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European Emergence of Ciprofloxacin-Resistant *Escherichia coli* Clonal Groups O25:H4-ST 131 and O15:K52:H1 Causing Community-Acquired Uncomplicated Cystitis[▽]

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A total of 148 *E. coli* strains displaying reduced susceptibility to ciprofloxacin (MIC ≥ 2 μg/ml) and causing uncomplicated urinary tract infections in eight European countries during 2003 to 2006 were studied. Their phylogenetic groups, biochemical profiles, and antibiotic susceptibilities were determined. Determination of the O:H serotype, pulsed-field gel electrophoresis (PFGE), randomly amplified polymorphic DNA (RAPD) PCR, and multilocus sequence typing provided additional discrimination. The majority (82.4%) of the microorganisms (122/148) carried resistance to two or more additional drugs, with the pattern ciprofloxacin–trimethoprim-sufamethoxazole–tetracycline–ampicillin being the most represented (73 strains out of 148; 49.3%). Extended-spectrum beta-lactamase production was detected in 12/148 strains (8.1%), with CTX-M-15 being the most-common enzyme. Six strains out of the whole collection studied (4.0%) contained a *qnrB*-like gene. Overall, 55 different PFGE or RAPD PCR profiles could be distinguished, indicating a substantial heterogeneity. However, about one-third (51/148) of the strains belonged to two clonal groups: O15:K52:H1 (phylogenetic group B2, lactose-nonfermenting variant, ciprofloxacin MIC of 16 μg/ml) and O25:H4 sequence type 131 (ST-131) (phylogenetic group D, ciprofloxacin MIC of ≥32 μg/ml). With the exception of Poland, strains of these two groups were isolated in samples from all participating countries but more frequently in samples from Spain and Italy. In some representative strains of the two main clonal groups, alterations in GyrA and ParC were the basic mechanism of fluoroquinolone resistance. In some members of the O25:H4 ST-131 group, displaying a ciprofloxacin MIC of >32 μg/ml, additional OmpF loss or pump efflux overexpression was found. In the Mediterranean area, strains belonging to these two clonal groups played a major role in determining the high rate of fluoroquinolone-resistant *E. coli* strains observed in the community.

Uncomplicated urinary tract infections (UTIs) are among the most prevalent infectious diseases mainly affecting women. Between one-quarter and one-half of all women experience a UTI at some time (27).

The overall etiology has not changed in recent years, and *Escherichia coli* remains by far the most-common uropathogen, accounting for >80% of all positive cultures (19). However, the management of these infections is becoming complicated due to the emergence of resistance to several first-line antimicrobial agents in this primary pathogen (13).

In fact, in *E. coli* bacteria, ampicillin and sulfamethoxazole-trimethoprim resistance rates have reached 20% to 50% worldwide. In Europe, increasing percentages of ciprofloxacin-resistant mutants have been observed in the community in Spain, Portugal, and Italy (10, 18).

The results of a recent surveillance survey, the ARESC (Antimicrobial Resistance Epidemiological Survey on Cystitis) study, have shown high rates (>10%) of ciprofloxacin-resistant *E. coli* in Spain, Italy, and Russia and the emergence of this problem in all countries participating into this epidemiological survey, with rates ranging from 1.4% (France) to 6.7% (Poland) (26).

The emergence and dissemination of fluoroquinolone-resistant *E. coli* strains might have two possible explanations: the selection of different mutant strains, often attributed to local antimicrobial-prescription habits, or, alternatively, clonal spread. The aim of this study was to assess the extent of clonality within the ARESC collection of ciprofloxacin-intermediate and -resistant *E. coli* strains in order to understand the basis for the emergence and dissemination of this pathogen in the community of eight European countries.

MATERIALS AND METHODS

Bacterial strains. One hundred forty-eight *E. coli* pathogens displaying reduced susceptibility to ciprofloxacin (MIC ≥ 2 μg/ml) were selected from among 1,941 *E. coli* strains causing community-acquired uncomplicated UTI. Strains were isolated during the years 2003 to 2006 from 32 widely dispersed centers in eight different European countries participating in the ARESC study (26) and then sent to the Institute of Microbiology, University of Genoa, Italy, where they were stored at −80°C for further analysis.

The contribution of each country was as follows: Spain, 55 strains; Russia, 41; Italy, 29; Germany, 9; Poland, 6; France, 5; Hungary, 2; and Austria, 1. One ciprofloxacin-resistant strain isolated in The Netherlands was not properly stored and was not available for this study.

All 148 isolates were reidentified to the species level by using an API 20E system (bioMèrieux, Marcy l'Etoile, France) at the Institute of Microbiology, University of Genoa, Italy.

For all *E. coli* strains, the ability to ferment lactose was evaluated by employing MacConkey agar plates (Oxoid, Milan, Italy).

The 148 *E. coli* strains studied were isolated from 148 women (mean age,

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44.1 ± 15.2 [mean ± standard deviation]) affected by uncomplicated UTI. A large percentage of these patients had experienced at least three episodes of infection (33.8%) and received at least a single course of antimicrobial therapy (38.1%) during the last year prior to the isolation of the *E. coli* strains studied.

Susceptibility tests. The MICs of ampicillin, amoxicillin-clavulanic acid, cefuroxime, ciprofloxacin, amdinocillin, nalidixic acid, nitrofurantoin, and trimethoprim-sulfamethoxazole were determined by the broth microdilution method, while the MICs of fosfomycin were determined by the agar dilution method in Mueller-Hinton agar supplemented with glucose-6-phosphate (25 μg/ml) according to the Clinical Laboratory Standards Institute (CLSI) recommendations (6).

Susceptibility to tetracycline was determined by using the disk diffusion test according to CLSI criteria (7).

Testing synergy between clavulanate and beta-lactams: double-disk assay and quantitative Etest. The double-disk synergy test was performed as a standard disk diffusion assay on Mueller-Hinton agar (Oxoid, Milan, Italy). Disks containing aztreonam, ceftazidime, cefepime, ceftriaxone, and cefotaxime (30 μg each) were placed at a 30-mm distance (center to center) around a disk containing amoxicillin (20 μg) plus clavulanic acid (10 μg). Enhancement of the inhibition zone toward the amoxicillin-plus-clavulanic acid disk, indicating a synergy between clavulanic acid and any one of the test antibiotics, was taken as presumptive evidence of extended-spectrum beta-lactamase (ESBL) production. The synergistic activity of clavulanate with both ceftazidime and cefotaxime was confirmed by means of Etest special strips (AB Biodisk, Solna, Sweden) containing ceftazidime-ceftazidime plus clavulanic acid and cefotaxime-cefotaxime plus clavulanic acid. The production of ESBLs was suggested if clavulanic acid caused a decrease of at least three dilutions in the MICs of the above-mentioned drugs.

Phylotyping. The *E. coli* phylogenetic group (A, B1, B2, or D) was determined by using an established multiplex PCR assay (5).

Molecular characterization of ESBL determinants. The 12 phenotypic putative ESBL producers and their transconjugants were screened for the presence of bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ genes by PCR using primers and conditions described by other authors (25).

The complete nucleotide sequences of group 1 $bla_{CTX\text{-}M}$ genes were determined on both strands by direct sequencing of the PCR products. The PCR products were purified by using a Wizard SV gel and PCR cleanup system (Promega, United States) and sequenced as previously described (3). The analysis and comparison of the nucleotide sequences were carried out with the help of programs available at the NCBI web interface (http://www.ncbi.nlm.nih.gov).

Transfer of ESBL resistance. Conjugation experiments were carried out between each ESBL-producing isolate and *Escherichia coli* K12 J53 Rif^r or Sm^r in broth medium. Transconjugants were selected on rifampin or streptomycin (256 μg/ml and 200 μg/ml, respectively) and either ceftazidime (2 μg/ml) or cefotaxime (2 μg/ml).

Screening for *qnr* genes. All isolates were screened for the presence of the *qnr* genes (*qnrA*, *qnrB*, and *qnrS*) by using previously described primers and amplification conditions (4). *E. coli* K12 J53 pmG252, pmG298, and pmG306 strains were used as positive *qnrA1*, *qnrB1*, and *qnrS1* controls.

PFGE. The genetic relatedness of *E. coli* isolates was determined by molecular typing using pulsed-field gel electrophoresis (PFGE).

Genomic DNA extracted and digested with XbaI (New England Biolabs, Inc., MA) as described by Maslow et al. (23) was separated by PFGE using a CHEF DRII device (Bio-Rad, Hercules, CA). The running conditions were as follows: 30 h at 11.3°C at a voltage of 170 V, ramped with an initial forward time of 5 s and a final forward time of 40 s. After electrophoresis, gels were stained with ethidium bromide and photographed.

The banding patterns were interpreted visually by following published guidelines (37).

RAPD. Randomly amplified polymorphic DNA (RAPD) analysis was performed by using, separately, two decameric primers, primer 1247 (5′-AAGAG CCCGT) and primer 1283 (5′-GCGATCCCCA), and PCR conditions described by Wang et al. (38). The RAPD patterns were considered to be different when the profiles differed by at least one band.

Serotyping. Serotyping was done by using *E. coli* O and H antisera purchased from Denka Seiken Co. Ltd. (Tokyo, Japan) and Statens Seruminstitut (Copenhagen, Denmark), respectively, according to the manufacturers' instructions. *E. coli* 2P9 (serotype O15:H1), *E. coli* 171-02, and *E. coli* TE-1 (serotype O25:H4) were used as controls.

PCR detection of the O15:K52:H1 clonal group. To confirm the serotype results for all lactose-nonfermenting *E. coli* strains (22), these were further investigated by gene-specific PCR to detect the O15:K52:H1 clonal group as described by Johnson et al. (16). A previously characterized O15:K52:H1 *E. coli* strain (*E. coli* 2P9) was used as the positive control (16). In addition, because members of the O15:K52:H1 clonal group were described as being not typeable by PFGE (32), all lactose-fermenting, PFGE-refractory strains were tested.

Sequence type (ST) determination. Multilocus sequence typing (MLST) was performed on 22 representative isolates of the O25:H4 clonal group characterized by nonidentical PFGE profiles, following the recommended procedure at the *E. coli* MLST web site (http://web.mpiib-berlin.mpg.de/mlst/dbs/Ecoli).

PCR amplification and DNA sequencing of *gyrA* and *parC*. The *gyrA* and *parC* gene fragments of five *E. coli* strains of the O25:H4 ST-131 clonal group (representing five nonidentical PFGE profiles, A, A1, A3, A6, and A7, and the three different levels of ciprofloxacin resistance found) and of one representative strain of the O15:K52:H1 clonal group (all members of this clonal group have the same RAPD profile and the same ciprofloxacin MIC) were amplified by using previously described PCR conditions and primers (39). PCR amplimer purification and sequencing and comparison of the nucleotide sequences were carried out as described above.

OMP profile and LPS analysis. Outer membrane proteins (OMPs) were prepared with *N*-lauryl-sarcosine by using a modification of the method of Sawai et al. (35). Samples were subjected to denaturing PAGE by using a discontinuous Tris-glycine buffer system in 12% polyacrylamide gels. Lipopolysaccharide (LPS) extracts were prepared by boiling bacterial OMP preparations for 10 min in sample buffer. After cooling, 50 mg of proteinase K was added and samples were incubated overnight at 55°C and boiled at 100°C for 10 min. LPS profiles were obtained by electrophoresis of the protease-resistant LPS preparations in 12% polyacrylamide gels and visualized by silver staining. *E. coli* strain HB 101 was used as a fluoroquinolone-susceptible control for OMP and LPS profile analysis.

Organic-solvent tolerance. Overexpression of AcrAB was measured indirectly by using the organic-solvent tolerance assay described by Wang et al. (39). The appearance of confluent growth in the presence of a 3:1 hexane/cyclohexane mixture was interpreted as being positive for AcrAB overexpression.

Statistical analysis. Statistical analysis was performed by using the Student *t*, Fisher's exact, and chi-square tests. The threshold for statistical significance was a P value of <0.001.

RESULTS

Clinical data. In comparison to the ciprofloxacin-susceptible counterparts isolated in the ARESC survey (1,793 strains), *E. coli* strains with reduced susceptibility to fluoroquinolones were significantly associated with recurrences, prior fluoroquinolone treatment, older age, and menopause ($P < 0.001$), while diabetes mellitus was not found to be a significant risk factor ($P = 0.1$).

Biochemical identification of the strains. The great majority of the strains (126/148; 85%) were able to ferment lactose on MacConkey agar plates, but an unusually high percentage of lactose-nonfermenting strains was observed (22/148; 15%). However, these 22 *E. coli* isolates were positive in an *O*-nitrophenyl-β-D-galactopyranoside test, indicating that lack of fermentation was not due to reduced β-galactosidase activity, but to other alterations (i.e., lack of permease).

More than half of the strains (91/148; 61.5%) belonged to two biochemical profiles (API codes 5144572, 58 strains, and 5044552, 33 strains). All the remaining isolates were distributed in 16 different biochemical profiles. The majority of lactose-nonfermenting strains (19/22) had the same biochemical profile, corresponding to the second-most-common one observed (5044552).

Susceptibility tests. Table 1 shows the susceptibility patterns of *E. coli* strains with reduced susceptibility to ciprofloxacin. Fosfomycin and amdinocillin emerged as the most-active drugs tested (97.9% and 91.8% of susceptible strains, respectively), followed by nitrofurantoin (86.3%).

The difference in the rates of fosfomycin and amdinocillin resistance between *E. coli* with reduced susceptibility to ciprofloxacin and the 1,793 susceptible strains collected was not

TABLE 1. Susceptibility of 148 ciprofloxacin-intermediate and -resistant *E. coli* isolates to selected drugs[a]

Antibiotic	MIC range	MIC_{50}	MIC_{90}	% S	% I	% R
Ciprofloxacin	2 to 256	32	128	0	2	98
Nalidixic acid	>128	>128	>128	0	0	100
Fosfomycin	≥1 to 256	2	8	97.9	1.4	0.7
Nitrofurantoin	2 to 256	16	64	86.3	7.5	6.2
Ampicillin	2 to 128	>128	>128	10.3	2.0	87.7
Amoxicillin-clavulanic acid	2/1 to 128/64	8/4	16/8	68.9	20.3	10.8
Amdinocillin	≥12 to >128	1	8	91.8	3.4	4.8
Cefuroxime	0.5 to 128	8	32	66.2	21.7	12.1
Trimethoprim-sulfametoxazole	≤0.015/0.30 to >16/304	>16/304	>16/304	30.2	0	69.8
Tetracycline[b]	NA	NA	NA	31.5	0	68.5

[a] MIC values are μg/ml. S, susceptible; I, intermediate; R, resistant; NA, not available.
[b] Susceptibility was determined by using the disk diffusion test.

statistically significant (P values, 0.7 and 0.06, respectively). The rates of resistance to all the remaining antimicrobials were significantly higher among *E. coli* strains categorized as intermediate or resistant to ciprofloxacin than among susceptible ones ($P < 0.0001$) (data not shown).

Among ciprofloxacin-nonsusceptible strains, MICs for this fluoroquinolone ranged from 2 to 256 μg/ml (modal MIC, 32 μg/ml).

The isolates characterized by the highest MICs (128 and 256 μg/ml) were isolated from Spain (8 strains), Russia (4), Italy, and Poland (2 each).

The majority (82.4%) of the microorganisms (122/148) carried resistance to two or more additional drugs, with the pattern ciprofloxacin–trimethoprim-sulfamethoxazole–tetracycline–ampicillin being the most-represented one (73 out of 148 strains; 49.3%).

ESBL production. Twelve (6 isolated in Italy, 3 in Spain, and 3 in Russia) out of 148 strains (8.1%) were ESBL positive by the double-disk synergy test and quantitative Etest. Molecular characterization by PCR showed that of the 12 putative ESBL producers (and their transconjugants), 2 carried a bla_{SHV} gene and 10 a $bla_{CTX\text{-}M}$ gene. Subsequent PCR and sequencing with group 1 blaCTX-M-specific primers showed the presence of CTX-M-15 in six isolates (four from Italy, two from Russia).

Plasmid-mediated ciprofloxacin resistance. Six strains out of the whole collection studied (4.0%) contained a *qnrB*-like gene. These strains, characterized by ciprofloxacin MICs ranging from 2 to 128 μg/ml, were isolated in Spain (3 strains), Italy (2), and Russia (1). One of the two Italian strains produced an SHV-type ESBL.

Isolates carrying *qnrA*-like or *qnrS*-like genes were not detected.

Phylogenetic groups. Approximately half of the strains (49.3%) belonged to phylogenetic groups B2 and D, and the remaining half (50.7%) to the less-virulent, commensal phylogenetic groups A and B1.

The most-common phylogenetic group was A (65/148; 44%), followed by D (37/148; 25%), B2 (36/148; 24.3%), and B1 (10/148; 6.7%). This ranking order was maintained for the three countries providing the largest numbers of isolates (Spain, Russia, and Italy).

The majority (35/36) of the strains belonging to phylogenetic group B2 had the same biochemical profile (API code 5144572), were able to ferment lactose, and were inhibited by a concentration of ciprofloxacin of ≥32 μg/ml.

The majority (21/22) of the lactose-nonfermenting *E. coli* strains belonged to phylogenetic group D, had the same biochemical profile (API code 5044552), and were inhibited by the same concentration of ciprofloxacin, 16 μg/ml.

The results of combing phylogenetic groups, biochemical profiles, lactose fermentation abilities, and susceptibility data showed the presence of two groups of microorganisms exhibiting the same set of characteristics and was suggestive of the possible presence of two main clones or clonal groups in our collection (Tables 2 and 3).

PFGE profiles. Finer resolution of the clonal relationship was obtained by PFGE analysis.

One hundred fifteen strains were typeable by PFGE analysis, while 33 were not. The high incidence of PFGE-refractory strains was related to the 22 lactose-nonfermenting *E. coli* strains; they were all not typeable, despite the introduction of technical modifications. Neither using different restriction enzymes nor changing enzymatic restriction and electrophoresis conditions helped to circumvent this problem.

Overall, 49 different profiles could be distinguished by PFGE, indicating a substantial heterogeneity among the ciprofloxacin-intermediate and -resistant isolates. The PFGE profile designated A was found in five strains circulating in Italy and Spain, and its A3 variation (differing by 5 bands from profile A) was found in five strains isolated in Spain and Russia; the remaining variations were found in 26 other microorganisms from Italy, Spain, France, Germany, Hungary, and Russia (Table 2).

RAPD PCR profiles. To assess the clonal relationships within lactose-nonfermenting strains, all 21 phylogenetic group D isolates and one group B2 isolate were subjected to RAPD PCR with two different primers. Overall, six different profiles could be distinguished by this method. The most-represented profile related to phylogenetic group D was found in 16 strains circulating in Spain, Germany, Italy, France, and Russia.

Serotyping. Only 35 lactose-fermenting, phylogenetic group B2 and 22 lactose-nonfermenting, phylogenetic group D strains were serotyped. All lactose-fermenting strains belonging to the first group had serotype O25:H4, and 16 out of 22 lactose-nonfermenting *E. coli* strains had serotype O15:H1.

TABLE 2. Phylogenetic groups, PFGE profiles, and geographic distribution of 126 lactose-fermenting *E. coli* strains with reduced susceptibility to ciprofloxacin

Phylogenetic group	PFGE profile[a]	Country (no. of strains)	Total no. of strains
B2	A	Italy (4), Spain (1)	5
	A3	Spain (3), Russia (2)	5
	Other A-related profiles	Spain (10), Russia (5), Italy (4), France (2), Germany (2) Hungary (1), Austria (1)	25
A	B	Spain	1
	Other B-related profiles	Hungary, Germany	2
	C	Spain	1
	Other C-related profiles	Spain	2
	D	Spain	1
	Other D-related profiles	Spain	2
	E	Spain	1
	Other E-related profiles	Spain	1
	F	Spain	1
	Other F-related profiles	Poland, Russia	2
	G	Spain	1
	Other G-related profiles 3	Spain	1
	H	Russia	2
	Other H-related profiles	Russia (3), Poland (1), Spain (1)	5
	I	Russia (2)	2
	Other I-related profiles	Russia (1), Spain (1)	2
	J	Italy	1
	Other J-related profiles	Italy	1
	K	Italy	2
	Other K-related profiles	Italy	1
	L	Russia	2
	Other L-related profiles	Russia	3
	M	Russia	1
	Other M-related profiles	Russia	2
	N	Russia	1
	Other N-related profiles	Russia	1
	Other unique profiles	Italy (6), Russia (5), Spain (5), Germany (2)	18
	NT	Spain (4), Russia (1)	5
D	O	Italy (2), Spain (1)	3
	Other unique profiles	Spain (5), Italy (4), Germany (1), Russia (1)	11
	NT	Russia, Spain	2
B1	P	Russia, Spain	2
	Other unique profiles	Poland (2), Italy (1), Spain (1)	4
	NT	Poland (2), Italy (1), Russia (1)	4

[a] NT, not typeable.

PCR detection of the O15:K52:H1 clonal group. The fumC primers were tested against all 22 lactose-nonfermenting, *E. coli* phylogenetic group D isolates and against all 11 lactose-fermenting, PFGE-refractory strains. PCR analysis demonstrated that the 16 lactose-nonfermenting, *E. coli* phylogenetic group D and serotype O15:H1 strains were O15:K52:H1 clonal group members (Tables 3 and 4). All remaining strains were negative.

MLST. To more rigorously assess the phylogenetic relationships within clonal group O25:H4, 22 isolates representing nonidentical PFGE profiles underwent MLST analysis. All 22 strains were found to belong to ST-131, indicating that all 35

TABLE 3. Phylogenetic groups, biochemical profiles, serotype, RAPD profiles, and geographic distribution of 22 lactose-nonfermenting *E. coli* strains with reduced susceptibility to ciprofloxacin

Phylogenetic group	Biochemical profile (API code)	Serotype O15:K52:H1	RAPD profile	Country (no. of strains)	Total no. of strains
D	5044552	+	Q	Spain (8), France (2), Germany (2), Italy (2), Russia (2)	16
		−	R	Russia	2
		−	S	France	1
	7044552	−	T	Germany	1
	5044572	−	U	Spain	1
B2	5144532	−	V	Russia	1

TABLE 4. Geographic distribution of *E. coli* strains belonging to clonal groups O25:H4 ST-131 and O15:K52:H1

Country (total no. of *E. coli* strains collected during the ARESC study)	No. of *E. coli* strains with ciprofloxacin MICs of ≥2 μg/ml (%)	No. of O25: H4 ST-131 and O15:K52:H1 *E. coli* strains/no. of *E. coli* strains with ciprofloxacin MICs of ≥2 μg/ml (%[a])
Austria (62)	1 (1.6%)	1/1
France (409)	6 (1.4%)	4/6
Germany (243)	9 (3.7%)	4/9
Hungary (52)	2 (3.8%)	1/2
Italy (239)	29 (12.2%)	10/29 (34.4%)
Poland (90)	6 (6.7%)	0/6
Russia (302)	41 (13.6%)	9/41 (21.9%)
Spain (515)	55 (10.7%)	22/55 (40.0%)

[a] Percentages were reported only for those countries providing more than 10 strains.

lactose-fermenting, phylogenetic group B2, serotype O25:H4 isolates belonged to the O25:H4 ST-131 clonal group. Members of this clonal group were found in all countries with the exception of Poland (Table 4).

Identification of mutations in *gyrA* and *parC* DNA. Five isolates belonging to the O25:H4 ST-131 clonal group and representative of three different levels of ciprofloxacin resistance (*E. coli* 77 and 138, ciprofloxacin MIC of 32 μg/ml; *E. coli* 94 and 120, ciprofloxacin MIC of 64 μg/ml; and *E. coli* 88, ciprofloxacin MIC of 128 μg/ml) and one isolate belonging to the O15:K52:H1 clonal group (*E. coli* 51, ciprofloxacin MIC of 16 μg/ml) were analyzed. All five assayed isolates belonging to the O25:H4 ST-131 clonal group possessed two mutations in *gyrA* and *parC*, resulting in alterations at positions Ser-83 and Asp-87 in GyrA and positions Ser-80 and Glu-84 in ParC, respectively, compared to the amino acids in wild-type *E. coli* K12. The O15:K52:H1 clonal group representative strain had two mutations in *gyrA* (the same observed in the other strains studied) but a single mutation in *parC*, resulting in an alteration at position Ser-80 (Table 5).

OMP profile, LPS analysis, and organic-solvent tolerance. The six strains chosen for DNA *gyrA* and *parC* sequencing were further characterized for OMP expression, LPS alteration, and organic-solvent tolerance.

All six *E. coli* isolates expressed both OmpC and OmpF, the same as the fluoroquinolone-susceptible control strain *E. coli* HB 101, and displayed the smooth LPS phenotype, with the exception of *E. coli* 94 and 120 (ciprofloxacin MIC of 64 μg/ml) that expressed only OmpC.

All strains tested grew on LB agar overlaid with 99% hexane; only one strain (*E. coli* 88, ciprofloxacin MIC of 128 μg/ml) was able to grow in a mixture of 3:1 hexane/cyclohexane, and this strain was designated organic solvent tolerant (Table 5).

DISCUSSION

Increasing resistance to first-line antibiotics has progressively complicated the management of urinary infection, necessitating revised empirical treatment approaches (27). Until recently, fluoroquinolones were reliably active against *E. coli* bacteria causing uncomplicated UTI worldwide. However, the prevalence of fluoroquinolone-resistant *E. coli* in the community has reached alarming levels in some parts of the world (18), jeopardizing their usefulness.

A further threat is the evidence that fluoroquinolone-resistant *E. coli* strains circulating in the community are often multiresistant (19) and can carry ESBL genes (2, 8), a feature that has been confirmed by our data (of the tested microorganisms, >80% were multiresistant and 8.1% produced ESBLs).

The most-common ESBLs found were CTX-M enzymes, including specifically CTX-M-15, which according to other epidemiological data indicates a global dissemination of this enzyme group, especially among urinary *E. coli* (2). This study provides further evidence of the dissemination of CTX-M-type ESBLs, most notably CTX-M-15, in the community.

Another alarming threat is the recent description of plasmid-mediated fluoroquinolone resistance (33). Even if this phenomenon is rare and its clinical impact is still unknown, this possibility has further complicated the scenario. Two out of the 29 Italian isolates tested (6.8%) carried a *qnrB*-like gene; this relatively high observed rate deserves further investigation in order to understand the true incidence of these genes in our country. To our knowledge, this is the first detection of a *qnr* gene in Russia, while in Spain and Italy, other authors have already described *qnrA*- and *qnrS*-like genes (1, 21). The ciprofloxacin MICs for these six strains ranged from 2 to 128 μg/ml; the coexistence of other mechanisms of resistance to fluoroquinolone has not been investigated, but it could be the reason for this variability (40). Only one of the six *qnrB*-positive strains found carried an ESBL. Studies looking for *qnr* genes only among ESBL producer strains can underestimate the true incidence of this trait.

It is reassuring that fosfomycin and amdinocillin are active against urinary *E. coli* strains, irrespective of other resistance traits (12, 19), a notion confirmed by our data.

The ARESC study's data confirmed high rates of ciprofloxacin resistance in countries such as Spain and Italy but showed

TABLE 5. Characterization of fluoroquinolone resistance mechanisms in selected ciprofloxacin-resistant UTI isolates[a]

Isolate	Clonal group	RAPD profile	PFGE profile	Ciprofloxacin MIC (μg/ml)	OMP profile	LPS type	Alterations in GyrA	Alterations in ParC	OST
51	O15:K55:H1	Q	NA	16	OmpC, OmpF	S	S83L D87N	S80I	−
77	O25:H4 ST-131	NA	A1	32	OmpC, OmpF	S	S83L D87N	S80I E84V	−
88	O25:H4 ST-131	NA	A7	128	OmpC, OmpF	S	S83L D87N	S80I E84V	+
94	O25:H4 ST-131	NA	A6	64	OmpC	S	S83L D87N	S80I E84V	−
120	O25:H4 ST-131	NA	A3	64	OmpC	S	S83L D87N	S80I E84V	−
138	O25:H4 ST-131	NA	A	32	OmpC, OmpF	S	S83L D87N	S80I E84V	−

[a] NA, not available; S, smooth; OST, organic-solvent tolerance.

also that the phenomenon is rampant in other European countries, most notably Russia (26).

A significant increase in the rates of fluoroquinolone resistance in all the countries participating in the ARESC study has also been recorded during the same period (2003 to 2006) by the EARSS (European Antimicrobial Resistance Surveillance System) network (http://www.rivm.nl/earss) among invasive isolates of *E. coli* in these countries, with the exception of Russia (this country did not participate to the EARSS network).

As expected, the rates of fluoroquinolone resistance observed among nosocomial invasive isolates were higher than those observed in our study, but similarly, Spain and Italy were the countries most affected in those years (with percentages ranging from 21% to 28%), while France was characterized by the lowest percentage of fluoroquinolone-resistant *E. coli* strains (from 9.3% in 2003 to 13.8% in 2006).

Our results are in accordance with previous data reporting that the major risk factors for fluoroquinolone-resistant *E. coli* are recent fluoroquinolone use, recurrent UTI, and older age (11, 12, 20, 41).

Although consumption has been reported as the main driving force selecting resistance for fluoroquinolones, a statistically significant correlation between consumption of quinolones and *E. coli* resistance to ciprofloxacin was not always found (11, 34). Some authors have therefore emphasized that the introduction of drug-resistant *E. coli* clonal strains into a community plays a greater role in changing the prevalence of drug-resistant UTIs than does the drug usage or the prescribing habits in that community (36).

To our knowledge, this is the first wide molecular analysis of European isolates with reduced susceptibility to ciprofloxacin causing uncomplicated cystitis.

Our data show that the spread of two ciprofloxacin-resistant clonal groups, O15:K52:H1 and O25:H4 ST-131, causing uncomplicated UTIs in the community has taken place, at least in some European countries, and this phenomenon has to be taken into account together with fluoroquinolone consumption to better understand the present scenario.

About one-third of the analyzed strains belonged to these two main clonal groups. The clonality was evident from their homogeneity with respect to phylogenetic group, uniform serotype, and biochemical profile and the similarity of their PFGE, MLST, or RAPD profiles.

E. coli ciprofloxacin-resistant strains have been reported to belong mainly to the commensal phylogenetic groups A and, to a lesser extent, B1 (17). In our collection of ciprofloxacin-resistant strains, clonal spread of O15:K52:H1 and O25:H4 ST-131 strains can explain the high percentage of strains belonging to the two virulent phylogenetic groups B2 and D (49.3%).

In Italy and Spain, strains belonging to these two clonal groups played an important role in determining the present high rate of resistance (34.4% and 40.0% of the fluoroquinolone-resistant strains isolated in Spain and Italy, respectively, belonged to these lineages) (Table 4), while in Russia and Poland, multiple clones belonging to the less-virulent, commensal phylogroups A and B1 have emerged as the major groups responsible for resistance, since they represented 65.8% and 100% (6 out of 6) of the isolates analyzed from these two countries.

In Spain, clonal spread of *E. coli* strains causing community-acquired UTI has already been observed by other authors (9), while in Italy this is the first documentation.

In Germany, a more-heterogeneous distribution was found, and in France, strains belonging to the two main clonal groups were detected, but at present they seem not able to spread in this country. The reasons why these clonal groups are capable of spreading elsewhere and have not duplicated this behavior in France are unknown. The differences observed among the participating countries cannot be ascribed to an unbalanced number of uropathogens versus colonizer strains. All ARESC participating laboratories used the same criteria for reporting significant organisms in urinary infections, and as a consequence, only true pathogens have been included in the study.

Possibly, different prescribing habits, hygienic food processing (fluoroquinolone-resistant *E. coli* strains may be acquired from exogenous reservoirs, including the food supply) (15), and total antibiotic consumption may all have contributed to this heterogeneous scenario in Europe.

The O15:K52:H1 clonal group is a globally distributed extraintestinal pathogen, often associated with resistance to multiple antimicrobial drugs (14). Members of this serotype first gained attention during a community outbreak of drug-resistant UTIs in London, England, in 1986 to 1987 (31). The serotype was subsequently identified among blood isolates in Copenhagen, Denmark, and urine and blood isolates in Spain (9, 29, 32), where, in comparison with other *E. coli* isolates, it more often infected younger hosts and caused pyelonephritis. Two major biotypes, differing only with respect to their lactose fermentation abilities, were identified (32). In our study, the members of the O15:K52:H1 clonal group were all lactose nonfermenting, resistant to fluoroquinolones, and not more related to recurrences or to infections in young women than the other ciprofloxacin-resistant strains studied. It is possible that strains belonging to this peculiar biotype and antibiotype could be less aggressive than O15:K52:H1 strains previously described.

Recently, the intercontinental emergence of the ciprofloxacin-resistant *E. coli* O25:H4 ST-131 clonal group producing CTX-M-15 and characterized by an extensive virulence profile has been described in the hospital and community settings of several countries, including France, Portugal, Canada, Korea, Spain, Lebanon, and Switzerland (28). In our study, we found members of this clonal group in additional countries, such as Italy, Russia, Hungary, Austria, and Germany, but only four strains isolated in Italy carried the CTX-M-15 enzyme.

Because of its wide distribution, the O25:H4 ST-131 clonal group represents a highly epidemic group that is able to acquire different mechanisms of resistance, sometimes including ESBL production.

The main mechanism of ciprofloxacin resistance in the O15:K52:H1 and O25:H4 ST-131 clonal groups involves mutations in both *gyrA* and *parC*. In particular, among the members of the O25:H4 ST-131 clonal group studied, the same mutations were found. The number of strains analyzed was limited; however, this additional information can contribute support to the hypothesis of a common origin for these microorganisms. While all members of the O15:K52:H1 clonal group studied

had a ciprofloxacin MIC not exceeding 16 μg/ml, more alarming is the spread of strains belonging to the O25:H4 ST-131 clonal group that can reach ciprofloxacin MICs of >32 μg/ml due to additional OMP loss or efflux pump overexpression.

The emergence of *E. coli* multidrug-resistant and virulent clonal groups that may be spreading rapidly in the community poses a significant public health threat (22, 28). The pandemic clonal dispersal of drug-resistant pathogens, related to unsuccessful antimicrobial treatment and clinical failures, has precedent in other species, such as methicillin-resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumoniae* (24, 30). The implementation of appropriate intervention is urgently needed in order to avoid further expansion of this phenomenon in *E. coli*.

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