Virulence Factors of *Escherichia coli* Isolates From Patients with Symptomatic and Asymptomatic Bacteriuria and Neuropathic Bladders Due to Spinal Cord and Brain Injuries

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Chronic bacteriuria is a common occurrence among spinal-cord injury patients and others with neuropathic bladders. If bacteria are present in the urinary tract, the patient may develop symptoms of infection or remain asymptomatic. We have compared virulence properties of 28 *Escherichia coli* isolates from patients with symptomatic urinary tract infections (UTI) and 29 *E. coli* isolates from patients with asymptomatic bacteriuria (ABU). Bacteria from patients with symptomatic UTI were more likely to be hemolytic than isolates from patients with ABU (P = 0.05) or fecal isolates obtained from healthy volunteers (P < 0.001). Bacteria from patients with symptomatic UTI were also more likely than strains isolated from patients with ABU (P = 0.08) or fecal strains (P < 0.001) to exhibit α-mannose-resistant hemagglutination of human erythrocytes. The results suggest that *E. coli* isolates from nonimmunocompromised patients who require intermittent catheterization and who develop symptomatic UTI may be distinguished from bacteria recovered from patients who remain asymptomatic and possibly from normal fecal *E. coli*.

Urinary tract infection (UTI) is a frequent medical complication during the initial medical and rehabilitation period after spinal-cord injury (SCI) and traumatic brain injury (TBI) and continues to be a problem throughout the life of many spinal-cord-injured individuals. The incidence of patients with neuropathic bladders frequently contains bacteria, and *Escherichia coli* is among the most frequent bladder colonizers (1, 5, 6). These bacteria may produce symptoms of UTI or may produce only asymptomatic bacteriuria (ABU). Bacteria associated with ABU are often left untreated and may even be beneficial in preventing symptomatic infection by more virulent organisms (16). Little is known about the virulence properties of *E. coli* bacteria that cause symptomatic UTI in patients with neuropathic bladders or how these strains may differ from benign colonizing strains. In the present study, approved by the Institutional Review Board of the hospital at which it was performed, we investigated selected virulence properties of *E. coli* isolates from SCI and TBI patients with UTI and ABU. Our hypothesis was that *E. coli* isolates from patients with symptomatic UTI possess virulence properties that may be used to distinguish them from other *E. coli* strains.

**MATERIALS AND METHODS**

**Patient characteristics.** Subjects were selected from patients admitted to a rehabilitation hospital between January 1994 and December 1996 with a diagnosis of SCI or TBI who were treated with intermittent catheterization and acquired at least one colonization of the urinary tract with *E. coli*. Twenty-five patients developed 28 symptomatic UTIs due to *E. coli* during that time. Of these 16 males and 9 females, 4 had TBI and 21 had SCI (12 tetraplegics and 9 paraplegics); the mean age was 39 (range, 7 to 75). Twenty-seven patients were colonized 29 times with *E. coli* but remained asymptomatic. Of these 15 males and 12 females, 1 had TBI and 19 had SCI (9 tetraplegics and 10 paraplegics); the mean age was 42 (range, 14 to 70). Symptomatic UTI was defined as a fever of at least 38.5°C (101°F) plus one or more of the following symptoms in a patient with a urine colony count of >100,000 CFU per ml: voiding between catheterizations which had not previously occurred, otherwise unexplained increase in spasticity, pyuria (>10 leukocytes per high-power field), or hematuria. Such UTIs were treated with an antibiotic chosen from the antibiogram report indicating sensitivities to antibiotics. All patients had documented sterile urine after discontinuation of the antibiotics.

**Collection of specimens.** All patients while on intermittent catheterization had a catheterized urine specimen collected under sterile conditions once weekly. Isolates from patients with symptomatic UTI used in this study were collected prior to initiation of antimicrobial therapy. These specimens were kept on ice and transported to the clinical microbiology laboratory within 20 min. Samples were streaked for single colonies on MacConkey agar, sheep blood agar, and Columbia CNA agar plates (BBL, Cockeysville, Md.). *E. coli* isolates were identified by standard microbiological methods. All *E. coli* isolates identified from colonizations where *E. coli* was the only species, or the predominant species (>99%), present were subjected to further analysis as described below. In five instances, isolates from the same patient were collected from separate infection or colonization events. Such isolates were verified as unique based on differences in antibiogram biotype and/or pulsed-field gel electrophoretic analysis of endonuclease-digested whole-cell DNA. The charts of the patients from whose specimens the *E. coli* isolates grew were also reviewed based on the criteria listed above to determine whether or not each patient had a UTI and was in fact treated. If the patient’s manifestations met the criteria and the patient was treated, the colonization was counted as symptomatic.

**Determination of phenotypes.** Methods used for assay of hemolysin expression, serum resistance, and α-mannose-resistant hemagglutination (MRHA) of human erythrocytes have been described previously and were used without modification (9, 12). Strains were identified as proteotrophic or auxotrophic by testing for capacity to grow on minimal salts agar supplemented with 0.4% glucose (4).

**Colony blot hybridization.** Colony DNA blots were prepared from a single colony of each isolate by the steam-alkaline lysis method of Maas (14). Hybridization probes were purified as restriction endonuclease fragments from recombinant plasmids or synthesized as PCR amplification products from plasmid-containing strains. Specific hybridization probes used for detection of *dru, pap, hly*, and *psh* genes have been described elsewhere (2, 9, 17). Probes specific for *focA* and *focC* were prepared by PCR amplification of recombinant-DNA strains HB101(pPIL110-54) and HB101(pANN801-13), respectively, kindly provided by J. Hacker. The sequences of PCR primers used were 5′-GACGTGGATACGA CGATTACTG 3′ and 5′-TACGCATAGGTATAGGTGAC 3′, respectively, of human erythrocytes. The probe for *ucaA* was prepared by PCR amplification of *Proteus mirabilis* HU1069 (3).

Primer sequences were 5′-TCTAAAAGCGATGTTAAGTAACTGAC 3′ and 5′-TATGACGTTACAATTACCTTACTGGAAA 3′. Probes were radiolabeled with a random priming kit (Pharmacia). Hybridizations were conducted at high stringency (in 1× SSC [0.15 M NaCl plus 0.015 M sodium citrate] at 68°C).

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TABLE 1. Frequency of virulence-associated phenotypes among *E. coli* isolates from patients with symptomatic UTI and ABU

<table>
<thead>
<tr>
<th>Patient status</th>
<th>Frequency of phenotype (%)</th>
<th>MRHA</th>
<th>Hly&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Serum resistance&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Antimicrobial resistance&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Auxotrophy&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>Symptomatic</td>
<td></td>
<td>13/28 (46)</td>
<td>10/28 (36)</td>
<td>17/28 (61)</td>
<td>10/27 (37)</td>
<td>10/27 (37)</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td></td>
<td>7/29 (24)%</td>
<td>4/29 (14)%</td>
<td>16/24 (67)</td>
<td>7/24 (29)</td>
<td>10/29 (34)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Hly, hemolysin expression.  
<sup>b</sup> Resistance to the bactericidal action of normal human serum.  
<sup>c</sup> Resistance to one or more of the antibiotics tested.  
<sup>d</sup> Requirement for one or more nutritional factors for growth.  
<sup>e</sup> *p* = 0.08 versus symptomatic isolates.  
<sup>f</sup> *p* = 0.05 versus symptomatic isolates.

**Antimicrobial resistance.** The disk diffusion method was used to determine antimicrobial susceptibility. Antimicrobials tested included amikacin, ampicillin, ampicillin-sublactam, augmentin, cefazolin, ceftriaxime, cefuroxime, cephalothin, ciprofloxacin, gentamicin, nitrofurantoin, norfloxacin, piperacillin, tetracycline, tobramycin and trimethoprim-sulfamethoxazole.

**Statistical methods.** Statistical comparisons were made by chi-square analysis.

**RESULTS**

Single urinary tract (UT) *E. coli* isolates from patients experiencing symptomatic UTI or ABU were examined for expression of selected virulence phenotypes and for the presence of virulence-associated genes. The results are summarized in Tables 1 and 2, respectively.

**Hemagglutination.** Isolates were tested for MRHA, the ability to agglutinate human erythrocytes in the presence of the receptor analog D-mannose. The MRHA phenotype was more frequent among symptomatic-UTI isolates than among ABU isolates. The frequencies of P pilus genes among symptomatic-UTI isolates and ABU strains were similar. Two *E. coli* isolates from patients with ABU possessed *pap* genes but did not express MRHA. One ABU isolate was MRHA<sup>+</sup> and possessed *dra*, but not *pap*, genes. Symptomatic-UTI and ABU isolates were also tested for the presence of other UT-associated adherence genes, including genes for type 1 (*pil*), S (*sfa*), Dr (*dra*), type 1C (*foc*), and Uca (*uca*) pilis. For all except *pil*, the probes detected homologous sequences at low frequency. One or more of the adherence genes were present in 48% of symptomatic-UTI isolates and 36% of ABU isolates. As reported in numerous other studies, type 1 pilus genes (*pil*) were found in nearly all isolates; due to the nearly ubiquitous nature of type 1 pilis and the *pil<sup>+</sup>* genotype among the *Enterobacteriaceae*, no further analysis was pursued (2).

**Hemolysin.** The Hly<sup>+</sup> phenotype was significantly more prevalent among *E. coli* isolates from patients with symptomatic UTI than among ABU strains. Isolates were also probed for hemolysin-specific DNA sequences. The frequency of *hly* genes among symptomatic-UTI strains was greater than that among ABU isolates, but the difference was not significant. Three Hly<sup>−*hly<sup>+</sup>* isolates and one that was Hly<sup>+</sup> but lacked *hly* were observed among *E. coli* isolates from patients with ABU.

**Serum resistance.** No significant difference was observed in frequency of resistance to human serum between symptomatic-UTI and ABU *E. coli* isolates. Values were similar to the reported frequency of 52% for normal fecal strains.

**Antimicrobial resistance.** No significant difference was observed in frequency of resistance to one or more antimicrobial agents between *E. coli* isolates from patients with symptomatic UTI and ABU strains. The number of antimicrobial agents (17 tested) to which these 49 isolates were resistant ranged from 1 to 9, with a mean of 4.3.

**Auxotrophy.** No significant difference was observed in frequency of requirement for nutritional supplements for growth between symptomatic-UTI and ABU isolates. Values are similar to reported values for frequency of auxotrophy among normal fecal *E. coli* isolates (35%) and isolates from non-SCI subjects with symptomatic UTI (58%).

**Associations.** The combined strain set (symptomatic UTI plus ABU), which represents *E. coli* isolates that colonize the neuropathic bladder, was analyzed for associations between virulence factors. This analysis revealed that *pap<sup>+</sup>* isolates were more frequently *hly<sup>+</sup>* and expressed the hemolytic phenotype (*hly* was present in 13 of 16 *pap<sup>+</sup>* isolates but only in 1 of 35 isolates lacking *pap*) (*P* < 0.005). A trend toward increased antimicrobial resistance among *pap<sup>+</sup>* strains was also noted (8 of 16 *pap<sup>+</sup>* strains versus 8 of 33 strains lacking *pap* were resistant) (*P* < 0.1). In addition, a strong association was observed between the auxotrophic phenotype and sensitivity to antimicrobial agents (16 of 18 auxotrophic isolates versus 15 of 29 prototrophic isolates were sensitive) (*P* < 0.01).

**DISCUSSION**

Hemolysin is a cytolytic enzyme secreted by many *E. coli* isolates from patients with extraintestinal infections. Our results suggest that hemolysin may contribute to clinical symptoms of UTI in SCI patients. This conclusion is consistent with results of an earlier study of UT colonization in young women without SCI wherein the hemolytic phenotype was found at significantly increased frequency among symptomatic-UTI versus ABU isolates (8).

**TABLE 2. Frequency of virulence genotypes among *E. coli* isolates from patients with symptomatic UTI and ABU**

<table>
<thead>
<tr>
<th>Patient status (no. of isolates)</th>
<th><em>pap</em>&lt;sup&gt;a&lt;/sup&gt;</th>
<th><em>pil</em>&lt;sup&gt;a&lt;/sup&gt;</th>
<th><em>sfa</em>&lt;sup&gt;a&lt;/sup&gt;</th>
<th><em>foc</em>&lt;sup&gt;a&lt;/sup&gt;</th>
<th><em>dra</em>&lt;sup&gt;a&lt;/sup&gt;</th>
<th><em>uca</em>&lt;sup&gt;a&lt;/sup&gt;</th>
<th><em>hly</em>&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptomatic (28)</td>
<td>9 (32)</td>
<td>27 (96)</td>
<td>4 (14)</td>
<td>4 (14)</td>
<td>3 (11)</td>
<td>3 (11)</td>
<td>10 (36)</td>
</tr>
<tr>
<td>Asymptomatic (29)</td>
<td>8 (27)</td>
<td>28 (96)</td>
<td>3 (10)</td>
<td>3 (10)</td>
<td>1 (3)</td>
<td>2 (8)</td>
<td>6 (21)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Genes: *pap*, P pilus; *pil*, type 1 pilus; *sfa*, S pilus; *foc*, type 1C pilus; *dra*, Dr pilus; *uca*, Uca pilis; *hly*, hemolysin.
Bacterial adherence is widely held to contribute to colonization of the normal UT. Our results suggest that D-mannose-resistant adherence and the pap* genotype also were associated with colonization of the UT in our patient group. The present results are in contrast with a previous study of catheter-associated E. coli bacteruria in geriatric subjects wherein no correlation was found between MRHA or pap genotype and UT colonization (15). However, these studies may not be directly comparable because subjects in the previous study had indwelling urinary catheters in place at least 30 days prior to the study, while the subjects in the present study were managed with intermittent catheterization. The frequency of the MRHA phenotype was also significantly greater among isolates from patients with symptomatic infection in our study group compared with the reported frequency of MRHA among E. coli in bowel flora of the general population (16%) (7, 10). One interpretation may be that D-mannose-resistant adherence genes among symptomatic-UTI isolates may reflect their possibly increased presence in the bowel flora of our study group. However, numerous studies have shown a low frequency of MRHA among fecal isolates from a variety of subject populations. Additional studies comparing the frequency of virulence factors among fecal isolates of SCI patients with those for other subject groups will be required to address this possibility.

Consistent with numerous published reports showing that E. coli type 1 pili contribute to bladder colonization, pil genes were detected in a majority of both symptomatic-UTI and ABU E. coli isolates in this study. Other UT-associated adherence genes representing S, type 1C, and Dr pili were found at a significantly different frequency among urine isolates from SCI and TBI subjects. Resistance, antimicrobial resistance, and auxotrophy, among symptomatic-UTI and ABU E. coli isolates were not significantly different. However, associations were found between these properties and other virulence-associated traits. We observed, as have others, a close correlation between pap and hly, most likely the result of these traits being linked genetically on the E. coli chromosome (9, 11). We also found a significant association between auxotrophy and sensitivity to antimicrobial agents commonly used for treatment of UTI. This finding suggests possible selection of auxotrophs among sensitive clinical isolates. The mechanism for selection is currently unclear. Our results did not confirm an increased frequency of auxotrophic bacteria among UT isolates compared with normal fecal E. coli isolates as reported in studies of non-SCI subjects (13).

In conclusion, we have determined that E. coli isolated from patients with symptomatic UTI undergoing intermittent catheterization may be distinguished from ABU isolates and normal fecal bacteria on the basis of their virulence characteristics. Our results point to the need for further research to determine if virulence factors such as hemolysin can guide the clinician in deciding whether or not to treat for E. coli colonization by indicating which isolates pose a higher risk of causing a symptomatic UTI under certain conditions. The results also suggest that future vaccines directed against selected bacterial virulence antigens may be of value in reducing the frequency of symptomatic UTI in this high-risk patient group.

ACKNOWLEDGMENTS

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REFERENCES