

Quinolone, fluoroquinolone and trimethoprim/sulfamethoxazole resistance in relation to virulence determinants and phylogenetic background among uropathogenic *Escherichia coli*

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Introduction: The goal of this study was to assess how resistance to quinolones, fluoroquinolones and trimethoprim/sulfamethoxazole relates to the virulence potential and phylogenetic background of clinical *Escherichia coli* isolates.

Methods: Among 150 uropathogens (21% resistant to quinolones, 12% resistant to fluoroquinolones and 29.3% resistant to trimethoprim/sulfamethoxazole), *E. coli* phylogenetic group, 15 virulence-associated genes and 7 O antigens were analysed. Clonal group A (CGA) and genomic PCR profiles were studied among trimethoprim/sulfamethoxazole-resistant isolates.

Results: Isolates susceptible to the three antimicrobial agents were significantly associated with phylogenetic group B2, whereas resistant isolates exhibited shifts to non-B2 groups (quinolone and fluoroquinolone-resistant isolates to group A; trimethoprim/sulfamethoxazole-resistant isolates to group D). Diverse virulence traits, including UTI-associated O antigens, were significantly less frequent among resistant isolates, particularly those resistant to fluoroquinolones (median score, 3.9 virulence factors/strain) and also to quinolones (5.2) or trimethoprim/sulfamethoxazole (6.4), as compared with the corresponding drug-susceptible isolates (median scores of 7.9, 8.6 and 7.9, respectively). Among 44 trimethoprim/sulfamethoxazole-resistant isolates, 3 (6.8%) belonged to CGA. All these 3 CGA strains caused pyelonephritis ($P=0.02$) and exhibited the consensus virulence profile of previously described CGA strains from abroad.

Conclusions: *E. coli* isolates resistant to quinolones, trimethoprim/sulfamethoxazole and especially fluoroquinolones were associated with reductions in virulence traits and shifts to non-B2 phylogenetic groups. Moreover, fluoroquinolone resistance usually occurred in low-virulence *E. coli* group A isolates rather than in isolates from groups B2 and D which had lost virulence traits. CGA accounted for 23% of trimethoprim/sulfamethoxazole-resistant *E. coli* producing pyelonephritis.

Keywords: *E. coli*, virulence traits, urinary tract infections

Introduction

Among human clinical isolates of *Escherichia coli*, rates of antimicrobial resistance have increased substantially during the past two decades, especially in some geographical areas. At the Hospital Vall d'Hebron in Barcelona, in 1987, 57% of *E. coli* isolates from urinary tract infections were resistant to ampicillin, 31% to

trimethoprim/sulfamethoxazole, 7% to the quinolone piperidic acid and none to the fluoroquinolone norfloxacin. By 1995 these prevalences had increased to 63, 37, 20 and 15%, respectively, and by 2002 to 67, 36, 24 and 19%. However, in a Spanish national surveillance study conducted in 2002 among *E. coli* causing community-acquired lower urinary tract infections,¹ resistance to fluoroquinolones approached 20% overall, but varied significantly

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according to sex (28.9% in men versus 19% in women), age (7.1% if <40 years, 15% if 41–60 years, 29.2% if 61–80 years and 33.7% if >81 years), type of urinary infection (24.8% if complicated versus 13.7% if uncomplicated) and geographic region (9.2% in Galicia and Asturias versus 33.3% in Aragon).

Yet, despite the increasing prevalence of resistant *E. coli*, some data suggest that they are less virulent than susceptible *E. coli*. In 1987 Johnson *et al.*² reported that *E. coli* that cause urosepsis in otherwise healthy patients with normal urinary tracts were usually P fimbriated and susceptible to antibiotics, whereas those from compromised patients often were non-P fimbriated but resistant to antibiotics. Shortly after, Picard *et al.*³ noted that carboxylesterase type B2 uropathogenic *E. coli* strains (which correspond to *E. coli* phylogenetic group B2) were more susceptible to antibiotics than carboxylesterase type B1 strains (which correspond to phylogenetic groups A, B1 and D). Others studies confirmed these results.^{4–7} Recently, it has been reported that *E. coli* resistant to quinolones and fluoroquinolones were less virulent than susceptible strains.^{8–10}

However, the relationship between resistance and virulence in *E. coli* is complex. For example, in 2001 Manges *et al.*¹¹ described a new *E. coli* clonal group, designated clonal group A (CGA), which is strongly associated with trimethoprim/sulfamethoxazole resistance and exhibits a robust and distinctive virulence profile. CGA is globally but heterogeneously distributed, affects diverse host populations, infects urinary and non-urinary tract sites, and seems to have emerged as a broadly disseminated epidemic clone.

To gain insight into the role that resistance, phylogenetic background and virulence traits play in the pathogenesis of extra-intestinal pathogenic *E. coli* (ExPEC) infections, we compared phylogenetic groups and virulence traits between *E. coli* urine isolates resistant and susceptible to quinolones, fluoroquinolones and trimethoprim/sulfamethoxazole. In addition, we studied the prevalence of CGA among the trimethoprim/sulfamethoxazole-resistant isolates.

Materials and methods

Isolates

A total of 150 *E. coli* isolates, including 50 each from (i) the urine of women suffering acute uncomplicated cystitis; (ii) the urine of patients with acute pyelonephritis; and (iii) the blood of patients with urinary-source bacteraemia, were selected at the Microbiology Laboratory of Hospital Vall d'Hebron in Barcelona. Only one isolate per patient was analysed.

Clinical criteria for cystitis included the presence of dysuria, urgency and frequency, with or without suprapubic pain or gross haematuria, and absence of flank pain or fever >38°C. Of the 50 cystitis isolates, 46 were from a national surveillance study regarding susceptibility of uropathogens causing community-acquired lower urinary infections¹; the remaining 6 were isolated during 2004, from outpatient women presenting to the emergency room who met the inclusion criteria. The median age was 38.9 years. *E. coli* isolated from patients with pyelonephritis or urinary bacteraemia were published previously.¹² Briefly, they were recovered from inpatients and patients attending at the emergency room, consecutively encountered between 1996 and 2003. Clinical information was obtained by medical record review. The pyelonephritis group comprised 11 men and 39 women (median age, 26 years), whereas the urinary bacteraemia group comprised 17 men and 33 women (median age, 71 years). Conventional

methods were used for urine cultures, blood cultures and *E. coli* identification.

Susceptibility to quinolones, fluoroquinolones and trimethoprim/sulfamethoxazole

Antimicrobial susceptibility was determined using standard disc diffusion techniques, according to the recommendations of NCCLS.¹³ The discs used were Neo-Sensitabs (Rosco, Taastrup, Denmark). For blood isolates the quinolone and fluoroquinolone agents tested were piperidic acid (30 µg) and ciprofloxacin (10 µg), and for urinary isolates piperidic acid (30 µg) and norfloxacin (10 µg). All isolates were tested to trimethoprim/sulfamethoxazole (5.2/240 µg). *E. coli* isolates were stored at –70°C in trypticase soy broth with 5% glycerol until use.

Phylogenetic analysis

The phylogenetic group of origin was determined by a triplex PCR assay,¹⁴ using a combination of three DNA markers (*chuA*, *yjaA* and the DNA fragment TSPE4.C2). The combination of PCR products obtained allowed the *E. coli* isolates to be classified into one of the four major *E. coli* phylogenetic lineages: A, B1, B2 and D.

Virulence genotypes

Fifteen virulence-associated genes were characterized by using previously described methods.¹⁵ The 15 genes included adhesins *papA* (P fimbriae structural subunit), *papG* (P fimbriae adhesin, with alleles I, II and III), *fimH* (type-1 fimbriae), *afa/draBC* (Dr-binding adhesins) and *sfafocDE* (S and F1C fimbriae); toxins *hlyA* (haemolysin) and *cnf* (cytotoxic necrotizing factor); siderophores *iutA* (aerobactin receptor) and *fyuA* (yersiniabactin receptor); capsule synthesis *KpsM* II; serum resistance-associated gene *traT*; invasion of brain endothelium *ibeA*; and pathogenicity island marker *malX*. A multiplex PCR assay, abbreviated to five pools, with appropriate positive and negative controls, was used.¹²

Determination of O antigens

The O antigens O1, O2, O4, O6, O7, O18 and O83 were determined by using antisera produced in the Spanish *E. coli* Reference Laboratory (LREC). These antigens are the most prevalent among uropathogenic *E. coli* in our area.^{16,17} Thus, they will be defined as UTI-associated O antigens.

Virulence factors score

A virulence score was calculated for each isolate as the sum of *pap* (which was counted only once regardless of the number of *pap* elements identified) and all other virulence-associated genes detected.

Detection of clonal group A

The presence of CGA among the 44 trimethoprim/sulfamethoxazole-resistant *E. coli* isolates was determined through the identification of a CGA-specific single nucleotide polymorphism (SNP).¹⁸ Briefly, primers CGAr (5'-CGTGCATCGCCGTTGGAAAG-3') and CGAf (5'-GCTATCTGGCAGACT-3') were used to detect the C288T CGA-specific *fumC* SNP. The amplification product was 175 bp. *E. coli* reference (ECOR) strains 46 and 47 were used as negative and positive controls, respectively.¹⁸

ERIC PCR

Clonal relationships among the 44 trimethoprim/sulfamethoxazole-resistant *E. coli* isolates (42 from this study and two reference strains) were assessed by studying enterobacterial repetitive intergenic consensus (ERIC) PCR patterns of genomic DNA, as generated using primers ERIC1R and ERIC2, as described previously.¹⁹

Data analysis

Images from ERIC PCR were captured digitally (Gel Compar II, version 3.0; Applied Maths, Sint-Martens-Latem, Belgium), enabling estimation of fragment molecular weights and normalization of gel images before the analysis. Bands were assigned initially by using the auto search facility of the software and later were checked manually. Similarity relationships were estimated by using the Pearson correlation coefficient, and cluster analysis was performed by using the Pearson coefficient and the unweighted pair group method with arithmetic averages (UPGMA). None of the ERIC patterns were shown by the software to be 100% similar, even though some were indistinguishable by visual inspection. Isolates with >93% similar ERIC profiles were assumed to be closely related, because in our previous experience, isolates with visually indistinguishable ERIC profiles, plus identical phylogenetic backgrounds and virulence profiles, showed this degree of computer-defined profile similarity.

Statistical methods

Comparisons of proportions involving two groups were tested using a χ^2 test when all expected cell frequencies were ≥ 5 , a χ^2 test with Yates' correction when any expected cell frequency was 3 or 4 and Fisher's exact test (2-tailed) when any expected cell frequency was <3. Comparisons involving virulence scores were assessed using the Mann-Whitney *U*-test. *P* values <0.05 were considered statistically significant.

Results

Quinolone and fluoroquinolone resistance

Of the total of 150 *E. coli* isolates studied, 31 (21%) were resistant to quinolones and 18 (12%) were resistant also to fluoroquinolones. The prevalence of quinolone resistance among the three clinical syndromes was 14% (cystitis), 20% (pyelonephritis) and 28% (urinary bacteraemia), whereas for fluoroquinolone resistance the prevalence was 10% (cystitis and pyelonephritis) and 16% (urinary bacteraemia).

Quinolone-susceptible isolates (Table 1) were statistically associated with the phylogenetic group B2 (81% versus 32% of resistant isolates; *P* < 0.001), whereas quinolone-resistant isolates were significantly associated with group A (36% versus 3% of susceptible; *P* < 0.001) and exhibited similar (albeit non-significant) shifts towards groups B1 and D (Table 1). All of the virulence determinants studied except *iutA* and *ibeA* occurred at least somewhat more frequently among susceptible than resistant isolates (Table 1). Most of these differences were statistically significant, including for *malX*, *papA*, *papG* allele III, *sfa/focDE*, *kpsM II*, *hlyA*, *cnfI*, *fyuA* and UTI-associated O antigens (Table 1). Accordingly, quinolone-susceptible isolates exhibited significantly higher virulence scores than did quinolone-resistant isolates (median score, 8.6 versus 5.2; *P* < 0.001).

Fluoroquinolone-susceptible isolates (Table 1) were also statistically associated with phylogenetic group B2 (79% versus 11% of resistant isolates; *P* < 0.001), whereas fluoroquinolone-resistant

isolates were significantly associated with group A (56% versus 4% of susceptible isolates; *P* < 0.001) and exhibited similar (albeit non-significant) shifts towards groups B1 and D. All of the virulence determinants studied except *iutA* occurred at least somewhat more frequently among fluoroquinolone-susceptible than fluoroquinolone-resistant isolates (Table 1). Most of these differences were statistically significant, including for *malX*, *papA*, *papG* allele II, *fimH*, *sfa/focDE*, *kpsM*, *hlyA*, *cnfI* and *fyuA*. Accordingly, fluoroquinolone-susceptible isolates exhibited a median virulence score of 7.9, which, although slightly lower than that of quinolone-susceptible isolates (8.6), was significantly higher than that of fluoroquinolone-resistant isolates (3.9; *P* < 0.001). Remarkably, while O-UTI antigens were present in 53% of the fluoroquinolone-susceptible isolates, they were absent from all fluoroquinolone-resistant isolates (*P* < 0.001).

Trimethoprim/sulfamethoxazole resistance

Of the 150 isolates studied, 44 (29.3%) were resistant to trimethoprim/sulfamethoxazole, with a distribution among the clinical syndromes of 26% in cystitis and pyelonephritis and 36% in urinary bacteraemia. The trimethoprim/sulfamethoxazole-susceptible and -resistant isolates showed differences in phylogenetic distribution similar to those observed with quinolone and fluoroquinolone resistance, with susceptible isolates being associated with group B2 (82% versus 43%; *P* < 0.001) and resistant isolates with group D (25% versus 8%, respectively; *P* = 0.003). Although only four virulence traits (*malX*, *sfa/focDE*, *hlyA* and *fyuA*) were significantly more prevalent among trimethoprim/sulfamethoxazole-susceptible than trimethoprim/sulfamethoxazole-resistant isolates, the aggregate virulence scores of trimethoprim/sulfamethoxazole-susceptible isolates were significantly higher (median score, 7.9 versus 6.4; *P* = 0.005). Likewise, the O-UTI antigens were approximately twice as prevalent among susceptible isolates compared with resistant isolates (57% versus 25%; *P* < 0.001).

Combined susceptibility phenotype

Additional significant differences were evident when the isolates were grouped according to their combined susceptibility phenotype for the three drug classes, i.e. quinolones, fluoroquinolones and trimethoprim/sulfamethoxazole (Table 2). At the extremes, the 'all susceptible' isolates appeared to be the most virulent (median virulence score 8.1) and exhibited a strong group B2 predominance, whereas those resistant to quinolones and fluoroquinolones but susceptible to trimethoprim/sulfamethoxazole appeared to be the least virulent (median score 2.8) and were all derived from group A. Between these extremes, the apparent degree of virulence of isolates resistant only to quinolones or trimethoprim/sulfamethoxazole was quite similar (median scores 7.1 and 7.8, respectively) and only slightly higher than that of isolates simultaneously resistant to both these drug classes (median score 6.2). Each of these subgroups comprised predominantly group B2 isolates, although to a lesser extent with combined quinolone and trimethoprim/sulfamethoxazole resistance (Table 2). However, with the addition of resistance to fluoroquinolones the inferred degree of virulence dropped dramatically (e.g. for resistance to all three drug classes, median score 4.2) and the phylogenetic background shifted towards group A. These shifts in inferred virulence and phylogenetic background associated with the addition of fluoroquinolone resistance were greatest among isolates susceptible to trimethoprim/sulfamethoxazole.

Virulence of resistant *E. coli*

Table 1. Distribution of phylogenetic groups and virulence determinants in relation to quinolone, fluoroquinolone and trimethoprim/sulfamethoxazole phenotypes among *Escherichia coli* isolates

Category	Specific trait	Prevalence of trait [no. (%)] or virulence score								
		Quinolone			Fluoroquinolone			Trimethoprim/sulfamethoxazole		
		Susceptible (n = 119)	Resistant (n = 31)	<i>P</i> ^a	Susceptible (n = 132)	Resistant (n = 18)	<i>P</i> ^a	Susceptible (n = 106)	Resistant (n = 44)	<i>P</i> ^a
Phylogenetic group	A	4 (3)	11 (36)	<0.001	5 (4)	10 (56)	<0.001	7 (7)	8 (18)	
	B1	6 (5)	4 (13)		7 (5)	3 (17)		4 (4)	6 (14)	
	B2	96 (81)	10 (32)	<0.001	104 (79)	2 (11)	<0.001	87 (82)	19 (43)	<0.001
	D	13 (11)	6 (19)		16 (12)	3 (17)		8 (8)	11 (25)	0.003
Virulence determinants	<i>malX</i>	102 (86)	10 (32)	<0.001	110 (83)	2 (11)	<0.001	92 (87)	20 (45)	<0.001
	<i>papA</i>	87 (73)	16 (52)	0.02	95 (72)	8 (44)	0.02	77 (73)	26 (59)	
	<i>papG</i> allele I	1 (1)	0		1 (1)	0		1 (1)	0	
	<i>papG</i> allele II	57 (48)	12 (39)		65 (49)	4 (22)	0.03	52 (49)	17 (39)	
	<i>papG</i> allele III	33 (28)	3 (10)	0.04	34 (26)	2 (11)		26 (25)	10 (23)	
	<i>fimH</i>	118 (99)	29 (94)		131 (99)	16 (89)	0.04	105 (99)	42 (95)	
	<i>afa/draBC</i>	12 (10)	0		12 (9)	0		9 (8)	3 (7)	
	<i>sfa/focDE</i>	69 (58)	8 (26)	0.001	75 (57)	2 (11)	<0.001	60 (57)	17 (39)	0.04
	<i>kpsM II</i>	101 (85)	15 (48)	<0.001	112 (85)	4 (22)	<0.001	86 (81)	30 (68)	
	<i>hlyA</i>	66 (55)	4 (13)	<0.001	68 (52)	2 (11)	0.001	55 (52)	15 (34)	0.05
	<i>cnfI</i>	50 (42)	3 (10)	0.001	51 (39)	2 (11)	0.02	42 (40)	11 (25)	
	<i>traT</i>	87 (73)	18 (58)		94 (71)	11 (61)		71 (67)	34 (77)	
	<i>iutA</i>	74 (62)	22 (71)		83 (63)	13 (72)		65 (61)	31 (70)	
	<i>fyuA</i>	110 (92)	23 (74)	0.01	122 (92)	11 (61)	0.001	99 (93)	34 (77)	0.01
<i>ibeA</i>	12 (10)	5 (16)		16 (12)	1 (6)		11 (10)	6 (14)		
O antigen	64 (54)	6 (20)	0.001	70 (53)	0	<0.001	59 (57)	11 (25)	<0.001	
Virulence score, median (range)		8.6 (2,12)	5.2 (1,10)	<0.001	7.9 (2,12)	3.9 (1,10)	<0.001	7.9 (1,12)	6.4 (1,11)	0.005

Data are presented as no. (%) of isolates. *malX*, marker for a pathogenicity-associated island from strain CFT073; *papA*, P fimbriae structural subunit; *papG*, P fimbrial adhesin molecule; *fimH*, type-1 fimbriae; *afa/draBC*, Dr-binding adhesins; *sfa/focDE*, S and F1C fimbriae; *kpsMII*, group II capsule synthesis; *hlyA*, haemolysin; *cnfI*, cytotoxic necrotizing factor 1; *traT*, serum resistance-associated outer membrane protein; *iutA*, aerobactin receptor; *fyuA*, yersiniabactin receptor; *ibeA*, invasion of brain endothelium. O antigen, O1, O2, O4, O6, O7, O18 and O83.

^a*P* values were determined using the Pearson χ^2 test for comparisons among phylogenetic groups or virulence determinants and antimicrobial resistance when all expected cell frequency were ≥ 5 ; χ^2 with Yates' correction when any expected cell frequency was 3 or 4; and Fisher's exact test (2-tailed) when any expected cell frequency was < 3 ; and Mann-Whitney *U*-test for comparisons among virulence scores. Only statistically significant differences ($P < 0.05$) are shown.

Clonal group A

According to SNP analysis, CGA accounted for 3 (7%) of the 44 trimethoprim/sulfamethoxazole-resistant isolates. The first of these CGA members was isolated in 1999 from a woman of 75 years of age, the second in February 2002 from a girl of 11 months of age and the third in November 2002 from a woman of 26 years of age, all suffering from pyelonephritis. All three strains were resistant to ampicillin but susceptible to extended-spectrum cephalosporins, carbapenems and β -lactamase inhibitor combination drugs, whereas the first two were susceptible and the third resistant to quinolones and fluoroquinolones. The three strains belonged to phylogenetic group D and had highly uniform virulence profiles that included, in all three, *papA*, *papG* allele II, *fimH*, *kpsM II*, *iutA* and *fyuA*, plus, in the first two isolates, *traT*.

ERIC PCR

At a delineation level of 93% similarity, 35 different ERIC patterns were generated for the 44 trimethoprim/sulfamethoxazole-resistant *E. coli* isolates (Figure 1). The three strains from CGA and the two reference CGA strains (*E. coli* SEQ102 and ECOR 47) shared a

unique ERIC pattern (95.4% similarity), which differed from the profiles of the other trimethoprim/sulfamethoxazole-resistant *E. coli* isolates (86% similar to the nearest neighbour in tree), and comprised cluster II. Two other multiple-isolate clusters were observed. Cluster I comprised two strains (IR62 and IR78), both from group B2 and with the same virulence profile, that included the presence of *malX*, *papA*, *papG* allele II, *fimH*, *sfa/focDE*, *kpsM*, *traT*, *iutA* and *fyuA*. Both produced urinary bacteraemia, in women of age 59 and 75 years, respectively. Cluster III comprised three strains (IR76, IR86 and IR59), all from group D, with a consensus virulence profile that included *papA*, *papG* allele II, *fimH*, *kpsM*, *iutA* and *fyuA*. All produced urinary bacteraemia, in men of age 93, 69 and 77 years, respectively. None of these 8 isolates (3 CGA, 5 non-CGA) were typeable with the seven O antisera used.

Discussion

Our findings suggest that, among human clinical *E. coli* isolates from UTI, resistance to quinolones, fluoroquinolones and trimethoprim/sulfamethoxazole is associated with a lower inferred

Table 2. Distribution of phylogenetic groups and virulence determinants according to combined quinolone, fluoroquinolone and trimethoprim/sulfamethoxazole phenotypes among *Escherichia coli* isolates

	Q-S FQ-S SXT-S isolates <i>n</i> = 94	Q-R FQ-S SXT-S isolates <i>n</i> = 8	Q-R FQ-R SXT-S isolates <i>n</i> = 4	Q-S FQ-S SXT-R isolates <i>n</i> = 25	Q-R FQ-S SXT-R isolates <i>n</i> = 5	Q-R FQ-R SXT-R isolates <i>n</i> = 14
Phylogenetic group						
A	3 (3)	0	4 (100) ^a	1 (4)	1 (20)	6 (43) ^a
B1	4 (4)	0	0	2 (8)	1 (20)	3 (21) ^b
B2	80 (85)	7 (88)	0 ^b	16 (64) ^b	1 (20) ^b	2 (14) ^a
D	7 (7)	1 (13)	0	6 (24) ^b	2 (40)	3 (21)
Virulence determinants						
<i>malX</i>	85 (90)	7 (88)	0 ^a	17 (68) ^b	1 (20) ^b	2 (14) ^a
<i>papA</i>	68 (72)	6 (75)	3 (75)	19 (76)	2 (40)	5 (36) ^b
<i>papG</i> allele I	1 (1)	0	0	0	0	0
<i>papG</i> allele II	46 (49)	6 (75)	0	11 (44)	2 (40)	4 (29)
<i>papG</i> allele III	26 (28)	0	0	7 (28)	1 (20)	2 (14)
<i>fimH</i>	94 (100)	8 (100)	3 (75) ^b	24 (96)	5 (100)	13 (93)
<i>afa/draBC</i>	9 (10)	0	0	3 (12)	0	0
<i>sfa/focDE</i>	56 (60)	4 (50)	0 ^b	13 (52)	2 (40)	2 (14) ^b
<i>kpsM II</i>	79 (84)	7 (88)	0 ^b	22 (88)	4 (80)	4 (29) ^a
<i>hlyA</i>	55 (59)	0 ^b	0 ^b	11 (44)	2 (40)	2 (14) ^b
<i>cnfI</i>	42 (45)	0 ^b	0	8 (32)	1 (20)	2 (14) ^b
<i>traT</i>	65 (69)	4 (50)	2 (50)	22 (88)	3 (60)	9 (64)
<i>iutA</i>	57 (61)	6 (75)	2 (50)	17 (68)	3 (60)	11 (79)
<i>fyuA</i>	88 (94)	8 (100)	3 (75)	22 (88)	4 (80)	8 (57) ^b
<i>ibeA</i>	9 (10)	2 (25)	0	3 (12)	2 (40)	1 (7)
O antigen	54 (58)	5 (63)	0	10 (40)	1 (20)	0 ^a
Virulence score, median (range)	8.1 (2,12)	7.1 (5,8)	2.8 ^b (1,4)	7.8 (2,11)	6.2 (2,10)	4.2 ^a (1,10)

Q, quinolone; FQ, fluoroquinolone; SXT, trimethoprim/sulfamethoxazole.

Virulence determinants are defined as follows: *malX*, marker for a pathogenicity-associated island from strain CFT073; *papA*, P fimbriae structural subunit; *papG*, P fimbrial adhesin molecule; *fimH*, type-1 fimbriae; *afa/draBC*, Dr-binding adhesins; *sfa/focDE*, S and F1C fimbriae; *kpsM II*, group II capsule synthesis; *hlyA*, haemolysin; *cnfI*, cytotoxic necrotizing factor 1; *traT*, serum resistance-associated outer membrane protein; *iutA*, aerobactin receptor; *fyuA*, yersiniabactin receptor; *ibeA*, invasion of brain endothelium. O antigen, O1, O2, O4, O6, O7, O18 and O83.

P-values were determined using the Pearson χ^2 test for comparisons between phylogenetic groups or virulence determinants and antimicrobial resistance when all expected cell frequencies were ≥ 5 ; χ^2 with Yates' correction when any expected cell frequency was 3 or 4; and Fisher's exact test (2-tailed) when any expected cell frequency was < 3 ; and Mann-Whitney *U*-test for comparisons among virulence scores.

^a*P* < 0.001 resistant versus all susceptible.

^b*P* < 0.05 resistant versus all susceptible.

virulence potential and with shifts away from phylogenetic group B2 towards groups A, B1 and/or D. However the magnitude of these shifts is dependent on the specific antimicrobial resistance pattern, it being greater for resistance to fluoroquinolones than for resistance to quinolones, or to trimethoprim/sulfamethoxazole, and varying, among fluoroquinolone-resistant isolates, according to the trimethoprim/sulfamethoxazole phenotype.

The observed depletion for phylogenetic group B2 among fluoroquinolones-resistant isolates is in agreement with Johnson *et al.*¹⁰ However, we did not find the increment in group D observed by those authors¹⁰ (among human but not animal isolates) and others.²⁰ Moreover, Johnson *et al.*¹⁰ found that 36% overall of their fluoroquinolones-resistant isolates belonged to groups A and B1 (27 and 9%, respectively), compared with 73% observed in our study (i.e. 56% plus 17%). However, in a more recent study involving cystitis isolates from Israel, Johnson *et al.*²¹ did also find that the majority (67%) of fluoroquinolone-resistant isolates were derived from group A. The significantly lower prevalence of most

virulence determinants and of UTI-associated O antigens that we observed among resistant isolates was also observed in previous studies,^{8,10,20} showing that these are general characteristics of fluoroquinolone-resistant human clinical isolates.

When the isolates were analysed according to combined resistance phenotypes for quinolones, fluoroquinolones and trimethoprim/sulfamethoxazole, despite the fact that the resulting subgroups were small, some additional interesting relationships emerged. First, among trimethoprim/sulfamethoxazole-susceptible isolates, the 4 isolates resistant to fluoroquinolones (and, hence, also to quinolones) derived exclusively from group A, whereas 7 of the 8 quinolone-resistant (but fluoroquinolone-susceptible) isolates were from group B2. Second, corresponding to these phylogenetic differences, 63% of our quinolone-resistant (but fluoroquinolone and trimethoprim/sulfamethoxazole-susceptible) isolates, but none of the corresponding fluoroquinolone-resistant isolates, exhibited UTI-associated O antigens. Third, however, despite their group B2 predominance, isolates resistant exclusively to quinolones did not

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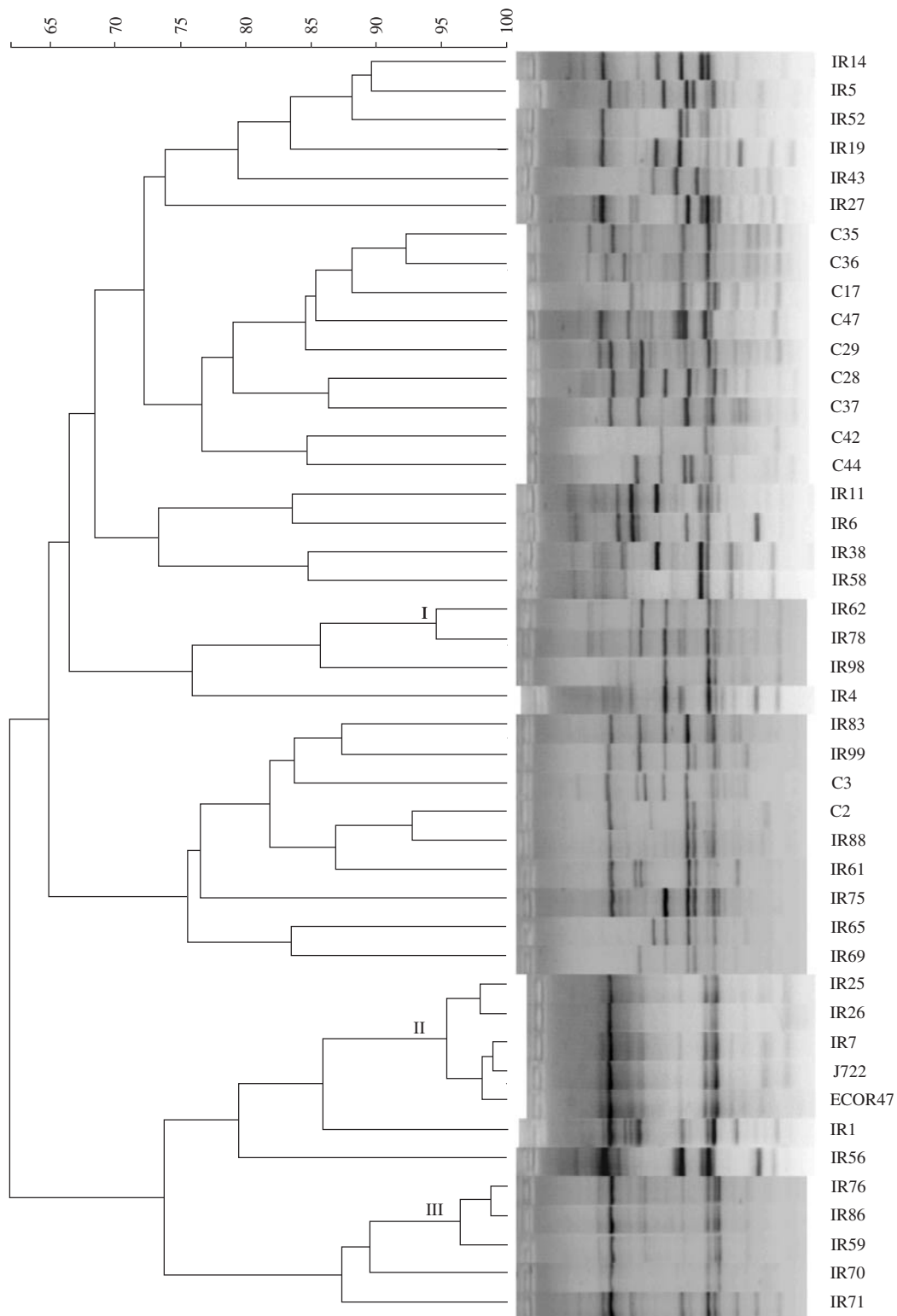


Figure 1. UGPMA clustering of ERIC profiles of 42 *Escherichia coli* isolates resistant to trimethoprim/sulfamethoxazole and two reference strains (J722 and ECOR 47).

carry the genes *hlyA* and *cnf1*, which also were reduced among the *E. coli* group B2 isolates resistant to nalidixic acid reported by Horcajada *et al.*²² Likewise, neither of the fluoroquinolone-resistant isolates from group B2 carried the genes *sfafocDE* and

hlyA, consistent with previously reported findings.²⁰ Fourth, resistance to fluoroquinolones seemed to be the main marker associated with a low inferred virulence potential. Isolates resistant to quinolones and fluoroquinolones but susceptible to trimethoprim/

sulfamethoxazole appeared to be the least virulent overall (median virulence score of 2.8, which is lower than the median scores of 3.2 among the 15 isolates from group A and of 4.0 among the 10 isolates from group B1). Moreover, among isolates resistant to quinolones, whether susceptible or resistant to trimethoprim/sulfamethoxazole, the added presence of fluoroquinolone resistance was associated with a significant drop in virulence characteristics.

Notably, the 'quinolone-only' resistant isolates (with retained susceptibility to fluoroquinolones and trimethoprim/sulfamethoxazole) were mainly derived from group B2, whereas isolates resistant to both quinolones and fluoroquinolones (but with retained susceptibility to trimethoprim/sulfamethoxazole) were derived from group A. This observation led us to suspect that these two subgroups represent distinct populations, rather than the fluoroquinolone-resistant isolates having emerged from the fluoroquinolone-susceptible population by acquisition of additional resistance capabilities. Quinolone-resistant isolates could have derived predominantly from susceptible *E. coli* group B2 isolates (possibly within a human host), whereas fluoroquinolone-resistant isolates could have derived from susceptible *E. coli* group A isolates (possibly within an animal host), by acquiring resistance mutations in *gyrA* (\pm *parC*) in the context of quinolone (or fluoroquinolone) exposure. Further study is needed to define the respective origins of these two groups of resistant isolates, so that appropriate preventive measures can be designed and implemented.

It is well known that most of the virulent *E. coli* extraintestinal isolates belong to the phylogenetic group B2 and (to a lesser extent) group D, whereas most commensal isolates belong to group A.²³ This fact led us to hypothesize that, since the use of fluoroquinolones could eliminate the susceptible bacteria in the intestine, due to the much higher number of commensals in this niche, the probability that they would emerge as fluoroquinolone resistant is much greater than for the few pathogens that also might be present. This could allow the less pathogenic resistant isolates from group A to cause disease, mainly in susceptible hosts (who also likely would be the most antibiotic-exposed).

In this study, trimethoprim/sulfamethoxazole-susceptible and -resistant isolates exhibited different phylogenetic backgrounds, with the resistant isolates being disproportionately from group D, as in a previous study.²⁴ These resistant isolates were also associated with a significantly decreased prevalence of certain virulence traits, resulting in a slightly (but still statistically significant) reduced inferred virulence potential compared with susceptible isolates (median virulence scores 6.4 versus 7.9, respectively). At first consideration, this would appear to conflict with the results of a previous study of cystitis isolates, in which trimethoprim/sulfamethoxazole resistance was not associated with a reduced prevalence of virulence factors.⁹ However, when we considered only the *E. coli* that were resistant exclusively to trimethoprim/sulfamethoxazole, the virulence differences in comparison with susceptible isolates virtually disappeared, suggesting that the virulence differences noted for the trimethoprim/sulfamethoxazole-resistant population as a whole were due largely to the confounding effects of concurrent quinolone and fluoroquinolone resistance.

Although CGA was one of the most prevalent clonal groups among the trimethoprim/sulfamethoxazole-resistant isolates, it accounted for only three (7%) of these isolates. The three CGA strains were essentially identical to American CGA strains,^{11,25} with respect to phylogenetic group D background, virulence traits, ERIC pattern and antimicrobial resistance profile, except that one

isolate was also resistant to fluoroquinolones. The latter isolate represents, to our knowledge, the second known instance of documented fluoroquinolone resistance in a CGA member, with the first such strain (strain ESB05-00) having been isolated in Calgary, Alberta, in 2000.²⁶ The fact that the first of the present three CGA strains was isolated in 1999, whereas the second and third were not isolated until 2001, and no additional CGA isolates were encountered through 2004, suggests that in our locale CGA does not seem to spread as an epidemic clone. In recent surveys, in the US, CGA has accounted for 15% of all trimethoprim/sulfamethoxazole-resistant *E. coli* isolates,²⁴ and for up to 50% of trimethoprim/sulfamethoxazole-resistant isolates producing acute uncomplicated cystitis and pyelonephritis in women,²⁷ whereas in Europe *E. coli* CGA has accounted for only 5.3% of total trimethoprim/sulfamethoxazole-resistant isolates.²⁴ In our study, while CGA accounted for 7% of trimethoprim/sulfamethoxazole-resistant isolates overall, it appeared to exhibit some degree of syndrome specificity, accounting for none of the trimethoprim/sulfamethoxazole-resistant cystitis and urinary bacteraemia isolates, but for 23% of trimethoprim/sulfamethoxazole-resistant pyelonephritis isolates ($P = 0.02$). This suggests that CGA is, at least in our locale, particularly pyelonephritogenic, as noted also for the closely related O15:K52:H1 clonal group.²⁸ Collectively, the available data indicate that CGA has a global but heterogeneous geographical distribution. Likely its dissemination and emergence has been more difficult in areas such as Spain where, since the mid-1980s, more than one-third of *E. coli* clinical isolates were resistant to trimethoprim/sulfamethoxazole,^{29,30} such that the ecological niche of resistant strains was already occupied.

In summary, we found that, among *E. coli* isolates from patients with various UTI syndromes, resistance to quinolones, fluoroquinolones and trimethoprim/sulfamethoxazole was associated with a decreased prevalence of virulence genes and shifts to non-B2 phylogenetic groups, with these changes being most marked among isolates resistant to fluoroquinolones. Strains from CGA accounted for 7% of our trimethoprim/sulfamethoxazole-resistant isolates, all from patients with pyelonephritis, suggesting that in our area CGA is particularly pyelonephritogenic.

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