Epidemiol. Infect. (2001), **126**, 19–23. Printed in the United Kingdom

P fimbriae and aerobactin as intestinal colonization factors for *Escherichia coli* in Pakistani infants

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(*Accepted 13 September 2000*)

SUMMARY

The carriage rate of a range of virulence genes was compared between resident and transient *Escherichia coli* strains obtained from the rectal flora of 22 home-delivered Pakistani infants 0–6 months old. Genes for the following virulence factors were assessed using multiplex PCR: P, type 1 and S fimbriae, three P fimbrial adhesin varieties, Dr haemagglutinin, K1 and K5 capsule, haemolysin and aerobactin. The *E. coli* strains examined here differed from those previously obtained from hosts in Western Europe in a lower prevalence of genes for P, S and type 1 fimbriae, K1 capsule and haemolysin. Nevertheless, genes for P fimbriae, the class II variety of *papG* adhesin, and aerobactin were significantly more common among resident than transient strains, as previously observed in a Swedish study. The results suggest that P fimbriae and aerobactin, previously implicated as virulence factors for urinary tract infection, might contribute to persistence of *E. coli* in the normal intestinal microflora.

INTRODUCTION

The intestinal microflora is a dynamic ecosystem with a constant turn-over of individual strains. In developed countries where the environmental exposure to pathogenic bacteria is low, individual *Escherichia coli* clones, or strains, persist for long periods of time in the colonic microflora [1]. In contrast, people in developing countries who are constantly exposed to a large variety of environmental bacteria exhibit a rapid turn-over of individual *E. coli* and other enterobacterial strains in their intestines [2].

The capacity of certain *E. coli* strains to persist in the human colon was demonstrated by Sears and coworkers over 50 years ago [3–5]. Strains resident in the intestinal microflora more often express P fimbriae than do transient strains [6–8]. Resident *E. coli* also frequently express O antigens associated with extra-intestinal infections [6, 9]. We recently showed that *E. coli* clones persisting in the intestinal microflora of Swedish schoolgirls not only carry the genes for P fimbriae, but also for K1 or K5 capsule, as well as the iron-chelator aerobactin [10].

Because virulence traits tend to occur in combinations, it is difficult to discern which factors are of primary importance for colonizing ability, and which are associated. We therefore investigated the carriage rate of a range of virulence genes in colonic resident and transient strains obtained from infants in Pakistan. Their *E. coli* strains differ in O antigen distribution from *E. coli* colonizing people in Europe and in the United States [2]. For example, O antigens associated with uropathogenicity, e.g. O1, O2, O4, O6, O7, O8, O16, O18, O25 and O75, are less frequent in colonic strains isolated in Pakistan compared with European strains whereas non-typable O antigens are more common in Pakistani than European *E. coli* strains [2, 11–13].

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In the present study, genes for P, type 1, S fimbriae and Dr haemagglutinin, the capsular types K1 and K5, aerobactin, haemolysin and three variants of the *papG* adhesin (class I–III) were identified in *E. coli* strains isolated from the rectal flora or Pakistani infants and their mothers. The carriage rate of these genes was compared between the Pakistani *E. coli* strains and two reference collections of *E. coli* obtained from Swedish children and adults. Secondly, the frequency of these genes was compared between resident and transient *E. coli* strains in the intestinal microflora of Pakistani infants.

MATERIALS AND METHODS

Bacterial strains

The resident and transient *E. coli* strains examined in this study derive from a longitudinal study of the intestinal microflora of 22 infants born in the urban slum of Lahore, Pakistan [2]. Eighteen of the infants were sampled for 6 months, 1 for 5 months and 3 for 1 month. All but one of the infants were partially breastfed for at least 2 months, none was exclusively breastfed. Rectal swabs were collected every second day during the first week, once a week during the first month, and thereafter once a month, and cultured on McConkey agar.

Three colonies were selected at random and identified as *E. coli* by biotyping [14]. All isolates were subjected to multilocus enzyme electrophoresis for determination of clonal (strain) identity [2]. Resident strains were defined as those isolated from an infant repeatedly over a period of at least 3 weeks and transient strains were those present only once, or on several occasions during a period of less than 3 weeks. Resident strains were shown more often than transient strains to express P fimbriae and mannose resistant adherence to the colonic cell line HT-29 [8]. Altogether, 23 resident and 130 transient strains were identified in the study, of which all resident and 123 of the transient strains were analysed here. A rectal swab was obtained from the mothers of 16 of the infants during delivery, and *E. coli* were isolated and identified as described above [2]. A total of 24 maternal *E. coli* strains were analysed.

Two collections of intestinal *E. coli* from Swedish hosts served as references for comparison of virulence gene distribution between Sweden and Pakistan. One consisted of 63 *E. coli* strains isolated from the rectal flora of Swedish school girls in the 1970s (of which 25 strains were resident and 38 transient in the intestinal microflora) [6]. The other consisted of a cross-sectional sample of the intestinal microflora of 20 Swedish adults (8 male, 12 female) which were healthy controls in a study of virulence gene carriage of *E. coli* in IgA deficiency (median age 42 years, range: 23–73).

Multiplex PCR for detection of virulence genes in *E. coli* strains

A multiplex PCR for the simultaneous detection of a range of *E. coli* virulence genes is described in detail elsewhere [10]. *E. coli* isolates were subjected to three sets of multiplex PCR reactions. In the first, the genes for P fimbriae (*papC*), type 1 fimbriae (*fimA*), S fimbriae (*sfaD/E*) and Dr haemagglutinin (*draA*) were assayed and in the second the three known varieties of the P-fimbrial adhesin gene *papG*: class I, class II and class III (class III previously being called *prs*) were detected. The third multiplex PCR identified genes for the capsular types K1 (*neuB*) and K5 (*kfiC*), aerobactin (*iutA*) and haemolysin (*hlyA*).

Statistical methods

Comparisons of proportions were performed using Fisher's exact test.

RESULTS

Virulence genes in strains from Pakistani infants and mothers

The frequency of a range of virulence genes in strains isolated from Pakistani infants and mothers was compared with two previously obtained collections of *E. coli* from Swedish schoolgirls and adults (Table 1). There were no significant differences in gene carriage rates for any of the tested traits between infant and maternal strains from Pakistan or between Swedish children's and adults' strains. In contrast, strains from Pakistan and Sweden differed in several respects. Genes for the following factors were significantly less common among Pakistani than among Swedish intestinal *E. coli* strains: P fimbriae ($P = 0{\cdot}0048$), type 1 fimbriae ($P < 0{\cdot}0001$), S fimbriae ($P < 0{\cdot}0001$), K1 ($P < 0{\cdot}0001$), and haemolysin ($P = 0{\cdot}018$). The most marked difference in carriage rates was noted for the K1 capsular gene *kfic* which was rare among the

Table 1. *Virulence genes carriage rates in intestinal* E. coli *from Sweden and Pakistan*

	Gene carriage rate (%)				
	Pakistani strains		Swedish strains		
Virulence factor (gene)	Infants ($n = 146$)	Mothers ($n = 24$)	Children* ($n = 63$)	Adults† ($n = 20$)	P value‡
P fimbriae (*papC*)	9·5	17	27	20	0·0048
Type 1 fimbriae (*fimA*)	49	67	81	90	<0·0001
S fimbriae (*sfaD-E*)	0	0	19	0	<0·0001
Dr haemagglutinin (*draA*)	1	0	0	0	1·00
K1 capsule (*neuB*)	1·5	4	17	25	<0·0001
K5 capsule (*kficC*)	4	8	8	5	0·40
Aerobactin (*iutA*)	47	50	35	55	0·28
Haemolysin (*hlyA*)	8	8	21	10	0·018

* Swedish schoolgirls [10].
† 20 controls Friman et al. (unpublished observations).
‡ P value for difference between Pakistani and Swedish *E. coli* strains.

Table 2. *Rates of carriage of adhesin genes in resident and transient* E. coli *strains from Pakistani infants*

	% positive strains		
Adhesins	Resident	Transient	P value
P fimbriae (*papC*)	26	6·5	0·01
Class I adhesin	0	0	
Class II	22	5·7	0·02
Class III	0	0	
None of the above	4	0·8	1·00
S fimbriae	0	0	
Type 1 fimbriae	48	50	1·00
Dr haemagglutinin	0	1	1·00

Table 3. *Carriage of genes encoding capsules, aerobactin and haemolysin in resident and transient* E. coli *strains*

	% positive strains		
Virulence factor	Resident	Transient	P value
Capsular antigens			
K1	0	1·6	1·00
K5	9	3·2	0·22
Other			
Aerobactin	70	42	0·01
Haemolysin	17	5·7	0·06

Pakistani strains, but present in one-fifth of the Swedish rectal *E. coli* strains.

Adhesin genes in resident and transient *E. coli* strains

Adhesin gene carriage rates were compared between resident and transient intestinal *E. coli* strains from Pakistani infants (Table 2). The P-fimbrial gene *papC* was carried four times more often by resident than by transient strains ($P = 0·01$). Eighty-four percent of the *papC*-positive strains carried the class II adhesin type, which was also significantly associated with persistence ($P = 0·02$). No class I- or class III-positive strains was found. One resident and one transient *papC*-positive strain carried neither of the three *papG* adhesin genes.

Genes for other virulence factors in resident and transient *E. coli* strains

The frequency of genes for haemolysin, aerobactin and the capsular types K1 and K5 in resident and transient *E. coli* strains is shown in Table 3. Genes encoding aerobactin were significantly more often found in resident than in transient strains ($P = 0·01$).

Combination of virulence genes in resident and transient *E. coli* strains

Carriage of combinations of genes for P fimbriae, capsule and aerobactin in resident and transient *E. coli* is shown in Table 4. A combination of P fimbriae and aerobactin was present in 22% of the resident compared with 2% of the transient strains ($P = 0·001$). In the subset of strains carrying neither P

Table 4. *Carriage of different combinations of virulence genes in resident and transient* E. coli *strains*

Virulence factor combination	No of strains	
	Resident ($n = 23$)	Transient ($n = 123$)
P + K1 + aerobactin	0	1
P + K5 + aerobactin	0	3
P + K5	1	0
P + aerobactin	5	2
K1 + aerobactin	0	1
K5 + aerobactin	0	1
P fimbriae	0	2
K5	1	0
Aerobactin	11	44
Neither P fimbriae, nor K1, K5 or aerobactin	5	69

fimbriae, nor K1 or K5, possession of the aerobactin gene *iutA* was significantly more common in resident than transient strains (69 % versus 39 %, $P = 0{\cdot}031$).

DISCUSSION

In the present study virulence gene carriage rates were compared between resident and transient *E. coli* strains from the rectal microflora of 0–6 months old Pakistani infants. Such genes tend to occur in fixed combinations in virulent *E. coli* clones, which makes it difficult to determine their independent contribution to persistence in the intestinal microflora. It is therefore of interest to note that strains of *E. coli* isolated in Pakistan differed markedly in carriage rates of some virulence factors from *E. coli* collected from people in Europe. Genes for type 1, P and S fimbriae, the K1 capsule and α-haemolysin were all less common among *E. coli* isolates from Pakistani infants as compared to Swedish subjects. In accordance, phenotypic expression of P fimbriae was seen in only 9 % of the Pakistani infant strains [8], as compared to 25 % of *E. coli* isolated from Swedish infants 0–18 months old [7]. Instead, the strains isolated from Pakistani infants quite often carried adhesins previously not characterized which mediated binding to cells of the human colonic cell line HT-29 [8].

Despite these differences, the same two bacterial factors were associated with intestinal persistence in the rectal flora of Pakistani infants, as previously identified in Swedish schoolgirls [10], namely aerobactin and P fimbriae. Thus, the combination of P fimbriae and aerobactin was significantly associated with persistence, being 11 times more common in resident than transient strains. *E. coli* resident in the intestinal microflora of Swedish schoolgirls generally had a combination of genes encoding P fimbriae, aerobactin and the K1 or K5 capsule, whereas transient strains showed neither of these traits [10]. Among the Pakistani *E. coli* strains examined here, such combinations were unusual, mainly due to the low frequency of the K1 capsule.

In both *E. coli* populations, the class II variety of the *papG* adhesin gene was the most common among P-fimbriated strains and was also significantly associated with intestinal persistence. In contrast, other adhesins which also mediate binding to human colonic cells, such as type 1 fimbriae, S fimbriae and Dr haemagglutinin [15] were not associated with persistence. It is interesting to speculate why P fimbriae seems to be so favourable for long-term colonization of the large bowel. P fimbriae, but not type 1 or S fimbriae also contribute to colonization in the rat [16, unpublished observations]. It may relate to the fact that intestinal *E. coli* seem to almost exclusively utilize host cell membrane lipids as their carbon source [17]. As the Galα1$\rightarrow$4Galβ moiety apparently occurs only in membrane-bound form [18, 19], binding to such receptors may place the bacteria in a favourable position in relation to their growth substrate. In contrast, terminal sialic acid, which is the receptor for S fimbriae, is present on mucin molecules [20] and mannose-containing receptors for type 1 fimbriae occur on secretory IgA [21]. Binding to these structures might be less favourable for long-term persistence. The results confirm that not mere adherence to intestinal cells, but rather adherence to certain specific receptor structures, is important for long-term persistence.

Aerobactin is the most effective siderophore synthesized by *E. coli*. Its affinity for iron is sufficiently high to enable it to sequester iron from protein carriers such as transferrin and lactoferrin [22, 23]. In strains of *E. coli* isolated from Swedish schoolgirls, aerobactin gene carriage was strongly linked to the carriage of P fimbriae as well as the K1 or K5 capsules. It was, thus, not possible to determine the independent importance of these traits for intestinal persistence [10]. In the present material, aerobactin was significantly associated with persistence in strains carrying neither genes for P fimbriae, nor the K1 or K5 capsules, which suggests that it might be an

intestinal colonization factor in its own right. Whether aerobactin is indeed required for survival in the large intestine can only be proven by allowing isogenic strains differing in this trait to compete for establishment in the intestinal microflora.

In summary, the present study shows that both P fimbriae and aerobactin are associated with intestinal persistence of *E. coli* in a geographical setting where the *E. coli* microflora differs from that of the industrialized Western societies. These results support the hypothesis that some bacterial traits contributing to extra-intestinal infections have, in fact, evolved primarily to increase the fitness of *E. coli* in its natural niche, the colon.

ACKNOWLEDGEMENTS

The present study was supported by the Swedish Medical Research Council (No. K98-06X-12612-01A), the Swedish Association for Research on Agriculture and Forestry, and by the Medical Faculty of Göteborg University.

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