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Characterization of Urinary *Escherichia coli* O75 Strains

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Forty-four *Escherichia coli* O75 strains from patients with urinary tract infections were characterized by a variety of methods to obtain evidence of their clonal distribution and uropathogenic properties. By K and H antigen typing, the strains were divided into the following serotypes: O75:K5:H$^-$ (18 strains), O75:K95:H$^-$ (10 strains), O75:K95:H5 (7 strains), O75:K100:H5 (4 strains), and O75:K$^-$:H55 (5 strains). Generally, biotyping proved to be of no discriminative value. With two exceptions the strains were found to be sensitive to the bactericidal effect of normal human serum. As shown by multilocus enzyme electrophoresis, the whole-cell protein profile (WCPP), and the patterns of the outer membrane proteins and lipopolysaccharides, all but the five O75:H55 strains were genetically closely related to each other and could be classified into one clonal group. The O75:K$^-$:H55 strains proved to be quite different and lacked type 1 fimbriae. All 17 K95 (H$^-$, H5) strains produced hemolysin and P fimbriae. Five of the O75:K5:H$^-$ strains were different from the other K5 strains by showing hemagglutinating properties, on the basis of the presence of the OX adhesin. The last two groups are suggested to be uropathogenic and are proposed to represent separate clonal groups or subgroups.

Escherichia coli strains have been serologically classified by many different O (somatic), K (capsular), and H (flagellar) antigens, giving rise to a large variety of different O:K:H serotypes (15). Only a relatively small number of particular O:K:H serotypes, however, were found to be associated with disease (15, 17). Serogroup O75 strains are among the most common cause of extraintestinal infections (6, 8, 11, 14, 18–20, 24–26). Usually, the O75 antigen is associated with capsular antigen K5 (6, 8–11, 19, 20, 24, 25).

Serotype O75:K95:H5 and O75:K100:H5 strains have only rarely been reported as causes of infection (16, 18, 19, 24, 25). It is possible that the pathogenicity of O75:K95 strains might have been underestimated. It is important to know, therefore, that among bacteriophage-detected K5 isolates, K95 strains may occur (9). It has recently been shown that among 44 K5 isolates, 11 strains carrying the K95 antigen could be identified by means of two bacteriophages (9).

Outer membrane proteins (OMPs) analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) are reported to be identical in O75 strains, irrespective of the strains' K and H antigens (1–3, 25). However, by multilocus enzyme electrophoresis (MLEE) distinct patterns for O75 strains have been shown (4, 19).

In the present study urinary tract *E. coli* isolates belonging to different O75:K:H serotypes were thoroughly characterized to obtain evidence of the possible existence of clonal groupings and evidence of the traits contributing to pathogenicity.

MATERIALS AND METHODS

The 44 bacterial strains were isolated from different patients with recurrent urinary tract infections (UTIs) in Rostock, Germany.

The API 20E kit (Bio Merieux SA, Lyon, France) was used for biotyping. Utilization of dulcitol, raffinose, and sorbose and a growth requirement for nicotinamide were also tested (2, 13).

Serotyping of O and H antigens was performed by bacterial agglutination techniques (11, 15).

For detection of the capsular antigens, K5-, K95-, and K100-specific bacteriophages and countercurrent immunoelectrophoresis were used (9, 10).

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

Hemagglutination (HA) of human OP1 and animal erythrocytes was performed in the presence of 1% mannose as described previously (12). Strains causing a mannose-resistant (MR) HA were defined as having P fimbriae. The specificity for P fimbriae (PF) was verified further by use of a PF-specific particle agglutination test (Orion Diagnostica, Espoo, Finland).

Resistance to the activity of serum was determined as described by Taylor (23).

The patterns of the O-antigenic lipopolysaccharides (LPS) and the OMPs as well as the whole-cell protein profiles (WCPPs) were determined by SDS-PAGE as described earlier (22). MLEE was performed in a starch gel under nondenaturing conditions as described previously. The following enzymes were tested by MLEE: aconitase; adenylate kinase; alcohol dehydrogenase; acid phosphatase; alkaline phosphatase; catalase; esterases; fumarase; glucose-6-phosphate dehydrogenase; glutamate dehydrogenase, NAD dependent; glutamate dehydrogenase, NADP dependent; glutamic oxaloacetic transaminase; glyceraldehyde phosphate dehydrogenase, NAD dependent; glyceraldehyde-phosphate dehydrogenase, NADP dependent; hexokinase; indophenol oxidase; isocitrate dehydrogenase; lactate dehydrogenase; leucine aminopeptidase; malate dehydrogenase; malic enzyme (ME; decarboxylating malate dehydrogenase); phosphoglucomutase; 6-phosphogluconate dehydrogenase; and phosphoglucose isomerase. A dendrogram was calculated by using average linkage cluster analysis.

RESULTS

The 44 *E. coli* O75 strains tested gave a very homogeneous picture when the fermentation reactions obtained by the API 20E system were considered. Only one pattern (code number 5.144.572) was found among the strains, thus giving no evidence of clonal groupings in relation to the O:K:H serotype. The substrates dulcitol, sorbose, and raffinose, which are known to be helpful in further differentiation were also tested, but even these were of no discriminative value. Only single strains had one or two different reactions.

Hemolysin production was observed only in strains of serotype O75:K95:H5/H$^-$. K5, K100 and K$^-$ strains did not show any hemolytic activity (Table 1).

MR HA of human OP1 and sheep erythrocytes was found for all K95 isolates (Table 1). The results correlated well with those of the PF test, thus providing evidence for the presence of P fimbriae in the K95 strains. All K100 and K$^-$ strains and the majority of the K5 strains proved to be negative under these conditions. However, a group of five O75:K5:H$^-$ strains was different from the others by exhibiting MR HA of human erythrocytes and by giving no reaction with sheep erythrocytes

TABLE 1. Comparison of the results of the individual typing methods[a]

Serial no.	Strain no.	Serotype	Hly	SS	MR HA	P	F1b	Xb	OMP	LPS	MLEE	WCPP	Type
1	16636	O75:K5:H$^-$	−	s	−	−	+	−	1	R	1	1	1.1
2	46/84	O75:K5:H$^-$	−	s	−	−	+	−	1	S	1	1	1.1
3	43/84	O75:K5:H$^-$	−	s	−	−	+	−	1	S	1	1	1.1
4	S 24	O75:K5:H$^-$	−	s	−	−	+	−	1	S	1	1	1.1
5	S 261	O75:K5:H$^-$	−	s	−	−	+	−	1	S	1	1	1.1
6	S 465	O75:K5:H$^-$	−	s	−	−	+	−	1	S	1	1	1.1
7	N 88	O75:K5:H$^-$	−	s	−	−	+	−	1	S	1	1	1.1
8	N 102	O75:K5:H$^-$	−	s	−	−	+	−	1	S	1	1	1.1
9	N 105	O75:K5:H$^-$	−	s	−	−	+	−	1	S	1	1	1.1
10	V 46	O75:K5:H$^-$	−	s	−	−	+	−	1	S	1	1	1.1
11	Z 32	O75:K5:H$^-$	−	s	−	−	+	−	1	S	1	1	1.1
12	Z 403	O75:K5:H$^-$	−	s	−	−	+	−	1	S	1	1	1.1
13	Z 231	O75:K5:H$^-$	−	s	−	−	+	−	1	S	1	1	1.1
14	J 29	O75:K95:H$^-$	+	s	+	+	+	−	2	R	1	1.1	1.3.1
15	Ki 37	O75:K95:H$^-$	+	s	+	+	+	−	1	S	1	1	1.3.1
16	S 98	O75:K95:H$^-$	+	s	+	+	+	−	3	S	1	1	1.3.1
17	Z 75	O75:K95:H$^-$	+	s	+	+	+	−	1	S	1	1.2	1.3.1
18	F 64	O75:K95:H$^-$	+	s	+	+	+	−	1	S	1	1	1.3.1
19	L 134	O75:K95:H$^-$	+	s	+	+	+	−	1	S	1	1	1.3.1
20	E 51	O75:K95:H$^-$	+	s	+	+	+	−	1	S	1	1	1.3.1
21	L 316	O75:K95:H$^-$	+	s	+	+	+	−	1	S	1	1	1.3.1
22	L 105	O75:K95:H$^-$	+	s	+	+	+	−	1	S	1	1	1.3.1
23	18488	O75:K95:H$^-$	+	s	+	+	+	−	1	R	1	1	1.3.1
24	E 36	O75:K95:H5	+	s	+	+	+	−	1	S	1	1	1.3.2
25	L 5	O75:K95:H5	+	s	+	+	+	−	1	S	1	1	1.3.2
26	L 59	O75:K95:H5	+	s	+	+	+	−	1	S	1	1	1.3.2
27	E 159	O75:K95:H5	+	s	+	+	+	−	1	S	1	1	1.3.2
28	E 164	O75:K95:H5	+	s	+	+	+	−	1	R	1	1	1.3.2
29	L460	O75:K95:H5	+	s	+	+	+	−	1	S	1	1	1.3.2
30	Zi 57	O75:K95:H5	+	s	+	+	+	−	1	S	1	1	1.3.2
31	F 147	O75:K100:H5	−	s	−	−	+	−	1	S*	1	1.3	1.2
32	N 175	O75:K100:H5	−	s	−	−	+	−	1	S	1	1.3	1.2
33	F 67 f	O75:K100:H5	−	s	−	−	+	−	1	S	1	1.3	1.2
34	1184	O75:K100:H5	−	s	−	−	+	−	1	S	1	1.3	1.2
35	Ki 31	O75:K$^-$:H55	−	s	−	−	−	−	1	R	2	2	2
36	N 50	O75:K$^-$:H55	−	s	−	−	−	−	4	S	3	3	3
37	N 216	O75:K$^-$:H55	−	s	−	−	−	−	4	R	3	3	3
38	J 23	O75:K$^-$:H55	−	s	−	−	−	−	4	S	3	3	3
39	R 1524	O75:K$^-$:H55	−	s	−	−	−	−	4	S	3	3	3
40	368/85	O75:K5:H$^-$	−	s	+	−	+	+	1	S	1	1	1.4
41	Z 27	O75:K5:H$^-$	−	s	+	−	+	+	1	S	1	1	1.4
42	Z 72	O75:K5:H$^-$	−	s	+	−	+	+	1	S	1	1	1.4
43	Z 86	O75:K5:H$^-$	−	r	+	−	+	+	1	S	1	1	1.4
44	Z 440	O75:K5:H$^-$	−	r	+	−	+	+	1	S	1	1	1.4

[a] Results include those for *E. coli* K test strains (14). Symbols and abbreviations: −, negative reaction; +, positive reaction; s, sensitive to normal human serum; r, resistant to normal human serum; MR HA, mannose-resistant hemagglutination; S, smooth type LPS; S*, smooth type LPS, monomodal; R, rough type LPS; Hly, hemolysin; SS, serum sensitivity; P, F1b, and Xb, type of fimbriae; in the case of OMP, MLEE, and WCPP numbers indicate provisional types; the serotype is the O:K:H type of the strains immediately after isolation (see text).

or by the PF test (data not shown). These strains carried another kind of adhesin (O75X), but this will not to be dealt with here.

Except for the K$^-$ strains, type 1 fimbriae were detected in all strains after growth in broth culture by mannose-sensitive HA of guinea pig erythrocytes.

After exposure to the bactericidal action of normal human serum, the strains were found to be sensitive in general. Only two O75:K5:H$^-$ strains carrying X adhesin (strains 43 and 44) proved to be resistant.

The lipopolysaccharide (LPS) patterns showed a typical ladder-like structure that was almost identical for the strains, with a bimodal band arrangement showing an accumulation of about 18 repeating units (RU). For four strains (strains 27, 30, 32, and 33) this accumulation spread about 19 RU. Strain 31 was monomodal and also had strong bands in the region of 1 to 15 RU. Another six strains (strain 1, 14, 23, 28, 35, and 37) proved to be rough by PAGE of LPS, which was a result of the long period of storage at room temperature. In Fig. 1 a representative of each of the three LPS patterns is given.

By OMP pattern analysis the strains could be divided into two groups. The majority formed a pattern resembling that of type 11 characterized by Achtman et al. (2) in the case of *E. coli* isolates containing the K1 antigen. A smaller group of four strains (strains 37 to 40) showed a pattern that was quite different from those of the other strains. We found two strains

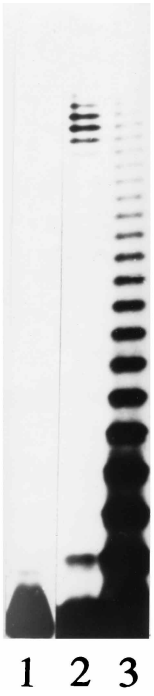

FIG. 1. LPS patterns by SDS-PAGE. Examples of each of the three patterns (S, S*, and R) listed in Table 1 are provided. Lane 1, R, rough type LPS; lane 2, S, smooth type LPS; lane 3, S* monomodal smooth type LPS.

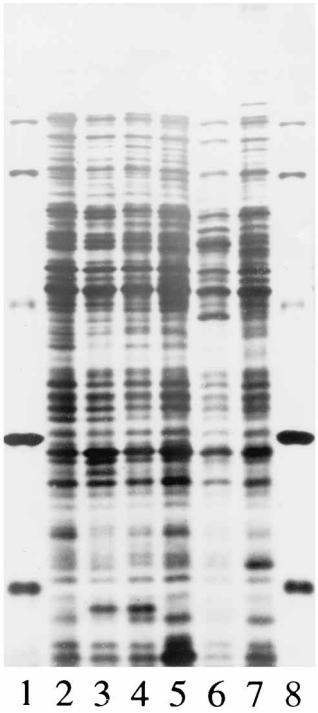

FIG. 2. WCPPs. Examples of each of the patterns listed in Table 1 are provided. Lanes 1 and 8, size standards of 21.5, 31.0, 45.0, 66.2, and 97.4 kDa; lane 2, strain 4, pattern 1; lane 3, strain 14, pattern 1.1; lane 4, strain 17, pattern 1.2; lane 5, strain 34, pattern 1.3; lane 6, strain 36, pattern 2; lane 7, strain 37, pattern 3.

(strains 14 and 17) which did not sort into one of the two groups and that differed from each other.

The much more complex WCPPs were found to be not completely identical among each other; i.e., very slight and insignificant differences were found to exist between practically all strains. These strains were grouped as pattern 1 in Table 1. However, K100:H5 strains 31 to 34 (pattern 1.3) and K95:H$^-$ strains 14 (pattern 1.1) and 17 (pattern 1.2) showed more significant differences from each other and from the main group. Very characteristic proved to be the differences in the K$^-$:H55 strains (strains 36 to 39 as a group [pattern 3] and strain 35 [pattern 2]). In Fig. 2 a representative of each of the individual patterns is shown.

Of 24 enzymes tested by MLEE, 10 proved to be monomorphic among the strains; an additional 3 yielded null alleles among all strains. A dendrogram (Fig. 3) calculated on the basis of these data showed a high relationship among all K5, K95, and K100 isolates. A few strains differed in the mobility of 1 of the 24 enzymes tested (acid phosphatase for strain 14 and hexokinase for strains 24, 27, 43, and 44; data not shown). According to the dendrogram, all these strains were grouped into type 1 by MLEE. The O75:K$^-$:H55 strains, however, produced different MLEE patterns, resulting in significant distances in the dendrogram. Strain 35, as a single strain, was clearly distinct from both groups of strains and thus was classified as type 2 by MLEE. The remaining four strains (strains 36 to 39) were identical to each other but were different from all other strains tested. Consequently, they were grouped into a third MLEE type (type 3).

DISCUSSION

E. coli strains of certain O:K:H serotypes that are predominant in extraintestinal infections are usually equipped with special traits that contribute to their pathogenicity. The occurrence of certain K and O antigens and special fimbriae, hemolysin production, and resistance to the bactericidal effect of serum have been described as important virulence factors (17). O75:K5 strains are often encountered in patients with pyelonephritis, cystitis, or bacteremia (6–8, 10, 14, 18–20, 24, 25). With regard to O75, it was shown in isogenic O75$^+$:K5 and O75$^-$:K5 strains that the O antigen contributes to the persistence in the mouse kidney and bladder (7). In another experimental mouse model, urinary and fecal O75:K95 and O75:K100 isolates proved to have no discernible virulence (26). O75:K100:H5 strains have also been reported as not being associated with any disease (2, 3). Interestingly, such strains contribute to natural immunity against *Haemophilus influenzae* type b by *E. coli* anticapsular antibodies as a result of the cross-reaction between *E. coli* K100 and *H. influenzae* type b (17).

In the present study 44 *E. coli* O75 strains isolated from patients with UTIs were characterized by various methods. According to the K and H antigens, the strains were divided into O75:K:H groups, as indicated in Table 1.

The biotypes determined with the API 20E system, which includes 21 fermentation reactions, showed a remarkable homogeneity among the strains. Even the utilization of dulcitol, raffinose, and sorbose and the requirement for nicotinamide for growth (tests for these are recommended for further differentiation of *E. coli* [2, 5, 13]) proved to be of no discriminative value. Thus, there was no correlation between serotype and biotype.

With respect to the virulence markers of the O75 strains described in this report, the K95 strains were outstanding because they were hemolytic and P fimbriated. Five O75:K5:H$^-$ strains, however, also differed from the others by hemagglutinating activity probably caused by the so-called OX75 adhesin (24). Serum-resistant strains were also found only in these

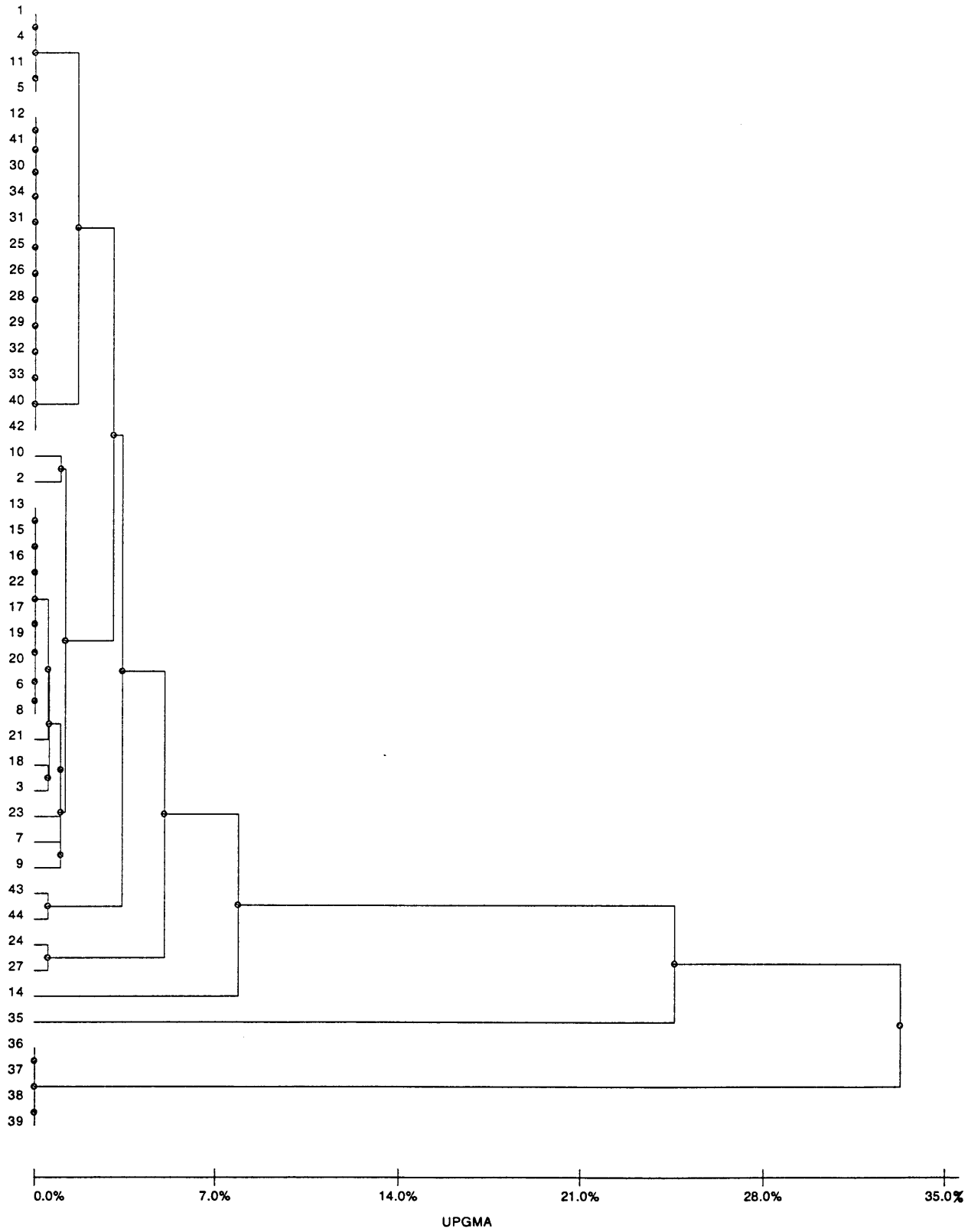

FIG. 3. Dendrogram calculated on the basis of the enzyme patterns. The ordinate indicates the strain numbers in Table 1. UPGMA, unweighted pair group average.

groups. Most of the O75:K5:H$^-$, O75:K100:H5, and O75:K$^-$:H55 strains are not equipped with such traits, apart from the formation of type 1 fimbriae, which are also missing from the K$^-$ group.

Hemolytic *E. coli* isolates mainly have serogroup antigens O2, O4, and O6. However, O18 and O75 strains from patients with UTIs were also found to produce hemolysin (2, 17), and certain O:K:H serotypes are more often hemolytic (2, 17, 24, 25). The results presented here indicate that hemolysis is associated only with O75:K95 strains. This is in agreement with the observations of van den Bosch et al. (26), while in other studies single strains of O75:K5:H$^-$ and O75:K100:H5 were found to be hemolytic (24, 25).

As a result of the adherence of bacteria to erythrocytes, HA has also been described for *E. coli* O75 strains (17–20, 24, 25). The agglutination of human OP1 erythrocytes is strongly associated with the presence of P fimbriae, which specifically recognize the GalA1→4Gal moiety as a receptor of the P blood group substance that is also present on uroepithelial cells. The MR HA of our O75:K95:H5 and O75:K95:H$^-$ strains proved to be of P specificity, which was also confirmed by the PF test. The hemagglutinating activity found in a small group of O75:K5:H$^-$ strains without P specificity seemed to belong to another kind of adhesin primarily termed O75X hemagglutinin (24). Further studies for a more detailed characterization of this adhesin are in progress.

With few exceptions, the O75 strains investigated in this study proved to be serum sensitive, independent of the O:K:H serotype. This result confirms the observations of Taylor (23).

By means of a complex physicochemical typing system consisting of analysis of LPS and OMP patterns, MLEE, and WCPPs (21), the strains could be unambiguously differentiated. The major group (types 1.1 to 1.4; Table 1) comprises nearly all O75:K$^+$:H5 and O75:K$^+$:H$^-$ strains. The overwhelming majority of the strains showed the same OMP pattern, which is in agreement with the results of studies of Väisänen-Rhen et al. (25) for O75 strains that had different K and H antigens and also with the results for fecal O75:K100:H5 isolates found by Achtman et al. (2). Furthermore, the O75 LPS patterns were very homogeneous except for those for strain 31, which proved to be monomodal, and for some rough strains, as mentioned above.

The results obtained by MLEE and from the WCPPs indicate a close genetic relationship among most strains investigated. For genetic and taxonomic reasons, a common descent and, therefore, the existence of one major O75 clone is suggested (Table 1, types 1.1 to 1.4). Although the K100 group showed an individual pattern by WCPPs (Table 1, type 1.3; Fig. 2), the differences were not significant enough to justify the classification into an individual clone. The electromorph variations of enzymes are highly conserved traits, contrary to the adaptive character of surface antigens (4, 27). The K$^-$:H55 strains represent a distinct clonal group (Table 1; type 3 by MLEE and type 3 by WCPPs). Another single isolate (strain 35) of the same serotype was clearly different from both groups by MLEE and WCPPs and had an OMP pattern different from that of type 3 strains (Table 1). Even more electrophoretic enzyme patterns in only a few O75 strains have been reported (4, 19).

From an epidemiological point of view and considering the occurrence of serologically (15) as well as chemically (9) different capsular antigens in the strains investigated, it seems reasonable to establish clonal and/or subclonal groups for serotypes O75:K5:H$^-$, O75:K95:H$^-$, O75:K95:H5, and O75:K100:H5 (2, 25). This seems to be especially justified for O75:K5:H$^-$ harboring the X adhesin, thus being in agreement with the results of Väisänen-Rhen (24).

From the results presented in this report it is concluded that *E. coli* O75 strains, especially those harboring virulence attributes like K antigens and P or OX fimbriae and producing hemolysin production, are regarded as uropathogenic; i.e., they are able to cause UTIs. In addition, O75 strains in general should not only be considered part of the fecal flora but should also be considered to be able to sustain already established infections in the bladder and kidney.

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REFERENCES

1. **Achtman, M., M. Heuzenroeder, B. Kusecek, H. Ochman, D. Caugant, R. K. Selander, V. Väisänen-Rhen, T. K. Korhonen, S. Stuart, F. Ørskov, and I. Ørskov.** 1986. Clonal analysis of *Escherichia coli* O2:K1 isolated from diseased humans and animals. Infect. Immun. **51:**268–276.
2. **Achtman, M., A. Mercer, B. Kusecek, A. Pohl, M. Heuzenroeder, W. Aaronson, A. Sutton, and R. P. Silver.** 1983. Six widespread bacterial clones among *Escherichia coli* K1 isolates. Infect. Immun. **39:**315–335.
3. **Achtman, M., and G. Pluschke.** 1986. Clonal analysis of descent and virulence among selected *Escherichia coli*. Annu. Rev. Microbiol. **40:**185–210.
4. **Caugant, D. A., B. R. Levin, I. Ørskov, F. Ørskov, C. Svanborg-Eden, and R. K. Selander.** 1985. Genetic diversity in relation to serotype in *Escherichia coli*. Infect. Immun. **49:**407–413.
5. **Crichton, P. B., and D. C. Old.** 1979. Biotyping of *Escherichia coli*. J. Med. Microbiol. **12:**473–486.
6. **Devine, D. A., L. Robinson, and A. P. Roberts.** 1989. Occurrence of K1, K5 and O antigens in *Escherichia coli* isolates from patients with urinary tract infections or bacteraemia. J. Med. Microbiol. **30:**295–299.
7. **Hagberg, L., R. Hull, S. Hull, J. R. McGhee, S. M. Michalek, and C. Svanborg-Eden.** 1984. Difference in susceptibility to gram-negative urinary tract infection between C3H/HeJ and C3H/HeN mice. Infect. Immun. **46:** 839–844.
8. **Kaijser, B., and U. Jodal.** 1984. *Escherichia coli* K5 antigen in relation to various infections and in healthy individuals. J. Clin. Microbiol. **19:**264–266.
9. **Nimmich, W.** 1994. Detection of *Escherichia coli* K95 strains by bacteriophages. J. Clin. Microbiol. **32:**2843–2845.
10. **Nimmich, W., G. Zingler, U. Falkenhagen, G. Naumann, and C. Bohnenstengel.** 1985. Vorkommen von *Escherichia coli* K1- und K5-Stämmen bei Patienten mit Harnweginfektionen. Immun. Infekt. **13:**15–20.
11. **Nimmich, W., G. Zingler, U. Falkenhagen, G. Naumann, and I. Kontny.** 1988. O- und H-Antigene von *Escherichia coli*-K5-Isolaten aus Urin. Z. Urol. Nephrol. **81:**257–261.
12. **Nimmich, W., G. Zingler, U. Falkenhagen, and G. Naumann.** 1990. Hämagglutinationsvermögen und Fimbrien-Antigene von aus Urin isolierten *Escherichia coli*-Stämmen. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. **272:**411–418.
13. **Nimmich, W., and G. Zingler.** 1984. Biochemical characteristics phage patterns, and O1 factor analysis of *Escherichia coli* O1:K1:H7:F11 and O1:K1:H$^-$:F9 strains isolated from patients with urinary tract infections. Med. Microbiol. Immunol. **173:**75–85.
14. **Ørskov, F., and I. Ørskov.** 1975. *Escherichia coli* O:H serotypes isolated from human blood. Acta Pathol. Microbiol. Scand. Sect. B **83:**595–600.
15. **Ørskov, F., and I. Ørskov.** 1984. Serotyping of *Escherichia coli*. Methods Microbiol. **14:**43–112.
16. **Ørskov, I., and F. Ørskov.** 1976. Five new *Escherichia coli* K antigens, K95, K96, K97, K98 and K100. Acta Pathol. Microbiol. Scand. Sect. B **78:**593–604.
17. **Ørskov, I., and F. Ørskov.** 1985. *Escherichia coli* in extra-intestinal infections. J. Hyg. Camb. **95:**551–575.
18. **Ørskov, I., F. Ørskov, A. Birch-Andersen, M. Kanamori, and C. Svanborg-Eden.** 1982. O, K, H and fimbrial antigens in *Escherichia coli* serotypes associated with pyelonephritis and cystitis. Scand. J. Infect. Dis. Suppl. **33:** 18–25.
19. **Pere, A., R. K. Selander, and T. K. Korhonen.** 1988. Characterization of P fimbriae on O1, O7, O75, rough, and nontypable strains of *Escherichia coli*. Infect. Immun. **56:**1288–1294.
20. **Sandberg, T., B. Kaijser, G. Lidin-Janson, K. Lincoln, F. Ørskov, I. Ørskov, E. Stockland, and C. Svanborg-Eden.** 1988. Virulence of *Escherichia coli* in relation to host factors in women with symptomatic urinary tract infection. J. Clin. Microbiol. **26:**1471–1476.
21. **Seltmann, G., W. Beer, and W. Voigt.** 1993. Die komplexe Typisierung von Bakterien mit Hilfe moderner physikochemischer Analysenverfahren. Bundesgesundhbleitsblatt **36:**313–318.

22. **Seltmann, G., W. Voigt, and W. Beer.** 1994. Application of physico-chemical typing methods for the epidemiological analysis of *Salmonella enteritidis* strains of phage type 25/17. Epidemiol. Infect. **113:**411–424.
23. **Taylor, P. W.** 1974. Sensitivity of some smooth strains of *Escherichia coli* to the bactericidal reaction of normal human serum. J. Clin. Pathol. **27:**626–629.
24. **Väisänen-Rhen, V.** 1984. Fimbria-like hemagglutinin of *Escherichia coli* O75 strains. Infect. Immun. **46:**401–407.
25. **Väisänen-Rhen, V., J. Elo, E. Väisänen, A. Siitonen, I. Ørskov, F. Ørskov, S. B. Svenson, P. H. Mäkelä, and T. K. Korhonen.** 1984. P-fimbriated clones among uropathogenic *Escherichia coli* strains. Infect. Immun: **43:**149–155.
26. **van den Bosch, J. F., P. Postma, P. A. R. Koopman, J. de Graaff, D. M. MacLaren, D. G. van Brenk, and P. A. M. Guinee.** 1982. Virulence of urinary and faecal *Escherichia coli* in relation to serotype, haemolysis and haemagglutination. J. Hyg. Camb. **88:**567–577.
27. **Whittam, T. S., M. L. Wolfe, I. K. Wachsmuth, F. Ørskov, I. Ørskov, and R. A. Wilson.** 1993. Clonal relationship among *Escherichia coli* strains that cause hemorragic colitis and infantile diarrhea. Infect. Immun. **61:**1619–1629.