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ARTICLES

Escherichia coli in Infants' Intestinal Microflora: Colonization Rate, Strain Turnover, and Virulence Gene Carriage

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ABSTRACT

Colonization by *Escherichia. coli* in infants might have decreased in the last decades, owing to changes in hospital routines and family lifestyle. In this study, the *E. coli* flora was characterized in 70 healthy Swedish infants followed for the first year of life. *E. coli* was isolated from rectal swabs obtained at 3 d of age and quantified in fecal samples collected at 1, 2, 4, and 8 wk of age and at 6 and 12 mo of age. Strains were typed using random amplified polymorphic DNA, and their virulence factor genes were identified by multiplex PCR. Colonization by *E. coli* occurred late; only 61% of the infants were positive by 2 mo of age. The turnover of individual strains in the microflora was slow (1.5 strains per infant during 6 mo, 2.1 during 1 y). Environmental factors, such as siblings, pets, or feeding mode, did not influence colonization kinetics or strain turnover rate. Genes encoding type 1 fimbriae, P fimbriae, and hemolysin were significantly more common in *E. coli* strains persisting for at least 3 wk in the microflora than in transient strains. The P-fimbrial class III adhesin gene was more common in *E. coli* from children who had a cat in their homes than in *E. coli* from children without pets ($p = 0.01$); this adhesin type is common in *E. coli* from cats. The late colonization and low *E. coli* strain turnover rate suggest limited exposure of Swedish infants to *E. coli*. Our results confirm that P fimbriae and other virulence factors facilitate persistence of *E. coli* in the human colonic microflora. **(*Pediatr Res* 54: 8–14, 2003)**

Abbreviations

CFU, colony-forming units
RAPD, random amplified polymorphic DNA

Escherichia coli is one of the first bacterial species to colonize the infant's intestines. In the 1970s, *E. coli* usually appeared in the baby's feces a few days after birth (1, 2), as a sign of its establishment in the intestinal microflora (3, 4). *E. coli* colonizing the newborn infant may originate in the maternal fecal flora (5), but *E. coli* strains are also commonly spread at maternity wards via the nursing staff, especially during periods of high bed-occupancy and staff workload (6). We have recently reported that *Staphylococcus aureus* has become a major colonizer of the infant gut (7), which may be a sign of reduced competition from other microbes. *E. coli* and other fecal bacteria might be less easily spread today, because of increased hygiene in hospitals and families.

Some *E. coli* strains persist in the intestinal microflora of an individual for months or years (resident strains), whereas others (transient strains) disappear within a few weeks (8). Resident *E. coli* strains display certain characteristics that enable them to persist in the intestinal microflora, *e.g.* the expression of P fimbriae and capacity to adhere to colonic epithelial cells (9–12). P fimbriae are composed of a fimbrial rod with a tip adhesin that exists in three varieties, termed papG classes I, II, and III. These recognize the Galα1→4Galβ disaccharide, with slight differences in binding specificity (13). The class II variety of the papG adhesin is common among *E. coli* causing pyelonephritis (14), whereas the class III variety is common in cystitis strains from humans (15), dogs (16), and cats (17). Intestinal persistence of *E. coli* has been linked to the class II variety of the adhesin (10). *E. coli* strains resident in the human

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colonic microflora also more commonly possess genes for other virulence factors, such as the iron-chelating compound aerobactin (9, 10), and the capsular types K1 and K5, compared with colonic transient strains (10).

The present study was designed to investigate the *E. coli* colonization pattern during the first year of life in Swedish infants born in the late 1990s, in relation to delivery mode and lifestyle factors, such as feeding pattern, family size, and pet ownership. Individual *E. coli* strains were identified, and their possession of genes for adhesins and other virulence factors were related to persistence in the intestinal microflora.

METHODS

Subjects. Seventy infants (35 girls and 35 boys) born in 1998–1999 at the Sahlgrenska University Hospital, Sweden, were included. They were part of a prospective birth-cohort study aiming to examine the relation between intestinal colonization pattern and allergy development, and 60 of 70 infants had at least one allergic parent. Information was obtained about siblings and pets, and the parents recorded the baby's feeding pattern. The records were checked by a study nurse who interviewed the parents by telephone at 6 and 12 mo. Informed consent was obtained, and the study was approved by the Medical Ethics Committee of Göteborg University.

Nine infants were delivered by cesarean section owing to signs of fetal asphyxia ($n = 3$), maternal infection ($n = 1$), preeclampsia ($n = 1$), myoma ($n = 2$), pelvic contraction ($n = 1$), or psychological reasons ($n = 1$). Most infants roomed in with their mothers at the maternity ward directly after delivery and left the hospital after an average of 2.5 d. Seven infants stayed a few days at the neonatal ward before being transferred to the maternity ward.

All mothers commenced breast-feeding, but five infants were breast-fed for less than 2 mo, and thereafter received commercial formula. Solids were mostly introduced by 4 mo of age, but 77% of the infants received breast milk in addition to solids until at least 6 mo of age. Fruits and vegetables were regularly introduced between 4 and 5 mo of age, porridge and gruel at 4–6 mo of age, and meat at 5–6 mo of age. Fish and egg were rarely introduced before 6 mo of age.

Isolation of E. coli from infants' intestinal microflora. A sample of the rectal flora was obtained 3 d after delivery using a cotton-tipped swab. The swab was put in COPAN's transportation medium (18) and transported to the laboratory within 20 h. The swab was streaked on a Drigalski agar plate (19), and the inoculate was spread to obtain free-lying colonies.

Fecal samples were obtained at 1, 2, 4, and 8 wk of age and at 6 and 12 mo of age. Feces was collected at home by the parents and put in a plastic bag in which an anaerobic atmosphere was generated (AnaeroGen Compact, Oxoid, Hampshire, U.K.). The samples were kept refrigerated until being transported to the laboratory where they were processed within 24 h after collection. Using this procedure, *E. coli* counts were not reduced compared with immediate culture as shown in preliminary experiments.

Feces was diluted serially, plated on Drigalski agar (19), and incubated aerobically overnight at 37°C. In each sample, individual colony types differing in size, shape, color. or mucoid appearance were separately enumerated and subcultured for purity, and their species identity was determined using the API20E biotyping system (API Systems SA, La Balme les Grottes, Montalieu-Vercieu, France). Colony types representing *E. coli* were saved at −70°C in Hogner's freezing medium (20) and analyzed further.

The limit of detection was 330 cfu/g feces (represented logarithmically as $10^{2.52}$). Subdominant strains could be identified if they differed in morphology and if their population numbers differed less than two log units from the dominant colony type. Colony types less frequent than this were overgrown and missed. One to six colony types were regularly identified in each sample.

Strain typing by RAPD. Different *E. coli* strains were identified by RAPD (21). A small amount of bacteria obtained from an overnight culture on tryptic soy agar (TSA) plates was added to 50 μL of HotStarTaq Master Mix, (Qiagen, Spånga, Sweden) containing 6 μM of the primer GTGATCGCAG. The PCR was started with a 95°C 15-min heat activation step for the polymerase, and continued with the following temperature profile: 94°C for 45 s; 30°C for 120 s; 72°C for 60 s for four cycles followed by 94°C for 5 s; 36°C for 30 s; 72°C for 30 s for 26 cycles (the extension step was increased by 1 s for every new cycle). The reaction was terminated at 72°C for 10 min and cooled to 4°C. The PCR products were separated on 8% ready-made Tris-Glycine gels and visualized by silver staining (21).

All *E. coli* isolates from one child were assayed together, and their PCR products were, when possible, separated on the same gel. Two isolates with identical profiles were considered to belong to the same strain. If two isolates showed minor differences in RAPD patterns, they were run together in a new PCR and thereafter assigned to the same or different strains.

Multiplex PCR for detection of virulence genes in E. coli strains. Virulence factor genes were identified using three sets of multiplex PCRs, the first identifying the genes for type 1 fimbriae (*fimA*), P fimbriae (*papC*), S fimbriae (*sfaD/E*), and Dr. hemagglutinin (*draA*); the second the class I, class II, and class III varieties of the P-fimbrial adhesin gene *papG*; and the third the genes for the capsule K1 (*neuB*) and K5 (*kfiC*), aerobactin (*iutA*), and hemolysin (*hlyA*) (10). The primer pairs used have been published previously (10, 22–25).

The multiplex PCRs were performed as previously described (10), but with slight modifications. Bacteria from colonies grown on TSA were added to a mixture containing HotStarTaq Master Mix (Qiagen) and 0.45 μM of each primer pair in a final volume of 50 μL. In the third multiplex PCR reaction, the concentration of $MgCl_2$ was increased from 1.5 to 2.0 mM. The PCR program was started with an initial heat activation step for the *Taq* polymerase at 95°C for 15 min. Thereafter, the PCR was run as described previously (10). PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide.

Statistical methods. Proportions were compared using Fisher's exact test. Population counts were compared using the Mann-Whitney *U* test.

RESULTS

Colonization by E. coli. Forty-two percent of the 70 infants were colonized by *E. coli* 3 d after birth. After this, further colonization occurred slowly. It took more than 2 wk before half of the infants had *E. coli* in their stools and by 2 mo only 61% were colonized. One child had not yet acquired any *E. coli* at 1 y of age, and another eight infants (11%), who had earlier been colonized with *E. coli*, were again negative by 1 y of age.

Infants delivered by cesarean section showed delayed acquisition of *E. coli* compared with vaginally delivered infants (Fig. 1*A*), although the difference between the groups did not reach statistical significance at any time. There was no difference in colonization rate between infants with or without siblings, with allergic or nonallergic parents, or between infants who grew up in a home with or without pets (Fig. 1, *B–D*).

Populations counts of E. coli. In colonized infants, the population counts of *E. coli* were high during the first 6 mo of life (Fig. 2). A significant decrease in *E. coli* population numbers occurred between 6 and 12 mo of age (from $10^{8.7}$ cfu/g to $10^{8.0}$ cfu/g, $n = 61, 60$, $p < 0.0001$). *E. coli* population levels did not differ at 6 mo of age between infants receiving or not receiving breast milk at that age ($10^{8.7}$ cfu/g feces *versus* $10^{8.6}$ cfu/g, $n = 47, 14$, $p = 0.72$).

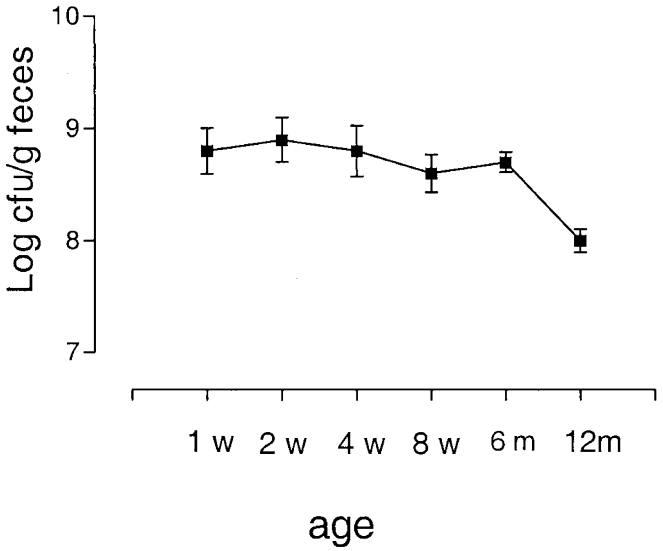

Figure 2. Total *E. coli* counts in fecal samples from culture-positive infants. The mean and SD is given for each time point.

Strain turnover. Individual strains of *E. coli* in an infant were identified by RAPD. Strain identities were not com-

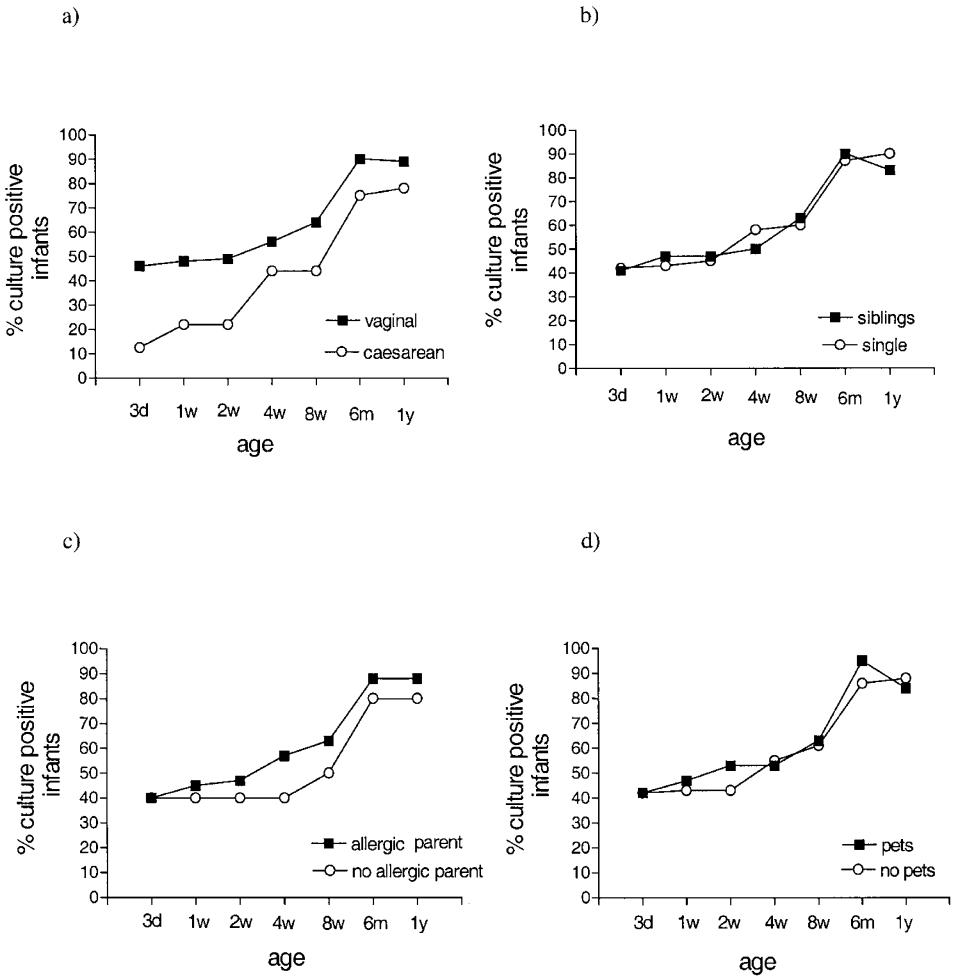

Figure 1. Intestinal *E. coli* colonization rate in infants delivered vaginally ($n = 61$) or by cesarean section ($n = 9$; *A*); infants with ($n = 30$) or without siblings ($n = 40$; *B*); infants with allergic ($n = 60$) or nonallergic parents ($n = 10$; *C*); and infants growing up in a home with ($n = 19$) or without pets ($n = 51$; *D*).

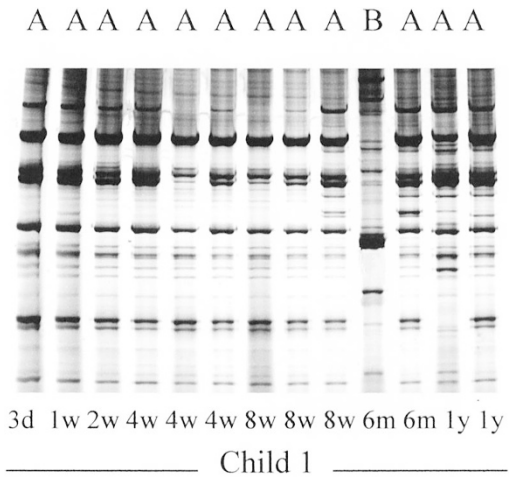

Figure 3. RAPD patterns of 13 intestinal *E. coli* isolates obtained on different occasions from a single child. Strain "A" was present on all sampling occasions, whereas strain "B" was present in the 6-mo sample only. The three isolates on the right end of the gel were reanalyzed and thereafter assigned to strain "A."

pared among infants. Figure 3 shows the RAPD profiles of the *E. coli* isolates obtained from one infant. Strain "A" was present on all sampling occasions from d 3 and onward, whereas another strain "B" was only present in the 6-mo sample. Although several colonies were often selected from a sample, because they differed in morphology and were suspected to represent different strains, they often turned out to be identical by RAPD (*e.g.* the three isolates in the 4-wk sample).

During the first 6 mo of life, 13% of the infants had no *E. coli*, 53% carried a single *E. coli* strain, 19% had two, 9% had three, and 7% had four to six strains. On average, 1.5 strains per child were identified during the first 6 mo.

During the first year of life, 1.4% had no *E. coli*, 39% carried a single strain, 31% had two strains, 16% had three strains, and 13% had four to six strains. On average, 2.1 different *E. coli* strains were found per infant during the first year of life. The diversity of the *E. coli* flora in colonized infants did not increase with age. Thus, at 3 d and at 12 mo of age, each infant positive for *E. coli* harbored on average 1.2 different strains. The mean number of strains isolated in the first year was equal in children who were delivered by cesarean section ($n = 9$) or vaginally ($n = 61$; 2.1 *versus* 2.1) as well as in children with ($n = 30$) or without ($n = 40$) elder siblings (2.2 *versus* 2.1). Infants growing up in a household with pets ($n = 19$) had no higher turnover rate of *E. coli* in their microflora than infants in pet-free households ($n = 51$; 1.8 *versus* 2.2; $p = 0.53$). Children of allergic parents ($n = 60$) had slightly, but insignificantly, fewer strains than children of nonallergic parents ($n = 10$; 2.0 *versus* 2.7; $p = 0.42$).

Five infants ceased breast-feeding before 2 mo of age, 11 between 2 and 6 mo, and 54 still received breast milk at 6 mo of age. Infants in the first and last groups acquired equally few *E. coli* strains during the period 2–6 mo (0.4 strains per infant).

Genes for adhesins and other virulence factors in intestinal E. coli strains. One isolate of each *E. coli* strain was analyzed by multiplex PCR for the detection of genes for virulence factors. In addition, several isolates from 17 strains were analyzed to assess the consistence of virulence gene carriage. All isolates of the 17 strains were identical with respect to virulence factor genes.

Among the 149 strains, genes encoding type 1 fimbriae were carried by 71%, genes for P fimbriae by 32%, and for S fimbriae by 26%. Not a single strain carried the genes for Dr hemagglutinin. Among the P-fimbriated strains, none carried the class I adhesin, 45% (21 of 47) carried the class II adhesin only, 34% (16 of 47) carried the class III adhesin only, and 8.5% (4 of 47) carried both the class II and class III adhesins, whereas 13% (6 of 47) lacked any of these adhesin varieties. Genes encoding the K1 capsule were found in 27%, the K5 capsule in 7%, aerobactin in 29%, and hemolysin in 23% of the strains.

Certain combinations of virulence genes tend to occur in combinations. We investigated virulence gene associations in the 149 *E. coli* strains. The P-fimbrial gene *papC* was significantly associated with the S-fimbrial gene *sfaD/E* ($p = 0.0021$), the hemolysin gene *hlyA* ($p < 0.0001$), the aerobactin gene *iutA* ($p = 0.0061$), and the gene for the K1 capsule *neuB* ($p = 0.0014$). *papG* class II was associated with *hlyA* ($p = 0.036$), *iutA* ($p = 0.0028$), and *neuB* ($p = 0.0001$), whereas *papG* class III was associated with *sfaD/E* ($p < 0.0001$) and *hlyA* ($p < 0.0001$). The S-fimbrial gene *sfaD/E* was significantly associated with *hlyA* ($p < 0.0001$) and *neuB* ($p = 0.019$). The aerobactin gene *iutA* was significantly associated with the K1 gene *neuB* ($p = 0.001$).

Strains recovered from 0- to 2-mo-old infants ($n = 71$) were compared with those recovered from 6- to 12-mo-old infants ($n = 78$). The former slightly more often possessed genes for K1 (34% *versus* 21%) and aerobactin (35% *versus* 23%) than the latter, but the differences were not significant ($p = 0.095$ and $p = 0.11$, respectively). The other virulence factor genes were equally common in early and late strains.

Strains first appearing in the microflora during exclusive breast-feeding ($n = 66$) were compared with strains acquired after termination of breast-feeding ($n = 48$). Genes for the K1 capsule were significantly more common in the strains acquired by exclusively breast-fed compared with weaned infants (32% *versus* 15%, $p = 0.047$). Carriage of genes for other virulence factors was similar in the two groups.

E. coli strains recovered from the five children who had a cat in the family carried *papG* allele III (42% *versus* 11%, $p = 0.011$) and the gene for hemolysin (59% *versus* 18%, $p = 0.021$) significantly more often than strains from children whose families had no pets. Strains isolated from children in households with a dog did not differ significantly from strains isolated from infants without pets.

Virulence factor genes in resident and transient E. coli strains. Forty-seven of the 70 infants harbored at least one *E. coli* strain that persisted in the microflora for at least 3 wk. These strains were classified as resident. Strains persisting for shorter periods were defined as transient, but strains appearing only in the 2-, 6-, or 12-mo sample were neither defined as resident nor transient, because several months passed between these sampling occasions. A total of 58 *E. coli* strains were classified as resident and 19 as transient. The mean time of

persistence for the resident strains was 30 wk. Because half of the strains were still present at 12 mo of age, this is an underestimation of the persistence time.

The carriage rate of different virulence factor genes in resident and transient strains is shown in Table 1. Genes for type 1 fimbriae, P fimbriae, and hemolysin were all significantly more common in resident than in transient strains. Furthermore, a combination of genes for P fimbriae and type 1 fimbriae was 7 times more prevalent among resident than among transient strains ($p = 0.0088$) and was, thus, more strongly associated with persistence than either P or type 1 fimbriae alone.

Several other combinations of virulence factor genes were associated with persistence, but in these cases the combination was not more strongly associated with persistence than one of the genes alone. Combinations of virulence factors that were more common in resident than transient strains included S fimbriae and hemolysin (12 of 58 *versus* 0 of 19, ratio >4.1, $p = 0.032$), P fimbriae and hemolysin (14 of 58 *versus* 0 of 19, ratio >4.8, $p = 0.016$), P and S fimbriae (11 of 58 *versus* 0 of 19, ratio >3.8, $p = 0.056$), and type 1 fimbriae and hemolysin (13 of 58 *versus* 0 of 19, ratio >4.5, $p = 0.030$). Overall, a combination of four or more virulence factors was detected in 36% of the resident *versus* 5.3% of the transient strains (ratio, 6.8; $p = 0.0088$). Conversely, only 5% of the resident strains carried neither of the tested virulence factors, as compared with 37% of the transient strains (ratio, 0.14; $p = 0.0015$).

DISCUSSION

In the present study, the intestinal *E. coli* colonization pattern was characterized in 70 healthy Swedish infants born in the late 1990s. *E. coli* is a bacterium which can only thrive in the intestines of man and other animals (26). It can, thus, be spread only via fecal contamination. Such contamination may occur during a vaginal delivery, and 45% of vaginally delivered, but only 12% of cesarean section delivered, infants were positive for *E. coli* by 3 d of age. This suggests that approximately one third of infants acquired *E. coli* from their mother at delivery, which fits well with other studies (27, 28).

In the 1970s, at least 70% of infants born in Western countries acquired *E. coli* during their first week of life (1, 2). Strains spread in the hospital milieu contributed significantly to colonization (29). In developing countries, almost all infants acquire *E. coli* in the first week of life (30, 31), including infants delivered by cesarean section (31, 32) In the present study, we noted less than 50% *E. coli* colonization by 1 wk of age. Spread of *E. coli* in maternity wards is restricted by rooming-in (33). We believe that rooming-in combined with early discharge from the hospital have reduced early colonization by *E. coli*.

It took 6 mo before (almost) all infants were colonized by *E. coli*, which is later than observed in the 1980s (34). This slow acquisition was not caused by the fact that most infants in our study had an allergic parent(s), because colonization occurred equally slowly in those with allergic and nonallergic parents. Siblings or pets in the household also did not influence *E. coli* colonization rate.

Once colonized, the studied infants displayed a slow turnover rate of individual *E. coli* strains in the microflora. During the first 6 mo of life, a mean number of 1.5 strains per infant were identified, which contrasts sharply to the 8.5 strains found on average in Pakistani infants (35). One may also compare the 2.1 different *E. coli* strains found during the first 12 mo in our study with the average 4.2 strains found in Swedish infants followed until 11 or 18 mo of age during the 1980s (36). Differences in sampling schedules and number of selected colonies preclude a direct comparison, but one may conclude that the turnover of individual *E. coli* strains in Swedish infants is at least as low as it was in the 1980s, and might even have further decreased since then.

We did not observe any greater number of *E. coli* strains in the microflora of children who shared households with older siblings or pets. We also did not observe any greater turnover of *E. coli* strains between 2 and 6 mo of age in weaned compared with breast-fed children, indicating that feeds were not a significant source of *E. coli* strains. In contrast, in a study performed in the 1980s breast-fed infants harbored fewer *E. coli* strains over time than bottle-fed infants (37).

The normal intestinal microflora is the major drive for the maturation of the intestinal immune system (38). A bacterial strain that colonizes the gut activates the gut immune system only transiently, even if it persists in the microflora. The secretory IgA induced in response to the bacterium coats it and prevents it from translocating across the mucosal barrier and, hence, from interacting further with the immune system (39). A constant turnover of strains in the microflora may, therefore, be needed to keep the immune system activated. In accordance, Pakistani infants, who are constantly exposed to new *E. coli* strains, have a strong secretory IgA response against a pool of *E. coli* O antigens already at 2 mo of age, whereas Swedish infants do not reach similar levels until 1–2 y of age (40). The total secretory IgA concentration in saliva also rises much more rapidly in Pakistani than Swedish infants (40).

E. coli population numbers remained high during the first 6 mo of life, but decreased 10-fold between 6 and 12 mo of age.

Table 1. *Carriage of genes encoding different virulence factors in resident and transient intestinal E. coli strains**

Virulence factor	% Positive strains: Resident ($n = 58$)	% Positive strains: Transient ($n = 19$)	Ratio	p value
Type 1 fimbriae	79	53	1.5	0.037
P fimbriae	41	11	3.7	0.014
Class II adhesin	22	5.3	4.2	0.17
Class III adhesin	16	5.3	3.0	0.44
S fimbriae	34	16	2.1	0.16
Capsule K1	38	26	1.5	0.42
Capsule K5	8.6	0	>1.6	0.33
Aerobactin	41	21	2.0	0.17
Hemolysin	28	0	>5.3	0.0082

* The carriage rate of genes encoding different *E. coli* virulence factors was assessed in 58 resident and 19 transient intestinal *E. coli* strains using multiplex PCR. The ratio between the occurrence of each virulence factor in resident and transient strains was calculated. If the denominator was zero, the next transient strain was assumed to posses the virulence factor in question to permit calculation of a ratio. The p values refer to differences in gene carriage rate between resident and transient strains.

The populations of facultative bacteria diminish as the anaerobic microflora becomes more complex (41, 42). This has been reported to occur already in the first weeks in earlier studies (30, 43). The late decrease in *E. coli* population numbers seen here could reflect delayed acquisition of a complex anaerobic microflora in the Swedish infants born today. We have noted very high fecal numbers of *S. aureus* (7) and coagulase-negative staphylococci (unpublished observations) in the infants examined here, which we interpret as a sign of reduced competition from other bacteria in the intestinal microflora.

Virulence genes were identified in the *E. coli* strains colonizing the infants' intestines. The carriage rates of these genes were similar to those observed in *E. coli* isolated from the intestinal flora of Swedish schoolgirls in the 1970s (10), but higher than among *E. coli* isolated from Pakistani infants (9), which confirms that commensal *E. coli* populations may differ in different geographic areas (35, 44).

Analysis of *E. coli* virulence gene carriage in relation to lifestyle factors revealed that *E. coli* acquired during exclusive breast-feeding significantly more often had the gene for K1 than those acquired during formula-feeding. This could be an age effect, as K1 tended to be more common in *E. coli* strains acquired during the first 2 mo than in strains acquired later, but it could also be the other way around; the tendency of K1 strains to colonize newborn infants could be related to the fact that they are often breast-fed. Previous studies have instead found the reverse: a decreased colonization by *E. coli* carrying K1 as well as P fimbriae in breast-fed infants (45, 46).

Another finding was that the class III variety of the papG adhesin located on P fimbriae, as well as the gene for hemolysin, were both significantly more common in *E. coli* in the microflora of children with a cat in the family, compared with *E. coli* isolated from children growing up in pet-free households. One may speculate that *E. coli* from a family cat could be transferred to the infant, because *E. coli* causing urinary tract infection in cats and dogs frequently carry the papG III adhesin type, and these strains arise from the intestinal flora of the animals (16, 17). We saw no effect on *E. coli* gene carriage by dog-ownership. Possibly the parents allow cats to have closer contact with the infant compared with dogs. Infants growing up with pets have lesser risk of developing allergy (47, 48), which might result from exposure to microbes with protective properties.

Resident and transient strains were compared for virulence gene carriage rates. We have previously shown that *E. coli* strains persisting in the intestinal microflora more often carry the genes for P fimbriae, K1 or K5 capsule, and aerobactin than transient strains in the same hosts (9, 10). In the present study, P fimbriae and type 1 fimbriae, and especially the combination of these two adhesins, were significantly associated with intestinal persistence. P fimbriae and type 1 fimbriae confer adherence to colonic epithelial cells (49), and type 1 fimbriae bind to the carbohydrate chains of secretory IgA, a major component of breast-milk (50). Interaction with secretory IgA seems to enhance intestinal survival of type 1-fimbriated *E. coli*, because these are reduced in IgA-deficient individuals (51).

The gene for hemolysin was also much more common among resident than transient strains. Hemolysin, which is toxic to a number of cell types (52), enhances colonization by diarrheogenic *E. coli* in the pig small intestine (53). Hemolysin might contribute to persistence by attacking enterocytes and releasing nutrients for the bacteria. In this context, it is interesting to note that *E. coli* in the large intestine appears to use membrane lipids as its main nutrient source (54). The association between hemolysin and persistence could also be indirect, as several different virulence factor genes occur together, sometimes carried on so-called pathogenicity islands in the genome (55–57). Indeed, 36% of the resident strains carried four or more virulence factor genes, compared with 5.3% of the transient strains. Pathogenic *E. coli* belong to certain phylogenetic subgroups (58). Further studies may determine whether the strains that are successful in colonizing the large intestine belong to the same subgroups, and whether they possess pathogenicity islands. These might rather be termed "fitness islands," as previously suggested (59), if they first aid in intestinal colonization and then confer pathogenicity to *E. coli* reaching extraintestinal sites.

CONCLUSIONS

In conclusion, the results of the present study demonstrate that *E. coli* is acquired late in Swedish infants today and that the turnover rate of individual strains is low, most likely because of a limited circulation of fecal bacteria in the Swedish hospitals and homes. This reduced bacterial exposure might have consequences for the maturation of the immune system and its function. Despite indications of poor competition within the intestinal ecosystem, *E. coli* strains that colonized for prolonged periods possessed certain traits that were much less common in transient strains. These traits, traditionally regarded as virulence factors, have most likely evolved to increase the fitness of *E. coli* in the human colon.

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