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P fimbriae and other adhesins enhance intestinal persistence of *Escherichia coli* in early infancy

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SUMMARY

Resident and transient *Escherichia coli* strains were identified in the rectal flora of 22 Pakistani infants followed from birth to 6 months of age. All strains were tested for O-antigen expression, adhesin specificity (P fimbriae, other mannose-resistant adhesins or type 1 fimbriae) and adherence to the colonic cell line HT-29. Resident strains displayed higher mannose-resistant adherence to HT-29 cells, and expressed P fimbriae ($P = 0{\cdot}0036$) as well as other mannose-resistant adhesins ($P = 0{\cdot}012$) more often than transient strains. In strains acquired during the first month of life, P fimbriae were 12 times more frequent in resident than in transient strains ($P = 0{\cdot}0006$). The O-antigen distribution did not differ between resident and transient strains, and none of the resident P-fimbriated strains belonged to previously recognized uropathogenic clones. The results suggest that adhesins mediating adherence to intestinal epithelial cells, especially P fimbriae, enhance the persistence of *E. coli* in the large intestine of infants.

INTRODUCTION

Adherence to host epithelial cells is a virulence factor in bacterial infection, but its role in the establishment and persistence of the normal flora is less well documented. The normal niche for *Escherichia coli* is the colonic microflora from which selected subgroups of strains may spread and cause extra-intestinal infections such as urinary tract infection or septicaemia [1, 2]. Pathogenic isolates are often characterized by the expression of adhesins that target them to different extraintestinal sites. Thus, P fimbriae, which bind to urinary tract epithelial cells [3, 4], are ubiquitous in *E. coli* isolates from pyelonephritis in hosts without predisposing conditions [5]. Similarly, S fimbriae, which bind to sialylated glycoproteins [6], are enriched in sepsis/meningitis isolates [7] and Dr adhesins in *E. coli* causing cystitis [8]. The same adhesins also enable *E. coli* to attach to human colonic epithelial cells [9, 10] which suggests these adhesins may promote colonization and long term persistence in the gut.

Intestinal *E. coli* may be divided into resident strains, which persist in the intestinal flora for extended periods of time, and transient colonizers [11, 12]. Resident strains more often carry P fimbriae than transient strains do [13, 14]. In addition, certain 'uropathogenic' O serotypes, namely O1, O2, O6, O7, O18, O25 and O75, are strongly associated with persistence [13]. Whether P fimbriae are of primary importance for colonization has not been resolved.

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Since P-fimbrial expression and other virulence factors, e.g. O-antigens and capsule expression, are genetically linked and tend to occur in fixed combinations in virulent *E. coli* isolates, it is difficult to assess the contribution of individual factors to colonizing ability in epidemiological studies. In experimental colonization of monkeys, P fimbriae did not increase the ability to colonize the intestine, but only to cause pyelonephritis [15]. In gnotobiotic rats, however, P fimbriae contributed to intestinal colonization [16].

Infants in Pakistan have a different colonization pattern than those born in Western countries. They show a rapid turnover of different *E. coli* strains probably due to a constant environmental exposure to enterobacteria [16a]. Many infants in developing countries are delivered at home, and are not exposed to *E. coli* selected for in the hospital milieu, which have an increased capacity to persist in the intestinal microflora compared with other *E. coli* strains [17]. Hospital strains often carry several virulence markers, including P fimbriae [18]. The aim of the present study was to investigate whether adhesin expression and *in vitro* adherence to the colonic epithelial cell line HT-29 correlate with the ability of *E. coli* to persist in the intestinal flora of Pakistani home-delivered infants.

METHODS

Subjects

Twenty-two infants born in the urban slum of Lahore, Pakistan, were included in the study [16a]. All infants were delivered at home with the assistance of traditional birth attendants. Eighteen of the infants were followed for 6 months, one was followed for 5 months and 3 infants were followed for 1 month only. The 4 dropouts were caused by family migration. All but 1 of the infants were partially breastfed for at least 2 months, and none was exclusively breastfed.

Informed consent from the parents was obtained and the study was approved by the ethic's committees of the Pakistani Medical Research Council and of Göteborg University, Sweden.

Sample collection and culture

The rectal flora of the infants was sampled within 2 h of delivery, every second day during the first week, weekly during the first month and monthly thereafter. In 16 cases, a sample of the rectal flora of the mother was obtained at parturition.

Sampling was performed with a cotton-tipped swab which was inserted through the anal canal, pressed against the mucosa and withdrawn. The swab was rolled over a Drigalski agar plate, the inoculum was spread with a sterile platinum loop and the plate was incubated aerobically at 37 °C overnight. The three last free-lying colonies were picked from the plate, which gives a 97% probability of including the dominant strain [19]. The isolates were subcultured to check for purity and transferred to stab agar and transported to Sweden for further analysis.

E. coli isolates were identified using biotyping as previously described [16a], using a panel of tests which permitted the identification of different biotypes of *E. coli* [20].

Strain identification by electromorphic typing

The strain identity of the *E. coli* isolates was determined using electromorphic typing for 8 different chromosomally encoded enzymes [21, 22], as described elsewhere [16a]. In each culture, *E. coli* isolates with a unique biotype and at least two isolates that shared a specific biotype were subjected to electromorphic typing. The following enzymes were assayed: malate dehydrogenase, 6-phosphogluconate dehydrogenase, adenylate kinase, phosphoglucose isomerase, glucose-6-phosphate dehydrogenase, mannose phosphate isomerase, phenyl-alanyl-leucine peptidase and leucyl-glycyl-glycine peptidase. *E. coli* isolates from each child were compared; those isolates that shared electrophoretic mobility for each of the eight enzymes tested were considered as one strain. Isolates from different children were not compared for electrophoretic type.

Definition of resident and transient *E. coli* strains

Strains isolated from one infant on at least two occasions were designated resident strains, provided that at least 3 weeks separated the first and last time of isolation. Transient strains were those found only once, and also, during the first month when sampling occasions were closely spaced in time, those found repeatedly during a period shorter than 3 weeks. These definitions are largely in agreement with those of Sears

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and colleagues [11], who defined transient strains as those colonizing for a few weeks only, and resident strains as those colonizing for longer periods. Similar definitions have been used by other authors [14, 17].

If a scheduled monthly sampling was missed, strains found only on the occasion immediately before or after this occasion could not be defined as transient or resident, and were omitted from this analysis. The same was true for isolates occurring only in the last sample obtained.

Serotyping

One isolate from each *E. coli* strain was serotyped (Adlerberth *et al.*, in manuscript) using antisera to 69 common *E. coli* O-antigens [23]. Selected *E. coli* strains ($n = 17$) were subjected to complete serotyping at Statens Seruminstitut, Copenhagen, Denmark.

Adhesin expression

The adhesin expression of *E. coli* isolates was analysed by agglutination of erythrocytes and Galα1-4Galβ-coated latex beads (Orion Diagnostics, Helsinki, Finland) after overnight culture on tryptic soy agar plates. For the detection of type 1 fimbriae, haemagglutination (HA) was tested after three passages in static Luria broth [24].

For HA, 25 μl of a thick bacterial suspension in PBS ($> 10^{10}$ bacteria/ml) was mixed on a microscope slide with 25 μl of a 3% suspension of human P_1, guinea-pig or chicken erythrocytes in PBS, or in PBS containing 2·5% methyl-α-D-mannoside. Agglutination was read by the naked eye after gentle tilting of the slide for 3 min, and designated as positive or negative.

An agglutination of guinea-pig erythrocytes in the absence, but not in the presence, of methyl-α-D-mannoside was termed mannose-sensitive (MSHA), signifying the expression of type 1 fimbriae. Agglutination of human P_1 or chicken erythrocytes both in the absence and presence of methyl-α-D-mannoside was designated as mannose-resistant (MRHA). Isolates causing MRHA of human P_1 erythrocytes were further tested for agglutination of human p erythrocytes negative for the Galα1-4Galβ-sequences, and Galα1-4Galβ-coated latex beads (Orion Diagnostics) [25]. An MRHA of human P_1, but not p erythrocytes, and/or an agglutination of Galα1-4Galβ-coated latex beads, defined the expression of P fimbriae [25]. All other MRHA patterns were grouped together and designated 'other MR'.

Adherence to cells of the human colonic carcinoma cell line HT-29

All isolates were tested for adherence to the human colonic epithelial cell line HT-29 [9] after overnight culture or tryptic soy agar and after 3 serial passages in static Luria broth (to maximize the expression of type 1 fimbriae). The bacteria were harvested, washed once in PBS and suspended at 5×10^9/ml in Hank's balanced salt solution (HBSS) as determined by optical density measurements (Vitatron, Hugo Tillqvist, Göteborg, Sweden).

HT-29 cells were cultured in Eagle's medium supplemented with 10% foetal calf serum, 2 mM L-glutamine and 50 μg/ml of gentamicin (Sigma Chemical Co., St Louis, Mo, USA). The cells were detached with buffer containing 0·54 mM EDTA, washed and suspended at 5×10^6/ml in HBSS. A mixture of 100 μl of bacterial suspension, 100 μl of cells and 300 μl HBSS, or HBSS containing 2·5% methyl-α-D-mannoside, was incubated with end-over-end rotation for 30 min at 4 °C. The cell were washed, fixed with neutral buffered formaline (Histofix, Histolab, Göteborg, Sweden) and examined by interference contrast microscopy ($500 \times$ magnification, Nicon Optiphot, Bergströms Instruments AB, Göteborg, Sweden). In each preparation, 40 cells were examined for the presence of adherent bacteria and the mean number of bacteria per cell was calculated.

All *E. coli* isolates were tested for adherence both in the absence and in the presence of methyl-α-D-mannoside. The mannose-sensitive adherence was calculated by substracting the number of bacteria adhering in the presence of methyl-α-D-mannoside (mannose-resistant adherence) from the number of bacteria adhering in the absence of methyl-α-D-mannoside (total adherence).

Statistics

For adhesin expression, the percentage of all isolates of a certain strain expressing each of the defined adhesins constituted the unit of observation on which the statistical analyses were based. For adherence, the mean MS or MR adherence of all isolates of a certain

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strain constituted the unit of observation. Mann-Whitney U-test was used for significance testing. Fisher's exact test was used for comparing O-serotype distribution between resident and transient strains.

RESULTS

Identification of resident and transient *E. coli* strains in the rectal flora of Pakistani infants

A total of 23 resident and 130 transient strains were identified; 20 strains could not be placed in either category. Eleven resident and 54 transient strains were acquired by the infants during their first month of life whilst 12 resident and 76 transient strains were acquired between 2 and 5 months' of age.

O-serotype distribution in resident and transient *E. coli* strains

The O-serotypes of resident and transient *E. coli* strains are shown in Table 1. Four of the resident strains (17%) and 19 of the transient strains (15%) belonged to the 10 'uropathogenic' serotypes O1, O2, O4, O6, O7, O8, O16, O18, O25 or O75. Thirty-nine per cent of the resident and 53% of the transient strains were non-typable with the 69 O-antisera used ($P = 0{\cdot}26$). Twenty-two per cent of the resident and 11% of the transient strains were 'rough', i.e. agglutinated spontaneously in saline ($P = 0{\cdot}29$).

Table 1. *O-serotypes of resident and transient intestinal* E. coli *strains in Pakistani infants 0–6 months of age*

	Number of strains (% of strains)	
O-antigens	Resident	Transient
O1	0	7
O2	0	1
O4	0	1
O6	1	1
O7	0	1
O8	0	3
O11	0	1
O12	0	1
O13	0	2
O15	1	2
O16	1	2
O17	1	4
O17/O77	0	2
O20	1	4
O21	0	2
O22	1	0
O25	1	1
O33	0	1
O42	0	1
O75	1	2
O77	0	1
O81	0	1
O85/O117	1	1
O86	0	2
O117	0	1
Typable	9 (39)	45 (36)
'Uropathogenic'	4 (17)	19 (15)
Non-typable	9 (39)	67 (53)
Rough	5 (22)	14 (11)
Total	23	126*

O-serotyping was performed using antisera to 69 O-antigens common among Swedish clinical isolates of *E. coli* [23]. Non-typable signifies that the isolates were not agglutinated by any of these antisera, and 'rough' that they agglutinated spontaneously in saline.

* Four transient strains were not typed.

The expression of adhesins in resident and transient *E. coli* strains

Three groups of adhesins were distinguished in agglutination tests using human P_1 and p, chicken and guinea-pig erythrocytes, and Galα1-4Galβ-coated latex beads: P fimbriae, other MR adhesins and type 1 fimbriae. Their haemagglutination patterns and their frequency among *E. coli* isolates are presented in Table 2.

A variable number of isolates of a given strain expressed a certain adhesin. Comparisons between resident and transient strains were therefore based on the mean expression of a certain adhesin among isolates of each strain. This measure was not affected by the fact that resident strains, on average, contributed a larger number of isolates than transient strains.

P fimbriae were expressed by an average 23% of resident strain isolates, compared with 7% of the transient strain isolates ($P = 0{\cdot}004$) (Fig. 1). Resident strains acquired during the first month of life expressed P fimbriae 12 times more often than transient strains isolated during the same period (28% *vs.* 2%, $P = 0{\cdot}0006$). In strains acquired later, P-fimbriation was twice as common in resident as in transient strains ($P = 0{\cdot}27$).

MR adhesins other than P fimbriae (P blood group independent MRHA of human erythrocytes and/or

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Table 2. *Definition of identified hemagglutination patterns, suggestive of adhesin groups*

Adhesin group	*n* (%)	Haemagglutination pattern*				Agglut. of Gal-Gal beads	Binding specificity
		Hum P_1	Hum p	Chicken	Guinea-pig		
P fimbriae	69 (10)	MR	—	—	—	+	Galα1-4-Galβ
Other MR†	74 (11)	MR	MR	—	—	−	Unknown
	60 (9·1)	—	—	MR	—	−	Unknown
	41 (6·2)	MR	MR	MR	—	−	Unknown
Total =	175 (27)	—	—	—	—	−	—
Type 1 fimbriae	275 (41)	—	—	—	MS	−	Mannose

* MR, agglutination not reversed in the presence of methyl-α-D-mannoside. MS, agglutination reversed by methyl-α-D-mannoside.

† Including 3 different HA patterns shown in the table.

Adhesins causing MRHA were detected using human P_1 and p erythrocytes, chicken erythrocytes and Galα1-4-Galβ-coated latex beads, after culture of the bacteria on tryptic soy agar (TSA). Type 1 fimbriae (MSHA) were detected using guinea-pig erythrocytes after three serial passages of the bacteria in Luria broth (L-broth).

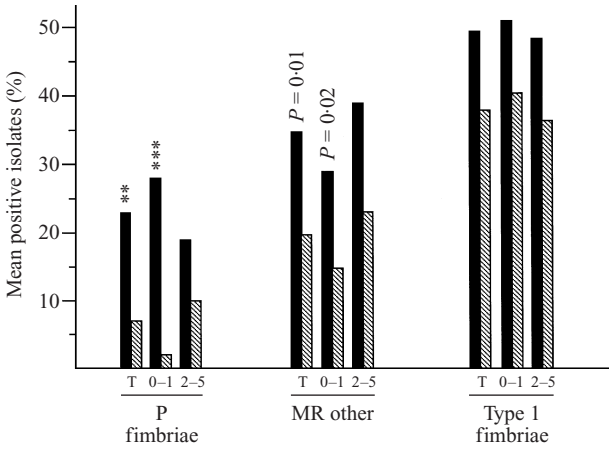

Fig. 1. Expression of different adhesins in resident and transient *E. coli* strains isolated from the rectal flora of Pakistani infants. The different groups of adhesins were defined by their hemagglutination patterns as shown in Table 2. The frequency of each group of adhesins among all isolates of each strain was determined, and constituted the unit of observation. Frequencies were compared between resident (black bars) and transient strains (shaded bars), using the total number of strains (T), strains acquired during the first month of life (0–1 months) or strains acquired later (2–5 months). Mann-Whitney U-test was used for test of significance.

an MRHA of chicken erythrocytes) were found in 35% of resident strain isolates, compared with 20% of the transient strain isolates ($P = 0{\cdot}01$) (Fig. 1). The difference was significant for strains acquired during the first month of life ($P = 0{\cdot}02$), but not for strains acquired later ($P = 0{\cdot}11$).

To exclude that the association between MR adhesins other than P fimbriae and persistence was secondary to a concomitant expression of P fimbriae in resident strains, adhesin expression was compared between resident and transient *E. coli* strains after omitting all strains expressing P fimbriae (7 resident and 10 transient strains). MR adhesins other than P fimbriae were found in 39% of the resident and in 18% of the transient strains not expressing P fimbriae. The difference was significant both in the material as a whole ($P = 0{\cdot}0088$) and in early acquired strains ($P = 0{\cdot}012$).

No significant difference in the expression of type 1 fimbriae was observed between resident and transient strains.

Adherence of *E. coli* to HT-29 cells as a function of adhesin expression

Adherence of *E. coli* isolates to HT-29 cells was tested after culture in broth and after culture on TSA. The mannose-sensitive (MS) and mannose-resistant (MR) adherence of individual isolates were determined, and analysed as a function of their adhesin expression (Table 3).

Isolates expressing P fimbriae or other MR adhesins displayed stronger MR adherence than MRHA-negative isolates, either after culture in broth or on TSA (Table 3). The strongest adherence was noted for isolated expressing P fimbriae simultaneously with other MR adhesins. Isolates expressing type 1 fimbriae (MSHA) attached to HT-29 cells in a mannose-sensitive manner (Table 3). These isolates showed a lower MR adherence to HT-29 cells than MSHA-negative isolates, both after culture on TSA (45% lower) and after culture in broth (33% lower) ($P = 0{\cdot}009$ and $P = 0{\cdot}11$, respectively).

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Table 3. *Adhesin expression and adherence to HT-29 cells of* E. coli *isolates*

		Adherence to HT-29 (bacteria/cell)			
		Mannose-resistant		Mannose-sensitive	
Adhesin group	*n* (%)	TSA culture	L-broth culture	TSA culture	L-broth culture
Mannose-resistant adhesins					
P fim	55 (8·3)	12	17	0	9·6
Other MR	161 (24)	7·8*	18	1·3	6·5
P fim + other MR	14 (2·1)	33	48	0	0
Not detected	429 (65)	4·3	9·8	1·4	7·0
Mannose-specific adhesins					
Type 1 fimbriae	275 (41)	4·3†	10	2·6‡	16‡
Not detected	395 (59)	7·8	15	0·2	0·7

The different groups of adhesins were defined by their haemagglutination patterns as explained in Table 2. Adherence of the bacteria to HT-29 cells was tested both after culture on TSA and after culture in broth. Mann-Whitney U-test was used for test of significance.

* $P < 0{\cdot}001$ compared with MRHA-negative isolates.

† $P < 0{\cdot}01$ compared with type 1 fimbriae-negative isolates.

‡ $P < 0{\cdot}001$ compared with type 1 fimbriae-negative isolates.

Table 4. *Adhesin expression and adherence to HT-29 cells of maternal* E. coli *strains*

		Adhesin expression (% of isolates)			Adherence (bacteria/cell)		
		MR*			MR		
Strains	*n*	P fim	Other MR	MS* type 1	L-broth	TSA	MS L-broth
Resident in child	5	40	43	47	12	7	7
Transient/not in child†	19	5·2	11	56	4	2	12
P value	—	< 0·05	< 0·05	NS	NS	< 0·05	NS

* MR, haemagglutination or adherence unaffected by methyl-α-D-mannoside. MS, haemagglutination or adherence abolished in the presence of methyl-α-D-mannoside.

† Maternal strains which only transiently or not at all colonized the infants.

The different groups of adhesins were defined by their haemagglutination patterns as shown in Table 2. Mann-Whitney U-test was used for test of significance.

Adherence to HT-29 cells of resident and transient *E. coli* strains

The mean MR or MS adherence of all isolates of a given *E. coli* strain constituted the unit of observation. Resident strains showed a higher average MR adherence than transient ones after culture either in broth or on TSA (Fig. 2*a*). MR adherence was weaker in strains acquired during the first month of life than in those acquired later ($P = 0{\cdot}053$, broth culture), while strains acquired during the first month displayed a higher MS adherence than those acquired later ($P = 0{\cdot}10$). No significant difference in mannose-sensitive adherence was observed between resident and transient strains (Fig. 2*b*).

Characteristics of *E. coli* strains transferred from mother to infant

Eight out of 24 *E. coli* strains isolated from maternal rectal samples at delivery were shown to appear later in the infants' rectal flora, five of which established as resident. The latter strains expressed P fimbriae as well as other MR adhesins significantly more often than maternal strains which never, or only transiently, colonized the infants (Table 4). Further, the strains

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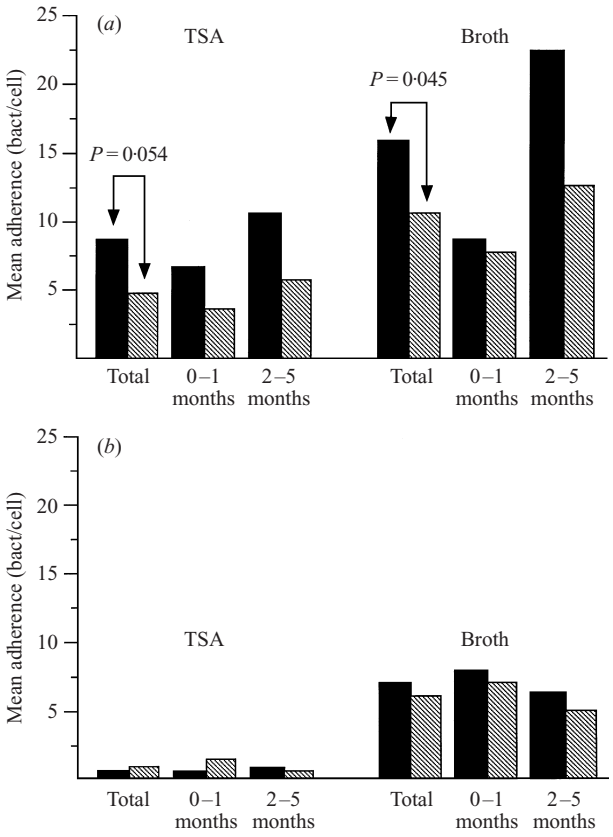

Fig. 2. (*a*) Mannose-resistant and (*b*) mannose-sensitive adherence to HT-29 cells of resident (black bars) and transient (shaded bars) *E. coli* strains, after culture on TSA overnight, or after 3 serial passages in static Luria broth. Comparisons between resident and transient strains were performed using the total number of strains (total), strains acquired during the first month of life (0–1 months) or strains acquired later (2–5 months). The mean adherence value for all isolates of a single *E. coli* strain constituted the unit of observation. Mann-Whitney U-test was used for test of significance.

which established as resident in the infants showed a three times higher mean MR adherence to HT-29 cells than the other maternal strains, although the difference was significant only after culture on TSA (Table 4).

Identification of uropathogenic clones among *E. coli* strains expressing P fimbriae

Certain P-fimbriated *E. coli* clones have an increased tendency to cause pyelonephritis [26]. To determine whether P-fimbriated resident *E. coli* strains belonged to such previously recognized uropathogenic clones, we typed all P-fimbriated strains for O, K and H antigen expression. As seen from Table 5, only one of the strains belonged to a previously recognized uropathogenic clone (O1:K1:H7) [26], and this was a transient strain.

Table 5. *Complete serotypes of resident and transient P-fimbriated* E. coli *strains*

Complete serotype*	
Resident strains	Transient strains
OR:K-:H10	OR:K28:H4
O6:K2:H10	O77:KN:H-
O15:K2:H18	O2:K5:H-
O44:K-:H18	O75:K-:H-
O75:KN:H-	OR:K2:H18
OR:K10:H-	OR:K-:H-
O15:K2:H18	O44:KN:H18
	O21:K-:HN
	O4:K12:H40
	O1:K1:H7

All *E. coli* strains with at least one isolate expressing P fimbriae were serotyped for their O, K and H antigens.
* R, rough, i.e. no O antigen was detected. N, non-typable. -, no antigen detected.

DISCUSSION

P fimbriae recognize the Galα1-4-Galβ receptor epitope in the globoseries of glycolipids in urinary tract epithelium [27] and bind to human intestinal epithelial cells and to the colonic HT-29 cell line [9, 10]. The ability to adhere to intestinal epithelial cells may contribute to their establishment and persistence in the large intestine and, indeed, the expression of P fimbriae has been associated with persistence of *E. coli* in the large intestine of Swedish infants [14] and schoolgirls [13]. The present study of Pakistani infants confirms the association of P-fimbrial expression with intestinal persistence of *E. coli*. Thus, resident strains in the infants' rectal flora expressed P fimbriae three times more often than transient strains. Further, strains isolated from the maternal rectal flora at parturition which established as residents in the baby, expressed P fimbriae 8 times more often than strains not passing over to the infant, or only establishing transiently.

Only one of the *E. coli* strains expressing P fimbriae belonged to a previously described 'uropathogenic' clone, O1:K1:H7 strain [26]. In contrast, in a Swedish neonatal ward, 23% of the neonates colonized with P-fimbriated *E. coli* harbour such clones [28]. Further, only 15% of the strains belonged to any of the 10 'uropathogenic' O groups that predominate among

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intestinal isolates from Western countries [29]. Isolates expressing these O antigens did not show any increased persistence as compared with those carrying other O antigens. Thus, the association between P fimbriae and persistence was not secondary to an association with certain serotypes.

Strains that established during the neonatal period seemed to be especially favoured by expressing P fimbriae. Such fimbriae were found 12 times more often in resident than in transient strains during the first month of life. Receptors for P fimbriae are present on colonic epithelial cells, but also in meconium [4, 30–32], which covers the intestinal epithelium at birth. Galα1-4-Galβ-containing glycolipids may also be more abundant in the intestinal epithelium of young infants as compared to older children [33]. These factors may explain that P-fimbriated strains are especially favoured early in life.

The association between P-fimbrial expression and intestinal persistence in this study was more clear cut than in previous studies involving Swedish infants [14]. The frequency of P-fimbrial expression among the resident strains was about equal (around 25%) in Sweden and Pakistan, but transient strains less often expressed P fimbriae in Pakistan compared to Sweden. If P fimbriae are indicative for *E. coli* of human intestinal origin, this may indicate exposure to a larger variety of *E. coli* strains in the Pakistani infants, many of which may derive from animals kept in the immediate surroundings.

A number of *E. coli* adhesins other than P fimbriae confer the capacity to adhere to human colonic cells and may thereby aid in intestinal colonization [10, 34]. In the present study, the expression of mannose-resistant adhesins other than P fimbriae was, indeed, associated with persistence. Since this heterogenous group of adhesins was defined only by an ability to agglutinate human and/or chicken erythrocytes, their identity remains to be determined. Of special interest are e.g. Dr and S-fimbrial adhesins, known to adhere to intestinal epithelial cells *in vitro* [10], and to be expressed by a fraction of intestinal *E. coli* [7, 8, 35, 36]. To determine the influence of S fimbriae and Dr adhesins on intestinal persistence, the *E. coli* strains in the present study will be investigated for their possession of the *sfa* and *afa* genes, coding for S fimbriae and Dr adhesins, respectively [37, 38].

Additional adhesins that could not be detected by the erythrocyte panel could be common among *E. coli* strains from Pakistani infants. Thus, many *E. coli* strains displayed a substantial capacity to adhere to HT-29 cells, despite an inability to cause haemagglutination with the erythrocyte species used. In contrast, HA-negative strains isolated from Swedish individuals are normally nonadherent (Wold, unpublished observation).

All MR adhesins described so far are associated with pathogenicity, and the high expression of MR adhesins in *E. coli* colonizing young Pakistani infants may represent a threat to health. Interestingly, *E. coli* isolates expressing type 1 fimbriae seemed to have down-regulated their MR adhesins, as indicated by the reduced MR adherence observed among type 1-fimbriated isolates. The MS adhesins of type 1 fimbriae bind to certain mannosylated glycoproteins including the oligosaccharide moieties of secretory IgA [39], and the presence of SIgA in the intestine favours the establishment of type 1-fimbriated *E. coli* [40]. Breastfed infants carry type 1-fimbriated strains more often than bottlefed infants [41]. The preference of type 1-fimbrial expression during breastfeeding may, thus, be the reason for the reduced expression of P fimbriae and other MR adhesins in *E. coli* from breastfed infants [42, 43] which, in turn, may be partly responsible for the protective effect of breastfeeding against urinary tract infection [44, 45] and neonatal septicaemia [46, 47].

We have previously proposed that some *E. coli* adhesins associated with extra-intestinal pathogenicity may have evolved to increase the persistence of *E. coli* in its normal habitat, the large intestine [13, 48]. The findings of the present study support this hypothesis. The extra-intestinal virulence conferred by certain *E. coli* adhesins may be 'coincidental' and driven by the adaptation for the colonic ecological niche.

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