Epidemiol. Infect. (1992), **108**, 415–421
Printed in Great Britain

The importance of P and type 1 fimbriae for the persistence of *Escherichia coli* in the human gut

K. TULLUS[1], I. KÜHN[2], I. ØRSKOV[3], F. ØRSKOV[3] AND R. MÖLLBY[2]

[1] *Department of Pediatrics, Karolinska Institute, S:t Göran's Children's Hospital, S-112 81 Stockholm, Sweden*

[2] *Department of Bacteriology, Karolinska Institute, S-104 01 Stockholm, and Swedish National Bacteriological Laboratory, S-105 21 Stockholm, Sweden*

[3] *Collaborative Centre for Reference and Research on Escherichia (World Health Organisation), Statens Seruminstitut, Copenhagen, Denmark*

(*Accepted 6 December 1991*)

SUMMARY

The faecal *Escherichia coli* flora was studied in 89 infants. Each infant was followed with a mean of 12 faecal samples (range 5–21) between 0 and 18 months of age. All isolates were assayed for P fimbriae and biochemically phenotyped and the persistence of each strain (phenotype) in the infant's gut was determined. In a subset of strains the occurrence of type 1 fimbriae and adherence to HeLa cells was studied. Thirty-one per cent of isolates belonging to strains colonizing for longer than 6 months expressed P fimbriae compared to 19% of the isolates from strains colonizing 1–6 months or transient strains colonizing less than 1 month. Type 1 fimbriae and adherence to HeLa cells occurred similarly often in all groups of strains. We conclude that P fimbriae, but not type 1 fimbriae or HeLa cell adherence seemed to contribute to the ability of the *E. coli* strain to colonize the human intestine.

INTRODUCTION

The faecal flora is the most important source for bacteria causing urinary tract infections (UTI) [1, 2]. *Escherichia coli* strains that colonize the intestine might subsequently colonize the periurethral area [3]; from that source certain strains invade the normally sterile urinary tract.

It has long been known that the aerobic human faecal flora consists of two types of strains; transients and residents [4–6]. Transient strains colonize only for short periods of time while resident strains persist longer. Recent data indicate that *E. coli* strains possessing type 1 fimbriae adhere to human colonic epithelial cells, and that both type 1 and P fimbriae bind to a substance loosely associated with the colonic cell surface [7]. Thus, both these fimbriae seemed to contribute to the colonization ability of *E. coli* in the human gut.

We have made a longitudinal study of the faecal *E. coli* flora of 89 healthy infants between 0 and 18 months of age [8]. All but four of the infants harboured at least one resident strain during the study period. The strain that first colonized the infant persisted longer than any other strain [8]. We here report the rate of P

and type 1 fimbriation and the ability to adhere to HeLa cells among resident and transient *E. coli* strains and relate this to the *in vivo* colonizing ability of the strain.

MATERIALS AND METHODS

Sampling

All 89 infants attending two particular Child Health Centers and born at Danderyd Hospital during 1 year were included in the study [8]. Faecal samples were collected from the infants at 6 days of age and at every visit to the Child Health Center until the age of 18 months for infants born during the first half of the study period and until 11 months of age for the other infants. A total of 1077 faecal samples was collected with a mean of 12 samples per child (range 5–21) [8].

The faecal samples were taken by cotton swab from faecal material or per rectum, transported in Stuart's medium [9] and cultivated overnight on CLED agar [10]. From each faecal sample six colonies were isolated using a method that gives a 99% probability that the most dominant aerobic species was selected [11]. The bacteria were identified by colony appearance and the Voges-Proskauer (VP) and indole reactions [12].

Biochemical fingerprinting

All isolates with different appearance and at least two isolates from each sample were further analysed using the Pheno Plate system as previously described [13, 14]. Isolates showing a correlation coefficient of more than 0·975 when compared were regarded as identical and assigned to the same phenotype [14].

When identical isolates were found in one infant, they were regarded as being the same strain, and identical strains from different infants were assigned to the same phenotype. A total of 209 different phenotypes was found. Each of 169 phenotypes colonized only 1 child, 31 were found in 2–5 infants and 9 in more than 5 infants. A total of 374 bacterial strains were found in the different children. Each child was colonized by a mean of 4·2 strains (range 1–10) [8]. Phenotypes found in more than one infant were called C strains (common phenotypes, previously called G phenotypes) and those colonizing only one infant in the study S (single) phenotypes. The minimum persistence time in the infant's intestine of each bacterial strain was determined. *E. coli* strains that persisted for less than 1 month were defined as transient strains and those more than 1 month as resident strains.

Adherence assays

All isolates were, immediately after arrival at the laboratory, subcultured on colonization factor antigen (CFA) agar [15] and tested for P fimbriation with the PPA-test (P-fimbriae-specific particle agglutination test) [16].

One isolate from all resident strains colonizing the infants longer than 6 months together with a similar number of transient strains were also analysed for their ability to cause mannose-sensitive haemagglutination (MSHA) and for their ability to adhere to HeLa cells. The haemagglutination was performed in round-bottomed microtitre plates using suspensions of 10^9 bacteria per ml in PBS. To each of the wells 50 μl of erythrocyte suspension and 50 μl of PBS or 2% D-mannose in PBS were added. The haemagglutination was determined after incubation at 4 °C for 1 h.

Table 1. *Percentage of isolates of each bacterial strain that expressed P fimbriae in relation to the persistence time of the strain in the faecal flora*

	Number of strains	Percentage of P fimbriated isolates within each strain					
		0	1–25	26–50	51–75	76–99	100
Transient*	265	78	5	3	1	0	13
Resident*							
1–6 months	68	71†	0	9	3	0	18
> 6 months	41	56‡	7	0	10	10	17
Total no.	374	75	4	4	2	0·5	14

* For definition see Materials and Methods.

† $P < 0{\cdot}05$ strains colonizing 1–6 months compared to transient strains.

‡ $P < 0{\cdot}001$ strains colonizing more than 6 months compared to the other two groups of strains.

Adhesion to HeLa cells was tested according to a method described by Cravioto and co-workers [17]. HeLa cells were grown in chambers 10 × 10 mm on microscope slides, bacterial suspensions were added to each well and the slides were incubated for 3 h at 37 °C in an atmosphere of CO_2 5% v/v. After washing with Hank's balanced salt solution, fixation with ethanol 70% and Giemsa staining, the slides were dried in air and observed by light microscopy for attachment of bacteria to HeLa cells.

Serotyping

In order to evaluate the phenotyping system 2 isolates from 33 of the strains colonizing for more than 6 months were serotyped. One isolate was chosen from the first time the strain was found in that infant and one isolate from the last occasion. If the strain varied in the expression of P fimbriae one isolate expressing P fimbriae and one which did not were serotyped. The serotyping was carried out according to standard methods [18].

Statistical analyses

The Chi-square test was used for the statistical analyses.

RESULTS

The expression of P fimbriae was variable for some of the bacterial strains (Table 1, Fig. 1). In Table 1 the relation between the duration of colonization of the infants and the rate of isolates from each strain that expressed P fimbriae is shown. The proportion of strains that never expressed P fimbriae was highest (78%) among the transient strains compared with strains that colonized for longer than 6 months (56%) ($P < 0{\cdot}001$).

The expression of P fimbriae varied most for the strains with longest persistence times, among which 73% were stably P positive or P negative compared to 91% for the transient strains (Table 1). This variation can in part be explained by the greater variability in P fimbriae expression that was found when more isolates of the same strain were analysed (Table 2). Forty-one per cent of the strains from

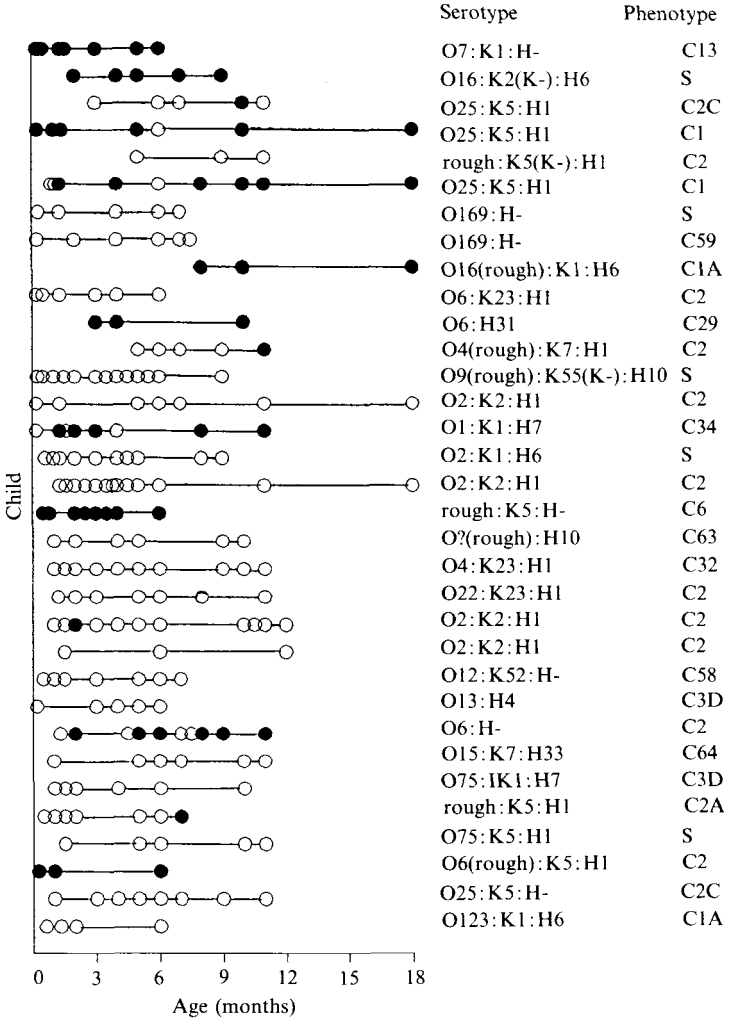

Fig. 1. Colonization patterns with *E. coli* strains persisting more than 6 months in the infant's intestine. Filled circles denotes isolates expressing P fimbriae and open circles isolates not expressing P fimbriae.

which more than 10 isolates were tested varied in their expression while only 5% of the strains tested less than 5 times did so.

To overcome the problems with variable P fimbriae expression the following analyses were made for isolates and not for strains. The rate of isolates expressing P fimbriae was significantly higher among the strains colonizing longer than 6 months compared with other strains (Table 3; $P < 0{\cdot}001$) while no difference was found between the strains colonizing less than 1 month and those colonizing 1–6 months. Two hundred and four (22·8%) of the 896 C strains (i.e. those strains that colonized more than one of the 89 infants studied) express P fimbriae compared to 72 (18·2%) of the 395 S strains (colonizing only one infant in the study). No statistically significant differences were found in the occurrence of MSHA or HeLa adherence between the different groups of strains (Table 4).

The serotypes were generally very stable among the isolates of the same

https://doi.org/10.1017/S095026880004992X Published online by Cambridge University Press

Table 2. *Relation between the percentage of P fimbriated isolates of each bacterial strain and the number of isolates tested of that strain*

Number of isolates tested from each strain	Total no. of strains	Percentage of P fimbriated isolates per strain					
		0	1–25	26–50	51–75	76–99	100
> 10	39	19	9	1	4	2	4
5–10	39	28	3	2	3	0	3
< 5	296	233	3	12	1	0	47

Table 3. *Expression of P fimbriae in relation to the persistence in the faecal flora*

	Total number of isolates	Number (%) of P fimbriated isolates
Transient*	300	56 (18·7)
Resident*		
Colonizing 1–6 months	739	141 (19·1)
Colonizing > 6 months	252	79 (31·3)
Total number	1291	276 (21·4)

* For definition see Materials and Methods: strains only colonizing at the last sampling occasion excluded from the analyses.

Table 4. *Mannose-sensitive haemagglutination (MSHA) and adherence to HeLa cells in relation to persistence in the faecal flora*

	Total number of isolates	MSHA percentage positive strains	HeLa percentage positive strains
Transient*	34	50†	32
Resident*			
> 6 months	35	37†	40
Total no.	69	43	36

* See Materials and Methods for definition.

† No statistical difference was found between the two groups. $P > 0{\cdot}05$.

phenotype. In 26 of the strains the first and the last isolate had exactly the same serotype while in 7 strains a difference in the O or K type was found (Fig. 1). This difference was always due to a loss or gain in the expression of the serotype (e.g. O16:K1:H6 became rough:K1:H6) and in no instance was a completely different serotype found. O-groups which have previously been associated with urinary tract infections (O1, O2, O4, O6, O7, O16, O18 and O75) [19] were found among 16 (46%) of the strains. In 10 strains most isolates were P fimbriated (Fig. 1). Five of these strains belonged to previously described pyelonephritogenic serotypes. O1:K1:H7, O6:K5:H1, O7:K1:H-, O16:K1:H6 and O16:K2:H6. The expression of P fimbriae was very high among the isolates of these strains and all isolates from 4 strains and 5 out of 8 isolates of the O1:K1:H7 strain expressed P fimbriae.

DISCUSSION

In the present study we followed the faecal *E. coli* flora of 89 infants for 12–18 months with 1077 faecal samples. The expression of P fimbriae in fresh faecal

isolates from infants was found to correlate with persistence of the strains in the faecal flora. Strains colonizing for longer than 6 months expressed P fimbriae more often than the other strains. There was also a tendency that strains colonizing several infants (thus seemingly of greater colonizing ability) more often expressed P fimbriae. The occurrence of type 1 fimbriae and adherence to HeLa cells did not differ between resident and transient strains.

Our data are in general agreement with those of Wold and coworkers (personal communication). In a study of the *E. coli* flora of 13 schoolgirls she found that 19% of resident strains expressed P fimbriae compared with only 2% of transient strains. Our somewhat higher rates may be due to the fact that we tested fresh isolates. Similar to our study, Wold found no difference for type 1 fimbriae.

Although the differences in the expression of P fimbriae reached statistical significance, the differences from a biological point of view are quite small. Thirty per cent of isolates with very long persistence in the faecal flora of the infants expressed P fimbriae compared to close to 20% of the other isolates. These results are somewhat surprising as adhesion to the gut wall has been thought to be one important factor for the organism to colonize and persist [20, 21]. Wold and colleagues found in a previous study that type 1 fimbriated strains bound both to the colonic cells and to a substance loosely associated to the epithelium while P fimbriated strains interacted with the loosely associated substance but not with the colonic cells [7]. Our results, thus, suggest that factors other than the adhesive properties of the bacteria are also of importance in colonization of the human gut. One such factor could be the ability of the bacteria to grow in mucus [22].

It is well known that P fimbriae are subject to phase variation [23] and this explains the variable expression of P fimbriae seen in some of our strains. Our isolates were tested immediately after arriving in the laboratory but after cultivation on a medium (CFA agar) known to enhance the production of P fimbriae. Similar results have been found in strains collected from a follow-up of elderly women with asymptomatic bacteriuria. In that study the expression of both type 1 and P fimbriae varied in 19 and 25% of the strains, respectively [24]. In our material the percentage of strains that were stably P positive or P negative, 89%, was surprisingly high.

In conclusion we found that the expression of P fimbriae, in contrast to type 1 fimbriae and to the property of adhesion to HeLa cells, was more common in strains with very long persistence (> 6 months) in the faecal flora of infants than in strains with shorter persistence. P fimbriae but not type 1 fimbriae therefore seemed to contribute to the ability of an *E. coli* strain to colonize the human intestine.

ACKNOWLEDGEMENTS

We thank Lena Gezelius and Mohammad Katouli for skillful technical assistance and Stefan B. Svenson for providing the PPA-kit.

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