The Role of Bacterial Virulence and Host Factors in Patients with *Escherichia coli* Bacteremia Who Have Acute Cholangitis or Upper Urinary Tract Infection

Ming-Cheng Wang,1 Chin-Chung Tseng,1 Chiung-Yu Chen,1 Jiunn-Jong Wu,2 and Jeng-Jong Huang1

Departments of 1Internal Medicine and 2Medical Technology, National Cheng Kung University Medical College, Tainan, Taiwan, Republic of China

We studied the pathogenic role of host and *Escherichia coli* virulence factors in the development of *E. coli* bacteremia in patients with acute cholangitis (AC) or upper urinary tract infection (UTI). Isolates recovered from 75 adult patients consecutively admitted to the hospital with *E. coli* bacteremia caused by AC (*n* = 24) or upper UTI (*n* = 51) were evaluated, as were 30 fecal strains isolated from healthy control individuals. Virulence genes of *E. coli* were detected by polymerase chain reaction analysis, including *papG* genes (classes I–III), *sfa/foc*, *fimH*, *afa*, *hlyA*, *cfsA*, and *iutA*. Our results show that biliary tract obstruction and urinary tract obstruction are important host factors for the development of *E. coli* bacteremia in patients with AC and upper UTI, respectively. With regard to *E. coli* virulence factors, the *papG* class II gene might play a more important role in the development of *E. coli* bacteremia in patients with upper UTI than in those with AC.

*Escherichia coli*, the predominant aerobic, coliform species in the healthy colon, is not a primary pathogen, but it can cause disease when it escapes from its usual gastrointestinal habitat. Invasion of the bloodstream is the most serious manifestation of *E. coli* infection and is commonly found among patients with urinary tract infection (UTI), biliary tract infection, or other intra-abdominal infection. *E. coli* is the species that most commonly causes bacteremia and sepsis in association with these infections [1, 2]. *E. coli* on the perineal skin can gain access to the urinary tract and proliferate there, especially when urine flow is disrupted by mechanical obstruction or neurologic dysfunction [3]. In the alimentary tract, *E. coli* typically ascends from the duodenum and may colonize the biliary tract [4].

Bacterial adherence to uroepithelial cells by fimbrial or nonfimbrial adhesins is considered to be an important pathogenic factor for UTI. The known fimbriae or adhesins of *E. coli* include P-, S-/F1C-, and type-1 fimbriae as well as afimbrial adhesins [3, 5]. Both host and *E. coli* virulence factors can contribute to the development of upper UTI, and less virulent strains can cause upper UTI in hosts with predisposing factors, such as diabetes with poor glycemic control, immunosuppression, or urinary tract obstruction [6]. The presentation of acute cholangitis (AC) may be similar to that of UTI. *E. coli* is the most commonly observed pathogen in studies of biliary bacteriology, on the basis of results of cultures of choledochal bile obtained from patients with or without AC or from patients with choledocholithiasis [7–10]. The incidence of bacteremia among patients with AC has varied from 0% to 75%, and *E. coli* has remained the pathogen most frequently associated with the disease [11–15]. Sakurai et al. [16]
found that adherence of *E. coli* to the epithelium of the human gallbladder correlated with the degree of epithelial damage. They also found that secondary bacterial infection is more likely to occur in patients with contaminated bile than in those without contaminated bile. There has been limited study of the role of adherence to biliary epithelium or other bacterial virulence factors of *E. coli* in the pathogenesis of AC. We attempted to identify the genotypes of fimbriae, adhesins, and other virulence factors of *E. coli* isolated from patients with *E. coli* bacteremia due to AC or upper UTI, compared with those of *E. coli* isolated from healthy controls, and we also examined the pathogenic role of host and *E. coli* virulence factors in both study groups.

**PATIENTS AND METHODS**

**Patients.** A total of 75 adult patients who were consecutively admitted to the hospital and who had *E. coli* bacteremia diagnosed at our institution and fulfilled the criteria for AC (*n* = 24) or upper UTI (*n* = 51) were included in the present study. A sufficiently detailed medical record and a stock of the causative *E. coli* strains isolated from blood cultures were available for all of these patients. Stool specimens also were obtained from 30 healthy individuals (15 men and 15 women). Stool was plated on MacConkey agar, which is used as a differential medium for *E. coli*. The *E. coli* strains were identified using standard methods and were stored in 20% glycerol at −70°C until they were used in subsequent analyses.

**Diagnostic criteria and patient selection.** A definite diagnosis of either AC or upper UTI with *E. coli* bacteremia was a prerequisite for inclusion in the study. The diagnostic criteria for AC included fever, abdominal pain in the right upper quadrant, and jaundice. The diagnostic criteria for upper UTI included fever (body temperature, ≥38.3°C), flank pain, and/or tenderness of the costovertebral angle, with or without disturbed urination. Patients were excluded from the study if they met the following criteria: (1) no mandatory renal or abdominal ultrasonographic study had been performed, (2) no radiological studies had been performed for indicated cases with suspected urinary or biliary tract obstruction, and (3) hematogenous infection was suspected. All blood cultures were performed on the first day of admission to the hospital. Bacteremia was defined by a blood culture that was positive for *E. coli* during an episode of AC or UTI. For culture, bile was obtained from the gallbladder or the common bile duct by use of a percutaneous catheter or endoscopic drainage or via intraoperative aspiration. Septic shock was defined as hypotension (mean arterial pressure, <70 mm Hg) with signs of inadequate organ perfusion. Disseminated intravascular coagulation (DIC) was defined as thrombocytopenia with consumption of plasma coagulation factors and production of fibrinogen degradation products.

**Definition of host factors predisposing to AC and UTI.** Detailed chart reviews were performed for the identification of host factors predisposing to AC or UTI. These factors included old age (≥60 years), sex, diabetes mellitus, immunosuppression, and urinary or biliary tract obstruction. Immunosuppression was diagnosed if the patient had neutropenia due to hematological disease, active systemic lupus erythematosus, liver cirrhosis, or advanced malignancy, or had received immunosuppressive therapy. Urinary tract obstruction was defined as (1) urine flow stasis due to an underlying urinary tract abnormality, or (2) radiological evidence of hydronephrosis or hydroureter. Urinary tract obstruction was first diagnosed by renal or bladder ultrasonography and then was confirmed by intravenous urography, retrograde or antegrade pyelography, and/or CT. Biliary tract obstruction was diagnosed by abdominal ultrasonography, endoscopic retrograde cholangiography, percutaneous transhepatic cholangiography, and/or abdominal CT.

**Detection of *E. coli* virulence determinants.** The genetic determinants for *E. coli* virulence were detected using PCR. The primer pairs that specifically discriminate for the 3 *papG* genes (class I, *papG*J96; class II, *papG*IA2; and class III, *prsG*J96) of P-fimbrial adhesins and the *sfa/foc* (encoding S-/F1C-fimbriae), *fimH* (encoding type-1 fimbrial adhesins), *afa* (encoding afimbrial adhesins), *hlyA* (encoding hemolysin), *cnfI* (encoding cytotoxic necrotizing factor 1), and *iutA* (encoding aerobactin receptor) genes have been described elsewhere [17–19].

**Amplification procedures.** DNA was prepared for amplification by use of the following method. Bacteria were first harvested from Luria-Bertani agar, suspended in 200 µL of sterile water, incubated at 100°C for 10 min, and centrifuged at 13,000 g. The supernatant was used as the DNA template. Amplification was performed in a 50-µL reaction mixture that contained 5 µL of the prepared template DNA, 50 pmol of each primer, 0.2 mM of each of the 4 deoxynucleoside triphosphates, and 1 U of DynaZyme II DNA polymerase (Finnzymes) in 1× PCR buffer II (Finnzymes). PCR was performed using a thermal cycler (Gene Amp, PCR System 9600; Perkin-Elmer). Multiply-primed PCR assay was used for the identification of the 3 *papG* alleles and *sfa/foc* genes, according to protocols described elsewhere [17–19]. The thermal-cycle protocols for *fimH*, *afa*, *hlyA*, *cnfI*, and *iutA* were as follows: denaturation at 95°C for 7 min, followed by 30 cycles each of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min; final extension was done at 72°C for 10 min. The PCR products were electrophoresed on agarose gels, stained with ethidium bromide, and photographed using UV transillumination.

The initial PCR results were duplicated to confirm the findings and to identify positive and negative strains. The PCR
products of positive strains were sequenced. If the results were identical to the corresponding regions of the expected gene sequence, these strains were used as positive control strains in each PCR assay. The negative strains were used as negative controls in each PCR assay. Positive and negative control strains for the traits of interest were included in each assay to confirm that PCR was working properly and to exclude contamination.

**Determination of nucleotide sequence.** The nucleotide sequence for the PCR products for each virulence gene was determined using the Sanger chain-termination sequencing method to confirm that amplification products obtained from PCR truly represented the expected sequences.

**Statistical analysis.** The $\chi^2$ test or Fisher’s exact test was used for categorical variables, whereas the Wilcoxon rank sum test was used for continuous variables between any 2 groups. Differences of virulence factors of *E. coli* among 3 groups were assessed by $\chi^2$ test in contingency tables. $P < .05$ was considered statistically significant. All statistical analyses were performed using JMP software (SAS Institute).

**RESULTS**

Seventy-six strains of *E. coli* isolated from the bloodstreams of 24 patients with 25 episodes of AC and 51 patients with upper UTI were evaluated, as were 30 fecal strains of *E. coli* isolated from healthy individuals. The healthy control group included 15 men and 15 women with a mean age of 32.8 years (range, 24–63 years). Comparisons between host factors and clinical features of patients with AC and those of patients with upper UTI are shown in table 1. More patients with AC were male (50% vs. 18%), but diabetes mellitus was more prevalent among patients with upper UTI (41% vs. 8%). In both the group with AC and the group with upper UTI, more than one-half of the patients were $\geq 60$ years of age (58% vs. 69%). All patients with AC had biliary tract obstruction, and 16 of 51 patients with upper UTI had urinary tract obstruction (100% vs. 31%; $P < .001$). No difference in the clinical features of *E. coli* bacteremia between the 2 study groups was noted, including the frequency of septic shock, DIC, WBC count, and C-reactive protein level.

A total of 25 episodes of AC with *E. coli* bacteremia occurred in 24 patients. The major causes of biliary tract obstruction included choledocholithiasis (in 19 patients), cholangiocarcinoma (in 2 patients), ampullary carcinoma (in 2 patients), and pancreatic head tumor (in 1 patient). Polymicrobial infection was noted in 11 episodes (44%), and pathogens other than *E. coli* that were isolated from blood cultures included *Enterococcus*, *Klebsiella*, *Aeromonas*, *Morganella*, and *Pseudomonas* species. Although bile culture was not performed for 4 episodes of AC, the other 21 episodes of AC for which results of bile cultures were positive were all polymicrobial, and *E. coli* was isolated in 16 (76%) of 21 episodes. Septic shock developed in

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AC $^a$ (n = 24)</th>
<th>Upper UTI (n = 51)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host factor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age $\geq 60$ years</td>
<td>14 (58)</td>
<td>35 (69)</td>
<td>NS</td>
</tr>
<tr>
<td>Male sex</td>
<td>12 (50)</td>
<td>9 (18)</td>
<td>.01</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2 (8)</td>
<td>21 (41)</td>
<td>.003</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>5 (21)</td>
<td>10 (20)</td>
<td>NS</td>
</tr>
<tr>
<td>Obstruction of the urinary or biliary tract</td>
<td>24 (100)</td>
<td>16 (31)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Clinical feature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Septic shock</td>
<td>11 (44)</td>
<td>17 (33)</td>
<td>NS</td>
</tr>
<tr>
<td>Disseminated intravascular coagulation</td>
<td>10 (40)</td>
<td>10 (20)</td>
<td>NS</td>
</tr>
<tr>
<td>WBC count, mean $\times 1000$ cells/mm$^3$ $\pm$ SD</td>
<td>14.3 $\pm$ 6.2</td>
<td>15.8 $\pm$ 9.2</td>
<td>NS</td>
</tr>
<tr>
<td>C-reactive protein level, mean mg/dL $\pm$ SD</td>
<td>125.3 $\pm$ 96.4</td>
<td>173.9 $\pm$ 125.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

NOTE. Unless otherwise indicated, data are the no. (%) of patients (for host factors) or the no. (%) of episodes (for clinical features). NS, not significant.

$^a$ A total of 25 episodes of AC occurred in 24 patients. Therefore, data on host factors were calculated using a denominator of 24 (the number of patients with AC), and data on clinical features were calculated using a denominator of 25 (the number of episodes of AC).
association with 11 episodes of AC (44%). Two patients with AC were transferred to other hospitals; the remaining 22 patients had 23 episodes of AC and either received a percutaneous catheter or underwent endoscopic drainage or intraoperative aspiration.

The major causes of urinary tract obstruction in the group with upper UTI included ureteral stones or tumors (n = 6), neurogenic bladder (n = 7), benign prostatic hypertrophy (n = 2), and uterine tumors with ureteral stretching (n = 1). All but 3 patients with urinary tract obstruction underwent Foley catheterization, retrograde ureteral catheterization, or percutaneous nephrostomy to relieve the obstruction. Of the 51 patients with upper UTI, 17 (33%) developed septic shock.

Comparisons of E. coli virulence factors among the 3 groups (AC, UTI, and control) are shown in table 2. Nearly all of the E. coli strains isolated from the 3 study groups had ≥1 adhesin detected (96%, 100%, and 93% of strains, respectively). The papG class I allele and the afa gene were not detected in any strains. There was a high prevalence of the genetic determinant of type 1 fimbrial adhesin (fimH) in all 3 groups (88%, 92%, and 90%, respectively). The prevalences of papG class II allele, genetic determinants of hemolysin (hlyA), cytotoxic necrotizing factors 1 (cnf1), and aerobactin receptor (iutA) were significantly higher in patients with upper UTI than in those with AC and healthy controls. However, no significant differences in these 4 genetic determinants were noted between the group with AC and the control group. Among the 3 groups, no significant differences were found in the genetic determinants of papG class III allele, S-fimbriae (sfa), F1C-fimbriae (foc), and type 1 fimbrial adhesin (fimH). The prevalence of papG class II gene was higher in the group with AC than in the control group (24% vs. 3%; P = .039), but it was lower in the group with AC than in the group with upper UTI (24% vs. 94%; P < .0001).

We further compared the host and E. coli virulence factors between 24 patients with 25 episodes of AC and 16 patients with upper UTI and urinary tract obstruction. There was no statistical difference between the host factors noted in the 2 study groups. With respect to virulence factors of E. coli, the prevalence of papG class II allele and that of hlyA gene were higher in patients with upper UTI who had urinary tract obstruction (81% and 56%, respectively) than in patients with AC (24% and 16%, respectively), but not for cnf1 and iutA genes.

**DISCUSSION**

AC is a serious biliary tract infection that is characterized by the clinical triad of fever, abdominal pain in the right upper quadrant, and jaundice. There are 2 factors necessary for the development of AC—namely, (1) the presence of microorganisms in the bile duct, mostly by the ascending route via the

<table>
<thead>
<tr>
<th>Virulence factor</th>
<th>AC (n = 25)</th>
<th>Upper UTI (n = 51)</th>
<th>Control (n = 30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any adhesin</td>
<td>24 (96)</td>
<td>51 (100)</td>
<td>28 (93)</td>
<td>NS</td>
</tr>
<tr>
<td>papG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class I</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>NS</td>
</tr>
<tr>
<td>Class IIa</td>
<td>6 (24)</td>
<td>48 (94)</td>
<td>1 (3)</td>
<td>&lt;.0001</td>
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<tr>
<td>Class III</td>
<td>2 (8)</td>
<td>4 (8)</td>
<td>1 (3)</td>
<td>NS</td>
</tr>
<tr>
<td>sfa (S-fimbriae)</td>
<td>2 (8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>.052</td>
</tr>
<tr>
<td>foc (F1C-fimbriae)</td>
<td>1 (4)</td>
<td>8 (16)</td>
<td>1 (3)</td>
<td>NS</td>
</tr>
<tr>
<td>fimH (Type 1 fimbrial adhesin)</td>
<td>22 (88)</td>
<td>47 (92)</td>
<td>27 (90)</td>
<td>NS</td>
</tr>
<tr>
<td>afa (Afimbrial adhesin)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>NS</td>
</tr>
<tr>
<td>hlyA (Hemolysin)b</td>
<td>4 (16)</td>
<td>23 (45)</td>
<td>2 (7)</td>
<td>.0003</td>
</tr>
<tr>
<td>cnf1 (Cytotoxic necrotizing factor 1)c</td>
<td>2 (8)</td>
<td>21 (41)</td>
<td>1 (3)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>iutA (Aerobactin receptor)d</td>
<td>13 (52)</td>
<td>40 (78)</td>
<td>14 (47)</td>
<td>.0069</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of episodes (for patients with AC) and no. (%) of patients (for patients with upper UTI and control subjects).

a For AC compared with upper UTI, P < .0001; for AC compared with control, P = .039; for upper UTI compared with control, P < .0001.

b For AC compared with upper UTI, P = .021; for upper UTI compared with control, P = .0003.

c For AC compared with upper UTI, P = .0032; for upper UTI compared with control, P < .0002.

d For AC compared with upper UTI, P = .032; for upper UTI compared with control, P < .0066.
in the urinary tracts of patients with local or systemic defects. Additionally, of 56 evaluable episodes of bacteremia that originated in the urinary tract were 77% positive for papG, which has toxic effects on polymorphonuclear leukocytes and renal tubular epithelium and is potentiated by P-fimbriae. It was identified that the prevalence of papG class II allele in patients with upper UTI was lower than those isolated from patients without urinary tract obstruction or other predisposing host factors, such as poor glycemic control or immunosuppression. Although cytotoxic necrotizing factor 1 (cnf1) and aerobactin (iapA), which have the ability to promote bacterial overgrowth and membrane damage in the kidney, were found more often in pyelonephritogenic E. coli strains than in cystitis and fecal strains, the importance of the role in patients who had upper UTI with or without E. coli bacteremia was not determined in the previous studies. Lastly, there were studies that reported the correlations between P-fimbriae and aerobactin production and the correlations between the production of hemolysin and cytotoxic necrotizing factor. The gene clustering carried by the pathogenicity islands of pathogenic strains of E. coli can explain the concurrent presence of the genetic virulence factors of papG class II, hlyA, cnf1, and iutA. This study showed that papG class II and hlyA are important virulence factors of E. coli for the development of bacteremia in patients who have upper UTI with or without urinary tract obstruction.

In conclusion, the present study shows that biliary tract obstruction and urinary tract obstruction are important host factors for the development of E. coli bacteremia in patients with AC or upper UTI, respectively. With regard to E. coli virulence factors, the papG II gene might play a more important role in the development of E. coli bacteremia in patients with upper UTI than in those with AC.

References

5. Tseng CC, Huang JJ, Ko WC, Yan JJ, Wu JJ. Decreased predominance of papG class II allele in Escherichia coli strains isolated from adults.