Escherichia coli Isolates Associated with Uncomplicated and Complicated Cystitis and Asymptomatic Bacteriuria Possess Similar Phylogenies, Virulence Genes, and O-Serogroup Profiles[▽]

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The genetic and serological characteristics of *Escherichia coli* isolates from patients with uncomplicated cystitis (UC), complicated cystitis (CC), and complicated asymptomatic bacteriuria (CASB) were determined. Phylogenetic group B2 was predominant in all categories. The prevalences of 14 out of 18 virulence factor genes were similar among the three categories, while *pap*, *iha*, *ompT*, and *PAI* were more frequently seen in isolates associated with UC than CC or CASB.

Escherichia coli is the microorganism most commonly isolated from patients with complicated as well as uncomplicated urinary tract infections (UTIs). Although the virulence factors (VFs) of *E. coli* from various clinical sources have been reported by a number of investigators, only a few studies have directed attention to complicated UTI and asymptomatic bacteriuria (ASB) (9, 18, 21).

Sandberg et al. investigated the virulence-associated properties of *E. coli* in women with uncomplicated and complicated pyelonephritis and showed that hemolysin production and mannose-resistant adhesins were less prevalent in isolates associated with complicated pyelonephritis than in those associated with uncomplicated pyelonephritis (19). On the other hand, Andreu et al. reported that similar O:K serogroups and VF properties were observed in isolates from uncomplicated cystitis (UC) and complicated UTIs, including both upper and lower UTIs (1).

ASB is a common disorder whose prevalence is reported to be 4 to 6% in healthy young adult women and which increases with the patient's age, up to 20% or more in ambulatory elderly women (7). However, little is yet known about the difference between symptomatic and asymptomatic UTI in terms of pathogenesis, natural history, and risk factors. Hull et al. compared virulence properties of *E. coli* isolates associated with symptomatic UTI and ASB with neuropathic bladder due to spinal cord and brain injury (8). Their results show that isolates associated with symptomatic UTI are more likely than those associated with ASB to be hemolytic and exhibit mannose-resistant hemagglutination of human erythrocytes. On the other hand, Geerlings et al. reported that the number of VFs in *E. coli* isolates from diabetic women with ASB is comparable to that found in noncompromised patients with ASB (4).

Thus, the difference in prevalence of VFs in *E. coli* isolates

associated with uncomplicated and complicated, or symptomatic and asymptomatic, UTI is controversial because previous reports have included various study designs, methods, definitions, and patient populations (1, 7, 8, 19).

Phylogenetically, *E. coli* strains are divided into four main groups designated A, B1, B2, and D (3, 6). It has been demonstrated that most *E. coli* strains responsible for UTI or other extraintestinal infections belong to group B2 or, less frequently, group D (17). But the distribution of phylogenetic groups, as well as of VFs, in isolates associated with asymptomatic UTI is not yet understood.

Our group also has shed some light on the phylogenetic and O-serogroup distributions and various VF gene profiles of acute uncomplicated UTI-associated isolates, in order to understand the specific characteristics of uropathogenic *E. coli* (UPEC) (13, 21, 23). To clarify the characteristic difference between *E. coli* strains causing uncomplicated and complicated, or symptomatic and asymptomatic, UTI, we determined the distributions of phylogenetic groups and O serogroups and the prevalence of 18 VF genes in a total of 283 *E. coli* isolates associated with three distinct categories: UC, complicated cystitis (CC), and complicated ASB (CASB). The 18 VF genes were selected as we surveyed their prevalence in acute uncomplicated UTI-associated isolates in other studies, and they turned out to be more frequently present in UTI-associated isolates than in fecal isolates.

Strains. A total of 283 *E. coli* strains, consisting of 153 associated with UC, 56 associated with CC, and 74 associated with CASB, were collected at Sapporo Medical University Hospital, Sapporo, and Kyoto Senbai Hospital, Kyoto, Japan, between 2000 and 2005. The categories CC and CASB consisted of isolates from patients with obstructive diseases such as neurogenic bladder, and benign prostate hypertrophy and non-obstructive diseases such as bladder tumor, urinary tract abnormality, and diabetes mellitus; patients with continuously indwelling urinary catheters or other instruments were excluded. Patients were diagnosed as having symptomatic cystitis (UC and CC) when they complained of one or more typical

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TABLE 1. VF gene primer sets for PCR

VF gene	Primer (forward/reverse)	Forward sequence	Reverse sequence	Product size (bp)	Reference
<i>afa</i>	afa 1/2	5'-GCTGGGCAGCAAATGATAACTCTC-3'	5'-CATCAAGCTGTTTGTTCGTCGCCG-3'	750	23
<i>aer</i>	aer 1/2	5'-TACCGGATTGTTCATATGCAGACCGT-3'	5'-AATATCTTCTCCAGTCCGGAGAAG-3'	602	23
<i>cnf1</i>	cnf 1/2	5'-AAGATGGAGTTTCTATGCAGGAG-3'	5'-CATTGAGAGTCTGCTCCCTCATTATT-3'	498	23
<i>sfa/foc</i>	sfa/foc 1/2	5'-CTCCGGAGAACTGGGTGCATCTTAC-3'	5'-CGGAGGAGTAATTACAAACCTGGCA-3'	410	23
<i>hly</i>	hly 1/2	5'-AACAAAGGATAAGCACTGTTCTGGCT-3'	5'-ACCATATAAGCGGTCTATCCCGTCA-3'	1,177	23
<i>pap</i>	pap 3/4	5'-GCAACAGCAACGCTGGTTGCATCAT-3'	5'-AGAGAGAGCCACTCTTATACGGACA-3'	336	23
<i>iha</i>	IHA F/R	5'-CTGGCGGAGGCTCTGAGATCA-3	5'-TCCTTAAGCTCCCGCGGCTGA-3'	827	10
<i>iroN</i>	IRONEC F/R	5'-AAGTCAAAGCAGGGGTTGCCG-3'	5'-GACGCCGACATTAAGACGCAG-3	665	10
<i>ompT</i>	ompT-f/r	5'-ATCTAGCCGAAGAAGGAGGC-3'	5'-CCGGGTCATAGTGITCATC-3'	559	11
<i>PAI</i>	RPAi f/r	5'-GGACATCCTGTTACAGCGCGCA-3'	5'-TCGCCACCAATCACAGCCGAAC-3'	930	12
<i>cvaC</i>	ColV-C f/r	5'-CACACACAAACGGGAGCTGTT-3'	5'-CTTCCCGCAGCATAGTTCAT-3'	680	12
<i>traT</i>	traT f/r	5'-GGTGTGGTGCGATGAGCACAG-3'	5'-CACGGTTCAGCCATCCTGAG-3'	290	12
<i>ibeA</i>	ibe f/r	5'-AGGCAGGTGTGCCCGCGTAC-3'	5'-TGGTGCTCCGGCAAACCATGC-3'	170	12
<i>FyuA</i>	FyuA f/r	5'-TGATTAACCCGCGACGGGAA-3'	5'-CGCAGTAGGCACGATGTTGTA-3'	880	12
<i>kpsMT</i>	kpsM 481f/880r	5'-CCATCGATACGATCATTTGCACG-3'	5'-ATTGCAAGGTAGTTCAGACTCA-3'	400	13
<i>pil</i>	fimH f/r	5'-CATTCGCCTGTAAAACCGCC-3'	5'-ATAACACGCCGCCATAAGCC-3'	207	22
<i>ETTT</i>	ETTT 6f/788r	5'-GCGGAAGTTTTGTATGATTGCCG-3'	5'-ATCAACCAGGAAAGCCAGTACG-3'	783	This work
<i>usp</i>	USP 81f/695R	5'-CGGCTCTTACATCGGTGCGTTG-3'	5'-GACATATCCAGCCAGCGAGTTTC-3'	615	This work

clinical symptoms such as dysuria, urinary frequency, lower abdominal pain or discomfort, sensation of residual urine, or pain during micturition, together with at least 10^5 CFU of *E. coli*/ml of urine. Patients without clinical history of urinary complications such as urolithiasis, vesicoureteral reflux, neurogenic bladder, benign prostate hypertrophy, urinary tract abnormalities, or malignant neoplasms, were classified as UC, and those who had one or more of these were classified as CC. CASB was defined, as cited by Geerlings et al., as the presence of at least 10^5 CFU *E. coli*/ml in a urine culture from patients with one or more urinary complications but without symptoms of UTI, as stated above (4).

Phylogenetic analysis and VF profiling by PCR. *E. coli* cells were harvested from Luria-Bertani agar after overnight incubation, suspended in 0.2 ml of sterile water, incubated at 95°C for 10 min, and centrifuged at $11,000 \times g$. The supernatant was used as the DNA template for PCR to detect phylogenetic groups and the following 18 VF genes: *aer* (aerobactin), *afa* (afimbrial adhesin), *cnf1* (cytotoxic necrotizing factor 1), *cvaC* (colicin V), *ETTT* (type III secretion system), *fimH* (type 1 fimbriae adhesin), *fyuA* (yersiniabactin receptor for ferric yersiniabactin uptake), *hly* (alpha hemolysin), *ibeA* (invasion of brain endothelium), *iroN* (catecholate siderophore receptor), *iha* (iron-regulated gene A homologue adhesin), *kpsMT* (group 2 capsule), *ompT* (outer membrane protease T), *PAI* (pathogenic island marker of CFT073), *pap* (P fimbriae), *sfa/foc* (S/F1C fimbriae), *traT* (serum resistance associates), and *usp* (uropathogenic specific protein). (Table 1) (10, 11, 12, 13, 22, 23). Phylogenetic grouping was determined by the use of a dichotomous decision tree, as previously reported by Clermont et al., using the results of PCR amplification of *chuA* and *yjaA* genes and DNA fragment TspE4.C2. Briefly, *chuA*⁺ *yjaA*⁺ strains were classified as B2, *chuA*⁺ *yjaA*⁻ strains were classified as D, *chuA*⁻ TspE4.C2⁺ strains were classified as B1, and *chuA*⁻ TspE4.C2⁻ strains were classified as A (3). Most of the genes were determined by multiplex PCR, and three (*hly*, *ompT*, and *ibeA*) were determined by single-primer PCR (Table 2). Amplification was performed in a 10-μl reaction mixture containing 1.4 μl of DNA template, 2.5 pmol of each primer (except for *PAI*, which was used at 5 pmol), 0.3 mM of

each deoxynucleotide triphosphate, 1 μl of 10× PCR buffer with 15 mM MgCl₂, and 0.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, New Jersey). The reaction was carried out in a PCR thermal cycler (Takara Bio Inc., Otsu, Japan) with the following schedule: preheating at 94°C for 10 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C or 55°C, and extension at 72°C for 1.5 min, with a final extension at 72°C for 7 min (Table 2). The PCR products were electrophoresed in 2% agarose gels, stained with ethidium bromide, and photographed under UV transillumination. PCR with single-primer sets was performed for negative data in multiplex PCR groups.

O serogrouping. O serogrouping of *E. coli* strains was carried out as previously described by Orskov et al., using diagnostic *E. coli* antiserum for serotyping of the *E. coli* O group (Statens Serum Institut, Copenhagen, Denmark) (16).

Statistical analyses. Comparisons of proportions were tested using the chi-square test or Fisher's exact test. *P* values of <0.05 were considered to indicate significance.

Prevalence of phylogenetic groups and O serogroups. Phylogenetic group B2 was most frequently discovered in all categories, as follows: UC, 71%; CC, 73%; CASB, 61%. There was no significant difference in the proportion of the four groups (A, B1, B2, and D) among these categories (Table 3). Although a relatively higher proportion of O6 was observed in CC isolates (UC, 8%; CC, 20%; CASB, 5%; *P* = 0.006), there

TABLE 2. Multiplex PCR grouping and annealing temperatures

Primer group	Gene(s)	Annealing temp (°C)
Multiplex		
1	<i>afa</i> , <i>aer</i> , <i>cnf1</i> , <i>sfa/foc</i> , <i>pap</i> , <i>fimH</i>	60
2	<i>PAI</i> , <i>ETTT</i> , <i>iroN</i> , <i>chuA</i> , <i>TspE4.C2</i>	60
3	<i>iha</i> , <i>usp</i> , <i>kpsMT</i> , <i>traT</i>	60
4	<i>fyuA</i> , <i>cvaC</i> , <i>yjaA</i>	55
Single		
	<i>hly</i>	60
	<i>ompT</i>	60
	<i>ibeA</i>	55

probably included many cases of upper UTI, which was defined as a fever of at least 38.5°C plus one or more of the symptoms of bacteriuria, pyuria, and hematuria. On the other hand, Graham et al. reported that *E. coli* strains from pregnant women with bacteriuria are less likely to carry adhesin-related and toxin genes than those from community-acquired cystitis patients (*pap*, 30% versus 60%; *sfa*, 26% versus 51%; F1C, 19% versus 36%; *cna1*, 26% versus 38%; and *aer*, 40% versus 64%), whereas no significant difference in the prevalence of *hly* (28% versus 30%) was seen between the two groups (5).

Taken together, although it is likely to require a greater number of VFs to cause uncomplicated or symptomatic UTI than complicated or asymptomatic infections, *E. coli* strains causing UTIs share similar characteristics and properties in terms of phylogeny, O serogroups, and VFs, as shown in the present study. In this regard, Svanborg and colleagues have recently proposed an interesting mechanism to explain the occurrence of ASB where Toll-like receptor 4 (TLR4) controls the initial mucosal response to UPEC, suggesting that the development of symptomatic infection would be more closely associated with variation of the host environment than with expression of bacterial VFs, since TLR4 mutant mice develop an asymptomatic carrier state resembling human ASB (20). They have shown that a clinical ASB-associated strain, *E. coli* 83972, is able to colonize the human urinary bladder without inducing an immune response, through the mutation of its genes encoding type 1 and P fimbriae (14). Thus, the development of symptomatic infection may be more closely associated with the variation in the host environment than the virulence of the microorganisms.

Finally, a recent report by Chen et al. has revealed 29 possible genes positively selective in UPEC strains as compared to traditional *E. coli* strains (2). These genes could be the candidates to be specific to uncomplicated or symptomatic UTIs. Further investigations would be required to clarify the difference in pathogenicity between uncomplicated and complicated, or symptomatic and asymptomatic, diseases with respect to both hosts and bacteria.

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