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ORIGINAL ARTICLE

Virulence factors of uropathogenic *Escherichia coli* of urinary tract infections and asymptomatic bacteriuria in children

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Background/Purpose: The clinical aspects of virulence genes of uropathogenic *Escherichia coli* (UPEC) are not fully understood. This study compared the presence of virulence genes in UPEC isolated from urinary tract infections (UTIs) and asymptomatic bacteriuria (ABU) in children.
Methods: The study included children with UTI ($n = 15$) or ABU ($n = 49$) treated at Chung-Ang University Yongsan Hospital between 2010 and 2011. The strains were acquired from each urine sample collected, and 18 major virulence genes were detected by polymerase chain reaction. Antimicrobial susceptibility of all UPEC isolates was determined.
Results: Sixty-four *E. coli* strains were isolated from the urine samples. The most commonly identified virulence gene in both groups was *fimH* (100.0% in the UTI group and 95.9% in the ABU group). The UTI isolates showed a higher prevalence of *papEF* and *fyuA*, and a lower prevalence of *feoB* than ABU isolates ($p < 0.01$ for all). The profile of virulence gene, $fimH^{+}kpsMTII^{+}feoB^{+}$ also showed a significant difference between the two groups ($p < 0.01$). Isolates from ABU were more resistant to most antimicrobials tested. The presence of *papEF, feoB,* and *fyuA* also correlated with the antimicrobial susceptibility of UPEC.
Conclusion: The virulence gene repertoire was different in the UPEC of UTI and ABU. The *papEF*, *feoB*, and *fyuA* genes showed meaningful differences between the two groups and may have an important role in the pathogenesis of overt UTI.

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Introduction

Urinary tract infection (UTI) refers to an infection with microbial pathogens at any site in the urinary tract, which includes the urethra, bladder, ureter, and kidneys. UTI is the most common bacterial infection in children; many affected children, particularly infants, have severe symptoms and lobar nephronia. Kidney scarring related to UTI has been linked to long-term morbidity.[1,2] By contrast, asymptomatic bacteriuria (ABU) is a condition in which bacteria subclinically colonize the urinary tract, closely resembling commensalism at other mucosal sites. ABU strains are well adapted for growth in the human urinary tract, without causing any clinical symptoms. Therefore, ABU strains are an interesting model for the study of mechanisms of commensalism and the driving forces between the pathogen and the host.[3,4]

Uropathogenic *Escherichia coli* (UPEC) are the most common bacterial pathogens that cause UTI and ABU in children. Extended-spectrum beta-lactamases-producing UPEC strains, which are increasing in prevalence worldwide, have an appreciable deleterious impact on the clinical management of UTI.[5,6] The UPEC strains harbor many genes that encode various virulence factors, which contribute to enhanced pathogenicity. The molecular characteristics and functions of these virulence genes have been well established.[7–12] The severity of UTI reflects the virulence profile or phenotype of the infecting strain, with many virulence factors being expressed fully at a higher frequency by strains causing UTI than by strains causing ABU.[13–15]

In this study, we sought to identify the prevalence and expression patterns of virulence genes in UPEC isolated from children in South Korea. We also compared the virulence gene repertoire in UPEC strains isolated from UTIs and ABU, with the aim of clarifying important virulence factors for the development of clinical diseases of the urinary tract in children.

Materials and methods

Participants

This study was performed at Chung-Ang University Yongsan Hospital between March 2010 and February 2011. All participants were children $<$18 years of age who lived in Seoul, Korea, during the study period. Inclusion criteria were fever with an axillary temperature of $\geq$38°C, positive urine culture ($>10^5$ colony-forming units/mL), and pyuria ($>$5 white blood cells/high-power field). Children who did not have any urinary symptoms (i.e., urgency, frequency, and dysuria) but had significant growth of *E. coli* ($>10^5$ colony-forming units/mL) were considered to have ABU. The urine samples were collected through midstream urine in toilet-trained children and by a sterile urine-collecting bag in others. The study protocol was reviewed and approved by the Institutional Review Board (No. 10-013-02-03) at Chung-Ang University Hospital, and written informed consent was obtained from the parents/guardians of all participants.

Detection of virulence genes

All *E. coli* isolates were identified by typical morphology, lactose fermentation, positive spot indole test, and VITEK 2-GN card (bioMérieux, Hazelwood, MO, USA). Only one isolate per patient was examined. Extraction and purification of DNA from all UPEC isolates were performed as described in the QIAamp Kit (Qiagen GmbH, Hilden, Germany). The presence of the following virulence genes was assessed by 18 simplex polymerase chain reaction (PCR):adhesion proteins (*papA, papC, papEF, papGI, papGII, papGIII, sfa, fimH, afa, bmaE*), toxins (*hlyA, cdtB*), siderophores (*fyuA, iutA, feoB*), capsule synthesis proteins (*kpsMTII, kpsMTIII*), and uropathogenic-specific protein (*usp*). Primer sequences for amplification of these individual virulence genes have been published in previous studies[16–19] and are listed in Table 1. PCR was consistently performed in a 20-μL reaction volume, with each reaction mixture containing 1.0 μL of DNA template, 10 μM of each primer, 2.0 μL of 10× *Taq* buffer, 2.5 mM of deoxynucleotide triphosphates, and 2.5 mM of *Taq* polymerase (iNtRON, Seoul, Korea). Thermal cycling was performed in a PTC-200 Peltier thermal cycler DNA engine (MJ Research, Watertown, MA, USA) under the following conditions: denaturation for 5 minutes at 94°C; 35 1-minute amplification cycles at 94°C, and additional amplification cycles for 1 minute at 55°C, 2 minutes at 72°C, and a final extension cycle for 10 minutes at 72°C. The PCR products were electrophoresed on agarose gels, stained with ethidium bromide, and photographed using a UV transillumination imaging system.

Antimicrobial susceptibility test

Antimicrobial susceptibility testing of all isolates to ampicillin, gentamicin, piperacillin, trimethoprim/sulfamethoxazole, tetracycline, amoxicillin/clavulanic acid, cefazolin, aztreonam, ciprofloxacin, cefepime, cefotaxime, tobramycin, piperacillin/tazobactam, and levofloxacin was performed using the VITEK 2 automated system (bioMérieux). In addition, *in vitro* antimicrobial susceptibility testing was performed by the broth microdilution method and the results were interpreted using the 2010 Clinical and Laboratory Standards Institute breakpoints.

Statistical analyses

All statistical analyses were performed using SPSS version 18.0 (SPSS, Chicago, IL, USA). The prevalence of virulence genes and antibiotic resistance patterns were compared between the two groups using Pearson Chi-square test and Fisher exact test. Continuous variables were compared with the Student *t* test and Mann–Whitney *U* test. A *p* value <0.05 was considered statistically significant.

Results

Clinical characteristics

A total of 64 patients were included in this study, of which 15 were diagnosed with UTI and 49 with ABU. In patients with