

## Characterisation of *Escherichia coli* adhesins in patients with symptomatic urinary tract infections

L CONVENTI,\* G ERRICO,† S MASTROPRIMIANO,† R D'ELIA,† F BUSOLO\*

From the \*Institute of Microbiology and †Department of Paediatrics, Faculty of Medicine, Padua University, Padua, Italy

**SUMMARY** The ability of bacteria to adhere to the epithelial cells of hosts has been shown to be mediated by adhesins. Many of these show readily demonstrable haemagglutinating activity. Of 109 *Escherichia coli* strains isolated from patients with symptomatic urinary tract infection, 11 (10.1%) were identified by their haemagglutinating properties as being P fimbriated, which was confirmed by the latex bead test. Other classes of adhesins, termed X and "other", were found in mannose resistant haemagglutinating *E coli* strains, which represented 4.6% (5) and 0.9% (1), respectively, of all the strains.

Type 1 fimbriae were found in 40.4% (44/109) of *E coli* strains grown on colonising factor agar (CFA) medium. This incidence was 12.8% higher (53.2%, 58/109) when the strains were grown on CFA supplemented with urea, which suggested that urea may modulate the expression of type 1 fimbriae. Conversely, this phenomenon was not seen in P fimbriated *E coli*.

Assays using trypsinised and non-trypsinised human erythrocytes showed no difference in the percentage of strains that haemagglutinated.

Regarding the clinical correlation of fimbriated *E coli* strains, the X mannose resistant haemagglutinating adhesins were also found to be of clinical relevance. P fimbriated *E coli* strains were isolated from five out of the eight patients with pyelonephritis.

Adherence to the host's epithelium has been recognised as an important initial step in bacterial infection. It is often mediated by fimbriae that recognise specific receptors in the epithelial cells, as has been shown with *Escherichia coli*.<sup>1</sup> Some types of fimbriae are well known, as is the cell receptor responsible for their attachment. The most common, type 1, fimbriae bind cellular receptors that contain  $\alpha$ -methyl-mannoside, and P fimbriae bind to neutral glycolipids of the globoseries, including globotetraacylceramide (minimal active receptor is  $\alpha$ -D Galp(1-4)- $\beta$ -Galp).<sup>2</sup> The virulence of *E coli* that causes pyelonephritis has been reported as being mediated by P fimbriae.<sup>3,4,5</sup> Other types of fimbriae have been described,<sup>6,7</sup> but their role is still under investigation.

A practical approach to studying the various types of fimbriae is to evaluate either their haemagglutination capacity or the competitive inhibition of the

reaction by receptor analogues. These techniques seem to be useful diagnostic tools for screening P fimbriated *E coli* strains from urinary tract infections, though several haemagglutination assays with erythrocytes obtained from different mammals are required.<sup>8</sup> To avoid these time-consuming tests, a simple and rapid commercial latex test kit may be used to identify P fimbriated strains.

*E coli* may harbour mannose resistant human haemagglutinins, generally termed X adhesins, that recognise receptors other than the P antigen of human erythrocytes.<sup>9</sup> Furthermore, mannose resistant adhesins defined as "others", which are characterised by haemagglutinating human erythrocytes, have been reported by some authors.<sup>10</sup> Evans *et al* also included types V G, VI G, and VII D in this group.<sup>11</sup>

The aim of the present study was to investigate in our patients: (1) either the incidence of the P and other types of fimbriated *E coli* strains or their clinical correlation with various forms of urinary tract infection and (2) the fimbrial "shift" phenomenon, which is mediated by urea.

Address for reprints: Dr L Conventi, Istituto di Microbiologia, Università' di Padova, Via A Gabelli 63, 35121 Padova, Italy

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## Patients, materials, and methods

### PATIENTS

We studied 109 patients with symptomatic urinary tract infection in Rovigo Hospital, Rovigo, Italy, from December 1986 to June 1987. They were different ages: 10 were children in the paediatric ward; 69 were adults, 40 of whom were pregnant women in the obstetric ward, and the other 29 were outpatients; and 30 were catheterised patients in the geriatric ward. Regarding the level of infection, six children had acute primary pyelonephritis, one of whom was an infant with grade IV vesicoureteral reflux; the other four children had recurrent cystitis. Only two pregnant women had primary pyelonephritis; the other adult and elderly patients had first episodes of cystitis.

### BACTERIA AND CULTURE MEDIA

We studied for the presence of fimbriae 109 strains of *E. coli* isolated from patients with symptomatic urinary tract infection. The strains were grown for 24 hours on colonising factor agar (CFA) containing 1% casamino acids (Difco, USA), 0.15% yeast extract (Difco), 0.005% magnesium sulphate, and 0.005% manganese chloride in 2% agar, as suggested by Evans *et al.*<sup>11</sup> In some experiments this medium was supplemented with 0.25% urea according to Ofek and Maayan.<sup>12</sup> *E. coli* strains C134, C1254, C1979, and C1212 (kindly supplied by Drs I and F Orskov of the Statens Serum Institut, Copenhagen, Denmark) were used as P fimbriated controls.

### ERYTHROCYTES

The cells were separated by low speed centrifugation, washed twice with 0.15 mol/l sodium chloride, and suspended to 4% in phosphate buffered saline (PBS) solution (0.15 mol/l sodium chloride and 0.02 mol/l phosphate buffer, pH 7.2). Guinea pig erythrocytes were used to show mannose sensitive haemagglutination. Erythrocytes from the human A blood group, previously trypsinised as described by Salit and Gotschlich,<sup>13</sup> were used to show mannose resistant haemagglutination fimbriated strains.

### HAEMAGGLUTINATION TEST

Bacterial cells suspended in PBS (pH 7.2) to an optical density of 0.4 using a wavelength of 540 nm, were mixed with an equal volume (20  $\mu$ l) of erythrocyte suspension on a glass slide at room temperature by intermittent rocking. Results were recorded after one minute as being positive or negative. The reaction was defined as being mannose resistant if the degree of haemagglutination was not affected by adding 1%  $\alpha$ -methyl-mannoside (Sigma, USA) to the 4% erythrocyte suspension, and was defined as mannose sen-

sitive if haemagglutination was inhibited or grossly reduced by the presence of mannoside.

### LATEX AGGLUTINATION TEXT

To identify P fimbriated *E. coli* strains we used a latex test kit (Kabivitrum, Sweden). In this test P fimbriae specific carbohydrate receptors are covalently bound to latex beads. When mixed with the latex suspension, P fimbriated *E. coli* give a strong agglutination reaction within seconds.

## Results

Table 1 shows that haemagglutination with guinea pig erythrocytes gave 58 (53.2%) positive reactions, compared with 50 (45.9%) obtained with human erythrocytes. All strains agglutinating guinea pig erythrocytes were mannose sensitive, whereas only 15 (13.8%) were mannose sensitive when agglutinated with human erythrocytes. Strains agglutinating human erythrocytes often did not agglutinate guinea pig erythrocytes, and vice versa.

After these preliminary studies, we undertook some experiments to analyse the influence of urea added to the growth medium, as suggested by Ofek and Maayan.<sup>12</sup> To do this we grew the *E. coli* strains on CFA and on CFA supplemented with 0.25% urea. Table 2 shows that 13% more guinea pig erythrocytes agglutinated with strains grown on CFA medium and urea than with the same strains grown in urea free medium ( $\chi^2 = 3.11$ ;  $p > 0.05$ ). Moreover, bacteria grown on CFA medium and urea produced more evident haemagglutination. To investigate the ability of urea to induce the type 1 gene expression in P fimbriated *E. coli*, 11 strains were cultivated by 15 consecutive passages on CFA medium with and without urea. Three out of the 11 P fimbriated strains also harboured type 1 fimbriae. The haemagglutination test performed using guinea pig erythrocytes showed that only these three positive strains were also positive after passage in medium that contained urea. Regarding treating erythrocytes with enzymes, the numbers of *E. coli* strains that showed haemagglutination activity were 54 (49.5%) for non trypsinised and 51 (46.8%) for trypsinised human erythrocytes. With trypsinised erythrocytes stronger reactions were

Table 1 *Haemagglutination of 109 strains of Escherichia coli with guinea pig and human erythrocytes*

|                   | No (%) of strains haemagglutinating with: |                    |
|-------------------|---|--------------------|
|                   | Guinea pig erythrocytes                   | Human erythrocytes |
| Mannose sensitive | 58 (53.2)                                 | 15 (13.8)          |
| Mannose resistant | 0   | 35 (32.1)          |

Table 2 Influence of urea and of trypsinisation of human erythrocytes on haemagglutination of 109 *Escherichia coli* strains

| Medium       | No (%) of strains haemagglutinating with: |                    |             |
|--------------|---|--------------------|-------------|
|              | Guinea pig erythrocytes                   | Human erythrocytes |             |
|              |   | Non-trypsinised    | Trypsinised |
| CFA          | 44 (40.4)                                 | 54 (49.5)          | 51 (46.8)   |
| CFA and urea | 58 (53.2)                                 | 54 (49.5)          | 51 (46.8)   |

CFA = colonising factor agar.

observed, but this method yielded three strains lacking haemagglutination ability.

Table 3 shows the types of mannose resistant adhesins classified on the bases of the results of agglutination tests. Eight (7.3%) of the 109 *E. coli* strains were P fimbriated, X adhesins were found in five (4.6%), and "other" adhesins alone in one strain. Strains can express various types of fimbriae, which makes their classification difficult. Three strains harboured type 1 and P fimbriae, and 19 (17.4%) harboured "other" and type 1 fimbriae.

Table 4 shows the clinical correlation between the type of adhesin and level of urinary tract infection. In our paediatric population three children (one of whom had vesicoureteral reflux) were affected by pyelonephritis caused by P fimbriated *E. coli*, three were affected by pyelonephritis caused by strains harbouring X fimbriae, and four had cystitis caused by type 1 fimbriated strains. The two pregnant women with pyelonephritis were colonised by P fimbriated *E. coli*. From the other patients with cystitis, of both sexes and all ages, we isolated the following strains: three autoagglutinating, 34 without demonstrable haemagglutinating activity, one with "other", and 58 with type 1 or mixed types of fimbriae.

Table 3 Types of mannose resistant adhesins demonstrable by haemagglutination with human erythrocytes in 109 *Escherichia coli* strains isolated from patients with urinary tract infection

| Type of adhesin:                  | No (%) of strains | References |
|-----------------------------------|-------------------|------------|
| P                                 | 8 (7.3)           | 5          |
| X*                                | 5 (4.6)           | 16         |
| "Other"†                          | 1 (0.9)           | 10         |
| Type 1 and P                      | 3 (2.8)           |            |
| Type 1 and "other"                | 19 (17.4)         |            |
| Autoagglutinating                 | 3 (2.7)           |            |
| No demonstrable haemagglutination | 34 (31.2)         |            |

\*All mannose resistant adhesins not including P fimbriae.

†Mannose resistant fimbriated strains agglutinating either human or guinea pig erythrocytes not including P fimbriae.

Table 4 Clinical correlation between type of adhesin harboured by *Escherichia coli* strains and level of urinary tract infection

| Type of adhesin:              | No (%) of strains from patients with: |                    |
|-------------------------------|---------------------------------------|--------------------|
|                               | Pyelonephritis (n = 8)                | Cystitis (n = 101) |
| I                             | 0                                     | 58*                |
| P                             | 5 (62.5)                              | 3                  |
| X                             | 3 (37.5)                              | 2                  |
| "Other"                       | 0                                     | 1                  |
| No demonstrable agglutination | 0                                     | 34                 |
| Autoagglutination             | 0                                     | 3                  |

\*Type 1 fimbriae only in 36 strains, type 1 and P or type 1 and "other" in 22.

## Discussion

The incidence (53.2%, 58/109) of the so-called "common", type 1, fimbriae that are apparently unrelated to the virulence of *E. coli* was lower than reported previously.<sup>13</sup> The incidence of P fimbriated strains (10%), however, was similar to their epidemiological distribution in Europe.<sup>14</sup> A clear correlation was established by Svenson *et al* between P fimbriation and its high incidence (more than 90%) in *E. coli* strains isolated from children with acute, febrile, and non-obstructed pyelonephritis.<sup>15</sup> In our paediatric population, however, the incidence of P fimbriation (50%) was lower than that reported by other authors.<sup>15,16</sup> Those authors indicated incidence of 10% for X adhesins and 5.2% for "others", but in our patients the incidences were 4.6% and 0.9%, respectively. If mixed fimbriae were considered, the incidence of "other" adhesins rose to 18.3%. X fimbriated *E. coli* strains were found in three out of eight patients affected by pyelonephritis (37.5%). According to other authors, these data suggest that mannose resistant adhesins may play an important part in human pyelonephritis.<sup>6</sup>

Ofek and Maayan found that *Klebsiella pneumoniae* can shift from a non-fimbriated to fimbriated phenotype, depending on the presence of urea in the growth medium.<sup>12</sup> We also studied the activity of urea in modulating the haemagglutination properties of our *E. coli* strains. Such a phenomenon was appreciable (40.4% versus 53.2%) in strains haemagglutinated with guinea pig erythrocytes, although this difference was not significant. Our findings indicated that this effect seemed to be limited to only the strains that were genetically able to produce type 1 fimbriae. That was because no P fimbriated strains became able to agglutinate guinea pig erythrocytes, even when grown in medium with urea, which suggested that urea did

not induce type 1 fimbriae codification in *E coli* strains carrying P fimbriae.

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