These negative results are probably not attributable to a lower sensitivity of tissue culture technique, since both methods proved equally sensitive when mice or cultures were inoculated with purified tachyzoites or bradyzoites (3). Possibly, only intracellular parasites were present in these negative cases and were not released from the amniotic fluid cells within 24 h of contact with the monolayers. Results of antigen determination were disappointing. Using a sensitive immunoassay that allows detection of 10 ng/ml (2), we could not demonstrate the presence of toxoplasma antigen in any of the amniotic fluid samples, even after concentration. These negative results suggest that, if antigen is present in amniotic fluid, it is at a very low concentration; moreover, its presence may be only transient due to either the rapid turnover of amniotic fluid or its destruction by proteolytic enzymes (4).

In conclusion, the tissue culture technique can be routinely used in addition to mouse inoculation for demonstration of *Toxoplasma gondii* in amniotic fluid. Since tissue culture results are obtained within a few days, the technique might provide early evidence of infection at a time when other parasitological methods are still inconclusive.

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Survey of Purported Virulence Factors of *Escherichia coli* Isolated from Blood, Urine and Stool

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One hundred randomly selected urinary and blood isolates and 50 stool isolates of *Escherichia coli* were analyzed for phenotypic characteristics which may contribute to their virulence potential. Bacteremic isolates were more likely to have K1 capsules and express mannose-sensitive hemagglutination compared to stool isolates. Blood-stream isolates more frequently contained complete O side-chains in their lipopolysaccharide layer and less frequently exhibited mannose-resistant hemagglutination when compared to urinary isolates. Total plasmid content, hemolysin, total colicin and colicin V production were not significantly increased in *Escherichia coli* from blood or urine when compared to those recovered from stool.

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Escherichia coli is the most common gram-negative bacillus causing infection of the bloodstream and urinary system. Immunotherapeutic approaches have been sought as adjuncts to conventional antimicrobial therapy in the management of these infections (1, 2). A greater knowledge of those determinants which contribute to the virulence of *Escherichia coli* may permit the development of vaccines which would interrupt the invasive potential of this organism. A large number of phenotypic characteristics of *Escherichia coli* have been reported which may contribute to its pathogenic potential in the urine and blood. Potential virulence determinants include certain outer membrane proteins (3), a complete (smooth) lipopolysaccharide (LPS) layer (1, 4), certain O serotypes (4), K1 and other K capsules (1, 5), hemolysin production (2, 6, 7), specific adhesins (6), plasmid content (4), certain colicins (8), and iron sequestration (9).

Many previous studies have relied upon results obtained using a limited number of genetically manipulated isolates of *Escherichia coli* (6, 7). While such experimental studies may provide valuable insights into the virulence potential of specific determinants, their relevance to human infection is difficult to ascertain. To define better the significance of these determinants we surveyed their relative frequency in clinical isolates obtained in an epidemiologically relevant manner.

Materials and Methods. One hundred randomly selected *Escherichia coli* isolates were recovered from

blood and urine specimens submitted to the clinical microbiology laboratory at Walter Reed Army Hosptial. Urinary isolates were derived from patients with symptomatic urinary tract infection. No attempt was made to determine the anatomic location within the genitourinary tract from which the urinary isolates originated. Fifty *Escherichia coli* isolates were collected from stool specimens from healthy individuals undergoing routine proctoscopic examination. A single colony was selected from each stool culture obtained.

The presence of complete (smooth) LPS was inferred from resistance to lysis by rough specific bacteriophages BR_{60}, Ffm, C_{21} and BR_2, and K1 specific lytic bacteriophages A-E (provided by Dr. B. Rowe, Central Public Health Laboratory, London, UK) were used to detect the K1 capsule using previously described methods (5). Hemolysin activity was observed on washed blood agar plates (Scott Laboratories, USA) after overnight incubation at 37 °C. Tests to detect colicin and colicin V production, and assays to determine mannose-sensitive (MSHA) and resistant (MRHA) hemmagglutination were performed using standard techniques (6, 8). Plasmis were isolated by the method of Kado and Liu (10) and characterized by agarose gel electrophoresis. Colony hybridization to detect the presence of genomic DNA homologous to the genes for colicin V and alpha-hemolysin production was performed using standard methods (11). DNA probes were prepared from cloned genes for colicin V (strain JK 492 [pKF 900], kindly provided by J. Konisky, Urbana, IL, USA) and hemolysin (strain WAM 298 [pWAM398], kindly provided by R. Welch, Madison, WI, USA).

The Kruskal-Wallis and Chi-squared tests were employed to determine statistical significance among variables using 2 X 2 or 2 X 3 contingency tables where appropriate.

Results and Discussion. The results are summarized in Table 1. The K1 capsule was significantly more common in extraintestinal isolates (35 % blood, 39 % urine) compared to stool isolates (18 %) [$p < 0.05$, $p < 0.01$]. There was no difference in K1 capsule production between blood and urinary isolates.

Urinary strains expressed rough LPS more frequently (38 %) than did stool (20 %) or blood isolates (21 %). There was no significant difference in the expression of hemolysin, total colicin, colicin V or plasmid content among urinary, stool or blood isolates of *Escherichia coli*. Results of the phenotypic assay for hemolysin production were remarkably congruent with results obtained using the DNA probe. There was only one discrepancy with a single stool isolate giving a positive result with the probe but negative results with the phenotypic assay. The colicin V probe was more sensitive than the phenotypic assay hybridizing with 14 blood, 10 urine and 3 (6 %) stool isolates. This compares with only 5 blood, 4 urine and 1 (2 %) stool isolate using the phenotypic assay.

Phenotypic expression of type I pili (MSHA) was found more frequently in blood isolates (91 %) than urinary (61 %) or stool isolates (66 %) [$p < 0.001$]. However, MRSA was significantly associated with urinary isolates compared to blood ($p < 0.0001$) or stool ($p < 0.05$) isolates. Urinary isolates showed an association between MRHA and rough LPS, 61 % of strains with MRHA having rough LPS compared to only 33 % of strains without MRHA ($p < 0.03$).

Table 1: Summary of purported virulence factors found in blood, urine and stool isolates of *Escherichia coli*.

Virulence factor	Number of strains giving positive reaction			Statistical analysis		
	Blood n = 100	Urine n = 100	Stool n = 50	Blood vs. urine	Blood vs. stool	Urine vs. stool
K1 capsule	35	39	9	p = NS	p < 0.05	p < 0.01
Smooth LPS	79	62	40	p < 0.01	p = NS	p < 0.05
Hemolysin	32	27	10[a]	p = NS	p = NS	p = NS
Mannose-sensitive hemagglutination	91	61	33	p < 0.001	p < 0.001	p = NS
Mannose-resistant hemagglutination	2	18	2	p < 0.0001	p = NS	p < 0.05
Colicin	24	21	16	p = NS	p = NS	p = NS
Colicin V (DNA probe)	14	10	3	p = NS	p = NS	p = NS
Colicin V (phenotypic assay)	5	4	1	p = NS	p = NS	p = NS
Plasmid content (mean ± SD)	2.8 ± 0.33	2.9 ± 0.36	2.7 ± 0.42	p = NS	p = NS	p = NS

[a]Isolates were detected by hemolysin on blood agar plates.

Previous investigations into the pathogenic significance of surface factors and extra-cellular products of *Escherichia coli* have studied well-defined strains and their isogenic variants lacking the specific determinant of interest, or have genetically transferred determinants into relatively avirulent strains (2, 3, 5, 7). In this manner, hemolysins, colicin V and various surface adhesins have been proposed as specific virulence factors of *Escherichia coli.* A survey of phenotypes among clinical isolates collected in an epidemiologically relevant manner provides complementary data to that derived from genetically manipulated strains and animal models.

In this study we compared *Escherichia coli* strains from systemic infection (blood) and non-systemic infection (urine), and non-pathogenic isolates from normal fecal flora, examining one isolate per patient. Our data show that there are significant phenotypic and genotypic differences among *Escherichia coli* isolated from blood and urine. Whereas isolates from both sites had a significantly higher frequency of K1 encapsulation than the non-pathogenic fecal isolates, blood isolates differed from uropathogenic *Escherichia coli* in their higher frequency of complete LPS and MSHA, and lower frequency of MRHA.

The high frequency of the rough LPS phenotype among urinary isolates has been previously observed (1, 12) and is important in that rough strains are more susceptible to serum and opsonophagocytic killing, even in the presence of K1 encapsulation (1, 5). This suggests that many uropathogenic *Escherichia coli* may be incapable of surviving in blood and disseminating. The urinary isolates in this study, as in other studies (6, 12), had significantly increased frequency of MRHA, which is associated with the ability of *Escherichia coli* to attach to host cells, an initial step in the infectious process.

A significant finding in this study was the similar rate of hemolysin activity among *Escherichia coli* isolated from the three sites. Hemolysin was detected in 20–32 % of all isolates. It has been proposed that hemolysin production by *Escherichia coli* could be a mechanism of scavenging iron under the iron-limited conditions that exist in the body. It is therefore surprising that we did not detect more hemolysin production, as O'Hanley et al. (2) did, in uropathogenic strains. Uropathogenic strains may differ considerably from bacteremic strains. Only 25 % of blood and spinal fluid isolates produced hemolysin versus 60 % of pyelonephritic strains in one study (12). The isolates examined in the current study may have greater iron-sequestering ability as a means of acquiring iron from extraintestinal sites (8, 9). The frequency of the colicin V production and total plasmid content did not differ in isolates derived from blood, urine or stool. While plasmid-mediated traits may be important in bacterial virulence (3, 8), they are not discerned by analyzing total plasmid content.

This study demonstrates that no single determinant is sufficiently frequent to warrant attention in vaccine development against invasive *Escherichia coli* infection. The significant differences in properties between uropathogenic and bacteremic isolates indicates a need for further studies to identify which uropathogenic strains are most likely to cause disseminated infection. We conclude that if vaccines designed to interfere with specific virulence determinants are developed for the prevention of urinary infections, such vaccines might not be effective for bacteremic infection with *Escherichia coli.*

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