fed (n=30)	26 (87)	8·72	13 (43)	7·89	8 (27)	10·24
Bottle fed (n=22)	20 (91)	8·92	10 (45)	8·69	3 (14)	10·01
Vaginal delivery (n=27)	24 (89)	8·85	16 (59)*	8·56	8 (30)	10·00
Caesarean section (n=25)	22 (88)	8·80	7 (28)*	8·19	3 (12)	10·39

*$p<0·03$. Only positive samples were used to calculate the median values.

Table 4 Colonisation at 30 days in infants born at full term

	Coliforms		*Lactobacilli*		*Bifidobacteria*	
	No (%) colonised	*Median log_{10} count/g wet weight of faeces*	*No (%) colonised*	*Median log_{10} count/g wet weight of faeces*	*No (%) colonised*	*Median log_{10} count/g wet weight of faeces*
All infants born at full term (n=46)	46 (100)	8·70	29 (63)	8·95	6 (13)	9·71
Breast fed (n=24)	24 (100)	8·65	14 (58)	8·77	5 (21)	9·48
Bottle fed (n=22)	22 (100)	8·73	15 (68)	9·11	1 (5)	10·30
Vaginal delivery (n=25)	25 (100)	8·55	17 (68)	9·05	4 (16)	9·40
Caesarean section (n=21)	21 (100)	9·04	12 (57)	8·21	2 (10)	10·12

Only positive samples were used to calculate the median values.

(Wyeth) at both 10 and 30 days; of the remainder, two were receiving Aptamil (Milupa), two were receiving Osterfeed (Farley), and three were receiving Cow and Gate Premium. In comparing the infants fed with SMA and those fed on other formula feeds, no difference in the pattern of colonisation was found at either 10 or 30 days of age.

COMPARISON OF INFANTS BORN AT FULL TERM AND PRETERM INFANTS

In comparing the two groups, at 10 days the prevalence of colonisation was significantly lower in the preterm group for both coliform organisms ($p<0·01$) and lactobacilli ($p<0·005$), but once again no difference in median counts was seen among those infants who were colonised. At 30 days, although almost all the infants were colonised with coliforms, there was still a highly significant difference in the rates of colonisation with lactobacilli ($p<0·001$).

PRETERM INFANTS

Tables 5 and 6 show the results for the preterm infants studied at 10 and 30 days of age, respectively, for mode of delivery, environment in which they were nursed, and whether they had been treated with antibiotics.

Although there was no significant difference in either prevalence of colonisation or median bacterial counts when modes of delivery were compared, the trends were similar to those seen among infants born at full term.

Of the six infants nursed in open cots at 10 days of age, two had been in cots for two days, one for four days, two for six days, and one for 10 days. Of the 17 infants nursed in open cots at 30 days of age, 15 had been in cots for seven days or more. There was a highly significant difference at 10 days in the prevalence of colonisation by lactobacilli of infants who were nursed in incubators compared with those in open cots ($p<0·001$). There was no difference in colonisation by coliform organisms, and no difference was found at 30 days. At 10 days those infants who had previously been treated with parenteral antibiotics had significantly reduced colonisation rates of lactobacilli ($p<0·01$). No difference in colonisation with

Table 5 Colonisation at 10 days in preterm infants

	Coliforms		*Lactobacilli*		*Bifidobacteria*	
	No (%) colonised	*Median log_{10} count/g wet weight of faeces*	*No (%) colonised*	*Median log_{10} count/g wet weight of faeces*	*No (%) colonised*	*Median log_{10} count/g wet weight of faeces*
All preterm infants (n=42)	26 (62)	8·45	6 (14)	7·72	3 (7)	8·98
Vaginal delivery (n=18)	14 (78)	7·55	5 (28)	7·84	3 (17)	8·98
Caesarean section (n=24)	12 (50)	8·58	1 (4)	7·60	0	0
Treated with antibiotics (n=30)	19 (63)	8·65	2 (7)*	8·22	1 (3)	9·48
Not treated with antibiotics (n=12)	7 (58)	6·80	4 (33)*	7·72	2 (17)	8·90
Nursed in incubator (n=36)	22 (61)	8·50	3 (8)**	7·60	1 (3)	8·83
Nursed in cot (n=6)	4 (67)	7·50	3 (50)**	7·84	2 (33)	9·23

*p=<0·01; **p=<0·001. Only positive samples were used to calculate the median values.

Table 6 Colonisation at 30 days in preterm infants

	Coliforms		*Lactobacilli*		*Bifidobacteria*	
	No (%) colonised	*Median log_{10} count/g wet weight of faeces*	*No (%) colonised*	*Median log_{10} count/g wet weight of faeces*	*No (%) colonised*	*Median log_{10} count/g wet weight of faeces*
All preterm infants (n=37)	35 (95)	8·90	7 (19)	7·18	4 (11)	9·18
Vaginal delivery (n=18)	17 (94)	8·90	3 (17)	8·01	1 (6)	8·95
Caesarean section (n=19)	18 (95)	8·88	4 (21)	6·89	3 (16)	9·41
Treated with antibiotics (n=32)	30 (94)	8·82	7 (22)	7·18	4 (12)	9·18
Not treated with antibiotics (n=5)	5 (100)	9·39	0	0	0	0
Nursed in incubator (n=20)	18 (90)	8·82	3 (15)	6·84	2 (10)	8·97
Nursed in cot (n=17)	17 (100)	8·91	4 (24)	7·59	2 (12)	9·18

Only positive samples were used to calculate the median values.

either coliforms or lactobacilli was found at 30 days.

Of the 36 infants who were nursed in incubators at 10 days of age, 27 had previously received treatment with antibiotics, as had three of the six infants nursed in open cots at 10 days. Because of the small number of infants who had not received treatment with antibiotics it was not possible to distinguish whether the place they were nursed or the antibiotics exerted the greater influence on early bacterial colonisation.

At 10 days, 23 of the preterm infants received expressed breast milk (a mixture of maternal and donor breast milk) only, five received a mixture of expressed breast milk and formula feed, and 14 received formula feed only. No difference in the pattern of bacterial colonisation was found among these three groups of infants, and no difference was found when the types of formula feed were compared. At 30 days of age nine infants received expressed breast milk from their own mothers, 11 received a mixture of expressed breast milk and formula feed, and 17 were fed with formula feed alone. Again no differences in the pattern of bacterial colonisation were found when types of feeding were compared.

Discussion

The results indicated that in most of these babies bifidobacteria were not usually found in the faeces. When they were detected, however, they were present in large numbers. Our findings are at variance with those of many previous studies,[5–7 13] and though it is possible that some of the earlier reports may have failed to discriminate between lactobacilli and bifidobacteria because of less precise microbiological identification methods, this is not likely to have been the case in those more recent studies that used gas liquid chromatography for the identification of bacteria.[14 15]

In addition, we were unable to confirm an inverse relationship between counts of bifidobacteria and lactobacilli, and coliform organisms, in contrast to some previous reports.[16 17] In view of the high counts of coliforms that we found, differences in methods are unlikely to account for this disparity and it is possible that there has been a change in the nature of early faecal colonisation in infants born in industrialised countries, an explanation that is supported by the findings of at least two recent studies.[8 9] There could be many reasons for such a change, ranging from differences in maternal dietary habits to changing obstetric practices, particularly regarding the use of vaginal antiseptics at the time of birth. Only one of the breast feeding mothers in our study used a nipple antiseptic preparation and this practice is unlikely, therefore, to have been an important factor in any ecological changes in colonisation with bifidobacteria.

The use of gas liquid chromatography for identification of bacteria allows us to state with confidence that lactobacilli were present in appreciable numbers in most of the infants born at full term by 30 days of age. In contrast to the many previous studies that have shown an association between colonisation with bifidobacteria and breast feeding, our findings indicate that the method of feeding was not related to colonisation with lactobacilli.

In our study there was, however, a significant difference when mode of delivery was considered, infants born at full term by the vaginal route being more likely to acquire lactobacilli by 10 days of age. It is possible, therefore, that an important contribution to the lactobacillary flora is acquired from the birth canal. No such relationship was found at 30 days, however, and it is likely that lactobacilli are also acquired from other sources during the first few weeks of life.

Although one study about the administration of lactobacilli to preterm infants has been published,[2] and at least one other group in the United States is actively concerned in preparing such a trial,[1] there have been few published reports before this to suggest that lactobacilli form an important part of the faecal flora of newborn infants. These findings, and those of other workers, indicate that factors such as prematurity, birth by caesarean section, parenteral antibiotic treatment, and being nursed in an incubator, may deprive infants of colonisation with lactobacilli during the first few days, and even weeks, of life.[15] It is not yet clear whether this deficiency is to the detriment of the infants.

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