JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2000, p. 201–209 Vol. 38, No. 1
0095-1137/00/$04.00+0


# *Escherichia coli* Serotype O15:K52:H1 as a Uropathogenic Clone

GUILLEM PRATS,[1]* FERRAN NAVARRO,[1] BEATRIZ MIRELIS,[1] DAVID DALMAU,[2] NURIA MARGALL,[1] PERE COLL,[1] ADAM STELL,[3] AND JAMES R. JOHNSON[3]

*Departament de Microbiologia, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma, 08025 Barcelona,[1] and Departament de Medicina, Unitat de Malalties Infeccioses, Hospital Mutua de Terrassa, 08221 Terrassa,[2] Spain, and Minneapolis Veterans Affairs Medical Center and Department of Medicine, University of Minnesota, Minneapolis, Minnesota[3]*

Received 27 July 1999/Returned for modification 23 September 1999/Accepted 8 October 1999

**To clarify the clinical and bacteriological correlates of urinary-tract infection (UTI) due to *Escherichia coli* O15:K52:H1, during a 1-year surveillance period we prospectively screened all 1,871 significant *E. coli* urine isolates at the Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, for this serotype and assessed the epidemiological features of community-acquired UTI due to *E. coli* O15:K52:H1 versus other *E. coli* serotypes. We also compared the 25 O15:K52:H1 UTI isolates from the present study with 22 O15:K52:H1 isolates from other, diverse geographic locales and with 23 standard control strains (8 strains from the ECOR reference collection and 15 strains of nonpathogenic O:K:H serotypes) with respect to multiple phenotypic and genotypic traits. Although *E. coli* O15:K52:H1 caused only 1.4% of community-acquired *E. coli* UTIs during the surveillance period, these UTIs were more likely to present as pyelonephritis and to occur in younger hosts, with similar risk factors, than were UTIs due to other *E. coli* serotypes. Irrespective of geographic origin, *E. coli* O15:K52:H1 strains exhibited a comparatively restricted repertoire of distinctive virulence factor profiles (typically, they were positive for *papG* allele II, *papA* allele F16, and *aer* and negative for *sfa*, *afa*, *hly*, and *cnf1*), biotypes, ribotypes, and amplotypes, consistent with a common clonal origin. In contrast, their antimicrobial resistance profiles were more extensive and more diverse than those of control strains. These findings indicate that *E. coli* O15:K52:H1 constitutes a broadly distributed and clinically significant uropathogenic clone with fluid antimicrobial resistance capabilities.**

Lineages of *Escherichia coli* which are overrepresented among clinical isolates from patients with urinary-tract infections (UTI) compared with the fecal flora and which exhibit virulence-associated phenotypic traits such as adhesins, toxins, and certain somatic or capsular polysaccharides are commonly designated "uropathogenic clones" (15, 18, 19, 25, 27, 35, 36, 43, 45, 47). Such virulent clones traditionally have been identified based on their O:K:H serotypes (8, 35, 36, 45), although more recently they have been defined by using multilocus enzyme electrophoresis (MLEE) and ribotyping (2, 8, 10, 18, 19, 27, 39).

Serotype O15:K52:H1, virtually unrecognized until recently, has not traditionally been regarded as one of the classic uropathogenic O:K:H serotypes, which include O6:K2:H1, O4:K12:H5, O1:K1:H7, and others (36, 45). However, O15:K52:H1 achieved notoriety in 1986 to 1987 when strains of this serotype that expressed P fimbriae, produced aerobactin, and displayed an unusual multiple antimicrobial resistance phenotype caused a large-scale epidemic of UTI, septicemia, and diverse other serious extraintestinal infections in south London, England (34, 38). The subsequent recognition of O15:K52:H1 as the second most common serotype among *E. coli* bacteremia isolates at a Copenhagen hospital (in which setting the organism usually arose from a urinary-tract source), together with the observation that the Copenhagen isolates exhibited the same virulence factor profile as the south London outbreak strains, provided further evidence of the pathogenic potential of *E. coli* O15:K52:H1 and suggested that this serotype might constitute a widespread virulent clone (33).

Reinforcing the uropathogenic-clone hypothesis for *E. coli* O15:K52:H1 and extending the known geographic range of this serotype to include southern Europe was the finding that in the early 1990s *E. coli* O15:K52:H1 (always from a urinary-tract source) accounted for 7 of 160 *E. coli* bacteremia isolates (4.4%) at the Hospital Mutua de Terrassa in Terrassa, Spain (9), a remarkable prevalence for an organism not generally recognized as a uropathogen. In view of these results, we performed a prospective epidemiological study at the Hospital de la Santa Creu i Sant Pau in Barcelona to determine the prevalence of this serotype as a cause of UTI and to characterize the clinical correlates of UTI due to such strains. We also investigated the virulence traits and other bacteriological characteristics of O15:K52:H1 strains from Barcelona, in comparison with those of O15:K52:H1 strains from other geographic locales and those of reference strains of *E. coli*.

(This work was presented in part at the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Ontario, Canada, 1997 [abstr. K-195]).

## MATERIALS AND METHODS

**Urine study.** A total of 24,462 urine specimens, comprising all urine specimens submitted to the clinical microbiology laboratory of the Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, from June 1994 through May 1995, were examined microscopically for epithelial cells, leukocytes, erythrocytes, and microorganisms by using both unstained centrifuged urine and Gram-stained uncentrifuged urine. Pyuria was defined as the presence of >5 leukocytes per 400× field in the sediment of 10 ml of centrifuged urine. Culture was done by inoculating 10 μl of urine onto CLED agar (Oxoid, Unipath Ltd., Basingstoke, England) and other media when suggested by the Gram stain results. After incubation at 37°C for 24 h, colonies were enumerated. Cultures were interpreted as positive or negative for UTI according to standard criteria endorsed by the

* Corresponding author. Mailing address: Departament de Microbiologia, Hospital de la Santa Creu i Sant Pau, Av. Sant Antoni Mª Claret, 167, 08025 Barcelona, Spain. Phone: 34 93 2919071. Fax: 34 93 2919070. E-mail: 2175@hsp.santpau.es.

American Society for Microbiology based on the colony count, the urinalysis findings, and patient-specific clinical data as provided on the request slip (16). Organisms were identified by standard methods (28). Significant *E. coli* isolates were serotyped as described below.

**Bacteremia isolates.** Blood culture was performed by using the BacT/ALERT blood culture automatic management and reading system (Organon Teknika, Turnhout, Belgium). All *E. coli* isolates were serotyped as described below.

**Serotyping.** Serotyping was done according to the methods of Orskov and Orskov (35) by using O15, K52, and H1 antisera provided by the Statens Seruminstitut (Copenhagen, Denmark). All significant *E. coli* strains isolated from urine and blood during the study period were tested for the O15 somatic antigen by slide and tube agglutination with O15 antiserum. Strains that were O15 positive were tested for the K52 capsular antigen by countercurrent immunoelectrophoresis and for the H1 flagellar antigen by slide and tube agglutination. All *E. coli* O15 strains that were negative for the K52 antigen in the initial screen were sent to the Statens Seruminstitut or the National Institute of Public Health and Environmental Protection (Bilthoven, The Netherlands) for serotyping. Nonencapsulated and nonmotile strains were designated as $K^-$ and $H^-$, respectively, whereas encapsulated strains for which the K or H antigen could not be typed were designated as KN or HN. *E. coli* O15 strains that had the K52 and/or the H1 antigen, and no other K or H antigen, were putatively considered to be O15:K52:H1.

**Clinical data.** Epidemiological data were collected from all patients with community-acquired *E. coli* UTI during a 3-month window within the study period and from all subjects with community-acquired UTI due to *E. coli* O15:K52:H1 at any point during the 1-year study period. (Community-acquired UTI was defined as a UTI episode in which, at the time the index urine sample was submitted, the patient either was not hospitalized or had been hospitalized for <48 h and had not been previously hospitalized during the preceding 28 days.) The data collected included age, gender, clinical manifestations of UTI (dysuria, frequency, urgency, fever, and fever plus flank pain or tenderness, i.e., pyelonephritis), and putative predisposing factors (sexual intercourse, pregnancy, and underlying urological or medical conditions). Data collection was done by medical record review or by interviews with the subject's primary physician, in accordance with the guidelines of the hospital's institutional review board.

**Other O15:K52:H1 strains and non-O15:K52:H1 controls.** For comparison with the O15:K52:H1 strains isolated in the present study, 22 other O15:K52:H1 strains were collected from various geographic sources, including seven bacteremia isolates from Terrassa, Spain (9), nine UTI isolates from Lugo, Spain (generously provided by J. Blanco), one ascitic fluid isolate from Barcelona, and five isolates of unknown clinical source from London (generously provided by I. Phillips) (38). Also studied were eight *E. coli* strains from the 72-member ECOR reference collection of natural *E. coli* isolates (32), which have been assigned to four major phylogenetic groups (A, B1, B2, and D) according to their relatedness by MLEE (14). The eight strains included ECOR strains 7 (group A; O85:HN), 10 (group A; O6:H10), 24 (group A; O15:$H^-$), 30 (group B1; O113:H21), 41 (group D; O7:$H^-$), 59 (group B2; O4:H40), 62 (group B2; O2:$H^-$), and 72 (group B1; O144:H8). (O:H serotypes for the ECOR strains were as determined by R. Wilson using standard methods.) ECOR strains were obtained from the American Type Culture Collection (Manassas, Va.) as ATCC 35326, ATCC 35329, ATCC 35343, ATCC 35349, ATCC 35360, ATCC 35378, ATCC 35381, and ATCC 35391, respectively. As putative nonpathogenic controls, 15 *E. coli* strains with O:K serotypes not corresponding to any recognized *E. coli* pathogenic group (43) were obtained from the National Institute of Public Health and Environmental Protection of The Netherlands. These included strains H93-425 (O122:KN), H93-428 (O145:$K^-$), H93-435 (O23:K18), H93-443 (O39:K1), H93-465 (O64:KN), H93-466 (O14:K1), H94-27 (O132:KN), H94-106 (O73:$K^-$), H94-122 (O5:$K^-$), H94-130 (O17:K1), H94-170 (O169:KN), H95-41 (O5:KN), H95-75 (O12:$K^-$), H95-84 (O108:$K^-$), and H95-133 (O69:KN).

**Antimicrobial resistance profiles.** Susceptibility to 27 antimicrobial agents was determined by a disk diffusion method. The agents assayed were ampicillin, amoxicillin-clavulanic acid, ticarcillin, piperacillin, cefazolin, cefuroxime, cefoxitin, cefixime, cefotaxime, ceftazidime, aztreonam, imipenem, chloramphenicol, tetracycline, streptomycin, kanamycin, neomycin, gentamicin, tobramycin, amikacin, sulfonamide, trimethoprim, trimethoprim-sulfamethoxazole, nalidixic acid, norfloxacin, ciprofloxacin, and nitrofurantoin. Procedures and interpretive criteria were as proposed for *Enterobacteriaceae* by the National Committee for Clinical Laboratory Standards (30). The antibiotic resistance score was the number of agents to which a strain was found to be resistant.

**Biotyping.** The biotype for 12 metabolic reactions was determined according to the system proposed by Richard (40). Similarities between pairs of strains with respect to 12-digit binary biotype results were scored by the simple matching coefficient, and strains were clustered on the basis of their degree of similarity by the method of weighted average linkage by using the TAXAN program (Information Resources Group, University of Maryland, College Park).

**Production of hemolysin and aerobactin.** Isolates were considered alpha-hemolysin positive if they showed clearing around or beneath bacterial colonies on 5% sheep blood agar after 18 h of incubation at 37°C (3). Aerobactin production was determined by using a standard cross-feeding bioassay in minimal medium agar containing 5 g of Casamino Acids/liter, 2 g of glucose/liter, 50 mg of thiamine/liter, 20 mg of tryptophan/liter, and 160 μM 2,2′-dipyridyl (17). The aerobactin-requiring strain *E. coli* CCUG 29422 was used as an indicator strain, and the pColV+ aerobactin-producing strain *E. coli* CCUG 29423 was used as a positive control. (All CCUG control strains in the present study were obtained from the Culture Collection, University of Göteborg, Göteborg, Sweden.)

**Expression of type 1 fimbriae and MRHA.** Expression of type 1 fimbriae was determined, as previously described, by 5% alpha-methyl-D-mannoside-sensitive agglutination of *Saccharomyces* (11). Expression of P and non-P mannose-resistant (NPMR) adhesins was determined by using microscope slide assays as previously described (20, 23) with plate-grown bacteria suspended in phosphate-buffered saline (pH 7.4) (PBS) plus 5% alpha-methyl-D-mannoside as the agglutinator, 5% human A1P1 erythrocytes in PBS as the agglutination substrate, pigeon egg white as a P adhesin-specific inhibitor, and PBS and 3% (wt/vol) bovine serum albumin in PBS (BSA) as negative control inhibitors. Slides were rocked at 4°C for at least 1 min and examined both grossly and microscopically for evidence of mannose-resistant hemagglutination (MRHA), which was graded by intensity as 0 to 5+. P-pattern MRHA was defined as a ≥3 level reduction in MRHA intensity in the presence of pigeon egg white compared with PBS or BSA. NPMR hemagglutination (HA) was defined as a ≤2 level reduction in MRHA intensity in the presence of pigeon egg white.

**Study of serum sensitivity.** Resistance to the bactericidal effect of normal human serum (RSB) was determined by a rapid turbidimetric assay. The growth of bacteria in the presence of 35% serum was tested by spectrophotometer absorbance values (Epy System; Sorin bioMedica, Saluggia, Italy) at 0, 30, 60, 90, 120, and 180 min, as described elsewhere (37). All test strains were compared with resistant and sensitive control strains CCUG 31246 and CCUG 31251, respectively.

**Multiplex PCR assays for virulence genes and *papA* alleles.** The genes encoding uropathogenic virulence factors such as pilus associated with pyelonephritis (*pap*), hemolysin (*hly*), aerobactin (*aer*), cytotoxic necrotizing factor 1 (*cnf1*), S and F1C fimbriae (*sfa*), and afimbrial and other Dr-specific adhesins (*afaI*) were amplified as previously described by Yamamoto et al. (48). Strains C7 (*pap sfa cnf1 hly*) and C149 (*afaI aer hly*), generously provided by S. Yamamoto, were used as positive controls (48). The presence of the three *papG* alleles was assessed by using an allele-specific PCR assay as previously described (21, 22). Control strains included wild-type strains IA2 (allele II), U5 (allele III), and J96 (alleles I and III), and recombinant strains JJ48 (allele I), HB101/pDC1 (allele II), and P678-54/pJFK102 (allele III) (21). Selected O15:K52:H1 strains were tested for the 11 known alleles of *papA* (which encodes PapA, the major structural subunit and major antigenic determinant of P fimbriae) by using an allele-specific multiplex PCR assay, as previously described (J. R. Johnson, F. Scheutz, C. C. Fasching, L. van Dijk, and W. Gaastra, Abstr. Am. Soc. Antimicrob. Annu. Meet., abstr. 12200, p. 147, 1998).

**Ribotyping.** DNA was extracted as described by Nastasi et al. (29). Purified DNA (4 mg) was digested with *Bgl*II under the conditions recommended by the manufacturer (Pharmacia Biotech, Uppsala, Sweden). DNA fragments were separated on a 0.6% agarose gel at 30 V overnight. Restriction fragments were transferred under vacuum to nylon membranes (Hybond-N; Amersham, Little Chalfont, England) by Southern blotting. Prehybridization and hybridization were performed as described elsewhere (13). The acetylaminofluorene-labelled 16S plus 23S rRNA from *E. coli* (Eurogentec, Liège, Belgium) was added to the hybridization solution. The hybridization reactions were visualized colorimetrically by immunoenzymatic detection as recommended by the manufacturer. *Xenorhabdus* sp. strain 278 chromosomal DNA (kindly provided by P. D. Grimont, Pasteur Institute, Paris, France) digested with *Eco*RI was used on each gel as a molecular size standard. Ribotyping patterns were compared with the Bio Image system (Genomic Solutions Inc., Ann Arbor, Mich.), and isolates were considered to be identical if there was complete concordance of profiles. Similarities in ribotyping patterns between two strains were scored by the Dice coefficient, and strains were clustered on the basis of their degree of similarity by the unweighted pair-group mean arithmetic method (UPGMA).

**PCR fingerprinting.** Selected strains were subjected to randomly amplified polymorphic DNA (RAPD) analysis using as a primer the decamer oligonucleotide 1290, as previously described (10, 46). Banding patterns in ethidium bromide-stained agarose gels were analyzed digitally by using the applications Molecular Analyst and Molecular Fingerprinting (Bio-Rad, Hercules, Calif.). Dendrograms were constructed by using UPGMA based on Pearson similarity coefficients as derived from pairwise comparisons between densitometric tracks representing each gel lane.

**Statistical analysis.** Differences involving categorical variables were tested for significance with Yates' corrected chi square or, when appropriate, with Fisher's exact test. Differences involving resistance scores were tested by the Mann-Whitney U test. The diversity ($H$) of genotypes or phenotypes was calculated as $H = (1 - \Sigma x_i^2)\ (n/n-1)$, where $x_i$ is the frequency of the $i$th type and $n$ is the number of types (31).

## RESULTS

**Prevalence of *E. coli* O15:K52:H1 in UTI.** The O15 antigen was detected in 25 (1.3%) of the 1,871 *E. coli* isolates obtained from the 3,664 urine specimens that were positive for UTI according to standardized criteria (16) among the 24,462 total

TABLE 1. Clinical manifestations in 429 community-acquired *E. coli* UTI episodes

| Clinical manifestation | % Strains with manifestation[a] | |
|---|---|---|
| | O15:K52:H1 ($n$ = 18) | Other serotypes ($n$ = 411) |
| Pyuria | 89 | 80 |
| Dysuria, frequency, or urgency | 62 | 44 |
| Fever | 56 | 39 |
| Pyelonephritis | 47[b] | 16[b] |

[a] Percentages are based on the number of cases evaluable, not on the total number of UTI episodes.
[b] For the proportion of strains with pyelonephritis, $P < 0.005$. For other manifestations, $P > 0.10$.

urine specimens tested during the 1-year study period. All 25 O15-positive *E. coli* strains met the study definition for O15:K52:H1. They either expressed all three antigens, i.e., were O15:K52:H1 ($n$ = 14) or were missing only the K or the H antigen, i.e., were O15:K$^-$:H1 ($n$ = 7) or O15:K52:H$^-$ ($n$ = 4). O15 strains were similarly prevalent among *E. coli* isolates from community-acquired UTI (18 of 1,259 [1.4%]) and nosocomial UTI (7 of 612 [1.1%]).

**Epidemiology.** To assess the clinical manifestations and underlying host characteristics associated with community-acquired UTI due to *E. coli* O15:K52:H1, a case-control study was done in which subjects who developed community-acquired UTI due to *E. coli* O15:K52:H1 at any time during the study ($n$ = 18) were compared with all evaluable control subjects who developed community-acquired UTI due to *E. coli* of other serotypes during a 3-month observation period ($n$ = 411). Compared with UTI episodes due to non-O15 *E. coli*, episodes due to *E. coli* O15:K52:H1 were significantly more likely to meet criteria for pyelonephritis (Table 1) and to occur in nonelderly hosts (Table 2) ($P < 0.005$ for both comparisons). The mean ages of case and control subjects were 38 years (range, 28 days to 83 years) and 55 years (range, 3 months to 97 years), respectively. Other underlying host characteristics, including predisposing urological and medical conditions, were similarly distributed among case and control subjects (Table 2).

TABLE 2. Associated host characteristics in 429 community-acquired *E. coli* UTI episodes

| Host characteristic | No. (%) with characteristic[a] | |
|---|---|---|
| | O15:K52:H1 ($n$ = 18) | Other serotypes ($n$ = 411) |
| Age of >65 yr | 3 (17)[b] | 213 (52)[b] |
| Female sex | 15 (83) | 334 (81) |
| Sexual intercourse | 4 (24) | 55 (14) |
| Any urological compromise | 3 (17) | 117 (28) |
| Nephrolithiasis | 1 (6) | 34 (8) |
| Instrumentation | 2 (12) | 41 (10) |
| Extrinsic compression | 0 | 30 (7) |
| Pregnancy | 2 (11) | 27 (7) |
| Any medical compromise | 5 (28) | 103 (25) |
| Diabetes mellitus | 2 (11) | 63 (15) |
| Others[c] | 3 (18) | 45 (11) |
| No risk factor[d] | 8 (44) | 180 (44) |

[a] All percentages are based on the number of cases evaluable, not on the total number of UTI episodes.
[b] For the proportion more than 65 years old, $P < 0.005$. For other characteristics, $P > 0.10$.
[c] Including cirrhosis, cancer, chronic renal failure, and corticosteroid treatment.
[d] No urological or medical compromise.

Of the 69 *E. coli* bacteremia isolates obtained during the study period, 2 (2.9%) were O15:K52:H1. (One of these was from a UTI source, the other from an unknown source.) Thus, during the study period O15 strains appeared to be as prevalent a cause of *E. coli* bacteremia (2.9%) as of UTI (1.3%).

**Virulence factor profiles.** The 25 O15:K52:H1 strains from the present study exhibited little diversity with respect to virulence factor profile ($H$ = 0.28). Nineteen (76%) displayed the group's modal profile, which included the presence of *papEF*, *papG* allele II, P pattern MRHA, type 1 fimbrial expression, *aer*, aerobactin production, and serum resistance, plus the absence of *afa*, *sfa*, *hly*, and *cnf*. Four of the remaining six O15 strains from the present study deviated from this pattern by lacking only a single trait, i.e., type 1 fimbriae, Aer, or RSB. All five *papEF*-positive O15:K52:H1 strains from the present study that were tested by multiplex PCR for the 11 known alleles of *papA* were positive for the F16 allele only (data not shown).

The 22 comparison O15:K52:H1 strains had virulence factor profiles similar to those of the 25 index O15 strains (Table 3). Thirteen (59%) exhibited the same modal profile, and seven of the remaining nine deviated from this profile by only one trait (i.e., by lacking RSB or type 1 fimbriae) (Table 3). When the prevalence of individual virulence traits was examined (Table 4), the O15:K52:H1 strains from the present study differed from the comparison O15:K52:H1 strains only with respect to serum resistance, which was slightly less frequent among the comparison O15:K52:H1 strains due to its absence from the four English strains.

Virulence factor profiles among the eight ECOR and 15 nonpathogenic serotype control strains were more diverse than those of the 47 O15:K52:H1 strains ($H$ = 0.74 versus $H$ = 0.43) and overlapped minimally with them (Table 3). Although the most common pattern among the 23 control strains was absence of all measured traits except type 1 fimbriae and serum resistance, this pattern accounted for only 9 (26%) of the control strains, but for only 1 (2%) of the 47 O15:K52:H1 strains ($P < 0.001$ for O15:K52:H1 strains versus controls). Conversely, only three (13%) of the control strains exhibited the modal pattern of the O15:K52:H1 strains ($P < 0.001$ for O15:K52:H1 strains versus controls).

The 47 O15:K52:H1 strains differed significantly both from the 8 ECOR strains and from the 15 other control strains in having a higher prevalence of *papEF*, P pattern MRHA, *papG*, *aer*, and aerobactin production. When the 23 control strains were combined, the lower prevalence of serum resistance among the O15:K52:H1 strains also was statistically significant (Table 4).

**Antimicrobial resistance.** Among the 25 *E. coli* O15:K52:H1 isolates from the present study, eight different antimicrobial resistance patterns were detected (Table 5). The predominant pattern included resistance to penicillins (compatible with plasmid-mediated beta-lactamase production, as supported in five strains by the detection in isoelectric focusing of an enzyme compatible with TEM-1 [data not shown]), accompanied by resistance to streptomycin, kanamycin, neomycin, sulfonamides, trimethoprim, tetracycline, and nalidixic acid. The 22 comparison O15:K52:H1 strains also exhibited diverse susceptibility patterns ($n$ = 10), ranging from fully susceptible (the predominant pattern) to multiply resistant, although none exhibited the predominant resistance pattern of the isolates from the present study. Compared with isolates from the present study, the other O15:K52:H1 strains had a lower prevalence of

TABLE 3. Virulence factors of all studied *E. coli* strains

| No. of strains with profile | | | | | | | | | Adhesin profile[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| O15:K52:H1 | | | | | Control | | All O15 strains ($n$ = 47) | All control strains ($n$ = 23) | | | | | | | | | | |
| Present study ($n$ = 25) | Lugo ($n$ = 9) | Terrassa ($n$ = 7) | Barcelona ($n$ = 1) | England ($n$ = 5) | ECOR ($n$ = 8) | Nonpathogenic ($n$ = 15) | | | *papEF* | *papG* allele | *afa* | *sfa* | MRHA | t1f | *hly* | aer | Aer | RSB |
| 19 | 7 | 5 | 0 | 1 | 2 | 1 | 32 | 3 | + | II | − | − | P | + | − | + | + | + |
| 2 | 0 | 1 | 0 | 4 | 0 | 0 | 7 | 0 | + | II | − | − | P | + | − | + | + | − |
| 1 | 1 | 1 | 0 | 0 | 0 | 0 | 3 | 0 | + | II | − | − | P | − | − | + | + | + |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | + | II | − | − | P | + | − | + | − | + |
| 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | + | II | − | − | P | + | + | + | + | + |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | + | − | + | − | NPMR | + | − | + | + | + |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | + | − | − | − | − | + | − | + | + | + |
| 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | − | − | − | − | NPMR | + | − | + | + | + |
| 1 | 0 | 0 | 0 | 0 | 1 | 5 | 1 | 6 | − | − | − | − | − | + | − | + | + | + |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | − | − | − | + | − | + | − | + | + | + |
| 0 | 1 | 0 | 0 | 0 | 3 | 6 | 1 | 9 | − | − | − | − | − | + | − | − | −/U | + |
| 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | − | − | − | − | − | + | − | − | − | − |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | − | − | − | − | − | − | − | − | U | + |

[a] All strains were *cnf* negative. t1f, type 1 fimbriae; Aer, aerobactin production; U, uninterpretable.

resistance to any antimicrobial agent (13 of 22 versus 23 of 25; $P = 0.01$), a lower median resistance score (3 versus 10; $P = 0.003$), and a lower prevalence of quinolone resistance (0 of 22 versus 15 of 25; $P < 0.001$). The Terrassa isolates, which were isolated in 1988 to 1991, i.e., chronologically between the English strains (1986 to 1987) and those from the present study (1994 to 1995), were generally quite sensitive (four of seven were fully susceptible). The hallmark $Amp^r$ $Str^r$ $Sul^r$ $Tmp^r$ $Tec^r$ $Chl^r$ pattern of the 1986-to-1987 London outbreak was confined to the English O15 strains (4 of 5 versus 0 of 42; $P < 0.001$).

The susceptibility patterns of the 23 control strains overlapped minimally with those of the 47 O15 strains. Compared with the O15 strains, the control strains exhibited less diversity of susceptibility patterns ($H = 0.73$ versus $H = 0.80$), a lower prevalence of any resistance (11 of 23 [48%], versus 36 of 47 [76%]; $P = 0.016$), and a lower median resistance score (7 versus 1; $P < 0.001$).

**Biotyping.** In a similarity dendrogram based on the 12-digit binary biotype code, all but 3 (i.e., 94%) of the 47 O15:K52:H1 strains were clustered together at the 83% similarity level, with no intermixed control strains (Fig. 1). The two major biotypes, which accounted for 41 (87%) of the O15:K52:H1 strains, differed only with respect to lactose fermentation. In contrast, biotypes were more numerous and more diverse among the control strains ($H = 0.84$ versus $H = 0.51$ for the O15:K52:H1 strains).

**Ribotyping.** Ribotyping was used as a molecular typing method for a subset of the O15:K52:H1 strains (nine from the present study and nine from other locales) and for the eight ECOR strains. All but 3 of the 18 O15:K52:H1 strains exhibited a single ribotype (A), which was unique to these strains (Fig. 2). The three other O15:K52:H1 strains (36-P, 8-P, and 42-P) exhibited unique ribotypes which clustered with ribotype A at homology levels of 89, 60, and 49%, respectively (Fig. 2). The farthest removed of these strains (42-P) was from the present study, had serotype O15:$K^-$:H1, lacked *pap*, and was *aer*$^+$; the intermediate strain (8-P) was from Lugo, had serotype O15:K52:$H^-$, and was *pap*$^+$ *aer*$^+$; and the closest strain (36-P) was from the present study, had serotype O15:K52:H1, and was *pap*$^+$ *aer*$^+$. All three strains had biotypes that fell within the major O15 biotype cluster (Fig. 1).

TABLE 4. Prevalence of virulence factors among O15:K52:H1 and control strains

| Virulence factor[a] | No. (%) of strains with virulence factor | | | | | |
|---|---|---|---|---|---|---|
| | O15:K52:H1 | | | Control | | |
| | Present study ($n$ = 25) | Other ($n$ = 22) | Total ($n$ = 47) | ECOR ($n$ = 8) | Nonpathogenic serotypes ($n$ = 15) | Total ($n$ = 23) |
| *pap*EF | 23 (92) | 20 (91)[b] | 43 (91)[c] | 3 (38) | 3 (20)[d] | 6 (26)[d] |
| MRHA or *pap*G | 23 (92) | 20 (91)[b] | 43 (91) | 3 (38)[c] | 1 (7)[d] | 4 (17)[d] |
| t1f | 23 (92) | 20 (91)[b] | 43 (91) | 8 (100)[e] | 15 (100)[e] | 23 (100)[e] |
| *aer* or Aer | 24 (96) | 20 (91)[b] | 44 (94) | 5 (63)[f] | 8 (53)[g] | 13 (57)[g] |
| RSB | 23 (92) | 16 (73)[b] | 39 (83)[h] | 8 (100) | 15 (100)[h] | 23 (100)[i] |

[a] t1f, type 1 fimbriae; Aer, aerobactin production.
[b] $P > 0.1$ for strains in the present study versus other O15 strains.
[c] $P < 0.01$ for total O15:K52:H1 strains versus ECOR strains.
[d] $P < 0.001$ for total O15:K52:H1 strains versus nonpathogenic serotypes or total control strains.
[e] $P > 0.1$ for total O15:K52:H1 strains versus ECOR strains, nonpathogenic serotypes, or total control strains.
[f] $P < 0.05$ for total O15:K52:H1 strains versus ECOR strains.
[g] $P < 0.01$ for total O15:K52:H1 strains versus nonpathogenic serotypes or total control strains.
[h] $P > 0.1$ for total O15:K52:H1 strains versus ECOR strains or nonpathogenic serotypes.
[i] $P < 0.05$ for total O15:K52:H1 strains versus total control strains.

TABLE 5. Resistance patterns of all studied *E. coli* strains

| No. of strains with pattern | | | | | | | | | Resistance pattern[a] |
|---|---|---|---|---|---|---|---|---|---|
| O15:K52:H1 | | | | | Control | | | | |
| Present study ($n$ = 25) | Lugo ($n$ = 9) | Terrassa ($n$ = 7) | Barcelona ($n$ = 1) | England ($n$ = 5) | ECOR ($n$ = 8) | Nonpathogenic ($n$ = 15) | All O15 strains ($n$ = 47) | All control strains ($n$ = 23) | |
| 12 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | Amp$^r$ Tic$^r$ Pip$^r$ Str$^r$ Kan$^r$ Neo$^r$ Tec$^r$ Sul$^r$ Tmp$^r$ Nal$^r$ |
| 3 | 1 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | Amp$^r$ Tic$^r$ Pip$^r$ Str$^r$ Sul$^r$ |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | Str$^r$ Tec$^r$ Sul$^r$ Tmp$^r$ Nal$^r$ |
| 1 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | Amp$^r$ Tic$^r$ Pip$^r$ Str$^r$ Tec$^r$ Sul$^r$ Tmp$^r$ |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | Amp$^r$ Tic$^r$ Pip$^r$ Str$^r$ Tec$^r$ Sul$^r$ Tmp$^r$ Nal$^r$ |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | Amp$^r$ Tic$^r$ Pip$^r$ Str$^r$ Kan$^r$ Neo$^r$ Chl$^r$ Tec$^r$ Sul$^r$ Tmp$^r$ Nal$^r$ Cip$^r$ |
| 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | Amp$^r$ Tic$^r$ Pip$^r$ Str$^r$ Chl$^r$ Sul$^r$ Tmp$^r$ |
| 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 | 0 | Amp$^r$ Tic$^r$ Pip$^r$ Str$^r$ Kan$^r$ Neo$^r$ Chl$^r$ Tec$^r$ Sul$^r$ Tmp$^r$ |
| 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | Str$^r$ Tmp$^r$ |
| 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | Str$^r$ Sul$^r$ Tmp$^r$ |
| 0 | 0 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | Amp$^r$ Tic$^r$ Pip$^r$ Str$^r$ Chl$^r$ Tec$^r$ Sul$^r$ Tmp$^r$ |
| 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | Amp$^r$ Tic$^r$ Pip$^r$ Str$^r$ Chl$^r$ Tec$^r$ Sul$^r$ |
| 2 | 5 | 4 | 0 | 0 | 3 | 9 | 11 | 12 | |
| 4 | 1 | 0 | 0 | 0 | 1 | 0 | 5 | 1 | Amp$^r$ Tic$^r$ Pip$^r$ |
| 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | Str$^r$ Nit$^r$ |
| 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | Str$^r$ Sul$^r$ |
| 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | Str$^r$ Tec$^r$ |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | Amp$^r$ Tic$^r$ Pip$^r$ Chl$^r$ Tec$^r$ Tmp$^r$ |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | Amp$^r$ Tic$^r$ Pip$^r$ Sul$^r$ Tmp$^r$ |
| 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | Str$^r$ Tec$^r$ Sul$^r$ |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | Tec$^r$ |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | Amp$^r$ Tic$^r$ Pip$^r$ Str$^r$ Sul$^r$ Tmp$^r$ |

[a] Resistance phenotypes are as follows: Amp, ampicillin; Tic, ticarcillin; Pip, piperacillin; Str, streptomycin; Kan, kanamycin; Neo, neomycin; Chl, chloramphenicol; Tec, tetracycline; Sul, sulfonamides; Tmp, trimethoprim; Nal, nalidixic acid; Cip, ciprofloxacin; Nit, nitrofurantoin.

In contrast to the O15:K52:H1 strains, the eight ECOR strains exhibited unique and highly diverse ribotypes ($H$ = 0.86 versus $H$ = 0.26 for the O15:K52:H1 strains). Seven of the eight ECOR strains were grouped loosely together in a cluster that was joined to the O15:K52:H1 cluster at a low similarity level of 18%. In contrast, ECOR strain 41 (group D) was placed in the same cluster as the O15:K52:H1 strains and was actually more similar to the farthest removed O15:K52:H1 strain (42-P) than this strain was to the other O15:K52:H1 strains.

**RAPD fingerprinting.** RAPD fingerprints were generated for five O15:K52:H1 strains, including two representatives of ribotype A (one from the present study and one from England) plus the sole representatives of each of the three outlier O15:K52:H1 ribotypes, and for five ECOR strains, i.e., strains 24 (group A), 30 (group B1), 41 (group D), 62 (group B2), and 72 (group B1) (14). Cluster analysis yielded groupings that were largely similar to those seen with ribotyping (data not shown). In replicate runs all the O15:K52:H1 strains (excepting occasionally outlier 42-P) clustered with one another and apart from the ECOR strains. The nearest neighbor to the O15:K52:H1 strains was either ECOR strain 41 (group D) alone, as in ribotyping, or a cluster containing ECOR strains 41 (group D) and 62 (group B2) (data not shown).

## DISCUSSION

In the present study we determined the prevalence of *E. coli* O15:K52:H1 as a cause of UTI and bacteremia during a 1-year comprehensive surveillance of all urine and blood isolates at a hospital clinical microbiology laboratory in Barcelona, Spain, and we assessed the clinical manifestations and underlying host characteristics associated with community-acquired UTI due to this organism. We also compared the O15:K52:H1 UTI isolates from the present study with O15:K52:H1 isolates from other locales in Spain and England and with two groups of non-O15:K52:H1 control strains, analyzing multiple phenotypic and genotypic traits. We found that although O15:K52:H1 strains caused only a small fraction of all UTI, when they did cause community-acquired UTI they behaved as aggressively as or more aggressively than other *E. coli* strains. We also found that O15:K52:H1 strains of diverse geographic origins were similar to one another but distinct from control *E. coli* strains with respect to virulence factor profiles, biotypes, ribotypes, and amplotypes, evidence that *E. coli* O15:K52:H1 constitutes a widely disseminated uropathogenic clone.

Several observations testify to the virulence of *E. coli* O15:K52:H1. Our finding that this organism accounted for only a small fraction (1.3%) of the total 1,871 *E. coli* isolates from patients with UTI during the 1-year prospective surveillance period might be interpreted as evidence that it is not a significant uropathogen. However, although precise data are lacking, 1.3% is probably higher than the frequency of most serotypes in the *E. coli* population as a whole, which has been estimated to contain as many as 1,000 distinct clones (41). Thus, O15:K52:H1 strains indeed may be overrepresented among UTI isolates, which is one criterion of uropathogenicity. Additionally, in our case-control study of community-acquired UTI, *E. coli* O15:K52:H1 strains exhibited a level of clinical virulence equal to or greater than that of other urinary *E. coli* strains. Compared with UTI episodes due to other *E. coli* strains, the O15-associated episodes were as likely to be accompanied by pyuria, voiding symptoms, and fever and were

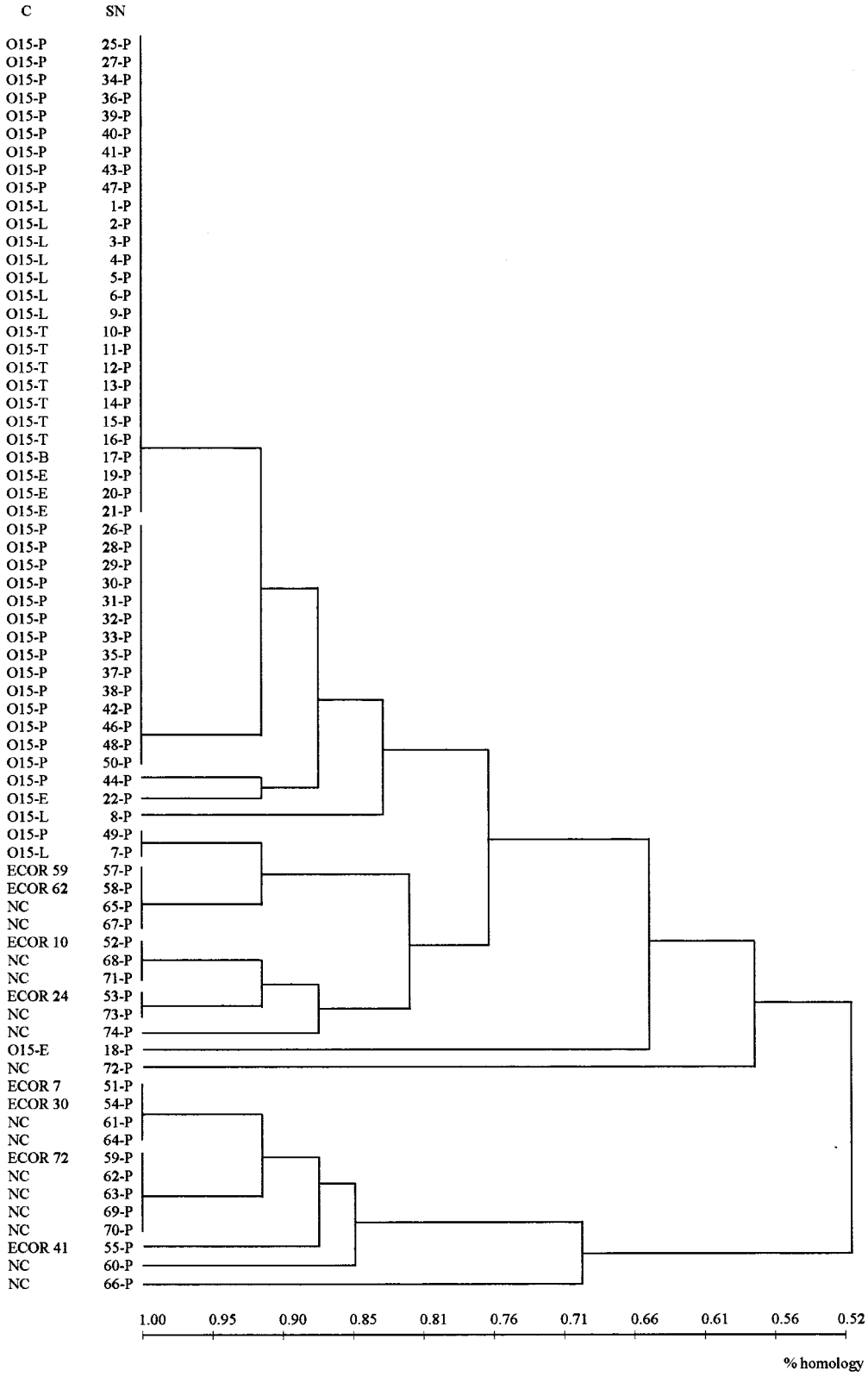


FIG. 1. Dendrogram depicting the degrees (percentages) of biotype homology among the 70 studied strains. The O15-P, O15-L, O15-T, O15-B, and O15-E, O15:K52:H1 strains were from the present study (P), Lugo (L), Terrassa (T), Barcelona (B), and England (E), respectively. NC, nonpathogenic control; C, category, SN, study number.

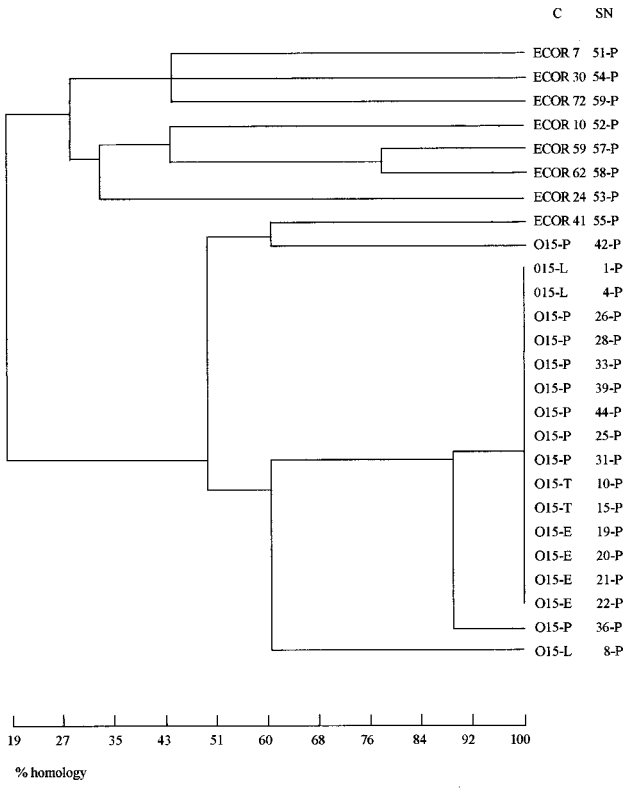


FIG. 2. Dendrogram depicting the degrees (percentages) of ribotype homology among the 27 studied strains. The O15-P, O15-L, O15-T, and O15-E, O15:K52:H1 strains were from the present study (P), Lugo (L), Terrassa (T), and England (E), respectively. C, category, SN, study number.

more likely to present as pyelonephritis and to occur in young hosts. Finally, in the present study *E. coli* O15:K52:H1 appeared to be as prevalent a cause of *E. coli* bacteremia (2.9%) as of *E. coli* UTI (1.3%), a finding consistent with previous reports of the substantial contribution of this serotype to *E. coli* bacteremia in Copenhagen (4.6%) and Terrassa (4.5%) (9, 33). Although based on small numbers (two bacteremia isolates), this observation is consistent with the hypothesis that this organism may have an invasive potential as great as or greater, on average, than that of other urinary *E. coli* strains.

The comparative homogeneity with respect to virulence factor profile, biotype, ribotype, and RAPD fingerprints observed among the 47 O15:K52:H1 strains examined in the present study is consistent with the hypothesis that these strains, whether from Barcelona, elsewhere in Spain, or England, are clonally derived. The finding of a common virulence factor profile and a fairly uniform biotype largely confirms the results of previous studies of O15:K52:H1 strains elsewhere and extends these observations to include Spanish O15:K52:H1 isolates. Novel aspects of the present study are the demonstration of *papG* allele II in *pap*-positive O15:K52:H1 strains; the finding of the F16 *papA* allele in Spanish clone members (which corresponds with the reported F16 fimbrial antigen positivity of Danish O15:K52:H1 strains [33]); the documentation of the absence of *sfa*, *afa*, and *cnf1*; and the ribotype and RAPD results, which provide the first direct genetic evidence of clonality among O15:K52:H1 strains.

Previous investigators have proposed (correctly, as it turns out) that O15:K52:H1 strains are clonally derived (33). This hypothesis has been based in part on the three-antigen serotype itself and in part on these strains' typical homogeneity with respect to virulence traits, biotype, plasmid profile, and (in some instances) antimicrobial resistance profile (33, 38). However, all of these traits are unreliable as indicators of clonality because of their instability, horizontal mobility, and/or nonspecificity (1, 4, 8, 24, 26, 41).

For example, the distinctive multiple antimicrobial resistance phenotype which initially was regarded as a hallmark of the outbreak strain in the London O15:K52:H1 epidemic of 1986 to 1987 (38) was supplanted by a less-resistant phenotype toward the end of that outbreak (34) and was not noted at all among the subsequently reported O15:K52:H1 bacteremia isolates from Copenhagen (33) or Terrassa.

Similarly, the existence of O15:K52:H1 strains that lack *pap* illustrates the variability of virulence factor profiles within a clone (41) and is consistent with the known localization of *pap* on pathogenicity-associated islands (PAIs) (5–7, 44). Although PAIs presumably provide a vehicle through which *pap* and other linked virulence genes can move en bloc into new *E. coli* lineages (7), they also can facilitate spontaneous deletion of virulence genes (6), as may have occurred in some O15:K52:H1 clone members.

Finally, the same O:K:H serotype sometimes occurs in strains from distantly related evolutionary lineages, presumably because of recombination or convergent evolution (8, 41). Thus, the previously available evidence for clonality among O15:K52:H1 strains, although strongly suggestive, has been inconclusive. Since both ribotyping and RAPD fingerprinting correspond to MLEE in defining clonal relationships (2, 10, 27, 39, 46), the present study provides genetic confirmation that O15:K52:H1 strains, irrespective of geographic locale, are clonally derived. Further support for this conclusion is provided by our observation that all O15 strains were refractory to pulsed-field gel electrophoresis (PFGE), an uncommon characteristic observed with certain serovars of *Salmonella* and certain phage types of *E. coli* O157:H7 (B. Swaminathan, personal communication). Analysis of polymorphism of chromosomal macrorestriction fragments by PFGE using standard methods (42) was unsuccessful, because all *E. coli* O15:K52:H1 DNAs were completely sheared during the PFGE process despite the introduction of technical modifications intended to circumvent this problem (not shown). In contrast, control *E. coli* strains of other serotypes were readily analyzed by PFGE (not shown).

The antimicrobial susceptibility results contrast with the results of the other bacteriological typing that was done on the O15 and control strains. Whereas the virulence factor profiles, biotypes, ribotypes, and RAPD amplotypes of the O15:K52:H1 strains were markedly less diverse than those of the control strains and were similar among O15 strains from different geographic locales, the resistance profiles of the O15 strains were actually more diverse than those of the control strains and differed significantly between locales. This is consistent with the expectation that among members of a clone the background genomic structure (as reflected in the ribotype or RAPD amplotype) should be the most highly conserved characteristic, followed by such potentially mobile or variably expressed traits as virulence factor profiles and biotypes, followed finally by the most mobile and selection-prone trait of all, antimicrobial resistance.

The seeming locale-specific nature of the resistance patterns of the O15 strains suggests that in different geographic regions clone members may have encountered distinctive selective environments to which they have adapted extensively, possibly over as short a time span as the past 50 years of antibiotic use by humans. Alternatively, locale-specific differences in the res-

ervoirs of mobile resistance elements available for acquisition by clone members could have driven divergent evolution of resistance patterns. Time of isolation also may be relevant, since the O15 strains from the present study, which were the most highly resistant of all, also were the most recently isolated, and hence may have had more opportunity to acquire resistance than O15 strains isolated earlier, which in contrast may have had more opportunity to lose resistance markers during subculture. Finally, anatomic site of infection may be relevant, since in several bacterial species the bacteremic strains are characteristically more antibiotic sensitive than the nonbacteremic strains (12). This would be fully consistent with the reported susceptibility of the Danish (33) and Terrassa bacteremia isolates.

Our inclusion of diverse members of the ECOR reference collection as control strains permits us to tentatively place the O15:K52:H1 clone on the *E. coli* evolutionary tree within ECOR group D (14). This hypothesis is consistent with the observed *pap*-positive but *hly*-, *sfa*-, and *cnf*-negative virulence profile of this clone, which is characteristic of ECOR group D strains and contrasts with the more extensive virulence factor profiles typical of ECOR group B2 strains (7, 39). It should be noted that the ECOR collection contains a single O15-positive strain, strain 24 (group A), which in the present study was only distantly related to the O15:K52:H1 strains according to ribotype homology (Fig. 2).

In summary, we have shown that strains of *E. coli* O15:K52:H1 constitute a genetically restricted clone that exhibits diverse antimicrobial susceptibility patterns but a limited range of virulence factor profiles and biotypes irrespective of geographic locale. Epidemiological data suggest that although the clone is an infrequent cause of UTI in a non-outbreak setting, when it does cause UTI it exhibits a level of virulence comparable to or greater than that of other *E. coli* strains, which qualifies it to be regarded as a uropathogenic clone.

## ACKNOWLEDGMENTS

We thank I. Phillips and J. Blanco for the gift of *E. coli* O15:K52:H1 strains from England and Lugo, Spain, respectively; S. Yamamoto for the gift of control strains C7 and C149; S. Clegg and S. Hull and R. Hull for providing the *papG* allele II and III controls, respectively; A. Andreu for the gift of the *Saccharomyces* sp. strain for determination of type 1 fimbriae; E. Martínez, P. Rodríguez, and M. E. Sabanés for clinical data collection; F. Scheutz, B. Olesen, F. Sanchez, and T. Llovet for scientific advice; and T. O'Bryan, J. Kavle, P. Delavari, and M. Español for technical assistance.

This work was supported by grant 95/1379 from the Fondo de Investigaciones Sanitarias de la Seguridad Social de España (G.P.), grant DK 47505 from the National Institutes of Health (J.R.J.), and VA Merit Review (J.R.J.).

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