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 D-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*.

**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 who developed symptomatic UTI or TDI. Subjects had a mean age of 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 catheterization, 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 antibiotic susceptibility, plasmid content, or hemagglutination properties. *E. coli* isolates were identified by standard plasmid methods. All *E. coli* isolates were identified by standard microbiological methods. *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 antibiotic susceptibility, plasmid content, or hemagglutination properties. *E. coli* isolates were identified by standard microbiological methods. *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 antibiotic susceptibility, plasmid content, or hemagglutination properties.

**Determination of phenotypes.** Methods used for assay of hemolysin expression, serum resistance, and D-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.

**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 *dra, pap, hly,* and *pl* genes have been described elsewhere (2, 9, 17). Probes specific for focal adhesion (focal adhesion) were amplified by PCR of recombinant-DNA strains HB101 (pPI110-54) and HB101 (pANN801-13), respectively, kindly provided by J. Hacker. The sequences of PCR primers used were 5’ GACGTTGATACGA CGATTACTG 3’ and 5’ TACGCATAGGTATAGGTGAC 3’. The probe for nucA was prepared by PCR amplification of Proteus mirabilis HU1069 (3). Primer sequences were 5’ CTCATAAGCGATGGTGTAATGAACTGTAGC 3’ and 5’ TATGACGGTACAATTACTTTTACTGGAAA 3’.

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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 urine 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.
RESULTS

Single urinary tract (UT) *E. coli* isolates from patients experiencing asymptomatic 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\(^+\) 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) pili. 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 pili and the pil\(^+\) genotype among the *Enterobacteriacea*, no further analysis was pursued (2).

**Hemolysin.** The Hly\(^+\) 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\(^-\) Hly\(^+\) isolates and one that was Hly\(^+\) but lacked *hly* were observed among *E. coli* isolates from patients with ABU.

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

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

**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>
<thead>
<tr>
<th>Patient status</th>
<th>Frequency of phenotype (%)</th>
<th>No. (%) of isolates with virulence gene*</th>
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<tbody>
<tr>
<td>Symptomatic (28)</td>
<td>MRHA 13/28 (46)</td>
<td>pap 9 (32)</td>
</tr>
<tr>
<td></td>
<td>Hly(^+) 10/28 (36)</td>
<td>pil 27 (96)</td>
</tr>
<tr>
<td></td>
<td>Serum resistance(^a) 17/28 (61)</td>
<td>sfa 4 (14)</td>
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<td></td>
<td>Antibacterial resistance(^b) 10/27 (37)</td>
<td>foc 4 (14)</td>
</tr>
<tr>
<td></td>
<td>Auxotrophy(^c) 10/27 (37)</td>
<td>dra 3 (11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>uca 3 (11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hly 10 (36)</td>
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<tr>
<th>Asymptomatic (29)</th>
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<tr>
<td>Hly(^+)</td>
<td>7/29 (24)(^d)</td>
<td>pap 8 (27)</td>
</tr>
<tr>
<td>Serum resistance(^a)</td>
<td>4/29 (14)(^d)</td>
<td>pil 28 (96)</td>
</tr>
<tr>
<td>Antibacterial resistance(^b)</td>
<td>16/24 (67)</td>
<td>sfa 3 (10)</td>
</tr>
<tr>
<td>Auxotrophy(^c)</td>
<td>10/29 (34)</td>
<td>foc 7/24 (29)</td>
</tr>
</tbody>
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* Genes: pap, P pilus; pil, type 1 pilus; sfa, S pilus; foc, type 1C pilus; dra, Dr pilus; uca, Uca pilus; hly, hemolysin.
Bacterial adherence is widely held to contribute to colonization of the normal UT. Our results suggest that α-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+ E. coli in bowel flora of the general population (16%) (7). One interpretation may be that α-mannose-resistant adherence contributes to E. coli uropathogenesis in this patient group. Alternately, the increased presence of MRHA+ bacteria 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, pili genes were detected in a majority of both symptomatic-UTI and ABU E. coli isolates in this study. Other UT-associated virulence genes representing S, type 1C, and Dr pili were found at reduced frequency in all SCI isolates, as has been reported for E. coli isolates from the UTs of non-SCI patients (10). E. coli strains were tested for the presence of uca genes, previously described only in P. mirabilis, because of reported similarities between the Uca structural protein and a protein associated with the E. coli G hemagglutinin (3). uca genes were found at low frequency among urine isolates from SCI and TBI subjects. Additional studies will be required to determine whether uca genes in E. coli promote uroepithelial cell adherence as they do in P. mirabilis.

The frequencies of other tested properties, including serum 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 auxotrophy 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 hemolysins 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