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Hemagglutination Typing of *Escherichia coli*: Definition of Seven Hemagglutination Types

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A hemagglutination (HA) typing system has been developed for demonstrating and characterizing the mannose-sensitive and mannose-resistant hemagglutinins produced by *Escherichia coli* isolated from human sources. HA typing is performed by testing CFA agar-grown *E. coli* cells for HA with human, bovine, adult chicken, African Green monkey, and guinea pig erythrocytes in the presence and absence of mannose. Seven major HA types, designated HA type I through HA type VII, have been defined according to the HA patterns produced by 1,334 test cultures consisting of 33 colonization factor antigen I (CFA/I)-positive enterotoxigenic *E. coli* (ETEC), 37 CFA/II-positive ETEC, 614 isolates belonging to the classical enteropathogenic *E. coli*, or EPEC, serogroups, 446 non-ETEC, non-EPEC stool isolates, and 204 bacteremia-associated *E. coli*. Facultatively enteropathogenic *E. coli* (FEEC) serogroups, which are the causative agents of extraintestinal infections but also sporadic cases of enteritis, comprised 38% of the stool isolates and 91% of the blood isolates examined. Previous observations concerning the HA patterns of CFA-positive ETEC and the EPEC were confirmed. A significant correlation was found between FEEC serogroups and the production of mannose-resistant HA with human, monkey, and usually chicken erythrocytes (the HA patterns designated HA type VI). A large majority (80.2%) of the FEEC strains belonging to the most frequently isolated serogroups from cases of bacteremia (O1, O2, O4, O6, O7, and O18) produced type VI HA patterns. Stool isolates belonging to these same serogroups were 59.2% positive for HA type VI patterns. In contrast, only 17.4% of the non-FEEC stool isolates and 1.9% of the EPEC isolates belong to HA type VI. Of the blood isolates, the HA type VI phenotype was two times more prevalent among K1-positive *E. coli* than among K1-negative *E. coli*, 70.6 versus 31.1%. These results suggest that surface-associated hemagglutinins of *E. coli*, many of which are known to be fimbriae, should be considered in addition to serotype (O:K:H antigenicity) in the description of isolates.

We previously described a hemagglutination (HA) typing system for *Escherichia coli* based upon tests for HA of human, bovine, chicken, and guinea pig erythrocytes in the presence and absence of mannose (11). The impetus for development of this HA typing system was that the fimbrial colonization factor antigens CFA/I and CFA/II of enterotoxigenic *E. coli* (ETEC) are detectable as mannose-resistant (MR) hemagglutinins (7). CFA/I fimbriae mediate MRHA of human, bovine, and chicken erythrocytes, and CFA/II fimbriae mediate that of only bovine and chicken erythrocytes. Neither CFA/I nor CFA/II exhibits mannose-sensitive (MS) HA. Thus, CFA-positive *E. coli* are clearly distinguishable from *E. coli* possessing only common fimbriae which mediate MSHA with human, chicken, and guinea pig, but not bovine, erythrocytes. As we reported (11), a significant proportion of enteropathogenic *E. coli* (EPEC; e.g., serogroups O55, O86, O111, O119) were found to produce MS-type hemagglutinin(s) detectable with chicken and guinea pig, but not human or bovine, erythrocytes, and this HA pattern was termed HA type III. Intestinal isolates belonging to the facultatively enteropathogenic *E. coli*, or FEEC, serogroups associated with sporadic diarrhea (4, 5) and extraintestinal infections such as meningitis, urinary tract infections, and bacteremia were also found to exhibit the type III HA pattern; however, a relatively small number of these *E. coli* were available at that time. The work reported here was carried out for several reasons. One purpose was to

expand the HA typing system to better characterize *E. coli* present in the normal intestine of humans (non-EPEC and non-ETEC, but including the FEEC). Another goal was to characterize the HA patterns of FEEC isolated from cases of bacteremia (mostly serogroups O1, O2, O4, O6, O7, and O18) since these are the serogroups most frequently associated with extraintestinal *E. coli* infections.

MATERIALS AND METHODS

Bacterial cultures. The ETEC isolates used in this investigation were either CFA/I- or CFA/II-positive *E. coli* which produce both heat-stable and heat-labile enterotoxins (7, 10, 11). The EPEC cultures (351 isolates) used in the previous investigation (11) were also used here to describe the results obtained with the expanded HA typing system. However, 263 additional EPEC isolates were either obtained from local sources or kindly contributed by other investigators upon request. *E. coli* isolates representing the non-ETEC, non-EPEC intestinal flora (446 isolates) were obtained mainly from the stools of healthy (nondiarrhea) individuals participating in studies in which EPEC or ETEC or both were sought (7, 11). Approximately 38% of these 446 isolates belong to the FEEC serogroups; this is a common observation and consistent with the idea that the normal flora population is the reservoir of these opportunistic pathogens. *E. coli* isolated from blood specimens (204 different strains isolated from cases of bacteremia) were used to represent extraintestinal FEEC, and 91% (105 of 115 serotyped) were confirmed as FEEC. The majority of these isolates were obtained by one of the authors (L.S.Y.) from clinical cases to be described elsewhere, and approximately 10% were isolated by another author (J.P.) (21, 22) and were originally selected as K1-positive *E. coli*. Serotyping of these *E. coli* isolates was performed, by standard methods, in Houston, using anti-O and anti-H sera obtained from the Center for Disease Control, Atlanta, Ga.

Culture conditions. Stock cultures were maintained on agar slants composed of 2% peptone–0.5% NaCl–2% agar at room temperature. All HA tests were performed as described below with cells grown for 18 to 24 h on CFA agar (9), which consists of 1% Casamino Acids (Difco) and 0.15% yeast extract (Difco) plus 0.05% $MgSO_4$ and 0.0005% $MnCl_2$, with 2% agar added (pH approximately 7.4). The culture conditions were selected to enhance production of fimbriae (8, 9, 13).

Assay for K antigens. Test strains were examined for production of capsular, or K, polysaccharide antigens as follows. Each test strain was grown for 18 h at 37°C in a large Roux bottle. The cells were harvested into 10 ml of phosphate-buffered saline, pH 7.2. Capsular polysaccharide was extracted by heating the cell suspensions at 60°C for 20 min in a large screw-cap tube, and the bacterial cells were removed by centrifugation at 18,000 × *g* for 20 min and discarded (19, 20). Virtually all of the bacteremia-associated *E. coli* were found to possess acidic polysaccharide antigens, according to the results obtained with several different assay systems. These assays were the HA inhibition test (2) and the erythrocyte sedimentation assay (2) performed with anti-K serum as well as with anti-*Neisseria meningitidis* group B (Difco) serum (21–23) in the case of the K1 antigen.

HA typing. Type A human blood was drawn from volunteers and placed into a tube containing 1.0 ml of 3.8% citric acid in distilled water per 9.0 ml of blood. Blood was diluted 1:4 with phosphate-buffered saline to test for HA and 1:4 with 1% mannose in phosphate-buffered saline to test for MRHA. The same procedure was used for blood freshly drawn from guinea pigs and for bovine, adult chicken, and African Green monkey erythrocytes obtained from Flow Laboratories, Inc., McLean, Va.

HA tests were performed by slide agglutination as follows. Bacterial cells from CFA agar cultures (see above) were picked up with a sterile wooden toothpick and mixed with a drop of the appropriate species of blood (approximately 20 μl) on a glass slide at room temperature. After observation for HA for about 1 min, the slides showing less than maximum HA were placed on the surface of ice and observed for a least 2 min, with intermittent mixing by rotation of the slide. Results were recorded at 4+ when the HA reaction was instantaneous and complete, involving all the erythrocytes. Lesser degrees of HA were recorded as 3+, 2+, 1+, or negative (11). HA was denoted as resistant (MRHA) if the same degree of HA occurred with and without mannose and as sensitive (MSHA) if HA was prevented or grossly reduced by the presence of mannose. For consistency each test strain was tested first with erythrocytes in phosphate-buffered saline in the following order: human, bovine, chicken, monkey, guinea pig. If positive for HA, the test was repeated with the appropriate species of erythrocytes in phosphate-buffered saline plus mannose. There was only one circumstance, which occurred infrequently, in which an HA pattern could not be obtained; a few rough (untypable) isolates of *E. coli* were found to produce MRHA with every species of erythrocyte tested, but this HA was not typical and therefore, was easily recognized.

RESULTS

HA typing of *E. coli* isolates with human, bovine, chicken, monkey, and guinea pig erythrocytes tested in the presence and absence of mannose. CFA-positive ETEC were tested mainly to discover whether CFA/I or CFA/II might be detectable by MRHA of monkey erythrocytes. *E. coli* possessing these fimbriae did not react with monkey erythrocytes, except that MSHA was produced by those strains which also produced MSHA with guinea pig erythrocytes (Table 1). It can also be seen that strains typed as HA type III-A and HA type IV-A (i.e., strains producing MSHA with guinea pig and chicken erythrocytes with or without MSHA of human erythrocytes, but no detectable MRHA) also produced MSHA with the monkey erythrocytes. None of the strains tested produced MSHA or MRHA exclusively with

TABLE 1. *Results obtained with expanded HA typing system using CFA-positive ETEC, EPEC, intestinal E. coli (non-ETEC, non-EPEC) isolates, and extraintestinal, bacteremia-associated isolates of E. coli*

HA type	HA with:[a]					CFA/I-positive *E. coli* (ETEC)	CFA/II-positive *E. coli* (ETEC)	EPEC	*E. coli* isolated from:	
	Hu	Bv	Ck	Mk	Gp				Stool[b]	Blood
I-A	R	R	R	–	–	30 (90.0)[c]	0	0	0	0
I-B	R	R	R	S	S	3 (9.1)	0	0	1 (0.2)	3 (1.5)
II-A	–	R	R	–	–	0	29 (78.2)	1 (0.2)	1 (0.2)	0
II-B	–	R	R	S	S	0	6 (16.2)	0	1 (0.2),	0
II-C	S	R	R	S	S	0	2 (5.4)	1 (0.2)	0	1 (0.5)
III-A	–	–	S	S	S	0	0	236 (38.4)	41 (9.2)	25 (12.3)
IV-A	S	–	S	S	S	0	0	60 (9.8)	69 (15.5)	14 (6.7)
IV-B	–	–	–	–	–	0	0	256 (41.7)	160 (35.9)	47 (23.0)
V-A	R	–	–	–	–	0	0	4 (0.6)	34 (7.6)	12 (5.9)
V-B	–	R	–	–	–	0	0	1 (0.2)	0	0
V-C	–	–	R	–	–	0	0	10 (1.6)	17 (3.8)	1 (0.5)
V-D	–	–	–	R	–	0	0	0	0	0
V-E	–	–	–	–	R	0	0	2 (0.3)	2 (0.4)	0
V-F[d]	R	–	S	S	S	0	0	12 (2.0)	18 (4.0)	5 (2.5)
V-G (other)						0	0	2 (0.3)[e]	2 (0.4)[f]	0
VI-A	R	–	R	R	–	0	0	5 (0.8)	42 (9.4)	37 (18.1)
VI-B	R	–	R	R	S	0	0	5 (0.8)	26 (5.8)	40 (19.6)
VI-C	R	–	–	R	–	0	0	0	9 (2.0)	7 (3.4)
VI-D	R	–	S	R	S	0	0	2 (0.3)	2 (0.4)	5 (2.3)
VI-E	R	–	R	R	R	0	0	0	15 (3.4)	0
VI-F	R	–	S	R	R	0	0	0	4 (0.9)	0
VI-G (other)[g]						0	0	0	0	4 (2.0)[h]
VII-A	R	R	–	–	–	0	0	9 (1.5)	0	0
VII-B	R	R	–	S	S	0	0	2 (0.3)	0	0
VII-C	R	R	S	S	S	0	0	2 (0.3)	0	0
VII-D (other)[i]						0	0	4 (0.6)[j]	2 (0.4)[k]	3 (1.5)[l]

[a] Erythrocytes tested: Hu, human group A; Bv, bovine; Ck, adult chicken; Mk, African Green monkey; Gp, guinea pig. R = MRHA; S = MSHA; – designates no HA.

[b] CFA-negative ETEC are not included here (see text).

[c] Numbers in parentheses are percentage of total for each group of *E. coli* listed.

[d] This HA pattern was originally classified as HA type III (minor).

[e] Two isolates with HA pattern SRSSS.

[f] Two isolates with HA pattern R---S.

[g] Monkey MRHA included in HA pattern but not included in HA types VI-A through VI-F.

[h] Two isolates with HA pattern S-RRS; one with --RR-; one with -RRR-.

[i] MRHA with both human and bovine erythrocytes plus other HA not listed, or miscellaneous HA patterns not listed otherwise.

[j] One isolate with HA pattern RRSSR; three with ----S.

[k] Two isolates with HA pattern ----S.

[l] Three isolates with HA pattern ----S.

monkey erythrocytes. Thus, inclusion of monkey erythrocytes in the HA typing system did not alter the results previously reported for HA types I through IV, and these HA type designations have been retained (11). The HA patterns grouped in type IV are those which have long been assumed to be typical of *E. coli*, i.e., cells with and without common fimbriae (11). In this study, approximately one-half of the stool isolates tested belong to HA type IV (Table 1).

Many *E. coli* isolates produce MRHA of human erythrocytes, which is a property of CFA/I, although they prove to be CFA/I-negative by serological tests and fail to produce MRHA with bovine erythrocytes. The results shown in Table 1 show that these *E. coli* can be differentiated into several different types according to their HA reaction with chicken, monkey, and guinea

pig erythrocytes. In particular, 203 of 300 CFA/I-negative *E. coli* which produced MRHA with human erythrocytes (67.7%) also produced MRHA with monkey erythrocytes; these are denoted HA type VI in Table 1. Since 98.1% of the monkey MRHA-positive isolates (203 of 207) are also human MRHA positive, it is likely that the same factor mediates both reactions, although this remains to be proven. The data also show that MRHA of guinea pig erythrocytes occurs independently of other MRHA reactions.

Classification of MRHA-positive *E. coli* not belonging to HA types I, II, or VI. A significant number of *E. coli* isolates (122 isolates listed in Table 1) produced MRHA with only a single species of erythrocytes, and 14 EPEC isolates produced MRHA with human and bovine but not chicken or monkey erythrocytes. Those isolates producing monospecific MR-type hemagglutinins are classified as HA type V. Note that of 87 human (only) MRHA-positive isolates, 37 (42.5%) also produced MSHA; 35 of these were classified as a distinct group (HA type V-F) because of an apparent association with EPEC serogroups (11). Chicken (only) MRHA-positive isolates were almost as frequent as HA type V-F isolates, but bovine (only) MRHA-positive and guinea pig (only) MRHA-positive isolates were rare and no monkey (only) MRHA-positive isolates were found among the 1,334 isolates tested.

Characterization of CFA-positive ETEC, EPEC, stool isolates, and extraintestinal isolates of *E. coli* according to HA type. Data concerning the distribution of the five major groups of *E. coli* HA typed by the expanded system are presented in Table 2. Major points of interest are as follows.

CFA/I-positive ETEC belong exclusively to the HA type I category, and only 10% of those tested produced MS hemagglutinin(s) in addition to CFA/I. CFA/II-positive ETEC belong exclusively to HA type II; only 21.6% of those tested produced MS hemagglutinin(s) in addition to CFA/II.

Analysis of 614 *E. coli* isolates belonging to the classical EPEC serogroups showed that 38.4% of these belong to HA type III, as opposed to only 9.2% of 446 non-ETEC, non-EPEC stool isolates. There is no particular association between any of the five *E. coli* groups and HA type IV or HA type V.

It is of interest that 45.6% of the extraintestinal *E. coli* isolates belong to HA type VI, i.e., produce MRHA with both human and monkey and usually with chicken erythrocytes. This is in contrast to 1.9% of the EPEC and 22% of the normal flora, which includes FEEC serogroups (approximately 38%). Note that the stool isolates representing the normal flora are rather unremarkable with respect to HA patterns.

HA patterns of *E. coli* FEEC serogroups isolated from stool and blood. The *E. coli* serogroups listed in Table 3 as FEEC have been documented as the causative agents of a variety of extraintestinal infections (5, 11, 14, 16, 18, 23, 31) and enteritis with or without extraintestinal involvement (4, 20, 26). In the present study, 184 stool isolates (excluding ETEC and EPEC) and 115 isolates from blood were serotyped, and 62.5 and 91.3%, respectively, were found to be FEEC on the basis of serogroup analysis. The major FEEC serogroups found among both the stool isolates and the bacteremia-associated isolates were O1, O2, O4, O6, O7, O15, O18, and O75. It may be noted that serogroup O4 was six times

TABLE 2. *Distribution of CFA-positive ETEC, EPEC, stool, and extraintestinal isolates of E. coli according to HA type: comparison of MS- and MR-type hemagglutinins of each group and further definition of terms*

HA type	Type of hemagglutinin produced[a]	CFA/I-positive ETEC	CFA/II-positive ETEC	EPEC	Stool isolates[b]	Extraintestinal isolates[c]
I	Hu, Bv, Ck-MR	33 (100)[d]	0	0 (0)	1 (0.2)	3 (1.5)
II	Bv, Ck-MR	0	37 (100)	2 (0.4)	2 (0.4)	1 (0.5)
III	Ck, Mk, Gp-MS	0	0	236 (38.4)	41 (9.2)	25 (12.3)
IV-A	Hu, Ck, Mk, Gp-MS	0	0	60 (9.8)	69 (15.5)	14 (6.7)
IV-B	None detectable	0	0	256 (41.7)	160 (35.9)	47 (23.0)
V	Monospecific-MR[e]	0	0	31 (5.0)	73 (16.2)	18 (8.9)
VI	Hu, Mk-MR	0	0	12 (1.9)	98 (22.0)	93 (45.6)
VII	Hu, Bv-MR	0	0	17 (2.7)	2 (0.4)	3 (1.5)

[a] Minor variations, particularly production of MS-type hemagglutinins in addition to the designated MR-type hemagglutinins, are designated by subgrouping (e.g., HA type I-B; see Table 1 for details and abbreviations).
[b] Stool isolates are non-ETEC, non-EPEC but do include FEEC serogroups (approximately 38%).
[c] Extraintestinal isolates of *E. coli* are primarily FEEC serogroups (approximately 91%).
[d] Numbers designate number of isolates in each HA type, with percentage data in parentheses.
[e] Example: HA type V-A—human (only) MR hemagglutinin.

TABLE 3. *HA type of FEEC and non-FEEC serogroups of E. coli isolated from stool and blood*

Isolates from:	Serogroup	No. of HA type:			
		III	IV	V	VI
Stool	O1	2	2	2	2
	O2	0	2	2	6
	O4	0	0	0	2
	O6	1	6	3	19
	O7	2	2	1	4
	O15	1	1	6	2
	O18	1	1	2	9
	O19	0	0	1	0
	O21	0	4	2	0
	O25	0	1	1	3
	O51	0	1	0	0
	O73	1	3	0	0
	O75	0	7	3	1
	O83	1	4	0	0
	O85	0	0	1	0
	All FEEC	9	34	24	48
	Non-FEEC	12	39	6	12
	% FEEC[a]	7.8	29.6	20.8	41.7
Blood	O1	0	4	1	14
	O2	0	1	1	8
	O4	0	0	0	12
	O6	0	2	3	18
	O7	2	1	0	8
	O15	0	1	3	3
	O18	0	1	0	5
	O19	0	0	0	0
	O21	1	0	0	2
	O25	0	0	0	2
	O51	0	0	0	0
	O73	0	0	0	0
	O75	0	5	4	1
	O83	0	2	0	0
	O85	0	0	0	0
	All FEEC	3	17	12	73
	Non-FEEC	4	2	1	3
	% FEEC	2.9	16.2	11.4	69.5

[a] Percent distribution among HA types III, IV, V, and VI; for further data on distribution, see Table 5. No isolates belonging to serogroups O27, O78, or O117 were found in these collections.

more prevalent in the group isolated from blood. The data in Table 3 show that serogroups O1, O2, O4, O6, O7, and O18 (hereafter referred to as the major FEEC serogroups) are primarily responsible for the observed correlation between source of isolation (blood) and HA type VI, since 69.5% of the blood isolates belong to this HA type.

Table 4 summarizes the relationship between production of specific HA patterns (particularly HA type VI versus other HA types), FEEC serogroups, and source of isolation. Note that isolates belonging to the major FEEC serogroups were 80.2% positive for HA type VI when isolated from blood and 59.2% positive for HA type VI when isolated from stool. FEEC in the intestine are also more likely to be found as HA type VI isolates than non-FEEC serogroups in the intestine (41.7 versus 17.4%, Table 4). In addition, there is an inverse relationship between the occurrence of HA type VI *E. coli* and HA type IV *E. coli* as a function of serogroup and source of isolation (Table 4); this is apparently the result of the close association between FEEC serogroup and HA type VI.

TABLE 4. *Summary data: distribution of FEEC and non-FEEC serogroups (number and percentage of isolates) according to source (blood versus stool) and HA type*

Group of *E. coli*	Source	No. of isolates	Distribution (%) among HA types:			
			III	IV	V	VI
Major FEEC serogroups[a]	Blood	81	2.5	11.1	6.1	80.2
	Stool	71	8.5	18.3	14.1	59.2
All FEEC isolates[b]	Blood	105	2.9	16.2	11.4	69.5
	Stool	115	7.8	29.6	20.8	41.7
FEEC plus non-FEEC	Blood	204	12.3	29.7	8.9	45.6
	Stool	446	9.2	51.4	16.2	22.0
Non-FEEC	Stool	69	17.4	56.6	8.7	17.4

[a] Major FEEC serogroups are O1, O2, O4, O6, O7, and O18.
[b] For other FEEC serogroups, see Table 3.

TABLE 5. *Relationship between production of K1 antigen and HA type in extraintestinal isolates of E. coli*

HA type	Distribution among isolates belonging to the designated HA types		Total no. (% K1 positive)
	K1 positive	K1 negative	
III	5 (7.4)[a]	36 (20.3)	41 (12.2)
IV	12 (17.6)	60 (33.9)	72 (16.7)
V	3 (4.4)	26 (14.7)	29 (10.4)
VI	48 (70.6)	55 (31.1)	103 (46.6)

[a] Numbers in parentheses indicate percent distribution.

Correlation between K1 antigen production and HA type in *E. coli* isolated from blood. The K1 antigen is considered by many to be a major virulence factor of the FEEC (23). In the present work, 245 isolates from blood were analyzed for K1 production, and 27.7% were found to be K1 positive. Table 5 shows the occurrence of K1 antigen production in relation to HA type with these isolates. It can be seen that most (70.6%) of the K1-positive isolates also belong to HA type VI, whereas the K1-negative isolates are evenly distributed with respect to HA type and in this respect resemble the general stool flora. Also, of 103 isolates belonging to HA type VI, 55 (46.6%) were K1 positive, and there

was no correlation seen between K1 production and any other HA type. These data (Table 4) and other studies to be reported elsewhere show that the K1 antigen is not responsible for HA type VI but that both of these characteristics are common to the FEEC.

DISCUSSION

The HA typing system described here is the extension of a protocol originally designed for the detection and presumptive identification of ETEC possessing CFA/I and CFA/II, which are fimbrial MR hemagglutinins (11). Two observations led us to expand the HA typing system to include tests with monkey erythocytes. First, *E. coli* isolated from non-ill individuals and particularly from extraintestinal sources were frequently observed to produce MRHA with human erythrocytes, although they were serologically negative for CFA/I and negative for MRHA with bovine or chicken erythrocytes or both. Second, *E. coli* belonging to the EPEC serogroups frequently failed to produce MSHA with human erythrocytes, although they were positive for MSHA with guinea pig erythrocytes, a test for common fimbriae. Inclusion of HA tests with monkey erythrocytes has provided additional criteria for HA typing and has led to the definition of seven major HA types which describe virtually all of the major HA patterns observed with human-associated *E. coli*. All except one of the HA types encompass more than one HA pattern, primarily because of the large number of *E. coli* isolates which produce MRHA with one or more species of erythrocyte plus MSHA with other species of erythrocyte. Nevertheless, each of the seven HA types can be defined in relatively simple terms. HA types I and II include the HA patterns produced by ETEC possessing CFA/I and CFA/II, respectively. *E. coli* belonging to HA types I and II always produce MRHA with bovine erythrocytes and never produce MRHA with monkey or guinea pig erythrocytes. Only 0.7% of 1,235 CFA-negative *E. coli* tested produced CFA-like HA patterns, presumably because these produce a combination of hemagglutinins which only rarely occur.

Strains designated as belonging to HA type III do not produce MRHA but do produce MSHA with chicken, monkey, and guinea pig erythrocytes. These are differentiated from strains which also produce MSHA with human erythrocytes (HA type IV-A); the laboratory strain K-12 belongs to HA type IV-A, and this strain is defined as possessing common fimbriae (11). In the survey reported here, 78.1% (236 of 302) of the isolates exhibiting HA type III belong to the classical EPEC serogroups. It is unclear why EPEC strains which possess fimbriae observable by electron microscopy and which produce MSHA with guinea pig erythrocytes should fail to react with human erythrocytes, and it is equally unclear whether or not this property is related to enteropathogenicity of the EPEC.

HA type V includes those HA patterns characterized by MRHA of only one species of erythrocyte. None of the five possible monospecific MRHA patterns were found in significant numbers except possibly human (only) MRHA (HA type V-A) isolates. It is important that these are distinguishable from *E. coli* belonging to HA type VI, which also produce MRHA with monkey erythrocytes and usually with chicken erythrocytes. In general, *E. coli* belonging to HA type V are unremarkable, with 68.2% of these occurring as normal stool flora. There is some evidence that monospecific MR-type hemagglutinins may not be fimbriate (6). We have examined HA type V-A *E. coli* by electron microscopy and these proved to be nonfimbriate.

HA type VI is complicated by the fact that not all (85%) of those isolates producing MRHA with human and monkey erythrocytes also produce MRHA with the chicken erythrocytes and by the fact that at least half of the HA type VI *E. coli* also produce MSHA or MRHA with guinea pig erythrocytes. It is probably significant that 45.6% of the extraintestinal isolates tested belong to HA type VI. It is more significant that 69.5% of bacteremia-associated FEEC and that 80% of those belonging to serogroups O1, O2, O4, O6, O7, and O18 belong to HA type VI. Several other investigators have cited the high incidence of *E. coli* producing MRHA with human erythrocytes among isolates from extraintestinal sources such as cerebrospinal fluid, urine, and blood (3, 15, 17), showing that the collection used here is not unusual in this respect. Czirók et al. (4, 5) and others (11, 20) have cited evidence that those *E. coli* which are a major cause of neonatal meningitis also cause sporadic, or nonepidemic, enteritis. These serogroups (e.g., O1, O18) are common in the normal flora of the bowel and are considered to be opportunistic pathogens, thus the term facultatively enteropathogenic *E. coli*, or FEEC (4). It will be of interest to determine the frequency of the HA type VI phenotype among FEEC isolated from urinary tract infections, especially because the human and monkey hemagglutinins are CFA like, i.e., share the property of mannose resistance which is the hallmark of the fimbrial CFAs of ETEC (11). The possible role of CFA-like fimbriae in adhesiveness for extraintestinal epithelial cells has already been proposed by Kal-

lenius and Mollby (15). It might also be speculated that MR-type hemagglutinins, fimbrial or otherwise, might contribute to one or more of the virulence-related properties thought to be mediated by the FEEC somatic antigens or capsular K antigens or both (28). These properties include "invasiveness" (14, 23), resistance to complement (12, 30), serum resistance (16), and resistance to phagocytosis (1, 31).

Finally, the findings reported here may be considered in relation to the general problem of defining pathogenicity of *E. coli*. *E. coli* serotyping as currently practiced provides no information about the possession of fimbrial antigens or surface-associated hemagglutinins. Our results indicate that there is a need for a serotyping system which would include these hemagglutinins, fimbrial or otherwise, in addition to O, K, and H antigens. We suggest that an HA typing system such as that described here might serve as the basis for selecting *E. coli* prototype strains for serological and antigenic classification of these surface-associated hemagglutinins.

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LITERATURE CITED

1. **Bortolussi, R., P. Ferrieri, B. Bjorksten, and P. G. Quie.** 1979. Capsular K1 polysaccharide of *Escherichia coli*: relationship to virulence in newborn rats and resistance to phagocytosis. Infect. Immun. **25**:293-298.
2. **Ceppellini, R., and M. Landy.** 1962. Suppression of blood group agglutinability of human erythrocytes by certain bacterial polysaccharides. J. Exp. Med. **117**: 321-338.
3. **Cravioto, A., R. J. Gross, S. M. Scotland, and B. Rowe.** 1979. Mannose-resistant hemagglutination of human erythrocytes by strains of *Escherichia coli* from extraintestinal sources: lack of correlation with colonisation factor antigen (CFA/I). FEMS Microbiol. Lett. **6**:41-44.
4. **Czirók, E., J. Madár, J. Budai, Z. Stverteczky, A. Kertész, I. Dombi, and L. Gyengesi.** 1976. The role in sporadic enteritis of facultatively enteropathogenic *Escherichia coli* serogroups. Acta Microbiol. Acad. Sci. Hung. **23**:359-369.
5. **Czirók, E., H. Milch, J. Madár, and G. Semjén.** 1977. Characterization of *Escherichia coli* serogroups causing meningitis, sepsis and enteritis. I. Serological properties and animal pathogenicity of O18, O78 and O83 isolates. Acta Microbiol. Acad. Sci. Hung. **24**:115-126.
6. **Duguid, J. P., S. Clegg, and M. I. Wilson.** 1979. The fimbrial and nonfimbrial haemagglutinins of *Escherichia coli*. J. Med. Microbiol. **12**:213-227.
7. **Evans, D. G., and D. J. Evans, Jr.** 1978. New surface-associated heat-labile colonization factor antigen (CFA/II) produced by enterotoxigenic *Escherichia coli* of serogroups O6 and O8. Infect. Immun. **21**:638-647.
8. **Evans, D. G., D. J. Evans, Jr., and H. L. DuPont.** 1977. Virulence factors of enterotoxigenic *Escherichia coli*. J. Infect. Dis. **136** (Suppl.):S118-S123.
9. **Evans, D. G., D. J. Evans, Jr., and W. Tjoa.** 1977. Hemagglutination of human group A erythrocytes by enterotoxigenic *Escherichia coli* isolated from adults with diarrhea: correlation with colonization factor. Infect. Immun. **18**:330-337.
10. **Evans, D. G., D. J. Evans, Jr., W. S. Tjoa, and H. L. DuPont.** 1978. Detection and characterization of colonization factor of enterotoxigenic *Escherichia coli* isolated from adults with diarrhea. Infect. Immun. **19**:727-736.
11. **Evans, D. J., Jr., D. G. Evans, and H. L. DuPont.** 1979. Hemagglutination patterns of enterotoxigenic and enteropathogenic *Escherichia coli* determined with human, bovine, chicken, and guinea pig erythrocytes in the presence and absence of mannose. Infect. Immun. **23**:336-346.
12. **Glynn, A. A., and C. J. Howard.** 1970. The sensitivity to complement of strains of *Escherichia coli* related to their K antigens. Immunology **18**:331-346.
13. **Guinée, P. A. M., W. H. Jansen, and C. M. Agterberg.** 1976. Detection of the K99 antigen by means of agglutination and immunoelectrophoresis in *Escherichia coli* isolated from calves and its correlation with enterotoxicity. Infect. Immun. **13**:1369-1377.
14. **Kaijser, B., L. A. Hanson, U. Jodal, G. Lidin-Janson, and J. B. Robbins.** 1977. Frequency of *E. coli* K antigens in urinary-tract infections in children. Lancet **i**:663-664.
15. **Kallenius, G., and R. Mollby.** 1979. Adhesion of *Escherichia coli* to human periurethral cells correlated to mannose-resistant agglutination of human erythrocytes. FEMS Microbiol. Lett. **5**:295-299.
16. **McCabe, W. R., B. Kaijser, S. Olling, M. Uwaydah, and L. A. Hanson.** 1978. *Escherichia coli* in bacteremia: K and O antigens and serum sensitivity of strains from adults and neonates. J. Infect. Dis. **138**:33-41.
17. **Minshew, B. H., J. Jorgensen, G. W. Counts, and S. Falkow.** 1978. Association of hemolysin production, hemagglutination of human erythrocytes, and virulence for chicken embryos of extraintestinal *Escherichia coli* isolates. Infect. Immun. **20**:50-54.
18. **Ørskov, F.** 1978. Virulence factors of the bacterial cell surface. J. Infect. Dis. **137**:630-633.
19. **Ørskov, F., I. Ørskov, B. Jann, and K. Jann.** 1971. Immunoelectrophoretic patterns of extracts from all *Escherichia coli* O and K antigen test strains. Correlation with pathogenicity. Acta Pathol. Microbiol. Scand. Sect. B **79**:142-152.
20. **Ørskov, I., F. Ørskov, B. Jann, and K. Jann.** 1977. Serology, chemistry, and genetics of O and K antigens of *Escherichia coli*. Bacteriol. Rev. **41**:667-710.
21. **Pitt, J.** 1978. K-1 antigen of *Escherichia coli*: epidemiology and serum sensitivity of pathogenic strains. Infect. Immun. **22**:219-224.
22. **Pitt, J.** 1979. Virulence of *Escherichia coli* K1 in adults. J. Infect. Dis. **139**:106-108.
23. **Robbins, J. B., G. H. McCracken, Jr., E. C. Gotschlich, F. Ørskov, I. Ørskov, and L. A. Hanson.** 1974. *Escherichia coli* K1 capsular polysaccharide associated with neonatal meningitis. N. Engl. J. Med. **290**:1216-1220.
24. **Salit, I. E., and E. C. Gotschlich.** 1977. Hemagglutination by purified type 1 *Escherichia coli* pili. J. Exp. Med. **146**:1169-1181.
25. **Salit, I. E., and E. C. Gotschlich.** 1977. Type 1 *Escherichia coli* pili: characterization of binding to monkey kidney cells. J. Exp. Med. **146**:1182-1194.
26. **Sarff, L., G. H. McCracken, Jr., M. S. Schiffer, M. P.**

Glode, J. B. Robbins, I. Ørskov, and F. Ørskov. 1975. Epidemiology of *Escherichia coli* K1 in healthy and diseased newborns. Lancet **i**:1099-1104.

27. **Silverblatt, F. J., J. S. Dreyer, and S. Schauer.** 1979. Effect of pili on susceptibility of *Escherichia coli* to phagocytosis. Infect. Immun. **24**:218-223.
28. **Stendahl, O., B. Normann, and L. Edebo.** 1979. Influence of O and K antigens on the surface properties of *Escherichia coli* in relation to phagocytosis. Acta Pathol. Microbiol. Scand. Sect. B **87**:85-91.
29. **Svanborg, E. C., and H. A. Hansson.** 1978. *Escherichia coli* pili as possible mediators of attachment to human urinary tract epithelial cells. Infect. Immun. **21**:229-237.
30. **Van Dijk, W. C., H. A. Verbrugh, M. E. van der Tol, R. Peters, and J. Verhoef.** 1979. Role of *Escherichia coli* K capsular antigens during complement activation, C3 fixation, and opsonization. Infect. Immun. **25**:603-609.
31. **Weinstein, R., and L. S. Young.** 1978. Phagocytic resistance of *Escherichia coli* K-1 isolates and relationship to virulence. J. Clin. Microbiol. **8**:748-755.