INFECTION AND IMMUNITY, Jan. 1984, p. 149–155
0019-9567/84/010149-07$02.00/0
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Vol. 43, No. 1

P-Fimbriated Clones Among Uropathogenic *Escherichia coli* Strains

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Received 6 July 1983/Accepted 5 October 1983

A total of 237 *Escherichia coli* strains isolated from the urine of patients with various forms of urinary tract infection or from feces of healthy children were analyzed for O group, possession of K1 capsule, type 1 fimbriae, P fimbriae, X adhesin, and production of hemolysin. Some of the strains were also analyzed for K and H antigens, outer membrane protein pattern, and plasmid content. P fimbriation, hemolysin production, and certain O groups were found to be significantly correlated with pyelonephritogenicity. Possession of type 1 fimbriae or of K1 capsule or plasmid content did not significantly correlate with virulence. Outer membrane protein patterns in 139 strains of the more common O groups were analyzed. Only one to three patterns, which varied between serotypes, were usually found within any one O group. Distinctive groups (clones) were found when the strains were grouped according to complete serotype, fimbriation, hemolysin production, and outer membrane protein pattern; also, the mean number of plasmids was typical of the strains in a given clone. Seven clones associated with pyelonephritis were found; together they accounted for 57% of the O serotypable strains from the pyelonephritis patients. The seven clones were P fimbriated but differed in their serotypes as follows: O1:K1:H7, O4:K12:H1, O4:K12:H5, O6:K2:H1, O16:K1:H6, or O18ac:K5:H7. All O1:K1:H7 strains observed fell into two clones according to the presence or absence of type 1 fimbriae and hemolysin production. One clone associated with cystitis was also found; this consisted of O6:K13:H1 strains lacking P fimbriae. Not a single representative of these eight clones was found among the fecal strains from the healthy children. They are proposed to represent virulent clones with special ability to cause human urinary tract infection.

Properties reportedly associated with virulence in uropathogenic *Escherichia coli* strains include certain O and K antigens (15, 29), production of hemolysin, and resistance to serum bactericidal activity (3, 5). Strains causing severe disease, i.e., pyelonephritis, adhere to human uroepithelial cells and cause mannose-resistant (MR) hemagglutination (HA) of human erythrocytes more frequently than strains from other levels of urinary tract infections (UTIs) or from feces of healthy children (16, 37). The adhesion is mediated by P fimbriae (20) that recognize a specific structure on target cells, the α-D-Gal-(1-4)-β-D-Gal moiety of the P blood group antigens (17). In addition, some less frequently occurring MR binding specificities, provisionally termed X adhesins (39), have been described in *E. coli* strains associated with human UTI (33, 40). Type 1 fimbriae that bind to mannosides (19) occur frequently on all *E. coli* strains and are seemingly unrelated to bacterial virulence in human UTI (11).

E. coli strains are serotyped according to their lipopolysaccharide, capsular antigens, and flagellar antigens into O, K, and H serotypes, respectively (7, 18, 29). Production of hemolysin and colicins, plasmid content, metabolic properties, and electrophoretic migration of outer membrane proteins have also been used for studying the relatedness of *E. coli* strains (1). On the basis of such parameters, bacterial isolates have been grouped into clones, i.e., groups of strains with more or less homogeneous properties (1, 25, 26). Such clones have been suggested to be of common evolutionary origin, although the possibility of convergent evolution based on the selective value of certain combinations of properties has not been excluded. As early as 1946 (36) it was suggested that special O:K:H serotypes were associated with extraintestinal infections. It was later shown that certain O:K:H types had a special connection to pyelonephritis (24), and recently the clonal origin of such types from pyelonephritis was suggested (28). The notion that virulence is a consequence of certain independent factors occurring together in highly defined bacterial clones is extended and supported by the present examination.

MATERIALS AND METHODS

Bacteria. A total of 237 *E. coli* strains were isolated from the urine of girls with acute pyelonephritis (n = 67), cystitis (n = 60), or asymptomatic bacteriuria (ABU) (n = 60) attending Aurora Hospital, Helsinki, between 1977 and 1982. Clinical symptoms and signs, ages, and previous histories of the patients have been described (8, 39; J. Elo, L. G. Tallgren, V. Väisänen, T. K. Korhonen, S. B. Svenson, and P. H. Mäkelä, Proceedings of the XIX Congres Societe Internationale D'Urologie, San Francisco, 1982, in press). In addition, 50 *E. coli* strains were isolated in 1982 from the feces of healthy children in Helsinki. Only one strain per person was used. The strains, stored in nutrient agar stabs at room temperature, were subcultured twice on nutrient agar plates for HA assays and twice in static broth for yeast cell agglutinations as previously described (19, 20, 39). For the isolation of cell envelopes and plasmids, the bacteria were cultured overnight in broth, diluted 1/10 in broth, and grown in flasks on a rotary shaker at 37°C to late-exponential growth phase.

Agglutination tests. MR HA was tested with human OP_1 and $O\bar{p}$ erythrocytes as previously described (20, 39). A strain agglutinating human OP_1 but not $O\bar{p}$ erythrocytes in an MR manner was defined as having P fimbriae, and one

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agglutinating both OP_1 and $O\bar{p}$ was defined as having X adhesin (39). The occurrence of P fimbriae on an X-positive (X^+) strain was determined by using a P-specific particle-agglutination kit (38). Type 1 fimbriation was determined by mannose-sensitive agglutination of *Saccharomyces cerevisiae* cells (OY ALKO AB, Helsinki, Finland) as already described (19). To measure MR HA only, all HAs were performed in the presence of 5% (wt/vol) α-methyl-D-mannoside (Sigma Chemical Co., St. Louis, Mo.).

Serotyping. O grouping (18) of the strains was performed in Helsinki in microtiter plates with 19 anti-O sera (Table 2) selected on the basis of reported association with urinary tract infections (29). The possession of K1 antigen was tested by latex agglutination (22) by using particles coated with immunoglobulins from the serum of horse 46 (received from J. B. Robbins, National Institutes of Health, Bethesda, Md.) immunized with *Neisseria meningitidis* group B. In addition, 87 of the strains were completely serotyped in Copenhagen.

Hemolytic activity. The production of hemolysin was tested on sheep blood agar plates. Strains having a clear halo after overnight culture at 37°C were defined as hemolysin positive.

Outer membrane proteins. Cell envelope extracts, mostly outer membranes, were obtained after light sonication (32). Samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis by the method of Laemmli (21), using 12% slab gels. The gels were stained with Coomassie brilliant blue R250. Proteins with apparent molecular weights between 30,000 (30K) and 43K were defined as major outer membrane proteins. Among these, OmpA protein was identified by its heat modifiability and its sensitivity to trypsin (1, 13, 14, 30); in sodium dodecyl sulfate-polyacrylamide gel electrophoresis it migrated fastest of the major outer membrane proteins. Protein K, which is associated with encapsulated *E. coli* strains, was identified by its resistance to trypsin and by lack of expression on cells grown at 28°C (1, 30, 31). Major proteins larger than OmpA or K were defined as porins (1, 13, 14, 30). They were resistant to trypsin and to heat and were dominant porin-like proteins on bacteria grown at 28°C. We also checked the outer membrane protein patterns of our K1 and K5 bacteria against those of the K1 and K5 clones of Achtman et al. (1).

Plasmid analysis. Plasmid DNA was isolated by using the method of Birnboim and Doly (2) with the slight modifications of Berman et al. (M. L. Berman, L. W. Enquist, and T. J. Silhavy, Course in Advanced Bacterial Genetics, Cold Spring Harbor Laboratory, 1981) and analyzed in a 1% agarose gel as described in reference 12. The differentiation between large and small plasmids was based on traces of chromosomal DNA (about 25 kilodaltons) so that plasmids larger than that were defined as large and the others small.

Statistical methods. The chi-square test was used to test for significant differences in proportions. A probability of <0.01 was termed significant.

RESULTS

Occurrence of adhesins. MR HA was significantly more frequent among strains isolated from the pyelonephritis patients (87%) than among strains from the other diagnostic groups (22 to 35%) (Table 1). Since MR HA may be caused by P fimbriae or a number of different X adhesins (20, 33, 39, 40), we distinguished these alternatives by using OP_1 and $O\bar{p}$ erythrocytes and the P-specific particle-agglutination kit. P fimbriation was significantly more frequent among pyelonephritis strains (76%) than among other strains (16 to 23%). Also, X adhesins occurred more frequently among pyelonephritis strains than among others (15% versus 6 to 13%), but the differences were not statistically significant, probably due to the small number of X^+ strains (totally 26 strains). It should be noted that five strains had both P fimbriae and X adhesin; three of them were from pyelonephritis patients.

TABLE 1. HA, hemolytic activity, and occurrence of K1 capsule in *E. coli* strains

Property	Occurrence (%) in all strains (n = 237)	Occurrence (%) in each diagnostic group			
		Pyelonephritis (n = 67)	Cystitis (n = 60)	ABU (n = 60)	Fecal (n = 50)
MR HA capacity	44	87[a]	35	25	22
P fimbriae	35	76[b]	23	18	16
X adhesin	11	15	13	8	6
Type 1 fimbriae	84	91	83	82	76
No MR HA adhesin or type-1 fimbriae	11	0[b]	12	15	18
Hemolysin production	30	60[a]	27	17	10
K1 capsular antigen	23	31	15	23	22

[a] $P < 0.001$, pyelonephritis versus cystitis, ABU, and fecal isolates.

[b] $P < 0.01$ pyelonephritis versus cystitis isolates, $P < 0.001$ pyelonephritis versus ABU and fecal isolates.

The majority of the strains (84%) produced type 1 fimbriae (Table 1). The frequency of type 1-fimbriated strains was slightly higher in the pyelonephritis group than in the other diagnostic groups, but the difference was not significant. It is interesting to note that none of the pyelonephritis strains was totally devoid of detectable adhesins, whereas 12 to 18% of the strains in the other groups lacked such adhesins.

Production of hemolysin. Hemolytic activity was significantly more frequent among pyelonephritis strains (60%) than among others (10 to 27%) (Table 1).

K1 antigen. About one-third (31%) of the pyelonephritis strains had the K1 capsule; this was only slightly more than in other diagnostic groups (15 to 23%) (Table 1).

O antigens. O grouping was performed with 19 antisera (Table 2). Grouping of O antigens in Table 2 was done on the basis of their occurrence among pyelonephritis strains. Thus, O groups 1, 2, 4, 6, and 16 comprised 58% of all strains isolated from pyelonephritis, whereas their proportion was 20 to 22% among strains from the other groups. Strains belonging to O groups 7, 18, and 75 were equally frequent (12 to 16%) in all diagnostic groups. Almost half of the fecal strains but only 16% of the pyelonephritis strains were nontypable with the antisera used. Strains belonging to O groups 8, 9, 22, 25, 50, 77, 83, 85, and 86 were significantly less frequent in pyelonephritis (3%) than in the other groups (15 to 23%). About 10% of the strains in each diagnostic group were rough or panagglutinating.

Association of O groups with agglutinins, hemolysin production, and K1 capsule. A high proportion of strains with O groups associated with pyelonephritis were P fimbriated (Table 3). Thus, 76% of the O1 strains as well as 54% of O2, 89% of O4, 54% of O6, and 100% of O16 strains had P fimbriae. In addition, P fimbriation was common in O groups 7 (83%) and 18 (62%), but less frequent in the other groups, including the rough (panagglutinating) and nontypable strains.

X adhesins occurred mainly in O groups 2 (31% of the strains had X) and 75 (44%). In addition, a few strains of O

TABLE 2. Frequency of O groups in diagnostic groups

O group	No. of strains	Occurrence (%) in all strains (n = 237)	Occurrence (%) in each diagnostic group			
			Pyelonephritis (n = 67)	Cystitis (n = 60)	ABU (n = 60)	Fecal (n = 50)
1,2,4,6,16	73	31	58[a]	20	22	20
7,18,75	34	15	15	13	16	12
8,9,22,25,50,77,83,85,86	33	14	3[b]	23	15	16
NT[c]	74	31	16[d]	32	35	46
Rough or panagglutinating	22	9	8	12	12	6

[a] $P < 0.001$, pyelonephritis versus cystitis, ABU, and fecal isolates.
[b] $P < 0.001$, pyelonephritis versus cystitis isolates.
[c] NT, Not typable with the antisera to the O groups mentioned, nor groups O100 and O119.
[d] $P < 0.001$, pyelonephritis versus fecal isolates.

groups 1, 4, 6, 18, and 85 had X adhesins. About 10% of nontypable or rough strains had X adhesins.

The frequency of type 1-fimbriated strains in O groups 1 and 25 and in the rough strains was low (50 to 55%) compared to the other groups, in which 67 to 100% of the strains had type 1 fimbriae.

Production of hemolysin was, as was P fimbriation, associated with the O groups that were most frequent in pyelonephritis (Table 3); more than half of the hemolytic strains belonged to these serotypes. Hemolysin production was most frequent in O groups 4 (78% were hemolysin positive), 6 (81%), 16 (100%), 18 (62%), and 22 (57%). One-fourth to one-third (23 to 31%) of the strains with O groups 1, 2, and 75 produced hemolysin.

Although the presence of K1 capsule was not clearly correlated with virulence (Table 1), it correlated with O group. Thus, 86% of O1, 54% of O2, 80% of O7, and 100% of O16 strains were K1. It should be mentioned that none of the 9 O4 strains, only 1 of the 26 O6 strains, and 1 of the 16 O75 strains had K1 capsule.

The frequent occurrence among the strains associated with pyelonephritis of P fimbriae, hemolysin production, and O groups 1, 2, 4, 6, and 16 suggested to us that these strains might represent distinct clones of closely similar bacteria, as recently shown for K1-capsulated bacteria causing neonatal meningitis (1). For a more detailed characterization of these strains, we analyzed the outer membrane protein patterns and the plasmid content of all the strains belonging to the positively identified O groups. In addition, the complete serotypes of 87 of the strains were determined.

Outer membrane protein patterns. Among the 139 *E. coli* strains analyzed, 15 distinct protein patterns were found (Fig. 1). Both OmpA and porins were identified in all patterns, but their apparent molecular weights varied considerably from pattern to pattern. In some patterns, two porins could be distinguished. K protein was found in all patterns except in one from a noncapsulated strain (lane 10 in Fig. 1). However, the other two noncapsulated strains in our material did have K protein.

Outer membrane protein patterns were very closely associated with the O groups of the strains, and only a few outer membrane protein patterns were seen within an O group. One or two major types were usually found (Table 4). Two patterns, 1 and 5, which seemed to correlate with the virulence properties of the strains were found in O1 strains. Pattern 1 was predominant among the O1 strains associated with pyelonephritis (eight of the nine pyelonephritis strains had this pattern), whereas five of the six fecal strains were of pattern 5. In O2 strains, some of which showed patterns 1 or 3, pattern 15 was predominant (9 of 13 strains). O2:K1 strains were of pattern 1 or 15; pattern 3 occurred in all three O2:non-K1 strains. Protein pattern 2 was predominant in O4 strains (six of nine strains), and pattern 3 was predominant in O6 strains (22 of 26 strains). All four O16:K1 strains and all 16 O75 strains had pattern 4, which also occurred in O18 strains (9 of 13 strains). Four of five O7 strains had pattern 6. All five O25 strains had pattern 3, whereas in the rest of the O groups (8, 9, 22, 50, 77, and 85) the protein patterns were heterogeneous or the number of the strains was too small for reliable conclusions or both. Heterogeneity was especially apparent in O22 strains (not included in Table 4), where all eight strains had different protein patterns (lanes 3, 4, 8, and 10 through 14 in Fig. 1).

Plasmids. No correlation between plasmid content and virulence of the strains was found. In all diagnostic groups the mean number of large plasmids per strain was one, whereas the mean number of small plasmids per strain was 1.9 among the pyelonephritis strains, 2.7 among the cystitis strains, 2.4 among the ABU strains, and 2.8 among the fecal strains. Thus, the most virulent strains had the least number

TABLE 3. Frequency of adhesins and hemolysin production in O groups

O group	No. of strains	% of strains with:			
		P fimbriae (n = 85)	Type 1 fimbriae (n = 198)	X adhesin (n = 26)	Hemolysin production (n = 68)
1,2,4,6,16	73	68	81	11[a]	52
7,18,75	34	41	97	24[b]	35
8,9,22,25,50,77,83,85,86	33	9	85	3	21
NT[c]	74	14	88	9	7
Rough or panagglutinating	22	32	55	9	27

[a] Four of these eight strains were O2.
[b] Seven of these eight strains were O75.
[c] NT, Not typable.

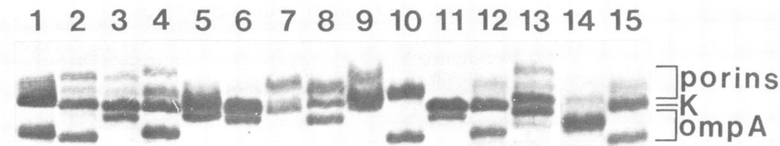

FIG. 1. Outer membrane patterns of *E. coli* strains. Identification of porins, protein K, and OmpA is described in the text. The nonidentity of patterns 1 and 15 was confirmed by running them in parallel.

of small plasmids. Generally, the plasmid contents of strains of the same O group were similar but not identical. The O1 strains were rich in large plasmids (1.3 per strain), and the O7 strains were rich in small plasmids (8.2 per strain). In contrast, the O4 strains had only a few small plasmids (0.4 per strain).

Clones. To recognize homogeneous groups of bacteria, i.e., bacterial clones, we arranged the strains by O, K, and H serotypes, fimbriation, hemolysin production, and outer membrane protein pattern. Only groups of three or more similar strains were regarded as clones. Many clones occurred among the pyelonephritis strains, and one clone that was apparently associated with cystitis was also detected (Table 5).

All the pyelonephritis-associated clones were P fimbriated. O1:K1:H7 strains grouped by type 1 fimbriation and hemolysin production fell into two clones. The two O4:K12 clones were distinguishable by their H antigen. The O6 clone associated with cystitis differed from the O6 clone associated with pyelonephritis in fimbriation (no P fimbriae in cystitis) and capsulation (K13 in cystitis, K2 in pyelonephritis).

All strains within a clone had the same outer membrane protein pattern; however, the same pattern could occur in several clones. The strains within a clone had slightly different plasmid patterns, which shows that the strains were not identical. Generally, however, the number of plasmids, especially the number of small ones, was rather similar within a clone (Table 5). In some clones, a few strains had the X adhesin, but the eight identified clones were mostly X^-. We did not include X adhesin as a criterion for a clone, since it obviously represents a group of heterogeneous adhesins (33, 40) and presently nothing is known about their genetic basis. The seven pyelonephritis-associated clones represented 29 (57%) of the pyelonephritis strains with positively identified O groups. The O75 strains, which all had the same outer membrane protein pattern, differed in their K or H antigen or hemolysin production and did not constitute a clone by the criteria used.

Although the full serotype was not available for every strain, the frequency of the clonal strains also in the other diagnostic groups could be estimated from comparisons of the known serotypes, fimbriation, hemolysin production, and outer membrane protein pattern. Only two of the strains belonging to the pyelonephritis-associated clones I and VII were found in other diagnostic groups (both were ABU strains); one pyelonephritis strain belonged to the cystitis-associated clone VIII.

DISCUSSION

Various properties have been associated with bacterial virulence in human extraintestinal infections. These possible virulence factors include certain O and K antigens (especially K1), hemolytic activity, adherence to epithelial cells, and P fimbriation (5, 15, 16, 29, 37). It has been suggested that such virulence factors and other traits occur together in well-defined phenotypes or clones (1, 27). Our results extend these observations and describe seven pyelonephritis- and one cystitis-associated clones (Table 5). The O:K:H serotypes were very similar to those described previously as pyelonephritis and cystitis types (28). In the present study the pyelonephritis-associated clones covered 43% of *E. coli* strains isolated from pyelonephritis cases in Helsinki. They are proposed to represent strains with a common evolutionary origin and to have specific properties that render them highly capable of causing human pyelonephritis.

Two properties that clearly correlated with the virulence of the strains were distinguished (Table 1): possession of P fimbriae, which mediate bacterial adhesion to human uroepithelial cells (20), and production of hemolysin. P fimbriation seems to be a major virulence factor in human childhood pyelonephritis, since all of the clones associated with pyelonephritis had P fimbriae (Table 5); one of the clones (I) lacked hemolysin production. Moreover, the O6:K13:H1 clone associated with cystitis lacked P fimbriae but produced hemolysin.

The special property of binding the bacteria to the P blood

TABLE 4. Frequency of different outer membrane protein patterns in various O groups

O group	No. of strains tested	Outer membrane protein pattern	No. of strains in each diagnostic group			
			Pyelonephritis (n = 51)	Cystitis (n = 35)	ABU (n = 31)	Fecal (n = 22)
1	21	No. 1	8		2	1
		No. 5	1	1	3	5
2	13	No. 1				1
		No. 3	1			2
		No. 15	5	2	1	1
4	9	No. 2	6	1		
		No. 3			2	
6	26	No. 1	1		1	
		No. 2	1		1	
		No. 3	12	8	2	
		No. 4			1	
7	5	No. 1		1		
		No. 6	1	1	2	
8	5	No. 7		2		
		No. 9			2	1
9	2	No. 6			1	
		No. 15				1
16	4	No. 4	4			
18	13	No. 1			2	2
		No. 4	5	2	2	
25	5	No. 3		3	2	
50	4	No. 15	1	1	1	1
75	16	No. 4	4	4	4	4
77	5	No. 1	1	1		
		No. 5		1		1
		No. 8				1
85	2	No. 4		1	1	

group-specific glycosphingolipids on human cells (20) suggest for P fimbriae a possible mechanism by which they promote an ascending infection in the urinary tract, simply by helping the bacteria to resist the normal cleansing action of urine flow in the ureters. P. fimbriae are determined by chromosomal genes and thus offer a reliable parameter for the classification of *E. coli* strains (34). In spite of reported variation in the expression of some fimbriae (35), all fimbrial types can be detected by proper subculturing.

Hemolysins, on the other hand, can have tissue-damaging properties (6), which may increase bacterial toxicity and invasiness and thereby contribute to the establishment of infection in the kidneys. A completely different virulence-enhancing mechanism of hemolysins may be their ability to increase the amount of iron available to the bacteria (23); however, it is not known whether this is a key point in human kidney infection as it apparently is in septic infection of mice (23).

Both P fimbriation and hemolysin production were found to be associated with certain O groups (groups 1, 2, 4, 6, and 16; Table 2) which have previously been reported as associated with human urinary tract infection (29). Our data suggest that the apparent association of these O groups with virulence may be indirectly due to their association with P fimbriae and hemolysin (Table 3). Achtman et al. (1) have demonstrated a clonal distribution of hemolysin production, and the data presented here as well as a recent analysis of the clones of Achtman et al. (B. Kusecek, H. Wloch, A. Mercer, V. Väisänen, G. Pluschke, T. K. Korhonen, and M. Achtman, submitted for publication) show that P fimbriation is strongly associated with certain bacterial clones. Thus, P fimbriae occurred here in O1:K1:H7, O4:K12:H1 and H5, O6:K2:H1, O16:K1:H6, and O18ac:K1:H7 clones (Table 5) as well as in the O7:K1 clone of Achtman et al., whereas they were absent in the O18:K1 clone of Achtman et al. and in our O6:K13:H1 clone (Table 5).

In our material, K1 antigen, which has earlier been reported as a virulence factor among uropathogenic *E. coli* (15), did not correlate significantly with pyelonephritis although the frequency of K1 strains was highest among pyelonephritis strains (Table 1). K1 capsule certainly is not related to virulence in human UTI as clearly as it is in newborn meningitis (1). However, the bacteria belonging to pyelonephritis clones all had acidic capsules, i.e., K1, K2, K5, or K12, and this suggests that possession of an acidic capsule may be a virulence factor in human UTI. It may increase virulence by increasing bacterial resistance to phagocytosis (4) and serum bactericidal effect (10). The possession of K2, K5, and K12 antigens was not determined for all our strains, and therefore their frequencies in the diagnostic groups could not be compared.

Another criterion found useful in defining clones was the outer membrane protein pattern. Achtman et al. (1) showed it to be a clonal property in K1-encapsulated *E. coli* strains. It has also been used in *Neisseria meningitis* serotyping (9). In the present material, the outer membrane protein patterns were closely associated with O groups so that one or two major patterns were usually found (Table 4). With the method used, 15 outer membrane protein patterns were defined (Fig. 1). OmpA, protein K, and porin(s) were tentatively identified and were found in all patterns, with the exception of protein K, which was missing in one pattern. Differences between patterns were easy to confirm by running samples in a single slab gel. We could not identify any specific outer membrane protein pattern as a virulence factor but rather their association with O groups seemed important;

TABLE 5. Proposed uropathogenic *E. coli* clones

Isolates from:	Clone	Serotype	No. of strains	No. of strains with				Outer membrane protein pattern	Mean no. of plasmids per strain (range)	
				P fimbriae	Type I fimbriae	X adhesin	Hemolysin		Large	Small
Pyelonephritis	I	O1:K1:H7	6[a]	6	6	0	0	No. 1	1.2 (1–2)	2.8 (1–6)
	II	O1:K1:H7	3	3	0	0	3	No. 1	1.7 (1–2)	2.3 (2–3)
	III	O4:K12:H1	3	3	3	0	3	No. 2	0.7 (0–1)	0
	IV	O4:K12:H5	3	3	3	0	3	No. 2	0.7 (0–1)	0.7 (0–2)
	V	O6:K2:H1	8	8	8	1	8	No. 5	0.5 (0–1)	4.1 (3–6)
	VI	O16:K1:H6	4	4	4	0	4	No. 4	1.3 (1–2)	3.0 (1–4)
	VII	O18ac:K5:H7	4[a]	4	4	0	4	No. 4	1.3 (1–2)	1.7 (1–2)
Cystitis	VIII	O6:K13:H1	5[b]	0	5	0	5	No. 5	0.8 (0–1)	2.0 (0–4)

[a] One strain was from ABU.
[b] One strain was from pyelonephritis.

however, in O1 strains the outer membrane protein pattern was different in strains from pyelonephritis cases or from feces (Table 4).

In our study, the number of plasmids per strain did not correlate with virulence. The average plasmid content was smallest in strains associated with pyelonephritis and largest in fecal strains, but the difference was small and variation between strains was large. However, a certain correlation could be seen between plasmid content and O group. Thus O7 strains were rich and O4 strains poor in plasmids. In general, the mean number of plasmids in a given clone was fairly constant (Table 5). Our ongoing studies have shown that a small plasmid (less than 2.8 megadaltons) is correlated with the X adhesins of the O75 strains (V. Väisänen-Rhen, unpublished data). Otherwise, no direct correlation was observed between possession of certain plasmids and the biological properties described here.

X adhesins were most frequent in O2 and O75 strains. We have recently demonstrated several distinct binding types among X adhesins, which therefore must be regarded as a working term for a group of adhesins. Thus, the X adhesin in one of the pyelonephritis strains described here shows blood group M specificity and binds to glycophorin A (40), whereas some X^+ strains with serotype O2 recognize neuraminyl α2-3 galactosides (33). Both of these adhesins have been tentatively identified as specific fimbriae (T. K. Korhonen and V. Väisänen-Rhen, unpublished data). Neuraminyl α2-3 galactose-binding specificity occurs in a few strains described here (33) and does not seem to correlate with virulence in human UTI but occurs more frequently among strains isolated from cases of newborn meningitis and sepsis (T. K. Korhonen, M. V. Valtonen, J. Parkkinen, V. Väisänen-Rhen, J. Finne, F. Ørskov, I. Ørskov, S. B. Svenson, and P. H. Mäkelä, submitted for publication). These binding specificities did not occur in the seven X^+ O75 strains whose receptors remain to be determined. A possible correlation of X adhesins with disease remains to be established; so far the total number of X^+ strains is too low to allow this. However, it is important to distinguish between X and P as it is obvious that MR HA of human erythrocytes is caused by several distinct binding properties of UTI-associated *E. coli*.

Type 1 fimbriae were not significantly more frequent in any diagnostic group (Table 1), nor were such fimbriae tightly associated with pyelonephritis clones; one clone (II) totally lacked type 1 fimbriae (Table 5). This is in agreement with previous reports, where no correlation between bacterial virulence in humans and type 1 fimbriae has been found (11, 27). Type 1 fimbriae have, in fact, been proposed to prevent adhesion to uroepithelial cells by trapping the bacteria in the mucus (27).

The concept of clonality in pathogenic *E. coli* strains (1, 25, 26) is a useful tool since it allows estimation of the importance of bacterial virulence factors in human UTI and simplifies the search for new virulence factors. With more strains and properties characterized, new clones will undoubtedly be identified. Similarly, the clones identified here will probably have other common features; we are currently analyzing the present strains for isoenzymes and colicin production.

ACKNOWLEDGMENTS

We thank John B. Robbins for horse 46 serum and Anna Pirkola and Bertil Cedergren for p̄ erythrocytes.

This study was supported by the Finnish Cultural Foundation, the Magnus Ehrnrooth Foundation, the Yrjö Jahnsson Foundation, the Joint Committee of the Nordic Natural Science Research Councils, and by the Swedish Medical Research Council.

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