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Hemagglutination of Human Type O Erythrocytes, Hemolysin Production, and Serogrouping of *Escherichia coli* Isolates from Patients with Acute Pyelonephritis, Cystitis, and Asymptomatic Bacteriuria

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The purpose of this investigation was to study potential virulence factors associated with *Escherichia coli* urinary pathogens isolated from patients with urinary tract infection. These factors were compared with characteristics of normal-flora *E. coli* isolated from stool specimens of healthy individuals without a history of urinary tract infection. The potential virulence factor focused on in this study was hemagglutination (HA) of human type O erythrocytes by *E. coli* urinary pathogens. A total of 265 strains of *E. coli* isolated from patients with urinary tract infections were tested for their ability to hemagglutinate human type O erythrocytes; of these, 148 (56%) were HA positive. Only 6 of 36 fecal *E. coli* strains (17%) isolated from healthy controls were HA positive. This significant association of the presence of hemagglutinin on *E. coli* that causes urinary tract infections indicates the likelihood that HA is a marker of virulence. Only 12% (5 of 43) of *Proteus mirabilis* and 3% (3 of 104) of *Klebsiella pneumoniae* urinary isolates were HA positive. There was a trend for HA-positive *E. coli* to be isolated from patients with kidney infections and positive tests for antibody-coated bacteria rather than bladder infections and negative tests for antibody-coated bacteria, although the difference was not statistically significant. There was a significant correlation ($P < 0.025$) between hemolysin production and HA; 67% (69 of 103) of the isolates that produced hemolysin also hemagglutinated human type O erythrocytes. There was no significant correlation between HA and motility, although there was a trend for flagellated organisms to be non-hemagglutinators. There was a marked correlation between the presence of hemagglutinin and the serogroup of the *E. coli* isolate; serogroups O4, O7, and O50 were almost always HA positive (57 of 63; 90%). In contrast, serogroups O8 and O25 were rarely HA positive (2 of 30; 7%).

Members of the family *Enterobacteriaceae* and particularly *Escherichia coli* are the most frequent organisms responsible for urinary tract infections. Several properties of *E. coli* strains may contribute to the pathogenicity of this organism; these include the presence of K_1 antigen (9-11), hemolysin production (1-3, 18, 23), and epithelial cell adherence characteristics (4, 5). In addition, the capability of *E. coli* strains to hemagglutinate erythrocytes appears to be associated with virulence (6, 8, 12, 16).

Our study further tests the hypothesis that hemagglutination (HA) of human type O erythrocytes is a marker of virulence. *E. coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* urinary pathogens and fecal *E. coli* isolates from individuals without a history of infection were compared in their hemagglutinating ability. The frequency of HA by *E. coli* isolates from patients with acute pyelonephritis (kidney tissue invasion with inflammation), patients with uncomplicated cystitis (superficial infection of the bladder usually without deep wall invasion), and patients with asymptomatic bacteriuria (tissue invasion may or may not have occurred) were compared to determine the significance of HA in tissue invasion. In addition, a comparison was made of the hemagglutinating ability of *E. coli* isolated from urines containing antibody-coated bacteria (ACB). The presence of ACB is a marker of an immune response and has been shown to indicate the occurrence of kidney tissue invasion and in some rare instances bladder wall invasion (22). If HA is a virulence marker related to aiding the organism in tissue invasion, then one might expect a higher frequency of hemagglutinating *E. coli* isolates from patients with acute pyelonephritis and from urines containing ACB. The occurrence of two other potential virulence factors, hemolysin production and mo-

tility, was compared with HA. In addition, the serogroup of *E. coli* was compared with the hemagglutinating ability of the organism. The sensitivity of the HA reaction to blocking by D-mannose, α-methyl-D-mannoside, D-mannoheptulose, and yeast mannan (sugars known to block some bacterium-erythrocyte interactions was tested (14, 15, 17).

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MATERIALS AND METHODS

Bacterial isolates and clinical diagnoses of patients. The bacteria characterized in these studies were isolates from adult female patients with urinary tract infections. The clinical diagnosis of acute pyelonephritis was based on the occurrence of fever, chills, and flank pain. Nausea and vomiting, as well as frequency, urgency, and dysuria, were frequent symptoms. Costovertebral angle tenderness was always found. The clinical diagnosis of cystitis was based on the occurrence of frequency, urgency, dysuria, and suprapubic pain; fever, chills, and flank pain were absent. Asymptomatic bacteriuria was diagnosed when the patient had ≥100,000 organisms per ml of urine in the absence of symptoms; urine cultures were repeated once and often twice to confirm the diagnosis of asymptomatic bacteriuria. Normal-flora *E. coli* organisms were isolated from stool specimens of healthy female individuals without a known history of urinary tract infection. All bacteria were subcultured in Todd-Hewitt broth and stored at −70°C.

Urine sediment FA test. Localization of the site of the infection was determined by the fluorescent-antibody (FA) method of detecting ACB in urine sediments; ACB occur in urines from patients with kidney infections but not uncomplicated bladder infections (22). The urines were centrifuged at 1,500 × *g* for 10 min. The supernatants were discarded, and the sediments were gently washed twice in phosphate-buffered saline (PBS) with the aid of a Vortex mixer. The washed sediments were treated with 0.2 ml of a 1:5 dilution of fluorescein-conjugated anti-human globulin of horse origin (Roboz Surgical Instrument Co., Washington, D.C.), incubated at 37°C for 30 min, and rewashed twice in PBS. During the second washing, the mixture was allowed to stand for 10 min before centrifugation. The sediment was Vortex mixed, and a 0.025-ml sample was spread on a slide, air dried, and examined microscopically for fluorescence (AO Spencer fluorolume illuminator, model 645) with a 50× oil objective. The intensity of fluorescence was recorded as negative (0) or positive with gradations of +, ++, +++, and ++++. An FA test was considered to be positive if at least 25% of the bacterial cells in each high-power field fluoresced. In addition to the clinical diagnoses and results of FA tests for ACB, the site of infection was determined by direct methods of ureteral catheterization or bladder washout catheterization for two patients.

Selection of media. Various organisms used in the HA tests were subcultured on 5% sheep blood agar (Trypticase soy agar base, BBL Microbiology Systems, Cockeysville, Md.), nutrient agar (Difco Laboratories, Detroit, Mich.), Trypticase soy agar (BBL), colonization factor antigen agar (8), Todd-Hewitt broth (Difco), and nutrient broth (Difco). Organisms were routinely grown on 5% sheep blood agar for HA studies unless specified otherwise. A chemically defined medium was used to determine whether medium-associated blood group-reactive substances were responsible for or masked HA. This medium was free of blood group-reactive substances. Hemagglutinating and nonhemagglutinating bacteria were subcultured three successive times on a synthetic medium consisting of 2% agar (Difco) in medium 199 (GIBCO Laboratories, Grand Island, N.Y.). The subcultured bacteria were tested for their ability to hemagglutinate erythrocytes.

HA test. Human type O, Rh-positive erythrocytes freshly collected periodically from two individuals were used for HA testing. The erythrocytes were washed three times and suspended to a 4% (vol/vol) concentration in PBS, pH 7.2 (PBS-FTA HA buffer, BBL). The bacteria were grown overnight on 5% sheep blood agar. Approximately 0.05 ml of PBS was dropped onto a cooled microscope slide. Colonies of bacteria were emulsified in PBS so that a heavy, milky-white suspension formed (10^9 organisms per ml). An equal volume of erythrocytes was added, and the suspension was gently mixed with a wood applicator. The slide was rotated, and macroscopic HA was observed within 1 min (14). Controls included bacteria suspended in PBS and erythrocytes suspended in PBS.

HA inhibition tests with sugars. The ability of 10^{-1} to 10^5 μM D-mannose, α-methyl-D-mannoside, and D-mannoheptulose and 2% yeast mannan (Sigma Chemical Co., St. Louis, Mo.) to inhibit HA was tested by using these sugars to pretreat either human type O erythrocytes or bacteria. Each sugar was diluted in a 4% suspension of erythrocytes to reach the desired concentrations of sugar; the mixture was incubated at 25°C for 10 min; the pretreated unwashed erythrocytes were then mixed with untreated bacteria in the HA test. In one experiment, the pretreated erythrocytes were washed twice in PBS before being used in the HA test. Similarly, bacteria were emulsified in 0.05 ml of PBS containing these various concentrations of sugars and incubated at 25°C for 10 min; the pretreated bacteria were mixed with untreated erythrocytes and read for HA or inhibition of HA.

Detection of hemolysin production. *E. coli* isolates were tested for hemolysin production on 5% human blood agar; erythrocytes used in the preparation of blood agar were washed three times in PBS. Human blood agar was prepared from type O, Rh-positive erythrocytes. Trypticase soy agar base was used for human blood agar. Subcultured bacteria were stabbed into blood agar plates with sterile toothpicks. The plates were incubated overnight at 37°C and observed for hemolysin production, as indicated by a clear zone surrounding the pinhead of growth.

Motility. *E. coli* strains were tested for motility by stabbing the isolates into motility test medium (BBL) containing 0.05 g of triphenyltetrazolium chloride per liter, which allowed better visualization of the spread

of growth. After overnight incubation at 37°C, organisms were reported as motile when their growth spread out from the stab; nonmotile strains and weakly motile strains were retested.

Serogrouping. *E. coli* isolates were routinely serogrouped. Organisms were subcultured in Todd-Hewitt broth, washed, and suspended in 5.0 ml of PBS. The organisms were heated at 100°C for 1 h, washed twice, and resuspended in 2.0 ml of PBS. A drop of the suspension of organisms was mixed with various O antisera (Difco). The plate was gently rocked and observed for agglutination. Agglutination with homologous antisera occurs within seconds. When the *E. coli* isolate appeared to be cross-reactive and agglutinated with more than one antiserum, various concentrations of the organism were tested to determine which antisera gave the strongest reaction. If the organism agglutinated with each of the O antisera, it was recorded as autoagglutinable. If it did not agglutinate with any of the antisera, it was classified as nontypable.

Statistical analysis. Significance levels were determined with the chi-square test, using the Yates continuity correction.

RESULTS

HA by *E. coli* urinary pathogens and normal fecal *E. coli* isolates. We tested the hypothesis that HA of type O erythrocytes is a marker of virulence. Results showed that *E. coli* urinary isolates hemagglutinated human type O erythrocytes significantly more often ($P < 0.001$) than did normal fecal *E. coli* isolates from individuals without a history of urinary tract infection. Of the *E. coli* isolates from patients with urinary tract infections, 56% (148 of 256) hemagglutinated human type O erythrocytes. In contrast, only 17% (6 of 36) of the normal fecal *E. coli* isolates hemagglutinated.

Studies have shown that *E. coli* HA of erythrocytes may be inhibited by sugars, particularly D-mannose or D-mannose derivatives, indicating the possible role of these sugars as receptors on the bacteria or erythrocyte surface. Fifty-two strains of hemagglutinating *E. coli* urinary isolates from patients with acute pyelonephritis, cystitis, and asymptomatic bacteriuria and six fecal *E. coli* isolates from control individuals were tested to determine whether HA was blocked by mannose or other sugars. HA by these strains of *E. coli* was not inhibited by treatment of bacteria or erythrocytes with 0.1 μM to 100 mM D-mannose (100 mM = 18.0 mg/ml), α-methyl-D-mannoside (19.4 mg/ml), D-mannoheptulose (21 mg/ml), 2-deoxy-D-glucose (16.4 mg/ml), D-galactose (18.0 mg/ml), L-fucose (16.4 mg/ml), and β-D-fructose (18.0 mg/ml) or 2% yeast mannan (20 mg/ml). In some experiments, the erythrocytes were washed to remove any unbound inhibitor. These erythrocytes were mixed with the bacteria and observed for hemagglutination. Washing the erythrocytes did not affect HA.

HA of human type O erythrocytes by other *Enterobacteriaceae*. The question was posed as to whether HA is a characteristic of *Enterobacteriaceae* urinary pathogens other than *E. coli*. Results showed that *E. coli* urinary isolates hemagglutinated human type O erythrocytes much more frequently than did *P. mirabilis*, *K. pneumoniae*, or other species of *Proteus*, *Enterobacter*, or *Citrobacter* combined (Table 1). Only 12% of the *P. mirabilis* and 3% of the *K. pneumoniae* urinary isolates hemagglutinated human type O erythrocytes.

HA and the presence of ACB in the urine. Results of FA tests for the detection of ACB in urine sediments indicate the anatomic site of urinary tract infection; positive FA tests are associated with kidney infections, and negative FA tests are associated with uncomplicated bladder infections (22). This study questioned whether HA was a virulence characteristic of *E. coli* isolates associated predominantly with kidney infection and positive FA results rather than with bladder infection and negative FA results. FA test results for 260 urines from patients with urinary tract infections were compared with the capability of *E. coli* urinary isolates to hemagglutinate human type O erythrocytes. A total of 61% (86 of 142) of the *E. coli* isolates associated with positive FA tests were HA positive, and 49% (58 of 118) of the *E. coli* isolates associated with negative FA tests were HA positive; this was not a significant difference ($P > 0.05$). In addition to these studies, results of FA testing of urines from 78 patients with well-documented acute pyelonephritis, cystitis, and asymptomatic bacteriuria were compared with the capability of *E. coli* isolates to hemagglutinate human erythrocytes (Table 2). Of the urines from pa-

TABLE 1. *HA of human type O erythrocytes by Enterobacteriaceae urinary pathogens*

Organism[a]	No. of isolates showing bacterial HA of human erythrocytes (%)	
	Positive	Negative
E. coli (265)	148 (56)[b]	117 (44)
P. mirabilis (43)	5 (12)	38 (88)
K. pneumoniae (104)	3 (3)	101 (97)
Other *Proteus* spp. (12)	2 (17)	10 (83)
Enterobacter (14)	3 (21)	11 (79)
Citrobacter (18)	1 (6)	17 (94)
Total (456)	162 (37)	294 (63)

[a] Number of isolates is given within parentheses.
[b] The significance of the difference between HA by *E. coli* compared with other *Enterobacteriaceae* is expressed as a *P* value for chi-square analysis ($P < 0.001$).

tients with acute pyelonephritis, 75% (21 of 28) contained ACB; 76% of the bacterial isolates from these ACB-positive urines were HA positive. Of the urines from patients with asymptomatic bacteriuria, 62% (18 of 29) contained ACB; 78% of the bacterial isolates from these ACB-positive urines were HA positive. Five percent of the urine isolates (1 of 21) from patients with clinical cystitis contained ACB, and the isolate from this urine was HA negative. It should be noted that a significant number of *E. coli* isolates from patients with cystitis were hemagglutinators (11 of 21; 52%), as were isolates from patients with acute pyelonephritis (21 of 28; 75%) and asymptomatic bacteriuria (20 of 29; 70%); there was no statistically significant difference in the number of positive HA tests for isolates from these three groups of patients.

Hemolysin production and HA. Another question was whether HA and hemolysin production were related characteristics of *E. coli* strains isolated from patients with infections rather than normal-flora *E. coli* strains. *E. coli* urinary isolates were more frequently hemolytic ($P < 0.001$) for human erythrocytes than were normal fecal *E. coli*, i.e., 38% (103 of 270) compared with 6% (2 of 36). Table 3 shows a correlation between hemolysis production and HA; 67% of the urinary pathogens that were hemolytic also hemagglutinated human erythrocytes.

HA and motility. *E. coli* urinary pathogens and normal fecal *E. coli* isolates were tested for motility. There was no relationship between pathogenicity and motility ($P > 0.2$); both urinary pathogens and normal fecal *E. coli* isolates were frequently motile, i.e., 66% (105 of 160) compared with 77% (27 of 35). Whether the presence of flagella inhibited or enhanced HA was determined (Table 4). Results showed a tendency for flagella to be associated with organisms that were HA negative, although this association was not statistically significant.

HA and serogroup of *E. coli*. There was a marked correlation of HA of erythrocytes and certain serogroups of *E. coli* urinary pathogens (Table 5). Serogroups O4, O7, and O50 almost always (57 of 63; 90%) hemagglutinated human erythrocytes. In contrast, serogroups O25 and O8 rarely (2 of 30; 7%) hemagglutinated human cells. In contrast to urinary pathogens, most normal fecal *E. coli* strains were nontypable with the antisera available, or they belonged to

TABLE 2. *Results of urine sediment FA test for the presence of ACB compared with the clinical diagnoses of the patients and the capacity of the urinary isolates to hemagglutinate human type O erythrocytes*

Diagnosis[a]	No. of patients (%) with:		
	ACB+[b]	HA+[c]	HA+ ACB+/ ACB+
Acute pyelonephritis (28)	21 (75)	21 (75)	16/21 (76)
Asymptomatic bacteriuria (29)	18 (62)	20 (70)	14/18 (78)
Cystitis (21)	1 (5)	11 (52)	0/1 (0)
Normal fecal flora (35)	ND[d]	6 (17)	ND

[a] Number of patients with each diagnosis is given within parentheses.
[b] ACB+, Positive FA test for the presence of ACB in the urine.
[c] HA+, Positive HA test.
[d] ND, Not done.

TABLE 3. *HA and hemolysis of human type O erythrocytes by E. coli urinary pathogens*

Hemolysin production	No. of isolates (%) showing HA		Total no. (%)
	Positive	Negative	
Positive	69 (67)[a]	34 (33)	103 (39)
Negative	84 (52)	79 (48)	163 (61)

[a] Chi-square value; $P < 0.025$.

TABLE 4. *HA of human type O erythrocytes by E. coli urinary pathogens compared with motility*

Motility	No. of isolates (%) showing HA		Total no. (%)
	Positive	Negative	
Positive	56 (53)[a]	49 (47)	105 (66)
Negative	37 (67)	18 (33)	55 (34)

[a] Chi-square value; $P > 0.05$.

TABLE 5. *HA of human type O erythrocytes by E. coli urinary pathogens compared with the serogrouping of the E. coli isolates*

Serogroup	No. of HA positive/total no. tested (%)
O7	22/23 (96)
O4	19/20 (95)
O50	16/20 (80)
O75	13/20 (65)
O22	5/8 (63)
O18	12/20 (60)
O2	9/16 (56)
O14	2/4 (50)
O15	7/14 (50)
O112	2/4 (50)
Autoagglutinators	6/12 (50)
O16	4/9 (44)
O6	8/21 (38)
O1	4/11 (36)
O86	4/12 (33)
O25	2/21 (10)
O8	0/9 (0)

serogroups different from the urinary isolates (Table 6).

Hemagglutinin detection in *E. coli* cultured in a medium free of blood group-reactive substances. Blood group-reactive substances are present in many complex bacteriological growth media. Organisms may pick up these blood group-reactive substances during growth on such media. These substances may mask the bacterial hemagglutinin, or they may coat the bacterial surface with hemagglutinating substances. Sixteen hemagglutinating *E. coli* and six non-hemagglutinating *E. coli* were subcultured successively three times on a chemically defined medium free of blood group-reactive substances. After subculture, the organisms were tested for HA. Hemagglutinating *E. coli* subcultured on the defined medium hemagglutinated as well as did organisms grown on blood agar, nutrient agar, Trypticase soy agar, colonization factor antigen agar, Todd-Hewitt broth, and nutrient broth. Non-hemagglutinating bacteria grown on these media did not hemagglutinate when subcultured on the chemically defined medium.

TABLE 6. *HA of human type 0 erythrocytes by normal fecal E. coli isolates compared with the serogrouping of the E. coli isolates*

Serogroup	No. of HA positive/total no. tested
Nontypable	2/14
O6	0/5
O3	0/2
O13	0/2
O18	1/2
O9	1/1
O1	0/1
O77	0/1
O19	1/1
O86	0/1
O21	0/1
O7	1/1
O124	0/1
O140	0/1
O8	0/1
Autoagglutinators	0/1

DISCUSSION

This study questions the relationship between HA and virulence. Previous studies have shown that the ability of *E. coli* to hemagglutinate was characteristic of strains that cause disease rather than normal-flora strains that are unassociated with disease (Table 7). In this study, 56% of the *E. coli* isolates from patients with urinary tract infections hemagglutinated human type O erythrocytes, whereas only 17% of normal fecal *E. coli* isolates from healthy individuals without a history of urinary tract infection hemagglutinated human type O erythrocytes. This marked association of hemagglutinating *E. coli* with infection or disease indicates the likelihood that HA of human erythrocytes is a marker of microbial virulence. HA is an indicator of the binding capacity of bacterial cells for membrane surfaces and may be related to the ability of strains to attach to epithelial cell surfaces enhancing colonization. However, the actual relationship of the capacity of an organism to hemagglutinate human type O erythrocytes and to attach to human uroepithelial cells is not known.

Mannose and mannose derivatives were used as inhibitors of the bacterium-erythrocyte interaction because studies have shown that pilus-mediated HA is mannose sensitive (15). We found that D-mannose, α-methyl-D-mannoside, D-mannoheptulose, and yeast mannan did not inhibit HA, regardless of whether the sugar was used to pretreat the bacteria or the erythrocytes. Similar results were observed by Ljungh et al. (12), who used human type O erythrocytes. Thus, HA of human type O erythrocytes by *E. coli* urinary pathogens is generally mannose resistant and may be unrelated to pili. Although these results indicate that the simple sugars tested are not part of the bacteria or erythrocyte receptor site, it is possible that a more complex form of the sugar is a part of the receptor site

TABLE 7. *HA of human and guinea pig erythrocytes by E. coli urinary pathogens and fecal E. coli strains*

Investigator (reference)	Source of erythrocyte	No. of *E. coli* HA^{+a} urinary pathogens/total no. tested (%)	No. of HA$^+$ fecal *E. coli* strains/total no. tested (%)	No. of MRHA^b *E. coli*/total no. tested (%)
Minshew et al. (13)	Human O	14/22 (64)	1/8 (12)	ND^c
C.P.G.	Human O	148/256 (56)	6/35 (17)	58/58 (100)
Ljungh et al. (12)	Human O or A	45/157 (29)	28/170 (16)	36/45 (80)
Vosti (24)	Human A	32/63 (51)	22/95 (23)	2/9 (22)
Svanborg Edén and Hansson (21)	Guinea pig	8/12 (67)	ND	1/8 (12)

[a] HA$^+$, Positive HA test.

[b] MRHA, Hemagglutination is not blocked by mannose. Concentrations of mannose used by the investigators were 18 mg/ml, 18 mg/ml, 12.5 μg/ml, and 25 mg/ml, respectively.

[c] ND, Not done.

which cannot be blocked by the simple sugar. The use of blood types other than O may give different results. Vosti (24), using human type A erythrocytes, and Svanborg Edén and Hansson (21), using guinea pig erythrocytes, found that most urinary isolates demonstrate mannose-sensitive HA, which indicates a pilus-mediated reaction.

The presence of hemagglutinin may convey invasive characteristics to *E. coli* strains. There was an increased frequency of HA-positive *E. coli* isolates from patients with acute pyelonephritis (75%) compared with HA-positive isolates from patients with cystitis (52%). These results are not surprising when the fact that most kidney infections occur by the ascending route is considered. Bacteria probably first colonize the bladder before the kidney. The hemagglutinin may aid the colonization of the bladder by the organism and may further give the organism a selective advantage in ascending the ureter to the kidney. The finding of an increased frequency of HA-positive *E. coli* isolates from urines containing ACB, a marker of kidney infection, lends further support to the possible role of HA as an indicator of invasiveness.

E. coli is the most common, but not the only, organism that causes urinary tract infections. Therefore, it was of interest to determine the capacity of other urinary pathogens to hemagglutinate. Results showed that *E. coli* urinary isolates hemagglutinated human type O erythrocytes much more frequently (56%) than did other urinary isolates tested (8%), including *P. mirabilis*, *K. pneumoniae*, and other species of *Proteus*, *Enterobacter*, and *Citrobacter*. Thus, the fact that *E. coli* is, by far, the most common cause of urinary tract infection and is also the most efficient hemagglutinator may be related to the greater ability of the organism to attach to membrane surfaces as compared with other *Enterobacteriaceae*.

HA is one of several potential virulence factors described for *E. coli*. Until now, no one has defined the relationship of HA to these other factors, including hemolysin production, motility, and serogrouping. Several studies have suggested that hemolysin production by *E. coli* might be an important virulence factor for strains that cause urinary tract infection (1–3, 18, 23). The finding of a significant association between hemolysin production and HA further substantiates the role of these factors in the virulence of *E. coli* urinary pathogens. Although HA and hemolysin production appear to be related phenomena, each factor can occur independently. The relationship of these two characteristics could be explained if they were controlled by the same plasmid or lysogenic bacteriophage. Studies have shown that plasmids may control the ability of an organism to hemagglutinate or hemolyze erythrocytes. Smith and Halls (19) found that, of 53 hemolytic *E. coli* strains isolated from epidemiologically unrelated sources, 10 were able to transfer their hemolytic ability to nonhemolytic bacteria. The K88 hemagglutinin of *E. coli* strains that causes porcine diarrhea and the colonization factor antigen-associated hemagglutinin of enterotoxigenic *E. coli* strains have also been shown to be governed by transmissible plasmids (7, 20). Certain somatic "O" antigenic groups of *E. coli* have been associated with strains isolated from various kinds of infections of humans or animals but are uncommon in *E. coli* strains of the normal enteric flora. Vosti (24) did not find a correlation between HA of human erythrocytes and serogrouping of *E. coli* urinary isolates; in addition, urine isolates of 10 common serogroups did not differ in their hemagglutinating ability from isolates of less common serogroups. In our study, a marked correlation was seen between HA and the serogroup of the *E. coli* urinary pathogen; serogroups O7, O4, and O50 were almost always HA positive, whereas serogroups O25 and O8 were rarely HA positive. The similarities of serogroups O7, O4, and O50 are not known, since information of the sugar constituents and linkages making up these O antigens is incomplete. However, the structure of the O8 antigen is known. This O antigen is made up of repeating mannose linkages, which may account for the inability of this organism to hemagglutinate. Most other O antigens are much more complex. These data suggest that the physical and chemical structure of the O antigen probably plays an important role in the interaction between *E. coli* and erythrocytes. Cross-reactivity among O antigens of *E. coli* is common. For example, *E. coli* O7, O4, and O25 are known to be cross-reactive antigens. The finding that serogroups O7 and O4 usually hemagglutinate human type O erythrocytes brings up the question of why serogroup O25 rarely hemagglutinates these cells. If the hemagglutinin of serogroups O7 and O4 is a part of the O antigen complex, then it appears to be separate or independent from the moiety that cross-reacts with serogroup O25.

Motility has been suggested as a possible virulence factor in urinary tract infections. However, there was no association between motility and virulence, since most of the *E. coli* urinary isolates and normal fecal isolates were motile. There was a tendency for flagella to be associated with organisms that were HA negative. Therefore, flagella may interfere with HA, pos-

sibly by sterically hindering the bacterium-erythrocyte interaction or blocking the hemagglutinin present on the bacterial cell surface.

In conclusion, *E. coli*-mediated HA of human erythrocytes was shown to be a marker of virulence. Results indicated a close correlation between HA by *E. coli* and pathogenicity. Other *Enterobacteriaceae* urinary pathogens usually did not hemagglutinate. There was a trend for HA-positive*E. coli* organisms to be isolated from patients with kidney infections rather than with bladder infections. In addition, most of the *E. coli* isolates from ACB-positive urines were also HA positive. There was a significant association between HA, hemolysin production, and the serogroup of the *E. coli* urinary pathogen, suggesting the role of multiple virulence factors in the pathogenesis of *E. coli* urinary tract infections.

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