VIRULENCE FACTORS OF UROPATHOGENIC *Escherichia coli* FROM A UNIVERSITY HOSPITAL IN RIBEIRÃO PRETO, SÃO PAULO, BRAZIL

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SUMMARY

The aim of the study was to determine the occurrence of virulence genes expressing fimbriae, production of hemolysin, colicin and aerobactin among a hundred *Escherichia coli* isolates obtained from in-and outpatients of a tertiary-care teaching hospital, between July and August 2000, showing clinical and laboratory signs of urinary tract infection (UTI). The presence of genes (pap, afa, sfa) for fimbriae expression was assayed using specific primers in a polymerase chain reaction. Among the isolates studied, the prevalence of the virulence factors was 96.0%, 76.0%, 24.0%, for hemolysin, aerobactin and colicin, respectively; the prevalence of genes coding for fimbrial adhesive systems was 32.0%, 19.0% and 11.0% for pap, sfa and afa respectively. The strains isolated from the outpatients displayed a greater number of virulence factors compared to those from hospitalized subjects, emphasizing the difference between these two kinds of patients.

KEYWORDS: *Escherichia coli*; Urinary infection; Virulence factors.

INTRODUCTION

*Escherichia coli* is one of the major causes of human infectious diseases and is also the most common cause of urinary tract infection (UTI)\(^9\). At least 10 to 20% of women experience an acute symptomatic UTI at some point during their lives\(^7\). The severity of the infection depends both on the virulence of the infecting bacteria and on the susceptibility of the host. Urinary infections most often occur in patients with anatomically and functionally normal urinary tracts, and involve spontaneous ascent of bacteria from the urethra to the bladder and in a few patients to the kidney\(^8\). Adhesion of *E. coli* to the uroepithelium may protect the bacteria from urinary lavage, increasing their ability to multiply and invade renal tissue\(^12\). The uropathogenic *E. coli* (UPEC) possess adherence factors called pili or fimbriae, which allow them to successfully initiate infections. The two main pili (type 1 and P) found in patients with UTIs, are morphologically similar but differ in their ability to mediate hemagglutination in the presence of mannose\(^20\). Specific adhesion is mediated by bacterial proteins termed adhesins which may or may not be associated with fimbriae. *Pap* (pyelonephritis-associated pili), *sfa* (S fimbrial adhesin) and *afa* (afimbrial adhesin) operons are most commonly found encoding respectively, P, S and Afa (also designated Dr hemagglutinin) adhesins\(^3,15\).

Besides bacterial adherence, several virulence factors may contribute to the pathogenicity of UPEC, including the production of α-hemolysin, colicin and aerobactin\(^4\).

In the present study, we analyzed urinary tract *E. coli* isolates to search for possible evidence of a correlation between biological characteristics that could represent pathogenicity traits of these strains.

MATERIAL AND METHODS

Study design and patients: Virulence factors of nosocomial and community-acquired infections data according to obtained from the Clinical Hospital of the School of Medicine of Ribeirão Preto (HCFMRP), concerning *E. coli* isolated from UTI in-and outpatients, were monitored and analyzed. The study was carried out from July to August 2000. HCFMRP is a university teaching tertiary care hospital.

Bacterial isolates: A total of 100 strains of *E. coli* isolated from 100 patients (only one isolate from each) distributed as: 13 subjects aged between 0 and 15 years (11 women and two men), 18 aged between 16 and 39 years (16 women and two men), and 69 aged > 40 years (40 women and 29 men), were analyzed. Thirty-three of the subjects were hospitalized (inpatients) and 67 were outpatients. UTI diagnoses were established by the hospital medical staff based on clinical symptoms and laboratory investigation. Urinary frequency, internal dysuria and suprapubic or pelvic pain are the characteristic symptoms of cystitis, in general acute pyelonephritis presents fever, flank pain, nausea and vomiting. The laboratory criterion for acute *E. coli* UTI was the presence of a positive culture response with at least 10\(^5\) CFU of *E. coli* per mL of clean-voided urine. Among the subjects there were 15 cases of...
pyelonephritis. *Escherichia coli* was identified with the use of standard methods. The strains were stored and subcultured, further analysis as previously described.

**Hemagglutination and expression of type 1 and P fimbriae:** The hemagglutinins were detected by agglutination of erythrocytes from humans blood group O and from guinea pig, in presence or in the absence of D-mannose.

**Hemolysin production:** Production of hemolysin was assayed by growing the isolates overnight (16 h), at 37 °C in Luria-Bertani Broth (LB), 50 µL are spot inoculated onto sheep blood agar base. The culture was incubated at 37 °C overnight (16 h) and hemolysin production was verified by the presence of a clear hemolytic halo around the orifices.

**Aerobactin production:** Production of aerobactin was assayed by growing isolated strains in LB medium containing 200 µM of α-α-dipryridyl at 37 °C for 24 h, without shaking. The growth was spun for three min (12000 g), supernatants were filtered through a nitrocellulose membrane (0.22 µm) and aliquots of 50 µL were added to orifices made in LA medium previously seeded with strain LG 1522. The plates were incubated at 37 °C for 48 h and the production of aerobactin was visualized by the growth of strain LG 1522 around the orifices.

**Colicin production:** Cultures were examined for colicin production by the overlay method, described previously, with indicator strain MA335 of *E. coli*.

**DNA extraction:** *E. coli* strains were grown in LB broth at 37 °C overnight (16 h). Bacteria were pelleted from 1.5 mL broth, suspended in 200 µL sterile distilled water, and boiled at 100 °C for 15 min. Following centrifugation of the lysate, a 150 µL sample of the supernatant was stored at –20 °C as a template DNA stock. Following centrifugation of the lysate, a 150 µL sample of the supernatant was stored at –20 °C as a template DNA stock.

**PCR:** Specific primers were used to amplify sequences of the *pap*, *sfa* and *afa* genes as indicated in Table 1. Primer sequences, predicted sizes of the amplified products, and specific conditions were described by LE BOUGUENEC et al.

**Table 1**

<table>
<thead>
<tr>
<th>Primer Oligonucleotide sequences (5'-3')</th>
<th>Size of amplicons</th>
</tr>
</thead>
<tbody>
<tr>
<td>pap 1 GACGGCTGTACTGCAGGCTGTGCGGCGG</td>
<td>328 bp</td>
</tr>
<tr>
<td>pap 2 ATATCCTTTTCTGCAGGGATGCAATA</td>
<td>410 bp</td>
</tr>
<tr>
<td>sfa 1 CTCCGGAGAAGCTGGGTGCACTTTCAC</td>
<td>750 bp</td>
</tr>
<tr>
<td>sfa 2 GCGAGGATCAAATTCAAAACTGGGCA</td>
<td></td>
</tr>
<tr>
<td>afa 1 GCTGGGCAGCAAAGCTGATAACTCTC</td>
<td></td>
</tr>
<tr>
<td>afa 2 CATCAAGCGTGTGTGTCTGCCGGCG</td>
<td></td>
</tr>
</tbody>
</table>

* pap, sfa and afa primers as reported by LE BOUGUENEC et al.

**RESULTS AND DISCUSSION**

Non properly managed from their onset, urinary tract infection can in time, become a real threat, capable of leading to renal failure. A better knowledge of the virulence characteristics of the microorganism causing the infection allows the clinician to anticipate the evolution of infection in the host.

The occurrence of virulence factors in the present study ranged from 11.0% for *sfa* to 96.0% for hemolysin. Among the adhesins, the P fimbriae was the most prevalent (42 strains), followed by type 1 fimbriae (25 strains) (Table 2), these results not agree with published reports, which emphasize the predominance of fimbriae type 1 among the UPEC strains.

**Table 2**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Outpatients</th>
<th>Hospitalized patients</th>
<th>Total of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolysin</td>
<td>64</td>
<td>32</td>
<td>96</td>
</tr>
<tr>
<td>Colicin</td>
<td>17</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>Aerobactin</td>
<td>53</td>
<td>23</td>
<td>76</td>
</tr>
<tr>
<td>P fimbriae</td>
<td>28</td>
<td>14</td>
<td>42</td>
</tr>
<tr>
<td>Type 1 fimbriae</td>
<td>20</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>pap</td>
<td>23</td>
<td>9</td>
<td>32</td>
</tr>
<tr>
<td>sfa</td>
<td>14</td>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td>afa</td>
<td>8</td>
<td>3</td>
<td>11</td>
</tr>
</tbody>
</table>

The presence of hemolysin and colicin, was related to tissue damage, and the prevalence of aerobactin which confers the capacity to bind iron was higher than previous reported ones. However, the high prevalence of these virulence factors among the isolates from pyelonephritis in the present report agrees with that reported by others. In general the virulence factors from outpatient isolates were found in more than double amounts compared with those from the nosocomial isolates (Table 2).

Based on the distribution of the various target sequences, the strains studied exhibited 10 most common virulence patterns, referred to as Ec followed by an Arabic numeral (Table 3). The strains isolated from patients admitted in hospital exhibited a great diversity of gene patterns, showing the Ec 1, Ec 2, Ec 3 and Ec 7 patterns in common with outpatients patterns and 14 new patterns that appear once a time, it means 43.7% of diversity in virulence patterns (results not showed), in agreement with other report. The presence of the *afa* operon together with aerobactin was detected in the same strain as the Ec 4 pattern (Table 3); this association had been previously reported. A co-dependence of these virulence patterns in a particular pathogenic pathway has been discussed but needs to be confirmed.

Out of the 100 uropathogenic *E. coli* isolates tested by PCR, 49 carried sequences related to the three adhesion-encoding operon families investigated. Twenty, 10 and six of the isolates respectively, exhibited *pap*, *afa* and *sfa* genotypes alone (Table 4). The distribution of the S fimbriae-encoding operons found among the isolates studied was lower than previously reported but agree with those reported by others. Regarding P fimbriae, pooled results from other studies indicate that among *E. coli* isolates from patients with pyelonephritis...
and cystitis, approximately 80% and 30% respectively, possess P
fimbriae. Although the frequency of \( \text{pap} \) in a cystitis strain agrees
with literature data, we found that only 26.6% of the pyelonephritis
strains contain \( \text{pap} \) sequences (Table 4). It is noteworthy that most of
the pyelonephritis strains studied were isolated from patients with
chronic infections and other diseases that complicate their clinical
pictures of. Additional epidemiological studies have to be carried out
to confirm this observation. However, these data agree with those
reported by USEIN et al.\textsuperscript{21} in Romania.

A total of 12 isolates carried both \( \text{pap} \) and \( \text{sfa} \) operons, and one
isolate carried both \( \text{sfa} \) and \( \text{afa} \) operons what it is new from other
reports\textsuperscript{1,3,15,18,21}, while the simultaneous presence of \( \text{pap-afa} \) operons
was not detected, differently from others\textsuperscript{3,15,18}. A small number of UTI
strains possessing \( \text{afa} \)-afimbrial adhesions have been reported\textsuperscript{1,3,15}.
Growing importance is attributed to the \( \text{afa} \) operon, which has been
implicated in the development of chronic interstitial nephritis\textsuperscript{9}. We
found a small increase in the percentage of \( \text{afa} \) PCR-positive strains in
isolates from patients with pyelonephritis (13.3%), compared with those
associated with cystitis (9.4%) (Table 4); however, this finding indicates
the need for studies on the association.

In conclusion, the strains isolated from patients attended at the
outpatient clinic exhibited a higher number of virulence factors per
strain and seemed to be more aggressive than strains from hospitalized
patients, emphasizing the importance of these infected patients that
arrive at a tertiary care teaching hospital. This study of the \( E. \text{coli} \)
strains isolated from UTI in this kind of hospital was meant as a step
towards improving the knowledge regarding their virulence genetic
determinants.

**RESUMO**

**Fatores de virulência de Escherichia coli uropatogênicas provenientes de um Hospital Universitário em Ribeirão Preto, São Paulo, Brasil**

O objetivo do trabalho foi determinar a ocorrência de fatores de
virulência, tais como, a expressão de fimbrias, produção de
hemolisina, colicina e aerobactina em 100 cepas de *Escherichia coli*
isoladas de pacientes ambulatoriais e hospitalizados de um hospital
universitário de nível de atendimento terciário, entre os meses de
julho e agosto de 2000, que apresentavam sinais clínicos e
laboratoriais de infecção do trato urinário (ITU). Foram pesquisados
os genes \( \text{pap} \), \( \text{afa} \) e \( \text{sfa} \) responsáveis pela expressão de fimbrias através
da técnica de PCR. A frequência dos fatores de virulência entre as
cepas estudadas foi de 96,0%, 76,0% e 24,0% para hemolisina,
aerobactina e colicina respectivamente, e a prevalência dos genes
para os sistemas de adesinas fimbriais foi de 32,0%, 19,0% e 11,0%
para os genes \( \text{pap} \), \( \text{sfa} \) e \( \text{afa} \) respectivamente. As cepas isoladas dos
pacientes ambulatoriais exibiram um número maior de fatores de
virulência quando comparadas com aquelas provenientes de
indivíduos hospitalizados.

**ACKNOWLEDGMENTS**

The authors thank Tania Marques for technical assistance. This
work is part of a thesis submitted by Edilene Santo to UNESP University
in partial fulfillment of the requirements for the Master’s degree.
REFERENCES


Received: 15 August 2005
Accepted: 26 April 2006