MAJOR ARTICLE

Escherichia coli Strains Belonging to Phylogenetic Group B2 Have Superior Capacity to Persist in the Intestinal Microflora of Infants

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Escherichia coli **strains segregate into 4 phylogenetic groups, designated "A," "B1," "B2," and "D." Pathogenic strains belong to group B2 and, to a lesser extent, group D, which more frequently carry virulence-factor genes than do group A strains and group B1 strains. This study investigated whether the capacity of *E. coli* to persist in the human intestine is related to its phylogenetic type. Resident ($n = 58$) and transient ($n = 19$) commensal *E. coli* strains isolated during a longitudinal study of 70 Swedish infants and previously tested for virulence-factor–gene carriage were tested for phylogenetic type. Of the strains resident in the intestinal microflora, 60% belonged to group B2, compared with only 21% of the transient strains ($P = .004$). In logistic regression, group B2 type predicted persistence in the intestinal microflora, independent of carriage of all investigated virulence-factor genes, including genes for P fimbriae ($P = .03$). Thus, group B2 strains appear to possess yet unidentified traits that enhance their survival in the human intestine.**

Escherichia coli is a member of the normal intestinal microflora of humans and of many animals [1]. It is also a common cause of extraintestinal infections, such as urinary-tract infection and septicemia [2, 3]. *E. coli* populations consist of stable genetic lineages termed "clones" or "strains" [4, 5]. Phylogenetic studies have shown that *E. coli* clones belong to 4 main phylogenetic groups, designated "A," "B1," "B2," and "D" [6]. Group B2 strains carry more virulence-factor genes than do the strains belonging to the other groups [7–9], and strains that cause extraintestinal infections belong mostly to group B2 and, to a lesser extent, group D [8, 10, 11]. According to some studies, commensal *E. coli* strains belong mostly to group A and group B1 [6, 8, 12], whereas another study reports a high proportion of group B2 strains in the intestinal microflora of humans [13].

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Commensal *E. coli* strains differ widely in their capacity to persist in the human intestine. Resident strains may persist in the intestinal microflora of an individual for months and years, whereas transient strains disappear within a few weeks and, in a strict sense, probably do not colonize [14–16]. Elsewhere, we have shown that adhesins and other virulence factors may contribute to the persistence of *E. coli* strains in the human intestine. Resident *E. coli* strains express P fimbriae [17, 18] and have genes for P fimbriae and type 1 fimbriae [19–21] more often than do transient strains. Genes encoding hemolysin [19–21], aerobactin [19, 20], and the capsular antigens K1 and K5 [20] are also enriched in resident strains. These findings have led us to suggest that uropathogenicity may be a side effect of *E. coli*'s adaptation to the intestinal milieu [17, 19–21].

The present study investigated whether *E. coli* strains belonging to "pathogenic" phylogenetic groups have superior capacity to persist in the intestinal microflora. *E. coli* strains isolated during a longitudinal survey of the commensal intestinal microflora of 70 Swedish infants were characterized with respect to phylogenetic type. Their persistence in the intestinal microflora, their virulence-factor–gene carriage, and their fecal population counts had been determined elsewhere [21]. The

colonizing capacity of individual *E. coli* strains could thereby be related to both their phylogenetic type and their virulence-factor–gene carriage.

SUBJECTS, MATERIALS, AND METHODS

E. coli strains. A total of 149 *E. coli* strains from 70 infants were examined. The infants participated in the ALLERGYFLORA study, a longitudinal study of how the presence of *E. coli* in the intestinal microflora relates to the development of allergy [21].

Rectal swabs were obtained 3 days after birth, and fecal samples were cultured at 1, 2, 4, and 8 weeks and 6 and 12 months of age. Fecal samples were diluted serially and were cultured on Drigalski agar. From dilutions with free-lying colonies, colonies with different morphologies were separately enumerated and subcultured. This procedure permitted the isolation of subdominant colony types present at counts up to 2 $\log_{10}$ units lower than those of the dominant colony type (data not shown). Colonies with different morphologies were subjected to biotyping by use of API20E (API Systems SA), for identification of *E. coli.*

All *E. coli* isolates were typed by use of random-amplified polymorphic DNA (RAPD). Isolates that had identical RAPD profiles and that were isolated from a single infant were considered to belong to the same strain. RAPD patterns were not compared between infants. After an isolate had been assigned a strain identity, the population counts of this strain during each sampling occasion were calculated on the basis of morphotype-based enumerations of individual isolates [21].

Strains that persisted in the intestinal microflora for >3 weeks were defined as resident, whereas strains that colonized for shorter periods were defined as transient [21]. Strains isolated on a single occasion at either 8 weeks, 6 months, or 12 months of age could not be classified, because of the length of the sampling intervals. Of the 149 strains examined, 58 were defined as resident and 19 as transient [21].

Each strain was characterized with respect to carriage of *fimH,* the gene encoding type 1–fimbrial adhesin, by polymerase chain reaction (PCR) using primers that have been published elsewhere [11]. The PCR conditions also have been described elsewhere [20].

In addition, each strain had previously been characterized, by multiplex PCR, with respect to carriage of the following virulence-factor genes: *papC* (encoding P fimbriae), the class I–III varieties of *papG* (encoding P-fimbrial adhesin), *sfaD/E* (encoding S fimbriae and F1C fimbriae), *draA* (encoding Dr-hemagglutinin), *neuB* (encoding K1 capsule), *kfiC* (encoding K5 capsule), *iutA* (encoding aerobactin), and *hlyA* (encoding hemolysin) [21]. The carriage rate for these virulence-factor genes, their association with persistence of the *E. coli* strains in the large intestine, and the colonization pattern of the cohort have been described elsewhere: according to Fisher's exact test, *papC* and *hlyA* were associated with persistence in the intestine [21], whereas carriage of *fimH* was not associated with persistence in the intestine.

Triplex PCR for phylogenetic classification of* E. coli *strains. Phylogenetic classification was performed by a 2-step triplex PCR [22]. A small amount of the bacterium was picked from a colony and was suspended in a PCR mixture containing 20 pmol of each primer pair, for the genes *chuA* and *yjaA* (in the first step) and for the DNA fragment TspE4.C2 (in the second step) [22]. HotStarTaq Master Mix (Qiagen) was added, and the PCR was performed as described elsewhere [22]. The amplified products were separated by electrophoresis on 2.5% agarose gels and were stained with ethidium bromide. The strains were assigned to phylogenetic groups, as follows: *chuA*$^+$/*yjaA*$^+$, group B2; *chuA*$^+$/*yjaA*$^-$, group D; *chuA*$^-$/TspE4.C2$^+$, group B1; and *chuA*$^-$/TspE4.C2$^-$, group A [22].

Statistical methods. Fisher's exact test was used for comparison of proportions, and the Mann-Whitney U test was used for comparison of population counts. A multivariate logistic regression model was used to evaluate the relative contributions that phylogenetic-group type and virulence-factor–gene carriage made to persistence (SPSS Analytics).

RESULTS

Of the 149 *E. coli* strains that were analyzed, 69 (46%) belonged to phylogenetic group B2, 43 (29%) to group A, 21 (14%) to group D, and 16 (11%) to group B1.

Population counts of* E. coli *strains, in relation to phylogenetic type. The fecal population counts of each *E. coli* strain, at various time points, were determined previously [21]. Because population counts decrease progressively with time as a more complex intestinal microflora develops [23, 24], analyses of the relation between strain characteristics and population counts must compare isolates from infants of the same age. In infants at 1, 2, 4, or 8 weeks or 6 months of age, the average fecal population counts of strains belonging to the 4 phylogenetic groups were not different (data not shown); at 1 year of age, the counts of strains belonging to group D were slightly higher than those of strains belonging to the other groups ($P = .03$) (figure 1).

Intestinal persistence of* E. coli *strains, in relation to phylogenetic type. Commensal strains that were repeatedly isolated over a period of ⩾3 weeks were defined as resident, whereas strains colonizing for shorter periods were defined as transient. The phylogenetic type of the 58 resident and the 19 transient strains is shown in figure 2. The majority (60%) of the resident strains belonged to phylogenetic group B2, compared with only 21% of the transient strains ($P = .004$); group

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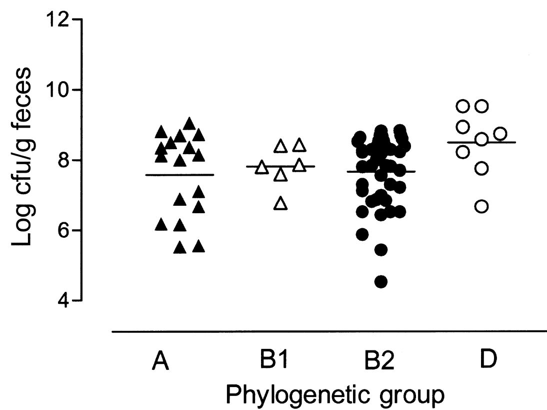

Figure 1. Population counts of *Escherichia coli* strains belonging to 4 phylogenetic groups—A, B1, B2, and D—in stool samples from 1-year-old Swedish infants. Each dot represents the population count for 1 strain; the horizontal bars indicate median values.

D was equally represented in the resident strains and in the transient strains; and groups A and B1 were more common in the transient strains than in the resident strains, although the differences did not reach statistical significance ($P = .1$ for group A; $P = .06$ for group B1).

Phylogenetic distribution of virulence-associated genes. Table 1 shows the relative frequency of virulence-factor genes within strains belonging to the 4 phylogenetic groups and provides the *P* values for carriage rates that are significantly higher or lower than those for carriage rates in the other phylogenetic groups combined (only *P* values $\leqslant .01$ have been given, to avoid potential problems of mass significance). Strains belonging to group A rarely carried genes for virulence factors other than type 1 fimbriae and aerobactin; furthermore, they carried *fimH*, the gene encoding type 1–fimbrial adhesin, significantly less frequently than did strains of the other phylogenetic groups. The genes for a number of virulence factors were significantly more frequent in strains belonging to group B2 (table 1).

PCR results indicated that, of the strains carrying *papC*, the gene encoding P fimbriae, none had the class I variety of the *papG* P-fimbrial adhesin, 45% (21/47) had the class II variety, 34% (16/47) had the class III variety, 8.5% (4/47) had both the class II and the class III varieties, and 13% (6/47) did not produce any adhesin-gene product; all strains carrying both the class II and the class III varieties of the *papG* adhesin belonged to phylogenetic group B2. No strain had *draA*, the gene encoding Dr-hemagglutinin.

Phylogenetic-group type and virulence-factor–gene carriage as determinants of persistence, in a logistic regression model. To investigate whether group B2 type per se was associated with persistence or was secondary to an accumulation of virulence-factor genes in this group, logistic regression analysis using phylogenetic type and carriage of the investigated virulence-factor genes as independent variables was performed. In univariate analysis, 2 variables—group B2 type and carriage of *papC*—were found to be significant determinants of persistence; in a multivariate logistic regression model using group B2 type and carriage of *papC* as independent variables, group B2 type was an independent predictor of persistence. Although the magnitude of the contribution that carriage of *papC* made to persistence was the same as that made by group B2 type, it did not reach significance as an independent colonization factor (table 2).

Virulence-factor–gene carriage rate in the resident and the transient strains belonging to the 4 phylogenetic groups. Although group B2 type was a strong predictor of persistence in the intestinal microflora, there were 23 resident strains belonging to other phylogenetic groups; in addition, 4 strains belonging to group B2 were transient. Figure 3 shows the prevalence of *papC*, *iutA*, and *hlyA* in the resident and the transient strains belonging to the 4 groups: within each group, these virulence-factor genes were more frequent in the resident strains than in the transient strains, although the differences were not significant; this finding was most notable for *hlyA*, which was carried only by the resident

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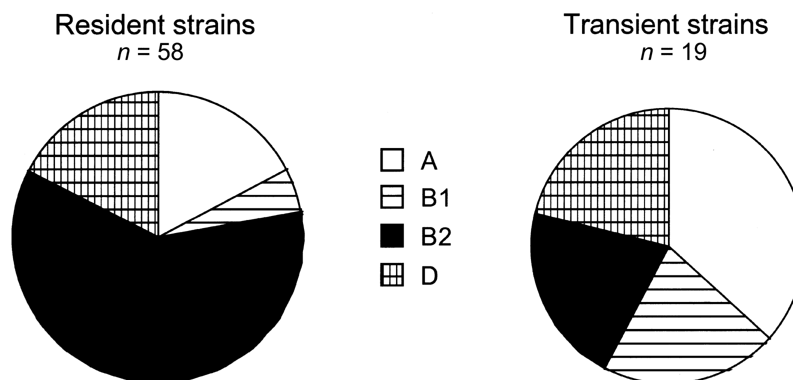

Figure 2. Distribution of 4 phylogenetic groups—A, B1, B2, and D—in resident and transient *Escherichia coli* strains isolated during a longitudinal study of the intestinal microflora of 70 Swedish infants followed during their 1st year of life.

Table 1. Distribution of virulence-factor genes in 149 commensal *Escherichia coli* strains, in each of 4 phylogenetic groups—A, B1, B2, and D.

Gene (virulence factor)	Strains positive, %			
	A (n = 43)	*B1* (n = 16)	*D* (n = 21)	*B2* (n = 69)
fimH (type 1 fimbriae)	76[a]	100	100	100
papC (P fimbriae)	7	0	33	54[b]
papG-II (class II)	2	0	19	29[c]
papG-III (class III)	2	0	0	28[b]
sfaD/sfaE (S/FIC fimbriae)	2	0	0	54[b]
neuB (capsule K1)	5	0	24	48[b]
kficC (capsule K5)	5	0	0	12
iutA (aerobactin)	26	0[d]	38	35
hlyA (hemolysin)	7	6	10	41[b]

NOTE. With the exception of *fimH*-gene carriage, which was determined in the present study, the virulence-factor–gene carriage of all strains had been analyzed in an earlier study [21]. The prevalence of each virulence-factor gene in a particular phylogenetic group was compared with its prevalence among strains of the other phylogenetic groups, by Fisher's exact test.

[a] Virulence-factor–gene prevalence significantly lower than those in other groups: $P < .001$.

[b] Virulence-factor–gene prevalence significantly higher than those in other groups: $P < .001$.

[c] Virulence-factor–gene prevalence significantly higher than those in other groups: $P < .01$.

[d] Virulence-factor–gene prevalence significantly lower than those in other groups: $P < .01$.

strains (figure 3). *fimH* was carried by all strains belonging to either group B1, group B2, or group D (table 1), and 80% (8/10) of the resident strains belonging to group A carried *fimH*, compared with 71% (5/7) of the transient strains belonging to that group (difference is not significant).

DISCUSSION

In the present study, we have identified the phylogenetic type of commensal intestinal *E. coli* strains from 70 Swedish infants who were followed from birth to 1 year of age and have related it to the strains' capacity to persist in the intestinal microflora. We have found that, compared with the transient strains, the strains that were resident in the intestinal microflora more often belonged to phylogenetic group B2, which previous studies have characterized as causing most extraintestinal infections; in fact, the B2 strains displayed such pronounced fitness for the intestinal milieu that they were almost always resident: of 39 group B2 strains, 35 were resident in the intestinal microflora of the infant from whom they were isolated.

Group B2 strains have an accumulation of virulence-factor genes, as has been demonstrated both elsewhere [25] and in the present study. However, multivariate logistic regression analysis showed that group B2 type contributed to persistence in the intestinal microflora independently of the virulence-factor genes that were investigated; thus, group B2 strains must possess traits other than the adhesins, capsules, aerobactin, and hemolysin that were investigated. In an earlier study, which used a rat model, one of us had observed that a pathogenic *E. coli* clone colonized much better in the intestine than did a nonpathogenic clone, regardless of adhesin expression [26]. Countless characteristics—including surface properties, preferred metabolic pathways, and resistance to toxic metabolites produced by other members of the intestinal microflora—may be of relevance to a strain's capacity to persist in the intestinal microflora. We propose that group B2 *E. coli* strains have evolved a perfect fitness for the milieu in the human intestine.

However, our results do not exclude the contribution that adhesins and other virulence traits make to the fitness of groups B2 strains in the human intestine. Half of the commensal group B2 strains had *papC*, the gene encoding P fimbriae, and P fimbriae permit *E. coli* to adhere to colonic epithelial cells [27, 28]. In a rat model, *E. coli* expressing P fimbriae has been found to colonize better than does the isogenic mutant lacking such adhesins [26]. In the present study, carriage of P-fimbrial genes seemed to contribute to persistence, although that contribution did not reach significance as an independent factor when we controlled for phylogenetic type. *hlyA*, the gene encoding hemolysin, also was enriched in the group B2 strains. Although hemolysin did not appear to be a predictor of persistence in the logistic regression analysis, all hemolysin-positive strains were resident in the intestinal microflora, regardless of phylogenetic type. If a larger set of strains had been studied, hemolysin might have been found to play a role in persistence in the intestine.

With regard to fecal population counts, we found only minor differences related to phylogenetic type. Similarly, we have observed that the resident strains have no higher fecal population counts than do the transient strains (authors' unpublished data), which suggests that the capacity to increase to high population counts in the intestinal milieu is unrelated to the capacity to persist over long periods in the intestine. The population counts of *E. coli* strains are inversely related to the complexity of the normal intestinal microflora, and the population counts of *E. coli* decline during late infancy, when an increasingly complex anaerobic intestinal microflora develops

Table 2. Relative contribution of phylogenetic group–B2 type and carriage of *papC*, the gene encoding P fimbriae, to persistence of *Escherichia coli* in the intestines of 70 Swedish infants.

Variable	β (SE)	*P*
Constant	0.2553 (0.3482)	
B2	1.4062 (0.6498)	.03
papC	1.3167 (0.8312)	.11

NOTE. The results were generated by a logistic regression model in which group B2 type and *papC* were the independent variables and persistence was the dependent variable.

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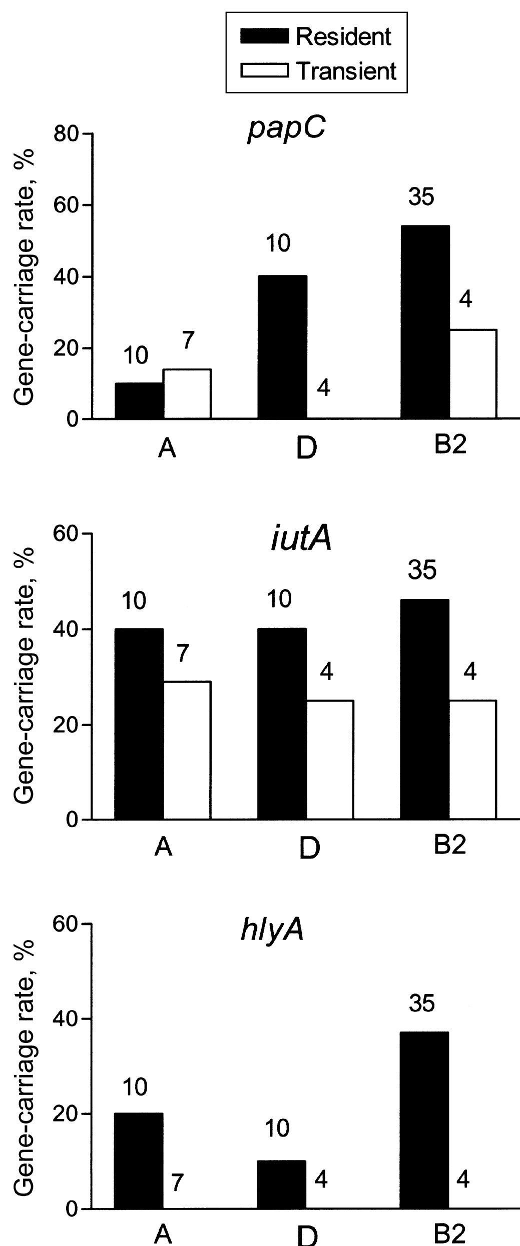

Figure 3. Carriage rate of *papC* (the gene encoding P fimbriae), *iutA* (the gene encoding aerobactin), and *hlyA* (the gene encoding hemolysin), in resident *(black bars)* and transient *(white bars) Escherichia coli* strains, in each of 4 phylogenetic groups—A, B1, B2, and D. The total number of strains belonging to each group is shown above the bars. None of the B1 strains possessed *papC, iutA,* or *hlyA.*

[29, 30]; our finding, in 1-year-old infants, that phylogenetic group D strains had significantly higher population counts than did strains belonging to the other groups could indicate that group D strains preferentially colonized infants who had a less complex intestinal microflora; however, it could also represent random variation that reached significance by chance.

The *E. coli* species has no natural niche other than the intestinal microflora of humans and animals. Strains belonging to phylogenetic groups A and B1 rarely cause disease in humans, and, in the present study, we have demonstrated that their capacity to persist in the human intestinal microflora also is deficient. Because most *E. coli* strains isolated from animal stools belong to these 2 groups [31, 32], we propose that their preferred niche is the commensal intestinal microflora of animals; they might produce adhesins that enable them to persist in the intestinal tract of these animals, and their metabolism might be adapted to the nutrients found there. When appearing in the human intestinal microflora, they mostly colonize only transiently.

A high proportion (46%) of all strains examined in the present study belonged to phylogenetic group B2. A similar frequency has also been reported for commensal *E. coli* strains from healthy American women [13], although other studies have found the commensal intestinal microflora to be dominated by groups A and B1 [8, 12]. We suggest that the infants examined in the present study were exposed chiefly to *E. coli* that was well adapted to the ecosystem in the human bowel, and elsewhere we have described the colonization pattern in these infants [21]; ~40% of them already harbored *E. coli* at 3 days after birth, and it probably had been acquired during birth, from the mother's perineal or vaginal microflora. Indeed, it has been reported that *E. coli* colonizing the vagina of pregnant women predominantly belong to group B2 [33]. In the 70 Swedish infants in the present study, acquisition of additional strains of *E. coli* occurred very slowly, suggesting that these infants had limited environmental exposure to *E. coli.* In contrast, Pakistani infants have been found to display an endless variety of new *E. coli* strains in their intestinal microflora during their first months of life, indicating that they have overwhelming exposure to *E. coli* from environmental sources [34]; accordingly, *E. coli* strains colonizing the intestines of these Pakistani infants are rarely of group B2 type (authors' unpublished data).

Taken together, our results suggest that group B2 *E. coli* strains have evolved characteristics that allow them to survive in the complex ecosystem of the human intestine. Some of these traits—for example, P fimbriae—clearly contribute to *E. coli*'s extraintestinal virulence. However, group B2 strains also appear to possess yet unidentified traits that enhance their survival in the human intestine. Whether these traits might also contribute to virulence remains to be determined.

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