Phylogenetic Groups and Virulence Factors in Pathogenic and Commensal Strains of *Escherichia coli* and Their Association with *bla*\_CTX-M

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**Abstract.** We compared the distribution of phylogenetic groups and nine virulence factors among the pathogenic (isolated from blood and urine) and commensal (isolated from feces of healthy individuals) strains of *Escherichia coli*, and also compared the occurrence of virulence factors according to the production of *bla*\_CTX-M among the pathogenic strains. A total of 550 non-duplicate *E. coli* isolates (145 from blood, 200 from urine, 205 from feces) were collected. Phylogenetic grouping and virulence genotyping were done by PCR for all isolates. For pathogenic strains, antimicrobial susceptibility tests and PCR for *bla*\_CTX-M were performed. The distribution of phylogenetic groups was similar between isolates from blood and urine: B2 (44.8%; 58.5%, respectively) > D (29.0%; 23.0%, respectively) > A (18.6%; 9.5%, respectively) > B1 (7.6% and 9.0%, respectively). Phylogenetic groups B2 and D were also frequent (22.9% and 21.0%, respectively) among isolates from feces. The prevalence of all virulence factors except S fimbrial adhesion was significantly higher in pathogenic strains than in commensal strains and they were most frequent in phylogenetic group B2. α-Haemolysin, yersiniabactin receptor, serum resistance-associated outer membrane protein (*traT*), and aerobactin receptor (*iutA*) were found to be independent predictors for pathogenicity, and of them, *iutA* and *traT* were significantly more common in *bla*\_CTX-M-1 group and *bla*\_CTX-M-9 group, respectively. Considering the possibility that these virulence genes, together with antimicrobial resistance genes, can spread to other strains, further study and ongoing surveillance seem to be required.

**Keywords:** *iutA*, *traT*, virulence factors, phylogenetic groups, *bla*\_CTX-M, *Escherichia coli*

**Introduction**

*Escherichia coli* is one of the most common isolates in clinical microbiology laboratories and is classified into three major groups: commensal strains, intestinal pathogenic strains, and extraintestinal pathogenic strains, according to their biological significance to humans [1]. Whereas most commensal strains belong to phylogenetic groups A or B1, extraintestinal pathogenic strains typically belong to phylogenetic groups B2 or D, and they possess more virulence factors than commensal strains [1]. However, only a few studies have been performed to determine which virulence factors are independent predictors of pathogenicity.

In regard to the association of genes for antimicrobial resistance and virulence factors, there are controversial reports. In contrast to positive associations between several antimicrobial resistance genes and the aerobactin receptor (*iutA*) and serum resistance-associated outer membrane protein (*traT*) [2], there are negative associations between quinolone-resistance and α-haemolysin (*hlyA*) and cytotoxic necrotizing factor type 1 (*cnf1*) in uropathogenic *E. coli* [3], and relatively low virulence in *bla*\_CTX-M-producing *E. coli* isolates [4]. Nowadays, multiresistant *E. coli* isolates with

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extended-spectrum \(\beta\)-lactamases (ESBLs) are found worldwide. Especially, CTX-M-type ESBLs are increasingly prevalent in Europe, Asia, and South America, where more than 50 allotypes have been reported, divided into six sub-lineages (groups 1, 2, 8, and 9, CTX-M-25, and CTX-M-45), with different groups prevalent in different countries [5-7]. In Korea, \(\text{bla}_{\text{CTX-M-15}}\) (\(\text{bla}_{\text{CTX-M-1} \text{group}}\)) and \(\text{bla}_{\text{CTX-M-14}}\) (\(\text{bla}_{\text{CTX-M-9} \text{ group}}\))-producing \(E. \text{coli}\) isolates are most common [8].

In this study, we investigated (1) the distribution of phylogenetic groups and virulence factors among pathogenic and commensal strains, (2) independent predictors for pathogenicity, and (3) the distribution of phylogenetic groups and virulence factors according to \(\text{bla}_{\text{CTX-M}}\) production in pathogenic strains.

**Materials and Methods**

**Bacterial strains.** A total of 345 non-duplicate, pathogenic \(E. \text{coli}\) isolates (145 blood and 200 urine samples from hospitalized patients) and 205 isolates from fecal samples of healthy humans were consecutively collected in 2008 at two Korean university hospitals. Positive urine culture was defined by a bacterial growth of \(\geq10^5\) CFU/ml [4].

**Phylogenetic grouping and virulence genotyping.** All isolates were assigned to one of the four main \(E. \text{coli}\) phylogenetic groups (A, B1, B2, D) by use of the multiplex PCR-based method of Clermont et al [9]. Briefly, the total DNA from \(E. \text{coli}\) isolates was extracted by boiling. The analysis for four main phylogenetic groups was determined by PCR using three published primers (\(chuA\), \(YjaA\), \(TspE4.C2\)).

All isolates were also screened for the nine virulence genes: two adhesion genes including \(S\) fimbrial adhesion (\(sfaS\)) and F1C fimbrial adhesin (\(focG\)), two toxin genes including \(hlyA\) and \(cwa1\), three siderophore receptor genes including \(iutA\), yersiniabactin receptor (\(fyuA\)), and siderophore receptor (\(iroN\)), gene encoding \(traT\); and pathogenicity island marker gene (\(PAI\)) [10,11]. Positive control strains were kindly provided by Professor James R. Johnson (University of Minnesota). PCR was done in a 50 \(\mu\)l reaction mixture containing 3 \(\mu\)l of template DNA, 0.6 \(\mu\)M of each primer, 50 \(\mu\)M of each dNTP, 1.25 U of \(h\)-Taq DNA polymerase, and 1X \(h\)-Taq PCR buffer (Solgent Co., Daejeon, Korea). The PCR steps were: activation (94°C, 15 min), 30 cycles of denaturation (94°C, 30 sec), annealing (63°C, 30 sec) and extension (72°C, 2 min), and a final extension interval (72°C, 5 min).

**Antimicrobial susceptibility test, ESBL confirmatory test, and PCR amplification of \(\text{bla}_{\text{CTX-M}}\).** For 345 pathogenic \(E. \text{coli}\) strains, susceptibility to 15 antimicrobial agents (ampicillin, piperacillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefazolin, cefoxitin, cefotaxime, ceftazidime, cefepime, amikacin, gentamicin, tetracycline, ciprofloxacin, imipenem, trimethoprim/sulfamethoxazole) was determined by VITEK 2 automated system (bioMérieux, VITEK) and the ESBL confirmatory test was performed according to the CLSI guidelines [12]. Presence of \(\text{bla}_{\text{CTX-M}}\) alleles (groups 1, 2, and 9) was determined by multiplex PCR [13].

**Statistical analyses.** The prevalence of each virulence factor in each group was compared using the Chi-square test (SPSS 12.0, SPSS Inc., Chicago, IL). Comparisons of aggregate virulence factor scores calculated by summing the number of the virulence factor genes present in each strain were assessed using the Mann-Whitney U test between pathogenic and commensal strains, and between \(\text{bla}_{\text{CTX-M}}\)-producers and non-producers [14,15]. To identify independent predictors of pathogenicity, multivariable logistic regression analysis was used [14]. The threshold for statistical significance was a \(p\) value \(<0.05\).

**Results**

**Distribution of phylogenetic groups and virulence factors in pathogenic and commensal strains.** The distribution of phylogenetic groups was similar between isolates from blood and urine: B2 (44.8%; 58.5%, respectively) > D (29.0%; 23.0%, respectively) > A (18.6%; 9.5%, respectively) > B1 (7.6% and 9.0%, respectively). However, isolates from feces revealed a different distribution: A (38.0%) > B2 (22.9%) > D (21.0%) > B1 (18.0%) (Table 1).
Table 1. Phylogenetic distribution of *E. coli* isolates according to the specimens.

<table>
<thead>
<tr>
<th>Phylogenetic group</th>
<th>Blood (n=145)</th>
<th>Urine (n=200)</th>
<th>Feces (n=205)</th>
<th>Total (n=550)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>27 (18.6%)</td>
<td>19 (9.5%)</td>
<td>78 (38.0%)</td>
<td>124 (22.5%)</td>
</tr>
<tr>
<td>B1</td>
<td>11 (7.6%)</td>
<td>18 (9.0%)</td>
<td>37 (18.0%)</td>
<td>66 (12.0%)</td>
</tr>
<tr>
<td>B2</td>
<td>65 (44.8%)</td>
<td>117 (58.5%)</td>
<td>47 (22.9%)</td>
<td>229 (41.6%)</td>
</tr>
<tr>
<td>D</td>
<td>42 (29.0%)</td>
<td>46 (23.0%)</td>
<td>43 (21.0%)</td>
<td>131 (23.8%)</td>
</tr>
</tbody>
</table>

Table 2. Comparison of prevalence of nine virulence factors between pathogenic and commensal *E. coli* strains.

<table>
<thead>
<tr>
<th>Virulence factor</th>
<th>Pathogenic strains (n=345)</th>
<th>Commensal strains (n=205)</th>
<th>p&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Expected odds ratio</th>
<th>p&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>sfaS</td>
<td>7 (2.0%)</td>
<td>2 (1.0%)</td>
<td>0.346</td>
<td>0.6</td>
<td>0.656</td>
</tr>
<tr>
<td>focG</td>
<td>26 (7.5%)</td>
<td>0 (0.0%)</td>
<td>0.000</td>
<td>2.7×10&lt;sup&gt;8&lt;/sup&gt;</td>
<td>0.998</td>
</tr>
<tr>
<td>hlyA</td>
<td>34 (9.9%)</td>
<td>2 (1.0%)</td>
<td>0.000</td>
<td>5.0</td>
<td>0.049</td>
</tr>
<tr>
<td>cnf1</td>
<td>35 (10.1%)</td>
<td>4 (2.0%)</td>
<td>0.000</td>
<td>0.9</td>
<td>0.859</td>
</tr>
<tr>
<td>iutA</td>
<td>227 (65.8%)</td>
<td>69 (33.7%)</td>
<td>0.000</td>
<td>1.8</td>
<td>0.011</td>
</tr>
<tr>
<td>fyuA</td>
<td>277 (80.3%)</td>
<td>81 (39.5%)</td>
<td>0.000</td>
<td>3.2</td>
<td>0.000</td>
</tr>
<tr>
<td>iroN</td>
<td>82 (23.8%)</td>
<td>19 (9.3%)</td>
<td>0.000</td>
<td>1.1</td>
<td>0.837</td>
</tr>
<tr>
<td>traT</td>
<td>239 (69.3%)</td>
<td>78 (38.0%)</td>
<td>0.000</td>
<td>2.1</td>
<td>0.001</td>
</tr>
<tr>
<td>PAI</td>
<td>177 (51.3)</td>
<td>48 (23.4)</td>
<td>0.000</td>
<td>1.3</td>
<td>0.406</td>
</tr>
</tbody>
</table>

<sup>a</sup>A p value of <0.05 was considered statistically significant by the Chi-square test.<br>
<sup>b</sup>A p value of <0.05 was considered statistically significant by multivariable logistic regression analysis.

Table 3. Distribution of phylogenetic groups among the *bla<sub>CTX-M</sub>*-producing, pathogenic *E. coli* strains.

<table>
<thead>
<tr>
<th>Phylogenetic group</th>
<th>*bla&lt;sub&gt;CTX-M&lt;/sub&gt;-1 group (n=17)</th>
<th>*bla&lt;sub&gt;CTX-M&lt;/sub&gt;-2 group (n=1)</th>
<th>*bla&lt;sub&gt;CTX-M&lt;/sub&gt;-9 group (n=23)</th>
<th>Total (n=41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4 (23.5%)</td>
<td>0 (0.0%)</td>
<td>2 (8.7%)</td>
<td>6 (14.6%)</td>
</tr>
<tr>
<td>B1</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>2 (8.7%)</td>
<td>2 (4.9%)</td>
</tr>
<tr>
<td>B2</td>
<td>11 (64.7%)</td>
<td>1 (100%)</td>
<td>9 (39.1%)</td>
<td>21 (51.2%)</td>
</tr>
<tr>
<td>D</td>
<td>2 (11.8%)</td>
<td>0 (0.0%)</td>
<td>10 (43.5%)</td>
<td>12 (29.3%)</td>
</tr>
</tbody>
</table>
Table 4. Prevalence of nine virulence factors among the pathogenic *E. coli* strains according to production of *bla*<sub>CTX-M</sub> group.

<table>
<thead>
<tr>
<th>Virulence factor</th>
<th>( bla_{CTX-M} )-producers</th>
<th>( bla_{CTX-M} )-non-producers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=41)</td>
<td>(n=304)</td>
</tr>
<tr>
<td></td>
<td>( bla_{CTX-M-1} ) group</td>
<td>( bla_{CTX-M-2} ) group</td>
</tr>
<tr>
<td></td>
<td>(n=17)</td>
<td>(n=1)</td>
</tr>
<tr>
<td><em>sfaS</em></td>
<td>0 (0.0%)</td>
<td>0.527</td>
</tr>
<tr>
<td><em>focG</em></td>
<td>1 (5.9%)</td>
<td>0.731</td>
</tr>
<tr>
<td><em>hlyA</em></td>
<td>1 (5.9%)</td>
<td>0.563</td>
</tr>
<tr>
<td><em>cnf1</em></td>
<td>1 (5.9%)</td>
<td>0.563</td>
</tr>
<tr>
<td><em>iutA</em></td>
<td>17 (100%)</td>
<td>0.003</td>
</tr>
<tr>
<td><em>fyuA</em></td>
<td>11 (64.7%)</td>
<td>0.086</td>
</tr>
<tr>
<td><em>irnN</em></td>
<td>3 (17.6%)</td>
<td>0.511</td>
</tr>
<tr>
<td><em>traT</em></td>
<td>15 (88.2%)</td>
<td>0.062</td>
</tr>
<tr>
<td><em>PAI</em></td>
<td>12 (70.6%)</td>
<td>0.098</td>
</tr>
</tbody>
</table>

* A p value of <0.05 was considered statistically significant by the Chi-square test.
The prevalence of all the virulence factors was highest in phylogenetic group B2 (3.9% for sfaS; 10.0% for focG; 11.4% for hlyA; 15.7% for cnf1; 67.2% for iutA; 90.4% for fyuA; 32.3% for iroN; 72.5% for traT; 81.7% for PAI) throughout all the isolates. Of the nine virulence factors, the prevalence of all but sfaS was significantly higher in pathogenic strains than in commensal strains; hlyA, fyuA, traT, and iutA were independent predictors for urinary tract or blood stream infection status (odds ratios of 5.0, 3.2, 2.1, 1.8, respectively, p <0.05) (Table 2).

The aggregate virulence factor scores were significantly higher in pathogenic strains than in commensal strains throughout all the phylogenetic groups (median scores of 3.0 and 1.0, respectively, p = 0.000) and also in the same phylogenetic group (median scores of 2.0 and 0.0 for group A; 1.0 and 0.0 for group B1; 4.0 and 3.0 for group B2; 3.0 and 2.0 for group D, respectively).

Distribution of phylogenetic groups and virulence factors according to blaCTX-M production. Of the 345 pathogenic strains, 41 (11.9%) were blaCTX-M-producers and most of them were blaCTX-M-9 group and blaCTX-M-1 group (23 and 17 isolates, respectively) (Table 3).

The phylogenetic distribution in the total of blaCTX-M-producers was similar to that seen in the total of pathogenic strains: B2 (51.2%) > D (29.3%) > A (14.6%) > B1 (4.9%). However, there was a difference between blaCTX-M-1 group-producers and blaCTX-M-9 group-producers; whereas the majority (67.4%) of blaCTX-M-1 group-producers belonged to phylogenetic group B2, followed by group A (23.5%), 43.5% of blaCTX-M-9 group-producers belonged to phylogenetic group D, followed by group B2 (39.1%) (Table 3).

The antimicrobial resistance rates of 12 out of 15 antimicrobial agents (ampicillin, piperacillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefazolin, cefoxitin, cefotaxime, cefazidime, cefepime, amikacin, gentamicin, ciprofloxacin) were significantly higher in blaCTX-M-producers vs blaCTX-M-non-producers (p <0.05, data not shown).

There was no difference in aggregate virulence factor scores between blaCTX-M-producers and blaCTX-M-non-producers (median scores = 3.0) among the pathogenic strains. The prevalence of each virulence factor was also similar irrespective of blaCTX-M production, but iutA and traT were significantly more common in blaCTX-M-1 group and in blaCTX-M-9 group, respectively, than in blaCTX-M-non-producers (100% vs 66.4%, p = 0.003, and 91.3% vs 66.4%, p = 0.014, respectively) (Table 4).

Discussion

The pathogenic strains causing urinary tract infection or bacteremia mostly belonged to groups B2 and D, which was concordant with previous studies [1,16]. However, in contrast to the previous studies in France and the USA, where most commensal strains were group A or B1 [1,16], as many as 43.9% of the commensal strains belonged to groups B2 or D. This difference can probably be attributed to the bacterial characteristics in different geographic regions under the influence of antibiotics usage or host genetic factors [16].

In this study, all the virulence factors except sfaS were found to be more prevalent in pathogenic strains than in commensal strains, as reported previously [2]. Of the 9 virulence factors examined, 4 virulence factors (hlyA, fyuA, traT, and iutA) were independent predictors of pathogenicity. This finding suggests that the combinatorial approach of virulence factors, including a toxin that can modulate the host signaling pathway (hlyA), two iron acquisition systems (iutA and fyuA) that allow bacteria to multiply in an environment of limited concentration of free iron such as in tissues and fluids of the host [17], and serum resistance (traT), may enable extraintestinal pathogenic E. coli to enter the primary infection sites (urinary tract or upper respiratory tract), spread to secondary internal organs, and survive in the blood stream, causing septicemia [17,18]. In addition, the prevalence of fyuA, traT, and PAI was significantly higher in E. coli isolates from urine than in isolates from blood (p <0.05, data not shown), as previously reported in E. coli strains from urosepsis [10]. We assume that these virulence factors are more associated with urinary tract infection than with infections at other sites. Further studies are needed.
to identify the role of virulence factors in the pathogenesis of septicemia according to the site of primary infection. The aggregate virulence factor scores were higher in pathogenic strains than in commensal strains or even in the same phylogenetic groups. Considering that a putative primary reservoir of extraintestinal pathogenic E. coli strains is within the human intestinal tract [1,18], it appears that on entry into the extraintestinal sites, strains with high aggregate virulence factor scores have superior opportunity in mutual competition with host defense mechanisms.

The prevalence of $\text{bla}_{\text{CTX-M}}$ alleles in 345 pathogenic strains was in line with the frequency of $\text{bla}_{\text{CTX-M}}$ in Korean populations; $\text{bla}_{\text{CTX-M-1}}$ group and $\text{bla}_{\text{CTX-M-9}}$ group were the two most common types, which are the two most common types of CTX-M-type ESBLs in clinical isolates of E. coli in Korea [8]. Based on our results, aggregate virulence scores were similar, irrespective of $\text{bla}_{\text{CTX-M}}$ production. However, two of the independent predictors for pathogenicity, $iutA$ and $traT$, which are involved in iron transport and increased serum survival, respectively, and are carried by CoLV plasmid [19,20], were significantly more common in isolates harboring the $\text{bla}_{\text{CTX-M-1}}$ group and the $\text{bla}_{\text{CTX-M-9}}$ group, respectively. The majority of the $\text{bla}_{\text{CTX-M-1}}$ group and the $\text{bla}_{\text{CTX-M-9}}$ group belonged to phylogenetic groups B2 and D, respectively, as reported previously [15,21]. Karisk et al [21] reported that $iutA$ was more common in isolates harboring $\text{bla}_{\text{CTX-M-15}}$ ($\text{bla}_{\text{CTX-M-1}}$ group), which belonged to phylogenetic group B2. Most septicemic strains contain CoLV plasmids that carry the aerobactin iron uptake system as well as genes coding for serum resistance [19,20]. A recent study of CoLV plasmid indicated that it belongs to the IncF incompatibility group [22], which is conjugative and also encodes type IV pili, which is important for adhesion to and invasion of epithelial cells by Salmonella typhimurium serovar Typhi [23].

In summary, in contrast to previous studies, as many as 43.9% of commensal strains also belonged to group B2 and D. Virulence factors $\text{blyA}$, $\text{fyuA}$, $\text{traT}$, and $iutA$ were found to be independent predictors for pathogenicity and, of them, $iutA$ and $\text{traT}$ were significantly more common in isolates harboring $\text{bla}_{\text{CTX-M-1}}$ group and $\text{bla}_{\text{CTX-M-9}}$ group, respectively. Considering the possibility that virulence genes, together with antimicrobial resistance genes, can spread to other strains by recombination of their plasmids, further study and ongoing surveillance seem to be required.

**Acknowledgement**

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**References**


