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Quinolone-Resistant Uropathogenic *Escherichia coli* Strains from Phylogenetic Group B2 Have Fewer Virulence Factors than Their Susceptible Counterparts

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The prevalence of 31 virulence factors was analyzed among nalidixic acid-susceptible and -resistant *Escherichia coli* strains from phylogenetic group B2. Hemolysin, cytotoxic necrotizing factor 1, and S and F1C fimbriae genes were less prevalent among nalidixic acid-resistant *E. coli* strains. Quinolone resistance may be associated with a decrease in the presence of some virulence factors.

Extraintestinal pathogenic *Escherichia coli* (ExPEC) strains have multiple virulence factors (VFs) that confer the potential for pathogenicity (6). Recently, extended virulence genotypes have been reported for ExPEC isolates from patients with diverse extraintestinal syndromes (9). *E. coli* strains derive from different phylogenetic groups (5). Pathogenic *E. coli* strains derive mainly from the more virulent phylogenetic group B2 (3, 7, 13).

Recent data suggest that quinolone-resistant ExPEC are less able to cause upper urinary tract infection and have fewer VFs than quinolone-susceptible *E. coli* (14, 15). Some studies have related quinolone resistance and low virulence with phylogenetic origin (8). However, in vitro studies (unpublished data) suggest a decreased pathogenicity of *E. coli* associated with the acquisition of quinolone resistance itself. To study whether the absence of VFs is associated with resistance specifically within phylogenetic group B2, we investigated the prevalence of 31 VFs among quinolone-resistant versus quinolone-susceptible *E. coli* urinary tract infection (UTI) isolates, all belonging to phylogenetic group B2 (the most virulent; not intrinsically related to quinolone resistance, as shown for phylogenetic group A) (10). The prevalence of the studied VFs according to susceptibility to ampicillin, cotrimoxazole, and gentamicin was also assessed.

E. coli strains isolated from urine from patients with acute pyelonephritis, acute cystitis, or acute prostatitis who presented at our department were identified by conventional biochemical tests. Cystitis, acute pyelonephritis, and acute prostatitis were defined as they were defined previously elsewhere (12). Fifty-three *E. coli* isolates causing acute pyelonephritis in women, 19 causing cystitis in women, and 13 causing prostatitis in men were analyzed.

Susceptibilities to nalidixic acid, ciprofloxacin, ampicillin, cotrimoxazole, and gentamicin were tested by the E-test method (AB Biodisk, Sölna, Sweden). All isolates were assigned to phylogenetic group B2 with the use of the multiplex PCR-based method (1), and all isolates belonged to different clones by Rep-PCR. Extended virulence genotypes, including 31 individual VFs and *papA* alleles, were determined by multiplex PCR assays and dot blot hybridization, as previously described (7). In addition, *sat* (secreted autotransporter toxin) was detected using previously described PCR conditions and primers (15). Each isolate was tested in duplicate, in parallel with appropriate positive and negative controls.

Statistical analyses were performed by using Fisher's exact and chi-square tests. Stratified analysis was performed by means of Mantel-Haenszel test. A P value of <0.05 was considered statistically significant.

The population studied included 64 nalidixic-susceptible isolates (cystitis, $n = 14$; pyelonephritis, $n = 38$; prostatitis, $n = 12$) and 21 nalidixic acid-resistant isolates (cystitis, $n = 5$; pyelonephritis, $n = 15$; prostatitis, $n = 1$). Among the 85 *E. coli* UTI isolates, each of the four drug resistance phenotypes studied was associated with a statistically significant shift in the prevalence of one or more of the studied VFs (Table 1). The greatest number of these shifts was observed with nalidixic acid resistance. Nalidixic acid resistance was associated with a significantly decreased prevalence of three factors, i.e., *sfa*/*foc* (S and F1C fimbriae), *hlyD* (hemolysin), and *cnf1* (cytotoxic necrotizing factor 1), and a significantly increased prevalence of six factors, *bmaE* (M fimbriae), *gafD* (G fimbriae), *iutA* (aerobactin system), *ireA* (siderophore receptor), *cvaC* (microcin V), and *iss* (increased serum survival) (Table 1).

Because of the disproportionate distribution of prostatitis isolates in the study population in relation to quinolone resistance and the known associations of clinical syndrome with both quinolone resistance and virulence (12, 14), stratified analysis was used to simultaneously assess the associations of resistance phenotype and clinical syndrome with virulence. This analysis showed a tendency to a lower prevalence of *hly*

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TABLE 1. Distribution of virulence factors according to antimicrobial resistance phenotype among 85 *E. coli* urine isolates from phylogenetic group B2

Drug (no. susceptible, no. resistant)[a]	Virulence factor[b]	No. (%) of isolates that were:[c]		*P* value[d]
		Susceptible	Resistant	
Nalidixic acid (64, 21)	*sfa/focDE*	46 (72)	7 (33)	(0.0015)
	bmaE	3 (5)	5 (24)	0.009
	gafD	3 (5)	5 (24)	0.009
	hlyD	42 (66)	7 (33)	(0.009)
	cnf1	39 (61)	7 (33)	(0.02)
	iutA	21 (33)	15 (71)	0.006
	ireA	15 (23)	11 (52)	0.012
	cvaC	10 (16)	9 (43)	0.009
	iss	9 (14)	14 (66)	<0.001
Ampicillin (30, 55)	K1 *kpsM*	13 (43)	7 (13)	(0.001)
Cotrimoxazole (59, 26)	*bmaE*	3 (5)	5 (19)	0.03
	gafD	3 (5)	5 (19)	0.03
	iroN	46 (78)	10 (38)	(<0.001)
	malX	53 (89)	19 (73)	(0.047)
Gentamicin (81, 4)	*fimH*	78 (96)	3 (75)	(0.04)
	iutA	32 (39)	4 (100)	0.01

[a] Susceptibility and resistance are as defined by E-test.

[b] Only those virulence factors that yielded a *P* value of <0.05 are shown. *sfa/focDE*, S and F1C fimbriae; *bmaE*, M fimbriae; *gafD*, G fimbriae; *fimH*, type 1 fimbriae; *hlyD*, hemolysin; *cnf1*, cytotoxic necrotizing factor 1; *iutA*, aerobactin receptor; *iroN* and *ireA*, novel siderophore receptors; *cvaC*, colicin (microcin) V; *iss*, increased serum survival; K1 *kpsM*, group 2 capsule (variant K1); and *malX*, pathogenicity island marker. No statistically significant association with resistance was noted for the following virulence factors: *papA*, P fimbriae structural subunit; *papC*, P fimbriae assembly; *papEF*, P fimbriae tip pilis; *papG*, P fimbriae adhesin (and alleles II and III); *sfaS*, S fimbriae; *focG*, F1C fimbriae; *afa/draBC*, Dr-binding adhesins; *iha*, putative adhesin-siderophore; *cdtB*, cytolethal distending toxin; *sat*, secreted autotransporter toxin; *fyuA*, yersiniabactin receptor; *kpsM* II, group 2 capsule (variant K2); *kpsMT* III, group 3 capsule; *rfc*, O4 lipopolysaccharide; *ibeA*, invasion of brain endothelium; *ompT*, outer membrane protease T; and *fliC*, flagellin.

[c] Numbers of isolates that were susceptible or resistant to drugs in column 1 are shown. Comparison groups varied by drug.

[d] *P* values (by Fisher's exact test or χ^2 test) are for comparisons of isolates susceptible and resistant to the indicated drug. Parentheses indicate negative associations of virulence factor with resistance.

and *cnf* in quinolone-resistant *E. coli* from cystitis (20%) and pyelonephritis (49%) than in their susceptible counterparts (40% and 69%, respectively; the *P* value was 0.08 between cystitis isolates and 0.23 for pyelonephritis isolates). Mantel-Haenszel stratified analysis showed that clinical syndrome was not a confounding factor (crude relative risk, 0.51 [95% confidence interval, 0.27 to 0.95]; adjusted relative risk, 0.52 [95% confidence interval, 0.27 to 0.99]). This fact suggests that quinolone resistance could be directly associated with virulence loss, as suggested in a previous study (15). Other studies have also found that resistance to quinolones is significantly more frequent among nonhemolytic *E. coli* isolates (11), although the mechanism is unknown. The *hly*, *cnf*, and *sfa* genes have been found in pathogenicity islands (PAI). PAI can be easily deleted from the chromosome, leading to mutants with reduced virulence (4). During the development of quinolone resistance, deletion and transposition of DNA regions may occur with the loss of PAI.

Recent studies have demonstrated a relationship between phylogenetic origin and antibiotic resistance and low prevalence of VFs (8, 10). In a previous study, phylotypes were not taken into account, and the presence of some low-virulence phylotypes, such as phylotype A, may explain the lower prevalence of VFs found among quinolone-resistant *E. coli* strains in our series. To test the hypothesis of the association between quinolone resistance and low virulence, independently of the phylogenetic origin and without its possible confounding effect on this association, we analyzed the prevalence of several VFs in quinolone-resistant and susceptible *E. coli* strains of phylotype B2 exclusively. The results suggested that quinolone resistance may be directly associated with virulence loss. However, a previous study showing that spontaneous quinolone-resistant mutants obtained from hemolytic quinolone-susceptible strains still produce hemolysin could suggest otherwise, although the authors used two mutants and a one-step selection method which does not mimic "in vivo" selection of mutants, which would have made the findings conclusive (11).

In countries with a higher prevalence of quinolone resistance, it is possible to find phylotype B2 *E. coli* strains resistant to quinolones in clinical settings. The higher prevalence of quinolone resistance in these countries has been related to the higher use of quinolones (2). Therefore, it is feasible that although phylotype B2 strains have harbored some VFs during their evolutionary history, in the presence of quinolones, they develop genetic changes leading to quinolone resistance and to a loss of VFs.

We performed statistical analyses by syndrome and demonstrated a tendency toward a lower prevalence of *hly* and *cnf* in quinolone-resistant *E. coli* from cystitis and pyelonephritis than in their susceptible counterparts. Isolates from prostatitis were not taken into consideration, since the majority of isolates were quinolone susceptible. However, the limited number of isolates by syndrome does not permit us to be conclusive regarding the possible association between quinolone resistance and fewer VFs confounded by syndrome.

To assess the possible relationship between fewer VFs in ExPEC and resistance to other antimicrobial agents, the prevalence of the studied VFs was also assessed according to susceptibility to ampicillin, cotrimoxazole, and gentamicin, with few differences among susceptible and resistant isolates to these antibiotics; the exceptions were the *iroN* and *malX* genes, which were less prevalent in cotrimoxazole-resistant strains.

Another interesting finding is that in quinolone-resistant *E. coli* strains, the aerobactin receptor gene *iutA* was significantly more prevalent than in their quinolone-susceptible counterparts. The same result occurred with other, less prevalent VFs, such as the *cva*, *bma*, *gaf*, *iss*, and *ire* genes, and it has been previously shown with the aerobactin gene (8). It is difficult to explain these findings, and further studies are needed to investigate the basis for their occurrence.

In conclusion, the study with the phylogenetic group B2 suggests that quinolone-resistant *E. coli* could be directly associated with low prevalences of hemolysin and *cnf1*.

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