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Virulence Factors in Urinary *Escherichia coli* Strains: Phylogenetic Background and Quinolone and Fluoroquinolone Resistance▽

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Quinolone- and fluoroquinolone-resistant *Escherichia coli* strains harbor fewer virulence factors than susceptible strains. The reasons underlying this correlation are incompletely understood. We investigated the phylogenetic background, the presence of the *papC*, *hlyA*, and *cnf1* (pathogenicity island II_{J96}-associated), *fimA*, *iss*, and *iutA* genes, and the presence of type 1 fimbriae, P fimbriae, and hemolysin in 243 urinary *E. coli* isolates resistant only to quinolones (8%), resistant to both quinolones and fluoroquinolones (51%), or susceptible to both drugs (41%). Group B2 accounted for 56% of the isolates, showing a significantly higher prevalence among fluoroquinolone-susceptible strains than among resistant strains (65% versus 50% [$P = 0.03$]). *hly* and *cnf1* were significantly more associated with susceptibility ($P < 0.001$) and with group B2 ($P < 0.001$ for group B2 versus groups A and D). However, within group B2, fluoroquinolone-resistant strains showed lower prevalences of *papC*, *hlyA*, and *cnf1* than their susceptible counterparts ($P < 0.001$). In contrast, the incidence of *iutA* appeared higher for refractory isolates, including group B2, than for susceptible isolates ($P < 0.001$). Only in group B2 did fluoroquinolone-resistant strains reveal a lesser ability to agglutinate *Saccharomyces cerevisiae* (7%) than quinolone-resistant (87%) and susceptible (80%) isolates, despite uniform possession of *fimA* genes. No similar contrast emerged for expression of hemolysin and P fimbriae. Mutations conferring quinolone and fluoroquinolone resistance may thus require a particular genetic background, not strictly correlated with phylogenetic groups. More interestingly, the mutational event itself can affect the expression of type 1 fimbriae, at least in the prevalent and complex B2 strains.

Urinary tract infections (UTIs) in humans are the most frequent bacterial disease, affecting both inpatients and outpatients. Especially the uncomplicated cases are mainly due to extraintestinal pathogenic *Escherichia coli* (ExPEC) (39). In recent years, management of UTIs has become increasingly problematic due to the emergence of resistance to first-line antibiotics among the causative bacteria, particularly among uropathogenic *E. coli* (UPEC) strains. This phenomenon involves quinolones (Q) and fluoroquinolones (FQ) (25), drugs of paramount importance in the treatment of several other infectious diseases. Indeed, the renal excretion of these molecules and the availability of oral and parenteral formulations have allowed them to compete with aminoglycosides and beta-lactams in the therapy of complicated UTIs, especially in the hospital setting. Their appropriate spectrum and good tolerability have also led to increased empirical adoption in uncomplicated infections, although their usage for these conditions in outpatients is still under debate.

In *E. coli*, as in other gram-negative bacteria, DNA gyrase, which codifies type II DNA topoisomerases, is the main target of Q and FQ, and mutation in *gyrA* is the most common way to acquire resistance. In this connection, several investigations have shown that Q- and FQ-resistant UPEC strains display overall reduced virulence and invade compromised patients. By contrast, susceptible *E. coli* strains are virulent and affect uncompromised hosts.

At present it is not clear if this relationship is due to the significant association between susceptible strains and phylogenetic group B2, the most virulent group. In other words, it remains undecided whether drug-refractory *E. coli* strains are intrinsically less virulent bacteria or if they become less virulent following acquisition of the *gyrA* mutation (18).

There is clear evidence that the relationship among virulence properties of *E. coli*, phylogenetic background, and antibiotic resistance is a complex phenomenon, resulting from their different interplays. Therefore, we thought that these possibilities were not mutually exclusive.

It is also possible that the geographic source of isolates represents an important additional element to be taken into consideration (9). The analysis of a collection of UPEC strains from a particular region may therefore be useful in order to correlate the patterns of antibacterial resistance with local trends in the human usage of antibiotics and/or consumption of animal products.

To clarify whether the lack of virulence factors (VFs) is directly associated with resistance or, instead, depends on a phylogenetic distribution, we analyzed VFs, resistance phenotype, and biotypes in a collection of 243 *E. coli* strains. The isolates were from urinary specimens obtained from inpatients and outpatients at our institution during 2005 and 2006. We considered VFs such as *fimA* and *papC* genes, whose products (type 1 fimbriae and P pili) are instrumental in the pathogenesis of UTIs. The presence of *hlyA* and *cnf1*

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TABLE 1. Distribution of phylogenetic groups and VFs among 243 *Escherichia coli* isolates from UTIs according to antimicrobial resistance phenotypes

Phylogenetic group or VF gene	No. (%) of *E. coli* isolates[a]				*P*[b]		
	Total (n = 243)	Susceptible (n = 101)	Q resistant (n = 19)	FQ resistant (n = 123)	Susceptible vs Q resistant	Susceptible vs FQ resistant	Q resistant vs FQ resistant
Phylogenetic groups							
A	46 (19)	16 (16)	3 (16)	27 (22)			
B1	19 (8)	5 (5)	3 (16)	11 (9)			
B2	136 (56)	66 (65)	8 (42)	62 (50)		0.030	
D	42 (17)	14 (14)	5 (26)	23 (19)			
VFs							
fimA	222 (91)	96 (95)	18 (95)	108 (88)			
papC	67 (28)	49 (48)	5 (26)	13 (11)		<0.001	
hlyA	50 (21)	44 (44)	2 (10)	4 (3)	0.009	<0.001	
cnf1	47 (19)	42 (42)	2 (10)	4 (3)	0.010	<0.001	
iss	75 (31)	31 (31)	12 (63)	32 (26)	(0.009)		[0.002]
iutA	143 (59)	32 (32)	14 (74)	97 (79)	(0.001)	(<0.001)	

[a] All susceptible isolates were susceptible to both quinolone and fluoroquinolones. Q resistant, resistant only to quinolone (pipemidic acid). FQ resistant, resistant both to quinolone and to fluoroquinolones (norfloxacin and ciprofloxacin).

[b] Only *P* values of <0.05(by the Fisher exact test) are shown. *P* values without parentheses indicate a positive association of the biotype or VF with susceptible isolates; those in parentheses indicate a negative association of the VF with susceptible isolates; those in brackets indicate a negative association of the VF with fluoroquinolone resistance.

was also investigated because of the physical linkage of these genes with *papC* in pathogenicity island II_{J96} (PAI II_{J96})-like domains, which are prevalent in UTI isolates (41), whereas *iutA* and *iss* were studied because they are known not to be linked to PAIs.

In order to further explore virulence itself, the phenotypic expression of type 1 fimbriae, P fimbriae, and hemolysin was also assessed in this collection.

MATERIALS AND METHODS

Patients and bacterial isolates. *E. coli* isolates were collected from July 2005 to November 2006 from urinary specimens that were either sent to the Clinical Microbiology Laboratory of San Martino Hospital by 16 internal medicine, 4 surgery, and 11 day care wards or collected from outpatients directly referred to our center. A single strain from each patient was analyzed.

Samples were derived either from clean-catch, midstream urine or from urinary catheters. Specimens were cultured (10 μl) on MacConkey agar. Lactose-fermenting, indole-positive colonies were evaluated by the BBL Crystal method (Becton Dickinson) to identify *E. coli*. Bacteria included in the study were from cultures yielding $>10^5$ CFU/ml.

Susceptibilities to pipemidic acid, norfloxacin, and ciprofloxacin were tested by disk diffusion techniques, using CLSI (formerly NCCLS) criteria (34).

Phylotyping, virulence genotyping, and phenotyping. *E. coli* isolates were grouped into phylogenetic biotypes (A, B1, B2, and D) according to the presence of the *chuA* and *yjaA* genes and TspE4.C2 (anonymous DNA fragment) by using multiplex PCR (6). Six ExPEC-associated VFs, *fimA*, *papC*, *cnf1*, *hlyA*, *iutA*, and *iss* (encoding, respectively, type 1 fimbriae, P fimbriae, cytotoxic necrotizing factor-1, hemolysin, the aerobactin iron transport system, and an increased-serum-survival factor), were also sought by using single PCR as described elsewhere (19, 37).

The presence of type 1 fimbriae was determined on the basis of mannose-sensitive agglutination of *Saccharomyces cerevisiae*, while P fimbriae were evidenced by P blood group-dependent hemagglutination, after in vitro passage to enhance their expression (1, 26). The production of hemolysin was evaluated visually by the appearance of a clear halo on 5% sheep blood agar plates after overnight culture at 37°C.

Statistical analysis. Statistical analysis was performed by using the Fisher exact and chi-square tests. The threshold for statistical significance was a *P* value of <0.05.

RESULTS

Characteristics of patients. A total of 243 UPEC isolates were analyzed. Of these, 191 derived from midstream urine and 52 were from urinary catheters; 150 patients were in the internal medicine ward (32% with urinary catheters) and 10 were in the surgery ward, while 83 specimens came from outpatients (43 in day care wards and 40 in our outpatient department). There were 55 males (23%) and 188 females (77%) (data not shown).

Q and FQ resistance and bacterial characteristics. Of 243 *E. coli* isolates, 19 (8%) were resistant only to the Q (pipemidic acid) while 123 (51%) were also resistant to FQ (norfloxacin and ciprofloxacin), while 101 (41%) were susceptible to both classes of drugs.

The incidences of global resistance to Q and FQ according to the different sources of specimens were 52% (no catheter) and 83% (urinary catheter); incidences according to the type of patient were 64% for inpatients (66% of those in the internal medicine ward and 42% of those recovering from surgery) and 46% for outpatients (43% of day care patients and 49% of the patients referred directly to our laboratory) (data not shown).

When the incidence of Q and FQ resistance was correlated with bacterial characteristics, i.e., phylogenetic groups and VFs, the following results were obtained (Table 1). A significant prevalence of phylogenetic group B2 (56%) was apparent with respect to the other biotypes (A, B1, and D) (Table 1). Despite the fact that B2 represented the most frequent group among the susceptible as well as the resistant bacteria, the prevalence of B2 strains was significantly higher among susceptible strains than among FQ-resistant strains (65% versus 50%, respectively; $P = 0.03$). Among susceptible strains, in fact, the frequencies of groups A, B1, and D were, respectively, 16%, 5%, and 14%, while among FQ-resistant strains these frequencies were 22%, 9%, and 19% (Table 1).

Susceptible isolates exhibited significant differences from

TABLE 2. Distribution of VFs among 243 *Escherichia coli* isolates from UTIs according to phylogenetic groups

VF	No. (%) of *E. coli* isolates					*P*[a]				
	Total (n = 243)	Group A (n = 46)	Group B1 (n = 19)	Group B2 (n = 136)	Group D (n = 42)	A vs B2	A vs D	B1 vsB2	B1 vs D	B2 vs D
fimA	222 (91)	38 (83)	19 (100)	130 (95)	35 (83)	0.008				0.014
papC	67 (28)	4 (9)	1 (5)	51 (37)	11 (26)	<0.001	(0.045)	0.004		
hlyA	50 (21)	2 (4)	1 (5)	46 (34)	1 (2)	<0.001		0.014		<0.001
cnf1	47 (19)	0 (0)	1 (5)	45 (33)	2 (5)	<0.001		0.014		<0.001
iss	75 (31)	19 (41)	10 (53)	36 (26)	10 (24)			[0.030]	[0.039]	
iutA	143 (59)	26 (56)	7 (37)	82 (60)	28 (67)				(0.049)	

[a] Only *P* values of <0.05 (by the Fisher exact test) are shown. *P* values without parentheses indicate a positive association of the VF with biotype B2; parentheses indicate a positive association of the VF with biotype D; brackets indicate a positive association of the VF with biotype B1.

both Q- and FQ-resistant isolates with regard to the distribution of *papC*, *hlyA*, and *cnf1*. The most significant differences emerged between susceptible and FQ-resistant isolates ($P < 0.001$): 48%, 44%, and 42% of the susceptible strains possessed *papC*, *hlyA*, and *cnf1*, respectively, whereas 11% of the FQ-resistant strains harbored *papC* and 3% possessed *hlyA* and *cnf1*. On the other hand, susceptible *E. coli* strains exhibited a significantly lower incidence of *iutA* (32%) than Q (74%)- and FQ (79%)-refractory strains ($P = 0.001$ for both comparisons). Q-resistant isolates exhibited a significant association with *iss* in comparison with susceptible isolates ($P = 0.009$) and particularly in comparison with FQ-resistant strains ($P = 0.002$). The presence of *fimA* did not differ according to the antimicrobial susceptibility phenotype (Table 1).

The Q- and FQ-resistant isolates from urinary catheters did not have lower incidences of VFs than resistant *E. coli* isolates obtained from midstream urine (data not shown).

Phylogenetic distribution of VFs. For all VFs considered, a very significant phylogenetic distribution emerged (Table 2). Despite the fact that *fimA* was detected at least in 83% of the isolates (groups A and D), some significant differences among the biotypes were observed with regard to the presence of *fimA*, which exhibited an association with group B2 ($P = 0.008$ for B2 versus A; $P = 0.014$ for B2 versus D). However, we found the highest differences among the biotypes with regard to the presence of *hly* and *cnf1*. In fact, *hly* and *cnf1* were associated with phylogenetic group B2; about one-third of B2 isolates possessed both traits (34% and 33%, respectively). *hly* and *cnf1* were nearly absent in the remaining groups, especially groups A and D ($P < 0.001$ for B2 versus A and for B2 versus D). In contrast, the presence of *papC* in group D was similar to that in group B2 (37% of group B2 versus 26% of group D isolates; *P*, not significant), while it was much lower in groups A and B1 ($P < 0.001$ for B2 versus A) (Table 2). A significant association was observed between the presence of *iss* and biotype B1 ($P < 0.05$ for B1 versus B2 and for B1 versus D). A similar association was observed between the presence of *iutA* and biotype D ($P < 0.05$ for B1 versus D) (Table 2).

Expression of VFs in relation to phylogenetic background. In order to assess the capability of *E. coli* isolates for phenotypic expression according to biotype, we evaluated the presence of type 1 fimbriae, P fimbriae, and hemolysin through mannose-sensitive agglutination, mannose-independent hemagglutination, and detection of hemolysis (Table 3). No isolates without *fimA*, *papC*, and *hlyA* genes showed the expected phenotype (data not shown). The number of isolates with phenotypic expression of type 1 fimbriae, P fimbriae, or hemolysin was calculated as a percentage of the number of isolates carrying the corresponding gene *fimA*, *papC*, or *hlyA*, respectively. The results showed that *fimA* was present in most isolates and type 1 fimbriae were expressed by the different groups with no significant difference. There was no difference in hemolysin expression among the four biotypes, though *hlyA* was associated only with biotype B2 (Table 2). The expression of P fimbriae in biotypes B2 and D (29% and 18%, respectively) did not permit a statistical correlation because of the small numbers in group D.

Virulence genotype in relation to antimicrobial resistance. The results from Tables 1 and 2 highlight a preliminary connection among susceptibility, VFs linked to PAI II_{J96}, and phylogenetic group B2, which, however, does not explain why 50% of the resistant isolates belong to B2 but only 3% have *hlyA* and *cnf1*. Therefore, to assess whether the absence of these virulence genes is also specifically related to drug resistance, we evaluated the frequency of each VF considered (*fimA*, *papC*, *hlyA*, *cnf1*, *iss*, and *iutA*) in susceptible or resistant (to Q and/or FQ, globally considered) *E. coli* isolates within each phylogenetic group (A, B1, B2, and D) (Table 4). The results indicate that within each biotype, as in the total strains

TABLE 3. Frequency of VF expression in relation to phylogenetic groups among *Escherichia coli* isolates from UTIs carrying *fimA*, *papC*, and *hlyA*

Phenotype or gene	No. of *E. coli* isolates[a]				
	Total	Group A	Group B1	Group B2	Group D
Phenotypic expression					
Type 1 fimbriae	115 (52)	18 (47)	12 (63)	63 (48)	22 (63)
P fimbriae	18 (27)	1 (25)	0 (0)	15 (29)	2 (18)
Hemolysin	47 (94)	2 (100)	1 (100)	43 (93)	1 (100)
Gene presence					
fimA	222	38	19	130	35
papC	67	4	1	51	11
hlyA	50	2	1	46	1

[a] Numbers in parentheses are percentages of strains carrying the single VF gene *fimA*, *papC*, or *hlyA* that express type 1 fimbriae, P fimbriae, or hemolysin, respectively. The presence of type 1 fimbriae, P fimbriae, and hemolysin was evaluated according to the occurrence of mannose-sensitive agglutination of *Saccharomyces cerevisiae*, hemagglutination, and hemolysis. No significant *P* value (<0.05) emerged from the comparisons of the four *E. coli* groups in relation to the expression of any VF.

TABLE 4. Distribution of VFs within each phylogenetic group according to antimicrobial resistance phenotype among 243 *Escherichia coli* isolates from UTIs

VF	No. (%)[a] of group A isolates (n = 46)		P^b	No. (%) of group B1 isolates (n = 19)		No. (%) of group B2 isolates (n = 136)		P	No. (%) of group D isolates (n = 42)	
	S (n = 16)	R (n = 30)		S (n = 5)	R (n = 14)	S (n = 66)	R (n = 70)		S (n = 14)	R (n = 28)
fimA	14 (87)	24 (80)		5 (100)	14 (100)	65 (98)	65 (93)		12 (86)	23 (82)
papC	0 (0)	4 (13)		0 (0)	1 (7)	45 (68)	6 (9)	<0.001	4 (29)	7 (25)
hlyA	2 (12.5)	0 (0)		0 (0)	1 (7)	41 (62)	5 (7)	<0.001	1 (7)	0 (0)
cnf1	0 (0)	0 (0)		0 (0)	1 (7)	40 (61)	5 (7)	<0.001	2 (14)	0 (0)
iss	5 (31)	14 (47)		1 (20)	9 (64)	22 (33)	14 (20)		3 (21)	7 (25)
iutA	4 (25)	22 (73)	(0.004)	0 (0)	7 (50)	22 (33)	60 (86)	(<0.001)	6 (43)	22 (79)

[a] Percentage of susceptible or resistant isolates with the indicated VF. S, susceptible to both Q and FQ; R, resistant. In each phylogenetic group (A, B1, B2, or D), resistant *E. coli* isolates include Q-resistant and FQ-resistant strains.

[b] P values (by the Fisher exact test) are for comparisons of isolates susceptible and resistant to the drugs within in a single biotype. Only P values of <0.05 are shown. Parentheses indicate a negative association of the VF with susceptible isolates. No comparison involving groups B1 and D yielded a P value of <0.05.

(Table 1), the prevalence of the *fimA* gene was identical in susceptible and resistant *E. coli* isolates. *papC*, associated with group D as well as with B2 (Table 2), showed the same prevalence in susceptible (29%) and resistant (25%) group D isolates. *hlyA* and *cnf1* were nearly absent in biotypes A, B1, and D (Table 2), independently of the phenomenon of resistance. Thus, besides the *papC* distribution in group D, it was possible to compare the frequencies of *papC*, *hlyA*, and *cnf1* according to the resistance phenotype only within group B2. In susceptible *E. coli* B2 strains, the incidences of *papC*, *hlyA*, and *cnf1* were, respectively, 68%, 62%, and 61%, whereas in resistant B2 isolates, the incidence was 9% for *papC* and 7% for *hlyA* and *cnf1* ($P < 0.001$).

Our results also indicate that in each phylogenetic group, the *iss* trait showed no significant differences between susceptible and resistant isolates, whereas the *iutA* trait, in groups A and B2, showed a significant shift toward the phenotype of resistance (in biotype A, $P = 0.004$; in biotype B2, $P < 0.001$) (Table 4).

Virulence phenotype in relation to antimicrobial resistance. To evaluate whether the capability for phenotypic expression in *E. coli* isolates, which has been shown not to be related to biotypes (Table 3), is instead related to resistance, we analyzed the presence of type 1 fimbriae, P fimbriae, and hemolysin in susceptible and resistant (to Q and/or FQ, globally considered) strains within groups A, B1, B2, and D. The number of isolates with phenotypic expression of type 1 fimbriae, P fimbriae, or hemolysin was calculated as a percentage of the number of isolates carrying the corresponding gene *fimA*, *papC*, or *hlyA*, respectively (Table 5). Only in group B2 did the expression of type 1 fimbriae greatly decrease, from 80% in susceptible strains to 17% in resistant strains ($P < 0.001$).

In groups A, B1, and D, the low level of expression of type 1 fimbriae seems not to be dependent on the phenomenon of resistance (P values for comparisons of susceptible and resistant isolates are not significant) (Table 5).

From these results, in the biotypes carrying the *papC* gene (biotypes B2 and D), it emerged that the low global expression of P fimbriae, 27% (Table 3), does not depend on the resistance phenomenon; in fact, the P values for comparisons of susceptible and resistant isolates are not significant.

It was impossible to evaluate the different levels of expression of *hlyA* between susceptible and refractory *E. coli* strains in either group B2 or non-B2 groups because of the very low

TABLE 5. Association of VF expression with antimicrobial resistance phenotypes within each phylogenetic group among 243 *Escherichia coli* isolates from UTIs carrying *fimA*, *papC*, or *hlyA*

Phenotype or gene	No. of *E. coli* isolates[a]							
	Group A		Group B1		Group B2		Group D	
	S	R	S	R	S	R	S	R
Phenotypic expression								
Type 1 fimbriae	7 (50)	11 (46)	5 (100)	7 (50)	52 (80)	11 (17)[b]	8 (67)	14 (61)
P fimbriae	0	1 (25)	0	0	14 (31)	1 (17)	1 (25)	1 (14)
Hemolysin	2 (100)	0	0	1 (100)	39 (95)	4 (80)	1 (100)	0
Gene presence								
fimA	14	24	5	14	65	65	12	23
papC	0	4	0	1	45	6	4	7
hlyA	2	0	0	1	41	5	1	0

[a] Numbers in parentheses are percentages of strains carrying the single VF gene *fimA*, *papC*, or *hlyA* that express type 1 fimbriae, P fimbriae, or hemolysin, respectively. The presence of type 1 fimbriae, P fimbriae, and hemolysin was evaluated according to the occurrence of mannose-sensitive agglutination of *Saccharomyces cerevisiae*, hemagglutination, and hemolysis. S, susceptible to both Q and FQ; R, resistant. In each phylogenetic group (A, B1, B2, or D), resistant isolates include Q-resistant and FQ-resistant strains.

[b] The P value (by Fisher's exact test) for comparison of expression of type 1 fimbriae by susceptible and resistant isolates within group B2 is <0.001, indicating a positive association of VF expression with susceptible isolates. No other comparison yielded a P value of <0.05.

TABLE 6. Association of VF expression with resistance to quinolone and fluoroquinolones among *Escherichia coli* isolates from UTIs belonging to phylogenetic group B2 and carrying *fimA*, *papC*, and *hlyA*

Phenotype or gene	No. of *E. coli* isolates[a]	
	Q resistant	FQ resistant
Phenotypic expression		
Type 1 fimbriae	7 (87)	4 (7)[b]
P fimbriae	0	1 (33)
Hemolysin	2 (100)	2 (66)
Gene presence		
fimA	8	57
papC	3	3
hlyA	2	3

[a] Numbers in parentheses are percentages of strains carrying the single VF gene *fimA*, *papC*, or *hlyA* that express type 1 fimbriae, P fimbriae, or hemolysin, respectively. The presence of type 1 fimbriae, P fimbriae, and hemolysin was evaluated according to the occurrence of mannose-sensitive agglutination of *Saccharomyces cerevisiae*, hemagglutination, and hemolysis.

[b] The *P* value (by Fisher's exact test) for comparison of expression of type 1 fimbriae by Q-resistant and FQ-resistant isolates is <0.001, indicating a positive association of VF expression with isolates resistant to Q. No other comparison yielded a *P* value of <0.05.

TABLE 7. Association of VF genes with resistance to quinolone and fluoroquinolones among *Escherichia coli* isolates from UTIs within phylogenetic group B2

VF	No. (%) of *E. coli* isolates		
	Q resistant (n = 8)	FQ resistant (n = 62)	P[a]
fimA	8 (100)	57 (92)	
papC	3 (37)	3 (5)	0.017
hlyA	2 (25)	3 (5)	
cnf1	2 (25)	3 (5)	
iss	5 (62)	9 (14)	0.006
iutA	5 (62)	55 (89)	

[a] Only *P* values (by Fisher's exact test) of <0.05 are shown. *P* values for comparison of Q-resistant and FQ-resistant isolates indicate positive associations of VFs with isolates resistant to Q.

incidence of the gene in groups A, B1, and D and in resistant isolates, including those of biotype B2.

Virulence phenotype and genotype of group B2 in relation to classes of resistance. Since among the resistant (globally considered) *E. coli* strains in group B2, 11 isolates (17%) still agglutinate *S. cerevisiae* (Table 5), we investigated the incidences of type 1 fimbriae, P fimbriae, and hemolysin in biotype B2 in order to determine whether diversity between the two different classes of resistance, resistance to Q and resistance to FQ, might provide a possible explanation (Table 6).

The additional results showed that of the 11 group B2 resistant strains showing mannose-sensitive agglutination, 7 strains were resistant to pipemidic acid alone and only 4 were also resistant to FQ, thus modifying the percentage of resistant *E. coli* B2 strains retaining the expression of type 1 fimbriae (7% of FQ-resistant strains). The percentage of *S. cerevisiae* agglutination among the 7 B2 strains resistant only to Q (87%) was similar to that detected for the susceptible strains (80%) (Table 5).

Despite the small sample size of the resulting Q resistance subgroup, which makes the statistical significance uncertain, an additional trend with an interesting biological significance emerged from the results. An important difference in the expression of type 1 fimbriae appears to exist, in fact, between the two classes of resistance (87% for Q resistance versus 7% for FQ resistance; $P < 0.001$). In contrast, no differences in the detection of hemagglutination or hemolysis emerged between the classes of resistance.

The results relating to phenotypic expression of type 1 fimbriae led us to investigate more closely the presence of VF genes in Q-resistant versus FQ-resistant *E. coli* isolates within biotype B2 (Table 7). The Q-resistant and FQ-resistant strains differed with regard to the presence of the *iss* and *papC* genes, which were more prevalent among the former (62% of Q-resistant versus 14% of FQ-resistant strains had *iss*; $P = 0.006$). Although a statistical limitation due to the small sample size still exists, these data confirmed the percentages of *iss* presence in Q-resistant versus FQ-resistant strains ($P = 0.002$) that were obtained without separating the phylogenetic backgrounds (Table 1).

DISCUSSION

Our findings confirmed the complexity of the association between antibacterial resistance and the properties of virulence in *Escherichia coli*.

Previous investigations clearly demonstrated that resistance of *E. coli* strains to several antimicrobial agents, e.g., ampicillin, aminoglycosides, and co-trimoxazole, does not significantly correlate with the presence of fewer VFs than in their susceptible counterparts (16, 23, 31). We therefore focused only on the characteristics of Q- and FQ-resistant UPEC strains and examined whether our results mimic or differ from those obtained in other regional settings.

We evaluated, in UPEC strains, the two different opinions currently under debate: the first assuming that the low frequency of certain VFs precedes resistance (22, 23, 33) and the second assuming that, vice versa, the low frequency of these VFs follows the acquisition of resistance (8, 16, 44). We explored a possible coexistence of both mechanisms.

As previously found in other studies (21), our results show that group B2 is globally the most frequent *E. coli* biotype in UTIs. The same prevalence does not hold true among Q- and FQ-refractory strains in studies that also consider different extraintestinal sources (17, 22).

A greater prevalence of biotype B2 was detected in susceptible than in FQ-resistant *E. coli* strains, and the association of certain virulence determinants with group B2 and with susceptible isolates was confirmed as well (22, 23, 33).

We also confirmed previous knowledge about the genes mostly present in all isolates (*fimA*), those nearly absent both in non-B2 isolates and in resistant isolates (*hlyA* and *cnf1*, which show a physical linkage in PAI II_{J96}) (4, 38, 41), and the intermediate percentages of *papC* presence (8, 16, 22, 23, 33, 44).

We might therefore agree with the idea that the higher incidence of VFs among susceptible *E. coli* isolates would depend on their phylogenetic distribution and that VFs would be intrinsic bacterial characteristics. Nevertheless, this relationship does not clarify the fact, previously found (33), that

despite the predominance of group B2 (50%), FQ-resistant UPEC strains did not harbor VFs such as *hlyA* (3%), *cnf1* (3%), and *papC* (11%).

This discrepancy was explained by the finding of lower incidences of *papC*, *hlyA*, and *cnf1* among refractory group B2 strains than among susceptible B2 strains, which seems to be due to a loss of the corresponding PAI, probably as a result of the mutation that causes resistance, as already assessed through the same approach (16). However, among our clinical urinary isolates, the high presence of susceptible *E. coli* strains lacking *papC* (52%), *hlyA* (56%), and *cnf1* (58%) might imply that the "lack" of PAI II_{J96} does not require the mutation leading to resistance. Experimental approaches suggested that even the "loss" of PAI, obtained in the presence of ciprofloxacin, does not require the mutation in the codon for Ser-83 of the *gyrA* gene in *E. coli* HC14366 and HC109 (40). Furthermore, in the clinical *E. coli* isolates that were rendered FQ resistant through selection in the presence of ciprofloxacin, no loss of PAIs occurred (20, 31). Moreover, *E. coli* 536 shows a loss of PAIs that appears without *gyra* mutation and without any antibiotics in the medium (32), indicating that their instability could be exacerbated by several stimuli (32), of which the presence of quinolone is just one (29).

Thus, we speculated that in UPEC, the lack of PAIs does not arise from the *gyrA* mutation but, instead, both are consequences of chromosomal characteristics. Although the instability and the loss of PAI_{536} and PAI II_{J96} have been demonstrated to be peculiar to single PAIs themselves (15, 41), we thought that these phenomena also required a specific genetic background. In *E. coli*, in fact, the occurrence of multiple insertional events (4) and the acquisition of VFs (10) have been shown to be restricted by a specific genetic structure.

Our results, moreover, showed that Q- and FQ-resistant isolates not harboring *papC*, *hlyA*, and *cnf1* are positively associated with the *iutA* gene, which is not located on PAIs. Since a similar relationship also emerged for *iss* and, through the investigations cited above, for other genes harbored outside the PAIs, such as *bmaE*, *gafD*, *ireA*, and *cvaC*, the hypothesis of a direct linkage between *iutA* and a resistance gene, occurring on the same plasmid, was discarded. Furthermore, in *Escherichia coli*, a plasmid-mediated aerobactin system and plasmid-mediated resistance to ciprofloxacin cannot have a considerable effect at the population level because of their rarity (42, 45). Thus, we believed *iutA* to be a chromosomal gene. The prevalence of *iutA* among clinical refractory isolates, even those in group B2, and the prevalence of *iss* among Q-resistant isolates, as well as the nearly total absence of genes linked to PAI II_{J96}, led us to believe that mutations in *gyrA* strictly require specific genetic characteristics. These are the genetic background of groups A, B1, and D and of a particular subgroup, or pathotype (5), of B2 resembling non-B2 isolates. From our results it emerged, in fact, that biotype B2 shows characteristics of flexibility, previously described as a bacterial mosaic-like genetic tool (12). In contrast, both susceptible and resistant isolates in non-B2 groups are more homogeneous, since they typically do not harbor PAI genes, *hlyA*, and *cnf1*. Since *papC* possesses a less consistent colocalization on PAIs (4), this VF shows an intermediate percentage of presence in group D isolates and in refractory isolates. We thus believe that in *E. coli* the association between Q and FQ resistance and the paucity of PAI-related genes does not depend on a phylogenetic distribution but instead is direct. A specific chromosomal background, only partially corresponding to the phylogenetic background, could therefore precede *gyrA* mutation.

We also demonstrated, for the resistant clinical *E. coli* isolates, decreased agglutination of *S. cerevisiae*, already previously described (2, 44) and explained through *gyrA* mutation affecting transcriptional events (3, 27, 28). Since we assessed the mannose-sensitive agglutination within each biotype, we found this phenomenon to be exclusive of group B2, and we could thus explain the more significant results with respect to those obtained without phylogenetic distinction in the previous works.

The hypothesis that lower expression of type 1 fimbriae would be an intrinsic characteristic of a group of strains was rejected. The different groups, in fact, showed the same capacity for phenotypic expression, and in particular, resistant *E. coli* non-B2 isolates, not carrying *hlyA* and *cnf1*, showed functional type 1 fimbriae. It thus emerged that low phenotypic expression of type 1 fimbriae depends neither on the genetic nor on the phylogenetic background but strictly on the phylogenetic background of B2 plus resistance. Among resistant *E. coli* strains, it was almost exclusively non-B2 groups that expressed type 1 fimbriae, except for B2 "quinolone-only"' resistant isolates, which carry a single mutation, conferring a low-level resistance that allows them to retain susceptibility to FQ (11, 43) and fimbrial phenotypic expression. This result would explain the relatively high number (11 strains; 17%) of resistant isolates still expressing type 1 fimbriae and would confirm that in UPEC strains, low expression of certain VFs follows acquisition of a certain type of resistance.

A common finding emerging from several aspects of our study is the complexity of lifestyle (of being and behaving) of *E. coli* isolates belonging to the B2 phylogenetic group. B2 appears to be the only biotype showing a strict relationship between resistance and low virulence, resulting from two different mechanisms that act simultaneously. This characteristic of group B2 can determine the relationship globally found in UPEC strains, because it concerns the most frequent biotype. We also supposed that the prevalence of group B2 among both uropathogenic and commensal *E. coli* isolates (21, 46), found recently to be independent of VF carriage (35), would be due to a superior adaptability, perhaps corresponding to the previously named "plasticity" of the species (5, 7, 15). In contrast, in sites other than the bladder and the gut, the presence of B2 strains, corresponding to disease (21) and to experimental lethality (24), depends on a high level of shared virulence factors.

Our results showing the high incidence of ciprofloxacin-resistant urinary *E. coli* isolates among outpatients and, especially, the same finding in the fecal microflora of children (36) (supposed to be naïve hosts with regard to FQ contact) make the natural mechanisms of resistance not very intelligible. However, this aspect led us to believe that, for B2 isolates, the advantage arising from an assumed instability is not, or not only, resistance itself. In fact, the reduced expression of adhesins, such as type 1 fimbriae, can be not a mere loss to pay out but, instead, a gain for the bacterial fine-tuning in the environment. Given the evolutionary success of *E. coli* B2 strains, the exclusive lack of type 1 fimbriae among the resistant strains could be regarded as a phase variation-like phenomenon (30), as can the low expression of P fimbriae among

susceptible isolates. The lack of fimbrial antigens, like the absence of VF traits linked to PAI II_{J96}, is irreversible but would be adapted to an appropriate context in which bacteria, without particular damage and through avoidance of host defenses, achieve new niches where they colonize or cause chronic infections (13, 14, 15), spreading possible resistance.

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REFERENCES

1. **Arthur, M., C. E. Johnson, R. H. Rubin, R. D. Arbeit, C. Campanelli, C. Kim, S. Steinbach, M. Agarwal, R. Wilkinson, and R. Goldstein.** 1989. Molecular epidemiology of adhesion and hemolysin virulence factors among uropathogenic *Escherichia coli*. Infect. Immun. **57:**303–313.
2. **Bagel, S., P. Heisig, and P. Wiedemann.** 1997. Fluoroquinolone resistance of *Escherichia coli* frequently is associated with decreased expression of type 1 fimbriae, abstr. C-37, p. 52. Prog. Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., Toronto, Ontario, Canada. American Society for Microbiology, Washington, DC.
3. **Bagel, S., V. Hullen, B. Wiedemann, and P. Heisig.** 1999. Impact of *gyrA* and *parC* mutations on quinolone resistance, doubling time, and supercoiling degree of *Escherichia coli*. Antimicrob. Agents Chemother. **43:**868–875.
4. **Bidet, P., S. Bonacorsi, O. Clermont, C. De Montille, N. Brahimi, and E. Bingen.** 2005. Multiple insertional events, restricted by the genetic background, have led to acquisition of pathogenicity island II_{J96}-like domains among *Escherichia coli* strains of different clinical origins. Infect. Immun. **73:**4081–4087.
5. **Bielaszewska, M., U. Dobrindt, J. Gartner, I. Gallitz, J. Hacker, H. Karch, D. Muller, S. Schubert, M. Alexander Schmidt, L. J. Sorsa, and J. Zdziarski.** 25 April 2007. Aspects of genome plasticity in pathogenic *Escherichia coli.* Int. J. Med. Microbiol. doi:10.1016/j.ijmm.2007.03.001.
6. **Clermont, O., S. Bonacorsi, and E. Bingen.** 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl. Environ. Microbiol. **66:**4555–4558.
7. **Dobrindt, U.** 2005. (Patho-)Genomics of *Escherichia coli*. Int. J. Med. Microbiol. **295:**357–371.
8. **Drews, S. J., S. M. Poutanen, T. Mazzulli, A. J. McGeer, A. Sarabia, S. Pong-Porter, Y. Rzayev, B. Willey, K. Green, and D. E. Low.** 2005. Decreased prevalence of virulence factors among ciprofloxacin-resistant uropathogenic *Escherichia coli* isolates. J. Clin. Microbiol. **43:**4218–4220.
9. **Duriez, P., O. Clermont, S. Bonacorsi, E. Bingen, A. Chaventre, J. Elion, B. Picard, and E. Denamur.** 2001. Commensal *Escherichia coli* isolates are phylogenetically distributed among geographically distinct human populations. Microbiology **147:**1671–1676.
10. **Escobar-Páramo, P., O. Clermont, A. B. Blanc-Potard, H. Bui, C. Le Bouguenec, and E. A. Denamur.** 2004. A specific genetic background is required for acquisition and expression of virulence factors in *Escherichia coli*. Mol. Biol. Evol. **21:**1085–1094.
11. **Everett, M. J., Y. F. Jin, V. Ricci, and L. J. V. Piddock.** 1996. Contributions of individual mechanisms to fluoroquinolone resistance in 36 *Escherichia coli* strains isolated from humans and animals. Antimicrob. Agents Chemother. **40:**2380–2386.
12. **Gal-Mor, O., and B. B. Finlay.** 2006. Pathogenicity islands: a molecular toolbox for bacterial virulence. Cell. Microbiol. **8:**1707–1719.
13. **Hacker, J., and J. B. Kaper.** 2000. Pathogenicity islands and the evolution of microbes. Annu. Rev. Microbiol. **54:**641–679.
14. **Hacker, J., U. Hentschel, and U. Dobrindt.** 2003. Prokaryotic chromosomes and disease. Science **301:**790–793.
15. **Hochhut, B., C. Wilde, G. Balling, B. Middendorf, U. Dobrindt, E. Brzuszkiewicz, G. Gottschalk, E. Carniel, and J. Hacker.** 2006. Role of pathogenicity island-associated integrases in the genome plasticity of uropathogenic *Escherichia coli* strain 536. Mol. Microbiol. **61:**584–595.
16. **Horcajada, J. P., S. Soto, A. Gajewski, A. Smithson, M. T. Jiménez de Anta, J. Mensa, J. Vila, and J. R. Johnson.** 2005. Quinolone-resistant uropathogenic *Escherichia coli* strains from phylogenetic group B2 have fewer virulence factors than their susceptible counterparts. J. Clin. Microbiol. **43:**2962–2964.
17. **Houdouin, V., S. Bonacorsi, P. Bidet, M. Bingen-Bidois, D. Barraud, and E. Bingen.** 2006. Phylogenetic background and carriage of pathogenicity island-like domains in relation to antibiotic resistance profiles among *Escherichia coli* urosepsis isolates. J. Antimicrob. Chemother. **58:**748–751.
18. **Johnson, J. R.** 2005. Virulence factors in *Escherichia coli*. J. Clin. Microbiol. **43:**6221–6222.
19. **Johnson, J. R., and A. L. Stell.** 2000. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J. Infect. Dis. **181:**261–272.
20. **Johnson, J. R., B. Johnston, M. A. Kuskowski, R. Colodner, and R. Raz.** 2005. Spontaneous conversion to quinolone and fluoroquinolone resistance among wild-type *Escherichia coli* isolates in relation to phylogenetic background and virulence genotype. Antimicrob. Agents Chemother. **49:**4739–4744.
21. **Johnson, J. R., K. Owens, A. Gajewski, and M. A. Kuskowski.** 2005. Bacterial characteristics in relation to clinical source of *Escherichia coli* isolates from women with acute cystitis or pyelonephritis and uninfected women. J. Clin. Microbiol. **43:**6064–6072.
22. **Johnson, J. R., M. A. Kuskowski, A. Gajewski, D. F. Sahm, and J. A. Karlowsky.** 2004. Virulence characteristics and phylogenetic background of multidrug-resistant and antimicrobial-susceptible clinical isolates of *Escherichia coli* from across the United States, 2000–2001. J. Infect. Dis. **190:**1739–1744.
23. **Johnson, J. R., M. A. Kuskowski, T. T. O'Bryan, R. Colodner, and R. Raz.** 2005. Virulence genotype and phylogenetic origin in relation to antibiotic resistance profile among *Escherichia coli* urine sample isolates from Israeli women with acute uncomplicated cystitis. Antimicrob. Agents Chemother. **49:**26–31.
24. **Johnson, J. R., O. Clermont, M. Menard, M. A. Kuskowski, B. Picard, and E. Denamur.** 2006. Experimental mouse lethality of *Escherichia coli* isolates, in relation to accessory traits, phylogenetic group, and ecological source. J. Infect. Dis. **194:**1141–1150.
25. **Karaca, Y., N. Coplu, A. Gozalan, O. Oncul, B. E. Citil, and B. Esen.** 2005. Co-trimoxazole and quinolone resistance in *Escherichia coli* isolated from urinary tract infections over the last 10 years. Int. J. Antimicrob. Agents **26:**75–77.
26. **Korhonen, T. K.** 1979. Yeast cell agglutination by purified enterobacterial pili. FEMS Microbiol. Lett. **6:**421–427.
27. **Kugelberg, E., S. Lofmark, B. Wretlind, and D. I. Andersson.** 2005. Reduction of the fitness burden of quinolone resistance in *Pseudomonas aeruginosa*. J. Antimicrob. Chemother. **55:**22–30.
28. **Leng, F., L. Amado, and R. McMacken.** 2004. Coupling DNA supercoiling to transcription in defined protein systems. J. Biol. Chem. **279:**47564–47571.
29. **López, E., M. Elez, I. Matic, and J. Blazquez.** 2007. Antibiotic-mediated recombination: ciprofloxacin stimulates SOS-independent recombination of divergent sequences in *Escherichia coli*. Mol. Microbiol. **64:**83–93.
30. **Manson, J. M., and M. S. Gilmore.** 2006. Pathogenicity islands integrase cross-talk: a potential new tool for virulence modulation. Mol. Microbiol. **61:**555–559.
31. **Martínez-Martínez, L., F. Fernandez, and E. J. Perea.** 1999. Relationship between haemolysis production and resistance to fluoroquinolones among clinical isolates of *Escherichia coli*. J. Antimicrob. Chemother. **43:**277–279.
32. **Middendorf, B., B. Hochhut, K. Leipold, U. Dobrindt, G. Blum-Oehler, and J. Hacker.** 2004. Instability of pathogenicity islands in uropathogenic *Escherichia coli* 536. J. Bacteriol. **186:**3086–3096.
33. **Moreno, E., G. Prats, M. Sabaté, T. Pérez, J. R. Johnson, and A. Andreu.** 2006. Quinolone, fluoroquinolone and trimethoprim/sulfamethoxazole resistance in relation to virulence determinants and phylogenetic background among uropathogenic *Escherichia coli*. J. Antimicrob. Chemother. **57:**204–211.
34. **NCCLS.** 2004. Performance standards for antimicrobial susceptibility testing: 14th informal supplement. Approved standard M100–S14. NCCLS, Wayne, PA.
35. **Nowrouzian, F. L., A. E. Wold, and I. Adlerberth.** 2005. *Escherichia coli* strains belonging to phylogenetic group B2 have a superior capacity to persist in the intestinal microflora of infants. J. Infect. Dis. **191:**1078–1083.
36. **Qin, X., Y. Razia, J. R. Johnson, J. R. Stapp, D. R. Boster, T. Tsosie, D. L. Smith, C. R. Braden, K. Gay, F. J. Angulo, and P. I. Tarr.** 2006. Ciprofloxacin-resistant gram-negative bacilli in the fecal microflora of children. Antimicrob. Agents Chemother. **50:**3325–3329.
37. **Rodriguez-Siek, K. E., C. W. Giddings, C. Doetkott, T. Johnson, M. K. Fakhr, and L. K. Nolan.** 2005. Comparison of *Escherichia coli* isolates implicated in human urinary tract infection and avian colibacillosis. Microbiology **151:**2097–2110.
38. **Sabaté, M., E. Moreno, T. Perez, A. Andreu, and G. Prats.** 2006. Pathogenicity island markers in commensal and uropathogenic *Escherichia coli* isolates. Clin. Microbiol. Infect. **12:**880–886.
39. **Sobel, J. D., and K. Donald.** 2005. Urinary tract infection, p. 875–883. *In* G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), Principles and practice of infectious diseases, 6th ed. Elsevier Inc., Philadelphia, PA.
40. **Soto, S. M., M. T. Jimenez de Anta, and J. Vila.** 2006. Quinolones induce partial or total loss of pathogenicity islands in uropathogenic *Escherichia coli* by SOS-dependent or -independent pathways, respectively. Antimicrob. Agents Chemother. **50:**649–653.
41. **Swenson, D. L., N. O. Bukanov, D. E. Berg, and R. A. Welch.** 1996. Two pathogenicity islands in uropathogenic *Escherichia coli* J96: cosmid cloning and sample sequencing. Infect. Immun. **64:**3736–3743.
42. **Valvano, M. A., R. P. Silver, and J. H. Crosa.** 1986. Occurrence of chromosome- or plasmid-mediated aerobactin iron transport systems and hemolysin production among clonal group of human invasive strains of *Escherichia coli* K1. Infect. Immun. **52:**192–199.

43. **Vila, J., J. Ruiz, F. Marco, A. Barcelo, P. Goni, E. Giralt, and T. Jimenez de Anta.** 1994. Association between double mutation in *gyrA* gene of ciprofloxacin-resistant clinical isolates of *Escherichia coli* and MICs. Antimicrob. Agents Chemother. **38:**2477–2479.
44. **Vila, J., K. Simon, J. Ruiz, J. P. Horcajada, M. Velasco, M. Barranco, A. Moreno, and J. Mensa.** 2002. Are quinolone-resistant uropathogenic *Escherichia coli* less virulent? J. Infect. Dis. **186:**1039–1042.
45. **Wang, M., J. H. Tran, G. A. Jacoby, F. Zhang, and D. C. Hooper.** 2003. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. Antimicrob. Agents Chemother. **47:**2242–2248.
46. **Zhang, L., B. Foxman, and C. F. Marrs.** 2002. Both urinary and rectal *Escherichia coli* isolates are dominated by strains of phylogenetic group B2. J. Clin. Microbiol. **40:**3951–3955.