Hemolysin and K Antigens in Relation to Serotype and Hemagglutination Type of *Escherichia coli* Isolated from Extraintestinal Infections

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Escherichia coli isolated from cases of bacteremia and from a variety of urinary tract infections were characterized according to serotype (O:H antigenicity), K type (possession of K1, K2, K3, K12, or K13), hemagglutination (HA) type, and production of beta-hemolysin. Results obtained with the bacteremia and urinary tract infection isolates were similar except for more hemolytic isolates from urine than from blood (42 versus 29%) and more K1⁺ isolates from blood than from urine (50 versus 29%). A close correlation was found between HA type VI (production of fimbriae which mediate mannose-resistant HA of human and African green monkey erythrocytes) and the production of hemolysin or K1 capsular antigen or both. Most (95 of 98, or 95%) of the HA type VI⁺ blood isolates and most (146 of 164, or 89%) of the HA type VI⁺ urine isolates produced hemolysin or K1 or both, in contrast to 22 and 26%, respectively, of those belonging to HA types other than HA type VI. Also, 76% of all hemolytic and 70% of all K1⁺ isolates belonged to HA type VI. Remarkably few of the HA type VI⁺ isolates (13%) and even fewer of the HA type VI⁻ isolates (3%) produced both K1 and hemolysin; these belonged mainly to serotypes O16:H6, O18:H7, and O2:H4. Other major serogroups were usually K1⁺/hemolysin⁻ (O1, O7) or K1⁻/hemolysin⁺ (O2, O4, O6). At least 74% (262 of 351) and possibly as many as 83% (293 of 351) of those isolates which produced mannose-resistant HA of human erythrocytes were classified as HA type VI⁺; 31 isolates produced mannose-resistant HA with all erythrocytes tested. Taking serogroup and serotype into consideration, we conclude that the *E. coli* fimbrial hemagglutinin(s) responsible for the HA type VI phenotype will prove to be the same as the virulence-associated mannose-resistant adhesins of uropathogenic *E. coli* which other investigators have characterized as unique fimbrial antigens detectable by mannose-resistant HA of human erythrocytes.

Hemagglutination (HA) typing is a simple, rapid, and economical technique for detecting bacterial surface-associated mannose-sensitive and mannose-resistant hemagglutinins of *Escherichia coli* (11, 12). Mannose-sensitive HA (MSHA) of guinea pig erythrocytes is generally accepted as a test for common fimbriae (type 1 pili) (7, 11). Erythrocyte species-specific mannose-resistant HA (MRHA) is a property associated with specific fimbrial antigens which are produced by enterotoxigenic *E. coli* and which function as colonization factors (8, 9, 11). Enterotoxigenic *E. coli* also exhibit tissue and host

specificity which is at least partly due to the different receptor, or binding, characteristics of the various fimbrial antigens (10, 28).

E. coli belonging to the normal flora of the human intestine are generally considered to be nonpathogenic, but in fact are opportunistic pathogens; the initiation of extraintestinal infection depends upon both predisposing host factors (13, 17, 34) and possession of one or more virulence factors by individual strains of *E. coli* composing the contaminating fecal inoculum (17, 28). Examples of these infections are neonatal meningitis caused by K1-positive *E. coli*

originating in the fecal flora of the mother (13, 31) and a wide spectrum of urinary tract infections (UTI) (14, 16, 20, 35).

Individual *E. coli* isolates from the normal intestinal flora may exhibit any one of a variety of HA patterns when tested for MSHA and MRHA with human, bovine, chicken, monkey, and guinea pig erythrocytes (11, 12). Despite this heterogeneity of *E. coli* with respect to HA, it was possible to describe a HA typing scheme by which virtually any isolate can be placed within one of seven major HA types and usually within 1 of 23 specific HA subtypes (12). Comparison of the HA types of *E. coli* isolated from cases of bacteremia with those of *E. coli* isolated from normal stool specimens led to the observation that 80% of the bacteremia-associated *E. coli* belonging to serogroups O1, O2, O4, O6, O7, and O18 also belong to HA type VI (12). This HA type designates MRHA with human and monkey but not bovine erythrocytes. In HA type VI, the reaction with chicken and guinea pig erythrocytes may vary, giving rise to eight different subtypes. However, HA type VI *E. coli* which are MSHA negative are heavily fimbriated when observed by electron microscopy.

Correlations between mannose-resistant adhesion of uropathogenic *E. coli* to uroepithelial and other related cells, fimbriae-mediated MRHA of human erythrocytes, female susceptibility to colonization by such *E. coli*, and the occurrence of UTI have been reported (2, 4, 18, 22, 27, 34, 35). The remarkable coincidence between the serotype-HA type relationships that we have reported (12) and those reported by other workers employing human erythrocytes to test for MRHA and *E. coli* from various extraintestinal sources (1, 3-5, 14, 16, 18, 27, 28, 30, 36, 38) indicates that many of these investigators have described clinical correlations between extraintestinal disease and HA type VI *E. coli*. However, we felt it necessary to examine a representative collection of uropathogenic *E. coli* isolates to make a valid comparison of our respective results. In addition, the growing list of evidence that MRHA-type fimbriae are important as colonization factors of uropathogenic *E. coli* led us to examine our isolates for possible relationships between serotype, HA type, and several other recognized surface-associated virulence factors, i.e., acidic polysaccharide antigens (K1, K2, K12, and K13) and hemolysin. The results of these studies are reported here.

MATERIALS AND METHODS

Bacterial cultures. The bacteremia-associated *E. coli* (216 isolates) were obtained from as many blood specimens from patients under chemotherapeutic

treatment at a large metropolitan hospital. The UTI isolates were obtained from 395 different urine specimens collected at a large hospital and at two different reference laboratories; these were from both symptomatic and asymptomatic cases and included both hospital-acquired and community-acquired infections. However, clinical information was not considered in this study. *E. coli* serotyping was performed according to standard methods in one laboratory with anti-O and anti-H sera obtained from the Center for Disease Control, Atlanta, Ga., and also at one reference laboratory. K typing has been described elsewhere; K1, K2, K3, K12, and K13 were sought, and appropriate reference and control cultures possessing these antigens were obtained as gifts from F. Ørskov and I. Ørskov (Statens Seruminstitut, Copenhagen S., Denmark) and from B. Kaijser (University of Göteborg, Göteborg, Sweden).

Culture conditions. Stock cultures were maintained on agar slants composed of 2% peptone-0.5% NaCl-2% agar at room temperature. HA tests were performed as described below with cells grown for 18 to 24 h on CFA agar (9). CFA agar consists of 1% Casamino Acids (Difco) and 0.15% yeast extract (Difco) plus 0.002% MgSO₄ and 0.005% MnCl₂ with 2% agar added, pH approximately 7.4.

Hemolysin production was determined with blood-agar plates (blood agar base medium [BBL Microbiology Systems] containing 5% sheep erythrocytes) as described by Minshew et al. (27). In preliminary tests, it was determined that unambiguous results were obtained only with inocula stabbed into the agar; hemolysis was in the form of a clear zone of lysis and was surface associated, i.e., nonfilterable, as was described by Smith in 1963 as beta-hemolysis (33).

HA typing. HA typing was performed as recently described (12). Citrated type A human blood and guinea pig blood were prepared in the laboratory, and bovine, chicken, and African green monkey erythrocytes were obtained from Flow Laboratories, Inc., McLean, Va. HA was denoted as R (MRHA) if the same degree of HA occurred with and without mannose and S (MSHA) if HA was prevented or grossly reduced by the presence of mannose. No extraintestinal isolates could be confirmed as belonging to HA type I or HA type II in this study; these are the HA types produced by CFA/I⁺ and CFA/II⁺ enterotoxigenic *E. coli*, respectively (11). However, 24 UTI isolates did produce MRHA with all erythrocytes tested, and this property could not be attributed to serotypic roughness as was previously found to be the case with fecal isolates (12).

RESULTS

HA types and the production of hemolysin and K1 antigen by *E. coli* isolated from blood and from urine. Table 1 shows the HA typing results obtained with 216 blood and 395 urine *E. coli* isolates. Only 45.3% of the blood isolates and only 41.5% of the urine isolates belong to HA type VI. However, this is twice the frequency of HA type VI *E. coli* found in a survey of intestinal normal flora *E. coli* (12).

TABLE 1. Analysis of 216 bacteremia-associated *E. coli* isolates and 395 *E. coli* isolated from urinary tract infections showing relationships between HA type, hemolysin production, and K1 antigen production

HA type	MRHA or MSHA with ^a :					Isolates from blood			Isolates from urine		
	Hu	Bv	Ck	Mk	Gp	Total	No. hemolytic	No. K1 ⁺	Total	No. hemolytic	No. K1 ⁺
III	—	—	—	S	S	25	1	2	50	16	4
IV-A	S	—	S	S	S	20	0	2	36	8	6
IV-B	—	—	—	—	—	51	1	11	70	2	2
V-A	R	—	—	—	—	12	0	4	26	3	0
V-B	—	R	—	—	—	0	0	0	1	0	0
V-C	—	—	R	—	—	2	0	0	5	1	0
V-D	—	—	—	R	—	0	0	0	0	0	0
V-E	—	—	—	—	R	0	0	0	0	0	0
V-F	R	—	S	S	S	6	2	1	12	5	4
V-G (other)						0	0	0	0	0	0
VI-A	R	—	R	R	—	34	24	17	43	31	16
VI-B	R	—	R	R	S	40	23	21	56	36	26
VI-C	R	—	—	R	—	9	1	6	4	3	1
VI-D	R	—	S	R	S	6	4	1	29	27	3
VI-E	R	—	R	R	R	0	0	0	14	11	1
VI-F	R	—	S	R	R	0	0	0	6	3	0
VI-G (other) (example)	R	R	S	R	R ^b	9	5	4	12	7	1
VII-A	R	R	—	—	—	0	0	0	0	0	0
VII-B	R	R	—	S	S	0	0	0	1	0	0
VII-C	R	R	S	S	S	0	0	0	1	0	0
VII-D (other) (example)	R	R	R	R	R	2	2	1	29 ^c	14	4

^a Erythrocytes tested: Hu, human; Bv, bovine; Ck, chicken; Mk, monkey; Gp, guinea pig; R, MRHA; S, MSHA; —, negative for HA.

^b Weak MRHA reaction with bovine cells, usually partially mannose sensitive.

^c HA patterns: RRRRR; RR--R, and RRSSR (24, 3, and 2 strains, respectively).

Also, 57 of 63 (90.5%) of the hemolytic blood isolates and 118 of 167 (70.7%) of the hemolytic urine isolates belong to HA type VI. Similarly, 49 of 70 (70.0%) of the K1⁺ blood isolates and 48 of 68 (70.6%) of the K1⁺ urine isolates belong to HA type VI. Overall, hemolysin production was more prevalent in the UTI cultures than in the bacteremia-associated cultures (42.3 versus 29.2%), and K1 production was more prevalent in the blood cultures (50.0 versus 29.3%), as might be expected from the results of previous studies.

Because of the above result, it was of interest to know the relationship between K1 and hemolysin production for *E. coli* from both sources, particularly for those belonging to HA type VI. The results of this analysis are shown in Table 2. Several interesting relationships are immediately evident. In the group of HA type VI⁺ blood isolates, 94.9% produced either K1 or hemolysin, but usually not both (13.3%). In contrast, in the HA type VI⁻ group of blood isolates, only 22% produced either K1 or hemolysin, and only one

of 118 of these cultures produced both K1 and hemolysin (0.8%). The same relationship can be seen with the HA type VI⁺ urine isolates, with 89.0% producing either K1 or hemolysin but usually not both (12.2%). In the group of HA type VI⁻ urine isolates, only 26% produced either K1 or hemolysin, with very few positive for both properties (3.9%). Overall, irrespective of source, 239 of 262 (91.2%) of the HA type VI⁺ *E. coli* produced K1 or hemolysin or both, whereas just 88 of 349 (25.2%) of the HA type VI⁻ *E. coli* produced K1 or hemolysin or both.

Relationship between HA type VI, hemolysin production, and possession of K1, K2, K12, and K13 antigens. It is generally agreed that acidic polysaccharide production is an important factor in the virulence of *E. coli* associated with extraintestinal infections and that in UTI the K antigens K2, K12, and K13 are important, as are hemolysin and K1 antigen production (see Discussion). The data presented in Table 2 indicated that K2, K12, and K13 production by these *E. coli* isolates might be an

TABLE 2. Definition of eight different phenotypes describing the relationship between HA type VI, hemolysin production, and possession of the K1 antigen in *E. coli* isolated from blood and from urine

Phenotype ^a	No. of blood isolates (%)	No. of urine isolates (%)
HA type VI ⁺ Hly ⁺ K1 ⁺	13 (13.3) ^b	20 (12.2) ^c
HA type VI ⁺ Hly ⁻ K1 ⁺	36 (36.7)	28 (17.0)
HA type VI ⁺ Hly ⁺ K1 ⁻	44 (44.9)	98 (59.8)
HA type VI ⁺ Hly ⁻ K1 ⁻	5 (5.1)	18 (11.0)
HA type VI ⁻ Hly ⁺ K1 ⁺	1 (0.8) ^d	9 (3.9) ^e
HA type VI ⁻ Hly ⁻ K1 ⁺	20 (16.9)	11 (4.8)
HA type VI ⁻ Hly ⁺ K1 ⁻	5 (4.2)	40 (17.3)
HA type VI ⁻ Hly ⁻ K1 ⁻	92 (78.0)	171 (74.0)

^a HA type VI⁻ isolates are those which belong to HA types III, IV-A, IV-B, V, or VII.

^b Percent distribution of 98 HA type VI⁺ blood isolates.

^c Percent distribution of 164 HA type VI⁺ urine isolates.

^d Percent distribution of 118 HA type VI⁻ blood isolates.

^e Percent distribution of 231 HA type VI⁻ urine isolates.

TABLE 3. K-antigen production in relation to HA type and production of hemolysin: difference in distribution observed between K1 and other (K2, K12, K13) K antigens

Group of isolates	% of isolates in group positive for:	
	K1 antigen	K2, K12, or K13
Blood HA type VI ⁺ Hly ⁺	22.8	47.4
Blood HA type VI ⁺ Hly ⁻	87.8	7.3
Blood HA type VI ⁻ Hly ⁺	16.7	33.3
Blood HA type VI ⁻ Hly ⁻	17.8	13.4
Urine HA type VI ⁺ Hly ⁺	16.9	56.8
Urine HA type VI ⁺ Hly ⁻	60.9	15.2
Urine HA type VI ⁻ Hly ⁺	18.4	40.8
Urine HA type VI ⁻ Hly ⁻	6.0	35.1

important factor to be considered in relation to hemolysin production; this proved to be the case (Table 3). For both the blood and the urine isolates, the number of strains positive for K2, K12, or K13 was lowest in the HA type VI⁺ Hly⁻ group, in which most strains produced the K1 antigen; K2, K12, and K13 production was most prevalent in the Hly⁺ groups, irrespective of HA type VI. An insignificant number, 0.8%, of all of the isolates proved to be positive for K3, and these K3⁺ strains were HA type VI⁺ Hly⁺. Overall, i.e., irrespective of source, K2, K12, and K13 production was approximately twice as prevalent among hemolytic isolates as among nonhemolytic isolates (50.4 versus 23.3%); this is not

much difference because 35.1% (64 of 182) of the urine HA type VI⁻ cultures were also positive for these K antigens (Table 3). In contrast, only 6% of the HA type VI⁻ UTI cultures were positive for K1.

Table 4 shows summary data for the above-described properties, taking into consideration each individual HA type. These data are important because both the bacteremia and the UTI cases which were the sources of these isolates were unselected in terms of severity. Also, it is not known how many of the bacteremia cases might have arisen from UTI, and the UTI cases included many varieties of cases (symptomatic, asymptomatic, hospital acquired, community acquired, etc.). This would naturally contribute to the heterogeneity of the collection. Note that for *E. coli* belonging to HA types III, IV-A, IV-B, and V, the percent distribution of the hemolytic, K1⁺, and K2/K12/K13⁺ phenotypes was less than the overall percent distribution, whereas the reverse was true for HA type VI⁺ isolates (Table 4).

***E. coli* serotype as the determining factor in the relationship between HA type, hemolysin production, and K-antigen production.** The available serotyping information for the blood and urine *E. coli* cultures is listed in Table 5, arranged according to HA type and hemolysin production. As can be seen, a relatively small number of serogroups predominate in three of the four phenotypes listed, whereas the other (HA type VI⁻ Hly⁻) group contains a heterogeneous assortment of serogroups and serotypes. Of 57 HA type VI⁺ Hly⁺ bacteremia-associated isolates, 45 belong to serogroups O2, O4, O6, O16, and O18, but only 13 are K1⁺; these belong to serogroups O2, O16, and O18. The few isolates belonging to the phenotype HA type VI⁻ Hly⁺ are similar. Note that serogroup O4 was associated with K3 and K12; O6 was associated with K2, K12, and K13; and O2 was associated with K1 and K13. With very few exceptions, the serotypes belonging to the phenotype HA type VI⁺ Hly⁻ are different, with O1:K1:H7 and O7:K1:H⁻ very prevalent in this group. Many of the predominant serogroups were consistent with respect to K antigen and hemolysin reaction, particularly O1:H7, O4:H1 or H5, and O7:H⁻. Within certain other serogroups, K antigen varied independently of hemolysin production, namely serogroups O2, O6, and O18, and O22 from UTI. Thus, the results shown in Tables 2 and 3 depend upon the relative proportion of certain major serogroups, mainly O2, O4, O6, O7, and O18 (and also O22 in the case of UTI), accounting for the inverse relationship between K1 and hemolysin produc-

TABLE 4. Distribution of *E. coli* isolated from blood and urine (611 isolates) and of hemolytic and K antigen-positive strains according to HA type

HA type	HAP ^a	No. of isolates (% distribution) identified as:				
		All	Hly ⁺	Hly ⁻	K1 ⁺	K2 ⁺ , K12 ⁺ , or K13 ⁺
III	(--SSS)	75 (12.3)	17 (7.4)	58 (15.2)	6 (4.3)	24 (11.7)
IV-A	(S-SSS)	56 (9.2)	8 (3.5)	48 (12.6)	8 (5.8)	13 (6.3)
IV-B	(-----)	121 (19.8)	3 (1.3)	118 (31.0)	13 (9.4)	27 (13.2)
V	(R----	64 (10.5)	11 (4.8)	53 (13.9)	9 (6.5)	18 (8.8)
VI	(R-RR-)	262 (42.9)	175 (76.1)	87 (22.8)	97 (70.3)	104 (50.7)
VII	(RR---	33 (5.4)	16 (7.0)	17 (4.5)	5 (3.6)	19 (9.3)
All		611 (100) ^b	230 (37.6)	381 (62.4)	138 (22.6)	205 (33.6)

^a Representative HA pattern; for details see Table 1.^b Overall percent distribution.

tion.

***E. coli* isolated from cases of neonatal meningitis.** Although only seven *E. coli* isolates from cases of neonatal meningitis were available for study, the results are worth noting (Table 6) because six of these belong to HA type VI, the seventh being an enteropathogenic *E. coli*, or EPEC, serogroup. At least five of the six HA type VI⁺ isolates possess K1 or hemolytic activity or both. Thus, in general these cerebrospinal fluid isolates resemble the bacteremia and UTI isolates.

DISCUSSION

E. coli isolated from cases of bacteremia, UTI, and neonatal meningitis exhibit several characteristics indicative of a complex system of virulence factors. These include hemolysin production, possession of acidic polysaccharide (or K) capsular antigens, and fimbriae with special adhesive properties, all of which are serogroup or serotype related (Table 7). Two other important factors shared by these *E. coli* are the ability to survive as members of the normal intestinal flora and an unusual ability to survive in extraintestinal sites such as the bloodstream, cerebrospinal fluid, and upper urinary tract. Our study, reported here, has confirmed the general observation that when a large number of individual isolates are examined, these virulence factors are found in a variety of combinations which are so numerous that any collection of extraintestinal isolates appears upon gross examination to be very heterogeneous. However, when any one major virulence factor, including any one of several major serogroups (e.g., O1, O2, O6, O18, O22), is examined relative to other major virulence factors, definite relationships are easily discerned. Numerous investigators have reported results obtained by this approach, usually taking into account a specific type of extraintes-

tinal infection; representative studies are cited in Table 7. As can be seen from the references cited, most if not all of the many virulence factors have been clearly identified with specific types of infection, for example, the K-1 antigen and neonatal meningitis. On the other hand, it may also be noted that no one virulence factor is restricted to a single type of extraintestinal infection, which one might expect from the opportunistic nature of these pathogens and the importance of host susceptibility factors.

The investigation reported here was prompted by several prior observations by ourselves and others. We noted a direct correlation between HA type VI and serogroup in bacteremia-associated isolates (12). Others have independently stated the idea that surface-associated adhesins and particularly fimbriae which mediate MRHA of human erythrocytes are virulence factors important in extraintestinal infections (2, 4, 18, 22, 27, 34). Our results document the existence of several important relationships which either have not been reported previously or have been observed and yet have received little attention or comment. For example, the vast majority (90%) of our hemolytic bacteremia-associated *E. coli* isolates belong to HA type VI, as well as 70% of those found to be positive for the K1 antigen. Seventy percent of the hemolytic and 70% of the K1⁺ UTI *E. coli* isolates were also typed as HA type VI. Most (95%) of the HA type VI⁺ bacteremia isolates proved to be K1⁺ or hemolytic or both, as were 89% of the HA type VI⁺ UTI isolates; this is in contrast to only 22 and 26%, respectively, of those isolates belonging to HA types other than HA type VI. Remarkably few HA type VI⁺ *E. coli* produced both K1 and hemolysin (13%), and even fewer of the non-HA type VI isolates had this property (only 3%). The basis for this relationship proved to be a negative correlation between hemolysin

TABLE 5. *E. coli* serotypes isolated from blood and urine: distribution with respect to production of hemolysin and HA type^a

Source	Serotype (no. of isolates)			
	HA type VI ⁺ Hly ⁺	HA type VI ⁺ Hly	HA type VI ⁻ Hly ⁺	HA type VI ⁻ Hly
Blood	O4:K12:H1, H5, or H ⁻ (10) O6:K2:H1 or H31 (9) O7:K2 or K ⁻ (8) O16:K1:H6 (5) O18:K1:H7 or H ⁻ (5) O6:K ⁻ :H?, H12, or H31 (5) O4:K3:H5 or H? (4) O2:K1:H4 (2) O2:K13:H1 or H ⁻ (2) O6:K13:H1 (2) Rxx:K1:H ⁻ (1) O11:K2:H1 (1) O21:K2:H5 (1) O25:K ⁻ :H ⁻ (1) O18:K ⁻ :H7 (1)	O1:K1:H7 (17) O7:K1:H ⁻ or H? (10) O2:K1:H7 (3) O15:K13, or K ⁻ :H1 (3) O18:K1:H7 or H14 (2) O75:K1:H ⁻ (2) O21:K ⁻ :H4 (1) Rgh:K1 or K2 (3)	O6:K13:H1 (2) O6:K ⁻ :H28 (1) O6:K ⁻ :H31 (1) O2:K1:H6 (1) O18:K1:H7 (1) O18:K1:H7 (1)	Rgh:K ⁻ or K13: H ⁻ or H? (43) O:K:H (misc.; one each x 24) O7:K ⁻ :H?, H6, H8, or H28 (9) O75:K2 or K ⁻ :H ⁻ or H? (7) O15:K ⁻ or K13:H ⁻ or H? (5) O6:K1:H1, H28, or H? (3) O75:K1:H?, H9, or H ⁻ (3) Rgh:K1:H ⁻ (6) O1:K1:H1 (2) O6:K12:H10 (2) O9:K ⁻ or K2:H ⁻ (2) O2:K1:H4 (1) O16:K1:H ⁻ (1) O18:K1:H? (1) O21:K1:H? (1) O83:K1:H?(1) O83:K1:H4 (1)
UTI	O1:K1:H7 (2) O18:K2, K13 or K ⁻ :H1, H14 or H ⁻ (7) O6:K2:H31 (2) O6:K13:H1 (2) O22:K2:H1 (2) O22:K ⁻ :H5 (1) O4:K3:H5 (2) O2:K3:H1 (1) O78:K12:H21 (1) O75:K13:H ⁻ (1) O85:K2:H2 (1) misc (UT) (6)	O1:K1:H ⁻ (1) O1:K1:H7 (1) O7:K1:H ⁻ (1) Rgh:K1:H ⁻ , or H7 (2)	O22:K1:H1 (2) O22:K ⁻ :H1 (3) O6:K ⁻ :H1 (3) O83:K ⁻ :H31 (1) O50:K ⁻ :H1 (1) Rgh:K2:H ⁻ (1)	O50:K?:H10 (2) Rgh:H ⁻ (6) O83:K1:H4 (1) Rgh:K ⁻ :H18 (1)

^a K⁻, Negative for K1, 2, 3, 12, and 13. UT or ?, untypable.TABLE 6. Preliminary survey of *E. coli* isolated from cerebrospinal fluid (neonatal meningitis) for HA type, hemolysin, and K1 production

Strain	Serotype	HA pat-tern ^a	Hemolysin
1	Rgh:K1:H ⁻	R-RR-	Negative
2	Rgh:K1:H6	R-RR-	Positive
3	O125:K70:H19	S-SSS	Negative
4	Not typed (K12 ⁺)	R-RR-	Positive
5	Not typed (K2 ⁺)	R-RR-	Positive
6	O16:K1:H6	R-RR-	Positive
7	O1:K?:H7	R-RR-	Negative

^a For details, refer to Table 1.

production and possession of the K1 antigen, coupled with a positive correlation between hemolysin production and possession of either K2, K12, or K13 (Table 3). This observation was confirmed when *E. coli* serogroup and serotype were taken into consideration. Although all of the O16:H6, many of the O18:H7, and a few of the O2:H4 isolates produced both K1 and hemolysin, these were exceptional. Serogroups O1 and O7 were strongly correlated with K1 production and lack of hemolysin activity; serogroup O4 (all 16 isolates) was found to be hemolytic and positive for either K3 or K12; most

TABLE 7. *Reported correlations between special surface-associated antigens or structures of E. coli and extraintestinal disease states*

Property of <i>E. coli</i>	Literature citation with properties in reference to:					
	UTI	Acute pyelonephritis	Neonatal meningitis	Bacteremia (general)	Bacteremia (adult)	Neonatal sepsis
0 groups 01, 07, 016, and 018	14, 28, 38	29, 34, 38	28, 31	12, 28	25	25
0 groups 02, 04, 06, 016, 018 and/or 075	1, 4, 11, 14, 17, 19, 22, 26, 31	16, 18, 23, 34, 38	4, 28, 31	4, 12, 24, 28, 38, 39	25	25
K1 antigen	13	15, 16, 23, 29	13, 26, 31, 32	12, 29, 40	25, 40	13, 25, 31, 32
K2, K12, K13	1, 3, 14, 16	16, 23	4	4, 12	25	25
Hemolysin	1, 3, 6, 27, 36			27		
MRHA of human erythrocytes	4, ^a 7, 15, ^a 19, 20, 21, 27, 35	18, ^a 35	4 ^a	4, ^a 12, ^a 22, ^a 27	12 ^a	
Adhesiveness	34, 35, 37, 19, 20, 21	2, 18, 34, 35, 37				
Fimbriation (piliation)	1, 19, 20, 21, 37	18, 37		12		

^a MRHA not caused by CFA/I.

of the O6 isolates (29 of 33) were hemolytic and produced either K2 or K13 or were K⁻. It may also be noted that serogroups O1, O2, O4, O6, O7, O16, and O18 were prominent in the HA type VI⁺ group, whereas the HA type VI⁻ group was composed of a variety of serogroups (mostly rough, untypable, or represented by single or very few isolates of unusual serotypes such as O6:K12:H10, O7:K⁻:H28, and O9:K1:H⁻).

In comparing the above results with the voluminous data on record (Table 7), we find good agreement. Several areas deserve special note; these are (i) reported correlations between *E. coli* fimbriae, MRHA, mannose-resistant adhesion to human uroepithelial cells, and special serogroups such as O4 in relation to UTI (2, 4, 7, 18, 19, 27, 34, 35); (ii) special O:K:H serotypes, for example O4:K12:H5, O1:K1, O2:K1:H4, and O6:K2:H1 in relation to UTI (1, 5, 14-16, 23, 30, 34, 38); (iii) serogroups and K antigen in relation to neonatal meningitis (13, 31); (iv) hemolysin production by serogroups O4 and O6 (1, 3, 36); and (v) special serogroups in relation to neonatal and adult sepsis (12, 25, 31).

Thus, there is general agreement that the surface-associated factors of extraintestinal isolates of *E. coli* which qualify as virulence-related factors include serogroup (specific O antigens); possession of capsular polysaccharide, or K, antigens; hemolysin; and fimbriae which mediate MRHA of human (and monkey) erythrocytes. In theory, these *E. coli* could be classified in terms of relative pathogenicity. It is safe to speculate that the most aggressive strains are those which belong to HA type VI and produce K1 or hemolysin or both. Supposedly secondary on the scale would be those which possess only

one of these properties (HA type VI fimbriae, hemolysin, or K1). The least aggressive would be those which belong to serotypes other than those which make up the first two groups and which are HA type VI⁻ Hly⁻ K1⁻. It will be interesting to compare, on a case-by-case basis, UTI infections according to predisposing host factors, the extent and severity of infection, and the infecting *E. coli* strain classified with respect to serotype, including K type, HA type, and production of beta-hemolysin.

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