ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2005, p. 26–31 Vol. 49, No. 1
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.1.26–31.2005

Virulence Genotype and Phylogenetic Origin in Relation to Antibiotic Resistance Profile among *Escherichia coli* Urine Sample Isolates from Israeli Women with Acute Uncomplicated Cystitis

James R. Johnson,[1,2]* Michael A. Kuskowski,[3,4] Timothy T. O'Bryan,[1,2] Raul Colodner,[5] and Raul Raz[6,7]

Medical Service[1] and Geriatric Research, Education, and Clinical Center,[3] Minneapolis VA Medical Center, and Departments of Medicine[2] and Psychiatry,[4] University of Minnesota, Minneapolis, Minnesota, and Microbiology Laboratory[5] and Infectious Diseases Unit,[6] HaEmek Medical Center, and Technion School of Medicine,[7] Afula, Israel

Received 10 May 2004/Returned for modification 16 August 2004/Accepted 12 September 2004

To clarify the virulence and phylogenetic implications of antimicrobial agent resistance in *Escherichia coli*, 100 *E. coli* isolates from urine samples of Israeli women with acute uncomplicated cystitis were analyzed by molecular phylotyping and virulence genotyping for comparison with resistance phenotypes. The differences between the isolates that were resistant and susceptible to trimethoprim-sulfamethoxazole and ampicillin were minimal. In contrast, ciprofloxacin resistance was associated with greatly reduced inferred virulence and categorical shifts away from the highly virulent phylogenetic group B2, which explained much of the virulence effect. The results of amplification fingerprinting suggested that most ciprofloxacin-resistant isolates represented unique clonal groups and were not derived from clonal groups with more highly virulent susceptible isolates. These findings suggest that virulence and antimicrobial resistance are not mutually exclusive in *E. coli* clinical isolates. Instead, the relationship between virulence and antimicrobial resistance varies according to the particular resistance phenotype; for ciprofloxacin resistance, the relationship is strongly influenced by phylogenetic background. The basis for the concentration of ciprofloxacin resistance in non-B2 phylogenetic groups remains unknown.

Acute cystitis is a common, morbid, and costly medical problem of adult women (5, 30, 36). Recently, managing acute cystitis has been increasingly complicated by the increasing prevalence of resistance to first-line antimicrobial agents among the causative microorganisms, most notably *Escherichia coli* (8). In many locales, including northern Israel, the prevalence of resistance to trimethoprim-sulfamethoxazole (TMP-SMZ) among uropathogens exceeds the 10 to 20% threshold at which authorities suggest using alternative empirical regimens, such as a fluoroquinolone, nitrofurantoin, or fosfomycin (9, 23, 33, 40). There is cause for concern that as the use of these agents increases, resistance to these agents will arise, increase, and eventually limit their use also (6, 7, 34).

An understanding of the microbiological basis for the emergence of antibiotic resistance among pathogenic *E. coli* strains is needed to guide efforts to interrupt this process. At this time, it is unknown to what extent today's drug-resistant clinical *E. coli* strains are simply antibiotic-resistant versions of the same virulent clones of extraintestinal pathogenic *E. coli* that constitute most of the susceptible bacterial population (12) or if they represent a phylogenetically different assortment of clones (16, 25, 31). It is also unknown how the intrinsic virulence of such strains compares with that of susceptible strains. Previous studies of antibiotic resistance phenotypes to antibiotics other than TMP-SMZ have suggested that the resistant bacterial population may largely consist of low-virulence opportunists whose success derives more from antibiotic resistance than from pathogenic capability (19, 22, 38). However, such associations may depend heavily on the ecological context; for example, among isolates from food animals, antimicrobial resistance and virulence are only weakly linked, if at all (15, 18). Furthermore, to the extent that resistance does correlate with reduced virulence, it is unknown whether emerging resistance is caused by novel low-virulence, resistant clones (22) or by putative exchange of virulence determinants for resistance elements within preexisting susceptible, virulent clones (26, 39).

To clarify these issues within a clinically well-defined population, we determined the phylogenetic background and virulence characteristics of 100 *E. coli* isolates from urine samples from women with acute, uncomplicated cystitis from an outpatient clinic in Afula, Israel. We then compared the TMP-SMZ-, ampicillin-, and ciprofloxacin-susceptible and -resistant bacterial populations and used experimental approaches to explore the mechanisms underlying the observed association between resistance and virulence.

MATERIALS AND METHODS

Patients and strains. The study population consisted of 100 premenopausal women (18 to 50 years of age) with uncomplicated acute cystitis who presented to the Infectious Diseases Outpatient Clinic during January to March 2001. Urine samples from the 100 women were obtained, and 100 pretherapy *E. coli* isolates from these urine samples were studied. Uncomplicated cystitis due to *E. coli* was defined as the presence of dysuria, frequent urination, and suprapubic tenderness in otherwise healthy premenopausal women without fever or loin pain and with clean-catch, midstream urine cultures yielding $\geq 10^3$ CFU of *E. coli*

* Corresponding author. Mailing address: Infectious Diseases (111F), Minneapolis VA Medical Center, One Veterans Dr., Minneapolis, MN 55417. Phone: (612) 467-4185. Fax: (612) 727-5995. E-mail: johns007@umn.edu.

TABLE 1. Virulence genotype in relation to antimicrobial resistance phenotype among 100 *E. coli* isolates from women with acute uncomplicated cystitis[a]

Drug (no. of susceptible isolates + no. of resistant isolates)	Trait	Prevalence of trait among isolates susceptible or resistant to drug (no. of isolates [%])		*P* value[b]
		Susceptible	Resistant	
TMP-SMZ (51 + 49)	*papG* allele III	13 (25)	4 (8)	(0.03)
	afa/*draBC*	2 (4)	12 (24)	0.004
	iutA	24 (47)	37 (76)	0.004
	traT	26 (51)	37 (76)	0.01
	malX	34 (67)	22 (45)	(0.04)
Ampicillin (8 + 92)	F12 *papA* allele	2 (25)	2 (2)	(0.03)
	traT	2 (25)	61 (66)	0.049
Ciprofloxacin (94 + 6)	*papA*[c]	46 (49)	0 (0)	(0.03)
	hlyD	44 (47)	0 (0)	(0.03)
	fyuA	78 (83)	0 (0)	(<0.001)
	malX	56 (60)	0 (0)	(0.006)

[a] Only traits or VFs with *P* values of <0.05 in comparison of susceptible and resistant isolates are shown. The genes encoding these traits or VFs follow: *papG* allele III (P-fimbria adhesin molecule variant III), *afa*/*draBC* (Dr-family adhesins), *iutA* (aerobactin receptor), *traT* (serum resistance associated), *malX* (pathogenicity island marker), F12 allele of *papA* (P-fimbria structural subunit [F12 variant]), *hlyD* (hemolysin), and *fyuA* (yersiniabactin receptor). The genes that were also detected but did not yield significant associations with antimicrobial resistance follow: *papG* allele II (31 isolates), *sfa*/*focDE* (S and/or F1C fimbriae) (37 isolates), *sfa5* (S-fimbria adhesin) (6 isolates), *focG* (F1C fimbriae) (27 isolates), *iha* (putative adhesin-siderophore receptor) (40 isolates), *fimH* (type I fimbriae) (98 isolates), *cnf1* (cytotoxic necrotizing factor) (34 isolates), *cdtB* (cytolethal distending toxin) (9 isolates), *ireA* and *iroN* (novel siderophore receptors) (51 and 78 isolates, respectively), *kpsMT* II and III (group 2 and 3 capsule synthesis) (69 and 4 isolates, respectively), K1 and K2 (variants of *kpsMT* II) (10 and 5 isolates, respectively), *rfc* (O4 lipopolysaccharide synthesis) (1 isolate), *cvaC* (colicin V) (8 isolates), *ibeA* (invasion of brain endothelium) (6 isolates), *ompT* (outer membrane protease T) (53 isolates), and *iss* (increased serum survival) (16 isolates). No isolates were positive for *bmaE* (M fimbriae), *gafD* (G fimbriae), or *papG* allele 1 (P-adhesin variant I).

[b] *P* values (by Fisher's exact test) for comparisons of susceptible versus resistant isolates. *P* values in parentheses indicate a negative association with resistance.

[c] For ciprofloxacin, results for *papC*, *papEG*, and *papG* were similar or identical to those shown for *papA*.

per ml (35). Exclusion factors were as follows: menopause, recurrent urinary tract infections, any anatomical or functional abnormality of the urinary tract, pyelonephritis, immunocompromised immune system, diabetes mellitus, and use of a urinary catheter. Consecutive clinic patients during the study period were assessed for study eligibility by medical record review by one of the investigators (R. Raz). The clinical microbiology laboratory bacteria collected from the urine samples from all eligible patients until approximately 50 isolates per primary susceptibility group, i.e., TMP-SMZ susceptible and resistant, had been accumulated. Isolates were collected and saved anonymously, without accompanying demographic or clinical data.

Urine samples were plated on Chromagar Orientation (Hy Laboratories, Rehovot, Israel) using a calibrated 1-μl bacteriological loop. Suspected *E. coli* isolates were identified using Microscan Urine Combo 2 panels (Dade-Behring, Fla.) and a Microscan Walkaway-96 apparatus. Susceptibility to TMP-SMZ was interpreted using the Microscan DMS software and National Committee for Clinical Laboratory Standards (NCCLS) breakpoints. Resistance to ampicillin, ciprofloxacin, ceftriaxone, and ceftazidime was screened for by agar dilution using NCCLS-specified breakpoints, with selective confirmation by disk diffusion and/or broth microdilution (27). *E. coli* ATCC 25922 was the reference strain.

Phylogenetic analysis and virulence genotyping. The isolates were assigned to one of the four main phylogenetic groups of *E. coli* (A, B1, B2, and D) (groups originally defined by multilocus enzyme electrophoresis [11]) by multiplex PCR (2). The isolates were also tested for 34 virulence-associated traits (hereafter referred to as virulence factors [VFs]) of extraintestinal pathogenic *E. coli* and, if positive for any *pap* (P-fimbria) element, for 13 *papA* (structural subunit) alleles, using established PCR and dot blot-based assays (20, 22). All testing was done in duplicate using independently prepared boiled lysates of each isolate, with appropriate positive and negative controls included in each run.

An aggregate virulence score was calculated as the sum of all VFs for which the isolates tested positive, with adjustment for multiple detection of the *pap*, *sfa*/*foc*, and *kps* operons.

O-antigen typing and RAPD analysis. O antigens were determined by the *E. coli* Reference Center, University Park, Pa., using 180 O-specific antisera by standard methods (29). Random amplified polymorphic DNA (RAPD) analysis was performed by using arbitrary decamer primer 1247 (1), with visual analysis of profiles in ethidium bromide-stained agarose gels.

Conjugation. Bacterial mating in broth was performed as previously described (21). Candidate donor strains were grown together overnight in nonselective broth medium with recipient strain JM109 (which was nalidixic acid resistant [Nal^r]) or a spontaneous Nal^r derivative of *E. coli* Reference (ECOR) strain 44 (13, 28), i.e., ECOR44 (Nal^r). Broth was then spread on agar plates supplemented with antimicrobial agents corresponding with resistance markers unique to the donor and recipient. Colonies from selective plates (putative transconjugants) were tested for antimicrobial susceptibility testing by disk diffusion. The genomic identity of the donor was confirmed by RAPD analysis, and the virulence genotype was assessed.

Statistical methods. For statistical analysis, isolates were regarded as independent samples, regardless of clonal background, since each isolate was derived from a unique host and represented a separate cystitis episode. Comparisons of proportions were tested using Fisher's exact test or Pearson's χ^2 test for unpaired comparisons and McNemar's test for paired comparisons (all tests were two-tailed tests). Comparisons of aggregate VF scores were tested using the Mann-Whitney U test. Multiple predictor variables were assessed simultaneously for their associations with VF score by using multiple linear regression. A *P* value of <0.05 was considered statistically significant.

RESULTS

Comparison of resistant and susceptible isolates. Of the 100 *E. coli* isolates from women with acute cystitis, 51 were susceptible and 49 were resistant to TMP-SMZ by design. Eight TMP-SMZ-susceptible isolates were susceptible to ampicillin, whereas six TMP-SMZ-resistant isolates were resistant to ciprofloxacin. No isolate was resistant to ceftriaxone or ceftazidime.

The differences in the prevalence of individual VFs (Table 1), aggregate VF scores (Table 2), and phylogenetic distribution (Table 3) according to susceptibility versus resistance to TMP-SMZ or ampicillin were minimal. Only five VFs differed significantly with the TMP-SMZ resistance phenotype, and only two VFs differed significantly with the ampicillin resistance phenotype. Of these VFs, several traits typically associated with chromosomal group B2, i.e., *papG* allele III (P-fimbria adhesin variant III), the F12 *papA* allele (P-fimbria

TABLE 2. Aggregate VF score in relation to antimicrobial resistance phenotype among 100 *E. coli* isolates from women with acute uncomplicated cystitis

Antibiotic (no. of susceptible isolates + no. of resistant isolates)	Aggregate VF score (median [range])		*P* value[a]
	Susceptible isolates	Resistant isolates	
TMP-SMZ (51 + 49)	9.0 (0–14)	7.0 (2–15)	NS
Ampicillin (8 + 92)	8.5 (6–12)	8.0 (0–15)	NS
Ciprofloxacin (94 + 6)	8.0 (0–15)	3.0 (2–5)	(0.001)

[a] *P* value (by Mann-Whitney U test) shown only where *P* was <0.05. The *P* value in the parentheses indicates a negative association with resistance. NS, not significant ($P > 0.10$).

structural subunit variant), and *malX* (a pathogenicity island marker [20]), exhibited weak associations with susceptibility. Conversely, three traits often associated with plasmids, i.e., *afa*/*dra* (Dr-binding adhesins), *iutA* (aerobactin receptor), and *traT* (serum resistance associated) (20), were associated with resistance (Table 1).

In contrast, despite the smaller numbers, ciprofloxacin resistance exhibited as many significant associations with VFs and phylogenetic distribution as did TMP-SMZ and ampicillin resistance, and several of these associations were the strongest observed (Tables 1 to 3). Specifically, ciprofloxacin resistance was associated with the absence of four traits typically associated with phylogenetic group B2, i.e., *papA*, *hlyD* (hemolysin), *fyuA* (yersiniabactin receptor), and *malX* (Table 1), with markedly lower aggregate VF scores (Table 2), and with a categorical shift away from phylogenetic group B2 to groups A and D (Table 3).

Phylogenetic distribution of VFs. The concurrent shifts in VF profiles (Tables 1 and 2) and phylogenetic distribution (Table 3) observed in association with resistance to TMP-SMZ, ampicillin, and ciprofloxacin suggested a possible underlying phylogenetic distribution of VFs. Consistent with this hypothesis, most individual VFs ($n = 16$) were associated with phylogenetic group B2 (Table 4). The only statistically significant exceptions were three VFs that were more prevalent in group D than in other groups; these traits included the F16 *papA* allele (26% for group D versus 5% for other groups [$P = 0.01$]), *afa*/*dra* (37% for group D versus 9% for other groups [$P = 0.005$]), and *traT* (84% for group D versus 58% for other groups [$P = 0.04$]). Consequently, aggregate VF scores differed markedly among the four phylogenetic groups; the aggregate VF scores were highest in group B2, intermediate in group D, and lowest in groups A and B1 (Table 5).

To assess whether the comparative paucity of VFs among ciprofloxacin-resistant isolates could be explained by these phylogenetic differences, two analytical approaches were used.

TABLE 4. Virulence genotype in relation to phylogenetic group among 100 *E. coli* isolates from women with acute uncomplicated cystitis[a]

Trait	Prevalence of trait (no. of isolates [%])		*P* value
	Group B2 ($n = 55$)	Other groups ($n = 45$)	
papA	36 (65)	10 (22)	<0.001
F10 + F14 alleles	7 (13)	0	0.016
F16 allele	1 (2)	8 (18)	0.01
papG allele II	22 (40)	9 (20)	0.049
papG allele III	17 (31)	0	<0.001
sfa/*focDE*	36 (65)	1 (2)	<0.001
sfaS	6 (11)	0	0.03
focG	27 (49)	0	<0.001
afa/*draBC*	3 (5)	11 (24)	0.009
hlyD	41 (75)	3 (7)	<0.001
cnf1	34 (62)	0	<0.001
cdtB	9 (16)	0	0.004
ireA	21 (38)	3 (7)	<0.001
iroN	43 (78)	8 (18)	<0.001
fyuA	53 (96)	25 (56)	<0.001
kpsM II	47 (85)	22 (49)	<0.001
ompT	39 (71)	14 (31)	<0.001
malX	53 (96)	3 (7)	<0.001

[a] Only traits or VFs yielding a significant (positive or negative) association with group B2 are shown. The genes encoding these traits or VFs follow: *papA* (P-fimbria structural subunit [and its alleles]), *papG* (P-adhesin molecule [with alleles II and III]), *sfa*/*focDE* (S and F1C fimbriae), *sfaA* (S-fimbria adhesin), *focG* (F1C fimbriae), *afa*/*draBC* (afimbrial and Dr-binding adhesins), *hlyD* (hemolysin), *cnf1* (cytotoxic necrotizing factor), *cdtB* (cytolethal distending toxin), *ireA* and *iroN* (novel siderophore receptors), *fyuA* (yersiniabactin receptor), *kpsMT* II (group 2 capsule synthesis), *ibeA* (invasion of brain endothelium), *ompT* (outer membrane protease T), *iss* (increased serum survival), and *malX* (marker for pathogenicity island from strain CFT073). Results for *papC* (chaperone) and *papEF* (tip pilins) were as shown for *papA*. The genes that were also detected but not did yield statistically significant associations with phylogenetic group B2 (so not shown) follow: *iha* (putative adhesin-siderophore receptor), *fimH* (type I fimbriae), *iutA* (aerobactin receptor), the K1 and K2 variants of *kpsMT* II, *kpsMT* III (group 3 capsule synthesis), *traT* (serum resistance associated), *rfc* (O4 lipopolysaccharide synthesis), *cvaC* (colicin [microcin] V), *ibeA* (invasion of brain endothelium), and *iss* (increased serum survival. No isolates were positive for *bmaE* (M fimbriae), *gafD* (G fimbriae), or *papG* allele I (P-adhesin variant I).

TABLE 3. Phylogenetic background in relation to antimicrobial resistance phenotype among 100 *E. coli* isolates from women with uncomplicated acute cystitis

ECOR[a] group	Prevalence of phylogenetic group (no. of isolates [%])							
	TMP-SMZ			Ampicillin[b]		Ciprofloxacin		
	Susceptible ($n = 51$)	Resistant ($n = 49$)	*P* value[b]	Susceptible ($n = 8$)	Resistant ($n = 92$)	Susceptible ($n = 94$)	Resistant ($n = 6$)	*P* value
A	6 (12)	14 (29)	0.046	1 (13)	19 (21)	16 (17)	4 (67)	0.01
B1	4 (8)	2 (4)		0	6 (7)	6 (6)	0	
B2	34 (67)	21 (43)	(0.03)	6 (75)	49 (53)	55 (57)	0	(0.007)
D	7 (14)	12 (24)		1 (13)	18 (20)	17 (18)	2 (33)	

[a] ECOR, *Escherichia coli* Reference.
[b] *P* values (by Fisher's exact test) shown only where $P < 0.05$. No comparison involving ampicillin yielded a *P* value of 0.05. *P* values in parentheses indicate a negative associations with resistance.

TABLE 5. Aggregate VF score in relation to phylogenetic group among 100 *E. coli* isolates from women with acute uncomplicated cystitis

Phylogenetic group (no. of isolates)	Aggregate VF score (median [range])	*P* value[a]
A (20)	4.1 (1–8)	(<0.001)
B1 (6)	4.5 (2–6)	(0.004)
B2 (55)	11.0 (0–15)	<0.001
D (19)	7.0 (3–10)	0.01

[a] *P* values (by Mann-Whitney U test) are for comparison of indicated group versus all other isolates. *P* values in parentheses indicate negative associations.

First, stratified univariate analysis was used to compare the aggregate VF scores with the ciprofloxacin phenotype in phylogenetic groups A and D, which contained all six ciprofloxacin-resistant isolates. A marginally significant association of ciprofloxacin resistance with reduced VF score was evident in group D alone (median score of 3.5 for ciprofloxacin-resistant isolates versus 7.0 for ciprofloxacin-susceptible isolates [$P = 0.047$]), but no association was apparent in group A alone (data not shown). Second, multiple linear regression was used to simultaneously evaluate ciprofloxacin phenotype and phylogenetic group as predictors of VF score. In the resulting model, phylogenetic group was an extremely strong predictor (beta weight of 0.70; $P < 0.001$), whereas ciprofloxacin resistance was a weak and only marginally significant predictor (beta weight of -0.14; $P = 0.049$), of VF score.

Conjugation. To assess whether TMP-SMZ and ampicillin resistance elements might be genetically linked with certain VFs (i.e., *afa*/*draBC*, *iutA*, and *traT*) on plasmids or other mobile elements, as suggested by the observed statistical associations (Table 1) and previously demonstrated in other collections (17, 21, 31), attempts were made to coordinately transfer resistance and VFs by conjugation. The 16 isolates that were resistant to both ampicillin and TMP-SMZ but were susceptible to nalidixic acid and were positive for *afa*/*draBC*, *iutA*, and/or *traT*, were mated with strains JM109 (*traT* positive) and/or ECOR44 (Nal^r) (*traT* negative) (13). For 4 of the 16 donor strains, transfer of multiple resistance markers was achieved. One of the transconjugants had acquired multiple VFs (*iutA*, *cvaC*, *iroN*, and *iss*) along with resistance markers. A derivative was recovered from another mating on selective plates; the derivative had acquired *iutA* and *traT*, in the absence of resistance markers, as defined by a standardized disk diffusion assay. No transfer of *afa*/*draBC* was observed.

Clonal analysis of ciprofloxacin-resistant isolates by RAPD profiling and O-antigen typing. To determine whether any VF-deficient ciprofloxacin-resistant isolates might be derived from the same clonal group as any VF-replete ciprofloxacin-susceptible isolates, which would support possible exchange of VFs for resistance, RAPD analysis and O-antigen typing were used to compare the 20 group A isolates and, separately, the 19 group D isolates. Among the group D isolates, 14 unique RAPD profiles were observed overall. Ten of these profiles were unique to individual isolates, whereas four profiles were shared among two or three isolates each. The two ciprofloxacin-resistant group D isolates constituted one of the four multiple-isolate RAPD clades, which was uniquely characterized by the O153 antigen and a *fim*, *iutA*, *kpsM* II VF profile. Thus, these isolates appeared to be related to one another but unrelated to other group D isolates ($P = 0.03$ for the prevalence of this clonal group among resistant versus susceptible isolates), evidence against the clonal commonality hypothesis.

Among the 20 group A isolates, 13 unique RAPD profiles were observed. Nine profiles were unique to individual isolates, whereas four profile were shared among two or three isolates each. Three of the ciprofloxacin-resistant group A isolates exhibited unique RAPD profiles. Of these isolates, two were O antigen nontypeable (as was the fourth ciprofloxacin-resistant isolate; these were the only O antigen nontypeable group A isolates). The third isolate was O8, as was a single ciprofloxacin-susceptible isolate which, however, differed from the O8 ciprofloxacin-resistant isolate by RAPD profile. The fourth ciprofloxacin-resistant isolate (strain 618; O antigen nontypeable) was similar by RAPD analysis to two ciprofloxacin-susceptible isolates from serogroups O8 and O9, respectively. Its VF profile (*fim*, *iroN*, and *iss*) differed substantially from that of the ciprofloxacin-susceptible O9 isolate (strain 654 [*fim*, *fyuA*, and *ompT*]) but represented a subset of the VF profile of the O8 ciprofloxacin-susceptible isolate (strain 633), which in addition to *fim*, *iroN*, and *iss* (as in strain 618) also contained *iha*, *iutA*, *cvaC*, and *traT*.

These findings suggested that strain 618 might represent a ciprofloxacin-resistant variant of the clonal group represented by strain 633 with multiple VFs deleted, albeit O antigen nontypeable instead of O8. To determine whether such a variant could be derived experimentally from strain 633, a spontaneous Nal^r derivative of strain 633 was selected by plating a dense suspension of strain 633 on Luria-Bertani agar containing nalidixic acid (32 μg/ml). The resulting Nal^r derivative demonstrated no loss of VFs compared with the parent strain. Efforts to derive a spontaneous ciprofloxacin-resistant mutant of strain 633 by similar methods were unsuccessful.

DISCUSSION

We found that among *E. coli* urine sample isolates from Israeli women with acute uncomplicated cystitis, resistance to TMP-SMZ, ampicillin, and ciprofloxacin exhibited distinctive patterns of association with virulence genotype and phylogenetic background. Resistance to TMP-SMZ and/or ampicillin was associated with borderline shifts away from phylogenetic group B2 and with modest decreases in one or more group B2-associated VFs that were balanced by modest increases in one or more other VFs, yielding no net change in the aggregate VF score. In contrast, resistance to ciprofloxacin was associated with a categorical shift away from group B2, total absence of several group B2-associated VFs, and no significant increases in any VF, resulting in extremely low aggregate VF scores. Moreover, most ciprofloxacin-resistant isolates appeared to be clonally distinct from ciprofloxacin-susceptible isolates and not derived from them.

That so few significant pathotypic or phylogenetic associations were observed with TMP-SMZ or ampicillin resistance and that the few observed shifts were comparatively modest and of borderline statistical significance cannot be attributed solely to sample size, since numerous highly significant differences were observed between the ciprofloxacin-susceptible and -resistant isolates and among the four phylogenetic groups,

despite the small numbers. Instead, the overall similarity of the TMP-SMZ- and ampicillin-susceptible and -resistant populations suggests that the resistant isolates were primarily derived from susceptible isolates via acquisition of transferable resistance elements, with little or no change in virulence profile. Indeed, consistent with previous reports (17, 31), in one isolate, ampicillin and TMP-SMZ resistance were coordinately transferred by conjugation along with specific VFs (*iutA* and *traT*), presumably on a mobile plasmid. The observed association of *afa*/*draBC* with ampicillin resistance is in agreement with previous findings from women with gestational pyelonephritis (10). However, a direct linkage was not demonstrated in either that study or the present study.

The preserved virulence and predominantly group B2 background of the present TMP-SMZ- and ampicillin-resistant *E. coli* isolates, in contrast to the reduced virulence and shifts away from group B2 previously associated with ampicillin and sulfonamide resistance in isolates from patients with urosepsis (17), may relate to differences in the respective study populations. The present study included only patients with no known compromising conditions, which presumably obliged most isolates to exhibit a threshold level of virulence to be included in the study, whereas the previous study included many compromised (and possibly heavily antibiotic-exposed) hosts, in whom low-virulence strains could readily cause disease (17).

In contrast to ampicillin and TMP-SMZ resistance, ciprofloxacin resistance was associated with markedly reduced inferred virulence and with categorical shifts away from phylogenetic group B2, consistent with previous reports regarding clinically less-well-defined fluoroquinolone-resistant *E. coli* strains from other contexts and locales (15, 22). How such presumably low-virulence strains could cause acute cystitis in otherwise healthy women is unclear. It is conceivable that these strains actually possessed unrecognized VFs that compensated for their lack of known VFs. However, there is evidence against this; an animal infection study found that experimental extraintestinal virulence was directly proportional to the number of conventional VFs detected, regardless of phylogenetic background or clinical source (14, 32). Comparative virulence assessment of the present ciprofloxacin isolates would be informative. If the isolates are indeed relatively avirulent, the fact that they caused acute cystitis may reflect enhanced host susceptibility. For example, there may have been an unrecognized lapse in host defenses, whether fixed or transient, or a high bacterial inoculum, possibly related to host behaviors, such as sexual activity, contraceptive practices, or prior antibiotic use (37). Similar considerations may also apply to the few susceptible isolates that exhibited low VF scores.

The marked phylogenetic differences noted between ciprofloxacin-resistant and -susceptible isolates suggest that these groups may represent, in part, distinct bacterial populations. Thus, the resistant isolates are not simply mutant derivatives of the susceptible isolates; accordingly, their relative lack of VFs does not necessarily represent loss of VFs in exchange for resistance (26, 39). Indeed, RAPD analysis and experimental data provided no evidence that mutation to quinolone or fluoroquinolone resistance caused loss of VFs. The predominance of phylogenetic groups A and D among the ciprofloxacin-resistant isolates suggested instead that fluoroquinolone resistance occurs more commonly among such strains, and based on the findings from RAPD analysis, possibly within specific clonal groups therein, whether due to greater ease of mutation to resistance or greater exposure to selection pressure among these clonal groups. Selection for ciprofloxacin resistance may have occurred in humans, e.g., within the predominantly non-group-B2 human gut flora (4, 13). However, the previously demonstrated similarity of ciprofloxacin-resistant human isolates to animal isolates from the ECOR collection (22) suggests a possible animal source, including perhaps antibiotic-exposed poultry (6), with subsequent food-borne transmission to humans (3, 24).

Limitations of this study include the small number of ampicillin-susceptible and ciprofloxacin-resistant isolates (which limited power for finding differences), the use of multiple comparisons (which increased the likelihood of type I errors), the fact that the isolates were from a single geographical locale, and the uncertain relationship between the molecular markers and in vivo virulence. As such, the findings regarding ciprofloxacin resistance in particular must be regarded as hypothesis generating, rather than definitive. Strengths and/or novel features of this study include the well-defined, homogenous clinical population, the analysis of TMP-SMZ resistance in relation to VF profiles and phylogenetic background, the comparative RAPD analysis of ciprofloxacin-resistant and -susceptible isolates, and the experimental assessment of genetic links between resistance and VFs.

In summary, we found that among *E. coli* isolates from women with uncomplicated cystitis, resistance to ciprofloxacin, but not to ampicillin or TMP-SMZ, was associated with marked reductions in inferred virulence and categorical shifts to non-B2 phylogenetic groups, which accounted for most of the virulence effects. This suggests that in *E. coli* the relationship between antimicrobial resistance and virulence varies according to resistance phenotype, with ciprofloxacin resistance usually occurring in intrinsically low-virulence non-B2 strains rather than via loss of VFs from virulent strains. The basis for the striking concentration of ciprofloxacin resistance within phylogenetic groups A and D remains to be determined but may include agricultural antimicrobial use. Confirmation of these findings in other populations is needed.

ACKNOWLEDGMENTS

This material is based upon work supported by the Office of Research and Development, Medical Research Service, Department of Veterans Affairs, National Institutes of Health grants DK-47504, and National Research Initiative (NRI) Competitive Grants Program/United States Department of Agriculture grant 00-35212-9408 (all to J.R.J.). Potential conflicts of interest include previous grant support and/or honoraria from Bayer, Wyeth-Ayerst, Ortho-McNeil, Merck, and Rochester Medical Group (all to J.R.J.).

Ann Emery (Minneapolis VA Medical Center) helped prepare the manuscript.

REFERENCES

1. **Berg, D. E., N. S. Akopyants, and D. Kersulyte.** 1994. Fingerprinting microbial genomes using the RAPD or AP-PCR method. Methods Mol. Cell. Biol. **5:**13–24.
2. **Clermont, O., S. Bonacorsi, and E. Bingen.** 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl. Environ. Microbiol. **66:**4555–4558.
3. **Cogan, T. A., S. F. Bloomfield, and T. J. Humphrey.** 1999. The effectiveness of hygiene procedures for prevention of cross-contamination from chicken carcasses in the domestic kitchen. Lett. Appl. Microbiol. **29:**354–358.
4. **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.
5. **Foxman, B., R. Barlow, H. D'Arcy, B. Gillespie, and J. D. Sobel.** 2000. Urinary tract infection: self-reported incidence and associated costs. Ann. Epidemiol. **10:**509–515.
6. **Garau, J., M. Xercavins, M. Rodriguez-Carballeira, J. Gomez-Vera, I. Coll, D. Vidal, T. Llovet, and A. Ruiz-Bremon.** 1999. Emergence and dissemination of quinolone-resistant *Escherichia coli* in the community. Antimicrob. Agents Chemother. **43:**2736–2741.
7. **Goettsch, W., W. van Pelt, N. Nagelkerke, M. G. R. Hendrix, A. G. M. Buiting, P. L. Petit, L. J. M. Sabbe, A. J. A. van Griethuysen, and A. J. de Neeling.** 2000. Increasing resistance of fluoroquinolones in *Escherichia coli* from urinary tract infections in The Netherlands. J. Antimicrob. Chemother. **46:**223–228.
8. **Gupta, K., T. M. Hooton, and W. E. Stamm.** 2001. Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. Ann. Intern. Med. **135:**41–50.
9. **Gupta, K., D. F. Sahm, D. Mayfield, and W. E. Stamm.** 2001. Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in women: a nationwide analysis. Clin. Infect. Dis. **33:**89–94.
10. **Hart, A., B. J. Nowicki, B. Reisner, E. Pawelczyk, P. Goluszko, P. Urvil, G. Anderson, and S. Nowicki.** 2001. Ampicillin-resistant *Escherichia coli* in gestational pyelonephritis: increased occurrence and association with the colonization factor Dr adhesin. J. Infect. Dis. **183:**1526–1529.
11. **Herzer, P. J., S. Inouye, M. Inouye, and T. S. Whittam.** 1990. Phylogenetic distribution of branched RNS-linked multicopy single-stranded DNA among natural isolates of *Escherichia coli*. J. Bacteriol. **172:**6175–6181.
12. **Johnson, J. R.** 1991. Virulence factors in *Escherichia coli* urinary tract infection. Clin. Microbiol. Rev. **4:**80–128.
13. **Johnson, J. R., P. Delavari, M. Kuskowski, and A. L. Stell.** 2001. Phylogenetic distribution of extraintestinal virulence-associated traits in *Escherichia coli*. J. Infect. Dis. **183:**78–88.
14. **Johnson, J. R., M. Kuskowski, E. Denamur, J. Elion, and B. Picard.** 2000. Clonal origin, virulence factors, and virulence. Infect. Immun. **68:**424–425.
15. **Johnson, J. R., M. A. Kuskowski, K. Owens, A. Gajewski, and P. L. Winokur.** 2003. Phylogenetic origin and virulence genotype in relation to resistance to fluoroquinolones and/or extended spectrum cephalosporins and cephamycins among *Escherichia coli* isolates from animals and humans. J. Infect. Dis. **188:**759–768.
16. **Johnson, J. R., A. R. Manges, T. T. O'Bryan, and L. R. Riley.** 2002. A disseminated multi-drug resistant clonal group of extraintestinal pathogenic *Escherichia coli* as a cause of pyelonephritis. Lancet **359:**2249–2251.
17. **Johnson, J. R., S. Moseley, P. Roberts, and W. E. Stamm.** 1988. Aerobactin and other virulence factor genes among strains of *Escherichia coli* causing urosepsis: association with patient characteristics. Infect. Immun. **56:**405–412.
18. **Johnson, J. R., A. C. Murray, A. Gajewski, M. Sulliman, P. Snippes, M. A. Kuskowski, and K. E. Smith.** 2003. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. Antimicrob. Agents Chemother. **47:**2161–2168.
19. **Johnson, J. R., I. Orskov, F. Orskov, P. Goullet, B. Picard, S. L. Moseley, P. L. Roberts, and W. E. Stamm.** 1994. O, K, and H antigens predict virulence factors, carboxylesterase B pattern, antimicrobial resistance, and host compromise among *Escherichia coli* strains causing urosepsis. J. Infect. Dis. **169:**119–126.
20. **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.
21. **Johnson, J. R., A. L. Stell, T. T. O'Bryan, M. Kuskowski, B. Nowicki, C. Johnson, J. M. Maslow, A. Kaul, J. Kavle, and G. Prats.** 2002. Global molecular epidemiology of the O15:K52:H1 extraintestinal pathogenic *Escherichia coli* clonal group: evidence of distribution beyond Europe. J. Clin. Microbiol. **40:**1913–1923.
22. **Johnson, J. R., C. Van der Schee, M. A. Kuskowski, W. Goessens, and A. Van Belkum.** 2002. Phylogenetic background and virulence profiles of fluoroquinolone-resistant clinical *Escherichia coli* isolates from The Netherlands. J. Infect. Dis. **186:**1852–1856.
23. **Kahlmeter, G.** 2000. The ECO-SENS project: a prospective, multinational, multicenter epidemiological survey of the prevalence and antimicrobial susceptibility of urinary tract pathogens–interim report. J. Antimicrob. Chemother. **46**(Suppl. S1)**:**15–22.
24. **Linton, A. H., K. Howe, P. M. Bennett, and M. H. Richmond.** 1977. The colonization of the human gut by antibiotic resistant *Escherichia coli* from chickens. J. Appl. Bacteriol. **43:**465–469.
25. **Manges, A. R., J. R. Johnson, B. Foxman, T. T. O'Bryan, K. E. Fullerton, and L. W. Riley.** 2001. Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group. N. Engl. J. Med. **345:**1007–1013.
26. **Martinez-Martinez, 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.
27. **National Committee for Clinical Laboratory Standards.** 2002. Performance standards for antimicrobial susceptibility testing; twelfth informational supplement (M100–S12), vol. M100–S12. National Committee for Clinical Laboratory Standards, Wayne, Pa.
28. **Ochman, H., and R. K. Selander.** 1984. Standard reference strains of *Escherichia coli* from natural populations. J. Bacteriol. **157:**690–693.
29. **Orskov, F., and I. Orskov.** 1984. Serotyping of *Escherichia coli*. Methods Microbiol. **14:**43–111.
30. **Patton, J. P., D. B. Nash, and E. Abrutyn.** 1991. Urinary tract infection: economic considerations. Med. Clin. North Am. **75:**495–513.
31. **Phillips, I., S. Eykyn, A. King, W. R. Grandsden, B. Rowe, J. A. Frost, and R. J. Gross.** 1988. Epidemic multiresistant *Escherichia coli* infection in West Lambeth health district. Lancet **1:**1038–1041.
32. **Picard, B., J. Sevali Garcia, S. Gouriou, P. Duriez, N. Brahimi, E. Bingen, J. Elion, and E. Denamur.** 1999. The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection. Infect. Immun. **67:**546–553.
33. **Raz, R., B. Chazan, Y. Kennes, R. Colodner, E. Rottensterich, M. Dan, I. Lavi, W. Stamm, and Israeli Urinary Tract Infection Group.** 2002. Empiric use of trimethoprim-sulfamethoxazole (TMP-SMX) in the treatment of women with uncomplicated urinary tract infections, in a geographical area with a high prevalence of TMP-SMX-resistant uropathogens. Clin. Infect. Dis. **34:**1165–1169.
34. **Sannes, M. R., M. San Roman, J. Moya, N. Mora, A. A. Eckhoff, D. N. Williams, J. R. Johnson, and P. K. Peterson.** 2003. Antimicrobial resistance among *Escherichia coli* causing urinary tract infections in Costa Rica: a clinical dilemma. Int. J. Antimicrob. Agents **21:**79–82.
35. **Stamm, W. E., G. W. Counts, K. R. Running, S. Fihn, M. Turck, and K. K. Holmes.** 1982. Diagnosis of coliform infection in acutely dysuric women. N. Engl. J. Med. **307:**463–468.
36. **Stamm, W. E., and T. M. Hooton.** 1993. Management of urinary tract infections in adults. N. Engl. J. Med. **329:**1328–1334.
37. **Stapleton, A., and W. E. Stamm.** 1997. Prevention of urinary tract infection. Infect. Dis. Clin. N. Am. **11:**719–733.
38. **Velasco, M., J. P. Horcajada, J. Mensa, A. Moreno-Martinez, J. Vila, J. A. Martinez, J. Ruiz, M. Barranco, G. Roig, and E. Soriano.** 2001. Decreased invasive capacity of quinolone-resistant *Escherichia coli* in patients with urinary tract infections. Clin. Infect. Dis. **33:**1682–1686.
39. **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.
40. **Warren, J. W., E. Abrutyn, J. R. Hebel, J. R. Johnson, A. J. Schaffer, and W. E. Stamm.** 1999. Guidelines for antimicrobial therapy of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. Clin. Infect. Dis. **29:**745–758.