Faecal colonization with P-fimbriated *Escherichia coli* between 0 and 18 months of age

By K. TULLUS,

Department of Pediatrics, Danderyd Hospital, S-182 88 Danderyd, Sweden

GUNILLA KÄLLENIUS AND R. MÖLLBY

Department of Bacteriology, National Bacteriology Laboratory, Stockholm, Sweden

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SUMMARY

A prospective study of faecal colonization with P-fimbriated *Escherichia coli* between 0 and 18 months of age was conducted in 751 healthy infants. The influence of breast-feeding and treatment with antibiotics on this colonization was studied. Colonization with P-fimbriated *E. coli* increased with age from 10% at 6 days to 30% at 18 months of age ($P < 0.01$). Breast-feeding influenced colonization at 6 weeks of age when breast-fed children harboured fewer bacterial species ($P < 0.001$) and fewer P-fimbriated *E. coli* ($P = 0.06$) than bottle-fed infants. Treatment with antibiotics increased the colonization rate with P-fimbriated *E. coli* at the age of 11 months ($P < 0.05$). However, this was not true for treatment with ampicillin, which increased colonization rate with Gram-negative species other than *E. coli* ($P < 0.05$). Fifty per cent (378) of all children were colonized and a quarter (183) had pure cultures of P-fimbriated *E. coli* in at least one faecal sample. The clinical importance of this colonization remains to be shown.

INTRODUCTION

Gram-negative infections among infants, cases of pyelonephritis as well as cases of septicaemia, usually develop with bacteria derived from the infected child's faecal flora (Vosti *et al.* 1964; Bennet, 1987). The infecting strain has in most cases been shown to be the dominating enterobacterial faecal strain (Källenius *et al.* 1981). Pyelonephritis in infancy is in close to 90% of the cases caused by *Escherichia coli* (Winberg *et al.* 1974) and 90% of these *E. coli* strains are P-fimbriated (Källenius *et al.* 1981). Gram-negative septicaemia or meningitis in small children is mostly caused by *E. coli* or *Klebsiella* sp. (Freedman *et al.* 1981).

It is well known that treatment with antibiotics can cause short-term changes of the faecal flora that normalizes within weeks after stopping the treatment (Bennet *et al.* 1986). The feeding habits of a child has also been shown to influence the faecal flora. It has been proposed that breast-fed children are colonized with possibly less virulent *E. coli* strains than bottle-fed infants (Gothefors, Olling & Winberg, 1975).

The aim of the present study was to follow the dominant aerobic faecal flora and especially colonization with P-fimbriated *E. coli* in children between the age of 6 days and 18 months. We also considered whether treatment with antibiotics or diarrhoea had any long term effects on the colonization with these possibly virulent strains and if breast or bottle feeding influenced this colonization.

MATERIALS AND METHODS

All children born at Danderyd Hospital during 1 year that belonged to two of the communities in the hospital's catchment area were followed and faecal samples obtained at the ages of 6 days, 6 weeks, 6 months and 11 months. From children born during the first half year of the study period a faecal sample was also obtained at the age of 18 months.

The faecal samples were taken at discharge from the hospital and at regular visits to the well-baby clinics. Parents at each well-baby clinic visit were interviewed by the staff about the feeding habits of the child, if the child had been treated with antibiotics and if the child had had any diarrhoea. These data were registered and sent to the laboratory together with the 11-month faecal sample.

Bacteriological methods

The faecal samples were taken by a cotton swab and transported in Stuart Medium (Gästrin, Kallings & Marcetic, 1968). The samples were cultured overnight on CLED agar. Six different bacterial colonies were selected using a method that gives a 99% possibility that at least one of the colonies is of the dominant aerobic strain (Carlsson *et al.* 1976). The bacteria were identified by colony appearance, and Voges-Proskauer (VP) and indole test reactions (Lennette, 1980). All colonies that were VP-negative and indole-positive were regarded as *E. coli*. After identification all *E. coli* strains were spread on colonization factor antigen (CFA) agar and tested for P-fimbriation with the PPA-test (Svenson *et al.* 1982).

Statistical methods

Chi-square test and Spearman's rank correlation test were used for the statistical analyses. A personal computer ABC 800 was used for the statistical analyses with help of the statistical programs MULREG 800 and IDA 800.

RESULTS

Seven hundred and fifty-one infants were included in the study. Three hundred and seventy-eight (50.3%) of these infants were colonized with a P-fimbriated *E. coli* strain in at least one faecal sample. Among 183 (24.4%) of the children this strain was found in pure culture, i.e. all six tested colonies were P-fimbriated.

Colonization with *E. coli*, P-fimbriated or not, increased with the age of the children. At 6 days 10.0% of the children were colonized with a P-fimbriated strain, while at 18 months 29.7% had such a strain in the faeces ($R_s = 1$, $P < 0.01$) (Fig. 1). The proportion of *E. coli* strains that expressed P-fimbriae increased from 18.3% at 6 days to 34.8% at 18 months. ($R_s = 1$, $P < 0.01$) (Fig. 1). A similar picture was found when only pure cultures were considered (Fig. 2).

Fig. 1. Occurrence of Gram-negative bacteria in relation to age of the infants. ○—○ denotes *E. coli*, △—△ other Gram-negative bacteria than *E. coli* and □—□ P-fimbriated *E. coli*.

Fig. 2. Pure culture of aerobic bacteria in relation to age of the infants. ○—○, denotes *E. coli*; △—△, other Gram-negative bacteria than *E. coli*; □—□, P-fimbriated *E. coli*; ●—●, Gram-positive cocci.

Colonization with P-fimbriated *E. coli* at 6 days of age significantly increased the risk of being colonized with P-fimbriated *E. coli* up to the age of 11 months (Table 1) ($P < 0.001$).

Breast-feeding was shown to influence colonization at 6 weeks. The children that were not breast-fed at this age were more often colonized with several bacterial species than were breast-fed children ($P < 0.001$) (Table 2) with increased colonization with both *E. coli* and other Gram-negative species. There was also a trend for *E. coli* strains found amongst bottle-fed infants more often to express P-fimbriae ($P = 0.06$) (Table 3). After this age breast-feeding could not be shown to influence faecal colonization with P-fimbriated *E. coli*.

Three hundred and twenty-seven (45.0%) of the 726 children for whom

Table 1. *Presence of P-fimbriated E. coli at 6 days of age in relation to the presence of P-fimbriated E. coli at later ages*

Children with P ⁺ <i>E. coli</i> at age	P ⁺ <i>E. coli</i> at 6 days of age (n = 73)	P ⁻ <i>E. coli</i> at 6 days of age (n = 658)
6 weeks	41 (56%)*	66 (10%)
6 months	32 (44%)*	136 (21%)
11 months	35 (48%)*	153 (23%)
18 months	12 (39%†)**	84 (29%†)

* $P < 0.001$; ** $P > 0.05$.

† Percentage calculated from the total numbers 31 and 290 respectively.

Table 2. *Breast feeding in relation to pure culture at 6 weeks of age*

Breast feeding	Total no. of children	Pure culture	Several bacterial species
Fully breast fed	612	496 (81%)	116 (19%)
Partly breast fed	74	43 (58%)	31 (42%)
Not breast fed	52	25 (48%)	27 (52%)

* $P < 0.001$.Table 3. *Breast feeding in relation to P-fimbriated E. coli at 6 weeks of age among children with E. coli*

Breast feeding	Total no. of children	P ⁺ <i>E. coli</i>	P ⁻ <i>E. coli</i>
Fully breast fed	384	81 (21%)	303 (79%)
Partly breast fed	59	12 (20%)	47 (80%)
Not breast fed	47	17 (36%)	30 (64%)

* $P = 0.06$.

complete data on antibiotic treatment was available received antibiotics at least once during the first 11 months of life. In Fig. 3 the relative proportions of different antibiotics are shown. Penicillin V, ampicillin, trimethoprim-sulfamethoxazole and erythromycin were most commonly used.

Treatment with antibiotics was shown to increase colonization with P-fimbriated *E. coli* at 11 months of age ($P < 0.05$) (Table 4). Ampicillin was the only antibiotic that had any other long term effect on the aerobic flora and was shown to increase the colonization with Gram-negative strains other than *E. coli* ($P < 0.05$) (Table 5). Before the age of 11 months treatment with antibiotics could not be shown to influence the colonization of the children.

Diarrhoea as reported by the parents had no influence on the faecal colonization with P-fimbriated *E. coli*.

Fig. 3. Treatment with antibiotics at the different ages among the 726 studied infants.

Table 4. *Treatment with antibiotics in relation to P-fimbriated E. coli at the age of 11 months*

Treatment	Number	P ⁺ <i>E. coli</i>	P ⁻ <i>E. coli</i>
Treated with antibiotics	218	81 (37%)*	137 (63%)
Not treated with antibiotics	398	112 (28%)	286 (72%)

* $P < 0.05$.

Table 5. *Treatment with ampicillin in relation to the occurrence of Gram-negative rods at the age of 11 months*

Treatment	Total no. of children	<i>E. coli</i>	Other Gram-negatives than <i>E. coli</i>
Ampicillin	66	50 (76%)*	36 (55%)*
Other antibiotics	160	141 (88%)	64 (40%)
No antibiotics	465	398 (86%)	181 (39%)

* $P < 0.05$; children treated with ampicillin in relation to the other children.

DISCUSSION

We found that faecal colonization with P-fimbriated strains increased with age during the first 18 months ($R_s = 1$, $P < 0.01$) and at the age of 18 months 29.7% of the children harboured such a strain in their faeces. This figure is surprisingly

high compared to previous figures found among children and adults where between 7 and 17% were colonized with such a strain (Källénius *et al.* 1981; Jacobson *et al.* 1985; Tullus *et al.* 1987). This high incidence of P-fimbriated strains at 18 months was partly an effect of increasing numbers of children being colonized with *E. coli* but the proportion of *E. coli* strains exhibiting P-fimbriae also increased with age ($P < 0.01$).

We have previously found that the first *E. coli* strain that colonizes a neonate has a tendency to become resident (Kühn, Tullus & Möllby, 1986). In this study children who at 6 days were colonized with a P-fimbriated strain had a strong tendency to continue to be colonized with P-fimbriated strains at 11 months.

Feeding habits but not antibiotic treatment or diarrhoea influenced the dominant aerobic faecal flora at the age of 6 weeks. Breast-fed children were more often colonized with fewer bacterial strains than were bottle-fed infants. This mixed flora was the reason why bottle-fed infants more often were colonized with other Gram-negative species than *E. coli* as previously has been found by other authors (Ørskov & Biering-Sørensen, 1975). Gothefors, Olling & Winberg, (1975) have previously suggested that breast-fed infants are colonized with possibly less virulent *E. coli* strains than bottle-fed infants and in our study there were also a statistically non-significant ($P = 0.06$) trend for bottle-fed infants more often to harbour P-fimbriated strains in their stools. It has been proposed that this inhibitory effect of breast milk could in part be due to prevention of bacterial attachment to epithelial surfaces by IgA antibodies in the milk (Gothefors, Olling & Winberg, 1975).

Treatment with antibiotics could only be shown to influence the faecal flora at the age of 11 months. Most changes in the faecal flora due to antibiotic treatment have been shown to be of short duration and our study design only discovered long term changes in the faecal flora. Treatment with antibiotics has been shown to increase the colonization with enterobacteria other than *E. coli* (Graham, Taylor & Davies, 1976; Goldmann, Leclair & Macone, 1978). Fairly soon after the cessation of antibiotic treatment the same normal flora is re-established (Bennet *et al.* 1986).

In our study ampicillin-treated children were less often colonized with *E. coli*, P-fimbriated or not, and they were more often colonized with other Gram-negative species. As most cases of extra-intestinal bacterial infections are caused by P-fimbriated *E. coli* strains this effect, previously regarded as unfavourable, might well be beneficial in respect to the development of such infections. Children treated with other antibiotics were, however, significantly more often colonized with P-fimbriated strains.

In our study 50 per cent of the infants were shown to be faecally colonized with P-fimbriated *E. coli* strains on one or more sampling occasions. The importance of this colonization for the later development of disease remains to be shown. Further characterization of the P-fimbriated strains is needed for a more accurate evaluation of which P-fimbriated strains are really virulent (Tullus *et al.* 1987).

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