Expression of DR-II Adhesins Encoded by the draE2 Genes in Escherichia coli Strains from Urinary Tract Infections—Related Bacteremia

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ABSTRACT
In this study we detected and determined the transcription of the draE2 gene encoding for the Dr-II adhesin, in relation to other adhesins to determine its role in urinary tract infection (UTI) and UTI-related bacteremia. PCR and RT-PCR of draE2 gene, in addition to pap gene, encoding P fimbriae (UTI-associated gene), and eaeA gene encoding intimin (non-UTI associated), was done on extracted DNA and RNA of 3 reference strains harboring the genes and E. coli isolated from clinical specimens of patients with UTI, 60% and 0%, 40% and 20%, 100% and 80%; patients with UTI associated blood stream infection, 100% and 100%, 100% and 100%, 100% and 76.5%; patients with non-UTI associated blood stream infection, 100% and 78.5%, 100% and 100%, 100% and 35.7%, and isolates from pus and sputum 0% and 0%, 0% and 0%, 100% and 100%. The high prevalence and expression of draE2 gene in addition to that of pap gene in isolates recovered from patients with bacteremia, UTI or non-UTI-related, highlights the possible involvement of these genes in this serious condition. Genotyping demonstrated the prevalence of multistrain involvement in these infections.

INTRODUCTION
Urinary tract infection (UTI), of which Escherichia coli is the major causative agent, are among the most common human infections. At least 10% to 20% of women experience an acute symptomatic UTI at some point during their lives.
The severity of the UTI depends both on the virulence of the infecting bacteria and on the susceptibility of the host. Factors contributing to the virulence of uropathogenic *E. coli* include hemolysin (encoded by the *Hly* gene), O serotypes, aerobactin (encode by *aer* gene), cytotoxic necrotizing factor-1 (encoded by *cnf-1* gene), and adhesins (encoded by *pap, afa, sfal, draE* genes).

Bacteremia can arise from different sources, but the most common one is the urinary tract (48% of cases). Like UTI, the most common causative agent of bacteremia is *E. coli* which accounts for 30% to 45% of episodes. The role of the above noted virulence factors in the etiology of *E. coli* bacteremia remains unclear. However, an earlier study from Boston favored the adhesin concept and showed that most bloodstream isolated *E. coli* had one or more adhesins predominantly *pap.* All episodes of bacteremia due to organisms lacking virulence factors involved patients who had defects in the mucocutaneous barriers at the primary site of infection or had systemic disease associated with decreased cellular defenses against infection, and the adhesin was detected in 96% of isolates from patients without any identified defect of host immunity. Therefore, adhesins are the most important among the virulence factors of *E. coli* in initiating the infection, whether it is a UTI or bacteremia; they allow attachment and colonization of the urinary tract.

The most significant fimbriae is P fimbriae, which is encoded by the *pap* operon. P fimbriae-mediated adherence is important in the virulence of *E. coli* in the urinary tract, especially in cases of pyelonephritis. There is a strong relationship between disease severity, development of bacteremia, and the presence of P fimbriae in human pyelonephritis. Its role in UTI related bacteremia is unclear.

A second fimbriae that plays an important role in the attachment of *E. coli* to numerous sites within the urinary tract, including the renal interstitium, Bowman's capsule, tubular basement membranes, and ureterial transitional epithelial cells, is the Dr Family of adhesins. This family of adhesins is associated with cystitis, in fact Dr-related sequences are present in 26% to 50% of cystitis patients isolates, but in only 6% to 26% of pyelonephritis, and 15% to 18% of fecal isolates. This Dr family of adhesins, including the 17-kDa Dr-II adhesin (afimbrial), encoded by the *draE2* gene, was not previously studied in UTI and UTI-related bacteremia as well as other infections and will be considered in this study.

A third fimbriae playing an important role in the intimate attachment of *E. coli* to epithelial cells, is intimin, encoded by *eaeA* gene that lies in the locus of enterocyte effacement. Intimin is an outer membrane protein, whose presence is an important virulence factor for *E. coli*, and it plays an important role especially in Enterohemorrhagic *E. coli* (EHEC) and Enteropathogenic *E. coli* (EPEC). Its role in UPEC is not known.

The aims of the present study were to demonstrate the possible role of the Dr-II adhesin, in comparison to P-fimbriae, a UTI-related adhesin, and the intimin, a non-UTI-related adhesin, encoded respectively by the *draE2, pap,* and *eaeA* genes in establishment of UTI and UTI-related bacteremia in infected patients with UPEC.

**MATERIALS AND METHODS**

**Bacterial Isolates**

Forty one *E. coli* isolates were obtained from the clinical microbiology laboratory at American University of Beirut Medical Center (AUBMC), a major tertiary care center in Lebanon. Ten isolates were isolated from patients with UTI, 14 from patients with UTI related
bacteremia and 17 from patients with non-UTI related bacteremia. Five additional control *E. coli* isolates were obtained from specimens other than urine (from pus and sputum). Reference strains including, *E. coli* 396 strain carrying the *pap* gene, CDC 0798 strain harboring the *eaeA* gene, and a standard in-house *E. coli* strain harboring the *draE2* gene, were also used as controls. Identification of isolates was done using the API E Kit (Biomerieux, Marcy L’Etoile, France).

**Antimicrobial Susceptibility Testing**

Antimicrobial susceptibility testing to the following antimicrobial agents: amikacin, ampicillin, amoxicillin, aztreonam, cefamandole, cefotaxime, cefoxitin, ceftazidime, cefuroxime, cephalothin, ciprofloxacin, gentamicin, imipenem, tazocin, tobramycin, and trimethoprim/sulfamethoxazole, was performed by the disk agar diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines.

**DNA Extraction and PCR**

Total DNA was extracted from the 41 isolates using the GFX (Amersham Biosciences, Piscataway, NJ). PCR conditions and primers used for the *eaeA* gene were described in a previous study. The *pap* gene was amplified using the primers and PCR conditions described by Le Bougueune et al. The *draE2* gene was amplified using the following primers designed by us: *draE2* 1: 5’ GAC AGT TTA CTG ATT CTG GGA 3’ and *draE2* 2: 5’ ACG TCC AAT AAT GCT TAC CC 3’. PCR was done in a 100 µL reaction mixture containing 10 µL total DNA (2 µg/µL), 0.5 µL of each primer, 200 µM of each dNTP, 10 µL PCR buffer and 2.5 U *Taq* DNA polymerase. PCR conditions consisted of 35 cycles, having each a denaturation step at 94°C for 1 minute, primer annealing step at 53°C for 2 minutes, and an extension at 72°C for 1 minute. The cycles were preceded by a denaturation step at 94°C for 13 minutes and followed by a final extension step at 72°C for 10 minutes. PCR was done for all reactions using the PTC-100 cycler (MJ Research Inc., Watertown, Mass) Amplicons of the *eaeA* (1110-bp), *pap* (328-bp), and *draE2* (620-bp) genes were subjected to electrophoresis on 1%-agarose (Sigma) gels in 1X Tris-borate-EDTA buffer at 117V for 45 minutes. Gels were stained with ethidium bromide (Sigma). Amplicons were detected on UV transiluminator and photographed using 667 Polaroid films.

**RNA Extraction and Reverse-transcription PCR**

RNA was extracted from each bacterial strain, using the RNeasy MiniKit (QIAGEN, Hilden, Germany) according to the manufacturers’ specifications and then subjected to reverse-transcription PCR (RT-PCR). The Ready-To-Go Kit (Amersham Biosciences) for c-DNA synthesis was used according to the manufacturers’ instructions. The primers, PCR conditions, electrophoresis, and detection of the amplicons were as described above for the DNA extracts. RNA extracts of each strain were run in PCR without the reverse transcription to check for DNA contamination.

**Random Amplified Polymorphic DNA (RAPD)**

Subtyping by RAPD was carried out on the 41 *E. coli* isolates in 100 µL reaction mixtures containing each: 10 µL of template DNA, 16 µL of dNTPs (0.2 mM), 10 µL of 10 X PCR buffer (100 mM TrisHCl [pH 8.3], 500 mM KCl, 4 mM MgCl₂), 1 µL of primer 1 (0.5 µM), 1 µL of primer 2 (0.3 µg/µL), 2.5 U of *Taq* DNA polymerase and 61.5 µL of nanopure sterile water. Positive and negative controls were included in every RAPD
**Table 1.** Overall Results of PCR and RT-PCR for *E. coli* Recovered from Different Study Groups

<table>
<thead>
<tr>
<th>Source of <em>E. coli</em></th>
<th>Number of isolates tested</th>
<th>Percentage of detected genes among</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>pap</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PCR</td>
</tr>
<tr>
<td>Urine</td>
<td>10</td>
<td>60%</td>
</tr>
<tr>
<td>UTI-associated blood isolates</td>
<td>17</td>
<td>100%</td>
</tr>
<tr>
<td>Non-UTI-associated blood isolates</td>
<td>14</td>
<td>100%</td>
</tr>
<tr>
<td>Controls (pus &amp; sputum)</td>
<td>5</td>
<td>0%</td>
</tr>
</tbody>
</table>

*pap gene encodes P fimbriae, eaeA encodes intimin, and draE2 encodes Dr-II adhesin.

run, the negative control consisted of sterile nanopure water while the positive control was *E. coli* ATCC 10536. The amplifications program, included the following steps: denaturation at 94°C for 3 minutes, annealing at 53°C for 1 minute and extension at 72°C for 1 minute, for 44 cycles. The cycles were followed by a final extension step at 72°C for 10 minutes. Amplicons were subjected to electrophoresis on 2% agarose gels as described above.

**RESULTS**

All of the 41 isolates (Table 1) obtained from urine and/or blood of patients with UTI or bacteremia with or without UTI, have a typical colonial morphology of *E. coli*. Antibiotic susceptibility testing has shown that these *E. coli* isolates have different patterns of susceptibility to the antimicrobial agents used (data not shown).

PCR data have shown that 4 of 10 (40%) urine isolates, all 17 (100%) UTI related blood isolates, all 14 (100%) non-UTI related blood, and none of the 5 control isolates, were PCR-positive for the *pap* gene (Table 1, Figure 1A). Six of 10 (60%) urine isolates, all 17 (100%) UTI related blood isolates, all 14 (100%) non-UTI related blood isolates and none of the 5 control isolates were PCR-positive for the *DraE2* gene (Table 1, Figure 1A). Finally, all 10 (100%) urine *E. coli* isolates, all 17 (100%) UTI related blood *E. coli* isolates, all 14 (100%) non-UTI related blood and all 5 (100%) control isolates were PCR-positive for the *eaeA* gene (Table 1, Figure 1A).

RT-PCR data for the *draE2* gene revealed that 2 of 10 (20%) urine isolates, all 17 (100%) and all 14 (100%) UTI related and non-UTI related blood isolates respectively were RT-PCR positive (Table 1, Figure 1B). RT-PCR data for the *pap* gene revealed that none of the 10 urine isolates, all 17 (100%) UTI related blood isolates and 11 of 14 (78.5%) non-UTI related blood isolates.
Discussion

*Escherichia coli* is the major causative agent of UTI and bacteremia.\(^7\)

Considering the high degree of morbidity and mortality of urinary tract infections, the subject of uropathogenic *E.coli* (UPEC) is receiving increasing attention.\(^1\)

In this study, we detected the presence and expression of 3 virulence genes (*pap, draE2* and *eaeA* genes), of which two, the *pap* and *draE2* genes, are associated with adherence of UPEC, while the third gene, *eaeA* gene, is associated with adherence of Enteropathogenic *E. coli* (EPEC) and used as a control gene.

Fimbriae-mediated adherence, involving P fimbriae, is important in the virulence of *E. coli* in the urinary tract, especially in cases of pyelonephritis. In fact, the proportion of *E. coli* strains expressing P fimbriae declines from a high of 70% among isolates from patients with pyelonephritis to 36% among isolates from patients with cystitis.\(^1\)

Among the *E. coli* urine isolates we obtained, 60% had the *pap* gene, but none expressed it. One of these isolates was obtained from a patient with pyelonephritis, and contrary to published data from other reports, it has the *pap* gene, but did not express it. While all, UTI and non-UTI related blood isolates had the *pap* gene and all expressed it. These observations suggest that *pap*-related adhesins are functionally significant not only for bacteremia arising from the urinary tract but also for those arising from the respiratory tract and possibly from other sites.

The Dr-II adhesin encoded by *draE2* gene, is a member of the Dr adhesin Family which are non-fimbriated adhesins expressed by most urinary *E. coli* isolates,\(^1\) and has only 20% identity to commonly studied Dr adhesins. The *draE2* gene sequence has already been published,\(^1\) however, no previous studies were done on its role in the pathogenicity of UPEC and bacteremia.
causing *E. coli* strains. Our data revealed that only 40% of urine *E. coli* isolates had the *draE2* gene and 20% of these isolates expressed it. While all, UTI and non-UTI related, blood isolates had and expressed this gene. These observations suggest that the *draE2* gene plays a certain role in the establishment of bacteremia due to UTI or to other site of infection, but its role in the development of UTI is unclear.

Intimin, encoded by *eaeA* gene, is responsible for adherence of EPEC and has not been previously studied as a virulence factor of UPEC. Our data revealed that all *E. coli* isolates, regardless of their source, had the gene, but only 80% of the urine isolates, 76.5% of the UTI related blood isolates, and 35.7% of non-UTI related blood isolates expressed this gene. These observations suggest that the *eaeA* gene may play a certain role in the pathogenesis of UPEC, maybe by facilitating adherence to uroepithelial cells. It also seems to contribute to the ability of UPEC strains to cause bacteremia.

In summary, our results support the hypothesis that the pathogenic potential of *E. coli* infecting the urinary tract site is closely related to the expression of adhesins that mediate colonization and

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**Figure 2.** (A) RAPD patterns of *E. coli* isolates obtained from urine of patients. Lane 1, 100-bp ladder; lane 2, negative control; lane 3, positive control; lanes 4-13, RAPD patterns of urine *E. coli* isolates. (B) RAPD patterns of *E. coli* isolates obtained from blood of patients with non-UTI. Lane 1, 100-bp ladder; lane 2, negative control; lane 3, positive control; lanes 4-13, RAPD patterns of blood *E. coli* isolates. (C) RAPD patterns of blood *E. coli* isolates from patients with UTI. Lane 1, 100-bp ladder; lane 2, negative control; lane 3, positive control; lanes 4-18, RAPD patterns of *E. coli* isolates.
facilitate subsequent invasion. This study has confirmed the importance of P fimbriae encoded by the \textit{pap} gene in the development of bacteremia whether it was from a urinary tract focus or not, and has identified possible associations between Dr-II adhesin, which was not previously investigated, and UTI-related as well as non-UTI-related bacteremia. Intimin appears to play a role in mediating UTI in UPEC. Genotyping data showed the involvement of multiple strains in these infections with no association of particular ones to UTI-related bacteremia. Additional studies are needed to determine transcription levels of these genes in relation to mediation of UTI and UTI-related bacteremia.

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**REFERENCES**