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Short Communication

Virulence Genes, Quinolone and Fluoroquinolone Resistance, and Phylogenetic Background of Uropathogenic *Escherichia coli* Strains Isolated in Japan

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SUMMARY: A total of 312 uropathogenic *Escherichia coli* strains were isolated from clinical specimens in 7 hospitals in Aichi Prefecture, Japan. Among them, 113 strains were resistant to quinolone, and 49 strains were resistant to fluoroquinolone. Phylogenetic group B2 was most prevalent in both susceptible strains (148 of 199 strains, 74.4%) and resistant strains (quinolone-resistant strains, 73 of 113 strains, 64.6%; fluoroquinolone-resistant strains, 40 of 49 strains, 81.6%). The resistant strains showed a significantly lower prevalence of virulence genes *papA*, *hlyA*, and *cnf1* than did the susceptible strains, and this observation was further obvious when compared within B2 group strains. Among the 40 fluoroquinolone-resistant strains belonging to group B2, 37 (92.5%) strains carried PAI*usp* subtype IIa, 36 strains of which carried E84V mutation in *parC*, whereas none of the 9 strains belonging to group D carried PAI*usp* subtype IIa, and only one strain carried the mutation. These observations indicate that the differences of phenotypes including resistance of quinolone and carriage of virulence genes are associated with the complex context of genetic background, including the phylogenetic group and PAI*usp* subtype.

Urinary tract infection (UTI) is one of the most commonly encountered diseases in clinical settings, and uropathogenic *Escherichia coli* (UPEC) is the major causative pathogen (1). First-line antibiotics for UTIs include quinolones (Qs) and fluoroquinolones (FQs). Recent surveys across Europe and the United States showed that the frequency of isolation of UPEC strains resistant to FQs is increasing year by year (2,3). Resistance to Qs and FQs in *E. coli* occurs by mutation in *gyrA* and/or *parC* genes or acquisition of plasmid-carrying resistance genes such as *qnrA*, *qnrB*, and *qnrS*.

Recent studies suggest that Q-resistant and FQ-resistant UPEC strains show reduced virulence and are less capable of causing upper urinary tract infection compared with susceptible strains (4–6). UPEC strains usually produce various virulence factors, thereby causing extraintestinal diseases (7,8). Several groups reported that a few virulence genes were less prevalent in resistant strains compared with susceptible strains (9–13). In addition to association with virulence factors, the relationship between phylogenetic group and resistance has also been reported. Horcajada et al. found that UPEC phylogenetic group B2 strains were more susceptible to antibiotics than were phylogenetic groups A, B1, and D strains (9). Other groups obtained similar results and mentioned that many FQ-resistant strains belonged to phylogenetic groups A and D (10,11). The findings in one study also suggested that resistance to Q and FQ could be associated with a specific genetic background (12).

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To gain a better understanding of the reasons underlying these correlations, more data from different geographic regions are required. Here we report the association among prevalence of virulence genes, phylogenetic background, and resistance to Q and FQ in UPEC strains isolated in Japan.

A total of 312 *E. coli* strains were isolated from urinary specimens in 7 hospitals in different geographical areas in Aichi Prefecture, Japan, from December 2007 to April 2008. Among the 312 patients (males 97 and females 215) aged 0 to 100 years (mean 60.4 years), 190 were outpatients and 122 were inpatients. The strains were identified as *E. coli* by the API20E system (bioMerieux Japan, Tokyo, Japan). *E. coli* ATCC25922 (American Type Culture Collection) was used as the control strain for susceptibility testing.

The minimum inhibitory concentration (MIC) of each antibiotic was determined by the agar dilution method according to the protocol recommended by the Clinical and Laboratory Standards Institute (CLSI). The antimicrobial agents were obtained from the following sources: nalidixic acid, Wako Pure Chemical Industries, Osaka, Japan; ciprofloxacin and levofloxacin, Daiichi-Sankyo Pharmaceutical Co., Tokyo, Japan.

Carriage of 8 kinds of virulence-associated genes was examined by the multiplex polymerase chain reaction (PCR) method as previously described (14). The 8 genes consisted of adhesion *papA* (fimbriae structural subunit), *fimH* (type-1 fimbriae), *sfa* (S fimbriae), *focG* (F1C fimbriae), *afa* (Dr-binding adhesions), *hlyA* (hemolysin), *cnf1* (cytotoxic necrotizing factor), and *iutA* (aerobactin receptor).

The phylogenetic group of each strain was determined by the triplex PCR method reported previously (15). *E. coli* strains were classified into 4 phylogenetic groups, A, B1, B2, and D, according to the pattern of PCR amplification. The

mosaic variations of PAI*usp* subtypes, Ia, Ib, IIa, and IIb, were also determined by PCR as described previously (16). All results were confirmed at least twice.

Statistical analysis of the obtained data was performed using statistical program SPSS for Windows version 11.0J (SPSS Inc., Chicago, Ill., USA). The comparison analysis was done by Pearson's χ^2 test and the Mann-Whitney U test. The difference was considered significant if the *P* value was <0.05.

Among the 312 UPEC strains examined, 113 strains were resistant to Q (nalidixic acid). Among the 113 resistant strains, 49 strains were resistant to FQ (ciprofloxacin and levofloxacin). Among these 49 resistant strains, 19 were from inpatients and 30 were from outpatients. The rates of the number of resistant strains in inpatients and outpatients were 15.6% (19 of 122 strains) and 15.8% (30 of 190 strains), respectively.

The phylogenetic group classification of susceptible and resistant strains is summarized in Table 1. The most prevalent group was B2 in both susceptible strains (148 of 199 strains, 74.4%) and Q-resistant strains (73 of 113 strains, 64.6%). The prominent feature was that all of the 113 Q-resistant strains belonged to either group B2 or group D, in contrast to several previous reports indicating that resistant strains exhibited shifts to non-B2 groups, including group A (10,13). These inconsistent observations indicate that the association of the resistance with phylogenetic group alone is a controversial matter which could be largely affected by other factors. Among the 49 FQ-resistant strains, 40 (81.6%) strains belonged to group B2, and 9 (18.4%) strains belonged to group D (Table 1).

The carriage of virulence genes in susceptible and resistant strains is also summarized in Table 1. Among the virulence genes examined, prevalence of *papA*, *hlyA*, and *cnf1* was significantly lower in resistant strains compared with susceptible strains. This observation was further obvious when the carriage rates were compared within the B2 group strains, as summarized in Table 2. This observation is consistent with previously published reports showing decreased prevalence of virulence factors in Q-resistant UPEC isolates (9–13).

Distribution of the mosaic variation of PAI*usp* subtypes of the FQ-resistant strains is summarized in Table 3. Among the 40 FQ-resistant strains belonging to group B2, 37 (92.5%) strains carried PAI*usp* subtype IIa. In contrast, none of the 9 FQ-resistant strains belonging to group D carried PAI*usp* subtype IIa. Among the 9 FQ strains belonging to group D, 8 (89%) strains were PAI*usp*-negative and one strain carried PAI*usp* Ib. Carriage of mutations conferring resistance among

Table 1. Distribution of phylogenetic groups and virulence genes among the susceptible and resistant strains

Phylogenetic group or VF gene	No. (%) of strains[1)]				*P*[2)]	
	Total (*n* = 312)	Susceptible (*n* = 199)	Q-resistant (*n* = 113)	FQ-resistant (*n* = 49)	Susceptible vs Q-resistant	Susceptible vs FQ-resistant
Phylogenetic groups						
A	12 (3.8)	12 (6.0)	0 (0)	0 (0)		
B1	21 (6.7)	21 (10.6)	0 (0)	0 (0)		0.037
B2	221 (70.8)	148 (74.4)	73 (64.6)	40 (81.6)		
D	58 (18.6)	18 (9.0)	40 (35.4)	9 (18.4)	<0.001	
Virulence genes						
papA	118 (37.8)	97 (48.7)	21 (18.6)	5 (10.2)	<0.001	<0.001
fimH	310 (99.4)	197 (99.0)	113 (100)	49 (100)		
sfa	75 (24.0)	53 (26.6)	22 (19.5)	15 (30.6)		
focG	24 (7.7)	20 (10.1)	4 (3.5)	1 (2.0)		
hlyA	64 (20.5)	56 (28.1)	8 (7.1)	2 (4.1)	<0.001	0.001
cnf1	83 (26.6)	63 (31.7)	20 (17.7)	13 (26.5)	0.011	
afa	14 (4.5)	8 (4.0)	6 (5.3)	3 (6.1)		
iutA	185 (59.3)	99 (49.7)	86 (76.1)	45 (91.8)		<0.001

[1)]: All susceptible isolates were susceptible to both quinolone and fluoroquinolones. Q-resistant, resistant only to quinolone (nalidixic acid). FQ-resistant, resistant to both quinolone and fluoroquinolones (ciprofloxacin and levofloxacin).
[2)]: Only *P* values of <0.05 (by Pearson's χ^2 test corrected continuously) are shown.

Table 2. Association between carriage of virulence genes and the resistance in phylogenetic groups B2 and D strains

VF gene	No. (%) of strains of group B2			*P*[2)]		No. (%) of strains of group D			*P*[2)]	
	S (*n* =148)	Q-R[1)] (*n* = 73)	FQ-R[1)] (*n* = 40)	S vs Q	S vs FQ	S (*n* =18)	Q-R[1)] (*n* = 40)	FQ-R[1)] (*n* = 9)	S vs Q	S vs FQ
papA	93 (62.8)	13 (17.8)	2 (5.0)	<0.001	<0.001	2 (11.1)	8 (20.0)	3 (33.3)		
fimH	147 (100)	73 (100)	40 (100)			18 (100)	40 (100)	9 (100)		
sfa	52 (35.1)	16 (21.9)	11 (27.5)			1 (5.6)	6 (15.0)	4 (44.4)		
focG	20 (13.5)	4 (5.5)	1 (2.5)			0 (0)	0 (0)	0 (0)		
hlyA	48 (32.4)	6 (18.2)	0 (0)	<0.001	<0.001	3 (16.7)	0 (0)	2 (22.2)	0.044	
cnf1	62 (41.9)	16 (21.9)	10 (25.0)	0.006		1 (5.6)	4 (10.0)	3 (33.3)		
afa	5 (3.4)	2 (6.1)	0 (0)			3 (16.7)	4 (10.0)	3 (33.3)		
iutA	80 (54.1)	55 (75.3)	36 (90.0)	0.004	0.003	11 (61.1)	31 (77.5)	9 (100)		

[1)]: Q-resistant, resistant only to quinolone (nalidixic acid). FQ-resistant, resistant to both quinolone and fluoroquinolones (ciprofloxacin and levofloxacin).
[2)]: Only *P* values (by Pearson's χ^2 test corrected continuously) <0.05 are shown.
S, susceptible; R, resistant.

Table 3. Distribution of PAI*usp*-subtypes in phylogenetic groups B2 and D strains

PAI*usp* subtype	Phylogenetic group	
	B2 (n = 40)	D (n = 9)
Ia	1	0
Ib	0	1
IIa	37	0
IIb	1	0
N.D.	1	8

N.D., PAI*usp*-negative.

Table 4. Mutation patterns of the FQ-resistant strains by phylogenetic group and PAI*usp* subtype

Phylogenetic group	PAI*usp* subtype	Total no.	Mutation			
			parC		*gyrA*	
			E84V	S80I	S83L	D87N
B2	Ia	1	1	1	1	1
	IIa	37	36	37	37	37
	IIb	1	1	1	1	1
	N.D.	1	1	1	1	1
D	Ib	1	0	1	1	1
	N.D.	8	1	8	8	8

N.D., PAI*usp*-negative.

the FQ-resistant strains is summarized in Table 4. A remarkable feature was that while 36 (97.3%) of the 37 strains of PAI*usp* subtype IIa in group B2 carried E84V mutation in *parC*, only one (11.1%) of the 9 strains of group D carried this mutation. Other mutations, S80I in *parC*, S83L in *gyrA*, and D87N in *gyrA*, were detected in all of the resistant strains. This result is consistent with a previous report by Takahashi et al., who also showed relatively high frequency of the mutation E84V in *parC* among the resistant strains of B2 PAI*usp* subtype IIa (13). These observations suggest that the occurrence of a certain mutation conferring resistance, e.g., E84V of *parC*, is actually associated with a specific genetic background, such as PAI*usp* IIa of group B2.

Our results were very consistent with earlier reports, including one from a Japanese group (13), except for the result regarding the relationship between the resistance and phylogenetic group. These observations suggest that although the carriage of virulence factors and acquisition of the resistance by mutation are actually associated with some specific genetic background of the strain, the relationship is complex. More intensive studies such as comparative genome analyses of strains with different phenotypes would be required to identify the genetic elements involved in the production of mutations that confer genotypic and phenotypic changes in virulence and antibiotic resistance. Our results provide background information for such further studies.

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