

## Characterization of *Escherichia coli* Wild-Type Strains by Means of Agglutination with Antisera Raised Against Cloned P-, S-, and MS-Fimbriae Antigens, Hemagglutination, Serotyping and Hemolysin Production\*

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### Summary

*E. coli* strains isolated from patients with urinary tract infections (UTI) very often possess mannose-sensitive (MS) and mannose-resistant (MR) adherence factors (fimbriae). According to their receptor specificity the mannose-resistant adhesins can be divided into several types, P, S, M and X. We have cloned the determinants of three groups of UTI *E. coli* adhesins, MS, P and S, and prepared specific antisera against the fimbriae antigens. 189 hemagglutination (HA<sup>+</sup>)-positive strains, 96 fecal isolates and 93 strains isolated from UTI have been tested with these specific antisera and further characterized by receptor specific HA, HA patterns and further of the "common O serogroups" 01, 02, 04, 06, 07, 08, 018, 025, 075, most prevalent in UTI, and hemolysin production.

68 (73%) of the UTI strains and 50 (52%) of the fecal isolates showed P-receptor specificity; 16 (17%) of the uropathogenic bacteria and 33 (34%) of the fecal strains exhibited S, M or X-fimbriae antigens. 24% of the P-hemagglutinating (P<sup>+</sup>) strains reacted with P (F8)-specific antiserum. In contrast, more than three quarter of the S<sup>+</sup>-strains were agglutinated by S-specific antiserum. HA-pattern VI and 018 antigen were found to be associated with P-fimbriae strains, whereas HA-pattern V and VII and the O antigens 02 (M-type), 06 and 018 (S-type) occurred most frequently in P<sup>-</sup>-strains. A high percentage of P-fimbriated strains showed mannose-sensitive hemagglutination and hemolysin production.

### Zusammenfassung

*E. coli*-Stämme, die von Patienten mit Urogenitaltraktinfektionen (UTI) isoliert werden, weisen oftmals Mannose-sensitive (MS) und Mannose-resistente (MR) Adhärenzfaktoren

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(Fimbrien) auf. Entsprechend ihrer Rezeptorspezifität können die MR-Adhäsine in verschiedene Gruppen, P, S, M und X unterteilt werden. Vor kurzem haben wir die Determinanten von drei Gruppen der UTI *E. coli*-Adhäsine, MS, P und S, kloniert und spezifische Antiseren gegen diese Fimbrienantigene hergestellt. 189 Hämagglutinations (HA<sup>+</sup>)-positive Stämme, 96 Isolate aus Stuhlproben und 93 Stämme von Patienten mit UTI, wurden mit diesen Fimbrienantigen-spezifischen Antiseren getestet. Sie wurden weiterhin bezüglich ihrer rezeptorspezifischen HA, ihres HA-Musters, dem Vorkommen der O-Serogruppen 01, 02, 04, 06, 07, 08, 018, 025, 075 ("common O-serogroups"), die bei Harnwegsisolaten vorherrschen, und der Hämolysebildung charakterisiert.

68 (73 %) der UTI-Stämme und 50 (52 %) der fäkalen Isolate zeigten P-Rezeptorspezifität; 16 (17 %) uropathogene Stämme und 33 (34 %) Stämme aus Stuhlproben prägten S, M oder X-Fimbrienantigene aus. 24 % der P-hämagglutinierenden (P<sup>+</sup>) Stämme reagierten mit P (F8)-spezifischem Antiserum. Im Gegensatz dazu reagierten mehr als drei Viertel der S<sup>+</sup>-Stämme mit dem S-spezifischen Antiserum. Das HA-Muster VI und 018-Antigen wurden meistens bei P<sup>+</sup>-Stämmen gefunden; die HA-Muster V und VII und die O-Antigene 02 (M-Typ), 06 und 018 (S-Typ) wurden vorzugsweise bei P<sup>-</sup>-Stämmen nachgewiesen. Ein hoher Prozentsatz von P-fimbrierten Stämmen zeigte Mannose-sensitive Hämagglutination und Hämolysebildung.

### Introduction

Bacterial adherence is an important step in the pathogenesis of various infectious diseases and is associated with specific bacterial surface antigens called adhesins (10, 28). Most of these adhesins consist of fimbriae (also called pili) and a binding part. Fimbriae are filamentous polymers of highly hydrophobic protein subunits (13, 14, 34). The binding parts interact with mammalian cells via specific eukaryotic receptors, and according to their capacity to agglutinate also erythrocytes of different vertebrate species they have been termed hemagglutination factors or hemagglutinins (5, 8).

*E. coli* isolates which cause urinary tract infections (UTI) possess different types of adhesins which can be characterized serologically by their hemagglutination (HA) pattern and receptor specificities (5, 26, 31). The ability of particular adhesins to mediate hemagglutination of erythrocytes can be abolished in the presence of 2 % D-mannose. These "mannose-sensitive hemagglutination" (MS)-antigens which include common type I fimbriae or F1A-antigens represent a serologically heterogeneous group (24) of adhesins found on *E. coli* cells from different sources.

In contrast, the "mannose-resistant hemagglutination" (MR)-antigens show binding properties independent of the presence of mannose, and several types of these adhesins can be distinguished. The P-fimbriae recognize a receptor, the  $\alpha$ -D-Gal-(1-4)- $\beta$ -D-Gal globoside, which is part of the human P blood group antigens (P-type adhesin; 16, 21, 33). Though all P-fimbriae adhesins react with the same eukaryotic receptor molecule they represent a group of serologically unrelated antigens termed F7-F13 (2, 22).

Another class of MR-adhesins, the S-fimbriae recognize a sialyl galactoside receptor molecule and this binding property can be inhibited by treatment of the erythrocytes with neuraminidase (17, 26). The adhesins which interact with glycophorin A are termed M-type fimbriae (32). MR-fimbriae which recognize other receptors than those of P-, S-, or M-adhesins are termed X-fimbriae antigens (20, 31).

Representatives of all three groups of these chromosomally encoded adhesins (MS, P, and S) have been cloned and genetically characterized (1, 9, 12, 20, 23, 30). In most cases these adhesins are associated with other characteristics assumed to be involved in virulence of uropathogenic *E. coli* strains (29). Thus, specific O-antigens like 01, 02,

04, 06, 07, 08, 018, 025, and 075, which have been termed "common O-serogroups" and K-antigens like K1, K5, K12, K13, K15 and some others are frequently associated with uropathogenic *E. coli*. These antigens can increase resistance to the bactericidal effect of serum and phagocytosis (11, 25). Furthermore, hemolysin formation (Hly<sup>+</sup>) was shown to contribute to *E. coli* virulence in different *in vivo* models (6, 7, 15).

In the present study we tested *E. coli* wild-type strains for the presence of the urinary virulence factors, e. g. "common O-serogroups", Hly<sup>+</sup>-production, and different adhesins. The distribution of these factors and their relation to the ability of strains to agglutinate with antisera prepared against cloned adhesin-determinants have also been elucidated.

## Materials and Methods

*E. coli* wild-type strains. In two previous studies (8, 11) 742 *E. coli* strains of fecal origin and 249 uropathogenic *E. coli* strains were tested for their ability to agglutinate human erythrocytes (HA). 96 of the fecal HA<sup>+</sup> strains and 93 HA<sup>+</sup> UTI strains are further characterized here. 52 fecal *E. coli* strains were isolated from healthy people or patients suffering from enteritis (Institut für Hygiene und Mikrobiologie, Univ. Würzburg, see 8) and 44 fecal strains were isolated from children suffering from diarrhoea (childrens hospital in Lima, Peru; see 27). 38 UTI strains originated from patients in Würzburg (8), and another 55 UTI strains were isolated from patients in London and Freiburg (11, 13) or received from F. Ørskov (Copenhagen), M. Achtman (Berlin), R. Marre (Lübeck) and H. Tschäpe (Wernigerode). All strains were identified by routine biochemical tests (10) and stored on nutrient agar (Difco).

*E. coli* K-12 strains carrying recombinant DNAs. In order to select for *E. coli* K-12 clones expressing P-, S-, and MS-fimbriae antigens cosmid gene banks were constructed from three *E. coli* wild-type strains (1, 9, Hacker et al., in preparation). These wild-type strains exhibited P-fimbriae of serogroup F8 (strains 764, 2980), S-fimbriae (strain 536), and MS-fimbriae (strains 2980, 536, see Table 1). After insertion of chromosomal DNA fragments of the wild-type strains into the cloning vector pJC74 *E. coli* K-12 strains could be obtained expressing the fimbriae antigens P (F8), S, and MS of the three parental strains. As demonstrated in Table 1 the fimbriated *E. coli* K-12 HB101 strains harbour the recombinant DNAs pANN820 (*mrhP*-F8), pANN801 (*mrhS*), and pANN830 (*msh*, see ref. 1, 9, and Hacker and Schmidt, unpublished results). The *E. coli* K-12 HB101 strain without a recombinant plasmid-DNA molecule did not show any fimbriae antigens. The protein subunits of the fimbriated *E. coli* K-12 strains are of the same molecular weight as those isolated from the wild-type strains, i. e. 21 kilodalton (kd) for the P (F8)-fimbriae (764), 16.5 kd for the S-fimbriae (536), and 17.0 kd for the MS-fimbriae (2980, data not shown).

*Media, chemicals, enzymes.* Cultures were grown in enriched nutrient broth or in Luria broth. For mannose-resistant hemagglutination, strains were grown on CFA plates as described previously (10), and for mannose-sensitive hemagglutination, strains were grown in liquid Luria broth. Antibiotics used were obtained from Bayer, Leverkusen, Fed. Rep. of Germany. All other chemicals were purchased from E. Merck A. G., Darmstadt, Fed. Rep. of Germany.

*Preparation of specific antifimbrial sera.* Rabbits were immunized intravenously five times at 4–5 days intervals with increasing volumes (0.25–1 ml) of formalin killed fimbriated *E. coli* K-12 clones carrying the recombinant DNAs pANN820, pANN801, and pANN830 (ca.  $5 \times 10^8$  bacteria/ml). The rabbits were bled 7 days after the last injection. *E. coli* K-12 specific LPS antibodies were removed by adsorption with heatkilled plasmid-free *E. coli* K-12 bacteria. Specific antifimbrial sera gave a strong reaction with the parental *E.*

Table 1. Agglutination of *E. coli* wild-type strains and *E. coli* K-12 strains carrying recombinant DNAs that code for different fimbriae antigen

<i>E. coli</i> strain	recombinant plasmid	Plasmid marker	Agglutination with					
			human erythr.	bovine erythr.	Sacch. cells	Antiserum against		
						HB101 pANN820 = Anti P(F8)	HB101 pANN801 = Anti S	HB101 pANN830 = Anti MS
HB101 (K-12)	-	-	-	-	-	-	-	-
764 (O18:K5:H:F8)	-	-	R <sup>1)</sup>	-	-	++	-	-
HB101 (K-12)	pANN820	Ap <sup>f</sup> , <i>mrh</i> -F8, <i>fim</i>	R	-	-	++++	-	-
536 (O6:K15:H31:S)	-	-	(R)	R	S <sup>1)</sup>	-	++	++ <sup>2)</sup>
HB101 (K-12)	pANN801	Ap <sup>f</sup> , <i>mrh</i> -S, <i>fim</i>	R	R	-	-	++++	-
2980 (O18:K5:H:F8)	-	-	R	-	S	++ <sup>3)</sup>	-	++
HB101 (K-12)	pANN830	Ap <sup>f</sup> , <i>msb</i> , <i>fim</i>	S	-	S	-	-	++++

<sup>1)</sup> R, mannose-resistant hemagglutination; S, mannose-sensitive hemagglutination

<sup>2)</sup> cross-reaction with MS-fimbriae of the wild-type strain 536 and cloned MS fimbriae of 2980

<sup>3)</sup> cross-reaction between P(F8)-fimbriae of the wild-type strain 2980 and cloned P(F8)-fimbriae of 764



*coli* wild-type strains and with the fimbriated recombinant *E. coli* K-12 clones, but not with the plasmid-free K-12 strain (see Table 1).

**Agglutination.** Bacterial agglutinations were performed with the adsorbed antisera on glass microscope slides. Hemagglutination (HA) patterns (see ref. 5) were determined with erythrocytes obtained either locally (human, bovine, chicken, and guinea pig) or from Flow Laboratories, Bonn, Fed. Rep. of Germany (African green monkey). Agglutination was assayed in phosphate-buffered saline with and without 2% mannose. HA-pattern IVa is defined as Msh with human, monkey, chicken, and guinea pig erythrocytes, pattern V as Mrh with human erythrocytes only, pattern VI as Mrh with human, monkey, and chicken erythrocytes, and pattern VII as Mrh with human and bovine erythrocytes. For identification of P-fimbriae hemagglutination tests were performed with  $\bar{p}$  erythrocytes (kindly supplied by S. Normark, Umeå), NN-erythrocytes (obtained locally) were used for detection of the M-receptor. Neuraminidase-treated erythrocytes were used to identify S-fimbriae (26, 32). MS agglutination with *Saccharomyces cerevisiae* cells was carried out to determine the presence of common type I (F1A) and other MS-fimbriae (24).

**Further characterization of wild-type strains.** The O antigens 01, 02, 04, 06, 07, 08, 018, 025, and 075, most prevalent in urine specimens (8), were identified by the agglutination method of Ørskov et al. (25). Antisera were prepared against the reference strains kindly supplied by F. Ørskov (Copenhagen). Hemolysin production of strains was assayed on meat agar plates containing washed human erythrocytes and confirmed in liquid tests (8).

## Results

### *Incidence of P-, Non-P-, and MS-fimbriae among fecal and UTI E. coli strains*

96 *E. coli* strains of fecal origin and 93 *E. coli* strains isolated from patients suffering from urinary tract infection were selected for their ability to agglutinate human erythrocytes. As summarized in Table 2, 50 bacterial strains (52%) of fecal origin and 68 UTI isolates (73%) showed MR<sup>+</sup>-hemagglutination with human erythrocytes, but failed to agglutinate  $\bar{p}$  erythrocytes indicative of the presence of P-fimbriae. 34% of the fecal strains and 17% of the UTI strains carry fimbriae other than P (S, M or X). 14% of the former and 10% of the latter isolates, respectively, showed hemagglutination of human erythrocytes in a mannose-sensitive manner, which corresponds to the HA-type IVa (5). Common type I fimbriae exhibiting a HA-pattern other than type IVa were produced by more than a half (56%) of the fecal and three quarter (76%) of the UTI isolates.

In order to get more information on the different fimbriae types, we tested the ability of the *E. coli* wild-type strains to agglutinate with sera raised against the cloned P(F8)-, S-, and MS-fimbriae determinants. 10 (11%) and 18 (19%) of the fecal and UTI strains, respectively, agglutinated with antiserum against P(F8)-fimbriae. On the other hand, 11 (12%) of the fecal and 24 (26%) of the UTI *E. coli* strains reacted with the S-specific antiserum. 32% of the fecal and 43% of the urinary strains agglutinated with MS-specific antiserum.

### *Incidence of fimbriae receptor specificities*

In order to estimate the different fimbriae receptor specificities we screened all *E. coli* wild-type strains with different types of red blood cells and found 118 out of 189 strains showing a P-specific hemagglutination (see Table 2 and 3, first line). 27 (24%) of these strains reacted with P(F8)-specific antiserum to the 018 strain 764. Surprising-

Table 2. Incidence of fimbriae types among fecal and UTI *E. coli* strains and frequency of crossreactivity with three established fimbriae-antisera

Fimbriae	Fecal strains (%) n = 96	UTI strains (%) n = 93
P-type <sup>1)</sup>	52	73
reactive in P-fimbriae (F8) antiserum	11	19
S-, M-, X-type <sup>2)</sup>	34	17
reactive in S-fimbriae antiserum	12	26 <sup>3)</sup>
MS-type (HA IVa) <sup>4)</sup>	14	10
MS-type (common type 1) <sup>5)</sup>	56	76
reactive in MS-fimbriae antiserum	32	43

<sup>1)</sup> Mannose resistant hemagglutination with human P<sup>+</sup>-erythrocytes, lack of hemagglutination with  $\bar{p}$ -erythrocytes

<sup>2)</sup> Mannose resistant hemagglutination with P<sup>+</sup>- and  $\bar{p}$ -erythrocytes

<sup>3)</sup> Crossreactivity with S-antiserum and S<sup>-</sup>-strains included

<sup>4)</sup> Mannose sensitive hemagglutination with human, chicken, monkey and guinea pig erythrocytes (HA pattern IVa, see *Evans and Evans*, 1983)

<sup>5)</sup> No hemagglutination with human erythrocytes, mannose sensitive hemagglutination with guinea pig erythrocytes and *Saccharomyces cerevisiae* cells (common type I fimbriae)

ly, 21 (18 %) of these P<sup>+</sup>-hemagglutinating strains also agglutinated with S-specific antiserum. In contrast only one out of the 49 P<sup>-</sup>-hemagglutinating isolates showed cross-reaction with P-specific antiserum, while 14 strains (29 %) were agglutinated by S-specific antiserum to strain 536 (Table 3).

From the 49 P<sup>-</sup>-hemagglutinating strains 12 (24 %) showed M-specific hemagglutination and 13 (27 %) exhibited a neuraminidase susceptible hemagglutination which is typical for S-fimbriae. Whereas only one of 12 M-hemagglutinating strains reacted with the S-specific antiserum raised against strain 536, 10 (77 %) of the S-hemagglutinating strains did so. 24 (49 %) of the P<sup>-</sup>-hemagglutinating strains do not belong to the M- or S-fimbriae type. Antisera-specific agglutination of these strains was very rare (1 P-agglutinating, 3 S-agglutinating isolates). While 62 % of the S-fimbriated strains were isolates from UTI cases, the majority of M-fimbriated strains and isolates with non-typable X-fimbriae (83 % of the former, 75 % of the latter) belonged to the fecal flora (data not shown).

#### *Fimbriae-type in relation to HA-patterns.*

Hemagglutination tests with five different species of erythrocytes have been carried out (for details see Materials and Methods and footnote 4 of Table 3) and HA-pattern V, VI and VII are characteristic for urinary pathogens (7). As listed in Table 3, P-fimbriated strains (both fecal and UTI) are most frequently associated with HA-pattern VI (81 % hemagglutination, 81 % P (F8)-specific serumagglutination, 86 % S-specific serumagglutination). In contrast, the incidence of HA-pattern VI is very low among P<sup>-</sup>-hemagglutinating strains, and ranges from 30 % (S-fimbriated, S-specific serumag-

Table 3. Incidence of different fimbriae and serological relationship to P (F8)- and S-antisera among *E. coli* wild-type strains. Relation to hemagglutination and hemolysin production

	P-fimbriae <sup>1)</sup> n = 118			S-fimbriae <sup>2)</sup> n = 13			M-fimbriae <sup>3)</sup> n = 12			X-fimbriae n = 24		
	not reac- tive n = 70 (%)	P(F8)- anti- serum n = 27 (%)	S- anti- serum n = 21 (%)	not reac- tive n = 3 (%)	P(F8)- anti- serum n = 0 (%)	S- anti- serum n = 10 (%)	not reac- tive n = 11 (%)	P(F8)- anti- serum n = 0 (%)	S- anti- serum n = 1 (%)	not reac- tive n = 20 (%)	P(F8)- anti- serum n = 1 (%)	S- anti- serum n = 3 (%)
HA-pattern V <sup>4)</sup>	8	4	5	—	—	30	42	—	—	75	—	33
HA-pattern VI <sup>4)</sup>	47	81	86	—	—	30	8	—	—	—	—	—
HA-pattern VII <sup>4)</sup>	5	15	10	33	—	40	42	—	100	25	100	66
hemolysin- production (Hly <sup>+</sup> )	73	85	81	33	—	80	42	—	—	15	100	33

<sup>1)</sup> Mannose resistant hemagglutination with P<sup>+</sup> erythrocytes, lack of hemagglutination with p erythrocytes

<sup>2)</sup> Neuraminidase sensitive hemagglutination

<sup>3)</sup> Mannose resistant hemagglutination with human MM erythrocytes, lack of hemagglutination with NN erythrocytes

<sup>4)</sup> HA-pattern according to *Evans and Evans* (1983). Strains were tested with human, bovine, chicken, monkey and guinea pig erythrocytes. Type V means Mrh with human erythrocytes only, type VI means Mrh with human, monkey and chicken erythrocytes, type VII means Mrh with human and bovine erythrocytes

glutinating strains) to zero (X-fimbriated strains with non-typable receptor) The M- and X-fimbriated isolates predominantly show HA-pattern V and VII.

*Fimbriae in relation to hemolysin production*

Hemolysin production has been demonstrated to contribute to virulence of uropathogenic *E. coli* strains (6), and hemolytic (Hly<sup>+</sup>) *E. coli* strains can be often isolated from patients with UTI. In this study Hly<sup>+</sup>-strains were frequently found among P<sup>+</sup>- and P<sup>-</sup>-fimbriated bacteria (80% and 69%), and especially those strains which also reacted with P- or S-specific antiserum showed the highest degree of hemolysin production (85% of P<sup>+</sup>-hemagglutinating and P-specific serumagglutinating strains, 80% of S<sup>+</sup>-hemagglutinating and S-specific serumagglutinating strains). X<sup>+</sup>-hemagglutinating strains from fecal sources contained a significant smaller portion of hemolytic isolates than uropathogenic bacteria (for details see Fig. 1 and Table 3).

*Fimbriae in relation to "common O-serogroups"*

As shown in Table 4, the nine O-serogroups 01, 02, 04, 06, 07, 08, 018, 025, and 075, which are characteristic for urinary pathogens (see 8) have been detected among the *E. coli* wild-type strains of the present study. Generally, these "common O-serogroups" are associated very frequently with P- and S-fimbriated bacteria (80% and 77%). Among P<sup>+</sup>-hemagglutinating strains serotype 018 was very frequently found, and from the 23 P<sup>+</sup>-hemagglutinating strains which exhibit P(F8)-specific serumagglutination 18 (67%) carried the antigen 018. The antigen 02 occurred predominantly in M-fimbriated strains, and strains with S-type fimbriae more often showed 06 and 018 antigens.

**Discussion**

*E. coli* antigens with hemagglutinating activity can be grouped by their receptor specificities. In this report 189 fecal and uropathogenic *E. coli* strains have been characterized in this way. In agreement with previous investigations (16, 18, 21, 33) P-type fimbriae were found to be the predominant MR<sup>+</sup>-adhesins among UTI *E. coli* strains. The fact that more than 50% of the hemagglutinating fecal *E. coli* strains (10% of the whole fecal *E. coli* population) exhibited P-specificity confirms the assumption that the human gut flora represents a pool of urinary pathogens (3, 8). With an incidence of nearly 10% the S-fimbriae represent the major part of Non-P (P<sup>-</sup>) adhesins among UTI strains. In addition these neuraminidase susceptible adhesins seem to play an important role in *E. coli* meningitis infections. Among such pathogens the incidence of S-fimbriated bacteria is much higher than among uropathogenic isolates (17, 18, 19, Hacker, unpublished results).

The agglutination of strains with antisera raised against cloned fimbriae antigens was another attempt in characterizing the UTI specific adhesins. Most wild-type strains produce more than one fimbria (9, 14) and the cloning of their determinants and its subsequent utilization for the production of antisera seems to be a good way to get pure and monospecific antibodies against a single fimbria type. The cloned fimbriae antigens P, S, and MS, used here, were derived from three different wild-type strains. The P-fimbriae determinant was cloned from a 018:K5:H<sup>-</sup> strain (1, Hacker et al., in preparation) and, as other 018:K5:H<sup>-</sup> strains tested (3) the antiserum of the cloned



Table 4. Incidence of different fimbriae and serological relationship to P(F8)- and S-antisera among *E. coli* wild-type strains. Relation to O-serogroups

	P-fimbriae <sup>1)</sup> n = 118			S-fimbriae <sup>2)</sup> n = 13			M-fimbriae <sup>3)</sup> n = 12			X-fimbriae n = 24		
	not reac- tive n = 70 (%)	P(F8)- anti- serum n = 27 (%)	S- anti- serum n = 21 (%)	not reac- tive n = 3 (%)	P(F8)- anti- serum n = 0 (%)	S- anti- serum n = 10 (%)	not reac- tive n = 11 (%)	P(F8)- anti- serum n = 0 (%)	S- anti- serum n = 1 (%)	not reac- tive n = 20 (%)	P(F8)- anti- serum n = 1 (%)	S- anti- serum n = 3 (%)
O-serogroup O1	4	—	5	—	—	—	—	—	—	5	—	—
O2	8	4	—	33	—	10	37	—	—	5	—	33
O4	8	4	20	—	—	10	—	—	—	—	—	—
O6	8	8	40	—	—	30	—	—	—	5	—	—
O7	4	—	5	—	—	—	—	—	—	5	—	—
O8	3	—	—	—	—	—	—	—	—	—	—	—
O18	7	67	15	—	—	30	—	—	—	—	—	—
O25	1	—	—	—	—	10	—	—	—	—	—	—
O75	4	4	5	—	—	—	19	—	—	20	—	—
common O-sero- group not typable	54	13	10	66	—	10	44	—	100	60	100	66

<sup>1)</sup> Mannose resistant hemagglutination with human P<sup>+</sup> erythrocytes, lack of hemagglutination with  $\bar{p}$  erythrocytes

<sup>2)</sup> Neuraminidase sensitive hemagglutination

<sup>3)</sup> Mannose resistant hemagglutination with human MM erythrocytes, lack of hemagglutination with NN erythrocytes

determinant cross-reacts with F8 fimbriae (Hacker, unpublished results). The strain 536 shows S-specific neuraminidase sensitive hemagglutination which is characteristic also for the cloned determinant (9). The cloned *msh*-determinant exhibits mannose sensitive hemagglutination also with human erythrocytes which is typical for "IVa" antigens (5, 24).

As can be seen in Fig. 1 the F8(P)-, S- and MS(IVa)-specific antisera cross react with a number of different strains, which also have been tested for their hemagglutination (HA)-pattern, presence of "common" O-antigens and hemolysin production. Most of the P<sup>+</sup>(F8)-serumagglutinating strains belong to the serogroup O18 and those so far tested carry the K5 antigen. Because all of these strains show the HA-pattern VI and hemolysin production, these strains represent a homogeneous group of isolates with identical or similar properties, called a clone (not identical to strains harboring recombinant DNAs). Several of such clones of uropathogenic and other extraintestinal *E. coli* strains have been described (18, 19, 33) and in our study 10% of the urinary isolates belong to the O18 : K5 : F8 : HAVI : Hly<sup>+</sup> group. The fact that more than three quarters of the P<sup>+</sup>HAVI<sup>+</sup> strains do not agglutinate with the P<sup>+</sup> (F8) antiserum supports the view that the P<sup>+</sup>HAVI<sup>+</sup> group is heterogeneous with respect to serological properties (see also ref. 2, 22).

While the P<sup>+</sup> (F8)-serumagglutination is restricted to P<sup>+</sup>-hemagglutinating isolates S<sup>+</sup>-serumagglutination has been identified among P- and Non-P-fimbriated strains as well.

It seems that one (or more) P-fimbriated strains which are associated with serogroups O4 or O6 and HA-pattern VI often cross react serum of S-type (see also Fig. 1). In addition, it is not surprising that the S-specific serum has a high affinity for S-fimbriated strains and O18 : K1 and O6 strains are predominantly reactive. The O18 : K1 strains belong to a clone often associated with cases of meningitis and sepsis (17, 18, 35) and Hly<sup>+</sup> O6 strains belong to the only Non-P UTI clone described by Väisänen-Rhen et al. (33). The S-specific serum did not cross react with M-fimbriated strains indicating that both fimbriae are unrelated with respect to either their receptor specificities or their serological properties.

From the X-hemagglutinating strains with non-typable receptor specificities only 29% belong to the "common O-serogroups" of urinary pathogens. Because more than 50% of these strains are fecal isolates selected from children with diarrhoea these MR-hemagglutinins may represent a group of CFA I/II independent adhesins which may recognize receptors located on intestinal cells as has been suggested by several authors (3, 4, 5, 8).

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